

Croatian ⁵⁹
jsa
2024 ¹⁹
International
Symposium on
Agriculture



Proceedings

Zbornik radova

11 – 16 February 2024, Dubrovnik, Croatia



Proceedings

59
Hrvatski

19
Međunarodni
Simpozij
Agronoma

Zbornik radova

Impressum

Izdavač Published by	Sveučilište u Zagrebu Agronomski fakultet, Zagreb, Hrvatska University of Zagreb Faculty of Agriculture, Zagreb, Croatia
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Oblikovanje - Designed by	Martin Šok, www.martinsok.com
Tisak – Press	Novi val d.o.o.
Naklada – Edition	15

ISSN 2459-5543

Web page <http://sa.agr.hr>

*Službeni jezici Simpozija su hrvatski i engleski.
The official languages of the Symposium are Croatian and English.*

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i
Fakultet agrobiotehničkih znanosti Osijek, Sveučilište Josipa Jurja Strossmayera u Osijeku

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organiziraju

59. hrvatski i 19. međunarodni simpozij agronoma
11. - 16. veljače 2024. godine, Dubrovnik, Hrvatska

Impressum

University of Zagreb Faculty of Agriculture
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under the auspices

Croatian Parliament
Ministry of Agriculture
Ministry of Economy and Sustainable Development
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In collaboration with

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Academy of Agricultural Sciences
Agricultural Institute Osijek
Association for European Life Science Universities (ICA)
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The effect of soil contamination on the chemical composition of goji berry (*Lycium Chinese*) fruits

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Abstract

A field study was conducted to evaluate the chemical composition of goji berry fruits, as well as the possibilities of goji berry cultivation on soils contaminated with heavy metals. The experiment was conducted on an agricultural field contaminated by the Non-Ferrous-Metal Works (NFMW) near Plovdiv, Bulgaria. The concentrations of macroelements, microelements, and heavy metals in goji berry fruits were determined by microwave mineralization. An atomic emission spectroscopy technique was used to define elements. Macronutrients (K, P, Mg, Ca) predominate in goji berry fruits, followed by Zn, Fe, Cu, and Mn. Contamination of the soil with heavy metals does not affect the quality of the goji berry. The high content of macroelements and trace elements and the negligible concentrations of toxic elements in cultivating goji berries in slightly polluted soil makes it possible to use these fruits (fresh or dried) as a food supplement supplying minerals to humans.

Keywords: *Lycium*, toxic elements, nutrients, polluted soils, Bulgaria

Introduction

The goji berry is the fruit of "*Lycium Barbarum*" or "*Lycium Chinense*", an Asian plant of the Solanaceae family. It originates from the temperate and subtropical regions of China, Mongolia, and the Himalayas in Tibet. The goji berry is a perennial, evergreen shrub with small red-orange fruits and yellow, kidney-shaped seeds. The most valuable part of the plant is its fruit (Amagase and Farnsworth, 2011). Bulgaria has the potential and conditions to produce and export goji berries, mainly because of the growing global interest in this fruit. For some time, small plantations of goji berry trees have been established on private farms in the country. Larger plantations have been established in the Plovdiv and Kyustendil regions. Many people also plant single pieces in their gardens and on smaller farms. The fruit contains significant amounts of macronutrients, carbohydrates, protein, fat, and dietary fiber (Wang et al., 2010; Mirecki et al., 2015). There are several publications on the mineral content of goji berry fruits grown in China (Chen et al., 2014; Nascimento et al., 2015), Italy (Niro et al., 2017), Spain (Llorent-Martínez et al., 2013) and Turkey (Endes et al., 2015). Balabanova et al. (2016) investigated the macro and trace element content of goji berry (*Lycium barbarum* L.) fruits grown in different geographical locations (China and Republic of Macedonia). The composition of goji berries and their beneficial properties are primarily due to the minerals contained in them, some of which are essential for the normal functioning of the human body (e.g., Ca, Mg, K, P, Fe, Zn, Cu, Cr, I, Se, etc.) (Seeram, 2010; Mirecki et al., 2015; Gogoasa et al., 2014). It is believed that goji berries can be considered one of the richest fruits containing Fe, Zn, Mn, Cu, and Cr. However, moderate consumption of goji berry fruit is recommended (Balabanova et al., 2016) as a dietary supplement because improper, excessive consumption may overload the body with certain minerals or nutrients, leading to undesirable side effects. Additionally, fruits may also contain some toxic elements (e.g., Hg, Pb, As, Cd, etc.) that can have adverse effects on human health (Gogoasa et al., 2014), as a result of geogenic or anthropogenic (soil and climatic) conditions, or the presence of some pollutants, treatments). While there are a few publications in the literature related to goji berry cultivation in Bulgaria, there is a need for more information regarding the composition of goji berry fruits, the tolerance of goji berries to heavy metals, and the possibilities for cultivation on soils contaminated with heavy metals. The present study aims to determine the nutrients and heavy metal content of goji berry fruit and to identify the possibilities of growing it on metal-contaminated soils.

Material and methods

The experiment was conducted on an agricultural field contaminated by the Non-Ferrous-Metal Works (NFMW) near Plovdiv, Bulgaria. Goji berry was used as a test plant. Goji berry was grown according to the generally accepted technology in areas at different distances from the NFMW (0.5 km, 2 km, 3.5 km, and 15 km). Soil samples were collected using a hand probe at a depth of 0 - 30 cm. One average sample was taken from each plot, formed from 10 stitches taken diagonally. Soil from all stitches from each plot was homogenized, air-dried, and sieved through a 2 mm sieve. Before heavy metals content detection and quantification, soil samples for total content determination were digested by aqua regia (ISO 11466). At the technological maturity stage, goji berry fruit samples were collected for analysis from each experimental plot. The fruits were analyzed fresh for macroelements, trace elements, and heavy metal contents by microwave mineralization. An atomic emission spectroscopy technique (AES) was used to define elements (Jobin Yvon Emission, model JY 38 S). The SPSS for Windows program was used in the statistical processing of the data.

Results and discussion

Soils

The physical and chemical properties of the soil samples are presented in Table 1. The soils are characterized by a slightly alkaline reaction, medium organic carbon content, and medium to high nutrient (N, P, K) availability. The total Zn, Pb, and Cd contents are high and exceed the maximum allowable concentrations (MAC) in the soils from the area 0.5 km (S1) and 2.0 km away from the NFMW (S2) (Table 1). The results presented in Table 1 show that with distance from NFMW - Plovdiv, there is a well-defined trend of decrease in the total content of heavy metals in soil. In soil samples taken from the area 0.5 km and 2.0 km away from the NFMW, values for Pb exceeding the MAC (100 mg kg⁻¹) were recorded. In the samples from 0.5 km (S1) and 2.0 km from the NFMW (S2), the Pb values ranged from 2509.1 mg kg⁻¹ to 4747.5 mg kg⁻¹. The Pb content in S3 (3.5 km away from KCM) reaches 83.1 mg kg⁻¹, while in the area 15 km away (S4), the Pb content decreases significantly to 24.6 mg kg⁻¹. Similar results were obtained for Cd and Zn. The reported values greatly exceed the MAC. At S1 (0.5 km from the NFMW), 2423.9 mg kg⁻¹ Zn and 64.3 mg kg⁻¹ Cd were recorded. At S2, 3440 mg kg⁻¹ Zn and 101.6 mg kg⁻¹ Cd were recorded, and in the area 3.5 km from the NFMW (S3), 215.3 mg kg⁻¹ Zn and 4 mg kg⁻¹ Cd were recorded. In the more remote location (15 km from the NFMW), 33.9 mg kg⁻¹ Zn and 2.7 mg kg⁻¹ Cd were detected. The Cu content in the soils of the study areas is significantly lower compared to the accepted Bulgarian MAC of 260 mg kg⁻¹ (except for the sample from S1). The observed difference between the samples from S1 and S2, taken at distances of 0.5 km and 2.0 km from the NFMW, is attributed to the spatial location of the soils relative to the NFMW and the contamination plume. S1 was sampled from the vegetated buffer zone constructed around the plant, where aerosols are trapped by the foliage of tree species and prevent transport by air. S2 was taken at a distance of 2 km from the plant in the direction of Asenovgrad to the hot-dip galvanizing plant in Asenovgrad, Plovdiv. On this side, there is no vegetated belt to stop aerosol pollutants, allowing unhindered transport of aerosol emissions over long distances from the plant.

Table 1. Soil characteristics

Parameter	Soil 1 (S1)	Soil 2 (S2)	Soil 3 (S3)	Soil 4 (S4)
	0.5 km	2 km	3.5 km	15 km
pH	7.8	7.3	7.6	7.5
EC, dS m ⁻¹	0.15	0.20	0.15	0.15
Organic content,%	1.56	2.39	2.24	1.54
N Kjeldal,%	0.34	0.17	0.22	0.13
Pb, mg kg ⁻¹	2509,1	4747,5	83.1	24.6
Cd, mg kg ⁻¹	64,3	106,1	4	2.7
Zn, mg kg ⁻¹	2423,9	3440	215.3	33.9
Cu, mg kg ⁻¹	222,1	289,5	71.3	16.0
Fe, mg kg ⁻¹	27388,4	20827,5	29581.9	27113.4

Mn, mg kg ⁻¹	1045,3	635,8	884,2	884,2
K, mg kg ⁻¹	8029,5	6358	6780	6780,0
Ca, mg kg ⁻¹	24355,8	34800	18065	16060,6
Mg, mg kg ⁻¹	12573,9	9402,5	10040,1	10040,0
P, mg kg ⁻¹	607,2	840	387,3	354,9

MAC (pH 6.0-7.4) – Pb-60 mg kg⁻¹, Cd-2.0 mg kg⁻¹, Zn-320 mg kg⁻¹

MAC (pH >7.4) – Pb-100 mg kg⁻¹, Cd-3.0 mg kg⁻¹, Zn-400 mg kg⁻¹

Goji berries

The content of macro, trace elements, and heavy metals in goji berry fruit is presented in Figure 1. The results showed that the macro elements (K, P, Mg, Ca) were predominant in goji berry fruits, followed by Zn and Fe. Cu and Mn. The toxic metals Pb and Cd are also present in goji berries. The conduction system causes the accumulation of heavy metals in the fruit and depends primarily on the variety and the elements considered. With distance from the source of contamination, there is a clear tendency for the heavy metal content of goji berry fruit to decrease (Fig. 1). The Pb content in goji berry fruit grown at a distance of 0.5 km (S1) and 2 km (S2) varied from 4.6 to 10.6 mg kg⁻¹, Zn from 31.1 to 74.6 mg kg⁻¹ and Cd from 0.2 to 1.4 mg kg⁻¹. The Pb content of goji berry fruit grown at a distance of 3.5 km from the NFMW (S3) decreased to 0.58 mg kg⁻¹, Zn to 14.1 mg kg⁻¹, and Cd to 0.10 mg kg⁻¹. In goji berries grown in the area 15 km away from the NFMW (S4), the values recorded were significantly lower - 0.19 mg kg⁻¹ Pb, 9.1 mg kg⁻¹ Zn, and the Cd content was below the limits of quantification with the method used. The toxic metal content of most foods is regulated by the European Union (EC) Directive, Commission Regulation (EC) No 1881/2006, 2006. According to European regulations, the Cd and Pb content of fruit must not exceed 0.05 mg kg⁻¹ fresh weight for Cd and 0.2 mg kg⁻¹ fresh weight for Pb. The measured concentrations of Pb and Cd in goji berry fruits grown at 3.5 and 15 km from the NFMW are below the maximum limits for Pb and Cd in food.

Similar results were found for micro and macro elements in goji berry fruit. With distance from the source of contamination, there was a clear trend towards a decrease in the micro and macro element content of goji berry fruits (except for Cu and Mn) (Fig. 1). The Cu content of goji berry fruits ranged from 3.7 mg kg⁻¹ (S4) to 14.1 mg kg⁻¹ (S3), Fe from 8.8 mg kg⁻¹ (S4) to 28.9 mg kg⁻¹ (S2), Mn from 2.5 mg kg⁻¹ (S4) to 6.6 mg kg⁻¹ (S3), K from 3,738.5 mg kg⁻¹ (S4) to 8,425.7 mg kg⁻¹ (S2), Ca from 119.6 mg kg⁻¹ (S4) to 227.9 mg kg⁻¹ (S2), Mg from 220.8 mg kg⁻¹ (S4) to 400.8 mg kg⁻¹ (S2) and P from 576.6 mg kg⁻¹ (S4) to 1,263.8 mg kg⁻¹ (S2).

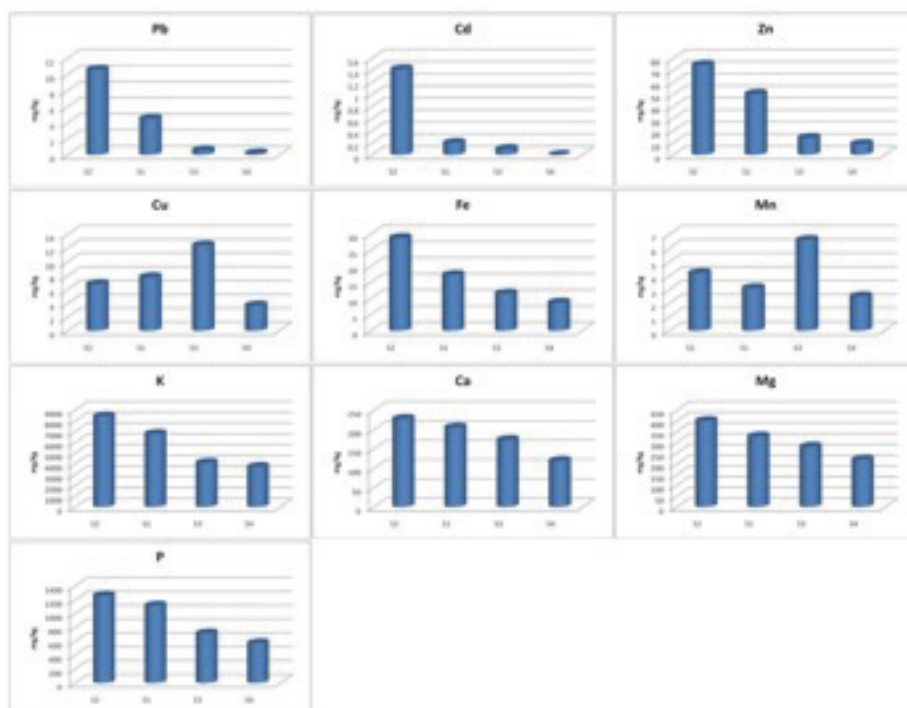


Fig.1. Content of macronutrient, trace element and heavy metal contents (mg kg⁻¹) of goji berry fruit

Table 2 presents the results obtained for the composition of the fruit composition of this study and the values obtained by other authors. The obtained results for goji berry composition are similar to the results of Endes et al. (2015) for goji berries grown in Turkey. The values for Fe, Mn, and Cu were not significantly different from the results of Niro et al. (2017) for the composition of goji berries from Italy. The Zn content was similar to China and Turkey, while the K content was similar to Macedonia. Ca and P contents were significantly lower than published values, while significantly higher values were obtained for Pb and Cd when goji berries were grown on highly contaminated soils. The comparative analysis shows that the toxic metals Cd and Pb are also present in fruits from Macedonia, China and Turkey. The Pb and Cd content in goji berry fruits ranged from 0.015-0.096 mg kg⁻¹ for the fruits from China, 0.008-0.028 mg kg⁻¹ for the fruits from Macedonia, 0.003-0.033 mg kg⁻¹ for the fruits from Spain and 1.3 mg kg⁻¹ for fruits from Turkey. It has been found that due to geogenic or anthropological reasons (soil and climatic conditions, presence of silting agents, improper processing, etc.) goji berry fruits may also contain some toxic elements (Hg, Pb, As, Cd, etc.) that have negative effects on the human body (Harmanescu et al., 2011; Gogoasa et al., 2014; Mihali et al., 2012).

Table 2. Content of heavy metals, trace and macro elements (mg kg⁻¹) in goji berry fruits

Element	Measured Values (this study)	Reference value				
		China (Balabanova et al.,2016)	Macedonia (Balabanova et al., 2016)	Turkey (Endes et al.,2015)	Italy (Niro et al.,2017)	Spain (Llorent-Martinez et al., 2013)
Pb	0.19-10.6	0.015-0.096	0.008-0.028	1.30		0.035-0.095
Cd	nd-1.4	0.003-0.033	0.055-0.093			0.090
Zn	9.1-74.6	10.4-110	7.45-10.8	8.27	5.0	10-15
Cu	3.7-12.4	8.91-14.3	7.45-10.8	10.10	3.0	1.8-8.5
Fe	8.8-28.9	63.3-82.6	20.9-60	45.77	9.0	35-72
Mn	2.5-6.6	5.94		5.28	2.0	6-12
K	3738.58425.7	2261-3877	3823-5047	1347.7	2762	13000-17000
Ca	119.6-227.9	507-1034	269-478	1003.4	266	330-710
Mg	220.8-400.8	1009-1402	632-825	806.9	127	775-1050
P	577.6-1263.8	1846-2541	2059-2781	1103.3	484	1300-2300

nd- not detectable

Goji berries from China are higher in Mg and Ca, while the opposite is true for K. K and Na play an essential role in regulating blood pressure and acid-base balance of the body (Clausen et al., 2013). According to Niro et al. (2017), K is the predominant element (276.2 mg 100 g⁻¹ and 881.9 mg 100 g⁻¹ for fresh and dried fruits, respectively), followed by Na in goji berry fruits grown in Italy. The obtained results showed that the K content of goji berry fruits grown in Bulgaria was in the range of 0.4-0.8% (3738.5-8425.7 mg kg⁻¹). The values obtained in this study are similar to the results of Balabanova et al. (2016) for K in goji berry fruits from Macedonia and China (0.2 - 0.5%) and much lower than the results obtained by Endes et al. (2015) for goji berry fruits from Turkey (1.5%) and by Llorent-Martinez et al. (2013) for fruits from Spain (1.3-1.7%).

It is known that goji berry fruits can be a good source of P and Ca, in addition to containing significant amounts of Mg, which is essential to prevent heart disease and growth retardation. The Mg content in the fruits ranged from 220.8 to 400.8 mg kg⁻¹. According to Balabanova et al. (2016), the Mg content in fruits from China and Macedonia ranged from 632 to 1452 mg kg⁻¹. In Turkey, the Mg content reached up to 806.9 mg kg⁻¹ (Endes et al., 2015), and in Spain it went up to 900 mg kg⁻¹ (Llorent-Martinez et al., 2013). Significantly lower results were found in fruits from Italy (127 mg kg⁻¹) (Niro et al., 2017). The values obtained in this study for P in fruits ranged from 577.6 to 1263.8 mg kg⁻¹. According to literature data, the P content in goji berry fruits ranged from 484 mg kg⁻¹ (Italy), 1103.3 mg kg⁻¹ (Turkey), 1840 mg kg⁻¹ (Spain), to 2234 mg kg⁻¹ (Macedonia), and 2258 mg kg⁻¹ (China) (Balabanova et al., 2016).

Differences were also observed in the Cu, Fe, Zn, and Mn contents in fruits (Bellaio et al., 2016; Endes et al., 2015; Llorent-Martínez et al., 2013). The obtained results showed significantly lower values for Fe, ranging from 8.8 to 28.9 mg kg⁻¹, compared to fruits from Turkey (45.77 mg kg⁻¹), Spain (55 mg kg⁻¹) and China (63-83 mg kg⁻¹) (Balabanova et al., 2016). The lowest values were found for Fe in fruits from Italy (9.0 mg kg⁻¹). The Fe content of 55 mg kg⁻¹ in fruits is considered higher concerning the food reference intake (Kulczynski and Gramza-Michałowska, 2016). The Zn content of goji berries ranges from 9.1 to 74.6 mg kg⁻¹. The metabolic role of this element is related to some protein syntheses. DNA and the functions of over 100 enzymes, Zn is also involved in critical cellular activities such as membrane transport, repair, and growth, especially in infants (Potterat, 2010; Amagase and Farnsworth, 2011). According to Balabanova et al. (2016), the Zn content in fruits ranges from 7.5 to 17.5 mg kg⁻¹. Studies by Endes et al. (2015) on the Zn content of Chinese goji berry fruits showed similar results (mean value of 8.27 mg kg⁻¹). According to Nascimento et al. (2015), fruit from China, Tibet, and other parts of Asia is characterized by a Zn content of less than 15 mg kg⁻¹. However, the data of Gogoasa et al. (2014) for Zn in Chinese goji berries showed significantly higher values of 90-130 mg kg⁻¹. The Cu content of the fruits ranged from 3.7-12.4 mg kg⁻¹. Endes et al. (2015) reported that the Cu content in goji berry fruits from Turkey reaches up to 10.10 mg kg⁻¹. Balabanova et al. (2016) indicated that the Cu content in goji berry fruits from Macedonia ranged from 7.45 to 10.8 mg kg⁻¹, and from 8.91 to 14.3 mg kg⁻¹ for fruits from China. Lower values were obtained for Cu in fruits from Spain (1.8-8.5 mg kg⁻¹) and Italy (3.0 mg kg⁻¹). It is essential to mention that Zn and Cu above specific concentrations established by regulations negatively affect the human body (WHO, 1996). The values obtained indicate that goji berry fruits do not contain excessive amounts of Zn and Cu (WHO, 1996). When comparing the composition of goji berry fruits grown in different countries, it is evident that fruits from Italy have the highest content of micro and macro elements (15422.9- 21167.7 mg kg⁻¹). The lowest range of micro and macro elements was found in fruits from Spain (3648 mg kg⁻¹). The composition of fruit grown in Bulgaria (4680.8) is similar to that of fruit from Turkey (4333.0 mg kg⁻¹).

Conclusions

It has been established that goji berry is a crop tolerant to heavy metals and can be successfully grown on soils contaminated with heavy metals. Goji berries can be an indicator of air (and soil) pollution. Elevated heavy metal content in soil does not affect the uptake of nutrients (Ca, Mg, and P) in goji berry fruit. Macronutrients (K, P, Mg, Ca) predominate in goji berry fruits, followed by Zn, Fe, Cu, and Mn. When goji berries are cultivated on lightly contaminated and unpolluted soils, the fruit contains negligible amounts of toxic elements (Pb and Cd).

Acknowledgment

The results presented in the paper are output from research projects KP-06-H54/7, Bulgarian National Science Fund, and “Possibilities for limiting the impact of mercury on the environment and human health”.

References

- Amagase H., Farnsworth N.R. (2011). A review of botanical characteristics, phytochemistry, clinical relevance in efficacy and safety of *Lycium barbarum* fruit (Goji). Food Research International. 44: 1702-1717.
- Balabanova B., Karov I., Mitrev S. (2016). Comparative analysis for macro and trace elements content in goji berries between varieties from China and R. Macedonia. Agricultural Science and Technology. 8: 79-84.
- Bellaio G., Carnevale E., Bona S. (2016). Preliminary studies on sensory, instrumental and chemical evaluation of dried goji (*Lycium barbarum* L.) berries. Acta Horticulturae. 1120: 515.
- Chen C., Shao Y., Li Y., Chen T. (2014). Trace elements in *Lycium Barbarum* L. leaves by inductively coupled plasma mass spectrometry after microwave assisted digestion and multivariate analysis. Spectroscopy Letters. 48: 775-780.
- Clausen M.J.V. and Poulsen H. (2013). Sodium/Potassium Homeostasis in the Cell. Ch. 3. In: “Metalloomics and the Cell (Metal Ions in Life Sciences 12),” L. Banci (Ed.), pp. 41. Springer, Dordrecht.

- Commission of the European Communities. (2006). Directive No1881/2006 on setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union. L 364,5-24.
- Endes Z., Uslu N., Ozcan M.M., Er F. (2015). Physico-chemical properties, fatty acid composition and mineral contents of goji berry (*Lycium barbarum* L.) fruit. Journal of Agroalimentary Processes and Technologies. 21: 36-40
- Gogoasa I., Alda L., Rada M., Negrea P., Negrea A., Bordean D.M., Draghici G.A., Gergen I. (2014). Goji berries (*Lycium barbarum*) as a source of trace elements in human nutrition. Journal of Agroalimentary Processes and Technologies. 20: 369-372.
- ISO. (1995). Soil Quality-Extraction of Trace Elements Soluble in Aqua Regia. [ISO 11466:1995]. Geneva, Switzerland: International Organization for Standardisation.
- Harmanescu M., Alda L., Bordean D., Gogoasa I., Gergen I. (2011). Heavy metals health risk assessment for population via consumption of vegetables grown in old mining area; a case study: Banat County, Romania, Chemistry Central Journal. 5: 64.
- Kulczynski K., and Gramza-Michałowska A. (2016). Goji Berry (*Lycium barbarum*): Composition and Health Effects – a Review. Pol. J. Food Nutr. 66(2): 67–75.
- Llorent-Martínez E.J., Fernández-de Córdova M.L., Ortega-Barrales P., Ruiz-Medina A. (2013). Characterization and comparison of the chemical composition of exotic superfoods. Microchemical Journal. 110: 444.
- Mihali C., Michnea A., Oprea G., Gogoasa I., Pop C., Senila M., Grigor L. (2012). Trace element transfer from soil to vegetables around the lead smelter in Baia Mare, NW Romania. Journal of Food, Agriculture & Environment. 10: 828-834.
- Mirecki N., Agic R., Sunic L., Milenkovic L., Ilic Z.S. (2015). Recommend caution when systematically using this fruit as a mineral Transfer factor as indicator of heavy metals content in plants. Fresenius Environmental Bulletin. 24: 4212-4219.
- Nascimento A.N., Silvestre D.M., Leme F.O., Nomura C.S., Naozuka J. (2015). Elemental Analysis of Goji Berries Using Axially and Radially Viewed Inductively Coupled Plasma-Optical Emission Spectrometry. Spectroscopy. 30: 36-41.
- Niro S., Fratianni A., Panfili G., Falasca L. (2017). Cinquanta and MD Rizvi Alam. Nutritional evaluation of fresh and dried goji berries cultivated in Italy. Italian Journal Food Science. 29: 401-408.
- Potterat O. (2010). Goji (*Lycium barbarum* and *L. chinense*): Phytochemistry, pharmacology and safety in the perspective of traditional uses and recent popularity. Planta Medica. 76: 7-19.
- Seeram N.P. (2010). Recent trends and advances in berry health benefits research. Journal of Agricultural and Food Chemistry. 58: 3869-3870.
- Wang C.C., Chang S.C., Inbaraj B.S., Chen B.H. (2010). Isolation of carotenoids, flavonoids and polysaccharides from *Lycium barbarum* L. and evaluation of antioxidant activity. Food Chemistry. 120: 184–192.
- World Health Organization. (1996). Trace elements in human nutrition and health. Geneva: WHO Library Cataloguing in Publication Data.

Utjecaj poboljšivača na fitoremedijaciju kadmija i žive iz tla pomoću trave *Miscanthus x giganteus*

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Sažetak

Cilj rada bio je utvrditi utjecaj različitih poboljšivača (otpadni mulj iz bioplinskog postrojenja, mikoriza, pepeo MxG) na fitoremedijaciju tla onečišćenog s kadmijem i živom pomoću energetske kulture miskantusa (*Miscanthus x giganteus* – MxG). Utvrđeno je da je onečišćenje visokim koncentracijama kadmija i žive nadjačalo utjecaj dodanih poboljšivača i negativno utjecalo na prinos. Prema izračunatom koeficijentu obogaćivanja miskantusa je pogodniji za korištenje u procesu fitostabilizacije i proizvodnji biomase na tlima umjereno onečišćenim teškim metalima.

Ključne riječi: otpadni mulj, mikoriza, pepeo, rizomi, teški metali, prinos

Uvod

Onečišćena tla mogu se iskoristiti za uzgoj energetskih kultura u svrhu proizvodnje biomase. Istovremeno, uzgajane energetske kulture uklanjaju onečišćivače iz tla procesom fitoremedijacije. Fitoremedijacija je okolišno prihvatljiva tehnologija koja koristi biljke za uklanjanje, degradaciju ili zadržavanje štetnih tvari u tlu (Morel i sur., 2006). Mehanizmi fitoremedijacije koji se koriste pri uklanjanju teških metala iz tla su fitostabilizacija (blokiranje onečišćivača u tlu), fitoekstrakcija (uklanjanje i akumulacija onečišćivača u biljci) i rizofiltracija (ekstrakcija i zadržavanje onečišćivača u podzemnoj biomasi) (Kisić, 2012). Potencijal za primjenu u procesu fitoremedijacije pokazala je energetska kultura miskantusa – *Miscanthus x giganteus* (MxG), koju karakterizira višegodišnji rast, visoka proizvodnja biomase, otpornost i prilagodljivost. Danas se miskantus uglavnom koristi u proizvodnji toplinske i/ili električne energije izravnim izgaranjem u obliku drvene sječke, peleta/briketa i bala (Bilandžija, 2015). Korištenje miskantusa u fitostabilizaciji Cd i Hg prethodno su proučavali Zgorelec i sur. (2020) i Bilandžija i sur. (2022). Miskantus se pokazao kao potencijalni kandidat za upotrebu u procesu fitostabilizacije i proizvodnje biomase na tlima onečišćenim umjerenim količinama ovih teških metala. Navedeno je dodatno potvrđeno istraživanjem energetskih karakteristika miskantusa pri čemu se pokazalo da onečišćenje tla s Cd i Hg nema značajan utjecaj na svojstva sagorijevanja biomase miskantusa nakon korištenja biljaka u procesu fitoremedijacije, no zbog akumulacije Cd iznad tipične vrijednosti za sigurno korištenje u proizvodnji čvrstih biogoriva sagorijevanjem biomase, biljke miskantusa moguće je koristiti isključivo u postrojenjima koja ograničavaju ponovnu emisiju teških metala u atmosferu. Nakon sagorijevanja kontaminirane biomase potrebno je provesti analizu sastava pepela u svrhu njegova zbrinjavanja na siguran i ekološki prihvatljiv način. U ovom radu nastavlja se istraživanje potencijala miskantusa kao akumulatora Cd i Hg u procesu fitoremedijacije te se nastoji utvrditi na koji način različiti poboljšivači (tlo bez poboljšivača – kontrola, otpadni mulj iz bioplinskog postrojenja, mikoriza i pepeo miskantusa) dodani u tlo utječu na taj proces.

Materijali i metode

Istraživanje je provedeno u završnoj godini trogodišnjeg eksperimenta koji se provodio na Agronomskom fakultetu Sveučilišta u Zagrebu u razdoblju od 2018. do 2021. godine. Presadnice miskantusa posađene su u eksperimentalne

posude (EP) u četiri tretmana i tri ponavljanja prema slučajnom bloknom rasporedu. U svim tretmanima korišteno je visoko onečišćeno tlo s Cd (100 mg kg^{-1} tla) i Hg (20 mg kg^{-1} tla), a postupak je opisan u Zgorelec i sur. (2020). Tretmani su se međusobno razlikovali po dodanom poboljšivaču: I – tlo bez dodatka poboljšivača, II – otpadni mulj iz bioplinskog postrojenja, III – mikoriza i IV – pepeo MxG. Kao prvi tretman u istraživanju korišteno je tlo bez dodatka poboljšivača s pokušališta Maksimir. Svaka EP ispunjena je s 18 kg tla . Drugi tretman bio je otpadni mulj iz fermentora bioplinskog postrojenja Agroproteinka. Sastav otpadnog mulja je analiziran i bakteriološki je bio ispravan. Dodano je 340 g otpadnog mulja po EP što odgovara dopuštenoj godišnjoj količini od $1,66 \text{ t ha}^{-1}$ poljoprivrednog tla prema Pravilniku o gospodarenju muljem iz uređaja za pročišćavanje otpadnih voda kada se mulj koristi u poljoprivredi (NN 38/08). U trećem tretmanu apliciran je MYKOFLOR®, komercijalni inokulum živih ektomikoriznih micelija suspenziranih u otopini AgroHydroGel® tvrtke Agroidea. Korišteno je 5 mL mikorize po EP. Četvrti tretman bio je pepeo MxG. Omjer tla i pepela bio je 75:25. Na dan žetve miskantusa (18.3.2021.) iz svake EP uzeti su uzorci nadzemne biomase i tla koji su pripremljeni za daljnje analize (HRN ISO 11464:2009). U laboratoriju Zavoda za opću proizvodnju bilja uzorcima nadzemne biomase izmjereni su visina, prinos i broj izboja po istraživanoj površini. Uzorcima nadzemne biomase i tla određen je udio vlage (HRN ISO 11465:2004), a ugljik (HRN ISO 10694:2004), dušik (HRN ISO 13878:2004) i sumpor (HRN ISO 15178:2005) određeni su metodom suhog spaljivanja (Vario Macro CHNS Analyzer, Elementar). Provedena je analiza na ukupne elemente u uzorcima tla (HRN ISO 13196:2013) metodom pXRF (Vanta VCR G2, Olympus). Analiza Cd i Hg provedena je na Institutu za medicinska istraživanja i medicinu rada (IMI), pri čemu je dio uzoraka tla mikrovalno razoren (Ultraclave, Milestone). Detekcija Cd odrađena je metodom ICP-MS (7500, Agilent Technologies), a Hg na uređaju Mercury Analyzer (AMA-254, Leco Inc). Za statističku analizu korišten je program SAS 9.1 (SAS Inst. Inc). Provedena je analiza varijance (One-way ANOVA) za ispitivanje varijabilnost unutar tretmana za sve ispitivane parametre. Za statistički značajne razlike ($p < 0,05$) proveden je Fisher-ov LSD post-hoc test. Kontrola kvalitete provedena je tijekom cijelog istraživanja, uključujući rukovanje uzorcima i laboratorijske analize. Za provjeru točnosti mjerenja tla upotrebljeni je referentni materijal ISE 989, a za provjeru točnosti mjerenja biljnog materijala referentni materijal IPE 225. Preciznost mjerenja provjeravana je replikacijom pojedinih mjerenja u tri ponavljanja.

Rezultati i rasprava

Prema podacima dobivenim analizom osnovnih fizikalno-kemijskih svojstava (Tablica 1) tlo je blago kiselo i dosta humozno, bogato opskrbljeno ukupnim dušikom (klasifikacija prema Woltmanu, Čoga i Slunjski, 2018), a vrlo slabo fiziološki aktivnim oblicima fosfora i kalija (Vukadinović i Lončarić, 1998). Utvrđena tekstura tla je praškasta ilovača (PrI).

Tablica 1. Osnovna fizikalno-kemijska svojstva tla prije postavljanja eksperimenta

pH_{KCl}	OT (%)	P_{AL} (mg 100 g ⁻¹)	K_{AL}	TN (%)	TC (%)	TS (%)	KIK (cmol ⁺ kg ⁻¹)
m. HRN ISO 10390:2004	m. HRN ISO 14235:2004	Škorić, 1982		HRN ISO 13878:2004	HRN ISO 10694:2004	HRN ISO 15178:2005	HRN ISO 11260:2004
6,23	3,8	4,4	7,8	0,23	2,48	0,049	18,9
Mehanički sastav tla, % (HRN ISO 11277:2004)							
Pijesak (%)	Prah (%)		Glina (%)		Teksturna oznaka		
2-0,063 mm	0,063-0,002 mm		< 0,002 mm		(FAO, 2006)		
14,4	75,7		9,9		PrI		

U Tablici 2 prikazani su rezultati statističke analize sadržaja odabranih mikro i makrohranjiva u tlu. Utvrđeno je pozitivno djelovanje dodanih poboljšivača na količine nekih hranjiva u tlu, u odnosu na njihov sadržaj na početku eksperimenta.

Tablica 2. Rezultati statističke analize sadržaja odabranih mikro i makrohranjiva u tlu

Tretman	0	I	II	III	IV	LSD
N (%)	0,230 ^{ab}	0,197 ^c	0,205 ^{bc}	0,212 ^{abc}	0,234 ^a	0,03
C (%)	2,5 ^b	2,5 ^b	2,5 ^b	2,6 ^b	5,7 ^a	0,4
S (%)	0,049 ^c	0,063 ^b	0,062 ^b	0,063 ^b	0,071 ^a	0,005
H (%)	0,23 ^c	1,13 ^b	1,15 ^b	1,17 ^b	1,45 ^a	0,1
Ca (%)	1,04 ^d	1,38 ^c	1,46 ^{bc}	1,50 ^b	1,67 ^a	0,1
Cu (mg kg ⁻¹)	27 ^c	34 ^b	34 ^b	33 ^b	40 ^a	3
Fe (%)	4,08 ^c	4,77 ^a	4,68 ^{ab}	4,73 ^{ab}	4,55 ^b	0,2
K (%)	1,35 ^b	1,47 ^a	1,44 ^a	1,47 ^a	1,39 ^b	0,04
Mn (mg kg ⁻¹)	995 ^b	1225 ^a	1207 ^a	1244 ^a	1171 ^a	82
Mo (mg kg ⁻¹)	< LOD ^a	0,14 ^a	0,22 ^a	0,14 ^a	0,31 ^a	0,4
Ni (mg kg ⁻¹)	44,7 ^c	63,6 ^{ab}	63,9 ^{ab}	64,4 ^a	59,2 ^b	5
P (mg kg ⁻¹)	150 ^c	175 ^{bc}	191 ^b	200 ^{ab}	234 ^a	34
Zn (mg kg ⁻¹)	97 ^b	771 ^a	917 ^a	1016 ^a	1040 ^a	355

0 – početno stanje (2018.), I – kontrola, II – otpadni mulj iz bioplinskog postrojenja, III – mikoriza, IV – pepeo MxG; Razlike između tretmana testirane su Fisher-ovim LSD post hoc testom. Vrijednosti označene različitim slovima (a, b, c) prema varijantama istraživanja statistički su značajno različite; $p < 0,05$; LSD – Fisher's least significant difference

Prinos suhe tvari kretao se od 30 g (I,IV) do 33 g (II,III), što odgovara vrijednosti od 5,0 t ST ha⁻¹ do 5,5 t ST ha⁻¹ u godini (Tablica 3). Ostvareni prinos sukladan je podacima o prinosu koji je ostvaren u prve dvije godine eksperimenta – između 3,6 t ST ha⁻¹ (III) i 5,3 t ST ha⁻¹ (IV) u 2018. godini te između 4,9 t ST ha⁻¹ (I) i 5,4 t ST ha⁻¹ (IV) u 2019. godini (Šestak i sur., 2022). U trećoj godini eksperimenta najviši prinos bio je u tretmanu III, dok je u prve dvije godine najviši prinos ostvaren za tretman IV. Visina biljaka kretala se između 86 cm (IV) i 100 cm (III) (Tablica 3) te je utvrđeno da nema značajnih razlika između tretmana, što je u skladu s rezultatima trogodišnjeg istraživanja u EP Bilandžije i sur. (2022) i Zgorelec i sur. (2020) na miskantusu uzgojenom u tlu onečišćenom različitim dozama Cd (0, 10 i 100 mg kg⁻¹ tla) i Hg (0, 2 i 20 mg kg⁻¹ tla).

Tablica 3. Srednje vrijednosti analize nadzemne biomase MxG

Tretman	I	II	III	IV
Prinos ST (g)	30 ^a	33 ^a	33 ^a	30 ^a
Visina biljke (cm)	90 ^a	92 ^a	100 ^a	86 ^a
Broj izboja	11 ^a	12 ^a	10 ^a	14 ^a
Udio vlage (%)	19 ^a	17 ^{ab}	18 ^a	15 ^b
N (%)	0,284 ^a	0,251 ^a	0,225 ^a	0,256 ^a
C (%)	47,92 ^a	48,52 ^a	47,98 ^a	45,95 ^b
S (%)	0,241 ^a	0,166 ^a	0,15 ^a	0,157 ^a
H (%)	8,09 ^b	8,33 ^a	8,19 ^{ab}	7,99 ^b

I – kontrola; II – otpadni mulj iz bioplinskog postrojenja, III – mikoriza, IV – pepeo MxG; Vrijednosti označene različitim slovima prema varijantama istraživanja statistički su značajno različite; $p < 0,05$

Broj izboja po biljci kretao se od 10 (III) do 14 (IV) (Tablica 3) što je usporedivo broju izboja u istraživanju Bilandžije i sur. (2022) i Zgorelec i sur. (2020) koji se kretao od 6 do 11 te manje od broja izboja koje su u svom istraživanju na miskantusu uzgojenom hidroponski uz dodatak različitih doza Cd (0, 0,75, 1,5, 2,25, 3 mg L⁻¹) utvrdili Arduini i

sur. (2006). Dodatak poboljšivača nije značajno utjecao na promjene u broju izboja. Sadržaj vlage kretao se od 15 % (IV) do 19 % (I) (Tablica 3) što je sukladno podacima za proljetnu žetvu Bilandžije i sur. (2018) i Lewandowskog i Heinza (2003) koji su u poljskim uvjetima utvrdili sadržaj vlage u biomasi miskantusa od 19 % i 18 % te rezultatima trogodišnjeg istraživanja kojeg su proveli Bilandžija i sur. (2022) na miskantusu uzgojenom u EP u tlu onečišćenom s Cd i Hg, u kojem se sadržaj vlage kretao od 10 % do 28 %. Sadržaj vlage utvrđen u ovom istraživanju smatra se poželjnim pri uzgoju miskantusa za proizvodnju energije jer kada je udio vlage niži od 20 % pri skladištenju nije potrebno naknadno sušenje (Lewandowski i Heinz, 2003), čime se povećava isplativost proizvodnje (Bilandžija i sur., 2018). Utvrđeno je da biomasa miskantusa uzgojenog u tlu s dodatkom pepela MxG sadrži značajno manje vlage u odnosu na kontrolu i miskantus uzgojen u tlu s dodatkom mikorize. U nadzemnoj biomasi nema značajnih razlika u sadržaju dušika i sumpora između tretmana. Statistički značajno niži sadržaj ugljika u odnosu na ostale tretmane zabilježen je za tretman s dodatkom pepela MxG, a dodatak otpadnog mulja iz bioplinskog postrojenja u tlu utjecao je na povećani sadržaj vodika u nadzemnoj biomasi miskantusa u odnosu na kontrolu i tretman s dodatkom pepela. Koncentracija Hg i Cd u nadzemnoj biomasi prikazana je u Tablici 4. U nadzemnom dijelu biljke Cd se kretao od 5,98 mg kg⁻¹ (II) do 7,12 mg kg⁻¹ (I) što je usporedivo s rezultatima Bilandžije i sur. (2022) i Zgorelec i sur. (2020) koji su u trogodišnjoj studiji utvrdili kretanje koncentracije Cd od 0,045 mg kg⁻¹ do 6,76 mg kg⁻¹. No ako se gledaju njihove koncentracije u trećoj godini studije (0,121 mg kg⁻¹ do 3,28 mg kg⁻¹) vidljivo je da su koncentracije Cd ustanovljene u ovoj studiji više. Utvrđeni sadržaj Cd prelazi tipičnu vrijednost za sigurno sagorijevanje biomase miskantusa (TMxG) kao čvrstog biogoriva (Tablica 4) koja prema HRN ISO 17225-1:2021 za Cd iznosi 0,1 mg kg⁻¹ i graničnu vrijednost za pelete od miskantusa (GMxG) koja prema HRN ISO 17225-6:2021 iznosi 0,5 mg kg⁻¹. Stoga se proizvedena biomasa može klasificirati kao opasan materijal čija uporaba u proizvodnji biogoriva zahtijeva korištenje suvremenih postrojenja koja sprječavaju ponovnu emisiju Cd.

Tablica 4. Srednje vrijednosti koncentracija Cd (u mg kg⁻¹) i Hg (u µg kg⁻¹) u nadzemnoj biomasi, njihovo odnošenje nadzemnom biomasom miskantusa (u g ha⁻¹) i koeficijent obogaćivanja (EC)

Kadmij	Tretman	Cd (mg kg ⁻¹)	RSD (%)	Oдноšenje (g ha ⁻¹)	EC (Cd)
	I	7,12 ^a	34	35,87	0,071
	II	5,98 ^a	7	32,35	0,060
	III	6,31 ^a	11	34,73	0,063
	IV	6,43 ^a	23	31,05	0,064
	TMxG	0,1	HRN ISO 17225-1:2021		
	GMxG	0,5	HRN ISO 17225-6:2021		
Živa	Tretman	Hg (µg kg ⁻¹)	RSD (%)	Oдноšenje (g ha ⁻¹)	EC (Hg)
	I	96,5 ^a	74	0,50	0,005
	II	41,8 ^a	5	0,23	0,002
	III	95,0 ^a	23	0,53	0,005
	IV	101,8 ^a	54	0,48	0,005
	TMxG	30	HRN ISO 17225-1:2021		
	GMxG	100	HRN ISO 17225-6:2021		

I – kontrola; II – otpadni mulj iz bioplinskog postrojenja, III – mikoriza, IV – pepeo MxG; RSD – relativna standardna devijacija, izračunata prema: $RSD = \frac{s}{\bar{x}} \cdot 100$; EC – koeficijent obogaćivanja, izračunat prema $EC = \frac{C_{\text{suha biljka}}}{C_{\text{suho tlo}}}$; TMxG – tipične vrijednosti za sagorijevanje miskantusa u proizvodnji biogoriva (HRN ISO 17225-1:2021); GMxG – granične vrijednosti za sadržaj u peletima (HRN ISO 17225-6:2021); Vrijednosti označene različitim slovima prema varijantama istraživanja statistički su značajno različite; $p < 0,05$

Koncentracija Hg se u nadzemnoj biomasi kretala između 41,8 µg kg⁻¹ (II) i 101,8 µg kg⁻¹ (IV) (Tablica 4). Utvrđena koncentracija Hg je viša u odnosu na rezultate Bilandžije i sur. (2022) i Zgorelec i sur. (2020) koji su utvrdili da

se koncentracija Hg kretala od 8,7 $\mu\text{g kg}^{-1}$ do 108,9 $\mu\text{g kg}^{-1}$, odnosno od 9,3 $\mu\text{g kg}^{-1}$ do 24,1 $\mu\text{g kg}^{-1}$ u trećoj godini studije. Sadržaj Hg utvrđen u ovoj studiji za sve tretmane prelazi TMxG koja prema HRN ISO 17225-1:2021 za Hg iznosi 30 $\mu\text{g kg}^{-1}$, a tretman IV prelazi i GMxG koja prema HRN ISO 17225-6:2021 iznosi 100 $\mu\text{g kg}^{-1}$. Odnosnje Cd kretalo se od 31,05 g ha^{-1} (IV) do 35,87 g ha^{-1} (I) što je sukladno s rezultatima istraživanja Barbu i sur. (2009) koji su utvrdili da miskantus Cd može akumulirati u količini od 35 do 55 g ha^{-1} te da je pogodan za uzgoj na siromašnim, kiselim tlima onečišćenim teškim metalima poput Pb i Cd. Barbu i sur. (2009) su utvrdili da miskantus nije pogodan za fitoekstrakciju Cd zbog niske akumulacije teških metala što je sukladno izračunatom koeficijentu obogaćivanja (Tablica 4) koji se u ovoj studiji kretao između 0,060 (II) i 0,071 (I), što prema Zgorelec (2009) ukazuje na slabu akumulaciju Cd nadzemnom biomasom. Odnosnje Hg kretalo se od 0,23 g ha^{-1} (II) do 0,53 g ha^{-1} (II) što je u skladu s rezultatima Bilandžije i sur. (2022) i Zgorelec i sur. (2020) u čijoj trogodišnjoj studiji se odnosnje Hg nadzemnom biomasom miskantusa kretalo do 0,79 g ha^{-1} godišnje. Koeficijent obogaćivanja za Hg u ovoj studiji kretao se od 0,002 (II) i 0,005 (IV), što znači da miskantus ne akumulira Hg u nadzemnoj biomasi (Zgorelec, 2009). Dobiveni rezultati u suprotnosti su sa Zhao i sur., (2019) koji su utvrdili da se za *M. sinensis* koeficijent obogaćivanja za Hg kretao između 0,365 i 225, pri čemu se koeficijent smanjivao povećanjem koncentracije Hg u tlu.

Zaključak

Pokazalo se da različiti poboljšivači dodani u tlo nisu imali značajan utjecaj na fitoremedijaciju kadmija i žive iz tla pomoću miskantusa. Onečišćenje teškim metalima u visokim koncentracijama nadjačalo je djelovanje dodanih poboljšivača i utjecalo na smanjenje prinosa. Pokazalo se da pri onečišćenju teškim metalima u koncentraciji kao što je 100 mg Cd kg^{-1} i 20 mg Hg kg^{-1} miskantus nije prikladan izbor za fitoekstrakciju jer nadzemnom biomasom godišnje odnosi između 31,05 – 35,87 g ha^{-1} Cd i 0,23 – 0,53 g ha^{-1} Hg. Prema izračunatom koeficijentu obogaćivanja u nadzemnoj biomasi (0,060 – 0,071 za Cd; 0,002 – 0,005 za Hg) zaključuje se da je miskantus pogodniji za korištenje u procesu fitostabilizacije i proizvodnji biomase na tlima umjereno onečišćenim teškim metalima jer se pri onečišćenju tla visokim koncentracijama teški metali u biomasi miskantusa akumuliraju u koncentracijama koje prelaze tipične vrijednosti za sigurno korištenje u proizvodnji čvrstih biogoriva.

Literatura

- Arduini I., Ercoli L., Mariotti M., Masoni A. (2006). Response of miscanthus to toxic cadmium applications during the period of maximum growth. *Environmental and Experimental Botany*. 55: 9–40.
- Barbu C. H., Pavel B. P., Sand C., Pop M. R. (2009). *Miscanthus sinensis giganteus* behaviour on soil polluted with heavy metals. *Metal Elements in Environment, Medicine and Biology*. In Proceedings of 9th International Symposium of Romanian Academy – Branch Cluj-Napoca, 21–24. Cluj-Napoca, Romania: Cluj University Press.
- Bilandžija N. (2015). The potential of *Miscanthus x giganteus* species as an energy crop in different technological and environmental conditions. Doctoral dissertation. Zagreb, Croatia: University of Zagreb, Faculty of Agriculture.
- Bilandžija N., Voća N., Leto J., Jurišić V., Grubor M., Matin A., Geršić A., Krička T. (2018). Yield and Biomass Composition of *Miscanthus x Giganteus* in the Mountain Area of Croatia. *Transactions of FAMENA*. 42: 51–60.
- Bilandžija N., Zgorelec Ž., Pezo L., Grubor M., Velaga A. G., Krička T. (2022). Solid biofuels properties of *Miscanthus X giganteus* cultivated on contaminated soil after phytoremediation process. *Journal of the Energy Institute*. 101: 131–139.
- Čoga L., i Slunjski S. (2018). *Dijagnostika tla u ishrani bilja: priručnik za uzorkovanje i analitiku tla*. Zagreb, Hrvatska: Agronomski fakultet Sveučilišta u Zagrebu.
- FAO (2006). *WRB – World Reference Base for Soil Resources, Guidelines for Soil Description*. Rome, Italy: Food and Agriculture Organization of the United Nations.
- ISO. (2004a). *Soil quality – Determination of dry matter and water content on a mass basis – Gravimetric method*. [HRN ISO 11465:2004]. Geneva, Switzerland: International Organization for Standardization.

- ISO. (2004b). Soil quality – Determination of effective cation exchange capacity and base saturation level using barium chloride solution. [HRN ISO 11260:2004]. Geneva, Switzerland: International Organization for Standardization.
- ISO. (2004c). Soil quality – Determination of organic and total carbon after dry combustion (elementary analysis). [HRN ISO 10694:2004]. Geneva, Switzerland: International Organization for Standardization.
- ISO. (2004d). Soil quality – Determination of organic carbon by sulfochromic oxidation (modified; titrimetric determination, Tjurin method, bichromate method). [HRN ISO 14235:2004]. Geneva, Switzerland: International Organization for Standardization.
- ISO. (2004e). Soil quality -- Determination of particle size distribution in mineral soil material – Method by sieving and sedimentation. [HRN ISO 11277:2004]. Geneva, Switzerland: International Organization for Standardization.
- ISO. (2004f). Soil quality – Determination of pH (modified; w/v = 1 : 2,5). [HRN ISO 10390:2004]. Geneva, Switzerland: International Organization for Standardization.
- ISO. (2004g). Soil quality – Determination of total nitrogen content by dry combustion (elemental analysis). [HRN ISO 13878:2004]. Geneva, Switzerland: International Organization for Standardization.
- ISO. (2005). Soil quality – Determination of total sulfur by dry combustion. [HRN ISO 15178:2005]. Geneva, Switzerland: International Organization for Standardization.
- ISO. (2009). Soil quality – Pretreatment of samples for physico-chemical analysis. [HRN ISO 11464:2009]. Geneva, Switzerland: International Organization for Standardization.
- ISO. (2013). Soil quality – Screening soils for selected elements by energy-dispersive X-ray fluorescence spectrometry using a handheld or portable instrument. [HRN ISO 13196:2013]. Geneva, Switzerland: International Organization for Standardization.
- ISO. (2021a). Solid biofuels - Fuel specifications and classes - Part 6: Graded non-woody pellets. [HRN ISO 17225-6:2021]. Geneva, Switzerland: International Organization for Standardization.
- ISO. (2021b). Solid biofuels – Fuel specifications and classes – Part 1: General requirements. [HRN ISO 17225-1:2021]. Geneva, Switzerland: International Organization for Standardization.
- Jeguirim M., Dorge S., Trouvé G. (2010). Thermogravimetric analysis and emission characteristics of two energy crops in air atmosphere: *Arundo donax* and *Miscanthus giganteus*. *Bioresource Technology*. 101: 788–793.
- Kisić I. (2012). *Sanacija onečišćenoga tla*. Zagreb, Hrvatska: Agronomski fakultet Sveučilišta u Zagrebu.
- Lewandowski I., and Heinz A. (2003). Delayed harvest of miscanthus—influences on biomass quantity and quality and environmental impacts of energy production. *European Journal of Agronomy*. 19: 45–63.
- Morel J.L., Echevarria G., Goncharova N. (ed.) (2006). *Phytoremediation of Metal-Contaminated Soils*. NATO Science Series IV: Earth and Environmental Sciences. Vol 68. Dordrecht, Netherlands: Springer.
- Pravilnik o gospodarenju muljem iz uređaja za pročišćavanje otpadnih voda kada se mulj koristi u poljoprivredi (NN 38/08).
- Šestak I., Bilandžija N., Perčin A., Fadljević I., Hrelja I., Zgorelec Ž. (2022). Assessment of the Impact of Soil Contamination with Cadmium and Mercury on Leaf Nitrogen Content and *Miscanthus* Yield Applying Proximal Spectroscopy. *Agronomy*. 12: 255.
- Škorić A. (1982). *Priručnik za pedološka istraživanja*. Zagreb, Hrvatska: Fakultet poljoprivrednih znanosti Sveučilišta u Zagrebu.
- Vukadinović V., and Lončarić Z. (1998). *Ishrana bilja*. Osijek, Hrvatska: Poljoprivredni fakultet Sveučilišta Josipa Jurja Strossmayera u Osijeku.

- Zgorelec Ž. (2009). Phytoaccumulation of Metals and Metalloids from Soil Polluted by Coal Ash. Doctoral dissertation. Zagreb, Croatia: University of Zagreb Faculty of Agriculture.
- Zgorelec Ž., Bilandžija N., Knez K., Galić M., Žužul S. (2020). Cadmium and Mercury phytostabilization from soil using *Miscanthus × giganteus*. *Scientific Reports*. 10: 6685.
- Zhao A., Gao L., Chen B., Feng L. (2019). Phytoremediation potential of *Miscanthus sinensis* for mercury-polluted sites and its impacts on soil microbial community. *Environmental Science and Pollution Research*. 26: 34818–34829.

Soil amendments influence on cadmium and mercury phytoremediation using energy crop *Miscanthus x giganteus*

Abstract

The aim of this paper was to determine the influence of different amendments (waste sludge from a biogas plant, mycorrhiza, MxG ash) on the phytoremediation of soil contaminated with cadmium and mercury using the energy crop miscanthus (*Miscanthus x giganteus* – MxG). It was found that contamination with high concentrations of cadmium and mercury overpowered the effect of added amendments and negatively affected the yield. According to the calculated enrichment coefficient, miscanthus is more suitable for use in the process of phytostabilization and biomass production on soils moderately polluted with heavy metals.

Keywords: sewage sludge, mycorrhiza, ash, rhizomes, heavy metals, yield

Does conservation tillage, in synergy with liming and fertilization, can affect selected soil properties?

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Abstract

The aim of this study is to determinate effect of different tillage systems, liming and soil fertilization on pH, SOC – soil organic carbon and CEC – cation exchange capacity. Soil samples were taken from 0-15 and 15 -30 cm. At a depth of 0-15 cm, tillage and liming had a significant effect on pH, and tillage and fertilization had a significant effect on CEC. None of the tested treatments had a statistically significant effect on SOC. At a depth of 15-30 cm, soil reaction was significantly affected by tillage, liming, and fertilization, CEC was significantly affected by tillage, fertilization, and liming and SOC content was significantly affected by fertilization and liming.

Keywords: soil tillage systems, mineral fertilization, liming, soil health, soil properties

Introduction

Conservation tillage (CT) represents a critical step towards the sustainable future of agriculture. It promotes soil health, reduces erosion, mitigates climate change, and significantly contributes to soil fertilization (cost savings for farmers) (Jug et al., 2018). There are three primary pillars of conservation tillage: 1) Reduced soil disturbance that represents one of the fundamental principles of conservation tillage. This typically involves a significant reduction in or elimination of traditional tilling or ploughing practices; 2) Retention of crop residues on the soil surface. This includes minimum of 30% of the plant residuals left behind after harvest and 3) Crop rotation and diversification as often seen as complementary practices. By rotating crops and diversifying planting schedules, farmers can further improve soil health, reduce the risk of pests and diseases, and maintain a balanced nutrient cycle (Jug et al., 2022; FAO, 2016). While there are challenges associated with this shift in agricultural practices, the long-term benefits in terms of environmental sustainability and resilience make conservation tillage a compelling choice. Soil health and climate change are intimately linked, and understanding how conservation tillage affects both is crucial in the context of sustainable agriculture and environmental responsibility. It also, helps maintain and improve soil health by preventing erosion, retaining organic matter, and enhancing nutrient cycling (Ligang et al., 2023; Jug et al., 2019). Simultaneously, it acts as a significant player in mitigating climate change by sequestering carbon, reducing energy consumption, and indirectly leveraging methane emissions (Hatano and Lipiec, 2004). Also, by minimizing soil disturbance, they reduce the disruption of the intricate web of microorganisms, earthworms, and beneficial soil organisms that contribute to soil fertility. This, in turn, maintains the integrity of vital soil properties such as pH, organic matter content, and cation exchange capacity (Ligang et al., 2023). Organic matter, primarily derived from crop residues, is retained in the soil. This, in essence, captures atmospheric carbon dioxide, sequestering it within the soil, thereby reducing the overall carbon footprint of agriculture (Bot and Benites, 2005; Đurđević et al., 2019). In comparison to conventional tillage methods, conservation tillage is more energy-efficient. The reduced need for heavy machinery and fuel consumption is not only economically beneficial but also ecologically responsible, given the associated reduction in carbon emissions. While conservation tillage holds immense promise, its full potential is realized when combine with different fertilization strategies and liming. Fertilization is the practice of adding essential nutrients to the soil to enhance plant growth, and liming represent traditional procedure for amelioration

of acid soils (Jug et al., 2022; Đurđević et al., 2011). When these practices are integrated, they influence selected soil properties in a synergistic manner, ultimately fostering a more productive and sustainable agricultural system (Jug et al., 2021). Considering the mentioned importance of conservation tillage practice to soil health the aim of this study is to determinate effect of different tillage systems, liming and soil fertilization practice on three key soil health indicators - pH, soil organic carbon (SOC), cation exchange capacity (CEC).

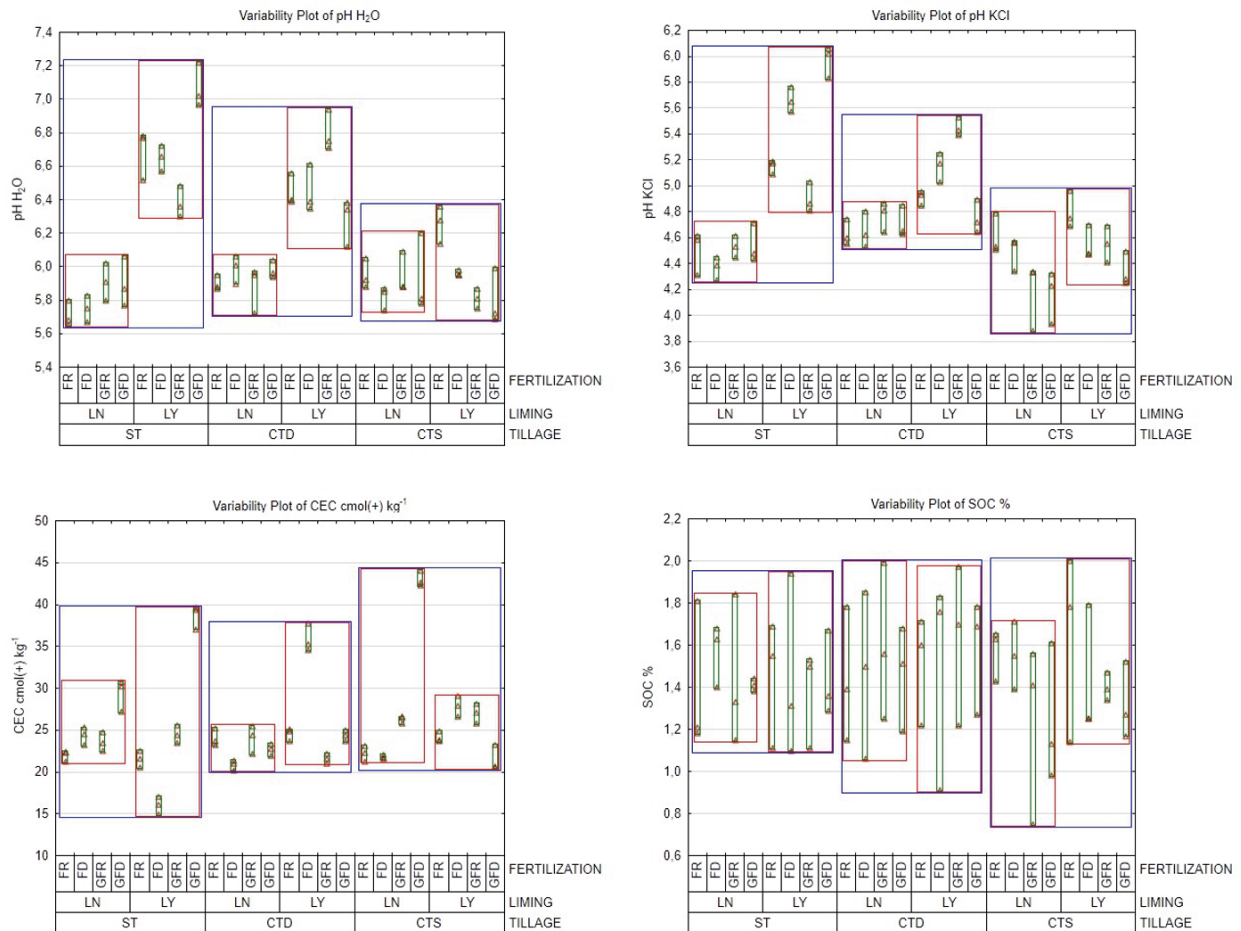
Material and methods

The research was conducted in the eastern part of Croatia (location Čačinci - 17° 86' 36" E, 45° 61' 32" N) on Stagnosol. Results in this paper include research of the second year 2022 where the impact of different tillage systems, liming and soil fertilization practice on key soil health indicators was investigated. First factor was the soil tillage treatments: conventional tillage (ST) - ploughing up to 30 cm depth, deep conservation tillage (CTD) - loosening up to 30 cm depth with a minimum of 30% soil coverage with crop residues and shallow conservation tillage (CTS) - loosening up to 10 cm depth with a minimum of 50% soil surface coverage with crop residues. Second factor included liming (LY) 4375 kg ha⁻¹ CaO (according to the recommendation for neutralizing the acid soil reaction, calculated with ALRxp computer program) (Đurđević et al., 2011) and treatment without liming (LN). Third factor was soil fertilization (FR - N₄₀ kg : P₂O₅-150 kg : K₂O₉₄ kg; FD - N₂₀ kg : P₂O₅-75 kg : K₂O₄₇ kg; GFR - N₄₀ kg : P₂O₅-150 kg : K₂O₉₄ kg + 150 kg Geo2_ soil microbial biomass activator; GFD - N₂₀ kg : P₂O₅-75 kg : K₂O₄₇ kg + 150 kg Geo2_ soil microbial biomass activator). The experiment was carried out on 24 plots (4 fertilisation treatments x 2 liming treatments x 3 tillage treatments) x 3 repetitions. The basic plot size was 320 m² (tillage treatment) and the size of the fertilization and liming plots was 80 m². One composite sample consists of 20 cores collected within a plot, with the sample recovery depth of 0-15 cm and 15-30 cm. Soil samples were taken after the harvest of soybean, before application of any kind of fertilization and prior to soil preparation for next crop (Đurđević, 2014.). Soil samples were stored in plastic bags, homogenized, dried, milled and analysed in a laboratory - pH - using a glass electrode in a 1:5 (volume fraction) suspension of soil, (in H₂O and KCl) (ISO, 2021); SOC - determination of organic and total carbon after dry combustion (elementary analysis) (ISO, 1995); CEC - determination of effective cation exchange capacity and base saturation level using barium chloride solution (ISO, 2018). ANOVA design with soil tillage and fertilization as given factors was used to test the influence of different soil tillage systems, liming and different fertilization levels on pH, soil organic carbon (SOC), cation exchange capacity (CEC). Mean values that were significant according to the performed F- test were compared using the LSD test at p < 0.05 level of significance for the investigated factors. Statistica software package, version 14.0.0. (TIBCO Software Inc., Palo Alto, CA, USA) was used to conduct the ANOVA analysis and graphic designs.

Results and discussion

The soil chemical health indicators under different tillage measures were analysed. The results show that active and exchangeable acidity were significantly affected by soil tillage and liming (p value <0.001), with average values of 6.13 (pH-H₂O) and 4.76 (pH-KCl). Significant interactions were observed between the tested factors. The highest measured values of active (7.07) and exchangeable acidity (5.97) were observed on conventional tillage with liming on the GFD fertilization, while the lowest value of active acidity was observed on conventional tillage without liming on the FR fertilization (5.71). The exchangeable acidity was lowest on CTS tillage without liming and on the GFD fertilization (4.16) (Graph 1). Conservation tillage can lower the soil pH due to decreased aeration. According to Yuan et al. (2022) conventional tillage disrupts the soil structure, reduces soil fertility, and increases soil porosity, leading to nutrient loss and a decrease in soil organic matter. On the other hand, conservation tillage can reduce wind and water erosion, provide shade, and improve soil moisture, and also reduce soil pH. However, it is important to note that the effect of conservation tillage on pH can vary depending on a number of factors, such as soil type, climate, and cropping system (Busari et al., 2015). The average CEC was 25.48 cmol⁽⁺⁾ kg⁻¹, and its variation was significantly affected by soil tillage and fertilization (p value <0.001). The highest CEC value was measured on CTS tillage, without liming on the GFD fertilization (42.99 cmol⁽⁺⁾ kg⁻¹), while the lowest CEC value was measured on conventional tillage, with liming on the FD fertilization (16 cmol⁽⁺⁾ kg⁻¹). None of the tested treatments had a statistically significant effect (p value >0.5) on the value of soil organic carbon, which averaged 1.46% (Graph 1). Conservation tillage can increase cation exchange capacity (CEC), especially in arid and semi-arid regions, primarily due to improved surface properties of soil particles. It promotes the formation of soil aggregates, which are clusters

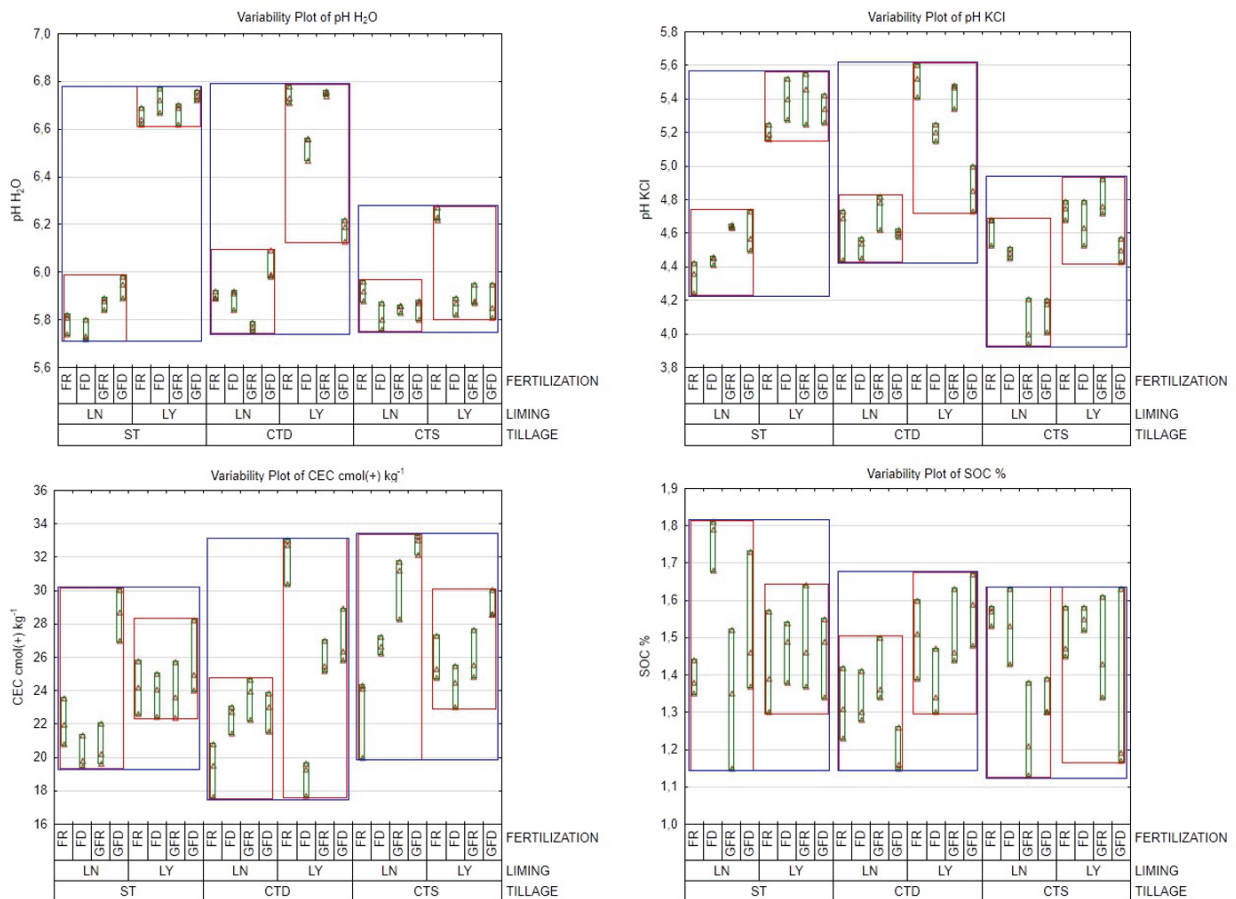
of soil particles that are held together by organic matter. Also, it can reduce the loss of soil organic matter (SOM), which is a major source of CEC in soil. Soil aggregates have a higher surface area than individual soil particles, which gives them a greater capacity to hold and exchange cations. A number of studies have shown that conservation tillage can improve CEC (Cooper et al., 2020). For example, according to Ligang et al. (2023) when applying conservation tillage practice, CEC was significantly higher in the top 15 cm of soil than in conventionally tilled soil. Another study by Busari et al., (2015) reported that effective cation exchange capacity (ECEC) were significantly higher at the end of the two years of study under CT in compare to conventional tillage.



Graph 1. Variability plots of pH (H₂O), pH (KCl), CEC and SOC (0-15 cm) (Conventional tillage (ST), deep conservation tillage (CTD), shallow conservation tillage (CTS); liming (LY), without liming (LN); FR - recommended fertilization FD - reduced fertilization by 50%, GFR - recommended fertilization + soil microbial biomass activator, GFD - reduced fertilization by 50% + soil microbial biomass activator)

At a depth of 15-30 cm, all the parameters tested were significantly affected by the treatments. Statistically significant interactions were observed between all treatments. The average active acidity was 6.13 and was significantly affected by soil tillage, liming, and fertilization. The highest value of active acidity was measured on the CTD, LY, GFR treatment and was 6.75, while the lowest value was measured in soil samples on the ST, LN, FD treatments and was 5.75. The average exchangeable acidity was 4.86 and the highest value (5.51) was measured in soil on the CTD, LY, FR treatments, while the lowest value (4.05) was measured on the CTS, LN, GFR. Cation exchange capacity was significantly affected by tillage, liming and fertilization (p value <0.001) and averaged 20.64 cmol(+) kg⁻¹. The highest CEC was measured on the CTS, LN, GFD treatment (32.81 cmol(+) kg⁻¹), and the lowest (18.87 cmol(+) kg⁻¹) on the CTD, LY, FD treatment. The average organic carbon content in the soil was 1.44% and was significantly affected by fertilization and liming (p value <0.005). All interactions of the investigated treatments were statistically significant.

The highest organic carbon content was measured in soil samples on the ST, LN, FD (1.76%), and the lowest on the CTD, LN, GFD (1.19%) (Graph 2). One of the main benefits of CT is that it can increase SOC content in the top layers of the soil. However, there is some evidence that CT may lead to lower measured values of SOC in deeper layers of the soil. CT may reduce the incorporation of organic matter into deeper layers of the soil. This is because CT practices such as no-till and reduced tillage disturb the soil less than conventional tillage (ploughing), which can make it more difficult for organic matter to be mixed into the soil profile. Also, CT may promote the decomposition of SOM in deeper layers of the soil. This is because CT practices can increase soil moisture and aeration, which can create more favourable conditions for the microbes that break down SOM (Li et al., 2020). Blanco-Canqui et al. (2018) reported that soil organic C mainly accumulates near the surface.



Graph 2. Variability plots of pH (H₂O), pH (KCl), CEC and SOC (15-30 cm) (conventional tillage (ST), deep conservation tillage (CTD), shallow conservation tillage (CTS); liming (LY), without liming (LN); FR - recommended fertilization FD - reduced fertilization by 50%, GFR - recommended fertilization + soil microbial biomass activator, GFD - reduced fertilization by 50% + soil microbial biomass activator)

Conclusions

The effect of tillage, liming, and fertilization on selected soil properties varied depending on the soil depth. At a depth of 0-15 cm, tillage and liming had a significant effect on pH, with the highest pH measured in conventional tillage with liming and the lowest pH measured in conservation tillage without liming. Tillage and fertilization had a significant effect on CEC, with the highest CEC measured in conservation tillage. At a depth of 15-30 cm, all of the parameters tested were significantly affected by the treatments. Soil reaction was significantly affected by tillage, liming, and fertilization, with the highest active acidity measured in conventional tillage and the lowest active acidity

measured in conservation tillage. CEC was significantly affected by tillage, fertilization, and liming, with the highest CEC measured in conservation tillage. Soil organic carbon content was significantly affected by fertilization and liming, with the highest organic carbon content measured in conservation tillage. It is important to note that the effectiveness of conservation tillage practices in synergy with liming and fertilization may vary depending on the specific soil type, climate, crop type, and local conditions. Therefore, further research, especially long-term trials, is needed.

Acknowledgement

This work has been fully supported by Croatian Science Foundation under the project “Assessment of conservation soil tillage as advanced methods for crop production and prevention of soil degradation – ACTIVEsoil” (IP-2020-02-2647).

References

- Blanco-Canqui H.O., and Ruis S.J. (2018). No-tillage and soil physical environment. *Geoderma*. 326: 164-200.
- Bot A., and Benites J. (2005). The importance of soil organic matter – Key to drought-resistant food and production. p 80. Rome, Italy: Food and Agriculture Organization (FAO) of the United Nations.
- Busari M.A., Kukul S.S., Kaur A., Bhatt R., Dulazi A.A. (2015). Conservation tillage impacts on soil, crop and the environment. *International Soil and Water Conservation Research*. 3: 119-129.
- Cooper R.J., Hama-Aziz Z.Q., Hiscock K.M., Lovett A.A., Vrain E., Dugdale S.J., Sünnerberg G., Dockerty T., Hovesen P., Noble L. (2020). Conservation tillage and soil health: Lessons from a 5-year UK farm trial (2013–2018). *Soil and Tillage Research*. 202: 104648.
- Đurđević B., Vukadinović V., Bertić B., Jug I., Vukadinović V., Jurišić M., Dolijanović Ž., Andrijačić M. (2011). Liming of acid soils in Osijek-Baranja County. *Journal of Agricultural Sciences*. 56: 187–195.
- Đurđević B. (2014). *Praktikum iz ishrane bilja*. p 71. Osijek, Hrvatska: Sveučilište J. J. Strossmayera u Osijeku, Fakultet agrobiotehničkih znanosti Osijek.
- Đurđević B., Jug I., Jug D., Bogunović I., Vukadinović V., Brozović B. (2019). Spatial variability of soil organic matter content in Eastern Croatia assessed using different interpolation methods. *International Agrophysics*. 33: 31-39.
- FAO. (2016). What is Conservation Agriculture? Available online: <http://www.fao.org/> (accessed on 20 October 2023).
- Hatano R., and Lipiec J. (2004). Effects of land use and cultural practices on greenhouse gases fluxes in soil. *Acta Agrophysica*. 109: 1–51.
- ISO. (1995). Soil quality – Determination of organic and total carbon after dry combustion (elementary analysis). [ISO 10694:1995(E)]. Geneva, Switzerland: International Organization for Standardization.
- ISO. (2018). Soil quality – Determination of effective cation exchange capacity and base saturation level using barium chloride solution. [ISO 11260:2018(E)]. Geneva, Switzerland: International Organization for Standardization.
- ISO. (2021). Soil quality – Soil, treated biowaste and sludge – Determination of pH. [ISO 10390:2021(E)]. Geneva, Switzerland: International Organization for Standardization.
- Jug D., Jug I., Brozović B., Vukadinović V., Stipešević B., Đurđević, B. (2018). The role of conservation agriculture in mitigation and adaptation to climate change. *Poljoprivreda*. 24: 35–44.
- Jug D., Đurđević B., Birkás M., Brozović B., Lipiec J., Vukadinović V., Jug I. (2019). Effect of conservation tillage on crop productivity and nitrogen use efficiency. *Soil and Tillage Research*. 194: 104327.

- Jug I., Brozović B., Đurđević B., Wilczewski E., Vukadinović V., Stipešević B., Jug D. (2021). Response of crops to conservation tillage and nitrogen fertilization under different agroecological conditions. *Agronomy*. 11: 1-18.
- Jug I., Jug D., Brozović B., Vukadinović V., Đurđević B. (2022). Osnove tloznanstva i biljne proizvodnje, Jug I. (ed.), p 527. Osijek, Hrvatska: Sveučilište J. J. Strossmayera u Osijeku, Fakultet agrobiotehničkih znanosti Osijek.
- Lal R., Minasny B., Morgan M. A., McBratney A. B., Bernhard A., Schrumpp M., Huggins D. R. (2015). Standardizing procedures for soil carbon monitoring: why and how? *Geoderma*. 252: 28-34.
- Li J., Wang Yk., Guo Z., Li J., Tian C., Hua D., Shi C., Wang H., Han J., Xu Y. (2020). Effects of Conservation Tillage on Soil Physicochemical Properties and Crop Yield in an Arid Loess Plateau, China. *Scientific Reports*. 10: 4716.
- Ligang L., Zhoubing G., Kaihua L., Qing Z., Junjun Z. (2023). Impact of conservation tillage on the distribution of soil nutrients with depth. *Soil and Tillage Research*. 225: 105527.
- Yuan J., Yan L., Li G. Sadiq M., Rahim N., Wu J., Ma W., Xu G., Du M. (2022). Effects of conservation tillage strategies on soil physicochemical indicators and N₂O emission under spring wheat monocropping system conditions. *Scientific Reports*. 12: 7066.

Influence of conservation tillage and fertilization on weed infestation and soybean yield

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Abstract

The aim of the experiment set up in Čačinci (17° 86' 36" E, 45° 61' 32" N, 111 m a. s. l.) on Stagnosol soil type in 2021 year was to determine the influence of conservation tillage and fertilization on soybean weediness and yield. Tillage treatments included conventional tillage (ST), deep (CTD) and shallow conservation tillage (CTS). Fertilization subtreatment were recommended amount (FR) and (HF) - 50% off. Tillage had a significant effect on soybean weediness. The highest average weed biomass (141.86 g m⁻²) and weed density (25.17 m⁻²) were recorded on CTS. Reduced fertilization resulted with the highest weed density (32.00 m⁻²) on CTD. Average soybean yields were the highest at ST (6.98 t ha⁻¹) without significant differences (p<0.05) compared to CTS (6.88 t ha⁻¹). Conservation tillage increased weediness without a significant decrease in soybean yield.

Keywords: soil tillage systems, mineral fertilization, weediness, *Glycine max* (L.) Merr.

Introduction

Nowadays, when agriculture is one of the most vulnerable sectors affected by climate change, conservation agriculture is recognized as one of the most effective methods of adaptation and mitigation of the negative consequences of climate change on plant production (Jug et al. 2018; Aune, 2012). It implies proper crop rotation, minimum soil disturbance and permanent soil coverage with at least 30% of crop residues (FAO, 2016). Conservation tillage is a suitable way to prevent soil degradation processes with preservation of soil health and fertility due to the positive effect on soil quality, water and nutrient conservation, yield stability, increased biodiversity while reducing production costs (Palm et al., 2013; Derpsch, 2005). Despite the numerous advantages of conservation tillage, the systematic introduction and acceptance of such production systems is still sometimes limited by certain factors that often relate to expected changes in the level of weediness of agricultural crops (Derksen, 1996; Derrouch et al., 2020). In addition to numerous available measures of sustainable weed management, weeds still represent a dominant limiting biotic factor in plant production, and conservation tillage certainly changes the composition of the weed flora and the intensity of weediness, but not necessarily to the detriment of agricultural crop. Besides tillage, fertilization is also a significant factor that contributes to the sustainability of crop production systems and also has an impact on crop weediness (Wan et al., 2012; Brozović et al., 2021). According to previous research, optimal fertilization can have a two-fold effect on weediness level. Due to the greater weed nutrient use efficiency related to the crops, the occurrence of weeds can be increased with optimal fertilization (Nie et al., 2009; Cheimona et al., 2016). However, it is also possible to reduce the occurrence of weeds through an increased competitive advantage of the crop as a result of optimal fertilization (Travlos et al., 2018; Kaur et al., 2018) and due to lower nutrient requirements of weeds suboptimal fertilization can result in a competitive advantage of weeds over the agricultural crops (Légère et al., 2008). The importance of soybean as an agricultural crop stems from its wide multiple uses as well as its positive agronomic effects (Stritongtae et al., 2021; Sudarić and Vratarić, 2008). Adverse weather conditions have been increasingly present in the soybean vegetation in recent years, which requires adoption of effective measures such as conservation tillage to maintain stability of soybean yield (Moraru and Rusu, 2012). Soybean is susceptible to

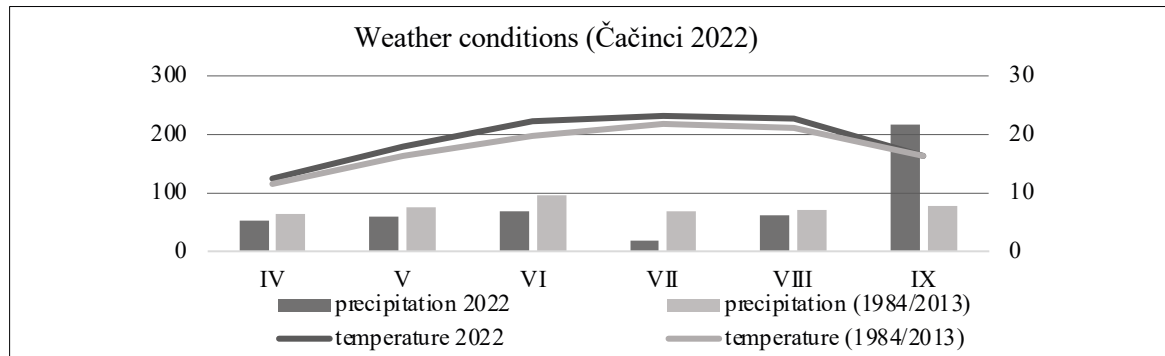
weeds (Gaweda et al., 2020) and weediness is one of the main causes of yield reduction (Wallace et al., 2018). Because of all the above, the aim of this study was to determine the influence of conservation tillage and fertilization systems on weediness and yield of soybeans.

Material and methods

The research with conservation tillage and fertilization was conducted in the eastern region of Croatia in Čačinci (17° 86' 36" E, 45° 61' 32" N, 111 m a. s. l.) on Stagnosol soil type (IUSS Working Group) in 2021 year with basic soil chemical properties: pH_{KCl/H₂O} (4.09/5.65), Hy-hydrolytic acidity (7.90 cmol⁽⁺⁾ kg⁻¹), AL_{p₂O₅} (10.37 mg 100 g⁻¹), AL_{K₂O} (15.63 mg 100 g⁻¹), SOM-soil organic matter (2.8 %). This paper presents the results of the second research year (2022) where the impact of different tillage and fertilization systems on weediness and soybean yield was investigated. The basic plot size was 160 m² (tillage treatment) and the size of the subtreatment (fertilization) plot was 80 m² (8 x 10 m). The soil tillage treatments were: conventional (ST) - plowing up to 30 cm depth, deep conservation tillage (CTD) - loosening up to 30 cm depth with a minimum of 30% soil coverage with crop residues and shallow conservation tillage (CTS) - loosening up to 10 cm depth with a minimum of 50% soil surface coverage with crop residues. Subtreatments included recommended fertilization (F) calculated with the ALRxp computer program for fertilizer recommendations (Vukadinović and Vukadinović, 2011) and suboptimal fertilization (HF) reduced by 50%. Soil tillage (plowing and loosening) was performed in autumn 2021 after the maize harvest. The winter furrow was closed in spring 2022 on ST tillage with a two pass of spike-tooth harrow combine with a hollow roller. Pre-sowing soil preparation was performed with one pass of a spike-tooth harrow combined with hollow roller. Fertilization was carried out in autumn prior to basic soil tillage in recommended amount (530 kg ha⁻¹ NPK – 0:20:30) and 50% reduced dose (265 kg ha⁻¹ NPK – 0:20:30). Combined with pre-sowing soil preparation recommended (150 kg ha⁻¹) and reduced amount (75 kg ha⁻¹) of KAN (27% N) were applied in spring. No-till seeder was used for soybean sowing (cultivar IKA) on April 12 with seeding rate of 600 000 seeds ha⁻¹. Chemical weed control was carried out uniformly on each investigated treatment and subtreatment twice and included pre-em treatment with application of 960 g l⁻¹ S-Metolachlor (1.2 l ha⁻¹) and Metribuzin 70 % (0.6 kg ha⁻¹) and post-em with 22.4 g l⁻¹ Imazamox, 480 g l⁻¹ Bentazon (1 l ha⁻¹) using sprayer. Soybean harvest was preformed manually on the whole investigated subplots and the rest of the experimental area was harvested using combine harvester. The yields have been recalculated to standard moisture content (9%). Weed assessment was carried out by weeds sampling in V3 (three unfolded trifoliolate leaves) and R7 (beginning maturity - one normal pod on the main stem has reached its mature pod color) soybean growth stages. The weed density, above-ground biomass, the number of weed species and weed coverage were determined at each treatment and sub-treatment. All classified weed species on the area of 0.25 m² in four repetitions were counted for weed density determination and cut off on the ground level, separated by different weed types and dried at 60 °C for 48 h to evaluate the aboveground biomass while weed coverage was determined visually. ANOVA design with soil tillage and fertilization as given factors was used to test the influence of different soil tillage systems and different fertilization levels on weed density, weed biomass, weed species number, weed coverage and soybean yield. Mean values that were significant according to the performed F- test were compared using the LSD test at p < 0.05 level of significance for the investigated factors. Statistica software package, version 14.0.0. (TIBCO Software Inc., Palo Alto, CA, USA) was used to conduct the ANOVA analysis.

Results and discussion

The lack of precipitation was pronounced during the soybean growing season from April to August in the conducted research (Graph 1). The amount of precipitation was lower compared to the multi-year average (1984/2013), with pronounced dry conditions in July, when only 18 mm of rain fell, which is almost 4 times less than the annual average (Graph 1). The lack of precipitation in the mentioned period was accompanied by above-average air temperatures, looking at the multi-year average (1984/2013). The dry period was interrupted in September, which was extremely wet, and the recorded amount of rain was almost three times higher than the average value (Graph 1).



Graph 1. Weather conditions at experimental site (Čačinci 2022)

During the research, the most numerous broadleaved annual was *Ambrosia artemisiifolia* L. while dominant perennial was *Calystegia sepium* (L.) R. Br. *Setaria viridis* (L.) P. Beauv. was the most present annual grass. In addition to the above, *Cirsium arvense* (L.) Scop., *Convolvulus arvensis* L., *Lythrum salicaria* L., *Mentha spicata* L., *Xanthium strumarium* L., *Setaria glauca* (L.) P. Beauv. and *Panicum capillare* L. were also determined weed species. Soil tillage significantly affected weed occurrence in V3 soybean growth stage (Table 1). Tillage had a significant effect on biomass, weed density and weed coverage in V3 soybean growth stage, while the average effect of fertilization on all investigated parameters of weediness was absent which is opposite to Tang et al. (2014) but consistent with Légère et al., (2008) who reported stronger effects of tillage on weed community than fertilization in soybean-maize crop rotation. The weed density was almost three times higher on CTS compared to ST in average (Table 1) but on deep conservation tillage systems (CTD) there was no statistically significant difference compared to ST. Higher weed densities, biomass and weed coverage are also confirmed by Légère et al., (2008); Romaneckas et al., (2021); Brozović et al., (2023) who investigated the impact of lower tillage intensity on weed community in agricultural crops. Interactions among tillage and fertilization existed in the case of weed density and coverage, which were the highest on shallow conservation tillage with suboptimal fertilization (CTS/HF) with statistically significant differences ($p < 0.05$) compared to conventional tillage and lower fertilization (ST/HF) (Table 1). Soybean weediness increased during the growing season (Table 2). A noticeable increase in all investigated parameters of weediness is a possible consequence of unfavorable weather conditions (lack of precipitation) (Graph 1) and it is known that stressful environmental conditions benefit weeds more than crops (Patterson, 1995) due to their ability to adapt to different adverse conditions (Radicetti and Mancinelli, 2021).

Table 1. Weed infestation of soybean in V3 growth stage

Tillage (T)	ST		CTD		CTS		Average (F)	
Fertilization (F)	FR	FH	FR	FH	FR	FH	FR	FH
WB (g m ⁻²)	3.87 ^{n.s.}	2.97	5.89	8.21	10.84	8.64	6.87 ^{n.s.}	6.61
Average (T)	3.42 ^B		7.05 ^A		9.74 ^A			
$F_{(T)}=11.753, F_{(F)}=0.060, F_{(T \times F)}=1.578$								
WD (m ⁻²)	36.00 ^b	25.33 ^c	23.67 ^c	33.00 ^{bc}	38.00 ^b	50.33 ^a	32.56 ^{n.s.}	36.22
Average (T)	30.67 ^B		28.34 ^B		44.17 ^A			
$F_{(T)}=9.670, F_{(F)}=1.335, F_{(T \times F)}=5.173$								
WSN (m ⁻²)	2.00 ^{n.s.}	2.33	1.67	1.67	2.67	1.67	2.11 ^{n.s.}	1.89
Average (T)	2.17 ^{n.s.}		1.67		2.17			
$F_{(T)}=1.800, F_{(F)}=0.800, F_{(T \times F)}=2.600$								
WC (%)	19.00 ^c	12.00 ^d	36.33 ^{ab}	33.00 ^{bc}	34.00 ^b	38.67 ^a	29.78 ^{n.s.}	27.89
Average (T)	15.5 ^B		34.67 ^A		36.34 ^A			
$F_{(T)}=131.591, F_{(F)}=2.627, F_{(T \times F)}=8.736$								

T-tillage, F-fertilization, ST-conventional tillage, CTD-deep conservation tillage, CTS-shallow conservation tillage, FR-recommended fertilization, FH-reduced fertilization, WB-weed biomass, WD-weed density, WSN-weed species number, WC-weed coverage, F(T)- F test for tillage, F(F)-F test for fertilization, F(TxF)-F test for tillage and fertilization interaction

In average, shallow conservation tillage (CTS) resulted in the highest weediness, and all investigated parameters were statistically significantly different ($p < 0.05$) compared to conventional tillage (ST) (Table 2).

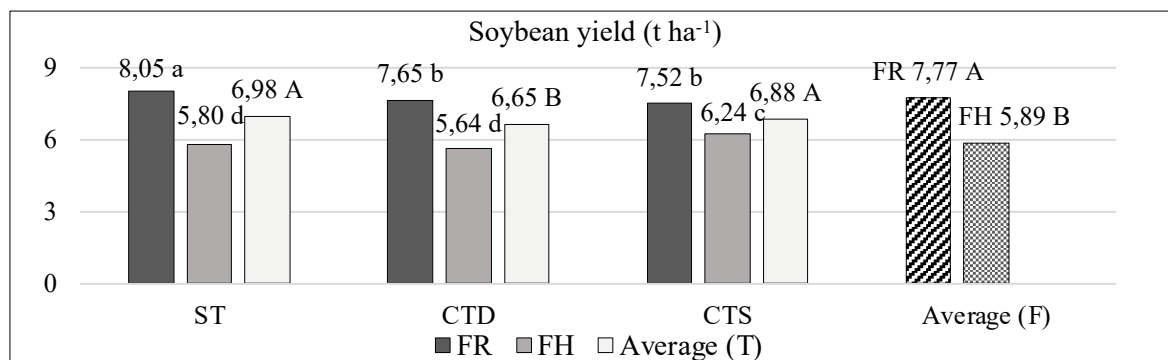
Table 2. Weed infestation of soybean in R7 growth stage

Tillage (T)	ST		CTD		CTS		Average (F)	
Fertilization (F)	FR	FH	FR	FH	FR	FH	FR	FH
WB (g m ⁻²)	20.28 ^{n.s.}	8.19	45.71	56.28	129.87	153.85	65.29 ^{n.s.}	72.77
Average (T)	14.24 ^C		51.00 ^B		141.86 ^A			
	$F_{(T)}=36.131, F_{(F)}=0.352, F_{(TxF)}=0.696$							
WD (m ⁻²)	18.33 ^b	16.00 ^{bc}	11.33 ^c	32.00 ^a	20.67 ^b	29.67 ^a	16.78 ^B	25.89 ^A
Average (T)	17.17 ^C		21.67 ^B		25.17 ^A			
	$F_{(T)}=9.926, F_{(F)}=38.423, F_{(TxF)}=20.406$							
WSN (m ⁻²)	3.33 ^c	2.33 ^{cd}	2.00 ^d	3.67 ^b	4.67 ^b	6.67 ^a	3.33 ^B	4.22 ^A
Average (T)	2.83 ^B		2.84 ^B		5.67 ^A			
	$F_{(T)}=36.125, F_{(F)}=8.000, F_{(TxF)}=9.125$							
WC (%)	33.00 ^c	18.67 ^d	38.00 ^b	40.33 ^{ab}	37.33 ^b	42.33 ^a	36.11 ^A	33.78 ^B
Average (T)	25.84 ^B		39.17 ^A		39.83 ^A			
	$F_{(T)}=24.856, F_{(F)}=1.627, F_{(TxF)}=10.937$							

T-tillage, F-fertilization, ST-conventional tillage, CTD-deep conservation tillage, CTS-shallow conservation tillage, FR-recommended fertilization, FH-reduced fertilization, WB-weed biomass, WD-weed density, WSN-weed species number, WC-weed coverage, F(T)- F test for tillage, F(F)-F test for fertilization, F(TxF)-F test for tillage and fertilization interaction

Increase in weed biomass, weed coverage and weed species number during the growing season on reduced tillage systems is also reported by previous research (Hofmeijer et al., 2019; Armengot et al., 2016; Sans et al., 2011). Optimal fertilization resulted with average decrease of weed density and weed species number but with higher weed biomass and weed coverage in maturity soybean growth stage (R7) (Table 2). Inconsistent influence of fertilization on weed infestation is a possible consequence of greater weed nutrient use efficiency (Nie et al., 2009; Cheimona et al., 2016) or lower nutrient requirements related to crops (Légère et al., 2008) and at the same time increased competitive advantage of the crop as a result of optimal fertilization (Travlos et al., 2018; Kaur et al., 2018). Increase in the average number of weed species on conservation tillage systems compared to conventional (ST) indicate the positive effects of reduced tillage on species diversity (Légère et al., 2008). The highest weed species number was recorded on shallow conservation tillage with reduced fertilization CTS/FH, almost three times higher compared to ST/FH while CTD/FH resulted with greater weed density compared to ST/FH. Tillage and fertilization had a significant impact on soybean yields. Average soybean yields were not significantly different ($p < 0.05$) among shallow conservation tillage (CTS) and conventional tillage (ST). Adequate soybean yields on reduced tillage systems are also reported by Čeřan et al., 2022. Reduced fertilization led to an average decrease in soybean yield and obtained yield was lower on FH for almost 2 t ha⁻¹. Significant interactions between tillage and fertilization in terms of yields were found. The highest soybean yield was achieved in ST/FR, while yields on CTD/FR and CTS/FR did not statistically significantly differ among themselves. Looking at soybean yields on reduced fertilization, the best was achieved on CTS/FH, and there were no statistically significant differences ($p < 0.05$) between ST/FH and CTD/FH. Soybean production is under the influence of soil fertility and available soil water content in relation to the soil tillage (Acharya et al., 2019). Drought

conditions correlated with high temperatures during the research certainly influences the yield formation (Basal and Szabo, 2020; Cotrim et al., 2021) but despite the present drought, soybean yields in this research were satisfactory.



Graph 2. Soybean yield on different tillage and fertilization treatments

Conclusion

Conservation tillage systems had the effect of increasing the level of soybean weediness. The average influence of fertilization on weediness was less expressed compared to soil tillage. Tillage and fertilization significantly affected soybean yield. The highest soybean yields were achieved on the conventional (ST) and shallow conservation tillage systems (CTS) despite the fact that this treatment had the highest weediness. Reduced fertilization led to a decrease in soybean yield on average, but not on the CTS tillage system, which can be recommended as substitution for plowing with the need for further research.

Acknowledgement

This work has been fully supported by Croatian Science Foundation under the project “Assessment of conservation soil tillage as advanced methods for crop production and prevention of soil degradation – ACTIVEsoil” (IP-2020-02-2647).

References

- Acharya B.S., Dodla S., Gaston L.A., Darapuneni M., Wang J.J., Sepat S., Bohara H. (2019). Winter cover crops effect on soil moisture and soybean growth and yield under different tillage systems. *Soil Tillage Research*. 195: 104430.
- Armengot L., Blanco-Moreno J., Bàrberi P., Bocci G., Carlesi S., Aendekerk R. (2016). Tillage as a driver of change in weed communities: A functional perspective. *Agriculture, Ecosystems & Environment*. 222: 276–285.
- Aune J.B. (2012). Conventional, Organic and Conservation Agriculture: Production and Environmental Impact. In *Agroecology and Strategies for Climate Change. Sustainable Agriculture Reviews*, 1st ed.; Lichtfouse, E., Ed.; Springer Dordrecht: Berlin, Germany. 8: 149–165.
- Basal O., Szabo A. (2020). Physiology, yield and quality of soybean as affected by drought stress. *Asian Journal of Agriculture and Biology*. 8: 247-252.
- Brozović B., Jug I., Jug D., Stipešević B., Ravlić M., Đurđević B. (2021). Biochar and Fertilization Effects on Weed Incidence in Winter Wheat. *Agronomy*. 11: 2028
- Brozović B., Jug I., Đurđević B., Ravlić M., Vukadinović V., Rojnica I., Jug D. (2023). Initial Weed and Maize Response to Conservation Tillage and Liming in Different Agroecological Conditions. *Agronomy*. 13: 1116.
- Cheimona N., Angeli C., Panagiotou E., Tzanidaki A., Drontza C., Travlos I., Bilalis D. (2016). Effect of different types of fertilization on weed flora in processed tomato crop. *Agriculture and Agricultural Science Procedia*. 10: 26–31.

- Cheţan F, Rusu T, Cheţan C., Urdă C., Rezi R., Şimon A., Bogdan I. (2022). Influence of Soil Tillage Systems on the Yield and Weeds Infestation in the Soybean Crop. *Land*. 11: 1708.
- Cotrim M.F., Gava R., Campos C.N.S., De David C.H.O., Reis I.D.A., Teodoro L.P. R. (2021). Physiological performance of soybean genotypes grown under irrigated and rainfed conditions. *Journal of Agronomy and Crop Science*. 207: 34-43.
- Derksen D.A., Blackshaw R.E., Boyetchko S.M. (1996). Sustainability, conservation tillage, and weeds in Canada. *Canadian Journal of Plant Science*. 76: 651–659.
- Derrouch D., Chauvel B., Felten E., Dessaint F. (2020). Weed Management in the Transition to Conservation Agriculture: Farmers' Response. *Agronomy*. 10: 843.
- Derpsch R. (2005). The extent of conservation agriculture worldwide. Implications and impact. In *Proceedings of the III World Congress on Conservation Agriculture*. Mkomva et al., 13-16. Nairobi, Kenya: African Conservation Tillage Network, Nairobi. 4–8 October 2005.
- FAO. (2016). What is Conservation Agriculture? Available online: <https://www.fao.org/>. (accessed on 20 October 2023).
- Gawęda D., Haliniarz M., Bronowicka-Mielniczuk U., Łukasz J. (2020). Weed Infestation and Health of the Soybean Crop Depending on Cropping System and Tillage System. *Agriculture*. 10: 208.
- Hofmeijer M.A.J., Krauss M., Berner A., Peigné J.; Mäder P., Armengot L. (2019). Effects of Reduced Tillage on Weed Pressure, Nitrogen Availability and Winter Wheat Yields under Organic Management. *Agronomy*. 9: 180.
- Légère A., Stevenson F.C., Ziadi N. (2008). Contrasting Responses of Weed Communities and Crops to 12 Years of tillage and Fertilization Treatments. *Weed Technology*. 22: 309–317.
- Jug D., Jug I., Brozović B., Vukadinović V., Stipešević B., Đurđević B. (2018). The role of conservation agriculture in climate change mitigation. *Poljoprivreda*. 24: 35-44.
- Kaur S., Kaur R., Chauhan B.S. (2018). Understanding crop-weed-fertilizer-water interactions and their implications for weed management in agricultural systems. *Crop Protection*. 103: 65-72.
- Moraru P. I., Rusu T. (2012). Effect of tillage systems on soil moisture, soil temperature, soil respiration and production of wheat, maize and soybean crops. *Journal of Food, Agriculture & Environment*. 10: 445-448.
- Nie J., Yin L. C., Liao Y. L., Zheng S. X., Xie, J. (2009). Weed community composition after 26 years of fertilization of late rice. *Weed Science*. 57: 256–260.
- Palm C., Blanco-Canqui H., De Clerck F., Gatere P. (2013). Conservation agriculture and ecosystem services: An overview. *Agriculture, Ecosystems & Environment*. 187: 87–105.
- Patterson D.T. (1995). Weeds in Changing Climate. *Weed Science*. 43: 685–701.
- Radicetti E. Mancinelli R. (2021). Sustainable Weed Control in the Agro-Ecosystems. *Sustainability*. 13: 8639.
- Romaneckas K., Kimbirauskienė R., Sinkevičienė A., Jaskulska I., Buragienė S., Adamavičienė A., Šaraukis E. (2021). Weed Diversity, Abundance, and Seedbank in Differently Tilled Faba Bean (*Vicia faba* L.) Cultivations. *Agronomy*. 11: 529.
- Sans F.X., Berner A., Armengot L., Mäder,P. (2011). Tillage effects on weed communities in an organic winter wheat–sunflower–spelt cropping sequence. *Weed Research*. 51: 413–421.
- Sudarić A., Vratarić M. (2008). Importance, achievements and trends in soybean breeding at the Agricultural Institute Osijek. *Sjemenarstvo*. 25: 3-4.
- Tang L.L., Cheng C.P., Wan K., Li R.h., Wang D.Z., Tao Y., Pan J.F., Xie J., Xie J., Chen F. (2014). Impact of fertilizing pattern on the biodiversity of a weed community and wheat growth. *PLoS One*. 9: e84370.
- Tibco Software Inc., Statistica (data analysis software system) 2020, version 14. <http://tibco.com>
- Travlos I.S., Cheimona N., Roussis I., Bilalis D.J. (2018). Weed-Species Abundance and Diversity Indices in Relation to Tillage Systems and Fertilization. *Frontiers in Environmental Science*. 6:

1-10.

Vukadinović V., Vukadinović V. (2011). Plant Nutrition, 3rd ed.; Faculty of Agriculture in Osijek: Osijek, Croatia. 73–200. (In Croatian).

Wallace J.M., Keene C.L., Curran W., Mirsky S., Ryan M.R., VanGessel M.J. (2018). Integrated Weed Management Strategies in Cover Crop-based Organic Rotational No-Till Corn and Soybean in the Mid-Atlantic Region. *Weed Science*. 66: 94–108.

Wan K., Tao Y. Li., Pan J. F., Tang L. L., Chen F. (2012). Influences of long-term different types of fertilization on weed community biodiversity in rice paddy fields. *Weed Biology and Management*. 12: 12-21.

World Reference Base for Soil Resources (2014). Update 2015-International Soil Classification System for Naming Soils and Creating Legends for Soil Maps; World Soil Resources Reports No. 106; FAO: Rome, Italy, 2015. Available online: www.fao.org/3/i3794en/i3794en.pdf (accessed on 20 October 2023).

The potential of medicinal and aromatic plants in the phytoremediation of heavy metal contaminated sites

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Abstract

Phytoremediation is a non-destructive method that utilizes the ability of plants to adsorb, degrade, transpire, or accumulate various types of pollutants from the environment. According to available studies, certain medicinal and aromatic plant species grown for the production of essential oils are able to remove heavy metals from the soil and water, which are then released when the plant material is processed. Therefore, they have great potential for use in phytoremediation while producing high-quality and uncontaminated essential oils as the final products. This review article summarizes the studies that have been conducted to clarify the possibility of using some medicinal and aromatic plant species in the phytoremediation of polluted soils.

Keywords: essential oils, green technology, heavy metals, hyperaccumulators, pollutants

Introduction

Soil pollution is the accumulation of natural or artificial substances in concentrations that are harmful to the growth and development of microorganisms, plants and animals. Pollutants include pesticides, industrial chemicals, products of industrial processes and combustion, radionuclides, pathogens, and heavy metals (cadmium [Cd], chromium [Cr], copper [Cu], mercury [Hg], nickel [Ni], lead [Pb], zinc [Zn], arsenic [As], etc.) (Gautam et al., 2023; Rashid et al., 2023). Soil can be contaminated with heavy metals from a variety of sources, including mining industry, industrial facilities, metal waste disposal, fossil fuel combustion, organic and mineral fertilizer application, pesticides, sewage sludge, wastewater irrigation, petrochemical releases, and atmospheric deposition (Alloway and Ayres, 1994). Environmental pollution by heavy metals is a major problem that threatens sustainable agricultural production and has a negative impact on human health (Tchounwou et al., 2012). The widespread presence of heavy metals in soils and water bodies necessitates the use of environmentally friendly, cost-effective, and sustainable approaches for their remediation, such as phytoremediation. Phytoremediation is an emerging technology in which plants are used to extract or remove pollutants from water or soil, or reduce their availability (Dheri et al., 2007; Cunningham and Berti, 2000). When food plants are used for phytoremediation, the problem of heavy metal contamination remains unresolved as they enter the food chain. On the other hand, many medicinal and aromatic plants could be suitable candidates for phytoremediation as they are non-food crops but are cultivated for the extraction of secondary metabolites (e. g. essential oils) (Gupta et al., 2013). In recent decades, numerous studies have been conducted confirming the successful phytoremediation of contaminated soils with medicinal and aromatic plants, as well as the increase in essential oil yields as the result of the exposure to the heavy metals. Moreover, some studies have shown an improved quality of essential oils and, most importantly, no toxic metals were detected in the essential oils or they reached insignificant levels that do not pose a risk to human health (Pandey et al., 2019; Mishra and Chandra, 2022). The main reason for obtaining heavy metal free essential oils appears to be the use of steam distillation for extraction (Bernstein et al., 2009).

This review article summarizes the most important and recent research findings on medicinal and aromatic plant species that have the potential to be used for phytoremediation of soils contaminated with heavy metals.

Principles of phytoremediation

Phytoremediation is a method in which plants are used to stabilize, remove, degrade or retain pollutants in soil, groundwater, surface water and the atmosphere, with the aim of restoring polluted ecosystems to their original state (Yan et al., 2020 and references herein). The advantage of phytoremediation compared to other methods of remediating contaminated soils lies in its high efficiency at low pollutants concentrations, its environmental friendliness, cost-efficiency, *in situ* implementation, esthetic acceptability, and public acceptance (Ali et al., 2013; Fatima et al., 2017). In addition, the use of phytoremediation has a positive effect on biodiversity, counteracts erosion, reduces the emission of soil particles into the air, and does not disturb the soil structure. On the other hand, the disadvantages or limiting factors in the application of phytoremediation are: the possibility of application only at the root depth, the impossibility of application at high pollutant concentrations, the slower remediation compared to physico-chemical methods, the unknown properties and toxicity of biodegradation products, the variable risk of release of pollutants into the food chain, the possibility of phytotoxicity, and the dependence on weather conditions, the season, and the stage of development of the plants (Ali et al., 2013; Pinto et al., 2015). Depending on the mechanism of action, the following phytoremediation techniques are distinguished: phytoextraction, rhizofiltration, rhizodegradation, phytostabilization, phytodegradation, and phytovolatilization (Sabreena et al., 2022 and references herein). Among green technologies to combat metal pollution, phytoextraction has received increasing attention since the discovery of hyperaccumulating plants, which are able to store high concentrations of certain metals in the above-ground, harvestable biomass (Vamerali et al., 2010). Hyperaccumulating plant species are characterized by a particular uptake of heavy metals, regardless of their concentration in the soil or tolerance to high concentrations of toxic substances in the root and aboveground biomass, and the ability to rapidly translocate elements through the root into the aboveground plant parts (Sytar et al., 2020).

Medicinal and aromatic plant species in phytoremediation

Medicinal and aromatic plants are the source of numerous active ingredients that can be used in the food, pharmaceutical and agricultural industries (Fierascu et al., 2021). Many of them are cultivated for the isolation of essential oils, a mixture of numerous combinations of bioactive compounds with a variety of structures, such as mono-, sesqui-, and di-terpenes, phenolic elements, sulfur-containing components, etc. (Masyita et al., 2022). Previous research has shown that cultivation on contaminated soils resulted in higher yields of essential oils and in some studies improved their composition. Moreover, they were also free of toxic metals and can be therefore used as a marketable product with economic benefits. In their review articles, Pandey et al. (2019) and Mishra and Chandra (2022) present the research results of numerous authors on the potential of certain medicinal and aromatic plant species for phytoremediation of heavy metals, highlighting those from the Lamiaceae, Poaceae, Asteraceae, and Geraniaceae families with the greatest efficiency.

The biological applications of Lamiaceae species are mainly related to their essential oils, which have various effects, such as antioxidant, antitumor, anti-inflammatory, sedative, etc. (Ramos da Silva et al., 2021 and references here). Some species from the Lamiaceae family have been studied for their phytoremediation potential. These include the species of the genus *Mentha*, which are characterized by high medicinal and aromatic value. Pandey et al. (2019) found that *Mentha* species accumulate most of the heavy metals in the root system, which are then transferred to a lesser extent to the aerial parts of the plant, from which the essential oils are then extracted, and therefore act as phytostabilizers in heavy metals – polluted areas. Prasad et al. (2010) investigated the effects of Cr and Pb on the yield and chemical composition of essential oils as well as the accumulation of heavy metals in three species: corn mint (*Mentha arvensis* L.), peppermint (*Mentha x piperita* L.) and water mint (*Mentha citrata* Ehrh., syn. *Mentha aquatica* L.). In *M. citrata*, the yield of essential oil decreased, while in *M. x piperita* it increased, which was explained by the authors as a consequence of the increase in *M. x piperita* aerial parts due to the application of Cr and Pb. The chemical composition of essential oils tested was altered, indicating the influence of heavy metals on the quality of the essential oils. However, the tested species were not found to be hyperaccumulators of Cr and Pb and therefore cannot be used for the purification of soil from these metals. In their study Kunwar et al. (2015) found that essential oil yield increased in spearmint (*M. spicata*) grown in soils with Cu, Cd and Pb, and that their concentrations in the essential oil were below the detection limit. In other study Zheljzakov and Nielsen (1996a) investigated the effects of soil pollution by a mining company with excessive amounts of Cd, Pb, Cu, Mn, and Zn on the growth and productivity of *M. x piperita* L. (cv Tundza and Clone No. 1) and *M. arvensis* var. *piperascens* Malinv. ex Holmes (cv Mentolna-14) and

found a decrease in fresh mass and essential oil yield. The extracted essential oils were not contaminated with heavy metals. Another highly valued members of the Lamiaceae family are basil (*Ocimum* L.). The species of the genus *Ocimum* have long been used as flavorings, as well as for various medicinal purposes (Zahran et al., 2020). Kunwar et al. (2015) reported the increase in essential oil yield of *O. basilicum* L. grown on soils with Cu, Cd, and Pb whose concentrations in the essential oils were below the detection limit. They also reported the changes in the chemical composition, which were mainly related to a significant increase in the essential oil component linalool. Prasad et al. (2011) reported the effects of Cr, Cd, and Pb uptake on decrease in linalool content and increase in estragole content in basil essential oil. The content of linalool and methyl chavicol (estragole) decreased in the presence of Ni, while the concentration of methyl eugenol concentration was increased. Siddiqui et al. (2013) analyzed the As accumulation potential of *O. tenuiflorum*, *O. basilicum* and *O. gratissimum* and the resulting effects on growth, biomass, essential oil yield and essential oil components. At lower As concentrations (mostly up to 25 μM) the yield of essential oil and its main components, such as eugenol, methyl chavicol, and linalool, increased in all three species and no detectable amount of As was found in the essential oil of any of the tested species. Tulsi (*O. tenuiflorum*) was found to have the ability to bind high amounts of Cr in the root and then in the leaves. Increased synthesis of eugenol in the essential oil was found due to Cr-induced stress (Rai et al., 2004). Angelova et al. (2016) reported that clary sage (*Salvia sclarea* L.) is a Pb hyperaccumulator and Cd and Zn accumulator which do not interfere with the plant development and in the presence of these metals produces an essential oil that is safe for use. The phytoremediation potential of lavender (*Lavandula angustifolia* Mill.) was investigated by Zheljzkov and Nielsen (1996b). The authors found that heavy metals (Cd, Pb, Cu, Mn, Zn, Fe) in soil and air do not affect the rate and extend of the developmental stages of lavender, the amount of branching, the yield of fresh inflorescences or the content and composition of essential oil. Despite the large amounts of heavy metals that accumulated in the inflorescence, the oil was not contaminated. Angelova et al. (2015) pointed out the potential of *L. vera* (syn. *Lavandula angustifolia* Mill.) to hyperaccumulate Pb, Cd, and Zn. In their study, neither negative effects on plant development nor on the quality and quantity of the essential oil were observed. In the study on horehound (*Marrubium vulgare* L.), lemon balm (*Melissa officinalis* L.) and Greek oregano (*Origanum heracleoticum* Benth.; syn. *Origanum vulgare* subsp. *hirtum*), Zheljzkov et al. (2008) concluded that these plant species can be grown on soils contaminated with heavy metals in the vicinity of the smelter and provide metal-free final commercial products.

Lydakiss-Simantiris et al. (2016) conducted a study on chamomile (*Chamomilla recutita* (L.) Rauschert, syn. *Matricaria chamomilla* L.), sage (*Salvia officinalis* L.) and thyme (*Thymus vulgaris* L.) grown in pots with soil contaminated with a wide range of Cd, Pb and Ni concentrations. The chemical composition of the essential oils did not change significantly under the influence of heavy metals and their concentrations in the extracted essential oils were within the permissible detection limits. The phytoremediation potential of chamomile was also investigated by Salamon et al. (2007). The authors analysed the ability of chamomile to accumulate Cd, Pb and Ni in different plant parts (roots, leaves and flowers). In general, the highest levels of heavy metals were found in the roots, followed by the leaves, while the lowest concentrations were found in the flowers. They also found that Cd was highly mobile, while Pb had the lowest mobility within the plants. The authors did not study the content of these heavy metals in the essential oils. Ghanavatifard et al. (2018) describe chamomile as a plant species that effectively tolerates Ni-induced stress. In their study, Száková et al. (2018) found changes in the abundance of essential oil compounds in *M. recutita* as a result of exposure to heavy metals (As, Cd, Pb and Zn), but no detectable amounts of these metals were found in the essential oil. Angelova et al. (2020) conducted a study on absinth (*Artemisia absinthium* L.), another plant species from the Asteraceae family, which was grown on soils contaminated with heavy metals such as Cd, Pb, and Zn. As a result of the exposure of the plants to heavy metals, the chemical composition of the essential oils was altered. In addition, the concentrations of these heavy metals in the leaves and flowers of the plant were much higher than in the essential oil, in which it was below the acceptable limits. Plant species from the Poaceae family have been reported to reduce the toxicity of heavy metals and facilitate the uptake of metals from contaminated sites (Patra et al., 2021). Vetiver (*Vetiveria zizanioides* (L.) Nash, syn. *Chrysopogon zizanioides* (L.) Roberty) is a perennial grass of the Poaceae family. It produces essential oils with antifungal, antibacterial, anticancer, antioxidant, and anti-inflammatory properties (Bharathi Raja et al., 2018). This plant species is known to be highly resistant to a wide range of soil pH, salinity, alkalinity, acidity, and heavy metal concentrations (Pandey et al., 2019). Danh et al. (2009) state that vetiver is an ideal plant species for cultivation in contaminated soils due to its tolerance to extreme environmental and weather conditions. Kafil et al. (2019) have reported that vetiver can be used in the treatment of polluted water. The removal of Cr and Pb from wastewater was investigated by Singh et al. (2015). Seventy-seven to seventy-eight percent of Cr and 80 to 94% of Pb were successfully removed, whereas most of the Cr was stored in the stem while most of

the Pb was found in vetiver roots. To evaluate the effects of Pb on vetiver growth and oil yield and composition, Rotkittikhun et al. (2010) conducted a study on hydroponically grown plants. Higher concentrations of Pb had a negative effect on the length of the shoots and roots, but no phytotoxic symptoms were observed. The presence of Pb affected the essential oil, i. e. it increased its yield (the highest was observed with 100 mg Pb for 5 weeks) and the number of its compounds, while the highest number was observed in plants grown in soil containing 1000 mg Pb/kg. Xia (2004) refers to the possibility of using vetiver as a phytostabilizer due to Pb storage in the root. Lemongrass (*Cymbopogon citratus* (DC.) Stapf) is a perennial medicinal and aromatic plant of the Poaceae family. It synthesizes essential oil known for its antimicrobial, insecticidal (Aluyor and Oboh, 2014), antidiarrheal, antifungal, and anti-inflammatory properties (Shah et al., 2016). Various studies have also shown its suitability for phytoremediation of sites contaminated with heavy metals. In the study by Israila et al. (2015), *C. citratus* was reported to accumulate metals (Cd, Ni and Pb). Khilji i Saljid (2020) suggest using East Indian lemongrass (*C. flexuosus* (Nees ex Steud.) Will. Watson) for extraction of heavy metals from tannery sludge. Lal et al. (2013) investigated the yield of essential oil and accumulation of heavy metals in *C. flexuosus* irrigated with wastewater and groundwater. A higher biomass and a higher yield of essential oil were obtained when plants were irrigated with wastewater alone or in combination with groundwater. The heavy metal concentration in the essential oils extracted from the plants irrigated with wastewater was present within the permissible values.

Conclusion

Due to the problem of the growing number of polluted areas in the world, interest in their remediation or restoration is increasing rapidly. The use of plants in the remediation of contaminated soils has many advantages. Some of these are the possibility of *in situ* use, the environmental friendliness, the positive impact on biodiversity and the safety of the method itself. However, the use of food crops in phytoremediation carries the risk of heavy metals or other pollutants entering the food chain, which poses a risk to human health. For this reason, it is advantageous to use medicinal and aromatic plants in phytoremediation that are not used directly as food but for the production of secondary metabolites, usually essential oils. For some of the medicinal and aromatic plant species such as vetiver, lemongrass, peppermint, etc., a positive effect of heavy metal contamination on the essential oil content was found, and the essential oils were not significantly contaminated with heavy metals. For vetiver, the potential for phytoextraction of zinc and copper and for phytostabilization of chromium was determined, while for corn mint the authors indicate the potential for phytostabilization of nickel and lead, and for basil the ability to phytostabilize chromium, cadmium, lead, and nickel. Some authors point to the ability of lavender to hyperaccumulate lead, cadmium and zinc and state that the essential oil is not contaminated with heavy metals. The use of medicinal and aromatic plant species in phytoremediation is of great importance from an environmental and economic point of view. Indeed, contaminated soils that are not suitable for the cultivation of agricultural food can be used for the cultivation of certain medicinal and aromatic plant species that produce uncontaminated, high-quality essential oils and at the same time remediate the contaminated soils. Further research is needed to determine the effectiveness of a larger number of plant species when using this method. Also, the composition and quality of essential oils extracted from plants grown on contaminated soils needs to be further investigated and analyzed to more accurately determine the safety of such production.

References

- Ali H., Khan E., Sajad M.A. (2013). Phytoremediation of heavy metals – Concepts and applications. *Chemosphere*. 91: 869 – 881.
- Alloway B.J., Ayres D.C. (1994). *Chemical principles of environmental pollution*. Glasgow, Scotland, UK: Blackie Academic and Professional, an imprint of Chapman and Hall.
- Aluyor E.O., and Oboh I.O. (2014). Preservatives: Traditional Preservatives – Vegetable Oils. In: *Encyclopedia of Food Microbiology* (Second Edition), Batt C. A., Tortorello M. L. (eds.), 137 – 140. Cambridge, Massachusetts, SAD: Academic Press.
- Angelova V.R., Grekov D.F., Kisyov V.K., Ivanov K.I. (2015). Potential of lavender (*Lavandula vera* L.) for phytoremediation of soils contaminated with heavy metals. *World Academy of Science, Engineering and Technology International Journal of Agricultural and Biosystems Engineering*. 9:

465 – 472.

- Angelova V., Ivanova R., Todorov G., Ivanov K. (2016). Potential of *Salvia sclarea* L. for Phytoremediation of Soils Contaminated with Heavy Metals. World Academy of Science, Engineering and Technology. International Journal of Agricultural and Biosystems Engineering. 10: 780 – 790.
- Angelova V. (2020). Heavy metal accumulation and chemical composition of essential oils of wormwood (*Artemisia absinthium* L.) cultivated on heavy metal contaminated soils. Series E. Land Reclamation, Earth Observation & Surveying, Environmental Engineering. 9: 44 – 52.
- Bernstein N., Chaimovitch D., Dudai N. (2009). Effect of Irrigation with Secondary Treated Effluent on Essential Oil, Antioxidant Activity, and Phenolic Compounds in Oregano and Rosemary. Agronomy Journal. 101: 1 – 10.
- Bharathi Raja M., Rajamani K., Suresh J., Joel J. J., Uma D. (2018). Chemical composition of Vetiver root oil obtained by using GCMS analysis. Journal of Pharmacognosy and Phytochemistry. 7: 1709 – 1713.
- Cunningham S.C., Berti W.R. (2000). Phytoextraction and Phytostabilization: Technical, Economic, and Regulatory Considerations of the Soil-Lead Issue. In: Phytoremediation of Contaminated Soil and Water, Terry N., Banuelos G. (eds.), 359 – 376. Boca Raton, USA: Lewis Publishers.
- Danh L.T., Truong P., Mammucari R., Tran T., Foster N. (2009). Vetiver grass, *Vetiveria zizanioides*: a choice plant for phytoremediation of heavy metals and organic wastes. International Journal of Phytoremediation. 11: 664 – 691.
- Dheri G.S., Brar M.S., Malhi S.S. (2007). Comparative Phytoremediation of Chromium-Contaminated Soils by Fenugreek, Spinach, and Raya. Communications in Soil Science and Plant Analysis. 38: 1655 – 1672.
- Fatima K., Imran A., Saeed M., Afzal M. (2017). Plant-Bacteria Synergism: An Innovative Approach for the Remediation of Crude Oil-Contaminated Soils. Soil and Environment. 36: 93 – 113.
- Fierascu R.C., Fierascu I., Baroi A.M., Ortan A. (2021). Selected Aspects Related to Medicinal and Aromatic Plants as Alternative Sources of Bioactive Compounds. International Journal of Molecular Sciences. 22: 1521.
- Gautam K., Sharma P., Dwivedi S., Singh A., Gaur V.K., Varjani S., Srivastava J. K., Pandey A., Chang J.S., Ngo H.H. (2023). A review on control and abatement of soil pollution by heavy metals: Emphasis on artificial intelligence in recovery of contaminated soil. Environmental Research. 225: 115592.
- Ghanavatifard F., Mohtadi A., Masoumiasl A. (2018). Investigation of tolerance to different nickel concentrations in two species *Matricaria chamomilla* and *Matricaria aurea*. International Journal of Environmental Science and Technology. 15: 949 – 956.
- Gupta A.K., Verma S.K., Khan K., Verma R.K. (2013). Phytoremediation Using Aromatic Plants: A Sustainable Approach for Remediation of Heavy Metals Polluted Sites. Environmental Science & Technology. 47: 10115 – 10116.
- Israila Y.Z., Bola A.E., Emmanuel G.C., Ola I.S. (2015). The effect of application of EDTA on the phytoextraction of heavy metals by *Vetiveria zizanioides*, *Cymbopogon citrates* and *Helianthus annuus*. International Journal of Environmental Monitoring and Analysis. 3: 38 – 43.
- Kafil M., Nasab S.B., Moazed H., Bhatnagar A. (2019). Phytoremediation potential of vetiver grass irrigated with wastewater for treatment of metal contaminated soil. International Journal of Phytoremediation. 21: 92 – 100.
- Khilji S. A., Sajid Z.A. (2020). Phytoremediation potential of lemongrass (*Cymbopogon flexuosus* Stapf.) grown on tannery sludge contaminated soils. Applied Ecology and Environmental Research. 18: 7703 – 7715.
- Kunwar G., Pande C., Tewari G., Singh C., Kharkwal G.C. (2015). Effect of Heavy Metals on Terpenoid Composition of *Ocimum basilicum* L. and *Mentha spicata* L. J Essential Oil Bearing

- Plants. 18: 818 – 825.
- Lal K., Yadav R.K., Kaur R., Bundela D.S., Khan M.I., Chaudhary M., Meena R.L., Dar S. R., Singh G. (2013). Productivity, essential oil yield, and heavy metal accumulation in lemon grass (*Cymbopogon flexuosus*) under varied wastewater–groundwater irrigation regimes. *Industrial Crops and Products*. 45: 270 – 278.
- Lydakakis-Simantiris N., Fabian M., Skoula M. (2016). Cultivation of medicinal and aromatic plants in heavy metal-contaminated soils. *Global NEST Journal*. 18: 630 – 642.
- Masyita A., Mustika Sari R., Dwi Astuti A., Yasir B., Rahma Rumata N., Emran T. Bin, Nainu F., Simal-Gandara J. (2022). Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives. *Food Chemistry*. 13: 100217.
- Mishra B., and Chandra M. (2022). Evaluation of phytoremediation potential of aromatic plants: A systematic review. *Journal of Applied Research on Medicinal and Aromatic Plants*. 31: 100405.
- Pandey J., Verma R. K., Singh S. (2019). Suitability of aromatic plants for phytoremediation of heavy metal contaminated areas: a review. *International Journal of Phytoremediation*. 21: 405 – 418.
- Patra D.K., Acharya S., Pradhan C., Patra H.K. (2021). Poaceae plants as potential phytoremediators of heavy metals and eco-restoration in contaminated mining sites. *Environmental Technology & Innovation*. 21: 101293.
- Pinto A.P., de Varennes A., Fonseca R., Martins Teixeira D. (2015). Phytoremediation of Soils Contaminated with Heavy Metals: Techniques and Strategies. In: *Phytoremediation: Management of Environmental Contaminants*, Ansari A., Gill S., Gill R, Lanz G. and Newman L. (eds.), Vol. 1, 133 – 158. Switzerland: Springer.
- Prasad A., Singh A.K., Chand S., Chanotiya C.S., Patra D.D. (2010). Effect of chromium and lead on yield, chemical composition of essential oil, and accumulation of heavy metals of mint species. *Communications in Soil Science and Plant Analysis*. 41: 2170 – 2186.
- Prasad A., Kumar S., Khaliq A., Pandey A. (2011). Heavy metals and arbuscular mycorrhizal (AM) fungi can alter the yield and chemical composition of volatile oil of sweet basil (*Ocimum basilicum* L.). *Biology and Fertility of Soils*. 47: 853.
- Rai V., Vajpayee P., Singh S.N., Mehrotra S. (2004). Effect of chromium accumulation on photosynthetic pigments, oxidative stress defense system, nitrate reduction, proline level and eugenol content of *Ocimum tenuiflorum* L. *Plant Science*. 167: 1159 – 1169.
- Ramos Da Silva L.R., Ferreira O.O., Cruz J.N., De Jesus Pereira Franco C., Oliveira Dos Anjos T., Cascaes M. M., Almeida Da Costa W., Helena De Aguiar Andrade E., Santana De Oliveira M. (2021). Lamiaceae Essential Oils, Phytochemical Profile, Antioxidant, and Biological Activities. *Evidence-based Complementary and Alternative Medicine: eCAM*. 14: 6748052.
- Rashid A., Schutte B.J., Ulery A., Deyholos M.K., Sanogo S., Lehnhoff E.A., Beck L. (2023). Heavy Metal Contamination in Agricultural Soil: Environmental Pollutants Affecting Crop Health. *Agronomy*. 13: 1521.
- Rotkittikhun P., Kruatrachue M., Pokethitiyook P., Baker A.J. (2010). Tolerance and accumulation of lead in *Vetiveria zizanioides* and its effect on oil production. *Journal of Environmental Biology*. 31: 329.
- Sabreena Hassan S., Bhat S. A., Kumar V., Ganai B.A., Ameen F. (2022). Phytoremediation of Heavy Metals: An Indispensable Contrivance in Green Remediation Technology. *Plants* 11: 1255.
- Salamon I., Labun P., Skoula M., Fabian M. (2006). Cadmium, lead and nickel accumulation in chamomile plants grown on heavy metal enriched soil. *Acta Horticulturae*. 749: 231 – 237.
- Shah G., Shri R., Panchal V., Sharma N., Singh B., Mann A.S. (2011). Scientific basis for the therapeutic use of *Cymbopogon citratus*, Stapf (Lemon grass). *Journal of Advanced Pharmaceutical Technology & Research*. 2: 3.
- Siddiqui F., Krishna S. K., Tandon P.K., Srivastava S. (2013). Arsenic accumulation in *Ocimum* spp.

- and its effect on growth and oil constituents. *Acta Physiologiae Plantarum*. 35: 1071 – 1079.
- Singh V., Thakur L., Mondal P. (2015). Removal of Lead and Chromium from Synthetic Wastewater Using *Vetiveria zizanioides*. *CLEAN – Soil, Air, Water*. 43: 538 – 543.
- Sytar O., Ghosh S., Malinska H., Zivcak M., Brestic M. (2021). Physiological and molecular mechanisms of metal accumulation in hyperaccumulator plants. *Physiologia Plantarum*. 173: 148 – 166.
- Szákóvá J., Dziaková M., Kozáková A., Tlustoš P. (2018). The risk element uptake by chamomile (*Matricaria recutita* (L.) Rauschert) growing in four different soils. *Archives of Environmental Protection*. 44: 12 – 21.
- Tchounwou P. B., Yedjou C. G., Patlolla A. K., Sutton D. J. (2012). Heavy Metals Toxicity and the Environment. *Experientia supplementum*. 101: 133 – 164.
- Vamerali T., Marianna B., Giuliano M. (2010). Field crops for phytoremediation of metal-contaminated land: A review. *Environmental Chemistry Letters*. 8: 1 – 17.
- Xia H. P. (2004). Ecological rehabilitation and phytoremediation with four grasses in oil shale mined land. *Chemosphere*. 54: 345 – 353.
- Yan A., Wang Y., Tan S. N., Mohd Yusof M. L., Ghosh S., Chen Z. (2020). Phytoremediation: A Promising Approach for Revegetation of Heavy Metal-Polluted Land. *Frontiers in Plant Science*. 11: 513099.
- Zahran E.M., Abdelmohsen U.R., Khalil H. E., Desoukey S.Y., Fouad M.A., Kamel M.S. (2020). Diversity, phytochemical and medicinal potential of the genus *Ocimum* L. (Lamiaceae). *Phytochemistry Reviews*. 19: 907 – 953.
- Zheljazkov V.D., Nielsen N.E. (1996a). Effect of heavy metals on peppermint and cornmint. *Plant and Soil*. 178: 59 – 66.
- Zheljazkov V.D., Nielsen N.E. (1996b). Studies on the effect of heavy metals (Cd, Pb, Cu, Mn, Zn and Fe) upon the growth, productivity and quality of lavender (*Lavandula angustifolia* Mill) production. *Journal of Essential Oil Research*. 8: 259 – 274.
- Zheljazkov V.D., Jeliaskova E.A., Kovacheva N., Dzhurmanski A. (2008). Metal uptake by medicinal plant species grown in soils contaminated by a smelter. *Environmental and Experimental Botany*. 64: 207 – 216.

Potencijal eteričnih ulja u inhibiciji rasta patogenih bakterija

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Sažetak

Eterična ulja su mješavine raznih hlapljivih kemijskih spojeva koje se dobivaju metodama destilacije i ekstrakcije iz različitih dijelova biljaka. Zbog dokazanih antimikrobnih i antioksidativnih svojstava imaju široku primjenu u medicini, poljoprivredi te kozmetičkoj i prehrambenoj industriji. Antimikrobni učinak eteričnih ulja ovisi o sastavu, koncentraciji i interakciji kemijskih spojeva eteričnih ulja kao i o vrsti mikroorganizma. Ovaj rad daje pregled dosadašnjih spoznaja o svojstvima i mehanizmima djelovanja eteričnih ulja na bakterijske stanice te ukazuje na potencijal primjene eteričnih ulja kao konzervansa u prehrambenoj i kozmetičkoj industriji.

Ključne riječi: eterična ulja, antimikrobno djelovanje, patogene bakterije, konzervansi

Uvod

Eterična ili esencijalna ulja dobila su naziv po svojem sastavu, tj. mogućnosti hlapljenja u eter. To su tekućine koje se sastoje od raznih hlapljivih spojeva te se imenuju po vrsti biljke iz koje su ekstrahirana. Dobivaju se iz različitih dijelova biljke poput lista, cvijeta, korijena ili stabljika gdje kao produkti biljnog metabolizma nastaju u specijaliziranim biljnim tkivima unutar uljnih žlijezda (Butnariu, 2018; Falerio 2011.). Količina ekstrahiranog eteričnog ulja ovisi o različitim čimbenicima poput biljne vrste, vremenu berbe, klimi, kao i dijelu biljke iz koje se ekstrahira. (Butnariu, 2018). Eterična ulja pokazala su se kao učinkoviti konzervansi u prehrambenoj i kozmetičkoj industriji (Ziosi i sur. 2013; Shaaban 2020). Dodatkom eteričnih ulja proizvod ostaje duže mikrobiološki ispravan te je ima jači antioksidativni učinak. Također, eterična ulja se koriste i kao „zeleni pesticidi“ u suzbijanju glodavaca, štetnika i mikroorganizama. Koriste se u ekološkoj proizvodnji hrane zbog izostanka negativnog učinka na okoliš, kao i manje mogućnosti razvoja otpornosti od strane štetnika zbog vrlo kompleksnog sastava (Sharma i sur. 2011). Zbog sve prisutnije pojave antibiotske rezistencije istražuje se primjena eteričnih ulja u medicini za tretiranje infekcija koje uzrokuju bakterije rezistentne na više klasa antibiotika ili se koriste u tretiranju različitih drugih oboljenja (Russo i sur. 2015). Cilj ovoga preglednog rada je istaknuti važnost uporabe eteričnih ulja u inhibiciji rasta patogenih bakterija s mogućom primjenom kao konzervansa u prehrambenoj i/ili kozmetičkoj industriji. Iako je teško sažeti i prezentirati sve utjecaje eteričnih ulja na mikroorganizme, u radu će biti navedeni do sada najvažniji poznati mehanizmi djelovanja pojedinih eteričnih ulja na pojedine patogene bakterije.

Uporaba eteričnih ulja kao konzervansa

Konzervansi su stvari kojima je glavni cilj inhibicija mikrobnog rasta te produljenje trajanja i očuvanje kvalitete proizvoda. Konzervansi trebaju biti netoksični, učinkoviti i kompatibilni s ostalim sastojcima proizvoda kojeg konzerviraju. Svaki konzervans kao primarnu zadaću ima produljiti trajnost gotovog proizvoda, bez promjene organoleptičkih osobina, kvalitete i zdravstvene ispravnosti. Najvažniji čimbenik kod odabira konzervansa je širok spektar djelovanja, tj. da djeluje inhibirajuće na više vrsta mikroorganizama odjednom. U industriji koriste se sintetički i prirodni konzervansi. Budući da su dokazani negativni učinci nekih sintetičkih konzervansa na zdravlje ljudi (Cui i sur. 2020), potražnja za prirodnim konzervansima u stalnom je rastu. Uporaba prirodnih konzervansa, poput eteričnih ulja i ekstrakata biljaka, kao alternativa sintetičkim konzervansima, vezana je prvenstveno uz njihova, antimikrobna svojstva i izostanak negativnih utjecaja na zdravlje ljudi u preporučenoj koncentraciji (Bassole i

Julirani 2012). Međutim nemaju svi kemijski spojevi unutar eteričnih ulja jednako antimikrobno djelovanje. Najjače djelovanje pokazuju fenoli, zatim aldehidi, ketoni, alkoholi, eteri i ugljikovodici (Bassole i Julirani 2012). Način na koji će eterično ulje djelovati na mikroorganizme ponajprije ovisi o interakciji spojeva od kojih je sastavljeno. Interakcija između spojeva eteričnih ulja može proizvesti četiri vrste učinka: a) indiferentni – spojevi ne utječu jedan na drugi; b) aditivni – kada je zbroj pojedinačnih učinaka jednak zajedničkom učinku; c) antagonistički – primjećuje se kada je učinak jednog ili oba spoja manji nego kada se primjenjuju pojedinačno; d) sinergijski – učinak kombiniranih tvari je bolji nego zbroj učinaka svake tvari pojedinačno (Chouhan i sur. 2017). Na djelovanje eteričnih ulja kao konzervansa može djelovati cijeli spektar uvjeta, primjerice sadržaj masti, interakcija terpenoidnih fenola s enzimima te varijabilnosti u sastavu samog proizvoda. Učinkovitost eteričnih ulja kao konzervansa ovisi o vrsti, rodu, soju i broju ciljanih mikroorganizama te uvjetima okoliša (Macwan i sur. 2016).

Mehanizmi djelovanja eteričnih ulja na bakterijske stanice

Budući da se kemijski sastav eteričnih ulja znatno razlikuje, ne postoji jedan specifičan mehanizam djelovanja eteričnih ulja na mikroorganizme. Antimikrobni mehanizmi eteričnih ulja ovise uglavnom o njihovim kemijskim spojevima, funkcionalnim skupinama i vrsti interakcija između sastojaka. Način na koji će eterično ulje djelovati na patogene bakterije određen je nizom biokemijskih procesa u stanici i raznolikosti kemijskih spojeva eteričnog ulja (Basavegowda i Baek 2021). Također, djelovanje eteričnih ulja je različito na Gram pozitivne u odnosu na Gram negativne bakterije. Gram negativne bakterije otpornije su na antibiotike od Gram pozitivnih bakterija, a istraživanja su pokazala jednak trend otpornosti i na djelovanje eteričnih ulja (Lewis 2013). Gram negativne bakterije imaju hidrofilne lipopolisaharide u vanjskoj membrani koje stvaraju barijeru ili ograničavaju difuziju makromolekula i hidrofobnih spojeva. Ovo svojstvo ujedno otežava ulaz molekula eteričnih ulja koje zbog svoje hidrofobnosti teže ulaze u periplazmatski prostor i inhibiraju stanicu (Hyltdgaard i sur. 2012). Općenito, eterična ulja djeluju na bakterijsku stanicu tako da se vežu za površinu stanice te oštećuju staničnu stjenku i membranu što rezultira nepovratnim oštećenjem i smrću stanice (Basavegowda i Baek 2021). Nakon što eterično ulje dođe u kontakt sa staničnom stjenkom ona postaje propusna za protone te dolazi do ometanja sinteze ATP-a i ravnoteže u stanici. Eterična ulja na stanicu patogena mogu djelovati kao blage kiseline jer snižavaju pH stanice propuštanjem protona kroz staničnu membranu. Fenol karvakrol, prisutan u eteričnom ulju origana, pokazao se izrazito učinkovit kod poremećaja pH stanice. Naravno, varijacije u djelovanju eteričnih ulja na stanične stjenke može se objasniti raznolikosti sastava eteričnih ulja (Faleiro i Miguel 2013). Međutim, najbitniji mehanizmi djelovanja eteričnih ulja vezani su uz inhibiciju staničnih ciklusa, sinteze proteina, lipida, replikacije DNA i koagulacije staničnog sadržaja (Basavegowda i Baek 2021). Također, pojedina eterična ulja, npr. ona izdvojena iz klinčićevca (*Syzygium aromaticum* (L.) Merr. & L.M.Perry), geranija (*Geranium robertianum* L.), lavande (*Lavandula angustifolia* Mill.), ruže (*Rosa damascena* L.) i ružmarina (*Rosmarinus officinalis* L.) imaju dokazan učinak na sustav međusobne komunikacije između stanica, poznat kao *Quorum sensing* kojim su regulirani različiti stanični procesi poput odgovora na stres, stvaranja biofilma, smrtnost stanica i virulencije (Faleiro i Miguel 2013).

Spojevi pojedinih eteričnih ulja odgovorni za antimikrobni učinak

S obzirom na sastav eteričnih ulja i načine antimikrobnog djelovanja, ključan faktor u antimikrobnom djelovanju je omjer aktivnih kemijskih spojeva eteričnog ulja. Spojevi koji sadrže hidroksilnu skupinu (-OH) izrazito su antimikrobnog djelovanja i učinkovitiji od spojeva s karbonilnom skupinom (C=O). Hidroksilna skupina ima sposobnost vezanja za aktivno mjesto na enzimima te uzrokuje promjene u njihovoj aktivnosti. Položaj hidroksilne skupine također utječe na inhibitorna svojstva eteričnih ulja. Alkilna supstitucija u fenolnom spoju povećava antimikrobno djelovanje (Macwan i sur. 2016). Fenoli poput karvakrola, eugenola, linalola i timola imaju najjače antimikrobno djelovanje (Burt 2007). Pri nižim koncentracijama djeluju na enzime koji sudjeluju u proizvodnji energije za stanicu, a pri višim koncentracijama denaturiraju stanične proteine (Tiwari i sur. 2009). Spojevi poput cimernog aldehida, citrala, timola, triciklena, flavesona, mircena, karvakrola, p-cimena, eugenola, c-terpinena, fenilpropanoida, β-selinena i kalamenina inhibiraju rast i uništavaju biofilmove patogena (Gadisa i sur. 2019). Česti spojevi raznih eteričnih ulja koja su pokazala baktericidno djelovanje su eugenol, cinamaldehyd i karvakrol, dok inhibicijski djeluju timol i (+)-karvon (Ebani i Mancianti 2020). U tablici 1. prikazani su najpoznatiji kemijski spojevi eteričnih ulja koji djeluju inhibitorno na odabrane vrste bakterija te vrijednosti minimalne inhibitorne koncentracije (MIK).

Tablica 1. Primjer minimalnih inhibitornih koncentracija (MIK) najčešćih kemijskih spojeva eteričnih ulja u *in vitro* testovima na odabranim bakterijama (Chouhan i sur. 2017.).

Kemijski sastojak	Vrsta bakterije	MIK ($\mu\text{L}/\text{mL}$)
Karvakrol	<i>Escherichia. coli</i>	0,225 – 5
	<i>Staphylococcus aureus</i>	0,175 – 0,450
Timol	<i>Escherichia. coli</i>	0,225 – 0,45
	<i>Staphylococcus aureus</i>	0,140 – 0,225
	<i>Bacillus cereus</i>	0,450
Eugenol	<i>Escherichia. coli</i>	1,0

Od svih načina interakcije spojeva eteričnih ulja, najpoželjnija je sinergija poput one između karvakrola i p-cimena, koji ima slaba antimikrobna svojstva te olakšava ulaz karvakrola u stanicu bubrenjem stijenke *B. cereus* (Ultee i sur. 2000). Timol i karvakrol mogu djelovati sinergički i antagonistički, ovisno o tome u kojim eteričnim uljima se nalaze te inhibiraju rast *Staphylococcus* spp., *Micrococcus* spp., *Bacillus* spp. i *Enterobacter* spp. Također kombinacija karvakrola i eugenola djeluje sinergički i antagonistički prema *E. coli*, *S. aureus* i *B. cereus* (Bassolé i Juliani 2012). Karvakrol i/ili timol također djeluju sinergijski s cinamaldehydima stvarajući pore na membranama stanice čime se olakša ulaz eteričnog ulja u stanice (Shaaban 2020). Sinergija eteričnog ulja timijana (*Thymus vulgaris* L.) i antibiotika ima inhibicijski učinak na sljedeće bakterije: *E. coli*, *Salmonella* spp. te *K. pneumoniae*. Također je sinergija eteričnog ulja timijana s eteričnim uljem origana (*Origanum vulgare* L.) i ružmarina učinkovito inhibirala rast vrsta *B. cereus*, *P. aeruginosa*, *E. coli* i *L. monocytogenes* (Macwan i sur. 2016). Ako se primjenjuje zasebno, utjecaj eteričnog ulja timijana na Gram-pozitivne bakterije jači je nego na Gram-negativne, a prvenstveno djeluju na efluks pumpu i strukturu lipopolisaharida (Fadli i sur. 2012). Kombinacija eteričnih ulja klinčića i ružmarina proizvela je inhibitorni učinak prema *S. aureus*, *S. epidermidis*, *B. subtilis*, *E. coli*, *P. vulgaris* i *P. aeruginosa* (Faleiro 2011). U radu Bozin i sur. (2016) istraživano je djelovanje eteričnog ulja timijana i origana dobivenih vodenom destilacijom (20 i 50 %) na višestruko otporne bakterije *P. aeruginosa* i *E. coli*. Zabilježeno je njihovo antibakterijsko djelovanje pri kojem je inhibicijska zona eteričnog ulja timijana za *P. aeruginosa* bila u rasponu od 12.0 ± 1.41 do 12.2 ± 0.45 , dok je za *E. coli* iznosila od 21.0 ± 1.00 do 29.4 ± 0.89 . Eterično ulje origana stvorilo je inhibicijsku zonu kod *P. aeruginosa* u rasponu između 14.2 ± 0.45 do 20.0 ± 0.71 , a kod *E. coli* 28.0 ± 1.87 do 50.0 ± 0.71 . Kombinacija eteričnih ulja eukaliptusa (*Eucalyptus globulus* Labill.) i paprene metvice (*Mentha x piperita* L.) povećava efikasnost djelovanja metilparabena i propilparabena prema *P. aeruginosa*, a u istom istraživanju dokazano je da kombinacija eteričnog ulja ružmarina, paprene metvice i origana povećava aktivnost diazolidinil uree koja se koristi kao česti konzervans u kozmetici (Patrone i sur. 2010). Antimikrobno djelovanje eteričnog ulja limuna (*Citrus limon* (L.) Burm. f.) i čajevca (*Melaleuca alternifolia* (Maiden & Betche) Cheel) na *P. aeruginosa* pojačano je u kombinaciji s eteričnim uljem ružmarina i/ili sintetičkim konzervansima (Kunicka-Styczyńska i sur. 2011.). Eterično ulje ruže je u *in vitro* istraživanjima pokazalo antimikrobno djelovanje na *E. coli*, *P. aeruginosa*, *B. subtilis* i *S. aureus* s MIK već od $0,25 \mu\text{L}/\text{mL}$ (Ulusoy i sur. 2009). Eterično ulje klinčića i cimetoanca (*Cinnamomum burmanni* (Nees & T.Nees) Blume) koncentracije 40, 20 i 10 % bilo je učinkovito prema *K. pneumoniae* i *E. coli* koje produciraju beta-laktamaze proširenog spektra, sa zonama inhibicije od 15.0 ± 1.00 do 25.3 ± 0.57 mm (Ginting i sur. 2021). Eugenol i cinamaldehyd, koji se nalaze u sastavu raznih vrsta eteričnih ulja, djeluju inhibitorno i na Gram pozitivne bakterije. Eugenol je pokazao inhibitorno djelovanje prema vrstama *L. monocytogenes* i *L. sakei*, dok je cinamaldehyd inhibirao rast *L. monocytogenes* (Gill i Holley 2006).

Zaključak

Eterična ulja obećavajuća su prirodna sredstva za borbu protiv patogenih bakterija, primijenjena samostalno ili u kombinaciji s drugim eteričnim uljima, sintetičkim konzervansima ili aditivima. Svoju primjenu su našla (ili će tek naći) u raznim područjima, od prehrambene industrije preko medicine i poljoprivrede pa sve do kozmetičke industrije. Ipak, treba istaknuti kako dostupna relevantna literatura uglavnom opisuje *in vitro* istraživanja. Iako su *in vitro* rezultati pokazali djelovanje na široki spektar bakterija, sada predstoji sustavnija provedba *in vivo* istraživanja.

Literatura

- Basavegowda N., and Baek K.H. (2021). Synergistic Antioxidant and Antibacterial Advantages of Essential Oils for Food Packaging App. *Biomolecules*. 11: 1267.
- Bassolé I.H., Juliani H.R. (2012). Essential oils in combination and their antimicrobial properties. *Molecules*. 17: 3989-4006.
- Bozin B., Mimica-Dukic N., Simin N., Anackov G. (2016). Characterization of the volatile composition of essential oils of some lamiaceae spices and the antimicrobial and antioxidant activities of the entire oils. *Journal of agricultural and food chemistry*. 54:1822-1888.
- Burt S.A., van der Zee R., Koets A.P., de Graaff A.M., van Knapen F., Gaastra W., Haagsman H.P., Veldhuizen E.J. (2007). Carvacrol induces heat shock protein 60 and inhibits synthesis of flagellin in *Escherichia coli* O157:H7. *Applied and environmental microbiology*. 73: 4484-4490.
- Butnariu M., Sarac I. (2018). Essential Oils from Plants. *J. biotechnol. biomed. sci*. 1: 35-43.
- Chouhan S., Sharma K., Guleria S. (2017). Antimicrobial Activity of Some Essential Oils-Present Status and Future Perspectives. *Medicines (Basel)*. 8/4: 58.
- Cui S.M., Li T., Wang Q., Ke He K., Mei Zheng Y., Yun Liang H., Ya Song L. (2020). Antibacterial Effects of Schisandra chinensis Extract on *Escherichia coli* and its Applications in Cosmetic. *Current microbiology*. 77: 865-874.
- Ebani V.V., Mancianti F. (2020). Use of Essential Oils in Veterinary Medicine to Combat Bacterial and Fungal Infections. *Veterinary sciences*. 7: 1-35.
- Fadli M., Saad A., Sayadi S. (2012). Antibacterial activity of *Thymus maroccanus* and *Thymus broussonetii* essential oils against nosocomial infection – bacteria and their synergistic potential with antibiotics. *Phytomedicine*. 19: 464-471.
- Faleiro M. L. (2011). The Mode of Antibacterial Action of Essential Oils. In: *Science against Microbial Pathogens: Communicating Current Research and Technological Advances* (Ed. Mendez-Vilas A.). Formatex. 1143-1156.
- Faleiro M.L., and Miguel M.G. (2013). Use of Essential Oils and Their Components against Multidrug-Resistant Bacteria, In: *Fighting Multidrug Resistance with Herbal Extracts, Essential Oils and Their Components* (Eds: Kumar Rai M. and Volodymyrivna Kon K.) Academic Press. 65-94.
- Gadisa E., and Usman H. (2021). Evaluation of Antibacterial Activity of Essential Oils and Their Combination against Multidrug-Resistant Bacteria. *International journal of microbiology*. 1-8.
- Ginting E.V., Retnaningrum E., Widiasih D.A. (2021). Antibacterial activity of clove (*Syzygium aromaticum*) and cinnamon (*Cinnamomum burmannii*) essential oil against extended-spectrum β -lactamase-producing bacteria. *Veterinary world*. 14: 2206-2211.
- Gill A.O., Holley R.A. (2006). Disruption of *E. coli*, *L. monocytogenes* and *L. sakei* cellular membranes by plant oil aromatics. *International journal of food microbiology*. 108: 1-9.
- Hyldgaard M., Mygind T., Meyer R. L. (2012). Essential oils in food preservation: Mode of action, synergies, and interactions with food matrix components. *Frontiers in microbiology*. 3: 12.
- Kunicka-Styczyńska A., Sikora M., Kalemba D. (2011). Lavender, tea tree and lemon oils as antimicrobials in washing liquids and soft body balms. *International journal of cosmetic science*. 33: 53-61.
- Lewis K. (2013). Platforms for antibiotic discovery. *Nature reviews Drug discovery*. 12: 371-387.
- Macwan S.R., Dabhi B.K., Aparnathi K.D., Prajapati J.B. (2016). Essential Oils of Herbs and Spices: Their Antimicrobial Activity and Application in Preservation of Food. *Journal of Current Microbiology and Applied Sciences*. 5: 885-901.
- Patrone V., Campana R., Vittoria E., Baffone W. (2010). *In vitro* synergistic activities of essential oils and surfactants in combination with cosmetic preservatives against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Current microbiology*. 60: 237-41.

- Russo R., Corasaniti M.T., Bagetta G., Morrone L.A. (2015). Exploitation of Cytotoxicity of Some Essential Oils for Translation in Cancer Therapy. *Evidence-Based Complementary and Alternative Medicine*. 6: 1-9.
- Shaaban H.A. (2020). Essential Oil as Antimicrobial Agents: Efficacy, Stability, and Safety Issues for Food Application. U: *Essential Oils – Bioactive Compounds* (ur. de Oliveira M.A., Costa W.A., Silva S.G.). *New Perspectives and Applications*. IntechOpen. 1-33.
- Sharma M.M., Haider S.Z., Andola H., Purohit V.K. (2011). Essential Oils as Green Pesticides: For Sustainable Agriculture. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 2: 100-106.
- Tiwari V.M., Wahr J., Swenson S. (2009). Dwindling groundwater resources in northern India, from satellite gravity observations. *Geophysical Research Letters*. 36: 18.
- Ultee A., Slump R.A., Steging G., Smid E.J. (2000). Antimicrobial Activity of Carvacrol toward *Bacillus cereus* on Rice. *Journal of food protection*. 63: 620-624.
- Ulusoy S., Boşgelmez-Tınaz G., Seçilmiş-Canbay H. (2009). Tocopherol, Carotene, Phenolic Contents and Antibacterial Properties of Rose Essential Oil, Hydrosol and Absolute. *Current microbiology*. 59: 554-558.
- Ziosi P., Manfredini S., Vertuani S., Ruscetta V., Radice M., Sacchetti G., Bruni R. (2013). Evaluating Essential Oils in Cosmetics: Antioxidant Capacity and Functionality. *Cosmetics & Toiletries*. 125: 32-40.

The potential of essential oils in inhibiting the growth of pathogenic bacteria

Abstract

Essential oils are mixtures of various volatile chemical compounds obtained by distillation and extraction methods from different parts of plants. Due to their proven antimicrobial and antioxidant properties, they are widely used in medicine, agriculture, and the cosmetic and food industries. The antimicrobial effect of essential oils depends on the composition, concentration and interaction of the chemical compounds of essential oils as well as on the type of microorganism.

This paper provides an overview of the current knowledge about the properties and mechanisms of action of essential oils on bacterial cells, highlighting their potential use as preservatives in the food and cosmetic industries.

Keywords: essential oils, antimicrobial activity, pathogenic bacteria, preservatives

Reciklirana voda i poljoprivreda – rješenje ili problem?

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Sažetak

Globalni učinci klimatskih promjena najizraženije se očituju u promjenama hidrološkog ciklusa. Lanac „Od polja do stola“ najznačajniji je svjetski potrošač vode, dok podatci UN-ove Organizacije za hranu i poljoprivredu pokazuju da potrošnja vode za potrebe poljoprivrede raste posljednjih desetljeća dvostruko brže od rasta stanovništva. Stoga Europska unija Zelenim planom potiče uporabu reciklirane vode, a financijskim alatima i novim pravnim okvirima nastoji osnažiti otpornost poljoprivrednog sektora na klimatske promjene te doprinjeti ostvarivanju ciljeva kružnog gospodarstva i održivosti. U ovom radu opisane su smjernice Europske strategije o recikliranju vode, konvencionalne i alternativne metode pročišćavanja otpadnih voda, kao i rizici korištenja reciklirane vode za potrebe navodnjavanja.

Ključne riječi: recikliranje vode, otpadne vode, navodnjavanje

Uvod

Europski parlament u listopadu 2023. godine amandmanom za Prijedlog direktive Europskog parlamenta i Vijeća o pročišćavanju komunalnih otpadnih voda naglasio je da je voda opće i javno dobro koje kroz svoje tri dimenzije, društvenu, gospodarsku i okolišnu, pripada svima te da je prirodni resurs koji je nužan, nezamjenjiv i neophodan za život na kojeg svaki čovjek ima pravo (Europski parlament, 2022).

Klimatske promjene podrazumijevaju promjene uobičajnih klimatoloških i hidroloških prilika na određenom području, a kontinuirani su proces koji se odvija kroz povijest naše planete. No tijekom proteklih desetljeća, bilježe se intenzivne promjene klimatoloških i hidroloških prilika u gotovo svim dijelovima svijeta koje prate do sada nezabilježene ekstremne vrijednosti temperatura zraka i količine padalina, pojave olujnih vjetrova te izrazito dugih sušnih razdoblja. Znanstvenici iz godine u godinu bilježe i porast prosječne globalne temperature što rezultira povećanjem temperatura mora i oceana te podizanjem njihovih razina (United Nations, 2023).

Brzini klimatskih promjena značajno doprinosi i povećanju koncentracije stakleničkih plinova koji se u najvećim količinama u atmosferu ispuštaju pri sagorijevanju fosilnih goriva te drugih ljudskih aktivnosti, kao što je deforestacija te intenzivna poljoprivreda i stočarstvo. Prema izvješću Svjetske meteorološke organizacije, tijekom 2022. godine izmjerene su do sada rekordne razine stakleničkih plinova. Izvješće naglašava da se koncentracija ugljikovog dioksida u odnosu na predindustrijsko razdoblje povećala za više od 50 %, a da se usporavanje trenda rasta ne nadzire. Također su naglasili da će daljnji porast CO₂ zasigurno za posljedice imati daljnje povećanje učestalosti pojave ekstremnih vremenskih uvjeta, odnosno pojave ekstremnih vrućina ili poplava, topljenje polarnog leda, smanjenje bioraznolikosti, pojavu velikih šumskih požara, gubitke i smanjenje prinosa u poljoprivredi i drugo. Navodi se i da se, usprkos svim upozorenjima, rezolucijama i dokumentima donešenim proteklih desetljeća, klima i dalje ubrzano mijenja te da se polovina planeta suočava s povećanim brojem poplava, a trećina s ekstremnim sušama. Svjetska meteorološka organizacija navodi da uz koncentraciju CO₂, ubrzano rastu i koncentracije metana te spojeva dušikovih oksida (World Meteorological Organization, 2023).

Ipak, uz sve loše klimatske pokazatelje, navodi se da podatci pokazuju da mjere zelenih politika, a naručito aktivnosti koje provodi Europska unija ipak daju rezultate, no da bi se za usporavanje negativnih klimatskih učinaka, njihova primjena morala značajnije i opsežnije primjenjivati na globalnoj razini. Naime, mjerama i zakonskom regulativom koja ide u smjeru održivosti i klimatske neutralnosti, Europa je kao **četvrti najveći proizvođač stakleničkih plinova**, nakon Kine, SAD-a i Indije, uspjela smanjiti proizvodnju ugljikovog dioksida sa 15,2 % iz 1990. na 7,3 % u 2019.

godini. Mehanizmi i aktivnosti dodatno su osnažene donošenjem globalnog Pariškog sporazuma 2015. godine koji je obvezao 194 države potpisnice na smanjenje emisija stakleničkih plinova za 40 % ispod razina iz 1990. do 2030. Taj je cilj dodatno povećan na 55 % do 2030 na konferenciji o klimi u Glasgowu 2021. godine, kojom prilikom je Europska unija ponovno istaknula svoj cilj klimatske neutralnosti do 2050. godine (European Environment Agency, 2023). Dodatnu nadu ulijeva istraživanje Europske komisije o stavovima mladih generacija o klimatskim promjenama prema kojima više od devedeset posto mladih prepoznaje klimatske promjene kao ključni problem za budućnost Europe, ali i njihovo zdravlje i dobrobit cjelokupne zajednice (EU Copernicus, 2023).

Europska komisija primjenom smjernica Europskog zelenog plana te promjenama u svim sektorima gospodarstva u smjeru digitalne i zelene tranzicije želi utjecati na postizanje održivosti i otpornosti kako bi ostvarila status prvog klimatski neutralnog kontinenta do 2050. godine, a time i dala doprinos usporavanju rasta prosječne globalne temperature koja danas iznosi 0,95 - 1,20 °C u odnosu na prosječne globalne temperature prije industrijske revolucije. Procjene ukazuju da će se nastavak porasta prosječne globalne temperature iznimno negativno odraziti na hidrološki ciklus i rezultirati smanjenjem količina dostupne vode za 40 % naročito u južnim europskim zemljama, što će, osim nestašica vode uzrokovati i neizbježne poremećaje u lancu proizvodnje hrane. Izvješće navodi da bi nedostatak vode u tim razmjerima za posljedicu moglo imati godišnje gospodarske gubitke i do 190 milijardi eura. Za ublažavanje svega navedenog, bit će nužno provoditi zahtjevne i skupe mjere i aktivnosti, stoga je imperativ današnjice poduzeti sve kako bi se porast globalne temperature usporio, a prirodni resursi očuvali i zaštitili u najvećoj mogućoj mjeri (European Union, 2005; International Monetary Fund, 2022).

Globalni učinci klimatskih promjena najizraženije se očituju u promjenama hidrološkog ciklusa, stoga među mjerama, aktivnostima i ciljevima, politike i strategije upravljanja i očuvanja vodnih resursa zauzimaju važno mjesto. U tom smjeru članice Europske unije poduzimaju intenzivne mjere u cilju promicanja recikliranja vode, odnosno upotrebe pročišćenih otpadnih voda pri čemu je navodnjavanje za potrebe poljoprivrede jedna od najznačajnijih. Uz racionalno upravljanje vodama, navodnjavanje poljoprivrednih površina korištenjem pročišćenih komunalnih otpadnih voda trebalo bi doprinjeti i smanjenju potreba za korištenjem spojeva dušika i fosfora u poljoprivredi jer se isti najčešće nalaze u povišenim koncentracijama u ispusnim vodama uređaja za pročišćavanje otpadnih voda (European Union, 2023).

Pročišćavanje otpadnih voda

Otpadne vode su vode koje nastaju korištenjem vode u kućanstvima, ugostiteljskim objektima ili industriji, a sadrže razne otopljene ili suspendirane biološke i kemijske onečišćujuće tvari kao što su mikroorganizmi, organske tvari, teški metali, ulja i masti, hranjive tvari i drugo. U svijetu se bez pročišćavanja ispušta oko 80 % otpadnih voda što najčešće uzrokuje negativne učinke po okoliš i zdravlje ljudi, stoga je važno sustavom javne kanalizacije prikupiti otpadne vode te ih primjenom fizikalnih, fizikalno-kemijskih, kemijskih i bioloških procesa pročistiti kako bi njihovo ispuštanje bilo što manje štetno za okoliš (Mishra i sur., 2023).

Navodnjavanje poljoprivrednih površina pročišćenim otpadnim vodama mora se provoditi sukladno Europskoj Uredbi (EU) 2020/741 i Direktivi 91/271/EEZ prema kojima se, u cilju postizanja ciljeva kružnog gospodarstva i promicanja provedbe ponovne uporabe vode, za potrebe navodnjavanja, otpadna voda treba pročišćavati prema manje ograničavajućim zahtjevima za uklanjanje hranjivih tvari. Naime, navodnjavanjem pročišćenim otpadnim vodama osiguravaju se dostatne količine vode, a biljkama osiguravaju i hranjive tvari kojima otpadne vode obiluju čime se u značajnoj mjeri može smanjiti ili u potpunosti zamjeniti korištenje umjetnih gnojiva što ima pozitivne učinke i na ekonomičnost poljoprivredne proizvodnje. Navodnjavanjem pročišćenim otpadnim vodama se također može i povećati godišnji prinos zemljišta jer se zbog cjelogodišnje dostupnosti pročišćene vode, u jednoj godini može na istom zemljištu imati više žetvi/berbi. Korištenje pročišćene otpadne vode za navodnjavanje može imati posebnu važnost u područjima koje se suočavaju s dugim i čestim sušnim razdobljima (Europski parlament, 2023).

Nažalost, podatci navode da se recikliranje vode na razini Europske unije provodi u prosjeku s oko 2,4 % ukupne pročišćene gradske otpadne vode. Podatci se razlikuju od članice do članice, a najuspješnije korištenje pročišćenih otpadnih voda bilježi se u mediteranskim zemljama, pojedine recikliraju i do približno 90 % pročišćene vode (Cipar), 60 % (Malta) te do 12 % (Italija i Španjolska). Postoje i primjeri članica koje ne posjeduju sustave za ponovnu upotrebu pročišćene otpadne vode (Interreg Baltic Sea Region, 2023).

Lazaridou i suradnici (2019) proveli su istraživanje u Grčkoj o spremnosti tamošnjih poljoprivrednika da koriste pročišćenu otpadnu vodu u svrhu navodnjavanja te utvrdili da je 64,2 % od ispitanih 302 poljoprivrednika spremno

koristiti pročišćenu otpadnu vodu. Naročitu spremnost pokazali su oni koji su svjesni njezine dobrobiti za okoliš. Stoga autori naglašavaju da je za povećanje udjela reciklirane vode u svrhe navodnjavanja potrebna dodatna edukacija i pružanje pravovaljanih informacija.

Pročišćavanje otpadnih voda podrazumijeva primjenu fizikalnih, kemijskih, fizikalno-kemijskih i bioloških postupaka, a proces pročišćavanja, ovisno o primjenjenim postupcima i učincima pročišćavanja, dijeli se na *preliminarno* (prethodno), *primarno*, *sekundarno*, *tercijarno* i *kvartarno* pročišćavanje (Slika 1).

Preliminarno (prethodno) pročišćavanje otpadnih voda

Preliminarni stupanj pročišćavanja otpadnih voda podrazumijeva mehaničko uklanjanje tzv. grubih čestica većih dimenzija koje mogu plutati na vodi, biti dispergirane ili se zbog veće gustoće talože u vodi. Obrada otpadne vode preliminarnim stupnjem podrazumijeva primjenu grubih (razmak od 50 do 100 mm), srednjih (razmak od 10 do 25 mm) i finih rešetki (razmak od 3 do 10 mm) koje vodu pročišćavaju tehnikom prosijavanja. Ovisno o razmaku između šipki na rešetki, učinak pročišćavanja može biti, ovisno o pojedinom parametru, od 10 do 20 %. U ovom stupnju pročišćavanja primjenjuju se postupci izdvajanja masti i ulja pomoću uređaja mastolov te izdvajanje grubih čestica pijeska i šljunka pomoću uređaja pjeskolov (Tedeschi, 1997).

Dodatni učinak izdvajanja masti i ulja može se postići upuhivanjem komprimiranog zraka u mastolove i pjeskolove pri čemu mjehurići zraka omogućuju dodatno izdvajanje nečistoća na površinu vode ili kružno kretanje vode koje potpomaže taloženje čestica na dno. Uz pjeskolov i mastolov, u ovom stupnju pročišćavanja moguće je primijeniti i postupak usitnjavanja čestica prolaskom otpadne vode preko sjekača. Smanjenje čestica nečistoća doprinosi njihovoj boljoj razgradnji u sljedećim postupcima pročišćavanja.

Većina tehnoloških linija u ovoj fazi pročišćavanja otpadnih voda ima mogućnost i primjene postupka izjednačavanja (egalizacije) kojim se, kod dotoka povećanih količina otpadnih voda, voda zadržava i pomalo upušta u sustav kako ne bi došlo do naglog razrjeđenja organske tvari u otpadnoj vodi, značajnije promjene pH vrijednosti vode što bi rezultiralo padom učinkovitosti sljedećih postupaka, naročito padom učinkovitosti biološke faze pročišćavanja otpadne vode (Tedeschi, 1997; Tušar, 2009).



Slika 1. Metode pročišćavanja otpadnih voda

Primarno pročišćavanje otpadnih voda

Primarno pročišćavanje otpadnih voda podrazumijeva primjenu fizikalnih postupaka pročišćavanja kojima se iz otpadne vode uklanjaju čestice organskih tvari većih dimenzija. Primarno pročišćavanje provodi se primjenom taloženja u taložnicama gdje nastaje primarni otpadni mulj koji se dalje odvodi na obradu. Uz taloženje, u taložnicama spori protok vode omogućuje dodatno izdvajanje tvari s manjom gustoćom od gustoće vode. Pojedine linije za pročišćavanje otpadnih voda u ovoj fazi vodu mogu pročišćavati i primjenom mikrosita čime se dodatno smanjuje udio čestica organskih tvari koje odlaze na sljedeći, sekundarni (biološki) stupanj pročišćavanja otpadnih voda (Tedeschi, 1997; Tušar, 2009).

Sekundarno pročišćavanje otpadnih voda

Sekundarno pročišćavanje otpadnih voda podrazumijeva primjenu biološke mase (mikroorganizme, gljivice, alge) raspršene u vodenom mediju ili u obliku biofilma na nosačima. Mikroorganizmi koji se koriste u ovoj fazi pročišćavanja mogu biti aerobni, anaerobni ili fakultativno aerobni, stoga je učestalost i dužina prozračivanja otpadne vode u ovoj fazi pročišćavanja od iznimne važnosti.

Ukoliko se biološka masa u ovoj fazi pročišćavanja otpadne vode nalazi raspršena u vodi, tehnologija se naziva pročišćavanje *aktivnim muljem*, dok u slučaju primjene biofilma, ista se primjenjuje prolaskom otpadne vode preko čvrstih podloga (pijesak, šljunak, glina, plastični materijali) na kojima su mikroorganizmi u obliku biofilma. U ovom stupnju obrade otopljene i suspendirane organske tvari koje se nisu izdvojile u prethodnim procesima mikroorganizmi koriste za svoj metabolizam i razmnožavanje čime se koncentracija organskih tvari u vodi smanjuje. Prozračivanje u ovoj fazi pročišćavanja vode ima dvojaku funkciju, omogućuje metabolizam aerobnih mikroorganizama te oksidira u vodi prisutne tvari što dalje doprinosi pročišćavanju otpadne vode. Prozračivanje vode također doprinosi uklanjanju hlapljivih tvari iz vode kao što su sumporovodik i amonijak te drugi, odnosno neugodnih mirisa koje njihova prisutnost u vodi uzrokuje.

Sekundarno pročišćavanje otpadnih voda može završiti sporim protokom vode kroz sekundarni taložnik, a ukoliko postoji mogućnost poželjno je sekundarnu obradu završiti prozračivanjem vode u tzv. oksidacijskim (stabilizacijskim) bazenima ili lagunama izvedenim u obliku plićih zemljanih bazena gdje se odvijaju prirodni procesi pročišćavanja otpadnih voda algama i bakterijama (Tedeschi, 1997; Tušar, 2009).

Tercijarno pročišćavanje otpadnih voda

Tercijarno pročišćavanje otpadnih voda podrazumijeva primjenu pojedinih fizikalnih, kemijskih ili dodatnih bioloških procesa, odnosno njihovih kombinacija u cilju uklanjanja hranjivih tvari (spojevi dušika i fosfora), mikroorganizama, pojedinih po okoliš i ljude štetnih kemijskih tvari te zaostale organske tvari. Primjena tercijarne obrade nužna je i kada se otpadna voda ispušta u prijemnik u kojem bi povišene koncentracije hranjivih tvari mogle uzrokovati ili pospješiti eutrofikaciju te manjak kisika i slične nepoželjne procese koji narušavaju kvalitetu vode.

Od fizikalnih postupaka, u tercijarnom stupnju pročišćavanja otpadne vode najčešće se koristi membranska filtracija primjenom polupropusnih sintetičkih membrana kojima se iz vode mogu ukloniti spojevi dušika i fosfora, mutnoća, otopljene soli, mikroorganizmi te druge toksične organske tvari poput spojeva benzena, pesticidi, teški metali i slično, adsorpcija na aktivnom ugljenu kojom je moguće ukloniti kemijske tvari koje uzrokuju pojavu neugodnih mirisa te organske spojeve iz skupine pesticida, fenola i slično te klasična pješčana filtracija kojima se iz vode uklanjaju zaostale flokule, talozi i zaostale čestice organskih tvari. Kemijski postupci u tercijarnoj obradi otpadnih voda podrazumijevaju doziranje pojedinih kemikalija koje će uzrokovati kemijske reakcije i nastanak novih spojeva (najčešće taloga) kojima se iz vode uklanjaju zaostale nepoželjne kemijske tvari poput fosfata, željeza, mangana i slično (Tedeschi, 1997; Tušar, 2009).

U kemijske postupke ubrajamo i postupke neutralizacije kada je cilj doziranjem kemikalija pH vrijednost izlazne vode dovesti u područje između pH 6 i 8. Među fizikalnim i kemijskim procesima koji se provode u tercijarnoj obradi su i postupci dezinfekcije kojima se smanjuje broj mikroorganizama u izlaznoj vodi i otpadnom mulju. Od fizikalnih postupaka dezinfekcije mogu se primijeniti membranska filtracija, UV zračenje te toplina, dok se među kemijskim postupcima najčešće primjenjuje doziranje preparata na bazi klora ili se provodi doziranjem ozona (Zagklis i Bamos, 2022).

Tercijarno pročišćavanje otpadnih voda biološkim postupcima najčešće se primjenjuje za uklanjanje spojeva dušika i fosfora, odnosno amonijaka, nitrata i nitrita te organskih fosfatnih estera, fosforata, polifosfata i ortofosfata. Proces uklanjanja spojeva dušika odvija se kroz oksidaciju amonijaka do nitrita (nitrifikacijom) odnosno redukcijom nitrita do plinovitog dušika (denitrifikacijom). Navedene biološke procese moguće je provoditi primjenom autotrofnih aerobnih bakterija, fakultativno anaerobnim bakterijama za procese nitrifikacije i denitrifikacije, odnosno primjenom aktivnog mulja obogaćenog polifosfat akumulirajućim organizmima za biološko uklanjanje spojeva fosfora (Tutić i sur. 2021).

Kvartarno pročišćavanje otpadnih voda

Najnovija znanstvena istraživanja ukazuju da otpadne vode pročišćene primjenom preliminarnog, prvog, drugog i trećeg stupnja, često i dalje sadrže tzv. mikroonečišćujuće tvari koje potječu od sredstava za čišćenje, farmaceutskih, kozmetičkih i higijenskih preparata te čestice mikroplastike i nanoplastike. Prisutnost navedenih tvari naročito predstavlja problem ukoliko se pročišćena otpadna voda reciklira. Stoga Europska komisija u svojim nedavno objavljenim dokumentima naglašava potrebu uvođenja kvartarnog stupnja pročišćavanja otpadnih voda čijom primjenom bi se navedene problematične komponente, primarno organske mikroonečišćujuće tvari opasne za zdravlje populacije i okoliš, uklonile iz vode u skladu s EU Direktivom 2000/60/EZ i Okvirnom direktivom o vodama, a na temelju načela predostrožnosti kojim bi bilo zajamčeno brzo djelovanje u identificiranom trenutku moguće opasnosti za zdravlje ljudi i životinja te okoliš (Europski parlament, 2023).

Prema usvojenoj dopuni Prijedlogu direktive Europskog parlamenta i Vijeća o pročišćavanju komunalnih otpadnih voda iz listopada 2023. godine, uređaji za pročišćavanje otpadnih voda koji prikupljaju otpadnu vodu s područja koje broji > 150.000 ES trebali bi osigurati mogućnost kvartarnog pročišćavanja otpadnih voda u cilju uklanjanja mikroonečišćujućih tvari jer navedeni sustavi u okoliš ispuštaju njihove značajne količine. Kod uređaja za pročišćavanje otpadnih voda manjih kapaciteta, kvartarni stupanj pročišćavanja treba primijeniti ukoliko procjene donesene na temelju jasno definiranih parametara ukažu na značajnu mogućnost pojave mikroonečišćujućih tvari u pročišćenim otpadnim vodama ili nedovoljan kapacitet razrjeđenja vodnog tijela u koje se pročišćene vode ispuštaju. Za uvođenje kvartarnog stupnja, Europska komisija predviđa prijelazno razdoblje od 15 godina od dana donošenja Uredbe kako bi se osigurala financijska sredstva za investicije u nove tehnologije i potrebnu opremu. Europska komisija također ističe da je prethodno potrebno utvrditi popis te analitičke metode kojima bi se mogla utvrditi prisutnost i pratiti učinkovitost njihova uklanjanja (Europski parlament, 2023).

Do danas su učinkovitost pri uklanjanju mikroonečišćujućih tvari te mikroplastike i nanoplastike iz vode pokazale metode dodatne koagulacije, membranske filtracije (reverzna osmoza), magnetska separacija, fotodegradacija, napredni oksidacijski procesi, postupci biološke i mikrobiološke razgradnje i slično (Massima Mouele i sur., 2021; Gao i sur., 2022).

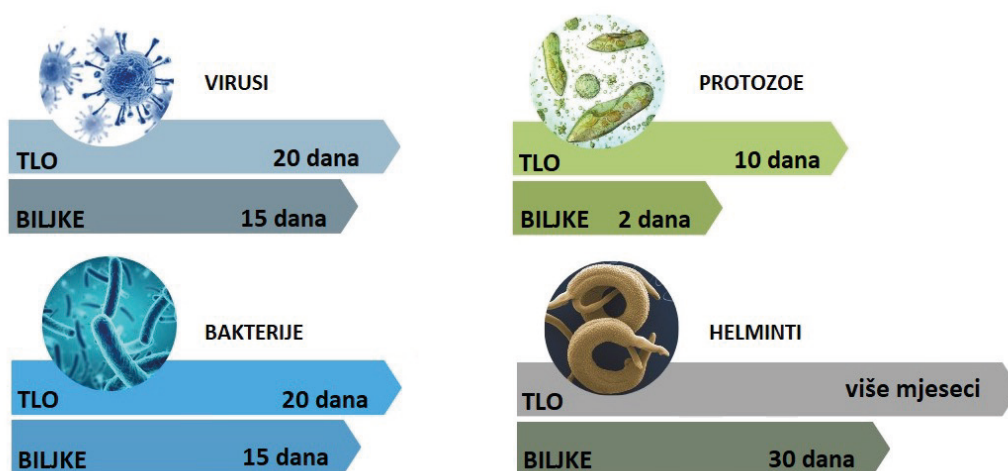
Alternativne metode pročišćavanja otpadnih voda

U alternativne metode pročišćavanja otpadnih voda ubrajaju se pročišćavanje otpadnih voda biljnim uređajima te pročišćavanje otpadnih voda membransko-biološkim reaktorima. Membransko-biološki reaktori (MBR) su uređaji u kojima se biološko pročišćavanje otpadnih voda provodi djelovanjem mikroorganizama, a potom se mikroorganizmi i ostaci onečišćujućih tvari od vode razdvajaju visokoselektivnim polupropusnim membranama. Navedeni postupak naročito je poželjan kada se želi postići visoka kvaliteta pročišćene vode. Biljni uređaji podrazumijevaju tehniku pročišćavanja kojom se otpadna voda učinkovito pročišćava na ekološki prihvatljiv način primjenom močvarnih biljaka zasađenih u plitkim betonskim spremnicima. Močvarne biljke (rogoz, šaš, trska, vodena leća i slične) sade se u bazene ispunjene poroznim materijalom, a otpadna voda se nakon mehaničke obrade (rešetanja) upušta u bazene gdje se fizikalnim postupcima (prolazak vode kroz porozne podloge), kemijskim reakcijama oksidacije i redukcije koje se odvijaju pod utjecajem zraka, odnosno u slojevima podloga bez zraka, te biološkim postupcima (mikroorganizmi koji se nalaze na biljkama i u sustavu korijenja močvarnih biljaka) pročišćava do visoke čistoće uz minimalne ekonomske troškove. Otpadne vode pročišćene s navedene dvije alternativne metode iznimno su pogodne za navodnjavanje jer su najčešće vrlo visoke kvalitete (Anić Vučinić i Ružinski, 2010; Pervez i sur., 2020; Vymazal i sur. 2021).

Rizici korištenja otpadnih voda za navodnjavanje

Korištenje pročišćenih otpadnih voda u poljoprivredi poželjno je zbog kontinuirane dostupnosti vode te prisutnosti hranjivih tvari (spojeva fosfora i dušika) te organskih tvari u vodi koji, korištenjem vode za navodnjavanje, obogaćuju tlo.

Ipak, korištenje pročišćene otpadne vode za navodnjavanje nosi određene rizike i opasnosti po ljudsko zdravlje i okoliš. Naime, otpadne vode, naročito ukoliko tijekom procesa pročišćavanja ne prolaze dezinfekciju, sadrže mikroorganizme koji mogu izazvati bolesti poput crijevnih virusa, hepatitisa i slično. Stoga je, za sigurno navodnjavanje poljoprivrednih površina, važno znati mjesto i način primjene reciklirane vode te njen mikrobiološki sastav i vrijeme preživljavanja u vodi prisutnih mikroorganizama na biljkama i na tlu. Najveći je rizik za ljudsko zdravlje ukoliko se navodnjavanje provodi pročišćenim otpadnim vodama koje sadrže helminte i patogene bakterije. Ispitivanjima je utvrđeno da je preživljavanje mikroorganizama duže na tlu nego na biljkama (Tedeschi, 1997). Prosječno vrijeme preživljavanja za pojedine skupine mikroorganizama prikazano je na Slici 2.



Slika 2. Prosječno vrijeme preživljavanja pojedinih mikroorganizama prisutnih u otpadnim vodama

Rizik korištenja pročišćenih otpadnih voda ovisi o uvjetima u kojima otpadne vode nastaju (mješanje s industrijskim i drugim opterećenim otpadnim vodama) te o primjenjenom stupnju pročišćavanja. Stoga, prilikom izrade planova recikliranja voda, iste primarno treba imati u vidu. Tako na primjer, nakon primarnog stupnja obrade otpadnih voda, u vodi zaostaje i do 70 % suspendiranih krutih tvari kao što su pijesak te masti i ulja, visoki udjeli patogenih i nepatogenih mikroorganizama, organskih i hranjivih tvari čije bi ispuštanje u značajnoj mjeri opteretilo poljoprivredne površine te u pitanje dovele sigurnost navodnjavanih kultura i njihovih plodova, odnosno osoba koje dolaze u dodir ili konzumiraju takve plodove. Navedeni rizici mogu se u značajnoj mjeri smanjiti ukoliko se za navodnjavanje koristi otpadna voda pročišćena tercijarnim i/ili kvartarnim stupnjem uz obveznu primjenu dezinfekcijskog postupka. Sigurnost primjene pročišćene otpadne vode za navodnjavanje dodatno se može postići i primjenom digitalnih alata kojima je moguće trenutno i kontinuirano mjerenje kemijskog sastava otpadnih voda.

Kako bi se pročišćena otpadna voda mogla koristiti u svrhu navodnjavanja važno je utvrditi sljedeće: (a) postoji li rizik za zdravlje ili okoliš u smislu pojave eutrofikacije, (b) udovoljava li voda zahtjevima utvrđenima u Uredbi (EU) 2020/741, (c) utvrditi usklađenost koncentracija mikroonečišćujućih tvari, mikroplastike i nanoplastike s preporukama, (d) utvrditi da minimalni ekološki protok prihvatnih vodnih tijela nije ugrožen i (e) postojanje dovoljnog kapaciteta za pročišćavanje ili skladištenje svih dolaznih komunalnih otpadnih voda za razdoblja u kojima se komunalne otpadne vode ne upotrebljavaju za navodnjavanje (Europski parlament, 2023).

Uz zdravstvene i okolišne rizike, potrebno je napomenuti da upotreba reciklirane vode nosi i ekonomske rizike. Qin i Horvath (2020) navode na primjeru Kalifornije da bi navodnjavanje recikliranom vodom uz miješanje s oborinskom vodom moglo povećati udio alternativne upotrebe vode u navodnjavanju poljoprivrednih zemljišta s trenutnih 0,82 % na 5,4 %. No, autori naglašavaju da bi upotreba reciklirane vode povećala godišnje troškove

pročišćavanja otpadnih voda za 900 milijuna dolara što bi se neminovno odrazilo i na cijenu poljoprivrednih kultura i prehrambenih proizvoda dobivenih uz navodnjavanje recikliranom vodom. Također navode da će recikliranje vode neizbježno pratiti i povećanje ukupnih emisija stakleničkih plinova u sektoru pročišćavanja komunalnih otpadnih voda.

Zaključak

Za potrebe poljoprivrede odlazi 70 % slatke vode koja se svakodnevno crpi iz vodnih tijela, a UN-ova Organizacija za hranu i poljoprivredu navodi da se u posljednjih 30 godina proizvodnja hrane povećala za više od 100 % što se odrazilo i na potrošnju vode za potrebe poljoprivrede. Navodnjavanje je osnovni i glavni alat kojim će poljoprivrednici, u desetljećima koja dolaze, nastojati ublažiti utjecaj klimatskih promjena na vodne sustave i povećati otpornost pri čemu je prioritet što manje zadirati u zalihe prirodnih voda te, u skladu s zelenim politikama, pri navodnjavanju koristiti obnovljive izvore energije i recikliranu vodu.

Otpadne vode smatraju se značajnim budućim izvorom vode za potrebe poljoprivrede, naročito u zemljama koje već sada pogađaju česta sušna razdoblja. Korištenjem reciklirane vode dobivene pročišćavanjem otpadnih voda, moguće je riješiti probleme nestašice vode, a istovremeno osigurati pouzdan i siguran izvor vode.

Uspješna upotreba reciklirane vode za potrebe navodnjavanja se provodi u nekoliko zemalja Europske unije među kojima prednjače zemlje na Mediteranu, Cipar, Malta te Španjolska i Italija.

Europska komisija odlučna je poticati i promicati korištenje pročišćene otpadne vode u poljoprivredi, a Uredbom o minimalnim zahtjevima za ponovnu upotrebu vode u poljoprivrednom navodnjavanju iz lipnja 2020. te dopunama Prijedloga direktive Europskog parlamenta i Vijeća o pročišćavanju komunalnih otpadnih voda iz listopada 2023. godine postavlja temelje za olakšavanje ponovne upotrebe vode u cijeloj Europskoj uniji, ali definira i tehnološke kriterije na temelju kojih će primjena reciklirane vode biti sigurna i uz najmanje rizike za zdravlje ljudi i okoliš.

Literatura

- Anić Vučinić A., Ružinski N. (2010). Obrada otpadnih voda biljnim uređajima. Zagreb, Hrvatska: Hrvatska sveučilišna naklada
- EU Copernicus: Climate Change (2023). <https://climate.copernicus.eu/>
- European Union: The Impacts and Costs of Climate Change (2005). https://climate.ec.europa.eu/system/files/2016-11/final_report2_en.pdf
- European Union: Water reuse (2023). https://environment.ec.europa.eu/topics/water/water-reuse_en
- Europska unija (1991). Direktiva 91/271/EEZ – pročišćavanje komunalnih otpadnih voda. <https://eur-lex.europa.eu/HR/legal-content/summary/urban-waste-water-treatment.html>
- Europski parlament (2020). Uredba (EU) 2020/741 Europskog parlamenta i Vijeća od 25. svibnja 2020. o minimalnim zahtjevima za ponovnu upotrebu vode. <https://eur-lex.europa.eu/legal-content/HR/TXT/?uri=CELEX:32020R0741>
- Europski parlament (2022). Pristup vodi kao ljudsko pravo: vanjska dimenzija Rezolucija Europskog parlamenta od 5. listopada 2022. o pristupu vodi kao ljudskom pravu: vanjska dimenzija (2021/2187(INI)). https://www.europarl.europa.eu/doceo/document/TA-9-2022-0346_HR.pdf
- Europski parlament (2023). Izvješće o Prijedlogu direktive Europskog parlamenta i Vijeća o pročišćavanju komunalnih otpadnih voda (preinaka). https://www.europarl.europa.eu/doceo/document/TA-9-2023-0355_HR.html
- European Environment Agency (2023). www.eea.europa.eu
- Gao W., Zhang Y., Mo A., Jiang J., Liang Y., Cao X., He D. (2022). Removal of microplastics in water: Technology progress and green strategies. *Green Analytical Chemistry*. 3: 100042.
- International Monetary Fund: Climate Change and Energy Security: The Dilemma or Opportunity of the Century? (2022). <https://www.imf.org/-/media/Files/Publications/WP/2022/English/wpiea2022174-print-pdf.ashx>

- Interreg Baltic Sea Region (2023). <https://interreg-baltic.eu/project-posts/the-new-regulation-aims-to-encourage-and-facilitate-water-reuse-in-the-eu/>
- Lazaridou D., Michailidis A., Mattas K. (2019). Evaluating the Willingness to Pay for Using Recycled Water for Irrigation. *Sustainability*. 11: 5220.
- Massima Mouele E.S., Tijani J.O., Badmus K.O., Perea O., Babajide O., Zhang C., Shao, T., Sosnin E., Tarasenko V., Fatoba O.O., Laatikainen K., Petrik L. (2021). Removal of Pharmaceutical Residues from Water and Wastewater Using Dielectric Barrier Discharge Methods—A Review. *International Journal of Environmental Research and Public Health*. 18:1683.
- Mishra S., Kumar R., Kumar M. (2023). Use of treated sewage or wastewater as an irrigation water for agricultural purposes - Environmental, health, and economic impacts. *Total Environment Research Themes*. 6: 100051.
- Pervez M.N., Balakrishnan M., Hasan S.W., Choo K.-H., Zhao Y., Cai Y., Zarra T., Belgiorno V., Naddeo V. (2020). A critical review on nanomaterials membrane bioreactor (NMs-MBR) for wastewater treatment. *NPJ Clean Water* 3: 43.
- Tedeschi S. (1997). *Zaštita voda*. Zagreb, Hrvatska: Hrvatsko društvo građevinskih inženjera.
- Tušar B. (2009). *Pročišćavanje otpadnih voda*. Zagreb, Hrvatska: Kigen.
- Tutić A., Zeko-Pivač A., Landeka Dragičević T., Šiljeg M., Habuda-Stanić M. (2021). Uklanjanje i uporaba fosfora iz otpadnih voda. *Hrvatske vode*. 29: 33-41.
- United Nations: What Is Climate Change? (2023). <https://www.un.org/en/climatechange/what-is-climate-change>
- Vymazal J., Zhao Y., Mander Ü. (2021). Recent research challenges in constructed wetlands for wastewater treatment: A review. *Ecological Engineering*. 169: 106318.
- Zagklis D.P., and Bampos G. (2022). Tertiary Wastewater Treatment Technologies: A Review of Technical, Economic, and Life Cycle Aspects. *Processes*. 10: 2304.
- Qin Y., Horvath A. (2020). Use of alternative water sources in irrigation: potential scales, costs, and environmental impacts in California. *Environmental Research Communications*. 2: 5.
- Water Reuse Europe (2023). European Parliament Adopts New Rules for Urban Wastewater - Water Reuse Europe. <https://www.water-reuse-europe.org/european-parliament-adopts-new-rules-for-urban-wastewater/>
- World Meteorological Organization: Greenhouse Gas Bulletin (2023). <https://public.wmo.int/en/greenhouse-gas-bulletin>

Recycled water and agriculture - solution or problem?

Abstract

The global effects of climate change have a strong and negative impact on the hydrological cycle. According to the UN Food and Agriculture Organization, water consumption for agricultural purposes has been growing twice as fast as population growth in recent decades. Therefore, the European Union via the Green Deal, i.e. legislation and financial tools, encourages the use of recycled water for the purpose of irrigation in order to strengthen the agricultural sector's resistance to climate change and contribute to achieving the goals of circular economy and sustainability. This paper describes the current European Strategy for water recycling and its usage for irrigation. Conventional methods for wastewater purification are described, as well as risks of recycled water usage for irrigation.

Keywords: water recycling, wastewater, irrigation

Flora makrofita dravskih mrtvica na području Virovitičko-podravske županije

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Sažetak

Makrofiti su često glavna komponenta akvatičnih ekosustava i važna karika u lancu prehrane. Ove biljke osiguravaju stanište mnogim beskralješnjacima (zooplankton) i drugim životinjskim organizmima. Vodeni ekosustavi su ugroženi zbog ljudskih aktivnosti, klimatskih promjena, a značajne štete dolaze i zbog pojave invazivnih vrsta. Iako makrofiti mogu činiti ekološki problem zbog masovne pojave, mogli bi pozitivno utjecati na poljoprivrednu proizvodnju: kao gnojivo, kompost, fitoremediatori, biomasa za biogoriva i drugo.

Ključne riječi: stajačice, makrofiti i njihov značaj

Uvod

Rijeka Drava, ukupne dužine 725 km, izvire u Italiji, a prolazi kroz Austriju, Sloveniju, Mađarsku i Hrvatsku. Nekadašnji dijelovi rijeke koji su sada potpuno ili djelomično odvojeni od glavnog toka korita nazivaju se mrtvice. U tim dijelovima voda gotovo da ne teče. Mrtvice kao poseban oblik staništa doprinose bioraznolikosti. Nema stvaranja novih mrtvica, te njihov broj opada. Razlog tomu je kanaliziranje i utvrđivanje rijeke, te mnogi agrikulturni zahvati (Egertson i sur., 2004), što u konačnici dovodi do manje biološke raznolikosti prostora uz rijeku.

Makrofiti su biljke različitih vodenih ekosustava (jezera, močvare, potoci, rijeke), rastu djelomično ili potpuno u vodi, ukorjenjuju se ili slobodno plutaju. Makrofite dijelimo na potopljene, plutajuće i izranjajuće. To su biljke čiji se životni ciklus potpuno ili periodički odvija u vodenom okruženju i rasprostranjene su u cijelom svijetu (Lesiv i sur., 2020). Sposobne su rasti i razmnožavati se i u vodenom i kopnenom okruženju. Prozirnost vode i količina hranjivih tvari utječu na pojavu makrofita (Bornette i Puijalon, 2009). Općenito koloniziraju plitke ekosustave gdje utječu na ekološke procese. Makrofiti i kemija vode usko su povezani, odnosno prisutni makrofiti i njihova količina ovise o kemijskim svojstvima vode (Lukacs i sur., 2011). Izostanak makrofita ukazuje na problem kvalitete voda. Ove biljke utječu na kruženje hranjivih tvari (Camargo i sur., 2003; Pott i Pott, 2003; Zelnic i sur., 2022) i na nekoliko drugih fizikalno-kemijskih svojstava kao što su: pH i alkalnost koji mogu biti rezultat njihovog metabolizma (Caraco i Cole, 2002), zatim na prijenos kemijskih elemenata iz sedimenta u vodu, te oslobađanje P i N koje mogu koristiti mikroalge i bakterije. Makrofiti su uz alge važan izvor hrane za vodene organizme zbog velike proizvodnje biomase. Izvor su organske tvari za vodene biljojede (Poi de Neiff i Casca, 2003; Mormul i sur., 2013). Primarni su proizvođač kisika putem fotosinteze i osiguravaju utočište mnogim beskralješnjacima. Pored toga, povećavaju heterogenost staništa vodenih ekosustava, utječu na brojnost životinja kojima osiguravaju stanište (Bakker i sur., 2013; Palmik, 2017) i bioraznolikost. Oni su bioindikator kvalitete vode, jer su osjetljivi na kemijske, fizičke i hidrološke promjene u vodi. Neki makrofiti imaju mogućnost akumulirati teške metale iz vodene sredine, pesticide (Alencar i sur., 2020) uključujući i herbicide kao glifosat (Poveda, 2022) te antibiotike korištene u veterinarske svrhe (Xian i sur., 2010). Koriste se i za zelenu gnojdbu, a korist toga ogleđa se u boljem rastu biljaka, toleranciji na abiotički stres, obrani od patogena i štetnika. Prema Shukla i suradnicima (2020.) kompost načinjen od pripadnika roda *Typha* utječe na rast biljaka te povećanje fotosintetske aktivnosti. Kompost povoljno utječe na strukturu i biologiju tla te na povećanje dobrih bakterija u tlu (Matsuoka i sur., 2020). Makrofiti imaju važnu ulogu u održavanju čistoće vode, odnosno važna su komponenta u očuvanju zdravlja ekosustava (Hamabata i Kobayashi, 2002). Bogatstvo vrsta ovisi o antropogenim faktorima. Faktor koji utječe na zajednicu makrofita je i visina vode koja je pod antropogenim utjecajem (hidromelioracije).

Materijal i metode

Istraživanje je obuhvatilo pet lokacija (Križnica, Graba, Neteča, Liman, Budakovačka bara) u Virovitičko-podravskoj županiji na potezu od 171-104 riječnog km tijekom ljetnih mjeseci 2021. godine. Biljne svojte determinirane su pomoću standardnih ključeva te je nomenklatura usklađena prema Nikolić (2019.). Florističkim istraživanjem determinirane su biljne svojte koje su karakteristične za prepoznavanje stanišnih tipova. Svi makrofiti determinirani su do taksonomske razine vrste, a *Typha* i *Sparganium* do roda. Florni elementi određeni su prema Horvatić (1963) i Trinajstić (1986): kozm – kozmopoliti, eur – euroazijski florni element, subeur – subeuroazijski florni element, cirk – cirkumholarktički florni element, subcirk – subcirkumholarktički florni element i subse – subsrednjoeuropski florni element. Životni oblici određeni su prema Pignatti (1982) i Rauš i Šegulja (1983): H – hidrofiti i G – geofiti.

Rezultati i rasprava

Na istraživanom području utvrđeno je 17 makrofita koji su razvrstani u 13 porodica (Tablica 1.), a 13 svojti prisutno je na svim lokalitetima. Hidrofitima pripada 71 % svojti dok 29 % svojti pripada geofitima. Kozmopoliti, odnosno biljke široke rasprostranjenosti (35 %) su najzastupljenije. Iza njih dolaze biljke koje pripadaju euroazijskom flornom elementu (23 %) te biljke cirkumholarktičke rasprostranjenosti (17 %). Ostali florni elementi zastupljeni su u manjem udjelu. Dominiraju dvosupnice (59 %), a jednosupnice su zastupljene u manjem obimu (41 %).

Vrsta *Ceratophyllum demersum* L. je potopljeni makrofit koji cvate ispod vode. Iznad vode cvatu *Myriophyllum spicatum* L., *Potamogeton lucens* L. i *Potamogeton perfoliatus* L., dok su *Potamogeton lucens* i *P. perfoliatus* indikatori su čistih voda. Plutajuće listove imaju *Nuphar lutea* (L.) Sm., *Nymphaea alba* L., i *Nymphoides pelata* (S. G. Gmel.) Kuntze. Uz rubove rijeka javljaju se *Nuphar lutea* i *Nymphaea alba* koje čine jednu od najčešćih zajednica vodenih vegetacija u našoj zemlji. *Nymphoides pelata* dobro podnosi gubitak vode. Njegova brojnost vremenom opada zbog antropogenih utjecaja. Makrofitima čiji listovi izlaze iz vode pripadaju *Hippuris vulgaris* L. i *Sparganium* sp. Slobodno plutajući makrofiti su *Lemna minor* L., *Lemna trisulca* L., *Hydrocharis morsus-ranae* L., *Salvinia natans* (L.) All. i *Spirodela polyrhiza* (L.) Schleid.

Lemna minor i *Lemna trisulca* zastupljene su na svim lokalitetima, ali *L. minor* dominira u ljetnim mjesecima u kojima je istraživanje i obavljeno.

Vodeni makrofiti prema Nacionalnoj klasifikaciji staništa pripadaju vegetaciji slobodno plivajućih flotalnih i submerznih hidrofitu (A 3. 2.), zakorijenjenoj vodenjarskoj vegetaciji (A 3. 3.) te tršćacima i rogozici (A 4. 1.).

Slobodno plivajući flotalni i submerzni makrofiti su *Lemna minor*, *Lemna trisulca*, *Spirodela polyrhiza* i *Salvinia natans*. Uz rubove vodenih površina prevladava *Hydrocharis morsus-ranae*, *Spirodela polyrhiza*, *Salvinia natans* i *Myriophyllum spicatum*.

Zakorijenjenu vodenjarsku vegetaciju čine biljke koje su ukorijenjene u dno, a listovi i cvjetovi im plutaju na površini vode ili strše u zrak. To su npr. *Nymphaea alba*, *Nuphar lutea*, *Myriophyllum spicatum* i *Ceratophyllum demersum*. *Potamogeton perfoliatus* i *Potamogeton lucens* su submerzni hidrofiti čiji cvatovi izviruju iznad površine vode.

Tablica 1. Popis i osnovna obilježja biljaka

Latinski naziv	Porodica	Morfotip	Životni oblik	Florni element
<i>Nymphaea alba</i>	Nymphaeaceae	D	H	Subse
<i>Nuphar lutea</i>	Nymphaeaceae	D	H	Eur
<i>Myriophyllum spicatum</i>	Haloragaceae	D	H	Subcirk
<i>Ceratophyllum demersum</i>	Ceratophyllaceae	D	H	Kozm
<i>Lemna minor</i>	Lemnaceae	M	H	Kozm
<i>Lemna trisulca</i>	Lemnaceae	M	H	Kozm
<i>Spirodela poyrhiza</i>	Lemnaceae	M	H	Kozm
<i>Salvinia natans</i>	Salviniaceae	D	H	Cirk

<i>Hydrocharis morsus-rane</i>	Hydrocharitaceae	M	H	Eur
<i>Mentha aquatica</i>	Lamiaceae	D	G	Eur
<i>Sparganium sp.</i>	Sparganiaceae	M	G	Subeur
<i>Phragmites australis</i>	Poaceae	M	G	Kozm
<i>Potamogeton perfoliatus</i>	Potamogetonaceae	D	H	Cirk
<i>Potamogeton lucens</i>	Potamogetonaceae	D	H	Cirk
<i>Typha sp.</i>	Typhaceae	M	G	Kozm
<i>Nymphoides peltata</i>	Menyanthaceae	D	H	Eur
<i>Hippuris vulgaris</i>	Hippuridaceae	D	G	Subcirk

D-dvosupnice; *M*-jednosupnice; *H*-hidrofiti; *G*-geofiti; *koz*m – kozmopoliti; *eur* – euroazijski florni element; *subeur* – subeuroazijski florni elementi; *cirk* – cirkumholarktički florni element; *subcirk* – subcirkumholarktički florni element; *subse* – subsrednjoeuropski florni element

Tršćaci i rogozici prepoznatljivi su po makrofitskoj vegetaciji koju čine biljke koje rastu uz rubove stajaćih voda: *Phragmites australis* (Cav.) Trin. ex Steud., *Mentha aquatica* L. i *Typha* sp. Pod vodom je korijenov sustav i podanak koji sprječavaju eroziju (Preininger i sur., 2018). Prisutnost *Phragmites australis* i *Typha* sp. zabilježena je na svim istraživanim lokalitetima. To su poželjne biljke jer imaju sposobnost akumulacije niskih koncentracija Cd i Pb (Borowiak i sur., 2016). Osim toga, tršćaci su važno gnjezdište za ptice.

Lokaliteti Križnica i Graba nalaze se s lijeve strane rijeke Drave. Na tim lokalitetima, zbog pada vode u Dravi, dolazi do zarastanja i smanjenja vodene površine. Ovdje se javljaju sljedeće vrste: *Nymphaea alba*, *Nuphar lutea*, *Myriophyllum spicatum*, *Ceratophyllum demersum*, *Lemna minor*, *Lemna trisulca*, *Spirodela poyrhiza*, *Salvinia natans*, *Hydrocharis morsus-rane*, *Mentha aquatica*, *Sparganium sp.*, *Phragmites australis*, *Potamogeton perfoliatus*, *P. lucens* i *Typha* sp.

Nizvodno od 140 rkm nalaze se Neteča i Liman u blizini naselja Detkovic. Na ta dva lokaliteta prevladavaju sljedeće biljne vrste: *Nymphaea alba*, *Nuphar lutea*, *Nymphoides peltata*, *Myriophyllum spicatum*, *Ceratophyllum demersum*, *Lemna minor*, *Lemna trisulca*, *Spirodela poyrhiza*, *Salvinia natans*, *Hydrocharis morsus-rane*, *Mentha aquatica*, *Sparganium sp.*, *Phragmites australis* i *Typha* sp.

Budakovačka bara je površinom veća od prethodno istraženih lokacija. Od vodene vegetacije najčešće vrste su *Nymphaea alba*, *Nuphar lutea*, *Nymphoides peltata*, *Myriophyllum spicatum*, *Ceratophyllum demersum*, *Lemna minor*, *Lemna trisulca*, *Spirodela polyrhiza*, *Salvinia natans*, *Hydrocharis morsus-rane*, *Hippuris vulgaris*, *Mentha aquatica*, *Sparganium sp.*, *Phragmites australis* i *Typha* sp.

Istraživanja provedena u sjeveroistočnom dijelu Slovenije uz rjeku Dravu (Zelnik i sur., 2022) pokazuju da prisutnost nekih invazivnih biljnih vrsta nije utjecala na prisutnost i pokrovnost hidrofita na tom području.

Zaključak

Makrofiti su važni vodeni organizmi staništa koji utječu na sastav faune, brojnost i raznolikost životinja, dostupnost hrane i skloništa te povećavaju heterogenost staništa. Navedeni makrofiti nastanjuju botanički važna područja Hrvatske. Pravilno upravljanje ovakvim ekosustavima i monitoring važne su mjere očuvanja i obnove prirodnih staništa i makrofitske vegetacije. Plići dijelovi mrtvica u potpunosti su prekriveni vodenom vegetacijom. Na mrtvicama koje su okružene poljoprivrednim površinama zamijećena je ubrzana eutrofikacija. Evidentan je utjecaj čovjeka, to su omiljena mjesta među ribičima, kupališta i vikendice. Mnogi znanstveni i stručni radovi govore o benefitima ovih biljaka koje bi mogle značajno pridonijeti poljoprivrednoj proizvodnji na način da utječu na poboljšanje strukture tla te kao gnojivo za povećanje prinosa. Ove biljke mogle bi utjecati i na smanjenje ugljičnog otiska te tako doprinijeti ublažavanju klimatskih promjena. Isto tako, važno je prepoznati invazivne vrste kako bi se smanjila opasnost od njihova širenja.

Literatura

- Alencar B.T.B., Ribeiro V.H.V., Cabral C.M., dos Santos N.M.C., Ferreira E.A., Francino D.M.T. (2020). Use of macrophytes to reduce the contamination of water resource by pesticides. *Ecological Indicators*. 109.
- Bakker E.S., Sarneel J.M., Gulati R.D., Liu Z., Donk E. (2013). Restoring macrophyte diversity in shallow temperate lakes: biotic versus abiotic constraints. *Hydrobiologia*. 710: 23-37.
- Bornette G., and Puijalon S. (2009). *Macrophytes: Ecology and aquatic plants*. Encyclopedia of life science (ELS), John Wiley & Sons, Chichester.
- Borowiak K., Kanclerz J., Mleczek M., Lisiak-Zielinska M., Drzewiecka K. (2016). Accumulation of Cd and Pb in water, sediment and two littoral plants (*Phragmites australis*, *Typha angustifolia*) of freshwater ecosystem. *Archives of environmental protection*. 42: 47-57.
- Camargo A.F.M., Pezzato M.M., Henry-Silva G.G. (2003). Limiting factors to the primary production of aquatic macrophytes. In Thomaz S. M., Bini L. M., eds. *Ecologia e Manejo de Macrófitas Aquáticas* Maringá: Eduem. p. 59-83.
- Caraco N.F., Cole J.J. (2002). Contrasting impacts of a native and alien macrophyte on dissolved oxygen in a large river. *Ecological Applications*. 12: 1496-1509.
- Egertson C.J., Kopaska J.A., Downing J.A. (2004). A century of change in macrophyte abundance and composition in response to agricultural eutrophication. *Hydrobiologia*. 524: 145-156.
- Hamabata E., and Kobayashi Y. (2002). Present status of submerged macrophyte growth in Lake Biwa: recent recovery following a summer decline in the water level. *Lakes Reservoir Research and Management*. 7: 331–338.
- Horvatić S. (1963). Vegetacijska karta otoka Paga s općim pregledom vegetacijskih jedinica Hrvatskog primorja. *Acta biologica* 4.
- Lesiv M.S., Polishchuk A.I., Antonyak H.L. (2020). Aquatic macrophytes: ecological features and functions. *Studia Biologica*. 14: 79-94.
- Lukacs B.A., Devai G., Tothmeresz B. (2011). Small scale macrophyte-environment relationship in an oxbow-lake of the Upper-Tisza valley (Hungary). *Community Ecology*. 12: 259-263.
- Matsuoka S., Kobayashi, Y., Hobara S., Osorno T. (2020). Identifying microbial drivers promoting plant growth on soil amended with composed aquatic plant: insight into nutrient transfer from aquatic to terrestrial systems. *Limnology*. 21: 443-452.
- Mormul R.P., Thomaz S.M., Vieira L.J.S. (2013). Richness and composition of macrophyte assemblages in Amazonian lakes. *Acta Scientiarum Biological Sciences*. 35: 343-350.
- Nikolić T. (2019.). *Flora Croatica: Vaskularna flora Republike Hrvatske*. Vol: 1-4.
- Nikolić T. (2020). *Flora Croatica Database*. Prirodoslovno-matematički fakultet, Sveučilište u Zagrebu.
- Palmik K. (2017). Effects of natural and anthropogenic pressures and disturbances on the macrophytes of lake Peipsi. *Eesti Maaülikool, Estonian University of Live Science*, Tartu.
- Pignatti S. (1982). *Flora d Italia*. I-III. Edagricole, Bologna.
- Poi de Neiff A.S., Casco S.L. (2003). Biological agents that accelerate winter decay of *Eichhornia crassipes* Mart. Solms. in northeastern Argentina. In Thomaz, S. M., Bini, L. M., ed. *Ecologia e Manejo de Macrófitas Aquáticas* Maringá: Eduem. p. 127-144.
- Pott V.J., and Pott A. (2003). Dynamics of aquatic vegetation in Pantanal. In Thomaz, S. M., Bini, L. M., ed. *Ecologia e Manejo de Macrófitas Aquáticas* Maringá: Eduem. p. 145-162.
- Poveda J. (2022). The use of freshwater macrophytes as a resource in sustainable agriculture. *Journal of Cleaner Production*. 369: 133247.
- Preininger T., Kirin T., Šestani G., Žeger Pleše I. (2018). Projekt “Trščaci – vrednovanje usluga slatkovodnih ekosustava”, Pregled i ocjena usluga ekosustava na trščacima Republike Hrvatske, Hrvatska agencija za okoliš i prirodu, Zagreb.

- Rauš Đ., and Šegulja N. (1983). Flora Slavonije i Baranje. Glasnik za šumske pokuse. 21: 179-211.
- Shukla A., Shukla S., Hodges A.W., Harris W.G. (2020). Valorization of farm pond biomass as fertilizer for reducing basin-scale phosphorus losses. Science of the Total Environment. 720: 137403.
- Trinajstić I. (eds.) (1975-1986). Analitička flora Jugoslavije 2. Institut za botaniku Sveučilišta u Zagrebu.
- Zelnik I., Germ M., Kuhar U., Gaberščik A. (2022). Waterbodies in the Floodplain of the Drava River Host Species-Rich Macrophyte Communities despite Elodea Invasions. Diversity. 14: 870.
- Xian Q., Hu L., Chen H., Chang Z., Zou H. (2010). Removal of nutrients and veterinary antibiotics from swine wastewater by a constructed macrophyte floating bed system. Journal of Environmental Management. 91: 2657-2661.

Flora of macrophytes of Drava oxbow lakes in the area of Virovitica-podravina county

Abstract

Macrophytes are often the main component of aquatic ecosystems and an important link in the food chain. These plants provide a habitat for many invertebrates (zooplankton) and other animal organisms. Aquatic ecosystems are threatened due to human activities, climate change, and significant damage is also caused by the appearance of invasive species. Although macrophytes can be an ecological problem due to their mass occurrence, they could have a positive effect on agricultural production: as fertilizer, compost, phytoremediators, biomass for biofuels and more.

Keywords: oxbow lakes, macrophytes and their importance



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Uzajamni fond – strategija za upravljanje rizikom u stočarstvu

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Sažetak

Cilj rada je istražiti mišljenje stočara o uzajamnom fondu kao inovativnom alatu za upravljanje rizikom. Istraživanje je provedeno među članovima Udruge Baby Beef. Anketni upitnik imao je nekoliko grupa pitanja: podaci o poljoprivrednom osiguranju; mišljenje o uzajamnom fondu; sociodemografski podaci i podaci o gospodarstvu. Prikupljeni podaci analizirani su deskriptivnom statistikom. U uzorku su ispitanici pretežno muškog spola (80%), u dobi između 40 i 49 godina (33%) i najviše sa završenom srednjom školom (43%). Polovica ispitanika primijenjuje osiguranje životinja. Nadalje, rezultati pokazuju kako 70 % ispitanika smatra da je uzajamni fond dobra strategija za upravljanje rizikom na gospodarstvu. Kao osnovna prepreka implementaciji uzajamnog fonda navodi se manjak informacija o uzajamnom fondu.

Ključne riječi: stočarstvo, uzajamni fond, upravljanje rizikom

Uvod

Stočarska proizvodnja u Hrvatskoj je u padu. Broj goveda u 2022. prema podacima DZS-a je u padu u odnosu na 2021. godinu za 1,34 %. U 2022. ukupan broj goveda je preko 421 tisuću grla, dok je u 2021. broj grla bio preko 427 tisuća. Na stočarsku proizvodnju utječu razni rizici. Prema Chand i sur. (2018) rizici u stočarstvu su vezani uz sam uzgoj stoke (primjerice pojava bolesti, uginuća i loša kvaliteta hrane), zdravlje životinja, tržište, ali i rizici vezani uz politiku i promjene zakona. Uz proizvodni rizik, promjene cijena inputa na tržištu, ali i outputa predstavljaju rizik za stočare (Meuwissen i sur., 2001).

Poljoprivrednici mogu primjenjivati razne strategije, poljoprivredno osiguranje, diversifikaciju proizvodnje ili izvora prihoda, prikupljanje informacija, ugovornu proizvodnju i slično (Njavro i Čop, 2021). Također, bankoosiguranje može predstavljati važni eksterni instrument za upravljanje proizvodnim, financijskim i cjenovnim rizicima (Ivanović i Marković, 2018). Meuwissen i sur. (2001) važnim strategijama u stočarstvu navodi proizvodnju s niskim troškovima, ugovaranje osiguranja, primjena strogih zoohigijenskih pravila, a najmanje važnim smatraju *off-farm* posao ili posao koji nije vezan uz gospodarstvo, te terminske ugovore i opcije.

Europska komisija veliku pažnju posvećuje upravljanju rizikom. U prošlom programskom razdoblju kroz Europski fond za ruralni razvoj (EFRR) financirane su tri podmjere, poljoprivredno osiguranje, alat za stabilizaciju dohotka i uzajamni fond. Poljoprivredno osiguranje je u primjeni u svim članicama EU, dok je alat za stabilizaciju dohotka i uzajamni fond u primjeni u ponekoj zemlji EU.

U prošlosti se uglavnom diskutiralo o zajedničkim osiguravajućim fondovima koji su bili organizirani na regionalnom (mikro) nivou i odnosili su se na fondove gdje su se sami poljoprivrednici štitili od vremenskih nepogoda, a osnovni njihov nedostatak je bio da svi ili većina poljoprivrednih proizvođača pretrpe gubitke u isto vrijeme (Marković, 2013). S druge strane, uzajamni fond predstavlja program osiguranja udruženih poljoprivrednika koji omogućuje isplatu naknada za materijalne gubitke uzrokovane nepovoljnim klimatskim prilikama, bolestima životinja i biljaka, najezdom nametnika, okolišnim incidentom i za značajan pad u njihovom dohotku (Krišto i sur., 2020). Dodatno, prema Uredbi (EU) br. 1305/2013 Europskog parlamenta i Vijeća uzajamni fond se definira kao financijska potpora poljoprivredniku u slučaju gubitka proizvodnje od 30 %, kada ostvaruje naknadu do 70 % za gubitke uzrokovane nepovoljnim klimatskim prilikama ili izbijanjem bolesti životinja ili biljaka ili najezdom nametnika ili okolišnim incidentom, a koji utječu na prinose na gospodarstvu, prihode i dohodak (Bergevoet, 2017).

Primjeri uzajamnih fondova u stočarstvu i biljnoj proizvodnji vidljivi su u Austriji, Belgiji, Nizozemskoj, Njemačkoj, Italiji i Francuskoj (Meuwissen i sur., 2013; Bergevoet, 2017; Cordier, 2017).

Prednost uzajamnih fondova je ta da osiguravaju rizike koje nije moguće osigurati klasičnim poljoprivrednim osiguranjem, odnosno sistemske rizike (primjerice cijena), te je trenutno preko EFRR dostupna financijska potpora za njihovo osnivanje. Nadalje, utječu na smanjenje asimetričnih informacija i moralnog hazarda, dok se kao izazovi mogu navesti povjerenje i solidarnost među članovima fonda (Bergevoet, 2017).

Cilj rada je istražiti mišljenje stočara o uzajamnom fondu kao strategiji za upravljanje rizikom u stočarstvu.

Materijal i metode

Anketno ispitivanje provedeno je na prigodnom uzorku članova udruge za tov i uzgoj junadi Baby-Beef (u nastavku Udruga). Udruga Baby Beef čini 90 % sektora tovne junadi u Hrvatskoj.

Udruga broji oko 300 članova. Svaki proizvođač je telefonski kontaktiran kako bi se objasnilo istraživanje i zamolilo za sudjelovanje u istraživanju. Istraživač je svakog ispitanika upoznao sa značenjem i funkcioniranjem uzajamnog fonda. U konačnici, ispitivanje je provedeno na 10 % članova Udruge, nositelja gospodarstava u srpnju i kolovozu 2023.

Anketni upitnik obuhvaćao je pitanja vezana uz zastupljenost osiguranja životinja i biljaka, mišljenje i sklonost ispitanika prema uzajamnom fondu, te socio-demografska pitanja i pitanja o gospodarstvu.

Prikupljeni podaci obrađeni su primjenom deskriptivne statistike i linearne regresije, a primjenom statističkog programa STATA verzija 16.

Rezultati i rasprava

Opis uzorka

Ukupan broj ispitanika je 30 tovljača junadi. U uzorku prevladavaju muškarci (80 %), u dobnoj skupini od 40 do 49 godina (33 %). Najveći udio ispitanika u uzorku završio je srednju školu (43 %) i fakultetsko obrazovanje (37 %). U najvećem udjelu (30 %) ispitanici imaju od 10 do 50 grla na gospodarstvu, dok u uzorku nema uzgajivača s manje od 10 grla na gospodarstvu. Polovica ispitanika se bavi samo tovom junadi, dok druga polovica je s diverzificiranom proizvodnjom, odnosno tov junadi i uzgoj žitarica. Više podataka o opisu uzorka dostupno je u nastavku, u tablici 1.

Tablica 1. Opis uzorka

Naziv varijable		Frekvencija	Udjel (%)
Spol ispitanika	Muško	24	80
	Žensko	6	20
Dob ispitanika	Od 18 do 29 godina	1	3
	Od 30 do 39 godina	8	27
	Od 40 do 49 godina	10	33
	Od 50 do 59 godina	7	23
	Više od 60 godina	4	13
Razina obrazovanja	Osnovna škola	3	10
	Srednja stručna sprema	13	43
	Viša stručna sprema	3	10
	Visoka stručna sprema	11	37
Broj grla na gospodarstvu	10-50	9	30
	51-100	5	17
	101-250	2	7
	251-500	7	23
	više od 500	7	23

Osnovna djelatnost na gospodarstvu	Tov junadi	15	50
	Uzgoj žitarica	0	0
	Diverzificirana proizvodnja	15	50

Ispitanici u 50 % slučajeva navode kako su primijenili osiguranje životinja, točnije osiguranje kapaciteta. Ostala polovica ispitanika kao najčešće razloge zašto ne primjenjuju osiguranje životinja navode neisplativost ugovaranja osiguranja, visoka premija i mala naknada u slučaju gubitaka i nepovjerenje u osiguravajuće kuće.

Od polovice uzorka koje osigurava životinje, njih 40 % ugovorilo je osiguranje u 2023. godini, dok je 27 % ugovorilo osiguranje u 2022. i 27 % prije više od dvije godine, dok je zadnji put ugovorilo osiguranje životinja prije dvije godine, odnosno 2021. godine, njih 7 %.

Obzirom da se ispitanici bave tovnom junadi ili uzgojem žitarica i stočarstvom, većina ispitanika (50 %) osigurava žitarice na gospodarstvu. U najvećem udjelu osigurava se biljna proizvodnja od rizika tuče, oluje i udara groma.

Uzajamni fond

Nakon upoznavanja ispitanika sa uzajamnim fondom, upitalo se ispitanike smatraju li uzajamni fond dobrom strategijom zaštite od rizika. Više od dvije trećine ispitanika smatra uzajamni fond dobrom strategijom, dok samo 3 % ispitanika istu ne smatra važnom, dok 27 % ispitanika nije sigurno, što je prikazano u sljedećoj tablici (Tablica 2).

Tablica 2. Članstvo u uzajamnim fondovima je dobra strategija zaštite od rizika

	Frekvencija	Udjel (%)
Da	21	70
Ne	1	3
Nisam siguran(a)	8	27
Ukupno	30	100

Više od polovice ispitanika iskazuje srednju vjerojatnost članstva u uzajamnom fondu (Tablica 3). Oko 23 % ispitanika iskazuje kako je mala sklonost ispitanika prema članstvu, dok 17 % ispitanika iskazuje veliku i izrazito veliku vjerojatnost članstva u uzajamnom fondu.

Tablica 3. Vjerojatnost članstva u uzajamnom fondu

	Frekvencija	Udjel (%)
Nikakva	2	6,67
Mala	7	23,33
Srednja	16	53,33
Velika	4	13,33
Izrazito velika	1	3,33
Ukupno	30	100

Za potrebe linearne regresije definirana je zavisna varijabla (vjerojatnost članstva u uzajamnom fondu), te nezavisne varijable (dob, spol, razina obrazovanja, broj grla na gospodarstvu i osnovna djelatnost na gospodarstvu) (Tablica 4).

Tablica 4. Utjecaj izabranih varijabli na vjerojatnost članstva u uzajamnom fondu

Vjerojatnost članstva u uzajamnom fondu	Koeficijent	Std.Err.	t-test	Signifikantnost*
Spol	0,043	0,401	0,11	0,915
Dob	-0,039	0,177	-2,22	0,036
Razina obrazovanja	-0,156	0,216	-0,72	0,477
Broj grla na gospodarstvu	0,222	0,123	1,81	0,083
Osnovna djelatnost na gospodarstvu	-0,191	0,165	-1,16	0,257
Broj opservacija (N)	30			
R-squared	0,2598			

* $p < 0,05$

Sve varijable prikazane u tablici četiri su kategoričke. Jaka korelacija ($>0,5$) između nezavisnih varijabli nije zabilježena, te su sve nezavisne varijable uključene u model.

Dokazano je kako ne postoje statistički značajne razlike ($p > 0,05$) svih ispitanika u odnosu na spol, razina obrazovanja, broj grla na gospodarstvu i osnovnu djelatnost na gospodarstvu.

Regresija je pokazala kako je varijabla dob jedina statistički značajna varijabla koja utječe na vjerojatnost članstva u uzajamnom fondu. Varijabla dob se interpretira kako se povećanjem dobi ispitanika povećava vjerojatnost članstva u uzajamnom fondu. Kao razlog navedenom ističe se kako u uzorku prevladavaju ispitanici iznad 40 godina starosti.

Tablica 5. Prepreke pri implementaciji uzajamnog osiguranja kod tovljača jundi

	Frekvencija	Udjel (%)
Nedovoljno informacija	18	60
Nedostatak povjerenja	15	50
Visoki administrativni troškovi	10	33
Visina premije	8	27
Složenost osiguranja	7	23

Dodatno, istražilo se koje su prema mišljenju ispitanika prepreke kod implementacije uzajamnog fonda, a što je prikazano u tablici 5. Ispitanici su mogli izabrati više odgovora ili upisati vlastiti odgovor. Ispitanici ističu kako najviše utječe manjak informacija o uzajamnom fondu (60 %), nedostatak povjerenja (50 %), dok 23 % njih smatra kako je složenost osiguranja jedno od ograničenja.

Zaključak

U radu je prikazana sklonost tovljača junadi prema uzajamnom fondu. Ukupan broj članova Udruge koji je sudjelovao u istraživanju je 30 ispitanika. Rezultati istraživanja pokazuju kako je samo polovica tovljača junadi do sada primijenila osiguranje životinja, te kako pretežno osiguravaju biljnu proizvodnju, obzirom se većina ispitanika bavi tovnom junadi i uzgojem žitarica. Veliki udio ispitanika, čak njih 70 % smatra kako je uzajamni fond dobra strategija za upravljanje rizikom na gospodarstvu. Od socio-demografskih varijabli i pitanja o gospodarstvu, samo dob utječe na slaganje s članstvom u uzajamnom fondu. Osnovna prepreka implementacije uzajamnog fonda po mišljenju ispitanika je manjak informacija o uzajamnom fondu. Ograničenje ovog istraživanja ogleda se u malom uzorku, odnosno niskoj sklonosti članova Udruge u sudjelovanju u istraživanju. Dodana vrijednost rada je pojasniti i približiti uzajamni fond kao inovativnu strategiju za upravljanje rizikom u poljoprivredi, te njezine prednosti i izazove pri implementaciji uzajamnog fonda.

Napomena

Rad je rezultat diplomskog rada studentice Dijane Pejazić, obranjen na Sveučilištu u Zagrebu Agronomskom fakultetu, dana 22.9.2023. pod mentorstvom prof.dr.sc. Maria Njavre i neposredne voditeljice dr.sc. Tajane Čop.

Literatura

- Bergevoet R. (2017). Study on risk management in EU Agriculture. Annex 3 – Case study 3, Consequential losses due to sanitary risks in the livestock sector: from insurance to mutual funds, Ecorys, Wageningen Economic Research, European Commission, Brussels.
- Chand S., Narayan P., Chaudhary K.R. (2018). Sources of risks in livestock production and their management strategies in northern India. *Indian Journal of Animal Sciences*. 88: 612-619.
- Cordier J. (2017). Study on risk management in EU Agriculture. Annex 5 – Case study 5 Critical issues for the implementation of a mutual fund compensating for production loss under Article 38 of Reg. (EU) No. 1305/2013, Ecorys, Wageningen Economic Research, European Commission, Brussels.
- Državni zavod za statistiku (2023). Baza Poljoprivreda, lov, šumarstvo i ribarstvo. www.dzs.hr
- Ivanović S., i Marković T. (2018). Upravljanje investicijama u agrobiznisu, Univerzitet u Beogradu, Poljoprivredni fakultet, Beograd – Zemun, Srbija.
- Krišto J., Njavro M., Čop T. (2020). Mutual Insurance as a Risk Management Tool in the Agricultural Sector – The EU Experience and Lessons for Croatia (in Croatian). 28. Tradicionalno savjetovanje Ekonomska politika Hrvatske u 2021. Hrvatska poslije pandemije, str. 254-290
- Marković T. (2013). Vremenski derivati i upravljanje rizikom u poljoprivredi, Univerzitet u Novom Sadu, Poljoprivredni fakultet, Novi Sad, Srbija.
- Meuwissen M.P., Huirne R.B.M., Hardaker J.B. (2001). Risk and risk management: an empirical analysis of Dutch livestock farmers. *Livestock production science*. 69: 43-53.
- Meuwissen M.P.M., Assefa T.T., van Asseldonk M.A.P.M. (2013). Supporting Insurance in European Agriculture: Experience of Mutuals in the Netherlands. *EuroChoices*. 12: 10-15.
- Njavro M., i Čop T. (2021). Upravljanje rizikom u poljoprivredi. Fakultetski priručnik. Njavro Đ., Njavro M. (eds.), Zagreb, Hrvatska: Mate d.o.o.
- Official Journal of the European Union (2013). Council Regulation 1305/2013/EC of 13/12/2017 on support for rural development by the European Agricultural Fund for Rural Development (EAFRD) and repealing. Raspoloživo: <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32013R1305>.

Mutual fund - risk management strategy in livestock

Abstract

This paper aims to investigate farmers' opinion about mutual funds as an innovative risk management tool. The study was conducted among Baby Beef Association members. The survey included several sets of questions: agricultural insurance data; opinions about mutual funds; sociodemographic data and farm data. The data collected were analysed using descriptive statistics. In the sample, respondents are mostly male (80%), between 40 and 49 years old (33%) and graduated from high school (43%). Only 50% of the sample has already applied for livestock insurance. In addition, the results show that 70% of respondents believe that a mutual fund is a good strategy for farm risk management. Lack of information about the mutual fund is cited as the main barrier to mutual fund adoption.

Keywords: livestock, mutual fund, risk management

Usporedba korisnosti vanjskotrgovinskih podataka na temelju zemlje partnera i na temelju podrijetla – na primjeru poljoprivrednim i prehrambenim proizvodima u Sloveniji

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Sažetak

Pojava poljoprivrednih proizvoda ukrajinskog podrijetla izazvalo je burne reakcije na slovenskom tržištu poljoprivredno-prehrambenih proizvoda. Svrha ovog rada je analiza robnih tokova odabranih poljoprivredno-prehrambenih proizvoda između Slovenije i Ukrajine na temelju proširene baze podataka o vanjskotrgovinskoj razmjeni. Rezultati analize pokazuju da je kod kukuruza, pšenice i pšeničnog brašna dovoljna kontrola uvoza po zemlji trgovačkom partneru, dok je manjkava kod pilećeg mesa, gdje je uvozni tok vidljiv tek analizom prema zemlji podrijetla. Količine uvezenih proizvoda ukrajinskog podrijetla bile su premale da bi imale značajan izravni utjecaj na tržišne bilance na slovenskom tržištu. Zbog neobjašnjivog viška izvoza ukrajinske robe nad uvozom, otkrivene su i neke nedosljednosti u podacima, a time i potreba za dodatnim kontrolama kvalitete podataka.

Ključne riječi: vanjska trgovina, podrijetlo robe, poljoprivredno-prehrambeni proizvodi, Ukrajina, Slovenija

Uvod

Slovenija je oduvijek bila neto uvoznik poljoprivredno-prehrambenih proizvoda (Travnikar i Bele, 2022) i poljoprivrednih inputa, te je vrlo osjetljiva na promjene koje se dešavaju na globalnim tržištima. Poljoprivredno-prehrambeni sektor u Sloveniji snažno osjeća sve iznenadne promjene ili poremećaje na opskrbnim pravcima, a to se najviše ogleda u pritiscima na cijene i njihovoj volatilnosti (Bedrač i sur., 2023). Od 2020. godine poljoprivreda se ponovno susrela s brojnim globalnim pritiscima koji značajno utječu na brojne odluke proizvođača i kupaca, a time i na promijene tržišnih puteva. Ograničenja trgovanja i intenzivni cjenovni pritisci započeli su s izbijanjem epidemije Covid-19. Nakon kratkotrajnog smirivanja, s ratom u Ukrajini pritisci se nastavljaju u još jačem obliku (Bele i Travnikar, 2022).

Aktualni potresi na europskim tržištima vezani uz ukrajinsko-ruski sukob rezultat su uvođenja „pojaseva solidarnosti“ za transport ukrajinskih poljoprivrednih proizvoda kroz EU te ograničenja uvoza određenih poljoprivrednih proizvoda u pet europskih zemalja koje graniče s Ukrajinom (Commission Implementing Regulation (EU) 2023/903..., 2023). Prema Finance (2023a), ova politička, nedovoljno promišljena i nekoordinirana mjera je utjecala na značajno smanjenje cijena žitarica što je i bila želja Europske komisije u borbi protiv inflacije. Međutim, to je istovremeno izazvalo znatnu ekonomsku štetu brojnim poslovnim subjektima u zemljama središnjeg i istočnog dijela EU.

Prema medijskim izvješćima, neki poljoprivredni proizvodi ukrajinskog podrijetla pojavili su se i na slovenskom tržištu krajem 2022. i u 2023. godini. Na to su ukazali i predstavnici prehrambenih kompanija (Finance, 2023a, 2023b) koji su izrazili bojazan od prodora jeftinije robe i narušavanja tržišnih ravnoteža, posebice u slučaju žitarica, brašna i mesa peradi. Upravo u slučaju pilećeg mesa pokazalo se da službena statistika nije zabilježila uvoz mesa ukrajinskog podrijetla u Sloveniju.

Službena statistika prikuplja podatke o vanjskoj trgovini prema dva odvojena sustava i to Extrastat (SURS, 2023a) te

Intrastat (SURs, 2023b). Prva je statistika robne razmjene s trećim zemljama (nečlanicama EU), a druga je statistika robne razmjene između članica EU. Prema Ekstrastat metodologiji, uvoz uključuje sve fizičke i pravne osobe koje su podnijele carinsku prijavu i obuhvaća sav uvoz, dok Intrastat uključuje podatke onih izvještajnih jedinica (tvrtki) koje su u prethodnoj kalendarskoj godini premašile vrijednosne pragove utvrđene Intrastat metodologijom. Izvještajne jedinice u oba sustava prijavljuju primitak i otpremu robe prema šiframa kombinirane nomenklature koju propisuje Europska komisija (Commission Implementing Regulation (EU) 2022/1998 ..., 2022).

Za robu koja na carinsko područje EU stiže iz trećih zemalja, carinska deklaracija se podnosi odmah u državi članici u koju ulazi ili se nakon obavljenog proвозnog postupka carinski postupak provodi naknadno u jednoj od drugih država članica EU, neovisno o tome koja država članica je krajnji primatelj robe (FURS, 2023). Podaci o carinskim deklaracijama prikazuju se u Ekstrastat bazi podataka pri čemu se kao zemlja trgovinski partner podrazumijeva i zemlja podrijetla. Pod podrijetlom robe smatra se zemlja u kojoj je roba u potpunosti proizvedena, odnosno u kojoj je obavljena posljednja važna faza proizvodnog procesa, kao što je prerada ili obrada.

U sustavu Intrastat (SURs, 2023b), u slučaju trgovine između država članica EU, kao trgovinski partner za uvoz pojavljuje se zemlja iz koje je roba otpremljena, a nije nužno u njoj i proizvedena. Od 2022. godine u Intrastat bazi su i podaci o podrijetlu robe, ali ti podaci nisu javno dostupni. S obzirom da javno dostupna baza vanjskotrgovinskih podataka pokazuje tijek robe isključivo preko trgovačkih partnera, ostaje skriveno podrijetlo robe iz trećih zemalja, koja je u Sloveniju uvezena posredno preko jedne od država članica EU.

Zbog toga je za cjelovitiji prikaz robnih tokova i objašnjenje tržišnih uvjeta nužan detaljniji pregled vanjskotrgovinskih podataka. Bele i Travnikar (2022), koji su se u svom istraživanju fokusirali na procjenu ranjivosti slovenskog prehrambenog sustava kroz prehrambene bilance i trgovinu s inozemstvom, također su bili suočeni s dilemom je li u intenzivnoj vanjskoj trgovini, podaci temeljeni na partnerske zemlje, daju pravu, sveobuhvatnu sliku.

Na analizu proširene vanjskotrgovinske baze podataka poticaj nam je bio interes javnosti za uvoz poljoprivredno-prehrambenih proizvoda iz Ukrajine. Prikazanim nalazima i rezultatima željeli smo skrenuti pozornost na važnost, točnog i sveobuhvatnog pregleda baza podataka, kako bi se stvorila stvarna slika o tome što se događa na slovenskom tržištu s kukuruzom, pšenicom, pšeničnim brašnom i pilećim mesom.

Materijal i metode

Osnova za istraživanje bili su podaci o vanjskotrgovinskoj razmjeni dobiveni od Zavoda za statistiku Republike Slovenije (SURs). To su kvantitativni podaci o uvozu i izvozu podrijetlom iz Ukrajine, i to za kukuruz, pšenicu, pšenično brašno i pileće meso na razini 4- ili 6-znamenaste šifre kombinirane nomenklature Europske unije (Commission Implementing Regulation (EU) 2022/1998 ..., 2022). U analizu smo uključili godišnje podatke za razdoblje od 2012. od 2021. godine kao i mjesečne podatke za razdoblje siječanj 2022. do uključivo srpanj 2023. godine. Niz mjesečnih podataka detaljniji je od javno dostupnih podataka jer osim podataka po trgovinskim partnerima sadrži i podatke o podrijetlu robe, a od 2022. godine također i u slučaju uključivanja iz Intrastat sustav.

Podatke o uvozu analiziranih proizvoda u 2022. i 2023. godini podijelili smo u dvije usporedne skupine. Prva skupina sadrži podatke prema Ukrajini kao trgovinskom partneru, dok druga skupina uključuje podatke prema Ukrajini kao zemlji podrijetla robe. Kako bismo dobili širu sliku dostupnosti analiziranih proizvoda ukrajinskog podrijetla na slovenskom tržištu, dodatno smo analizirali i podatke o izvozu analiziranih proizvoda.

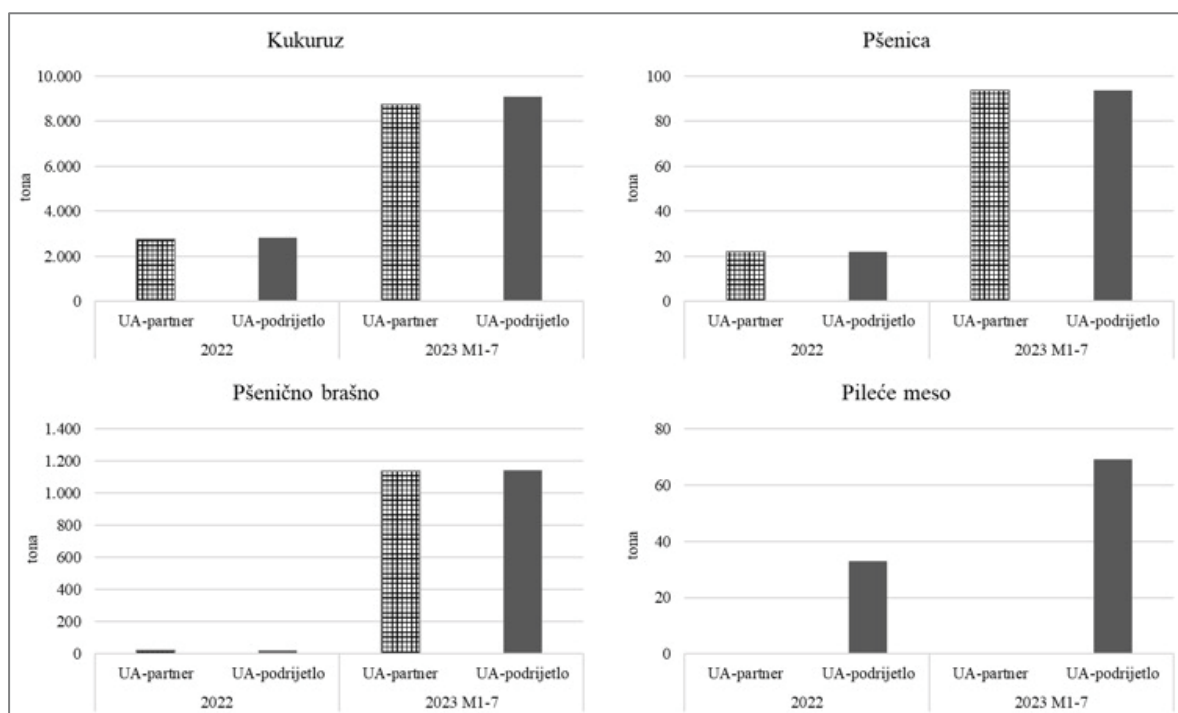
Podaci su prikladno podijeljeni u skupine i obrađeni pomoću alata MS Access, te analizirani pomoću zaokretnih tablica u alatu MS Excel. Rezultati analiza za pojedine proizvode prikazani su u grafičkom obliku.

Rezultati i rasprava

Slovenija tradicionalno nije uvoznik žitarica, mlinskih proizvoda i pilećeg mesa podrijetlom iz Ukrajine. Analiza podataka o vanjskotrgovinskoj razmjeni za dulje vremensko razdoblje (2012.-2021.) pokazala je da uvoza, uz izuzetak zanemarivih količina kukuruza i pšenice, u nekim godinama uopće nije bilo. Ipak, analiza mjesečnih podataka za razdoblje nakon 2021. ukazuje na pojavu uvoza poljoprivrednih proizvoda iz Ukrajine.

Na slici 1 prikazan je kumulativni uvoz kukuruza, pšenice, pšeničnog brašna i pilećeg mesa u Sloveniju u 2022. i u prvih sedam mjeseci 2023. godine. Prikazane su količine uvoza u kojima je Ukrajina bila trgovinski partner (izravni uvoz) i količine uvoza u kojoj je Ukrajina evidentirana kao zemlja podrijetla (također pokriva neizravni uvoz preko zemalja članica EU).

U razdoblju siječanj 2022. - srpanj 2023. najživlji uvozni tokovi zabilježeni su za kukuruz. To se događa od sredine 2022. Ipak, osjetan porast uvoza bilježimo tek početkom 2023. godine, a kumulativno je u 7 mjeseci ove godine uvezeno oko 9.000 tona kukuruza, što predstavlja oko 8 % ukupnog uvoza kukuruza u istom razdoblju.



Slika 1. Kumulativni uvoz kukuruza, pšenice, pšeničnog brašna i pilećeg mesa iz Ukrajine (UA) po uvoznom partneru i podrijetlu roba

Napomena: privremeni podaci za 2023.

Izvor: SURS, izračuni KIS

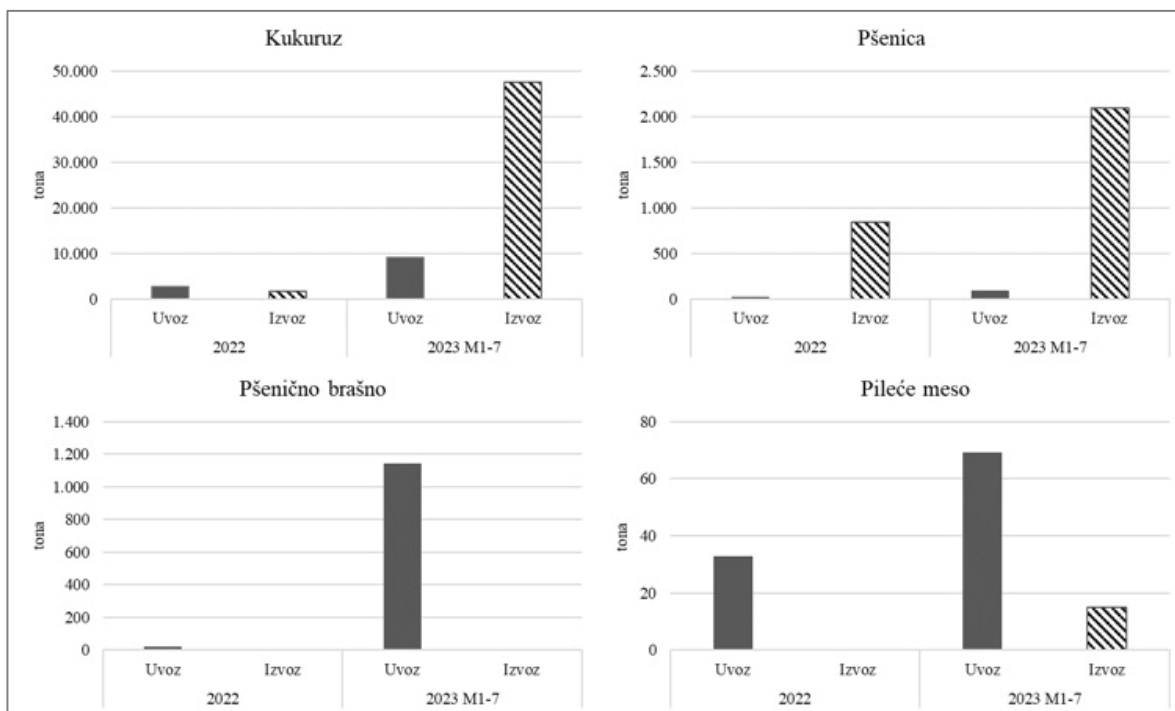
Uz iznimku manjih količina, uvoz kukuruza u Sloveniju evidentiran je putem carinskih deklaracija u sustavu Ekstrastat, što znači da je najveći dio kukuruza uvezen izravno iz Ukrajine kao zemlje partnera. U tom slučaju, za analizu uvoznih tokova dovoljni su podaci o vanjskoj trgovini po zemlji partneru i nisu potrebni dodatni podaci o podrijetlu robe.

U slučaju pšenice, uvoz iz Ukrajine zabilježen je samo u dva uzastopna mjeseca analiziranog razdoblja, tijekom kojih je ukupno uvezeno oko 120 tona. Uvoz pšeničnog brašna u većem je opsegu zabilježen tek od veljače 2023., no uvezene mjesečne količine bile su male i kretale su se između 100 i 300 tona. Kumulativno je u 7 mjeseci 2023. godine uvezeno oko 1.100 tona brašna, što predstavlja 6 % ukupnog uvoza pšeničnog brašna u istom razdoblju. U analiziranom razdoblju je kumulativno najmanje uvezeno pilećeg mesa i to oko 100 tona neto težine.

Slično kukuruzu, pšenicom i pšeničnim brašnom trgovalo se izravno između Ukrajine i Slovenije. Kod pilećeg mesa otkrivena je razlika u kumulativnom uvozu po zemlji partneru i zemlji podrijetla. Cjelokupni uvoz mesa odvijao se posredno preko trgovačkih partnera iz Nizozemske i Slovačke te je evidentiran u sustavu Intrastat.

Rezultati analize pokazuju da je kod kukuruza, pšenice i pšeničnog brašna dovoljna kontrola uvoza po zemlji trgovačkom partneru, dok je manjkava kod pilećeg mesa, gdje je uvozni tok bio vidljiv tek analizom detaljnije baze vanjskotrgovinskih podataka.

Potvrdivši da proširena baza podataka daje jasniju sliku uvoznih tokova, analizirani su i podaci na izvoznjoj strani. Slika 2 prikazuje kumulativni uvoz i izvoz kukuruza, pšenice, pšeničnog brašna i pilećeg mesa podrijetlom iz Ukrajine u razdoblju od 2022. do 2023. godine.



Slika 2. Kumulativni uvoz i izvoz kukuruza, pšenice, pšeničnog brašna i pilećeg mesa iz Ukrajine (UA) po podrijetlu roba

Napomena: privremeni podaci za 2023.

Izvor: SURS, izračuni KIS

Rezultati pokazuju da je tijekom analiziranog razdoblja pšenično brašno podrijetlom iz Ukrajine u potpunosti utrošeno na slovenskom tržištu. Slično je bilo i za pileće meso, gdje je u 2023. godini ipak zabilježen manji izvoz.

Međutim, rezultati za kukuruz i pšenicu ukazuju na neke nedostatke u kvaliteti podataka. Za oba proizvoda ukrajinskog podrijetla izvoz je u prvih sedam mjeseci 2023. uveliko premašio uvoz (u slučaju pšenice u 2022. godini). Kod kukuruza kumulativni izvoz iznosio je 47.500 tona i bio je za 38.500 tona veći od uvoza. Kod pšenice se bilježi izvoz od 2.000 tona, dok je uvoznosio svega 100 tona. Višak uvoza nad izvozom moguć je u kratkom roku, sve dok se uvezeni proizvodi skladište i kasnije izvoze. To se ne odnosi na analizirane proizvode, budući da u dužim vremenskim serijama mjesečnih podataka izvoz uveliko premašuje uvoz.

Nelogičnosti u podacima proizlaze iz činjenice da se carinske formalnosti za robu uvezenu iz trećih zemalja mogu uređivati u državi članici neovisno o tome je li ona namijenjena potrošnji u istoj zemlji ili je samo u tranzitu. Primjerice, za uvoz kukuruza i pšenice ukrajinskog podrijetla, carinski postupci obavljaju se u Sloveniji, a zatim se roba izvozi u Italiju, koja je glavni trgovinski partner. U ovom slučaju, količine izvoza jednake su količinama uvoza. Ukoliko dio robe ostaje za potrošnju na domaćem tržištu, izvozne količine su manje od uvoznih, ali nikako veće, što dovodi u pitanje vjerodostojnost službenih podataka o robnoj razmjeni.

Zaključak

Na temelju analize vanjskotrgovinskih podataka za četiri važna poljoprivredno-prehrambena proizvoda možemo iznijeti neke glavne nalaze i preporuke. Rezultati ukazuju na pojavu uvoza proizvoda ukrajinskog podrijetla u Sloveniju u razdoblju nakon početka rusko-ukrajinskog rata te povećan opseg uvoza nakon početka 2023. godine. Uvoz kukuruza, pšenice i pšeničnog brašna iz Ukrajine je uglavnom bio izravan, ali se pileće meso ukrajinskog podrijetla uvozilo posredno preko zemalja članica EU. Iako je samo u slučaju pilećeg mesa prisutnost uvoza iz Ukrajine otkrivena isključivo temeljem podacima upita o podrijetlu, ti se podaci ne smiju zanemariti u detaljnim analizama. Ovo je dokaz da samo prošireni vanjskotrgovinski podaci, koji daju informacije o trgovinskim partnerima i podrijetlu, pružaju cjelovit uvid u zbivanja na tržištima.

Izuzev kukuruza, zanemarive su uvezene količine pšenice, pšeničnog brašna i pilećeg mesa. Dodatne analize izvoza pokazale su da su količine proizvoda namijenjene slovenskom tržištu premale da bi imale veći izravni utjecaj na maloprodajne cijene. Naravno, to ne znači izostanak utjecaja na formiranje veleprodajnih cijena na domaćem i širem regionalnom tržištu. Utjecaj na kretanje cijena na slovenskom tržištu zbog izvoza žitarica i brašna iz Ukrajine u članice EU s kojima Slovenija ima tradicionalno jake poslovne veze svakako postoji, budući da se pad cijena kukuruza i pšenice podudara sa pojavom trgovine između Ukrajine i Slovenije te Ukrajine i susjednih zemalja.

Kao važan nalaz pokazala se i potreba provjere podataka o uvezenom kukuruzu i pšenici. Za oba proizvoda ukrajinskog podrijetla izvoz je znatno premašio uvoz, što ukazuje na nedosljednost u podacima. Analize vanjskotrgovinskih podataka vrlo su važne u teškim tržišnim uvjetima, ali i kao temelj za formuliranje tržišnih i cjenovnih mjera, te je ključno da su podaci dovoljno pouzdani za kvalitetne tržišne analize. U našem istraživanju službeni vanjskotrgovinski podaci pokazali su se manje pouzdanim, stoga administratorima vanjskotrgovinskih baza podataka preporučujemo dodatne kontrole podataka.

Literatura

- Bedrač M., Bele S., Brečko J., Hiti Dvoršak A., Kožar M., Ložar L., Moljk B., Travnikar T., Zagorc B., Očko J., Oblak O., Jagodic A., Kuhar A., Ščap Š., Žovlje S. Poročilo o stanju kmetijstva, živilstva, gozdarstva in ribištva : 2021. Ljubljana: Kmetijski inštitut Slovenije: Ministrstvo za kmetijstvo, gozdarstvo in prehrano, 2023. 264 str. Raspoloživo: https://www.kis.si/f/docs/Porocila_o_stanju_v_kmetijstvu/ZP_2021_splosno__priloge_6.9.2022.pdf
- Bele S., and Travnikar T. (2022). Slovenian agri-food trade with Ukraine and Russia. In: Societal changes and their implications on agri-food systems and Rural Areas (eds. Tomšič M., Novak A., Travnikar T., Juvančič L.), Joint Conference DAES and ÖGA, 22 – 23 September, Ljubljana, Slovenija: 13-14. Raspoloživo: <https://www.daes.si/storage/category/FuE9aHblO8pg8MSrObsxvwQLMI6TKaT3kwXkf47V.pdf>
- Commission Implementing Regulation (EU) 2022/1998 of 20 September 2022 amending Annex I to Council Regulation (EEC) No 2658/87 on the tariff and statistical nomenclature and on the Common Customs Tariff. (2022). Official Journal of the European Union, L 282. Raspoloživo: <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ%3AL%3A2022%3A282%3ATOC>
- Commission Implementing Regulation (EU) 2023/903 of 2 May 2023 introducing preventive measures concerning certain products originating in Ukraine. Official Journal of the European Union, L 114I. Raspoloživo: <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=OJ:L:2023:114I:TOC>
- Finance (2023a). Zakaj nekatere članice EU kar naenkrat prepovedujejo uvoz ukrajinskih žit. Ljubljana, Slovenija. Raspoloživo: <https://www.finance.si/finance/zakaj-nekatere-clanice-eu-kar-naenkrat-prepovedujejo-uvoz-ukrajinskih-zit/a/9012282>
- Finance (2023b). V primeru vdora ukrajinske pšenice na naš trg bomo pred težko dilemo, kaj storiti. Ljubljana, Slovenija. Raspoloživo: <https://agrobiznis.finance.si/agro-podjetnik/v-primeru-vdora-ukrajinske-psenice-na-nas-trg-bomo-pred-tezko-dilemo-kaj-storiti/a/9014048>
- FURS (2023). Uvozni postopki - podrobnejši opis. Finančna uprava Republike Slovenije, Ljubljana, Slovenija. Raspoloživo: https://www.fu.gov.si/carina/podrocja/uvoz_bлага?type=%253D03c749e18cc6336f120fa77b03603655%253Ded54bb1662cf86839384603c4c178a23%253D0ed72950819f475b8217685a8b08a06f#c4725
- SURS (2023a). Izvoz in uvoz blaga. Metodološko pojasnilo. Ljubljana, Slovenija: Statistični Urad Republike Slovenije. Raspoloživo: <https://www.stat.si/statweb/File/DocSysFile/8287>
- SURS (2023b). Intrastat navodila 2023. Ljubljana, Slovenija: Statistični Urad Republike Slovenije. Raspoloživo: https://stat.si/StatWeb/File/DocSysFile/12210/INTRASTATNAVODILA_2023.pdf

Travnikar T., and Bele S. (2022). Vulnerability of the Slovenian food system in connection with the war in Ukraine = Ranljivost slovenskega prehranskega sistema v povezavi z vojno v Ukrajini. *Journal of Central European Agriculture*. 23: 921-934.

UMAR (2023). Analiza zunanjetrgovinske menjave z Rusijo od začetka vojne v Ukrajini. *Kratke analize*. Ljubljana, Slovenija. Raspoloživo: https://www.umar.gov.si/fileadmin/user_upload/publikacije/kratke_analize/2023_9_Hribernik/Analiza_zunanjetrgovinske_menjave_z_Rusijo_od_zacetka_vojne_v_Ukrajini.docx

Comparison of the usefulness of foreign trade data based on partner country and on the country of origin - examples of agri-food products in Slovenia

Abstract

The appearance of agricultural products of Ukrainian origin triggered a response on the Slovenian agri-food market. The purpose of this paper is to analyse the trade flows of selected agri-food products between Slovenia and Ukraine based on an expanded database. The results of the analysis show that the use of foreign trade data based on the trading partner is sufficient in the case of the import of maize, wheat, and wheat flour, while the import of chicken meat was detected only with the analysis based on the origin of goods. Despite the perceived import flow of goods, the quantities of products of Ukrainian origin were too small to have a significant direct impact Slovenian market. Due to the inexplicable excess of exports of Ukrainian goods over imports, some inconsistencies in the data were discovered, and thus the need for additional data quality controls.

Keywords: foreign trade, origin of goods, agri-food products, Ukraine, Slovenia

Classification and financial analysis of climate change adaptation measures in Slovenian agriculture

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Abstract

The classifications developed so far for monitoring agricultural budgets are not designed to monitor adaptation to climate change. Therefore, we have developed a methodological framework that matches climate adaptation measures with the commonly used OECD methodology for monitoring agricultural policies. In Slovenia, the total budget expenditure on climate adaptation measures in agriculture over twenty years (2003-2022) amounted to EUR 517 million which is about 7% of the total budget expenditure on agriculture. The approach presented in the paper is a very good start for the development of a standardised classification of climate change adaptation in agriculture which can allow for cross-country analysis and comparability over time. These analyses are important to understand the relationship between the financial contribution of climate action in agriculture and the climate targets set.

Keywords: agricultural policy, agricultural budget, climate change adaptation measures

Introduction

In order to better understand the developments of agricultural policies it is necessary to monitor how and in what form the agricultural sector is supported. One of the cross-country approaches is the OECD classification — the Producer and Consumer Support Estimates and other related indicators (OECD PSE manual, 2016). This OECD approach is used in 54 countries, including the 38 OECD countries, the five non-OECD EU member states and 11 emerging economies (OECD, 2023). Rising impacts of climate change underscore the necessity of adaptation and reform of policies. Agricultural policies urgently need to be reformed to meet the challenge of providing adequate, affordable, safe and nutritious food for a growing world population (OECD, 2023). From this perspective, it is necessary to understand and monitor public funding for agricultural adaptation to climate change. A detailed budget analysis can help to understand the distribution of funding for climate adaptation measures and check whether the distribution corresponds to the priorities, climate targets and vulnerability of the individual agricultural sectors. A special report by the European Court of Auditors (ECA, 2022) states that 26% of all EU Common Agricultural Policy funds were spent on climate action in the period 2014-2020. However, the researchers found that reported EU spending was not always relevant to climate action and that climate reporting was generally overstated. It was recommended to improve climate reporting and link the contribution of the EU budget to its climate targets.

In reviewing the literature, we were surprised to find that there is no general (budget) classification of climate adaptation measures in agriculture, which is absolutely necessary for performing various data analyses, presenting them in longer time series and even for cross-country analyses (Erjavec et al., 2021; Volk et al., 2012; OECD, 2023). While overall budgetary support in agriculture is relatively well analysed and comparable across countries (distribution of funds to support producers, support for general services in agriculture, payments for natural disasters, etc.), little is known about the financial analysis of agricultural climate adaptation measures.

The main objective of our research was to link agricultural climate adaptation measures with budget expenditure in Slovenia. For this purpose, we developed a classification of climate adaptation in agriculture and corresponding classification keys, which were attached to the already developed and commonly used OECD methodology on budgetary indicators (OECD PSE manual, 2016).

Material and methods

The starting point for the classification of climate adaptation measures was taken from two research reports (Volk et al., 2012; EEA Climate Change Adaptation, 2019), which were expanded and slightly updated for the needs of the financial assessment. The database on the Slovenian agricultural budget, which is annually prepared by the Agricultural Institute of Slovenia for OECD reporting (estimation of support to producers) and for national reporting (estimation of total support to agriculture), was taken into account. Detailed information on payments for this database is obtained annually from the Ministry of Agriculture, Forestry and Food and the Agency for Agricultural Markets and Rural Development. In this way, we have financially evaluated all measures in Slovenian agriculture that have directly or indirectly contributed to climate change adaptation since 1992. Due to limited space in the paper, the analysis is only presented for the last twenty years (period 2003-2022). All relevant climate change adaptation measures (CCA) are categorised into six basic groups:

- CCA1 (adaptation of agricultural production and agrotechnical practises)
- CCA2 (irrigation and water management)
- CCA3 (physical protection against extreme weather events)
- CCA4 (protection against pests and diseases)
- CCA5 (income protection by reducing economic risks)
- CCA6 (general services, advisory services and co-operation).

Measures that are directly related to adaptation to climate change and ensure the protection of agricultural production and yields (preventive measures) are categorised in groups CCA1 to CCA4. Group CCA5 stands for the protection of producers' income (curative measures) and comprises the following subgroups of measures: crop and livestock insurance (CCA51), compensation for producers in the event of natural disasters (CCA52), payments for agricultural diversification (CCA53) and other income support for producers (CCA54; other non-climate-related PSE OECD payments). Group CCA6 includes general climate adaptation services (e. g. plant breeding, testing of animal breeds) and other climate-related activities (e. g. advisory services, co-operation).

The measures are divided into many subgroups, that are not presented in this paper. We only present the main groups of measures (CCA1, CCA2, CCA2, CCA4, CCA6). An exception is group CCA5, in which we only present the subgroups CCA51 and CCA52 (we have excluded other non-climate-related income support for producers). In addition, we have added defining keys to each individual measure, such as the associated groups/subgroups, the basic purpose of the measure (preventive/curative, etc.), which climate factor the measure is designed for (drought, frost, hail, etc.), which has enabled various analyses to be carried out over the years.

Results and discussion

In Slovenia, a total of EUR 6907 million, or an average of EUR 345 million per year was disbursed for agriculture over twenty years (2003-2022). This includes all measures financed from the European and national budgets. Of this, 7.5 % was paid out for climate adaptation measures in agriculture (totaling EUR 517 million in the period 2003-2022, or an average of EUR 36 million per year). Compared to the two decades, there was a slight increase of 1 % (from 7.0 % in the period 2003-2012 to 7.9 % in the period 2013-2022).

It can be said that the financing of climate adaptation measures is increasing (Figure 1), while at the same time, other support for agriculture is also increasing. Thus, the share of climate adaptation measures compared to all measures is similar (about 7%). It is interesting to note that this share is much lower than stated by the European Commission (26% at the EU CAP level, taking into account mitigation and adaptation measures), but the European Court of Auditors states that this share is inflated and the actual share of funding for climate measures in agriculture is lower (ECA, 2022). We would like to emphasise at this point that we have only considered agricultural measures or individual requirements within measures that contribute to climate change adaptation in the analysis. If climate change mitigation measures were included, this percentage would be somewhat higher.

Table 1. Overview of payments for climate adaptation measures in Slovenia; 2003-2022

	Payments (mill EUR)		Struct. (%)
	2003-2022	20-year average	
CCA1: Adaptation of agricultural production/agrotechnical practises	211.8	10.6	41
CCA2: Irrigation and water management	38.7	1.9	7
CCA3: Protection against extreme weather events	49.1	2.5	9
CCA4: Protection against pests and diseases	6.9	0.3	1
CCA6: General services, advisory services, co-operation on CCA	7.9	0.4	2
Preventive measures	314.4	26.2	61
CCA51: National co-funding crop and livestock insurance premiums	82.5	4.1	16
CCA52: Compensation for producers - natural disasters	120.1	6.0	23
Curative or incom protection measures (CCA51+CCA52)	202.6	10.1	39
TOTAL CCA measures	517.1	36.3	100
Total support to agriculture (EU + national funding)	6906.6	345.3	

In the following analysis, we have focussed only on groups that contribute to the adaptation of agricultural production to climate change (so-called preventive measures) and on measures that aim to secure income in the event of natural disasters and yield losses (so-called curative measures). EUR 314.4 million or EUR 26.2 million/year was paid out for the first group of adaptation measures, which corresponds to 61% of all CCA measures (Table 1, Figure 1). The rest of CCA funds (39%) is earmarked for curative measures in the event of disasters, of which 23% is for compensation to farmers for natural disasters and 16% for insurance premiums (subsidizing insurance premiums). On average, EUR 10 million are paid out for these measures, but the funds for compensation for natural disasters fluctuate significantly each year as they depend on the damage events in a given year. As a result, total disbursements for CCA measures also fluctuate, with a significant increase since 2018 (Figure 1). In the period 2003-2017, an average of EUR 22.5 million per year was disbursed for CCA measures; in the period 2018-2022, an average of EUR 35.8 million was disbursed, which is almost 60 % more funding. Funding has increased in all CAA groups, but most notably in group CCA3 (measures to protect against extreme weather events), where investment in animal housing and investment in crop production in protected areas (greenhouses) have increased significantly (EUR 1.1 million/year in the period 2003-2017, EUR 6.4 million/year in the period 2018-2022).

Most of the funds are earmarked for measures to adapt agricultural production and agrotechnical practices (CCA1), with an average of EUR 10.6 million per year (in the period 2003-2017: EUR 9.4 million/year, in the period 2018-2022: EUR 14.2 million/year). This group includes measures such as the use, management, and care of the soil (e.g. crop rotation, greening, reducing erosion, etc.), breeding and selection of suitable plant species and varieties, restoration of permanent orchards with more resistant varieties, selection of animals and optimization of animal husbandry, etc.

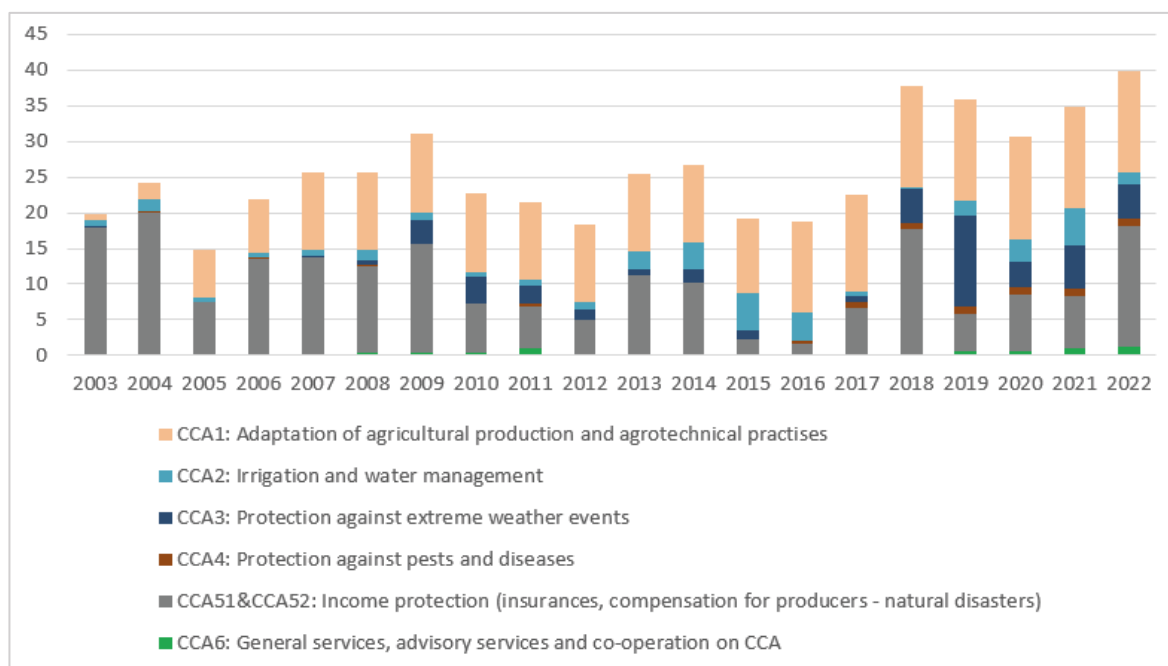


Figure 1. Budgetary payments for climate adaptation measures (mill EUR); 2003-2022

This is followed by measures to protect farmers' income (CCA51&CCA52, together averaging EUR 10.1 million/year). With these measures, the dilemma often arises as to whether these measures can be considered adaptation measures. According to various researchers and experts (IPPC, 2022), this group of measures is very important as it protects agricultural income in the event of crop failure (insurance and compensation); but also from the point of view that full adaptation of agriculture to climate change will not be possible. Other income payments (not explicitly related to climate adaptation) are also important for the longer-term maintenance of agricultural production, as they enable the longer-term stabilisation of agricultural income. If the mechanisms for income protection and risk management in agriculture (the entire CCA5 group) were not implemented, the number of agricultural producers (local and global) would fall sharply, resulting in a drastic decline in food production.

Significantly less is earmarked for other groups. For example, EUR 1.9 million per year was disbursed for CCA2 irrigation systems (adaptations, new investments, irrigation equipment, etc.) over the entire period analysed (in the period 2003-2017: EUR 1.7 million/year, in the period 2018-2022: EUR 2.5 million/year). Given the importance of water-related measures (especially given the fact that drought is a significant problem in Slovenia), we would have expected the funding of such measures to be prioritised more.

In the same way, we have also analysed these groups of measures according to the target of the climate event or according to which climate impact a particular measure is aimed at (Figure 2). Most of the budget is earmarked for measures to combat droughts (in the period 2003-2022: approx. 61% or EUR 15.9 million/year). This is followed by measures that target multiple climate factors and cannot be attributed to a single climate impact; on average over the period 2003-2022, these measures account for around 19% of payments or EUR 4.9 million/year. With a share of 6% or EUR 1.6 million of payments per year, they are followed by a group of measures aimed at hail and heat stress in animals. Around 3% of all payments, i.e. just under EUR 1 million per year, are paid for measures to combat frost and diseases. The rest, with a small percentage, are compensation in beekeeping due to unfavourable weather conditions (0.6%) and compensation due to flooding in agriculture (0.4%).

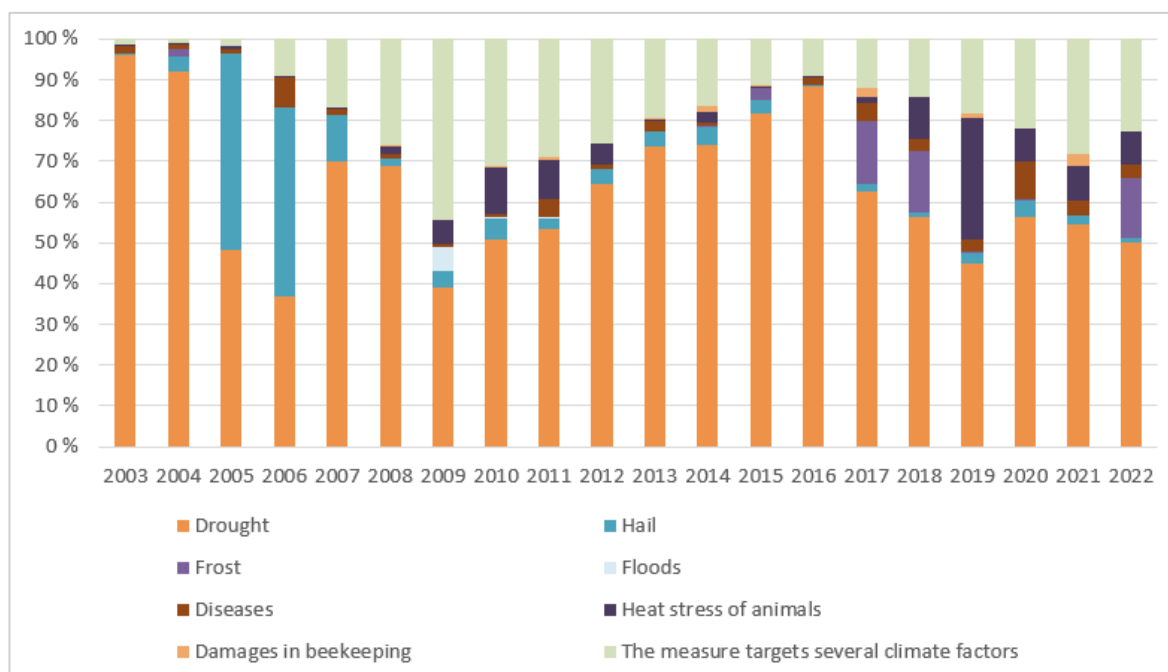


Figure 2. Structure of payments for climate adaptation measures, by target (%); 2003-2022

The structure of reported natural disasters in Slovenia is dominated by drought (58% of all compensation payments in the period 2003-2022), followed by hail (18%) and frost (13%). Since compensation for farmers after natural disasters (CCA52) is also included in the analysis, it is to be expected that most measures are targeted at drought. However even if we consider only the preventive measures, most of the measures are also aimed at drought (irrigation, soil moisture conservation measures, etc.), followed by measures that targets several climatic factors (plant breeding, greenhouse investments, etc.).

Conclusions

The European Court of Auditors (ECA, 2022) recommends obtaining scientific evidence for the climate contribution of EU agricultural policy. Monitoring budget expenditure on climate action is essential to increase the knowledge base for successful adaptation to climate change in agriculture. To this end, we have created a classification of climate change adaptation measures in agriculture (CCA measure classification) that is universal and can also be used by other countries when monitoring these measures. We have aligned the different climate identification keys from the CCA classification with the existing OECD classification for monitoring agricultural budgets. In this way, we analysed the financing of climate adaptation measures in agriculture in Slovenia since 1992.

In Slovenia, total budget expenditure on measures directly or indirectly related to climate change adaptation in agriculture over a twenty-year period (2003-2022) amounted to EUR 517 million, or an average of EUR 36 million per year, which is about 7% of total budget expenditure on agriculture. Over the years, a positive trend of increasing payments for all groups of climate adaptation measures can be observed, but their share of total funding for agriculture remains fairly constant (between 7% and 8%) as overall spending on agriculture also increases.

We estimate that the classification of climate change adaptation (CCA) measures presented here is a very good start for the development of a standardised classification that can allow monitoring of adaptation measures in agriculture across countries over time, ensure comparability between years and also easily update the database in the following years. For future contribution, it is recommended to extend the classification to include measures that contribute to climate change mitigation. This would allow a full financial assessment of the contribution of all climate actions in agriculture, which could be better linked to the defined climate and environmental targets.

References

- ECA. (2022). Climate spending in the 2014-2020 EU budget - Not as high as reported, Special report 09/2022. Luxembourg, European Court of Auditors. Available from: https://www.eca.europa.eu/lists/ecadocuments/sr22_09/sr_climate-mainstreaming_en.pdf
- EEA Climate Change Adaptation. (2019). Climate change adaptation in the agriculture sector in Europe. Copenhagen, Denmark: Publication Office, European Environment Agency. Available from: <https://euroseeds.eu/app/uploads/2019/09/Climate-change-adaptation-in-the-agriculture-sector-in-Europe.pdf>
- Erjavec E., Volk T., Rednak M., Ciaian P., Lazdinis M. (2021). Agricultural policies and European Union accession processes in the Western Balkans: aspirations versus reality. *Eurasian Geography and Economics*. 62: 46-75.
- IPCC. (2022). AR6 Climate Change 2022: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC). Cambridge, UK and New York, NY, USA: Cambridge University Press. Available from: <https://www.ipcc.ch/reports/>
- OECD PSE manual. (2016). OECD's producer support estimate and related indicators of agricultural support. Concepts, Calculations, Interpretation and Use (The PSE Manual). Paris, France: OECD Publishing. Available from: <https://www.oecd.org/agriculture/topics/agricultural-policy-monitoring-and-evaluation/documents/producer-support-estimates-manual.pdf>
- OECD. (2023). Agricultural Policy Monitoring and Evaluation 2023: Adapting Agriculture to Climate Change. Paris, France: OECD Publishing. Available from: https://www.oecd-ilibrary.org/agriculture-and-food/agricultural-policy-monitoring-and-evaluation-2023_b14de474-en
- Volk T., Rednak M., Erjavec E. (2012). Presoja ukrepov slovenske kmetijske politike z vidika podnebnih sprememb. *IB revija: za strokovna in metodološka vprašanja gospodarskega, prostorskega in socialnega razvoja Slovenije*. letn. 46: 75-84. Available from: <http://www.dlib.si/details/URN:NBN:SI:doc-U4RIAUNL>.

Ekonomska opravdanost primjene konzervacijske obrade tla u proizvodnji soje

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Sažetak

Soja je značajan izvor bjelančevina i ulja u ishrani ljudi, ali i proizvodnji hrane za stoku. Osim zrna, proizvodni značaj soje je i u obogaćivanju tla dušikom te se priznaje kao ekološki značajna površina u Programu izravnih plaćanja (za razdoblje 2015.-2020.). Istraživanjem su analizirani troškovi i koristi konzervacijske obrade tla u proizvodnji soje, kojom se nastoji poboljšati kvaliteta tla, optimizirati prinose i povećati profit. Istraživanjem je potvrđeno da ekonomska opravdanost primjene konzervacijske obrade tla u proizvodnji soje ovisi o sposobnosti realizacije prinosa koji su iznad prosječnih u našim proizvodnim uvjetima.

Ključne riječi: ekonomičnost, konzervacijska obrada tla, prihod, soja, troškovi

Uvod

Soja (*Glycine max.* (L.) Merr.) je značajan izvor bjelančevina i ulja u prehrani ljudi te proizvodnji stočne hrane. Sojina sačma spada u najkvalitetnija biljna bjelančevinasta krmiva (Pospišil, 2010.). Osim što je poželjna u prehrambenoj industriji, soja je vrlo poželjna i u plodoredu, zbog sposobnosti simbioze korijena sa kvržičnim bakterijama koje vežu dušik iz zraka i pretvaraju ga u oblik koji biljka može koristiti te na taj način obogaćuju tlo.

Prema Državnom zavodu za statistiku (DZS, 2023.) u razdoblju 2018.-2022. godine, površine pod sojom su povećane sa 77.087 na 91.000 ha, dok se prinos u istom razdoblju smanjio sa 3,2 na 2,1 t/ha. Povećanje proizvodnih površina pod sojom rezultat je više čimbenika: od relativno niskih troškova proizvodnje po jedinici površine (Ranogajec i sur., 2014.), povećane primjene u postranoj sjetvi (Iljković i sur., 2019), veće tržišne potražnje (Andrić i sur., 2022), korištenja biomase ili žetvenih ostataka u energetske svrhe (Kiš i sur., 2013). Također, u mjeri potpore „Zelena plaćanja“ za razdoblje 2015.-2020. godine (NN 21/2019) je soja uvrštena u popis osnovnih kultura koje fiksiraju dušik, te se priznaje kao ekološki značajna površina. U novom programskom razdoblju (2023.-2027. godine) nema više spomenute mjere Programa ruralnog razvoja iz prethodnog programskog razdoblja (2015.-2020. godine), ali jedna od operacija u kojoj poljoprivrednici mogu birati sjetvu soje je 31.05. Eko shema: Minimalni udio leguminoza od 20% unutar poljoprivrednih površina. Smanjeni prinos soje rezultat je vremenskih ekstrema koji su sve češći i uzrokuju velike štete na poljoprivrednim usjevima, poput suše (Vratarić i Sudarić, 2009), visokih temperatura zraka te suviškova vode (Marković i sur., 2022).

Konzervacijska poljoprivreda obuhvaća tehnološke mjere prilagodbe i ublažavanja klimatskih promjena, a predstavlja način gospodarenja agroekološkim sustavima s ciljem poboljšane i održive produktivnosti, povećanih profita i sigurnosti hrane (Jug i sur., 2018).

Košutić i sur. (2006) proveli su dvogodišnje istraživanje utjecaja konvencionalnog, konzervacijskog i nultog sustava obrade tla na prinos, utrošak energije i ljudskog rada u proizvodnji soje i ozime pšenice te zabilježili najveći urod soje kod nulte obrade tla. S obzirom na utrošak energije u proizvodnji, konzervacijski sustav obrade tla omogućio je uštedu od 31,8% kod soje. Troškovi konzervacijskog sustava obrade tla za soju su bili 20% niži od konvencionalnog sustava, a nulti sustav obrade tla za soju imao je za 34% niže troškove.

Primjenom konzervacijske obrade tla poboljšava se kvaliteta tla, optimiziraju prinosi i smanjuju troškovi poljoprivredne proizvodnje. Cilj ovog rada je utvrditi ekonomsku opravdanost primjene konzervacijske obrade tla u proizvodnji soje.

Materijal i metode

U radu su analizirani podaci sa eksperimentalnih lokaliteta Čačinci i Križevci na kojima se provodilo istraživanje u 2022. godini u sklopu HRZZ projekta "Procjena konzervacijske obrade tla kao napredne metode uzgoja usjeva i prevencije degradacije tla – ACTIVEsoil".

U ovom radu je izračun prihoda napravljen prema visinama prinosa i prosječnoj prodajnoj cijeni neposredno nakon žetve. U prihode su uračunata i prosječna izravna plaćanja za ratarsku proizvodnju u iznosu od 2.250,00 kn/ha (298,63 €). Doprinos pokriću varijabilnih troškova je izračunat kao razlika vrijednosti proizvodnje i varijabilnih troškova. Cijena koštanja je izračunata prema jednostavnoj djelidbenoj kalkulaciji, a kalkulacije su napravljene po *direct costing* metodi s obračunom samo izravnih troškova proizvodnje. Za kalkulacije, osim cijena inputa i outputa u poljoprivredi 2022. godine objavljenih u bazi Tržišnog cjenovno informacijskog sustava (TISUP), korištena su i izvješća Državnog zavoda za statistiku (DZS) te objave dobavljača i otkupljivača na tržištu RH. Troškovi proizvodnje dobiveni su množenjem utroška materijala i rada s tekućim cijenama po jedinici utroška. Utrošci materijala (sjeme, zaštitna sredstva i mineralna gnojiva) su dobiveni prema stvarnim podacima iz pokusa, dok su utrošci rada po radnim operacijama procijenjeni iz standarda (tehnoloških normativa) prema ranijim istraživanjima.

Trošak sata rada traktora i priključnih oruđa (Tablica 3) izračunat je prema sljedećoj shemi:

- A: cijena novog stroja na tržištu
- B: cijena nakon amortizacijskog razdoblja
- C: razlika u cijeni (A – B)
- D: amortizacijsko razdoblje
- E: godišnji iznos amortizacije (C / D)
- F: prosječno radnih sati godišnje
- G: amortizacija po satu rada (E / F)
- H: Godišnji iznos kamata na uložena sredstva (7%)
- I: kamata po satu rada (H / F)
- J: cijena plavog dizela
- K: potrošnja goriva po satu rada
- L: trošak goriva po satu rada (J * K)
- M: trošak održavanja (30% troškova goriva)
- N: trošak sata rada (G + I + L + M)

Normativi utroška rada ljudi i strojeva procijenjeni su prema tehnološkim zahtjevima kulture i tehničkim obilježjima mehanizacije. Normativi se ostvaruju u prosječnim proizvodnim uvjetima i s prosječnim naporom. Utrošci rada mehanizacije po jedinici površine određeni su brzinom rada i zahvatom koji definiraju teoretski učinak, a praktični učinak je određen stupnjem iskorištenosti radnog vremena koje se u ratarstvu kreće od 35-60% (Grgić i sur.,1999).

Kalkulacije su izrađene za prinose zabilježene u pokusima, sukladno različitim sustavima obrade tla i gnojidbi. Prikazana su tri različita sustava osnovne obrade tla (obrada tla oranjem te dubokim rahljenjem i plitkim rahljenjem), dva načina gnojidbe (prema preporuci i dvostruko manja) s kojima je u kombinaciji primjena poboljšivača tla (Geo2). Prema tome su dobivene cijene koštanja soje na razini zbroja ukupnih troškova mehanizacije i varijabilnih troškova (sjeme, gnojivo, zaštitna sredstva). Fiksni troškovi gospodarstva (najam, osiguranje i dr.) nisu uračunati. Uračunati su troškovi za sjeme, gnojivo i zaštitna sredstva, bez PDV-a.

Rezultati i rasprava

Podaci se odnose na cijene sjemena u vrijeme sjetve, mineralnih gnojiva i zaštitnih sredstava krajem prvog tromjesečja tekuće godine, a prodajne cijene neposredno nakon žetve u tekućoj godini (Tablica 1).

Tablica 1. Pregled cijena inputa, potpora i prodajnih cijena u proizvodnji soje

Opis	Naziv	jedinica mjere	2021.	2022.
Potpورا		€/ha	298,63	298,63
Prodajna cijena	€/kg	0,58	0,56	
Sjeme soje		€/ha	200,92	230,94
Gnojiva	Urea	€/kg	0,84	1,01
	KAN	€/kg	0,55	0,64
	NPK 7:20:30	€/kg	0,87	0,75
	NPK 15:15:15	€/kg	0,65	0,72
	FASTER	€/L	5,57	7,70
Zaštitna sredstva	Senat	€/kg	45,13	53,09
	Dual Gold 960 SC	€/kg	21,79	33,30
	Sirtaki	€/L	107,77	132,59
	Glyfon	€/0,1 L	7,03	7,35
	Benta Plus	€/kg	32,69	39,15
	Corum	€/kg	57,20	65,43
	Frontier	€/kg	40,88	43,85
	Dasch	€/kg	5,04	5,44
Ostalo	Plavi dizel	€/L	0,83	1,13

Izvor: TISUP i DZS

Za proračun cijene koštanja sata rada mehanizacije i obračun troškova mehanizacije za soju korištene su prosječne nabavne cijene mehanizacije za ratarsku proizvodnju, čija je nabavka preporučljiva i tehnološki dobro iskoristiva na obradivim površinama poljoprivrednih gospodarstava do 50 ha (Tablica 2).

Tablica 2. Pregled korištene vlastite poljoprivredne mehanizacije i nabavne cijene 2020. godine

	Nabavna cijena, €	Godine korištenja	Amortizacija, €
Traktor (114 kw)	39.847,24	10	3.437,52
Traktorski plug	2.389,01	12	154,84
Traktorska sijačica	2.787,18	12	210,14
Razbacivač gnojiva	1.513,04	12	103,97
Atomizer	2.070,48	12	133,83
Prorahljivač	2.787,18	12	188,02

Izvor: Prosječne vrijednosti korištene mehanizacije

Prema shemi prikazanoj u materijalima i metodama, proračunati su troškovi sata rada traktora i priključnog oruđa za 2022. godinu, te prikazani po radnim operacijama (Tablica 3).

Tablica 3. Troškovi sata rada traktora i priključaka po radnim operacijama

	Iznos, €/sat
Oranje	26,70
Zatvaranje brazde	26,70
Dublje rahljenje	27,43
Pliće rahljenje	27,16

Predsjetvena priprema	28,93
Sjetva	28,93
Kultiviranje	26,68
Gnojidba	26,68
Kalcizacija	26,68
Zaštita	26,76
Prijevoz	26,90
Ostalo	26,76

Troškovi rada mehanizacije (Tablica 4) proračunati su prema stvarnim utrošcima sati rada na dvije lokacije (Čačinci i Križevci) za 3 razine obrade tla: A1 je konvencionalna obrada tla oranjem, A2 uključuje duboko rahljenje tla i A3 je obrada s plitkim rahljenjem tla.

Tablica 4. Troškovi mehanizacije na eksperimentalnim lokacijama (€/ha)

Lokalitet	Čačinci			Križevci		
	A1	A2	A3	A1	A2	A3
Operacije						
Oranje	92,47	92,47	92,47	92,47	92,47	92,47
Zatvaranje brazde	75,83	0,00	0,00	75,83	0,00	0,00
Rahljenje	0,00	76,80	65,19	0,00	76,80	65,19
Predsjetvena priprema	37,57	37,57	37,57	46,29	46,29	46,29
Sjetva	44,09	44,09	44,09	46,29	46,29	46,29
Kultiviranje	42,69	42,69	42,69	42,69	42,69	42,69
Gnojidba	44,47	44,47	44,47	44,47	44,47	44,47
Zaštita	64,22	64,22	64,22	64,22	64,22	64,22
Prijevoz	64,56	64,56	64,56	64,56	64,56	64,56
Ostalo	42,81	42,81	42,81	42,81	42,81	42,81
Ukupno	508,68	509,66	498,05	519,60	520,58	508,97

Na pokusnim poljima su zabilježene razlike prinosa zrna što izravno određuje vrijednost proizvodnje (Tablica 5). U čak 17 od 24 pokusna polja na lokaciji Čačinci je zabilježen negativan doprinos pokriću. U strukturi troškova je najveći utjecaj troška gnojidbe i mehanizacije, ali razlike tehnologije ne utječu bitno na visinu ukupnog troška, koliko je to kod drugih agrotehničkih mjera. Na doprinos pokrića manje djeluje primjena mineralnog gnojiva, a odlučujući je utjecaj primjene poboljšivača tla koji čini najveće odstupanje u ukupnim troškovima pokusnih polja. U poljima gdje je primijenjen Geo2 dodatni trošak primjene je uzrokovao neekonomičnu proizvodnju u čak 11 od 12 pokusnih polja na kojima je primijenjen.

Tablica 5. Prihodi, troškovi i doprinos pokriću na lokaciji Čačinci

Polja/ Opis	Prinos zrna t/ha	Vrijednost proizvodnje €	Potpora €	Prihod €	Varijabilni trošak €	Doprinos pokriću €
1	3,26	1.817,24	298,63	2.115,87	1.627,66	488,21
2	2,37	1.321,12	298,63	1.619,75	1.356,17	263,57
3	1,83	1.020,11	298,63	1.318,73	1.751,09	-432,36

4	2,71	1.510,65	298,63	1.809,28	1.479,61	329,67
5	1,57	875,17	298,63	1.173,80	2.559,26	-1.385,46
6	1,80	1.003,38	298,63	1.302,01	2.287,77	-985,76
7	2,08	1.159,47	298,63	1.458,09	2.682,69	-1.224,60
8	3,77	2.101,53	298,63	2.400,16	2.411,21	-11,05
9	2,60	1.449,33	298,63	1.747,96	1.628,63	119,32
10	1,81	1.008,96	298,63	1.307,59	1.357,15	-49,57
11	2,35	1.309,97	298,63	1.608,60	1.752,07	-143,47
12	2,26	1.259,80	298,63	1.558,43	1.480,58	77,85
13	3,02	1.683,46	298,63	1.982,08	2.560,23	-578,15
14	2,49	1.388,02	298,63	1.686,64	2.288,75	-602,11
15	3,48	1.939,88	298,63	2.238,50	2.683,67	-445,16
16	2,42	1.348,99	298,63	1.647,62	2.412,18	-764,56
17	1,93	1.075,85	298,63	1.374,48	1.617,03	-242,55
18	2,97	1.655,58	298,63	1.954,21	1.345,54	608,67
19	2,57	1.432,61	298,63	1.731,24	1.740,46	-9,22
20	2,83	1.577,54	298,63	1.876,17	1.468,97	407,20
21	2,52	1.404,74	298,63	1.703,36	2.548,63	-845,26
22	2,75	1.532,95	298,63	1.831,57	2.277,14	-445,57
23	2,85	1.588,69	298,63	1.887,32	2.672,06	-784,74
24	2,90	1.616,56	298,63	1.915,19	2.400,57	-485,38

U većini polja (20 od 24) na lokaciji Križevci, neovisno o primijenjenoj agrotehnici su ostvareni pozitivni doprinosi pokriću (Tablica 6), što je najviše određeno višim prinosima zrna u odnosu na prethodno opisanu lokaciju. Izuzetak su pojedina pokusna polja na kojima su korišteni poboljšivači tla, a ostvareni su relativno niži prinosi (pokusna polja 3 i 11).

Tablica 6. Prihodi, troškovi i doprinos pokriću na lokaciji Križevci

Polja/ Opis	Prinos zrna t/ha	Vrijednost proizvodnje €	Potpora €	Prihod €	Varijabilni trošak €	Doprinos pokriću €
1	2,43	1.354,57	298,63	1.653,20	1.673,54	-20,35
2	2,52	1.404,74	298,63	1.703,36	1.347,65	355,72
3	2,59	1.443,76	298,63	1.742,39	1.796,98	-54,59
4	3,14	1.750,35	298,63	2.048,97	1.471,08	577,90
5	2,75	1.532,95	298,63	1.831,57	1.896,28	-64,70
6	4,89	2.725,86	298,63	3.024,49	1.570,38	1.454,11
7	4,22	2.352,38	298,63	2.651,01	2.019,71	631,30
8	4,79	2.670,12	298,63	2.968,74	1.693,81	1.274,93
9	6,39	3.562,01	298,63	3.860,64	1.674,52	2.186,12
10	3,11	1.733,63	298,63	2.032,25	1.348,62	683,63
11	2,57	1.432,61	298,63	1.731,24	1.797,95	-66,71
12	5,30	2.954,41	298,63	3.253,04	1.472,06	1.780,98

13	4,01	2.235,32	298,63	2.533,94	1.897,25	636,69
14	3,85	2.146,13	298,63	2.444,75	1.571,36	873,40
15	3,84	2.140,55	298,63	2.439,18	2.020,68	418,50
16	3,92	2.185,15	298,63	2.483,77	1.694,79	788,99
17	5,06	2.820,63	298,63	3.119,25	1.662,91	1.456,34
18	3,63	2.023,49	298,63	2.322,12	1.337,02	985,10
19	5,00	2.787,18	298,63	3.085,81	1.786,34	1.299,46
20	3,55	1.978,90	298,63	2.277,52	1.460,45	817,08
21	4,88	2.720,29	298,63	3.018,91	1.885,64	1.133,27
22	3,16	1.761,50	298,63	2.060,12	1.559,75	500,38
23	3,57	1.990,05	298,63	2.288,67	2.009,07	279,60
24	3,56	1.984,47	298,63	2.283,10	1.683,18	599,92

Zaključak

Velike su razlike prosječnih vrijednosti između lokaliteta pokusa. Prosječni prinosi na lokalitetu Čačinci su 2,55 t/ha, a u Križevcima 3,86 t/ha. Budući su provedeni isti postupci na dvije lokacije, a prinosi na tim lokacijama su drugačiji neovisno o dodatnim inputima (količina gnojiva, poboljšivač tla) ili izboru tehnologije (pliča ili dublja obrada tla), preporučuje se provesti dodatna istraživanja budući visina prinosa zrna i ulaganja u proizvodnju izravno određuju ekonomsku učinkovitost.

Na temelju ostvarenih rezultata u istraživanjima projekta ACTIVEsoil, može se zaključiti da ekonomičnost primjene konzervacijske obrade tla, koja se svrstava u tehnologije za ublažavanje klimatskih promjena u ratarskoj proizvodnji, uvelike ovisi o proizvodnosti, odnosno, o visini ostvarenog prinosa. Ekonomičnost se ostvaruje kod prinosa viših od prosječnih u uvjetima panonske podregije Republike Hrvatske. U protivnom, neke mjere koje se primjenjuju s konzervacijskom obradom tla (primjena poboljšivača tla) predstavljaju dodatni trošak koji rezultira gubicima proizvodnje.

Napomena

Ovaj je rad financirala Hrvatska zaklada za znanost projektom "Procjena konzervacijske obrade tla kao napredne metode uzgoja usjeva i prevencije degradacije tla – ACTIVEsoil" (IP-2020-02-2647).

Literatura

- Andrijanić Z., Matoša Kočar M., Brezinščak L., Pejić I. (2022). Trendovi proizvodnje soje u Hrvatskoj. Glasnik zaštite bilja. 4: 58-68.
- Državni zavod za statistiku (2023). Površina i proizvodnja žitarica i ostalih usjeva. Raspoloživo na: <https://podaci.dzs.hr/2022/hr/29384>
- Grgić Z., Šnajder I., Košutić S. (1999). Korištenje poljoprivredne mehanizacije na obiteljskom gospodarstvu. Aktualni zadaci mehanizacije poljoprivrede, Opatija 2.-5.02.1999., Zagreb: Agronomski fakultet. 29-34.
- Iljkić D., Kranjac D., Zebec V., Varga I., Rastija M., Antunović M., Kovačević V. (2019). Stanje i perspektiva proizvodnje žitarica i uljarica u Republici Hrvatskoj. Glasnik zaštite bilja. 42: 62-7.
- Jug D., Jug I., Vukadinović V., Stipešević B., Đurđević B. (2018). The role of conservation agriculture in mitigation and adaptation to climate change. Poljoprivreda. 24: (1).
- Kiš D., Sučić B., Šumanovac L., Antunović M. (2013). Energetska i fertilizacijska vrijednost žetvenih ostataka soje. Poljoprivreda. 19: 48-52.
- Košutić S., Filipović D., Gospodarić Z., Husnjak S., Zimmer R., Kovačev I. (2006). Usporedba različitih sustava obrade tla u proizvodnji soje i ozime pšenice u Slavoniji. Agronomski glasnik. 68: 381-392.

Marković M., Spišić M., Josipović M., Sudarić A., Matoša Kočar M., Bara, Ž., Japundžić Palenkić B., Stanisavljević A., Bošnjak D., Kojić A. (2022). Osnovni elementi navodnjavanja soje ovisno o klimatskim uvjetima i rokovima sjetve. *Glasnik Zaštite Bilja*. 4: 48-56.

Ministarstvo poljoprivrede. Tržišno informacijski sustav u poljoprivredi (TISUP). Raspoloživo na: <http://www.tisup.mps.hr/>

Ministarstvo poljoprivrede (2019). Pravilnik o provedbi izravne potpore poljoprivredi i IAKS mjera ruralnog razvoja. *Narodne novine NN 21/2019*.

Pospišil A. (2010). *Ratarstvo 1. dio*. Zrinski d.d., Čakovec

Ranogajec L., Kanisek J., Deže J. (2014). Ekonomski rezultati proizvodnje soje u Republici Hrvatskoj. *Zbornik radova 49. hrvatskog i 9. međunarodnog simpozija agronoma*. Dubrovnik, 16-21.02.2014. Osijek: Sveučilište Josipa Jurja Strossmayera u Osijeku, Poljoprivredni fakultet, 171-175.

Vratarić M., i Sudarić A. (2009). Abiotski činitelji u proizvodnji soje. *Glasnik Zaštite Bilja*. 32: 67-76.

Economic justification for the use of soil conservation tillage in soybean cultivation

Abstract

Soybean is an important source of protein and oil in human nutrition, but also in the production of livestock feed. In addition, the importance of soybean also lies in the enrichment of the soil with nitrogen, and it was recognized as an ecologically significant area under the Direct Payment Scheme (for the period 2015-2020). The study analyzed the costs and benefits of conservation tillage in soybean production, which aims to improve soil quality, optimize yields, and increase profits. The study confirmed that the economic justification for the use of conservation tillage in soybean production depends on the ability to achieve above-average yields under our production conditions.

Keywords: conservation tillage, costs, efficiency, income, soybean

Etičke implikacije korištenja umjetne inteligencije u poljoprivredi

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Sažetak

Društvo se trenutno nalazi pred novom znanstvenom revolucijom koju mu omogućava primjena sužene umjetne inteligencije. Ta će znanstvena revolucija pomoću AI-a utjecati na sve sfere ljudskog djelovanja, pa tako i na poljoprivredu. Međutim, povijest nam iznova pokazuje da nisu sve znanstvene revolucije imale isključivo pozitivan učinak na cjelokupno ljudsko društvo. Stoga nam preostaje zapitati se koje su potencijalne negativne strane primjene AI-a, kako bi se eventualni nedostaci preduhitili. Cilj je ovoga rada prikazati etičke implikacije korištenja AI-a u poljoprivredi, pritom prikazujući njezin utjecaj na društvo i ekosferu.

Ključne riječi: umjetna inteligencija, poljoprivreda, etika, ekosfera, privatnost

Uvod

Prije otprilike 10 000 godina došlo je do agrarne revolucije koja je u potpunosti izmijenila način ljudskog življenja i strukturu ljudskih odnosa (Harari, 2015). Sada, na počecima 21. stoljeća, nalazimo se pred novom revolucijom koja će iznova promijeniti ustroj društva. Tu nam novu društvenu revoluciju donosi razvoj umjetne inteligencije (eng. *AI*). No, između je prve agrarne revolucije i *AI* revolucije na poljoprivredu neposredno utjecao niz drugih povijesnih promjena poput industrijske revolucije (Allen, 1994; Hendrickson i James, 2005), zelene revolucije (Khush, 1999) i biotehnoške revolucije (Fukuyama, 2003). Potonja je ostavila traga u (bio)etici kroz razmatranja o posljedicama uporabe *GMO*-a u poljoprivredi, kako na poljoprivredne proizvođače i agronomsku struku (e.g. Šimić, 2002) tako i na potrošače i društvo u cjelini (Devos i sur. 2008). Etičke se debate o *GMO*-u proširuju i na fenomen generiranja velikih setova podataka (Šimić i sur., 2023) u „velikoj“ poljoprivredi biotehnoških multinacionalnih kompanija koje svoje ugovore o korištenju *GMO* tehnologije proširuju na prikupljanje podataka pomoću različitih platformi u njihovom vlasništvu time dodatno umanjujući autonomnost poljoprivrednih proizvođača (Carbonell, 2016). Uporabom *AI*-a za obradu velikih setova podataka, etička se pitanja dodatno umnožavaju i usložnjavaju (Ryan, 2019). U posljednjih je godinu dana došlo do ubrzanog razvitka raznovrsnih *AI* sustava koji su svoju prominentnost stekli zbog pristupačnosti. No, postoje i raznovrsni *AI* sustavi koji nemaju medijsku eksponiranost, ali koji će izgledno biti novi korak u sveopćoj znanstvenoj revoluciji koja će zahvatiti sva područja ljudskog djelovanja poput poljoprivrede. Međutim, pitanje je koja će biti bit ove znanstvene revolucije. Ako ispitamo bit agrarne revolucije, uočiti ćemo da je njezina bit bila održavanje na životu većeg broja ljudi od onoga što je prethodno bilo moguće, pa makar i u gorim životnim uvjetima (Harari, 2015). Stoga je nejasno kakva će biti bit primjene *AI*-a na područjima ljudskog djelovanja, a s time i koje će društvene posljedice biti. Dakle, u ovome će se radu ispitati kakve će etičke implikacije imati primjena *AI*-a na poljoprivredu. Naime, zbog manjka iskustva s ovakvim sustavima, nejasno je koji će aspekti tih sustava negativno utjecati na društvo. Analizirajući povijesna zbivanja industrijalizacije, pronalazimo kako znanstveni napredak nije uvijek bio svačiji boljitak. Npr., industrijalizacija je poljoprivrede izmijenila načine odvijanja proizvodnje koji su pak negativno utjecali na odlučivanje farmera na etičkoj razini (Hendrickson i James, 2005), ali etička pitanja ne polaze samo od farmera (proizvođača), već i od potrošača. Stoga upotreba *AI*-a u poljoprivredi ima kauzalne posljedice po društvo koje naizgled nisu uočljive. *AI* pomaže u kartiranju, uređivanju i izmjeni genoma, simuliranju proteinskih ovojnica, mjerenju saliniteta tla, raspršivanju pesticida i vode, prikupljanju plodova, nadzoru životinja itd. Iz tih je primjera vidljivo da *AI* omogućuje i novu biorevoluciju, ali unatoč svim pogodnostima, postoje razni problemi *AI*-a u koje spada privatnost podataka, ekološka održivost i njezin utjecaj na ekosferu.

Metode rada i izvori podataka

Prilikom analiziranja problema, u radu ćemo zauzeti metodološki pristup bibliografske analize na temelju čega ćemo prikazati etičke implikacije korištenja AI-a u poljoprivredi kroz pet ključnih tema: 1. privatnost i vlasništvo podataka, 2. neprovidnost i transparentnost AI sustava, 3. pristranost i pravičnost, 4. odgovornost, 5. održivost. Pritom ćemo se koristiti znanstvenim člancima iz područja agronomije i računarstva te studijama iz društveno-humanističkih znanosti (pravo, filozofija – etika). Kriteriji su za uključivanje radova u analizi bili: a) da su radovi iz područja agronomije i računarstva objavljeni u proteklih 30 godina, tj. da su relevantni za trenutnu problematiku; b) da u sebi sadrže ključne teme poput pitanja o privatnosti, transparentnosti, odgovornosti, održivosti, raspolaganju i reguliranju informacijama, etičnosti korporacija, negativnom utjecaju proizvodnje na ekosferu, povijesti industrijske revolucije, te o AI-u; c) da su navedeni podaci provjerljivi i da sadrže listu referenci, d) da su u svome opredjeljenju neutralni i da iznose objektivno stanje stvari. Na temelju tih ćemo kriterija iznijeti analizu pet ključnih tema o etičkim pitanjima upotrebe AI-a u poljoprivredi.

Rezultati i rasprava

Privatnost i vlasništvo podataka

Razmjena je podataka nešto na čemu počiva današnje informacijsko društvo i ono je unutar njega glavni predmet trgovanja. Informacija je ključna za društveni razvoj, ali ona ujedno predstavlja i rizik, a to važi i za sektor poljoprivrede. Naime, privatnost je u digitalnom društvu postala narušena i privatni su podaci postali robom te se stoga provode brojne rasprave o pravu da pojedinac bude „pušten na miru“, pravu pojedinca da kontrolira protok informacija i da skriva podatke o sebi itd. (Müller, 2023). S tim je problemom povezan i AI koji bi mogao posvuda pospješiti narušavanje privatnosti, pa tako i unutar poljoprivrede. Prikupljanje bi i uporaba velikih količina podataka u poljoprivredi pridonijelo točnijim i pouzdanijim modelima AI-a. No, poljoprivrednici su proizvođači zabrinuti za privatnost i povjerljivost svojih podataka, jer je nejasno što pružatelj podatkovnih usluga ima pravo činiti s prikupljenim podacima i koje su njegove obveze u vezi zaštite privatnosti i povjerljivosti podataka (Ryan, 2019). Ujedno postoji i zabrinutost oko toga tko bi bio vlasnikom podataka s farmi i tko bi sve imao kontrolu nad upotrebom tih podataka (Ryan, 2019). Takva bi situacija mogla dovesti do neravnoteže snaga između poljoprivrednika i pružatelja tehnologije ili usluga (Dara i sur., 2022.). Stoga, ključno je pitanje tko dobiva koju informaciju, na koji je način dobiva, kojim redoslijedom, o kome i čemu je dobiva, te posljednje, za koju svrhu (Beck, 1992). No, što to sve znači za pojedinca u informacijskom društvu? Beck predviđa da će unutar društva doći do sukoba zbog moći oko distribucije i koeficijenta distribucije tokova informacija (Beck, 1992). Naime, informacija je ključna za posjedovanje i povećanje onoga što Foucault naziva biomoći, koja se definira kao moć koja nastoji upravljati, optimizirati i umnožiti život podvrgavajući ga preciznim kontrolama i sveobuhvatnim regulacijama (Foucault, 1976). Stoga, prikupljanje privatnih podataka s farmi i pojedinaca koji rade na njima može imati dalekosežne pozitivne, ali i negativne posljedice te je stoga potrebno pravovremeno legislativno djelovati kako bi se osigurala transparentnost i zaštitilo pojedinca od predatorskih praksi.

Neprovidnost/transparentnost

Nailazimo i na etičke probleme neprovidnosti, transparentnosti te pristranosti. Naime, neprovidnost i pristranost ključna su pitanja koja pripadaju u etičnost korištenja velikih podataka (Šušnjara, 2019). Tehnikama se strojnog učenja detektiraju obrasci u podacima i obilježavaju se korisnima za odluke koje donosi sustav, dok istovremeno programer ne zna zapravo koji su obrasci u podacima korišteni, a svemu tome dodatni izazov predstavlja što se pristizanjem novih podataka sami obrasci unutar sustava iznova mijenjaju (Müller, 2023). Iz takvog načina rada proizlazi da ishod učenja nije transparentan za korisnika i programera, tj. on je neprovidan (Müller, 2023). Time što kod korisnika postoji nedostatak interpretabilnosti i razumljivosti ishoda AI modela, narušava se načelo transparentnosti (Dara i sur., 2022). No, kako uopće definiramo transparentnost? Transparentnost možemo definirati kao praksu otvorenosti prema politikama i djelovanjima prema dionicima kako bi se ostvarila poboljšana komunikacija, međusobno razumijevanje i suradnja, stoga ona predstavlja ključno etičko načelo u poljoprivrednim AI okruženjima gdje je interpretabilnost važna za razvoj i upravljanje (Dara i sur., 2022). Tako se npr., u svrhu smanjivanja utjecaja stakleničkih plinova, AI može koristiti za procjenu količine ugljičnog dioksida u proizvodnim pogonima, a poljoprivrednici trebaju znati prepoznati i suprotstaviti se ovakvim procjenama njihovoga ugljičnog

otiska ako smatraju da je procjena sustava netočna i nepravična. No, pitanje je kako postići navedenu transparentnost? Jedan bi od mogućih odgovora bila demokratizacija znanja, edukacija i mogućnost postojanja otvorenog pristupa određenog dijela sustava (tzv. *open-source*).

Pristranost/pravičnost

Pri uporabi AI sustava postoji i opasnost od statističke pristranosti, no postoje značajni naponi da se ona detektira i ukloni (Müller, 2023). Takvo se praćenje i ublažavanje svih oblika pristranosti u AI modelima u poljoprivredi referira kao pravičnost (Dara i sur., 2022), a postoje dvije vrste pravičnosti – tehnička i socijalna. Jedan od primjera tehničke pravičnosti autonomna je berba jabuka u kojoj AI može detektirati zrele jabuke. No, ako je AI model optimiziran za branje crvenih jabuka, on je pristran upravo prema takvim jabukama i ne detektira zeleni tip jabuka kao zrele i stoga ih ne ubire unatoč tome što su one zrele (Dara i sur., 2022). Ovakvi se tipovi pristranosti trebaju korigirati kako bi sustav prepoznao jabuke različitih boja i različite zrelosti te kako bi se izradio pouzdaniji i inkluzivniji model. Nadalje, imamo socijalnu pravičnost koja kod AI uporabe uključuje ljudska prava, demokratske vrijednosti i poštivanje različitosti. U poljoprivredi to se odnosi i na pitanje raspodjele moći nad pristupom, vlasništvom i kontrolom podataka između poljoprivrednih proizvođača i pružatelja podatkovnih usluga koja može biti zloupotrijebljena dijeljenjem podataka trećoj strani (Dara i sur. 2022). Navedeno ima potencijal stvoriti jaz i sukob između korporacija i pojedinca te se dovodi u pitanje način na koji će se osoba promatrati. Naime, osobe se promatraju kao entiteti koji imaju posebnu vrijednost – dignitet – što ih čini vrijednima i neprocjenjivima (Zagzebski, 2001). No, postoji opasnost da korporacije naruše ljudski dignitet uzimanjem osoba kao robe koju će prodati na tržištu. Također, pitanje je kakav će stav zauzeti poljoprivrednici i samo tržište prema samim plodovima koji trebaju biti prikupljeni, tj. što će biti s onim proizvodom za koje je AI sustav odredio da nije dovoljno kvalitetan za tržište, no i dalje je iskoristiv.

Odgovornost

Postoji i pitanje odgovornosti za AI i učinke njegova djelovanja. Korištenjem se AI tehnologija može dogoditi da se apliciraju nerazumne količine vode ili pesticida na određeno polje zbog sustavnih grešaka, stoga se s opravdanjem postavlja pitanje tko će biti odgovoran za gubitke prinosa i eventualnu ekološku štetu (Dara i sur., 2022). Nadalje, automatizirani strojevi poput robo-berača mogu uništiti biljke tijekom berbe zbog pogreške u sustavu ili lošega dizajna. Ovdje imamo problem manjka pravne jasnoće i transparentnosti o tome tko je odgovoran za loše upravljanje, pogrešne odluke i loše preporuke dobivenih od postojećih AI modela prilikom automatiziranog donošenja odluka (Dara i sur., 2022). Problem je i što velike kompanije koriste pravni vakuum pa se u ugovorima o korištenju AI tehnologija ograđuju od preuzimanja odgovornosti za eventualne štete na usjevima, zemlji, dobiti ili strojevima (Dara i sur., 2022). Međutim, prema OECD-u, organizacije koje razvijaju, postavljaju i upogonjuju AI sustave u digitalnoj poljoprivredi i ostalim srodnim sektorima trebale bi biti odgovorne za ispravno funkcioniranje AI alata i donošenje odluka (OECD AI, 2019). Stoga, odgovornost je za eventualne posljedice nužno pravno utemeljiti na temelju pravičnog suglasja.

Održivost

U današnjem društvu u kojem postoji strah od globalne ekološke katastrofe, G. Myerson, oslanjajući se na teoriju psihopatologije, izgradio je vlastitu teoriju ekopatologije kojom ispituje svakodnevne obrasce ljudskog ponašanja kako bi prikazao da gotovo pa svaki ljudski čin koji se naizgled čini bezazlenim ima nekakav ekološki utjecaj (Myerson, 2002). Navedeno važi i za područje AI-a čija će primjena imati nekoliko jasno uočljivih posljedica po ekosferu, ali ujedno i posljedice koje će od nas iziskivati poseban umni napor za uočavanje. Stoga, govoreći o održivosti i AI-u u poljoprivredi, može se govoriti o postojanju nekoliko aspekata. Prvi se aspekt tiče samog korištenja AI modela. Naime, AI modeli trebali bi biti jednostavni za korištenje i korisni za poljoprivrednike, jer ako AI ne može pružiti učinkovite preporuke za pomoć poljoprivrednicima, ili sustav nije jednostavan za korištenje te je manjkav u smislu dizajna i upotrebljivosti, on jednostavno nije održiv (Dara i sur., 2022). Upotrebljivost je AI softvera ključna za korištenje, predviđanje i davanje preporuka koje pametni informacijski sustavi pružaju u poljoprivredi. Nadalje, korisnost tehnologija temeljenih na AI-u za poljoprivrednike ovisi o točnosti i pouzdanosti preporuka radnji (Dara i sur., 2022), a mogući su problemi vezani uz implementaciju AI-a brojni. Greške u softveru, kvarovi hardvera i pristrana predviđanja samo su neki od primjera koji utječu na održivu implementaciju AI rješenja na poljoprivrednim

gospodarstvima. Tako npr., roboti, bespilotne letjelice i autonomna vozila u poljoprivredi mogu ispuštati otrovne kemikalije, što može dovesti do onečišćenja, primijeniti previše ili premalo vode na farmi zbog grešaka ili lošeg upravljanja AI sustavima ili algoritmima (Dara i sur., 2022). Posljednja stvar koja se treba razmotriti jest održivost okoliša. Treba ispitati kako sustavi AI-a prilikom izgradnje i primjene utječu na okoliš. Utjecaj strojeva koji koriste AI na okoliš jest dvojak – jedan počiva na softveru, a drugi na hardveru. Što se tiče samog softvera, opća je preporuka da se algoritmi od početka razvijaju i mjere na način da se smanji šteta za okoliš (Okengwu i sur., 2023). Također, preporuka je da se AI ne razvija konstantno ispočetka, već da postoji neka osnova baza iz koje se potom grade AI sustavi jer bi se time uštedjelo na potrošnji energije (Okengwu i sur., 2023), no to bi ujedno od kompanija zahtijevalo postojanje određene *open-source* baze podataka. Druga stvar koja se tiče softvera jest planirano zastarijevanje. Naime, trebao bi se pravno regulirati način na koji korporacije ažuriraju svoj softver kako bi se spriječilo namjerno usporavanje, ograničavanje i kvarenje samog softvera iz želje da si krajnji korisnik pribavi novi produkt. Takva bi zloupotreba znatno utjecala na ekološku održivost i ekosferu, a ona nije nešto nevideno. Sljedeća stvar koju treba uzeti u obzir jest sama građa AI sustava. Prvi problem na koji ovdje nailazimo jest da su za treniranje AI sustava potrebne ogromne količine podataka koje moraju biti pohranjene na određenim fizičkim mjestima. Naime, već sada postoji problem s bazama podataka koji troše ogromne količine vode, a pretpostavlja se da će AI sustavi situaciju učiniti još gorom. Postoje i drugi problemi s građom strojeva te je stoga potrebno dodatan oprez kako potencijalna nesreća ne bi imala devastirajuće učinke za poljoprivrednika i okoliš. Naravno, ovim se razmatranjem vraćamo i na prethodno pitanje odgovornosti – tko bi bio odgovoran u slučaju nesreće? Daljnji je problem s trenutnim AI sustavima iznimna energetska potrošnja, ali bi potencijalno rješenje za to bio analogan AI koji troše manje energije, no koji trenutno ima vlastite nedostatke (Ambrogio i sur., 2023).

Zaključak

Iz navedenih se etičkih problema uviđa da su oni međusobno povezani, stoga je nužan jedan holistički pristup. Naime, potrebna je suradnja između različitih struka kako bi se uspostavile određene maksime po kojima će funkcionirati AI i korporacije koje ih nude krajnjem korisniku. Potrebno je odrediti i pristup koji će društvo zauzeti prema okolišu – hoće li opći konsensus biti antropocentrički stav kojim će ljudi svemu ostalome negirati inherentnu vrijednost ili će pak zauzeti blaže pozicije poput patocentrizma ili biocentrizma. Naime, AI sadrži u sebi potenciju da poboljša kvalitetu života, ali je problem određivanja granica i nepoznavanja kakve bi dalekosežne posljedice imao AI na ekosferu i samog čovjeka. U radu je izrečeno da, osim navedenog, opasnost leži ujedno i u monopolizaciji tehnike i znanja. Postoji realna opasnost da će masovne korporacije proširiti svoj utjecaj i imati još veću nadmoć nad proizvođačima. No navedeno je moguće ograničiti raznim pravnim regulacijama na razini Europske Unije te nadopunom GDPR-a kojim se regulira nadzor nad privatnim podacima. Međutim, tu se također nalazi i potencijalni problem za transatlantske te euroazijske odnose, pa bi stoga kompanije mogle bojkotirati pravne izmjene kao što to danas čine pojedine kompanije. Također je potrebno uložiti napor u istraživanje novih tehnologija koje bi potencijalno mogle neutralizirati trenutne nedostatke AI sustava. Navedeno su sve problemi koji se moraju pažljivo razmotriti i detaljno legislativno urediti kako se AI revolucija ne bi isključivo iskoristila za održavanje na životu sve većeg broja ljudi, već i za opći boljitak društva. Ono što je sigurno, AI revolucija donosi nam jedan duboki lom s prošlošću i nepoznatim ostaje kakva će i kolika biti korporativna biomoc te kakav će stav društvo zauzeti spram etičkih dilema.

Literatura

- Allen R. (1994). Agriculture during the industrial revolution. In: The economic history of Britain since 1700: 96-123.
- Ambrogio S., Narayanan P., Okazaki A., Burr G.W. (2023). An analog-AI chip for energy-efficient speech recognition and transcription. *Nature*. 620: 768–775.
- Beck U. (1992). *Risk Society: Towards a New Modernity*. SAGE Publications.
- Carbonell I. (2016). The ethics of big data in big agriculture. *Internet Policy Review*. 5: (1).
- Dara R., Hazrati Fard S.M., Kaur J. (2022). Recommendations for ethical and responsible use of artificial intelligence in digital agriculture. *Frontiers in Artificial Intelligence*. 5: 884192.

- Devos Y., Maesele P., Reheul D., Van Speybroeck L., De Waele D. (2008). Ethics in the societal debate on genetically modified organisms: A (re) quest for sense and sensibility. *Journal of agricultural and environmental ethics*. 21: 29-61.
- Foucault M. (1978). *The History of Sexuality. Volume I: An Introduction*. New York: Pantheon Books.
- Fukuyama F. (2003). *Our posthuman future: Consequences of the biotechnology revolution*. Farrar, Straus and Giroux.
- Harari Y.N. (2015). *Sapiens: kratka povijest čovječanstva*. Zagreb: Fokus komunikacije.
- Hendrickson M.K., and James H. S. (2005). The ethics of constrained choice: How the industrialization of agriculture impacts farming and farmer behavior. *Journal of Agricultural and Environmental Ethics*. 18: 269-291.
- Khush G.S. (1999). Green revolution: preparing for the 21st century. *Genome*. 42: 646-655.
- Müller V.C. (2023). Ethics of Artificial Intelligence and Robotics. *The Stanford Encyclopedia of Philosophy (Fall 2023 Edition)*, Edward N. Zalta & Uri Nodelman (eds.), URL = <https://plato.stanford.edu/archives/fall2023/entries/ethics-ai/>
- Myerson G. (2002). *Ekologija i kraj postmoderne*. Zagreb: Naklada Jesenski i Turk.
- OECD AI. (2019). *OECD AI Principles Overview (2019)*. Raspoloživo: <https://oecd.ai/en/ai-principles>
- Okengwu U.A., Onyegbu L.N., Oghenekaro L.U. Musa M.O., Ugbari A.O. (2023). Environmental and Ethical Negative Implications of AI in Agriculture and Proposed Mitigation Measures. *Scientia Africana*. 22: 141-150.
- Ryan M. (2019). Ethics of using AI and big data in agriculture: The case of a large agriculture multinational. *The ORBIT Journal*. 2: 1-27.
- Šimić D. (2002). Poljoprivreda, biotehnologija i bioetika. In Lošinjski danji bioetike. Jurić, H. (ed.), 69–71. Zagreb, Croatia: Hrvatsko filozofsko društvo.
- Šimić D., Sviličić P., Galić V. (2023). Crop Production, Climate Change and Data Galore: Where are We Now?. Objavljeno u *Proceedings of International Summit on Renewable Energy (INSORE 2023)*, Kljak, K., Carović-Stanko, K. (eds.) p. 28, Agronomski fakultet Sveučilišta u Zagrebu, Zagreb.
- Šušnjara K. (2019). *Etičnost u korištenju velikih podataka*, Diplomski rad, Fakultet hrvatskih studija, Sveučilište u Zagrebu.
- Zagzebski L. (2001). The Uniqueness of Persons. *The Journal of Religious Ethics*. 29: 401–423.

Ethical implications of using artificial intelligence in agriculture

Abstract

Society is currently facing a new scientific revolution which is made possible by applying narrowed AI to it. This scientific revolution, which uses AI, is going to affect all spheres of human activity, including agriculture. However, history repeatedly shows us that not all scientific revolutions had a completely positive effect on the entirety of human society. Therefore, in order to prevent possible shortcomings, we are left with the question of what are the potential negative sides of using AI. The aim of this paper is to present the ethical implications of using artificial intelligence in agriculture, while also presenting its impact on society and the ecosphere.

Keywords: artificial intelligence, agriculture, ethics, ecosphere, privacy

Implementacija društvenih inovacija u hrvatskim perifernim ruralnim područjima

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Sažetak

Društvene inovacije u kontekstu ruralnog razvoja doprinose socioekonomskim promjenama u skladu s društvenim potrebama lokalnih zajednica. Njihova primjena uključuje tradicionalne načine proizvodnje u suvremenim sociokulturnim uvjetima života, što istodobno predstavlja poseban izazov. Cilj rada je analizirati učinke primjene društvenih inovacija u perifernim ruralnim mikroregijama u kontinentalnom i jadranskom području Hrvatske. Provedena je analiza implementacijskih procesa i to: identifikacija društvenih inovacija i njihovog doprinosa održivom razvoju. Provedena je komparativna analiza primjene šest društvenih inovacija povezanih s poljoprivrednim aktivnostima. Rezultati pokazuju društvene, ekološke i ekonomske učinke implementiranih inovacija koji diversificiraju gospodarske aktivnosti i utječu na razvoj ruralnih područja.

Ključne riječi: društvene inovacije, poljoprivreda, diversifikacija, ruralni razvoj

Uvod

Periferna ruralna područja izuzetno su važna za ruralni život, očuvanje tradicionalnog kulturnog naslijeđa, bioraznolikosti i ekosustava. Negativni ekonomski i demografski procesi vezani uz depopulaciju, deagrarizaciju, visoke stope nezaposlenosti i nedostatak socijalnih usluga posebno teške posljedice imaju upravo na ta područja. Istodobno, upravo ta područja imaju posebno značajnu ulogu u nacionalnim ekonomijama zbog njihove prirodne i proizvodne vrijednosti, kulturnog i tradicijskog naslijeđa te potencijala zapošljavanja. Stoga je u tim područjima posebno važno uvođenje društvenih inovacija koje predstavljaju nove načine suočavanja s razvojnim izazovima upotrebe državnih intervencija (Deže i sur., 2023) za stvaranje društvenih koristi i ekonomskih mogućnosti (Adams i Hess, 2010).

U ovom radu fokus je na područja s posebnim geografskim (otoci, brdsko-planinska područja), biološkim (poljoprivredna zemljišta visoke prirodne vrijednosti – *HNV eng. High Natural Value*¹) i ekonomskim obilježjima (poljoprivreda malih poljoprivrednih gospodarstava, niskog intenziteta), koja su obuhvaćena pojmom perifernih ruralnih područja. Ta područja imaju veliku biološku raznolikost i tradiciju poljoprivrednih praksi pri čemu imaju veliki potencijal razvoja lokalnih ekonomija, jer se mali kapacitet njihove proizvodnje prilagođava geografskim, biološkim i ekonomskim uvjetima.

Društvene inovacije odnose se na razvoj ili značajno unaprjeđenje strategija, koncepata, ideja, proizvoda ili usluga s ciljem da se naglase pozitivne socioekonomske promjene i/ili da se odgovori na značajne društvene potrebe. Mogu doprinijeti višim stopama zaposlenosti ili potrošnje u društvu te unaprijediti kvalitetu života (French i sur., 2014). U kontekstu revitalizacije ruralnih područja, s ciljem postizanja održivosti proizvodnih resursa, suradnje i povezivanja, te osiguravanja kanala znanja i informacija, društvene inovacije od velikog su značaja. U političkim dokumentima Europske komisije poseban naglasak stavlja se na društvena pitanja i inovacije, doprinos ublažavanju posljedica klimatskih promjena, okolišnim ciljevima, upravljanju rizicima i održivoj poljoprivrednoj proizvodnji (Ascani i De Vivo, 2020).

¹ Područja na kojima poljoprivredne aktivnosti doprinose bioraznolikosti, održanju kulturnog naslijeđa, visokokvalitetnih proizvoda i ruralnog zapošljavanja, a karakterizira ih niži intenzitet proizvodnje. Često su ta područja dijelovi zaštićenih područja u sklopu Natura 2000 staništa. Dodatno je njihova vrijednost u korištenju za rekreacijske svrhe te razvoj prirodnih i ruralnih turističkih praksi (<http://www.hnmlink.eu/>).

U ovom radu istražuju se društvene inovacije u perifernim ruralnim područjima Hrvatske s ciljem identificiranja njihovog doprinosa u tri stupa održivog razvoja: društvenom, ekonomskom i ekološkom.

Materijal i metode

Glavni izvori analiziranih podataka u ovom radu su edukativni materijali objedinjeni u virtualnoj banci studija slučajeva (*Virtual Case Study Bank*) koja je rezultat dvaju uzastopnih istraživačkih projekata programa ERASMUS+. Prvotni podaci prikupljeni su i analizirani u projektu HNV-Link u 10 europskih područja² gdje su prepoznate poljoprivredne i povezane djelatnosti visoke prirodne vrijednosti koje su implementirale društvene inovacije. Projektom su prikupljene i opisane inovacije koje podržavaju specifične lokalne poljoprivredne sustave i zajednice doprinoseći unapređenju socioekonomske i ekološke održivosti, a područja na kojima su inovacije primijenjene istaknuta su kao primjeri dobre prakse za učenje. Po završetku projektnih aktivnosti HNV-Link-a, nastavilo se s izradom edukativnih materijala u projektu RUR'UP - Innovative Education for Sustainable Development of Peripheral Rural Areas (2020.-2023.), unutar kojeg su se oblikovali edukacijski materijali i objedinili u virtualnu banku studija slučajeva o inovativnim rješenjima za razvoj perifernih ruralnih područja kako bi se mogli koristiti u svrhe učenja na primjerima dobre prakse. Edukacijski materijali korišteni su u pilot-projektu provedbe e-kolegija kojeg su provodila sveučilišta iz zemalja partnera na projektu³ korištenjem CPD (2022) platforme (*Teaching and Learning Online Courses*). Cjelokupna Virtualna banka studija slučajeva pohranjena je i na EPALE (2023) platformi (*Electronic Platform for Adult Learning in Europe*) koja je dostupna javnosti.

U ovom radu analizirani su odabrani slučajevi primijenjenih društvenih inovacija u perifernim ruralnim područjima Hrvatske, konkretnije na dalmatinskim otocima te u istočnoj Hrvatskoj, a koje su pridonijele ekonomskim, ekološkim i društvenim promjenama te su time prepoznate kao katalizatori gospodarskog razvoja na lokalnoj razini.

Društvene inovacije su analizirane s obzirom na njihove doprinose održivom razvoju. Korištena je metoda komparativne analize slučajeva; analizirani su slučajevi društvenih inovacija tekstualnom analizom materijala nastalih projektnim aktivnostima (HNV-Link 2019. i RUR'UP, 2023). Prvotno su identificirani indikatori održivog razvoja u svakom pojedinačnom slučaju, a potom su kodirani i grupirani prema pojavljivanjima u ključnim doprinosima društvenih inovacija - društvenim, ekološkim i ekonomskim koji predstavljaju tri stupa održivog razvoja. Svaka grupa obuhvaća pet indikatora koji su se u tekstu najčešće pojavljivali. Sveukupno, svaka od šest inovacija analizirana je s obzirom na 15 indikatora doprinosa održivom razvoju. Učinkovitost društvene inovacije zabilježena je potvrdno (.), dok je ostalo obilježeno negacijski (X). Pojavnost svakog pojedinog indikatora u svakoj grupi izražena je kao omjer parcijalne prisutnosti u odnosu na ukupni skup. Udjeli pojedinih doprinosa održivom razvoju - društveni, ekološki i ekonomski, prikazani su numerički (%) i grafički za dva analizirana područja Hrvatske.

Rezultati i rasprava

Društvene inovacije mogu se smatrati jednim od rješenja kako se suočiti s izazovima održivog razvoja perifernih ruralnih područja. Njihova primjena rezultat je aktivnosti lokalnih zajednica koje prepoznaju svoje potrebe i ostvaruju mogućnosti kojima doprinose društvenom, ekološkom i ekonomskom razvoju. Geografska raznolikost na nacionalnoj razini utječe i na oblike, tipove i doprinose društvenih inovacija održivom razvoju perifernih ruralnih područja. U radu su analizirana tri primjera dobre poduzetničke prakse u implementaciji društvenih inovacija u kontinentalnom području (KP₁-KP₃) i komparirana s tri primjera u jadranskom području Hrvatske (JP₄-JP₆), kako je prikazano u Tablici 1.

2 Bugarska, Hrvatska, Francuska, Grčka, Irska, Portugal, Rumunjska, Španjolska, Švedska i UK.

3 Zemlje partneri u projektu: Bugarska, Finska, Grčka, Francuska, Irska, Rumunjska i Hrvatska.

Tablica 1. Implementacija društvenih inovacija u kontinentalnom i jadranskom području Hrvatske

Područja	Nazivi
Kontinentalno područje	KP ₁ Kratki lanac opskrbe u okolici Osijeka
	KP ₂ Objekt za preradu poljoprivrednih proizvoda malih proizvođača
	KP ₃ Transfer znanja u suradnji s osječkim Sveučilištem
	JP ₄ Otok Murter: Akcijski plan održivog korištenja resursa
Jadransko područje	JP ₅ Dalmatinski otoci: organizacije s više dionika koje potiču proizvode i prakse HNV-a
	JP ₆ Dalmatinski otoci: HNV poljodjelstvo kao turistička djelatnost

Prema nazivima implementiranih društvenih inovacija prepoznaju se razlike u ciljevima, aktivnostima i potrebama lokalnog stanovništva koje su se razvile u skladu s geografskim specifičnostima perifernih ruralnih područja. Opisi inicijativa, aktivnosti i procesa upravljanja promjenama navedeni su kako slijedi:

KP₁ Kratki lanac opskrbe u okolici Osijeka – inicijativa se oslanja na strategiju participativnog sudjelovanja lokalnom razvoju i uključuje različite dionike za rješavanje problema pri horizontalnom umrežavanju ekoloških proizvođača i stvaranju preduvjeta za bolji plasman njihovih proizvoda. Rezultat provedbe inovativnog procesa su bili uspostava udruge ekoloških proizvođača u OBŽ i uspostave prodaje ekoloških proizvoda za Osijek i okolice u okviru osječskog kratkog opskrbnog lanca s Internet prodajom. Time je ostvarena baza za širenje mreže ekoloških proizvođača i različitih oblika prodaje kroz kratki lanac opskrbe čime je ostvaren rast prihoda, osnažena tržišna pozicija i njihova konkurentnost.

KP₂ Objekt za preradu ekoloških poljoprivrednih proizvoda – aktivnosti su usmjerene na povezivanje malih proizvođača s ciljem zajedničkog korištenja pogona za preradu primarnih ekoloških poljoprivrednih proizvoda izgrađenog Ivanovcima. Objekt je u vlasništvu Grada Valpova, uređen sredstvima EU projekta, certificiran za preradu ekoloških proizvoda te je predan na korištenje udruzi ekoloških proizvođača. Pogon je opremljen linijom za preradu soka od jabuka i drugog voća, pasirkom za rajčicu, kotlom za ukuhanje sokova i džemova, sušarom za voće i čajeve manjeg kapaciteta te pasterizatorom s linijom za punjenje staklenih boca i *bag-in-box* ambalažu. Postignuto je osnaživanje tržišne pozicije malih ekoloških poljoprivrednih proizvođača koji su stekli mogućnost da preradom proizvoda postignu veću dodanu vrijednost svoje proizvodnje.

KP₃ Transfer znanja u suradnji s osječkim Sveučilištem – suradnja putem zajedničkog projekta Sveučilišta Josipa Jurja Strossmayera, Tera Tehnopolis – ured za transfer tehnologija i Fakulteta agrobiotehničkih znanosti Osijek s ekološkim proizvođačima uz potporu lokalnih institucija. Cilj je bio istaknuti važnost ekološke poljoprivrede i uloge malih proizvođača u opskrbljivanju lokalnih tržišta te doprinijeti unaprjeđivanju kompetencija ekoloških proizvođača u skladu s njihovim potrebama. Aktivnosti su omogućile: razvoj preduvjeta za implementaciju novih tehnologija i inovacija u ekološkoj proizvodnji koji su rezultat znanstvenih istraživanja; koncipiranje razvoja marketinških strategija za lokalno tržište; poboljšanje preduvjeta za brendiranje ekoloških proizvoda, te podrška organiziranju zajedničkih prodajnih kanala ekoloških proizvođača. Kroz aktivnosti deset radionica educirano je između 15 do 30 sudionika o temama: solidarnost u proizvodnji i prodaji ekološke hrane, bioraznolikost obradivih površina, ekološki načini zaštite, ekološki uzgoj svinja i peradi, novi pristupi ispaši i hranidbi stoke, inovativni marketinški principi, razvoj tržišta ekološke hrane te kratkih lanaca opskrbe ekološki proizvedenom hranom.

JP₄ Otok Murter: Akcijski plan održivog korištenja resursa (Argonauta, 2022) - primjer je inovacije u ruralnom razvoju koja se provodi temeljem Akcijskog plana održivog korištenja resursa na području otoka Murtera, Jezera Vrana, te Nacionalnog parka Kornati. Promjene u pretvorbi tradicijske u modernu praksu HNV-a vođene su s ciljem prijenosa znanja o tradicijskim praksama koje nestaju zbog napuštanja poljoprivrede i starenja stanovništva u ruralnim područjima. Oživljeno je i aktivirano znanje i iskustvo lokalne zajednice prema smjernicama zaštite prirode i održivog korištenja njezinih resursa. Koncept održivog turizma nastao je sinergijom zaštite prirode i razvoja otoka Murtera. Uspostavljena je digitalna platforma koja uključuje otočane kako bi se ojačali kapaciteti dionika i razvilo participativno upravljanje lokalnim područjem. To je primjer prakse participativnog upravljanja zaštićenim područjima po uzoru na francuske ekomuzeje koji oživljavaju lokalne tradicionalne prakse održivog korištenja prirodnih resursa (Botica i sur., 2017).

JP₅ Dalmatinski otoci: organizacije s više dionika koje potiču proizvode i prakse HNV-a, na području dalmatinskih otoka postoji nekoliko LEADER organizacija koje neizravno promiču HNV (LAG-5, 2022). Surađujući s javnim,

privatnim i civilnim sektorom, ostvaruju suradnju u svrhu dobivanja EU sredstava za lokalne projekte koji potiču ruralni razvoj i održivost agroekosustava. Primjer za to je Slow Food Pelješac Convivium koji promiče kako brendirane domaće proizvode tako i korištenje hrane temeljeno na načelima dobre, svježe, lokalne i sezonske prehrane. Aktivnosti su usmjerene na promociju sezonskih prehrambenih proizvoda, zaštitu biološke raznolikosti, lokalnih recepata, namirnica te hrane koja je zaboravljena ili na neki način ugrožena. Istodobno se utječe na proizvođače i potrošače kroz promicanje organske poljoprivrede bez pesticida (Andlar i sur., 2017).

JP₆ Dalmatinski otoci: HNV poljodjelstvo kao turistička djelatnost. Prema Nicolle i sur., (2017), primjeri inovacija okupljaju različite dionike koji su povezani zajedničkim pristupom čija je bit obraditi poljoprivredna zemljišta svojih predaka i revitalizirati povijesnu baštinu. Jedan od hrvatskih primjera je Muzej maslinovog ulja (Muzej maslinovog ulja, 2022.) na otoku Braču. Osim razgledavanja, turisti mogu sudjelovati u radovima na maslinicima, te kušati i kupiti domaću hranu. Sljedeća inovacija je rasadnik autohtone vrste „Anemona“ kao primjer tradicionalne proizvodnje sadnica autohtonog područja u skladu s praksom HNV-a. Sudjeluju u očuvanju biološke raznolikosti te svojom ponudom i prodajom upotpunjuju turističke usluge otoka Korčule. Prema Roglič (2017), realizacija inovacija dugoročno ovisi o osiguranju financijskih sredstava za troškove provedbe akcijskih planova, edukacije te suradnje u implementaciji i brendiranju proizvoda. Proces upravljanja promjenama nisu dizajnirani za postizanje visoke prirodne vrijednosti (HNV) ili ciljeva očuvanja, ali je vjerojatno da će to biti dugoročno moguće postići kroz rezultate održavanja ekstenzivnih sustava ispaše i smanjenja udjela šikare. Potencijalno se upravljanje promjenama može prilagoditi posebnim ciljevima HNV-a uključivanjem nadležnih institucija za zaštitu okoliša u skladu s ciljevima Zelenog plana.

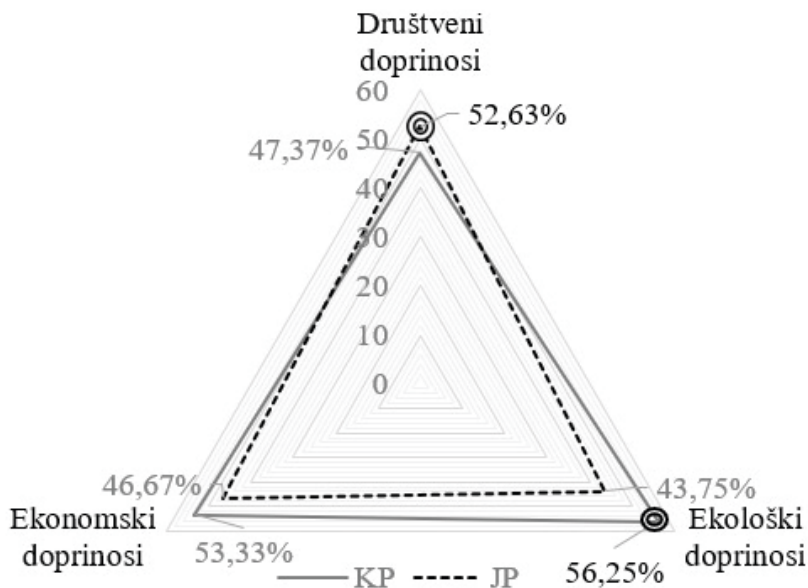
Prema rezultatima analize oblikovani su indikatori i identificirani su društveni, ekološki i ekonomski doprinosi za svaku inovaciju u istraživanju, te su potvrdno () ili negacijski (X) prikazani u Tablici 2.

Tablica 2. Doprinosi društvenih inovacija održivom razvoju u kontinentalnom i jadranskom perifernom ruralnom području Hrvatske

Doprinosi	Indikatori	Kontinentalno područje			Jadransko područje		
		KP ₁	KP ₂	KP ₃	JP ₄	JP ₅	JP ₆
Društveni doprinos	1. Odgovornost pojedinca	✓	X	✓	✓	X	✓
	2. Socijalna sigurnost	✓	X	X	X	✓	✓
	3. Smanjenje siromaštva	X	X	X	✓	✓	X
	4. Društveno umrežavanje	✓	✓	✓	✓	X	X
	5. Osobna samoodrživost	✓	✓	✓	✓	✓	✓
Ekološki doprinos	6. Zaštita staništa	X	✓	X	✓	✓	✓
	7. Ekološka proizvodnja	✓	✓	✓	X	✓	X
	8. Ekološka osviještenost	X	✓	✓	X	X	X
	9. Zaštita bioraznolikosti	X	✓	X	✓	✓	✓
	10. Smanjenje ekološkog otiska	✓	X	✓	X	X	X

Ekonomski doprinos	11.	Profitabilnost farme	✓	✓	✓	X	✓	X
	12.	Rast konkurentnosti	✓	X	✓	X	✓	X
	13.	Korištenje državnih potpora	X	X	X	✓	X	✓
	14.	Povezivanje gospodarskih subjekta	✓	✓	X	✓	X	✓
	15.	Javne investicije	X	X	✓	X	X	✓

Na osnovu prisutnosti pojedinih indikatora utvrđeno je da je u ukupnoj strukturi najprisutniji društveni doprinos (38%) u oba istraživana geografska područja, zatim slijedi ekološki doprinos (32%) i najmanji je ekonomski doprinos (30%). Sa svrhom prepoznavanja geografskih različitosti provedeno je sumiranje svih društvenih doprinosa kroz pet indikatora, od prvog do šestog primjera primjene inovacija, te su izračunati udjeli za svako geografsko područje. Istovrsni postupak primijenjen je za izračunavanje udjela ekološkog i ekonomskog doprinosa kako za kontinentalno (KP) tako i za jadransko (JP) područje Hrvatske. Rezultati komparativne analize prema društvenim, ekološkim i ekonomskim doprinosima održivom razvoju perifernih ruralnih područja potvrđuju razlike u njihovoj distribuciji, kako je prikazano u Grafikonu 1.



Grafikon 1. Komparacija društvenih inovacija prema grupama doprinosa održivom razvoju i geografskim područjima Hrvatske

Najveća je zastupljenost inovacija s ekološkim doprinosom (56.25%) u kontinentalnom području, zatim slijede ekonomski (53,33%) i društveni (47,37%). U jadranskom području prevladava društveni doprinos (52,63%), a slijede ga ekonomski (46,67%) i ekološki (43,75%).

Zaključak

Implementacija društvenih inovacija je sve aktualnija zbog ostvarivanja ciljeva održivog razvoja u perifernim ruralnim područjima. Rezultati komparativne analize šest primjera potvrđuju razlike u njihovim doprinosima održivom razvoju i prema geografskim područjima u kojima su nastale. Doprinosi su identificirani korištenjem indikatora koji su svrstani u tri skupine, tri stupa održivog razvoja: društveni, ekološki i ekonomski. U kontinentalnom području inovacije su imale značajniji ekološki doprinos kroz povezivanje ekoloških proizvođača u cilju unaprijeđena prodaje, izgradnji objekta za preradu ekoloških proizvoda i unaprjeđivanju kompetencija ekoloških proizvođača. Na jadranskom području društvene inovacije postigle su razvojne perspektive održivosti s društvenim doprinosima i to aktiviranjem znanja i iskustva lokalnog stanovništva prema zaštiti prirode i održivog korištenja resursa uspostavljanjem digitalne platforme za participativno upravljanje lokalnim održivim razvojem. Utvrđeni doprinosi društvenih inovacija održivom razvoju perifernih ruralnih područja nastajali su u skladu s lokalnim potrebama, te ih je zbog toga potrebno poticati i podržavati kroz aktivnosti komparativnih institucija privatnog i javnog sektora.

Napomena

Podaci o procesima implementacija društvenih inovacija u periferna ruralna područja prikupljeni su i kreirani kao virtualna baza podataka kroz projekt RUR'UP Innovative Education for Sustainable Development of Peripheral Rural Areas, KA2, Erasmus+2020. programa (<https://epale.ec.europa.eu/en/group/171142>).

Literatura

- Adams D., and Hess M. (2010). Social Innovation and Why it has Policy Significance. *The Economic and Labour Relations Review*. 21: 139–55.
- Andlar G., Roglić M., Pavlinović Mršić S. (2017). Inovacije za visoko vrijedne poljoprivredne površine na otocima. Raspoloživo na: http://www.hnmlink.eu/download/CR_NationalLanguageReport_innovationseminar_Dalmatianislands.pdf
- Argonauta (2022). Udruga za održivi razvoj zajednice pronalazanjem inovativnih rješenja. Raspoloživo na: <https://argonauta.hr/o-nama/>
- Ascani M., and De Vivo C. (2020). Social innovation and rural development in the documents on the future of EU policies. In *Green Metamorphoses: Agriculture, Food, Ecology: Proceedings of the LV Conference of SIDEA Studies*. Wageningen: 242–251.
- Botica I., Grgić J., Muštra, V., Pavlinović Mršić S., Roglić, M., Šimunović B. (2017). Learning Area “Dalmatian Islands” (Croatia) Innovation Experiences and Needs. Project: High Nature Value Farming Thematic Network (HNV-Link). Croatia: University of Split.
- CPD. (2022). Customizable Personal Development, Learn Online Is Managed by the ATU Teaching & Learning Centre (Galway-Mayo) and Is Hosted by HEANet Ireland. Raspoloživo na: <https://www.cpdlearnonline.ie/my/>
- Deže J., Sudarić T., Tolić S. (2023). Social Innovations for the Achievement of Competitive Agriculture and the Sustainable Development of Peripheral Rural Areas. *Economies*. 11: 209.
- EPALE. (2023). Electronic Platform for Adult Learning in Europe. Raspoloživo na: <https://epale.ec.europa.eu/en/group/171142>
- French J., Montiel K., Palmieri V. (2014). Innovation in Agriculture: A Key Process for Sustainable Development. Inter-American Institute for Cooperation on Agriculture. <https://repositorio.iica.int/bitstream/handle/11324/2607/BVE17038694i.pdf;jsessionid>
- HNV-Link (2019). High Nature Value Farming: Learning, Innovation and Knowledge. Raspoloživo na: <http://www.hnmlink.eu/>
- LAG. (2022). Local Action Group, LAG-5. Raspoloživo na: <http://www.lag5.hr>
- Muzej uja (2022). Raspoloživo na: <https://www.muzejuja.com/>
- Roglić M. (2017). Learning Area Dalmatian Islands Croatia, Innovation Experiences and Needs. Research Report. Supetar: LAG Brač. Hvar: LAG Škoji. Orebić: LAG 5.

Implementation of Social Innovations in Croatian Peripheral Rural Areas

Abstract

Social innovations in the context of rural development contribute to socioeconomic changes in accordance with the social needs of local communities. Their application includes traditional ways of production in contemporary socio-cultural living conditions, which at the same time represents a special challenge. The aim of the paper is to analyze the effects of the application of social innovations in peripheral rural microregions in the Continental and Adriatic areas of Croatia. An analysis of implementation processes was carried out, namely: identification of social innovations and their contribution to sustainable development. A comparative analysis of the application of six social innovations related to agricultural activities was carried out. The results show the social, ecological and economic effects of implemented innovations that diversify economic activities and influence the rural development.

Keywords: social innovation, agriculture, diversification, rural development

Pregled svinjogojskog sektora u Republici Hrvatskoj metodom mjesečnih ostataka

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Sažetak

Rad prikazuje pregled svinjogojskoga sektora u Republici Hrvatskoj metodom mjesečnih ostataka (eng. *Monthly remainders methodology*) u periodu od 2019. do 2023. godine. Metoda predstavlja analizu osnovnih proizvodnih ulaznih čimbenika u sektoru svinjogojstva kojom se izračunava preostali dio iznosa u EUR koji ostaje proizvođačima nakon što se troškovi osnovnih proizvodnih inputa oduzmu od prosječnih mjesečnih cijena svinjskoga trupa. Rezultati su uspoređeni sa predpandemijskom 2019. godinom jer u navedenoj godini nije bilo izrazitih tržišnih šokova. Na temelju analize 2021. i 2022. godina predstavljaju najizazovnije razdoblje u sektoru svinjogojstva zbog visokih ulaznih troškova i niske otkupne cijene trupa.

Ključne riječi: svinjogojski sektor, metoda mjesečnog ostatka, Republika Hrvatska

Uvod

Svinjogojski sektor predstavlja značajnu granu poljoprivredne proizvodnje koja ima duboke korijene u hrvatskoj tradiciji i kulturi, poglavito na području istočne Hrvatske (Grgić i sur., 2019). Recentne stalne pojave tržišnih šokova predstavljaju ogroman izazov razvoju svinjogojstva u Republici Hrvatskoj. Počevši s izbijanjem COVID-19 pandemije u 2020. godini došlo je do značajnih poremećaja na globalnom i jedinstvenom tržištu svinjskog mesa, s viškovima koji su dramatično smanjili cijene. Izrazito niske razine cijena zadržavale su se sve do veljače 2022. godine, kada je počeo postupan oporavak cijena svinjskoga mesa. Međutim, usprkos oporavku cijena mesa, proizvođači nisu osjetili olakšanje tijekom 2022. godine zbog pojave inflacije. Opći rast cijena, prvenstveno energenata zbog rata u Ukrajini, razlogom je znatnim povećanjem cijena žitarica i stočne hrane (Sohag i sur., 2023). U 2023. godini također se ističu povišeni osnovni proizvodni troškovi, prije svega visoka cijena prasadi. Dodatno, suočavamo se s novim vanjskim tržišnim šokom, a to je pojava afričke svinjske kuge (ASK), koja dodatno produbljuje već postojeće negativne trendove u domaćem sektoru svinjogojstva (Kranjac i sur., 2018., Grgić i sur., 2016).

Materijal i metode

Metoda mjesečnih ostataka (eng. *Monthly remainders methodology*) predstavlja ustaljenu metodu opservatorija tržišta mesa (eng. *Meat Market Observatory*) koji djeluje u sklopu Europske komisije odnosno DG-AGRI-ja. Njena svrha je praćenje i analiza osnovnih proizvodnih ulaznih čimbenika u sektoru svinjogojstva na razini Europske unije (EU). Kao takva, metoda omogućuje izračunavanje preostalog iznosa koji ostaje proizvođačima nakon što se troškovi osnovnih proizvodnih inputa oduzmu od prosječnih mjesečnih cijena svinjskoga trupa.

Osnovne proizvodne inpute čini cijena osnovnih sastojaka stočne hrane i cijena prasadi uz koje se usporedno prate cijene svinjskoga trupa. Prosječna mjesečna cijena osnovnih sastojaka stočne hrane izračunava se na temelju prosječnih mjesečnih cijena žitarica (stočnog ječma, pšenice i kukuruza) i sojine sačme u kojoj žitarice sudjeluju sa 85% udjela a sojina sačma s 15%. Prosječne mjesečne cijene prasadi, svinjskoga trupa i osnovnih sastojaka stočne hrane preuzete su sa DG AGRI-jeve platforme za praćenje cijena roba u poljoprivredi (eng. *DG AGRI commodity price monitoring: Monthly commodity dashboard*).

Pored cijena osnovnih inputa, metoda uključuje osnovne parametre ciklusa tova svinja od kojih neki predstavljaju

fiksne vrijednosti poput: početne težine prasadi, prosječnog dnevnog prirasta, razdoblja tova, konverzije hrane, randmana trupa, dok drugi predstavljaju izračunate vrijednosti poput: potrebne količine hrane za 100 kg težine trupa, konačne težine tovljenika, težine trupa, itd. S obzirom na ukratko opisanu metodologiju izrađen je pregled prosječnih mjesečnih cijena stočne hrane, prasadi, trupa, te preostalog iznosa u EUR koji ostaje proizvođaču nakon što se prosječne mjesečne cijene osnovnih proizvodnih inputa oduzmu od prosječnih mjesečnih cijena svinjskoga trupa u Republici Hrvatskoj od 2019. do zadnjih dostupnih podataka u 2023. godini

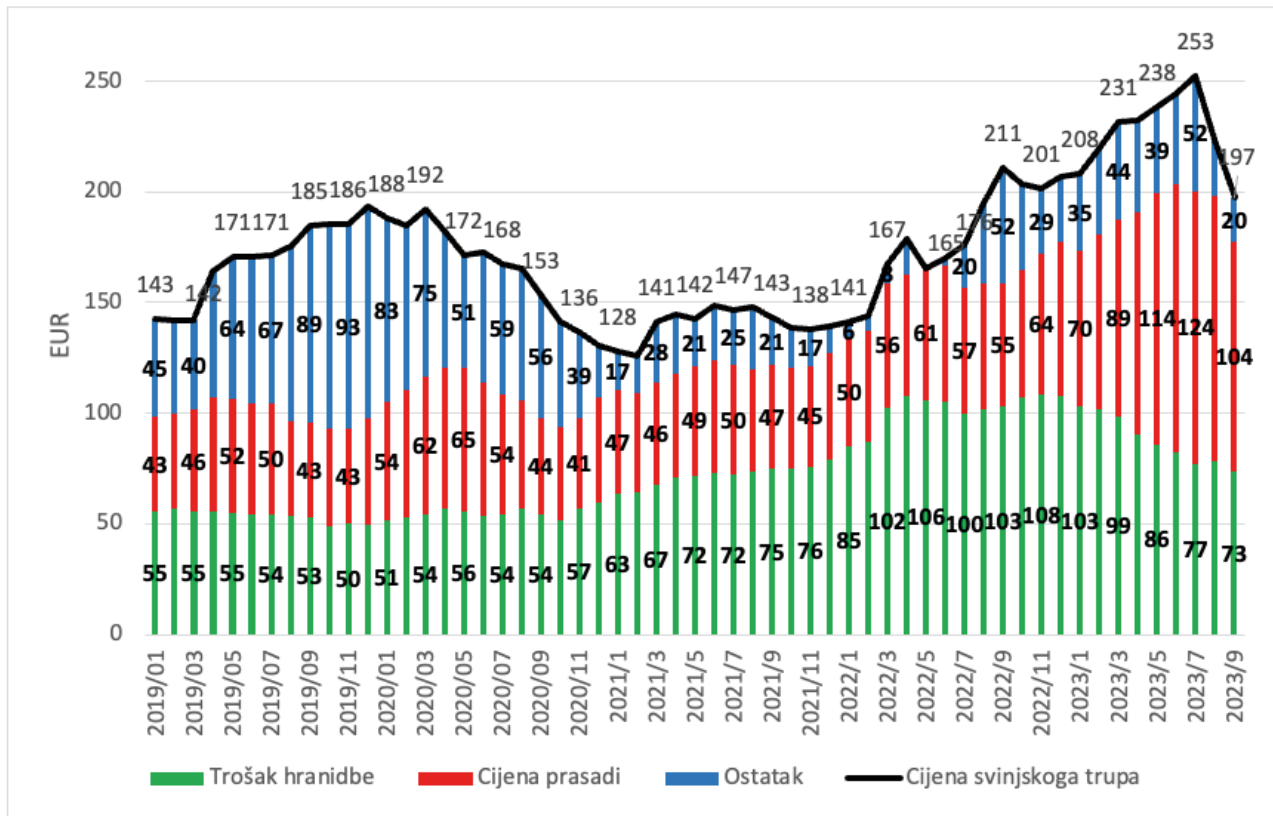
Čimbenici koji se koriste prilikom izračunavanja mjesečnih ostataka u tovu svinja:

- Početna težina prasadi PTP (kg) = fiksno = 25,00
- Prosječni dnevni prirast – PDP (kg) = fiksno = 0,8
- Trajanje tova (mjeseci) = fiksno = dani = 121,7
- Utrošak krmiva za prosječni dnevni prirast kg prirasta – konverzija – fiksno = 3,00
- Prirast trupa PT (indeks) = fiksno =0,78
- Završna težina ZT (kg) = izračun = Početna težina prasadi * (PDP * trajanje tova u danima)
- Težina trupa TT (kg) = izračun = Završna težina * Prirast trupa
- Utrošak krmiva UK (kg za 100 kg trupa) = PDP * trajanje tova u danima * konverzija) * 100 / Težina trupa
- Sastav smjese fiksno
- Prosječna cijena smjese PCS (euro/kg) = mjesečna cijena krmiva * udio krmiva u smjesi
- Trošak hranidbe TH = Prosječna cijena smjese PCS (eura/100kg težine trupa) *Utrošak krmiva UK (kg za 100 kg težine trupa)
- Cijena za prasid CP (eura za prase) = fiksno
- Ulazna vrijednost prasadi UVP(euro/100 kg trupa) = cijena za prasid * 100 / Težina trupa
- Cijena trupa CT = prosjek S,E i R klase trupa
- Mjesečni ostatak (euro/100 kg trupa) = Cijena trupa CT- (Cijena glavnih krmiva CGK + Ulazna vrijednost prasadi UVP)

Rezultati i rasprava

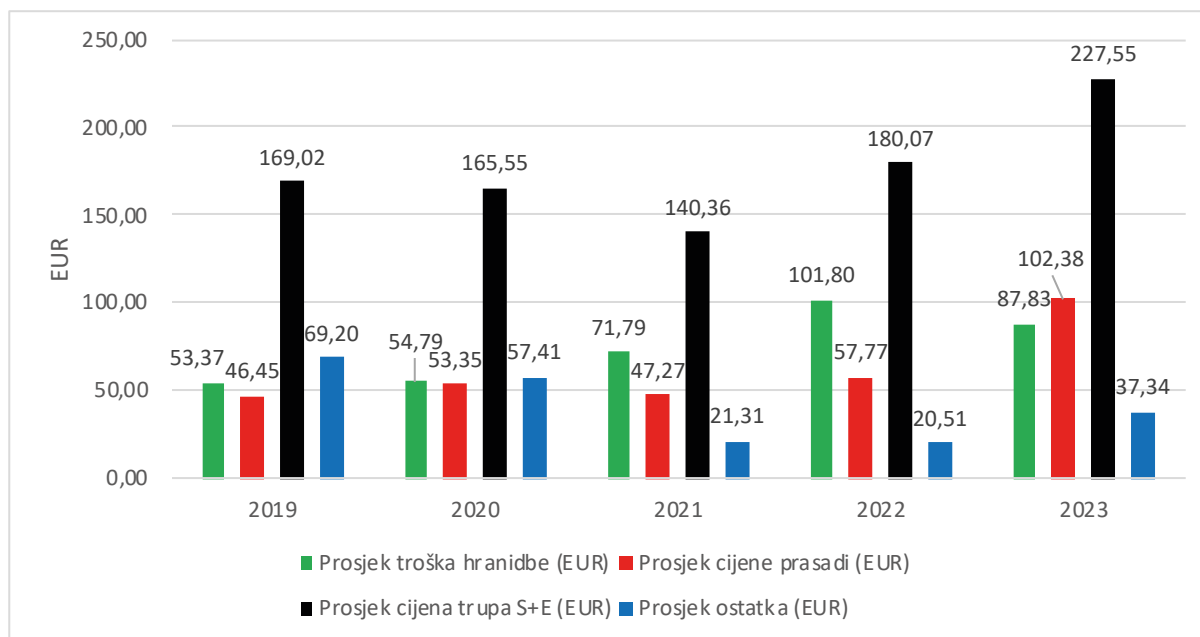
Rezultati u predpandemijskoj 2019. godini služe za usporedbu promatranih parametara sa ostalim analiziranim godinama, iz razloga jer u navedenoj godini nije bilo izrazitih tržišnih šokova i volatilnosti. Prosječni godišnji troškovi hranidbe u 2019. godini iznosili su 53,37 EUR/tovljeniku, prosječna godišnja cijena prasadi bila je 46,45 EUR/prasetu, prosječne godišnje cijene trupa S+E 169,02 EUR/trupu (NN., 2/2009), a prosječni godišnji ostatak proizvođaču iznosio je 69,20 EUR/tovljeniku. U narednoj 2020. godini, kada usporedimo vrijednosti godišnjih prosjeka promatranih parametara u odnosu na 2019. godinu ne uočavamo značajnija odstupanja iako je COVID 19 pandemija započela početkom godine.

No problemi vezani uz tržišne viškove na jedinstvenom tržištu počinju se javljati rujnu 2020. godine što je vidljivo iz Grafikona 1., gdje cijene trupa odnosno mesa počinju značajno padati. 2021. godina označava početak jedne od najgorih kriza sektora svinjogojstva od samostalnosti RH, troškovi hranidbe počinju rasti, te su u prosjeku za 34,5% viši, dok je prosječna godišnja cijena S+E trupa niža za 17 %, što rezultira 69,2% nižom vrijednosti ostatka proizvođaču u odnosu na 2019. godinu.



Grafikon 1: Pregled svinjogojskoga sektora RH od 2019.-2023. metodom mjesečnih ostataka
 Izvor: Izračun autora prema DG AGRI - Monthly commodity dashboard

Povijesno niske cijene svinjskoga mesa zadržavale su se sve do veljače 2022. godine, no oporavak cijene mesa nije rezultirao oporavkom sektora gdje vidimo kako je ostatak proizvođaču u sektoru svinjogojstva u prosjeku bio manji nego 2021. godine, te je u prosjeku na godišnjoj razini iznosio 20,51 EUR. Razlog tomu je siloviti rast cijena troška hranidbe zbog rata u Ukrajini, te je prosječni godišnji trošak hranidbe u 2022. godini bio za 90,7 % veći u odnosu na 2019. godinu. Tekuću 2023. godinu također obilježavaju relativno visoki troškovi hranidbe koji su za 64,5% viši u odnosu na 2019. godinu. Uz relativno visoke troškove hranidbe u 2023. godini dolazi do snažnog rasta cijena prasadi koja je u prosjeku viša za čak 120,4% u odnosu na predpandemijsko razdoblje. Ekstremno visoke ulazne troškove donekle ublažava cijena trupa koja je u prosjeku 2023. godine viša za 34,6% u odnosu na 2019. godinu, što rezultira nešto većim prosječnim ostatom proizvođaču koji prema do sada dostupnim podacima iznosi u prosjeku 37,34 EUR po tovljeniku na godišnjoj razini.



Grafikon 2. Prosječni godišnji iznosi promatranih vrijednosti svinjogojskog sektora po tovljeniku/trupu/prasetu u RH od 2019.-2023.

Izvor: Izračun autora prema DG AGRI - Monthly commodity dashboard

Iz Grafikona 2. možemo vidjeti prosjeke troška hranidbe, cijena prasadi, cijena trupa i mjesečnih ostataka za period od 2019.-2023. iz kojih se vidi kako su 2021. i 2022. godina bile najizazovnije godine u sektoru, s time da 2023. godina predstavlja blagi oporavak sektora zbog rasta prosječnih otkupnih cijena svinjskoga trupa do rujna 2023.

Zaključak

Prema dosadašnjim istraživanjima svinjogojski sektor od ulaska Hrvatske u Europsku uniju bilježi negativne trendove, te se očekuje daljnji nastavak i produbljivanje krize sektora. U prilog tome idu i rezultati ove analize metodom mjesečnih ostataka. Iz rezultata možemo zaključiti kako sektor posljednje tri godine ima izrazito niske prosječne mjesečne ostatke, uglavnom zbog visokih ulaznih troškova i niskih otkupnih cijena trupa (između 21,31 – 37,34 EUR) u odnosu na predpandemijsku 2019. godinu kada je prosječni godišnji mjesečni ostatak iznosio 69,20 EUR po tovljeniku.

Metoda mjesečnih ostataka naime ne uključuje sve vrste ostalih troškova na farmi, ali daje dobar uvid u kojim periodima se sektor nalazi u krizi.

Dodatno, Republika Hrvatska suočava se s novim egzogenim šokom, a to je pojava afričke svinjske kuge, koja će dodatno produbiti već postojeće negativne trendove u domaćem sektoru svinjogojstva, gdje je iznimno teško procijeniti cjelokupne i točne razmjere negativnih učinaka sektoru domaćem svinjogojstva u narednim godinama.

Literatura

- Europska komisija, DG AGRI (2023). Monthly commodity dashboard. Raspoloživo na: <https://agridata.ec.europa.eu/extensions/DashboardPrice/DashboardMarketPrices.html> (03.11.2023.)
- Grgić I., Krznar S., Bratić V. (2019). Poljoprivredna proizvodnja Republike Hrvatske prije i nakon pristupanja EU. U: Bilandžija, N., Kovačev, I., ur. Aktualni zadaci mehanizacije poljoprivrede. Zagreb: Sveučilište u Zagrebu, Agronomski fakultet, Zavod za mehanizaciju poljoprivrede, 487-496.
- Grgić I., Hadelan L., Prišenk J., Zrakić M. (2016). Stočarstvo Republike Hrvatske: stanje i očekivanja. Meso. 3: 256-262.

Kranjac D., Zmaić K., Erjavec E. (2018). Pregled i perspektiva tržišta svinjskog mesa u Republici Hrvatskoj-simulacija modelom parcijalne ravnoteže. *Agroeconomia Croatica*. 8: 75-83.

Narodne novine, (2009). Pravilnik o kakvoći svinjskih trupova i polovica. Raspoloživo na: https://narodne-novine.nn.hr/clanci/sluzbeni/2009_01_2_43.html

Sohag K., Islam M., Tomas Žiković I., Mansour H. (2023). Food inflation and geopolitical risks: analyzing European regions amid the Russia-Ukraine war. *British Food Journal*. 125: 2368-2391.

Outlook on the pork meat market in the Republic of Croatia using the monthly remainder method

Abstract

The paper presents an outlook of the pork meat sector in the Republic of Croatia using the monthly remainders methodology in the period from 2019 to 2023. The method represents an analysis of the basic production input factors in the pig farming sector, by which we calculate the remaining amount that remains for producers after the costs of basic production inputs are subtracted from the average monthly prices of pig carcasses. The results were compared with the pre-pandemic year 2019 because there were no significant market shocks in that year. Based on the analysis, the years 2021 and 2022 represent the most challenging period in the pig farming sector due to high input costs and low carcass purchase prices.

Keywords: Pork meat market outlook, Monthly remainder, Republic of Croatia

Sprječavanje prijevvara na tržištu ličkog krumpira sa oznakom zemljopisnog podrijetla primjenom pametnih ugovora

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Sažetak

Prijevare s hranom sve su učestaliji problem koji nanosi veliku štetu tržištu hrane. Projekt H2020 ALLIANCE¹ usmjeren je na sprječavanje prijevvara s hranom u opskrbnim lancima proizvoda s oznakom kvalitete kao što su ekološki proizvodi i proizvodi zaštićeni zemljopisnim oznakama. Za Hrvatsku, specifični cilj projekta ALLIANCE je poboljšanje sljedivosti ličkog krumpira zaštićenog oznakom zemljopisnog podrijetla (ZOZP) primjenom pametnih ugovora (*engl. smart contracts*). Tijekom 2023. godine provedena je prva faza projekta, tijekom koje su utvrđeni sljedeći izazovi u opskrbnom lancu ličkog krumpira ZOZP: mali broj proizvođača u sustavu zaštite, teško praćenje i sprječavanje zlouporabe imena „Lički krumpir“, nedostatak provjera krumpira izvan sustava certificiranja ZOZP, sustav sljedivosti temelji se na elementarnom vođenju dokumentacije u Word i Excel obrascima, nedostatak infrastrukture i znanja za digitalizaciju i implementaciju blockchain sustava sljedivosti.

Ključne riječi: EU H2020, prijevare s hranom, pametni ugovori, lički krumpir, ZOZP

Uvod

Prijevare s hranom postaju sve učestaliji problem koji ozbiljno narušava integritet tržišta hrane (Brooks i sur., 2022.). Procjenjuje se da trošak za globalno gospodarstvo iznosi između 40-50 milijardi dolara godišnje (Platonova, 2023). Prijevare s hranom definirane su kao namjerno mijenjanje svojstava i sastava namirnica korištenjem zamjenskih sirovina, aditiva te nedopuštenih tvari, čime se obmanjuju potrošači, smanjuje prehrambena vrijednost namirnice, te izlaže potrošače štetnom utjecaju na njihovo zdravlje (FAO, 2021). Prijevare hranom uključuju razne oblike krivotvorenja, patvorenja i lažnog označavanja prehrambenih proizvoda kako bi se ostvarila ne pripadajuća ekonomska korist.

Žrtve prijevare s hranom i krivotvorenja mogu biti različiti sudionici u lancima opskrbe kao što su distributeri, trgovci na malo te potrošači (Rizzutti, 2020). Svi oni suočavaju se s ekonomskim gubicima te zdravstvenim i sigurnosnim rizicima pri rukovanju ili konzumiranju lažnih proizvoda. Prijevvara s hranom posebno je značajna za segment prehrambenih proizvoda s oznakom kvalitete zbog viših profitnih marži u usporedbi s ostatkom tržišta hrane. Projekt ALLIANCE je usmjeren na sprječavanje prijevvara s hranom u opskrbnom lancu proizvoda s oznakom kvalitete kao što su ekološki proizvodi, proizvodi zaštićeni zemljopisnim oznakama. Sveučilište u Zagrebu Agronomski fakultet, Udruga proizvođača ličkog krumpira (UPLK) i Biotechnicon poduzetnički centar d.o.o. su među 24 partnera u ovom projektu kojeg koordinira University of Thessaly iz Grčke. Za Hrvatsku, specifični cilj projekta ALLIANCE je poboljšanje sljedivosti ličkog krumpira zaštićenog oznakom zemljopisnog podrijetla (ZOZP) primjenom pametnih ugovora (*engl. smart contracts*).

Krumpir se nalazi u top 10 najvažnijih usjeva na globalnoj razini i neizostavni je dio prehrane diljem svijeta. U Hrvatskoj se ukupna proizvodnja krumpira u 2021. godini odvijala na 8.786 ha s prosječnim prinosom od 14,5 t/ha i

1 A holistic framework in the quality Labelled food supply chain systems' management towards enhanced data Integrity and verAcity, interoperability, traNsparenCy, and tracEability

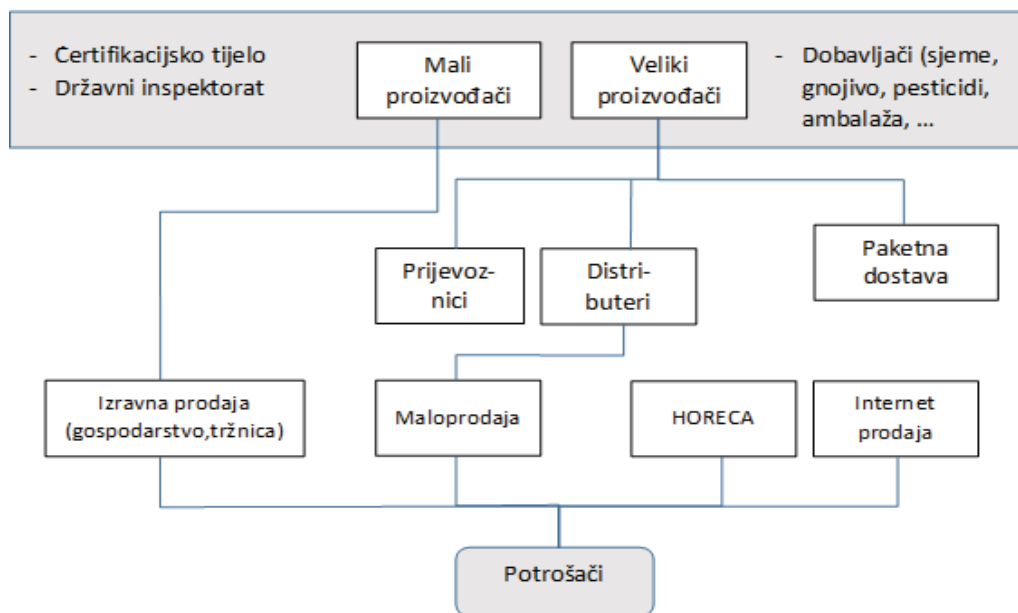
ukupnom proizvodnjom od 127.826 t. Iste godine, ukupne količine certificiranog ličkog krumpira ZOZP iznosile su svega 753 tone. Lički krumpir je registriran i zaštićen na zajedničkom tržištu EU, gdje je dobio oznaku zemljopisnog podrijetla u rujnu 2015. godine. Za proizvodnju ličkog krumpira koristi se sjemenski krumpir sorata Desire, Bintje ili Viktorija i drugih sorata sličnih karakteristika čiji sadržaj suhe tvari iznosi minimalno 19% (Udruga proizvođača ličkog krumpira (UPLK), 2014). Prema internim podacima Državnog inspektorata u razdoblju od 2020. - 2022. poljoprivredni inspektori obavili su dva nadzora ličkog krumpira ZOZP te su u jednom nadzoru na tržnici utvrdili zlouporabu naziva ličkog krumpira. Prema istom izvoru, najviše zlouporaba naziva zaštićenih oznaka je utvrđeno u ugostiteljskim objektima.

Metode i materijali

U razdoblju od veljače do travnja 2023. godine provedeno je kvalitativno istraživanje u vidu četiri dubinska intervjua s različitim dionicima u opskrbnom lancu ličkog krumpira ZOZP. Proveden je intervju s predstavnikom UPLK, zatim s dva proizvođača koji se nalaze u sustavu zaštite te s predstavnicom certifikacijskog tijela (Biotechnicon d.o.o.) nadležnog za obadva proizvođača, jedan proizvođač je član Udruge, dok drugi proizvođač sudjeluje u sustavu kontrole van Udruge. U analizi opskrbnog lanca ličkog krumpira korišten je pristup brze procjene lanca opskrbe (*eng. rapid supply chain appraisal*).

Rezultati istraživanja

Udruge proizvođača ličkog krumpira trenutno ima 7 članova, od kojih je samo jedan proizvođač u sustavu ZOZP. Također, postoje dva proizvođača koji nisu članovi Udruge, ali proizvode i prodaju krumpir pod ZOZP. Manji proizvođači krumpir proizvode i prodaju direktno na kućnom pragu ili na lokalnoj tržnici. Provjeru sukladnosti oznaka kvalitete u Hrvatskoj provode privatna certifikacijska tijela registrirana pri Ministarstvu poljoprivrede.



Slika 1. Opskrbni lanac ličkog krumpira ZOZP

Biotechnicon d.o.o. je certifikacijsko tijelo odgovorno za proces certificiranja ličkog krumpira sa ZOZP za oba ispitana proizvođača. Kontrolu valjanosti provodi i Inspekcija koja djeluje u sastavu Državnog inspektorata. Dva najveća proizvođača imaju vlastito sjeme, ali i otkupljuju manje količine sjemena od dobavljača. Ambalaža (vreće i kutije za krumpir) se dijelom nabavlja od domaćih dobavljača, a dijelom od uvoznika. Glavni kanali distribucije su supermarketi (LIDL, INTERSPAR), specijalizirane trgovine, restorani i online prodaja. Isporuka krumpira u distribucijske centre trgovačkih lanaca ide od proizvođača preko distributera ili preko transportne tvrtke. Online prodaja radi putem internetskih platformi i također putem tvrtki za dostavu paketa koje dostavljaju izravno potrošaču. (Slika 1).

Postojeća infrastruktura za praćenje blockchain sustava sljedivosti

Sustav sljedivosti temelji se na elementarnom vođenju dokumentacije u Word i Excel obrascima. Nedostaje infrastruktura kao što su IoT senzori, bežična komunikacija ili senzorske i nadzorne digitalne tehnologije koje se koriste za sustav certificiranja, nadzora i kontrole provedbe specifikacije za lički krumpir ZOZP. Osim infrastrukture nedostaje i znanja za digitalizaciju i implementaciju blockchain sustava sljedivosti. Samo najveći proizvođač ima opremu koja omogućuje online povezivanje senzora u skladištu. Skladište koje se koristi je javnog tipa, odnosno u njemu krumpir mogu skladištiti i drugi (manji) proizvođači - članovi udruge. Sustav ima režime hlađenja koji se automatski podešavaju u novoj hladnjači, te poluautomatski ili ručno u starijem dijelu skladišta gdje se također drži krumpir. Ugrađena je oprema za prozračivanje i ventilaciju. Na temelju rezultata analize lanca opskrbe definirane su potrebe za uspostavu djelotvornog suvremenog sustava sljedivosti koji bi sprječavao prijevare. S druge strane, projekt ALLIANCE nudi različita tehnološka rješenja kao potencijalne odgovore na uočene potrebe. U tablici 1 uparene su potrebe u sustavu sa tehnološkim rješenjima koja nude adekvatan odgovor na iste.

Tablica 1. ALLIANCE moguća tehnološka rješenja za Lički krumpir ZOZP

Identificirana potreba	ALLIANCE moguća tehnološka rješenja
Digitalizacija procesa	Blockchain platforma: Prva faza u digitalizaciji procesa je implementacija Blockchain platforme koja će omogućiti sigurno pohranjivanje dokumenata i digitalizaciju operativnih procesa. Kako: Zajedno s Blockchainom omogućiti korištenje IoT-a za digitalizaciju procesa prikupljanja podataka i automatizaciju analize podataka u kritičnim kontrolnim točkama. Krajnji potrošači mogu imati koristi od provjerenog proizvoda, pristupiti pouzdanim informacijama i povećati svoju lojalnost marki.
Prevenција prijevара s hranom	Sustav za rano upozoravanje i podršku odlučivanju temeljen na AI: Ovaj sustav omogućuje otkrivanje prijevara s hranom, interoperabilnost, sljedivost i praćenje te pametna rješenja za pakiranje/označavanje. Kako: AI analitika koristit će se za pružanje potpore informiranom donošenju odluka sudionicima opskrbnog lanca hrane, za poduzimanje protumjera i proaktivno sprječavanje prijevara s hranom te osiguranje zdravlja i javne sigurnosti.
Stjecanje znanja o tehnologijama koje se mogu primijeniti u poslovanju	Digitalna baza znanja za prijevare s hranom: omogućiti će razmjenu znanja, informacija, podataka, najboljih praksi, naučenih lekcija, dobro uspostavljenih procesa u prehrambenim lancima prehrambenih proizvoda s oznakom kvalitete. Kako: Staviti će se na raspolaganje sustav upravljanja znanjem.
Digitalizacija operativnih procesa korištenjem QR kodova	Blockchain platforma: Bilježenje svakog procesa u opskrbnom lancu hrane na jedinstvenoj stalnoj platformi. Kako: Digitalizirani procesi se bilježe i lako se mogu pružiti potrošačima kao dokaz autentičnosti.

Zaključak

Projekt ALLIANCE usmjeren je na sprječavanje prijevara s hranom u opskrbnim lancima proizvoda s oznakom kvalitete, a jedan od njegovih ciljeva je borba protiv prijevara u lancu opskrbe ličkim krumpirom ZOZP. Tijekom 2023. godine provedena je prva faza projekta, tijekom koje su utvrđeni sljedeći izazovi: mali broj proizvođača krumpira u sustavu zaštite ZOZP, teškoća u praćenju i sprječavanje zlouporabe imena „Lički krumpir“, nedostatak provjere krumpira izvan sustava certificiranja ZOZP, sustav sljedivosti temelji se na elementarnom vođenju dokumentacije u Word i Excel obrascima, nedostatak infrastrukture i znanja za digitalizaciju i implementaciju blockchain sustava sljedivosti. Rezultati ovog projekta će ponuditi rješenja za navedene izazove, uključujući primjenu suvremenih digitalnih tehnologija prilagođenih okruženju i karakteristikama lanca opskrbe Ličkim krumpirom. Kako bi se smanjile prijevare u prehrambenom lancu ličkog krumpira ZOZP, potrebno je učestalije provoditi nadzore, pogotovo u restoranima i tržnicama, te uvesti sustav kontrole sljedivosti koji uključuje sve sudionike opskrbnog lanca.

Napomena

This abstract is based on research undertaken as part on European Union's ALLIANCE project. "This project has received funding from the European Union's HE research and innovation programme under grant agreement No 101084188. Views and opinions expressed are, however, those of the authors only and do not necessarily reflect those of the European Union or the European Research Executive Agency (REA). Neither the European Union nor the granting authority can be held responsible for any use that may be made of the information the document contains."

Literatura

- Brooks C., Parr L., Smith J.M., Buchanan D., Snioc D., Hebishy E. (2021). A review of food fraud and food authenticity across the food supply chain, with an examination of the impact of the COVID-19 pandemic and Brexit on food industry. *Food Control* 130: 108171.
- FAO (2021). Food fraud – Intention, detection and management. Food safety technical toolkit for Asia and the Pacific No. 5. Bangkok
- Platonova M. (2023). „What Is Food Fraud, and How Can Nuclear Science Detect It?“. Raspoloživo na: <https://www.iaea.org/newscenter/news/what-is-food-fraud-and-how-can-nuclear-science-detect-it> (pristupljeno 01.07.2023).
- Rizzuti A. (2020). Food crime: A review of the UK institutional perception of illicit practices in the food Sector. *Social Sciences*. 9: 112.
- Udruga proizvođača ličkog krumpira (2014). Specifikacija proizvoda lički krumpir oznaka zemljopisnog podrijetla. Raspoloživo na: https://poljoprivreda.gov.hr/UserDocsImages/arhiva/datastore/filestore/106/Izmijenjena_Specifikacija_proizvoda.pdf (pristupljeno 20.12.2023).

Applying Smart-Contracts to fight Food Fraud in PGI Lika Potatoes

Abstract

Food fraud is an increasingly common problem that is causing great damage to the food market. The H2020 ALLIANCE project is aimed at preventing food fraud in quality-labelled supply chains, of organic, PDO and PGI products. For Croatia, ALLIANCE will enhance the traceability of the PGI Lika potatoes applying Smart-Contracts. In 2023, the first phase of the project was implemented, where the following weak points were identified: a small number of potato producers in the PGI protection system, difficult to monitor and prevent the misuse of the name 'Lički krumpir', verification of potatoes outside the PGI certification system is missing, opaque traceability documentation system based on a simple set of paper forms in Word or Excel sheets, lack of infrastructure and knowledge for digitization and implementation of the blockchain traceability system.

Keywords: EU H2020, food fraud, smart contracts, Lika potatoes, PGI

The influence of the region on the determination of typical family dairy farms in the Republic of Croatia

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Abstract

This paper presents the results of cluster analysis to determine typical farms, in which the variable region classified by NUTS2 was added in order to analyze the influence of that variable. The aim is to find the most representative typical dairy family farms that could be used to analyse the situation in the dairy sector in Croatia. On the basis of these information, we will further create farm models supported by the mathematical programming approach. Cluster analysis was performed on real data obtained from the Croatian Agency for Agriculture and Food. Hierarchical clustering and non-hierarchical clustering were performed using IBM SPSS Statistics.

Keywords: cluster analysis, typical farms, dairy sector, NUTS2 regions, farm model, Croatia

Introduction

The dairy sector in the Republic of Croatia has been characterized by negative trends for years, as shown by the decline in the number of farms, livestock, and the amount of milk production, while a positive effect is expected in the increase of milk yield per cow due to the introduction of new technologies (Mijić et al., 2021). During 2022, the decreasing trend continues. In the Republic of Croatia 405 425 t of milk were purchased, i.e. by 5,4% less than the previous year, with a decrease in the number of suppliers by 13,7%. A positive trend is the increase in delivered quantities per supplier by 12,3% (Croatian Agency for Agriculture and Food, 2023). Small farms with few animals are either closed or transferred to arable production (Mijić et al., 2021). The Croatian dairy sector is not expected to recover soon, and the simulation results indicate a further decline in the number of dairy cows and the amount of milk produced (Kranjac, 2020). The situation has been further aggravated in the last few years due to the consequences of the corona crisis and the war in Ukraine. The measures implemented obviously do not lead to improvement of the situation.

Before the actual creation and application of the model at the farm level, it is necessary to determine typical dairy farms in Croatia, i.e. farms should be grouped according to common characteristics (Chibanda et al., 2020). For this purpose cluster analysis could be used to obtain these groups of representative farms which are called typical farms (Pečnik et al., 2022). Cluster analysis solutions are not unique and depend on the application of different elements of the analytical procedure (e.g. hierarchical or non-hierarchical method, different methods of the hierarchical method). The final solution also depends on the variables that are used as a basis for measuring similarity of the group, so one should be careful about the impact of each decision when choosing variables.

In this paper we present one of the possible combination of variables of cluster analysis that has been conducted. Since the results of the cluster analysis are not unique, the cluster analysis in our holistic analysis of dairy sector will serve as the starting point for further analysis and defining final typical farms (production parameters, technologies etc.), which will further be modeled with the farm model. Typical farms will be defined in more detail at workshops with consultants and experts in the field and will be further adjusted and upgraded with the Slovenian farm model - SiTFarm (Žgajnar et al., 2022). For the workshop with consultants, several variants of cluster analysis will be created by changing the specified parameters.

This paper describes the procedure of cluster analysis for the case when, in addition to the previous 4 variables already analysed (Petrač et al., 2023) (Number of cows (NOC), Annual delivery of milk (ADOM), Number of plant cultures (NOPC) and Area under culture (AUC)), the variable Region (REG) was also added. This provides additional insight into where individual farms are located, and the technology of animal husbandry and technology of fodder production also depends on this.

Materials and methods

Cluster analysis was performed on real data obtained from the Croatian Agency for Agriculture and Food. The database consisted of 4198 dairy farms that supply milk in the Republic of Croatia. After arranging the obtained database (connecting data from different farm databases, removing duplicate and inactive farms, etc.) there were 3398 farms left for the analysis. Since in this analysis we focus on family farms, 67 of the farms have been excluded from the sample, since they have the status of a legal entity and form a special category. A separate cluster analysis will be done on them. Therefore, the final number of farms analysed is 3331. These are family farms that delivered milk and as such were included in the final register (Table 1).

IBM SPSS, Statistics V22.0 software package was used for statistical data processing and analysis. The variables and their descriptive statistics are presented in Table 1. Unlike the previous cluster analysis on these data (Petrač et al., 2023) when the quantitative variables NOC, ADOM, NOPC and AUC were used, here the qualitative variable Region (REG) was added. To define the mentioned variable, the national classification of statistical regions from 2021 was used - Level 2 Statistical Regions (HR NUTS 2), in which the Republic of Croatia is divided into 4 regions (Croatian Bureau of Statistics, 2021): Pannonian Croatia, Adriatic Croatia, City of Zagreb and Northern Croatia (Figure 1).

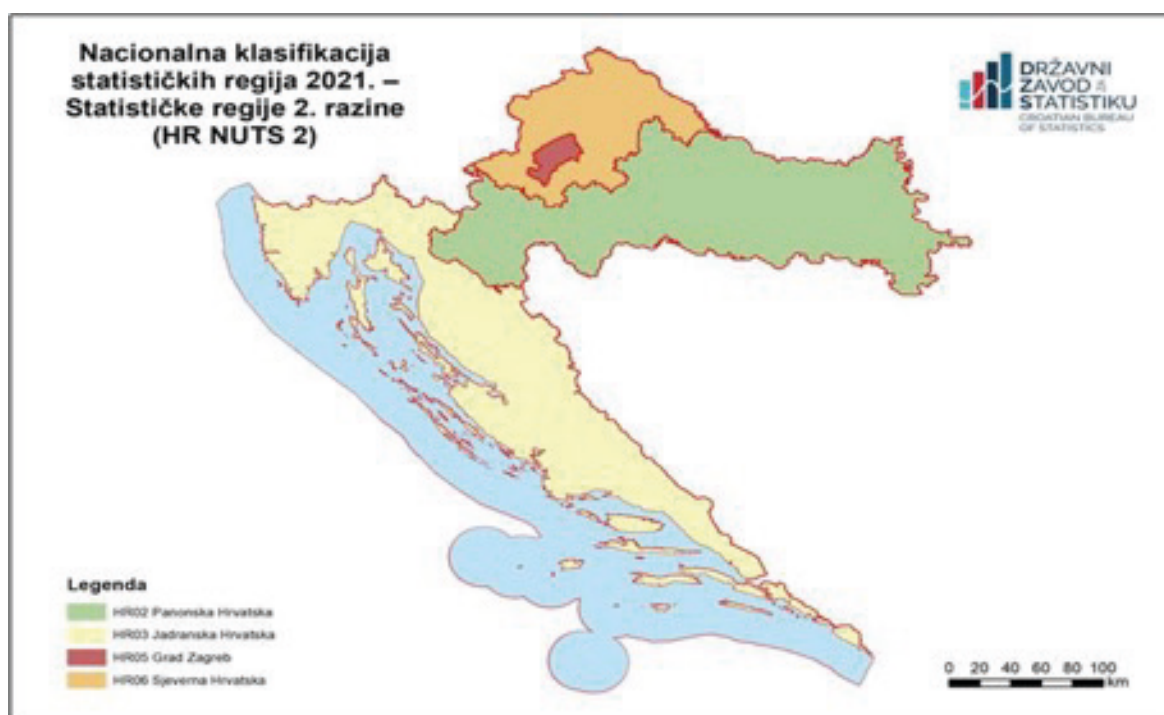


Figure 1. Level 2 Statistical Regions (HR NUTS 2)

Quantitative variables and their descriptive statistics are presented in Table 1. Several numerical characteristics of the variables (mean, standard deviation (SD), minimum (min) and maximum (max)) were determined for these 4 quantitative variables.

Table 1. Descriptive statistics for quantitative variables - 3.331 dairy farms

Variable Name	Variable	Mean	SD	Min	Max
NOC	Number of cows	14.78	20.47	1	456
ADOM	Annual delivery of milk (kg)	73 257	146 015	21	2 799 071
NOPC	Number of plant cultures	6.29	2.27	1	18
AUC	Area under culture (ha)	23.68	31.68	0.15	469.05

Source: Own calculations

The relative frequency was determined for the qualitative variable REG (see Figure 2). The most dairy farms are located in the Pannonian region (47%) and Northern Croatia (45%). Both regions are located in the continental part of the country and include eight and five counties of Croatia, respectively. The Adriatic region forms the coastal part of the country, including seven counties and 7% of farms are located in it. As expected, the least farms are located in the city of Zagreb, with about 1%.

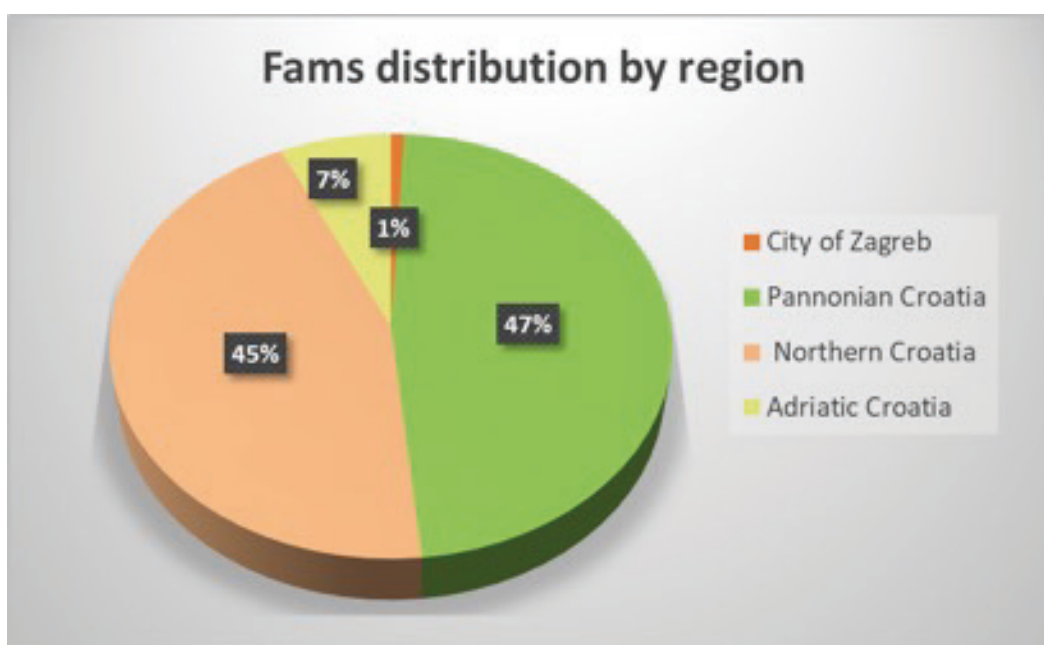


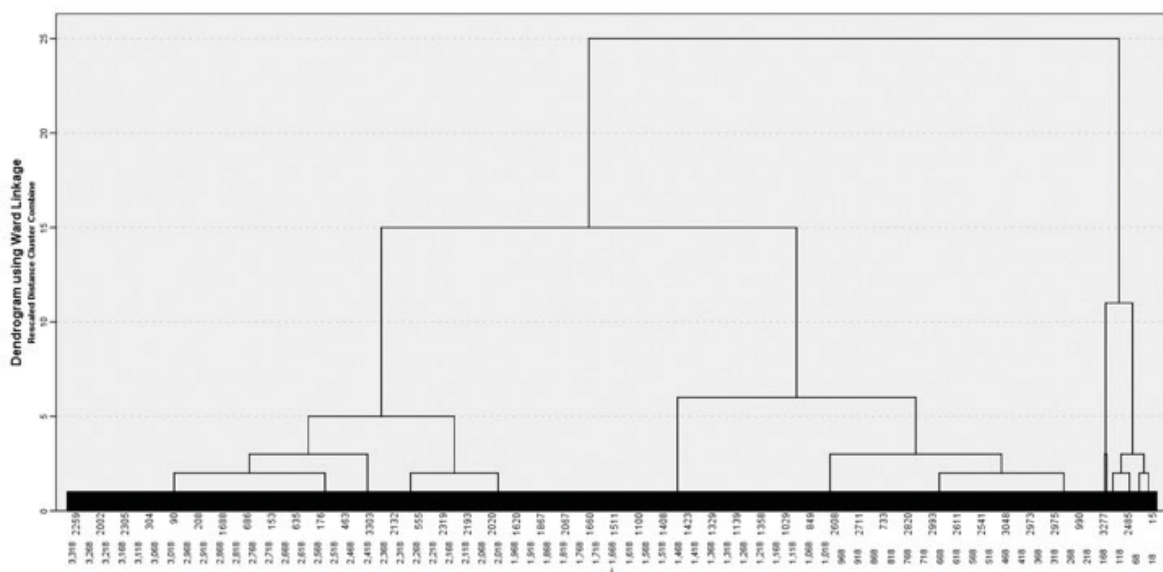
Figure 2. Descriptive statistics for qualitative variable (relative frequency)

The cluster analysis was first carried out in relation to all the mentioned variables. First, hierarchical (agglomerative) clustering was performed using Ward's method. The results of the agglomerative method can be graphically displayed in the form of a two-dimensional hierarchical diagram, known as a dendrogram. Then non-hierarchical clustering, i.e., k-means algorithm, was performed (Scitovski et al., 2020). The squared Euclidean distance was chosen as the distance measure. All algorithms were applied to standardized data. Among numerous solutions, finally one solution was chosen as the final one. Cluster analysis solutions are not unique and depend on the application of different elements of the analytical procedure (e.g. hierarchical or non-hierarchical method, different algorithms of the same method). One should be careful about the impact of each decision when choosing variables because the solution also depends on the variables that were used as a basis for measuring similarity. The final decision remains for users (the researchers and expert) to estimate. The experts will be the ones who will choose the cluster that best describes the real situation in the sector.

Results and discussion

Further we briefly present the main results. In the first part, we show the dendrogram, and further the results of the k-means algorithm will be shown, where we can see how the farms are distributed in 15 clusters, i.e. which farms belong to which cluster and what are its characteristics, how many farms are in which cluster, what is the average number of cows in each cluster as was the goal of this work, we see the representation of regions in each cluster.

The first step in this analysis was to determine the optimal number of clusters using the dendrogram (see Graph 1) using Ward's method, based on the squared Euclidean distance. The structure of the data was analyzed, i.e. different groups of farms were found, which share common characteristics. How heterogeneity within clusters increases, so the number of clusters decreases. The dendrogram helps in deciding on the optimal number of clusters, but the final decision remains for the researchers and expert to estimate. As apparent from the dendrogram, analysis suggests 15 clusters.



Graph 1. Dendrogram

After it was decided that there would be 15 clusters, using the k-means algorithm, the farms were distributed into 15 clusters. Table 2a and 2b show the structure of all clusters after the implementation of k-means.

As apparent from Table 2a, 836 farms (25%) belong to cluster 1. The average number of cows in this cluster (8.01) is smaller than the average number of cows in the Republic of Croatia (14.78), the average annual milk delivery (33870.71 kg) is lower than the national average of 73257.69 kg, the average area of land per farm (10.48 ha) is also smaller than the national average. This cluster consists mainly of farms from Northern Croatia (90%), and a small part of farms is from Adriatic region (10%). This implies that cluster 1 consists mainly of very small farms from Northern Croatia.

It can be seen that cluster 2 consists of 653 farms (20%) and is also relatively similar to cluster 1, but farms in cluster 2 have much more agricultural land as well as plant cultures than farms in cluster 1. Namely, there are many farms with a few cows and a lot of land, and these are not the farms whose primary activity is milk production. It can also be concluded that in this cluster the majority of farms are from Northern Croatia. However with a slightly smaller share of farms from Northern Croatia, and a larger share from Adriatic Croatia.

Cluster 3 consists of 621 farms (19%). The average number of cows in this cluster (9.84) is also smaller than the average number of cows in the Republic of Croatia (14.78), the average annual milk delivery (38071.60 kg) is lower than the national average of 73275.69 kg, the average area under culture (12.76 ha) is also smaller than the national average. Almost all of the farms in this cluster are from Pannonian region, and a very small part of farms are from City of Zagreb. This implies that cluster 3 consists of very small farms in Pannonian Croatia.

Again, cluster 4 is similar to cluster 3, with the only difference being that the farms in cluster 4 have much more

land and plant cultures. Namely, there are many farms in Pannonian region with a few cows and a lot of land, and these, obviously, are not the farms that are primarily active in milk production and dairy is not the only activity on these farms. From the fifth cluster onwards, there are smaller groups that represent 18% of the total number of farms. However, they are extremely important from the point of view of milk production. Even 57% of the total production of dairy cows takes place on these farms (Table 2a). With the exception of the fifth and eighth clusters, milk production per cow is significantly higher on all farms than in the first four clusters. The two largest farms are in special clusters (cluster 14 and 15). They are both located in Pannonian Croatia. The 6 largest Croatian farms (cluster 12 to 15) whose production is 5% in the total milk production in Croatia are also located in the Pannonian region (Table 2b).

Table 2a. Cluster structure

<i>Cluster</i>	<i>Number of farms</i>	<i>Average NOC</i>	<i>Average NOPC</i>	<i>Average AUC</i>	<i>Yield per cow</i>
1	836	8.01	4.75	10.48	4,186.47
2	653	9.27	8.22	15.12	4,103.21
3	621	9.84	4.01	12.76	3,917.06
4	611	11.08	7.02	21.84	4,055.33
5	221	18.75	10.11	48.25	4,529.97
6	213	32.41	6.15	39.43	5,600.62
7	93	56.49	7.01	67.82	6,621.60
8	35	33.60	8.83	175.84	4,423.23
9	32	88.66	7.44	119.00	7,797.23
10	5	166.80	5.80	98.52	7,747.71
11	5	204.00	8.20	226.84	6,950.93
12	2	230.50	4.50	204.51	9,123.60
13	2	107.50	7.50	388.93	6,309.97
14	1	317.00	8.00	469.05	8,829.88
15	1	456.00	6.00	370.27	5,614.38
Croatia	3331	14.78	6.29	23.68	4,329.80

*Legend: NOC - Number of cows, NOPC - Number of plant cultures
AUC - Area under culture (ha)*

Table 2a also contains data on milk yield per cow for each cluster (*Yield per cow* column). This data was calculated from the obtained data base and was not included as a variable in the cluster analysis.

Table 2b. Cluster structure by regions

<i>Cluster</i>	<i>Number of farms</i>	<i>Number of farms (%)</i>	<i>ADOM (%)</i>	<i>City of Zagreb</i>	<i>Pannonian Croatia</i>	<i>Northern Croatia</i>	<i>Adriatic Croatia</i>
1	836	25%	12%	0%	0%	90%	10%
2	653	20%	10%	0%	0%	80%	20%
3	621	19%	10%	1%	99%	0%	0%
4	611	18%	11%	3%	97%	0%	0%
5	221	7%	7%	0%	83%	17%	0%
6	213	6%	15%	0%	37%	60%	3%
7	93	3%	14%	0%	62%	33%	4%
8	35	1%	2%	0%	74%	14%	11%
9	32	1%	9%	0%	66%	28%	6%
10	5	0%	3%	0%	60%	40%	0%
11	5	0%	3%	0%	60%	40%	0%
12	2	0%	2%	0%	100%	0%	0%
13	2	0%	1%	0%	100%	0%	0%
14	1	0%	1%	0%	100%	0%	0%
15	1	0%	1%	0%	100%	0%	0%
Grand Total	3331	100%	100%				

Legend: ADOM - Annual delivery of milk

Conclusion

By applying cluster analysis to the data of dairy farms in Croatia, many different solutions could be obtained. By introducing the variable Region (REG), new and different solutions are obtained. As expected, the variable Region affects the new division of farms, and 15 clusters are obtained. Most of the farms are mainly distributed in the Northern and Pannonian regions. These are mostly small farms with low milk production. Since the farms of the Northern Region are mostly in hilly areas, they are limited in arable land availability. Unlike the Northern Region, the Pannonian region has a lot of arable land, so the farms in that area have more arable land on which to produce their own fodder for livestock and thereby reducing production costs. Therefore, it is not surprising that the largest farms are located in the same region, which are also the main carriers of the total milk production. Small farms are crucial for rural areas, especially from a socioeconomic aspect. On the other hand, they are not a key factor that contributes to the increase in production volume, food security, or the increase in market self-sufficiency. Typical farms have different levels of economic efficiency so the same measure is not equally effective for every typical farm. A certain policy measure that would increase the profitability of one typical farm, does not mean that it would increase the profitability of another type of farm. Such results could be useful to policy makers to get information on which typical dairy farm needs which type of measure and how much they can adapt to the given situation. Such more effective policy measures should encourage more economical farming.

References

- Chibanda C., Agethen K., Deblitz C., Zimmer Y., Almadani M. I., Garming H., ... Lasner T. (2020). The Typical Farm Approach and Its Application by the Agri Benchmark Network. *Agriculture*. 10: 646.
- Croatian Agency for Agriculture and Food. 2023. Annual report for 2022. Centre for Livestock Breeding, Cattle breeding.
- Croatian Bureau of Statistics. National classification of statistical regions 2021 (HR_NUTS 2021)?

- National Gazette (in Croatian) (125/2019). Retrieved 2023-10-10.
- Kranjac D., Zmaić K., Salputra G., Salamon P., Erjavec E. (2020). Production and trade impacts of CAP post 2021 reform scenarios on main Croatian crop and livestock sectors. *German Journal of Agricultural Economics*.
- Mijić P., and Bobić T. (2021). Milk production and challenges in transition from conventional to robotic milking in Croatia. *Acta Sci. Pol. Zootechnica*. 20: 59–64.
- Pečnik Ž., and Žgajnar J. (2022). Resilience of dairy farms measured through production plan adjustments = Odpornost kmetij s prirajo mleka z različnimi prilagoditvami proizvodnega načrta. *Journal of Central European Agriculture*. 23: 207-219.
- Petrač M., Zmaić K. and Žgajnar J. (2023). Typical family dairy farms in the republic of Croatia. 17th International Symposium on Operational Research in Slovenia, 31-34, Bled, Slovenia, September 20-22, 2023.
- Scitovski R., and Sabo K. (2020). Klaster analiza i prepoznavanje geometrijskih objekata. Sveučilište Josipa Jurja Strossmayera u Osijeku, Odjel za matematiku.
- Žgajnar J., Kavčič S., Tomšič M., Zagorc B., Brečko J., Hiti Dvoršak A., Moljk B., Verbič J., Bedrač M., Kožar Maja, Cunder T., Jerič D. (2022). Razvoj modela za sistematično spremljanje ekonomskega položaja in analizo vpliva kmetijske politike na ravni tipičnih kmetijskih gospodarstev : zaključno poročilo. Ljubljana: Biotehniška fakulteta, feb. 2022. 437 str., ilustr. [COBISS.SI-ID 97899267]

How would an agri-food chain with added value look like: a theoretical approach based on a case study from Slovenia

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Abstract

The proposed model of the agri-food chain with added value contains guidelines which, if the defined criteria are met, represent the existence of added value in the individual agri-food chains. The guidelines are based on indicators that have been created to assess or recognise the added value in the individual agri-food chains of livestock farming. The indicators and the guidelines derived from them are designed to cover three aspects of sustainable development (economic, social, environmental) and at the same time to analyse the existence of added value at the various stages of the individual agri-food chains (primary production, processing, marketing, distribution and consumption).

Keywords: food supply chain, added value, sustainability, Slovenia

Introduction

The paper presents a proposal for a model of a sustainable value-added agri-food chain based on the results of a targeted research project (CRP V4-2010) entitled: Evaluation of value-added food supply chains with the aim of identifying gaps and making recommendations for further development in Slovenia. Characteristics of value-added food supply chains are that they maintain positive social, environmental and social values which are integrated into production process from the primary producer to the final customer and ensure economic, social and environmental sustainability, thus forming a sustainable agricultural and food production system (Zidar et al., 2023). The aim of the project was to evaluate individual agri-food chains according to the existence of added value. The agri-food chain in Slovenia has been characterized by structural changes in individual sectors in recent years, linked to the development and economic performance of individual agri-food industries. Differences between individual agricultural industries relate to: the level of self-sufficiency in food, the level of purchase prices of agricultural products, the change in the share of employees in each chain, the wage inequality of employees by gender, the change in the share of organically raised animals and animals of indigenous breeds, etc. The assessment of individual agri-food chains on an aggregated level was carried out with the help of indicators of added value. In creating the indicators, we were guided by three aspects of sustainable development (economic, social, environmental), which is why the assessment of agri-food chains itself is based on the identification of economic, social and environmental added value. As pointed out by various authors Stevenson and Pirog (2008), Pirog and Bregendahl (2012), Stevenson et al. (2011), Prišenk (2015), Lev et al. (2015), Feenstra & Hardesty (2016), Vitterso et al. (2019), Malak-Rawlikowska et al. (2019), Reckinger (2022), value-added agri-food chains are those in which all actors in the individual chain recognise a certain stability and security. The business relationships between the strategic partners in the chain are based on common principles, which has an impact on the trust and satisfaction of all parties in the chain. All actors involved in the chain are treated equally, with all rights and obligations, which is also reflected in the distribution of the final price of the product, which is distributed proportionally among all actors. In practise, added value can also be expressed through agricultural products or food with clearly proven origin, through protected food labelling and through a set of correct business relationships between the different actors in the food chain (Stevenson and Pirog, 2008; Pirog and Bergendahl, 2012).

The main outcome of the paper is the presentation of the theoretical basis for the development of the agri-food chain with added value based on the achievement of the identified objectives. The objectives were identified on the basis of a multi-criteria analysis of the DEX model developed for the assessment of existing agri-food chains in Slovenia. With this contribution, we want to present the progress of the research work on the project, indicate the limitations we encountered during the research itself, and summarize the results of the project.

Materials and methods

The three aspects of sustainability (economic, social and environmental) were also decisive for the creation of a set of added value indicators and the construction of the DEXi model, whose evaluation parameters were divided into three groups: economic, social and environmental. The indicators that enable the evaluation of added value in livestock agri-food chains at the aggregate level were created on the basis of Slovenian and foreign literature in the field of indicators (Yakovleva, 2007; Rossi, 2020; Malak-Rawlikowska et al., 2019), national and international accepted legal goals ("From farm to fork" strategy, European Green Deal, EU biodiversity strategy until 2030, Sustainable Development Goal 17) and internationally accepted indicators of sustainable development (COSA, SAFA) (Zidar et al., 2023).

Multi-criteria The DEXi model was developed for the assessment of agri-food chains in the livestock sector, where we analysed the agri-food chain for cattle and pigs as well as the dairy production chain. The decision in favour of the livestock sector is primarily due to the availability of statistical data in the livestock chains, in contrast to the crop production sectors where no statistical data is collected for individual chains.

As the amount of statistical data (SORS¹, KIS²) relating to the primary sector (farmers) is extensive and easily accessible, many indicators have been designed to relate primarily to the primary sector in each chain. A major problem is obtaining data for all further stages of the food chain, as only these are not publicly available or cannot be obtained from public databases. The consequence of this gap in data availability is that this model is primarily used to assess the position of the primary sector (farmers) in each chain.

The DEXi software tool supports a theory based on multi-parameter decision making that emphasises the role of the decision maker in the decision-making process. DEX is the shell of an expert system that does not have a pre-prepared database or knowledge, but the user builds it himself with the help of the tools offered by DEX. The DEXi methodology basically consists of two parts:

1. Obtaining data and organizing knowledge: it helps the user in creating a "tree of criteria" and decision rules for the problem under consideration (it is a process of structuring the decision problem and expressing preferences).
2. Assessments and analyzes of variants (this is an assessment in accordance with the set criteria and decision rules)

The model itself is designed in the form of a "tree", where at the beginning each variant is described with the values of the criteria, which represent the "leaves of the tree". The "tree trunk" expresses the utility of the variant or the final evaluation of the model. The utility is then branched into a utility function, the purpose of which is to combine all the values of the individual parameters into a final estimate or utility of the variant. Parameters are those variables that illustrate the subproblems of the decision problem, or those factors that define the quality of variants (economic, social, environmental). In our case, each parameter consists of four indicators that have the same weight in the final evaluation of the parameter. The DEX method has so far been used in various analyzes in agriculture and agri-food (Prišenk et al., 2014; Pažek et al., 2014; Nikoloski et al. 2017; Pažek et al., 2018; Rossi et al. 2021; Puccinelli et al. 2022).

1 Statistical Office of the Republic of Slovenia

2 Kmetijski inštitut Slovenije/Agricultural Institute of Slovenia

Table 1. A set of indicators included in the DEXi model

ECONOMIC
The average ratio between the producer price index for agricultural products and the price index for agricultural inputs on an annual basis
Average annual change in the ratio between the average retail price of agricultural products and the own price (in per cent)
Average ratio between the average price of purchased agricultural products and own price
Average weekly change in the market price of the product on the representative market in %
SOCIAL
The average ratio between the average gross wage in each production chain and the average gross wage in agriculture
Average change in the share of the working population in a single agricultural activity compared to all activities combined in a single year (based on the share in 2012)
The average ratio between the wage index in each agricultural activity and the consumer price index
Average degree of self-sufficiency with individual products (between 2012 and 2021)
ENVIRONMENTAL
Food kilometres – average route/distance of imports in the last 10 years, route or distance between Ljubljana and the capital of each country (between 2012 and 2021)
Average annual change in the share of all domestic breeds of a single species compared to all bred animals of a single species in %
Average annual change in the number of livestock farms included in the animal welfare sub-measure in %
Average annual change in the proportion of organically reared animals of a single species compared to all farmed animals of that species in % (based on the situation in 2012)

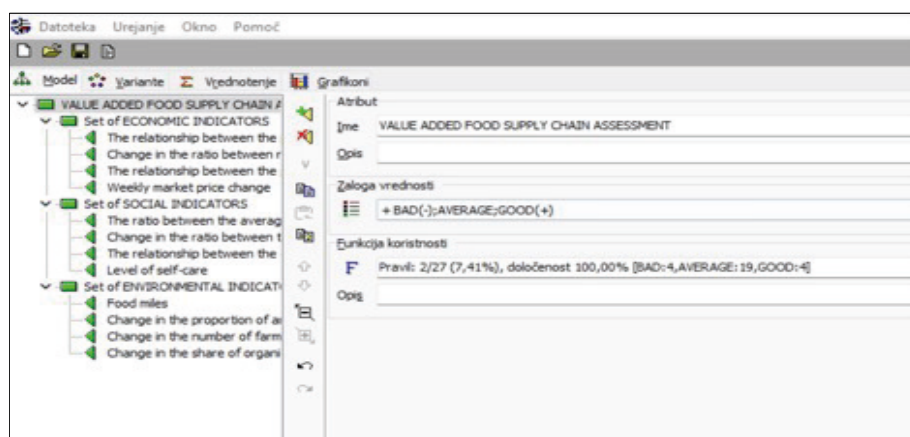


Figure 1. Assessment tree representation of the DEXi model

Results and discussion

Evaluation results with the DEXi multi-criteria model

On Figure 2 the evaluation results for each agri-food chains are presented. The discussion of individual chains follows separately in individual subsections below.

Variantna	Cattle	Pigs	Milk (dairy)
. VALUE ADDED FOOD SUPPLY CHAIN ASSESSMENT	AVERAGE	AVERAGE	AVERAGE
... Set of ECONOMIC INDICATORS	AVERAGE	AVERAGE	AVERAGE
... The relationship between the prices of agricultural products and the prices of agricultural inputs	0,99 and less	1,01 and more	1,01 and more
... Change in the ratio between retail price and production price	0,01 and more (positive vs	0,01 and more (positive val	- 0,01 and less (negative
... The relationship between the prices of purchased products and the own price	0,99 and less	0,99 and less	0,99 and less
... Weekly market price change	0,10 and more	- 0,01 and less (negative vs	Between 0 and 0,09
... Set of SOCIAL INDICATORS	GOOD	AVERAGE	AVERAGE
... The ratio between the average gross salary in each chain and the average gross salary in agriculture	0,99 and less	1,01 and more	1,00
... Change in the ratio between the working population in each chain and the working population in all activities in the Republic of Slovenia	0,01 and more (positive vs	- 0,01 and less (negative vs	- 0,01 and less (negative
... The relationship between the change in the wage levels and the change in the level of consumer prices	1,01 and more	1,00	1,00
... Level of self-care	More than 75 %	Less than 50 %	More than 75 %
... Set of ENVIRONMENTAL INDICATORS	AVERAGE	GOOD	GOOD
... Food miles	More than 600 KM	More than 600 KM	Between 300 and 600 KM
... Change in the proportion of animals of indigenous breeds compared to all animals combined	More than 5,01 %	More than 5,01 %	More than 5,01 %
... Change in the number of farms included in the animal welfare sub-measure	Between 0 and 5,00 %	More than 5,01 %	Between 0 and 5,00 %
... Change in the share of organically raised animals	More than 5,01 %	More than 5,01 %	More than 5,01 %

Figure 2. Evaluation of individual chains with the DEXI model

Chain with beef meat

In order to maintain the recognised added value in the cattle breeding chain, it is important to continue the trend of price development in which the retail price for beef will increase more than the own price for young fattening cattle. The same applies to the development of the share of employees in cattle breeding compared to all employees in the Republic of Slovenia, where the share of employees has increased in the period between 2012 and 2021. The share of employees indirectly has a significant impact on maintaining a high level of self-sufficiency in beef, with Slovenia achieving a self-sufficiency level of more than 100% in the period between 2012 and 2021. A positive feature of the cattle breeding chain, which adds value to the chain as a whole, is also the trend towards increasing the share of Zika cattle (native cattle breeds) and organically bred cattle in the total population of breeding cattle in Slovenia. Areas that represent a certain gap in value creation refer to the relationship between the costs of primary production itself and the prices received by primary producers. The prices of agricultural inputs are rising more intensively than the prices of cattle at producers. The purchase price of cattle is, on average, lower than the cost price of raising young fattened cattle. The average wage in cattle breeding is lower than the average wage in agriculture. Given that Slovenia is more than 100% self-sufficient in beef, it would be very important to shorten the average distances traveled when importing beef from abroad.

Chain with pork meat

In order to maintain the recognised added value in the pig farming chain, it is important to continue the trend of price development in the future, in which the retail price of pork will increase faster than the own price for rearing fattening pigs with compound feed. The same applies to the development of producer prices for pigs and prices for agricultural inputs. Another important feature of the pig farming chain is that average wages in pig farming were higher than wages in agriculture during the period under review. Regarding the environmental aspect of value creation in the pig farming chain, the share of Krškopolski pigs (native pig breeds) and organically reared pigs in the total population of reared pigs in Slovenia is increasing. In the future, it would be necessary to improve the relationship between the costs of the primary production itself (farming) and the prices that primary producers receive for their crops. The purchase price of pigs from producers was on average lower than the cost price for the rearing of fattening pigs with compound feed. The supply of pork in Slovenia is less than 40 %. The low percentage of self-sufficiency in pork is thus reflected in the dependence of the Slovenian market on the import of pork from abroad. The decline in the number of employees in pig farming compared to all employees in the Republic of Slovenia is also characteristic.

Milk production chain

In order to maintain the recognised added value in the milk production chain, it is important to maintain a high level of self-sufficiency in milk (on average more than 100 %). The price of cow's milk has risen faster for producers than the price of agricultural inputs. The added value of the chain is also given by the fact that the share of Zika cattle

(native cattle breed) and organically bred cattle in the total population of bred cattle in Slovenia is increasing. Areas that will need to be given more attention in the future, or that currently represent a certain gap in the creation of added value, relate to the relationship between the costs of primary production itself (cost price) and purchase or retail prices. The share of employees working in milk production in the total number of employees in the Republic of Slovenia declined.

Proposal for the development of an agri-food chain with added value

The proposal for a sustainable model of the animal husbandry chain with added value is developed with the help of indicators that are used to evaluate the individual chains. From these indicators, guidelines are derived that demonstrate the existence of added value in the chain by achieving the established criteria in each phase of the chain (primary production, processing, marketing and distribution, and consumption). Similar to the indicators, the targets are assumed to cover all three aspects of sustainable development, namely the economic, social and environmental aspects. Failure to achieve individual targets represents anomalies in certain sectors of the respective chain, which form the basis for finding or creating appropriate solutions.

Table 2. Targets in the chain representing added value identified in the extension of the DEXi model and additional indicators

GUIDELINES	
1.	The retail price of livestock products changes in harmony, or rises more intensively than the own price of livestock production, and it is important that this difference between the two prices (profit) is distributed among all links in the chain.
2.	The change in the price of agricultural products among growers is coordinated or more intense than the change in the prices of inputs in agriculture.
3.	The purchase price of agricultural products is higher than the own price of livestock production, which means that primary producers make a profit on sale.
4.	The change in the price of purchased agricultural products is coordinated with changes in the retail price (the ratio between prices does not deteriorate to the detriment of primary producers or the price of purchased agricultural products), and it is important that this difference between the two prices is distributed among all links in the chain.
5.	The market prices of produce (meat, milk and other products) on the representative market are harmonized with the average prices in the EU, i.e. they change at the same time and in proportion to the changes on the EU market.
6.	The share of the working population at each stage of the agri-food chain is maintained or increased, which can indirectly affect the preservation or increase of food security, the reduction of dependence on foreign trade, the arrangement and cultivation of the countryside, and the preservation of jobs in the countryside.
7.	Salaries in an individual activity in the chain are equal to or higher than the average salary in the sector to which this activity belongs (e.g. salaries in cattle breeding are higher than average salaries in agriculture).
8.	Changes in the level of wages at individual levels in the chain are coordinated (or more intense) with changes in the level of consumer prices.
9.	The amount of salary at each level in the chain does not differ depending on the gender of the employees.
10.	Changes in the level of salaries are coordinated between all individual levels in the chain, which means that salaries at all levels in the chain change proportionally.
11.	Food kilometers when importing goods from foreign markets or transporting goods should be as short as possible, with the average transport distance not exceeding 300 km.

12. The share of domesticated animals of indigenous breeds in each chain is increasing, and it is necessary to raise awareness among all involved stakeholders about the importance of preserving indigenous breeds, which are important from the point of view of preserving both the natural heritage and biotic diversity, as well as the various physical adaptations that individual breeds have developed and they are adapted to living in our agricultural environment.
 13. The number of farms included in the animal welfare sub-measure is increasing, which indirectly affects animal-friendly living conditions (production processes).
 14. The share of organically raised animals is increasing, which indirectly affects the production processes, which represent the sustainable management of natural resources and the enforcement of the principle of animal welfare.
 15. The presence or introduction of various national or local quality schemes that emphasize the specific properties of each product.
 16. The presence of tied income support from the strategic plan of the Common Agricultural Policy, which can only be approved for sectors and production or specific types of farming that are important for socio-economic or environmental reasons.
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Conclusion

So far, only individual examples of agri-food chains have emerged in Slovenia that have been indirectly identified as sustainable or as examples of chains with recognised added value, but no clear proposal for a sustainable model of agri-food chains with added value has been formulated.

The assessment of each agri-food chain using the DEXi model based on the defined indicators of added value showed clear differences in terms of the presence of added value at different stages of each agri-food chain (beef and pork chain, dairy production chain). The identified gaps in the provision of added value in each chain differed both in terms of the stage in the chain at which they occur and in terms of the form of recognised added value based on one of the three aspects of sustainable development (economic, social, environmental). In order to facilitate the assessment of the individual agri-food chains or to eliminate the anomalies identified, we have drawn up a proposal for a sustainable model of agri-food chains with added value with clearly defined objectives, based on the indicators used to identify the added value in the individual agri-food chains at the aggregate level. The defined objectives represent guidelines for achieving added value in livestock agri-food chains.

The limitations that we have identified in the creation of indicators for recognising added value in agri-food chains, i.e. in the assessment of the chains themselves, and which relate to the lack of statistical data for the secondary and tertiary sectors of individual agri-food chains, are consequently also reflected in the set of drafted objectives that we have covered in the proposal for a sustainable model of agri-food chains with added value. The challenges for the future will therefore be to create new objectives for the realisation of added value, mainly related to the secondary and tertiary sectors of individual agri-food chains.

Acknowledgment

The results presented in the paper are the part of the results of the research project CRP V4-2010, entitled: Evaluation of food supply chains with added value in order to identify gaps and make recommendations for further development in Slovenia.

References

Committee on Sustainability Assessment. Indicator Library. Available from:

<https://thecosa.org/about-cosa-indicators/>

Feenstra G., and Hardesty S. (2016). Values-based supply chains as a strategy for supporting small and mid-scale producers in the United States. *Agriculture*. 6: 39.

Food and Agriculture Organization of the United Nations (FAO). Sustainability Assessment of Food and Agriculture systems. Guidelines version 3.0 (2014). Available from: <https://www.fao.org/nr/>

sustainability/sustainability-assessments-safa/en/

- Lev L., Stevenson G.W., Clancy K., King R., Ostrom M. (2015). Values-based food supply chains. In K. Albala (Ed.), *The Sage Encyclopedia of Food Issues* [E-reader version]. pp. 1417-1419.
- Malak-Rawlikowska A., Majewski E., Wąs A., Borgen S.O., Csillag P., Donati M., Freeman, R., Hoang V., Lecoer J.L., Mancini M.C., Nguyen A., Saidi M., Tocco B., Torok A., Veneziani M., Vittersø G., Wavresky P. (2019). Measuring the economic, environmental, and social sustainability of short food supply chains. *Sustainability*. 11: 4004.
- Nikoloski T., Udovč A., Pavlovič M., Rajkovič U. (2017). Farm reorientation assessment model based on multi-criteria decision making. *Computers and Electronics in Agriculture*. 140: 237-243.
- Pažek K., Turk J., Hari S., Rozman Č., Prišenk J. (2014). Multi-criteria and econometric evaluation of dairy products. *Mljekarstvo/Dairy*. 64: (2).
- Pažek K., Irgolič A., Turk J., Borec A., Prišenk J., Kolenko M., Rozman Č. (2018). Multi-criteria assessment of less favoured areas: a state level. *Acta geographica Slovenica*. 58: 97-108.
- Pirog R., and Bregendahl C. (2012). *Creating change in the food system: The role of regional food networks in Iowa*. Center for Regional Food Systems, Michigan State University: East Lansing, MI, USA.
- Prišenk J. (2015). *The effects of value based agro-food chain on the socio-economic situation of dairy farms in mountain regions* (Doctoral dissertation). University of Maribor, Maribor.
- Prišenk J., Rozman Č., Pažek K., Turk J., Bohak Z., Borec A. (2014). A multi-criteria assessment of the production and marketing systems of local mountain food. *Renewable agriculture and food systems*. 29: 345-354.
- Puccinelli M., Fierro-Sañudo J. F., Bibbiani C., Fronte B., Maibam C., Dubois T., Pardossi A., Incrocci L., Rossi L. (2022). Multi-Criteria DEXi Analysis for the Selection of Crop Species for Saltwater Aquaponics. *Horticulturae*. 8: 703.
- Reckinger R. (2022). Values-based territorial food networks: Qualifying sustainable and ethical transitions of alternative food networks. *Regions and Cohesion*. 12: 78-109.
- Rossi L., Bibbiani C., Fierro-Sañudo J. F., Maibam C., Incrocci L., Pardossi A., Fronte B. (2021). Selection of marine fish for integrated multi-trophic aquaponic production in the Mediterranean area using DEXi multi-criteria analysis. *Aquaculture*. 535: 736402.
- Rossi, R. (2020). *European Union food system*. Available from: [https://www.europarl.europa.eu/RegData/etudes/BRIE/2020/652058/EPRS_BRI\(2020\)652058_EN.pdf](https://www.europarl.europa.eu/RegData/etudes/BRIE/2020/652058/EPRS_BRI(2020)652058_EN.pdf)
- Stevenson G.W., and Pirog R. (2008). Values-Based Supply Chains: Strategies for Agrifood Enterprises of the Middle. In T. A. Lyson, G. W. Stevenson, R. Welsh (Ed.), *Food and the Mid-level Farm: Renewing an Agriculture of the Middle* (Chapter 18) [E-reader version].
- Stevenson G.W., Clancy K., King R., Lev L., Ostrom M., Smith S. (2011). Midscale food value chains: An introduction. *Journal of Agriculture, Food Systems, and Community Development*. 1: 27-34.
- United Nations. *Sustainable development goals*. Available from: <https://sdgs.un.org/goals>
- Vittersø G., Torjusen H., Laitala K., Tocco B., Biasini B., Csillag P., Dubois de Labarre M., Lecoer J., Maj A., Majewski E., Malak-Rawlikowska A., Menozzi D., Torok A., Wavresky P. (2019). Short food supply chains and their contributions to sustainability: Participants' views and perceptions from 12 European cases. *Sustainability*. 11: 4800.
- Yakovleva N. (2007). Measuring the sustainability of the food supply chain: a case study of the UK. *Journal of Environmental Policy & Planning*. 9: 75-100.
- Bohanec M., and Rajkovic V. (1999). Multi-attribute decision modeling: Industrial applications of DEX. *Informatica (Ljubljana)*. 23: 487-491.

Citizen`s role in energy transition of Križevci Municipality, Koprivnica-Križevci County, Croatia¹

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Abstract

The main objective of the paper is to show how the citizens of the municipality of Križevci are involved in the energy transition, an important strategic development goal. Over the past three years, citizens have become one of the most important development actors and creators of a sustainable, energy-independent and climate-neutral town. They have helped to achieve the current state of the energy transition through self-mobilisation, a type of participation that is an indicator of endogenous development and a bottom-up approach. With the support of the local government, citizens have positively influenced all three aspects of sustainability through the activities of the KLIK energy cooperative: social, environmental and economic.

Keywords: endogenous development, bottom-up development, citizen participation, energy cooperative, energy transition

Introduction

The world today is shaken by an energy crisis triggered by the war in Ukraine, which is putting households, farms, businesses and other structures around the world under severe pressure. The rising prices of fossil fuels such as gas, gasoline, oil and others pose a major financial problem, but one must also consider the negative impact on the climate and the environment, particularly through the emission of greenhouse gases. Greenhouse gases such as carbon dioxide (CO₂) are released when fossil fuels are burned, the oil industry is an extremely large polluter and, together with transportation, has an extremely harmful effect on the climate. Due to the negative impact of fossil fuels on the environment and the current energy crisis, the world has realised that an energy transition is necessary. The European Union has defined important environmental goals with the European Green Deal, a package of policy initiatives aimed at putting the EU on the path to energy transition. One of the targets of the Green Deal sets 2030 as the first checkpoint for the energy transition, with the aim of reducing greenhouse gas emissions by 55% compared to 1990. The first target should be seen as a checkpoint for achieving the main objective of the Green Deal, which is to become the first climate-neutral continent by 2050. These goals can be achieved with the same approach that can be used to solve the energy crisis: the energy transition. The need to promote the energy transition in the form of using renewable energy sources instead of fossil fuels is becoming increasingly clear in the current circumstances. Renewable energy sources include solar energy, wind, water, geothermal energy, hydropower and thermal energy from the sea. Encouraged by the goals of the European Green Deal, various local communities have embarked on the path to energy independence and climate neutrality. One example is the energy transition of the municipality of Križevci² in Croatia, where citizens have decided to actively participate in the creation of a climate-neutral town. In 2020, 11 citizens mobilised themselves and founded the KLIK energy cooperative. Since then, they have organised more than 20 educational events and set up an energy and climate office where all citizens can receive free advice, administrative assistance and information on investing in photovoltaic systems. The role of citizens in the energy transition in Križevci is an example of endogenous development and the citizen participation in achieving the

¹ The work was made on the basis of Master thesis of Martin Topljak, defended on September 28th, 2023 at University of Zagreb, Faculty of Agriculture under the mentorship of Assoc. Prof. Nataša Bokan, PhD

² Križevci is a town in Koprivnica-Križevci County (Croatia) with the population of 18.949 (DZS, 2022).

municipality's most important strategic goal and of supporting the local government in implementing a bottom-up development approach aimed at energy independence and climate neutrality. Energy transition is the concept which makes energy independence possible, it implies change from using the non-renewable energy sources (fossil fuels) to renewable energy sources (RES), such as Sun, wind, bioenergy and others. Climate neutrality regarding energy consumption can also be reached through the energy transition, this is what makes this concept crucial for local communities striving to reach the Green Deal objectives.

Material and methods

The concepts of endogenous development, bottom-up development and participation in the context of rural development form the theoretical basis of the research. In order to determine the role of citizens in the energy transition in the municipality of Križevci, available data from the energy cooperative KLIK and qualitative material collected in student practise are used. Primary data are collected through the analysis of previously organised educational events and workshops, the secondary data used are from the annual reports of the cooperative, which show statistics and results (number of implemented photovoltaic systems, total power of implemented photovoltaic systems, project results, number of citizens in trainings, carbon dioxide savings). The research method is a qualitative research with participation.

Results and discussion

What is the energy transition?

The energy transition is defined today as the transition from an energy system dominated by fossil fuels to a system using predominantly renewable energy sources, with increased energy efficiency and better management of energy consumption, in order to separate economic growth and development from fossil sources. The energy transition entails numerous changes, the two most important of which are worth highlighting: first, the structure of electricity generation capacity is gradually changing, with an increasing share of renewable energy sources, mainly solar and wind. The second change is based on technical and technological progress in the transport sector (a major energy consumer), where electric vehicles are gradually replacing traditional fossil fuel-powered vehicles; the entire transition is accompanied by the digitalization of the energy sector, supported by new IT solutions. The energy transition will have an increasing impact on the economy, the environment, the energy sector and transport, as well as on other sectors and society as a whole. The energy transition is a process whose ultimate scope and impact on the economy and society are still unimaginable (Gelo, 2018). In recent years, the concept of energy transition has found a place in the energy policies of many countries, and the production of energy from renewable sources has become a priority of political programmes. The continued use of a large amount of energy from fossil fuels has an impact on climate change and an energy transition towards clean energy is necessary (Šafranec, 2021). The main reason for the energy transition is the pollution caused by burning fossil fuels, which leads to the release of carbon dioxide into the atmosphere. A direct consequence is global warming, which is caused by high emissions of greenhouse gases (Jurić, 2021).

The energy transition is closely linked to the objectives of the European Green Plan, which aims to make Europe the first climate-neutral continent, i.e. to avoid as many CO₂ emissions as are produced. It represents a growth strategy that aims to transform the Union into a modern, resource-efficient and competitive economy in which there will be no net emissions of greenhouse gases in 2050, economic growth will be decoupled from the consumption of energy from fossil sources and no single person or region will be neglected (European Commission, 2019). The energy transition in Križevci requires great and strong efforts, but its end result is the creation of a green, sustainable and energy-efficient town. To make the energy transition possible, everyone needs to be involved and contribute. Infrastructure projects such as large solar power plants would require large investors, which means that citizens are not involved. Woods (2020) describes this as participation, there are different ways of involving citizens in development, in the municipality of Križevci you can see the example of self-mobilisation, the citizens have identified a problem, they have created an energy cooperative through which they have an impact on the local community and get other citizens to achieve the goals of energy independence and climate neutrality. This is also an example of endogenous development, where local resources feed into local development, educated and informed citizens are an important

human resource and citizens are now ready for the necessary changes and next steps in the energy transition. The current state of the energy transition would not be possible without the active support of the local authorities, who support the work of the energy cooperative KLIK and provide grants for the introduction of photovoltaic systems in households, subsidising both the administrative costs and the costs of solar power plants. Here we see an example of bottom-up development, where the development projects are borne by the citizens and not the state. In the book *Rural Geography* (2020), Woods describes the transition from top-down to bottom-up development as a change in the management of rural development. Bottom-up development means the involvement of local communities who are encouraged to identify problems, find appropriate solutions, create and implement development projects. Thus the role of government has changed from a leading and key actor of rural development to a facilitator of rural development and regeneration.

Bottom-up and endogenous development

In recent times, the biggest shift has certainly been from a top-down approach to a bottom-up approach, the realization that rural development cannot be dictated by high institutional authorities but should be the result of local action (Ruganec and Bokan, 2021). Guided by a change in approach, the form and focus of rural development has changed; in most cases, the emphasis is no longer on attracting foreign investors but on improving local, endogenous resources, which is why we refer to this type of development as endogenous (Woods, 2020).

Endogenous development is a newer approach to agriculture and rural areas, where the focus is on local actors doing their own development, i.e. taking development initiatives (Ruganec and Bokan, 2021). An example of endogenous rural development is certainly the municipality of Križevci, where local actors are involved in the development and transformation of their town, encouraging the engagement of the local community and citizens. The most comprehensive rural development program is implemented by the European Union through the European Structural and Investment Funds (hereinafter: ESIF) (Woods, 2020). The actions of the energy transition actors in the municipality of Križevci depend on the financial resources they receive from various ESIF programs. One of the programs is the LIFE program, a financial instrument of the European Union for financing project activities in the field of environmental and climate protection (European Structural and Investment Funds, 2023).

Partnership

Since the energy transition of the city of Križevci, i.e. investing in renewable energy sources and initiating green projects, has been carried out according to the bottom-up development approach, it should be mentioned that the main actors of the energy transition are financed through various funds they receive by participating in tenders for financing projects and activities. As Woods (2020) points out when explaining bottom-up development, local development actors are also expected to raise funds through partnerships. The transition from top-down development to bottom-up development has meant that the state no longer has a monopoly on governance, it no longer delivers public goods but is a facilitator that enables communities to govern themselves. It is assumed that the legitimacy of governance results from active citizenship, the direct participation of citizens and stakeholders in governance activities (Woods, 2020). When formulating the Sustainable Development Goals, the United Nations (UN) defined the need for exchange, unification and transformation. The UN defines partnerships for sustainable development as voluntary and cooperative relationships between different groups in which all stakeholders work together to achieve common goals. The partners jointly carry out tasks and activities to achieve the set goals, share risks, resources and benefits, and share responsibilities (United Nations, 2023). Woods (2020) describes partnership as core to the idea of rural management and mentions how such relationships can manifest in different ways. The most important thing is that partnerships are created in such a way that the partners want to achieve certain goals by working together. The energy transition in the municipality of Križevci would not be possible without a strong partnership of local stakeholders. As already mentioned, the projects are funded by the European Union, but the advisory activities for citizens, the eco-festival and various other initiatives would not be possible without partnerships. The KLIK energy cooperative works together with various partners. The cooperation can be classified as a kind of executive partnership, as the main partner in the energy transition is the local government, i.e. the Municipality of Križevci, which has promoted the synergy of the local community by introducing the agenda “Križevci 2030 - an energy-independent city”, with the aim of achieving energy independence and organizing sustainable management of natural resources. In partnership with the city administration, the energy cooperative KLIK makes an exceptional

contribution by managing the energy and climate office, advising and informing citizens, implementing projects, organizing training and workshops and numerous other activities. Another example of a consultative partnership can be found in Križevci: citizens are involved in the town administration in various ways, the local government supports citizens' initiatives and listens to the needs of the community.

Križevci solar roofs

The solar roofs of Križevci can be described as a pioneering project of the energy transition in Križevci. In 2018, the Green Energy Cooperative from Zagreb presented the project to the municipality, received support from the Municipality of Križevci, involved the citizens and installed solar power plants on the roofs of two public buildings: Križevci Development Centre and Technology Park, and the Municipal Library „Franjo Marković“. Citizens were involved as investors, they invested through microloans, which means that the Križevci solar roof project was financed through crowdfunding. The idea to start the project was initially based on the desire to generate more energy from renewable energy sources. In cooperation with the Križevci Development Centre and Technology Park, a solar power plant was built on the roof of one of the centre's buildings. This project is the first group investment project in Croatia based on the microcredit model. As this method of utilising solar energy resources has been known for a long time, the Green Energy Cooperative aims to create new local values, new opportunities and new jobs with the implementation of this project (ZEZinvest, 2018). The Križevci solar roofs are a project that provides an alternative for citizens to actively participate in the energy transition in their community. Other development projects that increase the standard of living in the local community can be organised according to the same or a similar model (Car et al., 2020). This is also the first energy transition project in Križevci that enabled citizen participation. It created a basis for further development initiatives such as the establishment of a local energy cooperative, i.e. for endogenous development towards energy transition.

Križevci Laboratory for innovation in the climate sector

The Križevci Laboratory for Innovation in Climate (commonly known as: KLIK) was founded in Križevci in 2019. It is a community of citizens created within the transformation process of the Future Cities of Southeast Europe, where the citizens gathered in this community share a common goal of creating a resilient local community. KLIK was founded as „KLIK, Energy Cooperative“ and “Association Križevci Laboratory for Innovation in Climate“. Both organisations gather active enthusiasts who strive to encourage the local population to invest in renewable energy sources (RES). Citizen participation through self-mobilisation based on the existing social capital of the gathered enthusiasts created an organisation that became an important development actor in the town (KLIK, 2023). The energy cooperative KLIK, through its Energy and Climate Office, supports citizens in the implementation of renewable energy projects, informs them about available subsidies for the construction of solar power plants and collaborates with the local administration, which provides citizens with a subsidy for the construction of solar power plants and a subsidy for the creation of construction projects (administrative costs). Cooperation with the local government, i.e. the municipality of Križevci, is important for the development of the municipality. KLIK supports the municipality in achieving the goal of energy independence of the town by 2030, which shows that bottom-up development based on citizen participation is extremely important for achieving development goals. KLIK's activities include various training courses aimed at citizens, farmers, entrepreneurs and all interested parties. The training courses focus on energy efficiency, renewable energy sources, organic farming, waste management and other topics related to a sustainable way of living and working (KLIK, 2023).

Activities of KLIK, the energy cooperative

KLIK, the Energy Cooperative, supports the local government in achieving strategic goals and carries out regular activities as part of the daily work of the Energy and Climate Office. The activities through which KLIK fulfils its mission to promote the energy transition and the development of the municipality of Križevci include: free information to citizens about renewable energy, free administrative assistance in applying for funding, communication and cooperation with partners, participation in the Future Cities initiative, organisation of training courses, organisation of the Eco-Festival, strategic and operational planning with the local government. The KLIK energy cooperative organises occasional training sessions with the aim of informing citizens about various topics

related to energy efficiency, renewable energy and solar power plants, in addition to the advisory activities of the Energy-Climate Office and various project activities. In addition to the energy transition, the cooperative seeks to support the conversion of agriculture to sustainable agricultural practises, which is why educational activities also relate to organic and sustainable agriculture.

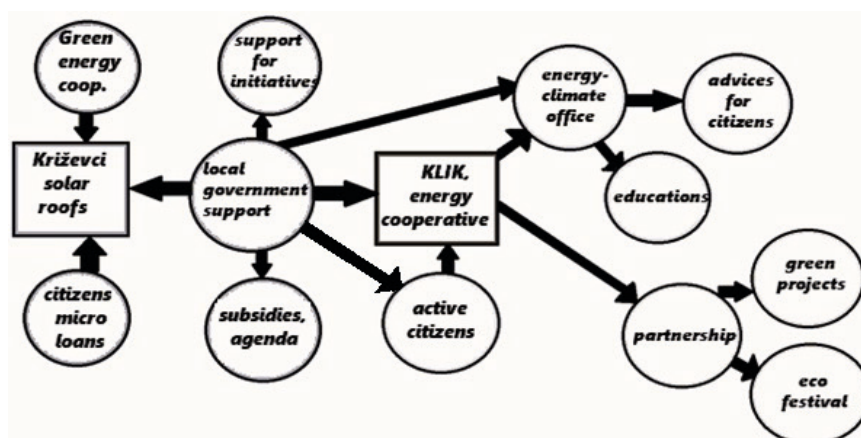


Figure1. Scheme of key actors and activities in energy transition of Križevci.

Source: own

The main actors in achieving the current state of the energy transition in the municipality of Križevci are KLIK, the Energy Cooperative, the Green Energy Cooperative and the local government, which strongly supports the energy transition.

Impact and results of KLIK, the energy cooperative

The energy cooperative KLIK, together with the municipality of Križevci, aims to achieve energy independence by 2030. To achieve this goal, considerable efforts and resources need to be invested, but the impact of the KLIK energy cooperative's activities in the municipality of Križevci is already visible. The most important results are: 277 kW of photovoltaics in households (500,000.00 euros of investment, half of which is covered by subsidies from the municipality), consultation of numerous citizens, 22 educational activities attended by almost 400 citizens (informed and educated citizens are a key resource for energy transition and development), the installed photovoltaics will produce about 288,791 kWh of electrical energy annually, which will result in a saving of 54 tonnes of carbon dioxide (CO₂). The impact is not only reflected in the areas of energy and ecology, but also in the strengthening of the local economy through the creation of 15 new jobs (designers for the installation of photovoltaic systems, fitters). The endogenous development promoted by the KLIK energy cooperative has a positive impact on all three components of sustainability: economic, ecological and social.

Conclusion

The actors involved in the energy transition in the municipality of Križevci are led by citizens who have founded the KLIK energy cooperative through self-mobilisation. Through their activities, they try to support the local government in achieving the strategic goal of energy independence. The local government supports bottom-up development activities and points out that citizens are the main actors in achieving the strategic goals. Move towards bottom-up development brought a change, the state no longer has a monopoly on administration and management. It can be concluded that the energy transition in Križevci was made possible by the transformation from national administration to local management, noting that the legitimacy of management comes from the participation of citizens in management activities. As the energy transition of Križevci Municipality still has not reached the desired level of creating energy-independent and climate neutral town, this opens up a space for further research questions on achieving the higher state of the energy transition, researching the dynamics of local actors, public, civil and private ones, in accomplishing the mentioned goals. Thus the findings from this study could be the starting point for

future studies on how social capital boosted through this successful project will be used and managed in future local policies and civil engagements or how will this experience shape or direct further civil engagement in Križevci and its rural hinterland.

Literature

- Car S., Pašičko R., Kordić Z. (2020). Zadrugarstvo i društveno odgovorno poslovanje. *Social Ecology: a journal for ecological thought and sociological research of the environment*. 29: 417-424.
- European Commission: European Green Deal. Available from:
<https://www.consilium.europa.eu/hr/policies/green-deal/> (Date of access: 10.06.2023.)
- Gelo T. (2018). Energetska tranzicija i novi model energetskeg tržišta. *EFZG Occasional Publications (Department of Macroeconomics)*. 1: 395-426. Available from: <https://ideas.repec.org/h/zag/chaptr/18-17.html> (Date of access: 12.06.2023)
- Jurić I. (2021). Energetska tranzicija na obnovljive izvore energije u posljednjih 10 godina s posebnim osvrtom na geotermalnu energiju. Master thesis, University of Zagreb, Faculty of Mining, Geology and Petroleum Engineering. Available from: <https://urn.nsk.hr/urn:nbn:hr:169:538200> (Date of access: 06.06.2023)
- KLIK (2023). O nama. Available from: <https://klikninaodrzivo.com/>. (Date of access 06.06.2023)
- Ruganec I., i Bokan N. (2021). Kratki lanci opskrbe u kontekstu endogenog ruralnog razvoja. *Agroeconomia Croatica*. 11: 72-82.
- Šafranec K. (2021). Energetska tranzicija Kine. Specijalistički diplomski stručni, University of Zagreb, Faculty of Economy. Available from:
<https://urn.nsk.hr/urn:nbn:hr:148:192417> (Date of access: 06.06.2023).
- United Nations: Partnership for the goals. Available from:
<https://www.un.org/sustainabledevelopment/globalpartnerships/> (Date of access: 12.06.2023).
- Woods M. (2020). Ruralna geografija: procesi, odjeci i iskustva u ruralnom restrukturiranju. Zagreb: Sveučilište u Zagrebu Agronomski fakultet.
- European Structural and Investment Funds: „Program LIFE“. <https://strukturnifondovi.hr/life/> (Date of access: 10.06.2023.)
- ZEZinvest: Križevci Sunny Roofs. <https://zezinvest.community/krizevacki-suncani-krovovi/> (Date of access: 14.06.2023.)

Uloga poljoprivredne savjetodavne službe u inoviranju poslovanja na gospodarstvu – percepcija poljoprivrednika u odabranim županijama

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Sažetak

Cilj rada je utvrditi percepciju poljoprivrednika o ulozi savjetodavne službe u inoviranju poslovanja na gospodarstvu. Empirijsko anketno istraživanje je provedeno 2022. godine na prigodnom uzorku od 100 nositelja obiteljskih poljoprivrednih gospodarstava (OPG) s područja pet županija. Ispitanici (43%) smatraju da su poljoprivredni proizvođači skloni uvođenju inovacija na gospodarstvo, dok njih 50% smatra za sebe otvorenim za inovacije u poljoprivredi. Najčešće uvedene inovacije u proteklih pet godina su „novi traktor“, „dron“ i „GPS navigacija“. Savjetodavna služba je kod 29% ispitanika prepoznata kao kanal informiranja o inovacijama. Usluge savjetodavne službe najčešće se koriste vezano uz promjene tehnologije proizvodnje i prijave na natječaje iz ruralnog razvoja i potpora u poljoprivredi. Ispitanici su suglasni s izjavom da su savjetnici dobro upoznati s inovacijama u poljoprivrednoj proizvodnji (64%), dok više od 50% ispitanih smatra da savjetnici uvijek daju korisne savjete te prenose inovacije i educiraju proizvođače.

Ključne riječi: savjetodavna služba, uloga, inovacije, poljoprivredno gospodarstvo

Uvod

Savjetodavna služba u poljoprivredi je organizacija ili institucija koja pruža stručne savjete i podršku poljoprivrednicima, ali i drugim dionicima u agrobiznisu i ruralnom području. Savjetodavne službe u poljoprivredi koriste rezultate znanstvenih istraživanja, iskustva terenskih stručnjaka i lokalno prilagođene prakse kako bi pružile relevantne i pouzdane savjete. Glavni cilj pružanja stručne podrške je poboljšanje produktivnosti, održivosti i profitabilnosti u poslovanju poljoprivrednih gospodarstava kroz prijenos znanja od istraživačkih institucija i stručnjaka do poljoprivrednika na lako razumljiv i primjenjiv način u svrhu poboljšanja životnog standarda poljoprivrednika.

Savjetodavne službe nude različite druge usluge kao što su: savjetovanje o tehnološkim postupcima (stručni savjeti u uzgoju biljaka, stočarstvu, navodnjavanju, zaštiti od bolesti i štetnika, upotrebi gnojiva i pesticida itd.); ekonomsko-financijsko savjetovanje (stručni savjeti o tržišnim prilikama, cijenama proizvoda, financijskom planiranju, upravljanju troškovima i prihodima te pomoć u donošenju odluka o ulaganju i diversifikaciji poslovanja, itd.); edukacija (organiziranje seminara, radionica, tečajeva i obuka o najnovijim trendovima, tehnologijama i praksama u poljoprivredi, itd.) te promocija inovacija i novih tehnologija u poljoprivredi (FAO, 2015).

Danas su savjetodavne službe u poljoprivredi organizirane kao javne ili privatne organizacije. Javne službe su u pravilu dio Ministarstva poljoprivrede (kao što je to u Hrvatskoj) i srodnih institucija s područja ruralnog razvoja, dok privatne službe mogu biti neovisne organizacije ili dio komercijalnih tvrtki s uslugom savjetovanja (Žutinić i Dekanić, 2010). Služba ima važnu ulogu u premošćivanju jaza između istraživača i poljoprivrednika pružajući im potrebno znanje i vještine za usvajanje novih tehnologija/inovacija i praksi pri tome jačajući sposobnosti poljoprivrednika pri donošenju odluka i vještina rješavanja problema (Altalb i sur., 2015).

Moderne savjetodavne službe dio su AKIS-a (Sustav prijenosa znanja i inovacija u poljoprivredi) te surađuju s drugim dionicima u poljoprivrednom sektoru, poput proizvođača inputa, kreatora politika, istraživača, medija i gospodarskih subjekata (Rivera, Qamar i Mwandemere, 2005).

Cilj rada je prikazati stavove poljoprivrednih proizvođača u pet odabranih županija o ulozi savjetodavne službe u inoviranju poslovanja na gospodarstvu.

Prema Hrvatskoj enciklopediji, inovacija je neka novina, uvođenje novih sustava i mjerila, razvoj i primjena novih rješenja, proizvoda, procesa i postupaka. Inovacija je potpuna novost ili novost za neku okolinu, a ostvaruje se prenošenjem tuđih iskustava koja u dotičnoj okolini dotad nisu bila korištena. Inovacija također može biti poboljšanje i unaprjeđenje već poznatih rješenja. Inovacija je rezultat istraživačke i razvojne sposobnosti pojedinca ili organiziranih skupina stručnjaka. Za razliku od znanstvenog otkrića, inovacija se može pravno zaštititi kao intelektualno vlasništvo (Hrvatska enciklopedija, 2021).

Materijal i metode

Empirijsko anketno istraživanje provedeno je na prigodnom uzorku od 100 poljoprivrednih gospodarstava koja se nalaze na području pet županija (Bjelovarsko-bilogorske, Zagrebačke, Sisačko-moslavačke i Požeško-slavonske te Grada Zagreba) a podaci su prikazani skupno. Istraživanje je započelo kontaktiranjem poljoprivrednika-poznanika, nakon čega se metodom snježne grude došlo do ostalih ispitanika. Istraživanje su proveli studenti-koautori ovog rada za potrebe stručnog projekta. Kriterij sudjelovanja u istraživanju bili su mjesto stanovanja (odabrana županija), punoljetnost ispitanika i nositeljstvo poljoprivrednog gospodarstva. Anketa je provedena licem u lice, od 15. studenog 2021. do 31. siječnja 2022. godine. Anketni upitnik sastojao se od 24 pitanja otvorenog i zatvorenog tipa. Pitanja iz anketnog upitnika su većinski preuzeta iz istraživanja Zrakić i sur. (2018), Zrakić Sušac i sur. (2022) dok je manji dio prilagođen ovom istraživanju. U prvom dijelu ankete pitanja se odnose na sociodemografska obilježja ispitanika i obilježja poljoprivrednih gospodarstava, dok se u drugom dijelu ankete propituje njihova sklonost unaprjeđenju proizvodnje kroz inovacije, potrebe za savjetodavnim uslugama, percepcija uloge službe u razvoju poljoprivrede, način komuniciranja sa službom i zadovoljstvo radom službe. Odgovori ispitanika obrađeni su primjenom jednovarijantne statističke analize.

Rezultati i rasprava

Pregled nekih dosadašnjih istraživanja

Utjecaj inovacija i transfera tehnologija na konkurentnost agrobiznisa u zemljama u razvoju istražuju Rambe i Khaola (2021). Prema rezultatima ankete provedene sa 268 ispitanika iz Južne Afrike i Zimbabvea, autori zaključuju kako transfer tehnologija značajno olakšava utjecaj inovacija na produktivnost i konkurentnost malih, srednjih i mikro poduzeća.

Briones-Peñalver i sur. (2018) istražuju utjecaj društveno odgovornog poslovanja na inovacije i suradnju u agrobiznisu na primjeru Španjolske. Koristeći model strukturnih jednadžbi zaključuju kako inovacije imaju pozitivan utjecaj na suradnju koja potiče društveno odgovorno poslovanje.

Odnos između inovacijskih sposobnosti i inovativnog učinka na uzorku od 300 poljoprivrednih poduzeća analiziraju Leo i sur. (2021). Autori zaključuju kako poljoprivredna poduzeća imaju ključnu ulogu u inovacijskom procesu pri čemu zahtijevaju specifičnu kombinaciju sposobnosti za različite karike u lancu vrijednosti. Tako primjerice, za tvrtke koje se nalaze na vrhu lanca vrijednosti i najudaljenije su od krajnjih potrošača, najvažnije su transakcijske i upravljačke sposobnosti. Za tvrtke najbliže krajnjim potrošačima, najvažnije su transakcijske i razvojne mogućnosti. Prepreke s kojima se susreće poljoprivreda i kako inovacije mogu pomoći u prevladavanju tih prepreka istražuju Gaffney i sur. (2019). Autori navode kako poljoprivredne inovacije i poboljšanje pristupa inovacijama korespondiraju sa dijeljenjem tehnologija (ekonomija dijeljenja).

Obilježja uzorka

Od ukupnog broja ispitanika, većina je muškaraca (70%). Prosječna dob ispitanika je 43,3 godine. Više od polovice (63) ispitanika završilo je srednjoškolsko obrazovanje, dok ih se 18 izjasnilo da imaju višu ili visoku školu. Takav podatak nije nužno pokazatelj svojstva populacije, nego posljedica odabira uzorka.

Značajan dio ispitanika (83%) navodi da je gospodarstvo registrirano kao OPG (obiteljsko poljoprivredno gospodarstvo) dok ih 12% navodi da je to obrt odnosno poduzeće (5%). U uzorku prevladavaju gospodarstva s 4 i više članova kućanstva (61%), dok njih 63% izjavljuje da im poljoprivreda nije primarni izvor prihoda. Prosječna veličina gospodarstva u istraživanju je 35,53 ha, dok su najveće prosječne površine zabilježene u Sisačko-moslavačkoj (68,2 ha) i Bjelovarsko-bilogorskoj županiji (61,8 ha). Najmanje su zabilježene na području Grada Zagreba i to 5,25 ha (Tablica 1).

Tablica 1. Socio-demografska i ekonomska obilježja uzorka

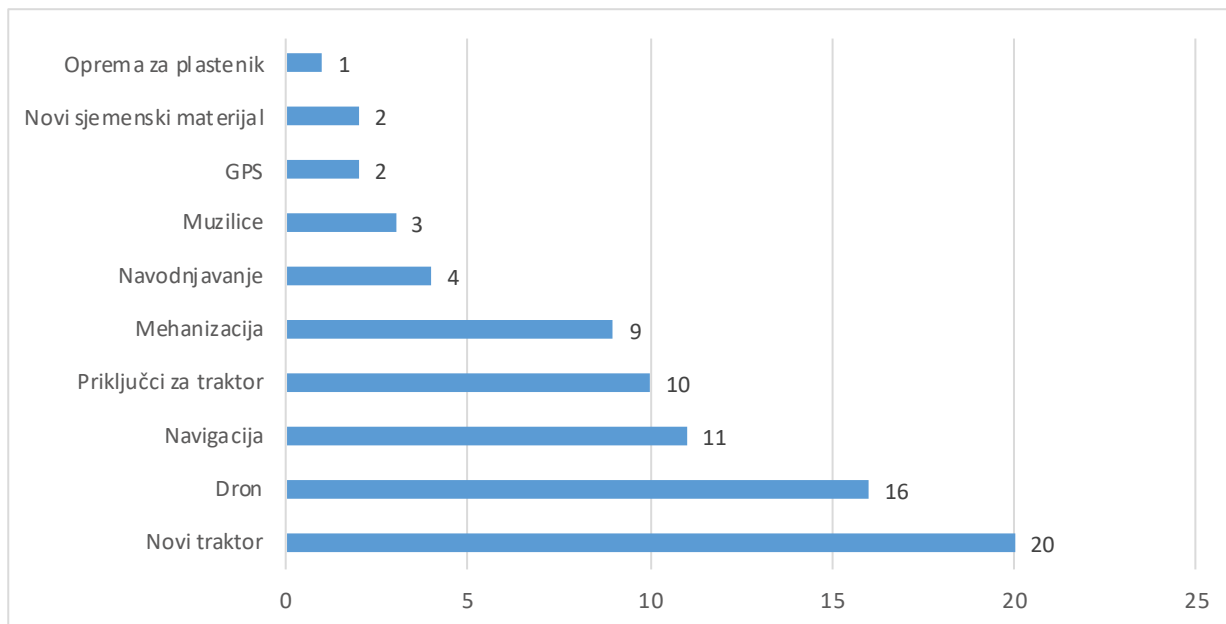
Pokazatelj		%
Stručna sprema	Osnovna škola i NKV	19
	Srednja škola	63
	Visoka ili viša škola	18
Poslovni oblik gospodarstva	OPG	83
	Obrt	12
	Poduzeće	5
Broj članova kućanstva	1 do 2	26
	3	13
	4 i više	61
Poljoprivreda kao glavni izvor prihoda kućanstva	DA	37
	NE	63
Prosječna veličina gospodarstva	35,53 ha	

U strukturi tipa proizvodnje na gospodarstvu većina je ispitanika odgovorila da ima biljnu (37%) odnosno mješovitu proizvodnju (37%), dok se 22% njih bavi stočarstvom odnosno ruralnim turizmom (4%).

Stavovi poljoprivrednika o inovacijama i ulozi savjetodavne službe

U ovom istraživanju veći dio ispitanika (43%) drži da su poljoprivredni proizvođači skloni uvođenju inovacija ili novih tehnologija na gospodarstvu dok ih 36% ima suprotno mišljenje (21% navodi da ne zna). Kod procjene vlastite sklonosti uvođenju inovacija, polovica njih smatra se otvorenim za inovacije, 18% njih nije skloni inovacijama, dok ih 32% ima indiferentan stav o tome („niti sam sklon niti nisam sklon“).

U posljednjih pet godina 20% ispitanika kao inovaciju koje je uvelo navodi „novi traktor“ dok ih 27% navodi da su od inovacija implementirali navigaciju i dron. U istraživanju Zrakić i sur. (2018), 30% ispitanika kao najzastupljeniju uvedenu novinu navodi poljoprivrednu mehanizaciju.



Graf 1. Inovacije u poslovanju u posljednjih pet godina (%)

O inovacijama proizvođači se mogu informirati kroz različite kanale. Najveći dio ispitanika u ovom istraživanju informira se komunicirajući s drugim proizvođačima (33%) i putem medija (31%). Internet kao kanal informiranja o inovacijama ističe 13% proizvođača dok njih 29% koristi mogućnost komunikacije sa savjetodavnom službom.

Na pitanje koliko često komuniciraju sa savjetodavnom službom, ispitanici u najvećoj mjeri (43%) odgovaraju da je to 1 do 3 puta godišnje, zatim do 4 do 10 puta godišnje (23%) dok njih 5% komunicira više od 10 puta. Najčešći kanali komunikacije su osobnim putem (44%) ili telefonom (36%), a kao mjesto komunikacije navode u najvećoj mjeri „prostorije savjetodavne službe“ (73%) ili „na gospodarstvu“ (14%).

Tablica 2. Domena poslovanja u kojoj poljoprivrednici najčešće koriste usluge savjetnika

Izjava	Vrlo često (%)	Često (%)	Rijetko (%)	Ne koristim (%)
Povećanje proizvodnje i proizvodnih kapaciteta	8	34	38	20
Promjena tehnologije proizvodnje	10	35	36	19
Prijava na natječaje za potpore iz Programa ruralnog razvoja	23	33	15	29
Uvođenje dopunskih djelatnosti na PG - Diversifikacija proizvodnje	8	27	22	43
Promocija i prodaja poljoprivrednih proizvoda	11	33	24	32
Upravljanje troškovima poslovanja i resursima na gospodarstvu (menadžment)	10	28	12	50

U Tablici 2 prikazana je distribucija odgovora ispitanih o domeni poslovanja u kojoj poljoprivrednici traže savjet. Kao i u prethodnom istraživanju (Zrakić Sušac i sur., 2022) najčešće su to promjene tehnologije proizvodnje i prijave na natječaje iz ruralnog razvoja i potpora u poljoprivredi.

Tablica 3. Stupanj slaganja s izjavama o ulozi savjetodavne službe u inoviranju proizvodnje

Izjava*	1+2 (%)	3 (%)	4+5 (%)
Savjetnici uvijek daju korisne savjete poljoprivrednicima. (N=83)	17	22	61
Savjetnici educiraju poljoprivredne proizvođače. (N=81)	19	26	56
Savjetodavna služba je najvažniji kanal za prenošenje inovacija do poljoprivrednika. (N=84)	17	29	54
Savjetnici su dobro upoznati s inovacijama u poljoprivrednoj proizvodnji. (N=83)	6	30	64
Prenošenje inovacija do poljoprivrednika je sastavni dio posla savjetnika. (N=84)	19	23	58
Savjetodavna služba je doprinijela da na vlastitom gospodarstvu uvedem inovacije u proizvodnju. (N=83)	23	29	48
Savjetodavna služba mi je pomogla da riješim neke probleme na gospodarstvu. (N=83)	12	42	46
Savjetodavna služba dobro informira poljoprivrednike o financiranju projekata iz EU fondova. (N=83)	25	35	40
Savjetodavna služba pomaže u razvoju poljoprivrednih gospodarstava. (N=81)	1	46	53
Od savjetnika uvijek saznam nešto novo što mi pomaže u radu i proizvodnji. (N=82)	32	26	42

*1-u potpunosti se ne slažem, 2-ne slažem se, 3-niti se slažem niti se ne slažem, 4-slažem se, 5-u potpunosti se slažem

Poljoprivredni proizvođači u najvećoj su mjeri suglasni s izjavom da su poljoprivredni savjetnici dobro upoznati s inovacijama u poljoprivrednoj proizvodnji (64%). Takav nalaz u skladu je s nalazima istraživanja Zrakić i sur. (2018). Visok stupanj suglasnosti (više od 50% ispitanih) zabilježen je i kod izjava da je savjetnici uvijek daju korisne savjete, prenose inovacije i educiraju proizvođače, pomažu u razvoju gospodarstva ali i da su važan kanal u prenošenju inovacija. Nešto manji, iako još uvijek relativno značajan, udio ispitanih drži da od savjetnika sazna nešto novo što pomaže u radu, da ih se dostatno informira o EU fondovima, te da savjetnici doprinose uvođenju inovacija i rješavanju problema.

U istraživanju poljoprivrednici iz svih pet županija ukazuju i na određene probleme i ograničenja u radu savjetodavne službe. Učestalo navode problem nedovoljnog broja savjetnika na terenu što posljedično utječe i na nezadovoljstvo raspoloživošću i dostupnošću savjetodavnih usluga.

Zaključak

Savjetodavna služba u poljoprivredi važan je posrednik i diseminacijski kanal informiranja o znanstvenim postignućima prema poljoprivrednim proizvođačima. Temelj rada savjetodavne službe je edukacija i cjeloživotno obrazovanje. Provedeno empirijsko istraživanje predstavlja nastavak prethodno provedenih istraživanja na drugim lokalitetima odnosno na drugim uzorcima.

Glavni nalazi istraživanja ukazuju da:

- se poljoprivredni proizvođači smatraju sklonima za uvođenje inovacija na svom gospodarstvu;
- u posljednjih pet godina inovacije koje su uvedene na gospodarstvu većinom su u domeni poljoprivredne mehanizacije (novi traktor) i popratnih uređaja (dron, navigacija);
- najvažniji kanali informiranja o novim informacijama i inovacijama su informacije od drugih proizvođača i iz medija dok 29% ispitanika navodi savjetodavnu službu;
- komunikacija sa savjetodavnom službom se provodi najčešće 1 do 3 puta godišnje (43%), najčešće osobno ili telefonom (80%);
- poljoprivrednici najčešće kontaktiraju savjetodavnu službu u domeni promjene tehnologije proizvodnje i prijave na natječaje iz ruralnog razvoja i drugih potpora;

- poljoprivredni proizvođači smatraju da su savjetnici dobro upoznati s inovacijama (64%), da daju korisne savjete, prenose inovacije i educiraju proizvođače, pomažu u razvoju gospodarstva ali i da su važan kanal u prenošenju inovacija;
- glavni percipirani problem u radu savjetodavne službe koji proizvođači navode su nedovoljan broj savjetnika na terenu.

Rezultati ovog istraživanja zbog odabira uzorka ne mogu predstavljati stavove i mišljenja populacije. Primarna svrha ovog, kao i prethodnih istraživanja, je aktualizirati problem uvođenja tehnoloških i drugih inovacija u poljoprivredi te potrebu za daljnjim istraživanjima.

Napomena

Istraživanje je provedeno u sklopu stručnog projekta „Mišljenja poljoprivrednih proizvođača o doprinosu savjetodavne službe u širenju inovacija na poljoprivrednim gospodarstvima“ na diplomskom studiju Agrobiznis i ruralni razvitak obranjenog 27.4.2022. godine grupe autora: Dorotea Blašković, Maja Celižić, Darko Stefan Derenj, Karla Pavlak, Helena Šiftar.

Literatura

- Altalb A.A.T., Filipek T., Skowron P. (2015). The Role of Agricultural Extension in the Transfer and Adoption of Agricultural Technologies. *Asian Journal of Agriculture and Food Sciences*. 3: 500-507.
- Briones-Peñalver A.J., Bernal-Conesa J.A.N., Nieto, C. (2018). Analysis of corporate social responsibility in Spanish agribusiness and its influence on innovation and performance. *Corporate Social Responsibility and Environmental Management*. 25:182-193.
- Gaffney J., Challender M., Califf K., Harden K. (2019). Building bridges between agribusiness innovation and smallholder farmers: A review. *Global food security*. 20: 60-65.
- Inovacija. *Hrvatska enciklopedija, mrežno izdanje*. Leksikografski zavod Miroslav Krleža, 2021. <http://www.enciklopedija.hr/Natuknica.aspx?ID=27516> (Pristupljeno 16.10.2023).
- FAO (2015). Training Manual on ORGANIC FARMING. chrome-extension://efaidnbmnnnibpcajpcgclefindmkaj/https://www.fao.org/fileadmin/templates/nr/sustainability_pathways/docs/Compilation_techniques_organic_agriculture_rev.pdf (Pristupljeno 12.12.2023).
- Leo R.M., Camboim G.F., Avila A.M.S., Reichert F.M., Zawislak P.A. (2022). Innovation capabilities in agribusiness: evidence from Brazil. *RAUSP Management Journal*. 57: 65–83.
- Rambe P. and Khaola P. (2022). The impact of innovation on agribusiness competitiveness: the mediating role of technology transfer and productivity. *European Journal of Innovation Management*. 25: 741-773.
- Rivera W.M., Qamar M.K., Mwandemere H.K. (2005). Enhancing coordination among AKIS/RD actors: an analytical and comparative review of country studies on agricultural knowledge and information systems for rural development (AKIS/RD). FAO, Rome, 2005. <http://www.fao.org/3/a-y9087e.pdf> [Pristupljeno 12.12.2023]
- Zrakić M., Lončar H., Išasegi V., Rukavina M., Žutinić Đ. (2018) Stavovi poljoprivrednika o inovacijama i uloji savjetodavne službe u njihovom širenju. *Agroeconomia Croatica*. 8: 64-74.
- Zrakić Sušac M., Oršolić M., Grgić I., Žutinić Đ. (2022) Percepcija važnosti inovacija i savjetodavne službe za razvoj poljoprivrednog gospodarstva. *Agroeconomia Croatica*. 12: 31-41.
- Žutinić Đ., Dekanić M. (2010). Uloga poljoprivredne savjetodavne službe u ruralnom razvoju vukovarsko-srijemske županije. *Agronomski glasnik*. 72: 239-260.

The role of the agricultural advisory service in farm business innovating - the perception of farmers in selected counties

Abstract

The objective of the paper is to determine the perception of farmers about the role of the advisory service in the farm business innovating. Empirical survey was conducted in 2022 on a suitable sample of 100 respondents from the area of five counties. The respondents (43%) believe that agricultural producers are inclined to introduce innovations in the production, while 50% of them consider themselves open to innovations in agriculture. The most frequently introduced innovations in the last five years are the “new tractor”, the drone and navigation. The advisory service was mentioned by 29% of the respondents as an information channel for innovations. The services of the extension service are most frequently used in the area of changes in production technology and in applying for rural development tenders and support in agriculture. Respondents agree with the statement that extension agents are well acquainted with innovations in agricultural production (64%), while more than 50% of respondents believe that extension agents always provide useful advice, communicate innovations and educate producers.

Keywords: advisory service, role, innovation, agricultural economy



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Očuvanje bioraznolikosti, genetika i
oplemenjivanje

Čimbenici koji utječu na priježetveno proključavanje i dormantnost sjemena pšenice

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Sažetak

Pšenica je jedna od osnovnih poljoprivrednih kultura na koju djeluju razni biotički i abiotički čimbenici. Utjecaj vlažnih uvjeta prije žetve može rezultirati priježetvenim proključavanjem sjemena (PHS) na matičnoj biljci. Veliki utjecaj na tolerantnost PHS imaju okolišni uvjeti, genotip, propusnost sjemene ovojnice, boja sjemena, aktivnost α -amilaze, te dormantnost sjemena koja je glavni čimbenik odgovoran za tolerantnost na priježetveno proključavanje kod sjemena pšenice u žetvenoj zriobi. Dormantnost (mirovanje) sjemena je korisno biološko prilagođavanje kojim se sprječava prijevremeno klijanje sjemena u nepovoljno doba godine. Rad će objasniti pojmove koji utječu na priježetveno proključavanje i dormantnost sjemena pšenice.

Ključne riječi: pšenica, priježetveno proključavanje, dormantnost

Priježetveno proključavanje (PHS)

Pšenica je jedna od najvažnijih žitarica u svijetu koja je podložna različitim biotičkim i abiotičkim čimbenicima koji značajno utječu na njezinu proizvodnju. Među tim čimbenicima, posebno se ističe temperatura i vlažni uvjeti prije žetve, što može rezultirati priježetvenim proključavanjem sjemena. Navedeni proces opisuje situaciju kada zrelo sjeme počinje proključavati na matičnoj biljci prije žetve (Gao i Ayele 2014). Tolerantnost na PHS može biti izazvana okolišnim uvjetima i genotipom, kod kojeg su posebno važni lokusi kvantitativnih svojstava (QTL), te njihovom interakcijom. Također, pored navedenih postoje još neki bitni čimbenici za tolerantnost na PHS, a to su: dormantnost, propusnost sjemene ovojnice, boja sjemena, aktivnost α -amilaza, razina endogenih hormona (Gao i sur., 2013). U ovome radu koriste se znanstveni pregledi dosadašnjih spoznaja o navedenoj temi.

Definicija dormantnosti i njezine kategorije

Dormantnost (mirovanje) sjemena je korisno biološko prilagođavanje kojim se sprječava prijevremeno nicanje sjemena u nepovoljno doba godine. Finch-Savage i Leubner-Metzger (2006) ističu da geni utječu na taj proces, ali i okolina ima značajan utjecaj posredovan hormonima poput abscisinske kiseline i giberelina. Sjeme može ostati dormantno radi preživljavanja nepovoljnih uvjeta poput suše, niske temperature ili nedostatka svjetla, ali kada se uvjeti poboljšaju, giberelin potiče klijanje i rast biljke.

Ovo prilagođavanje omogućava biljkama optimizaciju rasta i reprodukciju te prilagodbu ciklusa rasta prema trenutnim uvjetima. Međutim, nedostatak dormantnosti može dovesti do preuranjenog proključavanja, što je ozbiljan problem kod žitarica poput riže, pšenice, ječma i kukuruza (Bewley i Black, 1994), dok sjeme bez dormantnosti može imati smanjenu dugotrajnost (Clerckx i sur., 2003).

Prema Guberacu (2000), dormantnost sjemena može se podijeliti u tri kategorije: pravo (endogeno) mirovanje, prinudno (relativno) mirovanje i sekundarno mirovanje. Kod pravog mirovanja sjeme ne proključa čak ni kad su vanjski uvjeti za klijanje povoljni. Ovo stanje može spontano ili pod utjecajem vanjskih faktora prestati, odražavajući važnu ulogu u prilagodbi biljaka promjenjivim uvjetima okoline.

Prinudno (relativno) mirovanje je stanje u kojem sjeme ne klija zbog nepovoljnih uvjeta u okolišu. Sekundarno

mirovanje uzrokovano je kasnijim djelovanjem okolinskih čimbenika, pri čemu visoke temperature često igraju ključnu ulogu. Visoke temperature mogu izazvati promjene u ovojnici sjemena, čineći je nepropusnom za plinove i vodu te sprječavajući normalno klijanje.

Značajan broj znanstvenika navodi da postoji nekoliko načina prekidanja dormantnosti sjemena, a najčešći su: temperatura, odstranjivanje sjemenjače, tretiranje sjemena giberelinskom kiselinom (GA3), ispiranje inhibitora vodom, tretiranje sjemena pomoću svjetla, djelovanje kemikalija, suho čuvanje, prethodno hlađenje, kalijev nitrat (Zare i sur., 2011; Can i sur., 2009; Majd i sur., 2013; Baskin i Baskin 2003; Naredo i sur., 1998; Guberac, 2000).

Utjecaj sjemene ovojnice i boje sjemena na PHS

Sjemena ovojnica predstavlja prvi zaštitni sloj sjemena koji bi mogao spriječiti apsorpciju vode u sjeme kako bi se povećala tolerantnost na PHS. U trenutku kada sjeme počne upijati vodu dolazi do aktivacije α -amilaze u aleuronskom sloju, te počinje proces klijanja. Hoće li neka sorta biti tolerantna također ovisi i o rasporedu stanica epiderme. Stanice epiderme mogu biti čvršće raspoređene te na taj način mogu biti manje propusne za vodu, što sortu čini tolerantnijom na PHS (Wang i sur., 2008).

Osim sjemene ovojnice, bitan čimbenik za tolerantnost na PHS je boja sjemena. Linije s bijelom bojom sjemena općenito su osjetljivije na proklijavanje od onih s crvenom bojom sjemenom. Crvena i bijela boja odnose se na boju ovojnice sjemena koju kontroliraju tri nevezana gena. Geni koji kontroliraju PHS povezani su s genima za boju ovojnice sjemena, ili su blizu njih na istom kromosomu, tako da kada oplemenjivači provode selekciju na bijelu boju ovojnice sjemena, oni također neizravno provode selekciju na nižu dormantnost.

Sva tri zasebna gena za boju ovojnice sjemena moraju biti homozigotna za bijelu boju kako bi se razvila sorta pšenica s bijelom ovojnicom sjemena.

Crvena boja je dominantna u odnosu na bijelu, tako da jedan crveni gen rezultira pojavom crvene boje ovojnice. Stupanj crvene boje je aditivno uvjetovan, što znači da linije pšenice s tri crvena gena daju najtamnije crveno sjeme. Općenito, dormantnost je veća kod linija s većim brojem gena za crvenu ovojnicu sjemena. Postoje neke varijacije između linija koje imaju isti broj crvenih gena zbog različitih formi gena. Postoje također geni koji kontroliraju dormantnost sjemena, a koji su neovisni o genima za boju ovojnice sjemena. (Thomason i sur., 2019).

Prema istraživanju Groos i sur., (2002), veza između priježetvenog proklijavanja i boje sjemena pšenice može se objasniti plejotropnim učinkom gena koji kontroliraju pigmentaciju sjemena (R). Općenito, crvena boja sjemena pšenice, koja je kontrolirana genima za crvenu boju (R), prihvaćena je kao marker za otpornost na PHS. U njihovom istraživanju analizirane su 194 rekombinantne inbred linije koje su rezultat križanja dvaju kultivara, Renan i Récital, kako bi se utvrdili lokusi kvantitativnih svojstava (QTL) povezani s otpornošću na PHS i bojom sjemena.

Kultivar Renan, koji ima crvenu boju sjemena, pokazao se otpornim na PHS, dok je kultivar Récital, s bijelim sjemenom, izrazito podložan PHS-u. Nakon dvije godine istraživanja, identificirana su četiri QTL-a povezana s PHS i bojom sjemena. Tri od tih četiri QTL-a locirana su na dužim krakovima kromosoma 3A, 3B i 3D, blizu lokusa gdje su ranije pronađeni geni R i taVp1. Otpornost na PHS pripisuje se alelima naslijeđenim od kultivara Renan za ova tri QTL-a. Četvrti QTL za PHS i boju sjemena lociran je na kraćem kraku kromosoma 5A i pripisuje se alelu koji potječe od kultivara Récital.

Napredak u razvoju visokopropusnih molekularnih markera omogućio je identifikaciju lokusa povezanih s otpornošću na PHS na kromosomu 3B heksaploidne pšenice (Cabral i sur., 2014). Povezanost između PHS i boje sjemena može se objasniti uskom genetskom vezom između gena za boju sjemena i otpornosti na PHS ili putem plejotropnih učinaka gena za boju sjemena (Flintham, 2000; Lin i sur., 2016).

Aktivnost α -amilaze

Enzim α -amilaza je prisutan u širokom spektru biljaka te igra ključnu ulogu u mnogim fiziološkim procesima. Ovaj enzim sposoban je hidrolizirati α -1,4-glikozidne veze u molekulama škroba. Ekspresija α -amilaze u biljkama povezana je s njihovim metaboličkim procesima i može značajno utjecati na brojne aspekte biljnog razvoja. Ekspresija α -amilaze može utjecati na brzinu klijanja sjemena, tolerantnost biljaka na niske temperature te na ukupan prinos sjemena (Autio i sur., 2001).

Važno je napomenuti da regulacija ekspresije α -amilaze u biljkama strogo ovisi o prisutnosti fitohormona kao što

su ABA (abscisinska kiselina) i giberelin. Ovi fitohormoni igraju ključnu ulogu u kontroliranju kada i kako će se α -amilaza izražavati u biljkama, što ima dubok utjecaj na njihovu fiziologiju i razvoj.

Tijekom razvoja sjemena, fitohormon ABA inhibira ekspresiju amilaze. U pšenici su identificirana četiri izoenzimski oblika α -amilaze koje utječu na otpornost na PHS. Ta četiri izoenzima nazivamo: malt- α -amilaza (α -amilaza-1), čiji gen se nalazi na kromosomu 6; green- α -amilaza (α -amilaza-2), čiji gen se nalazi na kromosomu 7; α -amilaza-3, čiji gen se nalazi na kromosomu 5 i α -amilaza-4, koja ima dva člana s genima na kromosomima 2 i 3 (Zhang i sur. 2017).

Geni za α -amilazu-1 i α -amilazu-2 prisutni su u genomu B. Također, GA3 uključena je u regulaciju razine ekspresije α -amilaze-1 i α -amilaze-2 (Marchylo i sur., 1983).

Utjecaj biljnih hormona na PHS

Proteini i biljni hormoni igraju ključnu ulogu u regulaciji dormantnosti sjemena, kontrolirajući procese indukcije i prekida ovog stanja (Née i sur., 2017). Dva glavna antagonista u ovom procesu su biljni hormoni, abscisinska kiselina (ABA) i giberelini (GA), dok se od proteina mogu izdvojiti ABI3, ABI5, RGL2 (Gao i Ayele 2014). ABA ima pozitivan utjecaj na indukciju i održavanje dormantnosti sjemena, dok GA3 potiče klijanje sjemena (Finch-Savage i Leubner-Metzger 2006). Zanimljivo je napomenuti da gubitak dormantnosti sjemena nakon izlaganja hladnoj imbibiciji (upijanje vode pri nižim temperaturama), rezultira smanjenjem endogenih razina ABA. Ovo otkriće sugerira da snižena signalizacija ABA može biti ključni mehanizam koji potiče gubitak dormantnosti (Tuttle i sur., 2015). Ovaj mehanizam omogućava sjemenu da prepozna povoljne uvjete za klijanje i nastavi svoj razvoj. U svakom slučaju, razumijevanje kompleksnih interakcija između biljnih hormona, proteina i okolišnih čimbenika važno je za razvoj strategija za optimizaciju procesa klijanja sjemena i poboljšanje usjeva biljaka. Ova istraživanja pružaju važan uvid u regulaciju fizioloških procesa biljaka i mogu imati značajan utjecaj na poljoprivrednu proizvodnju i očuvanje biljnih vrsta.

U svom istraživanju su Liu i sur. (2013) identificirali značajne mehanizme koji reguliraju dormantnost sjemena pšenice putem biljnog hormona abscisinske kiseline (ABA). Njihova istraživanja ukazuju na to da ABA održava dormantnost sjemena pšenice putem nekoliko ključnih mehanizama:

- ABA utječe na pregradnju kromatina. Ovaj proces, poznat kao epigenetska modifikacija, može utjecati na dostupnost gena za transkripciju i time regulirati različite procese u biljci.
- ABA mijenja svojstva stanične membrane, što može utjecati na propusnost i funkcionalnost membrane. Ovo također može igrati ulogu u održavanju dormantnosti sjemena.
- ABA aktivira gene koji su uključeni u katabolizam giberelina (GA), drugog važnog biljnog hormona. GA je poznat po svojoj ulozi u stimuliranju klijanja sjemena, pa je ABA regulacijom GA gena ključan faktor u održavanju dormantnosti sjemena.

Lokusi kvantitativnih svojstava (QTL)

Priježetveno proklijavanje je kod pšenice kontrolirano kvantitativnim lokusima koji se nalaze na gotovo svim kromosomima (1A, 1B, 2A, 2B, 2D, 3A, 3B, 3D, 4A, 4B, 5B, 5D, 6A, 6B, 6D, 7A, 7B i 7D) (Munkvold i sur., 2009; Mares i Mrva, 2014; Xiao-bo i sur., 2008; Fofana i sur., 2009; Mohan i sur., 2009; Lohwasser i sur., 2005; Chao i sur., 2010). Također, nekoliko gena pokazali su se kao dobri kandidati za otpornost na priježetveno proklijavanje, uključujući i TaSdr, TaPHS1, TaMFT, TaVp-1, Tamyb10 i TaMKK3-A. Ovi geni pokazali su se kao vrlo vrijedni u programima oplemenjivanja (Ali i sur., 2019).

Zaključak

Klimatske promjene i rast svjetske populacije predstavljaju ozbiljne izazove za proizvodnju hrane. Utjecaj sve češćih vlažnih uvjeta pred žetvu, može rezultirati priježetvenim proklijavanjem sjemena na matičnoj biljci, koje pridonosi smanjenim prinosom te lošijom kvalitetom sjemena. Proučavanje čimbenika koji utječu na priježetveno proklijavanje ključno je kako bi se razvile sorte koje su većeg prinosa i kvalitete, te otpornije na različite stresne uvjete. Regulacijski mehanizmi priježetvenog proklijavanja i dalje ostaju nepoznanica te je zbog toga značajan napredak u poboljšanju svojstva otpornosti na priježetveno proklijavanje ograničen. Zbog toga su daljnja istraživanja i inovacije u oplemenjivanju pšenice nužni kako bi se osigurala održiva proizvodnja hrane za rastuću svjetsku populaciju.

Literatura

- Ali A., Cao J., Jiang H., Chang C., Zhang H.P., Sheikh S.W., Ma C. (2019). Unraveling molecular and genetic studies of wheat (*Triticum aestivum* L.) resistance against factors causing pre-harvest sprouting. *Agronomy*. 9: 117.
- Autio K., Simoinen T., Suortti T., Salmenkallio-Marttila M., Lassila K., Wilhelmson A. (2001). Structural and Enzymic Changes in Germinated Barley and Rye. *Journal of the Institute of Brewing*. 107: 19–25.
- Baskin C.C., and Baskin J.M. (2003). When breaking seed dormancy is a problem: try a move-along experiment. *Native Plants Journal*. 4: 17-21.
- Bewley J.D., and Black M. (1994). *Seeds: physiology of development and germination*. Springer Science and Business Media.
- Cabral A.L., Jordan M.C., McCartney C.A., You F.M., Humphreys D.G., MacLachlan R., Pozniak C.J. (2014). Identification of candidate genes, regions and markers for pre-harvest sprouting resistance in wheat (*Triticum aestivum* L.). *BMC plant biology*. 14: 1–12.
- Chao S., Xu S.S., Elias E.M., Faris J.D., Sorrells M.E. (2010). Identification of chromosome locations of genes affecting preharvest sprouting and seed dormancy using chromosome substitution lines in tetraploid wheat (*Triticum turgidum* L.). *Crop science*. 50: 1180-1187.
- Clerckx E.J., Vries H.B.D., Ruys G. J., Groot S.P., Koornneef M. (2003). Characterization of green seed, an enhancer of *abi3-1* in *Arabidopsis* that affects seed longevity. *Plant physiology*. 132: 1077-1084.
- Finch-Savage W.E., and Leubner-Metzger G. (2006). Seed dormancy and the control of germination. *New phytologist*. 171: 501-523.
- Flintham J.E. (2000). Different genetic components control coat-imposed and embryo-imposed dormancy in wheat. *Seed Science Research*. 10: 43–50.
- Fofana B., Humphreys D.G., Rasul G., Cloutier S., Brûlé-Babel A., Woods S., Somers D.J. (2009). Mapping quantitative trait loci controlling pre-harvest sprouting resistance in a red × white seeded spring wheat cross. *Euphytica*. 165: 509-521.
- Gao F., and Ayele B.T. (2014). Functional genomics of seed dormancy in wheat: advances and prospects. *Frontiers in Plant Science*. 5: 458.
- Gao X., Hu C.H., Li H.Z., Yao Y.J., Meng M., Dong J., Li X.Y. (2013). Factors affecting pre-harvest sprouting resistance in wheat (*Triticum aestivum* L.): a review. *Journal Animal and Plant Sciences*. 23: 556-565.
- Groos C., Gay G., Perretant M.R., Gervais L., Bernard M., Dedryver F., Charmet G. (2002). Study of the relationship between pre-harvest sprouting and grain color by quantitative trait loci analysis in a white × red grain bread-wheat cross. *Theoretical and Applied Genetics*. 104: 39–47.
- Guberac V. (2000). *Sjemenarstvo ratarskih kultura*, 8-9.
- Ju L., Jing Y., Shi P., Liu J., Chen J., Yan J., Sun J. (2019). JAZ proteins modulate seed germination through interaction with ABI 5 in bread wheat and *Arabidopsis*. *New Phytologist*. 223: 246-260.
- Lohwasser U., Röder M.S., Börner A. (2005). QTL mapping of the domestication traits pre-harvest sprouting and dormancy in wheat (*Triticum aestivum* L.). *Euphytica*. 143: 247-249.
- Majd R., Aghaie P., Monfared E.K., Alebrahim M.T. (2013). Evaluating of some treatments on breaking seed dormancy in Mesquite. *International Journal of Agronomy and Plant Production*. 4: 1433-1439.
- Marchylo B.A., Kruger J.E., Macgregor A.W. (1983). Production of multiple forms of α -amylase in germinated, incubated, whole, de-embryonated wheat kernels. *Cereal Chemistry*. 61: 305–310.
- Mares D.J., and Mrva K. (2014). Wheat grain preharvest sprouting and late maturity alpha-amylase. *Planta*. 240: 1167-1178.
- Mohan A., Kulwal P., Singh R., Kumar V., Mir R.R., Kumar J., Gupta P.K. (2009). Genome-wide QTL analysis for pre-harvest sprouting tolerance in bread wheat. *Euphytica*. 168: 319-329.

- Munkvold J.D., Tanaka J., Benscher D., Sorrells M.E. (2009). Mapping quantitative trait loci for preharvest sprouting resistance in white wheat. *Theoretical and applied genetics*. 119: 1223-1235.
- Naredo M.E.B., Juliano A.B., Lu B.R., De Guzman F.L.O.R.A., Jackson, M.T. (1998). Responses to seed dormancy-breaking treatments in rice species (*Oryza L.*). *Seed Science and Technology*. 26: 675-690.
- Née G., Kramer K., Nakabayashi K., Yuan B., Xiang Y., Miatton E., Soppe W.J. (2017). DELAY OF GERMINATION1 requires PP2C phosphatases of the ABA signalling pathway to control seed dormancy. *Nature communications*. 8: 72.
- Nukasani V., Potdukhe N.R., Bharad S., Deshmukh S., Shinde S.M. (2013). Genetic variability, correlation and path analysis in wheat. *Journal of Cereal Research*. 5: 48-51.
- Lin M., Cai S., Wang S., Liu S., Zhang G., Bai G. (2015). Genotyping-by-sequencing (GBS) identified SNP tightly linked to QTL for pre-harvest sprouting resistance. *Theoretical and Applied Genetics*. 128: 1385-1395.
- Liu A., Gao F., Kanno Y., Jordan M.C., Kamiya Y., Seo M., Ayele B.T. (2013). Regulation of wheat seed dormancy by after-ripening is mediated by specific transcriptional switches that induce changes in seed hormone metabolism and signaling. *PLOS One*. 8: e56570.
- Thomason W.E., Hughes K.R., Griffey C.A., Parrish D.J., Barbeau W.E. (2019). Understanding pre-harvest sprouting of wheat.
- Tuttle K.M., Martinez S.A., Schramm E.C., Takebayashi Y., Seo M., Steber C.M. (2015). Grain dormancy loss is associated with changes in ABA and GA sensitivity and hormone accumulation in bread wheat, *Triticum aestivum* (L.). *Seed Science Research*. 25: 179–193.
- Zare S., Tavili A., Darini, M.J. (2011). Effects of different treatments on seed germination and breaking seed dormancy of *Prosopis koelziana* and *Prosopis juliflora*. *Journal of Forestry Research*. 22: 35-38.
- Zhang Q., and C Li. (2017). Comparisons of copy number, genomic structure, and conserved motifs for α -amylase genes from barley, rice and wheat. *Frontiers in Plant Science*. 8: 1727.
- Wang X., Liu H., Liu G., Mia M.S., Siddique K.H., Yan G. (2019). Phenotypic and genotypic characterization of near-isogenic lines targeting a major 4BL QTL responsible for pre-harvest sprouting in wheat. *BMC plant biology*. 19: 1-10.
- Wang X.G., Ren J.P., Yin J. (2008). The mechanism on wheat pre-harvest resistant sprouting. *China Agriculture Sciences*. 24: 243-250.
- Xiao-bo R., Xiu-jin L., Deng-cai L., Jia-li W., You-liang Z. (2008). Mapping QTLs for pre-harvest sprouting tolerance on chromosome 2D in a synthetic hexaploid wheat× common wheat cross. *Journal of applied genetics*. 49: 333-341.

Factors affecting preharvest sprouting and wheat seed dormancy

Abstract

Wheat is one of the primary agricultural crops affected by various biotic and abiotic factors. The influence of moist conditions before harvest can result in pre-harvest sprouting (PHS) on the mother plant. Environmental conditions, genotype, seed coat permeability, seed color, α -amylase activity, and seed dormancy significantly impact PHS tolerance. Seed dormancy is a beneficial biological adaptation that prevents premature seed germination during unfavorable periods of the year. The paper will explain the terms that affect the preharvest sprouting and wheat seed dormancy.

Keywords: wheat, preharvest sprouting, seed dormancy

Korištenje IAKS mjera iz Programa ruralnog razvoja za očuvanje bioraznolikosti u Bjelovarsko-bilogorskoj županiji

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Sažetak

Zajednička poljoprivredna politika Europske unije sadrži niz mjera za očuvanje bioraznolikosti i poboljšanje usluga ekosustava, čiji je cilj potaknuti poljoprivrednike na prihvaćanje i korištenje određenih praksi i načina uzgoja koji će smanjiti negativni utjecaj poljoprivrede na bioraznolikost. Učinci ovise o educiranosti poljoprivrednika i razini prihvaćenosti mjera. Istraživano je korištenje IAKS mjera u Bjelovarsko-bilogorskoj županiji i provedeno je anketno istraživanje o informiranosti poljoprivrednika o značenju IAKS mjera za očuvanje bioraznolikosti. Poljoprivrednici su podnijeli 1.326 zahtjeva za potporama u 2022. godini. Od 209 ispitanika, njih 36,8 % je točno odgovorilo koji je cilj mjera, dok 34,2 % smatra da nisu dovoljno educirani i da su procedure i zahtjevi presloženi. Radi povećanja korištenja IAKS mjera preporuča se pojačati aktivnosti osvješćivanja i edukacije poljoprivrednika.

Ključne riječi: ruralni razvoj, poljoprivreda, bioraznolikost, informiranost poljoprivrednika

Uvod

Poljoprivredna djelatnost omogućuje opstanak brojnih biljnih i životinjskih vrsta, staništa, ekosustava i krajobraza, a način gospodarenja u poljoprivredi utječe na stanje prirode (Znaor i Karoglan Todorović, 2016). Intenzifikacija poljoprivrednih aktivnosti zadnjih desetljeća drastično je preobrazila funkcioniranje agroekosustava, ugrozila bioraznolikost i prouzročila da je 76 % poljoprivrednih staništa i 70 % vrsta koje ih nastanjuju u nepovoljnom stanju očuvanosti. Pokretači gubitka bioraznolikosti su različiti, ali glavni su homogenizacija krajolika, gubitak poluprirodnih staništa i povećana primjena gnojiva i pesticida na poljima. Trend gubitka bioraznolikosti u agroekosustavima izaziva zabrinutost zbog poremećaja usluga ekosustava bitnih za poljoprivrednu produktivnost, kao što su oprašivanje, održavanje staništa, formiranje tla i kontrola štetnika. Stoga je zaštita bioraznolikosti ključni korak u osiguranju dugoročne održivosti poljoprivrede (Séchaud i sur., 2022).

Zajednička poljoprivredna politika Europske unije danas zagovara višeznačnu ulogu poljoprivrede: gospodarsku, ekološku i društvenu. Poljoprivredno-okolišne mjere uvedene su 1992. godine radi poticanja praksi povezanih s koristima za bioraznolikost na europskim farmama, a slični oblici poticaja ostali su na snazi sve do danas. Osim doprinosa zaštiti okoliša i klime, cilj mjera je očuvanje i povećanje bioraznolikosti, poboljšanje strukture tla, smanjenje unosa gnojiva i pesticida, posebno u blizini osjetljivih vodnih tijela, te dobrobit životinja (Geppert i sur., 2023). Mjere doprinose diverzifikaciji poslovnih mogućnosti na poljoprivrednim gospodarstvima i podizanju kvalitete života u ruralnim zajednicama. Poljoprivrednici koji pristaju na poljoprivredno-okolišno-klimatske mjere dobrovoljno se obvezuju da će najmanje pet godina primjenjivati poljoprivredne prakse prihvatljive za okoliš koje su ambicioznije od relevantnih zakonskih obveza. Stoga, pojedinačne odluke poljoprivrednika igraju važnu ulogu u uspjehu ovih mjera (Hannus i Sauer, 2021). Dosadašnji sustav Zajedničke poljoprivredne politike nije postigao dovoljan i značajan učinak na bioraznolikost i okoliš.

Europski revizorski sud procijenio je pomaže li poljoprivredna politika Europske unije očuvati i povećati bioraznolikost (Europski revizorski sud, 2020). Utvrdio je da su ciljevi iz Strategije o bioraznolikosti oblikovani tako da je teško mjeriti napredak, učinak izravnih plaćanja je ograničen ili nepoznat, a prioritet su imale mjere kojima se ostvaruje

slabiji učinak. Sud je preporučio povećanje doprinosa za bioraznolikost kroz izravna plaćanja, a najprikladnije su poljoprivredno-okolišno i klimatske mjere, organska poljoprivreda i mjere u područjima ekološke mreže Natura 2000. Trenutačno se poljoprivrednici ne potiču na široko korištenje ovih mjera zbog manjka informacija, složenosti i nejasnoća u postupcima prijave, strogih smjernica, dugotrajnih nadzora, te visokog rizika od sankcija u slučaju nepridržavanja. Potrebno je unaprijediti i strukturirati pružanje informacija o ekološkim ciljevima i sadržajima financiranja te ih stalno ažurirati (Geppert i sur., 2023).

Program ruralnog razvoja Republike Hrvatske za razdoblje 2014.–2020., sadrži 19 mjera, od kojih su za očuvanje prirode i okoliša najvažnije dvije: Mjera 10 Poljoprivreda, okoliš i klimatske promjene, s 1 podmjerom i 17 tipova operacija i Mjera 11 Ekološki uzgoj, s dvije podmjere (Vakanjac i sur., 2022). IAKS (Integrirani administrativni i kontrolni sustav) koristi Agencija za plaćanja u poljoprivredi, ribarstvu i ruralnom razvoju za identifikaciju korisnika te obradu i isplatu zahtjeva za potporu, kao i nadzor nad provedbom svih potpora i mjera ruralnog razvoja. Cilj korištenja IAKS mjera je potaknuti poljoprivrednike da zaštite i poboljšaju okoliš na svom gospodarstvu prihvaćanjem određenih praksi i načina uzgoja kojima je moguće umanjiti ili zaustaviti negativni utjecaj poljoprivrede na prirodne resurse i bioraznolikost (Pokos, 2016).

Prema definiciji u Zakonu o zaštiti prirode („Narodne novine”, broj 80/2013., 15/2018., 14/2019. i 127/2019), bioraznolikost je sveukupnost svih živih organizama koji su sastavni dijelovi ekosustava, a uključuje raznolikost unutar vrsta, između vrsta, životnih zajednica te raznolikost ekosustava. Temeljni mehanizam u politici zaštiti prirode Europske unije za postizanje povoljnog stanja očuvanosti divljih vrsta i stanišnih tipova je uspostava ekološke mreže Natura 2000, koju čine područja važna za očuvanje ugroženih vrsta divlje faune i flore i stanišnih tipova (Ozimec i sur., 2022). Republika Hrvatska proglasila je područje ekološke mreže temeljem Uredbe o ekološkoj mreži i nadležnostima javnih ustanova za upravljanje područjima ekološke mreže („Narodne novine“, broj 80/2019. i 119/2023.). Ekološka mreža Natura 2000 obuhvaća 37 % kopnenog teritorija Republike Hrvatske. Čine ju 783 područja, od kojih 38 područja očuvanja značajna za ptice (POP) i 745 područja očuvanja značajnih za vrste i stanišne tipove (POVS). U Bjelovarsko-bilogorskoj županiji nalazi se, cjelovito ili djelomično, 17 područja ekološke mreže Natura 2000, koja obuhvaćaju 30,2 % ukupne površine županije (<https://zastita-prirode-bbz.hr/podrucja/ekolska-mreza>).

Informiranost poljoprivrednika o mjerama javnih politika, uključujući i mjere Zajedničke poljoprivredne politike usmjerene na očuvanje bioraznolikosti, preduvjet je učinkovitog korištenja javnog novca, a mjere, zahtjevi i procedure prijave, provedbe i kontrole mjera moraju biti prilagođeni poljoprivrednicima. U svrhu utvrđivanja koliko su poljoprivrednici na području Bjelovarsko-bilogorske županije upoznati s IAKS mjerama ruralnog razvoja, te koliko ih koriste, provedeno je istraživanje putem anketnog upitnika. Pretpostavka istraživanja bila je da su poljoprivrednici u Bjelovarsko-bilogorskoj županiji slabo ili vrlo malo upućeni u navedene mjere.

Materijal i metode

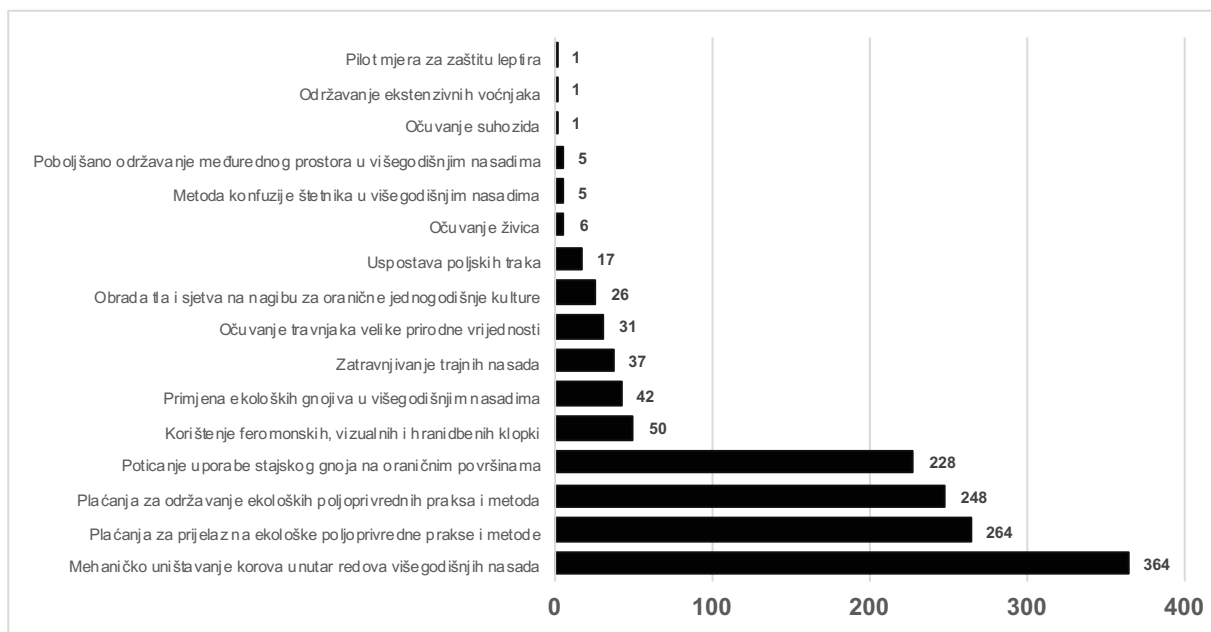
Izvor dijela korištenih podataka, primjerice broj zahtjeva za pojedinu IAKS mjeru te podaci o korištenju poljoprivrednog zemljišta u Bjelovarsko-bilogorskoj županiji je Agencija za plaćanja u poljoprivredi ribarstvu i ruralnom razvoju (<https://www.apprrr.hr/baza-korisnika-potpورا>). S obzirom da su ovi podaci razvrstani u Excel tablicama po naseljima, trebalo ih je dodatno obraditi i prilagoditi.

Ostali podaci, primjerice o dobi poljoprivrednika i njihovom stupnju obrazovanja preuzeti su iz Upisnika poljoprivrednika (<https://www.apprrr.hr/upisnik-poljoprivrednika>).

Anketni upitnik sastavljen je kako bi istražili razinu informiranosti poljoprivrednika o poznavanju ciljeva IAKS mjera ruralnog razvoja za očuvanje bioraznolikosti, korištenju mjera, te stavova o prednostima i preprekama za bolje korištenje ovih mjera. Upitnik je bio kreiran korištenjem Google obrazaca i sastojao se od pet pitanja: Na tri pitanja se odgovaralo s „Da“ ili „Ne“, dok su za dva pitanja sudionici imali mogućnost odgovora ili upisivanja vlastitog odgovora. Google obrasci su bili otvoreni za popunjavanje od 7. prosinca 2022. do 15. ožujka 2023. godine. Poziv, uz upute kako ispuniti anketni upitnik, poslan je na 400 e-mail adresa ciljane skupine poljoprivrednika koji su upisani u Agronet - digitalnu aplikaciju koju vodi Agencija za plaćanja u poljoprivredi ribarstvu i ruralnom razvoju.

Rezultati i rasprava

Prema podacima iz Upisnika poljoprivrednika, u Bjelovarsko-bilogorskoj županiji u 2022. godini ima 10.711 poljoprivrednika, odnosno 6,4 % od 166.430 poljoprivrednika u Republici Hrvatskoj. Jedinствeni zahtjev za potporu podnijelo je 8.417 poljoprivrednika. Za korištenje IAKS mjera čiji cilj je očuvanje bioraznolikosti, podnešeno je 1.326 zahtjeva (15,7 %). Prema broju zahtjeva (Grafikon 1.), najviše ih je za mjere: Mehaničko uništavanje korova unutar redova višegodišnjih nasada, Plaćanja za prijelaz na ekološke poljoprivredne prakse i metode, Plaćanja za održavanje ekoloških poljoprivrednih praksa i metoda, Poticanje uporabe stajskog gnoja na oraničnim površinama i Korištenje feromonskih, vizualnih i hranidbenih klopki.



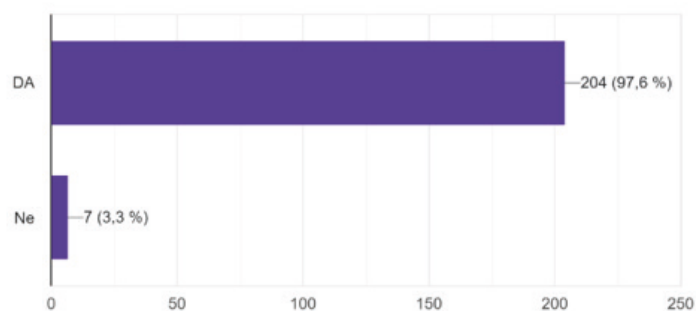
Grafikon 1. Struktura korištenja IAKS mjera u Bjelovarsko-bilogorskoj županiji prema broju podnesenih zahtjeva u 2022. godini (Izvor: Agencija za plaćanja u poljoprivredi, ribarstvu i ruralnom razvoju)

Prema strukturi korištenja mjera, 814 zahtjeva (61,4 %) odnose se na mjere M10.1. Plaćanja za poljoprivredno-ekološke i klimatske obveze, što je manje od 70,5 % zahtjeva na razini Republike Hrvatske. Broj podnijetih zahtjeva za mjere M.11. Ekološki uzgoj iznosi 512 (38,6 %), što je više od 29,5 % zahtjeva na razini Republike Hrvatske. Iz analize je izostavljena mjera Očuvanje ugroženih izvornih i zaštićenih pasmina domaćih životinja, za koju podaci nisu bili dostupni.

Prema načinu korištenja, 80 % poljoprivrednog zemljišta u Bjelovarsko-bilogorskoj županiji čine oranice i postoje još neiskorištene mogućnosti za korištenje IAKS mjera za očuvanje bioraznolikosti. Tip operacije: Očuvanje živica koristi svega 6 poljoprivrednika, a one pridonose očuvanju bioraznolikosti u monotonim poljoprivrednim krajobrazima jer živice služe za gnježđenje ptica i kao zaklon manjim sisavcima i divljači (Znaor i Karoglan Todorović, 2016). Tip operacije: Uspostava poljskih traka koristi svega 17 poljoprivrednika, a doprinosi očuvanju bioraznolikosti na oranicama jer nije dopuštena primjena pesticida, služi za ispašu pčela, kao zaklon divljači i manjim sisavcima, te obogaćuje plodored jer je svaki treći hektar oranice zasijan kukuruzom. Ovdje postoji zajednički interes u poboljšanju staništa i unaprjeđenju lovstva jer poljske ili cvjetne trake odvajaju površine pod monokulturom. Tako divljač ne bi izlazila iz šuma ili vodotoka u potrazi za hranom i pravila štetu na poljoprivrednim kulturama.

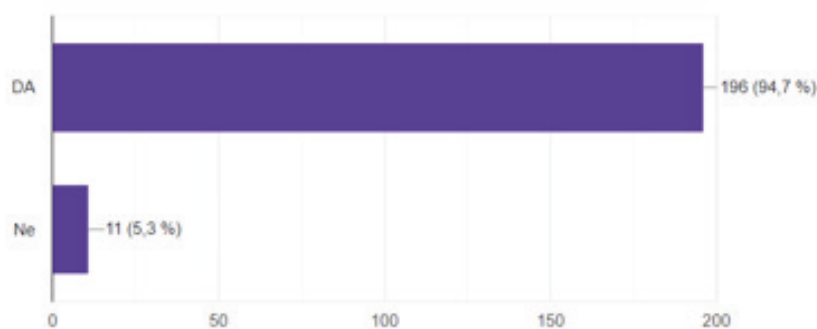
Na anketni upitnik za ciljanu skupinu poljoprivrednika u Bjelovarsko-bilogorskoj županiji, dostavljen na 400 e-mail adresa, pristigli su odgovori 209 ispitanika, što znači da je odaziv iznosio 52,3 %, dok 191 poljoprivrednik (47,8 %) nije dostavio odgovor.

Raspodjela odgovora ispitanika na pitanje broj 1: Jeste li upisani u Upisnik poljoprivrednika i podnosite li Jedinствeni zahtjev za potporu? (Grafikon 2.), pokazuje da ih je 204 (97,6 %) odgovorilo sa „Da“, a 7 (3,3 %) sa „Ne“.



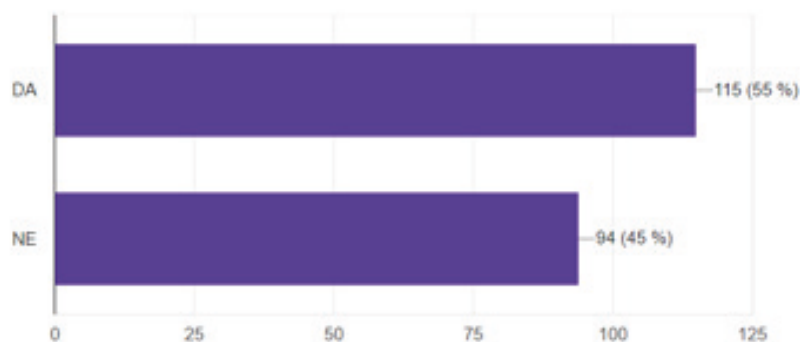
Grafikon 2. Raspodjela odgovora ispitanika na pitanje broj 1.

Raspodjela odgovora ispitanika na pitanje broj 2: Jeste li čuli za IAKS mjere ruralnog razvoja? (Grafikon 3.), pokazuje da ih je 196 (94,7 %) odgovorilo sa „Da“, a 11 (5,3 %) sa „Ne“.



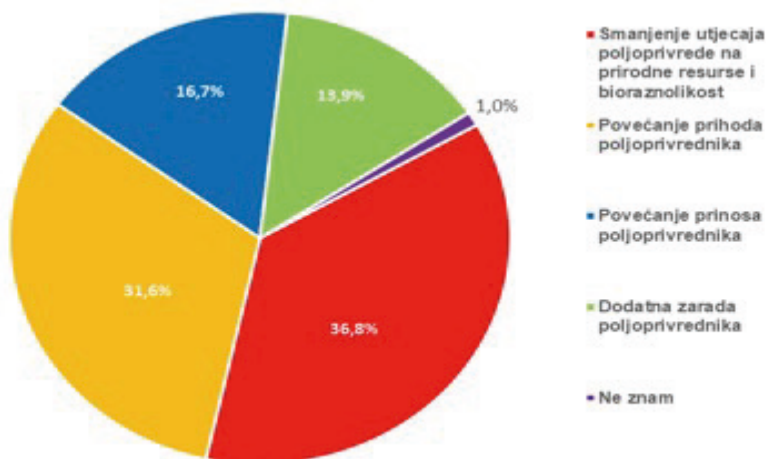
Grafikon 3. Raspodjela odgovora ispitanika na pitanje broj 2.

Raspodjela odgovora ispitanika na pitanje broj 3: Pri podnošenju Jedinstvenog zahtjeva za potporu prijavljujete li se za korištenje IAKS mjera ruralnog razvoja? (Grafikon 4.), pokazuje da ih je 115 (55 %) odgovorilo sa „Da“, a 94 (45 %) sa „Ne“.



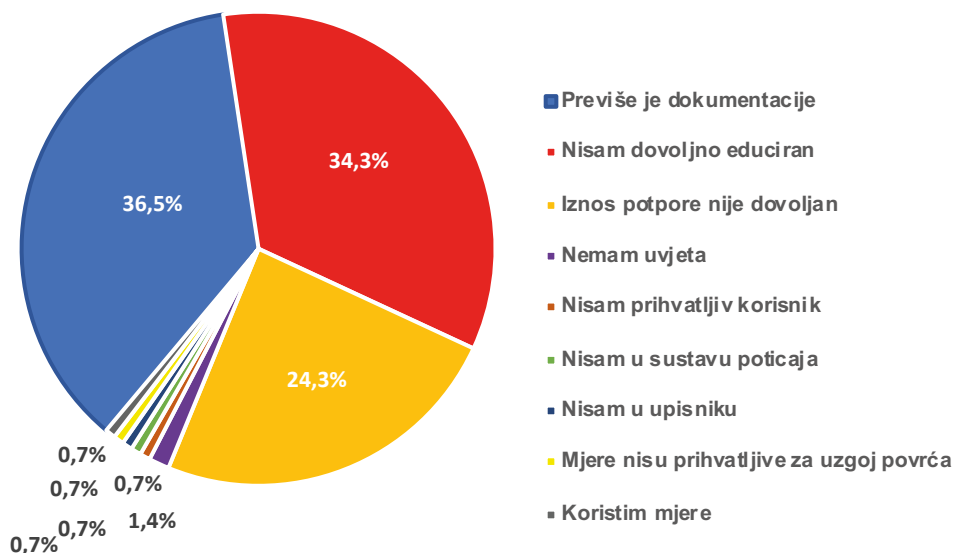
Grafikon 4. Raspodjela odgovora ispitanika na pitanje broj 3.

Raspodjela odgovora ispitanika na pitanje broj 4: Zna li koji je cilj IAKS mjera ruralnog razvoja? (Grafikon 5.) pokazuje da svega 77 ispitanika (36,8 %) zna točan odgovor: Smanjenje utjecaja poljoprivrede na prirodne resurse i bioraznolikost. Ostali odgovori ispitanika bili su: povećanje prihoda poljoprivrednika (65 odgovora, 31,6 %); povećanje prinosa poljoprivrednika (35 odgovora, 16,7 %); dodatna zarada poljoprivrednika (29 odgovora, 13,9 %) i ne znam (2 odgovora, 1,0 %).



Grafikon 5. Raspodjela odgovora ispitanika na pitanje broj 4.

Raspodjela odgovora ispitanika na pitanje broj 5: Zašto ne koristite IAKS mjere i navedite razloge nekorištenja? (Grafikon 6.) pokazuje da najviše ispitanika, njih 50 (36,5 %) smatra da je previše dokumentacije. Ostali odgovori ispitanika bili su: nisam dovoljno educiran (47 odgovora, 34,3 %); iznos potpore nije dovoljan (33 odgovora, 24,3 %); nemam uvjeta (2 odgovora, 1,4 %). Po jedan ispitanik (0,7 %) odgovorio je: nisam prihvatljiv korisnik; nisam u sustavu poticaja; nisam u upisniku; mjere nisu prihvatljive za uzgoj povrća; koristim mjere.



Grafikon 6. Raspodjela odgovora ispitanika na pitanje broj 5.

Rezultati anketnog istraživanja prema kojima je 55 % ispitanika podnijelo zahtjev za korištenje IAKS mjera ruralnog razvoja, a svega 36,8 % razumije cilj tih mjera, ukazuju na nedovoljnu educiranost i informiranost poljoprivrednika. Analizom dobne strukture poljoprivrednika utvrđeno je da ih je 47 % starije od 61 godine, a svega 14,6 % su

mlađi od 41 godine. Analiza strukture poljoprivrednika prema školskoj spremi pokazuje da ih je s nezavršenom osnovnom školom 5 %, s osnovnom školom 23 %, srednjom školom 33 % i visokoškolskim obrazovanjem 6 %, dok za preostalih 33 % poljoprivrednika nema podataka u Upisniku poljoprivrednika (<https://www.apprrr.hr/upisnik-poljoprivrednika/>).

Educiranost i informiranost poljoprivrednika problem je i na razini Europske unije, zbog slabog korištenja mjera za očuvanje bioraznolikosti (M.10), dok su poljoprivrednici više zainteresirani za olakšice koje su potpora dohotku i koje funkcioniraju po principu izravnih plaćanja (M.11). Na odluku poljoprivrednika za korištenjem pojedine mjere utječu iznos financijske potpore i očekivana korist za njegovo gospodarstvo. Rizici su povezani s dostupnošću informacija. Nedostatak ili ograničen pristup poljoprivrednika relevantnim i razumljivim informacijama stvara nesigurnost, što smanjuje motivaciju za prihvaćanjem korištenja mjera (Klebl i sur., 2023). Potrebno je pojačati aktivnosti osvješćivanja i edukacije poljoprivrednika radi porasta korištenja IAKS mjera za očuvanje bioraznolikosti, te unaprijediti i pojednostavniti postojeći složeni sustav prijave i nadzora korištenja mjera.

Zaključak

Korištenjem IAKS mjera poljoprivrednike se potiče na doprinos smanjenju negativnih utjecaja poljoprivrede na bioraznolikost i očuvanju prirode. Anketno istraživanje pokazalo je nisku razinu informiranosti poljoprivrednika jer svega 36,8 % ispitanika poznaje cilj IAKS mjera. Kao razlog nekorištenja mjera 36,5 % smatra kako postoji previše dokumentacije, a 34,2 % da nisu dovoljno educirani. Stoga je potrebno pojačati aktivnosti osvješćivanja i edukacije poljoprivrednika kako bi navedene mjere postigle željene ciljeve.

Literatura

- Europski revizorski sud (2020). Special Report 13/2020, Biodiversity on farmland: CAP contribution has not halted the decline. European Court of Auditors. Luxembourg.
- Geppert F., Bellingrath-Kimura S.D., Mouratiadou I. (2023). Fostering the implementation of nature conservation measures in agricultural landscapes: The NatApp. Sustainability. 15: 1-15.
- Hannus V., and Sauer J. (2021). Understanding farmers' intention to use a sustainability standard: The role of economic rewards, knowledge, and ease of use. Sustainability. 13: 10788.
- Klebl F., Feindt P.H., Pierr A. (2023). Farmers' behavioural determinants of on-farm biodiversity management in Europe: a systematic review. Agriculture and Human Values.
- Ozimec S., Florijančić T., Prlić D. (2022). Urban wildlife management and Natura 2000 areas in Osijek-Baranja County. Objavljeno u: *Congres Proceedings of 5th international Rural Tourism Congress - Rural Tourism: Quality, Sustainability, Inclusiveness*, Tubić D., Bakan R., Pleša Puljić N. (eds.), 656-665. Cavtat, Croatia: Veleučilište u Virovitici, Hrvatska udruga za turizam i ruralni razvoj "Klub članova selo".
- Pokos V. (2016). Poljoprivreda i očuvanje prirode. Glasnik zaštite bilja. 5: 4-13.
- Séchaud R., Van der Meer M., Fabian Y., Jeanneret P. (2022). What evidence exists on the effect of the main European lowland crop and grassland management practices on biodiversity indicator species groups? A systematic map protocol. Environmental Evidence. 11: 1-27.
- Uredba o ekološkoj mreži i nadležnostima javnih ustanova za upravljanje područjima ekološke mreže. „Narodne novine“, broj 80/2019. i 119/2023.
- Vakanjac D., Šimunija K., Bedeković M. (2022). The importance of agricultural sector for the economy of Croatia. Objavljeno u: *Congres Proceedings of 5th international Rural Tourism Congress - Rural Tourism: Quality, Sustainability, Inclusiveness*, Tubić D., Bakan R., Pleša Puljić N. (eds.), 347-358. Cavtat, Croatia: Veleučilište u Virovitici, Hrvatska udruga za turizam i ruralni razvoj "Klub članova selo".
- Zakon o zaštiti prirode. „Narodne novine“, broj 80/2013., 15/2018., 14/2019. i 127/2019.
- Znaor D., and Karoglan Todorović S. (2016). Poljoprivreda koja štiti prirodu - Zaštita prirode kroz mjere Programa ruralnog razvoja RH 2014.-2020. Ministarstvo zaštite okoliša i prirode, Avalon Foundation, Nizozemska ÖKL, Austrija i Ecologica, Hrvatska.

<https://www.aprrr.hr/baza-korisnika-potpura>

<https://www.aprrr.hr/upisnik-poljoprivrednika>

<https://www.zastita-prirode-bbz.hr/podrucja/ekoloska-mreza>

Usage of the IACS measures from the Rural Development Programme for biodiversity conservation in Bjelovar-Bilogora County

Abstract

The European Union's Common Agricultural Policy contains measures to conserve biodiversity and improve ecosystem services. The aim of the measures is to encourage farmers to accept and use certain practices and cultivation methods that will reduce the negative impact of agriculture on biodiversity. The effects of the measures depend on the education of farmers and the level of acceptance. The use of IACS measures in the Bjelovar-Bilogora County was investigated and a survey was conducted on the awareness of farmers about the meaning of IACS measures for the biodiversity conservation. Farmers submitted 1,326 applications for the payment of subsidies in 2022. Of the 209 respondents, 36.8% answered correctly what the goal of the measure was, while 34.2% responded that they were not sufficiently educated and that the procedure and requirements are rather complicated. In order to increase the use of IACS measures, it is recommended to increase the awareness and education activities of farmers.

Keywords: rural development, agricultura, biodiversity, informativeness of farmers



Session

4

Proceedings

Vegetable Growing, Ornamental, Medicinal and
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19
Međunarodni
Simpozij
Agronoma

Zbornik radova

Povrćarstvo, ukrasno, ljekovito i aromatično bilje

Utjecaj načina uzgoja na vegetativna i generativna svojstva graška (*Pisum sativum* L.)

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Sažetak

Grašak se uzgaja kao povrtna i ratarska kultura u cijelom svijetu. Osim okolišnih stresnih uvjeta na prinos graška utječe i konkurencija među biljkama, odnosno razmak u sjetvi. Za potrebe ovog istraživanja grašak je uzgajan iz sjemena na dva načina, sjetvom u redove i sjetvom u kućice. U svrhu izrade rada mjereni su sljedeći parametri: masa biljaka, visina biljaka, broj mahuna, broj zrna po mahuni i masa zrna. Biljke graška uzgojene sjetvom u kućice imale su 14,96 % više ukupne mase u odnosu na uzgoj u redovima. U pogledu visine nadzemnog dijela grašak uzgojen sjetvom u kućice bio je za 11,06 % viši te ujedno imao za 4,88 % veći broj mahuna u odnosu na sjetvu u redove. Kod uzgoja u kućicama broj zrna po mahuni bio je za 7,41 % veći nego kod graška uzgojenog u redovima, a postignuta je za 10,21 % veća masa zrna nego u redovima. Istovremeni uzgoj u jednakim uvjetima pokazao je da je uzgoj u kućicama dao biljke koje su ostvarile bolje rezultate prema svim mjerenim parametrima.

Ključne riječi: grašak, način sjetve, razmak biljaka, prinos

Uvod

Grašak (*Pisum sativum* L.) je jednogodišnja zeljasta biljka koja pripada porodici *Fabaceae* (Lešić i sur., 2004). Biljke iz porodice mahunarki imaju važnu ulogu u prehrani ljudi i životinja jer mogu zadovoljiti zahtjeve za visokim udjelom bjelančevina u hrani (Ayupov i sur., 2019). Grašak ima visoku prehrambenu vrijednost, uravnoteženi odnos bjelančevina i ugljikohidrata, a mlado zrno bogato je vitaminima, mineralima i sirovim vlaknima (Kanižai Šarić i sur., 2016). Redovita konzumacija zelenog graška može smanjiti rizik od raka, ponajviše zbog sadržaja antioksidanasa u grašku i njihove sposobnosti smanjenja upala u organizmu. Zeleni grašak također sadrži saponine, poznate po antikancerogenim učincima (Talaat i sur., 2022). Koristi se u svježem stanju ili se prerađuje zamrzavanjem, konzerviranjem i dehidracijom u nezreloj fazi kako bi se zadovoljili zahtjevi potrošača izvan sezone uzgoja (Dhal, 2017). Grašak se uzgaja kao povrtna i ratarska kultura u cijelom svijetu (Osmanović i sur., 2015). Zbog kratkoće vegetacije može rasti u širokom rasponu agroklimatskih zona što predstavlja potencijal za njegov uzgoj (Kanižai Šarić i sur., 2016), ali je osjetljiv na visoke temperature i stres od suše (Shaukat i sur., 2021). Najbolje uspijeva u uvjetima umjerene i vlažne klime jer je optimalna temperatura za njegov razvoj 18 °C (Osmanović i sur., 2015). Uspijeva na različitim tipovima tala i za rast mu najviše odgovaraju tla pH vrijednosti između 6,5 i 8. Grašak veći dio svojih potreba za dušikom može zadovoljiti putem simbiotskog odnosa s kvržičnim bakterijama i u plodoredu je dobar predusjev jer ostavlja poboljšani status hranjiva u tlu (Kanižai Šarić i sur., 2016). Najbolji predusjevi za grašak su rajčica, paprika, strne žitarice i krmne kulture (Matotan, 2004). Važna uloga u povećanju prinosa graška pripada oplemenjivanju i sjemenarstvu s ciljem stvaranja produktivnih sorti (Ayupov i sur., 2019). Skromni zahtjevi prema toplini omogućuju sjetvu rano u proljeće, a u područjima s blagom zimom kasno u jesen. Na području Slavonije sjetva najčešće započinje početkom ožujka i traje do kraja travnja (Lešić i sur., 2004). Osim stresnih uvjeta na prinos graška utječe i konkurencija među biljkama, odnosno razmak u sjetvi (Wilkins i sur., 1991). Kako bi se postigao maksimalan prinos biljke je neophodno uzgajati na optimalnoj udaljenosti jer će se na taj način između njih smanjiti konkurencija za svjetlošću, hranjivima i vodom (Devi i sur., 2022). Sjetva se najčešće obavlja pneumatskim ili mehaničkim sijačicama u redove razmaka od 15 cm do 20 cm te razmakom posijanog zrna u redu od 4 cm do 6 cm na dubinu od 3 cm do 5 cm (Matotan, 2004). Optimalan razmak za bilo koji usjev varira zbog uvjeta okoliša u kojem se uzgaja. Zbog toga nije moguće preporučiti optimalni razmak jer se grašak uzgaja u različitim godišnjim dobima

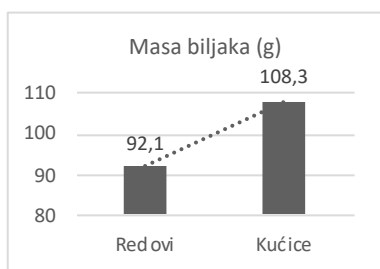
s različitim praksama na različitim tipovima tala (Devi i sur., 2022). Prema Biarness-Dumoulinet i sur. (1996) i Erac i sur. (1985) prinos graška se kreće od 1,5 t ha⁻¹ do 8,0 t ha⁻¹. Prosječna visina biljaka ovisno o genotipu poljskog graška se kreće od 50 cm do 200 cm (Tekeli, 1988). Cilj istraživanja bio je utvrditi utjecaj načina uzgoja na razvoj morfoloških i generativnih svojstava graška.

Materijali i metode

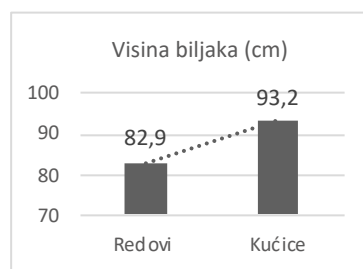
Istraživanje je provedeno u razdoblju od 24. ožujka do 28. lipnja 2023. na području Općine Klakar (Brodsko-posavska županija). Tlo prema teksturi pripada u klasu praškaste gline i sljedećeg je sastava: 0,99 % grubi pijesak, 4,90 % fini pijesak, 18,01 % grubi prah, 26,28 % fini prah i 49,82 % glina (ISO, 2009). Prema kemijskoj analizi utvrđeno je: humusa 2,41 % (ISO, 1998), CaCO₃ 2,09 (ISO, 1995), AL-P₂O₅ 8,36 mg 100g⁻¹, Al-K₂O 20,11 mg 100g⁻¹ (Egner, 1960), pH u KCl 6,41, pH u H₂O 7,36. (ISO, 2005). Za potrebe pokusa odabrana je srednje rana sorta graška Rondo. Pripada u visoke sorte sa stabljikom do 1 metra visine i mahunama koje mogu sadržavati do 15 zrna. Pokus je postavljen u vanjskim uzgojnim uvjetima. Grašak je uzgajan iz sjemena na dva načina, sjetvom u redove i sjetvom u kućice. Sjetva je obavljena u dvorede na međuredni razmak 20 cm. Posijano je po 10 sjemenki na razmak 8 cm u redu. Ukupno je posijano šezdeset biljaka. Prilikom uzgoja, postavljena je mreža koja je služila kao potporanj. Osim toga obavljeno je nagrtanje tla oko biljaka kako bi se spriječilo polijeganje. U drugom načinu sjetve formirane su kućice, unutar kojih su posijane po 4 sjemenke graška na razmak 8 cm i dubinu 5 cm. Formirano je ukupno petnaest kućica. Stabljike graška povezane su uz potporanj u obliku stošca kako bi ih se zaštitile od lomljenja i olakšao njihov rast u visinu. Sjetva na oba načina obavljena je 24. ožujka. Tlo je pripremljeno jesenskom obradom, a u proljeće je usitnjeno kopačicom. Temperatura zraka tijekom sjetve graška iznosila je 22 °C. Prosječna temperatura u mjesecu travnju iznosila je 21 °C, u svibnju 25 °C i u lipnju 28 °C. Navodnjavanje se provodilo prema potrebi. Nasumičnim odabirom izdvojeno je po deset biljaka iz oba načina uzgoja nakon čega je obavljeno mjerenje i vaganje prema mjerenim parametrima. Za potrebe istraživanja analizirali su se sljedeći parametri; masa biljaka, visina nadzemnog dijela, broj mahuna po biljci, broj zrna u mahuni i masa zrna po biljci. Masa se određivala na analitičkoj vagi Adventurer Pro proizvođača Ohaus. Duljina se mjerila pomoću pomične mjerke 1108 proizvođača Insize. Razlike u istraživanim svojstvima između dva načina sjetve obrađene su u programu Exel te izražene kao postotak.

Rezultati i rasprava

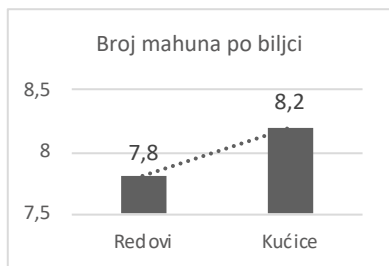
Oba graška niknula su 15. travnja, a formiranje vitica zabilježeno je 30. travnja. Razvoj mahuna je također započeo istovremeno 6. lipnja. Vrijeme berbe određeno je prema stupnju zriobe kada je zrno ispunilo mahunu, a prije nastupanja fiziološke zriobe. Prva berba mahuna kod oba načina sjetve je bila 19. lipnja, a druga berba je uslijedila tjedan dana kasnije, 28. lipnja. Rezultati dobiveni mjerenjima biljaka graška iz oba načina uzgoja prikazani su u grafikonima 1-5.



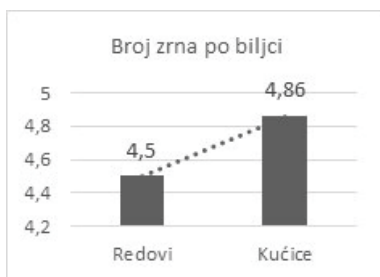
Grafikon 1. Prosječna masa biljaka (g)



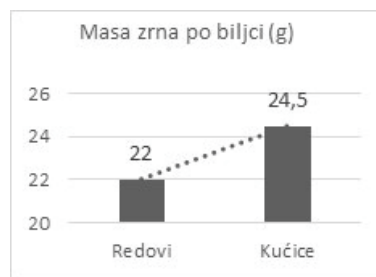
Grafikon 2. Prosječna visina biljaka (cm)



Grafikon 3. Prosječan broj mahuna po biljci



Grafikon 4. Prosječan broj zrna po biljci



Grafikon 5. Prosječna masa zrna (g) po biljci

Prema dobivenim rezultatima biljke graška uzgojene sjetvom u kućice i uzgajanih uz pomoć stošca imale su srednju ukupnu masu 108,3 g za razliku od biljaka uzgojenih sjetvom u redove i povezanih uz mrežu sa srednjom ukupnom masom 92,1 g (Grafikon 1), odnosno sjetvom u kućice dobiveno je 14,96 % viša ukupna masa biljaka. Masa pojedinačnih biljaka posijanih u redove kretala se u rasponu od 34 g do 137 g, a kod biljaka zasijanih u kućice od 65 g do 229 g. Uzgoj u redovima je zauzeo površinu od 8000 cm², a uzgoj u kućicama je zauzeo 7680 cm². Iz toga se vidi da je uzgoj u kućicama zauzeo manju površinu, a dao veću ukupnu masu. U pogledu visine nadzemnog dijela vidljivo je da je grašak uzgojen sjetvom u kućice imao za 11,06 % veću srednju visinu nadzemnog dijela biljke u odnosu na grašak iz redova (Grafikon 2). Visina nadzemnog dijela biljaka uzgojenih sjetvom u redove kretala se u rasponu od 63 cm do 93 cm, dok je kod biljaka uzgojenih sjetvom u kućice visina bila u rasponu od 60 cm do 118 cm. Ranija istraživanja pokazala su da biljke uzgojene na manjem međusobnom razmaku imaju veću visinu nadzemnog dijela jer zbog ograničenog prostora više rastu u visinu (Shaukat i sur., 2021). Uzgojem graška sjetvom u kućice dobiven je za 4,88 % veći broj mahuna u odnosu na sjetvu u redove (Grafikon 3). Najveći broj mahuna po biljci kod graška uzgajanog uz stožac bio je 16, a kod graška uzgajanog uz mrežu 11. Istraživanja koja su proveli Sajid i sur. (2012) pokazala su da se povećavanjem razmaka između redova značajno smanjio prinos mahuna graška. Rops (1997) je ustanovio da se prinos povećavao na manjem međurednom razmaku, a njegovim povećavanjem smanjivao se za 6 % do 17 % (Sajid i sur., 2012). Broj zrna po mahuni kod biljaka uzgojenih sjetvom u kućice i povezanih uz stožac bio je za 7,41 % veći nego kod graška uzgojenog sjetvom u redove (Grafikon 4). Posljednji mjereni parametar bila je masa zrna gdje je zabilježeno da je sjetvom u kućice postignuta za 10,21 % veća masa zrna nego sjetvom graška u redove (Grafikon 5). Najmanja masa zrna po biljci kod graška uzgajanog uz stožac bila je 15 g a najveća 56 g, dok je kod graška uzgajanog uz mrežu najmanja masa zrna po biljci bila 7 g, a najveća 39 g. Ustanovljeno je da su mahune biljaka uzgajanih u stošcu dale veću ukupnu masu, veći broj zrna, veću masu zrna, veću visinu i više mahuna po biljci.

Zaključak

Rezultati provedenog istraživanja pokazuju da je uzgoj u kućicama ostvario bolji rezultat u pogledu svih mjerenih parametara. Kako je obrazloženo, uzgoj u kućicama je postigao veći broj biljaka po površini i sukladno tome postignut je i veći prinos. Osim toga, takav uzgoj je omogućio veću uzgojnu površinu i osvjetljenost biljaka što je rezultiralo boljom fotosintezom i proizvodnjom asimilata i u konačnici boljim porastom biljaka i razvojem mahuna.

Literatura

- Ayupov D.S., Davletov F.A., Asylbaev I.G., Kuznetsov I. Yu., Akhmadullina I.I., Dmitriev A.M., Vakhitova R.K., Satarov M. Yu., Avsakhov F.F., Irgalina R. Sh. (2019). Selection of high-yielding, high-tech varieties of field pea (*Pisum sativum* L.). Legume Research. 42: 615-619.
- Biarness-Dumolin V., Denis J.B., Lejeune-Henaut I., Eteve G. (1996). Interpreting yield instability in pea using genotypic and environment covariant. Crop Science. 36: 115-120.
- Devi K.M., Chanu M.M., Abonmai T., Singh M.S. (2022). Effect of spacing on growth and green pod yield of pea (*Pisum sativum* L. subsp. Hortense) local cultivar Makhyatmubi. The Pharma Innovation Journal. 11: 3183-3186.
- Dhal R.K. (2017). Pea cultivation. Ludhiana, Punjab, Agricultural University Ludhiana.
- Egner H., Riehm H., Domingo W. R., (1960). Untersuchungen über die chemische Bodenanalyse als Grundlage für die Beurteilung des Nährstoffzustandes der Boden II. Chemische Extraktionsmethoden zu Phosphor- und Kaliumbestimmung, Landbr. Hogsk. Annlr. W.R. 26: 199-215.
- Erac A., and Ekiz H. (1985). Forage Crop Production. Ankar University Press, Ankara, Turkey.
- ISO. (2009). Soil quality - Determination of particle size distribution in mineral soil material Method by sieving and sedimentation. ISO 11277.
- ISO. (1998). Soil quality - Determination of organic carbon by sulfochromic oxidation. HRN ISO14235.
- ISO. (1995). Soil quality - Determination of carbonate content. Volumetric method. HRN ISO 10693.
- ISO. (2005). Soil quality - Determination of pH. International standard. ISO 10390.
- Kanižai Šarić G., Milaković Z., Rapčan I., Majić I., Šepuť J., Kojić D. (2016). Rast i prinosi graška (*Pisum sativum* L.) pod utjecajem bakterizacije i gnojidbe dušikom. Agronomski glasnik. 5-6: 205-214.
- Lešić R., Boroši J., Butorac I., Herak-Čustić M., Poljak M., Romić D. (2004.). Povrćarstvo. Čakovec, Zrinski d.d.
- Matotan Z. (2004.). Suvremena proizvodnja povrća. Zagreb, Nakladni zavod Globus.
- Osmanović S., Huseinović S., Šljivić E. (2015). Uticaj pH vrijednosti vodene otopine na klijavost graška. XX savetovanje o biotehnologiji. Zbornik radova. 20: 33-37.
- Rops A.H.J. (1997). Effect of wider row spacing and date of sowing on the yield of anning peas. Proefstation voor de Akkerbouw en de Groenteteelt in de Vollegrond, Lelystad. 81A: 215-216.
- Sajid M., Rab A., Noor-ul-Amin Fazali W., Jan I., Ahmad I., Khan I.A., Khan M.A. (2012). Effect of herbicides and row spacing on the growth and yield of pea. Pakistan Journal Weed Science Research. 18: 1-13.
- Shaukat S.A., Ahmad Z., Choudry Y.A., Shaukat S.K. (2021). Effect of different sowing dates and row spacing on the growth, seed yield and quality of off-season pea (*Pisum sativum* L. cv. Climax) under temperate conditions of Rawalakot Azad Jammu and Kashmir. Scientific Journal of Agricultural. 1: 117-125.
- Talaat I.M., El-Metwaly I.M., Dawood M.G., El-Awadi M.M., El-Rokiek K.G. (2022). Response of Pea Plants (*Pisum sativum*) to Nano-Salicylic Acid and Herbicides. Journal of Materials and Environmental Science. 13: 1218-1226.
- Tekeli A.S. (1988). Forage Legumes, Trakya University, Agricultural Faculty Press, Tekirdag, Turkey. 65: 23-24.
- Wilkins D.E., Kraft J.M., Klepper B.L. (1991). Influence of plant spacing on pea yield. Transaction of the ASAE. 34: 1957-1961.

The influence of cultivation methods on the vegetative and generative properties of peas (*Pisum sativum* L.)

Abstract

Peas are grown all over the world as a vegetable and field crop. In addition to external stress conditions, pea yield is also influenced by competition between plants, i.e. the sowing distance. For the experiment, peas were grown from seed in two ways: by sowing in rows and by sowing in houses. The following parameters were measured: plant mass, plant height, number of pods, number of grains per pod and grain weight. Pea plants grown by sowing in houses had a 14.96% higher total mass compared to those grown in rows. Regarding the height of the above-ground part of the plant, peas grown indoors had an 11.06% higher height of the above-ground part of the plant and a 4.88% higher number of pods than when sown in rows. When grown in houses, the number of grains per pod was 7.41% higher than in peas grown in rows, and a 10.21% higher grain mass was achieved than in rows. Simultaneous cultivation under the same conditions showed that cultivation in houses produced plants that achieved better results for all measured parameters.

Keywords: peas, sowing method, plant spacing, yield

The effect of nutrient solution concentration on the yield and bioactive compounds content of hydroponically grown wild rocket

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Abstract

The aim of this research was to determine the effect of nutrient solution concentration (25, 50 and 100%) on the yield and content of vitamin C, flavonoids, non-flavonoids, total phenols and total chlorophyll of wild rocket grown in floating hydroponics. The highest yield of leaves (3.2 kg m⁻²) was obtained when wild rocket was grown in a solution with a nutrient concentration of 50%. The highest amount of vitamin C (113.86 mg 100 g⁻¹ FW), total phenols (158.87 mg GAE 100 g⁻¹ FW), flavonoids (61.41 mg GAE 100 g⁻¹ FW), non-flavonoids (97.46 mg GAE 100 g⁻¹ FW) and total chlorophyll (0.51 mg g⁻¹ FW) was determined in the leaves of wild rocket grown in a 100% concentrated nutrient solution.

Keywords: *Diplotaxis tenuifolia* L., floating system, vitamin C, total phenols, total chlorophyll

Introduction

Wild rocket (*Diplotaxis tenuifolia* (L.) DC.) belongs to the group of leafy vegetables and is becoming increasingly popular due to its nutritional value, especially due to the high content of specialized metabolites, vitamin C and minerals (Nicoletti et al., 2007). Wild rocket leaves are characterized by a bitter or pungent taste due to their glucosinolate content and a strong, pungent aroma due to the release of volatile isothiocyanates (Caruso et al., 2018). It is a cool season crop showing a faster growth rate and development under increasing day length and temperatures in the range of 2 to 25 °C (Caruso et al., 2020). In order to avoid unfavorable outdoor production conditions, the production of wild rocket is increasingly practiced in a greenhouses where continuous cultivation takes place throughout the year under controlled conditions, which can ensure economically justified yield and high quality production (Pimpini et al., 2005). Considering the increasing demand for this species, its hydroponic cultivation is becoming more popular to eliminate the problems of conventional soil cultivation and achieve higher nutritional value and yield while reducing production costs (Nicola et al., 2005). The purchase of water-soluble salts for the preparation of the nutrient solution accounts for a large part of the cost of hydroponic cultivation. It is therefore necessary to research the possibility of reducing the concentration of nutrients in the solution without negatively affecting the quality and nutritional value of wild rocket.

Material and methods

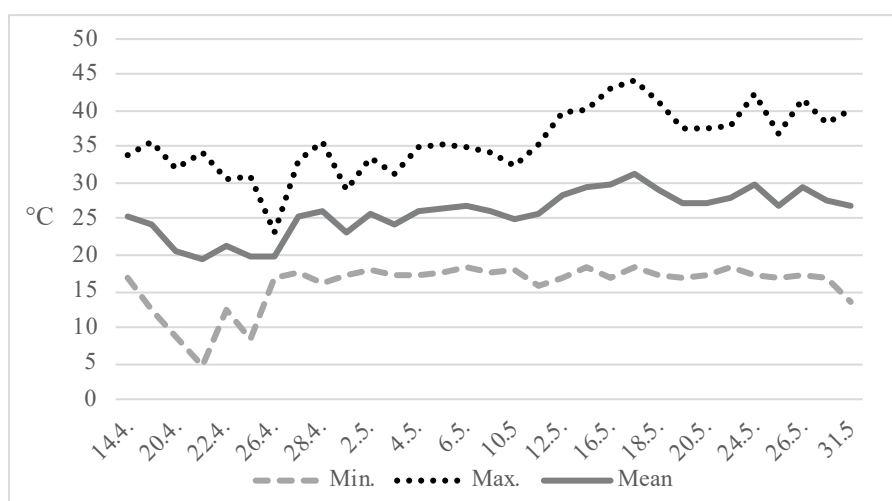
The trial was carried out in the greenhouse of Department of Vegetable Crops, University of Zagreb Faculty of Agriculture during the spring growing season of 2022. The experiment was set up in a floating system as a single-factor trial according to a randomized block design with three replications. It included wild rocket cultivar 'Themisto' (Rijk Zwaan) and 3 concentrations (25, 50 and 100%) of nutrient solution for leafy vegetables. Seeds of wild rocket were sown on April 8 in polystyrene boards filled with perlite of granulation 0 - 3 mm (Agroperl-G, Europerl, Croatia). One polystyrene board (0.96 m x 0.6 m) with 102 splits (17 cm long and 0.5 cm wide) represented scoring lot (0.57 m²). After sowing, the boards were placed in a dark place with a temperature and relative humidity suitable for germination (20°C and 65%) of wild rocket seeds. Germination was observed five days after sowing, i.e. on

April 13. The nutrient solution for the cultivation of wild rocket was prepared according to Tessi (2002.) and the polystyrene boards with the germinated plants were placed in the three basins with different concentrations of the nutrient solution. During vegetation, pH and electrical conductivity (EC) value of the nutrient solution in each basin as well as the temperature and relative humidity in the greenhouse were measured daily. A single harvest of wild rocket was carried out on May 8 (31 days after sowing) in basins with 100 and 50% concentrated standard nutrient solution, while the harvest in a basin with a 25% solution concentration was done 10 days later.

Chemical analyses of bioactive compounds were performed in laboratory of Department of Sustainable Technologies and Renewable Energy Sources, Faculty of Agriculture. Dry matter content (%) was determined according to the standard method (AOAC, 1995) and vitamin C content (mg 100 g⁻¹) according to the standard method (AOAC, 2002). The spectrophotometric method with the Folin–Ciocalteu reagent was used for the determination of phenolic compounds (mg GAE 100 g⁻¹), as described by Ough and Amerine (1988). Total chlorophylls (TCh) were determined by following the Holm (1954) and Wettstein (1957) as shown in Dujmović et al. (2023) and all measurements were performed on a Shimadzu 1900i spectrophotometer (Kyoto, Japan). The final content values were expressed in mg g⁻¹. The statistical program Windows SAS® Software v.14.3 (2017) was used for statistical procedures of the obtained results. The differences between the tested nutrient solution concentrations for all the observed traits were analyzed by analysis of variance, and the significant differences between the average values were tested by LSD test and were considered significantly different at $p \leq 0.05$.

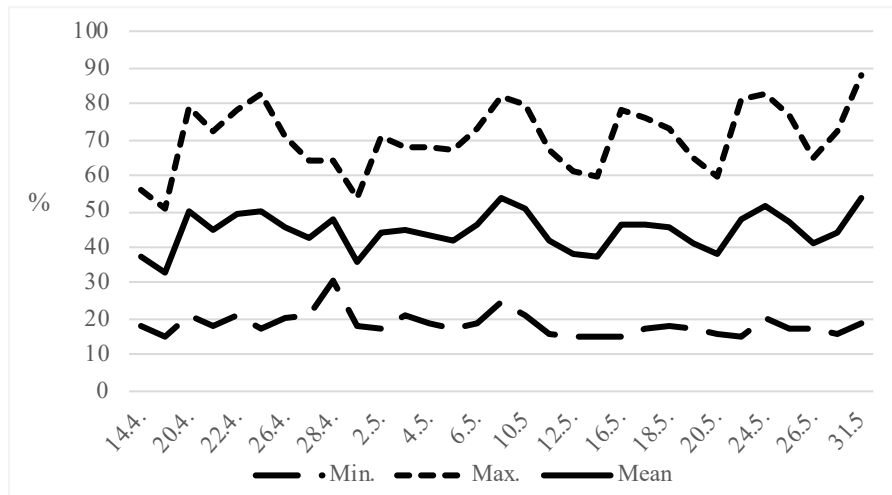
Results and discussion

During the research, minimum air temperature varied between 4.6 (April 21) and 18.5 °C (May 13), while the maximum daily air temperature ranged between 23.1 (April 26) and 44.1 °C (May 17). The mean daily air temperature in the greenhouse during the cultivation of wild rocket ranged from 19.5 to 31.3 °C, which is above the optimum values for the cool-season leafy vegetables (Graph 1). According to Toth et al. (2012.), the optimum range of day and night temperatures for rocket growth is 22 to 24 °C and 16 to 18 °C, respectively.



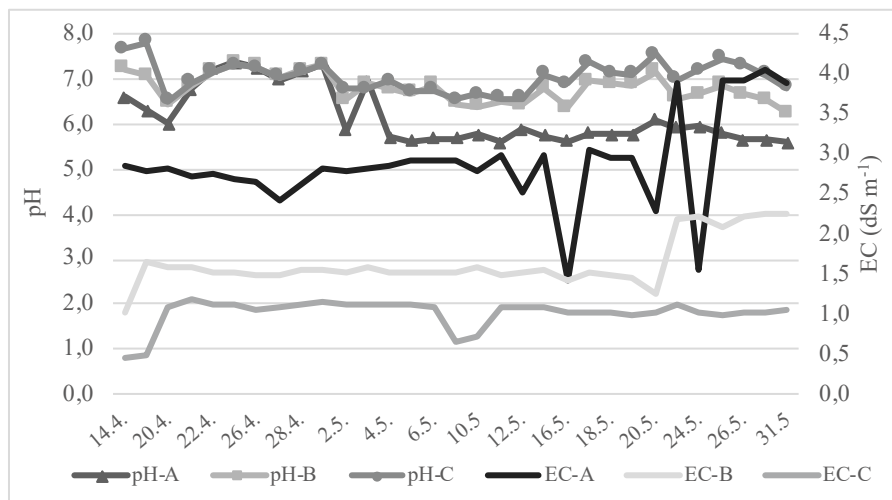
Graph 1. Minimum, mean and maximum air temperature (°C) in greenhouse during wild rocket cultivation, Zagreb, 2022.

Graph 2 shows the relative humidity during the cultivation of wild rocket in this research. It is an important abiotic indicator, as it has a significant effect on the intensity of transpiration and thus on the uptake of the nutrient solution. The minimum relative humidity varied from 15 and 31%, while the maximum relative humidity ranged between 51 and 88%. During the research, the mean relative humidity was between 33 and 54%, giving an average value of 45%, which is lower than the mean relative humidity (53.6%) in the spring growing season of rocket (*Eruca sativa*) in a study by Toth et al. (2012).



Graph 2. Minimum, mean and maximum relative humidity (%) in greenhouse during wild rocket cultivation, Zagreb, 2022.

During the cultivation of the wild rocket, the pH in basin A (100%) varied from 5.6 to 7.4, in basin B (50%) from 6.3 to 7.3 and in basin C (25%) from 6.5 to 7.8 (Graph 3). The highest average pH of the solution was in basin C (7.04) and the lowest in basin A (6.16); data not shown. The measured values were above the optimum for cultivated rocket (5.8 to 6.2) given by Toth et al. (2012).



A-100%, B-50%, C-25% nutrient solution concentration

Graph 3. pH and EC values of the nutrient solution in basins during the wild rocket cultivation

In the basin with a 100% nutrient solution concentration (A), the EC value varied from 1.44 to 4.06 dS m⁻¹, in basin B from 1.02 to 2.27 dS m⁻¹ and in the basin with the lowest nutrient solution concentration (25%) from 0.46 to 1.01 dS m⁻¹. According to Caruso et al. (2018) nutrient solution for wild rocket cultivation should have an EC ranging from 1.5 to 2.5 dS m⁻¹ and pH from 6.0 to 6.5. Toth et al. (2012) indicated 3.2 dS m⁻¹ as the optimum value for the cultivation of cultivated rocket using an adapted nutrient solution for leafy vegetables according to Pimpini et al. (2005). In this research, a nutrient solution with a lower EC value was used and prepared according to Tessi (2002). In the research by Bonasia et al. (2017), wild rocket was grown hydroponically, whereby a solution with a higher EC value (3.5 dS m⁻¹) resulted in a higher antioxidant capacity and a higher dry matter content as well as a lower nitrate content in the leaves.

Table 1 shows that in this research, the leaves of wild rocket grown in basin A with a 100% concentration solution

had the highest dry matter content (12.7%). Application of reduced solution concentration resulted with the lower dry matter content (7.7% (B) and 7.3% (C)) and was in accordance with the dry matter content (7.2%) in the research by Villani et al. (2023). Dry matter content indicates the nutritional quality of the plant material, suggesting that raw material with higher dry matter values contains a high amount of nutrients, mainly minerals, vitamins and other bioactive compounds (Dujmović et al., 2023). In a study by Toth et al. (2018), different EC values (3, 5, 7 and 9 dS m⁻¹) of the nutrient solution for rocket hydroponic cultivation resulted in dry matter content in range from 8.48 and 12.66%. The increase in dry matter due to the increase of EC value is consistent with the results of this research.

Caruso et al. (2018) reported that the harvest of wild rocket leaves occurs in the pre-flowering stage, 20 to 100 days after planting, depending on the growing season, environment conditions and market destination. In this study, the harvest of wild rocket grown in the spring period was carried out 31 days after sowing in basins with 100 and 50% concentrated nutrient solution, while the harvest in a basin with a 25% solution concentration was carried out 41 days after sowing. Wild rocket grown in a basin with a 25% concentration of the standard solution produced the lowest yield of 1.1 kg m⁻², while the highest yield (3.2 kg m⁻²) was obtained with a 50% concentration of the solution (Table 1). Toth et al. (2012) reported almost equal yield of cultivated rocket from hydroponic cultivation, where the highest yield (3.31 kg m⁻²) was achieved in the spring growing season and being 22 and 35 % higher than in the summer and autumn-winter period (2.60 and 2.14 kg m⁻²). Caruso et al. (2020) reported the lowest yield of wild rocket (0.9 kg m⁻²) in the autumn-winter growing season and 50 and 100 % higher yield in the spring and winter seasons, respectively.

Table 1. Yield and total dry matter content of wild rocket cultivated in different nutrient solution concentrations (NSC)

NSC	Yield (kg m ⁻²)	Dry matter (%)
A (100%)	1.8 ^b	12.7 ^a
B (50%)	3.2 ^a	7.7 ^b
C (25%)	1.1 ^c	7.3 ^b
Average	2.0	9.2

Different letters indicate significant differences between mean values according to LSD test, p ≤ 0.05

As shown in Table 2, the reduction in the concentration of the nutrient solution led to a decrease in the vitamin C content. Therefore, the highest vitamin C content in the leaves of wild rocket was determined at a concentration of 100 % and amounted to 113.86 mg 100 g⁻¹ FW (fresh weight). In the research by Villani et al. (2023), a positive effect of increasing the nutrient concentration on the vitamin C content of wild rocket was found, with the highest content being 69.4 mg 100 g⁻¹ FW, which is lower than the values obtained in this research. In the study by Hamilton and Fonseca (2010), garden cress, rocket and wild rocket were grown hydroponically at five EC values between 1.5 and 9.5 dS m⁻¹ and wild rocket was characterised by the highest vitamin C content in the range of 79.81 to 105.94 mg 100 g⁻¹ FW.

A statistically significant difference was found in the content of total phenols, flavonoids and non-flavonoids in the wild rocket leaves depending on the different concentrations of the nutrient solution. The content of total phenols ranged from 82.95 to 158.87 mg GAE 100 g⁻¹ FW, with the highest content being determined in the leaves of wild rocket grown in a basin filled with a 100% concentration solution. Villani et al. (2023) reported a lower content of total phenols in wild rocket leaves, in the range of 56 to 58 mg GAE 100 g⁻¹ FW. Bonasia et al. (2017) found differences in the content of total phenols in relation to the cultivation period, so wild rocket grown in the winter-spring period had a higher content of total phenols (101 mg GAE 100 g⁻¹ FW) than plants grown in the autumn-winter period (46.8 mg GAE 100 g⁻¹ FW). Dujmović et al. (2023) emphasizes that the phenolic content can depend on the plant species and organs, but also on the environmental conditions during production. In a study by Toth et al. (2018), a higher content of total phenols was found in the leaves of cultivated rocket, which ranged between 150.33 and 173.44 mg GAE 100 g⁻¹ FW depending on the EC value of the nutrient solution.

In this research, the highest content of flavonoids (61.41 mg GAE 100 g⁻¹ FW) and non-flavonoids (97.46 mg GAE 100 g⁻¹ FW) was found at the highest concentration of the nutrient solution. Nicoletti et al. (2007) point out that there is a seasonal effect on the flavonoid content of rocket, with higher values in spring period. According to Bennett et

al. (2006.) concentrations and structural classes of flavonoids are affected by various stresses such as light (especially UV light), nutrient supply and other growing conditions. The content of total non-flavonoids ranged from 51.38 to 97.46 mg GAE 100 g⁻¹ FW (Table 2). Toth et al. (2018) report the similar values of non-flavonoids content in the leaves of cultivated rocket ranging from 53.51 to 82.75 mg GAE 100 g⁻¹ FW.

Table 2. Ascorbic acid, total chlorophylls and phenols compound content and of fresh wild rocket cultivated in different nutrient solution concentrations (NSC)

NSC	AsA	TPC	TNFC	TFC	TCh
	mg 100 g ⁻¹ FW		mg GAE 100 g ⁻¹ FW		mg g ⁻¹ FW
A (100%)	113.86 ^a	158.87 ^a	97.46 ^a	61.41 ^a	0.51 ^a
B (50%)	82.97 ^b	103.23 ^b	55.75 ^b	47.49 ^b	0.40 ^b
C (25%)	67.23 ^c	82.95 ^c	51.38 ^c	31.58 ^c	0.44 ^b
Average	88.02	115.02	68.20	46.82	0.45

AsA - ascorbic acid; TPC - total phenols; TNFC - total non-flavonoid; TFC - total flavonoid; TCh - total chlorophylls content. Different letters indicate significant differences between mean values according to LSD test, $p \leq 0.05$

Photosynthetic pigments are molecules that play a crucial role in photosynthesis and are essential for the proper development and growth of plants. Customers associate vegetables with their specific colors, which provide information about the condition of the plant material. The color of the leaves can also be specific to different plant species and is related to the pigment composition, mainly the chlorophylls (Dujmović et al., 2023). Table 2 shows that in this research the wild rocket grown at 100 % nutrient solution concentration had the highest total chlorophyll content (0.51 mg g⁻¹ FW), while the rocket grown at a concentration of 50 and 25% had a statistically equal total chlorophyll content (0.40 mg g⁻¹ fw and 0.44 mg g⁻¹ FW, respectively). In a study by Toth et al. (2018), content of total chlorophylls of cultivated rocket varied from 0.72 to 0.90 mg g⁻¹ FW and the highest value was determined in control treatment (EC 3) indicating that an increase of EC value of nutrient solution led to a decrease in the content of total chlorophylls. Bonasia et al. (2017) point out that the chlorophyll content of rocket depends on the cultivation period and the hydroponic technique. Thus, a higher chlorophyll content was found in the leaves of rocket grown in the floating system than in the ebb and flow system.

Conclusions

In spring cultivation of wild rocket in a floating system, statistically justified differences were found between the tested nutrient solution concentrations for all traits. For the cultivation of wild rocket intended for sale in chain stores, the use of a 50% concentration of the nutrient solution can therefore be recommended to ensure rapid plant growth, an economically satisfactory yield and the quality of this leafy vegetable. A higher consumption of water-soluble salts when cultivating wild rocket using a 100% concentration of the nutrient solution can be justified by the higher price of nutritionally rich food. Therefore, in practice, both 50% and 100% nutrient solution concentration can be used to produce wild rocket of satisfactory quality.

Acknowledgement

The article is an excerpt from the diploma thesis of the student Gabrijel Antonina, mag. ing. agr. entitled „The effect of nutrient solution concentration on the chemical composition and yield of wild rocket in a floating hydropon“.

References

- AOAC (1995). Official Methods of Analysis, 16th ed.; Association of Official Analytical Chemists: Washington, DC, USA.
- AOAC (2002). Official Methods of Analysis, 17th ed.; Association of Official Analytical Chemists: Washington, DC, USA.
- Bennett R.N., Rosa E.A., Mellon F.A., Kroon P.A. (2006). Ontogenic profiling of glucosinolates,

- flavonoids, and other secondary metabolites in *Eruca sativa* (salad rocket), *Diplotaxis eruroides* (wall rocket), *Diplotaxis tenuifolia* (wild rocket), and *Bunias orientalis* (Turkish rocket). *Journal of agricultural and food chemistry*. 54: 4005-4015.
- Bonasia A., Lazzizzera C., Elia A., Conversa G. (2017). Nutritional, biophysical and physiological characteristics of wild rocket genotypes as affected by soilless cultivation system, salinity level of nutrient solution and growing period. *Frontiers in Plant Science*. 8: 300.
- Caruso G., Parrella G., Giorgini M., Nicoletti R. (2018). Crop systems, quality and protection of *Diplotaxis tenuifolia*. *Agriculture*. 8: 55.
- Caruso G., El-Nakhel C., Roupheal Y., Comite E., Lombardi N., Cuciniello A., Woo S.L. (2020). *Diplotaxis tenuifolia* (L.) DC. yield and quality as influenced by cropping season, protein hydrolysates, and Trichoderma applications. *Plants*. 9: 697.
- Dujmović M., Opačić N., Radman S., Fabek Uher S., Voća S., Šic Žlabur J. (2023). Accumulation of stinging nettle bioactive compounds as a response to controlled drought stress. *Agriculture*. 13: 1358.
- Hamilton J.M., and Fonseca J.M. (2010). Effect of saline irrigation water on antioxidants in three hydroponically grown leafy vegetables: *Diplotaxis tenuifolia*, *Eruca sativa*, and *Lepidium sativum*. *HortScience*. 45: 546-552.
- Holm G. (1954). Chlorophyll mutations in barley. *Acta Agriculturae Scandinavica*. 4: 457– 471.
- Nicola S., Hoeberechts J., Fontana E. (2005). Comparison between traditional and soilless culture systems to produce rocket (*Eruca sativa*) with low nitrate content. *Acta Horticulturae*. 697: 549-553.
- Nicoletti R., Raimo F., Miccio G. (2007). *Diplotaxis tenuifolia*: biology, production and properties. *European Journal Plant Science and Biotechnology*. 1: 36-43.
- Ough C.S., and Amerine M.A. (1988). *Methods for analysis of musts and wines*. 2nd ed.; John Wiley & Sons: New York, NY, USA.
- Pimpini F., Giannini M., Lazzarin R. (2005). *Ortaggi da foglia da taglio*. Veneto Agricoltura, Padova.
- SAS®/STAT 14.3 (2017). SAS Institute Inc.: Cary, NC, USA.
- Tessi R. (2002). *Colture fuori suolo in orticoltura e floricoltura*. Bologna, Edagricole.
- Toth N., Fabek S., Benko B., Žutić I., Stubljar S., Zeher S. (2012). Učinak abiotskih čimbenika, gustoće sjetve i višekratne berbe na prinos rige u plutajućem hidroponu. *Glasnik zaštite bilja*. 35: 24-34.
- Toth N., Jerončić L., Čoga L., Benko B., Fabek Uher S., Šic Žlabur J. (2018). Macroelements and specialized metabolites content under salt stress in hydroponically grown rocket. *Proceedings of 9th International Congress of Food Technologists, Biotechnologists and Nutritionists*. Croatian Society of Food Technologists, Biotechnologists and Nutritionists, Zagreb: 108-112.
- Villani A., Loi M., Serio F., Montesano F.F., D'Imperio M., De Leonardis S., Mulè G., Paciolla C. (2023). Changes in antioxidant metabolism and plant growth of wild rocket *Diplotaxis tenuifolia* (L.) DC cv Dallas leaves as affected by different nutrient supply levels and growing systems. *Journal of Soil Science and Plant Nutrition*. 23: 4115-4126.
- Wettstein D. (1957). Chlorophyll-letale und der submikroskopische Formwechsel der Plastiden. *Experimental cell research*. 12: 427-434.

Microelement content in hydroponically cultivated nettle (*Urtica dioica* L.) under varied irrigation intervals

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Abstract

Stinging nettle is a rich source of specialized metabolites, pigments, vitamins and minerals and can be used in gastronomy to diversify the human diet. Nettle plants were cultivated in a greenhouse using the ebb and flow hydroponic technique. The aim of the study was to investigate the influence of 24, 48 and 96 hour irrigation intervals on the content of dry matter, iron, zinc, manganese, copper, boron and molybdenum in nettle leaves. The highest dry matter content (21.3%) was found in plants cultivated with an irrigation interval of 96 hours. An irrigation interval of 24 hour resulted in the highest contents of iron (126.6 mg kg⁻¹ Fe), manganese (63.5 mg kg⁻¹ Mn) and molybdenum (2.2 mg kg⁻¹ Mo).

Keywords: ebb and flow technique, minerals, nutritional quality, plant nutrition, water stress

Introduction

A significant proportion of the world's population suffers from a lack of essential vitamins and minerals such as zinc, iron, magnesium, selenium and iodine (Krzepińko et al., 2019). This global problem, also known as hidden hunger, affects around two billion people, with inappropriate eating habits being a major factor (Saltzman et al., 2014). Institutions such as the World Health Organization (WHO) have worked to raise public consciousness, so more and more consumers are aware of the importance of a varied and balanced diet and pay attention to their food choices and the inclusion of novel plants in their diet. Wild green leafy plants are known for their rich content of phytonutrients (Grauso et al., 2020), which play an important role in the prevention of numerous chronic diseases (Opačić et al., 2022).

Nettle leaves (*Urtica dioica* L.) can be used as a green leafy vegetable in the human diet and are a good way to diversify that diet and provide the body with the desired amounts of compounds such as plant pigments, fatty acids, polyphenols, essential amino acids, vitamins and minerals, including microelements such as iron and zinc standing out the most (Rafajlovska et al., 2013; Đurović et al., 2017; Repajić et al., 2021). Due to its peculiarity (tendency to accumulate nitrates and heavy metals from the soil), it is recommended to cultivate nettle in agricultural production and not to collect it in the wild (Radman et al., 2021).

Hydroponic cultivation of nettle is emerging as a reliable method to obtain high-quality plant material while sustainably managing resources such as water and fertilizers (Radman et al., 2021; Opačić et al., 2022; Dujmović et al., 2023). Various hydroponic techniques have proven to be suitable for nettle production. By choosing the ebb and flow hydroponic technique, it is possible to regulate the supply of a nutrient solution to the plants, i.e. the plants are exposed to different irrigation intervals (controlled water stress). Research shows (Dujmović et al., 2023; Opačić et al., 2023) that nettle plants under water stress accumulate more specialized metabolites, resulting in higher nutrient quality, but such stress can potentially impair mineral uptake from the nutrient solution (Seleiman et al., 2021).

For this reason, a study was conducted to determine the influence of three different irrigation intervals on the content of dry matter and microelements (iron, zinc, manganese, copper, boron and molybdenum) in stinging nettle.

Material and methods

The study was conducted in the greenhouse at the Department of Vegetable Crops, University of Zagreb Faculty of Agriculture (Croatia) during winter – spring cultivation period (January – June 2022). The nettle plants were grown in a hydroponic system with ebb and flow. In the experiment, 10 nettle seeds per pot were sown on January 20 in polystyrene containers with 40 pots each, which were filled with a commercial substrate (Klasman Potgrond H). After sowing, containers were placed on benches (2m × 3m) and irrigated regularly. On March 14, each container was checked for the number of plants per pot and the plants were thinned to 3 per pot. From April 11 to the end of cultivation period, the plants were subjected to three different irrigation intervals (treatments): bench I received the nutrient solution every 24 hours, bench II every 48 hours and bench III every 96 hours. For each treatment, the nutrient solution was left on the benches for 1 hour and then drained.

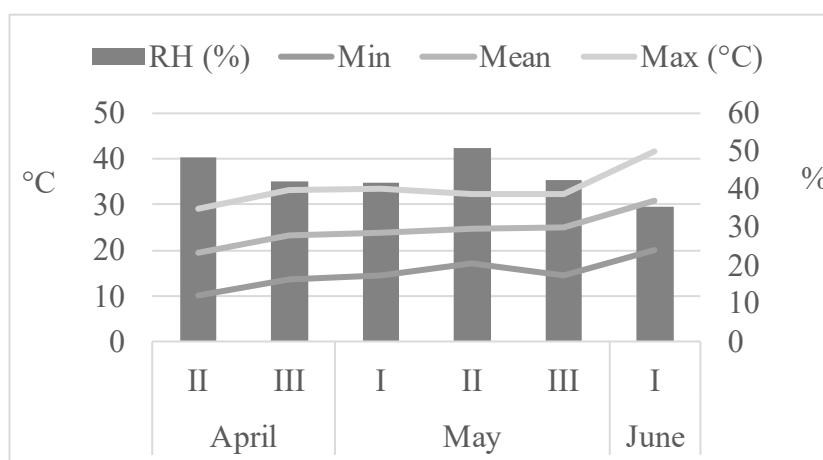
The content of the nutrient solution according to Lorenz and Maynard (1988), which was used for the nettle cultivation is listed in Table 1. The pH value of the solution was adjusted by adding HNO₃ (56%).

Table 1. Content of nutrient solution for nettle cultivation

Salts	mg L ⁻¹
KNO ₃	251.0
KH ₂ PO ₄	142.7
Ca(NO ₃) ₂ × 4H ₂ O	501.5
MgSO ₄ × 7H ₂ O	256.3
FeEDTA, 13%	12.8
H ₃ BO ₃	1.32
CuSO ₄ × 5H ₂ O	0.03
MnSO ₄ × 4H ₂ O	0.79
ZnSO ₄ × 7H ₂ O	0.11
Na ₂ MoO ₄ × 2 H ₂ O	0.02
EC	1.5
pH	5.8 – 6.2

EC – electric conductivity

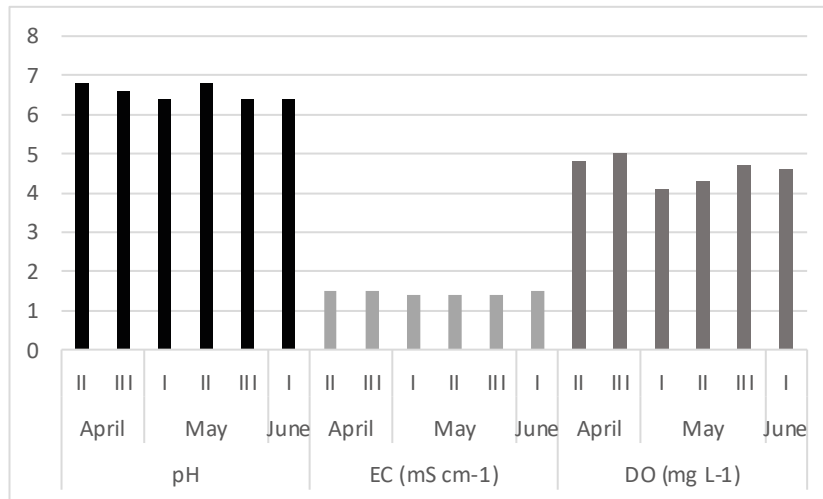
During the entire cultivation period, the abiotic factors of the greenhouse, such as air temperature (°C) and relative humidity (RH, %), were closely monitored using a table top thermohygrometer (Agrologistika d.o.o., Čakovec, Croatia) and are shown in Graph 1 as decadal values.



Graph 1. Abiotic factors of the greenhouse during nettle cultivation; RH – relative humidity

Air temperature values ranged from 10.3 to 41.6°C with an average value of 24.6°C, while relative humidity ranged from 32 to 53% with an average value of 43%.

Nutrient solution abiotic factors such as pH, electric conductivity (EC, mS cm⁻¹) and deluted oxygen (DO, mg L⁻¹) were also monitored (Graph 2) using a multiparameter instrument HI98194 (Hanna instruments, Romania) and are also presented as decadal values.



Graph 2. Abiotic factors of the nutrient solution during nettle cultivation; EC—electrical conductivity, DO—dissolved oxygen

The pH averaged 6.6, the EC value averaged 1.5 mS cm⁻¹, and the average value of DO in the nutrient solution was 4.6 mg L⁻¹.

The harvest was carried out before flowering phase on May 10 and the samples of nettle leaves were sent to the laboratory of the Department of Plant Nutrition for analysis of the dry matter and microelement content. The fresh plant samples were dried at 105°C until constant weight, then crushed and homogenized. The dry matter was determined by the gravimetric method according to HRN ISO 11465:2004. After digestion of the dry samples with HNO₃ and HClO₄, iron, zinc, manganese, copper, boron and molybdenum were determined using the atomic absorption spectrometer (AOAC, 2015).

The experiment was set up according to the randomized block design in three replicates, and the laboratory analyzes were performed in triplicate. Statistical data processing was performed in SAS software v. 14.3 (SAS, 2017) using the PROC GLM (general linear model) procedure. The results were subjected to one-way analysis of variance (ANOVA), and the differences between means were compared using the t-test (LSD) at the significance level p≤0.05.

Results and discussion

Table 2 shows the influence of 3 irrigation intervals (24 h, 48 h and 96 h) on the dry matter and microelements content of nettle cultivated in the hydroponic ebb and flow system. There were found statistically significant differences between the treatments, except for the zinc and boron content.

Dry matter (DM) is a key indicator of the quality of plant material and indicates the amount of essential components such as bioactive compounds and minerals and can be influenced by various factors such as cultivation period, water availability, temperature, etc. (Paulauskienė et al., 2021). The highest DM content was found in plants exposed to the longest irrigation interval (96 h) and amounted to 21.3% DM, while the 48 h irrigation interval resulted in the lowest DM content (17.8% DM). According to Dujmović et al. (2023), nettle cultivated in floating hydroponics with the same nutrient solution as in this study yielded 13.5 – 24.0% DM depending on the harvest. In a study by Paulauskienė et al. (2021), the DM content of wild- collected nettle varied between 20.5 and 24.4% DM depending on the time of harvest.

Table 2. Dry matter and microelement content of nettle

Irrigation interval	DM	Fe	Zn	Mn	Cu	B	Mo
	%	mg kg ⁻¹					
24 h	19.1 ^b ± 0.1	126.6 ^a ± 8.3	28.1 ^{ns} ± 1.5	63.5 ^a ± 3.1	11.9 ^a ± 0.4	34.5 ^{ns} ± 1.0	2.2 ^a ± 0.1
48 h	17.8 ^c ± 0.2	115.8 ^a ± 9.1	27.0 ^{ns} ± 1.7	47.9 ^b ± 0.3	13.3 ^a ± 1.8	34.3 ^{ns} ± 2.3	1.4 ^b ± 0.1
96 h	21.3 ^a ± 0.2	99.8 ^b ± 5.7	26.3 ^{ns} ± 0.1	41.8 ^c ± 1.7	9.3 ^b ± 0.7	31.6 ^{ns} ± 0.8	1.6 ^b ± 0.1
ANOVA	<.0001	0.0161	0.3280	<.0001	0.0140	0.1032	0.0003
LSD	0.3628	15.65	2.7483	4.0383	2.2838	3.0219	0.2431

DM – dry matter; ns—non significant. Results are expressed as mean ± standard deviation. Different letters indicate significant differences between mean values.

The most frequently observed deficiency of microelements is due to an inadequate supply of iron and zinc caused by inappropriate or insufficiently varied dietary habits (Saltzman et al., 2014). Green leafy vegetables such as kale and spinach are known to be fantastic sources of iron (Czarnowska-Kujawska et al., 2022). Previous research conducted by Upton (2013), Radman et al. (2016), Kregiel et al. (2018), and Radman et al. (2021) indicates that nettle is rich in both iron and zinc. The mineral content varied depending on factors such as the origin of the plant material (wild collected or cultivated) or the cultivation methods (open field or hydroponics). Since the lowest iron (Fe) content (99.8 mg kg⁻¹ Fe) in this study was found in plants exposed to a 96 h irrigation interval, this indicates that a longer irrigation interval had a negative effect on the Fe content in nettle. The highest value (126.6 mg kg⁻¹ Fe) was found in plants exposed to a 24 h irrigation interval, but this result was not statistically different from the 48 h irrigation interval. Nettle from floating hydroponics contained 79.2 - 89.5 mg kg⁻¹ Fe in a research by Radman et al. (2021) and Dujmović et al. (2023) reported 1.0 - 2.2 mg 100g⁻¹, which are lower values than in this study. On the other hand, according to Kregiel et al. (2018) Fe content of dried nettle leaves powder averaged 227.9 mg 100g⁻¹ Fe. The apparently large difference in the iron content of nettle plants from the above studies may be due to different geographical locations, different cultivation periods, whether the plant material was collected in the wild or from agricultural production, and whether it was grown in the open field or in hydroponics. All this can lead to very different amounts of absorbed iron.

An insufficient intake of zinc (Zn) can lead to frequent infections and a weakened immune system (Saltzman et al., 2014). Consuming foods rich in this microelement can strengthen the body's natural defenses against disease. In this study, the Zn content was between 26.3 and 28.1 mg kg⁻¹. The differences were not significant, although there was a tendency towards a lower Zn content in plants exposed to longer irrigation intervals. Paulauskienė et al. (2021) reported content of 12.7 – 34.3 mg kg⁻¹ Zn in nettle plants. According to Dujmović et al. (2023), the average Zn values in nettle from floating hydroponics was 0.5 mg 100g⁻¹.

The content of manganese (Mn) was highest in nettle plants exposed to a 24 h irrigation interval (63.5 mg kg⁻¹ Mn) and this content decreased with increasing irrigation interval suggesting that longer irrigation interval may have a negative effect on the Mn content in nettle plants. In floating hydroponics nettle Mn content varied from 0.1 - 1.3 mg 100g⁻¹ Mn as confirmed in research by Dujmović et al. (2023). According to Paulauskienė et al. (2021), wild-harvested nettle contained an average of 48.1 mg kg⁻¹ Mn, which corresponds to the average content determined in this study. The significantly lower amounts of Zn and Mn reported by Dujmović et al. (2023) in the floating hydroponics could be the result of higher temperatures of the nutrient solution as well as higher pH values of the solution during cultivation compared to the data in this study, which could have led to a lower uptake of these microelements by the nettle plants.

There was no significant difference in copper (Cu) content in plants exposed to an irrigation interval of 24 h and 48 h and irrigation interval of 96 h resulted in lowest Cu value (9.3 mg kg⁻¹ Cu). Paulauskienė et al. (2021) reported 10.2 – 18.4 mg kg⁻¹ Cu in wild-harvested nettle while Dujmović et al. (2023) analysed much lower Cu content in nettle leaves.

The boron (B) content of nettle varied between 31.6 and 34.5 mg kg⁻¹ B and the differences were not significant. These results were higher than those from the study by Dujmović et al. (2023), but in correlation with the B content in nettle collected in nature in May from the study by Paulauskienė et al. (2021), while plant material from the same study collected in the other months contained higher levels of Cu.

Molybdenum (Mo), the deficiency of which is rare, is associated with anti-inflammatory properties and helps the

body to process toxins through the liver (Johnson, 2023). The highest Mo content in this study was found in plants exposed to 24 h irrigation interval (2.2 mg kg⁻¹ Mo). The treatments with an irrigation interval of 48 and 96 h did not differ statistically (1.4 and 1.6 mg kg⁻¹ Mo). Dujmović et al. (2023) reported values of 0.0 – 0.1 mg 100g⁻¹ Mo in nettle cultivated in floating hydroponics.

Conclusions

A longer, 96 hours irrigation interval, had a negative effect on the iron, manganese and molybdenum content, but had a positive effect on the dry matter content of nettle cultivated in a hydroponic ebb and flow system. Given the crucial importance of incorporating iron and zinc-rich foods into diet, the study recommends adopting a 48 hour irrigation interval for ebb and flow cultivation of nettle. This approach ensures that the plant material maintains satisfactory microelement content while promoting a more efficient and sustainable use of nutrient solution.

Acknowledgement

This research was funded by Croatian Science Foundation (Hrvatska zaklada za znanost) within the project IP-2019-04-3325 URTICA-BioFuture, Nutritional and functional value of nettle (*Urtica dioica* L.) by application of modern hydroponic cultivation techniques.

References

- AOAC (2015). Official Method of Analysis of AOAC International, Gaithersburg, Maryland, USA.
- Czarnowska-Kujawska M., Starowicz M., Barišić V., Kujawski W. (2022). Health-Promoting Nutrients and Potential Bioaccessibility of Breads Enriched with Fresh Kale and Spinach. *Foods*. 11: 3414.
- Dujmović M., Opačić N., Radman S., Fabek Uher S., Čoga L., Petek M., Voća S., Šic Žlabur J. (2023). How to Increase the Nutritional Quality of Stinging Nettle Through Controlled Plant Nutrition. *Food Technology and Biotechnology*. 61(4).
- Durović S., Pavlič B., Šorgić S., Popo S., Savić S., Petronijević M., Radojkovi M., Cvetanović A., Zeković Z. (2017). Chemical composition of stinging nettle leaves obtained by different analytical approaches. *Journal of Functional Foods*. 32: 18-26.
- Grauso L., De Falco B., Lanzotti V., Motti R. (2020). Stinging nettle, *Urtica dioica* L.: Botanical, phytochemical and pharmacological overview. *Phytochemistry Reviews*. 19: 1341–1377.
- HRN ISO 11261:2004 (Soil quality - Determination of total nitrogen - Modified Kjeldahl method (ISO 11261:1995).
- Johnson L.E. (2023). Molybdenum Deficiency. Accessed on October 10 2023: <https://www.merckmanuals.com/professional/nutritional-disorders/mineral-deficiency-and-toxicity/molybdenum-deficiency>.
- Kregiel D., Pawlikowska E., Antolak H. (2018). *Urtica* spp.: Ordinary plants with extraordinary properties. *Molecules*. 23: 1664.
- Krzepiłko A., Prazak R., Skwaryło-Bednarz B., Molas J. (2019). Agronomic biofortification as a means of enriching plant foodstuffs with iodine. *Acta Agrobotanica*. 72(2).
- Lorenz O. A., and Maynard D. N. (1988). *Knotts Handbook for Vegetable Growers*. John Wiley Sons, New York.
- Opačić N., Radman S., Fabek Uher S., Benko B., Voća S., Šic Žlabur J. (2022). Nettle Cultivation Practices—From Open Field to Modern Hydroponics: A Case Study of Specialized Metabolites. *Plants*. 11: 483.
- Opačić N., Šic Žlabur J., Sikirić L., Petek M., Čoga L., Fabek Uher S., Benko B., Toth N., Voća S., Radman S. (2023). Specialized metabolites content in hydroponically grown nettle. *Acta Horticulture*. 1358: 333-340.
- Paulauskienė A., Tarasevičienė Ž., and Laukagalis V. (2021). Influence of harvesting time on the

- chemical composition of wild stinging nettle (*Urtica dioica* L.). *Plants*. 10: 686.
- Radman S., Žutić I., Fabek S., Toth N., Benko B., Čoga L. (2016). Influence of propagation method and fertilization on chemical composition and yield of stinging nettle. In Proceedings of the 51st Croatian & 11th International Symposium on Agriculture, Opatija, Hrvatska, 15–18 February 2016, pp. 192–196.
- Radman S., Javornik M., Žutić I., Opačić N., Benko B. (2021). Impact of different nutrient solution composition on stinging nettle growth and mineral content. In Proceedings of the VIII South-Eastern Europe Symposium on Vegetables and Potatoes 1320, Ohrid, North Macedonia, 24–26 September 2021., pp. 157–166.
- Rafajlovska V., Kavrakovski Z., Simonovska J., Srbinoska M. (2013). Determination of protein and mineral contents in stinging nettle. *Quality of Life (Banja Luka)-APEIRON*, 7 (1-2).
- Repajić M., Cegledi E., Zorić Z., Pedisić S., Elez Garofulić I., Radman S., Palčić I., Dragović-Uzelac V. (2021). Bioactive Compounds in Wild Nettle (*Urtica dioica* L.) Leaves and Stalks: Polyphenols and Pigments upon Seasonal and Habitat Variations. *Foods*. 10: 190.
- Saltzman A., Birol E., Wiesman D., Prasai N., Yohannes Y., Menon P., Thompson, J. (2014). 2014 global hunger index: The challenge of hidden hunger. Intl Food Policy Res Inst. Accessed on October 10 2023: https://ebrary.ifpri.org/utils/getfile/collection/p15738_coll2/id/128360/filename/128571.pdf
- SAS®/STAT 14.3. (2017). SAS Institute Inc., Cary, NC, USA
- Seleiman M.F., Al-Suhaibani N., Ali N., Akmal M., Alotaibi M., Refay Y., Dindaroglu T., Abdul-Wajid H.H., Battaglia M.L. (2021). Drought Stress Impacts on Plants and Different Approaches to Alleviate Its Adverse Effects. *Plants*. 10:259.
- Upton R. (2013). Stinging nettles leaf (*Urtica dioica* L.): Extraordinary vegetable medicine. *Journal of herbal medicine*. 3: 9-38.

Organic fertilization effects on chemical composition and morphological traits of cultivated nettle

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Abstract

Modern agricultural production is increasingly oriented towards sustainable cultivation guidelines, which include a reduced use of mineral fertilizers. This is a particular challenge for nitrophilous plant species such as stinging nettle, which requires significant amounts of nitrogen for its growth and development. Therefore, the aim of this study was to investigate the influence of two types of organic fertilization (compost and dehydrated organic fertilizer) compared to the control and the application of mineral fertilizers on the morphological characteristics, the amount of minerals and the content of vitamin C and antioxidant capacity of stinging nettle. Fertilization with dehydrated organic fertilizer showed a greater influence than mineral fertilization on several morphometric components investigated (number of leaves, leaf length and width) and on dry matter, but at the same time had a negative effect on magnesium uptake. The application of compost had a more favorable effect on the nitrogen and phosphorus values of the stinging nettle.

Keywords: *Urtica dioica* L., compost, dehydrated organic fertilizer, minerals, vitamin C

Introduction

Recognizing the urgent global threat to food security posed by climate change, with agriculture being the most vulnerable sector, as highlighted by Malhi et al. (2021), the European Union has taken proactive measures. In response to the challenges of the modern age, EU has launched a number of programs and strategies which aims to transform the existing economy into a resource-efficient and globally competitive one. In the focus of this comprehensive approach are numerous plans tailored for agriculture, strategically geared towards fostering the adoption of sustainable agricultural practices. The main objective of the “Farm to Fork” strategy is to promote organic farming with the aim of increasing the proportion of agricultural land in the European Union to 25% by 2030 (European Council, 2023). In addition, efforts will be made to reduce nutrient losses by at least 50 % and the use of mineral fertilizers by at least 20 % by 2030 (European Commission, 2020). The excessive use of mineral fertilizers in agriculture leads to an excess of nutrients in the environment, especially nitrogen and phosphorus, which pollutes the air, soil and water and has a negative impact on the climate. Excess nutrients also contribute to the decline of biodiversity in rivers, lakes, wetlands and oceans. For many years, agricultural practices have focused on increasing yields without considering product quality and the rational use of resources. But the goal of modern agriculture is to reduce the necessary resources without reducing yield and product quality (Bulgari, 2015).

There is a growing trend among people to explore alternative food sources and recognize the importance of a varied diet in terms of overall health. In this context, stinging nettle (*Urtica dioica* L.) is proving to be a valuable but often overlooked food source due to its rich content of specialized metabolites, showing promise for both nutritional and pharmaceutical purposes (Zeipiņa et al., 2014). In addition to its well-known use for medicinal and cosmetic purposes, nettle is also traditionally used as a green leafy vegetable in many Mediterranean and Eastern European countries. However, it is usually harvested in the wild, which raises concerns about its quality and undefined chemical composition (Di Virgilio et al., 2015; Radman et al., 2015; Opačić et al., 2022a). Stinging nettle is a plant species that requires sufficient amounts of nutrients (especially nitrogen) in the soil to grow and develop properly. Petek

(2019) points out that only a well-nourished plant can provide sufficient minerals for human nutrition, which can be achieved through optimal fertilization according to the rules of good agricultural practice. Therefore, it is necessary to investigate an alternative to soluble mineral fertilizers. Considering the context described above, the aim of this study was to investigate the effects of two types of organic fertilizers on nettle cultivation compared to conventional mineral fertilizers and without fertilization, focusing on the evaluation of morphological characteristics and chemical composition of cultivated nettle.

Material and methods

Experiment setup

The field trial was conducted at the Department of Vegetable Crops of the University of Zagreb Faculty of Agriculture. In this study, the effects of two organic fertilizers – compost (F1) and dehydrated organic fertilizer (F2) – together with a mineral fertilizer (F3) on the morphological characteristics, dry matter content, nitrogen and mineral content (phosphorus, potassium, calcium, magnesium), vitamin C and antioxidant capacity of nettle plants were investigated. No fertilization was applied to the control plots (F0). The experiment was laid out according to the randomized block design method with three replicates.

The organic compost fertilizer 'Lumbrical' – F1 (Niveleta 92. d.o.o.) is obtained from composted horse manure using the red earthworm *Lumbircus rubellus*. It has a long-lasting effect and contains 2.3 – 2.8% N, 1.3 – 1.8% P₂O₅, 2.4 – 2.8% K₂O, 1.5 – 1.9% CaO and 0.5 – 0.8% MgO. It contains 35 – 45% dry matter and its pH value is between 6.9 and 7.2. The dried, pelletized organic fertilizer 'Siforga' – F2 (Menon BV-Netherlands) was obtained by heat treatment of chicken manure and is intended for crops with high nitrogen requirements. It contains 90% dry matter, of which 60% is organic matter, 4% N, 3% P₂O₅, 7.4% K₂O, 8% CaO and 1% MgO. The pH value is 7. Mineral fertilizer NPK – F3 (Petrokemija d.d., Croatia) is a highly concentrated mineral fertilizer. Nitrogen is present in the form of ammonium (7% N), phosphorus (20% P₂O₅) is water-soluble and potassium is present as potassium chloride (30% K₂O). The seeds of stinging nettle *Urtica dioica* L. (B&T World Trade, France) were sown on February 25 in polystyrene containers with 106 pots filled with the commercial substrate Potgrond H (Klasmann-Deilmann GmbH, Germany). After 46 days in the greenhouse, the seedlings were planted out in the field on April 12 at a spacing of 25 × 15 cm (26 plants m⁻²). Two plants were planted at each planting site. All fertilizers (F1 – compost 'Lumbrical' 2.5% N; F2 – dehydrated organic fertilizer 'Siforga' 4% N; F3 – mineral fertilizer NPK 7% N) were applied at a rate of 100 kg N ha⁻¹, according to Opačić et al. (2022a). The organic fertilizers (F1 and F2) were applied immediately before sowing (12 April) in a total rate of 100 kg N ha⁻¹. The mineral fertilizer (F3) was divided into an initial fertilization at planting (40 kg N ha⁻¹) and the remainder into two additional fertilizations (30 + 30 kg N ha⁻¹ in the form of KAN fertilizer) on 5 and 26 May. The reason for this is its easy solubility and rapid release. After planting the seedlings, a drip irrigation system was set up and mechanical weed control was carried out during the growth period as needed.

Morphological analysis of plant material

The harvest of the nettle mass was carried out in the pre-flowering phase in such a way that the nettle was cut off above the lower two nodes to allow the plant to regrow. The basic morphological characteristics (height, number of leaves, length and width of leaves) were determined on 10 plants per replicate.

Methods of determining the mineral composition, ascorbic acid and antioxidant capacity of nettle

Prepared representative samples of plant material (leaves and the top part of the plant) were analysed at the Department of Sustainable Technologies and Renewable Energy Sources and Department of Plant Nutrition. Samples of plant material (dried at 105°C) were analysed in triplicate and the results presented as mean values. Prior to digestion the samples were re-dried in order to remove possible moisture gained before the analyses. After digestion of plant material with concentrated HNO₃ (MILESTONE 1200 Mega Microwave Digester), the phosphorus content was determined using a spectrophotometer, and potassium using a flame photometer, while calcium, magnesium, were analysed using an atomic absorption spectrophotometer (AAS), (AOAC, 1995). The ascorbic acid (AsA) content was determined in accordance with the AOAC (2002) standard method by employing titration with 2,6-dichloroindophenol (DCPIP). Detailed method is described in Dujmović et al. (2023). The antioxidant capacity

was determined spectrophotometrically using the ABTS radical cation [2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] (Re et al., 1999).

Statistical data processing

The obtained results of morphological and chemical analyzes were statistically processed in the SAS software system, version 9.4 (SAS/STAT, 2017) according to the PROC GLM (general linear model) procedure. The differences between the treatments for all measured properties were statistically processed by analysis of variance (ANOVA), and the established differences between mean values were compared by t-test (LSD) and are considered significantly different at $p < 0.0001$. In the tables the exponents of the different letters indicating the groups of samples are shown. The standard deviation is also expressed as the average deviation from the mean value for the morphology and chemical composition of the nettle.

Results and discussion

Temperatures of 20–25°C are recommended for the growth and development of plants (Opačić et al., 2022a). According to the Croatian Meteorological and Hydrological Service (2021), the average air temperature for the months of April, May and June was 9.8, 14.7 and 23.3°C, respectively. The maximum air temperatures for the same months were: 15.4, 20.2 and 29.2°C. The most precipitation fell in June (124 mm), twice as much in April (67.7 mm) and the least in June (13.2 mm), which favored the growth and development of nettles. Table 1 shows that the fertilizer treatments had no statistically justified influence on the morphological characteristics of the cultivated nettle. The height of the nettle varied from 20.3 (F0) to 29.7 cm (F3). Considering only the influence of organic fertilization, the application of dehydrated organic fertilizer 'Siforga' was more favorable (F2 - 29.2 cm) compared to the compost 'Lumbrical' (F1 - 22.7 cm), however this difference was not statistically justified. The measured height of the nettle was lower than in the study by Radman et al. (2022), in which a two-year-old nettle plantation was analyzed. Just like the height, the number of leaves was also lowest on the control plots (F0 - 23.3), while the nettle on the F2 treatment had the most leaves (28.0). Unexpectedly, the nettle had fewer leaves when the mineral fertilizer was applied (24.3) than with the two organic fertilizers (F1 - 25; F2 - 28.0). Compared to nettle grown using the hydroponic floating technique according to Opačić et al. (2022b), a slightly lower average height of nettle was determined in this study, but twice the number of leaves was measured. The reason for this is probably a much denser sowing in floating system, when the plants are elongated and produce fewer leaves that have a larger surface area. The shortest leaves were found in the nettle in treatments F0 and F1 (6.9 cm), followed by F3 (7.3 cm), while the longest leaves were found when the organic fertilizer 'Siforga' was applied (F2 - 7.5 cm). Similarly, the nettles in treatment F2 (5.7 cm) had the widest leaves, while the nettles in treatments F0 (5.2 cm) and F1 (5.0 cm) had the narrowest leaves. Although the fertilization had no statistical influence, it can be stated that the morphological characteristics were weakest in the control plots, while the organic fertilization with dehydrated organic fertilizer 'Siforga' showed the best effect, even more than the mineral fertilization.

Table 1. Morphological properties of cultivated nettle

Treatment	Height (cm)	Number of leaves	Leaf length (cm)	Leaf width (cm)
F0	20.3 ± 1.53	23.3 ± 1.15	6.9 ± 0.63	5.2 ± 0.38
F1	22.7 ± 4.73	25.0 ± 4.00	6.9 ± 0.63	5.0 ± 0.58
F2	29.2 ± 5.58	28.0 ± 9.54	7.5 ± 1.00	5.7 ± 0.63
F3	29.7 ± 5.75	24.3 ± 2.08	7.3 ± 0.58	5.5 ± 0.66
ANOVA	0.0983	0.73752	0.6624	0.4424
LSD	12.911	14.54	1.9661	1.5658

F0 – without application of fertilization (control); F1 – compost 'Lumbrical'; F2 – dehydrated organic fertilizer 'Siforga'; F3 – mineral fertilizer NPK 7-20-30. Results are expressed as mean ± standard deviation. Different letters indicate significant differences between means.

Table 2 shows the influence of organic fertilization on the amount of dry matter (DM), nitrogen (N) and minerals (P, K, Ca, Mg) in nettle leaves. It can be seen that there are significant differences ($p < 0.0001$) between the tested fertilizer treatments on the content of dry matter and macroelements in the nettle. The total dry matter content represents the basic chemical composition of the raw material, i.e. the content of all components such as proteins, carbohydrates, minerals and bioactive components except water. Therefore, plant materials with higher DM values have a higher nutritional quality (Dujmović et al., 2023). The lowest dry matter content was measured in the control varieties (F0 – 21.48%), while nettles fertilized with organic fertilizers had justifiably higher DM values (F1 – 23.39%; F2 – 24.47%). Mineral fertilizer led to a decrease in dry matter compared to both organic fertilizers (F3 – 22.54%).

The nettle leaves of the control varieties (F0) showed the statistically highest content of nitrogen (4.39%), phosphorus (0.59%), potassium (3.47%) and calcium (4.89), while the application of mineral fertilization (F3) led to a significant decrease in the macroelements mentioned (N – 3.13%; P – 0.49%; K – 2.76% Ca – 4.89%). Petek (2019) explains that in agricultural production, fertilization cannot be expected to have a strong influence on the mineral composition of plants under all conditions, because numerous different factors influence the nutrient uptake in the field. Comparing organic fertilization, significantly higher values for nitrogen and phosphorus were found when F1 fertilizer was applied, while no statistically justified difference was found for the macroelements K and Ca depending on the organic fertilizer. According to Petek (2019) organic fertilization increases the availability of phosphorus in soil as during the mineralization process a certain amount of P is released from organic compounds. In this research the application of fertilizer F1 proved to be the best for the amount of magnesium, with the highest values recorded (0.42%), while the lowest values were found in the nettle fertilized with dehydrated organic fertilizer (F2 - 0.37%). Unexpectedly, statistically lower magnesium levels were found in the plots where mineral fertilizers were applied (0.39%) than in the control (0.40%).

Table 2. Amount of dry matter, nitrogen and minerals in nettle (%)

Tretman	DM	N	P	K	Ca	Mg
F0	21.48 ^d ± 0.02	4.39 ^a ± 0.05	0.59 ^a ± 0.00	3.47 ^a ± 0.06	4.89 ^a ± 0.02	0.40 ^b ± 0.01
F1	23.39 ^b ± 0.01	3.61 ^b ± 0.05	0.58 ^a ± 0.00	3.06 ^b ± 0.02	4.57 ^b ± 0.02	0.42 ^a ± 0.01
F2	24.47 ^a ± 0.02	3.31 ^c ± 0.07	0.52 ^b ± 0.01	3.00 ^b ± 0.01	4.55 ^b ± 0.03	0.37 ^d ± 0.01
F3	22.54 ^c ± 0.01	3.13 ^d ± 0.02	0.49 ^c ± 0.01	2.76 ^c ± 0.00	4.36 ^c ± 0.03	0.39 ^c ± 0.01
ANOVA	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
LSD	0.0387	0.1365	0.0158	0.0881	0.0694	0.0137

F0 – without application of fertilization (control); F1 – compost 'Lumbrical'; F2 – dehydrated organic fertilizer 'Siforga'; F3 – mineral fertilizer NPK 7-20-30; DM – dry matter content. Results are expressed as mean ± standard deviation. Different letters indicate significant differences between means.

Table 3 shows the influence of fertilization on the content of ascorbic acid (AsA), known as vitamin C, and the antioxidant capacity of cultivated nettle. AsA has several important functions in the plant organism, such as reducing cell division, mitigating oxidative stress in plant cells (antioxidant properties), is a cofactor for enzymes and a precursor for the synthesis of oxalates and tartrates (Gaafar et al., 2020). Nettles fertilized with mineral fertilizer had statistically the least AsA (F4 – 71.89 mg 100g⁻¹ fw). This is consistent with other studies (Roumeliotis et al., 2021; Chadzinikolau and Formela-Luboińska, 2023), which proved that the nitrogen content has a negative effect on the accumulation of AsA, i.e. it leads to lower synthesis of AsA in the plant tissues.

Table 3. Ascorbic acid content and antioxidant capacity of nettle under different fertilization treatment

Treatment	AsA	ABTS
	mg 100g ⁻¹ fw	μmolTE L ⁻¹
F0	94.30 ^a ± 5.71	2421.12 ^a ± 1.37
F1	89.45 ^{ab} ± 5.30	2420.53 ^a ± 4.00
F2	110.69 ^a ± 13.48	2369.26 ^c ± 3.88
F3	71.89 ^b ± 3.56	2393.54 ^b ± 3.08
ANOVA	p<0.0025	p<0.0001
LSD	21.877	8.9208

F0 – without application of fertilization (control); F1 – compost 'Lumbrical'; F2 – dehydrated organic fertilizer 'Siforga'; F3 – mineral fertilizer NPK 7-20-30; AsA – ascorbic acid content. ABTS – antioxidant capacity. Results are expressed as mean ± standard deviation. Different letters indicate significant differences between means.

Accordingly, the highest AsA levels were found in the leaves of the plots to which dehydrated organic fertilizer was added (F2 - 110.69 mg 100g⁻¹ fw), but this was not statistically different from the slightly lower vitamin C levels of the plots treated with compost (F1 - 89.45 mg 100g⁻¹ fw) and without fertilizer (F0 - 94.30 mg 100g⁻¹ fw).

The antioxidant capacity of plant samples is directly related to the amount of bioactive compounds a plant contains. Total phenols and vitamins are among the most powerful antioxidants, so their content is generally decisive for the overall antioxidant capacity of plant samples (Opačić et al., 2023). Table 3 shows that fertilization had a statistically justified influence on the antioxidant capacity of stinging nettle. The lowest value was found in nettles with the application of F2 fertilizer (2369.26 μmolTE L⁻¹). Nettles fertilized with compost had a statistically significant higher antioxidant capacity (F1 - 2420.53 μmolTE L⁻¹), which was not statistically different from the highest value found in nettles from control plots (F0 - 2421.12 μmolTE L⁻¹). As with vitamin C, mineral fertilization led to a reduced antioxidant capacity (F3 - 2393.54 μmolTE L⁻¹).

Conclusions

Organic fertilization showed a profound influence on the morphological characteristics and chemical composition of nettles, proving the potential to replace mineral fertilizers with organic fertilizers and to grow nettles according to organic principles. In future research, it is necessary to investigate the influence of organic fertilization on the morphological properties and chemical composition of nettle through repeated harvest and on perennial crop.

References

- AOAC (1995). Official Methods of Analysis. 16th Edition, Arlington, USA.
- AOAC (2002). Official Methods of Analysis. 17th Edition. Association of Official Analytical Chemist, Washington DC, USA.
- Bulgari R., Cocetta G., Trivellini A., Vernieri P., Ferrante A. (2015). Biostimulants and crop responses: a review. *Biological Agriculture and Horticulture*. 31: 1-17.
- Chadzinikolau T., and Formela-Luboińska M. (2023). Nitrogen Metabolism and Antioxidant Capacity of Selected Vegetables from Organic and Conventional Crops *Applied Sciences*. 13: 11170.
- Croatian Meteorological and Hydrological Service (2021). Available from: https://meteo.hr/index_en.php
- Di Virgilio N., Papazoglou E.G., Jankauskiene Z., Di Lonardo S., Praczyk M., Wielgusz K. (2015). The potential of stinging nettle (*Urtica dioica* L.) as a crop with multiple uses. *Industrial Crops and Products*. 68: 42–49.
- Dujmović M., Radman S., Opačić N., Fabek Uher S., Mikulićin V., Voća S., Šic Žlabur J. (2022). Edible Flower Species as a Promising Source of Specialized Metabolites. *Plants*. 11: 2529.

- Dujmović M., Opačić N., Radman S., Fabek Uher S., Voća S., Šic Žlabur, J. (2023). Accumulation of Stinging Nettle Bioactive Compounds as a Response to Controlled Drought Stress. *Agriculture*. 13:1358.
- European Commission (2020). Available from: <https://eur-lex.europa.eu/legal-content/ENG/TXT/HTML/?uri=CELEX:52020DC0381&from=EN>
- European Council (2023). From farm to fork. Available from: <https://www.consilium.europa.eu/en/policies/from-farm-to-fork/>
- Gaafar A.A., Ali S.I., El-Shawadfy M.A., Salama Z.A., Sękara A., Ulrichs C., Abdelhamid M.T. (2020). Ascorbic acid induces the increase of 2ndary metabolites, antioxidant activity, growth, and productivity of the common bean under water stress conditions. *Plants*. 9: 627.
- Malhi G. S., Kaur M., Kaushik P. (2021). Impact of climate change on agriculture and its mitigation strategies: A review. *Sustainability*. 13: 1318.
- Opačić N., Radman S., Fabek Uher S., Benko B., Voća S., Šic Žlabur J. (2022a). Nettle Cultivation Practices—From Open Field to Modern Hydroponics: A Case Study of Specialized Metabolites. *Plants*. 11: 1-19.
- Opačić N., Šic Žlabur J., Sikirić L., Fabek Uher S., Benko B., Toth N., Radman S. (2022b). Morfološka svojstva koprive (*Urtica dioica* L.) u plutajućem hidroponu. In Book of Abstracts 57th Croatian and 17th International Symposium on Agriculture, Majić I., Antunović Z. (ed.), Fakultet agrobiotehničkih znanosti Osijek Sveučilišta J.J. Strossmayera u Osijeku, Osijek, pp.247-251.
- Ough C.S., and Amerine M.A. (1998). *Methods for Analysis of Musts and Wines*. Washington: J. Wiley & Sons.
- Petek M., Toth N., Pecina M., Karažija T., Lazarević B., Palčić I., Veres S., Čustić M. H. (2019). Beetroot mineral composition affected by mineral and organic fertilization. *PLoS One*. 14: e0221767.
- Radman S., Žutić I., Fabek S., Žlabur J.Š., Benko B., Toth N., Čoga L. (2015). Influence of nitrogen fertilization on chemical composition of cultivated nettle. *Emirates Journal of Food and Agriculture*. 27: 889–896.
- Radman S., Opačić N., Voća S., Petek M., Čoga L., Fabek Uher S., Benko B., Toth N., Šic Žlabur J. (2021). Održive poljoprivredne prakse u uzgoju koprive. In Book of Abstracts of 1st international conference Food & Climate Change, Šamec D., Šarkanj B., Petrić I. (ed.), Sveučilište Sjever, Koprivnica, Hrvatska, pp. 22.
- Radman S., Fabek Uher S., Opačić N., Žutić I., Benko B., Jurčić B., Šic Žlabur J. (2022). Primjena biostimulatora rasta u uzgoju koprive. *Glasnik zaštite bilja*. 45: 22-28.
- Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C.A. (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*. 26: 1231-1237.
- Roumeliotis C., Siomos A.S. Gerasopoulos D. (2021). Comparative nutritional and antioxidant compounds of organic and conventional vegetables during the main market availability period. *Nitrogen*. 2: 18-29.
- SAS®/STAT 14.3 (2017). SAS Institute Inc.: Cary, NC, USA.
- Zeipiņa S., Alsiņa I., Lepse L. (2014). Stinging nettle—the source of biologically active compounds as sustainable daily diet supplement. *Res. Rural Dev*. 20: 34-38.

The influence of the growing medium on the growth of *Capsicum annuum* L.

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Abstract

Pepper is a thermophilic culture that is very demanding to grow. It belongs to the group of fruitful vegetables, which for successful cultivation requires soil rich in nutrients and good water capacity. Considering the fact that the soils are more and more devastated and that due to unpredictable climate changes, growing in a protected area is increasingly being done. The goal of this research was to establish which growing medium gives the best results in terms of the development of pepper plants. The experiment was set up in 3 plots, each was consisted of 60 plants. During the research, following measurement were done; the length of the aerial part, the mass of the aerial part, the number of leaves, the number of nodes and the number of buds. Three growing mediums were used in the research; soil, ProLine Herb substrate and Substrate 5 PLUS Green fibre. As a result of the research, it was established that cultivation in the ProlineHerb substrate gave the best results according to all measured parameters.

Keywords: pepper, growing, substrate

Introduction

Pepper (*Capsicum annuum* L.) is originated from South America. Now is widely cultivated in almost all European countries. It appeared in Europe in the 15th century, and in our region it began to grow in the 16th century. In the world, largest quantities are produced in China, while the largest producer in Europe is Spain. In Croatia, pepper is produced mostly in the continental part (82 %) on only 4,500 ha, on which about 30,000 tons of fruit are produced (Parađiković, 2009). Pepper is a very demanding culture in terms of soil and growing conditions. Well drained moisture holding loamy soils are considered fit for optimal growth and production (Mali et al., 2019). Excessive use of soil with the use of chemical protection agents and mineral fertilizers led to poor soil quality. Abiotic stresses include salinity, drought and high temperature impair growth and yield (Lemoine et al., 2013). According to Khaitov et al. (2019) the soil in a greenhouse is prone to an impoverishment of organic carbon and other essential nutrients due to the high amount of crop removal and decomposition rate. Together with the high incidence of nematodes and weed pressure in the soil, this leads to considerable damage to the crops (Gilreath et al., 2005). Not well-developed root system in pepper reduces the ability to uptake maximum nutrients for normal growth. There are many factors having an impact on pepper yield and the most important constraints are associated with water or nutrient deficiency (Abayomi et al., 2012). Thus it is important to supply a sufficient amount of nutrients to the root zone to improve the nutrient use efficiency and crop yield (Khaitov et al., 2019). Year-round cultivation requires production in greenhouse conditions, which brings many problems. One of the biggest problem is soil devastation. To overcome these problems the implementation of soilless culture is mandatory (Rubio et al., 2011). Many types of substrates are currently available for the greenhouse industry, but many of them produce a significant amount of residues at the end of their useful life (Tzortzakis et al., 2008). Considering the guidelines for sustainable agriculture, it is important to choose substrates that are biodegradable and affordable. Our vegetable growers mainly use peat-based substrates. Most of them are produced on the north of the Europe where are the largest deposits of peat. The aim of this research was to find out which of these three growing mediums (soil, ProLine Herb substrate and Substrate 5 PLUS with clay+Green fibre) is the best for growing peppers.

Material and methods

Capsicum annuum L. Bibic F1 was used as the seed material. The Bibic F1 variety is a hybrid adapted for early greenhouse production. In the experiment three growing mediums were evaluated for their effects on sweet pepper growing; soil, ProLine Herb, Substrate 5 with clay + GreenFibre.

Growing mediums

Potgrond H 80

The substrate Potgrond H 80 consists of frozen black sphagnum peat and fine white sphagnum peat. The substrate is enriched with additional microelements, the pH of the substrate is 6.0. The structure of the substrate particles is up to 5 mm. (Klasmann) This substrate was used for seedlings production.

Soil

The soil used in this research belongs to powdery clay according to its mechanical composition. It was taken beside greenhouse of the Biotechnical department. The greenhouse is located at 45°09'57" N and 17°57'08" E and have an altitude of 87 m. The particle content determined after soil analysis (ISO, 2009) was as follows: 0.99 % coarse sand, 4.90 % fine sand, 18.01 % coarse powder, 26.28 % fine powder, 49.82 % clay. According to the chemical composition, the soil contained: humus 2.41 % (ISO, 1998), CaCO₃ 2.09 % (ISO, 1995), AL-P₂O₅ 8.36 mg/100g, AL-K₂O 20.11 mg/100g (Egner, 1960), pH KCl 6.41, pH H₂O 7.36. (ISO, 2005).

Substrate ProLine Herb

The substrate ProLine Herb is composed of white peat (up to 25 mm), black peat, GreenFibre and TerrAktiv high-quality green compost, which is biologically active, suppresses root diseases and enables a longer life of herbs in pots. TerrAktiv FT is added as organic fertilizer. The structure of the substrate particles is up to 25 mm. (Klasmann)

Substrate 5 with clay + GreenFibre

The basic substrate 5 with clay + GreenFibre is a substrate that can be used alone and can also be mixed with fertilizers. It is composed of medium GreenFibre, frozen through black peat, peat fibres and white peat (0-25 mm). pH value is 6.0. It is intended for plants grown in pots. (Klasmann)

Experimental place

Experiment was set up in the greenhouse of the Biotechnical Department, University of Slavonski Brod, between March and July of 2022. The greenhouse is located at 45°09'57" N and 17°57'08" E and have an altitude of 87 m. The climate of Brod-Posavina County is moderately warm and humid, with warm summers (Cfb according to Köppen) and moderately cold winters. Greenhouse indoor temperatures and relative humidity values during the experiment in 2022 were measured daily with H560 DewPoint Pro placed 1 m above the ground in the middle of the greenhouse. Outdoor temperature and relative humidity during the experiment were recorded by a meteorological station situated nearby the experimental field. The temperature ranged from 10°C in the morning to 32°C during the day. In this experiment the greenhouse was ventilated passively through side openings. The greenhouse dimensions are 30 m x 8 m with a height of 8 m. It is covered by low-density polyethylene (LDPE) film roof (200 µm thickness) with approximately 60% photosynthetically active radiation (PAR) transmittance. It has no heating or artificial light.

Experimental design

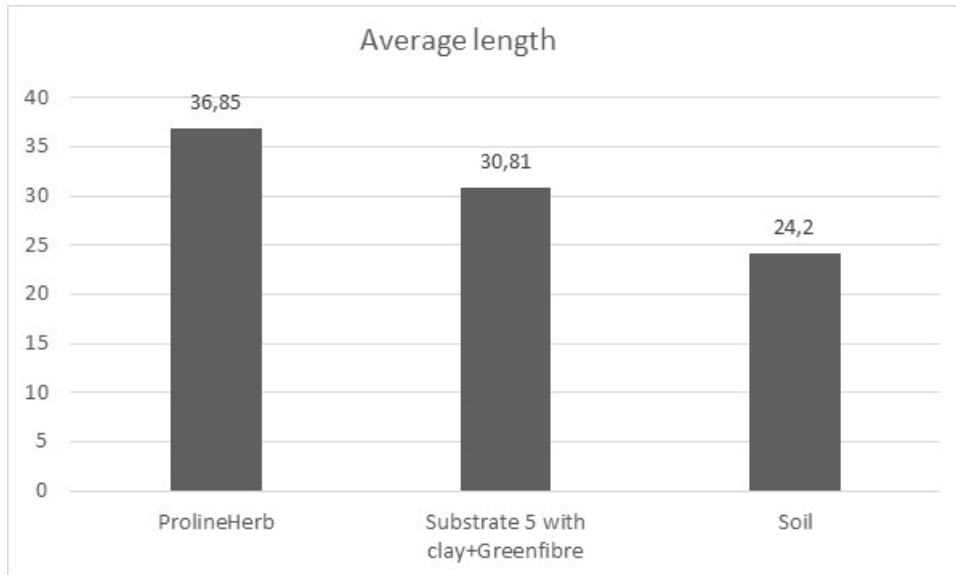
Seedlings were grown in 3 styrofoam container with 60 sowing places (Ø46,4 / 23,4 x 55 mm, 52 ml volume) filled with Potgrond H 80 substrate. After six weeks the seedlings grew to a satisfactory size and were transplanted into pots (Pöpellmann TEKU VCG 19). Sixty pots were filled with soil, sixty with substrate ProLine Herb and sixty with Substrate 5 with clay + GreenFibre.

Measurements

During the research, following measurement were done; the length of the aerial part, the mass of the aerial part, the length of the roots, the number of leaves, the number of nodes and the number of buds. At the end of growing 16 plants were randomly selected and measured. An electronic scale (PL3002, Mettler-Toledo International Inc., Greifensee, Switzerland) with a precision of 0.01 g was used to measure mass and a digital caliper (DIGI-MET 1226932-D, Germany) was used to measure length.

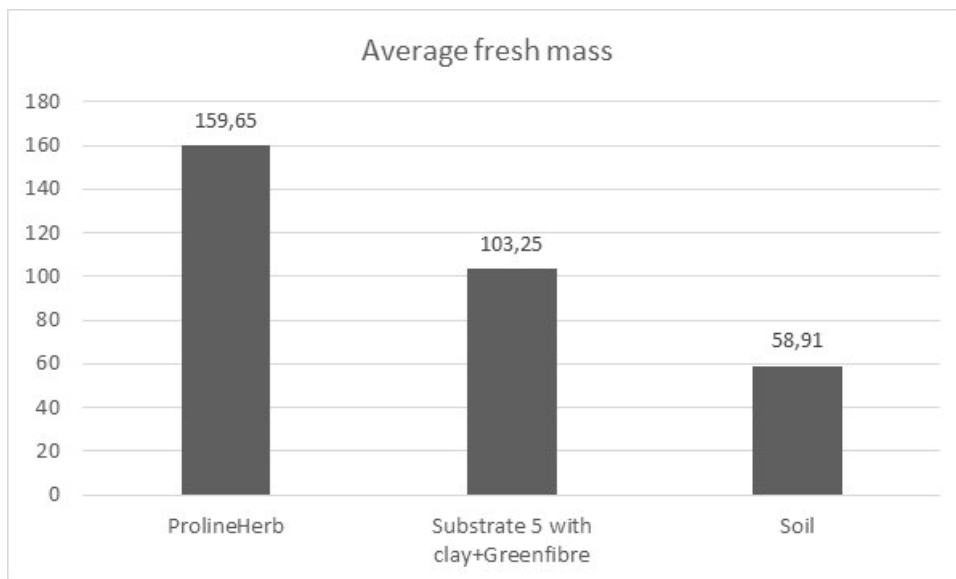
Results and discussion

According to the results of measuring the length of grown plants, it can be concluded that the best result was obtained with plants grown in ProLine Herb substrate and the worst result was achieved in soil cultivation (Graph 1). Similar results were obtained by Ikram et al. (2012) who found that physiological activity in successful growing produces new shoot and leaves. More number of shoot and leaves triggered the process of photosynthesis which resulted in accumulation of energy. Consequently to that simultaneously availability of moisture and nutrient through media resulted in higher length of plants..



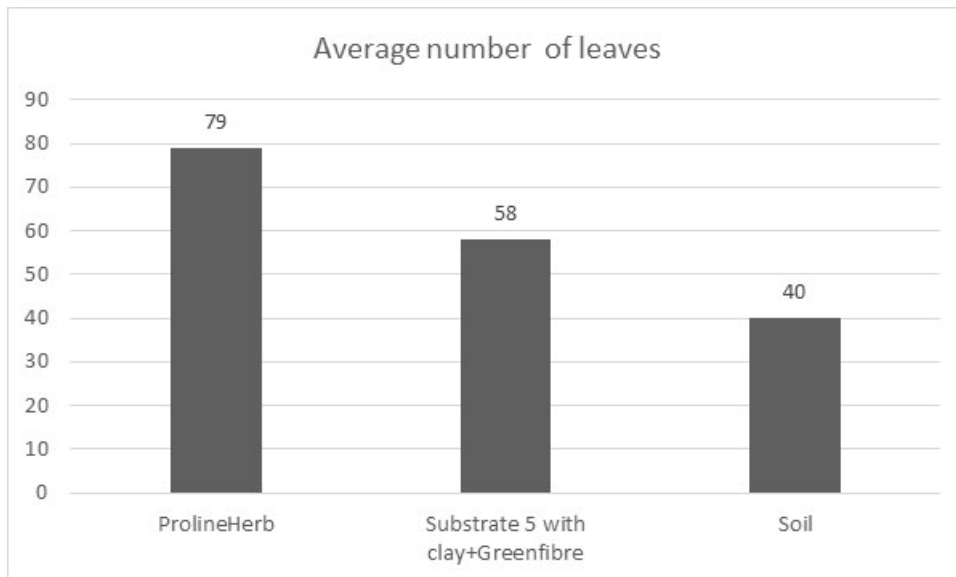
Graph 1. Average length of cultivated plants (cm)

The second measured parameter was the mass of fresh plants. According to the obtained measurement results, it was established that the highest average mass was achieved in plants grown in ProlineHerb substrate and worst result was obtained on plants grown in soil which can be seen in Graph 2.



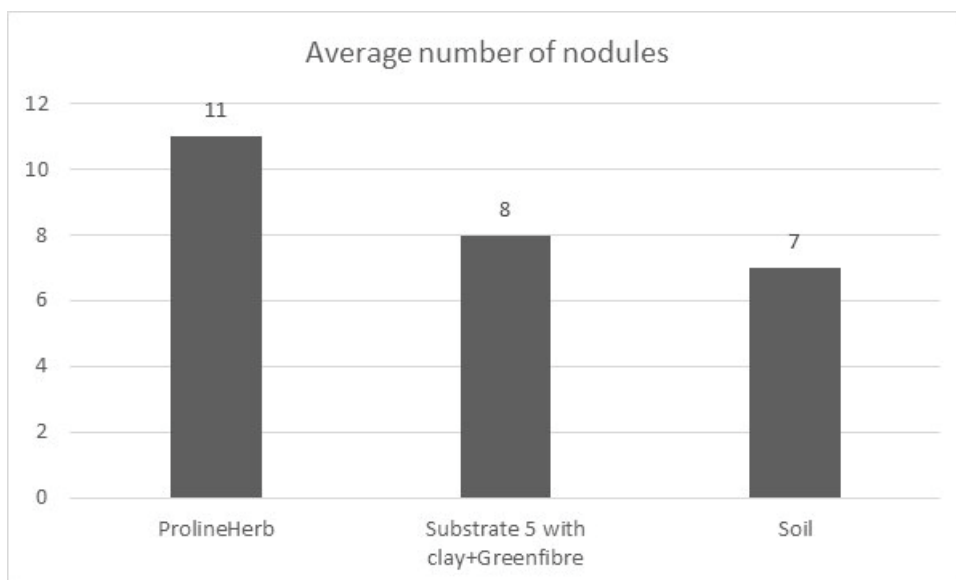
Graph 2. Average fresh mass per plant (g)

The third investigated parameter was the number of leaves. It was found that the highest average number of leaves was developed on plants grown on the ProlineHerb substrate, and the smallest on soil grown plants, which can be seen from Graph 3. Similar results were obtained by Pradnya et al. (2020) who said that physical and chemical activity in the media might be resulted in increase in rate of photosynthesis of the cuttings which triggered the maximum number of pepper leaves.



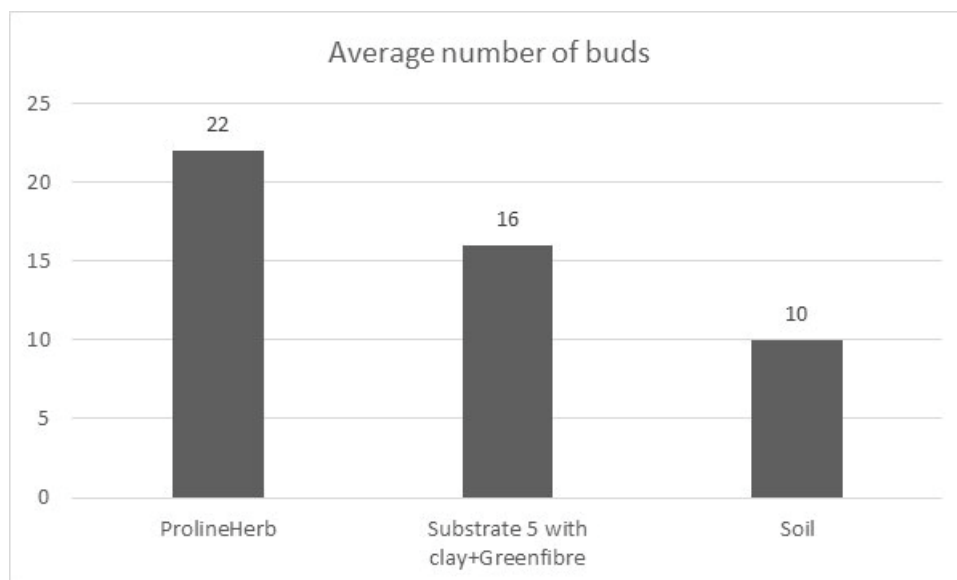
Graph 3. Average number of leaves

Furthermore, the number of nodes developed by the plants was examined. The highest number was found in plants grown in ProlineHerb substrate, and the smallest in soil, which can be seen in Graph 4.



Graph 4. Average number of nodules per plant

The last measured parameter was the number of buds. According to the obtained results, it was established that the highest number of buds developed on plants grown on the ProlineHerb substrate, and the smallest on plants grown in soil (Graph 5).



Graph 5. Average number of buds per plant

In open-field production, due to their shallow rooting system and short lifespan, nutrients released from soil fertility generally do not match the needs of vegetables (Liu et al., 2012; Tei et al., 2020). Large amount of chemical fertilizers is being applied to soil in order to increase a crop yield which have an enormous harmful impact to the environment and reduce fertilizer use efficiencies (Guignard et al., 2017). The root system is not well-developed in chilli pepper which reduces the ability to uptake maximum nutrients for normal growth. Thus, it is important to supply a sufficient amount of nutrients to the root zone to improve the nutrient use efficiency and crop yield (Khaitov et al., 2019). Many types of substrates are currently available for the greenhouse industry (Amor and Gomez-Lopez, 2019) but not all of them are good for all cultures and therefore before choosing a substrate, it should be checked the suitability for growing a certain crop. Nourizadeh et al. (2003) reported that the most high of cucumber, fresh weight of shoot plant, total yield, number of fruits at m² in every bush obtained from peat's substrate and the lowest yield was in the context of pure rice husk. Lee and Liao (2007) compared five substrates (chicken dung compost substrate, sugarcane residue, cattle dung compost, Taoki no.3 and hog dung compost) for growing peppers and found out that sugarcane gave the best results. Albaho et al. (2009) investigated the effect of three substrates on growing and yield of two green pepper cultivars reported that the pepper cultivars under study showed different responses to different cultural substrates. Thus, the physicochemical proprieties of the substrate could reduce plant growth, especially when the substrate has a low water-holding capacity and can alter root develop-ment under water stress conditions, reducing water potential in stems, leaves and fruit (Amor and Gomez, 2019). Good substrate must have excellent capacity characteristics for holding and releasing fertilizer than the others. The media with high organic matter content increases the water and nutrient holding capacity of the medium also high N content resulting in the vegetative growth of the plant (Joiner and Nell 1982). The result of this research was that the ProlineHerb substrate gave the best results in all measured parameters so it could be recommended for pepper growing.

Conclusions

It is a fact that year-round cultivation in greenhouses is necessary to make the facility profitable, but it is also a fact that the soil in such facilities is devastated and that it is necessary to find a quality substrate in which peppers can be grown successfully. It was found that according to all measured parameters, the best result was achieved when growing in ProlineHerb substrate.

References

Abayomi Y.A., Adolaju M.O., Egbewunmi M.A., Suleiman B.O. (2012). Effects of soil moisture contents and rates of NPK fertilizer application on growth and fruit yields of pepper (*Capsicum*

- spp) genotypes. International Journal of Agriculture Science. 2: 651-663.
- Albaho M., Bhat N., Abo H., Tomas B. (2009). Effect of three substrates on growth and yield of cultivars of *Capsicum annum* L. European Journal of Scientific Research. 28: 233-227.
- Amor F.M., and Gomez-Lopez M. (2009). Agronomical response and water use efficiency of sweet pepper plants grown in different greenhouse substrates. Horticultural Science 44: 810-814.
- Egner H., Riehm H., Domingo W.R. (1960). Untersuchungen über die chemische Bodenanalyse als Grundlage für die Beurteilung des Nährstoffzustandes der Boden II. Chemische Extraktionsmethoden zu Phosphor- und Kaliumbestimmung, Landbr. Hogsk. Annlr. W.R. 26: 199-215.
- Gilreath J.P., Santos B.M., Motis T.N., Noling J.W., Mirusso J.M. (2005). Methyl bromide alternatives for nematode and *Cyperus* control in bell pepper (*Capsicum annum*). Crop protection. 24: 903-908.
- Guignard M.S, Leitch A.R, Acquisti C., Eizaguirre C., Elser J.J., Hessen D.O., Jeyasingh P.D., Neiman M., Richardson A.E., Soltis P.S., Soltis D.E. (2017). Impacts of nitrogen and phosphorus: From genomes to natural ecosystems and agriculture. Frontiers in Ecology and Evolution. 5: 70-80.
- Ikram S., Habib U., Khalid N. (2012). Effect of different potting media combinations on growth and vase life of tuberose (*Polianthus tuberosa* L.). Pakistan Journal of Agricultural Sciences. 49: 121-125.
- ISO. (2009.). Soil quality. Determination of particle size distribution in mineral soil material Method by sieving and sedimentation. ISO 11277.
- ISO. (1998). Soil quality. Determination of organic carbon by sulfochromic oxidation. HRN ISO14235.
- ISO. (1995). Soil quality. Determination of carbonate content. Volumetric method. HRN ISO 10693.
- ISO. (2005). Soil quality. Determination of pH. International standard. ISO 10390.
- Joiner J.N., and Nell T.A. (1982). Fired montmorillonite clay as a propagation and hydroponic growing medium. Proceedings of the Florida State Hort. Society. 93: 214-215.
- Khaitov B., Umurzokov M., Cho K.M., Lee Y.J., Park K.W. (2019). Importance and production of chilli pepper; heat tolerance and efficient nutrient use under climate change conditions, Korean journal of agriculture science.
- Lee A.C., and Liao F.S. (2007). Effects of organic substrates, training system and plant density on the yield of sweet pepper (*Capsicum annum* L.) grown in basket culture under plastic house. Acta Horticulturae. 761: 533-538.
- Lemoine R., La Camera S., Dedaldecamp F., Allario T., Pourtau N., Bonnemain J.L., Laloi M., Coutos-Thevenot P., Maurousset L. (2013). Source to sink transport of sugar and regulation by environmental factors Frontiers in Plant Science. 4: 272.
- Liu Z., Ying H., Chen M., Bai J., Xue Y. Yin, Batchelor W.D., Yang Y., Bai Z., Du M. (2021). Optimization of China's maize and soy production can ensure feed sufficiency at lower nitrogen and carbon footprints. Nature Food. 2: 426-433.
- Mali S.S., Naik S.K., Jha B.K., Singh A.K., Bhatt B.P. (2019). Planting geometry and growth stage linked fertigation patterns: Impact on yield, nutrient uptake and water productivity of Chilli pepper in hot and subhumid climate. Scientia Horticulturae. 249: 289-298.
- Monirul I., Satyaranjan S., Hasanuzzaman A., Rahim A. (2011). Effect of spacing on the growth and yield of sweet pepper. Journal of Central European Agriculture. 12: 328-335.
- Nourizadeh M. (2003). The effect of different substrate cultivation on the growth, performance and quality of greenhouse cucumber in without soil cultivation system. M.Sc. Thesis, on Horticulture, Guilan University, Iran.
- Paradžiković N. (2009). Opće i specijalno povrćarstvo. Poljoprivredni fakultet u Osijeku, Osijek, Hrvatska, 224.

- Pradnya D.B., Kulkarni M.M., Khandekar R.G., Pawar C.D., Gokhale N.B. (2020). Effect of Different Potting Media on Sprouting, Survival and Growth Performance of Bush Pepper (*Piper nigrum* L.). International Journal of Current Microbiology and Applied Sciences. 11: 606-614.
- Rubio J.S., Pereira W.E., Garcia-Sanchez F., Murillo L., Garcia A.L., Martinez V. (2011). Sweet pepper production in substrate in response to salinity, nutrient solution management and training system. Horticultura Brasileira. 29: 275-281.
- Tei F., De Neve S., Haan J., Kristensen H.L. (2020). Nitrogen management of vegetable crops. Agricultural Water Management. 240: 106316.
- Tzortakis N.G., Economakis C.D. (2008). Impacts of the substrate medium on tomato yield and fruit quality in soilless cultivation Horticultural Science. 35: 83-89.

Effects of different soil amendments on Cd content in garlic

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Abstract

Cadmium (Cd) is considered as one of the most ecotoxic metals with harmful effects on humans, animals and plants, but its bioavailability may be reduced with different agrotechnical measures. Researchers are mainly focused on reducing Cd uptake by plants in soil contaminated with Cd. The aim of this research was to investigate the effect of repeated addition of different soil amendments (lime and lime + zeolite) on Cd uptake by different garlic varieties grown on uncontaminated field where excessive content of Cd in carrot was determined in previous years. Among the tested varieties (Garpek, Messidor, Gardos), regardless the soil amendment, Cd content was the highest in the variety Garpek, while the variety Messidor showed the lowest tendency to accumulate Cd. The Cd content in garlic was also significantly reduced after the second application of studied soil amendments, but the content of Cd in garlic cloves was reduced below the limit of European Commission Regulation 2023/915 only with variety Messidor after the second application of lime and/or lime + zeolite.

Keywords: cadmium, *Allium sativum*, zeolite, lime, variety

Introduction

European Commission Regulation 2023/915 (OJ EU L 119/103, 2023) sets maximum levels for certain contaminants in food, including Cd. The food monitoring in primary production in Slovenia have shown that elevated Cd levels in vegetable crops occur even where limits for soil Cd content are not exceeded (Žerjal et al., 2018). This happens because Cd is not an essential element for plants, but it is highly available and some plants have a greater affinity to adsorb Cd (Kabata-Pendias and Pendias, 2001; Kabata-Pendias and Mukherjee, 2007). Cd is considered to be one of the most ecotoxic metals, with harmful effects on humans, animals and plants. Globally on average, there is between 0.06 and 1.1 mg kg⁻¹ Cd in soil and it is an easily accessible element (Kabata-Pendias and Mukherjee, 2007). The presence of Cd in soil varies due to its different distribution in the earth's crust. Cd content in soil in Slovenia is higher than in other European countries due to parent material. According to the Geological Survey of Slovenia (n=817) the median Cd content of the surface soil layer (0-10 cm) is 0.47 mg kg⁻¹, so even a small increase of Cd (> 1 mg kg⁻¹) in soil can pose a potential risk for Cd uptake into plants (Gosar et al., 2019). The main anthropogenic sources of Cd in soil are industry, atmospheric deposition and phosphate fertilisers (Kabata-Pendias and Mukherjee, 2007). Therefore EU REGULATION 2019/1009 sets a limit for phosphate fertilisers at 60 mg Cd kg⁻¹ P₂O₅ (OJ EU L 170, 2019). Most of Cd contamination remains in the top 15 cm of soil (Kabata-Pendias and Mukherjee, 2007). The limit value for Cd in soil is set at 1 mg kg⁻¹ dry soil, the alert value at 2 mg kg⁻¹ dry soil and the critical value at 12 mg kg⁻¹ dry soil (UL RS, št. 68/96). Cd is most mobile in acid soils and is relatively immobile in alkaline soils at pH higher than 5 (Lo et al., 1992; Kabata-Pendias and Pendias, 2001). In-situ immobilization of Cd by amendments is one of the most widely adopted methods to remedy soil contamination. The following agronomic practices may be used to reduce Cd content in vegetable crops: elevating soil pH, elevating soil organic matter content, improving cation exchange capacity (CEC) of the soil by addition of Si and clay minerals rich materials because soils with a higher CEC can bind more Cd which reduces its availability (Lo et al., 1992). Effect of amendments on Cd concentration in plants also depends on soil type and amount of amendment, so it is necessary to select amendment according to the soil type (Keller et al., 2005). Plant species have different affinities for Cd uptake (Kabata-Pendias and Pendias, 2001; Aleksander et al., 2006; Fang et al., 2019). Vegetables from the *Brassicaceae*, *Asteraceae*, *Apiaceae* and *Convolvulaceae* families have higher Cd uptake, while vegetables from the *Liliaceae* and *Amaranthaceae* families have lower Cd

uptake (Fang et al., 2019). Leafy vegetables and vegetables such as turnips, carrots, potatoes are the main dietary sources of Cd (Kabata-Pendias and Pendias, 2001). Plant varieties also have different affinities for Cd uptake, e.g. Aleksander et al. (2006) reported the differences between the varieties of carrot and pea and Zhu et al. (2007) the differences between the asparagus bean varieties.

Most research is conducted on Cd contaminated soils in pot experiments, while there is a lack of experience in field management of soils with naturally higher but not excessive Cd concentrations. To study the possibilities for reducing the Cd content in vegetable crops produced in Slovenia, a long term experiment was set up to monitor the effect of repeated application of lime and zeolite on selected soil parameters and Cd content in the yield of garlic. In this paper we report the results of the first two years of the experiment.

Material and methods

In the field in Gradišče near Murska Sobota, Slovenia where the Cd content in carrots exceeded the maximum acceptable level in the past, a long-term experiment was set up in autumn 2021. The soil characteristics of the experimental field before the setup of the experiment are shown in Table 1. Soil is moderately acid (pH 5.4), with low organic matter (1.6 %) and low CEC (10.87 mmol 100 g⁻¹). The garlic is used as a test crop in this experiment.

The experiment is designed as a split-plot in which the main factor, alongside the control (C) where only mineral fertilisers containing N, K and P are applied in accordance with the recommendations for garlic, is represented by different soil amendments, which are applied annually: liming (L) with Calcevia Agro (Intercal) at 3 t ha⁻¹ and liming with Calcevia Agro at 3 t ha⁻¹ + zeolite flour (Montana) at 1 t ha⁻¹ (LZ). The subfactor in the experiment is represented by 3 garlic varieties: Messidor (Agri Obtentions), Garpek/Plavigar (Planasa) in Gardos (Planasa). The experiment is conducted in a completely randomized design with 4 replications. The subplot size is 0.8 m² with 40 plants per subplot.

The previous crop for 2021/22 experiment was buckwheat and for 2022/23 experiment rye. Before planting (autumn 2021 and 2022), soil samples were taken from each of the main factor plots at depths of 0-15 cm and 15-30 cm to determine pH, organic matter, available and total Cd. The whole experimental plot was fertilised with 120 kg K₂O ha⁻¹ (potassium chloride fertilizer) in autumn 2021 and with 42 kg N ha⁻¹, 21 kg P₂O₅ ha⁻¹, 51 kg K₂O ha⁻¹, 6 kg MgO ha⁻¹ and 66 kg SO₃ ha⁻¹ (complex NPK fertilizer with ammonia stabilizer DMPP) in autumn 2022. The soil amendments were then applied according to the experimental design and added into the soil mechanically. Garlic planting material was obtained from the supplies in Slovenia and prepared for planting. Cloves were planted manually around 10 days after the application of lime and zeolite. Additional N fertilizer was applied in spring, 80 kg N ha⁻¹, 32 kg P₂O₅ ha⁻¹, 40 kg K₂O ha⁻¹, 8 kg MgO ha⁻¹ and 12 kg SO₃ ha⁻¹ (complex NPK fertilizer with ammonia stabilizer DMPP) in March 2022 and 27 kg N ha (ammonium nitrate fertilizer) in March 2023. During the growing season the crop was hoed and weeded manually.

The garlic was harvested in July, according to the maturation of each variety. The yield was left to dry for about 1 month. The bulbs of each plot were then sorted into marketable and waste and weighed. 10 marketable bulbs from each plot were selected and 2 cloves from each bulb (20 cloves per sample) peeled and frozen until the analysis. For the determination of Cd fresh homogenised garlic cloves (weight 0.250 g) were digested using a high-pressure microwave oven (Milestone ETHOS 1600) with 5 ml 65% nitric acid (HNO₃, SUPRAPUR, Merck) and 1 ml 30 % hydrogen peroxide (H₂O₂, SUPRAPUR, Merck). The analysis of Cd in digested samples was performed by ICP-MS (Agilent 7900) using helium (He) collision mode to eliminate the possible interferences.

The collected data were, for each of the two years separately, statistically evaluated with R Commander (R version 4.2.1) using statistical model for split-plot design ANOVA and Tukey's multiple comparison test. All data points represent means ± standard deviations (SD) of four replicates for each treatment. Differences at p ≤ 0.05 were considered as statistically significant.

Table 1. The soil characteristics of the experimental field before the setup of the experiment*

Texture	pH (in KCl)	Organic matter (%)	CEC (mmol 100g ⁻¹)	Cd total (mg kg ⁻¹)	Cd available (mg kg ⁻¹)	P available (mg P ₂ O ₅ 100 g ⁻¹)	K available (mg K ₂ O 100 g ⁻¹)
SL	5.4	1.6	10.87	0.294	0.013	34.5	19.0

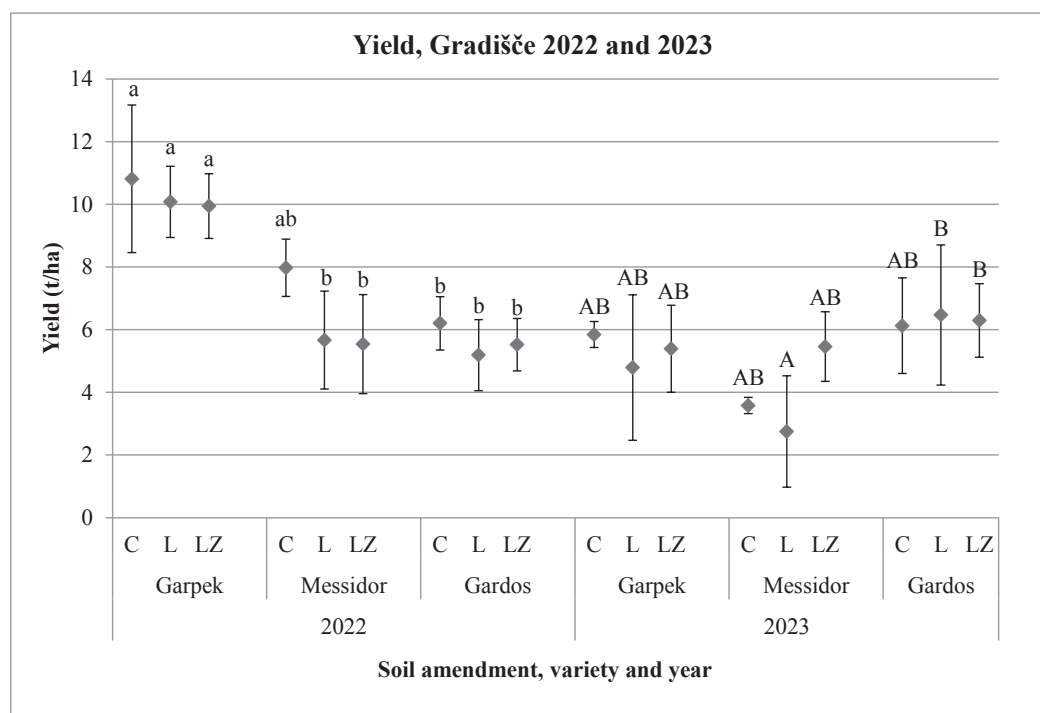
* The values refer to the soil layer 0-30 cm; the sample taken in October 2021.

Results and discussion

Graph 1 shows the yield in 2022 and 2023. In 2022, statistically significant the highest yields, regardless of soil amendment, were recorded for the variety Garpek, from 9,9 to 10,8 t ha⁻¹ of marketable bulbs. The yields of Messidor and Gardos varieties were between 5 and 8 t of marketable bulbs per ha. For soil amendments, marketable yields were the highest in the control plots, while there were no differences in yields between the plots treated with lime or lime + zeolite. The interaction soil amendment×variety was not statistically significant.

After the second application of soil amendments, the yields in 2023 were, regardless of soil amendment, statistically significant the highest for the variety Gardos, from 6,1 to 6,5 t ha⁻¹ of marketable bulbs. The yields of Garpek were between 4 and 6 t of marketable bulbs per ha. The yields of Messidor were between 2 and 6 t of marketable bulbs per ha. There were no statistical differences between the marketable yields at different soil amendments, t the interaction soil amendment×variety, as well, was not significant.

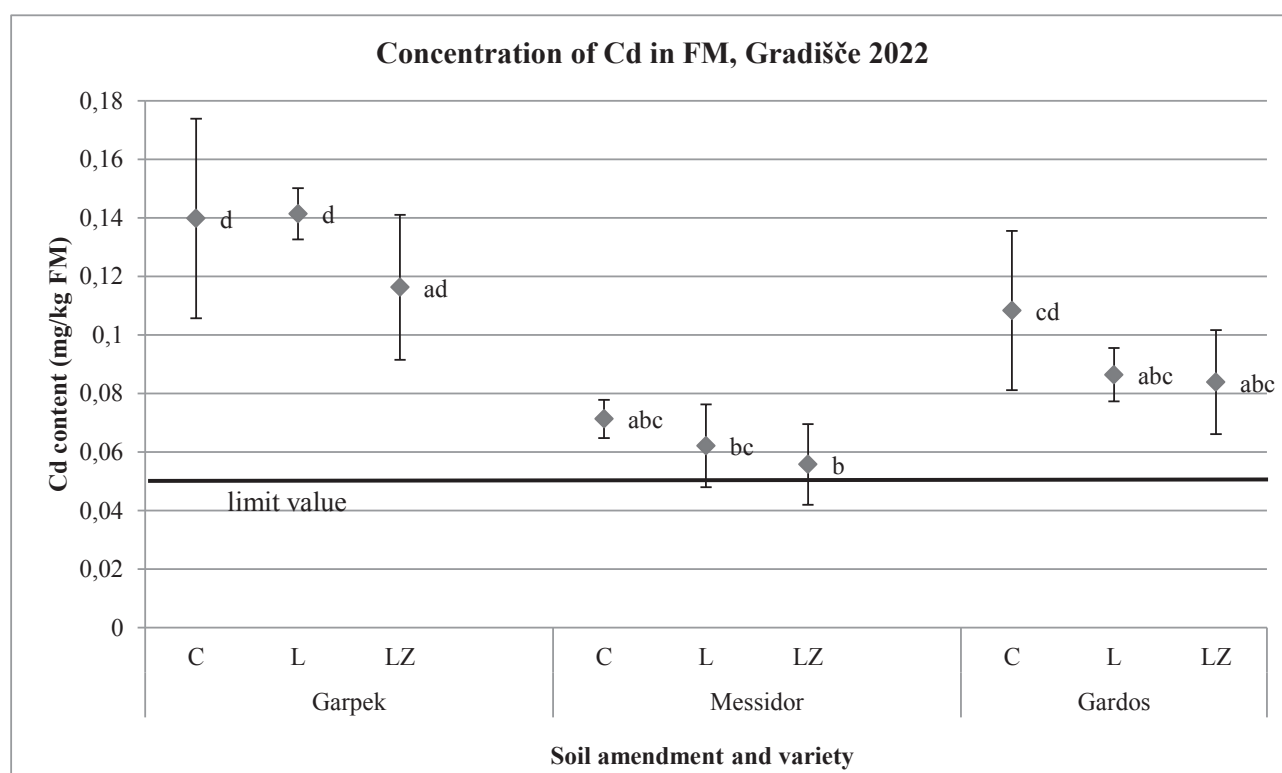
Zhang et al. (2023) reported that amendment of lime, biochar, by-products, manure, straw and combinations of them significantly increased soil pH and increased yield respectively, but it varied among crop types (rice, maize, wheat, fruits, vegetables, oil crops, tubers, tobacco, tea, cotton, grasses and others). From examining 175 studies on the effects of liming rate, lime application method, and liming material type on various soil chemical properties and crop yield Li et al. (2019) concluded that most important variables that drive changes in soil pH and crop yield are liming rate and crop species, respectively. Soil conditions, such as initial soil organic matter and soil pH, are more important for increasing soil pH in field-based experiments, while lime material type and application method are more important for improving crop yield.



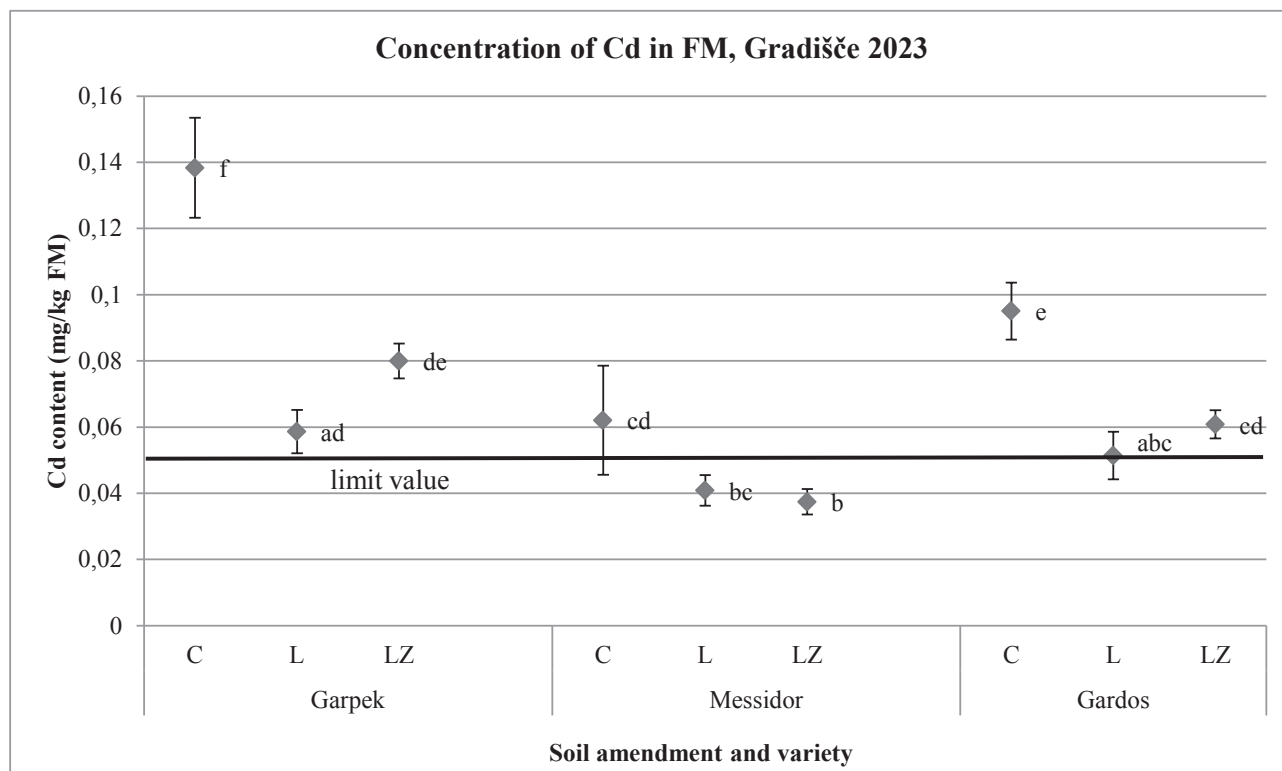
Graph 1. Average marketable yield in 2022 and 2023 with standard deviations. Different letters above the columns indicate significant differences ($p \leq 0.05$) between the marketable yields of different treatments (combination of soil amendment and variety) within each year (2022 - small letters and 2023 - capital letters).

The analysis of the Cd content (Graph 2) in the cloves showed that the limit value for Cd in fresh matter, set at 0.05 mg Cd kg⁻¹ fresh matter for garlic (OJ EU L 119/103, 2023), was exceeded in all the samples in year 2022. The differences between the varieties are statistically significant – Cd content is the highest for variety Garpek and the lowest for variety Messidor. The differences in the content of Cd in garlic cloves between different soil amendments were not significantly significant although it may be noticed that it is slightly lower at lime + zeolite amendment for all 3 varieties.

The analysis of the Cd content in the cloves of 2023 yield (Graph 3) showed that the limit value for Cd in garlic fresh matter was exceeded in all the samples for varieties Garpek and Gardos. For Messidor, amendments of lime and lime + zeolite reduced the Cd content below the limit value. The Cd content was significantly influenced by the soil amendment, variety and the interaction soil amendment×variety. There are significant differences between different treatments. In this year too, the Cd content was the highest for the control plots of variety Garpek and the lowest for the plots of variety Messidor amended with lime + zeolite. The Cd content in cloves of control plots was in the range of those from previous year while it was lower than the year before for the plots where lime and lime + zeolite were applied. Two subsequent applications with each of the amendments obviously influenced the Cd bioavailability more than a single application.



Graph 2. Average Cd content in 2022 yield with standard deviations. Different letters at the columns refer to significant differences ($p \leq 0.05$) between the Cd content of different treatments (combination of soil amendment and variety).



Graph 3. Average Cd content in 2023 yield with standard deviations. Different letters at the columns refer to significant differences ($p \leq 0.05$) between the Cd content of different treatments (combination of soil amendment and variety).

It may be noticed (Graph 2 and 3) that different garlic varieties differently accumulate Cd in the cloves. Regardless the soil amendment, in both years, the Cd content was by far the highest in the variety Garpek, while the variety Messidor showed the lowest tendency to accumulate Cd among the varieties tested. Rijavec (2020) also found a higher cadmium content in garlic in the variety Garpek compared to the variety Gardos. Aleksander et al. (2006) reported significant differences in Cd accumulation between varieties of carrot and pea. Differences in Cd content between asparagus bean cultivars with different seed coats (black, red and spotted), suggests that Cd accumulation is genetic-dependent (Zhu et al., 2007). Differences in cadmium accumulation have also been detected among other varieties of legumes, cereals, root and tuber crops.

Compared to the control, Cd concentration in garlic was also reduced by adding lime and zeolite (Graph 2 and 3) in both years, but the differences between the soil amendments were significant only in the second year of the experiment, i.e. after the second application of lime and/or lime + zeolite, what is in accordance with the expectations since lime and zeolite are known to elevate soil pH, and soil pH has significant role in plant uptake of Cd (Chang et al., 1982; Wang et al., 2014; Belimov et al., 2015; Lu et al., 2017). The effect of zeolite on reducing the Cd uptake was also reported by Ibrahim et al. (2023) for cowpea, they also observed that zeolite application significantly increased soil pH and EC.

Table 2. Statistical analyzes of yield and Cd content

	n	mean	sd	p	LSD
Yield 2022:					
Soil amendment				0.31	0.06
C	12	0.97	0.26		
L	12	0.81	0.29		
LZ	12	0.83	0.23		
Variety				0.00	0.12
Gardos	12	0.63	0.10		
Garpek	12	1.16	0.12		
Messidor	12	0.82	0.20		
Soil amendment×variety				0.27	ns
Yield 2023:					
Soil amendment				0.32	0.14
C	12	0.61	0.16		
L	12	0.57	0.25		
LZ	12	0.69	0.15		
Variety				0.00	0.12
Gardos	12	0.77	0.15		
Garpek	12	0.59	0.17		
Messidor	12	0.51	0.18		
Soil amendment×variety				0.09	ns
Cd 2022:					
Soil amendment				0.34	0.02
C	12	0.11	0.04		
L	12	0.10	0.04		
LZ	12	0.09	0.03		
Variety				0.00	0.02
Gardos	12	0.09	0.02		
Garpek	12	0.13	0.03		
Messidor	12	0.06	0.01		
Soil amendment×variety				0.71	ns
Cd 2023:					
Soil amendment				0.00	0.01
C	12	0.10	0.04		
L	12	0.05	0.01		
LZ	12	0.06	0.02		
Variety				0.00	0.01
Gardos	12	0.07	0.02		
Garpek	12	0.09	0.04		
Messidor	12	0.05	0.02		
Soil amendment×variety				0.00	*

Conclusions

This research investigated the effect of different soil amendments on Cd uptake by different garlic varieties grown in uncontaminated soil. Research showed that garlic varieties have different tendency to accumulate Cd. The significant effect of application of lime and/or lime + zeolite on reduction of Cd content in garlic cloves was achieved after the second application of both amendments. Anyhow the reduction of the content of Cd in garlic cloves below the limit of European Commission Regulation 2023/915 after two years was only observed with variety Messidor at both lime and/or lime + zeolite applications.

Acknowledgement

The authors thank to the farmer Drago Serec who enabled the setup of the experiment on his field and helped with needed machinery operation and Breda Vičar from Agricultural Forestry Institute Murska Sobota at the Agricultural Forestry Chamber of Slovenia, Mojca Škof from the Agricultural Institute of Slovenia and Suzana Poplašen for their help with organisation and maintenance of the experiment. The authors acknowledge the financial support from the Slovenian Research Agency, the research core funding No. P4-0431 (Next Generation Agriculture) and the Slovenian Ministry for Agriculture, forestry and food, the founding of public service in vegetables and herbs (grant no. 2330-22-000006 and 2330-23-000079).

References

- Alexander P.D., Alloway B.J., Dourado A.M. (2006). Genotypic variations in the accumulation of Cd, Cu, Pb and Zn exhibited by six commonly grown vegetables. *Environmental Pollution*. 144: 736-745.
- Belimov A.A., Puhalsky I.V., Safronova V.I., Shaposhnikov A.I., Vishnyakova M.A., Semenova E.V., Zinovkina N.Y., Makarova N.M., Wenzel W., Tikhonovich I. A. (2015). Role of Plant Genotype and Soil Conditions in Symbiotic Plant-Microbe Interactions for Adaptation of Plants to Cadmium-Polluted Soils. *Water Air Soil Pollution*. 226: 1-15.
- Chang A.C., Page A.L., Foster K.W., Jones T.E. (1982). A Comparison of Cadmium and Zinc Accumulation by Four Cultivars of Barley Grown in Sludge-Amended Soils. *Journal of Environmental Quality*. 11: 409-412.
- EC (2023). Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006. *OJ EU L 119/103*, 2023.
- EC (2019). Regulation (EU) 2019/1009 of the European Parliament and of the Council of 5 June 2019 laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009 and repealing Regulation (EC) No 2003/2003. *OJ EU L 170*, 2019.
- Fang H., Li W., Tu S., Ding Y., Wang R., Rensing C., Li Y., Feng R. (2019). Differences in cadmium absorption by 71 leaf vegetable varieties from different families and genera and their health risk assessment. *Ecotoxicology and Environmental Safety*. 184: 109593.
- Gosar M., Šajn R., Bavec Š., Gaberšek M., Pezdir V., Miler M. (2019). Geochemical background and threshold for 47 chemical elements in Slovenian topsoil. *Geologija*. 62: 7–59.
- Ibrahim E.A., El-Sherbini M.A.A., Selim E.M. (2023). Effects of biochar, zeolite and mycorrhiza inoculation on soil properties, heavy metal availability and cowpea growth in a multi-contaminated soil. *Scientific Reports*. 13: 1-10.
- Kabata-Pendias A., and Mukherjee A.B. (2007). *Trace Elements from Soil to Human*. Berlin, Heidelberg, Germany: Springer-Verlag.
- Kabata-Pendias A., and Pendias H. (2001). *Trace Elements in Soils and Plants*. 3rd ed. Boca Raton, USA: CRC Press LLC.
- Keller C., Marchetti M., Rossi L., Lugon-Moulin N. (2005). Reduction of cadmium availability to tobacco (*Nicotiana tabacum*) plants using soil amendments in low cadmium-contaminated

- agricultural soils: a pot experiment. *Plant and Soil*. 276: 69-84.
- Li Y., Cui S., Chang S.X., Zhang Q. (2019). Liming effects on soil pH and crop yield depend on lime material type, application method and rate, and crop species: a global meta-analysis. *Journal of Soils and Sediments*. 19:1393–1406.
- Lo K.S.L., Yang W.F., Lin Y.C. (1992). Effects of organic matter on the specific adsorption of heavy metals by soil. *Toxicological & Environmental Chemistry*. 34: 139-153.
- Lu J., Yang X., Meng X., Wang G., Lin Y., Wang Y., Zhao F. (2017). Predicting Cadmium Safety Thresholds in Soils Based on Cadmium Uptake by Chinese Cabbage. *Pedosphere*. 27: 475-481.
- Rijavec Ž. (2020). Vsebnost kadmija v različnih delih dveh sort česna (*Allium sativum* L.). Diplomsko delo, Univerza v Ljubljani. Available from: <https://repozitorij.uni-lj.si/IzpisGradiva.php?lang=slv&id=114463>.
- Uredba o mejnih, opozorilnih in kritičnih imisijskih vrednostih nevarnih snovi v tleh (Uradni list RS, št. 68/96, 41/04 – ZVO-1 in 44/22 – ZVO-2). Priloga 1: Meje in kritične imisijske vrednosti snovi v tleh. (1996). Št. 68 – 29. XI: 5774.
- Wang L., Xu Y., Sun Y., Liang X., Lin D. (2014). Identification of pakchoi cultivars with low cadmium accumulation and soil factors that affect their cadmium uptake and translocation. *Frontiers of Environmental Science & Engineering*. 8: 877–887.
- Zhang S., Zhu Q., de Vries W., Ros G. H., Chen X., Muneer M. A., Zhang F., Wu L. (2023). Effects of soil amendments on soil acidity and crop yields in acidic soils: A world-wide meta-analysis. *Journal of Environmental Management*. 345: 118531.
- Zhu Y., Yu H., Wang J., Fang W., Yuan J., Yang Z. (2007). Heavy metal accumulations of 24 asparagus bean cultivars grown in soil contaminated with Cd alone and with multiple metals (Cd, Pb, and Zn). *Journal of Agricultural and Food Chemistry*. 55: 1045-52.
- Žerjal E., Ivanoš J., Drame P. (2018). Obremenitev tal s kadmijem in drugimi težkimi kovinami. NLZOH Maribor. Available from: http://www.kgzs-ms.si/wp-content/uploads/2018/04/Cdindrugekovinevtleh_MSobota15022018.pdf.

Mogućnosti uzgoja matovilca u plutajućem hidroponu u proljetno-ljetnom razdoblju

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Sažetak

Sa svrhom produžene opskrbe tržišta matovilcem, istraživane su mogućnosti uzgoja u plutajućem hidroponu u proljetno-ljetnom razdoblju s ciljem utvrđivanja dinamike rasta i prinosa s obzirom na temperaturu hranive otopine i zasjenjivanje energetsom zavjesom. Veće vrijednosti relativne stope rasta morfoloških pokazatelja (visina i promjer rozete, masa biljke, rozete i korijena) u drugom tjednu nakon nicanja u odnosu na treći, ukazuju na brži i intenzivniji rast mlađih biljaka. Najveće vrijednosti većine promatranih morfoloških pokazatelja i prinosa ($1,112 \text{ kg m}^{-2}$) matovilca sorte 'Accent' ostvarene su pri uzgoju u hlađenoj hranivoj otopini prosječne temperature $25,2 \text{ }^{\circ}\text{C}$ uz prekrivanje energetsom zavjesama.

Ključne riječi: *Valerianella locusta*, temperatura hranive otopine, energetska zavjesa, dinamika rasta, prinos

Uvod

Matovilac (*Valerianella locusta*) je biljka blage klime, otporna na niske temperature zraka, pa se u Hrvatskoj tradicionalno uzgaja u razdoblju jesen-rano proljeće na otvorenim površinama i u negrijanim zaštićenim prostorima. Prema Lešić i sur. (2016) matovilac podnosi golomrazicu i do $-15 \text{ }^{\circ}\text{C}$, a klijanje i rast započinju pri temperaturi oko 1 , odnosno $5 \text{ }^{\circ}\text{C}$. Optimalna temperatura za rast je umjerena, između 10 i $18 \text{ }^{\circ}\text{C}$ (Osvald i Kogoj-Osvald, 2005; Lešić i sur., 2016). Veća hranidbena vrijednost od salate i atraktivnost tamnozelenih listova specifičnog okusa i umirujućeg djelovanja zbog sadržaja valerijanske kiseline, razlozi su da tržište, posebice restoranski sektor, traži cjelogodišnju opskrbu matovilcem. Međutim, proljetno-ljetni uzgoj matovilca je problematičan jer viša temperatura zraka i dugi dan imaju negativan učinak na prinos i kvalitetu.

Temperatura medija u kojem raste korijen može imati izravan utjecaj na njegov rast i usvajanje hraniva te na fiziološke procese, rast i kvalitetu biljke (Landhäuser i sur., 2001; Zhang i Dang, 2007). U hidroponskom uzgoju matovilca temperatura hranive otopine (HO) tijekom proljeća i ljeta može znatno odstupati od potrebne za optimalan rast biljaka, a temperatura HO viša od temperature zraka može izazvati stres u biljkama matovilca, s negativnim posljedicama na kvalitetu, zdravstvenu ispravnost i prinos (Cortella i sur., 2014). Pretpostavlja se da se u hidroponskom uzgoju s mogućnošću regulacije temperature HO može riješiti ovaj problem, odnosno osigurati uzgoj matovilca zadovoljavajućeg prinosa i kvalitete i tijekom razdoblja s temperaturom zraka višom od optimalnih vrijednosti za rast.

Dalla Costa i sur. (2011) istraživali su utjecaj temperature HO (15 , 20 i $25 \text{ }^{\circ}\text{C}$) na kvalitetu i prinos matovilca sorte 'Gala' u plutajućem hidroponu uz stalnu temperaturu zraka od $20 \text{ }^{\circ}\text{C}$. U masi korijena nije zabilježena velika razlika između biljaka uzgajanih u HO temperature 15 i $20 \text{ }^{\circ}\text{C}$, dok je temperatura HO od $25 \text{ }^{\circ}\text{C}$ značajno ograničila rast korijena. Najveći prinos matovilca ($1,560 \text{ kg m}^{-2}$) ostvaren je pri temperaturi HO od $20 \text{ }^{\circ}\text{C}$, pri $15 \text{ }^{\circ}\text{C}$ bio je značajno manji ($1,264 \text{ kg m}^{-2}$), dok je najmanji prinos ($0,897 \text{ kg m}^{-2}$) bio u najtoplijoj HO ($25 \text{ }^{\circ}\text{C}$). Biljke uzgajane u HO temperature $20 \text{ }^{\circ}\text{C}$ imale su značajno veći broj listova ($7,7$) od biljaka uzgajanih u HO temperature 15 i $25 \text{ }^{\circ}\text{C}$ ($6,6$ i $5,9$).

Visoki prinos matovilca $2,149 \text{ kg m}^{-2}$ u jesensko-zimskom roku, $1,815$ i $1,993 \text{ kg m}^{-2}$ u ljetno-jesenskom i proljetnom koji navode Fabek i sur. (2011), ukazuje na mogućnost uzgoja matovilca u plutajućem hidroponu pri različitim

temperaturnim uvjetima. Najkraće vegetacijsko razdoblje matovilca bilo je u proljetnom periodu (31 do 44 dana od sjetve do berbe) pri različitoj prosječnoj temperaturi zraka i HO 23,4 i 18,8 °C. Nešto duže vegetacijsko razdoblje 37 do 45 dana bilo je u ljetno-jesenskom razdoblju pri ujednačenijoj prosječnoj temperaturi zraka i HO 19,8 i 20,3 °C, a najdulje (80 dana) u jesensko-zimskom razdoblju pri jednakoj prosječnoj temperaturi zraka i HO 12,7 i 12,6 °C. Slične rezultate navode Toth i sur. (2012) u istraživanju uzgoja rige u plutajućem hidroponu. Najbrži rast rige (od sjetve do 1. berbe 22 dana, odnosno do 2. berbe 36 dana) s prinosom 2,60 kg m⁻² postignut je u ljetnom razdoblju pri različitoj temperaturi zraka i HO 28,1 i 25,1 °C.

Zasjenjivanje usjeva tijekom toplog i sunčanog vremena različitim tipovima mreža ili energetske zavjese ima sve češću primjenu u uzgoju povrća. Zasjenjivanjem se poboljšava mikroklima usjeva smanjenjem temperature lišća i brzine transpiracije, čime se ublažava toplinski stres (Aberkani i sur., 2008). Sanford (2019) navodi da polupropusne energetske zavjese imaju visoku refleksiju i otvorenu strukturu za ventilaciju, pa su idealne za zasjenjivanje biljaka i hlađenje zraka u plasteniku u vrućim klimatskim uvjetima. Tijekom ljetnih mjeseci s visokom razinom sunčeve svjetlosti (oko 1250 μmol m⁻²s⁻¹), u plastenik s dvostrukim PE-filmom ulazi 80 % sunčeve svjetlosti (oko 1000 μmol m⁻²s⁻¹) pa uz primjenu polupropusnih zavjesa (55 % propusnosti za svjetlost i 45 % zasjenjivanje) do biljaka dopire oko 550 μmol m⁻²s⁻¹, što je u potrebnom rasponu sunčeve svjetlosti (500 do 635 μmol m⁻²s⁻¹) za rast i razvoj biljaka.

Navedeno ukazuje na potrebu istraživanja uzgoja matovilca u plutajućem hidroponu u plasteniku s krovnom i bočnom ventilacijom, bez mogućnosti dopunskog hlađenja zraka tijekom proljetno-ljetnog razdoblja s ciljem utvrđivanja dinamike rasta i prinosa s obzirom na temperaturu hranive otopine i zasjenjivanje biljaka energetsom zavjesom.

Materijal i metode

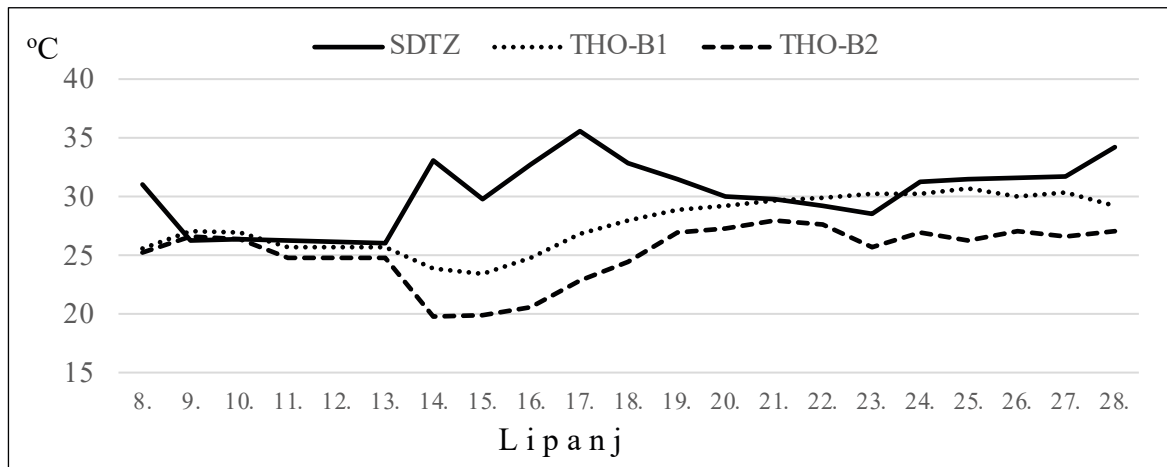
Istraživanje je postavljeno u proljetno-ljetnom roku uzgoja (sjetva 24. svibnja, berba 30. lipnja 2021.) u plutajućem hidroponu u plasteniku Zavoda za povrćarstvo Sveučilišta u Zagrebu Agronomskog fakulteta. Dvofaktorijski pokus s matovilcem sorte 'Accent' postavljen prema metodi slučajnog blokno rasporeda u tri ponavljanja uključivao je temperaturu hranive otopine (nehlađena i hlađena) i zasjenjivanje usjeva energetsom zavjesom (neprekriveno i prekriveno). Sorta matovilca 'Accent' sjemenske tvrtke „Enza Zaden“ razvija okrugle listove tamnozeleno boje u teškoj rozeti, a odlikuje se izrazito brzim rastom koji rezultira kratkom vegetacijom. U pokusu su korištene energetske zavjese Solaro 5220 DO tvrtke „Svensson“ izrađene od traka aluminijske i poliesterske (43 i 57 %). Sjetva uz utrošak sjemena od 7 g m⁻² je obavljena u proreze polistirenskih ploča (96 cm × 60 cm × 2,7 cm; površine 0,57 m²) ispunjene perlitom granulacije 0 do 5 mm. Do klijanja sjemena ploče su držane jedna na drugoj u plasteniku, a nakon klijanja (8. lipnja) po 6 ploča je raspoređeno u dva bazena (1,2 m × 3,0 m × 0,3 m, volumena 880 L) ispunjena hranivom otopinom za lisnato povrće prema Jensenu (Lorenz i Maynard, 1988). U oba bazena, s nehlađenom otopinom - bazen 1 (B1) i s hlađenom otopinom - bazen 2 (B2) po 3 ploče s matovilcem (50 % uzgojne površine bazena) zasjenjene su prekrivanjem energetsom zavjesama. Svaki od 4 tretmana bio je zastupljen s tri ploče, odnosno imao je tri ponavljanja.

Svakodnevno je u plasteniku očitavana minimalna i maksimalna temperatura zraka u proteklih 24 sata radi izračuna srednje dnevne temperature zraka (minimalna + maksimalna / 2). Nakon prijepodnevnog hlađenja hranive otopine u B2 potapanjem spremnika s ukupno 25 litara zaleđene vode, hranivim otopinama u oba bazena utvrđene su temperatura, pH i EC-vrijednost te koncentracija otopljenog kisika. Početna povišena pH-vrijednost hranive otopine (7,04 u B1 i 7,08 u B2) korigirana je dodavanjem 56 % HNO₃ u količini 15 ml (11., 14. i 15. lipnja) i 20 ml (23. lipnja). Od punog nicanja (10. lipnja) do berbe, svakih 7 dana, odnosno 17., 24. i 30. lipnja, provedene su analize morfoloških svojstava matovilca na reprezentativnom uzorku od 10 biljaka iz svakog tretmana pokusa. Utvrđivan je broj listova, visina, promjer i masa rozete, masa biljke, masa i dužina korijena. Na osnovu prikupljenih podataka, matematičkim izrazom za relativnu stopu rasta (RSR) prema Hoffmann i Poorter (2002) utvrđena je RSR za sva promatrana morfološka svojstva izuzev broja listova za razdoblja između 1. i 2. te 2. i 3. analize. Prilikom ručne berbe izmjerena je masa rozeta sa svake ploče temeljem koje je utvrđen prinos matovilca (kg m⁻²).

Prikupljeni podaci tijekom istraživanja statistički su obrađeni u programskom sustavu SAS, verzija 14.3 (SAS/STAT, 2017). Provedena je analiza varijance (ANOVA), a srednje vrijednosti su uspoređene t-testom na razini značajnosti p ≤ 0,05.

Rezultati i rasprava

U Grafikonu 1. prikazan je dnevni hodogram temperature zraka u plasteniku te nehlađene i hlađene hranive otopine (HO) u plutajućem hidroponu tijekom vegetacijskog razdoblja matovilca od nicanja do berbe.

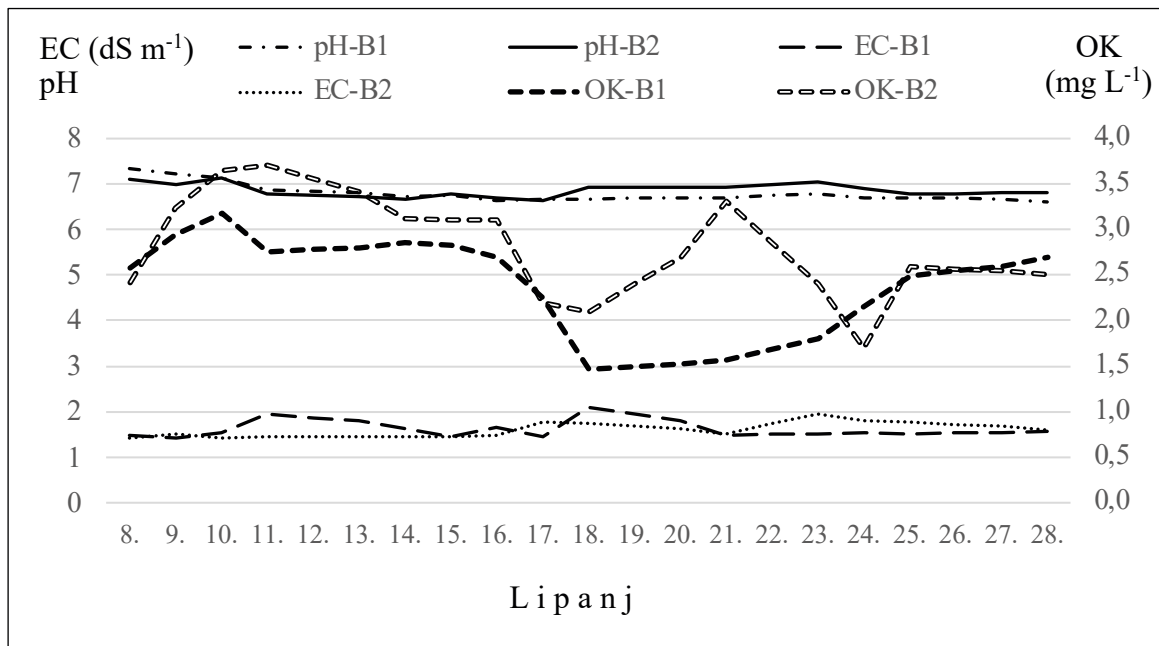


Grafikon 1. Temperatura zraka i hranivih otopina tijekom uzgoja matovilca

Legenda: SDTZ – srednja dnevna temperatura zraka, THO-B1 – temperatura nehlađene hranive otopine u bazenu 1, THO-B2 – temperatura hlađene hranive otopine u bazenu 2

Srednja dnevna temperatura zraka bila je u rasponu od 26,1 do 35,6 °C, s prosječnom vrijednosti od 30,3 °C, pa je značajno premašivala optimalnu temperaturu za rast matovilca od 10 do 18 °C koju navode Osvald i Kogoj-Osvald (2005) i Lešić i sur. (2016). Temperatura nehlađene HO u B1 bila je u rasponu od 23,4 do 30,7 °C, a hlađene u B2 od 19,8 do 28,0 °C te je s obzirom na ostvarene prosječne vrijednosti od 27,7 i 25,2 °C, temperatura hlađene HO bila niža za 2,5 °C. U usporedbi sa stabilnim temperaturnim uvjetima (20 °C temperatura zraka te 15, 20 i 25 °C temperatura HO u tri plutajuća hidropona) koje u istraživanju s matovilcem ostvaruju Dalla Costa i sur. (2011), temperatura zraka je značajno varirala i bila je viša za 10 °C, dok je temperatura HO u B1 premašivala sve testirane temperature, a u B2 najnižu i srednju, odnosno bila je identična najvišoj testiranoj temperaturi. Utvrđene temperaturne vrijednosti, također su bile manje povoljne za uzgoj matovilca od zabilježenih u istraživanju Fabek i sur. (2011) s obzirom na temperaturu zraka i HO 23,4 i 18,8 °C u proljetnom roku, odnosno 19,8 i 20,3 °C u ljetno-jesenskom.

Hodogrami pH i EC-vrijednosti hranivih otopina u oba bazena bili su vrlo ujednačeni (Grafikon 2.), s identičnim prosječnim vrijednostima pH 6,8 i EC 1,6 dS m⁻¹ koje su malo više, odnosno malo niže od preporučenih za lisnato povrće (pH 5,8-6,2 i EC 1,8 dS m⁻¹) koje navodi Jensen (Lorenz i Maynard, 1988). Od promatranih pokazatelja HO, koncentracija otopljenog kisika u oba bazena bila je najmanje stabilna, a hodogram ukazuje na značajnu ovisnost ovog svojstva o temperaturi hranive otopine. Koncentracija otopljenog kisika u HO u oba bazena bila je viša od minimalne (2 mg L⁻¹) koju za uzgoj lisnatog povrća preporučuju Goto i sur. (1996), pa biljke nisu pokazivale simptome nedostatka kisika koji se pojavljuju pri koncentraciji manjoj od 1 mg L⁻¹ (Gonnella i Serio, 2003). Na višu koncentraciju otopljenog kisika u B2 (2,8 mg L⁻¹) nego u B1 (2,4 mg L⁻¹), utjecala je niža temperatura hranive otopine u B2, što je sukladno navodu Morgan (2009) da viša temperatura hranive otopine rezultira smanjenim zadržavanjem kisika.



Grafikon 2. Koncentracija otopljenog kisika, pH- i EC-vrijednost hranivih otopina tijekom uzgoja matovilca
 Legenda: pH-B1 – pH nehladene hranive otopine (HO), pH-B2 – pH hladene HO, EC-B1 – EC nehladene HO, EC-B2 – EC hladene HO, OK-B1 – koncentracija otopljenog kisika nehladene HO, OK-B2 – koncentracija otopljenog kisika hladene HO

Interakcija istraživanih faktora imala je opravdan učinak na sva istraživana morfološka svojstva matovilca pri 3. analizi 20 dana nakon nicanja, dok je kod mlađih biljaka pri 1. i 2. analizi utvrđen djelomičan učinak (Tablica 1.). Kombinacija hladene HO i zasjenjivanja pri 3. analizi rezultirala je značajno većom visinom rozete (11,8 cm) i dužinom korijena (8,7 cm) u odnosu na ostale istraživane interakcije, dok je masa biljke, rozete i korijena (1,15, 1,07 i 0,12 g) bila statistički jednaka ostvarenim u hladenoj HO bez zasjenjivanja (1,36, 1,11 i 0,20 g) i značajno veća nego u kombinacijama nehladene HO. Ostvarena veća masa korijena u hladenoj HO, sukladna je navodu Dalla Costa i sur. (2011) da je kod temperature HO od 25 °C zabilježena značajno manja masa korijena u odnosu na HO temperature 15 i 20 °C. Po broju listova u rozeti (9,0) kombinacija hladene HO i zasjenjivanja pri 3. analizi nije se razlikovala od ostalih kombinacija s 8, 8,7 i 9,3 listova u rozeti, a što je više od utvrđenih 6,6, 7,7 i 5,9 pri temperaturi HO 15, 20 i 25 °C koje navode Dalla Costa i sur. (2011). Također, kod navedene kombinacije ostvaren je najveći promjer rozete (8,6 cm), statistički jednak kao kod kombinacije nehladene HO i zasjenjivanja (7,7 cm), a koji se značajno razlikovao od ostvarenog pri kombinacijama hranivih otopina bez zasjenjivanja.

Tablica 1. Utjecaj temperature hranive otopine i zasjenjivanja energetskom zavjesom na morfološka svojstva matovilca 7, 14 i 20 dana nakon nicanja

Tretman	Broj listova rozete	Visina rozete (cm)	Promjer rozete (cm)	Masa biljke (g)	Masa rozete (g)	Masa korijena (g)	Dužina korijena (cm)
1. analiza morfoloških svojstava matovilca 7 dana nakon nicanja							
B1×NP	4,0	2,4	2,5 b ^x	0,19 b	0,16 b	0,03 ab	6,57 a
B1×P	4,0	2,7	3,3 a	0,17 b	0,16 b	0,01 b	4,26 b
B2×NP	4,0	2,4	2,7 b	0,18 b	0,16 b	0,02 b	6,66 a
B2×P	4,0	2,5	2,7 b	0,24 a	0,20 a	0,04 a	6,57 a
2. analiza morfoloških svojstava matovilca 14 dana nakon nicanja							
B1×NP	6,9 a	6,6 b	6,5 b	0,48	0,42	0,06	5,9 b
B1×P	7,1 a	6,8 b	8,0 ab	0,61	0,54	0,07	7,8 ab
B2×NP	6,1 b	5,3 c	6,6 b	0,70	0,61	0,09	7,1 ab
B2×P	7,2 a	8,5 a	8,6 a	0,64	0,58	0,06	8,8 a
3. analiza morfoloških svojstava matovilca 20 dana nakon nicanja							
B1×NP	8,0 b	7,1 b	6,4 b	0,79 c	0,72 b	0,07 b	6,1 b
B1×P	8,7 ab	8,5 b	7,7 ab	0,90 bc	0,79 b	0,11 b	6,8 b
B2×NP	9,3 a	6,4 b	6,5 b	1,31 a	1,11 a	0,20 a	7,3 b
B2×P	9,0 ab	11,8 a	8,6 a	1,19 ab	1,07 a	0,12 a	8,7 a

Legenda: B1 – bazen bez hlađenja hranive otopine (HO); B2 – bazen s hlađenjem HO; NP – nepokrivanje energetskom zavjesom; P – pokrivanje energetskom zavjesom; ^xRazličita slova predstavljaju značajno različite prosječne vrijednosti prema *t*-testu, $p \leq 0,05$

Relativna stopa rasta (RSR) mase biljke, visine rozete, mase rozete, mase i visine korijena između 1. i 2. analize bila je prosječno 2,7, 6,2, 2,3, 5,4 i 4,4 puta veća nego u razdoblju između 2. i 3. analize (podaci nisu prikazani). Veće vrijednosti RSR navedenih morfoloških pokazatelja u razdoblju između 1. i 2. analize, u odnosu na razdoblje između 2. i 3. analize, ukazuju na brži i intenzivniji rast mlađih biljaka matovilca, što je sukladno rezultatima istraživanja Toth i sur. (2016) sa salatnom u plutaćem hidroponu.

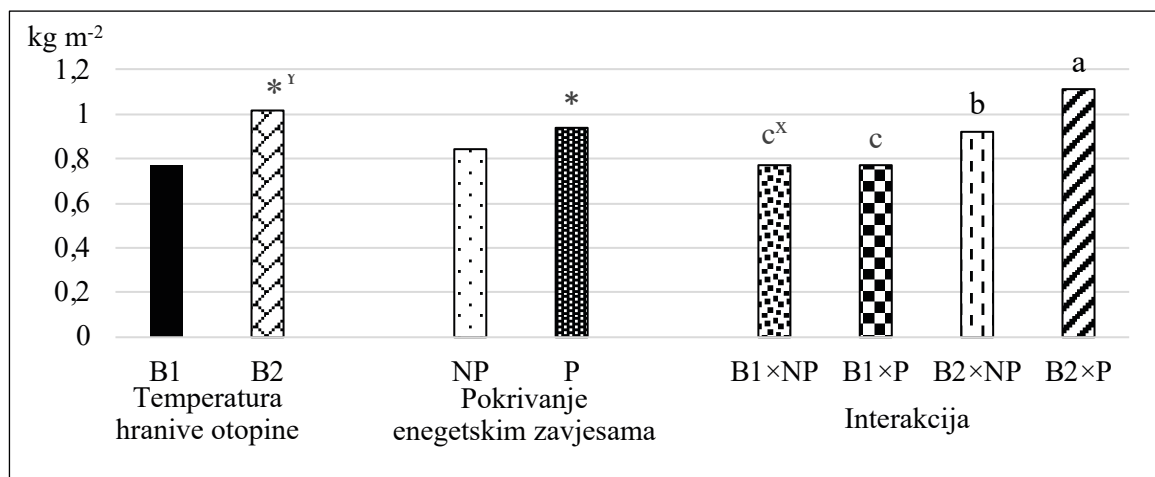
Istraživani faktori i njihove interakcije imale su opravdan utjecaj na prinos matovilca (Grafikon 3.). Niža prosječna temperatura (25,2 °C) hladene HO rezultirala je značajno većim prinosom matovilca (1,016 kg m⁻²) od ostvarenog (0,769 kg m⁻²) u nehladenoj HO prosječne temperature 27,7 °C. Također, matovilac pokriven energetskom zavjesom imao je značajno veći prinos (0,941 kg m⁻²) od nepokrivenog (0,844 kg m⁻²). Matovilac uzgajan pri kombinaciji hladene HO i pokrivanja energetskom zavjesom imao je najveći prinos (1,112 kg m⁻²), značajno veći od ostvarenog pri ostalim testiranim kombinacijama. Najmanji, statistički jednaki prinosi (0,769 kg m⁻²) ostvareni su pri kombinacijama s nehladnom HO.

Prinos od 0,919 kg m⁻², ostvaren u hladenoj HO temperature 25,2 °C bez zasjenjivanja i pri temperaturi zraka od 30 °C, bio je podjednak prinosu (0,897 kg m⁻²) koji su utvrdili Dalla Costa i sur. (2011) pri temperaturi zraka i HO od 20 i 25 °C te manji od ostvarenog pri istoj temperaturi zraka (20 °C) i nižoj temperaturi HO (15 i 20 °C), odnosno 1,264 i 1,560 kg m⁻². Međutim, prinos matovilca od 0,769 kg m⁻² iz nehladene HO temperature 27,7 °C bez zasjenjivanja, bio je manji u odnosu na ostvaren pri sve tri testirane temperature HO iz navedenog istraživanja. Najveći prinos matovilca ostvaren kombinacijom hladene otopine i zasjenjivanja (1,112 kg m⁻²) bio je 20 % veći, odnosno 29 % manji od prinosa koje Dalla Costa i sur. (2011) navode za HO temperature 25 i 20 °C.

Utvrđeni prinos, bez obzira na istraživane faktore, bio je manji u odnosu na prinos koji Fabek i sur. (2011) navode u istraživanju različitih rokova hidroponskog uzgoja matovilca. Tako je najveći prinos ostvaren kombinacijom hladene HO i zasjenjivanja (1,112 kg m⁻²) bio 44 i 39 % manji u odnosu na prinos od 1,993 i 1,815 kg m⁻² iz proljetnog i ljetno-jesenskog roka pri temperaturi zraka i HO 23,4 i 18,8 °C, odnosno 19,8 i 20,3 °C. Prinos iz ljetno-jesenskog roka iz istraživanja Fabek i sur. (2011) bio je veći i od najvišeg (1,560 kg m⁻²) ostvarenog u istraživanju Dalla Costa i sur.

(2011) pri gotovo jednakim temperaturnim uvjetima, odnosno pri istoj temperaturi zraka i HO od 20 °C.

Prinosi matovilca od 0,919, 1,264 i 1,993 kg m⁻² iz ovog te istraživanja Dalla Costa i sur. (2011) i Fabek i sur. (2011), ostvareni pri podjednakoj razlici između više temperature zraka i niže temperature HO (oko 5 °C), ukazuju da su razlike u visini prinosa vidljive iz njihovog odnosa (1:1,4:2,2), posljedica utjecaja temperaturnih uvjeta, odnosno temperature zraka i HO (30,1 i 25,2 °C; 20 i 15 °C; 23,4 i 18,8 °C) koje su bile iznad, odnosno bliže optimalnim vrijednostima potrebnim za rast matovilca.



Grafikon 3. Učinak temperature hranive otopine i zasjenjivanja energetskim zavjesama na prinos matovilca
 Legenda: B1 – bazen bez hlađenja hranive otopine (HO); B2 – bazen s hlađenjem HO; NP – nepokrivanje energetskim zavjesama; P – pokrivanje energetskim zavjesama; ^y – ANOVA; ^x Različita slova predstavljaju značajno različite prosječne vrijednosti prema t-testu, p≤0,05

Zaključak

Veća relativna stopa rasta visine i promjera rozete, mase biljke, rozete i korijena u drugom tjednu nakon nicanja u odnosu na treći, ukazuju na brži i intenzivniji rast mlađih biljaka. Najveće vrijednosti većine promatranih morfoloških pokazatelja i prinosa (1,112 kg m⁻²) matovilca ostvarene su pri uzgoju u hlađenoj hranivoj otopini temperature 25,2 °C uz prekrivanje energetskim zavjesama.

Literatura

- Aberkani K., Hao X., Gosselin A., de Halleux D. (2008). Responses of leaf gas exchanges, chlorophyll a fluorescence, and fruit yield and quality of greenhouse tomato to shading with retractable liquid foam. *Acta Horticulturae*. 797: 235-240.
- Cortella G., Saro O., De Angelis A., Ceccotti L., Tomasi L., Dalla Costa L., Manzocco L., Pinton R., Mimmo T., Cesco S. (2014). Temperature control of nutrient solution in floating system cultivation. *Applied Thermal Engineering*. 73(10): 1055-1065.
- Dalla Costa L., Tomasi N., Gottardi S., Iacuzzo F., Cortella G., Manzocco L., Pinton R., Mimmo T., Cesco S. (2011). The effect of growth medium temperature on corn salad *Valerianella locusta* (L.) Laterr. baby leaf yield and quality. *Horticultural Science*. 46: 1619-1625.
- Fabek S., Toth N., Benko B., Borošić B., Žutić I., Novak B. (2011). Lamb's lettuce growing cycle and yield as affected by abiotic factors. *Acta Horticulturae*. 893: 887-894.
- Gonnella M., and Serio F. (2003). Yield and quality of lettuce grown in floating system using different sowing density and plant spatial arrangements. *Acta Horticulturae*. 614: 687-692.
- Goto E., Both A.J., Albright L.D., Langhans R.W., Leed A.R. (1996). Effect of dissolved oxygen concentration on lettuce growth in floating hydroponics. *Acta Horticulturae*. 440: 205-210.

- Hoffmann, W.A., and Poorter, H. (2002). Avoiding bias in calculations of relative growth rate. *Annals of botany*. 90: 37- 42.
- Landhäusser S.M., DesRochers A., Lieffers V.J. (2001). A comparison of growth and physiology in *Picea glauca* and *Populus tremuloides* at different soil temperatures. *Canadian Journal of Forest Research*. 31: 1922–1929.
- Lešić R., Borošić J., Buturac I., Herak Ćustić M., Poljak M., Romić D. (2016). *Povrčarstvo*. III. dopunjeno izdanje. 624-629. **Čakovec**, Hrvatska: Zrinski.
- Lorenz O.A., and Maynard D.N. (1988). *Knotts Handbook for Vegetable Growers*. New York, USA: John Wiley Sons.
- Morgan L. (2009). Nutrient temperature – oxygen and Pythium in hydroponics. http://www.simplyhydro.com/nutrient_temp.htm
- Osvald J., and Kogoj-Osvald M. (2005). *Vrtnarstvo*. Splošno vrtnarstvo in zelenjadardstvo. 291-296. Ljubljana, Slovenija: Univerza v Ljubljani, Biotehniška fakulteta.
- Sanford S. (2019). Using curtains to reduce greenhouses heating and cooling costs. <https://farm-energy.extension.org/wp-content/uploads/2019/04/4.-A3907-03.pdf>
- SAS®/STAT 14.3 (2017). SAS Institute Inc.: Cary, NC, USA.
- Toth N., Fabek S., Benko B., Žutić I., Stubljar S., Zeher S. (2012). Učinak abiotičkih čimbenika, gustoće sjetve i višekratne berbe na prinos rige u plutajućem hidroponu. *Glasnik zaštite bilja*. 35: 24-34.
- Toth N., Vrhovec R., Borošić J., Benko B., Fabek S., Žutić I., Radman S. (2016). Cultivar and plant density impact on growth and morphometric characteristics of lettuce in floating system. *Acta Horticulturae*. 1142: 99-104.
- Zhang S. i Dang Q.L. (2007). Interactive effects of soil temperature and [CO₂] on morphological and biomass traits in seedlings of four boreal tree species. *Forest science*. 53: 453–460.

Possibilities of growing lamb's lettuce in a floating hydropon in the spring-summer period

Abstract

With the purpose of extending the market supply of lamb's lettuce, the possibilities of cultivation in a floating hydropon in the spring-summer period were researched with the aim of determining the dynamics of growth and yield with regard to the temperature of the nutrient solution and shading with an climate screens. Higher values of the relative growth rate of morphological parameters (rosette height and diameter, plant, rosette and roots mass) in the second week after emergence compared to the third, indicate a faster and more intense growth of younger plants. The highest values of most of the observed morphological parameters and yield (1.112 kg m⁻²) of the cv. 'Accent' were achieved during cultivation in a cooled nutrient solution with an average temperature of 25.2 °C and shading with climate screens.

Keywords: *Valerianella locusta*, nutrient solution temperature, climate screens, growth dynamics, yield

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Breakage susceptibility of maize kernel as a function of moisture content

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Abstract

The proportion of damaged kernels increases from harvest to the place where they will be used for a specific purpose. The aim of this study was to determine the breaking forces of a maize kernel that will be exposed to a different number of impacts (from 0 to 40). The forces at which the kernels were broken were determined at 5 moisture levels (approximately 10, 14, 18, 22 and 26%). With increasing in moisture, breaking force decreased and the number of undamaged kernels increased. Average breaking force differed significantly between moisture levels. The obtained data can help in estimating how many broken kernels can be expected when performing certain procedures at the moisture levels selected in the research.

Keywords: maize, kernel moisture, kernel breakage, breaking forces

Introduction

Maize kernel breakage is an important factor that, according to the available literature, has been well studied. There are factors that directly and indirectly affect the appearance of kernel breakage, and some create preconditions for the appearance of cracks and later kernel breakage. Between hybrids there is a difference in kernel structure, so hybrids with a higher proportion of hard endosperm are less susceptible to damage. During growing season, breakage susceptibility can be influenced by applying certain management practices. Among them nitrogen fertilization can influence breakage susceptibility. It has been proven that with an increase in the amount of added nitrogen, kernel hardness increases and breakage susceptibility decreases (Kneip and Mason, 1989). On the other hand, delayed planting, a large number of plants per unit area (high plant densities) and less added nitrogen increase susceptibility to breakage (Bauer and Carter, 1986). It is very important that maize plant is well supplied with water from silking to physiological maturity, i.e., that stressful conditions do not occur (Abendroth et al., 2011). Before harvest, air temperature and relative humidity affects grain moisture content (Jukić, 2004), and there are also differences in the structure of the ears, due to which some hybrids release moisture faster and some slower. In harvesting, it is important to determine moisture, because the adjustment of certain parameters of the thresher depends on kernel moisture content, which can again affect more or less damage to the kernel. During conditioning and storage, kernel moisture is also an important factor because it has been found that grain is damaged differently at different moisture levels. The equipment used during transportation in silos and warehouses, consisting of bucket elevators, screw and chain conveyors, can damage the grain during transfer (Boumans, 1980). An increase in the proportion of cracked and broken grains can cause problems during storage, on the one hand, due to increased moisture absorption, and on the other hand, due to increased in the presence of insects and molds on the grains (Fox and Manley, 2009). According to Chen et al. (2020) during harvesting and handling, grain kernels are subject to complex loading conditions consisting of a combination of impact, shear, and compression forces. The main damage mechanisms include impact, which causes external and internal cracks or even fragmentation of the kernel; attrition, which generates fine material; jamming, which deforms and breaks kernels due to high compressive forces; and fatigue, which produces broken kernels and fine material via repeatedly applied loads. The proportion of damaged kernels increases from harvest to the place or point where the grain will be used or processed. By studying the forces at which maize kernels break or crack, useful information can be obtained about when and how to carry out a certain procedure or operation. The data on crushing strength of the kernel and rupture energy are useful for designing size reduction machines. These

properties relate to the moisture content, type of the grain and grain orientation (Verma and Prasad, 2000). Babić et al. (2011) concluded that kernel compressive strength properties decreased as the moisture content increased. Data concerning the physical and mechanical properties of agricultural food materials are of importance to plant breeders, engineers, machine manufactures, food scientists, processors and consumers. Those properties are useful in postharvest unit operations for the design of cleaning, grading, sorting, transportation, handling, aeration, sizing, storing, size reduction, packaging and other processing equipment (Sharma et al., 2017). Maize kernel breakage is something that is inevitable in today's technologies used in harvesting, processing and storage, and that is why it is necessary to do everything to reduce the proportion of cracked and broken kernels as much as possible. The aim of this study was to determine the breaking forces of a maize kernel that will be exposed to a different number of impacts (from 0 to 40).

Material and methods

The study was conducted on one maize hybrid (PIONEER CORTEVA Agriscience; P9958). Maize grain samples were provided by the company EKOPATENT plus d.o.o., which conducts its own research with the aim of determining the impact of the application of different fertilizers on grain yield and soil properties. Before harvest, ears were taken manually from one field divided in four parts (four different variants of fertilization). The ears were hand-shelled to avoid kernel damage (kernels free from stress cracks). One grain sample (5 kg) was taken from each fertilization variant. Samples were cleaned of impurities and the moisture content was determined. Grain samples are naturally dried. From these samples, grain samples of 120 grains each were taken, which were placed in smaller paper bags which were kept at cool space (air temperature between 8 and 10°C). Samples of different moisture levels (10%, 14%, 18%, 22% and 26%) were prepared for measuring breaking forces at which kernels were broken. Considering the aforementioned moisture range, one part of the samples had to be conditioned (rehydrated) i.e., increase the moisture content in the kernel, one part of the samples was immediately ready for testing ($\approx 14\%$ moisture), and part of the samples had to dry naturally to a moisture content of approximately 10%.

The rehydration procedure was carried out by adding distilled water according to the following formula (1):

$$W = M_1 \times \frac{w_2 - w_1}{100 - w_2} \quad (\%) \quad (1)$$

where W – is the amount of water added (ml); w_1 – moisture content of the sample before rehydration (%); w_2 – moisture content of the sample after rehydration (%); M_1 – initial mass of sample (g).

Rehydration process lasted 72 hours. After rehydration, kernels were exposed to a different number of impacts (from 5 to 40). For this purpose, equipment was made, consisting of a metal stand, a metal tube, a metal base and a stick with a metal head, on the top of which there was an incised cross. Before exposure to impacts, kernel was placed in a groove located in a metal base in the position germ up side down. A stick with a metal head fell through a metal tube onto the kernel from a height of 20 cm. Kim (2000) also used similar equipment in his research. Weight of the stick was 25.5g. Each time when the stick fell on the kernel, kernel was acted upon with 0.05J [Potential (impact) energy; $W_p = 0.2m \times 0.0255kg \times 9.81 \text{ m/s} = 0.05J$]. This means that the kernels were acted upon from 0.25 to 2.00J. A total of 100 kernels, divided into 9 subsamples of 10 kernels each. Out of a total of 9 samples, in 8 samples, 10 kernels were exposed to impacts (from 5 to 40 impacts). In one sample, kernels were not exposed to impacts and immediately used to determine the breaking force. After impact exposure, the breaking force was determined in the aforementioned 8 samples. During impact exposure, part of the kernels cracked and it was not possible to determine the breaking force on such kernel or on their remains. Equipment used to determine the breaking force is shown in Figure 1. Maize kernel was placed between two parallel plates and compressed. The kernel was placed on the bottom plate, which was fixed (static), and in such a way that the germ was facing down (germ up side down). A small metal rod is attached under the upper plate, which is used to press the kernel. The upper plate was moved down by hand, and the force at which the kernel was broken was determined.

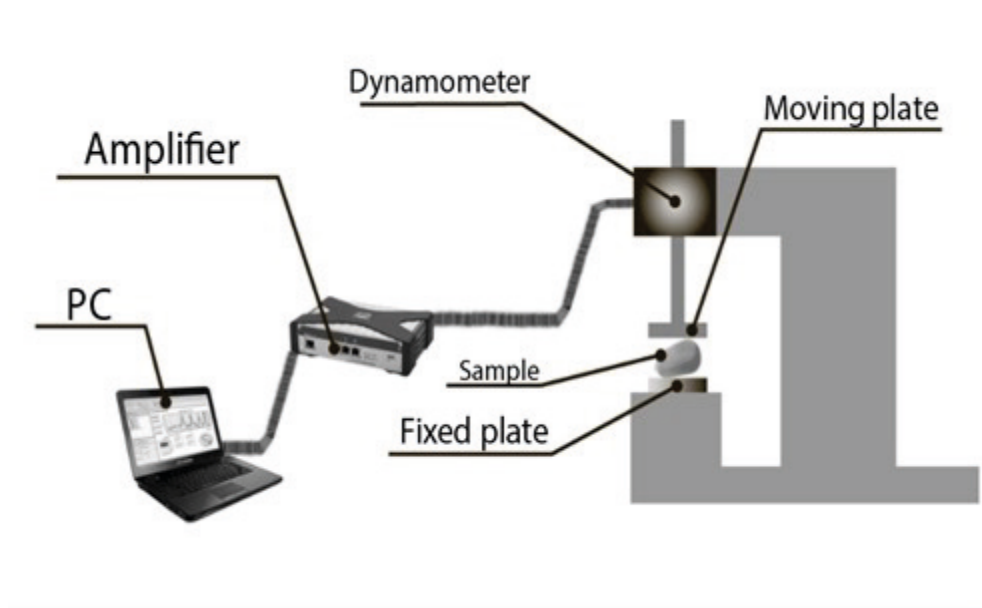


Figure 1. Equipment for measuring breaking force

The forces were measured by the data acquisition system, which included dynamometer HBM (Hottinger Baldwin Messtechnik GmbH, Darmstadt, Germany), amplifier HBM Quantum MX 840 B and personal computer (Figure 1). Balala et al. (2023) used a similar method of determining the breaking force and similar equipment. Statistical data analysis was performed using SAS software (SAS Institute, 2004).

Results and discussion

It is interesting to note that the force at which the kernels are broken decreases from the lowest to the highest moisture content. The average breaking force at which the kernels were broken at the lowest moisture (on average 11.76%) was 0.60 kN, and at the highest moisture (on average 25.98%) the average force was 0.41 kN (Figure 2). Likewise, it is interesting to note that the force at which the grain broke did not change much depending on the number of impacts (Table 1). Thus, it can be determined from Table 1 that the average force at which the kernels that were not exposed to impacts were broken was 0.46 kN, and that the average force at which the kernels were broken after 40 impacts was 0.45 kN, which is slightly less compared to kernels that were not exposed to impacts. But the truth is that the average force at 30, 35 and 40 impacts was obtained from less data because, many kernels did not withstand a greater number of impacts.

It can be seen from Figure 1 that there was a significant difference in the average forces between moisture levels chosen in this study. The average breaking force in this study (all kernel moisture levels, all kernels that were not exposed to impacts and all kernels that were exposed to impacts) was 0.46 kN. This can be useful information, provided that there is information about the forces acting on the kernel from harvest to processing, that is, where and when an increase in kernel breakage can be expected.

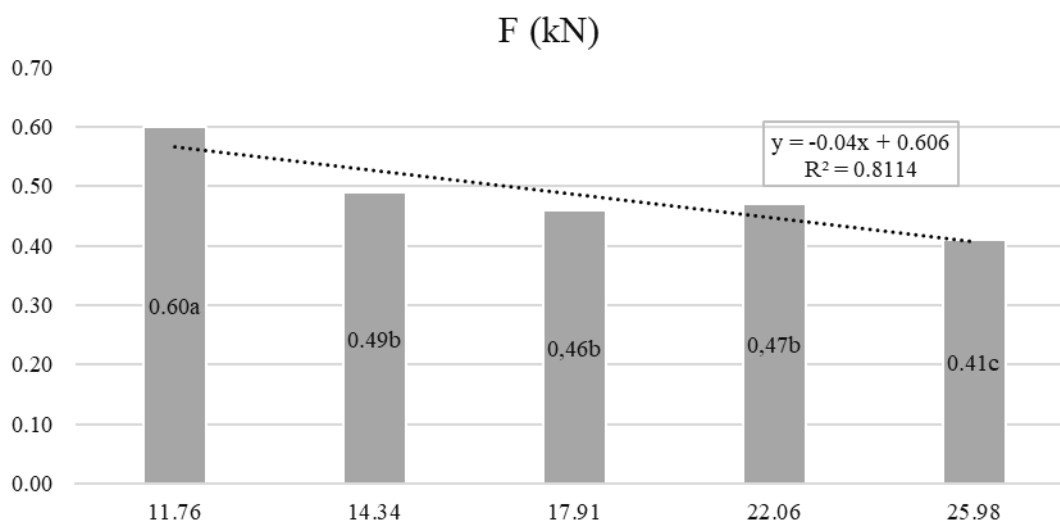


Figure 2. Breaking forces at different moisture content

From available literature, it can be determined that maize kernels are exposed 20 to 40 times to various mechanical influences (impacts, friction, falling from a height, etc.) from harvest to the place where they will be used for a specific purpose.

Trend that with increasing of kernel moisture content, decreased breaking forces is in agreement with the available literature (Verna and Prasad, 2000; Maksoud, 2009; Barnwal et al., 2012; Lupu et al., 2016; Chandio et al., 2021). In addition to the forces at which kernel breakage occurs it is also useful to determine how many kernels remain undamaged after being exposed to a certain number of impacts.

Table 1. Average number of undamaged grains and average force (F) at which kernels were broken in relation to number of impacts and potential energy (WP)

Number of impacts	Potential energy (WP)	Number of undamaged kernels	Force (F) (kN)
0	0.00	10.0	0.46
5	0.25	8.7	0.45
10	0.50	7.1	0.45
15	0.75	6.7	0.46
20	1.00	6.2	0.45
25	1.25	5.5	0.50
30	1.50	4.1	0.50
35	1.75	3.3	0.49
40	2.00	2.5	0.45

As can be seen from Table 1, the average number of undamaged kernels decreases with increasing number of impacts (from 5 to 40 impacts). Already after 5 impacts, out of 10 kernels that were exposed to impacts, an average of 1.3 kernels were no longer whole or were broken.

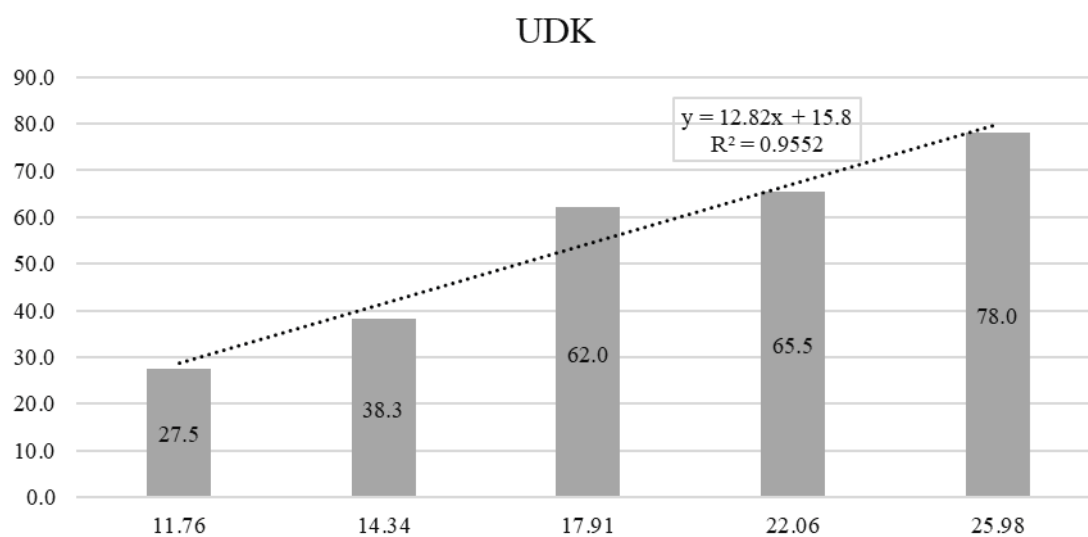


Figure 3. Number of undamaged kernels (UDK)

After 40 impacts, only an average of 2.5 grains out of 10 remained whole or not broken. By increasing kernel moisture, number of whole or undamaged grains increased (Figure 3).

At an average moisture content of 11.76%, the number of undamaged kernels was on average 27.5, while at an average grain moisture of 25.98%, the number of whole (unbroken) grains was on average 78.0 (Figure 3).

Conclusion

Based on the data obtained from the research, the following can be concluded:

1. With increasing in moisture, breaking forces at which the kernels were broken decrease.
2. With increasing in moisture, the number of whole (undamaged) kernels increases.
3. The average force at which the kernels that were not subjected to impacts were broken was slightly higher than the average force at which the kernels were broken after 40 impacts. However, fewer kernels remained undamaged (entirely) after 40 impacts without being destroyed. The average force did not decrease with increasing number of impacts.
4. The average number of undamaged (whole) kernels decreases with an increase in the number of impacts (from 5 to 40 impacts).

The obtained data can help in estimating how many broken kernels can be expected when performing certain procedures at the moisture levels selected in the research.

References

- Abdel Maksoud M.A.F. (2009): Mechanical properties of corn kernels. *Misr Journal of Agricultural Engineering*. 26: 1901-1922.
- Abendroth J.L., Elmore R.W., Boyer M.J., Marlay S.K. (2011). *Corn Growth and Development*. PMR 1009, Iowa State University Extension, Ames, Iowa.
- Babić LJ., Radojčin M., Pavkov I., Babić M., Turan J., Zoranović M., Stanisić S. (2013). Physical properties and compression loading behavior of corn seed. *International Agrophysics*. 27: 119–126.
- Balala M., Kovačev I., Čoga L., Slunjski S., Cella F., Sito S., Jukić Ž. (2023). Influence of nitrogen fertilization on the rupture force of maize kernels. *Proceedings of the 49th Symposium "Actual Tasks on Agricultural Engineering"*, Opatija, Croatia, 2023, pp. 303-313.

- Barnwal P., Kadam D.M., Singh K.K. (2012). Influence of moisture content on physical properties of maize. *International Agrophysics*. 26: 331-334.
- Bauer P.J., and Carter P.R. (1986). Effect of Seeding Date, Plant Density, Moisture Availability and Soil Nitrogen Fertility on Maize Kernel Breakage Susceptibility. *Crop science*. 26: 1220-1226.
- Boumans G. (1985). *Grain Handling and Storage*. Elsevier Science Publishers B.V.
- Chandio A.F., Li Y., Ma Z., Ahmad F., Naz Syed T., Ali Shaikh S., Tunio M.H. (2021). Influences of moisture content and compressive loading speed on the mechanical properties of maize grain orientations. *International Journal of Agricultural and Biological Engineering*. 14:41-49.
- Chen Z., Wassgren C., Kingsly Ambrose R.P. (2020). A review of grain kernel damage: mechanisms, modeling, and testing procedure. *Transactions of the ASABE*. 63: 455-475.
- Fox G., and Manley M. (2009). Hardness Methods for testing Maize Kernels. *Journal of agricultural and food chemistry*. 57: 5647-5657.
- Jukić Ž. (2004). *Water Release Rates of Maize Grain in the Field and Dryer in a Convection Drying Process*. PhD Thesis, University of Zagreb Faculty of Agriculture, Zagreb, Croatia.
- Kim T.H. (2000). *Physical changes in maize (Zea mays L.) grains during postharvesting drying*. A thesis presented in partial of the requirements for the degree of Doctor of Philosophy in Seed technology at Massey University.
- Kneip K.R., and Mason S.C. (1989). Kernel Breakage and Density of Normal and Opaque-2 Maize Grain as influenced by Irrigation and Nitrogen. *Crop Science*. 29:158-163.
- Lupu M.I., Pădureanu V., Canja C.M., Măzărel A. (2016). The effect of moisture content on grinding process of wheat and maize single kernel. *ModTech International Conference - Modern Technologies in Industrial Engineering IV*. IOP Conf. Series: Materials Science and Engineering. 145: 022024.
- SAS Institute 2004 (SAS/STAT)
- Sharma V., Basu S., Lal S.K., Anand A., Hossain F., Munshi A.D. (2017). Comparison of Physical and Physiological Properties of Specialty Maize Inbred Lines. *Chemical Science Review and Letters*. 6: 1758-1763.
- Verma, R.C., and Prasad S. (2000.) Mechanical and thermal properties of maize. *Journal Food Science Technology*. 37:500-505.

Prinos i hektolitarska masa zrna pšenice (*Triticum aestivum* L.) pod utjecajem vremenskih prilika tijekom višegodišnjeg razdoblja

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Sažetak

Pšenica (*Triticum aestivum* L.) je jedna od najvažnijih svjetskih usjeva za proizvodnju hrane. Prinos zrna je s ekonomskog aspekta najvažnije agronomsko svojstvo u proizvodnji pšenice, a veća vrijednosti hektolitarske mase ukazuju na veći prinos brašna. Opće je poznato da su prinos i hektolitarska masa pšenice značajno pod utjecajem genotipa, vremenskih prilika i primijenjenih agrotehničkih mjera tijekom uzgoja. Cilj istraživanja bio je utvrditi prinos i hektolitarsku masu deset analiziranih kultivara pšenice tijekom višegodišnjeg razdoblja (2015.–2019.). Kroz petogodišnji period najveći prosječan prinos (9,86 t ha⁻¹) postignut je 2017. koja je po količini oborina (435 mm) i srednjoj temperaturi zraka (8,5 °C) bila najsličnija višegodišnjem prosjeku (475 mm i 8,1 °C). Najmanji prinos (6,73 t ha⁻¹) ostvaren je 2016. godine tijekom koje je u vegetacijskom periodu zabilježeno 120 mm oborina više i 1,5 °C viša temperatura u odnosu na višegodišnji prosjek. U istom periodu, najveća razlika u hektolitarskoj masi zrna zabilježena je također 2017. (82,2 kg hL⁻¹), dok su 2015., 2018. i 2019. bile na istoj razini značajnosti s najmanjim vrijednostima za hektolitarsku masu (78,9–79,2 kg hL⁻¹) bez obzira na različitu distribuciju oborina i različite temperaturne varijacije tijekom vegetacijskih perioda.

Ključne riječi: pšenica, prinos, hektolitarska masa, kultivari, vremenske prilike

Uvod

Prinos pšenice je kompleksno kvantitativno svojstvo pod utjecajem različitih gena s aditivnim, dominantnim i epistatičkim učincima, kao i pod utjecajem različitih okolišnih čimbenika. Povećanje prinosa postiže se putem različitih komponenti, kao što su povećanje mase zrna, broja zrna po klasu i po biljci, te utjecaja na vanjske čimbenike (Španić, 2016). Klima je jedan od najvažnijih faktora, kako za formiranje tla, tako i za formiranje visokih prinosa i tehnoloških svojstava žitarica (Dudkina i Dolgopolova, 2022). Cilj uzgoja pšenice je razviti nove genotipove s poboljšanim karakteristikama, što doprinosi većem potencijalu za urod zrna, stabilnijim prinosima i boljoj kvaliteti pšeničnih proizvoda (Iljkić i sur., 2019). Temperatura igra ključnu ulogu u formiranju mase zrna, sintezi ugljikohidrata i proteina. Ozima pšenica zahtijeva izloženost niskim temperaturama, ali bez teških mrazeva u fazi rasta, kako bi se potakla reproduktivna faza u proljeće (Huzsvai i sur., 2022). Povoljne temperature tijekom faze nalijeivanja zrna za pšenicu obično uključuju dnevne temperature oko 25°C i noći hladnije od 10 °C, jer to potiče akumulaciju suhe tvari u zrnu pšenice (Španić, 2016). Tijekom vegetacije, visoke temperature, posebno iznad 30 °C, mogu negativno utjecati na prinos pšenice, dovodeći do sterilnosti klasića i manje hektolitarske mase (Pospišil, 2010). Kovačević (1998) je analizirao utjecaj oborina i temperatura na prinos kukuruza i pšenice u Istočnoj Hrvatskoj te je ustanovio da su varijacije u prosječnim prinosima povezane s vremenskim uvjetima te su iznosile do 19% za kukuruz i do 23% za pšenicu. Marijanović i sur. (2010) analizirali su utjecaj padalina i temperature na prinos pšenice u Istočnoj Hrvatskoj te su utvrdili da je u povoljnim godinama prosječni prinos iznosio 5,53 t ha⁻¹, dok je u nepovoljnim godinama bio za 24% niži, s indikacijama da umjerene padaline i blage zime povoljno utječu na prinos pšenice. Hektolitarska masa je važan parametar koji utječe na kvalitetu pšenice, a na nju također utječu svojstva genotipa, klimatski uvjeti i

agrotehničke prakse tijekom uzgoja (Jukić i sur., 2020). Cilj rada bio je ispitati utjecaj vremenskih uvjeta tijekom pet vegetacijskih sezona (2014./2015. – 2018/2019. godine) na prinose i hektolitarsku masu zrna ozime pšenice.

Materijal i metode

Tijekom petogodišnjeg razdoblja od 2015. do 2019. godine, na Poljoprivrednom institutu Osijek provedeni su sortni pokusi na 10 sorata pšenice. Sjetva je obavljena u optimalnim rokovima u listopadu, uz sjetvenu normu od 330 zrna/m². Sortni pokusi su postavljeni prema slučajnom blok rasporedu, s tri ponavljanja na parcelama veličine 7,56 m² (7 m duljine i 1,08 m širine). Obrada tla i priprema prije sjetve provedene su prema standardnim postupcima za uzgoj ozime pšenice. Ukupna količina primijenjenih gnojiva, uključujući osnovnu gnojivu i prihrane, varirala je ovisno o predkulturi i kretala se u rasponu od 101 do 114 kg N, 80 kg P₂O₅ i 120 kg K₂O ha⁻¹. Na pokusima se provodila standardna zaštita od korova i štetočina primjenom uobičajenih kemijskih preparata. Prinos zrna ocijenjen je žetvom cijele parcele uz korištenje specijaliziranog pokusnog kombajna, a vaganjem je određena ukupna masa koja je zatim preračunata na prinose ha⁻¹ uz pretpostavku da je vlaga zrna iznosila 13,5%. Osim toga, na uzorcima je izmjerena vlaga zrna i hektolitarska masa pomoću brzog analizatora zrna Infratec 1241 (Foss, Švedska). U istraživanju su korišteni podaci o mjesečnim količinama oborina (mm) i srednjim mjesečnim temperaturama zraka (°C) tijekom pet vegetacijskih godina, te višegodišnje prosječne vrijednosti s meteorološke postaje Osijek. Svi podaci obrađeni su uz pomoć računalnog softvera, dok je analiza varijance napravljena pomoću statističkog programa SAS Enterprise guide 7.1.

Rezultati i rasprava

Prosječne temperature i količina oborina za ispitivanu lokaciju Osijek prikazane su u tablicama 1 i 2 (Državni hidrometeorološki zavod, 2020). Prosječne temperature svih vegetacijskih sezona su bile toplije od višegodišnjeg prosjeka (tablica 1) i varirale su od +5% do +25%.

Tablica 1. Mjesečna temperatura zraka (°C) tijekom ispitivanog razdoblja

	Temperatura zraka (°C) za postaju Osijek										
	X	XI	XII	I	II	III	IV	V	VI	Prosjek	Suma
2014./2015.	13,3	8,3	3,5	2,9	2,5	7,5	12,1	17,8	20,8	9,9	88,7
2015/2016	11,1	7,5	3,2	0,8	6,9	7,5	13,1	16,5	21,0	9,7	87,6
2016/2017	10,4	6,2	-0,1	-5,1	4,2	9,5	11,3	17,5	22,4	8,5	76,3
2017/2018	11,8	6,7	3,6	4,2	0,9	4,6	17	20,6	21,7	10,1	91,1
2018/2019	14,4	7,6	1,5	0,5	4,2	9,1	12,8	14,0	23,1	9,7	87,2
VGP* 71.-01.	11,1	5,1	1,3	-0,2	1,8	6,4	11,2	16,7	19,6	8,1	73,0

Što se tiče količine oborina, dvije vegetacijske sezone imale su iznadprosječnu količinu oborina, dvije su imale prosječnu, dok je jedna imala ispodprosječnu količinu oborina (tablica 2).

Tablica 2. Mjesečna količina oborina (mm) tijekom ispitivanog razdoblja

	Mjesečna količina oborine (mm) za postaju Osijek									
	X	XI	XII	I	II	III	IV	V	VI	Suma
2014./2015.	87,9	8,8	66,0	73,7	57,1	50,5	12,9	113,4	17,1	487,4
2015/2016	142,1	45,1	1,9	67,0	68,3	68,2	39,8	63,1	99,5	595,0
2016/2017	65,4	57,1	0,5	25,2	74,4	67,6	49,7	50,6	45,4	435,9
2017/2018	68,7	33,0	51,7	61,7	70,2	83,4	21,0	27,4	126,8	543,9
2018/2019	12,2	25,2	26,7	42,4	26,8	8,4	68,6	150,8	112,8	473,9
VGP* 71.-01.	55,9	61,5	48,8	41,6	34,5	40,5	51,0	59,2	82,0	475,0

*VGP – višegodišnji prosjek 1971.-2001.

Prinos zrna pšenice je najvažnije svojstvo u proizvodnji, stoga je svakom proizvođaču primarni cilj ostvariti što bolje rezultate za to svojstvo. Prosječni ostvareni prinos analiziranih 10 sorata pšenice bio je 7,9 t ha⁻¹ (tablica 3.). Analizirane sorte su pokazale različite prosječne prinose tijekom petogodišnjeg ispitivanja. Sorta Ficko ostvarila je najveći prosječni prinos od 8,75 t ha⁻¹, dok je sorta Srpanjka imala najniži prosječni prinos od 7,22 t ha⁻¹.

Tablica 3. Prinos zrna ozime pšenice (t ha⁻¹) tijekom ispitivanog razdoblja

Sorta	2015	2016	2017	2018	2019	Prosjek ± SD
Žitarka	9,04	7,44	10,10	7,75	8,06	8,47 ± 1,09 ^{BC*}
Srpanjka	7,23	6,54	8,83	7,04	6,49	7,22 ± 0,95 ^A
Golubica	6,62	5,80	9,37	7,69	7,61	7,41 ± 1,34 ^A
Lucija	8,21	7,59	10,79	6,83	7,00	8,08 ± 1,61 ^{ABC}
Alka	5,91	5,59	9,55	7,41	8,13	7,31 ± 1,63 ^A
Ficko	9,70	8,20	10,75	8,64	6,45	8,75 ± 1,62 ^C
Renata	8,00	7,06	9,83	7,96	7,43	8,06 ± 1,07 ^{ABC}
Katarina	7,10	6,31	10,24	7,89	8,56	8,02 ± 1,50 ^{ABC}
Felix	7,69	6,74	9,46	8,08	7,20	7,83 ± 1,04 ^A
OS Olimpija	7,33	6,02	9,68	8,53	7,71	7,85 ± 1,36 ^{ABC}
Prosjek	7,68 ^B	6,73 ^A	9,86 ^C	7,78 ^B	7,46 ^B	7.90

*Razlika između vrijednosti u kolonama koje sadrže istu slovnju oznaku nisu statistički značajne ($p < 0,05$)

Najveći pojedinačni prinos zabilježen je 2017. godine, kada je sorta Lucija ostvarila prinos od 10,79 t ha⁻¹. S druge strane, najniži pojedinačni prinos zabilježen je 2016. godine, kada je sorta Alka imala prinos samo 5,59 t ha⁻¹. Na temelju podataka iz tablice 3, može se zaključiti da postoji statistički značajan utjecaj godina i sorti na prinos zrna ozime pšenice. Stošić i sur. (2017) navode da godina uzgoja ima najveći utjecaj na prinos pšenice, slijedi način obrade tla, a tek na kraju razina dušika u opskrbi. Drezner i sur. (2006) su tijekom 2005./2006. na 4 lokacije analizirali 13 kultivara pšenice i utvrdili značajan utjecaj okoline na prinos i parametre kvalitete te su izdvojili nekoliko kultivara koji su pokazivali najmanju varijabilnost prinosa. Ako usporedimo 2016. i 2017. godinu, kada je ostvarena najveća razlika u prinosu, utjecaj vremenskih prilika na prinos pšenice je značajan. U listopadu 2015./2016. godine, zabilježena je količina oborina od 142 mm (86,2 mm iznad višegodišnjeg prosjeka), što je pomaklo optimalne rokove sjetve i produljilo razdoblje od sjetve do nicanja, dok je u istom periodu 2016./2017., količina oborina iznosila je 65,4 mm (9,5 mm iznad višegodišnjeg prosjeka). U travnju 2015./2016. godine, zabilježena količina oborina bila je 11,2 mm manja, a temperature za 1,9°C više u odnosu na višegodišnji prosjek, što je nepovoljno za pšenicu koja se tada nalazi u fazi vlatanja, a koja je kritična jer tijekom nje dolazi do intenzivnog rasta biljne mase, određivanja broja klasića i broja cvjetova u klasićima. Za razliku od 2016., u travnju 2017. godine, temperature i oborine bile su gotovo na razini višegodišnjeg prosjeka. Hektolitarska masa zrna često se primjenjuje u kontekstu ocjene kvalitete zrna prilikom trgovine krušaricama. Na temelju podataka iz tablice 4, možemo zaključiti da je hektolitarska masa bila najveća u 2017. godini, dok je najmanja vrijednost hektolitarske mase ostvarena 2015., 2018. i 2019., pri čemu između tih godina nije bilo statistički značajnih razlika. Drenjančević i sur. (2017) su tijekom dvije vegetacijske godine (2013./2014. i 2014./2015.) proveli istraživanje na 120 kultivara krušne pšenice u agroekološkim uvjetima istočne Slavonije, pri čemu je ispitivan utjecaj klimatskih prilika na komponente prinosa krušne pšenice, te su utvrdili visoko značajne razlike između svih ispitivanih svojstava i značajan utjecaj dvije klimatski različite godine na sva ispitivana svojstva, osim za hektolitarsku masu. U našem istraživanju sorta OS Olimpija ostvarila je najvišu prosječnu hektolitarsku masu zrna (82,45 kg hL⁻¹), dok je sorta Alka imala najnižu (78,07 kg hL⁻¹) tijekom pet godina ispitivanja (tablica 4).

Tablica 4. Hektolitarska masa zrna ozime pšenice (kg hL⁻¹) tijekom ispitivanog razdoblja

Sorta	2015	2016	2017	2018	2019	Prosjek ± SD
Žitarka	84,00	79,6	82,33	78,30	82,00	81,25 ± 2,28 ^{EF*}
Srpanjka	76,15	79,9	80,75	77,70	76,40	78,18 ± 2,07 ^{AB}
Golubica	79,10	81,5	82,88	81,20	80,50	81,04 ± 1,39 ^{BCDE}
Lucija	78,55	79,0	81,03	77,90	77,10	78,72 ± 1,48 ^{ABCD}
Alka	74,43	77,5	80,30	78,90	79,20	78,07 ± 2,26 ^A
Ficko	83,30	82,8	83,40	79,30	78,00	81,36 ± 2,53 ^{DEF}
Renata	78,03	79,2	81,75	79,00	76,60	78,92 ± 1,89 ^{ABCDE}
Katarina	74,05	79,3	82,28	78,70	80,80	79,03 ± 3,11 ^{ABC}
Felix	78,65	80,6	83,58	80,10	78,20	80,23 ± 2,12 ^{DEF}
OS Olimpija	81,63	81,4	84,1	82,0	83,1	82,45 ± 1,13 ^F
Prosjek	78,79 ^A	80,08 ^B	82,24 ^C	79,31 ^A	79,19 ^A	79,92

*Razlika između vrijednosti u kolonama koje sadrže istu slovnju oznaku nisu statistički značajne ($p < 0,05$)

Kada promatramo godine, najveću hektolitarsku masu postigla je sorta OS Olimpija 2017. godine, a najnižu ostvarila je sorta Katarina 2015. u vegetacijskoj godini 2016./2017., tijekom koje je ostvarena najveća hektolitarska masa, zabilježeno je 499,9 mm oborina, što je 13,6 mm manje od višegodišnjeg prosjeka, uz nešto više temperature zraka od prosječne (tablica 1 i 2). U usporedbi 2017. godine s 2015., 2018. i 2019., tijekom vegetacije 2016./2017. godine količina oborina od vlatanja do zriobe bila je slična višegodišnjem prosjeku. Tijekom 2014./2015., količina oborina u fazi vlatanja bila je značajno niža od prosjeka, dok je u 2017./2018. količina oborina u istoj fazi bila ispod prosjeka, a 2018./2019. znatno viša od prosjeka. Đekić i sur. (2015) ispitivali su utjecaj godine na prinos i kvalitetu zrna ozime pšenice te navode da je utjecaj godine na hektolitarsku masu zrna ozime pšenice statistički značajan.

Zaključak

Temeljem provedenog petogodišnjeg istraživanja na 10 sorti ozime pšenice može se zaključiti da su vremenske prilike statistički značajno utjecale na ispitivane parametre. Prosječan prinos pšenice iznosio je 7,9 t ha⁻¹ a hektolitarska masa 79,92 kg ha⁻¹. Analizom varijance prinosa utvrđene su veće razlike između ispitivanih godina ($\Delta=3,13$ t ha⁻¹) nego između sorti ($\Delta=1,53$ t ha⁻¹), dok je najveća razlika utvrđena u interakciji godina x sorta ($\Delta=5,2$ t ha⁻¹). Što se tiče hektolitarske mase, analizom varijance utvrđene su veće razlike kod sorti ($\Delta=4,38$ kg hL⁻¹) nego između ispitivanih godina ($\Delta=3,45$ kg hL⁻¹), dok je najveće odstupanje također utvrđeno u interakciji godina x sorta ($\Delta=9,95$ kg hL⁻¹).

Literatura

- Drenjančević L., Petrović S., Rebekić A., Guberac S., Rukavina I., Guberac V. (2017). Utjecaj klimatskih prilika na komponente prinosa krušne pšenice. Zbornik radova 52. hrvatski i 12. međunarodni simpozij agronoma. Dubrovnik, 203–207.
- Drezner G., Dvojkovic K., Horvat D., Novoselovic D., Lalic A. (2007). Environmental impacts on wheat agronomic and quality traits. Cereal Research Communications. 35: 357--360.
- Državni hidrometeorološki zavod (2020). Meteorološki podaci, Klimatološko meteorološki sektor, Državna hidrometeorološka stanica Osijek-Klisa, Zagreb.
- Đekić V., Glamočlija Đ., Milovanović M., Staletić M. (2010). Uticaj godine na prinos i kvalitet zrna kragujevačkih sorti ozime pšenice. Zbornik PKB, 16 (1-2): 43–50.
- Huzsvai L., Zsembeli J., Kovács E., Juhász C. (2022). Response of winter wheat (*Triticum aestivum* L.) yield to the increasing weather fluctuations in a continental region of four-season climate. Agronomy. 12: 314.
- Iljić D., Grbeša A., Rukavina I., Jukić G., Šunjić K., Orkić V., Rastija, M. (2019). Utjecaj sorte na

- prinos, komponente prinosa, agronomska svojstva i kvalitetu zrna ozime pšenice. Zbornik radova 54. hrvatski i 14. međunarodni simpozij agronoma. Vodice, 309–313.
- IOP Conf. Series (2022). Earth and Environmental Science 954: 012024.
- Jukić Ž., Krička T., Pospišil A., Radiček T., Jukić K. (2020). Utjecaj različitih oblika zrna pšenice na hektolitarsku masu. Agronomski glasnik: Glasilo Hrvatskog agronomskog društva. 82: 3-16.
- Kovačević V. (1998). Oborinski i temperaturni režim kao čimbenici prinosa kukuruza i pšenice u Istočnoj Hrvatskoj i mogućnosti njihovom prilagođavanju, U: Maceljki, M. 36 (ur.) Poljoprivreda i proizvodnja hrane u novom europskom okruženju, Hrvatska akademija znanosti i umjetnosti, Zagreb, 189–194.
- Marijanović M., Markulj A., Tkalec M., Jozić A., Kovačević V. (2010). Impact of precipitation and temperature on wheat (*Triticum aestivum* L.) yields in eastern Croatia. Acta Agriculturae Serbica. 15: 117–123.
- Pospišil A. (2010). Ratarstvo I. dio, Zrinski d.o.o, Čakovec.
- Stošić M., Brozović B., Tadić V., Stipešević B., Jug D. (2017). The effect of soil tillage and nitrogen fertilization treatments on winter wheat grain yield. Romanian agricultural research. 34: 105–111.
- Španić V. (2016). Pšenica, (ed) Poljoprivredni institut Osijek, Osijek.

Yield and hectolitre mass of wheat grain (*Triticum aestivum* L.) under the influence of weather conditions over a multi-year period

Abstract

Wheat (*Triticum aestivum* L.) is one of the world's most important crops for food production. Grain yield is the most significant agronomic trait in wheat production from an economic perspective, while higher hectolitre mass values indicate a greater flour yield. It is widely recognized that both the yield and hectolitre mass of wheat are significantly influenced by genotype, weather conditions, and applied agronomic practices during cultivation. The aim of this research was to determine the yield and hectolitre mass of ten analysed wheat cultivars over a multi-year period (2015–2019). Over the five-year period, the highest average yield (9.86 t ha^{-1}) was achieved in 2017. This year had a rainfall amount of 435 mm and an average air temperature of $8.5 \text{ }^\circ\text{C}$, which was most similar to the multi-year average (475 mm and $8.1 \text{ }^\circ\text{C}$). The lowest yield (6.73 t ha^{-1}) was recorded in 2016, during which the vegetative period experienced 120 mm more rainfall and $1.5 \text{ }^\circ\text{C}$ higher temperatures compared to the multi-year average. In the same period, the greatest difference in hectolitre mass of grain was also observed in 2017 (82.2 kg hL^{-1}), while 2015, 2018, and 2019 had similar hectolitre mass values ($78.9\text{--}79.2 \text{ kg hL}^{-1}$), regardless of variations in rainfall distribution and temperature during the vegetative periods.

Keywords: wheat, yield, hectolitre mass, varieties, weather conditions

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Agronoma

Zbornik radova

Ribarstvo, lovstvo i pčelarstvo

Kakvoća pčelinjeg voska na međunarodnom tržištu

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Sažetak

Zbog nedostatka zakonske regulative i obvezne kontrole kakvoće, pčelinji je vosak česta meta patvorenja i rastući problem diljem svijeta. Cilj je ovog rada bio utvrditi kakvoću pčelinjeg voska na međunarodnom tržištu. Uzorci pčelinjeg voska (satnih osnova i blokova voska) prikupljeni su iz 33 zemalja (n=300) te analizirani metodom infracrvene (FTIR-ATR) spektroskopije. Rezultati su pokazali kako je 166 (55,3 %) uzoraka bilo patvoreno parafinom (51 %) i stearinom/stearinskom kiselinom (4,3 %). Udio parafina u patvorenim uzorcima kretao se od 3,5 do 100 %, a stearina/stearinske kiseline od 1,5 do 29,2 %. Najviši udjeli parafina (> 90 %) utvrđeni su u uzorcima iz Bosne i Hercegovine, Srbije i Kine, a stearin/stearinska kiselina su detektirani u uzorcima iz Belgije i Nizozemske. Rezultati ovog istraživanja ukazuju na hitnu potrebu za rješavanjem problema patvorenja pčelinjeg voska na međunarodnoj razini.

Ključne riječi: pčelinji vosak, kakvoća, patvorenje, međunarodno tržište

Uvod

Patvorenje pčelinjeg voska (satnih osnova) velik je i rastući problem diljem svijeta. Niz je čimbenika koji doprinose patvorenju, a primarno je to visoka cijena pčelinjeg voska, niska cijena lako dostupnih patvorina pčelinjeg voska (primarno parafina) te nedostatak zakonske regulative koja definira kriterije kakvoće i rutinsku kontrolu autentičnosti pčelinjeg voska (Svečnjak i sur., 2019a.). Danas se za patvorenje pčelinjeg voska koristi više od 15 supstanci različitog podrijetla (biljnog, životinjskog, mineralnog) (Bogdanov, 2017.). Najučestalije korištene patvorine pčelinjeg voska su parafin i stearin i/ili stearinska kiselina (Reybroeck i Van Nevel 2018.; Waś i sur. 2016.; Svečnjak i sur. 2015.; Maia i sur. 2013.; Serra Bonvehi i Orantes Bermejo, 2012.), no u posljednje vrijeme na tržištu prisutna i pojava višestrukog patvorenja, odnosno patvorenje kombinacijom dviju ili više patvorina (Tanner i Lichtenberg-Kraag, 2019.). Patvorenje uzrokuje degradaciju sastava pčelinjeg voska, a unosom patvorenog voska (satnih osnova) u košnice ugrožava se dobrobit pčelinje zajednice u vidu narušene kemijske komunikacije, destrukcije izgrađenog saća te narušenih uvjeta za normalan fiziološki razvoj legla (Chęć i sur., 2021; Reybroeck i Van Nevel 2018.; Wallner, 2005.). Također, utvrđeno je kako patvorenje pčelinjeg voska negativno utječe i na sastav i kavoću meda (Svečnjak i sur., 2019b.). Uz navedeno, patvorine pčelinjeg voska (posebice parafin) mogu sadržavati brojne kontaminante pa se patvorenjem u pitanje dovodi zdravlje pčela, ali i javno zdravlje budući da pčelinji vosak (saće) predstavlja prvu prirodnu ambalažu za med, odnosno medij u kojem se med skladišti i dozrijeva. Na ovu je problematiku nedavno ukazala Europska agencija za sigurnost hrane koje je nedavno provela detaljnu procjenu rizika koje patvorenje pčelinjeg voska predstavlja za zdravlje ljudi i pčela (EFSA, 2020).

U pčelarskom je sektoru pčelinji vosak klasificiran kao nusproizvod životinjskog podrijetla koji nije namijenjen prehrani ljudi (Uredba komisije 1069/2009), a koristi se u obliku satnih osnova i blokova voska (pretopljenog saća) za njihovu izradu te kao takav ne podliježe obaveznoj kontroli kakvoće niti ispitivanju autentičnosti prije stavljanja na tržište. S ciljem pronalaska pouzdanih metoda za otkrivanja patvorenja pčelinjeg voska u posljednja su dva desetljeća razvijene dvije instrumentalne analitičke metode za kvalitativno i kvantitativno otkrivanje patvorenja pčelinjeg voska: plinska kromatografija-spektrometrija masa (engl. *gas chromatography-mass spectrometry*, GC-MS) (Waś i sur., 2015; 2016.), plinska kromatografija s plameno-ionizacijskim detektorom (engl. *gas chromatography with flame ionization detector*, GC-FID) (Maia i Nunes, 2013.; Serra Bonvehí i Orantes Bermejo, 2012.)

i infracrvena spektroskopija s Fourierovom transformacijom (engl. *Fourier transform infrared spectroscopy*, FTIR)

(Maia i sur., 2013.; Svečnjak i sur., 2015.; 2019.). Navedene su metode pokazale pouzdane rezultate u otkrivanju pojedinih patvorina u pčelinjem vosku jer pružaju dobre granice detekcije ($LoD < 5\%$) u usporedbi s fizikalno-kemijskim metodama koje karakteriziraju visoke granice detekcije ($LoD = 5-50\%$) (Svečnjak i sur., 2021; Bernal i sur., 2005.). U otkrivanju patvorenja pčelinjeg voska posebice se ističe FTIR spektroskopija koju karakterizira niz prednosti, uključujući pouzdanost u detekciji svih vrsta patvorina. Iako je brojnim prethodno navedenim istraživanjima potvrđena prisutnost različitih patvorina pčelinjeg voska na EU tržištu (uglavnom na nacionalnoj razini), sustavno i sveobuhvatno istraživanje kakvoće (autentičnosti) pčelinjeg voska na međunarodnom tržištu dosad nije provedeno. Stoga je cilj ovog rada bio utvrditi kakvoću pčelinjeg voska na međunarodnom tržištu kvalitativnom i kvantitativnom FTIR spektroskopskom analizom (utvrđivanjem tipa i udjela patvorina) uzoraka pčelinjeg voska prikupljenih iz 33 zemalje svijeta.

Materijal i metode

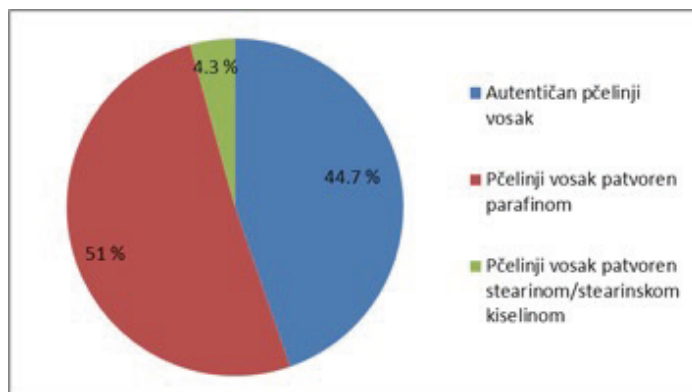
Ukupno 300 uzoraka pčelinjeg voska (satnih osnova i blokova voska namijenjenih za njihovu proizvodnju) prikupljeni su iz 33 zemlje svijeta; iz 17 zemalja članica Europske unije (Austrija, Belgija, Češka, Cipar, Francuska, Hrvatska, Italija, Latvija, Luksemburg, Mađarska, Nizozemska, Njemačka, Poljska, Rumunjska, Slovenija, Španjolska i Švedska), drugih zemalja s područja Europe koje nisu EU članice (Bosna i Hercegovina, Crna Gora, Srbija i Ujedinjeno kraljevstvo) te drugih zemalja/kontinenata (Australija, Brazil, Etiopija, Južna Koreja, Kamerun, Kanada, Kina, Kongo, Novi Zeland, Ruanda, Rusija i Vijetnam) u razdoblju od 2016. do 2019. godine. Uzorci su prikupljeni sukcesivno tijekom navedenog razdoblja s domaćeg i međunarodnog tržišta (od pčelara i proizvođača satnih osnova, s pčelarskih sajmova ili iz specijaliziranih pčelarskih trgovina). Uzorci su čuvani na tamnom mjestu pri sobnoj temperaturi (23 ± 2 °C) do analize.

Za kvalitativno i kvantitativno utvrđivanje patvorina u pčelinjem vosku primijenjena je metoda FTIR spektroskopije. Spektralna analiza je provedena sukladno metodologiji Svečnjak i sur. (2019a.). Infracrveni spektri prikupljenih uzoraka pčelinjeg voska s međunarodnog tržišta snimljeni su pomoću infracrvenog spektrometra Cary 660 (Agilent Technologies) s DTGS (deuterirani triglicin sulfat) detektorom, a za snimanje spektara primijenjena je tehnika jednorefleksijske prigušene totalne refleksije (engl. *attenuated total reflectance* – ATR) koristeći Golden Gate (Specac) ATR instrumentalni dodatak s dijamantom kao internim refleksijskim elementom. Apsorpcijski spektri ispitivanih uzoraka pčelinjeg voska snimljeni su na sobnoj temperaturi (23 ± 2 °C) pri spektralnoj rezoluciji od 4 cm^{-1} . FTIR-ATR spektri analiziranih uzoraka pohranjeni su pomoću softverskog paketa Agilent ResolutionsPro (version 5.3.0, Agilent Technologies), kojim je provedeno i kemometrijsko modeliranje te kvantitativna analiza podataka s ciljem utvrđivanja točnog udjela patvorina u pčelinjem vosku. Analiza i grafički prikaz podataka (distribucije uzoraka s obzirom na tip utvrđenih patvorina, razinu patvorenja i zemlju podrijetla) provedeni su programom Excel (Microsoft 365).

Rezultati i rasprava

Distribucija analiziranih uzoraka pčelinjeg voska prema tipu patvorina

Kako je prikazano na Grafikonu 1., rezultati su pokazali kako je ukupno 166 (55,3 %) od ukupno 300 analiziranih uzoraka pčelinjeg voska bilo patvoreno parafinom (51 %) i stearinom/stearinskom kiselinom (4,3 %). Manje od polovine analiziranih uzoraka (44,7 %; 134 uzoraka) identificirano je kao autentičan nepatvoreni pčelinji vosak, odnosno vosak koji sadrži $< 3,5$ % parafina i $< 1,5$ % stearina/stearinske kiseline (uvažavajući utvrđene granice detekcije za patvorine i prirodne varijacije sastava pčelinjeg voska iz prethodnih istraživanja - Maia i sur. 2013.; Svečnjak i sur. 2019a.; Svečnjak i sur. 2020.). Ovi rezultati potvrđuju kako je parafin najučestalija patvorina pčelinjeg voska na međunarodnom tržištu i u skladu su s rezultatima dosadašnjih istraživanja na nacionalnoj (Serra Bonvehí i Orantes Bermejo 2012., Maia i sur. 2013., Svečnjak i sur. 2015., Waš i sur. 2016.) i međunarodnoj razini (Tanner i Lichtenberg-Kraag 2019.) kojima je također potvrđena dominacija parafina kao najzastupljenije patvorine te sporadična pojava stearina/stearinske kiseline. U analiziranim uzorcima nisu utvrđene primjese drugih patvorina (goveđeg loja, karnauba voska i/ili drugih stranih tvari) niti kombinacije mješavina patvorina (višestruko patvorenje).



Grafikon 1. Zastupljenost autentičnog i patvorenog pčelinjeg voska na međunarodnom tržištu (n=300; uzorci prikupljeni iz 33 zemlje svijeta)

Distribucija analiziranih uzoraka pčelinjeg voska prema razini patvorenja

Tablica 1. prikazuje rezultate analize patvorenih uzoraka pčelinjeg voska (n=166) kategoriziranih s obzirom na razinu patvorenja parafinom i stearinom/stearinskom kiselinom. U 153 (92,2 %) od ukupno 166 patvorenih uzoraka utvrđena je prisutnost različitih udjela parafina (udio se kretao od 3,5 do 100 %), dok je stearin/stearinska kiselina utvrđena u znatno manjem broju uzoraka (n=13). Udio stearina/stearinske kiseline kretao se od 1,5 do 29,2 %. Rezultati su pokazali kako je većina patvorenih uzoraka (63,9 %) sadržavala niži udio patvorina (5 – 20 %), 17,5 % uzoraka sadržavalo je 20 – 40 % parafina ili stearina/stearinske kiseline, dok je 18,6 % uzoraka sadržavalo više od 40% parafina. Izuzetno visoka razina patvorenja (> 80 % parafina) utvrđena je u 15 % uzoraka.

Tablica 1. Kategorizacija patvorenih uzoraka pčelinjeg voska (n=166) prema razini patvorenja parafinom i stearinskom kiselinom

Razina patvorenja	Prisutnost parafina (broj uzoraka)	Prisutnost stearina/stearinske kiseline (broj uzoraka)	% uzoraka prema razini patvorenja
>80 %	25	–	15
60-80 %	2	–	1,2
40-60 %	4	–	2,4
20-40 %	19	10	17,5
3,5-20 %	103	3	63,9
Ukupno patvoreno (%)	92,2	7,8	100

Distribucija analiziranih uzoraka pčelinjeg voska prema zemlji podrijetla

U Tablici 2. prikazana je distribucija analiziranih uzoraka pčelinjeg voska prema zemlji podrijetla te tipu i udjelu utvrđenih patvorina. Spektralnom je analizom utvrđeno kako je većina analiziranih uzoraka satnih osnova (manjim dijelom i blokova voska namijenjenih za njihovu izradu) podrijetlom iz 17 zemalja članica EU te zemljama izvan EU bila patvorena parafinom, dok su stearin/stearinska kiselina utvrđeni u samo 13 uzoraka podrijetlom iz Belgije i Nizozemske. U većini tih uzoraka su utvrđene zabrinjavajuće količine stearina/stearinske kiseline (do 29,2 %) obzirom da je recentnim istraživanjima utvrđeno kako i male količine stearina u satnim osnovama (10 %) mogu uzrokovati visoke stope mortaliteta pčela u ličinačkoj fazi razvoja (Chęć i sur., 2021.; Reybroeck i Van Nevel 2018.).

Od brojnih zemalja Europe uključenih u ovo istraživanje, patvorine nisu utvrđene samo u uzorcima iz Luksemburga (n=1), Švedske (n=9) i Ujedinjenog kraljevstva (n=1). Uz navedene europske zemlje, nepatvoreni uzorci autentičnog pčelinjeg voska zapaženi su i na drugim kontinentima, a pritom se posebice se ističu uzorci pčelinjeg voska prikupljeni iz Novog Zelanda i Australije (n=25) u kojima nisu utvrđene primjese niti jednog tipa patvorine. Ovaj svijetli primjer slijede i uzorci iz afričkih zemalja (Kamerun, Kongo, Etiopija) te uzorci iz Kanade, Rusije i Vijetnama koji su također identificirani kao autentičan pčelinji vosak bez primjese patvorina.

Najviši udjeli parafina utvrđeni su u uzorcima podrijetlom iz Bosne i Hercegovine, Crne Gore, Srbije i Kine. Svi uzorci prikupljeni iz BiH (n=6) i većina uzoraka iz Srbije (n=11) bili su patvoreni različitim udjelima parafina, a posebice visokim udjelima parafina (> 90 %). Visoki udjeli parafina detektirani su sporadično i u jednom uzorku iz Poljske (99,6 %) i Crne Gore (95,3 %) te šest uzoraka iz Hrvatske (86,3 – 90,1 % parafina). Kineske satne osnove najbolje odražavaju zabrinjavajuću situaciju u vidu nekontroliranog plasmana satnih osnova upitnog kemijskog sastava na međunarodno tržište. Naime, rezultati analize satnih osnova prikupljenih s pčelarskog sajma u okviru Međunarodnog pčelarskog kongresa „Apimondia“ u Montrealu 2019. godine su pokazali kako je velik broj kineskih satnih osnova sadržavao visok udio parafina (> 90 %), a mnoge su osnove bile izrađene od 100 % parafina te sadržavale i umjetna bojila.

Tablica 2. Distribucija analiziranih uzoraka pčelinjeg voska (satnih osnova i blokova voska) prikupljenih s međunarodnog tržišta (n=300) s obzirom na zemlju podrijetla, tip i udio prisutnih patvorina

Zemlja podrijetla	Autentičan pčelinji vosak (n)*	Patvoreni pčelinji vosak (n)	Udio parafina (%)	Udio stearina / stearinske kiseline (%)
Australija (n=1)	1	–	–	–
Austrija (n=3)	1	2	5,1 – 19,6	–
Belgija (n=14)	6	8	9,5	1,5 – 27,9
Bosna i Hercegovina (n=6)	–	6	77,4 – 93	–
Brazil (n=1)	–	1	94,7	–
Češka (n=1)	–	1	4,7	–
Cipar (n=12)	6	6	4,1 – 28,2	–
Crna gora (n=1)	–	1	95,3	–
Etiopija (n=1)	1	–	–	–
Francuska (n=8)	5	3	3,7 – 7,2	–
Hrvatska (n=76)	11	65	3,6 – 90,1	–
Italija (n=20)	17	3	3,6 – 23,7	–
Južna Koreja (n=4)	3	1	3,9	–
Kamerun (n=6)	6	–	–	–
Kanada (n=4)	4	–	–	–
Kina (n=14)	3	11	5,8 – 100	–
Kongo (n=1)	1	–	–	–
Latvija (n=4)	3	1	11,6	–
Luksemburg (n=1)	1	–	–	–
Mađarska (n=2)	–	2	3,6 – 39,7	–
Nizozemska (n=14)	2	12	3,6 – 6,9	20 – 29,2
Novi Zeland (n=24)	24	–	–	–
Njemačka (n=3)	1	2	41,2 – 43,1	–
Poljska (n=28)	16	12	3,8 – 99,6	–
Ruanda (n=1)	–	1	7,1	–
Rumunjska (n=1)	–	1	11,2	–
Rusija (n=2)	2	–	–	–
Slovenija (n=22)	7	15	3,5 – 11,6	–
Srbija (n=12)	1	11	11,5 – 94,3	–
Španjolska (n=1)	–	1	6,5	–

Švedska (n=9)	9	–	–	–
Ujedinjeno Kraljevstvo (n=1)	1	–	–	–
Vijetnam (n=2)	2	–	–	–
Ukupno	134	166	3,5 – 100 %	1,5 – 29,2 %

Iz Tablice 2. je razvidno kako je većina uzoraka pčelinjeg voska patvorenih parafinom, neovisno o podrijetlu, sadržavala umjerene količine parafina (do 20 %). U francuskim i slovenskim uzorcima detektirani su relativno niski udjeli parafina koji su rijetko prelazili 10%, a slično je zamijećeno i u uzorcima iz Belgije, Češke, Latvije, Nizozemske, Rumunjske i Španjolske. Također, od ukupno 20 uzoraka prikupljenih iz Italije, 17 je nije sadržavalo patvorine, dok je u samo jednom uzorku detektirano više od 10 % parafina (23,7 %), a preostala dva uzorka sadržavala manje od 5 % parafina.

Radi malog broja uzoraka (≤ 3) prikupljenih iz pojedinih zemalja, nije moguće donijeti sud o stanju glede patvorenja u dotičnim zemljama, no generalno se može zaključiti kako pojavnost patvorenog pčelinjeg voska ne poznaje granice s obzirom da je ista utvrđena u zemljama diljem svijeta, s rijetkim izuzecima.

Zaključak

Rezultati istraživanja su pokazali kako je 166 (55,3 %) od ukupno 300 analiziranih uzoraka pčelinjeg voska prikupljenih iz 33 zemlje svijeta bilo patvoreno parafinom (51 %) ili stearinom/stearinskom kiselinom (4,3 %). Ovi rezultati potvrđuju kako je parafin najučestalija patvorina pčelinjeg voska na međunarodnom tržištu. Rezultati analize su također pokazali kako je u 153 (92,2 %) od ukupno 166 patvorenih uzoraka utvrđena prisutnost različitih udjela parafina (udio se kretao od 3,5 do 100 %), dok je stearin/stearinska kiselina utvrđena u znatno manjem broju uzoraka (n=13) podrijetlom iz Belgije i Nizozemske. Udio stearina/stearinske kiseline kretao se od 1,5 do 29,2 %. Većina patvorenih uzoraka (63,9 %) sadržavala je niži udio patvorina (5 – 20 %). Najviši udjeli parafina (> 80 % parafina) utvrđeni su u uzorcima iz Bosne i Hercegovine, Srbije i Kine. Patvorine nisu utvrđene samo u uzorcima iz Novog Zelanda, Australije, Kanade, Rusije, Vijetnama, afričkih zemalja (Kamerun, Kongo, Etiopija) te nekoliko europskih zemalja (Luksemburg, Švedska i Ujedinjeno Kraljevstvo).

Radi malog broja uzoraka (≤ 3) prikupljenih iz pojedinih zemalja, nije moguće donijeti sud o stanju glede patvorenja u dotičnim zemljama, no može se zaključiti kako pojavnost patvorina ne poznaje granice s obzirom da je ista utvrđena diljem svijeta. Nedostatak zakonske regulative glede kontrole kakvoće voska u pčelarskom sektoru te nekontroliran uvoz/izvoz satnih osnova svakako doprinosi patvorenju i diseminaciji kontaminiranog pčelinjeg voska diljem svijeta. Navedeno ukazuje na hitnu potrebu za izradom zakonske regulative koja definira kriterije kakvoće/čistoće za pčelinji vosak namijenjen primjeni u pčelarstvu uz obavezno rutinsko ispitivanje kakvoće prije stavljanja na tržište.

Literatura

- Bernal J.L., Jiménez J.J., Del Nozal M.J., Torbio L., Martin M.T. (2005). Physico – chemical parameters for the characterization of pure beeswax and detection of adulterations. *European Journal of Lipid Science And Tehnology*. 107:158–166.
- Bogdanov, S. (2004). Beeswax: Quality issues today. *BeeWorld*. 85(3), 46–50.
- Bogdanov S. (2017). Beeswax: Production, Properties, Composition and Control. *Beeswax book* (Chapter 1). *Bee Product Science*, pp. 1–18.
- Chęć M., Olszewski K., Dziechciarz P., Skowronek P., Pietrow M., Borsuk G., Bednarczyk M., Jasina G., Jasina J., Gagoś M. (2021). Effect of stearin and paraffin adulteration of beeswax on brood survival. *Apidologie*. 52:432–446.
- European Food Safety Authority (EFSA) (2020). Risk assessment of beeswax adulterated with paraffin and/or stearin/stearic acid when used in apiculture and as food (honeycomb). *EFSA Supporting Publication*. 17(5), 1–64.
- Maia M., Barros A.I.R.N.A., Nunes F.M., (2013). A novel, direct, reagent – free method for the detection of bee – wax adulteration by single – reflection attenuated total reflection mid –

- infrared spectroscopy. *Talanta*. 107: 74–80.
- Maia M., Nunes F.M. (2013). Authentication of beeswax (*Apis mellifera*) by high-temperature gas chromatography and chemometric analysis. *Food chemistry*. 136(2): 961–968.
- Reybroeck W., Van Nevel J. (2018). Effect of beeswax adulterated with sterin on the development of worker bee brood: results of a field trial. In Program & Abstract Book EurBee 8. 8th Congress of Apidology, Ghent, Belgium, 18-20 September 2018. p. 115.
- Serra Bonvehí J.S., Orantes Bermejo F.J. (2012). Detection of adulterated commercial Spanish beeswax. *Food Chemistry*. 132(1): 642–648.
- Svečnjak L., Baranović G., Vinceković M., Prđun S., Bubalo D., Tlak Gajger I., (2015). An approach for routine analytical detection of beeswax adulteration using FTIR-ATR spectroscopy. *Journal of Apicultural Science*. 59(2): 37–49.
- Svečnjak L., Chesson L.A., Gallina A., Maia M., Martenello M., Mutinelli F., Necasti Muz M., Nunes F.M., Saucy F., Tipple B.J., Wallner K., Waś E., Waters T.A. (2019a). Standard methods for *Apis mellifera* beeswax research. *Journal of Apicultural Research*. 58 (2): 1–108.
- Svečnjak L., Jović O., Prđun S., Rogina J., Marijanović Z., Car J., Matošević M., Jerković I. (2019b). Influence of beeswax adulteration with paraffin on the composition and quality of honey determined by physico – chemical analysis, ¹H NMR, FTIR-ATR spectroscopy. *Food chemistry*. 232: 286–294.
- Svečnjak L., Nunes F.M., Gracia Matas R., Cravedi J-P., Christodoulidou A., Rortais A., Saegerman C. (2021). Validation of analytical methods for the detection of beeswax adulteration with a focus on paraffin. *Food Control*. 120: 107503.
- Tanner N., Lichtenberg-Kraag B. (2019). Identification and Quantification of Single and Multi-Adulteration of Beeswax by FTIR-ATR Spectroscopy. *European Journal of Lipid Science and Technology*. 121: 1–10.
- Uredba komisije (EU) br. 1069/2009 o utvrđivanju zdravstvenih pravila za nusproizvode životinjskog podrijetla i od njih dobivene proizvode koji nisu namijenjeni prehrani ljudi te o stavljanju izvan snage Uredbe (EZ) br. 1774/2002 (Uredba o nusproizvodima životinjskog podrijetla)
- Wallner K. (2005). Foundation causing honeybee brood damage. In: Proceedings of ICPBR 9. International Symposium. York, United Kingdom. 12-14 October 2005, p.30.
- Waś E., Szcześna T., Raybak-Chmielewska H. (2015). Application of gas chromatography with the mass detector (GC-MS) technique for detection of beeswax adulteration with paraffin. *Journal of Apicultural Science*. 59(1): 143–152.
- Waś E., Szcześna T., Raybak-Chmielewska H. (2016). Efficiency of GC-MS method in detection of beeswax adulterated with paraffin. *Journal of Apicultural Science*. 60(1): 131–147.

Quality of beeswax on the international market

Abstract

Due to the lack of legislation and mandatory quality control, beeswax is a frequent target of adulteration and a growing problem worldwide. The aim of this study was to determine the quality of beeswax on the international market. Beeswax samples (comb foundations and beeswax blocks) were collected from 33 countries (n=300), and analysed by infrared (FTIR-ATR) spectroscopy. The results have revealed that 166 (55.3%) samples were adulterated with paraffin (51%) and stearin/stearic acid (4.3%). The proportion of paraffin in adulterated samples ranged from 3.5 to 100%, and of stearin/stearic acid from 1.5 to 29.2%. The highest proportions of paraffin (> 90%) were detected in samples from Bosnia and Herzegovina, Serbia and China, while stearin/stearic acid were detected in samples from Belgium and Netherlands. The results of this study indicate an urgent need to resolve the problem of beeswax adulteration on international level.

Keywords: beeswax, quality, adulteration, international market

Indeks kondicije i infestacija ljuštura kamenica, *Ostrea edulis* (Linnaeus, 1758) i *Magellana gigas* (Thunberg, 1793), polihetom *Polydora* sp. na području Medulinskog zaljeva

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Sažetak

Poliheti roda *Polydora* najčešći su uzrok vidljivih oštećenja na ljušturama kamenica što rezultira smanjenom tržišnom vrijednosti, a mogu uzrokovati slabiju kondiciju, usporeni rast i povećani mortalitet. Cilj ovog istraživanja bio je odrediti indeks kondicije i intenzitet infestacije kamenica, *Ostrea edulis* i *Magellana gigas*, polihetom *Polydora* sp. na području Medulinskog zaljeva kao pokazatelje njihove kvalitete. Uzorkovane su divlja populacija *M. gigas* te divlja i uzgojna populacija *O. edulis* u kolovozu 2023. godine. Najniži indeks kondicije bio je kod uzgojne populacije *O. edulis*, dok su vrijednosti bile slične kod *M. gigas* i divlje populacije *O. edulis*. Najniži prosječni broj poliheta po kamenici te niži broj infestiranih jedinki utvrđen je kod *M. gigas*. Vrlo niska negativna korelacija između indeksa kondicije i broja poliheta kod sve tri istraživane skupine pokazuje da utvrđeni stupanj infestacije ne utječe značajno na kondiciju jedinki.

Ključne riječi: europska plosnata kamenica, *Crassostrea gigas*, pacifička kamenica, kvaliteta kamenica, kondicija kamenica

Uvod

Europska plosnata kamenica (*Ostrea edulis* Linnaeus, 1758) autohtona je vrsta u Jadranu koja se kroz povijest uspješno uzgajala i sakupljala iz divljih populacija (Airoldi i Beck, 2007). Kako bi se zadovoljila potražnja kamenice na europskom tržištu, 1960-ih godina na područje Mediterana u uzgoj je uvedena pacifička kamenica, *Magellana gigas* (Thunberg, 1793), ranije poznata pod imenom *Crassostrea gigas* (Thunberg, 1793). Pacifička kamenica podnosi veća kolebanja okolišnih uvijeta i manje je osjetljiva u odnosu na europsku plosnatu kamenicu zbog čega se ubrzo nakon unosa uspjela proširiti i formirati stabilne populacije (Ezgeta-Balić i sur., 2019). Unatoč dugoj tradiciji uzgoja, zbog rastuće potražnje na tržištu, klimatskih promjena i strogih tržišnih zahtjeva za kvalitetom proizvodnja kamenica je sve izazovnije (MacKenzie i sur., 1997).

Kamenice se konzumiraju najčešće sirove, servirane na vlastitoj ljušturi zbog čega kvalitetom moraju zadovoljiti stroge tržišne i zdravstvene zahtjeve (Santeramo i sur., 2017).

Uspješan plasman i postizanje povoljne cijene na tržištu određuju vanjski izgled i veličina te količina i svojstva mesa. Oštećenja ljušture umanjuju tržišnu vrijednost proizvoda zbog estetskih nepravilnosti koje mogu izazvati gađenje potrošača iako kvaliteta mesa ne mora nužno biti umanjena (Loose i sur., 2013). Vidljiva oštećenja ljuštura najčešće su uzrokovana polihetima roda *Polydora*. Iako infestirane kamenice nisu rizične za ljudsko zdravlje, prisutnost mulja u kanalici izaziva neugodan miris i može promijeniti okus mesa (Morse, 2015). Osim smanjene tržišne vrijednosti, posljedice infestacije su povećani mortalitet i usporeni rast što uzrokuje značajne ekonomske gubitke (Sato-Okoshi, 2013).

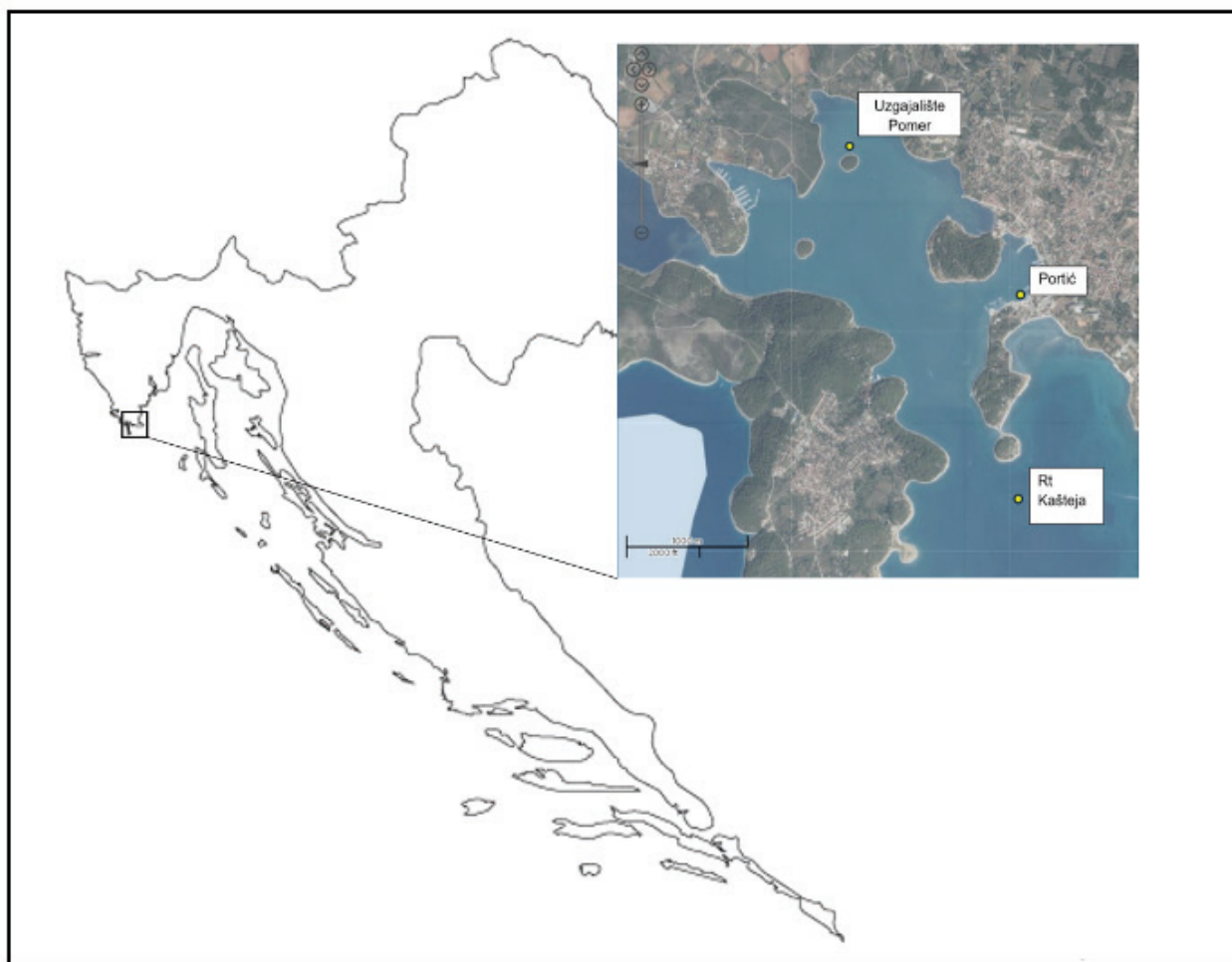
Za ocjenu kvalitete mesa, odnosno mekog tkiva kamenica, ključni su boja i količina mesa, odnosno mekog tkiva (Loose i sur., 2013). Postotak količine mesa unutar ljuštura iskazuje se kao indeks kondicije (Mann, 1978). Ovaj parametar varira ciklički tijekom godine ovisno o biotičkim i abiotičkim čimbenicima kao što su razdoblje mrijesta,

zdravstveno stanje, dostupnost hrane, temperatura, salinitet, pH, koncentracija otopljenog kisika, onečišćenje okoliša i dr. (Mann 1978; Gavrilović i sur., 2010). Osim što predstavlja odraz svih navedenih čimbenika, indeks kondicije se koristi kao pokazatelj tržišne kvalitete školjkaša i rentabilnosti uzgojnog područja (Marušić i sur., 2009).

Područje Medulinskog zaljeva ima izrazito dugu tradiciju izlova i uzgoja školjkaša (Hrs-Brenko, 1980). Trenutno se na području zaljeva nalaze dva uzgajališta školjkaša, a cijeli zaljev čini sastavni dio ekološke mreže Natura 2000. Unatoč dugoj tradiciji uzgoja i ekološkoj važnosti područja, podaci o infestaciji polihetima roda *Polydora* i kondicijskom stanju kamenica u Medulinskom zaljevu ne postoje. Stoga je cilj ovog rada istražiti spomenute parametre za europsku plosnatu kamenicu iz uzgojne i divlje populacije te pacifičku kamenicu iz divlje populacije na području Medulinskog zaljeva.

Materijal i metode

Uzorkovanje je provedeno 19. kolovoza 2023. godine na tri postaje u Medulinskom zaljevu (Slika 1). Na svakoj postaji prikupljeno je 30 kamenica. Na postaji „Rt Kašteja“ europske plosnate kamenice prikupljene su na 12 m dubine, na postaji „Portić“ pacifičke kamenice prikupljene su s područja izmjene plime i oseke, a europske plosnate kamenice iz uzgajališta u Pomeru prikupljene su s 3 m dubine.



Slika 1. Medulinski zaljev označenim postajama uzorkovanja

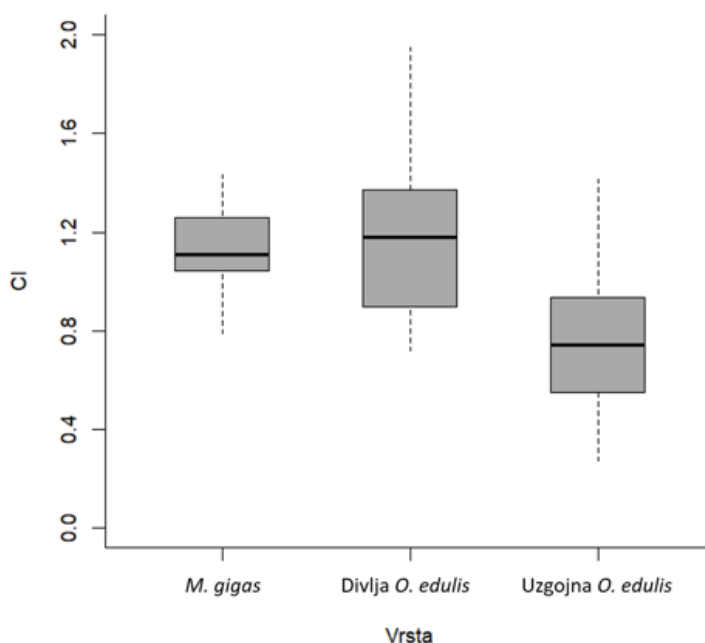
Za određivanje indeksa kondicije kamenice su očišćene od obraštaja, ocjeđene na upijajućem papiru te potom vagane digitalnom vagom preciznosti $\pm 0.01g$. Svaka kamenica je otvorena presjecanjem mišića aduktora, meso je odstranjeno i sušeno na $105^{\circ}C$ u trajanju od 24 sata. Indeks kondicije (CI) je računat po formuli (Marguš, 1985): $CI = \text{masa suhog mesa} / \text{masa cijelog školjkaša} \times 100$.

Kako bi se odredio broj poliheta u ljušturi, obje ljušture su odložene u staklene posude ispunjene 2M otopinom klorovodične kiseline, zatvorene poklopcem i ostavljene 48 sati da se otope. Nakon 48 sati otopina klorovodične kiseline je procjeđena kroz metalno cjedilo kako bi se prebrojali poliheti. Bilježen je broj poliheta za svaku kamenicu iz obje ljušture zajedno, na temelju čega je računat srednji broj parazita za svaku postaju po formuli: srednji broj parazita=ukupan broj parazita/ukupan broj kamenica. Također je izračunan postotak infestiranih kamenica za svaku postaju prema formuli: % infestiranih kamenica=broj infestiranih kamenica/broj pregledanih kamenica.

Rezultati su obrađeni računalnim programom R (2023). Tip distribucije određen je pregledom histograma i korištenjem Shapiro-Wilk testa. Za određivanje homogenosti varijanci modela korišten je Fligner-Killeen Test. Za statističku analizu indeksa kondicije korišten je Tukey post hoc test, dok je za analizu srednjeg broja parazita na različitim postajama korišten Dunn post hoc test budući da vrijednosti ne prate normalnu distribuciju. Za određivanje korelacije između broja poliheta u svakoj kamenici i CI korištena je Spearmanova korelacija.

Rezultati i rasprava

Srednja vrijednost CI bila je najniža kod *O. edulis* iz uzgojne populacije (0.75 ± 0.29), dok su ove vrijednosti bile slične kod *M. gigas* (1.14 ± 0.30) i divlje populacije *O. edulis* (1.18 ± 0.38) (Grafikon 1).



Grafikon 1. Indeks kondicije (CI) za *M. gigas* te uzgojnu i divlju populaciju *O. edulis*

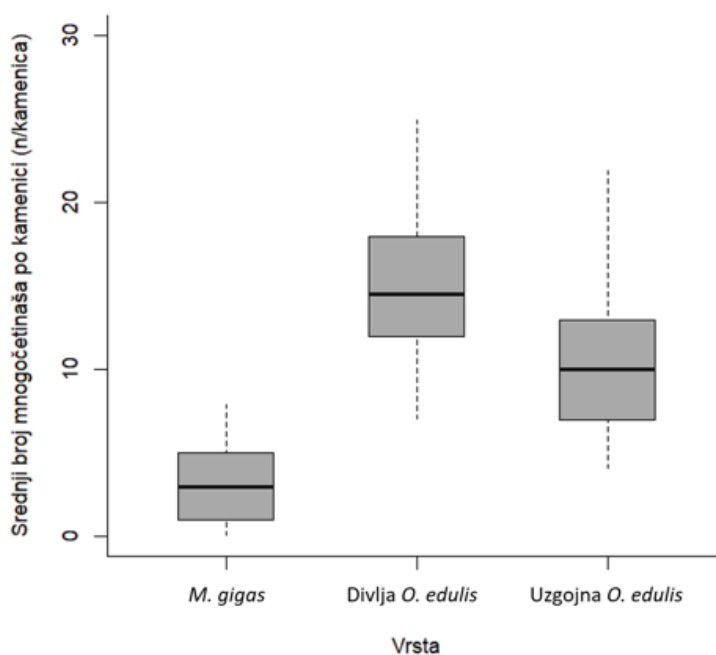
Tukey post hoc testom utvrđeno je da se indeks kondicije *O. edulis* iz uzgojne populacije statistički razlikovao od ostalih kamenica ($p < 0.05$) (Tablica 1).

Tablica 1. Parametri Tukey post hoc testa korištenog za statističku analizu indeksa kondicije (CI)

Usporedbe parova	diff	lwr	upr	p adj
Divlja <i>O. edulis</i> – <i>M. gigas</i>	0.03243	-0.1675	0.2325	0.9209
Uzgojna <i>O. edulis</i> – <i>M. gigas</i>	-0.3937	-0.6013	-0.1861	0.0001
Uzgojna <i>O. edulis</i> – Divlja <i>O. edulis</i>	-0.4261	-0.6337	-0.2186	0.00001

Iako vrsta *M. gigas* zbog obitavanja u pojasu izmjene plime i oseke dobiva hranu periodično, prosječna vrijednost CI u ovom istraživanju je slična ili viša u odnosu na vrstu *O. edulis* koja je konstantno uronjena. Mercado-Silva (2005) također nije utvrdio razlike u CI kod kamenica vrste *Crassostrea virginica* između konstantno uronjenih populacija i populacija koje obitavaju u pojasu izmjene morskih mjena. Kao objašnjenje autor navodi niz fizioloških prilagodbi na smanjeni period ishrane i razlike u dostupnosti hrane na različitim dubinama. CI se kod školjkaša mijenja s obzirom na sezonalnost i promjene u okolišnim čimbenicima, no najniži je neposredno nakon mrijesta (Gosling, 2003; Gavrilović i sur., 2010). Uz odgovarajuću količinu hrane, vrsta *M. gigas* se u sjevernom Jadranu mrijesti ljeti (Ezgeta-Balić i sur., 2020), a vrsta *O. edulis* u proljeće (Carlucci i sur., 2010). U ovom istraživanju zabilježena je jedna jedinka vrste *O. edulis* u sivom sjemenu, dok je u ostatku kamenica iste vrste konzistencija mesa bila vodenasta, a gonade su izostajale što je uobičajeno za period nakon mrijesta. Osim navedenog, na CI mogli su utjecati debljina i oblik ljuštura kamenica, koji je znatno varijabilniji u divljim populacijama velike gustoće zbog međusobnog prerastanja, dok se u uzgoju nastoji održati čim pravilniji i ujednačeniji. Kod *O. edulis* iz uzgojne populacije je pri otvaranju kamenica primjećena izrazita krhkost ljuštura. Na jedinkama s izrazito krhkim ljušturama, osim intenzivnije infestacije polihetima roda *Polydora*, zabilježena je infestacija spužvama roda *Cliona* i školjkašima vrste *Hiatela arctica* (Linnaeus, 1767) što je također moglo utjecati na CI. Osim toga, narušena čvrstoća i struktura ljuštura povećava podložnost kamenica predaciji i uzrokuje dodatne gubitke u proizvodnji (Handley, 1992).

Srednji broj poliheta po kamenici bio je najveći kod *O. edulis* iz divlje populacije (14.5 ± 5.16), a najmanji kod vrste *M. gigas* (3.00 ± 3.24), dok je vrste *O. edulis* iz uzgojne populacije iznosio 10.00 ± 4.40 (Grafikon 2).



Grafikon 2. Srednji broj poliheta po kamenici za *M. gigas* te uzgojnu i divlju populaciju *O. edulis*

Dunn post hoc testom utvrđene su statistički značajne razlike između svih analiziranih skupina ($p < 0.05$) (Tablica 2).

Tablica 2. Parametri Dunn post hoc testa korištenog za statističku analizu srednjeg broja poliheta po kamenici

Usporedbe parova	Uočena razlika	Kritična razlika	Statistički značaj testa (p<0.05)
<i>M. gigas</i> – divlja <i>O. edulis</i>	45.46667	15.43452	Značajno
<i>M. gigas</i> – uzgojna <i>O. edulis</i>	27.86026	16.01716	Značajno
divlja <i>O. edulis</i> - uzgojna <i>O. edulis</i>	17.60641	16.01716	Značajno

Postotak infestiranih jedinki je kod *M. gigas* iznosio 90%, dok je kod obje skupine *O. edulis* (uzgojne i divlje populacije) bio 100%.

Koeficijent Spearmanove korelacije između CI i broja poliheta u svakoj kamenici iznosio je -0.05 kod vrsta *C. gigas* i *O. edulis* iz uzgojne populacije te -0.07 kod *O. edulis* iz divlje populacije.

Iako čimbenici koji utječu na stupanj infestacije kamenica polihetima roda *Polydora* nisu potpuno razjašnjeni, smatra se da ona primarno ovisi o vjerojatnosti susreta poliheta i školjkaša, odnosno o režimu strujanja mora, zbog čega su općenito pridnene populacije podložnije infestacijama (Bower i sur., 1994). Navedeno podupiru izjave uzgajivača da su veći gubitci zbog parazitizma na pridnenim kulturama. Također, kamenice koje su ranije bile infestirane ovim parazitom imaju veću šansu za ponovu infestaciju (Cole, 2020). Periodično sušenje ljuštura kamenica sukladno ciklusima plime i oseke snižava intenzitet infestacija (Morse, 2015). Posljedično, *M. gigas* uzorkovana u pojasu izmjene morskih mjena u ovom istraživanju imala je najniži srednji broj parazita po kamenici i niži postotak infestiranih jedinki. Kod vrste *O. edulis* iz uzgojne i divlje populacije koje su obitavale u suspenziji, bliže sedimentu, utvrđen je značajno veći srednji broj poliheta po kamenici, a infestacija je kod obje skupine uzorka bila stopostotna. Usto, Lemasson (2019) je opisao preferencijalni parazitizam vrste *Polydora ciliata* (Johnston, 1838) prema vrsti *O. edulis*, dok *M. gigas* pokazuje veću otpornost.

Intenzivne infestacije polihetima roda *Polydora* uzrokuju velike gubitke energije kamenica u procesu zacjeljivanja ljuštura što rezultira slabijim rastom, povećanom osjetljivošću na bolesti, lošijom kondicijom i kvalitetom mesa te povećanim mortalitetom (Handley, 1992). S obzirom na razlike u promatranim parametrima, *M. gigas* iz divlje populacije pokazala je jednako, odnosno bolje kondicijsko stanje i niži supanj infestacije polihetima roda *Polydora* u odnosu na *O. edulis* iz divlje i uzgojne populacije. Međutim, stupanj infestacije kod sve tri istraživane skupine kamenica nije značajno utjecao na kondiciju školjkaša.

Zaključak

Utvrđene su značajne razlike u indeksu kondicije, srednjem broju parazita po kamenici i postotku infestiranih jedinki. Najniži indeks kondicije bio je kod uzgojne populacije *O. edulis*, dok su vrijednosti bile slične kod *M. gigas* i divlje populacije *O. edulis*. Najniži prosječni broj poliheta po kamenici te niži broj infestiranih jedinki utvrđen je kod *M. gigas*. Vrlo niska negativna korelacija između indeksa kondicije i broja poliheta kod sve tri istraživane skupine pokazuje da utvrđeni stupanj infestacije ne utječe značajno na kondiciju jedinki. Za potpunu analizu ovih parametara potrebno je provesti istraživanje koje obuhvaća sve sezone.

Literatura

- Airoldi L., and Beck M.W. (2007). Loss, status and trends for coastal marine habitats of Europe. *Oceanography and Marine Biology: An Annual Review*. 45: 345–405.
- Bower S.M. (1997). Synopsis of infectious diseases and parasites of commercially exploited shellfish: shell-boring Polychaetes of Abalone. *Annual Review of Fish Diseases*. 4: 1-199.
- Carlucci R., Sassanelli G., Matarrese A., Glove A., D'Onghia G. (2010). Experimental data on growth, mortality and reproduction of *Ostrea edulis* (L., 1758) in a semi enclosed basin of the Mediterranean Sea. *Aquaculture*. 306: 167e176.
- Cole S.M., Dorgan K.M., Walton W., Dzwonkowski B., Coogan J. (2020). Seasonal and spatial patterns of mudblister worm *Polydora websteri* infestation of farmed oysters in the northern Gulf of Mexico. *Aquaculture Environment Interactions*. 12: 297-314.

- EzgetaBalić D., Radonić I., Bojanić Varezić D., Zorica B., Arapov J., Stagličić N., Jozić S., Peharda M., Briski E., Lin Y., Šegvić Bubić T. (2020). Reproductive cycle of the non-native Pacific oyster, *Crassostrea gigas*, in the Adriatic Sea. *Mediterranean Marine Science*. 21: 10.12681/mms.21304.
- Ezgeta-Balić D., Šegvić – Bubić T., Stagličić N., Lin Y., Bojanić Varezić D., Grubišić L., Briski E. (2019). Rasprostranjenost nezavičajne vrste kamenice *Magallana gigas* (Thunberg, 1793) duž istočne obale. *Acta Adriatica*. 60: 137-146.
- Gavrilović A., Jug-Dujaković J., Ljubičić A., Conides A., Strunjak-Perović I., Topić Popović N., Čož-Rakovac R., Van Gorder S. (2010). Meat quality of European Flat Oyster *Ostrea edulis* in relation with variations of different environmental parameters in the Bay of Mali Ston. *Proceedings of the European Aquaculture Society Conference, Porto, October 5.-8. 2010*. 508-509.
- Gosling E. (2008). *Bivalve Molluscs Biology, Ecology and Culture*. Wiley-Blackwell. 456 pp.
- Handley S.J. (1992). The dynamics of spionid polychaete (mudworm) infestations of the Pacific oyster *Crassostrea gigas* (Thunberg) in northern New Zealand. Unpublished MSc. Thesis. University of Auckland 100pp.
- Hrs-Brenko M. (1980). Pomorski zaljev: uzgoj i istraživanja školjkaša. Prilozi o zavičaju, 2. Čakavski sabor – Katedra Pula. Pula.
- Lemasson A.J., and Knights A.M. (2019). Preferential parasitism of native oyster *Ostrea edulis* over non-native *Magallana gigas* by a Polydorid worm. *Estuaries and Coasts*. 42: 1397-1403.
- Loose S.M., Peschel A., Grebitus C. (2013). Quantifying effects of convenience and product packaging on consumer preferences and market share of seafood products: the case of oysters. *Food Quality and Preference*. 28: 492–504.
- MacKenzie C.L., Burrell Jr. V.G., Rosenfield A., Hobart W.L. (1997). *The History, Present Condition and Future of the Molluscan Fisheries of North and Central America and Europe*, vol. 3. Europe. U.S. Department of Commerce, Seattle, Washington. 240 pp
- Mann R. (1978). A comparison of morphometric, biochemical and physiological index of condition in marine bivalve molluscs. In: *Energy and environmental stress in aquatic systems*. Woods Hole Oceanographic Institution, Massachusetts. 484-497.
- Marguš D., and Teskeredži E. (1984). Indeks kondicije dagnji (*Mytilus galloprovincialis*) u estuariju rijeke Krke. *Morsko ribarstvo*. 1: 17-20.
- Marušić N., Vidaček S., Medić H., Petrak T. (2009). Indeks kondicije dagnji (*Mytilus galloprovincialis*) u uvali Budavai u zaljevu Raša. *Ribarstvo*. 67: 91-99.
- Mercado-Silva N. (2005). Condition index of the eastern oyster, *Crassostrea virginica* (Gmelin, 1791) in sapelo island georgia-effects of site, position on bed and pea crab parasitism. *Journal of shellfish research*. 24: 121–126.
- Morse D.L., Rawson P.D., Kraeute J.N. (2015). *Mud Blister Worms and Oyster Aquaculture*. Maine Sea Grant Publications. 46.
- R Core Team (2023). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Santeramo F.G., Carlucci D., De Devitiis B., Nardone G., Viscecchia R. (2017). On consumption patterns in oyster markets: the role of attitudes. *Marine Policy*. 79: 54–61.
- Sato-Okoshi W., Okoshi K., Abe H., Li J.-Y. (2013). Polydorid species (Polychaeta, Spionidae) associated with commercially important mollusk shells from eastern China. *Aquaculture*. 406–407: 153–159.

Condition index and infestation of the shell of the oysters, *Ostrea edulis* (Linnaeus, 1758) and *Magellana gigas* (Thunberg, 1793), with the polychaete *Polydora* sp. in the Medulin Bay

Abstract

Polychaetes from the genus *Polydora* are the most common cause of visible damage to oyster shells, resulting in reduced market value, and can cause poorer condition, slower growth, and increased mortality. The aim of this research was to determine the condition index and intensity of infestation of oysters, *Ostrea edulis* and *Magellana gigas*, by *Polydora* sp. in the Medulin Bay area as indicators of their quality. The wild population of *M. gigas* and the wild and breeding population of *O. edulis* were sampled in August 2023. The lowest condition index was found in the breeding population of *O. edulis*, while the values were similar in *M. gigas* and wild population of *O. edulis*. The lowest average number of polychaetes per oyster and the lowest number of infested individuals were found in *M. gigas*. The very low negative correlation between the condition index and the number of polychaetes in all three investigated groups shows that the established degree of infestation does not significantly affect the condition of the specimens.

Keywords: European flat oyster, *Crassostrea gigas*, pacific oyster, oyster quality, oyster condition

Contribution to the Characterisation of False Indigo Honey (*Amorpha fruticosa* L.)

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Abstract

False indigo (*Amorpha fruticosa* L.) is one of the nectariferous plants present mostly in the area of continental Croatia. It grows in semi-natural and natural habitats, mostly along river banks. Though its invasive growth nature, it is very important plant species for the bees because of its rich nectar production. The aim of this study was to contribute to the characterization of false indigo honey by covering melissopalynological, physicochemical, sensory aspect, so as antioxidant capacity (FRAP and DPPH assay) and total phenolic content. Analysed parameters showed that this unifloral honey type is valuable product, regardless of the invasiveness of the plant from which it originates.

Keywords: false indigo honey, characterization, sensory profile, pollen spectrum, physicochemical properties

Introduction

False indigo (*A. fruticosa*) was first used in Europe as ornamental plant, but due to its fast spreading because of production of numerous offsprings which have ability to disperse to a large distance it became invasive shrub (Richardson et al., 2000; Sârâteanu et al., 2008). It's characterised with 1 – 3(6) m high with impair-pinnate leaves, bright green, with 5 - 12 elliptic – oblong leaflets dotted on the back. Flowering period is from May to July and it grows in meadows, riverbanks and along the roads. Plant is indifferent to soil reactions and it can survive extreme climatic conditions (Dumitraşcu et al., 2010). Despite its invasive nature and negative impact upon environment (Sârâteanu, 2008) there is a wide range of uses of different plant organs from flower to roots (medicinal, food and industrial application). This leads that *A. fruticosa* is quite controversial plant because of its invasive nature and possibility for exploitation for various purpose (Grabić et al., 2022).

One of the positive sides of false indigo plant is production of nectar which is highly attractive to bees thus enabling production of honey whose characteristics need to be more described. Honey is a natural sweet substance produced by honey bees (*Apis mellifera* L.) from the nectar of plants, secretion of living parts of the plants or excretions of plant-sucking insects and its price depends on its botanical and geographical origin. Unifloral honeys stand out as valuable, high-quality honeys with higher price comparing to multifloral honeys. In Europe more than 100 species produce different unifloral honey types. Some of the most popular unifloral honey types, from the aspect of their abundance and commercial relevance, are lime, black locust, chestnut, honeydew honey etc. Because honey is often target of fraud, it is very important to determine its botanical origin, and that is one reason why it is important to know characteristic properties of unifloral honey types.

Due to the lack of information about unifloral honey from *A. fruticosa*, the aim of this study was to determine main pollen, physicochemical and antioxidant properties of this honey type and to contribute to its characterisation.

Material and methods

Samples

Seven samples of *A. fruticosa* honey was collected in continental Croatia in the beekeeping season 2018. Samples were provided from local beekeepers and they were placed in the glass bottles, labelled and stored in the dark at room temperature until analyses.

Melissopalynological analysis

Melissopalynological analysis was conducted according to DIN 10760:2002-05. After preparation of specimen, pollen grains were identified and then 500 pollen grains were counted. Analyses were done in duplicate and results were expressed as the percentage of pollen of the required plant species in relation to the pollen from nectar plants. Requirement for false indigo honey is more than 45% of *A. fruticosa* pollen grains (Ministry of Agriculture, Fisheries and Rural Development, 2009).

Physicochemical analyses

Determination of physicochemical parameters were conducted in accordance to official methods for honey analysis (International Honey Commission, 2009; AOAC International, 2002) soon after honey collection. Refractometric method was used for water content determination at DR-A1 refractometer (Atago, Tokyo, Japan) and conductometric method for electrical conductivity determination with SevenEasy conductometer (Mettler-Toledo GmbH, Zürich, Switzerland) in 20% honey solution (dry matter basis) using ultra-pure distilled water at 20°C (results expressed as milliSiemens per centimeter - mS/sm). Hydroxymethylfurfural (HMF) content was determined by spectrophotometric method after White using UV1800 spectrophotometer (Shimadzu, Kyoto, Japan) (results expressed as milligrams per kilogram of honey). Honey diastase activity was determined with Phadebas method where blue water-soluble fragments were determined using the spectrophotometer at 620 nm. Lovibond Honey Color-Pod (Amesbury, UK) was used for the determination of color grading and results were expressed as mm in the Pfund scale. The pH and free acidity, were determined using the titrimetric method with Mettler Toledo pH meter (GmbH, Zürich, Switzerland). Carbohydrate content was determined using Shimadzu (Kyoto, Japan) high pressure liquid chromatography (HPLC) system consisting of an LC-20AD Prominence solvent delivery module, a DGU-20A5R degassing unit, an SIL-10 AF automatic sample injector, and an RID-10A refractive index detector. The instrumental system was coupled with a computer equipped with LabSolutions Lite Version 5.52 software.

Phenolic content and antioxidant capacity

Phenolic content was determined using modified Folin-Ciocalteu method by Beretta et al. (2005) (mg gallic acid/kg honey). Ferric Reducing Antioxidant Power (FRAP) was determined according to Benzie and Strain (1996) method ($\mu\text{M Fe(II)}$). Antioxidant capacity by DPPH assay was determined using method described by Brand-Williams et al. (1995) and Beretta et al. (2005) where IC_{50} was calculated (honey concentration that causes a decrease in the initial DPPH radical concentration by 50%). All analyses were conducted in duplicates. Average values and standard deviations were calculated using Microsoft Excel 2021 (Microsoft Corp.) software.

Results and discussion

Melissopalynological analysis of false indigo honey samples showed that *A. fruticosa* pollen (Figure 1) is present in the range 43.7 – 59.0%. (Table 1). Requirement for classification of honey as unifloral is $\geq 45\%$ of specific pollen type (if not specified differently by national legislation (Ministry of Agriculture, Fisheries and Rural Development, 2009). Rašić et al. (2018.) analysed in their research honey from north-eastern Croatia where *A. fruticosa* pollen frequency of occurrence was around 70%. Two samples (A1 and A6) had slightly lower share of *A. fruticosa* pollen, but due to characteristic sensory and physicochemical properties they were included in this study. Other pollen types present in almost all samples were *Apiaceae*, *Brassica* sp., *Rosaceae*, *Robinia pseudoacacia* L. and *Trifolium* sp. Which were also present in *A. fruticosa* sample in the research of Sabo et al. (2008). In all samples honeydew elements were present in lower amount.



Figure 1. Pollen grain of *Amorpha fruticosa* (800× magnification)

Table 1. Pollen analysis of *Amorpha fruticosa* honey samples

Pollen type	%						
	A1	A2	A3	A4	A5	A6	A7
<i>Amorpha fruticosa</i> L.	43.7	45.8	59.0	46.2	49.8	43.8	54.0
Apiaceae	15.6	6.4	0.8	3.6	7.2	-	5.3
Asteraceae (other)	3.1	-	-	-	-	7.8	-
<i>Bellis</i> sp.	2.5	2.0	0.6	1.8	1.2	-	-
Boraginaceae	0.6	-	-	0.2	-	-	-
Brassicaceae (other)	-	-	-	-	-	-	3.7
<i>Brassica</i> sp.	4.2	7.4	4.2	5.8	-	12.8	3.7
<i>Carex</i> sp.*	-	-	-	-	-	-	-
<i>Castanea sativa</i> Mill.	-	-	-	5.0	4.2	2.4	-
<i>Cornus sanguinea</i> L.	-	10.4	3.0	6.4	-	7.2	-
<i>Euphorbia</i> sp.	-	-	-	-	-	2.2	2.0
<i>Fagopyrum</i> sp.	12.8	6.2	-	3.6	-	-	-
<i>Helianthus annuus</i> L.	-	-	2.4	-	-	-	-
<i>Loranthus europaeus</i> Jacq.	3.3	1.8	-	-	-	-	-
<i>Potentilla</i> sp.	-	1.8	-	-	-	-	-
Roasceae	5.0	7.4	5.2	4.2	4.8	4.2	-
<i>Robinia pseudocacia</i> L.	1.4	1.2	7.8	-	5.4	2.0	2.7
<i>Salix</i> sp.	-	-	-	-	7.0	5.8	-
<i>Taraxacum officinale</i> (L.) Weber	3.1	-	1.0	-	-	-	-
<i>Tilia</i> sp.	-	0.4	12.2	12.2	-	0.8	-
<i>Trifolium</i> sp.	-	-	2.4	3.8	9.4	6.4	22.6
HDE	+	+	+	+	+	+	+
Other	4.7	9.2	1.4	7.2	11.0	4.6	6.0

*nectarless pollen type

HDE (+) honeydew elements present

Results of physicochemical parameters are shown in Table 2. All analysed samples had water content below 20% (criteria prescribed by Ministry of Agriculture, 2015) which ranged from 15.5 – 18.1%. Water content mostly depends on beekeeping technology and weather conditions in the beekeeping season and it is important quality parameter. Electrical conductivity of analysed samples ranged from 0.14 – 0.28 mS/cm with average value 0.20 mS/

cm. These values are comparable with values for rape honey (Bilić Rajs et al., 2017) and black locust honey who is characterised with even lower electrical conductivity values (Uršulin-Trstenjak et al., 2017). In the research of Hrg Matušin (2018) obtained average value for electrical conductivity for this honey type were slightly higher (0.32 mS/cm), while Svečnjak et al. (2015) and Sabo et al. (2008) obtained similar results (0.22 mS/cm). (Parameters which indicate proper honey processing (hydroxymethylfurfural - HMF and diastase activity - DN) were in ranges prescribed by Ministry of Agriculture (2015). Color expressed as mm Pfund ranged from 10 – 30 with average value 19 which classifies false indigo honey in category of white honey types (Figure 2). This honey has from sensory aspect weak odour and pale yellow color with reddish reflection. Average pH and free acidity values were 3.91 and 9 mmol/kg, respectively, which is also similar to the values for black locust honey (Uršulin-Trstenjak et al., 2017), so as for false indigo honey reported by Svečnjak et al. (2015) and Sabo et al. (2008). Carbohydrate profile, fructose and glucose ratio (average value 1.3) showed that false indigo honey does not have high tendency to crystalize. The phenolic content and antioxidant capacity (FRAP and DPPH assay) are presented in

Table 2. Physicochemical parameters of *Amorpha fruticosa* honey ($n = 7$)

Parameter	Unit of Measurement	Average	Minimum	Maximum	SD *
Water content	%	17.0	15.5	18.1	0.91
EC*	mS/cm	0.20	0.14	0.28	0.06
Diastase activity (DN)	/	27.5	20.5	41.0	6.89
HMF	mg/kg	3.5	0	6.8	2.52
Color	mm Pfund	19	10	30	6.58
pH	/	3.91	3.71	4.05	0.12
Free acidity	mmol/kg	9	5.75	12.8	2.34
Fructose	g/100 g	39.9	39.1	40.9	0.62
Glucose	g/100 g	30.7	29.7	33.1	1.26
Sucrose	g/100 g	0.7	0.1	2.2	0.96
Maltose	g/100 g	2.6	1	2.2	0.96
Melezitose	g/100 g	0.6	0.1	1.1	0.44
Raffinose	g/100 g	0.2	0.1	0.2	0.05
Xylose	g/100 g	ND*	ND*	ND*	ND*
F+G	g/100 g	70.5	69	72.5	1.34
F/G	/	1.3	1.2	1.4	0.06

EC* - electrical conductivity; ND*—not detected; SD*—standard deviation.

Table 3. The phenolic content varied between 115.40 mg gallic acid/kg of honey and 160.46 mg gallic acid/kg of honey with an average of 131.81 mg gallic acid/kg of honey. FRAP values were between 66.83 μM Fe (II) and 226.00 μM Fe (II), with an average of 129.93 μM Fe (II), while DPPH-IC₅₀ values were from 22.02 mg/mL to 50.80 mg/mL with average value 40.11 mg/mL. Obtained results for phenolic content are higher than those for well-known honey types (sage, black locust and lime honey) reported by Flanjak et al. (2016). Average FRAP values were higher than those reported by Flanjak et al. (2016) for black locust honey (36.1 μM Fe (II)), but lower in comparison to other honey types in the same research. DPPH reported as IC₅₀ value (honey concentration that causes a decrease in the initial DPPH radical concentration by 50%) average value is comparable to those for lime honey, but higher than sage, chestnut and honeydew honey (Flanjak et al., 2016) which was expected as they are known for their high antioxidant activity.

Table 3. Phenolic content and antioxidant capacity of *Amorpha fruticosa* honey (n = 7)

Parameter	Unit of Measurement	Average	Minimum	Maximum	SD *
Phenolic content	mg gallic acid/kg honey	131.81	115.40	160.46	15.71
FRAP value	$\mu\text{M Fe (II)}$	129.93	66.83	226.00	50.80
DPPH -IC ₅₀	mg/mL	40.11	22.02	50.80	9.77

DPPH-IC₅₀ honey concentration that causes a decrease in the initial DPPH radical concentration by 50%.

* SD—standard deviation

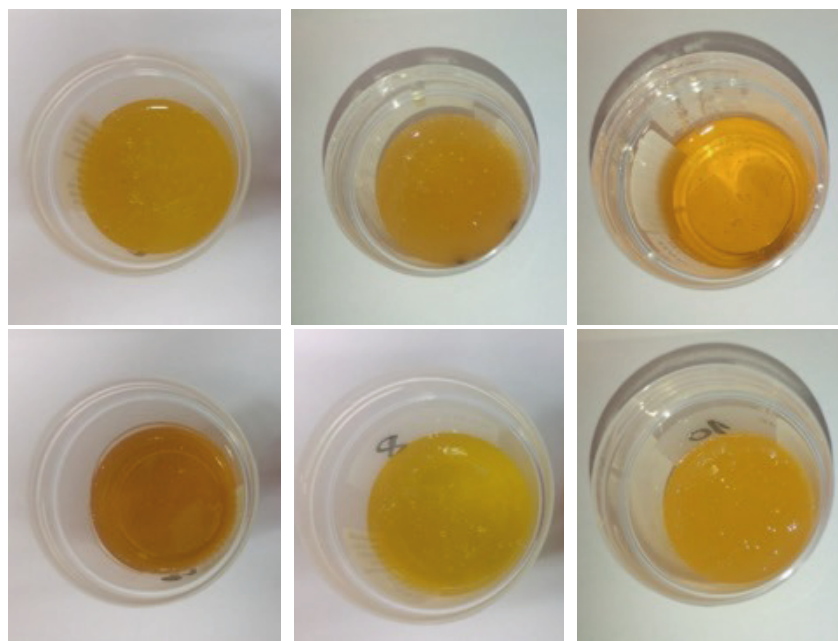


Figure 2. Color of analysed samples of *Amorpha fruticosa* honey

Conclusions

This research is contribution to the characterisation of false indigo (*A. fruticosa*) honey, covering melissopalynological, physicochemical and antioxidant aspect. This honey is characterised with low electrical conductivity values comparable with those for black locust and rape honey, low crystallisation tendency, weak odour and yellow color with reddish reflection. Its antioxidant capacity and phenolic content is not negligible, quite the opposite, in comparison to other well-known honey types some parameters are higher. False indigo honey abundance could be exploited in a good manner as it could represent cheap source of valuable nutrients. Taking into account *A. fruticosa* invasive nature and positive effects (e.g., honey production) there is arising need for smart management of spreading and growing areas for this plant.

References

- AOAC (2000). Official Methods of Analysis, 17th ed.; Rev. 1, Chapter 44; AOAC Official Methods, Suppl; Association of Official Analytical Chemists: Rockville, MD, USA.
- Benzie I.F.F., and Strain J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant power": The FRAP assay. *Analytical Biochemistry*. 239: 70–76.
- Beretta, G., Granata, P., Ferrero, M., Orioli, M., Facino, R.M. (2005). Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. *Analytica Chimica Acta*. 533: 185-191.
- Bilić Rajs B., Flanjak I., Mutić J., Vukojević V., Đurđić S., Primorac Lj. (2017). Characterization of Croatian Rape (*Brassica* sp.) Honey by Pollen Spectrum, Physicochemical Characteristics and

- Multielement analysis by ICP-OES. *Journal of AOAC International*. 100: 881–888.
- Brand-Williams W., Cuvelier M.E., Berset C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft und Technologie*. 28: 25–30.
- Deutsches Institut für Normung DIN:10760 (2002). Determination of relative pollen content of honey.
- Dumitrascu M., Grigorescu I., Nastase M., Dragota C., Kucsicsa G. (2010). The Main Environmental Driving Forces of the Invasive Plant Species in the Romanian Protected Areas. BALWOIS 2010 – Ohrid. Republic of Macedonia – 25. 29 May 2010. https://balwois.com/wp-content/uploads/old_proc/ffp-1600.pdf
- Flanjak I., Kenjerić D., Bubalo D., Primorac L. (2016). Characterisation of selected Croatian honey types based on the combination of antioxidant capacity, quality parameters, and chemometrics. *European Food Research and Technology*. 242: 467–475.
- Grabčić J., Ljevnaić-Mašić B., Zhan A., Benka P., Heilmeyer H. (2022). A review on invasive false indigo bush (*Amorpha fruticosa* L.): Nuisance plant with multiple benefits. *Ecology and Evolution*. 12: e9290, 1-16.
- Hrg Matušin Ž. (2018). Procjena kvalitete unifloernih vrsta meda s područja Republike Hrvatske u razdoblju od 2015. do 2016. *Proceeding-International Symposium on Agriculture, Vodice, Hrvatska*.
- International Honey Commission (2009). Harmonised methods of the European Honey Commission. <https://www.bee-hexagon.net/>. Accessed 01 November 2023.
- Ministry of Agriculture (2015). Regulation ordinance on the quality of honey. *Official Gazette* 30: 3–5.
- Ministry of Agriculture, Fisheries and Rural Development (2009). Ordinance on the quality of unifloral honey. *Of Gazette*. 122:15–16.
- Rašić S., Štefanić E., Antunović S., Jović J., Kristek S. (2018). Pollen analysis of honey from north-eastern Croatia. *Agriculture*. 24: 43-49.
- Richardson D.M., Pyšek P., Rejmánek M., Barbour M.G., Panetta F.D., West C.J. (2000). Naturalization and invasion of alien plants: Concepts and definitions. *Diversity and Distributions*. 6: 93–107.
- Sabo M., Vasić M., Banjari I., Flanja I., Bačić T. (2008). Melissopalynological, Physicochemical and Sensory Characteristic of Honey of Three Floral Species in Croatia. *Deutsche Lebensmittel-Rundschau: Zeitschrift für Lebensmittelkunde und Lebensmittelrecht*. 10: 79-83.
- Sărățeanu V., Horablaga M.N., Stroia M.C., Butnariu M., Bostan C. (2008). Approach on the shrub invasive species impact on western Romanian grasslands. *Research Journal of Agricultural Science*. 40: 315-318.
- Svečnjak L., Bubalo D., Baranović G., Novosel H. (2015). Optimization of FTIR-ATR spectroscopy for botanical authentication of unifloral honey types and melissopalynological data prediction. *European Food Research and Technology*. 240: 1101-1115.
- Uršulin-Trstenjak N., Levanić D., Grabar I., Koldenjak M., Bošnjir J. (2017). Physico-Chemical Profiles of Croatian Honey with an Overview of Its Consumption among Healthcare Students. *Journal of Applied Health Sciences*. 3: 51-59.

Seasonal video-monitoring of fauna and biodiversity in Martinska bay, a coastal Natura 2000 area near Šibenik

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Abstract

Monitoring of marine fauna with non-destructive methods may provide strategic data for the ecological management of policy important sites, such as Natura 2000 areas. In this paper, images from a stationary underwater camera at 5 meters depth in Martinska Bay (Šibenik, Croatia) were analyzed. Captured during daylight in selected weeks of January, April, July, and October 2019, the dataset included 91,758 photographs, with 2,911 removed due to camera obstructions. A total of 54 animal species were identified, 82% of which were fish, with the largest percentage (72.2%) being economically significant species. Invertebrate species were also recorded. Other faunal iconic items observed were: *Phalacrocorax aristotelis* (Linnaeus, 1761) with 11 recorded occurrences, *Caretta caretta* (Linnaeus, 1758) with 8 recorded occurrences, and *Pecten jacobaeus* (Linnaeus, 1758) with 2 recorded occurrences.

Keywords: Stationary video cameras, Natura 2000, seasonality, monitoring

Introduction

Natura 2000 is a coherent European ecological network composed of areas of inhabited types of natural habitats and habitats of wild species of interest to the European Union. It allows for the conservation or, where necessary, the re-establishment of a favourable natural state of certain types of habitats and habitats of species in their natural area of distribution. The monitoring of coastal areas is often poor or non-existent (Bakran-Petricioli, 2011), despite the legal obligation for the Natura 2000 ecological network (OG 127/19). Aguzzi et al. (2020) emphasize the importance of continuous surveillance of fragile and high-biodiversity ecosystems, such as those in the Natura 2000 network. Monitoring the biodiversity of residing and migrating fauna on a spatiotemporal scale can be crucial for the preservation of marine ecosystems, as it can serve as an indicator of ecosystem functioning (Tittensor et al., 2010; Costello and Chaudhary, 2017). Assessment of marine fauna populations using conventional sampling methods such as towed fishing gears or set nets can provide useful data, however, it is limited by the selectivity of the tool (Mallet and Pelletier, 2014). Video monitoring is a viable, cost-effective alternative to improve ecosystem management, especially in areas where fishing is not allowed (Aguzzi et al., 2020). Information obtained in high frequency with underwater cameras is highly valuable, as the abundance of marine fauna varies greatly over time (Guidetti et al., 2003; Azzurro et al., 2010). It has proven useful for monitoring the deep sea (Stefanni et al., 2022) and coastal areas (Francescangeli et al., 2022).

The aim of this research is to monitor the seasonal presence and frequency of various marine fauna in Martinska bay near Šibenik (East Adriatic) and to compare the results with previous research in the same area.

Material and methods

This research was conducted in Martinska Bay near Šibenik, in the Natura 2000 ecological network area (HR3000171) (Figure 1A, 1B). The area is combined with the mainland and covers a total of 4423.8 hectares. The mouth of the Krka River is a karst-type estuary with specific oceanographic characteristics: stratification of the water column, maximum underground temperature, and long periods of saltwater retention in the estuary (Aguzzi et al., 2020). The estuary is inhabited by a biocenosis of algae growing on the solid bottom of the infralittoral. Its depth limits are determined by the amount of light, which is often enough in these habitats. It spreads from the sea surface to a depth of 30 meters. A large number of primary producers is the basis of the lives of many consumers, and the biomass in these communities can reach very high values (Bakran-Petricioli, 2011).

This area is also protected as a Significant Landscape named "Channel – Port". A significant part of the Krka River watercourse is protected as a national park "Krka", which include the estuarine part of the park covering 13.64% of the total protected area (Aguzzi et al., 2020).

The photographs were collected using a stationary Hikvision video camera, model DS-2CD2020F-I, which was software programmed to capture images on every movement. The sampling rate was conducted in 30-minute intervals. The camera was cable-powered and it was deployed near the Martinska research station (by the Ruđer Bošković Institute; coordinates: 43°44'9.85"N and 15°52'37.62"E) at a depth of 5 m. (Figure 1).

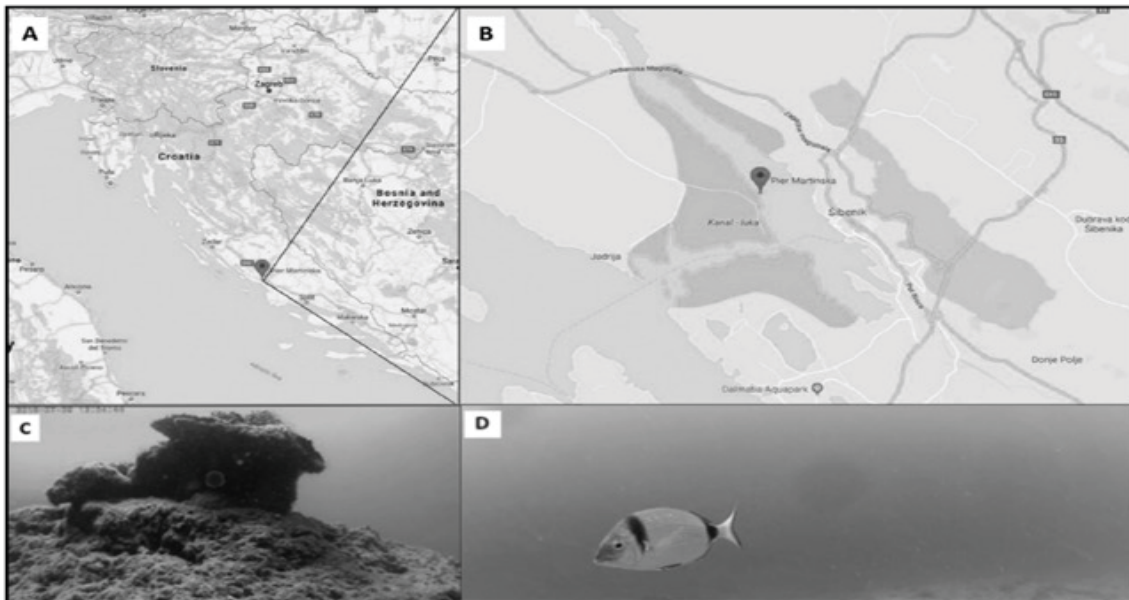


Figure 1. The research area in Croatia (A), a close-up of the locality of the video camera in relation to the estuary (B), a close sight of the underwater video camera placement, embedded and disguised in an artificial reef (C); and finally, an example of an imaged fish, *Diplodus vulgaris* (D).

The images were collected in daylight from morning to dusk, seasonally in 4 central months (January, April, July, and October of 2019).

Prior to the analysis, images that were unusable due to camera obstruction by organisms or blurring were eliminated. Species determination in each individual photograph was conducted according to Froese and Pauly (2009) and the precise classification of species relied on the observer's experience, therefore images were deemed appropriate for later analyses only when the morphological characteristics of the individuals were clearly visible. Less vagile benthic fauna members (such as polychaetas and sea urchins) were not recorded due to their high abundance and the impossibility of precise counting. Fish and other vagile species populations were analysed using the methodology of Aguzzi et al. (2011; 2012). To avoid errors in the determination of morphologically similar species based on photographs, in cases where species determination was not possible, the final determination was made at the genus level. The images where the identification of fish and/or other organisms could not be accurately determined and/or classified into the appropriate family were excluded from further analysis.

To avoid the multiple counting of the same individual, organisms that were clearly present in consecutive records were excluded from the count. When a shoal of fish with more than 100 individuals appeared in the photographs, it was recorded as 100 individuals, following the methodology of Condal et al. (2012). Commercially important species were determined according to the Regulation on the Form, Content, and Method of Keeping and Submitting Data on Catch in Commercial Sea Fishing (NN 38/2018), while other species were classified separately.

Results and discussion

During a four-month period of the year 2019 a total of 94 669 photographs were collected, of which 91 758 (97%) were usable, and 2911 (3%) were discarded due to water turbidity from primary production, sediment resuspension or camera malfunction. A total of 54 species of marine fauna were identified, of which 82% were fish. The largest percentage (72.2%) consisted of economically significant fish species and invertebrates (39 species) (Figure 2). Aguzzi et al. (2020) monitored the area of Martinska bay during a four-month period from January to April of 2018 and recorded fewer species of commercial importance (24 species), while the total number of recorded species was 35. The data confirm the importance of monitoring throughout the year for a more comprehensive sampling of richness present at the mouth of the Krka River and similarly in all coastal Natura 2000 areas.

The family Sparidae was the most common with 12 recorded species. The most represented economically important species was the blotched picarel *Spicara maena* (Linnaeus, 1758) (13168 individuals or 23% of the total number), followed by *Spicara smaris* (Linnaeus, 1758) with 11290 individuals (20% of the total number). *S. smaris* was most abundant in January (7070 individuals) and April (4565 individuals), while only 3 individuals were recorded in July and October. *S. maena* occurrences were elevated during all seasons except in summer when only 40 individuals were recorded. The most numerous non-economically significant species were *Serranus scriba* (Linnaeus, 1758) and *Serranus hepatus* (Linnaeus, 1758). In Aguzzi et al. (2020) *S. hepatus* was reported at high count numbers, with 11.5% of the overall fish count for this species. High occurrences of this member of the Serranidae family may be explained by its territorial behavioural regimen, therefore, this species tends to have a restricted range of movement, resulting in frequent appearances of the same individual in front of the camera.

In addition to the ichthyofauna, other species of organisms were also observed: the European shag (*Phalacrocorax aristotelis*, Linnaeus, 1761) with 11 recorded occurrences, the loggerhead sea turtle (*Caretta caretta*, Linnaeus, 1758) with 8 recorded occurrences, and the commercially important Mediterranean scallop (*Pecten jacobaeus*, Linnaeus, 1758) with two recorded occurrences. Three commercially significant species of cephalopods were present, comprising 19 recorded individuals (*Eledone* sp.; *Loligo vulgaris*, Lamarck 1798; *Sepia officinalis*, Linnaeus, 1758). One non-commercial crab species (*Maja crispata*, Risso 1827) with a total of 14 individuals, and two species of starfish with a total of 119 recorded individuals (*Echinaster sepositus*, Retzius 1783 and *Marthasterias glacialis*, Linnaeus 1758) were recorded.

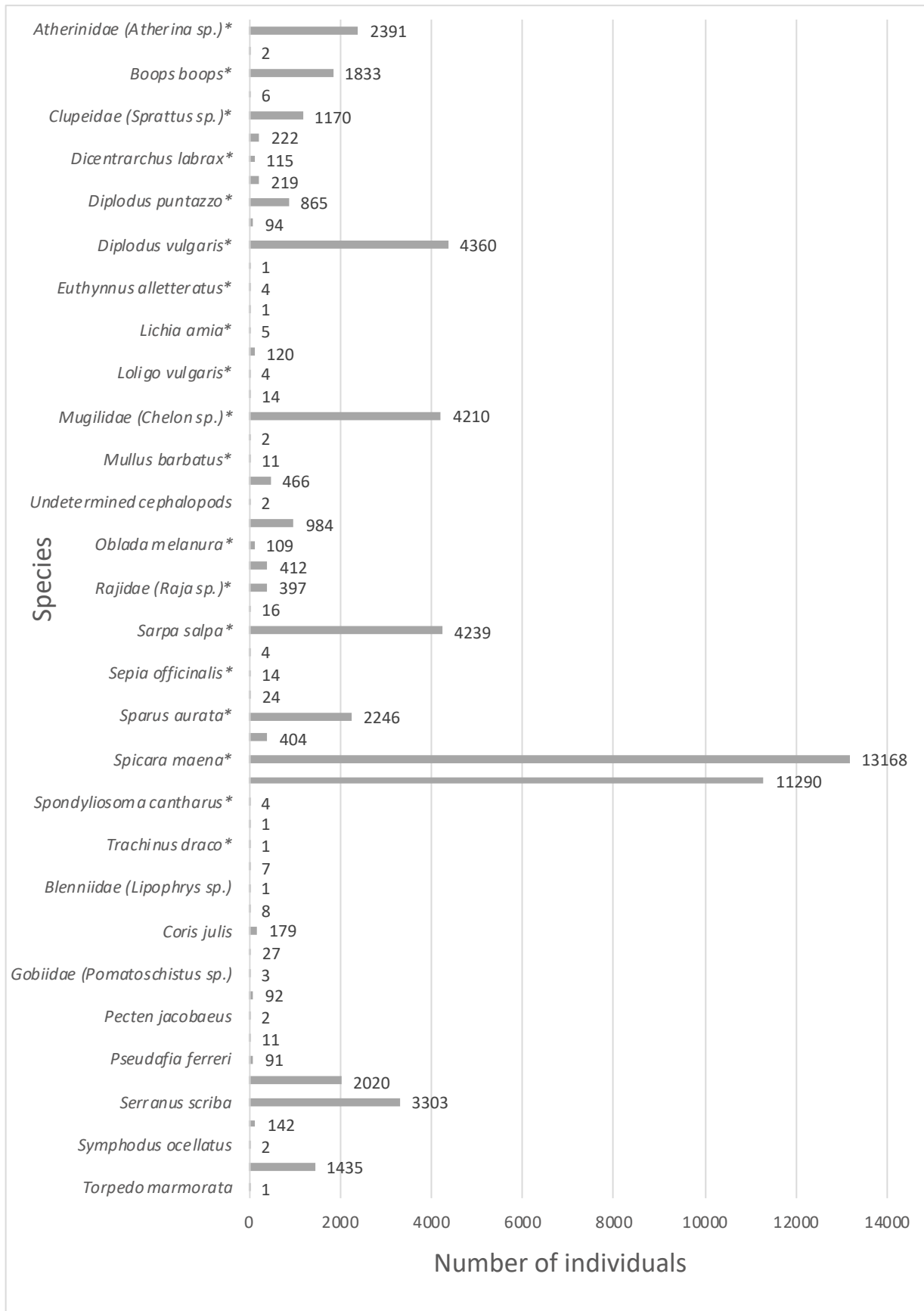


Figure 2. Total number of species in the four monitored seasons of 2019 (species with commercial significance are marked with an asterisk; protected species are marked with two asterisks).

The highest number of species was recorded in January, and the lowest in July (Figure 3). The lower species count in the summer and autumn period may be connected with antropogenic activities in this area (Radeljak and Pejnović, 2008). Tourism and boating are more frequent in summer and autumn than in winter and spring, thus affecting the number of species present in the studied area.

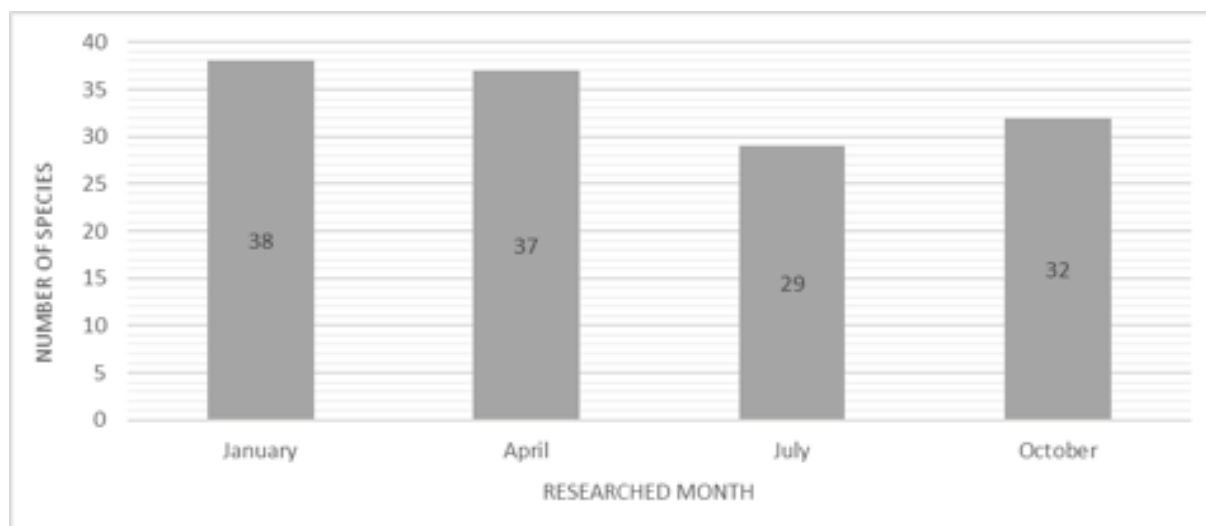


Figure 3. Seasonal species count in 2019 (January, April, July and October)

This paper presents the most comprehensive video-monitoring of the area and contributes to long-term data collection in Martinska bay. Various authors emphasized the importance of continuous monitoring through the adoption of new technologies (Rountree et al., 2020; Mirimin et al., 2021; Ruppert et al., 2019; Zaiko et al., 2018). This type of monitoring leads to a better understanding of the relevant processes in fish communities such as seasonal rhythms of species activity. The collected data suggest that the research area is an important habitat, a possible migration route of some species, and possibly a breeding ground for certain species, many of which are commercially important and some of them protected. The use of underwater cameras for the identification of marine fauna has proven highly beneficial due to the year-round data availability and the low percentage (1.6%) of undetermined individuals. The methodology used in this research can be helpful for future monitoring and consequently management plans.

Conclusions

Based on the results of this paper, a seasonal variation in species count can be noticed. The number of species is highest in winter and spring and decreases in summer and autumn. Several species appear to be territorial and linger in an area in front of the camera's field of view, while others can be seen in fewer numbers, appearing only once. For those species with rare occurrences, Marine Protected Areas such as Martinska bay possibly serve as a migratory route or feeding ground. While our study presents the first seasonal analysis of this type of monitoring for the area and shows clear variations, confirming precise seasonal patterns requires multiple years of observation. Continued monitoring is essential for this purpose.

References

- Aguzzi J., Company J.B., Costa C., Matabos M., Azzurro E., Manuel A., Menesatti P., Sarda F., Canals M., Delory E., Cline D., Favali P., Juniper S.K., Furushima Y., Fujiwara Y., Chiesa J.J., Marotta L., Bahamon N., Priede G.I. (2012). Challenges to the assessment of benthic populations and biodiversity as a result of rhythmic behaviour: Video solutions from cabled observatories. *Oceanography and Marine Biology: An Annual Review*. 50: 233-284.
- Aguzzi J., Iveša N., Gelli M., Costa C., Gavrilovic A., Cukrov N., Cukrov M., Cukrov N., Omanovic D., Štifanić M., Marini S., Piria M., Azzurro E., Fanelli E., Danovaro R. (2020). Ecological video

- monitoring of Marine Protected Areas by underwater cabled surveillance cameras. *Marine Policy*. 119: 104052.
- Aguzzi J., Mànuel A., Condal F., Guillén J., Nogueras M., Del Rio J., Costa C., Menesatti P., Puig P., Sardà F., Toma D., Palanques A. (2011). The new Seafloor observatory (OBSEA) for remote and long-term coastal ecosystem monitoring. *Sensors*. 11: 5850–5872.
- Azzurro E., Matiddi M., Fanelli E., Guidetti P., Mesa GL., Scarpato A., Axiak V. (2010). Sewage pollution impact on Mediterranean Rocky-reef fish assemblages. *Marine Environmental Research*. 69: 390–397.
- Bakran-Petricioli T. (2011). Manual for determination of marine habitats in Croatia according to EU Habitat Directive, Državni zavod za zaštitu prirode. Available from: <https://www.haop.hr/sites/default/files/uploads/publications/2018-01/Bakran-Petricioli%20-%20Prirucnik%20za%20morska%20stanista.pdf>
- Condal F., Aguzzi J., Sardà F., Nogueras M., Cadena J., Costa C., Del Río J., Mànuel A. (2012). Seasonal rhythm in a Mediterranean coastal fish community as monitored by a cabled observatory. *Marine Biology*. 159: 2809–2817.
- Costello M.J., and Chaudhary C. (2017). Marine Biodiversity, biogeography, deep-sea gradients, and conservation. *Current Biology*. 27: 511–527.
- Francescangeli M., Sbragaglia V., del Rio Fernandez J., Trullols E., Antonijuan J., Massana I., Prat J., Nogueras Cervera M., Mihai Toma D., Aguzzi J. (2022). Long-term monitoring of diel and seasonal rhythm of *Dentex dentex* at an Artificial Reef. *Frontiers in Marine Science*. 9: 837216.
- Froese R., Pauly D. (2009). FishBase. Available from: www.fishbase.org.
- Guidetti P., Terlizzi A., Frascchetti S., Boero F. (2003). Changes in Mediterranean Rocky-reef fish assemblages exposed to sewage pollution. *Marine Ecology Progress Series*. 253: 269–278.
- Iveša N., Gavrilović A., Cukrov N., Omanović D., Cukrov M., Piria M., Gelli M., Gobić K., Štifanić M., Marini S., Fanelli E., Aguzzi J. (2018). Upotreba podvodne video kamere za procjenu učestalosti pojavljivanja gospodarski važnih vrsta riba na postaji Martinska kraj Šibenika. 54. hrvatski i 14. međunarodni simpozij agronoma, 17. - 22. veljače 2019. godine, Vodice, Hrvatska. Zbornik radova 2019: 363-367.
- Mallet D., and Pelletier D. (2014). Underwater Video Techniques for Observing Coastal Marine Biodiversity: A review of sixty years of publications (1952–2012). *Fisheries Research*. 154: 44–62.
- Mirimin L., Desmet S., Romero D.L., Fernandez S.F., Miller D.L., Mynott S., Brincau A.G., Stefanni S., Berry A., Gaughan P., Aguzzi J. (2021). Don't catch me if you can – using cabled observatories as multidisciplinary platforms for Marine Fish Community Monitoring: An in-situ case study combining underwater video and environmental DNA data. *Science of The Total Environment*. 773: 145351.
- Radeljak P., and Pejnović D. (2008). Utjecaj turizma na održivi razvoj funkcionalne regije nacionalnog parka Krka. *Godišnjak Titius*. 329-361.
- Rountree R.A., Aguzzi J., Marini S., Fanelli E., De Leo F.C., Del Rio J., Juanes F. (2020). Towards an optimal design for ecosystem-level Ocean Observatories. *Oceanography and Marine Biology*. 79–105.
- Ruppert K.M., Kline R.J., Rahman M.S. (2019). Past, present, and future perspectives of environmental DNA (Edna) metabarcoding: A systematic review in methods, monitoring, and applications of Global Edna. *Global Ecology and Conservation*. 17.
- Stefanni S., Mirimin L., Stanković D., Chatzievangelou D., Bongiorno L., Marini S., Modica M.V., Manea E., Bonofiglio F., del Rio Fernandez J., Cukrov N., Gavrilović A., De Leo F.C., Aguzzi J. (2022). Framing cutting-edge integrative deep-sea biodiversity monitoring via environmental DNA and optoacoustic augmented infrastructures. *Frontiers in Marine Science*. 8: 797140.
- Tittensor D.P., Mora C., Jetz W., Lotze H.K., Ricard D., Berghe E.V., Worm B. (2010). Global patterns and predictors of marine biodiversity across taxa. *Nature*. 466: 1098–1101.

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Zaiko A., Pochon X., Garcia-Vazquez E., Olenin S., Wood S.A. (2018). Advantages and limitations of environmental DNA/RNA tools for marine biosecurity: Management and surveillance of non-indigenous species. *Frontiers in Marine Science*. 5: 322.

Morphometric characteristics of bluefish, *Pomatomus saltatrix* (Linnaeus, 1766) population inhabiting North Adriatic Sea

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Abstract

Bluefish, *Pomatomus saltatrix* (Linnaeus, 1766) is a thermophilic apex piscivore species that is increasingly common in fishing catches in the northern Adriatic. Since, according to the available literature, there is no data on the study of classic bluefish morphometry, the aim of this work was to determine the morphometric characteristics of bluefish in the northern Adriatic, as a basic tool for measuring variability in ecological functions among different populations. Research was conducted from April 2022 to April 2023 on 102 individuals of bluefish, 27 morphometric variables were measured, and ratios of morphometric parameters were calculated. Total body length (TL) of bluefish ranged from 31 to 84.8 cm (54.88 ± 12.08 cm). Statistical differences between sexes were recorded in the following ratios of morphometric parameters: second dorsal fin base length and total length (D2/TL, $t = 2.16$), anal fin base length and total length (LA/TL, $t = 1.99$), pre-anal length and total length (PA/TL, $t = 1.99$), maximum body width and total length (BW/TL, $t = 2.35$) and snout height and head length (SH/CL, $W = 941$) ($P < 0.05$). The catch of relatively large specimens compared to other studies probably derives from the unexploited population of bluefish in the eastern Adriatic.

Keywords: thermophilic species, fish morphometry, functional morphology, Mediterranean

Introduction

Bluefish, *Pomatomus saltatrix* (Linnaeus, 1766) is a pelagic, migratory and thermophilic apex piscivore that inhabits the Atlantic, Indian, and Pacific Oceans as well as the Mediterranean and Black Sea (Tortonese, 1986; Briggs, 1960). In the Mediterranean it is more common in the southern and eastern parts, but knowledge about its biology in the Mediterranean is very scarce (Tortonese, 1986). Some authors reported on the spawning and distribution of bluefish on the Catalan coast (Sabates and Martin, 1993) and Black sea (Gordina and Klimova, 1996). As for the Adriatic Sea, from 1887 to 2003 there were several records of its presence (Dulčić et al., 2005). Recently, Dulčić et al. (2005; 2019) reported unusually large catches of bluefish in Tarska Cove (northern Adriatic).

Morphometric analysis of fish presents an important tool for comparison of variability in ecological functions among different populations (Antonucci et al., 2009). Data regarding morphometric characteristics of bluefish in the Adriatic were not found in the available literature. In the Mediterranean and Black Sea some morphometric studies were carried out using the truss network method (Turan et al., 2006; Bal et al., 2021), while there are no references on classical morphometry. As the bluefish population obviously spread over the whole Adriatic in the last few decades, the aim of this paper was to investigate its morphometric characteristics, as a basic tool for measuring variability in ecological functions among different populations.

Materials and methods

In total, 102 bluefish samples were obtained from local fishermen operating in the area of the north Istria and Kvarner from April 2022 to April 2023. The sex of the fish was determined macroscopically by examining the shape and color of the gonads. Twenty seven morphometric variables were measured: TL- total body length, SL- standard length, FL- fork length, D1- first dorsal fin base length, D2- second dorsal fin base length, LA- anal fin base length, LP- pectoral fin base length, LV- ventral fin base length, LC- caudal fin length, PD- pre-dorsal length, PA- pre-anal length, PV- pre-ventral length, PP- pre-pectoral length, H- maximum body height, h- minimum body height, BW- maximum body width, CL- head length, CH- head height, ML- mouth length, EH- eye height, EW- eye width, PO- preocular length, SH- snout height, IO- interocular length, OLO- post-ocular length, MH- mouth height and MW- mouth width (Figure 1). Each morphometric variable was measured using an ichthyometer and a digital hand caliper with the precision of ± 0.1 mm. Ten morphometric parameters CH, ML, EH, EW, PO, SH, IO, OLO, MH, MW are expressed as a percentage of CL, while others are expressed as a percentage of TL. The coefficient of variation was calculated to analyze the dispersion of morphometric parameters and their ratios.

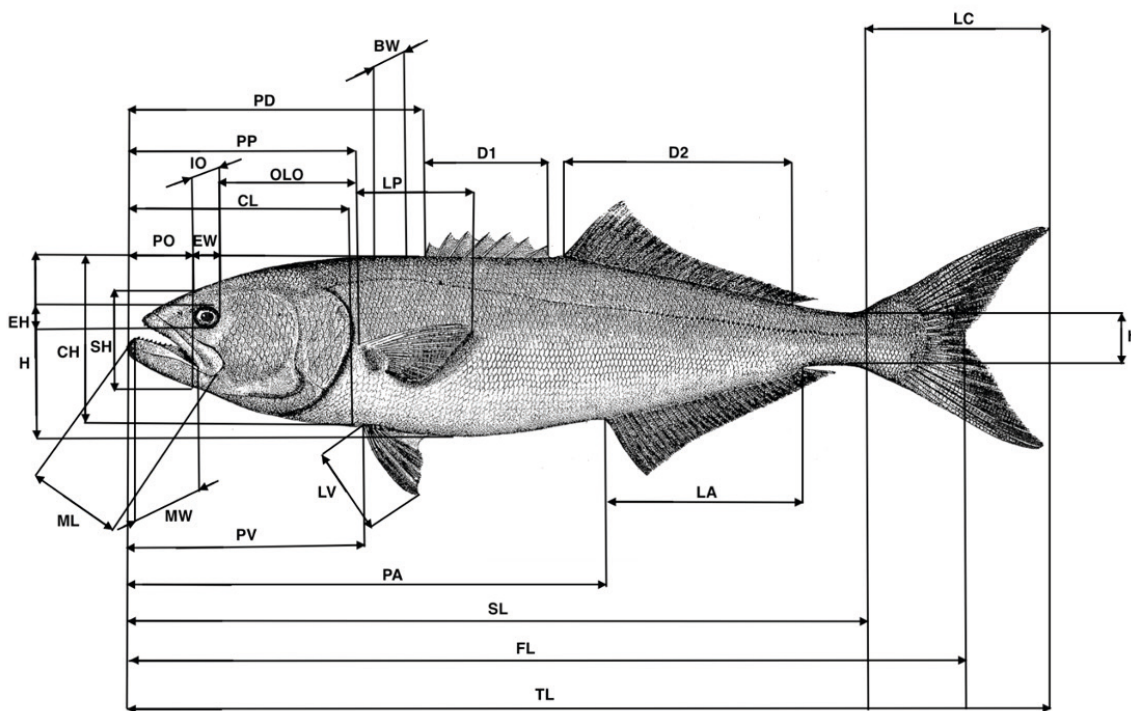


Figure 1. Stylised drawing of measured morphometric characteristics of bluefish, *Pomatomus saltatrix* (Linnaeus, 1766).

To process all data sets; minimum, maximum, average, and standard deviation values were calculated. In order to evaluate whether the data set is normally distributed, Shapiro – Wilk test ($p < 0.05$) was used. The Student t - test ($P < 0.05$) and Wilcoxon test ($P < 0.05$) were used to determine the statistical differences between morphometric characteristics between genders. All the data were analyzed using Microsoft Excel 2023 and RStudio 2021.09.0+351.

Results and discussion

Values of measured morphometric parameters are shown in Table 1. Shapiro – Wilk test showed that all measurements of morphometric characteristics did not have a normal distribution ($P < 0.05$), except the mouth height (MH) and mouth width (MW) ($P > 0.05$). No statistically significant differences between sexes were found for any morphometric parameter ($P > 0.05$).

Table 1. Values of morphometric parameters for all individuals and both sexes separately (min = minimum values,

max = maximum values, mean = average values, SD = standard deviation), Test score = values for comparing the average of males and females and percentage of the coefficient of variation (V).

	Sex	Min	Max	Mean	SD	Test score	P	V%
Total body length TL (cm)	M	32.5	84.8	54.98	11.61			21.11
	F	37	80.2	54.78	12.69	1290	0.92	23.16
	Total	31	84.8	54.88	12.08			22.02
Standard length SL (cm)	M	25.3	66.5	43.02	9.35			21.72
	F	28.4	63.1	42.82	10.30	1286	0.94	24.07
	Total	23.2	66.5	42.92	9.77			22.77
Fork length FL (cm)	M	29.2	76.3	49.52	10.59			21.39
	F	32.8	72.3	49.19	11.48	1290	0.92	23.34
	Total	27.3	76.3	49.36	10.98			22.24
First dorsal fin base length D1 (cm)	M	4.1	10.2	6.98	1.51			21.58
	F	4.2	10.5	7.04	1.63	1261	0.93	23.11
	Total	4.1	10.5	7.01	1.56			22.24
Second dorsal fin base length D2 (cm)	M	7.7	19.5	12.80	2.75			21.51
	F	7.9	18.8	12.58	3.00	1346	0.63	23.86
	Total	7.2	19.5	12.69	2.86			22.56
Anal fin base length LA (cm)	M	7	18	12.25	2.53			20.66
	F	7.6	17.5	11.96	2.72	1375	0.5	22.75
	Total	7	18	12.11	2.62			21.60
Pectoral fin base length LP (cm)	M	4.8	10	7.23	1.28			17.65
	F	5	10.4	7.27	1.46	1243	0.84	20.03
	Total	4.5	10.4	7.25	1.36			18.75
Ventral fin base length LV (cm)	M	3	7.2	5.08	1.01			19.93
	F	3.36	7.5	5.10	1.08	1284	0.95	21.24
	Total	3	7.5	5.09	1.04			20.48
Caudal fin length LC (cm)	M	7.2	19.7	11.82	2.38			20.12
	F	7.8	17.2	11.87	2.63	1254	0.89	22.21
	Total	7.2	19.7	11.84	2.49			21.05
Pre-dorsal length PD (cm)	M	10.4	28	17.24	3.64			21.10
	F	11.6	25.3	17.37	4.11	1242	0.83	23.64
	Total	9.7	28	17.30	3.85			22.27
Pre-anal length PA (cm)	M	16	44.8	27.63	6.06			21.93
	F	17.9	42.1	27.82	6.80	1226	0.87	24.45
	Total	14.8	44.8	27.72	6.40			23.09
Pre-ventral length PV (cm)	M	7.9	21.8	13.67	2.80			20.51
	F	9.3	20.5	13.80	3.22	1282	0.96	23.33
	Total	7.9	21.8	13.73	3.00			21.83
Pre-pectoral length PP (cm)	M	7.3	21	13.09	2.76			21.08
	F	8.7	19.6	13.17	3.04	1264	0.95	23.07
	Total	7.3	21	13.13	2.88			21.96

Maximum body height H (cm)	M	6.7	17.7	10.75	2.13			19.82
	F	7.5	15.5	10.82	2.04	1225	0.74	18.89
	Total	6.7	17.7	10.79	2.08			19.28
Minimum body height h (cm)	M	2.2	5.2	3.43	0.61			17.84
	F	2.5	4.6	3.40	0.61	1275	1	18.04
	Total	2.1	5.2	3.41	0.61			17.85
Maximum body width BW (cm)	M	2.9	8.9	5.19	1.23			23.78
	F	3.5	7.8	5.31	1.24	1198	0.61	23.34
	Total	2.9	8.9	5.25	1.23			23.48
Head length CL (cm)	M	7.5	19.4	12.12	2.55			21.07
	F	8.1	18	12.14	2.89	1271	0.98	23.80
	Total	7	19.4	12.13	2.71			22.32
Head height CH (cm)	M	6.4	16	9.86	1.95			19.73
	F	6.8	14.3	9.92	1.94	1241	0.83	19.59
	Total	6.3	16	9.89	1.94			19.57
Mouth length ML (cm)	M	3.3	9.2	5.50	1.26			22.93
	F	3.5	8.9	5.55	1.41	1223	0.73	25.38
	Total	3	9.2	5.52	1.33			24.04
Eye height EH (cm)	M	1.1	2.4	1.54	0.24			15.57
	F	1.1	2	1.53	0.22	1295	0.89	14.33
	Total	1.1	2.4	1.54	0.23			14.92
Eye width EW (cm)	M	1.2	2.3	1.62	0.24			14.73
	F	1.2	2.3	1.62	0.26	1239	0.81	16.33
	Total	1.1	2.3	1.62	0.25			15.45
Pre-ocular length PO (cm)	M	2.2	6.3	3.69	0.83			22.50
	F	2.4	5.6	3.68	0.91	1288	0.93	24.78
	Total	2.2	6.3	3.69	0.87			23.51
Snout height SH (cm)	M	3.4	10.1	5.50	1.25			22.69
	F	3.7	8.2	5.61	1.25	1178	0.52	22.33
	Total	3.1	10.1	5.56	1.25			22.42
Interocular length IO (cm)	M	2.2	6.3	3.74	0.84			22.44
	F	2.3	6	3.78	0.95	1258	0.92	25.01
	Total	2.2	6.3	3.76	0.89			23.62
Post-ocular length OLO (cm)	M	4.3	12.2	7.53	1.71			22.72
	F	4.6	12.3	7.52	2.01	1305	0.84	26.73
	Total	3.9	12.3	7.52	0.89			11.82
Mouth height MH (cm)	M	3.6	9	5.71	1.14			20.05
	F	3.9	8	5.78	1.15	-0.29	0.77	19.97
	Total	3.5	9	5.74	1.14			19.92
Mouth width MW (cm)	M	3	7.8	5.08	1.10			21.54
	F	3.4	8.5	5.11	1.10	-0.13	0.89	21.58
	Total	3	8.5	5.10	1.09			21.43

The ratios of morphometric parameters are displayed in Table 2. Shapiro – Wilk test showed that ratios of H/TL, h/TL, CH/CL, ML/CL, SH/CL and IO/CL didn't have a normal distribution ($P < 0.05$), while others did. T-test and Wilcoxon test showed statistically significant differences between sexes for the following ratios: D2/TL ($t = 2.16$), LA/TL ($t = 1.99$), PA/TL ($t = 1.99$), BW/TL ($t = 2.35$) and SH/CL ($W = 941$) ($P < 0.05$) (Table 2). In the available literature, we did not find the results of research on the classical morphometry of bluefish, so this paper very likely brings the first results on this topic.

Table 2. Values of ratios of morphometric parameters for all individuals and both sexes separately (min = minimum values, max = maximum values, mean = average values, SD = standard deviation), Test score = values for comparing the average of males and females (statistically significant differences in averages are marked with *, $P < 0.05$) and percentage of the coefficient of variation (V).

Ratios of morphometric parameters	Sex	Min	Max	Mean	SD	Test score	p	V%
SL/TL	M	76.49	79.94	78.05	0.95			1.21
	F	75.79	80.54	77.99	1.22	0.29	0.77	1.57
	Total	75.79	80.54	78.02	1.09			1.39
FL/TL	M	87.83	94.17	90.00	1.16			1.29
	F	87.65	92.25	89.75	1.11	1.08	0.28	1.24
	Total	87.65	94.17	89.88	1.14			1.24
D1/TL	M	11.55	14.63	12.69	0.69			5.42
	F	11.06	14.15	12.87	0.71	-1.35	0.18	5.48
	Total	11.06	14.63	12.78	0.70			5.47
D2/TL	M	21.42	25.78	23.28	0.77			3.31
	F	20.41	24.55	22.93	0.84	2.16*	0.03	3.65
	Total	20.41	25.78	23.11	0.82			3.54
LA/TL	M	19.39	24.62	22.32	1.69			7.55
	F	19.74	24.64	21.91	1.10	1.99*	0.04948	5.02
	Total	19.39	24.64	22.12	1.05			4.75
LP/TL	M	11.61	14.77	13.27	0.73			5.49
	F	11.38	15.26	13.38	0.75	-0.76	0.45	5.59
	Total	11.38	15.26	13.32	0.74			5.53
LV/TL	M	7.75	10.77	9.35	0.54			5.75
	F	8.29	10.39	9.36	0.49	-0.92	0.36	5.24
	Total	7.75	10.77	9.32	0.52			5.53
LC/TL	M	19.26	23.59	21.79	1.02			4.70
	F	18.99	23.64	21.73	0.99	-0.67	0.50	4.57
	Total	18.99	23.64	21.66	1.01			4.65
PD/TL	M	28.42	34.34	31.69	1.01			3.17
	F	29.98	33.39	31.68	0.86	-1.54	0.13	2.71
	Total	28.42	34.34	31.53	0.94			2.99
PA/TL	M	47.54	53.86	50.11	1.40			2.80
	F	47.27	52.96	49.42	1.34	-1.99*	0.04935	2.72
	Total	47.27	53.86	50.36	1.39			2.77

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	M	22.05	27.56	25.17	1.21			4.81
PV/TL	F	22.78	28.72	25.21	1.08	-1.24	0.22	4.26
	Total	22.05	28.72	25.07	1.15			4.59
	M	21.56	25.85	24.06	0.89			3.72
PP/TL	F	22.07	25.59	24.06	0.75	-1.38	0.17	3.12
	Total	21.56	25.85	23.94	0.83			3.47
	M	17.29	21.69	19.84	1.07			5.39
H/TL	F	16.08	25.00	20.00	1.65	1138	0.36	8.26
	Total	16.08	25.00	19.83	1.39			6.99
	M	5.60	7.30	6.43	0.41			6.43
h/TL	F	5.32	7.37	6.64	0.42	1174	0.50	6.35
	Total	5.32	7.37	6.29	0.42			6.60
	M	8.03	10.98	9.50	0.66			6.97
BW/TL	F	8.81	11.39	9.64	0.62	-2.35*	0.02	6.38
	Total	8.03	11.39	9.56	0.65			6.84
	M	20.40	23.69	22.27	0.66			2.98
CL/TL	F	20.35	23.95	22.15	0.69	-0.64	0.52	3.12
	Total	20.35	23.95	22.10	0.67			3.05
	M	74.58	96.25	81.77	3.69			4.51
CH/CL	F	71.59	94.12	82.58	4.90	1114	0.28	5.93
	Total	71.59	96.25	82.16	4.31			5.25
	M	42.37	50.00	45.24	1.56			3.44
ML/CL	F	41.67	54.10	45.56	2.06	1156	0.42	4.52
	Total	41.67	54.10	45.40	1.82			4.00
	M	10.14	16.25	12.96	1.36			10.52
EH/CL	F	9.14	16.05	12.91	1.65	-0.18	0.86	12.79
	Total	9.14	16.25	12.94	1.50			11.61
	M	11.49	16.05	13.56	1.21			8.89
EW/CL	F	10.80	16.05	13.60	1.28	0.18	0.86	9.39
	Total	10.80	16.05	13.58	1.24			9.10
	M	27.38	34.00	30.42	1.39			4.55
PO/CL	F	27.87	32.54	30.30	1.25	0.44	0.66	4.12
	Total	27.38	34.00	30.36	1.32			4.33
	M	41.53	52.06	45.42	2.44			5.38
SH/CL	F	42.14	51.22	46.46	2.34	941*	0.02	5.04
	Total	41.53	52.06	45.92	2.44			5.31
	M	27.70	35.16	31.87	1.83			5.75
IO/CL	F	26.44	36.59	31.47	2.92	1179	0.52	9.27
	Total	26.44	36.59	31.00	2.01			6.47

	M	54.69	67.24	61.98	2.76			4.45
OLO/CL	F	54.76	69.89	61.61	3.13	0.62	0.53	5.08
	Total	54.69	69.89	61.80	2.93			4.75
	M	38.51	53.41	46.24	2.65			5.73
MH/CL	F	40.74	54.55	47.66	3.06	-0.90	0.37	6.41
	Total	38.51	54.55	47.39	2.85			6.02
	M	36.50	48.98	41.08	2.78			6.78
MW/CL	F	32.70	49.42	42.13	3.06	-0.42	0.67	7.25
	Total	32.70	49.42	42.00	2.91			6.92

The total body length (TL) of the bluefish in this study ranged from 31 to 84.8 cm (54.88 ± 12.08 cm), which is similar to the findings of Dulčić et al. (2019). The average length of bluefish from the eastern Adriatic differs from the investigated populations in other areas of the Mediterranean. In size composition studies conducted on the Turkish coast of the Sea of Marmara (Kalayci et al., 2019) and the Black Sea (Ozdemir et al., 2009), the analyzed fish length ranged from 12.9 to 26.3 cm ($17, 7 \pm 0.086$ cm), respectively 9.2 – 23.4 cm (18.04 ± 0.223). Bluefish fishing in Turkish waters dates to 1959 (Turgan, 1959). Studies on the consumption of fish products in Turkey have shown that bluefish is one of the most preferred types of fish (Saka and Bulut, 2020), and the research conducted by Erdogan et al. (2011) showed that bluefish is the most preferred seafood for 39.34% of respondents. In 2002, the bluefish fishery in Turkey reached 25,000 tons, approximately half of the world's annual catch of this species (Akyol and Ceyhan, 2007), only to drop to 5,804 tons in 2021 (FAO, 2021). The consumers preference for bluefish, which resulted in long-term fishing pressure, is the reason for the decline in catches and the catch of smaller specimens in Turkish waters. On the contrary, in the eastern Adriatic large catches of bluefish were reported only recently by Dulčić et al. (2005 and 2019) and in 2021 the bluefish landings in Croatia were only 2 tonnes (FAO, 2021). The unexploited population of bluefish in the eastern Adriatic could be the reason for the catch of relatively large specimens in this study. In order to encourage the consumption of bluefish and further develop bluefish fishing in Croatia, it is necessary to educate the public and encourage fishermen to adapt their fishing tools for catching bluefish.

Conclusion

The relatively larger size of bluefish (31 to 84.8 cm in total length) compared to other studies probably derives from the unexploited population of bluefish in the eastern Adriatic. Bluefish from the North Adriatic showed statistically significant differences between sexes in the following ratios of morphometric parameters: D2/TL, LA/TL, PA/TL, BW/TL and SH/CL. The results of research into the classical morphometry of bluefish were not found in the available scientific literature, so this paper very likely brings the first results on that topic.

References

- Akyol O., and Ceyhan T. (2007). Exploitation and Mortalities of Bluefish (*Pomatomus saltatrix* L.) in the Sea of Marmara. Turkey Journal of Applied Biological Sciences. 1: 25-27.
- Antonucci F., Costa C., Aguzzi J., Cataudella S. (2009). Ecomorphology of morpho-functional relationships in the family of Sparidae: A quantitative statistic approach. Journal of Morphology. 270: 843-855.
- Bal H., Yanik T., Turker D. (2021). Assessment of morphological variation between stocks of bluefish, *Pomatomus saltatrix* (Actinopterygii, Perciformes, Pomatomidae), in the Aegean Sea, Black Sea, and Sea of Marmara. Acta Ichthyologica et Piscatoria. 51: 85–94.
- Briggs J.C. (1960). Fishes of world-wide (circumtropical) distribution. Copeia. 1960: 171-180.
- Dulčić J., Dragicević B., Matić-Skoko S., Pavičić M., Vrdoljak D. (2019). Bluefish (*Pomatomus saltatrix*) again in the fishing catches in the northern Adriatic. 42nd CIESM congress, Cascais Portugal 7-11 October 2019.
- Dulčić J., Pallaoro A., Kraljević M., Glamuzina B. (2005). Unusual catch of bluefish *Pomatomus saltatrix* (Pomatomidae) in Tarska cove (northern Adriatic). Cybium. 29: 207-208.
- Erdogan B.E., Mol S., Cosansu S. (2011). Factors Influencing the Consumption of Seafood in Istanbul, Turkey. Turkish Journal of Fisheries and Aquatic Sciences. 11: 631–639.
- FAO (2021). Available from: https://www.fao.org/fishery/statistics-query/en/gfcm_capture/gfcm_capture_quantity
- Gordina A.D., and Kimova T.N. (1996). On bluefish (*Pomatomus saltatrix* L.) spawning in the Black Sea. Marine and Freshwater Research. 47: 315-318.
- Kalaycı F., Yesilcicek T., Sahin C. (2019). Lüfer Balığı (*Pomatomus saltatrix* L., 1766)'nın Av Kompozisyonu, Gonadosomatik İndeks ve Kondisyon Faktörü. Journal of Anatolian Environmental and Animal Sciences. 4: 97-103.
- Özdemir S., Erdem Y., Özdemir Z.B., Erdem E. (2009). Karadeniz'de dip trolü ile Ekim ve Kasım aylarında avlanan lüfer (*Pomatomus saltatrix*, L.) balığının av verimi ve boy kompozisyonun karşılaştırılması. Erciyes Üniversitesi Fen Bilimleri Enstitüsü Dergisi. 25(1-2).
- Sabates A., and Martin P. (1993). Spawning and distribution of bluefish *Pomatomus saltatrix* (L.) in the northwestern Mediterranean. Journal of Fish Biology. 43: 109-118.
- Saka F., and Bulut M. (2020). Determination of fish consumption in Çanakkale. Marine Science and Technology Bulletin. 9: 7-14.
- Tortonese E. (1986). Pomatomidae. In: Whitehead P.J.P., Bauchot M.L., Hureau J.C., Nielsen J., Tortonese E. (Eds.). Fishes of the North-eastern Atlantic and Mediterranean. vol. 2. Unesco, Bungay: 812– 813.
- Turan C., Oral M., Öztürk B., Düzgüneş E. (2006). Morphometric and meristic variation between stocks of bluefish (*Pomatomus saltatrix*) in the Black, Marmara, Aegean and northeastern Mediterranean seas. Fisheries Research. 79: 139–147.
- Türkan G. (1959). About the biology of *Pomatomus saltatrix* L. (in Turkish). Hidrobiologi Mecmuası, İ.Ü. Fen Fak. Hidrobiologi Araş. Enst. Cilt. V:144-180.

Some eco-labelling and certification schemes in fisheries and aquaculture

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Abstract

The development of the fisheries and aquaculture sector has raised many sustainability issues. Effective, internationally recognised standards help to address various sustainability issues and promote sustainable and responsible fishing and farming practises. Standards in fisheries and aquaculture are critical to strengthening the resilience of the sector as a whole. As there are a multitude of private standards that can cause confusion among stakeholders (governments, fish producers, buyers, etc.), this paper aims to provide an insight into the main standards in fisheries related to environmental sustainability.

Keywords: sustainability, environmental standards, fishing industry, responsible fishing, farming

Introduction

Fisheries and aquaculture are an important sector for global economic development and food supply (FAO, 2022a). However, the rapid development of the sector has triggered many issues related to the economic and social viability, but also the environmental sustainability of the sector (FAO, 2022b; Garlock et al., 2022; Akbari et al., 2023; Bertheussen and Vassdal, 2023).

Standards in fisheries and aquaculture are essential for promoting the Blue Economy and the Sustainable Development Goals (SDGs) (Potts et al., 2016; FAO, 2018). Standards can cover different aspects of sustainability: environmental protection, food safety and quality, seafood traceability, labour ethics, etc., and can be developed by different public and private organisations (FAO, 2009; Washington and Ababouch, 2011; FAO, 2011; FAO, 2022b). Eco-labelling in the sector has developed significantly since the 1970s and the first ecolabels for tuna (Oosterveer, 2010; Gutierrez et al., 2016; Potts et al., 2016; Schiller and Bailey, 2021). Ecolabels confirm that a product has been produced in an environmentally responsible way and they can also improve a corporate reputation and avoid negative publicity (Murphy et al., 2021; FAO 2922b; Pierucci et al., 2022). Growing consumer awareness of sustainability issues has led producers and retailers to adopt the myriad of different public standards (UNEP, 2009; Hojnik et al., 2019; Penca, 2020; UNCTAD, 2020; Giacomarra et al., 2021; Bimbo et al., 2022)

As there are a multitude of private standards that can cause confusion among stakeholders (governments, fish producers, buyers, etc.), this paper aims to provide an insight into the most important standards in fisheries in the context of environmental sustainability.

International legal framework in eco-labelling

Following of the international and national law is an essential prerequisite for the award of an ecolabel. All fisheries should abide by international agreements, such as:

- the United Nations Convention (UN) on Fishing and Conservation of the Living Resources of the High Seas (1966)
- the UN Convention on the Law of the Sea (1982),
- Agenda 21 of the Rio Declaration, Section 17.1 (1992),
- the FAO Compliance Agreement (1993),
- the UN Fish Stocks Agreement (1995),

- the FAO Code of Conduct for Responsible Fisheries (1995),
- Reykjavik Declaration on Responsible Fisheries in the Marine Ecosystem (2001),
- the FAO Guidelines for the Ecolabelling of Fish and Fishery Products From Marine Capture Fisheries (2009),
- the FAO Guidelines for the Ecolabelling of Fish and Fishery Products From Inland Capture Fisheries (2011),
- the FAO International Guidelines on Bycatch Management and Reduction of Discards (2011),
- the FAO Voluntary Guidelines on the Responsible Governance of Tenure of Land, Fisheries and Forests in the Context of National Food Security (2012).

In 1996, the Committee on Fisheries (COFI) of the Food and Agriculture Organisation (FAO) first discussed the issue of eco-labelling, when some countries raised questions about the transparency and possible impact of the Marine Stewardship Council (MSC) certification scheme. In 2001, FAO Fisheries Technical Paper 422 Product certification and ecolabelling for fisheries sustainability was published. In 2003, it was agreed that FAO should adopt guidelines for fish and seafood eco-labelling to address the problem of the ineffectiveness of many voluntary private standards, and in 2005 the guidelines were adopted the Guidelines for the Ecolabelling of Fish and Fishery Products and the Technical Guidelines on Aquaculture Certification (Washington and Ababouch, 2011; Potts et al., 2016). The Technical Guidelines on Aquaculture Certification contain minimal requirement including animal health and welfare; food safety; environmental and socio-economic aspects.

In 2022, the FAO adopted the Framework for Environmental and Social Management (FESM), which replaces the Environmental and Social Management Guidelines adopted in 2015 and complements the Compliance Reviews Following Complaints Related to the Organization’s Environmental and Social Standards Guideline. In addition to the above documents, FAO has also published the following documents on environmental sustainability (FAO, 2023): Interim guidance: sustaining FAO’s commitment to Environmental and Social Standards during the COVID-19 pandemic (2020), Operational Guidelines for Stakeholder Engagement (2017) and FAO Environmental Impact Guidelines (1999).

All eco-labelling schemes should also comply with the rules and mechanisms of the World Trade Organization (WTO).

Private standards in fisheries and aquaculture

Public standards which relate to sustainability, safety and compliance with international treaties, are developed by national authorities (governments), while private standards are developed by non-governmental actors (Green, 2014) to solve the problems that are not or not satisfactorily solved by public regulatory frameworks (Berliner and Prakash, 2013). Public and private standards are voluntary but can become mandatory to target specific markets. The development of private standards is important for international fish trade and is encouraged by food industry coalitions, integration and complexity of supply chains, consumer demand for ethical products and corporate policies (Washington and Ababouch, 2011; Potts et al., 2016). Private environmental standards serve as insurance against bad publicity and boycotts, but can also provide an advantage over competitors (Sainsbury, 2010; Roheim et al., 2011; Thorlakson et al., 2018). Despite their sometimes questionable effectiveness (Hadjimichael and Hegland, 2016; Ramachandran and Parappurathu, 2020), there are large number of private standards worldwide..

Table 1. contains the list of public and private standards in the sector related to eco-labelling according to FAO (2009) and Washington and Ababouch (2011).

Table 1. The list of public and private standards (S), codes (C), guidelines (G), labels (L) and certificates (CS) in fisheries and aquaculture related to environmental issues according to FAO (2009) and FAO (2011)

Name	Type	Market orientation	Issue addressed				
			Food safety	Animal health	Environment	Social/ ethic	Food quality
Alter-Trade Japan (ATI)	C, L	Japan			+	+	?
Agriculture Biologique	S, L	Europe	+	+	+		
Aquaculture Stewardship Council (ASC)	C, S, L	global	+	+	+	+	+

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Bio Gro, New Zealand	S, L	global	+	+	+		
Bioland, Germany	CS, L	Europe	+	+	+		
BioSuisse	C, L	Switzerland	+	+	+		
COC-certified Thai shrimp, Thailand	S, I	Europe, USA	+	+	+	+	
Debio, Norway	CS, L	UK, Europe	+	+	+		
Fair-Fish	S, L	Switzerland		+	+	+	
Federation of European Aquaculture Producers (FEAP) code of conduct	C	Europe	+	+	+	+	+
Fishmeal and fish oil Code fo Responsible Practice (CORP)	C, CS	global	+		+		+
Friend of the Sea (FOS)	C, S	global			+		
Global Aquaculture Alliance (GAA)/Aquaculture Certification Council (ACC)	CS, L	USA	+		+	+	
GLOBALG.A.P	S, CS	Europe	+	+	+		+
International Federation of Organic Agriculture Movements (IFOAM)	S, L	UK, Europe	+	+	+	+	+
International Social and Environmental Accreditation and Labelling Alliance (ISEAL)	S, C, L	global			+	+	
Irish Quality salmon and trout	C, L	Europe	+	+	+		
ISO 22000	S	global	+		+		+
ISO 9001/14001	S	global			+		+
KRAV, Sweden	C, L	Europe	+	+	+		
Marine Stewardship Council (MSC)	C, S, L	global			+		
National Association for Sustainable Agriculture, Australia (NASAA)	C, L	global	+	+	+		
Naturland	CS, L	Europe	+		+	+	+
Norge seafood, Norway	S, L	Europe			+		
Peche responsible Carrefour	C, L	global			+		
Qualite aquaculture de France	S, L	France, EU			+		+
Scottish Salmon Producers' Organisation (SSPO), Code of Good Practice (COGP)	C, L	global	+	+	+		+
Seafood Watch	C, L	USA			+		
SIGES Salmon Chile	CS, L	Europe, USA	+	+	+		+
Shrimp quality guarantee ABCC, Brasil	CS, C, L	UK, Europe	+	+	+	+	+
Shrimp Seal of Quality, Bangladesh	S, L	global	+		+	+	+
Soil Association	S, L	UK	+	+	+	+	+
The Responsible Fishing Scheme	C, CS	UK			+	+	

Different eco-labelling and certification schemes in fisheries have different criteria, assessment procedures, levels of transparency, developers of standards (non-governmental organisations (NGOs), private companies, industry groups, etc.), and cover different topics: fishing techniques, bycatch, sustainability of stocks, conservation of ecosystems, etc. (Washington and Ababouch, 2011). Anyway, public and private eco-labelling and certification schemes must be clear, powerfull and in line with FAO Guidelines (FAO, 2011).

Naturland has been a regional standard for organic agriculture since 1982 and a global standard since 1986. The first private organic label standard in the fisheries and aquaculture sector was Naturland Standard for Organic Aquaculture, introduced in 1996, and for wild catch in 2006. Naturland was initially used for standardising carp aquaculture in Germany but has also developed a standard for sustainable capture fisheries that takes into account environmental aspects (fish stocks and fishing gear), but also social and economic aspects of fishing (Naturland, 2023). The standard for aquaculture includes mussels, shrimp, finfish, and macroalgae. The wild catch standard includes all freshwater and marine organisms.

In 1997, the Marine Stewardship Council (MSC) is founded as a global non-profit organisation by Unilever and the World Wildlife Fund (WWF). In 1998, the MSC established a global standard for eco-labelling of all wild caught

species (MSC, 2023). Founding stakeholders include civil society and the private sector. The MSC blue fish label encourages fisheries, retailers and restaurants, the supply chain and consumers towards a sustainable seafood market. The Global Aquaculture Alliance (GAA)/The Global Seafood Alliance (GSA) was founded in 1997 by shrimp farmers and aquafeed and seafood companies in the USA, but the Standards for Best Aquaculture Practices (GAA BAP) were not developed until 2004 (GSA, 2023). The GAA is an industry-led trade organisation in the aquaculture sector. Founding members include civil society, the private sector and producers.

EUREPGAP was founded in 1997 as an initiative of the Euro-Retailer Produce Group (EUREP) in Germany. In 2007, EUREPGAP was renamed the Global Partnership for Good Agricultural Practice (GLOBALG.A.P.). GLOBALG.A.P. is a label for smart agriculture and covers a wide range of aquatic organism groups. (GLOBALG.A.P., 2023)

ChinaG.A.P. was established in 2005 and has been certifying farming production in China since 2008, with specific checkpoints for some taxa (such as eel, tilapia, etc.) (Potts et al., 2016).

In 2008, Friend of the Sea (FOS) was established by the Earth Island Institute, initially for the Tuna Dolphin Safe Project. The first edition of the standards for both wild catch and aquaculture dates from 2013 and covers all species of fish, abalone, shellfish and crustaceans (FOS, 2023).

The Aquaculture Stewardship Council (ASC) was established in 2010 by the Dutch Sustainable Trade Initiative (IDH) and WWF Netherlands (ASC, 2023). The ASC has produced standards for fish farming for various taxa, as well as standards for shellfish, abalone and shrimp.

The International Federation of Organic Agriculture Movements (IFOAM) is an international organisation for organic agriculture founded in 1972. The aim of the organisation is to facilitate the transition to organic agriculture and sustainable production and consumption (IFOAM, 2023). The standardisation of organic production began in the 1970s, while the standards for aquaculture were developed in 2012.

The EU has its own ecolabel, the EU Ecolabel Flower, for a wide range of consumer products (EU, 2023). The idea of creating a European ecolabel for fisheries products dates to 1997 and the Communication from the Commission to the Council and the European Parliament – The future for the market in fisheries products in the European Union: responsibility, partnership and competitiveness (COM/97/0719 final). In 2005, the Communication from the Commission to the Council, the European Parliament and the European Economic and Social Committee – Launching a debate on a Community approach towards eco-labelling schemes for fisheries products (COM(2005) 275 final) highlighted the need for a consistent EU policy on eco-labelling in fisheries to solve the problem of the development of different eco-labels (Bloom, 2023). In 2016, the European Commission adopted a report on options for an EU eco-label scheme for fisheries and aquaculture products (COM(2016) 263 final). Nevertheless, private certification does not provide entry to some markets, e.g. the EU market, if the exporting country does not have a licence to export to the EU (Potts et al, 2016).

Conclusions

The growing demand for various high-quality seafood products has increased pressure on marine ecosystems, and this demand will probably increase in the future, posing various sustainability challenges. To address these challenges in the fisheries and aquaculture sector, effective, internationally recognised standards are essential. Standards can cover various aspects of sustainability: environmental issues, food safety and quality, seafood traceability, labour ethics, etc. As consumer awareness of sustainability issues and demand for ethical products increases, so does the number of different voluntary standards. The wide acceptance of environmental standards has increased their impact at the global level., but a large number of standards can be confusing for stakeholders. Governments, producers, retailers and consumers are trying to figure out which standard is the most beneficial from their perspective. Despite the large number of private standards, the confusion they create and their sometimes questionable effectiveness, they are becoming more common as they can fill the gaps in public standards and help companies improve their reputation and avoid negative publicity. Although standards are not mandatory, they can also become mandatory to address specific markets. Different standards and certification schemes in fisheries cover different topics and have different criteria, assessment procedures and levels of transparency, but whether public or private, eco-labelling schemes should be clear, powerful and comply with FAO Guidelines (FAO, 2011). Standards in fisheries are crucial not only for the conservation of ecosystems and biodiversity, but also for strengthening the resilience of the entire sector.

References

- Akbari N., Failler P., Pan H., Drakeford B., Forse A. (2023). The Impact of Fisheries on the Economy: A Systematic Review on the Application of General Equilibrium and Input–Output Methods. *Sustainability*. 15: 6089.
- ASC. (2023). Available from: [https://www.bloomassociation.org/en/a-european-eco-label-for-aquaculture-and-fisheries-products/](https://asc-aqua.org/producers/farm-standards/Berliner D., Prakash, A. (2013). Signaling environmental stewardship in the shadow of weak governance: The global diffusion of ISO 14001. <i>Law and Society Review</i>. 47: 345–373.</p><p>Bimbo F., Viscecchia R., De Devitiis B., Seccia A.R., De Boni A. (2022). How Do Italian Consumers Value Sustainable Certifications on Fish?—An Explorative Analysis. <i>Sustainability</i>. 14: 3654.</p><p>Bloom (2023). Available from: <a href=)
- Bertheussen B.A., and Vassdal T. (2023). Appropriation of economic values in a rights-based fishery. *Ocean & Coastal Management*. 237: 06537.
- EU (2023). Available from: https://environment.ec.europa.eu/topics/circular-economy/eu-ecolabel-home_en
- FAO (2009). Private standards in fisheries and aquaculture: current practice and emerging issues. *Globefish Research Programme Vol. 97*.
- FAO (2011). FAO Guidelines for the Ecolabelling of Fish and Fishery Products from Marine Capture Fisheries. Available from: <https://www.fao.org/3/i1119t/i1119t.pdf>
- FAO (2018). Achieving Blue Growth Building vibrant fisheries and aquaculture communities. Available from: <https://www.fao.org/3/ca0268en/CA0268EN.pdf>
- FAO (2022a). The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. Rome.
- FAO(2022b). Framework for environmental and social management. Rome.
- FAO (2023). Available from: <https://www.fao.org/environmental-social-standards/en/>
- FOS (2023). Available from: <https://friendofthesea.org/#>
- Garlock T., Anderson J.L., Asche F., Smith M.D., Camp E., Chu J., Lorenzen K., Vannuccini S. (2022). Global insights on managing fishery systems for the three pillars of sustainability. *Fish and Fisheries*. 23: 899–909.
- Giacomarra M., Crescimanno M., Vrontis D., Pastor L. M., Galati A. (2021). The ability of fish ecolabels to promote a change in the sustainability awareness. *Marine Policy*. 123: 104292.
- GLOBALG.A.P. (2023). Available from: https://www.globalgap.org/uk_en/who-we-are/about-us/
- Green J. F. (2014). Rethinking private authority. Princeton University Press. Available from: http://www.jstor.org/stable/j.ctt5h_hqdr
- GSA (2023). Available from: <https://www.globalseafood.org/>
- Gutierrez N.L., Defeo O., Bush S.R., Butterworth D.S., Roheim C.A., Punt A.E. (2016). The current situation and prospects of fisheries certification and ecolabelling. *Fisheries Research*. 182: 1-6.
- Hadjimichael M., and Hegland T.J. (2016). Really sustainable? Inherent risks of eco-labeling in fisheries. *Fisheries Research*. 174: 129-135,
- Hojnik J., Ruzzier M., Konečnik Ruzzier M. (2019). Transition towards Sustainability: Adoption of Eco-Products among Consumers. *Sustainability*. 11: 4308.
- IFOAM (2023). Available from: <https://www.ifoam.bio/>
- MSC (2023). Available from: <https://www.msc.org/>
- Murphy E.L., Bernard M., Gerber L.R., Dooley K J. (2021). Evaluating the role of market-based instruments in protecting marine ecosystem services in wild-caught fisheries. *Ecosystem Services*. 51: 101356.
- Naturland (2023). Available from: <https://www.naturland.de/en/naturland/who-we-are/history.html>

- Oosterveer P. (2010). Ecolabelling and seafood certification in equitable benefit sharing of Tuna fisheries.
- Penca J. (2020). Mainstreaming Sustainable Consumption of Seafood Through Enhanced Mandatory Food Labeling. *Frontiers in Marine Science*. 7: 598682.
- Peiró-Signes A., Miret-Pastor L., Galati A., Segarra-Oña M.V. (2022). Consumer Demand for Environmental, Social, and Ethical Information in Fishery and Aquaculture Product Labels. *Frontiers in Marine Science*. 9: 948437.
- Pierucci A., Columbu S., Kell L.T. (2022). A global review of MSC certification: Why fisheries withdraw? *Marine Policy*. 143: 105124.
- Potts J., Wilkings A., Lynch M., McFatridge, S. (2016). State of Sustainability Initiatives Review: Standards and the Blue Economy, International Institute for Sustainable Development, Winnipeg, Manitoba, Canada.
- Ramachandran C., and Parappurathu S. (2020). Who Should Certify the Sustainability of Our Fisheries? A Property Rights Perspective on Ecolabelling. *Current science*. 118: 1496-1499.
- Roheim C.A., Asche F., Santos J.I. (2011). The elusive price premium for ecolabelled products: Evidence from seafood in the UK Market. *Journal of Agricultural Economics*. 62: 655–668.
- Sainsbury K. (2010). Review of ecolabelling schemes for fish and fishery products from capture fisheries, FAO Fisheries and Aquaculture Technical Paper. 93p. Rome. Available from: <https://www.fao.org/3/i1433e/i1433e.pdf>
- Schiller L., and Bailey M. (2021). Rapidly increasing eco-certification coverage transforming management of world's tuna fisheries. *Fish and fisheries*. 22: 592–604.
- Thorlakson T., Hainmueller J., Lambin E.F. (2018). Improving environmental practices in agricultural supply chains: The role of company-led standards. *Global Environmental Change*. 48: 32–42.
- UNEP (2009). Certification and sustainable fisheries.
Available from: <https://wedocs.unep.org/20.500.11822/23019>
- UNCTAD (2020). The Framework for the Voluntary Sustainability Standards (VSS) Assessment Toolkit. Available from:
https://unctad.org/system/files/official-document/ditctabinf2020d5_en.pdf
- Washington S., and Ababouch L. (2011). Private standards and certification in fisheries and aquaculture: Current practice and emerging issues FAO Technical paper 553, FAO, Rome, Italy. Available from: <https://www.fao.org/3/i1948e/i1948e.pdf>

Morfometrijska obilježja sipe, *Sepia officinalis* Linnaeus, 1758, u Jadranskom moru

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Sažetak

Cilj rada je opisati morfometrijske značajke kljuna i sipovine vrste *Sepia officinalis* u Jadranu. Uzorci su prikupljeni tijekom 2017. i 2018. godine na području srednjeg Jadrana. Utvrđeno je da povećanjem dužine plašta kod oba spola na donjem i gornjem kljunu najviše raste dužina krijeste, a najsporije rostrum. Ustanovljene su statistički značajne razlike među spolovima za morfometrijske odnose širine sipovine i dužine plašta, širine unutarnjeg konusa i širine sipovine te širine vanjskog konusa i širine sipovine. Dobiveni rezultati mogu poslužiti za diferencijaciju kljunova i sipine kosti vrsta iz porodice Sepiidae, a korisni su i za istraživanja hranidbenih mreža, posebice prehrane predatora glavonožaca.

Ključne riječi: *Sepia officinalis*, morfometrija, kljun, sipovina, Jadransko more

Uvod

Obična sipa, *Sepia officinalis* Linnaeus, 1758, je vrsta glavonošca koja obitava na području sjeveroistočnog Atlantika, od Baltičkog i Sjevernog mora do Južne Afrike i Mediterana (Roper i sur., 1984). Živi na pjeskovitom i muljevitom dnu kontinentalnog šelfa, najčešće na dubinama od 100 do 200 metara (Guerra, 2006).

To je vrsta velikog gospodarskog značaja, jedna od najvažnijih među glavonošcima (Jereb i sur., 2015). Ulovi svih sipa čine 12 do 16 % ukupnog ulova glavonožaca ili između 300 000 i 500 000 tona godišnje, a najviše se love na području Azije, sjevero-zapadnog Indijskog oceana i na području Mediterana (FAO, 2023). Ukupni godišnji ulovi sipe u Jadranu 2020. godine iznosili su 2 147 tona, a najviše sipe lovi Italija (1 956 tona). Ulovi Republike Hrvatske 2020. godine su iznosili ukupno 103 tone (STECF, 2021).

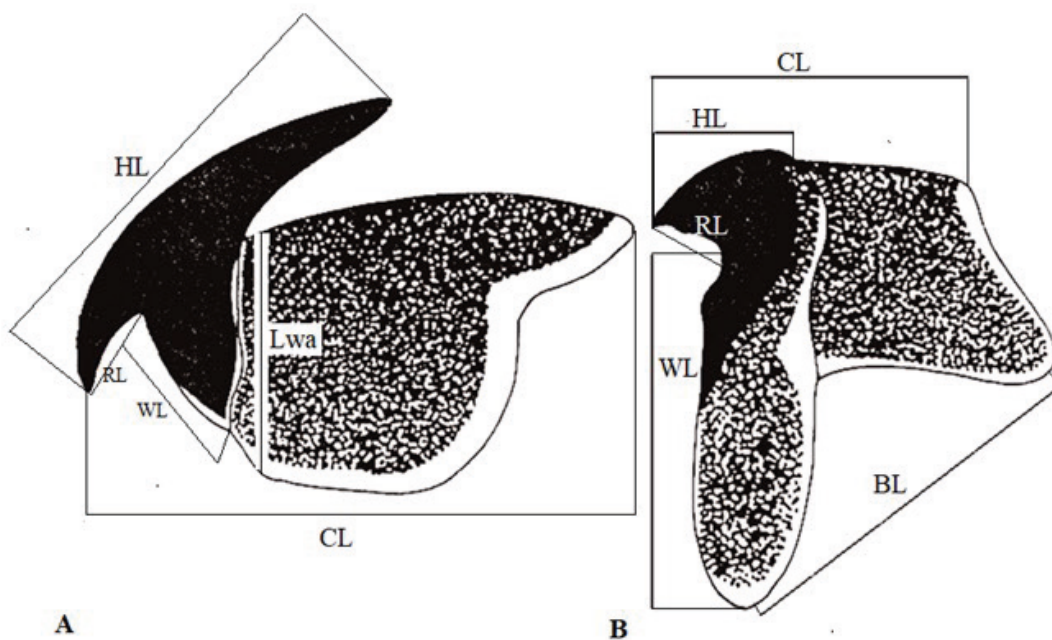
Na području Mediterana i istočnog Atlantika, sipa se najviše lovi pridnenom povlačnom mrežom kočom i jednostrukim mrežama stajačicama (Lefkaditou i sur., 2007). Na tržište se plasira svježa ili smrznuta i vrlo je cijenjena, poglavito u Japanu, Koreji, Italiji i Španjolskoj. U Jadranu se primarno lovi povlačnom mrežom kočom, kao ciljane vrsta ili prilov pridnenog ribolova. Lovi se i raznim vrstama ribolovnih alata u malom obalnom ribolovu, primjerice jednostrukim i trostrukim mrežama stajačicama, vršama i ostima, često kombiniranim s upotrebom svjetla.

Na području Jadrana dosadašnja istraživanja uglavnom su se bavila rasprostranjenošću, abundancijom i strukturom populacije ove vrste (vidi Krstulović Šifner i sur., 2011). Istraživana je i struktura sipovine *S. officinalis* (Čadež i sur., 2017; Ivanković i sur., 2010; Milovac i sur., 2014), ali prema dostupnoj literaturi do sada nisu detaljno istražena morfometrijska obilježja sipovine i kljuna ove vrste. Stoga je glavni cilj ovog rada bio ispitati i opisati međusobne morfometrijske odnose kljunova i sipovine kao i odnose mjerenih veličina ove dvije čvrste tvorbe i duljine plašta sipe. Sipovina i kljunovi su pouzdan alat za diferencijaciju glavonožaca što je osobito značajno kod istraživanja prehrane njihovih predatora obzirom da se radi o rijetkim tvrdim tvorbama u tijelu glavonožaca te se često mogu naći kao jedini njihov ostatak u probavilima predatora (Clarke, 1987; Neige i Boletzky, 1997). Ovakva istraživanja, osim što su značajna su za procjenu položaja i značaja vrste u hranidbenim mrežama, predstavljaju i važan alat za otkrivanje morfoloških varijacija među stokovima te indirektno služe boljem dugoročno održivom upravljanju ribolovnom resursima.

Materijal i metode

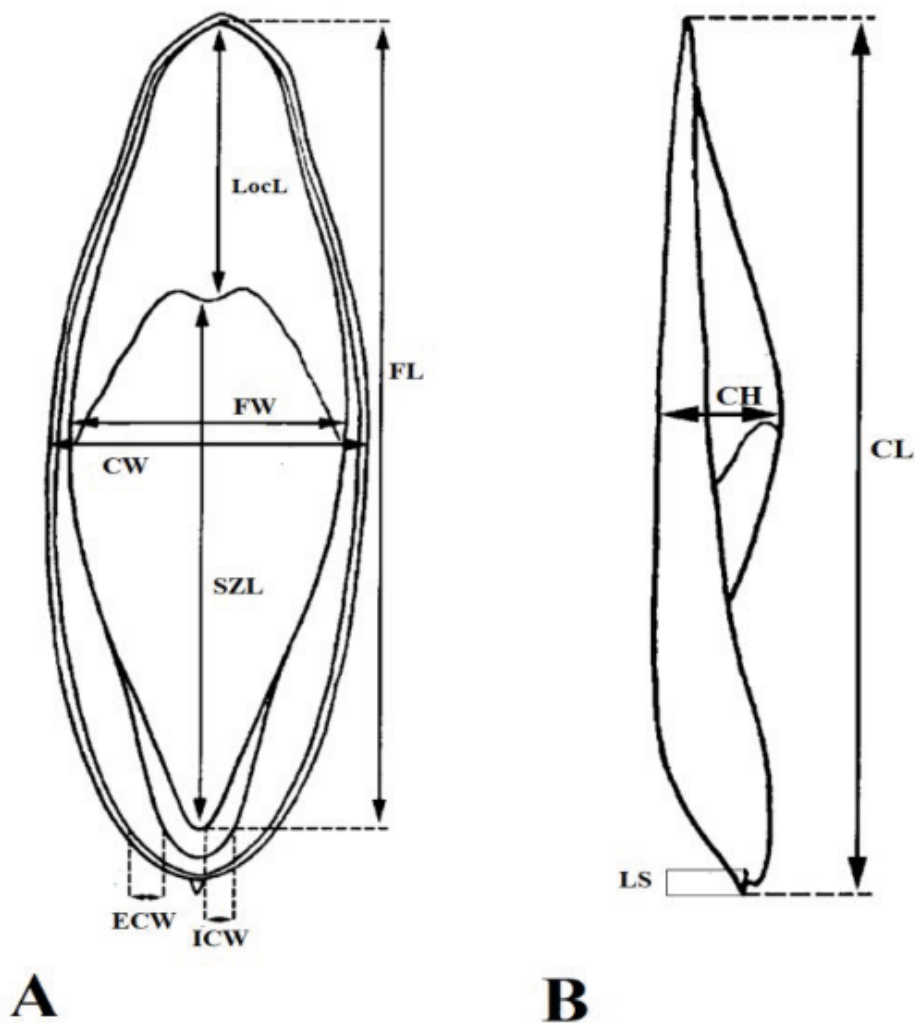
Za biometrijsku analizu sipe korišteni su uzorci ulovljeni u vodama otoka Visa, Palagruže i Hvara te uz obale Murtera i Primoštena tijekom 2017. i 2018. godine. Ribolovni alati koji su korišteni pri izlovu su migavica, kočca, trostruke jednododne mreže stajačice i poponica (tzv. mrižica).

Gornje i donje čeljusti, tzv. kljunovi, izolirani su iz analiziranih jedinki te su oprani i pohranjeni u označene epruvete s čistom vodom do obrade. Prilikom analize svi su kljunovi izvagani s točnošću od 0,01 g, a veličine na gornjem i donjem kljunu izmjerene su digitalnom mjerkom s točnošću od 0,01 mm. Izmjereno je ukupno 7 različitih morfoloških značajki (Slika 1). Odvojeno za ženke i mužjake napravljena je usporedba rasta pojedinih dijelova kljuna u odnosu na dužinu plašta jednostavnom linearnom regresijom: $y = ax + b$, gdje je graf linearne funkcije pravac čiji je nagib jednak a , odsječak na osi y jednak je b , y predstavlja mjereno morfometrijsko obilježje, a x dorzalnu dužinu plašta. Za svaki opisan odnos naveden je koeficijent determinacije (R^2) kao pokazatelj reprezentativnosti dobivenih regresija.



Slika 1. Mjerene morfometrijske značajke gornjeg A) i donjeg B) kljuna vrste *Sepia officinalis* (mm): dužina kape (HL), dužina krijeste (CL), dužina rostruma (RL), dužina krila (WL), širina čeljusti (JW), širina lateralne stijenke na gornjem kljunu (Lwa), dužina bazne linije na donjem kljunu (BL) (prema Mangold i Fioroni, 1966).

Sipovina vrste *S. officinalis* izolirana je iz jedinki u uzorcima, isprana u vodi, osušena te pohranjena u papirnate omotnice s pripadajućim identifikacijskim oznakama. Sve sipovine su izvagane, a prilikom analize na svakoj je digitalnom mjerkom s točnošću od 0,01 mm izmjereno 11 različitih veličina (Slika 2). Napravljena je usporedba međusobnih omjera pojedinih izmjerenih morfometrijskih značajki sipovine za mužjake i ženke, a uz to su izračunati omjeri između dužine i širine sipovine (CL i CW) i dužine plašta (DML). Dobivene morfometrijske vrijednosti ispitane su pomoću t-testa kako bi se utvrdile eventualne razlike između ženki i mužjaka.



Slika 2. Morfometrijske mjere sipovine vrste *Sepia officinalis* (mm): A) širina sipovine (CW), dužina i širina fragmokon (FL, FW), širina unutarnjeg i vanjskog konusa (ICW, ECW), dužina unutarnjeg konusa (ICL), dužina isprugane zone (SZL), dužina lokulusa (LocL), B) dužina sipovine (CL), visina sipovine (CH) i dužina bodlje (LS) (prema Guerra i sur., 2001).

Rezultati i rasprava

Analizirano je ukupno 45 primjeraka ($m/\bar{z} = 1,14$), dorzalne duljine plašta (DML) u rasponu od 83 do 283 mm i ukupne mase tijela (BW) od 86,6 to 1400 g. Masa analiziranih kljunova sipe kretala se od 0,05 do 0,21 g ($0,12 \pm 0,04$ g). U tablici 1 prikazane su izmjerene vrijednosti dužine pojedinih dijelova kljuna.

Tablica 1. Raspon (min-max), srednje vrijednosti sa standardnom devijacijom (\pm SD) i standardna pogreška (SE) morfometrijskih značajki donjeg (d) i gornjeg (g) kljuna (mm) sipe (puni nazivi kratica mjera kljunova navedenih u tablici nalaze se na slici 1).

značajka		raspon (mm)	\pm SD	SE
HL	d	3,28 – 12,65	$5,06 \pm 1,93$	0,26
	g	9,76 – 26,23	$13,28 \pm 3,46$	0,52
CL	d	7,86 – 26,23	$10,42 \pm 96$	0,45
	g	13,57 – 36,05	$18,17 \pm 4,58$	0,69

RL	d	2,21 – 6,17	$3,1 \pm 0,78$	0,12
	g	2,16 – 5,60	$3,52 \pm 0,76$	0,12
WL	d	6,94 – 21,19	$10,05 \pm 2,66$	0,4
	g	4,33 – 12,67	$6,05 \pm 1,5$	0,22
JW	d	2,19 – 6,05	$3,17 \pm 0,74$	0,11
	g	1,41 – 4,04	$2,32 \pm 0,53$	0,08
BL	d	1,77 – 26,68	$13,09 \pm 3,97$	0,6
Lwa	g	2,54 – 13, 86	$7,27 \pm 1,76$	0,26

Prema dobivenim korelacijama i vrijednostima koeficijenta a , kod oba spola se na donjem kljunu s porastom dužine plašta najviše povećava dužina krijeste (CL) i dužina bazne linije (BL) dok dužina rostruma (RL) i širina čeljusti (JW) imaju najsporiji rast (Tablica 2). Najbrži rast gornjeg kljuna s porastom dužine plašta kod mužjaka i ženki imaju dužina krijeste (CL) i dužina kape (HL), a najsporije s povećanjem dužine plašta raste dužina rostruma (RL) i širina čeljusti (JW).

Tablica 2. Linearna regresijska analiza za morfometrijske odnose mjera kljunova mužjaka i ženki sipe u odnosu na dužinu plašta (DML) odvojeno za donji (DK) i gornji kljun (GK), prikazano dobivenom formulama s koeficijentima determinacije (R^2) (puni nazivi kratica mjera kljunova navedenih u tablici nalaze se na slici 1).

	ženke	mužjaci
DK	CL = $0,057DML + 3,7076$, $R^2 = 0,7707$	CL = $0,0774DML + 1,9313$, $R^2 = 0,8200$
	BL = $0,092DML + 2,1136$, $R^2 = 0,2856$	BL = $0,0836DML + 3,9787$, $R^2 = 0,7637$
	WL = $0,0518DML + 3,0865$, $R^2 = 0,6039$	WL = $0,0675DML + 2,4146$, $R^2 = 0,7778$
	HL = $0,0349DML + 0,7773$, $R^2 = 0,6488$	HL = $0,0455DML - 0,1766$, $R^2 = 0,8269$
	JW = $0,0203DML + 0,9847$, $R^2 = 0,3079$	JW = $0,0175DML + 1,1349$, $R^2 = 0,8080$
	RL = $0,0093DML + 2,0438$, $R^2 = 0,0871$	RL = $0,0167DML + 1,11562$, $R^2 = 0,5895$
GK	CL = $0,0923DML + 7,4181$, $R^2 = 0,7451$	CL = $0,1186DML + 4,8991$, $R^2 = 0,7939$
	HL = $0,0893DML + 2,9347$, $R^2 = 0,7953$	HL = $0,087DML + 3,3257$, $R^2 = 0,8055$
	Lwa = $0,0426DML + 2,5549$, $R^2 = 0,5693$	Lwa = $0,0417DML + 2,4508$, $R^2 = 0,6601$
	WL = $0,0441DML + 1,3124$, $R^2 = 0,5144$	WL = $0,0379DML + 1,6024$, $R^2 = 0,8264$
	JW = $0,0134DML + 0,8451$, $R^2 = 0,2737$	JW = $0,009DML + 1,2835$, $R^2 = 0,4434$
	RL = $0,0122DML + 2,1359$, $R^2 = 0,1496$	RL = $0,0148DML + 1,8381$, $R^2 = 0,5062$

Unatoč velikom broju istraživanja vrste *S. officinalis* na području Atlantika i Mediterana (Boletzky, 1983; Relini i sur., 1999), jako malo je studija koje se obave analizama kljunova (Xavier, 2022). Na temelju istraživanja odnosa veličina dijelova kljuna i plašta za ovu vrstu na području središnjeg Mediterana (južno Tirensko more) ispostavilo se da krijesta gornjih i donjih kljunova raste brže od ostalih parametara (Giordano i sur., 2006), a isto pokazuju i rezultati ovog rada za ukupni uzorak te odvojeno za mužjake i ženke.

Analiza morfometrijskih odnosa sipovine ukazuje na značajne razlike između mužjaka i ženki za odnos širine sipovine i dužine plašta (CW/DML), odnos širine unutarnjeg konusa i širine sipovine (ICW/CW) te odnos širine vanjskog konusa i širine sipovine (ECW/CW) (Tablica 3). Za ostale odnose nije utvrđena razlika između spolova, a u prosjeku su oni veći kod ženki nego kod mužjaka, osim srednjih vrijednosti odnosa dužine bodlje i dužine sipovine (LS/CL) te širine fragmokona i širine sipovine (FWL/CL) koji su veći kod mužjaka.

Tablica 3. Morfometrijski odnosi sipovine za mužjake, ženke i ukupni uzorak *S. officinalis*; raspon % vrijednosti odnosa (raspon), srednja vrijednost sa standardnom devijacijom (\pm SD), standarna pogreška (SE), varijanca (V), značajnost razlike među spolovima prema t-testu (s), naznačena sa * (puni nazivi kratica mjera sipovine navedenih u tablici nalaze se na slici 2).

omjer (%)	spol	N	raspon	$\bar{x} \pm SD$	SE	V	s
CL/DML	♀	24	88,43 – 99,89	95,81 \pm 2,95	0,67	5,29	
	♂	21	80,37 – 98,75	95,10 \pm 4,59	1,14	5,03	
	ukupno	45	80,37 – 99,89	95,47 \pm 3,72	0,63	6,74	
CW/DML	♀	24	31,10 – 45,76	38,10 \pm 2,64	0,54	6,94	
	♂	21	28,89 – 40,27	35,33 \pm 2,43	0,53	6,88	*
	ukupno	45	28,89 – 45,76	36,81 \pm 2,88	0,42	7,82	
FL/CL	♀	24	90,56 – 96,54	92,46 \pm 1,33	0,27	2,74	
	♂	21	85,07 – 96,92	92,45 \pm 2,27	0,51	5,06	
	ukupno	45	85,07 – 96,92	92,46 \pm 1,81	0,28	3,96	
LS/CL	♀	24	4,00 – 9,29	6,96 \pm 1,35	0,32	19,42	
	♂	21	5,49 – 9,33	7,68 \pm 1,22	0,31	15,96	
	ukupno	45	4,00 – 9,33	7,27 \pm 1,28	0,21	17,67	
LocL/CL	♀	24	21,71 – 45,18	32,53 \pm 4,39	0,90	5,29	
	♂	21	23,06 – 44,40	32,19 \pm 5,33	1,16	5,03	
	ukupno	45	21,71 – 45,18	32,37 \pm 4,80	0,72	6,74	
FW/CW	♀	24	81,85 – 91,70	87,84 \pm 2,18	0,44	2,48	
	♂	21	86,48 – 92,41	88,95 \pm 1,57	0,34	1,77	
	ukupno	45	81,85 – 92,41	88,35 \pm 2,00	0,29	2,27	
SZL/CL	♀	24	48,08 – 69,52	60,92 \pm 4,86	0,99	7,98	
	♂	21	52,92 – 71,65	60,82 \pm 4,98	1,08	8,19	
	ukupno	45	48,08 – 71,65	60,88 \pm 4,86	0,72	7,98	
ICL/CL	♀	24	29,24 – 45,43	39,70 \pm 2,79	0,57	7,03	
	♂	21	31,27 – 47,25	38,58 \pm 3,06	0,66	7,94	
	ukupno	45	29,24 – 47,25	38,51 \pm 3,48	0,51	9,05	
ICW/CW	♀	24	6,92 – 15,81	10,76 \pm 1,66	0,34	15,49	
	♂	21	6,31 – 13,25	9,15 \pm 1,62	0,35	17,71	*
	ukupno	45	6,31 – 15,81	9,73 \pm 2,06	0,30	21,22	
ECW/CW	♀	24	24,31 – 36,06	28,73 \pm 3,24	0,66	11,29	
	♂	21	20,19 – 30,60	26,90 \pm 2,07	0,45	7,72	*
	ukupno	45	20,19 – 36,06	27,48 \pm 3,25	0,48	11,85	

Sipovina je prepoznata kao koristan alat za određivanje vrsta porodice Sepiidae (Neige i Boletzky, 1997). Rast sipovine povezan je s parametrima poput temperature, dostupnosti nutrijenata i dubine na kojoj jedinke obitavaju, međutim oni ne bi trebali u većoj mjeri utjecati na morfometrijske odnose. Khromov (1998) je dao detaljne opise i deskriptivna obilježja sipovine za diferencijaciju vrsta *S. officinalis* i *S. hierreda*. Morfometriju sipovine tih istih vrsta s područja senegalskih voda i obala Pirenejskog poluotoka opisali su Guerra i suradnici (2001) te također nalaze i razlike određenih omjera između mužjaka i ženki.

Zaključak

Rezultati ovog rada predstavljaju prilog poznavanju morfometrijskih obilježja sipe u Jadranu te ukazuju na specifičnosti vrste za analizirane odnose kao i na određene razlike među spolovima. Analiza morfometrije kljuna sipe pokazala je da se kod oba spola na donjem kljunu s porastom dužine plašta najviše povećava dužina krijeste i

dužina bazne linije dok dužina rostruma i širina čeljusti imaju najsporiji rast. Najbrži rast gornjeg kljuna s porastom dužine plašta kod mužjaka i ženki imaju dužina krijeste i dužina kape, a najsporiji dužina rostruma i širina čeljusti. Analizom morfometrije sipovine utvrđene su razlike među spolovima za odnos širine sipovine i dužine plašta, odnos širine unutarnjeg konusa i širine sipovine te odnos širine vanjskog konusa i širine sipovine.

Literatura

- Boletzky S.V. (1983). *Sepia officinalis*. U: Boyle P.R. (ur.), Cephalopod Life Cycles. Vol I. Species account. Academic Press, London, str. 31-52.
- Clarke M.R. (1987). Cephalopod biomass. Estimation from predation. U: Cephalopod Life Cycles. Vol. II. Comparative Reviews. Boyle P.R. (ur.), Academic Press, London, str. 221-237.
- Čadež V., Škapin S., Leonardi A., Križaj I., Kazazić S., Salopek-Sondi B., Sondi I. (2017). Formation and morphogenesis of a cuttlebone's aragonite biomineral structures for the common cuttlefish (*Sepia officinalis*) on the nanoscale: Revisited. *Journal of Colloid and Interface Science*. 508: 95-104.
- FAO (2023). FISHSTAT Plus: Universal software for fishery statistical time series, Version 2.3. Fisheries Department, Fishery Information, Data and Statistics Unit. Capture production 1950-2021. Dostupno na: <https://www.fao.org/fishery/en/topic/166235/en>.
- Giordano D., Bottari T., Busalacchi B., Jereb P., Ragonese S., Rinelli P. (2006). On the relationship between beaks and body size in *Sepia officinalis* from the Southern Tyrrhenian Sea (Central Mediterranean). *Cahiers de Biologie Marine*. 46: 35-42.
- Guerra Á. (2006). Ecology of *Sepia officinalis*. U: The cuttlefish *Sepia officinalis*: a working model in cephalopod research. *Vie et Milieu/Life & Environment*, str. 97-107.
- Guerra Á., Pérez-Losada M., Rocha F., Sanjuan A. (2001). Species differentiation of *Sepia officinalis* and *Sepia hierredda* (Cephalopoda: Sepiidae) based on morphological and allozyme analyses. *Journal of the Marine Biological Association of the UK*. 81: 271-281.
- Ivanković H., Tkalčec E., Orlić S., Gallego Ferrer G., Schauerl Z. (2010). Hydroxyapatite formation from cuttlefish bones: kinetics. *Journal of materials science. Materials in medicine*. 21: 2711-2722.
- Jereb P., Allcock L.A., Lefkaditou E., Piatkowski U., Hastie L.C., Pierce G.J. (2015). Cephalopod biology and fisheries in Europe: II. Species Accounts. ICES Cooperative Research Report br. 325, str. 67-87.
- Khromov D.N. (1998). Appendix I. Taxonomic keys. *Smithsonian Contributions to Zoology* 1, str. 140-156.
- Krstulović Šifner S., Peharda M., Vrgoč N., Isajlović I., Dadić V., Petrić M. (2011). Biodiversity and distribution of cephalopods caught by trawling along the Northern and Central Adriatic Sea. *Cahiers de Biologie Marine*. 52: 291-302.
- Lefkaditou E., Verriopoulos G., Valavanis V. (2007). VII. 9. Research on cephalopod resources in hellas. *State of Hellenic Fisheries*. 440: 62-68.
- Mangold K., and Fioroni P. (1966). Morphologie et biométrie des mandibules de quelques céphalopodes Méditerranéens. *Vie et Milieu*. 22: 1139-1196.
- Milovac D., Gallego Ferrer G., Ivanković M., Ivanković H. (2014). PCL-coated hydroxyapatite scaffold derived from cuttlefish bone: morphology, mechanical properties and bioactivity. *Materials science & engineering. Materials for biological applications*. 34: 437-45.
- Neige P., and Boletzky S.V. (1997). Morphometrics of the shell of three *Sepia* species (Mollusca: Cephalopoda): intra- and interspecific variation. *Zoologische Beiträge*. 38: 137-156.
- Relini G., Bertrand J., Zamboni A. (1999). Synthesis of the knowledge on bottom fishery resources in the Central Mediterranean (Italy and Corsica). *Biologia Marina Mediterranea*. 6: 680-686.
- Roper C.F.E., Sweeney M.J., Nauen C.E. (1984). Cephalopods of the world. An annotated and illustrated catalogue of species of interest to fisheries. *FAO Fisheries Synopsis No. 125, Vol. 3*: 1-277.

STECF-Scientific, Technical and Economic Committee for Fisheries. (2021). Stock assessments in the Mediterranean Sea 2021 – Adriatic and Ionian Seas (STECF-21-15). Publications Office of the European Union, Luxembourg, ISBN 978-92-76-46195-1, doi:10.2760/59806, JRC127766.

Xavier J.C., Golikov A.V, Queirós J.P, Perales-Raya C., Rosas-Luis R., Abreu J., Bello G., Bustamante P., Capaz J.C., Dimkovikj V.H, González A.F, Guímaro H., Guerra-Marrero A., Gomes-Pereira J.N, Hernández-Urcera J., Kubodera T., Laptikhovsky V., Lefkaditou E., i sur. (2022). The significance of cephalopod beaks as a research tool: An update. *Frontier in Physiology*. 13: 1038064.

Morphometric traits of the common cuttlefish, *Sepia officinalis* Linnaeus, 1758, in the Adriatic Sea

Abstract

The aim of the study is to describe the morphometric characteristics of the cuttlebone and beaks of the species *Sepia officinalis* in the Adriatic Sea. Samples were collected during 2017 and 2018 in the central Adriatic. It was found that in lower and upper beaks of both sexes, with an increase of the mantle length there is the highest increase of crest length, while the lowest increase for rostrum length was found. There are significant differences between sexes for cuttlebone width and dorsal mantle length, internal cone width and cuttlebone width, and external cone width and width of cuttlebone rations. The obtained results can be used for the differentiation of beaks and cuttlebones of species from the Sepiidae family, and are also useful in studies of food webs, especially feeding of the cephalopod predators.

Keywords: *Sepia officinalis*, morphometry, beaks, cuttlebone, Adriatic Sea

Content of calcium and magnesium in pollen from the area of Eastern Bosnia

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Abstract

Bee pollen is a high-quality supplement used in human and bee nutrition. Data about its mineral composition are important for proper assessment of its nutritional quality and safety. In this research we analysed the contents of calcium (Ca) and magnesium (Mg) in the bee pollen collected from the three locations in the area of Eastern Bosnia: Bijeljina, Lopare and Zvornik. Determination of calcium and magnesium was done by method of atomic absorption spectrophotometry after acid digestion ($\text{HNO}_3 + \text{HClO}_4$) of pollen. The lowest contents of both elements were found in the bee pollen from Zvornik (1350,18 mg kg⁻¹ for Ca, 541,59 mg kg⁻¹ for Mg), while the highest contents were found in the samples from Bijeljina (1643,91 mg kg⁻¹ for Ca, 670,29 mg kg⁻¹ for Mg). Determined statistically high significant differences in the content of both elements between pollen originating from all three tested apiaries indicated that the location of apiaries had significant impact on the content of calcium and magnesium in tested pollen samples. Considering that the location of the apiary was mainly determined by the predominant bee forage, it is necessary for future research to specify in more detail the honey plant species and their presence at any particular microlocation.

Keywords: bee products, pollen, minerals, bee forage

Introduction

Bee pollen is one of the most valuable apicultural products, directly produced by honey bees (*Apis mellifera*), which collect pollen from various flowering plants by their back legs (Altunatmaz et al., 2017). Bees use pollen as their primary food, during which pollen is mixed with bees oral secretion and some nectar. Finally, gotten bee pollen is not the same as flower pollen because it contains some nectar, saliva and honey (De-Melo et al., 2015). Therefore, the content of mineral elements in the bee pollen is lower than their amount in flower pollen (Stanciu et al., 2011).

According to Salzar-Gonzales and Diaz-Moreno (2016), bee pollen contains a variety of nutrients and compounds, including proteins (10-40%), carbohydrates (13-55%), lipids (1-13%), dietary fiber (14-20%), minerals, vitamins, and several other bioactive compounds. Mineral content in pollen is usually expressed as ash content (Human and Nicolson, 2006) or as the content of macro- and microelements (Kostić et al., 2015; Aylin et al., 2021). Pollen contains macronutrients such as potassium (K), phosphorus (P), calcium (Ca), sodium (Na), magnesium (Mg), and microelements like iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), and others (Salzar-Gonzales and Diaz-Moreno, 2016). According to Kostić et al. (2015), three most common elements in bee pollen are potassium (K), calcium (Ca) and magnesium (Mg). Campos et al. (2008) reported about the mineral content of bee pollen with the average percentage of four individual macroelements: K 60%, Mg 20%, 10% Na and Ca.

Calcium and magnesium are both alkaline-earth metals essential for the everyday bodily functions of humans and animals, particularly for the health of bones and teeth (Ciosek et al., 2021). Prolonged deficiency of both elements in the human body is responsible for osteoporosis (Farak et al., 2023). Furthermore, a huge lack of calcium leads to kidney and liver disorders, while excessive retention can result in kidney stones (Ali, 2023). Magnesium is present in over 300 enzymes involved in the metabolism of carbohydrates, proteins, and lipids (Swaminathan, 2003). Therefore, its deficiency in the human body, apart from osteoporosis, leads to various disorders such as muscle spasms, cardiovascular diseases, and diabetes (Ciosek et al., 2021). The recommended daily allowances (RDAs) in the human diet include 1000 mg of calcium and 300-400 mg of magnesium (Farak et al., 2023).

Bee pollen is a good source of minerals for humans, including calcium and magnesium. Although the content of individual elements can vary considerably, the order of mineral elements share in pollen is similar. Thus, in 27 bee pollen samples collected in Poland, South Korea and China, the decreasing order of macro- and microelement amounts was reported as K>Mg>Na>Ca>Fe>Mn>Zn>Cu (Szczesna, 2007). In another study, this line-up was K>P>Ca>Mg>Na>Fe>Mn>Zn>Cu (Morgano et al., 2012). In the case of 25 bee pollens examined in the research conducted in Serbia, the following trend was determined: K>Ca>Mg>Fe>Mn>Zn (Kostić et al., 2015). During the examination of 71 bee pollen samples in Spain, Valverde et al. (2023) observed a similar general trend regarding minerals: P>K>Ca>Mg>Na. The main reason for minor differences in the amount of mineral elements in pollen from different geographical areas is the fact that the mineral composition of bee pollen depends on its botanical origin and environmental conditions, such as geographic and climatic characteristics (Kostić et al., 2015; Liolios et al., 2019; Aylanc et al., 2021).

Additionally, climatic conditions during the harvesting period have a great influence on the mineral composition of bee pollen (Taha et al., 2020; Valverde et al., 2023). During the study done in Saudi Arabia, Taha et al. (2020) found the highest values of essential elements (Na, K, Ca, Mg, P, and Mn) in pollen loads trapped during the spring and winter seasons.

Bosnia and Herzegovina has suitable environmental conditions for beekeeping and the production of various bee products (honey, pollen, propolis, etc.). The quantification of macroelements such as Ca and Mg in bee pollen is valuable for the proper assessment of its quality and health indicators. Therefore, the aim of our study is to determine the content of calcium (Ca) and magnesium (Mg) in the bee pollen collected at three locations (Bijeljina, Lopare and Zvornik) in the northeastern part of our country, known as Eastern Bosnia.

Material and methods

This research was conducted in an area situated in the northeastern part of Bosnia and Herzegovina, commonly called Eastern Bosnia. It is characterized by a moderate continental climate and developed agricultural and industrial activities. The area dominates the plains with hills and low mountains (Motajica, Javornik). In the plains, especially in Semberija, the biggest plain in the area, located near the town of Bijeljina, intensive agricultural production is developed, which includes the application of pesticides and other chemical amendments (artificial fertilizers, nutritional supplements, etc.). Besides, the sampling area abounds in long-lasting and diverse bee forage during the whole year (particularly during the spring and early summer), which includes fruit, rapeseed, acacia, linden and meadow plants pasture.

Samples of pollen were collected at the three apiaries located in the municipalities of Bijeljina, Zvornik, and Lopare during the summer of 2022 (from July to August). The same type of pollen trap was used in all examined apiaries. This was an external pollen trap integrated into the subfield. These apiaries were located in the rural parts of the sampling area, far from industrial facilities, roads, and intensive agricultural production, which is ideal for organic agricultural production.

The apiary of Bijeljina is located in the village of Popovi (44°45'54.67"N, 19°17'38.51"E), at an altitude of 90 m. This apiary has a capacity of 150 bee colonies in LR hives. The most abundant forage for the bees during the sampling period was fruits, *Amorpha fruticosa* L., and poppy flowers. The second examined apiary belongs to the municipality of Zvornik and is located in the village of Drinjača (44°16'44.69"N, 19°8'8.88"E), where bee forage of meadow plants predominates. This apiary is located at an altitude of 200 m, and its capacity is 170 production bee colonies in LR hives. The third apiary of Lopare is in the village of Tobut (44°38'28.26"N, 18°55'5.98"E), at an altitude of 212 m. The apiary has a capacity of 60 bee colonies in LR hives, and it is characterized by predominant bee forage from various meadow plants.

Sampling of the pollen was done from three different beehives randomly selected in the apiary at each of the three sampling locations. The samples were collected using external pollen traps placed at the entrance of LR hives. An average of 100 g of bee pollen samples were taken from each beehive and kept in plastic containers at -4°C prior to analysis.

Quantification of calcium (Ca) and magnesium (Mg) in the samples of pollen was done by the method of flame atomic absorption spectrophotometry (AAAnalyst 400, Perkin Elmer USA). Previously, pollen samples were digested with concentrated nitric acid (HNO₃) with the addition of 70% perchloric acid, HClO₄ (Altunatmaz et al., 2017). Both examined elements were quantified in the air-acetylen flame at wavelengths of 422,67 nm for Ca and 285,21 nm

for Mg. Standard solutions were prepared using a single-element stock solution for AAS (1000 mg L⁻¹, Perkin Elmer, USA) and an appropriate diluent extractant. All analytical procedures were triplicated and made with glassware and plastic materials prewashed with 10% HNO₃. The obtained results were analyzed and graphically presented using the statistical software package SPSS 22 (IBM 2013).

Results and discussion

The measured contents of calcium and magnesium in the analyzed samples of bee pollen are represented in Table 1. The average contents of calcium and magnesium in the pollen for all three examined apiaries were in the range of 1350,18-1643,91 mg kg⁻¹ for Ca and 541,59- 670,29 mg kg⁻¹ for Mg. Considering the daily consumption of common 15-20 g doses, tested bee pollens can supply the RDAs of 1000 mg Ca and 300-400 mg Mg (Farag et al., 2023) for both elements at quite high rates.

Table 1. Measured contents of calcium (Ca) and magnesium (Mg) in bee pollen samples

Location	Ca (mg kg ⁻¹)			Mg (mg kg ⁻¹)		
	mean	±	SD	mean	±	SD
Bijeljina	1643.91	±	33.26	670.29	±	16.62
Lopare	1530.71	±	21.77	589.51	±	23.95
Zvornik	1350.18	±	11.13	541.59	±	37.31
F _{location} , P _{location}	347.62**, p<0.001			50.95**, p<0.001		
HSD _{location}	28.06			32.18		

The lowest contents of both elements were found in the bee pollen from Zvornik (1350,18 mg kg⁻¹ for Ca, 541,59 mg kg⁻¹ for Mg). On the other hand, the highest contents of calcium and magnesium were found in the pollen samples from Bijeljina (1643,91 mg kg⁻¹ for Ca, 670,29 mg kg⁻¹ for Mg). These findings are generally in the range of their contents found in Serbia by Kostić et al. (2015) during the study that included 25 bee pollen samples, in which 856-2032 mg kg⁻¹ for Ca, and 503-964 mg kg⁻¹ for Mg were determined. In addition, during the same research, in the area of Western Serbia, which borders the area of East Bosnia, a similar average calcium content (1447,98 mg kg⁻¹) was found, as in our study. However, the determined average content of magnesium (800,2 mg kg⁻¹) was slightly higher compared to our results.

Besides, a determined range of values for the content of calcium and magnesium (491,85-1472,1 mg kg⁻¹ for Ca and 271,1-1278,3 mg kg⁻¹ for Mg) in bee pollen from Turkey (Altunatmaz et al., 2017). During the examination of 71 bee pollen samples in Spain, Valverde et al. (2023) found 580-2800 mg kg⁻¹ for Ca and 360-1300 mg kg⁻¹ for Mg. All these findings, together with our results and the reports of other authors (Szczesna, 2007; Morgano et al., 2012; Kostić et al., 2015), indicate the general trend of a higher amount of calcium compared to magnesium in the bee pollen.

Additionally, we compared the determined contents of Ca and Mg in the analyzed samples using general linear models, and in the case of observed statistically significant differences, comparisons were made using the LSD (least statistical difference) test (Table 1). Analyzing the Ca contents in pollen originating from apiaries at different locations (Table 1), statistically highly significant differences in determined contents are observed (F= 347.62**, p<0.001). Furthermore, it was found that there was a higher content of calcium in pollen from Bijeljina compared to Lopare (p<0.001), as well as in pollen samples from Lopare compared to Zvornik (p<0.001).

Regarding Mg contents in pollen from the studied locations (Table 1), the same situation was observed as for Ca, i.e., there were statistically highly significant differences in the content of this element (F=50,95, p<0.001) between the locations. Besides, there is a higher content of magnesium in pollen from Bijeljina compared to Lopare (p<0.001), as well as in the samples from Lopare compared to those from Zvornik (p<0.001).

Conclusion

According to our results, bee pollen collected in the area of East Bosnia is a valuable source of calcium and magnesium, indicating its high nutritional quality due to the presence of these macroelements. Statistical analysis of the obtained

results revealed statistically highly significant differences in the content of both elements in pollen samples from different locations (apiaries). It is clear that climatic factors are not the primary reason for the variation in the Ca and Mg amounts in the examined pollen samples because all three of the investigated apiaries are located in the same agroecological area and are relatively close to one another. Besides, the technology of pollen collecting and handling is the same in all examined apiaries.

However, differences in bees forage at the investigated locations were probably the most likely reason for the determined differences in Ca and Mg amounts in the examined bee pollen samples. Therefore, it is necessary to continue with this research in terms of specifying the honey plants at each investigated microlocation. Also, it would be interesting to expand the research to include more apiaries with the same or different types of hives and pollen traps from the same or broader region.

References

- Ali A.A.H. (2023). Overview of the vital roles of macro minerals in the human body. *Journal of trace elements and minerals*. 4:100076.
- Altunatmaz S.S., Tarhan D., Aksu F., Barutcu U.B., Or M.E. (2017). Mineral element and heavy metal (cadmium, lead and arsenic) levels of bee pollen in Turkey. *Food Science and Technology*. 37: 136–141.
- Aylanc V., Falcao S.I., Ertosun, S., Vilas-Boas, M. (2021). From the hive to the table: Nutrition value, digestibility and bioavailability of the dietary phytochemicals present in the bee pollen and bee bread. *Trends in Food Science and Technology*. 109: 464–481.
- Ciosek Z., Kot K., Kosik-Bogacka D., Lanocha-Arendarczyk N., Rotter I. (2021). The Effects of Calcium, Magnesium, Phosphorus, Fluoride, and Lead on Bone Tissue. *Biomolecules*. 11: 506.
- Campos M., Bogdanov S., De Almeida-Muradian L. B., Szczesna T., Mancebo Y., Frigerio C., Ferreira F. (2008). Pollen composition and standardisation of analytical methods. *Journal of Apicultural Research*. 47: 154–161.
- De-Melo A.A.M., Estevinho M.L.M.F., Almeida-Muradian L.B. (2015). A diagnosis of the microbiological quality of dehydrated bee-pollen produced in Brazil. *Letters in Applied Microbiology*. 61: 477-483.
- Farag M.A., Abib B., Qin Z., Ze X., Ali S.E. (2023). Dietary macrominerals: Updated review of their role and orchestration in human nutrition throughout the life cycle with sex differences. *Current Research in Food Science*. 6: 100450.
- Human H., and Nicolson S.W. (2006). Nutritional content of fresh, beecollected and stored pollen of *Aloe greatheadii* var. *davyana* (Asphodelaceae). *Phytochemistry*. 67:1486-92.
- Kostić A.Ž., Pešić M.B., Mosić M.D., Dojčinović B.P., Natić M.M., Trifković J.Đ. (2015). Mineral content of bee pollen from Serbia. *Archives of Industrial Hygiene and Toxicology*. 66: 251–258.
- Morgano M.A., Martins M.C.T., Rabonato L.C., Milani R F, Yotsuyanagi K., Rodriguez-Amaya D.B. (2010). Inorganic bee pollens from Southeastern Brazil. *Journal of Agricultural and Food Chemistry*. 58: 6876-6883.
- Liolios V., Tananaki C., Papaioannou A., Kanelis D., Rodopoulou M.A., Argena N. (2019). Mineral content in monofloral bee pollen: investigation of the effect of the botanical and geographical origin. *Journal of Food Measurement and Characterization*. 13: 1674-1682.
- Salazar-Gonzalez C., and Diaz-Moreno C. (2016). The nutritional and bioactive aptitude of bee pollen for a solid-state fermentation process. *Journal of Apicultural Research*. 55: 161–175.
- Stanciu O.G., Margithas L.A., Dezmirean D., Campos M.G. (2011). A comparison between the mineral content of flower and honeybee collected pollen of selected plant origin (*Heliantus annuus* L. and *Salix* sp.). *Romanian Biotechnological Letters*. 16: 6291-6295.
- Swaminathan R. (2003). Magnesium metabolism and its disorders. *Clinical Biochemist Reviews*. 24: 47-66.

- Szczesna T. (2007). Concentration of selected elements in honeybee-collected pollen. *The Journal of Agricultural Science*. 51: 5-13.
- Taha E.K. A., and Al-Kahtani S. (2020). Macro- and trace elements content in honeybee pollen loads in relation to the harvest season. *Saudi Journal of Biological Sciences*. 27: 1797-1800.
- Valverde S., Tapia J.A., Perez-Sanz A., Gonzales-Porto A.V., Higes M., Lucena J.J., Martin-Hernandez R., Bernal J. (2023). Mineral composition of bee pollen and its relationship with botanical origin and harvesting period. *Journal of Food Composition and Analysis*. 119: 105235.

Settlement of *Ostrea edulis* on collectors of mussel socks in the southern Adriatic Sea: a pilot study

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Abstract

This experimental study was carried out in the southern Adriatic Sea (Mali Ston), to determine the distribution of settlement of the *O. edulis* together with other shellfish in the water column at different depths on collectors made of mussel socks. The results revealed significant differences between the abundance of settled European flat oysters compared to other settled bivalves. The higher recruitment rate of *O. edulis* was mainly observed at depths of 4, 5 and 6 metres, while *M. galloprovincialis* settled in the upper layer of the water column. The results point out that these collectors could be a suitable substrate for the settlement of *O. edulis* in the southern Adriatic.

Keywords: settlement, collectors, mussel socks, *Ostrea edulis*, Adriatic Sea

Introduction

While shellfish farming in the Adriatic has a centuries-old tradition, the huge potential of shellfish aquaculture in Croatia is insufficiently exploited. In retrospect, the classical technique of collecting European flat oyster larvae from the collector and rearing them in the natural environment until they reach consumption size is still used in these areas (Basioli, 1968, 1981; Zavodnik and Hrs-Brenko, 1987; Benović, 1997). The European flat oyster *O. edulis* is one of the shellfish species with the longest tradition of harvesting and aquaculture (Caceres-Martinez et al., 1993; Elizieère-Papayanni, 1993; Gouletquer and Heral, 1997; Gouletquer 2004; Colsoul et al., 2021).

Ostrea edulis is a sessile, filter-feeding bivalve mollusc with a distribution ranging from Norway to Morocco in the Atlantic and Mediterranean Seas and extending to the Black Sea (Perry et al., 2017). In the wild, *O. edulis* lives from the intertidal zone to depths of 90 m and on different types of bottoms. Larval development is strongly influenced by temperature and food (Korringa, 1957; Deksheniaks et al., 1996; Roncarati et al., 2023). Insufficient food quantity or quality significantly delays development (Deksheniaks, 1996; Robert et al., 2017). Higher temperatures in the water could lead to faster larval development (Korringa, 1940; Robert et al., 2017). During the pelagic period, the ocean current brings mature oyster larvae onto the collectors, signalling the start of the rearing process. The collectors are installed when there is a sufficient concentration of oyster larvae in the water column. Oysters are able to settle on different types of hard substrates such as rocks, gravel or silty sand with cultch (Shelmerdine and Leslie, 2009), but have different preferences for certain materials used to obtain abundant recruitment material. The most commonly used substrates for oyster recruitment include tree branches, empty mussel shells (cultch), ropes, coco ropes, hemp ropes, ribbons, so-called coupelles: Chinese hats, tubes and tiles - sometimes covered with quicklime (Basioli, 1981; Colsoul et al., 2020; Roncarati et al., 2023). Sheaves made of wooden branches were used in the southern Adriatic (Basioli, 1968, 1981; Benović, 1997), and nowadays collectors made of plastic nets are mostly used. *Ostrea edulis* has a short pelagic larval period and larval settlement plays an important role in connectivity and successful recovery of the stock. Wieczorek and Todd (1998) pointed out that larvae are unable to metamorphose unless they are attached to a suitable substrate. The availability of suitable settlement substrate is considered one of the most important factors in the recruitment success of European flat oyster populations (Korringa, 1946; Low et al., 2007; Smyth et al., 2018; Colsoul et al., 2021).

This study was conducted to determine the distribution of *O. edulis* spat together with other shellfish in the water column at different depths on collectors made of mussel socks in Bistrina Bay, Croatia.

Material and methods

This study was conducted in the southern Adriatic Sea, in the Bay of Mali Ston, Bistrina station (Figure 1). The Bay of Mali Ston is an elongated, sparsely populated, unpolluted area and a traditionally important shellfish farming area in the south-eastern part of the Adriatic coast. Three net collectors were deployed at a depth of 8 m in October 2022. Each collector consisted of 50 mussel socks of 6 m length. The pergolar nets are fixed at the ends with a 16 mm thick reinforcement bar, which was anchored in the park. At the bottom of the collector was an “anchor” made of stones to keep the collector stable in the sea. After 4 months, in February 2023, the shellfish were easily removed from the collectors. Samples of 30 mussel socks were taken from each collector, taking into account depth (1, 2, 3, 4, 5, 6 m). The collected bivalves were identified during the fieldwork, for certain smaller individuals the identification was done later in the laboratory according to Tebble (1966), Nordsieck (1969), Parenzan (1974, 1976).

Descriptive indicators (mean, standard deviation, median, minimum, maximum, lower quartile and upper quartile) were calculated for all bivalves. Due to the non-parametric nature of the data, Kruskal-Wallis ANOVA test was used to examine statistical differences between the abundance of bivalves settled. Furthermore, pairwise comparisons were performed and the significance of the differences between all pairs were examined, with special emphasise on *O. edulis*. All calculations were performed using the statistical data analysis software Statistica 14.0.1. (TIBCO Statistica, 2023). In addition, for each depth (1, 2, 3, 4, 5, 6 m), the proportion of European flat oysters was compared with the proportion of all other bivalves, and the significance of the differences in proportion was examined. Type I error was set at 5 % for all calculations.

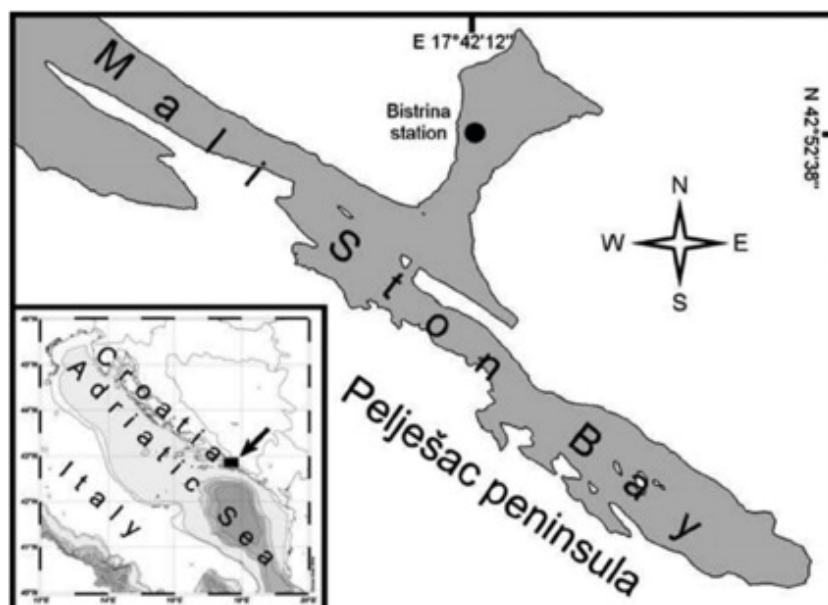


Figure 1. Geographic location of Mali Ston Bay and the Bistrina Station (map from: Duplić Radić, 2014).

Results and discussion

A total of 169 individuals were sampled, of which 6 species were presented: *Aequipecten opercularis* (AEQ), *Flexopecten glaber* (FLE), *Hiatella arctica* (HIA), *Limaria hians* (LIM), *Musculus subpictus* (MUS), *Mytilus galloprovincialis* (MYT), *Ostrea edulis* (OST) and *Parvicardium* sp. (PAR). The most abundant species was *Ostrea edulis* (60 %), followed by *Mytilus galloprovincialis* (24 %) and *Flexopecten glaber* (8 %). The descriptive indicators for sampling bivalves are shown in Table 1. As this study is preliminary, the descriptive indicators are given.

Significant differences between *O. edulis* and other settled shellfish: *A. opercularis*, *H. arctica*, *L. hians*, *M. subpictus* and *Parvicardium* sp. were identified (Table 2).

The exact timing for settling the collectors is crucial for the successful recruitment of oyster spat. If the collectors are set too early in the season, they may be heavily fouled before the start of the settlement season. Conversely, if collectors are set too late in the year, oyster recruitment may be missed (Manning, 1952; Morales-Alamo and

Mann, 1990). The peak of spawning colonisation depends on the ecological conditions of the site. In this study, the collectors were planted in October, when the peak of mussel settlement is expected.

Temperature is probably the most important controlling factor for recruitment (Spärck, 1951; Korringa, 1940, 1946). Under laboratory conditions, Hrs-Brenko (1989) observed *O.edulis* in the phase of settlement and found increased recruitment at temperatures of 20 to 22°C. The same authors observed in nature a large number of mature larvae *in situ* on the substrate at a temperature of 20°C, which is similar to the results observed in this experiment.

Numerous studies have already been conducted on settlement cues and the search of pelagic marine invertebrate larvae for suitable substrates (Lök and Acarli, 2006; Rodrigues et al., 2021). Larval settlement is generally a critical bottleneck in the life cycle of marine invertebrates. Although the preferred settlement substrate for European flat oyster larvae is the hard substrate (Ulanowicz et al., 1980; Smyth et al., 2018), mussel socks collectors, which were used in this study, could be a suitable substrate for European flat oyster settlement in the southern Adriatic. Recently, these type of collectors, which are made of mussel socks, have been used in the Bay of Mali Ston (Bratoš Cetinić and Bolotin 2016) and in the Bay of Kotor in Montenegro (Peraš et al., 2018).

Table 1. Descriptive indicators of bivalves abundance without considering depth of recruitment.

Species	Mean±SD	Median	Min.	Max.	Lower quartile	Upper quartile
AEQ	0.67±1.03	0.00	0.00	2.00	0.00	2.00
FLE	2.33±1.63	2.00	1.00	5.00	1.00	3.00
HIA	0.17±0.41	0.00	0.00	1.00	0.00	0.00
LIM	0.17±0.41	0.00	0.00	1.00	0.00	0.00
MUS	0.83±1.33	0.00	0.00	3.00	0.00	2.00
MYT	6.67±2.16	6.50	4.00	10.00	5.00	8.00
OST	17.20±9.45	18.50	6.00	27.00	7.00	26.00
PAR	0.17±0.41	0.00	0.00	1.00	0.00	0.00

Aequipecten opercularis (AEQ), *Flexopecten glaber* (FLE), *Hiatella arctica* (HIA), *Limaria hians* (LIM), *Musculus subpictus* (MUS), *Mytilus galloprovincialis* (MYT), *Ostrea edulis* (OST), *Parvicardium* sp. (PAR).

Table 2. Multiple Comparisons of p values between all bivalves.

Depend.:	OST	MYT	FLE	AEQ	PAR	LIM	MUS
MYT	1.00						
FLE	1.00	1.00					
AEQ	0.03*	0.19	1.00				
PAR	0.01*	0.04*	1.00	1.00			
LIM	0.01*	0.04*	1.00	1.00	1.00		
MUS	0.04*	0.23	1.00	1.00	1.00	1.00	
HIA	0.01*	0.04*	1.00	1.00	1.00	1.00	1.00

Aequipecten opercularis (AEQ), *Flexopecten glaber* (FLE), *Hiatella arctica* (HIA), *Limaria hians* (LIM), *Musculus subpictus* (MUS), *Mytilus galloprovincialis* (MYT), *Ostrea edulis* (OST), *Parvicardium* sp. (PAR); (*) statistically significant differences ($p < 0.05$).

The significance of the differences between the proportions of European flat oysters and other bivalves at different depths (1, 2, 3, 4, 5, 6 m) was investigated. The higher recruitment rate of *O. edulis* was mainly observed at depths of 4, 5 and 6 meters, while the Mediterranean mussel species (*M. galloprovincialis*) settled in the upper layer of the water column (Table 3). In this comparison, the 3 bivalves species (*L. hians*, *H. arctica* and *Parvicardium* sp.) were excluded due to their low abundance. These results are in agreement with those of other authors, as the behaviour

of Mediterranean mussel larvae is also influenced by gravity and light, namely positively phototactic (Bayne 1964). European flat oyster larvae are negatively phototactic, which is well documented by other authors (Cole and Knight-Jones, 1939; Cole, 1956; Walne, 1974; UMBSM, 2007) and was also demonstrated in this study.

Table 3. Significance of differences in proportions at a given depth between European flat oysters and other bivalves.

Depth (m)	OST-MYT	OST-FLE	OST-AEQ	OST-MUS
1	0.01*	0.77	0.62	0.58
2	<0.01	<0.01	0.59	0.55
3	0.56	0.26	0.43	0.38
4	0.27	0.55	0.05*	0.10
5	0.19	0.02*	0.25	0.50
6	0.19	0.03*	0.20	0.23

Ostrea edulis (OST), *Mytilus galloprovincialis* (MYT), *Flexopecten glaber* (FLE), *Aequipecten opercularis* (AEQ) *Musculus subpictus* (MUS); (*) statistically significant differences ($p < 0.05$).

Conclusion

The number of oyster larvae ready to settle and the availability of suitable substrates are among the most important factors determining recruitment success in oyster populations.

Considering that Bistrina Bay is a natural bed (“conservation” site) for the European flat oyster (*Ostrea edulis*), the following connection could be established. This pilot study has shown that collectors made of mussel socks are a suitable material for settlement of bivalves, especially oysters. In this study, collectors made of mussel socks, in the southern Adriatic Sea called ‘curtains’ were used for the first time and a new net was used. However, the settlement of *Ostrea edulis* could also take place on reused mussel socks, which could also serve as a suitable collector. Considering the reuse of the net, the final cost of oyster production could be reduced. Furthermore, the settlement of other bivalves on this type of collector shows that they do not compete with the oysters, as other bivalves settle in smaller quantities or in other depths of the water column. Further studies on settlement dynamics using the collectors of mussel socks could extend the duration of settlement. It would certainly be desirable to test other types of collectors to find the most suitable material for settling European flat oyster spat.

References

- Basioli J. (1968). Uzgoj školjkaša na istočnim obalama Jadrana. Pomorski zbornik. 6: 179-216.
- Basioli J. (1981). Uzgoj školjkaša na istočnoj obali Jadranskog mora s posebnim osvrtom na Malostonski zaljev. Zbornik radova savjetovanja i Malostonski zaljev prirodna podloga i društveno valoriziranje JAZU, Znanstveni savijet za zaštitu prirode. Dubrovnik, 268-281.
- Bayne B.L. (1964). Primary and secondary settlement in *Mytilus edulis* L. (Mollusca) Journal of Animal Ecology. 33: 513-523.
- Benović A. (1997). The history, present condition, and future of the molluscan fisheries of Croatia. The History, present condition, and future of the molluscan fisheries of north and central America and Europe. Volume 3, Europe. US Dept Commer, NOAA Technical Report 129: 217-226.
- Bratoš Cetinić A., and Bolotin J. (2016). Uzgoj školjkaša u Malostonskom zaljevu, More hrvatsko blago; Word Press, 891- 894 pp.
- Cáceres-Martínez J., Robledo J.A.F., Figueras A. (1993). Settlement of mussels *Mytilus galloprovincialis* on an exposed rocky shore in Ría de Vigo NW Spain. Marine Ecology - Progress Series. 93: 195–198.
- Cole H.A., and Knight-Jones E.W. (1939). Some observations and experiments on the setting and behaviour of larvae of *Ostrea edulis*. Journal Conseil International pour l’Exploration de la Mer. 14: 86–105.

- Cole H.A. (1956). Oyster cultivation in Britain. A manual of current practice. HMSO 43 pp.
- Colsou B., Pouvreau S., Di Poi C., Pouil S., Merk V., Peter C., Boersma M., Pogoda B. (2020). Addressing critical limitations of oyster (*Ostrea edulis*) restoration: Identification of nature-based substrates for hatchery production and recruitment in the field. *Aquatic Conservation: Marine and Freshwater Ecosystems*. 30: 2101–2115.
- Colsou B., Boudry P., Pérez-Parallé M.L., Bratos Cetinic A., Hugh-Jones T., Arzul I., Merou N., Wegner K.M., Peter C., Merk V., Pogoda B. (2021). Sustainable large-scale production of European flat oyster (*Ostrea edulis*) seed for ecological restoration and aquaculture: Reviews in *Aquaculture*. 13: 1423–1468.
- Deksheniaks M.M., Hofmann E.E., Klinck J.M., Powell E.N. (1996). Modeling the vertical distribution of oyster larvae in response to environmental conditions. *Marine Ecology – Progress*. 136: 97–110.
- Dupčić Radić I., Carić M., Najdek M., Jasprica N., Bolotin J., Peharda M., Bratoš Cetinić A. (2014). Biochemical and fatty acid composition of *Arca noae* (Bivalvia: Arcidae) from the Mali Ston Bay, Adriatic Sea // *Mediterranean Marine Science*. 15: 520-531.
- Elizière-Papayanni P. (1993). Coquillages. Informations techniques des services vétérinaires français, éditeur: I.T.S.V.F.: 522 pp.
- Gouletquer P., and Héral M. (1997). History, present conditions and future of the molluscan fisheries of North America and Europe. Marine molluscan production trends in France: from fisheries to aquaculture. *Marine Fisheries Review*, NOAA Technical Report NMFS. 129: 137-164.
- Gouletquer P. (2004). FAO Inland Water Resources and Aquaculture Service (FIRI) “Cultured Aquatic Species Information Programme – *Ostrea edulis*”, Cultured Aquatic Species Fact Sheets, FAO (Food and Agriculture Organization of the United Nations) Rome. http://www.fao.org/figis/servlet/static?dom=culturespecies&xml=Ostrea_edulis.xml
- Hrs-Brenko M. (1989). Collection of oyster and mussel seed on the Yugoslav Adriatic coast. U: *Aquaculture – a biotechnology in progress* (De Pauw, N., Jaspers, E., Ackefors, H., Wilkins, N., ured.). European Aquaculture Society, Bredene, Belgija, 327-334.
- Korringa P. (1940). Experiments and observations on swarming, setting and pelagic life in the oyster *Ostrea edulis*. *Archives Neerlandaises de Zoologie*. 5: 1-249.
- Korringa P. (1946). The decline of natural oyster beds. *Bacteria*. 10: 36–41.
- Korringa P. 1957. Water temperature and breeding throughout the geographical range of *Ostrea edulis*. Colloque international de biologie marine station biologique de Roscoff, 27 juin-4 juillet 1956. *L'Année Biologique*. 33: 1–17.
- Low P.J., Moore G., Smith I.P., Hannah F. (2007). Conservation of the native oyster *Ostrea edulis* in Scotland. *Communication Research Reports*. 25: 27–48.
- Lök A., and Acarlı S. (2006). Preliminary settlement studies of flat oyster (*Ostrea edulis*, L.) on oyster and mussel shell collectors in Karantina Island (Turkey). *Israeli Journal of Aquaculture*. 58:105-115.
- Manning J.H. (1952). Setting of oyster larvae and survival of spat in the St. Mary's River, Maryland, in relation to fouling of cultch. *Proceedings of the National Shellfisheries Association* 74-89 pp.
- Morales-Alamo R., and Mann R. (1990). Recruitment and growth of oysters on shellplanted at four monthly intervals in the lower Potomac River, Maryland. *Journal of shellfish research*. 9: 165-172.
- Nordsieck F. (1969). Die europäischen Meeremussheln (Bivalvia) vom Eismeer bis Kapverden, Mittelmeer und Schwarzes Meer. G. Fischer Verlag, Stuttgart, 256 pp.
- Parenzan P. 1974. Carta d'identita delle conchiglie del Mediterraneo. Vol. II. Bivalvi. Prima parte Bios Taras Edition Taranto, 277 pp.
- Parenzan P. 1976. Carta d'identita delle conchiglie del Mediterraneo. Vol. II. Bivalvi. Seconda parte Bios Taras Edition Taranto, 264 pp.

- Peraš I., Gvozdenović S., Petović S., Mandić M. (2018). Comparative analysis of bivalves diversity on experimental spat collectors. *Water Research and Management*. 8: 25-31.
- Perry F., Jackson A., Garrard S.L. (2017). *Ostrea edulis* Native oyster. In Tyler-Walters H. and Hiscock K. (eds) *Marine Life Information Network: Biology and Sensitivity Key Information Reviews*, [online]. Plymouth: Marine Biological Association of the United Kingdom. Available: <https://www.marlin.ac.uk/species/detail/1146>.
- Robert R., Vignier J., Petton B. (2017). Influence of feeding regime and temperature on development and settlement of oyster *Ostrea edulis* (Linnaeus, 1758) larvae. *Aquaculture research*. 48: 4756–4773.
- Rodriguez-Perez A., James M.A., Sanderson W.G. (2021). A small step or a giant leap: Accounting for settlement delay and dispersal in restoration planning. *PLoS ONE*. 16: e0256369.
- Roncarati A., Mosconi G., Palermo F.A., Magi G.E., Galosi L., Gennari L. (2023). Recruitment of Oysters by Different Collection Devices at a Longline Shellfish Farm in the Central Adriatic Sea. *Sustainability*. 15: 8685.
- Shelmerdine R.L., and Leslie B. (2009). Restocking of the native oyster, *Ostrea edulis* Shetland: Habitat identification study. *Scottish Natural Heritage Commissioned Report*, 396.
- Smyth D., Mahon A.M., Roberts D., Kregting L. (2018). Settlement of *Ostrea edulis* is determined by the availability of hard substrata rather than by its nature: Implications for stock recovery and restoration of the European oyster. *Aquatic Conservation: Marine and Freshwater Ecosystems*. 28: 662–671.
- Spärck R. (1951). Fluctuations in the stock of oyster (*Ostrea edulis*) in the Limfjord in recent time. *Rapports et Procès-verbaux des Réunions. Conseil Permanent International pour l'Exploration de la Mer*. 128: 27-29.
- Tebble N. 1966. *British Bivalve Seashells. A Handbook for Identification*. Trustees of the British Museum (Natural History), London, 212 pp.
- Ulanowicz R.E., Caplins W.C., Dunnington E.A. (1980). The forecasting of oysterharvest in central Chesapeake Bay. *Estuarine and coastal marine science*. 11: 101-106.
- UMBSM (2007). *Conservation of the Native Oyster in Scotland*. SNH Report 251. 186 pp.
- Zavodnik D., and Hrs-Brenko M. (1987). Allevamento di organismi attraverso i secoli sulle sponde dell'Adriatico orientale. *Atti del Museo Civico di Storia Naturale di Trieste*. 40: 25-53.
- Walne P. (1974). *Culture of bivalve molluscs: 50 years' experience at Conwy*. Farnham: Fishing News Books. 189 pp.
- Wieczorek S.K., and Todd C.D. (1998). Inhibition and facilitation of settlement of epifaunal marine invertebrate larvae by microbial biofilm cues. *Biofouling*. 12: 81–118.

Rasprostranjenost, dužinsko-maseni odnos i eustrongilidoza glavočića okrugljaka *Neogobius melanostomus* (Pallas, 1814) u donjem toku rijeke Kupe

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Sažetak

Cilj ovoga rada je utvrditi rasprostranjenost, vrijednosti dužinsko-masenog odnosa i faktora kondicije glavočića okrugljaka u donjem toku rijeke Kupe.

Uzorci su prikupljeni na tri lokaliteta metodom ribolova udicom tijekom srpnja i kolovoza 2021. godine. Ukupno je analizirano 115 jedinki u rasponu ukupne dužine tijela od 48,26 do 139,81 mm. Utvrđen je pozitivan alometrijski rast populacije s najvećim faktorom regresije $b=3,27$ na lokalitetu Lasinja, prosječnog faktor kondicije $K=1,12$. Na uzorcima s lokaliteta Novo selo Lasinjsko utvrđena je infestacija oblicima iz roda *Eustrongylides*. Istraživani lokaliteti trenutno su najzapadnija rasprostranjenost i područje pojavljivanja ove vrste u Hrvatskoj.

Ključne riječi: alometrijski rast, faktor kondicije, parazitizam, strana invazivna vrsta, rijeka Kupa, CPUE

Uvod

Glavočić okrugljak je bentoska, euritopna, ponto-kaspijska vrsta, a u Hrvatskoj nastanjuje vodotoke dunavskog sliva (Čaleta i sur., 2019). Prvi puta je utvrđen u rijeci Dunav (Mustafić i sur., 2005) te u rijeci Savi na lokalitetima Račinovci, Županja, Babina greda (Piria i sur., 2011), Slavonski Brod, Gornja Varoš i Jasenovac (Jakovlić i sur., 2015). Brojna populacija je utvrđena 2011. godine na 12 rkm rijeke Drave i kod Aljmaša u rijeci Dunav (Šanda i sur., 2013). Prema FISK (Fish Invasiveness Scoring Kit) metodi s 28 bodova ocijenjen je kao vrsta visokog rizika invazivnosti u Hrvatskoj (Piria i sur., 2016). Ova vrsta posjeduje više invazivnih osobina: visoku toleranciju na salinitet vode te nastanjuje morska, bočata i slatkovodna staništa (Skora i sur., 1993; Cross i Rawding, 2009), toleranciju na temperaturu od 1 do 30°C (Moskal'kova, 1996) te može nastanjivati staništa s niskom koncentracijom otopljenog kisika (Charlebois i sur., 1997). Vrsta se smatra otpornom na zagađenja (Pinchuk i sur., 2003), konzumira široki raspon hrane, iskazuje agresivno teritorijalno ponašanje i brine o gnijezdu s oplođenom ikrom (Balážová i Kováč, 2007).

U nedavnim istraživanjima na području Baltičkog mora glavočić okrugljak je utvrđen kao jedna od vrsta s najvećim utjecajem među 18 taksona invazivnih stranih vrsta (Ojaveer i Kott, 2015). Glavočić okrugljak je predator ikre zavičajnih vrsta i komercijalno vrijednih ribljih vrsta te često ima bolje kompeticijske sposobnosti zauzimanja staništa mrijesta, zaklona i hrane od zavičajnih vrsta (Lauer i sur., 2004; Sapota 2004; Balshine i sur., 2005; Karlson i sur., 2007; Lederer i sur., 2008).

Osim negativnog utjecaja ove vrste nakon koloniziranja novih staništa, glavočić okrugljak u novim staništima može utjecati na veću brojnost zavičajnih ribojednih vrsta riba (Oesterwind i sur., 2017) i ptica koje se njima hrane (Behrens i sur., 2019).

Analize ukazuju na potencijalno pozitivne utjecaje prisutnosti ove vrste na zlatnog vijuna (*Sabanejewia balcanica*) i negativne na običnog klena (*Squalius cephalus*) i velikog vretenca (*Zingel zingel*) u Hrvatskoj (Piria i sur., 2016). Zbog mogućih negativnih, ali i pozitivnih utjecaja na zavičajnu ihtiofaunu važno je utvrditi i pratiti rasprostranjenost i stanje glavočića okrugljaka u hrvatskim vodotocima. Cilj ovoga rada je utvrditi širenje, dužinsko-maseni odnos i faktor kondicije glavočića okrugljaka u donjem toku rijeke Kupe. Prijašnji radovi s podacima o prisutnosti, vrijednosti dužinsko-masenog odnosa i faktora kondicije za ovu vrstu u rijeci Kupi nisu poznati.

Područje istraživanja

Rijeka Kupa pripada dunavskom slivu, izvire u Gorskom Kotaru te joj je ušće kod grada Siska u rijeku Savu. Dužine toka od 296 km Kupa je najdulja rijeka koja cijelim svojim tokom prolazi kroz teritorij Republike Hrvatske. U gornjem toku rijeka Kupa je tipična krška rijeka kanjonskog tipa. Prema morfologiji, hidrološkim karakteristikama i sastavu riblje zajednice njezin donji tok počinje na istočnim rubovima grada Karlovca. Istraživano područje uključuje tri lokaliteta (Slika 1.) prema kordinatnom sustavu UTM (WGS84):

1. Novo Selo Lasinjsko (45°32'03"N, 15°49'16"E)
2. Lasinja (45°32'49"N, 15°51'42"E)
3. Desno Sredičko (45°31'47"N, 15°54'11"E).



Slika 1. Kartografski prikaz lokaliteta uzorkovanja i najzapadnije rasprostranjenosti glavočića okrugljaka u rijeci Kupi

Udaljenost od prvog do trećeg lokaliteta uzorkovanja iznosi približno 9600 m vodotoka. Svi lokaliteti nalaze se unutar područja Natura 2000 HR2000642 te su prema Nacionalnoj klasifikaciji staništa (NKS) stanišni tip A.2.3.2.2. srednji i donji tokovi sporih vodotoka.

Na prvom lokalitetu uzorkovanja supstrat je mulj s gustom submerznom vegetacijom i približne dubine 6 m. Drugi lokalitet je također muljevitog supstrata, ali s mjestimičnom rjeđom submerznom vegetacijom i približne dubine 4,5 m. Treći lokalitet uzorkovanja je šljunkovitog supstrata s gustom submerznom vegetacijom, približne dubine od 3 m. Od devet endemskih vrsta riba dunavskog sliva ovo područje rijeke Kupe nastanjuje njih sedam (Čaleta i sur., 2019). U neposrednoj blizini lokaliteta uzorkovanja utvrđene su tri invazivne vrste riba koje izazivaju zabrinutost u EU te jedna ponto-kaspijska vrsta riječni glavočić (*Neogobius fluviatilis*) (Petraović i sur., 2020).

Materijali i metode

Uzorcima su prikupljeni sveukupno 12 puta tijekom 11 različitih dana u mjesecu srpnju i kolovozu 2021. godine, odnosno na prvome lokalitetu tri puta, na drugome četiri te pet puta na trećem lokalitetu. Jedinke su lovljene ribolovnim štapom i metodom kavezne hranilice (feeder metoda). Kao prihrana i mamci su korištene larve *Musca domestica*.

Korištene su veličine udica 16 i 18. Sve ulovljene jedinke su determinirane prema ključu (Kottelat i Freyhof, 2007). Nakon determinacije sve jedinke glavočića okrugljaka su zamrznute istoga dana, a ostale vrste u najkraćem vremenu su vraćene u vodu na lokalitetu gdje su ulovljene. Prije mjerenja standardne (SL) i ukupne dužine (TL) te mase (W) tijela sve jedinke su odmrznute. Za izmjeru dužina tijela korištena je pomična mjerka preciznosti 0,01mm marke Westward, a za masu digitalna vaga marke FA-6405 preciznosti 0,01 g.

Dužinsko-maseni odnos izračunat je jednadžbom $W=a L^b$ (Ricker, 1975.), a faktor kondicije $K=100W/L^3$ (Ricker, 1975). Koordinate lokaliteta utvrđene su GPS Garmin uređajem te je napravljena analiza karte rasprostranjenosti pomoću programa QGIS, korištene su podloge OSM Standard, Vodena tijela Karta staništa 2016 i Područja prema Direktivi o staništima. Vrijednost ulova po jedinici napora (CPUE) izračunata je brojem svih jedinki ulovljenih jedinki u vremenu u minutama (Treer i Piria, 2019). Za izračun udaljenosti širenja vrste Hrvatskom korišten je "Daljinar rijeke Save i njenih plovnih pritoka" (Međunarodna komisija za sliv rijeke Save, 2005).

Nakon odmrzavanja u PVC vrećici uočeni su paraziti crvenkaste boje, duguljastog tijela.

Vizualnim pregledom nakon odmrzavanja i mjerenja jedinki, potvrđena je prisutnost parazita, crvenkaste boje i tankog duguljastog tijela u visceralnim šupljinama ulovljenih jedinki. Za determinaciju parazita korištena je binokularna lupa Zeiss Stemi 305, a determinirani su prema karakterističnim dvanaest kružnih cefaličnih papila u dva reda. U vanjskom redu vrhovi papila su poput kvržica, a u vanjskom redu vrhovi papila su nalik na bodlje (Pekmezci i Bolukba, 2021).

Rezultati

U donjem toku rijeke Kupe na lokalitetima Lasinja, Novo Selo Lasinjsko i Desno Sredičko utvrđena je prisutnost i brojna populacija glavočića okrugljaka.

Ukupno je analizirano 115 jedinki glavočića okrugljaka iz donjeg toka rijeke Kupe. Raspon mase ulovljenih jedinki je od 1,39 do 31,56 g, prosječne mase 10,51 g. Najmanja, najveća i prosječna vrijednost ukupne dužina tijela uzoraka su 48,26 mm, 139,81 mm i 92,48 mm.

Iz jednadžbe dužinsko-masenog odnosa očitana je vrijednost faktora regresije b koja iznosi 3,17 i pokazuje pozitivni alometrijski rast. Dobivena vrijednost koeficijenta determinacije $r^2=0,9513$ ukazuje da 95,13% varijacije mase potiče od varijacije ukupne dužine tijela.

Na cjelokupnom uzorku najmanji faktor kondicije K od 0,81 utvrđen je na lokalitetu Lasinja, dok najveći iznosi 1,45 na lokalitetu Novo Selo Lasinjsko. Prosječan faktor kondicije cjelokupnog uzorka iznosi $K=1,12$ (Tablica 1.).

Tablica 1. Raspon i prosječna masa, standardna i ukupna dužine tijela, dužinsko-maseni odnosi i ulov po jedinica napora glavočića okrugljaka prema lokalitetima.

Lokalitet	N	W(g) _{raspon} prosječna±SD	SL(mm) _{raspon} prosječna±SD	TL(mm) _{raspon} prosječna±SD	b	K	CPUE min
Novo Selo Lasinjsko	31	1,39-31,56	40,06-117,65	48,26-139,81	3,09	1,20	650
		12,31±8,43	79,18±19,93	92,48±23,73			
Lasinja	40	1,99-24,73	48,54-109,28	57,91-132,55	3,27	1,12	785
		11,04±7,38	78,28±18,11	93,34±21,83			
Desno Sredičko	44	1,8-18,81	45,86-102,19	55,18-121,74	3,10	1,05	1150
		8,78±5,31	75,50±15,14	90,22±18,11			
Ukupni uzorak	115	1,39-31,56 10,51±7,14	40,06-117,65 77,46±17,65	48,26-139,81 92,48±21,13	3,2	1,12	2585

Broj ulovljenih jedinki glavočića okrugljaka po jedinici vremena iznosi 115 jedinki u 2585 minuta, odnosno u 60 minuta prosječan broj ulovljenih jedinki iznosi 2,66 jedinki.

Osim glavočića okrugljaka u istraživanju je istom metodom ribolova jednakim ukupnim lovnim naporom ulovljeno je 10 različitih vrsta riba koje pripadaju srednjem i donjem toku rijeke Kupe, njih ukupno 336 jedinki: obična

uklija (*Alburnus alburnus*) 25,00%, plotica (*Rutilus virgo*) 15,48%, babuška (*Carassius gibelio*) 8,93%, mrena (*Barbus barbus*) 4,76%, krupatica (*Blicca bjoerkna*) 4,17%, nosara (*Vimba vimba*) 3,57%, bodorka (*Rutilus rutilus*) 2,68%, šaran (*Cyprinus carpio*) 1,19%, som (*Silurus glanis*) 0,30%, klen (*Squalius squalus*) 0,30%. Udio glavočića okrugljaka čini 34,23% u ukupnoj brojnosti ulova.

Na lokalitetu Novo Selo Lasinjsko 14. srpnja 2021. godine od 21 ulovljene jedinke glavočića okrugljaka njih 81,00% bili su infestirani ličinačkim stadijem oblića roda *Eustrongylides*. U ostalim uzorcima nije zabilježena infestacija. Infestirane jedinke imale su prosječnu vrijednost faktora kondicije $K=1,16$ i nižu vrijednost koeficijenta regresije $b=2,99$.

Rasprava

U Hrvatskoj je vrsta prvi puta utvrđena 2005. godine u rijeci Dunav (Mustafić i sur., 2005). Širenje Hrvatskom prema zapadu nastavilo se pritokom Dunava, odnosno rijekom Savom do Slavenskog Broda i kanala Sava-Odra (Piria i sur., 2011) gdje je i najzapadniji lokalitet rasprostranjenosti ove vrste. Vrsta je zabilježena kasnije u rijeci Savi kod Jasenovca (Jaković i sur., 2015). Od prvog nalaza u Hrvatskoj, u sljedećih 17 godina, glavočić okrugljak se proširio od ušća rijeke Save u Dunav do Novog Sela Lasinjskog u donjem toku rijeke Kupe, odnosno 702,7 km riječnoga toka. Širenje vrste može se očekivati uzvodno rijekom Kupom od Novog Sela Lasinjskog, ali i kanalom Kupa-Kupa do grada Ozlja. Brane MHE Ozalj i MHE Ilovac mogu predstavljati fizičku barijeru uzvodnog širenja vrste rijekom Kupom. U dosadašnjim višegodišnjim istraživanjima ihtiofaune rijeke Kupe kod MHE Ilovac nije utvrđena prisutnost glavočića okrugljaka (Petraović i sur., 2019., 2020., 2021). U budućnosti je vrlo vjerojatno širenje vrste i pritokama rijeke Kupe, odnosno rijekama Koranom, Dobrom, Kupčinom i Radonjom.

Razlika u vrijednostima najmanje ukupne dužine tijela glavočića okrugljaka utvrđene u ovom radu i uzoraka iz rijeke Save (Jakšić, 2016) mogu biti zbog selektivnosti ulova ribolovnim alatom, odnosno veličina udice koja je korištena u ovom istraživanju. Najveće ukupne dužine tijela uzoraka iz rijeke Kupe i rijeke Save, kao i faktora kondicije podjednake su vrijednosti. Vrijednosti koeficijenta regresije sa sva tri lokaliteta i cjelokupnog uzorka ukazuju na pozitivan alometrijski rast, odnosno veći rast u masu nego u dužinu i podjednake su vrijednosti kao i koeficijent regresije u rijeci Savi (Jakšić, 2016). Dobiveni rezultati dužinsko-masenih odnosa, koeficijentata kondicije i mrijest glavočića okrugljaka u rijeci Kupi ukazuju na dobre biotičke uvjete staništa i brzu prilagodbu.

Istraživanje parazitofaune kod glavočića okrugljaka u Baltičkom moru (Ojaveer i sur., 2020) potvrđuju ovu vrsta kao domaćina i međudomaćina 28 vrsta parazita, među kojima su i dvije vrste roda *Eustrongylides*. U prijašnjim radovima eustrongilidoza kod glavočića okrugljaka u Hrvatskoj nije opisana. Zbog specifičnog višestadijskog načina razvoja *Eustrongylides spp.* (Oraić i sur., 2023) visoki postotak infestacije može indicirati prehranu glavočića okrugljaka maločetinašima (*Oligochaeta*) na pojedinim lokalitetima u određeno razdoblje godine.

Infestacija ovim parazitima na lokalitetu Novo Selo Lasinjsko u manjem značaju je negativno utjecala na vrijednost dužinsko- masenog odnosa, ali ne i na faktor kondicije što indicira dobru prilagodbu glavočića okrugljaka na ovu vrstu parazita u donjem toku rijeke Kupe.

Zaključci

Glavočić okrugljak ima tendenciju brzog širenja i nastanjanja novih staništa te postizanja brojne populacije u hrvatskim rijekama i njihovim pritokama. Zabilježena prisutnost vrste na istraživanim lokalitetima potvrđuje širenje glavočića okrugljaka zapadno rijekom Kupom što je trenutno najzapadnija rasprostranjenost i područje pojavljivanja ove vrste u Hrvatskoj. Pozitivni alometrijski rast, brojnost i mrijest potvrđuju dobru prilagodljivost vrste i povoljne stanišne uvjete u hrvatskim vodotocima. Glavočić okrugljak može biti međudomaćin i paratenični domaćin oblića roda *Eustrongylides* te sudjelovati u njihovom prostornom širenju. Zbog negativnog utjecaja na zavičajne vrste, visoku rizičnost invazivnosti i prijenosa parazita potrebno je pratiti daljnje širenje vrste i njezin utjecaj na zavičajne vrste i stanište.

Literatura

- Balažova M., and Kovač V. (2007). Epigenetical context in the life-history of round goby *Neogobius melanostomus* from Slovak stretch of the Danube. *Freshwater Bioinvasions: Profiles, Distribution, and Threats*. Springer Verlag. 14: 275-288.
- Balshine S., Verma A., Chant V., Theysmeyer T. (2005). Competitive interactions between round gobies and logperch. *Journal of Great Lakes Research*. 31: 68–77.
- Behrens J., Mikael van D., Riikka P. Ann-Britt F. (2019). Policy brief: Round goby – a threat or a new resource? Nordic Council of Ministers. Nord No. 2019:037.
- Charlebois P.M., Marsden J.E., Goettel R.G., Wolfe R.K., Jude D J., Rudnika, S. (1997). The Round Goby, *Neogobius melanostomus* (Pallas), a Review of European and North American Literature. Zion, IL: Illinois-Indiana Sea Grant Program and Illinois Natural History Survey.
- Cross E.E., and Rawding R.S. (2009). Acute Thermal Tolerance in the Round Goby, *Apollonia melanostoma* (*Neogobius melanostomus*). *Journal of Thermal Biology*. 34: 85-92.
- Čaleta M., Marčić Z., Buj I., Zanella D., Mustafić P., Duplić A., Horvatić S. (2019). A review of extant Croatian freshwater fish and lampreys -Annotated list and distribution. *Croatian Journal of Fisheries*. 77: 137-234.
- Jakovlić I., Piria M., Šprem N., Tomljanović T., Matulić D., Treer T. (2015). Distribution, abundance and condition of invasive Ponto-Caspian gobies *Ponticola kessleri* (Günther, 1861), *Neogobius fluviatilis* (Pallas, 1814), and *Neogobius melanostomus* (Pallas, 1814) in the Sava River basin. Croatia. *Journal of Applied Ichthyology*. 31: 888-894.
- Jakšić G. (2016). Biološka, ekološka i genska obilježja invazivnih ponto-kaspijskih glavoča (Gobiidae) savskog sliva u Hrvatskoj. Disertacija. Sveučilište u Zagrebu, Agronomski fakultet, Zagreb 2016.
- Karlson A.M.L., Almqvist G., Sk'ora K.E., Appelberg M. (2007). Indications of competition between non indigenous round goby and native flounder in the Baltic Sea. *ICES Journal of Marine Science*. 64: 479–486.
- Kottelat M., and Freyhof J. (2007). *Handbook of European Freshwater Fishes*. Kottelat, Cornol, Switzerland and Freyhof, Berlin, Germany.
- Lauer T.E., Allen P.H., McComish T.S. (2004). Changes in mottled sculpin and Johnnydarter trawl catches after the appearance of round gobies in the Indiana water of Lake Michigan. *Transactions of the American Fisheries Society*. 133: 185–189.
- Lederer A.M., Janssen J., Reed T., Wolf A. (2008). Impacts of the introduced roundgoby (*Apollonia melanostoma*) on Dreissenids (*Dreissena polymorpha* and *bugensis*) and on macroinvertebrate community between 2003 and 2006 in the littoral zone of Green Bay, Lake Michigan. *Journal of Great Lakes Research*. 34: 690–697.
- Moskal'kova K.I. (1996). Ecological and morphophysiological prerequisites to range extension in the round goby *Neogobius melanostomus* under condition of anthropogenic pollutions. *Journal of Ichthyology*. 36: 584-90.
- Mustafić P. (2005). Indeks biotičkog integriteta riblje zajednice velikih rijeka Hrvatske. Doktorska disertacija, Zagreb. Sveučilište u Zagrebu Prirodoslovno-matematički fakultet.
- Ojaveer H., and Kotta J. (2015). Ecosystem impacts of the widespread non-indigenous species in the Baltic Sea: literature survey evidences major limitations in knowledge. *Hydrobiologia*. 750:171–185.
- Oraić D., Giovanna Zupčić I., Zrnčić S. (2023). Eustrongilidoza riba: pregledni članak. *Veterinarska stanica*. 54: 107-113.
- Pekmezci G.Z., and Bolkuba C.S. (2021). Morphological and molecular characterization of *Eustrongylides excisus* larvae (Nematoda: Dioctophymatidae) in *Sander lucioperca* (L.) from Northern Turkey. *Parasitology Research*. 120: 2269-2274.
- Petravić J., Jarnjak M., Kuri K., Andrašić M., Kaleb Vuletić I., Maruškić Kulaš M., Jajčević H., Jakšić,

- G. (2020). Fish assemblage of the artificial flood protection channel Kupa-Kupa. Zbornik radova. Sveučilište u Zagrebu, Agronomski fakultet, Zagreb 2020. str. 316-321.
- Petravić J., Kresonja M., Petravić M., Popović R., Mrakovčić M. (2020). Fish assemblage in the middle course of the Kupa river in the area of small hydropower plant Ilovac. Book of abstracts 10th symposium with international participation Kopački rit past, present, future 2020. Osijek.
- Pinchuk V.I., Vasil'eva E.D., Vasil'ev V.P., Miller P. (2000). *Neogobius melanostomus*. The freshwater fishes of Europe. Vol. 8: 293-345.
- Piria M., Jakšić G., Jakovlić I., Treer T. (2016). Dietary habits of invasive Ponto-Caspian gobies in the Croatian part of the Danube River basin and their potential impact on benthic fish communities. *Science of the Total Environment*. 540: 386-395.
- Piria M., Šprem N., Jakovlić I., Tomljanović T., Matulić D., Treer T., Aničić I., Safner R. (2011). First record of round goby, *Neogobius melanostomus* (Pallas, 1814) in the Sava River. Croatia. *Aquatic Invasions*. 6: 153-157.
- Piria M., Zannella D., Simonovic P., Copp G. (2015). Risk screening of non-native freshwater fishes in Croatia and Slovenia using the Fish Invasiveness Screening Kit. *Fisheries Management and Ecology*. 23: 21-31.
- Ricker W.E. (1975). Computation and Interpretation of Biological Statistics of Fish Populations. *Bulletin of Fishery Research Board Canada*. 191:382.
- Sapota M.R. (2004). The round goby (*Neogobius melanostomus*) in the Gulf of Gda'nsk – aspecies introduction into the Baltic Sea. *Hydrobiologia*. 514: 219–224.
- Skóra K.E., and Stolarski J. (1993). New fish species in the Gulf of Gdansk, *Neogobius* sp *Neogobius melanostomus* (Pallas 1811). *Bulletin of the Sea Fisheries Institute*. 1: 83-84.
- Šanda R., Balković I., Bogut I., Galović D., Vidaković J., Čerba D., Kovačević T.(2013). Nova saznanja o rasprostranjenosti invazivnih vrsta porodice Gobiidae. 9. Međunarodni gospodarsko-znanstveni skup o ribarstvu Vukovar. Postersko izlaganje.
- Treer T., and Piria M. (2019). Osnove primijenjene ihtiologije. Sveučilište u Zagrebu Agronomski fakultet, Zagreb 2019.

Distribution, length-mass ratio and eustrongylidosis of the roundhead *Neogobius melanostomus* (Pallas, 1814) in the lower reaches of the Kupa River

Abstract

The aim of this work is to determine the distribution, length-weight relationship and the condition factor of Roundhead goby (*Neogobius melanostomus*) in the lower reaches of the Kupa River. The samples were collected on three localities using the fishing gear method during July and August 2021. The total of 115 individuals were analyzed in the range of total body length from 48.26 to 139.81 mm. The positive allometric growth of the population was determined with the largest regression factor $b=3.27$ in the Lasinja locality. The average fitness factor of the entire sample is $K=1.12$, while the lowest fitness factor was determined in the locality Desno Sredičko $K=1.05$. The number of Roundhead roby caught per unit of time (CPUE) is 115 individuals in 2585 minutes. The samples from the Novo selo Lasinjsko locality were found to be infected with molds from the genus Eustrongylidae. The investigated localities are currently the westernmost distribution and area of occurrence of this species in Croatia.

Keywords: allometric growth, condition factor, parasitism, foreign invasive species, Kupa River, CPUE

Metode razrojavanja pčelinjih zajednica

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Sažetak

Pčele su socijalni kukci koji žive u velikim zajednicama, gdje svaka jedinka obavlja svoju ulogu. U povoljnim vremenskim uvjetima pčelinja zajednica brojčano raste te uslijed nedostatka prostora dolazi do rojenja pri čemu se zajednica dijeli na dva dijela. Rojenje brojčano oslabljuje zajednicu, a samim time i prinosi pčelinjih proizvoda su manji. Stoga rojenje u suvremenom pčelarstvu nije poželjno. Pčelari nastoje sve uzroke rojidbenog nagona svesti na minimum. Za povećanje pčelinjih zajednica služe se pouzdanim metodama razrojavanja koje im omogućavaju stvaranje novih pčelinjih zajednica u vremenu i količini koja im najviše odgovara.

Ključne riječi: *Apis mellifera*, pčelinja zajednica, rojenje, metode razrojavanja

Uvod

Uloga pčela u održavanju ekosustava i biološke raznolikosti je neprocjenjiva. U oprašivanju poljoprivrednih kultura posebno se ističe medonosna pčela (*Apis mellifera* L.), pčela koja danas nastanjuje gotovo sva područja svijeta u kojima se ljudi bave pčelarstvom. Najbolje je opisana i ekonomski gledano, za ljude je najvažnija vrsta pčele (Laktić i Šekulja 2008). Najpoznatije su pasmine europske medonosne pčele: siva pčela (*Apis mellifera carnica* Pollman, 1879), žuta talijanska pčela (*Apis mellifera ligustica* Spinola, 1806), tamna europska pčela (*Apis mellifera melifera* Linnaeus, 1758) i kavkaska pčela (*Apis mellifera caucasica* Pollman, 1889). Na području Hrvatske, koja ima dugu tradiciju pčelarenja, najzastupljenija je siva pčela, naša izvorna pasmina pčela. Uzgoj drugih pasmina u Republici Hrvatskoj zakonski je zabranjen (Brence-Lazarus, 1998). Po svojim osobinama spada među najbolje pasmine medonosne pčele (Čerimagić, 1990). Karakterizira je velika produktivnost, mala potrošnja hrane, dobro prezimljavanje, relativno blag temperament, brz proljetni razvoj i tolerantnost na bolesti (Jašić i sur., 2016). Pored izuzetno dobrih proizvodnih osobina pčeli *A. mellifera carnica* se pripisuje i manje poželjna osobina sklonosti rojenju (Plavša i Nedić, 2015). Tradicionalno pčelarenje s košnicama fiksnog volumena, bez pokretnog saća razvilo je kod sive pčele izraženu sklonost rojenju koje je u to vrijeme bila poželjna odlika, dok je u suvremenom načinu pčelarenja rojenje nepoželjna odlika. Prirodno rojenje brojčano oslabljuje pčelinju zajednicu, a samim tim i prinosi meda se smanjuju. Pčelarenjem s košnicama promjenljivog volumena i pokretnim saćem nastoji se suzbiti prirodno rojenje svim poznatim postupcima (Kezić i sur., 2014). Međutim, i suvremeni pčelar ima potrebu povećati svoj pčelinjak, obnoviti zimske gubitke ili ponuditi tržištu višak rojeva (Puškadija i sur., 2008). U suvremenom pčelarstvu primjenom pravilne tehnologije možemo proizvesti značajan broj pomoćnih pčelinjih zajednica (nukleusa) različitim metodama razrojavanja. Kako bi spriječili prirodno rojenje pčelinje zajednice u vrhuncu njenog razvoja, zajednicu umjetno dijelimo na dva ili više nukleusa. Proizvodnja i korištenje pomoćnih pčelinjih zajednica važna je mjera u tehnologiji pčelarenja. Cilj ovog rada je dati pregled istraživanja o rojidbenom nagonu pčelinjih zajednica i time doprinijeti boljem razumijevanju ponašanja pčela tijekom rojenja, opisati čimbenike koji potiču, odnosno suzbijaju rojidbeni nagon te navesti metode razrojavanja.

Rojenje i rojidbeni nagon

Rojenje je temeljni nagon za razmnožavanje pčelinje zajednice kojim dolazi do dijeljenja zajednice na dva dijela. Iz košnice izlazi dio pčela sa starom maticom, a sa pčelama koje ostaju u košnici ostaje nova matica. Bez rojenja, kao načina prirodnog razmnožavanja, pčele bi nestale, usprkos tome što pčelinja zajednica može živjeti neograničeno, jer se ne bi uvećavao njihov broj, a iz puno razloga pojedina zajednica može i propasti (Kezić i sur., 2014). Više je uzroka koji kod pčela potiču pojavu prirodnog nagona za rojenje. Nasljedna sklonost za rojenje, nedovoljna prisutnost

matičnog feromona, obilje paše, skučenost prostora u košnici, nedostatak prostora za polaganje jaja, smještaj nektara i gradnju novog saća te dob matice, samo su neki od njih (Plavša i Nedić 2015). Za sivu pčelu je svojstveno da u proljeće naglo povećava broj radilica. Pripreme za rojenje počinju približno dva do četiri tjedna prije izlaska prvog roja, tijekom razdoblja kad košnica postaje prenapučena uslijed intenzivnog rasta radiličke populacije (Winston 1987). Matični feromon je odgovoran za održavanje zajednice na okupu. Ukoliko se razvije preveliki broj pčela u košnici, smanjuje se frekvencija kontakata između matice i radilica zaduženih za prenošenje feromona na druge radilice. Prosljeđena doza feromona nije dovoljna i počinje uzgajanje nove matice što je prva faza ulaska pčelinjeg društva u rojdbeni nagon, dok je druga faza samo rojenje (Fefferman i Starks 2006).

Rojenje se događa kad je u prirodi obilje hrane, posebno peludne hrane koja doprinosi kvalitetnom radu mliječnih žlijezda. Prisutan višak matične mliječi u zajednici te nedostatak prostora u plodištu dovode do posebnog raspoloženja koje se odražava u nagonu za rojenje. Zajednice sa starijim maticama se češće roje. Prvi znak rojdbenog nagona je pojačana gradnja trutovskog saća iznad okvira. Pčele grade 10 do 20 matičnjaka u koje matica postupno polaže jaja, rijetko kada u sve odjednom. Matica je učestalo hranjena te leže puno jaja sve do tjedan dana prije rojenja. Pojavom rojdbenog nagona radilice potiču maticu na kretanje te je sve rjeđe hrane matičnom mliječi radi čega matica manje nese jaja i postaje vitkija i sposobna za let s rojem (Allen 1960). Istovremeno počinje se sama hraniti medom što joj povećava koncentraciju šećera u krvi neophodnu za rad letnih mišića.

Za lijepa vremena, nakon što se nasišu meda u dovoljnoj količini da imaju rezerve za nekoliko dana i poklope prve matičnjake, počinje rojenje. Kad izađe polovica pčela pridružuje im se i matica. Zatim se roj skupi na prikladnom mjestu, najčešće na obližnjem stablu, u obliku grozda. Taj prvi roj se naziva prvenac. Dok je dio pčela u izvidnici, u potrazi za prikladnim mjestom za podizanje novog gnijezda, što traje par sati do nekoliko dana, pčelar je u mogućnosti stresti roj. Roj slobodno može staviti u košnicu pored stare, bez bojazni da će pčele ulaziti u staru košnicu. Ukoliko matica ne može letjeti tada se roj vrati u staru košnicu (Katalinić i sur., 1968). Ako se pčelar nađe na pčelinjaku pri izlasku roja, treba suziti leto da se taj izlazak što više odulji. Tako se pčele kruženjem zamore i uhvate na niže mjesto. Dobro je također da ima pri ruci kavez u koji će uhvatiti maticu. Kavez može staviti na leto pripremljene košnice te će se roj vratiti i naseliti u tu košnicu (Belčić i sur., 1985). U novoj košnici roj aktivno gradi novo saće, a matica polaže jaja u nove stanice. Kako prve mlade pčele izlaze tek nakon 21. dana u prvim tjednima novi roj je brojčano slab i zajednica se često ne stigne dovoljno razviti i pripremiti za zimu.

U košnici iz koje je roj izašao u pogodnim vremenskim prilikama ima puno tek izleženih pčela koje brane matičnjake. Matice ne napuštaju matičnjake već ih pčele hrane kroz otvor na stjenci. Za ovog događanja se čuje pjevanje matice. Slobodna matica proizvodi visoko TI-TI-TI, a matice iz matičnjaka muklo KVA-KVA-KVA. U takvim uvjetima košnicu ubrzo napušta i drugi roj, drugak ili drugenac. Takav roj najčešće se podjeli na više manjih rojeva budući da se u njemu nalazi više mladih nesporenih matice. Za nekoliko dana se može izrojiti i treći, pa čak i četvrti roj, brojčano slabiji. U izobilju paše te kod pčela koje imaju izraženu sklonost rojenju može se dogoditi i da se prvenac izroji i dade novi roj, paroj. Ukoliko se u istoj sezoni izroji paroj, daje roj pčela koje se zovu bijele pčele (Katalinić i sur., 1968).

U vrijeme jakih proljetnih paša svaki pčelar želi imati dobro razvijenu pčelinju zajednicu koja će iskoristiti tu bogatu i često kratkotrajnu pašu. Rojenje je stoga u suvremenom pčelarstvu nepoželjno, jer oslabljuje pčelinju zajednicu te je proizvodnja meda nedostatna. Zajednice koje su se dva do tri puta dijelile jedva prikupe dovoljno hrane i za svoje potrebe. Kako bi se spriječilo rojenje mora se raditi protiv rojdbenog nagona, i prije i za vrijeme glavne paše. Matici je nužno osigurati dovoljno mjesta za polaganje jaja. Ako matica zaliježe bit će otvorenog legla koje će mlade pčele moći hraniti. Dodavanjem satne osnove mlade pčele će aktivirati voštane žlijezde gradeći saće, a dodavanjem izgrađenog saća sakupljačice će imati gdje odlagati nektar. Pravovremeno proširenje pčelinjeg gnijezda je vrlo djelotvorna mjera protiv ulaska pčelinje zajednice u rojdbeni nagon (Križ 2014).

Razrojavanje pčelinjih zajednica

Prirodno rojenje nastupa pod određenim uvjetima i u ograničenom vremenskom periodu. Osim negativnog utjecaja na prinose meda, prirodni roj često puta odlazi u potragu za novim mjestom gdje će se smjestiti, jer pčelar nije u mogućnosti stalno biti u pčelinjaku. Suprotno tome, kod umjetnog rojenja, odnosno razrojavanja pčelinjih zajednica, pčelar kontrolira kad će i koliki broj rojeva proizvesti i s kojim maticama. Posebno je važno planirati rojenje nakon što je glavna paša prošla, ali i dovoljno rano kako bi se novi rojevi razvili prije završetka pčelarske sezone i tako pripremili za uspješno prezimljavanje. Umjetni se rojevi mogu dobiti na dva načina: izdvajanjem dijela legla i pčela

s maticom iz osnovne zajednice ili stresanjem pretežno mladih pčela sa okvira s leglom u košnicu s pripremljenim okvirima ili satnim osnovama. Izdvajanjem dijela legla s pčelama osigurava se kontinuitet u razvoju zajednica, dok kod stresenog roja matica počinje iz početka sa polaganjem jaja, kao kod prirodnog roja (Kulinčević 2012). Kod pravilne primjene umjetnog rojenja prinosi meda na pčelinjim zajednicama se ne smanjuju (Laktić i Šekulja 2008). Postoji više razrađenih metoda za razrojavanje pčelinjih zajednica.

Taranova metoda

Taranova metoda razrojavanja temelji se na formiranju umjetnog roja bez uzimanja saća na način da se napravi kosi stalak, od daske širine 30 cm, a dužine 50 cm, pod kutom od 45°. Stalak se postavi ispod leta košnice koju želimo razrojiti, tako da bude odmaknut 10 cm od leta košnice, a onda se na donji dio kosine stalka stresa cijela pčelinja zajednica, zajedno s maticom (Relić 2006). Pritom stare pčele vratit će se u košnicu iz koje su strešene. Mlade pčele, uključujući i one aktivno rojevne, također se penju stalkom prema košnici, ali ih sam razmak (10 cm) do leta zaustavlja i one se malo pomalo sakupljaju u klupko ispod stalka gdje ostaju mirno visiti poput roja. Tako uhvaćeni roj se kasno poslije podne smjesti u novu košnicu kao i svaki drugi prirodni roj. Ako je u zajednici iz koje je istresen roj bila matica koja je zalijegala, takva matica mora biti u istresenom roju. Ako je bila nesparena, roj će prihvatiti i takvu maticu.

Pelletova metoda

Ovaj način razrojavanja primjenjuje se samo na jakim zajednicama. Na podnicu zajednice postavi se prazan nastavak. U taj se nastavak, u sredinu, stavi okvir s leglom i starom maticom te se još doda 9 okvira sa saćem i satnim osnovama. Na taj nastavak se stavi matična rešetka sa letom. Na matičnu rešetku se stavi drugi nastavak s praznim saćem i satnim osnovama. Na taj, medišni nastavak se postavi nastavak u kojem su okviri s leglom, pčelama i medom. Na mjesto okvira koji je prethodno uzet zajedno s maticom se dodaje jedan prazan okvir. Na tom trećem nastavku, bivšem plodištu, se obavezno otvara leto. Za 24 sata se u taj nastavak s otvorenim letom doda zreli matičnjak između okvira legla. Kada se matica izleže, kroz gornje leto odlazi na oplodnju. Nakon oplodnje matica polaže jaja u tom svom gornjem plodištu, dok će stara matica sve to vrijeme nesti jaja u svom nastavku koje se nalazi na podnici košnice (Laktić i Šekulja 2008). Za tri tjedna izleći će se u gornjem plodištu sve leglo koje potječe od stare matice. Kad je većina okvira popunjena leglom mlade matice, može se taj nastavak prenijeti na novo mjesto, jer je u njemu kompletan roj. Većina pčela koje potječu od stare matice vratit će se na staro mjesto, a mlade će nastaviti razvoj (Belčić 1985). Pelletovim načinom umjetnog razmnožavanja pčelinjih zajednica se ne smanjuju prinosi meda u osnovnoj zajednici (Relić 2006).

Belčićeva metoda

Ova se metoda primjenjuje u proljeće kad se pčelinje zajednice razvijaju u dva nastavka. Tri tjedna prije bagremove paše se pripremi treći nastavak sa izgrađenim saćem te se potraži matica. Pronađena matica sa dva okvira najmlađeg legla i pčela se prenese u pripremljeni nastavak. Umjesto dva okvira legla, pčelinjoj zajednici se vrata dva okvira s izgrađenim saćem. Košnicu, odnosno prva dva nastavka se prekrije s Hanemanovom rešetkom, sa zatvorenim letom. Na pregradu se postavi treći nastavak s maticom i dva okvira otvorenog legla i pčela i osam okvira saća. Tijekom tri tjedna, do početka bagremove paše, radilice su u dodiru s maticom jedino preko Hanemanove rešetke. Za to vrijeme sve se leglo u donjim nastavcima izleže, pa pčele imaju na raspolaganju dva cijela nastavka kao medišni prostor. Upravo taj veći medišni prostor i oslobađanje pčela od grijanja i othranjivanja legla rezultira povećanim prinosom. Na početku bagremove paše skine se gornji, treći, nastavak i odloži u stranu. Na Hanemanovu rešetku postavi se žičana mreža ili najlon te se nastavak s maticom vrati na svoje mjesto, s tim da se leto na pregradi otvori. Pčele koje od matice odu na pašu kroz novootvoreno leto na pregradi, više se neće vratiti matici. Kako su navikle u košnicu će ulaziti na donje leto, a postavljena žičana mreža onemogućavat će im pristup matici. Radi toga će treći nastavak ostati bez svih skupljačica koje će svojim radom u donjem medišnom dijelu košnice pridonijeti većem prinosu meda, od već započete bagremove paše. Iako odvojene od matice žičanom mrežom (najlonom), pčele će ostati motivirane za rad, jer će osjećati njezinu prisutnost. U najgornjem nastavku s maticom ostaju samo mlade kućne pčele. One njeguju leglo i u nešto ranijoj dobi postaju skupljačice na što ih prisile potrebe zajednice i njihov urođeni nagon. Nakon bagremove paše, oduzimanja i vrcanja meda, ako je u najgornjem nastavku mlada i kvalitetna

matica, ukloni se samo pregrada i matici omogući nesmetan pristup cijeloj košnici. Time se opet dobije normalna pčelinja zajednica (Belčić 1985).

Demarijeva metoda

Demarijev način ili demariranje je postupak u pčelarstvu koji se koristi prvenstveno za sprječavanje pojave rojidbenog nagona iako puno pčelara ovu metodu koristi i nakon što pčele izgrade matičnjake. Metoda je iznimno popularna, a ime je dobila po francuskom pčelaru Demareu, koji ju je 1884. godine počeo primjenjivati. Ovaj postupak razrojavanja mogu primjenjivati pčelari na košnicama s nastavcima jednakih visina. U plodištu se ostavlja matica s jednim do dva okvira mladog legla, a u gornji nastavak se premjeste svi ostali okviri s leglom i medom. Oba nastavka se dopune okvirima sa praznim saćem ili satnim osnovama. Između plodišta i gornjeg nastavka se umetne matična rešetka. Trutovima iz gornjeg nastavka treba omogućiti slobodan izlaz kroz otvoreno leto na matičnoj rešetki. Neki pčelari postavljaju iznad matične rešetke, ispod gornjeg nastavka s leglom i medom, nastavak s praznim saćem ili satnim osnovama. Donji nastavak predstavlja novoformirani roj, a gornji izrojenu zajednicu. Nakon deset dana potrebno je uništiti matičnjake u gornjem nastavku. Primjena ove metode omogućuje veći prinos meda, jer u košnici u narednom periodu, nema puno otvorenog legla pa će hraniteljice preuzeti ulogu skupljačica nektara. Također, primjena ove metode trebala bi spriječiti pojavu rojidbenog nagona. Ova metoda se može primijeniti čak i kod zajednice koja je ušla u rojidbeno stanje, pa i nakon što su pčele izgradile matičnjake. Prema Dolovcu, (2005) metoda se zasniva na odvajanju matice od legla prevješavanjem okvira, odnosno na razbijanju pčelinjeg legla, čime se stvara stanje izrojene zajednice. Ako se u košnici nađu zaleženi ili zatvoreni matičnjaci, uzme se jedan okvir s leglom i matičnjacima, tako što se ostave samo dva najbolja, i okvir stavi u sredinu donjeg nastavka. Na krajeve se stavi po jedan okvir s izgrađenim saćem, a ostatak nastavka se popuni sa satnim osnovama. Donji nastavak se prekrije matičnom rešetkom te se na njega postavi drugo plodište. Na drugi nastavak se stavi treći nastavak sa starim leglom. Drugi dan se u nastavcima iznad matične rešetke potraži matica. Ukoliko je pronađena, matica se odstrani. Ne pronalazak znači da je matica ostala u nastavku s matičnjacima. Matica koja se prva izleže će ju likvidirati. Nakon nekoliko dana bit će uništen i drugi matičnjak. Rojidbeni nagon će se zaustaviti. Demarijeva metoda kod zajednice koju je već zahvatio rojidbeni nagon se pokazala uspješnom. Zajednica nije brojčano oslabljena, a u košnici ostaje mlada matica (Čolak 2015).

Snelgrova metoda

Snelgrova metoda temelji se na primjeni Snelgrove daske. To je horizontalna pregradna daska ugrađena u okvir čije su unutarnje i vanjske dimenzije jednake dimenzijama LR – košnice. Uporabom ove pregradne daske, moguće je usmjeravanje pčela iz gornjeg nastavka u donji ili obrnuto. Osim leta, za ovu dasku karakteristično je i to da ima otvor u sredini veličine 15×15 cm, zatvoren dvostrukom mrežom. Pčele se usmjeravaju otvaranjem i zatvaranjem pojedinih leta pregradne daske. Na prednjoj strani pregradne daske nalaze se dva para leta širine 10 cm. Leta su izrezana tako da gornja leta usmjeravaju pčele u gornji nastavak, a donja leta ih usmjeravaju u donji nastavak. Na suprotnoj strani izgrađena su još po dva leta, širine 5 cm po istom principu kao ona na prednjoj strani. Ovom metodom se umnožavaju pčelinje zajednice nakon vrcanja bagremovog meda. Nastavak iz kojeg je izvrcan med se postavi na podnicu zajednice. U tom nastavku je predviđeno razvijanje novoformiranog roja. U njega se stavi oplodena matica u kavezu. Nastavak se prekrije matičnom rešetkom te se na rešetku stavi nastavak s medom. Na taj nastavak se postavi snelgrova daska. Potom se na pregradnu dasku stavi plodišni nastavak sa starom maticom i njezinim leglom. Na kraju se doda drugi izvrcani nastavak. Na stražnjoj strani snelgrove daske otvori se leto kroz koji će pčele, iz tog nastavka, izlaziti van. U košnicu će se vraćati na leto u podnici, kako su navikle ulaziti prije ove podjele. Uslijed toga roj na podnici s mladom maticom će dobiti sve pčele koje su pripadale osnovnoj zajednici. Nakon 15 do 20 dana roj će već imati 4 do 5 zaležanih okvira. Za to vrijeme brojčano će se razviti i gornja zajednica budući da je već imala leglo. Gornju zajednicu možemo premjestiti na novo mjesto te ju dobro prihranjivati dvadesetak dana. Prednost ove metode je i što se ne traži matica po okvirima, već se uspješno radi s kompletnim nastavkom (Laktić i Šekulja 2008).

Paketni rojevi

U suvremenom načinu pčelarenja povećanje broja pčelinjih zajednica preporučljivo je raditi paketnim rojevima i dodavanjem selekcionirane matice. Pri selekciji se uzimaju u obzir osobine pčelinje zajednice kao što su mirnoća

pčela, sklonost rojenju te otpornost na bolesti. Rojdbeni nagon ima izuzetno visok heritabilitet, što je negativna karakteristika sive pčele. Izlučivanjem iz uzgoja rojivih zajednica (matica), sklonost rojenju će biti kroz nekoliko generacija značajno potisnuta (Kezić i sur., 2014). Širenje bolesti i nametnika su stalna opasnost za pčele te pčelari ulažu veliki trud i materijalna sredstva u prevenciji i suzbijanju bolesti. Stoga se posebna pažnja pridaje istraživanjima i razvoju metoda testiranja i uzgoja pčela tolerantnijih na bolesti (Buchler 2000; Harris i Harbo 2000). U Hrvatskoj se zadnjih godina, uglavnom, koristi Multibox kutija, izrađena od plastične rešetke. Osim što se može višekratno koristiti, ova kutija sadrži i spremnik za tekuću hranu s polupropusnim čepom što osigurava hranu pčelama i do tjedan dana. Također, ovi paketi se mogu međusobno povezati i složiti u vozilo. Paketni rojevi pčela, teški 1,5 – 2,0 kg, formiraju se iz zdravih, dobro razvijenih zajednica s velikim brojem mladih pčela. Mlade pčele imaju veću vrijednost, manje su agresivne i puno je lakše njima manipulirati, pa je iz tog razloga pčele najbolje vaditi za lijepog vremena kad je većina skupljačica u paši. U međunarodnom prometu, trutovi nisu poželjni, što se rješava natresanjem pčela iz medišta, jer im se tako smanjuje broj u paketnim rojevima. U lokalnim uvjetima, trutovi se mogu odvojiti kombinacijom tri međusobno spojene nepotpune Multibox kutije, tako da se na donju, sa pčelama, stavi matična rešetka. U paketnom se roju ne bi smjela naći mlada nesparena matica, jer će rojevi s mladom nesparenom maticom uglavnom ubiti kasnije dodanu maticu u kavezu. Stoga se izbjegava formiranje paketnih rojeva iz zajednica koje su u uznepredovalom rojdbenom stanju. Pčele se natresaju s okvira iz jedne ili više zajednica pomoću sipaonika za pčele. Potrebno ih je dodatno prskati vodom ako temperatura prelazi 20 °C. Paket je potrebno ukloniti sa sunca i staviti ga na mirno mjesto. Sljedeće jutro se dodaje sparena matica, selekcionirana na poželjna svojstva, u kavezu. Kavez se na žici objesi uz hranilicu. Pčele odmah prihvaćaju novu maticu, što potvrđuju promjenom zujanja i formiranjem klupka oko nje. Tijekom prijevoza treba osigurati odgovarajuću ventilaciju i kontrolirati temperaturu. Temperatura iznad 37 °C je opasna za pčele. Pčele se rashlađuje raspršivanjem vode po transportnim kutijama. Poželjno je prije utovara navlažiti i pod transportnog vozila (Relić 2006). Prije stavljanja pčela u transport potrebno je osigurati sva propisana uvjerenja i certifikate (zdravstvena uvjerenja, uvjerenje o porijeklu) za stavljanje pčela u promet (Kezić i sur., 2014). Kad pčelar primi paketne rojeve, potrebno ih je ostaviti do sutra navečer da se umire i profunkcioniraju kao prirodan roj, pa ih tek onda, narednu večer smjestiti u košnicu. U protivnom, smještanjem tijekom dana, radi nedovoljne adaptiranosti, odnosno nedostatka vremena za preciznu orijentaciju moglo bi doći pri uzdizanju pčela oko košnice do njihovog ulijetanja u susjedne košnice ili da dio pčela odleti (Krstić 2006). Košnica treba imati pet okvira s nešto meda, a ostali prazni prostor služi za smještaj roja. Prije nego što se paketni roj istrese, potrebno je izvaditi kavez s maticom i objesiti u novu košnicu. Važno je zajednicu hraniti i ne uznemiravati je narednih 6 - 8 dana. Nakon tog razdoblja kavez se uklanja i provjerava stanje matice. Da se zajednica dobro razvija potvrđuje unos peludi potrebnog za leglo (Kezić i sur., 2014). Kod nukleusa formiranog od paketnog roja kritično razdoblje nastaje nakon 10-ak dana, jer nema dovoljno pčela koje bi hranile leglo i brinule se za maticu, zato što stare pčele počinju umirati, a nove se još nisu razvile. Stoga nukleuse s izraženim gubitkom pčela treba pojačati dodavanjem mladih pčela, uz neutralisanje njihovog mirisa, te im prvi mjesec osigurati dohranu (Puškadija 2008).

Zaključak

U suvremenom pčelarstvu prirodno rojenje pčelinjih zajednica nepoželjna je osobina. Kod prirodnog rojenja pčelar nema utjecaja niti na vremensko razdoblje u kojem se događa, niti na broj rojeva. Međutim, kod razrojavanja pčelar može organizirati kada će obaviti umjetno rojenje, s kojim maticama te odrediti koliko rojeva treba za povećanje svog pčelinjaka, a koliko za potrebe tržišta. Uglavnom kod pravilne primjene razrojavanja prinosi meda na pčelinjim zajednicama se ne smanjuju. Razrojavanje je poželjno vršiti samo na onim zajednicama koje su se kroz višegodišnje praćenje pokazale najboljim na pčelinjaku.

Literatura

- Allen M.D. (1960). The honeybee queen and her attendants. *Animal Behaviour*. 8: 201-208.
- Belčić J., Katalinić J., Loc D., Lončarević S., Peradin L., Sulimanović Đ., Šimić F., Tomašec I. (1985). Pčelarstvo. Nakladni zavod Znanje, Zagreb.
- Brence-Lazarus T. (1998). Uzgojno selekcijski rad u pčelarstvu Republike Hrvatske, Hrvatska Pčela. 4: 73-76.
- Buchler R. (2000). Design and success of a German breeding program for Varroa tolerance. *American*

- Bee Journal.140: 662-665.
- Čerimagić H. (1990). Pčelarstvo. NIP „ZADRUGAR“, Sarajevo.
- Čolak J. (2015). Demarijev princip protiv rojevog nagona. BH pčelar.
- Dolovac A. (2005). Savremeno pčelarstvo: Nauka i praksa. Bemust, Sarajevo.
- Fefferman H.N., and Starks T.P. (2006). A modeling approach to swarming in honey bees (*Apis mellifera*). *Insectes Sociaux*. 1: 37-45.
- Harris J.W., and Harbo J.R. (2000). Changes in reproduction of *Varroa destructor* after honey bee queens were exchanged between resistant and susceptible colonies. *Apidologie*. 31: 689-699.
- Jašić M., Šubarić D., Budimlić A., Filipi J., Nogić E., Elvedi, Miličević I, Šehić E. (2016). Uzgoj i selekcija matica sive pčele (*Apis mellifera carnica*). Udruženje za nutricionizam i dijetetiku „Hranom do zdravlja“, Tuzla.
- Katalinić J., Loc D., Lončarić S., Peradin L., Simić F., Tomašec I. (1968). Pčelarstvo. Nakladni zavod Znanje, Zagreb.
- Kezić N., Bubalo D., Grgić Z., Dražić M., Barišić D., Filipi J., Jakopović I., Ševar M., Krakar D., Tretinjak V. (2014). Priručnik konvencionalno i ekološko pčelarenje. Sveučilište u Zagrebu Agronomski fakultet, Interna skripta, Zagreb.
- Križ J. (2014). Sprječavanje rojenja i tihe paše. *Hrvatska pčela*. 133: 193 -194.
- Krstić M. (2006). Formiranje paketnih rojeva u komercijalne svrhe. Web pčelinjak popularizacija pčelarstva.
- Kulinčević J. (2012). Pčelarstvo. Partenon, Beograd.
- Laktić Z., and Šekulja D. (2008). Suvremeno pčelarstvo. Nakladni zavod Globus, Zagreb.
- Plavša N., and Nedić N. (2015). Praktikum iz pčelarstva. Univerzitet u Novom sadu, Poljoprivredni fakultet, Novi sad.
- Puškadija Z., Opačak A., Florijančić T., Jelkić D., Mijić P., Bošković I. (2008). Formiranje i njega nukleusa medonosne pčele (*Apis mellifera* L.). *Poljoprivreda*. 14: 1-4.
- Relić B. (2006). Pčelarstvo. Neron, Bjelovar.
- Winston M. (1987). *The Biology of the honey bee*. Harvard University Press Cambridge, Massachusetts London.

Methods of artificial swarming of honey bee colonies

Abstract

The Western honey bees are social insects which live in large colonies, where every bee has its role to fulfil. During favourable weather conditions the number of colony members increases, but the number of honey bee colonies does not. Propagation of honey bee colonies occurs through the act of swarming, during which the colony splits in half. One half leaves the hive with the old queen, while the latter half remains with a new, young queen. Swarming numerically weakens the colony, and along with it, the honey yield as well. Swarming is therefore undesired in modern beekeeping. Beekeepers endeavour to keep all the causes of the swarming tendency at a minimum. To increase the number of honey bee colonies they use reliable artificial swarming methods in order to create, new swarms and colonies in in particular period, and in numbers they require.

Keywords: *Apis mellifera*, honey bee colony, swarming, artificial swarming methods

Botaničko podrijetlo pčelinje peludi na području parka Maksimir

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Sažetak

Cilj je ovoga rada bio utvrditi botaničko podrijetlo pčelinje peludi na području parka Maksimir. Istraživanje je provedeno na pčelinjaku Agronomskog fakulteta gdje je odabrana jedna zajednica sive pčele (*Apis mellifera carnica*) na koju je bio postavljen unutarnji skupljač peludi. Pelud je skupljana jednom tjedno, tijekom travnja i svibnja. Nakon provedene melisopalinološke analize utvrđena je prisutnost peludi 20 biljnih vrsta. Tijekom travnja najzastupljenija je bila pelud divlje šljive (*Prunus cerasifera*) s udjelom od 22,53 % i divlje trešnje (*P. avium*) s 20,25 %, dok je u svibnju dominirala pelud dvogodišnjeg dimka (*Crepis biennis*) s 42,02 % i pajasena (*Ailanthus altissima*) s 17,03 %. Najveća i najmanja dnevna masa prikupljene pčelinje peludi ustanovljene su u svibnju i iznosile su 73,48 g, odnosno 11,32 g.

Ključne riječi: pčelinja pelud, *Apis mellifera carnica*, botaničko podrijetlo, Maksimir

Uvod

Medonosne pčele su jedne od rijetkih vrsta kukaca koji su djelomično domesticirani te su njezine koristi, kako izravno ili neizravno višestruke za čovjeka kroz prehranu i apiterapiju, tako i za ekosustav putem oprašivanja. Bez pčela propale bi brojne biljne zajednice te bi se cijeli prirodni ekosustav kakvog danas poznajemo značajno promijenio. Pelud predstavlja muške spolne stanice biljaka koje za razliku od ostalih pčelinjih proizvoda pčele ne sintetiziraju u svome tijelu kao što je to slučaj s pčelinjim voskom, matičnom mliječi i pčelinjim otrovom, već ga skupljaju u prirodi s prašnika cvjetova različitih biljnih vrsta. Peludna zrnca različitih vrsta biljaka znatno se razlikuju po veličini, odnosno njihova veličina je specifična za vrstu s koje dolaze. Peludno zrnce obavijeno je dvoslojnom staničnom stjenkom. Unutarnja stanična stjenka naziva se intina, a vanjska egzina. Egzina je građena od sporopolenina koji se očituje velikom otpornošću na različite fizikalno-kemijske čimbenike (kiša, vjetar, UV zračenje, mehanička oštećenja i sl.). Nadalje, na njegovoj površini nalaze se brojne pore i brazde te sloj balzama koji olakšava lijepljenje peludi na dlake pčela (Komosinska-Vassev i sur., 2015). Stoga je navedena građa egzine tipična za biljnu vrstu i od velike je pomoći u određivanju botaničkog podrijetla peludi. Također, ova je činjenica od velike važnosti jer se može iskoristiti i za utvrđivanje botaničkog podrijetla meda. Pčelinja pelud skupljena uz pomoć skupljača razlikuje se od izvorišne peludi na cvijetu, jer joj pčele prilikom skupljanja dodaju izlučevine žlijezda slinovnica i malo nektara, kako bi ju lakše povezale u kompaktnu masu i tako lakše donijele u svoju zajednicu. Postoji više tipova skupljača peludi, ali svi funkcioniraju na isti način. Naime, pčele prolaskom kroz perforiranu rešetku skupljača zapinju s peludnim teretima o rub rešetke i na taj način pelud biva skinuta s košarica njihovih stražnjih nogu.

Uz nektar, koji je izvor ugljikohidrata, pelud je glavni izvor bjelančevina za pčelinju zajednicu te joj osigurava optimalan omjer svih hranjivih sastavnica koje su joj potrebne za rast i razvoj. Sastav peludi uvelike ovisi o biljnom izvoru, zemljopisnom podrijetlu, klimi, tipu tla, sezoni i skupljačkoj aktivnosti pčelinje zajednice. Iz tog razloga pelud s različitih biljnih vrsta ima različitu hranidbenu vrijednost za pčele, a kvalitetna ishrana osnova je za normalan razvoj svih članova pčelinje zajednice (Prđun 2017., Prđun, 2018). Uzgoj medonosnih pčela postaje sve popularniji posljednjih godina i u urbanim sredinama. Tako se brojne svjetske metropole mogu pohvaliti urbanim pčelinjim zajednicama, koje njihovi građani drže u košnicama na balkonima i vrhovima zgrada. Iako ih u gradove donose pčelari, pčele se i same sve češće nastanjuju u gradskim sredinama. Pčele vrlo dobro uspijevaju u gradu, a razlog tome je što u gradovima postoji velika, a ponekad i veća bioraznolikost nego u nekim seoskim područjima (Brizanac,

2015). Parkovi i gradski vrtovi izvrstan su izvor nektara i peludi za pčele, jer se u njima često nalaze biljne vrste koje su jako zanimljive pčelama, posebice lipa, javor, divlji kesten i sl. Stoga je cilj ovoga rada bio melisopalinološkom analizom utvrditi koje biljne vrste medonosna pčela (*Apis mellifera carnica* Pollman 1879) posjećuje na području parka Maksimir.

Materijal i metode

Pokus je proveden na pčelinjaku Agronomskog fakulteta Sveučilišta u Zagrebu koji je smješten u sjeveroistočnom dijelu perivoja parka Maksimir (45°49'52" S, 16°01'47" I) (Slika 1a).



Slika 1a) pokusni pčelinjak Agronomskog fakulteta Sveučilišta u Zagrebu

1b) pokusna pčelinja zajednica s unutarnjim skupljačem peludi

U istraživanje je bila uključena jedna pčelinja zajednica sive pčele (*A. mellifera carnica* Pollman 1879) na koju je bio postavljen unutarnji skupljač peludi (Slika 1b). Pčelinja pelud je skupljana tijekom cijeloga dana, jednom tjedno, od početka travnja do kraja svibnja, u sedam navrata. Nakon skupljanja, pčelinja pelud je izvagana pomoću digitalne vage Mettler Toledo (0,1 mg – 200 g) i pohranjena u plastične bočice u zamrzivač na temperaturu od -18 °C do daljnje analize. Nakon svakog uzorkovanja, prikupljena pelud je razvrstana prema boji na poduzorke, nakon čega je svaki poduzorak izvagana te je određen njegov udio u skupnom uzorku. Poduzorci su smješteni u zasebne staklene posudice i ponovno vraćeni u zamrzivač na temperaturu od -18 °C.

Nakon razvrstavanja peludi prema boji, uslijedila je priprema za peludnu analizu. Za pripremu peludi za peludnu analizu bile su korištene dvije kuglice peludnog tereta od svake boje, a priprema je izvršena prema metodi Louveaux i sur. (1978.) i Barth i sur. (2010.). Nakon homogenizacije uzorci su bili osušeni u sušioniku na 40 °C te im je bila dodana kap glicerina želatine (Kaiser's Glycerin gelatine, Darmstadt, Germany) nakon čega su bili poklapani pokrovnim stakalcem.

Identifikacija peludnog tereta provedena je melisopalinološkom metodom prema von der Ohe i sur. (2004). Identifikacija i brojanje peludnih zrnaca izvršeno je pod svjetlosnim mikroskopom Carl Zeiss Axio (Jena, Germany) pri povećanju od 400 – 1 000 x te pomoću zbirke referentnih uzoraka peludnih zrnaca u obliku nativnih preparata (interna zbirka Zavoda za ribarstvo, pčelarstvo i specijalnu zoologiju, Sveučilište u Zagrebu Agronomski fakultet) i literature (von der Ohe i von der Ohe, 2003). Pritom je promatran oblik, boja i veličina peludnog zrnca, kao i struktura eksine te broj i mjesta pora klijanja.

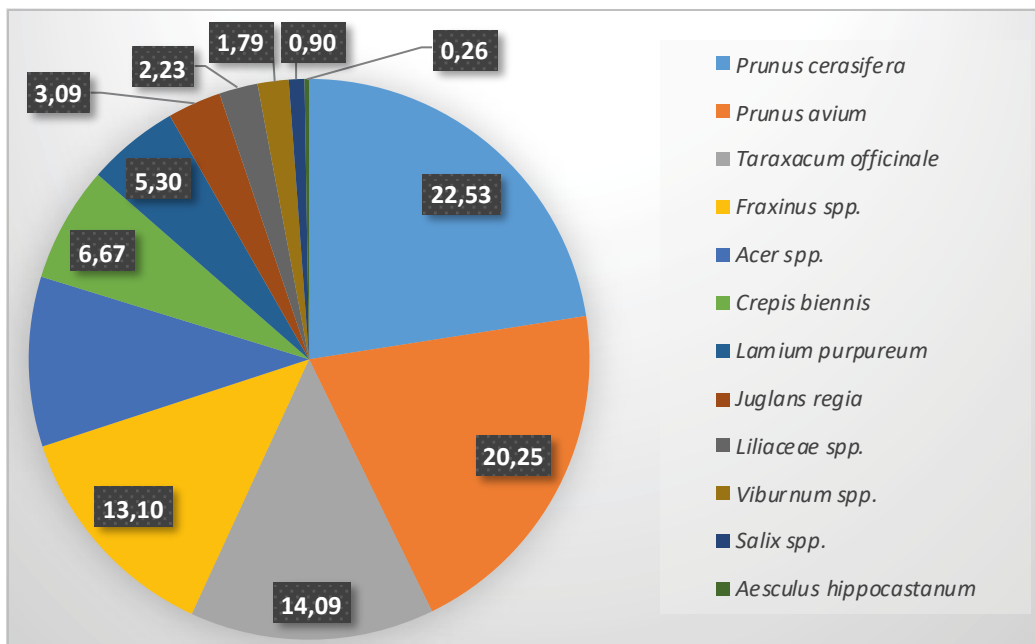
Dobiveni podatci su obrađeni u programu Microsoft Excel 2016.

Rezultati i rasprava

Pelud je jedna od najvažnijih komponenti za razvoj pčelinje zajednice jer pčelama osigurava izvor bjelančevina i minerala (Brodschneider i Crailsheim 2010). Bez dostatnih količina peludi, pogotovo u proljetnom dijelu godine pčelinja zajednica se neće moći dovoljno brzo razviti, stoga je bogata peludna paša neophodna za sve članove pčelinje zajednice i njezino normalno funkcioniranje. Peludnom je analizom na osnovu specifične strukture (unutarnja

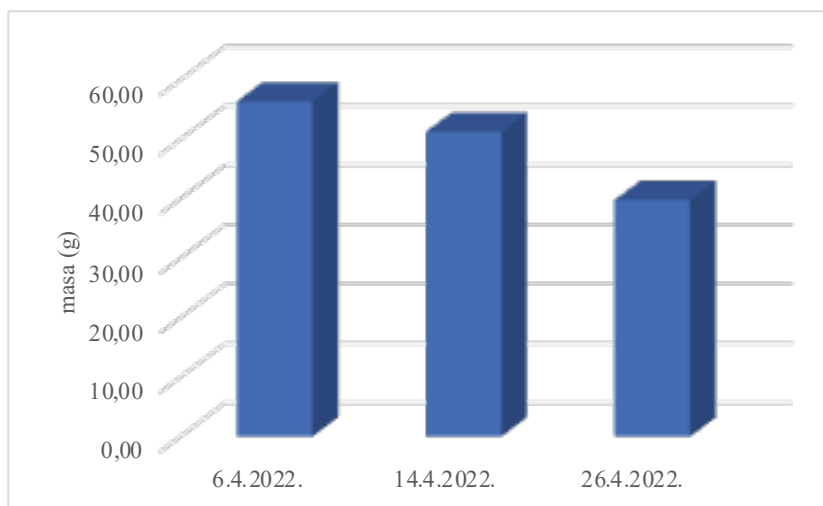
organizacija vanjskog sloja peludnog zrnca – eksine) i skulpture (vanjski izgled peludnog zrnca) peludnih zrnaca biljnih vrsta ukupno u ovom istraživanju identificirano botaničko podrijetlo njih 20 (Grafikoni 1 i 3).

Kao što je razvidno iz Grafikona 1 tijekom travnja ukupno je identificirano 12 biljnih vrsta s kojih su pčele skupljale pelud. S najvećim udjelom dominirala je pelud biljaka iz roda *Prunus*, odnosno pelud divlje šljive (*Prunus cerasifera*, 22,53 %) i divlje trešnje (*Prunus avium*, 20,25 %). Pelud ovih biljaka bogata je bjelančevinama i mastima (Prđun i Bubalo, 2021) te ih pčele posjećuju u velikom broju, budući da im je pelud neophodna za razvoj legla, koji je u proljeće vrlo intenzivan kod zajednica sive pčele. Pelud maslačka (*Taraxacum officinale*) bila je zastupljena s udjelom od 14,09 %, a pelud jasena (*Fraxinus spp.*) sa 13,10 %. Naime, maslačak kao medonosna biljna vrsta je također vrlo zanimljiv pčelama posebice u proljetnom dijelu godine zbog obilja peludi, ali i nektara kojeg ova biljka izlučuje, za razliku od jasena koji prvenstveno pčele posjećuju zbog peludne paše.



Grafikon 1. Udio uniflorne pčelinje peludi (%) u uzorku prikupljenom u travnju 2022. godine na području parka Maksimir

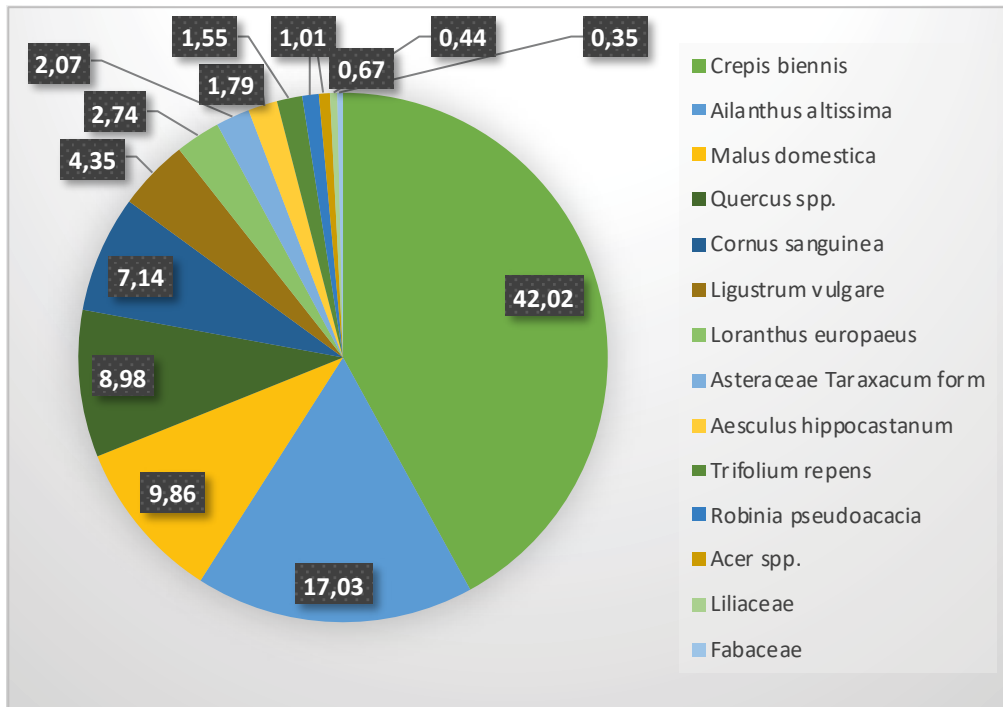
Pelud javora (*Acer spp.*) bila je zastupljena s udjelom od 9,79 %, pelud dvogodišnjeg dimka (*Crepis biennis*) s udjelom od 6,67 %, a crvene mrtve koprive (*Lamium purpureum*) 5,30 %. Biljne vrste koje su pčele također posjećivale tijekom travnja, ali u manjem udjelu su bile: orah (*Juglans regia*) s 3,09 %, biljke iz porodice Liliaceae s 2,23 %, udikovina (*Viburnum spp.*) s 1,79 %, vrba (*Salix spp.*) s 0,90 % te divlji kesten (*Aesculus hippocastanum*) s 0,26 %. Pelud divljeg kestena osim što je specifične tamno crvene boje ima i izuzetno dobra hranidbena svojstva, pa tako sadrži prosječno 27,26 % bjelančevina i 2,94 % mineralnih tvari (Prđun i Bubalo, 2021) te je vrlo zanimljiv izvor peludi za medonosne pčele u ovom razdoblju godine. Hušnjak (2020.) navodi da su na području Krapine tijekom travnja najposjećenije biljke na tom području bile trnina (*Prunus spinosa* L. 1753.) i vrba (*Salix spp.*). Promatrajući masu peludi prikupljene tijekom tri uzorkovanja u travnju iz Grafikona 2 je razvidno da je najviše prikupljeno pčelinje peludi na početku mjerenja, točnije 6. travnja gdje je ukupna masa iznosila 56,40 grama, slijedi uzorak od 14. travnja s masom od 51,27 grama. U posljednjem mjeranju 26. travnja prikupljena je najmanja količina pčelinje peludi od 39,80 grama.



Grafikon 2. Masa skupnih uzoraka pčelinje peludi skupljenih tijekom travnja 2022. godine na području parka Maksimir

Ovakav kontinuirani pad mase prikupljene pčelinje peludi posljedica je završetka cvjetanja biljaka iz roda *Prunus* (divlja trešnja i divlja šljiva) i maslačka (*T. officinale*) koje su pčelama bile izuzetno atraktivne u vrijeme njihove pune cvatnje, što se vidi i po količini skupljene peludi u prva dva mjerenja, kada su ove vrste peludi dominirale.

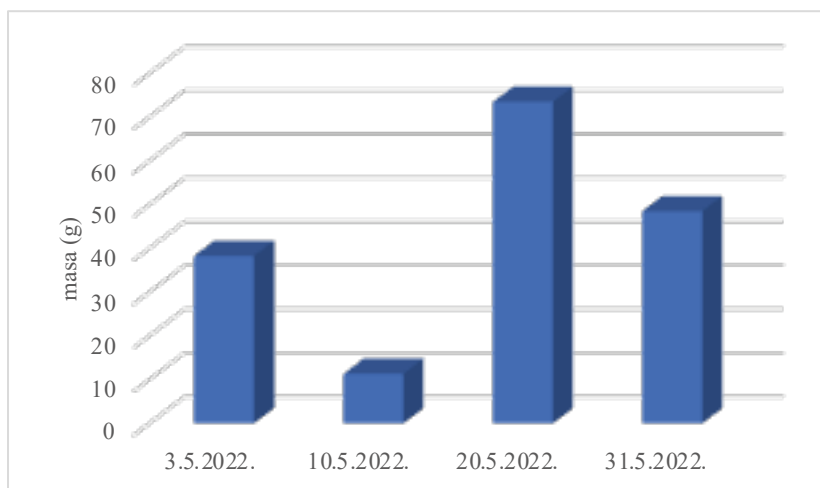
Promatrajući botaničko podrijetlo pčelinje peludi prikupljene tijekom svibnja iz Grafikona 3 je razvidno da je identificirano ukupno 14 biljnih vrsta. Najzastupljenija je bila pelud dvogodišnjeg dimka (*C. biennis*) kojeg su pčele tijekom istraživanja u svibnju prikupile u udjelu od 42,02 %. Pajasen (*Ailanthus altissima*) je invazivna biljna vrsta podrijetlom iz Kine i Sjeverne Koreje, a u Europu je unesena 1740. godine kao ukrasna vrsta (Nikolić i sur., 2014.). Jako je raširen u urbanim sredinama pa tako i u Zagrebu. Na rubnim dijelovima parka Maksimir tvori manje sastojine. Medonosna je biljna vrsta, a pčele s njega skupljaju nektar i pelud, stoga im je jako zanimljiv u ovom razdoblju godine, što se vidi i po udjelu prikupljene peludi koji je iznosio 17,03 %. Slijedi pelud jabuke (*Malus domestica*) s 9,86 %, zatim hrasta (*Quercus* spp.) s 8,98 % koji na području parka Maksimir zajedno s grabom zauzima značajnu površinu. Na rubnim dijelovima parka raširen je svib (*Cornus sanguinea*) i obična kalina (*Ligustrum vulgare*), biljne vrste koje su također atraktivne pčelama zbog nektara, ali i peluda. Tako je udio peludi sviba iznosio 7,14 %, a obične kaline 4,35 %.



Grafikon 3. Udio uniflorne pčelinje peludi (%) u uzorku prikupljenom u svibnju 2022. godine na području parka Maksimir

Od ostalih biljnih vrsta čija je pelud identificirana, a bila je zastupljena s udjelom manjim od 3 % su žuta imela (*Loranthus europeus* - 2,74 %), biljke iz porodice glavočika (*Asteraceae* - 2,07 %), divlji kesten (*A. hippocastanum* - 1,79%), bijela djetelina (*Trifolium repens* - 1,55 %), bagrem (*Robinia pseudoacacia* - 1,01 %), pelud biljaka iz porodice javora (*Acer spp.* - 0,67 %), pelud biljaka iz porodice ljiljanovki (*Liliaceae* - 0,44 %) te pelud biljaka iz porodice lepirnjača (*Fabaceae* - 0,35 %).

Sredina mjeseca svibnja razdoblje je bagremove paše, kada su pčele orijentirane na prikupljanje velikih količina nektara, koje ova biljka izlučuje, a unos peludi se smanjuje. Međutim, da bi održale razvoj legla pčele ipak moraju posjećivati peludonosne biljne vrste koje su im u tom trenutku na raspolaganju. Promatrajući masu peludi prikupljene tijekom četiri uzorkovanja u svibnju iz Grafikona 4 je razvidno da je u prvom uzorkovanju, 3. svibnja prikupljeno 38,28 grama pčelinje peludi. Međutim, kada je krenula bagremova paša količina skupljene peludi se naglo smanjila te je u drugom uzorkovanju, 10. svibnja iznosila samo 11,32 grama. Završetkom bagremove paše unos pčelinje peludi se naglo povećao te je 20. svibnja iznosio 73,48 grama, što je najveća ustanovljena masa tijekom cijelog istraživanja, dok je u posljednjem uzorkovanju masa pčelinje peludi iznosila 48,52 grama.



Grafikon 4. Masa skupnih uzoraka pčelinje peludi skupljenih tijekom svibnja 2022. godine na području parka Maksimir

Uspoređujući istraživanja utvrđivanja botaničkog podrijetla peludi, koja je proveo Chica (2012.) na pet biljnih vrsta (*Rubus* spp., *Echium* spp., *Cistus* spp., *Olea* spp. i *Quercus* spp.), u odnosu na ovo istraživanje proizlazi da se vrijeme potrebno za odvajanje peludnog tereta prema boji može smanjiti ukoliko se koriste klasifikatori, a samim time je i mogućnost pogreške znatno smanjena. Boja se peludi može mijenjati, ovisno o različitim vrstama peludnih zrnaca u istom peludnom teretu ili pod utjecajem atmosferske vlage ili tvari koje dodaju pčele prilikom prikupljanja i spremanja u košnicu. Svi ti čimbenici imaju veliki značaj, stoga oni mogu promijeniti izvornu boju peludi (Bassignani 2002).

Zaključak

Provedenim je istraživanjem utvrđeno kako su pčele na području parka Maksimir tijekom travnja i svibnja 2022. godine skupljale pelud s 20 različitih biljnih vrsta. Promatrajući botaničko podrijetlo u travnju, utvrđena je pelud divlje šljive (*P. cerasifera*), divlje trešnje (*P. avium*), maslačka (*T. officinale*), udikovine (*Viburnum* spp.), vrbe (*Salix* spp.), jasena (*Fraxinus* spp.), javora (*Acer* spp.), divljeg kestena (*A. hippocastanum*), mrtve koprive (*L. purpureum*), oraha (*J. regia*), dvogodišnjeg dimka (*C. biennis*) i biljaka iz porodice Liliaceae. Promatrajući botaničko podrijetlo u svibnju je utvrđena pelud jabuke (*M. domestica*), biljaka iz porodice Asteraceae, sviba (*C. sanguinea*), bagrema (*R. pseudoacacia*), žute imele (*L. europaeus*), bijele djeteline (*T. repens*), pajasena (*A. alltissima*) i kaline (*L. vulgare*). Na osnovu dobivenih rezultata može se zaključiti da na području parka Maksimir pčelinje zajednice imaju na raspolaganju dostatne količine raznovrsne peludi tijekom proljetnog razdoblja.

Literatura

- Barth O.M., Freitas S.A., Oliveira E.S., Silva R.A., Maester F.M., Andrella R.R.S., Cardozo M.B.Q. . (2010). Evaluation of the botanical origin of commercial dry bee pollen load batches using pollen analysis: a proposal for technical standardization. *Anais da Academia Brasileira de Ciências*. 82: 893-902.
- Bassignani V., (2002). Il polline, pp. 31-44. In: *Apicoltura, il sapore di una storia. I prodotti dell'apicoltura* (Sabatini A. G., Carpana E.).- Ist. Naz. Apicoltura, Edizioni Leader II, Bologna, Italy.
- Brizanic I. (2015). *Urbano pčelarstvo*. Završni rad. Sveučilište Josipa Jurja Strossmayera u Osijeku.
- Brodshneider R., and Crailsheim K. (2010). Nutrition and health in honey bees. *Apidologie*. 41: 278–294.
- Chica M. (2012). Authentication of Bee Pollen Grains in Bright-Field Microscopy by Combining One-Class Classification Techniques and Image Processing. *Microscopy research and technique*. 75: 1475–1485

- Hušnjak K. (2020). Botaničko i zemljopisno podrijetlo pčelinje peludi s područja Hrvatske. Diplomski rad, Sveučilište u Zagrebu, Agronomski fakultet.
- Komosinska-Vassev K., Olczyk P., Kafmierczak J., Mencner L., Olczyk K. (2015). Bee Pollen: Chemical Composition and Therapeutic Application. Evidence-Based Complementary and Alternative Medicine. 6: 297425.
- Louveaux J., Maurizio A., Vorwohl G. (1978.). Methods of melissopalynology. Bee World. 59: 139-157.
- Nikolić T., Mitić B., Boršić I. (2014). Flora Hrvatske: invazivne biljke, Zagreb.
- Prđun S. (2017). Skupljačka aktivnost pčelinje zajednice na paši i sastav nektara i meda unšijske mandarine (*Citrus unshiu* Marc.). Doktorska disertacija. Sveučilište u Zagrebu, Agronomski fakultet.
- Prđun S. (2018). Važnost peluda za pčelinju zajednicu. Hrvatska pčela. 137: 161-163.
- Prđun S., and Bubalo D. (2021). Utjecaj botaničkog i zemljopisnog podrijetla na fizikalno-kemijska svojstva pčelinje peludi. Hrvatska pčela. 140: 340-344.
- Von der Ohe K., and Von der Ohe W. (2003). Celle's Mellisopalynological Collection. Niedersächsisches Landesinstitut für Bienenkunde, Celle.
- Von der Ohe W., Persano Oddo L., Piana L., Morlot M., Martin P. (2004). Harmonised methods of melissopalynological analysis. Apidologie. 35: 18-25.

Botanical origin of the bee pollen in the Maksimir park area

Abstract

The aim of this study was to determine the botanical origin of bee pollen in the Maksimir park area. The study was conducted at the apiary of the Faculty of Agriculture, where a colony of honeybees (*Apis mellifera carnica*) was selected, and an front-mounted pollen trap was placed. Pollen was collected once a week during April and May. After conducting a melissopalynological analysis, the presence of pollen from 20 plant species was determined. In April, the most abundant pollen was from wild plum (*Prunus cerasifera*) with a contribution of 22.53% and wild cherry (*P. avium*) with 20.25%, while in May, the dominant pollen was from biennial hawkweed (*Crepis biennis*) with 42.02% and tree of heaven (*Ailanthus altissima*) with 17.03%. The highest and lowest masses of collected bee pollen were observed in May, amounting to 73.48 g and 11.32 g, respectively.

Keywords: bee pollen, *Apis mellifera carnica*, botanical origin, Maksimir

Seasonal changes in the prevalence of endoparasite *Pomphorhynchus laevis* in chub *Squalius cephalus* in the Sava River

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Abstract

Pomphorhynchus laevis is a freshwater fish endoparasite with a complex life cycle which includes an invertebrate as an intermediate host, and a final host (fish). The aim of this study was to investigate the seasonal changes in the prevalence of *P. laevis* in European chub, *Squalius cephalus*, in the Sava River at the Medsave sampling site, and to determine correlation between the parasites prevalence and of physical-chemical water quality parameters. Fish sampling and the measurement of temperature, dissolved oxygen concentration and pH was performed from July 2021 to June 2022. During four seasons, 190 fish individuals were sampled, out of which 68 individuals (36 %) were infested. The results show a seasonal infestation dynamic, with peaks in summer. The prevalence and water temperature were strongly positively correlated ($r= 0.79$), while the strong negative correlation was between prevalence and oxygen concentration ($r= -0.71$). In further research, it is necessary to determine the relationship between the fish size/age and the degree of infestation.

Keywords: fish parasites, water temperature, pH, dissolved oxygen, fish infestation

Introduction

Pomphorhynchus laevis is considered to be one of the most common and widespread species of Acanthocephala parasites of the freshwater fish in Europe (Kennedy, 2006). Due to its complex life cycle (Taraschewski, 2000). *P. laevis* requires an intermediate host for its development (commonly amphipods from the genus *Gammarus*) and a final host. The dominant final hosts in freshwater are barbel, *Barbus barbus* and chub, *Squalius cephalus* (Kennedy, 2006; Vardić Smrzlić et al., 2015), but may also be other species, such as rainbow trout, *Oncorhynchus mykiss* (Çevrimel et al., 2017), tench, *Tinca tinca*, and Atlantic salmon, *Salmo salar* (Yildiz et al., 2004).

Life cycle of the parasitic helminths from the Acanthocephala phylum is shown on figure 1. In the final host, the adult parasite lives in the intestine and reproduces sexually. The females lay eggs that are released with the feces of the host. The acanthor stage, developed from the released eggs, presents the infective form to the intermediate host, who orally ingest the parasite larvae (Taraschewski, 2000; Grabner et al., 2023). In the amphipod intermediate host, the parasite develops further through acanthella to the cystacanth stage, which is trophically transferred to the fish (Figure 1).

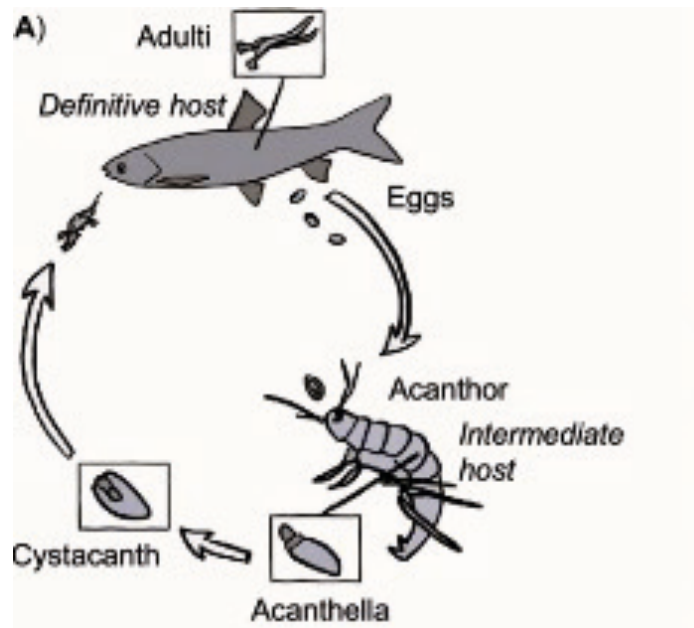


Figure 1. Life cycle of *Acanthocephalus Pomphorhynchus* sp. (Grabner et al., 2023).

Seasonal changes in the prevalence of parasites in aquatic ecosystems, i.e. the proportion of the host population infected with parasite (Sures, 2006) are directly related to biotic factors such as feeding habits, immune response, reproductive behaviour, and/or abiotic factors such as temperature, dissolved oxygen, seasonality, photoperiod, pH and pollution (Adams and Greeley, 2000; Jerônimo et al., 2022). According to Lal and Kumar (2015), temperature has the highest impact on parasite accumulation and is reflected in parasitological indicators such as prevalence and abundance. The aim of this study was to investigate the seasonal changes in the prevalence of *P. laevis* in chub inhabiting the Sava River, and to determine the correlation between the parasite prevalence and the physical-chemical water quality parameters.

Material and methods

Fish samples were collected monthly in the Sava River at the Medsave sampling site 45°50'04.0"N, 15°46'28.3"E from July 2021 to June 2022 in order to cover all four seasons. The Sava River, at this sampling site, is characterized by fast flowing water (mean annual discharge of 294 m³s⁻¹), mean annual water temperature of 14 °C, a rip-rap river bank, and a river bed covered with gravel. Sampling was performed by electro-fishing (Hans Grassl EL 63 II, 220/440 V, 17.8/8.9 A) with a Φ50-cm rounded stainless-steel anode and a 10-mm-mesh-size net was used for the sampling. During the field survey, dissolved oxygen (mg L⁻¹), pH and water temperature (°C) were recorded *in situ* using a portable multiparameter instrument (SI Analytics HandyLab 680). Each sampled fish stunned by electro anesthesia were euthanized, and transported to the laboratory for further processing. In the laboratory, prior the dissection, a total length (TL) and weight of fish (W) were measured. Following dissection, *P. laevis* specimens found in gut were collected, fixed in 96 % ethanol for morphological identification (Bauer, 1985) and molecular analyses (Vardić Smrzlić et al., 2015).

The morphological identification of the parasites was carried out using a „Nikon ECLIPSE Ci-L plus“ light microscope according to referent key for identification Petrochenko (1956) and Reier et al. (2019). In addition, molecular analysis for confirmation of morphological findings was performed using the molecular marker cytochrome c oxidase subunit I (COI). DNA extraction was done using the DNeasy® Blood & Tissue (Qiagen) kit from an individual specimen according to the manufacturer's instructions. PCR amplifications were performed using PCR Master Mix (EmeraldAmp Max, Takara) as it was published previously (Vardić Smrzlić et al. 2023), followed by gel electrophoresis and commercial sequencing (Macrogen, Netherlands). For phylogenetic tree construction was used sequences from the GenBank dataset under the following accession numbers: AY218096, AY423348-AY423353, EF051062-EF051071, KF559284-KF559300, LN994875-LN995000, LN994844-LN994873 and LN994840-LN994842.

Phylogenetic tree was constructed using MEGA 11 (Tamura et al., 2021) and the evolutionary history was inferred by using the Maximum Likelihood methods and the model TN93+G.

The prevalence of *P. laevis* infestation in chub was calculated according to formula (Tenny and Hoffman, 2023):

$$\text{Prevalence (\%)} = \frac{\text{number of infested fish}}{\text{number of fish in the population}} * 100$$

The correlation between prevalence and average seasonal water temperature, pH and dissolved oxygen was calculated using the Spearman's Rank correlation. Microsoft Excel (2016) was used for data processing.

Results and discussion

The mean water temperature at the sampling site was the highest in summer (23.1 °C), and the lowest in winter (9.07 °C). In spring, the mean water temperature was 21.43 °C, while in autumn was 12.43 °C. Seasonal mean pH was highest in winter (8.51), and lowest in summer (8.01). The highest mean seasonal dissolved oxygen concentration was 13.98 mg L⁻¹ in winter, while the lowest value was recorded in summer (6.64 mg L⁻¹), (Table 1).

Table 1. Seasonal changes in physical-chemical parameters at the Medsave sampling station (SD=standard deviation)

Season	Water temperatures (°C)	pH	Dissolved oxygen (mg L ⁻¹)
	Mean ± SD	Mean ± SD	Mean ± SD
Winter	9.07 ± 2.89	8.51 ± 0.12	13.98 ± 2.00
Spring	21.43 ± 6.72	8.17 ± 0.16	9.96 ± 1.94
Autumn	12.43 ± 6.49	8.25 ± 0.08	11.59 ± 0.79
Summer	23.1 ± 0.28	8.01 ± 0.16	6.64 ± 4.70

Similar to the fish weight (Table 2), the average TL values of fish were lowest in spring (14.28 ± 3.06 cm), and highest in summer (15.34 ± 3.58 cm) (Table 2).

Table 2. Average total length (TL) and fish weight (W) per seasons (SD=standard deviation)

Season	Total length (cm)	Fish weight (g)
	Mean ± SD	Mean ± SD
Winter	14.77 ± 3.79	35.72 ± 28.46
Spring	14.28 ± 3.06	35.65 ± 24.20
Autumn	14.91 ± 3.03	36.21 ± 30.59
Summer	15.34 ± 3.58	51.53 ± 38.69

During all four seasons, a total of 190 individuals of *S. cephalus* were sampled at the Medsave station, 68 of which (36 %) were infested with *P. laevis*, that was confirmed by both methods, morphologically and by molecular analysis (Figure 2) where the sequence analyses confirmed the identification. Phylogenetic tree based on COI sequence showed that *P. laevis* from Medsave was clustered within Ponto-Caspian – Black Sea – Balkans group, together with the other specimens from Croatia, Slovenia, Serbia, Romania and several specimens from Austria. This finding is consistent with the results of previous investigations of *P. laevis* in Croatia (Vardić Smrzlić et al., 2023).



Figure 2. Phylogenetic tree constructed on the basis of partial COI gene (535 bp) of *P. laevis* found on infested chub *S. cephalus* collected at Medsave sampling site (red dot) and other data available from the GenBank

A total of 53 chubs were sampled in winter, 18 of which were positive for the presence of *P. laevis* (Table 3). In spring, 58 chubs were sampled, of which 25 were infested. In the autumn, 50 individuals were sampled, of which only 10 were infested. During the summer season, a total of 29 chubs were sampled, of which 15 were positive for *P. laevis*. The highest prevalence was recorded in summer (51.72 %), while the lowest prevalence was only 20 % in fall. In winter, the prevalence was 33.96 % and in spring 43.10 % (Table 3).

The highest average number of parasites per fish was 3.94 in winter, while the lowest number was 1.9 in autumn. In spring this value was 2.64, and in summer 2.26 (Table 3).

Table 3. Prevalence of *Pomphorhynchus laevis* infestation in chub

Season	Number of sampled fish (N)	Number of infested fish (N)	Prevalence (%)	Average number of parasites per fish
Winter	53	18	33.96	3.94
Spring	58	25	43.10	2.64
Autumn	50	10	20	1.90
Summer	29	15	51.72	2.26

According to Spearman's correlation coefficient the prevalence and average seasonal water temperature were strongly positively correlated ($r = 0.79$), while a strong negative correlation was determined between prevalence and dissolved oxygen ($r = -0.71$). The correlation between prevalence and pH was negative ($r = -0.55$).

The results showed a seasonal infestation dynamic, with clear peaks of *P. laevis* infestation in summer, which is characteristic of acanthocephalan infestation in freshwater fish (Poulin, 2020). According to Poulin (2020) the main reason for this is temperature, when the dissolved oxygen concentrations are the lowest. Temperature is a key factor for parasite reproduction and growth (Franke et al., 2017) as well as for host feeding activity. This means that a higher temperature in combination with lower oxygen concentration in summer leads to a greater risk of infestation (Poulin, 2020), which was also shown in this study. In addition to above, temperature combined with changes in the fish diet (faster metabolism and a higher food intake) is an important factor in the spread of parasites in the parasite systems of acanthocephalans and fish (Kennedy et al., 1972). In species with a complex life cycle, such as *P. laevis*, increase in water temperature can accelerate/delay the development/reproduction of intermediate hosts, which is essential for parasite success (Jerônimo et al., 2022). This is related to the seasonal changes in intermediate host life cycle, because many gammarids breed in late summer, what may be reason why parasitic infections in fish show the highest prevalence and abundance during these periods (Kennedy, 2006). Characteristic native gammarids e.g. *Gammarus fossarum* are distributed in the Sava-upper flow, although downstream of the Sava River and Croatian part of the Danube River are almost completely inhabited by gammarid non-native species: *Chelicorophium sowinskyi* (Martynov, 1924), *C. curvispinum* (G.O. Sars, 1895), *Dikerogammarus haemobaphes* (Eichwald, 1841) and *D. villosus* (Sovinsky, 1894) (Žganec et al., 2009; Kralj et al., 2022). In the sampling area, the new *Gammarus* species *Synurella ambulans*, was also found recently (Redžović et al., 2023). As *P. laevis* is common in the Danube, Sava and its tributaries, it is possible that this species expanded its range from the largest Black Sea drainage rivers to their tributaries (Vardić Smrzlić et al., 2023).

Same as in our study, the study of the Svitava and Svratka rivers in the Czech Republic, showed the presence of *P. laevis* in all four seasons. In that study, the highest prevalence for this Acanthocephala species were observed in spring, when the water temperature was 10.5 °C. Authors (Lamková et al., 2007) investigated 19 collected fish with a TL of 239.13 ± 24.01 cm, and the prevalence of this parasite was 89 %. In summer, when the water temperature was 15.4 °C, 20 fish with a TL 248.49 ± 42.52 cm were collected, and the prevalence was 70 % Galli et al. (2001) conducted studies on 157 chub individuals with a TL of 26 ± 3.59 cm collected at four locations near Milan (northern Italy) from April 1997 to August 1998. The prevalence was 94 % at one of the four sites (Galli et al., 2001), what is significantly higher than the total prevalence recorded in this study. In both mentioned studies, the average TL and W of the sampled fish were higher than in our study. This could be one of the possible reasons for the higher prevalence of *P. laevis* found in Czech and Italy, since our samples had a lower TL. The second reason for these differences could be the number of samples. In our research, the highest prevalence was in summer, when the number of samples was the lowest (N= 29). It is possible that the summer temperature accelerates the infestation, but it is unlikely that recorded small number of parasites per infested fish in our research can significantly influence the fish survival in the following seasons. High prevalence at the proximity of Medsave station, when the small number of individuals was examined was found in research of (Marijić et al., 2013; Vardić Smrzlić et al., 2015). In spring 2005, 15 chubs were sampled, with a *P. laevis* prevalence of 86.7 %, while in autumn the same year, in 22 chubs that were sampled, the prevalence was lower than in spring (77.2 %) (Vardić Smrzlić et al., 2015). Same authors, during the spring 2006, sampled 15 individuals of chub at the same station, and the prevalence of parasite was lower than the previous year (73.3 %). In autumn 2006, 15 chubs were sampled at the Medsave station, 9 of which were infected with *P. laevis*, i.e. the prevalence was 60 % (Marijić et al., 2013; Vardić Smrzlić et al., 2015).

Considering the results the fact that this research was conducted on a larger number of fish within one year compared to previous research on the Sava River, it is necessary to examine in more detail the influence of the physico-chemical water quality parameters on the prevalence of parasites on a larger number of samples, and to determine the relationship between the size/age of the individual fish and the degree of infestation.

Conclusions

The prevalence of *P. laevis* in chub at the Medsave station showed seasonal infestation dynamic, with likely peak of infection in summer, while the lowest prevalence occurred in autumn. The highest prevalence in the summer is strongly related to the highest water temperatures, and consequently lower dissolved oxygen concentrations. In the same time, the feeding activity of fish is at its highest level. Future studies could benefit from a larger sample size and a more detailed analysis of the relationship between infestation and fish size and water quality parameters.

References

- Adams S.M., Greeley M.S. (2000). Ecotoxicological indicators of water quality: Using multi-response indicators to assess the health of aquatic ecosystems. *Water, Air, & Soil Pollution*. 123: 103-115.
- Çevrimel D.S., and Soylu E. (2017). The occurrence of *Pomphorhynchus laevis* (Acanthocephala) in cagereared rainbow trout (*Oncorhynchus mykiss*) from Işıklı Spring, Çivril, Turkey. *Ege Journal of Fisheries and Aquatic Sciences*. 34: 255-260.
- Franke F., Armitage S.A., Kutzer M.A., Kurtz J., Scharsack J.P. (2017). Environmental temperature variation influences fitness trade-offs and tolerance in a fish-tapeworm association. *Parasites & Vectors*. 10: 1-11.
- Galli P., Crosa G., Mariniello L., Ortis M., D'Amelio S. (2001). Water quality as a determinant of the composition of fish parasite communities. *Hydrobiologia*. 452: 173-179.
- Grabner D., Rothe L.E., Sures B. (2023). Parasites and pollutants: effects of multiple stressors on aquatic organisms. *Environmental Toxicology and Chemistry*. 42: 1946-1959.
- Jerônimo G.T., Cruz M.G., Bertaglia E.D.A., Furtado W.E., Martins M.L. (2022). Fish parasites can reflect environmental quality in fish farms. *Reviews in Aquaculture*. 14: 1558-1571.
- Kennedy C. R. (1972). The effects of temperature and other factors upon the establishment and survival of *Pomphorhynchus laevis* (Acanthocephala) in goldfish, *Carassius auratus*. *Parasitology*. 65: 283-294.
- Kennedy C. R. (2006). *Ecology of the Acanthocephala*. Cambridge University Press.
- Kralj T., Žganec K., Čuk R., Valić D. (2022). Contribution of alien peracarid crustaceans to the biocontamination of benthic macroinvertebrate assemblages in Croatian large rivers. *Limnetica*. 41: 181-199.
- Lamková K., Šimková A., Palíková M., Jurajda P., Lojek A. (2007). Seasonal changes of immunocompetence and parasitism in chub (*Leuciscus cephalus*), a freshwater cyprinid fish. *Parasitology Research*. 101: 775-789.
- Lal H., and Kumar H. (2015). Influence of temperature effect on nematodes infection in freshwater fish (*Labeorohita*). *Int J Appl Sci Technol*. 6: 5-7.
- Marijić V.F., Smrzlić I.V., Raspor B. (2013). Effect of acanthocephalan infection on metal, total protein and metallothionein concentrations in European chub from a Sava River section with low metal contamination. *Science of the Total Environment*. 463: 772-780.
- Petrochenko V. I. (1956). *Acanthocephala of domestic and wild animals*. Vol. 1. Isdatel'stvo Akademii Nauk SSR, Moscow, Russia. Russian: English translation by Israel Program for Scientific Translations Ltd., Jerusalem, Israel.
- Piria M., Simonović P., Zanella D., Čaleta M., Šprem N., Paunović M., ...Tree, T. (2019). Long-term analysis of fish assemblage structure in the middle section of the Sava River–The impact of pollution, flood protection and dam construction. *Science of the Total Environment*. 651: 143-153.
- Poulin R. (2020). Meta-analysis of seasonal dynamics of parasite infections in aquatic ecosystems. *International Journal for Parasitology*. 50: 501-510.
- Redžović Z., Erk M., Gottstein S., Perić M.S., Dautović J., Fiket Ž,...Cindrić M. (2023). Metal bioaccumulation in stygophilous amphipod *Synurella ambulans* in the hyporheic zone: The influence of environmental factors. *Science of The Total Environment*. 866: 161350.
- Reier S., Sattmann H., Schwaha T., Harl J., Konecny R., Haring E. (2019). An integrative taxonomic approach to reveal the status of the genus *Pomphorhynchus* Monticelli, 1905 (Acanthocephala: *Pomphorhynchidae*) in Austria. *International Journal of Parasitology: Parasites and Wildlife*. 8: 145-155.
- Sures B., Tarachewski H., Jackwerth E. (1994). Lead accumulation in *Pomphorhynchus laevis* and its hosts. *Journal of Parasitology*. 80: 355-357.

- Sures B. (2006). How parasitism and pollution affect the physiological homeostasis of aquatic hosts. *Journal Helminthology*. 80: 151-157.
- Tamura K., Stecher G., Kumar S. (2021). MEGA 11: molecular evolutionary genetics analysis Version 11. *Molecular Biology and Evolution*.
- Taraschewski H. (2000). Host-parasite interactions in Acanthocephala: a morphological approach. *Advances in parasitology*. 46: 1-179.
- Tenny S., Hoffman M.R. (2023). Prevalence. In: StatPearls. Treasure Island (FL): StatPearls Publishing. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK430867/>.
- Vardić Smrzlić I., Valić D., Kapetanović D., Marijić V.F., Gjurčević E., Teskeredžić E. (2015). *Pomphorhynchus laevis* (Acanthocephala) from the Sava River basin: new insights into strain formation, mtDNA-like sequences and dynamics of infection. *Parasitology International*. 64: 243-250.
- Vardić Smrzlić I., Čolić B., Kapetanović D., Šariri S., Mijošek T., Filipović Marijić V. (2023). Phylogeny and genetic variability of Rotifer's closest relatives Acanthocephala: an example from Croatia. *Hydrobiologia*. 1-16.
- Žganec K., Gottstein S., Hudina S. (2009). Ponto-Caspian amphipods in Croatian large rivers. *Aquatic Invasions*. 4: 327-335.
- Yildiz Y., Kabackci N., Yarim M. (2004). Pathological changes of tench intestines infected with *Pomphorhynchus laevis*. *Revue de médecine vétérinaire*. 155: 71-73.

Usporedba tri različite metode za ocjenu mase i volumena rogovlja srnjaka (*Capreolus capreolus* L.)

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Sažetak

Cilj istraživanja je usporedba službene CIC formule i skraćenih postupaka s koeficijentima 0,23 i 0,25 za ocjenu mase i volumena rogovlja srnjaka. Ukupno je analizirano 45 rogovlja srnjaka. Prema koeficijentu 0,25 dobiven je statistički značajno najveći broj točaka za masu i volumen rogovlja svih vrijednosnih razreda, zbog čega je on opravdano i uklonjen iz službene upotrebe. Prema našim rezultatima upotreba koeficijenta 0,23 je neopravdana za nekaptalne trofeje neto mase manje od 270 g jer se njegovom upotrebom dobiva značajno veći broj točaka za masu i volumen rogovlja. Potrebno je napraviti analizu na većem broju trofeja srnjaka iz različitih staništa s pravilnim i nepravilnim rezom lubanje.

Ključne riječi: rogovlje, trofej, srna obična, masa rogovlja, volumen rogovlja

Povijest razvoja formule za ocjenjivanje trofeja srnjaka

Ulovom divljači između ostalog lovac stječe i određene dijelove tijela koje zovemo lovačkim trofejama. Lovačke su trofeje, u užem smislu te riječi, određeni dijelovi tijela ulovljene divljači koje se ocjenjuju i vrednuju te koji nam mogu poslužiti kao pokazatelj njihovog općeg stanja u lovištu te kvalitete staništa i provedenih gospodarskih mjera (Frković, 1997). Kod srne obične (*Capreolus capreolus* L.) trofej ima samo muško grlo i to u obliku rogovlja. Vrednovanje i ocjenjivanje trofeja stara je disciplina koja je postojala i prije uvođenja u upotrebu međunarodnih formula i uputa (Krapinec i sur., 2019).

S prvim lovačkim izložbama ukazala se i potreba za jedinstvenim formulama za ocjenjivanje trofeja. Lovačka izložba 1910. godine u Beču (Frković, 1989) potaknula je stvaranje jedinstvenih formula za ocjenjivanje trofeja. Jedinstvene međunarodne formule za ocjenjivanje lovačkih trofeja donesene su na plenarnom zasjedanju Međunarodnog vijeća za zaštitu divljači i prirode (CIC) 1937. godine u Pragu.

Trenutnu formulu za ocjenjivanje rogovlja srnjaka osmislio je Bieger 1927. godine (Reichelt, 1986), a ona predviđa mjerenje mase i volumena rogovlja odvojeno. Biegerova formula za ocjenjivanje rogovlja srnjaka prihvaćena je na zasjedanju CIC-a u Pragu 1937. godine i konačno u Madridu 1952. godine. Do današnjeg dana ona je doživjela samo manje izmjene.

U prošlosti je mjerenje volumena rogovlja srnjaka bilo dosta teško zbog nedostatka hidrostatske vage. Zbog toga su se i razvili skraćeni postupci ocjene mase i volumena rogovlja uz pomoć koeficijenata, koji su pojednostavnili postupak ocjene. Jedan od takvih koeficijenata je koeficijent Voltza 0,225, odnosno 0,23 (Bieger i Nüßlein, 1977) te koeficijent Raića 0,25 za trofeje do 130 CIC točaka (Raić, 1966). Ni u današnje vrijeme mjerenje volumena nije lak posao, a ni potpuno točan posao (Frković, 1997). Zbog oblika ruža kod starih srnjaka ili zbog nepravilnih oblika ruža, mjerenje volumena rogovlja je otežano, a vrijednosti ocjene razlikuju se prema različitim komisijama ocjenjivača.

Pravilnik o trofejima divljači (Anon, 2021) propisuje način ocjenjivanja trofeja divljači, oblik obrasca trofejnih lista, oblik obrasca ocjemenog lista i način vođenja evidencije o trofejima divljači. U Republici Hrvatskoj trofeji divljači ocjenjuju se prema važećim pravilnicima, formulama i uputama CIC-a. Prema navedenom Pravilniku, uz uvjet da su mjerljivi svi elementi, CIC-ovom formulom mora se ocijeniti i izdati trofejni list za trofej grla s navršene dvije i više godine starosti. Kod ocjene trofeja srnjaka za trofeje čija neto masa nije veća od 250 g dopušta se upotreba faktora 0,23 za masu i volumen rogovlja.

Cilj istraživanja je usporediti ocjene za masu i volumen rogovlja srnjaka prema službenoj metodi CIC-a i skraćenom postupku upotrebom koeficijenata 0,23 i 0,25.

Ocjena mase i volumena trofeja srnjaka

Analiza je provedeno na ukupno 45 trofeja srnjaka. Trofeje su stečene od 2000. do 2014. godine i to na području državnog otvorenog lovišta broj: IX/21 – „Vidovača“, državnog otvorenog lovišta broj: IX/23 – „Vrebac“ i državnog otvorenog lovišta broj: IX/38 – „Marković rudine“. Sva lovišta su brdskog tipa, a srna obična je jedna od glavnih vrsta krupne divljači. Za uzgoj srne obične lovišta su II. brdskog bonitetnog razreda s krupnim predatorima, s matičnim fondovima od 128 grla (IX/21 – „Vidovača“, površine 11781 ha), 240 grla (IX/23 – „Vrebac“, površine 12661 ha) i 180 grla (IX/38 – „Marković rudine“, površine 10734 ha). Cilj lovnog gospodarenja u predmetnim lovištima je uzgoj zdrave i stabilne populacije srne obične.

Sve trofeje ocijenila je ista komisija za ocjenjivanje trofeja divljači prema pravilima i uputama CIC-a, odnosno prema formuli za ocjenjivanje srne obične koristeći hidrostatsku vagu i mjereći odvojeno masu i volumen trofeje (Frković, 2017). Trofeje su imale cijelu lubanju. Nakon ocjenjivanja trofeje su svrstane u vrijednosne razrede kao I. vrijednosni razred (zlato, $\geq 130,00$ točaka), II. vrijednosni razred (srebro, od 115,00 do 129,99 točaka), III. vrijednosni razred (bronca, 105,00 do 114,99 točaka) i nekapitalne trofeje ($\leq 104,99$ točaka). Kapitalne trofeje su trofeje ukupne ocjene iznad 105,00 CIC bodova.

Usporedno smo za određivanje broja bodova za masu i volumen zajedno, svaku od trofeja dodatno ocijenili po skraćenom postupku, odnosno svakoj od trofeja dodatno je pomnožena neto masa u gramima s koeficijentom $Votza$ 0,23 (Raić, 1970; Frković, 1997) i koeficijentom Raića 0,25 (Raić, 1966). U bazu podataka unesene su vrijednosti bodova za masu i volumen zajedno prema službenoj formuli za ocjenjivanje (Frković, 2017) te prema skraćenom postupku na osnovu koeficijenata 0,23 i 0,25. Razlike u dobivenim bodovnim vrijednostima testirane su analizom varijance (ANOVA) ponovljenih mjerenja. Levenov test homogenosti varijance nije bio statistički značajan. Stvarni koeficijent je izračunat za svaku trofeju kao omjer zbroja CIC bodova za masu i težinu prema službenoj formuli CIC-a sa neto masom trofeje. Deskriptivna statistika i ANOVA ponovljenih mjerenja su napravljeni u statističkom programu Statistica 13 (TIBCO Software Inc. 2018).

Analiza trofeja srnjaka

Analizom je obuhvaćeno ukupno 45 trofeja srnjaka, od toga 6 trofeja u I. kategoriji, 6 trofeja u II. kategoriji, 8 trofeja u III. kategoriji i 25 nekapitalnih trofeja. Prosječna neto masa (sa oduzetih 90 g zbog cijele lubanje) svih analiziranih trofeja iznosila je 337,90 g, s rasponom od 123,0 g do 593,0 g. Prosječni volumen rogovlja iznosio je 136,20 cm³ i kretao se u rasponu od 31,0 cm³ do 286,0 cm³. Prosječna konačna službena ocjena svih trofeja bila je 97,75 CIC točaka. Minimalna ocjena bila je 33,95 CIC točaka, a maksimalna 164,48 CIC točaka.

U tablici 1 su prikazane prosječne vrijednosti neto mase i volumena rogovlja te konačne (ukupne) ocjene u CIC točkama po vrijednosnim razredima trofeja.

Tablica 1. Prosječne vrijednosti za masu i volumen rogovlja te konačnu CIC ocjenu

Vrijednosni razred	Neto masa (g)	Volumen (cm ³)	Ocjena (CIC točke)
I.	522,83	221,66	146,91
II.	394,41	171,16	119,08
III.	368,00	157,75	110,97
Nekapitalne trofeje	270,32	100,41	76,60

U tablici 2 prikazana je usporedba prosječnih točaka za masu i volumen rogovlja, kao i raspon vrijednosti točaka od minimalnih do maksimalnih prema vrijednosnim razredima trofeja. Za kapitalne trofeje (vrijednosni razredi I., II. i III.) prosječni broj točaka za masu i volumen rogovlja po skraćenju metodi koeficijenta 0,25 bili su statistički značajno veći ($p=0,0006$) u odnosu na službenu metodu CIC-a i skraćeni postupak koeficijenta 0,23 (tablica 2). Za nekapitalne trofeje utvrđene su statistički značajne razlike za sve tri metode određivanja broja točaka za masu i

volumen rogovlja ($p=0,0000$).

Tablica 2. Usporedba prosječnih točaka za masu i volumen te minimalni i maksimalni broj točaka za masu i volumen (a,b,c vrijednosti označene različitim slovom značajno se razlikuju, $p \leq 0,05$)

Vrijednosni razred	Metode za masu i volumen rogovlja		
	CIC-a	Koeficijent 0,23	Koeficijent 0,25
	Prosjek \pm Std. Dev.		
I.	118,78 \pm 14,67 ^a	120,25 \pm 11,06 ^a	130,70 \pm 12,02 ^b
II.	90,79 \pm 3,70 ^a	90,71 \pm 7,96 ^a	98,60 \pm 8,66 ^b
III.	84,12 \pm 2,91 ^a	84,64 \pm 4,73 ^a	92,00 \pm 5,14 ^b
Nekapitalne trofeje	56,66 \pm 16,89 ^a	62,17 \pm 14,97 ^b	67,58 \pm 16,27 ^c
Sve trofeje	74,38 \pm 26,15 ^a	77,71 \pm 23,72 ^b	84,47 \pm 25,78 ^c

Zbog razlika u populacijama divljači sve formule imaju određene slabosti (Krapinec i sur., 2019) pa tako i ova formula za ocjenjivanje rogovlja srnjaka, kao i metode skraćenih postupaka ocjene rogovlja. Najveća kritika za Biegerovu formulu je da favorizira masu i volumen trofeje u odnosu na neke druge elemente trofeje (Stubbe, 1967., Reichelt, 1986., Frković, 1997). Prema Frkoviću (1997) točke za masu čine preko 30% ukupnih točaka trofeje, a one za volumen 40%.

Tablica 3. Prosječne vrijednosti razlike broja točaka službene metode CIC-a i skraćenog postupka (koeficijenti 0,23 i 0,25) te izračunati stvarni koeficijenti

Vrijednosni razred	Razlika CIC – „0,23“	Razlika CIC – „0,25“	Stvarni koeficijent
I.	-1,47	-11,93	0,23
II.	0,08	-7,81	0,23
III.	-0,52	-7,88	0,23
Nekapitalni trofej	-5,51	-10,91	0,21

U tablici 3 prikazane su prosječne vrijednosti razlike broja točaka službene metode CIC-a i skraćenog postupka određivanja broja točaka za masu i volumen rogovlja (koeficijenti 0,23 i 0,25). Prema rezultatima istraživanja najmanje razlike u broju točaka bile su kod koeficijenta 0,23. Najveće razlike u broju točaka za masu i volumen rogovlja bile su za koeficijent 0,25. Najveće razlike su bile za trofeje u I. vrijednosnom razredu – zlato (11,93 boda) i nekapitalne trofeje (trofeje \leq 104,99 točaka CIC bodova) u iznosu od 10,91 bodova. Koeficijent 0,25 bio je upravo namijenjen za skraćeni postupak ocjene mase i volumena rogovlja kod I. vrijednosnog razreda, odnosno kod trofeja ukupne ocjena iznad 130,00 CIC točaka (Raić, 1966). Zbog tako velikih odstupanja u broju točaka za masu i volumen rogovlja, nakon Međunarodne lovačke izložbe u Novom Sadu 1967. godine odustalo se od upotrebe koeficijenta 0,25, dok je koeficijent 0,23 ostao u upotrebi (Raić, 1970).

Za kapitalne trofeje (I., II. i III. vrijednosni razred) stvarni izračunati koeficijent za skraćeni postupak određivanja broja točaka za masu i volumen rogovlja iznosio je 0,23, čime je i potvrđena upotreba ovog koeficijenta za neslužbene ocjene u skraćenom postupku. Međutim nije opravdana njegova upotreba u vrednovanju nekapitalnih trofeja, gdje je stvarni izračunati koeficijent iznosio 0,21.

Prema važećem Pravilniku o trofejima divljači (Anon, 2021) za trofeje čija je neto masa manja od 250 g dozvoljava se upotreba faktora 0,23 za skraćeni postupka ocjene mase i volumena rogovlja. U našem istraživanju nekapitalne trofeje imale su prosječnu masu od 270,32 g, a prema našim rezultatima istraživanja službeno dozvoljeni koeficijent 0,23 je prevelik. Neslužbeno se za trofeje čija je ukupna ocjena manja od 105,00 CIC točaka mogao primjenjivati skraćeni postupak Voltza s koeficijentom 0,23 (Frković, 1989).

Nedostaci koeficijenta 0,23 su da lakše trofeje dobivaju niže vrijednosti, a teže trofeje dobivaju veće vrijednosti ocjena (Krapinec i sur., 2019.). Prema našim rezultatima istraživanja ovaj koeficijent daje za većinu vrijednosnih kategorija trofeja veće vrijednosti točaka za masu i volumen rogovlja s izuzetkom II. (srebro) i III. (bronca)

vrijednosne kategorije, ali te razlike nisu bile statistički značajne ($p=0,6512$). Analizom lakših trofeja utvrdili smo da ovaj koeficijent daje veće vrijednosti za masu i volumen rogovlja, ali te vrijednosti nisu bile statistički značajne u odnosu na službenu metodu CIC-a. Potrebno je napraviti usporednu analizu većeg broja trofeja srnjaka iz različitih staništa, odnosno bonitetnih razreda lovišta, te vodeći brigu o pravilnom i nepravilnom rezu lubanje o čemu su pisali Abramović (2017) te Krapinec i sur. (2019).

Zaključak

Za kapitalne trofeje srnjaka (vrijednosni razredi I., II. i III.) utvrđena je statistički značajna razlika između prosječnih broja točaka za masu i volumen rogovlja prema tzv. Bieger-ovoj formuli odnosno službenoj metodi CIC-a te koeficijenta 0,23 u odnosu na koeficijent 0,25. Prema skraćenoj metodi pomoću koeficijenta 0,25 dobije se statistički značajno veći broj točaka za masu i volumen rogovlja. Koeficijent Voltza 0,23 moguće je neslužbeno primjenjivati za skraćeni postupak ocjene mase i volumena rogovlja kod kapitalnih trofeja srnjaka, budući da razlike između ovog skraćenog postupka i uputa CIC-a nisu statistički značajne. Ipak za ovakvu preporuku je potrebno provesti istraživanje na većem broju trofeja stečenih na različitim staništima.

Koeficijent 0,23 nije preporučljivo koristiti u skraćenom postupku ocjene za masu i volumen rogovlja kod nekapitalnih trofeja čija je ukupna ocjena manja od 105,00 CIC točaka. Prema našim rezultatima za ocjenu nekapitalnih trofeja (neto masa manja od 270 g) faktor 0,23 je velik i njegovom upotrebom dobiva se značajno veći broj točaka za masu i volumen rogovlja u odnosu na službenu metodu CIC-a upotrebom hidrostatske vage. Rezultati pokazuju da za nekapitalne trofeje čija je masa manja od 270 g, realni faktor iznosio je 0,21. Istraživanje bi svakako trebalo ponoviti na većem broju nekapitalnih trofeja da bi se mogli donijeti valjani zaključci o opravdanosti korištenja faktora 0,23.

Literatura

- Abramović R. (2017). Gubitak mase prilikom sušenja i utjecaj reza lubanje na bodovnu vrijednost trofeja srnjaka (*Capreolus capreolus* L.). Završni rad, Veleučilište u Karlovcu, Karlovac, str: 32.
- Anon (2021). Pravilnik o trofejima divljači. Narodne novine. 24/2021. Zagreb.
- Bieger W., and Nusslein F. (1977). Die Bewertung der europäischen Jagdtrophäen. Sechste Auflage. Verlag Paul Parey. 80. Hamburg und Berlin.
- Frković A. (1989). Lovačke trofeje, obrada, ocjenjivanje i vrednovanje. Lovački savez Hrvatske. str: 232. Zagreb.
- Frković A. (1997). 60 godina privremene CIC formule za ocjenjivanje rogova srnjaka. Šumarski list. 9-10: 533-537.
- Frković A. (2017). Priručnik za ocjenjivanje lovačkih trofeja. Hrvatski lovački savez. str: 177. Zagreb.
- Krapinec K., Nikolić M., Bujanić M., Konjević D. (2019). Considerations in the study of trophies. The effect of skull cutting on the real value of roe buck trophies. Šumarski list. 143: 203-213.
- Raić L. (1966). Izmjene u formulama za ocjenjivanje trofeja. Lovački vjesnik. 9: 255.
- Raić L. (1970). Izmjene u formuli za ocjenjivanje srnećeg roga. Lovački vjesnik. 3: 70.
- Reichelt H. (1986). Kritische Bemerkungen zur internationalen Bewertungsformel für Rehgehörne. Beiträge zur Jagd- und Wildforschung. 14: 105-112.
- Stubbe C. (1967). Variationsbreite, Variabilität und Beziehungen zwischen einigen Körper- und Gehornmassen beim Rehwild. Z. Jagdwiss. 13: 53-62.
- TIBCO Software Inc. 2018: Statistica (data analysis software system), version 13. <http://tibco.com>.

Comparison of three different methods for evaluating the mass and volume of roe deer antlers (*Capreolus capreolus* L.)

Abstract

The aim of the research is to compare the official CIC formula and abbreviated procedures with coefficients 0.23 and 0.25 for evaluating the mass and volume of roe deer antlers. A total of 45 roe deer antlers were analysed. According to the coefficient of 0.25, the statistically significantly highest number of points was obtained for the mass and volume of antlers of all value classes, which is why it was justifiably removed from official use. According to our results, the use of a coefficient of 0.23 is unjustified for non-capital trophies with a net mass of less than 270 g, as it gives a significantly higher number of points for the mass and volume of antlers. It is necessary to make an analysis on a larger number of roe deer trophies from different habitats with regular and irregular skull cuts.

Keywords: antlers, trophy, roe deer, antler mass, antler volume

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Zbornik radova
Animalne znanosti

Proizvodni i klaonički pokazatelji tovljenika crne slavonske svinje držanih na otvorenom i na dubokoj stelji

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Sažetak

Istraživanje je provedeno na 60 životinja crne slavonske pasmine svinja uzgajanih u na otvorenome (n=30) i na dubokoj stelji (n=30). Nakon postizanja završnih težina u tovu te starosti od 18 mjeseci svinje su zaklane u komercijalnoj klaonici te su im određena svojstva polovica i parametri kakvoće mesa. Svinje držane na dubokoj stelji imale su veće prosječne dnevne priraste, veću završnu masu, deblju leđnu slaninu, tanji leđni mišić te niže pH vrijednosti, niže vrijednosti boje mesakao i veće otpuštanje mesnog soka u odnosu na svinje držane na otvorenom. Temeljem dobivenih rezultata zaključuje se da držanje na dubokoj stelji nije pogodan način tova crne slavonske svinje zbog lošijih proizvodnih i kvalitativno-tehnoloških svojstava svinja.

Ključne riječi: crna slavonska svinja, uzgoj na otvorenom, duboka stelja, klaonička svojstva, kakvoća mesa

Uvod

Crna slavonska pasmina svinja autohtona je hrvatska pasmina, nastala krajem 19. stoljeća na području današnje istočne Hrvatske. Odlikuje se visokom otpornošću na bolesti i uvjete držanja, kao i vrlo dobrom kvalitetom mišićnog i masnog tkiva (Senčić i Samac, 2017). Zbog svojih morfoloških i fizioloških svojstava, pogodna je za uzgoj u ekstenzivnim ili poluintenzivnim sustavima (Margeta i sur., 2013). Dominantan način njenog uzgoja je držanje na otvorenome gdje se hrani smjesom žitarica uz mogućnost ispaše ili dodatak voluminozne svježe hrane. Posljednja dva desetljeća došlo je do značajnog porasta interesa uzgajivača za držanjem crne slavonske svinje, što je rezultiralo i povećanjem brojnog stanja svinja ove pasmine (Margeta i sur., 2016). Zbog ograničenja koja se pojavljuju prilikom uzgoja crne slavonske svinje na otvorenome (nedostatak adekvatnih površina, biosigurnosni rizici) pokušavaju se pronaći alternativni sustavi držanja ove pasmine koji bi omogućili proizvođačima jeftiniji i jednostavniji uzgoj, a bez negativnih utjecaja na njena proizvodna i klaonička svojstva. Jedan od takvih sustava je i držanje na dubokoj stelji koji se u prošlosti prakticirao prilikom tova crnih slavonskih svinja (Vukina, 1961). Tov svinja na dubokoj stelji jedan je od načina držanja koji sve više dobiva na značaju, međutim, oprečnost rezultata istraživanja u pogledu proizvodnih svojstava ukazuju kako je potrebno uložiti više napora kako bi se optimizirao ovakav način tova. Prema brojnim autorima, svinje držane u tovu na dubokoj stelji imale su u završnoj fazi tova veću konzumaciju hrane, manji utrošak hrane za kg prirasta, te veći prirast i povoljnije iskorištenje hrane (Lambooi i sur., 2004).

Prednosti objekata za držanje svinja na dubokoj stelji su njihova manja cijena izgradnje (čak i do 40%) u odnosu na klasična tovišta jer se ne ugrađuje skupa oprema, rešetke, odvodni kanali ili izrađuju lagune. Nadalje, ističe se povoljan učinak na dobrobit i zdravlje svinja zbog približavanja prirodnim uvjetima i omogućavanje rovanja (Perez-Hernandez i sur., 2022). Usprkos tome, objekti za tov svinja na dubokoj stelji još uvijek nisu u značajnijoj mjeri zastupljeni u Republici Hrvatskoj iako su dostupne dovoljne količine stelje koja se proizvede na oranicama istočne Hrvatske. Za očekivati je u budućnosti povećanje broja ovakvih objekata, jersu pogodni za tov svinja na obiteljskim gospodarstvima. Pored toga, normativi i direktive Europske Unije u resoru svinjogojstva (Perez-Hernandez i sur., 2022) stavljaju naglasak na dobrobit i zdravlje životinja, upotrebu stelje ismanjenu proizvodnju i aplikaciju gnojovke što je poznato kao nitratna direktiva.

Cilj istraživanja bio je istražiti utjecaj načina držanja (ekstenzivni i intenzivni) na proizvodna svojstva i svojstva polovica te parametre kakvoće mesa tovljenika crnih slavonskih svinja.

Materijal i metode

Istraživanje je provedeno na 60 svinja crne slavonske pasmine, podijeljenih u dvije skupine po 30 jedinki oba spola, držanih u dva različita proizvodna sustava. Prva skupina držana je na otvorenome cijelo trajanje tova, a druga skupina držana je na dubokoj stelji u zatvorenom objektu. Površina po jednom tovljeniku iznosila je 15 m² za svinje držane na otvorenome, dok je svinjama držanima u zatvorenom prostoru omogućeno 2 m² poda sa steljom po tovljeniku. Svinje su ušle u tov s prosječnom dobi od 90 dana, a tov je trajao ukupno 450 dana do ciljane starosti svinja od 18 mjeseci. Cijelo razdoblje tova svinje su dobivale obrok od smjese žitarica koji je sadržavao 13% sirovih bjelančevina i 12,7 MJ/ME, u količini od 2 kg/dan. Tijekom tova provedena su kontrolna vaganja pokusnih životinja svaka tri mjeseca, kao i mjerenje utroška hrane. Nakon završetka tovnog razdoblja, svinje su transportirane u komercijalnu klaonicu gdje su omamljene s CO₂, usmrćene i klaonički obrađene. Na liniji klanja utvrđena su svojstva polovica: debljina leđnog masnog tkiva (debljina slanine u mm izmjerene zajedno s kožom na križima na najtanjemu mjestu, tj. gdje *musculus glutaeus* najviše zalazi u slaninu) i debljina mišića (debljina slabinskoga mišića u mm, izmjerenoga kao najkraća veza između prednjega (kranijalnog) završetka *musculus glutaeus medius* i gornjeg (dorzalnog) ruba kralježničkoga kanala. Zatim, dužina trupa „a“ (od os pubisa do atlasa) i dužina trupa „b“ (od os pubisa do 1. rebra) te dužina buta od prednjeg ruba *simfize pubis* do skočnog zgloba), zajedno s opsegom buta. Od parametara kakvoće mesa utvrđene su pH vrijednosti u *musculus semimembranosus* (MS) i *musculus longissimus lumborum* (LL), pomoću uređaja Mettler MP 120-B (Mettler-Toledo, Schwerzenbach, Švicarska). Krajnje pH vrijednosti (pH₂₄) bile su određene 24 h post mortem na istim mjestima kao pH₄₅. Boja mesa (CIE L*, a* i b*, CIE, 2007.) određena je pomoću kolorimetra Minolta CR-410 (Konica Minolta Sensing Ltd, Singapur, kalibriranom prema a keramičkoj bijeloj ploči (Y = 84,9; x = 0,32 i y = 0,3381), s izvorom svjetlosti D65 i standardnim dvostupanjskim promatračem. Boja je određena na presjeku mišića. Otpuštanje mesnog soka izmjereno je prema EZ drip metodi (Christensen, 2003)

Opisna statistika za svojstva polovica te parametre kakvoće mesa, uključujući utvrđivanje značajnosti utjecaja sustav držanja, obrađeni su pomoću *psych* i *multcomp* paketa (Revelle, 2017; Hothorn i sur., 2016) dok je homogenost varijance testirana Levenovim testom pomoću *car* paketa (Fox i sur., 2014) u programskom okruženju R (R Development Core Team, 2020.). Značajnost razlika između skupina testirana je t-testom.

Rezultati i rasprava

Proizvodni pokazatelji

Proizvodni rezultati svinja u tovu u dva različita sustava držanja prikazani su u tablici 1. Svinje držane na dubokoj stelji imale su statistički visoko značajno (p<0,001) veću završnu tjelesnu masu i ukupne priraste u odnosu na svinje držane na otvorenome. Dobiveni rezultati u skladu su s rezultatima Lebret (2008) te Margete i sur. (2017) o proizvodnim pokazateljima svinja držanih u različitim proizvodnim sustavima gdje autori ukazuju da se kretanjem svinja u otvorenom sustavu značajan dio energije iz obroka troši na uzdržne potrebe, što rezultira značajno manjim prosječnim dnevnim i ukupnim prirastima tjelesne mase. Svinje držane na dubokoj stelji provode znatno više vremena u odmoru i ležanju u odnosu na svinje držane na otvorenome zbog čega se većina energije iz obroka iskoristi za porirast tjelesne mase (Perez-Fernandez i sur., 2022). Zbog tih razloga, svinje držane na otvorenome imale su visoko statistički značajno (p<0,001) manje prosječne dnevne priraste i, na istoj razini statističke značajnosti, veći utrošak hrane za kg prirasta u odnosu na svinje držane na dubokoj stelji.

Tablica 1. Opisna statistika za svojstva kakvoće mesa istraživanih skupina svinja u različitim sustavima držanja

Svojstva	Sustav držanja						p
	Uzgoj na otvorenom			Duboka stelja			
	Mean	SD	SE	Mean	SD	SE	
Početna težina prasadi, kg	28,31	9,31	3,15	28,15	4,65	2,38	n.s.
Završna težina tovljenika, kg	143,28	9,87	3,45	223,41	11,64	4,27	***
Ukupni prirast, kg	114,97	6,32	2,54	195,26	7,71	3,86	***
Prosječni dnevni prirast, kg	0,255	0,02	0,16	0,434	0,26	0,54	***
Utrošak hrane za kg prirasta, kg	7,83	1,02	0,34	4,61	0,93	0,31	***

*** $p < 0,001$, n.s. – nema značajnosti

Klaonička svojstva

Klaonička svojstva svinjskih trupova te mišićnog i masnog tkiva istraživanih skupina svinja prikazana su u tablici 2. Svinje držane na otvorenome imale su statistički visoko značajno ($p < 0,001$) manju debljinu lednog masnog tkiva i veću debljinu mišića u odnosu na svinje držane na dubokoj stelji. Posljedično tome, mesnatost tovljenika držanih na otvorenome bila je statistički visoko značajno ($p < 0,001$) veća od mesnatosti tovljenika držanih na dubokoj stelji. Što se tiče dužine polovica, svinje držane na dubokoj stelji imale su statistički značajno ($p < 0,05$) duže polovice u odnosu na svinje iz otvorenog sustava. Dužina buta nije se statistički značajno razlikovala između ispitivanih skupina svinja, no opseg buta bio je statistički značajno veći ($p < 0,05$) kod svinja držanih na dubokoj stelji. U pogledu kvalitativnih pokazatelja mišićnog tkiva nisu utvrđene statistički značajne razlike između ispitivanih skupina svinja u pogledu pH vrijednosti 45 minuta nakon klanja niti u *musculus semimembranosus* (MS) niti u *musculus longissimus lumborum* (LL). Međutim, pH vrijednost 24 sata nakon klanja bila je statistički značajno veća ($p < 0,05$) u mišićju svinja držanih na otvorenom u odnosu na pH vrijednost mišića svinja držanih na dubokoj stelji. Vrijednost svjetline mišićja (CIE L*) bila je statistički značajno manja ($p < 0,01$) odnosno boja mišića je bila tamnija kod svinja držanih na otvorenome u odnosu na svinje iz zatvorenog sustava. Za ostale vrijednosti boje (crvenilo CIE a* i žutilo CIE b*) nisu utvrđene statistički značajne razlike između ispitivanih skupina svinja. Vrijednosti otpuštanja mesnog soka u *musculus longissimus lumborum* 24 sata nakon klanja bile su statistički značajno ($p < 0,01$) manje, a 48 sati nakon klanja statistički visoko značajno ($p < 0,001$) manje kod skupine svinja držanih na otvorenome u odnosu na skupinu svinja držanih na dubokoj stelji.

Tablica 2. Opisna statistika za klaonička svojstva istraživanih skupina svinja u različitim sustavima držanja

Svojstva	Sustav držanja						p
	Uzgoj na otvorenom			Duboka stelja			
	Mean	SD	SE	Mean	SD	SE	
Debljina ledne masnoće, mm	21,47	5,17	2,47	61,24	4,36	3,51	***
Debljina mišića, mm	58,63	8,24	1,61	39,86	6,48	1,12	***
Dužina polovica "a", cm	85,96	4,17	0,83	91,52	5,01	0,76	*
Udio mišićnog tkiva u trupovima, %	40,11	3,83	1,12	29,31	3,51	2,07	***
Dužina polovica "b", cm	102,98	4,86	0,99	107,64	6,13	0,93	*
Dužina buta, cm	35,89	3,21	0,64	37,36	2,83	0,61	n.s.
Opseg buta, cm	63,56	4,86	0,89	68,31	5,01	0,90	*
pH ₄₅ , MS	6,54	0,15	0,03	6,49	0,19	0,03	n.s.
pH ₄₅ , LL	6,41	0,19	0,03	6,24	0,17	0,03	n.s.
pH ₂₄ , MS	5,73	0,10	0,03	5,60	0,10	0,02	*
pH ₂₄ , LL	5,79	0,16	0,03	5,65	0,11	0,03	*
CIE L*	47,36	9,42	2,11	54,91	3,15	0,67	**

CIE a*	13,69	2,64	0,21	14,56	1,12	0,25	n.s.
CIE b*	5,24	1,16	0,22	5,91	0,98	0,14	n.s.
EZ ₂₄ drip, LL, %	1,74	0,17	0,09	2,68	0,14	0,07	**
EZ ₄₈ drip, LL, %	5,43	1,22	0,13	8,19	1,54	0,10	***

*** $p < 0,001$, n.s. – nema značajnosti MS - *musculus semimembranosus*, LL - *musculus longissimus lumborum*

Navedeni rezultati ukazuju na nepovoljan učinak držanja crnih slavonskih svinja u zatvorenom sustavu na dubokoj stelji na klaoničke pokazatelje trupova i mišićnog te masnog tkiva. Pretjerana zamašćenost trupa kod svinja držanih na dubokoj stelji posljedica je genetske predispozicije ove pasmine za većom zamašćenošću trupa (Samac i sur., 2015, Margeta i sur., 2016, Senčić, 2017). Uz nedostatak kretanja dolazi do povećanog taloženja masnog tkiva što rezultira manjim udjelom mišićnog tkiva, a to za posljedicu ima i slabiju preradbenu vrijednost crne slavonske svinje (Slika 1.).



Slika 1. Izgled presjeka trupa crnih slavonskih svinja držanih na otvorenom (lijevo) i na dubokoj stelji (desno)
(Izvor: Margeta, V.)

pH vrijednost mišićja u izravnoj je povezanosti s načinom držanja svinja (Kidega i sur., 2021). U slučaju crne slavonske svinje vrlo rijetko dolazi do pojave niskih pH vrijednosti i one su uglavnom u vrijednostima koje ukazuju na vrlo dobra kvalitativna svojstva mišićja (Kušec i sur., 2019). Rezultati našeg istraživanja na tragu su ovih zaključaka s tim da su bolji rezultati (veće pH vrijednosti 24 sata nakon klanja) zabilježeni kod svinja iz otvorenog sustava što se može tumačiti kao posljedica kretanja i manjeg utjecaja stresora pri držanju svinja na otvorenom. Na slične zaključke upućuju Baković i sur. (2016). Vrlo veliki utjecaj načina držanja na tehnološke pokazatelje mišićnog tkiva utvrđen je za boju mesa i otpuštanje mesnog soka. Svinje držane na otvorenom imaju tamniju boju mesa zbog kretanja i, posljedično tome, bolje prokrvljenosti mišićja (Vieira i sur., 2021). Također, kretanje svinja utječe na čvrstoću i gustoću mišićnih vlakana što rezultira manjim otpuštanjem mesnog soka koji se nalazi u mišićnom tkivu u intramolekularnom prostoru između miofibrilarnih bjelančevina topljivih u soli – miozina i aktina (Brewer i sur., 2001). Kušec i sur. (2019) ukazuju na značajno manji postotak otpuštanja mesnog soka kod crnih slavonskih svinja držanih na otvorenom u odnosu na svinje iz konvencionalnih proizvodnih sustava. Iz svega navedenog možemo zaključiti da držanje crnih slavonskih svinja u zatvorenom sustavu na dubokoj stelji, osim ostvarivanja većih prosječnih dnevnih prirasta i manjeg utroška hrane za kg prirasta, rezultira nepovoljnim pokazateljima kakvoće zaklanih svinjskih trupova kao i kvalitativnim i tehnološkim pokazateljima mišićnog i masnog tkiva. Sve navedeno utječe na smanjenu preradbenu vrijednost tovljenika crne slavonske svinje te se ovakav način držanja ne preporuča za tov crnih slavonskih svinja.

Zaključak

Usporedbom proizvodnih i klaoničkih pokazatelja utvrđeno je da tovljenici crne slavonske svinje držani na dubokoj stelji imaju značajno veće ukupne i prosječne dnevne priraste i značajno veću tjelesnu masu na kraju tova u odnosu na tovljenike držane na otvorenom. Međutim, debljina leđnog masnog tkiva mišića, kao i prosječna mesnatost trupova svinja držanih na dubokoj stelji u znatno su nepovoljnijem odnosu prema tovljenicima držanim na otvorenome. Pored toga, izražena zamašćenost trupa, svjetlija boja mesa te veće otpuštanje mesnog soka ukazuju da držanje tovnih svinja crne slavonske pasmine na dubokoj stelji nije zadovoljavajuća alternativa klasičnom načinu držanja na otvorenome ili u sustavima s ispustima. režim hranidbe za crne slavonske svinje u sustavu s dubokom steljom trebao bi biti drugačiji u usporedbi sa svinjama u otvorenom sustavu. Također, potrebno je više od 2 kvadratna metra smještajnog prostora po životinji.

Literatura

- Baković M., Gvozdanić K., Galović D., Radišić Ž., Margeta V. (2016). Analysis of carcass traits of Black Slavonian fattening pigs under extensive keeping conditions. *Krmiva*. 58: 3-8.
- Brewer M.S., Zhu I.G., Bidner B.D., Meisinger J., Mc Keith F.K. (2001). Measuring pork color: Effects of bloom time, muscle, pH and relationship to instrumental parameters. *Meat Science*. 57: 164-176.
- Christensen, L. B. (2003). Drip loss sampling in porcine m. longissimus dorsi. *Meat Science*. 63: 469-477.
- CIE (2007). CIE colorimetry – part 4: 1976.
- Fox J., Weisberg S., Adler D., Bates D., Baud-Bovy G., Ellison S., Heiberger R. (2014). Package ‘car’. Version 2.0-21.
- Hothorn T., Bretz F., Westfall P., Heiberger R.M., Schuetzenmeister A., Scheibe S., Hothorn M.T. (2016). Package ‘multcomp’. Simultaneous inference in general parametric models. Project for Statistical Computing, Vienna, Austria.
- Kidega K., Aliro T., Mugonola B., Ndyomugenyi E.K., Okello-Uma I. (2021). Effect of different floor types on growth performance of pigs and carcass back-fat thickness. *London Journal of Research in Science: Natural and Formal*. 21: 47-54.
- Kušec G., Gvozdanić K., Djurkin Kušec I., Margeta V., Galović D., Radišić Ž. (2019). Changes in carcass composition of Black Slavonian pigs and their crossbreds with Duroc during growth. *Journal of Central European Agriculture*. 20: 43-49.
- Lebret B. (2008). Effects of feeding and rearing systems on growth, carcass composition and meat quality in pigs. *Animal*. 2: 1548-1558.
- Margeta V. (2013). Perspektive uzgoja crne slavonske svinje u Hrvatskoj u kontekstu pristupanja Europskoj uniji. 48. *Hrvatski I*. 8: 17-22.
- Margeta V., Gvozdanić K., Galović D., Grčević M., Margeta P., Radišić Ž. (2016). Proizvodna i klaonička svojstva crne slavonske svinje u tovu do visokih završnih tjelesnih težina // Zbornik sažetaka *Krmiva 2016* // Zagreb: Krmiva. 2016. str. 67-68.
- Margeta V., Gvozdanić K., Kušec I.D., Margeta P., Kušec G., Radišić Ž. (2017). The effect of the acorn in feeding on the production and slaughter traits of crna slavonska pig. In 11th International Symposium Modern Trends in Livestock Production. 11.-13.10.2017., Beograd, Srbija. pp: 327-334.
- Pérez-Hernández P., Solís-Tejeda M.A., Lango-Reynoso F., Díaz-Rivera P., Aguilar-Ávila J., Asiain-Hoyos A. (2022). Deep litter pig production system as a sustainable alternative for small farmers. *Agrociencia*.
- Revelle W.R. (2017). Procedures for personality and psychological research.
- Samac D., Senčić Đ., Antunović Z., Steiner Z., Novoselec J., Klarić I., Bugarić E. (2015). Utjecaj završne tjelesne mase crnih slavonskih svinja na fizikalno-kemijska i senzorna svojstva kulena.

- Krmiva: Časopis o hranidbi životinja, proizvodnji i tehnologiji krme. 57: 17-22.
- Senčić Đ., and Samac D. (2017). Očuvanje biorazličitosti crnih slavonskih svinja kroz proizvodnju i vrednovanje tradicionalnih mesnih proizvoda. Poljoprivreda. 23 (2).
- Vieira C., Sarmiento-García A., García J.J., Rubio B., Martínez,B. (2021). Quality and Shelf Life of Fresh Meat from Iberian Pigs as Affected by a New Form of Presentation of Oleic Acid and an Organic-Acid Mix in the Diet. Foods. 10: 985.
- Vukina R. (1961). Praktično svinjogojstvo. Znanje, Zagreb.

Production and slaughter indicators of fattening black Slavonian pigs kept outdoors and on deep litter

Abstract

The research was conducted on 60 animals of the black Slavonian breed of pigs raised outdoor open (n=30) and on deep litter (n=30). After reaching the final weight in fattening and at the age of 18 months, the pigs were slaughtered in a commercial slaughterhouse and their carcass traits and meat quality traits were measured. Pigs kept on deep litter had higher average daily gains, higher final weight, thicker back fat, thinner back muscle and worse qualitative and technological indicators of muscle tissue (pH value, color, drip loss) compared to pigs kept in free range. Keeping them on deep litter is not a suitable way of fattening for Black Slavonian pigs.

Keywords: black Slavonian pig, outdoor keeping, deep bedding, carcass traits, meat quality

Koncentracija Fe, Zn i Cu u krvi alpina koza u ranoj laktaciji

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Sažetak

Cilj je ovoga rada utvrditi koncentraciju Fe, Zn i Cu u krvi alpina koza u ranoj laktaciji te ih usporediti s rezultatima dostupnih istraživanja. Istraživanje je provedeno na 14 koza u ranom stadiju laktacije, prosječne dobi 5 godine, u četvrtoj laktaciji. Utvrđene prosječne koncentracije Fe, Zn i Cu (1,87; 0,68; 1,14 mg L⁻¹) u krvi alpina koza nisu značajnije odstupale uspoređujući ih s dosadašnjim istraživanjima, osim utvrđenih nešto nižih koncentracija Zn. Niža koncentracija Zn u krvi koza može biti povezana s njegovim povećanim izlučivanjem mlijekom u ranom stadiju laktacije. Nije utvrđena značajna korelacija između mikroelemenata u krvi alpina koza u ranom stadiju laktacije.

Ključne riječi: alpina koza, Fe, Zn, Cu, krv, laktacija

Uvod

Značajniji uvoz alpina koza u Republiku Hrvatsku započeo je osamdesetih godina prošloga stoljeća i to na područje sjeverozapadne Hrvatske, gdje se i danas uzgajaju u najvećem broju (Antunović i sur., 2012). Danas se u RH prema podacima HAPIH-a (2023) uzgaja 4.427 uzgojno valjanih alpina koza (3.145 koza, 883 jarice i 129 jarčeva) kod 48 uzgajivača, s prosječnom veličinom stada od 92 grla. Od ukupne populacije uzgojno valjanih koza u RH alpina koza sudjeluje s 54,1 %. Brojne su funkcije koje u organizmu imaju Fe, Zn i Cu. Za željezo se može reći da je značajno za razvitak stanica, vezanje i transport kisika te prijenos elektrona, a u slučaju njegovog duljeg nedostatka dolazi do poremetnji imunološkog sustava (Oppenheimer, 2001; Maggini i sur., 2007). Cink je sastavni dio brojnih staničnih bjelančevina, ali je između ostaloga važan, i u ekspresiji gena (Song i sur., 2021), dok je bakar enzimatski i proteinski kofaktor sa značajnom ulogom u očuvanju imunostnog sustava (Wu i sur., 2020). Osim toga, Zn i Cu značajni su za reprodukciju i plodnost koza i jarčeva (Mayasula i sur., 2021). Kod koza se nedostatak mikroelemenata ispoljava subklinički odnosno teško se uočava, a značajno može utjecati na plodnost, pojavu pobačaja, rast i razvoj, pojačanu osjetljivost na bakterijske i parazitarne infekcije i dr. (Vázquez-Armijo i sur., 2011). Utvrđivanjem koncentracija mikroelemenata u krvi može se dobiti kvalitetna procjena opskrbljenosti životinja hranom (Herdt i Hoff, 2011). Međutim, King (1990) ističe da koncentracija Zn u krvi ne predstavlja njegovo pravo stanje u organizmu. Budući da je laktacija, osobito rani stadij, izrazito stresno razdoblje za životinju, potrebno je poznavati njihove hranidbene potrebe kao i metabolizam hranjivih tvari. U cilju zadovoljavanja potreba koza za Fe, Zn i Cu kroz hranu, osim njihove zastupljenosti u obrocima, pozornost treba usmjeriti i na njihovo različito sudjelovanje u brojnim metaboličkim procesima u organizmu koza (Antunović i sur., 2017; Derar i sur., 2022). Relativno je malo dostupnih podataka o vrijednostima Fe, Zn i Cu u krvi alpina koza izuzev onih koji su za cilj imali utvrditi samo koncentracije Fe (Antunović i sur., 2009; 2017). Stoga bi ovi rezultati doprinijeli kvalitetnijem poznavanju njihove koncentracije, osobito u ranom stadiju laktacije. Cilj je ovoga rada utvrditi koncentraciju Fe, Zn i Cu u krvi alpina koze u ranom stadiju laktacije te ih kritički usporediti s rezultatima dostupnih istraživanja.

Materijal i metode

Istraživanje je provedeno na 14 koza pasmine francuska alpine na obiteljskom gospodarstvu Đurković u Osječko-baranjskoj županiji. Koze su bile prosječne dobi 5 godina u četvrtoj laktaciji u ranom stadiju laktacije (40. dan ± 5 dana). Od ukupno 50 koza odabrano je 14 koza koje su bile zdrave i u dobroj tjelesnoj kondiciji. Koze su hranjene

krmnom smjesom sa 16 % sirovih bjelančevina u količini 1,50 kg/dan te sijenom lucerne kojega su uzimale po volji. Voda im je bila također ponuđena ad libitum. Uzorkovanje krvi provedeno je od svih koza uključenih u istraživanje (n=14) istovremeno tijekom jutarnje mužnje u sterilne vacuum epruvete Venoject® (Sterile Terumo Europe, Leuven, Belgium) iz jugularne vene (10 ml). Po transportu u laboratorij uzorci krvi su centrifugirani na 3000 okretaja/min pri čemu je izdvojen serum koji je potom zamrznut na -80° C. U odmrznutim uzorcima krvnogseruma utvrđene su koncentracije Fe, Zn i Cu koje su izražene mg L⁻¹. Uzorci hrane i krvi su razoreni sa 10 ml smjese 5:1 HNO₃ i H₂O₂ na 180°C tijekom 60 min u mikrovalnoj pećnici (CEM Mars 6). Koncentracije Fe, Zn i Cu u krvnom serumu, hrani i vodi koza utvrđene su hidridnom tehnikom na ICP (PerkinElmer Optima 2100 DV). Svi uzorci su analizirani u duplikatu. Koncentracija Fe, Zn i Cu u hrani i vodi koje su koze konzumirale prikazane su u tablici 1 i izražene su u mg kg⁻¹ suhe tvari.

Tablica 1. Koncentracija Fe, Zn i Cu u hrani i vodi alpina koza

Element, mg kg ⁻¹ ST	Vrsta hrane		Voda
	Krmna smjesa	Sijeno	
Fe	162,00	445,7	0,000003
Zn	61,73	20,99	0,03
Cu	9,30	5,62	0,03

ST- suha tvar

Dobiveni podaci o koncentraciji Fe, Zn i Cu u krvi alpina koza analizirani su deskriptivnom statistikom u programu SAS (9.4), procedurom MEANS te su izraženi kao srednja vrijednost, standardna devijacija (SD) te minimalna i maksimalna vrijednost (MIN i MAX). Povezanost između varijabli utvrđena je pomoću Pearsonovog koeficijenta.

Rezultati i rasprava

U tablici 2 prikazana je opisna statistika za koncentraciju Fe, Zn i Cu u krvi alpina koza u ranoj laktaciji.

Tablica 2. Opisna statistika koncentracija Fe, Zn i Cu u krvi alpina koza u ranoj laktaciji

Element, mg L ⁻¹	Srednja vrijednost	SD	MIN	MAX
Fe	1,87	0,93	0,99	4,65
Zn	0,68	0,11	0,54	0,96
Cu	1,14	0,45	0,41	2,30

SD- standardna devijacija, MIN- minimalna vrijednost, MAX-maksimalna vrijednost

Prosječna koncentracija Fe, Zn i Cu u krvi alpina koza je bila 1,87, 0,68 i 1,14 mg L⁻¹. Utvrđena je velika varijabilnost navedenih mikroelemenata u krvi, osobito Fe (0,99 do 4,65 mg L⁻¹). Do sličnih rezultata i njihovih varijacija u krvi lakon ovce u ranom stadiju laktacije (60. dan) došli su Antunović i sur. (2023). Prema NRC (2007) hranidbene potrebe koza u laktaciji za Fe su 35 mg kg⁻¹ hrane, a za Cu do 15 mg kg⁻¹ hrane. Underwood i Suttle (2001) iznose da su marginalne razine koncentracije Zn u krvnom serumu ovaca 0,4-0,6 mg L⁻¹, dok su normalne razine 0,8-1,2 mg L⁻¹ što ukazuje na manji nedostatak ovoga mikroelementa utvrđenog u predmetnom istraživanju. Naime, prema istim autorima hranidbene potrebe ovaca u laktaciji za Zn su 11,6-17,9 mg kg⁻¹ suhe tvari hrane, dok su te potrebe koze prema AFRC (1997) 50 mg kg⁻¹ suhe tvari hrane. Budući da je u obroku (krmna smjesa i sijeno) koza prosječno utvrđena količina Zn iznosila 81,72 mg kg⁻¹ suhe tvari hrane može se konstatirati da je opskrbljenost koza ovim mikroelementom bila dostatna (Tablica 1). Razlog nešto niže koncentracije Zn u serumu može biti pojačano izlučivanje ovoga mikroelementa mlijekom s obzirom da je istraživanje provedeno u ranom stadiju laktacije kada je i proizvodnja mlijeka najveća. Naime, Pehova i sur. (1999) su istaknuli da se značajne koncentracije Zn izlučuju kozjim mlijekom (3-8 mg L⁻¹), dok su Salama i sur. (2003) utvrdili da je to prosječna koncentracija od 3,7 mg L⁻¹ Zn. Kako ističu brojna istraživanja veliki je broj čimbenika koji utječu na koncentraciju mikroelemenata u organizmu životinja (McDowell, 2003; Haenlein i Anke, 2011; Vázquez-Armijo i sur., 2011; Silva i sur., 2018; Arthington i

Ranches, 2021). Tako, Lopes-Alonso i sur. (2012) kao značajne izvore varijabilnosti koncentracije mikroelemenata u krvi navode fiziološki stadij životinje, zatim razinu stresa, metode utvrđivanja, razinu i izvor mikroelemenata u hrani kao i njihove antagonisti i dr. Do sličnih rezultata za koncentracije Fe u krvi mliječnih koza ($1886,5 \pm 416,3 \mu\text{g L}^{-1}$) u istraživanjima provedenim u Austriji došli su Schweinzer i sur. (2017), te nešto nižim koncentracijama Cu i Zn u krvi ($0,8 \pm 0,2 \text{ mg L}^{-1}$ i $587,4 \pm 236 \mu\text{g L}^{-1}$). Slične koncentracije Cu i niže Fe ($0,83\text{-}1,08 \text{ mg L}^{-1}$ i $1284\text{-}1396 \mu\text{g L}^{-1}$) u krvi koza utvrdio je Baumgartner (2009.). Niže koncentracije Cu i Fe ($0,548 \pm 0,094$, $1,548 \pm 0,173 \text{ ppm}$) te više Zn ($0,864 \pm 0,211 \text{ ppm}$) u pokrajini Kašmir u Indiji u krvnom serumu koza su utvrdili Yattoo i sur. (2013). Slične koncentracije Zn u krvnoj plazmi u koza u Češkoj tijekom laktacije utvrdili su Pechova i sur. (1999).

Razlog nešto nižih vrijednosti Zn u krvi može se potražiti u činjenici da je rana laktacija koza razdoblje značajnog stresa za koze kada su i povećane potrebe navedenih mikroelemenata s obzirom na njihovo pojačano izlučivanje mlijekom.

Iako Haenlein i Anke, (2011) navode da određivanje koncentracije mikroelemenata u krvi životinja može biti nepouzdan pokazatelj u procjeni njihovoga sadržaja u hrani, Schweinzer i sur., (2017) ističu kako koncentracija Zn u krvnom serumu može biti dobar pokazatelj opskrbljenosti životinja. Ramirez-Perez i sur. (2000) također ukazuju da sadržaj nekih mikroelemenata u krvi životinja može biti pokazatelj njihovih unosa hranom.

Tablica 3. Povezanost između utvrđenih koncentracija Fe, Zn i Cu u krvi alpina koza u ranoj laktaciji

Element	Fe	Zn	Cu
Fe	1,000	-	-
Zn	0,409	1,00	-
Cu	-0,056	0,384	1,00

Analizom tablice 3 vidljivo je da nije bilo značajne povezanosti između istraživanih koncentracija Fe, Zn i Cu u krvi alpina koze. U istraživanju Antunovića i sur. (2023) provedenom s lakon ovcom u ranom stadiju laktacije također nisu zabilježene značajne povezanosti između utvrđenih koncentracija Fe, Zn i Cu u krvi.

Zaključak

Utvrđene koncentracije Fe i Cu u krvi alpina koza u ranoj laktaciji pokazatelj su dostatne opskrbljenosti organizma tim mikroelementima dok je za Zn utvrđen manji nedostatak u odnosu na referentne vrijednosti. Razlog niže koncentracije Zn u krvi koza može biti njegovo pojačano izlučivanje mlijekom u ranom stadiju laktacije kada je i pojačana sinteza i lučenje mlijeka. Stoga se pri određivanju koncentracija istraživanih mikroelemenata u krvi koza, uz utvrđivanje njihove koncentracije u hrani, u obzir mora uzeti i stadij laktacije.

Napomena

Istraživanja za ovaj rad su provedena u okviru Istraživačkog tima „Inovativni uzgojno-tehnološki postupci u animalnoj proizvodnji“ Fakulteta agrobiotehničkih znanosti Osijek.

Literatura

- AFRC (1997). Technical Committee on responses to nutrients, Report 10. The Nutrition of Goats. NCAB International. str. 118.
- Arthington J.D., and Ranches J. (2021). Trace Mineral Nutrition of Grazing Beef Cattle. *Animals*. 11: 2767.
- Antunović Z., Šperanda M., Mioč B., Novoselec J., Šperanda T. (2009). Determination of nutritional status of goat in organic production systems. *Tierartztliche Umschau*. 64: 18-23.
- Antunović Z., Novoselec J., Klir Ž. (2012). Ovčarstvo i kozarstvo u Republici Hrvatskoj – stanje i perspektive. *Krmiva*. 54: 99-109.
- Antunović Z., Šperanda M., Novoselec J., Đidara M., Mioč B., Klir Ž., Samac D. (2017). Blood

- metabolic profile and acid-base balance of dairy goats and their kids during lactation. *Veterinarski Arhiv*. 87: 43-55.
- Antunović Z., Klir Šalavardić Ž., Mioč B., Držaić V., Širić I., Đidara M., Zmaić K., Novoselec J. (2023). Koncentracije Fe, Zn i Cu u krvi lakon ovce u ranom stadiju laktacije. očetkom laktacije. Zbornik radova 58. hrvatskog i 18. međunarodnog Simpozij agronoma. Dubrovnik, Hrvatska, 11.-17.02.2023, str. 291-295.
- Baumgartner W. (ed.) (2009). *Clinical Propaedeutics of House Animals and Pets* (in German). 7th edn. Parey, Stuttgart. 525 p.
- Derar D., Ali A., Almundarij T., Abd-Elmoniem E., Alhassun T., Zeitoun M. (2022). Association between Serum Trace Elements Levels, Steroid Concentrations, and Reproductive Disorders in Ewes and Does. *Veterinary Medicine International*. 8525089.
- HAPIH (2023). Godišnje izvješće za 2022. godinu: Ovčarstvo, kozarstvo i male životinje. Hrvatska agencija za poljoprivredu i hranu. Osijek, 2022. str. 151.
- Haenlein G.F.W., and Anke M. (2011). Mineral and trace element research in goats: A review. *Small Ruminant Research*. 95: 2–19.
- Herdt T.H., and Hoff B. (2011). The use of blood analysis to evaluate trace mineral status in ruminant livestock. *The Veterinary Clinics of North America: Food Animal Practice*. 27: 255–283.
- King J.C. (1990). Assessment of zinc status. *Journal of Nutrition*. 120: 1474-1479.
- Lopez-Alonso M. (2012). Trace minerals and livestock: Not too much not too little. *International Scholarly Research Network ISRN Veterinary Science*. 704825, 18.
- Maggini S., Wintergerst E.S., Beveridge S., Hornig D.H. (2007). Selected vitamins and trace elements support immune function by strengthening epithelial barriers and cellular and humoral immune responses. *British Journal of Nutrition*. 98: 29-35.
- Mayasula V.K., Arunachalam A., Babatunde S.A., Naidu S.J., Sellappana S., Krishnan B.B., Rajendran U.S., Janardhan R.I., Bhatta R. (2021). Trace minerals for improved performance: a review of Zn and Cu supplementation effects on male reproduction in goats. *Tropical Animal Health and Production*. 53: 491.
- McDowell L.R. (2003). *Minerals in Animal and Human Nutrition*. 2nd Ed. Elsevier, str. 660.
- NRC (2007). *Nutrition Requirements of Small Ruminants*. The National Academies Press, Washington, D.C. str. 362.
- Oppenheimer S.J. (2001). Iron and its relation to immunity and infectious disease. *The Journal of Nutrition*. 131: 616-635.
- Pechova A., Misurova L., Pavlata L., Dvorak R. (1999). The influence of supplementation of different forms of zinc in goats on the zinc concentration in blood plasma and milk. *Biological Trace Element Research*. 132: 112-121.
- Salama A.A.K., Caja G., Albanell E., Such X., Casals R., Plaixats J. (2003). Effects of dietary supplements of zinc-methionine on milk production, udder health and zinc metabolism in dairy goats. *Journal Dairy Research*. 70: 9-17.
- SAS® 9.4 (2002-2012). SAS Institute Inc., SAS Campus Drive, Cary, North Carolina, USA.
- Schweitzer V., Iwersen M., Drillich M., Wittek T., Tichy A., Mueller A., Krametter-Froetscher R. (2017). Macromineral and trace element supply in sheep and goats in Austria. *Veterinarni Medicina*. 62: 62–73.
- Silva T.R., Soares P.C., Dantas A.F.M., Marques A.V.S., Filho E.F.O., Aguiar G.M.N., Marques A.L.A., Riet-Correa F. (2018). Serum and liver copper, iron, molybdenum and zinc concentration in goats and sheep in the state of Paraíba, Brazil. *Pesquisa Veterinaria Brasileira*. 38: 1313-1316.
- Song C., Gan S., He J., Shen X. (2021). Effects of nano-zinc on immune function in qianbei-pockmarked goats. *Biological Trace Element Research*. 199: 578-584.
- Ramirez-Perez A.H., Buntinx S.E., Rosiles R. (2000). Effect of breed and age on the voluntary intake and the micromineral status of non-pregnant sheep. II. Micromineral status. *Small Ruminant*

- Research. 37: 231–242.
- Underwood E.J., Suttle N.F. (2001). The mineral nutrition of Livestock. 3rd Ed. CABI Publishing, str. 601.
- Vázquez-Armijo R.R., García R.M., López D., Salem A.F.Z., Domínguez I.A., Pescador N., Tinoco J.L. (2011). Effect of season on serum copper and zinc concentrations in crossbred goats having different reproductive status under semiarid rangeland conditions in Southern Mexico State. *Tropical and Subtropical Agroecosystems*. 14: 331-335.
- Wu T., Song M.L., Shen X.Y. (2020). Seasonal dynamics of copper deficiency in Wumeng semi-fine wool sheep. *Biological Trace Element Research*. 197: 487-494.
- Yatoo M.I., Saxena A., Kumar P., Gugjoo M.B., Dimri U., Sharma M.C., Jhambh R. (2013). Evaluation of serum mineral status and hormone profile in goats and some of their inter-relations. *Veterinary World*. 6: 318-320.

Concentration of Fe, Zn and Cu in the blood of Alpine goats in the early stage of lactation

Abstract

The aim of this work is to determine Fe, Zn and Cu concentration in the blood of Alpine goats in early lactation and to compare it with the results of previous research. The study was performed on 14 goats in early lactation, average age 5 years, in the fourth lactation. The determined average concentrations of Fe, Zn, and Cu (1.87; 0.68; 1.14 mg L⁻¹, respectively) in the blood of Alpine goats did not differ significantly compared to previous studies, except for the determined slightly lower concentrations of Zn. The lower concentration of Zn in the blood of goats could be related to the increased excretion of Zn in milk during the early lactation period. No significant correlation was found between the microelements in the blood of Alpine goats in the early lactation phase.

Keywords: Alpine goats, Fe, Zn, Cu, blood, lactation

The use of bacteriocins of lactic acid bacteria in the prevention and treatment of mastitis

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Abstract

In most cases, mastitis results from the galactogenic infection of the animal's udder by pathogenic bacteria, most commonly *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, and *Streptococcus uberis*. However, the success of treating mastitis with antibiotics is limited today, as most of these bacterial species have become antibiotic-resistant. Therefore, bacteriocins of lactic acid bacteria, which have a high antibacterial potential against various pathogenic bacteria and do not lead to antibiotic resistance, have become the subject of intensive research in the prevention and treatment of mastitis. Effective bacteriocins for the prevention and treatment of mastitis include various forms of nisin, lactacin, and aureocin. The aim of this review is to critically compare the previous research results on the effect of various bacteriocins produced by lactic acid bacteria on the four most common causes of mastitis in dairy cows.

Keywords: mastitis, pathogenic bacteria, lactic acid bacteria, antibiotics, bacteriocins

Introduction

Mastitis arises as a result of the detrimental action of bacteria on the mammary gland, resulting in physical, chemical, or bacteriological changes in the milk and udder of the animal (Bačić, 2009). The most common causative agents of mastitis are bacterial species such as *Streptococcus (Str.) uberis*, *Staphylococcus (S.) aureus*, *Streptococcus spp.*, *Corynebacterium spp.*, *Enterococcus spp.*, *Trueperella pyogenes*, *Staphylococcus spp.*, *Escherichia coli*, pathogenic yeasts, *Streptococcus dysgalactiae*, and *Pasteurella spp.* (Cvetnić et al., 2016).

Bacterial resistance to drugs is caused by the excessive use of antibiotics in treatment of mastitis without an antibiogram. Therefore, there is emerged need for research in the field of applying new antimicrobial agents in the treatment of humans and animals without compromising their health. Due to their antibacterial properties, the effect of bacteriocins in the prevention and treatment of mastitis in dairy cows has recently been explored (Castelani et al., 2019). Bacteriocins are ribosomally synthesized antimicrobial peptides that have antibiotic-like properties, although they are not antibiotics. They are colorless, tasteless, and odorless substances with a broad pH range of activity. They are produced by many bacteria and are susceptible to the action of proteolytic enzymes and do not persist in the body for long, meaning they do not have a toxic effect on the human body (Perez et al., 2014), as well as on the body of animals. They can have a bacteriostatic or bactericidal effect, usually directed against related bacterial species or strains of the same species. Bacteria that synthesize bacteriocins have a specific immune system that protects them from the action of their own bacteriocins. Their advantage over antibiotics lies in the fact that they are peptides produced by simple biosynthesis during the primary phase of bacterial growth, whereas antibiotics are secondary metabolites (Cotter et al., 2005).

With the advancement of science and technology, research on the effect of bacteriocins intensifies and the discovery of novel bacteriocins is becoming more intense. The economic implications of mastitis as the most common disease on dairy farms provide the impetus for further research aimed at developing new technologies for antimicrobial therapy. Bacteriocins are considered an alternative with a range of advantages over conventional antibiotic therapy. Due to increased concerns about human health, mainly due to the emergence of antibiotic resistance in pathogenic bacteria, the development of alternative anti-infective agents is further encouraged (Bastos et al., 2015).

The aim of this manuscript is to critically compare the results of previous research on the effect of different bacteriocins produced by lactic acid bacteria on the four most common causative microorganisms for mastitis in dairy cows: *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae* and *Streptococcus uberis*.

Bacteriocins in the prevention and treatment of mastitis

A global problem in today's intensive milk production is mastitis, the most common and expensive disease in dairy animals. The use of antibiotics for mastitis treatment can lead to the presence of antibiotic residues in the bodies of treated cows or the passage of antibiotics into milk through the mammary gland and blood. Residual antibiotics, aside from being dangerous to human health, can cause issues in the processing of milk into fermented dairy products such as yogurt, cheese, and ice cream. For this reason and due to the emergence of antibiotic resistance in bacteria, there is a need to find alternative methods for mastitis prevention and treatment (Zou et al., 2018). Nascimento et al. (2004) mention the use of bacteriocins as one of the potential alternative antimicrobial agents. Numerous studies have demonstrated that specific bacteriocins, either on their own or in synergy with other bacteriocins, antibiotics, or previously applied agents, have a positive effect on the prevention and treatment of mastitis (Twomey et al., 2000; Wu et al., 2007; Pieterse and Todorov, 2010; Cuellar Lopez et al., 2016; Castelani et al., 2019).

Bacteriocins are usually effective against specific bacterial strains based on the target receptors of susceptible strains. In the diagnosis of mastitis, the causative bacterium must be identified for a valid, specific, pathogen-targeted approach. Bacteriocins quickly kill sensitive bacterial species through cell lysis, ensuring the impossibility of resistance development in pathogens. Antibiotics commonly used for this purpose have a broad-spectrum of activity, and act on all gram-positive or gram-negative bacteria, not just those causing the infection. In contrast, the advantage of bacteriocins lies in their specific spectrum of action. If a broader spectrum of action is required, or to ensure targeting of more than one pathogen, it is possible to combine two or three bacteriocins simultaneously. The minimum inhibitory concentration of bacteriocins should be determined to reduce the amount of bacteriocin used in the treatment product. Additionally, the bacteriocin should be active and stable in the target environment for a certain period of time in order to come into contact with potential pathogens (Pieterse and Todorov, 2010). Bacteriocins produced by lactic acid bacteria are considered safe for health (GRAS - generally regarded as safe) and are therefore much more acceptable for use compared to antibiotics. Antibiotic therapy during lactation requires a withdrawal period, resulting in economic losses. Residual bacteriocin in milk is more acceptable because digestive enzymes easily break down peptides. Therefore, the withdrawal period would be significantly shorter if bacteriocin therapy were used instead of antibiotic therapy (Pieterse and Todorov, 2010).

Determining methods of drug application in the mastitis treatment strategy is crucial, and using a teat sealant (an oil-based formulation that creates a physical barrier against infections in the teat canal and sinus) offers numerous advantages. It primarily acts as a physical barrier and prophylaxis. By combining the antimicrobial substance in the teat sealant, the inhibitor is localized in the teat canal, targeting pathogens that may be present near the teat opening and thus preventing bacterial colonization in the udder tissue. The stability of bacteriocins on the teat skin's surface is essential, but it should not cause irritation or allergic reactions to avoid worsening inflammation in the teat area. The most effective bacteriocins in mastitis treatment include various forms of nisin, lactocin, and aureocin (Pieterse and Todorov, 2010; Cuellar et al., 2016).

Nisin

Udder disinfectants are routinely used before and after milking cows to prevent the entry of pathogens into the teat canal. Chemical udder sanitization products can pose a significant problem if not completely removed before milking. Products containing bacteriocins have an advantage because they do not need to be completely removed due to their properties. For example, a germicidal formulation based on nisin, in comparison to conventional chemical treatments like iodophors and chlorhexidine, has proven to be more effective in preventing the development of mastitis. After one minute of exposure to the germicidal formulation, the initial pathogen count on the treated udder's surface was reduced by 61.8 to 98.6% (Sears et al., 1992). Furthermore, the germicidal formulation has a low potential (less than 1.0) for skin irritation on the udder after repeated exposure, compared to 1% iodophor and 5% chlorhexidine digluconate, which have a serious potential for causing irritation.

Nisin Z has proven to be highly effective in the treatment of subclinical mastitis in lactating cows (Wu et al., 2007).

Specifically, nisin therapy had a 90.1% cure rate for *Str. agalactiae*, 50% for *S. aureus*, 58.8% for coagulase-negative staphylococci, and 65.2% for all cases combined, whereas only 15.9% of untreated cows spontaneously recovered. The same authors mention the significant impact of nisin concentration, as at a nisin concentration of 2,500,00 IU, no irritations were observed, whereas at a concentration of 5,000,000 IU, irritations and visual changes in milk and udder quarters were noted. Since the presence of nisin was below the allowed limit of 500 mg/ml after 48 hours, Chinese authorities allowed the use of nisin as a biopreservative in milk.

It is a well-known fact that the combination of antimicrobial compounds can prevent the development of resistance, reducing side effects while simultaneously broadening the spectrum of their action. For example, the antimicrobial action of nisin, both when applied alone and especially in combination with another antimicrobial substance, has proven to be highly effective against antibiotic-resistant *Staphylococcus* spp. isolated from the milk of cows with mastitis (Castelani et al., 2019).

Lacticin

Lacticin 3147, produced by *Lc. lactis* spp. *lactis*, has a broad spectrum of antimicrobial activity and inhibits the growth of bacteria such as *Bacillus* spp., *Enterococcus* spp., *Pediococcus pentriceans*, *S. aureus*, *Strep. thermophilus*, *Clostridium* spp., *Listeria monocytogenes*, *Lactobacillus* spp., and most streptococci that cause mastitis. It has been the subject of research because of its inhibitory effect on pathogenic bacteria responsible for mastitis, such as *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus uberis*, and *Streptococcus dysgalactiae* (Ryan et al., 1998; Pieterse and Todorov, 2010). Its action is twofold: it acts as a physical barrier against infection in the udder area and inhibits all gram-positive bacteria that easily enter the teat canal. Ryan et al. (1998) demonstrated the exceptional potential of lacticin 3147 in teat sealant formulations, as well as its effective role in preventing new infections. Twomey et al. (2000) concluded that the concentration of bacteriocin is an important factor in determining the efficacy of udder healing in cows infected with the pathogenic bacterium *S. aureus*, based on the observed reduced antagonistic effect of bacteriocin at the same concentration and increased inoculation of *S. aureus* into the cow's udder.

Bacteriocins like lacticin 3147 can replace antibiotics in teat barrier formulations because no study has yet demonstrated resistance in mastitis-causing pathogens to lacticin 3147 (Pieterse and Todorov, 2010). Teat barrier formulations based on bismuth subnitrate in combination with lacticin 3147 have shown a significant protective role against pathogenic bacteria such as *Strep. dysgalactiae* and *S. aureus*. In 91% of udder quarters treated with the barrier containing lacticin 3147, new infections did not occur, compared to 33.3% of udder quarters treated with a barrier formulation without lacticin.

Aureocin

Aureocins are bacteriocins produced by various strains of the *S. aureus*. In the control of mastitis, staphylocins in their purified form can be used as antimicrobial substances produced by staphylococci (Nascimento et al., 2004). Most staphylocins are either lantibiotics (Pep5, epicidin 280, epidermin, epilancin K7) or belong to another group of bacteriocins (aureocins A70 and A53). Staphylocins can inhibit many bacterial species, including some pathogens, and therefore have practical applications as alternatives to antibiotics or as biopreservatives in the food industry (Ceotto et al., 2009). Although a significant number of bacteriocins have been described and studied, not all of them exhibit the same effectiveness. Aureocin A70 and A53, as well as epidermin, have shown the most significant potential in the prevention and treatment of mastitis caused by various strains of *S. aureus*, *Strep. agalactiae*, and *Strep. epidermis* (Coelho et al., 2007; Ceotto et al., 2012).

Conclusion

Considering the extensive costs of treating mastitis and the consequences of antibiotic use in its treatment, research aimed at finding sustainable and safe alternative therapies or antimicrobial substances should be strongly considered. In this regard, bacteriocins can be considered a promising treatment solution. In the future, research should focus on bacteriocins and their effects, as well as harness their potential in the prevention and treatment of mastitis, especially when antibiotics are not effective. In this context, various forms of nisin, lacticin, and aureocin hold the greatest potential.

References

- Bačić G. (2009). Dijagnostika i liječenje mastitisa u goveda. Veterinarski fakultet Sveučilišta u Zagrebu.
- Bastos M.C.F., Coelho M.L.V., Santos O.C.S. (2015). Resistance to bacteriocins produced by Gram-positive bacteria. *Microbiology*. 161: 683-700.
- Castelani L., Arcaro J.R.P., Braga J.E.P., Bosso A.S., Moura Q., Esposito F., Sauter I.P., Cortez M., Lincopan N. (2019). Activity of nisin, lipid bilayer fragments and cationic nisin-lipid nanoparticles against multidrug-resistant *Staphylococcus* spp. isolated from bovine mastitis. *Journal of Dairy Science*. 102: 1-6.
- Ceotto H., Dias R.C.S., Nascimento J.S., Paiva Brito M.A.V., Giambiagi-deMarval M., Bastos M.C.F. (2012). Aureocin A70 production is disseminated amongst genetically unrelated *Staphylococcus aureus* involved in bovine mastitis. *Letters in Applied Microbiology*. 54: 455-461.
- Ceotto H., Nascimento J.S., Paiva Brito M.A.V., Bastos M.C.F. (2009). Bacteriocin production by *Staphylococcus aureus* involved in bovine mastitis in Brazil. *Research in Microbiology*. 160: 592-599.
- Coelho M.L.V., Nascimento J.S., Fagundes P.C., Madureira D.J., Oliveira S.S., Paiva Brito M. A.V., Bastos M.C.V. (2007). Activity of staphylococcal bacteriocins against *Staphylococcus aureus* and *Streptococcus agalactiae* involved in bovine mastitis. *Microbiology*. 158: 625-630.
- Cotter P.D., Hill C., Ross R.P. (2005). Bacteriocins: Developing innate immunity for food. *Nature Reviews Microbiology*. 3: 777-788.
- Cuellar Lopez M.R., Rodríguez-Hernández A.I., Chavarría-Hernández N. (2016). LAB bacteriocin applications in the last decade. *Biotechnology & Biotechnological Equipment*. 30: 1039-1050.
- Cvetnić L., Benić M., Habrun B., Kompes G., Stepanić M., Samardžija M. (2016). Najčešći uzročnici mastitisa u krava i koza u Republici Hrvatskoj. *Veterinarska stanica* 47.
- Nascimento J.S., Fagundes P.C., Paiva Brito M.A.V., Santos K.R.N., Bastos M.C.V. (2004). Production of bacteriocins by coagulase-negative staphylococci involved in bovine mastitis. *Veterinary Microbiology*. 106: 61-71.
- Perez R.H., Zendo T., Sonomoto K. (2014). Novel bacteriocins from lactic acid bacteria (LAB): various structures and applications. *Microbial Cell Factories*. 13: 1-13.
- Pieterse R., and Todorov S.D. (2010). Bacteriocins – exploring alternatives to antibiotics in mastitis treatment. *Brazilian Journal of Microbiology*. 41: 542-562.
- Ryan M.P., Meaney W.J., Ross R.P., Hill C. (1998). Evaluation of Lacticin 3147 and a Teat Seal Containing This Bacteriocin for Inhibition of Mastitis Pathogens. *Applied and Environmental Microbiology*. 64: 2287-2290.
- Sears P.M., Wilson D.J., Gonzalez R.N. (1992). The potential role of antimicrobial proteins in the treatment of bovine mastitis, p. 138-142. In *Proceedings of the XVIIth World Buiatrics Congress and the XXVth American Association of Bovine Practitioners Conference*, vol. 2.
- Twomey D.P., Wheelcock A.I., Flynn J., Meaney W.J., Hill C., Ross R.P. (2000). Protection against *Staphylococcus aureus* Mastitis in Dairy Cows Using a Bismuth-Based Teat Seal Containing the Bacteriocin, Lacticin 3147. *Journal of Dairy Science*. 83: 1981-1988.
- Wu J., Hu S., Cao L. (2007). Therapeutic Effect of Nisin Z on Subclinical Mastitis in Lactating Cows. *Antimicrobial Agents and Chemotherapy*. 51: 3131-3135.
- Zou J., Jiang H., Cheng H., Fang J., Huang G. (2018). Strategies for screening, purification and characterization of bacteriocins. *International Journal of Biological Macromolecules*. 117: 781-789.

Ponašanje svinja u različitim sustavima uzgoja

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Sažetak

Ponašanje je način postojanja i reagiranja jedinke koje se može objektivno promatrati. To je također i prilagodba na djelovanje različitih vanjskih i unutarnjih čimbenika. Kod svinja je česta pojava specifičnih abnormalnih ponašanja poput grizanja repova i uški, zadavanja ozljeda, kanibalizma, izražavanja agresije koje najčešće nastaju kao posljedica prenapučenosti prostora, loših ambijentalnih uvjeta, loše hranidbe i raznih drugih čimbenika. Nadalje, ponašanje uzgajivaču ukazuje kakvog je zdravstvenog stanja životinja. Različiti sustavi uzgoja različito utječu na ponašanje svinja. Svinje držane u alternativnim sustavima uzgoja imaju bolje životne uvjete i mogućnost ispoljavanja ponašanja specifičnih za vrstu u odnosu na svinje uzgajane u intenzivnoj proizvodnji.

Ključne riječi: svinje, ponašanje, hijerarhija, slobodno držanje

Uvod

Ponašanje, ne samo svinja, već i ostalih životinjskih vrsta jedno je od glavnih karakteristika životinja. Svaka se životinjska vrsta ponaša u skladu sa svojim urođenim instinktima. Ponašanje je način postojanja i reagiranja jedinke, koje se može objektivno promatrati. To je također i prilagodba na djelovanje različitih vanjskih i unutarnjih čimbenika te djelovanja podražaja poput zvuka, prostora u kojemu borave, količine dobivene hrane i vode, topline, straha i brojnih drugih. Neophodno je spriječiti negativne utjecaje kao što su visoka temperatura, nedostatak vode, hrane i slično. Ponašanje životinja čovjeku daje do znanja njihovo viđenje o novonastaloj situaciji ili okolišu u kojemu se nalaze te osim toga daju uvid u njihovo zdravstveno stanje.

Svinje su izrazito društvene životinje koje su naviknute na skupni uzgoj. Društvenost je izrazito uočljiva prilikom igre dvaju ili više životinja. Svinje se na farmama drže u skupinama o čijem broju i sastavu odlučuje čovjek. Sastav skupine, veličina i brojnost ovise o članovima iste, kao i o uvjetima okoline. Hijerarhija je vrlo važna komponenta stabilnosti zajednice. Na hijerarhiju utječu starost jedinke, masa te karakter. Hijerarhija, kao i agresivnost prisutni su od samoga rođenja pa sve do izlaska iz uzgoja. Agresivnost i međusobno grizenje pokazatelji su borbenosti svinja. Svinje se vrlo lako prilagođavaju prostoru i uvjetima u kojima žive. Poznavanje ponašanja i navika svinja omogućuje osiguravanje adekvatnih uvjeta života usklađenih s načelima dobrobiti. Različiti podražaji iz okoline mogu promijeniti ponašanje svinja iz normalnog ponašanja u abnormalno.

Različiti sustavi uzgoja različito utječu na ponašanje svinja. Navedeno potvrđuje činjenica da svinje na otvorenom imaju bolje životne uvjete i mogućnost ispoljavanja ponašanja specifičnog za vrstu u odnosu na svinje uzgajane u intenzivnoj proizvodnji. Otvoreni sustav držanja omogućuje korištenje prirodnih resursa, poput zemlje i biljaka koji mogu pozitivno utjecati na njihovo ponašanje. Uz sve navedeno, životinja ima mogućnost izražavanja svog urođenog instinkta za traženje hrane, snalaženje u prostoru, rovanje, kaljužanje i slično.

Cilj ovoga rada je pobliže objasniti načine uzgoja svinja, njihove pozitivne i negativne strane kao i sam hijerarhijski poredak unutar skupina. Također, u radu će se pobliže objasniti različiti obrasci ponašanja koji ukazuju na dobro ili loše držanje i postupanje s njima.

Načini držanja svinja

Suvremeni načini držanja svinja najveću pozornost pridodaju dobrobiti životinja te postizanju zadovoljavajućih proizvodnih rezultata. Proizvodni rezultati ovise o načinu držanja, smještaju, mikroklimatskim uvjetima, hranidbi, njezi, reprodukciji, selekciji i zdravstvenom stanju životinje.

Otvoreni je sustav uzgoja svinja, među proizvođačima, postao vrlo popularan i zastupljen posljednjih nekoliko godina. Ovo je jedini sustav koji se smatra prirodnijim, u kojemu se svinje slobodno kreću i borave na pašnjacima ili u šumskim područjima, gdje se hrane različitim šumskim plodovima ili kombinacijom drugih krmiva i koncentrata. Razlozi popularnosti ovoga sustava leže u manjim troškovima smještaja, smanjenoj potrošnji energije te očuvanju prirodnih resursa i okoline (Luković, 2014). Kako bi se svinje mogle uzgajati na ovaj način uzgajivač mora posjedovati velike zemljišne površine. Osim za držanje, neophodno je imati dodatne površine za oporavak uništenog tla i odmor kako bi se prekinuo životni ciklus parazita i drugih nametnika. Sloboda kretanja i velike površine za držanje svinja predstavljaju najveću razliku u odnosu na intenzivni način uzgoja koji svinjama ograničava prostor za kretanje i igru (Chapinal i sur., 2010). Svinje su znatizeljne životinje sklone istraživanju, stoga mogućnost slobodnog kretanja ima pozitivan utjecaj na iskazivanje urođenih instinkta za pronalazak hrane i kretanje što je u skladu s njihovom dobrobiti. Prema Grimberg-Henrici i sur. (2016) treba osigurati minimalno 12 m² prostora po svinji za njihovo neometano kretanje i ležanje u slobodnom uzgoju. U takvim uvjetima skloništa imaju jako važnu ulogu za svinje. Naime, prilagođavajući se vremenskim uvjetima neophodno je izgraditi objekte ili skloništa za životinje kako bi se mogle skloniti od prejakog sunčevog zračenja ljeti, odnosno zaštititi se od hladnoće tijekom zimskog razdoblja.



Slika 1. Otvoreni sustav držanja svinja (Izvor: Margeta, V.)

Zatvoreni ili intenzivni sustav držanja podrazumijeva uzgoj velikog broja životinja u velikim proizvodnim sustavima. Ovakvi su sustavi većinom farme koje teže što većoj financijskoj dobiti, pri čemu je nerijetko ugrožena dobrobit životinja. Cilj intenzivne proizvodnje je u što kraćem vremenskom razdoblju proizvesti što više mesnatih svinja i potomaka uz minimalne utroške stočne hrane, vode, električne energije i ljudskog rada. Zbog porasta ljudskih potreba za animalnim proteinom i povećane potražnje za svinjskim mesom, svinje se drže u na rešetkastom ili polurešetkastom podu, u malim boksovima ili kavezima u kojima im je ograničeno kretanje, a socijalni kontakt ugrožen (Horback i sur., 2016). Kako bi se izbjegli zdravstveni problemi i ekonomski gubici, Verdon i sur. (2015) ističu kako je u zatvorenom sustavu držanja svinja neophodno odvojiti prostore za hranidbu, lijevanje i defekaciju. Ovakvim se načinom uzgoja narušava zdravstveno stanje životinje. Osim toga, učestala je pojava mnogobrojnih ozljeda, pojava agresivnosti, kanibalizma, grižnja repova i brojnih drugih abnormalnih obrazaca ponašanja. U intenzivnom se uzgoju primjenjuju i uzgojne prakse poput ranog odbića prasadi od krmače s ciljem bržeg postizanja određene tjelesne mase te naposljetku bržeg ostvarenja profita. Kako bi se ublažili uvjeti držanja svinjama u intenzivnom sustavu koji odstupaju od onih u prirodnom okruženju, potrebno je osigurati kvalitetnu hranidbu, uskladiti mikroklimu te provoditi higijenske zdravstvene programe suzbijanja bolesti (Ocepek i Andersen, 2022).

Alternativni sustavi uzgoja svinja

Alternativni sustavi uzgoja koji se koriste u svijetu, a također su zastupljeni i kod nas, su tov na dubokoj stelji i silvo-pastoralni sustav. Oba su sustava vrlo specifična i prihvaćena posebice u uzgoju autohtonih pasmina svinja (Wolfová i sur., 2017).

Tov na dubokoj stelji

Duboka stelja podrazumijeva posipavanje podnih površina u objektu različitim prirodnim materijalima poput slame, strugotine drveta ili piljevine. Ovaj način uzgoja pruža svinjama izražavanje prirodnog, urođenog ponašanja poput rovanja čime se smanjuje agresija, što ima pozitivan utjecaj na dobrobit životinja (Beattie i sur., 2000). Ovakav je uzgoj karakterističan za zemlje koje imaju specifične klimatske i geografske uvjete te ne mogu zadovoljiti visoke zahtjeve intenzivnog uzgoja. Stelja, osim što je podloga za igru, služi kao toplinski izolator te jako dobro apsorbira urin i vlagu iz izmeta. Količina apsorpcije ponajviše ovisi o vrsti materijala koji se koristi. U pogledu sposobnosti apsorpcije, najbolje je koristiti drvenu piljevinu, no nju je potrebno često mijenjati čime se uzrokuje uznemiravanje životinja, što dovodi do poremećaja u njihovoj stvorenoj rutini (Škorput, 2014).



Slika 2. Tov svinja na dubokoj stelji (Izvor: Margeta, V.)

Tov na dubokoj stelji se pokazao iznimno dobrim za zdravlje i dobrobit životinja, osobito u prasadi. Uz dobrobit životinja, smanjeni su troškovi zagrijavanja objekta (Gentry i sur., 2002). Proizvodnja i ekonomska svojstva puno su kvalitetnija u odnosu na intenzivnog uzgoja. Kod svinja držanih na dubokoj stelji razina stresa je niža u usporedbi sa svinjama držanih u intenzivnom sustavu. Također, su veći prosječni prirasti, veća je konzumacija hrane i u završnom tovu su veće tjelesne težine.

Silvo-pastoralni sustav

Silvo-pastoralni je sustav takozvani agrošumski sustav uzgoja u kojemu dolazi do kombinacije uzgoja domaćih životinja na pašnjacima ili u šumama. Karakteristika ovoga sustava je u tome što se ne koristi visoka tehnologija kao u slučaju intenzivnog sustava uzgoja, a upotrebljavaju se prirodni resursi. Osim toga, ovaj sustav ne zahtjeva velika financijska ulaganja te je ekološki vrlo prihvatljiv. U opisanom se sustavu uzgajaju autohtone pasmine svinja, poput turopoljske svinje, crne slavonske svinje i banijske šare svinje (Budimir i sur., 2013).

Prema Budimir i sur. (2013) negativne strane silvo-pastoralnog sustava su mogućnost prijenosa zaraznih bolesti ukoliko uzgajane svinje dođu u doticaj s divljim svinjama ili drugim životinjama, te križanje s divljim svinjama. Također, zbog svoje radoznalosti, a tijekom potrage za hranom, svinje mogu uništiti mlade biljke čime će uzrokovati velike štete.

Ponašanje svinja

Ponašanje i društvene karakteristike svake svinje ključne su za dobrobit životinja na farmama i drugim sustavima uzgoja. Keeling i Gonyou (2001) pod društvenim ponašanjem smatraju međusobno njuškanje koje ubrajamo u uobičajeni kontakt među svinjama. Takvo se ponašanje smatra međusobnim prepoznavanjem koje je važno za održavanje grupnih odnosa i kohezije. Pozitivnim se društvenim ponašanjem smatra i igranje svinja. Kod prasadi, međusobno igranje pomaže u stjecanju vještina za rješavanje sukoba i individualno prepoznavanje te istovremeno omogućuje jako povezivanje i stjecanje prijateljskih veza.

Kao i ljudi, svaka životinja ima individualan karakter po kojemu se razlikuju od drugih. Proučavanje karaktera pojedine životinje može uvelike pomoći u razumijevanju različitoga ponašanja i fiziološkog odgovora na različite izazove i uvjete, kao što su različiti sustavi držanja, prakse upravljanja ili veterinarske intervencije. U društvenih životinja, agresivnost i društvenost spadaju među najčešće analizirane osobine (Finkemeier i sur., 2018). Prema istraživanjima Finkemeier i sur. (2018), koja se temelje na različitim vrstama životinja poput: teladi, krava, koza, ovaca utvrđeno je da su društveni položaji povezani s različitim individualnim značajkama. Bolje razumijevanje ranog socijalnog ponašanja može pomoći u prepoznavanju pretpostavljenih pozitivnih društvenih ponašanja važnih za dobrobit. Primjerice, pretpostavlja se da neka društvena ponašanja, kao što su gruba igra ili 'tučnjava', pomažu prasadi kod razvijanja motoričkih i socijalnih vještina potrebnih za uspješnu borbu, favorizirajući tako optimalnu integraciju u buduće hijerarhijske društvene skupine (Horback, 2014). Uz sve navedeno i zdravstveno stanje ima velik utjecaj na ponašanje. Prasad ima veću društvenu interakciju s 42 dana starosti nego s 21 dan. Izražen je kontakt nosom, skloniji su međusobnoj igri i istraživanju prostora te su međusobno povezani. Muška prasad ima više društvenih interakcija od ženskih, to znači da su muške jedinke više uključene u igru i nisu sklone izbjegavanju drugih životinja.

Ženske životinje u skupini neaktivnih životinja većinu svoga vremena provedu ležeći, a manje vremena stojeći, dok se jedinke u skupini aktivnih razlikuju po tome. Neka nesocijalna ponašanja koja uključuju vrijeme provedeno za hranidbenim stolom, pojilicama ili sisajući nisu bila pod utjecajem spola. Bez obzira na spol, prosječnu masu ili prosječni dnevni prirast te su interakcije povezane s grupom u kojemu se nalaze pojedine jedinke. Prema tome, neaktivne životinje imale su manju tjelesnu masu te ostvarivale manje prosječne dnevne priraste (Weller i sur., 2019).

Analize ponašanja prasadi pokazale su pozitivna socijalna ponašanja, odnosno njuškanje i igru koja predstavlja veliki udio svih vrsta ponašanja, dok je udio agonističkih ponašanja i drugih vrsta iskazivanja agresije nizak. Sukladno tome, niska učestalost agonističkih interakcija u usporedbi s neagonističkim društvenim interakcijama zabilježena je u stabilnim društvenim skupinama mladih svinja (Oostindjer i sur., 2011). Visoka učestalost društvenog njuškanja i niska učestalost agonističkih ponašanja odražavaju snažnu društvenu koheziju i partnerske odnose unutar legla u mirnom i stabilnom društvenom kontekstu koji su uzrokovani optimalnim uvjetima okoline. Navedeni se podaci smatraju bitnim za dugoročnu dobrobit svinja na farmama.

Muške i ženske jedinke općenito imaju iste razine aktivnosti, međutim muške životinje su uključene u više društvenih interakcija nego ženske. Uočeno je kako su muške jedinke imale više agonističkih ponašanja i drugih društvenih ponašanja, naskakivanja i guranja te su bili uključeni u značajno više društvenih igara od ženki. Naskakivanje se često smatra seksualnim ponašanjem, ali se također pretpostavlja da igra ulogu u formiranju hijerarhije dominacije (Mesarec i sur., 2021). Nadalje, utvrđeno je da su nerastići češće uključeni u igru, svađu i guranje nego nazimice tijekom razdoblja prije i nakon odbijanja (Weller i sur., 2019). Ove razlike u ponašanju tijekom igre potvrđuju hipotezu da vrste kod kojih se mužjaci i ženke razlikuju u svom kasnijem društvenom okruženju pokazuju spolne razlike u svom ranom ponašanju u igri, posebno onoj povezanoj s borbom (Meaney, 1988).

U intenzivnoj proizvodnji, gdje se krmače drže zatvorene u individualnim boksovima, ograničava se njihovo prirodno ponašanje poput izgradnje gnijezda. Intenzivna proizvodnja narušava prirodno ponašanje prasadi, budući da je interakcija s krmačom ograničena. Kod sustava držanja koje pruža slobodu kretanja krmače i prasadi, prasad prati krmaču, istražuju okoliš te uči tražiti hranu i konzumirati je. Obogaćivanje prasilišta raznim materijalima, kao što su strugotine, treset, grane ili slama pomaže prasadi prilikom učenja pronalaska hrane i utječu na njihovu samostalnost u daljnjem životu (Morgan i sur., 2006).

odbiće izlaže prasad različitim stresorima. Promjena okoline, naglo odvajanje od krmače, promjena hranidbenih navika te miješanje s nepoznatom prasadi utječe na smanjenje rasta i povećava učestalost dijareje. U slobodnim uvjetima držanja miješanje prasadi različitih legala događa se postupno. Prema Pittsu i sur. (2000) miješanjem mlade prasadi rezultira smanjenom agresijom u odnosu na miješanje različitih skupina prasadi u starijoj dobi. Davanje prilika prasadi za druženje prije odbića može smanjiti agresivno ponašanje i nastanak ozljeda kad dođe vrijeme za odbiće i to zbog već uspostavljene društvene hijerarhije (Parratt i sur., 2006). Prvi tjedni života i način držanja imaju veliku ulogu za dobrobiti prasadi zbog toga što se ponašanje, kongitivne i socijalne vještine te regulacija stresa uče već u prvim danima života. Prvi sat nakon miješanja skupina je najagresivniji period koji traje sve do četvrtoga dana zajedničkoga života, nakon čega se postepeno smanjuje. U navedenom razdoblju dolazi do zadavanja kožnih ozljeda u borbi. Odmicanjem vremena i stvaranjem hijerarhijskoga odnosa smanjuje se agresija i borba te dolazi do privikavanja prasadi na zajednički život.

Kako bi se osigurao kvalitetan i adekvatan uzgoj životinja na farmama od velike je važnosti osigurati kvalitetan odnos čovjeka prema životinjama koje se uzgajaju. Unatoč mnogim genetskim i selekcijskim postupcima koje utječu na sami način uzgoja i mijenjaju cjelokupnu jedinku, jedan od najstresnijih događaja u životu životinja je neposredan kontakt s ljudima te nagle promjene u njihovom društvenom i fizičkom okruženju. Iako su neke životinje cijelog života izložene čovjekovoj prisutnosti i dalje osjećaju strah. Strah od čovjeka ponajprije se javlja uslijed negativnog iskustva ili nepravilne manipulacije životinjama. U većini slučajeva kada dolazi do kontakta s ljudima radi se o provođenju zootehničkih zahvata koje životinje doživljavaju kao negativno iskustvo (kao što su posjet veterinaru, liječenje, obuzdavanje ili udarci). Svinje imaju izrazito dobro pamćenje. Negativne interakcije pamte jako dobro te se ponašaju u skladu s prijašnjim iskustvima. Pamte bijele kute veterinaru, nanesenu bol, osjećaju pozitivnu ili negativnu energiju ljudi koji se s njima bave. Ponašanje uzgajivača i osoba koji se brinu o svinjama glavna je varijabla koja određuje strah životinja i ponašanje prema ljudima. Negativna iskustva, strah i nepravilno rukovanje imaju niz nepoželjnih posljedica za životinju i uzgajivače. Stoga, iznenadno, intenzivno i dugotrajno izazivanje straha predstavlja narušavanje dobrobiti, reproduktivnih sposobnosti, kvalitete proizvoda kao i profitabilnosti uzgajivača (Hemsworth i Coleman, 1998).

Zaključak

Svinje su vrlo inteligentne i društvene životinje koje pokazuju širok raspon ponašanja. Vole živjeti u skupinama gdje stvaraju jake veze s drugim svinjama i često pokazuju znakove uznemirenosti kada su odvojeni od svojih društvenih prijatelja. Međusobno komuniciraju koristeći različite vokalizacije kao što su roktanje, cviljenje ili mazanje. Oni također koriste govor tijela i izraze lica kako bi prenijeli emocije i namjere drugim svinjama. Gravidne krmače pokazuju specifično ponašanje gniježđenja prije prasnjenja skupljajući materijale poput slame kako bi izgradili gnijezdo za svoje praščiće. Svinje, posebno mlade, razigrane su životinje, uživaju trčati, istraživati i sudjelovati u borbama. Svinje su vrlo inteligentne životinje, često se uspoređuju sa psima u pogledu kognitivnih sposobnosti. Mogu rješavati probleme, naučiti nove zadatke i prilagoditi se svojoj okolini. Iako su svinje općenito poslušne životinje, mogu postati agresivne ako se osjećaju ugroženo ili isprovocirano. Prirodno su znatiželjne životinje i uživaju u istraživanju okoline pri čemu koriste svoje njuške za istraživanje objekata i okoline. Važno je napomenuti da se ponašanje svinja može razlikovati ovisno o čimbenicima kao što su njihova okolina, društvene interakcije i individualne osobine. Pravilna njega, socijalizacija i stimulativno okruženje mogu pridonijeti općoj dobrobiti i pozitivnom ponašanju svinja.

Literatura

- Beattie V., O'Connell N., Moss B. (2000). Influence of environmental enrichment on the behavior, performance and meat quality of domestic pigs. *Livestock Production Science*. 65: 71-79.
- Budimir K., Margeta V., Kralik G., Margeta P. (2013). Silvo-pastoralni način držanja crne slavonske svinje. *Krmiva: Časopis o hranidbi životinja, proizvodnji i tehnologiji krme*. 55: 151-157.
- Chapinal N., de la Torre J.L.R., Cerisuelo A., Gasa J., Baucells M.D., Coma J., Vidal A., Manteca, X. (2010). Evaluation of welfare and productivity in pregnant sows kept in stalls or in 2 different group housing systems. *Journal of Veterinary Behavior*. 5: 82-93.
- Finkemeier M.A., Langbein J., Puppe B. (2018). Personality research in mammalian farm animals: Concepts, measures, and relationship to welfare. *Frontiers in veterinary science*. 5: 131.
- Gentry J., McGlone M., Miller J., Blanton J. (2002). Alternative housing systems for pigs: Influences on growth, composition, and pork quality *Journal of animal science*. 80: 1781-1790.
- Grimberg-Henrici C.G.E., Vermaak P., Bolhuis J.E., Nordquist R.E., van der Staay F.J. (2016). Effects of environmental enrichment on cognitive performance of pigs in a spatial holeboard discrimination task. *Animal Cognition*. 19: 271-283.
- Hemsworth P., and Coleman G.J. (1998). *Human-Livestock Interactions: The Stockperson and the Productivity of Intensively Farmed Animals*. Knjiga. CABI Publishing.
- Horback K. (2014). Nosing around: play in pigs. *Animal Behavior and Cognition*. 2: 186-186.
- Horback K.M., Pierdon M.K., Parsons T.D. (2016). Behavioural preference for different enrichment objects in a commercial sow herd. *Applied Animal Behaviour Science*. 184: 7-15.

- Keeling L.J., and Gonyou H.W. (2001). Social behaviour in farm animals. Knjiga. CABI publishing.
- Luković Z. (2014). Držanje svinja na otvorenom. *Gospodarski list*. 62 – 63.
- Mesarec N., Skok J., Škorjanc D., Prevolnik Povše M. (2021). Group dynamics in a spontaneously established group of newly weaned piglets. *Applied Animal Behaviour Science*. 238: 105317.
- Morgan T., Pluske J., Miller D., Collins T., Barnes A.L., Wemelsfelder F, Fleming P.A. (2014). Socialising piglets in lactation positively affects their post-weaning behaviour. *Applied Animal Behaviour Science*. 158: 23-33.
- Ocepek M., and Andersen I.L. (2022). The Effects of Pen Size and Design, Bedding, Rooting Material and Ambient Factors on Pen and Pig Cleanliness and Air Quality in Fattening Pig Houses. *Animals*. 12: 1580.
- Oostindjer M., van den Brand H., Kemp B., Bolhuis J.E. (2011). Effects of environmental enrichment and loose housing of lactating sows on piglet behaviour before and after weaning. *Applied Animal Behaviour Science*. 134: 31-41.
- Parratt C.A., Chapman K.J., Turner C., Jones P.H., Mendl M.T., Miller B G. (2006). The fighting behaviour of piglets mixed before and after weaning in the presence or absence of a sow. *Applied Animal Behaviour Science*. 101: 54-67.
- Pitts A.D., Weary D.M., Pajor E.A., Fraser D. (2000). Mixing at young ages reduces fighting in unacquainted domestic pigs. *Applied Animal Behaviour Science*. 68: 191-197.
- Škorput D. (2014). Značajke držanja svinja na dubokoj stelji. *Gospodarski list*.
- Verdon M., Hansen C.F., Rault J.L., Jongman E., Hansen L.U., Plush K., Hemsworth P.H. (2017). Effects of group housing on sow welfare: A review. *Journal of animal science*. 93: 1999–2017.
- Weller J.E., Camerlink I., Turner S.P., Farish M., Arnott G. (2019). Socialisation and its effect on play behaviour and aggression in the domestic pig (*Sus scrofa*). *Scientific Reports*. 9: 1-11.
- Wolfová M., Wolf J., Krupová Z., Krupa E., Žáková E. (2017). Estimation of economic values for traits of pig breeds in different breeding systems: I. Model development. *Livestock Science*. 205: 79-87.

Behavior of pigs in different farming systems

Abstract

Behavior is an individual's way of being and reacting, which can be objectively observed. It is also an adaptation to the action of various external and internal factors. In pigs, there are frequent occurrences of abnormal behaviours such as biting tails and ears, inflicting injuries, cannibalism, expressions of aggression that most often occur as a result of overcrowding, poor environmental conditions, poor feeding and various other factors. Furthermore, the animal's behavior provides informs about the health status of the animal. Different farming systems have different effects on the behavior of pigs. Pigs kept in an open breeding system have better conditions for a healthy and normal life compared to pigs raised in intensive production.

Keywords: pigs, behavior, hierarchy, free range

The impact of inbreeding on birth weight in the Pag sheep breed

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Abstract

The aim was to estimate the impact of inbreeding on the birth weight (BW) in the population of Pag sheep breed undergoing selection for milk yield. All available pedigree records were used in the estimation of the coefficient of inbreeding (F_{PED}), but only lambs born after the year 2010 with number of equivalent generations ≥ 3 were used in the inferential statistical analysis. Impact of F_{PED} on BW was tested under the 5-way ANCOVA statistical model with gender, litter size, parity, season and flock used as categorical, and F_{PED} as numerical predictor. Although statistically insignificant, the estimated inbreeding depression (-0.001 kg for $\Delta F_{PED} = 1\%$, $P > 0.05$) indicates that inbreeding should be minimised in order to prevent a genetically influenced decrease of the BW. More evidence is needed to generalize this effect in sheep.

Keywords: Pag sheep, selection, inbreeding depression, birth weight

Introduction

The Pag sheep breed has great economical, traditional, cultural, demographic, and touristic importance for the residents of Croatia. In the last couple of decades, the population size has been estimated to be ~30,000 animals. The breed had a historical importance in wool production, but today, majority of breeders raise this breed for milk production due to high demands for extraordinary expensive Pag sheep cheese (~40 €/kg). The breed has also a significant importance in lamb meat production, since all non-replacement male and female lambs are slaughtered before the age of 45 days and sold as delicacy suckling lamb meat (also sold for the extremely high price (12-15 €/kg)). It is important to note that there is no surplus of the products of this sheep breed on the market, evidencing thus its' tremendous cost effectiveness. Due to the favourable situation on the dairy market, many of the dairy orientated Pag sheep breeders would like to permanently increase the milk production in their flocks via selection, so they enrolled the national selection program. Currently there are 34 flocks with ~4,500 animals undergoing selection based on BLUP genetic evaluation system (HAPIH, 2023). Breeding values for several dairy traits have been predicted under the test-day repeatability animal model (Špehar et al., 2020) and regularly provided to the breeders. More recently, a substantial number of animals in the breeding population have been genotyped with 50K ovine SNP bead chip in order to establish a genomic selection under the framework of the single-step genomic BLUP (Legarra et al., 2009). There is also intention to follow the basic principles of optimum contribution selection (OCS), in order to provide selection gain with minimal loss of genetic variability. In addition to preventing genetic erosion of the breed, the purpose OCS is to avoid unwanted and unpredictable consequences of inbreeding such as incidence of genetically influenced defects and inbreeding depression. Inbreeding can impair growth, milk production, health, fertility, and survival. The inbreeding depression has been reported in many sheep populations for different traits (e.g. Ercanbrack and Knight, 1991, Analla et al., 1998, Dario and Bufano, 2003, van Wyk et al., 2009). By taking into account the fact that inbreeding is inevitable in small populations, especially in the populations undergoing selection due to overuse of genetically superior animals that tend to be genetically related, the genomic OCS seems to be the best possible solution. However, in order to justify and setup this novel and comprehensive selection strategy, some scientific and pragmatic questions need to be answered. Among the numerous specific goals in the series of the studies conducted under the scope of the scientific project OPTI-SHEEP (CSF, IP: 2019-04-3559), the aim of this study was to estimate the impact of inbreeding on the birth weight (BW) in the subpopulation of Pag sheep breed to check if inbreeding can

severely impair this important trait related to survival and early lamb growth in extensive outdoor sheep breeding system.

Material and methods

All data used in the analysis were provided by the Croatian Ministry of Agriculture. The original dataset included a total of 281760 animals (rams, ewes, and lambs) born between 1981 and 2019. Number of equivalent generations (NEG) that was used as criterion for retaining animals in the inferential statistical analysis was calculated as the sum of the proportions of known ancestors of an individual over all traced generations as follows: $\sum_{i=1}^n \frac{1}{2^i}$ where n was the number of ancestors of individual i , and i was the number of generations between individual i and its ancestor i (Maignel et al., 1996). In this way, 1/2 was added for each known parent, 1/4 for each known grandparent, 1/8 for each known great-grandparent and so on. The coefficient of inbreeding (F_{PED}), representing the probability that two alleles chosen at random from the maternal and paternal haplotypes are identical by descent was estimated for all animals in the pedigree, but only subset of the animals with $NEG \geq 3$ and born after year 2010 ($n=17,355$) were used in the analysis of F_{PED} . This subset of the animals was additionally reduced to 4470 animals with reliably recorded phenotypes (flocks under selection) for purpose of the analysis of the impact of F_{PED} on BW. The pedigree analysis was conducted in the R programming environment (R Core Team, 2020) using package “optiSel” (Wellmann, 2021.). Preparation of phenotypic data, descriptive statistical analysis, and plotting of the results in preliminary analysis were carried out using several R packages such as “data.table” (Dowle and Srinivasan, 2021), “tidyverse” (Wickham et al., 2019), “pastecs” (Grosjean and Ibanez, 2018), “descriptr” (Hebbali, 2020) and “ggplot2” (Wickham, 2016). The inferential statistical analysis was conducted under the 5-way ANCOVA statistical model with gender, litter size, parity, season and flock used as categorical predictors, and F_{PED} as numerical predictor. Linearity of the data (i.e. presence of linear relationship between the predictor and the outcome in the model) and assumption that residual errors had a mean value of zero were tested graphically (Residual vs Fitted plot). The assumption that residual errors had constant variance were tested graphically (Scale-Location plot) and additionally using the Non-Constant Error Variance Test ($p = 0.14833$). All assumptions were met. Marginal means (a.k.a. LSM) from the above statistical model were estimated using the “emmeans” package (Lenth, 2022) and predictor effect plots using the “effects” package (Fox and Weisberg, 2018).

Results and discussion

The average F_{PED} in this population was estimated to be 6%. Majority of these animals were slaughtered before sexual maturity which (they did not enter the breeding population). The F_{PED} of breeding animals, i.e. animals recorded as sires and dams in the pedigree, was a bit lower ($F_{PED} = 5\%$) but still above the reports of F_{PED} for Spanish, French and some Italian dairy sheep breeds. The F_{PED} for Latxa Cara Negra from Euskadi, Latxa Cara Rubia, and Latxa Cara Negra from Navare was estimated at approximately 2% for all three breeds (Granado-Tajada et al., 2020). The estimates of F_{PED} for Basco-Béarnaise, Manech Tête Noire, Manech Tête Rousse, and Lacaune Confederation ranged from 2% to 3% (Rodríguez-Ramilo et al., 2019). The F_{PED} for Comisana, Massese, Delle Langhe, and Sarda was estimated at 1.8%, 3.9%, 6.0%, and 9.2%, respectively (Cortellari et al., 2022). The estimated F_{PED} for all categories in the population of Pag sheep falls below 6.25% (acceptable inbreeding level in small populations undergoing selection). The results of inferential statistical analysis obtained with ANCOVA statistical model are summarised in Table 1, Figure 1 and Figure 2. All categorical predictors had statistically significant impact on BW ($P < 0.001$), except the season ($P > 0.05$). The BW increased up to 4th parity, males were heavier than females, and singletons were heavier than twins. The estimated differences between classes of fixed predictors were in general agreement with many previous findings on those effects in sheep (e.g Držaić et al., 2021 in regard to parity, Babar et al., 2004 in regard to litter size and gender).

Table 1. Marginal means of the categorical predictors obtained with ANCOVA statistical model

Class	Mean	Standard error	Lower confidence interval	Upper confidence interval
Parity (P<0.001)				
1	2.95	0.03	2.90	3.01
2	3.12	0.03	3.07	3.17
3	3.22	0.03	3.17	3.27
4	3.28	0.03	3.22	3.33
5	3.27	0.03	3.22	3.33
6	3.27	0.03	3.21	3.33
7	3.27	0.04	3.20	3.34
8	3.26	0.04	3.18	3.33
9	3.21	0.05	3.12	3.30
Litter size (P<0.001)				
Singletons	3.46	0.02	3.43	3.49
Twins	2.95	0.04	2.88	3.03
Gender (P<0.001)				
Females	3.17	0.02	3.12	3.21
Males	3.25	0.02	3.20	3.29
Season (P>0.05)				
November	3.18	0.03	3.12	3.24
December	3.20	0.02	3.16	3.24
January	3.22	0.02	3.17	3.27
February	3.23	0.04	3.16	3.30

Due to too many classes, the effect of flock on BW was presented graphically (Figure 1.). It can be seen that BW varied quite a lot between the flocks. These huge discrepancies between the flocks were partially influenced by genotype but most probably, by considerably different nutrition (due to different natural vegetation between different areas of the Pag Island and supplemental nutrition in the last third of gestation provided by more conscious breeders).

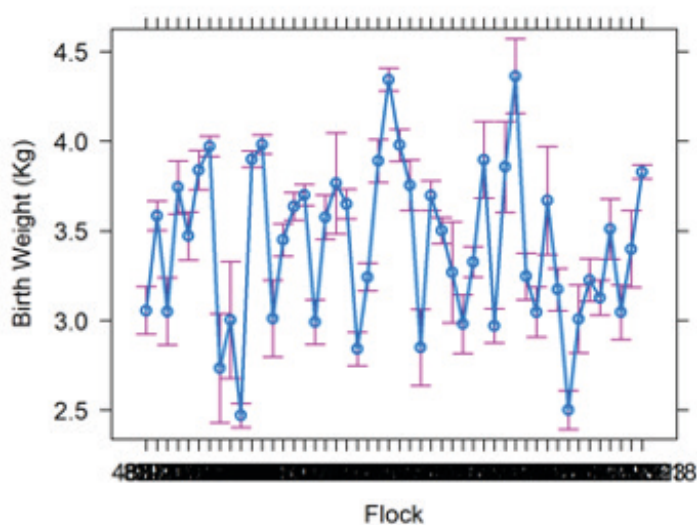


Figure 1. Estimates of flock effect on BW in the population of Pag sheep breed

The estimated slope of regression of BW on F_{PED} was negative, but it did not significantly differ from the 0 ($\beta_1 = -0.001$, $P > 0.05$). According to the obtained results, for every 10% increase in the F_{PED} , it is expected decrease of 0.01 kg in the BW (Figure 2). The obtained results are in general agreement (depression) with some previous reports on this phenomenon in other sheep populations, but they differ significantly in the estimated magnitude of the effect. The inbreeding depression of -0.006 kg, -0.007 kg, and -0.013 kg for $\Delta F_{\text{PED}} = 1\%$ was determined in Elsenburg Dormer sheep breed (van Wyk et al., 2009), Polish Olkuska breed (Drobik and Martyniuk, 2016), and Merino sheep breed (Analla et al., 1998), respectively. As it can be seen from the Figure 2, the confidence interval of the estimated regression slope was pretty wide, especially in the domain of the high values of F_{PED} , suggesting that the estimated line could take any other position in the shaded area. Based on this constellation of data, it is possible that inbreeding depression in this population was underestimated due to the small number of highly inbred animals (76% of animals had F_{PED} below 9%). Some experts in this scientific field argue that negative impact of inbreeding became evident only when F_{PED} exceeds 10%, but those claims have not been supported by firm scientific evidences and practically remain only on the spoken words. However, despite the fact that prevalence of lowly inbred animals makes our study inconclusive to some extent, we are glad that in this Croatian indigenous sheep population do not prevail highly inbred animals. This is a kind of evidence that majority of the breeders in this population take care to avoid very close inbreeding in their flocks, but relatively high overall inbreeding ($F_{\text{PED}} = 5\text{-}6\%$) still calls for attention. The results from this study related to inbreeding depression on BW support previous findings on this issue in sheep to some extent, but in order to be able to generalize this effect in sheep, more research need to be done. Inclusion of genomic data in the future studies in this population is expected to provide more reliable estimates of the overall inbreeding and inbreeding depression. The genomic information (SNP markers across the whole genome) will cancel out pedigree errors (common in outdoor raised and bred sheep populations), and provide realised (genomic based) relationship between animals under concern. Based on these results, we can argue that inbreeding in this population needs to be minimised at least in order to prevent genetically influenced decline in BW. In addition, other unpredictable unwanted side effects connected with inbreeding are additional reason to lower inbreeding in this small population undergoing selection on milk yield. In order to keep inbreeding as low as possible, more systematic approach in future breeding activities needs to be applied. According to the opinion of the experts in this scientific field, the most appropriate strategy would be the OCS (Meuwissen, 2009), preferably under the framework of genomic selection.

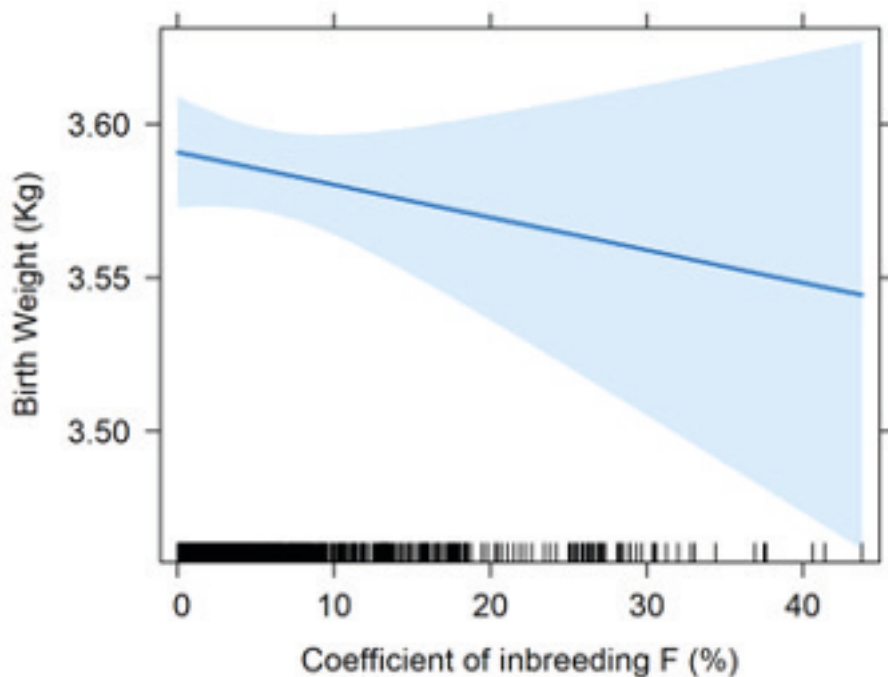


Figure 2. Estimate of inbreeding depression in the population of Pag sheep breed (blue areas below and above the regression line represent confidence interval)

Conclusion

These are first insights into direction and magnitude of impact of inbreeding on birth weight in the Pag sheep breed, which implicate presence of inbreeding depression, but more evidence is needed to generalise this effect. Future analysis on this phenomenon should include genome-wide genetic markers (SNPs) which will cancel out potential pedigree errors and should be conducted under the framework of animal genetic model, preferably using the single step GBLUP. In order to keep inbreeding as low as possible, and yet provide selection gain on milk yield, the optimum contribution selection should be implemented in practice. This selection strategy should guarantee selection gain with minimal loss of genetic variability, which is very important for long term viability of the breed (preservation of the “plasticity” of the genome which can respond to unpredictable changes of the environmental effects in the future).

Note

This study is extension of the study conducted by Bartol Smutni (final thesis, Bachelor's degree)

Acknowledgment

This study was Funded by Croatian Science Foundation (Genomic characterization, preservation and optimum contribution selection of Croatian dairy sheep, OPTI-SHEEP), grant number IP-2019-04-3559.

References

- Analla M., Montilla J.M., Serradilla J.M. (1998). Analyses of lamb weight and ewe litter size in various lines of Spanish Merino sheep. *Small Ruminant Research*. 29: 255-259.
- Babar M.E., Ahmad Z., Nadeem A., Yaqoob M. (2004). Environmental factors affecting birth weight in Lohi sheep. *Pakistan Veterinary Journal (Pakistan)*. 24: 5-8.
- Cortellari M., Negro A., Bionda A., Grande S., Cesarani A., Carta A., Macciotta N., Biffani S., Crepaldi P. (2022). Using Pedigree and Genomic Data toward Better Management of Inbreeding in Italian Dairy Sheep and Goat Breeds. *Animals*. 12: 2828.
- Dario C., and Bufano G. (2003). Efeito da endogamia sobre a producao de leite na raca ovina Altamurana.(Efecto de la endogamia sobre la produccion lactea en la raza ovina Altamurana). *Archivos de Zootecnia*, 401-404.
- Dowle M., and Srinivasan A. (2021). data.table: Extension of `data.frame`. R package version 1.14.2. <https://CRAN.R-project.org/package=data.table>
- Drobik W., and Martyniuk E. (2016). Inbreeding and its impact on the prolific Polish Olkusz sheep population. *Small Ruminant Research*. 137: 28-33.
- Držaić V., Širić I., Kasap A., Novoselec J., Klir Šalavardić Ž., Antunović Z., Mioč B. (2021). Utjecaj redosljedja janjenja na porodnu masu i prirast janjadi travničke pramenke. In *Proceedings of the 56. Croatian and 16. international symposium on agriculture*. Rozman V., Antunović Z (ed.), 584-588. Vodic, Croatia: Josip Juraj Strossmayer University of Osijek, Faculty of Agrobiotechnical Sciences Osijek.
- Ercanbrack S.K., and Knight A.D. (1991). Effects of inbreeding on reproduction and wool production of Rambouillet, Targhee, and Columbia ewes. *Journal of animal science*. 69: 4734-4744.
- Fox J., and Weisberg S. (2018). Visualizing Fit and Lack of Fit in Complex Regression Models with Predictor Effect Plots and Partial Residuals. *Journal of Statistical Software*. 87: 1–27.
- Granado-Tajada I., Rodríguez-Ramilo S.T., Legarra A., Ugarte E. (2020). Inbreeding, effective population size, and coancestry in the Latxa dairy sheep breed. *Journal of Dairy Science*. 103: 5215–5226.
- Grosjean P., and Ibanez F. (2018). pastecs: Package for Analysis of Space-Time Ecological Series. R package version 1.3.21. <https://CRAN.R-project.org/package=pastecs>

- Hebbali A. (2020). Descriptr. Available at: (Accessed December 2, 2022).
- Legarra A., Aguilar I., Misztal I. (2009). A relationship matrix including full pedigree and genomic information. *Journal of Dairy Science*. 92: 4656–4663.
- Lenth R.V. (2021). emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.7.1-1. <https://CRAN.R-project.org/package=emmeans>
- Maignel L., Boichard D., Verrier E. (1996). Genetic variability of French dairy breeds estimated from pedigree information. *Interbull Bulletin*. 14: 49–54.
- Meuwissen T. (2009). Genetic management of small populations: A review, *Acta Agriculturae Scandinavica, Section A — Animal Science*. 59: 71-79.
- R Core Team. (2020). R: A Language and Environment for Statistical Computing; R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org>
- Rodríguez-Ramilo S.T., Elsen J.M., Legarra A. (2019). Inbreeding and effective population size in French dairy sheep: Comparison between genomic and pedigree estimates. *Journal of Dairy Science*. 102: 4227–4237.
- Špehar M., Mulc D., Barać Z., Mioč B., Kasap A. (2020). Estimation of genetic parameters for dairy traits in Pag sheep with single and multi-trait test–day models. *Small Ruminant Research*. 183: 106029.
- Van Wyk J.B., Fair M.D., Cloete S.W.P. (2009). Case study: The effect of inbreeding on the production and reproduction traits in the Elsenburg Dormer sheep stud. *Livestock Science*. 120: 218-224.
- Wellmann R (2021). optiSel: Optimum Contribution Selection and Population Genetics. R package version 2.0.5. <https://CRAN.R-project.org/package=optiSel>
- Wickham H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York.
- Wickham H., Averick M., Bryan J., Chang W., D'Agostino McGowan L., François R., Golemund G., Hayer A., Henry L., Hester J., Kuhn M., Lin Pedersen T., Miller E., Milton Bache S., Müller K., Ooms J., Robinson D., Paige Seidel D., Spinu V., Takakashi K., Vaughan D., Wikle C., Woo K., Yutai H. (2019). Welcome to the tidyverse. *Journal of Open Source Software*. 4: 1686.

Morphometric characteristics of growing lambs as affected by litter size

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Abstract

The aim of the present study was to determine morphometric characteristics in growing lambs as affected by litter size. The study was conducted with 20 crossbred lambs (Tsigai × Ile de France, 8 twins and 12 singles) from birth to 90 days of age. Data on live weight, morphometric characteristics and indices were collected for each individual lamb after birth and at 30, 60 and 90 days of age. Litter size had an effect on morphometric characteristics, meaning that single lambs had a higher birth weights and grew faster than twin lambs. An effect of litter size needs to be included in the models used to analyse morphometric characteristics, especially during lamb growth.

Keywords: lambs, growth, morphometric characteristics, litter size

Introduction

World sheep production is mostly directed to meat production, especially lamb, which production is increasing in the world but decreasing in the European Union (EU). In 2021, the production of sheep meat amounted to 9,959,866.64 t in the world, 521,260 t in the EU and 5,610 t in the Republic of Croatia (Faostat, 2023). During the growth period, lambs are very sensitive and subjected to the effects of various non-genetic factors, especially feeding (Antunović et al., 2010). Live weight of lamb is one of the most important production traits used to monitor feeding strategies, veterinary issues, matting time, slaughter time and growth (Ibrahim et al., 2020), while morphometric characteristics provide a simple and objective way to assess body conformation and thus represent a very good trait for the usability of an animal (Marković et al., 2019). Results on phenotypic traits are very important for comparative studies on the body development of animals, and the study of morphometric characteristics allows the variation in the body size and shape to be analysed (Castillo et al., 2023). Litter size (LS) is defined as the number of lambs born per ewe lambing and is very important both from a zoological and economic point of view as it is important for the survival of the species and sheep production (Tao et al., 2021). On the other hand, litter size is one of the most important reproductive characteristics in terms of meat production, which has a direct impact on the profitability of lamb production, which is the most important sheep product in the Republic of Croatia (Kasap et al., 2016). The age of the dam and the number of lambs born and reared in the litter have an impact on body weight data, which is essential for accurate breeding value predictions in sheep destined for meat production (Notter and Brown, 2015).

The aim of the present study was therefore to determine morphometric characteristics, live weight and indices in growing lambs as affected by litter size.

Material and methods

The study was conducted at the farm of the Šmital family (Zbjeg near Slavonski Brod, Croatia). The study was carried out with 20 crossbred lambs between Tsigai and the Ile de France sheep breed for 90 days long period (from birth to the age of three months). Data on the live weight and morphometric characteristics of each lamb were collected up to 3 months of age. These measurements were taken during the growth of the lambs as follows: after birth and at 30, 60 and 90 days of age. Of the 20 lambs, 8 (3♂ and 5♀) were twins and 12 (4♂ and 8♀) singles. The gender ratio was similar between the groups. After lambing, the lambs were reared with their mothers and from 15 days of age had free access to good quality forage (hay of grass and clover mixture). From 30 days of age lambs were fed a mixture of

maize and oats twice a day and had free access to hay and fresh water. They occasionally grazed together with their mothers. Then the lambs were weaned at the age of 90 days.

The live weight (LW) of the lambs was determined using a livestock scale. The average daily weight gain (ADWG) of the lambs was estimated from the day 1 to 90 days of age according to the following equation: (final live weight - birth weight) / duration of the study. Lydtin's stick and measuring tape were used to determine the morphometric characteristics of body size. The wither height (WH) was measured with a Lydtin stick vertically from the ground behind the front hoof to the highest point of the withers. The body length (BL) was also measured with a Lydtin stick and was determined as the distance between the middle of the scapular-shoulder joint and the *tuber ischiadicum*. The width of the chest (CW) was measured with a Lydtin stick behind the shoulder and the depth of the chest (CD) from the highest point of the ridge to the lower edge of the sternum. The heart girth (HG) was measured with a measuring tape at the widest part of the chest, as was the body circumference (BC) at the widest part of the body. The leg circumference (LC) was measured with a measuring tape at the widest part of the leg and leg length (LL) was determined as a distance from the tuber calcanei to *tuber ischiadicum*. The shank circumference (SC) was measured in the middle of the right foreleg using a tape measure. Lambs were weighed a few hours after lambing to determine the birth weight of liveborn lambs. Then the lambs were weighed continuously every month until they were three months old, i.e. until they reached target slaughter weight. Based on morphometric characteristics, the indices were calculated according to Chiofalo et al. (2004) equations 1, 2, Ćinkulov et al. (2003) equations 3, 4, 5, and Marković et al. (2019) equation 6:

$$\text{Index of anamorphosis} = (\text{heart girth, cm})^2 / \text{wither height, cm} \quad /1/$$

$$\text{Index of body proportion} = (\text{body weight, kg} / \text{wither height, cm}) \times 100 \quad /2/$$

$$\text{Index of chest} = (\text{chest width, cm} / \text{chest depth, cm}) \times 100 \quad /3/$$

$$\text{Index of body compactness} = (\text{heart girth, cm} / \text{body length, cm}) \times 100 \quad /4/$$

$$\text{Index of muscularity} = (\text{heart girth, cm} / \text{wither height, cm}) \times 100 \quad /5/$$

$$\text{Index of body frame} = (\text{body length} / \text{wither height}) \times 100 \quad /6/$$

Data were analyzed with one-way ANOVA procedure of SAS[®]. Means were obtained using the MEANS procedure of SAS. Analysis of variance for all parameters (LW, WH, BL, CW, CD, HG, BC, LC, LL and SC) was carried out between both different litter size (single and twins) during growing period from birth to 90 days. Statistically significant differences in live weight and morphometric characteristics of growing lambs among litter size (singles or twins) were determined by the Tukey test. P-values were considered statistically significant when $p < 0.05$.

Results and discussion

The live weight and morphometric characteristics of the growing single and twin lambs are presented in Table 1. The most of traits shown were influenced by litter size, with the exception of CW and LC ($p \geq 0.07$) and SC in early age (≤ 30 days). Litter size had a greater influence on the birth weight of lambs, as reported by Ivanova and Raichev (2015), who found that the birth weight of single male lambs was 5.29 kg, and 4.65 kg in twin male lambs of the Ile de France breed. Compared to the present study, it can be seen that the present data show a very good LW of crossbreeds at birth, both in single and twin lambs. These significant differences continue to appear among singles and twins until up to 90 days of age. However, Ivanova and Raichev (2015) found a higher LW in lambs of Ile de France breed at 70 days of age, which differs from that of our study in singles (28.8 kg) and twins (22.16 kg). Birth weight is one of the most important factors influencing the growth of lambs until weaning, while lambs with higher birth weight gain weight faster (Chniter et al., 2009), which is in accordance with the present study where higher increase ($p < 0.05$) was found in lambs with higher birth weight. A lower LW could also be due to the behavior of twins and their mothers, as twin lambs suckle for a shorter time, while single lambs receive more attention from their mothers at birth and during lactation (Hinch, 1989), which means that singles have a stronger bond with their mothers compared to twins (Freitas-de-Melo et al., 2021). As reported by McGugh et al. (2017), the superior morphological development of single-born lambs at birth could not be caught up by multiple-born lambs, at least in the pre-weaning phase. The regular body development of lambs during rearing is determined by their body weight at birth, with single-born lambs usually having a higher body weight at birth and a faster growth rate than lambs from larger litters (Radzik-Rant et al., 2020).

Table 1. Effect of litter size on live weight and morphometric characteristics of growing Tsigai × Ile de France lambs

Trait, cm	Age of lambs, days				SEM	Litter size effect p-value
	1	30	60	90		
Live weight (LW), kg						
Single	5.28 ^a	11.45 ^a	16.58 ^a	23.08 ^a	0.705	0.023
Twins	4.46 ^b	9.85 ^b	12.10 ^b	16.94 ^b		
Wither height (WH), cm						
Single	40.04	45.71 ^a	51.63 ^a	55.04 ^a	0.638	0.042
Twins	38.94	43.69 ^b	47.94 ^b	51.31 ^b		
Body length (BL), cm						
Single	35.04 ^a	42.58 ^a	50.08 ^a	53.33 ^a	0.819	0.005
Twins	32.81 ^b	38.13 ^b	43.94 ^b	47.81 ^b		
Chest width (CW), cm						
Single	12.75	16.63	20.75	22.33	0.427	0.084
Twins	11.88	15.38	18.31	20.88		
Chest depth (CD), cm						
Single	10.33	14.83 ^a	16.29 ^a	17.25 ^a	0.308	0.035
Twins	9.63	13.38 ^b	14.81 ^b	15.63 ^b		
Heart girth (HG), cm						
Single	41.88 ^a	51.38 ^a	61.04 ^a	65.08 ^a	1.012	0.009
Twins	38.63 ^b	46.81 ^b	54.81 ^b	57.69 ^b		
Body circumference (BC), cm						
Single	42.38 ^a	55.83 ^a	66.50 ^a	70.92 ^a	1.208	0.010
Twins	39.75 ^b	51.25 ^b	58.25 ^b	61.25 ^b		
Leg circumference (LC), cm						
Single	22.75	29.92	36.58	40.17	0.751	0.068
Twins	21.00	27.86	33.25	36.13		
Leg length (LL), cm						
Single	17.33	19.33 ^a	22.00 ^a	25.42 ^a	0.330	0.042
Twins	17.19	18.44 ^b	20.13 ^b	22.88 ^b		
Shank circumference (SC), cm						
Single	7.53	7.74	7.92 ^a	8.13 ^a	0.028	0.022
Twins	7.40	7.68	7.80 ^b	7.94 ^b		

^{a, b} Values with different letters within the row are significant ($P < 0.05$); SEM-standard error of mean.

Body length, HG and BC differed between single and twin lambs at all ages, from birth to 90 days of age, which were higher in the single lambs ($p < 0.05$). It is well known that LW of small ruminants can be predicted by HG, BL and WH, therefore these characteristics are expected to follow the changes in LW. Accordingly, Markos et al. (2023) reported the highest correlation between LW and HG in both male and female lambs ($r=0.84$), while Klir Šalavardić et al. (2022) found the highest positive correlation between LW and HG ($r=0.97$) in goat kids compared to other correlations between morphometric characteristics and LW. Withers height, CD and LL differed between growing single and twin lambs at 30 days of age, being higher in single lambs ($p < 0.05$). Shank circumference differed ($p < 0.05$) between single and twin lambs only at 60 days of age.

Table 2 shows the morphometric indices of the growing single and twin lambs. The indices for body compactness

and chest did not differ ($p \geq 0.81$) depending on litter size. It is evident that some indices were higher in single lambs than in twins, such as the index of anamorphosis and body proportion. The muscularity index was higher in singles only after birth and at 90 days of age. A higher anamorphosis index, also called Baron-Crevat index or index of conformation (Marković et al., 2019), results from a better development of the respiratory and digestive system, while a higher body proportion index indicates a longitudinal development of the skeletal structure (Chiofalo et al., 2004). As reported by Marković et al. (2019), the higher the index of anamorphosis, the more robust the animal. Since the compactness index did not differ between singles and twins, this means that the muscle tissue content in the body was similar (Yáñez et al., 2004). The body frame index varied between 84.33 to 97.04 during growth, differing significantly only at 60 days of age and being higher in the single lambs. The body frame was rather short or breviline, the higher it gets, the more square the shape of the lambs (body frame index 97-103) (Marković et al., 2019).

Table 2. Effect of litter size on morphometric indices of growing Tsigai \times Ile de France lambs

Index	Age of lambs, days				SEM	Litter size effect p-value
	1	30	60	90		
Index of anamorphosis						
Single	43.82 ^a	57.89 ^a	72.34 ^a	76.98 ^a	1.511	0.005
Twins	38.50 ^b	50.43 ^b	62.87 ^b	64.90 ^b		
Index of body proportion						
Single	13.17 ^a	25.08	32.08 ^a	41.89 ^a	1.155	0.034
Twins	11.50 ^b	22.58	25.27 ^b	33.01 ^b		
Index of chest						
Single	123.86	112.58	127.64	129.59	1.167	0.810
Twins	123.52	115.19	123.57	133.70		
Index of body compactness						
Single	120.00	121.04	122.04	122.25	0.706	0.879
Twins	117.74	122.81	124.88	120.78		
Index of muscularity						
Single	104.61 ^a	112.65	118.29	118.25 ^a	0.893	0.005
Twins	99.34 ^b	107.30	114.48	112.44 ^b		
Index of body frame						
Single	87.55	93.36	97.04 ^a	96.92	0.741	0.002
Twins	84.33	87.35	91.72 ^b	93.18		

^{a, b} Values with different letters within the row are significant ($P < 0.05$); SEM-standard error of mean.

The total ADWG from birth to 90 days of age ($p < 0.05$) for single lambs was 197.87 g/day, while for twins it was 138.61 g/day (Figure 1). In the research of Ivanova and Raichev (2015), the ADWG from age of 30 to 70 days for single Ile de France male lambs was 344 g/day compared to twins, whose average ADWG was 265 g/day. The results of the present study showed that a significant difference in ADWG occurred at 30 days of age, being higher in single lambs than in twins. The highest growth was observed between the 60th and 90th day in single lambs and in the first 30 days in twins. The results showed that the lambs exhibited very high growth intensity during these periods. This can be explained by the high milk yield and the composition of the suckled milk at the age of the first days, as well as the supplementary feeding of the lambs (Panayotov et al., 2018) with hay *ad libitum* after the age of two weeks. In addition, after 30 days of age they were gradually fed a mixture of corn and oats as ADWG decreased, possibly due to adaptation to different diet such as concentrates and forage. However, after 60 days of age, ADWG increased indicating that the lambs had fully adapted to the solid feed.

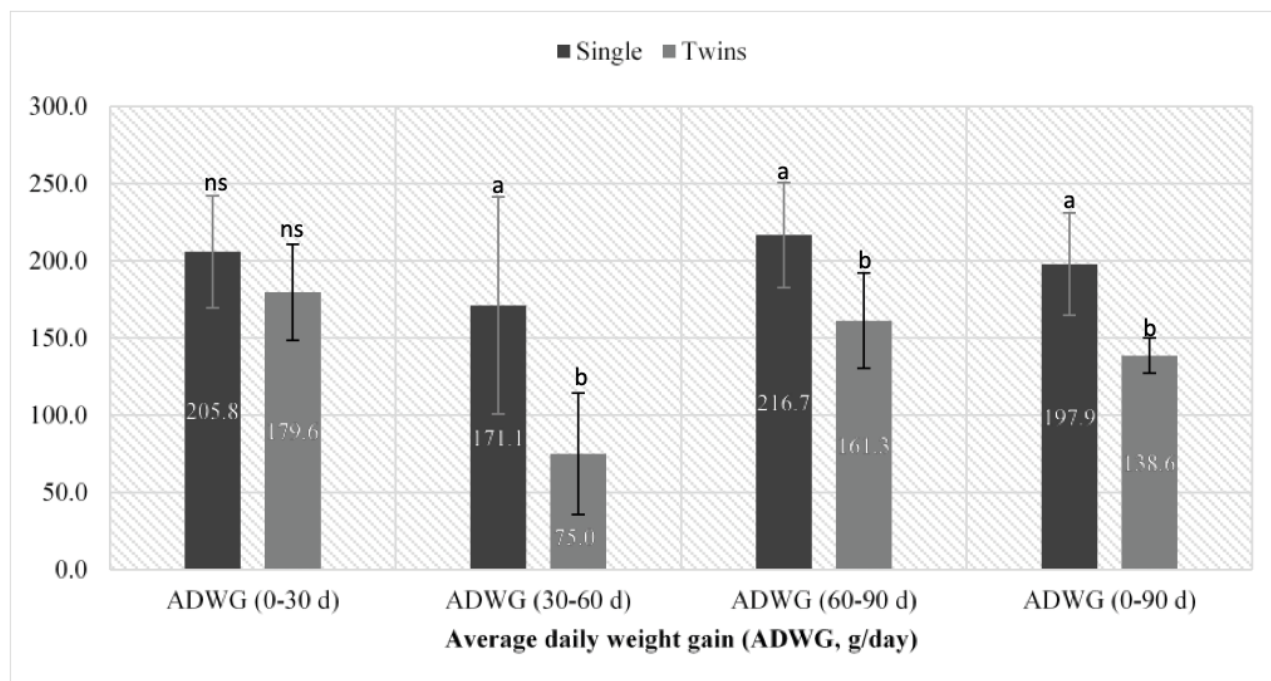


Figure 1. Average daily weight gain of single and twin Tsigai \times Ile de France lambs during different growth periods (0-30 days; 30-60 days; 60-90 days and 0-90 days); a, b-values with different letters are significant ($P < 0.05$), ns-not significant.

Conclusion

Litter size has a reliable influence on live weight and average daily weight gain as well as on the morphometric characteristics and their indices of growing lambs from birth to 90 days of age. Data confirmed that single lambs had a higher birth weight and grew faster compared to twin lambs, which was confirmed by higher values of morphometric characteristics and some indices such as the index of anamorphosis and the index of body proportion, which can determine the further breeding value for production traits. Therefore, the effect of litter size must be included in any model that analyzes morphometric characteristics, especially during lamb growth.

References

- Antunović Z., Šperanda M., Novoselec J., Đidara M. (2010). Nutrition of lambs and acid-base balance. *Krmiva*. 52: 333-338.
- Castillo P.E., Macedo R.J., Arredondo V., Zepeda J.L., Valencia-Posadas M., Haubi C.U. (2023). Morphological description and live weight prediction from body measurements of Socorro Island Merino lambs. *Animals*. 13: 1978.
- Chiofalo V., Liotta L., Chiofalo B. (2004). Effects of the administration of Lactobacilli on body growth and on the metabolic profile in growing Maltese goat kids. *Reproduction Nutrition Development*. 44: 449-457.
- Chniter M., Maali S., Hammadi M., Khorchani T., Harab H., Krit R., Hamouda M.B., Khaldi G., Nowak R. (2009). Effects of dam age, litter size and gender on birth weight of D'man lamb -consequence on lamb mortality. *Journal of Arid Land Studies*. 19-1: 169-172.
- Činkulov M., Krajinović M., Pihler I. (2003). Phenotypic differences between two types of Tsigai breed of sheep. *Lucrari științifice Zootnie și Biotehnologii*. 36: 395-299.
- Faostat (2023). Available online: <http://www.fao.org/faostat/en/#data/QP> (accessed on 26th October 2023).

- Freitas-de-Melo A., Ungerfeld R., Orihuela A. (2021). Behavioral and physiological responses to early weaning in ewes and their single or twin lambs. *Tropical Animal Health and Production*. 53: 150.
- Hinch G.N. (1989). The sucking behaviour of triplet, twin and single lambs at pasture. *Applied Animal Behaviour Science*. 22: 39–48.
- Ibrahim A., Budisatria I.G.S., Widayantim R., Atmoko B.A., Yuniawan R., Artama W.T. (2020). On-farm body measurements and evaluation of Batur Sheep on different age and sex in Banjarnegara District, Indonesia. *Advances in Animal and Veterinary Sciences*. 8: 1028–1033.
- Ivanova T., and Raicheva E. (2015). Weight growth of Ile de France lambs according to the genealogy line. *Bulgarian Journal of Agricultural Science*. 21: 409–412.
- Kasap A., Špehar M., Kaić A., Mioč B. (2016). Estimation of variance components for litter size in Romanov sheep. *Proceedings of the 51st Croatian and 11th International Symposium on Agriculture*, Pospišil M., Vnučec I. (ed.), 343-346. Opatija, Croatia: University of Zagreb, Faculty of Agriculture, Zagreb, Croatia.
- Klir Šalavardić Ž., Novoselec J., Ronta M., Antunović Z. (2022). Influence of pumpkin seed cake and extruded linseed on production traits of goat kids. *Krmiva*. 64: 13-22.
- Markos D., Masho W., Baye M., Bayou E. (2023). Morphometric traits and stepwise regression of indigenous sheep in southwestern region; Ethiopia. *Journal of Agriculture and Food Research*. 14: 100666.
- Marković B., Dovč P., Marković M., Radonjić D., Adakalić M., Simčić M. (2019). Differentiation of some Pramenka sheep breeds based on morphometric characteristics. *Archives Animal Breeding*. 62: 393-402.
- McHugh N., Pabiou T., McDermott K., Wall E., Berry D.P. (2017). Impact of birth and rearing type, as well as inaccuracy of recording, on pre-weaning lamb phenotypic and genetic merit for live weight. *Translational Animal Science*. 1:137–145.
- Notter D.R., and Brown D.J. (2015). Effects of birth-rearing type on weaning weights in meat sheep are systematically associated with differences in mean performance among flocks. *Genetics Selection Evolution*. 47: 57.
- Panayotov D., Sevov S., Georgiev D. (2018). Live weight and intensity of growth of lambs from Lacaune breed raised in Bulgaria. *Bulgarian Journal of Agricultural Science*. 24: 88-94.
- Radzik-Rant A., Rant W., Bryjak A., Niżnikowski R. (2020). The influence of birth type and sex on rearing of Polish Heath and Żelaźnieńska lambs. *Roczniki Naukowe Polskiego Towarzystwa Zootechnicznego*. 16: 9-20.
- Tao L., He X., Wang X., Di R., Chu M. (2021). Litter size of sheep (*Ovis aries*): Inbreeding depression and homozygous regions. *Genes*. 12: 109.
- Yáñez E.A., Ferreira A.C.D., Medeiros A.N., Pereira Filho J.M., Teixeira I.A.M.A., Resende K.T. (2004). Methodologies for ribeye area determination in goat. *Small Ruminant Research*. 66: 197-200.

Precizno svinogojstvo

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Sažetak

Precizna stočarska proizvodnja opisuje sustav upravljanja temeljen na neprekidnom automatskom praćenju i kontroli proizvodnje, reprodukcije, zdravlja i dobrobiti svinja u stvarnom vremenu te primjenu umjetne inteligencije. Danas postoji širok raspon dostupnih tehnologija, poput 2D i 3D kamera za procjenu tjelesne težine, ponašanja i aktivnosti, termalnih kamera za praćenje tjelesne temperature i utvrđivanje estrusa, mikrofona za praćenje kašlja i vokalizacije, različitih mjernih stanica za praćenje unosa hrane, tjelesne težine i hoda, radio frekvencijske identifikacijske tehnologije za individualnu identifikaciju i praćenje te brojne druge koje pomažu uzgajivačima u unaprijeđenju svinjogojske proizvodnje. Precizna svinjogojska proizvodnja pruža učinkovito i održivo korištenje resursa smanjenjem otpada, omogućuje rano otkrivanje bolesti na osnovu promjene aktivnosti i ponašanja životinja praćenih kamerama i različitim sensorima, pruža zaštitu životinja od okolišnih stresora te smanjuje manipulacije od strane čovjeka čime se poboljšava njihova dobrobit.

Ključne riječi: svinje, precizna poljoprivreda, upravljanje, dobrobit

Uvod

Uslijed kontinuiranog porasta broja stanovnika i povećanih potreba za proizvodima animalnog podrijetla, neophodno je primjenjivati nove tehnologije i prakse poput preciznog uzgoja stoke (PLF) kako bi svinjogojska proizvodnja bila održiva.

Intenzivan uzgoj svinja zahtijeva kontinuirani nadzor proizvodnje u svrhu prikupljanja informacija i praćenja zdravstvenog stanja te proizvodnih karakteristika životinja s ciljem ostvarenja boljih proizvodnih rezultata, veće ekonomske dobiti te osiguranja dobrobiti životinja. Precizno stočarstvo obuhvaća skup tehnologija praćenja u stvarnom vremenu usmjerenih na upravljanje vremenskom varijabilnošću najmanje upravljive proizvodne jedinice. Stoga, sustavi precizne stočarske proizvodnje teže postizanju potpuno automatiziranog kontinuiranog praćenja životinja primjenom novih tehnologija. Potrebne podatke moguće je prikupljati primjenom kamera, mikrofona, sustava za analizu zvuka ili bilo kojim drugim sensorom unutar proizvodne jedinice ili pak na životinji primjenom radio frekvencijske identifikacijske tehnologije, odnosno RFID senzora, akcelerometra i slično. Prikupljeni se podaci obrađuju i pohranjuju u sustave čime se dobiva cjelokupan pregled trenutnog stanja svake životinje na pojedinoj farmi. Jedan od ciljeva razvoja sustava precizne stočarske proizvodnje ogleda se u kontinuiranom i automatskom praćenju životinja u svrhu podrške poljoprivrednicima u upravljanju proizvodnjom kao što su strategije hranidbe, kontrola stope rasta i upravljanje zdravljem. S druge strane, benefiti ovoga sustava ogledaju se u smanjenju štetnih utjecaja na okoliš te povećanju profitabilnosti, učinkovitosti i održivosti farmi. Osim navedenog, u zadnje vrijeme sve je šira primjena umjetna inteligencije (AI) koja se može primijeniti na različite aspekte proizvodnje svinja kako bi se poboljšala učinkovitost, produktivnost i cjelokupno upravljanje. AI algoritmi mogu analizirati ponašanje svinja kako bi otkrili znakove bolesti ili nevolje. Promjene u obrascima kretanja ili prehranbenim navikama mogu ukazivati na zdravstvene probleme. Također, AI algoritmi mogu analizirati podatke iz različitih izvora, kao što su uvjeti okoliša, ponašanje svinja i zdravstveni zapisi, kako bi predvidjeli i spriječili izbijanje bolesti. Analizom povijesnih podataka, umjetna inteligencija može predvidjeti stope rasta pojedinačnih svinja, omogućujući optimiziranu praksu hranjenja i upravljanja Wang i sur., 2022).

Cilj ovoga rada je pobliže objasniti utjecaj precizne poljoprivredne proizvodnje na sektor svinjogojstva. U radu su opisane informacijsko komunikacijske tehnologije koje se primjenjuju u sektoru svinjogojstva, proizvodni procesi u kojima se primjenjuju te perspektiva razvoja svinjogojske proizvodnje njihovom primjenom.

Precizno svinjogojstvo

Danas postoji širok raspon dostupnih tehnologija osmišljenih za optimizaciju proizvodnih procesa u svinjogojskom sektoru (Benjamin i Yik, 2019), a neke od njih su: kamere za procjenu tjelesne težine, ponašanja i aktivnosti, termalne kamere za tjelesnu temperaturu, različite mjerne stanice za praćenje unosa hrane, tjelesne težine i hoda, mikrofoni za praćenje kašlja i vokalizacije, akcelerometri za aktivnost, senzori za utvrđivanje hromosti, radio frekvencijska identifikacija za individualnu identifikaciju i praćenje, GPS za lokaciju, bežični komunikacijski alati, internetske veze i pohrana u oblaku te brojni drugi. Prema Berckmansu (2014) kombinacijom svih raspoloživih hardvera s inteligentnim softverom moguće je dobiti širok raspon podataka na osnovu kojih se poljoprivredniku omogućuje automatsko praćenje životinja i stvaranje dodane vrijednosti pomažući u osiguravanju poboljšanog zdravlja, dobrobiti, prinosa i utjecaja na okoliš. Kroz tehnološku implementaciju, podaci se mogu prikupljati automatski pomoću kućnih upravljačkih računala ili unutar računala farme. Međutim, zbog slabije educiranosti o upravljanju pametnim tehnologijama i namjerama korištenja istih, većini uzgajivača svinja nedostaju potrebne informacije za ekonomično vođenje farme (Mahfuz i sur., 2022).

Primjena tehnologija precizne stočarske proizvodnje funkcionira na način da kada svinja u oboru doživi nepovoljne uvjete, javljaju se promjene u ponašanju kao biološki odgovor organizma na podražaj. Nakon toga, PLF sustav putem već spomenutih senzora uočava promjene čime utvrđuje elemente odgovorne za nastali problem. Zahvaljujući mogućnosti pojedinačnog nadzora, PLF pruža stalan dotok informacija o svakoj životinji. Analizom prikupljenih i dokumentiranih podataka pohranjenih u računalo tijekom određenog vremenskog razdoblja, moguće je izgraditi automatski klasifikator koji klasificira obrasce koji dovode do razlika u ponašanju zbog neprikladnih uvjeta (Berckmans, 2009), što također može poslužiti i za daljnju vizualizaciju situacije. Naime, od iznimnog je značaja pravilno dekodiranje i procjena vrijednosti prikupljenih podataka jer je zahvaljujući tome biološke reakcije životinja moguće koristiti kao senzor za dobivanje informacija o okolišu i podražajima.

Tehnologije preciznog svinjogojstva

Informacijsko komunikacijska tehnologija (Information and Communication Technology, ICT), podrazumijeva primjenu različitih uređaja, alata ili aplikacija za prikupljanje i razmjenu podataka. Primjena tehnoloških inovacija doprinosi razvoju precizne stočarske proizvodnje, a samim time i sektoru svinjogojstva. Svinjogojstvo danas, osim razvoju infrastrukture, veliku pozornost pridodaje primjeni tehnoloških znanja u svrhu praćenja i kontrole hranidbe, zdravlja, dobrobiti, reprodukcije te proizvodnih performansi životinja. Prema Mahfuz i sur. (2022) primjena ICT-a predstavlja važnu komunikaciju između farmera i posrednika povezanih s tržištem i potrošačima, što poboljšava proizvodnju te omogućuje sigurnost i sljedivost hrane.

Pametne tehnologije temeljene na ICT-u obuhvaćaju pametne ušne markice, senzore, IoT, dubinsko učenje, velike podatke i robotske sustave koji mogu izravno sudjelovati u radu poljoprivrednih aktivnosti te predstavljaju učinkovite alate za prikupljanje, obradu i analizu podataka s farmi (Mahfuz i sur., 2022). Budući da proizvodni rezultati ovise o različitim uvjetima držanja svinja, Connolly (2018) ističe kako digitalne tehnologije mogu biti iznimno korisne jer pružaju mogućnost poboljšanja i povećanja učinkovitosti proizvodnje uz povećanje stope sigurnosti.

Primjenom ICT tehnologije u uzgoju svinja, svi su parametri pod nadzorom i kontrolom softverskih aplikacija ili programabilnih inteligentnih računala (PIC) te je moguće njima upravljati. Kako Mahfuz i sur. (2022) navode, IoT tehnologija se također može koristiti za utjecaj na uzgoj svinja korištenjem senzora u stvarnom vremenu, analize podataka, informacijskih tehnologija i donošenjem odluka za poboljšanje zdravlja i dobrobiti svinja te učinkovitosti proizvodnje.

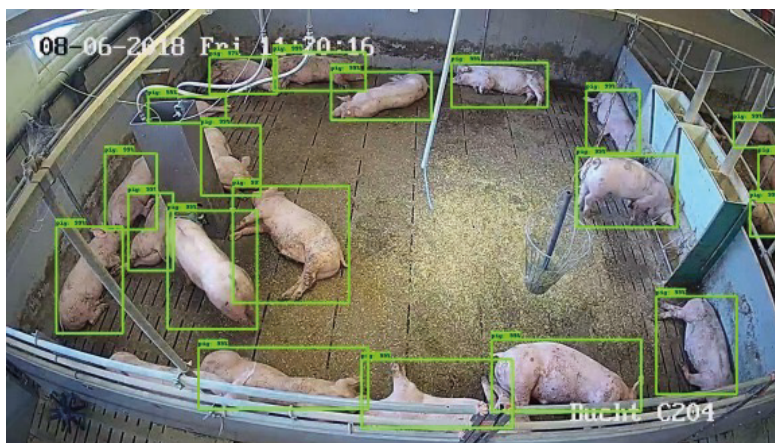
Senzori

senzori poput kamera, mikrofona, termometara te brojnih drugih, rade na principu prikupljanja okolišnih podražaja, odnosno „inputa“ poput topline, vlage, svjetlosti, kretanja i tome sličnih pojava. Prikupljeni se podaci pohranjuju u vanjskim pogonima ili ih senzori šalju na glavni procesor gdje se kombiniraju s pojedinačnim identifikacijama životinja, referenciranim opažanjima i proizvodnim podacima. Podaci se potom integriraju u algoritme za pružanje relevantnih informacija i upozorenja o zdravlju, produktivnosti i dobrobiti svinja. Algoritam čini određeni skup definiranih naredbi, operacija ili formula koji se koristi za rješavanje određenog problema ili skupine problema. U preciznoj se stočarskoj proizvodnji primjenjuju programski algoritmi. To su računalni postupci koji računalo

precizno kazuju koje je korake neophodno primijeniti u svrhu rješavanja problema. Sama vrijednost algoritma za farmere ovisi o njegovoj sposobnosti transformacije podataka sa senzora, odnosno značajne ili „input varijable“ u biološki ishod, odnosno ciljane ili „output varijable“. Output varijabla čini izlazne signale koji se pretvaraju u čitljivi podatak na zaslonu senzora koji je vidljiv i razumljiv čovjeku. Primjer značajnih varijabli može biti postotak vremena koji svinja provodi u ležanju kako bi se utvrdio biološki ishod hromosti ili pak frekventnost kašljanja za otkrivanje biološkog ishoda respiratorne bolesti.

Kamere

Praćenje kamerom se primjenjuje za detaljna promatranja učestalosti pojavljivanja određenih reakcija ponašanja, duljini trajanja te brzini njihovih izvođenja. Osim toga, kamere su od iznimnog značaja jer su u odnosu na druge senzorske sustave svima dostupne te na lak i jednostavan način prikupljaju informacije. Za proučavanje pojedinačnih bioloških odgovora poput agresivnih ili nekih drugih obrazaca ponašanja, snimke je moguće analizirati u usporenoj snimci ili pak kadar po kadar. Suprotno tome, ukoliko je potrebno provesti opću analizu aktivnosti kao što je klasificiranje ponašanja životinje u stajaćem i ležećem položaju, snimljeno se ponašanje može proučavati na ubrzanoj snimci. Danas su na tržištu dostupne brojne vrste kamere koje nude različite podatke i parametre slike, a neke od njih su CCD kamere, infracrvene, termalne, dubinske kamere te brojne druge. CCD kamere pružaju digitalne informacije, a razlikuju se dvodimenzionalne (2D) i trodimenzionalne (3D) (Marchant i sur., 1999). Njihovim postavljanjem iznad svakog boksa moguće je pružiti puno vidno polje (FoV) što uvelike ovisi o veličini boksa i specifikacijama pojedine kamere.



Slika 1. Automatsko otkrivanje položaja i držanja svinja pomoću 2D kamere
(Izvor: Riekert i sur., 2020)

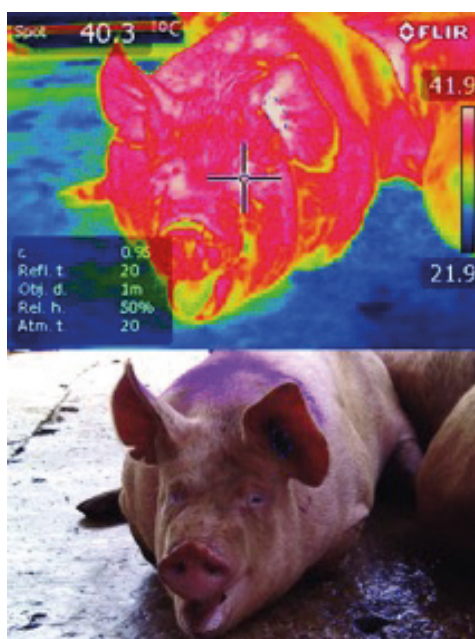
Navedeno je dovoljno učinkovito za potrebno prikupljanje slikovnih podataka, dok su istovremeno troškovi instalacije sustava svedeni na najmanju moguću razinu (Berckmans, 2014). Sustavi praćenja u stvarnom vremenu već su primijenjeni za istraživanje i kontrolu ponašanja pri hranjenju, za procjenu težine, za praćenje ponašanja tijekom konzumacije vode, identifikaciju i automatsko brojanje svinja te moguće sprječavanje agresivnih interakcija odvratanjem pozornosti (Tzanidakis i sur., 2021).

Mikrofoni

Mikrofoni su uređaji koji se koriste za proučavanje vokalizacije s ciljem lakše procjene dobrobiti životinja. Oni rade na principu prikupljanja zvučnih podražaja iz okoline pretvarajući ih u električne signale koji se potom obrađuju u računalnim sustavima. Kako Benjamin i Yik (2019) ističu, pravilnim prepoznavanjem vokalizacije moguće je rano utvrđivanje te pravovremeno otklanjanje štetnih čimbenika unutar pojedinih skupina životinja. Vokalizacija životinje može ukazivati na agresivno ponašanje unutar pojedinog boksa, može biti naznaka stresa ili signal pojave bolesti stoga se smatra jednim od indikatora psihološkog i fiziološkog stanja životinje.

Infracrvena termografija (IRT)

Budući da su svinje homeotermne životinje, emitiraju toplinu kroz temperaturne varijacije koje se mogu brzo identificirati pomoću toplinskih slika, odnosno infracrvenim kamerama. Infracrvena termografija (Infrared thermograph, IRT) je brza, beskontaktna i neinvazivna metoda praćenja temperature na površini nekoga tijela, objekta ili predmeta pomoću kamera, koje utvrđuju karakteristično infracrveno i toplinsko zračenje, dok CCD kamere mjere zračenje vidljivih traka (Arulmozhi i sur., 2021). Prema Kolarek (2018), infracrvene termovizijske kamere prikupljaju podatke o temperaturi objekta koje snima putem termičkog senzora, nakon čega slijedi elektronička obrada podataka. Nakon provedene obrade podataka izmjerene temperature objekta ili životinje, na zaslonu termovizijske kamere se prikazuju podaci u obliku infracrvene slike ili grafičkog prikaza koji se naziva termogram. Termogram se zatim računalnim programima detaljnije analizira, dok suvremene infracrvene kamere automatski pružaju osnovnu obradu.



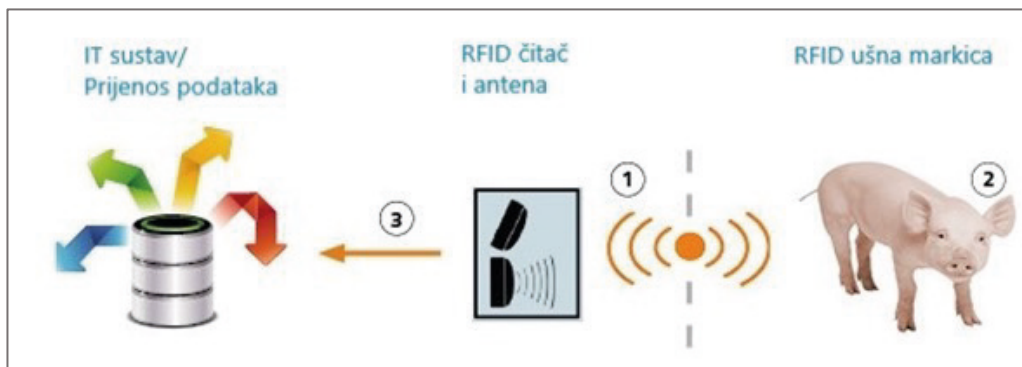
Slika 2. Primjena termovizijske kamere u svinjogojstvu
(Izvor: Flores-Peinado i sur., 2020)

U preciznoj se svinjogojskoj proizvodnji, prema Salles i sur. (2016), infracrvene termovizijske kamere koriste jer imaju mogućnost mjerenja promjene tjelesne temperature uzrokovane fiziološkim i patološkim procesima čime je moguće rano otkrivanje bolesti životinja ili rano utvrđivanje estrusa kod krmača i nazimica. Unatoč tomu, takve su kamere relativno skupe, stoga su i manje zastupljene u uzgoju.

Radio frekvencijska identifikacijska tehnologija (RFID)

Radio frekvencijska identifikacijska tehnologija (Radio Frequency Identification, RFID), predstavlja tehnički sustav koji pruža automatsko i beskontaktno prepoznavanje proizvoda, ljudi i životinja putem radio valova. U preciznoj se stočarskoj proizvodnji primjenjuje s ciljem praćenja životinja tijekom cjelokupnog proizvodnog vijeka. Ovaj se sustav, naime, sastoji od RFID oznake, čitača ili antene i sustava za obradu podataka, a radi na način da oznaka emitira signal čitaču putem mikročipa i namotane bakrene antene, nakon čega u sustavu upravljanja nastupa obrada podataka i započinje praćenje životinje. Prednosti RFID sustava ogledaju se u jednostavnom mehanizmu, niskoj cijeni koštanja i pouzdanoj korelaciji za identifikaciju objekta, no s druge strane, RFID ima ograničen domet, nema mogućnost istovremenog očitavanja više oznaka što smanjuje pouzdanost podataka zbog razigranosti i aktivnosti svinja koje se često nalaze okupljene u grupama (Arulmozhi i sur., 2021). Negativna strana je i ta što RFID s ušnom markicom, koji je ujedno i najzastupljeniji u uzgoju svinja, može postati nečitljiv uslijed prljanja, lomljenja ili istrošenosti, a nerijetko se mogu izgubiti zbog kidanja uha tijekom borbe ili igre. Stoga, RFID oznake moraju biti iznimno otporne jer su

često izložene ekstremnim vrućinama i hladnoći. U pogledu dobrobiti životinja RFID sustavi lošom primjenom mogu uzrokovati oštećenja uha i infekcije budući da se ušne markice mogu prenositi s jedne životinje na drugu.



Slika 3. Princip rada RFID (Izvor: www.purespekt.eu)

Akcelerometri

Prema Benjamin i Yik (2019) jedna od najboljih tehnologija za praćenje kretanja i ponašanja životinja su nosivi senzori koji sadrže akcelerometre. Akcelerometri su elektromehanički uređaji koji se koriste za mjerenje sile ubrzanja kao što su sile gravitacije i sile akceleracije. Naime, sile gravitacije su statične sile koje se očituju kada svinja leži, dok su sile akceleracije sile ubrzanja nastale uslijed kretanja životinja. Njihova se primjena u svinjogojstvu provodi s ciljem praćenja ponašanja životinja, ranog otkrivanja estrusa kod krmača i nazimica te utvrđivanja hromosti.

Primjena preciznog svinjogojstva u praksi

Prema Tzanidakis i sur. (2021) posljednjih su godina u svinjogojstvu uvedeni različiti sustavi temeljeni na kamerama, a razvojem tehnologije, sve je zastupljenija njihova primjena s poboljšanim rezultatima u praćenju i otkrivanju specifičnih parametara od interesa. Dosadašnjim je istraživanjima predložena identifikacija ponašanja životinja prilikom kretanja pomoću 2D kamera, IR kamera, GoPro ili web kamera (Kongsro, 2018). Posljednjih se godina, modernizacijom svinjogojstva nastoji označavanje svinja preusmjeriti na primjenu elektroničkih identifikacijskih uređaja među kojima je RFID, odnosno radio frekvencijski identifikator koji se ujedno i najviše primjenjuje, osobito u obliku ušnih markica. Kako Arulmozhi i sur. (2021) u svojem radu prema Granatosky (2020) i Supakorn i sur. (2018) ističu, kretanje se definira kao hodanje svinja, dok se nedostatak kretanja kao i svaka abnormalnost u kretanju naziva hromost ili šepavost. Hromost može biti uzrokovana načinom držanja, vrstom poda, higijenskim čimbenicima, nedostatkom adekvatne hranidbe ili može biti genetski uvjetovana (Arulmozhi, 2021). Pojavom hromosti značajno se narušava dobrobit svinja, a osim toga iziskuje značajne ekonomske gubitke. Stoga je od iznimnog značaja provoditi preventivne mjere u svrhu sprječavanja njenog nastanka, no ukoliko se ista pojavi, pravovremenim otkrivanjem i liječenjem se mogu spriječiti veće poteškoće pa i sami gubici. Procjena tjelesne težine svinja od iznimnog je značaja za praćenje proizvodnog ciklusa. Danas se procjena radi preko potpuno automatiziranog sustava primjenom 3D kamera s ciljem lakšeg praćenja žive težine i prirasta. Prema navodima Kongsro (2014), a kasnije i Cang i sur. (2019), 3D kamere rade na principu prikupljanja dubinskih i infracrvenih informacija putem senzora procjenjujući težinu s relativnom pogreškom 0,374 – 4,6 % čime je postignuta pouzdana 3D validacija na temelju dubinskih kamera u odnosu na 2D/CCD slike. Utvrđivanje tjelesne temperature svinja također je vrlo bitna stavka u praćenju proizvodnog procesa. Termalne se kamere koriste u raznim područjima svinjogojstva s ciljem otkrivanja bolesti i estrusa na temelju analize tjelesne temperature, učinkovitog praćenja zdravlja i dobrobiti. Osim toga, služe i kao alat za utvrđivanje tjelesne temperature umjesto rektalnih termometara, za otkrivanje upala i lezija te utvrđivanje čimbenika degradacije dobrobiti kao što su koncentracije plina i prašine nastale kao rezultat društvene aktivnosti. Naime, važno je zaključiti da se prednosti ovog sustava ogledaju u visokoj temperaturnoj točnosti, stabilnosti te jednostavnosti sustava. Mogućnost daljinskog rada u stvarnom vremenu uvelike olakšava primjenu istog na intenzivnim svinjogojstkim farmama. Kako Berckmans (2014) navodi, za posljedicu ima poboljšanje dobrobiti i produktivnosti svinja te optimiziranje korištenja izvora energije i hrane čime se poboljšava ekonomski status svinja. Infracrvena termografija predstavlja nedestruktivnu i beskontaktnu

metodu za određivanje tjelesne temperature. Osim toga, tehnologija digitalnog infracrvenog termalnog snimanja (DITI) može lako prepoznati i detektirati promjene temperature životinja (Mun i sur., 2022), stoga bi takve kamere mogle biti nova tehnologija za utvrđivanje estrusa kod krmača i nazimica. Njihovom bi se primjenom postotak uspješnosti utvrđivanja estrusa i pravovremenog osjemenjivanja krmača i nazimica uvelike povećao.

Zaključak

Danas precizna stočarska proizvodnja nastoji postići potpuno automatizirano kontinuirano praćenje svinja, koristeći tehnološka dostignuća kao dio procesa upravljanja. Stoga se posljednjih godina bilježi sve veći porast zainteresiranosti uzgajivača svinja za istu, no unatoč tomu još uvijek nije u širokoj primjeni. Precizna svinjogojska proizvodnja pruža učinkovito i održivo korištenje resursa smanjenjem otpada, omogućuje rano otkrivanje bolesti na osnovu promjene aktivnosti i ponašanja životinja praćenih kamerama i različitim sensorima, pruža zaštitu životinja od okolišnih stresora te smanjuje manipulacije od strane čovjeka čime se poboljšava njihova dobrobit. Stoga su njen razvoj i napredak od iznimnog značaja za bolju budućnost svinjogojske proizvodnje. Također, uspješna implementacija umjetne inteligencije u svinjogojskoj proizvodnji zahtijeva kombinaciju prikupljanja podataka, povezanosti i napredne analitike. Nadalje, etička razmatranja, privatnost podataka i dobrobit životinja treba uzeti u obzir pri integraciji AI tehnologija u uzgoju svinja.

Literatura

- Arulmozhi E., Bhujel A., Moon B.E., Kim H.T. (2021). The application of cameras in precision pig farming: An overview for swine-keeping professionals. *Animals*. 11: (8).
- Benjamin M., and Yik, S. (2019). Precision livestock farming in swine welfare: a review for swine practitioners. *Animals*. 9: 133.
- Berckmans D. (2014). Precision livestock farming technologies for welfare management in intensive livestock systems. *Revue scientifique et technique*. 33: 189–196.
- Cang Y., He H., Qiao Y. (2019). An Intelligent Pig Weights Estimate Method Based on Deep Learning in Sow Stall Environments. *IEEE Access*, 7.
- Connolly A. (2018.). Unlocking the Potential in Pigs with Digital Technology. Objavljeno 7. svibnja 2018. Dostupno na: <https://www.linkedin.com/pulse/unlocking-potential-pigs-digital-technology-aidan-connolly>. Pristupljeno: 30.3.2023.
- Flores-Peinado S., Mota-Rojas D., Trujillo M.E., Mora-Medina P. (2020). Physiological responses of pigs to preslaughter handling: infrared and thermal imaging applications. *International Journal of Veterinary Science and Medicine*. 8:71-84.
- Kolarek M. (2018). Analiza toplinskih postrojenja primjenom termovizijske kamere. Završni rad, Međimursko veleučilište u Čakovcu.
- Kongsro J. (2013). Development of a computer vision system to monitor pig locomotion. *Open Journal of Animal Science*. 3: 254–260.
- Mahfuz S., Mun H.S., Dilawar M.A., Yang C.J. (2022). Applications of smart technology as a sustainable strategy in modern swine farming. *Sustainability*. 14: (5).
- Marchant J., Schofield C., White R. (1999). Pig growth and conformation monitoring using image analysis. *Animals*. 68: 141–150.
- Mun H.S., Ampode K.M.B., Mahfuz S., Chung I.B., Dilawar M.A., Yang C.J. (2022). Heat detection of gilts using digital infrared thermal imaging camera. *Advances in Animal and Veterinary Sciences*. 10: 2142-2147.
- Riekert M., Klein A., Adrion F., Hoffmann C., Gallmann E. (2020). Automatically detecting pig position and posture by 2D camera imaging and deep learning. *Computers and Electronics in Agriculture*. 174.
- Salles M.S.V., da Silva S.C., Salles F.A., Roma Jr L.C., El Faro L., Mac Lean P.A.B., de Oliveira C.E.L., Martello L.S. (2016). Mapping the body surface temperature of cattle by infrared thermography.

Journal of Thermal Biology. 62: 63-69.

Supakorn C., Stock J.D., Garay E., Johnson A.K., Stalder K.J. (2018). Lameness: A principle problem to sow longevity in breeding herds. CAB Reviews: Perspectives in Agriculture Veterinary Science, Nutrition and Natural Resources 13.

Tzanidakis C., Simitzis P., Arvanitis K., Panagakis P. (2021). An overview of the current trends in precision pig farming technologies. Livestock Science. 249.

Wang S., Jiang H., Qiao Y., Jiang S., Lin H., Sun Q. (2022). The Research Progress of Vision-Based Artificial Intelligence in Smart Pig Farming. Sensors (Basel). 2022 Sep; 22: 6541.

Precision pig farming

Abstract

Precision livestock production describes a management system based on continuous automatic monitoring and control of pig production, reproduction, health and welfare in real time. Today, there is a wide range of technologies available, such as 2D and 3D cameras to assess body weight, behavior and activity, thermal cameras to monitor body temperature and determine estrus, microphones to monitor coughs and vocalizations, various measuring cells to monitor food intake, body weight and gait , radio frequency identification technologies for individual identification and tracking, and numerous others that help breeders improve pig production.

Keywords: pigs, precision agriculture, management, welfare

Proizvodni rezultati tova križevačke kukmice

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Sažetak

Cilj ovog rada bio je utvrditi proizvodne pokazatelje tova križevačke kukmice. Perad se držala podnim načinom uzgoja u ograđenim boksovima u zatvorenom prostoru. Praćene su vrijednosti tjelesne mase (g), konzumacija krmne smjese (g) i konverzija hrane (kg/kg). Rezultati mjerenja su zatim uspoređeni s rezultatima drugih autohtonih pasmina. Križevačka kukmica se kao pasmina odlikuje dobrom konverzijom (3,56 kg) i prirastom (781,47 g) na kraju razdoblja praćenja od 8 tjedana.

Ključne riječi: križevačka kukmica, tovnj pokazatelji

Uvod

Sve većim uzgojem linijskih hibrida u proizvodnji mesa i jaja došlo je do istiskivanja izvornih pasmina kokoši s poljoprivrednih gospodarstva i farmi (Andrašić, 2017). Križevačka kukmasta kokoš je hrvatska autohtona pasmina nastala uzgojno selekcijskim radom kojeg su započeli supružnici Josip i Katica Vojta iz Križevaca još davne 1984. godine (Pintić i sur., 2008). Domaće kokoši križevačkoga kraja planski su križane s pijetlovima orpington pasmine. Konačni rezultat selekcije je bila pasmina dobrog prinosa mesa i jaja, pogodna za tov. Na prostoru Veleučilišta u Križevcima se i dalje provodi uzgoj kukmice u čistoj krvi i vodi se briga o očuvanju pasmine. Prema proizvodnim svojstvima spada u srednje-teške pasmine kokoši. Masa pijetlova je oko 4 kg, a kokoši od 2,5 do 3 kg. Odlikuje se bijelom kožom te sočnim i vrlo ukusnim mesom. Ima srednje fini kostur. Meštović i sur. (2015) uz opis pasmine navode i tjelesne mase kokoši i pijetlova starosti 12 mjeseci koje su u njihovom istraživanju iznosile 2,95 odnosno 2,71 kg. Kokice pronešu sa 6 do 7 mjeseci i snesu godišnje 150 do 170 jaja, prosječne mase do 60 grama (Jelen, 2019).

Materijal i metode

Istraživanje je provedeno na prostoru Veleučilišta u Križevcima koji se bavi uzgojem križevačke kukmice te se brine o očuvanju ove pasmine. Formirane su tri skupine pilića križevačke kukmice. Skupine se razlikuju po datumu valjenja tako da je razmak između njih 1 tjedan. Broj jedinki u skupini bio je određen brojem jaja u trenutku nasađivanja na prostoru Veleučilista. Svi pilići držani su u istom objektu i smješteni u tri boksa neposredno jedan do drugoga. U hranidbi su korištene potpune krmne smjese i pilići su hranjeni *ad libitum*. Smjesa starter (22% SB, 12 MJ/kg, 4% SV, 5% SM, 6% SP) korištena je do 3 tjedna starosti pilića, a nakon toga postepeno je uvedena smjesa grover (16% SB, 11.50 MJ/kg, 2% SM, 12% SP). Prije svakog stavljanja hrane u hranilice, količina smjese se vagala preciznom vagom (Kern 440-51N), te se pratila tjedna konzumacija hrane. Vaganje pilića vršilo se na dan valjenja te nakon toga svakih tjedan dana preciznom vagom kroz period od 8 tjedana. Rezultati tova (tjelesna masa, konzumacija hrane i konverzija hrane) uspoređeni su s rezultatima tova drugih autora. Križevačka kukmica je također uspoređena s drugom autohtonom pasminom, posavskom kukmastom kokoši koja isto ima proizvodni potencijal (Barać i sur., 2011).

Rezultati i rasprava

Tablica 1. Prosječne tjedne mase po skupinama

Tjedan	Grupa	Prosječna masa (g)	SD	min	max	cv
Jednodnevni pilići	1 (N=23)	41,96 ^a	4,3	30	51	10,25
	2 (N=26)	42,73 ^a	3,49	37	52	8,18
	3 (N=38)	39,79 ^{ab}	3,39	32	47	8,51
	Prosjek	41,49				
1	1 (N=23)	85,22 ^{ab}	7,46	68	97	8,75
	2 (N=26)	87,8 ^a	5,42	75	99	6,17
	3 (N=37)	81,6 ^b	7,44	66	94	9,12
	Prosjek	84,88				
2	1 (N=23)	159,26 ^a	9,83	131	178	6,17
	2 (N=26)	164,12 ^a	13,09	138	193	7,98
	3 (N=35)	148,17 ^b	17,79	115	223	12,01
	Prosjek	157,18				
3	1 (N=23)	238,39 ^a	18,13	204	278	7,61
	2 (N=26)	243,77 ^a	22,76	202	289	9,34
	3 (N=35)	247,89 ^a	40,06	175	399	16,16
	Prosjek	243,35				
4	1 (N=23)	341,39 ^a	30,8	262	386	9,02
	2 (N=24)	344,63 ^a	29,54	286	391	8,57
	3 (N=35)	357,95 ^a	57,3	250	565	16,01
	Prosjek	347,99				
5	1 (N=23)	470,13 ^a	39,03	403	541	8,30
	2 (N=24)	411,54 ^b	50,89	294	513	12,37
	3 (N=35)	460,00 ^a	77,28	300	700	16,80
	Prosjek	447,22				
6	1 (N=23)	551,43 ^a	67,85	431	725	12,30
	2 (N=18)	525,00 ^a	71,2	350	650	13,56
	3 (N=35)	577,14 ^a	97,35	350	850	16,87
	Prosjek	551,19				
7	1 (N=23)	731,30 ^a	78,81	600	900	10,78
	2 (N=18)	633,30 ^b	72,65	500	750	11,47
	3 (N=35)	743,14 ^a	83,08	600	950	11,18
	Prosjek	702,58				
8	1 (N=23)	869,13 ^a	100,69	600	1100	11,59
	2 (N=18)	738,61 ^b	86,05	600	900	11,65
	3 (N=35)	861,14 ^a	115,16	630	1100	13,37
	Prosjek	822,96				

a, b, c - vrijednosti u istom redu tablice označene različitim slovima značajno se razlikuju, $P \leq 0,05$, SD- standardna devijacija, min- minimalna vrijednost, max- maksimalna vrijednost, cv- koeficijent varijacije

Prema podacima iz tablice 1. vidljivo je da su pilići u trećoj skupini imali veću masu u odnosu na ostale skupine u 4. tjednu praćenja. Nakon 8. tjedna najveću tjelesnu masu imaju prva skupina. Na dan valjenja pilića prosječna masa je bila 41,49 g. Na kraju 4. tjedna rezultati mjerenja pokazuju da je 3. skupina po prosječnoj masi pilića premašila prosječne mase prve i druge skupine. Između grupa nema značajnih statističkih razlika. Na kraju 8. tjedna istraživanja prosjek iznosi 822,96 g. Najveću prosječnu masu ima prva skupina i ona iznosi 869,13 g. Između prve i treće skupine nema značajnijih statističkih razlika dok druga skupina ima značajnu razliku u prosjeku mase. Tablica 2. pokazuje da treća skupina ima slabiju konverziju hrane na kraju 4. i 8. tj. praćenja. Napomena da se u 4. tjednu praćenja počeo javljati veći mortalitet u 2. skupini zbog bolesti. Vijednosti konzumacije i konverzije su najviši kod 2. grupe na kraju 8. tj., a razlog tome je i povećana smrtnog gdje je broj kljunova pao s 26 na 18. Mortalitet pilića na kraju 8. tjedna istraživanja bio je u prvoj skupini 0 %, u drugoj 30,80 % zbog oboljenja, a u trećoj 7,89 %. Na konverziju krmiva utječe i način držanja (Mikulski i sur., 2011). Pilići su cijelo vrijeme držani u zatvorenom prostoru, podnim načinom držanja u boksevima.

Tablica 2. Prosječni prirasti i konverzija hrane u 4. i 8. tjednu starosti pilića

Starost pilića	Proizvodni pokazatelji	Skupina			Prosjek
		1	2	3	
0 tjedana	prosječna masa jednodnevnih pilića (g)	41,96	42,73	39,79	41,49
4 tjedana	prosječna masa pileta (g)	341,39	344,63	357,95	347,99
	prosječni prirast pileta (g)	299,43	301,90	318,16	306,50
	konzumacija po piletu (g)	632,61	750,00	557,14	646,58
	konverzija (kg/kg)	2,11	2,48	1,75	2,11
8 tjedana	masa pileta sa 4 tjedna	341,39	344,63	357,95	347,99
	prosječna masa pileta (g)	869,13	738,61	861,14	822,96
	prosječni prirast pileta (g)	827,17	695,88	821,35	781,47
	konzumacija po piletu	2632,60	3354,17	2367,00	2784,59
	konverzija (kg/kg)	3,18	4,82	2,88	3,56

Usporedba proizvodnih pokazatelja križevačke kukmice i posavske kukmaste kokoši je prikazana u tablici 3. U prosjeku pilići posavske kukmaste kokoši imaju veću masu od križevačke kukmice. Do 4. tjedna starosti posavska kukmasta kokoš ima veću prosječnu masu, prirast i konzumaciju u odnosu na križevačku kukmicu. Mora se napomenuti da se zbog manjeg broja podataka za različite tjedne, mogućnost uspoređivanja na kraju 8. tjedna je dosta ograničena. Prosječna masa na kraju 8. tjedna je kod posavske 1071 g., dok je kod križevačke 822,96 g. Konzumacija po piletu za posavsku kukmastu kokoš do 4. tjedna iznosi 1071 g dok za križevačku iznosi 646,58 g. Veća konzumacija omogućuje i veće priraste kod posavske kukmaste kokoši.

Tablica 3. Usporedba križevačke i posavske kukmaste kokoši

Starost pilića	Proizvodni pokazatelji	Pasmine		
		Križevačka kukmica (VuK)	Križevačka kukmica*	Posavska kukmasta kokoš**
4 tjedana	masa jednodnevnog pileta (g)	41,49	40,39	45,54
	prosječna masa pileta (g)	347,99	323,92	431,07
	prosječni prirast pileta (g)	306,5	289,53	385,53
	konzumacija po piletu (g)	646,58	-	1071
	konverzija (kg/kg)	2,11	-	2,79
8 tjedana	prosječna masa pileta (g)	822,96	737,43	1078,14
	prosječni prirast pileta (g)	781,47	702,7	-
	konzumacija po piletu (g)	2784,59	-	-
	konverzija (kg/kg)	3,56	3,56	-

*Izvor: Jelen M. (2019.)

**Izvor: Jelenić, F. (2022.)

Tablica 3. pokazuje da posavska kukmasta kokoš ima bolje tovnne predispozicije u odnosu na križevačku kukmicu. Zbog malog broja radova na temu proizvodnih pokazatelja autohtonih pasmina kokoši, mogućnost usporedbe s drugim izvorima je vrlo ograničena.

Zaključak

Kada se uspoređuje s našom drugom autohtonom pasminom, križevačka kukmica ima bolju konverziju hrane (2,11 kg) u odnosu na posavsku (2,78 kg), ali ipak zaostaje po prosječnom prirastu pilića koji je kod križevačke 306,5 g, a kod posavske 385,53 g. Potrebna su daljnja istraživanja da bi se utvrdile mogućnost uspješne proizvodnje uzgojem hrvatskih autohtonih pasmina.

Literatura

- Andrašić Z. (2017). Zaštita autohtonih pasmina domaćih životinja kroz provedbu državnih potpora, Specijalistički diplomski stručni, Veleučilište u Križevcima.
- Barać Z., Bedrica Čaćić M., Dražić M., Dadić, M., Ernoić M., Fury M., Horvath Š., Ivanković A., Janječić Z., Jeremić J., Kezić N., Marković D., Mioč B., Ozimec R., Petanjek D., Poljak F., Prpić Z., Sindičić M. (2011). Zelena knjiga izvornih pasmina Hrvatske; Ministarstvo zaštite okoliša i prirode, Državni zavod za zaštitu prirode, Hrvatska poljoprivredna agencija.
- Jelenić F. (2022). Pokazatelji proizvodnje mesa pijetlova pasmine posavska kukmasta kokoš. Diplomski rad. Sveučilište u Zagrebu Agronomski fakultet, Zagreb.
- Jelen M. (2019). Pokazatelji proizvodnje mesa križevačke kukmaste kokoši. Diplomski rad. Sveučilište u Zagrebu Agronomski fakultet, Zagreb.
- Meštrović M., Janječić Z., Bedeković D., Duvnjak G. (2015). Fenotipska obilježja križevačke kukmaste kokoši: Zbornik radova XI. simpozija "Peradarski dani 2015."/ Balenović M., (ur.). Zagreb: Hrvatski veterinarski institut, Centar za peradarstvo, 2015. str. 125-128.
- Mikulski D., Celej J., Jankowski J., Majewska T., Mikulska M. (2011). Growth performance, carcass and meat quality of slower-growing and fast-growing chickens raised with and without outdoor access. Asian-Australasian Journal of Animal Sciences. 24: 1407-1416.
- Pintić V., Pintiće-Pukec N., Poljak F., Vojta K., Meštrović M., Flajpan S., Mihalić Z. (2008). Križevačka kukmica. Stočarstvo. 62: 55-57.

Fattening production results of the Križevci crested hen

Abstract

The aim of this study was to determine the production indicators of the Križevci turkey's fattening. Poultry was raised in a floor-rearing system in enclosed boxes indoors. Values of body mass (g), consumption of feed mixture (g), and feed conversion (kg/kg) were monitored. The measurement results were then compared with results from other indigenous breeds. The Križevci turkey breed stands out for its good feed conversion (3.56 kg) and growth (781.47 g) at the end of the 8-week monitoring period.

Keywords: Križevci crested hen, fattening indicators

Amino acid composition of meat from two local donkey breeds

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Abstract

Although donkey meat is still less common in human nutrition than other meat sources, its unique amino acid composition makes a valuable contribution to a balanced diet. This fact has led to increased research into the quality of donkey meat, with a focus on the amino acid profile. In this study it was found that donkey meat from the Istrian donkey and Littoral-Dinaric donkey breeds provides all essential amino acids, with leucine and lysine being the most abundant in this group in both breeds, followed by arginine, valine and isoleucine. Compared to other domestic animal meat (pork, beef and sheep), the results of the amino acid profile showed a higher number of detected essential amino acids. Namely, donkey meat contained 10-25% more essential amino acids compared to meat from other domestic animal species.

Keywords: meat, amino acid, Istrian donkey, Littoral-Dinaric donkey, nutritional value

Introduction

Amino acids are the basic building elements of proteins and play a crucial role in various biological processes in living organisms. They are essential for the growth, repair and maintenance of tissues, making them an important aspect of human nutrition. Recommendations have been issued for the daily intake requirements of essential amino acids for different population groups (FAO, 2007; FAO, 2013). Although donkey meat is less common in human diet than other meat sources, it offers a unique composition of amino acids that can contribute to a balanced diet. This has led to increased research into the quality of donkey meat, including its amino acid profile (Polidori et al., 2009; Polidori et al., 2015; Marino et al., 2022). In the past, donkey meat was obtained from animals slaughtered at the end of their working life and had less appreciated quality traits (less tender). Therefore, the meat was usually intended for the production of salami or other salted meat products. Nowadays, a greater proportion of younger animals is used for meat production with more favourable quality traits (more tender), and it is marketed as fresh meat. There are three autochthonous donkey breeds on the territory of Croatia, which belong to the group of endangered breeds due to the size of the population. Research into the phenotypes (Ivanković et al., 2000) and genetic structure of the local donkey breeds (Ivanković et al., 2002; Ivanković et al., 2022) is essential for the development of the program for their economic reaffirmation through recreation, tourism, but also the gastronomic utilization of their meat and milk. Some characteristics of the quality of carcasses and meat of local donkey breeds have already been determined (Ivanković et al., 2023), but in order to obtain complete information on the quality of meat, it is necessary to study the profile of amino acids in meat, especially essential amino acids. The aim of this study was therefore to investigate the amino acid composition of the meat of the Istrian donkey and the Littoral-Dinaric donkey breeds.

Material and methods

The research was approved by the Bioethical Committee for the Protection and Welfare of Animals at the Faculty of Agriculture, University of Zagreb (No.: 251-71-29-02/19-22-2). Twenty male donkeys aged four to six years were used in this study. The Littoral-Dinaric donkeys (LDD; n=10) came from traditional farms in the geographical region of Dalmatia and the Istrian donkeys (ID; n=10) originated from the region of Istria. The animals were kept in traditional husbandry, free range and with unrestricted seasonal access to pasture throughout before slaughter.

The chemical analysis of the meat was performed on a samples of *musculus longissimus thoracis* (MLT) from the 11th to 12th rib (weight 100 g) by near-infrared transmission spectrophotometry (NIRS) in the measuring range 850–1050 nm using a Food Scan instrument (Foss Electric A/S, Hillerød, Denmark).

Samples were minced, weighed 2.5 g and homogenised in 10 mL of highly purified water using a Stuart HSMI homogenizer. All samples were diluted 10x with highly purified water and spiked with the internal standard α -aminobutyric acid at a final concentration of 1 mM. A volume of 100 μ L of the prepared sample was used for further processing and analysis. Quantitative analysis of total amino acids was performed by the Waters AccQ Tag method according to the manufacturer's instructions (Waters) and includes the step of hydrolysis, derivatization of amino acids with the derivatization reagent AccQ-Fluor, and quantitative HPLC analysis using α -aminobutyric acid as an internal standard.

Statistical analysis of the results was performed using the statistical software IBM SPSS, version 21 (Armonk, New York, USA).

Results and discussion

The protein content (%) in the meat of the Littoral-Dinaric donkeys was higher (24.15 ± 0.28) than that in the meat of the Istrian donkey (23.65 ± 0.24), but the difference was not significant ($p=0.224$). The amino acid composition of meat from two local donkey breeds is presented in Table 1. The observed differences in the amino acid content of meat from two local donkey breeds were not significant.

Table 1. Amino acid composition (mg/100g meat) of meat from Istrian donkey and Littoral-Dinaric donkey (LS mean \pm s.e.) and share (%) in total amount of amino acids.

Amino acids	Istrian donkeys		Littoral-Dinaric donkeys	
	mg/100g meat	%	mg/100g meat	%
Arganine (Arg) ^E	14.38 \pm 1.10	7,4	15.36 \pm 1.10	8,05
Histidine (His) ^E	7.39 \pm 0.40	3,8	7.19 \pm 0.40	3,76
Isoleucine (Ile) ^E	9.48 \pm 0.62	4,87	9.63 \pm 0.62	5,04
Leucine (Leu) ^E	16.66 \pm 1.06	8,57	16.28 \pm 1.06	8,53
Lysine (Lys) ^E	18.00 \pm 1.20	9,26	17.66 \pm 1.20	9,25
Methionine (Met) ^E	4.71 \pm 0.68	2,42	2.98 \pm 0.68	1,56
Phenylalanine (Phe) ^E	8.14 \pm 0.51	4,18	7.96 \pm 0.51	4,17
Threonine (Thr) ^E	9.40 \pm 0.65	4,83	9.24 \pm 0.65	4,84
Valine (Val) ^E	9.68 \pm 0.64	4,98	9.92 \pm 0.64	5,2
Alanine (Ala) ^{NE}	12.80 \pm 0.75	6,58	12.60 \pm 0.75	6,6
Asp+Asn (Asparagine + Aspartic acid) ^{NE}	19.38 \pm 1.29	9,97	18.69 \pm 1.29	9,79
Glu+Gln (Glutamine + Glutamic acid) ^{NE}	30.96 \pm 1.97	15,93	30.18 \pm 1.97	15,82
Glycine (Gly) ^{NE}	10.04 \pm 0.67	5,16	10.08 \pm 0.67	5,28
Proline (Pro) ^{NE}	8.42 \pm 0.58	4,33	8.54 \pm 0.58	4,47
Serine (Ser) ^{NE}	8.01 \pm 0.52	4,12	7.79 \pm 0.52	4,08
Tyrosine (Tyr) ^{NE}	6.87 \pm 0.50	3,53	6.63 \pm 0.50	3,47
Amino acids total (AAT)	194,32		190,73	
Essential amino acids total (EAAT; %)	97.84	50,34	96.22	50,44

E = esencial amino acids, *NE* = non esencial amino acids

A protein source is an essential part of a healthy diet as it enables both the growth and maintenance of the many thousands of proteins encoded in the human genome. This donkey meat provided all the essential amino acids, with leucine (16.55 mg/100g in ID, 16.40 mg/100g in LDD) and lysine (17.87 mg/100g in ID, 17.78 mg/100g in

LDD) being the most abundant in this group in both donkey breeds, followed by arganine, confirming the results of previous studies on donkey meat (Polidori et al., 2009). Arganine was classified among the essential amino acids as by Hoffman et al. (2005), since arginine is considered a conditionally essential amino acid (Arienti, 2003) when endogenous synthesis is insufficient to meet metabolic needs, which is often the case during growth and under catabolic conditions (Franco and Lorenzo, 2014). In the fraction of non-essential amino acids, glutamine and glutamic acid together had the highest content in both breeds (30.64 and 30.50 mg/100g), followed by asparagine and aspartic acid (19.24 and 18.86 mg/100g), alanine (12.68 and 12.71 mg/100g) and glycine (9.72 and 10.33 mg/100g). Similar results for these aminoacids were reported by Polidori et al. (2009). Li et al. (2020) determined the amino acid composition of a local two-year-old donkey breed and found that lysine had the highest proportion of essential amino acids (14.63 mg/100g), which was also confirmed in this study.

The values of the essential amino acid were very similar to those of the study by Marino et al. (2022), who investigated an amino acid composition of donkey meat in two weeks of maturation process, where lysine, as the highest value amino acid in this study had 22.59 mg/100g and 21.58 mg/100g. Polidor et al. (2020) observed the highest essential amino acid result for the amino acid lysine (16.2 mg/100g muscle), which can be compared with our results (17.87 mg/100g in ID, 17.78 mg/100g in LDD). The share of essential amino acids in total amino acids in the meat of crossbred male donkeys (Martina Franca x Ragusana) determined by Polidori et al. (2020) was higher (52.2%) than in Croatian donkey breeds (50.49% and 50.31%). This result confirms the high biological value of donkey meat proteins.

The type and content of amino acids determine the nutritional value of proteins. As can be seen in Figure 1, the highest share of essential amino acids was found in donkey meat, namely lysine, leucine and arginine. Li et al. (2022) point out that the most important amino acids in donkey meat were alanine, lysine, glutamic acid, glycine and serine. Only one of these was an essential amino acid (lysine). However, when we compare donkey meat with pork, the total amino acid content was the highest in donkey meat. A similar ratio of essential to non-essential amino acids was observed in beef and sheep meat, and the highest share of amino acids was proline, asparagine and glutamic acid.

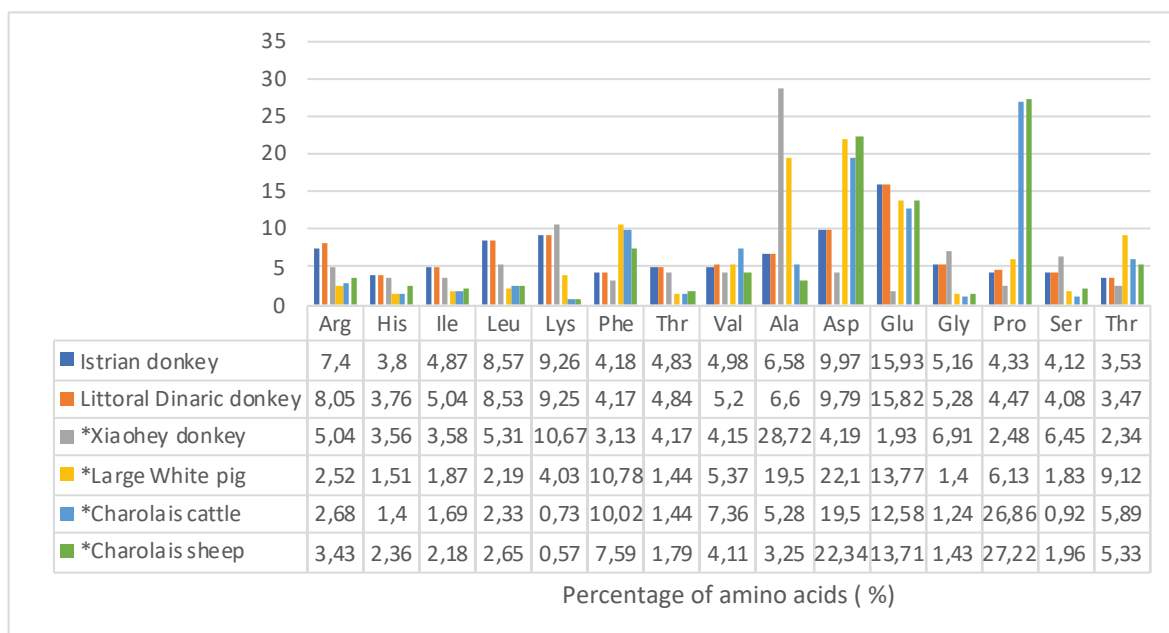


Figure 1. Share of amino acids of donkey, beef, sheep and pig meat (% in total AA content; * Li et al., 2020)

Conclusion

The studies carried out so far have shown that donkey meat is of good quality in terms of chemical composition, fatty acid profile and amino acid profile. Donkey meat appears to be a good source of proteins of high biological value due to its content of essential amino acids. The nutritional value of donkey meat differs significantly from that of other meats (pork, beef and sheep) where it has over 10-25% more essential amino acids, depending on the species it was compared with. Farmers could therefore work in short production chains to promote local donkey breeds.

Acknowledgements:

This research was funded by the Croatian Science Foundation (Genetic, economic, and social interactions of local breed conservation programs, GGD LocBreed), under grant number IP-2020-02-4860.

References

- Arienti G. (2003). *Le basi molecolari della nutrizione* (2nd Ed.). Padova, Piccin Nuova Libreria S.p.A.
- Food and Agricultural Organization/World Health Organization/UNU (2007). Protein and amino acid requirements for human nutrition. Report of a Joint FAO/WHO/UNU Expert consultation. UN University, Geneva.
- Food and Agricultural Organization (2013). Dietary protein quality evaluation in human nutrition. Report of an FAO Expert Consultation. Rome.
- Franco D., and Lorenzo J.M. (2014). Effect of muscle and intensity of finishing diet on meat quality of foals slaughtered at 15 months. *Meat Science*. 96: 327-334.
- Hoffman L., Kritzinger B., Ferreira A.V. (2005). The effects of region and gender on the fatty acid, amino acid, mineral, myoglobin and collagen contents of impala (*Aepyceros melampus*) meat. *Meat Science*. 69: 551-558.
- Ivanković A., Bittante G., Šubara G., Šuran E., Ivkić Z., Pečina M., Konjačić M., Kos I., Kelava Ugarković N., Ramljak J. (2022). Genetic and Population Structure of Croatian Local Donkey Breeds. *Diversity*. 14: 322.
- Ivanković A., Šubara G., Bittante G., Šuran E., Amalfitano N., Aladrović J., Kelava Ugarković N., Pađen L., Pečina M., Konjačić M. (2023). Potential of Endangered Local Donkey Breeds in Meat and Milk Production. *Animals*. 13: 2146.
- Ivanković A., Caput P., Mioč B., Pavić V. (2000). The Phenotype Features of Donkeys in Croatia. *Agriculturae Conspectus Scientificus*. 65: 99–105.
- Ivanković A., Kavar T., Caput P., Mioč B., Pavić V., Dovč P. (2002). Genetic diversity of three donkey populations in the Croatian coastal region. *Animal Genetics*. 33: 169–177.
- Li X., Amadou I., Zhou G-Y., Qian L-Y., Zhang J-L., Wang D-L., Cheng X-R. (2020). Flavour Components Comparison between the Neck Meat of Donkey, Swine, Bovine and Sheep. *Food Science of Animal Resources*. 40: 527-540.
- Marino R., della Malva A., Maggiolino A., De Palo, P., d'Angelo F., Lorenzo J.M., Sevi, A., Albenzo M. (2022). Nutritional Profile of Donkey and Horse Meat: Effect of Muscle and Aging Time. *Animals*. 12: 746.
- Polidori P., Cavallucci C., Beghelli D., Vincenzetti S. (2009). Physical and chemical characteristics of donkey meat from Martina Franca breed. *Meat Science*. 82: 469-471.
- Polidori P., Vincenzetti S., Pucciarelli S., Polzonetti V. (2020). Comparison of Carcass and Meat Quality Obtained from Mule and Donkey. *Animals*. 10: 1620.
- Polidori P., Pucciarelli S., Ariani A., Polzonetti V., Vincenzetti S. (2015). A comparison of the carcass and meat quality of Martina Franca donkey foals aged 8 or 12 months. *Meat Science*. 106: 6-10.

Cimet – mogući dodatak u hranidbi životinja

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Sažetak

Prirodni dodaci stoljećima su korišteni kao tradicionalni lijekovi i začini za hranu. Od 2006. godine, nakon zabrane korištenja antibiotika kao promotora rasta u Europskoj uniji, ovi spojevi dobivaju veću primjenu u industriji krmnih smjesa. Cimet može biti dobar potencijalni prirodni dodatak hrani za životinje jer sadrži niz bioaktivnih komponenti koje su odgovorne za njegovo antimikrobno, antioksidativno i protuupalno djelovanje. Istraživanja su pokazala kako cimet ima pozitivan učinak na tjelesne mase životinja, snižava razine masnoća u krvi, pozitivno utječe na sastav mikroflore u crijevima, te na histomorfologiju tankog crijeva. Za sada ne postoje preporučene koncentracije suplementacije cimetom, a u recentnim istraživanjima dodane koncentracije ovog prirodnog dodatka kretale su se u rasponu od 0,1 do 8,0 g/kg. Cilj ovog rada bio je prikazati cimet kao mogući prirodni dodatak u hranidbi životinja.

Ključne riječi: prirodni dodaci, cimet, hranidba životinja

Uvod

Biljke i začini korišteni su od davnina te se može reći kako su najstariji prijatelji čovječanstva (Nabavi i sur., 2015; Tipu i sur., 2006). Prirodni dodaci korišteni su kao začini za hranu i kao tradicionalni lijekovi stoljećima, ali njihova je primjena u industriji krmnih smjesa prilično nova, a veće značenje počinju doživljavati nakon zabrane korištenja antibiotika kao promotora rasta u Europskoj uniji, od 2006. godine, zbog njihovog štetnog djelovanja na ljudsko zdravlje odnosno zbog posljedičnog rizika prisutnosti rezidua antibiotika u životinjskim proizvodima (Hussein i sur., 2023; Odutayo i sur., 2021; Khafaji, 2018; Mehdipour i Afsharmanesh, 2018; Tipu i sur., 2006). Upravo se brojni bioaktivni spojevi u biljkama mogu koristiti kao alternativna zamjena za antibiotike (Hussein i sur., 2023; Qaid i sur., 2022; Saeed i sur., 2018; Simitzis i sur., 2014; Singh i sur., 2014). Biljke proizvode brojne bioaktivne fitokemijske metabolite kao što su alkaloidi, flavonoidi, flavoni, flavonoli, terpenoidi, tanini, lektini, kinini, kumarini, polipeptidi i eterična ulja koja se smatraju dobrim izvorima za nova antibakterijska sredstva i stupaju u interakciju s bakterijskim stanicama pomoću različitih mehanizama i načina djelovanja. Zapravo, za razliku od ograničenog, neobnovljivog kapaciteta sadašnjih antibiotika, biljke su stalni obnovljivi izvori života odnosno izvor bioaktivnih fitokemijskih molekula ogromne biološke raznolikosti na Zemlji (250.000 do 500.000 biljnih vrsta) te se općenito smatraju sigurnijima, ekološki prihvatljivima te relativno niske cijene koštanja, a posjeduju značajna antibakterijska, antioksidativna, antikancerogena, antifungalna, analgetska, protuupalna, hepatoprotektivna te imunostimulirajuća djelovanja upravo zbog prisutnosti brojnih fitokemikalija u svome sastavu (Alagbe, 2022; Błaszczuk i sur., 2021; Mohamed i sur., 2020; Balijepalli i sur., 2017; Nabavi i sur., 2015; Tipu i sur., 2006). Cilj ovoga radio bio je prikazati cimet kao mogući dodatak u hranidbi životinja.

Povijest i tradicionalna upotreba cimeta

Tisućama godina cimet je poznat kao jedan od najčešće upotrebljivanih začina te njegova upotreba datirati još od 2800 godine pr. Kr. kada se najviše upotrebljavao kao dio ulja za pomazanje koje je koristio i sam Mojsije u tu svrhu kako se spominje u Bibliji. Prvi zapisi o cimetu datiraju oko 2800 godine pr. Kr. gdje je opisan u jednoj od najstarijih knjiga o upotrebi biljaka u kineskoj medicini gdje se cimet spominje kao „kwa“, a tako se i danas zove u Kini (Borovec, 2005). U starom Egiptu se često koristio kao sredstvo za balzimiranje mumija te aromatiziranje pića i pri konzerviranju mesa, dok su Rimljani koristili njegova ljekovita svojstva za liječenje bolesti probavnog i dišnog

sustava te su ga često palili kako bih pročistili zrak u prostorijama. Plinije Stariji u prvom stoljeću pisao je kako je cimet bio 15 puta vrijedniji od srebra i zlata (Pathak i Sharma, 2021; Kumar i Kumari, 2019; Saeed i sur., 2018; Kawatra i Rajagopalan, 2015; Nabavi i sur., 2015; Singletary, 2008). Međutim, budući je bio vrlo skup i vrlo cijenjen začim, potraga za cimetom dovela je do istraživanja svijeta u 16. stoljeću pri čemu su portugalski konkvistadori otkrili *C. zeylanicum* kako raste na Šri Lanki (također poznatoj kao Cejlon), te je započeo uvoz začina u europske zemlje tijekom 16. i 17. stoljeća (Pathak i Sharma, 2021; Saeed i sur., 2018; Kawatra i Rajagopalan, 2015; Nabavi i sur., 2015). Cimet se tisućama godina koristi kao aroma te začim hrani u brojnim kuhinjama svijeta, dobiva se od kore cimetoanca te dolazi u dvije varijante na tržište, u obliku štapića ili mljeveni. Koristi se u pripremi čokolade, u žvakaćim gumama zbog ugodnog i osvježavajućeg učinka koji se razvija u ustima nakon konzumacije. Također se koristi u mnogim slasticama (pita od jabuka, krafne i kiflice s cimetom), pikantnim bombonima, čaju, vinu, vrućem kakau i likerima (osobito u Meksiku, glavnom uvozniku cimeta). Na Bliskom istoku često se koristi u slanim jelima od piletine i janjetine. U SAD-u, cimet se često koristi za aromatiziranje žitarica, jela od kruha i voća. Prah cimeta se u perzijskoj kuhinji koristi kao začim u različitim oblicima gustih juha, pića i slatkiša. Cimet se koristi i kao repelent za insekte. Također se koristi u ayurvedskoj i kineskoj medicini za liječenje kašlja, dijabetesa, groznice, nadutosti, probavnih smetnji, upala sinusa i grlobolje (Adedeji i sur., 2022; Pathak i Sharma, 2021; Mohamed i sur., 2020; Gaikwad i sur., 2020; Goel i Mishra, 2020; Saeed i sur., 2018; Balijepalli i sur., 2017; Nabavi i sur., 2015; Rao i Gan, 2014). Nadalje cimet je također sastavni dio biljnih pasta za zube kojima se smanjuje učestalosti zubnog karijesa, pokazuje blagotvorne učinke na oralno zdravlje i koristi se kod zubobolje, oralnih infekcija te za uklanjanje lošeg zadaha u ustima (Saeed i sur., 2018; Balijepalli i sur., 2017; Nabavi i sur., 2015; Rao i Gan, 2014). Eterično ulje cimeta uspješno se koristi u aromaterapiji (Nabavi i sur., 2015; Rao i Gan, 2014). Ulje iz kore cimeta se također koristi u proizvodnji parfema, kozmetike, šampona i toaletnih sapuna (Singletary, 2008), dok se drvo cimeta često koristi za izradu ukrasa, namještaja, ormara i šperploča te za izradu raznih tradicionalnih predmeta (Pathak i Sharma, 2021).

Botanički opis cimeta

Cimet pripada obitelji *Lauraceae* te se uglavnom uzgaja u tropskim i suptropskim područjima u Aziji, nešto u Južnoj i Srednjoj Americi te Australiji, a može se reći da je Šri Lanka glavna zemlja u uzgoju cimeta zajedno sa Sejšelima, Madagaskarom i Indijom (Ali i sur., 2021; Gaikwad i sur., 2020; Goel i Mishra, 2020; Kumar i Kumari, 2019; Rawat i sur., 2019; Khafaji, 2018; Vasconcelos i sur., 2018; Nabavi i sur., 2015). Cimet je prilagođen širokom rasponu klimatskih uvjeta. On zahtijeva toplu i vlažnu klimu s dobro raspoređenom godišnjom količinom oborina od oko 1250-2500 mm, te prosječnom temperaturom od oko 27°C. Stabla divljeg cimeta prilagođena su tropskim zimzelenim kišnim šumama. Najbolje raste na niskim nadmorskim visinama i obično se uzgaja bez sjene, ali budući je cimet u biti šumsko drvo, tolerira i laganu sjenu, te uspijeva i na 300-350 metara nadmorske visine. Dobro uspijeva na različitim tlima u tropima, ali vrsta tla ima izražen utjecaj na kvalitetu kore. Fina pjeskovita i šljunčana tla su bolja od stjenovitih i kamenitih podloga u Šri Lanki i Indiji, ali na Sejšelima i Madagaskaru preferiraju se ilovasta tla. Tlo za rast cimeta treba imati dobru drenažnu sposobnost, inače će korijenje istrunuti ako ostane u vodi dulje vrijeme. Smatra se da je cimet osjetljiv na slanost, a gorak proizvod proizlazi od vlažnih i močvarnih uvjeta (Rawat i sur., 2019; Nabavi i sur., 2015). Cimet je začim koji se uglavnom dobiva iz kore i listova cimeta, no glavni dio stabla koji se koristi kao začim je unutrašnja kora. To je zimzeleno drvo ili grm koje pripada obitelji *Lauraceae* koja obuhvaća oko 250 vrsta (Qaid i sur., 2022; Błaszczuk i sur., 2021; Christiany i sur., 2021; Gaikwad i sur., 2020.; Goel i Mishra, 2020; Kumar i Kumari, 2019; Rawat i sur., 2019; Khafaji, 2018; Vasconcelos i sur., 2018). Postoje četiri vrste cimeta:

- pravi cimet ili cejlonski cimet ili meksički cimet (*Cinnamomum zeylanicum*)
- indonezijski cimet (*Cinnamomum burmanni*)
- vijetnamski cimet (*Cinnamomum loureiroi*)
- cassia cimet ili kineski cimet (*Cinnamomum aromaticum*) (Kawatra i Rajagopalan, 2015; Nabavi i sur., 2015). U Tablici 1. prikazane su glavne značajke pojedinih vrsta cimeta prema Kawatra i Rajagopalan, (2015), no u literaturi se spominju i brojne druge vrste koje se obično prodaju kao cimet, poput *C. verum* (širilanka cimet), *C. citrodorum* (malabarski cimet), *C. Tamale* (indijski cimet) i dr. (Ali i sur., 2021; Błaszczuk i sur., 2021; Pathak i Sharma 2021; Yanakiev, 2020; Rawat i sur., 2019).

Tablica 1. Glavne značajke pojedinih vrsta cimeta (Kawatra i Rajagopalan, 2015).

	<i>Cinnamomum zeylanicum</i>	<i>Cinnamomum burmanni</i>	<i>Cinnamomum loureiroi</i>	<i>Cinnamomum aromaticum</i>
Zemlja porijekla	Šri Lanka	Indonezija	Vijetnam	Kina
Okus	Blago slatko	Ljut	Ljut i sladak	Ljut i gorak
Boja	Svijetlo do srednje crvenkasto smeđe boje	Tamno crvenkasto smeđa	Tamno crvenkasto smeđa	Tamno crvenkasto smeđa
Posebna obilježja	Najniži sadržaj kumarina	Visok sadržaj kumarina, jaka aroma	Jaka aroma, pikantan, visok sadržaj kumarina	Visok sadržaj kumarin, vrlo jak okus

Drvo cimeta je zimzeleno, naraste do oko 6-10 m visine te je jako razgranato. Grane su mu jake, a kora debela, glatka i obično crvenkasto smeđe boje, a unutrašnja kora je aromatična i ona se koristi kao začim. Ima kožaste listove, duge 11-16 cm, sa šiljastim vrhovima. Listovi koji su također aromatični, ovalnog su oblika i kožastog izgleda. Mladi listovi su crvenkaste boje, a starenjem postaju sjajno zeleni s tri istaknute bijele usporedne žile. Naličje lista je svijetlije boje (Borovac, 2005). Listovi se uglavnom koriste za destilaciju eteričnog ulja cimeta. Neugledni žuti cvjetovi neugodnog su mirisa, cjevasti sa 6 režnjeva, rastu na dugim metlicama kao i listovi. Plod je mala, mesnata bobica, duga 1-1,5 cm tamno ljubičaste do crne boje. Začinski oblik cimeta dobiva se uklanjanjem vanjske kore stabla, struganjem unutarnje kore koja se suši i melje u prah (Pathak i Sharma, 2021; Rawat i sur., 2019).

Cimet se sastoji od brojnih bioaktivnih spojeva; prirodnih antioksidansa, antimikrobnih i protuupalnih komponenti kao što su hlapljiva ulja, flavonoidi, kurkuminoide, kumarini, tanini, alkaloidi, ksantoni, terpenoidi, fenoli i drugi spojevi u značajnim količinama. Koncentracija hlapljivih spojeva u eteričnom ulju cimeta uglavnom ovisi o dijelovima biljke (lišće, kora, korijen, stabljika) iz kojih se ekstrahira. Cinamaldehyd (55-78%) glavni je spoj ekstrahirani iz kore, dok je eugenol (59-78%) glavni spoj koji se ekstrahira iz lišća, a manje važni sastojci zastupljeni u cimetu su cinzeilanin, cinzeilanol, arabinoksilan, 2'-benzoloksicinamaldehyd i 2'-hidroksi cinamaldehyd. Cimet sadrži i niz raznih smolastih spojeva, uključujući cinamat, cimetnu kiselinu i brojna eterična ulja, a upravo zbog cinamaldehyda, cimet ima ljut okus i jaku aromu (Alqhtani i sur., 2022; Ali i sur., 2021; Błaszczuk i sur., 2021; Christiany i sur., 2021; Pathak i Sharma, 2021; Yanakiev, 2020; Chowlu i sur., 2018; Khafaji, 2018; Mehdipour i Afsharmanesh, 2018; Vasconcelos i sur., 2018; Saeed i sur., 2018; Nabavi i sur., 2015). Brojna eterična ulja koja su utvrđena u cimetu uključuju trans-cinamaldehyd, eugenol, cinamil acetat, L-borneol, L-bornil acetat, β-kariofilen, kariofilen oksid, E-nerolidol, α-tujen, α-kubeben, terpinolen i α-terpin. Cimet ima visok sadržaj polifenola te antocijanidina (A i B procijanidin) (Ali i sur., 2021; Pathak i Sharma, 2021; Błaszczuk i sur., 2021; Christiany i sur., 2021; Mohamed i sur., 2020; Chowlu i sur., 2018; Nabavi i sur., 2015; Rao i Gan, 2014; Singh i sur., 2014; Symeon i sur., 2014). Cimet je poznat kao vrlo dobar izvor željeza, cinka, kalcija, kroma, mangana, magnezija, kalija i fosfora te vitamina C, K, E, B2 i B6 (Alqhtani i sur., 2022; Goel i Mishra, 2020; Rawat i sur., 2019; El-Kholy i sur., 2012).

Primjena cimeta u hranidbi životinja

Zbog svega prethodno navedenog, cimet može biti dobar potencijalni prirodni dodatak hrani za životinje jer sadrži niz bioaktivnih komponenti koje su odgovorne za njegovo antivirusno, antibakterijsko, antifungalno, antioksidativno, antidijabetičko, protuupalno i antihiperkolesterolemično djelovanje. Cimet se može koristiti i kao prirodni konzervans prehrambenih proizvoda te stimulans apetita, pomaže u probavi tj. izlučivanju probavnih enzima te može poboljšati performanse rasta životinja kao i prinos te kvalitetu finalnih proizvoda te poboljšati opće zdravstveno stanje životinja kroz njihovu zaštitu od različitih patogena poput *Escherichia Coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermis*, *Salmonella sp.*, i *Helicobacter pylori* (Mohamed i sur., 2020; Goel i Mishra, 2020; Chowlu i sur., 2018; Khafaji, 2018; Mehdipour i Afsharmanesh, 2018; Saeed i sur., 2018; Vasconcelos i sur., 2018; Balijepalli i sur., 2017; Rao i Gan, 2014; Symeon i sur., 2014). Hussein i sur. (2023) proveli su istraživanje s ciljem procjene učinka dodatka klinčića, paprene metvice, ulja cimeta i njihove mješavine krmnim smjesama tovnih pilića, na rast, biokemijske parametre krvi, i antioksidativnu aktivnost. Rezultati

su pokazali da su navedeni dodatci značajno ($P < 0,05$) poboljšali tjelesnu masu, prirast, omjer konverzije hrane; postotak abdominalne masti bio je manjih vrijednosti u skupinama s dodatkom eteričnih ulja cimeta u odnosu na kontrolnu skupinu. Pokusne skupine imale su veću aktivnost ukupnih proteina u serumu, glutationa (GPx) i lipoproteina visoke gustoće (HDL) od onih u kontrolnoj skupini, međutim, značajno je smanjena koncentracija kolesterola, triglicerida, lipoproteina niske gustoće (LDL) i koncentracije lipidne peroksidacije (MDA). Saied i sur. (2022) imali su za cilj istražiti učinak suplementacije ulja cimeta (500, 1000 i 1500 mg/kg) u hranidbi tovnih pilića na rast, osobine trupa, hemato-biokemijske parametre, imunološku funkciju te sastav crijevne mikroflore. Rezultati su pokazali da su skupine hranjene uz dodatak ulja cimeta imale veću tjelesnu masu, bolji prirast i konverziju hrane u odnosu na kontrolnu skupinu. Također, skupine hranjene s dodatkom ulja cimeta imale su niže koncentracije kolesterola, triglicerida, i lipoproteina niske gustoće od kontrolne skupine. Kod skupina pilića hranjenih uz dodatak ulja cimeta primijećeno je značajno smanjenje ukupnog broja mikroba u crijevu, ukupnog broja kvasaca i plijesni, *E. coli* i *Salmonella* u usporedbi s kontrolnom skupinom. Broj bakterija mliječne kiseline povećan je u cekumu pilića hranjenih uz dodatak ulja cimeta u usporedbi s onima iz kontrolne skupine. Oduyayo i sur. (2021) istraživali su učinak dodatka cimeta u prahu (2, 4, 6 i 8%) na histomorfologiju jejunuma, performanse rasta, hematološke i biokemijske parametre, karakteristike trupa te lipidni profil mesa prsa. Rezultati istraživanja su pokazali kako je dodatak cimeta u većim koncentracijama značajno ($P < 0,05$) utjecao na debljinu mišićne stijenke crijeva, visinu resica, te rezultirao većim dubinama kripi u odnosu na kontrolnu skupinu pilića. Tretmani su također rezultirali značajnim ($P < 0,05$) smanjenjem triglicerida te značajno smanjenim lipoproteinima niske gustoće u mesu prsa pilića. Adedeji i sur. (2022) u svom su istraživanju željeli utvrditi utjecaj dodatka praha cimeta u vodu (0 g, 0,5 g, 1,0 g, 1,5 g i 2,0 g), na hematološke i biokemijske pokazatelje krvi pijetlova. Rezultati istraživanja ukazuju na to da je dodatak cimeta značajno ($P < 0,05$) utjecao na sve promatrane hematološke i biokemijske parametre krvi pijetlova. Mehdipour i Afsharmanesh, (2018) istraživali su učinak dodatka ulja i praha cimeta (100 i 200 mg ulja/kg smjese te 1 i 2 g praha/kg smjese) te probiotika krmnim smjesama prepelica, na performanse rasta, hematološke i biokemijske pokazatelje krvi te utjecaj na sastav crijevne mikroflore prepelica. Rezultati su pokazali kako su skupine prepelica hranjene uz dodatak cimeta imale značajno ($P < 0,05$) bolje priraste tjelesne mase, povećani broj korisnih bakterija (*Lactobacillus*) u ileumu ($P < 0,05$), dok se broj štetnih koliformnih bakterija smanjio. Simitzis i sur. (2014) proveli su istraživanje dodatka eteričnog ulja cimeta (1 ml/kg koncentrirane hrane) na rast janjadi te kvalitetu njihova mesa. Dodavanje ulja cimeta nije značajno utjecalo na učinak rasta janjadi ($P > 0,05$) niti na pH vrijednosti mesa, boju mesa, sposobnost zadržavanja vode, te na vrijednosti intramuskularne oksidacije masti. El-Kholy i sur. (2012.) željeli su utvrditi učinak dodatka praha cimeta (500g, 1000g, 1500g) kunićima na njihove performanse rasta te reproduktivnu sposobnost kao i hematološke i biokemijske parametre. Rezultati istraživanja pokazali su kako su se u skupinama s dodatkom cimeta u prahu značajno ($P < 0,05$) povećale konačne tjelesne mase, dnevni prirasti te konverzija. Učinak tretmana cimetom na osobine trupa bio je statistički značajan ($P < 0,05$) jer je izazvao povećanje u randmanu trupa te masi unutrašnjih organa (slezena, bubrezi, jetra, srca i pluća). Značajno se povećao broj crvenih krvnih stanica (RBC), hematokrita, albumina i razine omjera albumin/globulin ($P < 0,05$). Kunići iz skupina koje su primale cimet imali su niže ukupne lipide, fosfolipide, trigliceride, lipoproteine visoke gustoće (HDL)-kolesterol i ukupni kolesterol u usporedbi s onima iz kontrolne skupine.

Zaključak

Provedena istraživanja uključivanja cimeta kao dodatka hranidbi u krmne smjese namijenjene hranidbi različitih vrsta i kategorija domaćih životinja jasno su pokazala njegove pozitivne utjecaje na performanse rasta kao i odabrane biokemijske i hematološke parametre. Dobiveni rezultati ukazuju na moguću primjenu cimeta kao prirodnog dodatka u hranidbi životinja, čijom se primjenom osigurava dodatna sigurnost, ali i kvaliteta te hranjivost finalnih proizvoda koji potencijalno mogu unaprijediti zdravlje potrošača takvih proizvoda.

Literatura

- Adedeji O.S., Oyetoro B.A., Ifanegan O.D., Sanni D.A. (2022). Dietary effect of cinnamon powder on hematology and serum biochemistry indices of cockerel chickens. *Bulgarian Journal of Animal Husbandry/Životnov Dni Nauki*. 59: 26-31.
- Alagbe J.O. (2022). Use of medicinal plants as a panacea to poultry production and food security: A review. *American Journal of Technology and Applied Sciences*. 1: 24-36.

- Ali A., Ponnampalam E.N., Pushpakumara G., Cottrell J.J., Suleria H.A.R., Dunshea F.R. (2021). Cinnamon: A natural feed additive for poultry health and production-A review. *Animals*. 11: 2026.
- Alqhtani A.H., Qaid M.M., Al-Garadi M.A., Al-Abdullatif A.A., Alharthi A.S., Al-Mufarrej, S.I. (2022). Efficacy of *Rumex nervosus* leaves or *Cinnamomum verum* bark as natural growth promoters on the growth performance, immune responsiveness, and serum biochemical profile of broiler chickens. *Italian Journal of Animal Science*. 21: 792-801.
- Balijepalli M.K., Buru A.S., Sakirolla R., Pichika M.R. (2017). *Cinnamomum* genus: A review on its biological activities. *International Journal of Pharmacy and Pharmaceutical Sciences*. 9: 1-11.
- Borovac M. (2005). *Začini i začinsko bilje*. Mozaik knjiga, Zagreb.
- Błaszczak N., Rosiak A., Kałużna-Czaplińska J. (2021). The potential role of cinnamon in human health. *Forests*. 12: 648.
- Chowlu H., Vidyarthi V.K., Zuyie R., Maiti C.S. (2019). Use of cinnamon in diet of broiler chicken-A Review. *Livestock Research International*. 6: 42-47.
- Christiany C., Sudrajat S.E., Rahayu I. (2021). The potency of *Cinnamomum zeylanicum* to prevent diseases: a review. *Eureka Herba Indonesia*. 2: 49-58.
- El-Kholy K.H., El-Damrawy S.Z., Seleem T.S.T. (2012). Rabbit productivity and reproductivity as affected by cinnamon (*Cinnamomum Zeylanicum*). *Egyptian Poultry Science*. 32: 691-703.
- Gaikwad D., Sharma N.R., Prabhakar P.K., Singh J. (2020). application of ginger and cinnamon in poultry nutrition on growth performance: a review. *Plant cell biotechnology and molecular biology*. 21:172-180.
- Goel B., and Mishra S. (2020). Medicinal and nutritional perspective of cinnamon: A mini-review. *European Journal of Medicinal Plants*. 31: 10-16.
- Hussein E.A., El-Kassas N.E., Alderey A.A. (2023). Effect of dietary supplementation of clove, peppermint, cinnamon oils and their blends on growth performance, carcass characteristics, blood biochemical parameters and antioxidant status of broiler chicks. *Egyptian Journal of Animal Production*. 60: 33-41.
- Kawatra P., and Rajagopalan R. (2015). Cinnamon: Mystic powers of a minute ingredient. *Pharmacognosy research*. 7: 1-6.
- Khafaji S. (2018). Study the effect of ceylon cinnamon (*cinnamomumzeylanicum*) powder on some physiological parameters in broiler chicks. *Journal of Global Pharma Technology*. 10: 236-242.
- Kumar S., and Kumari R. (2019). *Cinnamomum*: Review article of essential oil compounds, ethnobotany, antifungal and antibacterial effects. *Open Access Journal of Science*. 3: 13-16.
- Mehdipour Z., and Afsharmanesh M. (2018). Evaluation of synbiotic and cinnamon (*Cinnamomum verum*) as antibiotic growth promoter substitutions on growth performance, intestinal microbial populations and blood parameters in Japanese quail. *Journal of Livestock Science and Technologies*. 6: 1-8.
- Mohamed A.E., Abdur R., MM S.A. (2020). Cinnamon bark as antibacterial agent: A mini-review. *GSC Biological and Pharmaceutical Sciences*. 10: 103-108.
- Nabavi S.F., Di Lorenzo A., Izadi M., Sobarzo-Sánchez E., Daglia M., Nabavi S.M. (2015). Antibacterial effects of cinnamon: From farm to food, cosmetic and pharmaceutical industries. *Nutrients*. 7: 7729-7748.
- Odutayo O.J., Adeyemo A.A., Ibigbami D.J., Sogunle O.M., Olaifa R.O., Akinwale O.A., Orebiyi V.A., Ojebisi V.A., Joel A.O. (2021). Influence of cinnamon (*Cinnamomum cassia*) powder as additive on jejunal histomorphometry, growth performance, haemato-biochemical indices, carcass traits and breast meat lipid profile of broiler chickens. *Nigerian Journal of Animal Production*. 48: 167-184.
- Pathak R., and Sharma H. (2021). A review on medicinal uses of *Cinnamomum verum* (Cinnamon). *Journal of Drug Delivery and Therapeutics*. 11: 161-166.

- Qaid M.M., Al-Mufarrej S.I., Azzam M.M., Al-Garadi M.A., Alqhtani A.H., Al-Abdullatif A.A., Hussein E.O., Suliman G.M. (2022). Dietary cinnamon bark affects growth performance, carcass characteristics, and breast meat quality in broiler infected with *Eimeria tenella* Oocysts. *Animals*. 12: 166.
- Rao P.V., and Gan S.H. (2014). Cinnamon: a multifaceted medicinal plant. Evidence-Based Complementary and Alternative Medicine, 2014.
- Rawat I., Verma N., Joshi K. (2019). Cinnamon (*Cinnamomum zeylanicum*). Jaya Publishing House, New Delhi.
- Saeed M., Kamboh A.A., Syed S.F., Babazadeh D., Suheryan, I., Shah Q.A., Umar M., Kakar I., Naveed M., Abd El-Hack M.E., Alagawany M., Chao S. (2018). Phytochemistry and beneficial impacts of cinnamon (*Cinnamomum zeylanicum*) as a dietary supplement in poultry diets. *World's Poultry Science Journal*. 74: 331-346.
- Saied A.M., Attia A.I., El-Kholy M.S., Reda F.M., Nagar A.G.E.L. (2022). Effect of cinnamon oil supplementation into broiler chicken diets on growth, carcass traits, haemato-biochemical parameters, immune function, antioxidant status and caecal microbial count. *Journal of Animal and Feed Sciences*. 31: 21-33.
- Simitzis P.E., Bronis M., Charismiadou M.A., Mountzouris K.C., Deligeorgis S.G. (2014). Effect of cinnamon (*Cinnamomum zeylanicum*) essential oil supplementation on lamb growth performance and meat quality characteristics. *Animal*. 8: 1554-1560.
- Singh J., Sethi A.P.S., Sikka S.S., Chatli M.K., Kumar P. (2014). Effect of cinnamon (*Cinnamomum cassia*) powder as a phytobiotic growth promoter in commercial broiler chickens. *Animal Nutrition and Feed Technology*. 14: 471-479.
- Singletary K. (2008). Cinnamon: overview of health benefits. *Nutrition Today*. 43: 263-266.
- Symeon G.K., Athanasiou A., Lykos N., Charismiadou M.A., Goliomytis M., Demiris N., Ayoutanti A., Simitzis P.E., Deligeorgis S.G. (2014). The effects of dietary cinnamon (*Cinnamomum zeylanicum*) oil supplementation on broiler feeding behaviour, growth performance, carcass traits and meat quality characteristics. *Annals of Animal Science*. 14: 883-895.
- Tipu M.A., Akhtar M.S., Anjum M.I., Raja M.L. (2006). New dimension of medicinal plants as animal feed. *Pakistan Veterinary Journal*. 26: 144-148.
- Vasconcelos N.G., Croda J., Simionatto S. (2018). Antibacterial mechanisms of cinnamon and its constituents: A review. *Microbial pathogenesis*. 120: 198-203.
- Yanakiev S. (2020). Effects of cinnamon (*Cinnamomum* spp.) in dentistry: A review. *Molecules*. 25: 4184.

Cinnamon - a possible additive in animal feeding

Abstract

Natural supplements have been used for centuries as traditional medicines and food spices. Since 2006, after the ban on the use of antibiotics as growth promoters in the European Union, these compounds have been increasingly used in the animal feed industry. Cinnamon can be a good potential natural additive to animal feed because it contains a number of bioactive components that are responsible for its antimicrobial, antioxidant, and anti-inflammatory effects. Studies have shown that cinnamon has a positive effect on the body weight of animals, lowers blood fat levels, and has a positive effect on the composition of the microflora in the intestines, and on the histomorphology of the small intestine of broiler chickens. For now, there are no recommended concentrations of cinnamon supplementation, and in recent research, added concentrations of this natural supplement ranged from 0.1 to 8.0 g/kg. The aim of this paper was to present cinnamon as a possible natural additive in animal nutrition.

Keywords: natural additives, cinnamon, animal feed

Genomic inbreeding between flocks in Istrian Sheep

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Abstract

Population history, production management and selection pressure affect genome architecture, leading to reduced genetic diversity and increased frequency of runs of homozygosity (ROH). ROH has been used as a useful method for estimating the inbreeding coefficient at the genome level over the last decade. We analyzed 50k SNPs data from 636 animals to determine the distribution and level of ROHs in Istrian Sheep at flock and population level. A total of 15,817 ROHs were detected with an individual average of 24.11 segments. Most of the detected ROH segments belonged to the length category 4-8 Mb (42 %). The average inbreeding coefficients based on ROH (F_{ROH}) were 0.075 across population and in the range from 0.023 and 0.132 within the flock. Based on the length of ROH segments, some flocks show more recent inbreeding (long ROH segments) and some flocks show historical inbreeding (short ROH segments). The results obtained will serve for future mating plans in accordance with the basic principles of optimum contribution selection.

Keywords: Sheep, Flock, Runs of homozygosity, Genomic inbreeding

Introduction

The Istrian Sheep is a local Croatian sheep that is bred on the Istrian peninsula as a dual-purpose breed (milk and meat production) under traditional husbandry systems. In the last ten years, the population size of Istrian Sheep has slowly decreased and today there are about 1100 individuals recorded in the herd book (CAAF, 2022). However, despite the downward trend, many efforts are being made to preserve the breed. On the one hand, this happens through the continuous production of high-quality hard cheese, and in this sense it is important to increase productivity, on the other hand, the Istrian Sheep represents living monument of past centuries, which has a traditional and cultural significance for the inhabitants.

It is well known that the local (native) breeds comprise large neutral genetic variability and represent globally recognised valuable genetic resources for conservation. However, this poses a challenge for breeding programmes, as these populations are usually very small and inbreeding practices cannot be avoided. As a result, increased inbreeding may affect fitness and decrease the mean performance for economically important traits. In recent years, the availability of single nucleotide polymorphism (SNP) markers contribute to study the genetic diversity and population structure of sheep breeds in detail. One of the approaches widely used in genetic studies is the analysis of runs of homozygosity (ROH). ROH are contiguous segments of homozygous genotypes present in an individual that has inherited the same haplotype from both parents and which are identical by descent (IBD; Kirin et al., 2010). The use of ROH segments reveal information on population structure, demographic history and identification of traits associated with ROH regions. The extent and frequency of ROHs are useful to effectively estimate inbreeding, with longer ROH segments reflecting recent inbreeding, and shorter ROH segments ancient inbreeding.

The aim of this study was to quantify genomic inbreeding of Istrian Sheep from the ROH segments at flock level and population level as a preliminary step towards implementing the optimum contribution selection (OCS) approach.

Material and methods

Sample collection, SNP genotyping, and quality control

Ear tissue samples were collected from a total of 719 unrelated animals of Istrian Sheep from ten flocks and genotyped using the Illumina Ovine 50k SNPs array. SNPs at unknown positions and SNPs assigned to mitochondrial, X and Y chromosomes were removed and only autosomal SNPs were used in the analysis. The accuracy of SNP genotyping was assessed by applying a cut-off value of 0.7 for the GenCall score and 0.4 for the GenTrain score. Quality control was performed using PLINK v1.90 software (Chang et al., 2015) based on the following criteria: 1) SNP call rate \leq 90 %, 2) SNP minor allele frequency (MAF) \leq 5 %, and 3) animals displaying \geq 5 % missing genotypes. After quality control, a total of 636 sheep with 38,351 SNPs remained.

Runs of homozygosity and genomic inbreeding coefficient detection

ROH were detected by PLINK v1.90 software (Chang et al., 2015) with sliding window approach to detect autozygous segments. The criterion and thresholds to define a ROH were as follows: 1) the minimum length constituting ROH was set to 1 Mb; 2) at least 44 homozygous SNPs were included in a ROH as proposed Lencz et al., (2007); 3) the maximal gap between adjacent SNPs was 1 Mb; and 4) a minimum density of a SNP per ROH was 1 SNP per every 250 Kb (Meyermans et al., 2020). The number of allowed heterozygotes and missing genotypes in the ROH segment was set to 0 (Ferenčaković et al., 2013). ROH segments of five different lengths (1-4 Mb, 4-8 Mb, 8-16 Mb, 16-32 Mb and >32 Mb) were estimated at population and flock level.

Two genomic inbreeding coefficients were calculated, based on the observed versus expected number of homozygous genotypes (F_{HOM}) and based on the length of ROH segments (F_{ROH}). The individual genomic inbreeding coefficients were computed as the sum of the length of all ROH per animal divided by the total length of the autosomal genome covered by SNPs (2.64 Gb) as described by McQuillan et al. (2008).

$$F_{\text{ROH}} = \frac{\sum_i \text{length}(\text{ROH}_i)}{L(\text{auto})}$$

where $\sum_i \text{length}(\text{ROH}_i)$ is the length of ROHs, and $L(\text{auto})$ is the total length of the genome covered by the SNPs included in this chip. Results obtained from PLINK were analyzed using R (R Development Core Team, 2018).

Results and discussion

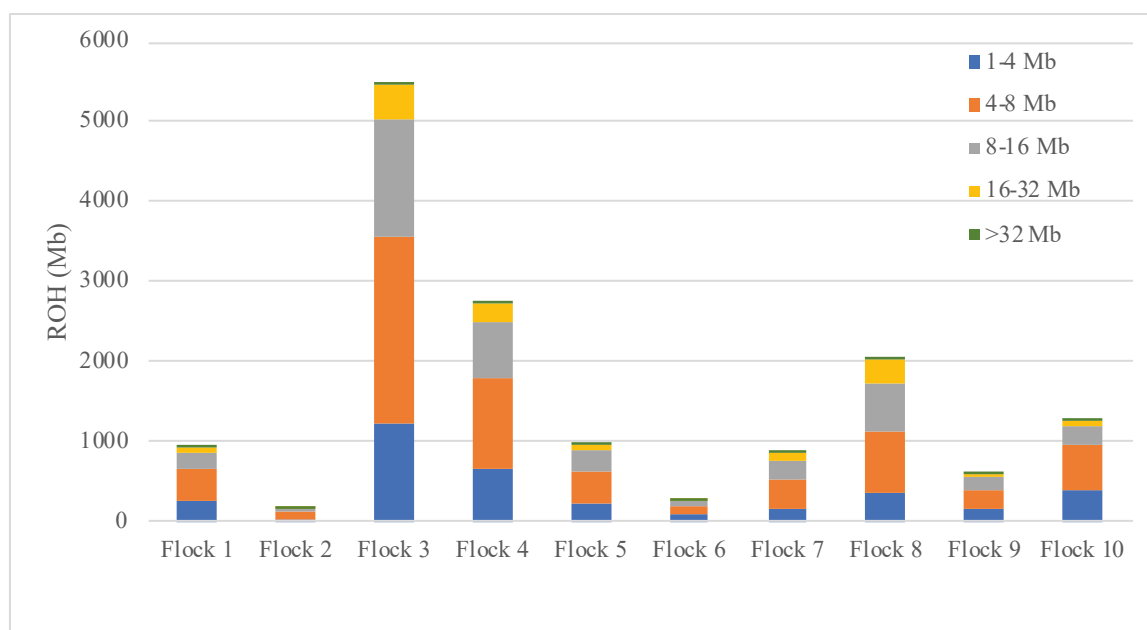
The distribution of ROH segments in Istrian Sheep according to their size are presented in Table 1. A total of 15,817 ROH segments were identified, with an individual average of 24.11 segments. Length of ROH segments ranged from 1.9 Mb to 70.10 Mb with an average length of 8.12 Mb. Most of the detected ROHs ranged from 4 to 8 Mb ($n = 6584$; 42 %) The remaining ROH segments 1-4 Mb, 8-16 Mb, 16-32 Mb and > 32 Mb ranged from 2 to 23 %. The prevalence of ROHs below 8 Mb in this population (Table 1) is in general agreement with many previous findings on this issue (e.g. the reports of Mastrangelo et al. (2018) for Italian breeds, Dzomba et al. (2021) for African sheep breeds, and Nosrati et al. (2021) for majority of worldwide sheep population).

Table 1. Descriptive statistics of ROH analysis in Istrian Sheep

	ROH length categories (Mb)				
	1-4	4-8	8-16	16-32	>32
n ROH	3715	6584	4022	1327	169
% n ROH	23.48	41.63	25.43	8.39	1.07
n Animal	614	626	566	365	102
% Animal	96.6	98.5	89.0	57.4	16.1
F_{ROH}	0.007	0.022	0.029	0.028	0.024

n ROH – number of ROH segments; % – frequency distribution of ROH and animal; n Animal – number of animals; F_{ROH} – ROH coefficient of inbreeding

The highest frequency of homozygous segments of 4-8 Mb was consistently present across all flocks in this population (Graph 1).



Graph 1. Classification of runs of homozygosity (ROH) size (in Mb) in five predefined categories in 10 flocks of the Istrian Sheep

The overall genomic $F_{ROH>4Mb}$ in this population was 0.075 and quite different from the reports of Mastrangelo et al. (2018), Ciani et al. (2020) and Drzaic et al. (2022) with $F_{ROH} = 0.028$, $F_{ROH} = 0.031$, and $F_{ROH} = 0.053$, respectively). However, a direct comparison of the results between the studies is difficult (due to different sample sizes, SNP chip density, set parameters for analysis, etc.). Moderate observed ($H_o = 0.382$) and expected ($H_e = 0.359$) heterozygosities were detected, and the genomic inbreeding coefficient based on these parameters (F_{HOM}) was estimated to be 0.060. The two estimates of genomic inbreeding were highly correlated ($r = 0.976$), so we decided to focus only on F_{ROH} in our later analysis. As it can be seen from Table 2, a relatively high level of inbreeding is present across the entire population of the Istrian Sheep ($F_{ROH} > 6.25\%$). The F_{ROH} coefficient is one of the most accepted tools for estimating autozygosity and detecting inbreeding effects, but it is even more important for managing breeding programs in many livestock species. This genomic parameter has ability to distinguish between recent and distant occurrence of inbreeding. To be more specific, short ROH segments are associated with historical (distant in time) inbreeding and long ROH segments are associated with recent (close in time) inbreeding. Taking into account the above philosophy, it can be concluded that some of the flocks show more recent inbreeding (e.g. Flock 8 and Flock 3) than the other (e.g. Flock 4 and Flock 10).

Table 2. Genomic inbreeding coefficient F_{ROH} by predefined ROH length category (in Mb) per flock in Istrian Sheep.

Flock	n	$F_{ROH\ 1-4}$	$F_{ROH\ 4-8}$	$F_{ROH\ 8-16}$	$F_{ROH\ 16-32}$	$F_{ROH\ >32}$	$F_{ROH-All}$
Flock 1	90	0.034	0.009	0.009	0.004	0.001	0.023
Flock 2	7	0.070	0.027	0.019	0.019	0.004	0.075
Flock 3	135	0.011	0.038	0.045	0.025	0.006	0.125
Flock 4	125	0.007	0.012	0.023	0.015	0.003	0.067
Flock 5	51	0.006	0.017	0.020	0.012	0.002	0.060
Flock 6	9	0.010	0.031	0.028	0.010	0.002	0.080
Flock 7	32	0.006	0.024	0.030	0.023	0.008	0.092

Flock 8	58	0.008	0.029	0.042	0.043	0.011	0.132
Flock 9	28	0.006	0.020	0.023	0.011	0.003	0.064
Flock 10	100	0.005	0.012	0.010	0.005	0.001	0.033

n – number of individuals

Global demographic and socio-economic changes in the last 50 years are among the main reasons which lead to erosion of population size and genetic variability in local (native) breeds. These results firmly evidence that the Istrian Sheep is undergoing these unfavourable changes that threaten its long-term viability. In order to preserve as much of the existing variability (gene pool) in this population as possible, it is necessary to pay more attention to future mating plans in this population, preferably following optimum contribution selection approach.

Conclusion

The study revealed that inbreeding in this population is relatively high and threatens to preservation of long term genetic variability. The estimated inbreeding at the flock level provides good information for implementation of optimum contribution selection in the population of Istrian Sheep. This selection approach, if conducted systematically, should provide selection progress of dairy traits with minimal loss of genetic variability.

Acknowledgment

This research has been fully supported by Croatian Science Foundation under the project Genomic characterization, preservation, and optimum contribution selection of Croatian dairy sheep (OPTI-SHEEP), grant number IP-2019-04-3559. The authors would also like to thank the Croatian Agency for Food and Agriculture (Pazin Substation) for their support in sampling.

References

- CAAF - Croatian Agency for Agriculture and Food (2022). Annual report. Sheep, goats and small animals breeding.
- Chang C.C., Chow C.C., Tellier L.C., Vattikuti S., Purcell S.M., Lee J.J. (2015). Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience*. 4: s13742-015.
- Ciani E., Mastrangelo S., Da Silva A. et al. (2020). On the origin of European sheep as revealed by the diversity of the Balkan breeds and by optimizing population-genetic analysis tools. *Genetic Selection Evolution*. 52: 2-14.
- Drzaic I., Curik I., Lukic B., Shihabi M., Li M.H., Kantanen J., Mastrangelo S., Ciani E., Lenstra J.A., Cubric-Curik V. (2022). High-Density Genomic Characterization of Native Croatian Sheep Breeds. *Frontiers in Genetics*. 13: 940736.
- Dzomba E.F., Chimonyo M., Pierneef R., Muchadeyi F.C. (2021). Runs of homozygosity analysis of South African sheep breeds from various production systems investigated using OvineSNP50k data. *BMC Genomics*. 22: 1-14.
- Ferenčaković M., Sölkner J., Curik I. (2013). Estimating Autozygosity from High-Throughput Information: Effects of SNP Density and Genotyping Errors. *Genetic Selection Evolution*. 45: 10.1186/1297-9686-45-42.
- Kirin M., McQuillan R., Franklin C.S., Campbell H., McKeigue P.M., Wilson J.F. (2010). Genomic runs of homozygosity record population history and consanguinity. *PLoS One*. 5: e13996.
- Lencz T., Lambert C., DeRosse P., Burdick K.E., Morgan T.V., Kane J.M., Kucherlapati R., Malhotra A.K. (2007). Runs of homozygosity reveal highly penetrant recessive loci in schizophrenia. *Proceedings of the National Academy of Sciences*. 104: 19942-19947.
- McQuillan R., Leutenegger A.L., Abdel-Rahman R., Franklin C.S., Pericic M., Barac-Lauc L., et al. (2008). Runs of homozygosity in European populations. *American Journal of Human Genetics*.

83: 359-372.

Meyermans R., Gorssen W., Buys N., Janssens S. (2020). How to study runs of homozygosity using plink? a guide for analyzing medium density snp data in livestock and pet species. *BMC Genomics*. 21: 2-14.

Nosrati M., Nanaei H.A., Javanmard A., Esmailizadeh A. (2021). The pattern of runs of homozygosity and genomic inbreeding in world-wide sheep populations. *Genomics*. 113: 1407-1415.

R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available online at <https://www.R-project.org/>.

Valorizacija sekundarnih animalnih proizvoda u funkciji održive proizvodnje mesa

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Sažetak

Predviđeni porast svjetske populacije od gotovo 35% u sljedeća tri desetljeća i prateće povećanje potražnje za bjelančevinama animalnog podrijetla rezultirat će povećanjem globalne proizvodnje mesa s današnjih 300 mil. tona na 470 mil. tona godišnje. Proporcionalno će nastati i veće količine sekundarnih animalnih proizvoda (SAP) poput mesa unutarnjih organa, ali i klaoničkog otpada te nusproizvoda životinjskog podrijetla. Osim u hrani za ljude, SAP su često glavni sastojak u hrani za kućne ljubimce, a mogu se koristiti i u farmaceutskoj i tekstilnoj industriji, kozmetici ili proizvodnji biogoriva. Boljim iskorištenjem SAP i valorizacijom njihovih prerađevina može se doprinijeti održivosti suvremene proizvodnje mesa, nutritivnom obogaćivanju hrane za ljude i boljem upravljanju prirodnim resursima.

Ključne riječi: prerada mesa, iznutrice, klaonički otpad, nutritivna vrijednost, profitabilnost

Uvod

Globalna proizvodnja mesa na dnevnoj bazi generira velike količine jestivih i nejestivih nusproizvoda, odnosno sekundarnih proizvoda animalnog podrijetla. Prema FAOSTAT-u (2020), oko 10 % ukupne svjetske proizvodnje svinjskog, goveđeg, ovčjeg, kozjeg i bivoljeg mesa (197 933 329 tona) otpada na jestive sekundarne animalne proizvode (SAP). Najčešći jestivi SAP nastali pri klaoničkoj obradi životinja su unutarnji organi poput mozga, jezika, jetre, pluća, srca, crijeva, bubrega, slezene, prsne žlijezde (brizle) i tripica, zajedničkog naziva "iznutrice". Pored toga, pri izradi mesnih proizvoda nastaju brojni drugi jestivi SAP kao što je kolagen i želatina, životinjske masti, krvni derivati te koža. Za razliku od jestivih SAP, čitave lešine i svi dijelovi trupa koji nisu namijenjeni ljudskoj prehrani se u Europskoj uniji nazivaju "nusproizvodi životinjskog podrijetla" (NŽP) i obično se prerađuju da bi se koristili kao hrana za kućne ljubimce, gnojivo ili gorivo. Korištenje ili odlaganje NŽP-a strogo je kontrolirano, a materijal namijenjen zbrinjavanju podijeljen je u kategorije sukladno riziku koji predstavlja za zdravlje životinja i ljudi (Official Journal of the European Union, 2009).

Iako u razvijenim zemljama uspješno posluju mnoga industrijska postrojenja za preradu i zbrinjavanje nusproizvoda animalnog podrijetla, raste globalna zabrinutost da su ti nusproizvodi najčešće nedovoljno iskorišteni (Toldrá i sur., 2012). Pored toga, proces prikupljanja, zbrinjavanja i neškodljivog uklanjanja NŽP-a iziskuje znatna financijska sredstva i na taj način predstavlja velike troškove u mesnoj industriji. Sa stanovišta higijenske i mikrobiološke kvalitete mesa, provedbe stručne klaoničke obrade trupova te primjena odgovarajućih uvjeta tijekom čuvanja jestivih SAP moraju biti standardni postupci u meso-prerađivačkoj industriji. Jedino tako SAP mogu biti vrijedan resurs snažnog ekonomskog potencijala u proizvodnji novih proizvoda i funkcionalnih sastojaka sa značajnom dodanom vrijednošću (Zhang i sur., 2010).

Uslijed indikacija o pojavi zdravstvenih problema dijela stanovništva povezanih s konzumacijom mesa i mesnih prerađevina te loših ekonomskih pokazatelja, tradicionalna tržišta mesa organa (iznutrica) i drugih jestivih SAP postupno su nestajala što je dovelo do povećanog fokusa na neprehrambenu upotrebu NŽP-a u proizvodnji hrane za kućne ljubimce i proizvodnji lijekova (Liu, 2002). Primjerice, prije samo nekoliko desetljeća jetra i bubrezi su u mnogim azijskim zemljama bili skuplji od odreska, a danas se smatraju nusproizvodom. Iako mesna industija konstantno ulaže velike napore u promotivne aktivnosti kako bi što bolje plasirala na tržište jestive SAP, potrebno je

provoditi aktivnosti usmjerene na kreiranje novih proizvoda s dodanom vrijednošću kako bi se taj ulog potpunije isplatio, a potrošači dobili nutritivno vrijedan proizvod bez negativnog učinka na zdravlje (Lynch i sur., 2018). Osim u prehrambene svrhe, SAP se od davnina koriste u proizvodnji raznovrsnih potrepština za svakodnevnu upotrebu, a njihova se važnost kroz povijest mijenjala. Proizvodi izrađeni od nejestivih SAP, najviše odjeća i obuća, još uvijek se smatraju luksuznim proizvodima s prepoznatljivom kvalitetom i dugotrajnošću. Kozmetička se industrija pak uvelike oslanja na različite SAP kako bi pripremila proizvode koji zadovoljavaju ljudske potrebe i sve zahtjevnije standarde tržišta (Toldrá i sur., 2016).

Cilj rada je opisati mogućnosti upotrebe najznačajnijih SAP u ljudskoj prehrani te suvremene i inovativne načine njihovog korištenja kao izvora funkcionalnih i tehnoloških dodataka u prehrambenoj industriji, a u svrhu poboljšanja održivosti i produktivnosti mesne industrije.

Hranjiva vrijednost iznutrica

Općenito govoreći, iznutrice odnosno meso unutranjih organa izvrstan su izvor proteina, minerala (željezo, fosfor, bakar, magnezij i selen), hormona, vitamina (vitamini A, B, D, E i K) te esencijalnih amino i masnih kiselina (Mullen i Álvarez, 2016) zbog čega se neke koriste kao lijekovi (Jayathilakan i sur., 2012). Stoga meso organa može biti zdrav i redovit dio uravnotežene prehrane pri umjerenoj konzumaciji. Suprotno popularnom mišljenju da životinjske masti nisu primjerene za ljudsko zdravlje, u novije vrijeme se smatra da su zasićene masti i kolesterol dio zdravog prehrambenog obrasca ukoliko ih ljudi ne konzumiraju u prekomjernim količinama (10% ili manje kalorija po individui dnevno, prema DGA 2020-2025). Uslijed visokog sadržaja vezivnog tkiva, aminokiselinski sastav mesa organa razlikuje se od onoga krtog mišićnog tkiva. Kao rezultat toga, SAP od svinjskih ušiju, nogica, pluća, želudca i tripica sadrže veću količinu prolina, hidroksprolina i glicina, te nižu razinu triptofana i tirozina nego klasično meso (Liu, 2002).

Jetra je organ s najvećom koncentracijom hranjivih tvari i izdašan je izvor vitamina A, folne kiseline, željeza, kroma, bakra i cinka (Williams, 2007). Primjerice, poznato je da divlji grabežljivci ponajviše preferiraju organe svog plijena i prvo jedu jetru zbog njezina bogatstva gore navedenim nutrijentima. Bubrezi su također bogati hranjivim tvarima i proteinima, posebno omega-3 masnim kiselinama. Meso mozga također sadrži visoke razine omega-3 masnih kiselina i hranjivih tvari poput fosfatidilkolina i fosfatidilserina. Srce je bogato folatima, željezom, cinkom, selenom, vitaminima B2, B6, B12 i koenzimom Q10 (CoQ10). Meso jezika je bogato kalorijama i masnim kiselinama, kao i cinkom, željezom, kolinom i vitaminom B12. Obzirom na mineralni sastav, meso svih iznutrica, osim tripica, sadrži više željeza i bakra od klasičnog krtog mesa (Mullen i Álvarez, 2016).

Uz podrijetlo organa, još jedan važan čimbenik koji treba uzeti u obzir prije uključivanja mesa organa u prehranu ljudi je dodana vrijednost konačnom proizvodu. Jednostavno rečeno, to je stupanj inovacije kojim sekundarni proizvod, koji bi se inače mogao smatrati otpadom, postaje prerađevina tj. prehrambeni artikal primamljiv potrošačima. Takva se dodana vrijednost može postići u smislu postojane stabilnosti na polici, poboljšanih tehnoloških funkcija (aroma, sredstva za vezivanje vode, emulgatori), bolje senzorske kvalitete (boja, tekstura, okus) i sl., a druga alternativa je proizvodnja funkcionalnih sastojaka poput bioaktivnih peptida i antioksidansa (Toldrá i sur., 2016).

Upotreba iznutrica u kulinarstvu

Diljem svijeta mnoge različite kulture imaju običaj koristiti trup životinje u cijelosti za hranu, uključujući korištenje krvi, kostiju i organa. U mnogim azijskim zemljama raznovrsno meso (tzv. „manje vrijedno meso“) smatra se delikatesom i osnova je mnogih tradicionalnih jela, a u drugim zemljama se njihova konzumacija povezuje s populacijom koja ostvaruje niža mjesečna primanja. U Kini mnogi kulinarski recepti prednost daju raznovrsnim vrstama mesa naglašenog okusa i arome, uključujući i meso organa, u odnosu na čisto meso tj. komadno meso (čitave mišiće ili odreske).

Goveđi jezik, organ koji u sebi krije mekano i sočno meso suptilnog okusa, izrazito je tražena delicija u Rusiji, Japanu, na Mediteranu te nekim južnoameričkim državama poput Brazila i Urugvaja. Jezik i jetra sastavni su dio mnogih meksičkih jela, kao što su putzaze (tripice i jetrica s rajčicama), lengua (jezik sa zelenim čilijem) i menudo norteña (juha od tripica). U Rusiji i Egiptu, dvama vodećim svjetskim uvoznicama jestivih iznutrica (glava, jetra, srce, bubrezi i jezik), kućanstva s nižim prihodima češće konzumiraju manje vrijedno meso i jestive organe te tako na jeftin način osiguravaju potrebe za visokokvalitetnim bjelanjčevinama i prehranom u cjelini (Lynch i sur., 2018).

Svinjski jezici najčešće se upotrebljavaju u proizvodnji toplinski obrađenih mesnih proizvoda, a mogu se konzumirati u izvornom obliku nakon kuhanja ili pirjanja.

Jetra je najrasprostranjeniji jestivi organ. Osim za pripremu u kulinarstvu, jetra se oristi u mnogim prerađenim mesnim proizvodima, kao što su jetrene kobasice i jetrena pašteta, pri čemu su gušća i pačja pašteta najcjenjenije i najskuplje delicije. Zbog blažeg okusa i teksture su janjeća, teleća i juneća jetra omiljena među potrošačima u Europi i Sjedinjenim Američkim Državama. Međutim, potrošači u jugoistočnoj Aziji pak preferiraju svinjska jetra. U Ujedinjenom Kraljevstvu je pita od bubrega nacionalni specijalitet. Bubrezi se inače mogu kuhati cijeli ili u kriškama, a obično se prže, peku na žaru ili pirjaju. Meso srca može se koristiti umjesto običnog mesa, ili češće kao sastojak kobasica i drugih mesnih prerađevina. Meso srca je vrlo zahvalna osnovna sirovina u kulinarstvu jer se cijela/narezana srca mogu pržiti, peći na žaru ili pirjati. Svinjska, teleća i janjeća pluća uglavnom se koriste za izradu raznih nadjeva te nekih vrsta kobasica i mesnih prerađevina (Darine i sur., 2010). Osim pluća, među najmanje popularne iznutrice pripada i slezena, koja se nakon usitnjavanja koristi u pripremi pita i kao začim u Ujedinjenom Kraljevstvu, te kao sastojak nekih mesnih prerađevina u SAD-u (Lynch i sur., 2018).

Predželuci goveda i janjadi (burag i kapura), često se upotrebljavaju kao hrana za ljude. Mogu se kuhati (tripice) ili se mogu koristiti u izradi kobasica i mesnih prerađevina. U nekim se zemljama životinjska crijeva nakon kuhanja koriste kao hrana. Životinjska se crijeva također koriste u hrani za kućne ljubimce ili za preradu u mesno brašno, loj ili gnojivo. Međutim, najvažnija upotreba crijeva kao sirovine je u proizvodnji ovitaka za kobasice.

Sekundarni animalni proizvodi mesne industrije kao funkcionalni i tehnološki dodaci hrani

Korištenje SAP kao izvora bioaktivnih peptida bilo je predmetom opsežnih istraživanja tijekom posljednjih godina, a u tome smislu najviše su istraženi krv i kolagen (Ryder i sur., 2016; Alao i sur., 2017). Bioaktivni peptidi su sekvence obično između 2 i 20 aminokiselina koje obavljaju biološku funkciju u jednome ili nekoliko fizioloških sustava ljudskog organizma. Neki se bioaktivni peptidi oslobađaju tijekom obrade hrane, za vrijeme fermentacije ili sušenja, dok drugi nastaju tijekom probave obroka. Prema tome, postoje bioaktivni peptidi s hipokolesterolemičnim, antioksidativnim i antitrombotskim učinkom koji moduliraju rad krvotoknog sustava, dok imunomodulacijski peptidi i oni koji vežu na sebe minerale utječu na rad gastrointestinalnog i imunološkog sustava. Neke skupine peptida mogu sudjelovati u višestrukim reakcijama sustava. Opioidni agonisti i antagonisti mogu djelovati na živčani, gastrointestinalni, i imunološki sustav, dok antimikrobni peptidi mogu modulirati gastrointestinalni i imunološki sustav (Lafarga i Hayes, 2014).

Kolagen je najzastupljeniji protein u mnogim SAP dobivenim iz mesne industrije. Glavni je sastojak kože, kostiju i hrskavice. Nutritivna vrijednost kolagena vrlo je niska jer mu nedostaju esencijalne aminokiseline, dok je s druge strane kolagen vrlo koristan kao izvor bioaktivnih peptida (Herregods i sur., 2011). Također je kao glavni sastojak želatine naveliko prisutan u prehrambenoj industriji, te izrazito bitan u proizvodnji slatkiša. Želatina se dobiva iskuhavanjem kolagenom bogatih dijelova životinjskih trupova: kože, rjeđe papaka, kopita i rogova. Kontroliranom se hidrolizom kolagen postupno prevodi u prah, a dodatkom vode poprma karakterističnu želatinoznu strukturu. Koristi se u proizvodnji gumenih bombona, želea te različitih preljeva za kolače kako bi krajnji proizvodi postigli željenu strukturu i oblik (Gómez-Guillén i sur., 2011).

Krv je važan izvor hemoglobina, a hem oblik željeza (polipeptid), kojeg ima uglavnom u hrani životinjskog podrijetla, apsorbira se puno bolje od željeza koje nije u obliku hema. Navedeni polipeptid može se generirati enzimskom hidrolizom hemoglobina (Nissenson i sur., 2003). Iako se stanična frakcija krvi koja sadrži crvene i bijele krvne stanice te trombocite može koristiti kao pojačivač boje kobasica, njezina primjena u prehrambenoj industriji je ograničena zbog tamne boje hemoglobina, štetnog utjecaja na senzorna svojstva ili čak higijensku kvalitetu proizvoda. S druge strane, proteini krvi zanimljivi su s tehnološkog aspekta u procesu prerade hrane obzirom da osiguravaju određena tehnološka svojstva gotovim prehrambenim proizvodima (Hsieh i Ofori, 2011). Dakle, imunoglobulini, fibrinogen i albumin krvnog seruma doprinose formiranju gel strukture i emulzifikaciji, dok drugi proteini plazme doprinose umrežavanju proteina, obogaćivanju proteina ili pjenjenju (Toldrá i sur., 2016). U neprehrambenom segmentu istraživanja učinaka SAP na ljudski organizam, utvrđeno je visoko antioksidativno djelovanje frakcija crvenih krvnih stanica ovaca, svinja, goveda i crvenog jelena, dok su bijele krvne stanice ovaca imale antimikrobno djelovanje protiv *E. coli*, *S. aureus* i *P. aeruginosa* (Bah i sur., 2016).

Percepcija potrošača o sekundarnim animalnim proizvodima

Unatoč nutritivnim prednostima i uglavnom blagotvornom utjecaju na zdravlje ljudi, SAP se ne smatraju posebno važnim dijelom prehrane u cijelom svijetu u usporedbi s tradicionalnim mesom tj. mišićima. U Europi je upotreba jestivih SAP u ljudskoj prehrani znatno smanjena tijekom 21. stoljeća (FAOSTAT, 2000). Najbolji primjer tome je Španjolska, gdje je potrošnja iznutrica smanjena s 1,15 kg na 0,86 kg po stanovniku u razdoblju 2004. - 2014. Postoje mnogi razlozi koji onemogućavaju postizanje punog potencijala iznutrica u prehrani, a najčešći su percepcija potrošača o bitnim značajkama visokokvalitetnog mesa, kulturološki ili tradicionalni običaji izravne konzumacije iznutrica, njihova prehrambena vrijednost, zatim vjerska ili etička ograničenja te pitanja javnog zdravlja kao što je goveđa spongiformna encefalopatija (BSE). Stoga je važno razumjeti percepciju potrošača kako bi se identificirali glavni čimbenici i prepreke češćoj konzumaciji iznutrica. Tada bi bilo moguće dodati vrijednost iznutricama njihovom konverzijom u prikladniju hranu prilagođenu trenutnim prehrambenim navikama ili kroz razvoj novih funkcionalnih sastojaka za prehranbenu industriju (Toldrá i sur., 2012).

Uoči uvrštavanja mesa organa u prehranu ljudi (ili kućnih ljubimaca), važno je znati iz kakvog sustava uzgoja potječu životinje čiji će organi biti namijenjeni ljudskoj konzumaciji. Pritom su životinje iz ekološke proizvodnje svakako u prednosti nad onima iz konvencionalne, pogotovo kada je riječ o intenzivnom sustavu uzgoja. Osim što je važno poznavati vrstu i kvalitetu krme za životinje, bitno je naglasiti da iznutrice namijenjene prehrani ljudi ne smiju potjecati od životinja neadekvatno tretiranih lijekovima, promotorima rasta i sl., i to ne samo tijekom njihova života, nego i neposredno prije klanja. Preporuka je da meso organa za ljudsku prehranu potječe od životinja uzgojenih na farmama s ekološkom proizvodnjom, što podrazumijeva držanje životinja na paši, odnosno pretežito na otvorenom tijekom čitavog života. Moguća je zabrinutost potrošača da će životinje koje su u nekom trenutku bile izložene toksinima i pesticidima (uglavnom iz konvencionalnih proizvodnih sustava) zadržati određeni dio izvorne toksičnosti u svojim organima. Međutim, činjenica je da organi poput jetre i bubrega zdravih životinja djeluju kao filteri koji izlučuju toksine i štetne produkte metabolizma iz organizma (tj. ne akumuliraju ih).

Senzorna svojstva ključni su element u izboru hrane, što objašnjava da određeni dio populacije nije spreman žrtvovati osjetilni užitak prilikom konzumacije određenog proizvoda, čak ni kada je riječ o poboljšanju zdravstvene ispravnosti namirnice, odnosno obroka. Niža percepcija senzorskih svojstava, sigurnosti i zdravstvene ispravnosti ovih proizvoda savršeno objašnjava negativan stav prema njima. No, relevantna prepreka potrošnji koja je uočena u svim ovim segmentima bila je socijalna komponenta; odnosno percepcija sekundarnih animalnih proizvoda kao značajno drugačijih od uobičajenih prehrambenih proizvoda animalnog podrijetla. Stoga je potrebno usredotočiti napore na inovacije u proizvodnji hrane i marketinške strategije za ovakve specifične proizvode kako bi se smanjili gore navedeni negativni učinci, i to pružanjem jasnih i pouzdanih informacija o njima i isticanjem pozitivnih aspekata povezanih s njihovom uporabom. U svakom slučaju, postoji značajan dio populacije za koji se jasno čini da daje prednost ovoj vrsti proizvoda, te koji cijeni njegova senzorna i nutritivna svojstva (Llauger i sur., 2021).

Zaključak

Sekundarni animalni proizvodi iz proizvodnje mesa svrstavaju se u kategoriju proizvoda manje vrijednosti te se na tržište mogu plasirati u svježem i ohlađenom stanju kada će se koristiti kao osnovne sirovine, ali i kao gotovi pripravci za upotrebu u mesnoj ili drugim prehrambenim i neprehrambenim industrijama. Pritom se ističu iznutrice (unutarnji organi) koje odlikuje visoka nutritivna vrijednost, dok pri umjerenoj konzumaciji mogu biti važan sastojak zdrave, uravnotežene prehrane. Diljem svijeta postoje znatne kulturološke razlike pri korištenju različitih organa kao i cjelovitih trupova životinja za pripremu obroka. U tome postoje prepreke i poteškoće, poput ekonomskih čimbenika u preradi, nepoznavanja inovativnih tehnika i metoda obrade te nedovoljne educiranosti potrošača i poznavanja tržišta, koje umanjuju iskorištenost i globalno rašireniju upotrebu sekundarnih animalnih proizvoda. Obzirom na ubrzani rast svjetske potražnje za proteinima animalnog podrijetla, učinkovito korištenje sekundarnih animalnih proizvoda, posebice mesa iznutrica, vrlo je važno za održivost i profitabilnost mesne industrije u cjelini.

Literatura

- Alao B.O., Falowo A.B., Chulayo A., Muchenje V. (2017). The potential of animal by-products in food systems: production, prospects and challenges. *Sustainability*. 9: 1089-1107.
- Bah C.S.F., El-Din A., Bekhit A., Carne A., McConnell M.A. (2016). Composition and biological activities of slaughterhouse blood from red deer, sheep, pig and cattle. *Journal of the Science of Food and Agriculture*. 96: 79–89.
- Darine S., Christophe V., Gholamreza D. (2010). Production and functional properties of beef lung protein concentrates. *Meat Science*. 84: 315–322.
- DGA (2022). Dietary guidelines for Americans 2020-2025. United States Department of Agriculture. Available from: https://www.dietaryguidelines.gov/sites/default/files/2020-12/Dietary_Guidelines_for_Americans_2020-2025.pdf
- FAO (2009). How to feed the world in 2050. *Population and Development Review*. Available from: https://www.fao.org/fileadmin/templates/wsfs/docs/expert_paper/How_to_Feed_the_World_in_2050.pdf
- FAOSTAT (2020). Food and Agriculture Organization of the United Nations. Data: Crops and livestock products. Available from: <https://www.fao.org/faostat/en/#data/QCL>
- Gómez-Guillén M.C., Giménez B., López-Caballero M.E., Montero M.P. (2011). Functional and bioactive properties of collagen and gelatin from alternative sources: A review. *Food Hydrocolloids*. 25: 1813–1827.
- Herregods G., Van Camp J., Morel N., Ghesquière B., Gevaert K., Vercruyse L., Dierckx S., Quanten E., Smagghe G. (2011). Angiotensin I-converting enzyme inhibitory activity of gelatin hydrolysates and identification of bioactive peptides. *Journal of Agricultural and Food Chemistry*. 59: 552–558.
- Hsieh Y.H., and Ofori J.A. (2011). Food-grade proteins from animal by-products. Their usage and detection methods. In: *Handbook of analysis of edible animal by-products*, Nollet L. M. L., Toldrá F. (Eds.) 3–11. Boca Raton FL, USA: CRC Press.
- Jayathilakan K., Khudisia S., Radhakrishna K., Bawa A.S. (2012). Utilization of by-products and waste materials from meat, poultry and fish processing industries: a review. *Journal of Food Science and Technology*. 49: 278–293.
- Lafarga T., and Hayes M. (2014). Bioactive peptides from meat muscle and by-products: generation, functionality and application as functional ingredients. *Meat Science*. 98: 227–239.
- Llauger M., Claret A., Bou R., López-Mas L., Guerrero L. (2021). Consumer attitudes toward consumption of meat products containing offal and offal extracts. *Foods*. 10: 1454-1471.
- Liu D.C. (2002). Better utilization of by-products from the meat industry 2002-10-01. *Extension Bulletins*. Food and fertilizer Technology Center for the Asian and Pacific region (FFTC publication database). Available from: https://www.ffc.org.tw/htmlarea_file/library/20110706135001/eb515.pdf
- Lynch S.A., Mullen A.M., O'Neill E., Drummond L., Álvarez C. (2018). Opportunities and perspectives for utilisation of co-products in the meat industry. *Meat Science*. 144: 62-73.
- Mullen A.M., and Álvarez C. (2016). Offal: types and composition. In: *The Encyclopedia of Food and Health*, pp 152-157. ISBN 0123849535 9780123849533.
- Nissenson A.R., Berns J.S., Sakiewicz P., Ghaddar S., Moore G.M., Schleicher R.B., Seligman P.A. (2003). Clinical evaluation of heme iron polypeptide: Sustaining a response to rHuEPO in hemodialysis patients. *American Journal of Kidney Diseases*. 42: 325–330.
- Official Journal of the European Union (2009). Regulation of the European Parliament and the Council (EC) No 1069/2009, L 300: 1–33.
- Ryder K., Ha M., El-Din Bekhit A., Carne A. (2015). Characterisation of novel fungal and bacterial protease preparations and evaluation of their ability to hydrolyse meat myofibrillar and connective tissue proteins. *Food Chemistry*. 172: 197–206.

- Toldrà F, Aristoy M.C., Mora L., Reig M. (2012). Innovations in value-addition of edible meat by-products. *Meat Science*. 92: 290–296.
- Toldrà F, Mora L., Reig M. (2016). New insights into meat by-product utilization. *Meat Science*. 12: 54-59.
- USDA (2011). United States Department of Agriculture. Where's the (Not) Meat? In: *Byproducts from beef and pork production*. A Report from the Economic Research Service. LDP-M-209-01. Available from:
https://www.ers.usda.gov/webdocs/outlooks/37427/8801_ldpm20901.pdf?v=116
- Williams P.G. (2007). Nutritional composition of red meat. *Nutrition & Dietetics*. 64: 113-S119.
- Zhang W., Xiao S., Samaraweera H., Lee E.J., Ahn D.U. (2010). Improving functional value of meat products. *Meat Science*. 86: 15–31.

Valorisation of secondary animal products in the sense of sustainable meat production

Abstract

The anticipated surge in the global population by nearly 35% over the next three decades, coupled with a heightened demand for animal-derived proteins, is poised to escalate worldwide meat production to an estimated 470 million tons annually. Consequently, a commensurate increase in secondary animal products (SAP) from the meat industry, encompassing internal organ meats (offal), slaughterhouse waste, and animal by-products (ABPs), is inevitable. Beyond their role in human consumption, SAPs often constitute a primary component in pet food and serve various other purposes. Optimizing the utilization of SAPs derived from meat production and effectively valorizing their products can significantly contribute to the sustainability of contemporary meat production practices, enhance the nutritional profile of human food, and...

Keywords: meat processing, offal, slaughterhouse waste, nutritional value, profitability

Povezanost nekih tjelesnih mjera i mliječnih odlika alpina koza

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Sažetak

Cilj rada bio je utvrditi povezanost nekih tjelesnih mjera s proizvodnjom mlijeka i morfološkim odlikama vimena koza. Istraživanjem je bilo obuhvaćeno 139 alpina koza, držanih u identičnim uvjetima smještaja i hranidbe, na kojima je provedena kontrola mliječnosti (A_{T4}) i u kojih su utvrđene tjelesne izmjere, kao i izmjere morfoloških odlika vimena. Značajne i pozitivne korelacije su utvrđene između dubine prsa, odnosno tjelesne mase i proizvodnje mlijeka (dnevne i laktacijske), kao i između širine prsa i laktacijske proizvodnje mlijeka. Opseg i širina prsa te tjelesna masa alpina koza bile su u značajnoj i pozitivnoj korelaciji s veličinom vimena (dubina i širina vimena) i sisa (duljina i širina sisa).

Ključne riječi: tjelesne mjere, tjelesna masa, proizvodnja mlijeka, morfologija vimena, korelacija

Uvod

Francuska alpska koza (alpina) nastala je križanjem švicarskih s lokalnim pasminama koza u Francuskoj (Porter i sur., 2016). Zbog visoke mliječnosti, uzgoj alpina koza danas je raširen po cijeloj Francuskoj i Sredozemlju, a početkom 19. stoljeća ta je pasmina uvezena i na područje Sjeverne Amerike te je nastavljeno širenje uzgoja po američkom kontinentu. Matična knjiga pasmine je iz 1930. godine, a pasmina je u potpunosti standardizirana nakon Drugog svjetskog rata. Alpina koze se mogu uzgajati u različitim okolišnim uvjetima postižući dobre proizvodne rezultate u ekstenzivnim i intenzivnim sustavima uzgoja budući da se odlikuju dobrom otpornošću i prilagodljivošću (Mioč i Pavić, 2002).

Prema tjelesnoj razvijenosti, alpina koze pripadaju u skupinu srednje razvijenih pasmina; tjelesna masa ženskih grla je između 50 i 80 kg, a muških između 80 i 100 kg. Pasmину odlikuje dobro razvijen trup s izraženim prsnim košem te dobro razvijenim nogama (Mioč i Pavić, 2002). Jedinke mogu biti s rogovima i bez njih, s bradom i resicama. Glava je srednje duga, čelo je široko, blago konkavnog nosnog profila. Uši su kratke i uspravne. Koža alpina je glatka i dobro prorasla dlakom koja je kod jarčeva nešto duža na vratu i plečkama. Vime je pravilno i dobro povezano s trbuhom te pogodno za strojnu mužnju (Porter i sur., 2016). Alpina koza je danas najmlječnija pasmina koza u Francuskoj te u laktaciji prosječno proizvede od 600 do 900 L mlijeka, s time da su nerijetko proizvedu i znatno više mlijeka (>1000 kg). Odlikuje se dobrom plodnošću (prosječno oko 180%), a oko 30% koza ove pasmine jari dva jareta već pri prvom jarenju (Mioč i Pavić, 2002).

Alpina koza je danas najbrojnija inozemna pasmina koza uzgajana u Republici Hrvatskoj i čini gotovo 30% (18 889 grla) ukupne populacije koza upisane u Jedinствени registar ovaca i koza Republike Hrvatske (HAPIH, 2023). Godine 2012. je donesen Program uzgoja koza u Republici Hrvatskoj (Mioč i sur., 2012) s ciljem unaprjeđenja kozarske proizvodnje. Ovim Programom definirane su, između ostaloga, i poželjne eksterijerne i proizvodne odlike alpina koza uzgajanih u Hrvatskoj, što je prikazano u tablici 1.

Tablica 1. Poželjne tjelesne mjere i proizvodne odlike alpina pasmine koza sukladno Programu uzgoja koza u Republici Hrvatskoj (Mioč i sur., 2012)

Odlika	Koze	Jarci
Visina grebena (cm)	70-80	70-80
Tjelesna masa (kg)	60-80	80-100
Plodnost (%)	170-190	
Proizvodnja mlijeka (L)	700-900	
Tjelesna masa jaradi u dobi od 45 do 60 dana (kg)	14-18	

Rezultatima ranijih istraživanja provedenih na različitim pasminama koza utvrđeno je da tjelesno razvijenije i superiornije životinje (unutar iste pasmine i dobi) proizvedu više mlijeka od slabije razvijenih te da tjelesne mjere koza, odnosno mjere njihove tjelesne razvijenosti mogu biti eksterijerni pokazatelji mliječnosti koza (Gall, 1980). Općenito, najpouzdaniji eksterijerni pokazatelji mliječnosti su veličina, izgled i razvijenost vimena te se svojstva morfologije vimena posljednjih godina sve češće uključuju u provedbu uzgojnih programa različitih mliječnih genotipova koza (Vrdoljak i sur., 2020). Stoga je cilj rada bio utvrditi povezanost nekih tjelesnih mjera s proizvodnjom mlijeka i morfološkim odlikama vimena alpina koza.

Materijal i metode rada

Istraživanjem je ukupno bilo obuhvaćeno 139 muznih, čistokrvnih alpina koza. Sva grla odabrana za istraživanje su iz istog stada, u vlasništvu obiteljskog poljoprivrednog gospodarstva u Međimurskoj županiji. Na svim istraživanim kozama primjenjivana je ista tehnologija uzgoja, odnosno proizvodnje (hranidba, način držanja, sezona pripusta/jarenja, trajanje razdoblja sisanja jaradi i dr.). Odabrana grla bila su različitog redoslijeda laktacije, pri čemu je zastupljenost koza različitog redoslijeda laktacije bila podjednaka (29 prvojarki, 31 koza u drugoj laktaciji, 37 koza u trećoj laktaciji, 42 koze u četvrtoj ili kasnijim laktacijama). Iz Matične evidencije Hrvatske agencije za poljoprivredu i hranu (HAPIH) prikupljeni su podaci o svakom istraživanom grlu (datum jarenja, dužina laktacije, redni broj laktacije, i dr.). Sezona jarenja u istraživanom stadu koza bila je od početka siječnja do kraja veljače, dok je prosječno trajanje razdoblja sisanja jaradi bilo 40 ± 3 dana.

Tijekom razdoblja mužnje (koje je u odabranom stadu započelo u ožujku, a završilo u studenom) na svim istraživanim kozama redovito je provedena kontrola mliječnosti primjenom A_{T4} metode (ICAR, 2016). Sukladno preporukama ICAR-a (2016), prva je kontrola mliječnosti provedena najranije 5, a najkasnije 30 dana po odbiću jareta (jaradi). Kontrola mliječnosti je obuhvaćala mjerenje količine namuzenog mlijeka (graduiranom menzuro) te je ukupna ostvarena laktacijska proizvodnja mlijeka za svako pojedino grlo utvrđena izračunavanjem (ICAR, 2016) na temelju podataka mjesečnih kontrola mliječnosti o dnevnoj količini proizvedenog mlijeka.

U cilju utvrđivanja povezanosti pojedinih tjelesnih mjera alpina koza s proizvodnjom mlijeka, sredinom razdoblja mužnje, prilikom provedbe četvrte kontrole mliječnosti koza na odabranom gospodarstvu, korištenjem Lydtinogovog štapa provedeno je mjerenje dubine i širine prsa, dok je pomoću mjerne vrpce utvrđen opseg prsa svake pojedine koze, i to na sljedeći način:

- širina prsa mjerena je iza lopatice, upotrebljavajući oba kraka mjerila koje je pri tome bilo postavljeno vodoravno;
- dubina prsa mjerena je iza lopatice (uzima se od grebena do donjeg ruba prsne kosti) koristeći oba kraka Lydtinova štapa, a pri tome je mjerilo okomito postavljeno na hrptu;
- opseg prsa mjereno je pomoću mjerne vrpce odmah iza lopatice.

Prilikom utvrđivanja mjera tjelesne razvijenosti utvrđena je i tjelesna masa koza pojedinačnim vaganjem na elektronskoj vazi preciznosti ± 100 grama.

Također, prilikom utvrđivanja ranije navedenih tjelesnih mjera i tjelesne mase koza, i to otprilike sat vremena prije početka večernje mužnje, na svim za istraživanje odabranim grlima mjerenjem su primjenom pomične mjerke, mjerne vrpce te kutomjera utvrđene morfološke odlike vimena i sisa, i to: opseg i širina vimena (mjereno u najširem dijelu); dubina vimena (udaljenost između abdominalnog zida i dna vimena); duljina i širina sise (mjereno u središnjem dijelu sise); kut sise (izraženo u stupnjevima ($^{\circ}$) u odnosu na okomitu liniju kroz sredinu vimena (linija između dviju polovica vimena, gledano sa stražnje strane) te udaljenost između vrhova sisa.

Statistička obrada podataka provedena je primjenom statističkog programa SAS STAT (2013.). Opisni statistički pokazatelji su izračunati primjenom procedure MEANS, dok su koeficijenti fenotipskih korelacija između istraživanih svojstava izračunate su primjenom Pearsonovog koeficijenta (procedura CORR).

Rezultati i rasprava

U tablici 2 prikazane su vrijednosti utvrđenih pokazatelja tjelesne razvijenosti istraživanih alpina koza. Najvarijabilniji pokazatelj tjelesne razvijenosti bila je tjelesna masa za koju je koeficijent varijabilnosti (CV) iznosio 12,86%, s najmanje varijabilno svojstvo bila je dubina prsa (koeficijent varijabilnosti 5,84%). U usporedbi s poželjnom tjelesnom masom alpina koza definiranom u Programu uzgoja koza u Republici Hrvatskoj (Tablica 1), prosječna tjelesna masa koza obuhvaćenih istraživanjem bila je nešto manja što se može objasniti činjenicom da je pojedinačno vaganje koza provedeno na početku razdoblja mužnje, odnosno prilikom provedbe prve kontrole mliječnosti (najranije 5, a najkasnije 30 dana po odbiću/odvajanju jaradi od koze/majke), odnosno svega nekoliko tjedana po očekivanom dostizanju vrha laktacijske proizvodnje alpina koza (uobičajeno između 4. i 6. tjedna po partusu). Poznato je, naime, da visokoproduktivna grla gube na tjelesnoj kondiciji, odnosno tjelesnoj masi u razdoblju između partusa i vrhunca laktacijske proizvodnje mlijeka (Datt i sur., 2023).

Tablica 2. Utvrđene mjere tjelesne razvijenosti i tjelesna masa istraživanih alpina koza

Pokazatelj	\bar{x}	SD	Min	Max	CV (%)
Dubina prsa (cm)	31,32	1,83	27,0	36,0	5,84
Širina prsa (cm)	20,15	1,73	16,0	29,0	8,59
Opseg prsiju (cm)	89,71	5,59	62,0	104,0	6,23
Tjelesna masa (kg)	55,29	7,11	29,35	89,65	12,86

\bar{x} – aritmetička srednja vrijednost; SD – standardna devijacija; min – najmanja vrijednost; Max – najveća utvrđena vrijednost; CV – koeficijent varijabilnosti.

U tablici 3 su prikazani opisni statistički pokazatelji proizvodnje mlijeka i morfoloških odlika vimena alpina koza. Najvarijabilnije svojstvo bila je dnevna proizvodnja mlijeka (CV=53,43%), dok je od analiziranih morfoloških odlika vimena, očekivano obzirom na zahtjevnost mjerenja, najvarijabilniji bio kut sisa (CV=47,10%). Ostvarena prosječna laktacijska proizvodnja mlijeka i trajanje laktacije istraživanih alpina koza (gotovo 900 kg mlijeka tijekom prosječnog trajanja laktacije od 294,7 dana) bila je znatno veća nego je utvrđeno za uzgojno-valjanu populaciju alpina koza u Hrvatskoj u 2022. godini: 676 kg mlijeka tijekom prosječnog trajanja laktacije od 243 dana (HAPIH, 2023.). U skladu s time utvrđena je visoka prosječna dnevna proizvodnja mlijeka (4,66 kg), odnosno gotovo dvostruko veća nego su istraživanjem mliječnosti uzgojno valjane populacije koza u RH tijekom ranog stadija laktacije (2,50 kg/dan) utvrdili Mioč i sur. (2008). Istraživanjem utvrđena veličina sisa (duljina i širina sisa) bila je usporediva s vrijednostima koje za alpina koze navode Rupp i sur. (2011), dok je utvrđena dubina vimena istraživanih alpina koza bila osjetno veća (20,04 : 15,82 cm) nego su u alpina koza ranije utvrdili Cividini i sur. (2016).

Tablica 3. Opisni statistički pokazatelji proizvodnje mlijeka i morfoloških odlika vimena alpina koza (n=139)

Pokazatelj	\bar{x}	SD	Min	Max	CV (%)
Dnevna proizvodnja mlijeka (kg)	4,66	2,49	0,95	5,10	53,43
Laktacijska proizvodnja mlijeka (kg)	898,95	303,7	351,0	1580,1	33,78
Dužina laktacije (dana)	294,7	25,56	166	323	8,67
Opseg vimena (cm)	56,05	12,75	33,6	67,0	22,75
Dubina vimena (cm)	20,04	3,92	14,10	26,80	19,56
Širina vimena (cm)	15,21	3,88	7,90	21,50	25,51
Udaljenost između vrhova sisa (cm)	12,22	3,28	7,2	20,0	26,84
Kut sisa (°)	42,64	20,10	10,0	72,0	47,10
Duljina sisa (cm)	5,43	2,08	2,1	10,0	38,30
Širina sisa (cm)	3,34	1,49	1,90	5,90	44,61

\bar{x} – aritmetička srednja vrijednost; SD – standardna devijacija; min – najmanja vrijednost; Max – najveća utvrđena vrijednost; CV – koeficijent varijabilnosti.

Koeficijenti fenotipskih korelacija između utvrđenih tjelesnih mjera, odnosno tjelesne mase i proizvodnje mlijeka alpina koza prikazani su u tablici 4. Između mjera tjelesne razvijenosti alpina koza i proizvodnje mlijeka (dnevna i laktacijska proizvodnja) utvrđeni su niski do umjereno visoki, pozitivni koeficijenti fenotipskih korelacija. Pritom su statistički značajne ($P < 0,001$) korelacije utvrđene između dubine prsa, kao i tjelesne mase koza i proizvodnje mlijeka (dnevne i laktacijske), odnosno između širine prsa i laktacijske proizvodnje mlijeka ($P < 0,05$). Trajanje laktacije, međutim, nije bilo u značajnoj ($P > 0,05$) korelaciji s analiziranim tjelesnim mjerama, kao niti s tjelesnom masom koza. Rezultati ovog istraživanja u skladu navodima Gall (1980., cit. Mioč i Pavić, 2002) kako tjelesni okvir životinje utječe na količinu proizvedenog mlijeka obzirom da veće životinje imaju veći kapacitet probavnih organa i shodno tome imaju veći kapacitet konzumacije hrane, a poznato je da je hranidba najvažniji negenetski čimbenik proizvodnje mlijeka. Nasuprot rezultatima ovog istraživanja, Tilki Yilmaz i Keskin (2021) su utvrdili postojanje pozitivne povezanosti opsega prsiju Kilis pasmine koza uzgajane u Turskoj i količine proizvedenoga mlijeka.

Kako je prikazano u tablici 4, tjelesne mjere koza, kao i njihova tjelesna masa bile su u pozitivnoj korelaciji s gotovo svim utvrđenim morfološkim odlikama vimena i sisa. Pritom su tjelesna masa, zatim opseg prsa te širina prsa bili statistički značajno povezani s dubinom i širinom vimena te s dimenzijama (veličinom) sisa (duljina i širina sisa). Širina prsa, međutim, nije bila u statistički značajnoj korelaciji ($P > 0,05$) niti s jednom morfološkom odlikom vimena i sisa alpina koza što se eventualno može objasniti činjenicom da je širina prsa bila slabo povezana ($r = 0,10$; $P > 0,05$) s količinom dnevno proizvedenoga mlijeka. Naime, upravo je količina u vimenu sintetiziranog mlijeka u (gotovo) linearnom odnosu s veličinom (volumenom) mliječne žlijezde (Gall, 1980., Vrdoljak i sur., 2020). Opseg vimena, međutim, kao jedna od mjera veličine vimena, u istraživanih grla nije bio u statistički značajnoj korelaciji niti s jednom tjelesnom mjerom. Navedeno je u suprotnosti s rezultatima istraživanja Merkhana (2019) provedenog na kozama lokalne pasmine Meritz. Nadalje, Makamua i sur. (2023) su utvrdili postojanje pozitivne i značajne korelacije između opsega vimena i drugih mjera vimena (dubina i opseg vimena) s tjelesnom razvijenošću sanskih koza, dok korelacije između dimenzija sisa i analiziranih tjelesnih mjera, nasuprot ovome istraživanju, nisu bile značajne. Jena i sur. (2019) navode značajnu pozitivnu korelaciju između mjera veličine vimena Beetal pasmine (uključujući i opseg vimena) i ocjene njihove tjelesne kondicije (sustav ocjenjivanja od 0 do 5), što je usporedivo sa svojstvom tjelesne mase koza, utvrđenom u ovom istraživanju.

Tablica 4. Koeficijenti fenotipskih korelacija između mjera tjelesne razvijenosti i proizvodnje mlijeka te morfoloških odlika vimena i sisa alpina koza

	Opseg prsa	Dubina prsa	Širina prsa	Tjelesna masa
Dnevna proizvodnja mlijeka	0,15	0,31**	0,10	0,32**
Laktacijska proizvodnja mlijeka	0,08	0,27**	0,22*	0,35**
Dužina laktacije	-0,08	0,09	0,13	0,14
Opseg vimena	0,13	0,04	0,03	0,17
Dubina vimena	0,28*	0,09	0,25*	0,26**
Širina vimena	0,36***	0,07	0,28**	0,34**
Udaljenost vrhova sisa	0,04	0,08	-0,07	0,12
Kut sisa	-0,08	0,04	0,05	-0,04
Duljina sisa	0,29**	0,04	0,25*	0,23*
Širina sisa	0,36**	-0,02	0,29**	0,20*

* $P < 0,05$; ** $P < 0,01$; *** $P < 0,001$.

Zaključak

Na temelju ovim radom utvrđenih koeficijenata fenotipskih korelacija može se zaključiti da alpina koze veće tjelesne mase te veće tjelesne razvijenosti (dublje i šireg grudnog koša) se odlikuju većom mliječnošću od lakših i tjelesno manje razvijenih koza (u pogledu razvijenosti prsa). Povezano s time, utvrđeno je da je tjelesna masa i tjelesna razvijenost alpina koza pozitivno povezana s veličinom njihova vimena (dubinom i širinom vimena) i sisa (duljina i širina).

Literatura

- Cividini A., Flisar T., Kovač M., Kompan D. (2016). Correlations between udder traits and their relationship with milk yield during first lactation in Slovenian Alpine goats. *Acta Agriculturae Slovenica*. 5: 113-117.
- Datt W., Bhatshwar V., Rai D.C. (2023). Importance of body weight, age and body condition in weaning of goat kids: a review. *Journal of Livestock Science*. 14: 71-77.
- Gall C. (1980). Relationship between body conformation and production in dairy goats. *Journal of Dairy Science*. 63: 1768-1781.
- HAPIH (2023). Ovčarstvo, kozarstvo i male životinje: Godišnje izvješće za 2022. godinu. Hrvatska agencija za poljoprivredu i hranu, Osijek.
- ICAR (2016). International agreement of recording practices. International Committee for Animal Recording, Rome, Italy.
- Jena S., Malik D.S., Kaswan S., Sharma A., Kashyap N., Singh U. (2019). Relationship of udder morphometry with milk yield and body condition traits in Beetal goats. *Indian Journal of Animal Sciences*. 89: 204–208.
- Makamua T.C., Madikadikea M.K., Kagisho M., Mokoena M., Tyasia T.L. (2023). Relationship between body measurement traits, udder measurement traits and milk yield of Saanen goats in Capricorn district of South Africa. *Revista mexicana de ciencias pecuarias*. 14: 423-433.
- Merkhan K.Y. (2019). Milk production and body weight at weaning and their relationships with body and udder measurements in Meriz goats. *Iraqi Journal of Agricultural Sciences*. 50: 480-486.
- Mioč B., and Pavić V. (2002). *Kozarstvo*. Hrvatska mljekarska udruga. Zagreb.
- Mioč B., Prpić Z., Vnučec I., Barać Z., Samaržija D., Pavić V. (2008). Factors affecting goat milk yield and composition. *Mljekarstvo*. 58: 305-313.
- Mioč B., Barać Z., Pavić V., Prpić Z., Mulc D., Špehar M. (2012). Program uzgoja koza u Republici Hrvatskoj. Hrvatski savez uzgajivača ovaca i koza. Zagreb.
- Porter V., Alderson L., Hall S.J.G., Sponenberg D.P. (2016). Goats. U: *Mason's World Encyclopedia of Livestock Breeds and Breeding*. Str. 350-352, CAB International.
- Rupp R., Clément V., Piacere A., Robert-Granié C., Manfredi E. (2011). Genetic parameters for milk somatic cell score and relationship with production and udder type traits in dairy Alpine and Saanen primiparous goats. *Journal of Dairy Science*. 94: 3629-3634.
- SAS STAT (2013). OnlineDoc® Software Release 9.4. SAS Institute Inc., Cary, North Carolina, USA.
- Tilki Yilmaz H., and Keskin M. (2021). Relationships between different body characteristics and milk yield traits in Kilis goats. *Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi*. 26: 272-277.
- Vrdoljak J., Prpić Z., Samaržija D., Vnučec I., Konjačić M., Kelava Ugarković N. (2020). Udder morphology, milk production and udder health in small ruminants. *Mljekarstvo*. 70: 75-84.

Relationship between some body measurements and dairy traits of Alpine goats

Abstract

The aim of the paper was to determine the relationship of some body measurements with milk yield and udder morphological traits of Alpine goats. The research included 139 Alpine goats, kept in identical housing and feeding conditions, on which milk yield control was performed (A_{T_4}) and in which body measurements, as well as measurements of the udder morphological traits were determined. Significant positive correlations were established between chest depth, body weight and milk yield (daily and lactation), as well as between chest width and lactation milk yield. Chest girth and chest width, as well as the body weight of Alpine goats, were significantly and positively correlated with udder (udder depth and width) and teat (teat length and width) size.

Keywords: body measurements, body weight, milk production, udder morphology, correlation

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Phenolic compounds evaluation in wines of Serbian autochthonous and local grapevine varieties

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Abstract

Qualitative and quantitative content of phenolic compounds in monovarietal wines of seven Serbian and two international varieties was determined and compared. The results showed that the variety influences the composition and content for most phenolic components determined in wines. The largest number of phenolic compounds (15) was detected in 'Prokupac' wine, while the smallest number (10) was found in 'Smederevka' wine. Hidroxybenzoic and hidroxicinnamic acids had the strongest discriminating effect. PCA indicated that phenolic composition depends on both the variety and the vintage, while in some cases their interaction was also manifested. From Serbian variety 'Prokupac' wine had the most specific phenolic profile.

Keywords: *Vitis vinifera* L., antioxidant activity, phenolic profile, PCA

Introduction

Wine is traditional and the most popular alcoholic beverage consumed worldwide. It is a rich source of phenolic compounds, which contribute to the mouth-feel sensations (Ma et al., 2014), color, flavor and aroma of wine (Morata et al., 2020). Biosynthesis of these compounds is determined by genes controlling the production of the individual enzymes involved in the relevant biosynthetic pathways (Makris et al., 2006). Further, several factors such as soil condition, climatic conditions and seasonal variation during grape development (Rodríguez-Montealegre et al., 2006), vineyard cultural and applied agronomical practices (Bešlić et al., 2015), can be affected the polyphenolic profile. The technological process and winemaking techniques can also play an important role in the final phenolic composition of the obtained wines (Zhang et al., 2021).

According to Peixoto et al. (2018), phenolic compounds found in wine can be classified in three main groups: phenolic acids (hydroxybenzoic and hydroxycinnamic acids), flavonoids (catechins, flavonols and anthocyanins) and proanthocyanidins. Phenols are not only closely related to wine quality but have also been shown to have health-promoting properties. Numerous studies shown that moderate consumption of wine is healthy, including antioxidant (Gutiérrez-Escobar et al., 2021; Nemzer et al., 2022; Radeka et al., 2022) and cardioprotective effects (Haseeb et al., 2019; Weaver et al., 2021).

Serbia has a long tradition in wine production. Due to its favorable climate and geological characteristics, it is an ancient wine growing region hosting a wide range of indigenous and/or traditional local grapevine varieties, most of which are not internationally recognized (Bešlić et al., 2012). In recent decades, the area under vineyard has significantly increased resulting in an increase in wine production, and a higher presence of these wines in the domestic and worldwide markets. This has led to renewed interest in indigenous grapevine varieties, because monovarietal wines produced from these varieties gain attention on the wine market, and with their authenticity contribute to the recognition of the region of their origin. In order to protect the authenticity of these wines, characterization and discrimination is an important issue, and evaluation of the phenolic profile seems to be a very suitable method of defining the authenticity of the individual wines (Kallitraka et al., 2007). According to Garrido and Borges, (2013) the phenolic composition of wines, which determines their organoleptic properties and provides

information about their primary characteristics, can be used as a fingerprint to distinguish them according to their origin in terms of grapevine variety, region and vintage.

Considering this, the aim of the present study was to determine total phenolic content (TPC), radical scavenging activity (RSA) and phenolic profile in wines of Serbian autochthonous or local grapevine varieties and to evaluate possible differences based on the detected polyphenols and PCA multivariate analysis. To the best of knowledge, this is the first comparative study of the phenolic profile of the Serbian monovarietal wines and well-known international wine varieties.

Material and methods

As a material in this two-year trial wine samples from 7 Serbian autochthonous and local and 2 international varieties from two vintages were investigated (Table 1). The wines were produced in the experimental winery of Faculty of Agriculture in Belgrade using small batches of 50 l. Grapes were harvested at the technological ripeness for each variety individually. The grape samples of each variety were processed immediately using small steel vats of 80 l capacity and 50 l double jacketed stainless steel fermenters. The vinification techniques were the same for all the red and white wines, respectively. Wine samples was prepared by procedure described by Pantelić et al. (2018)

Table 1. Description of the wines studied

Variety	Type of wine	Type of variety	Vineyard location
Cabernet Sauvignon	Red	International	South Serbia
Prokupac	Red	Autochthonous	South Serbia
Crna Tamjanika	Red	Autochthonous	East Serbia
Plovдина	Red	Local	South Serbia
Začinak	Red	Autochthonous	East Serbia
Chardonnay	White	International	South Serbia
Smederevka	White	Local	Central Serbia
Bela Tamjanika	White	Local	South Serbia
Kreaca	White	Autochthonous	North Serbia

In obtained wines total phenolic content (TPC), radical scavenging activity (RSA), and individual polyphenols were determined. Before the analysis all wine samples were filtered through a 0.45- μm PTFE filters and appropriately diluted. All procedures were described in Šuković et al. (2020). Spectrophotometric measurements (determination of TPC, and RSA) were carried out in triplicate on a GBC Cintra 6 UV-VIS spectrophotometer (GBC Scientific Equipment Ltd.). TPC were determined using Folin-Ciocalteu method, while RSA was evaluated using DPPH[·] assay. TPC and RSA results were expressed as equivalents of gallic acids and Trolox, respectively. Identification and quantification of the individual polyphenols was performed using a Thermo Scientific ultra-high performance liquid chromatography (UHPLC) system consisting of a quaternary Accela 600 pump and Accela Autosampler, connected to a linear ion trap-orbitrap (LTQ Orbitrap XL) hybrid mass spectrometer with heated-electrospray ionization probe (HESI-II, ThermoFisher Scientific, Bremen, Germany). Synchronis C18-column (100 \times 2.1 mm, 1.7 μm particle size) was used for the separations of compounds. The mobile phase consisted of water and acetonitrile (both with 0.1% formic acid). For the instrument control, data acquisition and analysis, Xcalibur software (version 2.1, Thermo Fisher Scientific, Waltham, MA, USA) was used. ChemDraw, the molecule editor program, (version 12.0, CambridgeSoft, Cambridge, MA, USA), was used as a reference library to calculate the exact (monoisotopic) masses of compounds of interest.

To establish the significance of differences between the varietal wines, analysis of variance (ANOVA) and Tukey's test for post hoc comparison et significance level of $P < 0.05$ was applied. The obtained data (44 variables) were used in a principal component analysis (PCA) to differentiate and discriminate the wine samples according to the variety, vintage and type of grape (white vs. red). All statistical analyses were performed using "Statistica" (Stat Soft software Inc., Tulsa, OK, USA) program package.

Result and discussion

As expected, red wines had a higher TPC and RSA, than white wines. Differences between years also manifested (Table 2). Both TPC and RSA values were found to higher in 2019 than in 2018. On the average, TPC and RSA in the samples of red wines were several times higher than in the white wines (Table 3). Differences for these two parameters were determined between red varieties (0.33-1.70 mg GAE g⁻¹ for TPC and 1.26-10.85 μ mol TE g⁻¹ for RSA) and it was fairly uniform across the white varieties (0.13-0.22 mg GAE g⁻¹ for TPC and 0.46-0.64 μ mol TE g⁻¹ for RSA). The strong linear relationship between TPC and RSA, pointing phenolics as the major contributors to the antioxidant potential of the wine samples studied.

Table 2. Total phenolic content (TPC) and radical-scavenging activity (RSA) in wines of studied grapevine varieties

Variety	TPC (mg GAE g ⁻¹)		RSA (μ mol TE g ⁻¹)	
	2018	2019	2018	2019
Cabernet Sauvignon	1.29±0.01	2.10±0.02	7.29±0.22	14.40±0.03
Prokupac	1.01±0.02	1.29±0.02	6.36±0.03	8.39±0.10
Crna Tamjanika	0.57±0.00	1.01±0.01	2.69±0.14	5.64±0.05
Plovdina	0.79±0.02	0.81±0.01	4.42±0.01	5.13±0.05
Začinak	0.24±0.00	0.42±0.01	1.05±0.05	1.47±0.05
Chardonnay	0.18±0.00	0.18±0.00	0.68±0.03	0.50±0.01
Smederevka	0.14±0.00	0.12±0.00	0.54±0.01	0.37±0.01
Bela Tamjanika	0.18±0.00	0.21±0.00	0.68±0.01	0.48±0.01
Kreaca	0.20±0.00	0.23±0.00	0.63±0.00	0.65±0.00

A total of 20 phenolic compounds in the wine samples were detected by the UHPLC-DAD method and divided into seven classes based on their structure (Table 3). The largest number of phenolic compounds (15) was detected in the wine of the 'Prokupac', while the smallest number (10) was found in the wine of the 'Smederevka'. In general, in our study, the red wines contained the highest concentrations of phenolics, while the white wines had lower values. Thus, our results are in line with expectations, considering that the pulp, skin, and seeds of grapes contain different classes and amounts of phenolic components (Pantelić et al., 2016), and that red wines are exposed to all parts of the grape during winemaking, while polyphenols in white wines mostly originating from the pulp (Gutiérrez-Escobar et al., 2021).

Table 3. Mean values of TPC (mg GAE g⁻¹), RSA (μmol TE g⁻¹) and phenolics (mg/l⁻¹) in monovarietal wines studied

Compounds	Red grapevine varieties					White grapevine varieties			
	Cabernet Sauvignon	Prokupac	Crna Tamjanika	Plovdina	Začinak	Chardonnay	Smederevka	Bela Tamjanika	Kreaca
TPC	1.70 ^a	1.15 ^b	0.79 ^b	0.80 ^b	0.33 ^c	0.18 ^c	0.13 ^c	0.20 ^c	0.22 ^c
RSA	10.85 ^a	7.38 ^b	4.17 ^c	4.78 ^{bc}	1.26 ^d	0.59 ^d	0.46 ^d	0.58 ^d	0.64 ^d
Hydroxybenzoic acids									
1. Gallic acid	12.38 ^a	11.57 ^a	2.68 ^b	2.37 ^b	3.26 ^b	0.29 ^c	4.85 ^b	4.38 ^b	0.32 ^c
2. Protocatechuic acid	0.81 ^{ab}	0.93 ^{ab}	0.53 ^{bcd}	0.39 ^{cd}	1.33 ^a	0.05 ^d	0.64 ^{bc}	0.34 ^{cd}	0.09 ^d
3. Gentisic acid	0.68 ^a	0.48 ^{abc}	0.17 ^{de}	0.27 ^{cd}	0.15 ^{de}	0.53 ^{ab}	0.36 ^{bcd}	0.03 ^e	0.35 ^{bcd}
4. Ellagic acid	4.44 ^a	3.41 ^a	0.15 ^b	0.33 ^b	0.32 ^b	-	0.31 ^b	0.21 ^b	0.07 ^b
5. Vanillic acid	-	-	0.28 ^a	-	-	-	-	0.06 ^b	-
Hydroxycinnamic acids									
6. Chlorogenic acid	-	-	-	0.005	-	-	-	-	-
7. Caffeic acid	0.89 ^{bc}	1.11 ^{ab}	0.40 ^{bc}	0.49 ^{bc}	1.01 ^b	1.84 ^a	0.29 ^c	0.40 ^{bc}	0.30 ^c
8. Ferulic acid	-	-	-	-	-	0.01	-	-	-
9. p-Coumaric acid	0.09 ^{bc}	0.12 ^{ab}	-	0.03 ^c	0.07 ^{bc}	0.16 ^a	0.06 ^{bc}	-	-
10. Sinapic acid	-	0.44 ^{ab}	-	-	-	-	-	0.13 ^b	0.92 ^a
Coumarins									
11. Aesculin	-	-	-	0.005	-	-	-	-	-
12. Aesculetin	-	0.38 ^a	-	0.09 ^b	-	-	-	-	-
Flavan-3-ols									
13. Catechin	5.15 ^a	1.75 ^{bc}	2.33 ^b	0.37 ^{cd}	0.47 ^{cd}	0.22 ^{cd}	1.06 ^{bcd}	4.08 ^a	0.11 ^d
14. Gallocatechin gallate	0.05	0.08	0.05	-	-	0.09	-	-	0.04
15. Gallocatechin	-	0.045	-	-	-	-	-	-	-
16. Epigallocatechin gallate	-	0.20 ^{ab}	0.13 ^b	-	-	0.48 ^a	-	-	0.06 ^b
Flavonols									
17. Quercetin 3-galactoside (Hyperoside)	0.02 ^c	0.09 ^c	0.33 ^a	0.01 ^c	0.23 ^{ab}	-	0.03 ^c	0.12 ^{bc}	-
Flavons									
18. Cynaroside	-	-	-	-	-	-	-	0.02	-
Dehydroflavonol									
19. Phlorizin	0.120 ^a	0.085 ^{ab}	0.055 ^{ab}	0.020 ^b	0.120 ^a	0.010 ^b	0.035 ^b	0.040 ^{ab}	0.005 ^b
Stilbens									
20. Oxyresveratrol	0.165 ^b	0.470 ^a	0.300 ^a	0.175 ^b	-	0.195 ^{ab}	0.075 ^b	0.120 ^b	0.175 ^b

^a Different letters in the same row denote a significant difference among varieties according to Tukey's test, $p < 0.05$.

Significant differences for most phenolic components were determined (Table 3). Hydroxybenzoic acids were the most abundant in analyzed red wines, whereby the highest concentration was found in wines of autochthonous variety 'Prokupac' and international variety 'Cabernet Sauvignon'. From this group, vanillic acid was found only in 'Tamjanika Crna' and 'Tamjanika Bela' wines, which can be related to their muscat aroma. From the group of hydroxycinnamic acids caffeic acid and p-coumaric acid were the dominant compounds present in highest concentration in 'Chardonnay' wine (1.84 mg l^{-1} and 0.16 mg l^{-1} , respectively). Of the remaining phenolic compounds, only catechin and phlorizin were present in all monovarietal wines, while the others showed varietal specificity. Our results are consistent with some previous studies (Mitić et al., 2010; Zhu et al., 2014; Ivanova-Petroleus et al., 2015; Šćepanović et al., 2019; Merkyté et al., 2020; Kroppek et al., 2023), which found that specific phenolic profile are suitable for the characterization of wines according to variety.

For clearer interpretation of the results, Principal Component Analysis (PCA) was performed presenting differences among grapevine varieties and years of vintage. With respect to PCA, the first two components obtained explained 47.1% of the total variability of the original data (27.4% was assigned to the first factor and 19.7% to the second factor). Besides TPC and RSA out of 20 phenolic components detected in wines, PC 1 was mostly described by hydroxybenzoic acids (gallic, protocatechuic and ellagic) and phlorizin (positive loading), while hydroxycinnamic acids (caffeic, ferulic and p-coumaric) and epigallocatechin gallate (negative loading) had high contributions within PC2 (Figure 1).

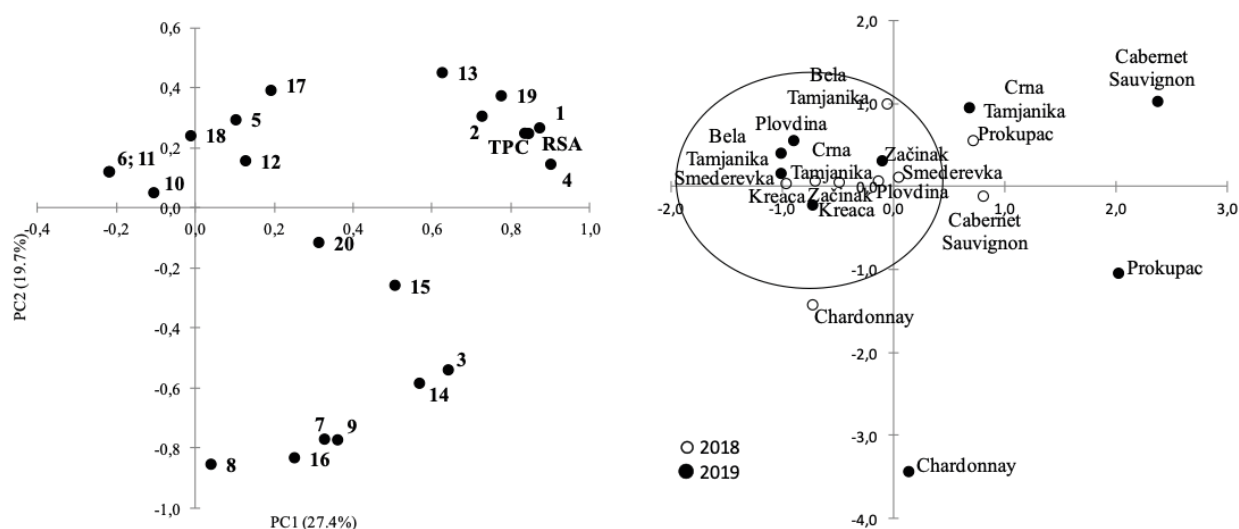


Figure 1. Loading plot representing phenolic compounds (represented by number from Table 3) and scatter plots representing variety and vintages, based on PCA

As can be seen in Figure 1, the distribution of samples into the score plot did not show any important grouping. Contrary to the results of Pantelić et al. (2018) and Šćepanović et al. (2019) in present study PCA not separated red vs. white wines. The wine of most varieties in both years showed little variability in terms of the phenolic profile and was closely located on the scatter plot forming one common group. From this group, the wines of 'Prokupac', 'Cabernet Sauvignon' and 'Chardonnay' stood out in both years and 'Crna Tamjanika' wine in 2019 year. Also, PCA showed that in addition to genetic potential of the variety, content of phenolic compounds in wines depends on vintage while in some cases interaction of these factors was manifested, which is in agreement with the results of Vilanova et al. (2009) and Lampíř and Pavloušek (2013). The influence of environmental factors was more pronounced in the varieties outside the group, while the varieties in the group showed greater stability in relation to the phenolic profile.

Conclusion

The phenolic composition of wines from some autochthonous and local Serbian varieties and its comparison with international varieties 'Cabernet Sauvignon' and 'Chardonnay' is reported for the first time. Obtained results give

important information for researchers, but also for the winemaking industry and consumers to understand the nature and content of phenolic compounds in different wine from Serbian varieties, as the most important components that influence the color, stability as well as sensorial properties of wines. Also, results are indicative of the polyphenolic richness of wine of autochthonous 'Prokupac' compared with the other varieties. This can be important information for winemakers in making decisions about the length of certain processes in winemaking, such as maceration time.

Acknowledgments

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia under projects No. TR31063 and 172017).

References

- Bešlić Z., Todić S., Korac N., Lorenzi S., Emanuelli F., Grando M.S. (2012). Genetic characterization and relationships of traditional grape cultivars from Serbia. *Vitis*. 51: 183-189.
- Bešlić Z., Pantelić M., Dabić D., Todić S., Natić M., Tešić Ž. (2015). Effect of vineyard floor management on water regime, growth response, yield and fruit quality in Cabernet Sauvignon. *Scientia Horticulturae*. 197: 650–656.
- Garrido J., Borges F. (2013). Wine and grape polyphenols-A chemical perspective. *Food Research International*. 54: 1844–1858.
- Gutiérrez Escobar R., Aliaño González M.J., Cantos Villar E. (2021). Wine polyphenol content and its influence on wine quality and properties: A review. *Molecules*. 26: 718.
- Haseeb S., Alexander B., Lopez Santi R., Sosa Liprandi A., Baranchuk A. (2019). What's in wine? A clinician's perspective. *Trends in Cardiovascular Medicine*. 29: 97–106.
- Ivanova-Petroleum V, Hermosín-Gutiérrez I., la Boros B., Stefova M., Stafilov T., Vojnoski B., Dörnyei A., Kilár F. (2015). Phenolic compounds and antioxidant activity of Macedonian red wines *Journal of Food Composition and Analysis*. 41: 1–14.
- Kallitraka S., Mamalos A., Makris D.P. (2007). Differentiation of young red wines based on chemometrics of minor polyphenolic constituents. *Journal of Agricultural and Food Chemistry*. 55: 3233–3239.
- Kropek M., Štefan M.B., Rajkovača K., Petković T., Cvetnić M., Bolanča T., Vladimir Knežević S. (2023). Comparative phenolic profiles of monovarietal wines from different Croatian regions. *Applied Science*. 13: 3031.
- Lampíř L., Pavloušek P. (2013). Influence of locality on content of phenolic compounds in white wines. *Czech Journal of Food Sciences*. 31: 619–626.
- Ma W., Guo A., Zhang Y., Wang H., Liu Y., Li H. (2014). A review on astringency and bitterness perception of tannins in wine. *Trends in Food Science & Technology*. 40: 6–19.
- Makris D.P., Kallithraka S., Mamaos A. (2006). Differentiation of young red wines based on cultivar and geographical origin with application of chemometrics of principal polyphenolic constituents. *Talanta*. 70: 1143–1152.
- Merkyte V., Longo E., Windisch G., Boselli E. (2020). Phenolic compounds as markers of wine quality and authenticity. *Foods*. 9: 1785.
- Mitić M.N., Obradović M.V., Grahovac Z.B., Pavlović A.N. (2010). Antioxidant capacities and phenolic levels of different varieties of Serbian white wines. *Molecules*. 15: 2016–2027.
- Morata A., Escott C., Banelos M. A., Loira I., del Fresno J.M., Gonzalez C., Suarez-Lepe J. A. (2020). Contribution of non- *Saccharomyces* yeasts to wine freshness. A review. *Biomolecules*. 10(1): 34.
- Nemzer B., Kalita D., Yashin A.Y., Yashin Y.I. (2022). Chemical composition and polyphenolic compounds of red wines: Their antioxidant activities and effects on human health—A review. *Beverages*. 8: 1.
- Pantelić M.M., Dabić Zagorac D.Č., Davidović S.M., Todić S.R., Bešlić Z.S., Gašić U.M., Tešić Ž.LJ.,

- Natić M.M. (2016). Identification and quantification of phenolic compounds in berry skin, pulp, and seeds in 13 grapevine varieties grown in Serbia. *Food Chemistry*. 211: 243–252.
- Pantelić M., Dabić Zagorac D., Gašić U., Jović S., Bešlić Z., Todić S., Natić M. (2018). Phenolic profiles of Serbian autochthonous variety 'Prokupac' and monovarietal international wines from the Central Serbia wine region. *Natural Product Research*. 32: 2356-2359.
- Peixoto C.M., Maria Inês Dias M.I., Alvesa J., Calhelha R.C., Barrosa L., Pinhob S.P., Ferreira I.C.F.R. (2018). Grape pomace as a source of phenolic compounds and diverse bioactive properties. *Food Chemistry*. 253: 132–138.
- Radeka S., Rossi S., Bestulić E., Budić-Leto I., Kovačević Ganić K., Horvat I., Orbančić F., Zaninović Jurjević T., Dvornik Š. (2022). Bioactive compounds and antioxidant activity of red and white wines produced from autochthonous Croatian varieties: Effect of moderate consumption on human health. *Foods*. 11: 1804.
- Rodríguez Montealegre R., Romero Peces R., Chacón Vozmediano J.L., Martínez Gascueña J., García-Romero E. (2006). Phenolic compounds in skins and seeds of ten grape *Vitis vinifera* varieties grown in a warm climate. *Journal of Food Composition and Analysis*. 19: 687–693.
- Šćepanović R.P., Wendelin S., Raičević D., Eder R. (2019). Characterization of the phenolic profile of commercial Montenegrin red and white wines. *European Food Research and Technology*. 245: 2233–2245.
- Šuković D., Knežević B., Gašić U., Sredojević M., Ćirić I., Todić S., Mutić J., Tešić Ž. (2020). Phenolic profiles of leaves, grapes and wine of grapevine variety Vranac (*Vitis vinifera* L.) from Montenegro. *Foods*. 9: 138.
- Vilanova M., Santalla M., Masa A. (2009). Environmental and genetic variation of phenolic compounds in grapes (*Vitis vinifera*) from northwest Spain. *Journal of Agricultural Science*. 147: 683–697
- Weaver S.R., Rendeiro C., McGettrick H.M., Philp A., Lucas S.J.E. (2021). Fine wine or sour grapes? A systematic review and meta-analysis of the impact of red wine polyphenols on vascular health. *European Journal of Nutrition*. 60: 1–28.
- Zhang P., Ma W., Meng Y., Zhang Y., Jin G., Fang Z. (2021). Wine phenolic profile altered by yeast: Mechanisms and influences. *Comprehensive Reviews in Food Science and Food Safety*. 20:3579–3619.
- Zhu F., Du B., Shi P., Li F. (2014). Phenolic profile and antioxidant capacity of ten dry red wines from two major wine-producing regions in China. *Advance Journal of Food Science and Technology*. 6: 344–349.

Influence of viticultural practices on Zlatarica vrgorska and Trnjak grape chemical composition grown in Vrgorac

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Abstract

Zlatarica vrgorska and Trnjak are native varieties characterized by vigorous growth. To improve canopy microclimate, yield and the accumulation of secondary metabolites, defoliation and cluster thinning are applied as standard practices. The aim was to investigate the impact of these practices on chemical composition of Zlatarica vrgorska and Trnjak grapes. For both varieties the treatment did not have significant impact on basic chemical parameters. Defoliation treatments on Trnjak variety had a positive impact on the accumulation of polyphenolic compounds. For Zlatarica vrgorska, the defoliation and cluster thinning treatments had a positive impact on the accumulation of volatile compounds.

Keywords: native varieties, defoliation, cluster thinning, grape secondary metabolites

Introduction

Vrgorac growing area belongs to Dalmatinska zagora subregion, characterized by Mediterranean climate and karst relief. However, due to the isolation by the mountain massifs, the favourable influence of the Adriatic sea on the climate is weaker in these inland areas (Maletic et al., 2015). The area is also known for its native varieties, and among them are Zlatarica vrgorska and Trnjak. Zlatarica vrgorska is a native variety characterized by late ripening and vigorous growth. Regarding the chemical composition, there is virtually no research. Trnjak is a late ripening red variety also characterized by vigorous growth.

Both varieties have vigorous growth that can affect the grape quality. Canopy microclimate can greatly influence the grape development and quality, thus influencing the wine quality. It is generally accepted that grape clusters growing in low sunlight exposure have lower soluble solids, higher acidity, and lower pH, alongside with delayed ripening (Dokoozlian and Kliewer, 1996, Smart et al., 1988). One of the common viticultural practices is defoliation, which can be carried out at different stages during vegetation and includes leaf removal of basal leaves. The results of leaf removal are influenced by timing, severity, grape varieties, and also the climate of the area in general and the particular season (Moreno et al., 2015). Besides the basal leaf removal, novel approach is the apical defoliation, which is carried out by removing leaves above fruiting zone. The main goal of apical defoliation is to delay sugar accumulation without affecting other secondary metabolites, such as polyphenols (Poni et al., 2018).

Cluster thinning is another standard viticultural practice used to prevent excessive yields and to improve grape quality. Like defoliation, it can be carried out at different stages (pre- or post-veraison) and it includes removing a proportion of the developing grape clusters. Cluster thinning can advance grape maturity and improve the accumulation of secondary metabolites, namely polyphenolic and volatile compounds (Carmona-Jiménez et al., 2021).

The aim of this study was to investigate the influence of cluster thinning and defoliation on grape chemical composition of Zlatarica vrgorska, and to investigate two types of defoliation, basal and apical, on the grape chemical composition of Trnjak.

Materials and Methods

Grape samples and experimental design

The experiment was carried out during 2021 growing season in two commercial vineyards in Vrgorac wine growing area, Dalmatia, Croatia. Vineyards of Zlatarica vrgorska included locations Topolac and Telčuša, while vineyards of Trnjak included locations Gašparuša and Miletuša. The experimental treatments for two grape varieties are shown in table 1.

Table 1. The experimental treatments carried out on Zlatarica vrgorska and Trnjak

Experimental treatment	Zlatarica vrgorska	Trnjak
C	Control	Control
T1	basal defoliation	basal defoliation
T2	basal defoliation and cluster thinning	apical defoliation

The experiment was designed by random block formation consisting of three replicates, each including 20 vines. The treatments were carried out during July before veraison. Defoliation and cluster thinning were manually performed. For defoliation 4-5 basal leaves, that is above fruiting zone, were removed. For cluster thinning treatment, all clusters, except the basal one, were removed from the shoots.

For chemical analysis ten clusters were randomly chosen. The berries were removed with small scissors and were left with attached pedicels. Part of the berries were immediately frozen for the analysis of secondary metabolites. The rest of the berries were used for basic parameter analysis (total soluble solids, titratable acidity, pH) according to the methods of International Organisation for Vine and Wine (OIV, 2019). The same samples were later used for organic acid analysis.

Analysis of polyphenolic compounds

The grape skins were manually removed from the pulp and freeze-dried. Freeze-drying was performed at 0.1 mbar for 72 h. Dry skins were grinded and obtained powder (500 mg) was extracted by a 10 mL of 70% aqueous ethanol, containing 1% formic acid for one day in the dark at room temperature. The extract was centrifuged in LC-321 centrifuge (Tehtnica, Železnik, Slovenia) for 20 min at 5000 rpm at room temperature. Supernatant was collected, concentrated under vacuum to remove ethanol (40 °C) on rotary evaporator and brought to final volume of 10 mL with a mobile phase A. The extract was filtered with Phenex-PTFE 0.20 µm syringe filter (Phenomenex, Torrance, CA, USA) and analyzed by HPLC.

Separation and identification of polyphenol compounds was performed to the method described by Tomaz and Maslov [16]. The analyses were performed on an HPLC Agilent 1100 (Agilent Technologies, Palo Alto, CA USA) comprising a binary pump, an auto-sampler, a diode array detector and Agilent 1200 fluorescence detector. Separation was performed on Luna Phenyl-Hexyl (Phenomenex, Torrance, CA USA) column (250 mm × 4.6 mm i.d., 5 µm particle size) with Phenyl guard column (4.0 × 3.0). Column was heated at 50 °C. The injection volume for all samples was 20 µL. Gradient consists of two phases: (A) water/phosphoric acid (99.5/0.5, v/v), and (B) acetonitrile/water/phosphoric acid (50/49.5/0.5, v/v/v). For detection and quantification of compounds, the chromatograms were recorded at 280, 360, and 518 nm by diode array detector and at excitation wave-length 225 nm and emission wavelengths at 320 nm by fluorescence detector. UV-VIS spectra were recorded in range of 200–700 nm. Quantification of non-commercial available standards of anthocyanins was made accordingly to the calibration curves of mal-vidin-3-O-glucoside. Samples were analyzed in triplicate.

Analysis of volatile compounds

SPME-Arrow extraction was carried out based on the method described by Šikuten et al. (2021). Briefly, SPME-Arrow extraction was conducted using an RSH Triplus autosampler (Thermo Fisher Scientific Inc., Brookfield, USA). Each sample weight of 100 mg was placed in a 20 mL headspace screw-top vial with a cap consisting of a PTFE/silicone septum

Separation and detection of the analytes was carried out by TRACETM 1300 Gas Chromatographer coupled with ISQ 7000 TriPlus quadrupole mass spectrometer (Thermo Fisher Scientific Inc., Bartlesville, OK, USA) equipped with TG-WAXMS A capillary column (60 m x 0.25 mm x 0.25 µm film thickness; Thermo Fisher Scientific Inc., Bartlesville, OK, USA). The volatile compounds injected into the inlet were delivered to the column in splitless mode, and helium was used as a carrier gas at a constant flow rate of 1 mL/min. The oven temperature program was as follows: the initial temperature of 40°C was maintained for 5 min, increased by 2°C every minute until the temperature reached 210°C, and held for 10 min. The MS spectra was recorded in the electron impact ionization mode (EI) at an ionization energy of 70 eV. The mass spectrometer performed in full scan mode in the range of 30–300 m/z. The obtained data was processed using ChromeleonTM Data System (Thermo Fisher Scientific Inc., Bartlesville, OK, USA). Identification of volatile compounds was achieved by comparing the recorded mass spectrum with the data available in the Wiley Registry 12th Edition/NIST Spectral Library.

Statistical analysis

ANOVA was used to test the significance of the experimental treatments on measured parameters. In the case of significant results obtained by ANOVA, means were compared using Duncan's multiple range test among experimental treatments. All the analysis were carried out using XLSTAT software (Addinsoft, USA).

Results and Discussion

Basic chemical parameters

The results of basic chemical parameters for Zlatarica vrgorska on two locations are shown in table 2. On both locations the experimental treatments did not differ in the content of total soluble solids (TSS), titratable acidity and pH. There were some differences in the content of organic acids. The experimental treatments on Topolac location differed in the content of tartaric acid, with control having the highest concentrations. On Telčuša location the experimental treatments differed in the content of malic acid, and again the control had the highest concentrations. This difference is caused by defoliation and exposure of clusters to the sun. It is known that higher radiation increases fruit cell respiration, with a greater consumption of organic acids (Baiano et al., 2015).

Table 2. Basic chemical parameters of Zlatarica vrgorska grown in two locations.

	TSS (Oe)	Titratable acidity (g/L)	pH	Citric acid (g/L)	Tartaric acid (g/L)	Malic acid (g/L)
Topolac						
C	79,333 a*	4,587 a	3,297 b	0,136 a	6,449 a	1,012 b
T1	72,333 a	4,170 a	3,277 b	0,089 a	5,354 b	1,059 ab
T2	84,000 a	4,450 a	3,400 a	0,120 a	5,762 b	1,204 a
Telčuša						
C	76,667 a	5,053 a	3,290 a	0,155 a	5,793 a	1,643 a
T1	81,667 a	5,143 a	3,437 a	0,176 a	5,731 a	1,281 b
T2	81,667 a	4,050 a	3,413 a	0,134 a	5,545 a	0,791 c

* Means with different superscript letters in the same row differ significantly ($p \leq 0.05$).

For Trnjak variety, on Gašparuša location the experimental treatments differed in all measured parameters, except in the content of citric acid (table 3.). Basal defoliation affected higher accumulation of sugar, while both defoliation treatments affected titratable acidity and organic acids by lowering their content. Different results were obtained for Miletuša location, where there was no statistically significant difference between experimental treatments in measured parameters. These results are in accordance with results obtained on Shiraz grape variety, where both apical and basal defoliation lowered the content of acids and improved sugar accumulation (Zhang et al., 2017). Furthermore, another research on Trnjak showed that defoliation performed before véraison can improve sugar accumulation and impact the acidity (Mucalo et al., 2021).

Table 3. Basic chemical parameters of Trnjak grown in two locations.

	TSS (Oe)	Titrateable acidity (g/L)	pH	Citric acid (g/L)	Tartaric acid (g/L)	Malic acid (g/L)
Gašparuša						
C	104,333 b*	5,993 a	3,420 c	0,363 b	5,857 a	1,720 a
T1	117,000 a	4,217 b	3,683 a	0,463 a	5,457 b	1,607 a
T2	106,000 b	4,360 b	3,640 b	0,423 ab	5,187 c	1,390 b
Miletuša						
C	95,333 a	3,927 b	3,463 b	0,238 c	5,590 a	1,435 a
T1	98,333 a	4,213 ab	3,540 a	0,403 a	5,151 a	1,538 a
T2	99,667 a	4,517 a	3,543 a	0,294 b	5,574 a	1,807 a

* Means with different superscript letters in the same row differ significantly ($p \leq 0.05$).

Polyphenolic compounds

The most abundant group of polyphenolic compounds were anthocyanins, followed by flavan-3-ols and flavonols. On both locations there were significant differences between control and defoliation treatments in the concentrations of all analyzed groups of polyphenolic compounds. The defoliation treatments significantly improved the content of anthocyanins, flavonols, and flavan-3-ols (Figure 1.).

However, the defoliation treatments gave different results depending on the location. On the Gašparuša location the higher anthocyanin and flavan-3-ol content was obtained by applying basal defoliation. The opposite results were obtained for Miletuša location, where apical defoliation gave better results. Regarding flavonols, on both locations the treatments of basal defoliation had higher content than apical defoliation. This is not surprising since the accumulation of flavonols is highly influenced by light (Flamini et al., 2013). By removing the leaves in the fruiting zone, the clusters were more exposed to the sunlight, which impacted the flavonol biosynthesis.

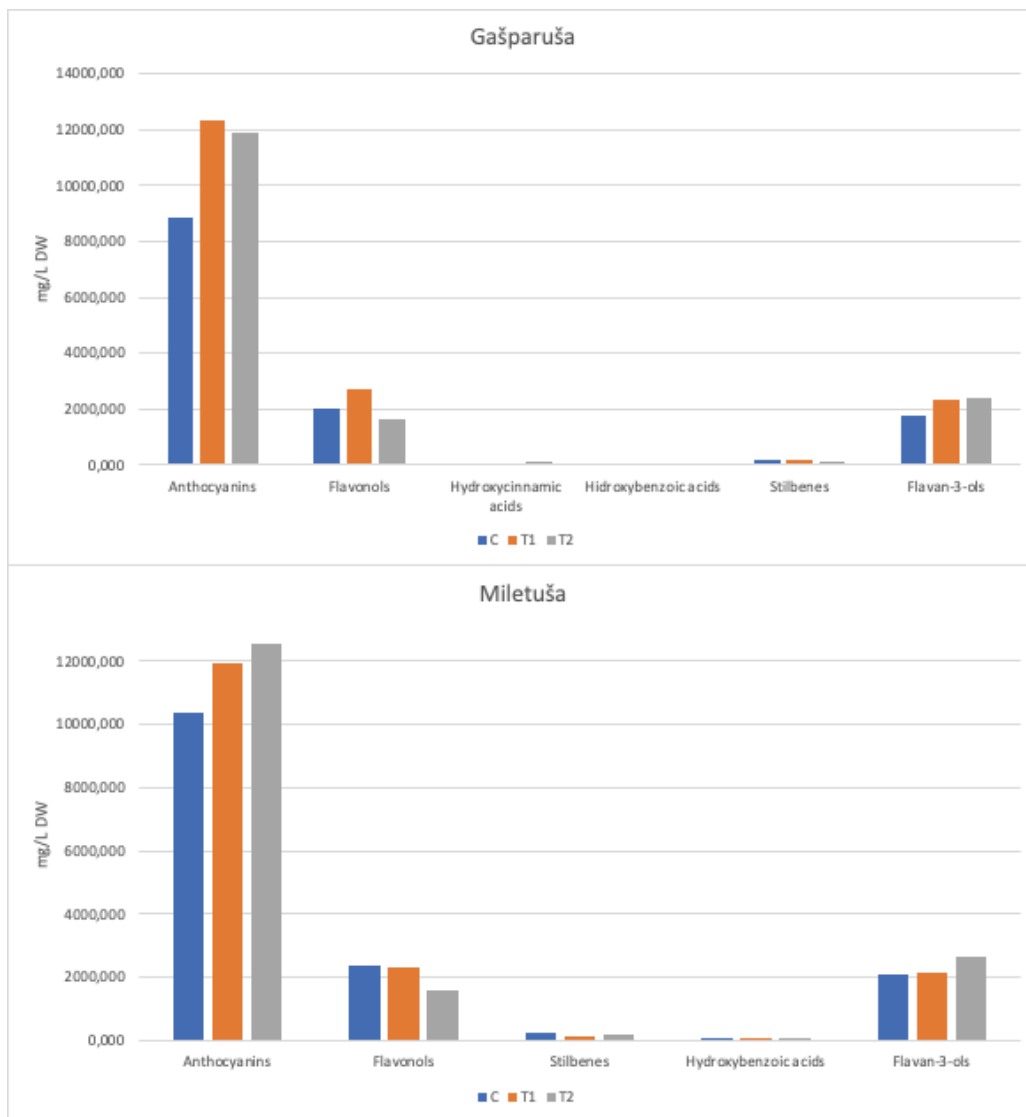


Figure 1. Main groups of polyphenolic compounds found in Trnjak grown on two locations under different defoliation treatments.

Volatile compounds

The most abundant group of volatile compounds were carbonyls, followed by alcohols. Other identified groups of volatiles found in higher content were volatile acids and monoterpenes. The results obtained on two locations were different with different impact of experimental treatments (Figure 2.).

On Topolac location the defoliation treatment and combined defoliation and cluster thinning positively impacted carbonyls and acids. The highest content of alcohols was recorded for combined defoliation and cluster thinning, followed by control. Similar results were obtained for Maraština, where defoliation before véraison also decreased the accumulation of alcohols, while cluster thinning had the opposite effect (Mucalo et al., 2022).

For Telčuša location, opposite results were obtained. The content of alcohols and carbonyls was the highest for control. When comparing treatments, combined defoliation and cluster thinning positively affected carbonyls, while negatively affecting the alcohols. Defoliation treatment, besides alcohols, also positively affected volatile acids.

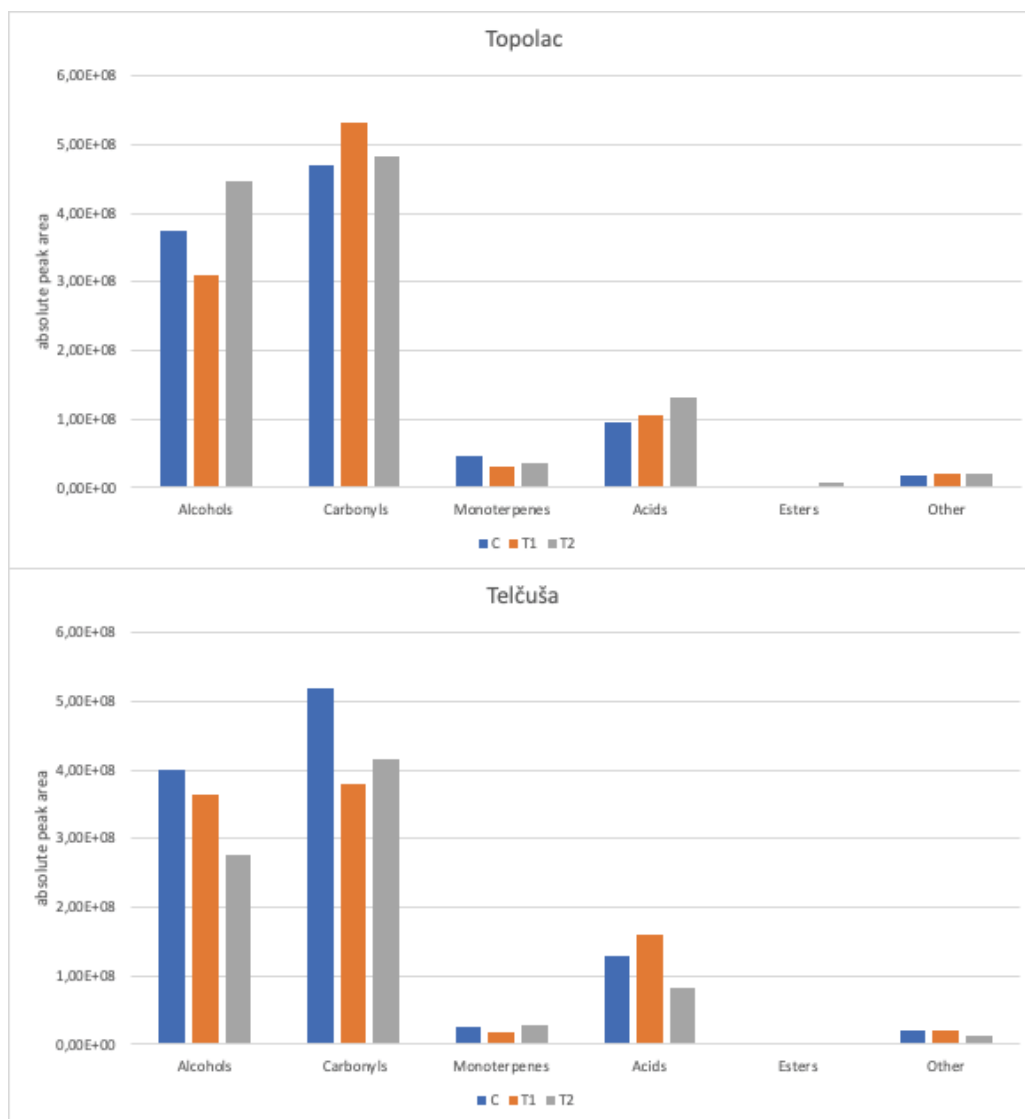


Figure 2. Main groups of volatile compounds found in Zlatarica vrgorska grown on two locations under defoliation and cluster thinning treatments.

Conclusion

Two native varieties, Zlatarica vrgorska and Trnjak, grown on two locations were subjected to different defoliation and cluster thinning treatments. The basic chemical parameters were not significantly impacted by the experimental treatments. However, depending on the location, the content of individual organic acids was slightly changed. The influence of experimental treatments on secondary metabolites was also measured. For polyphenolic compounds, analysed in Trnjak variety, defoliation treatments T1 and T2 on both locations positively influenced on accumulation of polyphenolic compounds, namely anthocyanins, flavonols and flavan-3-ols. The influence of T1 and T2 treatments on Zlatarica vrgorska were location dependent. On Topolac location, both treatments positively influenced the accumulation of volatile compounds. On the other hand, on Telčuša location treatments positively influenced the accumulation of only several groups of volatile compounds.

References

Baiano A., De Gianni A., Previtali M.A., Del Nobile M.A., Novello V. De Palma, L. (2015). Effects of defoliation on quality attributes of Nero di Troia (*Vitis vinifera* L.) grape and wine. Food Research

- International. 75: 260-269.
- Carmona-Jiménez, Y., Palma, M., Guillén-Sánchez, D. A. & García-Moreno, M. V. (2021). Study of the Cluster Thinning Grape as a Source of Phenolic Compounds and Evaluation of Its Antioxidant Potential. *Biomolecules*. 11: 227.
- Dokoozlian N.K. Kliewer W.M. (1996). Influence of light on grape berry growth and composition varies during fruit development. *Journal of the American Society for Horticultural Science*. 121: 869-874.
- Flamini R., Mattivi F., De Rosso M., Arapitsas P. Bavaresco L. (2013). Advanced Knowledge of Three Important Classes of Grape Phenolics: Anthocyanins, Stilbenes and Flavonols. *International Journal of Molecular Sciences*. 14: 19651-19669.
- Maletic E., Karoglan Kontic J., Pejic I., Preiner D., Zdunic G., Bubola M., Stupic D., Andabaka Z., Markovic Z., Simon S., Zulj Mihaljevic M., Ilijas I. Markovic D. (2015). Green book; Indigenous Grapevine Varieties of Croatia, Zagreb, Ministarstvo zaštite okoliša i prirode, Državni zavod za zaštitu prirode.
- Moreno D., Vilanova M., Gamero E., Intrigliolo D.S., Talaverano M.I., Uriarte D. Valdés M.E. (2015). Effects of Preflowering Leaf Removal on Phenolic Composition of Tempranillo in the Semiarid Terroir of Western Spain. *American Journal of Enology and Viticulture*. 66: 204-211.
- Mucalo A., Budić-Leto I., Lukšić K., Maletić E., Zdunić G. (2021). Early Defoliation Techniques Enhance Yield Components, Grape and Wine Composition of cv. Trnjak (*Vitis vinifera* L.) in Dalmatian Hinterland Wine Region. *Plants*. 10: 551.
- Mucalo A., Lukšić K., Budić-Let I., Zdunić G. (2022). Cluster Thinning Improves Aroma Complexity of White Maraschino (*Vitis vinifera* L.) Wines Compared to Defoliation under Mediterranean Climate. *Applied Sciences*. 12: 7327.
- Oiv (2019). Compendium of international of methods of wine and must analysis, France.
- Poni S., Gatti M., Palliotti A., Dai Z., Duchêne E., Truong T.-T., Ferrara G., Matarrese A. M.S., Gallotta A., Bellincontro A., Mencarelli F. Tombesi S. (2018). Grapevine quality: A multiple choice issue. *Scientia Horticulturae*. 234: 445-462.
- Šikuten I., Štambuk P., Karoglan Kontić J., Maletić E., Tomaz I. Preiner D. (2021). Optimization of SPME-Arrow-GC/MS Method for Determination of Free and Bound Volatile Organic Compounds from Grape Skins. *Molecules*. 26: 7409.
- Smart R.E., Smith S.M., Winchester R.V. (1988). Light quality and quantity effects on fruit ripening for cabernet sauvignon. *American Journal of Enology and Viticulture*. 39: 250-258.
- Zhang P., Wu X., Needs S., Liu D., Fuentes S. Howell K. (2017). The Influence of Apical and Basal Defoliation on the Canopy Structure and Biochemical Composition of *Vitis vinifera* cv. Shiraz Grapes and Wine. *Frontiers in Chemistry*. 5.

Influence of agro-ecological factors and pomotechnical measures on apple fruits

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Abstract

The apple is the most widespread fruit species, in Croatia grown mainly in the continental part. The most important agro-ecological conditions for growing apples are climate, soil and location. The development of the red colour of the skin in apples is related to the exposure of the fruit to light, temperature and nutrient conditions in the tree. Spring frosts damage the blossoms and high temperatures cause burns on the fruit. Foliar fertilisation has a significant influence on apple fruit quality. Summer pruning improves the quality of fruit on apple trees that are too tall and spindly. Mechanical thinning has a greater influence on the quality and stability of the yield than chemical thinning. Water dosage is very important in orchard irrigation. In recent years, the use of protective or anti-hail netting has become more common. Choosing the most effective net protection system is becoming more important than ever. The implementation of all agro-ecological and technical measures in apple orchards as well as the climate significantly influence the quality and fertility of apples.

Keywords: apple, climate, pruning, thinning, fruit yield

Introduction

Apple (*Malus x domestica* Borkh.) is the fourth most important fruit produced and eaten worldwide (Musacchi and Serra, 2018). In Croatia, it is the most important traditional fruit species, occupying about 22% of the total fruit growing area and 36% of the total fruit production (Cerjan et al., 2011). It is the most widespread fruit species grown in the continental part of Croatia, where the largest growing areas are located (Skendrović Babojelić et al., 2014).

The fruitfulness and quality of apple fruit depends on several factors, the most important of which are: orchard location, cultivar, the tree density, the cultivation method, the physical and chemical properties of the soil, the available amount of nutrients and water in the soil, and pomotechnical measures, the interactions of which can be highly competitive in modern plantations with a dense structure (Unuk et al., 2007a). The goal of apple cultivation is primarily to obtain high quality fruit with exceptional nutritional value.

Climatic factors and apple fruit

Temperature influences the intensity of many physiological processes such as photosynthesis, respiration, transpiration and the onset and duration of all phenophases. Apple requires an average annual air temperature of 8-12°C, an average air temperature during the growing period of 14-19°C and can tolerate temperatures as low as -28°C during dormancy.

Extremely high temperatures over a longer period of time mean extreme stress for the apple, which reduces the quality of its fruit. A sudden warming with extremely high air temperatures and low relative humidity without major precipitation leads to the appearance of burns on the fruit (Soldo, 2012). Croatia is located in the zone of temperate continental, continental and Mediterranean climate. The altitude and terrain have a significant influence on the microclimate in orchards. During the period of fruit ripening, temperature, precipitation, relative humidity and solar radiation are of utmost importance (Miljković, 2022).

The analysis of the effects of climate change on the territory of Croatia over a period of 10 years on different apple varieties showed that in all climatic zones there was an earlier onset of leaves and flowering of apples by 2–6 days,

which is a consequence of warmer winters and springs (Krulić and Vučetić, 2011). The apple cultivars Jonatan and Golden Delicious are more sensitive to temperature changes than old apple cultivars, and their shortened growing season was observed in inland Croatia (Vučetić, 2013). Climate changes with warmer winter and spring temperatures pose major challenges for apple production (Lempe et al., 2022).

The development of red skin colour in apples is related to the exposure of the fruit to light, temperature and nutritional conditions of the fruit tree. According to research by Dalhaus et al. (2020) on the effects of extreme weather conditions on apple fruit quality, it was found that fruit trees exposed to spring frosts during the flowering period, which partially damaged their flowers, showed significantly damaged fruit.

Soil, location and nutrition

Like most other fruit species, apples like deep soils with an even profile, sandy-loam composition, with sufficient humus and mineral content and good water capacity, soils with a pH of 5.5-6.5 that do not contain large amounts of physiologically active lime (not more than 5%). Based on a three-year study of apple production in semi-arid climates, Wang and Wang (2017) found that evapotranspiration is the cause of large moisture losses from the soil. They also found that the apple tree has very high transpiration, which, in combination with climatic conditions, significantly affects nature in relation to humid climatic regions. Wang and Wang (2017) found that apple requires more soil moisture than plum.

The best fruit quality is obtained on the southern, south-eastern and eastern sides with a slope of up to 15%, while the northern and north-western sides are unsuitable for apples as well as for most fruit species. In the continental part of Croatia, the most favourable altitudes for growing winter apples are between 120 and 600 m above sea level. The slope of the terrain of about 4% allows the most favourable use of machinery, easy drainage of surface water and excess soil water, and good illumination of the tree crowns.

Unuk et al. (2007b) found that fruit quality of the Fuji cultivar depended on the slope of the terrain (sloping and flat terrain) and the soil type (heavy clay soil at a depth of 60-80 cm, with marl as the initial substrate), as well as the position of the fruit in the canopy, and came to the following conclusions: On the flat terrain, fruit density was uniform in the upper and lower parts of the canopy, while on the sloping terrain, density was significantly higher in the lower part of the canopy. The average weight of fruit in the upper part of the canopy was lower on the flat terrain than on the sloping terrain, although the yield was higher on the sloping terrain. The average fruit mass in the lower part of the canopy was smaller and about the same in both microsites. Fruit colouration in the upper part of the canopy was excellent at both sites. Slightly poorer fruit colouration was observed in the lower zone of the crown, while fruit skin colouration was significantly poorer on the plateau.

A balanced, temporally and quantitatively defined nutrition of apples is the starting point for successful apple production in terms of fruit quantity and quality. Determining the optimal fertilisation of apples is one of the basic agrotechnical measures, without which the production and quality of apple fruits is significantly reduced (Ćosić et al., 2007).

Fertilisation is the most important measure for optimising soil fertility, and great emphasis is placed on rational fertilisation and the use of dosages and types of fertiliser that provide the best production results while not being a burden on the environment.

Wojcik et al. (2008) find that soil application of boron or foliar fertilisation significantly affects apple fruit quality and that application of 2 grammes of boron (B) per apple tree on clayey soil has a good effect on apple fruit quality. Foliar fertilisation has a significant effect on apple fruit quality.

Bennewitz et al. (2011) find that application of Ca, Mg and K (CaSO_4 , MgSO_4 and K_2SO_4) to the soil has statistically significant effect on apple fruit quality and that medium to low soil levels of Ca, Mg and K content is a factor that may affect fruit yield and quality in the future, especially considering that fruit trees have a significant demand for these elements.

Effect of pruning and thinning

Pruning is considered the most important technical measure, which, together with fruit thinning, is one of the most important procedures we can use to influence the scale of the yield. Pruning shapes the growth habit of young fruit

trees, provides a favourable balance between vegetative growth and fruitfulness for trees in full fruit and rejuvenates already exhausted plant parts in old fruit trees.

A fruit tree that is pruned regularly has an airy and well-lit crown, which has a positive effect on the quality of the fruit and the health of the fruit tree. In addition, light is also necessary for the formation of reproductive buds. Thickened and shaded tree crowns retain moisture for too long after rainfall, which is why fruit trees are susceptible to diseases (Jemrić, 2007).

The effects of summer apple pruning are generally attributed to improved light penetration and reduced carbohydrate uptake. Summer pruning causes a temporary loss of apical dominance unless thinning cuts are made. It also causes a temporary increase in cytokinin supply, probably mainly due to increased export from the roots. Both effects are probably due to a reduced availability of auxin (Saure, 2007).

In their research, Bhusal et al. (2017) find that summer pruning and reflective film improve fruit quality in apple trees that are too tall. High spindle planting systems achieve high quality fruit from plants that flower early and maintain high quality. Light distribution, flower bud diameter, fruit quality and leaf physiological characteristics were measured on different parts of the crown of trees treated with summer pruning. The application of summer pruning resulted in an increase in fruit size, more intense colour and higher soluble solids content in fruit from the lower parts of the crown. In addition, this treatment increased the photosynthetic activity of the leaves, the specific weight of the leaves and the diameter of the flower buds. Summer pruning and the application of a reflective film thus promote light penetration in over-tall, spindly trees and improve fruit quality in a high-density apple system.

According to the research by Keserović et al. (2012) on the effects of green pruning on reducing lushness and regulating fruitfulness of apples, pruning was carried out in early August 2011, removing up to 10% of the green mass from the trees. The experiment was conducted with the cultivars Idared, Gloster, Elstar and Jonagold and the green pruning carried out in the second or third week of August had a dampening effect on growth.

In the study by Pavičić et al. (2004), four different pruning of the apple cultivar Elstar in two years and their possible influence on improving the productivity of this cultivar were investigated (pruning A - load of 25 generative buds/tree, B - 50 generative buds/tree, C - 75 generative buds/tree and D-100 generative buds/tree). Cut A produced a higher fruit mass than cuts C and D. The fruiting efficiency and the alternation index showed no justifiable differences between the pruning methods. The variance of the alternation index was lowest in cut B. The cross-sectional area of the stem (TCSA) had a negative influence on fruiting mass in cut C. It was concluded that pruning of the aboveground part cannot provide adequate regulation of the yield of the apple cultivar Elstar (Pavičić et al., 2004).

In the research by Veličković et al. (2006), the two-year results of testing the influence of green pruning on the vegetative and reproductive potential of the apple cultivars Jonagold and Granny Smith were presented. For comparison, the trees of the control variant (without green pruning) were observed. Green pruning on the trees of the above-mentioned apple varieties was carried out in the first half of June and mid-August (first variant), i.e. only in the first half of June (II variant). It was found that green pruning has a positive effect on the parameters studied, which is why it is recommended that it be carried out regularly on trees of the genus. Double green pruning gave slightly more favourable results, but this need not be the rule for all varieties and different agro-ecological conditions. The cultivar Jonagold showed significantly less total acids compared to the cultivar Granny Smith, which is primarily due to its varietal characteristics. The complementary colour of the cultivar Jonagold was particularly evident in the double green cut on a regular palmette with sloping branches. The colour intensity of the fruits was evaluated on a scale from 0 to 5. It was found that the cultivar Jonagold had a significantly higher colour intensity than the cultivar Granny Smith, which is a consequence of its biological properties. Both green prunings had a significant influence on this parameter, but much better results were obtained with the double green pruning (Veličković et al., 2006).

In order to achieve regular fruiting in an apple orchard, it is necessary to carry out fruit thinning, the aim of which is to reduce the crop in the current year in order to increase the size and quality of the fruit on the tree and to allow better differentiation of generative buds, i.e. a better nature in the following year, especially if carried out up to 40 days after flowering (Arko and Poljak Laušić, 2008). Williams and Fallahi (1999) state that an excessive amount of 1-naphthylacetic acid (NAA) can lead to a reduced size of the fruits and thus we get abnormally small, so-called dwarf fruits. A similar effect is shown by naphthylacetamide (NAD), while in the Stopar et al. (2007) study on apple fruit thinning of the cultivar 'Gala' using naphthylacetic acid (NAA) and benzyladenine (BA) and their combinations, it is found that fruit weight and size increase significantly with the combination of NAA and BA. Link et al. (2000) conclude, based on thirty years of research, that increasing the intensity of fruit thinning produces both

negative and a number of positive results. These are mainly manifested in the reduced concentration of calcium and potassium in the fruit, which increases the tendency to develop physiological diseases. Mechanical thinning has a greater influence on the quality and stability of nature than chemical thinning.

In the study by Stajanko and Tojnko (2020) on chemical thinning of apple blossoms in organic farming, the following agents were used: 1% sodium chloride (NaCl), 1.5% NaCl, 1% acetic acid (CH_3COOH), 3% CH_3COOH , 3% calcium polysulphide (CaS_x), 1% rapeseed oil, 3% rapeseed oil, 3% sunflower oil, 3% soybean oil, 5% polysaccharide (dextrin) and polyoxyethylene (emulsifier 100). The results showed that 1% rapeseed oil and 3% acetic acid did not significantly thin the fruits, but the trees had the largest fruits and the highest average fruit weight. Both treatments resulted in a significant increase in average fruit weight compared to untreated trees. 1% CH_3COOH , 3% CaS_x , 3% sunflower oil and 5% dextrin had no significant effect on fruit thinning and only minimally reduced the number of fruits per tree. 3% rapeseed oil had the greatest effect on fruit thinning and at the same time the lowest average fruit size (kg) per tree, even lower than manually thinned trees.

So it is not the orchard that determines how many apples we will harvest in which year, but we need to use our knowledge to influence the coordination of pruning, feeding and thinning to successfully produce a financially profitable amount of apples each year.

Impact of irrigation and protective nets

Seasonal rainfall does not meet the water requirements of the trees, so irrigation of the orchard is necessary for optimal water supply to the fruit trees and for high quality fruit.

Du et al. (2017), in a study on water use efficiency through partial irrigation of apple root zone in arid northwest China, found that high irrigation volume (500 mm) improved apple texture compared to low irrigation volume (400 mm). Therefore, partial irrigation of the root zone at a high frequency is a promising technique to improve fruit characteristics (Du et al., 2017).

Jiang and He (2021) state that adequate irrigation can improve orchard productivity and fruit quality. In dense apple orchards, the water ratio is even more important. Most irrigation in orchards is based on grower experience, which can lead to over- or under-irrigation, and the use of soil moisture sensors for irrigation scheduling is very important for regulating the automatic system. The most favourable water temperature for irrigation is 25°C, and the difference between the water temperature and the temperature of the fruit tree must not exceed 10°C. The 'Drip-to-Drip' system has proven to be the most optimal for irrigation.

In recent years, the use of protective or anti-hail nets has become more common. Meteorological statistics show that hail is becoming more common, and global climate changes favour predictions that this trend will continue for many years. Choosing the most effective hail protection system is becoming more important than ever (Kantoci, 2007).

In European orchards, black and white nets are mostly used, and since 2007, red and green nets have also been used. Mupambi et al. (2018) state that protective nets, i.e. anti-hail nets, are increasingly used in apple orchards and that hail damage not only affects fruit production in the current growing season, but also the condition of the fruit in the next season by damaging flower buds that have developed in the current season, protective nets can also serve as an alternative to traditional fruit burn control strategies.

Conclusion

Agro-ecological (climate, soil, location) and technical auxiliary factors (pruning, fertilisation, irrigation, thinning of flowers and fruits, protective nets) are very important for apple cultivation, because the aim of any intensive production, including apple cultivation, is to obtain a sufficient quantity of natural and high-quality fruits that fulfil all nutritional values. Climate, as one of the agro-ecological factors, can significantly influence the quality and texture of apples. On the one hand, there is a risk of spring frosts that damage the blossoms already at the beginning of flowering, and on the other hand, high temperatures cause burns on the fruits. Pruning is one of the most important technical interventions that must be carried out regularly and, above all, professionally. By applying auxiliary techniques and selecting favourable agro-ecological factors, including, of course, those we can influence, such as soil and location, we can ultimately obtain a high-quality apple orchard with high-quality fruit and satisfactory fertility.

References

- Arko B., Poljak L.K. (2008). Prorjeđivanje plodova jabuke. *Glasnik zaštite bilja*. 31: 164-003.
- Bennewitz E., Cooper T., Benavides C., Losak T., Hlusek J. (2011). Response of “Jonagold” apple trees to Ca, K and Mg fertilization in an andisol in southern Chile. *Journal of Soil Science and Plant Nutrition*. 11: 71-81.
- Bhusal N., Han S.G., Yoon T.M. (2017). Summer pruning and reflective film enhance fruit quality in excessively tall spindle apple trees. *Horticulture, Environment, and Biotechnology*. 58: 560-567.
- Cerjak M., Vrhovec R., Vojvodi M., Mesić Ž. (2011). Analiza hrvatskog tržišta jabuka, Zbornik radova 46 hrvatskog i 16 međunarodnog simpozija agronoma, Pospišil (ed.) 311 – 314, Opatija, Croatia, University of Zagreb Faculty of Agriculture.
- Čosić T., Kovačević I., Poljak M., Herak Ćustić M., Petek M., Čoga, L., Njavro M., Slunjski S. (2007). Utjecaj gnojidbe dušikom na prirod jabuka Golden Delicious. 2. znanstveno-stručno savjetovanje hrvatskih voćara s međunarodnim sudjelovanjem : Zbornik sažetaka, Čuljat, Vanda (ur.). 40-41, Zadar, Hrvatska, Hrvatska voćarska zajednica.
- Dalhaus T., Schlenker W., Blanke M.M., Bravin E., Finger R. (2020). The effects of extreme weather on apple quality. *Scientific reports*. 10: 7919.
- Du S., Kang S., Li F., Du T. (2017). Water use efficiency is improved by alternate partial root-zone irrigation of apple in arid northwest China. *Agricultural Water Management*. 179:184-192.
- Jemrić T. (2007). Cijepljenje i rezidba voćaka. *Naklada Uliks*.
- Jiang X., and He L. (2021). Investigation of effective irrigation strategies for high-density apple orchards in Pennsylvania. *Agronomy*. 11: 732.
- Kantoci D. (2012). Navodnjavanje. *Glasnik zaštite bilja*. 35: 66-72.
- Keserović Z., Magazin N., Milić B., Dorić M., Bošnjak B., Gošić J. (2013). Gusta sadnja jabuke. Poljoprivredni fakultet, Novi Sad , Srbija.
- Krulić B., and Vučetić V. (2011). Phenological phases and winter dormancy of apple in Croatia. *Hrvatski meteorološki časopis*. 46: 35-43.
- Lempe J., Flachowsky H., Peil A. (2022). Exploring epigenetic variation for breeding climate resilient apple crops. *Physiologia Plantarum*. 174: e13782.
- Link H. (2000). Significance of flower and fruit thinning on fruit quality. *Plant growth regulators*. 31: 17-26.
- Miljković I. (2022). Istraživanje utjecaja klimatskih prilika na kemijski sastav i kvalitetu plodova sorti jabuka u Hrvatskoj. *Pomologia Croatica: Glasilo Hrvatskog agronomskog društva*. 26: 21-43.
- Mupambi G., Anthony B. M., Layne D. R., Musacchi S., Serra S., Schmidt T., Kalcsits L. A. (2018). The influence of protective netting on tree physiology and fruit quality of apple: A review. *Scientia Horticulturae*. 236: 60-72.
- Musacchi S., and Serra S. (2018). Apple fruit quality: Overview on pre-harvest factors. *Scientia Horticulturae*. 234: 409-430.
- Pavičić N., Jermić T., Blašković D., Skendrović M., Krstulović, A. (2004). Mesurolo-čimbenik redovitije rodnosti jabuke. *Poljoprivreda*. 10: 28-31.
- Saure M.C. (2007). Root pruning—a poorly understood management practice in fruit trees. *International Journal of Fruit Science*. 7: 43-56.
- Skendrović Babojelić M., Koren, P., Šindrak Z., Jemrić T. (2014). Pomološka svojstva i kakvoća ploda tradicionalnih sorata jabuka. *Glasnik zaštite bilja*. 37: 20-27.
- Soldo B., Ivandić K., Orešković M. (2012). Numerous landslides and landslide restorations—an example. *Acta Montan Slovaca*. 17: 257-262.
- Stajanko D., and Tojnko S. (2020). Kemijsko prorjeđivanje cvjetova jabuke u ekološkoj proizvodnji. *Glasnik Zaštite Bilja*. 43: 86-98.

- Unuk T., Čmelik Z., Schlauer B. (2007a). Crop load influences fruit quality of young 'Golden Delicious' apples. *Pomologia Croatica: Glasilo Hrvatskog agronomskog društva*. 13: 129-142.
- Unuk T., Čmelik Z., Tojnko S. (2007b). Kakvoća plodova jabuke sorte Fuji ovisi o čimbenicima staništa i položaja plodova u krošnji. *Pomologia Croatica: Glasilo Hrvatskog agronomskog društva*. 13: 197-210.
- Veličković M., Oparnica Č., Radivojević D., Zabrkić G. (2006). The effect of summer pruning on vegetative and reproductive potential of apple cvs Jonagold and Granny Smith. *Voćarstvo*. 40: 19-29.
- Vučetić V. (2013). Utjecaj klimatskih promjena na poljoprivrednu proizvodnju. Zbornik radova. *Agrometeorologija u službi korisnika: „Klimatske promjene i poljoprivreda“* (Vučetić, ed.) 1-2, Zagreb. Hrvatska, Hrvatsko agrometeorološko društvo.
- Wang D., and Wang L. (2017). Dynamics of evapotranspiration partitioning for apple trees of different ages in a semiarid region of northwest China. *Agricultural Water Management*. 191: 1-15.
- Williams K. M., and Fallahi E. (1999). The effects of exogenous bioregulators and environment on regular cropping of apple. *HortTechnology*. 9: 323-327.
- Wojcik P., Wojcik M., Klamkowski K. (2008). Response of apple trees to boron fertilization under conditions of low soil boron availability. *Scientia Horticulturae*. 116: 58-64.

Kvalitativni pokazatelji mošta sorata vinove loze prikladnih za ekološku proizvodnju na Veleučilištu u Križevcima

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Sažetak

Istraživani su međuvrsni hibridi djelomično ili potpuno otporni na određene bolesti vinove loze (Prinzival, Allegro, Primera, Bolero, Serena, Rondo, Sibera i Saphira) u Ampelografskom vrtu Veleučilišta u Križevcima. Nakon analiza uroda, parametara mošta te procjene prikladnosti tih sorata za ekološki uzgoj u regiji Središnja bregovita Hrvatska, podregiji Prigorje-Bilogora, vinogorju Kalnik tijekom vegetacija 2017. i 2023. može se zaključiti da su sorte prikladne za uzgoj i proizvodnju vina na području istraživanog vinogorja. Dobiveni rezultati prikazuju više koncentracije ukupne kiselosti za bijele sorte što je posljedica previsokog uroda te nepovoljnih klimatskih uvjeta vegetacijskih godina. Ukupna koncentracija fenola značajno se razlikovala između istraživanih sorata i godina na što je uz već navedene neprilike utjecao rok berbe.

Ključne riječi: vinova loza, međuvrsni hibridi, otporne sorte, ekološka proizvodnja, kvalitativni pokazatelji mošta

Uvod

Otporne sorte ponovo su dobile na značaju zbog strateških ciljeva Europske unije da se smanji uporaba sredstava za zaštitu bilja u poljoprivredi, pa tako i u vinogradarstvu koje je među najvećim potrošačima fungicida.

Uvođenje novih sorti/klonova u proizvodnju na određenom području uvijek predstavlja svojevrsni rizik, pa je nužno praćenje parametara mošta i vina te praćenje prikladnosti za uzgoj tijekom nekoliko vegetacijskih godina. Poznato je kako postoji veliki broj sorti vinove loze u uzgoju, a u novije vrijeme, posebice u ekološkoj proizvodnji, počinju se koristiti međuvrsni hibridi, odnosno sortiment djelomično ili potpuno otporan na pojedine (osnovne) bolesti vinove loze. Razlog sve veće popularnosti ovakvih sorti je i sve veći udio ekološke proizvodnje u kojoj je najveći rizik upravo pojava bolesti i mjere zaštite, posebno u klimatološki lošijim godinama.

Upravo zato se istražuje prikladnost novih sorata za uzgoj na pojedinom području tj. u vinogradarskim regijama u kojima do sada nisu bile prisutne u uzgoju ili su bile prisutne u zanemarivim količinama.

Na stvaranju i razvoju kvalitetnih sorti otpornih na gljivične bolesti oplemenjivački centri diljem svijeta rade već godinama. Oplemenjivači su međuvrsne križance dobivene prijašnjim oplemenjivačkim programima uzastopno povratno križali s *V. vinifera* sortama, pa se sa svakim sljedećim križanjem udio genoma vinove loze u potomcima povećavao (Štambuk i Karoglan Kontić, 2021). Optimalan rok berbe izuzetno je važan za kvalitetu i stil vina, na temelju određivanja koncentracije šećera, ukupnih kiselina, pH i fenolne zrelosti (Robinson i Harding, 2015; Herjavec, 2019).

U ovom radu analizirani su međuvrsni hibridi u Ampelografskom vrtu Veleučilišta u Križevcima u vinogradarskoj regiji Središnja bregovita Hrvatska (zona B), podregiji Prigorje-Bilogora, vinogorju Kalnik, na temelju baznog materijala proizvedenom na Institut für Rebenzüchtung, Geisenheim, u Njemačkoj.

Cilj ovog rada bio je usporediti kvalitativne pokazatelje mošta za dvije godine (2017. i 2023.) na osam ispitivanih međuvrsnih hibrida: Prinzival, Allegro, Primera, Bolero, Serena, Rondo, Sibera i Saphira te procijeniti njihovu prikladnost za uzgoj i proizvodnju vina na području vinogorja Kalnik.

Materijal i metode

Ampelografski vrt Veleučilišta u Križevcima podignut je 2014. godine. Formiran je uzgojni oblik Guyot, niskog opterećenja (jedan lucanj i jedan reznik, u prosjeku 10-12 pupova).

Rezidba vinograda u zrelo provedena je krajem veljače. Vegetacija ispitivanih sorti krenula je ranije od uobičajenih sorti za ovo područje (npr. 'Graševina' i 'Chardonnay'). Tijekom godine provedene su uobičajene mjere zelene rezidbe (plijevljenje, zalamanje zaperaka, vršikanje, defolijacija), održavanja tla (kombinacija malčiranja između redova i tretman herbicidima unutar reda), te redovite mjere zaštite od štetnika i bolesti.

Podloge na koje su cijepljeni međuvrtni hibridi odabrao je Institut für Rebenzüchtung, kao najprikladnije za uzgoj pojedine sorte. Sorta Prinzpal cijepljena je na podlogu *Vitis berlandieri* x *Vitis riparia* Kober 5BB, sorte Allegro, Bolero, Primera i Prinzpal na *Vitis berlandieri* x *Vitis riparia* SO4, a sorte Sibera, Serena i Rondo na *Vitis berlandieri* x *Vitis riparia* 125AA.

Istraživanje je obuhvatilo dvije vegetacijske sezone (2017. i 2023. g.) na pet bijelih (Primera, Prinzpal, Sibera, Serena i Saphira) i tri crne sorte (Bolero, Allegro i Rondo). U prvoj godini istraživanja berba je provedena 13. rujna, a u drugoj 25. rujna. Obje berbe provedene su ručno, na četiri slučajna uzorka sa po pet trsova istraživanih sorti, te je neposredno nakon berbe uslijedila analiza mošta.

Kako navode Schmid i sur. (2011) sorta Prinzpal selekcionirana je u Geisenheimu 1971. g., a na sortnu listu upisana 1999. g. Primera je jedna od najstarijih otpornih sorti selekcionirana 1952. g. u Geisenheimu, ima dobru otpornost na peronosporu i pepelnicu, u pravilu su dovoljna najviše dva tretmana fungicidima. Sorta Saphira posjeduje visoku toleranciju prema gljivičnim bolestima (posebno peronospori i botritisu, potrebna su do dva tretmana protiv pepelnice), nastala je 1978. g. Sibera ima vrlo dobru otpornost na hladnoću i također se pokazuje dovoljno tolerantna na peronosporu. Rahla struktura grozda čini ju slabije osjetljivom na botritis. Siberu je 1964. g. selekcionirao prof. Vilém Kraus u Lednicama u Češkoj. Serena je također nastala 1964. g. te kao i Sibera, ima vrlo dobru otpornost na hladnoću i također se pokazuje kao dovoljno tolerantna na peronosporu. Protiv pepelnice ovisno o vremenskim prilikama provode se najmanje dva tretmana. Sorta Bolero posjeduje visoku otpornost na gljivične bolesti i slabe je bujnosti, selekcionirana je 1982. g. Rondo je dobiven 1964. g., ima vrlo visoku otpornost na peronosporu pa se često koristi u ekološkoj proizvodnji vina. Rondo je otporan na niske temperature tijekom mirovanja, ali zbog ranog kretanja vegetacije postoji opasnost od proljetnog mraza. Sorta Alegro je nastala 1983. g. križanjem sorti *Chancellor* (Siebel 7053) x *Rondo*.

Prema podacima Državnog hidrometeorološkog zavoda (DHMZ) za Križevce u 2017. godini palo je 794 mm oborina, što je blizu višegodišnjeg prosjeka. Najkišnji mjesec bio je rujna sa 181,7 mm oborina u najvažnijoj fenofazi zrenja grožđa. U 2023. godini je tijekom prvih deset mjeseci palo 857,3 mm oborina što je više od višegodišnjeg prosjeka za Križevce. Najkišnji mjeseci bili su siječanj te svibanj i lipanj kada nastupaj fenofaza cvatnje i oplodnje što nije pozitivno utjecalo na vinovu lozu. I ostali mjeseci kada kreće dozrijevanje vinove loze bili su vlažniji od uobičajenog te s manje sunčanih sati. Ukupno gledajući obje istraživane godine nisu bile idealne za proizvodnju grožđa, pri čemu je 2023. godina bila klimatski lošija.

Osnovna analiza kvalitativnih parametara mošta provedena je prema referentnim metodama OIV-a (OIV, 2020). Ukupni fenoli mjereni su prema Folin-Ciocalteu metodi upotrebom spektrofotometra Lambda XLS+ (PerkinElmer) na 765 nm valne duljine. Rezultati su izraženi u ekvivalentima galne kiseline, GAE mg/L. Podaci su obrađeni statistički u aplikaciji Statistica 13.4.014 (2018), korištenjem metode Simple t-test, independent, by groups.

Rezultati i rasprava

U tablici 1 prikazani su rezultati analiza uroda i kemijske analize mošta. Više koncentracije ukupne kiselosti kod bijelih sorata (6,44 – 7,82 g L⁻¹) posljedica su nepovoljnih klimatskih prilika tijekom ispitivanih vegetacijskih godina te previsokog uroda (2,78 – 4,52 kg). Više koncentracije ukupnih fenola zabilježene su kod crnih sorata što je u pravilu u skladu s navedenim vrijednostima u literaturi (Jackson, 2019). Razlike u vrijednostima ukupnih fenola mogu se pripisati utjecaju vegetacijske godine, kao i stupnju zrelosti grožđa te roku berbe. Vrijednosti za bijele sorte kreću se u rasponu od 214 mg L⁻¹ do 519,03 mg L⁻¹, dok su vrijednosti za crne sorte u rasponu od 292,90 mg L⁻¹ do 799,55 mg L⁻¹. Vrijednosti ukupnih fenola nisu u skladu s navodima Reynolds i sur. (1986), ali su slične onim u istraživanjima Karoglan i sur. (2016) i Pintur (2022). Vidljivo je da u različitim vegetacijskim godinama odgoda roka berbe nije utjecala na rast ukupnih fenola kod svih sorata već samo kod sorti Serena i Saphira.

Tablica 1. Značajnost razlika uroda i kvalitativnih pokazatelja mošta istraživanih sorti s obzirom na različitu vegetacijsku godinu (N=4)

Parametri	Sorta	Godina		s \bar{x}	Razina značajnosti
		2017.	2023.		
Ukupni urod (kg)	Prinzipal	3,12 ^a	2,78 ^b	0,07	*
	Primera	3,64	3,56	0,04	NS
	Serena	3,84	3,70	0,10	NS
	Sibera	3,97 ^a	3,45 ^b	0,09	**
	Saphira	4,52	4,20	0,12	NS
	Allegro	4,11	3,95	0,10	NS
	Bolero	4,35	4,25	0,07	NS
	Rondo	3,71	3,68	0,13	NS
Ukupna kiselost (g L ⁻¹)	Prinzipal	6,82	6,99	0,08	NS
	Primera	6,94 ^a	6,55 ^b	0,06	**
	Serena	7,63 ^a	6,44 ^b	0,07	***
	Sibera	7,82 ^a	6,75 ^b	0,07	***
	Saphira	6,91	6,72	0,09	NS
	Allegro	5,90	5,74	0,07	NS
	Bolero	5,86 ^a	6,20 ^b	0,08	*
	Rondo	5,85 ^a	5,16 ^b	0,07	***
Reducirajući šećeri (g L ⁻¹)	Prinzipal	194,75 ^a	173,20 ^b	2,50	**
	Primera	182,75 ^a	165,52 ^b	1,70	***
	Serena	187,00 ^a	169,03 ^b	1,45	***
	Sibera	177,25 ^a	144,90 ^b	1,28	***
	Saphira	200,25	194,13	2,24	NS
	Allegro	187,00 ^a	194,48 ^b	1,76	*
	Bolero	184,75 ^a	177,43 ^b	1,52	*
	Rondo	183,25 ^a	153,98 ^b	1,26	***
Realna kiselost (pH)	Prinzipal	3,12	3,18	0,05	NS
	Primera	3,27	3,21	0,04	NS
	Serena	3,27	3,51	0,07	NS
	Sibera	3,23	3,27	0,05	NS
	Saphira	3,41 ^a	3,66 ^b	0,07	*
	Allegro	3,49 ^a	3,82 ^b	0,07	*
	Bolero	3,46	3,51	0,06	NS
	Rondo	3,41 ^a	3,64 ^b	0,03	**
Ukupni fenoli (mg L ⁻¹)	Prinzipal	384,88 ^a	234,00 ^b	5,25	***
	Primera	334,33 ^a	274,00 ^b	5,18	***
	Serena	443,63 ^a	519,03 ^b	5,66	***
	Sibera	436,94 ^a	214,00 ^b	5,20	***
	Saphira	345,90 ^a	371,00 ^b	3,66	**
	Allegro	553,09 ^a	292,90 ^b	3,29	***
	Bolero	590,39 ^a	253,53 ^b	2,77	***
	Rondo	799,55 ^a	550,03 ^b	2,65	***

^{a,b} Vrijednosti u istom redu tablice označene različitim slovima značajno se razlikuju; NS-nema značajne razlike ($P>0,05$); * $P<0,05$; ** $P<0,01$ *** $P<0,001$

Iz tablice 1 vidljivo je da je 2017. godine najveći urod po trsu imala sorta Saphira, a najmanji urod sorta Prinzipal. Dok je u 2023. godini najveći urod imala sorta Bolero, a najmanji opet sorta Prinzipal. Statistički značajne razlike u urodu uočene su kod sorti Prinzipal i Sibera.

Značajne razlike u koncentraciji ukupnih kiselina između dviju istraživanih vegetacijskih godina uočene su kod sorata Primera, Bolero, Serena, Rondo i Sibera, dok kod sorata Prinzipal, Allegro i Saphira nisu primijećene značajne razlike. U 2017. godini najviše koncentracije ukupnih kiselina u moštu bile su kod sorte Sibera ($7,82 \text{ g L}^{-1}$), a najniže kod sorte Rondo ($5,85 \text{ g L}^{-1}$ izražene kao vinska kiselina.) U 2023. g. najvišu ukupnu kiselost imala je sorta Prinzipal ($6,99 \text{ g L}^{-1}$), dok je sorta Rondo imala najnižu ($5,16 \text{ g L}^{-1}$).

2017. g. najviše reducirajućih šećera bilo je kod sorte Saphira (200 g L^{-1}), a 2023. g. kod sorte Allegro ($194,5 \text{ g L}^{-1}$). Najniže vrijednosti reducirajućih šećera zabilježene su kod sorte Sibera u obje godine (172 i $144,9 \text{ g L}^{-1}$). Kako je prikazano u tablici 1, značajne razlike uočene su kod svih istraživanih sorata osim kod sorte Saphira.

Najniže pH vrijednosti u moštu ispitivanih sorti u obje godine istraživanja zabilježene su kod sorte Prinzipal ($3,12$ i $3,18$), a najviše kod sorte Allegro ($3,49$ i $3,82$), dok su značajne razlike uočene kod sorata Allegro, Rondo i Saphira.

Najviše koncentracija ukupnih fenola ($799,55$ i 550 mg L^{-1}) zabilježene su kod sorte Rondo, dok su najniže vrijednost zabilježena kod sorte Primera ($334,33 \text{ mg L}^{-1}$) u 2017. godini i i Sibera (214 mg L^{-1}) u 2023. g. Koncentracije ukupnih fenola bile su značajne niže u 2023. g. za sve istraživane sorte.

Tablica 2. Vrijednosti kvalitativnih pokazatelja na ukupno istraženom uzorku i godinama

	Parametri	$s \bar{x}$	sd	$s \bar{x}$	min.	maks.	Cv
Ukupno (N=64)	Urod po trsu (kg)	3,80	0,47	0,06	2,66	4,88	12,40
	Ukupna kiselost (g L^{-1})	6,52	0,70	0,09	4,92	7,99	10,76
	Reducirajući šećeri (g L^{-1})	179,04	15,25	1,91	142,30	207,00	8,52
	Realna kiselost (pH)	3,40	0,22	0,03	2,99	3,88	6,32
	Ukupni fenoli (mg L^{-1})	412,32	154,72	19,34	199,10	803,54	37,52

U tablici 2 vidljivo je da su najveća odstupanja u ukupnim vrijednostima kvalitativnih pokazatelja za cjelokupno istraživanje (2017. i 2023. godina za sve istraživane sorte) zabilježena kod ukupnih fenola što je u skladu s vrijednostima prikazanim u tablici 1.

Zaključak

Na temelju dobivenih rezultata otpornih međuvrskih hibrida tijekom dvije klimatološki nepogodne vegetacije, može se zaključiti da su sorte prikladne za uzgoj i proizvodnju vina u regiji Središnja bregovita Hrvatska, podregiji Prigorje-Bilogora, vinogorju Kalnik. Daljnjim istraživanjem i redukcijom uroda te određivanjem različitih rokova berbe za istraživane sorte mogla bi se postići i znatno bolja kvaliteta uroda, te bi se na osnovi tih analiza mogle dati i točnije preporuke za proizvodnju određenog stila vina za pojedinu sortu. Istraživane sorte su dodatno zanimljive za uzgoj jer po svojim osobitostima pripadaju grupi otpornih sorata prikladnih za ekološki uzgoj.

Literatura

- Državni hidrometeorološki zavod – DHMZ. <https://meteo.hr/index.php>
- Herjavec S. (2019). Vinarstvo. Nakladni zavod Globus, Zagreb.
- Jackson R.S. (2019). Wine Science: Principles and Applications. Academic Press. 5th edition. London.
- Karoglan M., Osrečak M., Tomaz I., Sladić J. (2016). Utjecaj roka berbe na sadržaj polifenola i antocijana u grožđu crnih sorata vinove loze. *Journal of Central European Agriculture*. 17(3).
- OIV (2020). Compendium of international methods of wine and must analysis, Volume 1. Paris, France. Raspoloživo: <https://www.oiv.int/public/medias/7372/oiv-compendium-volume-1-2020.pdf>
- Pintur I. (2022). Utjecaj roka berbe na sadržaj polifenola i antocijana kultivarova Frankovka (*Vitis vinifera*, L). Diplomski rad. Sveučilište Josipa Jurja Strossmayera u Osijeku, Fakultet

agrobiotehničkih znanosti Osijek, Osijek.

Robinson J., and Harding J. (2015). The oxford companion to wine. Oxford University Press.

Schmid J., Manty F., Lindner B. (2011). Geisenheimer Rebsorten und Klone. Ralf Tempel. Foliant-Editioen. Geisenheim.

Statistica 13.4.0.14 (2018). TIBCO Software Inc. Pulo Alto, USA

Štambuk P., i Karoglan Kontić J. (2021). Razvoj sorata otpornih na uzročnike bolesti – važan korak ka smanjenju uporabe fungicida. Glasilo biljne zaštite. 21 (3).

Qualitative indicators of grape must suitable for organic production at the Križevci University of Applied Sciences

Abstract

Interspecies hybrids partially or completely resistant to certain vine diseases (Prinzival, Allegro, Primera, Bolero, Serena, Rondo, Sibera and Saphira) were investigated in the Ampelographic Garden of the Križevci University of Applied Science. After analyzing the yield, must parameters and assessing the suitability of those varieties for ecological cultivation in the region of Croatian Uplands Region, the Prigorje-Bilogora subregion, the Kalnik vineyard during the 2017 and 2023 growing seasons, it can be concluded that the varieties are suitable for growing and producing wine in the area of the investigated vineyard. The obtained results show a higher concentration of total acidity for white varieties, which is a consequence of too high a yield and unfavorable climatic conditions during the growing years. The total concentration of phenols differed significantly between the researched cultivars and years, which was influenced by the time of harvest in addition to the previously mentioned difficulties.

Keywords: grape vines, interspecies hybrids, resistant varieties, ecological production, qualitative indicators of must

Utjecaj odležavanja vina na organoleptičke karakteristike ‘Graševine’ Pavlović

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Sažetak

Cilj istraživanja bio je utvrditi potencijal odležavanja vina ‘Graševine’ Pavlović iz vinogorja Kutjevo. U tu svrhu odabrana su vina iz razdoblja od 6 godina i to od berbe 2017. do 2022. Sva vina osim berbe 2022. pripadala su u kategoriju tradiciionalnog izraza kvaliteteno vino i punjena su u pakovinu od 0,75 L. Evaluaciju je proveo panel od 5 ocjenjivača educiranih u skupu projekta „Uncorking rural heritage“. Vina su ocjenjena službenom „metodom 100 bodova“ te deskriptivno. Rezultati metode 100 bodova obrađeni su statistički. Deskriptori za arome i okus vina ‘Graševine’ određeni su općenito za graševinu neovisno o degustiranim uzorcima. Iako nisu posebno pripremane za odležavanje vina ‘Graševine’ Pavlović zadržala su izraženu svježinu i pitkost. Vina berbi 2019. i 2021. godine ocjenjena su znatno većim ocjenama nego prilikom puštanja vina u promet i prema broju ostvarenih bodova pripadaju u višu kategoriju, vrhunskih vina.

Ključne riječi: ‘Graševina’, Vinogorje Kutjevo, senzorna analiza, deskriptivna metoda

Uvod

Od devedesetih godina prošlog stoljeća u Hrvatskoj mnogi vinogradari postaju i vinari. Jedna od mladih vinarija vinogorja Kutjevo je i vinarija Pavlović u kojoj dominiraju vina sorte ‘Graševina’. Cilj rada je bio utvrditi utjecaj odležavanja na kakvoću vina koristeći različite metode organoleptičkog ocjenjivanja vina. Graševina je najzastupljeniji kultivar u Hrvatskoj, a područje Dunavskog sliva vjerojatno je mjesto podrijetla (Robinson i sur., 2012). U Republici Hrvatskoj, ‘Graševina’ se uzgaja dominantno u kontinentalnom području u vinogradarskim regijama Slavoniji i hrvatskom Podunavlju te Središnjoj bregovitoj Hrvatskoj, ali je uzgoj moguć i u regijama Hrvatska Istra i Kvarner te Dalmacija (Zakon o vinu). Najveći broj vrhunskih graševina nalazi se u vinogradarskoj podregiji Slavoniji (Sokolić 2006). koja danas odgovara zaštićenoj oznaci izvornosti ZOI Slavonija. Vina graševine su zelenkasto žute do žute boje. Ugodne su srednje izražene do izražene cvjetno – voćne arome. Prevladavaju arome jabuke, kruške, banane, citrusa i grejpa uz tragove cvjetnih mirisa, posebice bagrema. Prema Herjavec (2019) vina ‘Graševine’ najčešće su suha, blage gorčine umjerenih koncentracija alkohola i ekstrakta. To su harmonična vina, srednje dugog do dugog, čistog, blago gorkog okusa (Mirošević i sur., 2011; Mirošević i Turković 2003). Svakako kod karakterizacije pojedinog vina treba uzeti u obzir i utjecaj tehnoloških postupaka tijekom proizvodnje. Uparaba različitih komercijalnih kvasaca ima značajan utjecaj na voćne arome vina različitih klonova graševine (Tomašević i sur., 2023). Osim tehnoloških postupaka u proizvodnji na kakvoću vina utječu i okolinski uvjeti te godina berbe (Maletić i sur., 2008).

Provedena su mnoga istraživanja vezana za povoljni utjecaj odležavanje ili dozrijevanja vina, a kako bi se odvijali pozitivni procesi u boci vino je potrebno čuvati u adekvatnim uvjetima. Mnoga vina u hladnim podrumima svoj puni potencijal dosežu nakon više desetljeća ali na žalost većina vina nema zadovoljavajući tretman prilikom odležavanja ili arhiviranja. Iako prevladava opće mišljenje da bijela vina treba potrošiti dok su mlada i da su crna vina pogodna za čuvanje to je u biti refleksija preferencija većine potrošača (Jackson 2020). Korištenje drvenih bačava može biti jedan od postupaka koji povećava potencijal bijelih vina za odležavanje (Alanon 2018).

Senzorno ocjenjivanje vina predstavljaj detaljnu analizu svih dojmova koje vino ostavlja na naša osjetila vida, mirisa, okusa i opipa (Jackson 2017; Herjavec 2019). Iako je u Hrvatskoj uobičajene praksa brojčanog ocjenjivanja vina deskriptivna metoda značajno doprinosi razvoju vinskog vokabulara općenito i kod proizvođača i kod potrošača podižući kulturu pijenja vina na višu razinu.

Materijal i metode

Istraživanje je provedeno na vinima 'Graševine' vinarije Pavlović iz Češljakovaca u vinogorju Kutjevo. Nakon vinifikacije vino je napunjeno u boce za puštanje u promet, a iz posljednje serije punjenja isti su uzorci stavljeni u privatnu arhivu. Uzorci nisu posebno pripremani za arhiviranje. U tablici 1. prikazani su osnovni fizikalno kemijski parametri vina izuzeti iz rješenja za puštanje vina u promet. Analize je napravio Centar za vinogradarstvo, vinarstvo i uljarstvo Hrvatske agencije za poljoprivredu i hranu. Prema prikazanim podacima vidljivo je da se sadržaj alkohola kretao od 13,8 vol% u vinu berbe 2017 do 11,7 vol% u vinu berbe 2021. godine koje je jedinu u kategoriji polusuhih vina s reducirajućim šećerima od 6.2 g/L. Ukupna kiselost svih uzoraka kretala se od 5,1 do 6,3 g/L. Sva vina, osim berbe 2017. ocjenjena su ocjenama u rasponu od 75 do 79 bodova i pripadaju u kategoriju tradicionalnih izrata kvalitetno vino osim berbe 2017. godine koje pripada u kategoriju vrhunskih vina.

Tablica 1. Prikaz parametara sadržaja alkohola, ukupne kiselosti, te broja bodova po metodi 100 bodova vina 'Graševine' Pavlović, berbe: 2017., 2018., 2019., 2020., 2021., 2022.

Godina berbe	Alkohol (vol%)	Reducirajući šećer (g/L)	Ukupna kiselost (g/L)	Broj bodova
2017.	13,8	2,5	5,3	83
2018.	13,2	1,4	5,1	78
2019.	12,8	1,1	6,3	78
2020.	12,2	4,0	6,2	79
2021.	11,7	6,2	5,3	75
2022.	13,5	4,0	5,5	75

Izvor: Hrvatska agencija za poljoprivredu i hranu, Centar za vinogradarstvo, vinarstvo i uljarstvo

Za potrebe ovog istraživanja degustaciju vina proveo je panel od pet ocjenjivača. Svi ocjenjivači su prošli testiranje i edukaciju za organoleptičko ocjenjivanje vina u sklopu projekta: „Uncorking rural heritage: indigenous production of fermented beverages for local cultural and environmental sustainability“ programa EEA and Noreay Grants Found for Regional Cooperation. Nositelj projekta je University of Nova Gorica, a jedan od partnera je Polytechnic in Požega.

Uzorci su ocjenjivani metodom „100 bodova“ sukladno Pravilniku o vinarstvu (NN 81/2022), a nakon toga deskriptivno. Za vino 'Graševine' deskriptori su određeni prethodnim kušanjem pri čemu su ocjenjiva izdvojili deskriptore. Prilikom ocjenjivanja metodom 100 bodova dogovoreno je da se parametri izgleda vina ocjene maksimalnim brojem bodova. Ocjenjivanje metodom 100 bodova provedeno je u tri repeticije, a rezultati su statistički obrađeni analizom varijance (ANOVA), a razlike između razina signifikantnih faktora Fisherovim LSD testom uz Bonferronijevu korekciju pogreške, uz pomoć programa SAS System for Windows 9.3 (2012), (SAS Institute Inc., Cary, NC, USA).

Rezultati i rasprava

U tablici 2 prikazane su ocjene za svako pojedino senzorno svojstvo svih šest berbi vina graševine. Iz prikazanih podataka vidljivo je da su sva vina ocjenjena prosječnim ocjenama koje ih svrstavaju u kategorije tradicionalnih izraza „kvalitetno“ i „vrhunsko“ vino. Obzirom da uzorci nisu posebno pripremljeni za arhiviranje očekivan je pad organoleptičkih karakteristika. Statistički oprsavdane razlike utvrđene su samo u ukupnoj ocjeni vina koja su se grupirala u dvije skupine.

Tablica 2. Prikaz rezultata izgleda, mirisa, okusa, harmonije i ukupna ocjena vina 'Graševine' Pavlović, 2017., 2018., 2019., 2020., 2021. i 2022. godine

Berba		2017.	2018.	2019.	2020.	2021.	2022.
Izgled	Bistroća	5	5	5	5	5	5
	Boja	10	10	10	10	10	10
Miris	Čistoća	3	3	3	3	4	5
	Intenzitet	6	6	6	6	6	6
	Kvaliteta	10	10	12	12	12	12
Okus	Čistoća	4	4	5	5	4	5
	Intenzitet	6	6	7	7	7	7
	Trajnost	6	6	7	7	7	6
	Kvaliteta	16	16	19	19	19	19
Harmonija / opći dojam		9	9	9	9	10	10
Ukupna ocjena		75 a	77 a	83 b	78 a	86 b	85 b

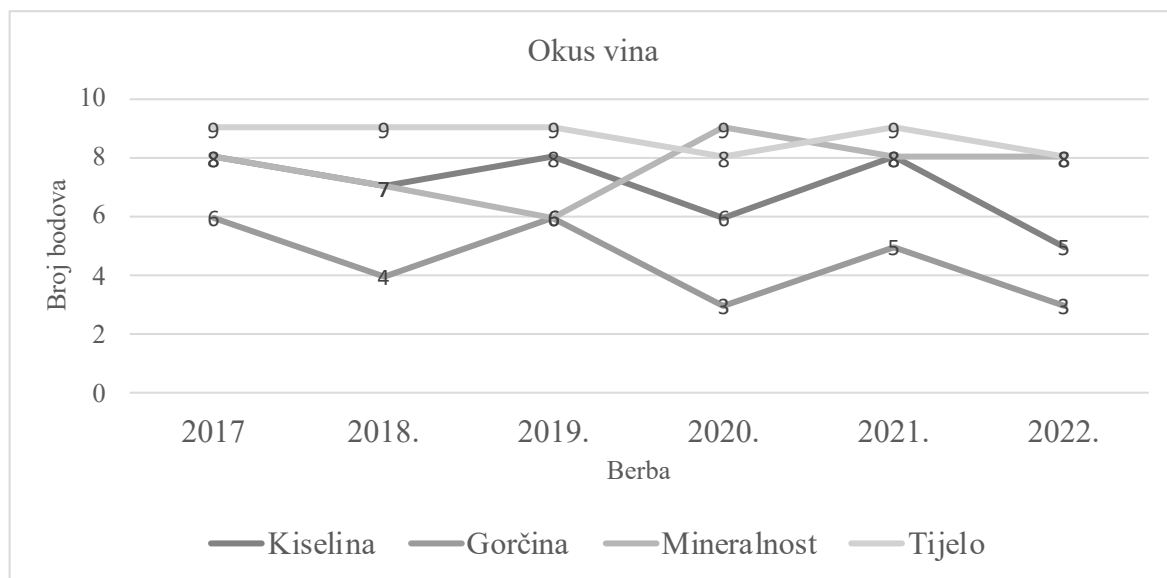
a, b – različita slova označavaju statistički signifikantne razlike između prosjeka tretmana kod $P < 0.05$ na osnovi Fisherova LSD testa

Tablica 3. Prikaz ocjena deskriptora arome vina 'Graševine' Pavlović; 2017., 2018., 2019., 2020., 2021. i 2022. godine

	2017.	2018.	2019.	2020.	2021.	2022.
Intenzitet	8	8	9	7	6	6
Herbalno	2	0	6	0	7	2
Cvijetno	0	0	0	0	7	7
Zelena jabuka	0	0	0	9	2	4
Zrela jabuka	10	8	9	3	9	6
Citrusi	1	4	1	5	4	3
Vino. breskva	0	0	0	4	2	5
Začini	3	3	0	0	0	1
Maslac	5	6	0	5	6	0

Tablica 3 prikazuje deskriptore aroma vina 'Graševine' Pavlović. Vidljivo je da u svim vinima dominira aroma zrele jabuke te citrusa. Tipičan deskriptor zelene jabuke prepoznat je u mlađim vinima berbi 2020., 2021. i 2022. godine. Iz prikazanih rezultata može se pretpostaviti da protekom vremena vina 'Graševine' Pavlović gube cvjetne arome kao i aromu vinogradarske breskve. Intenzitet arome kod svih uzoraka ocjenjen je visokim ocjenama, a od svih uzoraka ističe se vino berbe 2019, koje je ocjenjeno ocjenom 9 od 10 bodova.

Grafikon 1 prikazuje deskriptore okusa vina. Istaknuta su svojstva kiseline, gorčine, mineralnosti i tijela odnosno sklada okusa. Svi uzorci osim "mlade" 'Graševine' berbe 2022. ocjenjeni su vrlo visokom ocjenom od 9 bodova u svojstvu tijela. Zanimljivo je da i vina starijih berbi imaju visoku ocjenu u svojstvu kiseline odnosno svježine obzirom da vina nepripremljena za odležavanje često gube svježinu.



Grafikon 1. ocjene deskriptora okusa vina 'Graševine' Pavlović, , 2017, 2018., 2019., 2020., 2021., 2022. godine

Zaključak

Prema provedenom istraživanju može se zaključiti da postoji potencijal za dodležavanje vina 'Graševine' Pavlović. Vina berbi 2019. i 2021. godine ocjenjena su vrijednostima koje ih svrstavaju u višu kategoriju kakvoće proizvoda. Očekivano kod vina starijih godišta nisu zabilježeni deskriptori koji asociraju na svježinu (zelena jabuka) prisutnu kod mladih vina ali unatoč tome u okusu je utvrđena izrazita svježina kod svih uzoraka bez obzira na godinu berbe. Na temelju prikazanih rezultata vidljivo je da su vina zadržala jednaku ili veću razinu kakvoće u odnosu na trenutak kada su puštena u promet. U daljnjim istraživanjima trebalo bi uključiti i graševine drugih vinara vinogorja Kutjevo kako bi dobili sliku potencijala odležavanja vina 'Graševine' koja je najuže vezana na vinogorje Kutjevo.

Literatura

- Alanon M.E., Diaz-Morato M.C., Perez-Coello M.S. (2018). New strategies to improve sensorial quality of white wines by wood contact. *Beverages*. 4/4: 91.
- Herjavec S. (2019) *Vinarstvo*. Zagreb. Nakladni zavod Globus.
- Jackson R.S. (2017). *Wine tasting a profesional handbook*. Third ediotion. United Kingdom: Academic press, Elsevier Ltd.
- Jackson R.S. (2020). *Wine science, principles and applications*, fifth edition, United Kingdom: Elsevier inc.
- Maletić E., Karoglan Kontić J., i Pejić I. (2008). *Vinova loza, ampelografija, ekologija, oplemenjivanje*. Školska knjiga, Zagreb.
- Mirošević N., i Turković Z. (2003). *Ampelografski atlas*, Zagreb: Golden marketing Tehnička knjiga.
- Mirošević N., Vranić, I., Soldo Čamak, V., Božinović, T., Jelaska, V., Maletić, E., Premužić, D., Ivanković, Z., Brkan, B., Ričković, M., Bolić, J. (2011). *Kutjevačka Graševina Nadarbina Zlatne doline (Vallis aurea)*. Golden marketing - Tehnička knjiga, Zagreb.
- Narodne Novine: NN 81/2022, Službeni list Republike Hrvatske, Ministarstvo poljoprivrede, 1184.
- Robinson J., Harding J., Vouillamoz J. (2012). *Wine Grapes, A complete guide to 1368 vine varieties, including their origins and flavours*. Ujedinjeno kraljevstvo: Penguin Books .
- Sokolić I. (2006). *Veliki vinogradarsko vinarski leksikon*. Novi vinodolski, Sveučilišna knjižnica Rijeka.
- Tomašević M., Lukić K., Ćurko N., Jagatić-Korenika A-M., Preiner D., Tuščić V., Jeromel A., Kovačević Ganić K. (2023). The influence of Grape Clone and Yeast strains on varietal thiol concentrations and sensory properties of Graševina wines. *Foods*. 12.5: 985.

The influence of wine aging on sensory characteristics of 'Graševina' Pavlović

Abstract

The goal of the research was to determine the aging potential of 'Graševina' Pavlović wine from the Kutjevo vineyard. For this purpose, wines from a period of 6 years and from the harvest of 2017 to 2022 were selected. All wines, except for the 2022 harvest, belonged to the category of the traditional term quality wine and were filled in 0.75 L packaging. The evaluation was carried out by a panel of 5 evaluators trained as part of the "Uncorking rural heritage" project. The wines are rated using the official "100 point method" and descriptively. The results of the 100 points method were processed statistically. Descriptors for the aromas and flavors of Graševina wine are determined in general for 'Graševina' independently of the tasted samples. Although not specially prepared for the aging of 'Graševine' Pavlović wines, they have retained a pronounced freshness and drinkability. The wines of the 2019 and 2021 vintages were rated with significantly higher ratings when the wine was released and according to the number of points achieved, they belong to a higher category of premium wines.

Keywords: 'Graševina', Vinogorje Kutjevo, sensory analysis, descriptive method

Istraživanje generativnog potencijala sorata vinove loze ‘Graševina’ i ‘Kraljevina’

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Sažetak

Generativni potencijal je genetski definirano svojstvo sorte ka postizanju određenog prinosa. Ovo svojstvo izrazito varira ovisno o sorti a objektivno se utvrđuje mjerenjima prinosa, broja grozdova po trsu te izračunavanjem koeficijenta rodnosti. Cilj istraživanja bio je utvrditi i usporediti generativni potencijal sorata vinove loze ‘Graševina’ i ‘Kraljevina’. Istraživanje je pokazalo kako sorta ‘Graševina’ ima veće koeficijente rodnosti što je rezultiralo i većim brojem grozdova po trsu u odnosu na sortu ‘Kraljevina’ (‘Graševina’ prosječno 23.2 grozda po trsu, ‘Kraljevina’ 16,6 grozdova). ‘Kraljevina’ je imala značajno veću prosječnu masu grozda (203,06 grama naspram ‘Graševine’ sa 80,86 grama) što je u konačnici rezultiralo i većim prinosom po trsu (3,22 kg po trsu naspram ‘Graševine’ sa 1.88 kg). Navedenih pokazatelji u praksi mogu poslužiti u regulaciji prinosa kroz broj ostavljenih pupova rezidbom ili uklanjanja grožđa „zelenom“ rezidbom.

Ključne riječi: generativni potencijal, ‘Graševina’, ‘Kraljevina’, koeficijenti rodnosti

Uvod

Sorta ‘Graševina’ je najzastupljenija sorta u Republici Hrvatskoj sa 4347 hektara zasađenih površina dok je sorta ‘Kraljevina’ zastupljena sa svega 181 hektara, ali je najzastupljenija autohtona sorta sjeverozapadne Hrvatske (Vinogradarski registar, APPRRR, 2022).

Sorta ‘Graševina’ se prema literaturi spominje u Hrvatskoj još sredinom 19. st. (Trummer, 1854) te je pretpostavka je da je tada i počeo uzgoj ove sorte, prvo u sjeverozapadnoj Hrvatskoj a onda i dalje prema istoku (Maletić i sur., 2015a). Sorta ‘Graševina’ ne smatra se hrvatskom autohtonom sortom jer je raširena u svim vinogradarskim zemljama panonskog prostora od Italije pa do Rumunjske, što potvrđuju i genetičke analize (Maletić i sur., 2015a). Sorta je srednje do visoke rodnosti (Mirošević, 2003, Bisztray Gyorgy i sur., 2011). U lošijim klimatskim uvjetima i slabije ishranjenim vinogradima sjeverozapadne Hrvatske rodnost je slabija, a u toplijim područjima i boljim tlima Slavonije i Baranje značajno bolja (Maletić i sur., 2015a, Mirošević, 2003). Bez obzira na ekološke uvjete rodi redovito, a kvaliteta grožđa i vina je vrlo dobra (Maletić i sur., 2015a). Po fiziološkim karakteristikama pripada zapadnoeuropskoj grupi sorata (*Proles occidentalis, subproles gallica*) koje karakterizira slabija rodnost bazalnih pupova, zbog čega je rezidbom potrebno ostaviti veći broj pupova po trsu (Maletić i sur., 2015a).

Sorta ‘Kraljevina’ tipična je sorta starih vinograda sjeverozapadne Hrvatske. U drugoj polovici 19. stoljeća spominje se u brojnim literarnim izvorima kao sorta šireg zagrebačkog područja, Prigorja i Hrvatskog zagorja (Maletić i sur., 2015b), a navedena područja i danas predstavljaju najznačajniji areal njezine rasprostranjenosti. Danas je zastupljena i na području Plešivice i Pokuplja ali vrlo malo, uglavnom u starim vinogradima. Glavno gospodarsko obilježje sorte ‘Kraljevina’ je visoka i redovita rodnost (Maletić, 2015b). U vinogradarskoj praksi nije zahtjevna za uzgoj, ali zbog svoje izražene rodnosti pažnju treba obratiti na broj ostavljenih pupova prilikom rezidbe (Maletić i sur., 2015b). Prevelik broj ostavljenih pupova dovodi do previsokog prinosa grožđa a time i manje kvalitete vine. Bez obzira na prinos, kvaliteta vina je srednja (Mirošević, 2003).

Generativni potencijal je genetski definirano svojstvo sorte ka postizanju određene razine rodnosti (Maletić i sur., 2008). Ovo svojstvo izrazito varira ovisno o sorti, a objektivno se može utvrditi mjerenjima prinosa, broja grozdova po trsu, prosječne mase grozda, brojem ostavljenih pupova te rodni i nerodni mladica. Iz navedenih pokazatelja izračunavaju se koeficijenti rodnosti (potencijalni, relativni i apsolutni) kao pokazatelji generativnog potencijala

sorte, na osnovu čega se može planirati i sam postupak rezidbe kao primarni proces regulacije prinosa i kvalitete grožđa. Cilj istraživanja bio je utvrditi i usporediti generativni potencijal sorta 'Graševina' i 'Kraljevina' te utvrditi njihov utjecaj na prinos u novim proizvodnim nasadima zagrebačkog vinogorja i u istim proizvodnim uvjetima (klima, tlo, kategorija sadnog materijala, uzgojni oblik, održavanje vinograda).

Materijal i metode

Pokusni nasad nalazi se u sklopu vinogradarsko-vinarskog pokušališta Jazbina, Agronomskog fakulteta Sveučilišta u Zagrebu. Pokus je postavljen u nasadu sorata 'Graševina' i 'Kraljevina'. Vinograd se nalazi na nadmorskoj visini 280 m. Tlo u pokusnom nasadu predstavlja antropogeni pseudoglej na matičnom supstratu iluvijalnih ilovina. Reakcija tla je slabo kisela.

Vinogradi su posađeni 2016. godine od certificiranim sadnim materijalom (plava etiketa). Podloga na koju je cijepljena je SO4 (*Vitis berlandieri* x *Vitis riparia*). Razmaci sadnje su 2,1 metar između redova i 0,8 m između trsova u redu. Uzgojni oblik je jednostruki „Guyot“, pri čemu je zimskom rezidbom na lucnju ostavljeno 12 pupova, a na rezniku 2 pupa. Tehnologija uzgoja podrazumijeva standardne postupke održavanja vinograda (rezidba „u zrelo“, vezanje, uvlačenje mladica u žice, pljevljenje, vršikanje i berba). Istraživanja su provedena u berbi 2022. godine.

Za pokus je odabrano deset trsova od svake sorte. Netom prije cvatnje prebrojan je broj potjeralih mladica, broj rodni mladica i broj grozdova po mladici. Iz navedenih podataka izračunati su koeficijenti rodnosti: koeficijent potencijalne rodnosti (KpR, broj grozdova po pupu), koeficijent relativne rodnosti (KrR, broj grozdova po mladici) i koeficijent apsolutne rodnosti (KaR, broj grozdova po rodnoj mladici). Općenito, vrijednosti za koeficijent rodnosti mladica kreću se između 0,2 i 2, pri čemu se sorte sa vrijednosti do 0,5 smatraju sortama niskog KrR-a, do 1 srednjeg, do 1,5 visokog, a preko 1,5 vrlo visokog KrR-a (Maletić i sur., 2008).

U vrijeme tehnološke zrelosti sa svakoga trsa prebrojan je broj grozdova i izvagan prinos po trsu.

Podaci dobiveni istraživanjem statistički su obrađeni metotodom analize varijance tzv. ANOVA. Također je napravljena usporedba razlike srednjih vrijednosti pomoću „Duncan multiple range test“ metode: Statistička analiza provedena je u programu „XLSTAT“ tvrtke „Microsoft“.

Rezultati i rasprava

Iz podataka dobivenih na osnovu broja ostavljenih pupova, potjeralih mladica i broja grozdova po mladici izračunati su koeficijenti rodnosti i uspoređeni statističkom metodom ANOVA (Tablica 1).

Tablica 1. Koeficijenti rodnosti za sorte 'Graševina' i 'Kraljevina'.

Sorta	Koeficijent potencijalne rodnosti (KpR)	Koeficijent relativne rodnosti (KrR)	Koeficijent apsolutne rodnosti (KaR)
Graševina	1,677a*	1,756a	1,861 a
Kraljevina	1,159b	1,301b	1,575b
(Pr > F)**	0,004	0,007	0,021

*Srednje vrijednosti označene različitim slovima se statistički razlikuju uz $p < 0,05$.

** Razlika je signifikantna za $(Pr > F) < 0,05$ uz 95% vjerojatnosti

Statistička analiza potvrđuje da se sorte 'Graševina' i 'Kraljevina' značajno razlikuju po koeficijentima rodnosti. Koeficijent potencijalne rodnosti tj. broj grozdova po pupu veći je kod sorte 'Graševina' što što ukazuje da ukoliko se prilikom rezidbe ostavi isti broj pupova po trsu, kod sorte 'Graševina' u konačnici možemo očekivati veći broj grozdova po trsu.

Sorta 'Graševina' ima koeficijent relativne rodnosti (KrR) u prosjeku 1,76 što ju svrstava u sorte s vrlo visokim KrR-om, dok je sorta 'Kraljevina' imala koeficijent relativne rodnosti (KrR) u prosjeku 1,30, što ju svrstava u sorte visokog KrR-a, što potvrđuje i navode iz literature kako se radi o dvije sorte visokog rodnog potencijala. Koeficijent apsolutne rodnosti (KaR) kod sorte 'Graševina' je veći u odnosu na sortu 'Kraljevina' pa će u stvarnosti sorta 'Graševina' imati

i veći broj grozdova po rodnoj mladici od sorte 'Kraljevina'.

Uspoređujući koeficijente rodnosti ove dvije sorte možemo zaključiti da sorta 'Graševina' ima značajno veći generativni potencijal od sorte 'Kraljevina' u pogledu broja zametnutih grozdova na trsu. Međutim kako se radi o dvije sorte vrlo različite strukture grozda (dimenzije i mase grozda) koeficijente rodnosti ne možemo uzeti kao jedini pokazatelj koji utječe na ukupni rodni potencijal sorte.

Osim koeficijenta rodnosti, napravljena je i usporedba broja grozdova po trsu, prosječne mase grozda te prinosa po trsu između sorata 'Graševina' i 'Kraljevina' (Tablica 2).

Tablica 2. Broj grozdova po trsu, prosječna masa grozda te prinos po trsu za sorte 'Graševina' i 'Kraljevina'.

Sorta	Broj grozdova	Prosječna masa grozda (g)	Prinos po trsu (kg)
Graševina	23,20 a	203,06 a	1,86 b
Kraljevina	16,60 b	80,86 b	3,22 a
(Pr > F)**	0,005	<0,0001	<0,0001

*Srednje vrijednosti označene različitim slovima se statistički razlikuju uz $p < 0,05$.

** Razlika je signifikantna za $(Pr > F) < 0,05$ uz 95% vjerojatnosti

Sorta 'Graševina' imala je značajno veći broj grozdova po trsu (23,2 grozda po trsu) u usporedbi sa sortom 'Kraljevina' (16,6 grozdova po trsu) što je u skladu sa pokazateljima koeficijenta rodnosti.

Sorta 'Kraljevina' je imala značajno veću prosječnu masu grozda (203 g) u odnosu na sortu 'Graševina' (80,86 g). Prema Maletić i sur. (2008), sortu 'Kraljevina' možemo svrstati u sorte sa velikom masom grozda (160-240 grama), a 'Graševinu' sorata sa srednje velikom masom grozda (80-160 g).

Mjerenjem prinosa sorata 'Graševine' i 'Kraljevine' potvrđen je utjecaj genetski uvjetovanih karakteristika rodnosti na sam prinos. Sorta 'Graševina', iako vrlo visokog potencijala rodnosti pupova, ali zbog izrazito male mase grozda u konačnici postiže i niže prinose (manje od 2 kg po trsu). Sa tehnološkog aspekta ta je činjenica izrazito prihvatljiva jer se prema stručnim preporukama najbolja kvaliteta vina postiže upravo kod prinosa između 1.5 do 2.5 kilograma po trsu

Sorta 'Kraljevina' sa prinosom od 3.22 kilograma po trsu potvrđuje svoje karakteristike visokoprinodne sorte. Tome doprinosi visoki potencijal relativne rodnosti (broj grozdova po mladici), a posebice prosječna masa grozda, koja je 2.5 puta veće nego kod sorte 'Graševina'.

Zaključak

Istraživanje je pokazalo da su i sorta 'Graševina' i 'Kraljevina' sorte velikog potencijala rodnosti. Također, kod sorte 'Graševina' pokazalo se da je izbor uzgojnog oblika „guyot“ u uvjetima zagrebačkog vinogorja prihvatljiv za postizanje optimalnog prinosa grožđa, a time i potencijalne kvalitete vina. Kod sorte 'Kraljevina' navedeni sustav uzgoja nije prihvatljiv te bi za smanjenje prinosa i u konačnici postizanja bolje kvalitete vina trebalo primijeniti kraću razidbu sa manje ostavljenih pupova po trsu. Također, u godinama kada dolazi do velike rodnosti može se primijeniti postupak „zelene rezidbe“ koji uključuje uklanjanje grozdova kako bi se u konačnici postigao prinos od otprilike 2 kilograma po trsu.

Literatura

- Trummer F.X. (1854). Nachtrag zur Systematischen Classification un Beschreibung der im Herzogthume Steiermark vorkommenden Rebensorten, Graz.
- Mirošević N. (2003). Ampelografski atlas. Zagreb, Hrvatska, Golden marketing-Tehnička knjiga.
- Maletić E., Karoglan Kontić J., Pejić I. (2008). Vinova loza- biologija, ekologija, oplemenjivanje. Ćorić,

- S.(eds). Zagreb, Hrvatska, Školska knjiga.
- Bisztray Gyorgy, D., Cindrić, P., Edit, H., Ivanišević, D., Korać, N., Lazar, J., Medić, M., Szegedi, E. (2011). Sorte vinove loze, sadni materijal i bolesti. Hajdu, E., Cindrić, P. (eds), Budimpešta, Mađarska, Agroinform Kiado es Nyomda.
- Maletić E., Preiner D., Pejić I., Karoglan Kontić J., Šimon S., Husnjak S., Marković Z., Andabaka Ž., Stupić D., Žulj Mihaljević M., Merkaš S. (2015a). Sorte vinove loze Hrvatskog Zagorja. Maletić, E. (eds). Krapina, Hrvatska, Krapinsko-zagorska županija.
- Maletić E. (2015b.) Zelena knjiga: Hrvatske izvorne sorte vinove loze. Maletić, E., Karoglan Kontić, J., Ilijaš, I. (eds), Zagreb, Hrvatska, Državni zavod za zaštitu prirode.
- Vinogradarski registar- podaci iz vinogradarskog registra za 2022. godinu. Agencija za plaćanja u poljoprivredi, ribarstvu i ruralnom razvoju (<https://www.apprrr.hr/registri/>).

Investigation of the generative potential of the grape varieties ‘Graševina’ and ‘Kraljevina’

Abstract

Generative potential is the genetically defined ability of a variety to produce a certain yield. This characteristic varies greatly depending on the variety and is determined objectively by measuring the yield, the number of bunches per vine and by calculating the productivity coefficients. The aim of the study was to determine and compare the generative potential of the grape varieties ‘Graševina’ and ‘Kraljevina’. The results show that the grape variety ‘Graševina’ has higher productivity coefficients, which leads to a higher number of bunches per vine compared to the grape variety ‘Kraljevina’, while the grape variety ‘Kraljevina’ has a significantly higher average bunch mass, which ultimately leads to a higher yield per vine. The final results can be used in practice to regulate the yield by manipulating the number of buds left by winter pruning or the removal of grapes during vegetation.

Keywords: generative potential, ‘Graševina’, ‘Kraljevina’, productivity coefficients



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Pojavnost patule (*Camarosporium dalmaticum* (Thüm.) Zachos & Tzavella-Klonari) u uvjetima smanjene primjene bakra u masliniku Zadarske županije

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Sažetak

Cilj rada je bio utvrditi utjecaj bakra samostalno ili u kombinaciji za zeolitom u različitim dozama na pojavnost patule u masliniku na području Zadarske županije. Poljski pokus proveden je u sklopu projekta LIFE Microfighter, tijekom 2023. u masliniku površine 0,8 ha u mjestu Škabrnja. Procjena pojavnosti patule provedena je na četiri varijante s četiri ponavljanja na sorti Oblica. Određeni su začetnici zaraze: ubod maslinine muhe, maslininog svrdlaša ili mehaničkog oštećenja, te je procijenjen broj otpalih plodova uzrokovanih patulom. Podatci su obrađeni statističkim programom ARM7[®], analizom varijance (AOV). Srednje vrijednosti podataka rangirane su Student-Newman-Keuls testom rangova. Obzirom na uzročnika koji je prethodio pojavi patule, osim u slučaju svrdlaša, analiza je pokazala da ima statistički značajne razlike među varijantama u usporedbi s kontrolom (Cu0).

Ključne riječi: Škabrnja, maslina Oblica, pojavnost bolesti, IPM, zeolit

Uvod

Maslina (*Olea europaea* L.) je zimzelena drvenasta biljka koja pripada porodici *Oleaceae* i porijeklom je iz tropskih područja (Boskou i sur., 1996). Iako je uzgoj maslina rasprostranjen diljem svijeta, regija Mediterana još uvijek je glavno proizvodno područje koje čini oko 98 % svjetskog uzgoja masline (Ryan i Robards 1998). Proteklih je godina zabilježeno povećanje potražnje za maslinovim uljem i plodom masline, što za posljedicu ima povećanje proizvodnje stoga i potreba za primjenom sredstava za zaštitu bilja (SZB) raste. Iako je ostvaren napredak u istraživanju novih mogućnosti i metoda zaštite bilja, primjena kemijskih sredstava još dugo će ostati osnovni način zaštite poljoprivrednih kultura od štetnih organizama (Havranek i sur., 2014). Nekomolirano i prekomjerno korištenje kemijskih SZB dovodi do nakupljanja njihovih rezidua u tlu, vodi i hrani što u konačnici ima negativan utjecaj na cijeli ekološki sustav (Petrović i Godena 2023). Stoga se SZB prirodnog podrijetla nameću kao rješenje problema jer su biorazgradivi, ekološki prihvatljivi, jeftiniji te djeluju na štetne organizme preko nekoliko mehanizama djelovanja (Souto i sur., 2021). Proizvođači bilježe sve više problema s gljivičnim bolestima maslina (Havranek i sur., 2014), jedna od njih je i patula (*Camarosporium dalmaticum* (Thüm) Zachos & Tzavella-Klonari). Patula ili trulež ploda maslina poznata je u svim područjima uzgoja maslina. U pojedinim godinama značajna je bolest ploda masline. Zaraženi plodovi zbog promjene u izgledu nisu pogodni za konzerviranje, imaju manju tržišnu vrijednost i time daju lošiju kvalitetu ulja. Pojava bolesti je povezana s pojavom maslinine muhe (*Bactrocera oleae* Gmelin), a češće su napadnute sorte koje imaju krupniji plod. Prvi simptom patule je vodenasta pjega koja se uočava još na zelenim plodovima, nakon toga ona postaje nekrotična, svjetlosmeđe boje, udubljena nekoliko mm u tkivo ploda, zatim poprima tamniju boju. Pjega se širi do promjera 7 do 15 mm te je jasnim rubom odvojena od zdravog dijela ploda. U konačnici većina zaraženih plodova otpadne. Prvi korak u zaštiti masline od patule je suzbijanje maslinine muhe koja prenosi inokule, stoga je potrebno primjenjivati SZB poglavito fungicide na osnovi bakra (Cvjetković, 2010). Za očekivati je da i ostali štetnici ili mehanička oštećenja ploda kao i maslinina muha mogu dovesti do veće pojave patule. U sklopu projekta LIFE Microfighter (101074218-LIFE21-ENV-IT-LIFE MICROFIGHTER), u masliniku

je postavljen poljski pokus s ciljem da se uspoređi učinkovitost inovativnog okolišu prihvatljivijeg SZB, zeolita u kombinaciji s bakterijom *Pseudomonas* sp. DLS65 i bakra u kontroli patule.

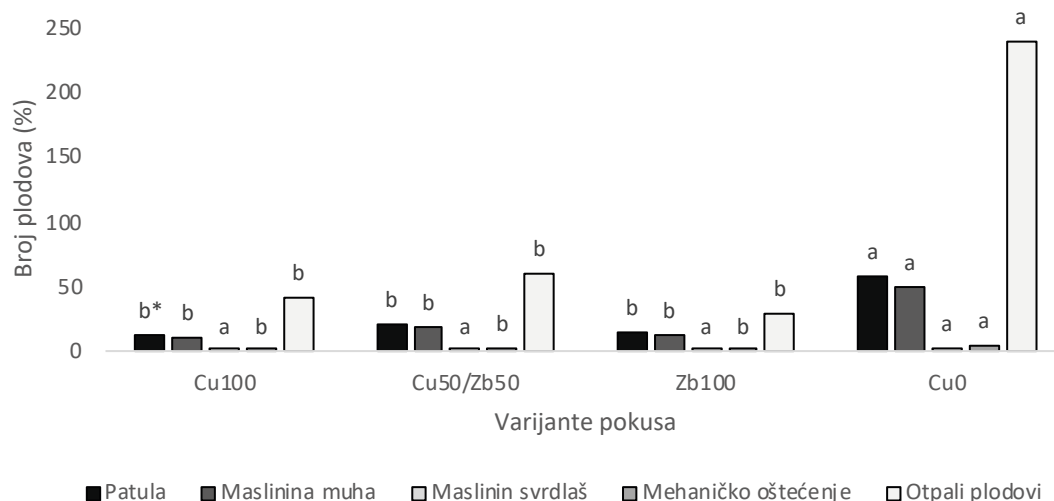
Materijali i metode

Istraživanje je provedeno u masliniku površine 0,8 ha (44.095018 N, 15.441517 E) u mjestu Škabrnja koje se nalazi na području Ravnih Kotara, Zadarska županija. Prema Köppenovoj klasifikaciji klima na području Ravnih Kotara zastupljena je umjereno topla vlažna klima s vrućim ljetom (Šegota i Filipčić, 2003). Prema podacima najbliže meteorološke postaje Pinova™, prosječna temperatura tijekom ljeta (lipanj-kolovoz) 2023., bila je 24,5 °C, dok je prosječna količina padalina za isto razdoblje iznosila 66,7 mm (Pinova-meteo, 2023). Mjesečna količina oborina tijekom ljetnih mjeseci posebice u lipnju i kolovozu bila je znatno veća nego prethodnih godina (DHMZ, 2023).

Prosječna starost nasada je 17 godina, a međusadni razmak stabala je 6x6 m. Uzgojni oblik maslina je slobodna vaza s 3 do 4 osnovne grane. Maslinik je u integriranom sustavu uzgoja. Pokus je postavljen po shemi slučajnog bloknoeg rasporeda. Pokusna površina maslinika podijeljena je u četiri ponavljanja, u kojima je bila zastupljena svaka od četiri varijante: 1. Cu100: ukupno 0,3 ha s tretmanom uobičajene doze bakra (4 kg/ha), 2. Cu50/Zb50: ukupno 0,3 ha s tretmanom u kombinaciji bakra i Zeo-Biopesticida u odnosu 50:50 (2 kg/ha bakar i 3 kg/ha zeolit), 3. Zb100: ukupno 0,1 ha tretmanom pune doze zeolita s bakterijom (6 kg/ha), te 4. Cu0: ukupno 0,1 ha, bez primjene tretmana zaštite. Tretiranje je obavljeno nošenim rasprskivačem AGP 100 - 440 dana 17. travnja 2023. u svrhu zaštite od paunovog oka (*Spilocaea oleaginea* Cast.). Plodovi masline sorte Oblica ocjenjeni su na pojavnosti patule u fenofazi razvoja plodova (BBCH skala 79, veličina ploda oko 90% konačne veličine; plod pogodan za branje zelenih maslina). Nasumičnim odabirom ubrano je 100 plodova sa svake osnovne parcelice, ukupno 1.600 plodova. Vizualnim pregledom svakog ploda određen je broj plodova zaraženih patulom. Također određen je mogući uzrok zaraze, ubodi: *B. oleae*, maslininog svrdlaša (*Rhynchites cribripennis*, Desbr) ili drugo mehaničko oštećenje. Isto tako procijenjen je broj otpalih plodova zbog napada patulom. Dobiveni podatci obrađeni su statistički programom ARM7®, (Grylling data management (2005.), analizom varijance (AOV) među podacima očitanih s varijanti pokusa. Srednje vrijednosti podataka rangirane su Student-Newman-Keuls testom rangova.

Rezultati i rasprava

Prikazane vrijednosti u Grafikonu 1. su zaraženi plodovi masline patulom ovisno o uzročniku koji je prethodio pojavi bolesti, (maslinina muha, maslinin svrdlaš ili mehaničko oštećenje), te prosječni broj plodova otpalih sa stabla na pojedinoj varijanti.



Grafikon 1. Zaraženi plodovi masline patulom ovisno o uzročniku koji je prethodio pojavi bolesti
*Vrijednosti koje slijede isto slovo ili simbol ne razlikuju se značajno ($P=0.05$, Student-Newman-Keuls).

Analiza je pokazala da postoje statistički značajne razlike među varijantama u usporedbi s kontrolom (Cu0) obzirom na uzročnika koji je prethodio pojavi patule, osim u slučaju svrdlaša. U Tablici 1. prikazan je postotni udio plodova zaraženih patulom te prosječni broj otpalih plodova po varijanti.

Tablica 1. Postotak zaraženih plodova masline patulom od različitih uzročnika po varijantama pokusa te prosječni broj otpalih plodova po varijanti, Škabrnja, Zadarska županija, 2023.

Varijanta	Patula	Maslinina muha	Maslinin svrdlaš	Mehaničko oštećenje	Otpali plodovi
Cu100	11,8b*	10,3b	0,5a	1b	41,3b
Cu50/Zb50	21,8b	18,5b	0,8a	2,5b	60b
Zb100	15,5b	12,8b	1,3a	1,5b	30b
Cu0	57a	50,5a	1,8a	4,8a	237,5a

*Vrijednosti koje slijede isto slovo ili simbol ne razlikuju se značajno ($P=0.05$, Student-Newman-Keuls).

U kontrolnoj varijanti (Cu0) statistička analiza je pokazala da je 57% plodova zaraženo patulom, dok je u varijanti Cu100: 11,8%, u varijanti Cu50/Zb50 21,8% i u varijanti Zb100 15,5% plodova zaraženo patulom. Iz rezultata je također vidljivo da je na kontrolnoj varijanti kod gotovo 50,5% plodova do infekcije patulom došlo uslijed uboda maslinine muhe, dok je kod ostalih varijanti taj broj znatno niži, Cu100 10,3%, Cu50/Zb50 18,5% i Zb100 12,8%. Nema značajnih razlika među varijantama kada je u pitanju pojava patule uzrokovana ubodom maslininog svrdlaša, stoga je on najrjeđi uzročnik zaraze. Prosječan broj otpalih plodova zaraženih patulom po varijantama pokusa znatno se razlikuje u usporedbi s kontrolnom (Cu0) koji iznosi 237,5.

Natprosječna količina oborina tijekom ljetnih mjeseci (DHMZ, 2023), a samim time i visoka razina relativne vlažnosti zraka 2023. godine pogodovali su infekciji plodova maslina te rastu i razvoju patule. Pregledom dostupne literature nisu nađena istraživanja o patuli pa tako ni podaci o utjecaju primjene bakra na pojavnost patule iako je bolest poznata već dugi niz godina u svim maslinarskim regijama. Međutim istraživanja Nigro i sur. (2018. i 2019.) navode da je kod antraknoze plodova maslina (*Colletotrichum* spp.), bolesti koja ima sličnu simptomatologiju kao patula, ključna primjena sistemskih fungicida u suzbijanju pojave bolesti. Zaključuju da je primjena sistemskog fungicida prije cvatnje ključna za smanjenje učestalosti antraknoze. Primjena mješavine tebukonazola + trifloksistrobina i mankozeba u fazi prije cvatnje, zatim bakrenog oksiklorida kod povećanja koštunice (rujan) pokazala se najboljom. Isti autori utvrđuju značajno smanjenje postotka latentne infekcije antraknozom, u usporedbi s netretiranom kontrolom i drugih varijanti, što ukazuje da je integrirani pristup korištenjem konvencionalnih fungicida i okolišu prihvatljivijih SZB učinkovit. Ovim preliminarnim istraživanjem pojavnosti patule u varijantama sa smanjenom uporabom bakra samostalno ili u kombinaciji sa zeolitom u usporedbi s kontrolnom, nisu utvrđene razlike u pojavnosti. Pojavnost patule nije jednaka svake godine, ona je posljedica prekomjernih padalina tijekom ljetnih mjeseci te se tada može očekivati u većem obimu. Iako je najučinkovitiji oblik suzbijanja patule kontrola populacije maslinine muhe nova rješenja u suzbijanju bolesti su uvijek nužna, osobito u godinama kada vremenski uvjeti pogoduju razvoju patogena.

Zaključak

Pojavnost patule nije značajno veća na varijantama gdje je korišten zeolit u usporedbi s varijantama gdje je korišten zeolit u kombinaciji s bakrom ili bakar samostalno, stoga možemo zaključiti da korištenje zeolita ili kombinacija smanjene koncentracije bakra (za 50%) i zeolita (za 50%) mogu imati jednaku učinkovitost u suzbijanju patule kao i sam bakar. Dakle, moguće je bakar zamijeniti s okolišu prihvatljivijim SZB što smanjuje i pojavu patule u godini s povišenim količinama oborina.

Napomena

Rad je izrađen u sklopu projekta LIFE Microfighter (101074218-LIFE21-ENV-IT-LIFE MICROFIGHTER). Zahvala kolegi Marku Zorici za pomoć pri postavljanju pokusa i očitavanju zaraze.

Literatura

- Boskou D., Blekas G., Tsimidou M. (1996). History and characteristics of the olive tree. *Olive Oil Chemistry and Technology*.
- Cvjetković B. (2010). Mikoze i pseudomikoze voćaka i vinove loze s opširnim prikazom zaštite 329-330 Čakovec: Zrinski.
- Državni hidrometeorološki zavod (DHMZ) (2023). Oborina i trajanje sijanja sunca, Raspoloživo: <https://meteo.hr/index.php> (pristupljeno 20.10.2023.)
- Gyrling data management (2005). Inc. ARM software revision 7.2.2. September 12, 2005. Brookings, south Dakota, USA. <https://www.gdmdata.com/> (pristupljeno: 9. 10. 2023.)
- Havranek J., Tudor Kalit M., Bažok R., Đugum J., Grbeša D., Hadžiosmanović M., Ivanković A., Jakopović I., Orešković S., Rupić V., Samaržija D. (2014). Sigurnost hrane od polja do stola. Zagreb: M.E.P.
- Nigro F., Antelmi I., Sion V., Pacifico A. (2019). Integrated approaches to control fungi affecting the canopy of olive trees. *IOBC-WPRS Bulletin*. 141: 14-18.
- Nigro F., Antelmi I., Sion V. (2018). Integrated control of aerial fungal diseases of olive. *Acta Hortic*. 1199, 327-332.
- Petrović E., i Godena S. (2023). Mogućnosti primjene *Trichoderma* vrsta u suzbijanju gljivičnih bolesti masline (*Olea europaea* L.). *Glasnik Zaštite Bilja*. 46: 32-38.
- Ryan D., and Robards K. (1998). Critical Review. Phenolic compounds in olives. *Analyst*. 123: 31R-44R.
- Souto A.L., Sylvestre M., Tölke E.D., Tavares J.F., Barbosa-Filho J.M., Cebrián-Torrejón G. (2021). Plant-Derived Pesticides as an Alternative to Pest Management and Sustainable Agricultural Production: Prospects, Applications and Challenges. *Molecules*. 26: 4835.
- Šegota T., and Filipčić A. (2003). Köppen's classification of climates and the problem of corresponding Croatian terminology. *Geoadria*. 8: 17-37.

Occurrence of drupe rot of olive (*Camarosporium Dalmaticum Thüm*) under conditions of reduced copper application in olive groves of Zadar County

Abstract

The aim of this study is to determine the influence of copper alone or in combination with zeolite in different doses on the occurrence of drupe rot of olive in olive groves in Zadar County. The field experiment was carried out as part of the LIFE Microfighter project, during 2023, in an olive grove of 0.8 ha in the village of Škabrnja. The evaluation of the appearance of drupe rot of olive was carried out on four variants with four repetitions on the Oblica variety. The initiators of the infection were determined: the sting of olive flies, olive fruit curculio or mechanical damage, and the number of fallen fruits caused by drupe rot of olive was estimated. The data were processed with the statistical program ARM7©, analysis of variance (AOV). Data means were ranked by the Student-Newman-Keuls rank test. Considering the causative agent that preceded the appearance of drupe rot of olive, except in the case of the olive fruit curculio, the analysis showed that there were statistically significant differences between the variants compared to the control (Cu0).

Keywords: Škabrnja, Oblica olive, incidence of disease, IPM, zeolite

Utjecaj različitih načina upravljanja vinogradom na populaciju američkog cvrčka (*Scaphoideus titanus*, Ball)

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Sažetak

Cilj rada bio je utvrditi populaciju američkog cvrčka u vinogradima s različitim načinom upravljanja uz pomoć žutih ljepljivih ploča tijekom tri godine. Ploče su postavljene u vinograde: redovita primjena insekticida, zapušteni vinograd, matični nasad podloga. Analizom ploča utvrđena je dominantno najveća brojnost odraslih jedinki u matičnom nasadu podloga (57/dan), dok je u zapuštenom vinogradu zabilježeno prosječno 15 odraslih po danu, međutim uočena je i velika bioraznolikost. Utvrđena je djelotvornost ciljanih kemijskih tretmana gdje je broj odraslih u vinogradima bio ispod 2/danu. Konstantnim praćenjem vektora i primjenom usmjerenih tretmana brojnost se može svesti na minimum.

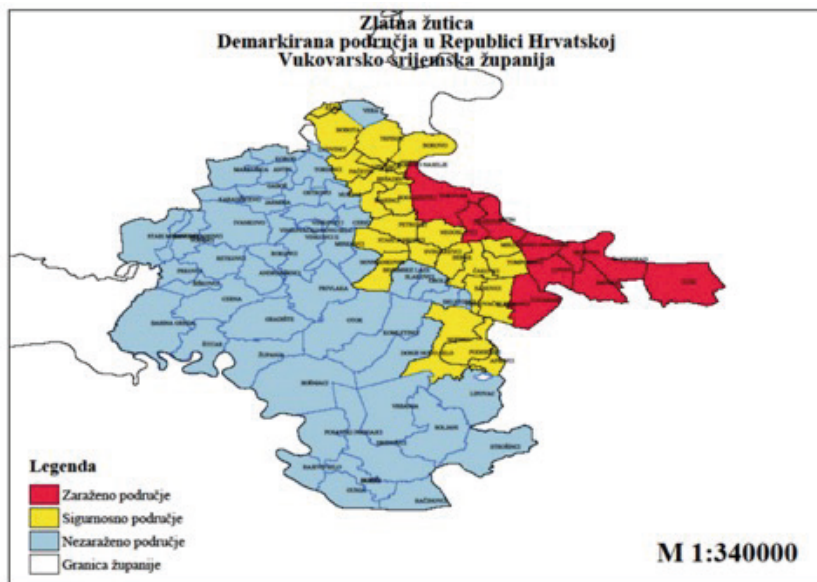
Ključne riječi: američki cvrčak, fitoplazma, zlatna žutica vinove loze, žute ljepljive ploče, vinograd

Uvod

Zlatna žutica vinove loze (Flavescence dorée) je karantenska bolest koja uzrokuje gubitke u prinosu i kvaliteti grožđa. Uzročnik bolesti je fitoplazma '*Candidatus Phytoplasma vitis*', a prenosi ju američki cvrčak (*Scaphoideus titanus* Ball) (Chuche i Thiery, 2014). Američki cvrčak pripada porodici Cicadellidae, potporodici Deltocephalinae. Široko je rasprostranjen u području srednje i južne Europe, međutim potrebno je naglasiti da je područje njegove rasprostranjenosti znatno veće u odnosu na rasprostranjenost zlatne žutice vinove loze (EFSA, 2020). U Hrvatskoj je bolest potvrđena u svim kontinentalnim županijama dok je na obalnom području potvrđena samo u istarskoj županiji (NN 58/2023). Trenutno se zlatna žutica vinove loze smatra najopasnijom bolesti vinograda u Europi brzo se širi i ubrzo poprima razmjere epidemije te pričinjava velike štete - gubitke u prinosu i trajne gubitke zaraženih trsova (Budinić i sur., 2021). Zbog njenog značaja donesen je niz zakonskih odredbi kojima se bolest pokušava staviti pod kontrolu. Zbog iznimne važnosti vektora i bolesti postupanje sa štetnim organizmom na području Europske unije regulirano je Provedbenom Komisije (EU) 2019/2072. U Republici Hrvatskoj, Ministarstvo poljoprivrede donijelo je „Naredbu o poduzimanju mjera za sprječavanje širenja i suzbijanje zlatne žutice vinove loze“ kojom su propisane mjere sprječavanja širenja, suzbijanja i iskorjenjivanja štetnog organizma kao i postupci u slučaju nalaza. Glavni način sprečavanja širenja bolesti je kontrola vektora za što je potrebno poznavati njegovu biologiju te redovito obavljati vizualne preglede biljaka radi određivanja početka tretiranja ličinki (Sarajlić i sur., 2021). Za praćenje populacije odraslog stadija američkog cvrčka najčešće se koriste žute ploče, iako nisu selektivne, zahtijevaju najmanje vremena za pregled (Chuche i Thiery, 2014). Akcijskim planom za sprječavanje širenja zlatne žutice vinove loze (2023.-2025.) regulirana je provedba i praćenje svih nužnih mjera kod sprječavanja širenja bolesti kod različitih načina gospodarenja vinogradima. Svake godine Ministarstvo poljoprivrede donosi Odluku o određivanju demarkiranih područja u kojima se provode mjere za sprječavanje širenja i suzbijanje zlatne žutice vinove loze (NN 58/23). Svi oni koji pripadaju demarkiranim područjima imaju obvezu tretiranja ličinki dva puta tijekom vegetacije, te prema potrebi i primjenu trećeg tretiranja usmjerenog protiv odraslih ako se utvrdi prisutnost odraslih stadija vektora dok su rasadnici i vinogradi s matičnim podlogama dužni obvezno provesti tri tretiranja godišnje protiv ovog štetnika (NN 63/2019).

Materijal i metode

Istraživanja su provedena tijekom tri godine (2021.-2023.). U istraživanju je praćena populacija odraslog stadija američkog cvrčka od srpnja do rujna svake godine. U istraživanje su uključeni vinogradi s različitim načinom gospodarenja: obrađivani vinograd, neobrađivani (zapušteni vinograd) i vinograd s matičnim nasadom podloga (poluzapušteni vinograd). Istraživanje je provedeno na području grada Iloka - vinogorje Srijem. Istraživani vinogradi nalaze se u demarkiranom području Vukovarsko-srijemske županije gdje je utvrđena prisutnost zlatne žutice vinove loze (Slika 1).



Slika 1. Karta demarkiranog područja u Vukovarsko-srijemskoj županiji u 2023. godini (Izvor: NN 58/2023)

Tijekom vegetacije vizualno su pregledavane biljke svake godine kako bi se utvrdio početak tretiranja usmjeren na ličinke američkog cvrčka. U obrađivanom vinogradu je primjenjena zaštita protiv ličinki i odraslih u skladu s preporukama za demarkirana područja (2 tretmana protiv ličinki + 1 tretman protiv odraslih) jedino je 2021. godine izostao treći tretman usmjeren protiv odraslih. U svaki vinograd je postavljeno pet žutih ploča, četiri ploče na svakom kutu vinograda i jedna u sredini. Pri postavljanju žutih ploča pazilo se na utjecaj rubnog reda te su žute ljepljive ploče postavljene nekoliko redova unutar vinograda. Ploče su zamjenjene svakih 14 dana. Analiza žutih ploča obavljena je pod Stereo zoom mikroskopom (Olympus SZ 51) u Laboratoriju za entomologiju, Fakulteta agrobiotehničkih znanosti Osijek. Rezultati su obrađeni u statističkom programu Statistica, napravljena je analiza varijance uz primjenu LSD testa značajnosti (TIBCO Software Inc., 2020).

Rezultati i rasprava

Najveći prosječan broj odraslog stadija američkog cvrčka po danu u 2021. godini zabilježen je u matičnom nasadu podloga koji se statistički značajno razlikovao od broja ulovljenih cvrčaka na žutim ljepljivim pločama u obrađivanom i zapuštenom vinogradu (Grafikon 1). Tijekom istraživanja u svim godinama je zabilježena visoka preferencija američkog cvrčka prema matičnoj podlozi (Kober 5BB) koja je visoko zastupljena u kontinentalnom dijelu države u odnosu na ostala dva vinograda. Najveća brojnost cvrčaka po danu u matičnom nasadu podloga (144) i obrađivanom vinogradu (22) zabilježena je u prvoj polovini kolovoza, dok je u zapuštenom vinogradu (39) najveća brojnost bila u drugoj polovini srpnja.

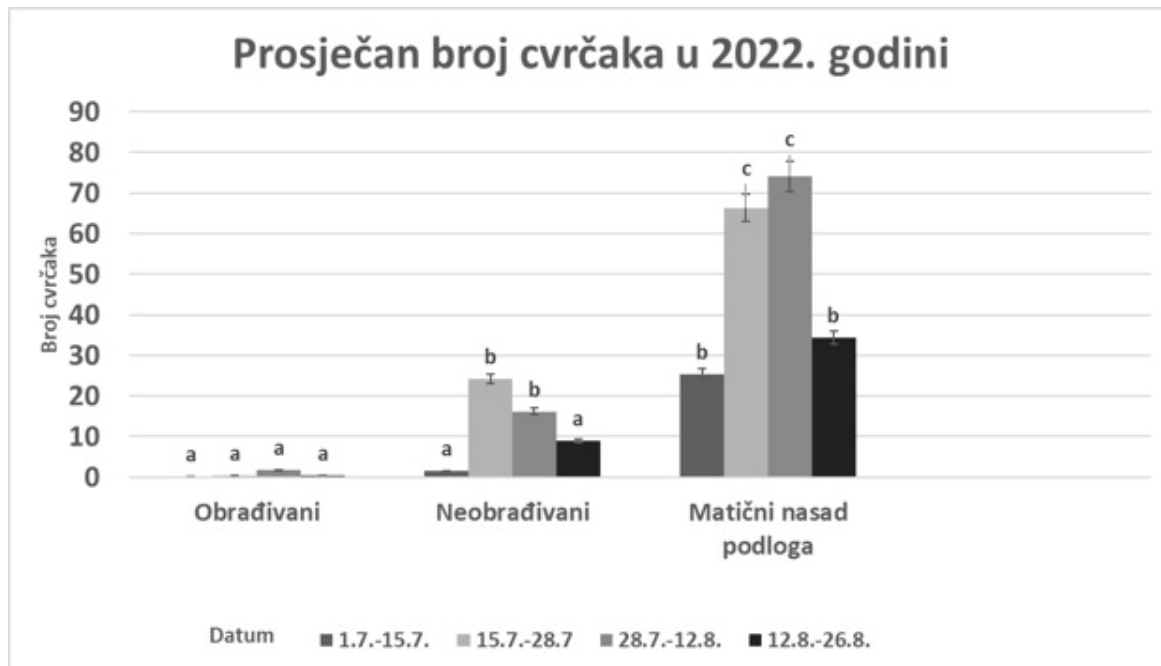
Budući da je 2021. godine izostalo tretiranje protiv odraslih stadija američkog cvrčka brojnost odraslih je bila izuzetno velika u kolovozu te je u prosjeku iznosila 22 cvrčka po danu dok je s redovnim tretmanima usmjerenim protiv ličinki i odraslih ostale dvije godine brojnost odraslih svedena na minimum. Ako usporedimo obrađivani vinograd s ostala dva vinograda gdje zaštita nije provedena ili je bila minimalna tijekom čitavog razdoblja istraživanja možemo utvrditi da su kemijski tretmani bili izuzetno učinkoviti protiv ličinki i odraslih stadija američkog cvrčka. Problem

za proizvođače predstavlja ukidanje određenih aktivnih tvari kemijskog podrijetla čime se smanjuje izbor zaštitnih sredstava i javljaju se ozbiljni problemi s upotrebom manje učinkovitih aktivnih tvari. Konstantno se pokušavaju pronaći nova rješenja zaštite od cvrčka, koja su ekološki prihvatljivija. U istraživanju Prazaru i sur. (2023.) ispitivana je učinkovitost kemijskih insekticida u odnosu na prirodne te je utvrđena slična učinkovitost (97% i 90%), međutim problem se javlja kod rezidualne učinkovitosti koja je izuzetno niska u poljskim uvjetima kod insekticida prirodnog porijekla, pretpostavlja se zbog visokih temperatura.



Grafikon 1. Prosječan broj odraslih cvrčaka po danu u vinogradu u 2021., lokacija

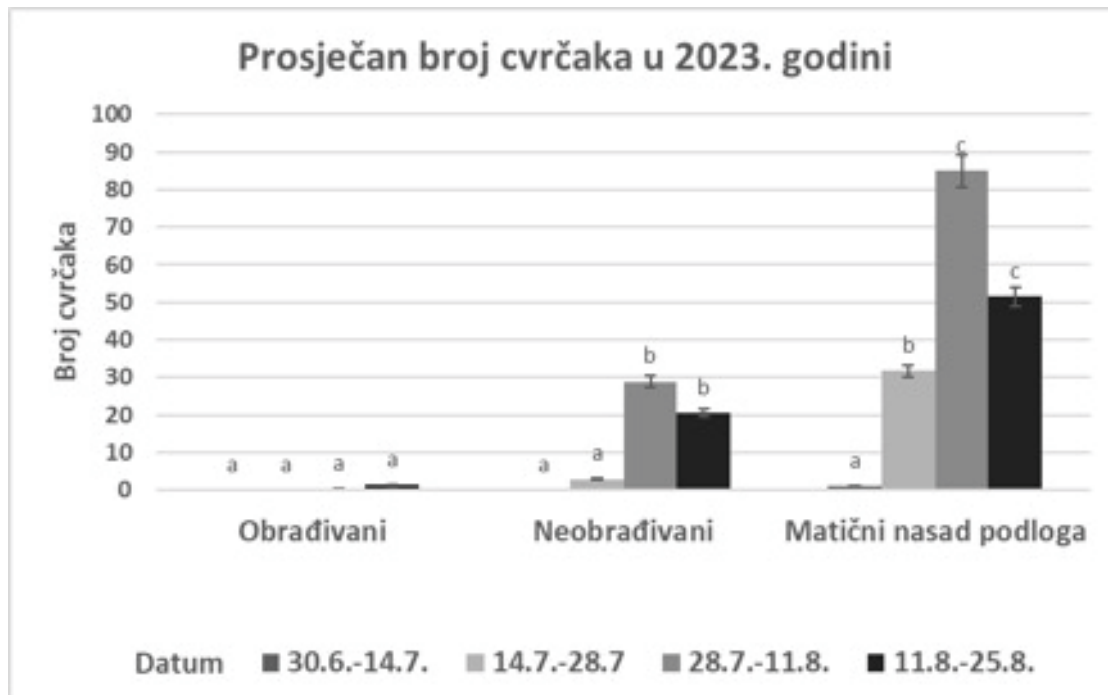
Tijekom 2022. godine također je zabilježena najveća brojnost odraslih stadija američkog cvrčka u matičnom nasadu podloga, međutim brojnost je bila skoro za 50% manja u odnosu na 2021. godinu uzimajući u obzir razdoblje vrhunca leta od polovine srpnja do polovine kolovoza (Grafikon 2). U obrađivanom vinogradu utvrđena je niska populacija odraslih stadija američkog cvrčka koja je bila i statistički značajna u odnosu na ostale vinograde u razdoblju vrhunca leta. Brojnost cvrčaka u neobrađivanom vinogradu bila je manja za preko 60% u odnosu na matični nasad tijekom vrhunca leta. Kod zapuštenog vinograda primjećena je velika biološka raznolikost na žutim ljepljivim pločama dok je u matičnom nasadu podloga dominirala pojava američkog cvrčka na pločama. Prema Döring i sur. (2019) uočeno je jasno povećanje bioraznolikosti u organskom vinogradarstvu na različitim trofičkim razinama u odnosu na konvencionalne, međutim potrebna su dodatna istraživanja mogućih interakcije sustava: upravljanja s različitim sortama, tipovima tala, matičnim podlogama i sustavima navodnjavanja kako bi se odredili učinkovitiji sustavi upravljanja vinograda. U istraživanju Dries i sur. (2021) utvrđeno je da različit genotip vinove loze različito utječe na sastav bakterijskih zajednica u tlu, a prema istraživanju Lòpez-Fernàndez i sur. (2017) vektori imaju potencijal za prijenos cijelih zajednica endofitnih bakterija između biljaka koji su korisni simbionti koji biljci osiguravaju zaštitu i homeostatsku stabilnost. Bakterijske zajednice pronađene su u svim dijelovima biljke neovisno na kojem dijelu biljke se vektor hranio.



Grafikon 2. Prosječan broj odraslih cvrčaka po danu u vinogradu u 2022., lokacija godini

U 2023. godini brojnost odraslih cvrčaka bila je manja tijekom srpnja u odnosu na 2022. godinu u svim ispitivanim vinogradima, međutim u zapuštenom vinogradu i matičnom nasadu podloga, utvrđena je veća brojnost odraslih tijekom kolovoza u odnosu na navedenu godinu te je bila i statistički značajna u odnosu na obrađivani vinograd (Grafikon 3). Brojnost odraslih se također statistički značajno razlikovala u kolovozu između neobrađivanog vinograda (29 i 20) i matičnog nasada podloga (85 i 52) kao i u drugoj polovini srpnja. Populacija američkog cvrčka u našem istraživanju varirala je iz godine u godinu. Utjecaj vremenskih uvjeta zauzima značajnu ulogu u razvoju kukaca. Danas postoje modeli koji na osnovi klimatskih podataka uspješno predviđaju pojavu američkog cvrčka. U istraživanju Falzoi i sur. (2014.) ispitivan je utjecaj različitih temperatura na embrionalni i postembrionalni razvoj američkog cvrčka gdje je utvrđeno da su više temperature u negativnoj korelaciji u embrionalnom razvoju dok su u pozitivnoj korelaciji s postembrionalnim razvojem.

Također su provedena i druga istraživanja utjecaja temperature i relativne vlage na ponašanje i razvoj američkog cvrčka gdje je utvrđeno da je američki cvrčak aktivniji od 18:00 do 08:00 sati, dok je skoro neaktivan tijekom perioda visokog intenziteta svjetlosti. Sezonski vrhunac leta događa se svake godine u različitim razdobljima, ovisno o srednjim vrijednostima temperature što potvrđuje i naše istraživanje. Dnevna povećanja ulova su u korelaciji s dnevnim minimalnim i maksimalnim temperaturama te su u negativnoj korelaciji s maksimalnim vrijednostima relativne vlažnosti. Kao rezultat toga ova vrsta se može okarakterizirati kao noćna ili krepuskularna, a vjerojatna je i bimodalna periodičnost leta (Lessio i Alma, 2004).



Grafikon 3. Prosječan broj odraslih cvrčaka po danu u vinogradu u 2023., lokacija godini

Tijekom 2023. godine zabilježena je izuzetno visoka populacija odraslih stadija u drugoj polovini kolovoza kod zapuštenog vinograda (20/dan) i matičnog nasada podloga (51/dan). Povećanje broja odraslih u navedenom razdoblju zabilježeno je i kod obrađivanog vinograda (1,36/dan) što ukazuje na dodatni problem jer izostaju kemijski tretmani. U istraživanju Bocca i sur. (2020.) utvrđen je dug životni vijek mužjaka (>40 dana) i ženki (>70 dana) te je posljedično s tim produljeno i razdoblje inokulacije odraslih i vremensko proširenje rizika od zaraze vinograda koji više nisu zaštićeni insekticidnim tretmanima. U navedenom istraživanju utvrđeno je polaganje jaja do kraja listopada iako je stopa polaganja jaja konstantna do rujna, a kasnije se smanjuje.

Zaključak

Američki cvrčak kao vektor fitoplazme koja uzrokuje zlatnu žuticu vinove loze ima ključnu ulogu u pristupu zaštite vinograda. Populacija vektora varira iz godine u godinu, a vrhunac populacije uglavnom se javlja u prvoj polovini kolovoza, međutim odstupanja se javljaju ovisno o načinu upravljanja vinogradom kao i vegetacijskoj sezoni. Preferencija američkog cvrčka prema matičnom nasadu podloga je visoko izražena i statistički značajna u odnosu na zapušteni i obrađivani vinograd. Od iznimne važnosti je primjena propisanih zaštitnih mjera protiv ličinki i odraslih kako bi se populacija vektora svela na minimum i spriječilo epidemijsko širenje bolesti.

Napomena

Istraživanja neophodna za ovaj rad dio su projekta hrvatsko-slovenskog bilateralnog projekta „Primjena biljnih ekstrakata u suzbijanju američkog cvrčka *Scaphoideus titanus* Ball“.

Literatura

- Bocca F.M., Picciau L., Alma A. (2020). New insights on *Scaphoideus titanus* biology and their implications for integrated pest management. *Entomologia Generalis*. 40: 337–349.
- Budinščak Ž., Ivančanin G., Plavec J., Križanac I. (2021). Američki cvrčak i zlatna žutica vinove loze. *Glasilo biljne zaštite*. 21: 387-392.
- Chuche J., and Thiéry D. (2014). Biology and ecology of the Flavescence dorée vector *Scaphoideus*

- titanus: a review. *Agronomy for Sustainable Development*. 34: 381–403.
- Döring J., Collins C., Frisch M., Kauer R. (2019). Organic and Biodynamic Viticulture Affect Biodiversity and Properties of Vine and Wine: A Systematic Quantitative Review. *American Journal of Enology and Viticulture*. 70: 221-242.
- Dries L., Bussotti S., Pozzi C., Kunz R., Schnell S., Löhnertz O., Vortkamp A. (2021). Rootstocks Shape Their Microbiome—Bacterial Communities in the Rhizosphere of Different Grapevine Rootstocks. *Microorganisms*. 9: 822.
- EFSA (European Food Safety Authority), Tramontini S, Delbianco A and Vos S, 2020. Pest survey card on flavescence dorée phytoplasma and its vector *Scaphoideus titanus*. EFSA supporting publication 2020:EN-1909. 36 pp.
- Falzo S., Lessio F., Spanna F., Alma A. (2014). Influence of temperature on the embryonic and post-embryonic development of *Scaphoideus titanus* (Hemiptera: Cicadellidae), vector of grapevine Flavescence dorée. *International Journal of Pest Management*. 60: 246-257.
- Lessio F., and Alma A. (2004). Seasonal and Daily Movement of *Scaphoideus titanus* Ball (Homoptera: Cicadellidae). *Environmental Entomology*. 33: 1689–1694.
- López-Fernández S., Mazzoni V., Pedrazzoli F., Pertot I., Campisano A. (2017). A Phloem-Feeding Insect Transfers Bacterial Endophytic Communities between Grapevine Plants. *Frontiers in Microbiology, Section Plant Pathogen Interactions*. 8: 834.
- NN 58/2023 Odluka o određivanju demarkiranih područja u kojima se provode mjere za sprječavanje širenja i suzbijanje zlatne žutice vinove loze.
- Prazaru S.C., D’Ambrogio L., Dal Cero M., Rasera M., Cenedese G., Guerrieri E., Pavasini M., Mori N., Pavan F., Duso C. (2023). Efficacy of Conventional and Organic Insecticides against *Scaphoideus titanus*: Field and Semi-Field Trials. *Insects*. 14: 101.
- Sarajlić A., Raspudić E., Majić I., Kujundžić T., Drenjančević M. (2021). Koliko znamo o američkom cvrčku? *Glasnik Zaštite Bilja*. 44: 93-99.
- Službeni list Europske unije (2019). Provedbena uredba Komisije (EU) 2019/2072 od 28. studenoga 2019. o utvrđivanju jedinstvenih uvjeta za provedbu Uredbe (EU) 2016/2031 Europskog parlamenta i Vijeća u pogledu zaštitnih mjera protiv organizama štetnih za bilje te o stavljanju izvan snage Uredbe Komisije (EZ) br. 690/2008 i izmjeni Provedbene uredbe Komisije (EU) 2018/2019. *Službeni list Europske unije*, 319, 276.
- TIBCO Software Inc. (2020). *Data Science Workbench*, version 14. <http://tibco.com>

Effects of different management regimes on the population of the American grapevine leafhopper (*Scaphoideus titanus*, Ball)

Abstract

The aim was to determine the population of the American grapevine leafhopper in vineyards with different management regimes with yellow sticky traps during the three years. The highest values were recorded in the rootstock of American *Vitis* sp., as confirmed by the analysis of yellow sticky traps (57 adults/day). The uncultivated vineyard had in average 15 adults per day, but also showed great biodiversity. The effectiveness of targeted chemical treatments was determined and the number of adults in this vineyard was below two. To keep the number of vectors to a minimum, it is important to monitor them constantly and apply targeted treatments against larvae and adults.

Keywords: american grapevine leafhopper, phytoplasma, *Flavescens doree*, yellow sticky traps, vineyard

Influence of Cannabidiol (CBD) biopolymer microparticles on *Botrytis cinerea*

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Abstract

Strawberry gray mold (*B. cinerea*) is a significant threat to strawberries, leading to serious crop reduction, especially in conditions of high humidity and heavy rains. The research goal is to determine the inhibitory effect of Cannabidiol (CBD) microparticles on the growth and development of *B. cinerea*. This research confirms the fungistatic and potentially fungicidal activity of CBD microparticles on *B. cinerea*. The control shows a high 86% of the mycelial growth area, whereas treatment with 1%, 2% or 3% CBD microparticles reduced the mycelial growth area about five-fold, 3.5-fold and six fold respectively. We conclude that Cannabidiol (CBD) biopolymer microparticles can be used as an ecological antifungal agent.

Keywords: CBD, biopolymer micro-particles, *Botrytis cinerea*, strawberry protection, fungistatic.

Introduction

Strawberries are a popular fruit all over the world all year round. They are used in fresh and processed products such as jams, ice cream, compote, etc. Strawberries are rich in antioxidants, vitamin C and fiber, which makes them a healthy food choice. The main center of strawberry cultivation in Croatia is the area around the capital Zagreb, the so-called “Zagreb ring”, and strawberries from the area of Vrgorac are also known. (Maretić and Duralija, 2014). Among the technological systems of strawberry cultivation, in terms of commercial production, the following systems of cultivation of monoecious cultivars are of greater importance: cultivation in the open with the application of planting beds with black PVC foil - cultivation in protected areas of PVC tunnels, greenhouses and greenhouses, cultivation in hydroponic greenhouses (Puljko, 2005).

The phytopathogenic fungus *B. cinerea*, known as the causative agent of gray mold, causes rot in a variety of plants, including strawberries, grapevines, raspberries, tobacco, soybeans, and others (Yunis and Elad, 1989). *B. cinerea* can survive as a saprophyte on plant residues and organic matter in the soil. Therefore, the inoculum (a potential source of infection) is always present in the crop, and the process of its formation, release and spread is continuous (Rotem et al., 1978). The phytopathogenic fungus *Botrytis cinerea*, the causative agent of strawberry gray mold disease, is considered the most dangerous pathogen of this fruit crop. It can reduce the strawberry yield to an unprecedented extent, especially if there are favorable conditions for the development of the fungus (frequent rains and high relative humidity). The total worldwide financial expenditure on gray mold control (through cultural measures, fungicides - botryticides, bio-control) easily exceeds the amount of 1 billion euros per year, but it is likely significantly higher (Dean et al., 2012). *B. cinerea* is a polyphagous and necrotrophic lifestyle, meaning it prefers to infect and grow on damaged or aged tissues, eventually causing tissue death. The inoculum (eg conidia) is very abundant and easily spread, and usually originates from infected plant tissue (Jarvis, 1962). *B. cinerea* mainly enters the host through wounds or natural openings (Holz et al., 2007). Infections of young and immature plant organs usually result in limited damage and latent infection (Dewey and Grant-Downton, 2016; Jarvis, 1962). Different types of dormancy have been described: (i) delay of conidia germination or growth arrest after germination (Jarvis, 1994), (ii) endophytic latent growth in the apoplast (Barnes and Shaw, 2003; Sowley et al., 2010), (iii) colonization falling off floral organs (eg petals) that continues with growth in ovaries or receptacles where growth stops (Bristow et al., 1986). Regardless of the type of infection, the pathogen generally enters a short asymptomatic, biotrophic phase at the beginning of the disease cycle (Veloso and van Kan, 2018). An aggressive necrotrophic phase usually follows a dormant or asymptomatic phase

after plant organs begin to senesce or mature, during which *B. cinerea* causes rapid decay of infected tissues (Elad et al., 2007). The most common route of plant infection with this pathogen is via conidia (Doehlemann et al., 2016). Pathogenic fungi and bacteria are becoming increasingly resistant to conventional preparations, and new strategies for pathogen control are sought to prevent human diseases, food contamination, or plant and animal diseases. This need includes the urgent identification of new active substances that achieve new modes of action and reduce pathogen resistance (Fisher et al., 2020). The industry of berry fruit protection products (strawberries) is today focused on the development of new and innovative formulations of plant protection and storage products. Karas et al. (2020), state the antimicrobial and antifungal properties of cannabinoids. *Cannabis sativa* L is known to have numerous active compounds representing different chemical substances. Some of them belong to primary metabolites, for example, amino acids, fatty acids and steroids, while cannabinoids, stilbenoids, flavonoids, lignans, terpenoids and alkaloids belong to secondary metabolites. Cannabinoids are usually a group of compounds with 21 carbons (C₂₁) and they are in the form of carboxylic acids. The best-known secondary metabolites of cannabis are cannabinoids (Ben Amer 2006). Classical cannabinoids are usually concentrated in a viscous resin produced in structures known as glandular trichomes. Cannabinoids are found in all parts of hemp organs, but the highest concentrations are found in the resinous exudate of flower tips (Yang et al., 2016). Research suggests that cannabinoids, such as CBD, have antimicrobial and antifungal properties that could be used to protect plants. The resistance of pathogenic fungi to traditional antifungal agents has stimulated the search for new alternative solutions and strategies. Numerous plant extracts and their pure compounds have been shown to be effective in controlling various pathogenic fungi. Cannabinoids and related compounds from the *Cannabis sativa* plant exhibit various attractive pharmacological and agronomic properties. Many of them have already been investigated in detail, while others, potentially useful as antifungal agents in agriculture, are still in the early stages of research. The application of new formulations of microparticles filled with cannabidiol (CBD) aims to increase the effect of berry fruit protection agents (strawberries) during growth and storage and their direct effect on *B. cinerea*. This work focuses on developing new formulations of biopolymeric microparticles filled with CBD to improve the protection of strawberries during growth and storage. A formulation of microparticles based on CBD with such capabilities would first of all be of great importance as a potential new product for the protection of strawberries during cultivation and storage. The encapsulation of CBD is intensively researched because it is necessary to create a formulation that will allow the antifungal activity of CBD to be maintained during storage, transport, and application. It is essential to choose the appropriate CBD carrier, and so far, with many studies, the alginate gel matrix, which was created by the ionic gelation process, has proven to be the best (Vincekovic et al. 2016). In our research, we created microparticles based on different concentrations of CBD and tested their antifungal effect on *B. cinerea*. According to our knowledge, this is a unique attempt to encapsulate CBD, and we also investigated the applicability of the formulation.

Materials and methods

Commercially available organic cannabis extract was used for the production of biopolymer microparticles. The producer is Zen CBD Farm. The oil is obtained by cold pressing and is produced in the Republic of Croatia. For the purpose of the research, an isolate of the pathogenic fungus *B. cinerea*, isolated from strawberry fruit, was used. First, molecular identification was performed using the conventional PCR method. After identification, the sample was sequenced to the species level at MacroGen Europe (Amsterdam, The Netherlands). The isolate of the pathogenic fungus *B. cinerea* that was identified and sequenced is kept in the collection of the Department of Phytopathology at the University of Zagreb, Faculty of Agriculture. The nutrient medium MEA (Malt extract agar) was previously prepared according to the manufacturer's instructions (Sigma–Aldrich, USA). The prepared biopolymer microparticles were sterilized with 70% technical alcohol. In the half-cooled nutrient medium, 6 grams of biopolymer microparticles were applied in each repetition at concentrations of 1%, 2% and 3% CBD. In the laminar (sterile conditions) nutrient medium with biopolymer particles were poured into a semiliquid state in Petri dishes, and circularly mixed for the purpose of even distribution among the dishes. Two types of biopolymer microparticles without CBD (ALG/Ca) and with CBD (ALG/(Ca + CBD)) were prepared by the ionic gelation procedure. The preparation of biopolymer microparticles is carried out under sterile conditions at room temperature. In order to prepare biopolymer microparticles filled with CBD, it is necessary to dissolve a certain amount of CBD (1,2, and 3 g) in 5 ml of 96% alcohol. The prepared solution is added to 95 ml of 1.5% sodium alginate solution, which creates a suspension that continues to be stirred for 30 minutes with gentle heating. The prepared suspensions are added with a syringe to a 2% solution of calcium chloride (CaCl₂). The biopolymer microparticles that were created almost

instantly contain CBD in a network of calcium alginate and are mixed on a magnetic stirrer (IKA topolino, USA) for about 30 min to further harden. They are then filtered through a single-layer muslin and washed 1x with 96% solution to sterilize them and 10x with sterilized distilled water to wash away excess CaCl_2 and ethanol (Figure 1.)

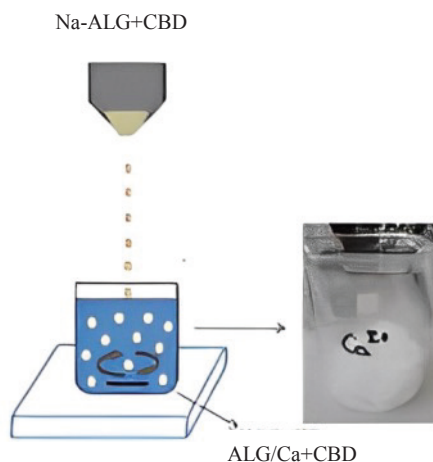


Figure 1. Schematic representation of the preparation of biopolymeric microparticles of calcium alginate filled with CBD

Testing of the antifungal activity of biopolymer microparticles filled with CBD was carried out using the adapted “poison food” method according to Martinko et al., (2022). Initially, the pathogen *B. cinerea* was inoculated onto a previously prepared MEA substrate using mycelial discs (\varnothing 5 mm) and incubated for 7 days at a temperature of 24°C in the dark.

Then, 6 grams of CBD-filled biopolymer microparticles of various concentrations were added to the dissolved and partially cooled MEA substrate. Microparticles were evenly added to sterile Petri dishes (\varnothing 9 cm) in a certain number of repetitions

These mycelial discs were placed in the center of Petri dishes containing MEA nutrient medium with 6 grams of CBD-filled biopolymer microparticles at different concentrations (test Petri dishes) and nutrient medium without microparticles (control Petri dishes). The mycelium was facing the substrate. Such prepared Petri dishes were incubated in a climatic chamber at a temperature of 24 °C in the dark. This experiment was set up in three treatments, each with 3 replicates and a control with 3 replicates (12 Petri dishes in total).

Formulations of microparticles prepared from different concentrations of CBD extract and a constant concentration of sodium alginate were used as treatments. The control sample did not contain microparticles. Conidia of *B. cinerea* are exposed: 1. distilled water, 2. biopolymer microparticles containing CBD (1%), 3. biopolymer microparticles containing CBD (2%), 4. biopolymer microparticles containing CBD (3%). After 7 days of incubation, the research results were read. To achieve this, the test and control Petri dishes were photographed. The photographs were further processed using the computer program ImageJ (Schneider et al., 2012), using the methodology described in Martinko et al. (2022.). Determination of the concentration of the spore suspension, i.e. determination of the number of spores in the suspension, was performed using a hemocytometer. Statistics were done by the Excell program. Results are significant.

Results and discussion

The results of testing the antifungal effect of different concentrations of CBD in biopolymeric microparticles on the phytopathogenic fungus *B. cinerea* are shown in Table 1 and Figure 2. On the variant with a substrate containing 1% of the particles, the fungus was inhibited by 76.8% compared to the control. On the variant with a substrate containing 2% biopolymeric microparticles of CBD, the fungus was inhibited by 70.7% compared to the control. The variant with a substrate containing 3% biopolymer microparticles of CBD was inhibited by 83.1% compared to the control variant. The interactions between lower concentrations of CBD and the fungus *B. cinerea* are complex and

depend on several factors. Smaller concentrations of CBD can slow the growth of the fungus, but will not completely stop its reproduction. This means that the fungus could continue to reproduce but over a longer period of time. CBD can affect various biological processes in the fungus, including its reproduction and growth. Depending on the specific mechanisms of action of CBD and how the fungus responds to these changes, higher concentrations of CBD do not necessarily mean greater efficacy. How CBD affects the signaling pathways in the fungus *B. cinerea* has not yet been investigated.

Table 1. The result of the inhibition of the fungus. \bar{x} (cm²) shows the area of mycelial growth calculated in the ImageJ program. I (%) shows the percentage of fungal growth inhibition calculated in Excel.

Treatment	Control + <i>B. cinerea</i>		Test % CBD + <i>B. cinerea</i>	
	0 %	1 %	2 %	3 %
\bar{x} (cm ²)	161,2 ± 1,1 ^a	37,5 ± 3,2 ^b	47,3 ± 2,8 ^c	27,4 ± 2,1 ^d
I(%)	0	76,8	70,7	83,1

*different letters indicate a statistically significant difference between mean values within the variants (Tukey test, $P < 0.05$).; I – inhibition index; SD- standard deviation.

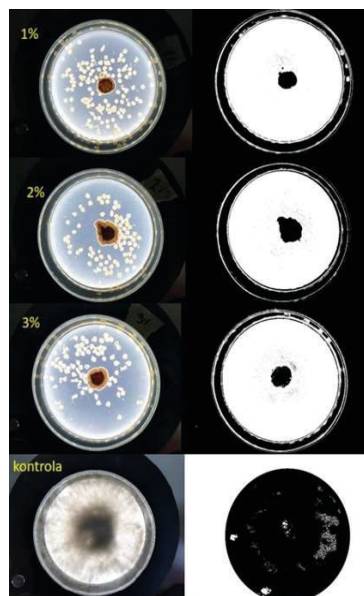


Figure 2. Presentation of the effect of biopolymeric microparticles filled with CBD on the mycelium of the phytopathogenic fungus *B. cinerea*. The surface covered with mycelium is shown in black. The control sample (control) is significantly different from the other treated samples. The 1% and 3% specimens show a lower percentage of surface area under mycelium than the specimen treated with 2% CBD microparticles.

The results of the suppression of spore development on the medium with different concentrations of CBD show a significant difference between the treated samples and the control (table 2). No sporulation was detected in the test specimen of *B. cinerea* with 1% CBD microparticles. Higher CBD concentrations of 2% showed 0.12×10^5 spores/mL of suspension, while a concentration of 3% showed 0.06×10^5 spores/mL of suspension. The control sample shows a significant no active spores in the value of 2.6×10^5 spores/mL suspension (Table 2).

Table 2. The result of the amount of spores in the suspension using a hemocytometer

Sample	Number of spores $\times 10^5$ / mL
Control	2,6
Test 1%	0
Test 2%	0,12
Test 3%	0,06

High concentrations of plant extracts can induce stress in the fungus *B. cinerea*, which can trigger its defense reaction. In response to stress, the fungus can accelerate its sporulation processes to ensure reproduction and survival. The concept of hormesis (Southam and Erlich, 1943) suggests that low doses of stress or toxic substances can induce positive biological responses, while high doses can be harmful. This can also be applied to *B. cinerea*. Lower concentrations of the extract may induce less stress and prevent sporulation, while higher concentrations may result in toxic stress that promotes sporulation. The fungus can develop adaptation mechanisms to survive in the presence of high concentrations of substances that should otherwise act as fungicides. This may include the development of resistance to substances or changes in biological processes that allow sporulation despite the presence of these substances. The results of this experiment are on track to confirm the antifungal effect of cannabidiol extract (CBD), but the inhibition of mycelial growth can be associated with various environmental factors. Petrić (2020) came to the conclusion that a higher concentration of essential oil does not necessarily mean a stronger antifungal effect, which can be related to a test sample of biopolymer microparticles filled with 1% CBD showing a higher inhibition than 2%. Spores were detected in the control sample using a hemocytometer, while in the test treatments with CBD, no significant sporulation occurred within the given time period of 7 days. The absence of sporulation confirms the fungistatic action of biopolymeric microparticles filled with CBD on the fungus *B. cinerea* isolated from strawberry fruits. In the research by Jelenić et al. (2020), the fungistatic effect of thyme oil was proven during a period of 96 hours on the fungus *B. cinerea*. It is assumed that biopolymer microparticles with CBD will have a prolonged effect, primarily due to the encapsulation process, which results in a slower release of the active substance. The assumption is in accordance with the results of De Angelis et al. (2022) who proved that encapsulated chemical fungicides have a longer and higher antifungal activity against the fungus *B. cinerea*. The results of this study are consistent with the results of Nachani et al. (2021) who observed that cannabinoids, such as cannabidiol (CBD), cannabigerol (CBG) and cannabichrome (CBC), can enhance the effects of caryophyllene oxide, an extremely potent antifungal agent. The lipophilic nature of hemp terpenes has the ability to disrupt the cell membrane, cause cell death, or inhibit the proliferation of certain pathogenic fungi (Appendino et al., 2008). According to Appendino et al. (2008), cannabinoids have shown strong activity against various strains of methicillin-resistant *Staphylococcus aureus* (MRSA) that are currently of clinical importance. Also, this research can be connected with experiments conducted on essential oils of different plant species. Essential oils are natural compounds obtained from plants, which are used in aromatherapy and cosmetics for their aromatic and therapeutic properties. It is known that essential oils containing certain terpenes have a fungistatic and fungicidal effect on pathogenic fungi of various genera (Grgić et al., 2016; Palfi, 2017).

Conclusion

The resistance of pathogenic fungi to conventional antifungal agents has stimulated the search for new alternative agents and strategies. Many plant extracts and their purified compounds have shown antifungal activity against a wide range of pathogenic fungi.

Cannabinoids and related compounds from *C. sativa* show many attractive pharmacological and agronomic properties. Many of them have been thoroughly investigated, while others, such as the potential of antifungal agents with targeted applications in agriculture, are still at a very early stage of research. The control sample shows a 0% inhibitory effect on the growth of the fungus. The test treatment with biopolymer microparticles in a percentage of 1% shows an inhibition of 76.8% compared to the control sample. Treatment with biopolymeric microparticles of CBD shows an inhibition of mushroom growth of 70.7% compared to the control, while treatment with biopolymeric microparticles of CBD shows an inhibition of 83.1% compared to the control specimen. No sporulation was detected in the test sample of *B. cinerea* with 1% CBD microparticles, while sporulation was detected in the other

concentrations, but significantly less than in the control sample. This study confirms the fungistatic and potentially fungicidal activity of CBD biopolymeric microparticles on *B. cinerea* isolated from infected strawberry fruit. New research on this topic is needed, including different concentrations of CBD-filled biopolymeric microparticles and *in vivo* methods

References

- Appendino G., Gibbons S., Giana A., Pagani A., Grassi G., Stavri M., Smith E., Rahman M.M. (2008). Antibacterial cannabinoids from *Cannabis sativa*: a structure-activity study. *Journal of natural products*. 71: 1427-30.
- Barnes S.E., and Shaw M.W. (2003). Infection of Commercial Hybrid Primula Seed by *Botrytis cinerea* and Latent Disease Spread Through the Plants. *Phytopathology*. 93: 573–578.
- Ben Amar M. (2006). Cannabinoids in medicine: A review of their therapeutic potential. *Journal of Ethnopharmacology*. 105: 1–25.
- Bristow P.R., McNicol R.J., Williamson B. (1986). Infection of strawberry flowers by *Botrytis cinerea* and its relevance to grey mould development. *Annals of Applied Biology*. 109: 545–554.
- De Angelis G., Simonetti G., Chronopoulou L., et al. (2022). A novel approach to control *Botrytis cinerea* fungal infections: uptake and biological activity of antifungals encapsulated in nanoparticle based vectors. *Scientific Reports*. 12: 7989.
- Dean R., van Kan J.A.L., Pretorius Z.A., et al. (2012). The top 10 fungal pathogens in molecular plant pathology. *Molecular plant pathology*. 13: 414–430.
- Dewey F.M., Grant-Downton R.T. (2016). *Botrytis*-Biology, Detection and Quantification.
- Doehlemann G., Ökmen B., Zhu W., Sharon A. (2017). Plant Pathogenic Fungi. *Microbiology spectrum*. 5: 5-1.
- Elad Y., Williamson B., Tudzynski P., Delen N. (Eds.). (2007). *Botrytis*: Biology, Pathology and Control. 412.
- Fisher M.C., Alastruey-Izquierdo A., Berman J., et al. (2022). Tackling the emerging threat of antifungal resistance to human health. *Nature reviews microbiology*. 20: 557–571.
- Grgić S., Ćosić J., Rebekić A. Vrandečić K. (2016). Utjecaj eteričnih ulja na porast micelija *Botrytis cinerea*. *Poljoprivreda*. 22: 29-33.
- Holz G., Coertze S., Williamson B. (2007). The Ecology of *Botrytis* on Plant Surfaces. *Botrytis: Biology, Pathology and Control*. 9–27.
- Karas J.A., Wong L.J.M., Paulin O.K.A., Mazeh A.C., Hussein M.H., Li J., Velkov T. (2020). The Antimicrobial Activity of Cannabinoids. *Antibiotics*. 9: 406.
- Jarwis W.R. (1962). The infection of strawberry and raspberry fruits by *Botrytis cinerea* Fr. *Annals of Applied Biology*. 50: 569–575.
- Jelenić J., Ilić J., Ćosić J., Vrandečić K. Velki M. (2020). Antifungalno djelovanje eteričnih ulja domicilnoga bilja hrvatske na uzročnika sive plijesni (*Botrytis cinerea*) s vinove loze. *Poljoprivreda*. 26: 58-64.
- Jarvis W.R. (1994). Latent infections in the pre-and postharvest environment. *Hort Science*. 29: 749-51.
- Martinko K., Ivanković S., Đermić E., Đermić D. (2022). In vitro antifungal effect of phenylboronic and boric acid on *Alternaria alternata*. *Arhiv za higijenu rada i toksikologiju*. 73: 83-87.
- Nachnani R., Raup-Konsavage W.M., Vrana K.E. (2021). The Pharmacological Case for Cannabigerol. *Journal of Pharmacology and Experimental Therapeutics*. 376: 204-212.
- Palfi M. (2017). Antifungalno djelovanje eteričnih ulja i njihovih komponenti na fitopatogene gljivice u in vitro uvjetima. / Ćosić, Jasenka ; Konjevoda, Paško (mentor); Osijek: Sveučilište Josipa Jurja Strossmayera u Osijeku, 2017. (doktorska disertacija).
- Petrić A., Ereš H., Vrandečić K. Ćosić J. (2021). Utjecaj eteričnih ulja na rast micelija *Globisporangium*

- ultimum* i *Globisporangium irregulare*. *Fragmenta phytomedica*. 35: 27-33.
- Puljko M. (2005). Suvremene tehnologije uzgoja jagoda. *Glasnik Zaštite Bilja*. 28: 5-8.
- Repajić M., Markov K., Frece J., Vujević P., Ćurić D., Levaj B. (2019). Studija kvalitete plodova više vrsta jagodastog voća tijekom skladištenja. *Glasnik Zaštite Bilja*. 42: 68-81.
- Rotem J., Cohen Y., Bashi E. (1978). Host and environmental influences on sporulation in vivo. *Annual Review of Phytopathology*. 16: 83-101.
- Schneider C., Rasband W., Eliceiri K. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*. 9: 671–675.
- Southam C.M., and Erlich J. (1943). Effects of extracts of western red-cedar heartwood on certain wood-decaying fungi in culture. *Phytopathology*. 33: 517–524.
- Sowley E.N.K., Dewey F.M., Shaw M.W. (2010). Persistent, symptomless, systemic, and seed-borne infection of lettuce by *Botrytis cinerea*. *European Journal of Plant Pathology*. 126: 61–71.
- Veloso J., and van Kan J.A.L. (2018). Many Shades of Grey in *Botrytis*-Host Plant Interactions. *Trends in plant science*. 23: 613–622.
- Vinceković M., Jalšenjak N., Topolovec-Pintarić S., Đermić E., Bujan M., Jurić S. (2016). Encapsulation of Biological and Chemical Agents for Plant Nutrition and Protection: Chitosan/Alginate Microcapsules Loaded with Copper Cations and *Trichoderma viride*. *Journal of agricultural and food chemistry*. 64: 8073–8083.
- Yang R., Berthold E.C., McCurdy C.R., da Silva Benevenuto S., Brym Z.T., Freeman J.H. (2020). Development of Cannabinoids in Flowers of Industrial Hemp (*Cannabis sativa* L.): A Pilot Study. *Journal of agricultural and food chemistry*. 68: 6058–6064.
- Yunis H., and Elad Y. (1989). Survival of dicarboximide-resistant strains of *Botrytis cinerea* in plant debris during summer in Israel. *Phytoparasitica*. 17: 13- 21.



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Rolled cheese with the addition of insects as an alternative source of protein

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Abstract

Rolled cheese is cheese from the area of Vojvodina and is produced with various additives. Alternative sources of protein are increasingly represented in animal and human nutrition. The aim of the work is to examine the chemical and sensory properties of rolled cheese without and with the addition of insects as an alternative source of protein (IASP), as well as the possibility of using IASP in human nutrition as an addition to cheese. Cheeses with the addition of IASP had a slightly higher protein content (21.45%) compared to the cheese without the addition IASP (20.69%). During the sensory evaluation, the rolled cheese without additives was better evaluated (23.27 points) compared to the cheese with the addition of IASP (21.45 points). The addition of IASP to rolled cheese had an effect on increasing the protein content, and the sensory properties of the cheese itself were not significantly changed.

Keywords: rolled cheese, edible insects, alternative protein, sensory evaluation

Introduction

Rolled cheese belongs to the group of cheeses of steamed dough or pasta filata and is produced from full-fat cow's milk. It has been traditionally produced in Vojvodina since the end of the 70s of the last century. It is believed that immigrants from the Czech Republic and Slovakia brought with them the production of cheeses, such as Parenica and Ošćepka, and that this is how traditional production developed in our region. Rolled cheese is also known as sheet cheese because the layering and sheeting of the dough are clearly visible when cut (Popović-Vranješ, 2014). The characteristic of this cheese is that the cheese dough is kneaded and stretched into layers, and after cooling it is shaped into a roll. This type of cheese is consumed after the production process because there is no ripening. There are various options for filling rolled cheese, such as: ham, kulen sausage, hot pepper, chives, olives, etc. It can also be smoked and then it gets a spicier and stronger taste. Furthermore, rolled cheese can be a dessert if it is filled with sweet additives, such as various dried fruits, fresh raspberries, chocolate, honey. The type of supplement depends on the taste and needs of consumers and the market itself.

In recent years, the use of alternative sources of protein in animal nutrition has been increasingly considered, due to the increase in the world population and the limited agricultural resources (Jozefiak et al., 2016). Proteins of animal origin are used in order to provide essential amino acids in the diets of non-ruminants, and they are usually obtained from by-products of slaughterhouses, dairies, fish processing, rendering products, etc. These nutrients must be used rationally due to their high price, so they are usually represented in a low percentage in animal feed mixtures (Stanković, 2013). Insects, due to the number of species, cheap, and efficient way of production, can be used as an alternative source of protein. The most important prerequisites for ensuring cost-effective insect-based protein production are their short reproductive cycle and high nutritional value (Hossain and Blair, 2007). Yang et al. (2006) found that edible insects contain good-quality fatty acids, especially long-chain omega-3 fatty acids such as alpha-linolenic acid, and eicosapentaenoic acid, and that different insect species have different fatty acid profiles.

The mealworm (*Tenebrio molitor*, *Celeoptera: Tenebrionidae*) is an edible insect that is distributed all over the world and is suitable for industrial production. It is used as a good substitute for conventional protein sources (Jajić et al., 2020). *T. molitor* are insects that infest finished and stored products, but many people do not consider them pests but grow their larvae as food for their pets, and in Europe, they are widely distributed as a supplement to human

nutrition (Caparros Megido et al., 2014).

The aim of this work is to examine the possibility of using IASP in human nutrition, as an addition to rolled cheese, as well as the chemical and sensory properties of rolled cheese without and with the addition of IASP.

Material and methods

Rolled cheese production

In the experimental dairy of the Agricultural School in Futog, rolled cheese was produced without and with the addition of insects as an alternative source of protein. Raw milk from the school farm was pasteurized in a duplicator (100 l) at 63 °C for 30 min, then cooled to 32 °C and a starter culture (mesophilic culture CHN11, CHR HANSEN, Denmark) was added. After 30 min, CaCl₂ (20 g) and rennet (according to the manufacturer's instructions) were added and mixed well. The coagulum was cut into cheese grain, the size of a wheat grain. The cutted curd was left to harden for 10 minutes, then it is heated to 42 °C and discharged into the pre-press where it was left for about 2 hours, in order to form a basque. Basque is left to ripen between 18 to 20 hours, on temperatures from 15 to 18 °C, in order to obtain adequate elasticity and acidity. Ripe basque had a pH of 5-5.2. Basque was cut into slices and then placed in hot water, heated to 75-78 °C, and mixed, a cheese dough was obtained, which was stretched on a cheese table and left to cool. When the cheese dough was cooled, it was divided into two parts, and then 5% IASP in the form of flour was added to the surface of one half, followed by the dough folding into a roll. The other half (control) was also folded into a roll.

Rearing of insects

The insects were obtained from the Department of Plant and Environmental Protection, Faculty of Agriculture, University of Novi Sad, Serbia. Mealworm specimens were maintained in an incubator under controlled conditions. The insects were grown on a food mixture that contained wheat bran, dried barley germs, dried oat germs, barley flakes, oat flakes and powdered beer yeast. Pieces of apple were spread over the food mixture to provide additional moisture to the insects. Before in the next step, the larvae were separated from the residual food and then deprived of food during the process 24 hours to eliminate the residual part that was in the gastrointestinal tract.

Preparing insects for rolled cheese production After that insects were sieved (2.5 mm pore diameter) and the remaining insect parts were removed with a weak air flow produced by a hair dryer. The sieved larvae were transferred to a sieve with smaller holes and the remains of insect bodies were removed with a weaker airflow. Afterward, the cleaned larvae were transferred into a 2-l plastic container and gently washed under a stream of water. Subsequently, the insects were placed in a container with boiling water and cooked for 180 s. The entire content of the cooking pot was then filtered through a sieve to remove water, and the larvae were spread in a thin layer on filter paper to evaporate excess water during 24 h. The dried insects were collected and placed on a new filter paper and allowed to dry for another 24 h. The dried insects were then ground.

Chemical and sensory analyses

Milk chemical composition was determined by the infrared test method (ISO 9622:2020) by Milcoscan FT in the Laboratory for Milk Quality Control, Faculty of Agriculture in Novi Sad. The laboratory is accredited in accordance with the international standard ISO 17025:2017.

The analysis of the cheese was carried out in the Laboratory for quality control of animal feed and animal products, at the Animal Husbandry Department of the Faculty of Agriculture in Novi Sad, using accredited methods. Water content (MC) was determined as weight loss after drying (AOAC Official Method 934.01). Protein (CP) was analyzed according to standard Kjeldahl method (AOAC Official Method 2001.11), while fat content (EE) was determined as petroleum ether extract (AOAC Official Method 991.36).

The sensory evaluation of the cheeses, two days after production, was performed by 11 participants using the scoring method 1 to 5. The following sensory characteristics of the cheese were evaluated: external appearance, consistency, color, odor, and taste. The maximum number of points was 25. Sensory evaluation of the cheese was carried out based on the Rulebook on evaluation of the quality of milk and milk products at the International Agricultural Fair in Novi Sad (2016).

Results and discussion

The milk did not contain antibiotics or other inhibitors. Table 1 shows the average chemical composition of raw milk for rolled cheese production.

Table 1. Average chemical composition of raw milk (N=5)

Trait	Mean	Minimum	Maximum	SD
Fat (%)	3.51	3.3	4.51	0.57
Protein (%)	3.25	2.88	3.45	0.28
Lactose (%)	4.5	4.38	4.97	0.26
SNF (%)	8.44	8.00	8.68	0.35
Casein (%)	2.51	2.16	2.63	0.23
Milk urea (mg/dl)	22.7	12.25	36.4	13.76

SD – standard deviations

The average chemical composition of the analyzed milk satisfied the criteria prescribed by the Rulebook on the quality of raw milk (2017), which states that cow's raw milk must have at least 3.2% milk fat and at least 3.0% protein. The content of dry matter without fat is slightly lower, 8.44%, compared to the 8.50% prescribed by the Rulebook (2017).

The average chemical composition of the analyzed rolled cheese, without and with the addition of insects as alternative protein sources, is shown in Table 2.

Table 2. Average chemical composition of rolled cheese without and with the IASP

Content	Rolled cheese	Rolled cheese with IASP
Dry mater (%)	48.14	49.30
Water (%)	51.55	50.97
Protein (%)	20.69	21.45
Fat (%)	21.02	20.82
Fat in dry mater (%)	43.28	42.56
Water in the non-fat dry mater (%)	65.40	64.56

IASP - insects as an alternative source of protein

Based on the average chemical composition, the rolled cheese can be declared as semi-fat semi-hard cheese. The Rulebook on the Quality of Milk Products and Starter Cultures (2014) prescribes that semi-fat cheese contains between 25 and 45% milk fat in dry matter, while for semi-hard cheeses the moisture content in the non-fat matter of the cheese is prescribed at 54 - 69%. Similar results were obtained by Popović-Vranješ et al. (2014), in their research.

The produced rolled cheese with the IASP had a slightly higher protein content (21.45%) compared to the cheese without addition (20.69%). This result was expected, based on previous research by Jajić et al. (2019) who found that the larvae of *T. molitor* mostly contain crude protein (55.83%) and crude fat (25.19%), as well as a low content of nitrogen-free extract in dry matter.

Table 3 presents the sensory score for the rolled cheese (control cheese) and the cheese with IASP. Generally, from the results it can be seen that the total score for the rolled cheese is higher (23.27 points) compared to the rolled cheese with IASP (21.45 points). The main difference between cheese samples were in the color, odor and taste. Rolled cheese got the lower score for appearance and consistency, but higher score for color, odor and taste

Table 3. Sensory analysis of rolled cheese with and without IASP

Trait	Rolled cheese	Rolled cheese with IASP
External appearance	4.55	4.64
Consistency	4.55	4.82
Color	4.91	4.00
Odor	4.64	4.09
Taste	4.55	3.91
Total	23.27	21.45

IASP - insects as an alternative source of protein

Conclusions

Larvae *T. molitor* are suitable for industrial production and are a good source of protein. The produced rolled cheese with the addition of IASP had an increased protein content. According to the sensory evaluation, it is rated lower, but still acceptable.

Further research is needed to determine the content of individual amino acids and fatty acids, and the possibility of further using IASP in human nutrition.

Acknowledgement

This research is financially supported by Ministry of Education, Science and Technological Development, Republic of Serbia (Contract number 451-03-47/2023-01/ 200117).

References

- AOAC International (2005). AOAC Official Method 934.01. Moisture in Animal Feed. Official Methods of Analysis of AOAC International, 18th Edition. Gaithersburg, MD (USA).
- AOAC International (2006). Method 991.36. Fat (Crude) in meat and meat products. Official Methods of Analysis of AOAC International. 18th Edition. Arlington, TX (USA).
- AOAC Official Method (2001). 11. Protein (crude) in animal feed, forage (plant tissue), grain and oilseeds. In: J.W. Horwitz, L. George (Editors). Official Methods of Analysis of AOAC International. Gaithersburg, MD (USA).
- Caparros Megido R., Sablon L., Geuens M., Brostaux Y., Alabi T., Blecker C., Drugmand D., Haubruge É., Francis F. (2014). Edible insects acceptance by Belgian consumers: promising attitude for entomophagy development. *Journal of Sensory Studies*. 29:14–20.
- Hossain S.M., and Blair R. (2007). Chitin utilisation by broilers and its effect on body composition and blood metabolites. *British poultry science*. 48: 33–38.
- Jajić I., Popović A., Urošević A., Krstović S., Petrović M., Guljaš, D (2019). Chemical composition of mealworm larvae (*TENEBRIO MOLITOR*) reared in Serbia. *Contemporary Agriculture*. 68: 23-27.
- Jajić I., Popović A., Urošević M., Krstović S., Guljaš D., Samardžić M. (2020). Fatty and amino acid profile of mealworm larvae (*TENEBRIO MOLITOR* L.). *Biotechnology in Animal Husbandry*. 36: 167-180.
- Jozefiak D., Jozefiak A., Kieronczyk B., Rawski M., Swiatkiewicz S., Dlugosz J., Engberg R.M. (2016). 1. Insects–A Natural Nutrient Source for Poultry–A Review. *Annals of Animal Science*. 16: 297-313.
- Popović-Vranješ A., Trivunović S., Boboš S., Pejanović R., Vlahović B., Jajić I., Pihler I. (2014). Production, processing and marketing of milk and indigenous dairy products in AP Vojvodina. Faculty of Agriculture, Novi Sad.

Rulebook on the quality of milk products and starter cultures, Official Gazette of SR, 33/2010, 69/2010, 43/2013 - dr. pravilnik i 34/2014.

Rulebook on the quality of raw milk, Official Gazette of SR, 106/2017.

Rulebook on evaluation of the quality of milk and milk products at the International Agricultural Fair in Novi Sad (2016).

International Organization for Standardization (ISO17025:2017) (2017). General requirements for the competence of testing and calibration laboratories. Geneva, Switzerland: International Organization for Standardization.

International Organization for Standardization [ISO 9622: 2020/IDF 141] (2020). Milk and liquid milk products — Guidelines for the application of mid-infrared spectrometr.. Geneva, Switzerland.

Stanković B.M. (2013). Uticaj smeša koncentrata sa različitim učešćem proteina i masti na prirast i konverziju hrane u ishrani mlađi šarana, (*Cyprinus carpio*, L., 1758), doktorska disertacija, Poljoprivredni fakultet, Beograd.

Yang L.F., Siriamornpun S., Li D. (2006). Polyunsaturated fatty acid content of edible insects in Thailand. *Journal of Food Lipids*. 13: 277-285.

The impact of cream ripening on butter characteristics

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Abstract

Butter is a dairy product with a high nutritional value, consisting of more than 80% milk fat, which imparts its characteristic flavor. The key technological operation in butter production is the preparation of the cream, involving its physical and/or biochemical ripening. In the production of sweet cream butter, physical ripening is a necessary process due to the crystallization of milk fat, which has a direct effect on the consistency and flavor of the butter. In the production of sour cream butter, both physical and biochemical ripening of the cream are carried out in order to develop a specific flavor. Improper cream ripening negatively impacts the sensory characteristics and the distribution of water and fatty acids in the butter.

Keywords: cream, butter, ripening, milk fat, butter quality

Introduction

Butter is a dairy product obtained by churning of cream, resulting in the formation of butterfat granules. Its popularity is evident from the fact that in Europe approximately 150 million liters of milk are produced annually, of which 29.4% (46 million liters) is used for butter production. After cheese, butter production accounts for the largest quantity of milk “consumed” (Cook, 2018). According to available data, the world’s largest butter producers are India, the EU-27, the United States, New Zealand, and Russia (www.statista.com).

According to the Codex Alimentarius (2011), butter is a dairy product obtained exclusively from milk and/or milk products, in the form of a water-in-oil emulsion. Butter must contain a minimum of 80% milk fat, up to 16% water, and up to 2% non-fat solids. For butter production, sweet cream, which is obtained by separation from the milk without fermentation, sour cream, which is obtained by fermentation using microbial dairy cultures after the separation process, and acidified sweet cream with added lactic acid can be used. These different sources contribute to variations in their sensory characteristics. Technological processes in butter production also play an important role. For example, the sensory characteristics are influenced by the ripening process of the cream, which can be either physical or biochemical. Physical ripening occurs at different temperatures, depending on the type of milk (cow, sheep, goat, buffalo) used for production and the type of butter desired. Unlike physical ripening, biochemical cream ripening is not mandatory, but contributes to the creation of aromatic compounds that are crucial for achieving the desired flavor of the butter. The goal of this manuscript is to consolidate significant research findings related to the optimization of physical and biochemical cream ripening, which are important for the physical and sensory quality of butter.

Cream ripening

The ripening of the cream plays a significant role in defining the quality of butter, whether it’s related to taste or consistency (Boode and Walstra, 1993). In particular, ripening influences the amount of aromatic compounds in the butter, primarily diacetyl and acetoin (Samaržija, 2011). During the ripening of the cream, the crystallization of the milk fat takes place. Crystallization is a process in which crystals form on the surface of the fat globules and penetrate into the junction of two milk fat globules (Figure 1). As the crystals penetrate the fat globule, liquid fat is released from the globule, resulting in the formation of a network of fat globules connected by crystals and coated with liquid fats (van Boekel and Walstra, 1981; Boode and Walstra, 1993; Buldo et al., 2013).

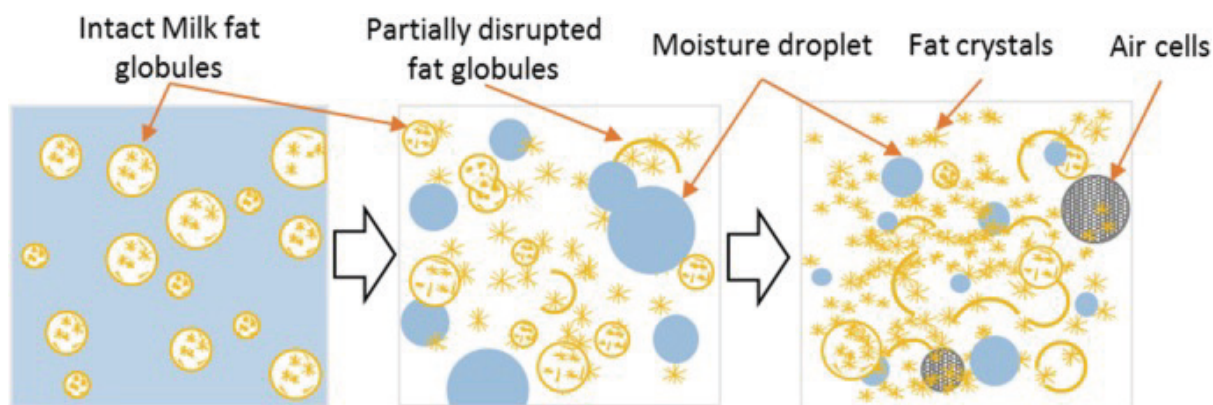


Figure 1. Formation of a network of fat globules (Source: Panchal and Bahndari, 2020)

The formation of fat agglomerates occurs in two phases, namely aggregation and fusion. When two fat globules come close to each other, the crystallized fats on the surface of one globule penetrate the membrane of the other fat globule (aggregation). During this process, the liquid fats within the globule surround the penetrating crystals, resulting in their fusion (Akoh, 2008). An optimal balance of crystallized and liquid fats enables the formation of fat agglomerates. Increasing the proportion of solid fats (capric, lauric, myristic, palmitic, stearic acids) makes agglomeration more likely. However, when the proportion of solid fats is too low or too high, the possibility of forming agglomerates decreases due to a lack of liquid fats (butyric, caproic, caprylic, oleic, linoleic, linolenic, and arachidonic acids) that could link the globules or a shortage of crystals that can penetrate the other globule (Buldo et al., 2013). The created crystals correlate with the composition and structure of the butter, influencing its physical properties such as hardness and spreadability, as well as sensory characteristics like appearance and taste. A wide variety of fatty acids with different melting points lead to the formation of crystals of various shapes and sizes. The polymorphism of the crystals formed during crystallization influences, for example, the final taste and sensory properties of the butter (Ceylan and Ozcan, 2020).

Physical cream ripening

Physical cream ripening is an essential step in butter production, and is only carried out in combination with biochemical ripening in the production of butter from sour (fermented) cream. The absence of physical cream ripening can lead to the appearance of unwanted butter properties. For example, due to the wide range of fats with different melting points present in the milk fat, fat may separate from the butter. Such butter can be either too hard or too soft, with an increased loss of fat in the buttermilk, which directly affects the water content in the final product (Deosarkar et al., 2016).

The principle of physical cream ripening involves applying different temperature-time regimes for cream ripening, such as cold-warm-cold ripening, to optimize the consistency properties of the butter. Some possible temperature-time solutions for physical cream ripening (Samaržija, 2011) include i) cold ripening at a temperature of 2-3 °C for 18 hours, ii) cold ripening at a temperature of 3-8 °C for 18 hours, iii) cold ripening at a temperature of 8-10 °C for 8 hours, and iv) warm ripening at a temperature of 19 °C for 8 hours.

The efficiency of the physical ripening of cream is achieved by combining lower and higher temperatures. For instance, with the cold-warm-cold ripening process. This process begins with cold ripening at a temperature of 6 °C to 8 °C for 2 to 3 hours, during which triacylglycerols with a high melting point form crystals on the surface. At the end of the first phase, the cream is gradually heated by mixing to a temperature of 18 °C to 23 °C, which initiates the second phase, warm ripening, which lasts for 2.5 hours. During this phase, the butter softens due to the melting of low-melting-point fatty acids, while saturated molecules such as palmitic and stearic fatty acids are deposited on the outer parts of the crystallized mass. Additionally, 2-5% of butter culture is added. The third phase is cold ripening at a temperature of >13 °C, lasting for more than 8 hours, leading to a decrease in pH value from 4.6 to 4.8.

Buldo et al. (2013) mention that the duration of ripening affects the churning time as well as the properties of cream and butter. Specifically, the churning time is inversely proportional to the ripening time. Measurement of viscosity at the phase inversion point showed that it increases with longer ripening periods. The formation of crystals within the milk fat globules does not affect viscosity, as it does not change the volume of the dispersed phase. The rapid increase in viscosity is a result of the formation of larger fat agglomerates and subsequently of butter granules. The duration of the ripening influences the proportion of solid fats in the cream, and therefore, the churning time (Figure 2). Longer cream ripening results in a higher proportion of solid fats and, consequently, shorter churning time (Buldo et al., 2013). The mixing process during ripening allows for better heat transfer, which promotes fat crystallization. Lee and Martini (2018) note that mixing during ripening also affects the proportion of solid fats in the cream (SFC). At lower ripening temperatures of 5 °C and 10 °C, the proportion of solid fats is higher than when ripening occurs at 15 °C. The intensity of the cooling of the cream during mixing affects the crystallization during the ripening of the cream. Slow cooling of the cream results in a higher proportion of solid fats with the formation of larger fat crystals that agglomerate more easily, while fast cooling often leads to the creation of smaller and narrower fat crystals that agglomerate less easily.

During ripening, under the influence of different temperatures, butter granules of different sizes are formed. This allows the influence of ripening on the size of the agglomerated particles and the butter granules to be observed. Longer ripening enables the formation of larger butter granules, with the size of the fat globules not significantly changing (Buldo et al., 2013).

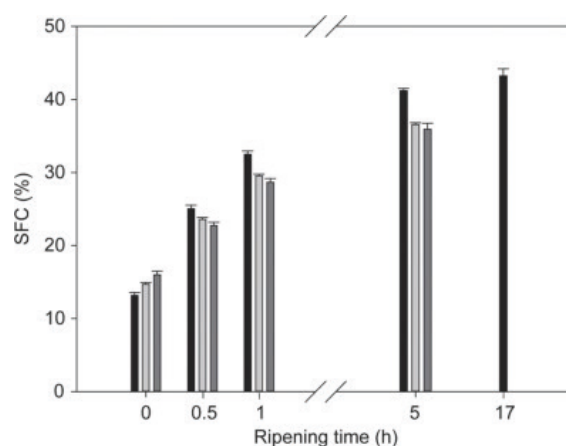


Figure 2. The influence of cream ripening duration on the proportion of solid fats (black), after 7 minutes of churning (light gray), and just before the formation of butter granules (dark gray) (Source: Buldo et al., 2013)

Biochemical cream ripening

The biochemical ripening of cream is the result of the action of a mesophilic butter culture, consisting of strains of lactic acid bacteria, including *Lactococcus* and *Leuconostoc*. These strains include *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis*, and *Leuconostoc mesenteroides* subsp. *cremoris* (Fearon, 2011). Their primary role is to produce the desired concentration of lactic acid and aromatic compounds in the cream, which will provide the butter with the desired sensory characteristics based on pH value and the presence of aromatic substances, primarily diacetyl and acetoin. In the production of aromatic butter, ripening is stopped at a pH value of 4.5, while for mildly aromatic butter at a pH of 5.0-5.3 (Samaržija, 2011).

Depending on the diet, the milk or cream for making butter has a different composition, and therefore, the optimal time and temperature for ripening must be determined accordingly. This results in distinguishing between “winter” and “summer” cream. In the colder part of the year, the cow diet primarily consists of hay and concentrated feeds; therefore so-called “winter cream” contains a higher proportion of short-chain and saturated fatty acids. On the other hand, with a diet of green forage, “summer cream” contains a higher proportion of long-chain and unsaturated fatty acids (Samaržija, 2011). Consequently, in the production of “winter” butter, cold-warm-cold cream ripening is applied to make the butter more spreadable, while warm-cold-cold cream ripening is used to achieve a firmer consistency for “summer” butter (Schäffer et al., 2001).

Sensory characteristics of butter

The sensory characteristics of butter, in addition to appearance, color, taste, and odor, also include consistency and texture. Butter should have a matte appearance without gloss or oily sheen (Kashaninejad et al., 2016). It is characterized by a pale yellow to yellow color and a plastic texture (low spreadability at refrigerator temperature). The presence of aromatic substances primarily depends on whether sweet cream (fruity flavor) or sour cream (diacetyl) is used for butter production. The intensity of the yellow color of butter is related to the content of carotenoids in the milk, whether they are naturally present in the milk or added as colourant.

Butter with desirable physical characteristics and texture must meet certain parameters. The physical properties of butter are determined by the proportion, size and shape of the liquid and solid fats in the cream. After pasteurization, the fats are in a liquid state, but each fatty acid present in the cream, of which there are about 450 known, has a different melting point ranging from -40 °C to 40 °C. During the physical cream ripening, the fat crystallization occurs, resulting in the formation of various forms of crystals by connecting fatty acids. Due to the formation of crystals with different densities and structures, it is desirable to determine the proportion of unsaturated and saturated fatty acids together with their hardening and solubility capabilities before physical cream ripening to determine the temperature and time for physical cream ripening that will yield high-quality butter with an optimal flavor (Samaržija, 2011). For this purpose, the iodine value is often determined (the number of grams of iodine needed to saturate the unsaturated bonds of fatty acids), which is directly correlated with the content of oleic acid. The iodine value of butter with optimal consistency is in the range of 32-37 (Božanić, 2012).

In general, hardness and spreadability are the most important physical characteristics of butter, and there is a proven high correlation between them. The most significant factors influencing these two physical characteristics are the proportion of fats in the form of the ratio between solid and liquid fats, the source of fats, and temperature (Glibowski et al., 2008). For butter, a smooth, slightly firm, and plastic texture is desirable, and it should provide resistance when cut. Besides ripening, the amount of fat also affects the hardness of the butter, with higher fat content resulting in greater hardness (Ceylan and Ozcan, 2020).

Apart from hardness, the most important characteristic of butter is spreadability. Pasteurization of milk for cream and butter production has been found to result in poorer spreadability compared to butter made from raw milk (Sert and Mercan, 2020). Butter spreadability can be improved through temperature regimens during cream ripening, depending on the type of milk used in butter production, and by enriching cream with low-melting-point fatty acids, primarily unsaturated fatty acids (Schäffer et al., 2001).

During ripening, the crystallization of milk fat occurs, and depending on the size and manner of crystal formation, the hardness of butter changes. Crystals are formed not only within fat globules but also in the continuous phase. An increase in the number of crystals outside the milk fat globules results in firmer-textured butter. However, untouched milk fat globules lead to better spreadability. The hardness of butter can be determined by the amount of incorporated air in the butter, with a lack of incorporated air resulting in a denser structure and greater hardness. On the other hand, a higher volume of air makes the butter softer (Buldo, 2016).

The cooling intensity during cream ripening also affects the hardness of butter. Slow cooling results in larger fat crystals and more contact points between the crystals, while rapid cooling leads to the formation of smaller crystals dispersed in the liquid phase. The temperature and duration of heat treatment of cream influence spreadability, with increased temperature and time resulting in decreased butter hardness (Rønholt et al., 2012).

The proportion of solid and liquid fats is the primary factor influencing spreadability. A higher proportion of solid fats in the butter leads to reduced spreadability. The fat composition also affects spreadability, with a positive correlation observed between the proportion of unsaturated fatty acids in milk fat (Ceylan and Ozcan, 2020). Hurtaud and Peyraud (2007) found that feeding dairy cows with seeds or meal of the Camelina plant (*Camelina sativa* L.) increased the proportion of unsaturated fatty acids and trans fatty acids in milk, while the proportion of short-chain fatty acids decreased. Consequently, the resulting butter was less hard and had better spreadability.

Conclusion

For the production of high-quality butter, it is not only crucial to start with high-quality raw materials, but also to pay attention to all stages of butter production. This primarily pertains to the ripening process, with the aim of obtaining a product of exceptional quality and desirable sensory characteristics. During the ripening phase, the formation of

milk fat crystals plays a crucial role in achieving the desired consistency and sensory characteristics of the butter. In addition to the crystal formation, the ripening process also generates aromatic compounds that give the butter its characteristic aroma and flavor.

Acknowledgements

The paper is an excerpt from the diploma thesis of Arijana Klindić, univ. bacc. ing. agr., entitled “Tehnologija proizvodnje i karakteristike maslaca”.

References

- Akoh C.C. (2008). Lipid-Based Emulsions and Emulsifiers. U: Food Lipids: Chemistry, Nutrition, and Biotechnology, Third Edition (ur. Akoh, C.C., Min, D.B.). CRC Press, Boca Raton, Florida, SAD. 90-91.
- Boode K., and Walstra P. (1993). Partial coalescence in oil-in-water emulsions 1. Nature of the aggregation. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*. 81: 121-137.
- Božanić R. (2012). Maslac. U: Mlijeko i mliječni proizvodi (ur. Tratnik, Lj., Božanić, R.). Hrvatska mljekarska udruga, Zagreb. 425-438.
- Buldo P. (2016). Butter: Properties and Analysis. U: Encyclopedia of Food and Health (ur. Cabaallero B., Finglas P.M. & Toldra). Elsevier, Oxford, United Kingdom. 535-540.
- Buldo P., Kirkensgaard J.J.K., Wiking L. (2013). Crystallization mechanisms in cream during ripening and initial butter churning. *Journal of Dairy Science*. 96: 6782-6791.
- Ceylan O., and Ozcan T. (2020). Effect of the cream cooling temperature and acidification method on the crystallization and textural properties of butter. *LWT*. 109806.
- Codex Alimentarius (2011). Codex Standard for Butter, Codex Standard 279-1971, Milk and Milk Products. World Health Organization and Food and Agriculture. Organisation of UN, Rim. 36-37.
- Cook E. (2018). Farm production, Milk. U: Agriculture, forestry and fishery statistics (ur. Cook, E.). Eurostat, 62-63.
- Deosarkar S.S., Khedkar C.D., Kalyankar S.D. (2016). Butter: manufacture. U: Encyclopedia of Food and Health (ur. Cabaallero B., Finglas P.M., Toldra F.). Elsevier, Oxford, United Kingdom. 529-534.
- Fearon A.M. (2011). Butter and Butter Products. U: Dairy Ingredients for Food Processing (ur. Fearon, R. C., Kilara, A.). Blackwell Publishing Ltd. 199-225.
- Glibowski P., Zarzycki P., Krzepakowska M. (2008). The Rheological and Instrumental Textural Properties of Selected Table Fats. *International Journal of Food Properties*. 11: 678-686.
- Hurtaud C., and Peyraud J.L. (2007). Effects of Feeding Camelina (Seeds or Meal) on Milk Fatty Acid Composition and Butter Spreadability. *Journal of Dairy Science*. 90: 5134-5145.
- Kashaninejad M., Razavi S.M.A., Mazaheri Tehrani M., Kashaninejad M. (2016). Effect of extrusion conditions and storage temperature on texture, colour and acidity of butter. *International Journal of Dairy Technology*. 70: 102-109.
- Lee J., and Martini S. (2018). Effect of cream aging temperature and agitation on butter properties. *Journal of Dairy Science*. 101: 1-12.
- Mallia S., Escher F., Schlichtherle-Cerny H. (2008). Aroma-active compounds of butter: a review. *European Food Research and Technology*. 226: 315-325.
- Panchal B., Bhandari B. (2020). Butter and Dairy Fat Spreads. In: Truong T., Lopez C., Bhandari B., Prakash S. (eds) *Dairy Fat Products and Functionality*. Springer, Cham.
- Rønholt S., Kirkensgaard J.J.K., Pedersen T.B., Mortensen K., Knudsen J.C. (2012). Polymorphism, microstructure and rheology of butter. Effects of cream heat treatment. *Food Chemistry*. 135: 1730-1739.

- Samaržija D. (2011). Fermentirana mlijeka, vrhnje i maslac, Agronomski fakultet Sveučilišta u Zagrebu.
- Schäffer B., Szakály S., Lőrinczy D., Schäffer B. (2001). Melting properties of butter fat and the consistency of butter. Effect of modification of cream ripening and fatty acid composition. *Journal of Thermal Analysis and Calorimetry*. 64: 659-669.
- Sert D., and Mercan E. (2020). Characterisation of physicochemical, microbiological, thermal, oxidation properties and fatty acid composition of butter produced from thermosonicated cream. *International Dairy Journal*. 104777.
- Van Boekel M.A.J.S., and Walstra P. (1981). Effect of Couette flow on stability of oil-in-water emulsions. *Colloids and Surfaces*. 3: 99-107.

Potencijalne rezidue u ovčjem i kozjem mlijeku u Hrvatskoj

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Sažetak

Posljednjih godina velika pozornost potrošača i prehrambene industrije u EU posvećuje se reziduama u hrani koje potencijalno ugrožavaju zdravlje ljudi. Cilj ovog istraživanja bio je utvrditi potencijalno opasne rezidue u ovčjem i kozjem mlijeku u Hrvatskoj. Istraživanje je provedeno u 15 stada ovaca i 12 stada koza tijekom tri godine. Od uzgajivača su uzeti podatci o bolestima koje se najčešće javljaju u njihovim stadima, vrsti lijeka i tko je liječio. Nadalje uzeti su i podatci o načinu provedbe preventivnih zahvata koji podrazumijevaju suzbijanje unutarnjih i vanjskih nametnika. U istraživanju su korišteni i podatci Središnjeg laboratorija za kontrolu kvalitete mlijeka Hrvatske agencije za poljoprivredu i hranu. Na temelju preliminarnih istraživanja i dostupnih literaturnih podataka možemo zaključiti da u Hrvatskoj postoji objektivan rizik od kontaminacije mlijeka ovaca i koza različitim vrstama rezidua. Poseban problem predstavljaju rezidue čija se prisutnost ne kontrolira sustavno ili se uopće ne kontrolira kao što je slučaj s mikotoksinima, hormonima, lijekovima za suzbijanje unutarnjih i vanjskih parazita.

Ključne riječi: rezidue, mlijeko, kontaminacija, mikotoksini

Uvod

Posljednjih godina velika pozornost potrošača i prehrambene industrije u EU posvećuje se reziduama u hrani koje potencijalno ugrožavaju zdravlje ljudi. Proizvodi animalnog podrijetla kao što su meso, mlijeko i jaja predstavljaju poseban rizik za zdravlje ljudi s obzirom da mogu biti kontaminirani brojnim reziduama. U prvom redu radi se o riziku kontaminacije antibioticima koji zaostaju nakon liječenja životinja. Chiesa i sur. (2021) navode da su antibiotici najveći zdravstveni i tehnološki problem u proizvodnji mlijeka. Pored antibiotika u mlijeku i mesu se mogu naći i druge rezidue, a koje u većim količinama mogu ugroziti zdravlje ljudi. Za razliku od proizvodnje kravljeg mlijeka, u proizvodnji ovčjeg i kozjeg mlijeka antibiotici se značajno manje koriste. S obzirom da se veliki dio ovaca i koza drži pašno stada su izložena invazijama različitih vrsta unutarnjih i vanjskih parazita tako da je njihova kontrola i suzbijanje sastavni dio takvog načina uzgoja. Jedziniak i sur. (2015) su utvrdili prisutnost antihelmintika u mlijeku ovaca i koza kao i u nekim mliječnim proizvodima. Pored antihelmintika u mlijeku se mogu naći i ostaci pesticida od kojih su najčešći insekticidi za suzbijanje insekata i ektoparazita (Hadžiosmanović i sur., 2002). Hormoni se u uzgojima ovaca i koza isključivo koriste za sinkronizaciju estrusa s ciljem ujednačenog perioda poroda i početka laktacije. U Francuskoj, najčešći način sinkronizacije estrusa u ovaca i koza je primjena intravaginalnih spužvica koje sadrže 45 mg fluorogeston acetata tijekom perioda od 11 dana. Zatim, 48 sati prije uklanjanja spužvica, životinjama se daje 400-600 IU ECG (Equine Chorionic Gonadotroping) i 50 µg kloprostenola (sintetski analog od prostaglandina F2α) primijenjen jednokratno (Corteel i sur., 1988). Malahova i Novopashina (2011) na temelju istraživanja provedenom na kozama zaključili su da uporaba pesara s gestagenom u trajanju od 16 dana zahtijeva davanje ECG (Equine Chorionic Gonadotropin) na dan uklanjanja pesara, što će značajno smanjiti troškove rada. Posljednjih godina u proizvodnji mlijeka koriste se visoko mliječne pasmine ovaca. Sinkronizacija se provodi isključivo tijekom suhostaja jer bi u suprotnom značajno porasla razina hormona u mlijeku koje se koristi u prehrani ljudi. Hranidba visoko mliječnih pasmina ovaca zahtijeva korištenje izbalansiranih obroka koji sadržavaju manji ili veći udio smjesa žitarica. U slučaju da žitarice sadrže veći udio bakra dugoročno se javlja rizik od trovanja koje najčešće završava uginućem životinja. U liječenju ovaca koriste se lijekovi na bazi amonijevog molibdenata (Mecitoglu i sur., 2017). Jaiswal i

sur. (2020) navode da nakon tretiranja ovaca amonijevim tetramolibdenatom karenca iznosi 10 dana ne navodeći za meso ili mlijeko. Pored navedenog, u mlijeku ovaca i koza mogu se naći i mikotoksini. Akinyemi i sur. (2020) su utvrdili mikotoksine u mlijeku koza. Autori navode da takvo mlijeko najviše ugrožava zdravlje djece. Bilandžić i sur. (2014) su utvrdili prisutnost aflatoksina M1 u dva uzorka kozjeg mlijeka u istraživanju provedenom u Hrvatskoj. U proizvodnji mlijeka dezinfekcija opreme koja se koristi u mužnji i preradi mlijeka sastavni je dio tehnološkog procesa. U slučaju nepravilnog korištenja, dezinficijensi i detergentsi mogu kontaminirati mlijeko te se ono ne smije koristiti u daljoj preradi. Prema Pravilniku o utvrđivanju sastava sirovog mlijeka (NN 136/2020) sirovo mlijeko ne smije sadržavati rezidue ili druge kontaminante u količinama većim od najvećih dopuštenih, ostatke nedopuštenih tvari, detergente i druge tvari koje mogu imati štetan učinak na zdravlje ljudi ili koje mijenjaju organoleptička svojstva mlijeka. Cilj ovog istraživanja bio je utvrditi koje se potencijalne rezidue mogu naći u mlijeku ovaca i koza u Hrvatskoj.

Materijali i metode

Istraživanje je provedeno u 15 stada ovaca i 12 stada koza tijekom tri godine. Na Pagu se nalazilo 9 stada ovaca uključenih u istraživanje, na Pelješcu jedno, dok je ostalih 5 bilo u kontinentalnom dijelu Hrvatske. Stada koza držana su intenzivno na području sjeverozapadne Hrvatske. U 5 stada ovaca mlijeko se proizvodi intenzivno, dok u ostalim ekstenzivno pritom su ovce tijekom cijele godine boravile na otvorenim površinama. Hranidba se temeljila na ispaši a tijekom zimskih mjeseci ovce su dohranjivane sijenom i žitaricama. U svim stadima koza mlijeko se proizvodi intenzivno. U takvom sustavu koze su držane isključivo stajski. Hranidba se temelji na sijenu, svježe pokošenoj travi i gotovim krmnim smjesama, a mužnja je isključivo strojna. Od uzgajivača su uzeti podaci o bolestima koje se najčešće javljaju u njihovim stadima, vrsti lijeka i tko je liječio. Nadalje, uzeti su i podaci o načinu provedbe preventivnih zahvata koji podrazumijevaju suzbijanje unutarnjih i vanjskih nametnika. U istraživanju su korišteni i podaci Središnjeg laboratorija za kontrolu kvalitete mlijeka Hrvatske agencije za poljoprivredu i hranu vezani uz prisutnost inhibitornih tvari u mlijeku ovaca i koza. Na temelju dobivenih podataka o vrsti lijekova i preventivnih preparata utvrđena je njihova karenca u mlijeku i potencijalni rizik za zdravlje ljudi.

Rezultati i rasprava

Istraživanjem je utvrđeno da se antibiotici u stadima ovaca i koza koriste vrlo rijetko. Najčešća uporaba je u slučaju upala mliječne žlijezde. U svim stadima uključenim u istraživanje suzbijanje parazita se provodi jednom godišnje. U niti jednom stadi odabir lijeka se ne temelji na koprološkoj pretrazi. Najčešće se koriste lijekovi na bazi albendazola i ivermektina. Istraživanjem je utvrđeno da trenutno u Hrvatskoj ne postoje antihelmintici koji su registrirani za korištenje kod ovaca i koza čije se mlijeko koristi u prehrani ljudi čime se povećava rizik od pojave rezidua u mlijeku. S obzirom da karenca kod pojedinih lijekova može iznositi od nekoliko dana do nekoliko tjedana važno je vrijeme tretiranja stada. U 7 stada ovaca i 5 stada koza utvrđeno je da su tretirana neposredno prije poroda čime se javlja rizik od pojave rezidua u mlijeku u prvim danima i tjednima laktacije. U tri stada janjad se odvaja neposredno nakon poroda kako bi se dobila što veća količina mlijeka za preradu, utvrđeno je da je u takvim stadima rizik za konzumacijom rezidua antihelmintika veća u odnosu na stada gdje mužnja počinje nakon odbića janjadi. Istraživanjem je utvrđeno da većina uzgajivača lijekove za suzbijanje parazita nabavlja ilegalno u susjednim zemljama što predstavlja dodatni rizik jer se radi o lijekovima koji nisu registrirani u Republici Hrvatskoj. Jedziniak i sur. (2015) su utvrdili prisutnost antihelmintika u mlijeku ovaca i koza kao i u nekim mliječnim proizvodima. Za suzbijanje ektoparazita kao što su šugarci i krpelji koristi se isključivo preparat na bazi foksima pritom je u uputama za korištenje naglašeno da se ne smije koristiti za liječenje životinja čije se mlijeko koristi za prehranu ljudi. U četiri stada ovaca došlo je do uginuća 7-12 životinja tijekom laktacije pritom je kao uzrok utvrđeno trovanje bakrom. U liječenju je korišten amonijev tetramolibdenat čija je uporaba zabranjena na razini EU (Sargison, 2008). Jaiswal i sur. (2020) navode da nakon tretiranja ovaca amonijevim tetramolibdenatom karenca iznosi 10 dana ne navodeći za meso ili mlijeko. Haskell i sur. (2005) navode da je minimalna karenca za mlijeko, u slučaju da je korišten amonijev molibdenat, 5 dana kod mliječnih ovaca. U tri stada u Hrvatskoj nije se poštivalo vrijeme karance za amonijev molibdenat koje navode Haskell i sur. (2005) i Jaiswal i sur. (2020). Mikotoksinima u ovčjem i kozjem mlijeku posvećuje se značajno manje pozornosti u odnosu na prisutnost u kravljem mlijeku. Postoji objektivni rizik od kontaminacije mlijeka ovaca i koza u Hrvatskoj jer je 2023. zabilježen slučaj uginuća 6 ovaca na Rabu (Kostelić, 2023), a kao uzrok utvrđena je intoksikacija visokim količinama mikotoksina deoksinivalenola podrijetlom iz kukuruza. U prilog tome govori i istraživanje Bilandžić i sur.

(2014) koji su utvrdili prisutnost aflatoksina M1 u dva uzorka kozjeg mlijeka. S obzirom da se na otocima ovce često dohranjuju kukuruzom postoji opravdana sumnja za kontaminaciju mlijeka različitim vrstama mikotoksina u stadima koja se muzu. Kao što je navedeno u stadima u kojima se koristi strojna mužnja svakodnevno se koriste dezinficijensi i detergentsi prilikom pranja sustava za mužnju. U Tablici 1. prikazani su rezultati analiza mlijeka ovaca i koza na prisutnost inhibitornih tvari (antibiotici, dezinficijensi, detergentsi) u Središnjem laboratoriju za kontrolu mlijeka (HAPIH) u razdoblju od 2018-2022.

Tablica 1. Rezultati ispitivanja uzoraka mlijeka ovaca i koza na prisutnost inhibitornih tvari

Godina	Ovce		Koze	
	Ispitano uzoraka	Pozitivnih na inhibitore	Ispitano uzoraka	Pozitivnih na inhibitore
2018	1438	9	1192	7
2019	1428	3	1215	4
2020	1318	4	1155	2
2021	1224	3	1069	5
2022	1121	9	995	1

Iz Tablice 1. vidljivo je da udio ovčjeg i kozjeg mlijeka u kojem je utvrđena prisutnost inhibitornih tvari vrlo mala iako i kao takva predstavlja rizik za zdravlje ljudi. U jednom stadu koza uključenom u istraživanje utvrđeno je da je do kontaminacije mlijeka detergentima došlo zbog kvara sustava za ispiranje nakon mužnje. Poseban problem predstavljaju neregistrirane sirane jer se u njima ne provodi nadzor vezan uz higijensku i zdravstvenu ispravnost mlijeka i mliječnih proizvoda.

Zaključak

Na temelju preliminarnih istraživanja i dostupnih literaturnih podataka možemo zaključiti da u Hrvatskoj postoji objektivan rizik od kontaminacije mlijeka ovaca i koza različitim vrstama rezidua. Poseban problem predstavljaju rezidue čija se prisutnost ne kontrolira sustavno ili se ne kontrolira uopće kao što je slučaj sa mikotoksinima, hormonima i lijekovima za suzbijanje unutarnjih i vanjskih parazita. Otežavajuća okolnost je da u Hrvatskoj veliki dio lijekova koji se koriste u ovčarstvu i kozarstvu nije registriran za životinje čije se mlijeko koristi u prehrani ljudi te nisu poznati podaci o karenci za mlijeko. Kako bi se izbjegla kontaminacija mlijeka na farmama koje koriste strojnu mužnju potrebno je redovito kontrolirati sustav za ispiranje i pritom se pridržavati uputa proizvođača o pravilnoj primjeni dezinficijensa i detergenata. U svrhu boljeg nadzora higijenske i zdravstvene ispravnosti kozjeg mlijeka potrebno je periodički provoditi analizu na prisutnost potencijalno opasnih rezidua koje nisu navedene u pravilniku. Posebna pozornost treba biti posvećena edukaciji proizvođača ovčjeg i kozjeg mlijeka o rizicima od kontaminacije mlijeka reziduama koje mogu ugroziti zdravlje ljudi, a naročito djece.

Literatura

- Akinyemi M.O., Braun D., Windisch P., Warth B. (2020). Assessment of multiple mycotoxins in raw milk of three different animal species in Nigeria. *Food Control*. 131.
- Bilandžić N., Božić Đ., Đokić M., Sedak M., Solomun Kolanović B., Kurtes Varenina I., Cvetnić Ž. (2014). Assessment of aflatoxin M1 contamination in the milk of four dairy species in Croatia. *Food Control*. 43: 18-21
- Chibundu N.E., Corteel J.M., Leboeuf B., Baril G. (1988). Artificial breeding of goats and kids induced to ovulate with hormones outside the breeding season. *Small Ruminant Research*. 1: 19-35.
- Chiesa L.M., Cesare F., Nobile M., Villa R., Decastelli L., Martucci F., Fontana M., Pavlovic R., Arioli F., Panseri S. (2021). Antibiotics and Non-Targeted Metabolite Residues Detection as a Comprehensive Approach toward Food Safety in Raw Milk. *Foods*. 10: 544.

- Corteel J.M., Leboeuf B., Baril G. (1988) Artificial breeding of goats and kids induced to ovulate with hormones outside the breeding season. *Small Ruminant Research*. 1: 19-35.
- Hadžiosmanović M., Mioković B., Njari B., Kozačinski L., Cvrtila Ž. (2002). Aktualna problematika veterinarsko-sanitarnog nadzora namirnica animalnog podrijetla. Veterinarski fakultet Sveučilišta u Zagrebu.
- Haskell S.R.R., Payne M., Webb A., Riviere J., Craigmill A.L. (2005). Antidotes in food animal practice. *JAVMA*. 226.
- Jaiswal S.K., Gupta R.K.S., Jaiswal S., Niyogi D. (2020). Copper Poisoning in Farm Animals: Diagnostic and Preventive Approaches. *Int.J.Curr.Microbiol.App.Sci*. 9(01): 2224- 2230.
- Jedziniak P., Olejnik M., Rola J.G., Szprengier-Juszkiewicz T. (2015). Anthelmintic residues in goat and sheep dairy products. *Bull Vet. Inst. Pulawy*. 59: 515-518.
- Kostelić A. (2023). Uginuće ovaca na Rabu uzrokovano mikotoksinom deoksinivalenol. Neobjavljeni podatci.
- Malahova L.S., Novopashina S.I. (2011). Stimulacija ohoty u koz v vesennij period [Stimulation of goat oestrus in spring]. *Sbornik nauchnyh trudov Vserossijskogo nauchno-issledovatel'skogo instituta ovcevodstva i kozovodstva*, 1 (4-1). Vol. 32-34.
- Mecitoglu Z., Topal O., Kacar Y., Batmaz H. (2017). Comparing the effects of treatment with ammonium molybdate versus ammonium molybdate and phenoxy-2-methyl-2-propionic acid on liver functions in natural copper poisoning of sheep. *Small Ruminant Research*. 93-96.
- Ministarstvo poljoprivrede (2020). Pravilnik o utvrđivanju sastava sirovog mlijeka. NN 136/2020.
- Sargison N. (2008). *Sheep Flock Health a Planned Approach*. Blackwell.

Potential residues in sheep and goat milk in Croatia

Abstract

In recent years, consumers and the food industry in the EU have been paying a lot of attention to food residues that potentially endanger human health. The aim of this research was to determine potentially dangerous residues in sheep and goat's milk in Croatia. The research was conducted in 15 flocks of sheep and 12 goats over three years. Information was taken from the breeders on the diseases that most often occur in their herds, the type of medicine and who treated it. In addition, data were also taken on the method of implementation of preventive procedures, which include the suppression of internal and external parasites. The research also used data from the Central Laboratory for Milk Quality Control of the Croatian Agency for Agriculture and Food. Based on preliminary research and available literature data, we can conclude that in Croatia there is an objective risk of contamination of sheep and goat milk with different types of residues. In order to better monitor the hygienic and health correctness of goat's milk, it is necessary to periodically carry out an analysis for the presence of potentially dangerous residues that are not specified in the rulebook. A special problem is represented by residues whose presence is not systematically controlled or is not controlled at all, as is the case with mycotoxins, hormones, drugs for controlling internal and external parasites, and cleaning and disinfecting agents for milking system.

Keywords: residues, milk, contamination, mycotoxins

Korištenje proteina sirutke u prehrani sportaša

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Sažetak

Pravilna prehrana je izuzetno važna u osoba koje se aktivno bave sportom. Osim zadovoljenja osnovnih potreba organizma za nutrijentima, ona mora sportašima osigurati i povoljan učinak na sportsku izvedbu te smanjiti predispoziciju za pojavu iscrpljenosti organizma, bolesti i ozljeda. Zbog toga, uz redovitu prehranu sportaši uzimaju različite dijetetske dodatke od kojih se najčešće koriste proteini sirutke budući da sadrže sve esencijalne aminokiseline koje poboljšavaju sintezu mišićnih proteina, odgovornih za formiranje i obnavljanje mišićne mase. Cilj ovog rada je prikazati nutritivnu vrijednost te potencijal korištenja proteina sirutke u prehrani sportaša.

Ključne riječi: proteini sirutke, dijetetski dodaci, prehrana sportaša

Uvod

Zbog izvrsnog aminokiselinskog sastava, lakoće apsorpcije i konzumiranja, proteini sirutke najčešći su dodatak prehrani sportaša, bilo rekreativaca ili osoba koje se profesionalno bave sportom. Oni sportašima osiguravaju brzi, visokokvalitetan izvor proteina i esencijalnih aminokiselina koji su ključni za sintezu mišićnog proteina odgovornog za formiranje i obnavljanje mišićne mase. Primjerice, za postizanje snažne stimulacije sinteze mišićnih proteina dovoljno je u organizam unijeti 25 g proteina sirutke odnosno 2,7 g leucina (Gorissen i sur., 2018.). Osim uobičajenih proteinskih prahova koji se prije konzumacije miješaju s vodom ili mlijekom ili se dodaju u različite oblike zobnih kaša, smoothieja i palačinki, danas se na tržištu mogu pronaći i „ready to eat“ proizvodi obogaćeni proteinima sirutke poput proteinskih pločica, pudinga i keksa. Ti se proizvodi uobičajeno koriste prije, tijekom i nakon sportskih izvedbi čijom se konzumacijom smanjuje mogućnost pojave iscrpljenosti organizma i sportskih ozljeda. Upravo je to razlog sve veće zastupljenosti dodataka u prehrani sportaša posljednjih godina.

Cilj ovog rada je prikazati nutritivnu vrijednost te potencijal korištenja proteina sirutke u prehrani sportaša.

Proteinski dodaci u prehrani sportaša

Zdrava i uravnotežena prehrana, često zanemarena, jedan je od najvažnijih čimbenika trenažnog procesa i postizanja sportskih rezultata. Tako, Matijević i Čutić (2016.) navode kako većina sportaša kao i opća populacija imaju nedostatnu i neadekvatnu količinu znanja o pravilnoj prehrani. Pravilna prehrana osim zadovoljenja osnovnih potreba organizma za nutrijentima, sportašima mora osigurati povoljan učinak na sportsku izvedbu te smanjiti mogućnost pojave iscrpljenosti organizma i ozljeda. Za funkcioniranje ljudskog organizma potreban je redovit unos hranjivih tvari čija količina raste s povećanjem tjelesne aktivnosti. To znači da su energetske potrebe aktivnih sportaša veće u odnosu na potrebe osoba koje prakticiraju sjedilački način života te umjereno aktivnih osoba. U tom smislu unesena hrana sportašu treba osigurati odgovarajući udio makro i mikronutrijenata (Jeukendrup i Gleeson, 2019). Ukoliko to nije moguće sportaši mogu posegnuti za mnogobrojnim dijetetskim dodacima od kojih su najčešći oni namjenjeni sportašima. Prema Lauritzen i Gjelstad (2023.) tzv. sportski dijetetski proizvodi uključuju najčešće proteine (primjerice aminokiseline razgranatog lanca (BCAA)) zatim ugljikohidrate (napitci) te njihove kombinacije (pločice, proteinski shakeovi).

Osim aktivnih sportaša te dodatke sve više konzumiraju i osobe koje se rekreativno bave sportom kao i „korisnici životnog stila“. Pod korisnicima životnog stila podrazumijevaju se osobe koje tjedno imaju manje od jednog treninga ili uopće ne treniraju, ali u svojoj prehrani koriste dodatke namijenjene sportašima (Karlund i sur., 2019). Pritom je važno navesti da osobe koje se rekreativno bave sportom ove dodatke u većini slučajeva koriste prema uputama koje

dobiju od prodavača ili putem web stranica pri čemu zanemaruju moguće posljedice do kojih može dovesti njihova neadekvatna konzumacija (Vasconcelos i sur., 2020). Upravo radi toga Saleh i Julien (2022) ukazuju na potrebu za značajnom ulogom zdravstvenih stručnjaka koji razmišljaju o kupnji ili korištenju dodataka prehrani u procjeni opravdanosti korištenja takvih proizvoda.

Europska agencija za sigurnost hrane (EFSA) dijetetske dodatke definira kao koncentrirane izvore hranjivih sastojaka, najčešće vitamina i minerala, ali i nekih drugih tvari s hranjivim ili fiziološkim učinkom koji se konzumiraju u određenim definiranim dozama (www.efsa.europa.eu). Na tržištu se najčešće nalaze u obliku tableta, kapsula, tekućina poznatog volumena i prašaka. Dodaci u prehrani sportaša prisutni na tržištu obuhvaćaju specijalizirane proizvode koji se koriste za unošenje jednog ili kombinacije više hranjivih sastojaka svakodnevno potrebnih tijelu kada ih nije praktično unijeti putem hrane. Najčešće su ti dodaci u obliku napitaka, gelova, pločica, tekućih obroka i praha u čijem se sastavu često nalaze i proteini sirutke. Dodaci na bazi proteina sirutke prihvaćeni su kao siguran dodatak i od strane Svjetske antidopinške agencije (Lam i sur., 2019).

Proteini sirutke

Prema Codex Alimentariusu (Codex STAN 289-1995) sirutka je tekući mliječni proizvod dobiven kao nusproizvod tijekom proizvodnje sira, kazeina ili sličnih proizvoda odvajanjem od grušta nakon zgrušavanja mlijeka i/ili proizvoda dobivenih od mlijeka djelovanjem enzima (sirila) ili kiseline. Sirutka u svom sastavu sadrži oko 4,86 g laktoze, 0,5 g proteina, i 0,06 g mliječne masti (Bendelja Ljoljić i sur., 2023). Upravo zbog svoje hranjive vrijednosti sirutka danas ima široku primjenu u prehrambenoj, a sve više se koristi i u farmaceutskoj industriji. Dodatno, zbog velikih količina sirutke koja nastaje nakon proizvodnje sira, ona predstavlja ekološki problem stoga je njeno iskorištavanje poželjno (Rako i sur., 2018).

Proteini sirutke tipični su globularni proteini koji imaju prilično jednaku raspodjelu niza neutralnih, nabijenih i nenabijenih te hidrofobnih i hidrofilnih aminokiselinskih ostataka (Chandrapala, 2018). Aminokiselinski profil ovih proteina znatno se razlikuje od aminokiselinskog profila kazeina. Proteini sirutke sadrže manje glutamina i prolina, a više aminokiselinskih ostataka u čijoj se strukturi nalazi sumpor (cistein i metionin). S obzirom na navedeni aminokiselinski sastav, imaju visoku biološku vrijednost te ih karakterizira lakoća njihove apsorpcije nakon konzumiranja. Navedenom najviše doprinosi udio slobodnih aminokiselina koji je četiri puta veći od onog u mlijeku pa ih ljudski organizam može lakše iskoristiti. β -laktoglobulin najzastupljeniji je protein sirutke, dobar je izvor peptida koji imaju širok spektar bioaktivnosti što znači da u tijelu djeluju antimikrobno, antihipertenzivno, antioksidativno, antikancerogeno i imunomodulatorno (Kalyankar i sur., 2016). α -laktalbumin osim što je bogat cisteinom sudjeluje u sintezi laktoze koja je izvor energije te se dovodi u vezu sa smanjenjem rizika od pojave nekih vrsta tumora (Ramos i sur., 2016). Proteini sirutke također uključuju imunoglobuline, albumine krvnog seruma, laktoperoksidazu i laktoferin.

Proteini sirutke na tržištu su prisutni u obliku koncentrata proteina sirutke, izolata proteina sirutke te hidrolizata sirutkinih proteina (Herceg i Režek, 2006). Koncentrat proteina sirutke dobiva se postupkom ultrafiltracije sirutke te naknadnim sušenjem retentata ili koncentrata. Koncentrat proteina sirutke je oblik sirutkinih proteina kojeg karakterizira najniža razina masti u usporedbi sa drugim dostupnim oblicima sirutke koji su našli svoju primjenu u prehrambenoj, ali i drugim industrijama. Osim smanjenog udjela masti, sadrži i reduciranu količinu laktoze, a najčešći udjel proteina iznosi 35 - 85% (O'Regan i sur., 2009). Koncentrat proteina sirutke karakterizira blagi do blago mliječni okus, a s obzirom da je bogat lizinom i aminokiselinama koje u svojoj strukturi sadrže sumpor može se koristiti kao dodatak prehrani koja se temelji primjerice na žitaricama koje karakterizira nedostatak lizina (Siso, 1996; Khaire i Gogate, 2019).

Izolat proteina sirutke je prah kod kojeg u suhoj tvari zaostaje i više od 90% proteina, a većinom se dobiva postupkom ionske izmjene. Kao takav sadrži više proteina te manji udio laktoze, mliječne masti i mineralnih tvari u usporedbi s koncentratom sirutkinih proteina. Karakterizira ga blagi do blago mliječni okus, a zbog povoljnog aminokiselinskog sastava ovaj pripravak pogodan je za konzumaciju u prehrani čiji je cilj smanjenje masnog tkiva, ali i kada je cilj izgradnja mišićne mase (Shankar i Bansal, 2013; Khaire i Gogate, 2019).

Hidrolizat proteina sirutke je proteinski prah dobiven procesom hidrolize pri čemu se proteini djelovanjem proteolitičkih enzima razgrađuju na peptide koji osim što su probavljiviji, njihovom konzumacijom smanjena je pojavnost alergijskih reakcija (Geiser, 2003; Khaire i Gogate, 2019). Hidrolizat u usporedbi s koncentratom i

izolatom proteina sirutke karakterizira bolja toplinska stabilnost, poboljšana probavljivost i apsorpcija, bolja svojstva emulgiranja i pjenjenja te duži rok trajnosti (Khan, 2013). Nedostatak ovog oblika proteina sirutke je gorčina koja je posljedica postupka hidrolize kroz koji prolaze proteini.

Učinak korištenja proteina sirutke na sportsku izvedbu

Proteinski dijetetski dodaci danas su jedan od najčešće konzumiranih dodataka u prehrani sportaša no posljednjih godina uobičajeno ih koriste i posjetitelji teretana te osobe koje prakticiraju aktivan način života (Samal i Samal, 2018). Primjerice, 2021. godine segment sportske hrane dominirao je globalnom prehrambenom industrijom s najvećim udjelom u prihodu više od 21,50% (<https://www.grandviewresearch.com/industry-analysis/whey-protein-market>).

Općenito, cilj je konzumacije proteinskih dodataka povećanje unosa hranjivih sastojaka u prehrani sportaša sa svrhom poboljšanja sportskih performansi te rast mišićne mase. Povišene fiziološke potrebe sportaša za proteinima javljaju se kod čestih, intenzivnih i duljih vježbanja radi održavanja odgovarajuće sinteze proteina, imunološke funkcije te proizvodnje energije. S obzirom da potrebe za proteinima rastu zajedno s povećanjem vremena i intenziteta treninga potrebno ih je unositi prije i nakon sportske izvedbe, ali i tijekom dana. To je važno kako bi se osigurala učinkovita opskrba esencijalnim aminokiselinama (Karlund i sur., 2019). U tom smislu proteini sirutke imaju važnu ulogu u sintezi proteina, povećanju čiste mišićne mase te metabolizmu ugljikohidrata koji su osnovni izvor energije za vrijeme treninga što rezultira poboljšanjem sportskih performansi (Vasconcelos i sur., 2020). Karakterizira ih visok udio esencijalnih aminokiselina (50%) te su bogat izvor aminokiselina razgranatog lanca (26%) (Lam i sur., 2019). Provedena su brojna istraživanja na temu dobrobiti konzumacije ovog dijetetskog dodatka. West i sur. (2017) svojim su istraživanjem utvrdili da brzo probavljivi proteini sirutke pojačavaju anabolizam što se može dovesti u vezu s poboljšanim izvedbama vježbi oporavka nakon intenzivnog treninga s otporom. Konzumacija proteina sirutke ima učinak na smanjenje povreda i poboljšanje tjelesnih performansi kod profesionalnih trkača maratona. Također je utvrđena i njihova uloga u fiziološkoj zaštiti, poboljšanju performansi u različitim vrstama aerobnih vježbi te porastu ukupne i mišićne mase (Huang i sur., 2017). Bergia i sur. (2018) proveli su meta-analizu utjecaja konzumacije proteina u kombinaciji sa ili bez smanjenja energetske unosa i treninga s otporom na promjenu tjelesne mase, nemasne mase tijela i masne mase tijela u žena. Na temelju provedene analize utvrdili su da dodatak proteina sirutke ima blagi utjecaj na povećanje nemasne mase tijela, ali bez utjecaja na masnu masu tijela odraslih žena. Pritom je povećanje nemasne mase tijela izraženije u kombinaciji treninga s otporom. Konzumacija proteina sirutke kao dodatka prehrani pokazala se učinkovitom i u osoba starije životne dobi. Finger i sur. (2014) proveli su analizu devet istraživanja (462 ispitanika) koji su uključivali osobe između 61. i 79. godine starosti. U tri istraživanja korištena količina proteina sirutke je temeljena na tjelesnoj masi sudionika dok su u ostalih šest istraživanja ispitanici konzumirali određenu dnevnu količinu sirutkinih proteina neovisno o masi tijela. Na temelju provedene analize, autori su zaključili da je konzumacija ovog dijetetskog dodatka u kombinaciji s treningom s otporom učinkovita kod postizanja povećanja nemasne mase tijela. Međutim, navode kako nema utjecaja na povećanje mišićne mase i snagu.

Fizička spremnost i sposobnost brzog oporavka izuzetno su važni za osobe koje se aktivno bave sportom. Primjerice, procjenjuje se da prilikom nogometnih utakmica oko 74,5% od ukupno potrošene energije dolazi iz aerobnog metabolizma dok anaerobni metabolizam čini samo 25,5% (Dheyongera i sur., 2016; Li i Sun 2019). Nogometaše pored izvrsne vještine i taktike treba karakterizirati i dobra aerobna izdržljivost te anaerobni sprint. Li i Sun (2019) ispitali su učinak proteina sirutke na aerobnu sposobnost nogometaša. Istraživanje je uključivalo 36 nogometaša nasumce podijeljenih u dvije skupine (kontrolna i pokusna). Kontrolna je skupina tijekom osmotjednog trenažnog procesa 1 sat prije i nakon treninga konzumirala čistu vodu dok je pokusna skupina unosila napitak od oligosaharida i proteina u omjeru 4:1 koji je sadržavao 25 g proteina. Također, ova skupina unosila je dodatno još 25 g proteina večer prije treninga. Na temelju dobivenih rezultata utvrđeno je da istovremeni unos oligosaharida i proteina sirutke može poboljšati anaerobne i aerobne tjelesne sposobnosti nogometaša i pospješiti fizički oporavak. Učinkovitost proteina sirutke na biokemijske parametre krvi uglavnom aminokiselina, kreatinin kinaze i mioglobina, a koji utječu na izvedbu i oporavak među sportašima potvrđena je i meta-analizom (Lam i sur., 2019).

Zaključak

Sportaši zbog povećane tjelesne aktivnosti imaju veće dnevne potrebe za energijom u odnosu na osobe koje se ne bave sportom. Ponekad, zbog životnog stila i nemogućnosti unosa tolikog volumena hrane potrebnog za zadovoljenje

tjelesnih potreba, posežu za dijetetskim dodacima od kojih su najzastupljeniji proteini sirutke. Temeljem brojnih znanstvenih istraživanja može se zaključiti da korištenje proteina sirutke kao dodatka u prehrani sportaša ima pozitivan učinak na poboljšanje sportskih performansi, snagu, smanjenje masne mase tijela i dr., ali uz prethodnu konzultaciju zdravstvenih stručnjaka i/ili nutricionista.

Literatura

- Bendelja Ljoljić D., Kalit S., Kazalac J., Dolencić Špehar I., Mihaljević Žulj M., Maslov Bandić L., Tudor Kalit M. (2023). The Potential of Using Istrian Albumin Cheese Whey in the Production of Whey Distillate. *Fermentation*. 9: 192.
- Bergia R.E., III, Hudson J.L., Campbell W.W. (2018). Effect of whey protein supplementation on body composition changes in women: a systematic review and meta-analysis. *Nutrition reviews*. 76: 539–551.
- Chandrapala J. (2018). Whey Wastes and Powders. *Microstructure of Dairy Products*. 261- 291.
- Codex Standard (1995). Standard for whey powders CXS 289-1995.
- Dheyongera G., Grzebyk K., Rudolf A.M., Sadowska E.T., Koteja P. (2016). The effect of chlorpyrifos on thermogenic capacity of bank voles selected for increased aerobic exercise metabolism. *Chemosphere*. 149: 383-390.
- Finger D., Goltz F.R., Umpierre D., Meyer E., Rosa L.H., Schneider C.D. (2015). Effects of protein supplementation in older adults undergoing resistance training: a systematic review and meta-analysis. *Sports medicine*. 45: 245–255.
- Garthe I., Ronald J., Maughan R.J. (2018). Athletes and Supplements: Prevalence and Perspectives. *International journal of sport nutrition and exercise*. 28:126-138.
- Geiser M. (2003). The wonders of whey protein. *NSCA's Performance Training Journal*. 2: 13-15.
- Gorissen S.H.M., Crombag J.J.R., Senden J.M.G., Huub Waterval W.A., Bierau J., Verdijk L.B., van Loon L.J.C. (2018). Protein content and amino acid composition of commercially available plant-based protein isolates. *Amino Acids*. 50:1685–1695.
- Herceg Z., i Režek A. (2006). Prehrambena i funkcionalna svojstva koncentrata i izolata proteina sirutke. *Mljekarstvo*. 56: 379-396.
- Huang W.C., Chang Y.C., Chen Y.M., Hsu Y.J., Huang C.C., Kan N.W., Chen S.S. (2017). Whey Protein Improves Marathon-Induced Injury and Exercise Performance in Elite Track Runners. *International Journal of Medical Sciences*. 14 : 648-654.
- Jeukendrup A., and Gleeson M. (2019). *Sport Nutrition- third edition*. Human Kinetics.
- Kalyankar S.D., Khedkar C.D., Patil A.M., Deosarkar S.S. (2016). Milk: Sources and Composition. In: *Encyclopedia of Food and Health* (Caballero, B., Finglas, P.M., Toldrá, F. (eds)). Academic Press, Elsevier publisher, London, UK, 741-747.
- Kårlund A., Gómez-Gallego C., Turpeinen A.M., Palo-Oja O.M., El-Nezami H., Kolehmainen M. (2019). Protein Supplements and Their Relation with Nutrition, Microbiota Composition and Health: Is More Protein Always Better for Sports people? *Nutrients*. 11: 829.
- Khair R.A., and Gogate P.R. (2019). Whey Proteins. In: *Proteins: Sustainable Source, Processing and Applications* (Galanakis, C.M. (ed)). Academic Press, Elsevier, United Kingdom, 193-223.
- Khan S.H. (2013). Whey protein hydrolysates: Techno-functional perspective. *Int. J. Applied Biol. Pharmaceut. Technol.* 4: 1-3.
- Lam F.C., Bukhsh A., Rehman H., Waqas M.K., Shahid N., Khaliel A.M., Elhanish A., Karoud, M., Telb A., Khan T.M. (2019). Efficacy and Safety of Whey Protein Supplements on Vital Sign and Physical Performance Among Athletes: A Network Meta-Analysis. *Frontiers in pharmacology*. 10: 317.
- Li J.W., and Sun L.L. (2019). Effect of Whey Protein on Aerobic Exercise Ability of Football Players. *Matrix Science Medica*. 3: 19-21.

- Matijević B., and Čutić, A. (2016). Značaj pravilne prehrane za očuvanje zdravlja sportaša i rekreativaca. 6. Međunarodni stručno-znanstveni skup, zaštita na radu i zaštita zdravlja. Zadar, Hrvatska.
- O'Regan J., Ennis M.P., Mulvihill D.M. (2009). Milk proteins. In: Handbook of Hydrocolloids (Phillips, G.O., Williams, P.A. (eds)). Woodhead Publishing Series in Food Science, Technology and Nutrition (Second Edition), Woodhead Publishing, Cambridge, UK, 298-358.
- Rako A., Tudor Kalit M., Kalit S., Soldo B., Ljubenković I. (2018). Nutritional characteristics of Croatian whey cheese (Bračka skuta) produced in different stages of lactation. *LWT*. 96: 657-662.
- Ramos O.L., Pereira R.N., Rodrigues R.M., Teixeira J.A., Vicente A.A., Malcata F.X. (2016). Whey and Whey Powders: Production and Uses. In: Encyclopedia of Food and Health (Caballero, B., Finglas, P.M., Toldrá, F. (eds)), Academic Press, Elsevier, Oxford, 498-505.
- Saleh K.K., and Julien S.G. (2022). Protein Supplement Perceptions, Use, and Associated Performance in Young Lebanese Resistance-Training Athletes. *Journal of nutrition and metabolism*. 4150620.
- Samal J.R.K., and Samal I.R. (2018). Protein Supplements: Pros and Cons. *Journal of Dietary Supplements*. 15: 365–371.
- Shankar J.R., and Bansal G.K. (2013). A study on health benefits of whey proteins. *International Journal of Advanced Biotechnology and Research*. 4: 15-19.
- Siso M.G. (1996). The biotechnological utilization of cheese whey: a review. *Bioresource technology*. 57: 1-11.
- Vasconcelos Q.D.J.S., Bachur T.P.R., Aragão G.F. (2020). Whey protein supplementation and its potentially adverse effects on health: a systematic review. *Applied Physiology, Nutrition, and Metabolism*. 46: 27-33.
- West D.W.D., Abou Sawan S., Mazzulla M., Williamson E., Moore, D.R. (2017). Whey Protein Supplementation Enhances Whole Body Protein Metabolism and Performance Recovery after Resistance Exercise: A Double-Blind Crossover Study. *Nutrients*. 9: 735.
- <https://www.grandviewresearch.com/industry-analysis/whey-protein-market>(pristupljeno 22.listopada 2023).
- www.efsa.europa.eu (pristupljeno 31.svibnja 2021).

Use of whey protein in athletes' nutrition

Abstract

Proper nutrition is extremely important for people who are actively involved in sports. In addition to satisfying the body's basic needs for nutrients, it must provide athletes with a favorable effect on sports performance and reduce the predisposition to the appearance of body exhaustion, illness and injuries. For this reason, in addition to regular nutrition, athletes take various dietary supplements, of which whey proteins are most often used since they contain all the essential amino acids that improve the synthesis of muscle proteins, responsible for the formation and restoration of muscle mass. The aim of this paper is to show the nutritional value and the potential of using whey protein in the nutrition of athletes.

Keywords: whey proteins, dietary supplements, athlete's nutrition

Preservation of Bioactive Compounds of Fresh Nettle Leaves by Modified Atmosphere

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Abstract

Fresh nettle leaves are a rich source of bioactive compounds while their preservation after harvest is a major challenge. The aim of this study was to determine the content of selected bioactive compounds and antioxidant capacity of cultivated fresh nettle leaves packed in different packaging materials (BOPP and LDPE) in a modified atmosphere (MA1 – 5/10% O₂/CO₂, MA2 – 5/5% O₂/CO₂) during a storage period of 9 days. The samples packed in MA1 had the highest AsA content in both packaging materials. Total phenolic content was best preserved in BOPP material packed in MA1. All samples were characterized by a high antioxidative capacity. The results indicate that the use of modified atmosphere and suitable packaging material can significantly contribute to the preservation of bioactive compounds in fresh nettle leaves.

Keywords: *Urtica dioica* L., antioxidants, packaging in modified atmosphere, packaging materials, storage.

Introduction

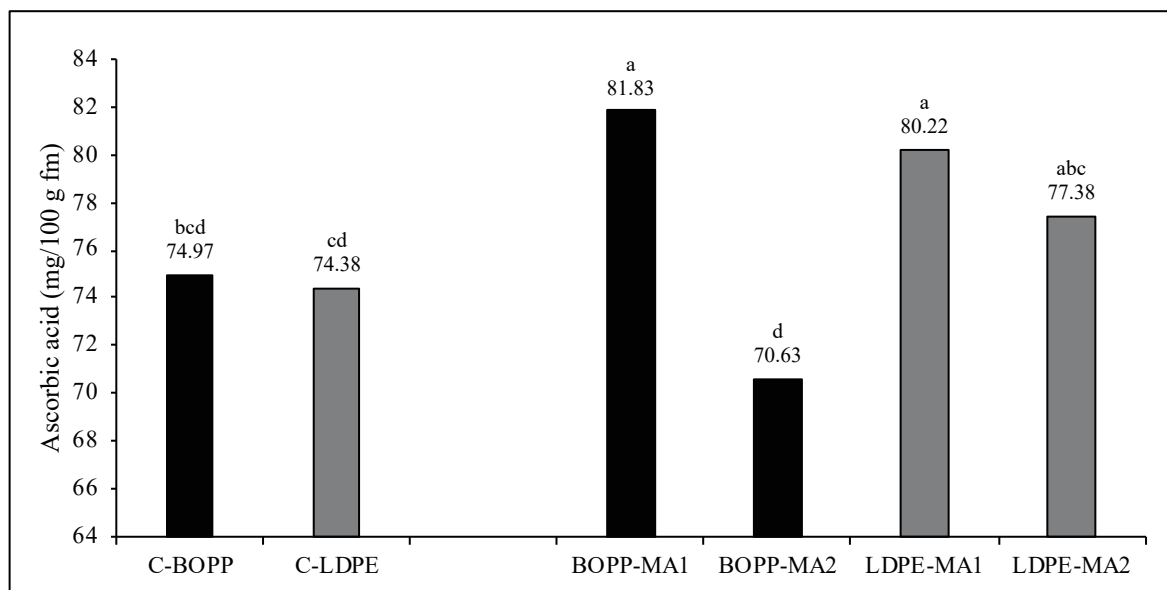
Numerous studies (Grauso et al., 2020; Repajić et al., 2021; Bhusal et al., 2022; Dujmović et al., 2023) have been conducted to determine the phytochemical composition responsible for the beneficial effects of nettle (*Urtica dioica* L.) on human health. This plant has been shown to have antioxidant and antimicrobial properties (Bhusal et al., 2022), which are associated with various bioactive compounds present in nettle, making it suitable for use in the pharmaceutical and food industries (Hulina, 2011). Nettle leaves contain a significant amount of ascorbic acid and phenolic compounds that are considered the most important antioxidants in neutralizing free radicals in the human body (Zehiroglu and Ozturk Sarikaya, 2019). In European countries, nettle is used to prepare various dishes such as cream soups, vegetable cakes, or aqueous herbal infusions (Said et al., 2015). However, there is still a lack of research on the possibility of packaging fresh nettle leaves like other green leafy vegetables. In the food industry, packaging of leafy vegetables is still a great challenge because numerous factors such as respiration, transpiration, and microbial development can disrupt organoleptic properties, accelerate spoilage of plant material, and increase degradation of bioactive compounds (An et al., 2009; Mogren et al., 2018; Schmitz et al., 2019). Therefore, hygienic conditions, temperature, relative humidity, gas composition, and packaging materials are very important during the storage period of packaged leafy vegetables. A modified atmosphere implies changes in gas proportions in a closed packaging with often reducing oxygen (O₂) content and increasing carbon dioxide (CO₂) content. Changes in O₂/CO₂ proportions reduce the respiration of plant material, slow down aging and spoilage, and extend the shelf life of fresh vegetables (Ščetar et al., 2010, Mattos et al., 2012). In view of the abovementioned, possible solutions for preserving nettle antioxidants as long as possible can be found in the packaging and storage under appropriate conditions. Therefore, the aim of this research was to determine the influence of different packaging materials and modified atmospheres on the preservation of AsA, phenolic compounds, and antioxidant capacity of nettle leaves during a storage period of 9 days.

Material and methods

A fresh nettle leaf used for packaging and analyses was grown in an ebb and flow hydroponic system at the University of Zagreb Faculty of Agriculture. Freshly harvested leaves at the pre-flowering phase (March 2023) were packed in a modified atmosphere (KM 20-3 (Germany), Dorado, Junior Digit (Italy)) at the University of Zagreb Faculty of Food Technology and Biotechnology. The plant material was packaged in two different materials: biaxially oriented polypropylene (BOPP) (Petruzalek d.o.o. Zabok, Croatia) and low-density polyethylene (LDPE) (Helana-pak d.o.o. Zagreb, Croatia) and two different gas compositions: MA1 – 5% O₂ and 10% CO₂, and MA2 – 5% O₂ and 5% CO₂. Control samples (C) were packed in both packaging materials under normal atmospheric conditions (21% O₂, 0.04% CO₂, and 78% N₂). Packaged nettle samples were stored in a cold chamber (T-05-1, MBFRIGO AE202017210) for 9 days at 4 ± 2 °C and relative air humidity of 60%. The content of selected bioactive compounds was determined. The AsA content (mg/100 g of fresh mass (fm)) was determined using a standard titration method (AOAC, 2002). Total phenolic compounds, total flavonoids (mg GAE/100 g fresh mass), and non-flavonoids (mg CTH/100 g fresh mass) were determined spectrophotometrically by the Folin–Ciocalteu colorimetric method according to Ough and Amerine (1988). The antioxidant capacity was determined spectrophotometrically using the ABTS method according to Miller et al. (1993). All data were analysed using the ANOVA procedure, and when differences between treatments were significant, Duncan's post hoc test was used to separate the means at $p \leq 0.05$. Statistics were performed by SAS software version 9.4. (SAS Institute Inc., USA).

Results and discussion

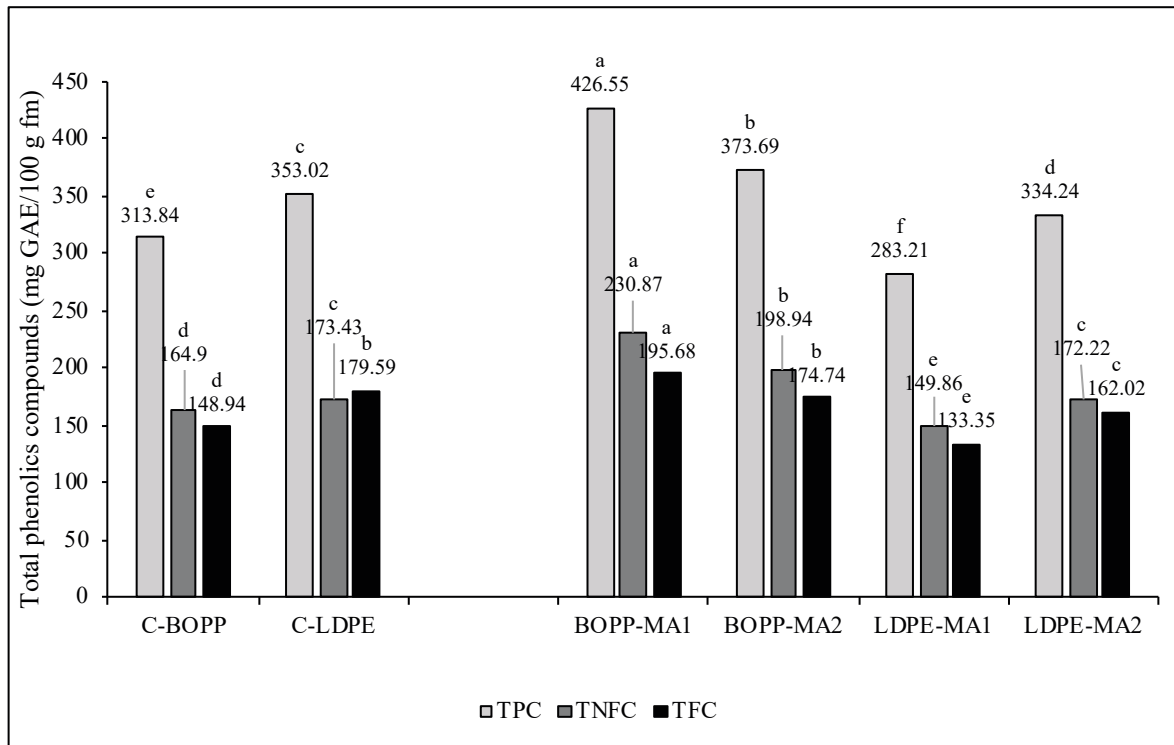
Ascorbic acid (AsA) is an essential vitamin that has potent antioxidant properties, contributes to immune defense, and is ingested through food (Carr et al., 2017). Being an unstable compound sensitive to abiotic factors, its preservation in harvested plant material is very demanding (Giannakourou et al., 2021). Therefore, in order to reduce its decomposition it is important to optimize storage conditions (Gil et al., 1999). The results of the AsA content in packaged fresh nettle leaves stored for 9 days are shown in Graph 1. The highest content of AsA was determined when MA1 (5% O₂ / 10% CO₂) was used with no influence of packaging material (81.83 and 80.22 mg/100 g for BOPP and LDPE, respectively). BOPP-MA1 and LDPE-MA1 had 9.2% and 7.9% more AsA than their matching control samples. Nettle leaves packed in BOPP with MA2 (5% O₂ / 5% CO₂) had the lowest AsA content. The AsA content of fresh nettle leaves previously reported in the scientific literature varies significantly with values ranging from 16.00 to 112.80 mg/100 g (Radman et al., 2015; Shonte et al., 2020; Dujmović et al., 2023). However, and to our knowledge, no available literature data exists on the influence of modified atmosphere and type of used packaging materials on AsA changes in packaged nettle leaves. The AsA values determined in this study are consistent with the results found in the aforementioned research with no differences compared to fresh plant material. Similar to the present study, Mathooko and Vilane (2003) and Moretti et al. (2003) reported that the content of AsA in spinach and collard leaves was well preserved when packed in modified atmosphere. The results of the present study show that higher CO₂ and lower O₂ levels can slow down the degradation of AsA in nettle leaves compared to normal atmosphere for samples packed in BOPP and LDPE packages. In addition, with appropriate packaging method, it is possible to preserve AsA content in packed nettle leaves for 9 days.



Graph 1. The content of ascorbic acid in fresh nettle leaves packaged in different packaging materials under modified atmosphere conditions.

C-BOPP – control sample with normal atmosphere packed in BOPP, C-LDPE – control sample with normal atmosphere packed in LDPE, MA1 – 5% O₂ and 10% CO₂, MA2 – 5% O₂ and 5% CO₂. The results are expressed as means and different letters in data labels (a-d) indicate significant differences between mean values.

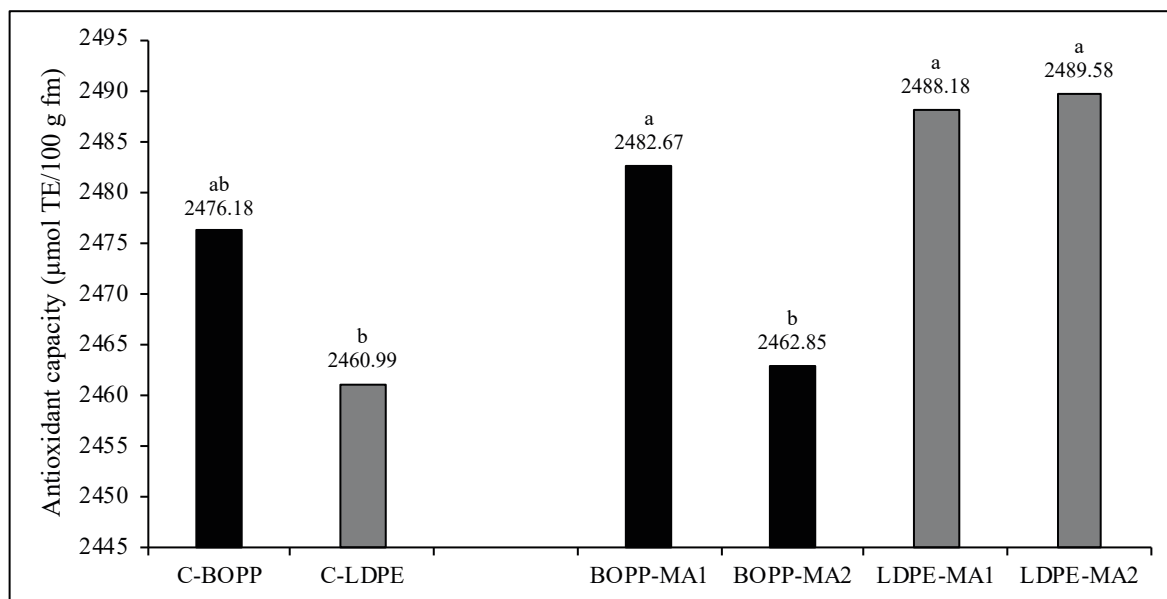
Phenols are compounds with significant antioxidant and antimicrobial properties that are important for both plants and humans (Zeb, 2020). The content of flavonoids and phenolic components in packaged leaves is presented in Graph 2. Regardless of gas composition, the BOPP material proved to be better at preserving all phenolic compounds, while regardless of material, nettle leaves packaged under modified atmosphere conditions contained more phenolic compounds compared to control samples. The content of all phenolic compounds (total phenolics, flavonoids, and non-flavonoids) was best preserved under modified atmosphere conditions with a gas composition of 5% O₂ and 10% CO₂ (MA1) packed in BOPP (426.55 and 230.87 mg GAE/100 g and 195.68 mg CTH/100 g, respectively). After 9 days of storage in BOPP-MA1, total phenolic compounds were 36% higher than in the control sample (C-BOPP). Some other studies have shown that elevated CO₂ increased the content of phenolic compounds and flavonoids in plant material (Ghasemzadeh and Jaafar, 2011, Ibrahim and Jaafar, 2012; Sgherri et al., 2017), which is also confirmed by the results of this research. This behavior may be due to the fact that phenolic compounds are derived from the phenylpropanoid pathway, and elevated CO₂ can increase phenylalanine lyase (PAL) activity which consequently can improve the production of total phenolics and flavonoids (Ibrahim and Jaafar, 2012). In addition, lower O₂ can slow down the enzymatic and oxidative degradation of phenolic compounds as suggested by some authors (Cheng et al., 2009; Kim et al., 2021).-



Graph 2. Total phenolic (TPC), total non-flavonoid (TNFC), and total flavonoid content (TFC) of fresh nettle leaves packaged in different packaging materials under modified atmosphere.

C-BOPP – control sample with normal atmosphere packed in BOPP, C-LDPE – control sample with normal atmosphere packed in LDPE, MA1 – 5% O₂ and 10% CO₂, MA2 – 5% O₂ and 5% CO₂. Results are expressed as means and different letters in data labels (a-f) indicate significant differences between mean values.

Regardless of the used material, nettle leaves packed under modified atmosphere manifested higher antioxidant capacity (Graph 3) than leaves packed under normal atmosphere. The highest antioxidant capacity was recorded in most of the samples with modified atmosphere, except for BOPP-MA2, while BOPP-MA1 and BOPP-MA2 did not statistically differ from their control sample (C-BOPP). In research by Modau et al. (2018), the antioxidant capacity of baby spinach leaves was well maintained under modified atmosphere at 9 days of storage, which is consistent with the results obtained in the present study. Elevated CO₂ can enhance antioxidant enzyme activity (Liang et al., 2021) and reduce oxidative stress (Abdelgawad et al., 2016), while lowering O₂ levels also slows down the oxidative processes, which helps to maintain the antioxidant capacity of plant material, explaining obtained results.



Graph 3. Antioxidant capacity of fresh nettle leaves packaged in different packaging materials under modified atmosphere conditions.

C-BOPP – control sample with normal atmosphere packed in BOPP, C-LDPE – control sample with normal atmosphere packed in LDPE, MA1 – 5% O₂ and 10% CO₂, MA2 – 5% O₂ and 5% CO₂. Results are expressed as means and different letters in data labels (a-b) indicate significant differences between mean values.

The significance of the influence of individual factors and their interaction on the content of bioactive compounds and antioxidant capacity of packaged nettle leaves is given in Table 1. Different gas compositions had a significant influence on the AsA content, while a selection of packaging materials and the interaction of tested factors (M x MA) had no effect on the AsA content. The factors and their interaction had a strong effect on the phenolic compounds, except for total flavonoids, which were not affected by different modified atmosphere compositions. As for the antioxidant capacity, the different materials had no influence, but the gas composition and the combination of material and gas influenced the antioxidant capacity.

Table 1. Significance of factors and their interactions ($p \leq 0.05$)

Factors	AsA	TPC	TNFC	TFC	Ant_cap
M	ns	≤ 0.0001	≤ 0.0001	≤ 0.0001	ns
MA	0.004	≤ 0.0001	≤ 0.0001	ns	0.0176
M x MA	ns	≤ 0.0001	≤ 0.0001	≤ 0.0001	0.0045

M – packaging material, MA – gas composition of modified atmosphere, M x MA – interaction of packaging material and gas composition of modified atmosphere, AsA – ascorbic acid, TPC – total phenolic content, TNFC – total non-flavonoid content, TFC – total flavonoid content, Ant_cap – antioxidant capacity, ns – non-significant.

Conclusion

In conclusion, the highest AsA content was measured in samples packed in MA1, no matter the used material, while the highest total phenolic compounds content was found in BOPP-MA1, and the highest antioxidant capacity in BOPP-MA1, LDPE-MA1, and LDPE-MA2. The results show that the use of an MA and suitable packaging material can significantly contribute to the preservation of certain bioactive compounds and antioxidant capacity of fresh nettle leaves during the storage period of 9 days. Finally, it should be emphasized that in order to preserve its antioxidants for as long as possible, it is advisable to pack and store fresh nettle leaves under appropriate conditions.

Acknowledgment

The results presented in the paper are output from research project IP-2019-04-3325 URTICA-BioFuture „Nutritional and functional value of nettle (*Urtica dioica* L.) by application of modern hydroponic cultivation techniques“ financed by the Croatian Science Foundation (Hrvatska zaklada za znanost—www.hrzz.hr).

References

- AbdElgawad H., Zinta G., Beemster G.T.S., Janssens I.A., Asard H. (2016). Future Climate CO₂ Levels Mitigate Stress Impact on Plants: Increased Defense or Decreased Challenge? *Frontiers in Plant Science*. 7.
- An D.S., Park E., Lee D.S. (2008). Effect of hypobaric packaging on respiration and quality of strawberry and curled lettuce. *Postharvest Biology and Technology*. 52: 78-83.
- AOAC (2002). Official methods of Analysis (17th ed.). Association of Official Analytical Chemists. Washington, USA.
- Bhusal K.K., Magar S.K., Thapa R., Lamsal A., Bhandari S., Maharjan R., Shrestha S., Shrestha J. (2022). Nutritional and pharmacological importance of stinging nettle (*Urtica dioica* L.): A review. *Heliyon*. 8: e09717.
- Carr A.C., and Maggini S. (2017). Vitamin C and Immune Function. *Nutrients*. 9: 1211.
- Cheng G., Jiang Y., Duan X., Macnish A.J., You Y. (2009). Effect of oxygen concentration on the biochemical changes of stored longan fruit. *Journal of Food Quality*. 32: 2–17.
- Dujmović M., Opačić N., Radman S., Fabek Uher S., Voća S., Šic Žlabur J. (2023). Accumulation of Stinging Nettle Bioactive Compounds as a Response to Controlled Drought Stress. *Agriculture*. 13: 1358.
- Ghasemzadeh A., and Jaafar H.Z.E. (2011). Effect of CO₂ Enrichment on Synthesis of Some Primary and Secondary Metabolites in Ginger (*Zingiber officinale* Roscoe). *International Journal of Molecular Sciences*. 12: 1101-1114.
- Giannakourou M.C., and Taoukis P.S. (2021). Effect of Alternative Preservation Steps and Storage on Vitamin C Stability in Fruit and Vegetable Products: Critical Review and Kinetic Modelling Approaches. *Foods*. 10: 2630.
- Gil M.I., Ferreres F, Tomas-Barberan F.A. (1999). Effect of postharvest storage and processing on the antioxidant constituents (flavonoids and vitamin C) of fresh-cut spinach. *Journal of Agriculture, Science and Technology*. 47: 2213-2217.
- Grauso L., Falco B., Lanzotti V., Motti R. (2020). Stinging nettle, *Urtica dioica* L.: botanical, phytochemical and pharmacological overview. *Phytochemistry Reviews*. 19: 1341-1377.
- Ibrahim M. H., and Jaafar H. Z. (2012). Impact of elevated carbon dioxide on primary, secondary metabolites and antioxidant responses of *Eleais guineensis* Jacq. (oil palm) seedlings. *Molecules*. 17: 5195–5211.
- Kim A-N., Lee K-Y., Rahman M.S., Kim H-J., Kerr W.L., Choi S-G. (2021). Thermal treatment of apple puree under oxygen-free condition: Effect on phenolic compounds, ascorbic acid, antioxidant activities, color, and enzyme activities. *Food Bioscience*. 39: 100802.
- Liang Z., Luo Z., Li W., Yang M., Wang L., Lin X., Li L. (2021). Elevated CO₂ Enhanced the Antioxidant Activity and Downregulated Cell Wall Metabolism of Wolfberry (*Lycium barbarum* L.). *Antioxidants*. 11: 16.
- Mathooko F.M., and Vilane B.R.T. (2003). Effect of Modified Atmosphere Packaging under Ice Cooling on the Postharvest Storage Life and Quality of Spinach (*Spinacea oleracea* L) Leaves. *Journal of Agriculture, Science and Technology*. 5: 61-79.
- Miller N.J., Diplock A.T., Rice-Evans C., Davies M.J., Gopinathan V., Milner A. (1993). A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clinical science*. 84: 407–412.

- Mogren L., Windstam S., Boqvist S., Vågsholm I., Söderqvist K., Rosberg A.K., Lindén J., Mulaosmanovic E., Karlsson M., Uhlig E., Håkansson Å., Alsanus B. (2018). The Hurdle Approach—A Holistic Concept for Controlling Food Safety Risks Associated With Pathogenic Bacterial Contamination of Leafy Green Vegetables. A Review. *Frontiers in Microbiology*. 24: 1965.
- Moretti C.L.; Araujo A.L., Mattos L.M. (2003). Evaluation of different oxygen, carbon dioxide and nitrogen combinations employed to extend the shelf life of fresh-cut collard greens. *Horticultura Brasileira*. 21: 676-680.
- Mudau A.R., Soundy P., Araya H.T., Mudau F.N. (2018). Influence of Modified Atmosphere Packaging on Postharvest Quality of Baby Spinach (*Spinacia oleracea* L.) Leaves. *HortScience*. 53: 224-230.
- Ough C.S., and Amerine M.A. (1988). *Methods for Analysis of Musts and Wines*, 2nd ed.; John Wiley & Sons: New York, NY, USA.
- Repajić M., Cegledi E., Zorić Z., Pedisić S., Elez Garofulić I., Radman S., Palčić I., Dragović-Uzelac V. (2021). Bioactive Compounds in Wild Nettle (*Urtica dioica* L.) Leaves and Stalks: Polyphenols and Pigments upon Seasonal and Habitat Variations. *Foods*. 10: 190.
- Schmitz F.R.W., Carvalho L.F.D., Bertoli S.L., Souza C.K. (2019). Effect of refrigerated storage conditions on leafy vegetables. *MOJ Food Processing & Technology*. 7: 75-77.
- Sgherri C., Pérez-López U., Micaelli F., Miranda-Apodaca J., Mena-Petite A., Muñoz-Rueda A., Quartacci M.F. (2017). Elevated CO₂ and salinity are responsible for phenolics-enrichment in two differently pigmented lettuces. *Plant Physiology and Biochemistry*. 115: 269-278.
- Shonte T.T., Duodu K.G., de Kock H.L. (2020). Effect of drying methods on chemical composition and antioxidant activity of underutilized stinging nettle leaves. *Heliyon*. 6: e03938.
- Zeb A. (2020). Concept, mechanism, and applications of phenolic antioxidants in foods. *Journal of Food Biochemistry*. 44: e13394.
- Zehiroglu C., and Ozturk Sarikaya S.B. (2019). The importance of antioxidants and place in today's scientific and technological studies. *Journal of Food Science and Technology*. 56: 4757-4774.

Utjecaj kvalitete mlijeka i tehnoloških postupaka proizvodnje na randman sira

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Sažetak

Cilj ovog rada je dati pregled čimbenika koji određuju randman u proizvodnji sira. Poznavanje vrijednosti randmana i čimbenika koji ga određuju može uvelike odrediti profitabilnost sirarske proizvodnje. Kvaliteta mlijeka i gotovo sve tehnološke faze proizvodnje mogu utjecati na randman sira. Najznačajniji utjecaj imaju hlađenje, standardizacija, homogenizacija, toplinska obrada i koagulacija mlijeka, količina i vrsta mljekarskih kultura i sirila, kiselost i obrada sirnog gruša te soljenje, prešanje i zrenje sira. Randman u sirarstvu je izuzetno važan uzevši u obzir da cijena sirovine čini i do 80% troškova proizvodnje. Međutim, treba biti oprezan jer korekcija nekih tehnoloških postupaka u cilju povećanja randmana može umanjiti kvalitetu sira, a time i profitabilnost.

Ključne riječi: sir, randman, tehnologija, kvaliteta mlijeka

Uvod

Osim ujednačene kvalitete gotovog proizvoda, konačni cilj tehnologije proizvodnje sira je zadovoljavajući prinos odnosno randman u proizvodnji sira. Randman u sirarstvu je izuzetno važan uzevši u obzir da cijena sirovine (mlijeka za proizvodnju sira) čini i do 80% troškova proizvodnje te je stoga cilj sirarske industrije prepoznati kako učinkovitije prerađivati mlijeko u sir (Havranek i sur., 2014). Stvarni randman sira definira se kao broj utrošenih kilograma mlijeka za proizvodnju jednog kilograma sira ili broj kilograma sira proizvedenih iz 100 kg mlijeka za sirenje. Osim stvarnog randmana koji se jednostavno dobiva vaganjem količine utrošenog mlijeka i količine proizvedenog sira, te stavljanjem te dvije vrijednosti u odnos, u sirarstvu se koriste i naravnati i teoretski randman. Važnost tih randmana ogleda se u činjenici da je randman promjenljiv ovisno o vrsti sira odnosno o njegovom sastavu kao i sastavu mlijeka za sirenje. Prema tome, naravnati randman uzima u obzir sadržaj vode i soli u siru, dok je za izračun teoretskog randmana važan udio kazeina i masti u mlijeku za sirenje. Omjer naravnatog i teoretskog randmana predstavlja efikasnost randmana (McSweeney, 2007; Abd El-Gawad i Ahmed, 2011; Havranek i sur., 2014).

Kemijski sastav mlijeka za sirenje važan je segment proizvodnje sira koji izravno utječe na randman sira kao i na učinkovitost proizvodnje, a time i na profitabilnost (Kalit i sur., 2021). Male razlike u randmanu sira pretvaraju se u velike razlike u dobiti. Osim kvalitete mlijeka, tehnološki postupci prerade mlijeka u sir od presudne su važnosti za količinu proizvedenog sira (McSweeney, 2007; Abd El-Gawad i Ahmed, 2011; Havranek i sur., 2014). Cilj ovog rada dati pregled čimbenika koji određuju randman u proizvodnji sira, odnosno prikazati utjecaj kvalitete mlijeka te pojedinih tehnoloških postupaka prerade mlijeka u sir.

Utjecaj kvalitete mlijeka na randman u proizvodnji sira

Proizvodnja sira je u suštini postupak prevođenja najvrjednijih sastojaka iz mlijeka u sir. Tu prije svega mislimo na prevođenje mliječne masti i proteina (kazein) u sir. Količina proteina i mliječne masti izravno utječe na kvalitetu sira, randman proizvodnje i iskoristivost masti i proteina iz mlijeka u sir (Vacca i sur., 2020).

Mliječna mast je najvarijabilniji sastojak mlijeka. Sadržaj mliječne masti u mlijeku ponajprije ovisi o pasmini, stadiju laktacije, stadiju mužnje i hranidbi. Što je neka pasmina ovaca ili koza mlječnija, u pravilu je mlijeko takve pasmine nižeg sadržaja mliječne masti. U početku laktacije sadržaj masti u mlijeku je niži, a kako laktacija napreduje sadržaj

masti u mlijeku se postepeno povećava. Stoga će randman sira biti veći ukoliko sirimo mlijeko manje mliječnih pasmina u kasnijoj laktaciji. Kad govorimo o stadiju mužnje, valja naglasiti da je mlijeko u početku mužnje manje masno od onog pod kraj mužnje. Od svih paragenetskih čimbenika, hranidba najviše utječe na sadržaj masti u mlijeku za sirenje, prije svega kroz unos voluminoznih krmiva bogatih vlaknima kao što su sijeno, djetelina, zrela paša itd. Takva voluminozna krmiva bogata su celulozom koja fermentacijom u buragu prelazi u spojeve koji predstavljaju prekursore za mliječnu i tjelesnu mast u organizmu preživača. U tom smislu problemi s niskim randmanom sira nastaju kada preživačima ne osiguramo dovoljno vlaknastih krmiva (nedostatak sijena zbog suše ili nekih drugih razloga) (Grbeša i Samaržija, 1994; Chamberlain i Wilkinson, 1998).

Iako sadržaj proteina (kazeina) u mlijeku varira ovisno o istim paragenetskim parametrima o kojima ovisi i varijacije u sadržaju masti, proteini mlijeka manje su varijabilan parametar od masti u mlijeku. Dok sadržaj masti u mlijeku ovisi o unosu vlakana u organizam preživača, sadržaj proteina ovisi o unosu energetskih, visoko-koncentriranih krmiva bogatih škrobom i proteinima (dušikom) kao što su različite žitarice (kukuruz, pšenica, ječam i zob) ili sojina sačma. Na sadržaj proteina u mlijeku povoljno utječe i unos svježe trave i leguminoze u organizam preživača. Prema tome, smanjenje proteina u obroku preživača, a time i sniženi randman sira, se događa ako su životinje energetski pothranjene ili je unos krme bogate proteinima (dušikom) nedostatan. Oslanjanje isključivo na pašu može u pojedinim dijelovima godine imati za posljedicu manjak masti i proteina u mlijeku za sirenje, a time i niži randman (Grbeša i Samaržija, 1994; Chamberlain i Wilkinson, 1998).

Za razliku od krava, iako ne postoje standardi za broj somatskih stanica u mlijeku ovaca i koza, nagli skok broja somatskih stanica u mlijeku preživača u pravilu upućuje na narušeno zdravlje vimena. Poremećaj sekrecije mlijeka kao posljedica stresa i narušeno zdravlje vimena kao posljedica infekcije i posljedično tome razvoja upalno-patogenih procesa u vimenu mogu uvjetovati smanjenje udjela masti i proteina, naročito kazeina (najvažnijeg proteina za proizvodnju sira) u mlijeku. Naime infekcijom vimena i pojavom upalno patogenih procesa u njemu smanjuje se udio onih sastojaka mlijeka koji se sintetiziraju u vimenu, a raste udio onih sastojaka mlijeka koji u mlijeko dopijevaju krvlju. Tako se sadržaj kazeina i masti smanjuje, a raste sadržaj dijela sirutkinih proteina koji u mlijeko dopijevaju krvlju kao što su albumin krvnog seruma i neki imunoglobulini (Jaeggi i sur., 2003; Abd El-Gawad i Ahmed, 2011; Bobbo i sur., 2016; Marti-DeOlives i sur., 2020).

Osim što se u mlijeku s povećanim brojem somatskih stanica smanjuje udio najvrjednijih sastojaka mlijeka za proizvodnju sira, mijenja se i njihov sastav tako da im je iskoristivost u preradi manja što rezultira otežanim izdvajanjem sirutke i sniženim randmanom, a dobiveni sir je lošijih senzorskih svojstava (Chen i sur., 2010; Bobbo i sur., 2016; Vacca i sur., 2020).

Utjecaj tehnoloških postupaka na randman sira

Gotovo sve tehnološke faze proizvodnje sira mogu utjecati na njegov randman. Najznačajniji utjecaj imaju hlađenje, standardizacija, homogenizacija, toplinska obrade i koagulacija mlijeka, količina i vrsta korištenih mljekarskih kultura i sirila, brzina zakiseljavanja i krajnja kiselost (pH vrijednost) gruš, obrada sirnog gruš, te soljenje, prešanje i zrenje sira. Sukladno tome različite kategorije sira s obzirom na vrstu mlijeka i udio vode u bezmasnoj tvari sira imaju različiti randman (Tablica 1).

Tablica 1. Potrebna količina mlijeka (kg) za proizvodnju 1 kg sira s obzirom na vrstu mlijeka i udio vode u bezmasnoj tvari sira

Naziv sira s obzirom na udio vode u bezmasnoj tvari sira	Vrsta mlijeka		
	Krvlje	Ovčje	Kozje
Ekstra tvrdi sir	≥14	7-8	≥15
Tvrdi sir	12-14	6-7	12-15
Polutvrdi sir	9-10	5-6	10-12
Meki sir	7-8	3-4	8-9
Svježi sir	3,5-4	2-3	4-5

Hlađenje i dulje čuvanje mlijeka u laktofrizu može umanjiti njegova svojstva grušanja i randman sira iz dva razloga. Prvi razlog je posljedica aktivnosti psihrotrofnih mikroorganizama u ohlađenom mlijeku, prije svega bakterije *Pseudomonas fluorescens*. Ovaj mikroorganizam izlučuje termostabilne proteolitičke, lipolitičke i fosfolipolitičke

enzime koji razgrađuju proteine i masti u mlijeku za sirenje čak i nakon provedenog postupka pasterizacije. Primjerice, aktivnost hidrolitičkih enzima bakterija iz ovog roda u sirovom ohlađenom mlijeku iznosi čak do 90%, a nakon pasterizacije između 60 i 70 % (Samaržija, 2021). Zbog razgradnje proteina i masti randman u proizvodnji sira se smanjuje. Upravo iz tog razloga u proizvodnji sira se ne preporuča čuvanje mlijeka u laktofrizu duže od 48 sati. Drugi razlog slabijeg grušanja mlijeka i smanjenja randmana u proizvodnji sira od mlijeka koje je prethodno duže vrijeme čuvano u laktofrizu je dezintegracija (odvajanje) β -kazeina iz kazeinske micelle. Kroz duže hlađenje mlijeka (48-72 sata) smanjuju se hidrofobne sile koje drže β -kazein u kazeinskoj miceli te je u tom slučaju moguće da čak do 20-ak molekula β -kazeina iz kazeinske micelle prijeđe u vodenu fazu mlijeka. Odvajanje molekula β -kazeina reverzibilan (povratan) je proces. Međutim, ukoliko se mlijeko naglo dogrijava nema dovoljno vremena da se izdvojene molekule β -kazeina vrate u micelle. Time micelle ostaju manje, gruš je mekši, a randman je smanjen jer odvojene molekule β -kazeina odlaze sirutkom (Decimo i sur., 2014; Havranek i sur., 2014).

Standardizacija mlijeka daje proizvođaču mogućnost manipuliranja u sastavu mlijekaza sirenje u cilju ispunjavanja zakonskih odredbi kategorizacije sireva ali i povećanja randmana. Standardizacija mlijeka podrazumijeva podešavanje omjera kazeina i masti na željeni omjer, primjerice 0,7:1 čime se postiže veća iskoristivost masti i kazeina u proizvodnji sira te se povećava randmana. Standardizaciju je moguće provesti obiranjem dijela vrhnja iz mlijeka za sirenje ili dodavanjem obranog mlijeka u mlijeko za sirenje (Abd El-Gawad i Ahmed, 2011; Kalit, 2015). Tako, standardizacija mlijeka za sirenje za proizvodnju Ličkog škripavca dodatkom obranog mlijeka u prahu povećava iskoristivost proteina iz mlijeka u gruš te povećava stvarni i naravnati randman (Kalit i sur., 2021). Dodatak obranog mlijeka u prahu u proizvodnji sira mekog argentinskog sira također povećava randman (Giménez i sur., 2023). Dodatak koncentrata proteina mlijeka također povećava randman sira (Guinee i sur., 2006; Ur-Rehman i sur. 2007; Francolino i sur., 2010) dok obiranje dijela vrhnja ima negativan učinak na randman u proizvodnji sira (Ur-Rehman i sur., 2007; Addis i sur., 2018; Kalit i sur., 2021).

Homogenizacija je podvrgavanje mlijeka visokim pritiscima u glavi homogenizatora i naglog prelaženja mlijeka iz uvjeta visokog tlaka (najčešće preko 300 bara) u uvjete normalnog tlaka što dovodi do pucanja i usitnjavanja globula mliječne masti na veličinu manju od 1 μ m. Time se dobiva mlijeko koje teže izdvaja vrhnje. Homogenizacijom se mijenja sastav membrana globula mliječne masti jer novonastale (manje) globule imaju relativno veću površinu, pa nema dovoljno vremena i prirodno prisutnih površinski aktivnih tvari da popune novonastale površine na novonastalim globulama. Zbog toga kazein preuzima ulogu emulgatora i popunjava novonastale površine. Tako homogenizirano mlijeko sadrži oko 8% manje kazeina u serum fazi mlijeka što je za sirenje nepovoljno. Dobiveni gruš je mekši (fini), glatke i elastične konzistencije koji zadržava više sirutke što većinom u sirarstvu nije poželjno jer je takav sir lošije kvalitete (Kalit, 2015). Međutim, randman je veći ukoliko se sir proizvodi iz homogeniziranog mlijeka jer se u grušu zadržava više masti, proteina i sirutke (Abd El-Gawad i Ahmed, 2011; Kalit, 2015; Vigneux i sur., 2022).

Aktivnost mljekarske kulture (porast kiselosti, odnosno pad pH vrijednosti mlijeka) potpomažu aktivnost sirila, a time mogu utjecati na čvrstoću gruša i konačni randman sira (Abd El-Gawad i Ahmed, 2011). Dobro je poznato da neka sirila daju mekši gruš, a time i manji randman. S druge strane, prevelika količina dodano sirila u mlijeko može u konačnici rezultirati manjim randmanom sira (Kalit, 2015).

Toplinska obrada mlijeka pri višim temperaturama (> 74 °C) može povećati randman kroz veće zadržavanje proteina i masti u siru. Međutim, pri tome dolazi do denaturacije molekule β -laktoglobulina što dovodi do njegove reakcije s κ -kazeinom i otežanog pristupa kimoza (sirila) specifičnom mjestu cijepanja na molekuli κ -kazeina što je prvi korak pri grušanju mlijeka. Isto tako toplinska obrada mlijeka (srednja i visoka pasterizacija) uzrokuju precipitaciju ionskog kalcija, važnog za grušanje mlijeka. Stoga dodavanje kalcij-klorida u prethodno toplinski obrađeno mlijeko pospješuje njegova svojstva zgrušavanja, a time povećava randman u proizvodnji sira (Lenoir i sur., 2000).

Za randman sira vrlo je važno odrediti vrijeme završetka koagulacije mlijeka. Ono se određuje uranjanjem ruke ili sirarske lopatice u gruš i njegovim podizanjem. Ukoliko gruš puca poput porculana s ravnim bridovima i glatkim površinama te ukoliko je sirutka bistra i zeleno-žute boje, proces koagulacije mlijeka je gotov. Ukoliko se gruš reže i miješa prerano, on će biti mekan, sklon trganju i lomljenju. S druge strane, prekasno rezanje gruša uvjetuje nastajanje gumastog gruša koji se teže reže što uvjetuje sporo nastajanje opne na sirnim zrnima te sirna zrna čini podložnim mehaničkim oštećenjima pri čemu se javljaju veći gubici masti i sirne prašine tijekom miješanja. Miješanje neposredno nakon rezanja takvog gruša je nepoželjno. Prerano i prekasno rezanje gruša ima za posljedicu povećan gubitak masti i sirne prašine sirutkom, odnosno niži randman u proizvodnji sira. U cilju postizanja boljeg randmana rezanje gruša treba provoditi ostrim sirarskim noževima, a ne žicama koje više trgaju nego što režu gruš. Trganjem

gruša također se povećavaju se gubici masti i sirne prašine što uvjetuje niži randman u proizvodnji sira. Rezanje, a kasnije miješanje sirnog zrna u sirutci treba biti lagano, osobito na početku dok sirno zrno još nije formiralo „opnu“. Nakon rezanja gruša na sirna zrna odgovarajuće veličine, zrno formira “opnu” ili gušći sloj kazeina kao posljedica gubitka sirutke i masti iz tih dijelova. Opna prijeći daljnji gubitak masti, ali sadrži male otvore kroz koje sirutka iz unutrašnjeg dijela zrna može biti istisnuta van. Kad se jednom formira opna miješanje sirnog zrna može se ubrzati što se neće negativno odraziti na randman sira (McSweeney, 2007; Abd El-Gawad i Ahmed, 2011; Havranek i sur., 2014).

Brzina zakiseljavanja i krajnja kiselost gruša prije svega određuju randman u proizvodnji svježih sireva kod kojih se zgrušavanje provodi djelovanjem mliječno kiselinskih bakterija. Ukoliko je brzina zakiseljavanja velika tada nastaje rastresiti, krpičasti gruš koji pliva u sirutci. Takav gruš teže je skupiti u sirnu maramu ili kalup za cijedenje svježeg sira, pri čemu dio kazeina u obliku finih flokula (krpica) može zaostati u sirutci. Stoga je za veći randman u proizvodnji svježeg sira bolje da se fermentacija odvija postepeno kroz 12-24 sata. Prebrza fermentacija posljedica je primjene prevelike količine kultura i/ili previsoke temperature fermentacije. Krajnja kiselost gruša također određuje visinu randmana. Za veći randman bolje je da se kiseli gruš reže pri što nižoj vrijednosti (ispod 4,6 pH jedinica). Međutim sirari često gruš režu pri većim pH vrijednostima (>4,6 pH jedinica) kako bi izbjegli prekiseo sir nakon njegova cijedenja na sobnoj temperaturi (kada se pH i dalje snižava). U tom smislu dobro je koristiti manje, za sirutku lako propusne kalupe čime se sir brzo iscijedi i ne dolazi do prekomjernog pada pH vrijednosti (Brule i sur., 2000).

Jedan od ciljeva prešanja je i izdvajanje zaostale sirutke. Prenaglo prešanje, odnosno preveliki pritisak na sir u početnoj fazi prešanja ili prekratko prešanje može rezultirati većim randmanom sira zbog njegove veće mase uslijed zadržavanja više sirutke. Međutim, zaostala sirutka uzrokuje pojavu prekiselog sira koji sporo zrije što u konačnici rezultira proizvodnjom sira drugorazredne kvalitete. Zbog toga je sir potrebno pravilno i odgovarajuće dugo prešati sukladno tehnološkom postupku proizvodnje za pojedinu vrstu sira. Soljenje sira uvjetuje dodatno izlaženje sirutke iz sirnog tijesta što produžava trajnost siru, ali smanjuje randman. Poznato je da svaki utrošeni kilogram soli u proizvodnji sira uvjetuje gubitak 2 litre sirutke iz sira. Iako manjim soljenjem možemo uvjetovati veći randman, to bi značajno narušilo kvalitetu sira stoga se ova mogućnost svodi samo na teoretsku (McSweeney, 2007; Havranek i sur., 2014). S druge strane Abd El-Gawad i Ahmed (2011.) navode da u proizvodnji nekih sireva inkorporacija više soli u sir povećava randman.

Ovisno o vrsti sira, odnosno dužini trajanja zrenja, sir tijekom zrenja gubi i do 20% svoje mase, ponekad i više. Gubitak mase sira tijekom zrenja posljedica je evaporacije vlage iz sira. Količina evaporirane vlage ovisi o formatu sira, kvaliteti i gustoći kore i relativnoj vlažnosti zraka u zronici. Sirevi manje mase imaju veću relativnu površinu kao i oni plosnatog oblika, dok sirevi velike mase u obliku cilindra s većom visinom (primjerice sir Grana Padano) imaju malu relativnu površinu. Vlaga se iz sira gubi kroz površinu, odnosno koru sira. Prema tome, što je relativna površina sira u odnosu na njegovu masu manja, gubici vlage su manji, a time je veći randman sira na kraju zrenja. Stoga nije preporučljivo proizvoditi sireve malih dimenzija koji se duže planiraju držati u uvjetima zrenja. Osim što takvi sirevi brzo kaliraju, što uzrokuje niski randman sira, sadržaj vlage u njima se smanjuje do te mjere da mnogi biokemijski procesi važni za nastajanje spojeva okusa i arome sira prestaju, a zrenje sira je usporeno ili čak zaustavljeno. Tekstura takvog sira je suha i rožnata (McSweeney, 2007; Abd El-Gawad i Ahmed 2011.). Drugi važan čimbenik intenziteta gubitka vlage u siru tijekom zrenja je relativna vlažnost zraka. Što prostorija za zrenje ima veću relativnu vlažnost zraka to će gubici vlage iz sira biti manji. Stoga je uputno u početku zrenja sir držati na nižim vrijednostima relativne vlažnosti zraka (70-80%) kako bi se kora sira što bolje osušila i formirala, a nakon toga, već napola zreli sir, možemo držati na višim vrijednostima relativne vlažnosti zraka (80-85%) kako bi daljnjim zrenjem čim manje kalirao, što osigurava veći randman. Za postizanje optimalne temperature i relativne vlažnosti zraka u prostorijama za zrenje sira koriste se sustavi mirnog hlađenja i odvlaživači za smanjivanje relativne vlažnosti zraka u prostoriji. Nadalje, potrebno je voditi računa da previsoka (>85%) relativna vlažnost zraka u prostoriji za zrenje sira može biti i uslijed gubitka vlage iz sireva koji zriju na policama. U tom je slučaju potrebno potrebno odstranjivati suvišnu vlagu iz zraka prostorije korištenjem odvlaživača (Havranek i sur., 2014).

Zaključak

Svakodnevno praćenje randmana u proizvodnji sira kroz sustav sljedivosti od velike je važnosti. To podrazumijeva svakodnevno bilježenje količine utrošenog mlijeka u proizvodnji sira te količine proizvedenog sira. Na taj način može se utvrditi na koje od opisanih čimbenika možemo djelovati kako bi povećali randman. Poznavanje vrijednosti

randmana u sirarskoj proizvodnji i čimbenika koji ga određuju može uvelike odrediti profitabilnost svake sirarske proizvodnje, o čemu učinkoviti sirari izrazito vode računa. Međutim, valja biti oprezan jer korekcija nekih tehnoloških postupaka u cilju povećanja randmana može umanjiti konačnu kvalitetu sira, a time i profitabilnost.

Literatura

- Abd El-Gawad M.A.M., Ahmed N.S. (2011). Cheese yield as affected by some parameters review. *Acta Scientiarum Polonorum, Technologia Alimentaria*. 10: 131-153.
- Addis M., Pes M., Fiori M., Nieddu G., Furesi S., Pirisi A. (2018). Effect of protein-to fat ratio of sheep milk on the composition, reological properties and yield of PDO Pecorino Romano cheese. *Small Ruminant Research*. 162: 1-7.
- Bobbo T., Cipolat-Gotet C., Bittante G., Cecchinato A. (2016). The nonlinear effect of somatic cell count on milk composition, coagulation properties, curd firmness modeling, cheese yield, and curd nutrient recovery. *Journal of Dairy Science*. 99: 5104-5119.
- Brule G., Lenoir J., Remeuf F. (2000). The casein micelle and milk coagulation. U: *Cheesemaking: From science to quality assurance*, Second edition, Eck, A. i Gills, J.C. (ed.), 7-40. New York, SAD: Intercept Ltd.
- Chamberlain A.T., and Wilkinson J.M. (1998). Manipulating the composition of milk. U: *Feeding the Dairy Cows*, 129-136. Hampshire, Velika Britanija: Chalcombe Publications.
- Chen S.X., Wang J.Z., Van Kessel J.S., Ren F.Z., Zeng S.S. (2010). Effect of somatic cell count in goat milk on yield, sensory quality, and fatty acid profile of semisoft cheese. *Journal of Dairy Science*. 93: 1345-1354.
- Decimo M., Morandi S., Silveti T., Brasca M. (2014). Characterization of gram-negative psychrotrophic bacteria isolated from Italian bulk tank milk. *Journal of Food Science*. 79: M2081-M2090.
- Francolino S., Locci F., Ghiglietti R., Iezzi R., Mucchetti G. (2010). Use of milk protein concentrate to standardize milk composition in Italian citric Mozzarella cheese making. *LWT-Food Science and Technology*. 43: 310-314.
- Lenoir J., Remeuf F., Schneid N. (2000). The use of powdered milk. U: *Cheesemaking: From science to quality assurance*, Second edition, Eck, A. i Gills, J.C. (ed.), 280-296. New York, SAD: Intercept Ltd.
- Giménez P., Peralta G.H., Batistela M.E., George G.A., Ale E.C., Quintero J.P., Hynes E.R., Bergamini C.V. (2023). Impact of the use of skim milk powder and adjunct cultures on the composition, yield, proteolysis, texture and melting properties of Cremoso cheese. *International Dairy Journal*. 140: 105595.
- Grbeša D., i Samaržija S. (1994). Hranidba i kakvoća mlijeka. *Mljekarstvo*. 44: 119-132.
- Guinee T.P., O’Kennedy B.T., Kelly P.M. (2006). Effect of Milk Protein Standardization Using Different Methods on the Composition and Yields of Cheddar Cheese. *Journal of Dairy Science*. 89: 468–482.
- Havranek J., Kalit S., Antunac N., Samaržija D. (2014). *Sirarstvo*. Zagreb, Hrvatska: Hrvatska mljekarska udruga.
- Jaeggi J.J., Govindasamy-Lucey S., Berger Y.M., Johnson M.E., McKusick B.C., Thomas D.L., Wendorff W.L. (2003). Hard Ewe’s Milk Cheese Manufactured from Milk of Three Different Groups of Somatic Cell Counts. *Journal of Dairy Science*. 86: 3082-3089.
- Johnston K.A. (2000). Control and recovery of fat and protein losses. U: *Practical guide for control of cheese yield*. International Dairy Federation.
- Kalit S., Tudor Kalit M., Dolenčić Špehar I., Salajpal K., Samaržija D., Anušić J., Rako A. (2021). The Influence of Milk Standardization on Chemical Composition, Fat and Protein Recovery, Yield and Sensory Properties of Croatian PGI Lički Škripavac Cheese. *Foods*. 10: 690-700.

- Kalit S. (2015). Opće sirarstvo. Objavljeno u Sirarstvo u teoriji i praksi, Matijević, B. (ed.), 29-46. Karlovac, Hrvatska: Veleučilište u Karlovcu.
- Martí-De Olives A., Peris C., Pilar Molina M. (2020). Effect of subclinical mastitis on the yield and cheese-making properties of ewe's milk. *Small Ruminant Research*. 84: 106044.
- McSweeney P.L.H. (2007). Cheese Yield. U: *Cheese Problems Solved*, McSweeney P.L.H (ed.), 100-114. Cambridge, England: Woodhead Publishing.
- Samaržija D. (2021). *Mljekarska Mikrobiologija*. Zagreb, Hrvatska: Hrvatska mljekarska udruga.
- Stocco G., Pazzola M., Dettori M.L., Cipolat-Gotet C., Summer A., Vacca G.M. (2019). Variation in caprine milk composition and coagulation as affected by udder health indicators. *International Dairy Journal*. 98: 9-16.
- Ur-Rehman S., Farkye N.Y., Considine T., Schaffner A., Drake M.A. (2003). Effects of Standardization of Whole Milk with Dry Milk Protein Concentrate on the Yield and Ripening of Reduced-Fat Cheddar Cheese. *Journal of Dairy Science*. 86: 1608–1615.
- Vacca G.M., Stocco G., Dettori M.L., Bittante G., Pazzola M. (2019). Goat cheese yield and recovery of fat, protein, and total solids in curd are affected by milk coagulation properties. *Journal of Dairy Science*. 103: 1352–1365.
- Vigneux M.P.B., Villeneuve W., Pouliot Y., Britten M. (2022). Increasing the proportion of homogenised fat in cheese milk: Effect on cheese-making properties. *International Dairy Journal*. 126: 105254.

The influence of milk quality and technological production procedures on the cheese yield

Abstract

This paper aims to provide an overview of the factors that determine efficiency in cheese production. Knowing the importance of cheese yield and the factors that determine it can greatly determine the profitability of cheese production. The quality of milk and almost all technological steps of production can affect the yield of cheese. The most significant influence is cooling, standardization, homogenization, heat treatment and coagulation of milk, amount and type of dairy cultures and rennet, acidity and treatment of cheese curds, salting, pressing, and ripening of cheese. Cheese yield is extremely important considering that the price of raw milk accounts for up to 80% of production costs. However, one should be careful because the correction of some technological procedures to increase the cheese yield efficiency can reduce the quality of the cheese, and thus the profitability.

Keywords: cheese, yield, technological, milk quality

Valorization of bioactive compounds of classic and quick-frozen nettle leaves through a combination of different pretreatments

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Abstract

A fresh nettle leaf is characterized by a high water content. The freezing method and the pre-treatment are key factors to preserve the nutritional and morphological characteristics of the raw material. Therefore, the aim of this study was to determine the content of bioactive compounds in nettle leaves frozen by quick and slow freezing with pretreatment by blanching and ultrasound after a storage period of 3 months. Quick frozen nettle leaves had significantly lower drip loss, while the analyzed bioactive compounds were significantly higher than in the classically frozen leaves. Some positive trends of ultrasound-assisted freezing on the preservation of bioactive compounds in nettle leaves can be observed. The quick freezing was found to be highly effective in maintaining the quality of frozen nettle leaves after three months of storage.

Keywords: bioactive compounds, drip loss, ultrasound-assisted freezing, polyphenolics, ascorbic acid

Introduction

Green leafy vegetables are one of the most demanding categories of fresh vegetables in terms of postharvest quality maintenance. Indeed, leafy vegetables in fresh state are characterized by an high water content (85-90%), which makes them a suitable for the development of microorganisms. Due to the high rate of transpiration and respiration, they lose their characteristic freshness and generally have a very short shelf life (Koukounaras et al., 2020; Martínez-Ispizua et al., 2022). In order to slow down the deterioration, loss of freshness, and color change of the plant tissue, and generally to preserve the original quality of the freshly harvested raw material, fresh leafy vegetables are often frozen. Freezing is one of the methods of food preservation that has the least impact on their qualitative properties, significantly affecting the extension of the shelf life by removing a medium suitable for the growth and development of microorganisms and enzyme activities (Mazzeo et al., 2015; González-Hidalgo et al., 2018). The choice of appropriate freezing conditions, i.e., temperature and rate of formation of ice fronts, is a key factor in the quality of the process itself. The main limiting factor for the quality of the freezing process is the result of the mobilization of water through the formation of ice crystals (Neri et al., 2020). In addition, crystallization of amorphous solutes leads to degradation of some bioactive compounds, deformation of the texture and structure, and loss of the characteristic color of the product. This is strongly influenced by the size and position of the ice crystals formed in the cell and intercellular spaces, depending on the type of freezing process used, which may result in more or less damage to the cell membranes and deterioration of the physical structure. Ultimately, this is also reflected in the reduced quality of the food after thawing (van der Sman, 2020). The faster and at a lower temperature freezing takes place (quick process), the more ice crystals are formed during freezing, which are smaller and more evenly distributed in the cells and intercellular spaces and thus have less negative impact on the physical and chemical properties of the food. In contrast, the slower method of freezing (classic method) forms a crystals of larger dimensions, which are more unevenly distributed and significantly affect the quality of the product (Bulut et al., 2018; Li et al., 2022). It is important to note that fresh green leafy vegetables must be pretreated by blanching before freezing to prevent browning of the tissue during the storage period. Recently, alternatives to thermal methods have been sought to preserve nutritional properties, and one of them is high-intensity ultrasound (Martínez et al., 2013; Chavan et al.,

2022). The aim of this study was to determine the content of bioactive compounds in fresh stinging nettle leaves frozen by quick and slow freezing with different pretreatment (blanching and ultrasonic) after a storage period of 3 months.

Materials and methods

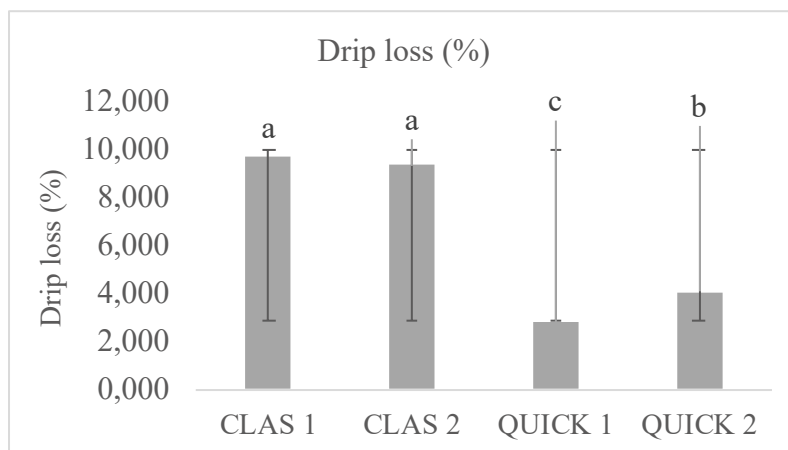
The fresh nettle leaf used for research purposes was cultivated within the project URTICA-BioFuture (IP-2019-04-3325). The nettle was grown in an ebb and flow hydroponic system, and the leaves were collected after the first harvest on June 15, 2023. For the purposes of freezing, the leaves were washed and part of the leaf biomass was separated for classical and quick freezing. In addition, before freezing, leaves were pretreated by blanching (100 °C, 2 min) and high-intensity ultrasound in an ultrasonic bath (Bandelin RK 103H, Germany) with a nominal power of 140 W and a frequency of 35 kHz for 2 min. After the pretreatments, part of the nettle leaf samples (50 g) were packed in polyethylene containers (average size 142 x 94 x 35 mm) with openings of 50 mm diameter and frozen at -18 °C for 24 h in a classical freezer (Lth 1, Gorenje, Slovenia), while for the quick freezing the same amount of leaf samples were frozen at -34 °C for 45 min in shock freezer (Tecnomac Sf 1, Italy) using carbon dioxide as cooling liquid (2200 g - R404A, GWP3922). After freezing, the samples were stored at -18 °C for 3 months. The content of bioactive compounds, namely ascorbic acid (AsA), was analyzed by titration with 2,6-dichloroindophenol according to the AOAC (2002) protocol, and the content of total phenols, non-flavonoids, and flavonoids was analyzed by the Folin-Ciocalteu reaction according to Ough and Amerine (1988). The drip loss (%) of cell water during thawing was also determined by weighing the plant material immediately after freezing (m_1) and after thawing (m_2) and calculating according to the following equation (1) by Xie et al. (2014).

$$\text{Drip loss (\%)} = \frac{m_1 - m_2}{m_1} \times 100$$

Results were statistically processed with the SAS® version 9.3 program package using the generalized linear model (PROC GLM) with replicates and interactions of pretreatment (blanching and VAE) and freezing method (classical, quick freezing). Means were compared by t-test and considered significantly different at $p \leq 0.01$.

Results and discussion

Graph 1 shows the drip loss (%) of frozen nettle leaves pretreated by different methods after 3 months of storage. According to the results, there are significant differences between the samples frozen classically and those frozen by quick freezing. Regardless of the pretreatment, significantly lower values of drip loss were observed in the samples frozen by the quick freezing method, which were even three times lower (QUICK 1 and QUICK 2) than in those frozen classically. The results obtained were to be expected, since a larger number of smaller ice crystals are formed during quick freezing due to the significantly lower temperatures, resulting in significantly less damage to cell structures, which translates into less loss of cell sap during thawing and, consequently, faster thawing and less changes in morphological properties (such as color, shrinkage, etc.) (Cheng et al., 2020; Chavan et al., 2022). Regarding the pretreatment method, no significant changes were observed in the classically frozen samples, while statistical differences were observed in the quick frozen samples. The samples pretreated by blanching (QUICK 1) had on average 47% less drip loss compared to the frozen nettle leaves pretreated by ultrasonication and quick frozen (QUICK 2). It can be concluded that the combination of blanching as a pretreatment and quick freezing as a freezing method contributed significantly to the lower drip loss and thus to better morphological characteristics of the frozen nettle leaves.



Graph 1. Drip loss (%) of the stinging nettle leaves pretreated by blanching and by ultrasound frozen by different methods (classical and quick) after 3 months of storage

CLAS 1- classically frozen samples pretreated by blanching; CLAS 2- classically frozen samples pretreated by high-intensity ultrasound; QUICK 1- quick-frozen samples pretreated by blanching; QUICK 2- quick-frozen samples pretreated by high-intensity ultrasound. The results are expressed as mean values and the standard deviation is indicated. Different letters indicate significant differences between the means at $p \leq 0.01$.

Considering the analyzed bioactive compounds of nettle leaves (Table 1) after 3 months of storage, it should be emphasized that the two varied factors, freezing method and pretreatment, significantly affected the content of specific compounds. The highest ascorbic acid (AsA) content was determined in leaves frozen by the quick freezing method and in samples pretreated by blanching and ultrasound (QUICK 1 and QUICK 2), with no significant difference between them. Regardless of the pretreatment method, the average AsA content was found to be 23% higher in the quick frozen samples than in the classically frozen ones. The pretreatment method also had a significant effect on the AsA content in nettle leaves, highlighting that blanching in combination with classical freezing resulted in the lowest AsA content. Blanching as a thermal process implies a short treatment at high temperatures (100 °C), which significantly affected the degradation of AsA content. Of the polyphenolic compounds, total phenolics (TPC), nonflavonoids (TNFC), and flavonoids (TFC) were analyzed. Both TPC, TNFC, and TFC levels were higher in the quick frozen nettle leaves than in the classically frozen ones. The TPC content was about 24% higher in the quick frozen samples than in the classically frozen ones, regardless of the pretreatment used. TNFC and TFC values varied slightly depending on the freezing method, as, for example, the highest TFC content was found in classically and quick frozen leaves, depending on the pretreatment. Pretreatment significantly influenced on the content of polyphenolic compounds, while some uniformity can not be single out. For example TPC and TFC were both the highest in samples pretreated by blanching and ultrasound (QUICK 1, QUICK 2 and CLAS 1), while for TNFC values some positive influence of ultrasound can be pointed out, since for sample QUICK 2 the highest TNFC values was determined. In general, some positive trends of ultrasound on the preservation of phenolic compounds in frozen nettle leaves can be noted. Ultrasound technology, specifically high intensity ultrasound, generally significantly favors the preservation of bioactive compounds including polyphenolics, vitamins (AsA content) etc. as well. Furthermore, ultrasound-assisted freezing significantly reduce the freezing time by shortening the water-ice crystal transition phase and thus allowing the preservation of nutritional quality of raw material with emphasis on bioactive compounds (Qui et al., 2020; Chavan et al., 2022). Also, the main advantage of ultrasound-assisted freezing is that allows formation of evenly distributed, fine ice nucleation in accelerated rate resulting in reduce of damage on the cell structures and thus maintaining the quality, both nutritional and morphological, of the frozen material (Zhang et al., 2017).

Table 1. The content of specific bioactive compounds in nettle leaves frozen with different freezing methods and pretreatments after 3 months of storage

Freezing method	AsA (mg/100 g fw)	TPC (mg GAE/100 g fw)	TNFC (mg GAE/100 g fw)	TFC (mg GAE/100 g fw)
Blanching				
CLAS 1	15.76±0.41 ^c	21.65±0.65 ^b	10.54±1.38 ^c	11.11±0.88 ^a
QUICK 1	21.07±0.69 ^a	25.77±0.82 ^a	12.68±0.89 ^b	13.09±0.61 ^a
Ultrasound treatment				
CLAS 2	19.17±0.79 ^b	21.86±1.06 ^b	13.28±0.33 ^b	8.58±1.10 ^b
QUICK 2	22.05±1.27 ^a	27.98±1.46 ^a	15.46±0.61 ^a	12.52±1.16 ^a
Pr≤F	0.0022	0.0029	0.0060	0.0213
F	0.0001	0.1035	0.0014	0.0306
PT	0.0034	0.0001	0.0052	0.0013
F×PT	0.0478	0.1647	0.9763	0.1312

AsA- ascorbic acid content; TPC- total phenol content; TNFC- total nonflavonoid content; TFC- total flavonoid content; CLAS- classical freezing; QUICK- quick-freezing; 1- blanching; 2- ultrasonic pretreatment; F- freezing; PT- pretreatment; F×PT- interaction between freezing method and pretreatment. Results are expressed as mean ± standard deviation. Different letters indicate significant differences between mean values.

Conclusions

The obtained results suggest that the quick freezing method is an efficient method for the preservation of nettle leaves during the storage period. All analyzed bioactive compounds, especially ascorbic acid content and polyphenols, were significantly higher in nettle leaves frozen by the quick freezing method than in leaves classically frozen. In addition, the leaves frozen by quick freezing had significantly lower drip loss compared to those frozen by the classical method, which favored the preservation of the morphologic properties of the frozen product. The pretreatment of nettle leaves with high-intensity ultrasound can be considered an efficient means to preserve the nutritional and morphological quality of the frozen material. However, more detailed studies are needed to optimize the ultrasound parameters, which would have an even greater effect on accelerating the freezing process and maintaining the quality of the final product.

Acknowledgement

The results presented in the paper are output from research project IP-2019-04-3325 URTICA-BioFuture financed by the Croatian Science Foundation „Nutritional and functional value of nettle (*Urtica dioica* L.) by application of modern hydroponic cultivation techniques“.

References

- AOAC (2002). Official methods of Analysis (17th ed.). Association of Official Analytical Chemists. Washington, USA.
- Bulut M., Bayer Ö., Kirtıl E., Bayındırlı A. (2018). Effect of freezing rate and storage on the texture and quality parameters of strawberry and green bean frozen in home type freezer. *International Journal of Refrigeration*. 88: 360-369.
- Chavan P., Sharma P., Sharma S.R., Mittal T.C., Jaiswal A.K. (2022). Application of High-Intensity Ultrasound to Improve Food Processing Efficiency: A Review. *Foods*. 11: 122.
- Cheng L., Wu W., An K., Xu Y., Yu Y., Wen J., Wu J., Zou Y., Liu H., Zhu J., Xiao G. (2020). Advantages of Liquid Nitrogen Quick Freezing Combine Gradient Slow Thawing for Quality Preserving of Blueberry. *Crystals*. 10: 368.
- González-Hidalgo I., Moreno D.A., García-Viguera C., Ros-García J.M. (2018). Effect of industrial

- freezing on the physical and nutritional quality traits in broccoli. *Food Science and Technology International*. 25: 56-65.
- Koukounaras A., Bantis E., Karatolos N., Melissas C., Vezyroglou A. (2020). Influence of Pre-Harvest Factors on Postharvest Quality of Fresh-Cut and Baby Leafy Vegetables. *Agronomy*. 10: 172.
- Li X.-Y., Yang X.-L., Gai R.-N., Dong Q.-X., Li L. (2022). Study of the dynamic characteristics of the food freezing process using a cryogenic immersion freezing tank. *Food Science and Technology*. 42: e19822.
- Martínez S., Pérez N., Carballo J., Franco I. (2013). Effect of blanching methods and frozen storage on some quality parameters of turnip greens (“grelos”). *LWT - Food Science and Technology*. 51: 383-392.
- Martínez-Ispizua E., Calatayud A., Marsal J.I., Basile F., Cannata C., Abdelkhalik A., Soler S., Valcárcel J.V., Martínez-Cuenca M.-R. (2022). Postharvest Changes in the Nutritional Properties of Commercial and Traditional Lettuce Varieties in Relation with Overall Visual Quality. *Agronomy*. 12: 403.
- Mazzeo T., Paciulli M., Chiavaro E., Visconti A., Fogliano V., Ganino T., Pellegrini N. (2015). Impact of the industrial freezing process on selected vegetables -Part II. Colour and bioactive compounds. *Food Research International*. 75: 89-97.
- Neri L., Faieta M., Di Mattia C., Sacchetti G., Mastrocola D., Pittia P. (2020). Antioxidant Activity in Frozen Plant Foods: Effect of Cryoprotectants, Freezing Process and Frozen Storage. *Foods*. 9: 1886.
- Ough C.S., and Amerine M.A. (1988). *Methods for Analysis of Musts and Wines*, 2nd ed.; John Wiley & Sons: New York, NY, USA.
- Qui L., Zhang M., Chitrakar B., Bhandari B. (2020). Application of power ultrasound in freezing and thawing Processes: Effect on process efficiency and product quality. *Ultrasonics Sonochemistry*. 68: 105230.
- van der Sman R.G.M. (2020). Impact of Processing Factors on Quality of Frozen Vegetables and Fruits. *Food Engineering Reviews*. 12: 399-420.
- Xie J., Zhao Y. (2014). Use of vacuum impregnation to develop high quality and nutritionally fortified frozen strawberries. *Journal of Food Processing and Preservation*. 28: 117-132.
- Zhang P., Zhu Z., Sun D.-W. (2017). Using power ultrasound to accelerate food freezing processes: Effects on freezing efficiency and food microstructure. *Critical Reviews in Food Science and Nutrition*. 58: 2842–2853.

Exploring the role of inulin as a nutraceutical for enhancing nutritional and health benefits - a review

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Abstract

This review explores the role of inulin, a natural prebiotic, delving into its transformative impact on the food industry, especially in creating functional products and assessing its impact on public health. Inulin, a versatile carbohydrate compound, not only acts as a fat replacer but also serves as a dietary fiber source, enhancing the overall nutritional value of the foods. Furthermore, inulin's fermentation by beneficial gut bacteria results in the production of short-chain fatty acids, contributing to colorectal cancer prevention and glycemic control, catering to the evolving preferences of health-conscious consumers.

Keywords: inulin, functional food, nutraceuticals, prebiotics

Introduction

Throughout history, developed civilizations have exhibited a significant level of fascination and concern regarding the quality and reliability of food sources. Historically, societies have long pondered the connection between nutrition and health. Even before the rise of nutritionism, philosophers like Hippocrates emphasized the importance of understanding the role of food in maintaining health and preventing diseases. He highlighted the significance of balancing nutrients to promote well-being (Adams, 1886). The human organism is composed of various substances that contribute to its unique chemical composition. Biologically active substances of natural origin play a pivotal role in maintaining normal physiological functions in plants, animals, and humans. These substances can also be utilized as external resources to achieve specific results in the organization of biological processes. As recognized more than 2000 years ago by Hippocrates, a fundamental connection exists between food and health. He emphasized that „... differences in diseases depend on the nutrients taken in...” (Andlauer and Fürst, 2002). Despite this understanding, the boundary between what constitutes food and what constitutes a potential cure remains a topic of ongoing research and discussion.

The term “nutraceuticals,” introduced in 1989 by the Foundation for Innovation in Medicine, an organization dedicated to advancing medical discovery, outlines an ever-growing realm within biomedical research (Alissa and Ferns, 2012). Broadly defined, nutraceuticals encompass bioactive compounds inherent to food, granting significant health benefits, including disease prevention, treatment, and the maintenance of essential physiological processes vital for human well-being (DeFelice, 1992). Beyond their nutritional content, nutraceuticals manifest indisputable pharmacological effects. When the safety and suitable bioavailability upon ingestion are demonstrated, they can show significant effectiveness in preventing and treating specific pathological conditions. Nutraceuticals operate in the realm denoted as ‘beyond diet, before therapy’ (Santini, 2014). Integrating these agents into daily dietary practices not only forestalls disease progression but could potentially lead to postponing the necessity for pharmaceutical interventions. Nutraceuticals appeal to individuals seeking non-pharmacological alternatives for managing health conditions, aligning with the concept of proactive medicine (Santini et al., 2017).

Functional foods and nutraceuticals

In the latter half of the 20th century, accompanied by increased food accessibility, lifestyle shifts, and the emergence of new dietary practices, notably characterized by the overconsumption of specific nutrients detrimental to human health (such as foods rich in saturated and unsaturated-trans fatty acids, as well as added and refined sugars), a necessity arose to conceptualize “optimal nutrition.” Optimal nutrition, the term highly popularized in the 1970s., focuses on a set of dietary guidelines advocating the modification, either reduction or augmentation, of specific foods or food components. Such modifications aim to enhance an individual’s overall health and well-being (Ridgway et al., 2019; Jiménez-Colmenero et al., 2010). This concept signifies the ability to optimize particular physiological functions through nutrition, thereby improving health and mitigating the risk of disease development. It was within this framework that the concept of „functional food“ emerged for the first time, gaining traction since the 1980s and serving as a pivotal catalyst for the development of innovative food products (Daliri and Lee, 2015). Functional food refers to food prepared using a scientific approach, wherein one or more nutraceuticals are incorporated, demonstrating established health benefits for a specific population (Kalra, 2003). Functional food, to gain acceptance, must adhere to the format of conventional food while incorporating added, substituted, or subtracted nutrients or other ingredients, facilitating consumption as part of the regular diet (Jiménez-Colmenero et al., 2001.; Siró et al., 2008). This evolution signifies a shift in the traditional conception of food, as functional food takes on the character of a “therapeutic ally,” addressing diverse individual needs (Kalra, 2003.). While recommended intake guidelines exist for numerous substances today, adherence to these guidelines is limited among the general population. A significant challenge to adherence lies in the extended duration of consumption required to yield positive effects, as foods rich in nutraceuticals must be consumed daily over an extended period to achieve the desired outcomes (Jiménez-Colmenero et al., 2010). Consequently, it is more plausible for consumers to meet their nutritional requirements by consuming foods that naturally incorporate these biologically active substances, thereby expanding the variety of available options.

Effect of dietary fibers as nutraceuticals

Dietary fibers are non-digestible carbohydrates that provide structural support to plants. There are two primary categories: insoluble and soluble fiber, both of which are crucial for a balanced diet (Dhingra et al., 2012). Insoluble fibers, including lignin, hemicellulose, and cellulose, absorb water, enhancing fecal bulk, and facilitating efficient movement through the digestive tract. Rich sources encompass wheat, whole grains, seeds, berries, nuts, and various fruits and vegetables, which are excellent sources of insoluble fiber. Soluble fiber, on the other hand, undergoes breakdown during its journey through the digestive tract, forming a viscous gel that impedes cholesterol absorption and regulates blood sugar levels. Water-soluble fibers consist of compounds such as fructans (e.g., inulin), pectins, gums (e.g., agar), and mucilage (as could be found in psyllium). Some fruits and vegetables, legumes (such as lentils, beans, chickpeas, green beans, and peas), and grains like oats and barley provide substantial sources of soluble fiber. Notably, human digestive enzymes cannot metabolize dietary fiber, allowing it to reach the large intestine. Insoluble dietary fiber contributes no caloric value, while soluble dietary fiber contains some calories. Furthermore, high-fiber sources could play a pivotal role in contemporary meat processing technology for several reasons: certain fibers offer prebiotic and probiotic functions, have favorable nutritional attributes, and some of them have the potential to comply with regulatory guidelines regarding enrichment claims (Tiefenbacher, 2017).

Inulin: Functional Properties and Applications

Inulin belongs to the group of oligosaccharides within the so-called fructan compounds that contain 2-60 fructose molecules interconnected by a $\beta(2\rightarrow1)$ bond and a terminal glucose molecule that is connected to fructan units by an $\alpha(1\rightarrow2)$ glycosidic bond (Esmailnejad Moghadam et al., 2019). The functionality of inulin is related to its degree of polymerization, which is highly variable among inulins and depends on many factors, such as the plant species from which it is isolated (Chi et al., 2011). As a carbohydrate, inulin is present in many plant species, such as Jerusalem artichoke, dahlia, and chicory root, which represent the three main commercial sources of inulin production (Rubel et al., 2014), while in nature they can be found in a large number of other plants such as unripe wheat, rye and unripe barley, onions, garlic, leeks, bananas, asparagus (González-Herrera et al., 2015). Chicory is a plant that has been exploited on an industrial scale for extracting inulin-type fructans. Inulin is one of the surface-active oligosaccharides

and has long been considered a functional food ingredient used in the food industry as a substitute for sugar or fat. Its adoption serves to enhance the nutritional profile of food products by reducing their caloric content (inulin typically possesses an energy content of 1.5 kcal/g), providing a source of dietary fiber, and offering prebiotic benefits. From a technological standpoint, inulin has proven to be an excellent gelling agent, viscosity modifier, and texturing agent in various foods (Tiefenbacher, 2017; Melilli et al., 2021). The effectiveness of inulin in fat replacement is closely linked to its capacity to form highly stable gels. However, the gel properties are highly dependent on temperature, concentration, and the degree of polymerization of the inulin compound (Yousefi et al., 2018). These molecules have the ability to create a robust interfacial film surrounding dispersed oil droplets, resembling networks of fat crystals in oil (McClements and Gumus, 2016). For instance, due to this similarity, inulin gels have been identified as an interesting ingredient in the preparation of new, functional meat products and contribute to flavor and creaminess similar to those containing animal fats (Yousefi et al., 2018). The advantage of using inulin is that, although proteins as emulsifiers have better emulsifying properties when used at low concentrations and create smaller oil droplets, these fructan-type molecules can generate emulsions that are stable to higher variations in environmental conditions such as pH, temperature (up to 80°C), and freezing (McClements and Gumus, 2016). The degree of polymerization is related to both the technological properties of inulin and the potential to cause positive health effects. Consumption of long-chain inulin, such as that isolated from the root of chicory (*Cichorium intibus*), offers a range of potential health benefits that will be discussed in detail later in this article.

Inulin and health claims

In the global market, particularly over the last two decades, it is estimated that nutraceuticals of natural origin, acting as bioactive compounds, and especially in the form of functional foods, have become integral components of the multi-billion dollar food industry (Awuchi and Okpala, 2022). The substantial economic expansion in this field has necessitated proper and legally defined nutritional labeling, along with assessments of health effects and recommendations regarding the consumption of functional foods containing nutraceuticals. Both the scientific community and regulatory authorities, including the European Food Safety Agency (EFSA), the American Food and Drug Administration (FDA), the World Health Organization (WHO), and individual national regulatory bodies, are actively engaged in addressing these issues. Inulin is a compound tested at high doses in animals with no reported toxic effects (Coussement, 1999). The US Food and Drug Administration (FDA) has categorized it as a substance “Generally Recognized as Safe” (GRAS).

Moreover, to date, only one prebiotic - inulin from chicory - has received an approved health claim from EFSA. The following wording reflects the scientific evidence: “Chicory inulin contributes to the maintenance of normal defecation by increasing stool frequency.” To achieve the intended effect, a daily intake of 12 grams of “native chicory inulin” is recommended (EFSA, 2015). However, concerning its laxative properties, inulin is generally well-tolerated at single doses of 5 grams, with a total daily intake reaching up to 15-20 grams. It’s worth noting that increasing flatulence may occur at higher doses (Yousefi et al., 2018).

Prebiotic Inulin: Impact on Gut Health

Increased awareness of the complex interactions between diet, the gut microflora of the gastrointestinal tract, and overall health have prompted the development of novel dietary approaches promoting the growth of specific beneficial bacterial groups (Boeckner et al., 2001). The definition of prebiotics varies across scientific and political spheres worldwide. However, it is generally accepted that almost all prebiotics can be classified as dietary fibers, but not all fibers are considered prebiotics. According to the latest description, a prebiotic is “a substrate selectively utilized by host microorganisms to foster a health benefit” (Carlson et al., 2017). To be categorized as a prebiotic, a food ingredient should display certain attributes, such as resistance to low stomach pH, resistance to hydrolysis by enzymes of the gastrointestinal tract (GIT), and resistance to absorption in the upper GIT. Additionally, it should possess the ability to be fermented by the intestinal microbiota, selectively stimulating the growth and metabolic activity of colonic bacteria, such as bifidobacteria and lactobacilli, while at the same time inhibiting the growth of pathogens. This selective stimulation should directly correlate with positive outcomes for the host’s health (Carlson et al., 2017; Kolida, 2002)

Given that the colonic bacterial microflora constitutes approximately 95% of the total cells in the human body and plays a pivotal role in the host’s nutrition, health, and disease, prebiotic fermentation should target desirable bacteria

in the human population, such as indigenous *Bifidobacterium* and *Lactobacillus*, known for their positive impact on the immune system of the GIT, lower-intestinal motility, plasma glucose and lipid level control, mineral absorption, and various intestinal diseases (Kolida, 2002; Scheid et al., 2013). The ultimate goal of nutritional supplementation/fortification with inulin is to modulate the intestinal microbiota, thereby enhancing digestive tract health and the overall state of the human organism (Kolida, 2002) while also leading to reducing the number of harmful species such as *Escherichia coli* and *Clostridium spp* (González-Herrera et al., 2015). Due to the susceptibility of bifidobacteria to changes when exposed to oxygen and heat, their application as food probiotics is limited compared to lactobacilli (which could be found as part of commercially available probiotics). Consequently, there has been substantial interest in bifidogenic factors in food that can withstand regular processing methods and exhibit efficacy within the human body following ingestion. Caused by the lack of prebiotic-hydrolyzing enzymes in the upper GIT, just like other insoluble dietary fibers, inulin passes through the upper GIT practically intact, and enters the large intestine, where it undergoes fermentation catalyzed by probiotic bacteria. This fermentation process yields short-chain fatty acids (SCFA) and gases, including methane, CO₂, and H₂. The fatty acids produced by this process are mainly butyrate, acetate, and propionates, which lead to a decrease in the pH level of the colon and serve as metabolic energy sources for colonocytes (Scheid et al., 2013).

The impact of SCFA on gastrointestinal health is evident in the energy generated within the intestinal mucosa by colonocytes upon absorption. Additionally, SCFA contributes to mitigating inflammatory processes by reducing or inhibiting the activity of pro-inflammatory nuclear factor (NF-κB), which inhibits the synthesis of pro-inflammatory cytokines such as interferon-γ (macrophage activator) and IL-2 (which promotes further production of Th1 and Th2 leukocytes and leads to exacerbation of immune response). The increased presence of propionate and acetate leads to increased secretion of anti-inflammatory cytokine IL-10, which downregulates the expression of Th1 cytokines and can block the expression of NF-κB (Roberfroid et al., 2010; Scheid et al., 2013; Pothuraju et al., 2021). Consequently, this secretion reduces the synthesis of IgE and promotes the induction of non-inflammatory immunoglobulins, specifically IgA (Esmailnejad Moghadam et al., 2019). Furthermore, the stimulation of mucin production by propionate and butyrate plays a role in preserving the mucosal layer, providing protection to the epithelium (Scheid et al., 2013). SCFA can induce an osmotic effect within the large intestine, playing a pivotal role in absorbing water from the intestinal lumen and enhancing intestinal peristaltic movement (Boeckner et al., 2001). This effect is highly beneficial for individuals struggling with constipation or obstipation issues, as well as patients with diabetes mellitus experiencing gastroparesis. However, it can pose challenges for consumers dealing with irritable bowel syndrome (IBS) (Tiefenbacher, 2017).

Role of inulin in colorectal carcinoma

Intestinal microbiota-derived metabolites, notably bile acids, hold a crucial role in human metabolism. Synthesized from cholesterol, bile acids are conjugated with glycine or taurine to form bile salts, stored in the gallbladder, which are then released into the duodenum during digestion, aiding in fat solubilization (Sayin et al., 2013). Bile salts exhibit potent antimicrobial efficacy by disrupting the cellular membrane structure and inducing DNA damage. Conjugated bile acids undergo reabsorption in the intestines through apical bile salt transporters, with a fraction (5-10%) remaining unabsorbed and subject to metabolism within the intestinal microbiota, particularly by “harmful” bacteria such as *E. Coli*, *Enterococcus faecalis*, and *Clostridia* strains. This metabolism results in the generation of secondary bile acids, which exhibit pronounced toxicity towards the proliferation and development of “beneficial” bifidobacteria and lactobacilli. The presence of secondary bile acids is associated with an elevated risk of cancer, specifically in the context of a high-fat diet (Pothuraju et al., 2021). In contrast, inulin exhibits a prophylactic effect against colorectal cancer by stimulating bifidobacteria activity (Esmailnejad Moghadam et al., 2019). Furthermore, inulin fermentation and subsequent short-chain fatty acid production constitute a significant anticancer mechanism. This process modifies colonic microbiota and reduces pH, preventing abnormal cell proliferation (Boeckner et al., 2001).

Effects of inulin on serum glucose in patients with type 2 diabetes

Inulin's resistance to human intestinal enzymes arises from its β(2→1) glycosidic bonds between fructose molecules. Its passage through the upper digestive tract provides a sense of fullness without adding calories, resulting in a low caloric value (1.5 kcal/g or 6.3 kJ/g). This value is associated with energy obtained from the absorption of short-chain

fatty acids in the colon (Yousefi et al., 2018; Shoaib et al., 2016). Top of Form Also, since the hormone insulin is not required for the inulin metabolism, it does not affect the postprandial blood glucose level, so people with diabetes tolerate it well (Rao et al., 2019). Based on scientific evidence, even the EFSA recognized inulin as a potent ally in which consuming foods containing non-digestible carbohydrates induces a lower postprandial glycemic rise after meals (EFSA, 2014). Inulin can reduce lymphocytic infiltration in pancreatic islets of Langerhans and increase the proliferation rate of β -cells, thereby improving insulin sensitivity and pancreatic β -cell function. A review of clinical trials also suggests that inulin supplementation has beneficial effects on metabolic syndrome in individuals with type II diabetes mellitus (Rao et al., 2019).

Inulin and its effect on mineral metabolism

Inulin fermentation in the large intestine enhances mineral absorption, primarily through a pH drop due to short-chain fatty acid production. In the upper gastrointestinal tract, inulin interacts with calcium (Ca^{+2}). Upon reaching the large intestine, this complex releases Ca^{+2} , increasing its availability for absorption (Miremedi et al., 2016). Studies suggest that SCFA, particularly butyrate, positively impacts the large intestine's surface, increasing epithelial cell count per crypt. This effect boosts calcium, magnesium, and iron absorption, a known mechanism inhibiting osteoporosis with prebiotic intake (Scheid et al., 2013).

Conclusion

Recognizing the potential of nutraceutical composition variations offers the industry a route for creating specialized functional foods aligning with evolving consumer preferences. This article delves into the positive aspects of inulin as a nutraceutical. It shows the dynamic relationship between diet, functional foods, and human health, highlighting the evolving significance of nutraceuticals and functional ingredients. Dietary fibers, notably inulin, emerge as versatile contributors to digestive health, gut microbiota modulation, and various physiological functions. Inulin's diverse properties, encompassing prebiotic effects, and favorable technological and functional attributes suitable for incorporation into specific food applications position it as a valuable component in functional foods prospectives. Utilizing inulin as a fat replacer proves effective in formulating low-calorie foods, mitigating the risk of hypercholesterolemia and hyperglycemia without adverse effects on consumer health. The evidence suggests that incorporating inulin in novel foods holds promise in preventing colorectal cancer, managing blood glucose levels in type 2 diabetes, and positively impacting mineral absorption, emphasizing its potential as an exciting player in proactive and personalized nutrition.

Acknowledgments

This research was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia in accordance with the Agreement on the realization and financing of scientific research work of SRO No. 451-03-47/2023-01/200022.

References

- Adams F. (1886). The genuine works of Hippocrates. Adams F. (eds.), 1: 61, 134. New York, USA: William Wood and Company.
- Alissa E.M., and Ferns G.A. (2012). Functional foods and nutraceuticals in the primary prevention of cardiovascular diseases. Journal of nutrition and metabolism. 2012. Available from: <https://www.hindawi.com/journals/jnme/2012/569486/>
- Andlauer W., and Fürst P. (2002). Nutraceuticals: a piece of history, present status and outlook. Food Research International. 35: 171-176.
- Awuchi C.G., and Okpala C.O.R. (2022). Natural nutraceuticals, especially functional foods, their major bioactive components, formulation, and health benefits for disease prevention-An overview. Journal of Food Bioactives. 19.
- Boeckner L.S., Schnepf M.I., Tunglund B.C. (2001). Inulin: a review of nutritional and health

- implications. *Advances in Food and Nutrition Research*. 43: 1-63.
- Carlson J.L., Erickson J.M., Hess J.M., Gould T.J., Slavin J.L. (2017). Prebiotic dietary fiber and gut health: comparing the in vitro fermentations of beta-glucan, inulin and xylooligosaccharide. *Nutrients*. 9: 1361.
- Chi Z.M., Zhang T., Cao T.S., Liu X.Y., Cui W., Zhao C.H. (2011). Biotechnological potential of inulin for bioprocesses. *Bioresource Technology*. 102: 4295-4303.
- Coussement P.A. (1999). Inulin and oligofructose: safe intakes and legal status. *The Journal of nutrition*. 129: 1412S-1417S.
- Daliri E.B.M., and Lee B.H. (2015). Current trends and future perspectives on functional foods and nutraceuticals. Beneficial microorganisms in food and nutraceuticals. *Microbiology Monographs*. 27: 221-244.
- DeFelice S.L. (1992). The nutraceutical initiative: a recommendation for U.S. Economic and regulatory reforms. *Genetic Engineering News*. 12: 13–15.
- Dhingra D., Michael M., Rajput H., Patil R.T. (2012). Dietary fibre in foods: a review. *Journal of food science and technology*. 49: 255-266.
- EFSA NDA Panel. (2014). Scientific opinion on the substantiation of a health claim related to non-digestible carbohydrates and a reduction of post-prandial glycaemic responses pursuant to Article 13(5) of Regulation (EC) No 1924/2006. *EFSA Journal*. 12: 3513.
- EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). (2015). Scientific Opinion on the substantiation of a health claim related to “native chicory inulin” and maintenance of normal defecation by increasing stool frequency pursuant to Article 13.5 of Regulation (EC) No 1924/2006. *EFSA Journal*. 13: 3951.
- Esmailnejad Moghadam B., Keivaninahr F., Fouladi M., Rezaei Mokarram R., Nazemi A. (2019). Inulin addition to yoghurt: Prebiotic activity, health effects and sensory properties. *International Journal of Dairy Technology*. 72: 183-198.
- González-Herrera S.M., Herrera R.R., López M.G., Rutiaga O. M., Aguilar C.N., Esquivel J.C.C., Martínez L.A.O. (2015). Inulin in food products: prebiotic and functional ingredient. *British Food Journal*. 117: 371-387.
- Jiménez-Colmenero F., Carballo J., Cofrades S. (2001). Healthier meat and meat products: their role as functional foods. *Meat Science*. 59: 5–13.
- Jiménez-Colmenero F., Sánchez-Muniz F.J., Olmedilla-Alonso B. (2010). Design and development of meat-based functional foods with walnut: Technological, nutritional and health impact. *Food chemistry*. 123: 959-967.
- Kalra E.K. (2003). Nutraceutical-definition and introduction. *AAPS PharmSci*. 5: 27-28.
- Kolida S., Tuohy K., Gibson G.R. (2002). Prebiotic effects of inulin and oligofructose. *British Journal of Nutrition*. 87: 193-197.
- McClements D.J. and Gumus C.E. (2016). Natural emulsifiers—Biosurfactants, phospholipids, biopolymers, and colloidal particles: Molecular and physicochemical basis of functional performance. *Advances in Colloid and interface Science*. 234: 3-26.
- Melilli M.G., Costa C., Lucera A., Padalino L., Del Nobile M.A., Conte A. (2021). Fiordilatte cheese fortified with inulin from cichorium intybus or cynara cardunculus. *Foods*. 10: 1215.
- Miremadi F., Sherkat F., Stojanovska L. (2016). Hypocholesterolaemic effect and anti-hypertensive properties of probiotics and prebiotics: A review. *Journal of Functional Foods*. 25: 497-510.
- Pothuraju R., Chaudhary S., Rachagani S., Kaur S., Roy H.K., Bouvet M., Batra S.K. (2021). Mucins, gut microbiota, and postbiotics role in colorectal cancer. *Gut Microbes*. 13: 1974795.
- Rao M., Gao C., Xu L., Jiang L., Zhu J., Che G., Law, B.Y.K., Xu Y. (2019). Effect of inulin-type carbohydrates on insulin resistance in patients with Type 2 diabetes and obesity: a systematic review and meta-analysis. *Journal of Diabetes Research*. 2019: 5101423.

- Ridgway E., Baker P., Woods J., Lawrence M. (2019). Historical developments and paradigm shifts in public health nutrition science, guidance and policy actions: a narrative review. *Nutrients*. 11: 531.
- Roberfroid M.B., Gibson G.R., Hoyles L., McCartney A.L., Rastall R., Rowland I., et al. (2010). Prebiotics effects: Metabolic and health benefits. *British Journal of Nutrition*. 104: 1–63.
- Rubel I.A., Pérez E.E., Genovese D.B., Manrique G.D. (2014). In vitro prebiotic activity of inulin-rich carbohydrates extracted from Jerusalem artichoke (*Helianthus tuberosus* L.) tubers at different storage times by *Lactobacillus paracasei*. *Food Research International*. 62: 59-65.
- Santini A. (2014). Nutraceuticals: An healthy bet for the future. *Journal of Food Research*. 3: 1-2.
- Santini A., Tenore G.C., Novellino E. (2017). Nutraceuticals: A paradigm of proactive medicine. *European Journal of Pharmaceutical Sciences*. 96: 53-61.
- Sayin S.I., Wahlström A., Felin J., Jäntti S., Marschall H.U., Bamberg K., Bäckhed F. (2013). Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. *Cell metabolism*. 17: 225-235.
- Scheid M.M.A., Moreno Y.M.F., Junior M.R.M., Pastore G.M. (2013). Effect of prebiotics on the health of the elderly. *Food research international*. 53: 426-432.
- Shoab M., Shehzad A., Omar M., Rakha A., Raza H., Sharif H.R., Niazi S. (2016). Inulin: Properties, health benefits and food applications. *Carbohydrate polymers*. 147: 444-454.
- Siró I., Kápolna E., Kápolna B., Lugasi A. (2008). Functional food. Product development, marketing and consumer acceptance – A review. *Appetite*. (51): 456–467.
- Tiefenbacher K.F. (2017). Chapter two - Technology of main ingredients: water and flours. In *Wafer and Waffle*, Tiefenbacher, K. F. (eds.), 86-97. United Kingdom: Elsevier, Academic Press.
- Yousefi M., Khorshidian N., Hosseini H. (2018). An overview of the functionality of inulin in meat and poultry products. *Nutrition & Food Science*. 48: 819-835.

Possible applications of encapsulation in fruit and vegetable technology - a review

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Abstract

This paper aims to present and summarize the current knowledges and experiences of encapsulation possibilities in fruit and vegetable technology. Encapsulation is widely used in the pharmaceutical industry, while in the food industry, its applications are still poorly investigated and applied for industrial purposes. In the fruit and vegetables technology encapsulation is mostly used for juices, extracts, functional drinks, jellies, jams and similar products. Vitamins, anthocyanins, beta carotene, curcumin, catechin, other antioxidants, aromas, essential oils, probiotics, omega fatty acids, and enzymes are mostly encapsulated compounds. Nowadays, especially great attention is given to the researches dealt with encapsulation and usage of bioactive compounds from waste obtained from fruit/vegetable processing. Encapsulation in fruit and vegetable technology can be applied in different ways. Encapsulated bioactive compounds commonly are added to fruit/vegetable products for fortification. The other way is encapsulation of valuable fruit or vegetable juices, extracts or isolated bioactive substances.

Keywords: encapsulation, fruit, vegetable, application, protection

Introduction

Encapsulation is a technique of packing or entrapping active compounds into the structure of coating material. It is mostly used in the pharmaceutical, and recently in the food industry. The most commonly encapsulated active materials in foods are vitamins, minerals, enzymes, probiotics, antioxidants, omega fatty acids, fish oil, essential oils and aromatic substances. The main aim of encapsulation is the formation of stable capsules from biopolymer materials with the encapsulated an active substances. Each capsule consists of a coating (wall material, carrier or matrix) and an active substance. The following reasons are commonly used for encapsulation in food technology: 1) increasing the stability of active substances sensitive to high temperatures, light, oxidation, etc., 2) masking of undesirable taste and smell of the active substance (e.g. fish oil, propolis); 3) improving solubility and achieving more homogeneous mixing when the active substance is dosed in small amounts and 4) controlled release and increasing the bioavailability of active compound in the human body. Protection of the active substance from external influences (heat, oxidation, moisture, oxidation, light, chemical reactions) is the main reason for encapsulation (Nedović et al., 2011; Wandrey et al., 2010; Tahmaz 2019).

Application of encapsulation in fruit and vegetable technology

Fresh tomatoes

Essential oils show high antimicrobial potential against spoilage and pathogenic microorganisms. In the free form, essential oils are highly volatile, unstable and often have a strong smell, which can affect the sensory properties of the product. Because of that, it is preferable to use it in encapsulated form. Encapsulation masks the smell, reduces the possibility of evaporation and oxidation, increases stability and enables controlled release. According to Amiri and Morakabati (2017), the essential oil of satureja (*Satureja khuzestanica*) encapsulated inside chitosan nanocapsules has a better power to inhibit the development of *Rhizopus stolonifer* and significantly higher antioxidant activity compared to unencapsulated oil. The extended shelf life of fresh tomatoes can be achieved by immersing them in

encapsulated satureja oil for 20 minutes. Tomatoes treated with encapsulated oil showed good viability without visible spoilage signs during 8 days of storage, while in samples without essential oils spoilage appeared after 4 days and after 7 days in the case when free/unencapsulated oil was used. Tomatoes treated with encapsulated oil soften more slowly. Higher firmness of treated tomatoes can be explained by the presence of chitosan capsules on the surface. According to Beyki et al. (2014), peppermint (*Mentha piperita*) essential oil prevents the development of molds and prolongs the viability of fresh tomatoes. While encapsulated satureja essential oil has been proven to inhibit the growth of *Rhizopus stolonifer*, peppermint oil encapsulated inside a chitosan and cinnamic acid nanogel inhibits the growth of *Aspergillus flavus*, and the inhibition effect increases by increasing oil concentration. Encapsulated oil has up to 4 times better effect of inhibiting the growth of *A. flavus* compared to the free. Complete inhibition could be achieved with a concentration of 800 ppm, while higher concentration (1000 ppm) increased the viability of fresh tomatoes for 30 days (Beyki et al., 2014).

Addition of encapsulated active compounds into juices and beverages

The aroma of fruit drinks can be improved by adding encapsulated lemon essential oil, which contributes to achieving a pleasant and fresh smell in the product. Due to the high amount of unsaturated fatty acids, this oil is sensitive to oxidative degradation. Encapsulation increases its stability, whereby its shelf life can be extended to 6 months. Aromatization of ice tea is suggested as an example of application. Encapsulated catechin and curcumin can be used in the technology of functional drinks (Peanparkdee et al., 2016). Encapsulated probiotics added to apple and orange juices have prolonged storage stability. It is interesting to note that juices with encapsulated probiotics have higher sugar concentrations, which can be explained by the fact that the sugars contained in the juices were less available to encapsulated probiotics (Tsen et al., 2004). Fruit products generally represent an acidic environment not suitable for the survival of probiotic bacteria, but encapsulation increases the stability of probiotics in acidic food (Tolve et al., 2016).

Carbonated soft drinks

Under microbial activity, soft drinks can change their appearance during storage and become opalescent. The addition of encapsulated enzyme glucose-oxidase can prevent this kind of spoilage. Glucose-oxidase nanocapsules dosed into soft drinks together with proteolytic enzymes (trypsin, chymotrypsin and papain) had a positive effect on physical properties, regulated the pH value, reduced the appearance of cloudiness and opalescence, and contributed to obtaining a clear drink (Sharma, 2012). Carbonated soft drinks contain glucose, fructose and sucrose in equal proportions (Idris et al., 2016). The high presence of glucose represents a suitable environment for the development of microorganisms. Glucose oxidase catalyzes the oxidation of glucose, resulting in gluconic acid. The encapsulated glucose-oxidase reduces the possibility of the development of microorganisms, thus preventing unwanted changes in the color, appearance and aroma of soft drinks. The optimal conditions for glucose oxidase activity are at a temperature of 40 °C and pH of 5.5, while its activity decreases at temperatures over 55 °C (Zia et al., 2007). For this reason, it is preferable to use nanoencapsulated enzyme, because in the encapsulated form glucose oxidase is protected from inactivation due to thermal denaturation and low pH value. This fact is very important in the production carbonated soft drinks and juices, which have a low pH value (carbonated soft drinks 2.5 and non-carbonated 3.3) and pass the pasteurization process at 70 °C. On the other hand, proteolytic enzymes have a positive effect on the quality of soft drinks, because they reduce turbidity, and help the clarification process (Sharma, 2012).

Encapsulation of juices, extracts and similar products

Plum juice encapsulated by spray drying showed significantly better stability of phenolic components compared to nonencapsulated. After 60 days of storage, encapsulation of plum juice can preserve about 85% of the total phenolic components present in fresh juice, while less than 65 % remains preserved in unencapsulated plum juice (Li et al., 2018). Encapsulated blueberry juice and extract can be added to biscuit dough before baking. Encapsulation can retain the antioxidant capacity of blueberry polyphenols and anthocyanins after baking at 200 °C. The best results for polyphenol stability were obtained by using an encapsulation by lyophilization, while anthocyanins content and total antioxidant capacity were higher in capsules obtained by co-extrusion (Tatar Turan et al., 2015). Cherry pomace extract, rich in anthocyanins and polyphenols, encapsulated within soy and whey proteins could be added

to the dough for cookies by replacing the 10-20 % of flour with the encapsulated extract. Dough with soy protein capsules had high firmness, viscosity and resistance to deformations, while the application of whey protein capsules resulted in a softer and less viscous dough prone to deformation (Petrović et al., 2018). Lončarević et al. (2018) added encapsulated blackberry juice (6-10 %) rich in polyphenols to the fortification of white chocolate. The addition of encapsulated blackberry juice to white chocolate can increase its antioxidant activity. The color of the chocolate depended on the concentration of added encapsulated juice. With a higher concentration of juice the solubility and viscosity of melted chocolate increased. Chocolate with blackberry juice had a fruity taste, their sweetness and shine on the surface were less intensive in comparison to the control, and all samples with encapsulated blackberry juice had a purple color.

The popular trend is the production of spherical macrocapsules (macrospheres) with encapsulated liquids, such as sauces, dressings, liquid extracts of spices (garlic and chili) in olive oil. Alginate macrocapsules with liquid spicy extracts stabilized with CaCl_2 are the most common examples. The addition of CaCl_2 enables the creation of a more compact structure. Many of these products are produced commercially. As the macrocapsules look like caviar spheres, the product is sold under the commercial name “*Caviaroli*” (Hochedel, 2020).

Jellies and jams

The nutritive value of fruit jelly can be improved with the addition of free or encapsulated probiotic bacteria (*L. acidophilus*). Probiotics encapsulated within chitosan capsules improve the texture of probiotic jelly. Fruit jelly with the addition of free probiotics has weak sensory properties, while the sensory properties of jelly with encapsulated probiotics are similar to jelly without the addition of probiotics (Talebzadeh and Sharifan, 2016). When probiotics are dosed in an encapsulated form, their stability in the products during storage increases. Shoaie et al. (2022) encapsulated probiotic bacteria *L. plantarum* into Na alginate and gum arabic. Obtained microcapsules were added to the rose petal jam. Results showed that encapsulated probiotics had improved survival in jam during a storage period of 90 days. Total bacterial count after storage was higher than that common recommended level, while sensory properties were acceptable with higher scores in comparison to control. Other physicochemical properties were very similar to the control.

Natural or artificial colors are used in the production of jelly products. For example, as a source of green color in the production of jelly candies, green alfalfa (*Medicago sativa*) extract (rich in chlorophyll) encapsulated in gelatin and agar can be used (Raei et al., 2017). Gummies with encapsulated betalains isolated from red Indian figs have an intense red-purple color that remains stable during 30 days of storage. Unlike artificial colors, encapsulated betalain not only gives an attractive color, but also increases the antioxidant activity (Otalora et al., 2018). Mihalcea et al. (2020) added encapsulated grape juice concentrate to the jelly. Grape juice was encapsulated into whey protein isolate-chitosan microcapsules by freeze drying with very high encapsulation efficiency (86.14 %). Results showed that jellies with encapsulated grape juice had higher anthocyanins stability and antioxidative activity during storage and slower release during simulated *in vitro* digestion test. Martins et al. (2020) added curcumin lipid microparticles to the formulation of low-sugar mango jam. Jam samples with encapsulated curcumin in a concentration of 4% had a more intense and stable color during storage period of 30 days. The sensory properties of jam with encapsulated curcumin were acceptable with higher scores for all sensory attributes in comparison to the control.

Anthocyanins from fruits and vegetables

Anthocyanins are red plant pigments soluble in water, with strong antioxidant properties. Due to their antioxidant capacity, anthocyanins are unstable compounds. Through oxidative decomposition, anthocyanins are transformed into colorless substances and lose their properties as red pigments. Decomposition of anthocyanin occurs under increased pH, high temperature, and exposure to light and oxygen. Different techniques can be used for their encapsulation, but spray drying is commonly used. In beverage technology, anthocyanins are encapsulated within maltodextrin and gum arabic (Peanparkdee et al., 2016). By applying microwave energy it is possible to encapsulate anthocyanins from fruit juices inside maltodextrin microcapsules. The significantly highest encapsulation efficiency (>70 %) can be achieved by microwave treatment in duration of 2 and 3 minutes at 330W. After applying the shorter treatments, maltodextrin could not be dissolved sufficiently, and anthocyanins remained on the capsule surface. Good encapsulation efficiency was achieved after 3 minutes, but the microcapsules had an unattractive red-brown color. As encapsulated anthocyanins have improved stability at 60 °C, obtained capsules can be used in the production of fruit juices and similar products that undergo evaporation or pasteurization (Abang Zaidel et al., 2015).

Bioactive compounds from fruit and vegetable waste

The wastes from fruit and vegetable (peel, seed, pulp, pomace) contain significant amounts of valuable active compounds (polyphenols, carotenoids and anthocyanins), which can be extracted and encapsulated for food fortification (Thakur and Boran, 2021). In the processing of tomato juice, pomace is separated and removed as waste, and a large amount of lycopene is lost. Tomato pomace as a by-product is rich in lycopene and beta carotene. Lycopene is known to be a strong antioxidant and very unstable in free form. It is possible to isolate an alcoholic extract rich in lycopene and beta carotene and encapsulate them by spray drying using gum arabic and inulin as carriers. Obtained capsules were suitable for the enrichment of many food products (Correa-Filho et al., 2019). The skin of the eggplant (*Solanum melongena*) is rich in anthocyanins, which give it a dark purple color. By peeling, the skin is separated as waste, resulting in the loss of anthocyanins. Encapsulated anthocyanins from eggplant skin can be used for coloring and fortification of various food products (Chatterjee and Bhattacharjee, 2015). Tran et al. (2022) reported that betalains from red dragon fruit peel encapsulated by freeze drying had improved stability at low pH, high temperature and light after 30 days of storage in comparison to unencapsulated. Vulić et al. (2019) studied benefits of encapsulated polyphenols and carotenoids from red papper waste. Results showed that encapsulation enhanced polyphenols stability. Encapsulation had protective effect against pH changes and enzymatic activity during *in vitro* digestion.

Vegetable proteins as coating materials

Pea, lentil, chickpea and bean proteins were mostly researched and used for encapsulation. Many studies supported the utilization of vegetable proteins as coating material because of their good protective effects, nutritional value and functional properties, such as solubility, emulsion stability and rheological properties (Wang et al., 2022; Islam et al., 2022; Chang et al., 2016). Pierucci et al. (2006) encapsulated ascorbic acid into pea proteins by spray drying. Obtained microcapsules had an irregular shapes, while retention of ascorbic acid was better in comparison to maltodextrin and carboxymethyl cellulose coatings. Garcia Segovia et al. (2021) reported that a higher concentration of pea proteins in an encapsulation mixture resulted in increasing encapsulation yield and better stability of beetroot juice encapsulated by spray drying. Lentil proteins in combination with maltodextrin and Na-alginate showed good encapsulation properties for canola oil, with 88 % encapsulation efficiency and good oxidative stability (Chang et al., 2016). Lentil protein isolates combined with carrageenan used for flaxseed oil encapsulation showed good oxidative stability of polyunsaturated fatty acids (Wang et al. 2022). Golzar et al. (2022) used kidney bean protein isolates with κ -carrageenan for the encapsulation of shrimp oil. Encapsulation efficiency was 43.99-89.25 %, and obtained microcapsules had good flowability. Retention of astaxantin and polyunsaturated fatty acids was significantly higher in comparison to free oil. Atli et al. (2023) used spray drying for the encapsulation of cumin seed essential oil into chickpea protein isolates and maltodextrin. Results showed very high encapsulation efficiency (90.9-98.4 %) and oil retention (86.6-96.4 %). Microcapsules had low moisture content and water activity (3.17-5.24 % and 0.19-0.25) and increased solubility at high pH values. Moser et al. (2020) used spray drying for the encapsulation of buriti (*Muritia flexuosa L*) into chickpea proteins alone or in combination with pectin. Oil extracted from the buriti palm tree contains a high amounts of tocopherols and carotenoids. Encapsulation improved the oxidative stability of buriti oil.

Conclusions

Encapsulation can be assessed as a process with promising applications in fruit and vegetable technology. As encapsulation is a relatively new process for applying in the food industry its benefits and commercialization have not been completely researched. The most encapsulation examples are still related to scientific research work in laboratories or pilot plants. Encapsulation in fruit & vegetable technology can have the following perspectives: 1) encapsulation of sensitive bioactive compounds and addition to fruits and vegetable products to enhance the nutritional value and the fortification with bioactive compounds that are not naturally presented in fruits and vegetables, 2) application of encapsulated essential oils as antimicrobial agents to prevent spoilage, 3) encapsulation of fruit and vegetable juices or extracts and their addition to the food, 4) utilization of waste from fruit and vegetable by encapsulation and 5) utilisation of vegetable proteins as coating materials.

References

- Abang Zaidel D.N., Makhtar N.A., Mohd J. Y.M., Muhamad I.I. (2015). Efficiency and thermal stability of encapsulated anthocyanins from red dragon fruit (*Hylocereus polyrhizus*) using microwave-assisted technique, *Chemical Engineering Transactions*. 43: 127-132.
- Amiri A. and Morakabati N. (2017). Encapsulation of *Satureja khuzestanica* essential oil in chitosan nanoparticles with enhanced antifungal activity. *World Academy of Science, Engineering and Technology. International Journal of Nutrition and Food Engineering*. 11: 331-336.
- Atli O., Karaca A.C., Ozcelik B. (2023). Encapsulation of cumin (*Cuminum cyminum L.*) seed essential oil in the chickpea protein-maltodextrin matrix. *ACS Omega*. 8: 4156-4164.
- Beyki M., Zhoveh S., Khalili S.T., Rahimi-Cherati T., Aballahi A., Bayat M., Tabatabaei M. (2014). Encapsulation of *Mentha piperita* essential oil in chitosan-cinamic acid nanogel with enhanced antimicrobial activity against *Aspergillus flavus*. *Industrial Crop and Production*. 54: 310-319.
- Chang C., Varenkovich N., Nickerson M.T. (2016). Microencapsulation of canola oil by lentil protein isolate-based wall materials. *Food Chemistry*. 212: 264-273.
- Chatterjee D., and Bhattacharjee P. (2015). Encapsulation of colour from peels of eggplant in calcium alginate matrix. *Nutrafoods*. 14: 87-96.
- Corrêa-Filho L., Lourenço S., Duarte D., Moldão-Martins M., Alves V. (2019). Microencapsulation of tomato (*Solanum lycopersicum L.*) pomace ethanolic extract by spray drying: Optimization of process conditions. *Applied Sciences*. 9: 612.
- Garcia-Segovia P., Igual M., Martinez-Monzo J. (2021). Beetroot microencapsulation with pea proteins using spray drying: Physicochemical, structural and functional properties. *Applied Sciences*. 11: 6658.
- Gulzar S., Balange A.K., Nagarajarao R.C., Zhao Q., Benjakal S. (2022). Microcapsules of shrimp oil using kidney bean protein isolate and k-carragenan as wall materials with the aid of ultrasonication and high pressure microfluidisation: Characteristics and oxidative stability. *Foods*. 11: 1431.
- Hochedel A.G. (2020). The Unique Luxury Magazine: Caviarolli: Exquisite magic in pearl form. Available from: <https://www.hochedel.ch/articles-en/caviaroli-gourmet-luxury-olive-oil-exquisite-magic-in-pearl-form-en/>. last accessed on 2023/10/26.
- Idris A.M., Vani N.V., Almutari D.A., Jafar M.A., Boreak N. (2016). Analysis of sugars and pH in commercially available soft drinks in Saudi Arabia with a brief review on their dental implications. *Journal of International Society of Preventive Community Dentistry*. 6: 192-196.
- Islam F., Ali Y.A., Imran A., Afzaal M., Zahra S.M., Fatima M., Saeed F., Usman I., Shehzadi U., Mehta S., Shah M.A. (2023). Vegetable proteins as encapsulating agents: Recent updated and future perspectives, *Food Science and Nutrition*. 11: 1705-1717.
- Li Y., Tang B.J., Lai P. (2018). Microencapsulation of plum (*Prunus salicina Lindl.*) phenolics by spray drying technology and storage stability. *Food Science and Technology Campinas*. 38: 530-536.
- Lončarević I., Pajin B., Fišteš A., Tumbas Šaponjac V., Petrović J., Jovanović P., Vulić J., Zarić D. (2018). Enrichment of white chocolate with blackberry juice encapsulate: Impact on physical properties, sensory characteristics and polyphenol content, *LWT Food Science and Technology*. 92: 458-464.
- Martins M.P., Geremias-Andrade I.M., Terreira L.S., Brito-Oliveira T.C., Pinho S.C. (2020). Technological and sensory feasibility of enrichment of low-sugar mango jam with curcumin encapsulated in lipid microparticles. *Food Science and Technology*. 41: 1-8.
- Mihalcea L., Barbu V., Enachi E., Andronolu D.G., Rapeanu G., Stoica M. Dumitrascu L., Stanciuc N. (2020). Microencapsulation of red grape juice by freeze drying and application in jellies formulation. *Food Technology and Biotechnology*. 58: 20-29.
- Moser P., Ferreira S., Nicoletti V.R. (2019). Buruti oil microencapsulation in chickpea protein-pectin matrix as affected by spray drying parameters. *Food Bioproduct processing*. 117: 183-193.

- Nedović V., Kalušević A., Manojlović V., Lević S., Bugarški B.(2011). An overview of encapsulation technologies for food application. *Procedia Food Science*, 11. International Congress on Engineering and Food (ICEF11): 1806-1815.
- Otálora M.C., de Jesús B.H., Perilla J.E., Osorio C., Nazareno M.A. (2018). Encapsulated betalains (*Opuntia ficus-indica*) as natural colorants. Case study: Gummy candies. *LWT Food Science and Technology*. 103: 222-227.
- Peanparkdee M., Iwamoto S., Yamauchi R. (2016). Microencapsulation: A Review of Application in the Food and Pharmaceutical Industry. *Reviews in Agriculture Science*. 4: 56–65.
- Petrović J., Pajin B., Lončarević I., Šaponjac V.T., Nikolić I., Ačkar Đ., Zarić D. (2018). Encapsulated sour cherry pomace extract: Effect on the colour and rheology of cookie dough. *Food Science and Technology International*. 25: 1-11.
- Pierucci A.P., Andrade L.R., Baptista E.B., Volpato N.A., Helena M., Roche-Leao M. (2006). New microencapsulation system for ascorbic acid using pea protein concentrate as coat protector. *Journal of Microencapsulation*. 23: 654-682.
- Raei A., Yasini Ardakani S.A., Daneshi M. (2017). Microencapsulation of the green pigment of alfalfa and its applications on heated food. *Journal of Food Process Engineering*. 40: e12529.
- Sharma K.R. (2012). Preparation of Emulsified Encapsulated Nanoparticles of Bovine Serum Albumin of Bound Glucose Oxidase and their Application in Soft Drinks/Non-Alcoholic Beverage. *Journal of Biotechnology & Biomaterials*. 2:1000126.
- Shoaei F., Hesmati A., Mahjub R., Garmakhanny A.D., Taheri M. (2022). The assessment of microencapsulated *Lactobacillus plantarum* survivability in rose petal jam and the changes in physicochemical, textural and sensorial characteristics during storage. *Scientific Reports Nature Portfolio* 12: 6200.
- Tahmaz J. (2019). Enkapsulacijske tehnike u prehrambenom inženjerstvu. Dobra knjiga Sarajevo.
- Talebzadeh S., and Sharifan A. (2016). Developing Probiotic Jelly Desserts with *Lactobacillus Acidophilus*. *Journal of Food Processing and Preservation*. 41: e13026.
- Tatar Turan F, Cengiz A., Kahyaoglu T., (2015): Evaluation of ultrasonic nozzle with spray drying as a novel method for the microencapsulation of blueberry's bioactive compounds, *Innovative Food Science and Emerging Technologies*. 32: 136-145.
- Thakur J., and Borah A. (2021). Microcapsules of bioactive compounds from fruits and vegetables waste and their utilization: A review, *The Pharma Innovation Journal*. 10: 151-157.
- Tolve R., Galgano F., Caruso M.C., Tchuembou-Magaia F.L., Condel N., Favati F., Zhang, Z. (2016). Encapsulation of health-promoting ingredients: Applications in foodstuffs. *International Journal of Food Sciences and Nutrition*. 67: 888–918.
- Tran U.P.N., Dang-Bao T., Le D.T.K., Huynh U.D.H., Nguyen T.T.H., Le T. M. (2022). Encapsulation of betalains extracted from red dragon fruit peel by freeze drying using microcrystalline cellulose and dragon fruit peel pectin as wall materials, *Chemical Engineering Transactions*. 97: 31-36.
- Tsen J.H., Lin Y.P., King V.A.E. (2004). Fermentation of banana media by using *j*-carrageenan immobilized *Lactobacillus acidophilus*. *International Journal of Food Microbiology*. 91: 215–220.
- Vulić J., Ševegelj V., Kalušević A., Lević S., Nedović V., Tumbaš Šaponjac V., Čanadanović-Brunet J., Četković G. (2019). Bioavailability and bioactivity of encapsulated phenolics and carotenoids isolated from red pepper waste. *Molecules* 24: 2837.
- Wandrey C., Bartkowiak A., Harding S.E. (2010). Materials for Encapsulation. In: *Encapsulation Technologies for Active Food Ingredients and Food Processing* (Zuidam N.J, Nedović V.A. eds.): 31-101.
- Wang Y., Ghash S., Nickerson M.P. (2022). Microencapsulation of flaxseed oil by lentil protein isolate-kappa carragenan based wall materials through spray and freeze drying. *Molecules*. 27: 3195.
- Zia M.A., Rahman K., Saeed M.K., Andaleeb F., Rajoka M.I., Sheikh M. ., Khan A.I. (2007). Thermal characterization of purified glucose oxidase from a newly isolated *Aspergillus Niger*. *Journal of Clinical Biochemistry and Nutrition*. 41: 132–138.

Utjecaj pektina i sadržaja šećera na senzorska svojstva džemova od borovnica

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Sažetak

Šećer, pektin i kiselina u uzajamnom djelovanju određuju konzistenciju džema, ali i druge kvalitativne karakteristike. Dodavanjem pektinskih preparata moguće je utjecati na konzistenciju džema, skratiti vrijeme pripreme, smanjiti udio dodanog šećera te povećati količinu gotovog proizvoda. U ovom radu istražuje se utjecaj različitih pektina u kombinaciji s različitim količinama dodanog šećera na osnovne karakteristike džemova. Senzorski panel sastojao se od 26 članova, studenata Enogastronomije koji poznaju metode senzorskog ocjenjivanja, te su ocjenjeni boja, miris, okus i konzistencija. Uzorcima su također izmjereni osnovni kemijski parametri: udio šećera i pH. Rezultati su pokazali kako su uzorci bez dodanog šećera najlošije ocjenjeni po svim kriterijima, ali i uzorci s visokim udjelom šećera dobili su slabije ocjene za okus jer slatkoća previše dominira. Najbolje rezultate ostvarili su džemovi sa udjelom šećera u rasponu od 33,8 °Bx do 45,8 °Bx, 2,8 – 3,1 pH i sa udjelom voća većim od 75 %.

Ključne riječi: borovnice, džem, pektin, šećer, senzorska svojstva

Uvod

U Europi je uzgoj borovnica započeo tek nakon 1960. godine i to u Njemačkoj, Nizozemskoj, Poljskoj i Rumunjskoj. U našim krajevima prve sadnice su uvezene 1962. te su posađene u Ljubljanskom barju (Oblak, 1996). Proizvodnja borovnica u svijetu ima značajan uspon, a u razdoblju od 2010. do 2019. je više nego udvostručena s 439.000 tona na gotovo milijun tona (USDA, 2021).

Plodovi borovnice izvrsnog su okusa i prepuni su hranjivih sastojaka, a 100 g borovnica sadrži samo 64 kcal. Borovnice sadrže visoku koncentraciju polifenola, osobito flavonoida i antocijana, te su time izvrstan izvor prehrambenih antioksidansa (Dragović-Uzelac, 2010). Utvrđeno je da antioksidansi generiraju različite pozitivne učinke na ljudsko zdravlje te da je konzumacija svježeg voća najbolji način unosa istih (Retamales & Hancock, 2018). Odličan su izvor vlakana, vitamina C, vitamina K, kalija i mangana. Borovnice mogu pomoći kod prevencije dijabetesa tipa 2, Alzheimerove bolesti i srčanih tegoba (U.S. HBC, 2023). Plodovi vrste *Vaccinium* imaju antimikotičke, antibakterijske i antivirusne učinke, za što su odgovorni fenolni spojevi i visoke razine organskih kiselina (Salunkhe & Kadam, 1995). Oko dvije trećine svjetske proizvodnje borovnica se stavlja na tržište u svježem obliku. Krupni plodovi su traženiji kod kupaca, lakše se beru i preferiraju se za svježju potrošnju, dok su sitniji plodovi bolji za preradu. Veličina ploda ovisi o sorti, agroekološkim i tehnološkim uvjetima uzgoja, a kod određivanja kvalitete svježih borovnica potrebno je obratiti pažnju na veličinu i čvrstoću ploda te koncentraciju topive suhe tvari (uglavnom šećera), kiselina i antioksidansa (Retamales & Hancock, 2018). Budući da su svježe borovnice relativno kratkog vijeka trajanja, prerada je jedan od načina da se sačuvaju njihove visoke nutritivne vrijednosti. Svježi plodovi borovnice, ali i džem od borovnica su bogat izvor fenolnih spojeva koji imaju jako antioksidativno djelovanje. Prema Prvulović (2021), fenolnih spojeva je više u džemu nego u svježim borovnicama (po jedinici mase) jer se procesom prerade gubi voda, a ne gube se bioaktivni spojevi. Zbog toga džem od borovnica može predstavljati važan izvor bioaktivnih spojeva u prehrani. Međutim, osim zdravstvenog aspekta izrazito je važan utjecaj dodataka na senzorska svojstva proizvoda te prihvatljivost od strane potrošača. Stoga je cilj ovog rada bio je odrediti utjecaj dodatka šećera i različitih vrsta pektinskih preparata na senzorsku prihvatljivost džemova od borovnice. U kontekstu propagiranja prehrane sa

smanjenim udjelom šećera, osobito zanimljivo je vidjeti percepciju mlađih potrošača na džemove bez dodatka šećera u odnosu na klasični proizvod sa šećerom.

Materijali i metode

Priprema uzoraka

Za pripremu džemova od borovnica korištene su zamrznute borovnice ubrane u sezoni 2022. na plantaži „Blueberry ranch“ u Ksajpi kraj Čakovca. Džemovi su pripremljeni na slijedeći način: zamrznute borovnice stavljene su u lonac na maceraciju i otapanje. Količina od 2 kg borovnica po jednom uzorku pomiješana je s predviđenom količinom šećera kako je navedeno u tablici 1 i ostavljena da odstoji minimalno četiri sata. Nakon maceracije pristupilo se kuhanju. Uzorci bez dodatnog pektina kuhali su se do prihvatljivog stupnja želiranja, odnosno kada je proba na hladnoj površini pokazala kompaktnu teksturu. Uzorci s dodanim pektinom kuhani su 45 - 60 minuta. Pektin i limunska kiselina (Šafram d.o.o.) su se dodavali pred kraj kuhanja. Količina pektina je varirala prema podacima prikazanim u tablici 1, a svim uzorcima je dodano 5 g limunske kiseline. Nakon kuhanja gotovi džem se punio u sterilizirane staklenke. Odmah nakon zatvaranja staklenke su stavljene na temperaturu od 90 °C na 15 minuta. Za usporedbu, u maloprodaji su kupljena još tri uzorka. Prilikom kuhanja koristila se uvijek ista količina borovnica i ista količina limunske kiseline. Parametri koji su varirani tijekom pokusa su: količina dodanog šećera (0, 500, 1000 i 1500 grama šećera na količinu od 2000 grama borovnica), te količina i vrsta dodanog pektina. Četiri uzorka su pripremljena bez dodanog pektina, u četiri je dodan srednje brzo želirajući HM pektin (Sosa Medium Rapid Set Pectin), u količini od 0,5 % i 0,8 %, a u dva uzorka dodan je amidirani LM pektin s dodanim kalcijem (Sosa Low Sugar Pectin), u količini od 0,8 %. U uzorke 5, 7, 8 i 9 je dodano 200 ml vode sukladno uputi proizvođača pektinskog pripravka. Točne količine ulaznih sastojaka navedene su u tablici 1.

Tablica 1. Ulazne sirovine za pripremu uzoraka

uzorak	masa borovnica (g)	masa šećera (g)	masa pektina (g)	masa limunske kiseline (g)	voda (ml)	vrsta dodanog pektina
1	2000	1.000	0	5	0	
2	2000	1.500	0	5	0	
3	2000	0	0	5	0	
4	2000	500	0	5	0	
5	2000	500	10	5	200	HM pektin
6	2000	1.000	10	5	0	HM pektin
7	2000	0	16	5	200	LM+Ca pektin
8	2000	500	16	5	200	LM+Ca pektin
9	2000	500	16	5	200	HM pektin
10	2000	1.500	16	5	0	HM pektin

Mjerenje osnovnih kemijskih parametara

Količina šećera u gotovom proizvodu mjerena je refraktometrom Milwaukee MA871 (Milwaukee Instruments, EU). Za mjerenje pH vrijednosti korišten je pH-metar Milwaukee pH51 (Milwaukee Instruments, EU).

Senzorska analiza uzoraka

Senzorski panel činili su studenti prve i druge godine preddiplomskog stručnog studija Enogastronomije na Fakultetu turizma i ruralnog razvoja u Požegi, koji su prošli obuku iz senzorskog ocjenjivanja. Redosljed kušanja određen je prema postotku šećera od najmanjeg prema najvećem kako količina šećera ne bi pretjerano oslabila senzorske sposobnosti ocjenjivača. Ocjenjivali su boju, miris, okus i konzistenciju. Ocjenjivanje se vršilo prema kriterijima navedenim u tablici 2.

Tablica 2. Kriteriji ocjenjivanja uzoraka

SVOJSTVO	KARAKTERISTIKA	BODOVI
BOJA	Nijansa i jakost boje karakteristični za odgovarajuću vrstu voća	4
	Nijansa karakteristična, ali nešto različitog intenziteta (neznatno izblijedila ili prejako obojena)	2 - 3
	Oslabljena ili pretamna nekarakteristična boja, po zagorenom	1
	Boja koja uopće ne odgovara vrsti navedenog voća	0
MIRIS	Izvrstan, harmoničan, karakterističan za odgovarajuću vrstu voća	4
	Karakterističan, ali neznatna razlika intenziteta (prejaka ili preslaba)	3
	Oslabljen, ali još karakterističan za proizvod	2
	Bez mirisa ili blago izražen strani miris (npr. po zagorenom)	1
	Pokvaren, po plijesni	0
OKUS	Karakterističan proizvodu, bez prigovora, harmoničan	6
	Karakterističan proizvodu, ali je intenzitet nešto slabije izražen	4 - 5
	Slabiji, ali još karakterističan proizvodu, ukupni utisak nije izrazito harmoničan	2 - 3
	Normalan okus navedene vrste voća s drugim blago izraženim priokusom (npr. po zagorenom)	1
	Pokvaren okus po plijesni i truleži	0
KONZISTENCIJA	Izvrсна, maziva, potpuno homogena	6
	Maziva, s vrlo slabo vidljivim odjeljivanjem tekućeg dijela (sineraze)	4 - 5
	Prekruta, slabo maziva ili preslaba, tekuća s vidljivim odjeljivanjem tekućeg sloja	1 - 3
	Potpuno nehomogena, neodgovarajuća	0

Rezultati i rasprava

Rezultati osnovnih kemijskih parametara prikazani su u tablici 3. U Republici Hrvatskoj proizvodi na bazi pektinskog gela moraju udovoljavati zahtjevima kvalitete prema Pravilniku o voćnim džemovima, želeima, marmeladama, pekmezu te zaslađenom kesten pireu (Narodne novine 84/2019). Sukladno navedenom Pravilniku, ako se izuzmu posljednja tri uzorka iz maloprodaje, samo uzorci broj 2 i 10 zadovoljavaju propisan udio topljive suhe tvari, a to su i uzorci kojima je dodana najveća količina šećera (tablica 3). Uzorci u koje je dodan pektin kraće su se kuhali jer je želirana konzistencija trebala biti postignuta pektinom. Uzorci 1 – 4 u koje nije dodavan pektin kuhali su se dulje što znači da je ispareno više vode te je konzistencija postignuta kombinacijom ugušćivanja i prirodno prisutnog pektina u borovnicama. Duljim kuhanjem, odnosno isparavanjem vode, povećava se količina šećera/suhe tvari, međutim ipak za uzorke 1, 3 i 4 to vrijeme nije bilo dovoljno da bi se postigla zadana vrijednost suhe tvari od 60 %. Ipak, uzimajući u obzir da se u uzorke bez šećera ili sa smanjenom količinom šećera smiju dodavati sladila (što u ovom slučaju nije učinjeno) i ovi uzorci bi se mogli deklarirati kao džemovi. Ukoliko to ne bi bio slučaj trebali bi se deklarirati na drugačiji način npr. voćni namaz od borovnica ili bilo koji drugi naziv koji dobro opisuje karakteristiku proizvoda.

Tablica 3. Rezultati osnovnih kemijskih parametara u gotovim džemovima

Uzorak	Topljiva suha tvar (°Brix)	pH
1	56,4	3,1
2	63,7	2,9
3	17,2	2,5
4	45,8	3,0
5	36,3	2,8
6	50,4	2,5
7	13,9	2,8
8	33,8	3,0
9	36,9	3,0
10	68,8	3,0
11	60,6	3,1
12	60	3,2
13	61,7	3,2

Tablica 4. Rezultati senzorskog ocjenjivanja uzoraka (rezultati su prikazani kao srednja vrijednost \pm standardna devijacija)

	Boja	Miris	Okus	Konzistencija	Ukupno
1	3,85 \pm 0,46	3,42 \pm 0,84	4,58 \pm 1,01	4,85 \pm 1,03	16,69 \pm 1,86
2	3,88 \pm 0,32	3,42 \pm 0,79	3,31 \pm 1,20	3,58 \pm 1,12	14,19 \pm 2,32
3	3,81 \pm 0,39	3,31 \pm 0,72	3,50 \pm 1,15	3,65 \pm 1,41	14,27 \pm 2,50
4	3,96 \pm 0,19	3,58 \pm 0,63	5,12 \pm 1,01	4,58 \pm 1,34	17,23 \pm 2,04
5	3,85 \pm 0,46	3,42 \pm 0,88	4,85 \pm 1,17	3,19 \pm 1,41	15,31 \pm 2,67
6	3,92 \pm 0,27	3,35 \pm 0,78	4,46 \pm 1,15	3,54 \pm 1,22	15,27 \pm 2,25
7	3,81 \pm 0,48	3,12 \pm 0,93	3,42 \pm 1,31	4,12 \pm 1,31	14,46 \pm 2,79
8	3,88 \pm 0,42	3,50 \pm 0,64	5,08 \pm 1,00	4,69 \pm 1,23	17,15 \pm 2,36
9	3,81 \pm 0,39	3,38 \pm 0,88	4,15 \pm 1,38	2,69 \pm 1,07	14,04 \pm 2,95
10	3,88 \pm 0,32	3,46 \pm 0,75	3,58 \pm 1,60	3,23 \pm 1,28	14,15 \pm 2,93
11	3,85 \pm 0,36	3,38 \pm 0,88	4,00 \pm 1,38	4,58 \pm 1,04	15,81 \pm 2,47
12	3,92 \pm 0,27	3,31 \pm 0,87	3,38 \pm 1,60	4,58 \pm 1,08	15,19 \pm 2,65
13	3,46 \pm 0,84	2,96 \pm 1,06	3,85 \pm 1,51	4,23 \pm 1,55	14,50 \pm 2,99

Rezultati senzorskog ocjenjivanja uzoraka prikazani su u tablici 4. Ocjene za boju su za sve uzorke bile ujednačene. Najbolje je ocjenjen uzorak 4 s prosječnom ocjenom 3,96, a najlošije uzorak 13 s prosječnom ocjenom 3,46 od maksimalnih 4 boda. Ocjene za miris su također ujednačene, te je najbolje ocjenjen uzorak 4 s prosječnom ocjenom 3,57, a najlošije uzorak 13 s prosječnom ocjenom 2,96.

Ocjene za okus su varirale. Najbolje je ocjenjen uzorak 4 s prosječnom ocjenom 5,12, a najlošije uzorak 2 s prosječnom ocjenom 3,31 (od mogućih 6 bodova). U svom istraživanju Guimarães i sur. (2014) utvrdila je značajnu razliku u ocjeni okusa za proizvode u kojima je bio korišten HM pektin u odnosu na proizvode u kojima je bio korišten LM pektin. U ovom slučaju dodani pektin, kao ni vrsta dodanog pektina, nisu utjecali na okus uzoraka. Uzorci s dodanim LM + Ca pektinom dobili su ocjene 5,07 za uzorak 8 i 3,42 za uzorak 7. Uzorak 8 je među najboljima, a uzorak 7 među najlošijima. Uzorci s dodanim HM pektinom po ocjeni okusa nalaze se između uzoraka bez dodanog pektina i uzoraka s dodanim LM + Ca pektinom. Tri najbolje ocjenjena uzorka; uzorak 4, 8 i 5, kojima je dodano samo 500 g šećera na 2000 g borovnica, imaju relativno nizak udio topive suhe tvari i to u rasponu od 33,8 °Bx do 45,8 °Bx.

Slabije su ocjenjeni uzorci bez dodanog šećera i uzorci s preko 60 °Bx. Prema količini dodanog šećera usporedivi su uzorci 4, 5, 8 i 9 jer je u njih dodano 250 g šećera na 1000 g borovnica. Uzorci 4 i 8 su najbolje ocjenjeni uzorci. U uzorak 4 nije dodan pektinski pripravak, u uzorak 8 je dodan LM + Ca pektin, a u uzorke 5 i 9, koji su među lošije ocjenjenim uzorcima, dodan je HM pektin. U uzorku 5 nije postignut odgovarajući stupanj želiranja. U uzorke 1 i 6 dodano je na 1000 g borovnica 500 g šećera (omjer 2:1). Uzorak 1 bez dodanog pektina je među najbolje ocijenjenim uzorcima, pogotovo u katgorijama okusa i konzistencije. Uzorku 6, kojem je konzistencija slabo ocjenjena, je kao i uzorku 5 dodan HM pektin kojim nije postignut odgovarajući stupanj želiranja. Uzorci 2, 10 i 13 mogu se nazvati „Ekstra džem od borovnica“ jer prema svim kriterijima zadovoljavaju uvjete iz Pravilnika o voćnim džemovima, želeima, marmeladama, pekmezu te zaslađenom kesten pireu (Narodne novine 84, 2019). U njih je dodano 750 g šećera na 1000 g voća. No, ovi uzorci imaju ujednačeno slabije ocjene, osobito iz kriterija okus uz komentare da šećer dominira nad voćnim okusom borovnica.

Najbolji uzorci 4, 8 i 5 imali su izmjereni pH 2,8 – 3,1, a radi se o uzorcima koji imaju izraženiju kiselinu i umjerenu količinu šećera.

Ocjene za konzistenciju su varirale. Najbolje je ocjenjen uzorak 1 s prosječnom ocjenom 4,85 a najlošije uzorak 9 s prosječnom ocjenom 2,69 (od maksimalnih 6 bodova). U uzorcima 9 i 10 dodano je više HM pektina, 0,8 % u odnosu na ukupnu količinu voća, nego u uzorcima 5 i 6 gdje je dodano 0,5 % HM pektina. Rezultat je čvršći gotovi proizvod koji se nije svidio kušačima. U uzorke 7 i 8 dodan je LM + Ca pektin i imao je pozitivan učinak na konzistenciju proizvoda. S druge strane, u svom istraživanju Guimarães, et al. (2014) utvrdila je da su uzorci u kojima je korišten HM pektin ocjenjeni bolje od uzoraka a u kojima je korišten LM pektin.

Najbolje ocjene dobili su uzorci 4 i 8 koji su u svih pet kategorija (boja, miris, okus, konzistencija, ukupno) bili u gornjoj polovini ljestvice. U oba uzorka dodana je ista, količina šećera, 250 g šećera na 1 kg voća. U skladu s Uredbom (EZ) br. 1924/2006 o prehranbenim tvrdnjama ovi proizvodi bi se mogli nazvati proizvodima sa smanjenim udjelom šećera. Najlošije ocjene dobili su uzorci 3 i 7 koji su u svih pet kategorija (boja, miris, okus, konzistencija, ukupno) bili u donjoj polovini ljestvice, ali niti u jednoj kategoriji najlošiji. To su uzorci u kojima nema dodanog šećera. Uzorak 3 ima 17,2 °Bx, a uzorak 7 ima 13,9 °Bx. Ovi proizvodi dobili su niske ocjene budući da uopće nisu slatki. Trebalo bi proširiti istraživanje na percepciju prema džemovima sa smanjenim udjelom šećera, ali uz primjenu različitih sladila koja bi nadomjestila željenu slatkoću u ovom tipu proizvoda. Uzorak 7 u koji je dodan pektin imao je bolju konzistenciju od uzorka u 3 bez dodanog pektina.

Zaključci

Džemovi bez dodanog šećera ostvarili su najlošije rezultate senzorskog ocjenjivanja po svim kriterijima bez obzira je li dodan pektin ili ne. Džemovi s najviše dodanog šećera ostvarili su također loše rezultate jer slatkoća šećera dominira nad voćnim okusom. Najbolje rezultate ostvarili su džemovi s udjelom šećera u rasponu od 33,8 °Bx do 45,8 °Bx, pH- vrijednosti od 2,8 – 3,1 s udjelom voća većim od 75 %. Dodavanje pektinskih preparata nije utjecalo na kvalitativne karakteristike gotovog proizvoda za parametre boja, miris i okus. Bolji rezultati za parametar konzistencije postignuti su dodavanjem LM + Ca pektina, u odnosu na HM pektin.

Literatura

- Dragović-Uzelac V., Savić Z., Brala A., Levaj B., Bursać Kovačević D., Biško A. (2010). Evaluation of Phenolic Content and Antioxidant Capacity of Blueberry Cultivars (*Vaccinium corymbosum* L.) Grown in Northwest Croatia. *Food technology and biotechnology*. 48: 214-221.
- Guimarães D.H.P., Alves G.L., Querido A.F. (2014). Influence of gelling substance on sensory quality blueberry (climax) jelly. *International Journal of Biotechnology and Food Science*. 2: 116-120.
- Oblak M. (1996). Ameriške borovnice: razvoj rastline in gojenje. Ljubljana, Slovenija: Kmečki glas.
- Pravilnik o voćnim džemovima, želeima, marmeladama, pekmezu te zaslađenom kesten pireu. Narodne novine 84, 2019. [datum pristupa 22.05.2023.].
https://narodne-novine.nn.hr/clanci/sluzbeni/2019_09_84_1726.html
- Prvulović D., Peić Tukuljac M., Kolarov R., Kolbas N., Kolbas A., Ljubojević M., Barać G., Ognjanov

- V. (2021). Chemical composition and antioxidant properties of blueberry fruits and jam. *Agriculture & Food*. 9: 78 – 85.
- Retamales J.B., and Hancock J.F. (2018). *Blueberries*, 2nd Edition. Boston (MA), USA: CABI.
- Salunkhe D. and Kadam S. (1995). *Handbook of Fruit Science and technology: production, composition, storage, and processing*. New York, USA: CRC Press.
- U.S. Highbush Blueberry Council, 2023., Blueberry formats. [datum pristupa 01.06.2023.]. <https://foodprofessionals.blueberry.org/blueberry-formats/>
- Uredba (EU) 1924/2006 Europskog Parlamenta i Vijeća o o prehrambenim i zdravstvenim tvrdnjama koje se navode na hrani, 20.12.2006. [datum pristupa 01.06.2023.]. <https://eur-lex.europa.eu/legal-content/HR/TXT/HTML/?uri=CELEX:02006R1924-2014121>
- USDA, Agricultural Research Service, 2021., Blueberries Around the Globe - Past, Present, and Future. [datum pristupa 25.05.2023.]. <https://www.fas.usda.gov/>

Influence of pectin type and sugar level on sensory properties of blueberry jam

Abstract

The interaction of sugar, pectin and acid determines the consistency of the jam, as well as other qualitative characteristics. By adding pectin preparations, it is possible to influence the consistency of the jam, shorten the preparation time, reduce the proportion of added sugar and increase the amount of the finished product. In this study, the influence of different pectins in combination with different amounts of sugar on the basic characteristics of jams is investigated. The sensory panel consisted of 26 members, students of Enogastronomy who were familiar with the methods of sensory assessment. Color, smell, taste and consistency of jams were rated. The basic chemical parameters of sugar content and pH were also measured. The results showed that the samples without added sugar were rated the worst by all criteria, but the samples with high sugar content also received low ratings for taste because sweetness dominated too much. The best results were achieved for jams with a sugar content ranging from 33.8 °Bx to 45.8 °Bx, 2.8 - 3.1 pH and with a fruit content of more than 75%.

Keywords: blueberries, jam, pectin, sugar

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Optimizacija energetske učinkovitosti procesa sušenja zrna kukuruza na osnovu temperaturnih, psihometrijskih i energetskih parametara

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Sažetak

U suvremenim industrijskim procesima, posebno u sektoru sušenja, energetska učinkovitost ključna je za održivost i ekonomsku isplativost. U ovom istraživanju razmatra se utjecaj različitih parametara, uključujući temperaturne, psihometrijske i energetske, na energetska učinkovitost sušare. Prvi segment istraživanja fokusira se na potrošnju goriva, promatrajući koliko goriva se troši u plamenicima tijekom različitih ciklusa sušenja. Drugi dio analize bavi se psihometrijskim vrijednostima, pružajući informacije o vlažnosti i drugim ključnim parametrima koji mogu imati utjecaj na energetska potrošnju i učinkovitost procesa sušenja. Na kraju, promatrana je potrošnja električne energije, kao dodatni pokazatelj energetske učinkovitosti i isplativosti procesa sušenja. Rezultati su pokazali da postoji povezanost između psihometrijskih parametara i potrošnje električne energije. Uzimajući u obzir navedeno, istraživanjem se nastoji predložiti optimalni uvjeti u procesu sušenja kako bi se postigla najveća moguća energetska učinkovitost.

Ključne riječi: sušenje, energija, energetska učinkovitost.

Uvod

Sušenjem se postižu konzerviranja svih poljoprivrednih proizvoda uklanjanjem suvišne vode. Ovaj postupak osigurava čuvanje proizvoda određeno razdoblje (cijelu godinu) bez promjena kao i mogućnost novog korištenja (Krička i sur., 2003; Krička i sur., 2021; Matin i sur., 2013; Matin i sur., 2017; Matin i sur. 2019; Matin i sur., 2023). Na učinkovitost sušenja utječe entalpija zraka, relativna vlažnost, protok zraka i konstrukcije sušara. U procesu sušenja voda se pomiče iz unutrašnjosti zrna prema njegovoj površini, a zatim s površine u zrak koji se suši (Mujumdar, 2000). Poljoprivredni materijal koji se suši u svojoj strukturi sadrži najviše vode u stanicama, a izdvaja se difuzijom, pa tako i brzina samog sušenja ovisi o tome. Brzina sušenja difuzijom je veća što je temperatura viša, manji zračni tlak, veća vlažnost te veći pad temperature od unutrašnjosti prema van.

Prema Katiću, (1997) sušare se mogu podijeliti prema vrsti i namjeni robe koja se suši, po načinu transporta robe kroz sušaru, prema načinu grijanja zraka za sušenje, prema vođenju zraka kroz sušaru.

Kukuruz se suši do 14 % vlažnosti, a ukoliko bi se presušio odnosno vlažnost bi mu bila niža od ravnotežne, čime bi se povećala cijena sušenja, naročito pošto su cijene goriva visoke. Taj isti problem također smanjuje količinu kukuruza koja se može osušiti u jednom danu te posljednično daje manju količinu kukuruza namijenjenu za tržište.

Sušenje zrna kukuruza može se provoditi pri različitim temperaturama i u raznim vrstama sušara, iako se većinom suši na 120°C u kontinuiranim sušarama. Osim navedenog, može se sušiti na visokim (150°C do 300°C), povišenim (80°C do 120°C), srednjim (40°C do 80°C) i nižim (0°C do 40°C) temperaturama. Prema Somer i sur., (2008) sušenje zrna kukuruza obavlja se na visokim temperaturama (do 260°C), dok sušenje na povišenim temperaturama (80°C do 120°C) obavlja se pretežno u saržnim sušarama sušarama. Isti autor navodi da se za kapacitet od 30 tona zrna, sušenje provodi maksimalno 48 sati na povišenim temperaturama fluida za sušenje od 90° C, a toplinska snaga agregata ne bi trebala biti veća od 200 kW. Prema tome, lako je i praktično riješiv problem sa energijama, jer takav

potrošač dnevno zahtijeva do 400 dm³ tekućeg ili oko 17 Nm³/h plinovitog goriva, pošto se kod nas energija za sušenje još uvijek većinom namiruje iz fosilnih goriva (nafta - plin).

Ovakva tehnologija energetski se vrlo nepovoljno odražava na bilancu sušenja. Potrošnja energije ovisi najviše od vlage zrna na koje se suši. Ako se suši zrno s 25% vlažnosti troši se oko 315 kWh/t zrna (1.130 MJ). Ako je vlažnost veća i iznosi 30%, troši se 510 kWh/t (1840 MJ), a kod 40% vlažnost zrna čak 690 kWh/t (2.500 MJ). To znači da se za prinos od 50 dt/ha zrna, samo za sušenje troši (30% vlažno zrno) 9.200 MJ/ha (2.576 kWh) ili 25% od ukupne potrošene energije (Katić, 1985).

Potrošnja plina je otprilike proporcionalna broju stupnjeva na koje se zrak zagrijava. Kada je temperatura okoline hladnija i kreće se oko 0°C, zagrijavanjem zraka od 0°C – 133°C koristi se oko 20% više energije nego kada se zrak zagrijava od 22°C – 133°C (Wilcke, 2001; Jakobović, 2018).

Dugi niz godina proces sušenja bio je usmjeren u istraživanje velikih proizvođača na smanjenja potrošnje energije. Tako su razvijane dvostupanjske sušare, dvoprolazne sušare i sušare sa djelomičnom recirkulacijom fluida te su tijekom vremena značajno snižene potrošnje goriva u procesima sušenja zrna. Međutim, pošto je doradbeno postrojenje (sušara) velika investicija, uglavnom se koriste sušare koje rade na jednoprolaznom principu fluida za sušenje, kod kojih je specifična potrošnja toplinske energije oko 5.000 kJ/kg isparene vode, dok se sezonska potrošnja kreće od 5.500 i 7.000 kJ/kg isparene vode, što ovisi od tehnoloških i organizacijskih okolnosti. Kod energetskih racionalnijih sušara druge generacije potrošnja energenta je manja za 15% do 20% (Babić, 2011).

S obzirom na sve navedeno cilj ovog rada je istražiti utjecaj različitih parametara, uključujući temperaturne, psihometrijske i energetske, na cjelokupnu energetska učinkovitost sušare, jer se određivanje energetske učinkovitosti temelji se na svemu navedenom.

Materijal i metode

Istraživanje je provedeno na kontinuiranoj, gravitacijskoj, direktnoj sušari smještenoj u kontinentalnoj Hrvatskoj (zapadnoj slavonskoj), a kao materijal za sušenje koristilo se zrno kukuruza. Cilj istraživanja je bio mjeriti različite parametre tijekom procesa sušenja i to potrošnju goriva, temperaturu zraka na termometrima i potrošnju električne energije.

Svi parametri su mjereni 9 puta kako bi se utvrdila varijabilnost prikupljenih podataka. Za svaku varijablu izračunati su sljedeći statistički parametri: srednja vrijednost i standardna devijacija.

Rezultati i rasprava

Sušare su veliki potrošači energije te smanjenje potrošnje energije po kilogramu isparene vode postalo vrlo važno za projektiranje energetski učinkovitijeg postrojenja, a taj problem je posebno izražen kod sušenja zrna kukuruza. U tablici 1. prikazana je potrošnja goriva u plamenicima tijekom različitih ciklusa sušenja.

Tablica 1. Potrošnja goriva u plamenicima tijekom različitih ciklusa sušenja

No	Plamenik dm ³ * 10 m ³
1	40.048,50
2	40.070,31
3	40.104,15
4	40.123,41
5	40.144,91
6	40.164,14
7	40.186,43
8	40.205,16
9	40.216,34
Srednja vrijednost	40.140,37
Standardna devijacija	58,76

Iz podataka tablice 1. je vidljiva je velika potrošnja goriva u 9 procesa sušenja. Prosječno je bila 40.140,37 dm³ Trošak energije u smislu topline predstavlja značajan dio ukupnih troškova sušenja, posebno u konvekcijskom sušenja, iako skoro 100% toplinske energije sadržane u gorivu prelazi u zrak za sušenje u kod direktnog sušenja za razliku od približno 70% kod indirektnog sušenja, a dodatni gubici topline u okoliš i ispuštaju se s ispušnim plinovima i smanjuju ukupnu toplinska učinkovitost do 60% ili manje. Stoga je grijač zraka u sušarama je potrebno opremiti dobrim plamenicima za tekuće gorivo kako bi izgaranje sadržavalo ugljičnog dioksida (CO₂) u dimnim plinovima bilo ispod 12%.

Toplina nakupljena u 1.000 kg zrna, koje je ugrijano na 60°C, iznosi oko 125.000 kJ. Oko 80%, tj. oko 100.000 kJ ove topline se prenosi na zrak u zoni hlađenja zrna u sušari. Za sušenje 1.000 kg zrna sa 35% vlage na 15%, potrebno je oko 1.370.000 kJ toplinske energije. Energija iskorištena grijanjem zraka u hladnjaku sušare iznosi oko 7% od ukupne toplinske energije koju dovodimo u sušaru, a ukoliko bi se iskoristila na navedeni način predstavljala bi isto toliko uštedu (Krička i sur., 1993).

U tablici 2. prikazana je temperatura zraka na termometrima, dok je u tablici 3. prikazane psihrometrijske vrijednosti istraživanih parametara u procesu sušenja.

Tablica 2. Temperatura zraka na termometrima

No	Ulaz zraka t ₁ °C	Izlaz zraka t ₂ °C	Prolaz zraka °C
1	109	40	63
2	110	40	62
3	111	40	62,50
4	110	40	63
5	106,50	39	62,50
6	113	39	64,50
7	107,50	39	64,50
8	109,50	39	64
9	110	39	64
Srednja vrijednost	109,61	39,44	63,33
Standardna devijacija	1,88	0,53	0,94

Temperature su mjerene pomoću termometara koji su postavljeni na sušari u određenim zonama sušenja. Iz tablice 2. je vidljivo da je prosječna temperatura na termometrima ulazu u sušaru bila 109,61°C, a na izlazu 39,44°C, dok je prolaz zraka bio 63,33°C što su zadovoljavajuće vrijednosti za daljnji postupak sušenja.

Tablica 3. Psihrometrijska vrijednost istraživanih parametara

No	t _s °C	t _v °C	φ (%)	x (g/kg)	h (kJ/kg)
1	32,50	29,50	80	26	99,44
2	30,50	28,75	88	25,25	97,35
3	26,50	26,50	100	23	84,79
4	30	28	86	24	92,13
5	29	27	85	22,50	87,93
6	30	29	93	26	98,39
7	28	28	100	25,75	96,30
8	29	28,50	96,50	25,25	97,35
Srednja vrijednost	29,44	28,16	91,06	24,72	94,21
Standardna devijacija	1,78	1,01	7,44	1,38	5,36

Tijekom ispitivanja temperatura suhog termometra iznosila je 29,44°C, vlažnog termometra 28,16 °C, a relativna vlaga iznosila je 91,06 %. Ulazom zraka u sušaru sadržaj vode u zraku bio je $x_0 = 24,72$ g/kg suhog zraka, sadržaj energije $h_0 = 94,21$ kJ/kg suhog zraka. Prema navedenim podacima garantirani utrošak toplinske energije u neposrednom radu pri sušenju kukuruza je 4 000 kJ (956 kcal) po kg isparene vode. Iz navedenih parametara možemo se zaključiti da je sušara nije bila dobro podešena.

Tablica 4. Potrošnja električne energije u procesu sušenja

No	kWh
1	16.077,50
2	16.078,40
3	16.079,60
4	16.080,40
5	16.081,10
6	16.081,90
7	16.082,70
8	16.083,40
9	16.083,80
Srednja vrijednost	16.080,98
Standardna devijacija	2,20

U tablici 4 prikazana je potrošnja električne energije prilikom sušenja. Uobičajena potrošnja električne energije kod sušenja kukuruza s toplim zrakom je 4-8 MJ (1,1-2,2 kWh) po kilogramu isparene vode (Nellist, 1987; Jokiniemi i Ahokas, 2014) što su niže vrijednosti nego dobivene u ovome istraživanju gdje je potrošnja električne energije bila značajno veća i iznosila je 16.080,98 kW u procesu sušenja što predstavlja veliku utrošak energije.

Zaključak

Rezultati istraživanja pokazuju da proces sušenja kukuruza prati visoka potrošnja goriva, s prosječnom vrijednošću od preko 40.000 dm³. To znači da trošak energije u obliku topline čini značajan dio ukupnih troškova procesa sušenja. Unatoč visokoj učinkovitosti direktnog sušenja gdje se gotovo sva toplina iz goriva prenosi na zrak za sušenje, postoje značajni gubici topline koji smanjuju ukupnu učinkovitost na 60% ili manje. Također, podaci ukazuju na to da sušara nije bila optimalno podešena, što je vidljivo iz nesklada između zabilježenih temperatura i potrošnje energije. Potrošnja električne energije tijekom sušenja bila je znatno veća od uobičajenih vrijednosti, što upućuje na potrebu za poboljšanjem energetske učinkovitosti. Na temelju toga, neophodno je preispitati i poboljšati postojeće procese sušenja kako bi se smanjila potrošnja energije i povećala ukupna energetska učinkovitost.

Napomena

Ovo istraživanje financirano je putem OP Konkurentnost i Kohezija – projekta „Razvoj inovativnih peleta iz šumske i/ili poljoprivredne biomase - INOPELET“ KK.01.2.1.02.0286

Literatura

- Babić M., Babić L., Radojčin M., Pavkov I., Bogićević M. (2011). Effect of combined technology of fruit and vegetables drying on equipment designing. *Journal on Processing and Energy in Agricultur.* 15: 244-247.
- Bill W. (2001). Saving Fuel in Corn Drying, Minnesota Extension Engineer, Minnesota.
- Jakobović J. (2018). Sušenje kukuruza direktnom sušarom” Seting” Delnice (Doctoral dissertation, Josip Juraj Strossmayer University of Osijek. Faculty of Agrobiotechnical Sciences Osijek.

- Jokiniemi H.T., Ahokas J.M. (2014). Drying process optimisation in a mixed-flow batch grain dryer. *Biosystems engineering*. 121: 209-220.
- Katić Z. (1997). Sušenje i sušare u poljoprivredi, Knjiga, Multigraf, Zagreb.
- Krička T. (1993). Utjecaj perforiranja zrna kukuruza na brzinu sušenja konvekcijom. Doktorska disertacija, Fakultet poljoprivrednih znanosti Sveučilišta u Zagrebu, Zagreb.
- Krička T., Jukić Ž., Voća N., Sigfild N., Zanuškar J. Voća S. (2003). Nutritional characteristics of soybean after thermal processing by toasting. *Acta Veterinaria*. 53: 191-197.
- Krička T., Grubor M., Vican, D., Matin A. (2021). Influence of drying air temperature on maize grain breakage. *Actual Tasks On Agricultural Engineering*. 409.
- Matin A., Krička T., Jurišić V., Bilandžija N., Kuže I., Voća N., Landeka M. (2013). Kvalitativne i energetske promjene ploda lješnjaka u procesu konvekcijskog sušenja, *Krmiva-časopis o hranidbi životinja, proizvodnji i tehnologiji krme*. 55: 11-19.
- Matin A., Krička T., Jurišić V., Voća N., Žunić J., Grubor M. (2017). Effects of different air drying temperature on sunflower seeds oil and ash content. *Journal on Processing and Energy in Agriculture*. 21: 5-8.
- Matin A., Majdak, T., Krička T., Grubor M. (2019). Valorization of sunflower husk after seeds convection drying for solid fuel production. *Journal of Central European Agriculture*. 20: 389-401.
- Matin A., Brandić I., Voća N., Bilandžija N., Matin B., Jurišić V., Antonović A., Krička T. (2023). Changes in the Properties of Hazelnut Shells Due to Conduction Drying. *Agriculture*. 13: 589.
- Mujumdar A. (2000). *Drying technology in agriculture and food science*. Plymouth: Science Publisher.
- Nellist M.E. (1987). Modelling the performance of a cross-flow grain drier. *Journal of Agricultural Engineering Research*. 37: 43-57.
- Somer D., Brkić M., Petrović J. (2008). Perspektiva sušenja zrna žitarica sa manjim kapacitetom sušare. *Savremena poljoprivredna tehnika*. 34: 129-135.

Optimizing the energy efficiency of the corn grain drying process based on temperature, psychrometric and energetic parameters

Abstract

In modern industrial processes, especially in the drying sector, energy efficiency is crucial for sustainability and economic profitability. In this study, the influence of various parameters, including temperature, psychrometric and energy parameters, on the energy efficiency of the dryer is investigated. The first part of the study focuses on fuel consumption, looking at how much fuel is consumed in the burners during the different drying cycles. The second part of the analysis looks at the psychrometric values, which provide information about humidity and other important parameters that can affect the energy consumption and efficiency of the drying process. Finally, electricity consumption was considered as an additional indicator of the energy efficiency and profitability of the drying process. The results showed that there is a correlation between the psychrometric parameters and electricity consumption. Considering the above, an attempt is made to propose optimal conditions for the drying process in order to achieve the highest possible energy efficiency.

Keywords: drying, energy, energy efficiency

Comparison of the physico-chemical properties of liquefied oak (*Quercus robur* L.) and walnut (*Juglans regia* L.) shells for use in the production of biocomposites

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Abstract

In this work, the physicochemical properties of oak (*Quercus robur* L.) and walnut (*Juglans regia* L.) shells and the chemical properties after their liquefaction were investigated by the thermochemical reaction method of biomass samples, glycerol (C₃H₈O₃) as solvent and sulfur-containing catalytic acid (H₂SO₄). The polymer properties of the liquefied biomass represent the percentage of liquefaction and insoluble residues, dry matter, and hydroxyl (OH) number. The percentage of oak liquefaction was 89.28 %, that of walnut shells 89.85 %. The insoluble residue of the oak was 10.75 %, that of the walnut shells 10.15 %. The dry matter content of the oak was 56.79 %, that of the walnut shells 53.94 %. The OH value of the oak was 851.72 mg KOH/G, that of the walnut shells 397.51 mg KOH/G. According to the hydroxyl value (OH value), both the oak and walnut shells have favorable liquefaction properties that can be used for the synthesis of many different products, such as biocomposites.

Keywords: chemical composition, oak, walnut shell, liquefaction percentage, number

Introduction

The increasing demand for a reliable and secure energy supply and concerns about the negative climate change caused by fossil fuels have triggered the development of renewable energy sources as an alternative. As a renewable source, the use of biomass is attracting interest due to its renewable properties and biodegradability, as well as the possibility of conversion into liquid and gaseous products that can be used for a wide range of chemicals, fuels and energy (Koruba et al., 2017).

Knowledge of the chemical composition and properties of biomass is the basis for the production of bioproducts, and its basic chemical components are cellulose (C), hemicellulose (P), lignin (L) and accessory substances (AS) (Pan and Shupe, 2009). One of the most important methods for converting biomass is thermochemical conversion, which includes liquefaction, pyrolysis and gasification and results in valuable bioproducts. During liquefaction, the sample becomes a viscous liquid, and almost all biomasses, whether from forestry or agriculture, can be successfully liquefied with alcohols or phenols (Pan and Shupe, 2009; Antonović et al., 2011; Barnes et al., 2017).

Lignin is the easiest to liquefy, followed by hemicellulose and cellulose. In general, a reaction with a higher reagent-solvent-sample ratio, a longer reaction time, a higher liquefaction temperature and a stronger acid catalyst leads to a higher liquefaction percentage. The commercial application of liquefied biomass depends on the solvent. For example, biomass liquefied with phenol can be used to develop cast products or resins as a primary adhesive for composite panels such as particleboard or plywood used in humid conditions (Antonović et al., 2011; Miyafuji, 2013; Antonović et al., 2017; Antonović et al., 2018).

Forest biomass accounts for almost 80 % of total biomass and is therefore of inestimable importance (Urbano and Keeton, 2017), while a considerable amount of walnut shells can be used as residues from the food industry as

building material or in the production of adhesives, plywood or paints. Compared to forest biomass, walnut shells contain a lower proportion of hygroscopic components and a higher proportion of hydrophobic components, which is why composites based on polymers from walnut shells, which must have a high durability, achieve a better economic value (Guengoer et al., 2019). Composites, which include biocomposites, consist of several components, one of which is of biological origin, with a clearly defined phase boundary. The aim of production is to obtain a new property, improve an existing one or achieve a property that another material has (Riedel, 2012).

How the 1:5 ratio between the solvent content and the content of the biomass sample at a liquefaction time of 120 minutes and a constant temperature of 150 °C influences the chemical composition of the liquefied oak and walnut shells was the aim of this research. The results showed a correlation between the properties of the above mentioned liquefied biomasses and their potential applications as material for biocomposites.

Material and methods

The oak samples were collected in the form of residues after processing the logs with a band saw by the company Bjelin Otok d.o.o., while the walnut samples were collected in an orchard in Sisak-Moslavina County (45° 04' 23" N 16° 22' 33" E). Sampling for laboratory analysis was carried out in September 2023.

The chemical composition of the samples was determined according to previous studies (Sluiter et al., 2005a; Sluiter et al., 2005b; Antonović et al., 2007; Sluiter et al., 2008; Antonović et al., 2008). The biomass samples were crushed in a hammer mill using a sieve with an opening of 1 mm. After shredding and sieving the samples, particles between 0.50 and 1 mm were selected for chemical analysis, and all analyzes were performed in five replicates.

The biomass samples were liquefied with glycerol (C₃H₈O₃) and sulfuric acid (H₂SO₄) (Antonović et al., 2019; Antonović et al., 2020). The samples were liquefied in 500 mL flasks with flat bottom connected to a condenser and placed on a heater and thermostat equipped magnetic stirrer. Liquefaction ratio was 1:5 ratio, i.e. 100 g of C₃H₈O₃ and 3 g of H₂SO₄ were added to the flask and then heated until solvent reached 150 °C, when 20.0 g of the sample was added to the flask and everything was liquefied for 120 minutes.

For liquefied samples, the insoluble residue was determined with a dilute dioxane-water solvent mixture (8:2), stirred for 60 minutes, filtered and rinsed until the filtrate was colorless. The residue was then dried at a temperature of 103±2 °C until a constant mass was reached (Antonović et al., 2020). The following formulas were used to calculate the liquefaction percentage and the insoluble residue:

$$\text{Liquefaction percentage (LP)} = 100 - \text{Insoluble residue (\%)}$$

$$\text{Insoluble residue (IR)} = \frac{\text{insoluble residue mass}}{\text{liquefied sample mass}} (\%)$$

The value of the hydroxyl (OH) number was determined in accordance with the investigations of Antonović et al. (2019) by weighing 1.5 to 2.5 g of the liquefied sample into two 250 mL Erlenmeyer flasks and adding 10 mL of the reagent mixture of reagent and phthalic anhydride. To determine the blank sample, only the reagent was measured in the third flask. Each flask was placed on a magnetic stirrer with a heater and connected to a cooler. Oil baths were placed on the magnetic stirrers to maintain a constant temperature of 115 °C, while the reagent was condensed with a condenser. The mixture was heated for 60 minutes from the first drop of reagent condensate. After cooling the mixture, pyridine (50 mL) was then added via a condenser. It was titrated with 0.5 N sodium hydroxide (NaOH) in the presence of phenolphthalein to the equivalence point, i.e. no bright red color was allowed to appear for 30 seconds. The Hydroxyl number was then calculated as follows:

$$\text{Hydroxyl (OH) number} = \frac{(B - A) \times c_{\text{NaOH}} \times 56.1}{m} \quad (\text{mgKOH/g})$$

A – volume of NaOH for sample titration (mL); B – volume of NaOH for blank titration (mL); cNaOH – molarity of NaOH (M); m – liquefied sample mass.

Results and discussion

The results presented in Table 1. show the chemical group composition of the analysed samples with particle sizes between 0.50 and 1.00 mm. It is desirable for the biomass to have the lowest possible proportion of moisture (MC), ash, volatiles and fixed carbon (Cfix) and a higher proportion of coke.

Table 1. Chemical composition of oak and walnut shells

Sample	Chemical component				
	MC (%)	Ash (%)	Coke (%)	Cfix (%)	Volatiles (%)
Oak	8,3 ± 0,05b	0,30 ± 0,03a	14,25 ± 0,29a	12,87 ± 0,27b	79,69 ± 0,22b
Walnut	12,29 ± 0,11a	1,29 ± 0,09b	17,21 ± 0,28b	15,95 ± 0,44a	70,89 ± 0,55a

Values in the same column of the table that are marked with different letters are statistically significantly different at the level ($P < 0.05$)

The MC content depends mainly on the air temperature and as one of the biomass most important properties have a great influence on fuel quality. In the oak samples it was 8.30 %, while in the walnut shell it was 12.29 %. A higher ash and MC content reduces the fuel quality, so a lower content in the biomass is desirable (Lebendig and Muller, 2022). The ash content in the oak samples was in line with the literature, where Núñez-Retana et al. (2019) reported an ash content between 0.30 and 0.50 %, while it was slightly lower in the walnut shells, as Yehorenko et al. (2022) reported a content of 1.60 to 1.70 %. The percentage of coke and Cfix represent the quantity of released energy during the combustion of biomass and represent its desirable property (Matin et al., 2013). In this study, the proportions of Cfix and coke in oak were consistent with the results reported by Ulusal et al. (2021), while in walnut shells they were slightly higher than the values reported by David et al. (2019). The volatile matter content in biomass is up to 80.0%, and higher content is associated with lower energy value of fuels (Haykırı-Açma, 2003). The oak sample analyzed had a lower volatile matter content than the 88.6% reported for oak by Núñez-Retana et al. (2019), while the walnut shell had less than the 80.21% reported by David et al. (2019).

The lignocellulosic composition of the oak and walnut shells samples analysed is shown in Table 2, which shows that the values determined are within the limits for forest biomass (Antonović, 2004) and for walnut shells (Demirbas, 2002; Yang and Qiu, 2010; Pirayesh et al., 2012; Queiros et al., 2020).

Table 2. Lignocellulose composition of oak and walnut shells

Sample	Chemical component			
	C (%)	L (%)	P (%)	AS (%)
Oak	49,15 ± 0,03b	25,80 ± 0,35a	21,20 ± 0,24a	1,79 ± 0,37b
Walnut	30,95 ± 0,15a	43,74 ± 0,82b	20,35 ± 0,59a	2,73 ± 0,09a

Values in the same column of the table that are marked with different letters are statistically significantly different at the level ($P < 0.05$)

During the study, the chemical properties of the liquefied oak and walnut shell samples (liquefaction percentage, insoluble residue, dry matter, and the hydroxyl (OH) number) were determined, and all these values determine the polymer characteristics of the liquefied samples. The values determined are listed in Table 3.

Table 3. Chemical properties of liquefied biomass from oak and walnut shells

Sample	Chemical component			
	LP (%)	IR (%)	DM (%)	OH-number (mg KOH/G)
Oak	89,28 ± 1,49a	10,75 ± 1,15a	56,79 ± 1,85b	851, 72 ± 3,55a
Walnut	89,85 ± 1,11a	10,15 ± 1,06a	53,94 ± 1,39a	397,51 ± 4,46b

Values in the same column of the table that are marked with different letters are statistically significantly different at the level ($P < 0.05$)

Conclusions

This research has shown that biomass of common oak and walnut shells can be efficiently liquefied with glycerol and sulphuric acid as solvent and catalyst and has a high liquefaction ratio. As the most important component of liquefaction, the hydroxyl (OH) number has an advantage over other liquefaction properties when it comes to synthesising into various bioproducts. There are large amounts of forest and agricultural biomass, but agricultural biomass is cheaper and available annually and therefore represents an interesting raw material source for the production of natural fibers or energy. The study shows that there are certain statistical differences between the biomass and liquefied samples of oak and walnut shells, but not so great as to exclude either of them from further development. As already mentioned, the hydroxyl (OH) number as the most important property, favors the liquefaction of the oak biomass, but with the indication that the liquefied walnut shell also offers great possibilities for scientific research and the development of new bioproducts, especially new ecological products such as biocomposites, biochemicals, foam or resin.

Acknowledgement

This research was financed through OP Competitiveness and Cohesion - project “Development of an innovative technical-technological line for the production of advanced bioadhesives based on liquefied wood – LiqWOODTech“ KK.01.2.1.02.0236

References

- Antonovic A., Jambrekovac V., Pervan S., Istvanic J., Greger K., Public A. (2008). A supplement to the research of native lignin of beech sapwood (*Fagus sylvatica* L.). Wood Research-Slovakia. 53: 55-68.
- Antonović A. (2004). Spektrofotometrijska analiza lignina bukovine. Magistarski rad. Sveučilište u Zagrebu, Šumarski fakultet. 1-214.
- Antonović A., Barčić D., Kljak J., Ištvanic J., Podvorec T., Stanešić J. (2018). The Quality of Fired Aleppo Pine Wood (*Pinus halepensis* Mill.) Biomass for Biorefinery Products. Croatian Journal of Forest Engineering: Journal for Theory and Application of Forestry Engineering. 39: 313-324.
- Antonović A., Ištvanic J., Medved S., Antolović S., Stanešić J., Kukuruzović J., Đurović A., Španić N. (2019). Influence of Different Wood Specie Chemical Composition on the Liquefaction Properties. 30th: 25.
- Antonović A., Jambrekovac V., Ištvanic J., Španić N. (2011). Liquefied wood-potential application in wood industry. Međunarodni znanstveno-stručni skup XIII. Ružičkini dani” Danas znanost-sutra industrija”, Vukovar, Hrvatska, 16. i 17. rujna 2010: 439-453.
- Antonović A., Jambrekovac V., Pervan S., Ištvanic J., Moro M., Zule J. (2007). Influence of sampling location on sapwood group chemical composition of beech wood (*Fagus sylvatica* L.). Drvna industrija. 58: 119-125.
- Antonović A., Krička T., Jurišić V., Šafran B., Antolović S., Stanešić J., Ištvanic J. (2020). Utjecaj različitih polihidričnih alkohola na utekućenje trave *Miscanthus x giganteus*. sa55: 553.
- Antonović A., Krička T., Matin A., Voća N., Jurišić V., Bilandžija N., Grubor M., Stanešić J. (2017). Lignocellulosic composition of some important oilseeds and grains Biomass in the Republic of Croatia. 52nd Croatian and 12th international symposium on agriculture: 623-627.
- Barnés M.C., de Visser M.M., Van Rossum G., Kersten S.R.A., Lange J.P. (2017). Liquefaction of wood and its model components. Journal of analytical and applied pyrolysis. 125: 136-143.
- David E., Kopac J., Armeanu A., Niculescu V., Sandru C., Badescu V. (2019). Biomass-alternative renewable energy source and its conversion for hydrogen rich gas production. In *E3S Web of Conferences* (Vol. 122, p. 01001). EDP Sciences.
- Demirbaş A. (2002). Fuel characteristics of olive husk and walnut, hazelnut, sunflower, and almond shells. Energy Sources. 24: 215-221.
- Güngör A., Akbay I.K., Özdemir T. (2019). Waste walnut shell as an alternative bio-based filler

- for the EPDM: Mechanical, thermal, and kinetic studies. *Journal of Material Cycles and Waste Management*. 21: 145-155.
- Haykırı-Açma H. (2003). Combustion characteristics of different biomass materials. *Energy Conversion and Management*. 44: 155-162.
- Koruba D., Piotrowski J.Z., Latosińska J. (2017). Biomass-alternative renewable energy source to the fossil fuels. In *E3S Web of Conferences* (Vol. 14, p. 02015). EDP Sciences.
- Lebendig F., and Müller M. (2022). Effect of pre-treatment of herbaceous feedstocks on behavior of inorganic constituents under chemical looping gasification (CLG) conditions. *Green Chemistry*. 24: 9643-9658.
- Matin A., Krička T., Jurišić V., Bilandžija N., Voća N., Mrkšić J. (2013). Energetska iskoristivost ljuške oraha i lješnjaka. In *48th Croatian and 8th International Symposium on* (Vol. 37): 836-840.
- Miyafuji H. (2013). Liquefaction of wood by ionic liquid treatment. *Ionic liquids–new aspect for the future–*. InTech, Croatia: 299-314.
- Núñez-Retana V. D., Wehenkel C., Vega-Nieva D. J., García-Quezada J., Carrillo-Parra A. (2019). The bioenergetic potential of four oak species from northeastern Mexico. *Forests*. 10: 869.
- Pan H., and Shupe T.F. (2009). “Louisiana Agriculture Fall, 2009,” *LSU AgCenter, Louisiana Agriculture*: Vol. 52: No. 4, Article 1: 30-31.
- Pirayesh H., Khazaeian A., Tabarsa T. (2012). The potential for using walnut (*Juglans regia* L.) shell as a raw material for wood-based particleboard manufacturing. *Composites Part B: Engineering*. 43: 3276-3280.
- Queirós C.S., Cardoso S., Lourenço A., Ferreira J., Miranda I., Lourenço M.J.V., Pereira H. (2020). Characterization of walnut, almond, and pine nut shells regarding chemical composition and extract composition. *Biomass Conversion and Biorefinery*. 10: 175-188.
- Riedel U. (2012). *Biocomposites: Long natural fiber-reinforced biopolymers*: 295-315.
- Sluiter A., Hames B., Ruiz R., Scarlata C., Sluiter J., Templeton D. (2005). Determination of ash in biomass (NREL/TP-510-42622). *National Renewable Energy Laboratory, Golden*: 19.
- Sluiter A., Hames B., Ruiz R., Scarlata C., Sluiter J., Templeton D., Crocker D.L.A.P. (2008). Determination of structural carbohydrates and lignin in biomass. *Laboratory analytical procedure*. 1617: 1-16.
- Sluiter A., Ruiz R., Scarlata C., Sluiter J., Templeton D.J.L.A.P. (2005). Determination of extractives in biomass. *Laboratory analytical procedure (LAP)*. 1617: 1-16.
- Ulusal A., Apaydın Varol E., Bruckman V.J., Uzun B.B. (2021). Opportunity for sustainable biomass valorization to produce biochar for improving soil characteristics. *Biomass Conversion and Biorefinery*. 11: 1041-1051.
- Urbano A.R., and Keeton W.S. (2017). Carbon dynamics and structural development in recovering secondary forests of the northeastern US. *Forest Ecology and Management*. 392: 21-35.
- Yang J., and Qiu K. (2010). Preparation of activated carbons from walnut shells via vacuum chemical activation and their application for methylene blue removal. *Chemical Engineering Journal*. 165: 209-217.
- Yehorenko T., Kizatova M., Sultanova M., Baikenov A., Saduakas A., Akzhanov N. (2022). Revealing the Features of the Composition of the Walnut Shell From the Point of View of the Possibility of Its Use in the Food Industry. *Eastern-European Journal of Enterprise Technologies*. 1: 115.

Utjecaj određenih inovativnih tehnika i tehnologija u poljoprivredi

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Sažetak

U radu su prikazane nove inovativne tehnike i tehnologije u poljoprivredi poput: umjetna inteligencija, mikroroboti, nanodronovi, senzori, Big data. Kroz rad pojašenja je umjetna inteligencija i njena upotreba u poljoprivredi, zatim međusobno povezivanje tehnike i tehnologije pomoću IoT-a, Big data i senzoričke. Kao posljednji tehnološki zamah predstavljena je nano tehnologija gdje se ista u poljoprivredi koristi u svrhu oprašivanja i buduće mikro zaštite poljoprivrednih kultura.

Ključne riječi: umjetna inteligencija, IoT, nanodronovi, Big data, senzori

Uvod

Kako ljudska populacija raste, raste i potreba za povećanjem proizvodnje hrane. Pritom treba obratiti pozornost na kvalitetu iste, očuvanje bioraznolikosti, ali i o očuvanju okoliša. Prema navodima autora Pepić (2023), poljoprivredna industrija znatno se transformirala u zadnjih 40-50 godina. Napredak strojeva proširio je omjer, brzinu i produktivnost sveukupne poljoprivredne opreme. Prethodno spomenutim dovodi do učinkovitijeg uzgoja uroda te je poljoprivreda u razvoju kojoj pridonose inovativne tehnologije. Agrowell (Poveznica 1., 2021) navodi kako je poljoprivreda podržana inovativnom tehnologijom poput digitaliziranih aplikacija, strojeva upravljanih daljinskim putem uz podršku umjetne inteligence (AI) što rezultira izvođenjem operacija bez ljudske pomoći. Za aktualno prikupljene podatke nužna je primjena senzora okoliša koji su stvarnome vremenu mogu bilježiti podatke o klimi i informacije o zahtjevima za dostatnu vlagu u tlu (Krevh, 2018; Lemić i sur., 2021; Poveznica 2., 2021). Jedna od suvremenih tehnologija je Internet od stvari (IoT, Internet of things). To je tehnologija koja omogućuje raznim alatima poput bespilotni zrakoplovi, senzori ili sateliti da se međusobno umreže i komuniciraju radi razmjene informacija kao i podataka korisnih za poboljšanje uvjeta razvoja usjeva. Farooq i sur. (2022). navode kako je IoT primjenjiv na različite domene primjene uključujući zdravstvo, pametnu mrežu i poljoprivredu. Prema (Köksal i Tekinerdogan, 2018) IoT omogućuje viziju pametne poljoprivrede kroz stvarno vrijeme prikupljanje podataka, analiziranje, obrada i omogućuje poboljšanje cjelokupnog upravljanja gospodarstvom kao pomoć poljoprivrednicima kako bi donosili bolje odluke. Prema Kumar i sur. (2022) nano dronovi se zbog svoje male veličine i male mase, obično koriste za biološki nadzor u poljoprivredi dok Gago i sur. (2020) navode kako se nano dronovi primjenjuju u preciznoj poljoprivredi te kod procjene vodnog stresa. Upotreba IoT-a rezultira prikupljanjem velikoga broja podataka koji se u IT sektoru zovu Veliki podaci (Big data). Koncept velikih podataka odnosi se na sve informacije i podatke prikupljene raznim tehnologijama koje pridonose lakšem odlučivanju pri izboru odluka tijekom proizvodnog procesa. Veliki podaci su vrlo različiti skup informacija jer potječu iz brojnih izvora te se upotrebom AI aplikacija omogućuje dobivanje rješenja odnosno praktičnih odgovora na postavljenje probleme (McCormick, 2021; Poveznica 2., 2021; Segal, 2022). Veliki podaci su postali sveprisutni, aplikacije za strojno učenje (ML) napreduju, AI se pojavljuje u svakodnevnim razgovorima, IoT je prisutan čak i u kućanskim aparatima. Poduzećima i organizacijama se sve više upravlja putem računalstva u oblaku, a računalstvo visokih performansi postupno je dostupno kao usluga (Rodríguez-Mazahua i sur., 2016). Tehnologije velikih podataka već su uvedene u poljoprivredne aplikacije (Astill i sur., 2020; Cockburn, 2020; Ip i sur., 2018; Kamble i sur., 2020; Kamilaris i sur., 2017; Lokers i sur., 2016; Pylaniadis i sur., 2021; Saiz-Rubio and Rovira-Mas, 2020; Verdouw i sur., 2019; Wolfert i sur., 2017). Osinga i sur. (2022) navode kako su se stvorili veliki sustavi s velikim podacima koji su specifičnih za poljoprivredu te je potreban pristup sustavnog razmišljanja za zajednički razvoj rješenja velikih podataka za rješavanje nesigurnosti poljoprivrednih sustava i izazova kod sigurnosti

hrane. Prema Pepić (2023) postoje dva glavna područja primjene velikih podataka: robotika i softveri za upravljanje (management software). Vangala i sur. (2020) navode kako senzori koji se koriste u poljoprivredi za svrhu primjene pametne i digitalne poljoprivrede zovu poljoprivredni senzori. Oni prikupljaju podatke za pomoć poljoprivrednicima u monitoringu i optimizaciji usjeva. Poljoprivredni senzori ugrađeni su i fiksirani u dronove, meteorološke stanice, te robote koji se koriste u poljoprivredi. Pomoću spomenutih senzora i mobilnih aplikacijama moguće je provoditi brojne agrotehničke operacije bez prisustva poljoprivrednika. Poljoprivredni senzor razmjenjuje podatke koristeći bežičnu vezu izravno putem Wi-Fi-ja ili putem mobilnog tornja (GPRS - General Packet Radio Service) pomoću aplikacije na mobilnom telefonu. Particio i sur. (2018) ističu brojne prednosti upotrebe poljoprivrednih senzora i to kroz uštedu radnih resursa (smanjen utrošak ljudskih radnih sati), bolju kontrolu potrošnje vode, mineralnih sredstava i sjemena (po jedinici površine). Prethodno navedeno dovodi do niže tržišne nabavna cijena te lakšeg rukovanja (senzori prostora) i održavanja strojeva (senzori za dojavu istrošenosti).

Senzori

Misra i sur. (2020) navode kako je upotreba senzora u stalnom porastu misleći na ranom izvješćivanju o problemima koji se odnose na zdravlje usjeva na gospodarstvima, čime se omogućuju rane provjere javnog zdravlja i sigurnosti. Prema Martić (2021) postoje razne podjele senzora prema vrsti i ostalim karakteristikama te se razlikuju po složenosti. Osnovna podjela senzora je prema prirodi izlazne veličine. Senzori mogu imati električni i digitalni i analogni izlazni signal. Xue i sur. (2020) ukazuju kako senzori u poljoprivredi daju podatke koji pomažu poljoprivrednicima da prate i optimiziraju svoje prinose i drže korak s promjenjivim čimbenicima okoliša i ekosustava. Senzori pametnog uzgoja pomažu identificirati životinje, pratiti stanje zdravlja, omogućuju poljoprivrednicima da „razumiju“ svoje usjeve i produktivnost, očuvaju resurse i zaštite ili upravljaju svojim usjevima od neželjenih utjecaja poput raznih eko katastrofa na okoliš. Patricio i sur. (2018) navode kako postoje razne namjene poljoprivrednih senzora koje mogu značajno pomoći pri radu, odnosno zadovoljiti sve veću potražnju za hranom uz minimalne resurse. Neki od senzora su: akustični senzor, senzor programiran na principu FPGA, optički senzori, ultrazvučni senzor dometa, senzori vlažnosti, senzor protoka zraka, elektrokemijski senzor, elektromagnetski senzor, senzor masenog protoka, senzor očitavanja korova, senzor brzine vjetera, senzor temeljen na vrtložnoj kovarijanci, senzor razine meke vode (SWLB), detekcija svjetla i domet (LIDAR), telematski senzor, senzor sadržaja vode u tlu, senzor lokacije, senzor vlažnosti lišća, senzor temperature, senzori vlage, PH senzor i optoelektronički senzor (Slika 1.).



Slika 1. Tipovi često korištenih senzora

Izvor: <https://ieeexplore.ieee.org/abstract/document/9741001>

AI

Primjena AI će u narednim godinama postepeno omogućiti zaštitu poljoprivredne proizvodnje od raznih poteškoća kao što su vremenski uvjeti, rast broja stanovnika i zabrinutost oko sigurnosti hrane (Sharma i sur., 2023). Eli-Chukwu i N. C. (2019) navode da korištenje najnovije tehnologije za učinkovitiju poljoprivredu je i dalje potrebno obzirom na zahtjeve stanovništva za kvalitetnijom hranom. Galić i sur. (2023) navode kako je prvi cilj u dokumentu

preporuke nacionalne posebnosti i ciljeva korištenja umjetne inteligencije u Republici Hrvatskoj potaknuti razvoj tehnoloških i industrijskih kapaciteta Republike Hrvatske i primjene umjetne inteligencije u svim područjima gospodarstva kao i u privatnom i javnom sektoru. AI primjena je značajno pripomogla kod tehnologije pametne poljoprivrede (Slika 2.). Prema Balafoutis i sur. (2017) tehnologija pametne poljoprivrede podijeljena je u tri kategorije, a to su: 1) tehnologija prikupljanja podataka (sadrži sva mjerenja, mapiranje, navigacijske i senzorske tehnologije), 2) tehnologija analize i procjene podataka (takve se tehnologije kreću od jednostavnih, upravljanih računalom do složenih upravljanja gospodarstvom i informacijskim sustavima koji sadrže puno različitih varijabli) i 3) tehnologija precizne aplikacije (ova kategorija sadrži sve aplikacijske tehnologije, usredotočujući se na tehnologije primjene i usmjeravanja s promjenjivom stopom).

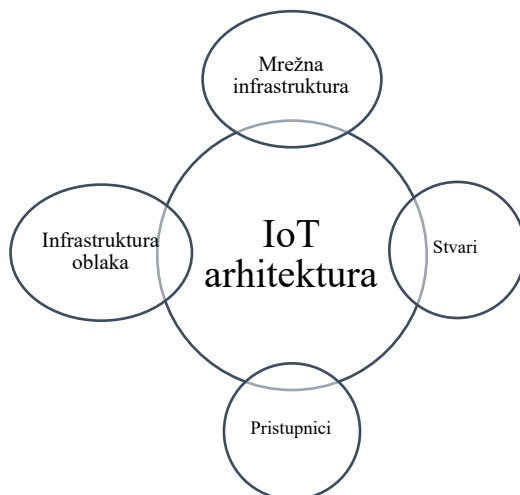


Slika 2. Primjer aplikacije koja se koristi za nadzor u pametnoj poljoprivredi

Izvor:http://breakthrough.unglobalcompact.org/site/assets/files/1332/hhw-16-0025-d_n_digital_agriculture.pdf

IoT

IoT jedna je od najsuvremenijih tehnologija današnjice, predstavlja široko područje rada i primjenu. IoT donosi velike promjene u komunikaciji i povezivanju modernoga društva kroz primjenu u logistici, praćenjem i kontrolom lanca snabdijevanja u stvarnome vremenu.. Prema Patel i sur. (2016) i Gelo, (2019) glavne karakteristike IoT-a su: 1) međusobna povezanost (sve može biti povezano globalnom informacijskom i komunikacijskom infrastrukturom), 2) usluge povezane stvarima (IoT pruža usluge poput zaštite privatnosti i semantičke dosljednosti između fizičkih i pridruženih virtualnih stvari), 3) heterogenost (uređaji koji koriste IoT su heterogeni jer se temelje na različitim hardverskim platformama i mrežama komunicirajući s drugim uređajima ili platformama kroz različite mreže), 4) dinamičke promjene (stanje uređaja se dinamički mijenja, kao i lokacija i brzina ili broj uređaja), 5) veliki razmjer: broj uređaja koji će komunicirati međusobno će biti veći od broja uređaja koji su trenutno spojeni na Internet, 6) sigurnost (uključena je sigurnost osobnih podataka i fizička sigurnost) te 7) povezivanje (omogućena je pristupačnost i kompatibilnost mreže što pruža zajedničko procesiranje podataka.). Prema Grganić, 2017; Manojlović, 2019; Ma, 2013. arhitektura IoT mreže se sastoji od više različitih slojeva. Prema Badžim (2018) općenito gledano arhitektura IoT-a je podijeljena u 3 sloja, a to su Percepcijski sloj, Mrežni sloj i Aplikacijski sloj. Khan i sur. (2012) spominju još neke arhitekture s više slojeva pa tako novija arhitektura ima 5 slojeva: Prvi sloj je Sloj uređaja koji se sastoji od objekata i senzora, drugi sloj je Mrežni sloj koji je odgovoran za spajanje sa drugim pametnim stvarima, mrežnim uređajima i serverima, treći sloj je Posrednički sloj (procesni sloj) njegova zadaća je spremanje, analiza i procesuiranje velikih količina podataka preuzetih iz Mrežnog sloja, četvrti sloj je Aplikacijski sloj kojim se usluge aplikacija približavaju korisnicima i upravlja se njihovim primjenama na temelju informacija procesuiranim u prethodnom sloju te peti sloj je Poslovni sloj, a on je odgovoran za upravljanje cjelokupnog sustava koji uključuju primjenu i usluge. Pojednostavljena shema prikazana je na slici 3.



Slika 3. IoT arhitektura

Izvor: <https://olevelexam.com/internet-of-things-and-its-applications/building-blocks-of-iot>

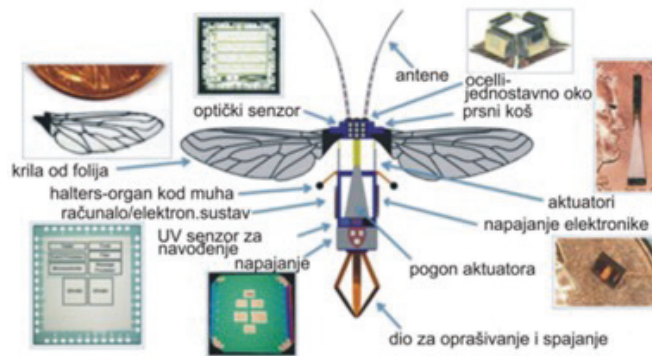
Nano dronovi

Modernizacija tehnologije u uzgoju bilja, uz ostale robote, obuhvaća mikrorobote i nano dronove. Moderna mehanizacija u uzgoju bilja itekako je olakšala i unaprijedila rad. Vukadinović (2016) navodi kako poljoprivrednici uz pomoć dronova – bespilotnih zrakoplova mogu uštedjeti vrijeme, odnosno u kratkom vremenu obaviti više posla. Pathak i sur. (2015) navode kako poljoprivredna industrija globalno sve više koristi bespilotnu tehnologiju za modernizaciju poljoprivrede. Oljača i sur. (2016) navode kako uloga mini bespilotnih letelica ili dronova se brzo počela koristiti sa vojne upotrebe za namjenu (75 % od ukupnog broja) u poljoprivredi, šumarstvu i time pomažući korisnicima da nadgledaju i kontroliraju velike površine različitih namjena, pri tome ostvarivati uštedu vremena i financijskih ulaganja u proizvodnju. Huang i sur. (2013) navode kako je mikrotehnoški napredak rezultirao širokom upotrebom bespilotne tehnologije u mnogim područjima znanosti. Prikupljeni daljinski očitani podaci pomoću nano dronova imaju visoku prostornu i vremensku rezoluciju, što pomaže u stvaranju preciznih, točnih i relevantnih procjena biofizičkih svojstava usjeva. Prema Harvard School of Engineering i Applied Sciences (SEAS) (Poveznica 3., 2013) uspješno je kreiran projekt RoboBee. RoboBee (Slika 4.) je nanodron pomoću kojeg je moguće praćenje okoliša, operacije potrage i spašavanja ili pomoć pri oprašivanju usjeva (Slika 5.).



Slika 4. Nano dron RoboBee

Izvor: <https://wyss.harvard.edu/technology/robobees-autonomous-flying-microrobots/>



Slika 5. Dijelovi RoboBee

Izvor: Nikolić, (2021)

Big data

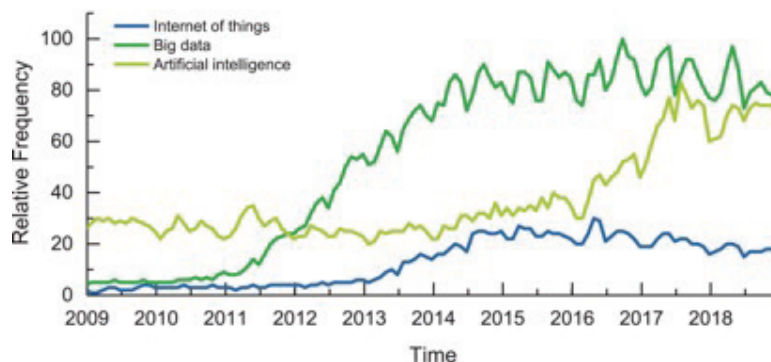
Arapović (2019) navodi kako pametna poljoprivreda predstavlja korištenje Big data (velikih podataka), informacijsko-komunikacijske tehnologije i aplikacija, u kombinaciji s naprednim i visokosofisticiranim uređajima i strojevima. Prema Perković (2020) Big data je od velikog značaja za industriju kojoj su podaci primarni dio poslovanja. Koristeći strojno učenje i umjetnu inteligenciju, značajno se mogu pojednostaviti i ubrzati poslovni procesi, uštedjeti vrijeme i novac. Prema Petrović i sur. (2023) postoje različite tehnologije koje se mogu koristiti za digitalizaciju poljoprivrede. U zavisnosti od potreba i mogućnosti treba procijeniti koja tehnologija je najbolja u određenim projektima (Slika 6.). Neke od najčešće primjenjenih tehnologija u projektima digitalizacije poljoprivrede su Big data analiza. Mitrović i sur. (2020) navode kako se Big data koristi za analizu velikih količina podataka i za izvlačenje značajnih informacija (u poljoprivredi, Big data se može koristiti za procijenu utjecaja vremenskih uvjeta, za predviđanje cijena i za poboljšanje procesa proizvodnje).



Slika 6. Big data podaci u poljoprivredi

Izvor: Bhat i sur., (2021)

Ukoliko se promatra relativni svjetski promet pretraživanja pojmova IoT, Big data i AI na Googleu tijekom posljednjeg desetljeća može se zaključiti kako su navedeni pojmovi sve više traženi naročito Big data i AI (Slika 7.). Misra i sur. (2020) navode kako je IoT prepoznat kao jedno od najvažnijih područja tehnologije budućnosti i privlači značajnu pozornost u širokom rasponu industrija. Uz implementaciju IoT infrastrukture u poljoprivredi, poljoprivrednici će biti učinkovitiji i povezani, dajući ogromne količine informacija analitičarima u vezi s prinosima usjeva, mapiranjem tla, primjenom mineralnih sredstava, vremenskim podacima, strojevima i zdravljem životinja. Napori koji vode ka jednostavnoj integraciji različitih IoT uređaja u smislu protoka podataka i uputa od gospodarstva do potrošačkog lanca važni su za dobivanje održivog i učinkovitog IoT sustava.



Slika 7. Big data podaci u poljoprivredi

Izvor: Bhat i sur., (2021)

Zaključak

Korištenje naprednih tehnologija poput AI-e, Big data analitike, senzora i nanodronova predstavlja paradigmu promjene u poljoprivredi. Navedene tehnologije osiguravaju dublje razumijevanje i precizno upravljanje poljoprivrednom proizvodnjom, što rezultira brojnim uštedama resursa. AI potaknuta dubokim učenjem i modeliranjem, omogućava naprednu analizu podataka koja se temelji na obilju parametara relevantnih za poljoprivredu, navedeno dovodi do preciznog predviđanja rasta usjeva, potreba za resursima i ranog otkrivanja bolesti, pružajući poljoprivrednicima alate za optimizaciju poljoprivrednih operacija i smanjenje gubitaka. Upotrebom Big data analitike dolazi do transformacije poljoprivredne proizvodnje putem integracije raznovrsnih podataka o vremenskim uvjetima, tlu, usjevima i agrotehničkim operacijama gospodarstva. Duboka analiza ovih podataka omogućuje bolje upravljanje resursima, smanjenje troškova i održivu praksu. Koristeći brojne senzore odnosno senzorsku mrežu postiže se učinkovit monitoring mikroklimatskih uvjeta, vlažnost tla, kvaliteta zraka i stanje usjeva u stvarnom vremenu. Upotrebom nanodronova omogućuje se futurističko oprašivanje potencijalno ugroženih usjeva te adekvatna zaštita istih. Integracija ovih naprednih tehnologija čini poljoprivredu učinkovitijom, smanjuje nepotrebne resurse, štiti okoliš i osigurava sigurnost opskrbe hranom u skladu s rastućim potrebama globalnog stanovništva. Znanstveni pristup upotrebi ovih tehnologija ključan je za kontinuirani napredak poljoprivrede i osiguranje održivosti poljoprivredne proizvodnje u budućnosti.

Literatura

- Arapović J. (2019). Mladi poljoprivrednici i " pametna" poljoprivreda, Diplomski rad, University of Zagreb. Faculty of Agriculture. Department of Agricultural Economics and Rural Development.
- Astill J., Dara R.A., Fraser E.D., Roberts B., Sharif S. (2020). Smart poultry management: Smart sensors, big data, and the internet of things. *Computers and Electronics in Agriculture*. 170: 105291.
- Badžim A. (2018). Internet stvari kao osnova pametnog grada, Diplomski rad, Sveučilište u Splitu, Ekonomski fakultet.
- Bhat S.A., and Huang N.F. (2021). Big data and ai revolution in precision agriculture: Survey and challenges. *IEEE Access*. 9: 110209-110222.
- Cockburn M. (2020). Application and prospective discussion of machine learning for the management of dairy farms. *Animals*. 10: 1690.
- Dujmenović I. (2020). Primjena Interneta objekata u logistici, Završni rad, Sveučilište Josipa Jurja Strossmayera u Osijeku, Fakultet elektrotehnike, računarstva i informacijskih tehnologija Osijek, Osijek.
- Eli-Chukwu N.C. (2019). Applications of artificial intelligence in agriculture: A review. *Engineering, Technology & Applied Science Research*. 9: (4).
- Farooq M.S., Sohail O.O., Abid A., Rasheed S. (2022). A survey on the role of iot in agriculture for the implementation of smart livestock environment. *IEEE Access*. 10: 9483-9505.

- Gago J., Estrany J., Estes L., Fernie A.R., Alorda B., Brotman Y., Medrano H. (2020). Nano and micro unmanned aerial vehicles (UAVs): a new grand challenge for precision agriculture?. *Current protocols in plant biology*. 5: (1).
- Galić A., Plietić S., Kovačev I., Čopec K., Brandić I. (2023). Primjena modela strojnog učenja u tehnologiji proizvodnje krmnih smjesa. *Krmiva: Časopis o hranidbi životinja, proizvodnji i tehnologiji krme*. 65: 69-76.
- Gelo D. (2019). *Internet of Things (IoT)-Izazovi i mogućnosti cyber sigurnosti povezane s IoT-om* (Doctoral dissertation, Algebra University College).
- Grganić D. (2017). *Povezivanje usluga temeljenih na lokaciji korisnika i IoT-a za nadzor stanja korisnika u stvarnom vremenu*, Diplomski rad, University of Zagreb. Faculty of Transport and Traffic Sciences. Division of Intelligent Transport Systems and Logistics. Department of Intelligent Transport Systems.
- Huang Y., Thomson S.J., Hoffmann W.C., Lan Y., Fritz B.K. (2013). Development and prospect of unmanned aerial vehicle technologies for agricultural production management. *International Journal of Agricultural and Biological Engineering*. 6: 1-10.
- Ip R.H., Ang L.M., Seng K.P., Broster J.C., Pratley J.E. (2018). Big data and machine learning for crop protection. *Computers and Electronics in Agriculture*. 151: 376-383.
- Kamilaris A., Kartakoullis A., Prenafeta-Boldú F.X. (2017). A review on the practice of big data analysis in agriculture. *Computers and Electronics in Agriculture*. 143: 23-37.
- Kamble S.S., Gunasekaran A., Gawankar S.A. (2020). Achieving sustainable performance in a data-driven agriculture supply chain: A review for research and applications. *International Journal of Production Economics*. 219: 179-194.
- Köksal Ö., and Tekinerdogan, B. (2019). Architecture design approach for IoT-based farm management information systems. *Precision Agriculture*. 20: 926-958.
- Krevh V. (2018). *Primjena bespilotnih letjelica u poljoprivredi*, Diplomski rad, Sveučilište u Zagrebu, Agronomski fakultet, Zagreb.
- Kumar A., Ahamad S., Kumar M., Bihari C., Singh S., Pandey V., Gautam P. (2022). An innovative drones technology in agriculture: A review, *The Pharma Innovation Journal*. 11: 279-286.
- Lemić D., Radanović R., Orešković M., Genda M., Kapor K., Virić Gašparić H. (2021). Dronovi kao moderan alat za suvremenu poljoprivredu. *Glasiilo biljne zaštite*. 21: 476-491.
- Lokers R., Knapen R., Janssen S., van Randen Y., Jansen J. (2016). Analysis of Big Data technologies for use in agro-environmental science. *Environmental modelling & software*. 84: 494-504.
- Manojlović M. (2019). *Analiza usluga mrežnih operatora temeljenih na IoT konceptu*, Završni rad, University of Zagreb. Faculty of Transport and Traffic Sciences. Division of Transport. Department of Information and Communications Traffic.
- Ma Z., Shang X., Fu X., Luo F. (2013). The architecture and key technologies of Internet of Things in logistics. In *International conference on cyberspace technology (CCT 2013)*, 464-468.
- Martić H. (2021). *Vrste i primjene senzora u pametnim okruženjima*, Sveučilište u Zagrebu, Fakultet prometnih znanosti, Završni rad, Zagreb.
- Misra N.N., Dixit Y., Al-Mallahi A., Bhullar M.S., Upadhyay R., Martynenko A. (2020). IoT, big data, and artificial intelligence in agriculture and food industry. *IEEE Internet of things Journal*. 9: 6305-6324.
- Mitrović Z., Rakićević A., Petrović D., Mihić M., Rakićević J., Jelisić E. (2020). Systems Thinking in Software Projects-an Artificial Neural Network Approach. *IEEE Access*, 8, 213619-213635.
- Nikolić G. (2021). Minijturni leteći roboti. *Polytechnic and design*. 9: 35-42.
- Oljaca M., Gligorevic K., Paic M., Dimitrovski Z. (2016). *Primena drona u poljoprivredi*.
- Osinga S.A., Paudel D., Mouzakitis S.A., Athanasiadis I. N. (2022). Big data in agriculture: Between opportunity and solution. *Agricultural Systems*. 195: 103298.

- Patel K.K., Patel S.M., Scholar P. (2016). Internet of things-IOT: definition, characteristics, architecture, enabling technologies, application & future challenges. *International journal of engineering science and computing*. 6: (5).
- Pathak H., Kumar G., Mohapatra S.D., Gaikwad B.B., Rane J. (2020). Use of drones in agriculture: Potentials, Problems and Policy Needs. *ICAR-National Institute of Abiotic Stress Management*. 4-5.
- Patrício D.I., and Rieder R. (2018). Computer vision and artificial intelligence in precision agriculture for grain crops: A systematic review. *Computers and electronics in agriculture*. 153: 69-81.
- Pylianidis C., Osinga S., Athanasiadis I. N. (2021). Introducing digital twins to agriculture. *Computers and Electronics in Agriculture*. 184: 105942.
- Pepić D. (2023). Utjecaj inovativne tehnike i tehnologije u suvremenoj poljoprivredi, Završni rad, Sveučilište J. J. Strossmayera u Osijeku, Fakultet agrobiotehničkih znanosti Osijek, Osijek.
- Petrović D., Stanimirović P., Vratonjić Gligorijević A. (2023). Izazovi u upravljanju projektima digitalizacije u poljoprivredi. <https://symorg.fon.bg.ac.rs/wp-content/uploads/2023/06/Odrzivo-upravljanje-poslovanje-Inovacije-softver-i-komunikacije-naslovna-i-sadrzaj.pdf>.
- Perković M. (2020). Umjetna inteligencija i big data tehnologija u industriji osiguranja, Diplomski rad, University of Zagreb. Faculty of Economics and Business.
- Saiz-Rubio V., Rovira-Más F. (2020). From smart farming towards agriculture 5.0: A review on crop data management. *Agronomy*. 10: 207.
- Segal T. (2022). "What is Big Data? Definition, How It Works, and Uses" <https://www.investopedia.com/terms/b/big-data.asp>
- Sharma S., Verma K., Hardaha P. (2023). Implementation of artificial intelligence in agriculture. *Journal of Computational and Cognitive Engineering*. 2: 155-162.
- Rodríguez-Mazahua L., Rodríguez-Enríquez C.A., Sánchez-Cervantes J.L., Cervantes J., García-Alcaraz J.L., Alor-Hernández G. (2016). A general perspective of Big Data: applications, tools, challenges and trends. *The Journal of Supercomputing*. 72: 3073-3113.
- Vangala A., Das A.K., Kumar N., Alazab M. (2020). Smart secure sensing for IoT-based agriculture: Blockchain perspective. *IEEE Sensors Journal*. 21: 17591-17607.
- Verdouw C., Sundmaeker H., Tekinerdogan B., Conzon D., Montanaro T. (2019). Architecture framework of IoT-based food and farm systems: A multiple case study. *Computers and Electronics in Agriculture*. 165: 104939.
- Vukadinović V. (2016). Dronovi u poljoprivredi. Dostupno na:
URL: http://tlo-i biljka.eu/Gnojidba/Zanimljivosti/Zanimljivosti_06-2016.pdf
- Wolfert S., Ge L., Verdouw C., Bogaardt M.J. (2017). Big data in smart farming—a review. *Agricultural systems*. 153: 69-80.
- Xue D., and Huang W. (2020). Smart agriculture wireless sensor routing protocol and node location algorithm based on Internet of Things technology. *IEEE Sensors Journal*. 21: 24967-24973.
- Poveznica 1. Agrowell (2021). (6.9.2023)
<https://agrowell.com.tr/what-is-agriculture-4-0-what-innovations-does-it-offer/>
- Poveznica 2. McCormick (2021). (06.09. 2023)
<https://www.mccormick.it/as/agriculture-4-0-what-is-it-and-what-are-its-tools-andbenefits/>
- Poveznica 3. (2. 11.2023)
<https://phys.org/news/2013-05-robobees-robotic-insects-flight-video.html>

Impact of specified innovative techniques and technologies in agriculture

Abstract

The paper presents new innovative techniques and technologies in agriculture such as: artificial intelligence, microrobots, nanodrones, sensors and Big data. Artificial intelligence and its use in agriculture are emphasized through the work, then the interconnection of techniques and technology using IoT, Big Data and sensors. As the last technological breakthrough, nano technology was presented, where it is used in agriculture for the purpose of pollination and future micro protection of agricultural crops.

Keywords: artificial intelligence, IoT, nanodrones, Big data, sensors

Energetski potencijal biomase vinograda i voćnjaka nakon uklanjanja nasada

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Sažetak

Nakon životnog ciklusa, značajnijeg obolijevanja trajnih nasada ili promjene potražnje na tržištu neophodno je provesti vađenje trsova/stabala. Navedenim zahvatom proizlazi velika količina biomase koju je moguće održivo zbrinuti procesom izravnog izgaranja. Pregledom literature je utvrđeno kako su dosadašnja istraživanja energetskeg potencijala trajnih nasada uglavnom bazirana na orezanoj biomasi u fazi mirovanja. Cilj rada je na odabranim sortama vinove loze, jabuke i šljive utvrditi energetski potencijal primarne energije po jedinici površine nakon vađenja cjelokupnog trsa ili stabla. U zadanim uvjetima najveći energetski potencijal moguće je ostvariti uklanjanjem voćnih vrsti (šljiva 159 GJ/ha; jabuka 248 GJ/ha), dok prosječni energetski potencijal uklonjenog nasada vinove loze iznosi 149 GJ/ha.

Ključne riječi: biomasa, trajni nasadi, vinova loza, jabuka, šljiva, energetska vrijednost

Uvod

Energija dobivena iz biomase predstavlja jedan od važnijih faktora u postizanju ciljeva Europske unije vezanih uz obnovljive izvore energije i klimatska pitanja do 2030. („Klimatski i energetski okvir do 2030.“) i 2050. („Europski zeleni plan“). U klasifikaciji poljoprivredne biomase, uz ostatke ratarske i stočarske proizvodnje, značajan potencijal proizlazi i iz vinogradarske i voćarske proizvodnje (Bilandžija, 2018). Godišnjim orezivanjem voćnjaka i vinograda stvaraju se značajne količine biomase koja se može učinkovito iskoristi u procesima proizvodnje obnovljive energije (Scarlat i sur., 2011). U kontekstu energetske valorizacije, rezidbu biomase različitih sorti voćaka i vinove loze, preporučljivo je provoditi tijekom faze mirovanja vegetacije (zrela rezidba). Biomasa Orezana biomasa tijekom vegetacije (zeleno rezidba) zbog svog sastava ne služi kao značajan izvor energije (Radojević i sur., 2007).

Količina rezidbenih ostataka ovisi o biološkim karakteristikama, vrsti/sorti, agrotehničkim mjerama, starosti nasada, načinu održavanja, bujnosti sorte i podloge te načinu rezidbe (Živković i sur., 2007). Očekivana količina orezane biomase kod vinove loze kreće se od 0,35 do 1,15 (kg/trsu), dok se u voćnjacima očekuje količina orezane biomase od 0,45 do 24,3 (kg/stablu) (Zajec, 2022). Autori Bilandžija i Sito (2013), strojeve i opremu za prikupljanje orezane biomase, dijele na zasebne strojeve za prikupljanje, iznošenje i usitnjavanje orezane biomase te one za simultanu rezidbu i usitnjavanje. Osim orezane biomase, značajan energetski potencijal može se očekivati i od biomase nakon uklanjanja/vađenja cjelokupnog trajnog nasada. Nasade vinove loze i voćaka potrebno je obnavljati tijekom određenog vremenskog perioda. Razlog tome može biti napad bolesti, završetak životnog ciklusa te promjena potražnje na tržištu. Životni vijek nasada, odnosno voćnjaka i vinograda, ovisi o vrsti/sorti višegodišnje kulture, njezinoj podlozi i području uzgoja.

Prema dostupnim podacima Hrvatskog zavoda za savjetodavnu službu (HZSS, 2006) životni vijek voćnjaka se kreće između 15 i 50 godina (kesten) ovisno o voćnoj vrsti. Gašparec - Skočić (2005), navodi kako je skoro 58% nasada vinove loze u Republici Hrvatskoj, preko 25 godina starosti. Uklanjanje trajnih nasada moguće je podijeliti obzirom na korištenu tehniku, odnosno (I) uklanjanje nadzemne i podzemne biomase, (II) uklanjanje nadzemne biomase i (III) integrirano uklanjanje i usitnjavanje nadzemne biomase (CIRCE i CERTH, 2019).

Cilj ovoga rada bio je utvrditi masu nadzemne biomase, udio vode i energetske vrijednosti te procijeniti energetski potencijal po jedinici površine, za odabrane sorte vinove loze, jabuke i šljive.

Materijali i metode

Uzorkovanje biomase je provedeno na četiri različite lokacije, odnosno Zagreb - Pokušalište Jazbina Agronomskog fakulteta u Zagrebu (vinova loza sorta „Frankovka“), Veliko Trgovišće - privatni nasad (vinova loza sorta „Chardonney“), poduzeće Fragarija d.o.o - Nedelišće (jabuka sorta „Zlatni delišes“) te Osijek - Poljoprivredni institut Osijek (šljiva sorta „Čačanska najbolja“). Nakon vađenja cjelokupnog trsa/stabla, motornom pilom je odvojen nadzemni i podzemni dio biljke te se pristupilo vaganju nadzemne biomase pomoću digitalne vage. Od svakog uzorka prikupljeno je približno 250 grama poduzoraka, koji su dopremljeni na Agronomski fakultet u Zagrebu gdje su stavljeni na sušenje 48 sati na 60°C te ponovno izvagani. Usitnjavanje uzoraka provedeno je korištenjem laboratorijskog mlina (Retsch GM 300), a analiza ogrjevne vrijednosti kalorimetrijom (IKA C200) sukladno normi (HRN EN 14918:2010). Energetski potencijal je dobiven umnoškom mase suhe tvari (kg ST) biomase i energetske vrijednosti (MJ/kg ST). Svako mjerenje i laboratorijska analiza provedena je u tri ponavljanja po istraživanoj sorti vinove loze, jabuke i šljive.

Rezultati i rasprava

U tablici 1 prikazane su osnovne značajke trajnih nasada unutar kojih je provedeno uzorkovanje biomase, odnosno istraživana sorta, starost nasada i korišteni uzgojni oblici. Na osnovu utvrđenog razmaka između i unutar redova izračunat je broj trseva/stabala po hektaru koji za vinovu lozu iznosi 4.229, jabuku 2.604 te šljivu 833.

Tablica 1. Osnovne značajke istraživanih trajnih nasada

Vrsta	Sorta	Starost nasada (godina)	Uzgojni oblik	Broj biljaka / ha
Vinova loza	„Frankovka“	13	Dvokraki Guyot	4629
	„Chardonney“	15	Dvokraki Guyot	4629
Jabuka	„Zlatni delišes“	20	Vretenasti grm	2604
Šljiva	„Čačanska najbolja“	22	Piramida	833

Tablica 2 prikazuje utvrđene udjele vode i mase svježe biomase (kg/trsu, stablu) te procijenjenu masu svježe biomase i masu ST biomase po jedinici površine. Sadržaj vlage u orezanoj biomasi se uobičajeno kreće između 35% i 40%, ponekad i preko 50%, što ovisi o vrsti nasada i meteorološkim uvjetima u kojima se rezidba provodi. Osim procesa industrijskog sušenja, izlaganjem biomase prirodnom sušenju u trajanju od 25 dana, moguće je smanjiti sadržaj vlage za 20% (Velázquez-Martí i sur., 2010). Usporedno s literaturnim navodom za orezanu biomasu, utvrđeni udjeli vode u ovome istraživanju se nalaze unutar gornje granice. Najviši udio vode je utvrđen kod šljive (47,05%), a najmanji kod vinove loze sorte „Frankovka“ (43,28%). Sukladno utvrđenoj masi svježe biomase (kg/trsu, stablu) najveća potencijalno dostupna količina utvrđena je kod šljive 62,51 (t/ha), a najmanja kod vinove loze sorte „Chardonney“ 13,23 (t/ha).

Tablica 2. Udio vode i potencijalna dostupnost biomase uklonjenih trajnih nasada

Vrsta	Sorta	Voda %	Masa svježe biomase (kg/trsu, stablu)	Masa svježe biomase t/ha	Masa ST biomase t/ha
Vinova loza	„Frankovka“	43,28	3,51	16,24	9,21
	„Chardonney“	44,85	2,86	13,23	7,29
Jabuka	„Zlatni delišes“	46,54	10,56	27,49	14,69
Šljiva	„Čačanska stara“	47,05	75,05	62,51	33,09

Donja ogrijeva vrijednost (LHV) predstavlja jedan od temeljnih parametara u procjeni energetskog potencijala. Bilandžija i sur. (2012) istražuju energetski potencijal orezane biomase u agroekološkim uvjetima Republike Hrvatske te utvrđuju donju ogrjevnu vrijednost za vinovu lozu (17,05 MJ/kg), jabuku (17,06 MJ/kg) i šljivu (17,12 MJ/kg). Uspoređujući literaturne podatke na orezanoj biomasi s analiziranim vrijednostima (tablica 3), na ukupnoj nadzemnoj biomasi može se uočiti blagi porast donje ogrjevne vrijednosti za vinovu lozu i šljivu. Sukladno

potencijalno dostupnoj biomasi, u zadanim uvjetima, najveći energetska potencija moguće je ostvariti uklanjanjem nasada šljive s 159 GJ/ha.

Tablica 3. Donja ogrjevna vrijednost i energetska potencijal uklonjenih trajnih nasada

Vrsta	Sorta	Donja ogrjevna vrijednost (MJ/kg ST)	Energetska potencijal	
			GJ/ha	MWh/ha
Vinova loza	„Frankovka“	18,18	167	46
	„Chardonnay“	17,86	130	36
Jabuka	„Zlatni delišes“	16,85	248	68
Šljiva	„Čačanka najbolja“	17,35	574	159

Zaključak

Dosadašnja istraživanja energetska potencijala iz vinogradarskog i voćarskog sektora uglavnom su vezana uz procjenu dostupnosti i korištenja orezane biomase. Uklonjeni trajni nasadi ukazuju na značajan potencijal u korištenju cjelokupne nadzemne biomase u procesu proizvodnje energije. Iako potencijalno dostupna biomasa nakon uklanjanja trajnih nasada ovisi o mnogobrojnim faktorima (vrsta/sorta, razmak između i unutar, uzgojni oblik, tehnika prikupljanja), temeljem provedenog istraživanja može se zaključiti kako je u zadanim uvjetima, najveći energetska potencijal moguće ostvariti nakon uklanjanja šljive, a potom jabuke i vinove loze.

Literatura

- Bilandžija N., Voca N., Kricka T, Matin A, Jurisic V. (2012). Energy potential of tree pruned biomass in Croatia. Spanish Journal of Agricultural Research. 10: 292-298.
- Bilandžija N., and Sito S. (2013). Poljoprivredna tehnika u proizvodnji energetske kulture *Miscanthus x giganteus*. 41th International symposium on Agricultural Engineering “Actual Tasks on Agricultural Engineering”, 41, 343-354.
- CIRCE i CERTH (2019). Biomasa iz poljoprivrednih rezidbenih ostataka i uklonjenih nasada (PROUN). Studija izvedivosti unutar projekta uP_running 2016-2019 (Horizon 2020).
- Gašparec - Skočić Lj. (2005). Hrvatska danas u području vinogradarstva i vinarstva. Glasnik zaštite bilja. 6: 16-19.
- HZSS (2006). Hrvatski zavod za savjetodavnu službu. Podizanje novih nasada voćnjaka (tehnološko-ekonomske smjernice), Zagreb.
- Radojević R., Živković M., Urošević M., Radojević D. (2007). Technological and technical aspects of using pruning residues of fruit trees and grapevine. J Agric Technic Energy Agric. 11: 32-36.
- Scarlat N., Blujdea V., Dallemand J.F. (2011). Assessment of the availability of agricultural and forest residues for bioenergy production in Romania. Biomass Bioenergy. 35: 1995-2005.
- Velázquez-Martí B., Fernández-González E., López-Cortés I., Salazar-Hernández D.M. (2011). Quantification of the residual biomass obtained from pruning of vineyards in Mediterranean area. Biomass Bioenergy. 35: 3453-3464.
- Zajec S.T. (2022). Prostorna distribucija i energetska potencijal orezane biomase na području Krapinsko-zagorske županije. Diplomski rad, Sveučilište u Zagrebu Agronomski fakultet.
- Živković M., Radojević R., Urošević M. (2007). Priprema i potencijal ostataka rezidbe u voćnjacima i vinogradima kao energetska materijala. Poljoprivredna tehnika. 3: 51 - 58. Novi Sad.

Energy potential of vineyards and orchards biomass after the plantation removal

Abstract

The removal of vines or trees at the end of their life cycle is mandatory if the permanent plantations are affected by serious diseases or if market demand fluctuates. This intervention yields a considerable amount of biomass that can be disposed of sustainably by direct combustion. The current state of the literature indicates that previous studies on the energy potential of permanent plantations have predominantly focused on pruned biomass during the dormant phase. The aim of this study is to determine the energy potential of the primary energy per unit area for specific grape varieties, apples and plums after removing the entire vine or tree. Under the given conditions, the highest energy potential can be achieved by removing fruit species (plum 159 GJ/ha; apple 248 GJ/ha). In comparison, the average energy potential of the removed vine plantation equals to 149 GJ/ha.

Keywords: biomass, permanent plantations, vines, apple, plum, energy value

Influence of soybean (*Glycine max*) and hemp (*Cannabis sativa*) stalks biomass to glycerol solvent ratio on liquefied properties

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Abstract

In this study, the properties of liquefied biomass from soybean (*Glycine max*) and hemp (*Cannabis sativa*) stalks were investigated using glycerol (GLI) as a solvent at different biomass to solvent ratios in the presence of sulfuric acid (H₂SO₄) as a catalyst under specific liquefaction conditions at a temperature of 150 °C for 120 min. The liquefaction percentage, the solids content and the hydroxyl number were determined as values reflecting the properties of the liquefied biomass. The liquefaction percentage ranged from 88.01 % to 92.20 % for soybeans and from 86.95 % to 91.97 % for hemp, depending on the biomass to solvent ratio. The solids content of the liquefied soybean biomass ranged from 44.99 % to 55.09 %, while for hemp it ranged from 59.49 % to 62.55 %. The hydroxyl value for liquefied soybean biomass ranged from 429.41 to 612.00 mg KOH/g, while the value for hemp ranged from 569.76 to 835.38 mg KOH/g. The best ratio of biomass to solvent, taking into account the degree of liquefaction and the hydroxyl number, was found to be 1:5 for both samples.

Keywords: *glycine max*, *cannabis sativa*, liquefied biomass, liquefaction percentage, solids content, hydroxyl number

Introduction

With the growth of the world's population and the rapid development of industry, the demand for energy is increasing, while natural resources are becoming increasingly scarce. The resulting energy crisis has led to a demand for renewable, natural fibers and biofuels to replace petroleum-based fibers and fuels (Mohan et al., 2006). One of the new sustainable alternatives to conventional fossil fuels is biofuel derived from biomass feedstocks. The use of various crops such as corn, wheat, rice, soybeans and sugarcane has been investigated as feedstock for biofuel production (Prade et al., 2011).

Soybean (*Glycine max*) is a cheap and renewable natural resource whose stalk is a rich agricultural lignocellulosic by-product that is planted around the world. China, a major agricultural country, produces over 16 million tons of soybeans annually. However, most soybean stalks are discarded or destroyed, resulting in the loss of a valuable resource and environmental pollution (Zhu et al., 2008). The development of renewable bioenergy technologies enables the conversion of soybean waste into value-added materials such as biochar (Huoliang K et al., 2011). Soybean stalks are mainly used as livestock feed, burned for energy on land or processed into compost (Zhu et al., 2008; Terashima et al., 2009). The current utilization of soybean stalks leads to resource loss and environmental pollution, especially considering the potential of the stalk, which is rich in cellulose, hemicellulose, proteins and other organic substances. According to recent research, soybean stalks can be used to produce natural cellulose fibers with a structure and properties similar to those of currently used cellulose fibers (Wang and Sain, 2007; Reddy and Yang, 2009).

Hemp (*Cannabis sativa*) is a plant in the *Cannabaceae* family that is cultivated for its fibers and edible seeds. Hemp is one of the fastest growing plants on earth (Zimniewska, 2022) and also has one of the strongest natural fibers (Schultz et al., 2020).

Hemp has a high biomass yield (4-6 tons ha) and a high content of cellulose and lignin (Bócsa and Karus, 1998). The primary chemical composition of hemp fiber includes cellulose (55-72%), hemicellulose (7-19%), lignin (2-5%) and pectin (4-8%) (Lewin and Pearce, 1985). Today, hemp is used in various industries; for example, hemp fibers are used in textile production (Soljacic and Cunko, 1994), pulp and paper production (Barbera et al., 2011; Kovacs et al., 1992), fiberboard production (Crowley, 2011), composite materials (Thomsen et al., 2006; Vignon et al., 1996), thermal insulation materials (Yates, 2006), and others. In addition, the stalks of industrial hemp can be used for methane and alcohol production (Kreuger et al., 2011).

Table 1 shows the chemical composition of *Glycine max* (Cabrera et al., 2015) and *Cannabis sativa* stalks (Tutt et al., 2011).

Table 1. Chemical Composition of *Glycine max* and *Cannabis sativa* stalks

Chemical component		<i>Glycine max</i>	<i>Cannabis sativa</i>
Cellulose	%	35,30	53,86
Hemicellulose	%	16,90	10,60
Lignin	%	21,70	8,76
Ash	%	10,60	5,25

Materials and methods

Samples of *Glycine max* and *Cannabis sativa* stalks were ground in a centrifugal mill. The reason for grinding the samples is to ensure uniformity in the liquefaction reaction and to achieve complete penetration of the solvent into the sample. The ground samples were sieved using an electromagnetic sieve shaker. The targeted particle size for further liquefaction processes ranged between 0.51-1.00 mm. The sieving time per sample lasted for 10 minutes, and the shaking frequency was set at 6 kHz.

The prepared samples were then subjected to a liquefaction process conducted according to the modified forest biomass liquefaction method (Antonović et al., 2008). The liquefaction of the samples was carried out in a 500 mL reactor equipped with a mixer and a Liebig condenser system, using glycerol as the solvent, with sample-to-solvent ratios of 1:5, 1:4, and 1:3. For example, in the 1:5 ratio, 20 g of finely ground biomass sample was dissolved in 100 g of glycerol. Sulfuric acid (H₂SO₄) was used as the acidic catalyst in an amount of 3% relative to glycerol. Furthermore, the finely ground samples of soybean and hemp stalks were added to the reaction solution only after the solution had reached a temperature of 150 °C. The liquefaction process occurred over 120 minutes with an electric heater supported by a thermostat.

The percentage of liquefied soybean and hemp stalks was determined by weighing 1 g of the sample into a beaker and adding 50 ml of distilled water. The beaker containing the mixture of distilled water and liquefied biomass was stirred with a magnetic stirrer for 60 minutes. The resulting liquid was filtered and the filter paper together with the residue was placed in a drying chamber at a temperature of 70 ± 2 °C for 3 hours. After drying, the sample was cooled in a desiccator and weighed on an analytical balance. The percentage of liquefaction of the liquefied biomass was determined using the following equation:

$$L.P. = 100 - \left(\frac{mfps - mfp}{ms} * 100 \right) [\%]$$

L.P. stands for the liquefaction percentage (%); mfps for the mass of the filter paper with the dried sample (g); mfp for the mass of the filter paper (g) and ms for the mass of the sample (g).

The solids content of the liquefied biomass is determined on an analytical balance with an accuracy of ± 0.1 mg. After measuring the mass of the empty watch glass, 1 g of the sample is weighed with the same accuracy, which represents the sample mass. The prepared samples are dried in a drying chamber at a temperature of 150 ± 2 °C for 24 hours. After drying, the samples are allowed to cool in the desiccator and then the dry samples are weighed together with the watch glass (mpz+s.uz). The percentage of solids in the liquefied biomass is determined using the equation below:

$$S. C. = \frac{mwgs - mwg}{ms} * 100 [\%]$$

S.C. is the solids content of the liquefied biomass, %; mwgs is the mass of the watch glass with the dried sample, g; mwg is the mass of the watch glass, g; and ms is the sample mass before drying, g.

The method for the determination of the hydroxyl number (OH number) was carried out according to a modified procedure specified in the ASTM D4274-05 standard. 25 ml of the prepared esterification reagent is added to a beaker with a lid using a pipette. Using an analytical balance with a measuring accuracy of ± 0.1 mg, weigh between 0.51 and 0.56 g of the liquefied biomass sample. The prepared samples are heated in a water bath to a temperature of 98 ± 2 °C, making sure that the sample is completely immersed in the beaker and that the beakers are tightly closed. The prepared samples are heated for a period of 15 minutes. During the entire process, a sufficient water level must be maintained in the bath so that the part of the beaker containing the sample is completely submerged. After heating phthalic anhydride and the sample, the contents of the beaker are cooled to room temperature and then 50 ml of pyridine and 10 ml of hot distilled water are pipetted. The mixture is stirred thoroughly and a magnet is added. The prepared sample is titrated using a titrator, which measures the pH of the sample in the glass vessel using a magnetic stirrer and a pH meter. The prepared sample, which has very low pH values, is titrated with a previously prepared standard solution of sodium hydroxide with a normality of 0.5 N, and the consumption is monitored until the electrode measures a neutral pH of 7. The entire procedure is carried out in a similar manner for a blank test, where only the esterification reagent is added without the sample and all subsequent steps are identical to the addition of the sample. The hydroxyl OH number of the liquid biomass is determined according to the following equation:

$$OH \text{ number} = \frac{(B - A) * N * 56,1}{m} [\text{mg KOH/g}]$$

Where A is the consumption of NaOH for the blank test, ml; B is the consumption of NaOH when the sample is added, ml; N is the normality of the NaOH solution; m is the mass of the liquefied biomass sample, g.

Results and discussion

In this study, the influence of different ratios of biomass to solvent on the liquefaction properties such as degree of liquefaction, solids content and hydroxyl-OH number was investigated. The results are shown in Table 2, where all values are given as the arithmetic mean and standard deviation of five measurements.

Table 2. Properties of the liquefied biomass polymers of *Glycine max* and *Cannabis sativa* as a function of the ratio between biomass and solvent.

Uzorak	GLI/biomasa	Liquefaction property					
		Liquefaction percentage		Solids content		OH number	
		%		%		mg KOH/g	
		\bar{x}	σ	\bar{x}	σ	\bar{x}	σ
<i>Glycine max</i>	1:5	92,20	0,71	55,09	0,68	612,00	34,18
	1:4	90,57	0,92	54,53	1,33	480,73	32,12
	1:3	88,01	1,24	44,99	0,89	429,41	39,48
<i>Cannabis sativa</i>	1:5	91,97	0,78	59,49	0,30	835,38	34,46
	1:4	88,89	0,35	59,55	0,28	790,39	29,87
	1:3	86,95	0,91	62,55	4,18	569,76	34,73

\bar{x} - arithmetic mean

σ - standard deviation

Since the OH number is the most important liquefaction property, as it provides information about the ability to bind liquefied biomass in further syntheses, the OH number takes precedence over other polymer liquefaction properties such as the liquefaction percentage and the solids content. Liquefaction percentage of the samples of *Glycine max* and *Cannabis sativa* strains at different biomass to glycerol ratios ranged from 86.95 % to 92.20 %, the solids content from 44.99 % to 62.55 % and the hydroxyl OH number from 429.41 to 835.38 mg KOH/g.

The liquefied samples with the lowest results were liquefied at lower biomass to solvent ratios when considering OH number, with the lowest value observed for the liquefied biomass of *Glycine max* (429.41 mg KOH/g) and a liquefaction percentage at approximately similar values for both samples *Glycine max* (88.01 %) and *Cannabis sativa* (86.95 %). The best results were obtained with samples liquefied at a ratio of 1:5, which makes them the most favorable for further application.

Based on the results presented, the liquefied biomass of *Cannabis sativa* stems with glycerol in a 1:5 ratio showed the best properties with the highest OH number value (835.38 mg KOH/g) and a high degree of liquefaction (91.97 %). The solids content fluctuated around the same values for each sample. Figure 1 shows that the solids content in the liquid biomass of *Cannabis sativa* stalks increases with decreasing biomass/solvent ratio, which is not the case for *Glycine max* samples.

A comparison with previous studies on other biomass types, such as liquefied *Miscanthus x giganteus* grass (Antonović et al., 2020), shows that *Glycine max* and *Cannabis sativa* have sufficient values of liquefaction properties for further applications.

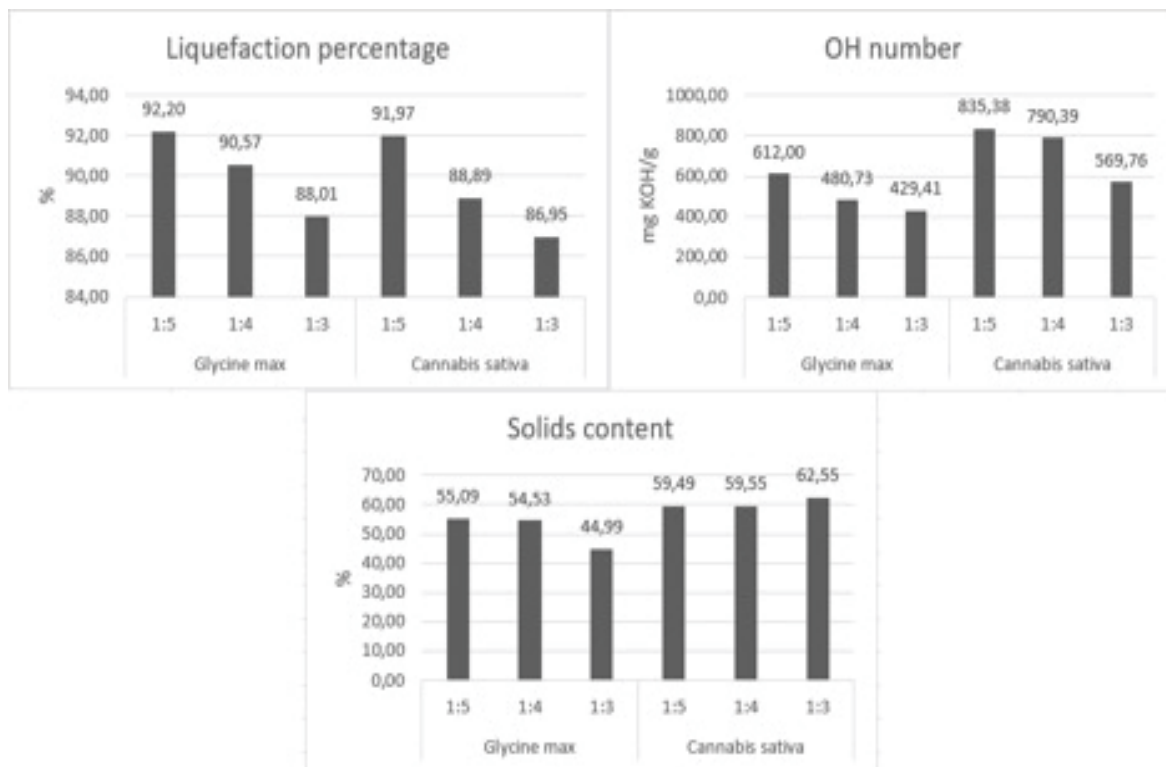


Figure 1. Liquefied biomass polymer properties of glycine max and cannabis sativa depending on different biomass to solvent ratio.

Conclusion

The best results for biomass liquefaction at different ratios with a solvent, considering the OH number as one of the key properties, were achieved with 1:5 ratio. A higher OH number indicates better polymerization properties, further development of chemicals, resins, and adhesives based on liquefied biomass of *glycine max* and *cannabis sativa*. It is noteworthy that all the ratios of biomass and solvent used in this study showed relatively high OH number values, which is a positive sign for further research.

An important positive factor contributing to the high OH number values is the solvent glycerol used for liquefaction. A higher ratio of biomass to solvent ensures more glycerol in a smaller amount of liquidized biomass. Glycerol, with three hydroxyl functional groups in one molecule, contributes to an increased OH number, which can be beneficial for achieving desired properties in chemicals and adhesives.

The results of the solids content in samples of liquefied biomass indicates that *glycine max* stalks samples at a ratio of 1:3 with a solvent have the least, but mutually similar values compared to other ratios. This value indicates the amount of the liquefied sample will remain and how much will evaporate in the event that this sample is used during the pressing of particle boards from shredded wood.

By the liquefaction percentage, we can conclude that it is not possible to achieve a high degree of liquefaction of 95 % or more with the liquefied biomass of *glycine max* and *cannabis sativa*. Positive results with fairly similar liquefaction percentage values are achieved by liquefaction of biomass at ratios of 1:5 with glycerol as the solvent.

Acknowledgement

This research was financed through OP Competitiveness and Cohesion - project “Development of an innovative technical-technological line for the production of advanced bioadhesives based on liquefied wood – LiqWOODTech“ KK.01.2.1.02.0236

References

- Antonović A. (2008). Istraživanja novih sustava formaldehidnih ljepila za drvene ploče modificiranih s utekućenim drvom, doktorska disertacija, Šumarski fakultet, Sveučilište u Zagrebu.
- Antonović A., Krička T., Voća N., Jurišić V., Matin A., Grubor M., Bilandžija N., Stanešić J., Ištvančić J. (2019). Kemijska karakterizacija utekućene trave *Miscanthus x giganteus*. U: Mioć B. & Širić I. (ur.) Proceedings 54th Croatian & 14th International Symposium on Agriculture.
- ASTM D4274-05 (2010). Standard Test Methods for Testing Polyurethane Raw Materials: Determination of Hydroxyl Numbers of Polyols.
- Barbera L., Pelach M.A., Perez I., Puig J., Mutje P. (2011). Upgrading of hemp core for papermaking purposes by means of organosolv process. *Industrial Crops and Products*. 34: 563–571.
- Batra S.K. (1986). Other long vegetable fibers - abaca, banana, sisal, henequen, flax, ramie, hemp, sunn, and coir. In M. Lewin & E. M. Pearce (Eds.), *Fiber chemistry*.
- Bocsa I., and Karus M. (1998). *The Cultivation of Hemp: Botany, Varieties, Cultivation and Harvesting*.
- Crowley J.G. (2011). The Performance of *Cannabis sativa* (Hemp) as a Fibre Source for Medium Density Fibre Board (MDF). *Irish Agriculture And Food Development Authority (Teagasc), Carlow, Ireland*, pp. 1–11.
- Cabrera E., Munoz M.J., Martin R., Caro I., Curbelo C., D'iaz A.B. (2015). Comparison of industrially viable pretreatments to enhance soybean straw biodegradability. *Bioresource Technology*. 194: 1–6.
- Kong H., He J., Gao Y., Wu H., Zhu X. (2011). Cosorption of phenanthrene and mercury (II) from aqueous solution by soybean stalk-based biochar. *Journal of agricultural and food chemistry*. 59: 12116-12123.
- Kovacs I., Rab A., Rusznak I., Annus S. (1992). Hemp (*Cannabis sativa*) as a possible raw-material for the paper-industry. *Cellulose Chemistry and Technology*. 26: 627–635.
- Kreuger E., Prade T., Escobar F., Svensson S.E., Englund J.E., Bjornsson L. (2011). Anaerobic digestion of industrial hemp-effect of harvest time on methane energy yield per hectare. *Biomass & Bioenergy*. 35: 893–900.
- Mohan D., Pittman C.U., Steele P.H. (2006). Pyrolysis of wood/biomass for bio-oil: a critical review. *Energy & Fuels*. 20: 848–889.
- Prade T., Svensson S.E., Andersson A., Mattsson J.E. (2011). Biomass and energy yield of industrial hemp grown for biogas and solid fuel. *Biomass and bioenergy*. 35: 3040-3049.
- Reddy N., and Yang Y. (2009). Natural cellulose fibers from soybean straw. *Bioresource Technology*. 100: 3593-3598.
- Schultz C.J., Lim W.L., Khor S.F., Neumann K.A., Schulz J.M., Ansari O., Burton R.A. (2020). Consumer and health-related traits of seed from selected commercial and breeding lines of industrial hemp, *Cannabis sativa* L. *Journal of Agriculture and Food Research*. 2: 100025.
- Soljačić I., and Čunko R. (1994). **Hrvatski tekstil kroz povijest**. *Tekstil* (43) 11, str. 584-602.
- Terashima N., Akiyama T., Ralph S., Evtuguin D., Neto C.P., Parkas J., Paulsson M., Westermarck U., Ralph J. (2009). "2D-NMR (HSQC) difference spectra between specifically ¹³C-enriched and unenriched protolignin of *Ginkgo biloba* obtained in the solution state of whole cell wall material," *Holzforschung*. 63: 379- 384.
- Thomsen A.B., Thygesen A., Bohn V., Nielsen K.V., Pallesen B., Jorgensen M.S. (2006). Effects of chemical-physical pre-treatment processes on hemp fibres for reinforcement of composites and for textiles. *Industrial Crops and Products*. 24: 113–118.
- Tutt M., and Olt J. (2011). Suitability of various plant species for bioethanol production. *Agronomy Research*. 9.
- Vignon M.R., Dupeyre D., Garcia Jaldon C. (1996). Morphological characterization of steam-

- exploded hemp fibers and their utilization in polypropylene-based composites. *Bioresource Technology*. 58: 203–215.
- Wang B., and Sain M. (2007). Isolation of nanofibers from soybean source and their reinforcing capability on synthetic polymers. *Composites Science and Technology*. 67: 2521-2527.
- Yates T. (2006). The use of non-food crops in the UK construction industry. *Journal of the Science of Food and Agriculture*. 86: 1790–1796.
- Zhu B., Fan T.X., Zhang D. (2008). Adsorption of copper ions from aqueous solution by citric acid modified soybean straw. *Journal of hazardous materials*. 153: 300-308.
- Zimniewska M. (2022). Hemp fibre properties and processing target textile: A review. *Materials*. 15: 1901.

Primjena sinteznog plina dobivenog rasplinjavanjem drvene biomase kao pogonskog goriva za četverotaktne Otto motore

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Sažetak

Biomasa predstavlja alternativu fosilnim gorivima, posebno zbog visokih cijena i negativog utjecaja na okoliš. Jedna od mogućnosti iskorištenja je rasplinjavanje biomase tj. njezino nepotpuno izgaranje, pri čemu nastaje smjesa zapaljivih plinova (sintezni plin) koju je moguće koristiti za pogon četverotaktnog Otto motora. U ovom radu su uspoređene karakteristike motora Briggs & Stratton, tip XP40 Sprint, nazivne snage 2,5 kW i radnog obujma 148 cm³, pogonjenog na benzin, propan butan plinsku smjesu i sintezni plin dobiven rasplinjavanjem drvene biomase u obliku peleta. Iako je ispitivani motor pogonjen sinteznim plinom ostvario samo 30% nazivne snage u odnosu na benzin, rezultati pokazuju da je moguće koristiti sintezni plin za pogon četverotaktnog Otto motora.

Ključne riječi: rasplinjavanje, sintezni plin, četverotaktni Otto motor, drveni peleti

Uvod

Visoka cijena fosilnih goriva kao i njihova dostupnost u današnje vrijeme, te utjecaj produkata izgaranja na okoliš, ponovno postavljaju pitanje zamjene fosilnih goriva alternativnim i obnovljivim izvorima energije. Kao jedno od relativno jeftinih, pristupačnih i ekološki prihvatljivih rješenja je korištenje biomase. Biomasa se sastoji od brojnih, raznovrsnih proizvoda biljnog i životinjskog porijekla, kao što su drvo, grane, grančice, kora i piljevina iz šumarstva, slama, stabljike kukuruza, stabljike suncokreta, ostaci orezivanja vinove loze i maslina, oguline jabuka iz poljoprivrede, životinjski otpad i ostaci sa stočnih farmi te komunalni i industrijski otpad (Šegon i sur., 2014).

Biomasa može pružiti gotovo sve što fosilna goriva pružaju, bilo gorivo ili kemijsku sirovinu. Osim toga, pruža dvije važne prednosti koje ju čine održivom sirovinom za proizvodnju sinteznog plina. Ne daje nikakav doprinos zagađenju atmosfere pri izgaranju, a njezina uporaba smanjuje ovisnost o neobnovljivim i često uvezenim fosilnim gorivima (Basu, 2010). Nedostatak biomase je njezina mala ogrjevna vrijednost u usporedbi s fosilnim gorivima i loša mogućnost implementacije na postojeće sustave. Jedna od mogućnosti primjene biomase na postojeće sustave motora s unutarnjim izgaranjem je rasplinjavanje drvnih peleta.

Prvi pokušaji komercijalne proizvodnje sinteznog plina počinju krajem 18. stoljeća kada gotovo istovremeno William Murdoch u Engleskoj i Philippe LeBon u Francuskoj koriste isti za rasvjetu (Schofield, 1947). Razvitkom industrijalizacije i potrebe za rasvjetom u prvoj polovici 19. stoljeća počinje se masovno upotrebljavati plinska rasvjeta na prirodni plin metan i sintezni plin dobiven rasplinjavanjem kamenog ugljena ili drva, a krajem 19. stoljeća počinje se koristiti i za pogon motora s unutarnjim izgaranjem jer u to vrijeme industrija prerade nafte nije mogla zadovoljiti potrebe za energijom. Rasplinjavanje je termokemijski proces nepotpunog izgaranja organskog materijala, koji je najčešće u obliku krutog goriva u kojem nastaje sintezni (generatorski) plin. Zbog visoke temperature nastaju zapaljivi plinovi: ugljikov monoksid (CO), vodik (H₂) i metan (CH₄), a u puno manjoj mjeri nastaju i razni ugljikovodici (uključujući i aromatske), kiseline i pepeo.

Da bi se osigurao pravilan rad rasplinjača, a time stvaranje sinteznog plina, potrebno je dovesti 20% do 45% kisika (O₂) od količine koja je potrebna za potpuno izgaranje. Reakcije rasplinjavanja mogu se odvijati indirektnim rasplinjavanjem kod kojeg se toplina razvija izvan rasplinjača i prenosi na njega, ili direktnim rasplinjavanjem kod kojeg se toplina stvara egzotermnim izgaranjem i djelomičnim izgaranjem unutar rasplinjača. Glavne reakcije rasplinjavanja su: dehidracija, piroliza, izgaranje i rasplinjavanje.

Primjer dobrog iskorištavanja biomase kao obnovljivog izvora energije su kombinirane termoelektrane-toplane (kogeneracija) u kojoj se sintezni plin nastao rasplinjavanjem biomase koristi za pogon motora s unutarnjim izgaranjem, a toplina koja nastaje hlađenjem plina i radom motora se može upotrijebiti za industriju ili zagrijavanje stambenih prostora. One su širokog raspona snaga, od nekoliko kW pa sve do nekoliko MW i mogu se kristiti za potrebe kućanstava. Istraživanje koje su proveli Shabbar i Janajreh (2013) pokazuje da učinkovitost istosmjernog rasplinjača uparenog s motorom s unutarnjim izgaranjem iznosi oko 80%.

Cilj rada je utvrditi mogućnost korištenja sinteznog plina dobivenog rasplinjavanjem biomase za pogon motora s unutarnjim izgaranjem, te izmjeriti i usporediti zakretni moment i snagu motora pogonjenog na benzin, propan butan plinsku smjesu i na sintezni plin.

Materijal i metode

Za ovo ispitivanje izabrano je indirektno rasplinjavanje drvnih peleta i dobiveni sintezni plin prolazi izmjenjivačem topline čija je uloga snižavanje temperature izlaznog plina i omogućavanje kondenzacije vlage i kiselina u posudi smještenoj iza samog izmjenivača. Tako očišćen sintezni plin se pomoću fleksibilnog crijeva dovodi u rasplinjač motora.

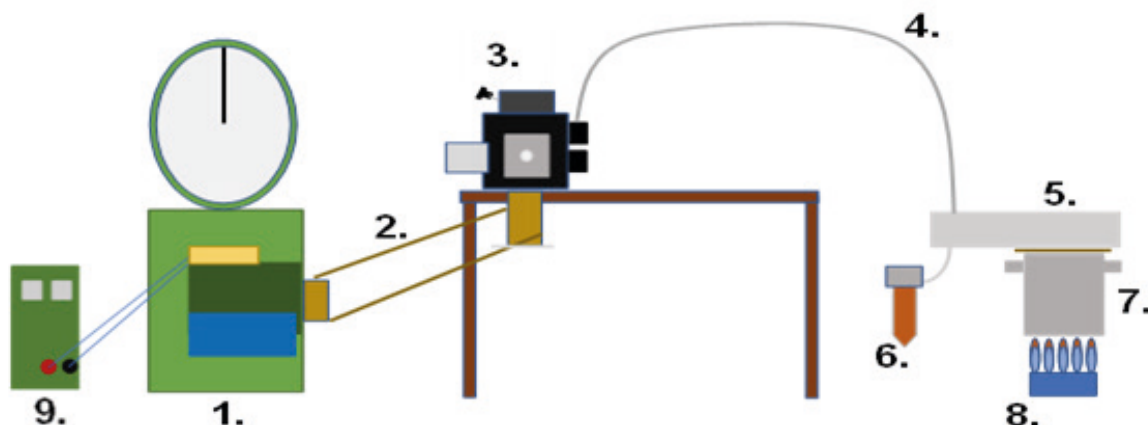
Peleti su načinjeni od miješanog crnogoričnog drva i kore, a proizvođač deklarira ogrjevnu vrijednost veću od 16,5 MJ/kg, sadržaj pepela ispod 0,7% i sadržaj vlage manji od 10%. Kao pogonski agregat je odabran jednocilindrični četverotaktni Otto motor Briggs & Stratton, tip XP40 Sprint, čije su tehničke karakteristike navedene u tablici 1. Prijenos zakretnog momenta s motora na mjernu kočnicu izveden je pomoću remenskog prijenosa.

Tablica 1. Karakteristike motora Briggs & Stratton XP40 Sprint

Radni obujam	148 cm ³
Promjer cilindra	65,08 mm
Hod klipa	44,45 mm
Najveća snaga	2,5 kW
Najveći zakretni moment	6,1 Nm
Kapacitet spremnika s gorivom	0,9 L
Masa praznog motora	9 kg

Mjerna kočnica je elektromagnetska, proizvođača Zollner & Co najveće mjerne snage 10 kW, a kočnicom se upravlja pomoću istosmjerne struje promjenom napona od 0 do 40 V.

Ostali korišteni uređaji su: digitalni multimetar točnosti $\pm 0,3$ V, digitalni brojač broja okretaja i regulator istosmjernog napona (Iskra). Shematski prikaz ovog pokusa je prikazan na slici 1.

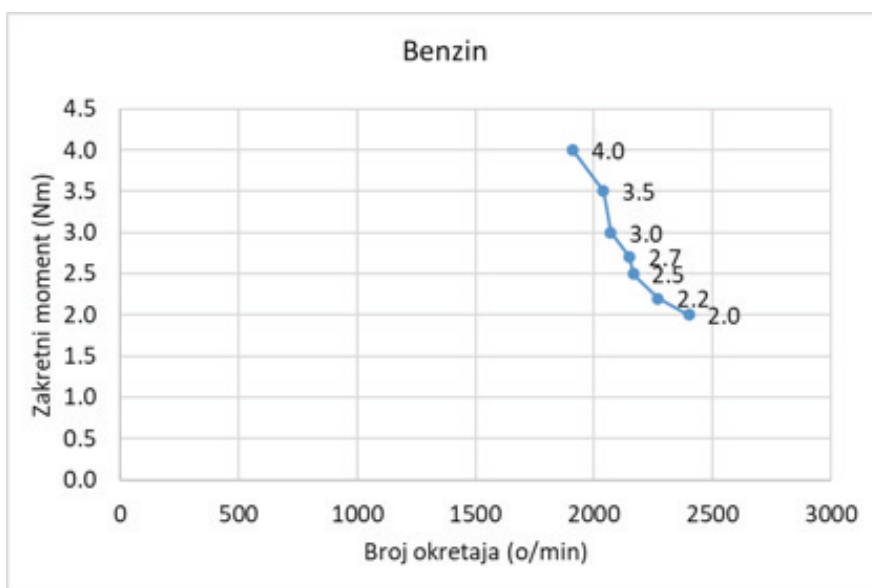


- | | |
|------------------------------------|--|
| 1. Mjerna kočnica | 2. Remenski prijenos |
| 3. Motor s unutarnjim izgaranjem | 4. Fleksibilno crijevo za dovod plina |
| 5. Hladnjak plina | 6. Posuda za sakupljanje plinskog kondenzata |
| 7. Rasplinjač drvene mase (posuda) | 8. Plinski plamenik |
| 9. Regulator istosmjernog napona | |

Slika 1. Shematski prikaz ispitivanje snage motora pogonjenog na sintezni plin

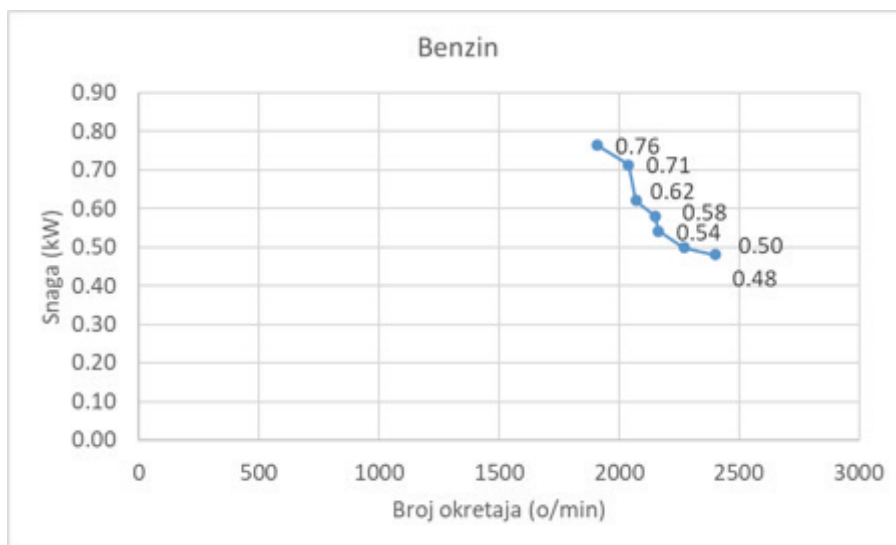
Rezultati i rasprava

Uz pogon na sintezni plin, za referentno ispitivanje korišteni su benzin Eurosuper 95 i plinska smjesa propan butan. Rezultati ispitivanja karakteristika motora pogonjenog benzinom Eurosuper 95 prikazani su u grafikonima 1 i 2. Od tri vrste goriva, najbolji rezultati su ostvareni s tim gorivom pri čemu je motor ostvario maksimalnu snagu od 0,74 kW i najveći moment od 4 Nm. Motor pogonjen benzinom Eurosuper 95 ostvario je svega 30% deklarirane snage i 65% maksimalnog deklariranog zakretnog momenta, a uzrok tome može biti istrošenost cilindra ili nekih drugi čimbenici, a treba uzeti u obzir i gubitke u remenskom prijenosu.



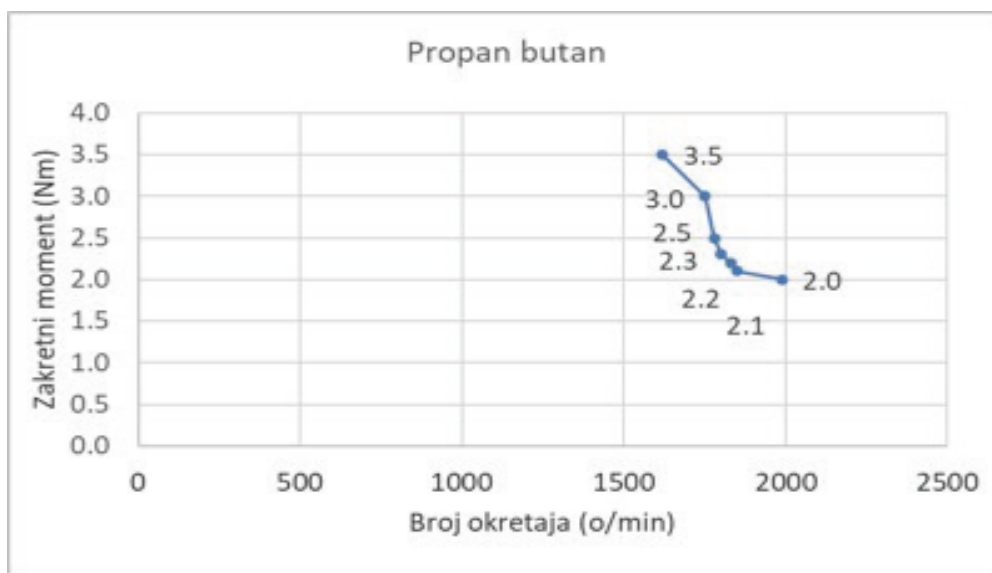
Grafikon 1. Izmjeren zakretni moment motora pogonjenog benzinom Eurosuper 95

Prema deklaraciji proizvođača novi motor bi trebao ostvarivati najveću snagu od 2,5 kW pri 4100 o/min. U stvarnim uvjetima korištenja ovaj motor ne može postići tako veliki broj okreta već najviše 3000 o/min. Uz to treba napomenuti da moment porastom broja okretaja opada što je vidljivo na provedenim mjerenjima.

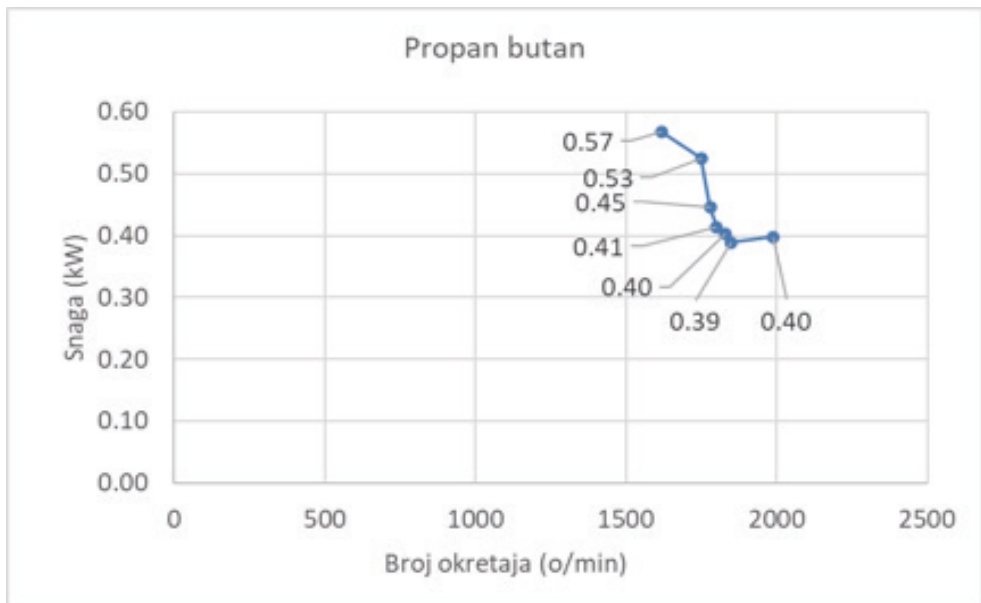


Grafikon 2. Izmjerena snaga motora pogonjenog benzinom Eurosuper 95

Pri pogonu motora na propan-butan plinsku smjesu došlo je do smanjenja zakretnog momenta i snage koji su iznosili 3,5 Nm odnosno 0,56 kW, što je vidljivo iz grafikona 3 i 4. Motor pogonjen propan-butan plinskom smjesom ostvario je 25% manju maksimalnu snagu u usporedbi s onim pokretanim na benzin, te 12,5% manji zakretni moment. Karakteristike motora pogonjenog propan-butan plinskom smjesom nisu se značajno razlikovale od onih kod motora pogonjenog na benzin. Dobivene razlike u podacima dobivenih mjerjenjem mogu biti prouzročeni pogrešnim omjerima zraka i plina prilikom ispitivanja. Pravilan omjer zraka i plina je od velike važnosti kako ne bi došlo do značajnijeg smanjenja zakretnog momenta i snage kao što se desilo u ovim mjerjenjima. Pogon na ovu vrstu goriva može biti prihvatljiv u slučajevima nestašice tekućih goriva poput benzina.



Grafikon 3. Izmjeren zakretni moment motora pogonjenog propan-butan plinskom smjesom

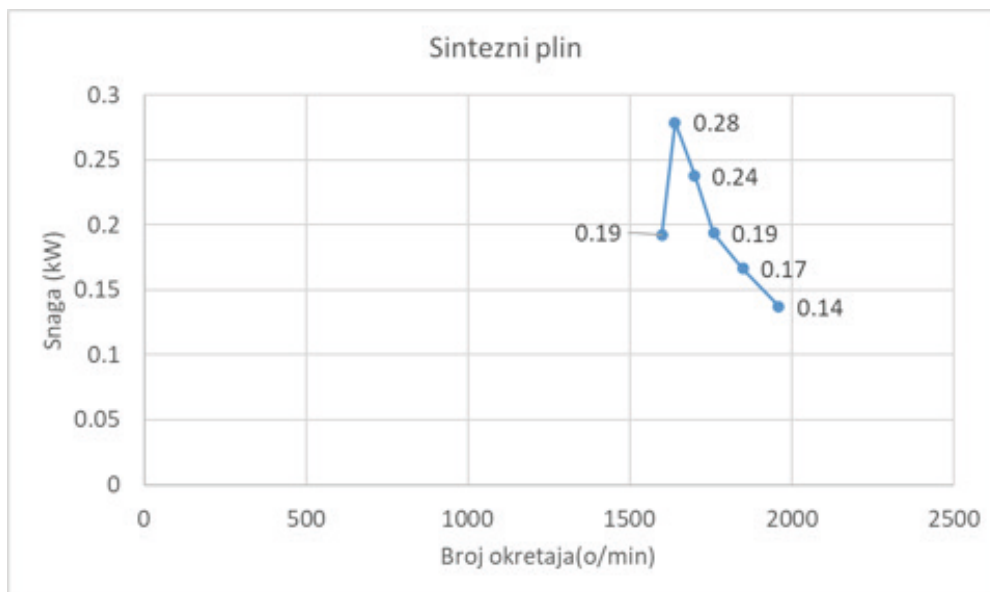


Grafikon 4. Izmjerena snaga motora pogonjenog propan-butan plinskom smjesom



Grafikon 5. Izmjeren zakretni moment motora pogonjenog sinteznim plinom

Motor pogonjen sinteznim plinom dobivenim rasplinjavanjem biomase ostvario je samo 37% snage u usporedbi s motorom pogonjenog benzinom, što se vidi u grafikonima 5 i 6. Ovakvi rezultati mogu biti posljedica lošeg rada rasplinjača. Prvi razlog može biti prekratko vrijeme zagrijavanja i rasplinjavanja. Proces rasplinjavanja trajao je samo 15 minuta što je jedva dovoljno da bi se iz cjelokupne mase uklonila voda (kondenzat) koji se nakupljao u posudi s kondenzatom. Drugi razlog može biti poddimenzioniran izmjenjivač topline koji ne može dovoljno ohladiti sintezni plin da se kondenzira sva vlaga iz peleta jer voda u motoru samo snižava ogrjevnu vrijednost plina.



Grafikon 6. Izmjerena snaga motora pogonjenog sinteznim plinom

Pošto sintezni plin s velikom količinom vlage ima smanjenu ogrjevnju vrijednost koja dodatno otežava rad motora, potrebno je povećati vrijeme sušenja biomase kada se plin ispušta u atmosferu dok se velika količina vlage iz biomase ne eliminira. Jaguaribe i sur. (2020), navode kako je potrebno minimalno vrijeme od 10 minuta da se uspostavi normalan rad rasplinjača te da je to vrijeme potrebno da se ostvari minimalna koncentracija od 10% ugljikovog monoksida potrebnog za rad motora. Čistoća samoga plina također je važan čimbenik jer može začeptiti ili zamastiti pojedine dijelove rasplinjača i motora. Takav onečišćen plin može utjecati na ventile motora koji svojim nepotpunim zatvaranjem onemogućuju pravilan rad motora. Nadalje, Yongseung (2012) navodi kako upotreba PID regulatora u sustavima rasplinjavanja uvelike olakšava i ujednačava rad rasplinjača na način da se regulacijom ulaza zraka zadržava temperatura potrebna za rasplinjavanje unatoč velikim oscilacijama u potrošnji sinteznog plina. Krivulje zakretnog momenta prikazane u grafikonu 5 i snage u grafikonu 6 gotovo su identične te pokazuju kako motor ostvaruje najveći moment od 1,7 Nm i najveću snagu od 0,28 kW pri 1640 okreta u minuti, a daljnjim porastom broja okreta moment i snaga opadaju.

Zaključak

Iako je pogonom na sintezni plin četverotaktni Otto motor pokazao izrazito veliko smanjene vrijednosti zakretnog momenta i snage u odnosu na pogon benzinom i propan-butan plinskom smjesom, ova mjerenja su pokazala da sintezni plin dobiven iz biomase ima potencijal kao alternativa fosilnim gorivima. Najbolje je koristiti biomasu s malom količinom vlage ili neposredno prije pokretanja rasplinjavanja dodatno posušiti biomasu. Potrebno je provesti daljnja istraživanja kako bi se ustanovila moguća poboljšanja u konstrukciji da bi se dobile što manje oscilacije u snazi i momentu motora pogonjenog na sintezni plin. Rasplinjavanje ne treba gledati kao staru i zastarjelu tehnologiju već kao tehnologiju koja uz današnje sustave samostalnog vođenja procesa može biti gotovo potpuno autonomna, te kao tehnologiju koja može koristiti goriva slabije kvalitete koja su pristupačna, relativno jeftina i iz obnovljivih izvora.

Literatura

- Basu P. (2010). Biomass gasification and pyrolysis: Practical design and theory. Cambridge, USA: Academic Press.
- Jaguaribe E.F., Rumão A.S., Silva F.S., Filho V.V.C., de Araújo Galdino W.V., Tavares de Luna F.E. (2020). Effects of biomass properties on the performance of a gasifier/genset system. Global Journal of Researches in Engineering: A Mechanical and Mechanics Engineering. 20: 21-31.
- Schofield M. (1947) Ex fumo dare lucem. Science Progress. (1933-), 35: 69-74.

Shabbar S., and Janajreh I. (2013). Thermodynamic equilibrium analysis of coal gasification using Gibbs energy minimization method. *Energy Conversion and Management*. 65: 755-763.

Šegon V., Šimek T., Oradini A., Marchetti M. (2014). Priručnik za učinkovito korištenje biomase. Jastrebarsko, Hrvatska: Hrvatski šumarski institut.

Yongseung Y. (2012). Gasification for practical applications. Rijeka, Hrvatska: InTechOpen.

Application of synthesis gas obtained by gasification of wood biomass as fuel for four-stroke Otto engines

Abstract

Biomass represents an alternative to fossil fuels, especially due to high prices and negative impact on the environment. One of the possibilities of utilization is the gasification of biomass, i.e. its incomplete combustion, whereby a mixture of flammable gases (synthesis gas) is created, which can be used to drive a four-stroke Otto engine. This paper compares the characteristics of the Briggs & Stratton engine, type XP40 Sprint, nominal power 2.5 kW and working volume 148 cm³, powered by gasoline, propane butane gas mixture and synthetic gas obtained by gasification of wood biomass in the form of pellets. Although the tested engine powered by synthesis gas achieved only 30% of the rated power compared to gasoline, the results show that it is possible to use synthesis gas to drive a four-stroke Otto engine.

Keywords: gasification, synthesis gas, four-stroke Otto engine, wooden pellets

Energy properties of the Virginia mallow (*Sida hermaphrodita*) residue from enzymatic fiber isolation

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Abstract

The fiber isolation process is based on the use of alkalis that have a negative impact on the environment. By employing more environmentally friendly methods and non-standard textile cultures, it is possible to mitigate these negative changes. The aim of this study is to research how the enzyme protease affects the energy properties of the residue from the fiber isolation process of Virginia mallow. The ash content, as an undesirable substance, is at its lowest in the control sample, with 2.26%. However, in the sample treated with the highest enzyme concentration (I-E1), there is an increase of 77.88% in ash content. The increase in ash content in this case does not have a negative impact on the heating value of sample I-E1, as it is 1.51 % higher than the control sample. For other analysis statistically there is significant difference between samples treated with highest enzyme concentrations (I-E1) and samples (III-E005) treated with lowest enzyme concentrations.

Keywords: biomass, proteases, circular economy, environmentally friendly

Introduction

Circular economy considers the impact of raw material consumption and waste generation on the environment, leading to the creation of alternative closed loops. The circulation of raw materials in these loops is based on production, consumption, reuse, refurbishment, and recycling, with the aim of extending the product's lifespan and reducing environmental pollution (Corona et al., 2019).

Textile industry has big negative impact on environment, especial acidic and alkali treatments (Phuong, 2003). Hydrolysis with acids or alkalis is a popular method for separating cellulose due to its high yield, purity, and ease of use. By using higher alkali concentrations and temperatures, more lignin can be removed to increase cellulose purity (Liu et al., 2021). For example, the Kraft process (16% alkalinity, 25% sulfidity) and the soda process (7% NaOH) can yield 91.8% and 60.7% cellulose from wheat straw (Sánchez et al., 2016). Athinarayanan et al. (2020) used two rounds of 4% NaOH treatment and bleaching with a 1:1 acetate buffer and 1.7% NaClO. However, extensive alkali uses and longer treatment times for lignin removal in cellulose isolation impose significant environmental stress and require improvement (Liu et al., 2021).

The use of enzymes in the textile industry offers several advantages over other chemicals. Enzymes are highly specific in their catalytic activity, allowing them to perform precise chemical reactions without affecting other molecules (Bilal et al., 2019). This enables the production of exceptionally pure and high-quality products. Enzymes can accelerate reactions much more efficiently than chemical catalysts and often require less energy. They frequently operate under mild conditions, such as low temperatures and neutral pH levels (Zhao et al., 2017). This can be particularly beneficial for sensitive compounds that may be damaged under aggressive conditions. Enzymes are biocatalysts derived from living organisms, typically microorganisms that can be cultivated and replenished (Robinson, 2015). They are not finite resources like many chemical catalysts, making them a more sustainable option. Enzymes are often cheaper to produce than chemical catalysts and can be used in low concentrations, leading to significant cost savings for biomass processing and other industrial processes (Perez et al., 2002). In the textile industry, a variety of enzymes

are used to enhance textile products. However, when it comes to fiber isolation, it has been determined that laccases, pectinases and peroxidases have the potential to be effective (Perez et al., 2002; Schimpf et al., 2013). These enzymes are produced by certain types of fungi and bacteria and can degrade lignin and pectin. They work in tandem to break down lignin polymer into smaller, more easily degradable fragments. It's important to note that the best enzymes for lignin degradation are peroxidases and laccases, produced by white-rot fungi, while the most effective enzyme for pectin degradation is pectinase, produced by filamentous fungi or molds (Perez et al., 2002; Shrestha et al., 2019). Except the use of novelty technologies, it is important to search for sustainable raw materials (Khadaka, 2021).

Due to its stem structure, it is soft woody herbaceous Virginia mallow (*Sida hermaphrodita*) is soft woody herbaceous energy crop (Banks et al., 2020). Because it is a fast-growing plant, and it can regrow after multiple cuttings and has a high yield potential it has attracted a lot of interest for research. Virginia mallow is ideally cultivated in less favorable farming land to avoid competing with conventional food plants, as it thrives in less fertile soils. Its adaptability extends to various climates, allowing growth on eroded slopes, chemically degraded areas, and even on dumps and landfills (Borkowska, 2012; Banks et al., 2020). Additionally, *Sida hermaphrodita* has a wide range of applications such as paper industry, fodder, textile industry, biocomposites production, phytoremediation, and honey production (Nahm & Morhart, 2018; Szwaja et al., 2019). Since the textile industry is in constant search for sustainable cultures to reduce pollution, unconventional textile cultures are being imposed. Virginia mallow stands out as an unconventional culture to produce textile fibers. Extracting the fibers from the Virginian mallow leaves a solid residue that can be disposed of, i.e., used to produce biofuel.

The aim of this paper is to examine the energy characteristics of the residual biomass of Virginia mallow from the process of fiber isolation using enzymes.

Materials and methods

Virginia mallow was harvested in November 2022 at the experimental field (Figure 1) of University of Zagreb Faculty of Agriculture. Biomass was roughly chopped to an average length of 5 cm and subjected to pretreatment with 5 % sodium hydroxide (NaOH), followed by enzymatic treatment. Enzymatic treatment (Figure 2) was carried out using proteases enzyme at three concentrations: 1%, 0.5%, and 0.05%, for a duration of 48 hours. Also, another sample was made with same pretreatment, but treatment was carried out with (H₂O) water. After enzymatic treatment, manual and mechanical cleaning was performed to separate the fibers from the residue. The residue obtained from fiber isolation (Figure 3) is subjected to research on energy characteristics. After air drying, residues were homogenized and crushed in a laboratory mill (IKA MF 10 basic, Germany). Proximate analysis determined the moisture content (HRN EN 18134-2:2015) using a laboratory dryer (INKO, Croatia), ash (HRN EN ISO 18122:2015) using a muffle furnace (Nabertherm, USA) and the content of fixed carbon (C_{fix}) and volatile matter (EN 15148:2009) which were calculated by calculation. Calorific value was determined using the EN 14918:2010 method using an adiabatic calorimeter (C6000, IKA Analysentechnik GmbH, Heitersheim, Germany). The calorific value is expressed in MJ/kg. Control sample was made for energy characteristics research and that is raw biomass from Virginia mallow.



Figure 1 Virginia mallow



Figure 2 Enzymatic treatment



Figure 3 Fiber residue

Results and discussion

Using various methods, the results for proximate analysis and higher heating value were obtained. Proximate analysis shows values for moisture content, ash, volatile matter, fixed carbon, and coke. All values are measured on dry matter.

Table 1. Proximate analysis of Virginia mallow residue

Sample	MC %	Ash %	VM %	Cfix %	Coke %	HHV MJ/kg
I-E1	7.96a ± 0.04	4.02a ± 0.13	78.14c ± 0.27	9.86a ± 0.21	13.90a ± 0.28	17.49a ± 0.003
II-E05	7.76a ± 1.46	2.52c ± 0.05	81.13b ± 2.22	8.58a ± 1.33	11.10b ± 1.31	17.45ab ± 0.01
III-E005	8.04a ± 0.02	3.00b ± 0.03	82.23ab ± 0.83	6.65b ± 0.83	9.66c ± 0.85	17.37b ± 0.12
IV-H2O	7.77a ± 0.02	2.47c ± 0.38	82.97a ± 1.51	6.79b ± 1.51	9.26c ± 1.23	17.36b ± 0.09
V-control	7.35a ± 0.03	2.68c ± 0.03	84.12a ± 0.05	9.18a ± 0.05	9.63c ± 0.02	17.23c ± 0.02

*MC – moisture content, VM – volatile matter, Cfix – fixed carbon, HHV – higher heating value; ± standard deviation, means followed by the different letter within the column are statistically different ($p < 0.05$).

Results from proximate analysis in table 1. shows that moisture content is highest in sample treated with enzymes III-E005 and the lowest in control sample, but there is no significant statistical difference. Compared to research that was based on fast pyrolysis, 6.41% was moisture content (Banks et al., 2020), in research by Szwaja (2019), in raw biomass moisture content was, 5.14%. Ash is undesirable content in biomass because is not combustible. High ash content can lead to reduction of fuel energy content (Bilandžija, 2018). Lowest ash content in this research, 2.47% is found in residue treated with water, and the highest, 4.02 % is the one treated with highest enzyme percent (I-E1). Statistical analysis shows that there is significant difference between I-E1 and II-E005 compared to other samples. In research by Banks et al. (2020), ash content was, 2.11%, ash content in research Szwaja et al., (2019) was, 1,71%.

Volatile matter, components that are released during burning at high temperature. The lowest, 78.14 % is found in residue with highest enzyme content (I-E1), and the highest, 84.12 % is in the control group, while, 79.74 % was found in research by Banks et al. (2020). Statistically there is a difference between samples. Research Szwaja et al., (2019) shows volatile matter of, 84.04% in raw biomass. Higher values of volatile matter indicate better combustion properties. Also, high fixed carbon content indicates a higher energy content, it shows quantity of carbon trapped in residues. In their research Banks et al. (2020), 18.15% was value of fixed carbon. Statistical data analysis suggests that there are significant differences between samples. While in this research the highest value is, 9.86% and it belongs to I-E1, the lowest value for fixed carbon is found in residue treated with enzyme III-E005. Coke content indicates positive properties because it represents quantity of energy released by combustion. Lowest vale for coke is, 9.26 % in residue treated with water and highest one, 13.90 % is in I-E1, and they have statistical difference between themselves.

Heating value is essential parameter for assessing energy characteristics. Heating value or calorific value is the amount of energy released during combustion, the highest HHV in this research is in the sample treated with enzyme, specifically I-E1 sample. Most of the samples are statistically different. In research by Banks et al. (2020), higher heating value was, 19.13 MJ/kg. Šiaudinis et al. (2015), did a research based on pellets from *Sida hermaphrodita*, 17.49 – 18.44 MJ/kg were a net calorific value.

Conclusion

Based on the research findings, the higher heating values (HHV) for all examined samples fall within acceptable ranges. The biomass residues obtained from enzymatic fibre isolation shows consistent HHV values, with slight variations noted in the E1 sample (containing a 1% enzyme solution). While there are subtle differences in this analysis, they do not raise significant concerns regarding the overall suitability of these biomass residues for various applications.

Acknowledgments

The research has received funding from the European Regional Development Fund via K.K.01.1.1.04.0091 project “Design of Advanced Biocomposites from Renewable Energy Sources – BIOCOMPOSITES”.

“Projekt razvoja karijera mladih istraživača – izobrazba novih doktora znanosti” (DOK-2020-01)

References

- Athinarayanan J., Alshatwi A.A., Periasamy V.S. (2020). Biocompatibility analysis of *Borassus flabellifer* biomass-derived nanofibrillated cellulose. *Carbohydrate Polymers*. 235: 115961.
- Banks S.W., Śnieg M., Nowakowski D.J., Stolarski M., Bridgwater A.V. (2021). Potential of Virginia mallow as an energy feedstock. *Waste and Biomass Valorization*. 12: 2375-2388.
- Bilal M., Zhao Y., Noreen S., Shah S.Z.H., Bharagava R.N., Iqbal H.M. (2019). Modifying biocatalytic properties of enzymes for efficient biocatalysis: A review from immobilization strategies viewpoint. *Biocatalysis and Biotransformation*. 37: 159-182.
- Bilandžija N., Krička T., Matin A., Leto J., Grubor M. (2018). Effect of harvest season on the fuel properties of *Sida hermaphrodita* (L.) Rusby biomass as solid biofuel. *Energies*. 11: 3398.
- Borkowska H., and Molas R. (2012). Two extremely different crops, *Salix* and *Sida*, as sources of renewable bioenergy. *Biomass Bioenergy*. 36: 234–240.
- Corona B., Shen L., Reike D., Carreón J.R., Worrell E. (2019). Towards sustainable development through the circular economy—A review and critical assessment on current circularity metrics. *Resources, Conservation and Recycling*. 151: 104498.
- Khadka R. (2021). The use of fibrous plants in the production of building materials. In *Proceedings of the International Scientific Conference “Rural Development”* (pp. 101-105).
- Liu Q., He W.Q., Aguedo M., Xia X., Bai W.B., Dong Y.Y., Song J.Q., Richel A., Goffin D. (2021). Microwave-assisted alkali hydrolysis for cellulose isolation from wheat straw: Influence of reaction conditions and non-thermal effects of microwave. *Carbohydrate Polymers*. 253: 117170.
- Nahm M., and Morhart C. (2018). Virginia mallow (*Sida hermaphrodita* (L.) Rusby) as perennial multipurpose crop: Biomass yields, energetic valorization, utilization potentials, and management perspectives. *Gcb Bioenergy*. 10: 393-404.
- Pérez J., Muñoz-Dorado J., De la Rubia T.D.L.R., Martínez J. (2002). Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview. *International microbiology*. 5: 53-63.
- Phuong L.N.T. (2003). The status and environmental problems of textile industry in Vietnam. In *Environmental Governance in Asia: New State-Society Relations*, INREF-AGITS Conference, Chiang Mai, 10-12 October 2003 (pp. 245-258).
- Robinson P.K. (2015). *Enzymes: principles and biotechnological applications*. *Essays in biochemistry*. 59: 1.
- Sánchez R., Espinosa E., Domínguez-Robles J., Loaiza J.M., Rodríguez A. (2016). Isolation and characterization of lignocellulose nanofibers from different wheat straw pulps. *International journal of biological macromolecules*. 92: 1025-1033.
- Schimpf U., Hanreich A., Mähner P., Unmack T., Junne S., Renpenning J., Lopez-Ulibarri R. (2013). Improving the efficiency of large-scale biogas processes: pectinolytic enzymes accelerate the lignocellulose degradation. *Journal of Sustainable Energy and Environment*. 4: 53-60.
- Shrestha S., Kognou A.L.M., Zhang J., Qin W. (2021). Different facets of lignocellulosic biomass including pectin and its perspectives. *Waste and biomass valorization*. 12: 4805-4823.
- Szwaja S., Magdziarz A., Zajemska, M., Poskart A., Musiał D. (2019). Virginia Mallow as an energy crop-current status and energy perspectives. In *IOP Conference Series: Earth and Environmental Science*. 214: 012078.
- Šiaudinis G., Jasinskas A., Šarauskis E., Steponavičius D., Karčauskienė D., Liaudanskienė I. (2015). The assessment of Virginia mallow (*Sida hermaphrodita* Rusby) and cup plant (*Silphium perfoliatum* L.) productivity, physico-mechanical properties and energy expenses. *Energy*. 93: 606-612.
- Zhao X., Liu W., Deng Y., Zhu J.Y. (2017). Low-temperature microbial and direct conversion of lignocellulosic biomass to electricity: Advances and challenges. *Renewable and Sustainable Energy Reviews*. 71: 268-282.

Primjena tehnologije udvojenih redova u sjetvi suncokreta

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Sažetak

Istraživanja su provedena na pokušalištu FAZOS-a sjetvom četiri hibrida suncokreta 21. travnja 2020. godine, u optimalnom agrotehničkom roku. Standardna sjetva obavljena je pneumatskom sijačicom OLT PSK4 na razmak redova 70 cm, a sjetva u *Twin Row* tehnologiji sijačicom MaterMacc *Twin Row-2* s razmakom udvojenih redova od 22 + 48 cm. U standardnoj sjetvi hibrid suncokreta NK *Neoma CL* u sklopu 65 675 biljaka ha⁻¹ ostvario je prinos suhog zrna od 4 145 kg ha⁻¹, a u sjetvi u udvojene redove ostvaren je prinos suhog zrna od 5 140 kg ha⁻¹ odnosno + 19,36 % više u odnosu na standardnu sjetvu. Hibrid *Sumiko HTS* ostvario je prinos suhog zrna od 3 772 kg ha⁻¹, a kod sjetve u udvojene redove 4 357 kg ha⁻¹ s prosječnom vlagom u berbi od 8,72 %. Slične rezultate ostvario je i hibrid SY *Diamantis CL*. Kod standardne sjetve hibrid suncokreta SY *Ivori CLP* ostvario je prinos zrna od 3 684 kg ha⁻¹, a kod sjetve u udvojene redove 4 649 kg ha⁻¹ s prosječnom vlagom u berbi od 9,94 %.

Ključne riječi: suncokret, sjetva, prinos, udvojeni redovi, sijačica

Uvod

Sjetva suncokreta na našem prostoru obavlja se na razmak redova od 70 ili 75 cm. Međutim već nekoliko godina provode se i znanstvena istraživanja sjetve suncokreta u udvojene redove, u svijetu poznata kao *twin row* tehnologija. Ovakva tehnologija sjetve omogućava bolji raspored biljaka unutar reda te veće iskorištenje sunčeve svjetlosti, hraniva i vlage iz tla i na većini površina doprinosi ostvarenju jednakog ili većeg prinosa zrna po hektaru. Standardnim sustavom sjetve suncokreta s razmakom redova od 70 cm može se zasijati 142 reda dužine 100 m. Primjenom tehnologije *twin row* s razmakom udvojenih redova od 22 cm može se posijati 284 reda dužine 100 m. Na tržištu poljoprivredne tehnike mogu se pronaći sijačice s razmacima udvojenih redova od 20, 22 ili 25 cm, a središnji razmak susjednih udvojenih redova iznosi 70 ili 75 cm tako da je obavljanje berbe moguće i sa standardnim beračima za suncokret. Sjetva u udvojene redove u SAD-u je u potpunosti razvijena, dok se kod nas *twin row* tehnologija obavlja na vrlo malim površinama prvenstveno u znanstvene svrhe. Upravo i cilj istraživanja bio je utvrditi razliku u visini prinosa pri sjetvi suncokreta u udvojene redove (*twin row*) i klasične sjetve.

Materijal i metode

Lokacija postavljenog istraživanja i osnovni podaci o sjetvi

Istraživanje je obavljeno na pokušalištu Fakulteta agrobiotehničkih znanosti Osijek – Tenja (45°31'1,83" s. z. š./18°46'37,5" i. z. d. - k. o. Tenja; kč. 1308 i 1309). Sjetva 4 hibrida suncokreta obavljena je 21. travnja 2020. u optimalnom agrotehničkom roku. Standardna sjetva na razmak redova od 70 cm obavljena sijačicom PSK4 OLT, a sjetva u udvojene redove (*Twin Row*), sijačicom MaterMacc *Twin Row-2*.

Opis tipa tla i kemijska analiza tla

Na pokušalištu *Tenja* prevladava eutrično smeđe tlo, koje pripada odjelu automorfnih tala, klasi kambičnih tala. Prema teksturi tlo pripada u praškaste ilovače te je malo porozno, s osrednjim kapacitetom tla za vodu u oraničnome i podoraničnom horizontu (Banaj, 2020). Tlo je alkalno u svim horizontima (pH u vodi 8,44 i KCl 7,55) s dosta humoznim oraničnim slojem (3,71 %). Sadržaj fosfora iznosio je 15,58 mg (umjerena opskrbljeno tlo), a kalija 24,29 mg na 100 g tla (dobro opskrbljeno tlo).

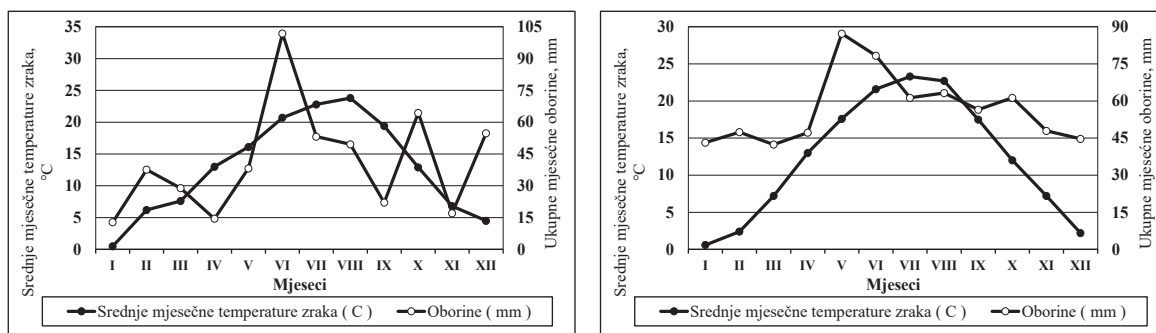
Klimatske prilike u vrijeme istraživanja

Klimatske prilike u vrijeme provođenja istraživanja u 2020. godini nisu značajnije odstupale od vrijednosti višegodišnjeg prosjeka. U Tablici 1. prikazane su srednje mjesečne temperature zraka i ukupne mjesečne količine oborina (mm) za vegetacijsku 2020. godinu.

Tablica 1. Klimatski uvjeti u 2020. godini izmjereno na GMP Osijek – aerodrom

Mjesec	Srednje mjesečne temperature zraka (°C)		Oborine (mm)	
	2020.	1991. - 2020.	2020.	1991. - 2020.
I.	0,5	0,6	12,8	43,2
II.	6,2	2,4	37,6	47,4
III.	7,6	7,2	28,9	42,4
IV.	13,0	13,0	14,5	47,2
V.	16,1	17,6	38,2	87,2
VI.	20,7	21,6	101,8	78,3
VII.	22,8	23,3	53,2	61,3
VIII.	23,8	22,7	49,6	63,2
IX.	19,4	17,5	22,1	56,5
X.	12,9	12	64,4	61,3
XI.	6,8	7,2	17,1	47,9
XII.	4,5	2,2	54,8	44,7
Ukupno IV.-IX.	19,3	19,3	279,4	393,7
Ukupno I.-XII.	12,9	12,3	495,0	680,6

Izvor: DHMZ (2021.); IV.-IX. – Vegetacija suncokreta



Grafikon 1. Klima dijagram prema Walteru za 2020. (lijevo) i 1991. - 2020. godine (desno)

Iz Tablice 1. i Grafikona 1. vidljivo je da je u prvom mjesecu 2020. godine nešto niža srednja mjesečna temperatura, a nakon toga zabilježeno je razdoblje s povećanim prosječnim srednjim mjesečnim temperaturama u odnosu na

višegodišnji prosjek u veljači i ožujku. U ta dva mjeseca zabilježena je razlika prosječne srednje temperature zraka u 2020. godini u odnosu na višegodišnji prosjek od + 3,8 °C. Ukupna zabilježena količina oborina u prva tri mjeseca bila je samo 59,63 % u odnosu na višegodišnjeg prosjek. Razdoblje od travnja do kolovoza bilo je po temperaturnim pokazateljima hladnije za -2,9 °C sa 193,2 litre oborina što je 14,81 % manje od višegodišnjeg prosjeka. U kolovozu i rujnu zabilježene su nešto više prosječne mjesečne temperature, posebice u rujnu (19,4 °C) što predstavlja povećanje za 2,1°C u odnosu na višegodišnji prosjek (17,5 °C). U pogledu količine oborina u kolovozu i rujnu zabilježena je manja količina oborina za 48,0 mm odnosno za 40,1 % u odnosu na višegodišnji prosjek. Ukupna količina oborina u vegetacijskom razdoblju iznosila je svega 279,4 mm ili 70,97 % u odnosu na višegodišnji prosjek. U razdoblju vegetacije suncokreta prosjek prosječnih mjesečnih temperatura u 2020. godini i višegodišnjem prosjeku iznosio je 19,3 °C. Autori Milošević i sur. (2015) te González i sur. (2013) navode da su prinosi zrna suncokreta te sadržaj ulja u zrnu u jakoj povezanosti s oborinama i temperaturama zraka tijekom perioda vegetacije. Pospišil (2008) konstatira da sjetva kasnih hibrida u pregustom sklopu rezultira s manjim glavama, manjim sjemenkama i manjim brojem sjemenki, a sjetva u rjeđim sklopovima stvara veće mogućnosti za razvoj bolesti i polijeganje. Navedenu tvrdnju opovrgavaju Ali i sur. (2012) gdje navode da je veći prinos zrna ostvaren s razmakom zrna unutar reda od 20 cm (3 470 kg ha⁻¹) za razliku s razmakom od 30 cm (2 690 kg ha⁻¹).

Rezultati i rasprava

Tablica 2. Utvrđeni sklop biljaka po ha⁻¹ kod ispitivanih hibrida u vrijeme žetve

Hibrid suncokreta	Sklop biljaka po ha ⁻¹ u vrijeme berbe suncokreta					
	Standardna tehnologija (70 cm) – teorijski sklop 70 580 (razmak u sjetvi 20,12 cm)			Tehnologija udvojenih redova (22*48 cm) – teorijski sklop 70 297 (razmak u sjetvi 40,40 cm)		
	\bar{x}	σ	KV (%)	\bar{x}	σ	KV (%)
<i>NK Neoma CL</i>	65 675	3734,537	5,69	64 610	4628,997	7,16
<i>Sumiko HTS</i>	61 202	3489,850	5,70	62 267	3504,265	5,63
<i>SY Diamantis CL</i>	62 906	4086,048	6,50	64 539	2627,320	4,07
<i>SY Ivori CLP</i>	60 279	3387,228	5,62	65 533	5747,639	8,77

Tablica 3. Ostvareni prinosi zrna (svedeno na vlagu 9 %) kg ha⁻¹ i vlaga zrna suncokreta kod standardne (S) i tehnologije sjetve u udvojene redove (TR) u vrijeme žetve

Hibrid suncokreta - sustav sjetve	Prinos zrna kg ha ⁻¹			Vlaga zrna (%)		
	\bar{x}	σ	KV (%)	\bar{x}	σ	KV (%)
<i>NK Neoma CL</i> (S)	4 145	182,899	4,41	9,28	0,277	2,99
<i>NK Neoma CL</i> (TR)	5 140	337,910	6,57	9,16	0,182	1,98
<i>Sumiko HTS</i> (S)	3 772	134,638	3,57	9,04	0,351	3,88
<i>Sumiko HTS</i> (TR)	4 357	376,525	8,64	8,72	0,259	2,97
<i>SY Diamantis CL</i> (S)	4 228	336,037	7,95	9,86	0,416	4,22
<i>SY Diamantis CL</i> (TR)	5 172	892,676	17,26	9,50	0,255	2,68
<i>SY Ivori CLP</i> (S)	3 684	165,190	4,48	10,32	0,581	5,63
<i>SY Ivori CLP</i> (TR)	4 649	347,364	7,47	9,94	0,321	3,23

S = standardna sjetva, *TR* = sjetva u udvojene redove

Iz Tablice 2. može se zaključiti da je u vrijeme berbe zabilježeno kod sva 4 hibrida smanjenje sklopa. Kod standardne sjetve između 4 905 do 10 301 biljaka, a kod tehnologije *twin row* u rasponu od 4 764 do 8 030 u odnosu na teorijski sklop biljaka ha⁻¹. Autori Banaj i sur. (2018) navode da od planiranog teorijskog sklopa pri sjetvi do berbe nedostaje

od 8 do 13 000 biljaka ha⁻¹. Standardnom sjetvom (Tablica 2. i Tablica 3.) hibrida suncokreta *NK Neoma CL*, ostvaren je prosječan sklop od 65 675 biljaka ha⁻¹ s prosječnim prinosom od 4 145 kg ha⁻¹. Dobiveni prinos je za -19,36 % niži od prinosa dobivenog u *twin row* sjetvi (5 140 kg ha⁻¹) s prosječnom vlažnošću zrna u vrijeme berbe od 9,16 %. Slične rezultate navode Blandino i sur. (2013) gdje su zabilježili da je na 66 % ispitivanih lokacija došlo do povećanja uroda zrna u sjetvi u udvojenim redovima u prosjeku 5,5 %. Kod hibrida suncokreta *Sumiko HTS*, standardnom sjetvom ostvaren je sklop u vrijeme berbe 61 202 biljaka ha⁻¹, s prinosom zrna od 3 772 kg ha⁻¹. To je za -13,43 % manje u odnosu na *twin row* sjetvu. Prosječna vlaga zrna kod standardne sjetve u vrijeme berbe iznosila je 9,04 %, a kod *twin row* sjetve 8,72 %. Kod *twin row* sjetve hibrida suncokreta *SY Diamantis CL*, pri sklopu od 64 539 biljaka ha⁻¹, ostvaren je prinos od 5 172 kg ha⁻¹ s prosječnom vlagom zrna u berbi od 9,50 %. Dobiveni prinos je za +18,25 % viši od prinosa ostvarenoga standardnom sjetvom. Kod hibrida suncokreta *SY Ivori CLP*, prinos zrna ostvaren sjetvom u udvojene redove bio je veći za +20,76 % odnosno +965 kg zrna ha⁻¹. Kod istog hibrida suncokreta, u standardnoj sjetvi ostvaren je sklop u vrijeme berbe od 60 279, a kod sjetve u udvojene redove 65 533 biljaka ha⁻¹. Zarea i sur. (2005) su istraživali utjecaj različitih tehnika sjetve i međurednog razmaka odnosno sklopa na prinos zrna suncokreta. Autori su utvrdili da se smanjenjem razmaka između redova povećava prinos. Također, rezultati su pokazali da su najveće prinose imali suncokreti posijani *twin row* sjetvom u cik-cak obliku pri sklopu od 8 biljaka m². Slična istraživanja u južnoj Rumunjskoj su proveli Ion i sur. (2015., 2018). Diepenbrock i sur. (2001) koji su zaključili da se optimalnim rasporedom biljaka na površini bolje iskorištava i vlaga u tlu, budući da biljke suncokreta imaju nerazgranati, vretenasti korijen sa samo jednom centralnom osi.

Zaključak

Na temelju dobivenih saznanja mogu se donijeti sljedeći zaključci;

- Temeljem meteoroloških podataka, prvenstveno promatrajući srednje mjesečne količine oborina možemo zaključiti da je u periodu vegetacije suncokreta u 2020. godini, na području pokušališta *Tenja* nedostajalo 114,3 mm oborina
- U standardnoj sjetvi s pneumatskom sijačicom *OLT PSK-4* hibrid suncokreta *NK Neoma CL* u sklopu 65 675 biljaka ha⁻¹ nakon nicanja ostvario je prinos naturalnog zrna od 4 145 kg ha⁻¹ s prosječnom vlažnošću u berbi od 9,28 %.
- Sjetvom istog hibrida u udvojene redove ostvaren je sklop od 64 610 biljaka ha⁻¹ s prinosom suhog zrna od 5 140 kg ha⁻¹ s prosječnom vlažnošću u vrijeme berbe od 9,16 % što čini povećanje u odnosu na standardnu sjetvu od +19,36 %.
- Standardnom sjetvom hibrid suncokreta *Sumiko HTS* ostvario je prinos suhog zrna od 3 772 kg ha⁻¹, a kod sjetve u udvojene redove 4 357 kg ha⁻¹ s prosječnom vlagom u berbi od 8,72 %.
- Kod hibrida suncokreta *SY Diamantis CL* pri standardnoj sjetvi u sklopu biljaka 62 906 ha⁻¹ ostvaren je prinos od 4 228 kg ha⁻¹, a u sjetvi u udvojene redove od 5 172 kg ha⁻¹.
- Standardnom sjetvom hibrid suncokreta *SY Ivori CLP* ostvario je prinos suhog zrna od 3 684 kg ha⁻¹, a kod sjetve u udvojene redove 4 649 kg ha⁻¹ s prosječnom vlagom u berbi od 9,94 %.

Literatura

- Ali A., Ahmad A., Khaliq T., Akhtar J. (2012). Planting density and nitrogen rates optimization for growth and yield of sunflower (*Helianthus Annuus L.*) hybrids. *The Journal of Animal & Plant Sciences*. 22: 1070-1075.
- Banaj A. (2020). Kvaliteta rada pneumatskih sijačica s podtlakom pri različitim sustavima sjetve kukuruza. Doktorska disertacija, Fakultet agrobiotehničkih znanosti Osijek, Sveučilište Josipa Jurja Strossmayera.
- Banaj A., Banaj Đ., Tadić V., Petrović D., Knežević D. (2018). Usporedba standardne i twin row sjetve suncokreta s obzirom na prinos. In N. Bilandžija (Ed.), *Proceedings of the 46th International Symposium - Actual Tasks on Agricultural Engineering* (pp. 79-88). Sveučilište u Zagrebu, Agronomski fakultet, Zavod za mehanizaciju poljoprivrede.
- Blandino M., Amedeo R., Giulio T. (2013). Aumentare la produttività del mais con alti investimenti e file binate. Un test in dodici località vocate conferma la validità delle nuove agrotecniche. *Terra e Vita, Tecnica e Tecnologia*. 7.

- Diepenbrock W., Long M., Feil B. (2001). Yield and quality of sunflower as affected by row orientation, row spacing and plant density. *Die Bodenkultur*. 52: 29-36.
- González J., Mancuso N., Ludueña P. (2013). Sunflower yield and climatic variables. *Helia*. 36: 69-76.
- Ion V., Băşa A.G., Dumbravă M., Epure, L.I. (2018). Results regarding yield components and grain yield at sunflower under different row spacing and nitrogen fertilisation conditions. *Scientific Papers. Series A. Agronomy*. 61: 247-254.
- Ion V., Dicu G., Basa A.G., Dumbrava M., Temocico G., Epure L.I., State D. (2015). Sunflower Yield and Yield Components under Different Sowing Conditions. *Agriculture and Agricultural Science Procedia*. 6: 44-51.
- Milošević D., Savic S.M., Stojanovic V., Popov-Raljic J. (2015). Effects of precipitation and temperatures on crop yield variability in Vojvodina (Serbia). *Italian Journal of Agrometeorology - Rivista Italiana di Agrometeorologia*. 20: 35-46.
- Pospišil M. (2008). Sjetva suncokreta. *Glasnik zaštite bilja*. 4: 95 - 100.
- Zarea M. J., Ghalavand A., Daneshian J. (2005). Effect of planting patterns of sunflower on yield and extinction coefficient. *Agronomy for sustainable development*. 25: 513-51.

Application of twin row technology in sunflower sowing

Abstract

Research was carried out at the FAZOS experimental field by sowing four sunflower hybrids on April 21, 2020, in the optimal agrotechnical period. Standard sowing was done with a pneumatic sowing machine *OLT PSK-4* at a row spacing of 70 cm, and sowing in *twin row* technology with a *MaterMacc Twin Row-2* sowing machine with a double row spacing of 22 + 48 cm. In standard sowing, the sunflower hybrid *NK Neoma CL* in a set of 65 675 plants ha⁻¹ achieved a natural grain yield of 4 145 kg ha⁻¹, and in twin row sowing, a dry grain yield of 5 140 kg ha⁻¹ was achieved, i.e. + 19,36 % more compared to standard sowing. The hybrid *Sumiko HTS* achieved a dry grain yield of 3 772 kg ha⁻¹ and when sown in twin rows achieved 4 357 kg ha⁻¹ with an average moisture content in harvest of 8,72 %. Similar results were achieved by the *SY Diamantis CL* hybrid. When standard sowing, the sunflower hybrid *SY Ivori CLP* achieved a grain yield of 3 684 kg h⁻¹, and when sowing in twin rows, 4 649 kg ha⁻¹ with an average moisture content in harvest of 9,94 %.

Keywords: sunflower, grain yield, twin rows, sowing machine

Primjena MaterMacc Twin Row–2 sijačice u sjetvi soje

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Sažetak

Prikazani su rezultati komparativnog istraživanja utjecaja načina sjetve četiri kultivara soje na pokušalištu *Tenja*. Sjetva u udvojene redove obavljena je na razmak 48 x 22, a standardna korištenjem pneumatske sijačice *PSK-4* na razmak redova od 70 cm. U standardnoj sjetvi kultivar soje *APSSB 21* u sklopu 506 017 biljaka ha⁻¹ ostvario je prinos suhog zrna od 4 115 kg ha⁻¹, a u sjetvi u udvojene redove ostvaren je prinos suhog zrna od 4 778 kg ha⁻¹ odnosno +16,11 % više u odnosu na standardnu sjetvu. Kultivar *APSSB 30* ostvario je prinos suhog zrna od 4 118 kg ha⁻¹, a kod sjetve u udvojene redove 4 878 kg ha⁻¹ s prosječnom vlagom 8,32 %. Slični rezultati zabilježeni su i kod kultivara *APSSB 45*. Pri sjetvi u standardne redove kultivar soje *ANNIKA* ostvario je prinos zrna od 4 054 kg ha⁻¹, a kod sjetve u udvojene redove 4 829 kg ha⁻¹ s prosječnom vlagom 8,86 %.

Ključne riječi: Soja, sjetva u udvojene redove, twin row sijačica, prinos

Uvod

Proizvodnja soje u periodu od 2014. do 2019. godine u svijetu (FAOSTAT, 2021) iznosila je između 117 i 121 milijun hektara s prosječnim prinosom od 2,60 do 2,86 t ha⁻¹. Najveći udio ukupne proizvodnje soje nalazi se u Sjevernoj i Južnoj Americi. Najveći svjetski proizvođači (FAOSTAT, 2021) soje su: SAD, Brazil, Argentina, Kina, Indija, Paragvaj, Kanada, Ukrajina, Rusija i Bolivija s ukupnom proizvodnjom od 94,48 % ukupne svjetske proizvodnje. U RH soja se sije u rasponu od 80 000 (2019) do 84 000 ha⁻¹ (2020) s prosječnim prinosima zrna od 2,2 do 3,2 t ha⁻¹ ovisno o meteorološkim pogodnostima u godini proizvodnje. U našim agroekološkim uvjetima, soja se može uzgajati u redovnim rokovima sjetve ili kao postrni usjev. Sjetva soje na prostorima Republike Hrvatske obavlja se na više načina. Najzastupljenija sjetva je žitnim sijačicama sa sjetvom svakog drugog reda, kao i sjetva pneumatskim sijačicama na međuredni razmak od 50 ili 70 cm. Sjetva soje u udvojene redove zastupljena je vrlo malo i prvenstveno je sastavni dio znanstvenih istraživanja. Cilj istraživanja bio je utvrditi razliku u visini prinosa sjetvom soje u udvojene redove (*Twin Row*) i sjetve soje na međuredni razmak od 70 cm.

Materijal i metode

Lokacija postavljenog istraživanja i osnovni podaci o sjetvi

Istraživanje utjecaja sustava sjetve na prinos zrna kg ha⁻¹ pri sjetvi četiri kultivara soje obavljeno je na pokušalištu Fakulteta agrobiotehničkih znanosti Osijek – *Tenja* (45°31'1,83" s. z. š./18°46'37,5" i. z. d. - k. o. *Tenja*; kč. 1308 i 1309). Sjetva 4 kultivara soje obavljena je 28. travnja 2021. u optimalnom agrotehničkom roku. Pokušna sjetva (standardna) na razmak redova od 70 cm obavljena sijačicom *PSK4 OLT*, a sjetva u udvojene redove (*Twin Row*), sijačicom *MaterMacc Twin Row-2*. Sijačica *PSK-4* bila je podešena na teorijsku sjetvu od 582 445 biljaka ha⁻¹ odnosno pri korištenju sjetvene ploče $n=120$ otvora \varnothing 4 mm ostvaren je razmak između biljaka od 2,438 cm u redu pri prijenosnim odnosom ($n_{kopač} - n_{sjetvena\ ploča}$) $i = 0,6667$. Teorijski broj biljaka po dužnom metru iznosio je 44,44 biljaka. Standardna sjetva obavljena je na razmak redova od 70 cm odnosno 142 reda ha⁻¹, a u *twin row* tehnologiji ostvareno je 284 reda ha⁻¹ s razmakom udvojenih redova od 22 cm. Za sjetvu u udvojene redove korištena je sijačica *MaterMacc Twin Row 2*, a sjetva je obavljena upotrebom sjetvene ploče $n=72$ otvora \varnothing 3,5 mm s prijenosnim odnosom ($n_{kopač} -$

$n_{\text{sjetvena ploča}} = 0,5296$. Kod ovog prijenosnog odnosa ostvaren je teorijski razmak od 4,760 cm. Na ovaj način ostvaren je teorijski sklop od 596 687 biljaka ha^{-1} . Kod ovog prijenosnog odnosa $i=0,5296$, po dužnom metru, teorijski broj biljaka iznosio je 23,11.

Opis tipa tla i kemijske analize tla

Na pokušalištu *Tenja* prevladava eutrično smeđe tlo, koje pripada odjelu automorfnih tala, klasi kambičnih tala. Prema teksturi tlo pripada u praškaste ilovače te je malo porozno, s osrednjim kapacitetom tla za vodu u oraničnome i podoraničnom horizontu (Banaj i sur., 2020). Tlo je alkalno u svim horizontima (pH u vodi 8,44 i KCl 7,55) s dosta humoznim oraničnim slojem (3,71 %). Sadržaj fosfora iznosio je 15,58 mg (umjereno opskrbljeno tlo), a kalija 24,29 mg na 100 g tla (dobro opskrbljeno tlo).

Klimatske prilike u vrijeme istraživanja

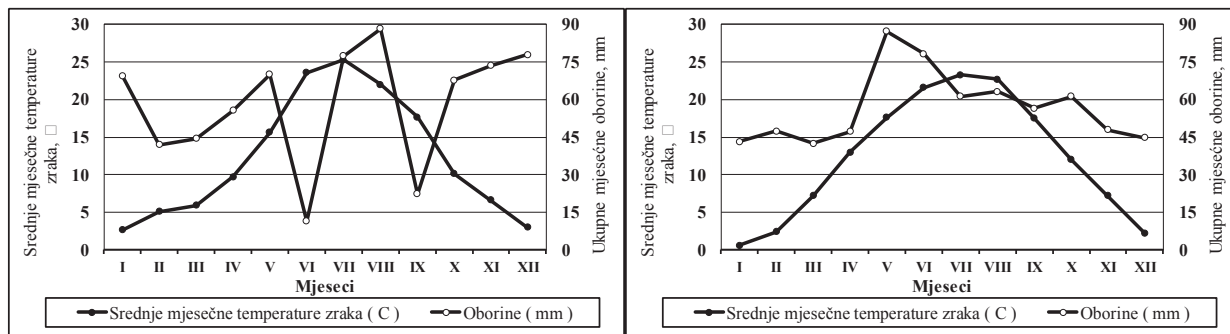
Klimatske prilike u vrijeme provođenja istraživanja u 2021. godini kao i vrijednosti višegodišnjih prosjeka temperatura i oborina prikazani su u Tablici 1. i Walterovim klimagramom (Grafikon 1.) za GMP Osijek – aerodrom (45°27'58" N, 18°48'28" E).

Tablica 1. Vrijednosti višegodišnjih prosjeka temperatura i oborina u 2021. godini izmjereni na GMP Osijek – aerodrom

Mjesec	Srednje mjesečne temperature zraka (°C)		Oborine (mm)	
	2021.	1991 - 2020.	2021.	1991 - 2020.
I.	2,6	0,6	69,6	43,2
II.	5,1	2,4	41,9	47,4
III.	5,9	7,2	44,4	42,4
IV.	9,7	13	55,7	47,2
V.	1,6	17,6	70,1	87,2
VI.	23,6	21,6	11,4	78,3
VII.	25,3	23,3	77,5	61,3
VIII.	22,0	22,7	88,3	63,2
IX.	17,7	17,5	22,2	56,5
X.	10,1	12	67,7	61,3
XI.	6,6	7,2	73,5	47,9
XII.	3,0	2,2	78	44,7
Ukupno IV.-X.	20,84	20,54	53,9	69,3
Ukupno I.-XII.	12,3	12,3	700,3	680,6

Izvor: DHMZ (2023.); IV.-X. – vegetacija soje,

Iz Grafikona 1. vidljivo je da u prva dva mjeseca vegetacije 2021. godine imamo razdoblje s povećanim srednjim mjesečnim temperaturama u odnosu na višegodišnji prosjek, posebice u veljači kada je razlika prosječne temperature zraka u 2021. godine u odnosu na višegodišnji prosjek iznosila +2,7 °C.



Grafikon 1. Klimagram prema Walteru za 2021. (lijevo) i 1991 - 2022. godine (desno)

U pogledu količine oborina mjesec srpanj je bio s većom količinom oborina za +22,6 % a kolovoz za +39,7 % u odnosu na višegodišnji prosjek. Srednja mjesečna temperatura u kolovozu bilo je za -0,7 °C niža, dok je rujna bio u granicama višegodišnjeg prosjeka (+0,2 °C). Količina oborina u rujnu 2021. godine bila je 34,3 mm manja od višegodišnjeg prosjeka. Ukupna količina oborina u vegetaciji soje u 2021. godini bila je za 15 mm manja u odnosu na višegodišnji prosjek (69,3 mm).

Postavljanje pokusa

Nakon nicanja usjeva unutar svakog sustava sjetve obilježeno po 48 parcela površine 5 m². Svaka parcela kao podvarijanta pokusa odijeljena je od drugih podvarijanti drvenim kolčićima radi lakšeg praćenja i mjerenja rezultata. Podvarijante su postavljene u središtu pokusne parcele udaljene od ruba parcele >20 metara kako bi se izbjegao njihov negativan učinak na rezultate pokusa. Nakon rezanja i zbrinjavanja stabljika soje u polju, sljedeći dan na FAZOS-u je odrađen izvršaj mahuna *Wintersteiger Ld 180* uređajem, te vaganje i mjerenje vrijednosti vlage i hektolitarske mase zrna soje.

Rezultati i rasprava

Veći broj autora (Mascagni i sur., 2008., Bruns, 2011) u nekoliko istraživanja na različitim tlima i klimatskim uvjetima navode rezultate prinosa zrna soje koji su veći u *Twin Row* tehnologiji u odnosu na standardnu sjetvu. Isto tako Mascagni i sur. (2008) navode povećanje prinosa sjemena soje od 12,6 i 13,1 % u sjetvi u udvojenim redovima u Louisiani.

Tablica 2. Utvrđeni sklop biljaka po ha⁻¹ kod ispitivanih kultivara u vrijeme žetve

Kultivar soje	Sklop biljaka po ha ⁻¹ u vrijeme žetve soje					
	Standardna tehnologija (70 cm) - teorijski sklop 582445 (razmak u sjetvi 2,438 cm)			Tehnologija udvojenih redova (22*48 cm) - teorijski sklop 596687 (razmak u sjetvi 4,760 cm)		
	\bar{x}	σ	KV (%)	\bar{x}	σ	KV (%)
APSSB 21	506 017	11073,270	2,19	515 176	12504,600	2,43
APSSB 30	511 022	16018,280	3,13	517 235	11396,330	2,20
APSSB 45	506 727	9623,578	1,90	516 738	9302,897	1,80
ANNIKA	505 662	9823,007	1,94	512 833	6262,506	1,22

Iz Tablice 2. vidljivo je da nisu ostvareni planirani teorijski sklopovi, a jedan od razloga je vrlo loša i krupna struktura pripreme tla kao i nešto niža klijavost kultivara ispitivanih soja.

Tablica 3. Ostvareni prinosi zrna (svedeno na vlagu 11 %) kg ha⁻¹ i vlaga zrna soje kod standardne (S) i tehnologije sjetve u udvojene redove (TR) u vrijeme žetve

Kultivar soje - sustav sjetve	Prinos zrna kg ha ⁻¹			Vlaga zrna (%)		
	\bar{x}	σ	KV (%)	\bar{x}	σ	KV (%)
APSSB 21 (S)	4 115	79,053	1,92	8,04	0,321	3,99
APSSB 21 (TR)	4 778	206,218	4,32	8,08	0,396	4,90
APSSB 30 (S)	4 118	126,022	3,06	7,84	0,207	2,64
APSSB 30 (TR)	4 878	103,604	2,12	8,32	0,377	4,53
APSSB 45 (S)	4 690	209,217	4,46	7,84	0,207	2,64
APSSB 45 (TR)	5 082	102,430	2,02	8,48	0,396	4,67
ANNIKA (S)	4 054	127,379	3,14	8,04	0,428	5,32
ANNIKA (TR)	4 829	105,107	2,18	8,86	0,279	3,15

S = standardna sjetva, TR = sjetva u udvojene redove

Gričar (2007) navodi povećanje prinosa za 17 % pri sjetvi u istoj tehnologiji udvojenih redova. Sjetva soje u udvojenim redovima u odnosu na sjetvu u jedan red tvori veći broj mahunica, te veći prinos (Bell, 2005). Slična razmišljanja s mogućnošću povećanja prinosa postoje i pri sjetvi kukuruza i suncokreta (Banaj i sur., 2019; Banaj i sur., 2021) u istoj tehnologiji u odnosu na standardnu sjetvu u jedan red (Ebelhar, 2010; Smith, 2010) što doprinosi razvoju navedene tehnologije. Sjetvom u udvojene redove brže se zatvara međuredni prostor, omogućava veću brzinu fotosinteze radi povećane lisne površine listova i poboljšava usvajanje hraniva i korištenja vode u tlu (Bruns, 2011). Uspješnost primjene tehnologije u udvojene redove ovisi o dostupnosti vode (Jamieson i sur., 1995), hranjivih tvari i temperatura. Ova tehnologija sjetve soje prihvaćena je među proizvođačima u Missisippiju i u velikom dijelu srednjeg juga Sjedinjenih Američkih Država (Mississippi, Arkansas, Louisiana, Missouri) kao strategija za maksimiziranje prinosa sjemena soje (Bowers i sur., 2000.; Bruns, 2011). Procijenjeno je da gotovo 80 % hektara soje u delti Missisippija 2010. posijano u tehnologiju udvojenih redova (Bruns, 2011).

Zaključak

Temeljem dobivenih saznanja mogu se donijeti sljedeći zaključci;

- U standardnoj sjetvi s pneumatskom sijačicom OLT PSK-4 kultivar soje APSSB 21 u sklopu 506 017 biljaka ha⁻¹ nakon nicanja ostvario je prinos suhog zrna od 4 115 kg ha⁻¹ s prosječnom vlažnošću u vrijeme berbe od 8,04 %.
- Sjetvom istog kultivara u udvojene redove ostvaren je sklop od 515 176 biljaka ha⁻¹ s prinosom suhog zrna od 4 778 kg ha⁻¹ s prosječnom vlažnošću od 8,08 % što čini povećanje u odnosu na standardnu sjetvu od +16,11 %.
- Standardnom sjetvom kultivara soje APSSB 30 ostvaren je prinos suhog zrna od 4 118 kg ha⁻¹, a kod sjetve u

udvojene redove 4 878 kg ha⁻¹ s prosječnom vlagom u berbi od 8,32 %.

- Kod kultivara soje *APSSB 45* pri standardnoj sjetvi u sklopu biljaka 506 727 ha⁻¹ ostvaren je od 4 690 kg ha⁻¹ a u *twin row* sjetvi od 5 082 kg ha⁻¹.

- Standardnom sjetvom kultivara soje *ANNIKA* ostvaren je prinos suhog zrna od 4 054 kg h⁻¹, a kod sjetve u udvojene redove 4 829 kg ha⁻¹ s prosječnom vlagom u berbi od 8,86 %.

- kod sva 4 kultivara soje pri sjetvi u udvojene redove ili u *twin row* tehnologiji ostvareno je povećanje prinosa zrna od 8,36 do 19,12 % odnosno došlo je do povećanja mase zrna za 393 do 775 kg ha⁻¹.

- Uvođenjem *twin row* tehnologije u sjetvi soje pruža se dodatna mogućnost financijskog osnaživanja poljoprivrednog gospodarstva.

Literatura

- Banaj A., Banaj Đ., Petrović D., Stipešević B., Tadić V. (2021). Sustavi sjetve kao čimbenik prinosa zrna u proizvodnji suncokreta. *Poljoprivreda (Osijek)*. 27: 84-90.
- Banaj A., Banaj Đ., Tadić V., Petrović D., Stipešević B. (2019). Utjecaj sustava sjetve na prinos zrna kukuruza različitih FAO grupa. *Poljoprivreda (Osijek)*. 25: 62-70.
- Bell A. (2005). Higher yields with twin-row soybeans. Available at <http://delta-farmpress.com/higher-yields-twin-row-soybeans> (verified 25 Feb. 2011). Delta Farm Press, Penton Media, New York.
- Bowers G.R., Rabb J.L., Ashlock L.O., Santini J.B. (2000). Row spacing in the early soybean production system. *Agronomy Journal*. 92: 524–531.
- Bruns H.A. (2011). Planting Date, Rate, and Twin-Row vs. Single-Row Soybean in the Mid-South. *Agronomy Journal*. 103: 1308-1313.
- Ebelhar M.W. (2010). Twin-row corn boosts yields. *Corn & Soybean Digest*. Available at <http://cornandsoybeandigest.com/corn/twin-row-corn-boosts-yields> (verified 25 Feb. 2011). Penton Media, New York.
- Grichar W.J. (2007). Row spacing, plant populations, and cultivar effects on soybean production along the Texas gulf coast. Available at www.plantmanagementnetwork.org/cm/. Crop Manage.
- Jamieson P., Martin R., Francis G., Wilson D. (1995). Drought effects on biomass production and radiation-use efficiency in barley. *Field Crops Research*. 43: 77–86.
- Mascagni H.J., Clawson E., Lancios D., Boquet D., Ferguson R. (2008). Comparing single-row, twin-row configurations for Louisiana crop production. *Louisiana agriculture*. 51: 16.
- Smith C. (2010). Twin-row corn. *Corn South*. Available at http://www.cotton-farming.com/home/Corn_South/issues/2010-01/2010_JanTwinRow-Corn.html (verified 25 Feb. 2011). One Grower Publ., Memphis, TN.

Application of the MaterMacc Twin Row–2 sowing machine in soybean sowing

Abstract

The results of a comparative study of the influence of the sowing method and four soybean cultivars at the *Tenja* experimental field are presented. Sowing in double rows was done at a spacing of 48 x 22, and standard sowing was done using a *PSK-4* pneumatic sowing machine at a row spacing of 70 cm. In standard sowing, the soybean cultivar *APSSB 21* within 506 017 plants ha⁻¹ achieved a dry grain yield of 4 115 kg ha⁻¹, and in double-row sowing, a dry grain yield of 4 778 kg ha⁻¹ was achieved, i.e. +16,11 % more compared to standard sowing. Cultivar *APSSB 30* achieved a dry grain yield of 4 118 kg ha⁻¹ and when sown in double rows 4 878 kg ha⁻¹ with an average moisture content of 8,32 %. Similar results were recorded with cultivar *APSSB 45*. When sowing in standard rows, soybean cultivar *ANNIKA* achieved a grain yield of 4 054 kg h⁻¹ and double rows achieved 4 829 kg ha⁻¹ with an average moisture content of 8.86 %.

Keywords: soybean, double rows sowing, twin row sowing machine, yield

Primjena modela umjetnih neuronskih mreža u modeliranju procesa sušenja zrna kukuruza

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Sažetak

U provedenom istraživanju, analizirana je kontinuirana, gravitacijska, direktna sušara locirana u zapadnoj slavonskoj. Primarni cilj bio je razviti model umjetnih neuronskih mreža (UNM) za procjenu mase zrna kukuruza na izlazu iz sušare koristeći različite parametre zraka u blizini sušare. Nakon preliminarnog obrade podataka, provedena je statistička analiza. Podaci su podijeljeni tako da je 70% korišteno za treniranje modela, dok je preostalih 30% služilo za testiranje. UNM model prikazao je visoku preciznost u procjeni mase zrna, što je potvrđeno visokim koeficijentom determinacije ($R^2 = 0,989$) i niskom prosječnom postotnom pogreškom ($MAE = 0,023$). Zaključno, UNM model pokazao se kao učinkovito sredstvo za modeliranje i procjenu izlaznih vrijednosti u odnosu na stvarne.

Ključne riječi: sušenje, strojno učenje, matematičko modeliranje, modeliranje.

Uvod

Sušenje žitarica predstavlja ključnu operaciju u pripremi gotovih proizvoda od žitarica. Kinetika procesa sušenja zrna direktno je povezana s prijenosom topline i mase između sušenog materijala i okoline. Nepotpuno kao i prekomjerno sušenje ima direktan utjecaj na kvalitetu žitarica. Ravnotežna vlažnost sušenog zrna smatra se najvažnijim parametrom prilikom kreiranja sustava za sušenje u industrijskim aplikacijama (Jimoh i sur., 2023). Cjelokupni proces izračuna kapaciteta sušare bazira se na zrnu kukuruza.

S obzirom da je sušenje žitarica energetski intenzivan proces u posljednje vrijeme se sve više koriste matematički modeli kako bi se analizirali i procijenili procesi sušenja žitarica u različitim fazama sušenja s ciljem poboljšanja kvaliteta zrna i smanjenja potrošnje energije (Myhan i Markowski, 2022). Cjelokupni sustavi sušenja imaju veliki potencijal za uštedu energije. Energetska učinkovitost procesa sušenja ovisi o gubicima topline koji su uzrokovani dimenzijama, dizajnu, raspodjeli protoka i količini zraka unutar sustava. Mjerenjem uvjeta vlažnosti i temperature zraka kao i mase zrna, prije i poslije sušenja moguće je odrediti parametre sušenja, ali i učinkovitost postrojenja (Jokiniemi i sur., 2011).

Nemogućnost kontrole uvjeta okoline glavni je ograničavajući faktor u postizanju samoprilagodljivog sustava sušenja. Kako bi se postigla precizna kontrola procesa sušenja, utjecaj uvjeta okoline potrebno je uzeti u obzir prilikom modeliranja procesa sušenja. U istraživanju mogućnosti predviđanja modeliranja budućih trendova procesa sušenja (Li i sur., 2020) razvijena su dva modela umjetnih neuronskih mreža za predviđanje učinkovitosti sušenja kukuruza. Statističkom analizom uspoređena su dva razvijena modela: jedan koji uzima u obzir uvjete okoline (IANN) i drugi koji je modeliran bez varijabli uvjeta okoline (OANN). Rezultatima je utvrđeno da je IANN model ima bolju prediktivnu sposobnost (Li i sur., 2020).

Umjetne neuronske mreže (UNM) predstavljaju klasu algoritama umjetne inteligencije koji su oblikovani prema strukturi i funkciji ljudskog mozga. UNM se koriste za različite zadatke, uključujući klasifikaciju, regresiju i prepoznavanje uzoraka. U regresijskim UNM-ima cilj je predvidjeti kontinuiranu izlaznu varijablu temeljem skupa ulaznih varijabli. Osnovna struktura regresijske UNM sastoji se od ulaznog sloja, jednog ili više skrivenih slojeva i izlaznog sloja. Svaki sloj sadrži skup čvorova, ili umjetnih neurona, koji su povezani s čvorovima u susjednim slojevima. Veze između umjetnih neurona su ponderirane, a težinski koeficijenti se prilagođavaju tijekom procesa treniranja kako bi se minimizirala pogreška između predviđenih i stvarnih izlaznih vrijednosti (Yang i Yang, 2014; Chamsaz i Yang, 2014).

S obzirom na navedeno cilj ovog istraživanja je razviti *UNM* model za procjenu mase zrna kukuruza na izlazu iz sušare temeljem različitih ulaznih parametara, kao i analizirati utjecaj ulaznih varijabli temperature zraka, tlaka, relativne vlažnosti zraka i entalpije. Također u istraživanju će se testirati robusnost i univerzalnost *UNM* modela kod različito mjernih uvjeta kako bi se osigurala pouzdanost i primjenjivost modela.

Materijal i metode

Istraživanje je provedeno na kontinuiranoj, gravitacijskoj, direktnoj sušari smještenoj u zapadnoj slavonskoj, a kao materijal za sušenje koristilo se zrno kukuruza. Cilj istraživanja je bio mjeriti različite parametre zraka u blizini sušare i procijeniti masu zrna kukuruza na izlazu iz sušare koristeći razvijeni *UNM* model. Svi parametri su mjereni 8 puta kako bi se utvrdila varijabilnost prikupljenih podataka. Kapacitet je mjereno utvrđivanjem mase suhe robe na silosnoj protočnoj vagi a masa jednog vaganja bila je 400 kg. Kontrolna mjerenja su izvršena povremeno tako je mjereno vrijeme između pojedinih vaganja. Prikupljeni podaci su preliminarno obrađeni (čišćenje podataka) kako bi se uklonile nepravilnosti i odstupanja. Nakon provedenog postupka, podaci su strukturirani u odgovarajuće formate. Za svaku varijablu izračunati su sljedeći statistički parametri: srednja, minimalna i maksimalna vrijednost, kao i standardna devijacija. Statistička analiza provedena je koristeći programski jezik Python putem biblioteka: pandas, numpy i Scikit-learn.

U okviru modeliranja primijenjena je podjela podataka gdje je 70% korišteno za učenje modela, dok je preostalih 30% alocirano za testiranje modela. Proces modeliranja provodio se kroz 100 000 iteracija treniranja. Arhitektura mreže dinamički je konfigurirana s nasumično odabranim brojem neurona u skrivenom sloju te s nasumično odabranim funkcijama aktivacije u skrivenom i izlaznom sloju. Izlazna vrijednost *UNM* modela izračunava se po idućoj jednadžbi (Murphy, 2012):

$$y = f\left(\sum_{i=1}^n w_i x_i + b\right)$$

Gdje je: y izlaz *UNM*, f aktivacijska funkcija, x_i ulazni podatak u model, w_i težinski koeficijent, b prag pristranosti.

Kako bi se utvrdila sposobnost razvijene *UNM* u modeliranju mase zrna kukuruza na izlazu potrebno je analizom pogrešaka izračunati parametre koeficijenta determinacije (R^2) i prosječne apsolutne greške (MAE). Koeficijent determinacije (R^2) predstavlja statističku mjeru za utvrđivanje pogodnosti regresijskog modela u opisivanju stvarnih podataka, a izračunava se (Cheng et al., 2014):

$$R^2 = 1 - \frac{\sum_{i=1}^n (y_o - y_p)^2}{\sum_{i=1}^n (y_o - \bar{y})^2}$$

Gdje je: R^2 – koeficijent determinacije; y_o – stvarna vrijednost; y_p – modelirana (procijenjena) vrijednost

Prosječna apsolutna pogreška (MAE) (2) predstavlja statističku mjeru za određivanje veličinu pogreške između stvarnih i predviđenih vrijednosti, a predstavlja prosjek apsolutnih razlika između stvarnih i procijenjenih vrijednosti i dobiva se pomoću jednadžbi (Schneider i Xhafa, 2022):

$$MAE = \frac{1}{n} \sum_{i=1}^n |y_o - y_p|$$

Gdje je: MAE – prosječna apsolutna pogreška; y_o – stvarna vrijednost; y_p – modelirana (procijenjena) vrijednost

Rezultati i rasprava

U tablicama 1. i 2. prikazana je statistička analiza ispitivanih parametara. Analizirane vrijednosti prikazane su u obliku prosječne, minimalne i maksimalne vrijednosti i standardne devijacije.

Tablica 1. Statistička analiza mjerenih parametara stanja zraka okoline sušare

Mjereni parametar	Varijabla	Prosjek	Min	Max	SD
Stanje zraka 50 m od sušare	t_{0v} (°C)	17,45	17,00	19,00	0,76
	P_0 (hPa)	1012,45	1012,00	1013,50	0,60
	φ_0 (%)	83,70	75,00	90,00	6,91
	x_0 (g/kg)	11,98	11,50	13,00	0,41
	h_0 (kJ/kg)	48,57	47,31	53,18	2,15
Stanje zraka uz sušaru	t_{0s} (°C)	19,69	18,50	21,50	1,00
	t_{0v} (°C)	17,81	17,00	19,00	0,65
	φ_0 (%)	82,88	78,00	90,00	4,05
	x_0 (g/kg)	12,13	11,50	13,00	0,52
	h_0 (kJ/kg)	49,56	46,89	53,18	2,04

t_0 – temperatura zraka; p_0 – početni tlak; φ_0 – relativna vlažnost zraka; x_0 – vlažnost zraka; h_0 – entalpija; SD – standardna devijacija.

Tablica 1 prikazuje parametre stanja zraka 50 metara od sušare i uz samu sušaru. Prosječna temperatura zraka 50 metara od sušare 17,45°C s relativnom vlažnošću od 83,70% i entalpijom od 48,57 kJ/kg, stanje zraka uz sušaru pokazivala je nešto više vrijednosti s prosječnom temperaturom od 19,69°C, relativnom vlažnošću od 82,88% i entalpijom od 49,56 kJ/kg. Uspoređujući ova dva mjerna mjesta, uz sušaru se bilježi viša temperatura a, nešto niža relativna vlažnost, što je bilo i za očekivati s obzirom na visoku temperaturu zraka.

Tablica 2. Statistička analiza mjerenih parametara na ulazu u sušaru i izlazu iz sušare.

Mjereni parametar	Varijabla	Prosjek	Min	Max	SD
Ulaz	θ_1 (°C)	19,46	15,90	21,70	1,76
	w_1 (%)	35,13	33,90	36,59	0,79
Izlaz	θ_1 (°C)	25,91	22,80	28,40	1,98
	w_3 (%)	14,37	12,80	15,15	0,75

θ_1 – temperatura zrna; w_1 – vlažnost zrna; SD – standardna devijacija.

U Tablici 2 analizirani su parametri temperature i vlažnosti zrna kukuruza na ulazu u sušaru i na izlazu iz sušare. Na ulazu, prosječna temperatura zrna iznosi 19,46°C s vlažnošću od 35,13%. Na izlazu, temperatura je prosječno 25,91°C s vlažnošću od 14,37%, što je zapravo došlo do navlaženja zrna u hladnjaku – tzv. „ispad sušare iz faze“ (L)

U Tablici 3 prikazana je prosječna vrijednost mase zrna na izlazu iz sušare. Varijabla koja se analizira je “Masa zrna na izlazu” izražena u tonama po satu.

Tablica 3. Prosječna vrijednost mase zrna kukuruza na izlazu iz sušare

Varijabla	Prosjek	Min	Max	SD
Masa zrna na izlazu t/h	26,49	26,13	27,57	0,43

Min – minimum; *Max* – Maksimum; *SD* – standardna devijacija

Prosječna masa zrna iznosila je 26,49 t/h, najmanja zabilježena masa je 26,13 t/h, dok je najveća zabilježena masa 27,57 t/h. Ova dobivena masa zrna je beskorektivnog faktora.

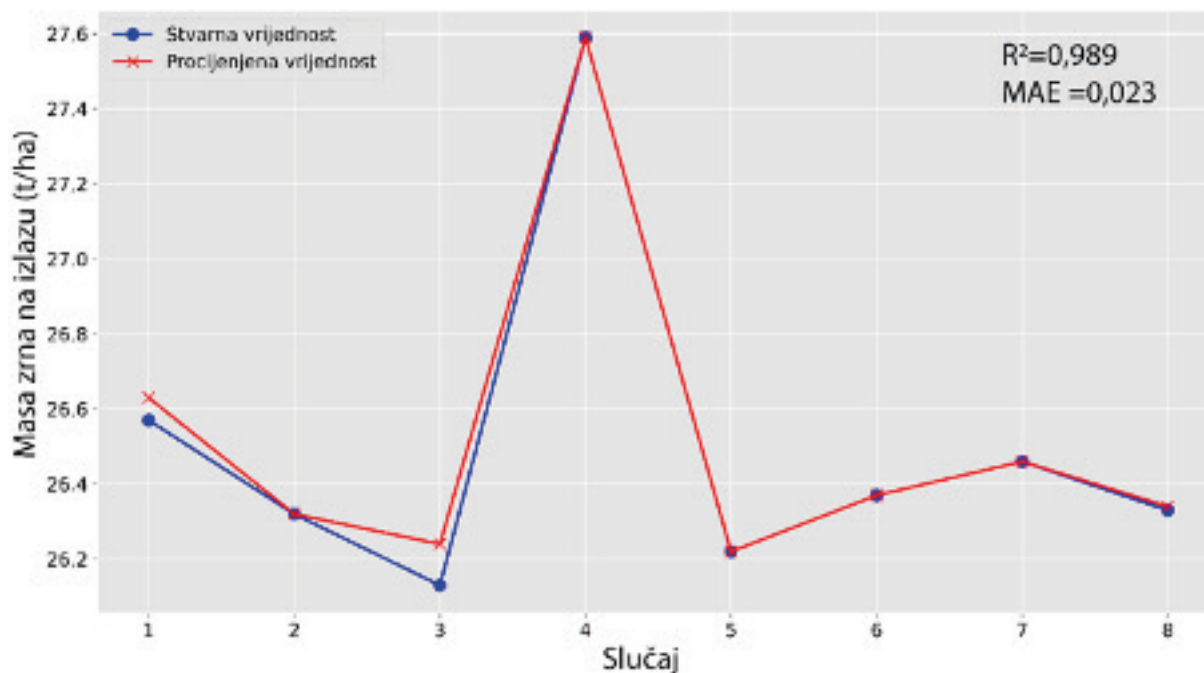
U tablici 4. prikazane su performanse razvijenog UNM modela kao koeficijenta determinacije prilikom učenja i treniranja modela kao i korištene aktivacijske funkcije prilikom modeliranja.

Tablica 4. Performanse razvijenog modela

Performanse učenja modela	Performanse testiranja modela	Pogreška učenja modela	Pogreška testiranja modela	Algoritam treniranja	Funkcija pogreške	Aktivacijska funkcija skrivenog sloja	Aktivacija funkcija izlaznog sloja
0,99	1,00	0,00	0,00	BFGS 19	SOS	Funkcija identiteta	Eksponecijalna

Razvijeni UNM model postigao je koeficijent determinacije 0,99 prilikom učenja i 1,00 prilikom testiranja. Ovo ukazuje na visoku točnost i nisku razinu pogreške modela. U modelu se kao aktivacijska funkcija koristi funkcije identiteta za skriveni sloj, dok u izlaznom sloju se koristi eksponencijalna funkcija.

Na slici 1. prikazane su dvije krivulje koje predstavljaju preklapanje između stvarnih i predviđenih vrijednosti mase zrna na izlazu putem modela UNM.



Slika 1. Prikaz preklapanja stvarne i procijenjene vrijednosti mase zrna na izlazu temeljem modela UNM

Krivulje u različitim bojama (slika 1.) prikazuju stvarne i modelirane vrijednosti. U većem broju slučajeva dolazi do preklapanja što sugerira na visoku sposobnost modela u procjeni mase zrna na izlazu iz sušare. Najveća odstupanja vidljiva su kod slučajeva 1. i 3. Statističkom analizom performansi UNM modela izračunat je koeficijent determinacije (R^2) i prosječna postotna pogreška (MAE). U ovom slučaju R^2 (0,989) i MAE (0,023) upućuju na visoku sposobnost modela u modeliranju i generiranju procjena s obzirom na visoku razinu generalizacije i preciznog modeliranja izlazne vrijednosti u odnosu na stvarne.

U svom istraživanju, autori Li i sur. (2020) primijenili su metodologiju umjetnih neuronskih mreža (ANN) s ciljem predviđanja ključnih indeksa industrijskog sušenja kukuruza, uzimajući pritom u obzir okolišne uvjete. Rezultati

su ukazali na veću preciznost predviđanja ANN modela koji uključuje ambijentalne uvjete u odnosu na model koji ih ne uzima u obzir. Kao ključne metrike za evaluaciju performansi modela, koristili su R^2 i MAE. Za sadržaj vlage, učinkovitost izmjenjivača topline i specifičnu potrošnju energije. R^2 vrijednosti bila je 0.98 – 0.99. S druge strane, MAE vrijednosti za navedene izlazne vrijednosti iznosile su 0.01-0.16. Niska razina pogreške modela potvrđuje mogućnost korištenja UNM modela, implicirajući njihovu široku primjenjivost u inženjerskoj praksi i modeliranju procesa sušenja.

Zaključak

Primjena UNM modela u modeliranju mase zrna kukuruza na izlazu iz sušare temeljem ulaznih parametara temperature zraka, tlaka, relativne vlažnosti zraka i entalpije postigla je visoku preciznost u procjeni mase zrna na izlazu. Po završetku detaljne obrade i analize podataka, UNM model je treniran koristeći 70% prikupljenih podataka, dok je preostalih 30% bilo namijenjeno za testiranje. Dinamička konfiguracija modela, uključujući nasumičan odabir broja neurona i funkcija aktivacije, pokazala je visoke performanse u modeliranju. Učinkovitost modela potvrđena je visokim koeficijentom determinacije ($R^2 = 0,989$) i niskom prosječnom postotnom pogreškom (MAE = 0,023), što sugerira na visoku preciznost modela u procjeni mase zrna. Ovi rezultati upućuju na to da je UNM model pouzdan alat za modeliranje i procjenu mase zrna na izlazu iz sušare.

Napomena

Ovo istraživanje financirala je Hrvatska zaklada za znanost u okviru projekta br. IP-2018-01-7472, "Zbrinjavanje mulja kroz proizvodnju energetske kulture" u okviru projekta „Projekt razvoja karijera mladih istraživača – izobrazba novih doktora znanosti“ uz su-financiranje od strane Europske unije, u okviru OP „Učinkoviti ljudski potencijali 2014-2020“ iz sredstava ESF-a.

Literatura

- Chamsaz M., Perai A.H., Asadpour S., Hosseini Shahidi R. (2011). Comparison of the 3-phase segmented linear regression and artificial neural network models to predict broiler hatchability. *Journal of Applied Poultry Research*. 20: 447-453.
- Cheng C.L., and Shalabh G. (2014). Coefficient of determination for multiple measurement error models. *Journal of Multivariate Analysis*. 126: 137–152.
- Jimoh K.A., Hashim N., Shamsudin R., Man H.C., Jahari M., Onwude D.I. (2023). Recent Advances in the Drying Process of Grains. *Food Engineering Reviews*. 15: 548–576.
- Jokiniemi T., Kautto K., Kokkin E., Ahokas J. (2011). Energy efficiency measurements in grain drying. *Agronomy Research*. 9: 69–75.
- Li B., Li C., Huang J., Li C. (2020). Application of artificial neural network for prediction of key indexes of corn industrial drying by considering the ambient conditions. *Applied Sciences (Switzerland)*. 10: (16).
- Murphy K.P. (2012). *Machine learning: a probabilistic perspective*. Cambridge, MA, MIT Press.
- Myhan R., and Markowski M. (2022). Generalized Mathematical Model of the Grain Drying Process. *Processes*. 10: (12).
- Schneider P., and Xhafa F. (2022). Anomaly detection: Concepts and methods. In P. Schneider & F. Xhafa (Eds.), *Anomaly Detection and Complex Event Processing over IoT Data Streams* (pp. 49-66). Academic Press.
- Van Rossum G., and Drake F.L. (2009). *Python 3 Reference Manual*. Scotts Valley, CA: CreateSpace.
- Yang Z.R., and Yang, Z. (2014). Artificial Neural Networks. In A. Brahme (Ed.), *Comprehensive Biomedical Physics* (pp. 1-17). Elsevier.

Application of the artificial neural network model in the modeling of the drying process of corn

Abstract

In the conducted research, a continuous direct gravity dryer in western Slavonia was analyzed. The primary objective was to develop an artificial neural network (UNM) to estimate the mass of corn kernels at the exit of the dryer using different air parameters near the dryer. After initial data processing, a statistical analysis was performed. The data was divided so that 70% was used for training the model, while the remaining 30% was used for testing. The UNM model showed high precision in estimating grain mass, as confirmed by a high coefficient of determination ($R^2 = 0.989$) and a low average percent error ($MAE = 0.023$). In conclusion, the UNM model proved to be an effective tool for modeling and evaluating output values against actual values.

Keywords: drying, machine learning, mathematical modeling, modeling.

Utjecaj procesa anaerobne fermentacije na sadržaj lignina u proizvodnji silaže i sjenaže miskantusa

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Sažetak

Predtretmani poljoprivredne biomase imaju ključnu ulogu u povećanju efikasnosti njenih energetske karakteristika. S pozicije proizvodnje bioplina odnosno procesa anaerobne digestije jedan od kriterija za veću proizvodnju imati će hemicelulozni sastav biomase, a ponajviše udio lignina. Stoga, cilj ovog istraživanja je analizirati utjecaj anaerobne fermentacije na udio lignina u silaži i sjenazi *Miscanthus x giganteus*. Rezultati pokazuju značajno smanjenje udjela lignina nakon procesa fermentacije, što implicira poboljšanu biorazgradivost materijala, a samim time i veću efikasnost proizvodnje bioplina, čineći *Miscanthus x giganteus* još prikladnijim za upotrebu kao obnovljivu energetske kulturu.

Ključne riječi: lignin, anaerobna fermentacija, silaža, anaerobna digestija, *Miscanthus x giganteus*

Uvod

Suočeni s mnogobrojnim izazovima u proizvodnji energije od strane Europske unije postavljeni su jasni ciljevi proizvodnje održive i obnovljive energije (Dominković i sur., 2018; Popescu i sur., 2022). U tom kontekstu, korištenje poljoprivredne biomase poput višegodišnjih energetske kultura imat će ključnu ulogu u toj tranziciji (Angelini, 2009; Naik, 2010). Međutim, pretvorba biomase u biogorivo predstavlja složen proces u kojem jednu od ključnih uloga ima i sam strukturalni sastav.

Strukturalni sastav biomase ovisi o sadržaju tri glavne komponente i to: celuloze, hemiceluloze i lignina (Luo i sur., 2022). Celuloza i hemiceluloza su polisaharidi koji se mogu razgraditi u jednostavne šećere koji se koriste kao bazne komponente za proizvodnju biogoriva. Kod proizvodnje tekućih i plinovitih biogoriva druge generacije poželjniji je niži udio lignina, te viši udjeli celuloze i hemiceluloze. Najznačajnija tekuća biogoriva jesu biodizel i bioetanol, dok je danas najznačajnije plinovito biogorivo bioplina (biometan) (Srivastava i sur., 2021). S obzirom da se proizvodnja bioplina temelji na procesu anaerobne digestije, odnosno na stupnju biorazgradivosti sirovine koji predstavlja poželjno svojstvo biomase, potrebno je sirovinu prilagoditi procesu proizvodnje bioplina.

Stupanj biorazgradivosti pojedine sirovine definiran je strukturalnim sastavom (sadržaj lignina, celuloze i hemiceluloze), a biomasa poput poljoprivredne energetske kulture miskantusa u svojem sastavu ima visoki udio lignina. Prilikom procesa anaerobne digestije (proizvodnje bioplina) i tijekom biološke razgradnje sirovine u samom procesu, visok udio lignina predstavlja nepoželjno svojstvo. Lignin, složeni organski polimer koji veže celulozu i hemicelulozu otežava pristup mikrobnim kulturama tijekom procesa razgradnje (Saini i sur., 2015). Kao moguće rješenje se nalaže provedba ranijih rokova žetve (jesenski rok), kada je udio lignina niži, udio vode viši, ali i prinosa nadzemne biomase u usporedbi sa zimskom ili proljetnom žetvom (Kiesel i Lewandowski, 2017). Huyen i sur. (2010) utvrdili su promjenu u udjelu lignina u različitim rokovima žetve miskantusa, gdje je sadržaj lignina u požetoj biomasi u jesen (studeni) iznosio 18,86 %, dok je nakon zimske žetve (veljača) bio zastupljen s 19,23 %.

S obzirom da je u ranijem roku žetve (jesen) povećan udio vlage u sirovini, potrebno ju je pravilno uskladištiti kako bi se mogla u kontinuitetu koristiti u proizvodnji bioplina. Skladištenje podrazumijeva posljednju fazu koja prethodi daljnjoj preradi sirovine i proizvodnji bioplina, to jest predstavlja mjesto gdje se biomasa čuva u adekvatnim prostorima i u određenom vremenskom razdoblju (Pakarinen i sur., 2008). Održiva proizvodnja obnovljive energije podrazumijeva i korištenje prirodnih procesa konzerviranja (skladištenja) sirovinske baze pa stoga anaerobna fermentacija visokovlažne sirovine, odnosno proizvodnja silaže i sjenaže višegodišnjih energetske kultura, predstavlja najpoželjniji oblik skladištenja. Silažom i sjenazom kao načinima skladištenja, omogućuje se neposredno

konzerviranje vlažnih sirovina gdje se pri anaerobnim uvjetima događa proces anaerobne fermentacije (Franco i sur., 2016). Nadalje, proces anaerobne fermentacije predstavlja jedan od načina pripreme sirovine za daljnju preradu pri čemu se u strukturalnom sastavu sirovine događaju određene fizikalne i kemijske promjene (Sun i sur., 2021). Stoga, proces anaerobne fermentacije može se smatrati kao jedan od načina biološkog predtretmana sirovine za daljnji proces prerade u svrhu proizvodnje veće količine energije po jedinici mase sirovine.

Materijal i metode

Žetva energetske kulture miskantusa odrađena je u drugoj dekadi mjeseca listopada, nakon prvog jesenskog mraza. Nakon prikupljanja biomase provedena je priprema uzoraka mljevenjem u skladu s preporukama za proizvodnju silaže i sjenaže. Odnosno, uzorci silaže (i) usitnjeni su koristeći mlin za usitnjavanje biomase te sito promjera otvora $\varphi=10$ mm, dok se za pripremu sjenaže (ii) koristio mlin za usitnjavanje bez sita čime su se dobile frakcije 1-5 cm. Tako pripremljeni uzorci koristili su se za daljnji proces anaerobne fermentacije. Uzorci silaže (i) konzervirani su u vakuum vrećicama (PA/PE), dok su uzorci sjenaže omotani u plastičnu foliju (PE). Na taj način pripreme uzoraka omogućeno je stvaranje povoljnih uvjeta za proces anaerobne fermentacije koja je trajala 60 dana. Nakon isteka vremenskog perioda skladištenja i kraja procesa anaerobne fermentacije uzorci su se pripremili za određivanje strukturalnog sastava koristeći uređaj za određivanje lignoceluloznog sastava (ANKOM 200).

Prilikom određivanja strukturalnog sastava (ANKOM Technology Methods), potrebno je odrediti udjele kiselih (ADF) i neutralnih (NDF) vlakana kao i udio kiselog lignina (ADL) koristeći navedene formule:

$$\%ADFdb = \frac{100 \cdot (w3 - (w1 \cdot c1))}{w2 \cdot DM}$$

$$\%NDFdb = \frac{100 \cdot (w3 - (w1 \cdot c1))}{w2 \cdot DM}$$

$$\%ADLdb = \frac{100 \cdot (w3 - (w1 \cdot c1))}{w2 \cdot DM}$$

Navedene vrijednosti koriste se prilikom izračuna udjela celuloze, hemiceluloze i lignina prema standardnim metodama te navedenim formulama:

$$\begin{aligned} \text{Udio celuloze } \%CEL &= \%ADF - \%ADL \\ \text{Udio hemiceluloze } \%HEM &= \%NDF - \%ADF \\ \text{Udio lignina } \%LIG &= \%ADL \end{aligned}$$

Rezultati i rasprava

Nakon provedene analize strukturalnog sastava uzoraka svježe biomase miskantusa (miskantus početno) prije procesa anaerobne fermentacije te silaže i sjenaže miskantusa nakon anaerobne fermentacije dobiveni podaci su statistički obrađeni. Statistička analiza provedena je koristeći program TIBCO STATISTICA 13.3.0 (StatSoft TIBCO Software Inc., Palo Alto, CA, USA). Za razumijevanje podataka provedena je statistička analiza prikazom srednjih vrijednosti s izraženim vrijednostima standardne devijacije i statističkom signifikatnosti između ispitivanih varijabli uzorka prema Tukey's HSD testu (Tablica 1.).

Tablica 1. Strukturalni sastav miskantusa prije i nakon anaerobne fermentacije

Uzorak	Udio celuloze (%)	Udio hemiceluloze (%)	Udio lignina (%)
Miskantus početno	38,5±0,009 ^a	24,5±0,001 ^a	15,4±0,013 ^c
Miskantus sjenaža	41,0±0,005 ^b	26,0±0,006 ^{ab}	12,5±0,006 ^b
Miskantus silaža	39,7±0,009 ^{ab}	26,8±0,012 ^b	9,7±0,005 ^a
Min.	0,385	0,245	0,097
Maks.	0,410	0,268	0,154

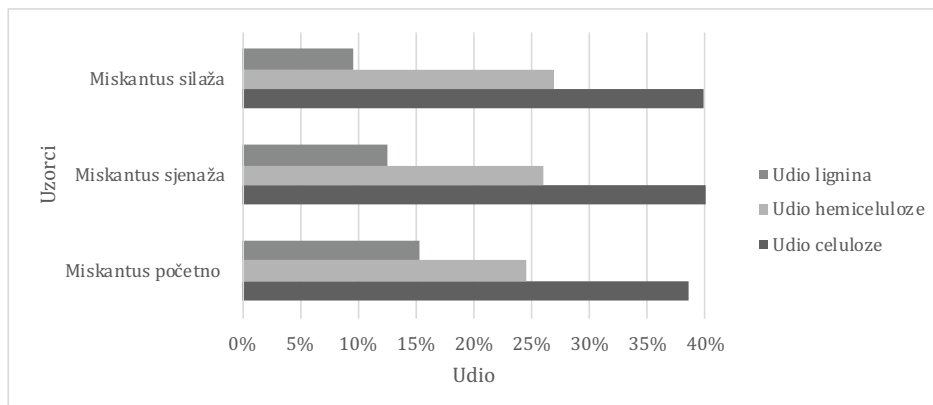
Statistička signifikatnost * $p \leq 0.01$ prema Tukey's HSD testu prikazana malim slovima u eksponentu

U okviru provedene analize, udjeli celuloze u uzorcima miskantusa variraju od 38,5±0,009 u početnom uzorku do 41,0±0,005 u uzorku sjenaže, s malom standardnom devijacijom koja ukazuje na relativnu konzistenciju vrijednosti. Udio hemiceluloze blago se povećava od početnog uzorka s 24,5±0,001 do uzorka silaže s 26,8±0,012, a standardne devijacije nešto su veće, što sugerira veću varijabilnost miskantusa u procesu anaerobne fermentacije. Udjeli lignina su se smanjili od 15,4±0,013 u početnom uzorku do 9,7±0,005 u uzorku silaže, što je statistički značajno smanjenje udjela lignina tijekom procesa anaerobne fermentacije.



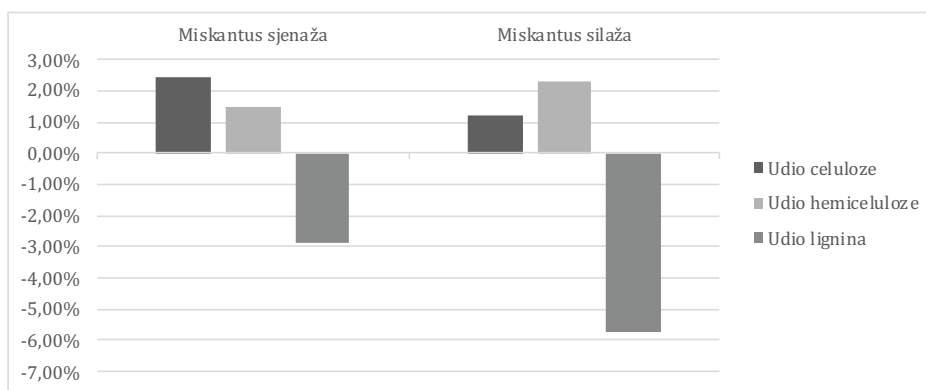
Slika 1. Korelacije između uzoraka i strukturalnog sastava

U početnom stanju miskantusa, postoji umjerena negativna korelacija između celuloze i hemiceluloze (-0.64), dok je korelacija između celuloze i lignina visoko pozitivna (0.80), sugerirajući da se s većim udjelom celuloze povećava i udio lignina. Za hemicelulozu i lignin, korelacija je blizu nule (-0.06), što ukazuje na to da promjene u udjelima ove dvije komponente nisu međusobno povezane. Miskantus sjenaža, pokazuje slabu negativnu korelaciju između celuloze i hemiceluloze (-0.25) i celuloze i lignina (-0.42). Hemiceluloza i lignin imaju vrlo visoku pozitivnu korelaciju (0.98), ukazujući na to da se povećanjem udjela jedne komponente gotovo proporcionalno povećava i udio druge. Za miskantus silažu, korelacija između celuloze i hemiceluloze je negativna (-0.54), što ukazuje na to da s povećanjem udjela jedne komponente dolazi do smanjenja druge. Odnos celuloze i lignina je pozitivan (0.69), sugerirajući da se povećanjem udjela celuloze povećava i udio lignina. Hemiceluloza i lignin pokazuju visoku negativnu korelaciju (-0.98), što ukazuje na to da s povećanjem udjela hemiceluloze značajno opada udio lignina.



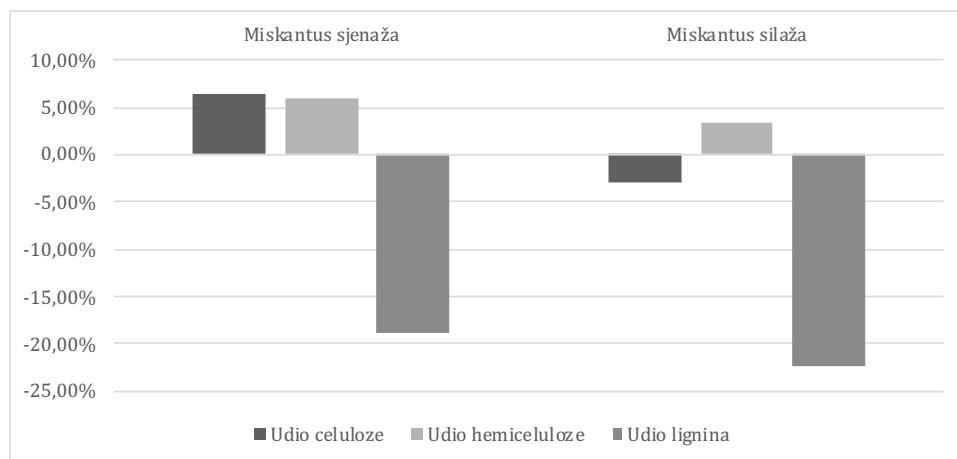
Grafikon 1. Udio lignina, hemiceluloze i celuloze prije i nakon anaerobne fermentacije

Iz navedenog grafikona možemo uočiti da početni uzorci miskantusa imaju značajan udio celuloze (38,50 %), koji se blago povećava u uzorcima miskantusa sjenaže (40,97 %) i silaže (39,73 %), nakon procesa anaerobne fermentacije. S druge strane, udio hemiceluloze se neznatno povećava od početnog uzorka (24,50 %) prema uzorcima sjenaže (25,97 %) i silaže (26,83 %). Značajno smanjenje udjela lignina uočeno je nakon procesa anaerobne fermentacije. Početni uzorak miskantusa sadrži 15,37 % lignina, uzorak sjenaže 12,47 %, dok uzorak silaže pokazuje najmanji udio lignina od 9,67 %. Smanjenje lignina kroz proces anaerobne fermentacije i proizvodnje silaže miskantusa dovelo je do najveće biorazgradivosti. Na temelju dosadašnjih spoznaja navedeni rezultati ovog značajnog smanjenja udjela lignina mogu efikasno utjecati na proces anaerobne digestije.



Grafikon 2. Razlika strukturalnog sastava nakon procesa anaerobne fermentacije

Prikazani grafikon detaljnije prikazuje smanjenje koje se dogodilo kod uzorka sjenaže i silaže miskantusa nakon procesa anaerobne fermentacije. Prema grafikonu uočava se povećanje udjela celuloze za 2,47 % i hemiceluloze za 1,47 %, dok se udio lignina smanjuje za 2,90 % kod sjenaže miskantusa. S druge strane, uzorak miskantus silaže pokazuje manje povećanje udjela celuloze od 1,23 % i veće povećanje udjela hemiceluloze od 2,33 %, uz znatno smanjenje udjela lignina od 5,70 %.



Grafikon 3. Postotno smanjenje udjela strukturalnog sastava

Grafikon 3. prikazuje odnos krajnjeg i početnog udjela unutar strukturalnog sastava miskantusa prije i nakon procesa anaerobne fermentacije. Uzorak sjenaze miskantusa pokazuje povećanje udjela celuloze za 6,41 % i hemiceluloze za 5,99 %, dok se udio lignina znatno smanjio za 18,87 %. Suprotno tome, uzorak miskantus silaže pokazuje smanjenje udjela celuloze za 3,01 %, ali povećanje udjela hemiceluloze za 3,34 % te smanjenje udjela lignina za 22,46 %.

Zaključak

Rezultati istraživanja ukazali su na promjene u strukturalnom sastavu miskantusa nakon tretmana anaerobne fermentacije. Značajno smanjenje udjela lignina kod silaže miskantusa doprinosi povećanju biorazgradivosti olakšavajući pretvorbu u bioplin i biometan. Takve promjene ključne su u kontekstu povećanja održive i obnovljive energije, ali i smjernica kako se biološkim procesima može utjecati na značajno smanjenje negativnih svojstava sastava biomase. Na taj način ostvaruje se povećana efikasnost proizvodnje obnovljivih izvora energije. Naglašavajući potencijalnu prednost ovog procesa kao oblika predtretmana miskantusa za povećanu učinkovitost proizvodnje bioplina putem anaerobne digestije, daljnja istraživanja treba usmjeriti upravo na daljnju provedbu procesa anaerobne digestije istraživane višegodišnje energetske kulture uksladištenom načinom silaže i sjenaze.

Napomena

Ovo istraživanje financirano je putem OP Konkurentnost i kohezija 2014-2020, projekta KK.01.1.1.07.0078 „Održiva proizvodnja bioplina zamjenom kukuruzne silaže poljoprivrednim energetskim kulturama“ i HRZZ „Projekt razvoja karijera mladih istraživača – izobrazba novih doktora znanosti“ DOK-02-2021

Literatura

- Angelini L.G., Ceccarini L., Nasso N., Bonari E. (2009). Comparison of *Arundo donax* L. and *Miscanthus × giganteus* in a long-term field experiment in Central Italy: analysis of productive characteristics and energy balance. *Biomass and Bioenergy*. 33: 635–643.
- Dominković D.F., Bačeković I., Pedersen A.S., Krajačić G. (2018). The future of transportation in sustainable energy systems: Opportunities and barriers in a clean energy transition. *Renewable and Sustainable Energy Reviews*. 82: 1823-1838.
- Franco R.T., Buffière P., Bayard R. (2016). Ensiling for biogas production: Critical parameters. A review. *Biomass and Bioenergy* 94: 94-104.
- Huyen T.L.N., Rémond C., Dheilly R.M., Chabbert B. (2010). Effect of harvesting date on the composition and saccharification of *Miscanthus x giganteus*. *Bioresource Technology*. 101: 8224-8231.
- Kiesel A., and Lewandowski I. (2017). *Miscanthus* as biogas substrate–Cutting tolerance and potential

- for anaerobic digestion. *Gcb Bioenergy*. 9: 153-167.
- Luo Z., Qian Q., Sun H., Wei Q., Zhou J., Wang K. (2022). Lignin-first biorefinery for converting lignocellulosic biomass into fuels and chemicals. *Energies*. 16: 125.
- Naik S.N., Goud V.V., Rout P.K., Dalai A.K. (2010). Production of first and second generation biofuels: a comprehensive review. *Renewable and sustainable energy reviews*. 14: 578-597.
- Pakarinen O., Lehtomäki A., Rissanen S., Rintala J. (2008). Storing energy crops for methane production: Effects of solids content and biological additive. *Bioresource Technology*. 99: 7074–7082.
- Popescu C., Panait M., Palazzo M., Siano A. (2022). Energy transition in European Union— Challenges and opportunities. *Energy Transition: Economic, Social and Environmental Dimensions*. 289-312.
- Saini J.K., Saini R., Tewari L. (2015). Lignocellulosic agriculture wastes as biomass feedstocks for second-generation bioethanol production: concepts and recent developments. *3 Biotech*. 5: 337-353.
- Srivastava N., Shrivastav A., Singh R., Abohashrh M., Srivastava K.R., Irfan S., Thakur V. K. (2021). Advances in the structural composition of biomass: Fundamental and bioenergy applications. *Journal of Renewable Materials*. 9: 615-636.
- Sun H., Cui X., Li R., Guo J., Dong R. (2021). Ensiling process for efficient biogas production from lignocellulosic substrates: Methods, mechanisms, and measures. *Bioresource Technology*. 342: 125928.

Effect of anaerobic fermentation on the lignin component in silage and haylage production from miscanthus

Abstract

Pre-treatments of agricultural biomass play a key role in increasing the efficiency of its energetic properties. With regard to biogas production, i.e. the process of anaerobic digestion, the hemicellulosic composition of the biomass, especially the lignin content, is one of the criteria for higher production. The aim of this study is therefore to analyze the influence of anaerobic digestion on the lignin content in *Miscanthus x giganteus* silage and haylage. The results show a significant decrease in the lignin content after the fermentation process, which means an improved biodegradability of the material and thus a higher efficiency of biogas production, making *Miscanthus x giganteus* even more suitable for use as a renewable energy crop.

Keywords: lignin, anaerobic fermentation, silage, anaerobic digestion, *Miscanthus x giganteus*

Precision urban farming

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Abstract

Agriculture will struggle to fulfil the needs of the world's growing population. Compared to conventional farming, urban farming offers a number of benefits, including increased productivity, enhanced sustainability and year-round access to fresh food and is defined as agricultural practice that takes place in cities and their immediate surrounding areas. Urban farming includes traditional farming, allotment gardens, rooftop gardens, hydroponics, aquaponics and indoor vertical farming. Smart technology integration contributes to increased sustainability and efficiency in urban farming.

Keywords: urban farming, precision farming, vertical farming, aquatic farming, smart technologies

Introduction

Urban farming is the agricultural practice that takes place in cities and their immediate surrounding areas and is becoming an increasingly important component in agriculture production (Rouquette, 2017). It includes traditional farming, allotment gardens, rooftop gardens, hydroponics, aquaponics and indoor vertical farming (Goldstein et al., 2016).

Some of the systems of urban farming are thousands of years old. Urban farming is primarily found in developing nations, although in the last several decades, cultivating food in the cities of developed countries has also grown increasingly common and is a strategy for adaptation and mitigation of climate change, one of the ways to alleviate the consequences of environmental degradation and improve living conditions (Kisić, 2018).

Urban farming implementation is heavily influenced by local factors, including climate and geographical conditions, planning strategies, local policies, local economics and tradition (Dubbeling et al., 2019; Taguchi and Santini, 2019; Wadumestrige Dona et al., 2021). The main barriers to the growth of urban agriculture in the world today is the conversion of land into construction zones or the practice of land grabbing, which has become very lucrative for investors (Kisić, 2018).

Urban farming is becoming increasingly popular because it encourages innovation and helps modernizing the agriculture industry. Smart technology integration contributes to increased sustainability and efficiency in urban farming (Khan et al., 2023). Precision urban farming uses sensors and automation to control optimal growing conditions such as temperature, humidity, light and nutrient levels (Oh and Lu, 2022).

Urban farming

Agriculture will struggle to fulfil the needs of the world population, with growth predicted to peak at over 11 billion by 2100 (UN, 2019). Lack of natural resources, changing demographics, climate changes and increasing waste are major challenges to feeding the world's exploding population that drive the use of new technologies to improve agriculture. Compared to conventional farming, urban farming offers a number of benefits, including increased productivity, enhanced sustainability and year-round access to fresh food as well as supporting/increasing biodiversity in cities, improving climate conditions and soil conservation, promoting waste recycling (Xi et al., 2021; Dubbeling et al., 2019; Taguchi and Santini, 2019; Wadumestrige Dona et al., 2021) and fostering social cohesion and benefiting urban residents' psychological, emotional, and physical health through the provision of jobs, training, and leisure activities (Açiksöz et al., 2021). According to Ilieva et al. (2022) many scholars are focused on health, economic and educational benefits of food-producing urban green spaces. Around one billion city people can be fed by urban and peri-urban farming, and furthermore can yield numerous positive social, economic and environmental effects (Pradhan et al., 2023).

Urban farming is defined in terms of applied production systems such as horticulture (greenhouse and container farming, hydroponics and aeroponics), aquaculture, animal husbandry and agroforestry and exercise intensive production methods, reuses natural resources and urban waste (Smit et al., 2001). According to Wadumestrige Dona et al. (2021) research, types of urban agriculture models recorded (where we basically distinguish the production of food for own use, the production of food with the aim of generating profit, and the production of food where the primary goal is not profit but education, socializing and recreation on private or public land/facilities) were community gardens (and allotment garden as its sub-category), home gardens, individual gardens, commercial farms that include agro-technology farms, plant nurseries, semi-commercial farms, fully commercial farms, market gardening, horticulture and micro-farms, then institutional gardens that include agriculture activities associated with religious places, schools, prisons, hospitals, senior centres, and public housing developments, then guerrilla gardens, then controlled environment farms which use soilless systems and finally urban parks or city gardens.

Any plot of land that is grown by a group of people is called a community garden. Community gardens and allotments are mainly used for food production but there are several ways that urban community gardening can enhance people's physical and mental well-being. In urban settings, community gardens foster social capital, offer chances for selflessness, and produce a sustainable and easily accessible source of food (Zutter and Stoltz, 2022; FAO et al., 2022).

A roof garden is an open space that is covered with edible herbs, medicinal herbs or spices, and is separated from the ground by a construction facility (Kisić, 2018). In order to address issues with food security and advance sustainable agriculture, rooftop farming is one of the present needs and solutions (Thapa et al., 2020). Rooftop farming technologies enhance the quality of environmental life by incorporating inactive building roofs into urban ecology (Hui, 2011 as cited in Açıksöz et al., 2021). Urban areas can benefit from enhanced storm-water management, better building temperature regulation, a decrease in the effects of urban heat islands, and an increase in the habitat for urban wildlife thanks to green roofs (Oberndorfer et al., 2007). Small-scale plants cultivating on one's own terraces, balconies, and other covered locations is one of the most popular types of urban farming (Kisić, 2018; FAO et al., 2022).

Vertical farming is the process of growing crops in layers that are stacked vertically (Purabi, 2022). Vertical farms use one of three soilless systems for providing nutrients to plants – hydroponic, aeroponic or aquaponic (Birkby, 2016). Modern vertical farming concepts use indoor farming techniques and controlled environment agriculture (CEA) technology, which allow control of all environmental factors, including temperature, artificial lightning, humidity and biofortification (Purabi, 2022). Currently, it is just as cost-effective, scalable, and environmentally sustainable as traditional farming methods. Opportunities and challenges of vertical farming could be of economic, environmental, social and political matter (Van Gerrewey et al., 2022). Vertical farming faces several obstacles that prevent it from becoming widely used, such as high starting costs, a lack of experience and the need for a controlled growth environment. However, it can shorten the food supply chain, cut down on pesticide and fertilizer use, reduce water consumption and land damage (Oh and Lu, 2022).

The third fundamental component are microorganisms due to their role in nutrient transformation (Baganz et al., 2021). In 2018, 49 % of the world's fish were produced by aquaculture (FAO, 2022).

Agroforestry, defined as deliberate blending of land-use systems based on forestry and agriculture, has several advantages that can help maintain the diversity of ecosystems and the mechanisms that support environmental quality and long-term sustainability when applied in conjunction with an environmentally conscious land management system resulting in a multitude of advantages, such as increased vegetable yields due to higher soil fertility, a longer harvest season and better produce quality (Muhie, 2022).

The practice of urban beekeeping has become more popular despite possessing distinct goals from the extensive professional or commercial one and more people are beginning to consume honey produced in urban areas. Integrating honeybees into urban settings will aid in the spread of biodiversity particularly by protecting pollinators in urban settings through conservation of green spaces habitats (Quiralte, 2023; Szczurek, 2023). In addition to offering excellent educational opportunities, urban apiculture fosters a connection with nature, helps urban areas to become more environmentally conscious, and raises environmental awareness. The benefits of urban beekeeping may increase with the use of specialized technological infrastructure that attracts younger generation into beekeeping (Szczurek, 2023).

Consumers and farmers often also create connections through Community Supported Agriculture (CSA) that has a great potential to create connections between dimensions of social, economic, and ecological sustainability. A

fundamental tenet of CSA is the connection of farmers and customers (members) via long-term agreements that include upfront payments to cover production costs (Egli et al., 2023).

Concepts encompassing a variety of activities, from knowledge-based solutions to nature-based technologies, make up smart urban farming practices and mostly refers to the integration of smart technologies including sensors, automated decision systems, information and communication technology infrastructure into agriculture. Smart farming techniques are used at almost every stage of production from soil tillage to harvesting (Açıksöz et al., 2021).

Smart technologies

Precision farming is an agricultural approach that maximizes crop yields, reduces waste, and boosts production by using cutting-edge technology and data analysis (Karunathilake et al., 2023). Artificial intelligence (AI), robotics, Big Data, Blockchain Technology and the Internet of Things (IoT) brought to the fourth industrial revolution (Industry 4.0) in 2011 (Liu et al., 2021 and Marr, 2020 as cited in Karunathilake et al., 2023) as opposed to Agriculture 1.0 as a labor-intensive and low-productive producing system, Agriculture 2.0 that increased productivity and reduced costs by using a variety of machines, fertilizers, and pesticides in the production process and Agriculture 3.0 which refers to the 1990s agricultural production period when multiple technological applications were included and Global Positioning System (GPS) signals were integrated (Açıksöz et al., 2021).

Novel trends in precision agriculture are Big Data, Machine Vision Technology, Internet of Things, Artificial Intelligence, Machine Learning (ML), and Deep Learning (DL), Guidance Systems, Blockchain Technology, Robotics and Autonomous Systems, Artificial Satellites, Unmanned Aerial Vehicles (UAVs) and Unmanned Ground Vehicles (UGVs), High-throughput Phenotyping and Telematics. For small-scale farmers, the initial cost of technology, worries about data privacy and incompatibilities with current farming techniques can be major obstacles for precision farming general adoption (Karunathilake et al., 2023).

According to Oh and Lu (2022) the state of the art technologies in vertical/urban farming are Big Data, Internet of Things for online and automated monitoring and smart systems with real-time hardware, and other technologies based on evaluation and validation of data by the automated monitoring and controlling system.

Smart agriculture can be viewed as a management approach that makes use of information and understandings gathered from both agri-food operations and research projects. Large volumes of data from many sensors and processes can be analysed and searched for interactions using artificial intelligence systems. "Artificial intelligence refers to systems designed by humans that, given a complex goal, act in the physical or digital world by perceiving their environment, interpreting the collected structured or unstructured data, reasoning on the knowledge derived from this data and deciding the best action (s) to take (according to pre-defined parameters) to achieve the given goal. AI systems can also be designed to learn to adapt their behaviour by analysing how the environment is affected by their previous actions" (STOA, 2023).

Application of robotics and autonomous systems enables automation, precision tasks and labour reduction while at the same time representing an expensive solution with restricted capacity to adjust to shifting field conditions (Karunathilake et al., 2023; Reshma et al., 2020).

Blockchain soon rose to prominence as a crucial technology in many precision farming applications and can be defined as decentralized (plus three more features as persistency, anonymity, auditability), distributed ledger that stores time-stamped transactions between numerous computers in a peer-to-peer network. Blockchain has the potential to improve precision farming in a number of ways and enable many applications such as smart farming, supply chain tracking and monitoring, finance management, data security, and assurance. Blockchain technology may be integrated with other systems in developing smart systems (Torky and Hassanien, 2020).

As Big Data technologies can handle massive volumes of data, academics are very interested in them. Big Data is used throughout the supply chain, not just in farming operations. New applications of big data in agriculture are continuously being developed. Some of methods, platforms or tools discussed in observed studies were: Zigbee protocol, wireless sensor node network, Regression model and Artificial Neural Network (ANN) models, Hadoop framework, 3d reconstruction, NoSQL database, Profile-based architecture, Hadoop and Hive tools, Internet of Things, Frame designation and design process, Data mining techniques (PAM, CLARA, and DBSCAN, IoT and cloud-based Big Data analysis, Wireless Sensor Network (WSN), High-Performance Computing (HPC) infrastructure (Sourav and Emanuel, 2021).

The Internet of Things can be defined as a network of intelligent, networked devices that can exchange important data with one another and with each other about the environment in which they function (Madakam et al., 2015). In the context of the Internet of Things, precision farming is defined by the gathering of data from multiple sensors placed across the farm. The characterization of these data is spatial, temporal, and semantic, which makes managing and implementing models and repositories more difficult. A lot of work is being done to solve this problem. Data management systems need to be capable of responding to the questions posed by farmers. The data generated from the farmer's questions by decomposition of the queries is used as input for Machine Learning models or Decision Support Systems (DSS), providing the farmer with a much more helpful and understandable response (De la Parte et al., 2023). Data mining, data analysis and decision support systems are becoming important tools for managing a variety of services in precision farming (Zhai et al., 2020).

Conclusions

Urban farming as agricultural practice that takes place in cities and their immediate surrounding areas includes traditional farming, allotment gardens, rooftop gardens, hydroponics, aquaponics and indoor vertical farming and will most likely play a significant role in agriculture's fight to feed a growing world's population. It is widely acknowledged that urban farming has many advantages over conventional farming, such as higher productivity, enhanced sustainability, shorter food supply chain, year-round access to fresh food and that at the same time it supports biodiversity in cities, improves climate conditions, contributes to soil conservation and promotes waste recycling but the question is whether and how it can overcome obstacles such as association with health risks as a result of crops grown in urban areas being exposed to higher pollution levels than crops grown in rural areas or, in cases such as vertical farming, high starting costs, a lack of experience and the need for a controlled growth environment. However, integrating smart technology makes urban farming more efficient and sustainable but for small-scale farmers starting cost of technology itself, software, hardware, equipment, technical support, training costs, concerns about data privacy and data quality due to incompatibilities with current farming systems can be major obstacles for precision farming widespread adoption. Better and less expensive technology solutions and more training and education may be an answer but are we going towards better technologies or just more user-friendly? Cutting-edge technology and data analysis include Artificial intelligence (AI), Robotics, Big Data, Blockchain Technology and the Internet of Things (IoT). As much as we know about them, some are as much unknown to us. Finally, what would be the main advantage of precise urban and peri-urban farming? In our opinion, the biggest impact will be on food security.

References

- Açıksöz S., Dal I., Özbek M.Ö. (2021). Smart Urban Agriculture. In *Developments in Engineering and Architecture*. Rangelov B., Berdenov Z. G., Efe R. (eds.), 102–114. Sofia, Bulgaria: St. Kliment Ohridski University Press.
- Baganz G.F.M., Junge R., Portella M.C., Goddek S., Keesman K.J., Baganz D., Staaks G., Shaw C., Lohrberg L., Kloas W. (2021). The aquaponic principle – It is all about coupling. *Reviews in Aquaculture*. 14: 252–264.
- Birkby J. (2016). Vertical Farming. In: ATTRA Sustainable Agriculture program. Mumma T. (ed.), 1–12. Butte, USA: National Center for Appropriate Technology.
- De la Parte M.S.E., Martínez-Ortega J-F, Díaz V.H., Martínez N L. (2023). Big Data and precision agriculture: a novel spatio-temporal semantic IoT data management framework for improved interoperability. *Journal of Big Data*. 10: 52.
- Dubbeling M., van Veenhuizen R., Halliday J. (2019). Urban agriculture as a climate change and disaster risk reduction strategy. *Field Actions Science Reports*. Special Issue 20 Urban Agriculture: Another Way to Feed Cities: 32–39.
- Egli L., Rüschoff J., Priess J. (2023). A systematic review of the ecological, social and economic sustainability effects of community-supported agriculture. *Frontiers in Sustainable Food Systems*. 7: 1136866.
- FAO (2022). The State of World Fisheries and Aquaculture. Rome, Italy: Food and Agriculture Organization. Available from:

- <https://www.fao.org/3/cc0461en/online/sofia/2022/world-fisheries-aquaculture.html>
- FAO, Rikolto, RUAFA. (2022). Urban and peri-urban agriculture sourcebook – From production to food systems. Rome, Italy: Food and Agriculture Organization and Rikolto. Available from: <https://www.fao.org/3/cb9722en/cb9722en.pdf>
- Goldstein B., Hauschild M., Fernández J., Birkved M. (2016). Urban versus conventional agriculture, taxonomy of resource profiles: a review. *Agronomy for Sustainable Development*. 36: 9.
- Ilieva R.T., Cohen N., Israel M., Specht K., Fox-Kämper R., Fargue-Lelièvre A., Poniz' y L., Schoen V., Caputo S., Kirby C. K., Goldstein B., Newell J.P., Chris Blythe C. (2022). The Socio-Cultural Benefits of Urban Agriculture: A Review of the Literature. *Land*. 11: 622.
- Karishma S. (2022). Agriculture in Urban Culture: A Review on Terrace Farming. *Advances in Crop Science and Technology*. 10: 489.
- Karunathilake E.M.B.M., Le A.T., Heo S., Chung Y.S., Mansoor S. (2023). The Path to Smart Farming: Innovations and Opportunities in Precision Agriculture. *Agriculture*. 13: 1593.
- Khan N., Lau T. C., Tan B.C. (2023). Adoption of smart urban farming to enhance social and economic well-being of elderly: a qualitative content analysis. *Food Research*. 7: 114–118.
- Kisić I. (2018). *Gradska poljoprivreda*. Zagreb, Croatia: Agronomski fakultet Sveučilišta u Zagrebu.
- Madakam S., Ramaswamy R., Tripathi, S. (2015). Internet of Things (IoT): A Literature Review. *Journal of Computer and Communications*. 3: 164–173.
- Muhie S.H. (2022). Novel approaches and practices to sustainable agriculture. *Journal of Agriculture and Food Research*. 10: 100446.
- Oberndorfer E., Lundholm J., Bass B., Coffman R.R., Doshi H., Dunnett N., Gaffin S, Köhler M, Liu K.K.Y., Rowe B. (2007). Green Roofs as Urban Ecosystems: Ecological Structures, Functions, and Services. *BioScience*. 57: 823–833.
- Oh S., and Lu C. (2022). Vertical farming - smart urban agriculture for enhancing resilience and sustainability in food security. *The Journal of Horticultural Science and Biotechnology*. 98: 133–140.
- Pradhan P., Callaghan M., Hu Y., Dahal K, Hunecke C., Reusswig F, Lotze-Campen H, Kropp J.P. (2023). A systematic review highlights that there are multiple benefits of urban agriculture besides food. *Global Food Security*. 38: 100700.
- Purabi B., Pritam G., Udit D. (2022). Vertical farming – an overview. *Plant Archives*. 22: 223–228.
- Quiralte D., Zarzo I., Fernandez-Zamudio M.-A., Barco H., Soriano J.M. (2023). Urban Honey: A Review of Its Physical Chemical and Biological Parameters That Connect It to the Environment. *Sustainability*. 15: 2764.
- Reshma R., Nikhil G., Aarya Y., Patil Y., Apurv, S. (2020). Precision Agriculture and Robotics. *International Journal of Engineering Research & Technology*. 9: 17–22.
- Rouquette L. (2017). *L'agriculture urbaine en Europe*. Bruxelles, Belgium: Pour la solidarité.
- Szczurek A., Maciejewska M., Batog P. (2023). Monitoring System Enhancing the Potential of Urban Beekeeping. *Applied Sciences*. 13: 597.
- Smit J., Nasr J., Ratta A. (2001). Urban Agriculture Yesterday and Today. In *Urban Agriculture: Food, Jobs and Sustainable Cities*. Smit J., Nasr J., Ratta A. (eds.), 1–31. New York, USA: UNDP, Urban Agriculture Network, Inc.
- Sourav A.I., and Emanuel A.W.R. (2021). Recent Trends of Big Data in Precision Agriculture: a Review. *OP Conf. Series: Materials Science and Engineering*. 1096: 012081.
- STOA (2023). Artificial intelligence in the agri-food sector. Bruxelles, Belgium: Scientific Foresight Unit. European Union. Available from: [https://www.europarl.europa.eu/RegData/etudes/STUD/2023/734711/EPRS_STU\(2023\)734711_EN.pdf](https://www.europarl.europa.eu/RegData/etudes/STUD/2023/734711/EPRS_STU(2023)734711_EN.pdf)
- Taguchi M., and Santini G. (2019). Urban agriculture in the Global North & South: a perspective

- from FAO. *Field Actions Science Reports*. Special Issue 20 Urban Agriculture: Another Way to Feed Cities: 12-17. Available from: <https://journals.openedition.org/factsreports/5536>
- Thapa S., Nainabasti A., Sashila Acharya S., Rai N., Bhandari R. (2020). Rooftop Gardening as A Need for Sustainable Urban Farming: A case of Kathmandu, Nepal. *International Journal of Applied Sciences and Biotechnology*. 8: 241–246.
- Torky M., and Hassanien A.E. (2020). Integrating Blockchain and the Internet of Things in Precision Agriculture: Analysis, Opportunities, and Challenges. *Computers and Electronics in Agriculture*. 178: 105476.
- UN (2019). *Global Issues: Population*. New York, USA: United Nations. Available from: <https://www.un.org/en/global-issues/population>
- Van Gerrewey T., Boon N., Geelen D. (2022). Vertical Farming: The Only Way Is Up? *Agronomy*. 12: 12010002.
- Wadumestrige Dona C.G., Mohan G., Fukushi K. (2021). Promoting Urban Agriculture and Its Opportunities and Challenges – A Global Review. *Sustainability*. 13: 9609.
- Xi L., Zhang M., Zhang L., Lew T., Lam, Y. (2021). Novel Materials for Urban Farming. *Advanced Materials*. 34: 202105009.
- Zhai Z., Martinez J.F., Beltran V., Martinez N.L. (2020) Decision support systems for agriculture 4.0: Survey and challenges. *Computers and Electronics in Agriculture*. 170: 105256.
- Zutter C., and Stoltz A. (2022). Community gardens and urban agriculture: Healthy environment/ healthy citizens. *International Journal of Mental Health Nursing*. 32: 1452–146.

Utjecaj laserskih tretmana na klijavost pšenice

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Sažetak

Cilj rada bio je utvrditi mogućnost primjene laserskog zračenja kao stimulatora klijavosti sjemena pšenice. Sjeme pšenice tretirano je laserima izlaznih snaga od 100 mW, 200 mW i 500 mW. Laserski tretmani provedeni su u trajanju od 30, 60 i 120 sekundi. Svaki tretman proveden je u 3 ponavljanja. Pri provedbi tretmana sjeme je pozicionirano u elementarnom sloju direktno ispod raspršenog snopa koherentne svjetlosti, unutar osvijetljenog područja. Količina isporučene energije kontrolirana je trajanjem izloženosti sjemena laserskom zračenju. Na temelju analize rezultata energije klijanja i standardne klijavosti vidljivo je da su najbolji rezultati postignuti tretmanima 100 mW laserom, među kojima se najboljim pokazao tretman u trajanju od 30 s. Tretmani 200 mW laserom u trajanju od 30, 60 i 120 s te 500 mW u trajanju od 60 i 120 s uzrokovali su smanjenje energije klijanja i standardne klijavost. Na osnovu dobivenih podataka vidljivo je da je nužno istražiti i pronaći ravnotežni ishod između izlazne snage lasera, fokusiranja laserskog snopa, vremena tretiranja materijala i biološkog efekta procesa kako bi se lasersko zračenje moglo uspješno koristiti u području biostimulacije.

Ključne riječi: energija klijanja, klijavost, laser, pšenica

Uvod

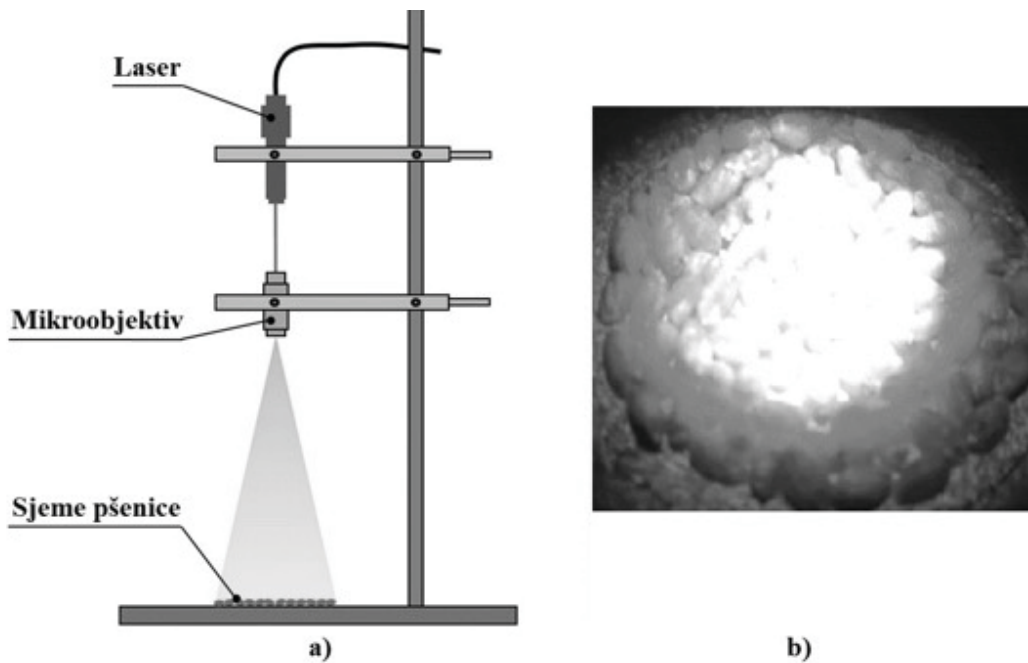
Pšenica (lat. *Triticum*) jedna je od najznačajnijih žitarica na svijetu. Od ukupnih poljoprivrednih površina, najveći dio opada na uzgoj pšenice a svjetska trgovina pšenicom je veća od trgovine svim drugim žitaricama zajedno (Shewry, 2009). Oko 70 % globalne proizvodnje ove kulture koristi se za ljudsku prehranu, 20 % za stočarstvo, a 2-3 % u industrijskom sektoru (farmaceutskoj, tekstilnoj, industriji papira te mnogim drugim industrijama). Potražnja za ovom žitaricom raste kako raste populacija a najveći dio proizvodnje služi za proizvodnju mlinskih proizvoda te za proizvodnju tjestenine, kruha i sličnih proizvoda (Shevkani i sur., 2016). Danas se s ciljem proizvodnje što kvalitetnijeg sjemenskog materijala koriste razne tehnologije od kojih većina može imati negativne posljedice za ljudsko zdravlje i okoliš. Kao alternativno rješenje za povećanje fiziološke kvalitete sjemena može se koristiti elektromagnetsko zračenje u vidu koherentnog svijetla. Laseri kao izvori koherentnog zračenja sve više dobivaju na značaju u području biostimulacije pri čemu biljnim stanicama predaju dodatnu energiju što za posljedicu može imati pozitivan utjecaj na energiju klijanja i standardnu klijavost sjemenskog materijala različitih biljnih vrsta. Budući da je interakcija između lasera i biljnog materijala složen proces, nužno je istražiti usklađivost između izlazne snage lasera, duljine tretmana i biološkog efekta procesa. Upravo iz tog razloga ovaj rad analizira mogućnost primjene lasera kao neinvazivne metode za unaprjeđenje poljoprivredne proizvodnje. Iako su pozitivna svojstva laserskog zračenja u pogledu klijavosti do sada opsežno analizirana i dokazana, od iznimne je važnosti pravilno primjenjivati ove tehnologije kako bi se postigli najbolji rezultati.

Materijal i metode

Za analize je korištena sorta pšenice Kraljica Poljoprivrednog instituta Osijek. To je visokorodna sorta koja u velikoj mjeri objedinjuje rodost i kakvoću, a spada među najraširenije sorte u proizvodnji u Republici Hrvatskoj. Njezin genetski potencijal za prinos je veći od 11 tona po hektaru, a spada u I. razred kakvoće. Hektolitarska masa zrna iznosi oko 81 kg/hl, a masa 1000 zrna u prosjeku iznosi 40 grama što ukazuje na solidnu kvalitetu zrna. Glavni elementi opreme za provođenje laserskog tretmana su izvori koherentne svjetlosti (laseri) i mikroobjektiv sastavljen od dvije optičke leće kojim se postiže širenje laserske zrake. Sjeme pšenice tretirano je trima izvorima koherentne svjetlosti izlaznih snaga od 100 mW (modeli: HLM1845), 200 mW (HLP18130) i 500 mW (HJ-308). Sjemenski materijal je

tretiran koherentnom svjetlošću stacionarno u elementarnom sloju unutar osvijetljenog područja promjera 5 cm, u trajanju od 30, 60 i 120 sekundi. Sva tri izvora koherentne svjetlosti imaju istu valnu duljinu od 650 nm, što znači da su u crvenom dijelu vidljivog spektra svjetlosti. Različite izlazne snage su korištene kako bi se utvrdio njihov utjecaj na energiju klijanja i standardnu klijavost.

Da bi se postiglo širenje koherentne svjetlosti u okviru zadanog radijusa udaljenost lasera i mikroobjektiva iznosila je 60 mm a udaljenost između mikroobjektiva i tretiranog sjemena 170 mm (slika 1).



Slika 1. a) Smještaj izvora koherentne svjetlosti i položaj mikroobjektiva; b) prikaz laserskog tretmana sjemena pšenice

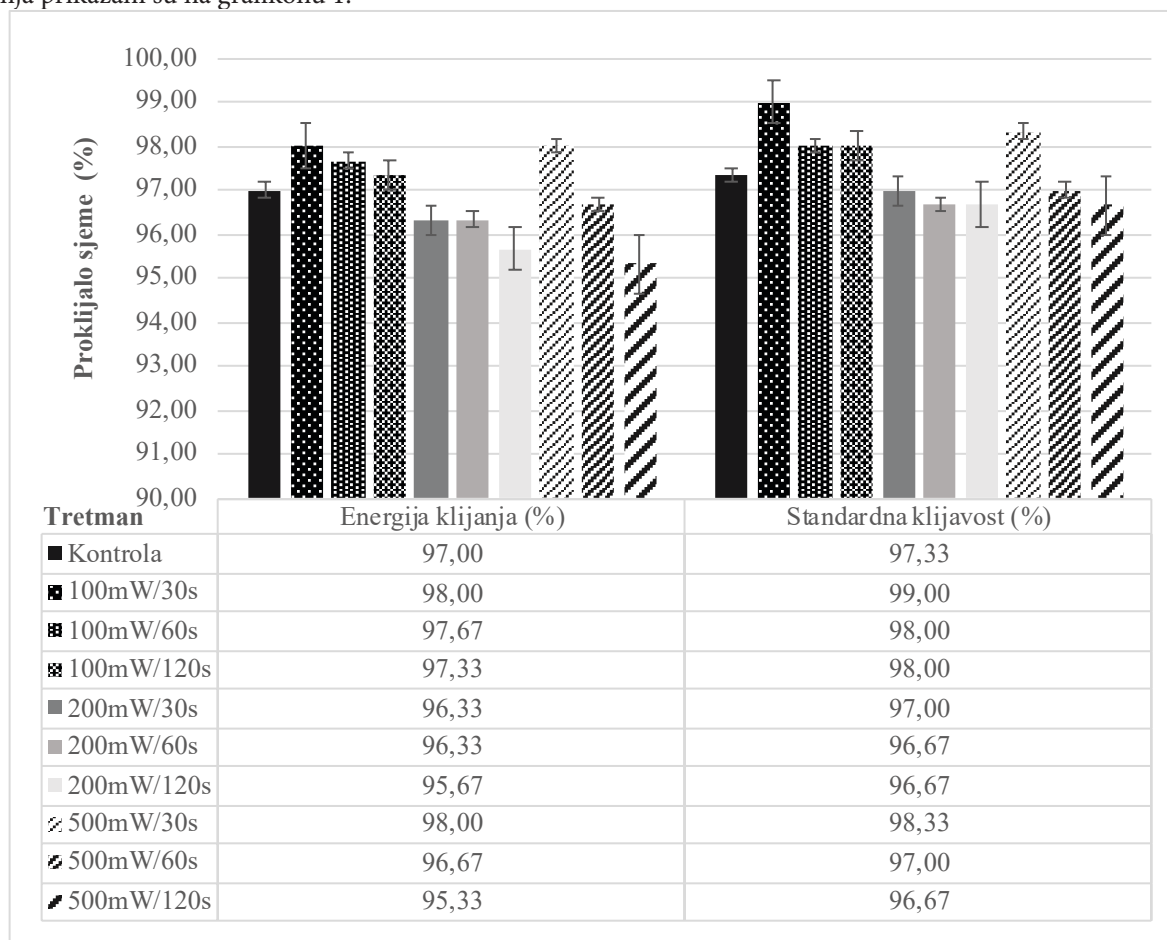
Postupak naklijavanja proveden je prema Pravilniku o temeljnim zahtjevima kakvoće, načinu ispitivanja, pakiranju i deklariranju sjemena poljoprivrednog bilja (NN 4/2005). Za potrebe analize energije klijanja i standardne klijavosti pripremljene su Petrijeve zdjelice s podlogom koja dobro upija vodu i koja je bez prisutnosti gljivica, bakterija ili toksičnih dodataka koji bi mogli utjecati na klijavost. Podloga je porozna, ali opet toliko zbijena da korijen raste na površini i ne prodire u nju i mora upijati dovoljno vode da ostane vlažna sve vrijeme ispitivanja, s pH vrijednošću između 6,0 i 7,5. Za vlaženje podloge korištena je destilirana voda bez prisutnosti organskih i anorganskih primjesa.

Kontrolni uzorak i tretirano sjeme pšenice pravilno su raspoređeni u Petrijeve zdjelice, u kojima je prethodno postavljena podloga navlažena s 2 ml destilirane vode. Nakon pripreme, Petrijeve zdjelice s uzorcima stavljene su u komoru za naklijavanje. Broj proklijalih sjemenki kontroliran je svakih 48 sati, pri čemu se proklijalom sjemenkom smatrala ona čiji je korjenčić ≥ 2 mm. Energija klijavosti određena se 6., a standardna klijavost 12. dan provođenja eksperimenta (ISTA, 2011).

Rezultati i rasprava

Svjetlosni podražaja imaju značajan utjecaj na žive organizme a tom činjenicom se može objasniti djelovanje koherentnog zračenja niskoenergetskih netermalnih lasera koji su korišteni u ovim pokusima. Unatoč tome što takvi laseri imaju znatno manju izlaznu snagu od termalnih oni ipak mogu imati određeni utjecaj na energiju klijanja i standardnu klijavost sjemena. S ciljem utvrđivanja svojstava koherentnog zračenja valne duljine 650 nm, provedena su mjerenja dozračene energije tretiranom materijalu. Izračunom energije predane zrnatom materijalu utvrđeno je da ona iznosi $46,73 \text{ Wsm}^{-2}$ za 100 mW laser, $93,47 \text{ Wsm}^{-2}$ za 200 mW laser i $233,68 \text{ Wsm}^{-2}$ za 500 mW laser. Iz rezultata je vidljivo da je količina energije koju odaju odabrani laseri relativno mala, pa se može zaključiti da je njihov utjecaj na sjeme pšenice temeljen isključivo na biostimulaciji. Rezultati analize standardne klijavosti i energije

klijanja prikazani su na grafikonu 1.



Grafikon 1. Energija klijanja i standardna klijavost pšenice

Prosječna vrijednost energije klijanja kontrolnog uzorka iznosila je 97,00 %. Nakon provedenih tretmana porast energije klijanja zabilježen je kod svih uzoraka tretiranih 100 mW laserom i to za 1,00 % kod uzorka tretiranih 30 s, 0,67 % kod uzoraka tretiranih 60 s i 0,33 % kod uzoraka tretiranih 120 s. Porast energije klijanja od 1% također je zabilježen i kod uzoraka tretiranih 500 mW laserom u trajanju od 30 s. Kod svih ostalih uzoraka zabilježeno je smanjenje energije klijanja. Najizrazitije smanjenje energije klijanja u iznosu od 1,67 % zabilježeno je kod uzorka tretiranih 500 mW laserom u trajanju od 120 s.

Prosječna vrijednost standardne klijavosti kod kontrolnog uzorka iznosila je 97,33 %. Kao i kod energije klijanja povećanje klijavosti zabilježeno je kod svih uzoraka tretiranih 100 mW laserom i to za 1,67 % kod uzorka tretiranih 30 s i 0,67 % kod uzoraka tretiranih 60 i 120 s. Povećanje klijavosti od 1,00 % također je zabilježeno i kod uzoraka tretiranih 500 mW laserom u trajanju od 30 s. Kod svih ostalih uzoraka zabilježeno je smanjenje klijavosti pri čemu su najveća smanjenja klijavosti od 0,66 % zabilježena kod tretmana 200 mW laserom u trajanju od 60 i 120 s te 500 mW laserom u trajanju od 120 s.

Uzevši u obzir izlazne snage lasera vidljivo je da je 100 mW laser uzrokovao najveći porast energije klijanja i standardne klijavosti. Rezultati dobiveni za tretman 100 mW laserom su u skladu s rezultatima koje iznose Gładyszewska (2006), Abu-Elsaoud i Tuleukhanov (2013), Hernandez i sur. (2016), Janayon i Guerrero (2019) te Hasan i sur. (2020) koji navode da tretman niskoenergetskim laserima uglavnom ima pozitivan utjecaj na povećanje energije klijanja i standardne klijavosti. Gładyszewska (2006), te Hasan i sur. (2020) navode biostimulaciju, tj. pretvorbu laserske svjetlosti u kemijsku energiju za kasnije korištenje, kao jednu od najznačajnijih mogućnosti primjene lasera u poljoprivredi. Hernandez i sur. (2010) navode da je apsorbirana energija elektromagnetskog zračenja u stanju unutar sjemena pokrenuti određene fiziološke i biokemijske procese. Dinoev i sur. (2004) iznose da čak i nefotosintetske stanice i materijali (npr. sjeme) imaju mogućnost apsorpcije svjetlosne energije te njeno pretvaranje u kemijsku

energiju za korištenje u daljnjem rastu i razvoju. Chen i sur. (2005) navode da zbog utjecaja laserskog zračenja na stanične molekule dolazi do povećane enzimske aktivnosti u sjemenu pri čemu se povećava biološka aktivnost, entropija kao i inicijalna energija koju sjeme sadrži.

Nasuprot navedenom, kod svih uzoraka tretiranih 200 mW laserom i kod uzoraka tretiranih 500 mW laserom u trajanju od 60 i 120 s došlo je do smanjenja energije klijanja i standardne klijavosti. To se može objasniti činjenicom da pojedine vrste zračenja mogu biti neprikladne jer imaju sposobnost da u potpunosti prodru u sjeme te nepovoljno djeluju na njegova reprodukcijska svojstva. Stoga je neophodno količinu dozračene energije prilagoditi ovisno o morfologiji materijala koji se tretira. Smanjenje energije klijanja i klijavosti je u skladu s rezultatima od Fanaro i sur. (2007) te te Zago i Rela (2007) koji navode da ukoliko unutarnji dio sjemena upije preveliku količinu energije, ta energija može oštetiti stanice embrija, izazivajući smrtnost ili mutagenost a time i smanjenje klijavosti.

Imajući u vidu do sada provedena istraživanja, potrebno je naglasiti da biostimulacija laserom još uvijek nije u potpunosti ispitana i potrebna su još mnoga ispitivanja kako bi se njezino pozitivno djelovanje moglo potvrditi i na drugim kulturama. Biostimulacija ne djeluje isto na sve kulture, i stoga je nužno istražiti i pronaći ravnotežni ishod između izlazne snage lasera, fokusiranja laserskog snopa, vremena tretiranja materijala i biološkog efekta procesa.

Zaključak

Na temelju dobivenih rezultata može se zaključiti da je najpovoljniji utjecaj na energiju klijanja i standardnu klijavost imao tretman 100 mW laserom. Tretman 100 mW laserom u trajanju od 30 s uzrokovao je najznačajniji porast energije klijanja u iznosu od 1.00 % i standardne klijavosti u iznosu od 1.67 %. Tretman laserskim zračenjem od 200 mW u trajanju od 30, 60 i 120 s te 500 mW u trajanju od 60 i 120 s nije pozitivno utjecao na klijanje, već da je uzrokovao smanjenje energije klijanja i standardne klijavost. Pri tome je najveće smanjenje energije klijanja iznosilo 1,67 % kod 500 mW lasera u trajanju od 120 s, a standardne klijavosti 0,66 % kod uzoraka tretiranih 200 mW laserom u trajanju od 60 i 120 s te 500 mW laserom u trajanju od 120 s. Razlog smanjenja klijavosti može se objasniti činjenicom da lasersko zračenje može izazvati i negativne promjene na sjemenskom materijalu, pa tako i uzrokovati ozbiljna oštećenja reproduktivnog dijela sjemena čime se smanjuje ili onemogućuje sposobnost klijanja.

Literatura

- Abu-Elsaoud A.M., Tuleukhanov S.T. (2013). Can He–Ne laser induce changes in oxidative stress and antioxidant activities of wheat cultivars from Kazakhstan and Egypt?. *Journal of Ecology of Health & Environment*. 1: 39-50.
- Chen Y.P., Liu Y.J., Wang X.L., Ren Z.Y., Yue M. (2005). Effect of microwave and He-Ne laser on enzyme activity and biophoton emission of *Isatis indigotica* Fort. *Journal of Integrative Plant Biology*. 47: 849–855.
- Dinoev S., Antonov M., Stoyanov T., Georgieva C. (2004). Spectral impact of lowpower laser radiation on wheat and maize parameters, *Prob. Engineer. Cybernet. Robotics*. 54: 74-85.
- Fanaro G.B., Silva P.V., Nunes T.C.F., Rogovschi V.D., Aquino S., Villavicencio A.L.C. H. (2007). Effects of Electron Beam Treatment in Soybean Grains Artificially Inoculated by *Phakopsora pachyrhizi*, *AccApp'07*, Pocatello, Idaho, July 29-August 2, 2007.
- Gładyszewska B. (2006). Pre-sowing laser biostimulation of cereal grains. *Technical Sciences*. 9: 33-38.
- Hasan M., Hanafiah M.M., Aeyad Taha Z., AlHilfy I.H.H., Said M.N.M. (2020). Laser irradiation effects at different wavelengths on phenology and yield components of pretreated maize seed. *Applied Sciences*. 10: 1189.
- Hernandez A.C., Dominguez P.A., Cruz O.A., Ivanov R., Carballo C.A., Zepeda B.R. (2010). Laser in agriculture. *International Agrophysics*. 24: 407-422.
- Hernandez A.C., Domínguez P.A., Cruz O.A., Podlesna A., Ivanov R., Carballo Carballo A., Pérez Reyes M.C., Sanchez Hernandez G., Zepeda Bautista R., López-Bonilla J.L. (2016). Laser biostimulation in seeds and plants *Gayana Bot*. 73: 132–149.
- ISTA (2011). *International Rules for Seed Testing*. International. Seed Testing Association,

Bassersdorf, Switzerland.

Janayon R.V B., and Guerrero R.A. (2019). Laser irradiation of mung bean (*Vigna radiata* L.) at two wavelengths for enhanced seedling development. *International Journal of Optics*. 3: 1-7.

Pravilnik o temeljnim zahtjevima kakvoće, načinu ispitivanja, pakiranju i deklariranju sjemena poljoprivrednog bilja. *Narodne novine* (2005.). Zagreb: Narodne novine d.d.

Shevkani K., Singh N., Bajaj R., Kaur A. (2016). Wheat starch production, structure, functionality and applications—a review. *International Journal of Food Science and Technology*. 52: 38–58.

Shewry P.R. (2009). “Wheat”, *Journal of Experimental Botany*. 60: 1537–1553,

Zago C., and Rela P.R. (2007). Technical Feasibility for Electron Beam Application on Maize Seeds Disinfection for Maize Cultivation in Brazil, *International Nuclear Atlantic Conference INAC 2007 Santos, SP, Brazil, September 30 to October 5, 2007 Associação Brasileira de Energia Nuclear – ABEN*.

Effect of laser treatments on wheat germination

Abstract

The aim of the work was to determine the possibility of using laser radiation as a stimulator for wheat seed germination. Wheat seeds were treated with lasers with an output power of 100 mW, 200 mW, and 500 mW. Laser treatments lasted 30, 60, and 120 seconds. Each treatment was performed in 3 replicates. During the treatment, the seed in the elemental layer is positioned directly under the scattering beam of coherent light in the illuminated area. The amount of energy delivered is controlled by the duration of exposure of the seed to the laser radiation. The analysis of the results of germination energy and standard germination shows that the best results were obtained in the treatment using a 100 mW laser, where the treatment with a duration of 30 s proving to be the best. Treatments with a 200 mW laser for 30, 60 and 120 s and 500 mW laser for 60 and 120 s resulted in a decrease in germination energy and standard germination. Based on the data obtained, it is clear that it is necessary to study and find a balance between the laser power, the focusing of the laser beam, the duration of the material treatment and the biological effect of the process, so that laser radiation can be successfully used in the field of biostimulation.

Keywords: germination energy, germination, laser, wheat

Inter- and intra-annual specificities of alfalfa and clover-grass mixtures yield prediction using Sentinel-2 satellite imagery

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Abstract

The goal of this research was to determine the relationship between NDVI and dry matter yield of forage crops between different dates intra- and inter-annually between the years 2021 and 2022. The research was conducted on an experimental field using two clover-grass mixtures and two alfalfa varieties. Field data was compared with satellite imagery and determination coefficient (R^2) was determined. This research showed that it is possible to forecast the yield using Sentinel-2 satellite images. The poor predictability of the 2021 NDVI before the first harvest indicates possible problems caused by NDVI saturation. The results showed that in perennial plantations of alfalfa and clover-grass mixtures, it is possible to predict the yield using Sentinel-2 satellite imagery within one season, but also between years.

Keywords: NDVI, Sentinel 2, yield prediction, alfalfa, clover-grass mixture

Introduction

Traditional yield assessment is based on destructive sampling which is to manually collect data samples in the field and weigh the samples to determine the yield but advances of remote sensing techniques provide more efficient way to monitor the crop growth and yield analysis. In the last few decades, satellite remote sensing has been widely used in agriculture (Feng et al., 2020). NDVI is the oldest and most commonly used vegetation index (Rouse et al., 1973; Panek and Gozdowski, 2020) and many studies emphasize the possibility of yield estimation in agriculture based on NDVI data (Smith et al., 1995; Singh et al., 2002; Becker-Reshef et al., 2010; Petersen, 2018; Yu and Shang, 2018). Usually univariate linear regression is used as the statistical model for predicting yields, and fairly reliable yield estimates with high R^2 have been achieved so far. Alfalfa yield was predicted in many studies using different vegetation indices and satellite data from various satellites like a Landsat, Quickbird, Sentinel but also using satellite data fusion (Pan et al., 2009; Chen and Zhang, 2023). Alfalfa and clover-grass mixtures represent an interesting model for study because they combine different botanical components that react differently to environmental conditions.

The goal of this research, which was made as part of the student's diploma thesis, is dual 1) to determine the relationship between NDVI and dry matter (DM) $t\ ha^{-1}$ between different dates within each of the years 2021 and 2022 and 2) between the years 2021 and 2022.

Material and methods

The research was conducted on an experimental field located in Zagreb near the Faculty of Agriculture at the experimental site Maksimir (λ 16°01'57.5"E, (ϕ) 45°49'43.0"N. The climate of that area is a moderate continental climate (DHMZ, 2022). The experiment was set up in the spring of 2020 on a 1 ha field which is divided into 16 plots with an area of 625 m² (25 m × 25 m) (Figure 1). The experimental scheme was set up according to the scheme of randomized block arrangement in four replications with four treatments. The treatments were determined by the different composition of clover grass mixtures and alfalfa and by different sowing rates (Table 1). In the experiment, the seeds of two varieties of alfalfa 'Sibemol' and 'Power 4.2' with a sowing rate of 25 kg ha⁻¹ and from two clover-grass mixtures "CutMax Original" were used (composition: 15% *Trifolium pratense*, 20% *Festulolium*, 20% *Lolium*

perenne, *Phleum pratense*, 20% *Dactylis glomerata*, 10% *Festuca arundinacea*), and “CutMax Alfa” (composition: 10% *Trifolium pratense*, 30% *Medicago sativa*, 25% *Festulolium*, 10% *Lolium perenne* 4n, 15% *Phleum pratense*, 10% *Dactylis glomerata*), both sowing rates 35 kg/ha. One treatment was sown on each plot according to the principle of random distribution.

P No.1	P No. 2	P No. 3	P No. 4	P No. 5	P No. 6	P No. 7	P No. 8
R-1	R-1	R-2	R-2	R-3	R-3	R-4	R-4
T-1	T-3	T-2	T-1	T-4	T-1	T-3	T-4
P No. 16	P No. 15	P No.14	P No. 13	P No. 12	P No. 11	P No. 10	P No. 9
R-1	R-1	R-2	R-2	R-3	R-3	R-4	R-4
T-2	T-4	T-4	T-3	T-2	T-3	T-1	T-2

Figure 1. Experimental scheme; P No – parcel number, R – repetition, T - treatment

In the following text, the mixture “CutMax Original” will be referred to as treatment no. 1, “CutMax Alfa” as treatment no. 2, variety ‘Power 4.2.’ as treatment no. 3, and the variety ‘Sibemol’ as treatment no. 4 (Table 1)

Table 1. Used mixtures and varieties expressed as treatments

Treatment	Name	Amount of seed
T-1	CutMax Original	35 kg ha ⁻¹
T-2	CutMax Alfa Protein	35 kg ha ⁻¹
T-3	Lucerna Power 4.2	25 kg ha ⁻¹
T-4	Lucerna Sibemol	25 kg ha ⁻¹

The collection of samples from the field was carried out on the day of mowing, which in the experiment from 2020 to 2022 was carried out every year on several occasions. The first cut was in May, and the second in June. For this research, data obtained from cutting on May 31, 2021, May 19, 2022, and June 28, 2022 were used. At each cut, the green plant mass (GM) was weighed from the lawn mower’s 2.35 m x 4,2 m surface area. (9.87 m²). From each weighing scale, a sub-sample weighing approx. 1 kg was collected and dried in an oven for 48 hours at 60 °C. The values obtained by weighing the weight of the subsamples were used to calculate the dry matter yield (DM) expressed in t ha⁻¹. Satellite images of the Sentinel 2 mission with atmospherically corrected surface reflectance (SS2MSI2A – Level2A) were downloaded from the Copernicus Open Access Hub website (<https://scihub.copernicus.eu/dhus/#/home>). The images were downloaded from March to June 2021 and 2022. The criteria for selecting suitable recordings was the absence of cloud cover and the requirement that they be as close as possible before the mowing date (Table 2). Calculation of the NDVI vegetation index was carried out in QGIS using a raster calculator. Two spectral bands (bands), band 4 (red) and band 8 (near infrared) were used. For this research, images with a spatial resolution of 10 m (pixel area 100 m²) were used. When overlaying the satellite image and the scheme of the experimental field, it was noticed that some pixels are not located with their entire area within the plot, which calls into question their representativeness. In order to exclude them from further analysis, a buffer zone of 6.5 meters from the edge inward was created within each plot. In this way, “clean zones” were defined within the parcels, into which pixels uncontaminated by the environment fell.

Table 2. Date of harvest and satellite images acquisition date with corresponding labels

Satellite image date	Harvest date	Label
27.05.2021		21NDVI1
	31.05.2021	21H1
09.06.2021		21NDVI2
	07.07.2021	21H2
	01.09.2021	21H3

15.05.2022		22NDVI1
	19.05.2022	22H1
26.06.2022		22NDVI2
	28.06.2022	22H2
	08.08.2022	22H3

For the purposes of statistical analysis and determining the existence of correlations within and between years, all data on yield measured in the field and in the laboratory, obtained by gravimetric measurements and expressed as mass of dry matter per unit area $ST\ t\ ha^{-1}$ as well as data on NDVI, obtained by computer by processing satellite images in QGIS, were tabulated by dates and parcels and analyzed in the MS Office Excel program. To determine the strength and direction of mutual linear connection, the determination coefficient (R^2) was determined and expressed as Pearson's linear correlation coefficient (r).

Results and discussion

NDVI values were calculated for a total of four dates throughout 2021 and 2022 within the period before the first and second mowing (Table 3).

Table 3. Average NDVI values per plot before 1st and 2nd harvest in 2021 and 2022

P.No	R	T	21NDVI1	21NDVI2	22NDVI1	22NDVI2
1	1	1	0.89	0.45	0.56	0.40
2	1	3	0.90	0.56	0.66	0.54
3	2	2	0.90	0.57	0.66	0.57
4	2	1	0.87	0.52	0.60	0.47
5	3	4	0.90	0.56	0.67	0.55
6	3	1	0.89	0.50	0.61	0.45
7	4	3	0.87	0.54	0.65	0.54
8	4	4	0.89	0.66	0.68	0.58
9	4	2	0.90	0.59	0.67	0.56
10	4	1	0.87	0.48	0.60	0.39
11	3	3	0.88	0.49	0.66	0.54
12	3	2	0.89	0.56	0.68	0.56
13	2	3	0.87	0.54	0.67	0.58
14	2	4	0.89	0.62	0.68	0.58
15	1	4	0.91	0.66	0.68	0.58
16	1	2	0.89	0.53	0.67	0.54
	average		0.89	0.55	0.65	0.53

The highest average value was recorded on 27/05/2021, and the lowest on 22/05/2022, which is expected because it is a recording of only three days after mowing. An insight at the results shows the expected annual dynamics of dry matter yield by mowing dates throughout the year, and the total average yields in 2021 were 5.57, 2.86 and 1.37 DM $t\ ha^{-1}$, respectively in 2022 6.43, 3.06 and 1.16 DM $t\ ha^{-1}$ (Table 4.).

Table 4. Dry matter yields per plots through three harvests in the years 2021 and 2022

P.No	R	T	Harvest date - yield DM [t ha ⁻¹]					
			21H1	21H2	21H3	22H1	22H2	22H3
1	1	1	5.33	3.04	0.37	4.10	0.75	0.32
2	1	3	4.04	2.73	0.54	6.45	3.61	0.51
3	2	2	4.85	2.17	1.57	7.62	4.28	1.48
4	2	1	5.78	3.79	0.22	5.64	1.14	0.17
5	3	4	4.30	2.66	1.47	8.05	4.17	1.31
6	3	1	5.91	1.95	0.23	4.28	1.17	0.18
7	4	3	4.33	3.80	1.54	6.31	3.79	1.49
8	4	4	4.61	3.98	2.24	5.59	4.52	2.06
9	4	2	7.63	3.76	1.76	7.46	4.34	1.46
10	4	1	6.46	1.60	0.44	4.50	0.39	0.29
11	3	3	5.44	2.34	3.26	7.70	4.34	2.45
12	3	2	6.95	1.73	2.50	6.85	2.37	1.63
13	2	3	5.09	2.04	1.55	7.16	3.54	1.32
14	2	4	5.23	3.12	1.62	7.38	4.44	1.51
15	1	4	5.83	4.60	1.15	7.92	3.24	1.04
16	1	2	7.40	2.39	1.45	5.91	2.87	1.33
average			5.57	2.86	1.37	6.43	3.06	1.16

Intra-annual relationships of NDVI and DM yield

Correlations of NDVI and dry matter yield within the season were always positive. The linear model almost does not fit the observed data comparing 21NDVI1 and 21H1 which was most likely caused by the saturation of NDVI, which on that date averaged a very high 0.89.

Table 5. In-season correlations between satellite NDVI data and harvest yield

correlation	equation	Coefficient of determination (R ²)
21NDVI1 vs 21H1	y=0.33x+5.28	10 ⁻⁵
21NDVI2 vs 21H2	y=8.67x+0.32	0.32
21NDVI2 vs 21H3	y=5.00x+1.39	0.12
22NDVI1 vs 22H1	y=29.02x+0.63	0.63
22NDVI1 vs 22H2	y=33.32x+18.59	0.69
22NDVI1 vs 22H3	y=14.00x+7.99	0.54
22NDVI2 vs 22H2	y=20.04x+7.53	0.80
22NDVI2 vs 22H3	y=7.85x+2.99	0.55

In 2022, the coefficients of determination show a high correlation (R²>0.54) even in cases of predictions almost three months in advance. The highest coefficient of determination was related to the satellite image on 26/06/2022 and the second section on 28/06/2022 (R²>0.80) (Table 5). Furthermore it is observable that coefficients of determination in 2021 were lower than in 2022 and reason may be in fact that for 2021 was used one NDVI date in June which was atypical. That year April was unusually cold and May very rainy which may influenced canopy composition.

Inter-annual relationships of NDVI and DM yield

For an insight into the possibility of success in predicting yield between years only one satellite imaging date was used, NDVI dated 9/06/2021. A low coefficient of determination of the vegetation index and yield was found in relation to the yield of the first and third period of the next year, but also a strong connection ($R^2=0.45$) with the second mowing period, which is probably explained by the phenological similarity, i.e. the time of year when the observation was made (Table 6).

Table 6. Inter-annual correlations of NDVI before 2nd harvest 2021 and

correlation	equation	Coefficient of determination (R^2)
21NDVI2 vs 22H1	$y=12.76x+0.62$	0.33
21NDVI2 vs 22H2	$y=16.32x+5.96$	0.45
21NDVI2 vs 22H3	$y=5.21x+1.72$	0.20

Conclusion

This research showed that it is possible to forecast the yield of alfalfa and clover-grass mixtures using Sentinel-2 satellite images. The poor predictability of the 2021 NDVI before the first harvest indicates possible problems caused by NDVI saturation. The results showed that in perennial plantations of alfalfa and clover-grass mixtures, it is possible to predict the yield using Sentinel-2 satellite imagery within one season, but also between years. The best forecast was achieved in 2022 before the second harvest $R^2 = 0.8$.

References

- Becker-Reshef I., Vermote E., Lindeman M., Justice C. (2010). A generalized regression-based model for forecasting winter wheat yields in Kansas and Ukraine using MODIS data. *Remote sensing of environment* 114, no. 6 1312-1323.
- Chen J., and Zhang Z. (2023). An improved fusion of Landsat-7/8, Sentinel-2, and Sentinel-1 data for monitoring alfalfa: Implications for crop remote sensing. *International Journal of Applied Earth Observation and Geoinformation*. 124: 103533.
- DHMZ (2022). *Klima Hrvatske*. [WEB] Web page: https://meteo.hr/klima.php?section=klima_hrvatska¶m=k1 [Accessed 14 June 2022].
- Pan G., Sun, G.J., Li F.M. (2009). Using QuickBird imagery and a production efficiency model to improve crop yield estimation in the semi-arid hilly Loess Plateau, China. *Environmental Modelling & Software*. 24: 510–516.
- Panek E., and Gozdowski D. (2019). Analysis of relationship between cereal yield and NDVI for selected regions of Central Europe based on MODIS satellite data. *Remote Sensing Applications: Society and Environment*. 17: 100286.
- Petersen L.K. (2018). Real-time prediction of crop yields from MODIS relative vegetation health: A continent-wide analysis of Africa. *Remote Sensing*. 10: 1726.
- Rouse Jr.J.W., Haas R.H., Schell J.A., Deering D.W. (1973). *Monitoring the vernal advancement and retrogradation (green wave effect) of natural vegetation* (No. NASA-CR-132982).
- Singh, R. A. N. D. H. I. R., Semwal D. P., Rai, A., Chhikara, R. S. (2002). Small area estimation of crop yield using remote sensing satellite data. *International Journal of Remote Sensing*. 23: 49-56.
- Smith R.C.G., Adams J., Stephens D.J., Hick P.T. (1995). Forecasting wheat yield in a Mediterranean-type environment from the NOAA satellite. *Australian Journal of Agricultural Research*. 46: 113-125.
- Yu B., and Songhao S. (2018). "Multi-year mapping of major crop yields in an irrigation district from high spatial and temporal resolution vegetation index." *Sensors*. 18: 3787.

Basic maintenance and service of the equipment on two biogas plants

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Abstract

The aim of this paper was to show how and the frequency of the scheduled maintenance and necessary replacements of worn-out parts and troubleshooting are carried out on two biogas plants located in the county of Slavonia and Baranja. The data were collected by the interview method with the plant managers. The research has shown that the personnel of both plants conduct all maintenance and service activities regularly which minimizes unexpected equipment failures and enables the plants to run efficiently.

Keywords: biogas plant, efficiency, scheduled maintenance, service

Introduction

Biogas is a gaseous fuel produced from biomass and other organic components, representing one of the most significant renewable energy sources (Beuk, 2023). It is a mixture composed mainly of methane (CH_4) and carbon dioxide (CO_2) mixed with water and other gases in traces such as hydrogen (H_2), hydrogen sulfide (H_2S), nitrogen (N_2), ammonia (NH_3), oxygen (O_2), carbon monoxide (CO), and volatile organic compounds (Afridi and Qammar, 2020). Biogas is created in the biological decomposition of biomass or through gasification.

Biomass is organic material such as manure, or sludge from urban wastewater treatment plants and industrial wastewater, household bio-waste, silage (corn, sorghum, rye, sugar beet), forest remains, etc., which can be used as substrates for biogas production.

Biogas production represents a more advanced technology that converts organic residues and waste into valuable organic fertilizer and renewable energy. It can be applicable continuously throughout the year in numerous sectors such as heating and cooling, electricity generation, transport, or even the chemical industry. The spectrum of possible applications of a biogas plant is broad, including landfills, wastewater treatment plants, agricultural farms, the pulp industry, paper, food, and beverage industry, etc. Biogas production, in addition to obtaining electricity and heat, contributes to the reduction of CH_4 emissions which is generated by disposing of organic waste (Beuk, 2023).

Biogas plants may be configured in different ways. Generally, they consist of a fermenter, a biogas storage, and a combined heat and power (CHP) unit. The fermenter produces biogas that is transferred to the biogas storage with a certain capacity that cannot be exceeded. If the stored biogas reaches the capacity limit, the exceeding quantity of biogas can be burned in the torch or transferred directly to the combustion process (Butemann and Schimmelpfeng, 2019). In cogeneration or CHP plants using gas engines, an internal combustion gas engine is connected to a generator to generate electricity, and the waste heat from the engine is used to generate thermal energy (hot water, or steam). This is known as one of the ways to increase the energy efficiency of energy supply to industrial consumers. If compared to a separate production of electricity and heat (such as a factory boiler room with a power plant and electrical distribution network), the higher energy efficiency of energy supply from the CHP system is evident because significantly less fuel is used for the same needs of the plant (Prvulovic et al., 2022).

To work optimally, a biogas plant should be maintained regularly. The strategy for scheduled maintenance activities is adjusted according to the number of operating hours of the biogas plant (Butemann and Schimmelpfeng, 2019). Besides regular operation, the state of biogas plant units can change over time due to natural processes or external

influences. External influences, divided into mechanical, thermal, chemical, or physical, can cause attrition and corrosion during use. Even when not in use, the condition of the units can deteriorate due to aging.

To remain optimally functional, the biogas plant and all related equipment must be adequately maintained. Inadequate maintenance leads to the deterioration of equipment and infrastructure over time which can result in decreased efficiency and ultimately plant failure. Also, biogas plant workers and staff must possess the necessary technical knowledge and skills to operate and maintain these systems effectively (Das, 2023; Russell, 2023).

Maintenance can be specified based on various criteria and various maintenance strategies are described in the literature (Butemann, 2021). Maintenance time can be chosen either systematically or randomly. When chosen randomly, maintenance is only conducted if necessary or if a flaw occurs. With the systematic approach, maintenance will be performed periodically during operations, depending on the condition of the checked unit part. Generally, maintenance strategies may be categorized into two main types - corrective and preventive maintenance (Schenk, 2013).

A Fundamental of Maintenance (DIN 31051) is a standard by the German Institute for Standardization that defines how major maintenance tasks should be performed. This standard defines four basic maintenance measures: inspection, service, repair, and improvement. Inspection is a measure taken to assess and detect the current state of an item with an examination of the attrition causes and to determine consequences for future operations. Service includes every operation that sustains the target state to postpone the degradation of parts. Repair is a measure for return in the functional and original condition, for example, replacing a machine component with an equivalent spare part. The improvement includes technical and administrative measures that can improve the reliability of the unit part without altering the required features (Anh et al., 2018).

In a biogas plant, routine maintenance comprises the following activities: a) the checkup of the battery, pressure of the cooling water, exhaust gas manifold, and fastenings, b) the replacement of oil, ignition plugs, and oil and air filters, c) the checkup of cooling water, the lambda sensor, exhaust gas back pressure, and d) the cleaning of the gas-air mixer (Butemann and Schimmelpfeng, 2020). The periods between the scheduled maintenance activities vary from around 500 to 2000 operating hours. For smaller CHP modules, maintenance cycles of up to 10 000 operating hours are now sometimes specified.

The aim of this paper was to show how and the frequency of the necessary replacements of worn-out parts and troubleshooting are carried out on two biogas plants.

Material and methods

The research was carried out at two biogas plants located in the county of Slavonia and Baranja, where the measures implemented for service and preventive maintenance of the components of the biogas plant were determined. One facility has a capacity of 1 MWel, while the other one of 2 MWel. The data were collected by the interview method with the plant manager.

Both biogas plants use the same substrates for anaerobic digestion – dairy cow manure, silages, and residual whey, and both work in the mesophilic temperature regime. The process is continuous and is comprised of three phases: a) preparation of substrates for fermentation that takes place in a pit equipped with mixers where the substrates are mixed until a homogenous mixture is obtained; b) dosage of homogenous substrates mixture in the fermenter, and c) fermentation of added mixture. The organic material that has fermented is poured from the digester into the post-digester through an overflow pipe, where in the last phase of decomposition of the organic material, biogas is produced and is collected above the fermentation mixture.

At the end of the fermentation phase, the obtained biogas is transported through the pipeline to the biogas engines, where it serves as an energy source for starting the engine and production of electricity and thermal energy. The produced thermal energy is used to a lesser extent in the technological process of biogas production, and a significant part represents the potential for use in district heating systems, greenhouses, pasteurization, drying, etc. The entire process ends with separating liquid and solid parts of the fermentation mixture. The liquid part is pumped into the lagoons while the solids (digestate) pass into the external storage, and are later used as valuable organic fertilizer on the surrounding agricultural land.

Table 1 represents the main facilities that are part of the biogas plants, their capacity, and related equipment.

Table 1. Major facilities and equipment of the biogas plants

	Facility	Quantity	Capacity	Equipment
Biogas plant I	Trench silo	1	35 000 t	-
	Vertical tank	2	50 m ³	-
	Horizontal tank	1	30 m ³	-
	Slurry pit with mixers	2	450 m ³	Two vertical mixers, two screw pumps
	Digestor/fermenter	2	3 800 m ³	One submersible and three transversal mixers, temperature and level sensors, pumps
				Temperature and level sensors;
				Postfermenter I:
	Postfermenter	2	4 400 m ³	two transversal and two submersible mixers;
				Postfermenter II:
				four submersible mixers
	Digestate storage tank	4	9 000 m ³	-
Biogas plant II	Trench silo	1	20 000 t	-
	Horizontal tanks	2	30 m ³	-
	Slurry pit with mixers	1	400 m ³	One vertical and one transversal mixer
	Digestor/fermenter	1	3 850 m ³	Two vertical and two transversal mixers
	Postfermenter	1	3 850 m ³	One transversal and two vertical mixers
	Digestate storage tank	1	10 500 m ³	-

Other components of the biogas plants are a pumping station, heat pipes, overflow pipe, desulfurizer, condensation shaft, digestate separator, biogas torch, heat exchanger skid, and biogas engine controlled by computer.

Results and discussion

A biogas plant is a system that includes different machines, devices, and equipment controlled by a comprehensive automated control system, featuring a variety of measuring and control devices. Such a plant aims to attain the lowest operational costs and maximum efficiency. To operate at maximum efficiency, each of the energy systems of the power plant must be optimized in its operational characteristics and maintained systematically (Harmon and Gaillard, 2010).

The optimization of the energy system is a continuous process because the operating characteristics of the various energy systems change with time.

Many industrial operators do not understand the complexities of these operations and do not adequately plan the extensive maintenance requirements and capital investments necessary to achieve maximum efficiency in their CHP plants.

At given biogas plants, where this research was conducted, all necessary maintenance activities are conducted regularly. As part of daily maintenance, visual inspections of equipment and facilities are carried out together with inspection of process parameters. In the case of unforeseen failures and deviations, the necessary operations are undertaken to

eliminate particular failures. Maintenance of all other facilities and equipment is performed at recommended time intervals which is called planned maintenance. Table 2 shows the planned maintenance and service of equipment and facilities at the biogas plants that were studied. Most of the scheduled maintenance activities and services are conducted once a year.

Table 2. Planned maintenance and service of equipment and facilities at the biogas plants

	Facility/equipment	Maintenance and service	The time interval between maintenance and service
Biogas plant I	Digestor/fermenter	Cleaning	Annual
	Transversal mixers	Oil exchange	Annual
	Submersible mixers	Oil exchange	Annual
	Pumps	Oil exchange	Annual
	Portable disk and screws	Replacement	Every two years
	Slurry pit with mixers	Service	Annual
	Compressor	Oil exchange	Annual
	Heat pipe distributor	Replacement	When required
	Valves	Replacement	When required
	Biogas blowers	Certified repairer service	Every three years
	Biogas purification system	Service	Annual
	Biogas engine	Valve adjustment, new spark plugs	2 000 working hours
	Biogas engine	Replacement	60 000 working hours
Spark plugs on a biogas engine	Cleaning	1 000 working hours	
Biogas plant II	Digestor/fermenter	Cleaning	Every three years
	Transversal and vertical mixers	Services and oil exchange	Every two years
	Pumps	Replacement of oil and blades	Annual
	Slurry pit	Cleaning	Annual
	Compressor	Oil exchange	Annual
	Heat spreader	Replacement	When required
	Valves	Replacement	When required
	Blower	Certified repairer service	Every three years
Biogas purification system	Service	Every two years	

Unlike other equipment and plant facilities, biogas engine has somewhat more complicated maintenance which is shown in Table 3. Proper care and maintenance of the engine will ensure many years of smooth operation (Ciolkosz, 2023).

Scheduled or preventive maintenance of the biogas engine relates to the various minor, intermediate, and major service procedures that must be followed at specific times following the manufacturer's guidelines, i.e. oil and filter changes. For the major overhaul, the engine is stripped down to the engine block and rebuilt with new or restored parts. For example, the gas engine Jenbacher undergoes a major overhaul after 60 000 hours of service (Clarke Energy, 2023) which is the case for the researched biogas plant I, while on the other biogas plant II, the biogas engine is replaced after 80 000 working hours.

By performing scheduled maintenance on the biogas engine, the maintenance costs are reduced, productivity is increased, and the life of the equipment is extended. This also optimizes the safety of the biogas engine (Eneria, 2022).

Unscheduled or corrective or curative maintenance refers to when the engine stops working or there are unforeseen issues or equipment breakdowns (Clarke Energy, 2023). It focuses specifically on solving the problems that have been identified and correcting the root cause of the failure (Eneria, 2022).

Table 3. Schedule maintenance and services of the biogas engines

	Working hours (h)	Maintenance activities
Biogas plant I	1 000	Spark plugs cleaning
	2 000	Adjusting the bearings and spark plugs cleaning
	10 000	Piston control
	20 000	Turbine and generator bearings replacement
	30 000	Cylinder head replacement
	40 000	Piston control
	50 000	Turbine and generator bearings replacement
	60 000	Biogas engine replacement
	1 000	Spark plugs cleaning
	2 000	Adjusting the bearings and spark plugs cleaning
Biogas plant II	10 000	Piston control
	30 000	Turbine and generator bearings replacement, cylinder head replacement
	40 000	Piston control
	50 000	Turbine and generator bearings replacement
	80 000	Biogas engine replacement

Conclusion

This manuscript analyzes the maintenance of the two biogas plants and their facilities and equipment. The scheduled maintenance of the biogas plants is carried out daily by inspecting the equipment and facilities and checking the process parameters, whereas in the occasion of unforeseen issues or equipment breakdowns, certain procedures are carried out. Also, scheduled maintenance includes monthly and annual inspections of the facilities and equipment and are conducted on both plants regularly.

Regular maintenance and servicing of the plant prolongs the durability of materials and equipment, thus directly affecting the success and efficiency of the entire biogas production process.

References

- Afridi Z.U.R., Qammar N.W. (2020). Technical Challenges and Optimization of Biogas Plants. *ChemBioEng Reviews*. 7: 119-129.
- Anh D.T., Dąbrowski K., Skrzypek K. (2018). The predictive maintenance concept in the maintenance department of the „Industry 4.0“ production enterprise. *Foundations of Management*. 10: 2080-7279.
- Beuk M. (2023). Izvješće o bioplinskim postrojenjima iz baze podataka Registra onečišćavanja okoliša Republike Hrvatske (ROO) za razdoblje 2017. - 2021. godine. Ministarstvo gospodarstva i održivog razvoja, Republika Hrvatska. Available from: file:///C:/Users/%C4%90ur%C4%91ica%20Kova%C4%8Di%C4%87/Desktop/Rad_bioplinsko%20postrojenje/Izve%C5%A1%C4%87e_Bioplinska%20postrojenja_2017-2021.pdf

- Butemann H. (2021). Modelle und Lösungsverfahren zur langfristigen Planung der Stromproduktion einer flexiblen Biogasanlage unter Berücksichtigung von Verschleiß. Faculty of Economics and Social Sciences, University of Hohenheim, Stuttgart, Germany.
- Butemann H., and Schimmelpfeng K. (2020). Long-term electricity production planning of a flexible biogas plant considering wear and tear. *Journal of Business Economics*. 90: 1289-1313.
- Ciolkosz D. (2023). Biogas Digesters – Engine Lubricating Oil. College of Agricultural Sciences, The Pennsylvania State University. Available from:
<https://extension.psu.edu/biogas-digesters-engine-lubricating-oil>
- Clarke Energy (2023). Using biogas for combined heat and power. Available from: <https://www.clarke-energy.com/2012/using-biogas-for-combined-heat-and-power/>
- Das N. (2023). Tackling Biogas Challenges in India: Why do 97% of biogas plants fail in India? *Gruner Renewable Energy*.
- DIN 31051 (2019). Fundamentals of maintenance. Germany: German Institute for Standardization.
- Eneria (2022). Services and maintenance of gas and biogas gensets. Available from:
<https://www.eneria.fr/en/services-parts/services-and-maintenance-of-backup-and-main-installations/multi-vendor-gas-and-biogas/>
- Harmon E., and Gaillard T. (2010). Energy decision management system. United States Patent.
- Prvulovic S., Micic I., Radosav D., Josimovic M., Juric S., Novakov V. (2022). Testing the energy efficiency of CHP engines and cost-effectiveness of biogas plant operation. *IET Renewable Power Generation*. 17: 555-562.
- Russell C. (2023). Do Biogas Professionals Need Skills. *Anaerobic Digestion at AD Energy*.
- Schenk M. (2013). *Instandhaltung technischer Systeme*. Heidelberg: Springer.

Poljoprivreda 4.0 u oblikovanju pametne poljoprivrede

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Sažetak

Poljoprivreda 4.0 predstavlja četvrtu poljoprivrednu revoluciju koja koristi digitalne tehnologije i kreće se prema pametnijem, učinkovitijem, ekološki odgovornijem sektoru poljoprivrede. Poljoprivredne tehnologije su se pojavile kako bi se poboljšala održivost i otkrile učinkovitije poljoprivredne metode. To obuhvaća sve procese digitalizacije i automatizacije u poslovanju i svakodnevnom životu, uključujući velike podatke, umjetnu inteligenciju, robote, internet stvari te virtualnu i proširenu stvarnost. Ovaj tehnološki napredak ima dubok utjecaj na učinkovit uzgoj i održavanje usjeva na obradivom zemljištu, omogućujući poljoprivrednicima korištenje većine resursa koji su im na raspolaganju.

Ključne riječi: poljoprivreda 4.0, digitalizacija, automatizacija, obrada podataka

Uvod

Pametna poljoprivreda detaljno se bavi različitim ključnim tehnologijama i specifičnim domenama za domenu Istraživanje poljoprivrede 4.0 te identificira i raspravlja o značajnim primjenama tehnologija poljoprivrede 4.0. U sustavima Poljoprivreda 4.0 (Agriculture 4.0), flote digitalizirane opreme koriste trenutnu infrastrukturu kao što je računalstvo u oblaku za povezivanje, prepoznavanje uvjeta obrade podataka različitih regija i zahtjeva za ulaznim materijalima i koordinaciju strojeva. Kao dio Poljoprivrede 4.0, internet stvari, umjetna inteligencija i nanotehnologija, između ostalog, dobivaju na snazi.

Poljoprivrednici su poboljšali kontrolu nad proizvodnjom životinja i uzgojem usjeva korištenjem inteligentnih poljoprivrednih tehnologija, čineći ih predvidljivijima i učinkovitijima. To je, u kombinaciji s rastućom potražnjom potrošača za poljoprivrednim proizvodima, pomoglo širenju tehnologije pametne poljoprivrede diljem svijeta. Pokret Industrija 4.0 predstavlja revolucionarnu snagu koja će značajno utjecati na industriju (Erdoğan, 2022). Kampanja se temelji na različitim digitalnim tehnologijama, uključujući internet stvari, velike podatke, umjetnu inteligenciju i digitalna ponašanja kao što su suradnja, mobilnost i otvorene inovacije. Osim uvođenja nove opreme i postupaka, pravi potencijal Poljoprivrede 4.0 u povećanju proizvodnje leži u sposobnosti prikupljanja, korištenja i razmjene podataka na daljinu (Zambon i sur., 2019). Inovativne aplikacije korištene u poljoprivredi trebaju omogućiti vlasnicima i upraviteljima pregled podataka na terenu ili putem uređaja (u stvarnom vremenu). Poljoprivreda 4.0 izgrađena je na četiri stupa: upravljanje temeljeno na podacima, proizvodnja temeljena na novim alatima, održivost i stručnost.

Industrija 4.0 revolucionizira i preoblikuje svaku industriju. To je strateška inicijativa koja kombinira nove disruptivne digitalne tehnologije kao što su internet stvari (Internet of Things-IoT), veliki podaci i analitika (big data and analytics-BDA), integracija sustava (system integration-SI), računalstvo u oblaku (cloud computing-CC), simulacija, autonomni robotski sustavi (autonomous robotic systems-ARS), proširena stvarnost (augmented reality-AR), umjetna inteligencija (artificial intelligence-AI), bežične senzorske mreže (wireless sensor networks-WSN), cyber-fizički sustavi (cyber-physical systems-CPS), digitalni blizanci (digital twin-DT) i aditivna proizvodnja (additive manufacturing-AM) kako bi se omogućila digitalizacija industrije (Aceto i sur., 2019).

Internet stvari

Poljoprivredni sustavi vođeni internetom stvari Internet stvari (IoT) je mreža međusobno povezanih računalnih uređaja, senzora, uređaja i strojeva koji su svi povezani na internet i imaju svoje jedinstvene identitete i kapacitete za daljinsko očitavanje i nadzor (Pylaniadis i sur., 2021). Mrežni sloj (komunikacija), percepcijski sloj (hardverski uređaji), sloj srednjeg softvera (upravljanje uređajima i interoperabilnost), servisni sloj (računalstvo u oblaku), aplikacijski sloj (integracija podataka i analitika) i sloj krajnjeg korisnika šest su slojeva IoT referentna arhitektura (korisničko sučelje). IoT uređaji na fizičkom sloju u poljoprivrednoj domeni prikupljaju podatke o karakteristikama okoliša i usjeva kao što su temperatura, vlažnost, pH vrijednost, razina vode, boja lišća, težina svježeg lišća te ima za cilj pružiti poljoprivrednicima alate za donošenje odluka i tehnologije automatizacije koje im omogućuju besprijekornu integraciju znanja, proizvoda i usluga kako bi povećali proizvodnju, kvalitetu i profit. Upravljanje farmom, kontrola navodnjavanja, razvoj usjeva, praćenje zdravlja i otkrivanje bolesti su prioritete.

Bežične senzorske mreže u poljoprivredi

Bežična senzorska mreža (WSN) je tehnologija koja se koristi u sustavu interneta stvari (IoT). Definiran je kao skup prostorno raspoređenih senzora za praćenje fizičkih uvjeta okoliša, privremeno pohranjivanje dobivenih podataka i prijenos informacija do središnje točke (Singh i sur., 2022).

Računalstvo u oblaku u poljoprivredi

Računalstvo u oblaku (CC) definirano je kao model za omogućavanje praktičnog, sveprisutnog mrežnog pristupa na zahtjev zajedničkom skupu konfigurabilnih računalnih resursa (npr. mreže, poslužitelji, pohrana, aplikacije i usluge) koji može se brzo osigurati i pustiti uz minimalan napor upravljanja ili interakciju pružatelja usluga, prema Nacionalnom institutu za standarde i tehnologije (NIST) (Mell i Grance, 2011). U sektoru poljoprivrede, računalstvo u oblaku je privuklo veliku pozornost u posljednjem desetljeću jer pruža: 1) jeftinu pohranu podataka prikupljenih iz raznih domena putem WSN-ova i drugih unaprijed konfiguriranih IoT uređaja, 2) velike računalne sustave za izradu inteligentne odluke pretvaranjem sirovih podataka u upotrebljivo znanje i 3) sigurnu platformu za razvoj IoT aplikacija temeljenih na poljoprivredi (Shi i sur., 2019). CC se koristi za razvoj različitih poljoprivrednih aplikacija u kombinaciji s IoT i WSN. CC tehnologija također se koristi za razvoj operativnih sustava upravljanja farmom (FMS) koji pomažu farmerima i upraviteljima farmi da učinkovitije prate aktivnosti farme (Wang i sur., 2017).

Edge/fog computing u poljoprivredi

Brza ekspanzija IoT-a rezultirala je eksplozijom senzora i pametnih uređaja, stvarajući ogromne količine podataka. Obrada i analiza tako velike količine podataka u stvarnom vremenu je teška jer opterećuje poslužitelj u oblaku i usporava vrijeme odgovora. Kad se radi s tako ogromnim skupom podataka, sam poslužitelj u oblaku neće moći ponuditi odgovore u stvarnom vremenu. Nadalje, budući da IoT aplikacije zahtijevaju stalnu razmjenu informacija između uređaja i oblaku, osjetljivi su na kašnjenje mreže, što CC čini neprikladnim za te aplikacije (Shi i sur., 2016). Uvođenje ideje rubnog računalstva ima potencijal za prevladavanje problema CC-a. Ova nova računalna arhitektura stavlja računalne resurse i resurse za pohranu (kao što su cloudlets ili fog nodes) bliže izvorima podataka poput mobilnih uređaja i senzora na rubu mreže. To omogućuje analitiku u stvarnom vremenu uz održavanje sigurnosti podataka na uređaju (Shi i sur., 2016).

Poljoprivredni roboti kombiniraju nove tehnologije kao što su računalni vid; bežične senzorske mreže (WSN), satelitske navigacijske sustave (GPS), umjetnu inteligenciju (AI), računalstvo u oblaku (CC) i Internet stvari (IoT) kako bi se poljoprivrednicima pomoglo poboljšati produktivnost i kvalitetu poljoprivrednih proizvoda. AARS u pametnoj poljoprivredi može biti mobilni ili fiksni (Sylvester, 2018). Mobilni AARS može se kretati po radnom polju. Bepilotna zemaljska vozila (UGV) i neidentificirana zračna vozila (UAV) dvije su vrste mobilnih AARS-ova. UGV bi trebala ispunjavati posebne zahtjeve, kao što su mala veličina, sposobnost manevriranja, otpornost, učinkovitost, sučelje prilagođeno ljudima i sigurnost, uz značajke potrebne za rad u polju, kako bi se poboljšali prinosi usjeva i produktivnost farme. Iako neki roboti uključuju sustave računalnog vida, većina tih robota dizajnirana je s jeftinim sustavom računalnog vida, kao što su tradicionalne RGB kamere, zbog poteškoća u uspostavljanju preciznog i pouzdanog sustava koji može zamijeniti ručni rad. Bepilotne letjelice (UAV), zrakoplovi su kojima se upravlja sa

zemlje te nemaju fizički prisutnog ljudskog pilota u letjelici. Postoji mnogo različitih vrsta bespilotnih letjelica (del Cerro i sur., 2021) ovisno o tehnologiji koja se koristi za let (struktura krila) i razini autonomije. UAV s fiksnim krilima (avioni), s jednim rotorom (helikopter), hibridnim sustavom (okomito uzlijetanje i slijetanje) i s više rotora primjeri su vrsta krila (dronovi). Poljoprivredne bespilotne letjelice s pravim sensorima (vizuelne, infracrvene, multispektralne i hiperspektralne kamere) mogu prikupljati podatke (vegetacija, lisna površina i indeksi refleksije) sa svojih polja za praćenje dinamičkih promjena u usjevima koje nisu vidljive s tla (Sylvester, 2018). Poljoprivrednici pomoću ovih podataka mogu zaključiti informacije o bolestima usjeva, nedostatku hranjivih tvari, razini vode i drugim karakteristikama poljoprivrednog rasta. Poljoprivrednici bi mogli planirati moguće agritehničke mjere koristeći ovo znanje (navodnjavanje, gnojidba, kontrola korova, itd.).

Veliki podaci i analitika u poljoprivredi

Brz napredak u IoT i CC tehnologijama uvelike je povećao količinu dostupnih podataka. Tekstualni sadržaj (strukturirani, polustrukturirani i nestrukturirani) i multimedijски sadržaj (npr. video zapisi, fotografije i audio) uključeni su u ove podatke, također poznate kao Big Data (BD) (Sivarajah i sur., 2017). Analitika velikih podataka praksa je analiziranja velikih količina podataka kako bi se pronašli skriveni obrasci, nepoznati odnosi, tržišni trendovi, preferencije klijenata i druge važne informacije (BDA). Koncept pametne poljoprivrede vođene BD-om vrlo je nov, ali je njegov trend dobar jer ima potencijal napraviti dramatičnu promjenu u lancu opskrbe hranom i povećati sigurnost hrane kroz veću produktivnost. Veliki podaci u poljoprivredi obično se generiraju iz različitih izvora u poljoprivredi, uključujući zemaljske senzore, zračna vozila i zemaljska vozila opremljena posebnim kamerama i sensorima; tijela državne uprave u obliku izvješća i propisa; privatne organizacije putem online web usluga; poljoprivrednici u obliku znanja stečenog anketama; i društveni mediji (Sivarajah i sur., 2017). Ovisno o poljoprivrednoj domeni, podaci mogu biti okolišni (vrijeme, klima, razina vlage itd.), biološki (bolest biljaka) ili geoprostorni, a dolaze u različitim količinama, brzinama i formatima. Informacije se prikupljaju i pohranjuju u računalnu bazu podataka, gdje se analiziraju korištenjem računalnih algoritama za karakteristike sjemena, vremenske prilike, svojstva tla (kao što je pH ili sadržaj hranjivih tvari), upravljanje marketingom i trgovinom, ponašanje potrošača i upravljanje zalihama (Singh i sur., 2022).

Umjetna inteligencija u poljoprivredi

Umjetna inteligencija (AI) proučava teorije i računalne sustave koji mogu obavljati aktivnosti za koje je potrebna ljudska inteligencija, kao što su osjetilna percepcija i donošenje odluka. AI, posebno u područjima strojnog učenja (ML) i dubinskog učenja (DL), smatra se jednom od primarnih sila koje pokreću digitalizaciju poljoprivrede u kombinaciji s CC, IoT i velikim podacima. Ove tehnologije imaju potencijal povećati proizvodnju usjeva, žetvu, preradu i marketing u stvarnom vremenu (Talaviya i sur., 2020). ML i DL algoritmi koriste se za određivanje različitih parametara kao što su detekcija korova, predviđanje prinosa i identifikacija bolesti u nizu inteligentnih poljoprivrednih sustava.

Strojno učenje u poljoprivredi

Strojno učenje u poljoprivredi obuhvaća nadzirano učenje (linearna regresija, regresijska stabla, nelinearna regresija, Bayesova linearna regresija, polinomijalna regresija i regresija potpornih vektora), i učenje bez nadzora (hijerarhijsko grupiranje, k-značenje klasteriranja, neuronske mreže (NN) otkrivanje anomalija, analiza glavnih komponenti, analiza nezavisnih komponenti, apriorni algoritam i dekompozicija singularne vrijednosti (SVD).

Detekcija korova, predviđanje prinosa usjeva, predviđanje bolesti i vremena (kiša), procjena svojstava tla (sadržaj vlage, vrsta, pH, temperatura, itd.), upravljanje vodom, određivanje količine gnojiva, te proizvodnja i upravljanje stočarstvom koriste tehnike strojnog učenja i algoritme (Liakos i sur., 2018).

Duboko učenje u poljoprivredi

Duboko učenje (DL) je proširenje klasičnog strojnog učenja (ML) jer je dodata "dubina" (složenost) modelu, može postići teške zadatke (predviđanja i klasifikacije) izvanredno dobro i brzo. Glavna prednost DL-a je učenje značajki, koje uključuje automatsko izdvajanje značajki (informacija visoke razine) iz velikih skupova podataka

(Kamilaris i Prenafeta-Boldú, 2018). Mreže dugotrajnog kratkoročnog pamćenja (LSTM), konvolucijske neuronske mreže (CNN), rekurentne neuronske mreže (RNN), generativne kontradiktorne mreže (GAN), mreže s radijalnom baznom funkcijom (RBFN), višeslojni perceptron (MLP), umjetna povratna veza neuronska mreža (ANN), samoorganizirajuće mape (SOM), mreže dubokog uvjerenja (DBN), ograničeni Boltzmannovi strojevi (RBM) i autokoderi primjeri su algoritama dubokog učenja (Canziani i sur., 2016). DL algoritmi se obično koriste u poljoprivredi za rješavanje problema povezanih s aplikacijama računalnog vida kojima je cilj predvidjeti ključne parametre kao što su prinosi usjeva, sadržaj vlage u tlu, vremenski uvjeti i uvjeti rasta usjeva; otkriti bolesti, štetnike i korove; i identificirati lišće ili biljne vrste (Kamilaris i Prenafeta-Boldú, 2018). Računalni vid je interdisciplinarno područje koje je posljednjih godina eksplodiralo u popularnosti zahvaljujući usponu CNN-a. Pruža metode i tehnike za preciznu obradu digitalnih slika i omogućava računalima da analiziraju i razumiju vizualni svijet (Canziani i sur., 2016). CNN-ovi, općenito poznati kao Convnet i njegovi derivati, najčešće su korišteni algoritmi dubokog učenja u poljoprivrednim aplikacijama.

Sustavi za podršku odlučivanju u poljoprivredi

Sustav za podršku odlučivanju (DSS) je pametan sustav koji pomaže dionicima i potencijalnim korisnicima u donošenju odluka kao odgovor na specifične potrebe i izazove nudeći operativne odgovore temeljene na smislenim informacijama dohvaćenim iz neobrađenih podataka, dokumenata, osobnog znanja i/ili modela (Terribile i sur., 2015). Količina podataka o poljoprivredi eksplodirala je kao rezultat pojave poljoprivrede 4.0. Platforme poput sustava za potporu odlučivanju u poljoprivredi (ADSS) neophodne su za pretvaranje ovih heterogenih podataka u praktično znanje kako bi se donijele precizne prosudbe utemeljene na dokazima o upravljanju farmama i rasporedu objekata (Terribile i sur., 2015).

Poljoprivredni kiberfizički sustavi

Kibernetičko-fizički sustav (CPS) je automatizirani distribuirani sustav koji integrira fizičke procese s komunikacijskim mrežama i računalnim infrastrukturama i jedna je od ključnih tehnologija Industrije 4.0 (Pivoto i sur., 2021). CPS koristi niz postojećih tehnologija, uključujući agentske sustave, IoT, CC, proširenu stvarnost, velike podatke i strojno učenje (ML) (Jimenez i sur., 2020). Skalabilnost, fleksibilnost, autonomija, pouzdanost, otpornost, sigurnost i sigurnost poboljšani su kao rezultat njegovog usvajanja.

Jedna od najtežih domena koja može imati koristi od CPS tehnologije je poljoprivreda. Poljoprivredni kiberfizički sustavi (ACPS) kombiniraju naprednu elektroničku tehnologiju s poljoprivrednom infrastrukturom kako bi stvorili integrirane sustave upravljanja farmama koji su u interakciji s fizičkim okolišem kako bi usjevi održali najbolji rast (Jimenez i sur., 2020). ACPS prikupljaju podatke visoke točnosti koji se odnose na klimu, tlo i usjeve i koriste ih za upravljanje zalijevanjem, vlagom i zdravljem biljaka. Pri projektiranju ACPS-a poseban naglasak treba staviti na autonomiju, robusnost i otpornost kako bi se nosilo s nepredvidivom prirodom okoliša i nepoznatim karakteristikama poljoprivrednih objekata.

Digitalni blizanci u poljoprivredi

Digitalni bliznac (DT) je dinamička virtualna replika stvarnog (fizičkog) objekta koji oponaša njegovo ponašanje i stanja u više faza životnog ciklusa objekata kombiniranjem podataka iz stvarnog svijeta, stimulacija i stroja modeli učenja s analitikom podataka kako bi se omogućilo razumijevanje, učenje i rasuđivanje (Verdouw i sur., 2021). Zbog napretka u tehnologiji poput internet stvari, velikih podataka, bežičnih senzorskih mreža i računalstva u oblaku, koncept DT-a postao je popularan. To je zbog činjenice da ove tehnologije omogućuju praćenje fizičkih blizanaca u stvarnom vremenu pri visokim prostornim razlučivostima korištenjem malih uređaja i daljinskog očitavanja, koji generiraju sve već tokove podataka (Pylianidis i sur., 2021). Zbog oslanjanja na prirodne okolnosti (temperatura, tlo, vlažnost), kao i na prisutnost živih i neživih fizičkih blizanaca (biljke i životinje), okvir je vrlo složen i dinamično područje (unutarnje gospodarske zgrade, uzgoj gredice, vanjska poljoprivredna polja, poljoprivredni strojevi). Neživi fizički blizanci izravno ili neizravno komuniciraju s biljkama i životinjama (živi fizički blizanci), što predstavlja više prepreka za DT u poljoprivredi, dok su neživi fizički blizanci fokus DT-a u drugim domenama kao što je proizvodnja. Uštede troškova, prevencija katastrofa, jasnije donošenje odluka i učinkovite operacije upravljanja sve su navedene prednosti DT aplikacija u poljoprivredi, koje se mogu primijeniti na niz poljoprivrednih potpodručja kao što su uzgoj biljaka i životinja, akvapionika, vertikalna poljoprivreda, sustavi usjeva i stočarstvo (Verdouw i sur., 2021).

Prijelaz na poljoprivredu 5.0

Poljoprivreda 4.0 nudi značajan potencijal za nadoknadu rastuće potražnje za hranom i pripremu za budućnost jačanjem poljoprivrednih sustava s WSN, IoT, AI i drugim tehnologijama. Dok se poljoprivreda 4.0 još uvijek provodi, o poljoprivredi 5.0 već se raspravlja. Poljoprivreda 5.0 nadograđuje se na poljoprivredu 4.0 uključivanjem načela industrije 5.0 za pružanje zdrave, pristupačne hrane, a istovremeno osigurava da se okoliši o kojima ovisi život ne degradiraju (Saiz-Rubio i Rovira-Más, 2020). Industrija 4.0 manje se fokusira na izvorna načela socijalne pravednosti i održivosti, a više na digitalizaciju i tehnologije vođene umjetnom inteligencijom za povećanje učinkovitosti i fleksibilnosti, stoga je Europska komisija službeno pozvala na Petu industrijsku revoluciju (industrija 5.0) 2021. godine (Xu i sur., 2021). Industrija 5.0 dodaje i proširuje koncepte industrije 4.0 naglašavajući usmjerenost na čovjeka, održivost i otpornost. To podrazumijeva poboljšanje suradnje čovjeka i stroja, smanjenje utjecaja na okoliš kroz kružno gospodarstvo i dizajniranje sustava s visokim stupnjem robusnosti kako bi se postigla idealna ravnoteža učinkovitosti i produktivnosti. Među tehnologijama koje omogućuju industriju su koboti (kolaborativni roboti), pametni materijali s ugrađenim bio-inspiriranim sensorima, digitalni blizanci, AI, energetski učinkovito i sigurno upravljanje podacima, obnovljivi izvori energije i drugi 5.0 (Xu i sur., 2021). Učinkovitost poljoprivredne proizvodnje i kvaliteta usjeva mogu se poboljšati u postavkama za poljoprivredu 5.0 delegiranjem ponavljajućih i dosadnih aktivnosti strojevima i onima kojima je potrebno kritičko razmišljanje ljudi. Iz tog razloga treba razviti poljoprivredne kibernetičke fizičke kognitivne sustave (CPCS) koji promatraju/proučavaju okoliš i provode odgovarajuće radnje, usporedive s onima uspostavljenima za proizvodni sektor. To može uključivati robote za suradničke farme koji rade na poljima kako bi pomogli uzgajivačima usjeva u dugotrajnim operacijama poput sjetve sjemena i žetve. Slično tome, digitalni blizanci u poljoprivredi 5.0 mogu dodati znatnu vrijednost prepoznavanjem tehničkih poteškoća u poljoprivrednim sustavima i njihovim bržim rješavanjem, otkrivanjem bolesti usjeva i izradom točnijih procjena uroda (Singh i sur., 2022). Ovo pokazuje da poljoprivreda 5.0 ima potencijal utrti put klimatski pametnoj, održivoj i otpornoj poljoprivredi. Također, Poljoprivreda 5.0 uključuje i postupni prijelaz na energetski pametno gospodarstvo. Kao rezultat toga, korištenje lokalno dostupnih obnovljivih izvora energije (sunce, vjetar, biomasa i geotermalna energija), zajedno s energetski učinkovitim tehnologijama, postalo je sve privlačnije u poljoprivrednom polju za smanjenje utjecaja rastućih troškova energije i doprinosu smanjenja klimatskih promjena.

Zaključak

Digitalne tehnologije, poput onih koje daje program Industrija 4.0, avangarde su modernog poljoprivrednog razdoblja, pružajući širok raspon inovativnih rješenja. Znanstvenici i istraživači integriraju disruptivne tehnologije u tradicionalne poljoprivredne sustave kako bi povećali prinose usjeva, smanjili troškove, smanjili otpad i održali inpute procesa. Činjenica je da su integracija velikih podataka i analitike, bežične senzorske mreže, kibernetičko-fizički sustavi i digitalnih blizansca u poljoprivredi još uvijek u povojima, a većina je još u fazi prototipa. Iako je poljoprivreda 4.0 tek u povojima treba se okrenuti poljoprivredi 5.0 jer ona nadograđuje poljoprivredu 4.0 poboljšanjem suradnje čovjeka i stroja, smanjenjem utjecaja na okoliš kroz kružno gospodarstvo i dizajniranjem sustava s visokim stupnjem robusnosti kako bi se postigla idealna ravnoteža učinkovitosti i produktivnosti.

Napomena

Ovo istraživanje financirao je Europski fond za regionalni razvoj putem KK.01.2.1.02.0286 projekta "Razvoj inovativnih peleta iz šumske i/ili poljoprivredne biomase-INOPELET".

Literatura

- Aceto G., Persico V., Pescapé A. (2019). A survey on information and communication technologies for industry 4.0: State-of-the-art, taxonomies, perspectives, and challenges. *IEEE Communications Surveys & Tutorials*. 21: 3467-3501.
- Canziani A., Paszke A., Culurciello E. (2016). An analysis of deep neural network models for practical applications. *arXiv preprint arXiv:1605.07678*.
- del Cerro J., Cruz Ulloa C., Barrientos A., de León Rivas J. (2021). Unmanned aerial vehicles in agriculture: A survey. *Agronomy*. 11: 203.

- Erdoğan M. (2022). Assessing farmers' perception to Agriculture 4.0 technologies: A new interval-valued spherical fuzzy sets based approach. *International Journal of Intelligent Systems*. 37: 1751-1801.
- Jimenez A.F, Cardenas P.F, Jimenez F, Ruiz-Canales A., López A. (2020). A cyber-physical intelligent agent for irrigation scheduling in horticultural crops. *Computers and electronics in agriculture*. 178: 105777.
- Kamilaris A., and Prenafeta-Boldú F.X. (2018). Deep learning in agriculture: A survey. *Computers and electronics in agriculture*. 147: 70-90.
- Liakos K.G., Busato P., Moshou D., Pearson S., Bochtis D. (2018). Machine learning in agriculture: A review. *Sensors*. 18: 2674.
- Mell P, and Grance T. (2011). The NIST definition of cloud computing, National Institute of Standards and Technology, Gaithersburg.
- Pivoto D.G., de Almeida L.F, da Rosa Righi R., Rodrigues J.J., Lugli A.B., Alberti A.M. (2021). Cyber-physical systems architectures for industrial internet of things applications in Industry 4.0: A literature review. *Journal of manufacturing systems*. 58: 176-192.
- Pylaniadis C., Osinga S., Athanasiadis I.N. (2021). Introducing digital twins to agriculture. *Computers and Electronics in Agriculture*. 184: 105942.
- Saiz-Rubio V., and Rovira-Más F. (2020). From smart farming towards agriculture 5.0: A review on crop data management. *Agronomy*. 10: 207.
- Shi W., Cao J., Zhang Q., Li Y., Xu L. (2016). Edge computing: Vision and challenges. *IEEE internet of things journal*. 3: 637-646.
- Shi X., An X., Zhao Q., Liu H., Xia L., Sun X., Guo, Y. (2019). State-of-the-art internet of things in protected agriculture. *Sensors*. 19: 1833.
- Singh G., Kalra N., Yadav N., Sharma A., Saini M. (2022). SMART AGRICULTURE: A REVIEW. *Siberian Journal of Life Sciences and Agriculture*. 14: 423-454.
- Sivarajah U., Kamal M.M., Irani Z., Weerakkody V. (2017). Critical analysis of Big Data challenges and analytical methods. *Journal of business research*. 70: 263-286.
- Sylvester G. (2018). E-agriculture in action: drones for agriculture. Food and Agriculture Organization of the United Nations and International Telecommunication Union.
- Talaviya T., Shah D., Patel N., Yagnik H., Shah M. (2020). Implementation of artificial intelligence in agriculture for optimisation of irrigation and application of pesticides and herbicides. *Artificial Intelligence in Agriculture*. 4: 58-73.
- Terribile F., Agrillo A., Bonfante A., Buscemi G., Colandrea M., D'Antonio A., Basile A. (2015). A Web-based spatial decision supporting system for land management and soil conservation. *Solid Earth*. 6: 903-928.
- Verdouw C., Tekinerdogan B., Beulens A., Wolfert S. (2021). Digital twins in smart farming. *Agricultural Systems*. 189: 103046.
- Zambon I., Cecchini M., Egidi G., Saporito M.G., Colantoni. A. (2019). Revolution 4.0: Industry vs. agriculture in a future development for SMEs. *Processes*. 7: 36.
- Wang J., Yue H., Zhou Z. (2017). An improved traceability system for food quality assurance and evaluation based on fuzzy classification and neural network. *Food control*. 79: 363-370.
- Xu X., Lu Y., Vogel-Heuser B., Wang L. (2021). Industry 4.0 and Industry 5.0—Inception, conception and perception. *Journal of Manufacturing Systems*. 61: 530-535.

Agriculture 4.0 in the development of Smart agriculture

Abstract

Agriculture 4.0 represents the fourth agricultural revolution that uses digital technologies and moves towards a smarter, more efficient, more environmentally responsible agricultural sector. Agricultural technologies emerged to improve sustainability and discover more efficient farming methods. This encompasses all digitization and automation processes in business and daily lives, including big data, artificial intelligence, robots, the Internet of Things, and virtual and augmented reality. These technological advances have a profound impact on efficient cultivation and maintenance of crops on arable land, enabling farmers to use more of the resources available to them.

Keywords: agriculture 4.0, digitization, automation, data processing

Zbrinjavanje komine masline sušenjem

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Sažetak

Prilikom proizvodnje maslinovog ulja nastaju dva nusproizvoda, komina masline i vegetabilna voda. Komina masline vrijedna je sirovina, ima mnogo potencijala za iskorištenje: proizvodnja energije, kompostiranje, proizvodnja kozmetičkih proizvoda, punila za polimere, kompost itd. Zbrinjavanje ovog otpada bez ikakvog tretmana uzrokuje ozbiljne ekološke probleme koji mogu imati veliki utjecaj na kopneni i vodeni okoliš.

Cilj ovog rada je opisati ostatke iz proizvodnje maslinovog ulja te navesti najzastupljenije načine uporabe komine masline, a fokus rada je stavljen na sušenje kao jednu od najčešćih i osnovnih tehnika dorade komine masline.

Ključne riječi: komina masline, vegetabilna voda, zbrinjavanje otpada, uporaba komine, sušenje

Uvod

U posljednje vrijeme sve je učestaliji pojam održivosti koji predstavlja održavanje ravnoteže u nekom sustavu ili procesu (Drljača, 2012). Glavni izvor energije još uvijek su fosilna goriva: nafta, ugljen i zemni plin, a globalnim razvojem dolazi do sve veće potrošnje goriva. Količine tih goriva su ograničene, potrošne i neobnovljive, a svojim izgaranjem proizvode štetne plinove koji utječu na klimatske promjene i globalno zatopljenje (Lay i Potočnik, 2002). Ekološka održivost odnosi se na uravnoteženo korištenje prirodnih resursa koji nisu potrošni već su obnovljivi i to na način koji ne šteti okolišu i koji ne ugrožava buduće generacije (Frajman-Jakšić i sur., 2010). Jedan od obnovljivih izvora energije je i poljoprivredna biomasa koja se osim za proizvodnju biogoriva koristi i kao kvalitetna sirovina za proizvodnju električne i toplinske energije.

Maslinarstvo je jedna od osnovnih grana poljoprivrede u mediteranskim zemljama već tisućama godina, a u Hrvatskoj je pretežito zastupljeno u priobalju, Dalmaciji i Istri (Čuka, 2002). Masline se uzgajaju za proizvodnju samog ploda odnosno stolnih maslina i/ili maslinovog ulja.

U svakoj industriji pa tako i u poljoprivrednoj nastaje određena količina više ili manje štetnog otpada. Poljoprivredni otpad obuhvaća rezidue koji zaostaju nakon uzgoja i obrade poljoprivrednih proizvoda. Komina masline i vegetabilna voda čine glavni otpad u maslinarstvu, a proizvedene količine ovise o primjenjenom procesu centrifugiranja ulja prilikom prerade maslina u maslinovo ulje. Sustavi centrifugiranja ulja mogu biti izvedeni kao sustavi s dva izlaza, odnosno dvofazni, čiji je nusproizvod žitka komina ili sustavi s tri izlaza, odnosno trofazni sustavi, čiji su nusproizvodi komina i vegetabilna voda (Koprivnjak i Červar, 2002).

Nusproizvodi ekstrakcije maslinovog ulja su vegetabilna voda bogata organskim tvarima i komina masline koja zbog svog kemijskog sastava predstavlja veliko opterećenje za okoliš. Veliku opasnost narušavanja kvalitete podzemnih i površinskih voda smanjenjem biološki dostupnog kisika predstavlja i visok sadržaj organskih spojeva poput fenola i lipida. Sirova komina ima fitotoksično djelovanje na rast korijena biljaka te inhibira enzimatsku aktivnost tla. Preradom jedne tone maslina nastaje opterećenje okoliša organskom tvari ekvivalentno dnevnoj aktivnosti 650 ljudi (Ačkar i sur., 2015).

Većina proizvođača ulja na našem području su mala obiteljska poljoprivredna gospodarstva koja su uglavnom u vlasništvu starijih osoba koje imaju ograničena znanja i informacije o gospodarenju otpadom te važnosti ovog problema pa upravo zbog toga često dolazi do nepropisnog odlaganja neprerađene komine u tlo. Područje Mediterana te priobalni dio Hrvatske zbog toga su razloga najviše zahvaćeni negativnim posljedicama na okoliš uzrokovanim takvom vrstom poljoprivredne proizvodnje (Kučić Grgić i sur., 2020).

Sustav koji omogućava ujedno i smanjenje otpada komine od masline i proizvodnju energije jest kogeneracijska

proizvodnja. U kogeneracijskim sustavima moguća je usporedna proizvodnja dva korisna oblika energije, toplinske i električne, iz jednog energenta (Çakir i sur., 2012).

Ovaj rad bavi se ostacima iz proizvodnje maslinovog ulja. Definiran je proces proizvodnje maslinovog ulja, nusproizvodi tog procesa te njihov utjecaj na okoliš. Također su navedeni najzastupljeniji načini uporabe komine masline, a fokus rada je stavljen na sušenje kao jednu od najčešćih i osnovnih tehnika dorade komine masline.

Proizvodnja maslinovog ulja i upravljanje otpadom u Hrvatskoj

Maslina (*Olea europaea L.*) je zimzelena biljna vrsta, tipičan predstavnik mediteranskog uzgojnog areala. Kultura masline je najmnogobrojnija i najperspektivnija poljoprivredna kultura u našem priobalju, a pretpostavlja se da je stara zapravo koliko i civilizacija (Gugić i sur., 2017). Područje rasprostranjenosti masline u Republici Hrvatskoj obuhvaća Istru, priobalni pojas Kvarnera i otoke te priobalni pojas Dalmacije s otocima. Radi uvođenja niza mjera državnih potpora i situacije na tržištu posljednjih nekoliko godina povećava se potražnja za maslinovim uljem i postizanjem veće cijene, stoga kontinuirano raste interes za proizvodnjom maslinovog ulja što je pozitivno utjecalo na razvoj maslinarstva u Republici Hrvatskoj (Ministarstvo poljoprivrede 2021).

Plod masline se čišćenjem i pranjem priprema za mehaničku dezintegraciju čiji produkt je maslinovo tijesto koje se dalje odvodi na separaciju ulja od biljne vode i krutih čestica. Izdvajanje ulja iz tijesta najčešće se provodi centrifugiranjem takozvanim dekanterima ili horizontalnim separatorima (kontinuirani postupak) ili hidrauličkom prešom (diskontinuirani postupak) (Drlječan 2014).

Proizvodnjom maslinovog ulja dobiva se velika količina otpada i to u obliku komine masline (kao čvrstog otpada) i vegetabilne ili biljne vode (kao tekuće faze). Mješavina vegetabilne vode i komine kao treća vrsta otpada dobiva se dvofaznim centrifugiranjem, odnosno procesom centrifugiranja u centrifugama s dva izlaza.. Kemijski sastav nastale otpadne vode i komine masline ovisi o vrsti masline, uvjetima uzgoja, porijeklu masline te o vrsti ekstrakcije (Dermeche i sur., 2013).

Mogućnosti iskorištenja nusproizvoda u proizvodnji maslinovog ulja

Otpadna voda dobivena preradom maslina je crveno-crne boje, visoke vodljivosti, visokog organskog opterećenja te niske pH-vrijednosti. U otpadnoj vodi kao glavni spojevi prisutni su voda, fenoli, šećeri, organske kiseline te mineralne hranjive tvari (Azabar i sur., 2013). Postoji znatna razlika između komine masline dobivene centrifugalnom ekstrakcijom u dvije faze od komine masline dobivene prešanjem i centrifugalnom ekstrakcijom u tri faze. Iz fizikalno-kemijskih analiza (tablica 1) koje su proveli Alburquerque i sur. ustanovljeno je da komina ima visok udio vode ($w = 55,6 - 74,5 \%$), vrlo visok udio organske tvari te je blago kisela, pH vrijednosti (4,8 – 6,5). Celuloza, hemiceluloza, lignin, masti i proteini ističu se kao glavni spojevi koji se nalaze u komini masline. Komina masline siromašna je mikronutrijentima i fosforom, a od mineralnih tvari ističu se kalij, kalcij i natrij te uglavnom organski dušik.

Tablica 1. Fizikalno – kemijski sastav komine masline

Parametri	Komina
Vlaga, %	55,6 – 74,5
pH	4,86 – 6,45
Električna vodljivost, dS/m	0,88 – 4,76
Pepeo, g/kg	24,0 - 151,1
Ukupni organski ugljik, TOC, g/kg	495,0 – 539,2
C:N omjer	28,2 – 72,9
Ukupni dušik, g/kg	7,0 – 18,4
Organska tvar, g/kg	848,9 – 976,0
Lignin, g/kg	323,0 -556,5
Hemiceluloza, g/kg	273,0 – 415,8
Celuloza, g/kg	140,2 – 249,0

Lipidi, g/kg	77,5 – 194,6
Proteini, g/kg	43,8 -115,0
P, g/kg	0,7 – 2,2
K, g/kg	7,7 -29,7
Ca, g/kg	1,7 – 9,2
Mg, g/kg	0,7 -3,8
Na, g/kg	0,5 -1,6
Fe, g/kg	78,9 – 1462,0
Cu, mg/kg	12,0 – 29,0
Mn, mg/kg	5,0 – 39,0
Zn, mg/kg	10,0 – 37,0

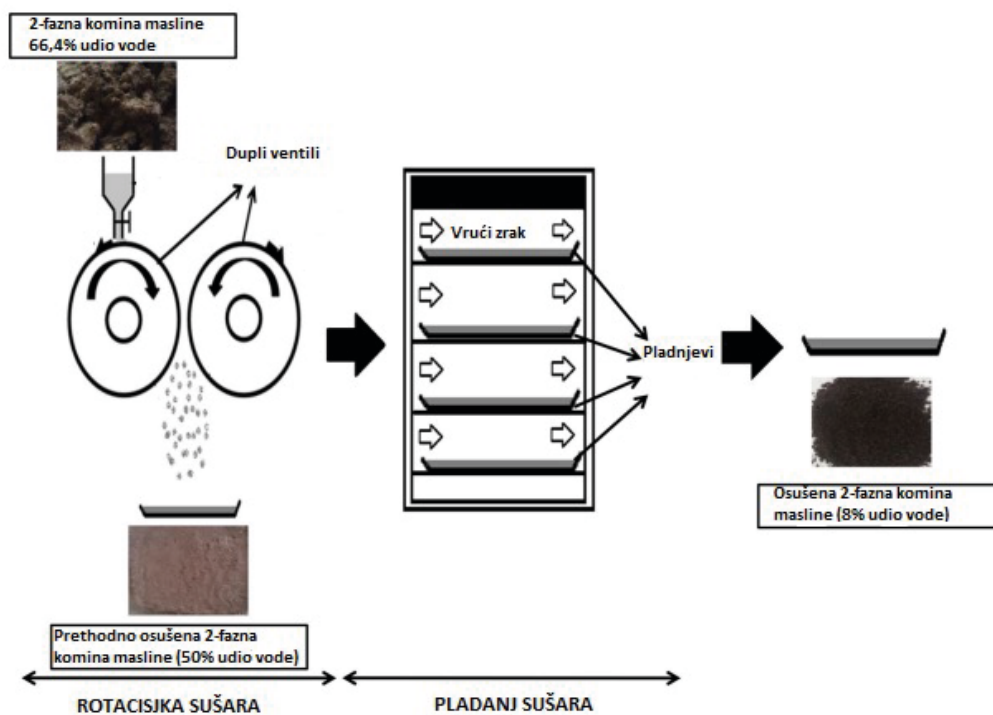
Izravno ispuštanje ovih nusproizvoda u tlo, bez prethodne obrade, ima štetne učinke na rast biljaka, mikrobnu aktivnost i fizikalno-kemijska svojstva tla (Ntougias i sur., 2013). Pojedini fenolni spojevi u maslinama su organske tvari koje su odgovorne za pigmentaciju, te štite biljku od bolesti i UV svjetla, a u netretiranoj komini nalaze se u visokim koncentracijama te primjenom u tlo inhibiraju klijanje sjemena biljaka. Uljni spojevi u vegetabilnoj vodi i komini mogu rezultirati smanjenjem pufernosti tla (Dermeche i sur., 2013).

Ovaj otpad također sadrži i vrijedne resurse koji mogu biti reciklirani, poput bioaktivnih spojeva: vitamin E, masne kiseline, određeni fenoli, a zbog čega se može koristiti u farmaceutskoj, prehrambenoj i kozmetičkoj industriji (Nunes i sur., 2018). Također, komina predstavlja značajan izvor vlakana i minerala, a bioaktivni spojevi komine su snažni antioksidansi i antimikrobna sredstva u hrani pa se smatra da mogu pridonijeti prevenciji kroničnih bolesti (Ribeiro i sur., 2020).

Za učinkovito korištenje biomase kao goriva potrebno je prethodno poznavanje sastava i svojstava materijala kako bi se mogle primijeniti tehnologije za njegovo učinkovito izgaranje, osiguravajući da emisije onečišćujućih tvari ostanu unutar prihvatljivih granica. Analiza fizikalnih i kemijskih svojstava otpadnog materijala poput ostataka nakon prerade maslina ključna je za procjenu njihovih potencijala za energetske svrhe (Caraschi i sur., 2019).

U sektoru poljoprivrede najznačajnije su slijedeće primjene komine: proizvodnja bioplina, proizvodnja peleta i briketa, proizvodnja energije u kogeneracijskom postrojenju te primjena zrele komine kao gnojiva (kompostiranje). Sadržaj vode u komini treba biti manji od 15 % da bi se mogla koristiti kao gorivo, a kako bi se to postiglo potrebno je biomasu podvrgnuti predtretmanu sušenja. Sušenje biomase uključuje nekoliko metoda, a izbor odgovarajuće ovisi o: početnom sadržaju vode biomase te željenom konačnom sadržaju vode, količini i vrsti biomase, lokaciji i prostoru te specifičnim zahtjevima korištenja osušene biomase (Saravanan i sur., 2021). Umjetno sušenje predstavlja praktično rješenje za biomasu visokog sadržaja vode i pogone velikih kapaciteta zbog svoje efikasnosti i brzine. Dok je kod manjih pogona proizvodnje ili biomase manje vlažnosti najisplativija metoda sušenje na otvorenom ukoliko je to moguće. Umjetno sušenje, odnosno sušenje u umjetnim uvjetima (sušionicima) za biomasu uključuje nekoliko vrsta sušara: rotacijske sušare, flash sušare, disk sušare, kaskadne i sušare s pregrijanom parom (Amos, 1999). Temperatura, debljina sloja i brzina strujanja zraka utječu na učinkovitost sušenja (Gögüs i Maskan, 2006).

Kominu iz dvofaznog sustava proizvodnje potrebno je prethodno centrifugirati ili pomiješati sa trofaznom kominom kako bi joj se smanjio udio vlage te kako bi se mogla sušiti u sušarama za kominu iz trofaznog sustava. Takvim procesom dolazi do velikih gubitaka vremena te se povećavaju troškovi (Bayasan i sur., 2020) Proces sušenja u dvije faze je jedan od načina sušenja dvofazne komine uz postizanje maksimalne energetske učinkovitosti. Komina se prvo osuši u rotacijskim sušarama, a zatim se suši u tray (pladanj) sušarama do konačnog sadržaja vode od 8% (slika 1.).



Slika 1. Sušenje komine iz dvofaznog sušenja

Torrecilla i sur. (2006) su također proveli sušenje komine do 8 % sadržaja vode te su zaključili da je prosječna potrošnja energije u rotacijskim sušarama oko 1,4 kWh/kg vode što je značajno više od troškova električne energije u sušarama s fluidiziranim pokretnim slojem koje zahtijevaju oko 1 kWh/kg vode. Sušenje komine na 8 % sadržaja vode primjenjuje se ukoliko se komina iz dvofaznog sustava proizvodnje koristi za ekstrakciju ostatka ulja i proizvodnju tzv. ulja od komine masline jer je za taj proces preporučena vlažnost komine od 7-10 % (Boskou, 2006). Optimalan sadržaj vode za korištenje komine u procesu izgaranja je 10-15 % s obzirom da veća vlažnost negativno utječe na učinkovitost procesa izgaranja, a jako mala vlažnost čini materijal presuhim i prašnjavim što može uzrokovati sigurnosne probleme poput opasnosti od požara (Galanakis, 2017).

Iz kogeneracijske proizvodnje može se opskrbiti električna energija potrebna za pokretanje sušare, a toplinska energija potrebna za sušenje biomase može se crpiti iz kogeneracijske otpadne topline.

Zaključak

Temeljem vlastitih istraživanja, a na osnovi literaturnih navoda može se zaključiti da se maslina najviše uzgaja u Europi, a najrasprostranjenija je na području Mediterana. Nakon proizvodnje ulja dobije se velika količina nusproizvoda u obliku komine koji se neadekvatno zbrinjava. Umjesto neadekvatnog zbrinjavanja ponuđen je način korištenja komine, koji bi mogao imati nekoliko pozitivnih učinaka. Korištenjem komine kao energenta, energija sadržana u komini pretvara se u električnu, tj. iz neiskoristivog u iskoristivi oblik energije. Nakon tještenja i proizvodnje ulja, u predviđenom načinu iskorištavanja, komina odlazi na sušenje, skladištenje i spaljivanje. Od svih dosadašnjih izvora energije najveći se doprinos očekuje od biomase. Posebnu važnost biomasa dobiva zbog problema stakleničkih plinova. Biomasa iz poljoprivrede, dosada se nije iskorištavala do kraja, nego su se neprimjerenim zbrinjavanjem, svake godine gubile značajne količine potencijalne energije.

Literatura

- Ačkar Đ., Šubarić D., Babić J., Miličević B., Jozinović A., Tarnai T. (2015). Dobra higijenska praksa u proizvodnji maslinovog ulja. Glasnik zaštite bilja. 38: 14-18.
- Alburquerque Méndez J.A., González J., García D., Cegarra Rosique J. (2004). Agrochemical

- characterisation of “alperujo”, a solid by-product of the two-phase centrifugation method for olive oil extraction.
- Amos W.A. (1999). Report on biomass drying technology (No. NREL/TP-570-25885). National Renewable Energy Lab.(NREL), Golden, CO (United States).
- Azbar N., Bayram A., Filibeli A., Muezzinoglu A., Sengul F., Ozer, A. (2004). A review of waste management options in olive oil production. *Critical Reviews in Environmental Science and Technology*. 34: 209-247.
- Baysan U., Koç M., Gungor A., Kaymak-Ertekin F. (2020). Investigation of drying conditions to valorize 2-phase olive pomace in further processing. *Drying Technology*. 40: 1-12.
- Boskou D. (2006). *Olive oil: chemistry and technology*. AOCS Publishing. New York.
- Çakır U., Çomaklı K., Yüksel F. (2012). The role of cogeneration systems in sustainability of energy. *Energy Conversion and Management*. 63: 196–202.
- Čuka A. (2002). Geographical basis for development of organic olive grow in Croatia. *Geoadria*. 7: 97-107.
- Dermeche S., Nadour M., Larroche C., Moulti-Mati F., Michaud P. (2013). Olive mill wastes: Biochemical characterizations and valorization strategies. *Process biochemistry*. 48: 1532-1552.
- Drljača M. (2012). Koncept održivog razvoja sustava i upravljanja. *Kvaliteta & izvrsnost*. 1: 20-26 i 110.
- Drljepan A. (2014). Postupci prerade maslina u ulje. Doktorski rad, Sveučilište Josip Juraj Strossmayer u Osijeku. Prehrambeno-tehnološki fakultet.
- Frajman-Jakšić A., Ham M., Redek T. (2010). Sreća i ekološka svjesnost – čimbenici održivog razvoja. *Ekonomski vjesnik: časopis Ekonomskog fakulteta u Osijeku*. 2: 467-482.
- Galanakis C.M. (2017). Olive mill waste: recent advances for sustainable management.
- Gögüs F., and Maskan M. (2006). Air drying characteristics of solid waste (pomace) of Olive oil processing. *Journal of Food Engineering*. 7: 378-382.
- Gugić M., Šarolić M. (2017). *Maslina i proizvodi*.
- Koprivnjak O., i Červar A. (2010). Proizvodne karakteristike maslinarsko-uljarske djelatnosti u Istarskoj županiji. *Agronomski glasnik*. 72: 125-142.
- Kučić Grgić D., Gavran M., Mikšić K., Škunca A., Očelić Bulatović V. (2020). Utjecaj komine masline na okoliš. *Kemija u industriji: časopis kemičara i tehnologa Hrvatske*. 69: 153-162.
- Murugan P., Saravanan D., Sudhakar K., Wilson V. (2021). Industrial and Small-Scale Biomass Dryers: An Overview. *Energy Engineering*. 118: 435-446.
- Ntougias S., Bourtzis K., Tsiamis G. (2013). The Microbiology of Olive Mill Wastes. *BioMed research international*. 2013 (1).
- Nunes M.A., Costa A.S.G., Bessada S., Santos J., Puga H., Alves C.R., Freitas V., Beatriz M., Oliveira P. (2018). Olive pomace as a valuable source of bioactive compounds: A study regarding its lipid-and water-soluble components. *Science of the total environment*. 644: 229-236.
- Potočnik V., and Lay V. (2002). Obnovljivi izvori energije i zaštita okoliša u Hrvatskoj. *Ministarstvo zaštite okoliša i prostornog planiranja Republike Hrvatske*. Zagreb.
- Ribeiro T., Oliveira A., Costa C., Nunes J., Vicente A., Pintado M. (2020). Total and Sustainable Valorisation of Olive Pomace Using a Fractionation Approach. *Applied Sciences*. 10: 6785.
- Torrecilla J., Aragón J., Palancar M. (2006). Improvement of fluidized-bed dryers for drying solid waste (olive pomace) in olive oil mills. *European Journal of Lipid Science and Technology*. 108: 913 - 924.

Removal of olive pomace by drying

Abstract

During the production of olive oil, two by-products are produced, olive pomace and vegetable water. Olive pomace is a valuable raw material, it has a lot of potential for use. Disposing of this waste without any treatment causes serious environmental problems that can have a major impact on the terrestrial and aquatic environment. The aim of this work is to describe the residues from the production of olive oil and to list the most common ways of recovering olive pomace, and the focus of the work is on drying as one of the most common and basic techniques for processing olive pomace.

Keywords: olive pomace, vegetable water, waste disposal, pomace recovery, drying

