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Zbornik radova

Plenarna izlaganja

The future prospects of the meat industry

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Abstract

Animal production and meat processing in general, regardless of their traditional nature and established social status, have a negative image because they are associated with a live animal, with handling practices and slaughter conditions, with the presence of blood, with environmental issues, and with religious, ideological, ethical, or moral concerns. Paradoxically, all of these negative factors associated with meat seem to have limited impact on meat consumption as it has increased in recent decades. However, such strong pressure on the meat industry opens up opportunities for alternatives. Proteins from meat can be replaced or substituted with proteins from plants, fungi, algae, or insects. Eventually, meat products could be produced through in vitro cultivation and three-dimensional printing techniques. Meat substitutes are expected to gain a higher market share in the future due to competition from low-quality cuts of meat, sausages, ground meat, and processed meat. On the other hand, synthetic meat and meat substitutes face significant barriers to commercialization and widespread adoption that will affect their presence, at least in the premium segment of the market. To meet the growing demand for protein and in the face of increasing competition from other sectors, the conventional meat industry will need to adopt new technologies and management systems to meet consumer demands and a changing market.

Keywords: meat, sustainability, reformulation, meat alternatives, industry 4.0, cultured meat

Introduction

Since the beginning of the 21st century, the meat industry has become increasingly global and interconnected, and worldwide consumption is on the rise. Compared to other commodities, meat is characterised by high production costs and high producer prices. Demand for meat is associated with higher incomes and a shift in food consumption driven by urbanisation, which favours a higher share of proteins from animal sources in the diet. Therefore, meat consumption is related to living standards, nutrition, livestock production, and consumer prices, as well as macroeconomic uncertainty and shocks to GDP. Current per capita meat consumption is around 34 kg and is projected to increase only slightly over the next decade (OECD/FAO, 2022). However, as the world population increases to over 8 billion people, meat production is also expected to increase. This is evident between 2016 and 2022, when global meat production increased from 317 million metric tonnes to about 345 million metric tonnes (OECD and FAO, 2022), despite growing concerns about the impact of meat on health and the environment. This was fuelled primarily by growing demand in developing countries, where populations are more affluent and urbanised. Meanwhile, markets for organic, grass-fed, and other specialty meats continued to grow as concerns about animal welfare and health increased. In response, major meat companies launched organic, green-fed and pasture-raised product lines. In addition, meat companies have begun to invest in plant-based alternatives and lab-raised meats, suggesting that they recognised that such options could reshape the market.

Consumer behaviour of meat and meat products is becoming less predictable and even controversial (de Araujo et al., 2022). According to analysts, consumers will increasingly demand fresh products with lower environmental impact, fewer antibiotics, and good animal welfare-all at low prices. In the past, meat was a prized food and eating it at certain times was a privilege that only someone with funds or who produced it themselves could afford. Nowadays, consumers are often confused due to the abundance of information (Sahin and Gul, 2022). Consumers expect government agencies to ensure food safety and science to provide definitive information about what is good and what is bad. However, such a clear distinction is rarely found (Spitery Cornish and Moraes, 2015). It is noted that the

food sector plays a critical role in both the economy and society, and food safety will become increasingly important in the coming years (Scherhaufer et al., 2020).

Relation of meat and meat producing system to humans

Meat is considered as basic food (Pereira and Vicente, 2013). It is a very important commodity and an essential component of a balanced and varied diet (Chouraqui, 2022). It may even have a positive effect on nutrient intake in terms of the profile of beneficial fatty acids, digestible protein, minerals, and vitamins (Binnie et al., 2014). Concerns about fat content in meat are not fully justified (Craigie et al. 2017), but lower consumption of red meat and animal fats is considered by some researchers to be a protective factor against colorectal cancer. Other negative factors such as obesity, smoking, and alcohol also play a role in human health (Azeem et al., 2016), especially when they all occur together. On the other hand, factors such as nutritional quality, chemical and biological hazards, animal welfare, beliefs, and fraud could influence consumers' perceptions of meat product safety. Meat consumers perceive sensory attributes positively and recognise the nutritional value but are still concerned about fat content.

Unable to distinguish between credible and unreliable sources of nutrition information, many participants blame policymakers for their confusion and express frustration and cynicism in the face of vague and often contradictory messaging (Spitery Cornish and Moraes, 2015). As beef, poultry, and pork producers try to figure out how to keep up with growing global demand for meat, policymakers and consumers worry about industry consolidation and its impact on the environment and human health. Currently, sustainable development efforts are colliding with the goals of animal welfare advocates. In recent decades, scientific findings have been continuously translated into animal welfare policies. Unfortunately, the proportion of people who actively harm farm animals rather than just slaughter them is increasing (Kolbe, 2018). Therefore, animal welfare and environmental impacts have become important factors in consumers' perceptions and as such are implemented in regulations. Images of carcass halves hanging from hanging rails in cold storage and meat processing plants often leave people feeling uncomfortable. These images evoke the notion that humans have no right to inflict suffering and death on other sentient creatures simply to obtain food. The currently growing vegan movement seeks to minimise the suffering of farmed animals as much as possible (Kolbe, 2018). Although the information consumers receive through the media has a strong influence on their perceptions and behaviours, there is a need to improve consumer knowledge about different aspects of food health (de Araujo et al., 2022).

New challenges in the meat processing

With the expected increase in meat production over the next decade and reported labour problems and viruses attacking human and animal health, there is a growing need for slaughterhouse automation (Ross et al., 2022). In addition, the integration of systems and greater efficiency thanks to more sophisticated logistics are also a means to reduce the negative impact of the industry on humans and realise sustainable development (Barbut, 2020). It is described that we are currently at the beginning of the fourth phase of the industrial revolution. The components of Industry 4.0, such as robotics, the Internet of Things, Big Data, augmented reality, cybersecurity, and blockchain, have recently transformed many industrial and manufacturing sectors, including agri-food, such as the meat industry. The need for digitised and automated solutions throughout the food supply chain has increased significantly during the pandemic COVID -19 (Echegaray et al., 2022). However, the problem should be considered more in employment policies. In developed countries, it is very difficult to find enough people for these jobs. Meat processing plants require physical labour in cold environments, work in noisy conditions that require workers to get up very early in the morning, and the plants are located outside of city centres, raising the issue of housing and travel. The interest of young adepts in apprenticeships such as butcher is declining. So there is a lack of young people to move and assure the field for the future. Every sophisticated system and advanced technology carries with it concerns about jobs and the quality of work in the resulting products. Every human advance in the past has naturally generated doubts and fears. Let us recall Karel Čapek's novel R.U.R. It is an immortal work in which the word robot first appeared and was included in the dictionary of all languages.

Many factors such as consumer acceptance, benefits and risks, high initial investments, and regulatory frameworks influence the adoption of novel technologies in the food industry and need to be considered and thoroughly analysed (Hassoun et al., 2023). Industry 4.0 has significant potential to improve meat processing, preservation, and analysis; reduce food waste and loss; develop safe, high-quality meat products; and prevent meat fraud. Despite the current

challenges, a large amount of literature shows that the meat sector can be highly automated through the use of smart technologies such as robots and smart sensors based on spectroscopy and imaging technology (Echegaray et al., 2022). Economic sustainability assessment is an important prerequisite for entrepreneurial activities. However, environmental sustainability assessment can be a strategic tool to increase product value and structure green marketing strategies (Stillitano et al., 2017).

A very detailed study (de Silveira et al., 2022), which could be considered fundamental, states that researchers describe Agriculture 4.0 differently and consider the adoption of new technologies as its pillar. They propose a definition of Agriculture 4.0 based on the results of the systematic literature review. In addition, this study provides a comprehensive overview of Agriculture 4.0 technologies in the agricultural production chain that are responsible for revolutionising the way commodities are produced, processed, traded, and consumed (de Silveira et al., 2022). There are five main dimensions: technological, economic, political, social, and environmental. These dimensions address a range of challenges that need to be studied, analysed, and addressed to help countries implement Agriculture 4.0 (de Silveira et al., 2022).

Nearly all sectors of the global meat industry are facing unprecedented challenges. Globally, meat companies are increasingly interconnected, with feed, livestock, and meat shipped across the planet. The global meat industry is struggling with increasing dependence on overseas trade, particularly in Asia. Greater reliance on overseas markets also means greater vulnerability to protectionist measures and other trade barriers. At the same time, the meat processing industry continues to lag behind other sectors in automation, largely due to the difficulty of designing machines that can handle the many sizes and shapes of animals and animal parts. Economic uncertainty, changes in supply and demand, and a growing role for technology are among the factors shaping the near future of the meat industry. Increasing global demand for meat raises environmental concerns. Many experts believe the meat industry is exacerbating climate change and threatening the world's food supply. Other experts disagree, saying animal agriculture is a relatively small source of greenhouse gases that are warming the planet. Critics of the industry say factory farming poses a risk to human health because of the routine use of antibiotics, the storage of large amounts of manure and the fact that it serves as an ideal incubator for pathogens that can pass from animals to humans. However, there are also some positive aspects, such as the elimination of antibiotics in poultry (Muaz et al., 2018) and beef with lower methane emissions (Thompson and Rowntree, 2020).

Are the reformulated recipes or various alternatives as cultured meat the answer?

Providing the world's growing population with sufficient sustainable and nutritious food is one of the greatest challenges. Dietary proteins are nutrients required for the maintenance of various functions and human health. Some conventional plant and animal protein sources are essential food ingredients, but reformulating recipes by replacing animal proteins with alternative proteins could be the answer. The further development required to expand our knowledge of bionanomaterials production, including food industry and biodegradable packaging materials, poses a challenge to various scientific fields. With the discovery of new nanoscale materials, new application areas will emerge (Małecki et al., 2021).

Reformulation of meat product recipes is a hot topic in many European countries. On the one hand, the protection of traditional food recipes is solved in Europe and regional production is promoted. On the other hand, the impact of processed meat and its consumption on human health remains a major concern. In a way, these are incompatible attitudes within the strategy for food quality and nutrition for the future. Another issue is sustainability, the effort to reduce the impact on the amount of emissions, the reduction of by-products in production, or the substitution for existing and new raw materials.

In recent years, scientists have addressed the issue of reducing the amount of salt (sodium) in meat products in their studies (Jůzl et al., 2018). Some studies deal with the elimination or reduction of the content of additives in meat products without major impact on quality parameters (Jůzl et al., 2019). Another issue is the reduced fat content and substitution for a healthier alternative. It can be an addition to the feed (Komprda et al., 2020) or directly during the technological process of processing and meat production (Komprda et al., 2021).

Many plant-based meat substitutes target meat eaters and have gained attention after appearing on fast-food restaurant menus. Surveys also show that younger consumers are more interested in these products. Meat companies have begun to respond to this emerging competition by investing in plant-based meat alternative start-ups or launching their own plant-based products. Meanwhile, livestock producers fear losing market share to plant-based

proteins after observing that cow's milk consumption is declining as soy, oat, almond, and other alternative products have captured about 10 percent of total milk sales in recent years (Haddon and Bunge, 2019). As a result, they have launched campaigns highlighting the purported benefits of animal meat and successfully lobbied for laws requiring that plant-based products be labelled as "imitation meat". The impact of additives on human health is strictly monitored, and labelling policy is a very important element of EU legislation (Hong et al., 2022). The fact that plant-based alternatives contain several unknown and highly processed ingredients such as soy leghemoglobin and expeller-pressed canola oil will also hold them back as consumers want fewer and recognizable ingredients.

Vegan alternatives and substitutes offer are some benefits as well as challenges. Benefits include new jobs, salary increases, better working conditions, entrepreneurial opportunities, easier access to protein, healthier products, and animal welfare. Challenges include reduced demand from animal meat producers, unemployment, low worker skills, high prices for alternative meats, and lower consumer acceptance. However, policy is critical to ensure that the transition process is inclusive and fair (Morais-da-Silva et al., 2022).

Cultured meat is a novel product of future organic food production and a breakthrough in the global food industry. Safety evaluation of cultured meat is important for its commercialization. The cultured meat industry is booming, but the potential benefits and the problems that may arise in the commercialization of cultured meat still need to be critically analysed (Ye et al., 2022). The development of economical and efficient bioreactor machines in which to grow the meat and the nutrient-rich liquid culture media is also a hurdle for lab-grown meat. And while the energy and resources required to grow it are certainly less than those needed to raise, feed and slaughter farm animals, it's not yet known exactly how much energy will be needed.

An important aspect for the future is the fact that not only the labels and labelling of food, but also the food packaging itself will be improved by new materials and production technologies. Much solved is the possibility of 3D food printing, the use of bio-protective films and biodegradability of packaging. Multimaterial 3D printers enable the production of recombined meats. Designing appetizing soft meat products is possible with this 3D printing technology but using low-temperature 3D printers. The application of heat- and cold-curing binders improves the rheology of the meat paste (Dick, 2019).

Some food analysts remain sceptical of predictions that lab-grown meat and plant-based meat alternatives will shake up the meat sector in the foreseeable future because of the difficulty of changing consumer habits, the unsafe nature of the products, and their higher cost. There is still a larger percentage of people who are not interested in limiting their meat consumption. Similarly, many consumers will try new products, but few will buy them again.

Conclusions

The global meat industry faces many challenges, from safety and authenticity scandals to animal and human health issues, animal handling and slaughter, and environmental impacts. Yet consumption continues to rise, especially in emerging and developing countries. More issues are emerging, such as higher production costs and competition from other proteins, while consumer concerns about food safety and animal welfare, as well as environmental impact, are becoming more prominent. The use of advanced technologies can lower costs and reduce waste, meat products can be prepared to meet consumer needs and attitudes, and nowadays meat can be produced in the laboratory. Alternative sources of protein such as plants, mushrooms, algae, or insects can be incorporated into meat products to make them healthier. Many animal husbandry techniques are available to reduce environmental impact, although ethical issues remain. The overall challenge for the meat industry is to meet consumer expectations and projected increases in meat consumption while supporting sustainable goals.

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Can the added values in supply chains be the solutions for improving the potential and functionality of the food sector?

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Abstract

This paper is part of the plenary lecture at the 58th Croatian and 18th International Symposium on Agriculture organised by the University of Zagreb, Faculty of Agriculture. The title of the presentation expresses the scientific question frequently asked in the last decade about the nature of values and their contribution to the improvement of food supply chains. The case studies were analysed based on Slovenian cases from the food sector. The aim of this paper is to present the types of values in food supply chains, to analyse different types of food supply chains in Slovenia and to discuss the results about bottlenecks and shortcomings. Multicriteria decision modelling was used as the method for evaluating food supply chains.

Keywords: supply chains, added-values, quality, food sector, Slovenia

Introduction

Various definitions of agri-food value chains can be found in the academic literature, varying from author to author or differing in certain details. Some of these definitions are listed below. Stevenson and Pirog (2008), Pirog and Bregendahl (2012), European Parliament (2013) state that value added is expressed in three ways:

- through agricultural products or foods made from raw materials, which demonstrate the origin of the food and thus the added value and consequently a higher price on the market,
- through protected food designations that express either geographic location, higher quality of raw materials and/or food safety, and
- as a network of proper business relationships and interactions between the different actors in the food chain.

Below, Stevenson and Pirog (2008), Pirog and Bregendahl (2012), Stevenson et al. (2011), and Stevenson (2013) developed a definition that defines value-added agri-food chains. The definition is essentially based on a description of the differences between chains that do not express value added and chains that do read:

- Value-added agri-food chains can be said to exist when the following important principles are taken into account and they differ from conventional agri-food chains in these segments:
- The business relationships between the strategic partners in the chain are based on common principles, primarily based on trust. In the chain, one of the strategic partners is the one who contributes a large part to the good and organized functioning of the chain.
- In the chain, the producers/farmers are treated equally as strategic partners with all rights and obligations in terms of risk taking, management and decision making.
- The obligations and rights in the chain apply equally to all actors in the chain.
- Coordination of actors in the chain is effectively coordinated at local, regional, national and/or international levels.

In addition to the primary positive impact of high value-added agri-food chains on intra-chain relationships, we can see that this type of chain also has a positive impact on the socio-economic status of the actors involved in the chain, and thus on the wider local environment.

In summary, high quality agri-food chains or value chains are those that maximize production value for the individual partners (actors) in the chain, and consumers of agri-food or food products from such agri-food chains are recognized as high quality, produced on the basis of environmentally friendly production systems, and with a positive indirect and direct contribution to the local economy and the community as a whole (Stevenson et al. (2011)). The primary producer itself also occupies an important position in such chains, or the farmer, who is seen as an important and equal strategic partner, taking risks and participating in management or decision-making. The business relationships between the actors of each chain must be correct, honest and based on trust, which means an equal and fair distribution of duties and rights.

Based on the assessments of agri-food chains conducted so far in the Slovenian and international space, it was found that so far they have been conducted mainly with qualitative methods (in the form of interviews), and much less empirical research examining the influences or links between different groups of actors and the agri-food value chains themselves. A large body of research has been published that examines the socioeconomic impacts of short agri-food chains on the development of local areas and local economies. Kneafsey et al. (2013) explain that the main social benefits of short food chains are the following:

- the development of trust relationships between consumers and buyers in various forms of "face-to-face" sales,
- the improvement of the social situation of all actors along the chain, and
- improving consumers' knowledge of nutritional, agricultural, and environmental issues (known as consumer education).

They also identified the economic benefits of short food chains:

- increase in local sales,
- lower unemployment in the region, and
- higher profits for producers/farmers or key players in the chain.

Using a variety of qualitative methods, the foreign authors found that consumers believe that by buying locally produced food, they are supporting local producers and thus helping to improve their socioeconomic situation.

The aim of this research was to define the added value of food chains based on individual indicators from different areas such as economic, social and environmental. Based on the data obtained from the set of indicators, we used the multi-criteria decision model DEX, which has proven useful in solving such research questions. In the context of individual evaluation of indicators, it allows us to identify those that show opportunities for improvement within food chains.

Material and methods

We used DEX® multicriteria decision modelling for the evaluation approach. The DEX method is commonly used in multicriteria decision analysis. It begins with the creation of a collection using software tools to model preference knowledge for multi-parameter decision making. The method DEX was developed in 1988 in cooperation with the Faculty of Organisational Sciences of the University of Maribor and the Jožef Štefan Institute in Ljubljana. Finally, the researchers became familiar with the subject of the computer programme DEXi, which was developed in 1999 with the help of the Ministry of Education and Sports; it is based on the method DEX and works in a developed environment. An important aspect of the programme is its freely accessible online version, which can be used anytime, anywhere and by anyone (Prišenk et al., 2022).

In creating the DEXI multicriteria model, we focused on the livestock and meat production sector and developed a food chain assessment model for beef, pork, and dairy production. The decision to focus on the livestock sector was primarily due to the availability of the data itself, as we did not collect data for individual chains (fruits, vegetables, ...) in the crop production sector. We chose a beef and pork chain because beef and pork are substitutes, which makes the comparison between the two chains meaningful.

The added value of the chain is a broad concept that considers several areas of sustainable development (economic, social, and environmental). These three aspects of sustainability were also key in the creation of the DEXI multi-criteria model, whose evaluation parameters or criteria were divided into three groups: Economic, Social and Environmental.

We decided to choose four indicators for each type of parameter (economic, social, environmental). All four indicators that make up a parameter have the same weight in the final evaluation, i.e. the weighted sum is distributed equally among all four indicators. Similarly, the influence of a single parameter on the final score is equal. In our case, all three parameters are equally important, so this weighted sum of individual preferences is not particularly important.

Table 1: The economic indicators used as sttributes in the DEXI model

ECONOMIC INDICATORS		
INDICATOR	DESCRIPTION OF THE INDICATOR AND METHODOLOGY	MEANING OF THE INDICATOR
The relationship between the prices of agricultural products and the costs of agricultural inputs	Average (multi-year) relationship between the producer price index for agricultural products and the annual cost index for agricultural inputs (between 2012 and 2021)	Value added as a positive economic situation for the primary producer (farmer) occurs when the cost of inputs increases more slowly than the price of agricultural products for producers. The ratios above 1.00 may represent the positive economic situation for the primary producer.
Change in the ratio between retail price and own price	Average annual change in the ratio of the average retail price of agricultural products to the price of production, on an annual basis, in %	Value added as a positive economic position for each participant in the chain (from primary producer to consumer) exists when the average annual change in the ratio is positive, which means that in the period from 2018 to 2021, the retail price of agricultural products increased on average faster than their own production costs.
The relationship between the prices of purchased products and the own price	Average (multi-year) ratio between average prices of purchased agricultural products and own price (between 2018 and 2021)	Added value as a favorable economic position for the primary producer arises when the prices of purchased agricultural products are higher than their own price. Since the goal of any producer is to make a profit, it is very important that the proceeds of sale are higher than the cost of production.
Weekly market price change	Average weekly change in product market price in %	Added value as a positive economic position for the primary producer occurs when the average weekly price change over a long period is positive.

Table 2: The social indicators used as sttributes in the DEXI model

SOCIAL INDICATORS		
INDICATOR	DESCRIPTION OF THE INDICATOR	MEANING OF THE INDICATOR
The ratio between the average gross wage in each chain and the average gross wage in agriculture	Average (multi-year) ratio between the average gross wage in each production chain and the average gross wage in the Republic of Slovenia (between 2014 and 2021)	It shows how much the primary producers in the considered chains are financially compensated compared to the average payments in agriculture, which, in case of an above-average payment in the single considered chain (beef, pork and dairy), indicates a certain added value of these chains.
Change in the ratio of employed persons in individual sectors of the economy to employed persons in all sectors of the economy in the Republic of Slovenia	Average annual change in the ratio of the labour force in individual sectors of the economy to the labour force in all sectors of the economy combined in the Republic of Slovenia, in % (based on the situation in 2012)	It shows the trend of change in the share of the employed population in each chain compared to the total employed population in the Republic of Slovenia. An added value is given if the trend of the change in the share in relation to the level or value of the ratio in 2012 is positive.
The relationship between the change in the wage level and the change in the consumer price level	Average (multi-month) relationship between the wage index for individual agricultural activities and the consumer price index (between 2014 and 2021)	This criterion expresses a certain added value for the subjects of each agricultural activity when their wages increase faster than the prices of consumer goods.
Level of self-care	Average degree of self-sufficiency with individual products or with products from individual sectors (between 2012 and 2021)	The degree of self-sufficiency indicates the extent to which domestic production (from the basic domestic product) covers domestic consumption (consumption for feed, food, and consumption in industry).

Table 3: The environmental indicators used as sttributes in the DEXI model

ENVIRONMENTAL INDICATORS		
INDICATOR	DESCRIPTION OF THE INDICATOR	MEANING OF THE INDICATOR
Food miles	Food kilometers - the average distance/distance of imports in the last 10 years, the distance or distance is calculated on the basis of the distance between Ljubljana and the capital of the respective importing country and on the basis of the share of imports from the respective country (between 2012 and 2021)	The path or distance traveled during the average transport when importing products in a single chain is an important indicator of the sustainability of the chain itself, since transport has a significant impact on CO2 emissions, the welfare of live animals during transport, the freshness of the products, etc.
Change in the proportion of animals of native breeds compared to all animals combined	Average annual change in the proportion of all native breeds of a given species compared to all bred animals of a given species in % (based on the situation in 2014)	The added value of a single chain is given if the value of the average change is positive, which means that the proportion of native races in a single chain increases compared to the situation in 2014.
Change in the number of farms included in the animal welfare submeasure	Average annual % change in the number of livestock farms included in the animal welfare sub-measure (based on the situation in 2015 and 2016, respectively)	There is an added value in a given chain if the value of the average annual changes is positive, which means that the number of farms included in the animal welfare sub-measure in each chain increases compared to the situation in 2015 or 2016.
Change in the share of organically raised animals	Average annual change in the proportion of organically kept animals of a single species compared to all animals kept of that species in % (based on the situation in 2012)	There is an added value in a single chain if the value of these average annual changes is positive compared to the situation in 2012, which means that the proportion of ecologically raised animals increases compared to the total population of a given raised species.

The multicriteria decision model is designed as a decision tree consisting of attributes at different levels. The attributes of the first qualification level are composed of the individual attributes of the second qualification level and the attributes of the third qualification level. With such an approach, one obtains the so-called decision tree, which is shown in Figure 1.

Attribute tree

Attribute

VALUE ADDED FOOD SUPPLY CHAIN ASSESSMENT
- **Set of ECONOMIC INDICATORS**
  - The relationship between the prices of agricultural products and the costs of agricultural inputs
  - Change in the ratio between retail price and own price
  - The relationship between the prices of purchased products and the own price
  - Weekly market price change
- **Set of SOCIAL INDICATORS**
  - The ratio between the average gross salary in each chain and the average gross salary in agriculture
  - Change in the ratio between the working population in each chain and the working population in all activitie
  - The relationship between the change in the wage levels and the change in the level of consumer prices
  - Level of self-care
- **Set of ENVIRONMENTAL INDICATORS**
  - Food miles
  - Change in the proportion of animals of indigenous breeds compared to all animals combined
  - Change in the number of farms included in the animal welfare sub-measure
  - Change in the share of organically raised animals

Figure 1: Attribute tree of DEX model.

Results and discussion

Beef food supply chain

Using the DEXI model, the beef chain was evaluated as an average agri-food chain based on the defined indicators and the established criteria. Two of the three parameters (economic, environmental), composed of individual indicators that together give the overall score of each chain, were rated as average, while the social parameter was rated as good.

As for the economic aspect of the added value in the cattle breeding chain, it was found in the changes in the ratio between the retail prices of beef and the own price of cattle breeding, as well as in the changes in the market price of the products.

However, the indicators of the average ratio between the prices of agricultural products and the cost of agricultural inputs, as well as the ratio between the prices of purchased products and own price, were evaluated as poor.

From all four economic indicators, it can be concluded that the position of primary producers or growers (farmers) in the beef chain could be better if one of the poorer rated indicators improves. This suggests a greater increase in the retail price of beef relative to its own price and an average monthly increase in the price of beef of about 1% per month, while the price of farm products increases more slowly on average than the cost of farm inputs or production inputs. By recording additional calculations along the chain, we might be able to draw conclusions about the economic proportionality or fairness among the links in the chain. Using the indicators of change in the ratio between the retail price and own price, we can see that, on the one hand, the retail price increases more than the own price, while, on the other hand, primary producers do not even cover the costs of primary production.

The social aspect of value added in the beef chain can be seen in the average change in the proportion of the working population in each agricultural activity compared to all activities combined, the average relationship between the wage index in each agricultural activity and the general consumer price index, and the degree of self-sufficiency in beef.

As for the environmental aspect of added value in the beef production chain, it was found in the average annual change in the proportion of all native breeds of a single species compared to the total livestock of a single species, and in the average annual change in the proportion of organically raised animals of a single species compared to the total livestock of that species.

Pork food supply chain

Using the DEXI model, the pork production chain was evaluated as an average agricultural food chain for animals based on the defined indicators and the established criteria. The groups of economic and social indicators representing the economic and social parameters were evaluated as average, while the evaluation of environmental parameters was good.

The economic aspect of the value added in the pork production chain was determined by the average ratio between the prices of agricultural products and the costs of agricultural inputs, as well as by the changes in the ratio between the retail prices of pork and the own price of pork production.

The average ratio between farm product prices and farm input costs calculated for a 10-year period (between 2012 and 2021) is 1.03, which means that pork prices have changed somewhat faster or more intensively than farm input costs over this period. However, it should be noted that these changes in favor of pork prices are minimal.

However, the indicators of the average relationship between the prices of purchased crops and own price, as well as the average changes in the market price of pork in the representative market, were evaluated as poor.

From all four economic indicators, it can be concluded that the average retail prices of pork have increased faster than the cost of production itself or the own price. This is also confirmed by the average relationship between the price indices for agricultural products and the price indices for agricultural inputs. On the other hand, even in the pork production chain, primary producers or breeders do not, on average, cover all the costs they incur in producing or breeding themselves by selling their products. Another interesting finding is that in the period from the beginning of 2020 to the end of 2021, the market price of pork in the representative market decreased, which contrasts with the price changes in other chains.

The social aspect of value added in the pork chain was determined by the average ratio between the average gross wage in the pork chain and the average gross wage in agriculture.

The environmental aspect of value added in the pork production chain was determined using the average annual change in the proportion of all indigenous breeds of a single species compared to all farmed animals of a single species, the average annual change in the number of livestock farms included in the animal welfare submeasure, and the average annual changes in the proportion of organically raised animals of a given species compared to all raised animals of that species.

Dairy food supply chain

Using the DEXI model, the dairy chain was evaluated as an average agri-food chain based on selected and defined indicators and established criteria. The groups of economic and social indicators (economic and sociological parameters) were rated as average, while the group of environmental indicators (environmental parameters) was rated as good.

The economic aspect of value added in the dairy chain was determined based on the average ratio between the prices of agricultural products (milk) and the costs of agricultural inputs.

Based on all four economic indicators, it can be observed that the market price of milk in the representative market has increased on average on a monthly basis in the period from the beginning of 2020 to the end of 2021, similar to the increase or change in the price of milk compared to the increase or change in the cost of agricultural inputs has also increased or changed on average. Taking into account these two positively evaluated attributes, the economic situation of each link in the chain should improve, but this is not certain, since in the period 2018 to 2021, the relationship between the retail price of milk and the own price of milk production is, on average, to the detriment of the average retail price of milk. Similar to the beef and pork chain, in the dairy chain, the purchase prices of milk from primary producers do not reach the prices of production or processing itself.

The social aspect of value creation in the dairy chain was found to be at the level of milk self-sufficiency.

In contrast to the positive or good evaluation of the indicator of self-sufficiency in milk, the indicators of the average ratio between the average gross wage in milk production and the average gross wage in agriculture and the average ratio between the wage index in milk production and the consumer price index were evaluated as average, which means that the situation indicated by the two indicators remained unchanged.

The evaluation of the environmental indicators in the dairy chain is very similar to that in the beef chain, since the

same data are used for three of the four indicators. Only for the indicator showing the average nutritional value kilometer of the imported products, or in the case of the dairy chain for the milk import, different data are used.

Conclusions

Based on the established criteria that we used to cover all three aspects of sustainable development (economic, social, environmental), we found that all three chains evaluated are very similar in terms of final score, but with the difference that they have different degrees of individual indicator added values. In all three chains, we found indicators that indicate the added value of the chains themselves, as well as those that hinder further development. For the future development of all agri-food chains, it is crucial to maintain the indicators that represent added value or positive practices, but at the same time to try to improve those that have been assessed as poor or inhibiting.

These indicators are mainly related to the economic and social status of primary producers or farmers. In all three chains, the purchase prices of products received by primary producers cannot cover all production costs with them (the purchase price is lower than their own price), which means that their existence depends on additional forms of employment or various forms of state support (direct payments,...). An important negative indicator is also the development of the number or percentage of employees in individual farms, which has been decreasing in recent years. One of the reasons for the low purchase prices of Slovenian products is the oversupply of products on foreign markets, where products are available at lower prices due to lower production costs and the oversupply itself, which leads to an increase in the volumes of products imported from abroad in particular in world trade. Import itself, or in particular the average length of import routes, is another important negative indicator in the three chains studied.

With the help of additional analyzes in the DEXi program, we were able to define those value-added indicators that represent an opportunity for the further or future development of all three chains, which would make the aforementioned chains more sustainable or whose (added) value would increase as a result. Above all, the improvement of socio-economic conditions in the form of more adequate payments is the key to reducing imports in the chains of cattle breeding and milk processing, since Slovenia can already meet the needs of the market with its own production and processing.

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Biostimulants as an integrated crop management tool

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Abstract

Plant protection is a significant component of modern agriculture. Different agricultural systems rely on integrated crop management (ICM) as a sustainable solution. The new ICM model provided a more holistic approach and promoted preventive measures and combinations of different management strategies. Therefore, plant biostimulants positively impacting nutrient use efficiency, tolerance to stress, crop quality, or soil health could be part of the ICM toolbox. This review will explore the agricultural functions of different biostimulants categories in tomatoes, wheat, and apples. Agricultural functions of biostimulants are classified according to ICM elements, confirming contribution to cultural control and preventive measures. More dedication from different stakeholders is needed to translate the enormous potential of biostimulants into farmer practices for various crops.

Keywords: integrated crop management, sustainable agriculture, biostimulant, abiotic stress, biodiversity

Introduction

Global agriculture and associated food systems face a significant challenge due to climate change and biotic and abiotic stressors (Arora, 2019; Oerke and Dehne, 2004). Simultaneously, it is necessary to satisfy increasing demands for food and make agricultural production more sustainable and environmentally friendly (FAO, 2018). Regarding crop protection, different low- and high-input agricultural systems need to rely on integrated crop management (ICM), referred to as integrated pest management (IPM) as well (Richard et al., 2021). Richard et al. (2021) suggested using the term Integrated Crop Management (ICM) to avoid any confusion with the definition of the term "pest" in Integrated Pest Management (IPM). This review will use IPM and ICM terms in the same context, referring to integrated pests, diseases, and weeds management.

Trends in modern agriculture indicated a need to clarify the ICM concept and include a sustainability aspect that covers economic viability, environmental safety, and social acceptability (Dara, 2019; Stenberg, 2017; Deguine et al., 2021).

In European Union (EU), IPM has been mandatory since 2014. IPM in Directive 2009/128/EC focused on eight principles (European Parliament and the Council, 2009), which do not address economic, social, and environmental aspects or organization between the relevant stakeholders (Barzman et al., 2015). Driven by Green Deal and Farm to Fork Strategy, the new Common Agricultural Policy (CAP) Strategic plans should contribute to the resilience of the EU farming systems and food security and enable the transition to sustainability over 2023-2027 (European Parliament and the Council, 2021). Member States should set eco-schemes in their strategic plans, including organic farming and IPM (European Parliament and the Council, 2021). In the adopted proposal for a new Regulation on the Sustainable Use of Plant Protection Products (SUR), the European Commission points out that increasing the implementation and uptake of IPM strategies will be vital in achieving the SUR objective (European Commission, 2022). Furthermore, the proposed SUR defines biological control agents and emphasizes preventive measures and non-chemical alternatives (European Commission, 2022).

The industry is crucial in promoting biological solutions, including plant biostimulants, for sustainable agriculture (Du Jardin, 2015; Rouphael and Colla, 2020; Ricci et al., 2019; EBIC, 2021). Although industry initially developed biostimulants for organic production, their use in other agricultural systems is increasing. The European market, approximately half of the global market, is estimated to be around USD 1.5-2 billion in 2022 (EBIC, 2021).

This review will explore definitions of biostimulants and their physiological and agricultural functions and group them according to ICM elements (Stenberg, 2017; Dara, 2019) on the example of tomato, wheat and apple.

Biostimulant Definition and European Regulatory Framework

Prof. V.P. Filatov introduced the concept of the "biogenic stimulant" theory in 1933, which Blagoveshchensky further developed in 1956 (Yakhin et al., 2017). According to Du Jardin (2015), Zhang and Schmidt introduced the first definition of "biostimulants" in a web journal in 1997, while Kaufmann et al. defined biostimulants in the scientific literature in 2007. Furthermore, Du Jardin (2015) concluded that the nature of biostimulants and physiological functions are diverse, definitions of economic and environmental benefits depend on agricultural and environmental policies and that effects of all biostimulants cover at least one or several agricultural functions.

Different categories of substances can be considered as plant biostimulants in publications and reviews: humic substances and fulvic acids, protein hydrolysates, free amino acids, and other N-containing compounds, seaweed extracts (hormone-containing products), inorganic compounds (salts), chitosan and other biopolymers, botanicals, carboxylic fatty acids, unsaturated fatty acids, phenolic acids, and phenolic aromatic acids containing several benzene rings and vegetable oil. In addition, biostimulants can also include microorganisms such as beneficial bacteria and fungi, including inoculants and plant-growth-promoting microorganisms (Yakhin et al., 2017; Du Jardin, 2015; Du Jardin, 2012; Calvo et al., 2014; Halpern et al., 2015).

In Europe, the first legal definition of biostimulants under the European Fertiliser Regulation (EU) No 2019/1009 is based on the agricultural function of the products (claims-based), stipulating that plant biostimulant refers to "a product stimulating plant nutrition processes independently of the product's nutrient content with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere: a) nutrient use efficiency, b) tolerance to abiotic stress, c) quality traits, and d) availability of confined nutrients in soil or rhizosphere" (European Parliament and Council, 2019). In the European Fertiliser Regulation (EU) No 2019/1009 (European Parliament and Council, 2019), the function of biostimulants is captured under the specific product function category six (PFC 6), as microbial and non-microbial plant biostimulants, while component material categories (CMC) addressed diverse nature of the substances.

Biostimulants are neither fertilizers nor plant protection products. The European Fertiliser Regulation (EU) No 1009/2019 distinguished biostimulants from plant protection products and provided guidance for products with one or more functions covered by the scope of the European Plant Protection Product Regulation (EC) No 1107/2009 (European Parliament and the Council, 2009a).

Du Jardin (2015) indicated that there was a growing consensus to separate biostimulants from biocontrol (plant protection products) while developing the EU regulatory framework. The EU regulatory framework narrows the products which could be registered as plant biostimulants when compared with the plant biostimulant definitions and categories in scientific reviews (Yakhin et al., 2017; Calvo et al., 2014; Halpern et al., 2015). Ricci et al. (2019) outlined some general principles for setting the biostimulant claims, which they proposed to incorporate into harmonized European standards (hEN) that are under the development of the European Committee for Standardization (CEN).

The European Fertiliser Regulation (EU) No 2019/1009 affixes the CE mark for biostimulant products and enables free movement in the single market compared to the registered products according to national legislations (European Parliament and Council, 2019).

Biostimulant claims in the context of ICM

Crop yield is a crucial parameter for farmers. Biostimulants positively impact yield; vegetables showed the highest yield benefits (+28%), followed by legumes, while yield response in fruits and cereals was less significant (Li et al., 2022).

Publications indicated the potential uses and benefits of various biostimulant categories in tomatoes, wheat, and apples (Table 1 and Table 2).

From the ICM perspective, the central claims in tomatoes, apples, and wheat (Table 1 and 2) refer to cultural control (nutrition efficiency improvement, abiotic stress tolerance, and crop quality improvement, growth) as part of the preventive measures within the new revised ICM (Dara, 2019; Stenberg, 2017). Some biostimulants, such as *Trichoderma sp.,* seaweed *Laminaria sp*., chitosan, or neem, can show multi-effects and address different ICM

elements, cultural control, and biocontrol, depending on the specific product claims (D'Addabbo et al., 2019; Agarwal et al., 2016; Butkevičienė et al., 2022; European Commission, Health and Food Safety Directorate General, 2022).

Additionally, European Biostimulant Industry Council (EBIC) (Ricci et al., 2019) proposed claims that could positively impact biodiversity, referring to the reinforcing biological regulation and interactions and improving biogeochemical cycles. Humic substances (Pane et al., 2016.), algal extracts (Renaut et al., 2019; Bentley et al., 2022), plant growth promoting rhizobacteria (PGPR), and other bacteria (Lee et al., 2022; Bellotti et al., 2022; Beceril-Espinosa et al., 2022.), and fungi (Derkowska et al., 2017) positively impact soil microorganisms in tomato and apples. Furthermore, abiotic stress tolerance (salinity, drought) claims in tomatoes and wheat (Table 1 and 2) are directly linked to climate change adaptation.

The queries in the scientific database (www.scopus.com) showed a continuous increase in the publications and interest of scientists in biostimulant topics. Simultaneously, industry members reported investment in R&D in this sector (EBIC 2021). Social and environmental trends include climate resilience, biodiversity, and greenhouse gas emissions into innovation and research, in addition to the usual drivers such as agronomic needs and human and environmental safety (FAO, 2018).

Table 1. Biostimulant categories and their agricultural functions in tomato, wheat, and apples

Crop	Tomato	Wheat	Apple
Biostimulant Category			
Humic substances	+	+	+
Protein Hydrolysates	+	+	+
Algal extracts	+	+	+
Botanical extracts	+	+	+
Inorganic substances	+	+	
Fungi	+	+	+
plant growth promoting rhizobacteria (PGPR)	+	+	+
Other bacteria	+	+	+
Chitosan	+		
Biowaste, bioslurry	+		
Agricultural functions	Suppression of parasitic weed, nematodes, suppressive for diseases, mechanism against bacterial and fungal diseases, induction of defence (secondary metabolites e.g.,, flavonoid synthesis), positive impact on yield, growth, seed germination quality parameters, macro- and micro-nutrient uptake, biodiversity (enhanced microbes, positive impact on mycorrhizal symbiosis, nutrient uptake), tolerance to abiotic stress (salinity, Fe-deficiency, drought).	Fungal activity, nematodes suppression, increase resistance against disease (plant defence), positive impact on growth, yield, quality parameters, tolerance to abiotic stress (salinity, drought), nutrient uptake (N, P), decrease N pollution (in wheat-rice rotation system).	Positive impact on quality parameters – secondary metabolites – phenolic compounds, anthocyanin (color), antioxidant potential (potentially plant defense), positive impact on growth, N, P, K uptake, increase in micronutrients (Fe, Mn), quality parameters (prevent physiological disorder of fruits, storage), biodiversity (positive impact on population of fungi, bacteria and actinomycetes in rhizosphere).

References: Disciglio et al., 2016; Pane et al., 2016; Choi et al., 2022; Zou et al., 2022; Rouphael et al., 2021; Buffagni et al., 2021; Celletti et al., 2020; Cozzolino et al., 2021; Ali et al., 2022; Bentley et al., 2022; D'Addabbo et al., 2019; Chantini et al., 2019; El Arroussi et al., 2018; Renaut et al., 2019; Agarwal et al., 2016; Mutale-Joan, 2021; Sudiro et al., 2022; Chele et al., 2021; Colman et al., 2019; Vieira et al., 2022; Papa et al., 2022; Joshi et al., 2021; Lee et al., 2022; Alzate et al., 2022; Bellotti et al., 2022; Beceril-Espinosa et al., 2022; Fragalà et al., 2022; Funes-Pinter et al., 2022; Pačuta et al. , 2021; Gaidau et al., 2017; Łangowski et al., 2022; Sharma et al., 2019; Szpunar-Krok et al., 2022; Mathlouthi et al., 2022; Butkevičienė et al. 2022; Chalfoun et al., 2018; Cheng et al., 2022; Blyuss et al., 2019;Swierczyński et al. 2021; Graziani et al. 2020; Kiczorowski, 2019; Soppelsa et al. 2018; Derkowska et al., 2017.

Table 2. Biostimulants categories and integrated crop management (ICM) elements in the example of tomato

Biostimulants Category	Integrated pest management (**IPM) Element /Agricultural function**	**Type of Application**	**References**
Humic Substances **Vermi-compost** **Compost tea**	**Biocontrol:** suppression of parasitic weed, suppressive for diseases **Cultural control:** biodiversity (enhanced microbes), nutrient uptake	Soil application Spraying	Disciglio et al., 2016; Pane et al., 2016.
Protein Hydrolysates	**Cultural control**: plant quality, nutrient uptake (N), tolerance to abiotic stress (salinity, Fe-deficiency, drought), growth	Spraying Root drench Substrate treatment at transplanting	Choi et al., 2022; Rouphael et al., 2021; Celletti et al., 2020; Cozzolino et al., 2021.
Algal extracts, seaweed	**Biocontrol:** nematode suppression, induction of defence mechanism against bacterial and fungal diseases **Cultural control:** growth, plant quality traits, biodiversity (positive impact on mycorrhizal symbiosis), tolerance to abiotic stress (salinity)	Spraying	Ali et al., 2022; Bentley et al., 2022; D'Addabbo et al., 2019;Chantini et al., 2019; El Arroussi et al., 2018; Renaut et al., 2019;Agarwal et al., 2016; Mutale-Joan, 2021; Cozzolino et al., 2021.
Botanical extracts	**Biocontrol:** suppression of nematodes **Cultural control:** tolerance to abiotic stress (drought)	Spraying	D'Addabbo et al., 2019; Sudiro et al., 2022.
Inorganic substances (silicates)	**Cultural control:** tolerance to abiotic stress (salinity), flavonoid synthesis	Spraying	Chele et al., 2021.
Chitosan	**Biocontrol:** impact on defence mechanisms **Cultural control:** growth	Spraying at transplanting	Colman et al., 2019.
Fungi and/ or fungi metabolites	**Cultural control:** growth, macro- and micro-nutrient uptake	Substrate Seed treatment (priming)	Vieira et al., 2022; Papa et al., 2022; Joshi et al., 2021.
Plant growth promoting rhizobacteria (PGPR)	**Cultural control:** growth, quality, soil nutrient availability, biodiversity (positive impact on soil bacteria)	Inoculation	Lee et al., 2022.

Other bacteria and/or metabolites	**Cultural control:** abiotic stress (salinity), growth, germination, plant quality (secondary metabolites), biodiversity (enrichment for operational taxonomic unit of plant-growth-promoting microbes, symbiotic relations)	Spraying	Alzate et al., 2022; Bellotti et al., 2022; Beceril-Espinosa et al., 2022.
Biowaste, bioslurry	**Biocontrol:** fungicidal potential **Cultural control:** seed germination, growth	Seed treatment	Fragalà et al., 2022; Funes-Pinter et al., 2022

Conclusions

Biostimulants are compatible with the ICM principles and should be promoted as a preventive tool in the ICM toolbox. The positive impact of biostimulants on biodiversity and climate adaptations are benefits that go beyond the new ICM paradigm and fit with the overall sustainability trends in the EU. More dedication from different stakeholders (scientists, industry, policymakers, farmers, advisory services) is needed to explore the enormous potential of biostimulants, including translation into the farmers' practices for various crops and local conditions.

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Heavy metals and nutrients in the leaves of Paulownia grown on polluted soils in Bulgaria

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Abstract

A field study was conducted to determine the content of heavy metals and micro and macroelements in Paulownia leaves grown on heavily metal-contaminated soils. The experiment plots were performed on an agricultural field contaminated by the Non-Ferrous-Metal Works near Plovdiv, Bulgaria, situated at different distances (0.1 km, 2.0 km, and 11 km) from the source of pollution. Contamination of Paulownia leaves with heavy metals was primarily by aerosol, and the amount depends primarily on the distance to the source of contamination and the heavy metal content of the soil. The increased heavy metal content of the soil resulted in less uptake of the nutrients Ca, Mg, and P in Paulownia leaves. It can be concluded that Paulownia is tolerant to heavy metals and can successfully be grown on heavy metal-contaminated soils. Paulownia can be used as an indicator plant for air (and soil) pollution and to build buffer strips around industrial plants.

Keywords: nutrients, Paulownia, toxic metals, soil contamination

Introduction

In Bulgaria, about 61% of the areas contaminated with heavy metals above the maximum permissible content of harmful substances in soil are located in the areas of metallurgical plants. In the regions of smelters, combined contamination of plants is observed - (i) uptake of accumulated heavy metals in the soil by the root system of plants and (ii) by aerosols from the atmosphere on the leaf surface. The aerosols‘ concentration varies with distance from the source, weather conditions and particle size. Larger particles settle quickly to the ground surface, in contrast to smaller particles which can be lifted by air currents to high altitudes and disperse over considerable distances. The height of smelter stacks, wind speed, rainfall and the character of the terrain also have an influence. Aerosols cling to plant leaves, and some trace elements may be adsorbed. Pb remains mainly as a surface deposit, while Zn and Cd penetrate partially into the leaves (Kabata-Pendias, 2001).

The area around a Non-Ferrous Metal Works-Plovdiv (NFMW) is one of the „hot“ ecological spots of the country. The plant‘s production activities are associated with the emission of industrial gases and dust (aerosols), which are the primary pollutants of the region. These emissions are dominated by aerosols of Pb and Zn, accompanied by Si, Cd, and Hg. The metals in the emissions are predominantly in the form of oxides. In recent years, there has been a downward trend in the amount of heavy metal and sulphur oxide emissions to the air as a result of improvements in Pb and Zn production technology at NFMW. However, accumulated heavy metals from previous years remain in the surface soil layer (Dimitrov et al., 2019).

Paulownia tomentosa (Thunb.) Steud and other tree species belonging to the genus *Paulownia* (*Paulownia fortunei* Hemsl., *Paulownia elongata* S.Y. Hu) have received increasing attention in recent years for cultivation in areas with aerosol and soil pollution (Doumett et al., 2008; Stankovic et al., 2009; Wang et al., 2010; Azzarello et al., 2011, Tzvetkova et al., 2015) as a result of their tolerance to heavy metals combined with rapid growth. The high biomass production of *Paulownia fortunei* Hemsl. within a short period results in significant contaminant removal from contaminated soil despite low metal uptake rates (Doumett et al., 2008). There has been a great deal of interest in the species *Paulownia elongata* and *Paulownia fortunei*, which in urban settings are grown in parks or boardwalks at the expense of their ornamental qualities. Owing to the large leaf surface, which is heavily girdled on the underside, these species absorb significant amounts of sulphur dioxide in combination with dust particles from the air (Stankovic et al., 2009). The most important characteristic of these species is their extremely rapid growth, which places them

among the fastest-growing trees in the world (Stankovic et al., 2009; Wang et al., 2010).

The aim of the present work was to conduct a comparative study in order to determine the heavy metal (Pb, Cd, Hg), micro (Fe, Cu, Zn, Mn), and macro (N, P, K, Ca, Mg) element contents in the leaves of *Paulownia tomentosa x fortunei*, as well as the possibilities to use the Paulownia trees for phytoremediation of heavy metal contaminated soils.

Material and methods

Paulownia (*Paulownia tomentosa x fortunei*) was used as a test plant. The Paulownia, selected by BIOTRI OOD, Bulgaria, was cultivated according to the generally accepted technology in areas at different distances from the pollution source NFMW - Plovdiv (0.1 km, 2 km and 11 km). The field trials were laid out using the block method in four replications with an experimental plot size of 100 m^2. Planting of Paulownia seedlings was carried out in spring on the previously prepared plots on each sampling location. The plots were ploughed to a depth of 40 - 60 cm to break up the soil and eradicate the rhizomes of perennial weeds. After the initial tillage, the land was disked, and planting holes were dug. Six saplings were planted in a 2x3 m scheme (distance of 2 m between plants of each row and 3 m between rows) in each 100 m^2. At the end of the growing season, leaves from 3 Paulownia trees from each experimental plot were collected for analysis. Leaves were taken from the lower, middle and upper parts of the tree canopy on the east, west, north and south sides. Samples were dried at room temperature without pre-washing the leaves until an air-dry mass was obtained, then dried at 75°C and analysed. Soil samples were also collected from individual plots prior to planting the Paulownia seedlings. Soil samples 1 and 2 are located at the same distance from NFMW - Plovdiv (0.1 km). Sample number 1 was collected from a site located south of the NFMW - Plovdiv and sample number 2 from a site located southwest of the NFMW - Plovdiv. Sample 3 was taken at a distance of 2 km from NFMW - Plovdiv and sample 4 from the experimental field of the Agricultural University-Plovdiv (11 km). Soil samples were collected using a hand probe at a depth of 0 - 30 cm. One average sample was taken from each plot, which was 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 (Pb, Cd, Hg, Fe, Cu, Zn, Mn) were digested by aqua regia (ISO 11466), while for mobile metals forms (Pb, Zn, Cd, Cu) determination digestion was performed by 0.005 M DTPA (ISO 14870). Element content (Pb, Cd, Hg, Fe, Cu, Zn, Mn, P, K, Ca, Mg) in plant samples previously before detection was mineralised by microwave method. An atomic emission spectroscopy technique (AES) was used for all elements determination (Jobin Yvon Emission, model JY 38 S).

Results and discussion

To elucidate the extent of soil contamination with heavy metals and their localisation in the leaves of Paulownia, soil samples were collected from the areas at different distances from the NFMW (0.1 km, 2.0 km and 11 km). The physical and chemical properties of the soil samples are presented in Table 1. The soils are characterised by a slightly alkaline reaction, medium organic carbon content and medium to high nutrient (N, P, K) availability. Total Zn, Pb and Cd contents are high and exceed the maximum allowable concentrations (MAC) (Table 1).

Table 1. Soil characteristics of the study areas

Parameter	Soil 1 (0.1 km)	Soil 2 (0.1 km)	Soil 3 (2 km)	Soil 4 (11 km)
pH	7.32	7.84	7.63	7.52
EC, dS m^{-1}	0.20	0.15	0.15	0.15
Org.content,%	2.39	1.56	2.24	1.54
N Kjeldal,%	0.17	0.34	0.22	0.13
P, mg kg^{-1}	733	607	823	355
K, mg kg^{-1}	6094	8030	5648	6780

Ca, mg kg^{-1}	51981	24356	33262	16061
Mg, mg kg^{-1}	10674	12574	10799	10040
Pb, mg kg^{-1}	26221	2509	2429	24.6
Cu, mg kg^{-1}	2610	222	170	16.0
Zn, mg kg^{-1}	10596	2424	2476	33.9
Cd, mg kg^{-1}	357	64.3	45.0	2.7
Fe, mg kg^{-1}	33008	27388	21207	27113
Mn, mg kg^{-1}	1216	1045	844	884
Hg, mg kg^{-1}	2.50	0.58	0.83	1.33

MAC according to Bulgarian legislation (Regulation No. 3 of 1 August 2008 on the norms for the permissible content of harmful substances in soils) (pH 6.0-7.4) – Pb -60 mg kg^{-1}, Cd-2.0 mg kg^{-1}, Zn-320 mg kg^{-1}, Cu-150 mg kg^{-1}, Hg -1.5 mg kg^{-1}
MAC (pH >7.4) – Pb -100 mg kg^{-1}, Cd – 3.0 mg kg^{-1}, Zn -400 mg kg^{-1}, Cu-300 mg kg^{-1}, Hg - 1.5 mg kg^{-1}

The results presented in Table 1 show that with distance from NFMW - Plovdiv, there is a well-defined tendency for decreasing the total content of heavy metals in the soil. In soil samples taken from the area 0.1 km and 2.0 km away from the NFMW, values for Pb exceeding the MAC (100 mg kg^{-1}) were recorded. In soil samples from S1 and S2 (0.1 km from the NFMW), Pb values ranged from 2509 mg kg^{-1} to 26221 mg kg^{-1}. The Pb content in soil from S3 (2 km away from the NFMW) reached 2429 mg kg^{-1}, while in the area 11 km away from the NFMW (S4), the Pb content decreased to 24.6 mg kg^{-1}. Similar results were obtained for Cd and Zn. The reported values significantly exceed the MAC. Soil 1 (0.1 km from the NFMW) recorded 10596 mg kg^{-1} Zn and 357 mg kg^{-1} Cd, soil 2 recorded 2424 mg kg^{-1} Zn and 64.3 mg kg^{-1} Cd, and the area 2 km from the NFMW (soil 3) recorded 2476 mg kg^{-1} Zn and 45 mg kg^{-1} Cd. In the more remote area (11 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 was significantly lower compared to the accepted MAC of 300 mg kg^{-1} (except for the sample from soil 1). The difference observed between the S1 and S2 spaced at the same distance (0.1 km) from the NFMW is due to the spatial location of the points relative to the NFMW. Soil 1 and soil 2 were taken at a distance of 0.1 km from the NFMW (along the length of the NFMW). Soil 1 is located opposite the lead raw material storage facilities. The warehouses are semi-open, allowing the drift of dry concentrates containing primarily PbS, ZnS, and CdS to the adjacent areas (soil 1). The second sample is located opposite the plant portal and relatively distant from the stockpiles.

Table 2 presents the results obtained for the mobile forms of Pb, Zn, and Cd in the investigated soils. The table also gives the percentage of the mobile forms relative to the total amount of the elements in the soil.

Table 2. Mobile forms (DTPA - extracted) of Pb, Zn, and Cd (mg kg^{-1}) in soils of the study area

Soils	Pb		Zn		Cd	
	mg kg^{-1}	%*	mg kg^{-1}	%*	mg kg^{-1}	%*
S1 (0.1 km)	584	2.2	469	4.4	81.9	22.1
S2 (0.1 km)	849	33.8	237	9.9	37.0	57.5
S3 (2 km)	895	36.9	412	16.6	26.6	61.1
S4 (11 km)	2.1	8.5	2.4	7.1	0.67	24.8

** % = (DTPA extracted / total content)*100*

The results for mobile forms of metals determined by DTPA showed that mobile forms of Cd in contaminated soils accounted for the highest proportion of its total content, followed by Pb and Zn. Notably, the content of mobile forms of heavy metals in soil from soil 1 is the lowest, which is contrary to expectations. This is the soil with the highest heavy metal content, and logically the amount of mobile forms of metals should be highest there. An explanation of this fact should be sought in the location of the sampling points and the possibilities of chemical processes involving

Pb. As already noted, the main cause of contamination at the S1 is due to the drift of dry concentrates from the semi-open storage areas, containing mainly PbS (galena). PbS is known to be readily oxidised by oxygen in the air to $PbSO_4$, which binds with CO_2 in the presence of moisture to $PbCO_3$:

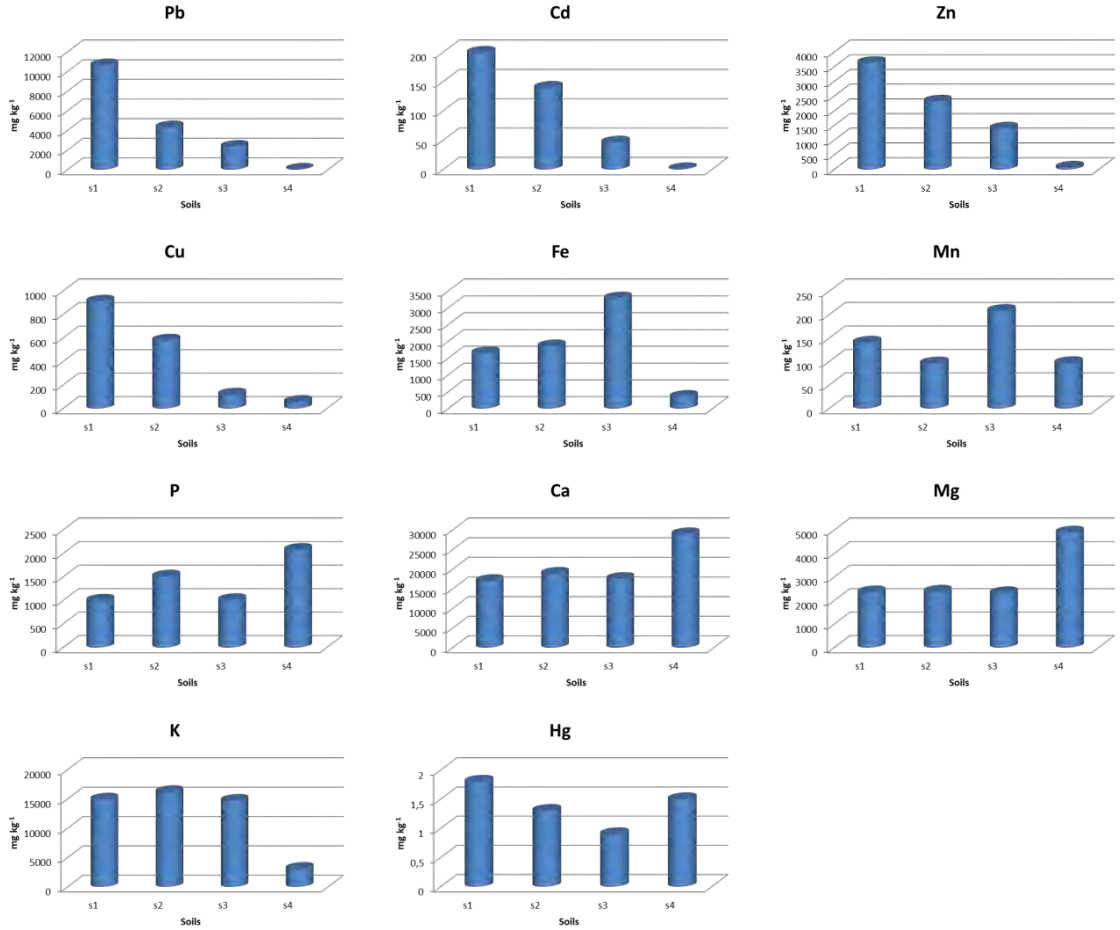
$$PbS + 2O_2 = PbSO_4$$

$$PbSO_4 + H_2O + CO_2 = PbCO_3 + H_2SO_4$$

The ratio between the amounts of Pb compounds determines the soil's total Pb content and the amount of exchangeable and available forms to plants. Drier and better-aerated soils stimulate the formation of the highly soluble lead sulphate, which readily passes into the soil solution and decreases total Pb, and increases its mobile forms (Kabata Pendias, 2001). The above processes are less likely in waterlogged soils, as confirmed by higher contamination levels. This is generally confirmed by the results presented in Tables 1 and 2 and explains the low content of mobile forms of Pb in the S1.

Soil conditions and plant type influence the uptake of heavy metals into plants, and their accumulation in plant organs depends on their content in the soil. Plants grown on contaminated soils accumulate heavy metals and accumulate them in their vegetative organs. Most of them get into the roots of plants as they come into contact with the contaminated soil. In some plants, however, a substantial fraction of heavy metals accumulate in the aboveground mass (Peng et al., 2006).

Figure 1 presents the results obtained for the heavy metal, micro and macro element contents in the aboveground mass (leafage) of Paulownia.



Graph.1. Content (mg kg^{-1}) of heavy metals, micro and macroelements in the leafage of Paulownia grown in the region of NFMW

As the source of contamination became more distant, there was a clear trend towards a decrease in the heavy metal content of paulownia leaves. The amount depends primarily on the distance to the pollution source and the heavy metal content of the soil. The Pb content in Paulownia leaves grown at a distance of 0.1 km (S1 and S2) ranged from 4319 to 10649 mg kg^{-1}, Zn from 2318 to 3635 mg kg^{-1} and Cd from 139 to 199 mg kg^{-1}. The Pb content of the Paulownia leaves from S3 (2 km NFMW) decreased to 2341 mg kg^{-1}, Zn to 1397 mg kg^{-1} and Cd to 45.9 mg kg^{-1}. In the Paulownia grown in the area 11 km from the NFMW, the recorded values were lower: -16.9 mg kg^{-1} Pb, 69.2 mg kg^{-1} Zn, and 0.43 mg kg^{-1} Cd. The obtained results strongly indicate that heavy metal contamination is primarily aerosol and the amount depends mainly on the distance to the source of contamination and the heavy metal content of the soil.

Similar results were obtained by Stankovic et al. (2009), who suggested that the accumulation of Pb in Paulownia foliage is of anthropogenic origin and due to vehicular traffic. The efficiency of Paulownia in the uptake and accumulation of Zn from contaminated soils was found by Azzarello et al. (2012) and Wang et al. (2010). Wang et al. (2010) found 3000 mg kg^{-1} Zn in the leaves of *P. fortunei* grown on soils with a Zn content of 1000 mg kg^{-1}.

The Cu content of Paulownia leaves ranged from 57.5 mg kg^{-1} to 911 mg kg^{-1}. These results are higher than the results of Madejon et al. (2014), who reported that the Cu content in paulownia leaves was low and ranged from 15 to 40 mg kg^{-1}. These values are higher than the upper limit of the range of sufficient or typical values (5-30 mg kg^{-1}, Kabata-Pendias (2001) and Mills and Jones (1991) (15 mg kg^{-1}) and significantly exceed the values suggested by Element Concentration Cadasters in Ecosystems (ECCE) (2-20 mg kg^{-1}) (Lieth and Markert, 1990). Presented results indicate the ability of Paulownia to accumulate copper in its tissues and are in agreement with the results of Jiang et al. (2012), who found that *P. fortunei* is a Cu-tolerant plant and can accumulate Cu in the aerial parts.

The Fe content in Paulownia leaves was extraordinarily high and ranged from 351 (S4) to 3281 mg kg^{-1} (S3) and exceeded the values given in ECCE (5-200 mg kg^{-1}) (Lieth and Markert, 1990). Lower content of Fe were found in Paulownia leaves by Stankovic et al. (2009) (105-362 mg kg^{-1}) in urban environments.

The Mn content reached 208 mg kg^{-1} and it was within the range of values given in ECCE (1-700 mg kg^{-1}) (Lieth and Markert, 1990).

Plants can absorb and accumulate mercury from the environment. The mercury content in Paulownia leaves varies within a relatively narrow range from 0.9 to 1.8 mg kg^{-1}.

The highest values for Ca, Mg, and P, were found in the leaves of Paulownia grown 11 km from the NFMW (S4). Ca, Mg, and P contents reached 28934 mg kg^{-1}, 3835 mg kg^{-1} and 2081 mg kg^{-1}, respectively. In the leaves of Paulownia grown at a distance of 0.1 km (S1 and S2) and 11 km (S3), the Ca, Mg, and P contents were lower and ranged from 14720 to 16209 mg kg^{-1} for Ca, from 1174 to 2354 mg kg^{-1} for Mg and from 1000 to 1512 mg kg^{-1} for P. The K content in leaves of Paulownia grown at 0.1 and 11 km spacing ranged from 3104 mg kg^{-1} (S4) to 16209 mg kg^{-1} (S2).

According to Madejon et al. (2014), there was no significant difference between the macronutrient contents of Paulownia grown on contaminated and non-contaminated soils. However, according to Kabata-Pendias (2001), heavy metals can change plants‘ nutrient uptake. The results confirm that high heavy metal content in the soil leads to less uptake of nutrients Ca, Mg, and P.

Paulownia can be used as an indicator plant for air (and soil) pollution. Differences in the heavy metal content in the leaves of Paulownia grown at different distances from the NFMW indicate significant aerosol pollution in the NFMW area, which decreases with increasing distance. Aerosol pollutants can spread over hundreds of kilometres, depending on weather conditions, wind direction, and wind speed. It is, therefore, necessary to conduct comprehensive monitoring over a significant area around the sources of pollution. Such monitoring should include determination soil and air physico-chemical parameters and a range of biological parameters (e.g. tree species such as Paulownia). Paulownia may be recommended for the construction of protective belts around industrial plants.

Conclusions

Based on the obtained results, the following more important conclusions can be drawn:

1. Paulownia is tolerant to heavy metals and can be successfully used in the phytoremediation of heavy metal-contaminated soils.
2. Paulownia can be used as an indicator plant for air (and soil) pollution and to build buffer strips around industrial plants.
3. Increased heavy metal content in soil results in less uptake of nutrients Ca, Mg and P in Paulownia leaves.

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Influence of heat stress on chloroplast pigments and proline content in apple leaves

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Abstract

Apple crops prefer moderate temperatures along with medium to high humidity. Dry climate and long-term high temperatures during summer can negatively affect the synthesis of chloroplast pigments in the apple leaf, which negatively affects the assimilation processes. One of the defense strategies against heat stress is the synthesis and accumulation of free proline, which is an important osmoregulator. The aim of the research was to determine the content of free proline and chloroplast pigments in the leaves of thirty-three apple varieties grown at two climatically different localities in Croatia, Tenja and Donja Zelina. The leaves were sampled after a long-term hot period, at the end of June 2021. In average for all the examined apple varieties, the location significantly influenced the content of free proline, chlorophyll a, total chlorophylls and the ratio of total chlorophylls and carotenoids. A significant influence of the variety on the accumulation of proline and the content of carotenoids was determined, which points to the genetic specificity in the response to high temperatures. Future research that will link the physiological response of apple to heat stress with the pomological properties of the fruit, should help producers in choosing varieties which are more suitable for cultivation in particular climatic conditions.

Keywords: apple varieties, heat stress, osmolyte accumulation, photosynthetic pigments

Introduction

The apple (*Malus domestica* Borkh.) is an important fruit, commonly consumed as unprocessed fresh fruit but also as canned, dried and frozen products. Since fresh apples are available worldwide all year round, their taste and texture are important (Doores and Splittstoesser, 1983; Sugiura et al., 2013). Cultivated apple is known for almost 3000 years and is spread by Romans troughtout Europe and Asia (Korban and Skirvin, 1984).

According to Food and Agricultural Organisation, it is estimated that apple production in Europe in 2020 was 17,535,979 tons over area of 984,509 ha. In 2020, apple production in Croatia was estimated to 63,610 tons harvested on an area of 4,360 ha (https://www.fao.org/faostat/en/#data/QCL).

Crop growth, yield and quality is expected to be under influence of global climate changes (Hay and Porter, 2006). Atmospheric CO_2 content raised from pre-industrial levels of 280 ppm over 410 ppm in 2021, and projections to the end of this century suggest that atmospheric CO_2 will be 700 ppm or more, whereas global temperature will increase by 1.8 – 4.0 °C, depending on the greenhouse emission scenario (Solomon, 2007; Bala, 2013; Friedlingstein et al., 2022). Plants with C3 photosyntetic pathways, such as apple, respond to elevated CO_2 through photosyntesis, stomatal conductance and decreased transpiration (Long et al., 2004; Long et al., 2006). In many crops, an elevated temperature reduces pollen viability and decreases the percentage of seed and fruit set (DaMatta et al., 2010).

Water deficit also influences various physiological and biochemical processes in plants (Šircelj et al., 2005). Along with the increased formation of free radicals and reactive oxygen species (ROS), high temperatures accompanied with lack of water, affects metabolism and increases the contents of soluble carbohydrates, free proline and other aminoacids (Hanson and Hitz, 1982; Chaves, 2003; Pinheiro et al., 2004). Changes in the ratios of photosynthetic pigments have been suggested as early indicators of oxidative stress (Darrall and Jager, 1984). Amino acids and carotenoids have protective role in photosynthetic apparatus against high light and reactive oxygen species, so their content increases with the intensity of slowly developing oxidative stress (Šircelj et al., 1999). A significant reduction

in chlorophyll content under heat and water stress conditions has been reported for many plant species (Mibei et al., 2017; Shivakrishna et al., 2018), including apple trees (Bai et al., 2019; Kovalikova et al., 2020; Mihaljević et al., 2021).

In Croatia, approximately hundred varieties of apples are currently grown with a large variability in tolerance to the negative effects of heat stress. Thus, the aim of this study was to determine the content of free proline and chloroplast pigments in the leaves of thirty-three apple varieties grown at two climatically different localities in Croatia.

Material and methods

Plant material

The experiment with thirty-three apple varieties differing in ripening period was settled in two climatically different localities: Experimental station Tenja (Faculty of Agrobiotechnical sciences Osijek), location Tenja (45.514342 N, 18.785161 E; Osijek-Baranja County; Croatia) and Experimental station Donja Zelina (Croatian Agency for Agriculture and Food), location Donja Zelina (45.921960 N, 16.225111 E; Zagreb County; Croatia). The orchards were established in 2020, and protective nets and irrigation system were installed. All cultivars were planted in four replicates by the method of random block design, where each cultivar in the particular block was represented by three plants. Conventional methods of cultivation and pest protection were applied during the growing season. Young apple leaves were sampled for laboratory analysis on June 28, 2021.

The determination of chlorophyll and carotenoid content

Apple leaves were grounded in liquid nitrogen and 0.05 g of powder was weighed in the 15 mL threaded plastic tubes by adding the $MgCO_3$ powder to neutralize acidity, along with the 10 mL of acetone. The samples were homogenized on a vortex mixer and then centrifuged at 4000*g* at 4°C for 10 minutes. The absorbance of supernatants were measured in a glass cuvette, at the wavelengths of 662, 644, and 440 nm. The concentration of chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Chl a+b), and the carotenoids (Car) were calculated using Holm-Wettstein's equations (Holm, 1954; Wettstein, 1957).

The determination of proline content

The proline content was analyzed according to Carillo and Gibon (2011) with minor modifications. Approximately 0.5 g of leaf powder was extracted with the 1.5 mL of 70% ethanol. The samples were homogenized on a vortex mixer and then centrifuged at 3500*g* at 4°C for 15 minutes. Supernatant was treated with reaction mixture containing 1% ninhydrin acid in 60% acetic acid and 20% of ethanol. After homogenization, the extracts were incubated for 20 minutes at 95°C. After cooling, the concentration of proline was determined by measuring the absorbance at 520 nm and compared with the absorbance of a range of standard proline solutions containing 0 – 45 nmol proline mL^{-1}.

Data analysis and processing

The concentrations of proline and chloroplast pigments were measured with Varian Cary 50 UV-VIS spectrophotometer using the Cary WinUV software. The results obtained from the four replicates were analyzed using the SAS Software 9.1.3 (2002 to 2003, SAS Institute Inc., Cary, USA). The analysis of variance (ANOVA), F test, and Fisher's LSD test (least significant difference) were used.

Results and discussion

Temperature is one of the climatic factors which has a great influence on the growth and development of plants. The range of optimal values is specific for a particular species and it was considered as a limiting factor influencing the quality and quantity of yield, in a large number of agricultural species (Bita and Gerats, 2013; Hatfield and Prueger, 2015).

In cultivars phylogenetically and through selection adapted to grow in a moderate climate, leaf overheating often occurs during hot and dry days. Excessively high leaf temperatures may negatively affect photosynthetic reactions

what can limit the growth, development and survival of plants (Seemann et al., 1984). Under such conditions, the effectiveness of the photosynthesis decreases as a result of physiological and biochemical changes in the functioning of the enzymes involved in the synthesis of chloroplast pigments (Ashraf and Harris, 2013).

The average daily air temperature measured in the orchard at Tenja experimental site during the second half of June was 25°C, while the average of maximum temperatures was 35°C (data not shown). As a result of high daytime temperatures and dry air, the average of maximum leaf temperature was 39°C with maximum reaching 45°C.

At Donja Zelina experimental site, the average daily air temperature measured during aforementioned period was 24°C, while the average of maximum temperatures was 33°C. The average of maximum leaf temperature was 38°C with maximum reaching 42°C.

According to the F test, in average for all the examined apple varieties, a location significantly influenced the content of chlorophyll a, total chlorophylls and the ratio of total chlorophylls and carotenoids in apple leaves (Table 1).

Table 1. The influence of locality on the content of free proline (Pro) and chloroplast pigments (Chl - chlorophyll, Car - carotenoids) in apple leaves (FW – fresh weight), in average for 33 varieties. The data are means of four replicates; ANOVA, F test. The values marked with different letters (A and B) differ according to the LSD test ($p \leq 0.05$).

	Pro nmol g^{-1} FW^{-1}	Chl a mg g^{-1} FW^{-1}	Chl b mg g^{-1} FW^{-1}	Chl a+b mg g^{-1} FW^{-1}	Car mg g^{-1} FW^{-1}	Chl a/b	Chl a+b/ Car
Tenja	283^{B}	0.552^{A}	0.175	0.727^{A}	0.410	3.34	2.04^{A}
Donja Zelina	475^{A}	0.501^{B}	0.165	0.667^{B}	0.434	3.32	1.65^{B}
F Value	248.17	8.77	0.61	6.06	1.93	0.18	15.05
p	<0.0001	0.0033	0.4363	0.0145	0.1656	0.6705	<0.0001

The content of chlorophyll a, total chlorophylls and the ratio of chlorophylls and carotenoids were significantly higher in apple leaves at the Tenja location. In average for both localities, variety of apple significant influenced on the content of carotenoids and both pigments' ratios (chlorophyll a/b and total chlorophylls/carotenoids, Table 2). From the data obtained, it is evident that the concentration of chlorophyll in apple leaves depends more on the environmental conditions than on the variety itself.

Table 2. The influence of variety on the content of free proline (Pro) and chloroplast pigments (Chl - chlorophyll, Car - carotenoids) in apple leaves (FW – fresh weight), in average for both localities. The data are means of four replicates; ANOVA, F test, LSD test ($p \leq 0.05$).

VARIETY	Pro nmol g^{-1} FW^{-1}	Chl a mg g^{-1} FW^{-1}	Chl b mg g^{-1} FW^{-1}	Chl a+b mg g^{-1} FW^{-1}	Car mg g^{-1} FW^{-1}	Chl a/b	Chl a+b/ Car
Allegro	366.19	0.506	0.150	0.656	0.327	3.39	2.25
B. Lochbuie	373.77	0.645	0.207	0.852	0.417	3.09	2.31
B. Mariri Red	503.16	0.510	0.162	0.671	0.380	3.15	2.26
Bay 3341	411.17	0.505	0.147	0.652	0.473	3.42	1.36
C. Crisp	270.25	0.568	0.162	0.730	0.504	3.48	1.50
Collina	449.81	0.493	0.140	0.633	0.473	3.51	1.40
Freya	356.62	0.500	0.135	0.635	0.419	3.71	1.52
Fuji Fubrax	371.35	0.638	0.195	0.833	0.516	3.27	2.10
G. Rush Coop	449.27	0.695	0.206	0.901	0.481	3.38	2.04
G. Dark Ann	378.83	0.457	0.148	0.604	0.414	3.10	1.49

G. S. Schniga	377.66	0.526	0.164	0.690	0.388	3.21	1.76
G. S. Schnico	421.09	0.536	0.169	0.705	0.390	3.19	1.94
Galval	378.37	0.532	0.164	0.695	0.489	3.26	1.43
G. Galaxy	353.96	0.498	0.180	0.678	0.334	3.03	2.11
Orion	309.79	0.539	0.172	0.711	0.376	3.24	2.04
Granny Smith	505.47	0.421	0.129	0.550	0.345	3.25	2.14
G. Smoothee	273.76	0.443	0.199	0.641	0.205	2.88	3.22
Rozela	417.99	0.488	0.133	0.621	0.420	3.70	1.47
J. Novajo	285.28	0.559	0.159	0.718	0.427	3.52	1.77
W. Jonaprince	321.71	0.498	0.141	0.639	0.279	3.55	2.41
Karneval	422.69	0.538	0.287	0.825	0.532	2.97	2.05
Merkur	311.02	0.556	0.195	0.752	0.383	3.21	2.03
Opal	351.78	0.426	0.220	0.645	0.426	3.34	1.84
S. C. Sandidge	372.17	0.515	0.160	0.676	0.366	3.23	2.01
Pinova	422.59	0.532	0.145	0.677	0.403	3.70	1.67
R. Idared	365.54	0.579	0.200	0.780	0.365	2.95	2.20
R. Topaz	314.68	0.520	0.162	0.683	0.434	3.59	1.64
Reglindis	410.66	0.545	0.189	0.733	0.445	3.44	1.86
Jeromine	398.16	0.570	0.170	0.739	0.548	3.38	1.38
Santana	438.49	0.483	0.179	0.661	0.533	3.05	1.34
Sirius	410.77	0.575	0.165	0.740	0.421	3.51	1.74
Roats King R. D.	394.70	0.507	0.153	0.660	0.525	3.35	1.26
Topaz	313.93	0.483	0.128	0.612	0.490	3.82	1.25
LSD	131.86	/	/	/	0.128	0.504	0.764
F Value	1.55	1.46	0.83	1.18	2.81	1.72	2.36
p	0.0371	0.0606	0.7355	0.2405	<0.0001	0.0128	<0.0001

Since carotenoids have a unique antioxidant role in photosystems, especially in the dissipation of excess energy and neutralization of triplet oxygen (Rmiki et al. 1999), a reduced ratio of chlorophyll and carotenoids may indicate photooxidative stress due to high temperatures and irradiation. One of the functions of the protective net in orchards is to reduce the amount of direct sun irradiation that reaches the surface of the leaves. When temperature rises above 40°C, plants which are adapted to moderate climate conditions suffer with higher amount of water loss through stomata. This may reduce the chlorophyll content, which leads to a decline of photosynthesis and respiration rates (Kumar et al., 2012; Gosavi et al., 2014). According to Sharkey (2005), the temperature of the leaves exposed to full sunlight can be significantly above ambient temperature, and it depends on the various factors that affect the transpiration, such as air and soil humidity, air flow, an exposure and inclination of the orchard, etc. Kalcsits et al. (2017) reported that at an ambient temperature of 29°C, the leaves' temperature of apple variety Honeycrisp was 36°C.

Proline is a water-soluble amino acid which is a known cellular osmotic regulator (Kumar et al., 2012). It is also involved into the ROS detoxification mechanisms through the stabilization of antioxidative enzymes (Bandurska, 1993). The accumulation of free proline occurs in plant tissues in response to stresses resulting in low water potential such as heat, drought, salinity and cold (Anaytullah, 2007; Verslues et al., 2006;). Thus, the biosynthesis and accumulation of free proline is often used as the physiological index of stress tolerance (Solomon et al., 1994).

In our research, the locality significantly influenced on the content of free proline in apple leaves with 60% higher accumulation determined in leaves sampled at Donja Zelina as compared to Tenja (Table 1). In average for both localities, the variety significantly influenced on the free proline content (Table 2). In general, late season ripening

apples accumulate higher amount of free proline in their leaves in comparison to early-ripening varieties. Varieties Braeburn Mariri Red and Granny Smith stood out by the highest accumulation of free proline in leaves, which was above 500 nmol g^{-1} FW^{-1}.

The capacity of proline accumulation can vary from one species to another as well as between the varieties within the same species, as a result of breeding process based on obtaining desirable traits (Hayat et al., 2012; Verbruggen and Hermans, 2008). Beside the genetic base of free proline accumulation, it also depends upon the level and duration of stress conditions. Therefore, an increased accumulation of proline in apple leaves is a potential early indicator of heat stress, which can be used in the selection of varieties more resistant to stressful growing conditions.

Conclusions

Physiological and biochemical reactions of apple trees to water and heat stress are quite variable which could be associated with cultivar, time of the year, previous intensity and duration of stress as well as other environmental conditions. Understanding the early response of particular apple variety to heat stress, will have profound importance in breeding program with the aim of creating new generations of resistant crops. According to the obtained results, varieties Braeburn Mariri Red and Granny Smith can be recommended as a good genetic base for further selection. Future research that will link the physiological response of the apple to heat stress with the quality of the pomological properties of the fruit, will help producers in choosing varieties which are more suitable for cultivation in particular climatic conditions.

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Značajke dinamike podzemnih voda na području Bid-bosutskog polja

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Sažetak

Cilj rada je prikazati dinamiku podzemnih voda na području Bid-bosutskog polja, smještenog na području istočne Slavonije. U razdoblju istraživanja (2003-2021) primijećen je blagi trend porasta količine oborina od 0,06 mm godišnje te trend porasta prosječnih mjesečnih temperatura zraka za 0,094 °C godišnje. Podaci praćenja razine podzemne vode u vodonosniku dubine 4 m tijekom razdoblja istraživanja (2003-2021) pokazuju prosječni trend sniženja od 8,8 cm godišnje.Također, od 2015. godine prati se i razina podzemne vode u vodonosniku dubine 15 m te izmjerene vrijednosti (2015-2021) pokazuju prosječni trend sniženja od 23,3 cm godišnje. Za isto sedmogodišnje razdoblje, trend sniženja razine podzemne vode u vodonosniku dubine 4 m iznosi 22,7 cm godišnje.

Ključne riječi: dinamika podzemnih voda, sniženje razine, piezometar, poljoprivreda, analiza trenda

Uvod

Razina podzemne vode i njezina vremenska varijabilnost ima značajan ekonomski utjecaj (Asmuth i Knotters, 2004). Najvidljiviji ekonomski učinci dinamike podzemnih voda su pojave poplava ili suša (Baker i sur., 1988; Jarett, 1991). U poljoprivrednim područjima visoke razine podzemnih voda mogu prouzročiti propadanje usjeva i značajno otežati upotrebu poljoprivredne mehanizacije, dok preniske razine i izostanak kapilarnog dizanja podzemne vode do zone rizosfere mogu značajno sniziti prinose (Kroes i sur., 2000). Naime, plitki akviferi podzemne vode osiguravaju kontinuiranu opskrbu vodom u zoni korijena putem kapilarnog dizanja (Chen i Hu, 2004) te tako osiguravaju dodatni izvor vode za procese transpiracije poljoprivrednih usjeva (Karimov i sur., 2014).

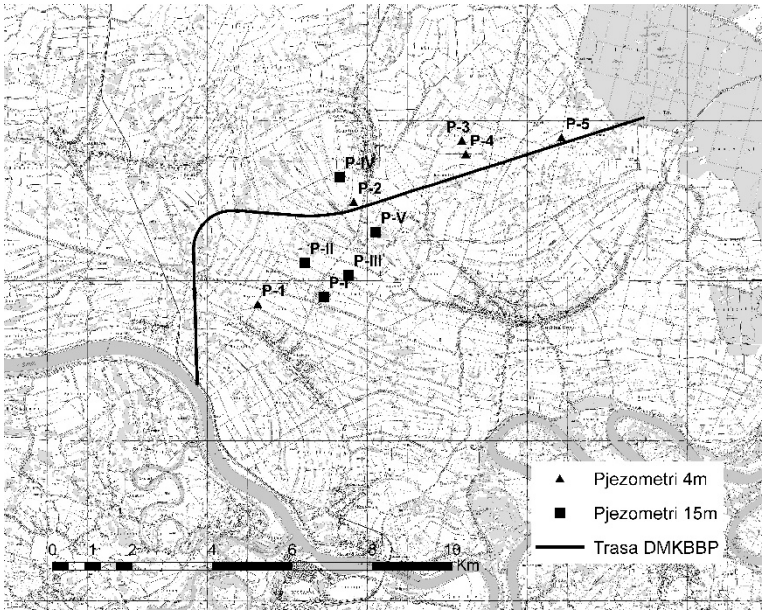
Promjena balansa između oborina, otjecanja i evaporacije u nekoliko zadnjih desetljeća pripisuje se antropogenim klimatskim promjenama (Farzana i sur., 2021). Akviferi podzemnih voda sporije reagiraju na klimatske promjene od površinskih voda, ali dugoročno gledano javljaju se značajne promjene njihove prosječne razine (Franssen, 2009). Unutar hidrološkog ciklusa, podzemne vode zadnje reagiraju na pojavu suše. Smanjenje rezervi podzemne vode vrlo je česti i opasni oblik suše (hidrološke suše), a definira se kao sniženje razine podzemne vode ispod njene dugoročne prosječne razine (Bonacci, 2019). Kad nastupe relativno kraća sušna razdoblja, sustavi podzemnih voda mogu ublažiti nedostatak infiltracije oborina. Budući da se razina podzemne vode ne spušta neposredno nakon nepovoljnih klimatskih prilika, promjena režima podzemne vode se ne događa uvijek, ali kad do toga dođe, često slijede duga razdoblja koja obilježava značajan pad razine podzemne vode.

Stjecanje temeljnih znanja o interakciji promjenjivih klimatskih uvjeta, navodnjavanja, kvalitete vode, režima razine podzemnih voda i njihovog učinka na uzimanje vode putem korijena ključno je za održivu poljoprivredu (Askari i sur., 2014).

Materijali i metode

Bid-bosutsko polje je dio prostrane savske doline, a određeno je slivnim područjem vodotoka Bid. Područje je u hidropedološkom pogledu vrlo heterogeno pri čemu dominiraju hidromorfna tla sa specifičnim načinima vlaženja, različitom stratigrafskom i teksturnom građom soluma te neujednačenom vertikalnom i horizontalnom vodopropusnošću profila (Petošić, 2002). Utvrđeno je postojanje plitkog talnog vodonosnika dubine 4 m i dubljeg

podtalnog vodonosnika područja dubine 15 m čije razine pokazuju čvrstu korelaciju (r = 0,75-0,94) (Mustać i sur., 2011). Za analizu osnovnih klimatskih parametara (oborine i temperature zraka) korišteni su podaci s meteorološke postaje Gradište kod Županje. Praćenje dinamike (razine) podzemnih voda vršeno je pomoću 5 hidropedoloških piezometara dubine od 4 m (oznake: P-1 do P-5) i 5 vrlo dubokih piezometara dubine od 15 m (oznake: P-I do P-V). Piezometri su postavljeni na području Bid-bosutskog polja, a pokrivaju površinu od oko 7200 ha (slika 1). Na hidropedološkim piezometrima dubine od 4 m mjerenja su vršena ručno svakih 10 dana. Na vrlo dubokim piezometrima dubine od 15 m mjerenje dnevne vrijednosti razine podzemne vode vršeno je pomoću limnigrafa. Podaci o vodostaju rijeke Save dobiveni su s vodomjerne postaje Sava-Slavonski Šamac.

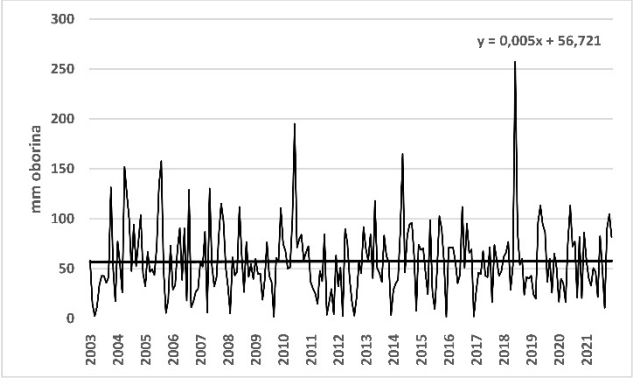


Slika 1. Područje istraživanja s prikazom ugrađene mjerne opreme

Rezultati i rasprava

Oborine

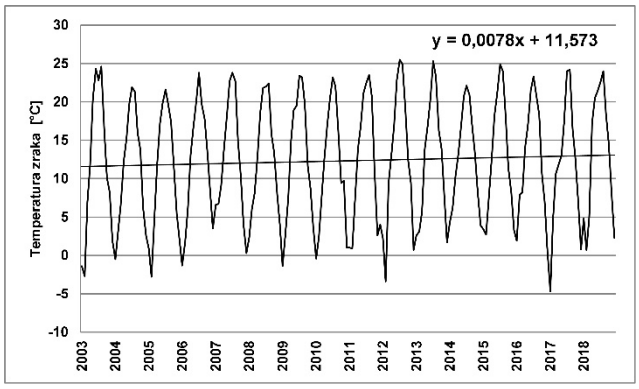
Na slici 2 prikazane su mjesečne vrijednosti oborina tijekom razdoblja istraživanja 2003.-2021. Iz formule trenda (slika 2) može se iščitati pozitivan trend porasta količine oborina od 0,06 mm godišnje, što je u skladu s podacima Perčec Tadić i sur., 2014. Međutim, valja ukazati na stvarnu situaciju na terenu, gdje je prisutna pojava sve učestalijih i duljih sušnih razdoblja tijekom godine kao i povremenih rekordnih količina padalina (npr. 258 mm, lipanj, 2018).



Slika 2. Mjesečne vrijednosti oborina (mm) tijekom razdoblja istraživanja

Temperature zraka

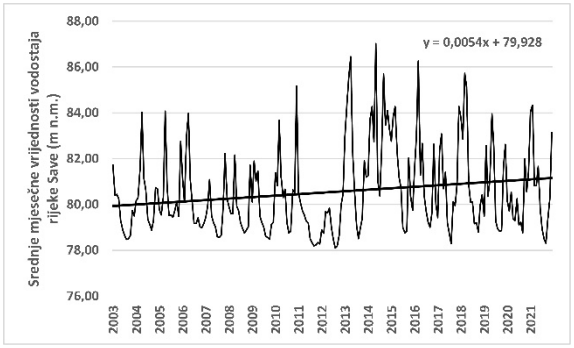
Iz formule trenda na slici 3 može se očitati porast prosječnih mjesečnih temperatura zraka od 0,094 °C godišnje u razdoblju od 2003. do 2021. godine. Navedena mjerenja su izvršena u relativno kratkom vremenskom razdoblju (19 godina) i na osnovu njih nije zahvalno iznositi definitivne zaključke, ali su svakako indikativna te u skladu s domaćim i globalnim klimatskim trendovima.



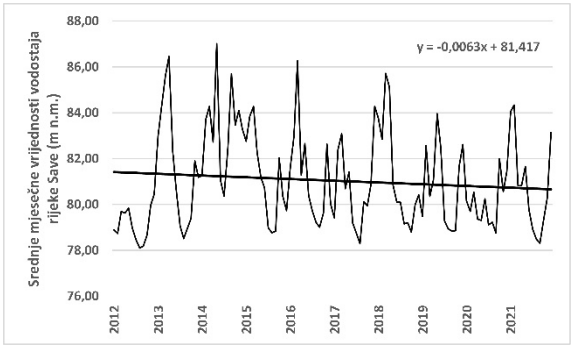
Slika 3. Prosječne mjesečne temperature zraka (°C) na području istraživanja

Vodostaj rijeke Save

Monitoringom vodnog režima na istraživanom području koji se vrši od 2003. godine (Petošić i sur., 2004), generalno je utvrđeno da dominantan utjecaj na kolebanje razine podzemne vode u talnom plitkom akviferu istraživanog područja imaju količina i raspored oborina, prostrano slivno područje, a posebice dinamika vodostaja u glavnom vodotoku područja, odnosno rijeci Savi. Na slici 4 prikazane su prosječne mjesečne vrijednosti vodostaja rijeke Save u razdoblju istraživanja (2003-2021). Iz formule trenda primjetan je trend blagog godišnjeg porasta razine vodostaja rijeke Save od 6,5 cm godišnje. Međutim, na slici 5 prikazane su iste vrijednosti za vremensko razdoblje od zadnjih 10 godina (2012-2021) gdje je primjetan trend sniženja vodostaja rijeke Save od 7,6 cm godišnje, što vjerodostojnije opisuje trenutnu situaciju na samom terenu. Budući da je prosječna visina terena istraživanog područja 82 m n.m. te u slučaju kada vodostaj rijeke Save padne ispod navedene vrijednosti (što se gotovo u pravilu javlja u ljetnom dijelu godine), rijeka Sava sve više gubi prihranjujući, a pojačava drenirajući utjecaj na plitki talni vodonosnik istraživanog poručja.



Slika 4. Prosječne mjesečne vrijednosti vodostaja rijeke Save (2003-2021; m n.m.)

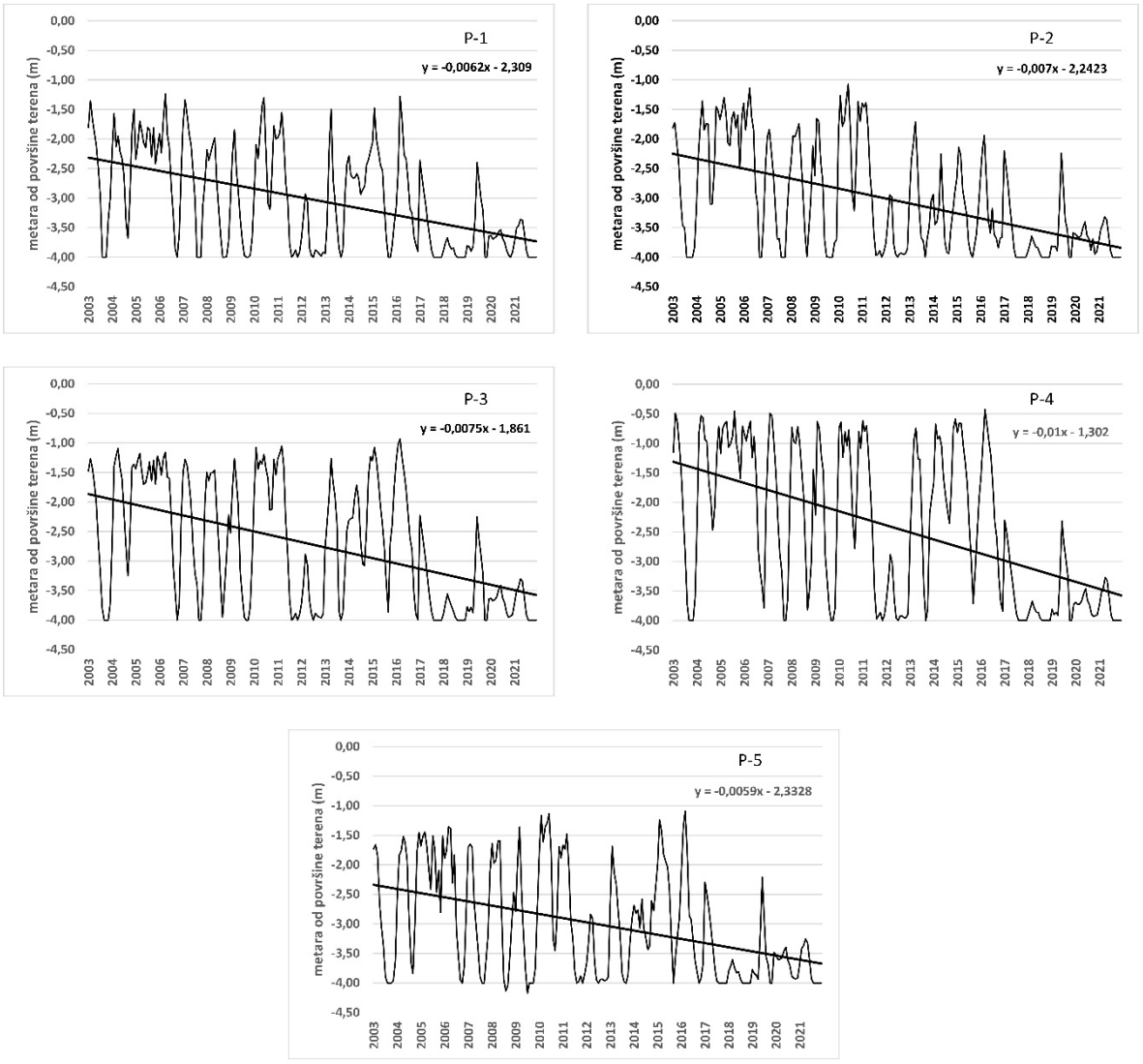


Slika 5. Prosječne mjesečne vrijednosti vodostaja rijeke Save (2012-2021; m n.m.)

Dinamika podzemnih voda na području istraživanja

Na slici 6 prikazana je dinamika srednjih mjesečnih vrijednosti razine podzemne vode u hidropedološkim piezometrima (4 m dubine) na području istraživanja (2003-2021). Temeljem prikazanoga uočljiv je godišnji trend sniženja razine podzemne vode kako slijedi (P-1: 7,4 cm godišnje; P-2: 8,4 cm godišnje; P-3: 9,0 cm godišnje; P-4:

12,0 cm godišnje; P-5:7,1 cm godišnje), odnosno prosječno godišnje sniženje razine podzemne vode od 8,8 cm.

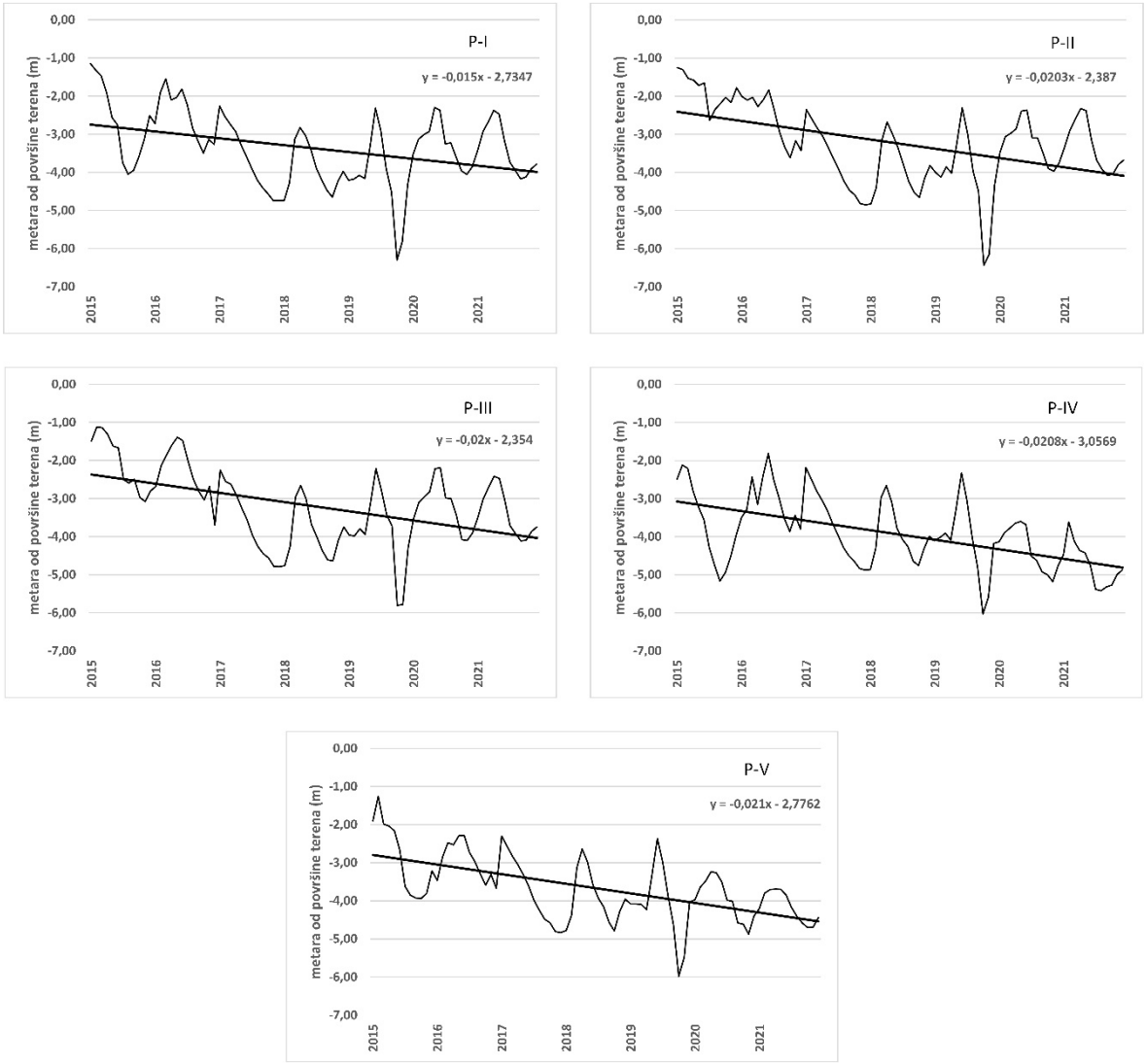


Slika 6. Dinamika srednjih mjesečnih vrijednosti razine podzemne vode u hidropedološkim piezometrima (4 m dubine) na području istraživanja

Valja naglasiti da se na ovim piezometrima mjerenje moglo vršiti samo dok razina podzemne vode ne padne ispod 4 m dubine od površine terena. Zbog sve učestalije pojave sniženja razine podzemne vode ispod 4 m dubine u odnosu na površinu terena, 2014. godine na području istraživanja ugrađeno je 5 dodatnih vrlo dubokih piezometara dubine 15 m.

Iz formula trenda (slika 7) na ovim vrlo dubokim piezometrima može se iščitati godišnji trend sniženja razine podzemne vode kako slijedi (P-I: 18,0 cm godišnje; P-II: 24,4 cm godišnje; P-III: 24,0 cm godišnje; P-IV: 25,0 cm godišnje; P-V: 25,2 cm godišnje), odnosno prosječno sniženje razine podzemne vode na motrenom području od 23,3 cm godišnje.

Primjetno je kako su vrijednosti trenda sniženja razine podzemne vode u vrlo dubokim piezometrima zamjetno više u odnosu na hidropedološke piezometre, što je povezano i s kraćim vremenom motrenja (2015-2021). Dakle, kada se u razmatranje uzme samo posljednjih 7 godina opažanja (2015-2021) na hidropedološkim piezometrima od 4 m dubine dobije se prosječno godišnje sniženje razine podzemne vode na području istraživanja od 22,7 cm, a na vrlo dubokim piezometrima (15 m dubine) 23,3 cm godišnje. Ovo ubrzanje trenda sniženja razine podzemnih voda kako u talnom, a isto tako i u podtalnom dijelu vodonosnika je posebno zabrinjavajuće jer ukazuje na sve brže i jače isušivanje soluma poljoprivrednih tala.



Slika 7. Dinamika srednjih mjesečnih vrijednosti razine podzemne vode u vrlo dubokim piezometrima (15 m dubine) na području istraživanja

Zaključak

Višegodišnja istraživanja s kontinuiranim praćenjem (monitoringom) razine podzemnih voda u pedosfernom (talnom) do 4 m dubine, kao i u podtalnom dijelu do 15 m dubine ukazuju na trend njihovog sniženja (opadanja).

Trend sniženja razine podzemnih voda na istraživanom području Bid-bosutskog polja posebno je prisutan i zabrinjavajući u posljednjem intervalu monitoringa, odnosno u razdoblju od 2015-2021. godine.

Ova negativna pojava povezna je s većim brojem čimbenika od čega prednjače klimatski i hidrološki, a posebice hidrogeološki čimbenici vezani za sve izraženiji drenirajući, a sve slabiji prihranjujući utjecaj glavnog vodotoka područja, rijeke Save.

Rješenje navedene problematike valja tražiti kroz ažurnu primjenu Melioracijskog kanala Bid-bosutskog polja, u vidu oplemenjivanja malih voda i kroz navodnjavanje uzgajanih poljoprivrednih kultura.

Napomena

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Characteristics of groundwater dynamics in the Bid-Bosut field

Abstract

The aim of the paper is to show the dynamics of groundwaters in the Bid-Bosut field, located in Eastern Slavonia. During research period (2003-2021), a mild trend of an increase in annual precipitation by 0.06 mm per year was noted, whereas average monthly air temperatures exhibited a rising trend of 0.094 °C per year. Monitoring data of the groundwater level in the 4 m deep aquifer during the research period (2003-2021) shows an average lowering trend of 8.8 cm per year. Also, since 2015, the groundwater level in the 15 m deep aquifer has been monitored, and the measured values (2015-2021) show an average lowering trend of 23.3 cm per year. For the same seven-year period, the trend of lowering the groundwater level in the 4 m deep aquifer is 22.7 cm per year.

Keywords: groundwater dynamics, level lowering, piezometer, agriculture, trend analysis

Isolation and characterization of a highly active keratinase from *Bacillus mycoides* S3021 with promising potential for waste sheep wool processing

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Abstract

Enormous amounts of organic waste rich in hard-to-degrade structural proteins, especially keratin are generated daily, and effective biotechnological solutions for their degradation is lacking. A new strain S3021 with high keratinolytic activity, identified as *Bacillus mycoides*, was isolated from keratin enriched soil. Biochemical characterization of this strain revealed the presence of a highly active keratinase with a molecular weight of ~35 kDa and a wide pH and temperature range with an optimum at pH 8 and 55 °C. This is the first reported keratinase from *B. mycoides*, and these results suggest isolate S3021 as a promising source of enzymes for processing of waste sheep wool and other keratinous wastes.

Keywords: sheep wool, keratin, *Bacillus mycoides*, keratinase

Introduction

Every year different organic wastes and by-products are produced in enormous quantities. A lot of fibrous proteins such as collagen, elastin and keratin are found in wastes from slaughterhouses, meat and poultry plants (e.g. viscera, skin remains, meat trimmings, bones, blood, bristles, horns, hooves, animal hair, feathers, etc.) or the wool textile industry (poor-quality raw wool, inappropriate for spinning), which must be properly managed (Callegaro et al., 2019;). Keratinolytic microorganisms and their enzymes can play an important role in degradation of keratin-rich wastes from various industries, through environmentally friendly processes. Due to inefficient use of keratin-rich waste and its accumulation, it requires environmentally friendly, economically viable and safe methods for decontamination and disposal (Petek and Marinšek, 2021).

Sheep wool fibres are composed of structural proteins, keratins, which are divided into three groups: alpha-, beta- and gamma-keratins. Alpha-helixes are dominant motifs of alpha- keratins, compactly packed and stabilised by a high degree of inter- and intramolecular disulphide bonds, non-covalent hydrogen bonds, and hydrophobic interactions, with low sulphur content and a molecular weight of 60 to 80 kDa. Beta-keratins contain beta-sheets as dominant motifs, and non-structural gamma-keratins with high sulphur content and a molecular weight of 11 to 28 kDa. Alpha- keratins are found in the cortex of wool-fibre representing 50-60% of wool fibres. Beta-keratins have primarily a protective function and are present in small amounts in the cuticle. Gamma-keratins represent 20-30% of the wool fibre and, due to their high content of cysteine and tyrosine, which act as disulphide cross-linkers, help hold the keratin superstructure together and make it water-insoluble, enzymatically resistant, and mechanically stable. Regardless of its compact structure, keratin-rich waste material does not accumulate in nature, which proves the presence of keratinolytic microorganisms and their enzymes (Daroit and Brandelli, 2014; Callegaro et al., 2019). These enzymes are called keratinases and are predominantly extracellular enzymes. Keratinases are the only group of proteases that can degrade keratin and belong mainly to the serine or metallo-proteases with a wide pH and temperature range (Vidmar and Vodovnik, 2018).

There is a lack of highly active keratinolytic microorganisms capable of breaking down the hard structure of wool keratin. Therefore, the aim of our study was to isolate new highly keratinolytic microbial strains that could be further used in biotechnological approaches for waste sheep wool processing, such as wool composting and biogas

production. In this paper, we present only part of the results of our study, which focused on the highly active isolate S3021 identified as *Bacillus mycoides*.

Material and methods

Isolation and identification of bacteria

Microorganisms were isolated from the sheep wool compost pile and the soil under the pile. Sheep wool and soil were mixed with saline solution and thus prepared samples were analysed for proteolytic activity on skim milk agar (SM) at 30°C, 55°C and room temperature. The proteolytic microorganisms were then inoculated on keratinolytic activity agar plates with wool keratin as the sole source of carbon and nitrogen (KAA-WK) (1.5 g l^{-1} $K_2HPO_4 \times 3H_2O$; 0.025 g l^{-1} $MgSO_4 \times 7H_2O$; 0.025 g l^{-1} $CaCl_2$; 0.015 g l^{-1} $FeSO_4 \times 7H_2O$; 0.005 g l^{-1} $ZnSO_4 \times 7H_2O$; 3 g l^{-1} wool keratin; 20 g l^{-1} agar technical) (Iglesias et al., 2017). The KAA-WK plates were incubated under the same conditions as during isolation. The clearing zones produced by the keratinolytic microorganisms were recorded daily. The isolates with visible clearing zones at three consecutive inoculations on KAA-WK were selected for further studies. DNA was extracted using the DNeasy UltraClean Microbial Kit (Qiagen, Hilden, Germany). The 16S rRNA genes of the bacterial isolates were amplified by PCR using Supercycler SC300T (Kyratec, Queensland, Australia) with primers fD1 (Weisburg et al., 1991) and 1492R (Lane, 1991). PCR products were sequenced with the same primers by Microsynth AG (Balgach, Switzerland). Sequences were compared with the 16S rRNA database of type and reference bacterial strains in the NCBI database. Isolates with higher keratinolytic activity than the positive control (*Streptomyces fradiae*, DSM 40758) were selected for whole genome sequencing (WGS). Whole genome sequencing was performed by Novogene (Beijing, China) using Illumina MiSeq paired-end technology. Final identification was performed by calculating the average nucleotide identity (ANI) (Richter & Rossello-Mora, 2009) for whole-genome comparisons with selected *Bacillus*-type strains using pyani (Pritchard et al., 2016).

Determination of keratinolytic activity

Keratinolytic activity was tested by detecting the increase in soluble proteins using the modified method of Bohacz and Kowalska (2019). Isolates were cultured in keratinolytic activity broth with raw wool as the sole source of carbon and nitrogen (KAB-RW) (KAA without agar; 1.5 g l^{-1} raw wool) for 6 days at 30 °C and 150 rpm. Culture samples were centrifuged (14 000 X g, 5 min) and supernatants were analysed for soluble protein concentration and used as an enzyme source for keratinolytic activity testing. Soluble protein concentration was determined by the measuring absorbance at 280 nm (de Paiva et al., 2019). Absorbance was measured using the Spark 10M microplate reader (Tecan, Austria). Soluble protein concentration was calculated using the bovine serum albumin (BSA) standard curve. To test keratinolytic activity 500 µl of supernatant was mixed with 500 µl of 1% (w/v) wool keratin (TCI, Japan) in 50 mmol l^{-1} TRIS-HCl buffer (pH 8.0). The mixtures were incubated at 55 °C and 50 rpm for 1 hour. The reactions were then terminated by adding 1 ml of 10% (w/v) trichloroacetic acid (TCA), and the mixtures were incubated at 4 °C for 30 min. Controls were treated the same way, except that the TCA was added before enzyme. The supernatants of the centrifuged mixtures (14 000 X g, 3 min) were used to detect the concentration of soluble proteins according to the method of de Paiva (2019). One unit of keratinolytic activity (U/mg) was defined as the amount of enzyme per mg of soluble protein in the culture supernatant that increases the absorbance at 280 nm by 0.01 in one hour under the assay conditions.

Purification and characterization of the enzyme

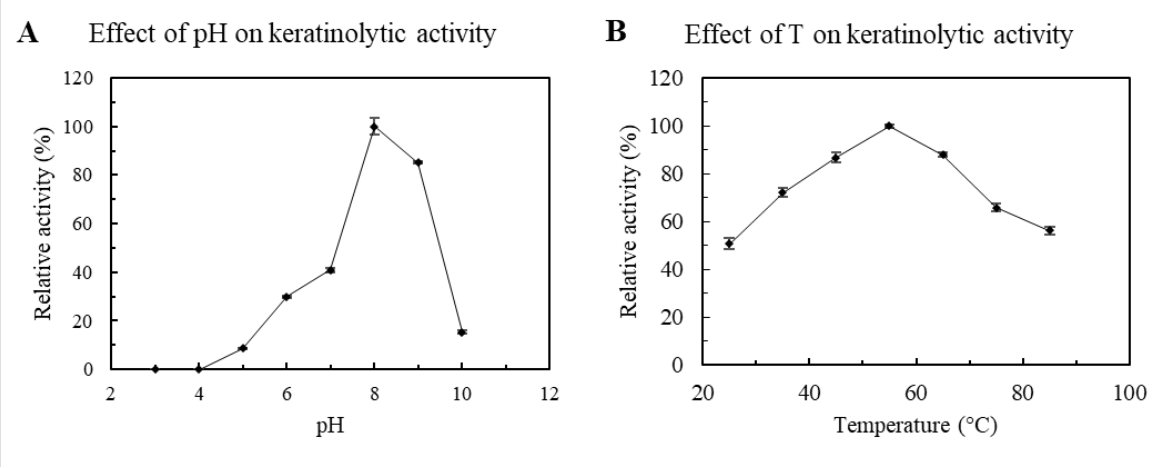
Microbial cultures were centrifuged (10 000 X g, 20 min) and filtered (filter with 0.2 µm pore size). We loaded approximately 100 ml of each supernatant (pH approximately 8.5) onto a 1 ml CIMmultus™ QA-1 anion exchange column (BIA Separations, Slovenia) using the 10 ml mobile phase pump of a Knauer Smartline HPLC system (Knauer, Germany) equipped with a 2550 UV/VIS detector with a deuterium and halogen lamp. The column was equilibrated in 25 mmol l^{-1} TRIS pH 8.5 (mobile phase A1). We collected the flow-through fraction and refrigerated it until further use. After loading, the column was washed with mobile phase A1 and the bound material was eluted with a gradient of 0% - 50% mobile phase B (25 mmol l^{-1} TRIS, 2 mol l^{-1} NaCl, pH 8.5) in 6 minutes followed by a steeper gradient of 50% - 100% mobile phase B in 1 minute. We manually collected the eluted fractions and stored them until further use. We monitored absorbance at 226 nm and 280 nm and maintained the flow rate at 5 ml per minute through the

experiment. We further fractionated the total collected flow-through from the anion exchange chromatography in a second chromatography step using the CIMmultus™ SO3-1 cation exchange column from the same manufacturer. We adjusted the pH of the load to 6.5 with 1 mol l^{-1} HCl and loaded it onto the column, equilibrated in 25 mmol l^{-1} TRIS pH 6.5 (mobile phase A2). We collected the flow-through and then eluted the bound material using mobile phase A2 and the same mobile phase B and gradient elution program as above. All collected fractions were tested for keratinolytic activity using the method of Bohacz and Kowalska (2019). All fractions were separated by SDS-PAGE using a 4% stacking gel and a 12% separating gel according to the method described by Laemmli (Laemmli, 1970). Samples were mixed with loading buffer (0.5 mmol l^{-1} Tris-HCl, pH 6.8; 20 % (v/v) glycerol; 2 % (w/v) SDS; 5 % (v/v) β-mercaptoethanol; 0.006 % (w/v) bromophenol blue) and heated at 100 °C for 5 min before electrophoresis. After electrophoresis, the gel was stained with 0.1 % (w/v) Coomassie Brilliant Blue R-250 in water/methanol/acetic acid 60:30:10 and destained in the same solution without dye.

The temperature and pH optimum of keratinase was determined using the method described above, except that the reactions were incubated at different temperatures from 25–85 °C and 1 % (w/v) wool keratin solutions were prepared with different buffers: pH 3.0 (0.1 M glycine-HCl buffer), pH 4.0 and 5.0 (0.1 M acetate buffer), pH 6.0 and 7.0 (0.1 M phosphate buffer), pH 8.0 and 9.0 (0.1 M Tris-HCl buffer) and pH 10.0 (glycine-NaOH buffer) (Gegeckas et al., 2014; Santan et al., 2013). The effect of different inhibitors (EDTA, β-mercaptoethanol), detergents (Tween 20, Triton X-100, SDS), organic solvents (DMSO), oxidizing agents (H2O2, NaClO), monovalent (K^+) and divalent metal ions (Mg^{2+}, Zn^{2+}, Cu^{2+}, Mn^{2+}) on keratinolytic activity was investigated by pre-incubating the culture supernatant with these chemicals at 55 °C for 30 min. After incubation, 500 µl of 1% (w/v) wool keratin (TCI, Japan) in 50 mmol l^{-1} TRIS-HCl buffer (pH 8.0) was added to the reactions and keratinolytic activity was detected by the method described above. The keratinolytic activity detected without additions was considered to be 100 % (Gegeckas et al., 2014; Fang et al., 2013; Santan et al., 2013)

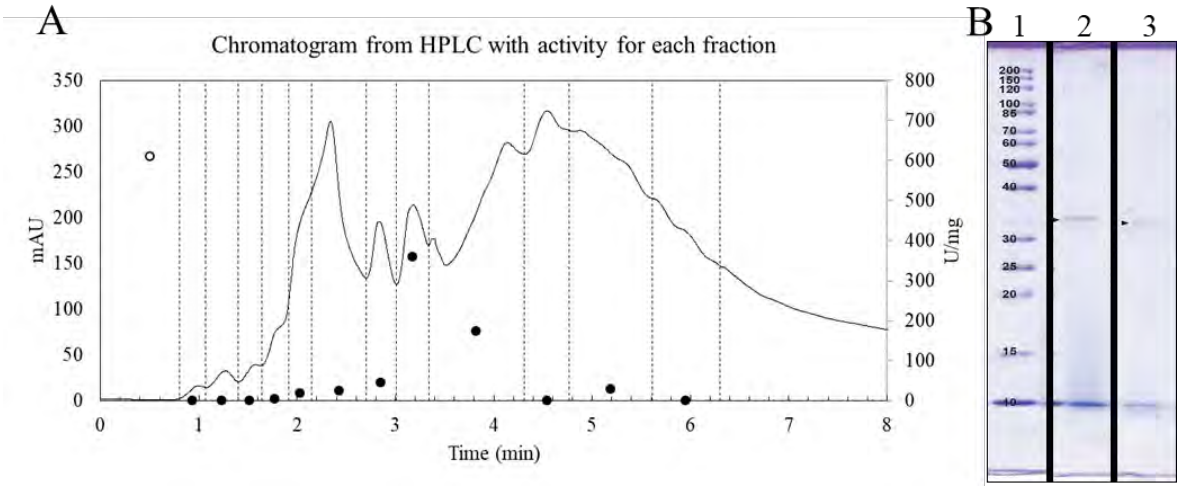
Results and discussion

Isolate S3021 was one of 116 keratinolytic bacterial strains isolated from waste sheep wool compost pile and the soil under the pile, that could grow in a basal salt medium with wool keratin as the sole source of carbon and nitrogen, indicating that sheep wool and wool keratin enriched soil could be a reservoir of keratinolytic microorganisms. The isolation of various bacterial (Queiroga et al., 2007; Infante et al., 2010; Iglesias et al., 2017) and fungal isolates (Abdel-Gawad, 1997) from sheep wool or soil enriched with wool keratin have already been reported before. Of our 116 isolates, 60 isolates were isolated at 30 °C, 12 isolates at 55 °C, and 44 isolates at room temperature. Thirty eight isolates were selected for 16S rRNA sequencing, which revealed that 33 isolates belonged to the genus *Bacillus*, three isolates belonged to the genus *Stenotrophomonas* and one isolate to the genus *Pseudomonas* and *Streptomyces*. 18 isolates that showed better keratinolytic activity than positive control (*Streptomyces fradiae*) were selected for whole-genome sequencing. According to the WGS results our isolates are divided into 5 bacterial species (*Bacillus subtilis, B. altitudinis, B. mycoides, B. wiedmannii* and *Streptomyces coelicoflavus*). In this paper we are focused on the isolate S3021, which was identified as *B. mycoides* and belongs to the larger *Bacillus cereus* group. The keratinolytic activity of *B. mycoides* has not been reported yet by now. The pH value showed a large effect on the keratinolytic activity of *B. mycoides* S3021, which was detected between pH 5.0 and 10.0 with an optimum at 8.0 although high activity (above 85%) was also detected at pH 9.0 (Graph 1.A), whereas activity below pH 7.0 and above pH 9.0 was significantly lower (below 40%). Temperature did not have such a large effect on activity. It was detected in a broad range between 25 °C and 85 °C (above 50% relative activity at all tested temperatures) with an optimum at 55 °C and almost 90% relative activity at temperatures 35 °C and 65 °C (Graph 1.B).



Graph 1. **A** *Effect of pH on keratinase activity of B. mycoides S3021. The activity assay was performed at 55 °C in buffers with pH ranging from 3.0 to 10.0;* **B** *Effect of temperature on keratinase activity. The activity assay was performed in Tris-HCl buffer with pH 8.0 at temperatures ranging from 25 to 85 °C. The specific activity of 529.3 U mg^{-1} was recorded as 100%. Each value represents the mean ± SD of three replicates.*

The first step of purification with an anion exchange column did not yield keratinolytic fractions, but the concentration of soluble proteins in the flow-through was significantly reduced (probably due to the removal of impurities, oligopeptides and keratin degradation products present in the supernatant of the microbial culture) so we chose second step of purification of total flow-through on a cation exchange column. Keratinolytic activity was detected in fractions 8 and 9 with specific activities of 359.9 U mg^{-1} and 173.3 U mg^{-1}, respectively, while the specific activity of the load on the column was 609.8 U mg^{-1} (Graph 2.A). The load and all fractions from cation exchange column were analysed by SDS-PAGE. The molecular weight of the single monomeric protein in fraction 8 and in the load on the column was determined to be ~35 kDa (Graph 2.B). The molecular weights of known keratinases range from18 kDa to 240 kDa (Brandelli et al., 2010). Among *Bacillus* species different sizes have been reported, 26 kDa in *B. licheniformis* (Yoshioka et al., 2006), 30 kDa in *B. megaterium* (Radha and Gunasekaran, 2007), 65 kDa in *B. pumilus* (Kumar et al., 2008), and 69 kDa in *B. subtilis* (Balaji et al., 2008). Gegeckas et al. (2014) reported the isolation and characterization of the 39 kDa keratinolytic proteinase BtKER from *Bacillus thuringiensis* AD-12, which also belongs to the *Bacillus cereus* group. Biochemical activity assays of BtKER revealed optimal activity at pH 7 and activity of more than 80% at pH 8. However, a larger difference appeared at the temperature optimum, where BtKER has an optimum at 30 °C, which is much lower than that of our isolate, and almost no activity at temperatures above 50 °C.



Graph 2. **A** *Chromatogram of B. mycoides S3021 from cation exchange chromatography with marked fractions (separated by vertical lines), and corresponding keratinolytic activities (closed circles), the activity of the load*

on the column is also shown (open circle); **B** *SDS-PAGE showing keratinase from B. mycoides S3021 purified by anion and cation exchange chromatography.* **Lane 1** *Prestained protein ladder with labelled molecular weights in kDa;* **lane 2** *10x concentrated flow-through from the anion exchange column (load on the cation exchange column);* **lane 3** *Fraction 8 from cation exchange chromatography (~35 kDa)*

Table 1. Effect of inhibitors, detergents, organic solvents and metal ions on the keratinolytic activity of *Bacillus mycoides* S3021 expressed as a percentage of the activity of the untreated control. The specific activity of 529.3 U mg^{-1} was recorded as 100%. Values represent the mean ± SD of three replicates.

	Concentration	Residual activity (%)
Control	-	100,00
EDTA	5 mmol l^{-1}	-6,57 ± 0,13
SDS	1% (w/v)	8,63 ± 3,64
β-mercaptoethanol	5 mmol l^{-1}	20,43 ± 1,46
Triton X-100	1% (v/v)	21,09 ± 0,48
Tween 20	1% (v/v)	**106,69** ± 1,81
H_2O_2	1% (v/v)	95,54 ± 1,28
NaClO	1% (v/v)	-11,75 ± 0,95
DMSO	1% (v/v)	**112,01** ± 0,14
K^+	5 mmol l^{-1}	73,31 ± 0,22
Mg^{2+}	5 mmol l^{-1}	41,80 ± 0,39
Zn^{2+}	5 mmol l^{-1}	-8,47 ± 0,62
Cu^{2+}	5 mmol l^{-1}	4,61 ± 0,67
Mn^{2+}	5 mmol l^{-1}	4,48 ± 0,89

The addition of various inhibitors, detergents and oxidizing agents decreased the keratinolytic activity in most cases, with the exception of H_2O_2, which had no effect, while Tween 20 and DMSO slightly increased the keratinolytic activity up to 7% and 12%, respectively (Table 1). All of tested metal ions decreased the enzyme activity. The study showed that the activity was strongly inhibited by Zn^{2+}, Cu^{2+} and Mn^{2+}, while the addition of K^+ and Mg^{2+} decreased the activity for up to 30% and up to 60%, respectively. Bradelli et al. (2010) reported that the presence of divalent metal ions often stimulates the keratinases, which did not happen in our case. Gegeckas et al. (2014) also reported decreased keratinolytic activity after addition of most metal ions, but these effects were smaller in their case than in ours, and the addition of Mn^{+2} even increased the enzyme activity for up to 12%.

Conclusions

The new highly keratinolytic bacterial strain S3021 was isolated at 30 °C from the soil under the sheep wool compost pile. After whole genome sequencing, it was identified as *Bacillus mycoides*. Biochemical characterization of its enzymes showed a wide pH and temperature range for keratinolytic activity, with an optimum at pH 8.0 and 55 °C. Two-step purification of the proteins in the culture supernatant by anion exchange chromatography followed by cation exchange chromatography yielded two fractions with keratinolytic activity. SDS-PAGE analysis showed the presence of ~35 kDa keratinase in active fractions. Addition of various inhibitors, detergents, organic solvents and metal ions mostly inhibited keratinolytic activity, with the exception of H_2O_2, which showed no effect, and DMSO and Tween 20, which slightly increased activity compared with the control.

Further work will be focused on the use of isolate S3021 alone or in mixed cultures with other keratinolytic isolates from our extended study for processing the waste keratinous material, particularly waste sheep wool. Isolate S3021, with its wide pH and temperature range, offers promising prospects for use as a starter culture for waste sheep wool composting (bioaugmentation) and microbial or enzymatic pretreatment of waste sheep wool for biogas production. The wide temperature range is particularly advantageous for composting, as the keratinolytic enzymes can be active at all stages of composting from mesophilic to thermophilic (up to 70 °C), which is very important for the hygienization of the substrate.

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Usporedba audiovizualne procjene i analize karbonatnosti tla temeljem reakcije na klorovodičnu kiselinu

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Sažetak

Karbonati su, većinom kao kalcit ($CaCO_3$), prisutni u mnogim tlima, gdje utječu na razne procese i značajke: pH, pristupačnost hranjiva, strukturu itd. Njihov sadržaj se često ispituje Scheiblerovim kalcimetrom, koji mjeri volumen ugljikovog dioksida oslobođenog u reakciji tla s 10%-tnom klorovodičnom kiselinom. Međutim, već i samim opažanjem intenziteta te reakcije (šum i pjena), može se pokušati procijeniti stupanj karbonatnosti tla. U ovom radu, procijenjena je karbonatnost 22 uzorka tla s udjelima karbonata od 3,5% do 57,1%. U 90,1% slučajeva (20 uzoraka), procjena je odgovarala interpretaciji rezultata dobivenih kalcimetrom. U jednom uzorku je došlo do podcjenjivanja, a u drugom do precjenjivanja karbonatnosti tla. Prema tome, audiovizualna procjena je načelno bila pouzdan i brz indikator okvirnog sadržaja karbonata u tlu.

Ključne riječi: karbonatna tla, kalcijev karbonat, kalcit, $CaCO_3$, Scheiblerov kalcimetar

Uvod

Karbonati, kao spojevi koji sadrže karbonatni anion, su prisutni u brojnim tlima. Pritom je najčešći kalcijev karbonat ($CaCO_3$), i to uglavnom kao kalcit (znatno rjeđe aragonit). Može se, međutim, pojaviti i kalcijev-magnezijev karbonat (dolomit, $CaMg(CO_3)_2$). Ponekad se u tlu nalaze i natrijev karbonat (Na_2CO_3) ili, u anaerobnim uvjetima, siderit ($FeCO_3$) (Pernar, 2017.).

Karbonati tla se najčešće dijele na primarne i sekundarne (FAO, 2006., Doner i Lynn, 1989.). Primarnima smatramo sve one koji su formirani geogenezom, odnosno koji potječu iz matičnog supstrata tla. U sekundarne ubrajamo karbonate koji se formiraju pedogenezom (u obliku bijelih nakupina, prevlaka, (rizo)konkrecija i sl.), i to otapanjem, premještanjem i naknadnom reprecipitacijom primarnih karbonata, ranije formiranih sekundarnih karbonata, ali i biogenetskih karbonata (Zamanian i sur., 2016.). Biogenetski karbonati su oni koji potječu od kostura i ljuštura različitih organizama.

Karbonati imaju važnu ulogu u pedogenetskim, ali i brojnim drugim kemijskim procesima u rizosferi tla (Loeppert i Suarez, 1996.). Oni utječu na fizikalne, kemijske i biološke značajke tla, a time i na njegovu sveukupnu plodnost. Kao prvo, karbonati u otopini tla djeluju kao pufer, odnosno ublažavaju promjene pH vrijednosti tla (Salomons i Mook, 1986.). Karbonatna tla uvijek imaju bazičan pH i adsorpcijski kompleks na kojem dominira ion Ca^{2+} (zastupljenost i do 80%), kao jedan od elemenata ključnih za ishranu bilja (Pernar, 2017.). U skladu s navedenim je i zasićenost adsorpcijskog kompleksa karbonatnog tla bazičnim kationima uvijek jednaka 100% (Škorić, 1991.). Dok je prosječni udio kalcija u tlu 2-20 g/kg, on u karbonatnom tlu raste iznad 100 g/kg (Pernar, 2017.). Posljedično se u karbonatnim tlima nerijetko javljaju problemi s dostupnošću nekih drugih makrohranjiva, poput fosfora, ali i raznih mikrohranjiva, poput cinka, željeza i bakra (Virto i sur., 2011., Zamanian i sur. 2016.).

Obzirom da se Ca^{2+} ioni čvrsto vežu na adsorpcijski kompleks tla, oni pospješuju flokulaciju (koagulaciju) tla, zbog čega imaju izrazito povoljnu ulogu u povezivanju primarnih čestica tla u mikroagregate i stabiliziranju strukture tla, a time i u poboljšanju vodozračnog režima te smanjenju erodibilnosti tla (Doner i Lynn, 1989., Virto i sur., 2011.). Kalcijevi karbonati, dakle, imaju izraženo povoljan učinak na ukupna fizikalna svojstva tla. S druge strane, tla bogata natrijevim karbonatom u pravilu imaju nepovoljna fizikalna, ali i kemijska, svojstva. Razlog tome je u činjenici

da ioni natrija, koji se znatno slabije vežu na adsorpcijski kompleks tla, umjesto flokulacije uzrokuju disperziju (dezintegraciju) agregata tla, čime se narušava struktura tla (Husnjak, 2014.). Nadalje, u natrijem bogatim tlima, reakcija tla nije samo slabo alkalna, kao što je slučaj u tlima bogatim kalcijem, već raste iznad pH vrijednosti 8 (Pernar, 2017.). Posljedično se, u sklopu agromelioracija kiselih tala, u pravilu koriste karbonatni materijali koji sadrže kalcij (zbog čega se i sam postupak naziva kalcizacija ili kalcifikacija tla) – npr. vapno (CaO) ili mljeveni vapnenac ($CaCO_3$).

Vapnenci, koji (uz sporadične dolomite) prekrivaju oko 40% površine Hrvatske i predstavljaju daleko najzastupljenije stijene u zemlji, građeni su gotovo isključivo od kalcita (Husnjak, 2014.). Međutim, obzirom da se tijekom dugotrajne pedogeneze kalcit iz ovih stijena otopio, tla razvijena na njima su u pravilu nekarbonatna. Iza vapnenaca, u slijedu zastupljenosti matičnih supstrata Hrvatske, slijede les (19%) i fluvijalni sedimenti (17%), na kojima se nalaze uglavnom karbonatna tla (Husnjak, 2014.). Osim navedenih, značajan karbonatni matični supstrat predstavlja i lapor. Unatoč dominaciji karbonatnih susptrata, u Hrvatskoj su tla (barem u njihovom površinskom sloju) dominantno kisela, zatim neutralna, a tek na kraju alkalna (Husnjak, 2022.). Naime, karbonati se, u uvjetima vlažne klime, otapaju i postupno ispiru iz tla oborinama, zbog čega onda dolazi i do acidifikacije tla (Pernar, 2017.). Stoga su u Hrvatskoj tla, koja su još uvijek karbonatna, genetski mlada, vlažena visokom podzemnom vodom i/ili razvijena u semihumidnom području istočne kontinentalne Hrvatske. Svejedno, velik broj tipova tala (ili njihovih podjedinica) su karbonatni: rendzina, černozem, regosol na lesu ili laporu, karbonatni koluviji, karbonatni fluvisoli i humofluvisoli, te karbonatne ritske crnice i eugleji (Škorić i sur., 1985.).

Sadržaj karbonata u tlu može se precizno odrediti različitim laboratorijskim metodama, od kojih je jedna od često korištenih ona volumetrijska, koja mjeri volumen ugljikovog dioksida (CO_2) oslobađenog prilikom reakcije karbonata i 10% otopine klorovodične kiseline (HCl). Za jednostavniji i brži uvid u okvirni sadržaj karbonata u tlu, odnosno za testiranje da li je tlo uopće karbonatno ili nije, moguće je procijeniti karbonatnost temeljem bilježenja samog intenziteta reakcije tla (šum i/ili pjena) na dodavanje 10% HCl. Ovo je postupak koji se obično obavlja prilikom zaprimanja uzorka tla u laboratoriju, ako ne već i prilikom standardnog opisa profila tla na terenu (FAO, 2006.). Međutim, audiovizualna reakcija nije uvijek i u potpunosti precizan indikator količine ukupnih karbonata u tlu (Soil Survey Division Staff, 1993., FAO, 2006., Zamanian i sur., 2016.).

Cilj ovog rada je usporediti interpretacije rezultata analize karbonatnosti 22 uzorka tla, dobivene putem dviju gore navedenih metoda, kako bi se utvrdilo u kojoj mjeri audiovizualna procjena sadržaja karbonata u tlu odgovara interpretaciji temeljenoj na laboratorijskom mjerenju, odnosno stvarnom sadržaju ukupnih karbonata u tlu. U slučaju dokazano dobre podudarnosti, brza procjena karbonatnosti može, za određene potrebe i u određenim uvjetima, zamijeniti laboratorijsku analizu sadržaja karbonata u tlu.

Metode i materijali

U laboratoriju je analizirano 22 uzorka karbonatnih tala različitih tekstura, uzetih iz arhive Zavoda za pedologiju Agronomskog fakulteta u Zagrebu. Uzorci su bili u obliku zrakosuhe sitnice, prethodno pripremljeni u skladu s HRN ISO 11464:2009. Svaki uzorak je analiziran na sadržaj ukupnih karbonata, i to najprije kvalitativno (prelijevanjem uzorka s 10%-tnom otopinom HCl), a zatim i kvantitativno (prelijevanjem uzorka s 10% HCl i mjerenjem oslobođenog CO_2 pomoću kalcimetra). Analiza svakog uzorka je provedena u dva ponavljanja (u radu su prikazane prosječne vrijednosti).

Kvalitativna analiza temelji se na audiovizualnom opažanju, odnosno na bilježenju intenziteta reakcije (šum, pjena) do koje dolazi nakon kapanja 10% HCl na uzorak tla. Pritom tu reakciju uzrokuje CO_2, koji u obliku plina, kao nusprodukt reakcije, izlazi iz uzorka u atmosferu, u skladu s donjom formulom:

$$CaCO_3 + 2HCl \leftrightarrow H_2O + CO_2(g) + Ca^{2+} + 2Cl^-$$

Interpretacija karbonatnosti uzorka, temeljem intenziteta gore opisane reakcije, rađena je prema kriterijima prikazanim u Tablici 1. Kako bi se mogla provesti usporedba dviju metoda, rezultati kvantitativne analize su interpretirani prema istoj tablici, odnosno u skladu s FAO (2006).

Tablica 1. Procjena karbonatnosti temeljem reakcije tla na 10%-tnu otopinu HCl (modificirano prema FAO, 2006).

$CaCO_3$ (%)	Interpretacija	Opis reakcije
0	Nekarbonatno	Nema vidljive niti čujne reakcije
<2	Blago karbonatno	Šum bez vidljive reakcije
2-10	Srednje karbonatno	Šum i pjena
10-25	Jako karbonatno	Jaka reakcija, mjehurići stvaraju tanku pjenu
>25	Vrlo jako karbonatno	Vrlo jaka reakcija, mjehurići stvaraju debelu pjenu

Volumetrijska metoda, kojom je provedena kvantitativna analiza, temeljena je na modificiranoj normi HRN ISO 10693:2014. U sklopu te analize se, pomoću Scheiblerovog kalcimetra, mjeri volumen CO_2 oslobođenog u reakciji tla i 10% HCl. Na taj način se neizravno (računski) određuje sadržaj ukupnih karbonata u tlu temeljem volumena plina koji se u reakciji oslobodio, kako slijedi:

$$w(CaCO_3) = (V(CO_2) \cdot f) / m$$

gdje je:

$w(CaCO_3)$ = udio karbonata (u pravilu kalcijevih) u tlu (%)

$V(CO_2)$ = na kalcimetru očitan volumen CO_2 (cm^3)

m = masa uzorka tla (0.50-5.00 g)

f = tablični faktor za preračunavanje mase oslobođenog CO_2 u postotni udio ukupnih karbonata u tlu, i to pri određenom tlaku (mmHg) i temperaturi zraka (°C)

Rezultati i rasprava

Procjena sadržaja karbonata na temelju intenziteta reakcije uzoraka tla s 10% HCl, kao i prosječni sadržaji karbonata određeni kalcimetrom, prikazani su u Tablici 2. Primjeri različitih intenziteta reakcije uzoraka na dodavanje kiseline prikazani su na Slici 1.

Tablica 2. Usporedba interpretacije sadržaja ukupnih karbonata procijenjenog audiovizualno temeljem intenziteta reakcije tla s 10% HCl i određenog laboratorijskom analizom pomoću Scheiblerovog kalcimetra.

Oznaka uzorka	%	Interpretacija sadržaja karbonata	
		Audiovizualna procjena	Laboratorijska analiza
1	53,8	vrlo jako karbonatno	vrlo jako karbonatno
2	57,1	vrlo jako karbonatno	vrlo jako karbonatno
3	49,6	vrlo jako karbonatno	vrlo jako karbonatno
4	7,0	srednje karbonatno	srednje karbonatno
5	14,5	jako karbonatno	jako karbonatno
6 *	3,5	jako karbonatno	srednje karbonatno
7	27,9	vrlo jako karbonatno	vrlo jako karbonatno
8	22,0	jako karbonatno	jako karbonatno
9	16,7	jako karbonatno	jako karbonatno
10	53,2	vrlo jako karbonatno	vrlo jako karbonatno
11	15,8	jako karbonatno	jako karbonatno
12	22,1	jako karbonatno	jako karbonatno
13	21,1	jako karbonatno	jako karbonatno

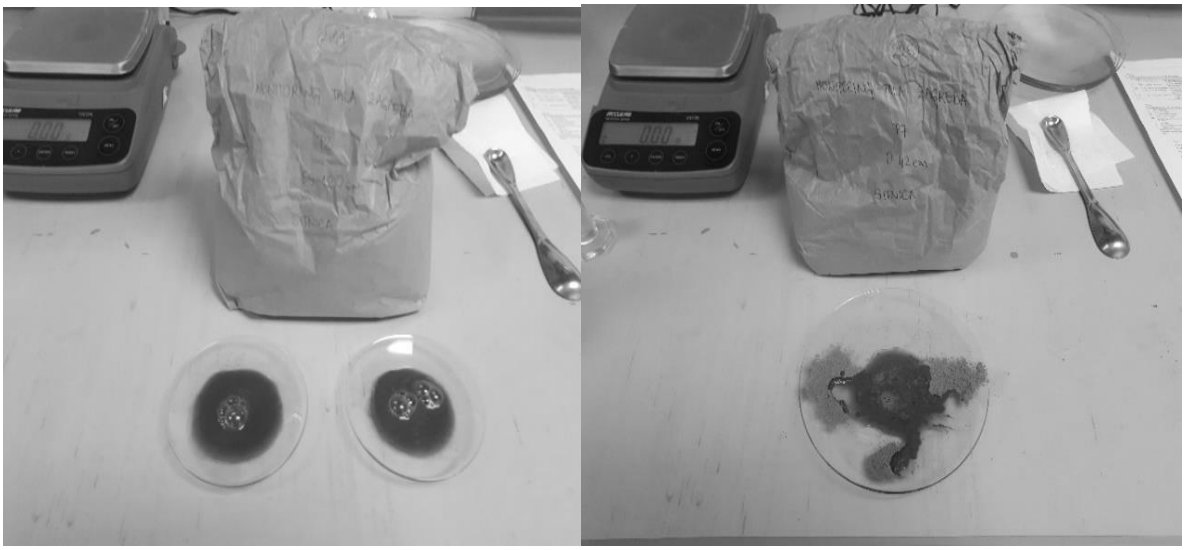
14	7,5	srednje karbonatno	srednje karbonatno
15	5,5	srednje karbonatno	srednje karbonatno
16	9,9	srednje karbonatno	srednje karbonatno
17	14,1	jako karbonatno	jako karbonatno
18	14,0	jako karbonatno	jako karbonatno
19	19,5	jako karbonatno	jako karbonatno
20 *	8,4	blago karbonatno	srednje karbonatno
21	20,0	srednje karbonatno	jako karbonatno
22	10,6	jako karbonatno	jako karbonatno

** Interpretacije sadržaja temeljene na procjeni i analizi se ne podudaraju*

Kao što se može uočiti u Tablici 2, kalcimetrom izmjereni udio karbonata kretao se od 3,5% do 57,1%, pri čemu je pet uzoraka interpretirano kao vrlo jako karbonatno, 11 kao jako karbonatno, a 6 kao srednje karbonatno. Vidljivo je da je, u gotovo svim uzorcima, procjena karbonatnosti odgovarala interpretaciji temeljenoj na analitičkim rezultatima (Tablica 2). Manja odstupanja zabilježena su samo za 2 uzorka, pri čemu je u jednom (Uzorak 20) došlo do podcjenjivanja, a u drugom (Uzorak 6) do precjenjivanja stvarne karbonatnosti tla (Tablica 2).

Neki istraživači (npr. Rowell, 1994.) navode da bi se svi uzorci s intenzivnom reakcijom na 10% HCl trebali smatrati vrlo jako karbonatnima (odnosno da nebi trebalo pokušavati razlikovati „jako karbonatne" od „vrlo jako karbonatnih" uzoraka) jer se fine razlike u reakciji takvih tala na kiselinu teško zamjećuju golim okom. U našem slučaju, takvi problemi ipak nisu zabilježeni, obzirom da su nepodudaranja između procjene i analize karbonatnosti zabilježena kod uzoraka sa sadržajem karbonata od 3,5% i 8,4% (dakle isključivo u srednje karbonatnim uzorcima). Štoviše, svi vrlo jako karbonatni uzorci kvalitativnom analizom su ispravno procijenjeni kao takvi (Tablica 2).

a) Uzorak 2 (vrlo jako karbonatno tlo) *b) Uzorak 5 (jako karbonatno tlo)*



c) Uzorak 15 (srednje karbonatno tlo) *d) Uzorak 20 (blago karbonatno tlo)*

Slika 1. Fotoaparatom zabilježena reakcija pojedinih uzoraka na 10% otopinu HCl.

Razlozi zbog kojih audiovizualna reakcija ne mora uvijek biti precizan indikator količine ukupnih karbonata u tlu mogu biti različiti. Tako dolomit na kiselinu reagira znatno slabije nego kalcit, karbonatna reakcija je u pravilu intenzivnija u teksturno lakšim tlima, a sekundarni karbonati reagiraju znatno burnije od primarnih (Soil Survey Division Staff, 1993., FAO, 2006.). Ovdje u primarne karbonate, osim onih geogenetskih, treba ubrojiti i biogenetske, koji nemaju tako velik utjecaj na otopinu tla, kao što ga imaju pedogenetski karbonati (Zamanian i sur., 2016.).

Treba imati na umu i da na burnost reakcije mogu utjecati i vlažnost tla te temperatura (Soil Survey Division Staff, 1993.), što može biti posebno izraženo kada se test provodi u terenskim uvjetima, odnosno na tlu u prirodnom stanju. Kao što je ranije istaknuto, u ovom istraživanju, uzorci su testirani u laboratoriju, i to u obliku zrakosuhe sitnice.

Obzirom na sve gore navedeno, jasno je da bi laboratorijsku analizu sadržaja karbonata u tlu bilo uputno provoditi paralelno s određivanjem mehaničkog (po mogućnosti i mineralnog) sastava tla, i to nakon morfološkog opisa tla, koji uključuje i opažanja vezana za sadržaj i svojstva sekundarnih karbonata u tlu.

Zaključak

Usporedbom audiovizualne procjene sadržaja ukupnih karbonata, temeljene na reakciji tla na 10% HCl, s interpretacijom rezultata laboratorijske analize Scheiblerovim kalcimetrom, utvrđeno je da je procjena bila dobar indikator okvirnog sadržaja karbonata u tlu. Od ispitanih 22 uzorka, za njih 20 je procjena odgovarala interpretaciji laboratorijskih rezultata. Stoga se može zaključiti da audiovizualno opažanje intenziteta reakcije tla na 10% HCl može osigurati brzu i jednostavnu ocjenu karbonatnosti tla, odnosno uštedu vremena i resursa.

Manja odstupanja u audiovizualnoj procjeni karbonatnosti zabilježena su za 2 uzorka, pri čemu je u jednom došlo do podcjenjivanja, a u drugom do precjenjivanja stvarne (kalcimetrom analizirane) karbonatnosti tla. Pritom su nepodudaranja između procjene i analize zabilježena u srednje karbonatnim uzorcima, dok su jako i vrlo jako karbonatni uzorci kvalitativnom analizom ispravno procijenjeni kao takvi.

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Comparison of audiovisual assessment and analysis of soil carbonate content based on reaction to hydrochloric acid

Abstract

Carbonates, mostly calcite ($CaCO_3$), are present in many soils, where they influence various processes and features: pH, nutrient availability, structure, etc. Their content is often analyzed with the Scheibler calcimeter, which measures the volume of CO_2 released in the reaction of soil and 10% HCl. However, just by observing the intensity of this reaction (effervescence, foam), one can try to estimate the approximate amount of $CaCO_3$. Herein, $CaCO_3$ content in 22 soil samples with 3,5% to 57,1% carbonates was assessed. In 90,1% of cases (20 samples), the assessment corresponded to the interpretation of the results obtained with the calcimeter. In one sample the carbonate content was underestimated and in the other it was overestimated. Hence, the audiovisual assessment generally proved as a reliable and quick indicator of the $CaCO_3$ content.

Keywords: calcareous soils, calcium carbonate, calcite, $CaCO_3$, Scheibler calcimeter

Agri-environmental Policies in the Western Balkans: soil conservation

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Abstract

Although all of the Western Balkan countries are at different stages of development, they face similar challenges in policy reform and harmonisation, information systems and economic development, coupled with general underdevelopment and limited institutional capacity, which continue to obstruct the reform processes of the Western Balkans. The Action Plan for the Implementation of the Sofia Declaration on the Green Agenda for the Western Balkans 2021-2030 has put forward several objectives that are in line with EU policies for soil conservation. To this end, the establishment of the Soil Partnership for Western Balkans as an open forum for gathering of all stakeholders in the region would be the perfect entity to discuss and exchange knowledge, data, best practices and experiences about sustainable soil management. This process started in 2021 under the leadership of the Regional Rural Development Standing Working Group in South Eastern Europe (SWG).

Keywords: soil conservation, partnership, Western Balkans

Introduction

The Western Balkans (WB) shows a great variety of climate, soil, and geomorphological characteristics. Although the region was blessed by some of the most fertile soils in Europe, soil is a limited natural resource in the Western Balkans and its availability for biomass production and ecosystem functions and services is decreasing (Zdruli et al., 2022). The region is characterized also by several natural constraints that include salinity, sodicity, poor drainage and texture conditions, shallowness and stoniness and other nature based and human induced limitations.

In addition to the policy requirement for integrating the WB countries into the EU, extended regional cooperation is most important for future relations with the EU. The process of establishment of the Soil Partnership for Western Balkans as an open forum for gathering of all stakeholders in the region started in 2021 under the leadership of the Regional Rural Development Standing Working Group in South Eastern Europe (SWG). The first goal was to make a first integrated assessment of soil degradation process in the Western Balkans and develop policy recommendations for a wider implementation of sustainable soil management practices. Other important objectives include the strengthening of institutional and technical/professional capacities of the region to assist the implementation of the new Soil Strategy for 2030 as well as work towards harmonization of the guidelines, methods, measurements, and introduction of soil protection indicators to enhance implementation of sustainable soil management practices. Furthermore, the enrichment and availability of soil data and information, along with awareness raising in the area of soil protection and land use are crucial components.

Material and methods

This paper presents an updated analysis of state of the art of soil management and conservation policies in the Western Balkans region. The paper was prepared using data and information from nominated soil experts from six countries in the region (Albania, Bosnia and Herzegovina, Kosovo*[1], North Macedonia, Montenegro and Serbia) working together in the implementation of the Action Plan for the Implementation of the Sofia Declaration on the Green Agenda for the Western Balkans 2021-2030. This process started in 2021 under the leadership of the Regional Rural Development Standing Working Group in South Eastern Europe (SWG).

Another important relevant source of information derived within the frame of the project titled "Rural development through integrated forest and water resource management in Southeast-Europe: Rural perspectives: qualification, reintegration and (self) employment", jointly implemented by the Regional Rural Development Standing Working Group (SWG) in South-Eastern Europe and GIZ and supported by the German Federal Ministry for Economic Cooperation and Development (Cukaliev et al., edts, 2018).

Results and discussion

An analysis of Corine Land Cover 2018 data shows that Western Balkans is a very rich and diverse region. Some conclusions can be drawn:

- Forest and semi-natural areas have the largest share of the land in the region (55.5%), followed by Agricultural areas with 40.04%.
- Montenegro has the largest share of Forest and semi natural areas in the region (79.17%), and Serbia has the largest portion of Agricultural areas (55.13%).
- More than 2.8 % of WB land is used as artificial areas, which includes built-up areas and unbuilt surfaced areas such as transport networks and associated areas.

Soil threats in the Western Balkans are complex, and although they are unevenly spread, their dimension is regional, and they are frequently inter-linked. When many threats occur simultaneously, the combined effect tends to aggravate soil degradation (Jones *et al.*, 2005). Based on a first assessment of the status and trend of soil degradation in the region the main degradation processes include land take, soil sealing, contamination, loss of organic carbon and erosion. These pressures will only increase in the future and climate change will aggravate the situation as the region could become hotter and drier (Table 1).

Table 1. Summary of soil threats status in WB

Soil treats		ALB	BIH FBIH	BIH RS	KOS*	MNE	MKD	SRB
Land take and soil sealing	In densely populated parts of WB countries soil sealing is one of the most threatening phenomena.	↑↓	↑↓	↘	↘	↘	↘	↘
Contamination	Soil contamination is a problem in some part of WB countries. The most frequent contaminants are heavy metals and mineral oil.	↑↓	↑↓	↘	↘	=	↘	=
Organic carbon change	The loss of organic carbon is evident in most agricultural soils.	=	=	↘	↘	↘	↘	↘

1 This designation is without prejudice to positions on status and is in line with UNSCR 1244/1999 and the ICJ Opinion on the Kosovo declaration of independence.

Soil erosion	Water erosion is active in all the cultivated mountainous areas and wind erosion in lowland areas.	↘	↘	↘	↘	↘	↘	↘

Legend: ALB-Albania, BIH FBIH-Bosnia and Herzegovina, Federation of Bosnia and Herzegovina, BIH RS-Bosnia and Herzegovina, Republic of Srpska, KOS-Kosovo, MNE- Montenegro, MKD- North Macedonia, SRB-Republic of Serbia*
Stable =, Variable ↑↓, Improving ↗, Deteriorating ↘

The magnitude of these pressures is largely conditioned by the lack of awareness and knowledge about sustainable soil management, the lack of a legislative framework (EU and national), division of responsibilities and lack of cooperation among the institutions. In order to tackle these challenges, well equipped and trained administrative steering is needed (institution building), while it is equally important to increase institutional cooperation, information development and data exchange.

Legal framework and Strategic documents for soil management in Western Balkans

The EU's soil policy aims to improve soils by taking measures and actions to protect and restore soils, and ensure that they are used sustainably, putting forward a vision to achieve healthy soils by 2050, improving the framework for soil monitoring in Europe, developing the knowledge base and supporting soil research and raising awareness about the vital importance of soils. In November 2021, European Commission adopted the new EU Soil Strategy for 2030 - Reaping the benefits of healthy soils for people, food, nature and climate (COM (2021) 699 final). This Soil Strategy therefore builds on and will significantly contribute to several of the objectives of the Green Deal. Strategy is complementary to other European initiatives such as the next Zero Pollution Action Plan, the EU's biodiversity strategy for 2030 and the "Farm to Fork" Strategy, as well as with the sustainable development goals (SDGs). This Strategy sets ambitious and necessary goals, which must be met urgently. The new EU Soil Strategy derives a long-term vision for soil from the ambition of the European Green Deal and lists the key existing objectives and targets for the medium and long term where soil policy is called to contribute. The new EU Soil Observatory will collect policy-relevant data and develop indicators for the regular assessment and progress towards the ambitious targets of the Green Deal (Panagos and Montanarella, 2021).

Legal framework for soil conservation in WB

The WB countries have incorporated provisions from the EU legal framework in their legislation, such as the Water Framework Directive, the Nitrate Directive, Biodiversity Protection, Agrobiodiversity, the Habitats and Bird Directive etc. Countries address the environmental requirements with the cross-compliance that is set as a Code of Good Agricultural Practices (CGAP). Most of the countries foresee cross-compliance in their legal documents, but it has either not been prepared yet, or it has not been properly implemented. Cross compliance is essential for going further in the environmentally friendly agriculture, particularly in the agri-environmental measures. Moreover, it is an essential tool for applying the "polluter pays" principle in the agri-environmental policy. Therefore, the legal framework enables implementation of the cross-compliance, but due to the weak institutional capacities and other reasons, it cannot be fully implemented (Cukaliev et al., edts, 2018).

Legal framework for soil protection and management in Western Balkans is not limited to one regulation but relies to a number of by-laws regulating spatial planning, protection of the environment and water, agricultural land, forests and forest land and so on. Most countries in the region has a law on agricultural land, while some have the law on soil protection. A downside in the existing legal regulations of the countries is the lack of an integrated systemic approach to soil management in the manner which will take into account the adaptations of the soil to the local climate conditions, types of the soil and other details regarding the land management.

The most important conclusion from the review of the legal framework for soil management in WB is that there is no country in the region with a comprehensive legal framework that covers soil protection, restoration, sustainable use

and monitoring, and that would set the necessary conditions to integrate soil protection in other policy areas, and thus ensure soil protection and restoration in other policy areas. Another reason for this is the lack of a comprehensive and coherent framework of the EU policy for protection of the soil and its resources, which is a key gap that reduces the effects of the existing incentives and measures and limits the capacity of both the European Union and the Western Balkans countries, which are striving to reach the Green Agenda Goals, UN Sustainable Development goals, No net land take by 2050 goal, land degradation neutrality and other objectives related to development of green infrastructure and bio-economy.

Strategic documents for soil management in WB

With the view to harmonization with the EU policy, all Western Balkan countries have set soil-related goals in their respective strategic documents regarding agriculture and rural development. These documents, inter alia, focus on agroecology and they provide a good basis for development of soil protection policy and related measures for integration of the environment in the agricultural policies. Regardless of the differences between the analyzed countries, it is worth noting that in their strategic documents related to agriculture and environmental protection all the countries present very similar strategic priorities, objectives and measures. Most of them are a result of EU rapprochement. As regards agriculture and rural development, strategies in all the countries mentioned set priorities and activities aimed at soil management, as follows:

- Restoration, preservation and improvement of the ecosystem through sustainable management of natural resources and climate actions,
- Improved management of natural resources and resource efficiency ensuring sustainability of the environment,
- Introduction of agricultural methods that protect the environment and mitigate climate influence (environmentally friendly practices),
- Introduction of EU accession policies related to natural resources management and climate actions focused on sustainable use and management of soils, forest and water resources and waste management, as well as ameliorated use of pollution prevention laws and regulations,
- Reversed trend of environmental degradation (soil erosion, water pollution and biodiversity loss) due to unsustainable soil management and agricultural practice,
- Organic production, control, certification and organic production control system,
- Revitalization and pasture preservation,
- Control of non-selective conversion of agricultural soil for other land uses,
- Preservation of the landscape and agricultural ecosystems, agricultural surfaces of high natural value and their respective resources,
- Awareness raising on climate change, its impacts and mitigation measures, and on the methods for protection from such change,
- Implementation of the reciprocal harmonization as a mechanism that links direct payments with harmonization between farmers and basic environmental standards, including requirements for maintenance of the soil in a good agricultural and ecological state.

With the view to harmonization with the requirements set in the new EU Soil Strategy for 2030, it is necessary to harmonize and finalize the goals set within the strategic documents of the Western Balkans countries.

The IPARD Agri-environment-climate measures: example of the Republic of Serbia

The Agri-environment-climate measure in IPARD III Program for the Republic of Serbia (2021-2027) consists of four operations that aim to enhance cultivation methods adapted to the environment and the features of landscapes, and to enhance the Republic of Serbia biodiversity and ecosystem services, promote water conservation and water quality improvement, and contribute to climate change adaptation and mitigation principally by sustainable input use and improving soil management. Supporting more sustainable practices and management of agricultural land in rural areas will contribute to the maintenance of a favorable landscape and environment for enhanced public goods.

The 4 operations consist of the following: Operation 1 - Crop rotation on arable land; Operation 2 - Grassing in inter-row area in permanent crop land; Operation 3 - Establishment and maintenance of pollinator strips; Operation 4 - Sustainable management of meadows and pastures. These operations are also directly relevant to the achievement of the vision and strategic goals of the National Agriculture and Rural Development Strategy which underlines the importance of a more responsible and sustainable use of resources and agricultural practices. Furthermore, the operations are highly relevant and complementary to the 5 Pillars of the Green Agenda for the Western Balkans.

Soil Indicators for Western Balkans policy support

Agri-environmental indicators (AEIs) track the integration of environmental concerns into the Common Agricultural Policy (CAP) at the EU, national and regional levels. In the context of monitoring the status of natural resources, land-use change, soil cover, soil quality and soil erosion are included among the 28 AEIs.

In WB countries there is nearly no structured soil monitoring or regular reporting on state of soil. Data availability is very diverse in the WB countries. In some countries the set of soil data can be covered to a large degree, although with a different frequency and sometimes diverging national methodology. Through a combination of statistical and environmental data sources, a lot of information is available or at least possible to collect. All available data has been put together in a targeted effort for reporting to international conventions or other obligations. Some AEIs related to soil are also being monitored in the Western Balkans (Table 2).

Table 2. Threats, issues and indicators related to soil in WB countries

Threat/issue	Indicator
Soil erosion	Estimated soil loss by wind erosion
	Estimated soil loss by water erosion
Decline in soil organic matter	Topsoil organic carbon content (measured)
	Soil organic carbon stocks (measured)
	Peat stock
Soil contamination	Heavy metal contents in soils
	Progress in management of contaminated sites
Soil sealing	Land take [to urban and infrastructural development]
Decline in soil biodiversity	Species diversity
	Soil microbial respiration
Soil salinization	Salt profile
	Exchangeable sodium percentage
Desertification	Land area at risk of desertification
	Land area burned by wildfire
Landslide	Occurrence of landslide activity
	Volume/mass of displaced material
	Landslide hazard assessment

In order to establish an indicator-based reporting system in the Western Balkans, it is necessary to consider what are the regional aspects that need to be taken into account when selecting a set of indicators for monitoring soil status.

Conclusions

Soils play a crucial role in achieving the ambitious target of the European Green Deal on a climate-neutral EU by 2050. Implementation of a soil protection framework to ensure healthy soils is a priority for the implementation of the Green Agenda for the Western Balkans. This requires coherent action across a broad policy base. Soil degradation is a trans boundary issue, therefore tackling it requires a well-coordinated regional approach where all stakeholders have equal responsibly to ensure that soils are managed sustainably and the 2030 Agenda for Sustainable Development goals are met.

The agri-environmental measures in place in WB countries and entities, mostly financed through national programs, refer to organic farming, protection of animal and plant genetic resources incl. bees, grazing or revitalization of extensive pastures. In most of the countries in the region, there are neither defined requirements for complying with the Statutory Management Requirements (SMR), nor with the good agricultural and environmental conditions (GAECs) in terms of environmental protection.

Agri-environmental indicators and monitoring of farming practices, impact on the environment, responses of society, and effectiveness of agri-environmental measures need to be further developed. Generally, there is a low level of environmental awareness among farmers. Big challenge and issue in some of the countries of Western Balkans is the LPIS system (Land Parcel Identification System) for all the agricultural plots as a major part of the agri-environmental payments. The Agricultural Advisory Extension Services in the region have had a very important role in farmer education and implementation of the agro-environmental and rural development measures.

The subsidies (IPARD and direct payments) present a good mechanism for support to the implementation of the good agricultural practices for soil conservation, they involve principles of the EU Common Agricultural Policy (CAP) and environment protection through direct support and rural development measures for farmers. In addition to constant work in the process of harmonization of national regulation with the EU standards, additional programs and measures are needed, as well. From the regional perspective, the highest priority is set on the issues of capacity building, awareness raising, and improvement of data availability and transparency.

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Agrarna ekonomika i ruralni razvoj

Mogući učinci djelovanja Europskog zelenog plana na sektor poljoprivrede

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Sažetak

Poljoprivredni sektor je u središtu interesa kad se rade procjene korisnosti strategije od polja do stola i strategije za bioraznolikost, kao važnih dijelova Europskog zelenog plana. Dosad je objavljeno nekoliko istraživanja koja procjenjuju učinke Zelenog plana i Zajedničke poljoprivredne politike (2021.-2027.) na okolišne i socioekonomske čimbenike. Stoga je cilj ovog rada: ustanoviti potencijalne učinke Zelenog plana na a) agrookolišne ciljeve, b) dohodak poljoprivrednika i c) opskrbu hranom u budućnosti. Rezultati pokazuju da bi primjena novih mjera mogla pozitivno utjecati na agrookolišne ciljeve, ali i pozitivno i negativno na dohodak poljoprivrednika i opskrbu hranom.

Ključne riječi: agrookolišni ciljevi, socioekonomski učinci, strategija bioraznolikosti, strategija od polja do stola

Uvod

Poljoprivredna proizvodnja je dosegla vrlo visoku razinu produktivnosti i kvalitete, što omogućuje prevladavanje kontinuiranog globalnog demografskog rasta. Međutim, zbog intenzivnog korištenja zemljišta i pritiskom na prirodne resurse poljoprivreda stvara negativne učinke na klimu, okoliš i biološku raznolikost (Mikuš i sur., 2020.)

Rastući ekološki i klimatski problemi traže učinkovitija rješenja za gospodarske aktivnosti, uključujući i poljoprivredu. Smjer europske poljoprivrede od posebne je važnosti i nastoji popularizirati određene poljoprivredne prakse u tu svrhu. Godinama se provode strategije ili programi održivog razvoja koji su, unatoč pokretanju željenog smjera promjena, još uvijek nedostatni u smislu uočenih potreba.

Opredjeljenje Europskog zelenog plana za razvoj ugljično neutralnog gospodarstva do 2050. godine, a osobito strategija od „polja do stola" i strategija za bioraznolikost do 2030. imat će značajni utjecaj na poljoprivredni sektor. ZPP se nastoji uklopiti novim i ambicioznijim agrookolišnim mjerama i instrumentima u postizanju ciljeva Zelenog plana. Različitim stručnim i znanstvenim studijama procjenjuju se učinci tj. izvjesnost postizanja ciljeva Zelenog plana kao što su: smanjenje upotrebe pesticida, povećanje površina pod ekološkom proizvodnjom, dobrobit životinja itd. Istovremeno, vodi se javni diskurs o potencijalnoj ugroženosti poljoprivrednih dohodaka i opskrbe hranom. Stoga je cilj našeg rada ustanoviti potencijalne učinke Zelenog plana na a) agrookolišne ciljeve, b) dohodak poljoprivrednika i c) opskrbu hranom u novom višegodišnjem programskom razdoblju, ali i daljnjoj budućnosti.

Materijal i metode

Rad se temelji na analizi sadržaja sekundarnih izvora, ponajprije znanstvenih i stručnih studija autora Peeters i sur., 2020., Ministarstva poljoprivrede Sjedinjenih Američkih Država (USDA, 2020.), Francuskog instituta za održivi razvoj i međunarodne odnose (IDDRI, 2021.), Zajedničkog istraživačkog centra Europske komisije (EC JRC, 2021.) i Sveučilišta Wageningen (WUR, 2021.), koje ispituju socioekonomske i okolišne učinke strategije „od polja do stola" i strategije za bioraznolikost koje su dio Zelenog plana. Za potrebe ovog rada izdvojena su tri elementa koji će zbog primjena mjera iz spomenutih strategija najviše biti podložni promjenama: agroekološki čimbenici (emisije štetnih plinova, biološka raznolikost), dohodak poljoprivrednika i sigurnost opskrbe hranom. Studije se očituju i o nekim drugim specifičnim učincima.

Značaj ZPP-a u okviru Zelenog plana

U skladu s ciljevima Europske komisije, Europski zeleni plan je nova strategija rasta. Njegov cilj je izgraditi moderno, resursno učinkovito i konkurentno gospodarstvo koje će dosegnuti nultu stopu emisije stakleničkih plinova do 2050. uz gospodarski rast koji uključuje optimalno korištenje prirodnih resursa. U okviru Zelenog plana Komisija je usvojila nekoliko priopćenja koja se bave pitanjima relevantnim za poljoprivredu i ruralna područja: strategiju „od polja do stola" i strategiju za bioraznolikost do 2030. Strategija od polja do stola nastala je kao potreba za temeljnom transformacijom u prehrambenom sustavu i, po prvi put u kontekstu EU-a, prepoznato je da se prehrambeni lanac treba rješavati kao cjelina, uz paralelno razmatranje ekonomskih, ekoloških i društvenih pitanja (Wrzaszcz i Prandecki, 2020.). Strategijom za bioraznolikost nastoji se spriječiti gubitak vrsta, šumske požare i nesigurnost opskrbe hranom, razviti otpornost na klimatske promjene i zaštititi divlje vrste životinja.

Zajednička poljoprivredna politika prilagođava se novim izazovima s kojima se suočava europska poljoprivreda. Prijedlogom Komisije uvedene su dvije glavne novine: novi model koji državama članicama daje veću fleksibilnost u ostvarivanju onih specifičnih ciljeva ZPP-a koje smatraju najvažnijima za svoje potrebe unutar okvira izvedbe i poboljšanu zelenu arhitekturu. Zelena arhitektura sastoji se od sljedećih elemenata: obvezni standardi kojih se moraju pridržavati svi poljoprivrednici koji primaju plaćanja ZPP-a, nova eko-shema za nagrađivanje poljoprivrednika koji dobrovoljno usvoje ekološki prihvatljiviju praksu i praksu koja se financira kao dio 1. stupa ZPP-a, nastavak dobrovoljnih agrookolišno-klimatskih mjera, podrška proizvodnim i neproizvodnim investicijama, edukacija i savjetovanje (Matthews, 2021.).

Potencijalni učinci

Poljoprivrednici tvrde da su voljni učiniti više za okoliš i klimu, ali samo ako za to dobiju naknadu. Dok se proračun ZPP-a za razdoblje 2021.-2027. možda nije smanjio u nominalnom smislu, primjena Zelenog plana u početku može imati negativne učinke na prihode poljoprivrednika. To je ono što najviše zabrinjava većinu dionika poljoprivrednog sektora. Ograničenje korištenja inputa, izdvajanje zemljišta u neproizvodne svrhe i viši standardi dobrobiti životinja vjerojatno će povećati njihove troškove. Također, poljoprivrednici će se morati više angažirati za izravna plaćanja iz proračuna ZPP-a koja u prosjeku čine 50 % prihoda od poljoprivrede diljem EU-a. Budući da se mnoga poljoprivredna gospodarstva trenutno bore s niskom profitabilnošću, na te se dodatne zahtjeve gleda sa strepnjom. Naime, i dosadašnja istraživanja (Pawlowska i Grochowska, 2021.) pokazuju da uvođenje zelenih praksi nije značajno djelovalo na zaštitu okoliša, kao što niti zelena plaćanja nisu u potpunosti nadoknadili poljoprivrednicima gubitak prihoda zbog primjene zelenih praksi (raznolikost usjeva, ekološki značajne površine, očuvanje travnjaka visoke vrijednosti).

Poljoprivredni sektor je u središtu interesa kad se rade procjene korisnosti Europskog zelenog plana. Dosad je objavljeno nekoliko istraživanja kojima se procjenjuju učinci Zelenog plana i Zajedničke poljoprivredne politike (2021.-2027.) na okolišne i socioekonomske čimbenike. Tako Peeters i sur. (2020). navode da bi se izdvajanja iz dohotka za hranu krajnjeg potrošača povećala u skladu s poboljšanom nutritivnom kvalitetom proizvoda. Smatraju da se na to ne treba nužno gledati kao na negativni učinak već kao na priliku za ponovno uspostavljanje ravnoteže u distribuciji dodane vrijednosti duž lanca opskrbe hranom. Istovremeno potrošačima se nudi prihvatljiva cijena, bolja kvaliteta hrane veće dodane vrijednosti, uz smanjivanje otpadaka od hrane. Pravednijoj raspodjeli dodane vrijednosti i primjerenijoj raspodjeli dohodaka poljoprivrednicima pogodovat će kratki lanci opskrbe hranom tipični za agroekološku proizvodnju. Drugo, povećana opskrba visokokvalitetnom, lokalnom i sezonskom hranom doprinijet će boljoj raspodjeli hrane čime će se smanjiti rasipanje hrane. Treće, poticanje agroekoloških prehrambenih sustava će (pre)odgojiti potrošače prema vrijednostima kao što su sezonalnost proizvodnje ili izbjegavanje masovne kupnje pretjerano prerađene hrane, te će ih osvijestiti da mogu imati aktivnu ulogu u poticanju lokalnog socioekonomskog bogatstva, te u očuvanju vlastitog zdravlja i zdravlja okoliša. Na taj će način potrošači saznati i koja je „tamna strana" jeftine hrane (neuravnotežena distribucija dodane vrijednosti, nepravedno nagrađivanje poljoprivrednika, degradacija okoliša, granično ili ilegalno iskorištavanje sezonskog i migrantskog rada).

Studija USDA (Beckem et al., 2020.) i primijenjeni modeli GTAP-AEZ (the Global Trade Analysis Project–AgroEcological Zones) i IFSA (*The International Food Security Assessment*) upućuju da bi mjere održivosti hrane i poljoprivrede kao što su smanjenje upotrebe tradicionalnih poljoprivrednih inputa zemljišta, gnojiva, antimikrobnih sredstava i pesticida u EU dovele do smanjenja poljoprivredne proizvodnje i konkurentnosti EU na izvoznim tržištima. Nadalje, intenziviranje opskrbe hranom u EU vjerojatno bi rezultiralo povećanjem cijena što bi negativno

utjecalo na potrošački dohodak, smanjilo sigurnost hrane i smanjilo BDP diljem svijeta. U tri scenarija analizirana u ovoj studiji, procijenjeni učinci usvajanja strategija „od polja do stola" i bioraznolikosti uključuju: smanjenu poljoprivrednu proizvodnju u regijama koje su usvojile strategije, povećane cijene poljoprivrednih proizvoda, te smanjenje BDP-a i blagostanja diljem svijeta. Modeli otkrivaju da što su mjere strategija koje ograničavaju upotrebu poljoprivrednih inputa raširenije, ti utjecaji postaju izraženiji, s posljedicama na međunarodnu nesigurnost opskrbe hranom. Isto tako, naglašava se da s ograničenjem trgovine na lokalnu opskrbu, rizik za udaljenije regije postaje veći u pogledu opskrbe hranom. Također, autori smatraju da će posljedice strategija ovisiti o stupnju u kojem drugi prihvate ovu ili slično usklađene strategije. Na primjer, udio EU-a u gubitku blagostanja iznosi 88 % kada EU sama usvoji strategije, ali iznosi 18 % kada se strategije usvajaju na globalnoj razini.

Zanimljivo je da s druge strane studija IDDRI-a, (Schiavo et al., 2021.) proturječi studiji USDA. Naime, rezultati primijenjenog modela GlobAgri-AgT (*Biomass balance models*) pokazuju da široka tranzicija prema agroekologiji koja uključuje cijeli prehrambeni lanac EU-a, čak i gubitak i rasipanje hrane, ne bi dovela u pitanje izvozni potencijal EU-a i doprinijela bi smanjenju uvoznih potreba EU-a. Kao rezultat toga, EU bi se od neto uvoznika pretvorila u neto izvoznika kalorija, ali uz uvjet da stanovništvo EU prihvati drastične promjene u prehrambenim navikama (uravnoteženija dijeta s nižim udjelom kalorija i smanjenim udjelom životinjskih i prerađenih prehrambenih proizvoda). To znači da u isto vrijeme EU može zadržati svoju nepromijenjenu stvarnu veličinu poljoprivrednih površina, zadržati izvozni potencijal izražen u količini, smanjiti ovisnost o uvozu i preinačiti sustav poljoprivredne proizvodnje s pozitivnim učincima na biološku raznolikost, emisiju stakleničkih plinova, zaštitu prirodnih resursa, ali i ljudskog zdravlja.

U studiji EC JRC (Barreiro-Hurle et al., 2021.) putem CAPRI modela autori ispituju potencijal ambiciozne provedbe ZPP-a za postizanje ciljeva Europskog zelenog plana koji su ukorijenjeni u održivoj poljoprivrednoj praksi. Rezultati prije svega upućuju na to da ih ZPP ne može postići sama te ukazuju na potrebu uključenja svih sektora. Postizanje ekoloških ciljeva zahtjeva primjenu modela zelene arhitekture. Naglašava se potreba populariziranja precizne i ekološke poljoprivrede i povećanje proizvodnosti u ekološkom uzgoju. Pretpostavlja se da će klimatske politike ograničiti negativne učinke na zapošljavanje i život poljoprivrednika koji bi proizašli iz katastrofalnih utjecaja globalnog zatopljenja na zemljišne i vodene resurse.

U studiji WUR- a (Bremmer et al., 2021.) korišten je model AGMEMOD. Prema procjeni na makro razini provedba ciljeva strategije „od polja do stola" i biološke raznolikosti rezultirat će smanjenjem proizvedenih količina po usjevu u cijeloj EU u prosjeku u rasponu od 10 % do 20 %. Obujam proizvodnje može pasti i do 30 % za neke usjeve kao što su jabuke, ali postoje i usjevi kod kojih proizvodnja gotovo ne trpi posljedice strategije „od polja do stola" kao što je šećerna repa. Proizvedeni obujam višegodišnjih nasada smanjit će se više nego jednogodišnjih nasada. Poskupjet će proizvodi poput vina, maslina i hmelja. Kao posljedica toga, međunarodna trgovina će se značajno promijeniti: utvrđeno je da će se izvoz EU-a smanjiti, a uvoz u EU povećati (količina uvoza proizvoda može se udvostručiti). Provedba cilja povećanja površina pod ekološkom proizvodnjom na 25 % rezultirat će padom proizvodnje manjim od 10 % i dogoditi se zajedno s povećanjem cijena od nešto manje od 13 %. Povećanje površina pod ekološkom proizvodnjom moglo bi pridonijeti smanjenju ukupne uporabe i rizika od pesticida te smanjenju gubitaka hranjivih tvari. Za jednogodišnje usjeve to je doista slučaj, ali rezultati pokazuju kako je za neke višegodišnje usjeve upravo suprotno. U takvim slučajevima ne bi postojao poticaj za prelazak na ekološku proizvodnju sa stajališta održivosti. U tablici 1 prikazana su glavna predviđanja promatranih studija.

Tablica 1. Učinci Zelenog plana na poljoprivredu: usporedba istraživanja

Autori	Agrookolišni ciljevi	Dohodak poljoprivrednika	Opskrba hranom	Specifični učinci
Peeters i sur. (2020.)	- pravednija raspodjela dohotka - jačanje napora u borbi protiv klimatskih promjena, zaštiti okoliša i očuvanju bioraznolikosti. - rezultati će ovisiti o nastojanju pojedinih država članica da smanje negativan utjecaj poljoprivrede na okoliš.	- poboljšanje kratkih lanaca opskrbe hranom pozitivno će utjecati na dohodak poljoprivrednika.	- povećana ponuda kvalitetne, lokalne i sezonske hrane pogodovat će uspostavljanju ravnoteže ponude čime se smanjuje bacanje hrane.	- poticanje agroekoloških prehrambenih sustava će (pre)odgojiti potrošače prema vrijednostima kao što su sezonalnost proizvoda ili izbjegavanje masovne kupnje pretjerano prerađene hrane.
USDA, 2020.	- ne raspolažu s podacima na kojima bi temeljili svoje projekcije.	- pad dohotka poljoprivrednika u EU, porast dohotka poljoprivrednika u regijama izvan EU (npr. EFTA članice).	- troškovi za hranu se povećavaju, siromašnija kućanstva će patiti od smanjenja kupovne moći, - nesigurnost opskrbe hranom se povećava za 2 %.	- otporan prehrambeni sustav može imati koristi od većeg ulaganja u inovativno poljoprivredno istraživanje i razvoj, gdje se održivost postiže stalnom prilagodbom novim i različitim izazovima kroz znanost, inovacije i usvajanje od strane dionika.
IDDRI 2021.	-transformacija poljoprivrede rezultira pozitivnim učincima na bioraznolikost, smanjenje emisije stakleničkih plinova, zaštitu prirodnih resursa, ali i ljudskog zdravlja.	- nema projekcija	-poboljšanje doprinosa opskrbi hranom kalorijama i proteinima uz istovremeno ostvarivanje agroekoloških ciljeva.	-uz promjenu prehrambenih navika domaćeg stanovništva (smanjeni udio soje, uljarica i prerađevina u prehrani), neto izvoz iz EU će rasti.
JRC, 2021.	- strategija utječe na smanjenje emisije štetnih plinova od 20 % do 30 %.	- očekuje se porast dohodaka poljoprivrednika zbog porasta cijena.	- pad proizvodnje, povećanje cijena za potrošače.	- pad stope BDP-a, negativni učinci preneseni na potrošače.
WUR, 2021.	- na makrorazini, povećanje površina pod ekološkom proizvodnjom pridonijet će ispunjavanju ekoloških ciljeva kao što su smanjenje upotrebe pesticida smanjenje okolišnih i klimatskih rizika.	-dohodak ima tendenciju pada, troškovi nadmašuju prihode, iako se smanjuje udio gnojiva i pesticida.	- smanjenje proizvedenih količina po usjevu u EU za 10 do 20 % - povećanje površina pod ekološkom proizvodnjom rezultira padom proizvodnje i porastom cijena.	- izvoz iz EU-a opada, dok se uvoz u EU povećava.

Izvor: Autori temeljem analize studija

Ako se Zeleni plan nastoji uspješno provesti bez poticanja političkih protupokreta koji odbacuju potrebu za promjenom, potrebno je riješiti potencijalni kompromis između ekoloških i klimatskih mjera te prihoda poljoprivrednih gospodarstava. Zeleni plan može stvoriti nove izvore prihoda za poljoprivrednike, npr. kroz proizvodnju industrijskih sirovina za bioekonomiju, biomase za energiju ili uzgoj ugljika kao novi poslovni model. Također, treba naglasiti da poboljšanje učinkovitosti resursa i promicanje kružnog gospodarstva može biti korisno istovremeno i za poljoprivrednike i za okoliš (Matthews, 2021.). Hoće li se taj potencijal ostvariti uvelike ovisi o sadržaju i ambiciji nacionalnih strateških planova ZPP-a. Trebalo bi poduzeti i druge korake kako bi se olakšala zelena tranzicija. Potrebna su mnogo veća ulaganja u istraživanje i inovacije kako bi se više primjenjivale ekološki prihvatljive prakse. Potrebne su inovacije koje se temelje na molekularnoj genetici i vertikalnoj poljoprivredi. Postojeće potpore dohotku u okviru ZPP-a mogle bi se bolje usmjeriti i pravednije raspodijeliti regulacijom putem zakonodavnih propisa kako bi se zaštitile ranjivije skupine poljoprivrednika.

Procjenu učinaka provedbe politike ZPP-a nakon 2022. u Hrvatskoj obrađuje rad Kranjac i sur., 2021. putem modela AGMEMOD. Rezultati su slični onima u studiji Europske komisije koja koristi CAPRI model (Barreiro-Hurle et al., 2021.). Naime, smanjenje udjela izravnih plaćanja dodijeljenih osnovnoj potpori dohotku utječe na različite sektore različitim razinama intenziteta. Ima snažan učinak na proizvođače goveda, usjeva, ovaca i maslina jer izravna plaćanja predstavljaju veliki udio u njihovim prihodima dok su sektori goveda i ovaca još su osjetljiviji zbog vezane potpore.

Zaključak

Cilj ovog rada bio je ustanoviti potencijalne učinke strategije od polja do stola i strategije za bioraznolikost, kao dijelova Europskog zelenog plana, na agrookolišne ciljeve, dohodak poljoprivrednika i opskrbu hranom u budućnosti. U radu je analiziran sadržaj pet studija koje se bave mjerenjem učinaka Zelenog plana na sektor poljoprivrede putem modela GTAP-AEZ, GlobAgri-AgT, CAPRI i AGMEMOD. Rezultati četiriju studija (osim USDA) pokazuju da zeleni prijelaz poljoprivrede rezultira pozitivnim učincima na bioraznolikost, na smanjenje emisije stakleničkih plinova, zaštitu prirodnih resursa i ljudskog zdravlja. Projekcije kretanja dohodaka poljoprivrednika s u nekim studijama (Peeters at al. 2020. i JRC, 2021.) pokazuju porast zbog poboljšanja kratkih lanaca opskrbe, a s druge strane pad zbog povećanja cijena inputa (USDA, 2020. i WUR, 2021.). Opskrba hranom neće biti ugrožena uz vrlo zahtjevne uvjete kao što su promjena prehrambenih navika stanovništva i značajna ulaganja u inovativno poljoprivredno istraživanje i razvoj, gdje se održivost postiže stalnom prilagodbom novim i različitim izazovima kroz znanost, inovacije i usvajanje od strane dionika.

Napomena

Rad je nastao temeljem diplomskog rada studentice Dore Brkljačić obranjenog dana 27. 9. 2022. godine na Agronomskom fakultetu u Zagrebu.

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Possible effects of the European Green Deal on the agricultural sector

Abstract

The agricultural sector is at the center of interest when assessing the usefulness of the Farm to Fork Strategy and the Biodiversity Strategy, as important parts of the European Green Deal. So far, several studies have been published that assess the effects of the Green Deal and the Common Agricultural Policy (2021-2027) on environmental and socioeconomic factors. Therefore, the aim of this paper is to: determine the potential effects of the Green Deal on a) agroecological objectives, b) farmers' income and c) food supply in the future. The results show that the implementation of new measures could have a positive impact on agroecological goals and both positive and negative impact on farmers' income and food supply.

Keywords: agroecological goals, socioeconomic effects, Biodiversity Strategy, Farm to Fork strategy

Bioekonomija i ruralni razvoj: akteri i potencijal

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Sažetak

Cilj ovog rada je istražiti društvenu dimenziju teritorijalnog kapitala Hrvatske za razvoj bioekonomije. Analizom baza partnera europskih i nacionalnih znanstvenih i istraživačkih projekata iz područja bioekonomije koji su uključivali Hrvatsku kao projektno područje, u periodu od 2016. do 2022., rezultati analize ukazuju da je u tom periodu provedeno 59 projekata u području bioekonomije koji okupljaju 38 aktera tj. nositelja znanja za sektor bioekonomije u Hrvatskoj. Za ostvarenje daljnjeg potencijala bioekonomije potrebno je ispitati i načine i intenzitet suradnje između aktera kako bi se postavili uvjeti za ostvarivanje funkcionalnog ekosustava za održivu bioekonomiju.

Ključne riječi: bioekonomija, multisektorski pristup, nositelji znanja, inovacije, suradnja.

Uvod

Bioekonomija obuhvaća ekonomske, društvene i okolišne inovativne oblike proizvodnje i korištenja resursa uzimajući u obzir ekološka ograničenja i odmak od proizvodnje temeljene na intenzivnoj upotrebi fosilnih goriva. Ona predstavlja korištenje obnovljivih bioloških kopnenih i morskih izvora, poput usjeva, šuma, riba, životinja i mikroorganizama za proizvodnju hrane, materijala i energije (Europska komisija, 2012.). Negativne posljedice neodrživog gospodarenja resursima uzrokuju ekstremne vremenske nepogode, utječu na ljudsko zdravlje i ograničavaju dostupnost resursa. Kako bi se zaštitilo prirodno bogatstvo na europskoj i globalnoj razini, osigurao prijelaz na resursno učinkovito gospodarstvo koje neće negativno utjecati na okoliš i klimatske promjene i zaštita ljudi od pritisaka povezanih s okolišem, razvoj politika u području održivog gospodarenja resursima postaje od strateške važnosti brojnih međunarodnih institucija. U tom kontekstu, Europska komisija je u veljači 2012. godine po prvi puta predstavila Strategiju za bioekonomiju za osiguranje pametnog zelenog rasta u Europi. Strategija i akcijski plan izneseni su pod nazivom *Innovating for Sustainable Growth: a Bioeconomy for Europe*. Suvremena bioekonomija nastoji pronaći održiva rješenja za konvencionalne proizvode na bazi fosilnog ugljika kroz, primjerice, korištenje biogoriva ili bioplastike (Kulišić, 2020.). Bioekonomija obuhvaća ekonomske, društvene i okolišne aspekte Zelenog plana Europske unije razvijajući inovativni pristup korištenju resursa i proizvodnji uz uvažavanje ekoloških ograničenja i smanjivanje upotrebe fosilnih goriva. Razvoj bioekonomije nosi za sobom veliki potencijal za ostvarenje ekonomskog rasta i otvaranje novih radnih mjesta u ruralnim, otočnim i industrijskim regijama, smanjenje ovisnosti o fosilnim gorivima te osiguranje ekonomske i ekološke održivosti primarne proizvodnje i prerađivačke industrije.

Ulaganje u istraživanja i inovacije na području bioekonomije smatra se prilikom za Europu da unaprijedi održivo upravljanje prirodnim resursima i otvori nova diversificirana tržišta za hranu i proizvode na prirodnoj bazi uz pomoć financiranja zelenih projekata kroz različite fondove EU. Za uspostavljanje funkcionalnog ekosustava bioekonomije, smatra se kako su razvoj strategija i ulaganja u inovacije potrebni na svim razinama u EU, ali i da ostvarenje kružnosti u biomasi ne znači i ostvarivanje održivosti bioekonomije. Iz tog razloga se u ažuriranoj Strategiji europske bioekonomije iz 2018. naglašava potreba sistemskom promjenom kroz razvoj nacionalnih i regionalnih strategija za bioekonomiju koje će obuhvatiti svih 17 ciljeva održivog razvoja Ujedinjenih naroda, tzv. *Sustainable Development Goals* (Ujedinjeni narodi, 2015.).

Sustav znanja i inovacija u poljoprivredi u Hrvatskoj sastoji se od mnogobrojnih dionika iz privatnog i javnog sektora s bogatim iskustvom u obrazovanju i istraživanju, međutim veze između njih su slabe te ne postoji efektivan način razmjene znanja, inovacija i rezultata (Jelaković, 2021.). Identificiranje nositelja znanja za bioekonomiju predstavlja priliku za jačanje njihovih kapaciteta, poticanje participativnih procesa i multidisciplinarnih istraživanja te pomaže

pri kreiranju ciljeva i implementacije Nacionalne strategije za bioekonomiju. Prelazak na održivo gospodarstvo temeljeno na biološkim resursima podrazumijeva sinergiju javnog i privatnog sektora suradnjom i razmjenom znanja različitih dionika i donositelja odluka. Generiranje znanja kroz multisektorsku suradnju predstavlja politički čin koji zahtijeva od pojedinaca i organizacija da "priznaju svoju ulogu u pokretanju društvenih i političkih promjena te da se pozabave napetostima i kompromisima koji u njima postoje" (Wyborn i sur., 2019.). Također, tema bioekonomije vrlo je kontroverzna budući da propituje neograničeni gospodarski rast, i time zahtijeva da se više dionika usuglasi oko inicijalno suprotstavljenih stajališta kako bi se postiglo zajedničko razumijevanje i ciljevi (D'Amato i sur., 2022.).

Prema Kulišić (2020.), Hrvatska pripada grupi zemalja Europske unije s ispodprosječnom produktivnošću rada unutar bioekonomije, što je uvjetovano visokom koncentracijom zaposlenosti u radno intenzivnijim sektorima proizvodnje biomase, kao što su poljoprivreda, šumarstvo i akvakultura. Prema izvještaju u sklopu projekta BIOEAST (2021.), u posljednjem desetljeću Hrvatska je bila među zemljama s niskom razinom razvoja bioekonomije u usporedbi s ostalim zemljama na razini EU i makroregijom BIOEAST[1]. Tranzicija prema bioekonomiji u RH prilično je spora, između ostaloga, zbog ograničenog pristupa financijskim resursima, nedostatka političkog okvira za bioekonomiju, nedostatka sinergije između znanosti i praktičnog znanja. Navedeni faktori zajedno dovode do niskog inovacijskog učinka koji je također umjeren i ispod prosjeka EU, što se smatra jednom od prepreka koja nije dopuštala ubrzanje razvoja bioekonomije temeljene na znanju. S obzirom na to da je Hrvatska zemlja s velikim potencijalom pretvaranja svoga poljoprivredno-prehrambenog sektora u suvremenu djelatnost koja potiče gospodarski rast, stvara radna

mjesta i ostvaruje prihod u ruralnim zajednicama (Kulišić, 2020.), potrebno je omogućiti adekvatne resurse koji mogu te potencijale ispuniti.

Dakle, bioekonomija predstavlja inovativno rješenje na razini Europske unije za prelazak na održivo, energetski i resursno učinkovito gospodarstvo. Kako bi se ostvarili zadani ciljevi na nacionalnoj razini, smatramo da je potrebno identificirati ključne aktere za tranziciju društva i ekonomije prema bioekonomiji. Jedan od tih aktera su nositelji znanja koji pomažu u učinkovitijem rješavanju društvenih problema, omogućuju brži razvoj inovacija i tehnologija te uspješniju primjenu novih znanja.

Primarni cilj ovog rada bio je identificirati nositelje znanja za sektor bioekonomije u Hrvatskoj što bi moglo poslužiti kao potpora izradi nacionalne strategije za bioekonomiju.

Materijal i metode

U prvoj fazi rada provedeno je *desk* istraživanje gdje su se identificirali projekti koji doprinose razvoju bioekonomije te s njima povezane institucije i pojedince kao nositelje znanja. Za ovo istraživanje relevantnima smo razmatrali isključivo projekte s početkom provedbe od 2016. godine, jer se to smatra razdobljem kada bioekonomija kakvu danas definiramo postaje prepoznata u Republici Hrvatskoj te su projekti takvom konceptu i prilagođeni. Budući da programi financiranja unutar Europske unije u najvećoj mjeri doprinose financiranju razvojnih projekata, kao primaran izvor informacija koristile su se baze projekata iz programa Obzor 2020 u sklopu Agencije za mobilnost i programe EU te programa BBI-JU (*Bio-based Industries Joint Undertaking*), javno-privatnog partnerstva između Europske unije i konzorcija za bioindustriju. Ostali projekti i nositelji znanja pretraživali su se preko službenih mrežnih stranica institucija i poduzeća koji su spomenuti kao partneri. Navedeni projekti su također financirani ili sufinancirani iz proračuna Europske unije, najčešće iz operativnih programa Obzor 2020 i Konkurentnost i kohezija 2014. – 2020. Uz projekte iz prve faze koji se fokusiraju isključivo na preradu primarnih proizvoda u proizvode visoke dodane vrijednosti, u drugoj fazi mapiranja stvorena je dodatna, komplementarna baza projekata. Takvi projekti svojim ciljevima doprinose sustavnom i održivom razvoju bioekonomije, te se oni zajedno s pripadajućim dionicima također smatraju relevantnima. U trećoj, završnoj fazi mapiranja institucije i druge nositelje smo kategorizirali u četiri skupine - gospodarski, javni, znanstveno-obrazovni i civilni sektor.

Što se tiče gospodarskog sektora, često se navodi kako mala i srednja poduzeća imaju više mogućnosti za postizanje veće razine fleksibilnosti i brzine odgovora na promjene u odnosu na velika poduzeća. S obzirom na to, očekuje se da će industrije i poslovne organizacije biti ključne za razvoj bioekonomije (D'Amato i sur., 2020.). Druga skupina aktera, javne ustanove, mogu biti od velike važnosti za razvoj bioekonomije u pogledu savjetovanja, administrativne i financijske podrške te organizirano sustavno djelovanje na lokalnoj ili regionalnoj razini. Treća grupa aktera, znanstveno-obrazovni sektor, primarnu ulogu ima u stvaranju inovacija koje doprinose razvitku bioekonomije. Četvrta skupina aktera, civilno društvo putem nevladinih organizacija predstavlja neformalni sustav građanske

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povezanosti i inicijativa, nastalih u svrhu ostvarenja (uglavnom) društveno korisnih ciljeva. Kao takve, djeluju kao posrednici između javnih vlasti i građana (Visinski, 2004). S obzirom na to, mogu pozitivno utjecati na razvitak bioekonomije te doprinijeti ekološkoj i socijalnoj održivosti. Na temelju prikupljenih podataka stvorena je baza nositelja znanja za sektor bioekonomije u Republici Hrvatskoj. Potrebno je napomenuti kako je mapiranje dinamičan proces te ovim radom ne namjeravamo prikazati konačni popis nositelja znanja, već započeti proces mapiranja koji zahtijeva redovito ažuriranje podataka i daljnje promišljanje vrste aktera koji mogu doprinijeti širenju i prihvaćanju bioekonomije kao novog načina razmišljanja i proizvodnje.

Rezultati i rasprava

Analizom izvora iz prve faze mapiranja identificirano je ukupno 29 znanstvenih projekata čiji je cilj pretvorba nusproizvoda iz primarne proizvodnje u proizvode dodane vrijednosti. Oni služe kao inovativna rješenja s ciljem pretvorbe ostataka iz primarne proizvodnje, kao što su kukuruz, žitarice, sjemenke bundeve, voće, ostaci poljoprivredne proizvodnje, sirutka, životinjski stajnjak, gljive i mikroalge u proizvode s dodanom vrijednošću, kao što su biougljen, bioplin, bioplastika, biokompoziti, biopeleti, inovativni proizvodi na bazi sirutke te zamjena štetnih tvari u prirodnoj kozmetici na bazi ostataka gljiva i mikroalgi.

Iz te baze podataka identificirano je 12 gospodarskih subjekata koji na različite načine doprinose razvoju sektora bioekonomije. Polovica uključuje inovativna mala i srednja poduzeća, koja su, osim na poljoprivredu, ribarstvo i šumarstvo, usmjerena i na proizvodnju bioplastike. Dodatno, postoji nekolicina velikih industrijskih pogona koji svoje poslovanje usmjeravaju prema načelima kružne bioekonomije kroz sudjelovanje u razvojnim projektima koji su također većinom povezani s proizvodnjom bioplastike iz mikroalgi ili ostataka gljiva. Proizvodnja biopeleta, biognojiva, namještaja iz ostataka poljoprivredne i šumarske proizvodnje također je zastupljena u identificiranim razvojnim projektima. U manjoj mjeri zastupljena je proizvodnja farmaceutika i tekstila koji također imaju veliki potencijal stvaranja dodane vrijednosti.

U drugoj fazi mapiranja stvorena je komplementarna baza projekata i inicijativa. Ta se baza ne odnosi izravno na preradu primarnih proizvoda, međutim identificirane institucije i projekti također imaju važnu ulogu u osnaživanju sektora bioekonomije na nacionalnoj i europskoj razini. Identificirano je dodatnih 30 projekata i 22 pripadajuće institucije koje doprinose razvoju bioekonomije kroz ispitivanje tržišnih potencijala za obnovljive izvore energije, poticanje sinergije znanosti i praktičnog znanja, osnaživanje civilnog sektora, edukaciju, održivo zbrinjavanje otpada, povezivanje s akterima na nadnacionalnoj razini, logističku podršku organiziranjem sabirnih centara za biomasu te prirodno graditeljstvo.

Govoreći o znanstveno-obrazovnom sektoru, tijekom mapiranja zabilježeno je 12 institucija koje doprinose razvoju bioekonomije, kao voditelji ili partneri u projektima, koji su većinom usmjereni na pretvorbu nusproizvoda iz primarne proizvodnje u proizvode visoke dodane vrijednosti. Od ukupnog broja aktivnih istraživača u projektima iz područja bioekonomije s Agronomskog fakulteta dolazi njih šest, a po troje istraživača dolazi s Prehrambeno-biotehnološkog fakulteta i Hrvatskog šumarskog instituta. U manjoj mjeri i s manjim intenzitetom sudjelovanja po projektima, s osam predstavnika zastupljen je javni sektor na regionalnoj, županijskoj i gradskoj razini. Takve javne ustanove i razvojne agencije imaju veliku ulogu u provođenju poduzetničkih i razvojnih projekata, povećanju konkurentnosti, pružanju savjetodavnih usluga te kontinuiranom stručnom usavršavanju. Veće uključivanje javnih ustanova u projekte povezane s bioekonomijom znatno bi doprinijelo regionalnoj konkurentnosti i ostvarenju bioekonomije koja poznaje i vodi računa o mogućnostima i resursima na lokalnoj ili regionalnoj razini.

Od prethodno navedenih sektora u bioekonomiji, civilni sektor najmanje je zastupljen, no dijelom je to posljedica uzorkovanja u našem istraživanju. Budući da smo nositelje znanja identificirali prvenstveno iz dviju baza projekata (iz programa Obzor 2020 i BBI-JU), udruge koje nisu među partnerima financiranih projekata nismo ovom analizom zahvatili. Stoga je važno naglasiti da zasigurno postoje brojne organizacije civilnog društva u Hrvatskoj koje nisu spomenute u ovom istraživanju, a koje izravno ili neizravno utječu na inkluzivan i održiv razvoj bioekonomije. Neke od njih su energetske zadruge i zadruge općenito, kratki lanci opskrbe kao što su grupe solidarne razmjene, ekološke udruge i druge udruge čiji rad indirektno podupire tranziciju prema bioekonomiji, primjerice, kroz projekte revitalizacije lokalnih zajednica ili projekte koji osnažuju marginalizirane društvene skupine. Važno je naglasiti i kako su programi financiranja unutar Europske unije uglavnom usmjereni na istraživanja i tehnološke inovacije te u fokus stavljaju gospodarsku i znanstvenu zajednicu, a razvojni projekti civilnog sektora u području bioekonomije, koji su financirani iz drugih izvora, ovim radom nisu obuhvaćeni. Dodatna istraživanja bila bi korisna kako bi se

istražilo na koje načine i koji drugi akteri bi mogli izravno i neizravno doprinositi bioekonomiji u Hrvatskoj. Stoga bi u daljnjem istraživanju i mapiranju aktera trebalo identificirati i šire indirektne promicatelje bioekonomskih praksi.

Predstavnici prethodno navedenih sektora za bioekonomiju uz broj projekata na kojima su sudjelovali prikazani su u nastavku u Tablici 1.

Tablica 1. Nositelji znanja za razvoj bioekonomije u Republici Hrvatskoj, prema broju projekata.

R. br.	Gospodarski sektor	**Javni sektor - javna uprava**	**Javni sektor -znanstveno-obrazovne institucije**	**Civilni sektor**
1.	Mi-plast Ltd. (9)	Hrvatska poljoprivredna komora (2)	Agronomski fakultet (9)	SDEWES - Međunarodni centar za održivi razvoj energetike, voda i okoliša (2)
2.	Bio-mi Ltd. (6)	Regionalna razvojna agencija Međimurje (1)	Fakultet elektrotehnike, strojarstva i brodogradnje (7)	Hrvatski drvni klaster (2)
3.	Particula Group (4)	Razvojna agencija Sjever (1)	Fakultet agrobiotehničkih znanosti Osijek (3)	DOOR - Društvo za oblikovanje održivog razvoja (1)
4.	IPS konzalting (3)	Razvojna agencija Vukovarsko-srijemske županije (1)	Prehrambeno – biotehnološki fakultet u Zagrebu (2)	Zelena energetska zadruga (1)
5.	EcoCortec (2)	Ministarstvo poljoprivrede (1)	Fakultet šumarstva i drvne tehnologije (2)	ZMAG – Zelena mreža aktivističkih grupa (1)
6.	SAPONIA d.d. (2)	Grad Rijeka (1)	Energetski institut Hrvoje Požar (2)	Eko Kvarner (1)
7.	INA d.o.o. (1)	Razvojna agencija Zagreb (1)	Prehrambeno-tehnološki fakultet Osijek (2)	
8.	Croatian Woods Ltd. (1)	Ministarstvo znanosti i obrazovanja (1)	Hrvatski šumarski institut (1)	
9.	Centar kompetencija d.o.o. za istraživanje i razvoj Vinkovci (1)		Hrvatska agencija za poljoprivredu i hranu (1)	
10.	Kutjevo d.d. (1)		Veleučilište u Karlovcu (1)	
11.	Zagrebački holding d.o.o. (1)		Institut za političku ekologiju (1)	
12.	Centar kompetencija - hrana i bioekonomija d.o.o. (1)		Tekstilno-tehnološki fakultet (1)	

Izvor: vlastito istraživanje.

Zaključak

Mapiranjem smo utvrdili da na ukupno 59 bioekonomskih projekata sudjeluje 38 aktera, od kojih su brojniji oni iz gospodarskog i znanstveno-obrazovnog sektora, te na pojedinim projektima sudjeluje istovremeno više nositelja znanja. Na temelju provedenog mapiranja možemo zaključiti kako u Hrvatskoj postoje određeni institucionalni i ljudski resursi koji su započeli razvijati bioekonomiju no da je taj stupanj razvoja vrlo ograničen. Među zahvaćenim projektima i akterima iznimno je nisko zastupljena javna uprava i civilno društvo. Potrebno je napomenuti kako rezultati dobiveni istraživanjem projekata ne daju sveobuhvatan prikaz relevantnih aktera za bioekonomiju, jer razmatramo isključivo one koji su sudjelovali u razvojnim projektima, dok svakako postoji niz aktera koji doprinose na druge načine. Za daljnja istraživanja bilo bi korisno istražiti i na koji način su navedeni nositelji znanja povezani te kojim mehanizmima i aktivnostima se može stvoriti viša razina suradnje i razmjene znanja među navedenim akterima i institucijama. Nadalje, bilo bi korisno evaluirati ulogu savjetodavne službe i ostalih državnih i javnih tijela u generiranju znanja prema primarnim proizvođačima i ostalim akterima s obzirom na to da tijekom provođenja ovog mapiranja nisu u značajnoj mjeri spomenuta. Stvaranje sustava koji uspješno razvija održivu bioekonomiju leži u sinergiji svih sektora i međusobnoj razmjeni znanja i multisektorskoj suradnji. Naposljetku, važno je nastaviti istraživati ulogu poljoprivrednika u procesu razmjene i primjene bioekonomskih znanja te načine na koje mogu biti aktivno uključeni u proces stvaranja bioekonomije na razini svojih lokalnih zajednica.

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Bioeconomy and Rural Development: actors and potential

Abstract

The goal of this paper was to identify knowledge holders for the bioeconomy sector in Croatia. The objective is to analyse the bioeconomy projects in the period 2016-2022 and to extract the knowledge holders within the project partners. The results show there were 59 projects comprising 38 knowledge holders for the bioeconomy sector, mostly in the economic and scientific sector. In future studies it is necessary to examine the nature and intensity of cooperation between bioeconomy actors. For future bioeconomy development it would be necessary to explore the nature and intensity of cooperation among those actors in order to build fruitful conditions for sustainable development of bioeconomy.

Keywords: bioeconomy, multisectoral approach, knowledge holders, innovation, cooperation

Poljoprivredno-prehrambeni proizvodi u agregiranom poljoprivrednom dohotku Ličko-senjske županije

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Sažetak

Cilj rada je utvrditi stanje poljoprivrede na području Ličko-senjske županije te izračunom agregiranog poljoprivrednog dohotka odrediti najvažnije poljoprivredno-prehrambene proizvode. Rezultati istraživanja pokazuju da su, prema udjelima u agregiranom poljoprivrednom dohotku, najdohodovnije djelatnosti Ličko-senjske županije ovčarstvo, govedarstvo u sustavu krava-tele, proizvodnja krumpira, šljiva i meda. U intervjuu s voditeljicom LAG-a Lika potvrđeni su navedeni rezultati te je naglašena želja lokalnih poljoprivrednika za povećanjem poljoprivrednog dohotka preradom proizvoda na vlastitim gospodarstvima. Zbog administrativnih prepreka i poreznih obaveza broj registriranih prerađivača još uvijek je relativno mali u odnosu na mogućnosti.

Ključne riječi: poljoprivreda, Ličko-senjska županija, tradicionalni proizvodi, agregirani poljoprivredni dohodak

Uvod

Ličko-senjska županija (LSŽ) prostorno je najveća županija u Hrvatskoj s 5.350,50 km² što čini 9,46% hrvatskog državnog teritorija. U LSŽ-u je 80,4% površine po reljefu planinski kraj stoga je poljoprivredna proizvodnja organizirana na krškim poljima (Gacko, Ličko i Krbavsko). Važnost poljoprivrede ogleda se u prvom strateškom cilju ove županije, a to je stvaranje uvjeta za uravnotežen i održiv gospodarski razvoj zasnovan na razvoju malih gospodarstava, ekološki prihvatljive prerađivačke industrije, poljoprivrede i turizma (Razvojna strategija LSŽ, 2010.). Tradicionalno se uz ovaj prostor veže ekstenzivna stočarska i biljna proizvodnja uz prepoznatljive ličke proizvode poput janjetine, krumpira, šljiva, kupusa i meda. Zbog aktualnih promjena u lancu proizvodnje i potrošnje hrane upitno je jesu li ovi proizvodi i u novije doba predvodnici ličke poljoprivrede, odnosno koji je njihov doprinos agregiranim ekonomskim poljoprivrednim pokazateljima gradova i općina LSŽ.

Ciljevi rada su: (a) odrediti vodeće poljoprivredne djelatnosti i vodeće JLS-ove obzirom na udio u agregiranom poljoprivrednom dohotku, (b) provjeriti dobivene rezultate intervjuom s voditeljicom LAG-a Lika.

Materijal i metode

U radu je provedena analiza sekundarnih izvora podataka koji se značajnim dijelom odnose na statističke podatke iz Upisnika poljoprivrednika. Proizvodnja na korištenim poljoprivrednim površinama LSŽ-a utvrđena je prema podacima Agencije za plaćanja u poljoprivredi, ribarstvu i ruralnom razvoju (APPRRR, 2022a) i dokumentu „Tražene kulture na jedinstvenom zahtjevu za potporu“ za 2021. godinu. Brojnost i najzastupljenije vrste stoke određene su prema podacima Jedinstvenog registra domaćih životinja (HAPIH, 2022) dok je broj pčelinjih zajednica određen prema podacima Sektora za označavanje i registraciju životinja i upravljanje podacima u stočarstvu. Agregirani dohodak poljoprivrede JLS-ova određen je prema utvrđenim proizvodnim resursima čije su vrijednosti pomnožene s očekivanim dohotkom po hektaru biljne kulture odnosno po grlu stoke navedenima u Katalogu kalkulacija poljoprivredne proizvodnje (Ministarstvo poljoprivrede, 2019). U Katalogu se financijski učinci pojedine vrste poljoprivredne proizvodnje određuju izračunom pokrića varijabilnih troškova - PVT (*engl. Gross Margin Calculations*). Iako PVT nije istoznačnica za dohodak, u praksi se često zbog nemogućnosti realnog utvrđivanja

fiksnih i/ili indirektnih troškova poslovanja upravo PVT iskazuje kao dohodak bez uključenih indirektnih troškova. Zaključci istraživanja provjereni su i prokomentirani intervjuom s voditeljicom LAG-a Lika.

Rezultati rada

Poljoprivreda Ličko-senjske županije

Poljoprivreda je u Lici oduvijek bila od presudne važnosti za njezino stanovništvo. Kako navodi Holjevac (2009) čak 92,6% stanovnika županije početkom dvadesetog stoljeća bavilo se poljoprivredom i živjelo od nje. Najzastupljenija žitarica bila je jari ječam, a najzastupljenija okopavina krumpir. Stočni fond činile su uglavnom ovce, goveda, svinje i konji. Najzastupljenija pasmina goveda bila je domaća buša (94% svih goveda), relativno slabih proizvodnih učinaka, ali naviknuta na zahtjevne planinske uvjete. Oštra klima i reljefno nepogodan gorski krajolik s dominantnom krškom podlogom znatno su ograničavali ratarsku proizvodnju jer je za obradu povoljne zemlje bilo samo u rijetkim visokim poljima u kršu. Unatoč tome, brojni planinski pašnjaci i proplanci pružali su povoljne uvjete za uzgoj stoke, osobito velikog broja ovaca pa su se Ličani tradicionalno više bavili stočarstvom nego ratarstvom (Holjevac, 2009). Županijska razvojna strategija LSŽ-a iz 2010. godine navodi kako je poljoprivredna proizvodnja organizirana na malim parcelama što za posljedicu ima ekstenzivan način proizvodnje i nisku dohodovnost po jedinici kapaciteta. Izostanak intenzivnije gospodarske aktivnosti doveo je do ekološke očuvanosti područja pogodnog za razvoj ekološke poljoprivrede i stočarstva (Starčević i sur., 2018), a samo poljoprivredno zemljište kao temeljni prirodni resurs u LSŽ je nezagađeno i pogodno za razvoj ekološke poljoprivrede (Razvojna strategija LSŽ, 2010). Veliki razvojni problem, osim loše demografske strukture i rascjepkanosti poljoprivrednog zemljišta, predstavlja i nedostatak prerađivačkih kapaciteta poljoprivrednih proizvoda što potvrđuje razmjerno mali broj objekata registriranih za preradu poljoprivrednih proizvoda. Na području LSŽ, 12 je odobrenih objekata i 113 registriranih objekata u poslovanju s hranom životinjskog podrijetla. Od toga je 86 objekata registrirano za proizvodnju meda, 9 za preradu mlijeka na gospodarstvu te 6 za proizvodnju mlijeka, sira i vrhnja (Ministarstvo poljoprivrede, 2022).

Prema podacima iz Upisnika poljoprivrednika za 2021. godinu (APPRRR, 2022b) na području LSŽ je ukupno 5.195 poljoprivrednih gospodarstava što je rast od 11% u proteklih pet godina. Prema obrazovnoj strukturi najveći je udio nositelja gospodarstava sa srednjoškolskim obrazovanjem (56%). Udio visokoobrazovanih nositelja je 12%, nositelja sa završenom osnovnom školom je 25% dok njih 7% nema završeno osnovnoškolsko obrazovanje. Obzirom na dobnu strukturu poljoprivrednika, prevladavaju poljoprivrednici stariji od 65 godina kojih je 39%. Značajan udio u strukturi imaju i poljoprivrednici između 56 i 65 godina (23%), ali je primjetan i visoki udio poljoprivrednika mlađih od 40 godina koji iznosi 17% što je tri postotna boda više od prosjeka Republike Hrvatske.

U ARKOD je upisano 55.313 hektara poljoprivrednog zemljišta. Prema vrsti uporabe najviše je krških pašnjaka koji s 27.456 ha zauzimaju gotovo polovinu korištenog poljoprivrednog zemljišta (APPRRR, 2022c). U 2020. godini ukupna površina zemljišta LSŽ pod ekološkom proizvodnjom iznosila je 17.746 ha što predstavlja 32% ukupno korištenog zemljišta. Istovremeno, udio ekoloških površina u Hrvatskoj bio je 7% što ukazuje da je važnost ekološke proizvodnje za LSŽ značajno veća u odnosu na hrvatski prosjek (DZS, 2021).

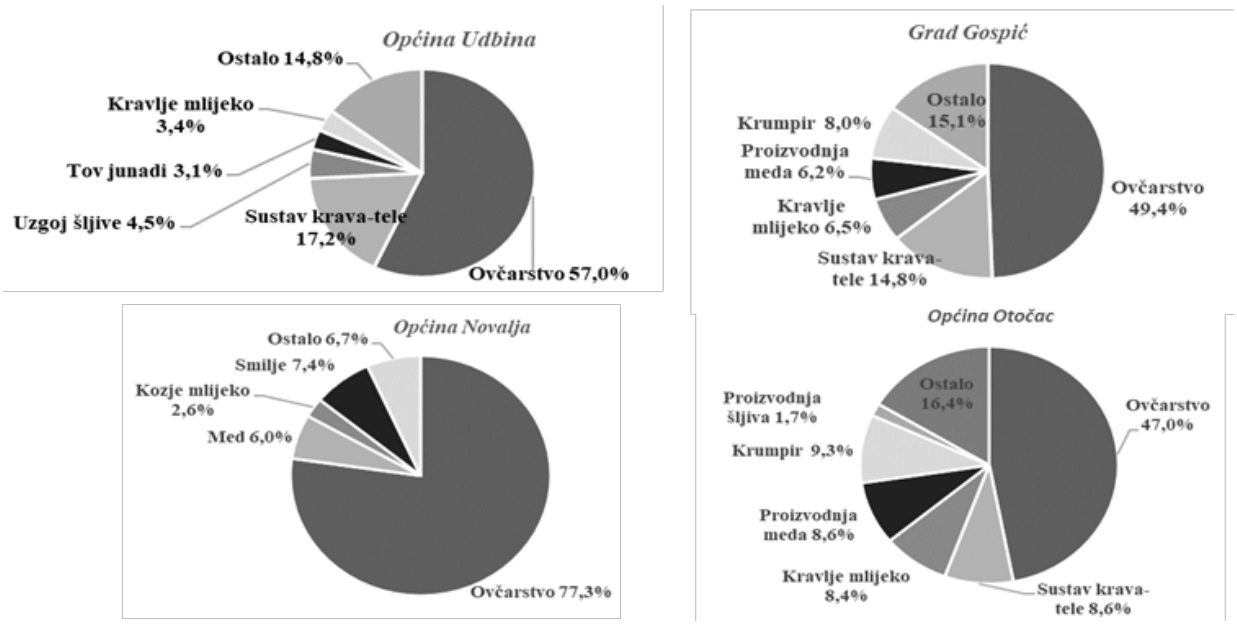
Na području svih JLS-ova LSŽ prevladavajuća poljoprivredna djelatnost je stočarstvo što je razvidno iz tipologije poljoprivrednih gospodarstava dobivenoj primjenom FADN kalkulatora pri izračunu standardnog ekonomskog rezultata. U svim općinama i gradovima LSŽ vodeći je tip pod brojčanom oznakom 4. „Stočarstvo - govedarstvo, ovčarstvo i kozarstvo".

Prema relativnoj vrijednosti standardnog ekonomskog rezultata najveća poljoprivredna gospodarstva, s najvećim prihodovnim potencijalom, nalaze na području općina Udbina i Karlobag. Ove dvije općine ujedno su jedine JLS na području Županije čija je prosječna ekonomska veličina gospodarstva veća od prosječne ekonomske veličine poljoprivrednih gospodarstava Jadranske Hrvatske koja iznosi 12.127 eura/poljoprivredno gospodarstvo (FADN, 2018).

Prevladavajuće biljne kulture i vrste stoke prema udjelu u poljoprivrednom dohotku

Proizvodnja ovčjeg mlijeka i mesa, uzgoj goveda u sustavu krava-tele te proizvodnja meda, najdohodovnije su stočarske proizvodnje na području JLS-ova, dok u biljnoj proizvodnji- prevladava proizvodnja krumpira. U ukupnom dohotku svih JLS-ova najzastupljenija je proizvodnja ovčjeg mlijeka i mesa s udjelom od 23,6% (Karlobag) do 77,3%

(Novalja). Uzgoj koza slabije je prisutan na području LSŽ osim u općini Karlobag gdje čini 21,6% poljoprivrednog dohotka. Govedarstvo u sustavu krava-tele prisutno je u dohotku svih JLS-ova osim u Novalji. Njegovi se udjeli u ukupnom dohotku kreću od 6,7% (Senj) do 36,9% (Vrhovine). Značajan je i doprinos proizvodnje meda koja, primjerice, u poljoprivrednom dohotku grada Senja čini udio od 29,2%. Proizvodnja kravljeg mlijeka zastupljena je u ukupnom dohotku poljoprivrede Županije s prosječno 5,9%. Što se tiče biljne proizvodnje, najzastupljenija stavka poljoprivrednog dohotka je proizvodnja krumpira *čiji je udio* 10,7% u općini Lovinac, 9,6% u općini Brinje te 9,3% u Otočcu. Važniju biljnu proizvodnju čini proizvodnja šljiva koja je u općinama Udbina, Donji Lapac i Plitvička Jezera zastupljena s više od 2% ukupnog agregiranog dohotka. Od ostalih kultura ističe se jedino proizvodnja smilja koja poljoprivrednom dohotku grada Novalje doprinosi sa 7,4%. Na slici 1 prikazani su udjeli pojedinih vrsti poljoprivrednih djelatnosti u dohotku odabranih JLS-ova.



Slika 1. Struktura poljoprivrednog dohotka prema djelatnostima u odabranim JLS na području LSŽ (2021)

Intervju s voditeljicom LAG-a Lika

Intervju s ciljem osvrta na aktualnosti i trendove poljoprivredne proizvodnje proveden je s Ivanom Starčević, voditeljicom LAG-a Lika. Poljoprivredu LSŽ karakterizira ekstenzivno stočarstvo u kojem se odvija promjena proizvodne orijentacije ličkih poljoprivrednika. Dolazi do gašenja velikog broja mliječnih farmi te je sve manji broj proizvođača mlijeka. Udaljenost od otkupljivača mlijeka pa samim time i otežana mogućnost isporuke mlijeka, niske otkupne cijene i radno intenzivan uzgoj, neki su od razloga napuštanja te proizvodnje. Stoga se poljoprivrednici okreću onim oblicima stočarske proizvodnje koje su isplativije i manje radno zahtjevne, a trenutno je to govedarstvo u sustavu krava-tele. Janjetina, kupus, šljive, krumpir, med i sir tradicionalni su lički proizvodi. Voditeljica LAG-a navela je da su ovi proizvodi i u aktualno doba najznačajniji lički proizvodi čija proizvodnja nema izraženijih oscilacija. Jedino što kontinuirano opada jest proizvodnja sira. Osim smanjenja broja mliječnih krava, razlog tome je i potreba registracije objekata za preradu čemu lički poljoprivrednici nisu skloni. Umjesto toga poljoprivrednici se odlučuju na držanje goveda u sustavu krava-tele na što ih motiviraju i zadovoljavajuće potpore po hektaru pašnjaka kao i potpore za autohtone pasmine goveda poput buše. Voditeljica LAG-a ističe zainteresiranost poljoprivrednika za preradu svojih proizvoda, međutim navodi da se još uvijek radi o razmjerno malom broju proizvođača. Ipak, prerada je i dalje zastupljenija među dopunskim djelatnostima OPG-ova u odnosu na ruralni turizam za koji smatra da bi mogao biti prisutniji obzirom na ekološke potencijale prostora. Voditeljica LAG-a zalaže se za pojednostavljivanje uvjeta registracije prerađivačkih objekata i predlaže izradu vodiča u kojem će se poljoprivrednicima na jednostavan način objasniti koraci u postupku registracije. Zaprekom za preradbene aktivnosti na gospodarstvima smatra i važeći porezni sustav koji zahtjeva od poljoprivrednika koji pokreću preradu upis u Registar poreznik obveznika što sa

sobom povlači i obaveze plaćanja poreza i doprinosa. Voditeljica je navela i oznake kvalitete kojima se označavaju neki od ličkih proizvoda. Zaštićenom oznakom izvornosti (ZOI) zaštićena je paška janjetina, sol i paški sir, a lički krumpir, škripavac i janjetina zaštićeni su Zaštićenom oznakom zemljopisnog podrijetla (ZOZP). Certificiranih proizvođača za proizvodnju navedenih proizvoda je svega nekoliko (janjetina – jedan proizvođač, sol – jedan proizvođač, lički krumpir – dva proizvođača, paški sir – pet proizvođača), dok za proizvodnju ličkog škripavca još nema certificiranih proizvođača. Lokalni poljoprivrednici, prema riječima voditeljice LAG-a, pokazuju zainteresiranost za certificiranje, međutim rijetko se odlučuju zbog straha od dodatnih kontrola, dodatne papirologije, ali i financijskih izdataka koje certificiranje podrazumijeva. Voditeljica se osvrnula konkretno na certificiranje ličkog škripavca koji je u prosincu 2021. godine dobio europsku zaštićenu oznaku zemljopisnog podrijetla, navodeći kako proizvođači pokazuju zanimanje te ih je već nekoliko iskazalo interes uključivanja u sustav certificirane proizvodnje. Međutim cijeli postupak odvija se presporo jer se od poljoprivrednika zahtjeva registracija objekata za preradu na što se slabo odlučuju jer to traži dodatna financijska ulaganja. Ono što također predstavlja prepreku certificiranju jest i činjenica da su potencijalni proizvođači uglavnom mali OPG-ovci s malim količinama proizvodnje koji svoje proizvode uspješno plasiraju i bez certificiranja. Osim europskih oznaka kvalitete, LAG Lika 2018. godine razvio je regionalnu oznaku kvalitete Lika Quality s ciljem promicanja tradicionalnih ličkih proizvoda natprosječne kvalitete te brandiranja Like kao gastro-destinacije. U sustav se mogu uključiti prehrambeni proizvodi, ali i suveniri i rukotvorine. Voditeljica LAG-a Lika navodi da je trenutno u sustavu Lika Quality 80 proizvođača sa 183 proizvoda u 12 kategorija. Najviše ih je iz kategorije suveniri (15 proizvođača) te meso i mesne prerađevine (11), voće (11) i povrće (11). Proizvođači su tako dobili priliku da se njihovi proizvodi plasiraju na šira tržišta. Primjerice, u NP Plitvička jezera postoji prodajno mjesto ovih proizvoda, a pokrenuta je i poljoprivredna zadruga Lika COOP koja otkupljuje proizvode od lokalnih proizvođača te ih plasira na tržište. Voditeljica LAG-a navodi kako su od prošle godine proizvodi Lika Quality u prodaji na nekoliko brodova Jadrolinije na međunarodnim linijama što znači da se prodaju i izvan područja Like i Hrvatske, a isto tako neke od njih moguće je pronaći i u trgovačkim lancima.

Zaključak

Ličko-senjska županija tradicionalno se identificira po poljoprivredno-prehrambenim proizvodima poput janjetine, krumpira, sira i meda. I u novije doba ovi su proizvodi značajno prisutni u strukturi ličkog prehrambenog sustava. Općina Udbina najznačajnija je lička općina prema prosječnoj ekonomskoj veličini poljoprivrednih gospodarstava. Na području ove općine 57% ukupnog poljoprivrednog dohotka stvara se u sektoru ovčarstva, a značajnije udjele ostvaruju i govedarstvo u sustavu krava-tele (17%) te proizvodnja **šljiva (5%). Slična struktura poljoprivrednog dohotka razvidna je i u drugim ličkim općinama** uz nešto izraženije udjele ovčarstva u općini Novalja (77%) i proizvodnju krumpira u općini Otočac (9%). Voditeljica LAG-a Lika navodi zabrinjavajuće trendove smanjenja proizvodnje sira koji proizlaze iz opadanja govedarstva mliječnog tipa koje ustupa pred rastućim govedarstvom u sustavu krava-tele. Unatoč povećanom zanimanju poljoprivrednika za preradu vlastitih poljoprivrednih proizvoda, broj registriranih prerađivača još je uvijek premalen u odnosu na potencijale područja. Uzrok tome je nesklonost malih poljoprivrednika za dodatne obaveze, pravne, financijske i tehnološke, koje podrazumijeva registracija njihovih OPG-ova za preradu poljoprivrednih proizvoda.

Napomena

Ovaj rad nastao je temeljem diplomskog rada Matee Đapić: Struktura standardnog ekonomskog rezultata i poljoprivrednog dohotka jedinica lokalne samouprave Ličko-senjske županije, obranjenog 13.7.2022. na Sveučilištu u Zagrebu Agronomski fakultet pod mentorstvom izv.prof.dr.sc. Larija Hadelana.

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Agricultural and food products of Lika-Senj County in the aggregated agricultural income

Abstract

The aim of the paper is to estimate the state of agriculture in the Lika-Senj County and to determine the most important agricultural and food products by calculating their share in aggregated agricultural income. According to research results, the most income-generating activities of the Lika-Senj County are sheep farming, cattle breeding in the cow-calf system, production of potatoes, plums and honey. In an interview with the head of LAG Lika, the stated results were confirmed and the wish of local farmers to increase agricultural income by processing products on their own farms was emphasized. Due to administrative obstacles and tax obligations, the number of registered processors is still relatively small compared to the possibilities.

Keywords: Agriculture, Lika-Senj County, traditional products, aggregated agricultural income

Ponuda zdravstvenog turizama u Osječko-baranjskoj županiji

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Sažetak

Zdravstveni turizam posljednjih godina eksponencijalno raste i postaje sve relevantniji za mnoge turističke destinacije. Promjene u životnim stilovima, zdravstvene politike, briga za zdravlje, trendovi i prakse protiv starenja povećali su interes za ovaj brzorastući segment. Cilj rada je utvrditi postojeće stanje zdravstvenog turizma s posebnim naglaskom na medicinski turizam u Osječko-baranjskoj županiji. Rezultati istraživanja sugeriraju na značajne potencijale u smislu ključnih čimbenika uspjeha, a koji se lociraju u širokoj lepezi usluga i pružatelja zdravstveno-turističkih proizvoda udruženih u klaster „Pannonian Health". No, kako bi se ostvario puni potencijal te kako bi Osječko-baranjska županija bolje iskoristila prilike i odgovorila na izazove ovog novog, globalnog i promjenjivog oblika selektivnog turizma, potrebno je sustavno raditi na promociji i projekte uskladiti s budućim nacionalnim strategijama, planovima i programima.

Ključne riječi: zdravstveni turizam, medicinski turizam, zdravstveno-turistički proizvod, klaster „Pannonian Health", Osječko-baranjska županija

Uvod

Zdravstveni turizam postaje brzorastući trend kako u svijetu, tako i u Hrvatskoj. No, unatoč rastućem društvenom, gospodarskom i političkom interesu za globalno tržište zdravstvenog i medicinskog turizma, ono ostaje još uvijek relativno neistraženo u stručnoj literaturi. Zdravstveni turizam je složeni turistički proizvod koji obuhvaća velik broj specijaliziranih sadržaja i usluga na putovanjima motiviranim potrebom za unapređenjem zdravlja i poboljšanjem kvalitete života (Telišman-Košuta i Marušić, 2018:4). Svjetska turistička organizacija i europska komisije za putovanja (2018) medicinski turizam definiraju kao one vrste turizma kojima je primarna motivacija doprinos tjelesnom, mentalnom i/ili duhovnom zdravlju kroz medicinske i wellness aktivnosti. Zdravstveni turizam može se i definirati kao privremena promjena stalnog boravka ljudi i odlazak u povoljno klimatsko ili kupališno mjesto radi zdravstvene preventive, kurative i rehabilitacije (Vukonić i Čavlek, 2001). Miličević (2013) govori kako zdravstveni turizam danas podrazumijeva pružanje zdravstvenih usluga koje se mogu kretati u rasponu od najsloženijih kirurških intervencija u specijaliziranim zdravstvenim ustanovama, preko tradicionalnih lječilišnih tretmana u lječilištima i toplicama pa sve do raznovrsne spa i wellness ponude u cilju pomlađivanja odnosno putovanja radi duhovnog uzdizanja. Općeprihvaćena definicija Svjetske turističke organizacije navodi da je zdravstveni turizam složena gospodarska aktivnost u kojoj bitno mjesto zauzima stručno i kontrolirano korištenje prirodnih ljekovitih činitelja, postupaka fizikalne medicine i programiranih fizičkih aktivnosti u svrhu održavanja i unapređenja fizičkog, mentalnog i duhovnog zdravlja turista te poboljšanja kvalitete njihovog života (UNWTO, 2018). Prema izvješću Europske komisije za putovanja, medicinski turizam posebno je potaknut preopterećenim zdravstvenim sustavima. Naime, posljednjih nekoliko desetljeća rashodi za dugotrajnu skrb porasli su u mnogim gospodarstvima diljem svijeta, posebice sa starenjem stanovništva koje zahtijeva sve više zdravstvene i socijalne skrbi (European Travel Commission, 2022). U Strategiji razvoja turizma Republike Hrvatske do 2020. godine te Nacionalnom programu – akcijski plan za razvoj zdravstvenog turizma prepoznaju se oblici zdravstvenog turizma kao kompleksnog turističkog proizvoda i to: wellness (odvija se pretežito u hotelima i lječilištima), lječilišni (odvija se u specijalnim bolnicama i lječilištima) i medicinski turizam (odvija se medicinskim ordinacijama, privatnim klinikama/poliklinikama). Svi navedeni selektivni oblici usmjereni su na poboljšanje fizičkog stanja pojedinca (turista), ali i korištenja svih medicinskih usluga za održavanje

zdravlja. Također, ovi oblici nalaze se na dvije paralele – „bolest-zdravlje-wellness" i „liječenje-prevencija-promocija". Medicinski turizam zbirni je pojam koji obuhvaća putovanja primarno motivirana korištenjem medicinske usluge (većeg ili manjeg stupnja složenosti) kao što su primjerice kirurški zahvati te stomatološke, kozmetičke, rehabilitacijske i druge alternativne usluge. Medicinski turizam dakle, kao važan oblik zdravstvenog turizma, može se definirati kao dio gospodarskog prosperiteta posjećene zemlje, pružanje zdravstvenih usluga zajedno s turističkim kulturnim i tradicionalnim baštinama (Bookman, 2007). Medicinski turizam i u hrvatskim okvirima mogao bi biti odgovor na produljenje kratke turističke sezone i razvijati se izvan granica povezanih s turizmom temeljenog na konceptu "sunce i more". Evidentno je kako turistički sektor u Hrvatskoj značajno doprinosi BDP-u. U 2019. taj je udio iznosio 24,3%, a prognoza za 2028. godinu iznosi 31,7%. No, u 2020. uslijed pandemije COVID-19 taj se udio smanjio na 10,2 % cjelokupnog gospodarstva, što uzrokuje ozbiljan pad ukupnog broja doprinosu turizmu i turističkom zapošljavanju (-15,6%). Iako se turistički sektor poboljšao tijekom godina, još uvijek nedostaju različiti turistički proizvodi poput zdravstvenog turizma, uključujući medicinski turizam (Barač Miftarević, 2022:123).

Materijal i metode

Metodologija istraživanja u ovom radu oslanja se na dosadašnje teorijske spoznaje o zdravstvenom turizmu i polazi od hipoteze da pružanje zdravstvenih usluga zajedno s postojećom turističkom kulturnom i tradicionalnom baštinom doprinosi brendiranju i gospodarskom rastu županije. Na temelju prikupljenih sekundarnih podataka i pregleda literature u radu su se koristile metode analize, sinteze i komparacije različitih oblika zdravstvenog turizma. U interpretaciji rezultata primijenjene su znanstvene metode deskripcije, dedukcije i analize.

Rezultati i rasprava

Danas, zdravlje je postalo široko prihvaćeno kao potrošačko dobro i smatra se dijelom opće kvalitete života. Nadalje, povećani troškovi zdravstvene skrbi na zapadu i globalizacija naveli su pojedince da zdravstvenu uslugu potraže u drugim zemljama koje nude kvalitetu usluge i pristupačne cijene (Omay i Cenzig, 2013, Krajnović i suradnici, 2013). Putovanje, kao element liječenja i oporavka, jedan je od najvažnijih ciljeva zdravstvenog turizma te neki autori smatraju kako će se tijekom putovanja zbog liječenja (ili oporavka) povećati aktivnosti u slobodno vrijeme ovisno o zdravstvenom stanju osobe. Budući da se trenutno financijsko stanje i životni problemi navode kao glavni čimbenici koji dovode do visoke razine životnog stresa, zdravstveni turizam je izbor za sve one osobe koji odluče izbjegavati svakodnevni stresni život, posvećujući više brige svom zdravlju i dobrobiti života općenito (Hofer i sur, 2012). Zdravstveni turizam veže se za putovanja u lječilišta ili druge destinacije. Njegova primarna svrha odnosi se na poboljšanje fizičkog stanja pojedinca kroz kontrolirano fizičko vježbanje, terapije, nadzor i kontrolu prehrambenih navika i medicinskih usluga u cilju poboljšanja i održavanja zdravlja. U Nacionalnom programu – akcijski plan za razvoj zdravstvenog turizma (2014) apostrofira se na obilježja postojeće prakse i tržišne trendove, a koji upućuju na četiri glavne domene ključnih činitelja uspjeha u zdravstvenom turizmu. Ključni faktori uspjeha prikazani su u Tablici 1.

Tablica 1. Ključni faktori uspjeha u zdravstvenom turizmu (Ministarstvo turizma, 2014:27)

Usluge	Razina cijena
	Kvalifikacije i iskustvo liječnika i stručnog osoblja
	Mogućnost komunikacije s liječnikom i osobljem na stranom jeziku Mogućnost konzultacije i detaljno objašnjenje zahvata prije dolaska
	Transparentna informacija o sadržaju kupljenih usluga
	Postojanje jasnih procedura u slučaju komplikacija
	Opći odnos prema pacijentu i pratitelju
Obilježja institucije/centra	Akreditacija i certifikacija, ovisno o potražnji prosuditi o međunarodnim ili domaćim certifikatima
	Ugled institucije/centra
	Osiguranje institucije u slučaju neželjenog ishoda zahvata/tretmana
	Visoki higijenski standardi u funkciji sprečavanja bolničkih infekcija
	Kontinuirano osuvremenjivanje opreme
	Smirujući i opuštajući ambijenti prostora, uključeni elementi lokalnog identiteta
	Okolišno odgovorna praksa
Obilježja destinacije	Poželjan imidž destinacije
	Dostupnost destinacije, uključujući prometne veze, ulaz u zemlju
	Dokazana i priznata ljekovita svojstva prirodnih činitelja
	Raspoloživost kvalitetnih kapaciteta i stručnog osoblja za rekuperaciju nakon zahvata
	Raspoloživost kvalitetnog, cjenovno prihvatljivog smještaja za pratitelje – Raspoloživost institucija primarne zdravstvene zaštite u destinaciji ili u neposrednoj blizini
	Raspoloživost raznovrsnih kulturnih, sportsko-rekreacijskih i zabavnih sadržaja
	Razvojna koncepcija destinacije, odnosno nepostojanje konfliktnih (zagađivačkih) djelatnosti u bližem okruženju
	Komunalna uređenost, razvijeni infrastrukturni sustavi, pješačke zone
	Okolišno odgovorna praksa
	Dostupnost besplatnog WIFI-a
Promocija i prodaja	Raspoloživost incoming turističkih agencija i facilitatora u medicinskom turizmu
	Mogućnost organizacije i plaćanja zdravstvenog i turističkog dijela boravka na jednom mjestu ('one-stop-shop')
	Atraktivne, informativne i ažurne Internet stranice destinacije i zdravstveno-turističkih centara
	Efikasna nacionalna promocija

Zdravstveni turizam bilježi sve veću ekspanziju, širi lepezu zdravstvene ponude od rekreativnih oblika wellness turizma do lječilišnog i bolničkog turizma, odnosno zdravstvenih ustanova koje pružaju zdravstveno-turističke usluge. Tome u prilog svjedoči činjenica da više od 10% klijenata putuje na različite zdravstvene tretmane u inozemstvo (Geić i sur., 2010). U prvih sedam mjeseci 2022. godine u Osječko-baranjskoj županiji zabilježeno je 58.291 noćenja što županiju pozicionira u sam vrh kontinentalnog turizma (HTZ, 2022). No, iako Osječko-baranjska županija generira respektabilne prihode od ostvarenih noćenja trenutno ne postoje standardi statističkog praćenja medicinskog turizma. Zdravstveni turizam pronalazi svoje mjesto u turističkoj ponudi Osječko-baranjske županije prije svega kroz tradicionalni oblik lječilišnog turizma (lječilište Bizovačke toplice) no prati i druge oblike – medicinski turizam, wellness i fitness. Iako se dosadašnji turistički razvitak OBŽ-a temeljio na valorizaciji prirodnih resursa, tradiciji, kulturno-povijesnim sadržajima, gastonomiji i zadovoljavajućem kapacitetu turističko-ugostiteljskih

objekata evidentno je da ima potencijala i za razvoj medicinskog turizma. U Tablici 2. prikazane su usluge i pružatelji usluga zdravstvenog turizma u Osječko-baranjskoj županiji.

Tablica 2. Usluge, oblici i pružatelji usluga zdravstvenog turizma u OBŽ (izrada autora)

PRUŽATELJ USLUGE	OBLIK ZDRAVSTVENOG TURIZMA	USLUGE
Poliklinika Lege Artis	Medicinski turizam	Ortopedija kirurgija, estetska kirurgija, opća kirurgija, laserska tehnologija
Poliklinika Slavonija	Medicinski turizam	Radiološka i laboratorijska dijagnostika, barokomora, estetska kirurgija
Poliklinika Rosso	Medicinski turizam	Estetska kirurgija, plastična kirurgija glave i vrata, ginekološka dijagnostika
Poliklinika Novoselec	Medicinski turizam	Fizikalna i manualna terapija, medicinska gimnastika
Poliklinika Osijek za internu medicinu, oftalmologiju i neurologiju	Medicinski turizam	Internistički i neurološki pregledi, oftalmologija, ultrazvučna dijagnostika, estetika
Poliklinika Sunce, Specijalna bolnica Agram u Osijeku	Medicinski turizam	Estetska kirurgija, mali operativni zahvati, intervencijska radiologija
Oftamološka poliklinika Dr. Balog	Medicinski turizam	Oftalmološka kirurgija, estetska kirurgija
Centar prirodnog zdravlja Organicos	Wellness	Holistički „tijelo-um-duh“ program, program učenja o zdravoj prehrani
Specijalistička dermatološka ordinacija Dr. Ageel	Medicinski turizam	Dermatološka i estetska kirurgija
Dental centar Čes	Medicinski turizam	Protetika, stomatologija, oralna kirurgija
Ordinacija dentalne medicine Saša Đukić	Medicinski turizam	Implantologija, estetska dentalna medicina
Park prirode Kopači Rit	Wellness	Holistički wellness, obiteljski spa ceentri, zdrava hrana seoskog domaćinstva, terapeutski učinak
Ordinacija dentalne medicine Siber	Medicinski turizam	Paradontologija, estetska stomatologija
Fizioterapeutske usluge i masaža Physio AT	Wellness	Medicinski wellness, masaže, tretmani tijela
Fizioterapeutske usluge i masaža Studio Vitalis	Wellness	Masaža, elektroterapije
Eccentric centar za dijagnostiku, terapiju i trening	Wellness	Holistički programi, fizički izazovi, učenje o zdravom životu
Lječilište Bizovačke toplice	Lječilišni turizam	Medicinska rehabilitacija, fizikalna terapija, lječilišne usluge

Zdravstveni turizam Osječko-baranjske županije temelji se na ponudi medicinskih i ostalih usluga koje svojom kvalitetom, stručnim i educiranim osobljem i cijenom mogu konkurirati europskim turističkim zdravstvenim destinacijama. Cilj rada bio je istražiti postojeću ponudu zdravstvenog turizma odnosno usluge, oblike i pružatelje zdravstveno-turističkih proizvoda udruženih u klaster „Pannonian Health" (iako na području županije egzistira još nekolicina privatnih poliklinika i ordinacija, one nisu udružene u klaster te stoga nisu uzete u uzorak). Provedenim istraživanjem, utvrđeno je kako na području Osječko-baranjske županije djeluje jedanaest privatnih poliklinika i ordinacija koje pružaju medicinske usluge, usluge estetske kirurgije te široku lepezu stomatoloških usluga. Nadalje, pet pružetelja usluga profiliralo se u domeni wellness turizma koji kupcima zdravstveno-turističkih usluga nude holističke programe, wellness tretmane/programe, masaže, učenje o zdravom životu. U sferi lječilišnog turizma predvodnik je lječilište Bizovačke toplice koje svojom dugom tradicijom i termalnim izvorom već dugi niz godina pruža usluge iz područja fizikalne medicine, rehabilitacije, lječilišnih usluga i u novije vrijeme robotske neurorehabilitacije. Osim lječilišta, svi pružatelji usluga koncentrirani su u gradu Osijeku, središtu i nositelju gospodarskih aktivnosti županije. Kao što je prethodno spomenuto, navedene zdravstvene ustanove udružene su u klaster „Pannonian Health" koji egzistira od 2018. godine, a primarni cilj usmjeren je na razvoj i promociju grada Osijeka i Osječko-baranjske županije kao nove destinacije zdravstvenog turizma na karti Europe. Aktivnosti klastera usmjerene su na unaprjeđenje zdravstvene zaštite ljudi i kvalitete života općenito te unaprjeđenje cjelokupne ponude i usluga u turizmu, promicanje tradicionalnih vrijednosti u cilju brendiranja regije (HTZ, 2018). Osim postojećih kapaciteta, perspektive medicinskog turizma svakako treba promatrati i u kontekstu popratnih sadržaja (kulture i tradicije) kao „odmorišnih elemenata". Osječka Tvrđa, park prirode i najvrjedniji zoološki rezervat u Hrvatskoj - Kopački Rit, Ergela lipicanaca u Đakovu, biciklistička staza kroz Baranju, bogata gastronomska i enološka ponuda te sada već prepoznatljive manifestacije poput Đakovačkih vezova, Pannonian Challenga i brojnih drugih. Na domaćem tržištu Osječko-baranjska županija sve je više prepoznata kao destinacija kvalitetne i raznolike ponude. S druge strane, Turistička zajednica OBŽ-a apostrofira da na stranim emitivnim tržištima treba napraviti veće iskorake prvenstveno kroz veća ulaganja u promotivne aktivnosti. Promocijske napore treba usmjeriti prema susjednim zemljama poput Srbije, Bosne i Hercegovine te Mađarske (Turistička zajednica Osječko-baranjske županije, 2022). Konačno, napravljen je još jedan važan iskorak u smislu komuniciranja i promocije jedinstvenog imidža destinacije u okviru platforme HeadOnEast Croatia/Visit Slavonia Baranja.

Zaključak

Zdravstveni turizam nametnuo se kao odgovor na potrebe i izazove s kojima s pojedinac danas, u smislu zdravlja i dobrobiti života općenito, suočava. U najširem smislu zdravstveni turizam predstavlja putovanje pojedinca u druge zemlje/ u cilju traženja medicinske skrbi, a doprinosi tjelesnom, mentalnom i/ili duhovnom zdravlju. Locira se u širokoj lepezi, od oblika wellness turizma do lječilišnog turizma, odnosno medicinskih usluga koje pružaju zdravstvene ustanove (medicinske, stomatološke, estetske i druge usluge). Destinacije u nastajanju sve više iskorištavaju ovaj tržišni segment u potrazi za diverzifikacijom, povećanjem prihoda te rješavanjem sezonalnosti. Imajući u vidu ključne čimbenike za razvoj zdravstvenog turizma (usluge, obilježja institucije, obilježja destinacije, promocija), ali i dosadašnju turističku ponudu, evidentno je da Osječko-baranjska županija ima značajne potencijale i za razvoj ovog segmenta turizma. Provedeno istraživanje pokazuje da u gradu Osijeku i šire trenutno djeluje sedemnaest zdravstvenih ustanova (lječilište Bizovačke toplice, privatne ordinacije, poliklinike, centri specijalizirani za usluge rehabilitacije, fizioterapije te holistički pristup). Navedene ustanove od 2018. godine djeluju kroz klaster „Pannonian Health" sa zajedničkim ciljem brendiranja i promocije županije kao prepoznatljive turističke destinacije zdravstvenog turizma na karti Europe. Aktivnosti klastera koreliraju sa zajedničkim europskim ciljevima zacrtanim u strateškim dokumentima, a koji su usmjereni na unaprjeđenje zdravstvene zaštite ljudi i kvalitete života općenito. Iako su napravljeni određeni pomaci u smislu komuniciranja i promocije jedinstvenog imidža destinacije u okviru platforme HeadOnEast Croatia/Visit Slavonia Baranja i kroz ulaganja u ICT rješenja upravljanja destinacijom, potrebno je i dalje sustavno raditi na promociji i vidljivosti te projekte uskladiti s nacionalnim strategijama, planovima i programima. Konačno, kroz sinergiju svih raspoloživih resursa, zdravstveni turizam može biti pokretač gospodarskog rasta i razvoja te poluga zapošljavanja ovog dijela Hrvatske.

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Health tourism offer in Osijek-Baranja County

Abstract

Health tourism has been growing exponentially in recent years and is becoming increasingly relevant for many tourist destinations. Changes in lifestyles, health policies, health care, anti-aging trends and practices have increased interest in this fast-growing segment. The aim of the work is to determine the current state of health tourism with special emphasis on medical tourism in Osijek-Baranja County. The results of the research suggest significant potential in terms of key success factors, which are located in a wide range of services and providers of health-tourism products united in the "Pannonian Health" cluster. However, in order to realize its full potential and for the Osijek-Baranja County to make better use of opportunities and respond to the challenges of this new, global and changing market segment, it is necessary to systematically work on promotion and align projects with future national strategies, plans and programs.

Keywords: health tourism, medical tourism, health-tourism product, "Pannonian Health" cluster, Osijek-Baranja County

Stavovi ispitanika prema tradicionalnoj obradi mesa u Slavoniji i Baranji

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Sažetak

U Slavoniji i Baranji tradicionalna obrada mesa (kolinje) ne predstavlja samo potrebu za preživljavanjem kroz konzumaciju mesne hrane bogate mastima i proteinima u zimskim mjesecima. Ono tradicionalno, a i danas, predstavlja povezivanje ljudi, očuvanje običaja, starih zanata prerade mesa kroz neke nove prakse, povratak selu kao sinonimu djetinjstva i zdrave hrane. U cilju utvrđivanja u kojoj se mjeri i dalje održava tradicija kolinja u Slavoniji i Baranji te identificiranja stavova o tradicionalnim načinima obrade svinjskog mesa (kolinje), provedeno je *online* anketno istraživanje na uzorku od 889 ispitanika s područja Osječko-baranjske županije. Istraživanjem su utvrđene statistički značajne razlike u stavovima ispitanika vezanim za tvrdnje kolinje doprinosi očuvanju tradicije, kulture i običaja Slavonije i Baranje ($p < 0,01$) i kolinje doprinosi povezivanju ruralnog stanovništva ($p < 0,01$) u odnosu na spol ispitanika i tvrdnji kolinje doprinosi deruralizaciji ($p < 0,05$) i danas je uzgoj svinja skuplji, te samim time i obrada mesa ($p < 0,01$) u odnosu na ekonomski status obitelji ispitanika.

Ključne riječi: tradicija, kolinje, svinjsko meso, Osječko-baranjska županija

Uvod

Osječko-baranjska županija, općenito je poznata kao nizinski kraj u kojem prevladavaju poljoprivreda i prehrambena industrija. Istočna Hrvatska obiluje raznim tradicionalnim proizvodima od kojih se posebno ističu svinjski suhomesnati proizvodi. Tradicionalni proizvodi čine kulturu, identitet i nasljeđe jedne zemlje (Karabasil i sur., 2018.; Lukač, 2021.). Tradicija u proizvodnji mesa i suhomesnatih proizvoda duboko je ukorijenjena na Balkanu, a odvija se unutar manjih obiteljskih gospodarstva koja imaju važnu ulogu unutar lokalne i regionalne zajednice (Karabasil i sur., 2018.; Kumalić, 2018.). Tradicionalna obrada mesa ili kolinje važna je kulturna komponenta društvenog života, jer stvara i jača solidarnost u zajednici te doprinosi procesu socijalizacije koji se prenosi društvenim normama i ritualima te pomaže u jačanju neformalnih društvenih mreža i pridonosi promicanju društvene kohezije (Žmegeč, 1998.; Kumalić, 2018.). Tradicionalno kolinje široko je rasprostranjena društvena praksa na zapadnom Balkanu koja okuplja obitelji, susjede i prijatelje te pridonosi porastu društvenog kapitala i ima važan kulturni, društveni i ekonomski utjecaj na obitelji i zajednice (Kumalić, 2018.). Autor nadalje navodi da se u Bosni i Hercegovini tradicionalno klanje životinja kod kuće uglavnom prakticira kao sezonsko klanje u kršćanskim zajednicama i kao vjersko klanje u muslimanskim zajednicama. U istraživanju koje je proveo Kumalić (2018.) ispitanici su istaknuli važnost tradicionalnog kolinja za jačanje društvenog kapitala jer je to prigoda za obiteljsko i šire društveno okupljanje kao i podjelu mesa nakon kolinja kojom se naglašava socijalna osjetljivost (prilikom kolinja od strane kršćanskih i muslimanskih zajednica) doprinoseći jačanju susjedskih, prijateljskih i obiteljskih odnosa.

Cilj ovoga rada bio je ispitati stavove ispitanika o tradicionalnim načinima obrade svinjskog mesa (kolinja) i istražiti u kojoj mjeri se i dalje održava tradicija kolinja u Slavoniji i Baranji.

Materijal i metode

Primarni podatci prikupljeni su putem metode ankete, a kao instrument istraživanja korišten je *online* anketni upitnik. Istraživanje o preferencijama sudionici itraživanja provedeno je u razdoblju od prosinca 2021. do siječnja

2022. putem *online* anketnog softvera tvrtke Qualtrics LLC. Anketni upitnik ispunilo je ukupno 1.049 ispitanika s područja Osječko-baranjske županije. Od ukupnog broja ispitanika iz daljnje analize isključeno je 160 ispitanika zbog nepotpuno ispunjenog anketnog upitnika. Cjelokupnim anketnim upitnikom istražuju se potrošačke preferencije potrošača u Osječko-baranjskoj županiji prema svježem mesu i suhomesnatim proizvodima te stavovi ispitanika o tradicionalnoj obradi mesa (kolinju) u Slavoniji i Baranji. Cjelokupni upitnik sadržavao je ukupno 38 pitanja otvorenog i zatvorenog tipa podijeljenih u nekoliko skupina, a na kraju anketnog upitnika dodana su pitanja koja se odnose na sociodemografska obilježja ispitanika. U radu u prikazani samo dijelovi vezani za stavove ispitanika o tradicionalnoj obradi mesa u Slavoniji i Baranji (mjereno korištenjem ljestvice slaganja s pet modaliteta odgovora: 1 – uopće se ne slažem, 5 – u potpunosti se slažem). Analiza podataka provedena je u statističkom programskom paketu IBM SPSS Statistic V26. U analizi podataka korištene su metode deskriptivne statistike (postotci, frekvencija, aritmetička sredina, standardna devijacija) kako bi se opisao uzorak, od neparametrijskih testova korišten je hi - kvadrat test ($\chi 2$) kojim se željelo utvrditi postoji li razlika između promatranih frekvencija i očekivanih frekvencija pitanja vezanog uz tradicionalni način obrade svinjskog mesa (obavljate li u vašem domaćinstvu kolinje ili idete kod rodbine/prijatelja na kolinje?) s obzirom na njihova sociodemografska obilježja (dob, mjesto stanovanja, razina obrazovanja, zanimanje i ekonomski status ispitanika). Od parametrijskih testova korišten je Studentov t – test (independent t- test) i jednosmjerna analiza varijance (oneway ANOVA) u svrhu utvrđivanja razlika u stavovima ispitanika vezano za tradicionalni način obrade svinjskog mesa (kolinje).

Rezultati i rasprava

Ciljana skupina ispitanika bili su ispitanici koji konzumiraju svježe svinjsko meso i suhomesnate proizvode s područja Osječko-baranjske županije. Od ukupnog broja ispitanika (n = 889), njih 66,6 % bilo je ženskoga, a 33,4 % muškoga spola. Većina ispitanika dolazi sa seoskog područja (43,9 %) i iz gradskih naselja (43,6 %), a svega 12,5 % iz prigradskih naselja. U istraživanju većinom prevladavaju studenti (55,9 %) i zaposleni (34,4 %) ispitanici između 18 – 34 godine starosti (84,6 %). Ispitanici su se u većoj mjeri izjasnili da imaju završenu srednju školu (36,9 %) i visoku školu ili stručni studij (33,4 %) te da je njihov ekonomski status obitelji oko prosjeka (60,0 %). 71,9 % (n = 639) ispitanika se izjasnilo da obavlja kolinje u svom domaćinstvu ili ide na kolinje kod rodbine ili prijatelja, a svega 28,1 % (n = 250) ispitanika se izjasnilo da ne radi i ne ide kod rodbine ili prijatelja na kolinje. Prema podatcima prikazanim u Tablici 1. rezultati hi-kvadrat testa pokazali su da postoji statistički značajna povezanost između sudionika istraživaja koji poštuju narodni običaj (kolinje) i dobi ispitanika ($\chi 2 = 40{,}712$, $p = 0{,}01$), mjesta stanovanja ($\chi 2 = 114{,}899$, $p < 0{,}01$) razine obrazovanja ($\chi 2 = 40{,}712$, $p < 0{,}05$) i zanimanja ispitanika ($\chi 2 = 114{,}899$, $p < 0{,}01$). Sudionici istraživanja su zatim izrazili svoje mišljenje na sljedećih šest tvrdnji: kolinje doprinosi očuvanju tradicije, kulture i običaja Slavonije i Baranje (T1), kolinje doprinosi deruralizaciji (T2), kolinje doprinosi povezivanju ruralnog stanovništva (T3), danas je uzgoj svinja skuplji, te samim time i obrada mesa (T4), smatram da je važno znati mjesto i način proizvodnje svinjskog mesa i suhomesnatih proizvoda (T5) i lokalno proizvedena hrana potiče održivost poljoprivrednih gospodarstava i podupre lokalne uzgajivače (T6). Na temelju odgovora ispitanika izračunate su aritmetičke sredine (M) i standardna devijacija (SD).

Tablica 1. Distribucija odgovora s obzirom na pitanje vezano uz obavljanje tradicionalnog načina obrade svinjskog mesa ovisno o socio-demografskim karakteristikama ispitanika (%)

Socio-demografska obilježja		N	Obavljate li u vašem domaćinstvu kolinje ili idete kod rodbine/prijatelja na kolinje?		χ2	p
			Da	Ne		
Dob	18-24	501	61,2	44,0	40,712	0,000**
	25-34	251	27,1	31,2		
	35-44	75	7,5	10,8		
	45-54	34	2,8	6,4		
	55+	28	1,4	7,6		
Mjesto stanovanja	Gradsko	388	34,1	68,0	114,899	0,000**
	Prigradsko	111	11,00	16,4		
	Seosko	390	54,9	15,6		
Razina obrazovanja	Osnovna škola	7	0,5	1,6	9,505	0,023*
	Srednja škola	328	38,7	32,4		
	Viša škola/Stručni studij	297	34,3	31,2		
	Magisterij/Doktorat	257	26,6	34,8		
Zanimanje	Student	497	61,7	41,2	33,559	0,000**
	Zaposlen	306	30,0	45,6		
	Nezaposlen	69	7,4	8,8		
	Umirovljenik	17	0,9	4,4		
Ekonomski status	Znatno ispod prosjeka	16	1,7	2,0	3,160	0,531
	Ispod prosjeka	71	8,6	6,4		
	Oko prosjeka	533	60,6	58,4		
	Iznad prosjeka	246	26,9	29,6		
	Znatno iznad prosjeka	23	2,2	3,6		

*** p<0,01; * p<0,05*

Ispitanici su se u najvećoj mjeri složili s tvrdnjama lokalno proizvedena hrana potiče održivost poljoprivrednih gospodarstava i podupre lokalne uzgajivače (T6) (M = 4,58, SD = 0,678), smatram da je važno znati mjesto i način proizvodnje svinjskog mesa i suhomesnatih proizvoda (T5) (M = 4,32, SD = 0,887) i kolinje doprinosi očuvanju tradicije, kulture i običaja Slavonije i Baranje (T1) (M = 4,27, SD = 0,963). Najmanji stupanj slaganja ispitanici su iskazali prema tvrdnji kolinje doprinosi deruralizaciji (iseljavanju sela) (M = 2,28; SD = 1,147). Prema istraživanju Lončarić i sur. (2015.) sudionici istraživanja smatraju tradicijske proizvode važnima za širu zajednicu, s posebnim naglaskom na njihovu ulogu u očuvanju kulture i baštine, kao i potrebu za većom prepoznatljivošću i ugledom u društvu. Stanovnici u Istočnoj Hrvatskoj imaju pozitivan stav prema tradicionalnim proizvodima svoje regije (Lončarić i sur., 2021.). Vandecandelaere (2013.) i Kumalić (2018.) nadalje ističu kako je razmjena hrane stara koliko i čovječanstvo te da se prodajom lokalne proizvedene hrane uspostavlja izravan kontakt između poljoprivrednika i potrošača te stvara povjerenje i međusobna korist među njima. Sudionici istraživanja u Hrvatskoj u istraživanju Jelić Milković (2022.) visokim ocjenama su ocijenili tvrdnje lokalno proizvedena hrana pomaže očuvanju tradicionalnih pasmina i načina proizvodnje (M = 4,22, SD = 0,821), lokalno proizvedena hrana je zdravija i prirodnija (slijedi izvorni postupak proizvodnje) (M = 3,95, SD = 0,898), preferiram kupnju lokalno proizvedene hrane iako je ponekad skuplja (M = 3,74, SD = 0,934). Što je potvrđeno i istraživanjem Januš i sur. (2022.) anketiranjem potrošača suhomesnatih proizvoda u Osječko-baranjskoj županiji. Podjelom potrošača u tri klastera autori su došli do zaključka da osim cijene, boje i načina proizvodnje suhomesnatih proizvoda ispitanicima je važno i porijeklo i da su suhomesnati

proizvodi proizvedeni s domaćom crvenom paprikom i češnjakom.

Kako bi utvrdili postoje li statistički značajne razlike u stavovima ispitanika prema tvrdnjama vezanim za tradicionalnu obradu mesa u Slavoniji i Baranji s obzirom na socioekonomske karakteristike ispitanika korišten je t – test za nezavisne uzorke i jednosmjerna analiza varijance (ANOVA) što je prikazano u Tablicama 2. i 3.. Prema rezultatima t – testa prikazanima u Tablici 2. vidljivo je da postoji statistički značajna veza između ponuđenih tvrdnji i spola ispitanika. Statistički značajna razlika uočena je kod tvrdnji kolinje doprinosi očuvanju tradicije, kulture i običaja Slavonije i Baranje (T1) ($t = 5,311$, $p < 0,01$) i tvrdnje kolinje doprinosi povezivanju ruralnog stanovništva (T3) ($t = 5,659$, $p < 0,01$). Ostale tvrdnje nisu se pokazale statistički značajne u odnosu na ponuđene tvrdnje. Prema srednjim vrijednostima prikazanim u Tablici 2. vidljivo je da se ispitanici muškog spola više slažu sa spomenutim tvrdnjama. Muški se ispitanici više slažu s tvrdnjama da je danas uzgoj svinja skuplji, a samim time i obrada mesa (T4) ($M = 4,02$, $SD = 0,539$), također smatraju da je važno znati mjesto i način proizvodnje svinjskog mesa i suhomesnatih proizvoda (T5) ($M = 4,34$, $SD = 0,925$) dok se ispitanici ženskog spola više slažu s tvrdnjom da lokalno proizvedena hrana potiče održivost poljoprivrednih gospodarstava i podupire lokalne uzgajivače (T6) ($M = 4,60$, $SD = 0,676$).

Tablica 2. Testiranje razlika aritmetičkih sredina tvrdnji s obzirom na spol ispitanika

	Muško		Žensko		t – test	p
	M	SD	M	SD		
T(1)	4,50	0,855	4,16	0,994	5,311	0,000**
T(2)	2,29	1,296	2,28	1,066	0,125	0,901
T(3)	3,97	0,970	3,57	1,007	5,659	0,000**
T(4)	4,02	0,953	3,91	0,874	1,687	0,092
T(5)	4,34	0,925	4,30	0,878	0,571	0,568
T(6)	4,56	0,681	4,60	0,676	-0,810	0,418

*** p<0,01; * p<0,05*

U Tablici 3. prikazani su rezultati testiranja aritmetičkih sredina tvrdnji s obzirom na ekonomski status obitelji te je vidljivo da ekonomski status obitelji ima utjecaj na mišljenje ispitanika po pojedinim tvrdnjama. Statistički značajna razlika uočena je kod tvrdnji kolinje doprinosi deruralizaciji (T2) ($F = 2,953$, $p < 0,05$) i danas je uzgoj svinja skuplji, te samim time i obrada mesa (T4) ($F = 3,663$, $p < 0,01$). Ispitanici koji su se izjasnili da im je ekonomski status obitelji znatno ispod prosjeka više se slažu s navedenim tvrdnjama. Dok se ispitanici čija su primanja znatno iznad prosjeka više slažu s tvrdnjom smatram da je važno znati mjesto i način proizvodnje svinjskog mesa i suhomesnatih proizvoda (T5) iako za ovu tvrdnju nije utvrđena statistički značajna razlika.

Tablica 3. Testiranje razlika aritmetičkih sredina s obzirom na ekonomski status obitelji

	Znatno ispod prosjeka		Ispod prosjeka		Oko prosjeka		Iznad prosjeka		Znatno iznad prosjeka		F	p
	M	SD	M	SD	M	SD	M	SD	M	SD		
T(1)	4,50	0,632	4,25	0,996	4,24	0,969	4,34	0,906	4,26	1,421	0,642	0,633
T(2)	3,06	1,652	2,24	1,075	2,27	1,106	2,23	1,132	2,74	1,738	2,953	0,019 *
T(3)	4,00	1,155	3,75	1,065	3,67	0,982	3,74	1,010	3,87	1,392	0,723	0,577
T(4)	4,50	0,730	3,82	1,060	3,93	0,873	3,93	0,896	4,43	0,992	3,663	0,006 **
T(5)	4,25	1,238	4,27	0,925	4,29	0,904	4,37	0,801	4,65	0,935	1,292	0,272
T(6)	4,81	0,403	4,61	0,665	4,56	0,698	4,61	0,627	4,61	0,891	0,752	0,557

*** p<0,01; * p<0,05*

Zaključak

Prema rezultatima ovog istraživanja, možemo zaključiti da su ispitanici svjesni značenja kolinja za ruralnu zajednicu te također prepoznaju važnost lokalno proizvedene hrane. Većina ispitanika (71,9 %) se izjasnila da obavlja kolinje u svom domaćinstvu ili ide na kolinje kod rodbine ili prijatelja. Istraživanjem je utvrđena statistički značajna povezanost između dobi, mjesta stanovanja, razine obrazovanja i zanimanja sudionika istraživanja koji poštuju narodni običaj (kolinje). Rezultati istraživanja pokazali su da se muški ispitanici više od ženskih slažu s tvrdnjama kolinje doprinosi očuvanju tradicije, kulture i običaja Slavonije i Baranje i tvrdnje kolinje doprinosi povezivanju ruralnog stanovništva. Međutim, ispitanici slabijeg ekonomskog statusa smatraju da kolinje doprinosi deruralizaciji sela te da je proizvodnja i obrada mesa skuplja.

Danas je kolinje i mesne prerađevine koje pri kolinju nastaju, svojevrstan brend visokovrijedna i prepoznatljiva delicija Slavonije i Baranje. Međutim, prema rezultatima istraživanja vidljivo je da kolinje doprinosi i povezivanju ruralnog stanovništva, očuvanju tradicije, kulture i običaja Slavonije i Baranje. Te se može zaključiti da kolinje u Slavoniji i Baranji ne služi isključivo kao priprema mesnih zaliha za zimu već je ima dublji značaj kada je u pitanju povezivanje ljudi, očuvanje običaja i stvaranje dodatne vrijednosti.

U vremenu sve većeg starenja i izumiranja ruralnih područja, pogotovo onih perifernih, ne samo u Republici Hrvatskoj, već i u cijeloj EU, zadržavanje stanovništva, poglavito onog mlađeg u ruralnim područjima i očuvanje tradicije i običaja postaje imperativom.

Napomena

Rad je proizašao iz diplomskog rada „Odnos potrošača prema tradiciji kolinja i kvaliteti suhomesnatih proizvoda" autorice Tea Januš, studentice diplomskog sveučilišnog studija „Agroekonomika" na Fakultetu agrobiotehničkih znanosti Osijek Sveučilišta Josipa Jurja Strossmayera u Osijeku.

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Respondents' attitudes towards traditional meat processing in Slavonia and Baranja

Abstract

In Slavonia and Baranja, the traditional processing of meat ("kolinje") has long ceased to be just a natural will to survive, eating meat dishes rich in fat and protein to get through the winter months. Today, every winter it stands for the connection of people, the preservation of customs, the old craft of meat processing through some new practises, the return to the village as a synonym for childhood and healthy food. In order to determine the extent to which the tradition of "kolinje" (backyard pig slaughter) is still maintained in Slavonia and Baranja, and to identify possible differences in respondents' attitudes towards the traditional way of processing pork ("kolinje") with regard to various socio-demographic variables, an online survey was conducted among a sample of 889 respondents from the area of Osijek-Baranja County. The survey revealed statistically significant differences in the respondents' attitudes with regard to the claims that "kolinje" contributes to the preservation of the traditions ($p < 0.01$), culture and customs of Slavonia and Baranja and that "kolinje" contributes to the socialisation of the rural population ($p < 0.01$), with regard to the gender of the respondents and with regard to the claims that "kolinje" contributes to de-ruralisation ($p < 0.05$) and that pig farming is more expensive today, and thus meat processing ($p < 0.01$) with regard to the economic status of the respondent's family.

Keywords: tradition, countybackyard pig slaughter ("kolinje"), pork meat, Osijek-Baranja County

Proposal of farm-level indicators for assessment of agricultural sustainability - case of Slovenia

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Abstract

Paper aims to present the outcomes and the process of compiling a set of indicators of sustainable agriculture in Slovenia at farm level. In the framework of two participatory workshops, a set of 29 topics and 90 indicators at farm level was proposed by all three aspects of sustainability (economic, environmental and social). Almost one fifth of proposed indicators can be based solely on FADN (Farm Accountancy Data Network) data, 18% on other existing farm-level databases and 22% on combined data sources. Around 41% of proposed indicators would need to be based on additional data (on-farm questions or measurements). Further prioritization of proposed set of topics and indicators is recommended. Synchronisation with the process of conversion of FADN to FSDN would enable abundant synergies, content and cost-wise.

Keywords: sustainable agriculture, indicators, agricultural holdings, FADN

Introduction

Sustainable agriculture is one of the key long-term priority goals of the European Union (EU), aiming at a higher quality of life. In addition to the economic function, agriculture has many other, socially and environmentally important functions, such as preservation of cultural landscape and cultural heritage, maintenance of ecosystem functions in the environment, preservation of biodiversity, protection of natural values, impact on social balance, etc. (Klemenčič et al., 2008). In the current strategic documents, Slovenia emphasizes sustainable agriculture, which, in addition to the basic function of food production, also provides public goods related to the protection of the environment and nature, food safety and balanced spatial development. (Rural Development Program..., 2019; Resolution MAFF, 2020; Public debate on ..., 2020).

Modern agricultural policy creation and decision making requires a reliable system of capturing real environmental, economic, social and other data. In the field of agri-food sector, Slovenia still does not have a unified, integrated database at various levels, which would enable systematic, effective monitoring and evaluation of sustainable orientation of farms and formulation of evidence-based sectoral development policies. Especially, there is a lack of sustainability data and indicators at farm level, despite the growing data needs.

Following the example of good practice from FLINT project (Vrolijk and Poppe, 2021) and activities of conversion and expansion of FADN microeconomic database with the environmental and social indicators to FSDN (Farm Sustainability Data Network), we propose the FADN as a starting point for the compilation of sustainability topics and farm-level indicators also for Slovenia. The aim of the paper is to present the key outcomes of this process.

The primary aim of the paper is to present the results and findings of the national project "Establishment of a reference agricultural holdings system for the purpose of permanent monitoring of indicators of sustainable agriculture".

Materials and methods

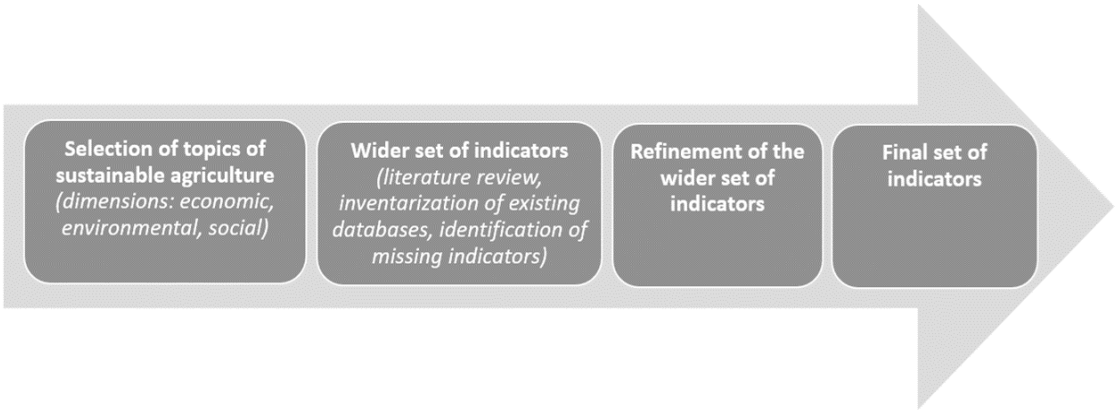
The proposal of farm level indicators is based on literature review and the outcomes of two participatory workshops which were held in Ljubljana, in February and September 2019, with the attendance of project team members, experts in specific analyzed agricultural sustainability topics.

For each individual proposed indicator of sustainable agriculture at farm level, a detailed methodology was prepared for the systematic collection of data, calculation of indicator and interpretation of the results (Bele, 2022). Missing

data for the proposed indicators, which are not collected within already existing databases, are proposed to be gathered with the additional questionarries and measurements on the reference agricultural holdings.

Results and discussion

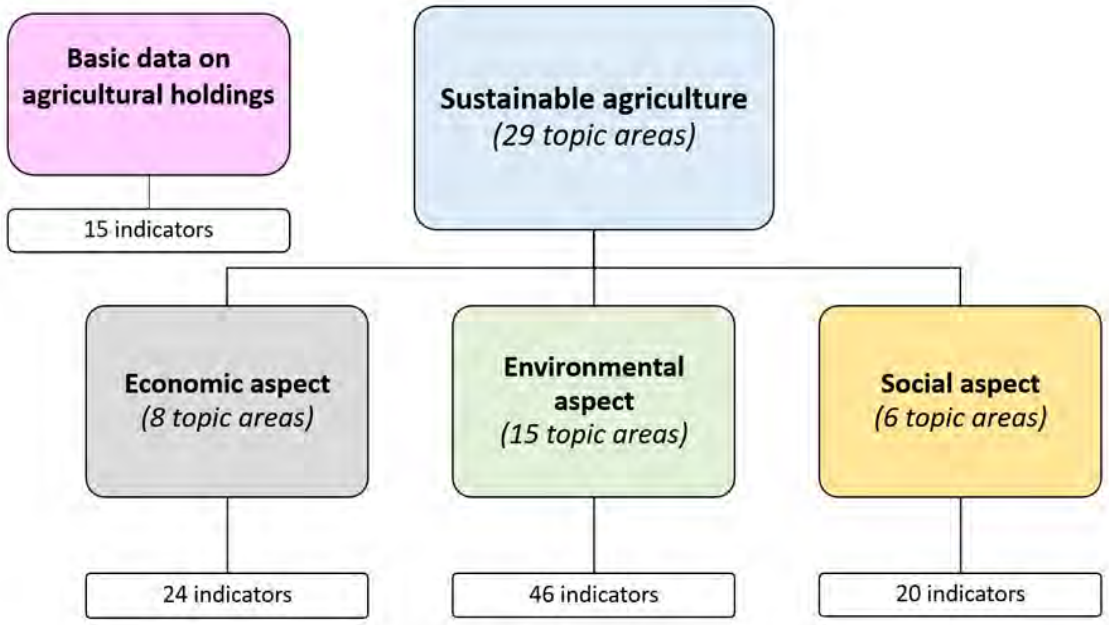
Two participatory workshops (Bertoncelj et al., 2022) were held to define the key topics of sustainable agriculture that could contribute to specific objectives of Slovenian agricultural policy (Resolution MAFF, 2020) and a set of indicators at the level of agricultural holdings. At the first workshop (Picture 1), based on the review of the FLINT outcomes (Kelly et al., 2015; Final publishable summary report, 2016; Vrolijk and Poppe, 2021), relevant literature, as well as based on the review of the available farm-level databases for the Slovenian agriculture, key topics and an initial (broader) set of indicators, considering by all three aspects of sustainability (economic, environmental and social) were proposed.



Picture 1: Methodological approach for the selection of indicators of sustainable agriculture at the level of agricultural holdings
Source: Own compilation

At the second workshop, the initial set of proposed topics and indicators was thoroughly reviewed in terms of their relevance for the Slovenian agricultural policy and feasibility of data collection (Bertoncelj et al., 2022). Some topics were merged, some were added or excluded, and the initial (broader) set of indicators was refined. After some additional refinements, a final (shorter) set of the farm-level indicators was proposed. Detailed description sheets were prepared for each agricultural sustainability topic and indicator. The topic descriptions include, among others, the relation and expected contribution of the individual topic to specific objectives of the Slovenian agricultural policy, while the indicator description sheets includes detailed methodology for their calculation and monitoring, as well as the required data sets at the farm level (Bele, 2022).

In total, 29 topics are proposed (Picture 2), covering all three aspects of sustainability (economic, environmental and social). The proposed set of topics and indicators is neither comprehensive nor definitive, and may be adapted depending on policy needs and the feasibility of the data collection at farm level. For the assessment of the economic aspect of sustainable agriculture, 8 topics and 24 indicators are proposed. Within the environmental aspect, 15 topics and 46 indicators are identified, while for the social aspect of sustainable agriculture, 6 topics and 20 indicators are proposed.



Picture 2. Number of topics and indicators according to the aspects of sustainable agriculture Source: Own calculations

Within the economic aspect of sustainable agriculture, the following topics are proposed: Farm income level, Farm income stability, Farm vitality and resilience, On- farm innovations, Protected agricultural products and foodstuffs, Marketing channels and producer groups, Parcel fragmentation of the farm, Farm modernization.

The environmental aspect of sustainable agriculture is described with the following topics: Permanent grassland, Sowing structure, Biodiversity, Use of plant protection products, Nutrient content and soil acidity, Indirect energy consumption, Direct energy consumption, Production of energy from renewable sources, Agricultural practices to reduce nutrient losses on the farm, Agricultural practices to reduce soil erosion, Increasing legume cultivation, Intensity of greenhouse gas emissions in agriculture, Greenhouse gas emissions per hectare of arable land, Irrigation of agricultural land, Soil carbon sinks.

The social aspect of sustainable agriculture is proposed in the following topics: Farm advisory services, Farm education and training, Farm ownership and management, Social inclusion and participation, Employment and working conditions, Quality of life.

In addition to the proposed set of indicators and topics of sustainable agriculture, it is proposed to collect descriptive set of data about agricultural holdings, which are important for the interpretation of the proposed farm-level indicators of sustainable agriculture. Descriptive dataset includes data about farm's production resources, production type, production intensity, farm owner/manager, and about agricultural household.

A descriptive sheet was created for each proposed topic of sustainable agriculture, and an indicator sheet was created for each proposed indicator within an individual topic. The descriptive sheet describes the relevance of each topic for the Slovenian agricultural policy and its contribution to achieving the policy goals. In addition, for each sustainability topic, individual indicators of sustainable agriculture at the farm level are proposed and additional information is defined (e.g. required and already available data for the creation of these indicators, methodology for their creation, etc.). As mentioned above, the indicator sheets present in more detail each of the proposed indicators and the methodology for their preparation and monitoring (Bele, 2022).

Indicator sheets are a tool for presenting, preparing, and monitoring individual indicators, and the structure of the indicator sheets is defined in advance to ensure quality and consistency as much as possible (USAID, 2016). Indicator sheets therefore contain essential information about the indicator, data required, the collection methods, indicator preparation/calculation methodology and the interpretation of the indicator results in the context of pursuing the goals defined for the selected indicator. To make it easier to understand the content of the individual indicator sheet,

the topic areas addressed by each proposed sustainable agriculture indicator at the farm level are described in more detail.

The structure of the indicator sheet for each indicator proposed for sustainable agriculture monitoring at the farm level is as follows (Bele, 2022):

1. General information about the indicator: author of the indicator, name and code of the indicator, aspect of sustainable agriculture (economic, environmental, social), specific topic area;
2. Groups of objectives and specific objectives of the agricultural policy: link to the objectives defined in the MAFF Resolution (2020);
3. Definition and explanation of the indicator: brief content (description) of the indicator and contribution to the defined specific objectives of sustainable agriculture in Slovenia at the level of agricultural holdings (Resolution MAFF, 2020);
4. Required/used data: indicating whether the data for the creation of the proposed indicator are already collected in existing databases, e.g. FADN or other already existing databases (e.g. administrative data).If the data exist in the FADN database, indication of whether they are accessible:
5. within the FADN standard results (RI/CC 1750, 2020) or
6. within the so-called FADN basic data (RI/CC 1680, 2021 and Regulation 2015/220, 2021).
7. In the case that the data required for the compilation of the proposed indicators are not collected in the already existing databases, it is necessary to define the questions/measurements that need to be additionally collected/performed at farms.
8. Calculation methodology:
9. Calculation of the indicator at the farm level;
10. Unit of measurement;
11. Administrator of the (existing) database;
12. Frequency of measurement: how many times per year, how many years;
13. Interpretation of the indicator: interpretation of the values/results for the proposed indicator at the farm level in the context of pursuing the defined goals of sustainable agriculture in Slovenia (Resolution MAFF, 2020).
14. Reference values, if available: e.g. comparison with statistical average for Slovenia, the European Union.
15. Resources.

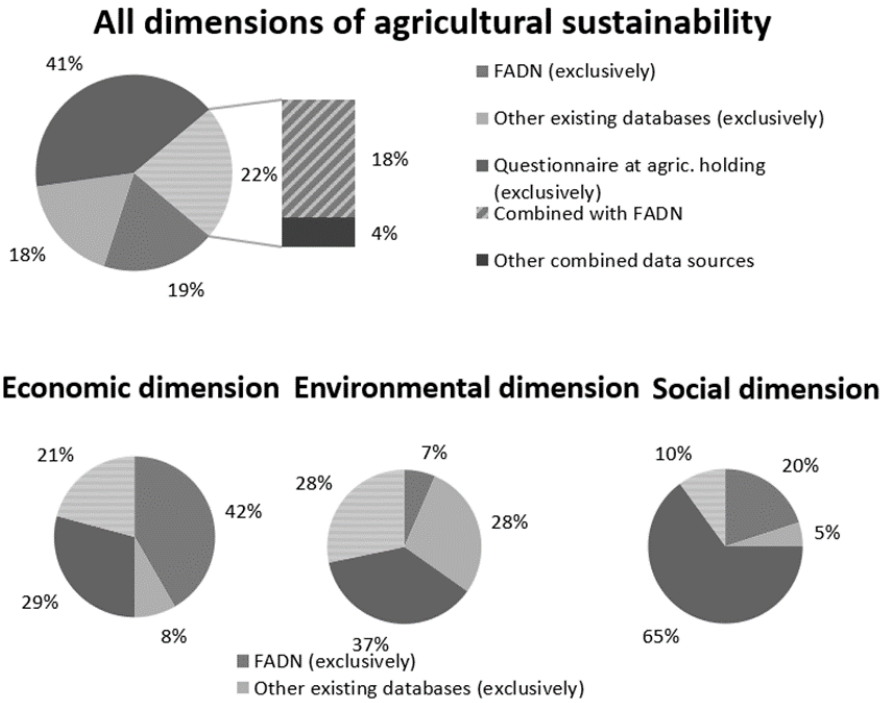
Table 1. Number of indicators according to the aspects of sustainable agriculture and the availability of data sources required for their preparation.

Availability of data	Economic aspect	Environmental aspect	Social aspect	Total
FADN database	10	3	4	17
Other (existing) databases	2	13	1	16
Questionnaire at agric. holding	7	17	13	37
Combined data sources	5	13	2	20
- Combined with FADN	4	11	1	16
- Other combined data sources	1	2	1	4
Total	24	46	20	90

Source: Own calculations

It is estimated that nearly one fifth of the proposed indicators can be prepared exclusively on the basis of FADN data, another 18% based solely on other existing databases (e.g., administrative databases), and 22% of the proposed

indicators based on combined data sources. About 41% of all proposed indicators should be prepared solely on data collected with additional on-farm questions/measurements on farms. Almost 18% of the proposed indicators could be prepared based on a combination of FADN data with other databases or with additional on-farm questions/measurements on selected agricultural holdings. FADN data, exclusively or in combination with other data sources, can thus be used to prepare only about 37% of all proposed indicators for monitoring sustainable agriculture.



Picture 3: Availability of data for indicators of sustainable agriculture: By dimensions and data sources
Source: Own calculations

The highest share of proposed sustainable agriculture indicators for which additional on-farm data would need to be collected is within the social aspect of sustainable agriculture (65%), and the lowest is within the economic aspect (29%). Within the environmental aspect, additional on-farm data collection would be required to prepare 37% of the proposed indicators. As expected, the largest share of indicators for the preparation of which only FADN data can be used (without combining with other data sources) is proposed for the economic aspect of sustainable agriculture (42%) and the lowest for the environmental aspect (7%). Within the social aspect of sustainable agriculture, FADN data alone can be used for one fifth of the proposed social indicators.

Conclusions

It can be concluded that the proposed list of sustainability topics and related indicators for Slovenia is neither comprehensive nor definitive. Therefore, it is strongly recommended that the list be further prioritised according to strategic policy needs of the country and the feasibility of collecting data at the farm level, and that it be refined with the participation of key stakeholders in the country. It is recommended to synchronise these activities with the process of converting FADN to FSDN (2022), which would allow strong synergies content- and cost-wise. The conversion of FADN to FSDN will allow better understanding of the sustainability challenges due to the increased data knowledge about Slovenian and EU agriculture.

Acknowledgement and disclaimer

Paper presents the results of the project "Establishment of a reference agricultural holdings system for the purpose of permanent monitoring of indicators of sustainable agriculture" financed by the Ministry of Agriculture, Food and Forestry of the Republic of Slovenia and Slovenian Research Agency. The authors would like to thank project partners and associates for their valuable inputs and contributions to the proposed set of farm sustainability topics and indicators.

An extended abstract of the paper was presented at the joint Conference of the Slovenian Association of Agricultural Economists (DAES) and the Austrian Association of Agricultural Economists (ÖGA) „Societal Changes and Their Implications on Agri-Food Systems and Rural Areas", Ljubljana, September 22 – 23, 2022 (conference of proceedings published: ISBN 978-961-94943-1-8).

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Pozicioniranje craft lager piva pomoću metode višedimenzionalnog skaliranja

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Sažetak

Cilj je ovog rada utvrditi tržišnu poziciju craft lager piva u odnosu na industrijska lager piva. Online anketno ispitivanje provedeno je na uzorku od 114 potrošača piva na zagrebačkom tržištu. Za utvrđivanje tržišne pozicije lager piva korištena je metoda višedimenzionalnog skaliranja. Craft lager piva uključena u istraživanje (Zlatni medvjed i Zmajsko pivo IPA) imaju jasno različitu tržišnu poziciju u odnosu na industrijska lager piva (PAN, Ožujsko i Karlovačko). Kako bi utvrdili po čemu se razlikuju istraživane marke piva potrošači su dodatno, na temelju vlastite percepcije, ocijenili ključna organoleptička obilježja (okus, miris i pitkost) istraživanih piva. Prema svim obilježjima craft lager piva su bolje ocjenjena u odnosu na tri industrijska lager piva (Karlovačko, Pan i Ožujsko). Rezultati istraživanja daju informacijsku podlogu proizvođačima craft piva za oblikovanje marketinške komunikacije i drugih marketinških aktivnosti.

Ključne riječi: pozicioniranje, višedimenzionalno skaliranje, lager pivo, craft pivo

Uvod

Pozicioniranje proizvoda je jedna od najvažnijih strateških marketinških odluka. Može se definirati kao određivanje jasnog, uočljivog i poželjnog mjesta za proizvod u odnosu na konkurentske proizvode u glavama ciljnih potrošača (Kotler i sur., 2006.). Svrha pozicioniranja je omogućiti ciljnim potrošačima jasno i pozitivno razlikovanje proizvoda tvrtke od drugih proizvoda kako bi ih se potaknulo na kupnju tih proizvoda. Uspješno pozicioniranje je ključna pretpostavka za dugoročni uspjeh tvrtke na današnjim konkurentskim tržištima.

Za pozicioniranje proizvoda odnosno izradu percepcijskih mapa često se koristi metoda višedimenzionalnog skaliranja (engl. multidimensional scaling, skr. MDS). MDS omogućuje određivanje relativnog odnosa između objekata nekog seta podataka u prostoru (Pećina, 2006.). Prednost te metode je što su obilježja objekta nepoznata, pa ispitanik ocjenjuje samo subjektivno doživljenu sličnost odnosno različitost između objekata. Ujedno, to je i manjkavost jer stvara poteškoće u interpretaciji rezultata, što je moguće prevladati korištenjem dodatnih metodoloških postupaka (Backhaus i sur., 2016.).

Predmet ovog rada je pozicioniranje craft lager piva na hrvatskom tržištu pomoću metode višedimenzionalnog skaliranja. Craft pivo ili kako se još naziva zanatsko pivo poteklo je iz Amerike, a označava pivo koja je proizvela mala nezavisna pivovara. U proizvodnji craft piva koristi se samo prirodni sastojci. U proizvodnji industrijskih piva, uz prirodne sirovine koriste se ekstrakti, razni aditivi i druge zamjenske supstance što ih čini jeftinijim od craft piva (Bais, 2017.). Lager piva, za razliku od ale piva su svijetla i lakša, imaju izračenu gorčinu i aromu po hmelju, a kad se natoče u času daju bogatu pjenu. Osnovna tehnološka razlika između ale piva i lager piva je u vrsti kvasca koji stvaraju pivo. Kvasci koji se koriste za lager imaju slabiju toleranciju na alkohol. Počinju fermentirati pri temperaturi od 6-8°C i zato se taj proces naziva hladnim vrenjem, a završava pri temperaturi od 9-18°C (Živčić, 2018.).

Posljednjih desetak godina u Hrvatskoj se događa „revolucija" craft piva. Započela je kuhanjem piva u kuhinjama i garažama, a nastavljena otvaranjem većeg broja zanatskih (craft) pivovara. U početku su zanatske pivovare većinom nudile Ale tip piva. Danas se uz Ale sve više nude i kvalitetna lager piva. Ukupna proizvodnja craft piva u Hrvatskoj je razmjerno mala i procjenjuje se na oko 2% ukupnog tržišta (Kelin, 2018.), pri čemu manji dio otpada na lager pivo.

Većina potrošača piva u Hrvatskoj ima naviku pijenja lager piva i preferira taj tip piva. Stoga postoji značajan potencijal za povećanje prodaje craft lager piva na domaćem tržišu. Žele li zanatske pivovare iskoristiti taj potencijal

trebaju diferencirati svoju tržišnu poziciju u odnosu na konkurenciju. Pretpostavka za to je da potrošači različito percipiraju tržišnu poziciju craft lager piva u odnosu na industrijska lager piva.

Cilj je ovog rada utvrditi tržišnu poziciju craft lager piva u odnosu na industrijska lager piva.

Materijal i metode

Za potrebe ovog istraživanja odabrano je pet marki piva, dva craft lager piva (Zmajsko pivo IPA i Zlatni medvjed) i tri industrijska lager piva (Karlovačko, Ožujsko i PAN). Fokus grupa provedena je na uzorku od 10 ispitanika kako bi se prikupile informacije za kreiranje ankete. Anketno istraživanje je provedeno online u travnju 2020. godine na uzorku od 114 ispitanika, potrošača piva. Anketa je izrađena u Googleovoj aplikaciji Google Forms te distribuirana putem društvenih mreža.

Za pozicioniranje 5 marki lager piva korištena je metoda MDS. Prema Backhaus i sur., (2016.) postupak MDS analize obuhvaća slijedeće korake: mjerenje sličnosti, izbor mjere udaljenosti, pronalaženje međusobnih odnosa između grupa, broj i interpretacija dimenzija te agregiranje ispitanika. Za mjerenje sličnost između objekta korištena je skala sličnosti od sedam stupnjeva. Kao mjera udaljenosti korištena je euklidiska metrika, a za pronalazak optimalne konfiguracije stress parametri. Broj dimenzija je određen na temelju mogućnosti interpretacije rezultata. Za agregiranja ispitanika korišten je tzv. Replicirani MDS uz pomoć aplikacije POLYCON (Backhaus i sur., 1996., Schober Gerold, 1995.).

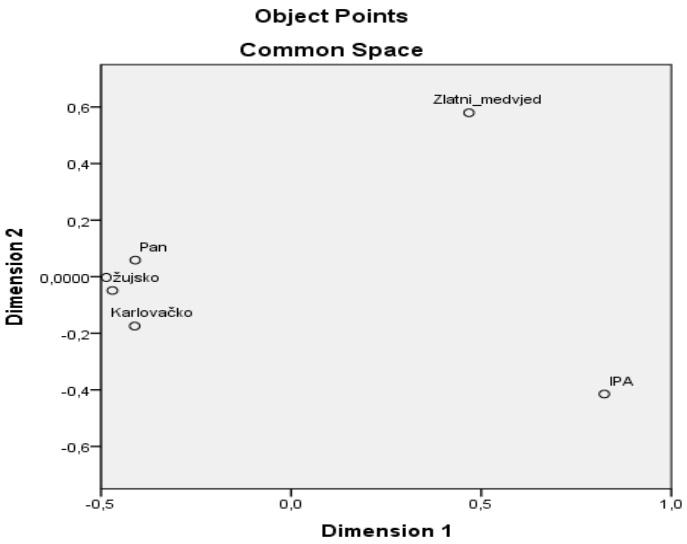
Obrada podatak provedena je u SPSS programu.

Rezultati i rasprava

U istraživanju su sudjelovali potrošača piva s područja Republike Hrvatske koji poznaju razlike između craft i industrijskog piva. U uzorku je bilo 57,5% žena i 42,5% muškaraca. Prema dobi najviše su zastupljene skupine između 21-30 godina (46,9%), između 31-40 godina (24,8%) te 41-50 godina (21,2%). Najviše ispitanika ima završenu višu ili visoku školu (56,6%), zatim magisterij i doktorat (26,5%) te srednju školu (16,8%). Najviše je ispitanika u skupini s obiteljskim dohotkom većim od 10.000 kn mjesečno (54%), zatim od 6.001 do 10.000 kn mjesečno (31,0%) te od 4.001 do 6.000 kn mjesečno (10,6%). U uzorku je razmjerno više mlađih i visoko obrazovanih ispitanika nego u populaciji, što je uobičajena pojava kod on line anketnih istraživanja.

Za određivanje sličnosti odnosno različitosti marki piva prema percepciji ispitanika korištena je sedam stupanjska rating skala (1 – potpuno slične do 7 – potpuno različite). Najsličnija piva su Ožujsko i Karlovačko (srednja vrijednost sličnosti/različitosti 2,55) te Pan i Ožujsko (srednja vrijednost 2,66), a najviše različite Ožujsko i Zmajska piva IPA (srednja vrijednost 4,09) te Pan i Zmajska piva IPA (srednja vrijednost 4,03). Međusobne udaljenosti 5 marki piva prikazane su u obliku matrice udaljenosti, koja je poslužila kao ulazni set podataka za MDS.

MDS analizom je obuhvaćeno 5 slučajeva (marki piva) i jedan izvor podataka (matrica udaljenosti). Niske vrijednosti Stress I – 0,0087, Stress II – 0,0182 pokazuju da je prilagođavanje vrlo dobro odnosno da su originalne blizine elemenata dobro predstavljene kao udaljenost u višedimenzionalnom prostoru. Na temelju koordinata svake od pet marki piva konstruiran je percepcijski prostor (dijagram zajedničkog prostora, Grafikon 1.) u kojem su pozicionirane marke piva.

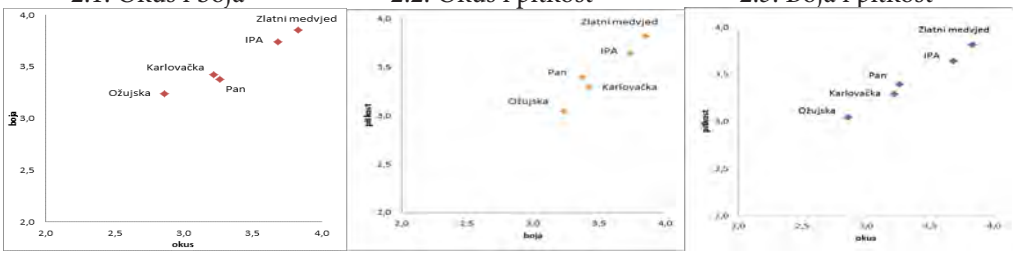


Grafikon 1. Pozicioniranje 5 marki lager piva (percepcijska mapa)

Jedan od najvažnijih kriterija valjanosti konačnog MDS rješenja predstavlja mogućnost njegove interpretacije. Percepcijska mapa pokazuje da ispitanici vrlo slično percipiraju 3 industrijska lager piva PAN, Ožujsko i Karlovačko. Obje marke craft piva se jasno razlikuju od industrijskih marki piva prema dimenziji 1 i međusobno se nalaze blizu po toj dimenziji. Po drugoj dimenziji marka piva Zlatni medvjed se značajnije razlikuje od industrijskih piva, dok je razlika između marke IPA i industrijskih piva manja.

U drugom koraku, potrošači su na temelju vlastite percepcije ocijenili organoleptička obilježja (okus, miris i pitkost) pet istraživanih piva. Za ocjenjivanje je korištena skala od pet stupnjeva (1-jako loše; 5 – jako dobro). Dobiveni podaci su korišteni za diskriminaciju istraživanih marki piva.

2.1. Okus i boja 2.2. Okus i pitkost 2.3. Boja i pitkost



Grafikon 2. Ogranoleptička obilježja marki piva

Pivo Zlatni medvjed ima najbolji okus (3,82) i najbolju boju (3,85), a tek nešto slabije ocjene dobilo je pivo IPA (okus 3,68 i boja 3,74). Industrijska piva su lošije ocjenjena: Karlovačko (okus 3,22 i boja 3,42), Pan (okus 3,26 i boja 3,38) i Ožujsko (okus 2,86 i boja 3,24).

Kod ocjene piva s obzirom na obilježja okus i pitkost vidljive su također različite pozicije craft i industrijskih lager piva. Najbolje su ocjenjene marke piva Zlatni medvjed (okus 3,82 i pitkost 3,80) i IPA (okus 3,68 i pitkost 3,64). Pan (okus 3,26 i pitkost 3,39) je prema pitkosti nešto bolje ocjenjen u odnosu na Karlovačko (okus 3,22 i pitkost 3,31) i Ožujsko pivo (okus 2,86 i pitkost 3,04).

S obzirom na obilježja boja i pitkost ponovo su izražene preferencije potrošača prema craft pivima. Ispitanici procjenjuju da pivo Zlatni medvjed ima najbolju pitkost (3,80) i najbolju boju (3,85). Nešto je slabije ocjenjena marka IPA (pitkost 3,64 i boja 3,74). Slijede pivo Pan (pitkost 3,39 i boja 3,38), Karlovačko (pitkost 3,31 i boja 3,42) i Ožujsko (pitkost 3,04 i boja 3,24).

Zaključak

Za utvrđivanje tržišne pozicije lager piva korištena je metoda višedimenzionalnog skaliranja. Dvije marke craft lager piva uključene u istraživanje (Zlatni medvjed i Zmajska piva IPA) imaju jasno različitu tržišnu poziciju u odnosu na industrijska lager piva (PAN, Ožujsko i Karlovačko). Pri tome se marka IPA po jednoj dimenziji jasno razlikuje od industrijskih marki piva, a pod drugoj nešto manje. Marka piva Zlatni medvjed se jasno razlikuje od industrijskih lager piva po obje dimenzije. Pozicije tri industrijska lager piva ispitanici slično percipiraju. Kako bi utvrdili po čemu se razlikuju istraživane marke piva potrošači su dodatno, na temelju vlastite percepcije, ocijenili ključna organoleptička obilježja (okus, miris i pitkost) istraživanih piva. Prema svim obilježjima najbolje je ocjenjena marka piva Zlatni medvjed. Nešto je lošije ocjenjena marka IPA, ali ipak bolje od tri industrijska lager piva (Karlovačko, Pan i Ožujsko). Provedeno istraživanje je pokazalo da ispitanici različito percipiraju craft i industrijske lager piva pri čemu craft piva bolje ocjenjuju. Rezultati istraživanja daju informacijsku podlogu za oblikovanje marketinške komunikacije i drugih marketinških aktivnosti za proizvođače zanatskog piva.

Napomena:

Podaci iznijeti u ovom radu predstavljaju rezultate istraživanja provedenog za potrebe izrade diplomskog rada studenta Ante Kraljevića na Agronomskom fakultetu Sveučilišta u Zagrebu pod nazivom: Pozicioniranje proizvoda pomoću metode višedimenzionalnog skaliranja na primjeru craft lager piva.

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Positioning of craft lager beer with the method of multidimensional scaling

Abstract

The aim of this paper is to determine the market position of craft lager in comparison to industrial lager. An online survey was conducted among a sample of 114 beer consumers in the Zagreb market. To determine the market position of lagers, the method of multidimensional scaling was used. The craft lagers included in the survey (Golden Bear and Zmajsko pivo IPA) have a significantly different market position than the industrial lagers (PAN, Ožujsko and Karlovačko). In order to determine how the studied beer brands differ, consumers additionally evaluated, based on their own perception, the main organoleptic characteristics (taste, smell and drinkability) of the studied beers. According to all characteristics, the craft lagers are rated better than three industrial lagers (Karlovačko, Pan and Ožujsko). The results of the study provide craft beer producers with an information base for designing marketing communication and other marketing activities.

Keywords: positioning, multidimensional scaling, lager beer, craft beer

Potencijal korištenja baza podataka za praćenje ekonomskih pokazatelja na poljoprivrednom gospodarstvu – primjer Slovenije

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Sažetak

Brzo donošenje odluka na razini poljoprivrednih gospodarstava je posebno važno u dinamičnim tržišnim uvjetima. U tu svrhu ispitane su i evaluirane baze podataka iz područja poljoprivrede koje mogu pomoći poslovnom odlučivanju na gospodarstvu. Za analizu su odabrane četiri baze podataka, SiStat, FADN, modelne kalkulacije KIS i Farm Manager KGZS. Ocjenjivali su se dostupnost, relevantnost, reprezentativnost i ažurnost. Rezultati istraživanja pokazuju da su korisnije one baze podataka koje detaljnije prate podatke na razini gospodarstva (FADN) i/ili poljoprivredne proizvodnje (modelne kalkulacije i Farm Manager). Za ocjenu ekonomskih pokazatelja manje su prikladne administrativne baze podataka (SiStat), jer kada su podaci agregirani smanjena je uporabna vrijednost za potrebe poljoprivrednih gospodarstava.

Ključne riječi: poljoprivredno gospodarstvo, baze podataka, evaluacija, Slovenija

Uvod

Važnost baza podataka najčešće se spominje u vezi kreiranja javnih politika, a osnova za odluke temeljene na činjenicama su kvalitetni podaci (Lovec i sur., 2021.). U istraživanju upotrebljivosti slovenskih baza podataka ocjenjuju da je slovenska poljoprivreda iznimno bogata mikropodacima, što međutim ne jamči korisnost baza podataka za donošenje odluka na mikrorazini - na razini poljoprivrednog gospodarstva.

Od 2020 godine poljoprivreda se ponovno susrela s brojnim globalnim pritiscima koji značajno utječu na odluke, a time i na ekonomski rezultat poljoprivredne proizvodnje. Intenzivni cjenovni pritisci na tržišta započeli su s izbijanjem epidemije Covid-19. Nakon kratkotrajnog smirivanja situacije, dolazi od rata u Ukrajini te se pritisci nastavljaju u još jačem obliku (Bele i Travnikar, 2022.). U neizvjesnim uvjetima proces odlučivanja na poljoprivrednim gospodarstvima postaje još teži, naročito ako se ne raspolaže s dovoljno kvalitetnim i ažurnim podacima. Unatoč brojnim bazama podataka u poljoprivredi, malo je onih koje na adekvatan način zadovoljavaju potrebe planiranja te donošenje odluka na poljoprivrednim gospodarstvima u kratkom roku.

Zbog navedenih uvjeta na poljoprivrednim tržištima, potreba za podacima na svim razinama je znatno porasla. Pritom su uočeni nedostaci u osiguravanju relevantnih i ažuriranih podataka, što je potaknulo autore ovog rada na reviziju baza podataka za potrebe poljoprivrednih gospodarstava. Posebna pozornost posvećena je javnoj dostupnosti i prikladnosti baza podataka za praćenje ekonomskih pokazatelja.

Baza podataka Statističkog ureda Republike Slovenije (SURS) predstavlja najopsežniji administrativni izvor podataka u Sloveniji. Ova baza uključuje i podatke o poljoprivredi. Na temelju portala SiStat (SURS, 2022.) za analizu u poljoprivredi najvažnija su sljedeća područja: (a) poljoprivreda, šumarstvo i ribarstvo, (b) industrija, (c) cijene i inflacija, (d) plaće i troškovi rada te (e) socijalna zaštita.

Prema Volk i suradnici (2017.), administrativni podaci se često temelje na periodičnom prikupljanju i dobrovoljnom sudjelovanju i prijenosu podataka. Stoga Rednak i suradnici (2016.) upozoravaju da se prilikom korištenja administrativnih baza podataka korisnici susreću s pogreškama u izvješćivanju, s nedostatkom kontrole nad ukupnošću i usklađenošću podataka, a s time i s njihovom usporedivošću. Primjer korištenja administrativnih baza podataka za izračun jednog od ekonomskih pokazatelja je ekonomska veličina poljoprivrednih gospodarstava, na temelju standardnog dohotka poljoprivrednih gospodarstava (EC, 2008.). Ovaj je pokazatelj, međutim, prilično robustan,

jer agregiran na razinu veličinskih razreda daje uvid samo u strukturne promjene na makro razini. Administrativne baze podataka uglavnom ne sadrže podatke koji bi se koristili za praćenje brojnih drugih ekonomskih pokazatelja i na taj način omogućili praćenje stanja i planiranje u poljoprivrednom gospodarstvu. Na ovaj problem upozoravaju i Rednak i suradnici (2016).

Prema nalazima Lovec i suradnika (2021) je sustav poljoprivrednih knjigovodstvenih podataka (FADN) među bazama podataka za potrebe mikro i makroekonomskih analiza organizacijski najpogodniji za korištenje. U slučaju FADN-a, to nije administrativna baza podataka, već rezultati periodičkog istraživanja. FADN baza podataka važna je jer je zbog usporedivosti europske metodologije i ima dugi vremenski niz podataka. Na makrorazini je posebno pogodna za komparativne analize po državama članicama, po regijama, po ekonomskim klasama kao i po vrstama proizvodnje. Njezinu korisnost potvrđuju kako analize Europske komisije tako i mnoge međunarodne analize (Martinovska-Stojčeska i sur. 2008., Cagliero i sur. 2011., EC Dashboard, 2022., Slijper i sur. 2022., Vaccaro i sur. 2022.).

FADN baza podataka pogodna je i za korištenje na mikrorazini jer u osnovnoj metodologiji (EC, 2019.) uključuje podatke i standardne rezultate po nekoliko sadržajnih cjelina: prikaz prihoda (proizvodnja, ostale djelatnosti, subvencije), prikaz troškova (varijabilni, amortizacija, plaće, najamnine, kamate i ostalo) te izračune ekonomskih pokazatelja (bruto dodana vrijednost, neto dodana vrijednost, neto prihod gospodarstva i ostalo). Time je omogućena komparativna analiza (benchmarking) i savjetovanje između poljoprivrednika sa istom vrstom proizvodnje gdje uče i razmjenjuju iskustva. Zbog toga je ova baza važan izvor podataka i u slučaju nacionalnih analiza (Kožar, 2022.). Nažalost, u Sloveniji FADN baza podataka ima reputaciju nedovoljno visoke razine kvalitete podatka (Kožar, 2017.) zbog problema kao što su procjena dugotrajne materijalne imovine, ažurnosti, točnosti i korektnosti podataka, kao i nedostatka znanja i svijesti korisnika za upotrebu FADN-a.

Uz FADN bazu podataka, u kojoj se evidentiraju troškovi i prihodi prema stvarnim događajima, poljoprivrednim gospodarstvima mogu koristiti i razni modeli. Među njima su i „modelne kalkulacije" Kmetijskega inštituta Slovenije (Rednak, 1998., Moljk i sur., 2021., Zagorc i sur., 2022.), koje predstavljaju sustav kalkulacija za praćenje troškova i ekonomskih pokazatelja za pojedine biljne i stočarske proizvode, ali također omogućuju procjene za potrebe poljoprivredne politike, procjene različitih naknada i slično (Cunder T. i sur., 2007., Sušin J. i sur., 2019.).

Osnova za izračun modela je implementirana baza podataka koja se temelji na mnogim bazama podataka i drugim izvorima. Važan dio su različite administrativne baze podataka (statistički podaci: SURS-a, EUROSTAT-a i Europske komisije), podaci državnih tijela (npr. Ministarstvo financija, Ministarstvo okoliša, Zavod za makroekonomske analize-UMAR, Agencija za plaćenja u poljoprivredi-ARSKTRP i druge). Osim navedenog, kontinuirano se prikupljaju brojni specifični podaci kao što su dobavljači materijala, opreme i usluga, FADN podaci, registri, mediji i drugo. Dio baze „modelnih kalkulacija" su i podaci raznih istraživanja i mjerenja, kataloški podaci i slično. Na temelju objavljenih rezultata online (KIS, 2022.), posebice iz analitičkog prikaza troškova, poljoprivrednim gospodarstvima je omogućen ažuriran pregled visine prosječnih cijena i troškova, ali i količina upotrebe proizvodnih inputa i rada. Jednostavan prikaz kalkulacija također može poslužiti menadžerima poljoprivrednim gospodarstvima za usporedbu vlastite proizvodnje prema pretpostavljenoj tehnologiji u kalkulacijama ili kao primjer, analitički prikaz, za procjenu vlastitih troškova proizvodnje (Zagorc i sur., 2021.) i očekivanog prihoda, te procjena dohotka na razini bruto i neto dodane vrijednosti.

„Modelne kalkulacije" su izvor podataka za menadžere poljoprivrednih gospodarstava, međutim za izračun proizvođačkih cijena, prihoda i troškova potrebno je imati i određena ekonomska znanja, sposobnosti i vještine. Stoga je u sklopu aktivnosti na projektu „Razvoj cjelovitog modela poljoprivrednih gospodarstava i povezanih baza podataka za potporu odlučivanju u slovenskoj poljoprivredi (Volk i sur., 2017.)" izrađen model za ekonomsko savjetovanje poljoprivrednih gospodarstava. Pilot model temeljen na slučaju prerade mlijeka prvenstveno koristi FADN bazu podataka, a dopunjen je i podacima iz drugih baza podataka. Unatoč tome, menadžer ili neki drugi korisnik mora u velikoj mjeri pronaći i prikupiti mnoge podatke, posebice tehnološke.

Nedavno je razvijen novi online model, a s njim i nova baza podataka Farm Manager (Farm Manager, 2022.). Sustav se temelji na kombinaciji gore opisanog pilot modela i kalkulacija pokrića varijabilnih troškova (Jerič i sur., 2011.) kojeg je pripremila poljoprivredna komora-KGZS za potrebe savjetovanja i planiranja na poljoprivrednim gospodarstvima.

Materijal i metode

Istraživanje je započeto pregledom svih javno dostupnih baza podataka koje su djelomično ili u potpunosti relevantne za ocjenu produktivnosti i ekonomske uspješnosti poljoprivrednog sektora. Za daljnju analizu odabrane su četiri baze podataka, i to: baza Statističkog ureda Republike Slovenije (SiStat), baza Sustav poljoprivrednih knjigovodstvenih podataka (FADN), „modelne kalkulacije" Kmetijskega inštituta Slovenije (MK KIS) i sustav „Farm Manager" poljoprivredne komore (FM KGZS).

Prikladnost korištenja baza podataka za potrebe na mikrorazini, odnosno za poljoprivredna gospodarstva, ocijenjena je sa četiri kriterija: dostupnost, relevantnost, reprezentativnost i ažurnost podataka. Dostupnost podataka razmatra se sa tehničkog i sadržajnog stajališta odnosno koliko je napora potrebno da korisnici (menadžeri poljoprivrednih gospodarstava) dođu do odgovarajućih baza podataka, a relevantnost pokazuje u kojoj se mjeri podaci prvenstveno odnose na potrebe poljoprivrednih gospodarstava. Kriterij reprezentativnosti povezan je sa kvalitetom podataka relevantnih za poljoprivredna gospodarstva odnosno kako se poljoprivredna gospodarstva mogu uspoređivati sa drugima. Kriterij ažurnosti podataka pokazuje jesu li podaci aktualni za potrebe korisnika (koliki je vremensko kašnjenje podataka). Kriteriji su razmatrani na tri razine i to V-visoka razina, S-srednja razina i N-niska razina. Ocjene po pojedinim kriterijima prikazane su u obliku matrice.

Rezultati i rasprava

Općenito, za statističku bazu podataka (SiStat) smatra se da je jednostavna za korištenje i dostupna velikom broju korisnika. Ipak, sa sadržajnog gledišta, pristup podacima koji su menadžerima poljoprivrednih gospodarstava potrebni za procjenu ekonomskih pokazatelja često je ograničen i stoga najmanje prikladan. U relevantnim područjima korisnicima su dostupni samo izvedeni podaci, kao što su indeksi, ali malo je podataka dostupno u obliku apsolutnih vrijednosti, kao što su cijene proizvodnih inputa. Povjerljivost podataka i statističke procjene - aproksimacije, uobičajene su u SiStat-u, i proizlaze iz (pre)malog broja izvještajnih jedinica, a uz nedostatak dodatnih periodičnih istraživanja ne postoji i cjeloviti snimak stanja. Dakle, prikaz podataka ponegdje nije dovoljno točan i reprezentativan u odnosu na potrebe tržišno orijentiranih gospodarstava. Takav primjer su neki proizvodni inputi u poljoprivredi, koji ne uključuju podatke o troškovima transporta i marže, što su uz cijenu materijala stvarni trošak poljoprivrednog gospodarstva. Problematična je i rigidnost u prilagodbi uzorka izvještajnih jedinica, a koja se pojavljuju na tržištu. Različita razina rigidnosti uočava se i u brzini davanja informacija, što je posebno problematično u vrijeme brzih promjena na tržištima. U stabilnim uvjetima, kašnjenje od dva ili tri mjeseca podataka može se smatrati dovoljno ažurnim, ali ne i u vrijeme intenzivnih promjena.

Problem s detaljnijim FADN podacima je taj što nisu lako dostupni menadžerima poljoprivrednih gospodarstava odnosno dostupni su samo kroz javne službe. Očekivano, podaci FADN baze relevantni su za poljoprivredna gospodarstva koja vode knjigovodstvo FADN i za koja su implementirani ekonomski pokazatelji. Međutim, baza je manje relevantna za ostala poljoprivredna gospodarstva, jer ne omogućuje opsežniji pregled kvantitativnih podataka i definiranje podataka odvojeno po vrstama biljne ili stočarske proizvodnje.

Relevantnost i reprezentativnost podataka FADN raste tijekom godina, ali ipak postoje pojedina područja gdje se, zbog malog broja poljoprivrednih gospodarstava u uzorku, pojavljuju manje točni i pouzdani podaci te su zbog toga ocijenjeni sa razredom S. Svinjogojstvo je jedan od takvih primjera. Tamo gdje je uzorak dovoljno velik, pojedino poljoprivredno gospodarstvo može se uspoređivati sa sličnim unutar iste vrste proizvodnje u zemlji i izvan nje, a pri tome se mogu dobiti i usporedivi podaci iz FADN baze podataka drugih zemalja.

Općenito, smatra se da je točnost i pouzdanost podataka karakteristična posebno za starije zemlje EU, u kojima je FADN u upotrebi već nekoliko desetljeća i gdje je FADN djelomično mapiranje dvojnog knjigovodstva koje poljoprivredna gospodarstva vode. Napominje se da FADN baza podataka nije ažurna (razred N), budući da postoji i dvogodišnje razdoblje između prijave podataka i prikaza rezultata. Korisnost FADN baze podataka na mikro razini može se poboljšati nadogradnjom i uključivanjem brojnih tehnoloških i kvantitativnih podataka.

Tablica 1. Upotrebljivost baza podataka za procenjivanje ekonomskih pokazatelja sa stajališta poljoprivrednog gospodarstva

baza podataka	kriterij			
	dostupnost podataka	relevantnost podataka	reprezentativnost podataka	ažurnost prikaza
SiStat	S	N	N	S
FADN	N	S	S	N
MK KIS	S	V	S	V
FM KGZS	N	V	S	S

Izvor: vlastito iztraživanje
Legenda: V-visoka razina, S-srednja razina, N-niska razina

Dostupnost podataka MK KIS za poljoprivredna gospodarstva ocijenjena je srednjom. Unatoč brojnim podacima, takva ocjena proizlazi iz prikaza izvedenih podataka. Zbog povjerljivosti ulaznih podataka, neki podaci nisu javno dostupni te stoga nisu u cijelosti prikazani. Međutim, manji dio podataka je neprikladan za daljnju upotrebu jer su manje točni (malo izvještajnih jedinica, preuzak skup podataka, podaci temelje na procijeni) i stoga manje su pouzdani ili se podaci pokazuju na previše agregiranoj razini. Unatoč tom nedostatku, ovi podaci su lako dostupni menadžerima poljoprivrednih gospodarstava jer se javno objavljuju. Na osnovu zahtjeva menadžera MK KIS prilagođava se poljoprivrednim gospodarstvima, a time i mogućnost usporedbe podataka i rezultata. Time se relevantnost baze podataka povećava i visoka je. Reprezentativnost i ažurnost podataka također je visoka jer je MK KIS osnova za stalno praćenje stanja u poljoprivredi i vrlo se često ažurira, najčešće mjesečno, kod pojadinačnih podataka par puta godišnje.

Slično kao u slučaju baze MK KIS, i za bazu FM KGZS očekuje se visoka razina relevantnosti i reprezentativnosti podataka. Baza je namijenjena poljoprivrednim gospodarstvima i poljoprivrednim savjetnicima, pa se itekako očekuje i njena visoka razina dostupnosti. Međutim, budući da je baza relativno nova i još u fazi razvoja, podaci su manje dostupni nekim grupama korisnika. Pravodobnost uključivanja i ažuriranja podataka ocjenjuje se srednjom, budući da online objava prati brzinu prijenosa izračuna iz tiskanog oblika u digitalni. Tako se ažuriranja i dopune provode periodički kao izrade obračuna pokrića ili se provode tijekom konzultacija te drugih povremenih poslova. U budućnosti, razvojem i dopunama sustava izračuna pokrića, očekuje se i poboljšanje dostupnosti i ažurnosti ovih podataka.

Rezultati pregleda upotrebljivosti baza podataka za potrebe izračuna ekonomskih pokazatelja na poljoprivrednim gospodarstvima u Sloveniji u skladu su s rezultatima istraživanja Lovec i sur. (2021.), Volk i sur. (2016.) i Kožar (2017.).

Zaključak

Analiza prikladnosti baza podataka za potrebe praćenja, planiranja i donošenja poslovnih odluka temeljenih na ekonomskim pokazateljima na razini poljoprivrednog gospodarstva pokazala je da SiStat baza podataka nije najprikladnija. Glavni nedostaci su mogućnost usporedbe podataka jednog poljoprivrednog gospodarstva s agregiranim poljoprivrednim podacima.

Primjerenijim se čine baze podataka modela za praćenje stanja u poljoprivredi, simulacije i savjetovanje (MK KIS i FM KGZS). Djelomično je prikladna i FADN baza podataka, gdje su ekonomski pokazatelji sastavni dio standardnih rezultata, ali samo za gospodarstva koja su u sutavu. Korisnost FADN baze podataka na mikro razini može se poboljšati očekivanom nadogradnjom i proširenjem sustava FADN-a. Nadogradnjom i uključivanjem brojnih tehnoloških i kvantitativnih podataka, uz procjene ekonomske učinkovitosti, moći će se procijeniti ekološki i društveni učinci.

Tijekom istraživanja uočeno je da se kod brojnih korisnika modeli za praćenje i/ili planiranje poljoprivredne proizvodnje ne mogu dovoljno primijeniti. Naime, prikupljanje pojedinačnih podataka, poput cijena ili tehnoloških parametara, nije toliko zahtjevno, ali je zahtjevnije daljnje korištenje za izračune ekonomskih pokazatelja, pogotovo ako korisnici nemaju odgovarajuća ekonomska znanja. Stoga je potrebno kontinuirano informirati i upućivati korisnike podataka (menadžere poljoprivrednih gospodarstava) o brojnim potencijalnim mogućnostima koje

baze podataka mogu ponuditi u donošenju odluka. Osim toga, aktivan pristup menadžera mogao bi pridonijeti poboljšanju kvalitete baza podataka.

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The potential of using databases for monitoring economic indicators on an agricultural farm – Slovenia example

Abstract

Quick decision-making at the farm level is particularly important under difficult market conditions. To this end, agricultural databases that can be helpful in economic decision-making at the farm level were examined and evaluated. Four databases were selected for analysis (SiStat, FADN, "calculations KIS" and "Farm Manager KGZS"). Accessibility, relevance, representativeness, and up-to-dateness were evaluated. The main findings are that those databases that track data at the level of the farm (FADN) and/or agricultural production level (calculations KIS and Farm Manager KGZS) are more useful. Administrative databases (SiStat) are less suitable for evaluating on-farm economic indicators because data at more aggregate levels reduce their usefulness for farm needs.

Keywords: farm household, data bases, evaluation, Slovenia

Ekonomska efikasnost sektora višegodišnjih nasada u Hrvatskoj korištenjem FADN sustava

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Sažetak

Hrvatska ima povoljne ekološke uvjete za uzgoj višegodišnjih nasada. Ipak svega 2% ukupno korištene poljoprivredne površine otpada na voćnjake a na vinograde i maslinike po 1%. U radu su analizirani podatci komercijalnih poljoprivrednih gospodarstava FADN sustava u tipu maslinarstvo, različite vrste trajnih nasada, vinogradarstvo i vinarstvo te voćarstvo. Kao najkonkurentniji tip gospodarstva izdvojio se vinogradarsko-vinarski koji ostvaruje dva i pol puta veću neto dodanu vrijednost u odnosu na europski prosjek. Gospodarstva u tipu voćarstvo ostvaruju pet puta manju neto dodanu vrijednost od europskog prosjeka te zahtjevaju dodatne napore za povećanje produktivnosti.

Ključne riječi: FADN, maslinarstvo, vinogradarstvo, voćarstvo

Uvod

U Republici Hrvatskoj se 2021. godine koristilo ukupno 1.476.351 ha poljoprivredne površine, od čega je na voćnjake otpadalo 2% a na vinograde i maslinike po 1% površine (DZS, 2022). Hrvatska ima vrlo povoljne pomoekološke uvjete za uzgoj voćaka a tradicija uzgoja voćaka duga je više stoljeća (Čmelik, 2010). Istraživanje Očić i sur. (2017) pokazalo je da u Hrvatskoj najekonomičnije posluju gospodarstva u tipu vinogradarstvo i vinarstvo, dok ona u tipu voćarstvo imaju najniži koeficijent ekonomičnosti i ujedno su najzaduženija. Rezultati FADN sustava (EC, 2021) navode da je prosječna neto dodana vrijednost po jedinici rada u Europskoj uniji 22.500 EUR-a, a najnižu imaju tipovi: vinogradarstvo i vinarstvo, voćarstvo i maslinarstvo (osim vinarstva). Rosić i sur. (2016) navode da najmanju vrijednost ukupnih prihoda imaju tipovi vinogradarstvo i vinarstvo, te voćarstvo i maslinarstvo koji ujedno bilježi i najnižu vrijednost ukupnih troškova. Istraživanje provedeno u Italiji (De Salvo i sur., 2013) na uzorku uzgajivača jabuka i grožđa implicira da klimatske promjene uzrokuju smanjenje godišnjih neto prihoda proizvođača. Prihodi koji se ostvaruju u poljoprivrednoj proizvodnji su jako promjenljivi i do 20% poljoprivrednika svake godine bilježi pad prihoda iznad 30%. Neki sektori, kao npr. voćarstvo i maslinarstvo bilježe i veće varijacije od toga (EC, 2017).

Materijal i metode

U radu su analizirani podaci o komercijalnim poljoprivrednim gospodarstvima obuhvaćeni Sustavom poljoprivrednih knjigovodstvenih podataka u Republici Hrvatskoj (FADN - farm accountancy data network) od 2016. do 2019. godine. Komercijalno gospodarstvo predstavlja ono čija je ekonomska veličina veća od 4.000 EUR-a, odnosno ono koje je dovoljno veliko da osigura glavnu aktivnost poljoprivrednika i razinu prihoda dostatnu za održavanje vlastite egzistencije i egzistencije obitelji. Uzorak je obuhvaćao 164 do 197 poljoprivrednih gospodarstava, ovisno o godini, podijeljenih u četiri tipa – maslinarstvo, različite vrste trajnih nasada, vinogradarstvo i vinarstvo i voćarstvo. U tip različite vrste trajnih nasada spadaju gospodarstva koja se bave uzgojem višegodišnjih kultura u zaštićenom prostoru (staklenici, plastenici) a obuhvaćaju voćne kulture (prvenstveno agrume i jagodasto voće izuzev jagoda). Sustav poljoprivrednih knjigovodstvenih podataka je europski sustav prikupljanja podataka s ciljem godišnjeg određivanja prihoda poljoprivrednih gospodarstava te poslovne analize poljoprivrednog gospodarstva. Podaci sustava su bitni i za razvoj i praćenje učinka Zajedničke poljoprivredne politike (ZPP) (Uredba Vijeća (EZ) br. 1217/2009).

Cilj rada je deskriptivnom metodom istraživanja usporediti proizvodne i ekonomske pokazatelje tipova maslinarstvo, različite vrste trajnih nasada, vinogradarstvo i vinarstvo te voćarstvo.

Rezultati i rasprava

Broj gospodarstava u uzorku se kretao od 164 do 197 ovisno o godini. Najviše gospodarstava pripada tipu voćarstvo te vinogradarstvo i vinarstvo (31, odnosno 30%), s najmanje (13%) različitim vrstama trajnih nasada (Tablica 1).

Tablica 1. Broj gospodarstava u uzorku

	2016	2017	2018	2019
Maslinarstvo	49	53	52	42
Različite vrste trajnih nasada	23	25	24	22
Vinogradarstvo i vinarstvo	58	60	59	47
Voćarstvo	57	54	62	53
Ukupno	187	192	197	164

Izvor: FADN sustav

Promatrajući ukupan uzorak vidljivo je postupno povećanje ukupno korištenih poljoprivrednih površina (Tablica 2). Ukupna korištena poljoprivredna površina (KPP) predstavlja ukupnu površinu u vlasništvu i zakupu nositelja i/ili članova gospodarstva, bez šuma i ostalog nepoljoprivrednog zemljišta, uključujući putove, gospodarska dvorišta i slično. Prosječna veličina gospodarstva u EU je 37 ha (2018. godine), a gledano po tipu sektor višegodišnjih nasada nalazi se na začelju sa prosječno 12 ha (EC, 2021), te se sva gospodarstva iz uzorka u svim promatranim godinama nalaze ispod europskog prosjeka korištenih poljoprivrednih površina.

Tablica 2. Ukupno korištena poljoprivredna površina (KPP), ha

	2016	2017	2018	2019
Maslinarstvo	5,76	6,53	6,53	6,56
Različite vrste trajnih nasada	4,33	5,45	6,48	7,27
Vinogradarstvo i vinarstvo	7,12	6,51	9,25	10,74
Voćarstvo	5,84	6,44	5,88	6,15
Prosječno	5,76	6,23	7,04	7,68

Izvor: FADN sustav

Udio korištenih poljoprivrednih površina u najmu u ukupno korištenim kreće se od prosječno 14% kod tipa različite vrste trajnih nasada do 30% kod tipa vinogradarstvo i vinarstvo. Kako je u EU-27 više od polovice korištenog poljoprivrednog zemljišta u najmu, vidi se da i po ovom pokazatelju gospodarstva iz uzorka odstupaju od europskog prosjeka. Udio unajmljenog zemljišta u ukupno korištenom pokazuje velike varijacije u EU-27, u Slovačkoj, Bugarskoj, Francuskoj i Malti više od 80% KPP je u najmu dok je u Irskoj i Portugalu manje od četvrtine (EC, 2021).

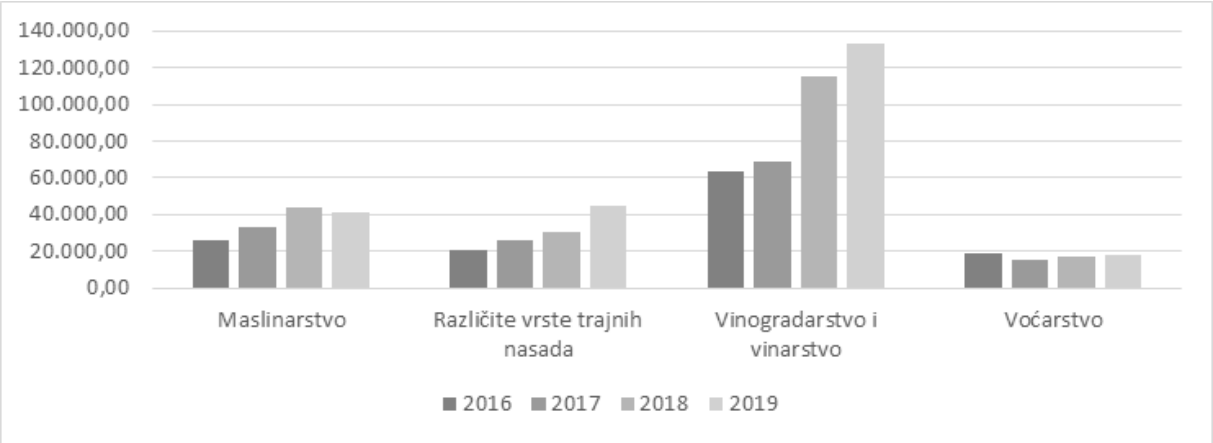
Najviše jedinica rada koriste gospodarstva tipa vinogradarstvo i vinarstvo (2,25 do 3,08 ovisno o godini), a najmanje tipa voćarstvo (1,40 do 1,62). U Republici Hrvatskoj 1 AWU je definiran kao 1.800 radnih sati godišnje (Tablica 3). Istraživanje Rosić i sur. (2016) navodi da tip voćarstvo i maslinarstvo ima najmanju potrebu za radom u odnosu na druge tipove gospodarstava. 2018. godine se u EU-27 koristilo prosječno 1,6 jedinica rada po gospodarstvu, dok je tip voćarstvo i maslinarstvo koristio nešto manje (1,4) (EC, 2021). Može se zaključiti da hrvatska gospodarstva iz uzorka troše više jedinica rada u odnosu na europski prosjek, tip vinogradarstvo i vinarstvo i više nego duplo.

Tablica 3. Ukupni rad / Total labour hour, AWU

	2016	2017	2018	2019
Maslinarstvo	1,53	1,90	1,84	1,91
Različite vrste trajnih nasada	1,58	1,68	2,04	2,15
Vinogradarstvo i vinarstvo	2,25	3,08	3,06	2,45
Voćarstvo	1,57	1,40	1,62	1,55
Prosječno	1,73	2,01	2,14	2,01

Izvor: FADN sustav

Prosječan ukupan prihod promatranih gospodarstava (ukupna prosječna vrijednost biljne i stočarske proizvodnje, uključujući i proizvode potrošene na gospodarstvu za ishranu stoke) u promatranom periodu raste. Najmanje ukupne prihode bilježe gospodarstva koja pripadaju tipu voćarstvo (15.750,01 do 18.773,29 EUR ovisno o godini), a najveće ona koja pripadaju tipu vinogradarstvo i vinarstvo (63.703,23 do 133.232,09 EUR), kod kojih je vidljivo veliko povećanje prihoda u zadnjoj promatranoj godini (Grafikon 1). Prosječni ukupni prihod gospodarstava u EU-27 2018. godine iznosio je 83.300 EUR-a. Gledajući po tipu najveće prihode bilježe svinjogojska i peradarska gospodarstva, dok najniže bilježe tipovi vinogradarstvo (bez vinarstva) i voćarstvo i maslinarstvo, prosječno 40.400 EUR (EC, 2021). Uspoređujući s europskim prosjekom (83.300 EUR) analizirana vinogradarsko-vinarska gospodarstva ostvaruju 38% veće prihode dok voćarska ostvaruju 80% niže prihode u 2018. godini.



Grafikon 1. Ukupni prihodi gospodarstava, EUR
Izvor: FADN sustav

Najveća zabilježena neto dodana vrijednost (FNVA=(ukupni prihodi uvećani za isplaćene potpore (bez investicijskih) i umanjeni za intermedijarnu potrošnju) – prosječni troškovi amortizacije) bila je kod tipa vinogradarstvo i vinarstvo (33.366,13 do 107.966,46 EUR ovisno o godini) a najniža kod tipa voćarstvo (4.273,17 do 13.893,95 EUR). Prosječna FNVA Europske unije iznosi 35.300 EUR, a Hrvatska se nalazi među četiri zemlje sa najnižom neto dodanom vrijednošću (Hrvatska, Rumunjska, Poljska i Slovenija) (EC, 2021). Može se zaključiti da tip vinogradarstvo i vinarstvo ima dva i pol puta veću FNVA od prosjeka EU-27, dok tip voćarstvo ima pet puta manju vrijednost.

Promatrajući neto dodanu vrijednost/jedinici rada (AWU) vidi se isti obrazac, najmanju FNVA/AWU pokazuju gospodarstva tipa voćarstvo. Kod gospodarstva tipa različite vrste trajnih nasada vidi se izuzetno nizak iznos 2017. godine koji je praćen velikim rastom u sljedećim godinama. S druge strane najveći zabilježeni iznosi su kod gospodarstava u tipu vinogradarstvo i vinarstvo (Tablica 4). U EU-27 vinogradarska, voćarska i maslinarska gospodarstva nalaze se ispod prosjeka Unije sa oko 19.000 EUR/AWU (EC, 2021).

Tablica 4. Neto dodana vrijednost po AWU / Farm Net Value Added / AWU, EUR/AWU

	2016	2017	2018	2019
Maslinarstvo	8.001,50	8.493,00	8.699,46	10.565,20
Različite vrste trajnih nasada	2.956,07	843,50	6.785,64	13.303,61
Vinogradarstvo i vinarstvo	9.226,20	13.373,73	25.431,77	27.536,96
Voćarstvo	1.453,52	9.076,23	5.211,38	7.301,00
Prosječno	5.409,32	7.946,62	11.532,06	14.676,69

Izvor: FADN sustav

Promatranjem ukupnih potpora koja gospodarstva primaju (prosječne ukupne potpore bez investicijskih potpora, uključujući izravna plaćanja, premije, proizvodno vezane potpore i ostale isplaćene potpore) vidi se da najveće su iznose primala gospodarstva tipa vinogradarstvo i vinarstvo, a najmanje različite vrste trajnih nasada. Kod tipa vinogradarstvo i vinarstvo ističe se 2017. godina u kojoj je došlo do velikog povećanja primljenih iznosa. Razlog tome su promjene u Vinskoj omotnici kao i činjenica da su se isplate sredstava ugovorenih unutar Vinske omotnice 2016. godine vršile 2017. i 2018. godine (Glas Slavonije, 2016) (Tablica 5).

Tablica 5. Ukupne potpore, bez invest. / Total subsidies, excl. Invest., EUR

	2016	2017	2018	2019
Maslinarstvo	2.631,75	4.452,11	4.999,65	9.455,41
Različite vrste trajnih nasada	745,51	1.514,12	2.139,66	4.491,74
Vinogradarstvo i vinarstvo	2.301,29	13.606,84	10.295,48	8.464,16
Voćarstvo	1.519,91	10.883,70	3.764,35	6.644,61
Prosječno	1.799,61	7.614,19	5.299,79	7.263,98

Izvor: FADN sustav

Najniži udio ukupnih potpora (bez investicijskih) u neto dodanoj vrijednosti (FNVA) imaju gospodarstva tipa vinogradarstvo i vinarstvo (7% 2016. godine do 26% 2017. godine), a najviši gospodarstva u tipu voćarstvo (36% 2016. godine do 78% 2017. godine. Ovo je u skladu s brojnim dosadašnjim istraživanjima (Očić i sur., 2018; Rosić i sur., 2016.; Juračak i sur., 2017.; Agrosynergie, 2011.) koja navode da su o potporama najmanje ovisna povrćarsko-cvjećarska i vinogradarsko-vinarska gospodarstva.

Iznosi primljenih izravnih plaćanja se kod promatranih tipova povećavaju tijekom godina, da bi 2019. godine došlo do stagnacije (različite vrste trajnih nasada) ili laganog pada (vinogradarstvo i vinarstvo, voćarstvo), uz iznimku maslinarstva koje bilježi daljnji rast. Prosječni udio izravnih plaćanja u neto dodanoj vrijednosti EU-27 je 28%, najveći je u Litvi (70%), najniži u Nizozemskoj (9%), dok je u Hrvatskoj oko 39%. Gledajući po tipu gospodarstva može se zaključiti da je udio izravnih plaćanja u FNVA visok za tipove gospodarstava koji bilježe niske prihode. Trajni nasadi se nalaze ispod prosjeka Unije sa udjelom oko 18% (EC, 2021). Najniži udio izravnih plaćanja u FNVA imaju gospodarstva u tipu vinogradarstvo i vinarstvo (1 do 3%), a najviši u tipu voćarstvo (14 do 28%), koji su ujedno i jedini tip koji je u 2018. godini bio iznad prosjeka Unije u tipu trajnih nasada (Tablica 6).

Tablica 6. Udio izravnih plaćanja u FNVA, %

	2016	2017	2018	2019
Maslinarstvo	9%	22%	12%	13%
Različite vrste trajnih nasada	12%	20%	11%	6%
Vinogradarstvo i vinarstvo	1%	3%	3%	2%
Voćarstvo	16%	14%	28%	19%
Prosječno	9%	15%	13%	10%

Izvor: vlastiti izračun na temelju podataka FADN sustava

Zaključak

U radu su analizirani podatci komercijalnih poljoprivrednih gospodarstava koja su obuhvaćena Sustavom poljoprivrednih knjigovodstvenih podataka u razdoblju od 2016. do 2019. godine. Gospodarstva su podijeljena u četiri tipa: maslinarstvo, različite vrste trajnih nasada, vinogradarstvo i vinarstvo te voćarstvo. Broj gospodarstava u uzorku se kretao od 164 do 197, ovisno o godini, a najveći dio ih je pripadao tipovima voćarstvo i vinogradarstvo i vinarstvo. U uzorku je vidljiv postupan rast korištenih poljoprivrednih površina po gospodarstvu, ali još uvijek nije dostignut prosjek Europske unije. Istovremeno je prosječno 14% korištenih poljoprivrednih površina (različite vrste trajnih nasada) do 30% (vinogradarstvo i vinarstvo) bilo u najmu, što je također ispod prosjeka Europske unije. Najviše jedinica rada (AWU) koriste gospodarstva tipa vinogradarstvo i vinarstvo a najmanje ona tipa voćarstvo. Sva analizirana gospodarstva koriste više rada u odnosu na europski prosjek, ona u tipu vinogradarstvo i vinarstvo 119% više. Svega 20% prosječnih europskih prihoda ostvaruju gospodarstva u tipu voćarstvo, dok vinogradarsko-vinarska postižu 38% više ukupnih prihoda od europskog prosjeka. Također, tip vinogradarstvo i vinarstvo ima dva i pol puta veću neto dodanu vrijednost u odnosu na europski prosjek, dok gospodarstva tipa voćarstvo imaju pet puta manju. Isti obrazac je vidljiv kada se promatra neto dodana vrijednost po jedinici rada (FNVA/AWU). Po ovom pokazatelju gospodarstva tipa vinogradarstvo i vinarstvo nalaze se 34% iznad europskog prosjeka, dok su ona tipa voćarstvo 73% ispod njega. Iz ovoga se vidi da se vinogradarsko-vinarska gospodarstva po produktivnosti mogu nositi sa izazovima Europske unije, dok su voćarska daleko manje produktivna. Najmanje iznose ukupnih potpora bez investicijskih primala su gospodarstva u tipu različite vrste trajnih nasada, a najveće vinogradarsko-vinarska gospodarstva koja ujedno imaju najniži udio ukupnih potpora u neto dodanoj vrijednosti (FNVA), što je u skladu s brojnim istraživanjima koja navode da je vinogradarsko-vinarski tip gospodarstava najmanje ovisan o potporama. Također gospodarstva ovoga tipa imaju najniži udio izravnih plaćanja u FNVA, a ona u tipu voćarstvo najviši pa se može zaključiti da su voćarska gospodarstva najovisnija o izravnim plaćanjima te su ujedno najmanje produktivna. Da bi voćarska gospodarstva postala konkurentnija na tržištu potrebno je raditi na povećanju ostvarenih prihoda i produktivnosti rada. Jedna od mogućih preporuka je ne pristupanje uzgoju voćarskih kultura 'hobistički' jer uzgoj voćarskih kultura zahtjeva adekvatno znanje i inovativan stručan pristup za postizanje konkurentnost na tržištu. Pored navedenog bio bi potreban i kvalitetan marketinški pristup koji bi omogućio postizanje većih prodajnih cijena.

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Economic efficiency of perennial plantations in Croatia using the FADN system

Abstract

The Republic of Croatia has favorable environmental conditions for perennial plantations. However, only 2% of the total used agricultural area is under orchards and 1% each under vineyards and olive trees. The paper analyzed the data of commercial farms of the FADN system in the type of olive growing, different types of perennial plantations, viticulture and wine growing, and fruit growing. As the most competitive, there was viticulture and wine type of farm, achieving two and a half times higher net added value compared to the European average. Farms in the fruit growing type achieve five times lower net added value than the European average and require additional efforts to increase productivity.

Keywords: FADN, olive growing, viticulture, fruit growing

ORIGINAL SCIENTIFIC PAPER

Ekonomski učinci konzervacijske obrade tla

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Sažetak

Konzervacijskom poljoprivredom se nastoje ublažiti klimatske promjene, a konzervacijskom obradom tla poboljšati kvaliteta tla, optimizirati prinose i povećati profit. Istraživanjem su analizirani troškovi i koristi konzervacijske obrade tla u proizvodnji kukuruza. Za tu su se svrhu provodila tri različita sustava obrade tla (osnovna obrada tla oranjem, dubokim i plitkim rahljenjem) i gnojidba (konvencionalna i dvostruko manja) u kombinaciji sa primjenom poboljšivača tla i kalcizacijom. Istraživanjem CVP analizom je potvrđeno da ekonomičnost primjene konzervacijske obrade tla ovisi o sposobnosti realizacije prinosa koji su iznad prosječnih u našim proizvodnim uvjetima Panonske podregije Republike Hrvatske.

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

Uvod

U doba globalnih ekoloških problema je održiva proizvodnja hrane jedan od najvećih izazova. Problemi sa kojima se suočava suvremena poljoprivredna proizvodnja po pitanju klimatskih promjena su erozija tla, gubitak bioraznolikosti, smanjivanje biljne proizvodnje, nedovoljna proizvodnja krmiva, smanjena dostupnost i kvaliteta vode, gubitak plodnosti tla, zaslanjivanje tala, gubitak organskog ugljika u tlu (Jug i sur., 2022). Konzervacijska poljoprivreda je jedna od tehnoloških mjera prilagodbe i ublažavanja klimatskih promjena. Osnova konzervacijske poljoprivredne proizvodnje je gospodarenje postavljeno na tri temeljna postulata, koji kontekstualno objedinjuju klimu-tlo-biljku, uz uvažavanje agroekoloških i socio-ekonomskih razlika (Jug i sur., 2018). Konzervacijska obrada tla predstavlja način gospodarenja agrookolišnim sustavima s ciljem poboljšane i održive produktivnosti, povećanih profita i sigurnosti hrane. Primjenom konzervacijske obrade tla poboljšava se kvaliteta tla, optimiziraju prinosi i smanjuju troškovi poljoprivredne proizvodnje. Cilj ovog rada je utvrditi troškove i koristi konzervacijske obrade tla u proizvodnji kukuruza na eksperimentalnim parcelama.

Materijal i metode

U radu su analizirani podaci sa eksperimentalnih lokaliteta Čačinci i Križevci na kojima se provodilo istraživanje u 2021. godini u sklopu HRZZ projekta "Procjena konzervacijske obrade tla kao napredne metode uzgoja usjeva i prevencije degradacije tla – ACTIVEsoil". Izračun prihoda napravljen je prema visinama prinosa i prosječnoj prodajnoj cijeni neposredno nakon žetve. U prihode su uračunata i prosječna izravna plaćanja za ratarsku proizvodnju. Cijena koštanja je izračunata prema jednostavnoj djelidbenoj kalkulaciji pri čemu su svi troškovi prikazani u realnim iznosima prema cijenama na tržištu iz podataka Tržišno informacijskog sustava u poljoprivredi pri Ministarstvu poljoprivrede (TISUP). Troškovi proizvodnje dobiveni su množenjem utrošaka 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 dobiveni iz standarda (tehnoloških normativa) prema ranijim istraživanjima. Cijene osnovnih inputa i outputa za proizvodnju kukuruza dobivene su prema arhivi podataka TISUP-a. U prihode su uračunata i prosječna izravna plaćanja za ratarsku proizvodnju u iznosu od 2.250,00 kn/ha.

Trošak sata rada traktora i priključnih oruđa (Tablica 3) izračunat je za 2020. godinu, 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 utrošaka rada ljudi i strojeva dobiveni 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 do 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 i plitkim rahljenjem), dva načina gnojidbe (konvencionalna i dvostruko manja) s kojima je u kombinaciji primjena poboljšivača tla i kalcizacija. Prema tome su dobivene cijene koštanja kukuruza na razini zbroja ukupnih troškova mehanizacije (amortizacija i gorivo, usluga kombajniranja) i varijabilnih troškova (sjeme, gnojivo, zaštitna sredstva), bez uračunatih troškova vlastitog rada. Fiksni troškovi gospodarstva (najam, osiguranje i dr.) nisu uračunati. Uračunati su stvarno plaćeni troškovi za sjeme, gnojivo i zaštitna sredstva, bez obračuna PDV-a. Troškovi kalcizacije su proračunati kao ¼ ukupnog troška, budući se učinci kalcizacije pretpostavljeno protežu na 4 godine.

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 kukuruza

	Jedinica mjere	2020.	2021.
Sjeme kukuruza	HRK/ha	450	480
Mineralna gnojiva			
Urea	HRK/kg	2,48	6,34
KAN	HRK/kg	1,75	4,13
NPK 0:20:30	HRK/kg	3,10	6,55
GeO2	HRK/kg	6,42	6,42
Zaštitna sredstva			
Lumax	HRK/litri	118,50	120,00
Glyphogan	HRK/kg	51,00	54,00
Glifosat	HRK/litri	70,00	71,00
Adengo	HRK/0,1 l	16,61	16,65
Laudis	HRK/litri	410,00	410,00
Plavi dizel	HRK/litri	6,26	6,26
Prodajna cijena	HRK/kg	1,11	1,45

Izvor: TISUP

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

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

	Nabavna cijena, HRK	Godine korištenja	Amortizacija, HRK
Traktor (114 kw)	684.000	10	55.900,00
Traktorski plug	18.000	12	1.166,67
Traktorska sijačica	21.000	12	1.583,33
Razbacivač gnojiva	11.400	12	783,33
Atomizer	15.600	12	1.008,33
Prorahljivač	21.000	12	1.416,67

Prema shemi prikazanoj u materijalima i metodama, proračunati su troškovi sata rada traktora i priključnog oruđa.

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

	Iznos, HRK/sat
Oranje	313,04
Zatvaranje brazde	313,04
Dublje rahljenje	304,96
Pliće rahljenje	302,96
Predsjetvena priprema	295,53
Sjetva	295,53
Kultiviranje	285,77
Gnojidba	285,77
Kalcizacija	285,77
Zaštita	284,44
Prijevoz	327,25
Ostalo	284,44

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

Tablica 4. Troškovi mehanizacije na eksperimentalnim lokacijama (1 ha)

Lokalitet	Čačinci			Križevci		
Operacije	A1	A2	A3	A1	A2	A3
Oranje	1.355,14	1.355,14	1.355,14	1.355,14	1.355,14	1.355,14
Zatvaranje brazde	1.111,29	0,00	0,00	1.111,29	0,00	0,00
Rahljenje	0,00	1.067,34	908,87	0,00	1.067,34	908,87

Predsjetvena priprema	479,75	479,75	479,75	591,05	591,05	591,05
Sjetva	562,91	562,91	562,91	620,60	620,60	620,60
Kultiviranje	571,53	571,53	571,53	571,53	571,53	571,53
Gnojidba	595,35	595,35	595,35	595,35	595,35	595,35
Kalcizacija	637,87	637,87	637,87	637,87	637,87	637,87
Zaštita	853,32	853,32	853,32	853,32	853,32	853,32
Prijevoz	981,75	981,75	981,75	1.309,00	1.309,00	1.309,00
Ostalo	568,88	568,88	568,88	568,88	568,88	568,88
Ukupno	7.717,77	7.673,83	7.515,35	8.214,02	8.170,08	8.011,60

Na pokusnim poljima su zabilježene razlike prinosa zrna što izravno određuje vrijednost proizvodnje (Tablica 5). U strukturi troškova je najveći utjecaj troška mehanizacije, ali tehnološke razlike 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 kalcizacije i nakon toga poboljšivača tla. Kalcizacija i poboljšivači tla su glavni razlozi većih odstupanja u ukupnim troškovima pokusnih polja. U većini polja (10 od 12) gdje je primijenjen Geo2 dodatni trošak primjene je uzrokovao neekonomičnu proizvodnju. Proizvodnja je neekonomična na svim pokusnim poljima kod primjene kalcizacije.

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

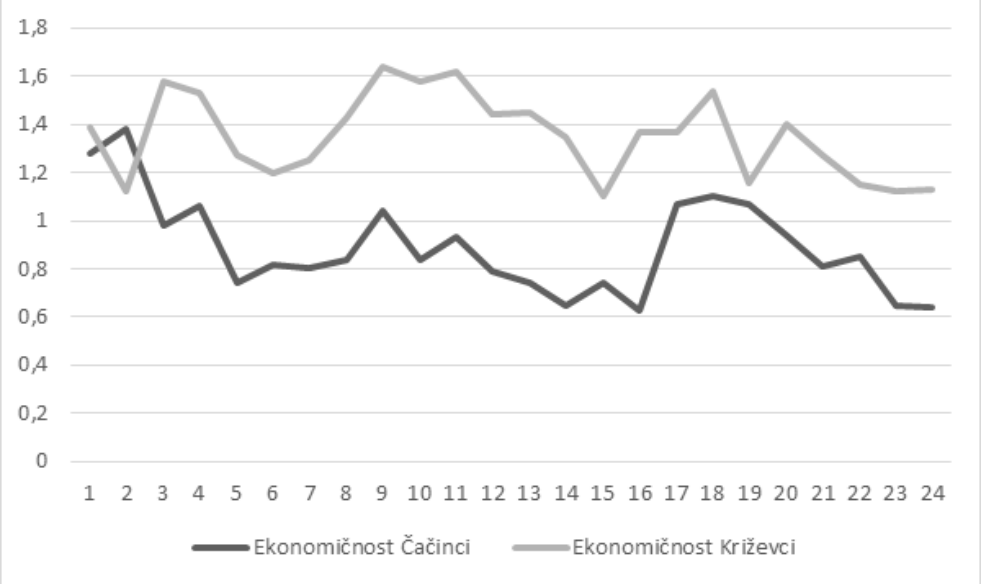
Polja/ Opis	Prinos zrna t/ha	Vrijednost proizvodnje HRK	Potpora HRK	Prihod HRK	Varijabilni trošak HRK	Doprinos pokriću HRK
1	9,46	13.719,05	2.250,00	15.969,05	12.515,65	3.453,40
2	8,76	12.700,86	2.250,00	14.950,86	10.851,11	4.099,74
3	7,54	10.926,18	2.250,00	13.176,18	13.445,65	-269,47
4	7,05	10.222,29	2.250,00	12.472,29	11.781,11	691,18
5	8,47	12.280,54	2.250,00	14.530,54	19.534,79	-5.004,25
6	8,58	12.448,11	2.250,00	14.698,11	17.870,25	-3.172,14
7	9,76	14.152,63	2.250,00	16.402,63	20.464,79	-4.062,16
8	9,32	13.513,87	2.250,00	15.763,87	18.800,25	-3.036,38
9	7,42	10.763,46	2.250,00	13.013,46	12.471,71	541,75
10	4,69	6.794,63	2.250,00	9.044,63	10.807,17	-1.762,54
11	7,06	10.238,52	2.250,00	12.488,52	13.401,71	-913,19
12	4,86	7.048,31	2.250,00	9.298,31	11.737,17	-2.438,86
13	8,37	12.129,81	2.250,00	14.379,81	19.490,85	-5.111,04
14	6,38	9.257,23	2.250,00	11.507,23	17.826,31	-6.319,08
15	8,84	12.814,38	2.250,00	15.064,38	20.420,85	-5.356,48
16	6,61	9.584,26	2.250,00	11.834,26	18.756,31	-6.922,05
17	7,57	10.977,35	2.250,00	13.227,35	12.313,23	914,12
18	6,49	9.416,08	2.250,00	11.666,08	10.648,69	1.017,38
19	8,21	11.906,73	2.250,00	14.156,73	13.243,23	913,50
20	5,94	8.615,70	2.250,00	10.865,70	11.578,69	-712,99
21	9,23	13.379,55	2.250,00	15.629,55	19.332,37	-3.702,83

22	8,75	12.680,62	2.250,00	14.930,62	17.667,83	-2.737,21
23	7,59	11.005,78	2.250,00	13.255,78	20.262,37	-7.006,59
24	6,69	9.695,52	2.250,00	11.945,52	18.597,83	-6.652,31

U svim poljima na lokalitetu Križevci, neovisno o primijenjenoj agrotehnici su ostvareni pozitivni doprinosi pokrića, odnosno proizvodnja je ekonomična (Tablica 6), što je najviše određeno višim prinosima zrna u odnosu na prethodno opisanu lokaciju.

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

Polja/ Opis	Prinos zrna t/ha	Vrijednost proizvodnje HRK	Potpora HRK	Prihod HRK	Varijabilni trošak HRK	Doprinos pokriću HRK
1	12,37	17.931,67	2.250,00	20.181,67	14.529,16	5.652,51
2	8,37	12.139,63	2.250,00	14.389,63	12.856,64	1.532,99
3	15,25	22.116,98	2.250,00	24.366,98	15.459,16	8.907,82
4	13,04	18.910,42	2.250,00	21.160,42	13.786,64	7.373,78
5	12,66	18.352,62	2.250,00	20.602,62	16.207,34	4.395,28
6	10,43	15.124,08	2.250,00	17.374,08	14.534,82	2.839,26
7	13,27	19.237,47	2.250,00	21.487,47	17.137,34	4.350,13
8	13,71	19.885,17	2.250,00	22.135,17	15.464,82	6.670,36
9	14,81	21.472,21	2.250,00	23.722,21	14.485,22	9.237,00
10	12,39	17.964,96	2.250,00	20.214,96	12.812,70	7.402,26
11	15,67	22.723,88	2.250,00	24.973,88	15.415,22	9.558,66
12	12,11	17.566,25	2.250,00	19.816,25	13.742,70	6.073,56
13	14,63	21.208,43	2.250,00	23.458,43	16.163,40	7.295,03
14	11,93	17.305,36	2.250,00	19.555,36	14.490,88	5.064,49
15	11,40	16.525,24	2.250,00	18.775,24	17.093,40	1.681,84
16	13,05	18.919,30	2.250,00	21.169,30	15.420,88	5.748,43
17	12,03	17.437,38	2.250,00	19.687,38	14.326,74	5.360,64
18	11,92	17.278,20	2.250,00	19.528,20	12.654,22	6.873,98
19	10,61	15.380,99	2.250,00	17.630,99	15.256,74	2.374,25
20	11,54	16.729,50	2.250,00	18.979,50	13.584,22	5.395,28
21	12,49	18.115,49	2.250,00	20.365,49	16.004,92	4.360,57
22	9,85	14.279,58	2.250,00	16.529,58	14.332,40	2.197,18
23	11,49	16.662,05	2.250,00	18.912,05	16.934,92	1.977,14
24	10,36	15.024,36	2.250,00	17.274,36	15.262,40	2.011,96



Grafikon 1 Kretanje koeficijenta ekonomičnosti po lokacijama i pokusnim poljima
Izvor: Vlastita kalkulacija

Zaključak

Na temelju ostvarenih rezultata u prvoj godini istraživanja može se zaključiti kako konzervacijska obrada tla može biti dobra mjera za ublažavanje klimatskih promjena u suvremenoj ratarskoj proizvodnji, ali ekonomičnost njene primjene ovisi o sposobnostima ostvarenja prinosa iznad prosječnih u uvjetima Panonske podregije Republike Hrvatske. U protivnom, neke mjere koje se primjenjuju s konzervacijskom obradom tla (kalcizacija i primjena poboljšivača tla) predstavljaju dodatni trošak koji rezultira gubicima proizvodnje.

Napomena

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Cost-benefit analysis of conservation soil tillage

Abstract

Conservation agriculture aims to mitigate climate change, while conservation soil tillage improves soil quality, optimizes yields and increases profits. The research analyzed the costs and benefits of conservation soil tillage in corn production. For this purpose, different agrotechnics (tillage by plowing, deep and shallow undermining) and fertilization (conventional and twice less) were carried out in combination with the application of soil improvers and calcification. The research has confirmed that the economics of applying soil conservation tillage depends on the ability to realize yields that are above average in our conditions.

Keywords: conservation soil tillage, cost, efficiency, income, maize

Application of the LEADER Approach in Western Balkan Countries - Challenges and opportunities in the framework of the regional cooperation and EU integration perspective

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Abstract

The development of the wider rural economies is one of the major challenges of the Western Balkan countries/territories in preparing rural communities to shape their own future. The objective of this paper is to assess the current state of LEADER-like initiatives in WB countries/territories, understand the state of the affairs and identifying specific national challenges and needs using a comparative framework. Desk research was used to collect existing secondary data and information on LEADER initiatives in project countries/territories. A content analysis, interviews with identified resource persons and case studies were used to illustrate the diversity of institutions, policy approaches, types of partnerships and practices related to LEADER in WB countries/territories. The findings reveal that Western Balkan countries/territories have made substantial progress in aligning their long-term strategic documents and administrative structures with the EU requirements. However legal, institutional and financial fragmentation remains a challenge for the development of LEADER in Western Balkans.

Keywords: LEADER, Local Action Groups, Western Balkans, rural areas.

Introduction

The LEADER approach (in French Liaisons Entre Actions de Developpement de ÍÉconomie Rurale, Links between actions for the development of the rural economy) is the main program implementing the rural development policy of the EU. The program builds on recent theories of neo-endogenous development (Cañete et al, 2018) supporting the ideology of participative democracy and using various principles of development namely bottom-up approach, multi-sectorial approach, public–private partnerships, inter-territorial cooperation and networks, use of endogenous resources, innovation diffusion economic diversification. LEADER remain a cost-effective measures Rural Development and Agricultural Fund contributing to the empowerment of rural population to participate in the Local Action Groups for a long-term sustainable growth in rural areas.

This approach has already attracted the interest of the local stakeholders in the process of creating a local partnership between the public, the private and the civil sector, but also the interest of the governments. After 2004 a mainstreaming of LEADER was witnessed in European rural space as a key approach to stabilization and development of rural areas (Dax et al., 2016) the LEADER. In the current EU programming period, Community-led Local Development (CLLD) was introduced to promote an integrated approach to territorial development (Servillo and de Buijn, 2018).

In EU the LEADER functions under conditions of limited capital resources and high levels of migration with the

consequent ageing of the population (Cañete et al, 2018). In order to save the environmental, social and economic sustainability of rural areas, EU's Regional Policy and Rural Development Policy is applied using several measures. In Candidate countries, LEADER is becoming an integral part of the IPARD in the Western Balkans. The major challenges of the Western Balkan countries/territories is the governance support. EU accession process is at various stages, therefore countries/territories commitment to rural development differ and depends on progress made under IPARD, own national agriculture and rural development programmes and financing as well as local government engagement in territorial and participatory processes.

There exist a very limited literatures assessing the progress that the Western Balkan countries/territories have achieved in adjusting their rural development policies to the CAP, the paper presents a comparative analyses of the current state of LEADER-like case studies in WB countries/territories. Considering the limited comparative analyses (Konečný, 2018; Chevalier et al., 2017; Dax and Oedl-Wieser, 2016) the study fills the existing gap by describing the state of the affairs and identifying specific national challenges and needs as well as illustrate LEADER approach as perceived or experienced in Western Balkans.

The reminder of the paper is organised as following. Section two describes the review of literatures. Section 3 describes the material and methodological approach. Section 3 present the main results of the study. Section 4 summarises the main conclusions and recommendations.

Theoretical review

LEADER is supported by theory of neo/endogenous development (Ray, 2000) where extra-local factors are of primary importance for enabling communities in aiming for a better future. The basis for sustaining growth are both the exogenous and endogenous institutions of rural development (High and Nemes, 2007).

The basic principles of LEADER are questioned by few authors. Canette et al (2018) is critic on the priority given to the main underlined value of LEADER. According to the authors territorial efficiency prevailed over territorial equity. Neoliberal ideology and "economists" dominance as well as limited funding has weakened the role of LEADER in addressing territorial inequalities, especially in remote areas which are subject to population ageing. Empirical studies show that LEADER is not implemented consistently among all EU members. Konečný (2018) identifies differences in the implementation of this method between the old and new member states, mainly related with the length and experience in EU membership and access to LEADER. The difference lies in the institutional embeddedness, scope, way of implementation and impact. Polleramn et al (2020) argue that the differences in implementing LEADER are related to the countries socio-economic structures and different concept given at local and/or rural institutional settings at the local level. In post-socialist members of European Union the LEADER is seen as a financial source rather than as a platform of communication (Konečný, 2018) or social innovation (Navarro et al, 2018).

In the case of Western Balkans, there is lack of literature in observing the LEADER implementation and the operational performance of Local Action Groups (LAGs). Few authors has been focusing in documenting the initiatives on LEADER during the recent decade. Earliest literature provided by Hoti and Kurti (2012) and Tahiraj (2013) explored the development of LAGs in Albania in the beginning of 2010. Krasniqi (2020) found that in Kosovo the development of the LEADER is yet at an early stage. Both articles identifies the dynamic development and the potential perceived by local actors and donors (central and local government are yet not aware of the importance), however this development is expected to lose if financial incentives are not in place. The absence of funding make relations and presence of stakeholders inactive. The comparative findings presented at this article fills a large gap on regards to the literature studying LEADER implementation in Western Balkans and using a common assessment framework provided a comparative analysis of the current situation. In addition, it adds empirical evidences to the existing literature observing the case of post-socialist countries.

Material and methods

The study is based on the use of qualitative methods. The research design is based on various steps. Desk research was used to collect existing secondary data and information on LEADER initiatives in project countries/territories. A content analysis was performed to examine how the LEADER and/or certain aspects of it (LAGs, social networks, local partnerships, territorial development etc.) are treated by national strategic, programming and regulatory documents. To provide an overall insight into the policy and legislative framework related to the LEADER initiative, the desk research involved assessment of relevant policy documents of all the line ministries responsible for/in charge of dealing with certain matters.

Primary data collection was done based on various methods. Two participatory workshops were used to consult and collect information from policy makers and practitioners. In addition, in order to obtain primary data and to ensure that all relevant information was covered, interviews were carried out with identified resource persons. In order to document the interviews, asemi-structured questionaire was used, where participants were encouraged to discuss all issues related to LEADER they considered important. Sampling was purposive. The resource people were selected in light of their responsibility and position, knowledge level or practical experience from the field, leadership role and influence, control over information (representative[s] of the Ministries of Agriculture or other relevant Governmental bodies, representatives of LAG-like partnerships and other practitioners).

Case studies were used to illustrate the diversity of institutions, policy approaches, types of partnerships and practices related to LEADER in WB countries/territories and experiences gained by policy makers, experts and stakeholders. To ensure a balanced sample of case studies, the themes were not predefined. Case studies were documented and structured in order to allow for comparative assesment. Data analyses was done based on content analysis of the quesitonaires filled and meeting notes from participatory meetings.

Results

The policy, institutional, legal and financial settings of LEADER in Western Balkans

The **hierarchy of strategic documents** governing the implementation of the LEADER programme in Western Balkan countries/territories includes: i. strategies for agriculture and rural development, ii. mid-term programmes, iii. IPARD II programmes and 4) strategic and/or programming documents of other ministries or governmental bodies, most of which are in charge of regional development, local self-governance and civil society. It has been already stated in previous reports on agricultural policies in the Western Balkan countries/territories (WBC) (Volk et al, 2017), that all of them have made substantial progress in aligning their long-term strategic documents and administrative structures with the EU requirements. All the newly developed Strategies for Agriculture and Rural Development in Western Balkans countries/territories recognise the importance of LEADER and envisage measures and activities to enhance its implementation.

With regard to the format and content of the local strategies for rural development, Macedonia and Kosovo* are the only countries where these issues are regulated by rulebooks. Based on the information provided in the national reports, only in Serbia attempts have been made to introduce the Law on the Planning System. The Law is drafted but has not been adopted yet.

The legal settings of LEADER in Western Balkans

The legal frame for the implementation of LEADER initiatives in WB countries/territories is composed of numerous laws and regulations within three pillars: a) providing the legal basis for LEADER-like support measures, b) the regulations related to the establishment of LAGs, and c) the regulatory base for the formulation of strategic and planning documents. Although significant progress has been made in developing the necessary regulatory framework, in almost all countries there are legislative gaps and lack of procedures in regard to LEADER.

The legal framework for implementing the LEADER measure in all countries is defined by laws on agriculture which provide the basis for the policy formulation. For the time being, in Albania neither the national nor the local government authorities have adopted procedures that facilitate LEADER-like actions and development of LAGs. A part of the countries has implemented laws on LAGs. For instance, in Albania Law no 36 of 14.04.2022 on „Organisation and functioning of LAGs“ define the main sources of support. Yet in IPARD II no support was provided. However, the rulebooks that govern the registration of LAGs, the methodology for preparing local strategies for rural development and their content, are still not developed in most countries, with the exception of Macedonia and Kosovo*. The preparation process for the necessary documents is in progress in Serbia.

Due to the absence of rulebooks on LAGs, the majority of existing local partnerships in the region (except Macedonia and Kosovo*) are registered in accordance with the national Law on Associations, as non-profit associations. This regulatory gap has resulted in a situation where it is not uncommon to find registered partnerships proclaiming themselves as “Local Action Groups”, i.e. using the term as a part of their official names. Apart from this, national experts have raised concerns that this type of entity is not likely to be suitable to represent “real” LAGs, due to the constraints related to fiscal burdens, governing structures etc.

There are a range of laws in WB countries/territories regulating certain aspects of intra and inter-municipality co-operation (including cross-border), and among actors from the civil society, public and private sectors, among which the most relevant ones are the Law on Local Self-government and the Law on Regional Development (both existing in all countries). The Law on Local Self-government provides the legal basis for local government units and inter-municipal co-operation in performing activities of common interest. The Law on Regional Development sets up the legal and institutional frame for planning, coordination and realization of development activities and support to inter-municipal, inter-regional, cross-border and international co-operation.

The instititional settings of LEADER in Western Balkans

The main authorities dealing with RDP, and hence LEADER-like activities in WB, are the national ministries of agriculture (MA) with their organizational units: i. departments responsible for programming (i.e. acting as a managing authorities) and ii. paying agencies as executive bodies responsible for the financial management of RDPs. In addition to MAs, in some countries there are other entities at a regional level mandated to manage certain activities related to LEADER:

- In Albania and Serbia there are Regional Development Agencies (RDA) running the networking activities among local and regional stakeholders, being also eligible to elaborate and to implement local development strategies in the respective regions.
- In Bosnia and Herzegovina, the main responsibility for implementation of LEADER-like activities lies with the cantonal ministries (divisions).
- In Serbia, the Provincial Secretariat of Agriculture of the Autonomous Province of Vojvodina is responsible for planning support measures, as well as for coordinating the work of LAGs on its territory.

The financial settings of LEADER in Western Balkans

The funding of the LEADER Approach is of particular importance in the Western Balkan countries/territories. However, the state of affairs differs from country/territory to country/territory and is not always related to the accession progress or candidate status. In the period of the current assessment, the funding of LEADER-like projects comes from different sources such as national governments, local authorities, but mostly from the CSOs, foundations that are funded by different donors (Bogdanov et al, 2018).

Even though the strategic and programme documents of the National Ministries recognize the importance of LEADER, the analysis of the budgets allocated to support LEADER-like activities shows that they are reluctant to invest for this purpose. Even in those places where such initiatives used to exist, funding from the state budget has ceased (Serbia and the Republic of Srpska), with the exception of Kosovo*.

International organizations, through donor projects, remain the dominant source of funding regarding the implementation of LEADER-like activities and the creation of LAG-like partnerships in the Western Balkan countries/territories. Major donor organizations involved in supporting the LEADER-like activities are the following: the European Commission, UNDP, USAID, SIDA, Swiss Development Cooperation, GIZ.

Table 1. Comparative analyses of strategic, regulatory, government and capacity development LEADER in Western Balkans

Country/ Territory	Strategic and programming framework	Regulatory framework		Government funding support	Capacity development		
		Rulebooks on selection of LAGs	Rulebooks on Local strategies		MoA	CSO Rural development networks established	LAG-like partnerships
Albania	Yes	No	No	No	No	Albanian Rural Development Network	4 LAG-like partnerships, operate as CSOs
Federation of Bosnia and Herzegovina	At a national, but not on entity level; low progress at cantonal level	No	No	No	No	"Rural development network in BiH"	3 LAG-like partnerships operate as CSOs
Republic of Srpska	Yes	No	No	No	No	"Rural Development Network of Bosnia and Herzegovina"	3 LAG-like partnerships operate as CSOs
Kosovo*	Yes	Yes	Yes	Yes	Yes	Network of Organizations for Rural Development of Kosovo*	30 LAG-like partnerships are formally established
Macedonia	Yes	Yes	Yes	No	No	Rural Development Network of the Republic of Macedonia	16 LAG-like partnerships operate as CSOs
Montenegro	In progress	No	No	No	No	No	Yet no LAG-like partnership
Serbia	Yes	In progress	In progress	Until 2013; Currently on the territory of AP Vojvodina	One person nominated to deal with LEADER	Two networks operate: The Network for Rural Development of Serbia and the National LEADER Network (NLN).	No reliable national data on the number of active LAGs or LAG-like partnerships

Source: Bogdanov et al, 2018.

The previous initiatives practicing LEADER-like activities in the WB countries/territories differ from country/territory to country/territory. The majority of the organizations currently performing as LAG-like partnerships were set-up within donor projects. As a result of donors' activities, considerable human resources have been trained and made aware about LEADER, regulatory documents have been drafted, and pilot activities on establishing LAG-like partnerships, preparing local strategies etc. have been carried out (Bogdanov et al, 2018). However, the number, capacities, structures and activities of these organizations differ considerably, depending on the specific country context.

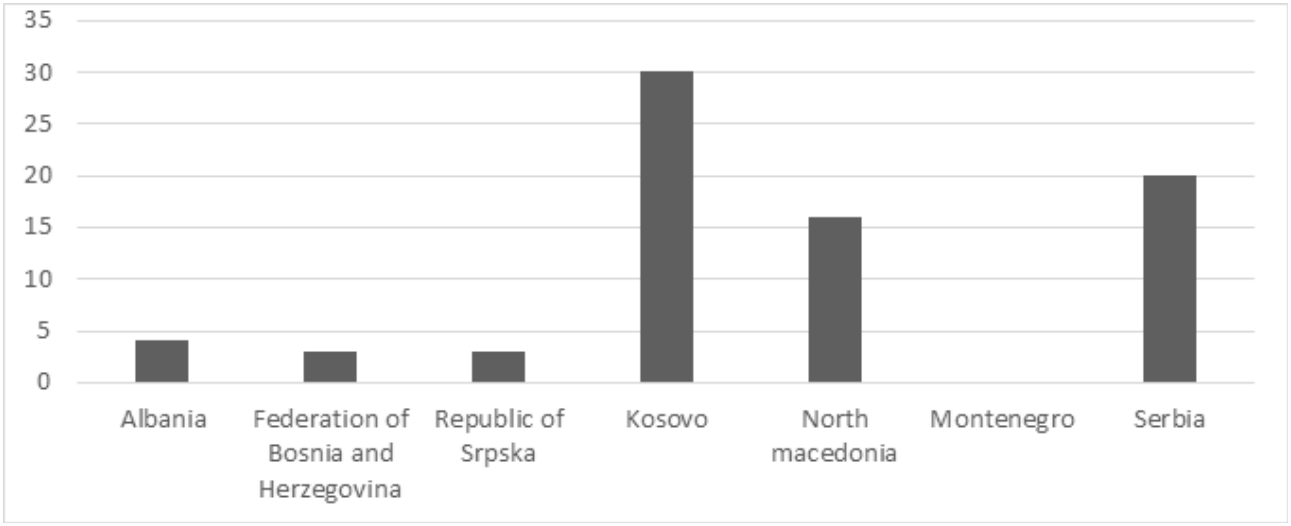

Figure 1. Number of LAGs existing partnership in Western Balkans in 2018
Source: Bogdanov et al, 2018.

In 2018 the situation in country level appeared as following. Kovovo, North Macedonia and Serbia appeared the countries with the largest number of LAG/like initiatives in 2018. At the rest of the countries, especially for the case of Bosnia and Herzegovina, Albania and Montenegro foremost, there are verz few initiatives following LEADER principles.

Conclusions

In Western Balkans the institutional, strategic and regulatory framework for introducing LEADER remains a prerequisite for the development of LAGs. LEADER is a part of the national strategic and programming documents and envisaged in IPARD. With the exception of Federation of Bosnia and Herzegovina, where the entities are responsible for the implementation of the LEADER programme on their territories, all the other countries/territories have adopted programs through which this support will be implemented.

The existing LAG-like partnerships in all countries/territories are registered as Civil Societz Organisations. The rulebooks on local development strategies are adopted only in Kosovo and Macedonia, while those in Serbia are in a process of being prepared. Considering the existing stage of legal development, the adoption of secondary legislation is needed, i.e. rulebooks and regulations on Local Action Groups encompassing guidelines for potential LAG constituents on their setting up, role, functioning and accreditation, including start-up toolkits for devising Local Development Strategies (LDSs).

The national ministries in Serbia and the Republic of Srpska had started to finance LEADER and LAG-like partnerships and eventually stopped funding; only the responsible Ministry in Kosovo is currently providing support. Most of the LEADER-like activities in all countries/territories up to now are supported by different donor organizations, including local resource organizations, local governments and networks. The support is usually limited in terms of sources of funding and the restricted timeframe, causing discontinuity in the process of implementing a full-fledged LEADER method. Moreover, the financial and technical support gap discourages stakeholders from remaining involved in LEADER, making it highly unlikely to mobilize enough local participation and support. This may especially affect the already established LAG-like partnerships which are still very new and fragile.

The newly formed LAG-like partnerships are only in the early stage of a process meant to fully respond to the needs and interests of the diverse rural stakeholders. The use of LEADER Measure in the IPARD III programming period remain a very important step to enhance capacity building. There is a permanent need for strengthening the local human capital capable of managing the LAGs, implementing Local Development Strategies, absorbing the funds dedicated to the LEADER measure in Western Balkan countries/territories. Local government is crucial for enabling coordination efforts, given the weak collective action in local level. Therefore, the pace of decentralisation and the empwoerement of Municipakities would be a second pivotal factor (after the creation of stable financial mechanisms) for the development of the LEADER.

The study is limited in illustrating the morphology of the LAGs in Western Balkans. The relational and power division as well as the institutional seetings in LAGs is crucial for observing socioeconomic differences in territories. Analzses using qualitative apporach (Gema and Masot, 2020) and use of participatory methods (Sisto et al, 2018) are required to explore the attitudes, opinions and expectations of LAGs members, considering their representation and interests in participating in the LAG.

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Session 3

Proceedings

Genetics and Plant Breeding

58 hrvatski 18 međunarodni simpozij agronoma

Zbornik radova

Genetika i oplemenjivanje bilja

Detection of GMOs in samples of food and animal feed sold in Bosnia and Herzegovina (BiH) market

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Abstract

Although according to the legislation in BiH, up to 0.9% of genetically modified (GM) ingredients in food is allowed, due to insufficient control, it is possible that food containing GM elements above the permitted threshold may be imported or even produced. In this paper, the presence of genetic modifications in random samples of corn, rice, soybean and animal feed products was analysed. A qualitative PCR method was used to determine the existence of traces of GMO. The research results of all food products are negative, which means that the samples did not contain GMOs, while the existence of genetic modifications was confirmed in all animal feed samples.

Keywords: genetically modified organisms, real-time PCR, soy, corn, rice

Introduction

Genetically modified organisms, are any organisms whose genetic material has been changed by methods of genetic engineering, usually by introducing genes from viruses, bacteria, plants or animals into an often unrelated species. Physiological barriers that naturally prevented this were overcome by new biotechnological achievements, enabling the transfer of genetic material between literally all living organisms (Maghari and Ardekani, 2011). Although fear and skepticism about new technologies in the production of food for humans and animals is extremely high in Europe, some countries have decided to allow the cultivation of genetically modified crops. However, the trend of cultivating GMOs in the EU has been declining since 2013-2015 when five countries cultivated GMOs (Spain, Portugal, Slovakia, Czech Republic and Romania), while in 2021 GMOs are effectively cultivated only in Spain (Dabrowska-Klosinska, 2022). BiH legislation allows not to label a product as a GM product, if traces of GMOs do not exceed 0.9% for each food component or for food consisting of one ingredient, provided that it is secondary or technically unavoidable. This means that BiH has adapted to EU laws, which set this threshold. Maize, soybeans and rice are among the most commonly modified crops and the three most popular kinds of GM plant food (with a share of 79.38%) in research studies (Shen at al., 2022). Testing human and animal food samples containing these crops can often yield positive results. The most sensitive and reliable method of detecting GMO in food is the polymerase chain reaction (PCR) method (Holst-Jensen et al., 2003). With this method specific sequences of DNA are amplified using a pair of short DNA sequences (primers) that flank the region to be amplified (Gachet et al., 1999). There is a large number of different genes of interest that are used for transfer to targeted organisms. Therefore, instead of searching for a specific gene, it is simpler to search for a sequence that contains promoters or terminators widely used in the creation of gene constructs. The most commonly used promoter of transferred gene transcription, in the target organism, comes from cauliflower mosaic virus (CaMV P35S), and the most common terminator is TNOS from the bacterium *A. tumefaciens* (Želježić D., 2004).

There are two main PCR analytical methods for detecting GMOs in food. Qualitative (which gives the answer as to whether or not there are GMOs in the sample) and quantitative method that tells what the percentage of GMOs is in the food sample (Schreiber, 1999).

In this paper, an analysis of food samples from BiH market was carried out using real-time qualitative PCR method in order to determine whether they contain and/or come from and/or consist of GMOs.

Material and methods

The analysis for the existence of traces of GMOs in food was carried out in the Laboratory for GMOs at the Federal Agro-Mediterranean Institute in Mostar na Buna, which is on the list of Authorized laboratories in Bosnia and Hercegovina for the examination, control and monitoring of genetically modified organisms and products that consist of, contain or they come from GMOs.

The samples for analysis were purchased randomly from the free sale from markets in BiH. Two samples of corn were taken: canned and polenta; three samples of rice: grain rice, crackers and integral rice flour; two soy samples: soy flakes and soy cream for cooking; three samples of animal feed, all containing soy, sunflower and corn.

DNA from food and feed samples was extracted using GMO Extraction Kit – ThermoFisher. The DNA isolated from the collected samples was subjected to a "Real time PCR" test, which amplifies DNA regions with GMO elements if the samples contain them. The qualitative PCR method for detecting the presence of GMOs in food and animal feed was made according to the TaqMan™ GMO Screening Kit which enables analysis of DNA extracted from food and feed samples for the presence of the main transgenic regulatory regions. This real-time PCR kit contains all reagents (except the template DNA) required for the detection of genetically modified organisms. In addition, the kit contains an Internal Positive Control (IPC) to identify possible PCR inhibition.

During the analysis, four real-time PCR reactions were performed. Each of these reactions amplifies two independent regions in such a way that a single multiplex PCR reaction uses two channels in the thermal cycler (FAM and VIC). These amplification reactions are described as: a) P35S/CaMV reaction uses two Taqman probes, one labeled with FAM that detects the CaMV P35S regulatory element, and the other VIC that detects specific amplification of CaMV virus genom region; b) TNOS/*A. tumefaciens* reaction uses two TaqMan probes. FAM- detects a TNOS regulatory element transcript, while the other VIC detects a transcript from a genomic region specific to the bacterium; c) P34S/FMV reaction includes two TaqMan probes: FAM – amplification of P34 S from Figwort Mosaic Virus (FMV), and the other VIC – detects amplification of a genomic region specific to the virus; d) PLANT/IPC also includes two TaqMan probes: FAM – detects plant DNA, VIC – detects internal positive control used to exclude the presence of inhibitors in the sample.

Results and discussion

The results (Table 1.) show that no food sample contains transgenic materials while transgenic materials were detected in all feed samples which is not strange considering that in Bosnia and Herzegovina there is a ban on the import of GMO food for humans, while the import of GMO soybeans for feeding livestock is allowed. The world market is saturated with feed containing GMOs. Up to 90% of world produced GMO crops (in the USA up to 95%) are used for livestock feed (Lucht, 2015).

Although this research did not find samples of food that were positive for GMOs, one study in Bosnia and Herzegovina showed positive GM elements in different soybean samples, some of which were labeled as "GMO free" (Čosić et al., 2020) and in another research 19 samples were positive: four samples of soybean meal and six samples of feed mixtures imported from abroad, as well as nine samples of soybean that were taken from fields in the entity of the Republic of Srpska (Trkulja et al., 2015). This is probably due to poor control of the import of food and seed material in Bosnia and Herzegovina.

Table 1. The results obtained after Real-Time PCR amplification

Sample	Plant Master Mix		P35S Master Mix		TNOS Master Mix		P34S-FMV Master Mix		Interpret.
	Plant	IPC	P35S	CaMV	TNOS	*A. tum.*	P34S	FMV	
1.Animal feed	+	+	+	-	+	-	+	-	GMO detected
2. Polenta	+	+	-	-	-	-	-	-	No GMO detected
3. Rice flour	+	+	-	-	-	-	-	-	No GMO detected
4. Animal feed	+	+	+	-	+	-	+	-	GMO detected
5. Animal feed	+	+	+	-	+	-	+	-	GMO detected
6. Soy flakes	+	+	-	-	-	-	-	-	No GMO detected
7. Rice crackers	+	+	-	-	-	-	-	-	No GMO detected
8. Grain rice	+	+	-	-	-	-	-	-	No GMO detected
9. Caned corn	+	+	-	-	-	-	-	-	No GMO detected
10. Soy cream	+	+	-	-	-	-	-	-	No GMO detected

Producers of the positive tested feed samples are unknown and there was no label indicating that this was a GMO product. Without quantitative PCR analysis, we cannot be sure that these samples are within the permissible threshold of 0.9%. If it is determined that it is above the threshold, it is up to the authorities to establish better quality control in the production, import and labelling of animal feed.

The main goal of introducing mandatory labeling is to inform consumers and users about the product, so that consumers will be able to protect their basic right to choose, that is, they will be able to make their own decision whether or not they want to buy and consume food containing GMOs. The same rules regarding the obligation of monitoring and labeling (declaration) also apply to animal feed (including various types of concentrated animal feed containing GM soybeans), in order to provide farmers with accurate information about the composition and properties of the animal feed that they use for feeding domestic animals.

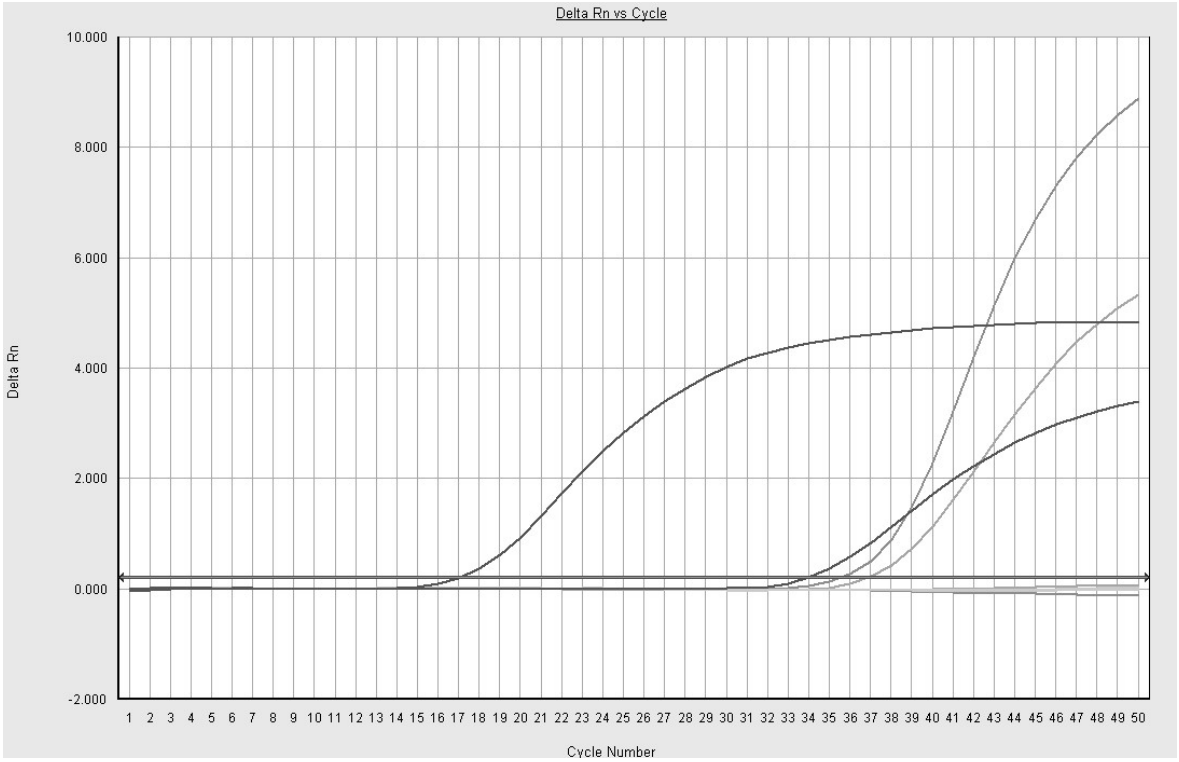

Figure 1. The example of detected GMO in animal feed sample. Fluorescence-labelled probes are amplified and reaction can be seen as distinct curves

Conclusions

There was no GMO traces found in samples of food collected randomly from market shelfs in Bosnia and Herzegovina. All samples of animal feed tested positive to GM events. There is a need for constant monitoring of food for GMO ingredients, so that the production and/or importation of GMO food can be prevented.

Regarding GMO animal feed, importation is allowed, but cultivation should not be carried out according to BiH laws, so fields suspected of such production should be controlled. Imported animal feed should be constantly monitored in order to determine its adequate labeling. Also, producers should be regularly checked for the content of GMOs in the feed they produce, as well as the correct labeling of the same.

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Session 4

Proceedings

Biodiversity Conservation

58 hrvatski 18 međunarodni simpozij agronoma

Zbornik radova

Očuvanje bioraznolikosti

Plant genetic resources conservation: case study of *Teucrium* spp. in Croatia

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Abstract

The genus *Teucrium* is a source of beneficial bioactive phytochemicals and its use is well known in the traditional medicine of many countries. Studies on the wild populations of *Teucrium* species are scarce. Croatian flora numbers 12 accepted species of this genus, including a recently discovered hybrid between *T. montanum* and *T. polium*. This study aimed at sampling of *T. montanum* and *T. polium* in Split-Dalmatia County in an area where the two species grow in sympatry and the occurrence of hybrids is more likely. We collected six populations of both *T. polium* and *T. montanum* and found potential hybrids at five sites. The goal of our future research is to confirm the hybrids at the genetic level. This work contributes to *in situ* conservation by providing new insights into the status of wild populations of *Teucrium* species, supports *ex situ* conservation by including seed accessions to the Collection of Medicinal and Aromatic Plants; and enables their further characterization and evaluation for use in future breeding programs.

Keywords: biodiversity, medicinal and aromatic plants, germander, Lamiaceae, ecogeographical survey

Introduction

Croatian flora is one of the richest in Europe, owing to its particular geographical position on the border between the continental and Mediterranean areas, separated by a higher mountain belt. One of the most numerous in species is the Lamiaceae family, which is also considered one of the richest in medicinal plants (Nikolić and Rešetnik, 2007). Among them are the species of the genus *Teucrium*. The genus includes perennial shrubs, subshrubs and herbaceous perennials, some evergreen, rarely annual or biennial, growing mostly on open rocky soils with plenty of sun.

Although this genus is cosmopolitan with more than 250 species, it is mainly distributed in the Mediterranean area (96% of the total number of species), throughout Europe and in Central Asia (Euro+Med, 2006; Gagliano Candela et al., 2021). The East Mediterranean seems to be the centre of origin of the genus, while the Western Mediterranean is its recent centre of speciation (Navarro and El Oualidi, 2000).

The genus *Teucrium* in Croatia includes 12 accepted species (Table 1.), including one hybrid (*T. x rohlenae* K. Malý), which was recently recorded in the Croatian flora, and a total of seven subspecies. One of the species is endemic to the Balkans (*T. arduinii* L.) (Nikolić, 2022), while others are distributed in various parts of the Europe, N Africa, Middle East and W Asia (Euro+Med, 2006).

The species of the genus *Teucrium* are mainly used in traditional medicine of the Middle East and the Mediterranean region, as well as in some Balkan countries. Since ancient times, the species of this genus have been used against a wide range of diseases (including gastrointestinal, respiratory, urinary, circulatory, diabetes, etc.), due to their biological properties, such as antimicrobial, anti-inflammatory, antispasmodic, anti-malaric, etc. However, the negative side effect of excessive consumption has also been recognized, as the presence of neo-clerodane diterpenoids can lead to liver damage and should therefore be avoided (Gagliano Candela et al., 2021).

Table 1. Species of genus *Teucrium* in Croatia.

	Distribution	
	Croatia	Entire area
Teucrium arduinii L.	coast and its hinterland, southern islands	Balkans
Teucrium aureum Schreb.	very rare	W Mediterranean
Teucrium botrys L.	sporadical records throughout Croatia	W Mediterranean, Balkans, UK, NW Africa
Teucrium chamaedrys L.	abundant throughout the whole Croatia	Mediterranean, middle Europe, Balkans, NW Africa, W Asia
Teucrium flavum L.	entire coast, hinterland and islands	Mediterranean (E to W), NW Africa
Teucrium fruticans L.	entire coast and islands, but less frequent	W Mediterranean, NW Africa
Teucrium marum L.	Lika County (only one record)	middle Mediterranean countries (France, Italy, SE Balkans), Tunisia
Teucrium montanum L.	coast, hinterland, islands at higher elevations	Mediterranean and middle European countries, Balkans, Middle East
Teucrium polium L.	coast, hinterland, islands, (abundant)	W Mediterranean, N Africa SW Asia
Teucrium scordium L.	coast, hinterland, islands and continental Croatia	almost whole Europe (except far N), Middle East, and N Africa
Teucrium scorodonia L.	higher mountains	W, middle and N Europe, Balkans, Tunisia
Teucrium x rohlenae K. Malý	Dalmatian hinterland (two records)	Montenegro

Although known in folk tradition for their medicinal properties, in Croatia they are among the less frequently used medicinal plants. Folk medicine of Bosnia and Herzegovina and Croatia mentions the use of *T. polium* and *T. arduini* for stomach disorders, *T. montanum* for liver and stomach disorders, *T. chamaedrys* for spleen disorders, cough, diarrhea, metabolic disorders and as a diuretic, *T. marum* for biliary disorders and *T. scordium* for diarrhea (Redžić, 2007).

Recent studies on chemical diversity continue to confirm the species of this genus as a valuable source of useful bioactive compounds. A phytochemical analysis of the essential oil of *T. montanum* and *T. polium* revealed that sesquiterpenes such as germacrene D, β-caryophyllene, and β-pinene are the most important compounds in the essential oils of the aerial parts (Zbiljić et al., 2021). Another study showed that *T. polium, T. flavum, T. montanum,* and *T. chamaedrys* have similar essential oil compositions characterized by a high content of sesquiterpenes with antiphytoviral activity (Bezić et al., 2011). In addition, the species *T. arduini, T. chamaedrys, T. montanum,* and *T. polium* are a rich source of various natural acetylcholinesterase inhibitors and antioxidants that may be useful in the prevention and treatment of Alzheimer's disease and other related disorders (Vladimir-Knežević et al., 2014). As aromatic plants, the insecticidal activity of *Teucrium* species essential oils has also been confirmed in several studies, focusing on *T. polium* (reviewed in Gagliano Candela et al., 2021).

There are very few systematic studies on natural *Teucrium* populations. In accordance with the scope of our project,

the research area of this study was limited to Split-Dalmatia County. Six *Teucrium* species have been previously recorded in this area (*T. scordium, T. fruticans, T. flavum, T. chamaedrys, T. montanum* and *T. polium*), the last two of which are among the most common (Nikolić, 2022). Also, ethnobotanical surveys revealed that along most of the Croatian coast, *T. montanum* is mainly used (under the name 'trava iva') in the form of an infusion as a panacea 'for cleansing the body' and also for common cold; while on the islands and in the lower coastal areas, where *T. montanum* is rare, *T. polium* is used instead under the same name (Luczaj et al., 2021). Otherwise, the habitats of *T. polium* and *T. montanum* overlap, just as their flowering time, from June to August. Both species are semi-woody, evergreen small shrubs.

Interestingly, two populations of individuals with intermediate morphological and anatomical characteristics of both *T. montanum* and *T. polium* were discovered relatively recently at two sites in Croatia, in Split-Dalmatia County. Previously, this taxon had been detected only at one site in Montenegro and described as *T.* × *rohlenae* K. Malý. All morpho-anatomical analyses supported the separation of two species, *T. polium* and *T. montanum*, and showed the intermediate position of the new natural hybrid in the flora of Croatia found at the two mentioned sites (Zbiljić et al., 2021).

Considering that we can expect hybrid individuals in all areas where *T. montanum* and *T. polium* grow sympatrically, the main objective was to investigate their potential habitats in the wider area of Split-Dalmatia County and to sample these species in order to gain deeper knowledge about their hybridization patterns and frequency. We focused on sampling populations of the species *T. polium* and *T. montanum* in parts of their distribution area where these two species grow sympatrically and the occurrence of hybrid individuals is more likely.

Material and Methods

Ecogeographical survey and collection of plant material of *T. montanum, T. polium* and *T.* × *rohlenae* for morphological and genetic analyses was performed within Split-Dalmatia County (Figure 1.). Prior to the collection missions the analyses of available data were undertaken, in order to find potential locations with the most suitable habitats for targeted species. The first collecting mission was carried out in June 2021. Besides *Teucrium* species, plant material of two other important medicinal and aromatic species (*Salvia officialis* L., *Helichrysum italicum* Roth.) was also collected if present at any of the examined locations, for further analyses. Voucher specimens were deposited in the Herbarium ZAGR of the Faculty of Agriculture, University of Zagreb.

Seeds collecting was performed during expedition in September 2022 and was carried out at confirmed localities as well as some newly found. Along with the seeds of *Teucrium* species (*T. montanum, T. polium*, *T. chamaedrys* and *T. flavum*), seeds of other available important medicinal and aromatic species (e.g. *Salvia officialis, Satureja montana* etc.) were also collected. After the expedition, seeds were cleaned out of the plant material and prepared for storage. Altogether 18 seed accessions were stored in the Collection of Medicinal and Aromatic Plants of the Department of Seed Science and Technology, University of Zagreb Faculty of Agriculture. Within the Collection, plant genetic resources of medicinal and aromatic plants are systematically collected, characterised, maintained, evaluated, documented and regenerated for future introduction into agricultural production and breeding programmes (Šatović et al., 2016). Recorded plant species locations were entered in Flora Croatica Database (Nikolić, 2022).

Results and Discussion

Within the area of Split-Dalmatia County, we found a total of six sites where both *T. polium* and *T. montanum* populations co-occur, and an occurrence of hybrids resembling *T. x rohlenae* at five of these sites (Figure 1). One additional location where all three species were found was only about 1 km distant from the previous site, so we assume it as the same population. The sites recorded in this study confirm two previously reported sites for Croatia (Zbiljić et al., 2021) and three that were not previously recorded. We sampled approximately 25 individuals per population of both *T. polium* and *T. montanum*, as well as an available number of hybrid individuals (1-25), depending on the site.

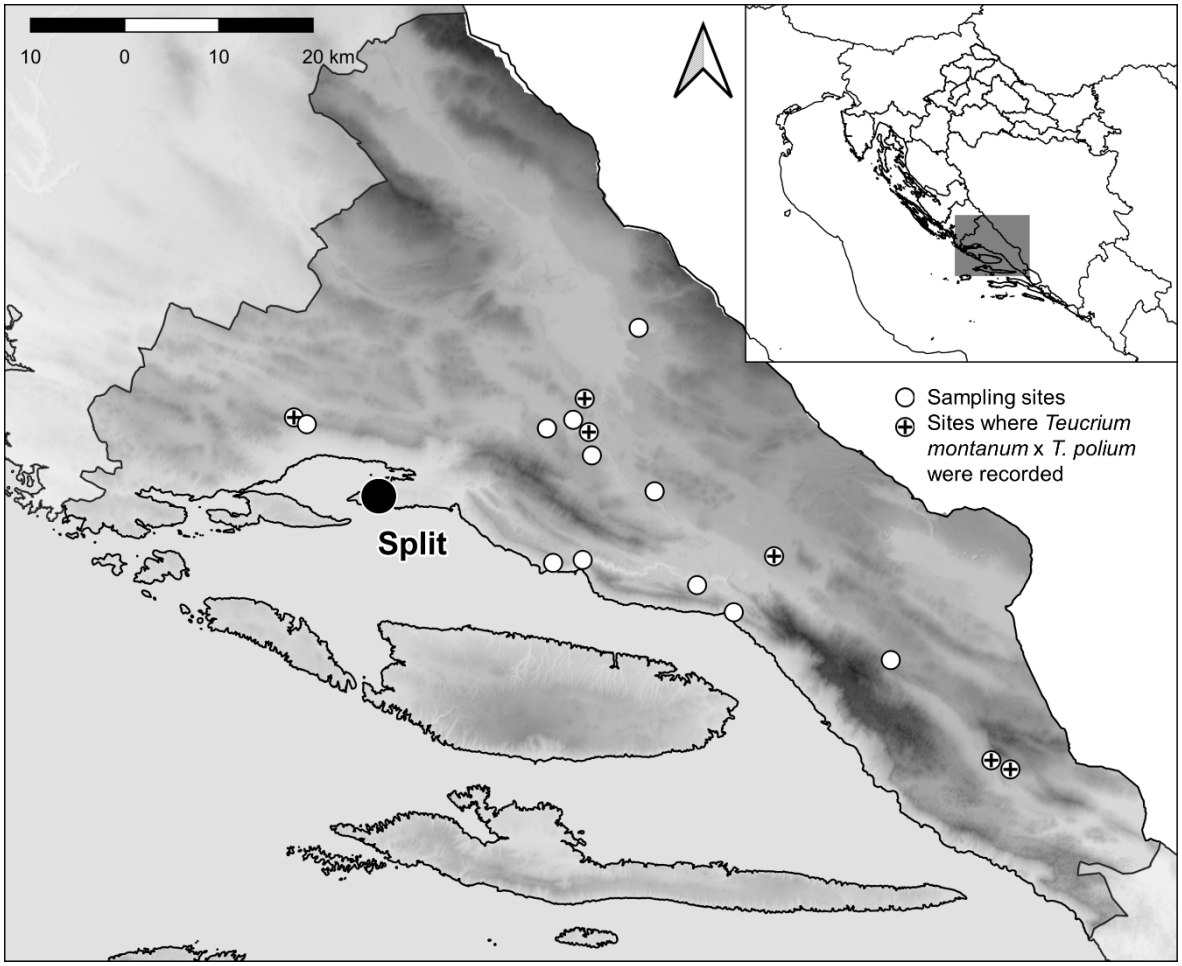

Figure 1. *Map of sampling locations in Split-Dalmatia County showing locations where putative hybrids were detected.*

The very high incidence of hybrids at sites where *T. polium* and *T. montanum* co-occur (five of the six sites) suggests that these two species hybridize frequently under natural conditions where their ecological requirements are met.

In addition, we also observed 'hybrid' individuals that were morphologically more similar to either *T. polium* or *T. montanum*. We hypothesize that this is the result of back-crosses between hybrids and their parental species. The goal of our future research is to confirm the hypothesis of the existence of hybrids and their back-crosses on the genetic level, using two complementary molecular methods: Simple Sequence Repeats (SSRs) and Amplified Fragment Length Polymorphisms (AFLP).

Zbiljić et al. (2021) performed detailed morpho-anatomical analysis, which indicated to the existence of natural hybridization between *T. polium* and *T. montanum.* This hypothesis was also confirmed by biochemical analysis of essential oil, which separated the two species *T. polium* and *T. montanum* and revealed the intermediate position of the putative hybrids (Zbiljić et al., 2021). On the other hand, some other studies show that the essential oil composition of the two species is not clearly distinguishable, as they contain similar compounds and a similar pattern of variability (Bezić et al. 2011; Marčetić et al. 2018). Therefore, more research on the phytochemistry of these species is needed. Natural hybrids may also have potential value for agronomy. Therefore, future research should focus on analyzing their phytochemical diversity and evaluating their potential for future breeding programs.

One of the long-term goals of plant genetic resource conservation and sustainable use of valuable species is their introduction into agricultural production. Currently, there are only a few studies documenting the cultivation of *Teucrium* species in Europe and Mediterranean countries, and these are related to their use as ornamental plants, due to their decorative properties. Some *Teucrium* species are used as ornamentals with functional benefits, e.g., *T. fruticans* has been shown to be a salt-tolerant species while retaining its ornamental value (Cassaniti et al. 2009).

T. scorodonia is cultivated in many places as an ornamental plant in gardens and also naturalizes in some regions (Gagliano Candela et al., 2021). To the best of our knowledge, there is no organized cultivation in Croatia. However, there is a potential for cultivation of the species of this genus, which can be used both as an ornamental and aromatic plants.

Conclusion

The main result of this study was finding of hybrid individuals between *T. montanum* and *T. polium* at three new sites in Split-Dalmatia County.

The professional contributions of this work include a broader knowledge of different *Teucrium* species and the status of their natural populations valuable for *in situ* conservation. In addition, this study contributes to *ex situ* conservation by including the collected accessions in the Collection of Medicinal and Aromatic Plants and thereby enabling their further characterization and evaluation, as well as their use in future breeding programs, taking into account the potential for cultivation of Croatian native species.

Acknowledgments

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Germination of *Salvia sclarea* L. accesions from the Collection of Medicinal an Aromatic Plants

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Abstract

The aim of the work was to determine the germination and germination energy of 32 clary sage (*Salvia sclarea* L.) accessions collected in the period 1998-2009 and preserved in the Collection of Medicinal and Aromatic Plants of the Department of Seed Science and Technology, University of Zagreb Faculty of Agriculture. The highest germination was observed in accessions which have been preserved since 2009 (90 and 94 %) and 2008 (76, 70, 68 and 62 %). It can be concluded that clary sage seeds have very good germination, that they tolerate storage very well, that they have been stored in good conditions and that regeneration in plant gene banks should not be carried out often.

Keywords: clary sage, germination energy, seed conservation, genebanks

Introduction

Plant productivity in agroecosystems is determined by seeds, which are the most important criterion for plant existence and dispersal, and rapid seed germination, which ist is the main criterion for plant establishment and the most important stage in the plant life cycle (Bentsinka and Koornneef, 2008; Sedghi et al., 2010). For accessions kept in the gene banks and seed collections, it is important to regularly check the germination of the preserved accessions (Government of the Republic of Croatia, 2021). Since many plant species as well as certain local plant populations are threatened with extinction due to various disasters and the replacement of local populations by new, high-yielding cultivars, plant gene banks have been established worldwide (NN 89/02). The Croatian Bank of Plant Genes (HBBG) was established in 1991 as a national project to organise activities related to conservation and sustainable use of plant genetic resources in the Republic of Croatia (Kolak and Šatović, 1996; Kolak et al., 2004). Different climate, relief and soil types in Croatia are the main reason for the richness of Medicinal and Aromatic Plant (MAP) species, whose natural populations have a great biological diversity at the morphological, biochemical and genetic levels, and whose collection and use has a very long tradition in Croatia (Šatović et al., 2016). The basic MAP collection was established in 1998 at the Department of Seed Science and Tecnology, University of Zagreb Faculty of Agriculture while the Working Group for Medicinal and Aromatic Plants was established in 2006 (Šatović et al., 2016; CPGRD, 2022). Within this collection, plant genetic sources of medicinal and aromatic plants are systematically collected, characterised, preserved, evaluated, documented and regenerated for introduction into agricultural production and breeding programmes (Šatović et al., 2016). The procedure for inclusion in the collection includes cleaning, moisture determination, drying, vitality testing, packaging and registration. The accessions are stored in classical ex situ rooms for medium-term seed storage, in a cold chamber at + 4 °C, in three-layer bags or jars. The vitality of the seeds of each accession is regularly monitored and, if necessary, regeneration is carried out (Šatović et al., 2016). In total, more than 4,411 accessions are stored in the HBBG, of which 2,604 are medicinal and aromatic plants (CPGRD, 2022). About 80 % of the collected material is wild material of Croatian origin, taken during collection missions or donated, while foreign samples come from other national gene banks, botanical gardens and research institutes (Brezovec et al., 2006; Šatović et al., 2016)

Clary sage (*Salvia sclarea* L.) is a biennial or perennial aromatic medicinal plant belonging to the Lamiaceae family (Peana and Moretti, 2002). It is cultivated for its high-quality essential oil, which is used in the perfumery and cosmetics industries, as a flavour enhancer, in tobacco products and in the production of liqueurs (Carrubba et al., 2002). Although cultivation in the Mediterranean region has been documented for centuries, the popularity of

this plant has increased in recent years (Yaseen et al., 2014). The growing demand for clary sage partly reflects its therapeutic properties, which include the ability to lower blood pressure, treat anxiety, stress, menstrual disorders and various skin conditions (Džamić et al., 2008; Yang et al., 2014). Numerous studies have found that the essential oil of the aerial part of the plant is rich in linalool and linalyl acetate, which have antioxidant, antimicrobial and cytotoxic effects (Tadtong et al., 2012).

The aim of the work is to determine the germination and germination energy of clary sage (*Salvia sclarea* L.) accessions from the Collection of Medicinal and Aromatic Plants of the Department of Seed Science and Technology, University of Zagreb Faculty of Agriculture.

Material and methods

Seeds of 32 accessions of clary sage from the Collection of Medicinal and Aromatic Plants of the Department of Seed Science and Technology of the University of Zagreb, Faculty of Agriculture, within the framework of the National Program for the Conservation and Sustainable Use of Plant Genetic Resources for Food and Agriculture of the Republic of Croatia, were used for this study.

Germination and germination energy testing was conducted under controlled laboratory conditions at the University of Zagreb Faculty of Agriculture, Department of Seed Science and Technology, in 2021 as per the ISTA (1993) Rules. Before the experiment, the seeds were surface-sterilized with 3% Izosan for three minutes and rinsed under running water. They were then rinsed in distilled water and dried to optimum moisture.

Due to the limited amount of seeds each accession was germinated in two replicates, two Petri dishes with 25 seeds each. The seeds were germinated on germination paper (Munktell 21/N, 580x580mm, 80g/qm) in 10-cm-diameter Petri dishes (Steriplan®, DURAN®, DWK Life Sciences GmbH, Germany). Petri dishes with seeds were for germination placed in a germination chamber at a constant temperature (25 °C ± 1 °C) with a photoperiod of 16 h of light and 8 h of darkness. The number of germinated seeds (seeds with a root size ≥ 2 mm) was determined every other day over a period of 21 days.

At the end of the study, the following parameters were calculated:

Germinability (G, %) represents the number of germinated seeds in percentage (Scott et al., 1984.) and was calculated according to the formula:

$$G = \frac{n_k}{n} \times 100$$

where n_k indicates the number of germinated seeds, and n is the total number of seeds in the experiment.

Mean germination time (MGT, day) is the germination time in days and was calculated

according to the formula:

$$MGT = \frac{\sum_{i=1}^{k} n_i \, t_i}{\sum_{i=1}^{k} n_i}$$

where t_i is the time from the beginning of the experiment to the observation time (i^{th}), n_i is

the number of germinated seeds in the ith time, and k is the last day of germination (Ranal et al., 2009.).

Results and discussion

In this study, the germination rate and germination energy of 32 accessions of clary sage from the Collection of Medicinal and Aromatic Plants were tested over 21 days.

At the end of the research, the germinability and the mean germination time were calculated. Table 4 shows that of the 32 accessions, the seeds of 28 accessions germinated, while four accessions did not have a single germinated seed.

Table 1. A list of clary sage accessions with information on the location and year of collection, germinability (G, %) and mean germination time (MGT, day)

No.	Accession	Location of collection	Year of collection	Germinability (G, %)	Mean germination time (MGT, day)
1	MAP00002	Slovakia	1996	22	9.54
2	MAP00141	Czech Republic	1998	0	/
3	MAP00377	Italy	1999	2	7.00
4	MAP02196	Kaštel stari	2008	34	2.97
5	MAP02197	Šolta	2008	8	2.00
6	MAP02198	Šolta	2008	62	2.14
7	MAP02199	Šolta	2008	68	2.15
8	MAP02200	Hvar	2008	36	2.00
9	MAP02201	Korčula	2008	68	2.29
10	MAP02202	Korčula	2008	76	2.06
11	MAP02203	Korčula	2008	34	3.32
12	MAP02204	Brač	2008	52	3.35
13	MAP02205	Brač	2008	70	4.37
14	MAP02206	Vrgorac	2008	0	/
15	MAP02207	Murter	2008	50	2.35
16	MAP02208	Murter	2008	20	2.65
17	MAP02209	Živogošće	2008	38	2.58
18	MAP02210	Trogir	2008	20	4.67
19	MAP02211	Trogir	2008	4	4.00
20	MAP02212	Vrpolje	2008	12	7.84
21	MAP02213	Trogir	2008	0	/
22	MAP02214	Trogir	2008	20	5.80
23	MAP02216	Šibenik	2008	20	8.59
24	MAP02217	Prokljansko jezero	2008	42	2.86
25	MAP02218	Žirje	2008	44	2.09
26	MAP02219	Dugi otok	2008	62	4.08
27	MAP02220	Brač	2008	34	6.90
28	MAP02271	Split	2008	20	9.25
29	MAP02386	Dugi otok	2009	94	3.07
30	MAP02392	Dugi otok	2009	0	/
31	MAP02393	Tribunj	2009	90	2.49
32	MAP02405	Stankovci	2009	50	3.27

Based on the research conducted, clary sage seeds were found to have very good germination considering the past years the seeds were stored.

The highest percentage of germination (Table 1) was observed in accessions 2386 (94 %) from Dugi otok and 2393 (90 %) from Tribunj, stored since 2009, slightly less in accessions 2202 (76 %) from Korčula, 2205 (70 %) from Brač, 2201 (68 %) from Korčula, 2199 (68%) from Šolta, 2219 (62 %) from Dugi otok and 2198 (62 %) from Šolta, stored since 2008. The other samples had a germination rate of about 50 % or less. Although accession 2197 had only eight germinated seeds, it had the fastest average germination time of two days, next to accession 2200 (Table 1). Besides

them, the fastest germination time was observed in accessions 2218 (2.09) from Žirje, 2209 (2.58) from Borovik, 2208 (2.65) from Murter and 2196 (2.97) from Kaštel Stari, which have been kept since 2008. Although accession 2386 had the highest percentage of germination (94 %) compared to the less germinated samples, it had a slightly longer germination time (3.07).

The results of this study suggest that the seeds were stored under good conditions and that regeneration in plant genebanks does not need to be carried out often.

A similar study was conducted by Žutić and Dudai (2008) who, among others, investigated the germination of Dalmatian sage (*Salvia officinalis* L.) cultivar "Moran" at 25 °C with constant lighting, stored at 6 °C and aged from 0 to 15 years. The highest germination and vigour were found in 3 and 8 year old seeds (81 and 73 %) and the authors concluded that ageing of sage seeds is a relatively slow process.

Conclusion

The research revealed that the seeds of clary sage showed very good germination, which means that it tolerates storage well, i.e. that it was stored under good conditions, so that regeneration in plant genebanks does not have to be carried out often. Out of 32 research accessions, nine had a germination level of more than 50%.

Acknowledgments

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Zbornik radova

Povrćarstvo, ukrasno, ljekovito i aromatično bilje

Količina mikroelemenata u mladim izdancima brokule, poriluka i cikle

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Sažetak

Mladi izdanci smatraju se funkcionalnom hranom zbog visokog sadržaja minerala i bioaktivnih spojeva, a sadrže manje nitrata u odnosu na biljke u kasnijoj fenološkoj fazi. Cilj istraživanja bio je utvrditi utjecaj 3 podloge (komercijalni supstrat, mješavina komercijalnog supstrata i perlita, juta) na količinu suhe tvari i mikroelemenata u mladim izdancima brokule, poriluka i cikle. Najveća prosječna količina suhe tvari (7,82 % ST) i mikroelemenata u svježoj tvari (st) mladih izdanaka utvrđena je pri uzgoju na juti. Primjena jute rezultirala je najvećom količinom suhe tvari (9,77 % ST) i cinka (0,72 mg Zn 100 g^{-1} st) u izdancima poriluka te najvećom količinom željeza (2,12 mg Fe 100 g^{-1} st) u izdancima cikle.

Ključne riječi: *Brassica oleracea* var. *italica*, *Beta vulgaris* var. *conditiva*, *Allium ampeloprasum* ssp. *porrum*, mineralni sastav, alternativni supstrati

Uvod

Novija kategorija biljnih namirnica koja se ističe kao funkcionalna hrana, zbog pozitivnog utjecaja na ljudsko zdravlje, mladi su izdanci različitih vrsta povrća, aromatičnog bilja, samoniklog jestivog bilja i žitarica. Mladi se izdanci konzumiraju u fazi kotiledona i djelomično ili potpuno razvijenih prvih pravih listova, a odlikuju se bogatim nutritivnim sastavom, raznolikim bojama te različitim teksturama i okusima koji doprinose poboljšanju okusa i izgleda jela (Marchioni i sur., 2021.; Renna i sur., 2017.). Nedavna istraživanja pokazuju da mladi izdanci sadrže veću količinu minerala i bioaktivnih spojeva, a manje nitrata u odnosu na biljke u kasnijoj fenološkoj fazi (Pinto i sur., 2015.; Xiao i sur, 2012.). Odabir podloge za uzgoj može značajno utjecati na nutritivnu vrijednost i prinos mladih izdanaka (Di Gioia i sur., 2016.). Najčešće korištene podloge za uzgoj mladih izdanaka su treset i mješavine supstrata s tresetom, no njihov nedostatak je visoka cijena pa su proizvođači u potrazi za održivijim podlogama s nižom cijenom. Alternativne podloge koje se mogu koristiti za uzgoj mladih izdanaka su kora drveta, kokosova vlakna, pijesak, perlit, vermikulit te nusproizvodi u industriji vlakna i tekstila, poput vlakana pamuka, konoplje i jute (Kyriacou i sur., 2016.; Bulgari i sur., 2021.). Cilj ovog rada bio je utvrditi utjecaj tri različite podloge na količinu suhe tvari i mikroelemenata mladih izdanaka brokule, poriluka i cikle.

Materijal i metode

Istraživanje je provedeno tijekom proljeća 2019. godine u Zavodu za povrćarstvo Agronomskog fakulteta u Zagrebu metodom slučajnog bloknog rasporeda u 3 ponavljanja. U istraživanje su uključene vrste: brokula (*Brassica oleracea* L. var. *italica* Plenck) 'Calabrese', poriluk (*Allium ampeloprasum* L. ssp. *porrum*) 'Doreamon' i cikla (*Beta vulgaris* var. *conditiva* Alef.) 'Red lady'; Rem Sprout (Italija), a korišteno je sjeme organskog porijekla. Testirane su 3 podloge za uzgoj mladih izdanaka: komercijalni supstrat za uzgoj presadnica 'Klasman Potgrond H' (S), mješavina komercijalnog supstrata 'Klasman Potgrond H' i perlita 'Agroperl'(Europerl) u omjeru 5:1 (S+P) te četveroslojna juta (J) rezana na odgovarajuće dimenzije za uzgojne posude (26 cm × 36 cm × 6,3 cm). Prije postavljanja pokusa provedeno je namakanje sjemena cikle u trajanju od 24 sata radi poboljšanja klijanja. Zbog različite dužine proizvodnog ciklusa uzgajanih vrsta, sjetva poriluka i cikle provedena je 30. svibnja u količini od 196 g m^{-2}, odnosno 356 g m^{-2}, dok je sjetva brokule bila 3. lipnja u količini od 263 g m^{-2}. Zbog održavanja optimalnih uvjeta (tama, visoka vlaga) nakon sjetve, sjeme je prekriveno papirnatim ubrusima koji su svakodnevno prskani vodom, a zatim su uzgojne posude prekrivene poklopcima do faze nicanja. Nakon uklanjanja ubrusa i poklopaca, svakodnevno se provodilo zalijevanje

podloga količinom od 100 do 200 ml vode po uzgojnoj posudi. Tijekom uzgoja kontinuirano je mjerena temperatura i relativna vlaga zraka.

Berba mladih izdanaka započela je 10. lipnja berbom izdanaka brokule i poriluka na svim podlogama, a nastavila se berbom cikle 12. lipnja (S i S+P) i 14. lipnja (J). Reprezentativni uzorci biljnog materijala osušeni su na 105 °C, samljeveni, homogenizirani te analizirani u triplikatu u laboratoriju Zavoda za ishranu bilja Agronomskog fakulteta gdje je utvrđena količina suhe tvari i mikroelemenata (željezo, cink, mangan i bakar). Određivanje suhe tvari (%) provedeno je gravimetrijskom metodom prema normi HRN ISO 11465:2004 (HZN, 2004.). Mikroelementi su određeni atomskom apsorpcijskom spektrofotometrijom, nakon digestije s koncentriranom HNO3 i HClO4 (Milestone 1200 Mega Microwave Digester), AOAC (1995.). Količina mikroelemenata mladih izdanaka izražena je u mg 100 g $^{-1}$ svježe tvari (st), budući da se mladi izdanci konzumiraju u svježem stanju te kako bi se unos minerala konzumacijom izdanaka istraživanih vrsta usporedio s preporukama za potreban dnevni unos minerala. Statistička obrada podataka provedena je u programu SAS Software v. 9.4 (2013.). Razlike između istraživanih vrsta za sva promatrana svojstva podvrgnute su jednosmjernoj analizi varijance (ANOVA), a srednje vrijednosti uspoređene su t-testom (LSD) na razini signifikantnosti $p \leq 0,05$.

Rezultati i rasprava

U tablici 1 prikazana je količina suhe tvari i mikroelemenata u mladim izdancima brokule, poriluka i cikle te je vidljiv opravdan utjecaj testiranih podloga na istraživana svojstva. Najveća količina suhe tvari (9,77 % ST) utvrđena je pri uzgoju poriluka na juti kao podlozi, dok je uzgoj mladih izdanaka brokule na podlozi S+P rezultirao najmanjom količinom suhe tvari (4,93 % ST). Količina suhe tvari ostvarena uzgojem izdanaka brokule na podlogama S i S+P u skladu je s rezultatima istraživanja Opačić i sur. (2016.) koji su utvrdili 5,08 % ST u izdancima brokule uzgojenim na supstratu 'Klasman Potgrond H'.

Najveća količina željeza utvrđena je u izdancima cikle uzgojenim na juti (2,12 mg Fe 100 g^{-1} st), dok je u izdancima brokule i poriluka uzgojenima na podlozi S+P utvrđena jednaka i statistički najmanja količina željeza (0,96 i 0,97 mg Fe 100 g^{-1} st). Utvrđena prosječna količina željeza u mladim izdancima brokule (1,02 mg Fe 100 g^{-1} st) uzgojenim na podlozi S veća je od vrijednosti (0,77 mg Fe 100 g^{-1} st) u istraživanju Opačić i sur. (2016.). U istraživanju De la Fuente i sur. (2019.) utvrđene su količine željeza 0,39 mg 100 g $^{-1}$ st te količine cinka 0,16 mg 100 g^{-1} st u uzgoju 4 vrste mladih izdanaka iz porodice *Brassicaceae* u hidroponskom uzgoju, a bile su manje u odnosu na rezultate dobivene u ovom radu. Primjena jute kao podloge rezultirala je najvećom količinom cinka u izdancima poriluka (0,72 mg Zn 100 g^{-1} st), dok je najmanja količina cinka utvrđena uzgojem izdanaka brokule na podlozi S+P. Najveća količina mangana utvrđena je u mladim izdancima cikle uzgojene na juti (0,94 mg Mn 100 g^{-1} st) i bila je statistički jednaka vrijednosti utvrđenoj na komercijalnom supstratu (0,84 mg Mn 100 g^{-1} st). Jednaka i statistički najmanja količina mangana utvrđena je u izdancima brokule uzgojene na podlogama S i S+P. Količina cinka i bakra u izdancima brokule uzgojenim na podlozi S u skladu su s rezultatima istraživanja Paradiso i sur. (2018.).

Tablica 1. Količina suhe tvari i mikroelemenata u mladim izdancima

Vrsta	Varijanta	Suha tvar	Fe	Zn	Mn	Cu
		% ST	mg 100 g^{-1} st			
Brokula	S	5,07 ^{h}	1,02 ^{f}	0,35 ^{f}	0,36 ^{f}	0,04 ^{b}
	S+P	4,93 ^{i}	0,96 ^{g}	0,31 ^{g}	0,36 ^{f}	0,04 ^{b}
	J	6,98 ^{d}	1,39 ^{c}	0,49 ^{d}	0,47 ^{e}	0,06 ^{b}
	Prosjek	**5,66**	**1,12**	**0,38**	**0,40**	**0,05**
Poriluk	S	8,43 ^{b}	1,09 ^{e}	0,46 ^{de}	0,88 ^{b}	0,13 $^{n.s.}$
	S+P	7,49 ^{c}	0,97 ^{g}	0,45 ^{e}	0,74 ^{d}	0,12
	J	9,77 ^{a}	1,43 ^{b}	0,72 ^{a}	0,86 ^{bc}	0,15
	Prosjek	**8,56**	**1,16**	**0,54**	**0,83**	**0,13**
Cikla	S	6,06 ^{f}	1,39 ^{c}	0,58 ^{c}	0,84 ^{a}	0,14 $^{n.s.}$
	S+P	5,35 ^{g}	1,30 ^{d}	0,59 ^{c}	0,75 ^{d}	0,14
	J	6,86 ^{e}	2,12 ^{a}	0,66 ^{b}	0,94 ^{a}	0,12
	Prosjek	**6,09**	**1,60**	**0,61**	**0,84**	**0,13**

Različita slova predstavljaju značajno različite prosječne vrijednosti prema LSD testu, p≤0,05
ST – suha tvar, st – svježa tvar

S obzirom na podlogu, statistički najveća količina suhe tvari svih uzgajanih vrsta mladih izdanaka ostvarena je uzgojem na juti (J), dok je primjena supstrata i perlita kao podloge rezultirala najmanjom količinom suhe tvari (Tablica 2). Veće vrijednosti suhe tvari ostvarene uzgojem na juti u skladu su s istraživanjem Di Gioia i sur. (2016.). Najveća količina željeza, cinka i mangana u mladim izdancima testiranih vrsta utvrđena je na juti (1,65 mg Fe, 0,62 mg Zn i 0,76 mg Mn 100 g^{-1} st). dok se uzgoj na podlozi S nije statistički razlikovao od uzgoja na podlozi S+P (0,46 mg Zn i 0,45 mg Mn 100 g^{-1} st). Primjena podloge S+P negativno je utjecala na količinu željeza i mangana u izdancima svih uzgajanih vrsta, s prosječnim vrijednostima 1,08 mg Fe i 0,62 mg Mn 100 g^{-1} st. Nije utvrđena statistički opravdana razlika u količini bakra između testiranih podloga za uzgoj mladih izdanaka povrtnih vrsta.

Tablica 2. Prosječne vrijednosti suhe tvari i mikroelemenata mladih izdanaka ostvarene na različitim supstratima

Varijanta	Suha tvar	Fe	Zn	Mn	Cu
	% ST	mg 100 g^{-1} st			
S	6,52 ^{b}	1,17 ^{b}	0,46 ^{b}	0,69 ^{b}	0,10 $^{n.s.}$
S + P	5,92 ^{c}	1,08 ^{c}	0,45 ^{b}	0,62 ^{c}	0,10
J	7,82 ^{a}	1,65 ^{a}	0,62 ^{a}	0,76 ^{a}	0,11

Različita slova predstavljaju značajno različite prosječne vrijednosti prema LSD testu, p≤0,05
ST – suha tvar, st – svježa tvar

Neovisno o podlozi, najveća prosječna količina suhe tvari (8,56 % ST) utvrđena je u mladim izdancima poriluka, a najmanja u izdancima brokule (5,66 % ST); (Tablica 3). U istraživanju Xiao (2013.) utvrđena je 30 % manja količina suhe tvari u izdancima cikle od vrijednosti ostvarene u ovom radu (6,09 % ST). Najveća prosječna količina željeza i cinka utvrđena je u izdancima cikle (1,60 mg Fe i 0,61 mg Zn 100 g^{-1} st), dok su izdanci brokule imali prosječno najmanje željeza i cinka (1,12 mg Fe i 0,38 mg 100 g^{-1} st). Prosječne vrijednosti količine mangana u izdancima poriluka i cikle nisu se statistički razlikovale (0,83 i 0,84 mg Mn 100 g^{-1} st), a najmanja količina je utvrđena u izdancima brokule (0,40 mg Mn 100 g^{-1} st) što je više od vrijednosti izdanaka brokule dobivene u istraživanju Opačić i sur. (2016.). Prema podacima USDA (2018.) svježi poriluk u tehnološkoj zrelosti sadrži manje cinka (0,12 mg Zn 100 g^{-1} st) i mangana (0,48 mg Mn 100 $^{-1}$ st) u odnosu na mlade izdanke. Mladi izdanci brokule u ovom istraživanju imali su prosječno najmanju količinu bakra (0,05 mg Cu 100 g^{-1} st), što je u skladu s rezultatima istraživanja Opačić i sur. (2016.) te Paradiso i sur. (2018.). U izdancima poriluka i cikle utvrđena je jednaka količina bakra.

Tablica 3. Prosječne vrijednosti suhe tvari i mikroelemenata mladih izdanaka brokule, poriluka i cikle

Vrsta	Suha tvar	Fe	Zn	Mn	Cu
	% ST	mg 100 g^{-1} st			
Brokula	5,66 ^{c}	1,12 ^{c}	0,38 ^{c}	0,40 ^{b}	0,05 ^{b}
Poriluk	8,56 ^{a}	1,16 ^{b}	0,54 ^{b}	0,83 ^{a}	0,13 ^{a}
Cikla	6,09 ^{b}	1,60 ^{a}	0,61 ^{a}	0,84 ^{a}	0,13 ^{a}

Različita slova predstavljaju značajno različite prosječne vrijednosti prema LSD testu, p≤0,05
ST – suha tvar, st – svježa tvar

Prema preporuci EU (2011.) preporučena dnevna vrijednost unosa željeza, cinka, mangana i bakra za odrasle osobe iznosi 14 mg Fe, 10 mg Zn, 2 mg Mn i 1 mg Cu. U ovom radu najveća količina željeza (2,12 mg Fe 100 g^{-1} st) i mangana (0,94 mg Mn 100 g^{-1} st) utvrđena je u izdancima cikle uzgajane na juti te se konzumacijom 100 g tako uzgojenih izdanaka može podmiriti 15,14 % dnevnih potreba za željezom i čak 47 % dnevnih potreba za manganom. Konzumacijom 100 g izdanaka poriluka uzgojenih na juti može se podmiriti 7,2 % dnevnih potreba za cinkom, dok se 14 % dnevnih potreba za bakrom može podmiriti konzumacijom 100 g izdanaka cikle uzgojenih na podlozi S ili S+P.

Zaključak

Upotreba jute kao podloge rezultirala je značajno višim vrijednostima suhe tvari te svih mikroelemenata stoga se može preporučiti za uzgoj mladih izdanaka. Primjena mješavine komercijalnog supstrata i perlita (S+P) kao uzgojne podloge negativno je utjecala na količinu suhe tvari, željeza i mangana u mladim izdancima. Neovisno o podlozi, mladi izdanci cikle izdvajaju se kao bolji izvor željeza, cinka, mangana i bakra u odnosu na ostale testirane vrste.

Napomena

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Microelements content of broccoli, leek and red beet microgreens

Abstract

Microgreens are considered functional food due to high content of minerals and bioactive compounds and contain fewer nitrates compared to plants in a later phenological phase. The aim of this research was to determine the effect of 3 tested growing media (commercial seedling substrate, a mixture of commercial substrate and perlite, burlap) on the amount of dry matter and microelements in broccoli, leek, and reed beet microgreens. The highest average values of dry weight (7.82% DW) and microelements of microgreens, expressed in fresh weight (fw), were determined when grown on burlap. Cultivation on burlap resulted in the highest amount of dry weight (9.77% DW) and zinc (0.72 mg Zn 100 g^{-1} fw) in leek microgreens and the highest amount of iron (2.12 mg Fe 100 g^{-1} fw) in red beet microgreens.

Keywords: *Brassica oleracea* var. *italica*, *Beta vulgaris* var. *conditiva*, *Allium ampeloprasum* ssp. *porrum*, alternative growing media, mineral content

ORIGINAL SCIENTIFIC PAPER

The effect of sowing density on morphological traits and yield of hydroponically grown spinach

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Abstract

The aim of this research was to determine the effect of sowing density on the morphological traits and yield of hydroponically grown spinach. During a harvest conducted 32 days after sowing, morphological characteristics were analysed and spinach yield was determined. No significant effect of sowing density on spinach rosette height was found, while statistical differences between the tested treatments were determined for other morphological traits and yield. The highest weight of spinach rosettes (6.5 g) was obtained at the lowest sowing density, while the highest yield (2 kg m^{-2}) was obtained at the highest sowing density.

Keywords: *Spinacea oleracea* L., floating system, rosette mass and height, leaves number

Introduction

Recently, the consumption of young spinach in combination with other leafy vegetables has been continuously increasing in Croatia. Also, in greenhouses, it is increasingly common to replace conventional cultivation of plants on the soil with hydroponic cultivation which reduces soil degradation, the accumulation of harmful organisms and harmful effects on the environment, and increases yield and quality (Kacjan Maršić, 2017.). According to Öztekin et al. (2018.), higher yield and nutritional value of spinach are obtained in floating hydroponics and the cultivation is shorter compared to soil cultivation. The same authors report that yield and quality of leafy vegetables are influenced by cultivation techniques, sowing density, concentration of macronutrients in the nutrient solution, length of the production cycle, and other factors. According to Toth et al. (2012.), in hydroponic cultivation, it is necessary to determine the optimal composition of the nutrient solution, sowing density, and appropriate assortment depending on the growing period to achieve higher yield and quality for each type of leafy vegetable. Janeczko and Timmons (2019.) state that the sowing density and the volume of the growing pots influence the morphological characteristics of cut leafy vegetables. For the cultivation of spinach in floating hydroponics, different data on sowing density are given. Cocetta et al. (2007.) indicate 1150 seeds m^{-2} for sowing density of spinach, while Öztekin et al. (2018.) in their study indicate 957 plants m^{-2} as a plant density of spinach. The aim of this research was to determine the effect of sowing density on the morphological characteristics and yield of spinach using the floating hydroponic technique during the spring-summer growing period.

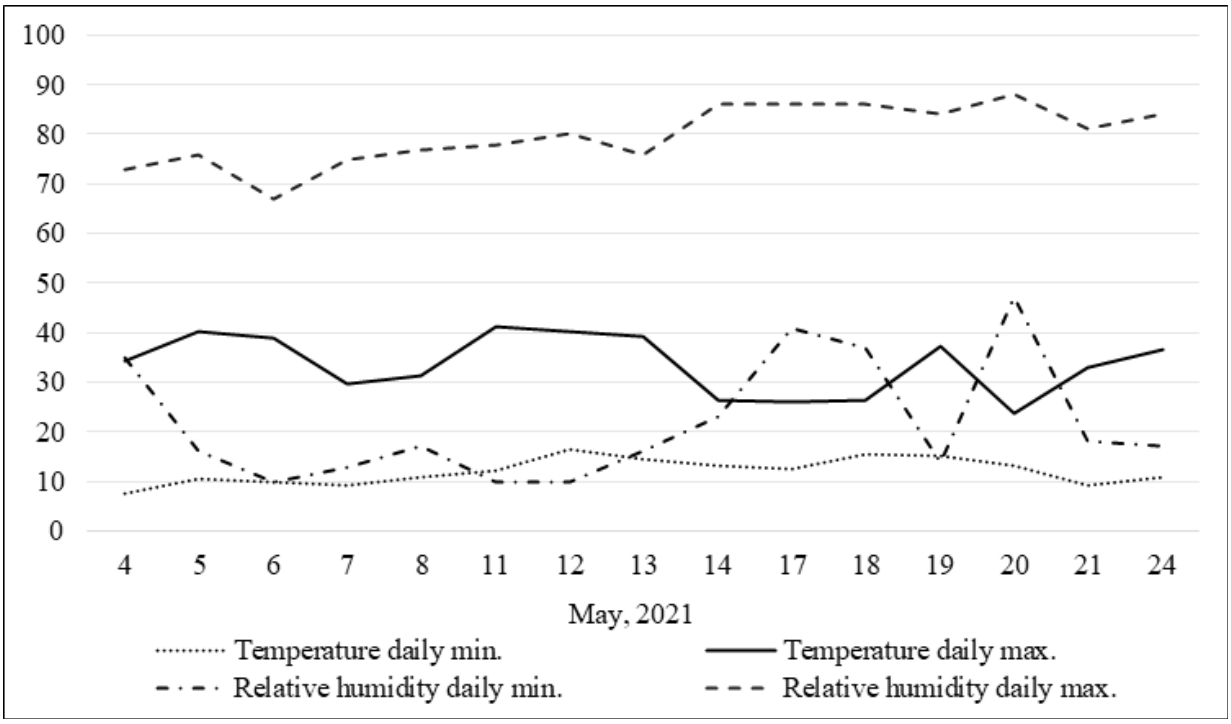
Material and methods

The research was set up in the greenhouse at the University of Zagreb Faculty of Agriculture in Zagreb during the spring-summer growing season of 2021. A one-factorial experiment was set up according to the randomized complet block design in 3 replications with the spinach cultivar 'Eagle F1' (Rijk Zwaan). Seeds were sown on April 23. 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 m^2). Boards were filled with perlite of granulation 0 - 6 mm. Three sowing densities were tested: A - 1252, B - 2505 and C - 3758 seeds m^{-2}, i.e. 21.3, 42.7 and 64.2 g m^{-2}) and covered with perlite of finer granulation (0-3 mm). Before sowing, the mass of 1000 seeds of cultivar 'Eagle F1' was determined (17.2 g). After sowing, the boards were placed in a dark place with a temperature suitable for germination (21°C, relative humidity 42 to 60%) of spinach seeds. Germination was detected on May 4, when the nutrient solution for growing spinach was prepared according to the Tessi (2002.) and the polystyrene boards with the germinated spinach were placed in the pool with the nutrient

solution. The spinach harvest was performed on May 25, i.e. 32 days after sowing. On 10 plants per repetition of each sowing density, next morphological characteristics of spinach were analysed: mass and height of the rosette, number of leaves, and the yield was determined. The statistical program Windows SAS® Software v.9.3 (2010.) was used for statistical procedures of the obtained results. The differences between the tested sowing densities 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

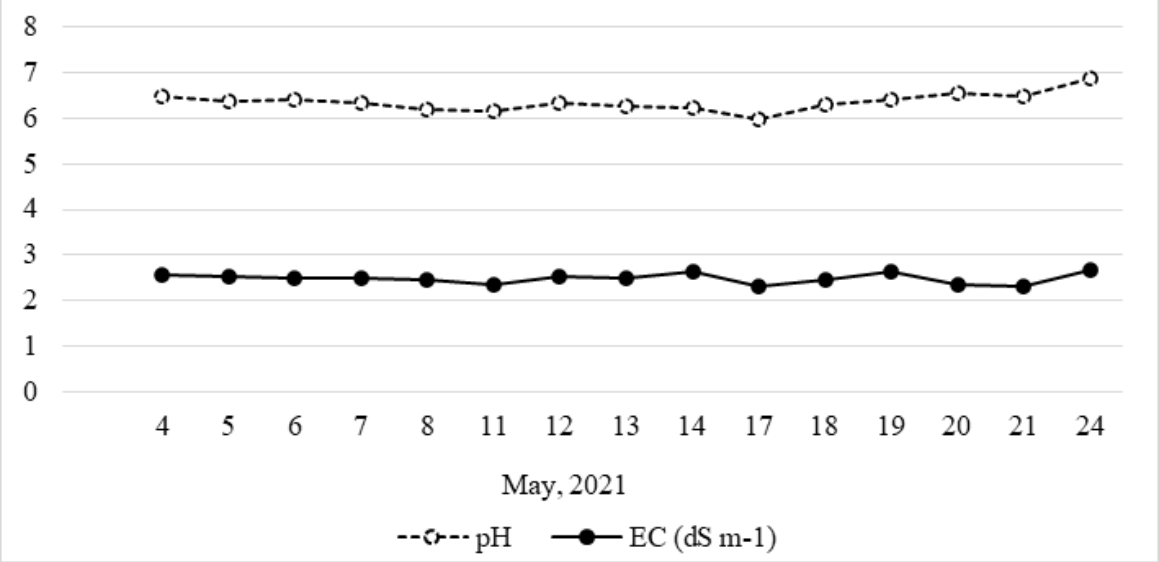
During the experiment (from May 4 to 25), the minimum and maximum temperature and relative humidity (graph 1) were measured daily in the greenhouse equipped with side and roof ventilation. The lowest minimum air temperature (7.5°C) was measured at the beginning of cultivation (May 4) and the highest maximum air temperature in the greenhouse (41.3°C) was measured on May 5 and 11. The values of minimum and maximum relative humidity ranged from 10 to 47% and from 73 to 88%, respectively. The average daily value of relative humidity varied between 38.5 and 67.5% during the reaearch (data not shown).



Graph 1. Minimum and maximum air temperature (°C) and relative air humidity (%) in greenhouse during spinach cultivation, Zagreb, 2021.

According to Albright (2005.), the optimal air temperature for hydroponic cultivation of spinach is 25°C (day) and 20°C (night), while the preferred relative humidity is 30% to 70%. Gent (2017.) recommends temperatures of 17 to 22°C with horizontal circulation, installation of shading and humidifiers. During the research, the average daily air temperature varied between 18.6 and 28.4°C (data not shown), which is higher than the optimal values for spinach growth (15 to 18°C) reported by Lešić et al. (2016.).

During spinach cultivation, the nutrient solution pH varied from 5.99 to 6.88 (Graph 2) and corresponded to the optimal values of 5.8 to 6.2 reported by Toth et al. (2012.) and 6.0 to 7.0 reported by Sharma et al. (2018.). According to Albright (2005.), the optimum values for pH and electrical conductivity (EC) of the nutrient solution in spinach cultivation are 5.8 and 1.2 dS m^{-1}, respectively. During cultivation, the EC value of the nutrient solution varied slightly, i.e., it was in the range of 2.30 to 2.66 dS m^{-1}, which was slightly different from the values (1.8 to 2.3 dS m^{-1}) reported by Sharma et al. (2018.).



Graph 2. pH and EC values of nutrient solution during spinach cultivation

The results of morphological characteristics and yield of spinach depending on the tested sowing densities, are presented in Table 1. The highest rosette mass (6.5 g) was measured at the lowest sowing density (A 21.3 g m^{-2}), while the smallest rosette mass (4.1 g) was determined at the highest seeding density (C 64.2 g m^{-2}). In the study conducted by Toth et al. (2016) on the influence of fluid drilling of germinated spinach seeds on agronomic traits in spring growing period, higher spinach rosette mass (8.45 g) was obtained.

Rosette height varied from 14.0 (A 21.3 g m^{-2}) to 17.0 cm (C 64.2 g m^{-2}). It can be assumed that the highest seeding density (C) resulted with elongation of plants due to an insufficient amount of light. The spinach rosette height achieved in this study, regardless of seeding density, was significantly lower than the value of 26.96 cm to 28.04 cm reported by Gaikwad and Mallick (2020.) in the study of growing spinach with different hydroponic systems. Lamut (2011.) reported that the average height of spinach plants grown in a commercial substrate (7.5 cm) was lower than that of plants grown in a floating hydroponic system (14.7 cm), regardless of the substrate used.

At the lowest sowing density (A 21.3 g m^{-2}), the highest average number of leaves (7.2) was found and was statistically equal to the number of rosette leaves (6.7) of plants grown at a sowing density of 42.7 g m^{-2} (B). The obtained results are in agreement with the values reported by Lamut (2011.) when spinach was grown in a floating hydroponic system, where the number of spinach rosette leaves at harvest varied from 6.4 in the cultivar 'Matador' to 7.0 in the cultivar 'Spokane'. In a study, Shah et al. (2009.) compared the cultivation of spinach in two nutrient solutions in a non-circulating hydroponic system and found that the maximum number of leaves (12.44 and 12.33) was obtained in plants grown in 50% and 100% Cooper's solution, respectively.

Table 1. The effect of sowing density on morphological properties and yield of spinach in floating hydropon

Sowing density			Rossete			Yield, kg m^{-2}
	Seeds m^{-2}	g m^{-2}	Mass, g	Height, cm	Number of leaves	
A	1252	21.3	6.5 a	14.0 n.s.	7.2 a	1.1 b
B	2505	42.7	5.5 ab	16.9	6.7 ab	1.6 ab
C	3758	64.2	4.1 b	17.0	6.5 b	2.0 a
		average	**5.37**	**16.0**	**6.8**	**1.56**

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

The highest yield (2.0 kg m^{-2}) was obtained at the highest sowing density (C 3758 seeds m^{-2}). In the study by Cocetta et al. (2007.), the average yield of spinach in floating hydroponics at a sowing density of 1150 seeds m^{-2} varied from 1.1 to 1.5 kg m^{-2} depending on the nutrient concentration in the solution. The same spinach yield (1.1 kg m^{-2}) was obtained in this research with the lowest spinach sowing density (A 21.3 g m^{-2}), i.e. sowing 1252 seeds m^{-2}. Sowing densities B (2505 seeds m^{-2}) and C (3758 seeds m^{-2}) resulted in higher and statistically equal yield. However, in this research, even the lowest yield obtained with the lowest sowing density is higher than the values reported by Öztekin et al. (2018) and Ranawade et al. (2017.) for spinach grown hydroponically (0.904 and 0.934 kg m^{-2}). Also, Toth et al. (2016.) reported that fluid drilling of germinated spinach seeds with the substrate cover resulted in a yield of 0.7 kg m^{-2}. In the mentioned study, the length of spinach vegetation in conventional cultivation in the soil in the spring growing season was 48 days. In the study by Brandenberger et al. (2007.), the length of spinach cultivation in the greenhouse varied from 37 (spring) to 53 days (autumn). A higher yield was obtained in the autumn growing season (2.09 kg m^{-2}) than in the spring growing period (1.65 kg m^{-2}). In this research, which was conducted in the spring growing season, the length of spinach vegetation was slightly shorter (32 days), with an average yield 1.56 kg m^{-2}. This suggests that a higher yield of spinach in floating hydropon could be expected in the autumn growing period.

Conclusions

In the research conducted in the spring growing period using floating hydroponic system, statistically justified differences between the tested sowing densities were found for all traits except rosette height. The highest sowing density resulted in the highest rosette height and yield, but also in elongation of the plants and their lower mass. The average yield of spinach in the study was 1.56 kg m^{-2}, and statistically equal spinach yield (2.0 and 1.6 kg m^{-2}) was found at the highest and medium tested sowing densities tested (3758 and 2505 seeds m^{-2}, respectively). In the cultivation of spinach in a floating hydroponic system, a sowing of 2505 seeds m^2 could be recommended to obtain an acceptable yield.

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Agronomic traits of hydroponically grown nettle under conditions of two irrigation intervals

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Abstract

The ebb and flow system is a hydroponic technique for growing plants that allows rational water use and management of water stress to obtain plant material rich in bioactive compounds. The risk with this technique is the lower yield and the deterioration of the morphological characteristics of the cultivated plants that can occur. The aim of this study was to determine the effect of 2 intervals of irrigation intervals on the agronomic traits of stinging nettle in 2 harvest periods. The combination of 48 hours of water stress and the 2nd harvest period gave the highest yield (1.21 kg m^{-2}), while the combination of 72 hours of water stress and the 1st harvest period resulted with the lowest nettle yield (0.65 kg m^{-2}).

Keywords: ebb and flow technique, irrigation frequency, yield, morphological characteristics, *Urtica dioica* L.

Introduction

Pronounced climate changes in the form of extreme weather conditions such as drought, environmental pollution with emphasis on groundwater contamination, and the general decrease in the availability of water suitable for growing food are among the major challenges facing modern agriculture (Kheradmand et al., 2014.). For this reason, the demand for sustainable production methods and management of important resources, such as water, is increasing, and growers are turning to hydroponic cultivation in greenhouses. Various hydroponic techniques allow the control and management of abiotic factors that directly affect the success of agricultural production. Through appropriate agrotechnical measures, higher yields and products with distinct nutrient quality can be obtained, all with precise and balanced plant nutrition and rationalized water use (Resh, 2013.).

For hydroponic cultivation of medicinal and aromatic plants, the nutrient film technique, floating hydroponics, aeroponics and the ebb and flow system are recommended. Various authors (Ferrarezi et al., 2015.; Yang et al., 2018.) point to ebb and flow as a water-saving technique suitable for rationalized fertilizer consumption and precise adjustment of nutrient solution rations to the needs of the cultivated species and the targeted end product. The mentioned technique works on the principle of time intervals in which the nutrient solution is available for the plants. In the period between two fertigations, the plants are exposed to water stress, which can affect the morphological and nutritional characteristics of the plants (Gent and McAvoy, 2011.; Opačić et al., 2022a). Water stress positively affects the amount of bioactive compounds produced by plants, and such plant material is rich in various phytochemicals. Since even controlled water stress can lead to yield loss, it is extremely important to find the right balance between induced stress depending on the plant species, which gives the plant material a richer content of bioactive compounds, and satisfactory yield (Ahanger et al., 2017.).

Stinging nettle (*Urtica dioica* L.) is considered a functional food rich in essential minerals, vitamins, phytochemicals, and other specialized metabolites and has been successfully grown using the floating hydroponics technique (Pagliarulo et al., 2004.; Hayden, 2006.; Radman et al., 2014.; Radman et al., 2021.; Opačić et al., 2022b). There is a lack of studies on the morphological characteristics, yield, and nutrient quality of stinging nettle under conditions of controlled water stress in the ebb and flow system. For this reason, a research was carried out with the aim of determining the agronomic traits of hydroponically grown nettle under conditions of different irrigation intervals during two harvest periods.

Material and methods

The study was conducted in an unheated greenhouse at the University of Zagreb Faculty of Agriculture experimental station during the winter-spring growing season of 2022. Nettle was grown using the ebb-and-flow technique. The influence of 48- and 72-hour irrigation intervals during the first and second harvest periods on the agronomic traits of nettle plants was studied. The experiment was laid out according to randomized complete block design in three replicates with water stress and harvest as factors. Seeds were sown on January 20, 2022, in polystyrene containers (dimensions 31 cm × 53 cm) containing 40 pots filled with commercial substrate (Klasman Potgrond H) and placed on ebb and flow benches (dimensions 2 m × 3 m; 33 containers per bench) after sowing and irrigated regularly. Nettle seeds (B&T World Seeds, France) were sown at a rate of 10 seeds per pot and thinned to 3 plants after germination to achieve uniformity in the experiment. Complete emergence was recorded on February 14, and thinning of the plants occured on March 14. From sowing until April 10 (when it was estimated that they had reached the appropriate size and crop density), the plants were watered as needed according to temperature and relative humidity (RH) in the greenhouse. On April 11 (81 days after sowing), treatments with nutrient solution (NS) according to the recipe of Johnson (Table 1; Lorenz and Maynard, 1988.), began with different irrigation intervals according to the following schedule:

Bench 1 - the interval for ebb and flow of NS was set to every 48 hours.

Bench 2 - the interval for ebb and flow of the NS was set to every 72 hours.

Table 1. Composition of the nutrient solution in mg L^{-1}

Macroelements	
KNO_3	251.0
KH_2PO_4	142.7
$Ca(NO_3)_2 \times 4H_2O$	501.5
$MgSO_4 \times 7H_2O$	256.3
Microelements	
FeEDTA 13%	12.8
H_3BO_3	1.32
$CuSO_4 \times 5H_2O$	0.03
$MnSO_4 \times 4H_2O$	0.79
$ZnSO_4 \times 7H_2O$	0.11
$Na_2MoO_4 \times 2\ H_2O$	0.02
Acid	
HNO_3	concentration – 56 %
EC (mS cm^{-1})	1.5
pH	5.8 – 6.2

The nutrient solution was brought to the plants in a volume of 120 L and remained on the bench for 1 hour, after which it was drained. This was repeated every 48 (bench 1) and 72 (bench 2) hours throughout the experiment. During cultivation, the abiotic factors of the greenhouse (air temperature, RH) and the nutrient solution (pH and EC values) were monitored regularly using a tabletop thermohygrometer (Agrologistika d.o.o., Croatia) and multiparameter meter (Hanna instruments HI98194, Romania).

Harvests of nettle herb were made in the pre-flowering stage on May 10 and June 8, and the morphological characteristics of 30 representative plants per treatment were analyzed. These included the following measurements: plant height (cm), number of leaves, and leaf width and length (cm), from which leaf area index (LAI) was calculated using the formula leaf length × width (cm^2), and total yield (kg m^{-2}) was also measured. To ensure retrovegetation,

nettle plants were cut above the first two nodules.

Statistical data processing was performed in SAS software v. 14.3 (2017.) using the PROC GLM (general linear model) procedure. The results were subjected to two-factor analysis of variance (ANOVA), and the differences obtained between the means were compared using the t-test (LSD) at the significance level $p \leq 0.05$.

Results and discussion

The abiotic factors of the greenhouse and NS are shown in Table 2 as decade values for the period from the second decade in February (greenhouse) i.e. the second decade in April (NS) to the first decade in June.

Table 2. Abiotic factors of the greenhouse and nutrient solution during nettle cultivation in the ebb and flow system

Period	Greenhouse				Nutrient solution	
	Temperature (°C)			RH (%)	pH	EC (mS cm^{-1})
	Min.	Max.	Mean			
February II	4.5	29.9	17.2	53		
February III	8.7	29.7	19.1	47		
March I	8.0	23.1	15.6	35		
March II	10.6	32.1	21.3	32		
March III	13.3	35.1	24.1	33		
April I	14.8	32.0	23.4	42		
April II	16.8	32.9	24.8	36	6.8	1.5
April III	12.9	32.0	22.5	48	6.6	1.5
May I	17.7	35.2	26.4	46	6.4	1.4
May II	17.2	40.6	28.9	42	6.8	1.4
May III	16.7	40.1	28.4	48	6.4	1.4
June I	16.2	38.2	27.2	52	6.4	1.5
Average	13.1	33.4	23.2	43	6.6	1.5

Minimum greenhouse air temperature during nettle cultivation ranged from 4.5°C (February II) to 17.7°C (May I), and maximum temperature ranged from 23.1°C (March I) to 40.6°C (May II). Average air temperature span was from 19.6°C (April II) to 24.9°C (May III) with an average of 23.2°C, which according to Opačić et al. (2022a) is within the range of optimal temperatures for nettle growth and development (20 to 25°C).

The average minimum temperature was 13.1°C, while the average maximum temperature reached 33.4°C, which is above the optimal range and can cause reduced growth of nettle plants (Opačić et al., 2022b). The average value of RH varied between 32 and 53%, which corresponds to the values recommended by Toth et al. (2012.) for RH during the cultivation of rocket in unheated greenhouse. The same authors recommend a solution pH of 5.8 to 6.2, but the value of 6.6 in this study was slightly higher. The average EC value was 1.5 mS cm^{-1}, which is the recommended value for the Johnson nutrient solution accourding to Lorenz and Maynard (1988.).

Table 3 shows the influence of irrigation intervals and harvests and their interaction on the agronomic traits of nettle grown in the ebb and flow system. A justified influence of irrigation intervals as the main factor on plant height, number of leaves, and yield is evident, while a statistically justified influence on LAI was not found. Depending on the harvest period, there were statistically justified differences in the number of leaves and yield, while no justified influence of this factor on plant height and LAI was detected.

Table 3. Influence of irrigation intervals, harvests and their interaction on agronomic traits of hydroponically grown nettle

		Plant height (cm)	Leaf number/ plant	LAI (cm^2)	Yield ($kg\ m^{-2}$)
Irrigation interval	48 h	33.2 ± 0.62^{a}	11 ± 0.84^{a}	23.5 ± 2.07^{ns}	1.06 ± 0.16^{a}
	72 h	19.6 ± 0.41^{b}	10 ± 1.19^{b}	20.0 ± 5.73	0.66 ± 0.09^{b}
Harvest	1st (1)	25.9 ± 3.18^{ns}	11 ± 0.34^{a}	22.5 ± 4.51^{ns}	0.76 ± 0.14^{b}
	2nd (2)	26.8 ± 2.99	10 ± 1.15^{b}	21.0 ± 4.77	0.96 ± 0.09^{a}
Interaction	48 h1	33.0 ± 1.14^{a}	11 ± 0.20^{a}	24.1 ± 1.18^{ns}	0.91 ± 0.05^{b}
	48 h2	33.4 ± 2.08^{a}	10 ± 1.11^{a}	22.9 ± 2.86	1.21 ± 0.09^{a}
	72 h1	18.8 ± 0.22^{b}	11 ± 0.42^{a}	20.9 ± 6.47	0.62 ± 0.03^{d}
	72 h2	20.3 ± 0.98^{b}	9 ± 0.40^{b}	19.0 ± 6.11	0.71 ± 0.02^{c}

Results are expressed as the mean value of triplicate ± standard deviation. Mean values followed by the same letter within each column do not differ significantly according to LSD test ($p \leq 0.05$); ns – non significant

The interaction between different irrigation intervals and harvest periods had a significant influence on most of the observed traits (plant height, number of leaves and yield), while for LAI the influence was not statistically significant.

An interval of 48 hours irrigation interval resulted in statistically significant taller nettle plants (33.2 cm) compared to an interval of 72 hours (19.6 cm). Considering only the harvest period, relatively taller plants were measured after the 2nd harvest (26.8 cm) compared to the 1st harvest (25.9 cm), but this difference was not statistically justified. Considering the irrigation interval and harvest period, the tallest plants were measured at 48 hours interval in both harvests (48 h×1= 33.0 cm, 48 h×2= 33.4 cm). The combination 72 h×1 resulted in the lowest plants (18.8 cm), but statistically not different from the 72 h×2 combination (20.3 cm). Radman et al. (2014.) found that the height of nettle plants grown in floating hydroponics in two different substrates at 3 different seeding densities was greater on average after the first harvest, which is contrary to the results of this study.

According to Chidiac (2017.), the height of lettuce shoots depends on the water stress interval, and a longer interval has a negative effect on growth, which is consistent with this study, where plants exposed to a longer period without nutrient solution were shorter than those that received nutrient solution more frequently. Osakabe et al. (2014.) found that water stress reduces plant growth by decreasing the opening of stomata, which reduces CO_2 uptake and thus photosynthetic activity.

When considering each factor, the highest number of leaves (11) was determined on plants grown under shorter irrigation interval, while irrigation interval of 72 hours resulted in plants with an average of 10 leaves. An identical result was obtained with harvest as the second factor. In the interaction of these two factors, the combinations 48 h×1 (n=11), 48 h×2 (n=10), and 72 h×1 (n=11) belonged to the highest statistical rank, while the lowest number of leaves was counted in plants in the combination 72 h×2 (n=9). Radman et al. (2014.) reported higher average number of nettle leaves after the second harvest. Mahmood et al. (2004.) found that water stress in the form of withholding irrigation until the lower 2-3 leaves of the plants wilted reduced the number of leaves in arugula and canola plants during 6 treatment cycles. In this study, a significantly lower number of leaves was seen only in the second harvest in combination with 72-h irrigation interval, which could be due to the temperature and RH in the greenhouse (Table 2), i.e., water stress did not occur in plants in the other treatments. According to Ncise et al. (2020.), different intervals of water stress (5, 14 and 21 days) in hydroponic cultivation of *Tulbaglia violacea* significantly affect the number of leaves, with a higher number of leaves recorded at intervals of 5 and 14 days compared to 21 days.

Regarding the tested factors and their interaction, no statistically significant differences were found, but there is still evidence of some trend. Plants exposed to 48 hours irrigation interval and plants after 1st harvest were found to have relatively higher LAI (23.5 cm^2 and 22.5 cm^2, respectively). As expected, the 48 h×1 interaction resulted in plants with the relatively highest LAI (24.1 cm^2), while the 72 h×2 combination resulted in the relatively lowest LAI (19.0 cm^2). Leaf surface area, on which the intensity of photosynthesis and transpiration largely depends, can be smaller to adapt plants to water stress, as a reduction in leaf surface area may prevent water loss through transpiration (Mahmood et al., 2004.). The same authors note that the lowest LAI value of arugula plants was recorded during the

longest drought period to which the plants were exposed, but there was no significant difference compared to the control.

The main factor, irrigation interval, had a significant effect on nettle yield in the ebb and flow system, and the highest yield was recorded in plants subjected to 48 hours irrigation interval (1.06 kg m^{-2}), while the yield was 38% lower in plants subjected to 72 hours irrigation interval. Considering only the harvest, the 1st harvest yielded 21% less than the 2nd harvest (0.96 kg m^{-2}). In the interaction of the tested factors, the highest yield was determined at the combination 48 h×2 (1.21 kg $m^{-}2$), while the yield was almost two times lower at combination 72 h×1 (0.65 kg m^{-2}). In Leskovar and Piccinni (2005.) study, deficit irrigation based on evapotranspiration affected the yield of spinach grown in the soil, with the lowest yield obtained at the highest water stress. Ors and Suarez (2017.) reached a similar conclusion when growing spinach in the open field under water and salt stress. The results of Radman et al. (2021.) indicated that nettle yield in floating hydroponics was higher after the 2nd harvest than after the 1st harvest. In the study by Radman et al. (2014.), the highest yield of nettle grown in floating hydroponics was obtained after the third harvest. The yield obtained after each harvest and number of harvests depend, among other factors, on the growing season and abiotic factors during cultivation.

Conclusion

A longer irrigation interval (72 h) negatively affected plant height, number of leaves, and yield. Plants had a greater number of leaves in the first harvest period, but a higher yield was observed in the second harvest. As expected, the irrigation interval had a greater effect on nettle agronomic traits than the harvest subfactor. The interaction between the irrigation interval of 48 hours and the 2nd harvest period resulted in the highest yield. Considering that water stress affects not only the agronomic traits but also the nutritional value of the plant material, future research should be focussed on the effect of different irrigation intervals on the nutritional quality of fresh nettle leaves.

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Effect of biofortification on dwarf french bean, pea and tomato with selenium enriched irrigation

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Abstract

Selenium (Se) deficient diet is a serious problem in some parts of the world. Agronomic biofortification of food crops can be a solution. Irrigation water containing 0, 100 and 500 μg L^{-1} Se was applied to green beans, peas and tomatoes in a greenhouse experiment with automatic irrigation system. The concentration and uptake of Se in the fruit, shoot and root of the vegetables, as well as the translocation factors were determined in order to evaluate the efficiency of biofortification. The 100 μg L^{-1} treatment increased Se concentration and uptake by two orders of magnitude compared to control, and the 500 μg L^{-1} treatment multiplied the values of 100 μg L^{-1} treatment 5-8 times on average depending on the vegetable species. As a result of the treatments, the Se concentration in the shoot and fruit increased compared to the root. Irrigation with Se-enriched water might be a feasible method for biofortification.

Keywords: vegetables, enrichment, fertigation, translocation, distribution

Introduction

Selenium (Se) is essential trace mineral for humans and animals. Crops grown in certain areas might have a low Se content, which also affects the Se intake of the human and livestock population living there (Gissel-Nielsen et al., 1984.). Proper Se intake is important for healthy growth and the normal function of the immune and cardiovascular system, reproductive organs and muscle activity. It also defend against carcinomas and the spread of certain infections (Rayman, 2012.; Koszta et al., 2012.; Tóth and Csapó, 2018.). The deficiency can be fatal in extreme cases. Animals may also suffer from metabolic diseases and liver damage due to low Se intake (Hartikainen, 2005.). Producing Se-enriched functional foods is a possible solution to compensate low Se intake in humans (Newman et al., 2019.; Schiavon et al., 2020.). Se can be applied to the soil or to plant surface, and irrigation water can also be enriched in order to enhance Se uptake by crops (Edelstein et al., 2016.; Foroughbakhch Pournavab et al., 2020.). Se biofortification of various crops has been extensively studied (Wiesner-Reinhold et al., 2017.; Manojlović et al., 2019.; Lončarić et al., 2021.). Green peas and green beans are very rich in protein and essential amino acids, while tomato is excellent source of vitamins, carotenoids and lycopenes. Thus all these vegetables play an important role in human nutrition due to their mineral content, and therefore may serve as good target plants for biofortification (Poblaciones et al., 2013.; Nithiyanantham et al., 2012.; Perveen et al., 2015.). Se biofortification of tomato have been exhaustively studied, while less research has been reported about peas and beans. Se fertigation during the whole vegetation period of crop grown in soil medium has so far been investigated for tomato (Edelstein et al., 2016; Foroughbakhch Pournavab et al., 2020). Green beans, peas and tomatoes are widely grown and consumed almost all over the world. In 2020, the total production was 1.60, 19.9 and 187 million tons on 0.255, 2.53 and 5.05 million hectares, respectively (FAO, 2022.).

The aim of the experiment was to study the effects of elevated Se levels in the irrigation water. In this paper, effect on the concentration, translocation, distribution and uptake of Se by the fruit, shoot and root of dwarf french bean, pea and tomato plants was investigated in order to evaluate the efficiency of Se biofortification.

Material and methods

The effect of Se-enriched irrigation water was studied on dwarf french bean (*Phaseolus vulgaris* L.) cv. 'Golden Goal', pea (*Pisum sativum* L.) cv. 'Rajnai törpe' and tomato (*Solanum lycopersicum* L.) cv. 'Mano' in a greenhouse at the Experimental Station of the Center for Agricultural Research in Őrbottyán, Hungary. A pot experiment was performed using the top 0-20 cm layer of three types of soil from Hungary according IUSS Working Group (2015): sand [Mollic Umbrisol (Arenic)], silty sand (Luvic Calcic Phaeozem) and silt (Calcic Chernozem). However, the effect of different soil types on the test parameters is not analysed, so these data served as repetitions in the process of the statistical analysis.

Volume of the pots was 10 L and four holes were drilled in the bottom to allow leached water to escape. The bottom of the pots was filled with 4-8 mm gravel, which was covered with a fine synthetic fiber and 10 kg of soil.

Tomato seedlings were grown in a commercial substrate until 6-8 leaf stage, after which they were planted into pots (1 seedling per pot) on 24 May 2018 having a growing period of 88 days. Germinated seeds of dwarf french bean (3 per pot) and pea (1 per pot) were directly sown on 7 May and 11 April 2019 and were harvested on 20 June and 4 July, respectively.

The application of Se-enriched irrigation water started three weeks after planting/sowing. The experiment was set up in three replicates with three treatment levels: Se-0: control, Se-1: 100 and Se-2: 500 μg Se L^{-1} in the form of Na_2SeO_4 in the irrigation water. Thus the number of pots was 3 Se levels x 3 soil types (not evaluated here) x 3 replications = 27 pots for each vegetable. The water was applied with an automatic irrigation system. Individual drip stakes were placed in each pot. Volume of irrigation water was adjusted according to the requirements of the plants: soil moisture content of six selected pots of each vegetable culture was monitored every hour. Fertilization was carried out weekly by mannually adding 200 ml Hoagland's nutrient solution per pot.

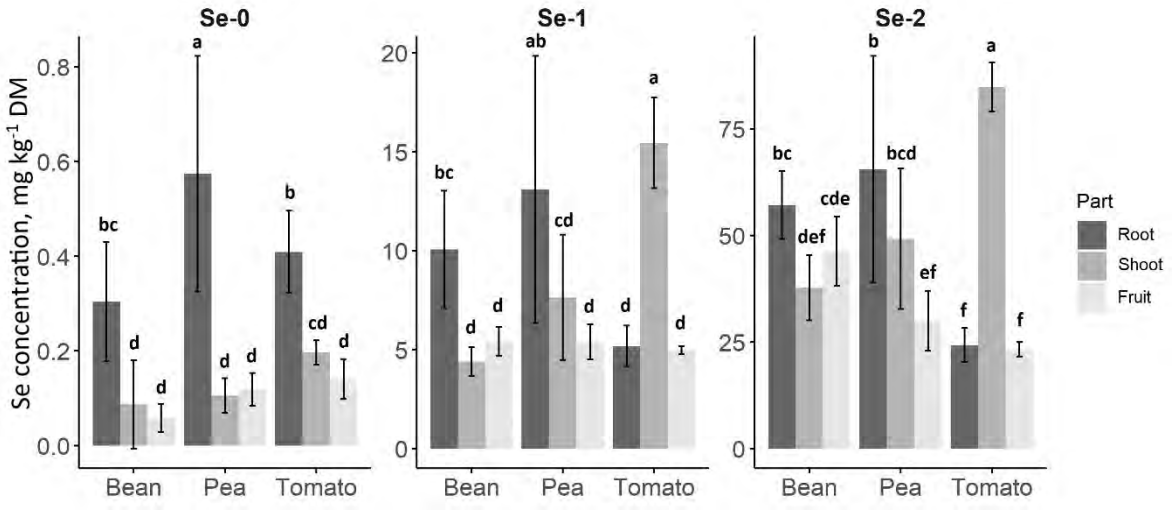
After harvest, root, shoot (stem+leaves) and fruit were separated. Fresh mass and dry matter (DM) were determined. Tomato and green bean fruits were milled and freeze-dried at -70°C, 200 Pa for 72 hours. Other plant parts were were dried at 40°C in a laboratory dryer for two days. Se content of dried samples were measured using inductively coupled plasma mass spectrometer (ICP-MS) after HNO_3 + H_2O_2 digestion. The Se translocation factors for the pairs of different plant parts were calculated as the ratio of the concentrations of the corresponding parts. The amount of Se absorbed by each plant part was calculated by multiplying the dry weight and the Se concentration of the respective plant part. Other details of the experiment can be found in earlier published papers (Ragályi et al., 2021 and 2022.).

One and two-factor factorial analysis of variance was used to evaluate the plant parameters. The factors are clearly defined in the appropriate place of results and discussion. The level of significance was set to a 95% confidence interval (p<0.05). Significantly different groups were determined using Tukey's HSD post hoc test. Statistica v.13 (StatSoft Inc.) software was used for all the statistical calculations. Data visualization was made with *R* statistical software (R Core Team 2022.) using *ggplot2* package (Wickham, 2016.).

Results and discussion

Statistically significant difference developed as a result of each treatment level, i.e. between Se-0 and Se-1, as well as Se-1 and Se-2 (thus between Se-0 and Se-2) concerning the Se concentration of bean root, pea fruit and all parts of the tomato. For bean shoot and fruit, as well as pea shoot and root, Se concentrations differed significantly only in the Se-2 treatment compared to Se-0 or Se-1 treatment levels.

Graph 1 shows the comparison of the Se concentrations in the different parts of the studied vegetables. Different letters indicate significant differences between Se concentrations of different parts of studied vegetables within each treatment level. In the case of Se-0 treatment, for all three crops, the Se concentration in the root was significantly higher than in the above ground parts of the plants. Se in the root reached a higher concentration in the green pea than in the other two crops. In the control Se-0 treatment, the concentrations remained below 1 mg kg^{-1} in all cases, which corresponds to the findings of Marschner (2012.). There was no significant difference between the Se concentration measured in the shoot and fruit of each plant in the control. As a result of Se enrichment, a higher concentration of Se developed in tomato shoot compared to the root and fruit, whereas in the case of green bean and pea, the highest concentration was still found in the root. In the Se-2 treatment, no significant difference could be detected between the Se concentrations measured in the bean root and fruit.



Different letters within each treatment level denote significant differences between mean values based on Tukey's HSD, $p \leq 0.05$. Error bars represent standard deviations.
Graph 1 Se concentration in plant parts of different vegetables

Table 1 shows the translocation factors for the vegetables at the different Se treatment levels. The shoot/root (root-to-shoot) translocation of tomato was significantly the most efficient for the Se-1 and Se-2 treatments, and comparing the control treatments, it was also the highest for tomato, although this latter was not statistically verified. The fruit/shoot translocation factor was the highest in bean. No significant difference was found between the fruit/root translocation factors, however the data varied greatly. The value was the highest in the case of pea treated with 100 µg Se L^{-1}, while on average the values of tomato were higher than the other two vegetables.

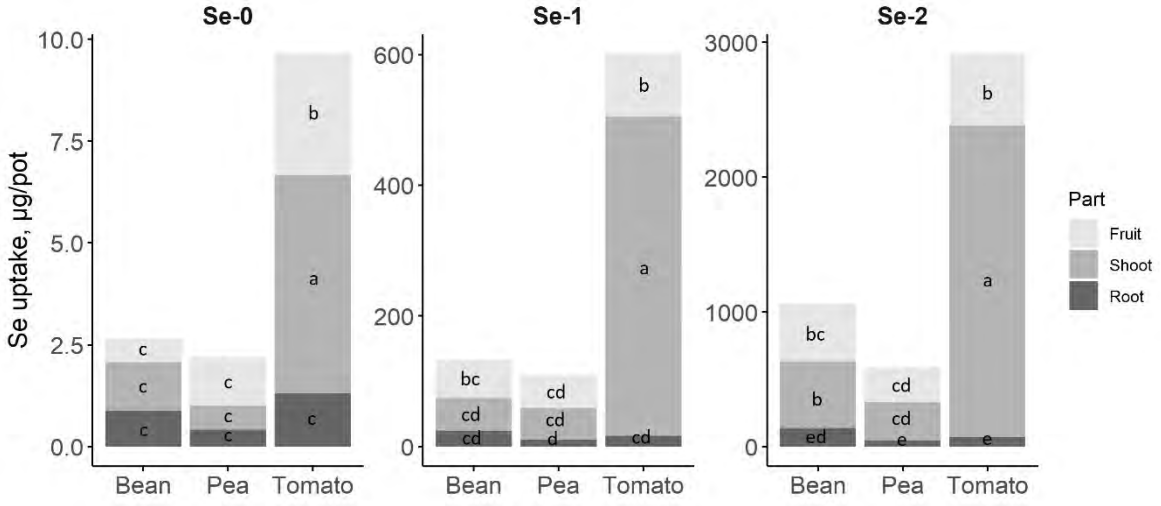
Graph 2 shows the amount of Se taken up by vegetables per pot, which is the Se concentration in D.M. multiplied by dry yield. Naturally, the biomass production of the crops is quite different according to their biological capabilities, which increased the differences in uptake.

The y-axis shows that Se-1 treatment increased Se uptake by two orders of magnitude, and Se-2 treatment also multiplied Se-1 treatment values: this increase was 7.6 times for green beans, 5.1 times for peas, and 4.8 times for tomatoes in the average of plant parts. Mostly, the highest amount was taken up by the shoot, but in the Se-0 treatment in pea the fruit (i.e. grain) took up the most Se, and in the Se-1 treatment, in the case of green bean and pea almost the same amounts were taken up by shoot and fruit (Graph 2).

Table 1. Se translocation factors for bean, green pea and tomato

Vegetable	Se dose	Shoot/Root	Fruit/Shoot	Fruit/Root
			ratio	
Bean	Se-0	0.342^{b}	1.04^{abc}	0.187^{a}
	Se-1	0.457^{b}	1.26^{a}	0.568^{a}
	Se-2	0.664^{b}	1.24^{a}	0.812^{a}
Pea	Se-0	0.207^{b}	1.22^{ab}	0.245^{a}
	Se-1	1.21^{b}	0.780^{abcd}	1.14^{a}
	Se-2	0.815^{b}	0.665^{cd}	0.496^{a}
Tomato	Se-0	0.503^{b}	0.703^{bcd}	0.361^{a}
	Se-1	3.11^{a}	0.328^{d}	0.998^{a}
	Se-2	3.60^{a}	0.275^{d}	0.987^{a}

Different letters indicate significant differences within each column (Tukey's HSD, $p < 0.05$).



Graph 2. Amount of Se absorbed by vegetables per pot and distribution in plant parts

Bitterli et al. (2010.) reports plant Se accumulation, root to shoot transfer and relationship between Se accumulation in plants and Se concentrations of growth media in detail for various plant species. Examining the diverse plant species, the accumulation of Se can vary more than two orders of magnitude among different plants grown in a media with the same Se concentration, however in this present study, vegetables had similar Se contents (Graph 1). The authors also state, that more Se accumulates in the leaves than in other plant parts, but differences are generally small and only rarely higher than one order of magnitude in the case of a given plant species. This fact is also confirmed by the present experiment, since the largest variation among the translocation factors shown in Table 1 was indicated by the shoot/root ratio of pea in the Se-0 treatment showing 5-fold difference.

The discussion of the results of the green beans and peas in the present experiment is difficult due to the lack of similar experiments. Arvy (1993.) examined green bean Se uptake and translocation in green beans at young stage during a few hours after treatment. However, to the best of our knowledge, the translocation and distribution of Se in the different plant tissues of mature beans has not been investigated so far.

Se enrichment of green peas with leaf spray has been investigated (Poblaciones and Rengel, 2018.; Hegedűsová et al., 2017.), as well as Se intake of pea seedlings through roots (Łukaszewicz et al., 2018.). Kádár (2009.) applied extremely high treatments of 30, 90 and 270 kg ha^{-1} Se to peas. Even with the 30 kg ha^{-1} treatment, the Se concentration increased more than 100 times, with a similar concentration in the stem and in the grain, while the crop practically perished at the Se load of 270 kg ha^{-1}. The author also found that pea grain is genetically protected against Cr(III), Cr(VI), Pb and Cu treatments, whereas the Zn enrichment was a maximum of 1.7 times, and the Se accumulation was 283 times compared to control. Although Se is not essential for plants, it is structurally similar to sulphur, which is essential, and plants have only a limited ability to distinguish between the two (Terry et al., 2000.).

In the present experiment, the enrichment of tomato with Se was effective, as Graph 1 clearly shows that the concentration rises remarkably as a result of the treatments. Similar results were obtained by Edelstein et al. (2016.) who found that Se accumulation of all (edible and non-edible) tissues of tomato linearly increased with rising Se concentrations in irrigation water, which were 0, 0.5, 1.0 and 1.5 mg Se L^{-1}. However, there was a difference in the extent of the effect, because the slope of the increase in concentration caused by Se treatment was the steepest in the case of the shoot, followed by the root and the fruit, which also corresponds to the results of the present experiment. Similar results were obtained by Foroughbakhch Pournavab et al. (2020.), who investigated the effect of 0, 2 and 5 mg L^{-1} Se enriched irrigation water on tomato Se concentration. In contrast, Carvalho et al. (2003.) found that the Se concentration of the fruit was less than 1% compared to nonedible parts.

Conclusions

The results support the feasibility of Se biofortification through irrigation with Se-enriched water for green bean, pea and tomato. However, based on the amount of Se absorbed by the examined vegeta-

bles, as well as the amounts of production, tomato biofortification can be recommended primarily. Further studies are needed to explore the relationship between the applied treatment doses and the fruit Se content in more detail.

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Vinogradarstvo, vinarstvo i voćarstvo

Utjecaj defolijacije i prorjeđivanja grozdova na kemijski sastav grožđa sorte Zlatarica vrgorska u vinogorju Vrgorac

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Sažetak

Ampelotehnički zahvati defolijacije i prorjeđivanja grozdova na kvalitativni potencijal grožđa. Cilj ovog istraživanja je utvrditi utjecaj rane defolijacije i prorjeđivanja grozdova na kemijski sastav grožđa i gospodarske karakteristike sorte 'Zlatarica vrgorska'. Sadržaj organskih kiselina utvrđen je pomoću HPLC uređaja, dok je analiza hlapljivih spojeva provedena pomoću plinske kromatografije. Tretmani su značajno utjecali na udio kožice i sjemenke u grozdu, kemijske pokazatelje kakvoće mošta (sadržaj šećera i ukupnih kiselina, sadržaj vinske i jabučne kiseline, asimilirajući dušik, sadržaj hlapljivih spojeva). Dobiveni rezultati istraživanja potvrđuju opravdanu primjenu provedenih zahvata i njihov pozitivan utjecaj na gospodarske karakteristike sorte.

Ključne riječi: Zlatarica vrgorska, defolijacija, prorjeđivanje grozdova

Uvod

Zlatarica vrgorska autohtona je sorta vinove loze koja se uzgaja na području vrgorskog polja. Vinogorje Vrgorac se nalazi u podregiji Dalmatinska zagora. Prvi spomen sorte potječe iz 1821. godine (Riterr von Heintl, 1821.). Zlatarica ima identičan genetski profil kao i talijanska sorta Francavidda (Schneider i sur., 2014.). Riječ je sorti lokalnog značaja koja se rijetko uzgaja na području Brindisija u Apuliji. Zlatarica je vrlo cijenjena na području Vrgorca zbog dobrih i stabilnih priroda i daje lagana i užitna vina. Sorta je relativno osjetljiva na pepelnicu i sivu plijesan. Defolijacija je zahvat zelene rezidbe kojim se odstranjuju bazalni ili vršni listovi u različitim fenofazama razvoja vinove loze. Zahvatom se utječe na mikroklimu trsa (Sabbatini i Howell, 2010.). Postiže se bolja prozračnost i osvijetljenost grožđa. Primjenom defolijacije smanjuje se pojava zaraženosti gljivičnim bolestima, ali i efikasnija primjena sredstava za zaštitu bilja (Bubola, 2015.). Termin rana defolijacija odnosi se na defolijaciju trsova prije ili neposredno nakon cvatnje. Uslijed uklanjanja fotosintetski najaktivnijih listova, dolazi do nedostatka asimilata što uzrokuje slabiju oplodnju, zametanje manjeg broja boba i rjeđe grozdove (Caspari i sur., 1998.). Primjenom rane defolijacije možemo uspješno kontrolirati prirod nasuprot kvalitete mošta kod sorata velikog generativnog potencijala (Poni i sur., 2006.). Osrečak i sur. (2011.) istraživali su primjenu djelomične defolijacije na sortama Traminac i Manzoni. Osim na polifenolni sastav, primijenjeni zahvati nisu imali značajniji utjecaj na ostale promatrane parametre. Bubola i Prešurić (2011.) su utvrdili kako defolijacija nije značajnije utjecala na prirod i kemijske pokazatelje grožđa kod sorte Malvazija istarska. Prorjeđivanjem grozdova mijenjamo odnos između broja grozdova i lisne površine, odnosno povećava se lisna površina naspram broja grozdova (Karoglan Kontić i Mirošević, 2008.). Prorjeđivanje grozdova je iznimno skup zahvat koji iziskuje dosta radne snage te ga treba primjenjivati selektivno (Diago i sur., 2010.). U većini istraživanja prorjeđivanje grozdova utječe na povećanje sadržaja šećera i smanjenje ukupne kiselosti (Wang i sur., 2018.). Obradović i sur. (2020.) utvrdili su kako proječivanje grozdova nije utjecalo na većinu kemijskih pokazatelja kakvoće grozda kod sorte Chardonnay, a Škrab i sur. (2021.) su utvrdili da prorjeđivanje grozdova nije imalo značajnijeg utjecaja na prirod i kemijske parametre mošta kod sorte Ribolla Gialla. Defolijacija i prorjeđivanje grozdova mogu imati značajan utjecaj na sadržaj hlapljivih spojeva (Feng i sur., 2015., Reynolds i sur., 2007.).

Cilj istraživanja je utvrditi utjecaj rane defolijacije i prorjeđivanja grozdova na kemijski sastav grožđa i gospodarske karakteristike sorte 'Zlatarica vrgorska'.

Materijal i metode

Pokusni vinograd nalazi se u vinogorju Vrgorac, lokalitet Topolac. Uzgojni oblik je dvostrani kordonac. Vinograd je posađen 2012. godine na podlozi *Vitis berlandieri x Vitis riparia* SO4 cijepljenjem na sadnom mjestu. Sklop sadnje je 2 x 0,9 m. Pokus je postavljen slučajnim bloknim rasporedom s tri pokusne varijante 2020. godine. Na samom lokalitetu vinograda nalazimo aluvijalna tla na jezerskim taložnima visoko vapnene ilovače. Aluvijalna karbonatna tla u Vrgorskom polju su dosta duboka, hladna i lagana za obradu. Kapacitet za vlagu i zrak im je dobar. Svaka varijanta ima tri ponavljanja s po deset trsova. Pokusi su uključivali kontrolu (K), defolijaciju (Def) i prorjeđivanje grozdova (Def+Pr). Provedena je bazalna defolijacija po završetku cvatnje (E-L 31, prema Coombe (1995.) u obje pokusne varijante koje su uključivale defolijaciju. Uklonjena su četiri bazalna lista, dok su kod prorjeđivanja grozdova uklonjeni svi osim bazalnog grozda na mladici.

U tehnološkoj zrelosti utvrđen je prirod po trsu. Nakon obavljene berbe odvojen je prosječni uzorak od 10 grozdova po repeticiji. Na izdvojenim uzorcima grožđa su utvrđeni sadržaj šećera (Oe^0), ukupna kiselost ($g\ L^{-1}$), sadržaji hlapljivih spojeva i pojedinačnih organskih kiselina, slobodnog amino dušika (FAN) određen metodom derivatizacije uz *o*-ftaldialdehid i N-acetil cistein kao derivatizacijske reagense, amonijaka (NH_4) određen primjenom ion selektivne elektrode, i ukupnog dušika (YAN) iskazan kao zbroj vrijednosti FAN i NH_4 i pH vrijednost. Isto tako, provedena je osnovna analiza mehaničkog sastava grozda (% udio peteljke i boba) prema Prostoserdovu (1946.).

Osnovni fizikalno kemijski pokazatelji kvalitete mošta, sadržaj ukupnih topivih tvari (°Oe), sadržaj ukupnih kiselina i pH vrijednost određeni prema (OIV, 2022). Sadržaj pojedinačnih organskih kiselina (vinske, jabučne i limunske) u moštu određen je pomoću HPLC-a (High-Performance Liquid Chromatography, Agilent 1100 Serie, Waldbronn, Germany) iz prosječnog uzorka svježe iscijeđenog, centrifugiranog (13000 x g, centrifuga VWR Mega Star 600R) i pročišćenog mošta (Zoecklein i sur., 1995.).

Analiza hlapljivih spojeva provedena je primjenom metode opisane u Šikuten i sur. (2021) na vezanom sustavu plinski kromatograf (Thermo Scientific Trace 1300) - spektometar masa (Thermo Scientific ISQ 7000) uz prethodnu izolaciju analita mikroekstrakcijom na čvrstoj fazi u izvedbi klina (engl. Solid Phase Microextraction Arrow) pomoću automatiziranog sustava za pripravu uzoraka. Za čvrstu fazu korišten je sustav CAR-PDMS-DVB. U posudicu za uzorke dodano je 100 mg suhe kožice. Prije same adsorpcije na čvrstu fazu, uzorak je uravnotežen pri 60 °C u trajanju od 10 min. Adsorpcija analita provedena je pri 60 °C u trajanju od 49 min. Desorpcija je provedena u injektoru plinskog kromatografa pri 250 °C u trajanju od 7 min. Kromatografska analiza provedena je pomoću TR-Wax kolone (60 m x 0,25 mm x 0,25 μm) uz temperaturni program u rasponu temperatura od 40 do 210 °C. Snimanje spektara masa provedeno je praćenjem struje svih iona u rasponu od 30 do 300 m/z dok je energija elektrona bila 70 eV. Identifikacija je provedena pomoću usporedbe vremena zadržavanja, retencijskih indeksa te usporedbom spektara masa s onima u NIST 17 i Wiley 12 bazi podataka.

Statistička obrada podataka provedena je analizom varijance (ANOVA). Analizom varijance ustanovljena je značajna razlika između ispitivanih klonova i njihovih testiranih vrijednosti. Dobiveni rezultati obrađeni su uz pomoć SAS v 9.3 statističkog softvera (2012, SAS Institute Inc., NC, SAD). Usporedba srednjih vrijednosti klonskih kandidata provedena je Duncan's Multiple Range testom.

Rezultati i rasprava

Mehanički pokazatelji grozda (udio kožice i sjemenki u grozdu) (tablica 1.) bili su pod značajnim utjecajem provedenih ampelotehničkih tretmana. Prosječno najmanji udio kožice i najviši udio sjemenki zabilježen je u kontrolnom tretmanu dok su provedeni tretmani utjecali na značajno povećanje udjela kožice i smanjenje udjela sjemenki u grozdu. Veći udio kožice u odnosu na meso i sjemenke pozitivno utječe na kvalitetu zbog povećanja površine kožice u odnosu na volumen bobice (Mirás-Avalos i sur., 2019.)

Tablica 1. Usporedba srednjih vrijednosti uvometrijskih i mehaničkih pokazatelja

Pokusna varijanta	Masa grozda (g)	Masa boba (g)	Masa peteljkovine (g)	Udio mesa u grozdu (%)	Udio kožice u grozdu (%)	Udio peteljkovine u grozdu (%)	Udio sjemenki u grozdu (%)
K	442.38a	429.81a	12.57a	85.89a	9.74b	2.86a	1.51a
Def	375.31a	364.89a	10.42a	85.58a	10.11a	2.84a	1.47b
Def+Pr	368.71a	358.52a	10.19a	85.84a	10.09a	2.74a	1.33c

**srednje vrijednosti označene različitim slovima između sorata razlikuju se na razini p<0.05 korištenjem Duncan's multiple-range testa*

Zahvati defolijacije i prorjeđivanja grozdova utjecali su na značajno povećanje sadržaja šećera (od 78.33 do 84.33 Oe^0) i smanjenje ukupnih kiselina (4.27 do 3,41 g L^{-1}) između kontrole (K) i tretmana kako slijedi (Def, Def+Pr) (tablica 2.). Utvrđeno je u skladu s istraživanjima Wanga i sur. (2018.) koji su koji su vršili prorjeđivanje grozdova uklanjanjem svih grozdova osim bazalnih u E-L 31.

Tablica 2. Usporedba srednjih vrijednosti promatranih gospodarskih svojstava i kemijskih pokazatelja kakvoće mošta

Pokusna varijanta	Sadržaj šećera u moštu (Oe^0)	Ukupna kiselost (g L^{-1})	pH	Prirod (kg trs^{-1})	Broj grozdova trs^{-1}
K	78.33b	4.27a	3.61a	3.28a	9.8a
Def	84.33a	3.86ab	3.67a	2.85a	8.8ab
Def+Pr	84.33a	3.41b	3.66a	2.97a	8.4b

**srednje vrijednosti označene različitim slovima između sorata razlikuju se na razini p<0.05 korištenjem Duncan's multiple-range testa*

Provedeni ampelotehnički zahvati značajno su utjecali na smanjivanje sadržaja vinske kiseline (od 4.98 do 4.33 g L^{-1}). Kod provedenog tretmana defolijacije (Def) utvrđen je najviši sadržaj jabučne kiseline (0.52 g L^{-1}) u odnosu na ostala dva tretmana. Budući da su provedeni zahvati uključivali uklanjanje dijela fotosintetski aktivnog lišća, dobiveni rezultati su očekivani (Caspari i sur., 1998.). Naime organske kiseline se velikim dijelom sintetiziraju u listovima te translociraju u bobe tijekom dozrijevanja grožđa (Burbidge i sur., 2021.). Provedena istraživanja su u skladu s istraživanjima Bubole i sur. (2012.) na sorti Malvazija istarska, a u suprotnosti s istraživanjima Obradović i sur. (2020.) na sorti Chardonnay.

Tablica 3. Usporedba srednjih vrijednosti sadržaja pojedinačnih organskih kiselina

Pokusna varijanta	Vinska kiselina (g L^{-1})	Jabučna kiselina (g L^{-1})	Limunska kiselina (g L^{-1})
K	4.98a	0.46ab	0.11a
Def	4.33c	0.52a	0.12a
Def+Pr	4.69b	0.37b	0.36a

**srednje vrijednosti označene različitim slovima između sorata razlikuju se na razini p<0.05 korištenjem Duncan's multiple-range testa*

Asimilacijski dušik predstavlja zbroj vrijednosti slobodnog amino dušika i amonij iona. Sve navedene vrijednosti su na donjem pragu te apsolutno nedostatne za pravilno odvijanje fermentacije (Butzke, 1998.). Donje granica vrijednosti YAN-a su od 150 do 200 mg L^{-1} (Bell i Henschke, 2005.). Mošt tretmana defolijacija i prorjeđivanje (Def+Pr) imao je značajno nižu koncentraciju asimilacijskog dušika (151.54 mg L^{-1}) u odnosu na defolijaciju (Def).

Tablica 4. Usporedba srednjih vrijednosti slobodnog amino dušika (FAN), amonij iona (NH_4^+) i asimilacijskog dušika (YAN)

Pokusna varijanta	FAN (mg L^{-1})	NH_4^+ (mg L^{-1})	YAN (mg L^{-1})
K	52.80a	110.33a	163.11a
Def	52.31a	106.00a	158.15ab
Def+Pr	55.67a	95.87b	151.54b

**srednje vrijednosti označene različitim slovima između sorata razlikuju se na razini p<0.05 korištenjem Duncan's multiple-range testa*

Analizom sadržaja hlapljivih spojeva u grožđu utvrđen je sadržaj 40 pojedinačnih organskih spojeva koji su podijeljeni u 7 grupa hlapljivih spojeva (tablica 5.). Provedeni tretman defolijacije za razliku od defolijacije i prorjeđivanja grozdova značajno je utjecao na sadržaj aldehida, alkohola, monoterpena, seskviterpena. Dobiveni rezultati su u skladu s rezultatima Bureau i sur. (2000.) i Škrab i sur. (2021.).

Tablica 5. Usporedba srednjih vrijednosti grupa hlapljivih spojeva izraženi u apsolutnoj površini pikova ($\times 10^5$)

Pokusna varijanta	Aldehidi	Alkoholi	Ketoni	Monoterpeni	Kiseline	Seskviterpeni	Ostali	UKUPNO
K	2560,5b	243,6b	24,89a	5,20b	12,69a	7,69b	4,40a	2859,0b
Def	2826,1a	372,8a	2,65c	6,00a	11,07b	8,32a	3,30c	3230,3a
Def +Pr	2542,5b	219,3c	16,97b	5,40b	9,91c	6,77c	3,80b	2804,7b

**Prosječne vrijednosti označene različitim slovima ukazuju na značajne statističke razlike između pokusnih varijanata (uz p > 0,05), korištenjem Duncan's multiple range testa*
***rezultati usporedbe srednjih vrijednosti hlapljivih spojeva su izraženi u veličini pika*

Zaključak

Tretmani defolijacije i defolijacije i prorjeđivanja grozdova kod sorte Zlatarica vrgorska značajno su utjecali na povećanje udjela kožice i smanjenje udjela sjemenki u grozdu. Kad je riječ o gospodarskim karakteristikama, tretmani su značajno utjecali na povećanje sadržaja šećera, smanjenje sadržaja ukupnih kiselina i broja grozdova po trsu. Analizom sadržaja pojedinačnih organskih kiselina utvrđeno je da su tretmani značajno utjecali na smanjenje sadržaja vinske kiseline, dok je kod defolijacije zabilježen prosječno najviši sadržaj jabučne kiseline. Provedeni zahvat defolijacije značajno je utjecao na sadržaj hlapljivih spojeva za razliku od tretmana defolijacije i prorjeđivanja. Ovo istraživanje temeljeno je na jednogodišnjem pokusu, a koji bi trebalo primijeniti u višegodišnjem istraživanju kako bi dobili što relevantniji prikaz utjecaja provedenih ampelotehničkih zahvata na karakteristike sorte Zlatarica vrgorska.

Napomena

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Influence of defoliation and grape thinning on the chemical composition of grapes of a grapevine variety Zlatarica vrgorska in the winegrowing area Vrgorac

Abstract

Agronomic practices such as defoliation and grape thinning can have a great impact on the grape quality. Aim of this research is to explore the impact of early defoliation and grape thinning on chemical composition and economic characteristic of grapevine variety Zlatarica Vrgorska. Content of the organic acids has been determined with HPLC method, while the analyses of the volatile compounds was done with the gas chromatography. The results of uvometric and mechanic indicators showed that the obtained agronomic practices significantly influenced on the share of skins and seeds in grapes, sugar content, total acidity, tartaric and malatic acid, yeast assimilable nitrogen and volatile compounds. The obtained results of the research confirm the reasoned application of the defoliation and grape thinning and their positive impact on the economic characteristics of the variety.

Keywords: Zlatarica vrgorska, defoliation, grape thinning

Impact of late winter pruning of Portugieser grapevines (*Vitis vinifera* L.) on yield components and grape composition

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Abstract

Postponing pruning until early spring has been shown to be an effective way of delaying vine phenology. We investigated the effects of the timing of late winter pruning on 'Portugieser' vines in northwestern Croatia (2021), on yield, berry primary and phenolic composition. Late winter pruning (at the time of budbreak of basal buds and when two to three leaves were separated) was compared with standard winter pruning. Late winter pruning at budbreak resulted in grapes with the highest total phenolic and anthocyanin content and the lowest sugar and flavonol content at harvest, while late winter pruning performed when two to three leaves were separated had the highest titratable acidity and lowest total phenolic content.

Keywords: grapevine, anthocyanins, late pruning, polyphenols

Introduction

Winter pruning is a canopy management procedure usually carried out during dormancy. Its main purpose is to maintain the training system of the vine and to regulate the yield and vegetative growth. It is known that winter pruning performed late in the season, when buds are already beginning to swell, delays bud-break by a few days, helping to avoid spring frosts, which is especially important in regions with cool climates (Poni et al., 2022).

Increased temperatures in spring and summer often accelerate flowering, ripening and harvest date, while the final stage of grape ripening (from veraison to harvest) usually occurs in the hottest months of July and August, resulting in grapes with higher sugar content, lower acidity and poor color development. This is a climate change-related phenomenon that is of increasing concern to grape and wine producers around the world. To mitigate these effects, several approaches exist in different wine regions of the world, including postverasion trimming and leaf removal apical to the cluster zone (Caccavello et al., 2017, Palliotti et al., 2013) and preveraison application of auxin (Böttcher et al., 2022).

One of the approaches to deal with high temperatures during ripening is to delay the onset of bud break by delaying winter pruning, with the assumption that this will delay vine phenology, delay grape ripening and cause the final stage of sugar and phenolic ripening to shift to cooler conditions after high summer temperatures. Delaying bud break by pruning at this time can be explained by apical dominance, in which basal bud growth is suppressed by bud break of apical buds of the cane, but then basal buds are forced to break after pruning (Friend and Trought, 2007, Keller, 2015). Therefore, postponing the winter pruning date seems to be an effective way to delay fruit ripening and improve grape chemical composition (Frioni et al., 2016, Frioni et al., 2019, Gatti et al., 2016, Gatti et al., 2018, Moran et al., 2019). Late winter pruning can have an effect on crop yield and improve grape and wine characteristics (Netzer et al., 2022). In warmer climates, late winter pruning can be an effective tool to improve vine water status due to delayed canopy development (Netzer et al., 2022).

Although late winter pruning is a promising tool to delay ripening, its effect depends largely on the timing of pruning (Frioni et al., 2016). The later in the season winter pruning is performed, the more it can affect floral primordium differentiation, flower development, fruit set and fertilization (Frioni et al., 2016). Extremely late winter pruning can result in unacceptably low yield. Repeating the treatment following years may further delay vine phenology, as repeating the same late pruning treatment on the same vines may result in excessive use of available reserves in

the spring to support vegetative growth (Gatti et al., 2018). The aim of our study was to investigate the effect of late winter pruning on yield, berry primary and phenolic composition of field-grown 'Portugieser' (*Vitis vinifera* L.) grape variety.

Materials and Methods

A one-year study (2021) was conducted on 'Portugieser' (*Vitis vinifera* L.) grape variety grown in a vineyard in north-western Croatia. The study was conducted on the Jazbina experimental field of the Department of Viticulture and Enology, Faculty of Agriculture, University of Zagreb (long. 45°51'N, lat. 16°0'E). The soil type was anthropogenic pseudogley with clay texture. The vines were trained on the bilateral Guyot system, 80 cm above the ground. The rows were oriented northeast-southwest, with 2.1 m between rows and 1.2 m between vines (4000 vines/ha). The vines were grown using cultural practices common to continental Croatia.

Three pruning treatments were applied at different phenological stages based on the modified Eichhorn and Lorenz (E-L) scale (Coombe, 1995): E-L 4 (budbreak of basal buds), E-L 9 (two to three leaves were separated), E-L 1 (winter bud phase- control). The study was set in a randomized block design with three blocks per treatment, each block containing 6 vines. Regardless of timing, all vines were pruned with 2 spurs with 2 buds and 2 canes with 10 buds, which had around 24-25 buds.

Yield was measured on the vine basis, together with counting the number of clusters per vine. Samples of grapes from all three treatments were harvested at the same time. Must samples were produced from the harvested grapes by crushing them. The basic parameters of the must are total soluble solids, titratable acidity and pH. The soluble solids content was measured with a hand-held refractometer and pH was determined with a pH meter. The titratable acidity was determined using the coloration pattern volumetric method according to the O.I.V. (O.I.V., 2001).

Randomly selected 100 berries per block were stored at -20°C. The grape skins were manually removed from the frozen berries and freeze dried. To obtain a powder, the skins were grinded with the MiniG Mill (SPEX Sample Prep, Meutchen, USA), and were stored at -20°C until analysis. The extraction of the phenolic compounds was carried out according to the method described by Tomaz et al. (2016). The separation, identification, and quantification of the phenolic compounds from berry skin extracts was performed on an Agilent 1100 series system (Agilent, Germany), equipped with DAD, FLD, and coupled to an Agilent Chem Station data-processing station according to the one described by Tomaz and Maslov (2016). The results are expressed in mg/kg dry weight (DW) of skin. All data were analyzed using the one-way ANOVA procedure, and when differences between treatments were significant, Tukey's test at $P>0,05$ was used to separate the means, using the XLSTAT software v.2020.3.1. (Addinsoft, New York, NY, USA).

Results and Discussion

Late winter pruning performed at the time of the budbreak increased yield, which was attributed to a higher average cluster weight (Table 1), a concept also seen in Friend and Trought (2007), who demonstrated that pruning around budburst may improve yield in cultivars that have unbalanced crop load because of poor fruitset. Winter pruning at E-L 4 appeared to stimulate yield production and increase cluster weight, as late winter pruning could have triggered preferential bursting of buds with different inherent fertility (Petie et al., 2017). On the other hand, late winter pruning performed when two to three leaves were separated did not affect yield and cluster weight (Table 1). Contrasting results of late winter pruning regarding the effect of timing of treatment on yield have been reported, which demonstrates a complex interaction between vine growth, phenology and the environment. In the research of Petrie et al. (2017), the yield variation in the 'Shiraz' vines were primarily caused by changes in bunch number and berry mass. Late winter pruning performed 1, 2 or 3 weeks after budbreak reduced the yield of cv. 'Malbec' in Israel (Netzer et al., 2022). On the other hand, in the research by Frioni et al. (2016), winter pruning performed at budbreak did not affect vine growth, yield and grape composition at harvest, but when performed in May, yield per vine decreased due to lower berry and cluster number, as a result of source limitation to the developing cluster primordia. Late pruning can lead to reduced yield compared to standard winter pruning, possibly due to reduced bud fertility, fewer clusters per vine and smaller berries (Gatti et al., 2018, Zheng et al., 2017).

Treatment with late winter pruning reduced grape sugar content regardless of timing (Table 1), confirming the findings of other researchers that delayed winter pruning can delay fruit ripening (Allegro et al., 202, Buesa et al.,

2022, Falginella et al., 2022, Gatti et al., 2018). Late winter pruning performed at the time of the budbreak also reduced the total acidity of the must (Table 1). Similar to our results, late winter pruning of cv. Merlot delayed grape ripening and lowered sugar levels and acidity at harvest (Friend and Trought, 2007). Late pruning treatments can result in shorter shoot lengths and delayed phenological stages for the early ripening cultivars (Ferrara et al., 2022).

Table 1. Yield and basic composition of 'Portugieser' grape juice from different late pruning treatments (2021)

	Control	LWP1	LWP2
Yield /vine (kg)	2,47 b	3,23 a	2,65 ab
Cluster weight (g)	134 b	189 a	153 b
Soluble solids (°Oe)	106 a	93 c	101 b
Titratable acidity (g/L)	8,2 b	6,7 c	9,3 a
pH	3,38 a	3,38 a	3,27 a

LWP1- late winter pruning performed at budbreak, LWP2- late winter pruning performed when two to three leaves were separated, data were analysed through one-way ANOVA, means are separated with Tukey's test at P>0,05

Late winter pruning at the time of budbreak resulted in grapes with higher total phenolic and anthocyanin content and lower flavonol content at harvest, whereas late winter pruning performed when two to three leaves were separated had higher titratable acidity, fenolic acids and flavan-3-ols content, and lower total phenolic content compared with standard winter pruning (Table 2). Late winter pruning, regardless of timing, increased the total stilbene content of 'Portugieser' grape skin. Similar results were shown by others. Buesa et al. (2022) showed that late pruning just before budbreak delayed grape ripening, resulting in grapes with higher anthocyanin concentration for similar sugar content at harvest.

Table 2. Polyphenolic composition of 'Portugieser' grape skin from different late pruning treatments (2021)

	Control	LWP1	LWP2
Delphinidin-3-O-glucoside	1445,12 a	1217,88 c	1341,98 b
Cyanidin-3-O- glucoside	472,65 b	231,15 c	613,73 a
Petunidin-3-O- glucoside	2374,16 a	2064,76 b	1921 b
Peonidin-3-O- glucoside	1753,82 a	1146,07 b	1709,8 a
Malvidin-3-O- glucoside	8704,9 b	10116,63 a	7116,68 c
Delphinidin -3-O-(caffeoyl) glucoside	119,31 a	114,58 b	99,56 c
Peonidin-3-O-(acetyl) glucoside	117,86 a	86,27 c	94,03 b
Malvidin-3-O-(acetyl) glucoside	1390,37 b	1796,89 a	1139,77 c
Delphinidin -3-O-(coumaroyl) glucoside	44,78 a	33,02 b	31,36 b
Malvidin-3-O-(caffeoyl) glucoside	330,39 b	388,45 a	228,75 c
Peonidin-3-O-(coumaroyl) glucoside	328,77 a	308,62 b	245,52 c
Malvidin-3-O-(coumaroyl) glucoside	1479,85 b	2345,22 a	1225,4 b
Total anthocyanins	18561,98 b	19849,54 a	15767,58 c
Myricetin-3-O- glucoside	456,32 b	503,8 a	448,46 b
Myricetin -3-O-glucuronide	35,39 b	57,37 a	8,58 c
Quercetin-3-O-galactoside	158,83 b	126,62 c	201,23 a
Quercetin -3-O- glucoside	5343,69 b	4958 c	5721 a
Quercetin -3-O- glucuronide	77,18 b	88,7 a	64,61 c

Kaempferol-3-O-rutinoside	408,76 b	399,12 b	513,56 a
Kaempferol -3-O- glucoside	201,34 a	219,71 a	192,66 a
Kaempferol -3-O- glucuronide	14,74 a	6,96 c	12,69 b
Izorhamnetine-3-O- glucoside	75,24 a	77,73 a	59,12 b
Total flavonols	6771,49 a	6438,01 b	6764,87 a
Caftaric acid	94,22 c	202,41 b	366,72 a
Protocatechuic acid	25,48 b	32,96 a	31,86 a
Total phenolic acids	119,7 c	235,57 b	398,58 a
Viniferin	3,7 b	3,57 b	4,22 a
Resveratrol-3-O- glucoside	281,47 b	302,51 a	309,52 a
Total stilbenes	285,17 b	306,8 a	313,74 a
Gallocatechin	150,41 c	240,69 a	220,95 b
Epigallocatechin	170,6 c	210,34 b	258,63 a
Procyanidin B1	150,45 b	176,89 b	222,41 a
Procyanidin B3	13,18 a	14,84 a	15,3 a
Catechin	72,92 c	120,98 b	164,38 a
Procyanidin B2	52,21 b	56,72 b	72,19 a
Epicatechin	2,07 b	2,76 b	12,79 a
Total flavan-3-ols	611,84 c	823,22 b	966,95 a
Total phenolic concentration	26350,18 b	27652,22 a	24211,42 c

LWP1- late winter pruning performed at budbreak, LWP2- late winter pruning performed when two to three leaves were separated, data were analysed through one-way ANOVA, means are separated with Tukey's test at P>0,05

Netzer et al. (2016) found that late winter pruning increased anthocyanin content in grapes, possibly due to the lower number of clusters per vine, resulting in a distribution of more assimilates to each cluster, or to better exposure of clusters to sunlight due to the more compact canopy. Performing late winter pruning when shoots were 10 cm long decreased sugar content, increased total acidity, and increased anthocyanin content in the study by Palliotti et al. (2017). According to Frioni et al. (2016), late spur pruning in May increased total anthocyanin and phenolic content, possibly as a result of smaller berry size. On the other hand, late winter pruning a few weeks after budbreak did not affect sugar, total acidity and total anthocyanin content of 'Maturana' grapes grown in the Rioja region of Spain (Zheng et al., 2017), and late winter pruning performed at the time of sap bleeding stage did not affect total anthocyanin and flavonol concentrations in 'Merlot' grapes from Treviso, Italy (Falginella et al., 2022).

Conclusion

Late winter pruning is a cost-effective canopy management practice that allows grape growers to cope with frost but also to postpone grape ripening to cooler temperature conditions. This study suggests that postponing the winter pruning date tends to have positive effects on grape quality compared to standard winter pruning. Late winter pruning can delay the ripening and harvest date of 'Portugieser' grapes without negatively affecting the basic fruit composition, as delaying pruning to budbreak resulted in grapes with the highest total phenolic and anthocyanin content and the lowest sugar content at harvest. More in-depth studies are needed to evaluate how the interaction between environmental conditions, variety, and the timing of late winter pruning affects the accumulation of sugars and berry color during ripening.

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Total phenolic content and antioxidant capacity of Teran red wine: influence of pre-fermentative mash procedures

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Abstract

Six vinification treatments, including control treatment (K7) were carried out to investigate: the impact of 48-h pre-fermentative mash cooling (8 °C) followed by 13-days maceration (C15), 28-days maceration (C30) and *saignée* technique (CS15); and the impact of 48-h heating (50 °C) followed by 13-days maceration (H15) and 28 days (H30) on total phenolic content (TPC) and antioxidant capacity (AC) in Teran wines. TPC was analysed by a method based on Folin–Ciocalteu reagent and AC was determined by FRAP and ORAC assay. The results showed higher TPC and AC in all treatments compared to the control wine (K7). The highest TPC was found after *saignée* technique applied (CS15). Pre-fermentative heating and 30-day maceration treatment (H30) showed the strongest antioxidant capacity, with both FRAP and ORAC assay.

Keywords: Teran red wine, pre-fermentative procedures, prolonged maceration, total phenolic content, antioxidant capacity

Introduction

Phenolic compounds are the main class of secondary metabolites in plants. Wine is one of the beverages with the highest phenolic content (Drosou et al., 2015) which also plays a major role in some organoleptic characteristics of wine, such as colour and astringency (Bai et al., 2013). The final phenolic content in wine depends on the type of soil, climate, grape, harvest, and pre- and post-harvest treatments (Bai et al., 2013; Muñoz-Bernal et al., 2021). The health-promoting properties, including antimicrobial, anticarcinogenic, and antioxidant properties (Büyüktuncel et al., 2014), of phenolic compounds are influenced by their structure, solubility, conjugation with other polyphenols or other compounds (Singh et al., 2018; Zeb, 2020). The antioxidant activity of phenolic compounds is attributed to the capacity of scavenging free radicals, donating hydrogen atoms, electrons, or chelate metal cations. Many studies have shown a strong and positive correlation ($p \leq 0.05$) between the phenolic compound contents and the antioxidant potential (Plavša et al., 2012; Minatel et al., 2017). The interest in natural antioxidants has increased in recent years because of their presumed safety and potential nutritional and therapeutical effects. Among natural antioxidants, red wine has attracted particular interest due to a high content of biologically active compounds (Büyüktuncel et al., 2014). This implies that the antioxidant capacity of wine, an important property closely related to the total amount of phenols, varies with grape varieties, vintages, weather, and wine-making procedures (Lissi et al., 2014), pre-fermentative procedures, fermentation/maceration, and maturation. Maceration is a critical step to obtain the best characteristics of wine since a large number of phenolic compounds come from seeds and skins (Aleixandre-Tudo and du Toit, 2018; Muñoz-Bernal et al., 2020). Also, extended (prolonged) maceration is a widely used winemaking technique, based on extended the contact of grape solids and wine after the end of fermentation (Sacchi et al., 2005). This technique has been used to alter the mouthfeel of the wines, possibly by facilitating proanthocyanidin extraction and the formation of polymeric pigments (Casassa et al., 2013). Additionally, different pre-fermentative enological techniques have been used and studied to understand their effects on the color and phenolic compound composition of the wine (Heredia and Guzman-Chozas, 1993; Cejudo-Bastante et al., 2014).

One example is cold pre-fermentative maceration, also known as cold soaking or cryomaceration, which could be adopted to extract water-soluble compounds in the absence of alcohol at low temperatures (5 - 10 °C), mainly phenolic and volatile compounds (Heredia et al., 2010; Barros et al., 2022). Another non-conventional technique, called thermovinification, entails short heating of the skins from 50 to 80 °C extracting them with the juice, pressing, and then cooling before fermentation. If heating at the same temperature before fermentation is extended for a longer period (for instance, up to 24 h), the process is called pre-fermentative heat treatment (Escudier et al., 2008; Rossi et al., 2022). The heat damages the hypodermal cell membranes, releasing anthocyanins, and it also denatures polyphenol oxidase, preventing browning. Since there is no alcohol present at the time of heating, it would not be expected to increase tannin extraction (Sacchi et al., 2005). Furthermore, frequently in use is the *saignée* technique, also known as pre-fermentation juice runoff. The juice is removed before fermentation, thus increasing the skin-to-juice ratio, thereby enhancing the extraction of phenolic compounds and stabilizing the apparent color of the wine (Sacchi et al., 2005). Teran (*Vitis vinifera* L.) is the most widespread red autochthonous cultivar on the Istrian peninsula, in Croatia (Plavša et al., 2012; Rossi et al., 2022). This study aimed to investigate how pre-fermentative cooling or heating procedure, *saignée* technique, and various maceration durations affect the total phenolic content (TPC), and antioxidant capacity (AC) in Teran red wines.

Material and methods

The grapes of *cv*. Teran (*Vitis vinifera* L.) was grown in wine-growing hill Western Istria, the town of Poreč, in a typical Istrian terroir. The harvest was held in 2020 when the sugar content was measured at 18.9 °Brix, 8.0 g L^{-1} of total acidity expressed as tartaric acid, and pH 3.2. On the same day, manually harvested grapes were destemmed and crushed with standard equipment and homogenized. Red grape mash was equally divided according to the plan of the experiment (Table 1).

Table 1: Overview of the experiment: pre-fermentative procedures, winemaking techniques and maceration duration in Teran wine treatments

Treatment*	Pre-fermentative procedure (48 hours)	Fermentation and maceration			Maceration duration + pre-fermentative procedure
		Winemaking technique	**Maceration/ fermentation temperature**	**Maceration duration**	
CS15	Cooling - at 8 °C	*Saignée*	24 °C	13 days	15 days
C15		Prolonged maceration		13 days	15 days
C30				28 days	30 days
H15	Heating - at 50 °C			15 days	15 days
H30				28 days	30 days
K7	/	Standard		7 days	/

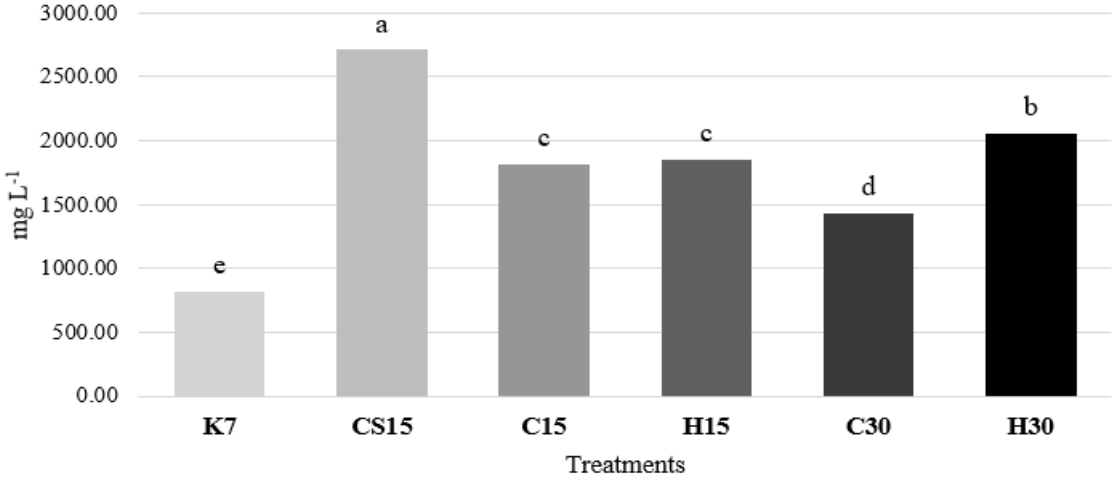
**C-cooling; H-heating; S-saignée; K-contol; 7,15,30 – days of maceration duration in total*

Five vinification treatments were submitted to pre-fermentative mash cooling at 8 °C (cryomaceration) or mash heating at 50 °C for 48 hours, proceeded with fermentation at 24 °C and followed by prolonged maceration in two periods of duration: 13 days, respectively 15 days in total including pre-fermentative procedure (C15; H15) and 28 days, in total 30 days (C30; H30). In one of the treatments with pre-fermentative mash cooling, the *saignée* technique was performed before fermentation. A proportion (33 %) of the total juice quantity was racked and concentrated mash proceeded with fermentation (24 °C) and prolonged 13-days maceration, 15 days in total (CS15). This experiment has also included a control treatment (K7), with a standard 7-day maceration and a maceration/ fermentation at temperature of 24 °C. Every treatment was performed in three replicats in 220 L-stainless steel tanks. All grape mashes were inoculated with 30 g hL^{-1} of selected *Saccharomyces cerevisiae* dry yeast (Fermol Mediterranee, AEB), and chaptalized with 3 kg hL^{-1} of saccharose. After the end of maceration, mashes were pressed and wine was racked in clean tanks. After six months the wine were bottled and stored prior to analysis. The total phenolic content of each wine was determined by the Folin–Ciocalteu colorimetric method (Singleton and Rossi, 1965) using a Cary 50 UV/Vis spectrophotometer (Varian Inc., Harbour City, CA, USA), at the Institute of Agriculture and Tourism in Poreč. The absorbance was measured against a blank at a wavelength of 765 nm, and the results were expressed as

gallic acid equivalents in mg L^{-1} of wine (mg GAE L^{-1}). While analysis of the antioxidant capacity in the wines were performed at the Faculty of Food Technology and Biotechnology in Zagreb by the ferric reducing/antioxidant power (FRAP) assay, and the oxygen radical absorbance capacity (ORAC) assay. The FRAP assay was conducted according to the method of Benzie and Strain (1996) and the results are expressed in mmol L^{-1} $FeSO_4 \times 7H_2O$. The ORAC assay was performed according to Ninfali et al. (2005), as briefly described in Mazor Jolić et al. (2011). Fluorescence was measured by a Varian Cary Eclipse Spectrofluorometer (Palo Alto, CA, USA). The results were calculated as ORAC values using the difference in the area under the fluorescein decay curve between the blank and the sample. The results are expressed as mmol L^{-1} of Trolox equivalents (TE). Analyses of antioxidant capacity by FRAP and ORAC assays were conducted in triplicate. One-way analysis of variance (ANOVA) and Fisher's least significance difference (LSD) test were used to compare mean values ($p < 0.05$). Statistics were performed using Statistica 10.0. software (Sta-Soft Inc. Tulsa, OK).

Results and discussion

Obtained results showed that total phenolic content (TPC) in all treatments was significantly higher in comparison to the control treatment (K7), where 821.52 mg L^{-1} was measured ($p < 0.05$) (Figure 1).



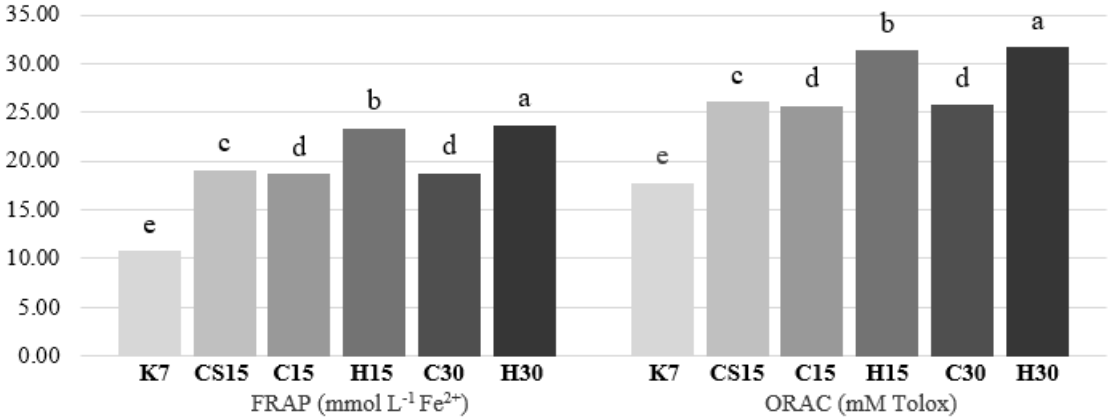
Lower-case letters above column represent significant differences at p < 0.05 level according to the LSD test.

Figure 1: Total phenolic content (mg L^{-1}) in Teran wines, 2020.

These results are in accordance with Rossi et al. (2020), who investigate similar vinification procedures and prolonged macerations on Teran wine of grapes from the same vineyard. In our investigation, the highest content of phenols, 2710.61 mg L^{-1}, was found in treatment submitted to pre-fermentative mash cooling, *saignée* technique, and prolonged post-fermentative 15-day maceration (CS15). This is confirmed by the fact that removing the juice with a certain level or ratio would theoretically increase the concentration of water-soluble compounds in the juice (Wu et al., 2017; Cheng and Watrelot, 2022). Regarding TPC among treatments with pre-fermentative mash cooling or heating and prolonged post-fermentative 15-day maceration (C15 and H15), a significant difference was not evident. Possibly, because the extraction of phenols did not reach the maximum within 15 days of maceration. This is in correspondence with results published by Plavša et al. (2012), where TPC increases significantly with the length of maceration and 20-day-long maceration shows higher values than 15-day-long maceration. But the difference was seen in respect of our treatments submitted to prolonged post-fermentative 30-day maceration (C30 and H30), where the heating procedure in H30 exhibited a greater effect on TPC in comparison to the cooling procedure in C30. In our previous study, dealing with total and free anthocyanins, C30 also showed lower values in comparison to other treatments (Orbanić et al., 2022).

According to the FRAP assay, values in all treatments were statistically higher compared to the control wine (K7). Treatment that underwent pre-fermentative heating and prolonged 30-day maceration (H30) provided significantly the highest antioxidant capacity, 23.67 mmol L^{-1} Fe^{2+}, with respect to the control treatment where 10.77 mmol L^{-1}

Fe^{2+} was measured. An identical situation was obtained with ORAC assay, values ranged from 17.76 mM Trolox found in K7 to 31.67 mM Trolox in H30 treatment (Figure 2).



Lower-case letters above column represent significant differences at $p < 0.05$ level according to the LSD test.

Figure 2: Antioxidant capacity in Teran wines (2020) analysed by FRAP and ORAC assay

This could be explained by the fact that the heating process is not effective in extracting tannins, which are responsible for phenolic stability (Sacchi et al., 2005). Therefore, providing direct condensation and co-pigmentation reactions of tannins with flavanol-3-ol monomers, anthocyanins, anthocyanin-derived pigments, and oligomers is decreased (Smith et al., 2015). Consequently, some phenolic compounds stay unbound thus enhancing antioxidant activity, with the hydrogen atoms of the adjacent hydroxyl groups (o-diphenol), located in various positions of the rings A, B, and C, with the double bonds of the benzene ring, and with the double bond of the oxo functional group (-C=O) of some flavonoids (Minatel et al., 2017). When correlation coefficients (r) were examined, a very strong correlation between FRAP and ORAC at 0.998 ($p < 0.05$) was noted. The results of correlation analysis showed that these methods are almost comparable in characterizing antioxidant capacities. Other researches using antioxidant capacity measurement methods have also shown a high correlation (Radeka et al., 2022; Tahmaz and Söylemezoğlu, 2022). Moreover, the positive linear correlation of moderate strength among TPC and antioxidant capacity by FRAP assay was determined, exhibiting an r value of 0.657 ($p < 0.05$) (Figure 3), providing evidence that the predominant source of antioxidant activity derives from phenolic compounds in wine (Paixao et al., 2007). A similar correlation was obtained in the case of ORAC assay and TPC, with an r value of 0.627 ($p < 0.05$) (Figure 4). These results could be explained with consideration that phenolic subgroups, possible synergy, and antagonism among them, degree of polymerization, and radical molecules contained in wine have different influences on antioxidant capacity (Di Majo et al. 2008). Additionally, Arnous et al. (2002) reported that polymeric and other types of pigments may not have similar antioxidant characteristics in comparison with monomeric anthocyanins.

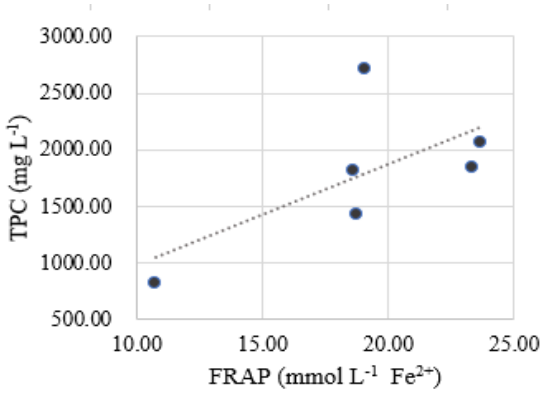


Figure 3: Correlation among TPC and FRAP values in Teran wines (r = 0.657)

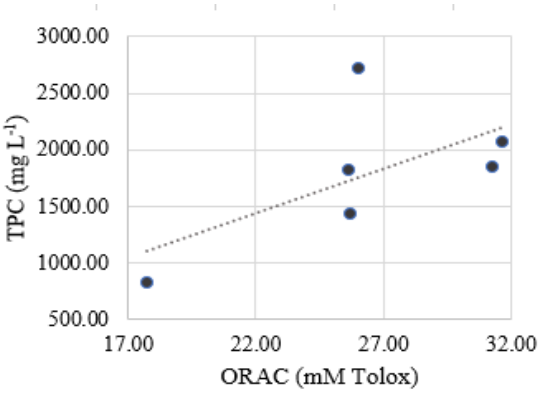


Figure 4: Correlation among TPC and ORAC values in Teran wines (r = 0.627)

Conclusions

Applied pre-fermentative procedures demonstrated a significant impact on total phenolic content as well as on the antioxidant capacity of Teran red wines when compared with wine produced by standard technique. Obtained evidence could be employed in applying investigated vinification procedures in the winemaking industry to produce Teran and other red wines of the desired style, thus providing diversification of the wine market. Furthermore, with considerable bioactive content, which acts as added value, the beneficial properties of moderate consumption of wine are enhanced.

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Sadržaj ukupnih fenola i antioksidativni kapacitet vina sorte ´Teran´: utjecaj predfermentativnih tretmana masulja

Sažetak

Provedeno je šest vinifikacijskih tretmana, uključujući kontrolu (K7) kako bi se istražio: utjecaj predfermentacijskog hlađenja masulja (48 h; 8 °C) koji se nastavio maceracijom od 13 dana (C15), 28 dana (C30), te *saignée* tehnologijom (CS15); i utjecaj tretmana grijanja (48 h; 50 °C), koji se nastavio maceracijom od 13 (H15) i 28 dana (H30) na koncentraciju ukupnih fenola (TPC) i antioksidativni kapacitet (AC) vina ´Teran´. TPC određeni su metodom koja se temelji na reakciji s Folin–Ciocalteu reagensom, a antioksidativni kapacitet metodama FRAP i ORAC. Prema dobivenim rezultatima, vrijednosti TPC i AC u vinima svih tretmana više su u odnosu na kontrolni tretman (K7). Najviše TPC dobiveno je u tretmanu sa *saignée* tehnologijom (CS15). Tretman koji uključuje predfermentativno zagrijavanje i produženu maceraciju od 30 dana (H30) pokazao je najveći antioksidativni kapacitet, koristeći obje metode (FRAP i ORAC).

Ključne riječi: Teran, predfermentacijski tretmani, produljena maceracija, ukupni fenoli, antioksidativni kapacitet

Application of zinc formulations of microalgae (Chlorella) in blueberry cultivation

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Abstract

American blueberry is a perennial fruit species, the fruits of which are rich in antioxidants that have a positive effect on human health. During cultivation, it is necessary to pay attention to quality nutrition, protection and agrotechnical measures to achieve the highest quality fruits. This research aims to investigate the effects of foliar application of biopolymer formulations filled with microalgae and zinc sulfate during the cultivation of two blueberry cultivars ('Duke' and 'Aurora') on the quality of the fruit. After the harvesting, in samples of blueberry fruits, some chemical properties were determined (anthocyanins, flavonoids and polyphenols). The results obtained showed that the application of certain bipolymer formulations increased the amount of polyphenols by 17%, flavanoids by 7,2%, and anthocyanins by 26% in the blueberry fruits of selected blubbery cultivars.

Keywords: encapsulation, blueberry, microalgae, microparticles, zinc, foliar

Introduction

Blueberry(*Vaccinium corymbosum* L.) is an increasingly popular food due to its favorable nutritional characteristics. It has numerous positive nutritional and pharmacological properties, such as a high proportion of vitamins, minerals and secondary metabolites; polyphenols and antioxidants. The presence of secondary metabolites in the diet contributes to the normal functioning of the organism, reducing the chance of oxidation processes and the development of numerous diseases. It occurs as a perennial, bushy plant. The main reason for growing this blueberry is its fruit, a berry composed of an exocarp and a mesocarp. It is suitable for consumption in fresh and dried form, freezing and processing (Nikolić et al., 2015). The leading world producers are the USA (294,000 tons), Canada (146,370 tons) and Peru (180,300 tons), while 720 tons of blueberries were grown in Croatia in 2020 (FAOSTAT,2022). The most commonly cultivar are 'Duke', 'Aurora', 'Patriot', 'Bluecrop', 'Nui' and 'Hannah's Choice' (Nikolić et al., 2015). Species of the genus *Vaccinium* began to be collected and experimentally cultivated as early as the beginning of the 18th century (Trehane, 2004), and experienced the greatest commercial success in the period between 1995 and 2005, when the area of total blueberry plantations increased by 90% (Zhao, 2007).

Blueberries require special climatic and environmental conditions that must be ensured for profitable production and the highest quality. Blueberry fruit has a low energy value and is rich in nutritious and bioactive components such as flavonoids, phenolic acids, vitamins and minerals (Nikolić et all, 2015, Nile et all., 2014). It has high antioxidant properties thanks to a large amount of phenolic compounds (Soče, 2019). The antibacterial, antimutagenic, anti-inflammatory and anti-cancer effects of certain active components of blueberry have also been confirmed (Landete, 2012). They have a favorable effect on maintaining the health of the circulatory system and improving vision (Nabavi, 2018). Flavonoids in food are responsible for pigments, aroma, annulation of oxidation and protection against the deactivation of enzymes and vitamins (Yao et al., 2004). The most abundant flavonoids in strawberry fruits are anthocyanins, and it has been proven that blueberry anthocyanins and other polyphenols have beneficial effects on reducing hyperglycemia, body weight and cholesterol accumulation (Roopchand et al., 2013). The nutritional composition of crops, including blueberries, can be improved by appropriate nutrition. One of the ways is the feeding of microalgae, which gives satisfactory results as a supplement in the food industry and agriculture.

According to the systematics of microalgae, they are prokaryotic and eukaryotic photosynthetic microorganisms. Their cells have a simple structure, that ensures rapid and successful growth in unfavorable conditions, which is

why they are found in diverse and extreme ecosystems; deserts, thermal springs and under the ice of Antarctica. (Heimann et all. 2015). Microalgae are attracting attention as a high-potential raw material for biofuel production (Sikirić et al., 2019). They are a potential source of numerous bioactive and energetic substances such as polymers, peptides, fatty acids, carotenoids and sterols. Their photosynthetic activity is high, which is why they perform rapid growth and biomass synthesis (Kim, 2015). They have a lot of potential as sources of carotenoids, vitamins and phenols, and as natural antioxidants, they can cancel lipid peroxidation in food and serve as a natural preservative (Vadlja, 2019). The production of microalgae itself is simple, and high yields are achieved with minimal investment. Microalgae derivatives are completely biodegradable and have no harmful effects on the environment (Čevid, 2016).

Biopolymer formulations of microcapsules have numerous applications; in food, cosmetic and agricultural production. Various bioactive components can be encapsulated and thus ensure their gradual release, and depending on their size, the speed of release can be determined. When it comes to agricultural production, the application of encapsulated bioactive components is a relatively new method of treatment, and their effect is still being tested. Biopolymer formulations of microcapsules can be used as a supplement, which achieves continuous release, i.e. a longer supplement effect (Vinceković et al., 2016 and Vinceković et al., 2017). Polymers for the preparation of biopolymer formulations may include proteins (gelatin, gluten, casein), carbohydrates (starch and its derivatives, gum arabic, xanthan gum, agar, dextran, alginates, cellulose derivatives), waxes and lipids (glycerides of palmitic and stearic fatty acids, acetyl -alcohol, beeswax) and synthetic polymers (polyvinyl alcohol, polyacrylic acid, polystyrene, polyurethanes and polysiloxanes) (Petrović, 2010).

The aim of this study is the folliar application of microcapsules formulations through the process of spraying them over the crown of the shrub so that the plant could absorb nutritiens through the leaves. With foliar application fertilization are 8-20 times greater than nutrient uptake from the soil through the roots, reducing the consumption of the applied agent.

Material and methods

Plant material and experimental design

The experiment was conducted at the blueberry plantation of Fragaria Ltd in Velika Ludina (45°36'10.1"N 16°36'14.8"E) in 2021. The experiment was set up according to the method of randomized block design in four replicates. Each experimental row consisted of 80 blueberry plants (20 in each repetion), and each treatment was applied to 5 plants in each row.

Treatments: The biopolymer formulations filled with microalgae and zinc sulfate were prepared in the laboratory of the Department of Chemistry, Faculty of Agriculture, University of Zagreb. The control treatment was distilled water, the second treatment is a 10% Chlorella algae solution, and the remaining two treatments were two types of microcapsules; sodium alginate microcapsules with zinc sulfate $ZnSO_4 \times 7H_2O$ and sodium alginate microcapsules with both zinc sulfate $ZnSO_4 \times 7H_2O$ and microalgae solution. The microcapsules were prepared using the ionic gelation technique at room temperature by adding a solution of the carrier of the active substance, sodium alginate, with a Büchi-Encapsulator B-390 (BÜCHI Labortechnik AG, Switzerland) into a solution of zinc sulfate heptahydrate (1 mol dm^{-3})(Vinceković et all. 2016).

Each cultivar of blueberries was treated three times (April 16, May 11, and June 1 in 2021). The foliar application was carried out with a manual pressure sprayer (LUX TOOLS Cl), and about 200 mL of the preparation was used per plant. The preparation contained 10 g of microcapsules per plant (200 g per repetition i.e.800 g per treatment. The application solution was prepared by putting 800 g of capsules and 5.2 L of distilled water into the canister so that there would be a total of 6 L of the treatment agent. The doser of the manual pressure sprayer is 3 L, which was enough for 2 rows of application, i.e. repetitions.

Fruit sampling: Ripe fruits were harvested for the 'Duke' on June 21 and 28 and for the 'Aurora' on August 2 and 9, 2021. For each treatment, at each harvest, an average sample of about 500 g was taken. Samples were stored in a refrigerator at the Department of Pomology Faculty of Agriculture until analysis. The dependent variables in each of the analyzes represented the average of the values of 2 measurements (1st harvest and 2nd harvest.).

Analysis of total polyphenols, flavanoids and anthocyanins: Total polyphenols were determined based on the colorimetric reaction of the Folin-Ciocalteu reagent with some reducing reagents (polyphenolic compounds). The absorbance intensity of the resulting blue coloration at 765 nm is determined spectrophotometrically (Ough et all.,

1988), whereby the intensity of the coloration is directly proportional to the proportion of polyphenolic compounds in the tested sample (Singleton et all., 1965). Results are expressed as gallic acid equivalents (GAE) [mg EGAL/100 g FW]. Total flavonoids (Ivanova et al. 2010) are determined spectrophotometrically Results are expressed as quercetin equivalent (QE) [mg QE/100 g FW] at a wavelength of 360 nm. Anthocyanins are determined by the method according to Giusti and Wrolstad (2001). Results are expressed as equivalent of cyanidin-3-glucoside (C3GE) [mg C3GE/100 g FW] at 520 nm and 700 nm. All spectrophotometric measurements were done by Shimadzu UV-1900i spectrophotometer.

Statistical analysis: The data were presented as mean ± standard deviation. Differences between means of examined chemical parameters of blueberry cultivars were compared by the Kolmogorov-Smirnov test and Wilcoxon W test ta at a significance level of 95%, with a risk level of 5%. The data analysis was done in using SPSS Statistics, version 24.0.) were used. Considering the small samples, one of the non-parametric statistical procedures was used to test each hypothesis, although the Kolmogorov-Smirnov test indicates the normality of the distribution of the results in all measurement subjects for the cultivar.

Results and discussion

The results of chemical analyzes of blueberry fruits for phenols can be found in Tables 1 and 2. According to the authors Dongnan et al. (2017) the average amount of polyphenols in the fruits of the 'Duke' is 380 mg/100 g FW. The Mann Whitney U test and the Wilcoxon W test determined a statistically significant difference in the amount of polyphenols in the fruits between the 'Aurora' and 'Duke', and it can be determined with 99% certainty that the fruits of the 'Aurora' have a higher amount of polyphenols compared to the fruits of the 'Duke', with 1% risk (Table 3.).

Table 1. Polyphenols, Flavanoids and Anthocyanins content in blueberries 'Duke'

Treatment	Polyphenols mg EGK/100 g FW		Flavonoids mg QE/100 g FW		Anthocyanins mg C3GE/100 g FW	
	1.harvest	2.harvest	1.harvest	2.harvest	1.harvest	2.harvest
Control	293.82	183.23	205.63	136.61	136.59	69.74
Microalgae solutions	317.59	173.86	217.64	136.64	144.59	71.26
Microcapules zinc	298.98	182.29	221.60	138.44	136.86	72.54
Microcapsules microalgae and zinc	288.01	167.11	209.39	126.83	139.02	65.54

Table 2. Polyphenols, Flavanoids and Anthocyanins content in blueberries 'Aurora'

Treatment	Polyphenols mg EGK/100 g FW		Flavonoids mg QE/100 g FW		Anthocyanins mg C3GE/100 g FW	
	1.harvest	2. harvest	1.harvest	2.harvest	1.harvest	2.harvest
Control	276.52	248.50	184.73	190.75	70.94	66.78
Microalgae solutions	256.19	261.81	197.56	219.64	71.24	73.07
Microcapules zinc	323.79	274.60	218.22	219.28	94.45	81.84
Microcapsules microalgae and zinc	326.30	305.52	229.08	222.41	95.66	91.38

Table 3. Statistical analyzes of polyphenols saturation results concerning cultivar

Cultivar	N	M	C	SD	U	W	p
'Aurora'	16	286.88	286.05	29.15			
'Duke'	16	237.99	240.85	30.56			
Total	32				30.00	166.00	0.00**

Legend: N-sample size; M – arithmetic mean; C- median; SD- standard deviation; U- Mann Whitney U test; W- Wilcoxon W test; p- statistical significance
*Note: ** $p < 0.01$; * $p < 0.05$*

According to the authors Dongnan et al. (2017), the average amount of flavonoids in the fruits of the 'Duke' is 220 mg/100 g^{-} FW. The Mann Whitney U test and the Wilcoxon W test showed a statistically significant difference in the amount of flavonoids in the fruits between the cultivars Aurora' and 'Duke', and it can be determined with 99% certainty that the fruits of the 'Aurora' have a higher amount of flavonoids, compared to the fruit 'Duke', with a 1% risk (Table 4).

Table 4. Statistical analyzes of flavonoid results with regard to cultivar

Cultivar	N	M	C	SD	U	W	p
'Aurora'	16	212.74	206.69	27.31			
'Duke'	16	174.10	174.34	16.64			
Total	32				21.00	157.00	0.00**

Legend: N-sample size; M – arithmetic mean; C- median; SD- standard deviation; U- Mann Whitney U test; W- Wilcoxon W test; p- statistical significance
*Note: ** $p < 0.01$; * $p < 0.05$*

The Mann Whitney U test and the Wilcoxon W test determined a statistically significant difference in the amount of anthocyanins in the fruits between the cultivars 'Aurora' and 'Duke', and it can be determined with 99% certainty that the fruits of the cultivar 'Duke' have a higher amount of anthocyanins compared to the fruits of 'Aurora' varieties, with 1% risk (Table 5).

Table 5. Statistical analyzes of anthocyanin results with regard to cultivarvariety

Cultivar	N	M	C	SD	U	W	p
'Aurora'	16	82.22	77.79	13.26			
'Duke'	16	104.54	105.16	15.69			
Total	32				31,00	167,00	0,00**

Legend: N-sample size; M – arithmetic mean; C- median; SD- standard deviation; U- Mann Whitney U test; W- Wilcoxon W test; p- statistical significance
*Note: ** $p < 0.01$; * $p < 0.05$*

Analysis of variance did not establish a statistically significant difference in the amount of polyphenols in the fruits between treatments, and with 95% confidence, it can be determined that there is no difference in the amount of phenols concerning the different treatments (Table 6). Although the results themselves showed some differences (treatments with capsules with zinc and microalgae in the cultivar 'Aurora'), the sample itself was not large enough to prove significant differences through statistical analysis.

Table 6. Statistical analyzes of polyphenols results concerning the type of treatment.

Treatment	N	M	C	SD	H	p
Control	8	250.27	243.58	30.12		
Microalgae solutions	8	259.84	264.83	34.02		
Microcapsules Zn	8	269.91	273.10	35.73		
Microcapsules Alge/Zn	8	269.72	264.78	53.97		
Total	32				1.55	0.67

Legend: N-sample size; M - arithmetic mean; C - median; SD - standard deviation; H - Kruskal-Wallis test; p-statistical significance.
*Note: ** p < 0.01; * p < 0.05*

The analysis of variance did not establish a statistically significant difference in the amount of flavonoids in the fruits between treatments, and with 95% confidence, it can be determined that there is no significant difference in the amount of flavonoids concerning the different treatments (Table 7).

Table 7. Statistical analyzes of flavanoids results concerning of treatment

Treatment	N	M	C	SD	H	p
Control	8	179.47	176.86	18.19		
Microalgae solutions	8	197.77	195.98	36.50		
Microcapsules Zn	8	199.46	196.57	22.89		
Microcapsules Alge/Zn	8	196.98	191.08	37.58		
Total	32				2,99	0,39

*Legend: N-sample size; M - arithmetic mean; C - median; SD - standard deviation; H - Kruskal-Wallis test; p-statistical significance. Note: ** p < 0.01; * p < 0.05*
*Note: ** p < 0,01; * p < 0,05*

The analysis of variance did not establish a statistically significant difference in the amount of anthocyanins in the fruits between treatments, and with 95% certainty, it can be determined that there is no significant difference in the amount of anthocyanins concerning the different treatments (Table 8).

Table 8. Statistical analyzes of anthocyanin results concerning the type of treatment

Treatment	N	M	C	SD	H	p
Control	8	86.01	80.60	22.75		
Microalgae solutions	8	91.13	81.15	24.51		
Microcapsules Zn	8	96.42	93.84	11.72		
Microcapsules Alge/Zn	8	97.93	97.71	13.02		
Total	32				2,79	0,43

*Legend: N-sample size; M - arithmetic mean; C - median; SD - standard deviation; H - Kruskal-Wallis test; p-statistical significance. Note: ** p < 0.01; * p < 0.05*
*Note: ** p < 0,01; * p < 0,05*

Although the results themselves showed some differences (treatments with capsules with zinc and capsules with zinc and microalgae in the variety 'Aurora'), the sample itself was not large enough to prove significant differences through statistical analysis which could be explained because of the weather extremes through the all 2021.

The conducted research opens up the potential for additional investigations of biopolymer formulations of microalgae because they are understudied and have great potential for application in sustainable fruit productionv

Conclusions

The results of this research showed an increased proportion of total polyphenols, flavanoids and an increased proportion of anthocyanins in blueberry fruits of the 'Aurora' cultivar foliar treated with biopolymer formulations with a solution of zinc sulfate $ZnSO_4 \times 7H_2O$ and biopolymer formulations with a solution of *Chlorella microalgae*.

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Pomološka svojstva višnje sorte 'Oblačinska' na vlastitom korijenu i na podlozi 'Rašeljka'

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Sažetak

Višnja (*Prunus cerasus*, L.) uzgaja se na različitim vegetativnim i generativnim podlogama. Na rast i razvoj, kao i na pomološka svojstva utječu genotip, podloga, te agroekološka obilježja. Cilj rada je bio utvrditi postoje li razlike među mjerenim pomološkim svojstvima ploda višnje 'Oblačinske' uzgajane na vlastitom korijenu i uzgajane na podlozi 'Rašeljka'. Mjerenja pomoloških svojstava su pokazala veću masu ploda kod višnje 'Oblačinska' na podlozi 'Rašeljka' (3.54 g) u odnosu na istu na vlastitom korijenu (2.87 g). Širina i visina ploda su bile veće kod višnje 'Oblačinska' na podlozi 'Rašeljka'.

Ključne riječi: višnja, podloga, pomološka svojstva

Uvod

'Oblačinska višnja' predstavlja populaciju velikog broja klonova (genotipova), (Milatović i Nikolić, 2011.). Prema navodima Mišića (1989) "Oblačinska višnja", danas predstavlja heterogenu populaciju koja je uglavnom, nastala vegetativnim razmnožavanjem izdancima. Ne treba, međutim, isključiti mogućnost utjecaja generativnog način razmnožavanja na širenje ove populacije. Stablo ove višnje je slabe do umjerene bujnosti, loptaste do široko piramidalne krune, vrlo pogodno za gustu sadnju. (Milutinović i Nikolić, 1997.). Zbog redovite rodnosti, visokih uroda i kvalitete ploda ubrzo se proširila u uzgoju (Puškar, 2005). Kako se lako razmnožava izdancima, 'Oblačinska višnja' se često uzgaja na vlastitom korijenu. Otporna je prema niskim zimskim temperaturama i proljetnim mrazevima. Također je otporna na sušu. Cvjeta srednje rano. Samooplodna je. Rađa dobro i obilno (15 – 30 t/ha). U odnosu na standardne sorte višanja kao što su 'Rexelle' i 'Haimanova konzervna', Oblačinska dozrijeva 10 - 15 dana ranije od istih, ali je unutar populacije Oblačinske višnje zabilježeno različito dozrijevanje. Vrijeme zriobe je genetski uvjetovano, ali i varira pod utjecajem vanjskih čimbenika. Oblačinska sazrijeva u drugoj polovici lipnja (Šoškić, 2008.). Plod je okruglastog do okruglastokolačastog oblika, prilično ujednačene krupnoće i vremena zriobe. Pokožica je tanka, tamnocrvene boje. Meso je crveno, srednje čvrsto, sočno, kiselkastog ukusa, aromatično i vrlo kvalitetno. Sok je tamnocrvene boje. Koštica je sitna i lako se odvaja od mesa. Plod se lako odvaja od peteljke. Otvor ploda po odvajanju peteljke ostaje zatvoren, te se sok ne gubi. Relativno je dobre transportablinosti i pri običnim uvjetima može se čuvati u svježem stanju 3 - 5 dana. Pogodan je za preradu u najrazličitije proizvode (Nikolić i sur., 2011), imajući u vidu da je plod 'Oblačinske višnje'sitan, ali sa izraženim kvalitetom mesa i soka. Izuzetno je dobra za proizvodnju sokova, kompota i za konditorsku industriju (Mratinić, 2010).

Od generativnih podloga za višnju, najčešće se koriste divlja trešnja i rašeljka, te sjemenjaci kulturnih sorata višnje. Danas se u svijetu koristi relativno veliki broj vegetativnih podloga za višnju, porijeklom od trešnje, rašeljke, obične višnje, stepske višnje, kao i međuvrsnih hibrida (Mratinić, 2010). Vegetativne podloge za višnju su 'F 12-I' 'Colt', 'St. Lucia 64', klon višnje 'Stocton Marello', 'Oblačinska višnja', 'Avalska višnja' i drugi tipovi domaće višnje. Pored navedenih, kao podloge za višnju koriste se i izdanci višnje (Miljković, 1991). 'Rašeljka' je botanički bliža višnji nego trešnji. Koristi se kao podloga za višnju, na suhim tlima, manje plodnim, ali dovoljno dubokim, kao i izrazito vapnenim tlima. Ne podnosi zbijena, kisela, vlažna tla i na njima brzo strada. Otporna je prema suši. Sorte cijepljene na njoj postižu manju bujnost, kraće su dugovječnosti. U proizvodnim nasadima kao podloga za višnju se koristi sjemenjak 'Rašeljke' (*Prunus mahaleb* Mill.) koja je dobra podloga za višnju u sušnijim uvjetima, jer ima snažno razgranat korijen, dobro podnosi sušu, lakša skeletoidna tla (Vrsaljko i sur., 2010). Između sorti višanja s jedne strane i podloga s druge strane postoji vegetativna podudarnost. Anderson i sur. (1993) zaključuju da podloge utječu na raniju ili kasniju cvatnju, na količinu uroda i krupnoćuploda. Pored navedenog, različita istraživanja govore o

tome kako podloga ima značajan utjecaj na urod višanja, kao i tip tla i drugi ekološki činitelji (Perry i sur., 1993). Poznavanje biologije višnje, kao i njenih zahtjeva prema agroekološkim uvjetima je bitno zbog izbora sorte, izbora proizvodnog prostora, izbora podloge, uzgojnog oblika, održavanja tla, kao i kvalitete plodova. Višnja najbolje uspijeva na dubokim, dobro dreniranim tlima, ilovaste, pjeskovito ilovaste do glinasto ilovaste teksture. Višnja na podlozi 'Rašeljka' pliće rasprostire korijenovu mrežu, nego na trešnji kao podlozi . Glavninu korijena (75%) stablo višnje na podlozi 'Rašeljka', rasprostire do dubine od 40 cm. U dubljim tlima postoje povoljni uvjeti za bolju opskrbu vodom i hranjivima, odnosno za dublje rasprostiranje korijena.

Višnja za dobar rast i rodnost zahtjeva slabo kiselu do neutralnu pH reakciju tla (Miljković, 1991). Optimalna pH vrijednost tla za uzgoj višanja se kreće između 5.5 i 7.0. Višnja se može uzgajati i na težim i zbijenijim i drugim manje povoljnim tlima ukoliko se izabere odgovarajuća podloga. Cilj rada je bio utvrditi postoje li razlike među mjerenim pomološkim svojstvima ploda 'Oblačinsk' uzgajane na vlastitom korijenu i uzgajane na podlozi 'Rašeljka'.

Materijal i metode

Istraživanje je provedeno u višnje 'Oblačinska' starosti 15 godina. Voćnjak se nalazi u općini Ljubinje, na jugoistoku Bosne i HercegovineJedan dio stabala u voćnjaku višnje 'Oblačinska' je uzgojen na vlastitom korijenu (izdanak), drugi dio stabala višnje 'Oblačinska' je uzgojen na podlozi 'Rašeljka' (*Prunus mahaleb*, Mill.). Uzgojni oblik je vaza. Razmak sadnje višnje 'Oblačinske' bio je 4 ×2.5 m, a pravac redova je sjever-jug. U voćnjaku je primjenjena zimska rezidba, zaštita od bolesti i štetočina i redovita gnojidba, a navodnjavanje je omogućeno sustavom kap po kap. Iz voćnjaka su uzeta dva prosječna uzorka tla, sa dubine 0–30 cm. Prvi prosječni uzorak tla uzet je s lokaliteta gdje je posađena višnja 'Oblačinska' na vlastitom korijenu, a drugi s lokaliteta gdje je 'Oblačinska' posađena na podlozi 'Rašeljka'. Analize tla su obavljene u pedološkom laboratoriju Agronomskog i prehrambeno-tehnološkog fakulteta Sveučilišta u Mostaru, prema sljedećim metodama: reakcija tla (pH) – potenciometrijski, ukupni karbonati – metodom *Scheibler*, humus prema *Kotzmannu*, dušik metodom po *Kjeldahl*-u, a fiziološki aktivni fosfor i kalij prema *Al* metodi (Amonim - laktat – octena kiselina kao sredstvo za ekstrakciju fosfora i kalija).Za pomološka mjerenja, po potpuno slučajnom rasporedu odabrano je po pet stabala Oblačinske na vlastitom korjenu i pet stabala na podlozi ašeljka. Stabla su odabrana slučajnim odabirom iz različitih dijelova voćnjaka. Sa svakog stabla ubrano je po 30 plodova u fenofazi pune zrelosti. Masa ploda (g) je mjerena na analitičkoj vagi (Kern 440 -45). Visina i širina ploda (mm) je mjerena uz pomoć ručnog digitalnog pomičnog mjerila (Hm Mullner werkzeuge). Provedena je deskriptivna statistika i T- test (Knezović, 2019.) u MS Excelu 2019.

Rezultati i rasprava

Analiza tla

Temeljem rezultata analize prosječnih uzoraka tla, vidno je da je uzorak tla uzet sa lokaliteta gdje je posađena 'Oblačinska' na podlozi 'Rašeljka', alkalne reakcije (pH 7.38). Sadržaj ukupnog vapna u uzorcima tla nije visok. Uslijed redovite gnojidbe organskim i mineralnim gnojivima tla su dobro opskrbljena humusom i osnovnim biljno hranidbenim elementima (NPK), naročito ukupnim dušikom i fiziološki aktivnim fosforom (Tablica 1).

Tablica 1.: Rezultati kemijskih ispitivanja uzoraka tla u voćnjaku'Oblačinske' višnje

'Oblačinska' višnja	pH		Ukupni $CaCO_3$ (%)	Sadržaj humusa (%)	Ukupni N (%)	Fiziološki aktivni (mg/100 g tla)	
	H_2O	KCl				P_2O_5	K_2O
Vlastiti korijen	7.76	7.09	4.0	3.57	0.48	31.50	22.48
'Rašeljka'	8.20	7.38	3.78	4.10	0.36	32.90	24.60

Pomološka svojstva

U ablici 2 istaknute su minimalna i maksimalna vrijednost aritmetičke sredine, standardne devijacije i varijacijski koeficijent (%) 'Oblačinske' višnje uzgajane na vlastitom korijenu i na podlozi 'Rašeljka'. Iz prikazanih podataka može se uočiti da je najvarijabilnije svojstvo bila masa ploda .

Tablica 2. Pomološka svojstva'Oblačinske' višnje

		Min	Max	Mean	s	CV(%)
'Rašeljka'	Visina(mm)	10.46	17.94	14.77	0.08	0.57
	Širina(mm)	13.68	21.72	17.57	0.11	0.60
	Masa(g)	2.26	5.34	3.54	0.04	1.08
Vlastiti korijen	Visina(mm)	11.27	16.43	13.35	0.07	0.52
	Širina(mm)	12.62	18.92	15.50	0.08	0.52
	Masa(g)	1.71	4.02	2.87	0.03	1.14

Min - minimalna vrijednost; Max - maksimalna vrijednost; Mean-srednja - vrijednost; s - standardna devijacija; CV - varijacijski koeficijent (%)

Masa ploda

Nakon provedenog T testa za masu ploda utvrđene su značajne razlike između prosječnih vrijednosti za kombinaciju 'Oblačinske' i podloge (t_{exp} 13.49**; p<0.01). Masa ploda utvrđena za višnju 'Oblačinska' na podlozi 'Rašeljka' (3.54 g), značajno se razlikovala u odnosu na višnju 'Oblačinska' na vlastitom korijenu 2.87 g (Tablica 3).

Širina ploda

Analizom podataka pomoću T testa za svojstvo širina ploda utvrđene su značajne razlike između prosječnih vrijednosti (t_{exp} 15.90**; p<0.01). Najveća širina ploda izmjerena je za višnju 'Oblačinska' na podlozi 'Rašeljka' i iznosila je 17.57 mm i visoko značajno se razlikovala u odnosu na višnju 'Oblačinska' na vlastitom korijenu koja je iznosila 15.58 mm (Tablica 3).

Visina ploda

Korištenjem T testa utvrđene su značajne razlike između prosječnih vrijednosti visine ploda za analiziranu sortu višnje, odnosno kombinacije 'Oblačinske' višnje i dvije podloge (t_{exp} 11.83**; p<0.01). Značajno viša vrijednost visine ploda utvrđena je kod višnje 'Oblačinska' na podlozi 'Rašeljka' (14.77 mm) u odnosu na višnju 'Oblačinska na vlastitom korijenu 13.55 mm (Tablica 3).

Tablica 3. Prosječne vrijednosti pomoloških svojstava ploda 'Oblačinsk'

Podloga	Masa (g)	Širina (mm)	Visina (mm)
'Rašeljka'	3.54	17.57	14.77
Vlastiti korijen	2.87	15.58	13.55
t	13.49**	15.90**	11.83**
p^{exp}	0.01	0.01	0.01

*** - visoko značajne razlike uz nivo značajnosti p 0.01*

Kod ispitivanih stabala prosječna masa ploda višnje 'Oblačinska' na podlozi 'Rašeljka' je bila 3.54 g, a na vlastitom korijenu prosječna masa ploda je iznosila 2.87 g, što je slično s istraživanjima drugih autora.

Prema istraživanju (Puškar, 2005.) u predselekcijskom radu 1993. godine masa ploda istraživanih stabala višnje 'Oblačinska' kretala se od 2.49 do 4.66g. Pavičević (1976.) navodi da u ovisnosti o intenzitetu oplodnje i godine, masa

ploda kod 'Oblačinske' višnje na vlastitom korijenu, može iznositi od 2.8 do 4.9 g. Puškar (2002.) navodi da se masa ploda višnje 'Oblačinska', uzgajane na vlastitom korijenu kretala između 2.2 i 3.9 g. Nikolić i sur. (2005./1) navode da je kod ispitivanih klonova višnje 'Oblačinska' masa plodova varirala od 2.62 do 3.52 g. Šebek (2019.) na osnovu svojih istraživanja navodi da je višnja 'Oblačinska' uzgajana na vlastitom korijenu imala masu ploda od 3.0 g.

Prema dobivenim podatcima, prosječna širina ploda za višnju 'Oblačinska'na podlozi 'Rašeljka' je iznosila 17.57 mm i bila je veća u odnosu na prosječnu širinu ploda kod 'Oblačinske' na vlastitom korijenu, gdje je iznosila 15.58 mm. Puškar (2002.) piše da se širina ploda istraživanih tipova 'Oblačinske' (na vlastitom korijenu) kretala između 16.1 i 20.1 mm, a visina ploda se kretala od 12.9 do 16.7 mm. Trajkovski (2016.) navodi vrijednosti prosječne širine plodova višnje 'Oblačinska' (na podlozi 'Rašeljka') između18.38 i 16.57 mm, dok za prosječnu visinu plodova navodi vrijednosti od 14.67do 16.16 mm. Milošević i Milošević (2012.) su na osnovu isvojih istraživanja utvrdili prosječnu visinu ploda višnje 'Oblačinska' na vlastitom korijenu, 14.85 mm i prosječnu širinu ploda od 15.50 mm.

Milutinović i sur. (1980.) piše da se širina ploda kretala od 16.0 do 19.7 mm, a visina od 14.5 do 17.00 mm.

Kiprijanovski i sur. (2018.) su uočili veću masu i dimenzije ploda kod višnje 'Oblačinska' cijepljene na podlogu 'Rašeljka' u odnosu na stabla na vlastitom korijenu. Također, ta stabla bila tolerantnija prema suši, hladnoći, karbonatima. Prosječna težina ploda je iznosila 2.96 g na 'Rašeljci' do 2.64 g na vlastitom korijenu. Različita istraživanja govore da podloga ima značajan utjecaj na urod višanja, kao i tip tla i drugi ekološki činitelji (Perry i sur., 1993.), Između sorti višanja s jedne strane i podloga s druge strane postoji vegetativna podudarnost. Pod podudarnošću se podrazumijeva dobro sjedinjenje između podloge i plemke, koja je uvjetovana anatomsko - fiziološkim svojstvima (Mratinić, 2010.). Anderson i sur., (1993.) navode da podloga utječe na raniju ili kasniju cvatnju sorte, na količinu njihova uroda i veličinu ploda. ploda kao i njegove dimenzije ovise o sorti i podlozi, čemu u prilog Rakonjac i sur. (2016.) navodi da su prosječna masa plodova sorata višnje 'Meteor koraii', 'Kelleris 14', 'Rexelle', 'Hajmanova konzervna' cijepljenih na podlozi 'Rašeljka' bile manje vrijednosti u odnosu na prosječne mase plodova s stabala koja su bila cijepljena na podlogu divlje trešnje. Kopytowski i sur. (2010.) navode kako su sorte višanja cijepljene na sjemenjak 'Rašeljke' imale najmanje prosječne vrijednosti visine,širine i mase plodova u odnosu na plodove onih stabala koja su bila cijepljena na podloge divlja trešnja i 'F12/1'. Važan čimbenik u izboru podloge može biti njezina prilagodljivost različitim tipovima tla i/ili otpornost na nepovoljne abiotičke i biotičke uvjete (Lang i sur., 1997.). Podloga, njeno prokorjenjivanje, raspoloživost biljno hranidbenih elemenata i njihova pokretljivost u tlu mogu biti odgovorni za masu ploda (Thouraya i sur., 2016.).

Zaključak

Mjerenja su pokazala da je veća masa ploda bila kod višnje 'Oblačinska' na podlozi 'Rašeljka' (3.54 g) u odnosu na istu sortu na vlastitom korijenu (2.87 g). Također izmjerena je veća širina i visina ploda za višnju 'Oblačinska' na podlozi 'Rašeljka' u odnosu na istu na vlastitom korijenu. Iz navedenog je uočljivo da je podloga utjecala na pomološka svojstva ploda 'Oblačinske' višnje. Podloga 'Rašeljka' se pokazala boljom podlogom za višnju 'Oblačinska' u danim uvjetima uzgoja, pa se ova podloga može preporučiti za širenje u proizvodnji.

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Pomological properties of Oblačinska variety on own root and grafted on ahaleb

Abstract

Sour cherry (*Prunus cerasus*, L.) is grown on different vegetative and generative rootstocks. The growth and development of the variety, as well as the pomological properties are influenced by the genetic properties, the rootstock, and the agroecological characteristics. In this paper, the goal was to determine whether there are differences between the measured properties of fruit 'Oblačinska' grown on its own root and grown on a 'Mahaleb'. Measurements properties showed a higher fruit weight of the 'Oblačinska' on the rootstock (3.54 g) compared to the same one on its own root (2.87 g). The fruit width (mm) and fruit height (mm) were greater for the sour cherry 'Oblačinska' grown on a rootstock 'Mahaleb'

Keywords: sour cherry, rootstock, pomological properties

Duljina maceracije i vrsta kvasaca kao čimbenici kemijskog sastava vina 'Trnjak' (*Vitis vinifera* L.)

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Sažetak

U istraživanju je analiziran utjecaj različitih sojeva kvasaca i duljina maceracije (8 i 12 dana) na osnovni kemijski sastav i aromatski profil vina 'Trnjak'. Cilj rada bio je utvrditi razlike između kontrolnog tretmana s epifitnim kvascima i sekvencijalne inokulacije sojeva *Lachancea thermotolerans* i *Saccharomyces cerevisiae* te tretmana sa *S. cerevisiae* na sastav vina. Rezultati su pokazali utjecaj sekvencijalne fermentacije na rast ukupne kiselosti te smanjenje pH vrijednosti, čime se postižu svježina i stabilnost koje doprinose kakvoći i dugovječnosti crnih vina južnih vinogorja. Duljina maceracije i vrste kvasaca značajno su utjecali na koncentracije ukupnih aldehida, estera i hlapljivih fenola.

Ključne riječi: *Saccharomyces cerevisiae, Lachancea thermotolerans,* sekvencijalna inokulacija, aromatski spojevi

Uvod

Na kvalitetu vina utječu mnogi čimbenici počevši od zemljopisnog podrijetla grožđa, sastava mošta, procesa vinifikacije i mikrobne aktivnosti različitih vrsta kvasca. Vino je složena mješavina kemijskih spojeva među kojima su i hlapljivi spojevi arome koji se prema podrijetlu mogu podijeliti na primarne ili sortne, sekundarne ili fermentacijske te tercijarne ili arome starenja, a koji su ujedno pod jakim utjecajem aktivnosti kvasaca tijekom proizvodnje vina (Belda i sur., 2017.). Saccharomyces cerevisiae tradicionalno je najčešće korištena vrsta kvasca u proizvodnji vina zbog iznimnih fermentabilnih sposobnosti u velikom rasponu uvjeta provođenja alkoholne fermentacije. Tijekom posljednjeg desetljeća, uloga ne-Saccharomyces kvasaca u proizvodnji vina se povećala, jer su brojna istraživanja potvrdila njihove jedinstvene enološke karakteristike koje utječu na poboljšanje kvalitete i aromatskog profila vina (Rojas i sur., 2001.; Jolly i sur., 2003.; Swiegers i sur., 2005.; Domizio i sur., 2007.; Viana i sur., 2008.;)

Lachancea thermotolerans je ne-Saccharomyces vrsta kvasaca poželjna zbog pozitivnog doprinosa senzornim svojstvima vina (Capece i Romano, 2019.) i proizvodnje uravnoteženih vina. L. thermotolerans u sekvencijalnoj inokulaciji sa S. cerevisiae pridonosi snižavanju pH vrijednosti i povećanju ukupne kiselosti temeljem sinteze L-mliječne kiseline. L. thermotolerans osigurava alkoholnu jakost u rasponu od 5 do 9 vol. %, pa čak i 10 vol. % (Hranilović i sur., 2018.), ali se sekvencijalno inokulira sa S. cerevisiae za potpunu fermentaciju šećera (Morata i sur., 2019.). Osim što može preživjeti nekoliko dana pri 9 vol. % alkohola (Kapsopoulou, 2007.), postojana je i kad fermentacijom dominira S. cerevisiae (Mills i sur., 2002.). Kao što je već dokazano, maceracija značajno utječe na kemijski sastav i senzorna svojstva crnog vina. Mnoga su istraživanja pokazala da se poželjna razina trpkoće, aroma i karakter vina mogu postići kontroliranjem uvjeta maceracije, kao što je duljina trajanja i temperatura procesa. Osim toga, spojevi poput tanina, terpena, estera i slično, dolaze u dodatne interakcije tijekom procesa maceracije (Cheynier i sur., 2006.).

Trnjak crni je sorta s, još uvijek, nedovoljno istraženim podrijetlom. Smatra se autohtonom sortom Imotske krajine i zapadne Hercegovine. Ističe se manjim prinosom i izrazito kvalitetnim sastavom mošta i vina. Uz visoku koncentraciju šećera od 20 - 26 % i nižu do srednju koncentraciju ukupne kiselosti (4,5 - 6 gL^{-1}) ovisno o godini i terminu berbe, karakterizira ga i visoka koncentracija polifenola. Vina su stoga puna, ekstraktna, s koncentracijom alkohola od 12 - 15 vol. %, intenzivno rubinske boje i vrlo ugodne arome (Mirošević i Turković, 2003., Sokolić, 2006.,

Maletić i sur., 2015.), a ponekad neharmonična zbog niske ukupne kiselosti.

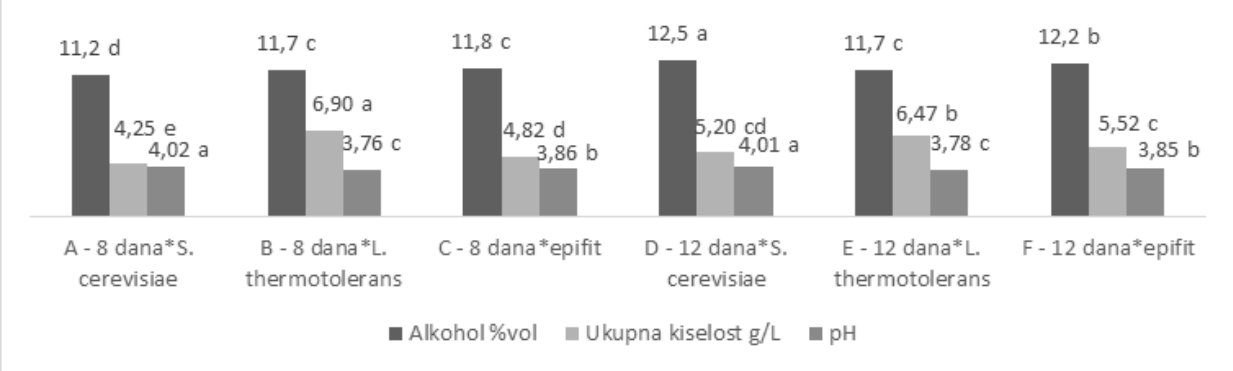
Cilj ovog rada bio je utvrditi razlike između tretmana s epifitnim kvascima, sekvencijalne fermentacije s kvascima L. thermotolerans i S. cerevisiae te tretmana sa S. cerevisiae obzirom na duljinu maceracije, na kemijski sastav vina 'Trnjak'.

Materijali i metode

Grožđe sorte ´Trnjak´ s položaja Potpolje (Mostarsko vinogorje, BiH) berbe 2019, korišteno je u istraživanju. Ukupno 150 kg masulja ravnomjerno je podijeljeno u 50 L-posude na maceraciju u trajanju 8 dana (A, B, C), te na maceraciju u trajanju od 12 dana (D, E, F). Masulj je sulfitiran s 5 %-tnom H_2SO_3. Tretmani A i D kao kontrolna varijanta sulfitirani su s 50 mL 5 %-tne H_2SO_3; tretmani B i E su sulfitirani s 10 mL 5 %-tne H_2SO_3 te inokulirani s kvascem *L. thermotolerans* (Laktia, Lallemand; 15 g 50 kg^{-1}), a nakon 24 h uslijedila je dodatna inokulacija sa *S. cerevisiae* (BDX, Lallemand; 15 g 50 kg^{-1}). Tretmani C i F sulfitirani su s 50 mL 5 %-tne H_2SO_3 te inokulirani sa *S. cerevisiae* kvascem (Fermol Premier Cru, AEB; 15 g 50 kg^{-1}). Nakon 8 dana maceracije i alkoholne fermentacije, masulj tretmana A, B i C prešan je hidrauličnom prešom te je mošt u fermentaciji svakog pojedinog tretmana raspodijeljen u tri ponavljanja, otakanjem u staklene posude volumena 10 L s vrenjačom, u kojima je nastavljena alkoholna fermentacija. Kod tretmana D, E, F odrađen je isti postupak nakon 12 dana maceracije. U prvom pretoku, nakon završene fermentacije, izvršena je korekcija sulfita s 1 mL L^{-1} 5 %-tne H_2SO_3 u svim tretmanima. Nakon drugog pretoka analizirani su osnovni kemijski parametri koncentracije alkohola, ukupne kiselosti i pH vrijednost prema metodama O.I.V-a (2012.). Analiza hlapljivih organskih spojeva vina provedena je primjenom vezanog sustava plinske kromatografije (Thermo Scientific Trace 1300), spektometar masa (Thermo Scientific ISQ 7000) uz prethodnu izolaciju analita mikroekstrakcijom na čvrstoj fazi u izvedbi klina (engl. *Solid Phase Microextraction Arrow*) pomoću automatiziranog sustava za pripravu uzoraka (Jagatić Korenika i sur., 2022.). Kao čvrsta faza korišten je sustav CAR-PDMS-DVB. U posudicu za uzorke dodano je 5 mL vina i 2,5 g NaCl. Prije same adsorpcije na čvrstu fazu, uzorak je uravnotežen pri 55 °C u trajanju od 10 min. Adsorpcija analita provedena je pri 55 °C u trajanju od 60 min. Desorpcija je provedena u injektoru tekućinskog kromatografa pri 250 °C u trajanju od 7 min. Kromatografska analiza provedena je pomoću TR-Wax kolone (60 m x 0,25 mm x 0,25 μm) uz temperaturni program u rasponu temperatura od 40 do 210 °C. Snimanje spektara masa provedeno je praćenjem struje svih iona u rasponu od 20 do 500 m/z dok je energija elektrona bila 70 eV. Identifikacija je provedena pomoću usporedbe vremena zadržavanja, retencijskih indeksa te usporedbom spektara masa s onima u NIST 17 i Wiley 12 bazi podataka. Rezultati su prikazani kao zbroj pojedinačnih vrijednosti za grupe aromatskih spojeva (ukupni aldehidi, esteri, viši alkoholi, terpeni, hlapljivi fenoli, masne kiseline laktoni, C13 norisoprenoidi i ostali spojevi). Statistička analiza obuhvačala je faktorijelnu ANOVU s ciljem ispitivanja utjecaja duljine maceracije, kvasaca kao i njihove interakcije na sadržaj različitih grupa aromatskih spojeva u vinu. Za analizu ukupne varijabilnosti sadržaja pojedinih grupa aromatskih spojeva i osnovnih parametara kakvoće vina kod analiziranih uzoraka vina korištena je analiza glavnih komponenti (PCA) primjenom statističkog programa XLSTAT v.2022.1.1. (Addinsoft).

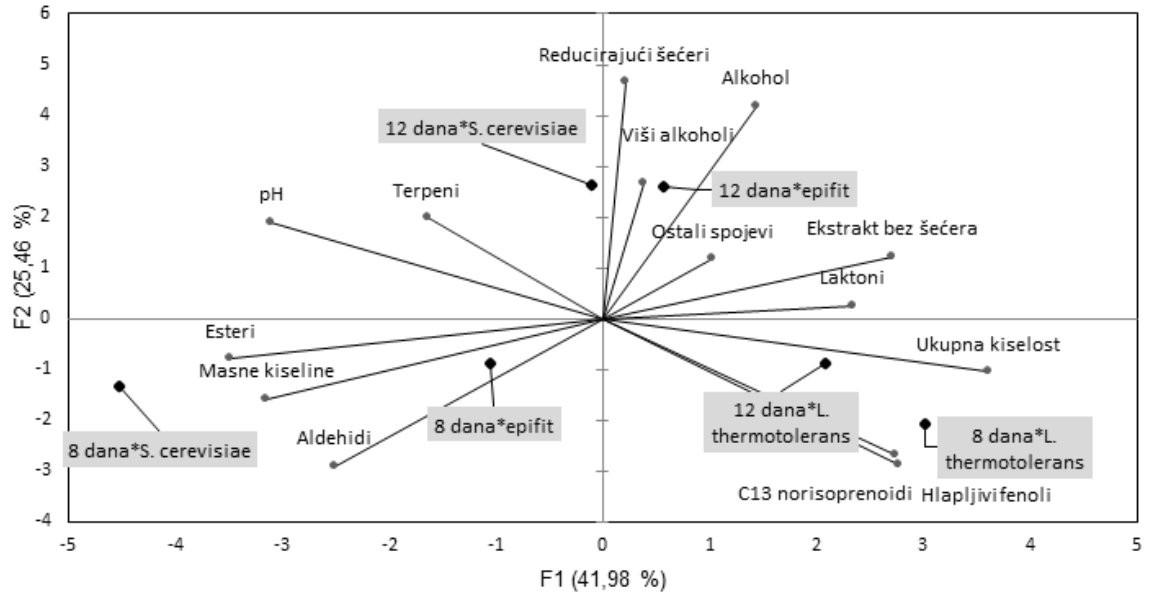
Rezultati i rasprava

Rezultati analize osnovnih kemijskih parametara u vinu (Grafikon 1) prikazuju značajan utjecaj *L. thermotolerans* x *S. cerevisiae* na povećanje koncentracije ukupne kiselosti, uz značajno smanjenje pH vrijednosti (tretmani B i E), što je u skladu s prethodnim istraživanjima (Morrata i sur., 2019., Comiti i sur., 2011., Dutraive i sur., 2019., Benito, 2018., Gobbi i sur. 2013.), čime se postiže stabilnost vina, što je iznimno važno za vina južnijih vinogorja s nižim ukupnim kiselinama i povišenom pH vrijednošću. Koncentracija alkohola bila je značajno najniža kod istih tretmana, što je u skladu s istraživanjima (Gobbi i sur., 2019., Dutraive i sur., 2019).



Grafikon 1. Utjecaj trajanja maceracije o primijenjenih kvasaca na osnovni kemijski sastav vina sorte 'Trnjak' (srednje vrijednosti označene različitim slovima razlikuju se uz p<0,05 na temelju Duncan's multiple range testa)

Nekoliko istraživanja izvijestilo je o značajno nižim koncentracijama alkohola (od 0,2 do 0,9 vol. %) kod sekvencijalnih inokulacija *S. cerevisiae* i *L. thermotolerans,* u odnosu na njihove kontrolne varijante sa *S. cerevisiae,* čime se naglašava potencijal *L. thermotholerans* u proizvodnji vina s nižom koncentracijom alkohola (Kapsopoulou i sur., 2007., Gobbi 2013., Benito i sur., 2016., Benito i sur., 2015). Duljina maceracije nije utjecala na pH vrijednost vina što je u skladu s Yilmaztekin i sur. (2015.), u istraživanju tri duljine maceracije (5, 10, 15 dana). Prema istom istraživanju najviša koncentracija reducirajućih šećera i ukupne kiselosti je kod vina maceriranog 5 dana, i njihove razine nisu u korelaciji s vremenom maceracije, kao koncentracija alkohola koja je bila viša s produljenjem vremena maceracije. U ovom istraživanju duljina maceracije od 12 dana značajno je utjecala na koncentraciju alkohola, reducirajućeg šećera i ekstrakta bez šećera i ukupnih kiselina (Grafikon 2). Koncentracija aldehida povezana je sa stupnjem zrelosti, tretmanima prije fermentacije, enzimatskom oksidacijom i razgradnjom lipida grožđa, kao i sortom. Uspoređujući ukupne koncentracije aldehida kod sorata ´Babić´, ´Blatina´, ´Frankovka´ i ´Trnjak´, u istraživanju Korenika i sur. (2021.), najviše koncentracije su u vinima ´Trnjak´, dok između ostalih nema značajnih razlika. Koncentracija ukupnih aldehida u tretmanu s *L. thermotolerans* u istom istraživanju bila je značajno viša u odnosu na kontrolu, što nije u skladu s ovim istraživanjem, gdje je koncentracija aldehida značajno niža (Tablica 1). Terpeni i C13 norisoprenoidi su skupine aromatskih spojeva koji čine sortnu aromu vina i odlikuju se cvjetnim i voćnim aromama, a oslobađaju se u mošt tijekom procesa primarne prerade u slobodnim ili hlapljivim te vezanim oblicima.



Grafikon 2. Analiza osnovnih komponenti (PCA) - grupe aromatskih spojeva i osnovni parametri kakvoće vina ´Trnjak´

U ovom istraživanju zabilježen je utjecaj sekvencijalne fermentacije s *L. thermotolerans* na C13 norisoprenoide što je u skladu s rezultatima Korenika i sur. (2021.). Viši alkoholi i esteri kao spojevi fermentacijske arome mogu biti pod utjecajem vrste kvasaca i uvjeta fermentacije (Padilla i sur., 2016.). *L. thermotolerans,* kao niti dvije duljine maceracije nisu utjecali na razlike u koncentracijama viših alkohola prema našem istraživanju, iako se *L. thermotolerans* smatra umjerenim proizvođačem viših alkohola (Balikci i sur., 2016.). Značajno najniža koncentracija estera zabilježena je u tretmanu B, kao i u istraživanju Korenika i sur. (2021.), gdje su najviše koncentracije estera povezane s kontrolnim tretmanom vina ´Trnjak´. Međutim, kod produljene maceracije, u tretmanu E, zadržana je najviša koncentracija u odnosu na tretmane D i F. Koncentracija masnih kiselina povezana je s početnim sastavom mošta i uvjetima fermentacije. Ovi spojevi opisani su voćnim, mliječnim i masnim aromama (Rocha i sur., 2004.). U istraživanju Yilmaztekin i sur. (2015.) najviše koncentracije masnih kiselina pronađene su u petodnevnoj maceraciji, pri 10 dana maceracije koncentracija se smanjuje, pa opet raste nakon 15 dana maceracije. Njihova koncentracija u ovom istraživanju bila je viša kod maceracije od 8 dana, te je potvrđena značajna razlika pod utjecajem duljine maceracije. Na smanjenu koncentraciju masnih kiselina značajno može utjecati sekvencijalna fermentacija s *L. thermotolerans* (Comitini i sur., 2000.; Korenika i sur., 2021.), što nije statistički potvrđeno u ovom istraživanju. Laktoni su važni spojevi primarne arome grožđa, posebno u vinima Rizlinga rajnskog, gdje doprinose sortnoj aromi (Ribéreau-Gayon i sur., 2006.). Prema rezultatima ovog istraživanja najviša koncentracija laktona bila je u tretmanu B, što je također u skladu s istraživanjem Korenika i sur. (2021). Hlapljivi fenoli su značajni spojevi arome dobiveni iz ekstrahiranih frakcija grožđa ili vina (Fereira i Lopez, 2019.). Najviše koncentracije potvrđene su u tretmanima B i E, slično kao u istraživanju Korenika i sur. (2021.). Prema Yilmaztekin i sur. (2015) koncentracija hlapljivih fenola se smanjuje s duljinom maceracije (10 i 15 dana), što je u skladu s ovim rezultatima gdje su značajno niže koncentracije bile zastupljene nakon maceracije od 12 dana.

Tablica 1. Utjecaj duljine maceracije, različitih kvasaca Koncentracije grupa aromatskih spojeva (**µg**/L) u vinima ´Trnjak´

Tretman	Aldehidi	Esteri	Viši alkoholi	Terpeni	Hlapljivi fenoli	Masne kiseline	Laktoni	C13
Trajanje maceracije								
8 dana	327,2 a	10476,1 a	49065,9	182,2	194,0 a	5388,2 a	60,2	6,4
12 dana	237,5 b	9186,1 b	67877,5	181,9	117,7 b	4608,3 b	59,0	5,9
	**	*	n.s.	n.s.	**	*	n.s.	n.s.
Vrsta kvasaca								
Epifit	287,7 a	9857,5 b	84267,2	181,4	59,1 b	5145,3 ab	65,3 a	5,9 b
L.t. x S.c.	262,5 b	8196,1 c	46462,3	171,8	349,9 a	4592,3 b	61,1 a	7,3 a
S. cerevisiae	296,9 a	11439,8 a	44685,6	192,9	58,4 b	5257,7 a	52,4 b	5,3 b
	*	**	n.s.	n.s.	**	n.s.	*	*
Trajanje maceracije * vrsta kvasca								
8 dana*epifit	355,9 a	14349,8 a	47025,3 a	197,8 a	55,4 de	6072,5 a	49,2 b	5,5 b
8 dana*L.t.xS.c.	298,9 b	6828,2 d	50939,0 a	182,6 ab	477,9 a	4833,1 b	66,8 a	7,8 a
8 dana*S.c.	326,7 ab	10250,5 b	49233,4 a	166,0 ab	48,5 e	5258,7 ab	64,6 a	5,7 b
12 dana*epifit	237,8 c	8529,9 c	42345,8 a	188,0 ab	61,4 cd	4442,9 b	55,5 b	5,0 b
12 dana*L.t.xS.c.	226,0 c	9564,0 b	41985,5 a	160,9 b	221,0 b	4351,4 b	55,4 b	6,7 ab
12 dana*S.c.	248,6 c	9464,4 bc	119301,0 a	196,8 a	69,6 c	5031,8 b	65,9 a	6,0 b
maceracija*kvasac	n.s.	**	n.s.	*	**	n.s.	*	n.s.

*ANOVA: n.s. – nije signifikantno, *-signifikantno (p<0,05), **-visoko signifikantno (p<0,01); srednje vrijednosti pojedinih grupa hlapljivih organskih spojeva označene različitim slovima unutar tretmana i interakcije značajno se azlikuju uz p<0,05 na temelju Duncan's multiple range testa*

Zaključak

Prema dobivenim rezultatima istraživanja učinka duljine maceracije i kvasaca na sastav vina ′Trnjak′ uočljivi su učinci sekvencijalne inokulacije *S. cerevisiae* x *L. thermotholerans* na povećanje ukupne kiselosti i ekstrakta bez šećera te smanjenje koncentracije alkohola i pH vrijednosti, što je važno za stabilnost i svojstva crnih vina. Duljina maceracije značajno je utjecala na koncentraciju alkohola, reducirajućih šećera i ekstrakta bez šećera. Sekvencijalna fermentacija utjecala je na rast koncentracije viših alkohola, laktona i C13 norisoprenoida te na pad koncentracije estera. Koncentracije viših alkohola i terpena nisu se značajno razlikovale s obzirom na tretmane. Duljina maceracije značajno je utjecala na koncentraciju aldehida, estera, hlapljivih fenola i masnih kiselina, pri čemu su njihove koncentracije bile značajno niže nakon 12 dana maceracije.

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Maceration lenght and yeast species as chemical composition factors of Trnjak wine (*V. vinifera* L.)

Abstract

The research analyzed the influence of different yeast strains and lengths of maceration (8 and 12 days) on the basic chemical composition and aromatic profile of wine made from Trnjak variety. The aim of the study was to determine the differences between the control treatment with epiphytic yeasts and the sequential inoculation treatment with *Lachancea thermotolerans* x *Saccharomyces cerevisiae* strains and the treatment with *S. cerevisiae* on the wine composition. The results showed the influence of sequential fermentation on the increase of total acidity and the reduction of pH values, which result in freshness and stability that contribute to the quality and longevity of red wines from southern vineyards. The length of maceration and the types of yeasts significantly influenced the concentrations of total aldehydes, esters and volatile phenols.

Keywords: *Saccharomyces cerevisiae, Lachancea thermotolerans,* sequential inoculation, aromatic components

From the sea to the mountain: morphological variability of *Rosa canina* L. fruits from the Mediterranean part of Croatia

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Abstract

Dog rose (*Rosa canina* L.) is a wild fruit species widely distributed in Croatia in forest edges and agricultural areas. This research discusses the morphological variability of the dog rose fruits in the Mediterranean part of Croatia. Fruits were collected *in situ*, in four different ecological areas: the coastal region (Split and Klis) and the hinterland (Neorić and Kamešnica). Morphological characterisation of the fruits (fruit weight, fruit length, fruit width, fruit shape index) was performed. The results showed that the highest average weight (1.59 g) and fruit width (1.59 mm) was measured in the population Kamešnica, and the highest average height (20.13 mm) and fruit index (shape index; 1.74) was determined in the population Klis. The greatest variability was determined for the trait fruit weight with a coefficient of variation of 41.58% (Klis); 41.03% (Split); 37.61% (Neorić) and 34.55% (Kamešnica). The obtained results indicate the need to expand the phenotype research on more populations in the Mediterranean part of Croatia, and to determine the influence of habitat, population size and climatic conditions on populations variability.

Keywords: *dog rose*, fruits, phenotype, populations

Introduction

Dog rose (*Rosa canina* L.) is a deciduous perennial shrub naturally distributed in Europe, Asia, and North Africa (Šegota and Limić, 2018; Franjić, 2021). The genus *Rosa* includes 37 species in the Croatian flora (Nikolić, 2018; Tomljenović et al., 2020), and species *Rosa canina* is one of the most widespread wild species of the genus *Ros*a in the entire Republic of Croatia (Tomljenović et al., 2021). It grows in thickets, open areas, forest edges, various types of oak forests, hedges, etc. It can grow as a solitary plant or in small groups and belongs to the Eurasian floristic element (Franjić, 2021). Dog rose grows in the continental part of Croatia as well as on the Adriatic coast and islands (Kovačić et al., 2008) and in the mountains. On the Adriatic coast it can be found on stony and sun-exposed slopes (Šindrak et al., 2012; Tomljenović et al., 2020) and on mountains it grows up to 1400 m altitude on the Velebit mountain (Kremeret et al., 2021). Dog rose grows up to 3 m tall, with long, quite thick, erect, spreading branches. Rose (''brier, rose Brier hip, dogberry, hip tree, dog rose, hep tree, hip fruit, wild brier") are the fruits of *Rosa* genus in the Rosaceae family (Demir, 2001). The red fruit is a 1.5-2 cm long aggregate with a large number of angular, hairy nutlets. The rose hip is a pseudocarp or false fruit, consisting of fleshy walls surrounding a cavity containing the single-seeded fruits or achenes (Igual et al., 2021; Franjić, 2021) In the Dalmation region dog rose blooms in May and June, and the fruit ripens from August to October (Šindrak, 2012; Šegota and Limić, 2018). Rose fruits were used as food from Neolithic Ages, (Godwin, 1975) but recently, interest in rose hips as a small fruit crop has increased (Uglla et al., 2005). The utilization of dog rose fruits has a long tradition, it is used in tea, jam, dessert soups, syrup, marmalade, jelly etc. Rose hips have a higher content of vitamin C than any other commonly available fruit or vegetable (Demir, 2001; Šegota and Limić, 2018). The rosehip fruit is known to strengthen the body's defenses against infections and especially against colds (Shnyakina and Malygina, 1975; Demir, 2001). Although dog rose is researched, cultivated and improved all over the world, it is poorly researched in the Mediterranean part of Croatia (Šindrak et al., 2012; Tomljenović et al., 2019; Tomljenović et al., 2020). Large differences between plants are observed in nature, especially in fruit size and color, and often at very small habitats. (Šindrak et al., 2012). The aim of this study was to determine the morphological characteristics of the fruits from the dog rose populations in Mediterranean part of Croatia.

Material and methods

Populations from four different geographic areas of Croatia were selected and sampled. Fruit samples were collected *in situ* in the middle Dalmatian archipelago: Split (L1) (20-60 m a.s.l.), Klis (L2) (100-200 m a.s.l.), Neorić (L3) (300-600 m a.s.l.) and Kamešnica (L4) (800-1300 m a.s.l.) (Figure 1). Fruits of dog rose were harvested during September 2022. At each location 10 shrubs were selected, and 30 healthy and undamaged fruits per shrub were collected. Four quantitative properties were evaluated: fruit weight, fruit length, fruit width and fruit shape index. Fruit weight (g) was measured using a digital scale (Metler Toledo; ± 0.01 g). Fruits were scanned using an Epson Perfection V700 Photo Scanner, and length (mm), width (mm) and fruit shape index (mm) were determined using the WinSEEDLE Pro 2019a 32-bit analysis software. Data obtained from this study were analyzed using Statistica software version 11.0 (StatSoft Inc., USA). Analysis of variance (ANOVA) was performed to determine the difference among accessions in pomological fruit properties. Differences among means were considered significant at $P \leq 0.05$ using Tukey's honest significant difference test.

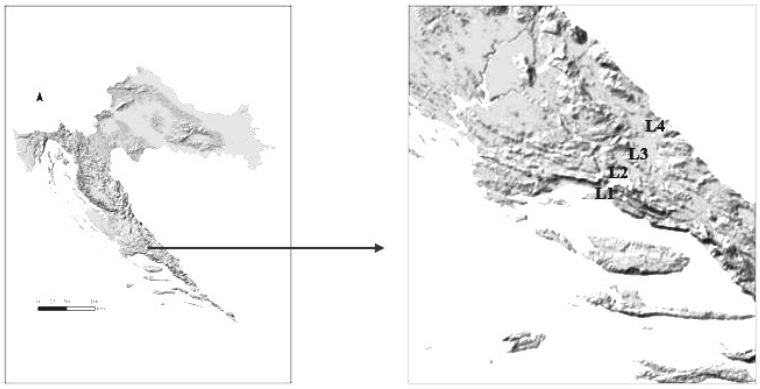

Figure 1. Sampling locations of investigated populations: Split (L1), Klis (L2), Neorić (L3) and Kamešnica (L4).

Results and discussion

The results of the descriptive statistical analyses for each investigated population and statistically significant difference (P≤0,05) between the populations are shown in Figure 2.

The fruit weight varied from 1.41 g to 1.59 g. The highest value was measured in the population Kamešnica, L4 (1.59 g), and it did not differ from the fruit weight of the Split, L1 (1.57 g). The fruit weight of populations Neorić, L3 and Klis, L2 were smaller than Kamešnica and Split. The fruit weight values obtained in this study are in accordance with the results of fruit weight (1.28 to 2.38 g) obtained in the study by Tomljenović et al. (2021) for dog rose populations in the Mediterranean part of Croatia. Fruit weight can vary widely and is affected by many factors such as: variety, growing conditions (Najda and Buczkowska, 2013) and altitude and climatic conditions (Ghiorghiță et al., 2012; Tomljenović, 2022).

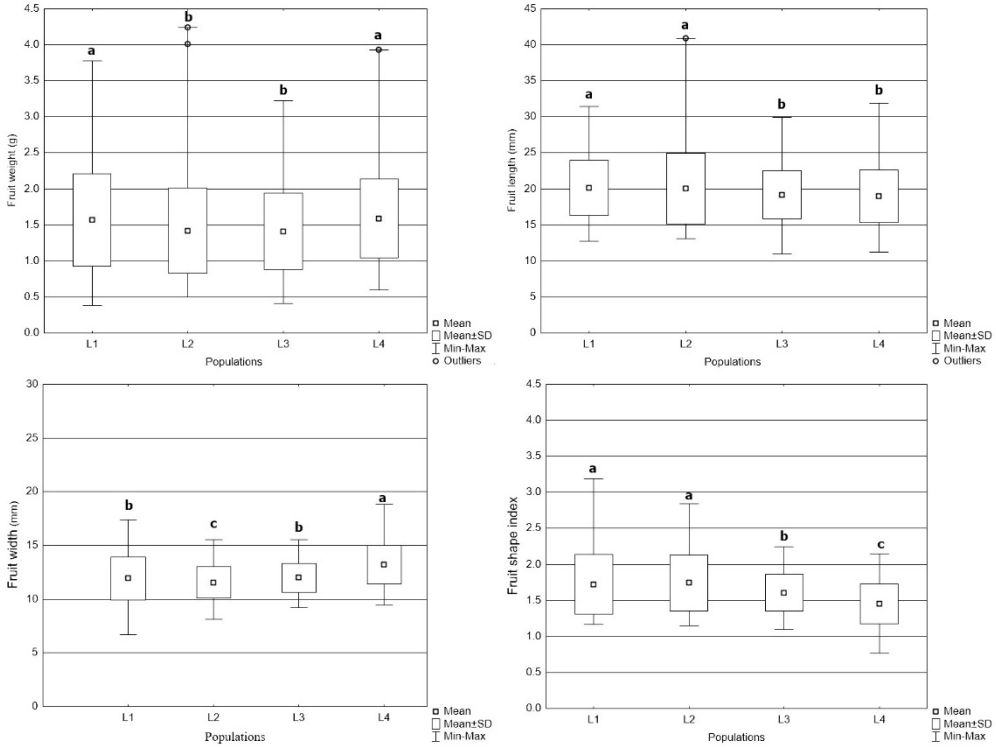

Figure 2. Descriptive statistics for analysed traits of dog rose fruits from four studied populations: Split (L1); Klis (L2); Neorić (L3) and Kamešnica (L4).

The average fruit lenght varied from 18.99 mm (Kamešnica, L4) to 20.13 mm (Split, L1), while the average fruit width varied from 11.55 mm (Klis, L2) to 13.21 mm (Kamešnica, L4). There were no significant differences in the fruit lenght between the populations Split (L1) and Klis (L2), nor were there any differences between the populations Neorić (L3) and Kamešnica (L4). Greater variability within populations was found for fruit lenght compared to fruit width. The average lenght of the fruit determined in this study is in accordance with the results of the study by Tomljenović et al., (2021) in which the values ranged from 17.94 to 21.93 mm. Celik et al., (2015) and Ersoy and Özen (2016) state in their research that fruit length is a trait with high variability. The highest shape index, and thus the most elongated fruit shape, was found in the populations Split, L1 (1.73) and Klis, L2 (1.74), while the lowest values were found in the populations Neorić, L3 (1.61) and Kamešnica, L4 (1.45), whose fruits were round to an oval shape (Figure 3). Results of our study for shape index is in accordance with result of dog rose fruits in the Mediterranean part of Croatia (Tomljanović et al., 2019).

Within populations, statistically significant differences at the significance level of $P \leq 0.05$ were found for all fruit properties studied (Table 1). In the Split population (L1), fruit weight varied from 0.77 g to 2.25 g, fruit length from 15.33 mm to 25.84 mm, fruit width from 9.24 mm to 14.27 mm and shape index from 1.34 to 2.44. In the Klis population (L2), fruit weight varied from 0.86 g to 2.09 g, fruit length from 15.15 mm to 30.50 mm, fruit width from 10.26 mm to 13.48 mm and shape index from 1.48 to 2.34. Fruit weight in the Neorić population (L3) varied from 0.89 g to 2.15 g, fruit length from 15.85 mm to 24.87 mm and fruit width from 1.17 mm to 13.40 mm. The fruit shape index varied from 1.31 to 1.94. In the population Kamešnica (L4), the fruit weight varied from 1.03 g to 2.43 g, the fruit length from 13.71 mm to 27.04 mm, the fruit width from 10.67 mm to 15.53 mm and fruit shape index from 0.89 to 1.82 (Table 1).

Table 1. Quantitative properties and coefficient of variation (CV) of dog rose fruit from four studied populations: Split (L1); Klis (L2); Neorić (L3) and Kamešnica (L4).

Number of accessions	Fruit weight (g)	Fruit length (mm)	Fruit width (mm)	Fruit shape index
		Split (L1)		
L1_RC001	0.77±0.2g	16.94±1.14gh	9.65±0.91de	1.77±0.14def
L1_RC002	1.43±0.36def	16.63±0.96gh	12.5±1.13b	1.34±0.09g
L1_RC003	1.96±0.48bc	21.49±1.89d	13.41±1.06ab	1.60±0.14ef
L1_RC004	2.25±0.77ab	21.18±2.06def	14.27±1.71a	1.49±0.12ef
L1_RC005	1.18±0.4f	24.08±2.82b	10.52±1.35cd	2.31±0.33b
L1_RC006	1.22±0.26ef	15.33±1.49h	11.04±1.00c	1.39±0.11f
L1_RC007	2.45±0.42a	25.84±2.35a	12.89±0.97b	2.00±0.11c
L1_RC008	1.74±0.36cd	19.70±1.4ef	13.26±0.91b	1.49±0.12f
L1_RC009	1.55±0.27de	17.74±1.43fg	12.63±0.96b	1.41±0.13f
L1_RC010	1.14±0.24f	22.0±3.15c	9.24±1.31e	2.44±0.22a
CV (%)	41.03	19.00	16.65	23.77
		Klis (L2)		
L2_RC001	1.03±0.20cd	24.10±3.52b	10.28±1.04f	2.34±0.27a
L2_RC002	1.07±0.23cd	17.68±1.53ef	10.74±1.09ef	1.65±0.12c
L2_RC003	2.09±0.52a	22.19±1.65c	13.48±1.11a	1.65±0.11c
L2_RC004	1.21±0.32c	17.28±1.11ef	11.71±0.98d	1.48±0.08d
L2_RC005	1.62±0.30b	18.99±1.19de	12.68±0.81ab	1.50±0.10d
L2_RC006	1.64±0.29b	16.73±1.02fg	11.39±0.73de	1.47±0.07d
L2_RC007	0.86±0.17d	15.15±1.37g	10.26±0.73f	1.48±0.13d
L2_RC008	2.25±0.88a	30.50±5.04a	12.58±1.44bc	2.24±0.27a
L2_RC009	1.19±0.30cd	16.99±1.15efg	11.85±1.02cd	1.44±0.08d
L2_RC010	1.23±0.19c	20.74±1.34cd	10.50±0.89f	1.99±0.17b
CV (%)	41.53	24.48	12.58	22.25
		Neorić (L3)		
L3_RC001	1.63±0.26b	21.49±1.55bc	12.15±0.98ab	1.78±0.15bc
L3_RC002	2.15±0.56a	24.87±1.80a	12.88±1.17a	1.94±0.12a
L3_RC003	1.37±0.23c	19.83±2.11cd	11.56±0.98bc	1.72±0.20c
L3_RC004	0.96±0.18d	15.47±1.10ef	11.32±0.80bc	1.37±0.11e
L3_RC005	1.58±0.40b	18.54±1.84d	13.08±1.12a	1.42±0.08e
L3_RC006	1.11±0.26cd	17.68±1.39de	11.17±0.66c	1.58±0.11d
L3_RC007	1.38±0.23bc	16.53±2.12e	12.71±0.68a	1.31±0.20e
L3_RC008	1.02±0.18d	19.67±1.33d	10.36±0.75d	1.90±0.13ab
L3_RC009	2.01±0.56a	22.07±2.11b	13.40±1.06a	1.65±0.15cd
L3_RC010	0.89±0.24d	15.85±1.63f	11.26±0.93e	1.41±0.13e
CV (%)	37.61	17.32	10.99	15.98
		Kamešnica (L4)		
L4_RC001	2.43±0.48a	27.04±2.24a	15.53±1.22a	1.75±0.16a
L4_RC002	1.46±0.28d	20.18±1.69b	12.22±1.00d	1.66±0.13b

L4_RC003	1.09±0.15e	17.64±1.31d	11.44±0.51e	1.53±0.10c
L4_RC004	1.12±0.19e	13.71±1.42f	15.41±1.10a	0.89±0.06e
L4_RC005	2.14±0.39bc	19.80±1.47b	13.87±0.95b	1.43±0.09cd
L4_RC006	1.48±0.22d	18.42±1.15cd	12.40±0.85d	1.49±0.09cd
L4_RC007	1.45±0.26d	17.95±1.17d	12.80±0.93cd	1.40±0.07d
L4_RC008	1.49±0.32cd	15.65±1.33e	13.44±0.98b	1.17±0.07d
L4_RC009	2.19±0.21b	20.17±0.98b	14.33±0.73b	1.41±0.09cd
L4_RC010	1.03±0.20e	19.36±1.13bc	10.67±0.73f	1.82±0.12a
CV (%)	34.55	19.06	13.46	19.20

The values are given as mean ±SD, n=30. Different lower-case letters in each column indicate significant difference between accessions within population for P≤0.05 by the Tukey.

Of all the investigated properties, the highest variability was found for fruit weight. The coefficient of variation (CV) was 41.58% for Klis (L2), followed by Split, L1 (41.03%), Neorić, L3 (37.61%) and Kamešnica, L4 (34.55%). Furthermore, for fruit length, the highest CV was recorded in the population Klis, L2 (24.48%) and the lowest in Neorić, L3 (17.32%). For fruit width, the highest CV value was found in the population Split, L1 (16.65%), and the lowest in Neorić, L3 (10.99%). The variability of the fruit shape index was the highest in the Split (L1) (23.77%), and the lowest in the Neorić (L3) (15.98%) population (Table 1).

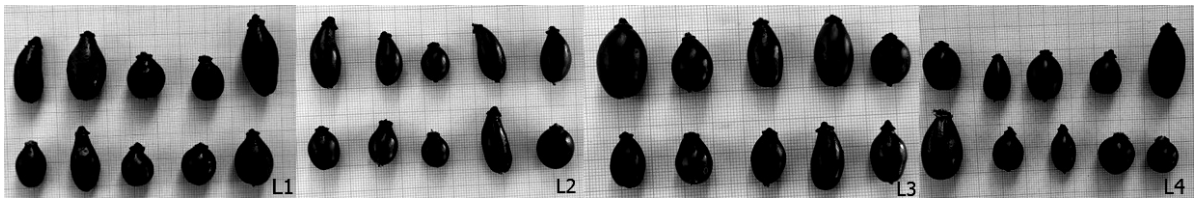

Figure 3. Fruits of investigated populations: Split (L1), Klis (L2), Neorić (L3) and Kamešnica (L4).

Conclusion

Variability in fruit properties was recorded between and within populations. The highest variability was found for fruit weight. According to the data on fruit shape index, Split (L1) and Klis (L2) populations have more elongated fruits, while Neorić (L3) and Kamešnica (L4) have rounder fruits. It is necessary to include a larger number of populations in the research and to study the influence of habitat, population size and climatic conditions on population variability. Additional physicochemical and molecular studies aimed at selecting the best samples for use in the food and pharmaceutical industries are also needed.

Acknowledgment

We appreciate the technical assistance of Radojka Plećaš and Sandra Đirlić. This research was funded through European Regional Development Fund by the Operational program Competitiveness and Cohesion 2014-2020 within the project CEKOM 3LJ - Centre of Competence 3LJ KK.01.2.2.03.0017".

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58 hrvatski 18 međunarodni simpozij agronoma

Zbornik radova

Ribarstvo, lovstvo i pčelarstvo

Mehanička filtracija u akvakulturi

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Sažetak

U ovom radu su opisane metode mehaničke filtracije, odnosno izdvajanja krutih čestica iz vode koje se primjenjuju u održavanju kvalitete vode u različitim akvakulturnim proizvodnim sustavima i u pročišćavanju otpadnih voda. To se postiže gravitacijom, kojom se odvajaju taložive čestice specifične težine veće od vode, filtracijom u užem smislu kojom se odvajaju suspendirane čestice i flotacijom kojom se odvajaju čestice manje specifične težine od vode. Taložive čestice uklanjaju se u bazenima za taloženje, cijevnim taložnicima i hidrociklonima. Filtri kojima se odvajaju suspendirane čestice imaju određenu vrstu sita, poput mrežice ili membrane, medij u obliku zrnaca pijeska ili plastike, ili neki porozni medij koji propušta vodu a zadržava mehaničke čestice. Najfinije čestice suspendiranog otpada izdvajaju se procesom flotacije pomoću struje zraka. Za obradu otpadnih voda iz akvakulturnih sustava koriste se i umjetne močvare.

Ključne riječi: akvakultura, sedimentacija, filtracija, flotacija

Uvod

Kruti akvakulturni otpad uglavnom se sastoji od nepojedene hrane, fecesa i ostalih metaboličkih izlučevina te je bogat spojevima s ugljikom, dušikom i fosforom (Dauda i sur 2019; Timmons i sur., 2001; Cripps i Bergheim, 2000; Van Gorder i Jug-Dujaković, 1996). Jedna od glavnih karakteristika krutog akvakulturnog otpada je veličina, odnosno specifična težina njegovih čestica pa se može podijeliti na plivajući, raspršeni ili suspendirani i taloživi (Timmons i sur., 2001). Količina i karakteristike otpada nastalog prehranom ovise o uzgajanoj vrsti, njenoj veličini, načinu uzgoja i karakteristikama hrane (Amirkolaie, 2011).

Zbrinjavanje krutog otpada ovisi o vrsti uzgojnog sustava. Ribnjaci, odnosno zemljani bazeni (*pond*), su statični i nemaju posebnu opremu za pročišćavanje vode. Mehanička filtracija u njima nije primjenjiva. Oni se uglavnom oslanjaju na unutarnje procese u kojima se kruti otpad taloži na dno ribnjaka i akumulira tijekom vremena. Jedini način uklanjanja krutog otpada je uklanjanjem mulja, što se radi nakon dva ili više ciklusa uzgoja ribe. Nedostatak aktivne tehnike zbrinjavanja otpada, odnosno mehaničke i biološke filtracije u ribnjacima ograničio je njihovu iskoristivost na ekstenzivne i poluintenzivne sustave (Dauda i sur., 2019).

Za razliku od ribnjaka, gdje se kruti otpad taloži unutar sustava, protočni sustav ima visoku razinu izmjene vode, gdje se većina proizvedenog otpada protokom ispušta iz uzgojnog sustava. U većini protočnih sustava vrijeme zadržavanja vode kraće je od sat vremena (Dauda i sur., 2019). Mehanička filtracija nije primjenjiva unutar samog uzgojnog sustava, ali je njena primjena moguća u tretmanu izlazne, odnosno otpadne vode iz uzgojnog sustava (uzgojnih bazena). Izdvajanjem krutih čestica iz otpadne vode protočnog sustava teško je i skupo upravljati zbog velike brzine vode s niskom koncentracijom otpada (Dauda i sur., 2019).

Kod djelomično protočnih, odnosno otvorenih recirkulacijskih sustava i zatvorenih recirkulacijskih sustava, mehanička filtracija jedan je od neophodnih postupaka održavanja optimalne kvalitete vode u uzgojnim prostorima. Taložnice, mehanički filtri i površinski odjeljivači pjene (*foam fractionator*) neophodne su komponente takvih sustava (Jug-Dujaković i sur., 2008; Gavrilović i Jug-Dujaković, 2018). U takvim sustavima je potrebno ispuniti dvije osnovna grupe uvjeta za održanje kvalitete vode: (I) uvjete koje zahtijeva uzgajana vrsta te (II) uvjete koje zahtijeva odvijanje pojedinačnih procesa i funkcioniranje samih komponenti sustava (Colt, 2006). Ovo podrazumijeva uklanjanje krutih čestica koje bi eventualno mogle naškoditi uzgajanom organizmu te uklanjanje čestica koje bi mogle smanjiti učinkovitost neke komponente u sustavu, poput biofiltra ili UV reaktora.

Kako bi se količina otpada održala na razini prihvatljivoj za ispuštanje u recipijent ili recirkuliranje te da bi se mogao primijeniti pravilan i učinkovit postupak upravljanja otpadom i/ili njegova tehnološka obrada, potrebno je poznavati njegove karakteristike (Cripps i Bergheim, 2000). Brojni autori navode kako kruti otpad iz protočnih i djelomično protočnih sustava nije jednostavno ukloniti zbog njegove male koncentracije u vodi. Recirkulacijski sustavi ispuštaju male količine koncentrirane otpadne vode čije je pročišćavanje lakše i jeftinije (Van Gorder i Jug-Dujaković, 1996). Kelly i sur. (1997) i Bergheim i sur. (1998) dokazali su da učinkovitost mehaničke filtracije raste s koncentracijom otpada i da se može očekivati povećanje njene uspješnosti ukoliko se poveća koncentracija otpada u izlaznoj vodi. Twarowska i sur. (1997) naglašavaju prednost kontinuiranog predtretmana koji će koncentrirati otpad. Zbog posebnih karakteristika veličinskih frakcija krutog otpada on se vrlo često koncentrira u dva odvojena toka taloživih i raspršenih čestica koje se zatim posebno obrađuju.

Izdvajanje krutih čestica gravitacijom

Izdvajanje krutih čestica gravitacijom temelji se na sedimentaciji. To je proces u kojem se čestice koje imaju veću masu ili specifičnu gravitaciju od vode, talože da bi se mogle izdvojiti. Karakteristika ovog procesa jest brža sedimentacija gušćih i većih čestica iz vode od manjih odnosno manje gustih. Zato je potrebno održati veličinu čestica te omogućiti njihovo izdvajanje iz vode što je prije moguće, posebno prije nego dođe do pumpanja vode te minimalizirati bilo kakve turbulencije prije samog izdvajanja čestica (Timmons i sur., 2001). Sedimentacija je najčešća i najpoznatija metoda uklanjanja čestica u akvakulturi. Ujedno i najjeftiniji proces jer koristi silu gravitacije, a njezini nedostatci su nisko opterećenje te neučinkovito tretiranje čestica manjih od 100 μm.

Bazeni za taloženje mogu biti i kružni i pravokutni, a zajedničko im je obilježje da su dizajnirani tako da je turbulencija i resuspenzija tekućine minimalna čime se osigurava učinkovito uklanjanje taloživih čestica, prikupljanje i pražnjenje nataloženog mulja i njegovo ugušćivanje u što manjem volumenu. U pravilno dizajniranim taložnicima razlikuju se četiri zone; ulazna, taložna, zona mulja i izlazna zona (Timmons i sur., 2001; Cripps i Bergheim, 2000). Sedimentacija se odvija u zoni taloženja, a nakon odvajanja od vode čestice se akumuliraju u zoni mulja. Pročišćena tekućina se u pravilu skuplja preko poprečnog presjeka bazena te se onda ispušta. Bazeni za taloženje su jako učinkoviti ako su pravilno dizajnirani i ako se s njima pravilno upravlja. Njihove prednosti su to što zahtijevaju mali unos energije, relativno su jeftini za instalaciju i upravljanje, ne zahtijevaju nikakve posebne operativne vještine te ih se može lako nadodati unutar postojećih ili novih postrojenja, a nedostaci su nisko hidrauličko opterećenje i slabo uklanjanje manjih čestica (ispod 100 μm). Uz to, ono što predstavlja prepreku primjene bazena za taloženje jest njihovo čišćenje te činjenica da im je potreban veliki prostor što može biti jako skupo (Timmons i sur., 2001).

Cijevni taložnici su razvijeni unaprjeđivanjem bazena za taloženje. Dizajnirani su tako da se smanji površina i vrijeme potrebno za taloženje. To su tankovi u koje su umetnute ploče ili cijevi pod kutom od 45°–60° ispod kojih je smješten ulaz vode. Kako voda protječe, čestice se zadržavaju na pločama, a pročišćena voda izlazi na vrhu. Brzina vode kroz cijev se mora regulirati da bi se izbjegla turbulencija. Kada se cijevi napune česticama, voda prestaje prolaziti kroz njih, a proces prestaje sve dok se cijevi ne očiste. Veće cijevi zahtijevaju manje čišćenja, ali imaju manji učinak taloženja, dok manje cijevi imaju puno bolji učinak, ali su njihovo čišćenje i održavanje zahtjevniji. Najveća mana cijevi je što se ne mogu same dovoljno očistiti pa ih treba čistiti i ručno (Timmons i sur., 2001).

Izdvajanje sedimentacijom se primjenjuje i u hidrociklonskim separatorima gdje se voda s česticama krutog otpada podvrgava centrifugalnom ubrzanju pa se čestice brže odvajaju od tekućine. Postoje dvije vrste hidrociklonskih separatora, vrtložni i radijalni. Vrtložni separatori funkcioniraju tako što se voda ubrizgava tangencijalno u vanjskom dijelu jedinice što dovodi do kruženja vode oko centralne osi bazena. Ova primarna rotacija stvara sekundarni radijalni tok prema centru tanka čija inercija pomaže u skupljanju čestica. Vrtložni separatori se u pravilu koriste kod tretiranja vode u kojoj su čestice visoke specifične gravitacije (2,65 veće od gravitacije vode) (Andoh, 1998). U radijalnom hidrociklonu se voda ubrizgava u središtu i onda teče prema rubovima, pri čemu se tok usporava prikupljajući čestice (Davidson i Summerfelt 2005). Radijalni separator se pokazao učinkovitiji i s manje oscilacija, uklanja do 48 % čestica za razliku od kružnog koji uklanja do 23 % (Davidson i Summerfelt 2005). U odnosu na bazene za taloženje i cijevne taložnike, hidrocikloni mogu obraditi veću količinu vode u manjem vremenu zbog bržeg protoka, ali upravo zbog tog kontinuiranog toka troše veliku količinu vode koju je potrebno nadomjestiti u sustavu, ne mogu ni izdvojiti čestice manje od 50 μm i relativno su skupi (Timmons i sur., 2001).

Izdvajanje krutih čestica filtracijom

Filtracija u užem smislu riječi podrazumijeva izdvajanje čestica iz tekućine pomoću neke vrste perforirane pregrade koja zadržava čestice koje su veće od njenih šupljina, a propušta tekućinu. Proces filtracije se provodi u različitim filterskim kućištima pomoću sita, granularnih ili poroznih materijala (Timmons i sur., 2001).

Primjena sita za filtriranje je jako popularna jer zahtjeva minimalan trud i mali prostor. U takvim filtrima se upotrebljava sito sitnih pora, mikrosito, koje zadržava suspendirane čestice, a propušta tekućinu. Veličina čestica koje se mogu profiltrirati ovisi o veličini pora sita. Talog se zatim odvaja pomoću mlaznica koje ispiru začepljeno sito malom količinom vode pod jakim pritiskom. Faktor koji čini svaki sitasti filtar različitim i izazovnim za projektiranje je upravo proces prikupljanja čestica s površine mikrosita (Losordo i sur., 1999). Ovisno o tome kakva se kvaliteta vode želi osigurati koristi se sito veličine pora od 60 do 200 μm, prosječno mikrosito ima otvore između 40 do 100 μm pri čemu je uspješnost odvajanja čestica između 30-80 % (Timmons i sur., 2001).

Najčešći sitasti filtar u akvakulturi je bubanj filtar (*drum filter*) u kojemu voda ulazi u njegovu otvorenu bazu i prolazi pročišćena kroz mrežicu postavljenu na oplošje bubnja. U većini slučajeva se bubanj pokreće tek kad se mrežica začepi česticama koje se zatim pomoću mlaznica ispiru u unutarnji kanal za prikupljanje otpada. Bubanj filtar se u redovnom procesu kompletno rotira svake dvije do tri minute. Ukoliko dolazi do češće rotacije znači da je sustav preopterećen te da je potrebna promjena ili popravak filtra (Timmons i sur., 2001). Bubanj filtri imaju širok raspon primjene, zauzimaju malo prostora i praktični su za održavanje, a njihov učinak usko je povezan s parametrima poput hidrauličkog opterećenja, veličine pora mreže, koncentracije čestica i intenziteta ispiranja (Xiao i sur., 2019). Za primjenu rotirajućeg bubanj filtra u recirkulacijskom sustavu u smislu ograničenja troškova kao najekonomičnija komponenta preporuča se manji filtar koji radi s kontinuiranim ispiranjem (Dolan i sur., 2013).

Alternativa rotirajućem bubanj filtru su disk filtri koji su u pravilu dosta jeftiniji od bubnjeva. U disk filtar voda ulazi s jedne strane, prolazi kroz nekoliko rotirajućih diskova s mrežicom i izlazi pročišćena na drugoj strani. Njihova mana jest ta da se zbog vertikalne orijentacije mikrosita čestice mogu zadržati dugo vremena prije uklanjanja pa može doći do pucanja i razbijanja čestica (Timmons i sur., 2001).

Sitasti filtri s nagnutim pojasom sastoje se od spremnika s vodom u kojem je pod određenim kutom postavljena mreža u obliku trake. Voda s jedne strane ulazi u spremnik kroz koji se giba mrežasta traka i izlazi pročišćena na drugoj strani. Na traci se zadržavaju čestice koje se mlazom vode ispiru u kanal za prikupljanje otpada. Zabilježen protok ovih filtara je i veći od 7500 L/min, ali je malo podataka o njihovim radnim karakteristikama (Losordo i sur., 1999). Prednost ovakvih filtara je nježna obrada čestica i jednostavno održavanje, a nedostatak veći kapitalni troškovi (Timmons i sur., 2001).

Filtar s paraboličnom mrežom je metalna mrežasta struktura visoke čvrstoće, krutosti i nosivog kapaciteta, jednostavna oblika i rada bez potrošnje energije i niskih troškova održavanja (Xiao i sur., 2019). Chen i sur., (2015.) su istraživali primjenu ovakvog filtra u recirkulacijskom sustavu i zaključili da su brzina ulaznog protoka i brzina uklanjanja krutih čestica obrnuto proporcionalne. Tijekom rada površinu mreže je potrebno ručno ribati jednom na sat, te pratiti brtvljenje i pravovremeno provjeriti sigurnost mreže kako bi se spriječilo oštećenje koje bi utjecalo na ukupni učinak obrade vode (Xiao i sur., 2019.).

Pješčani filtar je posuda pod tlakom u koju ulazi i prolazi voda kroz kvarcni pijesak za finu filtraciju, veličine pora do 20 μm. Filtri s granulama pijeska mogu biti dizajnirani tako da voda protječe, obično odozgo prema dole, kroz gusti sloj pijeska u nekoj tlačnoj posudi ili tako da voda ulazi odozdo i održava zrnca pijeska raspršena u takozvanom fluidiziranom sloju. I jedni i drugi su česti u akvakulturi, ali im je osnovni nedostatak brzo čepljenje i odvijanje biofiltracije (Timmons i sur., 2001). Ovi se filtri obično koriste u recirkulacijskim akvakulturnim sustavima i imaju prednosti poput niske cijene, jednostavne konstrukcije, visoke učinkovitosti uklanjanja suspendiranih čestica, učinkovitog ispiranja, dok nedostaci uključuju visoki tlak ispiranja, filtracijski materijal koji se lako stvrdne i visoke troškove održavanja. Uz to, pijesak je potrebno često provjeravati kako bi se na vrijeme uklonio površinski plutajući mulj i poboljšao ukupni sustav za pročišćavanje vode (Xiao i sur., 2019). Upravo zbog prečeste potrebe za ispiranjem Timmons i sur. (2001.) ne preporučuju upotrebu pješčanih filtara za mehaničku filtraciju u akvakulturi, osim u slabo opterećenim sustavima.

Granularni filtar je zatvorena posuda ispunjena plastičnim zrncima različitih oblika koje plutaju unutar posude i pokreću se ovisno o protoku vode čiji je ulaz smješten pri dnu. Pri dnu i vrhu filtra su postavljene perforirane ploče koje služe za zaustavljanje zrnaca. Dok filtar radi zrnca se nalaze u fluidiziranom sloju priljubljena uz gornju ploču koja im ne dopušta da isplutaju iz filtra. S nakupljanjem suspendiranih čestica smanjuje se veličina šupljina među

zrncima što dovodi do lijepljenja sitnijih čestica i potrebe za ispiranjem. Filtri manjeg volumena se obično ispiru tako da se zaustavi protok vode i isprazni cjelokupan volumen vode, a granule ostaju na donjoj ploči. Kod filtara većeg volumena se zaustavlja protok vode, a volumen koji je ostao u filtru se miješa propelerom ili upuhivanjem zraka pri čemu se krute otpadne čestice odvajaju od zrnaca i talože na dnu filtra. Zatim se iz donjeg dijela ispušta voda s tako koncentriranim otpadom. Za ovakvo ispiranje potrebna je mala količina vode (Van Wyk i sur., 1999).

Porozni filtri u pravilu imaju čvršće medije od sitastog filtra te manje pore od granularnih medija. Zbog vrlo sitnih pora, odnosno mogućnosti zadržavanja vrlo finih čestica ovi se filtri obično upotrebljavaju nakon nekog drugog oblika mehaničke filtracije u sustavima koji zahtijevaju vodu gotovo bez ikakvih čestica, poput sustava za inkubaciju jaja i ličinki. Štoviše, u takvim se sustavima ovi filtri često upotrebljavaju i za obradu ulazne vode. Njihova osnovna prednost je uklanjanje čestica manjih od 1 μm, a glavni nedostatak brzo čepljenje i potreba za ispiranjem, odnosno izmjenom uloška (Timmons i sur., 2001). U filtre s poroznim medijem ubrajaju se filtri s dijatomejskom zemljom i različitim ulošcima (Timmons i sur., 2001.). Noviji filtri s dodatkom zeolita i aktivnog ugljena imaju i dodatnu primjenu; mogu smanjiti do 92,82 % ukupne koncentracije amonijaka i povećati koncentraciju otopljenog kisika za 54,23 % (Zahidah i sur., 2018.).

Izdvajanje krutih čestica flotacijom

Flotacija je postupak izdvajanja vrlo finih čestica iz tekućine njihovim isplivavanjem na površinu pomoću mjehurića zraka. Propuhivanjem zraka kroz tekućinu na mjehuriće zraka se hvataju čestice koje zatim zajedno njima isplivaju na površinu tekućine. U akvakulturi se ova metoda odvajanja krutog otpada od vode odnosi uglavnom na uklanjanje nakupina proteina i masti iz nepojedene hrane, a odvija se procesima odstranjivanja zrakom, odnosno obiranja proteina, ili frakcioniranjem pjene. Proces se provodi tako da voda teče kroz kontaktnu komoru odozgo prema dole, a mjehurići zraka upuhani pri dnu komore struje u suprotnom smjeru. Površinski aktivne tvari, poput proteina i masti, pričvrste se na mjehuriće i s njima putuju prema površini vode. Tu mjehurići pucaju, a tvari se nakupljaju u obliku pjene koja se zatim izdvaja kao otpad. Ovaj proces ovisi o promjeru mjehurića, omjeru zraka i vode, koncentraciji krutih čestica i njihovim površinskim kemijskim svojstvima (Cripps i Bergheim, 2000; Timmons i sur., 2001; Brambilla i sur., 2008). Kao i filtri s poroznim medijem, zbog mogućnosti izdvajanja jako sitnih čestica ovakvi filtri se obično upotrebljavaju nakon nekog drugog oblika filtracije u sustavima koji zahtijevaju vrlo kvalitetnu vodu. Uz pomoć flotacije može se značajno smanjiti koncentracija otpadnih čestica iz sustava što dovodi do smanjenja količine organske tvari koja prolazi kroz bakterijsku razgradnju te ima veliki utjecaj na koncentracija dušika u sustavu (Sadeghi i sur., 2018).

Umjetne močvare

Umjetne močvare su plitki sustavi za pročišćavanje otpadnih voda koji su zasađeni močvarnim biljkama koje oponašaju prirodnu močvaru i oslanjaju se na prirodne procese za pročišćavanje. Umjetna močvara ima prednosti u odnosu na ostale sustave obrade otpada jer zahtijeva malo ili nimalo energije. Umjetne močvare postaju staništa različitim organizmima i mogu biti oku ugodne (Miller i Semens, 2002). Izlazna voda šalje se u močvaru u kojoj rastu močvarne biljke u poroznom zemljanom supstratu. Voda prokapava kroz porozni supstrat i tako se filtrira i pročišćava. Biljke uzimaju i iskorištavaju hranjive tvari iz otpadne vode, dok korijen biljaka utječe na pojačavanje procesa pročišćavanja (Lekang, 2007). Veliki nedostatak umjetnih močvara je nizak hidraulički kapacitet u odnosu na količine vode u akvakulturi (Lekang, 2007) te potreba za više kopnene površine od alternativnih sustava (Miller i Semens, 2002). Ipak tehnologija umjetnih močvara postaje sve važnija u recirkulacijskim akvakultuirnim sustavima jer su se močvare pokazale kao dobro uhodana i ispaltiva metoda (Turcios i Papenbrock, 2014).

Zaključak

Poznavanje karakteristika krutog otpada osnovni je uvjet odabira pravilnog i učinkovitog postupka njegova uklanjanja. Odabir mehaničkih filtara ovisi o veličini čestica, protoku, dimenziji uređaja, učestalosti i trajanju ispiranja te potrošnji energije. Čestice krutog akvakulturnog otpada se ovisno o njihovim svojstvima izdvajaju se iz uzgojne vode procesima gravitacije, filtracije ili flotacije. Gravitacijska sedimentacija je najčešća i najpoznatija metoda mehaničke filtracije u akvakulturi, a ujedno je i najjeftinija jer koristi silu gravitacije. Međutim, njen nedostatak je nisko opterećenje te neučinkovito tretiranje malih čestica. Filtracijom se iz vode uklanjaju suspendirane i raspršene

čestice, a koriste se različiti sitasti filtri te filtri s granularnim ili poroznim medijem. Sitasti filtri imaju visoke hidrauličke kapacitete, zauzimaju malo prostora te imaju mali gubitak vode za ispiranje otpada. Granularni filtri su učinkoviti u uklanjanju suspendiranih čestica, ali se u njima obično odvija i biološka filtracija što se može iskoristiti u slabo opterećenim sustavima. Fini otpad se iz uzgojne vode uklanja ili pomoću filtara s poroznim medijem ili flotacijom. Unatoč brojnim metodama otlkanjanja krutog otpada, potrebno je razvijati intenzivne uzgojne sustave s naglasakom na kontroli nastanka otpada boljom formulacijom hrane i optimizacijom hranidbe.

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Mechanical filtration in aquaculture

Abstract

This paper describes the methods of mechanical filtration, i.e. the separation of solid particles from water, which are used in maintaining the quality of breeding water in different

aquaculture production systems and/or purification of waste water. Gravity separation removes settleable waste in settling basins, tube settlers and hydrocyclones. Filtration separate suspended particles in filters that have some kind of sieve, like a mesh or a membrane, a bead medium like sand or plastic, or a porous medium. The finest particles of dissolved and floating waste are separated by the flotation process using an air stream. Constructed wetlands are also used for waste treatment from recirculating aquaculture systems.

Keywords: aquaculture, sedimentation, filtration, flotation

ORIGINAL SCIENTIFIC PAPER

Cephalopods in the bottom trawl catches of the northern and central Adriatic Sea

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Abstract

Bottom trawl catches of cephalopods in the northern and central Adriatic Sea were analysed using data collected during the MEDITS 2016 expedition. A total of 19 species were registered and the most represented were: *Alloteuthis media, Loligo vulgaris, Illex coindetii, Todaropsis eblanae and Eledone moschata.* The largest number of species was found at depths of 100-200 m and the lowest at 10-50 m. The highest mean abundance index of cephalopods was in 100-200 m depth stratum. The most abundant species in depths 10-50 m was *L. vulgaris*, in strata 50-100 m and 100-200 m *A. media*, and in stratum 200-500 m *I. coindetii.* The highest abundance indices were registered in Croatian fishing zones C and D. The obtained results could serve as a basis for improved long-term sustainable management of this group of marine invertebrates.

Keywords: cephalopods, Adriatic Sea, abundance, depth distribution, fishing zones

Introduction

Cephalopods are bioecologically and commercially very important group of marine organisms. Because of their abundance and wide distribution, they are significant components of marine food webs, both as predators and as prey. Therefore, the extent of their exploitation and the status of their populations are critical to the overall sustainability of marine ecosystems (Vidal et al., 2014). Cephalopods are widely distributed throughout the Mediterranean, including the Adriatic Sea, and they have been used for human consumption since ancient times. The first studies on cephalopods in the Adriatic date back to the 18th century (see details in Mandić, 1984) and these animals have been of interest to scientists in the area ever since (Krstulović Šifner et al., 2011; Quetglas et al., 2019). Because cephalopods are valuable to fisheries, it is important for management to increase general knowledge about them. For this reason, we aimed to provide an insight into the distribution and abundance of fished cephalopod species in the eastern part of the Adriatic Sea, i.e. in Croatian waters, both by depth and by fishing zone as defined by Croatian Low (NN, 5/2011). This study is a contribution to a better understanding of the cephalopod assemblages in different areas of the northern and central Adriatic and provides valuable information for a better future management of this group of animals.

Materials and methods

Samples were collected in the summer during the MEDITS 2016 expedition in the northern and central Adriatic Sea (GSA 17) (Figure 1) according to a stratified sampling scheme at the following depth strata: 10-50 m, 50-100 m, 100-200 m, and 200 to 500 m. The fishing tool used for sampling was an experimental trawl net named GOC 73 (Fiorentini et al., 1999). All catches were standardised using the swept-area method, and abundance and biomass indices (N/km^2 and kg/km^2) were used to represent the density of caught populations (Sparre and Venema, 1998). Details of the MEDITS (engl. *MEDiterranean International Trawl Survey*) protocol can be found in Bertrand et al. (2002). All cephalopods caught were determined to the species level using the FAO key (Mangold and Boletzky, 1987), counted and weighed.

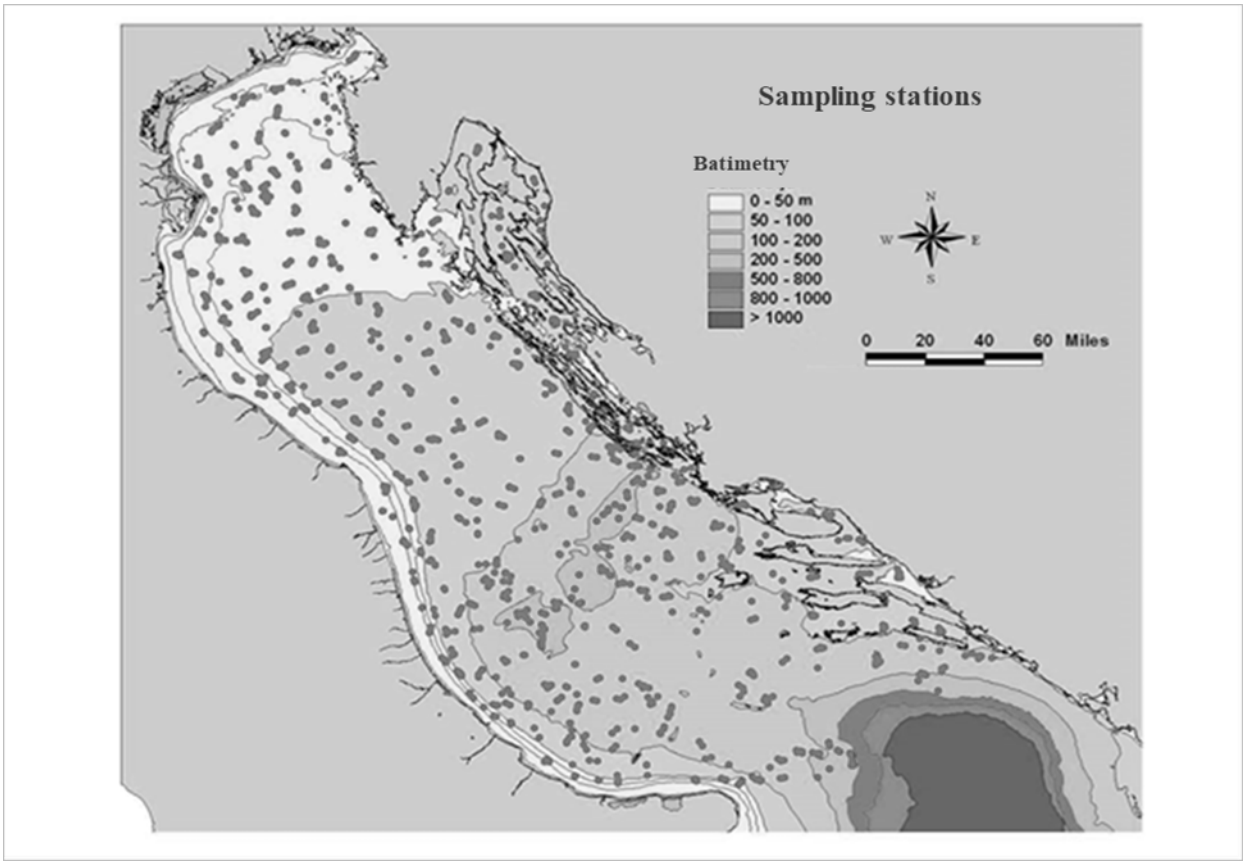

Figure 1. MEDITS sampling stations in the northern and central Adriatic Sea (GSA 17); sampling stations presented by dots

The data obtained during the expedition were used to represent the cephalopod fauna caught in GSA 17 and for analysis by depth and fishing zone in the territorial waters of the Republic of Croatia, i.e. in zones A, B, C, D, G and EF, where the last two zones have similar ecological characteristics and were therefore analysed together (Figure 2). The zones of the current exclusive economic zone (H, I, J and K), where cephalopods are of less importance for bottom trawling, were excluded from the analyses.

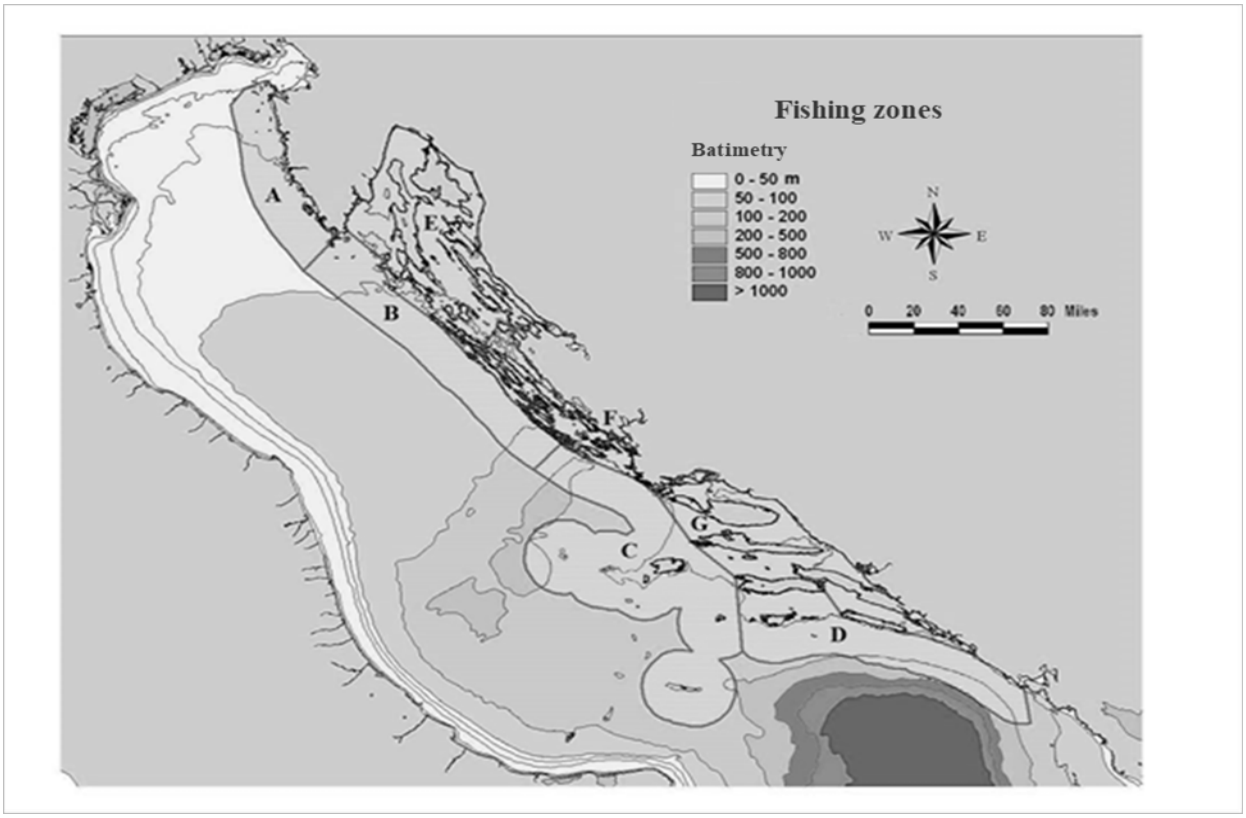

Figure 2. Fishing zones of the Republic of Croatia analysed in the study (A, B, C, D, EF and G)

Results and discussion

Abundance and biomass indices of cephalopods

During the MEDITS 2016 expedition, a total of 19 cephalopod species were registered, belonging to 5 different families: 3 species Sepiidae, 7 species Sepiolidae, 2 species Loliginidae, 3 species Ommastrephidae and 4 species Octopodidae (Table 1).

Table 1. The list of cephalopod families and species registered during MEDITS 2016 in GSA 17 with indicated mean abundance and biomass indices (N/km^2 and kg/km^2)

Family	Species	Abundance index (N/km^2)	Biomass index (kg/km^2)
Sepiidae	*Sepia elegans* Blainville, 1827	103.05	0.945
	Sepia officinalis Linnaeus, 1758	3.93	0.686
	Sepia orbignyana Férussac, 1826	7.88	0.257
Sepiolidae	*Rossia macrosoma* (Delle Chiaje, 1830)	1.99	0.054
	Sepietta neglecta Naef, 1916	2.14	0.008
	Sepietta obscura Naef, 1916	0.71	0.002
	Sepietta oweniana (d'Orbigny, 1841)	9.55	0.066
	Sepiola intermedia Naef, 1912	0.51	0.001
	Sepiola robusta Naef, 1912	0.51	0.001
	Sepiola rondeletii Leach, 1817	1.17	0.007
Loliginidae	*Alloteuthis media* (Linnaeus, 1758)	1623.68	3.647
	Loligo vulgaris Lamarck, 1798	726.00	10.952
Ommastrephidae	*Illex coindetii* (Vérany, 1839)	897.53	20.697
	Todarodes sagittatus (Lamarck, 1798)	0.51	0.044
	Todaropsis eblanae (Ball, 1841)	153.64	8.005
Octopodidae	*Eledone cirrhosa* (Lamarck, 1798)	24.01	2.305
	Eledone moschata (Lamarck, 1798)	266.43	23.138
	Octopus salutii Vérany, 1839	0.20	0.004
	Octopus vulgaris Cuvier, 1797	5.86	2.505

Considering both indices, the most represented species were *Illex coindetii* (897.53 N/km^2, 20.70 kg/km^2), *Loligo vulgaris* (726 N/km^2, 10.95 kg/km^2) and *Eledone moschata* (266.43 N/km^2, 23.14 kg/km^2). On the other hand, considering only the abundance index, *A. media* was the most abundant of all cephalopod species (1623.68 N/km^2). Nevertheless, due to its small body size the biomass index was relatively low (3.65 kg/km^2), and unlike the three previously mentioned species, it is not considered one of the commercially important species in the Adriatic despite its abundance. Relatively low values of the abundance index for the commercially important species *Octopus vulgaris* can be attributed to the fact that this species is caught in the Adriatic Sea mainly with lures, hooks and lines, pots and spears, and only sporadically with bottom trawl nets (Grubišić, 1988). Similarly, *Sepia officinalis* is caught with other fishing tools besides trawls. The main fishing season of this species corresponds to its reproductive season, which is spring, while it is caught sporadically during other seasons (De Mauro et al., 2007).

Distribution of cephalopods by depth stratum

Depth distribution analysis showed that cephalopods are most abundant in stratum 100 to 200 m (7367.64 N/km^2). The second most important depths are in stratum 10 to 50 m (3736.93 N/km^2), followed by stratum 200-500 m where the highest mean biomass index was also registered (3096.45 N/km^2; 127.72 kg/km^2). This probably indicates that the distribution depends on their life stages and that, in general, larger individuals of some cephalopod species prefer deeper waters (Figure 3).

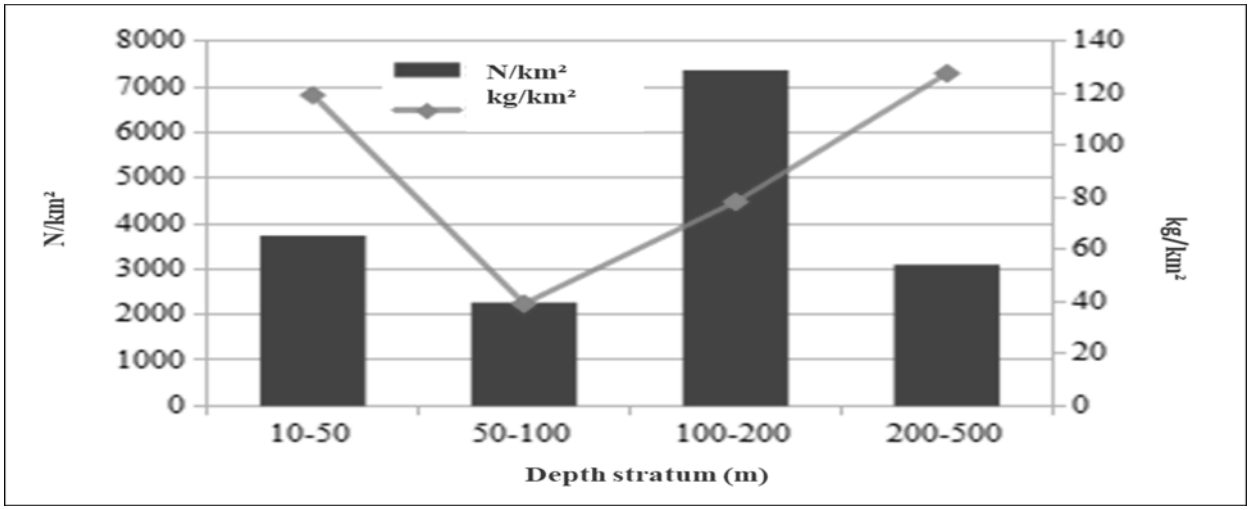

Figure 3. Abundance and biomass indices of cephalopods by depth strata (N/km² and kg/km²)

In shallow waters, 10-50 m, the lowest number of species was caught, i.e. a total of 8 species. The most abundant among them were *L. vulgaris*, *A. media* and *E. moschata*.

At stratum 50-100 m, 15 cephalopod species were registered. The most abundant were *A. media* (970.04 N/km²) and *E. moschata* (262.56 N/km²). *E. moschata* was also the most represented species regarding the biomass indices at depths 10-50 m and 50-100 m, with 75 kg/km² and 12,39 kg/km², respectively.

The largest number of cephalopod species was recorded in stratum 100-200 m, 16 in total, were the most abundant species were *A. media* and *I. coindetii*, and the highest biomass index was found for *I. coindetii* and *T. eblanae*.

At the deepest stratum, 200-500 m, only 9 cephalopod species were registered. The most abundant were the members of Ommastrephidae family, e.g. *T. eblanae* and *I. coindetii*. Still, majority of the commercially important species were not fished at these depths, i.e. *O. vulgaris*, *L. vulgaris*, *E. moschata* and *S. officialis*.

It is obvious that in the middle strata (50-100 m and 100-200 m) the species diversity is higher and the species are more abundant, while in the shallow and deepest strata (10-50 m and 200-500 m) the number of species is much lower but their relative weight is higher. This refers mainly to *E. moschata* in the shallow waters, and Ommastrephidae in the deepest stratum and, to a lesser extent to *E. cirrhosa*.

Distribution by fishing zone

The distribution of cephalopods in Croatian fishing zones was analysed and presented (Figure 4). In the fishing zone D, open southern parts of the Croatian Adriatic, the highest mean abundance and biomass indices of cephalopods were recorded. Among all cephalopods, the most representative species in this zone were *Illex coindetii*, *Alloteuthis media* and *Todaropsis eblanae*. It was found that the second most important regarding cephalopods was the zone C, i.e. the area of the open central Adriatic Sea, also the area well known for the very intensive commercial, bottom-trawl fishery. In this is area the species *A. media*, *Eledone cirrhosa* and *I. coindetii* were mainly caught. Almost as important is the fishing zone G, i.e. the channel area of the Central Adriatic Sea, where the most abundant were *Loligo vulgaris* and *E. moschata*. In the fishing zone A, covering an area along the western coast of the Istrian peninsula, the biomass index was very high (100.29 kg/km²), in contrast to the abundance index. The main reason for this are very large catches of the large sized Musky octopus, *E. moschata*, a species very common in this area. The same species was very abundant in zones E and F, i.e. in the channel areas of the northern Adriatic. However, zones E and F, and especially the zone B, i.e., the open northern Adriatic, had lower mean abundance and biomass indices, indicating the lower commercial importance of cephalopods for bottom trawling in these zones compared to the others zones described previously.

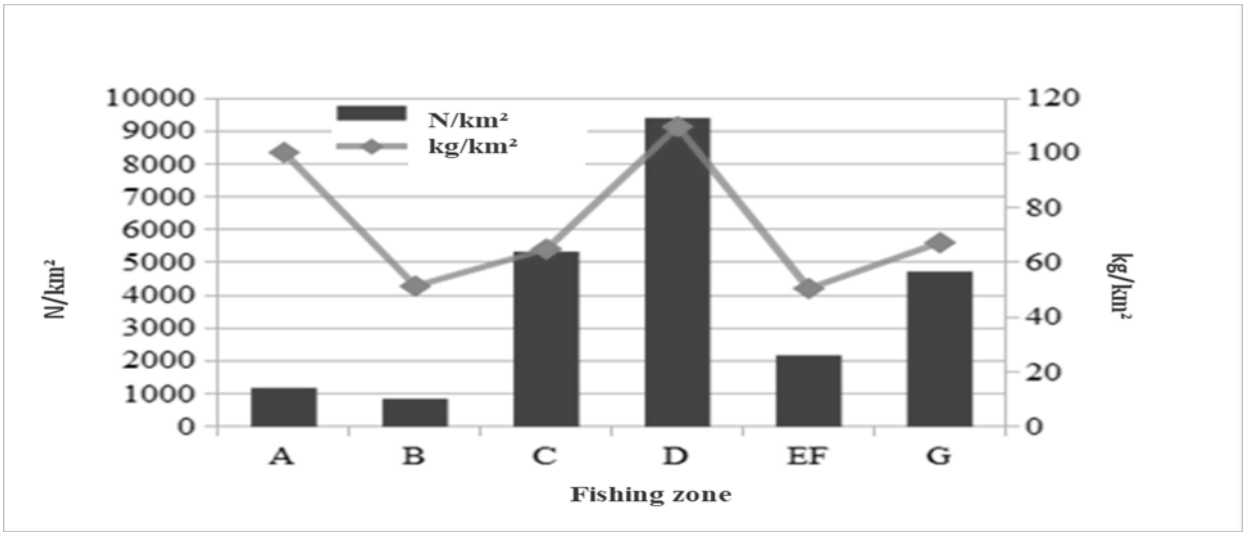

Figure 4: Distribution of cephalopods in the Croatian fishing zones expressed with abundance and biomass indices (N/km², kg/km²).

Considering only the commercially most important species, the situation is as follows: *S. officinalis* was most represented in fishing zones A (12.48 N/km²) and D (1.96 kg/km²) by number and weight, respectively. *L. vulgaris* was most represented in the fishing zone G (2382.62 N/km², 30.72 kg/km²). *I. coindetii* was most abundant in the fishing zone D (4439.59 N/km², 69.49 kg/km²). *E. moschata* had the highest abundance index in fishing zones EF (463.60 kg/km²), and the highest biomass index in the zone A (77.37 N/km²). *Eledone cirrhosa* was most abundant in the fishing zone C (84.2 N/km², 7.83 kg/km²) and *Octopus vulgaris* in the fishing zone B (27.77 N/km², 14.79 kg/km²).

Conclusion

Cephalopods are distributed in all depth strata in GSA17, and in all fishing zones in the Croatian part of the northern and central Adriatic. In the bottom trawl catches dominate commercially important species, such as *Eledone moschata* and *Illex coindetii*, but results also indicate very high abundances of some other species, e.g. *Alloteuthis media* and *T. eblanae*, which are not considered as commercially important. The obtained results may be useful in improving the management of cephalopod stocks in the Adriatic Sea by providing a better insight into their depth distribution and abundance in different fishing areas.

Acknowledgement

The data used in this study were obtained in the MEDITS expedition 2016, in the scope of the MEDITS programme. We want to express a sincere gratitude to our dear colleague Stjepan Jukić-Peladić who passed away recently. Thanks to his great engagement, in 1996 the Republic of Croatia joined this important international scientific program which continues to this day.

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Glavonošci u koćarskim lovinama sjevernog i srednjeg Jadrana

Sažetak

Ulovi glavonožaca u sjevernom i srednjem Jadranu analizirani su iz podataka prikupljenih pridnenom povlačnom mrežom koćom tijekom ekspedicije MEDITS 2016. Registrirano je ukupno 19 vrsta, a najzastupljenije su bile: *Alloteuthis media*, *Loligo vulgaris*, *Illex coindetii*, *Todaropsis eblanae* i *Eledone moschata*. Najveći broj vrsta pronađen je na dubinama 100-200 m, a najmanji na 10-50 m. Najveći prosječni indeks brojnosti glavonožaca je utvrđen u dubinskom pojasu 100-200 m. Najzastupljenija vrsta na dubinama 10-50 m bila je *L. vulgaris*, na 50-100 m i 100-200 m *A. media*, a na 200-500 m *I. coindetii*. Najveća brojnost glavonožaca zabilježena je u hrvatskim ribolovnim zonama C i D. Dobiveni rezultati mogu poslužiti kao osnova za bolje dugoročno održivo upravljanje ovom skupinom morskih beskralježnjaka.

Ključne riječi: glavonošci, Jadransko more, učestalost, rasprostranjenost, ribolovne zone

Utjecaj uvjeta skladištenja na promjene dielektričnih svojstava i pH vrijednosti mesa trlje blatarice, *Mullus barbatus*

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Sažetak

U ovom istraživanju su praćene promjene dielektričnih svojstava i pH vrijednosti kao pokazatelja svježine trlje skladištene u prikladnim i neprikladnim temperaturnim uvjetima u odnosu na promjene organoleptičkih svojstava. Elektrovodljivost je značajno brže opadala s vremenom skladištenja u neprikladnim uvjetima, dostignuvši donju razinu prihvatljivosti nakon četiri dana. Vrijednosti očitane torimetrom su u prikladnim uvjetima skladištenja treći i četvrti dan skladištenja pokazale blagi pad, ali je prema izmjerenim vrijednostima riba ostala unutar kategorije „vrlo svježa". Razina pH se tijekom pokusa u prikladnim uvjetima, nakon početnog pada od dana ulova, nije značajno mijenjala, za razliku od neprikladnog skladištenja gdje je varirala kroz dane (od 7,336 do 6,833). Promjene organoleptičkih svojstva pratile su promjene dielektričkih svojstava i pH vrijednosti koje su se pokazale validnim metodama brzo za rutinsko praćenje promjene svježine trlje skladištene na ledu.

Ključne riječi: morska riba, senzorska ocjena, kvaliteta, organoleptička svojstva, svježina ribe

Uvod

Kvarenje ribe započinje vrlo brzo nakon ulova, pogotovo kod visokih temperatura (Berkel i sur., 2004), a rezultat je enzimatske autolize, oksidacije i mikrobnog razvoja (Huss i sur., 1995). Sprječava se, odnosno usporava, na nekoliko načina: hlađenjem, smrzavanjem, sušenjem, soljenjem, dimljenjem, konzerviranjem, fermentacijom, no za bilo koji oblik konzerviranja nužno je hlađenje neposredno nakon ulova (Ghaly i sur., 2010). Niska temperatura smanjuje mikrobnu aktivnost koja uzrokuje kvarenje i tako održava kvalitetu ribe (Ashie i sur., 1996). Tehnike skladištenja trebaju spriječiti kvarenje uzrokovano mikrobnom aktivnošću, a istovremeno zadržati kvalitetu i nutritivnu vrijednost ribe (Ghaly i sur., 2010). Kvaliteta ribe, odnosno njena svježina, se često kvantificira procjenom senzorskih pokazatelja (izgled, miris, tekstura i dr.) te mjerenjem biokemijskih i fizikalnih svojstava ribe (Franceschelli i sur., 2021).

Cilj ovog rada je usporediti promjenu svježine trlje blatarice, *Mullus barbatus* (Linnaeus, 1758), kao jedne od najzastupljenijih vrsta u koćarskom ulovu na istraživanom području (Gavrilović i sur., 2022), mjerenjem dielektričnih svojstava kože, pH vrijednosti mesa i senzorskom ocjenom tijekom skladištenja u različitim teperaturnim uvjetima.

Materijal i metode

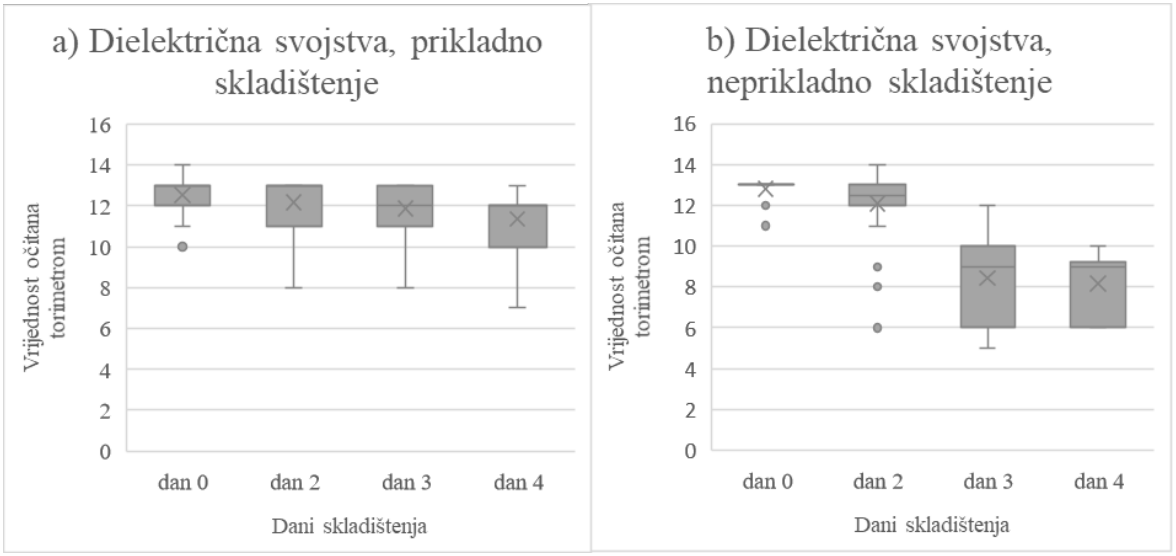
Jedinke trlje su prikupljane koćarenjem u Neretvanskom kanalu, odnosno ribolovnoj podzoni G5, tijekom svibnja i studenog 2021. godine. Sva riba je neposredno nakon ulova hlađena potapanjem u mješavinu morske vode s ledom do sortiranja. Iz ukupnog ulova, pri svakom uzorkovanju koje je obavljano, tijekom sortiranja izdvojene su po 22 jedinke trlje koje su stavljene na ljuskasti led u samodrenirajuće polistirenske kutije i pohranjene u brodski hladnjak do istovara. U prvom tretmanu (svibanj) riba je nakon istovara skladištena u prijenosnom sanduku koji zadržava hladnoću („jacera") u kojoj je led mijenjan i dodavan samo prva dva dana skladištenja (neprikladni uvjeti skladištenja). U drugom tretmanu (studeni) riba je skladištena u hladnoj komori gdje je po potrebi dodavan led (prikladni uvjeti skladištenja) kako bi se očuvala konstantna teperatura. Temperatura u sanduku kod neprikladnog

skladištenja se kretala od 5°C tijekom prva dva dana do 17°C četvrtog dana skladištenja. U drugom tretmanu, prikladnog skladištenja u hladnoj komori održavana je konstantna temperatura od 2°C. Mjerenje dielektričnih svojstava i vrijednosti pH mesa ribe te senzorska ocjena obavljeni su neposredno po početku skladištenja (kasni večernji sati na dan izlova– nulti dan) te potom drugog, trećeg i četvrtog dana skladištenja. S obzirom da je mjerenje obavljeno u noćnim satima krajem nultog dana, prvo iduće mjerenje obavljeno je drugog dana skladištenja. Dielektrična svojstva trlje su mjerena torimetrom (Distell Fish freshness meter, Model Torrymeter) na dorzalnoj bočnoj strani tijela, neposredno iza škržnog poklopca i iznad bočne pruge (Distell, 2011). Mjerenje je izvršeno na način da su se elektrode uređaja prislonile na svaku jedinku tri puta prije mjerenja iduće jedinke te je kao vrijednost eletrovodljivosti zabilježen prosjek tri očitanja. Elektrode torimetra su između svakog očitanja na istoj jedinki i između mjerenja različitih jedinki prebrisane čistim papirnatim ubrusom kako bi se smanjila moguća greška pri mjerenju. Bilježene su vrijednosti očitane torimetrom na skali od 0 do 18, pri čemu više vrijednosti označavaju svježiju ribu (Distell, 2011). Mjerenje pH vrijednosti mesa trlje je izvršeno digitalnim multiparametarskim uređajem (pH 70 Vio DHS) s ubodnom pH i temperaturnom sondom (XS Instruments). Vrijednosti su mjerene na dorzalnom mišiću ribe neposredno iza glave, na način da je tijekom svakog mjerenja sonda ubodena na drugo mjesto, odnosno ni jedno naknadno mjerenje nije provedeno na mjestu uboda prethodnog mjerenja kako bi se izbjegle greške. Paralelno su praćena organoleptička svojstva skladištene ribe. Senzorska ocjena svježine ribe obavljena je prema metodi koju su opisali Parlapani i sur. (2015), pri čemu su obučeni članovi tima ocjenjivali vanjski izgled, kožu, sluz, oči i miris ribe. Sva senzorska svojstva ocjenjivana su ocjenom od 1 do 5 prema deskriptivnoj skali, pri čemu je ocjena 5 bila za najsvježiju ribu. Riba s prosječnom ocjenom 3 smatrana je minimalno prihvatljivom za tržište, odnosno ribom čiji je rok upotrebe na granici prihvatljivosti.

Razlike u vrijednostima elektrovodljivosti i pH mesa između svih dana mjerenja unutar jednog tretmana su ispitane analizom varijance ponovljenih mjerenja (repeated measures ANOVA) (razina značajnosti 0,05). Nakon toga je proveden Tukey HSD post hoc test za višestruko uspoređivanje parova dana kako bi odredili između kojih točno dana postoji značajna razlika u mjerenjima. Razlika u vrijednostima elektrovodljivosti i pH mesa pojedinih dana skladištenja između tretmana je ispitana T-testom (razina značajnosti 0,05). Svi podaci su obrađeni u programu Microsoft Excel, a statističke analize su provedene u statističkom programu PAST 4.05 (Hammer i sur., 2001).

Rezultati i rasprava

Praćenjem dielektričnih svojstava trlje utvrđen je trend pada srednjih vrijednosti očitanih torimetrom u oba uvjeta skladištenja (Grafikon 1). Analizom varijance utvrđena je značajna razlika između dana skladištenja kod oba uvjeta skladištenja ($p<0,05$). Tukey HSD post hoc testom je zabilježen značajan pad vrijednosti između pojedinih dana skladištenja. Značajna razlika je utvrđena između dana ulova i trećeg i četvrtog dana prikladnog skladištenja te između drugog i četvrtog dana prikladnog skladištenja. Prilikom neprikladnog skladištenja, postoje značajne razlike između svih dana istraživanja osim dana ulova i drugog dana skladištenja te trećeg i četvrtog dana skladištenja (Tablica 1). T-test je pokazao da nema razlike u dielektričnim svojstvima ribe između tretmana na dan ulova i na drugi dan skladištenja dok je kasnije ta vrijednost značajno niža kod neprikladno skladištene ribe (Tablica 2).



Grafikon 1. Kretanje vrijednosti dielektričnih svojstava trlje kroz vrijeme skladištenja u prikladnim (a) i neprikladnim (b) uvjetima

Tablica 1. Dobivene p-vrijednosti Tukey HSD post hoc testa između pojedinih dana mjerenja za dielektrična svojstva u prikladnim (iznad dijagonale) i neprikladnim uvjetima skladištenja (ispod dijagonale). Statistički značajne razlike su označene s *.

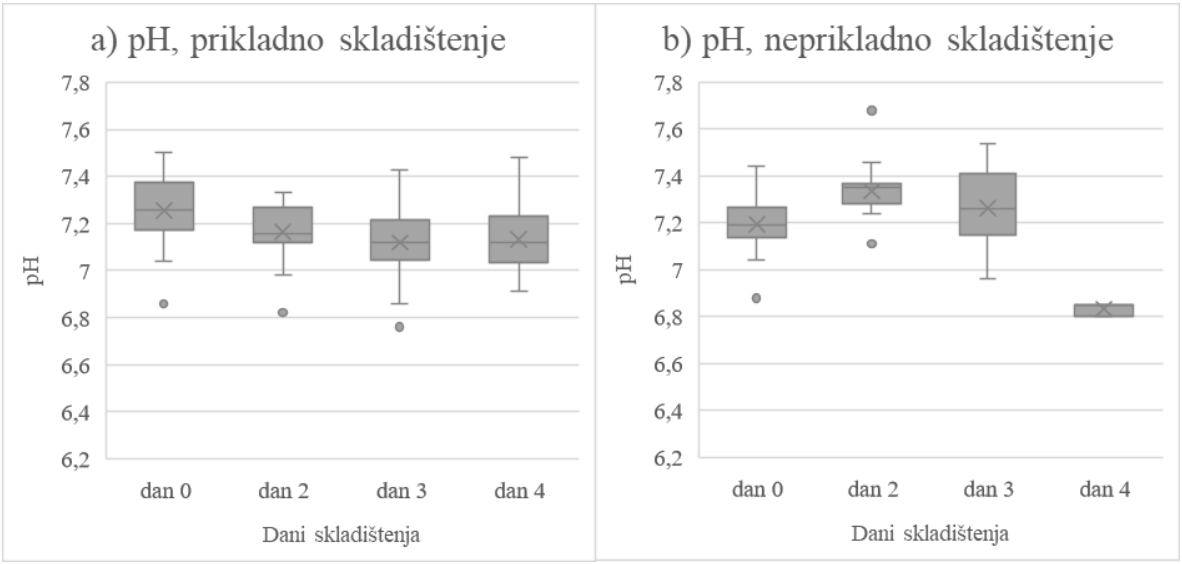
Dielektrična svojstva	Dan 0	Dan 2	Dan 3	Dan 4
Dan 0		0,345	0,011*	<0,01*
Dan 2	0,380		0,472	<0,01*
Dan 3	<0,01*	<0,01*		0,069
Dan 4	<0,01*	<0,01*	0,959	

Distell priručnik (2011) za vrste iz reda Perciformes (*Pagellus coupei*, validno *Pagellus bellottii*) navodi i kategorizira vrijednosti dielektričnih svojstava izmjerenih torimetrom na sljedeći način: 11-13 je vrlo svježa riba; 7-10 indicira pad svježine; 4-6 je gotovo pokvarena riba. Prema ovom priručniku vrijednosti ispod 4 označavaju nejestivu ribu. Tijekom istraživanja je prosječna vrijednost dielektričnih svojstava na dan ulova u oba tretmana iznosila više od 12 i do četvrtog dana je pala na 11,364 u prikladnim uvjetima te na 8,111 u neprikladnim uvjetima. Ove vrijednosti pokazuju da je tijekom prikladnog skladištenja trlja ostala vrlo svježa i nakon četiri dana, dok je u neprikladnim uvjetima svježina pala blizu donje granice prihvatljivosti.

Tablica 2. Srednje vrijednosti izmjerenih dielektričnih svojstava i pH tijekom trajanja pokusa (T-test). Statistički značajne razlike su označene s *.

	Dan 0		Dan 2		Dan 3		Dan 4	
	Diel. svojstva	pH	Diel. svojstva	pH	Diel. svojstva	pH	Diel. svojstva	pH
Prikladno skladištenje	12,530	7,256	12,182	7,165*	11,879*	7,121*	11,364*	7,132*
Neprikladno skladištenje	12,820	7,195	12,091	7,336*	8,439*	7,265*	8,111*	6,833*

Analizom varijance je utvrđena statistički značajna razlika izmjerenih pH vrijednosti između dana skladištenja kod oba tretmana ($p<0,05$). Tukey HSD post hoc test je pokazao da ta značajna razlika postoji između dana ulova i svih dana mjerenja kod prikladnog skladištenja. Pri neprikladnom skladištenju, pH vrijednost je značajno viša drugi dan skladištenja u odnosu na dan ulova, te onda značajno pada u četvrtom danu skladištenja (Grafikon 2, Tablica 3). Razlika vrijednosti pH između tretmana je provjerena T-testom i bila je značajna kroz sva tri dana skladištenja: drugi i treći dan je vrijednost pH bila značajno veća, a četvrti dan značajno manja u uvjetima neprikladnog skladištenja (Tablica 2).

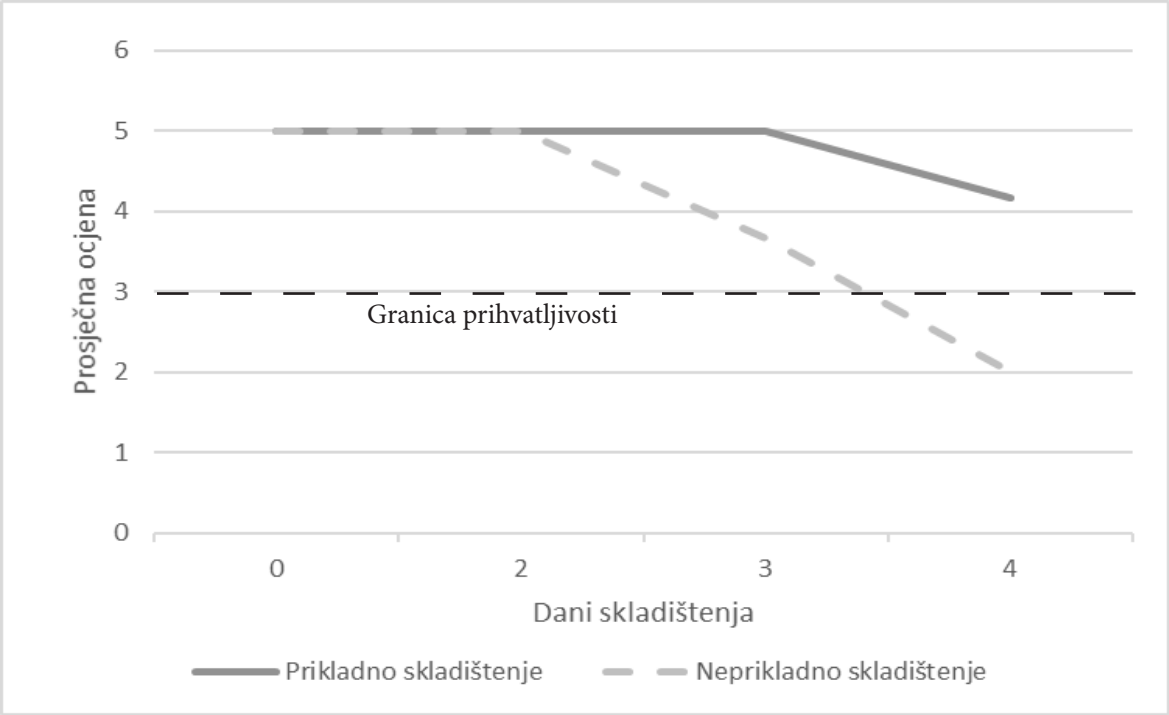


Grafikon 2. Kretanje pH vrijednosti trlje kroz vrijeme skladištenja u prikladnim (a) i neprikladnim (b) uvjetima

Tablica 3. Dobivene p-vrijednosti Tukey HSD post hoc testa između pojedinih dana mjerenja za pH u prikladnim (iznad dijagonale) i neprikladnim uvjetima skladištenja (ispod dijagonale). Statistički značajne razlike su označene s *.

pH	Dan 0	Dan 2	Dan 3	Dan 4
Dan 0		<0,01*	<0,01*	<0,01*
Dan 2	0,011*		0,161	0,401
Dan 3	0,362	0,367		0,949
Dan 4	<0,01*	<0,01*	<0,01*	

Senzorska svojstva su također pokazala karakteristične negativne promjene nakon četiri dana neprikladnog skladištenja: neugodan miris, zamućena sluz, pucanje kože na trbušnom području, mutne oči. Po dobivenoj ocjeni ta riba više nije bila prihvatljiva za tržište. Na prikladno skladištenoj ribi takve negativne promjene nisu uočene (Grafikon 3).



Grafikon 3. Promjena senzorskih svojstava tijekom skladištenja u oba tretmana

Kretanja torimetrijskih vrijednosti su slična onima kakve su zabilježili Šimat i sur. (2009) u istraživanju utjecaja skladištenja na dielektrična svojstva lubina, *Dicentrarchus labrax*. Pri niskim temperaturama su dielektrička svojstva i senzorske osobine kroz dulje razdoblje upućivala na visoku kvalitetu ribe, dok je pri višim temperaturama kvaliteta naglo opadala. Ustanovili su i da torimetar može pokazivati prihvatljivu kvalitetu ribe čak i kad po senzorskim osobinama riba padne ispod granice prihvatljivosti, što je slično neprikladno skladištenim trljama u ovom istraživanju. Kod ulovljene i skladištene ribe vrijednost pH prvo pada zbog stvaranja mliječne kiseline usred stresa, zatim neko vrijeme stagnira i onda s autolitičkim procesima raste, što pogoduje umnožavanju bakterija i vodi ka kvarenju (Daskalova 2019; Kyrana i sur., 1997; Abbas i sur., 2008). Viša vrijednost pH u drugom i trećem danu kod neprikladno skladištene ribe može upućivati na početnu fazu kvarenja. Nagli pad pH u posljednjem danu mjerenja pokazuje nastanak kiselih raspadnih produkata, odnosno posljedica je posljedica raspadanja unutarnjih organa, odnosno probavnog trakta i istjecanja želučanog sadržaja što je utjecalo na pH cijele ribe. Vrijednost pH kod prikladno skladištene ribe je pala nakon dana ulova i zatim stagnirala kroz cijelo razdoblje skladištenja, što znači da je očuvana svježina ribe i u tom razdoblju nije započeo proces kvarenja.

Zaključak

Praćenjem organoleptičkih, dielektričnih svojstava i vrijednosti pH je utvđeno da primjereno pothlađena skladištena svježa riba zadržava odgovarajuću kvalitetu kroz razdoblje od najmanje četiri dana (razdoblje skladištenja ribe u ovom istraživanju). Kod neprikladno skladištene ribe zabilježene su izraženije promjene u praćenim parametrima, koji su bili u skladu sa senzorskim pokazateljima kvalitete. To potvrđuje da metode mjerenje promjene dielektričkih svojstava i pH vrijednosti mogu biti korištene za brzu rutinsku ocjenu svježine ribe na tržištu.

Napomena

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Changes in flesh dieletric properties and pH values of red mullet, *Mullus barbatus*, under different storage conditions

Abstract

In this study, the values of dielectric properties and pH were monitored as indicators of red mullet freshness and compared with the organoleptic properties of the fish under suitable and unsuitable storage conditions. The conductivity of the fish decreased much faster under unsuitable conditions, reaching the lower acceptable value after four days. The same conductivity value decreased slightly under suitable conditions on the third and fourth day, but still remained in the "very fresh" category. Similarly, pH remained constant after an initial decrease on the day of capture under appropriate storage conditions, but fluctuated for several days under improper storage conditions (from 7.336 to 6.833). The readings for both parameters changed in accordance with changes in the organoleptic properties of the fish. Monitoring of dielectric properties and pH proved to be a reliable method for routine evaluation of freshness of red mullet stored on ice.

Keywords: marine fish, sensory evaluation, quality, organoleptic properties, fish freshness

Presence of different life stages of European eel (*Anguilla anguilla* Linnaeus, 1758) in the catch at the mouth of river Neretva

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Abstract

The aim of this study was to determine the presence of sedentary yellow and migrating silver eels in the catch at the mouth of river Neretva in different seasons, describe their basic morphological characteristics, and to compare the results with the available literature. Samples were collected in the spring (March and May), summer (June and July), and Autumn (September and November) of 2021, using two traditional fishing tools – eel traps with a mesh size 12 mm and fyke nets with a mesh size 24 mm. The life stage (yellow or silver eel) was determined using quantitative and qualitative criteria. A total of 61 specimens were caught, 13 of which were silver, and 48 were yellow eels. Yellow eels were present in the catch during the entire sampling period: 21 specimens were caught in the spring, 13 in the summer, and 14 in the autumn. All 13 specimens of silver eels were recorded in autumn when they usually start the spawning migration towards the Sargasso sea. The average length of silver eels was 47.18±11.69 cm and the average weight was 235.08±235.12 g. The mean length and weight of yellow eels were the smallest in the summer (38.31±5.32 cm and 101.00±56.79 g) and the largest in the spring (41.43±5.91 cm and 129.90±69.20 g). The importance of continuous monitoring of the eel population is emphasized, including the determination of relevant biological characteristics such as age and growth, and more detailed determination of the escapement rate of silver eels.

Keywords: European eel, Neretva, Eastern Adriatic sea, Eel monitoring

Introduction

European eel (*Anguilla anguilla* L.) is a catadromous species inhabiting the eastern Atlantic from Scandinavia to Morocco including the Mediterranean, Adriatic, and the Black sea with the connected freshwater bodies (Deelder, 1984; Rochard and Elie, 1994). It presented the target species in European fisheries since olden times throughout its distribution (Kettle et al., 2008; Gabriel and Wendt, 2003; Koch, 1925; Van Dam, 1998). Modernization of fishing tools, poor control, increase in fishing pressure, environmental changes, loss of habitat, disease, dam construction, and pollution led to a rapid decline in its populations (ICES, 2020). Consequently, recruitment decreased by roughly 95% (Wgeel, 2008). This caused the eel to be listed in the IUCN Red List as Critically Endangered since 2008 (ICES, 2020).

In the eastern Adriatic, the river Neretva and its delta are very well known for eel fishing. In the 1930s, total annual eel landings in Croatia were between 62 and 93 metric tons (Morović, 1948), and in 2020 reported landings dropped to only 388kg (EUROSTAT, 2022). Yellow sedentary and silver migratory are two life stages of eels that are commonly fished in the river Neretva (Morović, 1965; Glamuzina et al., 2022). Today, there are only 13 legal fishing areas for silver eel in the autumn, while the yellow eel is fished for by the local communities with traditional fishing tools (Glamuzina et al., 2022).

The establishment of continuous monitoring to improve local management plans is emphasized in order to restore abundance of European eel (Feunteun 2002). Spatial and temporal movements of different life stages and monitoring of silver migratory eels have been in focus for over 20 years (e.g. Lambert and Feunteun 1998; Acou et al., 2005; Barry et al., 2016; Verhelst et al., 2022), but are still missing in some localities. The transition from yellow to silver eel

involves a series of gradual morphological and functional changes in order to prepare the eels for seaward migration (Durif et al., 2000). These changes include enlargement of the eyes (Pankhurst, 1982), differentiation of the lateral line (Zacchei and Tavolaro, 1988), change in integument color and structure (Fontaine, 1994), increase in fat content (Larsson et al., 1990), etc. The use of only one of the characters is seldom satisfactory for classifying the individuals as completely yellow or silver, as some individuals do not clearly fit the two groups (Feunteun et al., 2000). Therefore, methods have been developed to distinguish yellow from silver eels, by examining a combination of the above characteristics (e.g. Cottrill, 2002; Acou et al., 2005; Durif et al., 2005). The aim of this study was to determine the presence of sedentary yellow and migrating silver eels in the catch at the mouth of river Neretva in different seasons, describe their basic characteristics, and compare the results with the available literature.

Material and methods

Samples were collected at the mouth of river Neretva in the south Adriatic. Two traditional fishing tools were used – eel traps with mesh size 12 mm and fyke nets with mesh size 24 mm, in the spring (March and May), Summer (June and July), and Autumn (September and November) of 2021. The eels that were caught were weighed to the nearest gram, total length was measured to the nearest millimeter, and eye height and eye width were measured to the nearest 0.1 millimeters. The coloration of the skin and differentiation of the lateral line were recorded according to Acou et al. (2005).

The life stage was determined by qualitative (skin coloration and lateral line differentiation) and quantitative criteria (ocular index) according to Acou et al. (2005). The ocular index was computed using the formula (Pankhurst, 1982): $OI=\{[(A_R+B_R)/4)]\times[(A_L+B_L)/4]\times\pi/TL\}\times100$, where OI is the ocular index, A and B are the horizontal and vertical eye diameter, TL is the total body length and R and L are the right and left eye diameters.

Threshold values of OI for determining the life stage were set to 6.5 (Pankhurst, 1982) instead of 8.0 (Marchelidon et al. 1999, Acou et al. 2003) because three different criteria were used for the identification of silver stage eels. The number of yellow and silver eels in different seasons during the sampling was reported. Statistical differences in weight and total length were determined by ANOVA test.

Results and discussion

A total of 61 specimens of European eel were caught at the mouth of Neretva river, 13 (21%) of which had an OI above the threshold of 6.5 (Fig. 1). Eye enlargement is one of the first changes to occur during the metamorphosis, with contrasting skin coloration and lateral line differentiation occurring later (Durif et al., 2000; Han et al., 2003). Although it is difficult to determine the exact order of silvering stages (Acou et al., 2005), three specimens of yellow eels with the OI over 5.0 and one with the OI over 6.0 were recorded in June, which is approximately the period in which the silvering process usually begins (Fontaine, 1994). This could indicate that the metamorphosis should have started around the time they were captured.

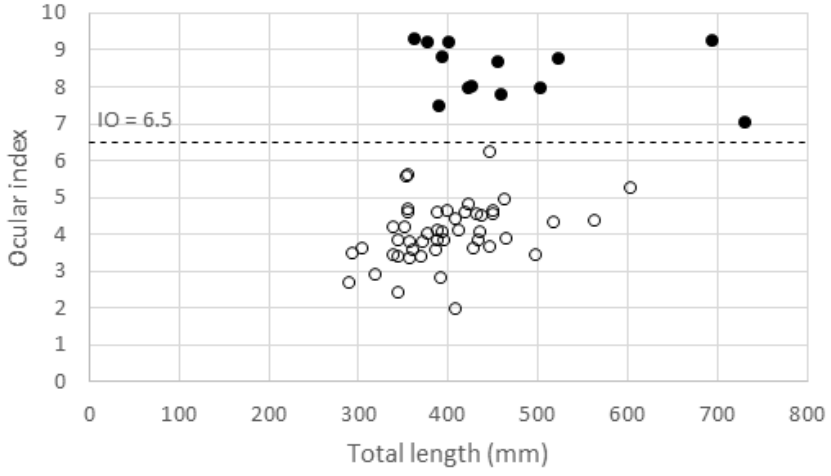

Figure 1. Values of the ocular index of yellow (empty circles) and silver eels (black circles) with the threshold line set at 6.5 (Pankhurst, 1982).

All eels that had an OI greater than a threshold value of 6.5 had contrast coloring on dorsal and ventral sides and a differentiated lateral line (Fig. 2) making them fully differentiated silver eels. No yellow eels that had a differentiated lateral line or contrast coloration were found. In research conducted on three rivers in France, Frémur, Oir, and Loire, Acou et al. (2005) also found that among yellow eels the most numerous were specimens without any silvering criteria (OI, lateral line differentiation, or contrast pigmentation). The absence of yellow eels with at least one of the silvering criteria present in this research could be related to the sampling location and sampling period. Further research is needed to understand the extent to which silver eels in the Neretva delta are descended from yellow eels living in the estuary and inland yellow eels coming from freshwaters.

Figure 2. Silver eel caught at the mouth of river Neretva with visible contrast coloring and a differentiated lateral line.

Yellow eels were present in the catch during the entire sampling period: 21 specimens were caught in spring, 13 in the summer, and 14 in the autumn. All 13 specimens of silver eels were recorded in autumn, in November. This is consistent with the literature as it is the time of migration of silver eels toward the Sargasso sea (Deelder, 1984). Morović (1955) claimed that in the lower flow of river Neretva the autumn catch consisted exclusively of silver eels in the years 1943, 1946, and 1947. Glamuzina et al. (2022) reported the presence of only a small percentage (9.8-16.4%) of silver eels in the autumn/winter catch during 2016-2019, with the dominance of the yellow eels throughout the year in Baćinska lakes and the Parila lagoon, also located at the mouth of river Neretva. Changes in presence of different life stages in the aforementioned area are impossible to trace due to an approximately 70-year gap in available data, but a decrease in the share of silver eels in the autumn catch can be noted.

Righton and Metcalfe (2011) hypothesized that the silvering of the eels can be a reversible process. Svedäng and Wickström (1997) suggested that the possible reason for temporary arrest or regression of the maturation process is insufficient fat accumulation during the yellow eel feeding stage. The feeding habits of eel in the river Neretva and its delta are poorly documented but it is generally considered that they mostly prey upon benthic fauna and small fish (Ali, 2017). Reduced food availability is possible due to a poor state of benthic and fish communities documented in the Parila lagoon (Prusina et al., 2017) accompanied by increased competition for food following recent changes in species composition (Glamuzina et al., 2021). Anyway, for validation of this claim further research is needed to determine the overlap in the diets of the eel and newly arrived species.

The mean length and weight of yellow eels were the smallest in summer (38.31±5.32 cm and 101.00±56.79 g) and the largest in spring (41.43±5.91 cm and 129.90±69.20 g) (Table 1). These results agree with the findings of Glamuzina et al. (2022), although the authors did not specify the life stage. Silver eels had statistically significant higher length and weight compared to yellow eels caught in summer and autumn determined by ANOVA test ($p<0.05$). Morović (1955) states that silver eels analyzed during sampling in 1943, 1946, and 1947 had a mean length of 49-56 cm and a mean weight of 206-266 g which is slightly higher than those in this research. Glamuzina et al. (2022) found that the most abundant size classes of all eels caught were between 30 and 45 cm or 50-150 g which coincides with the findings of this paper. Large differences in the size of silver eels in this paper were noticed, ranging from 36.2 to 73.0 cm (73 to 861 g). It is still not exactly known why some eels reach the silver stage at a smaller size (Righton and Metcalfe, 2011), but it is taught that males usually reach it at a younger age (Rossi and Collombo, 1976). Also, some

large specimens (over 4 kg) were recorded, which allegedly never sexually matured and have remained in the yellow stage (Morović, 1973).

Table 1. Length and weight values of yellow and silver eels during the sampling period (Min-minimal value, Max-maximal value, Mean-average value, SD- standard deviation)

Season	Life stage	Number of specimens (n)	Total length (cm)			Weight (g)		
			Min	Max	Mean ± SD	Min	Max	Mean ± SD
Spring		21	34.4	60.3	41.43±5.91	57	394	129.90±69.20
Summer	Yellow eel	13	29.4	51.8	38.31±5.32	47	267	101.00±56.79
Autumn		14	29.1	56.4	39.21±7.11	41	289	105.93±62.28
	Silver eel	13	36.2	73.0	47.18±11.69	73	861	235.08±235.12

Another factor that needs to be taken into account is that the Neretva delta periodically undergoes the process of salinization, especially during the summer when saltwater can protrude up to 25 kilometers upstream (Ljubenkov and Vranješ, 2012). This phenomenon creates a large transitional area between freshwater and seawater (Glamuzina and Dobroslavić, 2020). Salinity and oxygen saturation can influence the sex ratio of eels in different environments (Morović, 1957). Because females grow faster and undergo metamorphosis later (Bauchot, 1986), the sex ratio can affect the size and proportion of yellow and silver eels. Other factors that can influence growth, maturation, and escapement are the presence of parasites, water pollution, and diseases (Quadroni et al., 2013), which have all been documented at the studied area (e.g. Mateljak and Matić, 2011; Radočaj et al., 2022).

In order to investigate the potential changes in the presence of different life stages of eel at the mouth of the river Neretva, it is necessary to establish continuous monitoring and cooperation with local fishermen. Also, the need for a description of relevant biological characteristics such as age and growth and determination of the escape rate of silver eels for the investigated locality is highlighted.

Conclusions

Yellow European eel was present in the catch at the mouth of the Neretva river in spring, summer, and autumn, while silver eels were present only during the autumn of 2021. All collected silver eels were found to be fully differentiated by qualitative (skin coloration and lateral line differentiation) and qualitative (ocular index) criteria. Yellow eels did not show any changes in the aforementioned criteria, which means that no specimens in transition between stages were found. Silver eels had statistically significant higher values of length and weight compared to yellow eels caught in summer and autumn.

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Fizikalno-kemijska svojstva meda od divlje trešnje (*Prunus avium* L.)

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Sažetak

Divlja trešnja (*Prunus avium* L.) najznačajnije je listopadno stablo iz porodice Rosaceae. Budući da u povoljnim vremenskim uvjetima izlučuje značajne količine nektara zanimljiva je pčelinja paša. Cilj je ovoga rada bio odrediti fizikalno-kemijska svojstva meda od divlje trešnje. Istraživanje je provedeno na pet uzoraka meda prikupljenog 2022. godine s područja Karlovačke županije. Prosječna vrijednost električne provodnosti iznosila je 0,89 mS/cm, a HMF-a 1,59 mg/kg. Udio fruktoze prosječno je iznosio 37,58%, a glukoze 28,03%, dok je njihov omjer bio 1,34. Na osnovu boje ovaj med pripada u skupinu svjetlijih vrsta s 53,7 mm Pfundove ljestvice. Ovo preliminarno istraživanje prikazuje vrijednosti odabranih fizikalno-kemijskih parametera meda od divlje trešnje. Međutim potrebna su daljnja istraživanja koristeći dodatne analitičke metode kako bi se dobio potpuni fizikalno-kemijski profil.

Ključne riječi: divlja trešnja, fizikalno-kemijska svojstva, med

Uvod

Divlja trešnja (*Prunus avium* L.) najznačajnije je listopadno stablo iz porodice Rosaceae, a rasprostranjena je po brdskim bjelogoričnim šumama na području Europe, zapadne Azije i sjeverne Afrike. Brzorastuća je vrsta koja uglavnom raste na osami i šumskim rubovima, a rjeđe se javlja u gustim mješovitim sastojinama (Santi i sur., 1998., Popović i Kerkez, 2016.). Njezina cvatnja se odvija krajem ožujka ili početkom travnja, ovisno o nadmorskoj visini, a u povoljnim vremenskim uvjetima izlučuje značajne količine nektara. Cvjetovi su dvospolni, jednodomni, pravilni, promjera 2,5-3,5 cm, skupljeni u štitaste cvatove (Slika 1.a). Dvostrukog su ocvijeća, čaška je građena od pet duguljastih, unazad povinutih lapova cjelovitog ruba, vjenčić je sačinjen od pet obrnuto jajastih, bijelih latica veličine 1-1,5 cm. Tučak ima dva sjemena zametka, prašnici su mnogobrojni sa žutim prašnicama i bijelim prašničkim nitima. Cvatnja traje oko deset dana (Kremer i Krušić Tomaić, 2015.). Primjenom suvremenih tehnoloških radnji u pčelarstvu, a jedna od njih je i korištenje polunastavaka kao medišnog prostora, kod košnica nastavljača omogućava dobivanje uniflornih vrsta meda koje do sada nisu bile prepoznate upravo iz razloga što pčele na okvirima s većom površinom saća miješaju nektar većeg broja različitih biljnih vrsta te je na taj način teže postići uniflornost. To se posebice očituje kod biljnih vrsta koje nemaju jak medonosni potencijal. Najvažniji uvjeti za uspješno pčelarenje osim jakih pčelinjih zajednica su dobre pašne prilike, pravilni razmještaj pčelinjih zajednica na paši te pravovremena primjena tehnologije. Fizikalno-kemijska i senzorska svojstva meda (nektarni med ili medun/medljikovac) koriste se za određivanje njegove kvalitete, a na njih uvelike utječu vrsta nektara, tip tla, klimatski uvjeti te postupci prije i nakon vrcanja meda (Persano Oddo i sur., 1995; Gomes i sur., 2010.). Iako je divlja trešnja izvorna biljna vrsta na europskim prostorima njezin med još uvijek nije prepoznat kao unifloran, te o njemu nema dostupnih znanstvenih podataka. Stoga je cilj ovog preliminarnog istraživanja bio utvrditi odabrana fizikalno-kemijska svojstva meda od divlje trešnje i na taj način dobiti prva saznanja o ovom medu.

Materijal i metode

U ovom istraživanju analizirano je ukupno pet uzoraka meda od divlje trešnje (Slika 1.b) Uzorci meda prikupljeni su tijekom travnja 2022. godine od lokalnih pčelara s područja Karlovačke županije čije su pčelinje zajednice (*Apis mellifera carnica* Pollman, 1879.) bile smještene u brdskim područjima na kojima se nalaze značajne sastojine divlje

trešnje. Na početku cvatnje na svakoj je lokaciji dodan okvir sa satnom osnovom u odabrane pčelinje zajednice kako bi se dobio što uniflorniji uzorak meda. Nakon prikupljanja, uzorci su bili pohranjeni u staklenkama i držani na tamnom mjestu na 4°C do daljnjih analiza. Određivanjem botaničkog podrijetla analiziranih uzoraka meda prema metodi Von der Ohe i sur. (2004) potvrđena je njegova uniflornost (> 45% peludnih zrnaca divlje trešnje).

Slika 1. a) Divlja trešnja (Prunus avium L.); b) med od divlje trešnje

Fizikalno-kemijski parametri određeni su u skladu s Pravilnikom o kakvoći uniflornog meda (2009) i Pravilnikom o medu (2015., 2017.). Sadržaj vode određen je refraktometrijskom metodom uz pomoć prijenosnog refraktometra Mettler Toledo (Refracto 30 PX) na 20 °C, dok je električna provodnost utvrđena uz pomoć prijenosnog konduktometra Mettler Toledo (EL3) u 20 % vodenoj otopini meda na 20 °C, pri čemu se 20 % odnosi na suhu tvar meda. Sadržaj hidroksimetilfurfurala (HMF) određen je metodom po Whiteu pomoću Shimadzu (Kyoto, Japan) spektrofotometra (UV-1800). Mjerenja apsorpcije otopina meda određena su na 284 nm i 336 nm, a rezultati su izraženi u mg/kg meda. Analiza ugljikohidrata provedena je na uređaju za visokotlačnu tekućinsku kromatografiju (HPLC) (Shimadzu Corp., Kyoto, Japan) koji se sastoji od detektora indeksa loma (RID-10A), HPLC kolone (Agilent Zorbax NH_2, 4,6x250 mm, veličina čestica 5 μm), kvartarne pumpe (LC-20AD), pećnice (CTO-20AC) te autosamplera (SIL-10AF). Identifikacija i kvantifikacija je provedena pomoću software-a (LabSolution Lite Version 5.52) koji kroz usporedbu vremena zadržavanja i površina pikova ugljikohidrata iz meda i standarda uglijkohidrata izračunava konačne koncentracije koje su izražene u %. Za određivanje boje meda korišten je Lovibond Honey Color-Pod, a rezultati su izraženi u mm Pfund ljestvice. Podatci o fizikalno-kemijskim parametrima analizirani su deskriptivnom statistikom pomoću programa Microsoft Office Excel 2018.

Rezultati i rasprava

Rezultati odabranih fizikalno-kemijskih svojstava meda od divlje trešnje prikazani su u Tablici 1. Sadržaj vode je jedan od najvažnijih parametara kvalitete meda, a njezin udio u istraživanim uzorcima kretao se od 16,4% do maksimalnih 18,5% te je u prosjeku iznosio 17,8%. Ovaj parametar u medu uvelike ovisi o tehnologiji pčelarenja, tipu košnice, snazi pčelinje zajednice i vremenu vrcanja meda. S druge strane, sadržaj vode može se razlikovati između meda ovisno o izvoru (nektar/medna rosa) ili vremenskim uvjetima na području u kojem se med proizvodi. Stoga se vrijednosti ovog parametra mogu mijenjati unutar sezone, ali i iz godine u godinu (Fallico i sur., 2004; Majewska i sur., 2019.). Električna provodnost je fizikalno-kemijski parametar koji je u korelaciji s botaničkim podrijetlom meda i koristi se za identifikaciju meda u kombinaciji s melisopalinološkom (peludnom) analizom. Izravno je povezana sa sadržajem minerala i organskim kiselinama u medu (Habib i sur., 2014; Da Silva i sur., 2016.).

Tablica 1. Deskriptivna statistika fizikalno-kemijskih svojstava meda od divlje trešnje

Uzorak	Voda (%)	El.provodnost (mS/cm)	HMF (mg/kg)	Fruktoza (%)	Glukoza (%)	F/G	Boja (mm Pfund)
1	16,4	0,77	0,45	37,99	27,83	1,37	55,0
2	18,2	0,77	2,77	37,68	28,29	1,33	42,5
3	18,0	1,06	0,00	38,85	28,64	1,36	63,0
4	18,0	0,85	1,43	36,77	27,53	1,34	52,0
5	18,5	0,99	3,29	36,59	27,85	1,31	56,0
Prosjek	17,8	0,89	1,59	37,58	28,03	1,34	53,7
Min.	16,4	0,77	0,00	36,59	27,53	1,31	42,5
Maks.	18,5	1,06	3,29	38,85	28,64	1,37	63,0
SD	0,82	0,13	1,43	0,93	0,44	0,02	7,45

**SD -standardna devijacija*

Vrijednosti električne provodnosti kod meda od divlje trešnje kretale su se od 0,77 do 1,06 mS/cm, a u prosjeku je iznosila 0,89 mS/cm. Na osnovu dobivenih rezultata vidljivo je da med od divlje trešnje ima višu prosječnu električnu provodnost od 0,80 mS/cm, što je prema međunarodnim standardima granica razlučivanja između nektarnog meda i meduna. Stoga se med od divlje trešnje može svrstati u skupinu nektarnih vrsta meda kod kojih vrijednosti električne provodnosti osciliraju, tj. mogu prelaziti iznad 0,80 mS/cm, kao što je med od planike (*Arbutus unedo*), vrijesa (*Erica* spp.), lipe (*Tilia* spp.) i vrijeska (*Calluna vulgaris*) (Pravilnik o medu, 2015.). Hidroksimetilfurfural (HMF) jedan je od najvažnijih parametara za određivanje svježine meda (Ruoff i sur., 2007.; Zhu i sur., 2020.), kao i trajanja i uvjeta skladištenja te ima tendenciju rasta tijekom procesa zagrijavanja (dekristalizacije) meda i/ili njegovim dugotrajnim skladištenjem. U analiziranim uzorcima udio HMF-a se kretao od 0,00 do 3,29 mg/kg s prosječnom vrijednosti od 1,59 mg/kg. HMF je prirodno prisutan u svježem medu u niskim udjelima, no njegov udio ovisi i o vrsti meda, njegovoj pH vrijednosti, udjelu kiselina i vlage te izloženosti svjetlu. S obzirom na sadržaj glavnih šećera, udio fruktoze prosječno je iznosio 37,58%, a glukoze 28,03%, dok je njihov omjer bio 1,34. Na osnovu boje, med od divlje trešnje pripada skupini svjetlijih vrsta meda (svijetlo jantarna) s prosječno 53,7 mm Pfunda (42,5 mm - 63,0 mm).

Zaključak

Temeljem rezultata preliminarnog istraživanja odabranih fizikalno-kemijskih parametara meda od divlje trešnje, isti se zbog prosječno više električne provodnosti od 0,89 mS/cm svrstava u skupinu uniflornih vrsta meda iako je nektarnog podrijetla, a prelazi granicu razlučivosti između nektarnog meda i medljikovca koja iznosi 0,80 mS/cm. Vrijednost HMF-a prosječno je iznosila 1,29 mg/kg. Prema boji med od divlje trešnje svrstava se u skupinu svjetlijih vrsta meda (svijetlo jantarni). Udio fruktoze prosječno je iznosio 37,58 %, a glukoze 28,03%, dok je njihov omjer bio 1,34. Daljnjim je istraživanjima potrebno na većem broju uzoraka upotpuniti podatke o fizikalno-kemijskim svojstvima ove još uvijek nedovoljno istražene i raritetne vrste meda.

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Physicochemical properties of wild cherry honey (*Prunus avium* L.)

Abstract

The wild cherry (*Prunus avium* L.) is the most important deciduous tree of the Rosaceae family. Since it secretes considerable amounts of nectar in favorable weather conditions, it is an interesting honey bee forage. The aim of this study was to determine the physicochemical properties of wild cherry honey. The study was conducted on five honey samples collected during April 2022 in Karlovac County. The average value of electrical conductivity was 0.89 mS/cm, and HMF content was 1.59 mg/kg. The average fructose content was 37.58% and glucose 28.03%, while their ratio was 1.34. Based on the color, this honey belongs to the group of lighter honey types with 53.7 mm Pfund scale. This preliminary study revealed the data on selected physicochemical parameters of wild cherry honey, but further studies using additional analytical methods are required to obtain a complete physicochemical profile of this honeytype.

Keywords: wild cherry, physicochemical properties, honey

A review of the studies on bluefin tuna in the eastern Adriatic Sea

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Abstract

The aim of this paper is to provide a review of studies related to bluefin tuna (BFT) made in the eastern Adriatic Sea. BFT is an important food source and early studies were mostly related to fishing. BFT has wide spatial distribution, and for management purpose the International Commission for Conservation of Atlantic Tunas (ICCAT) was established in late 1960s. After the development of BFT farming, many studies were made on BFT in captivity. Croatian participation in ICCAT enabled participation of Adriatic scientists in common BFT studies on wide regional scale and stimulated local BFT research in the Adriatic Sea such as studies on fish parasites, molecular & genetic and nutritional studies.

Keywords: Bluefin tuna, studies, fishing, biology, Adriatic Sea

Introduction

Bluefin tuna (BFT), *Thunnus thynnus*, L., is one of the large pelagic fishes that have been the focus of interest of fishermen and researchers in the Mediterranean, especially in the Adriatic Sea, for decades due to their exceptional biology and economic value. Tuna farming brought dramatic changes in the fishing strategies as the majority of fish caught are transfered to rearing cages for further breading rather than landed and/or sold directly. Such a practice also had an impact on stock management. In this review some specific information on fishing and farming practice with its socio-economic and environmental impacts are discussed with special emphasises on Adriatic environment. Considering the long tradition of fishing for BFT and the great importance of this species for commercial fishing and farming in the eastern part of the Adriatic Sea, the Republic of Croatia has simbolicaly presented the silhouette of this amazing fish on its coins of 2 HRK (Fig. 1).

Figure 1. Croatian coin of 2 HRK with silhouette of bluefin tuna.

Material and methods

In this paper, the authors describe the historical work of fisheries experts on the eastern Adriatic coast and review the most of available information on various aspects related to bluefin tuna fishing practices, its biology, ecology, management and farming. A large number of older references, describes the great importance of BFT for local fishing society and scientific communities. Recent PhD Thesis and studies on BFT, published in collaboration with scientists from other regions, are reviewed also and continued interest and research efforts of scientists in the eastern Adriatic dedicated to the study of BFT are documented.

Results and discussion

Studies on bluefin tuna in 19th and at the beginning of the 20th century

A significant amount of knowledge concerning BFT life history and tuna fishing in the eastern Adriatic Sea by the ancients does exist. Some biological studies of the sea fauna and the fisheries of the Adriatic, during Austro-Hungarian presence, were published by foreign scientists (Faber 1883). Many important scientific documents dealing with BFT trap fishery as the most ancient industrial acitvity in the fisheries of Adriatic were updated by Di Natale (2018). Concerning the trap fishery activities along the north-eastern Adriatic, Kirsch (1900) provided very useful information on how the trap fishery operated between the XIX and the XX centuries, with a detailed description of methods and techniques applied (Fig. 2). Due to geomorphological characteristics of the north-eastern Adriatic rocky coast (e.g. Kvarner and Rijeka Bay, Velebit channel, Cres and Krk islands) tuna's migration occured along the coast and more than 75% of tuna catches in 10-year period before II Wold War were obtained by trap fishery in this area. At the beginning of II World War 28 active tuna traps operated in this area, while after the war the number of active tuna traps decreased (Basioli, 1962).

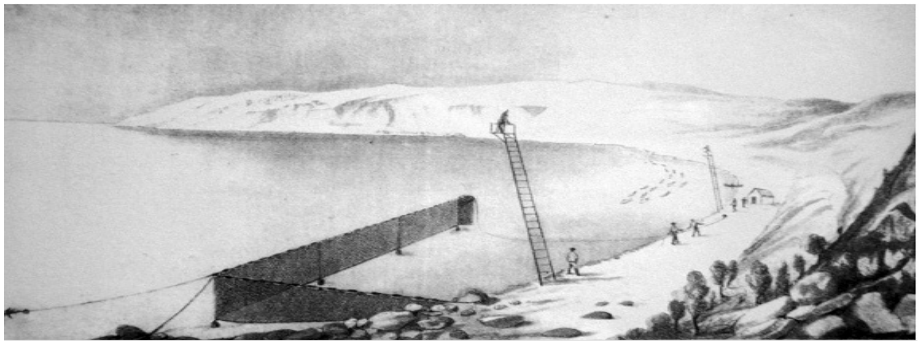

Figure 2. The coastal tuna trap fishery along the north-eastern Adriatic between the second part of the XIX century and the first part of the XX century (Kirsch, 1900).

One of the first Croatian fisheries scientists to devote part of his work at the Institute of Oceanography and Fisheries in Split (IOR) before World War II, describing the BFT fishing on the eastern Adriatic coast, was Tonko Šoljan (1930). In his book "Fishes of the Adriatic" Šoljan (1948), morphometric and meristic characteristics necessary for the identification of tunas and other fish species are available. Fishing techniques with different fishing gears targeting BFT in the Adriatic Sea were also the subject of studies conducted after the World War II by other fishery experts and scientists in Croatia (Basioli, 1962; Morović, 1971).

Josip Basioli, in his book "Tuna fishing on the Adriatic" (1962, in Croatian), described in detail all local fishing gears used for tuna fishing, including the appearance of the first purse seiners used for catching tuna and other large pelagic fish in the open sea. He also presented statistical data on their catches in certain areas and on certain islands along the eastern Adriatic coast, as well as the legislation in force at that time for catching large pelagic fish.

Morović (1971) in his book "Tuna and its Life" (in Croatian) has collected most of the current biological knowledge about tunas, including their geographical distribution and systematic classification, and describes for the first time the biometric characteristics of tuna body, as well as their growth, sexual maturity and reproduction. He also describes the predators, parasites, migrations, and feeding habits of BFT. The aroused scientific interest in studing the biology and ecology of BFT helped and enabled the research vessel "PREDVODNIK" owened by Institute of Oceanography and Fisheries in Split (IOR) equipped with purse-seine net and the so-called "Puratić block" or power block for fisheries research on pelagic fish (Fig. 3).

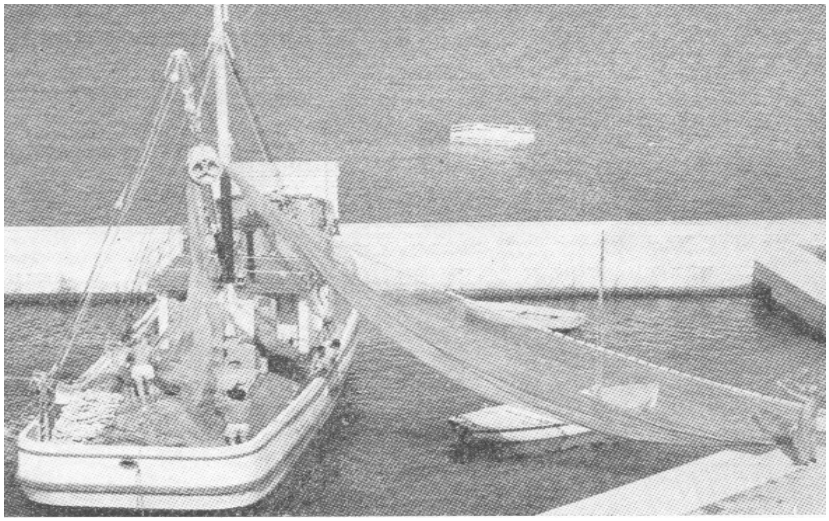

Figure 3. Research vessel PREDVODNIK of Institute of Oceanography and Fisheries with Puratić-block and purse-seine net (From: Morović, 1971; Foto: M. Alajbeg)

Otherwise, "Puratić block" is an invention of the Croatian emigrant to the USA, M. Puratić, from the Adriatic island of Brač who worked as a fisherman on tuna fishing vessels in the Pacific. He invented how to facilitate the demanding operation of purse seine nets lifting out of the sea on the fishing vessel, and his novelity is now an indispensable device in BFT purse seine fishing (Viličić, 1985).

Another Croat living in the USA, Ante Viličić, worked as a fisherman in the purse seine tuna fishery in the Pacific, where he gained valuable experience and became an expert in purse seine tuna fishing. After returning from the Pacific to the Adriatic Sea, he transfered his knowledge and experience on the eastern Adriatic. He published the book "Historical development of modern tuna fishing" (Viličić, 1985), describing the construction of the first fishing vessel in the Adriatic equipped with a purse seine net for tuna fishing, as well as experiences and statistical data of the tuna catches obtained. He pioneered the further development of BFT purse seine fishing in the open Adriatic Sea.

However, competition between modern fishing vessels equipped with purse-seine nets and traditional tuna fishing gears (e.g. tuna traps, beach seines etc.) resulted in many conflicts among fisherman (Basioli, 1962). Purse-seine fishing vessels were catching tuna schools far from the shore and therefore impacted abundance of tunas near shore. Consequently, development of purse seine fishing fleet resulted in gradual decline of tuna trap fishery and other old traditional tuna fishing gears used only in near shore areas. These old traditional fishing gears targeting large pelagic fish, together with modern, more effective fishing gears and techniques targeting BFT and other marine species, were described in "Fishing gears and techniques" by Cetinić and Swiniarski (1985).

The wide spatial distribution and long-distance migrations of BFT and other tuna species in the Mediterranean Sea and Atlantic Ocean highlighted the need for an international approach to further studies and management, and eventually the International Commission for the Conservation of Atlantic Tunas (ICCAT) was established in the late 1960s. As a BFT scientist D. Morović participated in the first ICCAT meeting hold in Rome, December 1-6, 1969 (Morović, 1971). After him, the monitoring of tuna catches on the east coast of the Adriatic Sea at the IOR in Split was taken over by the Chilean-born scientist Alegria-Hernandez (1984; 1990). After her return to Chile, fisheries scientist V. Tičina was engaged in more detailed studies of BFT biology and fisheries in the 1990s. These studies eventually resulted in his master's thesis "Morphological and nutritional characteristics and fishing of tuna (*Thunnus thynnus* L.) in the Adriatic Sea" (Tičina, 1994) and publications (Tičina, 1993, 1997, 1999), including study on the age and growth of tuna caught in the Adriatic Sea (Tičina and Kačić, 1998). However, there were no studies on sport-fishing of BFT during the XX century. At the end of 20th century, in 1997 the Republic of Croatia became a full member of the ICCAT, and V. Tičina became a member of the Standing Committee on Research and Statistics (SCRS) and served as the national BFT scientist of the Republic of Croatia in SCRS until 2010.

Studies on bluefin tuna at the beginning of the 21st century

Starting from 1st decade of XXI century, Katavić headed Croatian delegation to ICCAT in period 2001-2013, and in parallel was involved in coordination and research activities focused on BFT related studies. Thanks to his involvement two hystoric ICCAT sessions were held in the Republic of Croatia (Dubrovnik, 2006 and 2018 respectively). Providing the Vice –Chair to the General Fisheries Commission for the Maditerranean (GFCM) he also strenghten regional studies on BFT at ICCAT sessions, acting on behalf both respectve regional bodies.

At the very beggining of XXI century, Tičina and Kačić (2001) studied usefulness of hydroacoustic devices (e.g. commercial searchlight sonar) in tuna fishing operations in the Adriatic. V. Tičina as a leading BFT scientist from IOR, dedicated great part of his career to BFT research and together with his coleauges from the eastern Adriatic, published a large number of research papers in ICCAT publication - Collective Volume of Scientific Papers during the first decade of the XXI century (Katavić et al., 2002; Miyake et al., 2003; Tičina, 2007; Tičina et al., 2002, 2003, 2004a, 2005, 2006). They joined the work of an international group of BFT scientists under the framework of ICCAT Bluefin Year Program (BYP). Acting as BYP tagging coordinator, Tičina and his colleagues conducted research related to captive BFT tagging to investigate the post-tagging mortality and growth performance of BFT during aquaculture activities (Tičina et al., 2004b, 2007). Following BYP, the ICCAT launched Atlantic-Wide Research Programme for Bluefin Tuna (GBYP) in which Katavić served as an external expert aiming to improve knowledge and understanding of the Atlantic BFT stocks and migrations.

Croats returning 1996 to their homeland from Australia brought with them their experience in tuna aquaculture and decided to start this activity on the eastern Adriatic coast. Since the end of the 20th century, this extremely profitable activity has developed very rapidly on the eastern coast of the Adriatic Sea. In the first decade of the XXI century, fisheries-based BFT aquaculture became an important new activity in marine aquaculture, spreading rapidly throughout the Mediterranean (Miyake et al., 2003; Tičina, 2008). Given the domestication of tuna as a new species in Mediterranean aquaculture and the fact that caught live bluefin tuna is gaining importance as seed fish in cage farming, the need to study the relationship between the economic activities of fisheries and aquaculture is coming to the fore (Katavić and Tičina, 2005). As a result, there has been very good cooperation between the Croatian Ministry of Agriculture and scientists from the Laboratory of Fisheries Science and Management of Pelagic and Demersal Resources and the Laboratory of Aquaculture of the IOR. This eventually led to their joint participation in a larger number of ICCAT studies (e.g. BFT stock assessments), as well as in many international scientific meetings and a larger number of jointly published scientific papers on this topic.

Katavić et. al. (2002) undertake a pioner studies with caged juvenile BFT in the Adriatic Sea indicating a significant increase of average daily weight gain compare to wild fish. In the later studies, the research provides important information on the growth performances of BFT juveniles caged in the Adriatic under specific conditions (Katavic et al., 2003a, 2003b, 2003c; Tičina et al., 2007). However, due to the fact that fish were not landed in the same year when they were caught, BFT farming caused difficulties in terms of catch statictics (i.e. gap in data collection) and consequently difficulties to BFT stock assessment and management measures based on multi-year catch data. IOR's scientists studied this catch data issue, and eventually in close cooperation with country administration in 2005 developed projects (called: PUT and PRUT) aimed to monitor and follow captured small BFT used as seed fish in farming process up to their harvest/landing. Outcomes of studies made within framework of projects PUT and PRUT improuved accuracy of ICCAT BFT catch statistics from the Adriatic Sea.

In the second decade of XXI century, the biometric characteristics of BFT originating from the Mediterranean Sea (Tičina et al., 2011), and migration pattern of tagged BFT juveniles were studied. Further research efforts were made on use of a stereoscopic underwater camera system, as a non-invasive tool, in estimating the number and biomass of caged live BFT (Grubišić et al., 2013a; Katavić et al., 2016; Tičina et al. 2016). In respecting specifity of BFT stock in the Adriatic and farming practice applied, a novel Adriatic L-W algorithm were determined (Katavić, et al., 2018). Comparing size and weight distribution of caged fish large variation among farms were adressed to difference in husbandry and environmental conditions (Katavić et al., 2013, 2017).

Considering that BFT farming highly depends on the availability of small pelagic fish used as feed, studies were made with the aim of estimating the type and amount of food needed (Miletić, 2011; Miletić et al., 2019), as well as possible improvements in feeding zootechnics and sustainability of tuna farming (Mišlov Jelavić et al., 2012). The spontaneous spawning of BFT in cages and the spatial distribution of the earliest developmental stages were studied also (Grubišić et al. 2013b; Džoić at al. 2017).

Successful ICCAT management measures related to BFT stocks resulted in increased small BFT abundance in the Adriatic Sea. Eventually, scientists and fisherman noted also that behavior of numerous BFT affect other fishing activities, particularly small pelagic purse-seine night fishing with artificial lights. The influence of presence of BFT within purse-seine fishing grounds affecting the catch of small pelagic fish and damage of fishing nets was studied (unpublished). However, on the other hand, increased abbundance of BFT in the Adriatic Sea acted in favour of sport and recreational tuna fishing, known as Big Game Fishing (BGF). BGF tournaments become very popular events in Croatia, but given a very low quota allocated to BGF, this type of fishing is mainly based on the catch-and-release practice. In cooperation with ICCAT-GBYP and the Croatian Association for Sport Fishing at Sea and BFT farmers, in 2017 IOR's scientists studied effects of different types of hooks used in BGF with aim to reduce post-hooking mortality and suffering of BFT relesed (Grubišić et al. 2018).

Research on the impact of BFT farming on the marine ecosystem (Matijević et al., 2012; Stagličić et al., 2017; Šegvić et al., 2011; Tičina et al., 2020) were also important contributions to overall BFT studies on the eastern Adriatic Sea. Within framework of international cooperation, scientists from IOR also contributed to various international studies on tuna biology and ecology at large spatial scale in the Atlantic and the Mediterranean (Rooker et al., 2008; Brophy et al., 2015; Druon et al., 2016; Deguara et al., 2017; Corriero et al., 2020). In addition, it should be mentioned that BFT has also been used as an object for the study on fish parasites that it also hosts (Mladineo at al., 2008; Stanić and Mladineo, 2020).

Recently, BFT has been often used as object in various genetic studies in the Adriatic Sea, resulting in PhD thesis of young IOR's scientists. In her studies on BFT, Trumbić (2015) established a normalized mixed tissue of BFT cDNA library, pyrosequenced, assembled and annotated. It was used as a proposal for the construction of a BFT-specific DNA microarray. The relationship between the BFT host and the digenean trematode *Didymosulcus katsuwonicola* was further investigated using DNA microarrays and transmission electron microscopy. Furthermore, Lepen Pleić (2018) cloned the first full-length mRNA and gDNA sequences of BFT in order to obtain molecular characterization and expression analysis of three pro-inflammatory cytokines IL-1β, TNFα1 and TNFα2 in cage-reared BFT. The expression analysis was performed in peripheral blood leukocytes stimulated in vitro with LPS, Poly I:C and parasite protein extract; and in gill tissue during natural parasite infection. In order to find out whether survival or mortality through the BFT farming cycle can be monitored at the genetic level and linked to several genes related to immunity and stress response, IOR's researchers defined a set of 13 EST-SSRs for BFT, analysing wild vs. farmed adult BFT specimens (Radonić et al., 2020).

Finally, considering the fact that bluefin tuna is an important food for human consumption, special attention was paid to the fact that BFT is a long-lived species whose tissues are susceptible to bioaccumulation of various organic pollutants and heavy metals present in the marine environment. Therefore, IOR's scientist in collaboration with scientists from the Institute of Medical Research and Occupational Health in Zagreb and the Department of Marine Sciences and Applied Biology in Alicante, were studying this issue also (Fernandez-Jover et al., 2020; Klinčić et al., 2020; Kljaković-Gašpić and Tičina, 2021). This is particularly important for BFT from the Mediterranean Sea, highlighting importance to study BFT produced by tuna farming aquaculture activities in the Adriatic Sea, as one of the most enclosed parts of the Mediterranean. This is one of the reasons why BFT from eastern part of the Adriatic Sea should be better studied than those from other areas. Doing so, further studies may also reveal other ecological patterns of BFT.

Conclusions

Based on the review made, it is understood that BFT inspired a large number of experts and scientists working in the eastern Adriatic Sea to study its morphology, behaviour, biology, ecology and its life traits. Since ancient times BFT represented an important food source for coastal fishing communities, at the beggining studies on different fishing practices and types of fishing gears targeting BFT were the in the focus. The importance of studies in XX century related to BFT fishery was emphasized by high commercial value and the ability for capture BFT.

Due to BFT's long distance migrations very wide spatial distribution and multiple stock-users, need for an international management of this valuable sea resource resulted in establishment of ICCAT in 2nd half of XX century, higlighting the necessity for international stock assessments. Therefore, emphasis was given to studies that monitored BFT fishing activities in order to obtain regional statistical catch database and eventually provide management measures for sustainable BFT fishing.

Development of BFT farming practices in the Adriatic Sea at the begginning of XXI century, opened to Adriatic scientists a new study area, with a lot of chalenges and research opportunities coupling fisheries and aquaculture scientists. Therefore, many studies made in XXI century were mostly related to captive BFT kept in growth-out floating cages. Croatian active participation in ICCAT and SCRS offered to the Adriatic scientists' possibilities for developing international cooperation, enabling their participation in common BFT studies on regional, wide spatial scales, as well as fueling BFT research in the Adriatic environment.

More recently, BFT in the Adriatic Sea is ferquently used as an object of studies on fish parasites, molecular and genetic studies, as well as a studied food item important in human consumption. As a comercially very important large pelagic fish, BFT will certainly continue to play important role in fishery-based aquaculture (e.g. tuna farming) and sport fishing and inspire scientist and experts in their future studies.

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Shellfish biodiversity on larvae mesh bag collectors in two Natura 2000 sites, Pantan (HR3000430) and the Cetina estuary (HR3000126)

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Abstract

The paper presents research of the shellfish community on shellfish larvae collectors set up in two areas of the ecological network Natura 2000, Pantan (HR3000430), and the Cetina estuary (HR3000126). The collectors were set up with the purpose to collect data on bivalve biological diversity, to record emergence of invasive species and to collect juveniles of critically endangered species *Pinna nobilis*, Linnaeus 1758. Analysis of the shellfish larvae collectors resulted in the determination of 19 species from 11 families. During collector processing, none of the *P. nobilis* juveniles were found, but the cosmopolitan and invasive species *Styela plicata* was determined for which there are indications of being a reservoir of the *Haplosporidium pinnae* parasite which is detected as the probable cause of *P. nobilis* mass mortality. Further monitoring and analysis of the bivalve community and the occurrences of invasive species is recommended.

Keywords: biodiversity, Cetina estuary, Pantan, shellfish larvae collectors, invasive species

Introduction

Considering the multiple dimensions and complexity of life forms, there is no single measure to express biological diversity, but the number or heterogeneity (Sodhi and Ehrlich, 2010). With climate change as one of the main factors causing species extinction and ecosystem degradation, population growth, unsustainable use of resources, pollution, and the spread of invasive species further affect coastal areas and living communities (Ninan, 2009). A variety of bivalves inhabit the mouth of the Cetina River, characterized by a salty lagoon, sandy and muddy bottom, among which was also a population of now critically endangered *Pinna nobilis* species (Šašić Kljajo et al. 2009, Kurtović Mrčelić, 2020). Pantan as a Mediterranean estuarine marsh and lagoon with vegetational, ornithological, and ichthyological value on a national and international level prior to the mortality events also had recorded *P. nobilis* species by the Public Institution Sea and Karst (Cvitanić, 1999, Kurtović Mrčelić personal communication). In order to preserve and/or restore biological diversity in a state of natural balance in Natura 2000 sites it is important to document and analyze the changes in the bivalve community and record the progress in efforts to recover native species such as *P. nobilis* and at the same time provide an early alert for invasions (Official gazette 80, 2013; Kersting and Hendriks, 2019a).

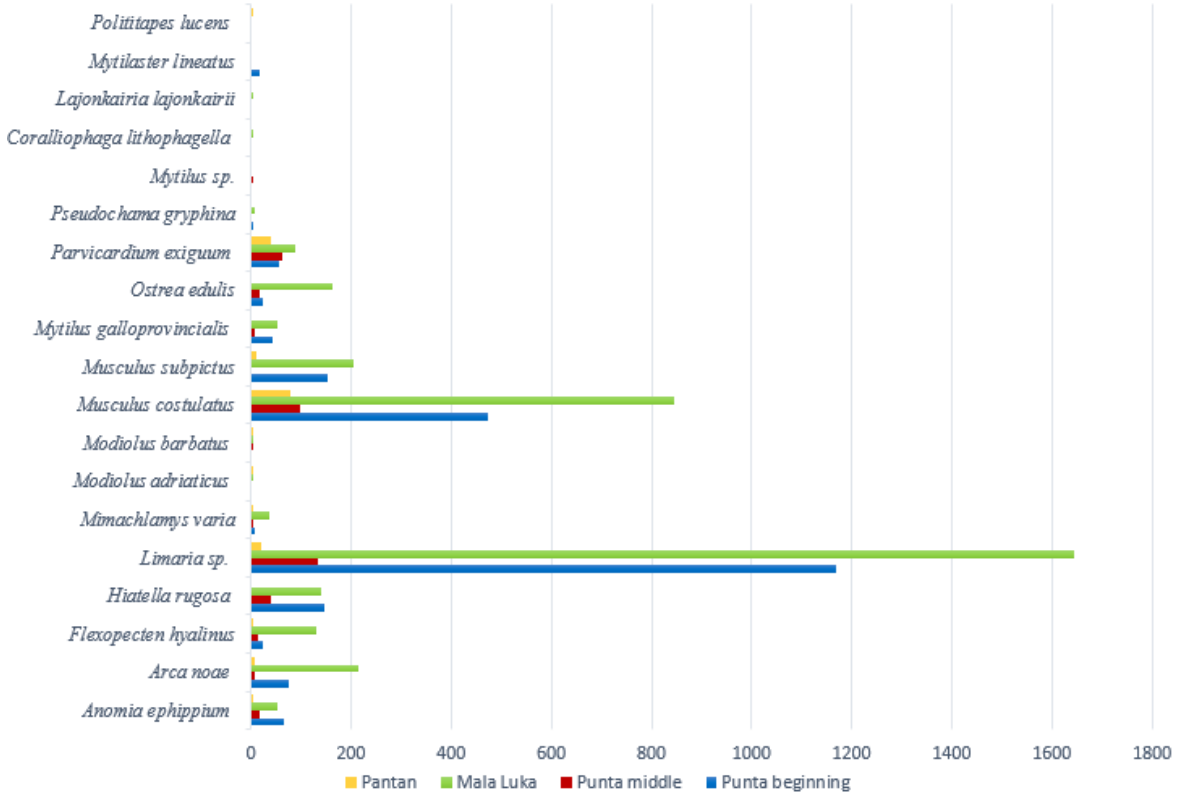
Material and methods

In the Natura 2000 site Cetina estuary (HR3000126), the larvae mesh bag collectors were placed in three locations with three replicas at different depths: Punta middle - 3 collectors at a depth of 2 m, Punta beginning - 6 collectors at a depth of 2 and 4 m, Mala Luka - 15 collectors at depths of 2, 4, 6, 8 and 10 m. In the Natura 2000 area Pantan (HR3000430), three replicas with one mesh bag collector were placed at a depth of 2 m. The mesh bag collectors were placed on the main rope lines placed vertically fixed to a small concrete mooring on the seabed and marked with a buoy on the surface. One mesh bag collector consists of a polyethylene bag for vegetables in which 3 more

polyethylene bags for vegetables were installed. The mesh bag collectors were 2 m apart on a rope line, while the rope lines were 10 m apart. The mesh bag collectors were collected by SCUBA divers after being submersed for around 6 months from June to November 2021. As soon as collected, the mesh bag collectors were placed in plastic "tubs" with seawater with a volume of 80 L. All fouling organisms found on the mesh bag collectors were manually separated and placed in a plastic tub following the filtration on a 500 µm sieve and conservation in 500 ml vials with 96% ethanol to do further laboratory analyses. In the laboratory analysis, all the species were sorted, counted, and determined with the help of an Olympus SZX10 stereo microscope. Determination was done according to Nikiferos (2005) and Giannuzzi-Savelli et al. (2001) while for the need of classification and nomenclature the European register of marine species was implemented (Costello et al. 2002). Following the laboratory analysis of the samples, all obtained quantitative and qualitative data were entered into the Microsoft Excel program. Statistical package Primer 6 with Permanova (Clarke and Warwick, 1994) was used for further statistical data analysis.

Results and discussion

During the research, a total of 6414 individuals were recorded on the bivalve larvae mesh bag collectors. A total of 19 bivalve species from 11 families were determined (Graph 1.), and no juvenile individuals of the *P. nobilis* species. The non-native invasive species *Stylea plicata* was found at the Pantan location at a depth of 2 m. Species with commercial value such as *Mytilus galloprovincialis*, *Ostrea edulis, Mimachlamys varia, Modiolus barbatus* and *Arca noae* were recorded. The most numerous species were *Limaria sp., Musculus costulatus, Muscuslus subpictus, Hiatella rugosa, Parvicardium exiguum* and *A. noae*. The smallest number of bivalves was recorded at the Pantan location, while the largest number was recorded at the Mala Luka location. A total of 3,585 bivalves were counted at the Mala Luka location, and the bivalve species *Limaria sp., M. subpictus, M. costulatus* and *A. noae* were the most numerous. A total of 171 individuals were recorded at the Pantan location, and the M. *costulatus* and *P. exiguum* were the most prevalent species. In terms of the number of registered bivalves, Punta beginning is the second most numerous location, and the species with highest numbers were *Limaria sp.* and *M. costulatus*. In Punta middle, the most abundant species were *Limaria sp., M. costulatus* and *P. exiguum*.



Graph 1. The total number of bivalves recorded by species at the locations Punta beginning, Punta middle, Mala Luka and Pantan.

The results of the analysis of shellfish biodiversity on a larvae mesh bag collector from the Cetina estuary area are presented in presence/absence Tables numbered 1, 2, and 3, and for the Pantan area in Table 4.

Table 1. Biodiversity of bivalves on a mesh bag collector in the area of the Natura 2000 ecological network - Cetina estuary (Punta beginning - PP, Punta middle - PS).

BIVALVE SPECIES	PP1 2m	PP1 4m	PP2 2m	PP2 4m	PP3 2m	PP3 4m	PS1 2m	PS2 2m	PS3 2m
Anomia ephippium	+	+	+	+	+	+	+	+	+
Arca noae	+	+	+	+	+	+	+	+	+
Flexopecten hyalinus	+	-	+	-	+	-	+	+	+
Hiatella rugosa	+	+	+	+	+	+	+	+	+
Limaria sp.	+	+	+	+	+	+	+	+	+
Mimachlamys varia	-	+	+	-	+	-	+	-	-
Modiolus adriaticus	-	-	-	-	-	-	-	-	-
Modiolus barbatus	-	-	-	-	-	-	-	+	+
Musculus costulatus	+	-	+	-	+	-	+	+	+
Musculus subpictus	+	-	+	-	+	+	-	-	-
Mytilus galloprovincialis	+	+	+	+	+	-	+	+	-
Ostrea edulis	-	+	+	+	+	+	+	+	+
Parvicardium exiguum	+	-	+	+	+	+	+	+	+
Pseudochama gryphina	+	-	+	-	-	-	-	-	-
Mytilus sp.	-	-	-	-	-	-	+	-	+
Coralliophaga lithophagella	-	-	-	-	-	-	-	-	-
Lajonkairia lajonkairii	-	-	-	-	-	-	-	-	-
Mytilaster lineatus	-	-	-	-	+	-	-	-	-
Polititapes lucens	-	-	-	-	-	-	-	-	-

Table 2. Biodiversity of bivalves on a mesh bag collector in the area of the Natura 2000 ecological network - Cetina estuary (Mala Luka – ML).

BIVALVE SPECIES	ML1 4m	ML2 6m	ML1 8m	ML1 10m	ML2 2m	ML2 4m	ML2 6m	ML2 8m	ML2 10m
Anomia ephippium	+	+	+	+	+	+	+	+	-
Arca noae	+	-	-	+	+	-	+	+	+
Flexopecten hyalinus	+	+	+	+	-	+	+	+	+
Hiatella rugosa	+	+	+	+	+	+	+	+	+
Limaria sp.	+	+	+	+	+	+	+	+	+
Mimachlamys varia	-	-	+	+	+	+	-	+	+
Modiolus adriaticus	+	-	-	-	-	-	-	+	-
Modiolus barbatus	-	-	-	-	-	-	-	+	-
Musculus costulatus	+	+	+	+	+	+	+	+	+
Musculus subpictus	+	+	+	+	-	+	+	+	-
Mytilus galloprovincialis	+	-	+	-	+	+	-	+	+
Ostrea edulis	-	+	+	+	+	+	+	+	+

Parvicardium exiguum	+	+	+	-	+	+	+	-	+
Pseudochama gryphina	-	-	-	-	-	-	-	-	-
Mytilus sp.	-	-	-	-	-	-	-	-	-
Coralliophaga lithophagella	-	-	-	-	-	-	-	-	-
Lajonkairia lajonkairii	-	-	-	-	-	-	-	-	-
Mytilaster lineatus	-	-	-	-	-	-	-	-	-
Polititapes lucens	-	-	-	-	-	-	-	-	-

Table 3. Biodiversity of bivalves on a mesh bag collector in the area of the Natura 2000 ecological network - Cetina estuary (Mala Luka – ML).

BIVALVE SPECIES	ML3 4m	ML 3 6m	ML3 8m	ML3 10m
Anomia ephippium	-	+	+	+
Arca noae	-	+	+	+
Flexopecten hyalinus	+	+	+	+
Hiatella rugosa	+	+	+	+
Limaria sp.	+	+	+	+
Mimachlamys varia	-	+	+	+
Modiolus adriaticus	-	-	-	-
Modiolus barbatus	-	-	-	-
Musculus costulatus	+	+	+	+
Musculus subpictus	+	-	+	+
Mytilus galloprovincialis	+	-	-	-
Ostrea edulis	+	+	+	+
Parvicardium exiguum	+	+	+	+
Pseudochama gryphina	-	+	+	-
Mytilus sp.	-	-	-	-
Coralliophaga lithophagella	-	-	+	-
Lajonkairia lajonkairii	-	-	+	-
Mytilaster lineatus	-	-	-	-
Polititapes lucens	-	-	-	-

Table 4. Biodiversity of bivalves on a mesh bag collector in the area of the Natura 2000 ecological network - Pantan (Pantan – P).

BIVALVE SPECIES	P1 2m	P2 2m	P3 2m
Anomia ephippium	-	+	+
Arca noae	+	+	-
Flexopecten hyalinus	-	+	+
Hiatella rugosa	-	-	-
Limaria sp.	+	+	+
Mimachlamys varia	-	-	+

Modiolus adriaticus	+	-	-
Modiolus barbatus	+	+	+
Musculus costulatus	+	+	+
Musculus subpictus	+	-	-
Mytilus galloprovincialis	-	-	-
Ostrea edulis	-	-	-
Parvicardium exiguum	+	+	+
Pseudochama gryphina	-	-	-
Mytilus sp.	-	-	-
Coralliophaga lithophagella	-	-	-
Lajonkairia lajonkairii	-	-	-
Mytilaster lineatus	-	-	-
Polititapes lucens	+	-	-

Based on the available literature in the area of the Cetina estuary, before this research, there were no studies on the acceptance of bivalve larvae on the mesh bag collectors. However, a survey of bivalve communities in the sediment of the Cetina estuary was carried out with the aim of assessing the populations of commercially important species, where 32 species were collected by hydraulic dredge, including *M. galloprovincialis, O. edulis, A. noae, Chlamys sp., Modiolus adriaticus, M. barbatus*, which were also recorded in this research. In the mentioned research, the area showed a high spatial heterogeneity of the habitat and the diversity of bivalves, unlike other investigated areas of the eastern Adriatic, however, according to the authors, the methodology is not suitable for assessing bivalve biodiversity but contributes to knowledge about their distribution (Peharda et al., 2010). Another study conducted in the Adriatic Sea in the Bay of Bokokotor with mesh bag collectors (Peraš et al., 2018) detected a total of 15 species of bivalves including *Acanthocardia sp., Anomia ephippium, Aequipecten opercularis, C. varia, Chlamys multistriata, Flexopecten glaber, Lima hians, H. rugosa, Modiolarca subpicta, M. galloprovincialis*, O. *edulis, P. nobilis, Pecten jacobaeus, P. radiata,* and *Tapes decussatus*, of which seven are the same as in this research. Marčeta et al. (2022) conducted research in the northwestern Adriatic where they analyzed the settlement of bivalve larvae on collectors that were installed at two locations, Pellestrina and Caleri. A total of 28 species of bivalves were determined on 140 installed collectors. The most abundant species in the spring-summer period were *M. galloprovincialis, F. glaber, M. varia* and *A. opercularis*, and in the summer-autumn period *Anadara transversa* and *F. glaber*. In addition, the non-indigenous species *Arcuatula senhousia* was discovered. The collectors that we analyzed in this research were immersed for six months and 19 species of bivalves were determined. No invasive bivalve species was found on a total of 27 collectors installed in four locations, however, an invasive species of ascidiacea *S. plicata* was found (Fig. 1).

Figure 1. An individual of the invasive species Styela plicata found in the location Pantan.

It was detected that *S. plicata* as an invasive species with an increasing number in all seas can represent a potential threat to native organisms such as *P. noblis* and potentially be a frequent natural reservoir and carrier of haplosporidia. With the help of molecular tests, increasing number of haplosporidia was discovered in ascidiacea of the genus *Styela* which serve as the primary host, whom they leave when they come to a new environment and find a new host (Messerman and Bowden, 2016). Reduction or removal of these species may reduce the infectious pressure of haplosporidia parasite if present (Messerman and Bowden, 2016). While settling of *P. nobilis* larvae on mesh bag collector is a complex process under influence of abiotic and biotic parameters that generate high variability in settlement rates, therefore larval settlement trends can be assessed only through longer data sets (Kersting and Garcia-March, 2017) with further research in invasive species presence.

Conclusions

The research presents qualitative and quantitative data on the bivalve larvae settlement on a mesh bag of bivalve larvae collectors installed in the areas of the ecological network Ušće Cetine (HR3000126) and Pantan (HR3000430). Obtained data are significant for assessing the state of biodiversity in the mentioned areas and as an early alert for invasive species. All results are useful for future program planning and the implementation of habitat protection in the areas of the ecological network which are also habitats of *P. nobilis* species. Further monitoring, research, and analyses are needed.

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ORIGINAL SCIENTIFIC PAPER

Masena zastupljenost hrskavičnjača u ribarskom ulovu na području ribolovne podzone G5, Istočni Jadran

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Sažetak

Hrskavičnjače se smatraju najugroženijom skupinom morskih riba na Mediteranu te je cilj ovog rada bio istražiti njihovu masenu zastupljenost u ribolovnom ulovu različitih ribarskih alata na području ribolovne podzone G5. Uzorci su prikupljani tijekom studenog 2020. te siječnja, ožujka, svibnja, lipnja i studenog 2021. godine. U ukupnom ulovu svih ribolovnih alata koji je iznosio 1684,5 kg identificirano je 90 vrsta morskih organizima (koštunjače, hrskavičnjače, glavonošci i rakovi), pri čemu je masena zastupljenost hrskavičnjača bila 12%. U ovisnosti o ribolovnom alatu, maseni udio ove skupine varirao je od 5 do 53%. Najveću masenu zastupljenost u ukupnom ulovu svim ribolovnim alatima imali su pas mekuš, *Mustelus mustelus* (Linnaeus, 1758), mačka mrkulja, *Scyliorhinus stellaris* (Linnaeus, 1758) te pas mekuš pjegavi, *Mustelus punctulatus* (Risso, 1827). Utvrđene su ukupno četiri jedinke osjetljivih vrsta hrskavičnjača čiji je maseni udio iznosio 1,1% ukupnog ulova: jedna žutuga dračorepa, *Bathytoshia centroura* (Mitchill, 1815), jedna žutuga, *Dasyatis pastinaca* (Linnaeus, 1758) te dva goluba ćukana, *Aetomilaeus bovinus* (Geoffroy Saint-Hilaire, 1817). S obzirom da je ovo istraživanje provedeno na ograničenom području jedne podribolovne zone, potrebno je istražiti i druga ribolovna područja u Jadranu kako bi se dobile potpunije informacije o prisutnosti hrskavičnjača, posebno osjetljivih vrsta, u ribarskom ulovu.

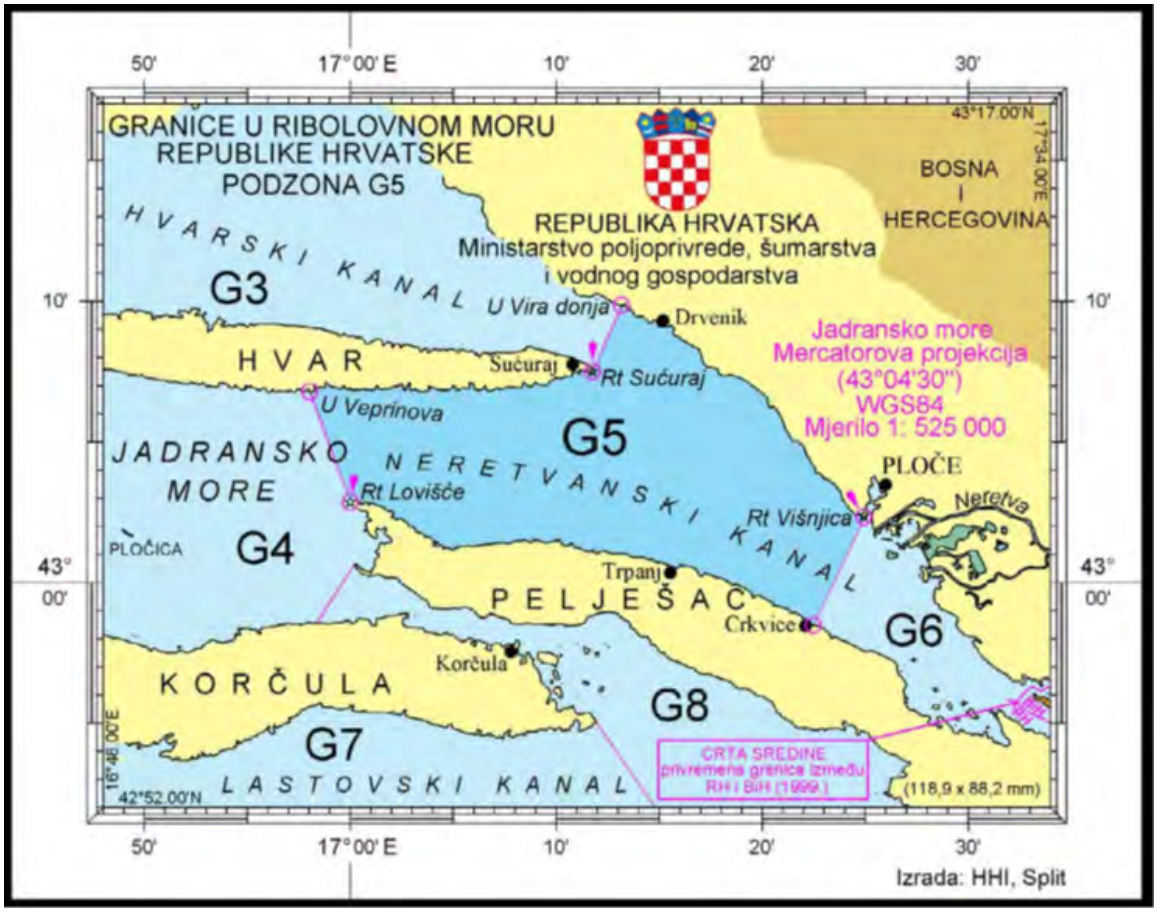
Ključne riječi: Jadrnsko more, hrskavičnjače, osjetljive vrste, ribolovni alati

Uvod

Životni ciklus hrskavičnjača karakteriziraju spori rast, kasno spolno sazrijevanje, niska plodnost i dugi životnog vijeka (Damals i Vassilopoulou, 2011), što doprinosi sporijem obnavljanju populacije (Dulvy i sur., 2014). U Jadranskom moru je zabilježeno ukupno 59 vrsta ove skupine (Ćetković i sur., 2021). Većina morskih pasa te morske mačke i raže vršni su grabežljivci i imaju važnu trofičku funkciju u morskom ekosustavu (Barausse i sur., 2014). Različiti čimbenici kao što su onečišćenja i uništavanje staništa mogu utjecati na brojnost hrskavičnjača (Barausse i sur., 2014). Brojni autori smatraju kako je ova skupina zbog prekomjernog izlova (komercijalnog ribolova i prilova) najugroženija skupina morskih riba na Mediteranu (Dulvy i sur., 2014; Barausse i sur., 2014; Serena i sur., 2020). Danas još uvijek nema dovoljno informacija o prisutnosti hrskavičnjača u ribarskom ulovu na području Jadranskog mora kako bi se mogla procijeniti njihova ugroženost. U literaturi se nalaze podatci o istraživanjima provedenim u Istri (Iveša i sur., 2021a) te na crnogorskom dijelu istogčnog Jadrana (Ćetković i sur., 2021). Prema podatcima na području Istre, hrskavičnjače su značajno zastupljene u ulovu različitih ribolovnih alata, iako veliki dio jedinki završava kao odbačeni ulov,odnosno prilov (Iveša i sur., 2021 a). Cilj rada bio je utvrditi zastupljenost hrskavičnjača u ulovu različitim ribolovnim alatima na području ribolovne podzone G5 u istočnom Jadranu.

Materijal i metode

Istraživanje je provedeno tijekom zajedničkog terenskog rada s ribarima s području Općine Gradac, grada Ploča i Trpnja u sklopu projekata „Ribarsko-znanstvena mreža Grada Ploča“ i „Ribarsko-znanstvena suradnja u Općini Gradac“. Područje istraživanja obuhvaća ribolovnu podzonu G5 (Slika 1).



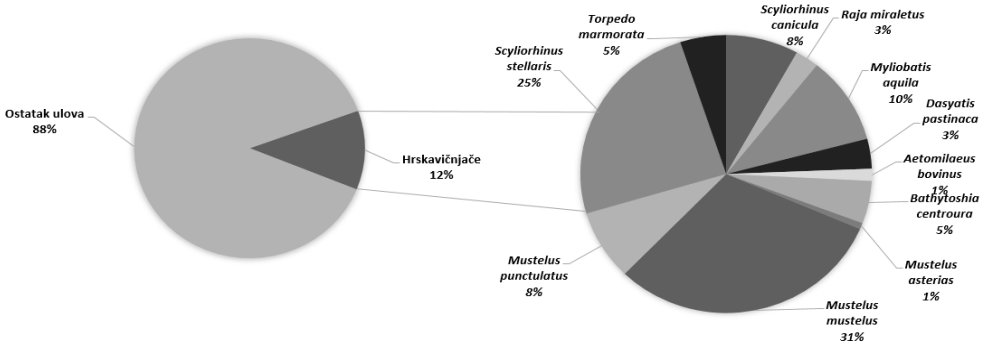
Slika 1. Karta s prikazom položaja ribolovne podzone G5 (Izvor: NN 5/2011)

Uzorkovanje je obavljano tijekom studenog 2020. te siječnja, ožujka, svibnja, lipnja i studenog 2021. godine korištenjem pet ribolovnih alata: pridnene povlačne mreže koće, jednostruke mreže stajaćice - prostice, trostrukih mreža stajaćica - listarice i poponice te parangala. Analiza koćarskog ulova obavljena je ukupno 11 puta (u studenom 2020., ožujku, svibnju i studenom 2021.), pri čemu je trajanje potega iznosilo dva do četiri sata. Sve stajaćice, bez obzira na vrstu, polagane su nakon zalaska sunca a podizane u ranim jutarnjim satima. Analiza ulova prostica obavljana je u studenom 2020. te svibnju, lipnju i studenom 2021., dok je ulov listarica analiziran u studenom 2020. godine, a poponica u siječnju 2021. Ulov parangala (ukupno 4000 udica) analiziran je u svibnju 2021. godine, pri čemu je vrijeme stajanja u moru iznosilo od pet do sedam sati. Nakon podizanja svakog ribolovnog alata sve ulovljene jedinke (koštunjače, hrskavičnjače, glavonošci i rakovi) su nakon determinacije vagane. Potom je detaljno analizirana masena zastupljenost za svaku pojedinačnu vrstu hrskavičnjača u ukupnom ulovu te prema pojedinom alatu prema formuli: masena zastupljenost = ukupna masa jedne vrste/masa ukupnog ulova x 100. Za obradu podataka korišten je Microsoft Exel (2016).

Rezultati i rasprava

U ukupnom ulovu svih ribolovnih alata zajedno utvrđeno je 90 vrsta morskih organzima iz skupina: koštunjače, hrskavičnjače, glavonošci i rakovi. Maseni udio hrskavičnjača iznosio je 12 % ukupnog ulova, dok je ostatak otpadao na ostale skupine (88 %) (Grafikon 1). Utvrđeno je jedanaest vrsta hrskavičnjača: žutuga dračorepa, *Bathytoshia centroura* (Mitchill, 1815), žutuga, *Dasyatis pastinaca* (Linnaeus, 1758), golub ćukan, *Aetomilaeus bovinus* (Geoffroy Saint-Hilaire, 1817), golub, *Myliobatis aquila* (Linnaeus, 1758), raža modropjega, *Raja miraletus* (Linnaeus, 1758), pas mekuš zvjezdasti, *Mustelus asterias* (Cloquet, 1819), pas mekuš, *Mustelus mustelus* (Linnaeus, 1758), pas mekuš pjegavi *Mustelus punctulatus* (Risso, 1827), mačka bljedica, *Scyliorhinus canicula* (Linnaeus, 1758), mačka mrkulja, *Scyliorhinus stellaris* (Linnaeus, 1758), drhtulja, *Torpedo marmorata* (Risso, 1810). Najveće masene udjele među

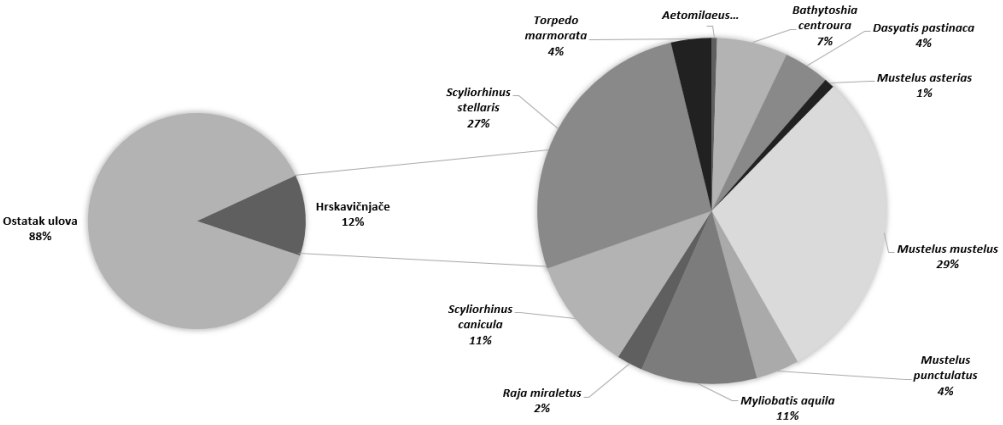
hrskavičnjačama imali su pas mekuš s 31 % i mačka mrkulja s 25 %, a najmanje golub ćukan i pas mekuš zvjezdasti sa svega 1%.



Grafikon 1. Masena zastupljenost hrskavičnjača u ukupnom ulovu svih ribolovnih alata na području ribolovne podzone G5 tijekom razdoblja istraživanja (studeni 2020. godine te siječanj, ožujak, svibanj, lipanj i studeni 2021. godine)

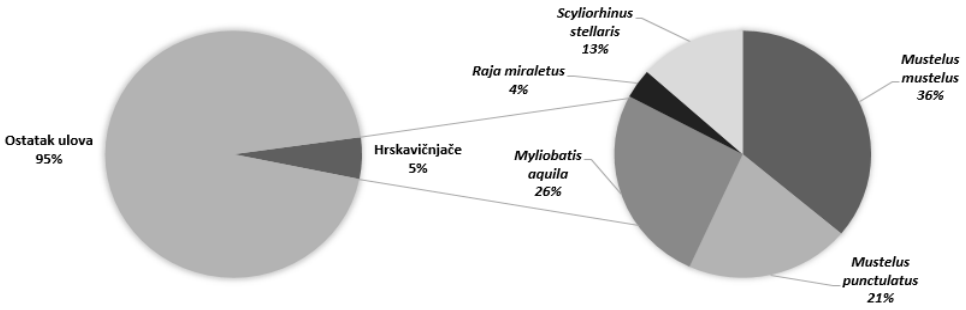
U ukupnom ulovu hrskavičnjača, utvrđene su tri vrste koje prema Pravilniku o obliku, sadržaju i načinu vođenja i dostave podataka o ulovu u gospodarskom ribolovu na moru (NN 38/2018) pripadaju osjetljivim vrstama. Iako se radi o svega četiri jedinke (jedna žutuga dračorepa od 10 kg, jedna žutuga od 6,4 kg te dva goluba ćukana od 2,1 i 07 kg), maseni je udio osjetljivih vrsta zbog njihove veličine iznosio 1,1% ukupnog ulova.

Ukupni ulov pridnene povlačne mreže koće sastojao se od 61 vrste morskih organizama. Maseni udio hrskavičnjača iznosio je 12%, dok je ostatak otpadao na ostale skupine organizama. Najveće masene udjele među hrskavičnjačama imali su pas mekuš s 29 % i mačka mrkulja s 27 %, dok je najmanji maseni udio imao pas mekuš zvjezdasti sa svega 1 % (Grafikon 2).



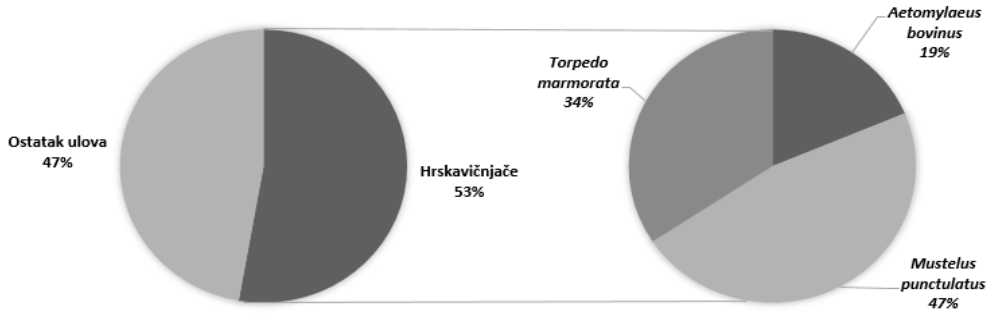
Grafikon 2. Masena zastupljenost hrskavičnjača u ukupnom ulovu pridnene povlačne mreže koće na području ribolovne podzone G5 tijekom razdoblja istraživanja (studeni 2020., ožujak, svibanj i studeni 2021.)

U ukupnom ulovu jednostruke mreže stajaćice (prostice) utvrđeno je 56 vrsta morskih organizama. Utvrđene su svega četiri vrste hrskavičnjača čiji je maseni udio iznosio 5 %, dok je ostatak otpao na koštunjače, glavonošce i rakove. Najveći maseni udio među hrskavičnjačama imao je pas mekuš s 36 %, a najmanji raža modropjega s 4% (Grafikon 3).



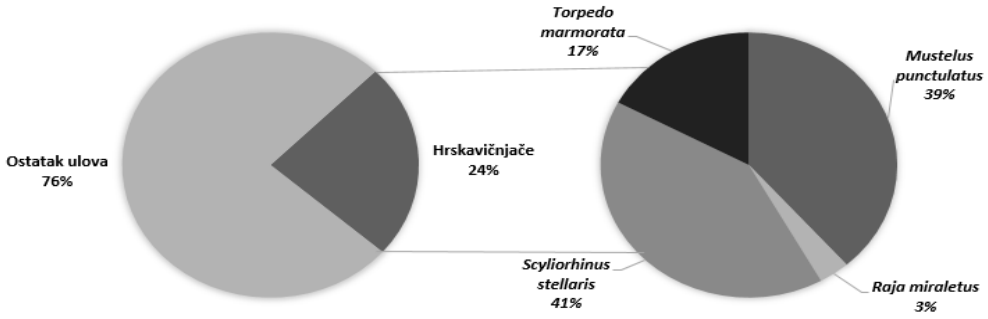
Grafikon 3. Masena zastupljenost hrskavičnjača u ukupnom ulovu prostice na području ribolovne podzone G5 tijekom razdoblja istraživanja (studeni 2020., svibanj lipanj i studeni 2021.)

U trostrukoj mreži stajačici popinici utrvđeno je 12 vrsta morskih organizama od čega je 53 % otpadalo na hrskavičnjače, a ostatak na koštunjače, glavonošce i rakove. U mreži poponici bile su zastupljene tri vrste hrskavičnjača: pas mekuš pjegavi s 47 %, drhtulja s 34% te golub ćukan s 19 % masenog udjela među hrskavičnjačama (Grafikon 4).



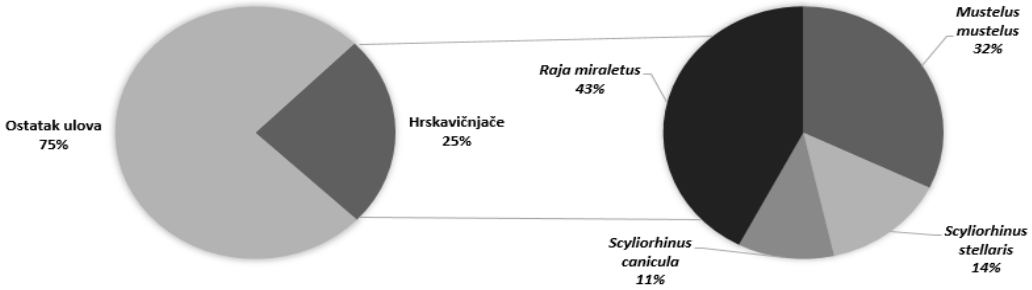
Grafikon 4. Masena zastupljenost hrskavičnjača u ukupnom ulovu poponice na području ribolovne podzone G5 tijekom razdoblja istraživanja (siječanj 2021.)

Ukupni ulov trostruke mreže stajaćice (listarice) sastojao se od 31 vrste od čega su četiri pripadale skupini hrskavičnjača. U ulovu ove stajaćice zastupljenost hrskavičnjača iznosila je 24%, dok je masena zastupljenost vrsta iz ostalih skupina iznosila 76 % . Najveću masenu zastupljenost imali su mačka mrkulja s 41% i pas mekuš pjegavi s 39 % (Grafikon 5).



Grafikon 5. Masena zastupljenost hrskavičnjača u ukupnom ulovu listarice na području ribolovne podzone G5 tijekom razdoblja istraživanja (studenom 2020.)

U ukupnom ulovu parangala hrskavičnjače su bile zastupljene s 25 %, dok je ostalih 75 % otpadalo na koštunjače, glavonošce i rakove. Utvrđeno je ukupno devet vrsta morskih organizama, od čega četiri vrste hrskavičnjača: raža modropjega s 43 %, pas mekuš s 32 %, mačka mrkulja s 14 % zastupljenosti i mačka bljedica s 11 % masenog udjela među hrskavičnjačama (Grafikon 6).



Grafikon 6. Masena zastupljenost hrskavičnjača u ukupnom ulovu parangala na području ribolovne podzone G5 tijekom razdoblja istraživanja (svibanj 2021.)

Iz prikazanih rezultata možemo vidjeti da je na području ribolovne podzone G5 udio ostalih skupina morskih organizama u ukupnom ulovu svih ribolovnih alata zajedno veći u odnosu na udio hrskavičnjača. Među hrskavičnjačama u gotovo svim ribolovnim alatima najveću masenu zastupljenost imali su pas mekuš, pas mekuš pjegavi, mačka mrkulja, a zatim slijede mačka bljedica, drhtulja, raža modropjega, golub, žutuga i pas mekuš zvjezdasti. U istraživanju Iveše i sur. (2021b) na području Medulinskog zaljeva tijekom 2018. godine maseno najzastupljenije hrskavičnjače u ulovu bile su mačka mrkulja i pas mekuš, dok su u manjem postotku bili su pristuni drhtulja i golub. Za razliku od istraživanja iz 2018. godine, Iveša i sur. (2021a) su tijekom 2021. godine utvrdili da su na području Istre brojčano najzastupljenije hrskavičnjače drhtulja, mačka bljedica i mačka mrkulja, dok je najmanju zastupljenost imao pas mekuš. Marino i sur., (2018) navode da je na području sjevernog Jadrana pas mekuš najzastupljeniji, zatim slijedi pas mekuš pjegavi, dok se pas mekuš zvjezdasti smatra rijetkim. Na području ribolovne podzone G5 također su najzastupljeniji bili pas mekuš i pas mukaš pjegavi, dok je prisutnost psa mekuša zvjezdastog bila mala.

Brojni istraživači navode kako dolazi do pada brojnosti hrskavičnjača na Mediteranu i kako ih je potrebno dodatno zaštiti (Dulvy i sur., 2014; Serena i sur., 2020). Dulvy i sur. (2014) kao glavni razlog opadanja populacija ove skupine riba navode negativan utjecaj ribolova. Međutim, isti autori navode kako osim ribolova postoje i drugi čimbenici koji mogu utjecati na pad brojnosti ovih vrsta riba to su promjene u sastavu bioloških zajednica, degradacija staništa i različita onečišćenja (Dulvy i sur., 2014).

Zaključak

Maseno najzastupljenije vrste hrskavičnjača u ribolovnim alatima na području ribolovne podzone G5 su pas mekuš i mačka mrkulja. Sukladno rezultatima, prisutnost osjetljivih vrsta hrskavičnjača gotovo je zanemariva jer su tijekom kompletnog razdoblja istraživanja utvrđene svega četiri jedinke hraskavičnjača (jedna žutuga dračorepa, dvije žutuge i dva goluba ćukana). S obzirom da je ovo istraživanje provedeno na ograničenom području jedne podribolovne zone, potrebno je istražiti i druga ribolovna područja u Jadranu kako bi se dobile potpunije informacije o prisutnosti hrskavičnjača u ribarskom ulovu. Također bi bilo uputno provesti dodatna istraživanja kojima bi se proučili zdravstveni status, biologija i populacijska dinamika ovih vrsta riba.

Napomena

Istraživanje je provedeno u sklopu Projekata "Ribarsko-znanstvena mreža Grada Ploča" u sklopu Mjere I.3., "Partnerstvo između znanstvenika i ribara za razdoblje 2020.-2022." i „Partnerstvo između znanstvenika i ribara u Općini Gradac" u sklopu Mjere I.3.

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Weight representation of cartilaginous species in the catch in the fishing subzone G5 area, Eastern Adriatic

Abstract

Cartilaginous species are considered the most endangered group of marine fishes in the Mediterranean. For this reason, the aim of this study was to investigate their weight representation in the catch of different fishing gears in the area of the fishing subzone G5. The sampling was performed during November 2020, and January, March, May, June, and November 2021. The total catch of all fishing gears together was 1684,5 kg, and 90 species of marine organisms were determined (bony fishes, cartilaginous fishes, cephalopods, and crustaceans). Weight representation of cartilaginous fishes in the total catch was 12%, and it varied from 5 to 53% depending on the fishing gear. Among cartilaginous species, common smooth-hound, *Mustelus mustelus* (Linnaeus, 1758), nursehound, *Scyliorhinus stellaris* (Linnaeus, 1758), and blackspotted smooth-hound, *Mustelus punctulatus* Risso, 1827, had the highest weight representation. Four specimens of vulnerable cartilaginous fish species, representing 1,1% of the total catch, were determined: one Roughtail stingray, *Bathytoshia centroura* (Mitchill, 1815), one common stingray, *Dasyatis pastinaca* (Linnaeus, 1758), and two bull rays, *Aetomilaeus bovinus* (Geoffroy Saint-Hilaire, 1817). Given that this research was conducted in a limited area of one fishing subzone, it is necessary to investigate other fishing areas in the Adriatic in order to obtain detailed information on the presence of cartilaginous fishes in the catches.

Keywords: Adriatic Sea, cartilaginous fishes, vulnerable species, fishing gears

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Nutritional value of whey after the production of Trappist cheese

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Abstract

The aim of this paper is to present the results of research, obtained for the nutritional values of whey by-products in the production of Trappist cheese. It is a famous cheese produced by the monks of the monastery "Marija Zvijezda" from Banja Luka in cooperation with the cooperative Livač. Whey is a well-known by-product of cheese production. New trends in nutrition require balanced nutritional values, and as a contribution to this is important to know the same about whey. Traditional cheeses and whey have high nutritive and health benefits. This paper presents the nutritional benefits of whey thats remains after the production of Trappist cheese. The content of whey in the dry residue is: fat - 0.41 g/100 g of whey, saturated fatty acids - 0.20 g, carbohydrates - 4.80 g, protein - 0.92 g and NaCl content is 0.11 g. The energy value of whey is 27 kcal or 112 kJ.

Keywords: nutritional value, whey, cheese Trappist

Introductions

Todays we all follow a fast lifestyle and this causes stress. On this occasion, people are paying more and more attention to proper nutrition. Milk and dairy products belong to the category of basic foods in human diet. A balanced diet has become an integral part of life and the declaration of finished products. The indigenous dairy products are of special importance within that category. They are foods that not only satisfy the basic needs for nourishment, but at the same time are part of the material culture and tradition of a particular country.

Milk and dairy products have always been part of the diet with a beneficial effect on humans. Nowadays, we strive for new goals, namely the the prevention of certain diseases and the maintenance of health through nutrition (Grerman et al, 2003). On the market today, there is a wide range of milk and dairy products whose functional properties are based on their natural high-value nutrient composition (Tudor and Havranek, 2009). Whey has been a well-known by-product in the cheese production since the beginning of cheese making, i.e. for more than 8,000 years. Hypocrites (460 BC) underlined the worthiness of whey and suggested it as a therapy for the treatment of tuberculosis, skin diseases, digestive problems and jaundice (Blazic et al., 2017). In the 18th century in Switzerland, Austria and Germany whey was used to treat diarrhoea, dysentery, and some forms of poisoning. It was a common belief then that whey had diuretic properties and invigorating effect on the body (Elatar et al., 2010). Whey is a greenish-yellow liquid that originates of vitamin B2 (riboflavin), and lags behind after coagulation casein milk during cheese production. Depending on the method of coagulation of casein, differ acid (action acid) and sweet whey (by enzyme action). Whey is a product rich in lactose, proteins, vitamins and minerals. Minerals include: potassium, sodium, calcium, magnesium, chlorides and phosphates which are present in whey in almost the same amount as in milk. The content of lactic acid in whey varies depending on the storage period and conditions (Lučić, 1983; Zandona et al., 2021).

Today, the annual growth of whey production is 2% on the global level, and is growing in parallel with the annual growth of milk and cheese production (Tratnik, 2003). The rapid growth of global markets for food ingredients, including whey-based protein powders, which are 'among the winners of several new nutrition trends and food developments' (Vik and Kvam, 2017). An indication of this trend is the large and growing market for functional food ingredients, i.e. probiotics, proteins and amino acids, phytochemicals and plant extracts, prebiotics, fibres and specialty carbohydrates, omega-3 fatty acids, carotenoids, vitamins, and minerals. In 2018, these were estimated to be worth US$ 68.6 billion worldwide, rising to US$ 94.2 billion by 2023 (PR Newswire, 2018).

It is also important to emphasize that whey has only 25 kcal (105 kJ). Therefore, whey is a low-calorie, highly nutritive product which makes it an ideal ingredient of various diets and a highly valuable component in the food processing industry (Jarc et al., 1994). Nutritional value of 3 kg of whey is equivalent to the nutritional value of 1 litre of milk (Popović-Vranješ and Vujičić, 1997). The most important whey protein-based products are: protein concentrates, isolates and hydrolysates of whey proteins (Jovanović et al., 2005). Whey protein concentrates have functional properties, they are completely digestible and suitable for cheese production (Božanić et al., 2000). Whey proteins should have a special place in the nutritional programme. Whey proteins have long been used in the dairy industry because of their nutritional and functional properties. Recently, they have also been used in other food processing industries, such as baby food, bakery, and meat industries (Herceg and Režek, 2006).

Whey is a by-product in the technological process of cheese or casein production, so it has a very variable composition. One example of the average proportions of whey ingredients is shown in Table 1.

Table 1. Approximate composition of separated whey, %

Contense	Cheese whey, %	HCL acid Casein whey, %
Tal solid	6	6.4
Water	94	93.6
Fat	0.05	0.05
True protein	0.6	0.6
Lactose	4.5	4.6
Ash (Minerals)	0.5	0.8
Lactic acid	0.05	0.05

Source: Dairy Processing Handbook (2020)

Whey contains about 50% of the dry matter mainly lactose and whey proteins as a whole, soluble minerals and B vitamins, while vitamin C is broken down already during cheese production (Tratnik, 1998). In general, whey has high nutritional value and is easily digested and assimilated. It is also considered an excellent source of functional proteins and a rich source of vitamins B, minerals (Ca, P, Na, K, Cl^-, Fe, Cu, Zn and Mg) and lactose (Macwan et.al 2016; Papademas et al., 2019).

Because of excellent nutritional and functional properties of whey solids, a substantial portion of whey is processed into whey powder, while the remainder is used for the production of sweet whey powder, demineralised whey, delactosed whey, whey protein concentrate whey protein isolate or lactose (Çelik and Önür, 2016).

Cheese is a food of high nutritional value and can occupy an important place in a healthy and well-balanced diet (Gregurek, 2015). The nutritional value of cheese is determined by many parameters; the most important is the quality of the milk from which the cheese is produced, then the technological process of cheese production and finally the process of cheese ripening. As such, cheese is a very good source of protein and milk fat. On the other hand, it is a good source of fat-soluble vitamins and in the case of mature cheese (especially with a longer ripening period) we have a slightly lower lactose content, which makes it suitable for the diet of consumers who are intolerant to lactose (Thomas et all, 2000). This strong market trend is strongly driven by the rise of 'functional nutrition', which refers to the increased engineering and reengineering of foods in coevolution with changing corporate strategies, trends in food, diets and health trends, and new food and nutrition policies (Vik and Kvam, 2017). Thermal instability of whey proteins relative to casein is attributed to the absence of phosphorus, low proline content, and higher content of cystine, cysteine, and methionine (Popović-Vranješ and Vujičić, 1997).

Lactose is the main ingredient in whey dry matter (Table 2) and has multiple role. It is easily digestible even for an infant, as evidenced by breast milk, which contains much more lactose (about 6-7%) than cow's milk (about 4.6-4.9%) (Tratnik et al., 1988.).

Table 2. Composition of sweet whey, before and after demineralisation

Whey	Dry metter %	Protein %	Ash %	Ca mg/100g	Lactose %	Lactic acid %
Sweet whey	5.40	0.76	0.45	43.35	4.10	0.11
Demineralized	4.73	0.52	0.02	1.90	4.10	0.02

Source: Tratnik et al., 1988

The average content of whey dry residue is: 70% lactose (depending on the acidity of whey), 14% proteins, 9% minerals, 4% fats, and 3% lactic acid, Blažić et al., 2018).

The nutritional value of Trappist cheese is: milk fat content = 34.5 g/100 g of cheese, saturated fatty acids contain 24.7 g, carbohydrates 0.8 g, protein 27.5 g and NaCl content 2.05 g. The energy value of Trappist cheese is 424 kcal or 1758 kJ (Budimir, 2021).

Table 3. Analysis of the composition of Trappist cheese Marija Zvijezda

Parameter	Determined value (g/100g)
Fat content	34.5
Saturated fatty acid content	24.7
Carbohydrate content	0.8
Sugar content	0.0
Protein content	27.5
NaCL	2.05
Energy value in 100 g:	424 kcal ili 1758 kJ

Source: Budimir, 2021

The beginning of cheese production in Marija Zvijezda monastery is dated to 1872, in a small dairy built by Father Franz, which he called the „Swiss". However, this cheese plant production did not last long due to animal diseases that caused the lack of milk. The production of the original cheese began in 1882 when in the monastery in Banja Luka arrived Father Ignatius from the French monastery „Port-du-Salut". The cheese was very well known and it was awarded with numerous prizes at fairs in Europe. At first, the monks processed the milk from their own farm and later they started to buy it from local farmers (Božinović, 2009). The secret was passed orally from brother to brother, or they took it with them into the grave. Kirin (2003) wrote about this: "Even though in cheese making industry the Trappist cheese was dominantly present for the entire century, in our literature there are very little data about the technological process of making this cheese". Due to the secrecy of making cheese, there is no description of the original Trappist cheese from Banja Luka, so it can only be speculated (Budimir and Bagarić, 2015). This secrecy draws the following conclusion: "The quality of the cheese and the art of its production are predicated largely on the method of its preparation. Specifically, a dozen specialists participated in the production of the cheese. The cornerstone was set on March 18, 1893. In this branch, besides other buildings, a cheese making plant was opened. The local people brought the milk and the monks processed it into cheese and butter (Božinović, 2009).

Cheese production in both branches was developed successfully. Up to now, there were no reliable data on the organoleptic properties of the original Trappist cheese, and the secrecy of its production does not allow us to obtain an insight into the production technology. Mainly, the research was done to study the chemical composition, quality and organoleptic characteristics of semi-hard cheese, produced in the former communist dairies. Cheese production was revived again in 2008 when Father Tomislav went to France in the monastery of Mont-des-Cats and learned the technique of cheese making and brought back the recipe for cheese production (Budimir, 2012).

Material and Methods

The research and sampling were conducted on the farm agricultural cooperative Livač, located in Aleksandrovac, Laktaši municipality, Bosnia and Herzegovina. The cooperative is engaged in the production of raw milk. Since 2008, in the newly built area for cheese production, in cooperation with the Trappist monks, Trappist cheese by Marija Zvijezda has been produced, according to a recipe owned by the Marija Zvijezda monastery. Currently, about 2.5 tons of Trappist cheese are produced per month. The cheese factory has HACCP and ISO 2008:2009 certificates and is under the supervision of a veterinary inspection. The cheese factory is licensed to export to the European Union market and as such complies all applicable hygiene and health regulations.

Physical-chemical tests were performed at the Veterinary institute "Slaven", Veterinary diagnostic laboratories, which are accredited laboratories for food testing in RS and Bosnia and Herzegovina.

The tests were performed in order to establish: the fat content according to the method of the Bosnia and Herzegovina Official Gazette (No. 85/13, MI-258), saturated fatty acids content (according to previously method), content of carbohydrates (according to internal method UMH 356), and sugar content (according to internal method). As for the protein content, the tests were carried out based on the Bosnia and Herzegovina Official Gazette, No. 85/13, Annex II, Section V and the NaCl content was determined according to the internal method.

Energy level was calculated based on the method of the Bosnia and Herzegovina Official Gazette, No. 68/13.

Results and Discussion

Trappist cheese belongs to the group of semi-hard cheeses, that are easy to cut. Unlike the products present on the market under the same name, which are either too soft or too hard to cut. Unfortunately, since there has been no possibility of banning the use of the name, here are several varieties of this cheese on the market. Whey used in the production of Trappist cheese belongs to the group of sweet whey (due to the actions of enzymes).

Lactose as the most important ingredient in whey has a multiple role. It stimulates intestinal peristalsis and supports the absorption of calcium and phosphorus, ensures optimal levels of magnesium and digestion of fats and other nutrients in the body. It also establishes a slightly acidic reaction in the intestines, thus preventing the growth and multiplication of harmful bacteria. After coagulation of casein, the whey proteins remain in the whey. The share of minerals in whey is very variable, which is a consequence of various biochemical processes in cheese production technology.

Table 4. Analysis of the composition of whey after productions of Trappist cheese Marija Zvijezda

Parameter	Determined value (g/100g)
Fat	0.41
Saturated fatty acid	0.20
Carbohydrate	4.80
Lactose	4.80
Protein	0.92
NaCL	0.11
Energy value in 100 g:	27 kcal ili 112 kJ

Table 4. shows that in 100 g of whey from Trappist cheese Maria Zvijezda contains 0.41 g of fat content. The content of saturated fatty acids in whey by-products of Trappist cheese is 0.2 g / 100 g of whey. The obtained result are wery low, if we compare them with that of cheese. In the tested samples, a carbohydrate content of 4.8 g / 100 g of whey was obtained. The protein content of the samples was 0.92 g / 100 g. The salt content was 0.11 g in 100 g of whey, and the sugar content was 4.8 g in the samples. The energy value of 100 g of whey by-products in the production of Trappist cheese Marija Zvijezda is 27 kcal, or 112 kJ.

There are many possibilities to use whey; in cottage cheese production, fermented and probiotic dairy products, and whey protein concentrates. In addition to their high nutritional value due to the high content of essential amino acids, whey proteins are widely used in the food industry due to their significant functional properties. The main

reasons for the insufficient use of whey for food purposes are attributed to the greater amount of water, and in dry matter, the greater amount of minerals, which causes technological problems and reduced production efficiency.

Conclusions

Mankind is bacoming increasingly aware of the importance of taking good care of nature, origins, safety and nutritional value of traditional products, which are gradually taking a special place in the nutrition of modern man. The consumption of traditional cheeses and whey are among the key elements of a well-balanced diet. The production of whey-based dairy products is possible due to the excellent functional properties of whey proteins, which allow them to fit into many products. They are calssified as value-added products since they have a positive effect on overall health by reducing the risk of diseases. There are many possibilities for the use of whey: in cottage cheese production, fermented and probiotic dairy products, and whey protein concentrates. In addition to their high nutritional value due to the high content of essential amino acids, whey proteins are widely used in the food industry due to their significant functional properties.

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Nutritivna vrijednost sirutke nakon proizvodnje sira Trapista

Sažetak

Cilj ovoga rada je prikazati rezultate istraživanja, nutritivne vrijednosti sirutke koja nastaje u procesu proizvodnje sira Trapist. Trapist je čuveni sir kojeg proizvode redovnici samostana „Marija Zvijezda" iz Banja Luke u suradnji sa zemljoradničkom zadrugom „Livač". Sirutka je poznat nus proizvod koji nastaje u proizvodnji sireva. Novi trendovi u ishrani ljudi zahtjevaju izbalanisirane nutritivne vrijednosti, stoga je važno poznavati iste kod sirutke. Tradicionalni sirevi i sirutka pozitivno utječu na zdravlje ljudi. U radu su prikazane hranjive dobrobiti sirutke nakon proizvodnje sira trapista. Sadržaj pojedinih sastojaka u 100 g sirutke (u suhoj tvari) iznosi: mliječna mast - 0.41 g, zasićene masne kiseline - 0.20 g, ugljikoihidrati - 4.80 g, proteini - 0.92 g i NaCl - 0.11 g. Energetska vrijednost sirutke je 27 kcal ili 112 kJ.

Ključne riječi: nutritivna vrijednost, sirutka, sir Trapist

Koncentracije Fe, Zn i Cu u krvi lakon ovce u ranom stadiju laktacije

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Sažetak

U Republici Hrvatskoj povećan je interes uzgajivača za lakon ovcom. Cilj rada je utvrditi koncentracije Fe, Zn i Cu u krvi lakon ovce u ranom stadiju laktacije i usporediti ih s drugim rezultatima istraživanja. Istraživanje je provedeno na 30 ovaca prosječne dobi od 4 godine u u trećoj laktaciji tj., ranom stadiju laktacije. Utvrđene prosječne koncentracije Fe, Zn i Cu (2,81, 0,91 i 0,97 mg L^{-1}) u krvi lakon ovaca nisu značajnije odstupale u usporedbi s dosadašnjim rezultatima. Nije utvrđena značajna korelacija između količine utvrđenih mikroelemenata u krvi lakon ovce u ranom stadiju laktacije. Nešto viša koncentracija Fe u krvi ukazuje na obrok bogat Fe, ali i promjenama u metabolizmu Fe s obzirom na pojačanu sintezu mlijeka i veći genetski potencijal za mliječnost lakon ovce.

Ključne riječi: lakon ovca, Fe, Zn, Cu, krv, laktacija

Uvod

U Republici Hrvatskoj (RH) sve je veći interes uzgjivača za mliječnim pasminama ovaca i proizvodnjom ovčjeg mlijeka. Shodno tome, povećan je interes uzgajivača za kupnjom lakon pasmine ovaca. Tijekom 2021. godine u Republici Hrvatskoj otkup ovčjeg mlijeka provodilo je 11 mljekara i 6 registriranih obiteljskih sirana. Tako je tijekom 2021. godine u RH isporučeno ukupno 2,76 mil kg ovčjeg mlijeka od 333 proizvođača. U skladu s Pravilnikom o utvrđivanju sastava sirovog mlijeka (NN 136/2020) higijenska kvaliteta ovčjeg mlijeka ocijenjena na temelju utvrđivanja ukupnog broja mikroorganizama je vrlo dobra i glavnina se svrstava u I. razred (92,65 %). Uzgoji lakona u RH organizirani su u kontinentalnom dijelu bogatom žitaricama s obzirom na potrebu korištenja kvalitetnih krmiva pri balansiranju obroka u cilju ispoljavanja genetskog potencijala (Antunović i sur., 2022.a). Prema podacima Hrvatske agencije za hranu i poljoprivredu-HAPIH (2022.) u 2021. godini u RH od ukupnog broja uzgojno valjanih ovaca (44.229 grla) na uzgojno valjane lakon ovce otpada 5,21 %, odnosno 2.405 grla. Županije s najvećim brojem uzgojnih grla lakona su Karlovačka (1.085), Virovitičko-podravska (591) te Vukovarsko-srijemska (191). Manji uzgoji lakon ovce zastupljeni su u Istarskoj (168), Bjelovarsko-bilogorskoj (156) i Brodsko-posavskoj županiji (155), a najmanji u Osječko-baranjskoj županiji (59). Veći broj lakon ovce u RH ukazuje na povećan interes uzgajivača što je i potvrđeno povećanjem njihovoga broja u protekle tri godine za 54 %. Naime, prema podacima HAPIH-a (2022.) 2019. se uzgajalo 1.559 grla lakona i to kod 5 uzgajivača, a 2021. godine već 2.405 grla lakona kod 10 uzgajivača. Lakon ovca (Lacaune) je poznata francuska pasmina čiji je genetski potencijal za mliječnost značajno unaprijeđen kvalitetno provođenom selekcijom na mliječnost. Osim visoke proizvodnje mlijeka, ona ima i naglašenu proizvodnju mesa. U laktaciji od 180 do 200 dana daje oko 200 litara mlijeka, s tim da najbolja grla daju i preko 400 litara mlijeka (Mioč i sur., 2007.). U Francuskoj se kontrola mliječnosti lakona sustavno provodi na 512.000 grla (prosječna mliječnost je 292,6 l mlijeka kroz 168 dana; http://en.france-genetique-elevage.org/Lacaune-dairy-line.html). U RH se pri provođenju kontrole mliječnosti kod lakon ovce u 2021. godini na 749 zaključenih laktacija, pri dužini laktacije od 172 dana i razdoblju mužnje 40 dana, utvrdilo ukupno 433,3 kg mlijeka (325,9 kg mlijeka u razdoblju mužnje te 107,4 kg posisanog mlijeka) ili dnevno 2,5 kg mlijeka po kontroliranoj uzgojno valjanoj lakon ovci (HAPIH, 2022.). Kako je lakon ovca relativno nova pasmina u našim uzgojima potrebno je istražiti i njen metabolički profil pa tako i sadržaj mikroelemenata u krvi tijekom laktacije. Osobito je to naglašeno tijekom rane laktacije kada je i najveća količina izlučenog mlijeka čemu se treba posvetiti dodatna pozornost (Antunović i sur., 2022.b). Stoga se

rani stadij laktacije posebice proučava u cilju spoznaja i pokušaja stabilizacije brojnih metaboličkih procesa. Fe, Zn i Cu trebaju biti zastupljeni u hrani u cilju zadovoljavanja njihovih potreba kao i njihovoga raznorodnog sudjelovanja u brojnim metaboličkim procesima u organizmu ovce (Yokus i sur., 2004.; Antunović i sur., 2021.a; Derar i sur., 2022.). Istraživanje samo nekih koncentracija makroelemenata u krvi lakon ovce u RH proveli su Antunović i sur. (2022.b) dok istraživanja o koncentracijama mikroelemenata u krvi lakon ovaca nema u dostupnoj literaturi. Cilj je ovoga rada utvrditi koncentracije Fe, Zn i Cu u krvi lakon ovce u ranom stadiju laktacije te dobivene koncentracije usporediti s dostupnim istraživanjima.

Materijal i metode

Utvrđivanje koncentracija Fe, Zn i Cu provedeno je u krvi lakon ovaca na obiteljskom gospodarstvu Orkić d.o.o. u Gundincima. Odabrano je 30 ovaca u 3 laktaciji koje se bile na početku laktacije (60. dan). Prosječna dob ovaca bila je 4 godine. Ovce su hranjene peletiranom krmnom smjesom sa 15% sirovih bjelančevina u količini 1,00 kg, 600 g/dan smjesom žitarica (1/3 zob i 2/3 ječam) te sijenom lucerne po volji. Ovce su stočnu sol i vodu imale po volji. Uzorkovanje krvi provedeno je za vrijeme jutarnje mužnje u sterilne vacum epruvete Venoject® (Sterile Terumo Europe, Leuven, Belgium) iz jugularne vene (10 ml). Po transportu u laboratorij krvni uzorci su centrifugirani na 3000 okretaja/min pri čemu je odvojen serum koji je smrznut na -80°C. Nakon odmrzavanja seruma 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 HNO_3 i H_2O_2 na 180°C tijekom 60 min u mirkovalnoj pećnici (CEM Mars 6). Koncentracije Fe, Zn i Cu utvrđene su na ICP (PerkinElmer Optima 2100 DV). Svi uzorci su analizirani u duplikatu. Koncentracije Fe, Zn i Cu u hrani ovaca prikazane su u tablici 1 i izražene su u mg kg^{-1} suhe tvari.

Tablica 1. Koncentracija Fe, Zn i Cu u hrani ovaca

Element, mg kg^{-1} ST	Vrsta hrane			
	Krmna smjesa	Smjesa žitarica	Sijeno	Stočna sol
Fe	377,70	129,90	91,96	37,55
Zn	165,80	20,93	9,58	0,34
Cu	24,50	4,66	5,49	0,43

ST - suha tvar

Rezultati istraživanja utvrđeni su deskriptivnom statistikom u programu SAS (9.4), procedurom MEANS te su izraženi kao srednja vrijednost, standardna devijacija (SD), minimalna i maksimalna vrijednost (MIN i MAX), standardna pogreška srednje vrijednosti (SEM) i koeficijent varijacije (CV). Povezanost između varijabli utvrđene su pomoću Pearsonovog koeficijenta.

Rezultati i rasprava

U tablici 2 prikazana je opisna statistika za koncentracije Fe, Zn i Cu u krvi lakon ovaca u ranoj laktaciji.

Tablica 2. Opisna statistika koncentracija Fe, Zn i Cu u krvi lakon ovaca u ranoj laktaciji

Element, mg L^{-1}	Srednja vrijednost	SD	MIN	MAX	SEM	CV, %
Fe	2,81	1,13	1,71	6,50	0,25	40,26
Zn	0,91	0,22	0,66	1,81	0,05	24,60
Cu	0,97	0,20	0,50	1,34	0,05	20,99

SD - standardna devijacija, SEM - standardna pogreška srednje vrijednosti; MIN - minimalna vrijednost; MAX - maksimalna vrijednost; CV - koeficijent varijacije

Analizom tablice 2 vidljivo je da je prosječna koncentracija Fe, Zn i Cu u krvi lakon ovaca bila 2,81, 0,91 i 0,97 mg L^{-1}. Nešto viši koeficijent varijacije utvrđen je za koncentracije Fe što ukazuje na izraženu varijabilnost ovoga elementa.

Brojni su čimbenici koji utječu na metabolizam mikroelemenata stoga su njihovi metabolički putevi i razumijevanje djelovanja vrlo zahtjevni. jedni od važnih čimbenika su: fiziološki stadij životinje, razina stresa, metode utvrđivanja, razina i izvor mikroelemenata u hrani kao i njihovi antagonisti i dr. (Lopes-Alonso i sur., 2020.). Miglio i sur. (2018.) su u istraživanjima s lakon ovcom u laktaciji utvrdili prosječne koncentracije Fe u krvi od 26,6 mmol L^{-1} te Zn od 78,9 μg L^{-1}. Niže koncentracije Zn i Cu (0,79 i 0,75 mg kg^{-1}) te više koncentracije Fe (117,33 mg kg^{-1}) u krvi križanih ovaca (Sakiz x Awassi) tijekom laktacije u Turskoj u usporedbi s predmetnim istraživanjem utvrdili su Youkus i sur. (2004.). U istraživanju Antunovića i sur. (2021.b) provedenom u RH s travničkom pramenkom u ranoj laktaciji (60. dan), koje su boravile na pašnjaku, utvrđene su niže koncentracije za Fe, Zn i Cu (561,79; 630,02 i 867,06 μg L^{-1}). Niže prosječne koncentracije Fe (1790,7 μg L^{-1}), Zn (664,0 μg L^{-1}) i Cu (0,7 mg kg^{-1}) u serumu ovaca u usporedbi s predmetnim istraživanjem u Austriji utvrdili su također Schweinzer i sur. (2017.). U istraživanjima u Turskoj s Buchi ovcama u laktaciji utvrđene su niže koncentracije Zn i Cu u krvi (Kulcu i Yur, 2003.).

Herd i Hoff (2011.) te Pugh i Baird (2012.) u serumu ovaca utvrdili su sljedeće koncentracije: za Fe od 900 do 2700 te od 700 do 1960 μg L^{-1}, za Zn od 550 do 1200 te od 700 do 1400 μg L^{-1}, a za Cu od 0,75 do 1,7 te od 0,57 do 0,76 mg L^{-1}. Međutim, Page i sur. (2018.) ističu da se odgovarajuće koncentracije Cu u krvi ovaca kreću od 0,70 do 1,00 μg dL^{-1}, koncentracije Fe od 116 do 122 μg dL^{-1}, a koncentracije Zn od 0,8 do 1,2 μg dL^{-1}. Navedeno ukazuje da su utvrđene koncentracije Zn i Cu u krvi lakon ovaca u ranoj laktaciji u predmetnom istraživanju bile u okviru referentnih vrijednosti dok su koncentracije Fe bile nešto više. Navedeno ukazuje na kvalitetnu nutritivnu opskrbu ovaca tijekom laktacije s istraživanim mikroelementima (Tablica 1.). Razlog viših koncentracija Fe u krvi ovaca može biti pod utjecajem više različitih čimbenika, prije svega o primijenjenoj metodi utvrđivanja, ali se može povezati i s pojačanim izlučivanjem Fe putem mlijeka jer je u tom razdoblju i najviša sekrecija mlijeka. U istraživanjima provedenim u Litvi, Autukaitė i sur. (2021.), također ističu različite promjene u koncentracijama Cu u krvi ovaca koje ovise i o pasmini (safolk, merino i crnoglava litvanska ovca) te da su koncnetracije Cu ovisne ne samo o stresu uzrokovanom promjenom kompozicije obroka nego i o temperaturi okoliša, ali i drugačijem fiziološkom odgovoru različitih pasmina ovaca. U istraživanjima s lakon ovcom u laktaciji provedenim u Italiji, Miglio i sur. (2018.) su utvrdili da odvijanje različitih promjena u serumu lakon ovaca u usporedbi s drugim pasminama ovaca, na što još utječu i dob te fiziološki status.

Tablica 3. Korelacijska povezanost između utvrđenih koncentracija Fe, Zn i Cu u krvi lakon ovce u ranoj laktaciji

Element, mg L^{-1}	Fe	Zn	Cu
Fe	1,000	-	-
Zn	0,140	1,00	-
Cu	-0,181	0,155	1,00

Analizom tablice 3 vidljivo je da nije bilo značajne povezanosti između istraživanih koncentracija Fe, Zn i Cu u krvi lakon ovce. U istraživanju Antunovića i sur. (2021.a) provedenom s travničkom pramenkom utvrđena je značajna povezanost između Cu:Zn (r = 0,301; P=0,002) i Fe:Cu (r = 0,395; P=0,010) dok povezanost između Zn:Fe nije bila značajna (r = 0,161; P= 0,308).

Zaključak

Utvrđene koncentracije Fe, Zn i Cu u krvi lakon ovaca ukazuju na njihovu kvalitetnu opskrbu u ranoj laktaciji putem hrane. Nešto više koncentracije Fe u krvi ovaca ukazuju na bogatstvo krmiva Fe, ali i promjene u metabolizmu Fe s obzirom na pojačanu sintezu mlijeka u ranoj laktaciji i utjecaj pojačane proizvodnje mlijeka ove mliječne pasmine.

Napomena

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Concentrations of Fe, Zn and Cu in the blood of Lacaune sheep in the early stage of lactation

Abstract

In the Republic of Croatia, farmers interest in Lacaune sheep has increased. The aim of this study was to determine the concentrations of Fe, Zn and Cu in the blood of Lacaune sheep in the early lactation period and to compare them with available research. The present study was conducted with 30 sheep aged about 4 years in the third lactation, i.e., early lactation. The average concentrations of Fe, Zn and Cu (2.81, 0.91 and 0.97 mg L^{-1}, respectively) determined in the blood of Lacaune sheep were not significantly different from previous results. No significant correlations were found between the determined microelements in the blood of Lacaune sheep in the early stages of lactation. A slightly higher concentration of Fe in the blood indicates a diet rich in Fe, but also changes in Fe metabolism with regard to increased milk synthesis and higher genetic potential for milk production in Lacaune sheep.

Keywords: Lacaune sheep, Fe, Zn, Cu, blood, lactation

Prinos i kvaliteta djetelinsko-travne smjese crvene djeteline i klupčaste oštrice u različitim agroekološkim uvjetima

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Sažetak

Istraživanje produktivnosti i kvalitete djetelinsko-travne smjese (DTS) crvene djeteline i klupčaste oštrice provedeno je tijekom dvije godine na dvije lokacije u RH.

Prosječni prinos suhe tvari (ST) tijekom dvije godine istraživanja na lokaciji Ogulin iznosio je 8,3 t ST ha^{-1}, dok je u Novom Marofu utvrđen prosječni prinos od 6,9 t ST ha^{-1}. Produktivnost DTS na pojedinoj lokaciji značajno je ovisila o vegetacijskoj sezoni, pri čemu je značajno viši prinos ST ($P<0,05$) utvrđen na lokaciji Ogulin samo u jednoj godini istraživanja.

Iako je utvrđen značajan utjecaj vegetacijske sezone na pokazatelje hranjive vrijednosti DTS-a, reakcija DTS-a na ekološke uvijete u pojedinoj vegetacijskoj sezoni mijenjala se ovisno o lokaciji istraživanja.

Ključne riječi: klupčasta oštrica, crvena djetelina, djetelinsko-travna smjesa, kvaliteta, prinos

Uvod

Čimbenici koji utječu na prinos i kvalitetu krme s travnjaka mogu se grupirati u tri međusobno povezane grupe: **čimbenici biljke (genetski, fiziološki, anatomski), čimbenici okoline (klima i tlo) te agrotehnički zahvati u proizvodnji voluminozne krme.** Međusobno povezani oni čine sustav proizvodnje voluminozne krme. U sklopu razvoja i evaluacije sustava proizvodnje voluminozne krme na razini određenog agroekološkog područja ili pak na razini farme, a u cilju povećanja konkurentnosti farmi, potrebno je sustavno provoditi istraživanja produktivnosti i kvalitete vrsta i kultivara za proizvodnju voluminozne krme.

Čimbenici okoline znatno utječu na prinos (Frankow-Lindberg i sur., 2009.) i kvalitetu voluminozne krme (Buxton i Fales, 1994.). Oni modificiraju brzinu rasta i razvoja, utječu na kemijski sastav biljnih dijelova i morfologiju. Klimatski faktori poput temperature i oborine modificiraju utjecaj stadija razvoja biljke na sadržaj neprobavljivih neutralnih detergent vlakana (NDV) (Nordheim-Viken i Volden, 2009.). K tome, reakcija travnih vrsta na zahvate managementa poput frekvencije defolijacije mijenja se s obzirom na klimatske uvjete (Volesky i Anderson, 2007.). Općenito govoreći, u uvjetima klimatskih promjena, u kojima je veća vjerojatnost pojave suše, produktivnost čistih kultura krmnih trava, mahunarki te samoniklih poluprirodnih travnjaka se smanjuje (Breitsameter i sur., 2014; Hoffstätter-Müncheberg i sur., 2014.). U istraživanju utjecaja količine oborina na prinos ST klupčaste oštrice i trstikaste vlasulje Kohoutek i sur. (2012.) su utvrdili da je na prinos ST značajnije utjecala količina oborina u vegetacijskom sezoni nego ukupna godišnja količina oborina. Petrychenko i sur. (2012.) navode da se tijekom sušnih godina produktivnost većine DTS-a na nizinskim livadama i poplavnim livadama smanjuje za 1-36%. U velikom broju istraživanja utvrđen je utjecaj lokacije na kvalitetu biljne mase poput sadržaja NDV-a (Brink i sur., 2010.) ili sadržaja vodotopivih ugljikohidrata (Perčulija i sur., 2005.; Tremblay i sur., 2005.).

Istraživanjem produktivnosti i kvalitete vrsta i kultivara za proizvodnju voluminozne krme u različitim širokim agroekološkim uvjetima dobivaju se informacije o njihovom potencijalu i ulozi u sustavu proizvodnje voluminozne krme na određenom agroekološkom području ili na razini farme. Cilj ovoga istraživanja bio je utvrditi prinos suhe tvari i kvalitetu krme djetelinsko travne smjese crvene djeteline i klupčaste oštrice u različitim agroekološkim uvjetima, tijekom dvije godine na dvije lokacije, koje se razlikuju kako po klimatskim tako i po pedološkim karakteristikama.

Materijal i metode

Istraživanje produktivnosti i kvalitete smjese crvene djeteline i klupčaste oštrice je provedeno tijekom dvije vegetacijske sezone (2012. i 2013. godine) na dvije lokacije (Novi Marof i Ogulin) u Republici Hrvatskoj. Klimatski podaci (DHMZ, 2022.) za obje lokacije i vegetacijske sezone u obje godine istraživanja prikazani su u grafikonu 1. Tlo na pokusnoj parceli lokacije Ogulin sadržavalo je 0,71 mg P_2O_5 kg^{-1} tla, 2,25 mg K_2O kg^{-1} tla, 0,18 % ukupnog dušika, 3,53 % humusa, a reakcija tla je iznosila pH 5,72 (H_2O) odnosno pH 4,46 (KCl). Tlo pokusne površine u Novom Marofu sadržavalo je 0,06 mg P_2O_5 kg^{-1} tla, 1,18 mg K_2O kg^{-1} tla, 0,11 % ukupnog dušika, 2,03% humusa, a reakcija tla je iznosila pH 6,42 (H_2O) odnosno pH 5,07 (KCl).

Na obje lokacije osnovna obrada tla obavljena je oranjem na dubinu 25-30 cm, u jesen 2011. godine. Predsjetvena obrada izvršena je frezom (N. Marof) i roto drljačom (Ogulin). Gnojidba je na obje lokacije iznosila 350 kg ha^{-1} NPK 15:15:15. Sjetva je izvršena 14.3.2012 (N. Marof) i 21.3.2012 (Ogulin). Na obje je lokacije posijana je djetelinsko travna smjesa crvene djeteline (*Trifolium pratense* L., cv. Viva; 7,5 kg ha^{-1}) i klupčaste oštrice (*Dactylis glomerata* L., cv. B15; 10 kg ha^{-1}). Sjetva je na obje lokacije obavljena žitnom sijačicom na razmak između redova od 11 cm. Nakon sjetve tlo je povaljano. Uzorkovanje biljnog materijala izvršeno je u fazi početka cvatnje crvene djeteline. Kod svih tretmana uzorci su uzeti destruktivnom metodom, košnjom biljne mase sa površine 0,3 m^2, električnim škarama (Gardena), na 10 slučajnih mjesta po osnovnoj parceli. Pokošena zelena masa je izvagana te je uzet poduzorak za kemijske analize.

Uzorci su osušeni u sušioniku na temperaturi 60 °C, u trajanju od 48 sati, te samljeveni u mlinu, na veličinu čestica 1 mm. Utvrđivanje hranjivosti rađeno je na uprosječenim prosječnim godišnjim uzorcima DTS-a na svakoj lokaciji, ovisno o broju otkosa i relativnom udjelu pojedinog otkosa u ukupnom godišnjem prinosu ST. Analiza hranjivosti krme utvrđena je korištenjem NIR spektroskopije. Uzorci su skenirani u infracrvenom elektromagnetskom spektru, u rasponu valnih duljina 1100 - 2500 nm, u intervalima po 2 nm, korištenjem programa ISI SCAN. Svaki uzorak skeniran je dva puta.

Prosječni godišnji prinos ST smjese na svakoj lokaciji izračunat je kao zbroj prinosa ST pojedinih otkosa u jednoj godini istraživanja. Ukupno je tijekom vegetacijske sezone 2012. godine na obje lokacije skinuta po 3 otkosa dok je tijekom 2013. godine skinuto po 2 otkosa.

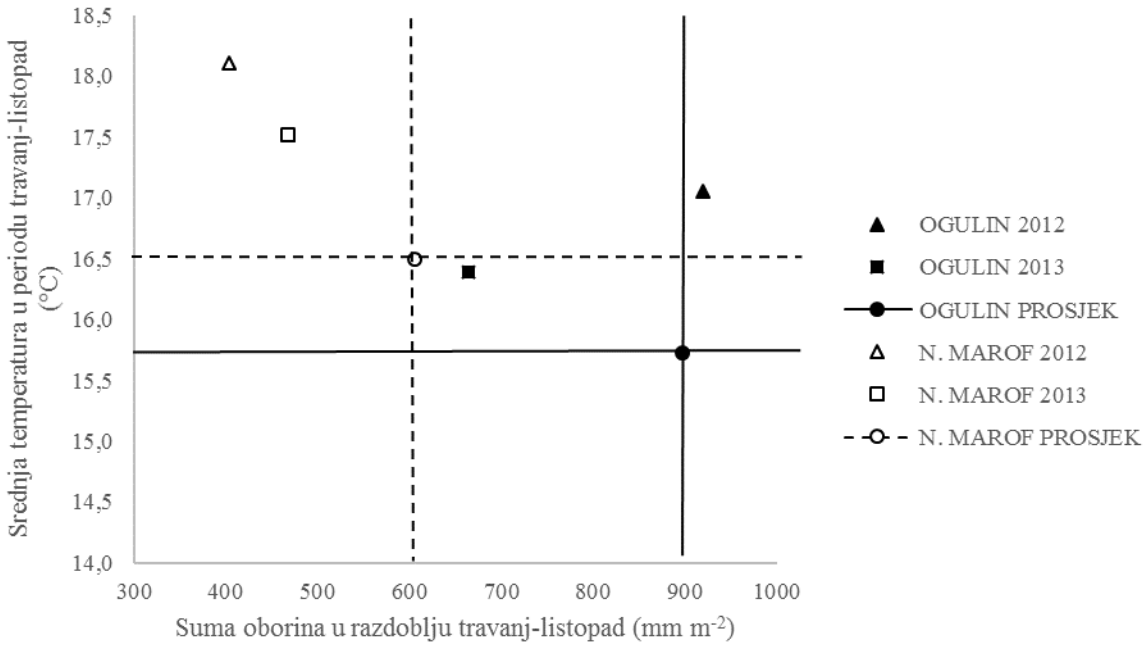
Pokus je bio postavljen po potpuno slučajnom rasporedu. Statistička analiza podataka izvedena je analizom varijance (ANOVA) i testom usporedbe srednjih vrijednosti korištenjem statističkog programa SAS (SAS, 1999.).

Rezultati i rasprava

Uspoređujući klimatološke podataka (DHMZ, 2022.) na lokacijama na kojima je provedeno istraživanje, Ogulin u usporedbi sa Novim Marofom, ima za 0,4 °C nižu srednju godišnju temperaturu zraka i 622 mm višu godišnju količinu oborina, odnosno nešto hladniju i vlažniju klimu (podaci nisu prikazani). Na obje lokacije, ako se uzimaju podaci za cijelu godinu, 2012. godina se može se okarakterizirati kao toplija i sušnija od višegodišnjeg prosjeka, dok je 2013. godina bila nešto toplija, no uz nešto veću količinu oborina od višegodišnjeg prosjeka.

Međutim, uspoređujući srednje temperature i količine oborina tijekom vegetacijske sezone 2012. i 2013. godine na lokacijama Ogulin i Novi Marof (Grafikon 1), razvidno je da se obje godine na lokaciji Novi Marof mogu okarakterizirati kao toplije i sušnije od prosjeka. Nedostatak oborina tijekom vegetacijskog perioda 2012. godine iznosio je 201 mm, donosno 138 mm tijekom 2013. godine, dok je prosječna temperatura vegetacijskog perioda u 2012. godini bila 1,6°C viša, a u 2013. godini 1°C viša u usporedbi sa višegodišnjim prosjekom.

Na lokaciji Ogulin, 2012. godina bila je toplija (+1,3°C) uz vrlo mali višak oborina (+ 22 mm) tijekom vegetacije u usporedbi sa prosjekom, dok je 2013. godina bila toplija (+0,7°C) i sušnija (- 233 mm) u usporedbi sa višegodišnjim prosjekom (Grafikon 1).



Grafikon 1. Srednje temperature i sume oborina tijekom vegetacijskog perioda (travanj-listopad) na istraživanim lokacijama (Ogulin i N. Marof) u 2012. i 2013. godini i višegodišnji prosjeci (DHMZ, 2022.)

Prosječni prinos ST DTS-a utvrđen tijekom dvije godine istraživanja na dvije lokacije iznosio je 7,6 t ST ha^{-1}. Za usporedbu, prosječni prinos ST smjese klupčaste oštrice i crvene djeteline u režimu korištenja sa tri otkosa godišnje iznosio je 8,9 t ST ha^{-1} (Farnham i George, 1994.). Bošnjak i sur. (2013.) su na Medvednici (638 m nadmorske visine) u godini punog korištenja smjese klupčaste oštrice i crvene djeteline utvrdili prinos ST od 13,7 t ST ha^{-1}.

Agroekološki uvjeti lokacije istraživanja značajno su utjecali na prinos ST DTS-a crvene djeteline i klupčaste oštrice (P=0,03; Tablica 1). Prosječni prinos ST utvrđen na lokaciji Ogulin tijekom dvije godine istraživanja bio je za 21 % viši u usporedbi sa lokacijom Novi Marof. Međutim, reakcija DTS-a u prinosu ST na pojedinim lokacijama nije bila identična u obje vegetacijske sezone. Tijekom 2012 godine prinos ST utvrđen na lokaciji Ogulin bio je za 2,9 t ST ha^{-1} odnosno 47 % veći (P=0,0138) u usporedbi sa lokacijom Novi Marof (Tablica 1). Tijekom 2013 godine nije utvrđena signifikantna razlika između lokacija u prinosu ST (P=0,9213). Ovakav rezultat jasno ukazuje na važnost količine oborina tijekom vegetacijske sezone u formiranju prinosa travnjaka, kao što to u svom radu ističu i Kohoutek i sur. (2012.) naglašavajući važnost količine oborina tijekom vegetacijske sezone.

Uspoređujući prosječne vrijednosti prinosa ST DTS-a na obje lokacije u vegetacijskim sezonama 2012 i 2013 nije utvrđen signifikantni utjecaj vegetacijske sezone (P=0,9533) na prinos ST DTS-a crvene djeteline i klupčaste oštrice (Tablica 1). Međutim, utvrđena je razlika u reakciji DTS-a na klimatske uvjete tijekom 2012. i 2013. godine unutar istraživanih lokacija. Tako je na lokaciji Ogulin utvrđen 17 % manji prinos ST DTS-a u 2013. godini u usporedbi sa godinom sjetve (P=0,0051) dok se prinos ST DTS-a utvrđen na lokaciji Novi Marof u 2012 godini nije značajno razlikovao (P=0,1106) u usporedbi sa prosječnim prinosom ST utvrđenim na istoj lokaciji tijekom 2013. godine (Tablica 1).

Tablica 1. Prinos ST (t ha^{-1}) DTS-a crvene djeteline i klupčaste oštrice na lokacijama Ogulin i Novi Marof tijekom 2012. i 2013. godine

Lokacija/Godina	2012	2013	Prosjek	SEM†
Novi Marof	6,14	7,56	6,85	0.49
Ogulin	9,03	7,53	8,28	0.19
Prosjek	7,59	7,54		
SEM	0,51		0,40	
SEM††	0,49	0,20		

† standardna pogreška srednjih vrijednosti godina unutar lokacije
†† standardna pogreška srednjih vrijednosti lokacija unutar godine

Osim u slučaju sadržaja sirovih bjelančevina (SB) i metaboličke energije (ME), utjecaj lokacije na ostale pokazatelje kvalitete DTS-a crvene djeteline i klupčaste oštrice ovisio je o klimatskim uvjetima pojedine vegetacijske sezone (Tablica 2). Tako je utjecaj lokacije na sadržaj SB potpuno izostao (P>0,05) u obje godine istraživanja, dok je u obje godine istraživanja, značajno veći (P<0,05) sadržaj ME utvrđen na lokaciji Ogulin (Tablica 2). Utjecaj lokacije na sadržaj ST, sadržaj NDV, vodotopivih ugljikohidrata (VUH) i probavljivost organske tvari (OT) bio je izraženiji tijekom 2013. godine (Tablica 2), pri čemu je na lokaciji Ogulin utvrđen veći sadržaj NDV i VUH i ME, te veća probavljivost OT u usporedbi sa lokacijom Novi Marof.

Utjecaj vegetacijske sezone na kvalitetu biljne mase smjese klupčaste oštrice bio je različit na pojedinim lokacijama (Tablica 2). Tako je na lokaciji Novi Marof, utjecaj godine na pokazatelje kvalitete gotovo u potpunosti izostao, dok je na lokaciji Ogulin utjecaj godine na kvalitetu biljne mase bio značajan (Tablica 2). Tijekom 2013. godine DTS na lokaciji Ogulin sadržavao je manje ST, manje SB, veći sadržaj VUH i ME, te veću probavljivost OT (P<0,05) u usporedbi sa 2012. godinom.

Tablica 2. Pokazatelji hranjivosti DTS-a crvene djeteline i klupčaste oštrice na lokacijama Ogulin i Novi Marof tijekom 2012. i 2013. godine

Pokazatelji hranjivosti		ST	NDV	SB	VUH	POT	ME
Godina/lokacija		g kg^{-1} SM	g kg^{-1} ST				MJ kg^{-1} ST
2012	Novi Marof	185,0	641,3	142,3	56,3	563,3	9,6
	Ogulin	207,4	665,2	145,7	47,1	680,0	10,2
2013	Novi Marof	190,7	636,8	132,8	48,8	633,3	9,5
	Ogulin	156,8	668,8	122	64,5	691,7	10,4
Signifikantnost							
lokacije u 2012		NS	NS	NS	NS	NS	**
lokacije u 2013		*	**	NS	*	***	***
godine u Novom Marofu		NS	NS	NS	NS	NS	NS
godine u Ogulinu		***	NS	*	***	*	*
Prosjeci							
	Novi Marof	187,8	639,1	137,6	52,6	598,3	9,6
	Ogulin	182,1	667,0	133,8	55,8	685,9	10,3
Signifikantnost		NS	**	NS	NS	*	**
	2012	196,2	653,3	144,0	51,7	621,6	9,9
	2013	173,8	652,8	127,4	56,7	662,5	9,9
Signifikantnost		NS	NS	**	NS	NS	NS

*NS – nije signifikantno; *, **, *** - signifikantno pri P<0,05, P<0,01, P<0,001, respektivno; SM – svježe mase;*

Zaključci

U uvjetima nedostatka oborina i visokih temperatura tijekom vegetacijskog perioda, farmeri moraju računati na značajan pad produktivnosti smjese crvene djeteline i klupčaste oštrice. Iako dominantno pod utjecajem fenofaze u trenutku košnje, kvaliteta biljne mase kao reakcija na klimatske uvjete vegetacijske sezone znatno varira i mijenja se ovisno o agroekološkim uvjetima pojedine lokacije.

Napomena

Istraživanje je provedeno u sklopu projekta „Primjena novih tehnologija proizvodnje voluminozne krme u cilju povećanja konkurentnosti obiteljskih mliječnih farmi“ Vijeća za istraživanje u poljoprivredi (VIP) Ministarstva poljoprivrede, ribarstva i ruralnog razvoja RH, tijekom 2011-2013. godine.

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Yield and quality of clover-grass mixture of red clover and orchardgrass in different agroecological conditions

Abstract

Research on the productivity and quality of grass-clover mixture (GCM) consisted of red clover and orchardgrass was carried out during two years at two locations in the Republic of Croatia.

The average dry matter yield (DMY) during the two years of investigation at Ogulin location was 8.3 t DM ha^{-1}, while in Novi Marof 6.9 t DM ha^{-1}. The productivity of GCM at a particular location was significantly depended on the growing season, whereby a significantly higher DMY ($P>0.05$) was determined at the Ogulin location only in one year of the study.

Although a significant influence of climatic factors on the nutritional value of grass-clover mixture was determined, the reaction of grass-clover mixture to ecological conditions in a particular growing season varied depending on the location of the study.

Keywords: orchardgrass, red clover, grass-clover mixture, quality, yield

Utjecaj gnojidbe dušikom na probavljivost i sadržaj metaboličke energije krme samoniklog poluprirodnog pašnjaka

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Sažetak

Gnojidba dušikom (N) je vrlo bitna agrotehnička mjera u proizvodnji krme na travnjacima koja utječe na produktivnost travnjaka, botanički sastav i hranjivu vrijednost krme. Cilj ovog istraživanja bio je utvrditi utjecaj primjene N na probavljivost organske tvari (POT) i sadržaj metaboličke energije (ME) u dostupnoj biljnoj masi samoniklog poluprirodnog pašnjaka zajednice *Arrhenaretheretum medioeuropaeum*. Istraživana su tri gnojidbena tretmana: N35 (35 kg N ha^{-1} god^{-1}), N100 (100 kg N ha^{-1} god^{-1}) i N150 (150 kg N ha^{-1} god^{-1}), tijekom tri vegetacijske sezone. Gnojidba N nije utjecala na POT i sadržaj ME u dostupnoj biljnoj masi samoniklog poluprirodnog pašnjaka. Izostanak reakcije travnjaka u POT kao i sadržaju ME (P>0,05) utvrđen je u svim godinama (P>0,05), ukazujući da se takva reakcija može očekivati u širokom rasponu klimatskih uvjeta. Međutim, utvrđen je jak utjecaj vegetacijske sezone na istraživana svojstva u svim istraživanim razinama N gnojidbe (P<0,0001).

Ključne riječi: dušik, gnojidba, poluprirodni pašnjak, probavljivost, metabolička energija

Uvod

Primjena N ima ključnu ulogu u proizvodnji voluminozne krme na travnjacima. Vrlo je dobro poznato da primjena N rezultira povećanjem produktivnosti travnjaka (Schils i Snijders, 2004; Annicchiarico i Tomasoni, 2010; Abraha i sur., 2015.). Osim što promovira rast biljne mase, N također utječe na botanički sastav travnjaka (Kalmbacher i Martin, 1996.), ali i na hranjivu vrijednost voluminozne krme. Povećanje N gnojidbe rezultira povećanjem sadržaja sirovih bjelančevina (SB) u biljnoj masi (Pelletier i sur., 2009.) i smanjenjem sadržaja vodotopivih ugljikohidrata bez utjecaja na sadržaj strukturnih ugljikohidrata u voluminoznoj krmi (Enriquez-Hidalgo i sur., 2018; Cinar i sur., 2020.).

Primjena N nema utjecaja na probavljivost organske tvari (OT) čistih kultura trava i djetelinsko travnih smjesa (DTS) (Abraha i sur., 2015; Enriquez-Hidalgo i sur., 2018.) ili je on vrlo mali. Valk i sur. (1996.) zaključuju da da se POT trava većim dijelom mijenja pod utjecajem stadija rasta nego pod utjecajem N gnojidbe .

U preglednom radu Peyraud i Astigarraga (1998.) navode da se sa smanjenjem primjene N sadržaj energije voluminozne krme ne mijenja ili se mijenja vrlo malo. Međutim, u nekim je radovima utvrđeno smanjenje sadržaja ME sa povećanjem N gnojidbe kod trstikaste vlasulje (Wolf i von Boberfeld, 2003.) i talijanskog ljulja (Abraha i sur., 2015.).

Dok je utjecaj primjene N na kvalitetu krme sijanih travnjaka vrlo dobro istražen, naročito sa aspekta sadržaja sirovih bjelančevina (SB), vrlo je malo radova koji opisuju utjecaj primjene N na kvalitetu krme poluprirodnih travnjaka u uvjetima napasivanja. Cilj ovoga rada je utvrditi utjecaj primjene N na probavljivost OT i sadržaj ME krme samoniklog poluprirodnog pašnjaka.

Materijal i metode

Istraživanje je provedeno tijekom tri godine na pokusnoj površini pokušališta „Centar za travnjaštvo" Sveučilišta u Zagrebu Agronomskog fakulteta (636 m n.v.). Pokusni pašnjak pripada travnjačkoj zajednici *Arrhenaretheretum medioeuropaeum* (Hulina, 1984.), a prije početka eksperimenta koristio se kombinirano, košnjom i napasivanjem govedima.

Istraživan je utjecaj gnojidbe N na probavljivost organske tvari i sadržaj metaboličke energije u dostupnoj biljnoj masi. Gnojidbeni tretmani su uključivali tri razine primjene N u količini od 35 kg N ha^{-1} god^{-1} (N35), 100 kg N ha^{-1} god^{-1} (N100) i 150 kg N ha^{-1} god^{-1} (N150). Kod svih tretmana, početkom proljetnog porasta biljne mase, primijenjeno je 500 kg ha^{-1} kompleksnog gnojiva NPK 7:20:30. Na taj način, uz 100 kg ha^{-1} P_2O_5 i 150 kg ha^{-1} K_2O primijenjeno je i 35 kg N ha^{-1}, što je kod tretmana N35 bila i ukupna godišnja količina N te tijekom vegetacije nije bilo primjene dodatnih količina N. Kod ostalih tretmana (N100 i N150) ostatak N bio je primijenjen korištenjem KAN-a (27 % N) u tri jednake aplikacije nakon prva tri turnusa napasivanja u svakoj godini istraživanja.

Za napasivanje su korištene ovce Charolais pasmine, prosječne tjelesne mase 70 kg. Intenzitet napasivanja bi je određen visinom tratine prije i nakon turnusa napasivanja. S napasivanjem se počinjalo kada je prosječna visina tratine dosegla 13-15 cm, dok je kraj napasivanja bio pri ciljanoj prosječnoj visini tratine od 5 cm na kraju turnusa napasivanja. Visina tratine utvrđivana je mjernim štapom.

U cilju utvrđivanja probavljivosti OT i sadržaja ME, prije početka svakoga turnusa napasivanja uzeti su uzorci biljne mase košnjom na visinu od 5 cm. Uzorci su uzeti sa 3 slučajno odabrana mjesta po osnovnoj parceli (27,5 m^2), upotrebom okvira površine 0,3 m^2. Za košnju su korištene ručne škare.

Uzorci su sušeni u sušioniku na temperaturi 60°C tijekom 48 sati nakon čega su samljeveni u mlinu čekićaru (Christy, Model 11) na veličinu čestica od 1 mm.

Probavljivost OT utvrđena je izračunom nakon *in vitro* razgradnje biljne mase korištenjem automatskog sustava proizvodnje plina (Cone i sur., 1996.) . Kumulativna proizvodnja plina matematički je podijeljena koristeći trofazni model (Groot i sur., 1996; Cone i sur., 1997.) korištenjem programa NLREG (Sherrod, 1995.). Detalji inkubacijskog postupka opisani su u radu Bošnjak (2008.).

Probavljivost OT (POT) je izračunata korištenjem formule (Gosselink i sur., 2004.):

$$POT = 300 + 1{,}616 \times GP20 + 0{,}332 \times SB$$

gdje je: GP20 – količina proizvedenog plina (ml g^{-1} OT) nakon 20 h inkubacije; SB – sadržaj sirovih bjelančevina (g kg^{-1} suhe tvari (ST)). Sadržaj SB utvrđen je metodom po Kjeldahlu (AOAC, 2012.).

Sadržaj ME je izračunat korištenjem sljedeće formule (Farina i sur., 2011.):

$$ME\ (MJ\ kg^{-1}\ ST) = [POT\ (\%) \times 0{,}16] - 1{,}8$$

Podaci su obrađeni analizom varijance, u statističkom programu SAS, koristeći MIXED proceduru (SAS, 1999.).

Rezultati i rasprava

U 2002. godini prosječna godišnja temperatura zraka iznosila je 7,9 °C, što je za 1,3 °C toplije od višegodišnjeg prosjeka, uz ukupnu godišnju količinu oborina od 1251,2 mm, što je 20,2 mm više od višegodišnjeg prosjeka. Srednja godišnja temperatura zraka za 2003. godinu iznosila je 7,6 °C, što je 1 °C više u odnosu na višegodišnji prosjek. Ukupna količina oborina u 2003. godini je bila znatno ispod višegodišnjeg prosjeka, sa ukupno izmjerenih 889,5 mm oborina, odnosno 38 % manje od višegodišnjeg prosjeka. U 2004. godini srednja godišnja temperatura zraka iznosila je 6,6 °C, što je identično višegodišnjem prosjeku temperature, sa ukupnom godišnjom količinom oborina od 1283,5 mm, što je 52 mm više od višegodišnjeg prosjeka.

Prosječno za sve gnojidbene tretmane, POT je iznosila 741,97 g kg^{-1} OT. Za usporedbu, Abraha i sur. (2015.) su utvrdili prosječnu POT talijanskog ljulja u uvjetima frekventne defolijacije od 798 g kg^{-1} OT dok su Enriquez-Hidalgo

i sur. (2018.), također u uvjetima napasivanja, utvrdili POT biljne mase engleskog ljulja od 770 g kg^{-1} OT te smjese engleskog ljulja sa bijelom djetelinom od 800 g kg^{-1} OT.

Primjena N nije utjecala na POT (P>0,05, Tablica 1). Ovi rezultati i na primjeru poluprirodnog pašnjaka potvrđuju navode i zaključke iz ranijih istraživanja u kojima primjena N nije utjecala na POT kako u uvjetima niže primjene N, do 60 kg N ha^{-1} (Abraha i sur., 2015.) tako i u uvjetima vrlo visoke gnojidbe N i primjene do 240 kg N ha^{-1} god^{-1} (Enriquez-Hidalgo i sur., 2018.).

Nesignifikantna interakcija N × godina (P>0,05) ukazuje da je ovakva reakcija travnjaka u POT dostupne biljne mase bila slična u svim istraživanim godinama.

Međutim, POT dostupne biljne mase bila je različita u pojedinim godinama istraživanja (Tablica 1), ukazujući na veliki utjecaj okolišnih čimbenika na POT dostupne biljne mase poluprirodnog pašnjaka. Razlika u POT biljne mase poluprirodnog pašnjaka između pojedinih vegetacijskih sezona iznosila je 54,4 g kg^{-1} OT (Tablica 1).

Tablica 1. Utjecaj N gnojidbe na probavljivost OT (POT) u dostupnoj biljnoj masi poluprirodnog pašnjaka

		POT (g kg^{-1} OT)		
Gnojidba	**2002**	**2003**	**2004**	**Prosjek**
N35	709,3	765,4	743,2	739,3
N100	717,5	773,8	748,9	746,8
N150	709,8	760,5	749,0	739,8
Prosjek	712,2[c]	766,6[a]	747,1[b]	
Izvor varijabilnosti	**Signifikantnost**	**P**		
N	NS	0,1009		
Godina	***	<0,0001		
N × Godina	NS	0,6792		

a, b, c – prosjeci označeni različitim slovima statistički se razlikuju (P<0,0001); NS – nije signifikantno

Prosječni sadržaj ME u dostupnoj biljnoj masi poluprirodnog pašnjaka u ovom istraživanju iznosio je 10,07 MJ kg^{-1} ST. U istraživanju Abraha i sur. (2015.) sadržaj ME u biljnoj masi talijanskog ljulja u uvjetima frekventne defolijacije iznosio je 11,26 MJ kg^{-1} ST.

Gnojidba N nije utjecala (P>0,05) na sadržaj ME u dostupnoj biljnoj masi poluprirodnog pašnjaka (Tablica 2), pri čemu se su se prosječne vrijednosti sadržaja ME između gnojidbenih tretmana kretale unutar 0,12 MJ kg^{-1} ST. Ovakav rezultat u skladu je s ranijim istraživanjima koja su saželi Peyraud i Astigarraga (1998.), koji navode da sa smanjenjem primjene N sadržaj energije voluminozne krme ostaje gotovo nepromijenjen. S druge strane, unatoč činjenici da je sadržaj ME u istraživanju Wolf i von Boberfeld (2003.) utvrđivan u uvjetima odgođenog korištenja i u kasnoj fazi rasta i razvoja trstikaste vlasulje, autori su utvrdili trend pada sadržaja ME s povećanjem N gnojidbe od 0 do 150 kg N ha^{-1} god^{-1}. Slično tome, ali u uvjetima frekventne defolijacije talijanskog ljulja, Abraha i sur. (2015.) su utvrdili pad sadržaja ME sa povećanjem gnojidbe N već i pri manjoj količini primijenjenog N (60 kg N ha^{-1} god^{-1}), pri čemu je sadržaj ME bio za 0,48 MJ kg^{-1} ST niži u usporedbi sa kontrolom i 0,27 MJ kg^{-1} ST niži u usporedbi sa primjenom 30 kg N ha^{-1} god^{-1} .

Ovakva reakcija travnjaka u sadržaju ME na primijenjeni N bila je slična u svim godinama istraživanja, na što ukazuje nesignifikantna interakcija N × Godina (P<0,05; Tablica 2). To ukazuje na stabilnost reakcije travnjaka na primijenjeni N u sadržaju ME, odnosno da se slična reakcija može očekivati u širokom rasponu klimatskih uvjeta.

Međutim, uspoređujući prosječne vrijednosti sadržaja ME u pojedinim godinama (Tablica 2), prosječno za sve gnojidbene tretmane, rezultati ukazuju na vrlo veliki utjecaj klimatskih uvjeta na sadržaj ME u krmi poluprirodnog pašnjaka. Razlika u prosječnom sadržaju ME između vegetacijskih sezona utvrđena u ovom istraživanju iznosila je i do 0,88 MJ kg^{-1} ST (Tablica 2).

Tablica 2. Utjecaj N gnojidbe na sadržaj ME u dostupnoj biljnoj masi poluprirodnog pašnjaka

	ME (MJ kg^{-1} ST)			
Gnojidba	**2002**	**2003**	**2004**	**Prosjek**
N35	9,55	10,45	10,09	10,03
N100	9,68	10,58	10,18	10,15
N150	9,56	10,37	10,19	10,04
Prosjek	$9,59^{c}$	$10,47^{a}$	$10,15^{b}$	
Izvor varijabilnosti	**Signifikantnost**	**P**		
N	NS	0,1050		
Godina	***	<0,0001		
N × Godina	NS	0,6807		

a, b, c – prosjeci označeni različitim slovima statistički se razlikuju (P<0,0001); NS – nije signifikantno

Zaključak

Primjena do 150 kg N ha^{-1} god^{-1} ne utječe na probavljivost OT i sadržaj ME u dostupnoj krmi poluprirodnog pašnjaka. Izostanak učinka N gnojidbe na probavljivost OT i sadržaj ME ostaje stabilan bez obzira na klimatske uvijete pojedinih vegetacijskih sezona. Međutim, bez obzira na N gnojidbu, probavljivost OT i sadržaj ME krme sa poluprirodnog pašnjaka znatno variraju između pojedinih vegetacijskih sezona.

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The effect of nitrogen fertilization on the digestibility and metabolic energy content of forage from indigenious semi-natural pasture

Abstract

Nitrogen (N) fertilization is a very important part of grassland management, which affects the grassland productivity, the botanical composition and forage nutritional value. The aim of this research was to determine the influence of N application on the digestibility of organic matter (POT) and the metabolic energy content (ME) of indigenous semi-natural pasture which belongs to the *Arrhenaretheretum medioeuropaeum* community. Three fertilization treatments were investigated: N35 (35 kg N ha^{-1} $year^{-1}$), N100 (100 kg N ha^{-1} $year^{-1}$) and N150 (150 kg N ha^{-1} $year^{-1}$), during three vegetation seasons. N fertilization did not affect the POT neither ME content of the available forage from semi-natural pasture. The absence of a sward reaction in POT as well as in ME content ($P>0.05$) was found in all years ($P>0.05$), indicating that such a reaction can be expected in a wide range of climatic conditions. However, a strong influence of the growing season on the investigated properties was found in all the investigated levels of N fertilization ($P<0.0001$).

Keywords: nitrogen, fertilization, semi-natural pasture, digestibility, metabolic energy

Tehnologija uzgoja, reprodukcijske i zdravstvene odlike angus pasmine u sustavu krava-tele

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Sažetak

U posljednjih nekoliko godina zamjetan je trend porasta populacije goveda pasmine angus u Hrvatskoj. U svrhu opisa tehnologije uzgoja, te utvrđivanja reprodukcijskih i zdravstvenih odlika goveda ove pasmine, korišteni su podaci prikupljeni tijekom petogodišnjeg razdoblja s gospodarstva na području Korduna koje uzgaja 230 angus krava. Uspješnost koncepcije iznosila je, ovisno o godini, od 65 do 96%, dok je u praćenom razdoblju evidentirano ukupno 760 teljenja uz 37 mrtvorođene teladi. Od utvrđenih bolesti, u teladi se najčešće javlja proljev (23 slučaja), pneumonija (13 slučajeva), upala pupka (5 slučajeva) te upala karpalnog zgloba (3 slučaja). Nije utvrđena pojava zaraznih bolesti, dok se odrasla grla redovito tretiraju protiv parazita. Angus pasmina ima dobre reprodukcijske pokazatelje te odličnu otpornost na pojavu bolesti.

Ključne riječi: angus, sustav krava-tele, telad, reprodukcijske odlike, zdravstvene odlike

Uvod

Stanje govedarske proizvodnje u nekoj zemlji, kao najvažnije grane stočarske proizvodnje, jest važan pokazatelj životnog standarda, ali i stanja ukupne poljoprivredne proizvodnje (Uremović, 2004.). U Republici Hrvatskoj je, nažalost, već duži niz godina prisutan negativan trend u mliječnom govedarstvu prvenstveno kao posljedica relativno niskih i nekonkurentnih otkupnih cijena kravljeg mlijeka. Međutim, posljednjih godina je vidljiv rastući trend mesnog govedarstva, pri čemu je osobito izražen interes uzgajivača za sustavom krava-tele. Svakako, interes za sustavom krava-tele je potaknut i činjenicom da u Republici Hrvatskoj postoji puno neiskorištenih poljoprivrednih površina koje ne mogu poslužiti kao ratarska zemljišta, već kao pašnjaci. Također, ovaj sustav uzgoja zahtjeva manje radne snage (što nije nevažno osobito u današnjim prilikama nedostatka (kvalitetne) radne snage na domaćem tržištu), i ujedno ne zahtjeva velike troškove mehanizacije, kao ni izgradnje građevinskih objekata (Uremović i sur., 2002.).

Usporedno s rastućim interesom broja uzgajivača koji se bave uzgojem goveda u sustavu krava-tele, posljednjih godina zamijećen je porast broja uvezenih grla angus pasmine u Hrvatskoj. Stoga je cilj ovog rada prikazati najvažnije odlike tehnologije uzgoja angusa, kao i reprodukcijske te zdravstvene odlike angus pasmine u sustavu krava-tele.

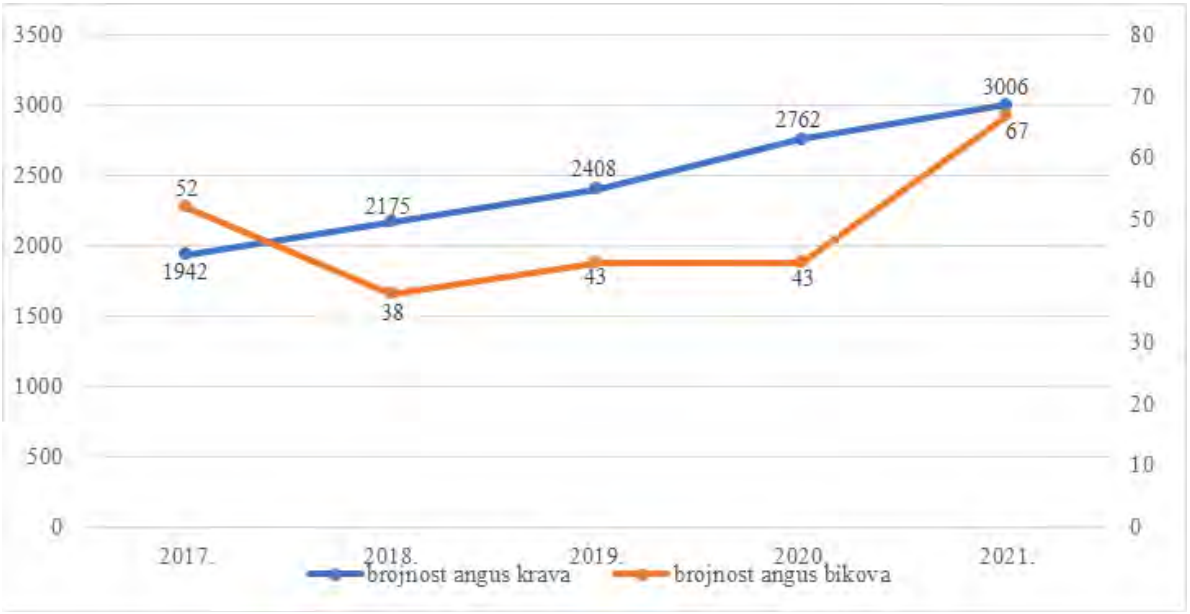
Odlike angus pasmine goveda

Pasmina goveda angus je nastala u Škotskoj u cilju formiranja ranozrelog, otpornog, mesnog goveda koje se može uzgajati na pašnjacima. Genetski je bezrogo govedo (pogoduje ekstenzivnom načinu držanja) i nižeg je rasta (krave u grebenu dostižu visinu od 125 do 130 cm, a bikovi do 140 cm). Angus je jednobojno govedo koje se pojavljuje u dva tipa: crnom i crvenom. Crna boja dlake je dominantna, češća je u uzgoju, traženija na tržištu te postiže bolju otkupnu cijenu. Angus govedo je umjereno razvijenog tjelesnog okvira s dobro razvijenom muskulaturom, dugačkog, širokog i zaobljenog trupa, kratkih nogu s dobro razvijenim mišićima (osobito u predjelu butova), kratkog i mišićavog vrata s malom i širokom glavom (Caput i sur., 2010.). Masa odraslih krava je od 550 do 700 kg, rasplodni bikovi mogu težiti do 1000 kg, dok je optimalna masa junica i junaca u završnom tovu od 500 do 600 kg. Junice u tovu ostvaruju dnevni prirast od 850 do 950 g, a bikovi od 1000 do 1200 g (Caput i sur., 2010.).

Porodna masa teladi je mala (30-35 kg) pa se pasmina odlikuje lakim teljenjem. Mliječnost krava je niska, ali dovoljna za othranu teleta. Procijenjena mliječnost angus krava po laktaciji jest u prosjeku 1800-1900 kg (Przysucha i sur., 2016.). Telad je vitalna i ostaje uz majku, bez potrebe za dodatnom njegom (Caput i sur., 2010.). Ova je pasmina idealna za uporabna križanja kako s mesnim, tako i s mliječnim pasminama goveda u cilju dobivanja križanaca pogodnih za tov, s dobrom kvalitetom mesa (Ivanković i Mijić, 2020.)

Obzirom na područja gdje su izvorno nastale, grla ove pasmine su otporna na nepovoljne vremenske uvjete te izražene temperaturne razlike. Kako je angus ranozrela pasmina, junice se za prvi pripust pripremaju već u dobi od 14 mjeseci, a nakon teljenja, krava se za novi reproduktivni ciklus priprema već nakon mjesec dana. Zahvaljujući paši i slobodnom načinu držanja, meso angusa idealne je masnoće i mramoriranosti, bez suvišne potkožne masti, te je sočno i mekano, specifičnog okusa i mirisa.

U Grafikonu 1. prikazano je brojno stanje krava i licenciranih bikova angus pasmine u razdoblju od 2017. do 2021. godine. Primjetan je gotovo linearan trend povećanja brojnosti čistokrvnih krava angus pasmine, dok broj bikova ima cikličko kretanje uz vidljivo povećanje tijekom posljednje dvije godine.



Grafikon 1. Linearni prikaz brojnosti krava i bikova angus pasmine u Hrvatskoj u razdoblju od 2017. do 2021. godine (Izvor: Ministartsvo poljoprivrede, 2022.)

Osnovne značajke uzgoja sustava krava-tele

Sustav krava-tele podrazumijeva uzgojni sustav držanja goveda mesnog ili kombiniranog proizvodnog tipa koji se temelji na slobodnom držanju na ispaši (Perčulija, 2011.). Namijenjen je isključivo proizvodnji teladi ili junadi za tov, a sve mlijeko koje krava proizvede, namijenjeno je hranidbi teladi - sisanjem (Knežević i sur., 2005.). Odluka o odabiru pasmine trebala bi se zasnivati na mogućnostima i prihodima koje određeno područje može pružiti, odnosno površini i kvaliteti raspoloživih pašnjaka, oranica i livada. Primjerice, u slučaju izdašnih i obilnih pašnjaka, u obzir dolaze pasmine većeg kapaciteta rasta te viših prirasta, i obrnuto. Ovaj sustav u posljednje vrijeme je sve više zastupljen u proizvodnji govođeg mesa jer su njegove značajke jednostavnost držanja životinja, manji troškovi hranidbe, niska ulaganja u objekte, kao i smanjena potreba za radnom snagom. Osnova sustava krava-tele je jeftinija hranidba koja se temelji na voluminoznim krmivima (paša, sijeno i sjenaža), a u manjoj mjeri na krepkim krmivima.

Tehnologiju uzgoja sustava krava-tele čini nekoliko faza koje zajedno čine zatvoreni proizvodni ciklus, i to: 1. razdoblje teljenja, 2. razdoblje pripusta, 3. faza potvrde graviditeta, 4. faza odbića teladi, 5. faza razvrstavanja po spolu i kategorijama unutar stada, 6. razdoblje suhostaja. Za sustav krava-tele poželjne su krave sa izraženom dugovječnošću i dobrim reproduktivnim odlikama (redovita pojava fertilnog estrusa i dobra koncepcija). Osim toga, kao važni čimbenici uspješne proizvodnje u sustavu krava-tele ističu se odlike vanjštine krava, i to: veličina okvira,

kapacitet rasta, bezrožnost (šutost), građa tijela, nogu, vimena i sapi. U sustavu krava-tele prakticira se prirodni pripust i umjetno osjemenjivanje goveda. Kod prirodnog pripusta najćešće se primjenjuje haremski pripust te korištenje licenciranih bikova (uzgojno pozitivno ocijenjeni).

Tehnologija uzgoja angusa u sustavu krava-tele

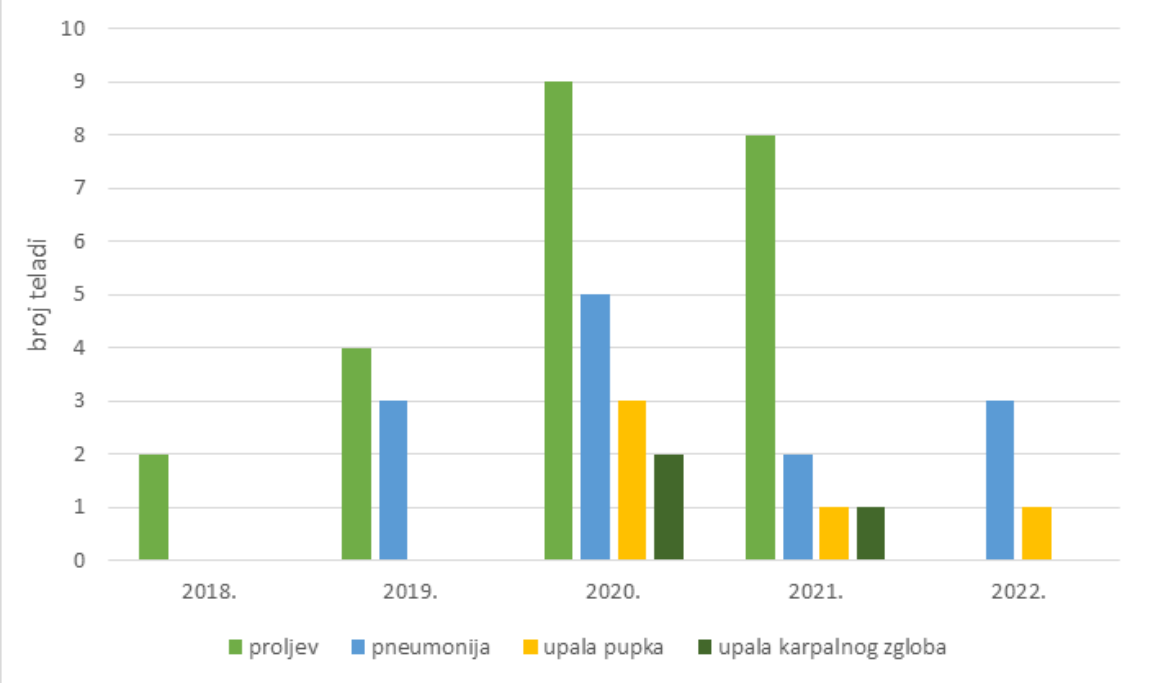
U sustavu krava-tele goveda su tijekom cijele godine na pašnjacima, čime se omogućuje njihov stalni boravak u prirodnom okruženju. Čak se niti junadima u završnom tovu ne ograničava kretanje, već se drže slobodno, na otvorenom. Kod ovakvog načina držanja smanjeni su troškovi ulaganja u objekte i hranu, a dobrobit životinja, kao i kvaliteta završnog proizvoda je veća. Jednom mjesečno provodi se vaganje cijelog stada kako bi se utvrdila masa svake pojedine jedinke i onda uskladila hranidba prema hranidbenim potrebama. Na ovakav način lakše se prati prirast životinja, graviditet krava, tjelesna masa junica pred prvi pripust te završna masa junadi za klanje. Uzgojno selekcijskim radom na gospodarstvu se odabiru životinje s boljim odlikama fenotipa. Selekcijski pritisak je najveći na krave, bikove rasplodnjake te junad koja je će se koristiti za obnovu stada. Izlučuje se svaka jedinka s uočenom eksterijernom manom: krivi stav nogu, loši papci i mane sustava za kretanje, zatim, nepoželjne su krave s teškim telenjima, one u kojih se javljaju pobačaji i mrtvorođena telad, no nisu poželjne niti one koje rađanju blizanace. Trenutno se u praćenom stadu krave i bređe junice tele u jesenskom (rujan-listopad) i proljetnom (veljača-svibanj) razdoblju. No, cilj je sva teljenja prebaciti na proljetno razdoblje kako bi krave i novorođena telad mogli u većoj mjeri koristiti prirodni izvor hranjivih tvari (pašu). Kako je ovaj sustav namijenjen uzgoju tovne junadi za klanje i prodaju gotovog proizvoda (mesa), najčešće se obavlja se kastracija muške teladi. Istraživanja su pokazala kako je meso kastrirane junadi sočnije i mekše, sadrži više intramuskularne masti uz smanjenu pojavnost visoke pH-vrijednosti i smanjenu pojavnost tamne boje mesa koja može odbiti potrošača prilikom kupnje (Morgan i sur., 1993.). Također, kastracijom se postiže smanjenje agresije mužjaka, lakša manipulacija i izbjegavanje naskakivanja na junice u slučaju zajedničkog držanja (Kos, 2008.). Kastracija se u praćenom stadu redovito provodi gumicama za kastraciju i izvodi se u prva dva tjedna života. To se radi tako što se gumicom stavljenom na korijen mošnje prekine dotok krvi u sjemenike. Telad se na praćenom gospodarstvu odbija s tjelesnom masom oko 200 kg, pri dobi od 5,5 do 7 mjeseci.

Hranidba

Temelj hranidbe ovog sustava uzgoja su pašnjaci, kao najbolji, najprirodniji i najjeftiniji izvor hranjivih tvari. S obzirom na moguća sušna ljeta i zime siromašne pašnjacima stado se tijekom cijele godine dodatno prihranjuje krmivima na hranidbenom stolu. Krave i tovna junad dobivaju izmješani obrok koji se sastoji od livadnog sijena, slame, ječmenog i stočnog brašna. Omjer voluminoze i žitarica za krave je 70:30 dok junad u tovu dobiva manji udio voluminoze, a veći udio koncentrata (60:40). Tijekom ljetnih mjeseci u hranidbi tovne junadi koristi se i svježi pivski trop kao izvor relativno jeftinog proteina. Za razliku od životinja držanih u zatvorenom objektu ili na vezu, životinje držane u sustavu krava-tele se dosta kreću te tako imaju i puno veće energetske potrebe organizma. Potrebe za bjelančevinama, s druge strane, nisu toliko izražene jer se u ovom sustavu ne radi o velikoj proizvodnji mlijeka. Važno je da sve dobne kategorije dobiju dovoljnu količinu hranjivih tvari kako bi junad u tovu postigla zadovoljavajuće dnevne priraste i kvalitetu mesa, krave zadovoljile svoje uzdržne potrebe i potrebe teladi tijekom laktacije, dok je za junice namijenjene rasplodu potrebno da se dovoljno tjelesno razviju za prvi pripust. Pitka voda i mineralne kocke za lizanje su stalno dostupni svim kategorijama goveda u praćenom stadu.

Zdravstveno stanje promatranog stada

Bolesti angus goveda nisu česte, tako da se ova pasmina, općenito, smatra jednom od otpornijih pasmina goveda. Najčešće je potrebno intervenirati u teladi starosti do dva mjeseca zbog pojave proljeva, zatim pneumonija, upala pupka i upala karpalnog zgloba, no obolijeva relativno mali broj teladi, uglavnom ona slabija i manje vitalna telad (Grafikon 2.).

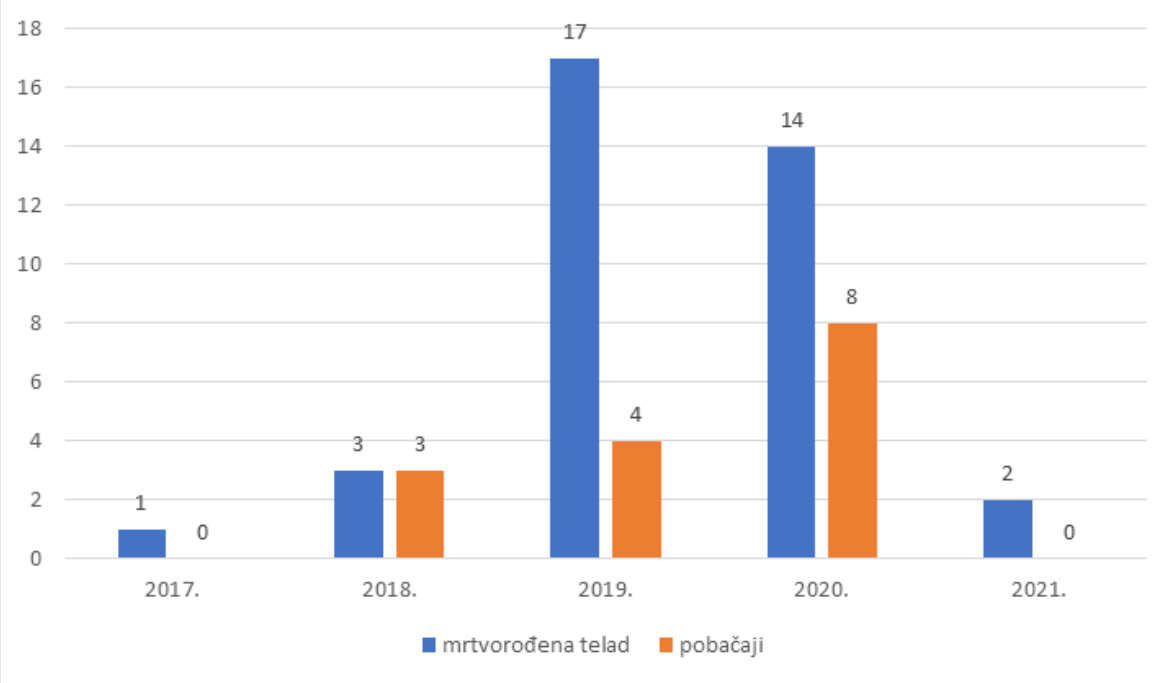


Grafikon 2. Evidentirane bolesti angus teladi u praćenom stadu

Odrasle jedinke su dobrog zdravstvenog stanja, osim što je u stadima redovita pojava bolesti moraksela tj. zaraznog keratokonjuktivitisa (Slika 1.).

Slika 1. Razvoj bolesti moraksela u goveda (stadij 1-3)
Izvor: (Cornell Cooperative Extension, 2022)

Na praćenom gospodarstvu u posljednjih je pet godina zabilježeno 37 mrtvorođene teladi te 15 pobačaja od kojih niti jedan nije prepoznat kao zarazan (Grafikon 3.)



Grafikon 3. Pojavnost mrtvorođene teladi i pobačaja na praćenom gospodarstvu

Reprodukcijske odlike

Angus pasmina, kako je ranije navedeno, spada u skupinu ranozrelih goveda (Ivanković i Mijić, 2020). Ranozrele pasmine mogu se prvi put pripustiti već nakon navršenih 16-18 mjeseci. Prilikom prvog pripusta, pažnja ne smije ostati samo na spolnoj zrelosti, nego i na fizičkoj jer ukoliko dođe do preranog pripusta, javljaju se brojni problemi poput zakržljalosti i poteškoća pri teljenju, smanjenog lučenja kolostruma i mlijeka, i sl. Uzgajivači se drže pravila da junice idu na prvi pripust kada dostignu 2/3 svoje završne mase. Mladi bikovi za reprodukciju počinju se koristiti već nakon navršene godine dana, ali se pritom koriste na manjem broju plotkinja. Kod ove pasmine, kako cijelo vrijeme boravi na otvorenom, prakticira se prirodni pripust, i to haremskim načinom, a umjetno se osjemenjuje tek manji broj krava (one koje nisu ostale gravidne uz bika). Na predmetnoj farmi mjesec dana nakon sezone pripusta obavlja se pregled ultrazvukom, kako bi se utvrdio broj gravidnih krava.

Važni parametri na koje je potrebno obratiti pažnju kod reprodukcije stada su: dob junica pri prvoj koncepciji, dob krava pri prvom teljenju, trajanje graviditeta, trajanje servis perioda, interval teljenja te prosječna proizvodnja tijekom života (Konjačić i sur., 2006.). Na predmetnoj farmi minimalna dob junica za prvu oplodnju je 15 mjeseci, ali pritom junica treba težiti minimalno 350 kg. Prosječna dob krava pri prvom teljenju je dvije godine, a gravidnost u prosjeku traje 280 dana, odnosno devet mjeseci. Servis period (razdoblje od teljenja do ponovne koncepcije) može trajati od 45 dana pa do četiri mjeseca, dok interval između teljenja iznosi od 11 do 14 mjeseci. Trenutno je u praćenom stadu angusa raspon starosti krava od 5 godina do 11 godina, a čak i starije krave se još uvijek koriste u reprodukciji. U Tablici 1. prikazani su prikupljeni reprodukcijski podaci predmetnog stada koji ukazuju na povoljne reprodukcijske odlike angus pasmine (u posljednjih pet godina).

Tablica 1. Reproduktivni podaci praćenog stada angus goveda tijekom posljednjih pet godina

Godina	Broj rasplodnih krava	Broj rasplodnih bikova	Broj krava u prirodnom pripustu	Broj krava umjetno osjemenjenih	Broj i udio potvrđeno gravidnih krava	Broj teljenja	Udio intervencija pri teljenju (%)	Liječenje teladi u prva dva mjeseca starosti (grla)
2018.	52	2	50	0	50 (96%)	47	1	2
2019.	267	4	202	32	234 (88%)	118	1,5	7
2020.	312	4	196	7	203 (65%)	290	4	19
2021.	271	3	205	11	216 (80%)	216	1,40	12
2022.	220	3	181	5	186 (85%)	89	1	4

Zaključak

Hrvatski uzgajivači prepoznali su potencijal i prednosti angus pasmine te radi toga populacija ima pozitivan trend. Reproduktivne odlike goveda na praćenom gospodarstvu su bile zadovoljavajuće: od ukupnog broja krava u pripustu ostaje gravidno 80-96% krava (izuzetak je bila 2020. godina kada je koncepcija iznosila samo 65%). Tijekom posljednjih pet godina u praćenom stadu angus goveda bilo je svega 15 pobačaja i 37 mrtvorođene teladi. U odraslih jedinki nije bilo problema sa zdravstvenim statusom, tek se u nekoliko jedinki pojavio zarazni keratokonjuktivitis (moraksela) koji se liječio antibioticima. Tijekom praćenog razdoblja u teladi se tijekom prva dva mjeseca najčešće pojavljivao proljev, zatim pneumonija, upala pupka te upala karpalnog zgloba. Nije bilo pojave zaraznih bolesti, dok su odrasla grla redovito tretirana protiv parazita. Angus pasmina se na praćenom gospodarstvu pokazala kao izuzetno prilagodljiva te postiže odlične rezultate obzirom na relativno nepovoljne vremenske uvjete na području Korduna.

Napomena

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Breeding technology, reproductive and health traits of the Angus breed in the cow-calf system

Abstract

In the last few years, there has been a noticeable trend towards an increase in the population of Angus cattle in Croatia. For the purpose of describing the breeding technology and determining the reproductive and health characteristics of cattle of this breed, data collected during a five-year period from one farm in the Kordun area that breeds 230 Angus cows were used. Conception success was, depending on the year, from 65 to 96%, while a total of 760 calvings with 37 stillborn calves were recorded in the monitored period. Among the established diseases, diarrhea (23 cases), pneumonia (13 cases), navel inflammation (5 cases) and carpal joint inflammation (3 cases) are the most common in calves. The occurrence of infectious diseases has not been determined, while adult throats are regularly treated against parasites. The Angus breed has good reproductive indicators and excellent resistance to disease.

Keywords: angus, cow-calf system, calves, reproductive traits, health traits

Polimorfizmi MTNR1A gena u populaciji dalmatinske pramenke

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Sažetak

Cilj je bio utvrditi frekvencije genotipova i alela na lokusima 606 i 612 MTNR1A gena te distribuciju janjenja po sezonama u ovaca pasmine dalmatinska pramenka. U tu je svrhu genotipizirano 40 jedinki PCR-RFLP metodom i utvrđeni su sljedeći genotipovi na lokusu 606: CC 0,10; CT 0,48 i TT 0,42, a na lokusu 612: GG 0,90 i GA 0,10. Na osnovu utvrđenih genotipova frekvencije alela bile su sljedeće: C 0,34; T 0,66; G 0,95 i A 0,05. Bez obzira na utvrđeni genotip na istraživanim lokusima, dominantna sezona janjenja bila je zima. Nejednaka prevalencija povoljnih alelnih varijanti na oba istraživana lokusa otežava donošelje zaključka o genetskoj preddisponiranosti dalmatinske pramnke za vansezonska janjenja.

Ključne riječi: genotip, alel, MTNR1A, dalmatinska pramenka, janjenje

Uvod

Sezonska reprodukcija česta je odlika većine divljih vrsta koja omogućuje partus u optimalno doba godine (obično u proljeće) te rast mladunčadi u povoljnim temperaturnim uvjetima uz dostupnost dostatnih količina hrane prije sljedeće zime (Thiéry i sur., 2002.). Proces pripitomljivanja doveo je do gotovo potpunog gubitka navedene prilagodbe u goveda i svinja, ali je ona uglavnom još uvijek zadržana u većine pasmina ovaca, koza i konja koji potječu s umjerenog klimatskog područja. Stoga je, u većine pasmina ovaca razdoblje spolne aktivnosti regulirano duljinom dana, odnosno fotoperiodom, a informacije o promjeni fotoperioda organizam prima putem hormona melatonina. Količina izlučenog melatonina ovisi o izmjenama dana i noći jer je trajanje sekrecije melatonina u pozitivnoj korelaciji s brojem noćnih sati (Karsch i sur., 1984.). Melatonin na ciljna tkiva i organe djeluje putem specifičnih G-proteinskih receptora, MT1 i MT2, koji imaju visok afinitet za melatonin, a od kojih je samo MT1 receptor uključen u regulaciju reprodukcijskih funkcija (Dubocovich i sur., 2003., Mura i sur., 2010.). Gen koji kodira MT1 receptor ili MTNR1A gen u ovaca se nalazi na 26 kromosomu između mikrosatelita CSSM43 i BM6526 (Messer i sur., 1997.). Navedeni gen se sastoji od dva egzona podjeljena s velikim intronom, a polimorfna mjesta na pozicijama 606 i 612 (Reppert i sur., 1994.) drugog egzona omogućila su detekciju poželjnih alela u različitih pasmina ovaca (Chu i sur., 2006.; Carcangiu i sur., 2009.; Mateescu i sur., 2009.; Mura i sur., 2010.; Martínez-Royo i sur., 2012.; Saxena i sur., 2014.; Avanus i Altinel, 2016.; Vlahek i sur., 2017.; Držaić i sur. 2016., 2017., 2019. i 2020.; Starič i sur., 2020.). Detekcijom poželjnih varijanti MTNR1A gena i odabirom jedinki s favoriziranim genotipovima nastoje se ublažiti izravne posljedice sezonske reprodukcije kao što su neravnomjerna ponuda ovčjih proizvoda tijekom godine, osobito janjećeg mesa i ovčjeg mlijeka. Sezonska ponuda ovčjih proizvoda prisutna je i u Hrvatskoj obzirom da većinu uzgajanih pasmina odlikuje sezonska reprodukcijska aktivnost. Skupini takvih pasmina pripada i dalmatinska pramenka koja se uzgaja na širem priobalnom području Dalmacije, u Dalmatinskoj zagori, Bukovici, te na nekim srednjedalmatinskim otocima (Brač, Hvar, Vis, Kornati i dr.). Dalmatinska pramenka pripada skupini kasnozrelih ovaca, a ovce uglavnom pripuštaju ujesen tako da se većinom ojanje u razdoblju od siječnja do ožujka (Caput i sur., 2010.). Iako pasminu odlikuje sezonska reprodukcijska aktivnost, navedeni autori, naglašavaju mogućnost dvokratnog godišnjeg janjenja ili tri janjenja u dvije godine u boljim hranidbenim uvjetima. Utvrđivanje poželjnih alelnih varijanti MTNR1A gena omogućilo bi uvid u gentsku predispoziciju pasmine za izvansezonsku reprodukciju. Stoga je cilj predmetnog istraživanja bio utvrditi frekvencije genotipova i alela na lokusima 606 i 612 MTNR1A gena PCR-RFLP metodom te distribuciju janjenja po sezonama u stadu ovaca dalmatinske pramenke.

Materijal i metode

Predmetnim je istraživanjem bilo obuhvaćeno 40 odraslih, tjelesno potpuno razvijenih i zdravih ovaca pasmine dalmatinska pramenka koje su odabrane iz stada s ukupno oko 200 rasplodnih grla s područja Vrlike. Kriterij za odabir rasplodnih ovaca bio je postojanje podataka o barem tri janjenja. Za potrebe izolacije DNK uzet je uzorak tkiva s ruba ušne školjke, a podatci o datumu rođenja pojedine jedinke te podatci o janjenjima (redoslijed i datum janjenja) ustupljeni su od strane Ministarstva poljoprivrede Republike Hrvatske. Izolacija DNK iz tkiva provedena je GenElute® Mammalian Genomic DNA Miniprep Kit-om (Sigma-Aldrich, Saint Louis, MO, USA), a uspješnost izolacije i kvaliteta DNK provjereni su elektroforezom na 1% gelu agaroze (Lonza, Rockland, ME, USA). Lančana reakcija polimeraze (eng. *polymerase chain reaction* PCR) provedena je prema protokolu proizvođača Promega sa GoTaq® G2 Hot Start Green Master Mix (Promega Corporation, Madison, WI, USA). PCR u ukupnom volumenu od 25 µL uključivao je 1,0 µL tj. 50 ng genomske DNK, 12,5 µL GoTaq® G2 Hot Start Green Master Mix, 0,75 µL pojedine oligonukleotidne početnice 10 µM, i 10,0 µL vode slobodne od nukleaza. Oligonukleotidne početnice: prema naprijed (eng. *forward*): 5'-TGTGTTTGTGGTGAGCCTGG-3' i prema natrag (eng. *reverse*): 5'-ATGGAGAGGGTTTGCGTTTA -3' (Sigma-Aldrich) preuzete su od Messer i sur. (1997.). Uvjeti PCR reakcije bili su sljedeći: reakcija aktivacije Taq polimeraze na 94˚C/5 min, 35 ciklusa umnažanja ciljne DNK sekvence (94˚C/30 s, 55˚C/30 s, 72˚C/45 s) te krajnje produljenje umnožene željene sekvence DNK na 72˚C/5 min. Umnažanje ciljnog DNK odsječka provedeno je u 35 ciklusa. Navedeni uvjeti omogućili su amplifikaciju glavnog dijela drugog egzona MTNR1A gena (GenBank Acc. No. U14109) duljine 824 bazna para (bp; Reppert i sur., 1994.). Provjera uspješnosti PCR reakcije obavljena je pomoću elektroforeze na 1% gelu agaroze paralelno s biljegom od 100 bp (PCR 100bp Low Ladder; Sigma-Aldrich). Nakon uspješne PCR amplifikacije genotipizacija za lokus 606 i lokus 612 MTNR1A gena provedena je PCR-RFLP metodom s *RsaI* i *MnlI* restrikcijskim endonukleazama (New England Biolabs, Beverly, MA, USA). Restrikcijsko cijepanje PCR produkata provedeno je u ukupnom volumenu od 20 µL sadržavajući 10 µL PCR produkta i 10 µL reakcijskog miksa pojedine restrikcijske endonukleaze. *RsaI* reakcijski miks sastojao se od: 5,85 µL demineralizirane vode, 2,0µL 10 x pufer T, 2,0 µL 0,1% BSA i 0,12 µL *RsaI* 1U, a *MnlI* reakcijski miks od: 6,88 µL demineralizirane vode, 3,0 µL 10 x NEB pufera i 0,12 µL *MnlI* 5U. Inkubacija PCR produkata i restrikcijskog miksa je provedena u vodenoj kupelji u ukupnom trajanju od 5 sati na 37˚C za obje restrikcijske endonukleaze. Rezultati enzimatskog cijepanja analizirani su elektroforezom na 3% gelu agaroze paralelno s biljegom od 50 bp (DNA Step Ladder, Promega Corporation, Madison, WI, USA). Genotipizacija uzoraka na lokusu 606 bila je: CC (267 bp, 23 bp), CT (290 bp, 267 bp, 23 bp) i TT (290 bp), a na lokusu 612: GG (236 bp, 67 bp), GA (303 bp, 236 bp, 67 bp) i AA (303 bp) (Carcangiu i sur., 2009.; Držaić i sur., 2017., 2019., 2020.).

Frekvencije alela i genotipova te frekvencije janjenja po sezonama za utvrđene genotipove izračunate su korištenjem statističkog programa R (R Core Team, 2008). Sezone su definirane na sljedeći način: proljeće (od 21. 3. do 20. 6.), ljeto (od 21. 6. do 20. 9.), jesen (od 21. 9. do 20. 12.) i zima (od 21. 12. do 20. 3.). Testiranje odstupanja frekvencija utvrđenih genotipova od očekivanih za populaciju u Hardy-Weinbergovoj ravnoteži u promatranom uzorku izvršeno je Hi-kvadrat testom ($\chi 2$).

Rezultati i rasprava

Kod svih istraživanih ovaca, analizom polimorfizama na osnovu dužine restrikcijskih fragmenata (RFLP), identificirani su polimorfizmi MTNR1A gena na pozicijama 606 i 612 referentne sekvence (U14109). U tablici 1 prikazane su utvrđene frekvencije genotipova i alela na lokusima 606 i 612 MTNR1A gena i rezultati Hi-kvadrat testa. Na lokusu 606 MTNR1A gena utvrđena su sva tri genotipa, a najzastupljeniji je bio heterozigotni genotip CT s ferkvencijom 0,48, zatim homozigotni genotip TT s frekvencijom 0,42, dok je frekvencija homozigotnog genotipa CC bila 0,10. Na drugom istraživanom lokusu (lokus 612) utvrđena su samo dva genotipa i to homozigotni genotip GG i heterozigotni genotip GA s frekvencijama 0,90 i 0,10. Genotip AA na lokusu 612 MTNR1A gena nije utvrđen u istraživanim uzorcima ovaca dalmatinske pramenke. Utvrđena je viša frekvencija alela T (0,66) u odnosu na alel C (0,34) na lokusu 606, te znatno viša frekvencija alela G (0,95) u odnosu na alel A (0,05) na lokusu 612.

Tablica 1. Utvrđene frekvencije genotipova i alela na lokusima 606 i 612 MTNR1A gena te rezultati χ2-testa

Frekvencija genotipova						Frekvencija alela				HWE	
Lokus 606			Lokus 612			Lokus 606		Lokus 612		Lokus 606	Lokus 612
CC	CT	TT	GG	GA	AA	C	T	G	A	χ^2	χ^2
0,10	0,48	0,42	0,90	0,10	0,00	0,34	0,66	0,95	0,05	0,155^{nz}	0,111^{nz}

HWE – Hardy-Weinberg equilibrium; $\chi^2_{0,05,\,1} = 3,84$; nz – nije značajno

Chu i sur. (2006.) navode značajno više frekvencije C i G alela u poliestričnih pasmina (Small tail Han i Hu ovca) u odnosu na sezonski poliestrične pasmine (Suffolk, Dorset i njemački merino). Navedeno potkrepljuju Saxena i sur. (2014.) navodeći visoke frekvencije C (0,87) i G (0,88) alela u poliestričnih Chokla ovaca. Više frekvencije C i G alela utvrđene su i u Sarda ovaca (0,66 i 0,78; Carcangiu i sur., 2009.) te u turske Kivircik pasmine (0,68 i 0,89; Avanus i Altinel, 2016.) koje odlikuje duža pripusna sezona u odnosu na tipične sezonski poliestrične pasmine ovaca. I dok su u poliestričnih pasmina i pasmina s produženom pripusnom sezonom utvrđene više frekvencije alela G na lokusu 612 MTNR1A gena, u divlje ovce (*Ovis Gmelini Musimon*) koju odlikuje izrazita sezonska pojava estrusa i janjenja utvrđene su više frekvencije alela A (0,66) u odnosu na alel G (0,34) te posljedično i više frekvencije genotipa AA (0,50) u odnosu na genotip GG (0,18; Carcangiu i sur., 2010.). U sezonski poliestričnih pasmina ovaca, kao što je travnička pramenka utvrđene su jednake frekvencije C (0,50) i T (0,50) alela te znatno više frekvencije G (0,88) u odnosu na A (0,12) alel (Držaić i sur., 2016.). Nadalje, u ovaca dalmatinske pramenke Vlahek i sur. (2017.) navode veću frekvenciju alela T (0,72) na lokusu 606 i veću frekvenciju alela G (0,90) na lokusu 612 uz izostanak genotipa AA, što je potvrđeno predmetnim istraživanjem (Tablica 1). U istarske ovce, također sezonski poliestrične pasmine, utvrđene su više frekvencije T (0,63) u odnosu na C (0,37) alel te više frekvencije G (0,80) u odnosu na A (0,20) alel (Držaić i sur., 2020.). U slovenskih pasmina ovaca: istarske pramenke i bovške ovce utvrđene su više frekvencije C (0,59) u odnosu na T (0,41) alel te više frekvencije G (0,64 i 0,68) u odnosu na A (0,36 i 0,32) alel (Starič i sur., 2020.), dok su u jezersko-solčavske pasmine isti autori utvrdili više frekvencije alela T (0,64) i alela G (0,64) premda razlike u frekvencijama alela koje autori navode nisu bile toliko izražene kao u ranije navedenim istraživanjima. Razlike u frekvencijama alela na istraživanim lokusima MTNR1A gena u različitih pasmina ovaca posljedica su različitog selekcijskog pritiska koji je prisutan u pojedinim uzgojima (Martínez-Royo i sur., 2012., Starič i sur., 2020.).

Iz rezultata χ2-testa je razvidno da utvrđeni genotipovi MTNR1A gena u istraživanoj populaciji ovaca dalmatinske pramenke na lokusima 606 i 612 statistički ne odstupaju značajno od očekivanih frekvencija genotipova populacije u Hardy-Weinbergovoj ravnoteži (Tablica 1). Navedeno je i očekivano obzirom da se radi o najbrojnijoj populaciji ovaca u Hrvatskoj u koje zbog ekstenzivnog sustava uzgoja nije provođena intenzivnija selekcija. Distribuciju genotipova na lokusima 606 i 612 MTNR1A gena unutar Hardy-Weinbergove ravnoteže navode Saxena i sur. (2014.) za Chokla pasminu, Držaić i sur. (2016.) za travničku pramenku, Držaić i sur. (2019.) za cigaju te Držaić i sur. (2020.) za istarsku ovcu. Distribucija frekvencija janjenja ovaca dalmatinske pramenke temeljem genotipa na istraživanim lokusima MTNR1A gena po sezonama prikazane su u tablici 2. U istraživanoj populaciji evidentirana su ukupno 142 janjenja, a od toga 88 janjenja bilo tijekom zime (61,97%), 53 tijekom proljeća (37,33%), dok je jedno janjenje (0,7%) evidentirano tijekom jeseni.

Tablica 2. Distribucija frekvencija janjenja dalmatinske pramenke po sezonama obzirom na genotipove lokusa 606 i 612 MTNR1A gena

Genotip		Sezona janjenja								UKUPNO	
		Proljeće		Ljeto		Jesen		Zima			
		n	%	n	%	n	%	n	%	n	%
Lokus 606	CC	7	4,93	0	0,00	0	0,00	9	6,34	16	11,27
	CT	31	21,83	0	0,00	1	0,70	36	25,35	68	47,89
	TT	15	10,57	0	0,00	0	0,00	43	30,28	58	40,85
Σ		53	37,33	0	0,00	1	0,70	88	61,97	142	100,00
Lokus 612	GG	47	33,10	0	0,00	1	0,70	78	54,93	126	88,73
	GA	6	4,23	0	0,00	0	0,00	10	7,04	16	11,27
	AA	-	-	-	-	-	-	-	-	-	-
Σ		53	37,33	0	0,00	1	0,70	88	61,97	142	100,00

S obzirom na genotip na lokusu 606 po broju janjenja dominirale su jedinke heterozigotnog genotipa CT sa 68 janjenja, zatim jedinke homozigotnog genotipa TT sa 58 janjenja te jedinke homozigotnog genotipa CC sa 16 janjenja što je sukladno utvrđenim frekvencijama genotipova na navedenom lokusu (Tablica 1). Jedinke sva tri genotipa na lokusu 606 MTNR1A gena najveći broj janjenja ostvarile su tijekom zime. Navedena distribucija janjenja po sezonama utvrđena je i s obzirom na genotip na lokusu 612 gdje je dominantna sezona janjenja, bez obzira na genotip, bila zima. Po broju janjenja dominirale su jedinke GG genotipa zimi (78 janjenja) i u proljeće (47 janjenja) što je sukladno visokim frekvencijama navedenog genotipa u istraživanoj populaciji ovaca dalmatinske pramenke.

Carcangiu i sur. (2009.) navode veći udio janjenja Sarda ovaca CC i GG genotipa u razdoblju rujan-prosinac (68% i 80%) u odnosu na razdoblje siječanj-travaj (35% i 20%), dok za ovce TT i AA genotipa navode veći udio janjenja u razdoblju siječanj-travanj (55% i 78%) u odnosu na razdoblje rujan-prosinac (45% i 22%). Autori dalje navode podjednake udjele janjenja ovaca heterozigotnih genotipova CT i GA u oba razdoblja. Nadalje, jedinke GG genotipa Sarda ovaca odlikuje veća plodnost te brža pojava estrusa nakon proljetnog janjenja i tretmana melatoninom (Mura i sur., 2019.) te značajan utjecaj mužjaka na pojavu estrusa tijekom anestralnog razdoblja bez obzira na dob i tjelesnu masu ovaca (Luridiana i sur. 2015.). Izostanak jasnijeg utjecaja istraživanih polimorfizama MTNR1A gena u ovaca dalmatinske pramenke na pojavu janjenja uvelike je uvjetovan menadžmentom u komercijalnom stadu iz kojeg su prikupljeni uzorci. U proizvodnji mesa uzgajivačima je cilj da se ovce janje tijekom zime i u rano proljeće kako bi glavnina janjadi za tržište bila spremna u vrijeme Uskrsa kad je i najveća potražnja za janjećim mesom. Uz navedeno, distribucija genotipova na istraživanim lokusima nije bila ujednačena, tko je na lokusu 606 zastupljenost CC genotipa bila samo 0,10, dok je u istoj frekvenciji bio zastupljen genotip GA na lokusu 612, a genotip AA je izostao što dodatno otežava interpretaciju dobivenih rezultata.

Zaključak

Obje povoljne varijante alela MTNR1A gena za mogućnost vansezonske reprodukcije (606 C i 612 G) nisu bile dominantne na oba lokusa, već samo na lokusu 612, što otežava donošenje zaključka o podobnosti pasmine za provedbu selekcije na van-sezonska janjenja. Nadalje, zastupljenost genotipova nije bila ravnomjerna već je bila izrazita prevalencija genotipova CT i GG što je imalo dodatni nepovoljni učinak u donošenju zaključaka. Daljnja istraživanja koja bi uključivala genotipiziranje većeg broja jedinki, a osobito onih kod kojih su povijesno poznate incidencije vansezonskih janjenja, značajno bi doprinjela boljem razumijevanju ove problematike i donošenju konkretnijih zaključaka o vezi između genotipa i fenotipa u dalmatinske pramenke.

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MTNR1A gene polymorphisms in Dalmatian pramenka

Abstract

The aim was to determine the frequency of genotypes and alleles at loci 606 and 612 of the MTNR1A gene and the distribution of lambing by season in Dalmatian pramenka. For this purpose, 40 individuals were genotyped using the PCR-RFLP method and the following genotypes were determined at loci 606: CC 0.10, CT 0.48, and TT 0.42 and loci 612: GG 0.90 and GA 0.10. Based on the determined genotypes, allele frequencies were as follows: C 0.34, T 0.66, G 0.95, and A 0.05. Regardless of genotype at the loci, the predominant lambing season was winter. Different prevalence of the favorable allelic variants at both loci makes it difficult to draw a firm conclusion about the genetic predisposition of Dalmatian pramenka for out-of-season lambing.

Keywords: genotype, allele, MTNR1A, Dalmatian pramenka, lambing

Estimation of inbreeding coefficient and generation interval in Pag Sheep – baseline for development of optimum contribution selection

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Summary

The aim of the study was to estimate coefficient of inbreeding (F_{PED}) and generation interval (GI) in the population of Pag sheep breed in order to provide a scientifically based background for implementation of optimum contribution selection. The average F_{PED} was 6%, with inconsistent trend in the last decade, and GI was estimated to be 4.44 years. The F_{PED} was higher than reported for majority of few well known European dairy breeds, while the GI was in line with the reports. The estimated F_{PED} calls for attention of the breeders to keep it as low as possible in the future, preferably by following the basic principles of the optimum contribution selection.

Keywords: Pag sheep, inbreeding, generation interval, selection

Introduction

The Pag sheep breed with an estimated population size of 30,000 animals has great economical, traditional, and cultural importance for residents of the Pag island. Dairy products of the breed have a tremendous market niche for long time now (milk ~1.7 €/L, cheese ~30 €/kg) which is the main reason why the breeders seek to permanently increase the milk production in their flocks via selection. Pedigree and performance recording in this population started about two decades ago in order to use BLUP in genetic evaluation for dairy traits (test-day repeatability animal model; Špehar et al., 2020.). Substantial part of the phenotyped population has been recently genotyped (50K SNP chip) for purpose of transition to genomic selection (single-step genomic BLUP; Legarra et al., 2009.). Inbreeding is inevitable in small populations, especially under severe selection pressure due to overuse of genetically superior animals. The rationale behind this claim relies on the fact that animals that share common genes, have similar breeding values, and therefore tendency to inter-mate. In order to control inbreeding while providing selection gain, future mating schemes should have a low expected inbreeding coefficient of the offspring, which is a consequence of the kinship between the parents (Legarra et al., 2009.). Generation interval (GI) represents an important population parameter for predicting response to selection and provides an indication about dynamics of generations change in the population under concern (Boichard et al., 1997.; Gutiérrez et al., 2003.). The shorter the GI, the faster selection gain is expected in the population. However, this is the true only under assumption that selection intensity remains the same, which cannot be obtained because these two parameters are tightly related to each other in undesirable manner. The optimum contribution selection seems to be the best possible selection strategy in the future for this population in order to provide selection gain while minimising loss of genetic variability. In the era of "genomics", the best possible solution seems to be the genomic selection. However, prior to transition to this complex strategy, numerous questions about existing state of the population need to be answered. The level and trend of inbreeding and GI are very important population parameters from this point of view so their estimation represents the main goals of this study.

Material and methods

Pedigree records were provided by the Croatian Ministry of Agriculture. Initial pedigree comprised of 281760 animals born from 1981 to 2019, but after comprehensive quality control of the pedigree, only subset of the animals with

sufficient pedigree information was used in estimation of coefficient of inbreeding and GI. Number of fully traced generations (NFG) represented the number of the last ancestral generation with known sire and dam. Number of equivalent generations (NEG) was obtained as the sum of the proportions of known ancestors of an individual over all traced generations as follows: . where was the number of ancestors of individual , and was the number of generations between individual 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. Pedigree completeness index (PCI) or harmonic mean of the pedigree completeness of the parents was calculated using the following formula: with and being proportions of paternal and maternal ancestors estimated based on: where was the ratio of known to unknown ancestors in each generation and was the number of generations (MacCluer et al., 1983.). Completeness was calculated for individuals and for groups of individuals in each ancestral generation representing the proportion of known ancestors in each generation (Figure 1). Reference population was set to animals born after 2010 with constraint imposed on animals with *NEG*≥3. The generation interval was calculated as the average age of the parents at birth of their offspring, and coefficient of inbreeding (F_{PED}) as the probability that two alleles chosen at random from the maternal and paternal haplotypes are identical by descent. The pedigree analysis was conducted in the R programming environment (R Core Team, 2020.) using package "optiSel" (Wellmann, 2021.). Preparation of data, descriptive statistical analysis, and plotting of the results were carried out using "data.table" (Dowle and Srinivasan, 2021.), "tidyverse" (Wickham et al., 2019.), "pastecs" (Grosjean and Ibanez, 2018.), "descriptr" (Hebbali, 2020.) and "ggplot2" (Wickham, 2016.).

Results and discussion

The coefficient of inbreeding (F_{PED}) obtained from genealogical records is accurate insofar as the quality of the pedigree. Therefore, a thorough control of the pedigree completeness was carried out in order to establish a reference population with sufficient ancestral information. Rams and ewes had practically the same completeness of the pedigree (Figure 1). It is visible that 100% of individuals have known both ancestors in the first generation, almost 99% of them in the second, 85% in the third, 56% in the fourth and 31% in the fifth. The average NEG, NFG, NMG, and PCI in the reference population was 3.96, 2.48, 6.9, and 0.73, respectively (Table 1). The obtained quality control parameters of the pedigree kind of guarantee that F_{PED} and GI in this population was estimated with sufficient dose of certainty. The estimated average of F_{PED} in the reference population was 6% (Table 1) with three quarters of population having F_{PED} below 1% (Table 2). Different quality of the pedigree between different populations often poses huge limits to fair comparisons of estimates of F_{PED} obtained in different studies. Many studies on sheep breeds do not impose any restrictions based on the pedigree quality parameters while estimating F_{PED}, which often results with severe underestimation of the true value of $F_{PED.}$ Our estimate might be to some extent comparable to F_{PED} obtained in Italian (Cortellari et al., 2022.), Spanish (Granado-Tajada et al., 2020.), and French (Rodríguez-Ramilo et al., 2019.) dairy sheep breeds. However, missingness of pedigree information in the two latter studies was tackled by using methodology proposed by VanRaden (1992.) which allows estimating inbreeding coefficients in populations with missing genealogy, by assuming that the F_{PED} of individuals with missing parents are equal to the mean of the inbreeding coefficients of those animals with known parents and born during the same year. The estimated F_{PED} for Delle Langhe, Comisana, Massese and Sarda was estimated at 6%, ~2%, ~4%, and ~9%; for Latxa Cara Negra from Euskadi, Latxa Cara Rubia, and Latxa Cara Negra from Navarre ~2%, ~2%, and ~2%; and for Basco-Béarnaise, Manech Tête Noire, Manech Tête Rousse, Lacaune Confederation, and Lacaune Ovitest ~3%, ~3%, ~2%, ~2%, and ~3%, respectively.

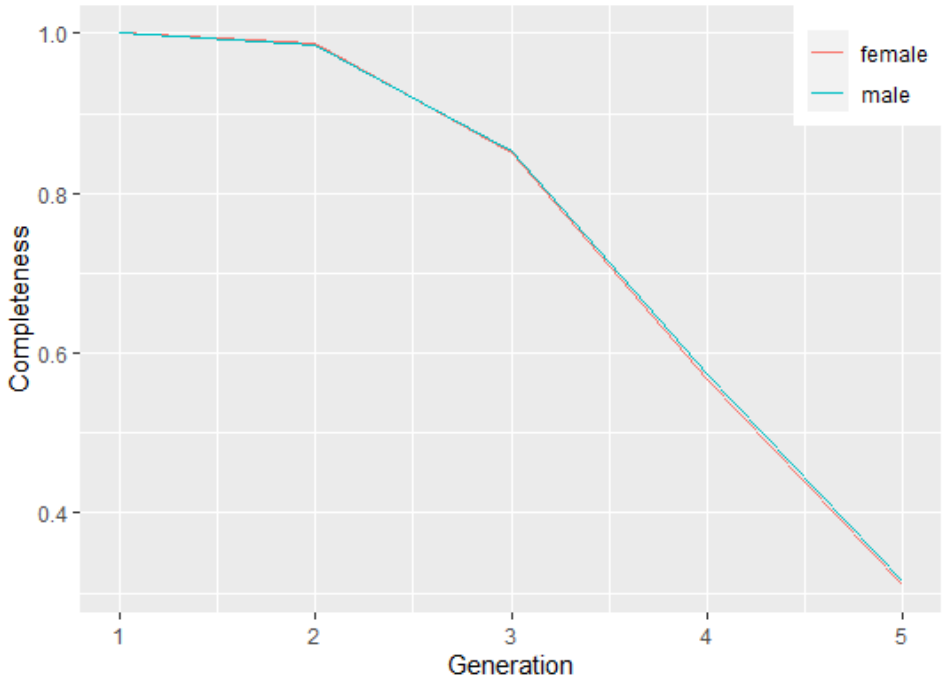

Figure 1. Completeness of the pedigree in the reference population.

Table 1. Quality control parameters of the pedigree, coefficient of inbreeding, and generation interval estimated in the reference population of Pag sheep breed

	NEG	NFG	NMG	PCI	F_{PED}	GI
min	3.00	1.00	3.00	0.32	0	1
max	6.43	5.00	11.00	1.00	0.44	14.5
average	3.96	2.48	6.9	0.73	0.06	4.44

NEG - number of equivalent generations; NFG - number of fully traced generations; NMG - number of maximum known generations; PCI - pedigree completeness index, F_{PED} – coefficient of inbreeding, GI – generational interval

Table 2. Distribution of the coefficient of inbreeding (F_{PED}) in the reference population

Class of F_{PED}	Frequency	Percent (%)
0.0000-0.0875	12323	76.43
0.0875-0.1750	2760	17.12
0.1750-0.2625	682	4.23
0.2625-0.3500	339	2.10
0.3500-0.4375	19	0.12
Total	16123	100

Trend of inbreeding in the examined period was not completely conclusive, since it actually decreased up to 2016, and thereafter increased (Figure 2). However, this small increase of the F_{PED} in the last five years does not represent a serious issue, since it did not exceed the 6.25% which is considered as an acceptable level of inbreeding in small populations undergoing selection. From all these results pertaining to F_{PED}, it seems that inbreeding in this population has been in acceptable level regardless of selection on the milk production. Breeders in this population are aware of potentially negative impacts of inbreeding (inbreeding depression) and they make efforts to avoid close inbreeding in their flocks by replacing their sires from other, usually neighbouring flocks (unofficial circulation of the rams). According to some preliminary results obtained in this sheep populations, it looks that inbreeding does not directly

threaten to the main selection goal in this population (milk production). However, there are serious indications that it has a substantial impact on birth weight, which can indirectly impact profitability of this breed. Regardless of being based on comprehensive and deep statistical analysis, these statements still need to be proven under the framework of genetic animal model level prior to generalisation.

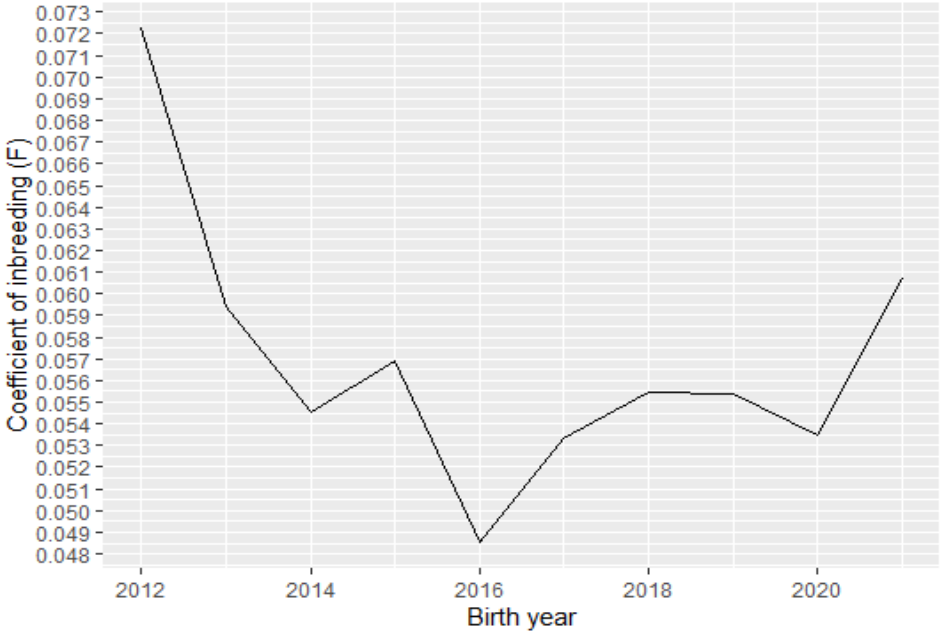

Figure 2. Trend of inbreeding in the reference population of Pag sheep.

The estimated GI in this population was 4.44 years (Figure 3), which goes in line with reports of the studies conducted on the above discussed Spanish and French dairy sheep breeds. On the other side, the GI was notably above reports for sheep meat breeds worldwide, where the average GI generally range between 2.0 and 3.8 years (e.g. Rafter et al., 2022.; Hashemi and Ghavi Hossein-Zadeh, 2020.). The obtained GI in Pag sheep breed was expected for us by taking into consideration that ewes in this dairy orientated sheep population stay in production as long as possible, in some flocks practically until they die (it is not unusual that ewes reach 10-12 years of age). The culling rate in this population is generally obviously low, which can be considered as a flaw from selection point of view, but this is tricky, and it does not automatically imply lower selection gain, because lower culling rate leaves "more room" for higher selection intensity. To be more precise, by simply looking at the breeder's equation it can be superficially concluded that lower GI automatically and unconditionally leads to the faster genetic gain. Since selection gain depends also on selection intensity, you always need to think of that by lowering GI you automatically pose restrictions to selection intensity. Therefore, it is necessary to find a balance between GI and selection intensity that will result with maximal response to selection in this population which will be one of our goals in the future along with the finding solutions to maximally reduce loss of genetic variation by following the principals of optimum contribution selection. Since the products of this sheep population has an extraordinary market niche, investments to genomic selection seems to be reasonable and economically justified. By using genomic information with all historical data collected in this population, selection gain could be achieved much faster, particularly via more efficient selection of replacement sires that have been sub-optimally selected as confirmed by Kasap et al. (2022.).

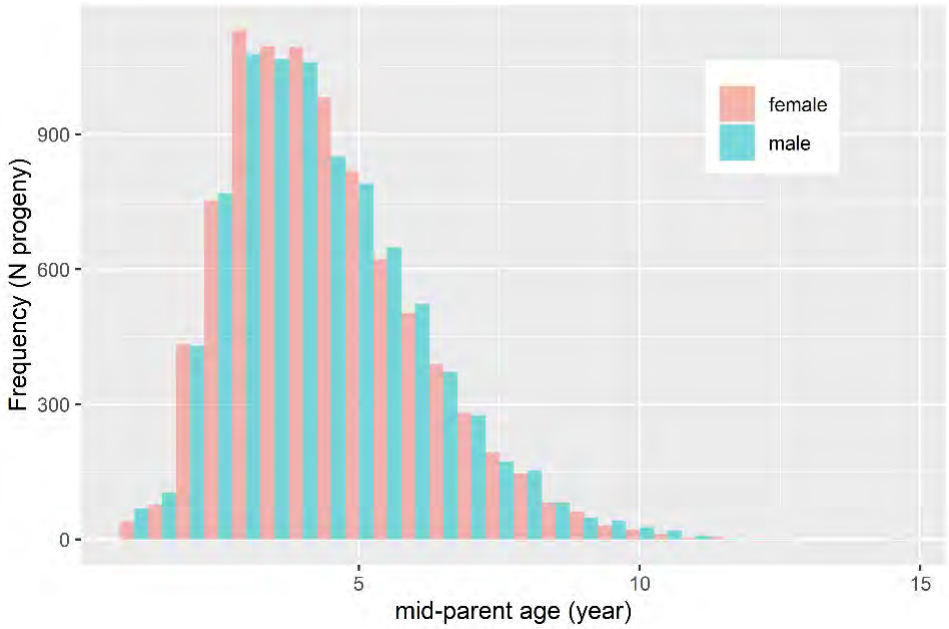

Figure 3. Distribution of mid-parent age in the reference population (n_{female}=8770, n_{male}= 8585)

Conclusion

The results represent an important scientific background about past breeding activities in the Pag sheep breed, and should serve as a baseline to upgrade the existing breeding program. The estimated inbreeding coefficient calls for attention of the breeders to keep it as low as possible in the future. The optimum contribution selection is the most appropriate selection strategy for populations under selection in order to balance selection gain with minimal loss of genetic variability, so we advocate hereby its usage in the future for this very important indigenous sheep breed.

Acknowledgment

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Odnos između boje mesa pilećih prsa i pH, gubitka mesnog soka i profila masnih kiselina

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Sažetak

U radu se istražuje kvaliteta prsnog mesa Ross 308 brojlera u dobi od 42 dana. U istraživanju je analizirano 30 uzoraka. Nakon klasifikacije prema boji mišića utvrđeno je 20 uzoraka blijedog, mekanog i vodnjikavog (BMV) i 10 normalnog. Tamnog, čvrstog i suhog mesa (TČS) nije bilo. Za vrednovanje kvalitete mesa korišteni su sljedeći pokazatelji: boja (CIE L*,a* b*), pH vrijednost, gubitak mesnog soka (%) i profil masnih kiselina. Pri klasifikaciji prsnog mesa prema boji upotrijebljeni su sljedeći pokazatelji: CIE L* od 44 do 53 meso normalnih svojstava, CIE L*> 53 BMV i CIE L*< 44 TČS meso. Vrijednosti boje mesa bile su statistički značajno veće kod BMV mesa u odnosu na meso normalnih svojstava (CIE L* 57,01 : 50,08; P<0,001). Nisu ustanovljene statistički značajne razlike u pH vrijednostima i gubitku mesnog soka (%) između ispitivanih skupina mesa. Normalno meso sadržavalo je statistički značajno manje ukupnih zasićenih masnih kiselina (∑SFA), a više ukupnih n-6 i n-3 polinezasićenih masnih kiselina (∑n-6 PUFA i ∑n-3 PUFA) u odnosu na BMV meso.

Ključne riječi: brojlersko meso, boja mesa, pH, masne kiseline

Uvod

Kvaliteta mesa je širok pojam i s prehrambenog i ekonomskog stajališta vrlo značajan. Općenito smatra se da su boja i nježnost mišića dva najznačajnija pokazatelja kvalitete brojlerskog mesa. S obzirom da je izgled mesa važan za potrošače, proizvođači vode računa o boji mesa i eventualnim nepoželjnim pojavama koje bi mogle utjecati na plasman i cijenu brojlerskog mesa na tržištu (Petracci i sur., 2004.). Neki autori ističu povezanost boje svježeg prsnog mesa s fizikalnim svojstvima (Van Lacck i sur., 2000.; Qiao i sur., 2001.). Galobart i Morgan (2014.) te Soares i sur. (2009.) su također istakli da se boja mesa (CIE L* vrijednost) može koristiti pri vrednovanju kvalitete posebice pri pojavi blijedog, mekanog, i vodnjikavog mesa (BMV). Karakteristike BMV mesa su niža pH vrijednost, bljedoća (veća L vrijednosti), povećani kalo kuhanja i povećani gubitak mesnog soka, te smanjena nježnost mišićnih vlakana (Petracci i sur., 2004.). Lee (2022.) smatra da se boja mesa može koristiti kao osnovni pokazatelj kvalitete brojlerskog mesa pri obradi i trženju. Cilj našeg istraživanja bio je ustanoviti povezanost svjetline (CIE L*) na pH vrijednosti i gubitak mesnog soka, te profil masnih kiselina u prsnom mišiću brojlera.

Materijal i metode

U istraživanju je korišteno 30 uzoraka prsnog mesa Ross 308 brojlera koji su klasificirani na osnovi vrijednosti boje mesa. Boja mesa mjerena je u klaonici za perad 24 sata nakon hlađenja trupova. Za klasifikaciju prsnog mesa u grupe korištene su slijedeće vrijednosti za boju: meso normalnih svojstava CIE L* od 44 do 53, BMV CIE L* > 53 i TČS (tamno, vrsto i suho) CIE L*< 44 (Soares i sur., 2009.). Boja prsnog mesa mjerena je pomoću kalorimetra Minolte Chroma CR400 (Minolta Camera Co. Ltd., Osaka, Japan). Uređaj je kalibriran na bijeloj ploči s izvorom svjetlosti D65 uz standardni kut promatranja od 2°. Vrijednosti pH mjerene su uporabom digitalnog Mettler MP 120-B pH metra (MettlerToledo, Schwerzenbach, Switzerland), a gubitak mesnog soka (EZ-Drip Loss) određen je metodom Christensena (2003.). Za određivanje profila masnih kiselina uzorci su pripremljeni na mikrovalnom uređaju MARS 6 (CEM Corporation, Matthews, NC, SAD) primjenom mikrovalova snage 1200 W. Ukupno je

analizirano 30 uzoraka. Na kraju procesa razaranja, uzorci su ekstrahirani u pentanu, prebačeni su u vijalicu te analizirani na plinskom kromatografu SCION 436-GC (SCION Instruments, Goes, Nizozemska) opremljenim s plameno-ionizacijskim detektorom (FID- flame ionization detector). Za separaciju masnih kiselina korištena je FAMEWAX (Restek Corporation, Bellefonte, PA, SAD) kapilarna kolona (30 m x 0,32 mm (unutarnji promjer) x 0,25 µm (debljina filma)). Volumen uzorka za injektiranje bio je 1 µL, a radni uvjeti sljedeći: temperatura injektora: 230^{O}C, temperatura detektora 230^{O}C, protok plina nosioca (vodik) 2.5 mL/min. Temperaturni program pećnice programiran je na sljedeći način: od 50 do 160^{O}C: 20^{O}C/min, od 160 do 225^{O}C: 10^{O}C/min uz zadržavanje na 225^{O}C devet minuta. Ukupno trajanje analize je 21 minutu. Za identifikaciju pojedinačnih masnih kiselina u kromatogramu korištena je standardna smjesa 37 masnih kiselina (Food Industry FAME Mix, Restek Corporation, Bellefonte, PA, SAD). Sve analize rađene su u dva ponavljanja. Rezultati istraživanja obrađeni su korištenjem statističkog programa TIBCO® Data Sciencis version 14.0.0.15 (©1984-2020 Tibco Softwere Inc.), a prikazani su u tablicama i to kao srednja vrijednost () i standardna devijacija (sd). Razlike među skupina utvrđene su analizom varijance (ANOVA) i testirane su t-testom na razini P<0,05; P<0,01 i P<0,001.

Rezultati i rasprava

Klasifikacijom uzoraka na osnovu svjetline mišićnog tkiva prsa, utvrđeno je 10 uzoraka normalnih svojstava (33,3%), 20 uzoraka BMV mesa (66,7%) i niti jedan uzorak TČS. U tablici 1 prikazani su pokazatelji kvalitete prsnog mišićnog tkiva brojlera koji su bili predmet našeg istraživanja. CIE L* vrijednost prsnog mesa koje je klasificirano kao meso normalnih svojstava, bila je niža od vrijednosti za BMV meso (CIE L* 50,08 : CIE L* 57,09) i razlika je bila statistički visoko značajna (P<0,001). Vrijednosti CIE a* (stupanj žutila) i CIE b* (stupanj crvenila) razlikovale su se između normalnog i BMV mesa. Stupanj žutila bio je veći kod normalnog u odnosu na BMV meso (CIE a*=1,14 : CIE a*=0,80; P=0,171). Vrijednost stupnja crvenila bila je veća kod BMV mesa u odnosu na normalno meso (CIE b*=19,31 : CIE b*=18,43; P=0,694). Veće vrijednosti pH zabilježene su kod mesa normalnih svojstava u odnosu na BMV meso (pH=6,13 i BMV pH=5,99; P=0,071). Gubitak mesnog soka (%) bio je veći kod BMV u odnosu na normalno meso, no razlike nisu bile statistički značajne (1,44% : 0,99%; P=0,143).

Tablica 1. Pokazatelji kvalitete prsnog mesa brojelerskih pilića (±sd)

Pokazatelji	Normalno (L 44-53)	BMV (L >53)	P vrijednost
CIE L*	50,08±2,33^{b}	57,09±3,01^{a}	<0,001
CIE a*	1,14±0,71	0,80±0,56	0,171
CIE b*	18,43±4,22	19,31±6,23	0,694
pH	6,13±0,11	5,99±0,21	0,071
Gubitak mesnog soka (%)	0,99±0,52	1,44±0,85	0,143

eksponenti ^{a,b} iznad brojeva u redu označavaju značajnost na razini od P<0,001

Soares i sur. (2009.) klasificirali su uzorke prsnog mišića brojlera na identičan način, stim da su navedeni autori u svom istraživanju ustanovili 27% uzoraka BMV i 41% uzorak mišića normalnih svojstava, što je različito od naših rezultata. Petracci i sur. (2004.) su klasificirali uzorke mesa prema boji, uzimajući u obzir i veličinu standardne devijacije pri klasificiranju: CIE L*<50 (TČS), 50≥ L≤56 (normalno meso), i CIE L* >56 (BMV), što su veće granične vrijednosti od naših u radu. Petracci i sur. (2004.) su u svom radu naveli sljedeće vrijednosti za klasifikaciju mesa prema boji, TČS uzorci bili su oni s CIE L*48,29, CIE a* 2,96 i CIE b* 1,07. Za uzorke normalnih svojstava vrijednosti CIE L* iznosile su 53,51, CIE a* 2,05 i CIE b* 1,52. Uzorci klasificirani kao BMV imali su CIE L* 57,53, CIE a* 1,63 i CIE b*2,08. Navedene vrijednosti razlikuju se od naših, posebice za CIE b* vrijednost odnosno stupanj žutila koje su u našem radu znatno veće. Soares i sur. (2009.) utvrdili su da je CIE L* vrijednost veća u uzorcima BMV mesa u odnosu na normalno i TČS meso. U našem radu također je ustanovljena veća CIE L* vrijednost za 7 jedinica u BMV u odnosu na normalno meso. Statistički značajne razlike u pH vrijednostima prema boji klasificiranog prsnog mesa navode Soares i sur. (2009.). Autori ističu da je normalno meso imalo u prosjeku pH vrijednost 5,95, BMV 5,67 a TČS 6,10. Vrijednosti pH u našem radu veće su kod normalnog i BMV mesa u odnosu na rezultate prethodno spomenutih autora. Petracci i sur. (2004.) su utvrdili pH vrijednosti za normalno i BMV meso koje su bliže našim rezultatima. Autori zaključuju da je CIE L* vrijednost >56 u prsnom mesu povezana s nižom konačnom vrijednosti pH i lošijom sposobnosti vezanja vode. Tamnija boja CIE L*<50 povezana je s većom pH vrijednošću.

Ovi rezultati sukladni su s navodima Fletcher i sur. (2000.), Van Lacck i sur. (2000.) i Qiao i sur. (2001.). Mir i sur. (2017.) ističu da nakon klanja, biokemijske promjene utječu na konverziju mišića u meso, određujući njegovu kvalitetu. Temperatura trupa post mortem, utječe na pojavu rigor mortis i fizikalno kemijske promjene koje vode mesu BMV svojstava tijekom post mortalne glikolize. Pri nastajanju rigor mortisa dvovalentni kationi Mg2+ i Ca2+ neutraliziraju negativno djelovanje reaktivnih grupa na proteine. Vrijednost pH direktno utječe na pokazatelje kvalitete kao što su sposobnost vezanja vode, boja, sočnost i održivost. Vrijednosti pH brojlerskog mesa je u funkciji pretvorbe glikogena u mliječnu kiselinu nakon klanja. Identifikacija boje mesa brzi je način procjene pH vrijednosti. Ako je meso tamne boje imat će visoku pH vrijednost, a ako je jako svijetle boje pH vrijednost će biti niska (Anadon, 2002.). Povezanost između boje i pH vrijednosti prsnog mesa objasnio je Fletcher (1999.). Varijacije boje prsnog mesa uzrokovane uglavnom pH vrijednostima utječu na održivost, razvoj mirisa, gubitak mesnog soka, sposobnost zadržavanja vode i kalo kuhanja (Alen i sur., 1998.). Barbut (1993.) navodi da meso svjetlije od normalnih vrijednosti ima početni pH 5,8, gubitak mesnog soka 3,34% i kalo kuhanja 32,09 %. Navedeni autor također je ustanovio da prsno meso s niskim pH vrijednostima ima manju sposobnost vezanja vode što rezultira s povećanom vrijednošću kala kuhanja, gubitka mesnog soka i smanjenom nježnošću. Van Lacck i sur. (2000.) su ustanovili razliku u biokemijskim svojstvima normalnog i BMV mesa. Kod BMV mesa prsa pH vrijednost je bila za 0,26 jedinica niža, a CIE L* vrijednost je bila za 4 jedinice viša u odnosu na normalno meso (pH=5,70 : 5,96; L=60,0 : 55,1). BMV meso je imalo niže CIE a* vrijednosti od normalnog mesa (1,2 : 2,0; P=0,20), a u CIE b* vrijednostima nisu ustanovljene razlike između BMV i normalnog mesa. Vrijednost gubitka mesnog soka bila je veća kod BMV u odnosu na normalno meso (1,34: 0,87). Autori su ustanovili negativnu korelaciju između pH i CIE L* vrijednosti (r=-0,76) u prsnom mesu brojlera. Wideman i sur. (2016.) također smatraju da pH vrijednost ima značajan utjecaj na boju prsnog mesa. Visoka pH vrijednost u tamnom mesu utječe na razvoj nepoželjnog mirisa i skraćuje trajnost proizvoda. Longergan i sur. (2003.) su ustanovili da prsno meso s prosječnom pH vrijednosti 5,82 ima u prosjeku CIE L* vrijednost 43,34. Soares i sur. (2003.) smatraju da nenormalne vrijednosti boje mesa mogu utjecati na fizikalno kemijske promjene uzrokovane oksidacijom lipida. Pri tome aktivnost enzima PLA2 (fosfolipaze A2) i Ca2+ zajednički iniciraju pojavu degenerativnih procesa u mesu.

U tablici 2. prikazan je profil masnih kiselina u mesu prsa koje je klasificirano kao meso normalnih ili BMV svojstava. Istraživanje je pokazalo statistički visoko značajnu razliku (P<0,001) u ukupnim zasićenim masnim kiselinama (∑SFA) između normalnog i BMV mesa (29,31% : 31,71%), a najzastupljenija je bila palmitinska masna kiselina (20,20% : 22,11%; P<0,001). Utvrđena je statistički značajna razlika u palmitoleinskoj masnoj kiselini s manjim vrijednostima u normalnom mesu (2,40% : 3,18%). Linolna masna kiselina bila je statistički značajno više zastupljena (31,03%:22,82%; P<0,001) kao i eikozadienska masna kiselina (0,94 : 0,66; P=0,039) u normalnom nego u BMV mesu. ∑n-6 PUFA bila je također veća u normalnom u odnosu na BMV meso (37,01% : 28,20%; P<0,001). α-linolenska masna kiselina, jedina iz skupine n3 PUFA, činila je 1,12% u normalnom i 1,02% u BMV mesu (P>0,05). Omjer ∑n-6/∑n-3 PUFA u našem radu bio je statistički značajno veći kod mesa prsa normalnih svojstava u komparaciji s BMV mesom (33,0 : 27,64).

Tablica 2. Profil masnih kiselina u prsnom mesu brojelerskih pilića (±sd)

Masna kiselina	Normalno (L 44-53)	BMV (L >53)	P vrijednost
Palmitinska (C16:0)	20,20±1,34[b]	22,11±1,34[b]	**<0,001**
Stearinska (C18:0)	9,11±1,15	9,60±1,09	0,265
ΣSFA	**29,31±1,71[b]**	**31,71±1,65[a]**	**<0,001**
Palmitoleinska (C16:1)	2,40±0,54[b]	3,18±0,95[a]	***0,024***
Heptadecenska (C17:1)	0,64±0,16	0,71±0,19	0,321
Oleinska (C18:1)	33,76±2,02	33,37±6,44	0,854
Eikozaenska (20:1)	0,29±0,26	0,42±0,13	0,092
ΣMUFA	**37,09±2,02**	**37,68±6,76**	0,790
Linolna (C18:2 n-6)	31,03±5,82[a]	22,82±4,11[b]	**<0,001**
Eikozadienska (C20:2 n-6)	0,94±1,23[a]	0,66±0,37[b]	***0,039***
Dihomo-γ-linolenska (20:3 n-6)	0,84±0,16	0,86±0,27	0,790
Arahidonska (C20:4 n-6)	4,20±1,14	3,86±1,08	0,425
Σn-6 PUFA	**37,01±6,29[a]**	**28,20±4,95[b]**	**<0,001**
α-linolenska (C18:3 n-3)	1,12±0,23	1,02±0,21	0,229
Σn-3 PUFA	**1,12±023**	**1,02±0,21**	**0,229**
Σn-6 PUFA/Σn-3 PUFA	**33,0±9,25[a]**	**27,64±4,93[b]**	***0,034***

eksponenti [a,b] iznad brojeva u redu označavaju značajnost na razini od P<0,05; P<0,001

Rezultati našeg istraživanja o profilu masnih kiselina u normalnom i BMV prsnom mesu, razlikuju se od rezultata koje navode Soares i sur., (2009.) u pogledu sadržaja PUFA. Qiao i sur., (2002.) u istraživanju odnosa boje i sastava mišićnog tkiva prsa brojlerskih pilića navode statistički značajno veći sadržaj palamitinske masne kiseline u prsnom mesu pilića koji su na liniji klanja imali BMV meso u odnosu na meso mormalne boje (P<0,05). Nadalje autori navode da BMV meso sadrži manji udio arahidonske, a veći udio stearinske masne kiseline u odnosu na meso normalne boje (P>0,05). Njihovi rezultati sukladni su s našima.

Zaključak

Na temelju rezultata naših istraživanja može se zaključiti sljedeće: kod BMV mesa utvrđene su niže pH vrijednosti i veći gubitak mesnog soka (%) u odnosu na meso normalnih svojstava (P>0,05). Profil masnih kiselina također se razlikovao između normalnog i BMV mesa. Normalno meso sadržavalo je više ∑n-6 PUFA (37,01% : 28,20%; P<0,01) i ∑n-3PUFA (1,12% : 1,02%; P<0,05) od BMV mesa. Navedeno je utjecalo na širi omjer ∑n-6 PUFA/∑n-3PUFA (33,00 : 27,64; P=0,034). Istraživanje je pokazalo da su se na temelju procjenjene klasifikacije s obzirom na svjetlinu mesa, potvrdile razlike u kvalitativnim pokazateljima između mesa normalnih i BMV svojstava.

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The relationship between chicken breast meat color and pH, drip loss and fatty acid profile

Abstract

The paper investigates the quality of breast meat of Ross 308 broilers at the age of 42 days. In the research was analyzed 30 samples. After classification according to muscle color, 20 samples of pale, soft and exudative (PSE) and 10 normal were determined. There was no dark, firm and dry meat (DFD). The following indicators were used to evaluate meat quality: color (CIE L*, a* b*), pH value, drip loss (%) and fatty acid profile. When classifying breast meat according to color, the following indicators were used: CIE L* from 44 to 53 meat with normal properties, CIE L*> 53 PSE and CIE L*< 44 DFD meat. Meat color values were statistically significantly higher in PSE meat compared to meat with normal properties (CIE L* 57.01:50.08; P<0.001). No statistically significant differences were found in pH values and drip loss (%) between the examined meat groups. Normal meat contained statistically significantly less saturated fatty acids (∑SFA) and more total n-6 and n-3 polyunsaturated fatty acids (∑n6 PUFA and ∑n3 PUFA) compared to PSE meat.

Keywords: broiler meat, colour meat, pH value, fatty acids

Indukcija prasenja primjenom sintetskih analoga prostaglandina F2α

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Sažetak

Cilj ovoga rada je utvrditi utjecaj indukcije prasenja primjenom alternativnih metoda aplikacije sintetskih analoga PGF2α - cloprostenola (75 µg intra muskuarno-IM i 37,5 µg para vulvalno-PV) na pokazatelje veličine legla (ukupno opraseno - UO, živoopraseno - ŽO, mrtvo opraseno - MO i avitalno - AP prasadi). Indukcija prasenja primjenom cloprostenola 115. dana graviditeta kod visokoplodnih krmača nije imala negativnih učinaka na svojstva veličine legla (UO; MO; ŽO; AP) te nije bilo razlike između broja MO i AP kod različitih načina aplikacije. PV aplikacijom cloprostenola postiže se jednaki učinak kao i kod IM primjene dvostruko većih doza, bez negativnih učinaka na svojstva veličine legla.

Ključne riječi: indukcija, prasenje, krmače, prostaglandin F2α, prasad

Uvod

Hormonska indukcija prasenja primjenjuje se na velikim svinjogojskim farmama s ciljem skraćivanja trajanja razdoblja prasenja u tjednim ciklusima što utječe na manju varijaciju u starosti prasadi te olakšava provođenje zoohigijenskih i biosigurnosnih mjera na farmi provođenjem načela „sve unutra sve van" (Calderón Díaz i sur., 2018). Primjenom sintetskih analoga prostaglandina F2α u kombinaciji s oksitocinom mogu se postići ciljana prasenja u određeno doba dana čime se omogućuje veći nadzor i pažnja od strane radnika na farmi što može značajno smanjiti broj mrtvorođene prasadi (Boonraungrod i sur., 2018; Tospitakkul i sur., 2019). To napose dolazi do izražaja kod krmača koje imaju otežano prasenje i zahtijevaju pomoć pri prasenju. S obzirom da se većina uginuća u prasilištu pojavljuje u prvih 12-24h sata nakon poroda, ova mjera može biti koristan alat u menadžmentu na farmi ako se primjenjuje na pravilan način. U suprotnom javljaju se negativni efekti koji se očituju u povećanju broja mrtvorođene i avitalne prasadi, uginuća ili prijevremenog izlučenja krmača uslijed problema s zostajanjem posteljice i/ili pojavom endometritisa (Monteiro i sur., 2022). Kao uobičajna metoda indukcije provodi se jednokratna aplikacija prostaglandina F2α intramuskularno iza baze uške. U novije vrijeme istražuju se i alternativni načini aplikacije te razdvojena primjena s ciljem smanjenja primjenjene doze i boljeg odgovora organizma. Cilj ovoga rada je utvrditi utjecaj alternativnih metoda aplikacije sintetskih analoga prostaglandina F2α s ciljem indukcije prasenja kod krmača i nazimica te istražiti njihov utjecaj na pokazatelje veličine legla s naglaskom na broj mrtvorođene i avitalne prasadi.

Materijal i metode

Istraživanje je provedeno na farmi svinja u sklopu koje se nalazi reprocentar kapaciteta 1.000 krmača. Za proizvodnju prasadi na predmetnoj farmi se koriste hibridne krmače (Danbred). Farma primjenjuje tjedni ritam prasenja pri čemu se najveći dio prasenja odvija u periodu petak-ponedjeljak, a manji dio sporadično tijekom cijelog tjedna. Istraživanje je provedeno u dva uzastopna tjedna, a uključivalo je ukupno 72 krmače (36 krmača u svakom tjednu). Krmače unutar svakog tjedna podijeljene su u tri skupine (po 12 u svakoj) s obzirom na način aplikacije sintestskog analoga prostaglandina F2α (cloprostenol) i to: skupinu koja je dobila cloprostenol intra muskularno u dozi od 75 µg (IM), zatim skupinu u kojoj je cloprostenol apliciran para vulvalno u dozi od 37,5 µg (PV) i kontrolnu skupinu koja nije dobivala sredstvo za indukciju prasenja (KONT). Za indukciju prasenja korišten je preparat trgovačkog naziva PGF2 Vexy forte (VeJx-Pharma GmbH, Schwarzenborn, Njemačka) koji sadrži 0,25 mg/ml cloprostenola ili ekvivalent 0,263 mg kloprostenolnatrija, a primjenjivan je dan prije očekivanog prasenja (115. dan graviditeta).

Nakon završetka prasenja utvrđen je broj ukupno oprasene (UO), broj živooprasene (ŽO), mrtvo oprasene (MO) i broj avitalne (AP) prasadi. Kao avitalna prasad smatrana je sva prasad lakša od 650 g i koja nakon poroda unutar 8h nije bila sposobna za samostalno sisanje. Za promatrane parametre broja prasadi izračunata je opisna statistika, a za testiranje utjecaja različitih načina primjene cloprostenola analiza varijance pri čemu je način primjene sintetskog analoga PGF2α uključen u model kao fiksni utjecaj, a tjedan prasenja kao slučajni utjecaj. Za statističku obradu korišten je programski paket SAS v 9.4 (SAS, 2013) i pripadajuće procedure (PROC MEANS i PROC GLM).

Rezultati i rasprava

U navedenom istraživanju su korištene Danbred hibridne krmače i nazimice za koje je karakteristična visoka plodnost. Prema podacima uzgojne kompanije DanBred International očekivani broj UO prasadi je 14,8 te broj odbijene prasadi 12,7 po leglu. U navedenom istraživanju broj UO kretao se od 12,7 u skupini kod koje je porod bio induciran paravulvalnom (PV) primjenom cloprostenola do 13,42 u kontrolnoj skupini. U skupini krmača kod kojih je cloprostenol primijenjen intramusluklarno (IM) zabilježeno je 13,04 UO prasadi (tablica 1.)

Tablica 1. Opisna statistika za pokazatelje veličine legla krmača bez i nakon idukcije prasenja primjenom cloprostenola kod raztličitih načina aplikacije

Pokazatelj	Srednja vrijednost	Standardna devijacija	Minimum	Maksimum	Koeficijent varijacije
Kontrolna skupina – bez primjene PGF2a					
Ukupno opraseno	13.42	1.28	10.0	15.0	9.56
Živo opraseno	11.88	1.45	9.0	14.0	12.24
Mrtvo opraseno	1.0	0.98	0	3.0	97.8
Avitalno	0.54	0.66	0	2.0	121.48
Intramuskularna primjena PGF2a					
Ukupno opraseno	13.04	2.14	8.0	17	16.38
Živo opraseno	11.79	1.86	6.0	14	15.81
Mrtvo opraseno	0.71	0.81	0	2	113.85
Avitalno	0.54	0.66	0	2	121.48
Paravulvalna primjena PGF2a					
Ukupno opraseno	12.70	1.94	9.0	16	15.29
Živo opraseno	11.52	1.81	8.0	14	15.67
Mrtvo opraseno	0.48	0.73	0	2	152.74
Avitalno	0.70	0.76	0	3	109.95

Broj prasadi u leglu je u velikoj mjeri i pod utjecajem okolišnih čimbenika odnosno uvjetovan je tehnološkim postupcima na samoj farmi. Navedeno može dijelom objasniti i nešto niže vrijednosti broja UO prasadi u ovom istraživanju u odnosu na vrijednosti koje navodi uzgojna komanija DanBred International.

Nadalje, broj prasadi u leglu ovisi i o redoslijedu prasenja odnosno udjelu prvopraskinja i višepraskinja u stadu. Istraživane skupine su činile prvopraskinje i višepraskinje od drugog do šestaog graviditeta. Prosječni udio prvopraskinja u svakoj skupini je bio oko 40%, nadalje 30-40% su bile drugo- i trećepraskinje, a ostatak su činile višepraskinje. Najveći broj peto- i šestopraskinja bio je u PV skupini (15% u odnosu na 10% u KONT i IM skupini) što dijelom objašnajva i razlike u broju UO prasadi između istraživanih skupina. Pojedinačno najveći zabilježeni broj UO prasadi u leglu bio je 17 (IM skupina) što potvrđuje da navedene hibridne krmače imaju veliki genetski potencijal za svojstvo plodnosti.

Rezultati nekih istraživanja ukazuju da indukcijom prasenja se značajno smanjuje stopa MO prasadi (Gokuldas i sur.,

2015; Nguyen i sur., 2011) te da onda ima opravdanje u uzgojima kod kojih se javlja veliki broj mrtvorođene prasadi kao posljedica nemogućnosti nadgledanja i pomoći kod prasenja (Nguyen i sur., 2011). Budući da na predmetnoj farmi nema organizirana dežurna služba, prasenja koja se događaju izvan radnog vremena (od 16h pa do 7h ujutro slijedećeg dana) nisu pod nadzorom radnika što povećava mogućnost ugibanja prasadi tijekom i neposredno nakon prasenja.

Nasuprot tome, indukcija prasenja može dovesti do povećane stope MO ukoliko se provodi prerano, a javlja se kao posljedica produjenja intervala između istiskivanja plodova (Tospitakkul i sur., 2019) i produljenju perioda prasenja (Tospitakkul i sur., 2019; Smith i sur., 1982) te do pojave većeg broja mrtvorođene prasadi i njihove manje porodne težine (Mils i sur., 2021; Olson i sur., 2009; Gunvaldsen i sur., 2007).

Prosječno trajanje graviditeta na predmetnoj farmi iznosi 116 dana (evidencija farme), a aplikacija cloprostenola izvršena je 115. dana graviditeta.

Prosječan broj MO u predmetnom istraživanju kretao se od 0,48 (PV skupina) do 1,0 (KONT skupina) i nije se značajno razlikovao između istraživanih skupina. Unatoč tome leglo s pojedinačno najvećim brojem MO prasadi (3) zabilježeno je u KONT skupini. Navedeno je u skladu s rezultatima ranijih istraživanja u kojima nije utvrđen utjecaj indukcije prasenja na stopu MO (Boonraungrod i sur., 2018; Mils i sur., 2021; Otto i sur., 2017; Gunvaldsen i sur., 2007; Olson i sur., 2009; Fraser i sur., 1984) a napose ako se primjenjuje unutar 2-3 dana prije očekivanog termina prasenja (Monteiro i sur., 2022).

Nadalje, indukcija prasenja ≥3 dana prije očekivanog prasenja rezultira manjom porodnom masom prasadi (Monteiro i sur., 2022; Mils i sur., 2021; Olson i sur., 2009; Gunvaldsen i sur., 2007). Prasad s malom porodnom masom (<800g) slabije napreduje i sklonija je obolijevanju i uginuću tijekom razdoblja dojenja i odgoja. U navedenom istraživanju sva prasad koja je bila lakša od 650g i pokazivala slabe znakove vitalnosti svrstana je u skupinu AV prasadi. Takve prasadi je bilo prosječno od 0,54 (KONT i IM skupina) do 0,7 (PVskupina) po leglu. Razlike u broju AV prasadi između istraživanih skupina nisu utvrđene što je u skladu s razultatima ranijih istraživanja (Tospitakkul i sur., 2019; Otto i sur., 2017; Decaluwé i sur., 2014; Kaeoket i sur., 2006; Stone i sur., 1987) kada je indukcija primjenjena unutar 2-3 dana prije očekivanog termina prasenja.

Tablica 2. Utjecaj indukcije prasenja primjenom različitih načina aplikacije cloprostenola na pokazatelje veličine legla

Pokazatelj	Intramuskularno		Paravulvalno		Kontrola	
	LSM	SE	LSM	SE	LSM	SE
Ukupno opraseno (UO)	13.04	0.37	12.70	0.38	13.42	0.37
Živo opraseno (ŽO)	11.79	0.35	11.52	0.36	11.88	0.35
Mrtvo opraseno (MO)	0.71	0.17	0.48	0.18	1.0	0.17
Avitalno (AP)	0.54	0.14	0.70	0.14	0.54	0.14

Zaključak

Indukcija prasenja primjenom cloprostenola (sintetskih analoga PG2α) 115. dana graviditeta kod visokoplodnih krmača nije imala negativnih učinaka na svojstva veličine legla (UO; MO; ŽO; AP). Nadalje, način aplikacije cloprostenola (IM vs. PV) nije imao utjecaja na broj MO i AP. PV aplikacijom cloprostenola postiže se jednaki učinak kao i kod IM primjene dvostruko većih doza, bez negativnih učinaka na pokazatelje veličine legla.

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Sara Mišan, Antun Kostelić, Zoran Luković, Bruna Tariba, Jelena Ramljak, Sven Menčik, Matija Brajković, Krešimir Salajpal

Induction of farrowing using synthetic analogues of prostaglandin F2α

Abstract

The aim of this paper is to determine the effect of farrowing induction using alternative methods of administration synthetic analogs of PGF2α - cloprostenol (75 μg intramuscularly-IM and 37.5 μg para vulval-PV) on litter size indicators (total born - UO, born alive - ŽO, still born - MO and non vital piglets - AP). Induction of farrowing by administration of cloprostenol on the 115th day of pregnancy in high-fertility sows had no negative effects on litter size indicators (UO; MO; ŽO; AP) and there was no difference between the number of MO and AP in different ways of application. The PV administration of cloprostenol achieves the same effect as IM administration of double doses without negative effects on litter size indicators.

Keywords: induction, farrowing, sows, cloprostenol, piglets

Povezanost kvalitete silaže i zdravlja životinja

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Sažetak

Cilj ovoga rada bio je opisati potencijalne zdravstvene opasnosti za zdravlje životinje koje mogu nastati prilikom hranidbe životinja loše pripremljenom te pokvarenom silažom. Ključne opasnosti vezane su uz porast broja nepoželjnih mikroorganizama kao što su enterobakterije, listerija, klostridije te kvasci i plijesni. Uz njih opasnost mogu predstavljati i mikotoksini kao produkti razvoja plijesni koji mogu preživjeti proces siliranja. Naposljetku, riječ može biti i o kemijskim opasnostima povezanim s nepoželjnim tijekom fermentacije silaže, kao što su visoke razine maslačne kiseline, biogenih amina, amonijaka, nitrata i nitrita. Kako bi se izbjegli svi prethodno navedeni neželjeni ishodi u procesu siliranja nužna je stroga kontrola sigurnosti i kvalitete siliranog biljnog materijala.

Ključne riječi: silaža, siliranje, nepoželjni mikroorganizmi, kemijske opasnosti, kontrola

Uvod

Proces siliranja se koristi za konzerviranje stočne hrane više od 3000 godina (**Àvila i Carvalho, 2019**). Proizvodnja silaže ima dugu tradiciju s dokazima o očuvanim biljnim materijalima kao hrani u starom Egiptu, 1500-1000 pr.Kr., također, Grci i Rimljani su cijenili silažu što se može iščitati u starim spisima toga doba koji objašnjavaju da je nepropusno, hermetičko zatvaranje bilo preduvjet uspješnog očuvanja silaže (Alonso i sur., 2013.). Iako su osnovni principi spremanja silaže bili primjenjivani stoljećima, ozbiljnija primjena spremanja silaže u stočarskoj proizvodnji dogodia se 1950-ih godina, kada se oživila intenzivna stočarska proizvodnja (Alonso i sur., 2013.; Driehuis i Elferink, 2000.). Trenutno u svijetu kao i kod nas je siliranje krme jedan od najprikladnijih oblika konzerviranja hrane za preživače, a višestruko korisni učinci silaže u obrocima goveda razlog su njene upotrebe kontinuirano tijekom cijele godine kao dominantnog krmiva u obroku (Domaćinović i sur., 2022.; Domaćinović i sur., 2022a; Àvila i Carvalho, 2019.; Alonso i sur., 2013.; Dunière i sur., 2013.; Driehuis i Elferink, 2000.).

Siliranje je tehnologija konzerviranja usjeva koja se temelji na prirodnoj mliječnoj kiseloj fermentaciji u anaerobnim uvjetima. Primarna svrha siliranja te proizvodnje silaže je da krma ostane dostupna tijekom cijele godine za korištenje kao izvor hrane visoke hranjive vrijednosti za preživače, čime se poboljšava ekonomska i ekološka održivost proizvodnih sustava (Alonso i sur., 2013.; Dunière i sur., 2013.; Driehuis i Elferink, 2000.).

Proces siliranja uključuje mnoge zahvate koje treba pažljivo provesti i kontrolirati kako bi se osigurala poželjna fermentacija u silosu uz minimalne ekonomske gubitke i zdravstvene rizike kako za životinje tako i za ljude (Dunière i sur., 2013.; Wilkinson, 1999.). Optimalna zrelost usjeva za siliranje je prvi uvjet za proizvodnju dobre silaže. Upravo optimalno vrijeme žetve odražava se na sadržaj suhe tvari siliranog materijala (32-37%) te pogodnost sirovine za sam proces siliranja i udio pojedinih dijelova biljke kao i na sadržaj hranjivih tvari što posljedično na kraju utječe na energetsku vrijednost silaže te njenu ukupnu hranjivu vrijednost (Kung i sur., 2018.).

Uz dobru agrotehniku te vremenske prilike u vrijeme proizvodnje, hranjivu vrijednost npr. silaže kukuruza uvjetuje i optimalni trenutak skidanja biljke, kvaliteta prerade odnosno način spremanja biljke te vrlo bitan odnos pojedinih dijelova biljke u masi (Domaćinović i sur., 2022.; Domaćinović i sur., 2022a). Optimalan trenutak skidanja biljke kukuruza za silažu je vrijeme završetka mliječne i početak voštane zriobe zrna (Domaćinović i sur., 2022.; Garcia, 2016.). Glavni princip siliranja je brzo postizanje niskog pH pomoću mliječno kiselinskog vrenje i održavanje anaerobnih uvjeta u silosu. Upravo mliječno-kisele bakterije imaju ključnu ulogu u osiguravanju uspjeha postupka siliranja (Driehuis i sur., 2018.; Kung i sur., 2018.; Pahlow i sur., 2003.). Mliječno-kisele bakterije koriste vodotopive

ugljikohidrate za proizvodnju mliječne kiseline te manjim dijelom octene kiseline, a upravo je mliječna kiselina vrlo važan konzervans koji konzervira silirani biljni materijal (Àvila i Carvalho, 2019.; Driehuis i sur., 2018.).

Zbog sve veće upotrebe silaže diljem svijeta, potrebno je osigurati dobru kvalitetu proizvedene silaže. Upravo ispravno pripremljena silaža izvrsna je hrana koja ne predstavlja opasnost po zdravlje životinja, a preko njihovih proizvoda i na zdravlje ljudi (Driehuis i sur., 2018.; Dunière i sur., 2013.). Poželjnu mikrofloru silaže čine bakterije mliječno-kiselinskog vrenja, u nešto manjoj mjeri tu su prisutne i bakterije octenog te propinskog vrenja, no svakako nisu zanemarive i one nepoželjne bakterije maslačnog vrenja, proteolitičke bakterije, plijesni i kvasci koje su često nezaobilazni pratitelji gotovo svake silaže (Àvila i Carvalho, 2019.; Queiroz i sur., 2018.; Driehuis i Elferink, 2000.). Nepoželjni mikroorganizmi se javljaju u silaži kada pH vrijednost nije dovoljno niska, odnosno kada nisu postignuti anaerobni uvjeti te zadovoljeni ostali okolišni uvjeti (temperatura i vlažnost) (Àvila i Carvalho, 2019.). Spomenuti nepoželjni mikroorganizmi su štetni za hranidbenu kvalitetu silaže, primjerice kvasci i bakterije maslačnog vrenja te one mikroorganizme koji mogu biti štetni za zdravlje životinja ili njihovih konačnih proizvoda (mlijeka), kao što su *Clostridium botulinum*, *Clostridium spp.*, *Bacillus spp.*, *Listeria monocytogenes*, *Escherichia coli* i druge vrste *Enterobacteriaceae* te različite vrste plijesni (Driehuis i sur., 2018.; Queiroz i sur., 2018.; Wilkinson, 1999.). Opasnost za zdravlje može prouzročiti sam mikroorganizam ili njegov metabolit, kao što su npr. mikotoksini koje proizvode plijesni (Driehuis i sur., 2018.; Kung i sur., 2018.).

Cilj ovoga stručnog rada bio je opisati potencijalne zdravstvene opasnosti za zdravlje životinja koje mogu nastati prilikom hranidbe životinja loše pripremljenom te pokvarenom silažom.

Nepoželjni mikroorganizmi u silaži i njihovi produkti

Mikroflora silaže ima ključnu ulogu u uspješnom konzerviranju biljnog materijala siliranjem. Nepoželjni mikroorganizmi koji se mogu javiti u silaži su uključeni u anaerobno kvarenje (npr. klostridije i enterobakterije) ili u aerobno kvarenje (npr. kvasci, plijesni i listerija). Mnogi od tih mikroorganizama kvarenja osim što smanjuju hranidbenu vrijednost silaže, imaju štetan učinak na zdravlje životinja i/ili kvalitetu mlijeka te posljedično tome zdravlje ljudi kao krajnjih konzumenata takvih proizvoda. Nepoželjni mikroorganizmi također potiču i ekonomske gubitke na farmi (Dunière i sur., 2013.; Driehuis i Elferink, 2000.). Enterobakterije su gram-negativne anaerobne bakterije u procesu siliranja. Njihova uloga je u redukciji nitrata u amonijak preko nitrita u ranoj fazi procesa siliranja, uz proizvodnju dušikovog oksida međutim, njihov rast i sposobnost preživljavanja se smanjuju kako pH vrijednost siliranog materijala opada (Queiroz i sur., 2018.; Driehuis i sur., 2018.; Wilkinson, 1999.). Bitni čimbenici koji određuju proliferaciju enterobakterija u silaži su uvjeti u silosu. Kada je stopa zakiseljavanja niska uvjeti za rast enterobakterija su povoljni te životinje koje konzumiraju takvu silažu često imaju crijevne poremećaje te učestaliju pojavu mastitisa (Ogunade i sur., 2017.; Wilkinson, 1999.). Najvažniji predstavnik iz ove skupine s gledišta rizika za zdravlje životinja je *E. coli*. *E. coli O157:H7* je gram negativna bakterija koja proizvodi Shiga toksin te je povezana s hemorgaijskim kolitisom i hemolitičko-uremičkim sindromom kod ljudi koji se zaraze konzumacijom mlijeka onečišćenog fecesom zaraženih krava. Goveda su zapravo glavni rezervoari ovoga patogena te se smatra kako su 30% goveda njegovi asimptomatski nositelji. Silaža je obično kontaminirana gnojidbom stajskim gnojem te vrlo često zagađenom vodom za navodnjavanje usjeva. Snižavanje pH vrijednosti uspješno eliminira *E. coli* iz silaže (Queiroz i sur., 2018.; Ogunade i sur., 2017.; Dunière i sur., 2013.).

Listeria je fakultativni anaerob, gram-pozitivna bakterija koja je široko rasprostranjena u okolišu. Riječ je o patogenu koji uzrokuje teške sistemske infekcije (listerioze) kod ljudi i životinja s visokom stopom smrtnosti, a njegova sve veća učestalost veliki je javno zdravstveni problem (Àvila i Carvalho, 2019.; Driehuis i sur., 2018.; Queiroz i sur., 2018.; EFSA i ECDC, 2016.). Listerioza se često smatra bolešću koja se prenosi hranom kod preživača, pri čemu je silaža glavni izvor hrane jer je obično upravo ona dominantna komponenata obroka preživača. Zbog visoke tolerancije na stresne uvjete, *L. monocytogenes* je sposobna preživjeti dulje vrijeme u okruženjima u kojima se inače ne može uzgajati, kao što su loše zbijanje silaže tijekom pripreme te ulazak zraka tijekom fermentacije siliranog materijala i relativno visoke pH vrijednosti koje mogu pospješiti njen rast i razvoj (Àvila i Carvalho, 2019.; Driehuis i sur., 2018.; Driehuis i Elferink, 2000.; Wilkinson, 1999.). U brojnim istraživanjima pokazana je uzročna veza između hranidbe nekvalitetnom silažom i prevalencije listerioze kod goveda, ovaca i koza (Driehuis i sur., 2018.; Queiroz i sur., 2018.; Ho i sur., 2007.). *Listeria monocytogenes* iz silaže preživi prolazak kroz gastrointestinalni trakt životinje i izbacuje se fecesom van organizma životinje (Unnerstad i sur., 2000.; Nightingale i sur., 2004.; Vilar i sur., 2007.). Kontaminacija sirovog kravljeg mlijeka bakterijom *L. monocytogenes* je povezana s pojavom visokih razina *L. monocytogenes* u

silaži, a prijenos u mlijeko najvjerojatnije se javlja tijekom mužnje ili u slučajevima njezine povezanosti s pojavama mastitisa. S obzirom da je *L. monocytogenes* osjetljiva na toplinu učinkovito se inaktivira adekvatnom pasterizacijom mlijeka, ali ona može preživjeti u mekim sirevima i mliječnim proizvodima koji nisu podvrgnuti takvim tretmanima pasterizacije (Àvila i Carvalho, 2019.; Driehuis i sur., 2018.; Queiroz i sur., 2018.; Driehuis i Elferink, 2000.).

Clostridium vrste su gram-pozitivne, obligatne anaerobne bakterije koje stvaraju spore. Klostridije za svoj rast zahtijevaju relativno visoke pH vrijednosti (>4,5) i visoku vlažnost krme (>70%) (Driehuis i sur., 2018.; Queiroz i sur., 2018.; Driehuis i Elferink, 2000.). Nepatogene klostridije koje se obično pojavljuju u silaži uključuju *C. tyrobutyricum*, *C. butyricum* i *C. sporogenes* (Driehuis, 2013.; Pahlow i sur., 2003.). Patogene klostridije uključuju *C. perfringens*, *C. difficile*, *C. tetani* i *C. botulinum*, a od njih samo vrsta *C. botulinum* je povremeno povezana sa silažom (Àvila i Carvalho, 2019.; Driehuis i sur., 2018.).

Vrsta *C. botulinum* je heterogena skupina bakterija koja ima sposobnost proizvodnje neurotoksina botulinuma, uzročnik botulizma. Botulinum je proteinski toksin i vjeruje se da je najjači toksin u prirodi (Driehuis i sur., 2018.). Botulizam je teška bolest i kod ljudi, ali i kod životinja. Botulizam kod goveda je intoksikacija uzrokovana konzumiranjem kontaminirane hrane (ili vode). Na sreću, *C. botulinum* ima ograničenu toleranciju na kiseline te ne raste u dobro pripremljenoj i fermentiranoj silaži. Međutim, pojava životinjskog botulizma uzrokovanog silažom kontaminiranom bakterijom *C. botulinum* gotovo uvijek se pripisuje lošoj pripremi silaže te vrlo često prisutnosti različitih uginulih životinja (npr. glodavaca ili ptica) u pripremljenoj silaži (Queiroz i sur., 2018.; Driehuis i Elferink, 2000.; Wilkinson, 1999.). Klostridije štetno utječu na kvalitetu silaže. Proteolitička aktivnost klostridija u silaži može rezultirati ekstenzivnom razgradnjom biljnih proteina do amonijaka što može imati negativne posljedice na zdravlje životinja (Wilkinson, 1999.).

Kvasci su fakukltativno anaerobni eukariotski mikroorganizmi. Vrste koje se javljaju u silaži uključuju rodove *Candida*, *Hansenula*, *Saccharomyces* i *Torulopsis*, a njihova aktivnost u siliranom materijalu se smatra nepoželjnom (Driehuis i Elferink, 2000.). Pod anaerobnim uvjetima kvasci fermentiraju šećere do etanola i CO_2. Proizvodnja etanola u silaži ne samo da snižava količinu šećera dostupnog za proizvodnju kiseline već također povećava gubitke suhe tvari tijekom procesa siliranja (Àvila i Carvalho, 2019.; Driehuis i Wikselaar, 2000.). Pod aerobnim uvjetima kvasci oksidiraju mliječnu kiselinu uzrokujući porast pH vrijednosti silaže što je okidač za rast i razvoj nekih drugih štetnih mikroorganizama (Dunière i sur., 2013.). Kvasci su danas prepoznati kao najznačajniji mikroorganizmi odgovorni za otpočinjanje aerobnih procesa kvarenja silaže (Driehuis i Elferink, 2000.).

Plijesni su eukaritski obično aerobni mikroorganizmi. Rast plijesni u silaži obično je ograničen na površinske slojeve silaže te često upućuje na loše zbijanje te pokrivanje silaže. Uz prethodno navedeno, plijesni se razvijaju tijekom uznapredovalih stadija aerobnog kvarenja silaže. Rodovi plijesni koji se najčešće izoliraju iz silaže su: *Penicillium*, *Fusarium*, *Aspergillus*, *Mucor* te *Trichoderma* (Křížová i sur., 2021.; Ulrikh i Smolovskaya, 2021.; Alonso i sur., 2013.; Àvila i Carvalho, 2019.; Driehuis i sur., 2018.). Plijesni u silaži dovode do smanjenja njezine kvalitete i ješnosti te također mogu imati negativan utjecaj na zdravlje životinja budući da veliki broj plijesni proizvodi različite vrste toksina (Dunière i sur., 2013.; Driehuis i Elferink, 2000.). Mikotoksini su poznati kao molekule niske molekularne težine obično su to vrlo stabilne molekule (Křížová i sur., 2021.; Ulrikh i Smolovskaya, 2021.). Oni nastaju uglavnom sekundarnim metabolizmom određenih filamentoznih gljiva, koje rastu pod utjecajem specifične temperature i vlage te uzrokuju ozbiljne rizike za zdravlje životinja i ljudi (Křížová i sur., 2021.; McElhinney i sur., 2015.). Ovisno o vrsti i količini mikotoksina prisutnog u silaži negativni utjecaji na zdravlje životinja mogu varirati, od blagih probavnih smetnji, manjih problema s plodnošću te smanjena imunost, do ozbiljnih oštećenja jetre ili bubrega te učestalih pobačaja kod životinja. Uz negativne utjecaje na zdravlje životinja mikotoksini se iz silaže mogu prenijeti na mlijeko ili meso životinja koje su takvu silažu konzumirale (Křížová i sur., 2021.; Àvila i Carvalho, 2019.; Wambacq i sur., 2016.; McElhinney i sur., 2015.; Driehuis i Elferink, 2000.). Mikotoksini koji se najčešće susreću u siliranom materijalu su: aflatoksini, ohratoksini, trihoteceni, fumonizini, zearalenon, mikofenolna kiselina i rokvefortin (Àvila i Carvalho, 2019.; Ogunade i sur., 2018.; Driehuis, 2013.). Kada se govori o prisutnosti mikotoksina u siliranom materijalu uvijek se naglašava potreba za dobrim načinima upravljanja silosima odnosno cijelim procesom siliranja od punjenja silosa do upotrebe silaže za hranidbu životinja kako bih se izbjegla i prevenirala pojava mikotoksina u siliranom materijalu (Wambacq i sur., 2016.).

Kemijske opasnosti

Zbog procesa fermentacije silaže, koncentracije nitrata u silaži su snižene u odnosu na njihovu koncentraciju u usjevima tijekom žetve. Nitrati se razgrađuju u ranim fazama procesa siliranja pod utjecajem enterobakterija i bakterija mliječne kiseline. Krajnji produkti degradacije nitrata u silaži su amonijak i dušikov oksid. Pozitivna posljedica redukcije nitrata u dušikov dioksid je sprečavanje rasta klostridija. Međutim, ako dušikov dioksid bude u potpunosti razgrađen do amonijaka pH vrijednost silaže može ostati relativno visoka (>4,5) i posljedično tome zakiseljavanje siliranog materijala može biti nedovoljno za sprečavanje rasta klostridija tijekom perioda skladištenja osobito kod niskih koncentracija suhe tvari u siliranom materijalu (Driehuis i sur., 2018.; Wilkinson, 1999.). Proizvodnja dušikovog oksida i dušikovog dioksida u silaži predstavlja ozbiljan rizik za zdravlje životinja u smislu pojave respiratornih smetnji. Udisanje ovih plinova oštećuje plućno tkivo i ozbiljno remeti funkciju disanja životinje dovodeći potencijalno do njenoga gušenja (Driehuis i sur., 2018.). Povišene koncentracije amonijaka u silaži mogu oštetiti jetru životinja ako one takvu silažu učestalo konzumiraju. Neka su pak istraživanja dovela u vezu povišene koncentracije amonijaka u silaži s pojavom šepavosti životinja no definitivni zaključci tek trebaju biti potvrđeni u budućim epidemiološkim studijama (Driehuis i sur., 2018.; Wilkinson, 1999.). Maslačna kiselina može se javiti u nedovoljno fermentiranoj silaži. Ona pod pod utjecajem klostridija nastaje uslijed fermentacije u vodi topljivih ugljikohidrata i mliječne kiseline. Uz maslačnu kiselinu spomenute bakterije dovode i do proizvodnje amina, amonijaka, ugljičnog dioksida, hidrogena i octene kiseline u silaži. Rizik za zdravlje životinja vezan uz maslačnu kiselinu u nedovoljno fermentiranoj silaži većinom se odnosi na ketozu povezanu izravno s konzumacijom silaže koja sadrži maslačnu kiselinu, ali i neizravno sa smanjenim energetskim unosom zbog konzumacije takve silaže (Driehuis i sur., 2018.). U silaži se mogu pojaviti i biogeni amini, što može imati nepovoljan utjecaj na konzumaciju hrane i zdravlje životinja. Ovi spojevi nastaju hidrolizom peptidnih veza te uključuju enzimatsku dekarboksilaciju slobodnih aminokiselina djelovanjem biljnih proteaza i peptidaza te bakterijskih enzima. Najznačajniji biogeni amini koji se mogu naći u silaži su: histamin, tiramin, putrescin, kadaverin, spermin i spermidin. Svi se oni mogu akumulirati u mliječnim proizvodima osobito siru te negativno utjecati na zdravlje konzumenata takvih proizvoda (Gardini i sur., 2016.). Glavni rizični čimbenik za produkciju amina u silaži je sporo zakiseljavanje siliranog materijala nakon siliranja. Amini nastali u ranim fazama siliranja vjerojatno su posljedica dekarboksilacije aminokiselina uz pomoć enterobakterija tijekom inicijalne aerobne faze siliranja, dok su oni amini koji nastaju kasnije tijekom siliranja rezultat posljedičnog rasta proteolitičkih vrsta klostridija u siliranom materijalu (Driehuis i sur., 2018.; Dunière i sur., 2013.).

Zaključak

Proces siliranja temelji se na mliječno-kiselom vrenju u anaerobnim uvjetima čime se čuva nutritivna vrijednost te higijenska ispravnost siliranog materijala. Međutim, u silaži se mogu pojaviti neželjeni mikroorganizmi koji uzrokuju degradaciju i kvarenje silaže što može značiti velike gospodarske gubitke tijekom procesa siliranja. Uz štetne mikroorganizme i različiti kemijski spojevi mogu dovesti do kvarenja silaže. Silaža može biti prijenosnik patogenih mikroorganizama koji prijete zdravlju životinja i ljudi. Kako bih se izbjegli svi prethodno opisani neželjeni ishodi u procesu siliranja nužna je redovita kontrola sigurnosti i kvalitete siliranog materijala odnosno kontrola njene zdravstvene i hranjive vrijednosti, čime se ograničava mogućnost pojave razbolijevanja životinja te posljedični prijenos patogena na ljude kroz kontaminaciju cijelog hranidbenog lanca.

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The connection between silage quality and animal health

Abstract

This manuscript aimed to describe the potential health hazards for animal health that may arise when feeding animals with poorly prepared and spoiled silage. The key dangers are related to the increase in the number of undesirable microorganisms such as enterobacteria, listeria, clostridia, and yeasts and molds. In addition to them, mycotoxins can also pose a danger as products of mold development that can survive the ensiling process. Finally, there may be chemical hazards associated with undesirable silage fermentation processes, such as high levels of butyric acid, biogenic amines, ammonia, nitrates, and nitrites. To avoid all previously mentioned unwanted outcomes in the ensiling process, strict control of the safety and quality of the ensiled plant material is necessary.

Keywords: silage, ensiling, undesirable microorganisms, chemical hazards, control

Procjena sadržaja Imunoglobulina G (IgG) u kolostrumu krmača BRIX refraktometrom

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Sažetak

Cilj istraživanja bio je utvrditi mogućnost primjene digitalnog Brix refraktometra kao jedne od metoda za mjerenje koncentracije imunoglobulina G (IgG) u kolostrumu krmača te njegovu usporedbu s rezultatima dobivenim laboratorijskim pretraživanjem uzoraka kolostruma krmača metodom radijalne imunodifuzije. Brix refraktometar pruža prihvatljivu metodu za procjenu koncentracije IgG kod kolostruma krmača. Ova metoda ima prednosti u odnosu na druge metode procjene koncentracije IgG, s obzirom da je Brix refraktometar jeftin, lako dostupan, manje krhak i manje osjetljiv na varijacije u temperaturi kolostruma, godišnje doba i druge čimbenike, te zahtijeva minimalnu opremu i obuku.

Ključne riječi: krmače, kolostrum, imunoglobulini, BRIX refraktometar

Uvod

Prva cjelovita hrana koja je dostupna novorođenoj prasadi je kolostrum. Kolostrum se izlučuje iz mliječne žljezde odmah nakon prasenja i unutar nekoliko sati njegov sastav se mijenja vrlo brzo te dalje predstavlja mlijeko krmače (Barrington i sur., 2001.). Kvaliteta kolostruma je važan faktor koji utječe na zdravlje novorođene prasadi (Rolinec i sur., 2012.).

Kolostrum sadrži imunološki funkcionalne proteine, imunoglobuline (Ig), masti, minerale i vitamine. Najvažniji sastojci kolostruma su imunoglobulini (Ig), važni u neutralizaciji toksina, virusa i bakterija. Oni predstavljaju pasivnu imunološku zaštitu jer obnavljaju i jačaju imunološke funkcije. Novorođenoj prasadi koja tijekom prvih tjedana života ne primi kolostrum prijeti slab prirast tjelesne mase te, u većini slučajeva, takva prasad ne preživi (Kirovski i sur., 2014.). Kako bi se osiguralo preživljavanje i željeni zdravstveni status prasadi, ključno je da prasad na vrijeme posisa kolostrum odgovarajuće kakvoće u pogledu sadržaja hranjivih tvari (Quesnel, 2011.). Jedna od značajnih metoda koja može pomoći u definiranju proizvodno-tehnoloških parametara jest procjena kvalitete kolostruma neposredno nakon prasenja krmača.

Danas su dostupne različite metode u svrhu procjene kvalitete kolostruma, međutim nisu sve od tih metoda pogodne za upotrebu na farmama. Glavni razlog tome je to što bi metoda procjene kvalitete kolostruma na farmi trebala bi biti brza, jednostavna za korištenje, cijenom prihvatljiva i točna.

Najpreciznija metoda vrednovanja sadržaja u IgG u kolostrumu je radijalna imunodifuzija (RID). Nažalost, RID je laboratorijska metoda koja zahtjeva oko 18 do 24 sata za utvrđivanje rezultata, te kao takva nije primjenjiva na farmama (Gelsinger i sur., 2015., Cabrera i sur., 2012.).

Vizualna procjena, kao što je viskoznost ili gustoća je najčešći način za procjenu kvalitete kolostruma, ali nije dobar pokazatelj razine antitijela jer komponente kolostruma poput masti i proteina mogu promijeniti vizuelni izgled kolostruma, ali ne i odražavati razinu antitijela.

Procjena kvalitete kolostruma pomoću hidrometra/colostrometra je jeftina, nudi brze rezultate i jednostavna je za korištenje. Ona mjeri specifičnu težinu kolostruma pomoću plutajućeg stakla. Specifična gustoća je učinkovit način za mjerenje razine antitijela, jer antitijela predstavljaju veliki dio proteina u kolostrumu, a proteini čine visok postotak ukupne suhe tvari koja visoko korelira sa specifičnom težinom. Hidrometri/kolostrometri su učinkovitiji od vizuelne

procjene kvalitete kolostruma, ali su temperaturno osjetljiviji i zahtjevaju veću količinu kolostruma potrebnu za testiranje. Osim toga plutajuće staklo je krhko, lako se razbije i skupo je za zamjenu (Heinrichs i Jones, 2011.).

Nova metoda za mjerenje kvalitete kolostruma, koja može pomoći proizvođačima u praksi, je mjerenje pomoću refraktometra. Refraktometrija je optička pojava koja se zasniva na prelamanju svjetlosnih zraka na granici dviju različitih sredina u kojima se svijetlost rasprostire različitom brzinom. Rezultat mjerenja je indeks prelamanja, n. Refraktometrijski određena suha tvar predstavlja u vodi rastvorljive supstance. Suha tvar se određuje specijalnim instrumentom - refraktometrom koji je podesan za brzu analizu sirovina i sadržaja suhe tvari. Prednost ove metode je u brzini, jednostavnosti i upotrebi malih količina uzorka (Chigerwe i Hagey, 2014.).

Brix refraktometri, odnosno „Brix" skala se obično koristi za mjerenje šećera u sadržaju voća, povrća, javorovom sirupu, medu i vinu. Studije objavljene u posljednjih nekoliko godina su podržale valjanost korištenja Brix refraktometra za mjerenje ukupne suhe tvari u kolostrumu, koji je tada u korelaciji s IgG sadržajem. Refraktometri su dovoljno točni, izdržljivi, cjenovno pristupačni a rezultati su gotovo trenutni i ne ovise o temperaturi kolostruma, te zahtjevaju samo nekoloko kapi uzorka. Brix refraktometri mogu biti optički i digitalni. Digitalni refraktometar ima sve prednosti optičkoga, ali daje digitalno očitanje i eliminira potrebu za tumačenje linije razgraničenja. Nekoliko kapi se stavi u za to predviđeno mjesto na uređaju, a Brix vrijednost se prikaže na digitalnom očitanju (Quigley i sur., 2013.).

Cilj ovog istraživanja je procjeniti upotrebljivost digitalnog Brix refraktometra za mjerenje kvalitete kolostruma u usporedbi s laboratorijskom pretragom RID.

Materijal i metode

Istraživanje je provedeno na dvije komercijalne reprodukcijske farme u istočnoj Hrvatskoj (farma A i farma B) koje se nalaze u sustavu dva različita hibridna programa (C i D). Obje farme bave se proizvodnjom i uzgojem prasadi za tov do prosječne tjelesne težine od 25 kg. Na svakoj farmi odabrano je 120 plotkinja (nazimica i krmača) koje su korištene za potrebe istraživanja. Osnovni kriterij prilikom odabira plotkinja bio je redni broj prasenja. Kao kriterij odabira u obzir se uzimala veličina legla, stopa smrtnosti unutar laktacije, porodna i završna težina legla i sastav hrane. Od svake krmače, nulti sat od početka prasenja, kroz tri ciklusa uzimanja uzoraka, iz jedne od prednjih sisa uzeto je (izmuzeno rukom) u sterilnu epruvetu ukupno 10 ml kolostruma za refraktometrijsku analizu. Uzimanje uzoraka provedeno je lkroz tri ciklusa prasenja. Kolostrum je odmah nakon uzimanja pohranjen na temperaturi od -20°C. Osim navedenih 640 uzoraka kolostruma za refraktometriju, prikupljeno je i ukupno 100 uzorka za radijalnu imunodifuziju (RID), paralelno s uzorcima za mjerenje pomoću digitalnog Brix refraktometra. Ovi uzorci uzimani su u isto vrijeme na dvije navedene farme od po 50 uzoraka na svakoj farmi također u 3 navrata od krmača koje su različitog broja prasenja. Smrznuti uzorci kolostruma korišteni su za laboratorijske analize u kojima se utvrđivao kemijski sastav kolostruma i sadržaj imunoglobulina G (IgG).

Laboratorijske analize provedene su u laboratoriju za biokemiju Odjela za biologiju Sveučilišta Josipa Jurja Strossmayera te laboratoriju MEGGLE Hrvatska d.o.o. Osijek.

Nakon što su prikupljeni i zamrznuti svi uzorci kolostruma krmača tijekom jednog od ukupno tri uzorkovanja, uzorci za refraktometriju koji su smrznuti na -20°C otopljeni su polagano na sobnu temperaturu. Nakon otapljanja sadržaj epruvete lagano se izmiješao okretanjam 10-12 puta kako bi se sadržaj kolostruma prije mjerenja refraktometrom dobro izmiješao za točnu mjeru kvalitete uzorka. Na isti način pripremljeni su i uzorci za kemijsku analizu kolostruma, a za radijalnu imunodifuziju kolostrum se odmrzavao u toploj vodenoj kupki. Neposredno prije RID analize, kolostrum se miješao nekoliko minuta.

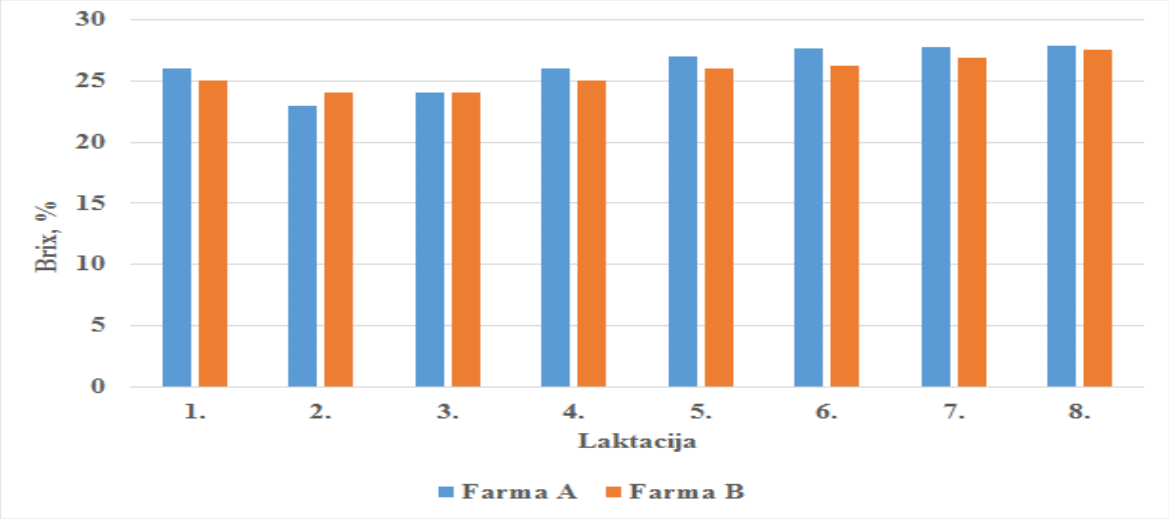
Sadržaj ukupne suhe tvari i udjel imunoglonulina G (IgG) u kolostrumu krmača utvrđen je pomoću digitalnog refraktometra HANNA HI 96801 Refractometer (mjerno područje 0 – 85% Brix, proizvođača HANNA Instruments, Woonsocket RI USA, Made in Romania, SN A0078364). Kemijska analiza kolostruma izvršena je u laboratorijskim uvjetima na uređaju MILKOSCAN FT120. Statistička analiza podataka provedena je pomoću SAS® Stat softvera (SAS® System, License No. 0092661001). GLM precedura (SAS Institute Inc.) korištena je za utvrđivanje razlika u sastavu nutrijenata u kolostrumu s obzirom na cikluse skladištenja uzoraka. Model za procjenu je opisan jednadžbom:

$$Yij = \mu + Fi + eij$$

gdje je Yij zavisna varijabla; μ srednja vrijednosti; Fi fiksni učinak zavisne varijable; eij pogreška zavisne varijable. (Morrilli i sur., 2012). Primjenom procedure CORR utvrđeni su koeficijenti korelacija između IgG utvrđenih RID i Brix metodom.

Rezultati i rasprava

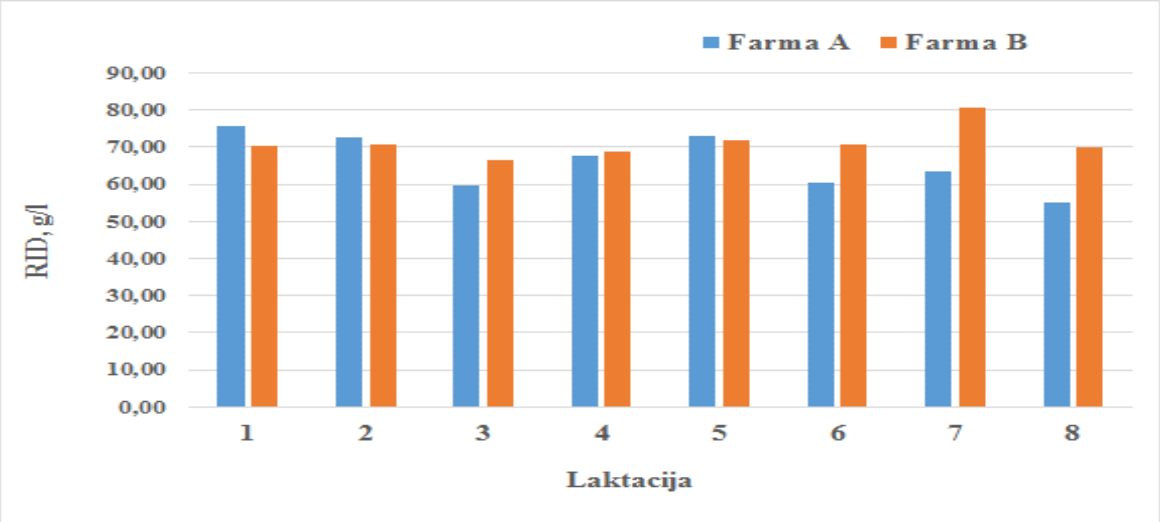
Vrijednosti udjela imunoglobulina G dobivene mjerenjem ukupne suhe tvari kolostruma pomoću Brix refraktometra prikazane su u grafikonu 1.



Grafikon 1. Brix vrijednosti utvrđene u kolostrumu krmača na farmi A i B

Prosječne Brix vrijednosti utvrđene analizom uzorka kolostruma kroz 8 laktacija na dvije različite farme ukazuju da je kolostrum krmača s farme A imao nešto više vrijednosti u odnosu na kolostrum krmača s farme B, no te vrijednosti nisu bile u rangu statističke značajnosti. Zamjetan je blagi pad vrijednosti Brix-a u prve dvije laktacije, nakon čega je uslijedilo linearno povećanje vrijednosti, što je u skladu s prethodnim istraživanjima (Bielmann i sur., 2010., Bartier i sur., 2015., Quesnel, 2011.). Prosječne Brix vrijednosti utvrđene na obje farme ukazuju da je kolostrum ispitivanih krmača bio u granicama kvalitetnog do vrlo kvalitetnog (>20%) što ukazuje na dobru genetsku osnovu stada i uspješno menadžiranje istog (Quigley i sur., 2013.).

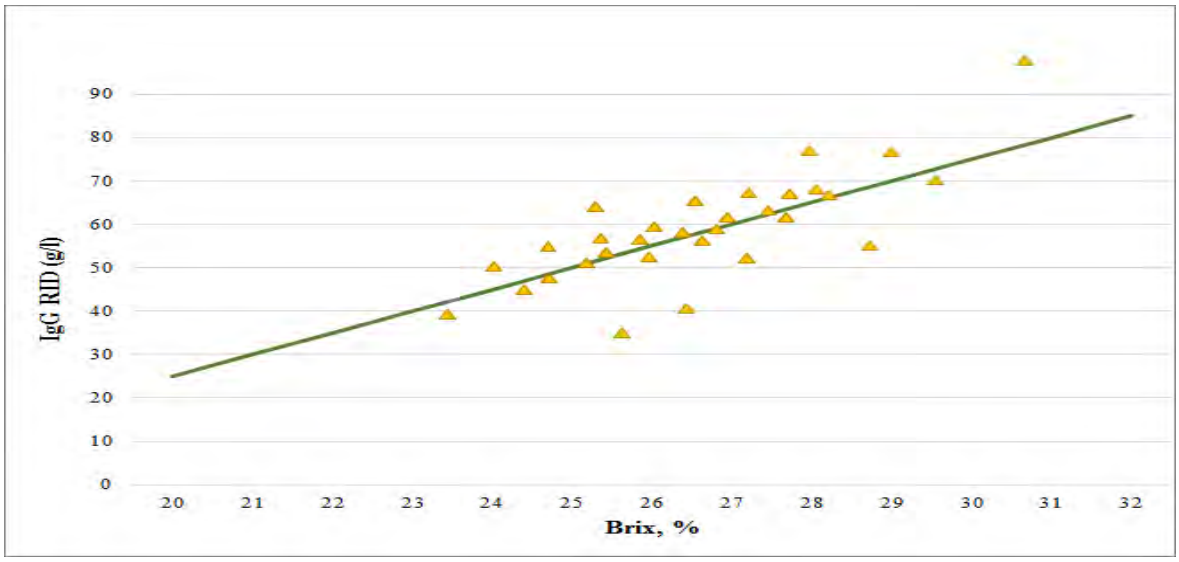
Ukupna količina serumskih imunoglobulina u kolostrumu krmača dvaju genotipova s dvije farme određena je metodom radijalne imunodifuzije (RID). Vrijednosti RID-a (serumske koncentracije imunoglobulina) iskazuju se u gramima po litri. Na grafikonu 2. prikazane su vrijednosti serumske koncentracije imunoglobulina dobivene iz uzoraka kolostruma krmača s farmi A i B.



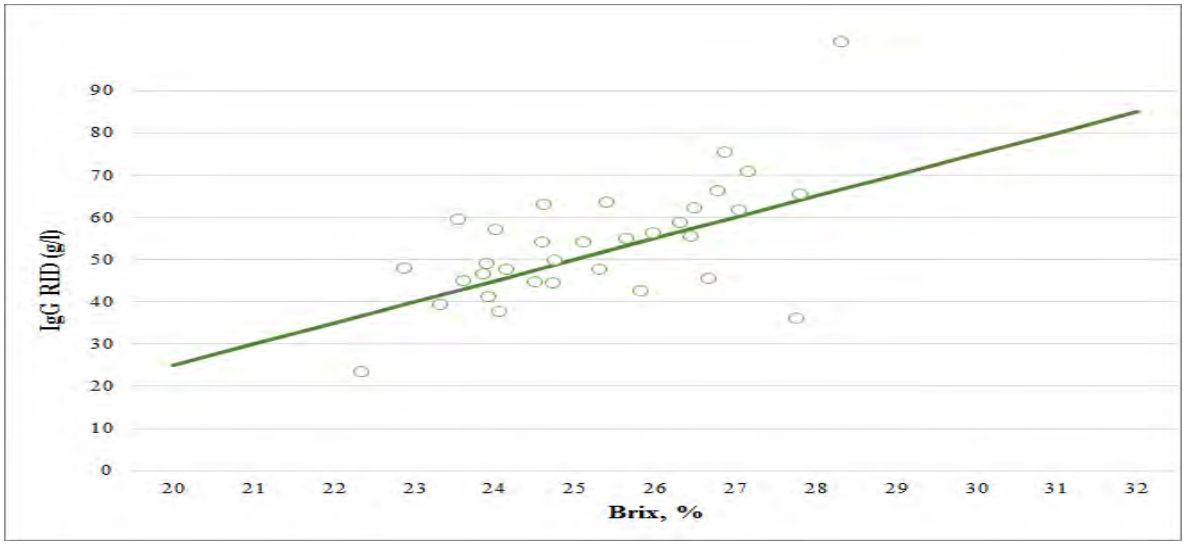
Grafikon 2. RID vrijednosti kolostruma na farmama A i B

Zamjetna je ujednačenost RID vrijednosti kolostruma kod krmača s obje farme, s tim da je na farmi A utvrđen blagi pad vrijednosti od 5. laktacije na dalje, međutim, utvrđene razlike nisu bile na razini statističke značajnosti. RID vrijednosti utvrđene u našem istraživanju kreću se između 60 i 80 g/l.

Odnos između koncentracija IgG dobivene RID metodom i vrijednosti dobivenih Brix refraktometrijom prikazan je u grafikonu 3. i 4.



Grafikon 3. Odnos između Brix i RID IgG mjerenja kolostruma krmača na farmi A



Grafikon 4. Odnos između Brix i RID IgG mjerenja kolostruma krmača na farmi B

Kao što se jasno vidi iz grafikona, usporedba dvaju metoda određivanja sadržaja i koncentracije imunoglobulina G rezultirala je jasnim linearnim odnosom između dobivenih vrijednosti. Koeficijent korelacije za ispitivana svojstva bio je vrlo visok (r^2=0.78). Do istih odnosa u svojim istraživanjima došli su Argüello i sur. (2005.), Chigerwe i Hagey (2014) te Colemann i sur. (2015.). Navedene usporedbe ukazuju da Brix refraktometar pruža prihvatljivu procjenu IgG kod kolostruma krmača.

Zaključak

Prosječne Brix vrijednosti utvrđene na obje farme ukazuju da je kolostrum ispitivanih krmača bio u granicama kvalitetnog do vrlo kvalitetnog (>20%) što ukazuje na dobru genetsku osnovu stada i menađment istog. Utvrđen je linearni porast RID vrijednosti kolostruma kod krmača oba hibrida na ispitivanim farmama. Možemo ga tumačiti porastom rednog broja prasenja. RID vrijednosti utvrđene u našem istraživanju kreću se između 50 i 80 g/l što ukazuje, na dobru kvalitetu kolostruma. Usporedba dvaju metoda određivanja sadržaja i koncentracije imunoglobulina G rezultirala je jasnim linearnim odnosom između dobivenih vrijednosti. Koeficijent korelacije za ispitivana svojstva bio je vrlo visok (r2=0.78). Brix refraktometar pruža prihvatljivu procjenu IgG u kolostrumu krmača. Ova metoda ima prednosti u odnosu na druge metode procjene koncentracije IgG, s obzirom da je Brix refraktometar jeftin, lako dostupan, manje krhak i manje osjetljiv na varijacije u temperaturi kolostruma, godišnje doba i druge čimbenike, te zahtijeva minimalnu opremu i obuku. Procjena kvalitete kolostruma može se obaviti kod same krmače neposredno nakon poroda, čime se poboljšava vjerojatnost da proizvođači donose pravovaljane i pravovremene odluke.

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The Assessment of Immunoglobulin G (IgG) content of sow colostrum using BRIX refractometer

Abstract

The aim of the research was to determine the possibility of using the digital Brix refractometer as one of the methods for measuring the concentration of immunoglobulin G (IgG) in sow colostrum after comparison with the the radial immunodiffusion method. The Brix refractometer provides an acceptable method to estimate of IgG in sow colostrum. This method has advantages over other methods of estimating IgG concentration, as the Brix refractometer is inexpensive, readily available, less fragile and less sensitive to variations in colostrum temperature, season and other factors, and requires minimal equipment and training.

Keywords: sows, colostrum, immunoglobulins, BRIX refractometer

Dresurno jahanje: kraj znanja početak je zlostavljanja konja

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Sažetak

U posljednjih nekoliko desetljeća u Olimpijskoj konjičkoj disciplini - dresurnome jahanju, pojedini nastupi dresurnih jahača pridonose većim potrebama za promjenama pravilnika Međunarodne konjičke federacije (FEI-a) s aspekta dobrobiti konja. Zlostavljan je onaj konj s kojim se neadekvatno postupa u treningu prakticiranjem dvojbenih metoda. Najpoznatiji je rollkur ili hiperfleksija vrata uz pomoć sile koja uzrokuje mentalnu i fizičku nelagodu. Nepravilno korištenje pomoćne opreme (žvale, vođice, mamuze, bičevi) također je povezano sa zlostavljanjem zbog svog upitnog načina i rezultata djelovanja. Trenutno se naglasak u raspravama stavlja na prekomjerno stezanje prekonosnog remena koji ograničava pokrete usta konja uz svrhu postizanja veće kontrole jahača nad konjem. Cilj ovoga rada je ukazati na posljedice korištenja nepravilnih metoda i tehnika u dresurnome jahanju na osnovi rezultata brojnih znanstvenih istraživanja iz polja veterine, etologije i biomehanike konja.

Ključne riječi: dresurno jahanje, zlostavljanje, rollkur, prekonosni remen

Uvod

Dresurno jahanje kao Olimpijska konjička disciplina predstavlja umijeće treninga konja i jahača. Prvotno je bilo sastavni dio vojne obuke u nekim europskim zemljama, a kasnije je prepoznato kao disciplina konjičkog sporta. U povijesti je smatrano umjetnošću, a s pravom nosi epitet „kraljice" konjičkoga sporta. Prvi jahači parovi nastupali su 1912. godine na Olimpijskim igrama u Stockholmu, ali tadašnje izvedbe po mnogočemu i ne nalikuju današnjima. Izmjene u načelima treniranja konja i posljedično drugačije izvedbe Ödberg i sur. (1999.) pripisuju prestanku održavanja i njegovanja tradicije, stvaranju pasmina s drugačijim morfološkim obilježjima i samoj popularizaciji sporta. Promatrajući današnju konjičku industriju, koja je svojevrsno prezasićena i intenzivirana, metode treninga formirale su se s ciljem što brže i efikasnije obuke. Na natjecanjima su vidljivi znakovi takovih metoda jer je u posljednjih 25 godina dokazan sve veći stupanj fleksije u vratu (Lashley i sur., 2014.). Također, Dyson i Pollard (2021.) navode kako na međunarodnim natjecanjima Grand prix razine uočavaju neke od znakova boli i nelagode u konja: pozicioniranje glave ispod okomice, otvaranje usta uz odvajanje zubi i mahanje repom. Najdvojbenija metoda, koja za Van Weerena (2013.) svoje korijene vuče iz treninga preponskih konja tijekom šezdesetih godina prošloga stoljeća, a danas je pretežito pripisana dresurnom jahanju, jest rollkur. Rollkur predstavlja presavijanje vrata konja i zadržavanje u toj biomehanički neprirodnoj poziciji tijekom treninga. Nekolicina je metodu vrlo kratko podupirala zbog raznih teorija o svojevrsnom razgibavanju i postizanju premoći nad konjem sve dok nije bila službeno zabranjena. Prema Kienapfelu (2014.) iz rollkura također proizlaze i ne nužno dobri, ali ekspresivniji pokreti prednjih nogu koje današnji suci nagrađuju i cjelokupno dresurno tržište cijeni. Nadalje, s obzirom da je negativan poticaj prema Merkies i sur. (2017.) učestala sastavnica treninga konja, nije neobično da su se oduvijek treneri često odlučivali na uporabu agresivnijih metoda ili pomagala. Zato je i uporaba pomoćne opreme (pomoćnih vođica, bičeva, mamuza) vrlo učestala u treningu i povezana je sa zlostavljanjem. Primjerice Gehlen i sur. (2021.) tvrde kako veliki broj jahača konja postavlja u nepravilan, prestegnut položaj i uopće ga ne izmijeni neovisno o otežanom disanju konja. Sljedeći problem, kojega javnost trenutno proziva, jest prekomjerno stezanje prekonosnog i podbradnog remena koji ograničava pokrete usta konja i smanjuje mogućnost opiranja. Relativno novija istraživanja (McGreevy i sur., 2012.; Fenner i sur., 2016.; Uldahl i Clayton, 2018.) prikazuju mnoge štetne učinke prekonosnog remena koji pridonose potrebi za boljom regulacijom pravila na natjecanjima.

Rollkur ili hiperfleksija vrata

Prvi prikazi rollkura u modernome dresurnom dobu potaknuli su javnost na burnu reakciju. Na inicijativu brojnih znanstvenika, profesionalnih trenera, jahača i ljubitelja konja rollkur je FEI službeno zabranio 2010. godine uz zaključak kako nije u skladu s dobrobiti zbog svojih raznih učinaka i jer se prikupljenost konja postiže uporabom sile. Unatoč tome, izvan samih natjecanja kontrola uporabe rollkura je gotovo nemoguća pa je stoga i dalje aktualna tema mnogih, involviranih u ovaj sport. Neupitna je važnost kontinuiranog obrazovanja konjičke zajednice o štetnim učincima na konja ali kako Borstel i sur. (2009.) iznose i o opasnosti te metode i za jahače zbog straha i veće reaktivnosti konja. Kako je rollkur uzrokovan primjenom sile jahača, može se povezati s tvrdnjom McGreevya i sur. (2012.) da konji pri velikom pritisku na žvalu uz pritisak prekonosnog remena i pritiska na tjeme izbjegavaju bol postavljanjem glave ispod okomice. Čak su i Christensen i sur. (2014.) u vrlo niskim pozicijama vrata detektirali veliku silu pritiska na žvalu, a veliki će pritisak, ovisno o trajanju, za Mellora (2020.) potencijalno uzrokovati oštećenja tkiva i bol. U istraživanju Borstela i sur. (2009.) konji koji su imali priliku izbjegavali su rollkur, a oni koji su bili prisiljeni, pokazali su znakove nelagode kao što su uši postavljene unatrag, mahanje repom i izražena aktivnost usta. Poznato je kako su uši konja jedan od načina izražavanja emotivnih stanja. Uši postavljene unatrag negativan su znak, a prema Dyson i Pollard (2021.) uši postavljene unatrag kao i prevelika aktivnost u ustima pripadaju jednom od 24 specifičnih znakova tijekom jahanja koji su uzrokovani boli lokomotornog sustava. Nadalje, Heuschmannu (2006.) su pokreti repa vrlo dobar pokazatelj napetosti u konja zbog povezanosti složenih struktura kralježnice i prenošenja sila kroz istu. No, isključujući rollkur, razlozi za mahanje repom mogu biti i snažnije komande jahačevih nogu te veća uporaba mamuza i biča (Kienapfel i sur., 2014.). Anatomski promatrano, s obzirom na mehanički neprirodan položaj vrata i samih gornjih dišnih puteva, dolazi do efekta gubitka daha i otežanog disanja (Petsche i sur., 2010.; Mellor, 2020.). Djelomično na to upućuje istraživanje Sleutjensa i sur. (2012.) kada su konji postavljeni u rollkur imali glasnije udisaje i izdisaje iako tim istraživanjem ipak nisu dokazane značajno drugačije vrijednosti izmjerenih plinova u arterijskoj krvi. Biokemijska analiza krvi van Oldruitenborgh-Oosterbaana i sur. (2006.) kod konja koji su jahani u rollkur poziciji nije pokazala povećanu razinu stresa iako su uočili veće količine laktata u krvi što upućuje na veće radno opterećenje. Niti van Breda (2006.) na temelju izmjerenih otkucaja srca nije utvrdila kako elitni dresurni konji nakon uporabe rollkura, na koji su doduše naučeni, pokazuju znakove stresa za razliku od rekreativnih konja. Također, u rollkur poziciji, ograničeno je vidno polje i percepcija konja, a neopiranje i naviku na agresivne metode u treningu potencijalno bi mogao objasniti fenomen naučene bespomoćnosti koja za Halla i sur. (2008.) podrazumijeva pasivno prihvaćanje neugode i nemogućnost povezivanja ponašanja i ishoda za određeno ponašanje. Kada je u pitanju utjecaj na kretnje konja, Rhodin i sur. (2009.) nisu utvrdili kako značajno utječe na duljinu koraka što objašnjavaju velikom mobilnošću i kapacitetom dresurnih konja za održavanje istih raspona kretnji u različitim pozicijama glave i vrata. Oprečno je mišljenje Heuschmanna (2006.) koji ističe smanjenu aktivnost u stražnjim nogama koja bi se mogla povezati s Kienapfelovim mišljenjem (2014.) o izričitoj ekspresiji u pokretima prednjih nogu koje bi moglo utjecati na prividno manju aktivnost u stražnjim nogama. Niti teorije nekih jahača o većoj elastičnosti u leđima nisu bile potvrđene u istraživanju Rhodina i sur. (2009.). Kada je u pitanju utjecaj na vrat, za Heuschmannu (2006.) su mišići i ligamenti gornjeg dijela preistegnuti i pod pritiskom, a prema Kienapfelu (2014.) mišići donjeg dijela vrata su pod rizikom od grčenja. O zdravstvenim posljedicama govore Dippel i sur. (2019.) koji ujedno potvrđuju kako je hiperfleksija povezana s izdužavanjem nuhalnog ligamenta i stvaranjem pritiska u regiji nuhalne burze preko atlasa (četiri puta više nego u normalnoj poziciji glave i vrata) i aksisa (dva puta više nego u normalnoj poziciji glave i vrata) pa je povećan rizik za pojavu nuhalnog burzitisa. Drugi negativni utjecaji nakon dugoročne uporabe rollkura nisu dovoljno istraženi, ali je unatoč tome Međunarodna konjička organizacija (FEI) rollkur zabranila. U Priručniku za stewarte (FEI, 2022.) poglavlje koje govori o zlostavljanju konja nalaže da "stewart mora diskretno i taktično reagirati jer prevelika i dugotrajna fleksija vrata nisu dozvoljene".

Pravilna uporaba pomoćne opreme u treningu

Idealna pozicija glave i vrata tema je rasprave raznih znanstvenika i jahača jer varira u ovisnosti o konformaciji konja i stupnju njegove utreniranosti. Primjerice, konji i u previsokim pozicijama glave i vrata koje su često povezane s pojmom prikupljenosti, mogu pokazivati znakove napetosti (Smiet i sur., 2014.). Također je opisan negativan utjecaj previsoko postavljenog vrata jer konji tada, u lošem smislu, spuštaju leđa (Heuschmann, 2006.; Rhodin i sur., 2009.) pa i nije neobično kako se u dresurnom treningu teži spuštanju glave i vrata jer tada potiču podizanje leđa i angažman stražnjih nogu. Ujedno, smatra se kada konj pravilno spušta glavu - traži i prihvaća žvalu, postiže se kontakt s ustima.

Kontakt s ustima često je krivo percipiran pojam te se opisuje raznim definicijama. Primjerice, jahači često mogu zamijeniti pravilan kontakt s pritiskom na usta konja i pomicanje prema žvali radi olakšanja (McLean i McGreevy, 2010.). Ni za Ödberga i sur. (1999.) kontakt ne znači silovito oslanjanje na žvalu te se postiže jedino putem kvalitetnog sjedišta i nogu jahača koji rezultiraju razgibanim i prikupljenim konjem. Održavanje pravilne pozicije za dresurnoga konja (nos na okomici ili ispred okomice) u suštini je moguće s vrlo malom primjenjenom silom na vođice (Piccolo i Kienapfel, 2019.). Stoga je svrha pomagalima, koji se uz poticanje angažmana stražnjeg dijela tijela koriste i za ograničenje pokreta glave, postavljanje konja u željeni položaj, vrlo upitna. Može se pretpostaviti kako prividno smanjuju potrebnu silu za postavljanje konja u pravilan položaj. Sljedeće kritike glede uporabe pomoćne opreme proizlaze iz općenito nepravilnog korištenja koje može dovesti do ozljeda i pretjeranog opterećenja u radu (Gehlen i sur., 2021.). Isti autori istraživanjem su utvrdili kako je čak polovica ispitanika pri lonžiranju konja postavilo u nepravilan, prestegnut položaj te ga nije izmijenilo usprkos pojavi otežanog disanja konja. Za sam pritisak na žvalu McGreevy (2011.) tvrdi kako pomoćne vođice ne stvaraju konstantan pritisak te tako ne mogu zamijeniti nježnu ruku jahača. Za Clayton i sur. (2011.) najveći je pritisak upravo pri uporabi neelastičnih pomoćnih vođica te se zato smatra kako bi trebale imati elastičnu komponentu zbog manje mogućnosti oštećenja tkiva. S druge strane, pojedini pozitivni učinci objašnjavaju učestalost uporabe pomagala u treningu ili rehabilitaciji konja. Primjerice, Roepstorff i sur. (2002.) tvrde kako je pravilna uporaba pomoćnih vođica u jahanju u pozitivnoj korelaciji s većim stupnjem opterećenja stražnjeg dijela trupa odnosno angažmana konja. Bystörm i sur. (2006.) su također dokazali kako uporaba pomoćnih vođica u kombinaciji s običnim vođicama uzrokuje veću silu potiskivanja stražnjih nogu i manji kut u skočnom zglobu pri fleksiji što ukazuje na angažman. Rhodin i sur. (2009.) smatraju kako stabiliziraju stražnji dio leđa konja iako negativno utječu na generalnu mobilnost u leđima. Nadalje, mamuze i bičevi se koriste za poticanje aktivnosti konja putem omogućenih preciznijih komandi nogu kao što su naznačenje smjera kretanja i promjene u kretnji (Lemon i sur., 2020.; Uldahl i Clayton, 2018.). McLean i McGreevy (2010.) uporabu mamuza i biča, kao i metode korištenja nejasnih komandi, uporabu elektrošokera i druge, svrstavaju u metode treninga koje negativno utječu na dobrobit, jasnoću i cilj obuke. Nedostatna su istraživanja glede uporabe različitih tipova mamuza, ali Lemon i sur. (2020.) ističu mamuze s rotirajućom komponentom zbog povećavanja rizika od ozljeda kože i gubitka dlake. FEI (2022.) nalaže da je ''stewart dužan obavijestiti suca ukoliko uoči svježu krv u ustima ili u području mamuza kod konja te će isti biti odmah elimiriran" uz to što prema Priručniku za stewarte (FEI, 2022.) poglavlje o agresivnom jahanju potiče stewarta na intervenciju i u zagrijavalištu jer je ''pretjerana uporaba mamuza, biča ili žvale zabranjena".

Problem prekomjerno stegnutoga prekonosnog remena

Prekomjerno stezanje prekonosnog remena prisutno je u svim konjičkim disciplinama, ali je osobito često u elitnom dresurnom jahanju (McGreevy, 2011.). Doherty i sur. (2016.) su na natjecanjima zabilježili samo 7% pravilno stegnutih prekonosnih remena, a naročito stegnuti bili su kod mladih konja. Istraživanje tih autora relativni je prikaz lošije izvedbe Pravilnika FEI-a (2022.) koji nalaže da ''prekonosni remen ne smije biti toliko stegnut da nanosi bilo kakvu vrstu štete i nelagode konju". Lošija izvedba potencijalno se može pripisati materijalu od kojeg je napravljen prekonosni remen, odnosno pretežito koži koja ima svojstvo djelomičnog rastezanja. Prema Clayton i Williams (2022.) prekonosni remen uopće nije nužna sastavnica uzde, ali su mu svrhe koje spominju Weller i sur. (2020.) od velikog značaja jer služi za kontrolu otvaranja usta konja, sprječavanje prebacivanja žvale preko jezika i općenitoga osiguravanja učinka žvale. Poznato je da je izričito otvaranje usta na natjecanjima nepoželjno, a prema načelima klasičnog dresurnog jahanja poželjno je nježno žvakanje žvale. Mehanizam agresivnoga zatvaranja usta konja u suprotnosti je sa zaključkom Dyson i Pollard (2021.) da je otvaranje usta uz odvajanje zubi pokazatelj boli i nelagode kod konja. Nadalje, Randle i McGreevy (2013.) za takozvano osiguravanje učinka žvale ukazuju da su zbog većeg pritiska konji osjetljiviji na žvalu u ustima (Randle i McGreevy, 2013.; Potz i sur., 2014.). Dodatno bi trebalo istražiti imaju li takvi učinci psihološku ili/i anatomsku prirodu. Upravo pravilno stegnut prekonosni remen prema tradicionalnoj metodi podrazumijeva prostor za dva prsta između nosne kosti i samoga remena. Problem je i u samom dizajnu prekonosnoga remena, kao primjerice švedskog na kojeg se dresurni jahači često odlučuju iako on povećava silu stezanja do dva puta i može vrlo lako konju uzrokovati štetu i nelagodu (McGreevy, 2011.; Doherty i sur., 2016.; Dyson i sur., 2020.). Prema Uldahl i Clayton (2018.) potencijalna šteta ogleda se u uzrokovanju oralnih lezija. Za McGreevya i sur. (2012.) konj je u vrsti fiziološkog stresa tijekom prekomjernog stezanja zbog uočenih nižih temperatura lica koje ukazuju na slabiju mogućnost prokrvljavanja tkiva. Fenner i sur. (2016.) navode etološki indikator stresa, a to je veća želja za zijevanjem, žvakanjem i lizanjem nakon otpuštanja remena, što se

vjerojatno događa zbog ranije nemogućnosti za isto. Određena opredjeljenja smatraju kako bi se i same žvale trebale izbaciti iz uporabe, no Robinson i sur. (2021.) su utvrdili kako pri uporabi uzdi bez žvale sile ne nestaju, nego se jednostavno premještaju na druge strukture lica što je također pitanje dobrobiti. Težnja ka boljoj kontroli jačine stezanja prekonosnog remena na natjecanjima i uporaba anatomski dizajniranih uzdi koje se nude na tražištu jedni su od načina zadovoljavanja uvjeta dobrobiti konja u sportu.

Zaključak

Kada je dresurno jahanje doživjelo svojevrsnu preobrazbu iz umjetničke i tradicijske izvedbe u Olimpijski sport, pojavile su se ubrzane metode obuke konja koje pridonose kompetitivnoj prirodi čovjeka. Posljednjih je nekoliko desetljeća ovaj sport pod utjecajem ubrzane popularizacije i komercijalizacije, a problematika seže i do nekih sudaca koji subjektivno nagrađuju spektakularne kretnje ispred tehničke pravilnosti izvedbi. Rollkur je najdvojbenija i ujedno zabranjena metoda na natjecanjima, ali ona nije kompletno iskorijenjena u treningu konja. Važno je kontinuirano obrazovanje kompletne konjičke zajednice o svim negativnim učincima rollkura jer prema raznim autorima negativno utječe na konja u anatomsko-fiziološkom, biomehaničkom i etološkom pogledu. Nedovoljna je i obrazovanost i kontrola unutar zajednice u pogledu korištenja pomoćne opreme u treningu koja ima svoje pozitivne učinke na angažman konja, ali i one potencijalno negativne na zdravlje i dobrobit. U raspravi o dobrobiti nisu isključene uporaba mamuza i bičeva, metode korištenja nejasnih komandi, uporaba elektrošokera, zlouporaba medikamenata i druge. Mamuze su u dresurnome sportu obvezne na pojedinim kategorijama, a potencijalno predstavljaju alat kojim se može naškoditi konju. Dodatna istraživanja treba provesti glede različitih tipova mamuza i učestalosti ozljeda kod konja pri korištenju istih. Trenutno najaktualnija tema u dresurnoj zajednici jest prekomjerno stezanje prekonosnog remena. Uz fiktivno pozitivne učinke kao što je povećanje osjetljivosti konja na žvalu i demobilizacija usta, dokazana je slabija prokrvljenost tkiva lica, pojave oralnih lezija te negativni učinci u etološkom smislu. Dostupnost različitih alata na današnjem tržištu dodatno pridonosi razlikovanju u načelima rada s konjima u već postojećim različitim sistemima treniranja. Na temelju znanstvenih istraživanja o pravilnom postupanju s konjima može se zaključiti da je prioritetno obrazovanje prvenstveno amaterskih sudionika s posebnim naglaskom na potrebi stručne edukacije svih profesionalaca involviranih u ovaj sport.

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Dressage riding: the end of knowledge is the beginning of abuse of horses

Abstract

In the last few decades in the Olympic equestrian discipline – dressage riding, individual performances of dressage riders contribute to greater need for the regulations by Federation Equestre Federation (FEI) from the aspect of horse welfare. The abused horse is the one who is inadequately treated in training by using controversial methods. The most famous one is rollkur or hyperflexion of the neck by using force, which causes mental and physical discomfort. Improperly used auxiliary equipment (bits, reins, spurs, whips) is also associated with training abuse because of its questionable mechanism and result of an action. The currently discussed method is overtightening the noseband which reduces the movement of the horse's mouth for the purpose of gaining more control. The aim of this paper is to point out the consequences of using incorrect methods and techniques in dressage riding based on the results of numerous scientific researches in the fields of veterinary medicine, ethology and horse biomechanics.

Keywords: dressage riding, abuse, rollkur, noseband

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Zbornik radova

Hrana i kvaliteta hrane

Ponašanje potrošača u konzumaciji kozjeg mlijeka i mliječnih proizvoda

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Sažetak

U svrhu utvrđivanja potrošačkih navika u pogledu kozjeg mlijeka i proizvoda od kozjeg mlijeka provedeno je *online* anketno ispitivanje 353 ispitanika. Gotovo polovica (47,9%) ispitanika ne konzumira kozje mlijeko, 30,8% ispitanika ne konzumira kozji sir, dok ih čak 60,9% ne konzumira ostale mliječne proizvode. Pritom žene rjeđe konzumiraju mlijeko, kozji sir i druge kozje proizvode od muškaraca. Potrošači najčešće kupuju kozje proizvode u supermarketima, kao i izravno na gospodarstvima koja se bave uzgojem koza, dok je način nabave kozjeg sira pod značajnim utjecajem mjesta odrastanja. Najčešći razlog nekonzumacije kozjeg mlijeka, sira i mliječnih proizvoda su miris, okus i aroma tih proizvoda (39%), dok 37% ispitanika nije imalo prilike konzumirati kozje proizvode.

Ključne riječi: kozje mlijeko, kozji sir, kozji mliječni proizvodi, konzumacija

Uvod

Proizvodnja kozjeg mlijeka u svijetu je u posljednjih dvadesetak godina, prema dostupnim FAO (2022.) podacima, povećana za više od 50% (sa 13 milijuna tona 2000. godine na oko 20 milijuna tona 2020. godine) te se danas više od 90% svjetske proizvodnje kozjeg mlijeka ostvaruje u zemljama u razvoju (Azija i Afrika). Pritom je važno istaknuti da kozje mlijeko i njegovi proizvodi (kao što su sir, jogurt i drugi) imaju trostruko (globalno) značenje u ljudskoj prehrani: (1) hrana za gladne i pothranjene ljude u zemljama u razvoju više nego što je to slučaj sa kravljim mlijekom; (2) liječenje ljudi koji pate od alergija na kravlje mlijeko i gastrointestinalnih poremećaja, što je značajan dio populacije razvijenih zemalja; i (3) zadovoljenje gastronomskih potreba potrošača gurmana, što predstavlja sve veći tržišni udio u mnogim razvijenim zemljama (Haenlein, 2004.). Naime, probavljivost, viši pufemi kapacitet i terapeutske vrijednosti, samo su neke karakteristike prema kojima se kozje mlijeko značajno razlikuje od ostalih vrsta mlijeka (Antunac i sur., 2000.). Potrošački trendovi, naime, prolaze kroz važne promjene jer hrana s posebnim svojstvima kvalitete postaje sve popularnija, osobito među potrošačima u razvijenim zemljama (Seregi i Kovács, 2016.).

Imajući u vidu sve navedeno, cilj je ovog rada bio utvrditi ponašanje potrošača u kupnji i konzumaciji kozjeg mlijeka i proizvoda od kozjeg mlijeka.

Materijal i metode

U svrhu izrade ovog rada provedeno je anketno istraživanje kojim su bili obuhvaćeni potencijalni potrošači kozjeg mlijeka, odnosno proizvoda od kozjeg mlijeka. Anketni upitnik je sadržavao pitanja o ponašanju ispitanika u pogledu kupnje i konzumacije kozjeg mlijeka i proizvoda od kozjeg mlijeka. Anketno istraživanje izrađeno je pomoću *Google forms* alata, te je anketni upitnik postavljen *online*, kao i poslan na adrese elektroničke pošte potencijalnih ispitanika. Također, anketni upitnik je podijeljen preko raznih društvenih mreža kao što su Facebook, WhatsApp i Viber. Anketno istraživanje se provodilo, odnosno bilo je online dostupno u razdoblju od 15. svibnja do 15. lipnja 2020. godine, a ukupno je u anketnom istraživanju sudjelovalo 353 punoljetnih ispitanika, različitih spolnih i dobnih skupina, stupnja obrazovanja te mjesta stanovanja.

U zasebnoj skupini pitanja prikupljeni su podaci o socio-demografskim obilježjima ispitanika, kao što su: spol, dob, mjesto odrastanja, stupanj obrazovanja, radni status, broj članova kućanstva i mjesečna primanja kućanstva.

Za analizu prikupljenih podataka korištenja je procedure FREQ statističkog programa SAS STAT (2015) pri čemu su rezultati analizirani primjenom statističkog χ^2 (hi-kvadrat) testa.

Tablica 1. Sociodemografska obilježja ispitanika

Obilježja ispitanika		**N**	**%**
Spol	Muško	114	32,3
	Žensko	239	67,7
Dob	18 – 29	159	45
	30 – 45	134	38
	46 – 60	52	14,7
	60+	8	2,3
Mjesto odrastanja	Selo	203	57,5
	Grad	150	42,5
Završeno školovanje	OŠ	6	1,7
	SSS	136	38,5
	VŠS	65	18,4
	VSS	146	41,4
Radni status	Zaposlen	235	66,6
	Nezaposlen	14	4
	Učenik/student	94	26,6
	Umirovljenik	10	2,8
Kućanstva s djecom mlađom od 10 godina	Da	90	25,5
	Ne	263	74,5
Mjesečna primanja kućanstva	Do 4.000	14	4
	4.001 – 8.000	91	25,8
	8.001 – 12.000	92	26,1
	12.001 – 16.000	82	23,2
	Više od 16.000	74	21

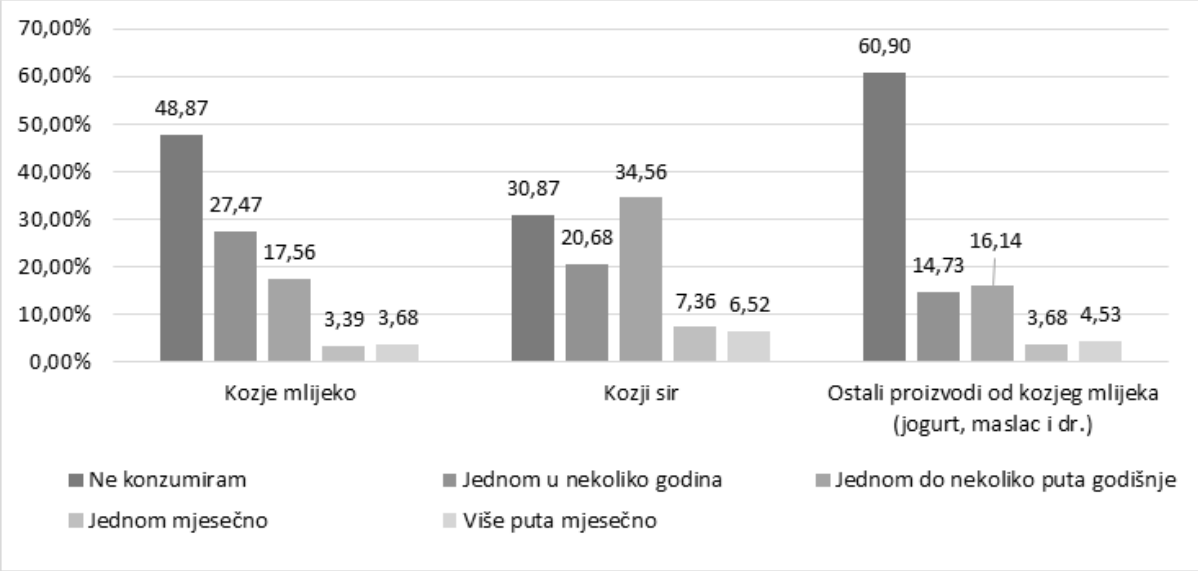
Izvor: anketno istraživanje.

Kao što je razvidno iz podataka prikazanih u tablici 1, u istraživanju je bio zastupljen veći udio ispitanika ženskog spola (67,7%), dok je većina ispitanika bila u dobi do 45 godina (83,0%). Najzastupljeniji stupanj obrazovanja bila je visoka stručna sprema (41,4%), dok je 38,5% ispitanika imalo završenu srednju školu. Čak dvije trećine (66,6%) ispitanika su zaposleni. Gotovo četvrtina ispitanika živi kućanstvima s djecom mlađom od 10 godina. Zastupljenost pojedinih kategorija kućanstava ispitanika s obzirom na mjesečna primanja bila je podjednaka (od 21,0 do 26,1%, izuzev kategorije do 4.000 kn mjesečno kojih je svega 4,0 %).

Rezultati i rasprava

Na temelju podataka prikazanih u grafikonu 1 vidljivo je da ispitanici najmanje konzumiraju proizvode od kozjeg mlijeka kao što su, primjerice, jogurt, maslac i drugi proizvodi (215 ispitanika ili njih 60,9% uopće ih ne konzumira), a gotovo svaki drugi ispitanik (njih 47,9%) ne konzumira kozje mlijeko. Navedeno je usporedivo sa rezultatima istraživanja Paskaš i sur. (2020.) gdje se više od polovice ispitanika izjasnilo da ne kupuje kozje mlijeko, odnosno proizvode od kozjeg mlijeka. Između analiziranih kozjih proizvoda (Grafikon 1), ispitanici najčešće konzumiraju sir što je i očekivano s obzirom na činjenicu da se većina kozjeg mlijeka, i to ne samo u Hrvatskoj, prerađuje u sir (Prpić i Mioč, 2004., Nayik, 2022.). Prethodno navedenim istraživanjem provedenim u Vojvodini (Paskaš i sur., 2020.), kao

i istraživanjem provedenim na području Mediterana (Tütenk i sur., 2022.) također je utvrđeno kako je sir najčešće konzumirani kozji proizvod. S obzirom na učestalost konzumacije (Grafikon 1), najviše ispitanika konzumira kozje mlijeko svega jednom u nekoliko godina (27,47% ispitanih), dok kozje sireve najviše ispitanika konzumira jednom do nekoliko puta godišnje (34,56% ispitanih).



Grafikon 1. Učestalost (%) konzumacije kozjeg mlijeka i proizvoda od kozjeg mlijeka

Prema provedenoj analizi križanjem varijabli spola ispitanika i učestalosti konzumacije kozjeg mlijeka, uočena je statistički značajna razlika (χ^2=17,2631; P=0,0017) u distribuciji odgovora ispitanika obzirom na spol. Naime, kao što je razvidno iz podataka prikazanih u tablici 2, razvidno je da žene rjeđe konzumiraju kozje mlijeko od muškaraca. Naime, čak 53,3% ženskih ispitanica (125 od ukupno njih 239) uopće ne konzumira kozje mlijeko, dok je to u slučaju muških ispitanika 38,6% (44 od ukupno 114 muških ispitanika). Istraživanjem provedenim u Americi McLean-Meyinsse i Cavalier (2004.) su također utvrdili da muški ispitanici znatno češće konzumiraju kozje mlijeko nego žene. Sličan trend je uočen u pogledu učestalosti konzumacije kozjeg sira obzirom na spol ispitanika u predmetnom istražicvanju (χ^2=28,8778; P<0,001), kao i pogledu konzumacije ostalih mliječnih proizvoda obzirom na spol ispitanika (χ^2=11,7356; P=0,0194).

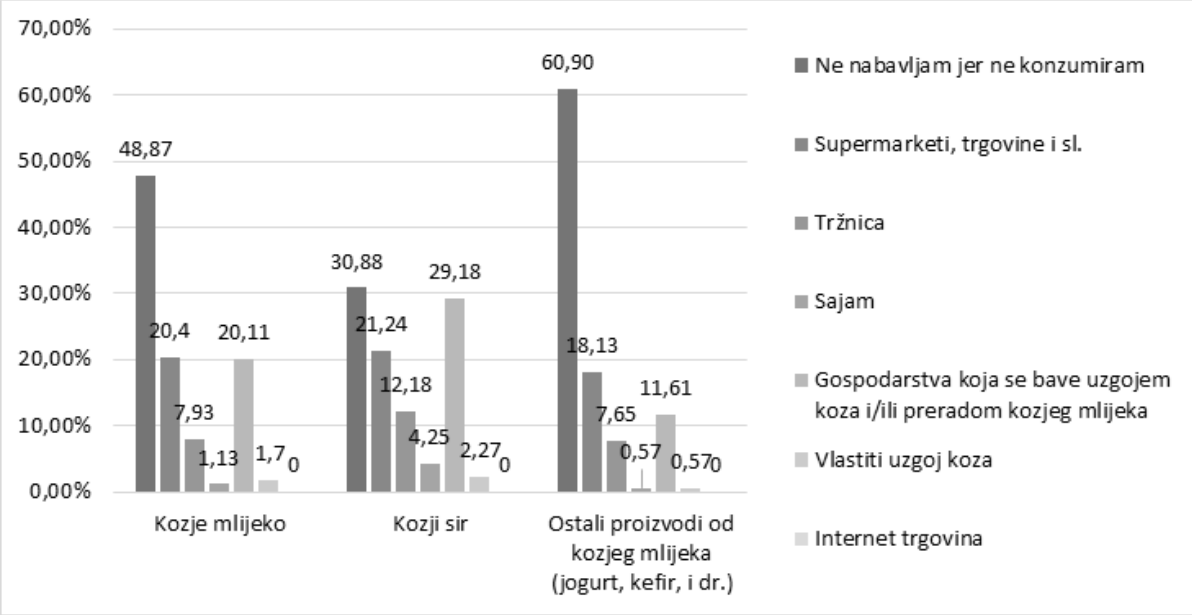
Tablica 2. Učestalost konzumacije kozjeg mlijeka obzirom na spol ispitanika

Spol ispitanika	Muškarci	Žene	Ukupno
Ne konzumiram	44a (12,46%)b	125 (35,41%)	169 (47,87%)
Jednom u nekoliko godina	34 (9,63%)	63 (17,85%)	97 (27,48%)
Jednom do nekoliko puta godišnje	26 (7,36%)	36 (10,2%)	62 (17,56%)
Jednom mjesečno	1 (0,28%)	11 (3,12%)	12 (3,40%)
Više puta mjesečno	9 (2,55%)	4 (1,13%)	13 (3,68%)
Ukupno	114 (32,29%)	239 (67,71%)	353 (100%)

[a]*Broj ispitanika.*
[b]*Vrijednost u zagradi predstavlja učestalost ispitanika izraženu kao udio od ukupnog broja ispitanika.*

Iz podataka prikazanih u grafikonu 2 razvidno je da najveći broj ispitanika nabavlja kozje mliječne proizvode u supermarketima, odnosno na gospodarstvima koja se bave uzgojem koza, dok su ostali izvori nabave kozjih proizvoda među ispitanicima znatno manje zastupljeni. Rezultati ovog istraživanja su u skladu sa rezultatima istraživanja provedenog u Turskoj (Tütenk i sur., 2022.) gdje najveći broj ispitanih kozje mlijeko i proizvode od kozjeg mlijeka

kupuje u supermarketima. Naime, u većini visokorazvijenih zemalja supermarketi su dominantan prodajni kanal za potrošače lokalne hrane (Jones i sur., 2004., Onozaka i sur., 2010.), među koje svakako spadaju kozje mlijeko i sir. U Hrvatskoj je izravna prodaja na gospodarstvu sve popularniji kanal prodaje koji potrošačima omogućuje svježinu proizvoda, veću kakvoću proizvoda, osobni kontakt s proizvođačem, uvid u proizvodni proces te pozitivnije kupovno ozračje (Kovačić, 2005.). Nitko od ispitanika ne kupuje kozje proizvode putem internet trgovine (Grafikon 2) što upućuje na slabu ponudu tih proizvoda na internetu koji je nakon pandemije COVID-19 sve popularniji kanal prodaje.



Grafikon 2. Mjesta kupnje kozjeg mlijeka i proizvoda od kozjeg mlijeka (u %)

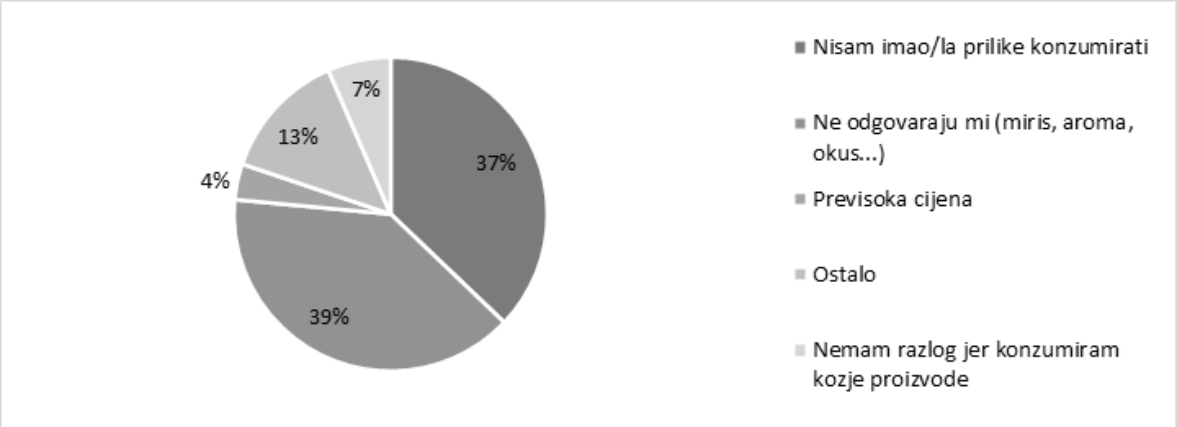
Prema provedenoj analizi križanjem varijabli mjesta odrastanja ispitanika i načina nabave kozjeg sira, uočena je statistički značajna razlika (χ^2=23,2158; P=0,0016) u distribuciji odgovora ispitanika obzirom na mjesto odrastanja. Iz podataka u tablici 3 je razvidno da ispitanici koji potječu iz grada statistički češće nabavljaju kozji sir u supermarketu (26%, odnosno njih 39 od ukupno 150) u odnosu na ispitanike koji potječu sa sela (17,7%, odnosno njih 36 od ukupno 203). Također, značajno više ispitanika koji potječu sa sela nabavlja kozji sir na gospodarstvu koje se bavi uzgojem koza, odnosno preradom kozjeg mlijeka u sir (30%, odnosno njih 65 od ukupno 203) u odnosu na ispitanike koji potječu iz grada (25% njih 38 od ukupno 150).

Tablica 3. Načini nabave kozjeg sira obzirom na mjesto odrastanja ispitanika

Mjesto odrastanja	Grad	Selo	Ukupno
Ne nabavljam jer ne konzumiram	37[a] (10,48%)[b]	72 (20,40%)	109 (30,88%)
Supermarketi, trgovine i sl.	39 (11,05%)	36 (10,20%)	75 (21,25%)
Tržnice	26 (7,37%)	17 (4,81%)	43 (12,18%)
Sajam	10 (2,83%)	5 (1,42%)	15 (4,25%)
Gospodarstva koja se bave uzgojem koza/proizvodnjom sira	38 (10,76%)	65 (18,41%)	103 (28,05%)
Vlastiti uzgoj koza	0 (0%)	8 (2,27%)	8 (2,27%)
Internet trgovina	0 (0%)	0 (0%)	0 (0,00%)
Ukupno	150 (42,49%)	203 (57,51%)	353 (100%)

[a]*Broj ispitanika.*
[b]*Vrijednost u zagradi predstavlja učestalost ispitanika izraženu kao udio od ukupnog broja ispitanika.*

Kao najčešći razlog nekonzumacije analiziranih kozjih proizvoda (Grafikon 3) ističu se miris, aroma i okus tih proizvoda (138 ispitanika, odnosno 39% ispitanih, Grafikon 3). Naime, okus i miris kozjeg mlijeka je izraženiji, osobito u odnosu na kravlje i ovčje mlijeko, zbog veće prisutnosti slobodnih i kratkolančanih masnih kiselina, a posebno kapronske, kaprilne i kaprinske (Antunac i sur., 2000.). Čak 131 ispitanik (37% ispitanih) nije imao prilike konzumirati neke od navedenih kozjih proizvoda što upućuje na slabu ponudu i nemogućnost nabave tih proizvoda (Grafikon 3). Tome u prilog idu i rezultati ranije objavljenog anketnog istraživanja Prpić i sur. (2022.) prema kojem čak 91,2% ispitanika smatra da bi kozje proizvode (u ovom slučaju proizvode hrvatskih izvornih pasmina koza) trebalo dodatno promovirati. Slično zaključuju Güney i Ocak (2013.) koji su upravo kao glavni razlog nekonzumacije kozjeg mlijeka i proizvoda od kozjeg mlijeka utvrdili slabu dostupnost tih proizvoda. Previsoka cijena proizvoda bila je razlog nekonzumacije u svega 4% ispitanih (Grafikon 3) čime se može objasniti činjenica da ovim istraživanjem nije utvrđena statistički značajna (P>0,05) povezanost između mjesečnih prihoda domaćinstva i učestalosti konzumacije kozjih proizvoda. Također, prema istraživanju Güney i Sangün (2019.) cijena je najmanje izražen čimbenik nekonzumacije kozjeg mlijeka, kao i proizvoda od kozjeg mlijeka.



Grafikon 3. Razlozi nekonzumacije kozjih proizvoda

Zaključak

Rezultati provedenog anketnog istraživanja upućuju na značajne mogućnosti povećanja potrošnje kozjeg mlijeka i proizvoda od kozjeg mlijeka obzirom na činjenicu da velik dio ispitanika ne konzumira navedene kozje proizvode. Također, više od trećine ispitanih nije niti imalo prilike konzumirati kozje proizvode što upućuje na potrebu dodatne promocije kozjeg mlijeka i proizvoda od kozjeg mlijeka i edukacije potrošača (primjerice, naglašavanje dijetetskih i terapeutskih odlika kozjeg mlijeka) te povećanja ponude tih proizvoda na tržištu. Ovo istraživanje pruža informativnu podlogu proizvođačima, kao i prerađivačima kozjeg mlijeka u svrhu planiranja proizvodnje, promocije i distribucije njihovih proizvoda.

Napomena

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Consumer habits regarding goat's milk and dairy products

Abstract

In order to determine consumer habits regarding goat milk and goat milk products, an online survey of 353 respondents was conducted. Almost half (47.9%) of respondents do not consume goat's milk, 30.8% of respondents do not consume goat's cheese, while even 60.9% of them do not consume other dairy products. At the same time, women consume milk, goat cheese and other goat products less frequently than men. Consumers most often buy goat products in supermarkets, as well as directly at farms that breed goats, while the method of obtaining goat cheese is significantly influenced by the place where the consumer grew up. The most common reason for not consuming goat's milk, cheese and dairy products is the smell, taste and aroma of these products (39%), while 37% of respondents did not have the opportunity to consume goat products.

Keywords: goat's milk, goat's cheese, goat dairy products, consumption

The potential of using *Saccharomyces boulardii* yeast in fermented milk products

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Abstract

Probiotic fermented milks are a group of functional food that, in addition to their nutritional value, contain certain components considered beneficial to human health. For their production, besides probiotic bacterial strains, yeasts - most commonly *Saccharomyces boulardii (S. boulardii)*, are used too in the form of an secondary culture. The biotherapeutic effect of this yeast on the human body has been most studied. *S. boulardii* is also considered a potential natural preservative due to its antagonistic effect against other microorganisms. Its survivability and its effects on organoleptic properties have been studied in the production of yogurt from cow's and goat's milk, kefir, and acidophilic fermented milk. Based on all these factors, these products can be considered as new functional dairy products.

Keywords: probiotic microorganisms, functional food, fermented milk, antagonistic action, *Saccharomyces boulardii*

Introduction

The modern way of life, in which stress together with unbalanced and irregular eating habits prevail, has a significant impact on people's health. For this reason, the number of diseases related mainly to the digestive system and increased body weight has increased recently. The food industry was the first to recognize the need to solve and prevent these problems by producing foods that, in addition to nutritional value, have functional value as well. The functional value of food refers to its components that are considered to promote health, general well-being or reduce the risk of disease occurrence by acting on one or more functions in the body (Samaržija, 2015a). To this end, the dairy industry often enriches its products with probiotic microbial cultures that have a positive health effect. Although strains of bacterial species from the genera *Lactobacillus, Bifidobacterium, Streptococcus, Pediococcus, and Leuconostoc* are most commonly used as probiotic cultures, recently more and more attention has been paid to the study and application of yeasts with probiotic potential. *Saccharomyces boulardii* is a unique probiotic and biotherapeutic yeast that has been successfully used as an oral substitute for the treatment of various digestive disorders (Lazo-Vélez et al., 2018). However, the use of *S. boulardii* in the food industry has only recently begun, and relatively few data are available on its potential in preventing digestive disorders and the onset of other diseases, and even fewer on its role and use in the manufacture of dairy products. Therefore, the aim of this article is to consolidate, comparatively and critically, the knowledge on the probiotic properties of *S. boulardii* and its use in the production of fermented milk.

Yeasts as probiotic cultures

Yeasts are a large, heterogeneous group of microorganisms and due to their different biological effects they are of great interest to today's food industry. The most common yeasts used in the food industry are those belonging to the *Ascomycota* group, among which the most scientifically studied species are those of the *Saccharomyces* genus. They are used for various fermentations, for the production of proteins and vitamins, and for biological control of pathogens. They are adaptable to changing of aerobic conditions (Deak, 2006; Hatoum et al., 2012). Yeasts can be non-fermentative, facultative fermentative, or obligate fermentative, usually with aerobic metabolism. In case of anaerobic metabolism, alcoholic fermentation occurs. They are also competitive to nutrients, tolerant to high concentrations of ethanol and a wide range of pH values; from 3 to 8 (Czerucka et al., 2007). They have the ability to acidify the medium in which they grow, they form and secrete mycotoxins and antibacterial components (Viljoen,

2006). All metabolic properties of yeasts are considered to be the basis for their probiotic activity. The antagonistic properties of yeasts are important in biotechnology, medicine, veterinary medicine, and other industries. They also contribute to food safety due to their inhibitory effects on pathogenic microorganisms (Niamah et al., 2017). In addition, yeasts' metabolic activity can influence the sensory quality of fermented foods and their shelf life.

Saccharomyces boulardii

Although bacteria are most commonly used, *Saccharomyces boulardii* is a yeast species that has been extensively studied in numerous clinical trials for its probiotic activity. The yeast *Saccharomyces boulardii* was isolated from the tropical litchi fruit (*Litchi chinensis*) in Indochina in 1923 by the French scientist Henri Boulard. Namely, he observed that the local population treated diarrheal diseases with litchi and mangosteen (*Garcinia mangostana*) (Liu et al., 2016). The probiotic properties of the yeast *S. boulardii* as a separate species or variety of the species *S. cerevisiae* are still the subject of numerous scientific investigations. In the literature, the name *S. boulardii* or *S. cerevisiae* var. *boulardii* is often used equally for this yeast species.

S. boulardii is able to assimilate and ferment carbohydrates and is naturally resistant to antibiotics. The optimal growth temperature of *S. boulardii* is 37°C, which is also the physiological temperature of the human body. It also tolerates low pH values of 2 to 4 and is resistant to bile salts and pancreatic juices. For this reason, *S. boulardii* has great potential for use as a probiotic. Moreover, it has no pathogenic properties and the products of its own metabolism show antipathogenic effects (Czerucka et al., 2007; Yerlikaya, 2014; Liu et al., 2016). Since the 1960s, many experimental and preclinical studies have demonstrated its anti-inflammatory, antimicrobial, enzymatic, metabolic, and antitoxic effects (Czerucka et al., 2007, Yerlikaya, 2014; Liu et al., 2016). *S. boulardii* is the only probiotic yeast whose effect has been scientifically demonstrated in numerous studies, and it is now considered one of the most effective probiotic microorganisms (Liu et al., 2016). There are numerous reliable clinical studies describing its therapeutic effect on various digestive disorders in humans such as diarrhea (Rolfe, 2000; Kotowska et al. 2005; Hatoum et al., 2012) and other disorders of the gastrointestinal system (Guslandi et al., 2003; Garcia et al., 2008; Kelesidis and Pothoulakis, 2011).

Application of S. boulardii in the production of fermented milk

The most common form of probiotic availability in the human diet are dairy products, especially fermented milk, and encapsulated probiotic bacterial strains available in dietary supplements. Compared to the rest of the food industry, the dairy industry is the fastest to bring new functional foods to market. Probiotic bacterial strains, which are used in the form of cultures in the production of fermented milk, are most commonly used for this purpose. Yeasts are rarely used as part of the starter culture compared to lactic acid bacteria. The main reason *S. boulardii* is part of the secondary microbial culture is that it cannot ferment lactose. Indeed, yeasts can only develop in a dairy product if lactic acid bacteria break down lactose into glucose and galactose or if sugar is intentionally added to the product (Parrella et al., 2012).

Among other things, it is known that the yeast *S. boulardii* has the ability to be highly proteolytic due to the production of several different peptidases that can also degrade milk proteins (Niamah, 2017). In addition, this yeast species has the ability to produce high concentrations of vitamin B complex. Based on these capabilities, it is believed that the use of *S. boulardii* yeast in the dairy industry may serve to enhance the growth of starter cultures bacteria in milk (Niamah, 2017). However, many very complex studies are conducted on its use as a probiotic culture (Yerlikaya, 2014). As a probiotic yeast, *S. boulardii* has been tested in the production of yogurt, kefir and acidophilic fermented milk, as well as UHT milk and ice cream.

Yogurt

Yogurt is recognized worldwide as a healthy food with beneficial health effects and, along with other types of fermented milk, is the most popular carrier of probiotics in foods (Oliveira et al., 2009; Allgeyer et al., 2010; Cruz et al., 2010a; Mortazavian et al., 2010; Marafon et al., 2011; Oliveira et al., 2011). The global yogurt market is constantly growing, as many consumers associate it with a positive effect on their own health. Yogurts are often fortified with probiotic bacteria, so they could be considered functional foods. However, some studies show that most probiotic products do not retain their probiotic properties until the end of their shelf life (Cruz et al., 2010b). Therefore, an

alternative solution was found in the form of probiotic yeasts such as *Saccharomyces boulardii* as follows.

Lourens-Hattingh and Viljoen (2001), for example, used the probiotic strain *S. boulardii* ATCC 74012 to produce yogurt (standard, UHT, and with fruit) in addition to yogurt culture. During 29 days of storage, an unchanged number of yeasts (7.6 log10 cfu/ml) was detected in standard and UHT yogurt, while in fruit yogurt the initial yeast population increased to 8.1 log10 cfu/ml. The authors attributed the increase in the number of *S. boulardii* yeasts in fruit yoghurt to the greater availability of fermentable sugars, as well as sucrose and fructose derivatives present in the fruit supplement. Notwithstanding the fact that *S. boulardii* does not metabolize lactose, it is able to utilize available organic acids, galactose and glucose derived from bacterial metabolism of lactose by yogurt culture composition in milk. The above study confirmed the survivability of the probiotic strain *S. boulardii* ATCC 74012 in yogurt during 29 days of storage in a number greater than 10^6 cfu/ml, which confirmed the potential of using the above yeast in the production of functional yogurt. A similar study was conducted for standard yogurt where milk was inoculated with the preparation of yogurt culture and *S. boulardii* in concentrations of 1%, 2% and 3%, respectively (experimental group), and by the use of the yogurt culture solely (control group). The rate of milk acidification was lower in the experimental groups of yogurts than in the control group, and the experimental yogurts had higher proteolytic activity due to the production of biopeptides than the yogurts of the control group (Niamah, 2017). Also, the addition of *S. boulardii* in yogurt production has a significant effect on the water retention capacity of the final product. Thus, the water retention capacity of yogurt with the addition of 3% of the S. boulardii preparation was 50% and that of the control yogurt was 40%. In addition, the positive effect of *S. boulardii* yeast on the rheological properties of yogurt was also noted. The determined population of live yeasts of more than 10^6 CFU/g in the yogurt during 21 days of storage met the criteria for its inclusion in the group of probiotic yogurts (Niamah, 2017).

Compared to yogurt made from cow's milk, in which the *S. boulardii* count of 10^7 cfu/g remains unchanged for up to 29 days of storage, the suitability of goat's milk for the production of probiotic yogurt with *S. boulardii* yeast is significantly lower (Karaolis et al., 2013). However, in the above study, the incubation temperature was higher than the optimal temperature for *S. boulardii*, which could be the reason for the weaker growth. However, *S. boulardii* was found to improve the survival of bacteria of standard yogurt culture for 28 days in yogurt prepared from goat milk. In addition, a much milder "goat" taste and odor were noted in yogurt samples with the addition of yeast. During storage, the sensory characteristics of the goat yogurts, especially those with added yeast, also changed as the taste of ethanol was strongly perceived. Although ethanol is the expected metabolite of yeast, its excessive concentration proved to be off-putting to consumers.

Recent research shows that *S. boulardii* in encapsulated form has a better survival rate during the shelf life of yogurt, as well as resistance to gastric pH, bile and salts, and certain digestive enzymes, and the ability to survive in the colon (Rodriguez et al., 2017).

Kefir

Kefir is a traditional fermented milk produced in Eastern Europe. It is considered a natural probiotic because the benefits it brings to human health have been known since ancient times. Like other probiotic fermented milks, kefir contains live microorganisms capable of competing with pathogenic microorganisms and participating in the establishment of the balance of the gut's desirable microbiota. Since kefir cultures do not normally contain *S. boulardii* yeast, a study of their effect and functionality under kefir production conditions was conducted by Ivanova et al., 2012. In the aforementioned study, 7 batches of kefir were produced, and in 6 of them, *S. boulardii* yeast (0.08 mg) was added in addition to the kefir culture (6 g kefir grains). Overall, the results showed that experimental kefir with the addition of *S. boulardii* did not differ from classically prepared kefir. In addition, an improvement in taste was observed, leading the authors to conclude that there is nothing stands in the way of the industrial use of such cultures in the production of kefir (Ivanova et al., 2012).

Acidophilic fermented milk

In general, acidophilic-fermented milk is fermented milk that, in addition to *Lactobacillus acidophilus* bacteria, also contains lactose-fermenting yeasts that have a strong antibacterial effect against some mycobacteria (Samaržija, 2015b). There is very little literature data on the use of *S. boulardii* yeast in the production of fermented milk, especially acidophilic-fermented milk. Kalpana (2008) experimentally prepared acidophilic-fermented milk from

sterilized skim milk to which 2% *S. boulardii* culture (5 different isolates) was added before incubation. At the end of incubation, *L. acidophilus* (NDC 15) was added at a concentration of 1% and the sample was incubated for additional 12 hours. It was found that *S. boulardii* utilized the lactic acid well for its growth and multiplication, maintaining its population above 10^6 cfu/ml. Moreover, a synergistic behavior was observed between the yeasts and the *L. acidophilus* culture, whose growth was enhanced, while no negative sensory changes were observed.

Conclusion

Saccharomyces boulardii yeast is a unique probiotic and biotherapeutic yeast that has been successfully used as an oral substitute to treat various digestive disorders in humans. There is relatively little research on the potential of *S. boulardii* yeast in the production of fermented milk. However, previous scientific research suggests that *S. boulardii* yeast has a positive effect on the sensory characteristics and growth of bacterial species commonly used in the production of fermented milk. Moreover, *S. boulardii* has a good survivability of more than 10^6 cfu/ml in yogurt and kefir made from cow's milk, which is sufficient to confirm its probiotic effect. Considering the potential of this yeast species, it would certainly be useful to carry out further research, focusing on finding strains that have a higher survival rate in fermented dairy products, produce less ethanol, with less proteolitic activity, and contribute to the creation of desirable sensory characteristics.

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ORIGINAL SCIENTIFIC PAPER

Analysis of components in the press fibre of lucerne (*Medicago sativa* L.) varieties

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Abstract

The aim of the present research is to study the pressed fibre fraction of 4 alfalfa varieties (Plato-Rhizo, Hunor, Tápiói, Olimpia) produced during green biorefining. The lignocellulosic components of the pressed fibre were investigated by HPLC and the protein and photosynthetic pigment content were determined by spectrophotometric method. The highest levels of glucan (36.13 w/w%), xylan (15.72 w/w%) and Klason lignin (16.84 w/w%) were measured for the Plato-Rhizo variety. The protein content ranged from 25.79 mg/g to 35.68 mg/g. No significant differences were observed between varieties for chlorophyll-a, chlorophyll-b and carotenoid content. Our results show that the Plato-Rhizo variety stands out.

Keywords: alfalfa, fibre, lignocellulosic components, protein, photosynthetic pigments

Introduction

Today, there is a clear increase in the world's population (Alexandratos and Bruinsma, 2012). This implies a need to increase the available food supply, which can only be done in a limited area, and therefore puts increased pressure on agriculture (Hobbs, 2007; Pereira, 2017). Agricultural production generates significant amounts of by-products from the harvesting of crops, which need to be used wisely (Paul and Dutta, 2018). Today, the circular economy system is becoming more widely known, the idea being to recycle used products and by-products as much as possible or to use their materials to produce a product with a higher added value (Tisserant et al., 2017; Luttenberger, 2020). In the shadow of circular economy, there is also green biorefining (Velvizhi et al., 2022). Green biorefining uses whole green plant biomass. The main product of the process is the leaf protein concentrate (LPC), along with other useful by-products such as brown juice and press fibre (Andersen and Kiel, 2000; Xiu and Shahbazi, 2015; Kamm et al., 2016; El-Ramady et al., 2020a). The brown juice can be used as a natural biostimulant and plant conditioner (Kisvarga et al., 2020). Pressed fibre has a wide range of uses, which is confirmed by its inherent biological values (Xiu and Shahbazi, 2015; El-Ramady et al., 2020b; Csatári et al., 2022). The main constituents of the pressed fibre are lignocellulosic components, which are the materials of the cell wall (Tungland and Meyer, 2002). The lignocellulosic components include lignin, cellulose, hemicellulose, which are insoluble fibres (Zhang et al., 2020; Csatári and Kovács, 2022). It is an excellent raw material for bioethanol production, biogas production and pharmaceutical products (Xiu and Shahbazi, 2015). The protein in pressed fibre has a favourable amino acid profile and is therefore also a popular feed ingredient (Damborg et al., 2018). The use of the 5-carbon sugars, xylose and arabinose, in lignocelluloses as natural sweeteners is well known in the food industry (Pol and Mars, 2021). Photosynthetic pigments in plants not only play an essential role in plants, but their consumption also has positive effects on human and animal health. Chlorophylls have excellent antioxidant and anti-mutagenic properties and their consumption can also be a preventive solution against cardiovascular diseases and chronic diseases (Pareek et al., 2017; Kaszás et al., 2018). Carotenoids also have anticarcinogenic effects and can reduce the development of cardiovascular and eye diseases (Ramel et al., 2012; Rosas-Saavedra and Stange, 2016; Langi et al., 2018).

The aim of the present research is to investigate the biological values of the pressed fibres obtained from 4 alfalfa varieties by wet fractionation, including the quantification of lignocellulosic components (glucan, xylan, arabinan, Klason lignin), the determination of protein content and the measurement of photosynthetic pigment content, in a circular econometric approach. A better understanding of these may provide a basis for their future valorisation.

Material and methods

The alfalfa varieties are grown in small plot experiments in the experimental area of the University of Debrecen. Alfalfa seeds at 25 kg/ha were sown in March 2018 in chernozem soil. The size of plots was 10.0 m^2. The plots were set up in 3 replicates per variety, the plots followed the random block arrangement. The cultivation of the plots was carried out according to the general agronomic practices such as irrigation, fertilization, weed control. The varieties used in this study were Plato-Rhizo (PLR), Hunor (HUN), Tápiói (TAP) and Olimpia (OLI). The date of the alfalfa harvest was October 2019 in a green bud stage. Harvesting took place in the morning hours, followed by laboratory processing. In the present study, fresh green biomass was used to produce pressed fibre using a twin screw juicer (Angel Juicer 5500, Angel Ltd., Czech Republic), which was frozen at -21 °C and then lyophilized. The inherent values of the resulting pressed fibre were subsequently investigated.

Lignocellulosic components were determined according to Sluiter et al. (2005). Briefly, the process consists of a two-step acid hydrolysis of pressed fibre samples to remove water-soluble sugars by vacuum filtration into solution, the amount of which is determined by HPLC. The residue on the filter contains the water-insoluble components. The glucan, xylan, arabinan and Klason lignin contents were determined using the appropriate equations.

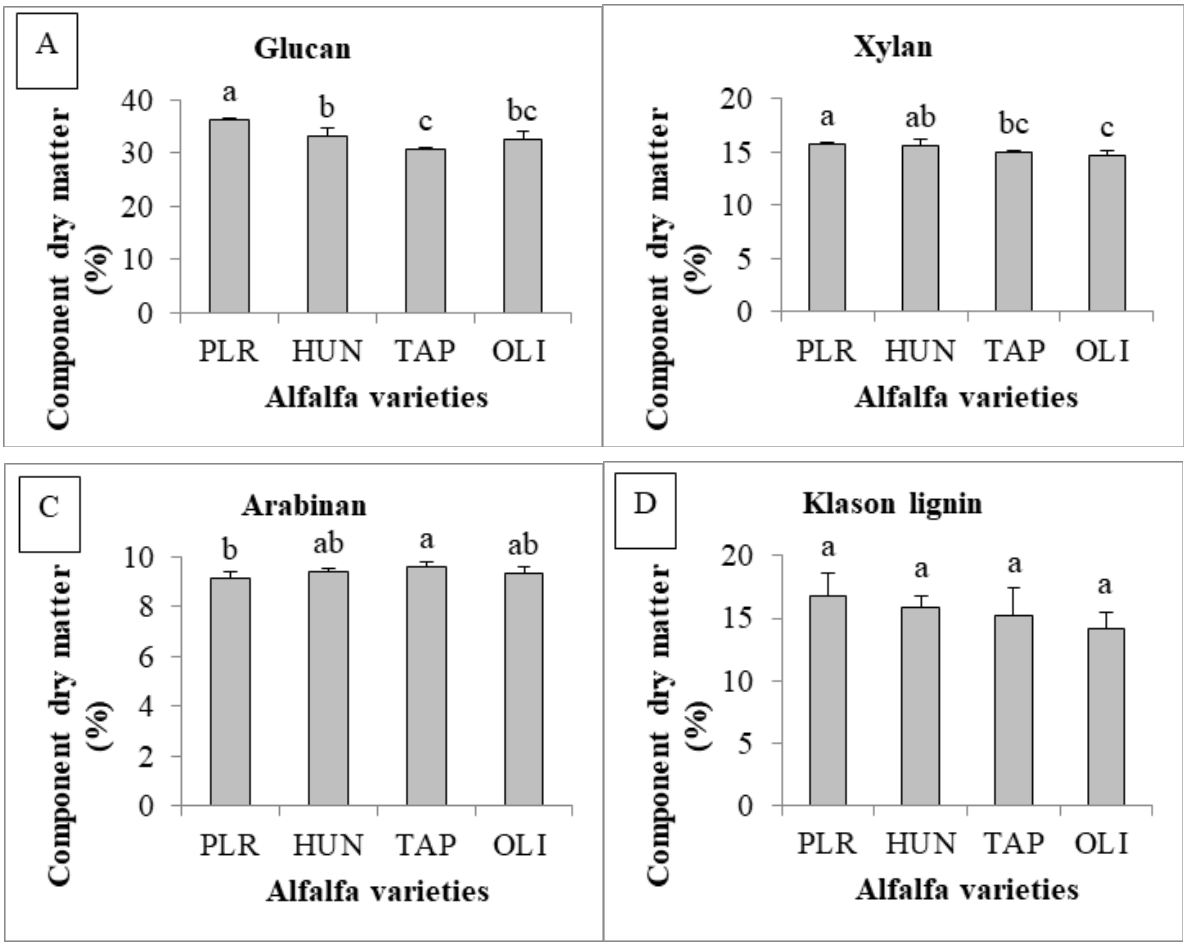
For the measurement of protein content 20 mg of lyophilized, powdered fibre sample was added to 1000 µL of protein solubilising solution (3.5 M urea, 1M thiourea, 0.1 M NaOH and 0.6% NaCl), vortexed for 1 min and incubated in an ultrasonic water bath for 1 hour. The sample was then centrifuged at 10,000 rpm. Then 90 µL NaCl was added to 10 µL supernatant followed by 1 mL Bradford reagent and the absorbance was measured at 595 nm (Ultrospec 2100 pro, Amersham BioSciences spectrophotometer) (Bradford, 1976).

Determination of photosynthetic pigment content from lyophilized, powdered fibre samples based on Duma et al. (2014). Accurate measurement of 10 mg of plant samples in an Eppendorf tube. The pigment content of the samples was extracted with 2 ml of 96% ethanol. After the addition of ethanol, the samples were shaken vigorously. This was followed by incubation in an ultrasonic bath for 60 minutes under dark conditions. The samples were then centrifuged at 13,000 rpm for 5 min on an Eppendorf Centrifuge 5415 R. An Ultraspec 2100 pro (Amersham BioSciences) spectrophotometer was used for the determination of photosynthetic pigments. Absorbances were measured at the following wavelengths: 665 nm, 649 nm, 495 nm, 480 nm and 440 nm.

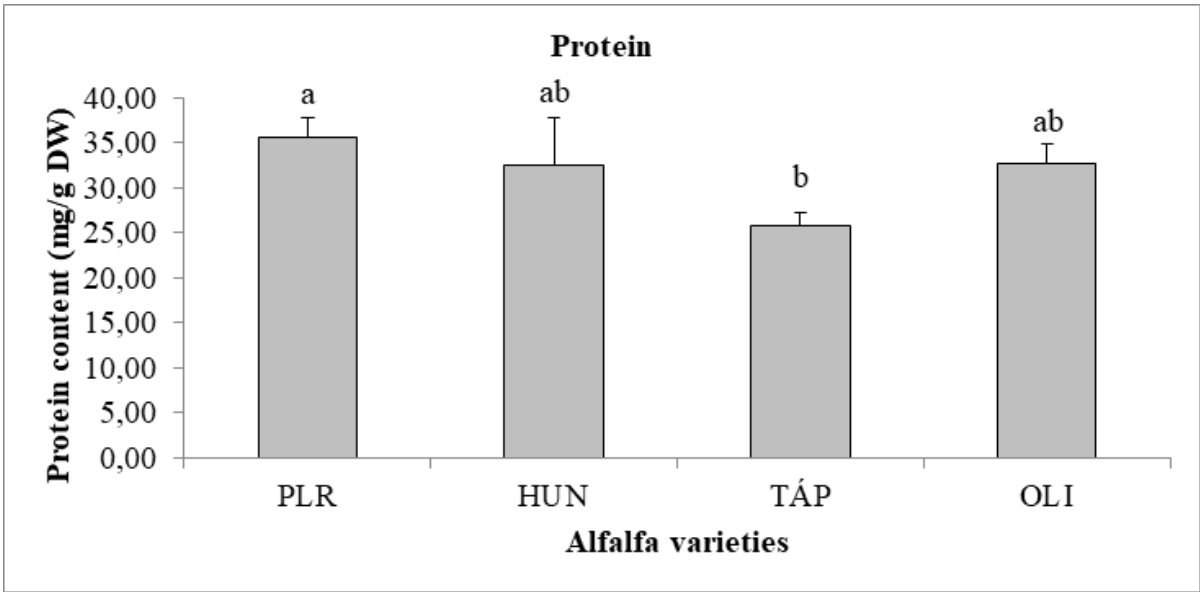
In the statistical analysis, a one-way ANOVA was used. Before running the ANOVA, a test of normality and Levene's Test of Equality of Variances were performed. The means were compared by Tukey's Honestly Significant difference (HSD) test at $p < 0.05$. The program that we used was IBM SPSS Statistics 24 (IBM Corp, Armonk, NY, USA).

Results and discussion

In Graph 1, the lignocellulosic components are observed. The Plato-Rhizo (PLR) variety had a significantly higher glucan content of 36.13 w/w% compared to the other varieties. In addition, a significantly lower glucan content was obtained for the Olimpia (OLI) variety (30.57 w/w%). Between the two extremes, the Hunor (HUN) variety was found to have a glucan content of 33.25 w/w%. (Graph 1A). As for the xylan content, it can be repeated that the Plato-Rhizo (PLR) variety had a significantly higher content (15.72 w/w%) compared to the Olimpia (OLI) (14.66 w/w%) and Tápiói (TAP) (14.94 w/w%) varieties. (Graph 1B). Graph 1C shows the arabinan content, with a significantly higher value (9.59 w/w%) for the Tápió (TAP) variety compared to the Plato-Rhizo variety (9.14 w/w%). For Klason lignin content, no significant differences were observed between varieties. The highest Klason lignin (16.84 w/w%) content was observed for the Plato-Rhizo (PLR) variety and the lowest (14.1 w/w%) for Olimpia (OLI). This is shown in Graph 1D. Comparing our results with Xu et al. (2002) and Duncan and Schilling (2010), the values measured for the lignocellulosic components are similar. It is worth mentioning that we measured a higher arabinan content compared to our previous experiments (Csatári et al., 2022).



Graph 1 shows the protein content of the pressed fibre. The Plato-Rhizo (PLR) variety had a significantly higher protein content (35.68 mg/g) compared to the Tápiói (TAP) variety (25.79 mg/g), the Hunor (HUN) variety 32.54 mg/g and the Olimpia (OLI) variety 32.65 mg/g. Compared to our previous studies, lower values are observed for protein content (Csatári et al., 2022).



Graph 2. Average protein content of alfalfa varieties (n = 3) ± Standard deviation. Different letters above the columns refer to significant differences (p ≤ 0.05). DW = Dry weight; PLR = Plato-Rhizo variety, HUN= Hunor variety, TÁP = Tápiói, OLI = Olimpia.

The Table 1. shows the photosynthetic pigment content in the pressed fibre for each alfalfa variety. For chlorophyll-a, no significant differences were observed between varieties. The highest value (1.510 mg/g) was measured for the Plato-Rhizo (PLR) variety and the lowest (1.028 mg/g) for the Olimpia (OLI) variety. No significant difference was found for chlorophyll-b. Again, the highest value was observed for the Plato-Rhizo (PLR) variety (0.858 mg/g), while the lowest value was observed for the Tápiói (TAP) variety (0.712 mg/g). No statistically verifiable difference was observed for carotenoids. The highest value (0.786 mg/g) is observed for the Plato-Rhizo (TAP) variety and the lowest (0.560 mg/g) for the Olimpia (OLI) variety. For xanthophylls, a statistically verifiable difference is observed. The xanthophyll content of Plato-Rhizo (PLR) is significantly higher (0.188 mg/g) than that of Olimpia (OLI) (0.031 mg/g). Dziwulska-Hunek et al. (2022) measured chlorophyll-a content of 1.429 mg/g, chlorophyll-b content of 0.419 mg/g and carotenoid content of 0.183 mg/g for alfalfa plants. Their results show that chlorophyll-a and carotenoids are similar to our values, but chlorophyll-b content is lower than in our case. Han et al. (2022) measured chlorophyll-b content similar to ours, but higher chlorophyll-a and carotenoids.

Table 1: Photosynthetic pigments of alfalfa varieties

Alfalfa variety	Chlorophyll-a	Chlorophyll-b	Carotenoid	Xanthophyll
PLR	1.510 ± 0.499 ^{a}	0.858 ± 0.269 ^{a}	0.786 ± 0.256 ^{a}	0.188 ± 0.085 ^{a}
HUN	1.488 ± 0.088 ^{a}	0.811 ± 0.039 ^{a}	0.747 ± 0.033 ^{a}	0.102 ± 0.037 ^{ab}
TÁP	1.144 ± 0.119 ^{a}	0.712 ± 0.120 ^{a}	0.620 ± 0.110 ^{a}	0.119 ± 0.026 ^{ab}
OLI	1.028 ± 0.031 ^{a}	0.810 ± 0.053 ^{a}	0.560 ± 0.065 ^{a}	0.031 ± 0.010 ^{b}

For Photosynthetic pigments, results are expressed as mg/g DW (n = 3) +Standard deviation. Different letters in the columns refer to significant differences (p ≤ 0.05). PLR = Plato-Rhizo variety, HUN= Hunor variety, TÁP = Tápiói, OLI = Olimpia

Conclusions

In the present study, the biological value of one of the by-products of green biorefining, the pressed fibre, was investigated in 4 alfalfa varieties (Plato-Rhizo, Hunor, Tápiói, Olimpia), including lignocellulosic components, protein content and photosynthetic pigments. The Plato-Rhizo variety had on average 3-5,5% higher glucan content.

For xylan and Klason lignin content, the Plato-Rhizo (PLR) variety also showed the highest measured content. However, for arabinan content, the Plato-Rhizo (PLR) variety was inferior to the other varieties, with the highest value being found for the Tápiói (TAP) variety. For protein content, the Plato-Rhizo (PLR) variety was 3-10% higher than the other varieties. For photosynthetic pigment content, the highest values were also observed for the Plato-Rhizo (PLR) variety. Based on our results, we can say that the Plato-Rhizo (PLR) variety stands out from the other varieties. Overall, pressed fibre, often undervalued, has value as a by-product and the right plant and variety can determine its future use, including potential use in the food industry, but the use of lignocelluloses for energy purposes could provide an opportunity to diversify pressed fibre use, including for biofuel production, reducing pressure on fossil fuels.

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Probiotic dairy-based desserts with cocoa addition

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Abstract

Dairy based-desserts include a large group of different products such as cream, pudding, mousse and ice cream. To improve their nutritional value and sensory properties, probiotic bacteria can be added, in addition to cocoa, fruits and cereals. Puddings and mousses with coccoa have been proven as an excellent medium for the growth and multiplication of probiotic bacteria, which could be the main reason for the growing demand for this type of product. The aim of this article is to give an overview of the research on the use of probiotic bacteria in the production of dairy-based desserts with added cocoa.

Keywords: probiotic bacteria, dairy-based desserts, cocoa

Introduction

Due to their sensory and nutritional properties, dairy-based desserts are consumed by consumers of all ages (Nastaj et al., 2007; Toker et al., 2013; Szwajgier and Gustaw, 2015; Aguilar-Raymundo and Ve'lez-Ruiz, 2018; Zarzycki et al., 2019). Marketing activities also contribute to their popularity, which is why there are a large number of different types of desserts based on milk and milk ingredients on the market today (Buriti and Saad, 2014). The advantages of consuming dairy desserts are: easy to use, long shelf life, can be a complete meal, can be used as an additive in the preparation of desserts, can have a reduced fat content and provide a source of nutrients through the addition of nutrients (Early, 1998). Nowadays, it is common to add different ingredients to dairy desserts to improve their sensory properties and their beneficial effects on human health, such as cereals and fruits as well as cocoa which is the most popular. Probiotic bacteria can also be included and in this sense such desserts are categorized as functional dairy desserts. The addition of probiotic bacteria could be one of the reasons for the increasing consumption of this type of product. The aim of this article is to give an overview of the research on the use of probiotic bacteria in the production of dairy-based desserts with added cocoa.

Dairy-based desserts

For many years, dairy-based desserts were not considered nutritionally valuable, but the perception of this type of product has changed. Namely, in recent years, dairy desserts have become one of the better examples of healthier diets (Saunders, 2011, Verruck et al., 2019) due to the high quality proteins, fats, carbohydrates, vitamins and minerals in their composition, which is why they are consumed by all age groups of consumers (Ferrar et al., 2011, Verruck et al., 2019). Their high nutritional value is one of the most important factors that play a role in consumer choice. In addition, dairy desserts with a lighter texture, such as puddings and mousses, are acceptable foods not only for the younger population but also for older people who have difficulties with swallowing (Quinchia et al., 2011, Verruck et al., 2019). In addition, dairy desserts have a long shelf life, can be used as a supplement when preparing desserts, are a meal in themselves and provide refreshment when chilled (Early, 1998). Dairy-based desserts, according to Codex Alimentarius (2015), include a large group of different products, including ready-to-eat flavoured dairy desserts, dessert mixes, frozen dairy desserts, flavoured and/or frozen yoghurt, ice cream, frozen milk, junket, Dulche de leche, pudding, mousse and many others. Dairy-based desserts are products made by transforming milk into a semi-solid form (smooth texture, „on the spoon“) with the addition of milk powder and non-dairy ingredients. They are produced by the process of heat treatment of milk and/or whey and added ingredients, which, through a special technological process, must obtain a firm, thick and/or foamy consistency and have a characteristic taste, smell and colour, depending on the basic raw material used or ingredient added (Official Gazette 133, 2007).

Probiotic

According to the World Health Organisation (WHO) and the Food and Agriculture Organisation (FAO), probiotics are defined as living microorganisms that, when consumed in appropriate amounts, have a positive health effect on the host (FAO/WHO, 2001). Experts from the International Scientific Association for Probiotics and Prebiotics recommend using the term probiotics only for products in which the microorganisms contained have a sufficient number of well-defined strains that contribute to the well-being of the host. In this sense, candidate probiotic strains can be divided into three categories: I) strains with no health effect , II) strains with possible health effect and III) strains that can be used for the prevention and treatment of diseases (Hill, 2014). The best known probiotics to date are lactic acid bacteria (LAB) and bifidobacteria, but some slow-growing strains of bacteria, as well as yeast strains, have been found to have possible health benefits when consumed appropriatelyLAB strains used as probiotics include *Lactobacillus acidophilus*, *Lacticaseibacillus rhamnosus* (GG), *Lactobacillus* ssp. and *Bifidobacterium*. Slow-growing bacteria that have a probiotic effect include strains of the bacteria *Bacillus cereus*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus pumilus*, *Bacillus clausii* and *Bacillus coagulans* (Hong et al., 2005; McHugh et al., 2017), as well as the yeast *Saccharomyces boulardii*.

Probiotics are usually supplied to the body in two ways: through the consumption of fermented foods or through dietary supplements. It should be noted that for clinical efficacy, the product to which probiotic bacteria are added must provide the samesufficient conditions for growth and temporary colonisation in the gut. Probiotics used as food supplements consist of freeze-dried bacteria in the form of powder, capsules or tablets. Milk has been shown to be the best transport medium for probiotics, as 100 times less viable bacteria are needed when they are in a milk medium than when they are in a frozen or dried medium (Khalighi et al., 2016). In this sense, dairy-based desserts are also a good medium for the growth and multiplication of probiotic bacteria (Table 1).

Table 1. Milk desserts with the addition of different strains of probiotic bacteria

Dairy-based dessert type	Probiotic strain	Author
Pudding	*Lacticaseibacillus casei*Shirota	Gul (2017)
Pudding with the addition of cocoa	*Lactobacillus acidophilus* LAFTI L10 *Bifidobacterium animalis* ssp. *lactis* LAFTI B94 *Lacticaseibacillus casei* LAFTI L26 *Lacticaseibacillus rhamnosus* GG	Irkin and Guldas (2011)
Mousse with the addition of cocoa	*Lacticaseibacillus paracasei* subsp. *paracasei* LBC 82 *Lacticaseibacillus paracasei* subsp. *paracasei* LBC 81 *Lacticaseibacillus paracasei* subsp. *paracasei* NCDC 022	Aragon-Alegro et al. (2007), Cardarelli et al. , (2008), Valencia et al. (2016), Patel, et al. (2008)
Rice pudding	*Lactobacillus acidophilus* LA-5 *Bifidobacterium bifidum* BB-12	Ozcan et al. (2010)
Pudding with the addition of cereals	*Lactobacillus acidophilus* La5 and 1748 *Bifidobacterium animalis* Bb12 *Lacticaseibacillus rhamnosus* GG	Helland et al. (2004)
Fermented dessert	*Lactobacillus acidophilus* MJLA1 *Bifidobacterium* spp. BDBB2 Mixed culture *Lactobacillus acidophilus* SAB 440-A and *Bifidobacterium animalis* subsp. *lactis*	Shah and Ravula (2000), Tavares Estevam et al. (2017)

Source: according to Verruck et al. (2019)

Probiotic dairy-based desserts with added cocoa

The production technology of all dairy desserts is similar, and the individual steps depend on the type of product. With the exception of ice cream, mousse and pudding with added cocoa are the best-known representatives of desserts made from dairy products. This is supported by the antioxidant properties of cocoa and its beneficial effects on cardiovascular diseases, inflammatory processes and cancer (Andújar et al., 2012). Due to its foamy texture, the production technology of mousse is somewhat more demanding than that of pudding. The ingredients for the production of mousse are cream cheese, butter, cream, skimmed milk, skimmed milk powder, sugar, chocolate, cocoa powder as well as emulsifiers and stabilisers. Mousse is characterised by a light, foamy texture. Therefore, it is desirable that the proportion of milk fat is more than 12%, the non fat dry matter 7-12% and the added sugar 8-15% (Early, 1998). The technology of pudding production involves mixing dry (powdered) ingredients that are added to the mixture of milk and cream. Industrial production of pudding uses whole or skimmed milk mixed with cream, sugar or sugar substitutes (natural or artificial sweeteners), additives such as starch and gelatin, and other ingredients prescribed by Codex Standard 192-1995. Eggs may also be added to the pudding, and flavourings and colourings are added in the production of some types of pudding (fruit, chocolate, vanilla, caramel, hazelnut) to give a distinctive taste and appearance (Chandan and Kilara, 2016). The resulting mixture is then heat-treated, filled into jars, cooled and stored. Apart from the fact that the best known representatives of dairy desserts are those with added cocoa, these products have also been shown to be a good medium for the growth and multiplication of probiotic bacteria, as they are favoured by a pH above 6, a moisture content of more than 70% and the absence of competing microorganisms (Silva et al. , 2012; Morais et al., 2014, 2015; Valencia et al., 2016). Due to their functional properties, the production of probiotic dairy desserts represents an increasingly important segment of the dairy industry. The fortification of various dairy desserts with probiotic bacteria and the increasing acceptance by consumers show the potential to include this type of product in the daily diet. This is confirmed by the study of Ares et al. (2008), in which consumers concluded that dairy desserts contribute most to the maintenance of health and are credible carriers of functional claims. At the same time, it should be taken into account that the possible health benefits of probiotics for human health can only be achieved if sufficient numbers of live bacteria are supplied. It is generally believed that it is necessary to supply the body with 10^8 - 10^{10} live bacteria per day through various products. Irkin and Guldas (2011) in their research describe the sustainability and sensory properties of probiotic pudding with the addition of cocoa and the addition of: I) *Bifidobacterium animalis* ssp. *lactis* LAFTI B94 DSL, II) *Lactobacillus acidophilus* LAFTI L10 DSL, and III) *Lacticaseibacillus casei* LAFTI L26 DSL. The number of live bacteria in all samples of the probiotic pudding with added cocoa was 10^8 - 10^9 during 25 days of storage at a temperature of 4°C. Changes in sensory properties were only found in the pudding with added cocoa, to which the strain *Lacticaseibacillus casei* LAFTI L26 DSL was added. The appearance of syneresis was also observed in this pudding during prolonged storage, while the sample with the addition of *Bifidobacterium animalis* ssp. *lactis* LAFTI B94 DSL showed more pronounced sensory properties compared to the control sample (Irkin and Gludas, 2011). Rosa et al. (2016) conducted a study on the effect of the probiotic bacterium *Lactobacillus acidophilus* in milk desserts with added cocoa on the presence of pathogenic bacteria. For research purposes, 9 samples were prepared with the addition of: I) *Lactobacillus acidophilus* LA -05, II) *Escherichia coli* O157:H7 (CDC EDL -933), III) *Salmonella* spp. ATCC 00150, IV) *Staphylococcus aureus* ATCC 00358, V) *Bacillus cereus* ATCC 14579, VI) *Lactobacillus acidophilus* LA -05 and *Escherichia coli* O157:H7 (CDC EDL -933), VII) *Lactobacillus acidophilus* LA -05 and *Salmonella* spp. ATCC 00150, VIII) *Lactobacillus acidophilus* LA -05 and *Staphylococcus aureus* ATCC 00358 and IX) *Lactobacillus acidophilus* LA -05 and *Bacillus cereus* ATCC 14579. The prepared samples were stored at a temperature of 8 °C and analysed during 24, 48 and 72 hours, i.e. after 7 and 28 days. The results of the study showed that the total number of pathogens did not increase beyond the safety level, which the authors interpret as a possible effect of bacteriocins or microbial competition. The physicochemical properties of probiotic dairy desserts depend on the probiotic strain added and whether the strain was added as a monoculture or mixed culture. The ingredients added in the production process influence the metabolism of the probiotic bacteria, especially the pH of the product, which is a consequence of the production of lactic and citric acid (Buriti and Saad, 2014). For example, Helland et al. (2004) in their study found that *Lacticaseibacillus rhamnosus*GG was responsible for the highest production of lactic acid (10 g/kg) and citric acid (1,819 g/kg), while the lowest amount of lactic acid (5 g/kg) was found in puddings with the addition of the strain *Lactobacillus acidophilus* 1748. A similar study was conducted by Aragon-Alegro et al. (2007) on mousse with the addition of cocoa and probiotics (*Lacticaseibacillus paracasei* LBC 82) and/or synbiotics (inulin). Samples I) mousse without additives (control sample), II) mousse with added probiotics, III) mousse with added probiotics and prebiotics, were analysed at a

temperature of 4°C for 28 days. Sensory analysis and pH verification were performed on the 1st, 7th, 14th, 21st and 28th day of storage. It was found that the pH changed the most when the probiotic *Lacticaseibacillus paracasei* LBC 82 and the prebiotic inulin were used. On the first day of storage, the pH of the mousse was 6.21 and on the 28th day it was 5.37, while the pH of the control sample was 6.22 on the first day of storage and 6.01 on the 28th day. Mousse with the addition of cocoa and the probiotic *Lacticaseibacillus paracasei* LBC 82 changed the pH from 6.26 (1st day of analysis) to 5.67 (28th day of analysis). The mousse with the addition of cocoa proved to be an excellent base for the probiotic strain *Lacticaseibacillus paracasei* LBC 82, while the addition of the prebiotic inulin did not affect the viability of the probiotics nor did their combination affect the sensory properties of the product.

Valencia et al. (2016) conducted a study on the microbiological and sensory properties of a creamy chocolate dessert with the addition of the probiotic strain *Lacticaseibacillus paracasei* subsp. *paracasei* LBC 81 and fructooligosaccharide prebiotics. Three samples were prepared to perform the analyses: I) without the addition of probiotics and prebiotics (control sample), II) with the addition of *Lacticaseibacillus paracasei* subsp. *paracasei* LBC 81 (probiotic sample), III) with the addition of *Lacticaseibacillus paracasei* subsp. *paracasei* LBC 81 and fructooligosaccharides (synbiotic sample). The analyses of all three samples included the determination of pH, acidity, syneresis index and viability of the number of bacteria in the product for 28 days. The samples were also sensory evaluated to assess consumer acceptance of the product and intention to purchase this type of product. The product analyses were carried out on the 1st, 7th, 14th, 21st and 28th day. The storage time has an influence on the decrease in pH, i.e. the increase in acidity in the probiotic and symbiotic sample. Thus, on day 1 and day 28, the pH of the control sample is 6.6 and 6.8, that of the probiotic is 6.1 and 5.0, and that of the symbiotic sample is 6.1 and 5.1. In the analysis, no syneresis was observed during the 28-day storage for all three types of samples, which is a consequence of the addition of xanthan gum. On a scale of 1 to 9, the average score for all three samples is 7, which corresponds to very good consumer acceptance. Also on a scale of 1 to 5, the evaluators gave an average score of 4, which means that they would gladly buy one of the products. The number of viable cells of *Lacticaseibacillus paracasei* subsp. *paracasei* LBC 81 remained constant during the storage period. That is, the number of viable cells in the probiotic sample was 8.3 log cfu/g on day 1 and 8.9 log cfu/g on day 28, and 8.5 log cfu/g and 8.7 log cfu/g in the symbiotic sample.

Conclusion

Dairy-based desserts with the addition of cocoa belong to a group of dairy products that are extremely popular with consumers of all ages due to their recognisable sweet taste and tender texture. The enrichment of dairy-based desserts with probiotic bacteria, especially with the addition of cocoa, and their possible beneficial effects on human health are leading to increasing consumption of this type of product. In this sense, there is great potential for the development and production of new types of probiotic dairy-based desserts.

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Utjecaj postupka prerade na sadržaj bioaktivnih spojeva u prahu maline

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Sažetak

Cilj ovoga rada bio je utvrditi promjene sadržaja bioaktivnih komponenti tijekom proizvodnje suhog praha maline kao i utjecaj temperature sušenja na kvalitetu finalnog proizvoda. Plodovi malina s početnim udjelom vode od 87,17 % osušeni su u laboratorijskom sušioniku „INKO" ST40 pri temperaturi od 40 °C i atmosferskom tlaku 1013 hPa do postizanja sadržaja vode od približno 15 %. Iz dobivenih rezultata vidljivo je da tijekom proizvodnje suhog praha u finalnom proizvodu došlo do smanjenja udjela vitamina C za 85,59 %, ukupnih fenola za 32,32 %, flavonoida za 40,09 %, neflavonoida za 24,61 %, antocijana za 2,78 % i antioksidacijskog kapaciteta za 18,98 %. Stoga se može zaključiti da parametri sušenja značajno utječu na sadržaj analiziranih bioaktivnih spojeva.

Ključne riječi: Malina, Himbo-Top, zamrzavanje, sušenje, prah, bioaktivni spojevi

Uvod

Malina (*Rubus idaeus*), pripadnica obitelji Rosaceae, izaziva je veliko zanimanje ne samo zbog karakterističnog okusa i arome već i zbog bogatstva bioaktivnih spojeva za koje je dokazano da blagotvorno djeluju na zdravlje. Bioaktivni spojevi kao što su flavonoidi, neflavonoidi, antocijani i vitamin C imaju važnu ulogu u antioksidativnom djelovanju i inhibicijskim učincima na pretilost, rak, upale, neuralne degeneracije i druge bolesti (Rao i Snyder, 2010; Beekwilder i sur., 2005; Seeram i sur., 2006). Međutim, svježi su plodovi maline podložni brzoj degradaciji zbog osjetljive strukture i visokog sadržaja vode. Zbog toga se većina malina prerađuje u proizvode kao što su sokovi, džemovi, želei, vina ili se konzervira zamrzavanjem ili sušenjem. Sušenje se smatra jednom od tradicionalnih, ali najučinkovitijih tehnika konzerviranja lako kvarljivog voća. Smanjenjem sadržaja vode u proizvodu zaustavlja se mikrobiološka aktivnost i biokemijski procesi koji mogu uzrokovati kvarenje, a ujedno se smanjuje i masa proizvoda što olakšava njegovo pakiranje i daljnju manipulaciju. Suhi plodovi malina najčešće se melju u prah koji se kao takav dodaje u različite prehrambene proizvode kao što su čajevi, peciva, žitarice, mliječni proizvodi, konditorski proizvodi i slično. Konvekcijsko sušenje je jedna od najčešćih tehnika sušenja koja se primjenjuje na maline, a sam proces sušenja treba voditi ovisno o karakteristikama sirovine uz pravilno održavanje temperature, relativne vlage i brzine radnog medija (zraka) (Lato i sur., 2012; Pliestić, 2020). Maline kao jednu od zahtjevnijih voćnih kultura za sušenje potrebno je sušiti na nižim temperaturama jer visoke temperature mogu uzrokovati nepoželjne gubitke u vidu degradacije vitamina, gubitaka biološki aktivnih komponenti te promjena u boji i okusu proizvoda. Stoga je glavni cilj ovog istraživanja bio utvrditi promjene sadržaja bioaktivnih komponenti i antioksidacijske aktivnosti tijekom proizvodnje suhog praha, kao i utjecaj termičkog procesa konvekcijskog sušenja na kvalitetu finalnog proizvoda.

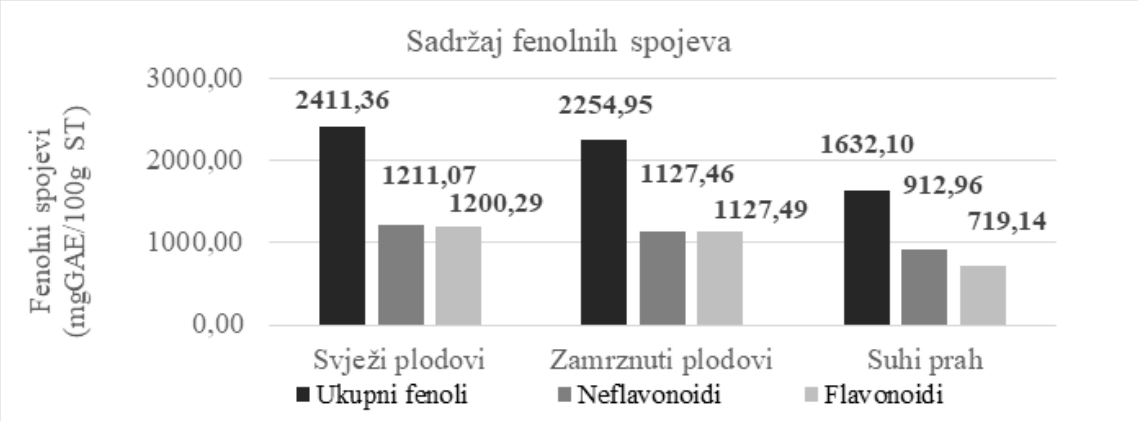
Materijal i metode

Za analize je korištena sorta malina Himbo-Top®. To je jesenska sorta nastala križanjem sorti 'Autumn Bliss' i 'Raftazer' a na tržištu je prisutna od 2008. godine. Plodovi korišteni za analize ubrani su u optimalnom roku berbe (lipanj – listopad 2021. godine) na području grada Zagreba. Nakon berbe dio plodova je odvojen za analizu u svježem stanju, a dio je smrznut i čuvan na temperaturi od -18 ^{0}C do daljnje prerade. Nakon odmrzavanja plodovi su osušeni i samljeveni u prah. Sviježi plodovi maline korišteni u pokusu imali su vlažnost od 87,17%, prosječnu masu od 4,02 g te prosječnu širinu od 17,85 mm i visinu od 16,28 mm. Plodovi su sušeni postupkom konvekcijskog sušenja u laboratorijskom sušioniku „INKO" ST40 (Hrvatska) pri temperaturi od 40 °C i atmosferskom tlaku 1013 hPa do postizanja sadržaja vode od približno 15 %. Postupak sušenja trajao je 42 sata tijekom čega je svakih sat

vremena mjerena promjena mase uzorka, kako bi se utvrdio tijek procesa sušenja. Prosječna masa plodova stavljenih na sušenje iznosila je 230 g po repeticiji. Analize su provedene u 4 repeticije (n=4). Tijekom sušenja brzina radnog medija bila je u prosjeku 1,0 ms^{-1} i mjerena je anemometrom Windmesser (Njemačka). U postupku sušenja plodovi su postavljeni jednako udaljeni jedan od drugog na rešetkastom okviru dimenzija 420 x 300 mm. Početna, kao i konačna vlaga plodova maline određena je prema standardnoj metodi (AOAC, 1995) sušenjem u laboratorijskoj sušnici na temperaturi 105 °C tijekom 90 (60 + 30) minuta do konstantne mase. Osušeni plodovi usitnjeni su u prah (veličine čestica manje od 1 mm) štapnim mikserom „Mixsy Zepter International" (Švicarska), te su skladišteni u staklenoj ambalaži u hladnom prostoru. Analize udjela bioaktivnih spojeva provedene su na sviježim i zamrznutim plodovima te na suhom prahu. Od bioaktivnih spojeva, određen je sadržaj ukupnih fenolnih spojeva, ukupnih flavonoida, neflavonoida (mg GAE 100 g^{-1} suhe tvari) i antocijana (mg/kg) prema metodi Ough i Amerine (1988) spektrofotometrijski (Shimadzu UV 1650 PC), te sadržaj vitamina C (mg 100 g^{-1} suhe tvari) standardnom metodom (AOAC, 2002). Antioksidacijski kapacitet određen je ABTS metodom (Miller i sur., 1993; Re i sur., 1999). Sušenje te analize svježih i zamrznutih plodova kao i praha provedeni su u laboratoriju Zavoda za poljoprivrednu tehnologiju, skladištenje i transport Agronomskog fakulteta Sveučilišta u Zagrebu.

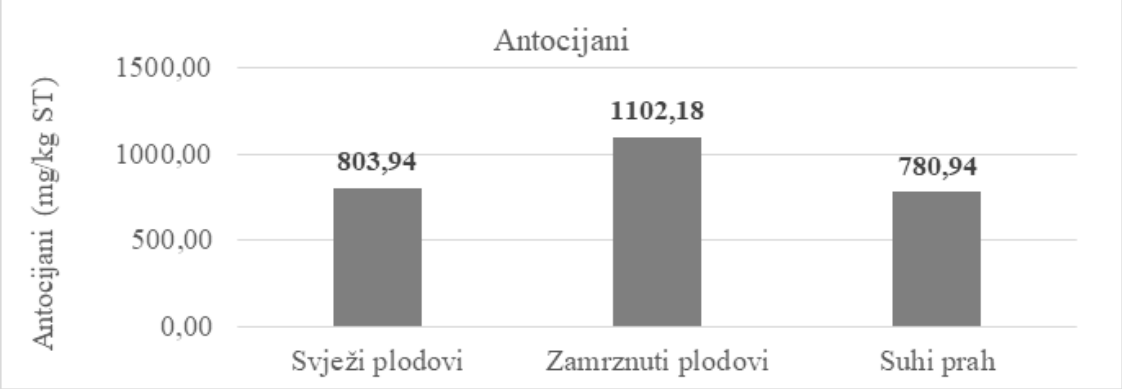
Rezultati i rasprava

Zbog lakše usporedivosti dobivenih rezultata konačni sadržaj fenolnih spojeva, antocijana i vitamina C preračunat je na suhu tvar uzorka.



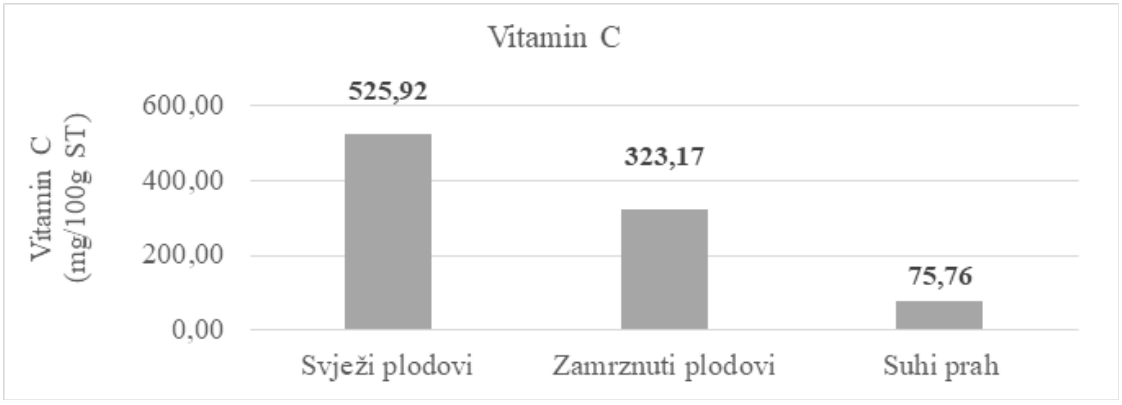
Grafikon 1. Sadržaj fenolnih spojeva (mgGAE/100g ST)

Rezultati određivanja ukupnih fenola, flavonoida i neflavonoida prikazani su na Grafikonu 1. Kahkonen i sur. (1999) u svom radu iznose da biljne sirovine koje sadrže ukupne fenole u koncentracijama većim od 20 mg/g suhe tvari predstavljaju bogat izvor tih biološki aktivnih komponenti. Sukladno tome možemo reći da svježi plodovi maline korišteni u ovom istraživanju predstavljaju bogat izvor fenolnih spojeva. Iz rezultata je vidljivo da je postupak zamrzavanja utjecao na smanjenje ukupnih fenola za 6,48 %, neflavonoida za 6,90 % i flavonoida za 6,06 %. Dobiveni rezultati su u skladu s rezultatima od Kivi i sur. (2014) koji navode da postupak zamrzavanja može utjecati na smanjenje udjela fenolnih spojeva. Pored toga, proces sušenja uzrokovao smanjenje ukupnih fenola za 32,32 %, neflavonoida za 24,61 % i flavonoida za 40,09 % u odnosu na svježe plodove što je vjerojatno posljedica njihove toplinske degradacije.



Grafikon 2. Sadržaj antocijana (mg/kg ST)

Sadržaj antocijana (Grafikon 2) u svježem uzorku maline iznosio je 803,94 mg/kg ST, nakon zamrzavanja povećao se na 1102,18 mg/kg ST, a nakon sušenja smanjio na 780,94 mg/kg ST. Prema Beekwilder i sur. (2005) prosječni sadržaj antocijana u svježem plodu maline iznosi 200 do 300 mg na 100 g svježe tvari što su nešto manje vrijednosti od onih dobivenih u ovom istraživanju. Ancos i sur. (2000) analizirali su utjecaj zamrzavanja na rane i kasne sorte maline te su otkrili da u ranim sortama zamrzavanje rezultira povećanim sadržajem antocijana, dok kod kasnijih sorti uzrokuje smanjenje. Isti autori su navode da pH ploda, udjel organskih kiselina i koncentracija šećera mogu značajno utjecati na očuvanje antocijana tijekom zamrzavanja. Scibiz i Mitek (2007) navode da je povećani udio antocijana u zamrznutom proizvodu rezultat njihove lakše ekstrakcije zbog utjecaja zamrzavanja na tkivo biljnog materijala. Usporedbom sadržaja ukupnih antocijana vidljivo je da je njihova najniža koncentracija utvrđena u suhim uzorcima. Dobiveni rezultati potvrđuju podatke o njegovoj podložnosti degradaciji pri izloženosti određenim faktorima kao što je povišena temperatura.



Grafikon 3. Sadržaj vitamina C (mg/100 g ST)

Beekwilder i sur. (2005) navode da je vitamin C jedan od spojeva koji posjeduje antioksidativna svojstva i može činiti oko 20 % ukupnog antioksidacijskog kapaciteta plodova maline. Tijekom prerade voća i povrća vitamin C se može lako razgraditi stoga mnoge studije o metodama konzerviranja hrane uzimaju upravo vitamin C kao pokazatelja kvalitete (Santos i Silva, 2008). Ancos i sur. (2000) navode da plod maline može sadržavati oko 20 do 30 mg vitamina C na 100 g svježeg uzorka što su skoro u pola manje vrijednosti od rezultata dobivenih ovim istraživanjima. Isti autori također navode da se sadržaj vitamina C može smanjiti do 50 % nakon skladištenja u zamrznutom stanju što je u skladu s rezultatima dobivenim u ovom istraživanju gdje je zabilježeno smanjenje udjela vitamina C u iznosu od 38.55 % u odnosu na svježe plodove. Smanjenje udjela vitamina C od 85,59 % tijekom postupka sušenja može se objasniti činjenicom da je on nestabilan pri povišenim temperaturama, lako se razgrađuje oksidacijom, i gubi se tokom prerade, a njegov udio se smanjuje i tijekom skladištenja. Sveukupno gledajući može se zaključiti da zamrzavanje i sušenje dovode do značajnog gubitka sadržaja vitamina C u plodovima maline.

Grafikon 4. Antioksidacijski kapacitet (µmol/100g ST)

Antioksidacijski kapacitet (Grafikon 4) u svježem uzorku maline iznosio je 1919,97 µmol TE/100g ST, nakon zamrzavanja se smanjio i iznosio je 1798,65 µmol TE/100g ST, a nakon sušenja 239,13 µmol TE/100g ST što je smanjenje od 87,55 % u odnosu na svježi uzorak. Imajući na umu da se antioksidacijski kapacitet prvenstveno odnosi na sadržaj pojedinih antioksidacijskih spojeva (vitamini, fenoli, biljni pigmenti) smanjenje udjela tih spojeva u uzorku značajno utječe i na smanjenje antioksidacijskog kapaciteta. To je vidljivo i iz rezultata ovih istraživanja koja su pokazala da osušeni prah sadrži najmanji udio vitamina C, fenolnih spojeva i antocijana a sukladno tome i najmanji antioksidacijski kapacitet.

Zaključak

Temeljem dobivenih rezultata može se zaključiti kako su svježi plodovi maline bogat izvor različitih biološki aktivnih spojeva i visokog su antioksidacijskog kapaciteta. Analizom kemijskog sastava zamrznutih plodova te suhog praha vidljivo je da je proces zamrzavanja utjecao na smanjenje udjela većine biološki aktivnih spojeva. Sušenje je uzrokovalo značajnu degradaciju svih analiziranih spojeva kao i smanjenje antioksidacijskog kapaciteta iz čega je vidljivo da parametri sušenja značajno utječu na sadržaj analiziranih biološki aktivnih spojeva.

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The influence of processing method on the content of bioactive compounds in raspberry powder

Abstract

The aim of this work was to determine the changes in the content of bioactive components during the production of dry raspberry powder and the influence of drying temperature on the quality of the final product. Raspberry fruits with an initial water content of 87,17 % were dried in a laboratory dryer "INKO" ST40 at a temperature of 40 °C and an atmospheric pressure of 1013 hPa until a water content of approximately 15 % was reached. From the obtained results it is evident that during the production of dry powder in the final product there was a decrease in the proportion of vitamin C by 85.59 %, total phenols by 32,32 %, flavonoids by 40,09 %, non-flavonoids by 24,61 %, anthocyanins by 2,78 % and antioxidant capacity by 18,98 %. From which it can be concluded that drying parameters significantly affect the content of individual bioactive compounds.

Keywords: Raspberry, Himbo-Top, freezing, drying, powder, bioactive compounds

Evaluation and gastronomic repositioning of beef from local cattle breeds

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Abstract

Local cattle breeds represent not only a genetic heritage, but also have significant economic and, above all, gastronomic potential. A considerable part of the public does not see the potential of these breeds for the preservation of the identity of the region, especially traditional and refined gastronomy. The observed opportunities for valorization and gastronomic repositioning of beef from local cattle breeds force the conclusion that it is necessary to involve all stakeholders in the food pathway to add value to the meat and provide a comprehensive gastronomic experience to the local population and visitors. The gastronomic valorization of Istrian cattle beef is an example of the successful transformation of lower value added meat into high quality meat that is recognized at the international level. The evaluation program for Buša breed beef is still in its infancy, as there are essential elements that need to be functionally linked. The quality of the meat should be at a high level, but also the traceability and presentation of the meat should be appropriate.

Keywords: local breeds, cattle, beef, gastronomy, evaluation

Introduction

In the second half of the 20th century, populations of local cattle breeds have undergone major structural and functional changes, and some of the local breeds were so neglected in terms of breeding and utilisation that they were pushed to the limits of biological survival. The trend of erosion of genetic resources, including cattle breeds, can be observed at national, regional and global levels. Of the total number of mammalian breeds used in agriculture, cattle breeds account for 22.90% (FAO, 2019). Although the number of extinct cattle breeds is relatively low (12.8%), a large number of breeds are endangered (21.1%), and for most cattle breeds the endangerment status is unknown (53.8%; FAO, 2019).

Local breeds reared in traditional farming systems contribute to maintain socio-cultural traditions and local identities (Zander et al., 2013; Marsoner et al., 2018) and support the resilience of agroecosystems and the conservation of traditional landscapes, which are often biodiversity hotspots (Fadlaoui et al., 2006). In addition to economic value, the importance of autochthonous breeds in preserving regional identity, living culture, and habitat biodiversity is also emphasized. Society as a whole now recognizes and appreciates the importance of local breeds and supports their preservation. This is also reflected in direct public (*governmental*) support for the preservation of autochthonous breeds and their inclusion in events and promotion of specific regions. However, it should be noted that the support system is necessary in the early stages of conservation and preservation of autochthonous breeds, i.e. in the phase of development, valorization and promotion of raw materials and products from these breeds. Only a balanced policy of support and development of economic programs, where incentives are compensated by the new added value of the product, ensures continuous progress and an incentive for the private sector to realize its economic benefits in the real sector (*breeding, processing, etc.*).

The value of local breeds as a genetic resource is particularly emphasized and, accordingly, the most common research in the scientific sense aims at learning more about the genetic structure, its peculiarities and phenotype. For local cattle breeds, registries have been established, breeding programs have been set up, and recognized breeding measures have been implemented to evaluate phenotype (*evaluation of young bulls and heifers*), pedigree (*maintenance of a balanced distribution of lines, verification of parentage*), and monitoring of certain production

indicators. However, the conservation of local cattle breeds *in vivo* depends largely on the motivation of breeders of local breeds, consumers of their products, other economic entities in the "food chain", and the support of the local community for programs to conserve local cattle breeds. Complete and systematic information of the society (*community*) about the direct and indirect benefits (values) and potential of local cattle breeds shapes their attitude towards this issue. Depending on the level of information, the community may actively support programs to conserve local cattle breeds or, in certain situations, may refuse to support such initiatives if it does not see the justification and sustainability of such programs. There are also situations where the community perceives the justification and sustainability of these programs partly in a way that they do not understand their complexity and believe it's enough to give just direct financial support to solve the threatened situation. In most cases, this support has proven to be a "palliative" and communities give up after a certain period of time, considering these programs unpromising.

Previous studies focusing on the conservation, breeding, rearing, and profitability of endangered breeds have recommended that products such as meat from endangered livestock breeds could be sold as lucrative niche products (Verrier et al., 2005; Lauvie et al., 2011; Lambert-Derkimba et al., 2013; Tienhaara et al., 2015). This commercialization strategy, it is argued, would compensate producers for the higher rearing costs associated with these breeds or feeding environment, making the rearing of old multipurpose breeds more profitable for farmers while helping to stabilise current populations (Lauvie et al., 2011). Tienhaara et al. (2015) found that a larger number of Finnish consumers were willing to pay a tax to support native breeds via a national conservation programme than were willing to pay a higher price for "Finncattle" meat products. Customers are willing to pay, but they want a guarantee of quality for the product they paid for. The quality guarantee in this case is not the breed per se, although breed characteristics have some influence, but the quality of the meat that results from the cattle's high-quality nutrition. In such cases, an enlightened producer looks for a guarantee of quality in one of the established quality marks that are provided by the production specification of the product itself, or simply has confidence in the producer himself.

Valorization of local cattle breeds

The valorization of local breeds, especially in terms of their phenotype, is a continuous process within the program for the conservation of local breeds, as it serves as a control for breeding direction. Evaluation of genetic structure based on pedigree is continuous, while review of genetic structure is conducted at targeted intervals or as needed to closely monitor and correct population genetic trends. Economic valorization of local cattle breeds is important for their proper positioning. The ways of valorizing local breeds are diverse and can be either collective actions or individual initiatives. Collective actions involve farmers grouped in cooperatives or associations, as well as companies in charge of processing the products (official label for a product of higher quality) and individual initiatives are mainly based on direct sale, organic farming, or both (Lambert-Derkimba et al., 2013).

Recognition of actual use potential, while respecting economic and social trends, often leads to repositioning of use value, which can significantly improve the sustainability of breeding programs. There are well-known examples around the world of breeds that have been pushed to the limits of biological viability and economically repositioned, functionally and biologically reactivated. For example, the breeding of the Hungarian gray (*Magyar Szürke*) has been revitalized after it was included in the composition of organic production, conservation of national park ecosystems, quality beef production programs, etc. Examples of Aubrac breed (Béranger et al., 1970) or Tarentaise breed (Beaufort PDO cheese; Lambert-Derkimba, 2007), also indicate the potential of local breeds, which, if recognized and properly positioned, can be sufficiently profitable.

Croatian autochthonous cattle breeds were formed in the past as multipurpose breeds. The working function was extremely important, while the potential for meat or milk production was not developed in breeding because the breeding (*feeding*) environment did not support high milk yields and high daily gains. Adaptability to modest housing and feeding conditions was important, and informal selection modestly respected the breeder' preferences. The influence of public administration was limited and was mainly through the import and distribution of quality bulls. Therefore, the characteristics of local breeds is acording with the farmers needs (*preferences for working animal characteristics resulted in coarser muscle fibers, slower growth dynamics, coarser carcass confirmation, lower ability to accumulate intramuscular fat, etc.*) than with the preferences of contemporary butchers or consumers.

Evaluation of local cattle breeds in today's world should take into account their unique characteristics, according to criteria they have developed themselves, which may or may not correspond to the criteria applicable to conventional cattle breeds. For example, Istrian cattle or Buša cattle can be evaluated according to the principles of ICAR, but

their valorization should be done in accordance with the breed standards and should not be considered with the standards of commercial breeds. When the production indicators of local cattle breeds are evaluated and compared with the indicators of commercial breeds, it is often concluded that local breeds are not sufficiently productive and competitive and should be replaced by commercial allochthonous meat or dairy breeds. Evaluation of conservation measures for agricultural genetic resources requires information on the use and non-use value of plant varieties and animal breeds, as well as preferences for *in situ* and *ex situ* conservation conservationandusingcost-benefit analysis in policy evaluation may shift the priorities of agricultural genetic resource conservation policies (Pouta et al., 2014).

It is understandable that each breed of cattle has a certain production potential, but the question is whether it is sufficient to achieve a reasonable profit and ensure the sustainability of this production. Istrian cattle and Slavonian-Sirmian Podolian cattle are breeds with a larger frame, a relatively modest potential for milk production and a recognized potential for meat production. Buša is a breed with a smaller frame, compared to the frame of a breed with a more favorable potential for milk production than for meat production. When the above breeds are compared to commercial cattle breeds, the lower productivity level would usually be the reason for a less knowledgeable producer to reject the local cattle breeds as a production genotype. However, when they look at the breed as a whole and compare the production indicators relative to the weight of the animal, the local breeds take on a different meaning. It is also necessary to look at the production indicators and relate them to the quantity and, especially the quality of feed for animals kept on dry karst pastures, lawns and arable land. In such conditions, the breeds that provide high production indicators under the conditions of intensive production and feeding show relatively poor results, sometimes even lower than those of local breeds. The fact that the costs of health protection and treatment of local breeds are several times lower should also be taken into account. Finally, the calculation is not complete without taking into account the ecological landscape function of the local breeds, which produce on pastures, grasslands and arable land, where profitable intensive livestock production is not possible.

Gastronomic positioning of meat from local cattle breeds

The gastronomic valorization and positioning of the meat of local cattle breeds is complex because it requires the connection of all stakeholders of the "food chain" from breeders to consumers, including stakeholders involved in the transport of the animals, the slaughter, primary cutting, the maturation of the meat, the preparation of culinary cuts of meat, the chefs who prepare the meat, and those who present the meat (waiters). There are three basic principles to consider when positioning meat, which are important for meat quality, meat quantity, and continuity of meat availability. Understanding the supply chain from producer to consumer is an important step (Ligda and Casabianca, 2013). In the case of local breeds, the main points to consider include the capacity to provide a regular supply of products to the market, to gain a distinct position in the market and to identify new potential markets. In this process, all local stakeholders, even if they have different short-term objectives, according to their position as farmers, processors, retailers or consumers, should try to compromise, with the aim of achieving the main objective (common to all actors in the medium and long term) of providing a sustainable future for local breeds and for the region (Ligda and Casabianca, 2013). The continuity of quality must be unquestionable in all cases, while the principle of quantitative availability is used as a marketing tool in such cases. This is because meat that is not always available according to the rule of supply and demand achieves a higher price due to the increased interest of end consumers. This is precisely in this fact that the difference in price is hidden, justified by non-availability and recognized quality.

The consumer is the last and most important link in the "food chain" and it is he who makes the final judgment about the quality of a given food product. Consumers form their opinions based on basic organoleptic criteria (*color, taste, smell, softness, juiciness, etc.*) and other available information about the meat (*breed, farm location, feeding conditions, sex, age, etc.*). The fact that consumers are informed about meat can have a significant impact on modeling their criteria for "meat quality" and they accept slightly darker, harder, or less juicy meat. Consumers do not significantly change their criteria for meat quality and, for example, rarely accept very hard or dark meat (*association with old or unhealthy food*). Such assumptions were created in the meat market during the "forcing" of non-native, highly productive breeds, which in fact created certain trends that are not justified from today's perspective. In the last decade, local cattle breeds in Croatia have tried to position themselves in the production of quality beef by considering and promoting quality in the whole social and economic context. Following the principles of short supply chains, preference for local ingredients, comprehensive consumer information, spatial experience through local gastronomy, originality of meat and refined traditional gastronomy, an attempt has been made to reposition the

meat of local products. The result is that Istrian beef is the first meat in Croatia protected by a Protected Designation of Origin (PDO; EU Commission Implementing Regulation 2022/226; *https://eur-lex.europa.eu/*).

Breeders of local cattle breeds are the starting point for the gastronomic positioning of their beef, because through feeding and housing they can significantly influence the conformation and fatness of the carcass, the marbling and the overall quality of the meat. Local cattle producers who understand the cause-and-effect relationship between farm management and carcass/meat quality can achieve high and expected quality standards that enable other participants in the "meat route" to achieve high quality standards, all the way to the consumer, who rightfully has high expectations and a comprehensive food experience. To achieve the desired quality, certain guidelines are set for breeders, especially in terms of feeding, taking into account sex, age and season, to achieve the desired marbling, color, juiciness and flavor of the meat when the animal is slaughtered. For example, the specification for Istrian beef contains basic guidelines for feeding Istrian cattle, including guidelines for the use of fermented or concentrated feed. In Buša production, it is common to produce veal under extensive and grazing husbandry, which is justified given the allometry of tissue growth. The guidelines for such husbandry systems emphasize the need to keep calves together with cows and to use cow's milk to feed the calves. Considering consumer preferences for veal, the production of veal based on the Buša genotype seems likely and justified, while Istrian cattle are preferable for beef production due to their favorable growth ability.

Slaughterhouses share responsibility in the gastronomic positioning of local breed meat for the process of preparing cattle for slaughter and primary processing of the carcass, which significantly affects the quality of the carcass and meat. For example, improper loading, transporting or unloading of animals can cause stress that leads to a decrease in glycogen reserves in the muscles, which are necessary for post-mortem processes in the meat. This then negatively affects the color of the meat and the pH_{24} after chilling, making the meat less suitable to go through the "aging" process. Butchers are responsible for the primary processing of the carcass, the "cleanliness of the cuts" and the chilling of the halves. Poor initial carcass processing, and especially slow cooling of halves, have a significant impact on the shelf life of the meat and the microbiological integrity of the meat. After the carcasses are cooled, the halves are quartered or assembled, depending on the needs of restaurant chefs. Before or immediately after carcass cutting (*confectioning*), beef must undergo a process of dry or wet aging, at an interval appropriate to the structure of the beef cut (hardness of dominant muscles), age, and sex of the animal. Maturation is a common process that "softens" the meat through lipolytic and proteolytic processes and releases certain flavors. It must be performed on local breed beef to achieve the desired quality. Because chefs look for prepared meat when preparing meat directly in restaurants, butchers are often have the task of cutting larger cuts of meat into smaller cuts, suitable for different forms of preparation, either as fresh raw meat or for thermal preparation.

Restaurant chefs are the most important link in the gastronomic positioning of local breed meats, because the thermal processing of the meat or without it (e.g. carpaccio, sausages) makes the final product that is evaluated by the consumer. The question often arises whether to maintain traditional gastronomy or introduce modern culinary creations. However, since it is necessary to highlight the quality of meat with local breeds of cattle, this is only possible by refining traditional gastronomy, i.e. introducing modern gastronomic practices while maintaining a touch of originality in the creation of dishes. In doing so, preference should be given to the combination of different local products in order to achieve the full effect of local flavors. For example, you can combine "Boškarin beef" with local wine (Malvazia) and local olive oil. Gastronomic creations from the meat of local breeds should be regularly supplemented to keep consumers interested in new tastes. The synergistic effect of the characteristics of local agricultural foods has long been described by the French with the word "terroir" (*territory*). This synergy is crucial for positioning the region as a gastronomic region, i.e. the tastes and smells of the territory combined in traditionally prepared dishes or their modern derivatives provide uniqueness and harmony.

Proper presentation of meat (*dishes*) is very important for the full experience of meat dishes. Modern consumers, especially in restaurants with better gastronomic position, are interested in more information about meat. It is desirable to emphasize the originality of the meat and its special characteristics and, if available, to indicate the origin of the meat. Complete information about meat increases the consumer's confidence in the quality of food, and such a consumer is willing to repeat the positive experience. An uninformed food establishment employee can create doubt in the consumer's mind about the credibility of the information with a poor or incorrect presentation of the prepared meat.

Media channels (*local, regional and national; digital or printed*) are important for the gastronomic positioning of the meat of local breeds, especially from the point of view of presenting its values, but also informing the whole

community about the importance of preserving local cattle breeds and their economic valorization. In this context, it should be noted that the information from the program itself must be shared in a credible and timely manner with the media, which will recognize the importance of the project and provide the entire public with quality information to ensure visibility and the possibility that the economic valorization programs are recognized. The collaboration with the media and being consistent in informing and presenting the results of the program definitely exerts a high-quality "social pressure" that ultimately allows all stakeholders to recognize the value of the program and participate constructively in its implementation.

Gastronomic positioning of meat of local breeds of cattle is focused not only on restaurants, top gastronomic offer and tourism, but also on developing the habit of local consumers to consume locally produced meat of local breeds. The interest of the local community is very important for the complete positioning of the meat of local breeds of cattle. It is important that local consumers realize the importance of preserving local breeds, not only for the sake of the breed itself, but also for the other benefits that this preservation brings. In this case, the consumer feels connected to the product and the breed, is aware of their contribution to the programs, and is willing to pay for that added value. The consumption of all other local products in daily life is of great importance. The knowledge of the value of the products, their interconnectedness, the willingness to innovate and develop while maintaining tradition in all aspects, from primary production to consumption, is ultimately united in the "table culture" of a region that can ultimately sell its rooted values as its "way of life" (liftstyle).

Conclusion

Local cattle breeds can compensate for the loss of their local function through direct and indirect use values. Today's consumers expect added value from the food on offer and especially appreciate local products. Because of its special quality and meaty character, the meat of local cattle breeds can be appropriately positioned through a gastronomic offer that adds value to it, so that all stakeholders in the "food chain" from the producer to the consumer obtain greater benefits

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Vrednovanje i gastronomsko repozicioniranje mesa izvornih pasmina goveda

Sažetak

Izvorne pasmine goveda ne predstavljaju samo genetsko nasljeđe već imaju značajan gospodarski, posebice gastronomski potencijal. Značaja dio javnosti ne sagledava potencijale ovih pasmina u očuvanju identiteta područja, posebice tradicionalne i oplemenjene gastronomije. Sagledane mogućnosti vrednovanja i gastronomskog repozicioniranja mesa izvornih pasmina goveda nameću zaključak o potrebi uključivanja svih dionika u putu namirnice kako bi se meso oplemenilo dodanom vrijednošću te domaćem stanovništvu i posjetiteljima pružio puni gastronomski doživljaj. Gastronomska valorizacija mesa istarskog goveda primjer je uspješne tranzicije mesa niže dodane vrijednosti u meso visoke vrijednosti prepoznato na međunarodnoj razini. Program vrednovanja mesa buše je u začetku uz prisutnost bitnih elemenata koje treba funkcionalno povezati. Kakvoća mesa treba biti na visokoj razini, no istu treba pratiti primjerena sljedivost i prezentacija mesa.

Ključne riječi: izvorne pasmine, goveda, meso, gastronomija, vrednovanje

Quality and nutritional properties of venison

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Abstract

The term venison in literature can be used in different meanings. Originally, ´venison´ referred to meat of any large game species suitable for human consumption. However, it should be noted that game meat comes not only from wild ungulates and other species, but also from farmed game animals. Nowadays term ´venison´ is used to define meat of antler ungulates, i.e. free-living or farmed cervids. Although production and consumption of venison are generally still modest, the both trends are constantly increasing. The most numerous cervids used for venison production in different systems worldwide are red deer (*Cervus elaphus*), fallow deer (*Dama dama*), elk (*Cervus canadensis*), axis deer (*Axis axis*) and reindeer (*Rangifer tarandus*). During last decade numerous studies have been published presenting different aspects of venison quality and factors affecting it. Thus, the aim of this paper was to give review of the quality and nutritional properties of venison.

Keywords: game meat, red deer, fallow deer, protein, fatty acids

Introduction

Due to world population growth, demand for animal protein increases steadily. For example, during last six decades global meat consumption has increased from 23 kg per capita annualy in 1961 to 43 kg in 2022 (OECD/FAO, 2022). In response to increased meat demand, alternative meat sources are imerging. Alternative animal species include horses, pigeons, ratites (mainly ostrich) and wild or farmed game species (Polawska et al., 2013). The term venison refers to meat derived from cervids (wild or farmed) and rates of global trade of venison are increasing (Kudračova et al., 2018). During last fifthy years trade has doubled and is currently around two million tonnes (Costa et al., 2016).

Meat is an important source of protein in the human diet, but during several last decades red meat has been considered as possible health risk. Health concerns were rising from food safety crises and livestock diseasses (such as ´mad cow´ disease) as well as recommendations to limit red meat intake due to its high fat and cholesterol content (Binnie et al., 2014). Venison is imerging as solution and non-traditional alternative to red meat from domestic ruminants. Alternative meat sources are preferred by so-called ″modern attentive consumers″ i.e. 41-50 year old consumers belonging to a middle cultural and professional status (Polawska et al., 2013). In general, meat from antlered ungulates is known to be lean and low in fat. Due to grass-based diet and limited contact with pharmaceuticals used in domestic animal disease treatments, venison is considered organic and safe for consumption (Barton et al., 2014; Wiklund et al., 2014). Consumer nowadays are highly interested in animal welfare, additives used in meat production and environmental pollution (Volpelli et al., 2003). Regading these aspects, vension harvested from free-living animals meets all the requirements. In case of deer farming there are some activities that are and can be questionable. These include castration of stags, intensive feeding and, as a next step human involvement in reproduction in form of artificial insemination, embryo transfer, vaccination etc. The use of hormones to increase the growth rate of deer has been rejected by all deer industries around the world. All of the above, if accepted as management practices in deer farming could contribute to damaging consumers´ perception of venison and its image as an organic and safe product (Hoffman and Wiklund, 2006).

Venison production and consumption

Venison is produced from cervids, farmed or harvested during hunting. Depending on the region of the world, the ratio between the number of wild and farmed deer differs. New Zeland and Australia are well knownfor their

established deer farming systems . Althoughg deer for centuries were kept for antler velvet in Asia (mainly China), modern deer farming began at early 1970s in New Zealand, where deer farming was first legalized (Janiszewski et al., 2008; Kudračova et al., 2018). The estimated number of deers farmed globaly and the number of deer farms are presented in Table 1.

Table 1. Number of cervid animals and farms worldwide

Country	Number of animals on farms	Number of farms	Wild deer population	Number of wild cervids harvested annually
New Zealand[1]	1.700.000	3.000	250.000[d]	70.000[d]
Australia[2]	200.000	1.400	2.000.000	120.000[c]
Canada[3]	162.000	2.200	400.000	12.000[b]
Europe[4]	280.000	10.000	2.000.000	450.000[a]
USA[5]	250.000	10.000	25.000.000	6.300.000[e]

[1]Hoffman and Wiklund (2006); [2]https://www.deerfarming.com.au/wp-content/uploads/2016/11/DFH03-Introduction.pdf; [3] Hoffman and Wiklund (2006); [4] https://www.fedfa.com/fedfa-members/
[5]https://deerfarmer.com/resources/elk-farming-north-america-history;[a] https://www.tandfonline.com/doi/abs/10.2478/v10043-010-0038-z; [b]red deer and moose; https://www2.gnb.ca/content/dam/gnb/Departments/nr-rn/pdf/en/Wildlife/2020-big-game-report.pdf; [c] https://www.austdeer.com.au/@news/2022/10/19/1385200/2021-deer-harvest-report; [d] https://www.tandfonline.com/doi/pdf/10.1080/03014223.1993.10420359; [e] https://deerassociation.com/the-biggest-buck-harvest-in-21-years/

Red deer (*Cervus elaphus*) and fallow deer (*Dama dama*) are the most abundant cervid species in Europe, New Zealand and Australia. In the Nordic countries, Russia and Alaska, reindeer (*Rangifer tarandus*) are reared in semi-domesticated systems. Wapiti (*Cervus canadensis*), fallow deer, sixa deer (*Cervus nippon*) and axis deer (*Axis axis*) are present on farms in North America and in the USA, deer farming in one of the fastest growing industries in rural areas (Hoffman and Wiklund, 2006; Danszkiewicz et al., 2015; Kudračova et al., 2018).

Worldwide, deer are kept in different farming conditions, some of which are very similar to those in nature, while others are very similar to intensive livestock production systems (Volpelli et al., 2002). But farming has contributed to making venison more accesible to consumers and to a renewed interest in deer meat. However, in Europe,the majority of venison meat (two-thirds) is harvested during hunting (Kudračova et al., 2018). Likewise, venison consumption in EU varies from 0.08 kg/capita/year (Poland, Portugal), 5.7 kg/capita/year in France up to 8.4. kg/capita/year in Andalusian hunter families (Milczarek et al., 2021).

Venison quality and nutritional properties

Meat quality includes physical, chemical and organoleptic meat attributes that have affect consumer purchasing decision and satisfaction (Purslow, 2017). These attributes are influenced by animal species/breed, sex, muscle type, nutrition, pre-slaughter handling and post-mortem changes in the meat such as pH, temperature and ageing) (Kudračova et al., 2018). Besides all the factors previously mentioned, the quality of venison is also influenced by the production system (wild vs farmed), season, environment and climate. Thus, venison quality can be very variable (Milczarek et al., 2021).

Among the physical parameters of meat quality, the ultimate pH value (pH_u; 24h after slaughter) is one of the most important. This value is associated with meat colour, tenderness and water-holding capacity. After slaughter deer meat has pH_{45} from 6.5 to 7.2 and within 24-48 h *post mortem* this value is in range of 6.0-5.4. The rate of post-mortem pH decline in deer meat is highly variable and depended on complex interactions between previously mentioned factors (Volpelli et al., 2003). Namely, in farm animals, a characteristic relationship has been found between *ante-mortem* handling and *post-mortem* pH_u due to glycogen depletion (more stress pre-mortem, less pH_u decline). However, this is not case for wild or farmed deer and it is suggested that more complex interactions occur than are known for domestic farm animals (Pollard et al., 2002). Farm-raised deer were reported to have a higher percentage of meat with pH_u >6.0 (57%), while meat samples from wild harvested deer were all in the pH_u <6.0 category (Danszkiewicz et al., 2015).

The colour of venison is intense red and as such is typical feature of deer meat. This is one of potential confunding physical characteristcs of venison quality, as consumers associate dark red colour with meat originating from older animals and dark, firm and dry defect of meat (Kudračova et al., 2018). Values of lightness (L^*) and chromaticity (a^*, b^*) in venison strongly depend on the production system. Indeed, wild, grass-fed deer have darker meat than farmed ones finished on concentrate. As wild animals are more physically active, higher content of muscle myoglobin is responsible for the darker colour than farm-raised deer (Danszkiewicz et al., 2015). Also, low colour stability is characteristic of deer meat. . This is thought to be due to high content of oxidants, like iron and copper, in venison (Ramanzin et al., 2010). It is recommended to modify feeding regime in farming systems and implement a pasture-based finishing diet instead of concentrate feeding or supplement deer diet with vitamin E (Hoffman and Wiklund, 2006). Reported values of red deer meat colour parameters ranged between 29.88-39.7 for lightness (L^*) , 9.35-15.6 for redness (a^*) and 4.10-12.2 for yellowness (b^*), regardless of farming system, sex, age or anatomic position of muscle (Volpelli et al., 2003; Purchas et al., 2010; Bureš et al., 2014; Serrano et al., 2018). For fallow deer, the following values of colour parameters are reported: 29.88-40.01 for lightness, 11.9-16.37 for redness and 6.2-11.13 for yellowness, regardless of farming system, sex, age or anatomic position of muscle (Danszkewicz et al., 2015; Piaskowska et al., 2015; Stanisz et al., 2015).

Analyses showed that meat obtained from farmed-raised deer usually has lower drip loss, higher thawing loss and lower cooking loss than meat harvested from wild ones. However, there are major variations in results depending on sex, muscle type and production system (Kudračova et al., 2018). Reported values of drip loss for red deer and fallow deer meat range between 1.09-3.29%, cooking loss between 25.63-33.45%, thawing loss between 9.2-14.67% and shear force between 19.17-53.7 N (Purchas et al., 2010; Bureš et al., 2015; Piaskowska et al., 2015; Stanisz et al., 2015; Ludwiczak et al., 2017). Regarding tenderness rating according shear force values of beef (Silva et al. 2015), venison tenderness ranges from tender to tough. Effect of different *post-mortem* methods to improve venison tenderness has been studied (Hutchinson et al., 2014). It was found that venison from pelvic-suspended deer carcasses was significantly more tender than venison from carcasses hung by Achilles tendon. There are limited data regarding different ageing treatments on venison quality. It was reported that prolonged storage (21 days, 4°C) results in increased cooking loss and reduced shear force of venison (Kim et al., 2017).

Regarding chemical composition, deer meat is preferable in human diet and has beneficial nutritional properties. Generally, venison has less calories (cca. 100 kcal/100 g meat) than meat of domestic animals (114-231 kcal/100 g meat) due to low content of fat. Average content of fat in venison is less than 3% (Hoffman and Wiklund, 2006). Venison can be considered as a rich source of protein (20-25%), with glutamine, aspartic acid, lysine, leucine and alanine being the most abundant aminoacids. Reported ash content in venison is 1-2% (Strazdina et al., 2013; Daszkiewicz et al., 2015; Serrano et al., 2019). Venison, like the meat of other game species, is often associated with higher shear force values and is less tender. This can be attributed to collagen content and its lower solubility. Increased collagen content in meat may also imply lower nutritional value, as collagen lacks some aminoacids, i.e. tryptophan (essential) and tyrosine (non-essential) (Volpelli et al., 2003; Purslow, 2005).

Fat content in meat increases with the age of deer. Concentrate-fed and farm-raised deer usually have higher fat content than those pasture-fed or in the wild (Vollpeli et al., 2003). The venison intramuscular fat content ranges between 0.35-10.9 g/100 g lean meat and it is usually several time lower than in domestic ruminants (Bureš et al., 2014; Kudračova et al., 2018). Compared to beef, venison has higher protein content and about four times lower fat content (Bureš et al., 2015). From a nutritional perspective, t the fatty acid composition of meat has important role in human diet. The most abundant fatty acids in venison are C16:0, then C18:1n-9, C18:2n-6 and C18:0, while in beef they are C18:1n-9, C16:0 and C18:0 (Bureš et al., 2015). Considering individual fatty acids, venison has considerably higher content of essential C18:2n-6 and C18:3n-3 than beef, especially venison produced in grass-based systems and in wilderness (Purchas et al., 2010; Bureš et al., 2015). It was found that venison in general has lower content of conjugated linoleic acid (CLA) than conventionally produced beef. This is considered to be due to lower intramuscular fat content (IMF) of venison meat which mainly consists of triglycerides, target lipids for CLA deposition (Bureš et al., 2015; Kudračova et al., 2018). Considering sums of total (FA), saturated (SFA), mono- (MUFA) and poly- (PUFA) unsaturated fatty acids, it was found that venison has lower content of SFA and MUFA than beef (Bureš et al., 2015). Venison can have up to three times higher content of PUFA (mainly C20:4n-6, C20:5n-3 and C22:n-6) than beef (Polowska et al., 2013; Bureš et al., 2015). Despite similar content of precursors (C18:2n-6 and C18:3n-3) in synthesis, PUFA content varies in meat of red and fallow deer. These differences in content of individual PUFA are probably result of differences in activity of desaturases and elongation enzymes during conversion processes (Cherfaoui et al., 2012).

Beside content of individual fatty acids and they sums, the nutritional aspect of fat and fatty acids in meat is estimated through specific ratios, i.e. n-6/n-3 and PUFA/SFA. Recommended value for n-6/n-3 ratio is less than 4.0 and in wild harvested or grass-fed venison this ratio ranges from 2.1-3.3, while for meat from concentrate-finished systems it ranges between 4.5-9.6 (Volpelli et al. 2003, Bureš et al., 2015). Recommended value of PUFA/SFA ratio is above 0.4 and in venison ranges between 0.18-0.84, depending on production system. Higher PUFA/SFA ratio was reported in venison (0.68-0.84) than in beef (0.18-0.22) (Bureš et al., 2015; Kudračova et al., 2018).

Fatty acids affect meat firmness and flavour while during heat treatment lipid oxidation, Maillard reactions and similar processes produce volatile and odours components. Contribution of MUFA and PUFA to meat flavour is crucial (Wood et al., 2004). Higher PUFA content in grass-based diet results in development of game aroma, while concentrate-besed diet results in lower PUFA content and less ´gamey´ flavour (Wiklund et al., 2003; Bureš et al., 2015). However, higher PUFA content is also associated with lower oxidation stability of meat and meat products (Polowska et al., 2013). Regarding game aroma and flavour, consumer preferences can be positive and negative (Wiklund et al., 2003).

Beside production system of venison, fatty acid content differs according to sex. Namely, higher PUFA content and PUFA/SFA ratio were found in meat of male than female deer. Age should be also considered when analysing fatty acid composition since it was found that venison from older animals has higher MUFA and lower PUFA content. This is result of higher IMF content in older animals and changes in lipid fraction ratios with age (Purchas et al., 2010; Piaskowska et al., 2015).

In terms of mineral content, venison has higher levels of iron, potassium, phosphorous, zinc, copper and calcium than meat of domestic ruminants or other wild species (Vengušt and Vengušt, 2004; Grace et al., 2008). Mineral composition of venison is result of different plants grazed and browsed in natural habitat and mineral composition of soil where plants grow (Kudračova et al., 2018). Besides minerals, muscle and organs (mainly liver and kidney) of deer can be source of toxic elements, such as cadmium, lead or mercury. These elements are components of some plants (holm oak) and mushrooms eaten by deer and slightly higher concentrations can be found in tissues of older animals. However, available results regarding this matter show that heavy metals in venison are below limiting values in most cases (Kudračova et al., 2018).

Conclusions

Trends production and consumption of venison meat are positive and meat of different deer species can be considered as good alternative to red meat of domestic animal species. From a nutritional point of view, venison is a very valuable source of macro- (high protein content, low fat content, favourable fatty composition) and micro- (iron, zinc, copper) nutrients. Venison is dark red in colour and physical parameters such as thawing loss, cooking loss and tenderness can differ depending on the sex, age and production system (free-living or farmed). Future research is are needed to cover all aspects of venison quality, especially sensory characteristics and the effects of different production systems.

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Kakvoća i nutritivna svojstva mesa jelenske divljači

Sažetak

Pojam ´meso divljači´ **u** literaturi se koristi u različitim značenjima. Izvorno se 'meso divljači' odnosilo na meso bilo koje vrste krupne divljači koje je dobiveno lovom divljih **životinja** i bilo je prikladno za ljudsku prehranu. No, treba napomenuti da se meso divljači ne proizvodi samo od divljih, slobodnoživućih papkara i drugih vrsta, već i od farmski uzgojene divljači. Meso jelenske divljači je meso različitih vrsta jelena, slobodnoživućih ili farmski uzgojenih. Iako su proizvodnja i potrošnja mesa jelenske divljači općenito još uvijek skromni, oba trenda su u stalnom porastu. Najbrojnije vrste jelena u svijetu koje se koriste za proizvodnju mesa u različitim sustavima su jelen obični (*Cervus elaphus*), jelen lopatar (*Dama dama*), kanadski jelen (*Cervus canadensis*), jelen aksis (Axis axis) i sob (*Rangifer tarandus*). Tijekom posljednjeg desetljeća objavljena su brojna istraživanja koja prikazuju različite aspekte kvalitete mesa divljači i **čimbenike** koji je određuju. Stoga je cilj ovog rada bio dati prikaz kakvoće i nutritivnih svojstava mesa jelenske divljači.

Keywords: meso, jelen obični, jelen lopatar, fizikalna svojstva, kemijski sastav, nutritivna vrijednost

Komparativna analiza sadržaja kolesterola i masnih kiselina u kokošjim i fazanskim jajima

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Sažetak

Jaje je animalna namirnica,bogata hranjivim tvarima kao što su proteini, masti i vitamini. Osim kokošjih jaja koja su prisutna na tržištu, mogu se u prehrani ljudi koristiti i jaja drugih vrasta peradi, kao na primjer fazanska jaja. Stoga je cilj ovog istraživanja bio analizirati sadržaj kolesterola i masnih kiselina u kokošjim i fazanskim jajima. Za analizu sadržaja masnih kiselina i kolesterola uzorkovano je 20 jaja (10 kokošjih i 10 fazanskih). Kokoši i fazanke hranjene su istom krmnom smjesom za nesilice, izbalansiranom na 17,30% sirovih proteina. Uzorci su pripremljeni i analizirani prema priznatim znanstvenim metodama, a rezultati ukazuju da je sadržaj kolesterola veći u fazanskim u odnosu na kokošja jaja (913,61 mg/100 g : 865,73 mg/100g; P>0,05). Sadržaj ∑SFA i ∑n-6 PUFA bio je statistički značajno manji kod kokošjih jaja u odnosu na jaja fazanki (P<0,05), dok je sadržaj ∑MUFA i ∑n-3 PUFA bio statistički značajno veći kod kokošjih jaja u odnosu na fazanska jaja (P<0,05). Omjer Σn-6 PUFA/Σn-3 PUFA bio je vrlo visok kod obje vrste jaja. Značajno veći omjer utvrđen je kod fazanskih jaja u usporedbi s kokošjim jajima (15,56 : 9,64; P<0,05). Ovi rezultati ukazuju da krmne smjese koje su se koristile u hranidbi kokoši i fazanki nisu sadržale krmiva koja mogu povećati udio poželjnih n-3 PUFA u jajima.

Ključne riječi: jaja, kokoši, fazanke, kolesterol, masne kiseline

Uvod

Jaja sadrže vrlo kvalitetne hranjive sastojke, a proizvode se po cijelom svijetu. Jaja kozumira veliki broj ljudi, gotovo svakodnevno. Jaja su namirnica koja je prema cijeni dostupna svima, dozvoljena u svim vjeroispovjestima i načinima prehrane. Jaja proizvode različite vrste peradi (kokoši, patke, pure, guske, prepelice, fazani i dr.). U prehrani stanovništva najčešće se na tržištu nalaze jaja podrijetlom od kokoši, no često su u restoranim delikatesa i jaja drugih vrsta peradi. Fazani su važna vrsta ptica za lov, no njihova jaja mogu biti vrlo korisna namirnica u svakodnevnoj prehrani ljudi (Gugała i sur., 2019.). Kvaliteta jaja uvjetuje njegovo prihvaćanje do strane potrošača. Kada se opisuje kvaliteta jaja onda se prvenstveno misli na pokazatelje vanjske i unutarnje kvalitete (Narushin, 1997.). Važan pokazatelj unutarnje kvalitete jaja je kemijski sastav. Ukoliko promatramo samo djelovanje konzumiranog jaja kao hrane na zdravlje konzumenta, tada nam je važan sastav masnih kiselina i sadržaj kolesterola u žumanjcima, jer utječe na funkciju kardiovaskularnog sustava kod ljudi (Simopoulos, 2016., Stupin i sur., 2018.). Masne kiseline su elementi staničnih membrana koji utječu na propusnost hranjivih sastojaka u stanicu (Ziemlański, 1997.). U zadnje vrijeme puno se pozornosti posvećuje ulozi polinezasićenih masnih kiselina (PUFA) u ljudskoj prehrani. Posebno su važne omega-3 (n-3) masne kiseline i to eikosapentaenska (EPA) i dokozaheksaenska (DHA). Osim što je važno u jajima imati više ukupnih n-3 PUFA, u obroku je važno imati što manji omjer n-6/n-3 PUFA (Kralik i sur., 2021.). U prehrani ljudi ovaj omjer je vrlo poremećen upravo zbog konzumacije takozvane „brze hrane“ koja je bogata osim sa zasićenim masnim kiselinama i polinezasićenim masnim kiselinama n-6 skupine, dok je sadržaj n-3 PUFA osobito EPA i DHA vrlo mali. Konzumacija ove hrane dovela je do neravnoteže u omjeru n-6/n-3 PUFA, koji se sa 1:1 ili 4:1 (optimalni omjer) povećao na 20:1 (Alagawany i sur., 2019.). S obzirom da ljudska prehrana treba biti što raznovrsnija, osim kokošjih jaja ljudi mogu i trebaju konzumirati i jaja drugih vrsta peradi, te je cilj ovog rada bio

usporediti sadržaj kolesterola i masnih kiselina u kokošjim i fazanskim jajima, pri konzumaciji standardne krmne smjese za nesilice.

Materijal i metode

U istraživanju su korištena jaja kokoši nesilica i jaja fazanki (*Phasianus colchicus*) iz kontroliranog uzgoja, koje su hranjene standardnom komercijalnom smjesom za nesilice. Krmna smjesa za kokoši nesilice izbalansirana je na 17,30% sirovih proteina. Nasumičnim odabirom uzorkovana su jaja za potrebe analize profila masnih kiselina i kolesterola u žumanjcima. Na ukupno 20 žumanjaka (10 kokošjih i 10 fazanskih) određen je profil masnih kiselina. Za određivanje profila masnih kiselina uzorci su pripremljeni na mikrovalnom uređaju MARS 6 (CEM Corporation, Matthews, NC, SAD) primjenom mikrovalova snage 1200 W. Na kraju procesa razaranja, uzorci su ekstrahirani u pentanu, prebačeni su u vijalicu te analizirani na plinskom kromatografu SCION 436-GC (SCION Instruments, Goes, Nizozemska) opremljenim s plameno-ionizacijskim detektorom (FID- flame ionization detector). Za separaciju masnih kiselina korištena je FAMEWAX (Restek Corporation, Bellefonte, PA, SAD) kapilarna kolona (30 m x 0,32 mm (unutarnji promjer) x 0,25 μm (debljina filma)). Volumen uzorka za injektiranje bio je 1 μL, a radni uvjeti sljedeći: temperatura injektora: 230^{O}C, temperatura detektora 230^{O}C, protok plina nosioca (vodik) 2.5 mL/min. Temperaturni program pećnice programiran je na sljedeći način: od 50 do 160^{O}C: 20^{O}C/min, od 160 do 225^{O}C: 10^{O}C/min uz zadržavanje na 225^{O}C devet minuta. Ukupno trajanje analize je 21 minutu. Za identifikaciju pojedinačnih masnih kiselina u kromatogramu korištena je standardna smjesa 37 masnih kiselina (Food Industry FAME Mix, Restek Corporation, Bellefonte, PA, SAD). Na ukupno 12 žumanjaka (6 kokošjih i 6 fazanskih) određen je sadržaj kolesterola, koristeći modificiranu metodu Albuquerque i sur. (2016.). Protokol pripreme i očitanja kolesterola bio je sljedeći: 5 ml 0,4 M KOH u etanolu dodano je u 0,5 g žumanjka i otopina je dobro promućkana u vortexu. Uzorci su inkubirani u vodenoj kupelji na 50°C u trajanju od 30 minuta. Nakon hlađenja na sobnoj temperaturi, ekstrakcija kolesterola obavljena je dva puta s 10 ml n-heksana. Ekstrakti su kombinirani i alikvot od 3 ml je osušen i nadopunjen s 3 ml mobilne faze. Shimadzu HPLC sustav opremljen UV-VIS detektorom SPD-10AV VP i SIL-10AD VP auto-injektorom, Shimadzu Shim-pack GIST (250 x 4,6 mm I.D., veličina čestica 5 μm) korišten je za odvajanje i kvantificiranje kolesterola. Mobilna faza bila je otopina izopropanol: acetonitril (50:50 v/v). Prije uporabe, mobilna faza je filtrirana kroz membranski filter od 0,20 μm i otplinjena u ultrazvučnoj kupelji. Temperatura kolone bila je 37°C, brzina protoka 1,2 ml/min i ubrizgani volumen 10 μL. Količina kolesterola utvrđena je pomoću UV-VIS detektora postavljenog na valnoj duljini od 210 nm. Ukupno vrijeme analize bilo je 10 minuta. Rezultati istraživanja obrađeni su korištenjem statističkog programa TIBCO® Data Sciencis version 14.0.0.15 (©1984-2020 Tibco Softwere Inc.), a prikazani su u tablicama i to kao srednja vrijednost () i standardna devijacija (sd). Razlike među ispitivanim skupina utvrđene su studentovim t-testom na razini P<0,05; P<0,01 i P<0,001.

Rezultati i rasprava

U tablici 1 prikazane su vrijednosti kolesterola u žumanjcima kokošjih i fazanskih jaja. Sadržaj kolesterola bio je ujednačen, odnosno između grupa nije utvrđena značajna razlika (P=0,333). Prisutnost kolesterola u jajima je neophodna jer kolesterol ima primarnu funkciju osigurati energiju za rast embrija. Upravo radi toga je vrlo teško hranidbom utjecati na njegovo smanjenje.

Tablica 1. Sadržaj kolesterola u jajima (±sd)

Vrsta jaja	Sadržaj kolesterola mg/100 g žumanjka
Kokošja	865,73 ± 42,93
Fazanska	913,61 ± 57,94
P-vrijednosti	0,333

n.s. P>0,05

Sadržaj kolesterola u kokošjim jajima kreće se između 150 i 200 mg po jajetu. Ova činjenica stvorila je negativnu percepciju kod nekih potrošača u pogledu njegove hranjive vrijednosti. U svom radu na temu konzumacija jaja i rizik od kardiovaskularnih bolesti i dijabetesa Li i sur. (2013.) istaknuli su da su jaja izazvala kontroverzna mišljenja

među nutricionistima i stručnjacima u zdravstvenim agencijama zbog sadržaja zasićenih masti i kolesterola. Zbog navedenog ljudima se preporučavalo da što manje konzumiraju jaja. Povećanu konzumaciju jaja upravo zbog sadržaja kolesterola, smatrali su uzrokom pojave kardiovaskularnih bolesti kod ljudi. Međutim, razna istraživanja potvrđuju da kolesterol iz jaja ima ograničen učinak na razinu kolesterola u krvi ljudi, a samim tim i ograničenu odgovornost za pojavu kardiovaskularnih bolesti (Stupin i sur., 2018.). Na sadržaj kolesterola utjecaj ima veličina jaja odnosno veličina žumanjka (Genchev, 2012.). Konzumiranje jednog do dva jaja dnevno nije povezano s povećanjem kolesterola u krvi kod ljudi ili povećanim rizikom od kardiovaskularnih bolesti (Seuss-Baum i sur., 2011.), stoga osobe koje nisu kardiovaskularni bolesnici mogu nesmetano u svakodnevnoj prehrani konzumirati jaja. Vrijednosti sadržaja kolesterola kokošjeg jajeta u našem istraživanju manje su od onih koji u svom radu navode Kaźmierska i sur. (2005.). Isti autori navode da je sadržaj kolesterola u fazanskim jajima 682 mg/100 g žumanjka, što je manje u odnosu na naše istraživanje. Gugała i sur., (2019.) navode veći sadržaj kolesterola u žumanjcima fazanskih jaja (1046 mg/100g) u usporedbi s našim rezultatima (913,61 mg/100 g).

U tablici 2 prikazan je sadržaj masnih kiselina u žumanjcima kokošjih i fazanskih jaja. Uočeno je da fazanska jaja imaju statistički značajno veći sadržaj ∑SFA i ∑n-6 PUFA u odnosu na kokošja jaja (P<0,05), dok je sadržaj ∑MUFA i ∑n-3 PUFA bio statistički značajno veći kod kokošjih jaja (P<0,05)

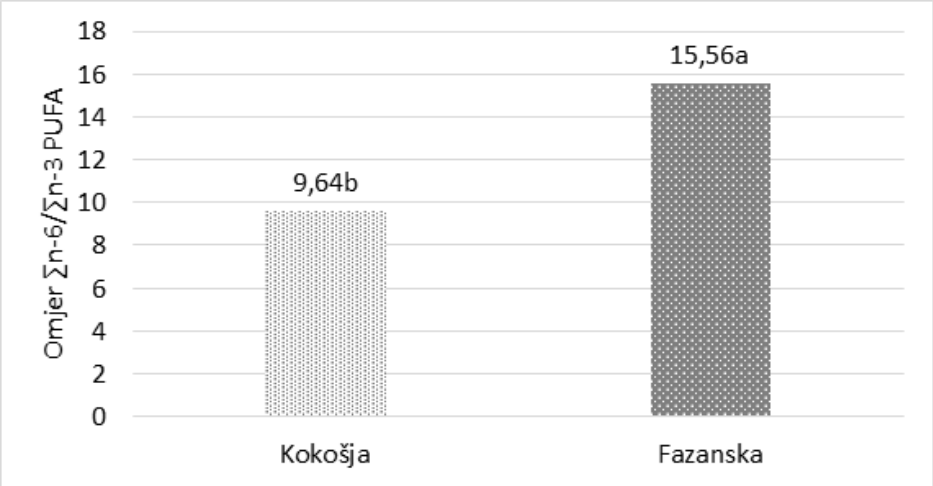
Tablica 2. Usporedba sadržaja masnih kiselina u žumanjcima kokošjih i fazanskih jaja (% od ukupnih masnih kiselina;)

Masne kiseline	Kokošja jaja	Fazanska jaja
Σ SFA	33,09[b]	38,67[a]
Σ MUFA	38,99[a]	31,65[b]
Σ n-6 PUFA	25,28[b]	27,87[a]
Σ n-3 PUFA	2,62[a]	1,79[b]

Eksponenti [a,b,] u redovima iznad srednjih vrijednosti pokazuju značajnu razliku između skupina na razini P<0,05
SFA= zasićene masne kiseline; MUFA= mononezasićene masne kiseline; PUFA= polinezasićene masne kiseline.

Rezultati našeg istraživanja kod kokošjih jaja sukladni su navodima Kralik i sur. (2020.) koji su kod kokošjih jaja podrijetlom od kokoši hranjenih konercijalnom krmnom smjesom utvrdili udio ∑SFA 31,65%, ∑MUFA 36,65%, ∑n-6 PUFA 28,82%, i ∑n-3 PUFA 2,43%. Rezultati koje navode Gugała i sur., (2019.) za sadržaj ∑SFA (34,79%), ∑n-6 PUFA (16,51%) i ∑n-3 PUFA (1,32%) u fazanskim jajima su niži, te nisu sukladni našim rezultatima.

Na grafikonu 1. prikazan je omjer ∑n-6 PUFA/∑n-3 PUFA u žumanjcima kokošjih i fazanskih jaja. Iz grafikona je vidljivo da fazanska jaja imaju statistički značajno nepovoljniji omjer ∑n-6 PUFA/∑n-3 PUFA u usporedbi s kokošjim jajima (P<0,05).



Grafikon 1. Omjer Σn-6 PUFA/Σn-3 PUFA u žumanjcima jaja

Gugała i sur., (2019.) navode da je u žumanjcima fazanskih jaja pri hranidbi smjesom izbalansiranom na 20,9% sirovih proteina omjer Σn-6 PUFA/Σn-3 PUFA iznosio 12,51. Usporedba naših rezultata sa podatcima gore spomenutih autora ukazuje na različit omjer Σn-6 PUFA/Σn-3 PUFA. Pretpostavka je da razlika u rezultatima proizlazi iz činjenice da životinje nisu konzumirale hranu istog sastava, a u nizu istraživanja potvrđeno je da na sadržaj masnih kiselina najveći utjecaj imaju krmiva koja se koriste u krmnoj smjesi.

Zaključak

U prehrani ljudi jaja sudjeluju s visokovrijednim bjelančevinama, esencijalnim masnim kiselinama, vitaminima i mineralima. Ljudi koji brinu o svom zdravlju imaju velika očekivanja od proizvođača hrane te su u svojim očekivanjima prema hrani vrlo zahtjevni i ne očekuju samo da hrana mora biti sigurna i zdrava, već i ukusna. Na tržištu prehrambenih proizvoda najčešće se u ponudi nalaze kokošja jaja, no u gastronomskoj ponudi moguće je konzumirati i jaja druge peradi kao što su fazanska jaja. Iz rezultata našeg istraživanja možemo istaknuti da su jaja kokoši imala niži sadržaj kolesterola u žumanjcima u odnosu na jaja fazanki. Statistički značajno manje ∑SFA i Σn-6 PUFA imala su jaja podrijetlom od kokoši u odnosu na jaja fazanki ($P<0,05$). Manji sadržaj ∑MUFA i Σn-3 PUFA bio je kod jaja fazanki u odnosu na kokošja jaja. Upravo radi navedenog, omjer Σn-6 PUFA/Σn-3 PUFA bio je povoljniji kod kokošjih jaja u odnosu na jaja fazanki (9,64 : 15,56; $P<0,05$). Rezultati istraživanja ukazuju da kokoši nesilice i fazanke nisu hranjene smjesama koje u svom sastavu sadrže krmiva koja mogu povećati sadržaj poželjnih n-3 PUFA. Mišljenja smo da je potrebno organizirati radionice, gdje bi se proizvođačima jaja ukazalo koja je važnost odabira krmiva kojima se hrani perad, upravo radi njihovog utjecaja na profil masnih kiselina u jajima.

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Comparative analysis of cholesterol and fatty acid content in hens and pheasant eggs

Abstract

Egg is an animal food, rich in nutrients such as proteins, fats and vitamins. In addition to hen eggs that are present on the market, eggs of other types of poultry, such as pheasant eggs, can also be used in human nutrition. Therefore, the aim of this research was to analyze the content of cholesterol and fatty acids in hen and pheasant eggs. 20 eggs (10 chicken and 10 pheasant) were sampled for analysis of fatty acid and cholesterol content. Hens and pheasants were fed the same feed mixture for layers, balanced at 17.30% crude protein. The samples were prepared and analyzed according to recognized scientific methods, and the results indicate that the cholesterol content is higher in pheasant eggs compared to hen eggs (913.61 mg/100 g : 865.73 mg/100 g; $P<0.05$). The content of ∑SFA and ∑n-6 PUFA was statistically significantly lower in hen eggs compared to pheasant eggs ($P<0.05$), while the content of ∑MUFA and ∑n-3 PUFA was statistically significantly higher in hens eggs compared to pheasant eggs ($P<0.05$). The Σn-6 PUFA/Σn-3 PUFA ratio was very high in both types of eggs. A significantly higher ratio was found in pheasant eggs compared to hen eggs (15.56 : 9.64; $P<0.05$). These results indicate that the feed mixtures used in the feeding of hens and pheasants did not contain components that could increase the proportion of desirable n-3 PUFA in eggs.

Keywords: eggs, hens, pheasant, cholesterol, fatty acids

Bioaktivni peptidi iz sira

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Sažetak

Bioaktivni peptidi podrijetlom iz kazeina i proteina sirutke najbrojnija su skupina bioaktivnih tvari sadržanih u mliječnim proizvodima. To su fragmenti nastali cijepanjem proteina, sastavljeni od 3 do 20 aminokiselina, koji nastaju tijekom probave u humanom organizmu ili tijekom procesa prerade mlijeka. Sirevi, uz fermentirana mlijeka predstavljaju glavne izvore bioaktivnih peptida. Brojna istraživanja pokazala su da su sirevi dobar izvor antihipertenzivnih, antitrombotičnih, opioidnih, antimikrobnih, imunomodulatornih, antioksidativnih peptida i peptida koji vežu mineralne tvari. Zbog navedenih bioaktivnih peptida sir se svrstava u skupinu funkcionalne hrane. Cilj ovog rada je dati pregled najznačajnijih bioaktivnih peptida podrijetlom iz sira te prikazati utjecaj tehnološkog procesa proizvodnje na njihov nastanak.

Ključne riječi: sir, bioaktivni peptidi, proteoliza, zrenje

Uvod

Bioaktivni peptidi podrijetlom iz kazeina i proteina sirutke najbrojnija su skupina bioaktivnih tvari prisutnih u mliječnim proizvodima. Definirani su kao specifični proteinski fragmenti sastavljeni od 3 do 20 aminokiselina koji pozitivno utječu na fiziološke i metaboličke funkcije ili stanje tijela i mogu imati pozitivne učinke na ljudsko zdravlje (Park i Nam, 2015.). Pokazuju antihipertenzivnu, antitrombotičnu, opioidnu, antimikrobnu, imunomodulatornu, antioksidativnu, antikancerogenu aktivnost te aktivnost s učinkom vezanja mineralnih tvari. Upravo aminokiselinski sastav i sekvence određuju njihovu biološku aktivnost. Većina bioaktivnih peptida prisutnih u mlijeku nalazi se u neaktivnom obliku unutar nativne sekvence proteinske molekule. Svoju aktivnost mogu ispoljiti tek nakon hidrolize proteina iz kojeg potječu: enzimatskom hidrolizom djelovanjem probavnih enzima (*in vivo* ili *in vitro)*, mikrobnom fermentacijom s proteolitičkim mljekarskim kulturama, enzimatskom hidrolizom enzimima izvedenim iz mikroorganizama i biljaka te enzimatskom hidrolizom kombinacijom probavnih enzima i proteolitičkih mljekarskih kultura ili enzima izvedenih iz mikroorganizama i biljaka (Korhonen i Pihlanto 2006., Korhonen 2009., Lisak Jakopović i sur., 2019).

Kravlje, kozje i ovčje mlijeko pripadaju skupini kazeinskih mlijeka, gdje je udio kazeina u ukupnom udjelu proteina iznad 75 %. Kazein se u sve tri vrste mlijeka smatra dobrim izvorom bioaktivnih peptida (Akuzawa i sur. 2009., Tratnik i Božanić 2012.). Velika većina bioaktivnih peptida u mlijeku potječe iz α_{S1}-kazeina, potom iz β-kazeina i α_{S2}-kazeina (Baum i sur., 2013). Iako proteini sirutke čine oko 20 % proteina u ukupnom udjelu proteina kravljeg, ovčjeg i kozjeg mlijeka također se smatraju dobrim izvorom bioaktivnih peptida pa mogu poslužiti kao izvor prethodno navedenih peptida, s izuzetkom antitrombotičnih peptida (Tratnik i Božanić 2012., Mann i sur. 2019.). Brojna znanstvena istraživanja ukazuju na potencijalni ili stvarni učinak bioaktivnih peptida mlijeka u ljudskom organizmu kroz pozitivan učinak na krvožilni, živčani, imunološki i probavni sustav. U tom smislu, ACE (engl. Angiotensin I-Converting Enzyme) - inhibitorni peptidi čine najveću i najznačajniju skupinu bioaktivnih peptida. Među njima, posebno mjesto zauzimaju tripeptidi Val-Pro-Pro (VPP) i Ile-Pro-Pro (IPP) čiji je antihipertenzivni učinak dokazan u mnogim *in vivo* i *in vitro* istraživanjima provedenim na humanim i životinjskim modelima. Zbog dokazanog pozitivnog učinka danas se mogu naći u mnogim komercijalno dostupnim proizvodima kao što su fermentirana mlijeka *Calpis*, *Evolus* i *Prodiet F200* (Lisak Jakopović i sur., 2019.). Stvorena je baza bioaktivnih peptida podrijetlom iz mlijeka različitih sisavaca, uključujući kravlje, ovčje i kozje mlijeko, a sačinjena na osnovi rezultata dosadašnjih znanstvenih istraživanja i dostupna na http://mbpdb.nws.oregonstate.edu/. Kako bi se pregledao ovaj veliki skup podataka, bioaktivni peptidi navedeni u literaturi vizualno su preslikani na matične proteinske sekvence. Upisivanjem željene proteinske sekvence peptida u tražilicu, ukoliko je peptid prisutan u bazi, omogućen je uvid u neke od karateristika traženog bioaktivnog peptida, kao što su vrsta mlijeka iz kojeg potječe, vrsta i interval proteina

iz kojeg potječe, jedna ili više funkcija koje iskazuje, naziv i vrstu istraživanja u kojima je izoliran i identificiran, kao i nazive autora provedenih istraživanja (Nielsen i sur., 2017.). Enzimatska hidroliza proteina mlijeka, bilo da je riječ o kazeinu ili proteinima sirutke, najčešće se događa tijekom procesa fermentacije, te stoga unutar skupine mliječnih proizvoda sirevi, uz fermentirana mlijeka predstavljaju glavne izvore bioaktivnih peptida (Korhonen i Pihlanto 2006.; Samaržija 2015.; Pihlanto 2016.). Cilj ovog rada je dati pregled najznačajnijih bioaktivnih peptida podrijetlom iz sira te prikazati utjecaj tehnološkog procesa proizvodnje na njihov nastanak.

Antihipertenzivni peptidi

Sir se smatra dobrim izvorom antihipertenzivnih peptida, odnosno ACE (engl. Angiotensin I-converting enzyme)-inhibitornih peptida. Iako velika većina sireva može biti izvor ACE- inhibitornih peptida koji iskazuju potencijalni ili dokazani bioaktivni učinak, zbog dokazane važnosti trajanja zrenja na količinu i vrstu stvorenih ACE-inhibitornih peptida, ekstratvrdi, tvrdi i polutvrdi sirevi smatraju se boljim izvorom spomenutih peptida. U kategoriji ekstratvrdih sireva, dobar izvor snažnih ACE-inhibitornih peptida su talijanski sirevi Parmiggiano Reggiano i Grano Padano. Basiricò i sur. (2015.) ispitali su prisutnost 8 snažnih ACE-inhibitornih peptida (VPP, IPP, RYLGY, RYLG, AYFYPEL, AYFYPE, LHLPLP i HLPLP) u uzorcima Parmigiano Reggiana prije i nakon izlaganja *in vitro* uvjetima probave. Iz uzoraka, prije nego su izloženi *in vitro* uvjetima probave, izolirani su i identificirani sljedeći ACE-inhibitorni peptidi: VPP , IPP , LHLPLP i HLPLP . Iz uzoraka, izloženih *in vitro* uvjetima probave, izolirana su i identificirana još dva ACE-inhibitorna peptida: AYFYPEL i AYFYYPE . Stuknyte i sur. (2015.) ispitali su prisutnost ACE-inhibitornih peptida u uzorcima Grano Padana prije i nakon izlaganja *in vitro* uvjetima probave. Iz uzoraka, prije nego su izloženi *in vitro* uvjetima probave, izolirani su i identificirani sljedeći ACE-inhibitorni peptidi: VPP , IPP , HLPLP i LHLPLP . Iz uzoraka, izloženih *in vitro* uvjetima probave u želudcu, izolirani su i identificirani peptidi: VPP, IPP, HLPLP i novonastali RYLG . Iz uzoraka, izloženih *in vitro* uvjetima probave u crijevima, izolirani su i identificirani peptidi: HLPLP i LHLPLP. Martini i sur. (2020.) iz sira Parmigiano Reggiano prije nakon zrenja identificirali su 26 bioaktivnih peptida, a nakon *in vitro* probave njih 52. Nadalje, u kategoriji tvrdih sireva dobrim izvorom antihipertenzivnih peptida pokazali su se Emmentaler i Cheddar. U istraživanju kojeg su proveli Gagnaire i sur. (2001.) od 91 peptida utvrđenog u Emmentaleru, 28 ih je pokazalo različite biološke učinke, među kojima su neki iskazali antihipertenzivni učinak u *in vitro* uvjetima. Saito i sur. (2000.) navode kako se u kategoriji polutvrdih sireva Gauda pokazala kao izvor nekoliko bioaktivnih peptida od kojih su -CN f(1-9) i β-CN f(60-68). Uz Gaudu ACE-inhibitornu aktivnost iskazali su i Edam i Havarti (Saito i sur., 2000.) te Tulum sir (Öztürk i Akin, 2021.). Antihipertenzivne peptide podrijetlom iz proteina sirutke, a izolirane i identificirane iz Cheddara i Fete čine: β-Lg f(147-148), Lf f(288-289) i Lf f(319-320) (Pripp i sur., 2006.). Dodatak mljekarske kulture *Lactobacillus helveticus* LH-B02 povećava sadržaj ACE-inhibitornih peptida podrijetlom iz β-kazeina u proizvodnji brazilskog Prato sira posebice β-CN (f194–209) (Baptista i sur., 2018).

Opioidni peptidi

Različite vrste sireva mogu biti dobar izvor opioidnih peptida, a osobito sirevi s plemenitim plijesnima i polutvrdi sirevi. Iako se njihova prisutnost u siru smatra poželjnom, kod previsokih koncentracija učinak nekih opioidnih peptida na tvorbu različitih vrsta bioaktivnih peptida sličan je učinku nekih imunomodulatornih peptida. Tako tijekom zrenja Cheddara i Crescenze nastaje frakcija β-CN f(58-72) koja uključuje opioidni peptid β-CM-7 zaslužan za selektivni inhibitorni utjecaj na endopeptidaze i aminopeptidaze bakterija mliječne kiseline prisutnih tijekom zrenja u spomenutim vrstama sira (Smacchi i Gobbetti, 2000.; Sienkiewicz-Szłapka i sur., 2009.). U istraživanju koje su proveli Sienkiewicz-Szłapka i sur. (2009.) dokazana je prisutnost opioidnih peptida s agonističkim i antagonističkim učinkom u tri polutvrda sira (Gauda, Edam i Kasztelan) te u dva sira s plemenitim plijesnima (Brie i Rokpol). Izolirane i identificirane peptide s agonističkim učinkom čine: YPFPG i YPFPGPI , dok one s antagonističkim učinkom čine: YIPIQYVLSR i SRYPSY . Iako su spomenuti peptidi pronađeni u svim vrstama sireva, značajno viša koncentracija peptida s agonističkim učinkom pronađena je u sirevima s plemenitim plijesnima, dok je značajno viša koncentracija peptida s antagonističkim učinkom pronađena u polutvrdim sirevima.

Antimikrobni peptidi

Među antimikrobnim peptidima posebno važnima smatraju se peptidi koji iskazuju potencijalni ili dokazani

antibakterijski učinak. U istraživanju kojeg su proveli Rizzello i sur. (2005.) ispitana je prisutnost antibakterijskih peptida u 9 vrsta talijanskih sireva (Parmigiano Reggiano, Caciocavallo, Gorgonzola, Crescenza, Pecorino Romano, Fossa, Canestrato Pugliese, Mozarella, Caprino del Piemont) koji su se razlikovali po vrsti mlijeka za sirenje, korištenoj mikrobnoj kulturi i vrsti sirila te trajanju zrenja. Među spomenutim sirevima u Parmigiano Reggianu, Gorgonzoli i Fossi nisu nađeni antibakterijski peptidi što bi se moglo pripisati intenzivnoj proteolizi koja se odvija tijekom zrenja ovih sireva. U ostalim sirevima pronađene su različite vrste i količine pojedinih antibakterijski peptida, a neke od izoliranih i identificiranih peptida čine: GLSPEVLNENLL i RFVVAPFPE iz Pecorino Romana, MAIPPKKNQD i FVAPFPEVFG iz Canestrato Pugliesea, RPKHPIK i GLPQE iz Caciocavalloa, YQEPVLGPVRGPFPIIV i MPIQAFLL iz Crescenze te YPFTGPIPN i MPIQA iz Caprino del Piemontea.

Imunomodulatorni i antioksidativni peptidi

Dobar izvor imunomodulatornih pepida predstavljaju sirevi s dugim trajanjem zrenja, poput primjerice sira Parmigiano Reggiano. Iako se njihova prisutnost u siru smatra poželjnom, kod previsokih koncentracija određeni imunomodulatorni peptidi mogu imati inhibitorni učinak na proteolitičku aktivnost bakterija mliječne kiseline, a samim time i negativan učinak na tvorbu ostalih vrsta bioaktivnih peptida u siru (Smacchi i Gobbetti, 2000., Summer i sur., 2017.). Neke od imunomodulatornih peptida izoliranih i identificiranih iz Parmigiano Reggiana različite zrelosti čine: RPKHPIKHQGL , FFVAPFPEVFGK i YQEPVLGPVR (Summer i sur., 2017.). Izvor antioksidativnih peptida najčešće predstavljaju sirevi sa srednjim ili dugim trajanjem zrenja i sirevi s plemenitim plijesnima. Tako, Akuzawa i sur. (2009.) navode kako je dobra antioksidativna aktivnost, a samim time i prisutnost antioksidativnih peptida zabilježena u Gaudi, Parmesanu i Camembertu nakon predviđenog vremena zrenja za svaku od navedenih vrsta sireva, pri čemu se nastali antioksidativni učinak pripisuje proteolitičkoj aktivnosti koja se odvija tijekom zrenja.

Utjecaj tehnološkog postupka proizvodnje sira na sadržaj i vrstu bioaktivnih peptida

Vrsta i količina bioaktivnih peptida prisutnih u nekom siru velikim djelom ovisi o uvjetima tijekom zrenja, dužini trajanja zrenja te karakteristikama mlijeka iz kojeg je sir proizveden. Mikrobna fermentacija korištenjem proteolitičkih mikrobnih kultura podrazumijeva korištenje mljekarskih mikrobnih kultura, najčešće bakterija mliječne kiseline (BMK) i njihovih enzima gdje se u procesu fermentacije iz proteina mlijeka oslobađaju bioaktivni peptidi (Choi i sur., 2012.). Mljekarske kulture imaju posebnu važnost u tvorbi bioaktivnih peptida u tijekom zrenja različitih vrsta sireva (Griffiths i Tellez, 2013.; Pihlanto, 2016.). Naime, Kocak i sur. (2020.) navode da vrsta dodane mljekarske kulture utječe na sadržaj pojedinih bioaktivnih peptida u bijelom kozjem siru u tipu feta sira. Rezultati istraživanja pokazali su da najveći sadržaj ACE- inhibitornih peptida sadrži sir proizveden uz dodatak *Lactobacillus casei***,** a antioksidativnih peptida uz dodatak *Lactobacillus bulgaricus.* Sirevi koji su proizvedeni iz nepasteriziranog mlijeka imaju veći sadržaj bioaktivnih peptida zbog intenzivnijeg procesa proteolize uslijed prisutnosti autohtonih proteolitičkih bakterija mliječne kiseline (Gómez-Ruiz i sur., 2004; Pisanu i sur., 2015). Bütikofer i sur. (2007.) navode da termički neobrađeno mlijeko u proizvodnji sireva rezultira većim sadržajem antihipertenzivnih peptida VPP-a i IPP-a.

Postupak zrenja sira smatra se najznačajnijim izvorom bioaktivnih peptida s obzirom da se tada odvijaju proteolitičke reakcije, odgovorne između ostalog i za formiranje karakteristične arome i teksture sira. Proteolitičke reakcije predstavljaju hidrolizu kazeina do peptida i aminokiselina djelovanjem sirišnih enzima, prirodnih proteinaza mlijeka, proteolitičkih enzima bakterija mliječne kiseline i proteolitičkih enzima nestarterskih bakterija mliječne kiseline (Havranek i sur., 2014.; Vrdoljak i sur., 2022.). U istraživaju kojeg su proveli Bütikofer i sur. (2007.) dokazana je pozitivna korelacija između dužine trajanja zrenja pojedinih vrsta sireva i sadržaja antihipertenzivnih peptida VPP-a i IPP-a. Analizom klasično proizvedenog Emmentalera starosti 4, 8 i 12 mjeseci utvrđena je pozitivna korelacija između trajanja zrenja i količine stvorenih tripeptida, pa je u 4 mjeseca starom Emmentaleru utvrđena najmanja količina VPP-a i IPP-a, dok je u 12 mjeseci starom Emmentaleru utvrđena najveća količina VPP-a i IPP-a. Öztürk i sur. (2022.) također navode da se sadržaj bioaktivnih peptida povećava tijekom zrenja, te zaključuju da vrlo zreli Tulum sir može imati pozitivne učinke na ljudsko zdravlje čime ga svrstavaju u kategoriju funkcionalne hrane. Međutim, tijekom zrenja Gruyer sira, sadržaj VPP i IPP se smanjuje što se pripisuje različitostima u tijeku odvijanja proteolitičkih procesa tijekom zrenja različitih vrsta sireva, odnosno činjenici da se neki peptidi akumuliraju u siru tijekom zrenja, a neki se dalje razgrađuju do aminokiselina (Bütikofer i sur., 2007.; Taivosalo i sur., 2018.).

Zaključak

Vrsta i sadržaj bioaktivnih peptida prisutnih u nekom siru ovisi o tehnološkom procesu proizvodnje sira, što uključuje vrstu mlijeka, termičku obradu mlijeka, korištenje mljekarskih kultura te dužinu trajanja zrenja. Brojna *in vivo* i *in vitro* istraživanjima pokazala su da su sirevi dobar izvor antihipertenzivnih, antitrombotičnih, opioidnih, antimikrobnih, imunomodulatornih, antioksidativnih peptida i peptida koji vežu mineralne tvari. Zbog navedenih bioaktivnih peptida sir se svrstava u skupinu funkcionalne hrane.

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Bioactive peptides from cheese

Abstract

Bioactive peptides from casein and whey proteins are the most numerous group of bioactive substances in dairy products. They are fragments consisting of 3 to 20 amino acids and are formed by the degradation of proteins during digestion in the human body or during milk processing. Cheese and fermented milk are the main sources of bioactive peptides. Numerous studies have shown that cheese is a good source of antihypertensive, antithrombotic, opioid, antimicrobial, immunomodulatory, antioxidant, and mineral-binding peptides. Due to the mentioned bioactive peptides, cheese is classified as a functional food. The aim of this article is to give an overview of the most important bioactive peptides from cheese and to show the influence of the technological production process on their formation.

Keywords: cheese, bioactive peptides, proteolysis, ripening

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Precizna poljoprivreda i održiva proizvodnja

Uzroci i mogućnosti sprječavanja nastajanja otpada od hrane

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Sažetak

Sve veće količine generiranog otpada od hrane odbačene na odlagališta velika su prijetnja u postizanju održivog razvoja, stabilnosti tržišta hrane, rastu ljudske populacije i dobrobiti ljudi. Odbačena hrana ima dalekosežne posljedice, kako na nacionalnoj tako i na globalnoj, ali i okolišnoj razini. Sve je manje obradivih poljoprivrednih površina, smanjuju se zalihe fosilnih goriva i sve je teže proizvesti hranu. Poljoprivredna proizvodnja se okreće obnovljivim izvorima energije i održivoj proizvodnji uz maksimalno iskorištavanje dostupnih resursa. Uzimajući u obzir sve to potpuno je neprihvatljivo proizvoditi velike količine otpada od hrane, dok se istovremeno bilježi stalan porast gladi. S obzirom da su potrošači odgovorni za veliki stupanj odbačene hrane, institucije uz pomoć znanosti orjentiraju se na istraživanja koja mogu ponuditi moguća rješenja u preventivnom djelovanju ili barem djelomičnom zaustavljanju negativnog trenda u bacanju hrane.

Ključne riječi: otpad od hrane, gubici, mogućnosti smanjivanja, planiranje

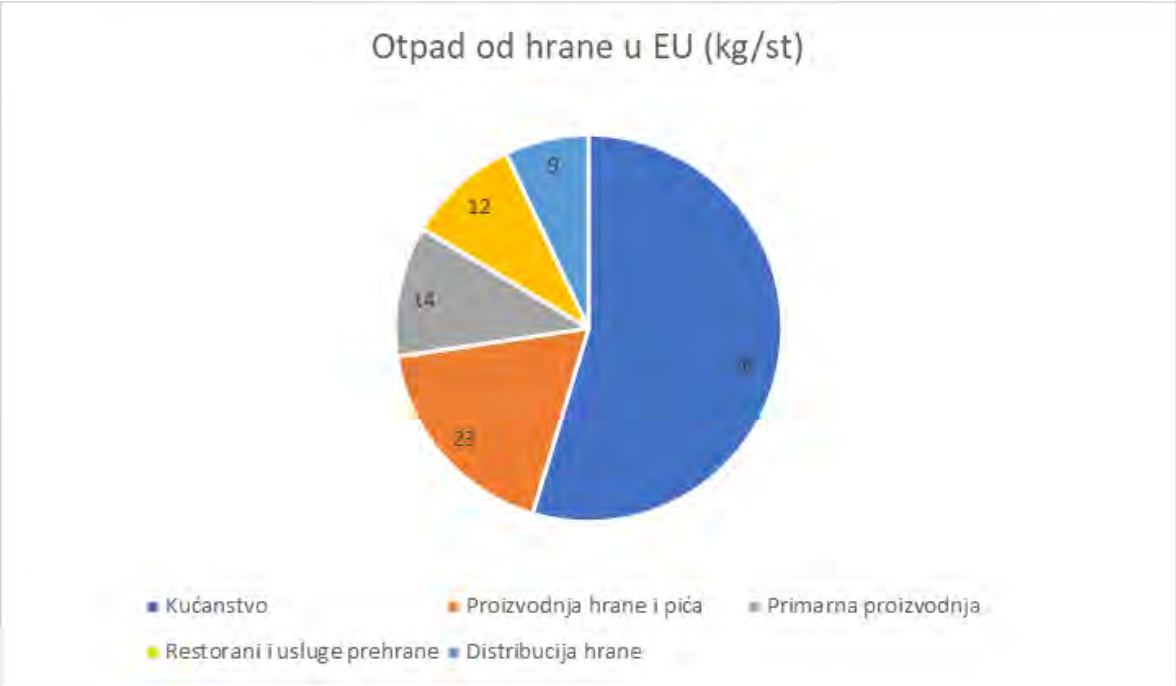
Uvod

Diljem svijeta posljednjih godina velika pozornost pridaje se otpadu nastalom od hrane. Sve veće količine generiranog otpada od hrane važan su pokazatelj neodrživosti sustava, ne samo kod potrošača nego i u sektoru poljoprivredne proizvodnje. Korištenje ograničenih proizvodnih resursa u vidu obradivih površina, fosilnih goriva i mineralnih gnojiva svakako bivaju uzalud utrošeni ukoliko se koriste za proizvodnju hrane koja se u konačnici ne konzumira (Kymäläinen i sur., 2021). Osim iskorištavanja neobnovljivih resursa, proizvodno-prehrambeni sustavi značajno doprinose povećanoj emisiji staklečničkih plinova, gubitku bioraznolikosti i iscrpljivanju vodnih zaliha što je cijelom svijetu u cilju izbjeći (Garnet, 2013).

Bacanje hrane događa se u svim fazama, od proizvodnje i prerade preko distribucije do prodaje i konzumacije. Trećina proizvedene hrane u jednoj godini bude bačena, uglavnom zbog prekomjerne kupovine u kućanstvima ili slabog razumijevanja značenja roka trajanja, ali i neprivlačnog izgleda voća i povrća. Najveće količine otpada od hrane stvaraju se u kućanstvima odnosno kod krajnjih konzumenata (Parfitt i sur., 2010). Prema podacima FAO-a, svake se godine u svijetu baci gotovo 1,3 milijarde tona proizvedene hrane (Gustafsson i sur., 2011; Reset, 2020). Prema podacima Eurostata u Europskoj uniji (EU) 2020. godine bacilo se približno 60 milijuna tona hrane, odnosno 127 kilograma po stanovniku, od čega više od 50% dolazi iz kućanstava. Procjenjuje se kako time EU svake godine gubi otprilike 130 milijarde eura. Istovremeno, smatra se kako je gotovo 821 milijun ljudi kronično pothranjen dok su s druge strane siromaštvo i glad u stalnom porastu (UN, 2021).

U Hrvatskoj postoje samo procjene koliko se hrane baci, i one se kreću između 380.000 i 400.000 tona godišnje. Svaki stanovnik u 2019-oj godini u prosjeku je odbacio 71 kilogram hrane, a 76% bačene hrane bilo je iz kućanstava (Ilakovac i sur., 2020).

Iz Grafikona 1 vidljivo je kako rješavanje problema s otpadom od hrane iz kućanstava predstavlja veliki izazov ne samo za Hrvatsku već i za ostale zemlje EU. Dobivene količine otpada od hrane u kućanstvima gotovo dvostruko su veće od količine otpada od hrane koji proizlazi iz sektora primarne proizvodnje i proizvodnje prehrambenih proizvoda. Također, kada je riječ o sektoru restorana i distribucije hrane, treba uzeti u obzir dobivene rezultate s obzirom da je riječ o godini u kojoj je vladao učinak blokade zbog COVID-19 krize koja je zasigurno smanjila posjete ugostiteljskih objekata.



Grafikon 1. Količine proizvedenog otpada od hrane po stanovniku u Europskoj uniji 2020. godine u kilogramima (Izvor: Eurostat, 2022).

Iz svega prikazanog proizlazi kako je neophodno pronaći mjere kojima bi se potaknulo podizanje svjesti kod ljudi kako bi dobiveni brojevi u budućnosti bili što manji. Kućanstva su odgovorna za veliki udio ukupnog otpada od hrane i važna su žarišna točka po pitanju rješavanja odlaganja otpada od hrane (Nguyen i sur., 2022).

Sve je više literaturnih istraživanja o razlozima stvaranja otpada od hrane u kućanstvu (Boulet i sur., 2021.; Roodhuyzen i sur., 2017.; Schanes i sur., 2018.) koja pokušavaju utvrditi zašto u kućanstvu stvaramo tolike količine odbačene hrane. Ispitivanjem stručnjaci pokušavaju ponuditi rješenja koja bi utjecala na promjenu ponašanja pojedinaca što bi rezultiralo minimaliziranjem odbačene hrane koja najčešće završi u mješanom komunalnom otpadu (Aschemann-Witzel i sur., 2019; Borg i sur., 2022).

Općenito, kućanstvima je dostupno tek nekoliko opcija zbrinjavanja otpada od hrane. Međutim, glavno rješenje nije utvrditi poboljašanja u vidu odlaganja otpada od hrane već znatno smanjiti proizvedeni otpad ili ga svesti na najmanju moguću razinu (Ladele i sur., 2021). Takvim mjerama mogu se pružiti praktična znanja o čimbenicima koji mogu promicati održive prakse nastajanja otpada od hrane u kućanstvima, i u konačnici pridonijeti smanjenim količine odbačene hrane na odlagalište.

Cilj ovog rada je prikazati neke od mogućnosti smanjenja nastajanja otpada od hrane kao i percepciju učinkovitost različitih rješenja za problem prekomjernog bacanja hrane.

Uzroci nastajanja otpada od hrane

Otpad se može različito kategorizirati ovisno o tome u kojoj fazi nastaje, pa se tako razlikuje „gubitak" hrane koji se događa prije nego što hrana stigne do potrošača i „otpad od hrane" u koji se ubraja hrana koja je zdravstevno ispravna za konzumaciju, ali se svejedno odbacuje (FAO, 2013). Gubitak hrane jest smanjenje njezine kvalitete, što ju čini neprikladnom za ljudsku konzumaciju. Gubici hrane prisutni su u segmentu proizvodnje i distribucije u opskrbnom lancu hrane, a u najvećoj mjeri radi se o svježim proizvodima. Svake godine baci gotovo polovica svježeg voća i povrća u svijetu. Razmjeri problema su također znatno veći u razvijenom svijetu, gdje potrošači oko 10 puta više bacaju hranu u odnosu na siromašnije regije (Gustafsson i sur., 2011). S druge strane, pojam otpada od hrane odnosi se na hranu koja je trentuno ili je u određenom trenutku bila prikladna za ljudsku upotrebu, ali zbog ljudkog faktora ili isteka roka trajanja bude odbačena. Do stvaranja otpada od hrane dolazi u završnim fazama opskrbnog lanca hrane, najčešće u fazama krajnje potrošnje (Europska komisija, 2020; Makhal i sur., 2020).

Kada je riječ o proizvodnji, uzorci gubitaka mogu biti vezani uz ne pravovremeno odrađenu žetvu ili neodgovarajuće rukovanje proizvodima nakon žetve. Tako se bilježe gubici i prilikom neadekvatnog skladištenja pojedinih sirovina zbog neodgovarajuće skladišne infrastrukture koja može značajno skratiti rok trajanja hrane (Raak i sur., 2017).

Slijede prerada/proizvodnja i pakiranje, gdje su gubici česti zbog skladišnih prostora u kojima nedostaje protok zraka što dovodi do kvarenja namirnice. S druge strane, problem mogu biti i neadekvatna ambalažna pakiranja unutar koji se počinju stvarati mikroorganizmi čime se ubrzava proces kvarenja. U fazi distribucije i dolaska proizvoda na police trgovina, hrana se odbacuje zbog visokih zahtjeva i potreba potrošača da prehrambeni proizvodi prije svega zadovoljavaju estetske standarde u vidu boje, oblika i veličine (Makhal i sur., 2020).

U fazi potrošnje, rasipanje je često uzrokovano lošom kupnjom i ne planiranjem obroka što rezultira prekomjernom kupnjom (kupnja velikih pakiranja zbog niže cijene). Najčešće odbacivanje hrane događa se zbog pogrešnog shvaćanja oznake datuma (datumi „upotrijebiti do" i „najbolje upotrijebiti do") i lošeg skladištenja proizvoda u domaćinstvu (Canali i sur., 2017; Nguyen i sur., 2022).

Mogućnosti sprječavanja nastajanja otpada od hrane

S obzirom na velike količine odbačene hrane u kućanstvima, Europska komisija predložila je nekoliko koraka koji bi mogli pridonijeti u smanjivanju bačene hrane. Prva od mjera uključuje planiranje obroka i odlazak u kupovinu u skladu s tim. Ukoliko dođe do stvaranja zaliha ideja je povezati se s potencijalnim donatora hrane preko organizacija za pomoć gladnima poput banaka hrane. Još jedna mjera koja uključuje „pametnu kupovinu" jest provjera zaliha iz kućanstva, odnosno rotacija onih koje su blizu isteka roka trajanja.

Utvrđivanje razlike između „upotrijebiti do" i „najbolje upotrijebiti do". „Upotrijebiti do" je odrednica roka trajanja koji se odnosi na sigurnost hrane s mikrobiološkog gledišta. Korištenje takvog roka trajanja namijeneno je za lako kvarljive namirnice koje mogu biti brzo pokvarljiva s čime predstavljaju neposrednu opasnost za ljudsko zdravlje. Označavanje namirnica izrazom "najbolje upotrijebiti do" označava datum do kojeg hrana zadržava svoja najbolja svojstva kod pravilnog čuvanja. Što podrazumijeva kako je namirnica, bez obzira na istek roka, i dalje zdravstveno ispravna za konzumaciju, ali smanjenog okusa ili teksture (Stenmarck i sur., 2016).

Recikliranje otpada od hrane za hranjenje životinja ili stvaranje komposta, bioenergije i prirodnih gnojiva još su neke od mogućnosti kako bi se izbjeglo bacanje ili odlaganje proizvedenog otpada od hrane čime se može značajno smanjiti teret za okoliš (Garnett, 2013). Procjenjuje se da bi se smanjenjem bacanja hrane za 15% moglo prehraniti više od 25 milijuna Amerikanaca svake godine, a ne treba ni zanemariti značajne ušteta za kućni budžet (Stenmarck i sur., 2016).

Zaključak

Pravilno rukovanje otpadom od hrane u središtu je interesa javnosti, političkih programa i brojnih strategija. Rezultati takvog globalnog stanja jesu edukacije i buđenje svjesti kod ljudi kako bi otpada u kućanstvima bilo sve manje. Planski odlazak u nabavku hrane, kupovina namirnica u rinfuzi kao i kontrola zaliha stvorenih u kućanstvu samo su neki od preventivnih mjera u smanjenju odbačene hrane. S druge strane, do problema odbacivanja hrane u kućanstvu dolazi zbog ne poznavanja terminologije „upotrijebiti do" i „najbolje upotrijebiti do" gdje prvi termin označava lako kvarljive namirnice, a drugi informira potrošača kako su mogćue promjene u okusu ali one su svejedno zdravstveno ispravne. Svakako se potrebno orjentirati na edukaciju mlađe populacije koja bi mogla biti ključna u stvaranju generacije „Z„, koja inovativnim, malim izumima može odgovoriti na izazov globalnog bacanja hrane kao i o ekološkoj i održivoj budućnosti.

Napomena

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Causes and ways to avoid food waste

Abstract

Increasing amounts of food waste sent to landfills are a major threat to sustainable development, food market stability, population growth, and human well-being. Discarded food has far-reaching consequences, both at the national and global levels and at the environmental level. There is less and less arable land, fossil fuel supplies are decreasing and it is becoming increasingly difficult to produce food. Agricultural production is turning to renewable energy sources and sustainable production with maximum use of available resources. Given all of this, it is completely unacceptable to produce large amounts of food waste at a time when hunger is steadily increasing. Since consumers are responsible for a large part of the food thrown away, institutions, with the help of science, are oriented towards research that can offer possible solutions to prevent or at least partially stop the negative trend in food waste.

Keywords: food waste, losses, ways to reduce, planning

ORIGINAL SCIENTIFIC PAPER

Primjena modela umjetnih neuronskih mreža u predviđanju otpada od hrane na području Europske unije

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Sažetak

Porastom stanovništva dolazi do stvaranja sve većih količina otpada od hrane. Za planiranje smanjenja i stvaranja mehanizama rješavanja otpada od hrane potrebno je različitim modelima predvidjeti količinu otpada od hrane. Na stvaranje otpada od hrane utječu brojni socioekonomski i društveni čimbenici. Kao alat za nelinearno modeliranje i predviđanje nude se modeli u obliku umjetnih neuronskih mreža. U ovom istraživanju kreiran je model umjetnih neuronskih mreža za predviđanje ukupne količine otpada od hrane na području Europske unije. Provedenom analizom pogodnosti (R^2=0,99), utvrđena je mogućnost razvijenog za predviđanje ukupne količine otpada od hrane.

Ključne riječi: otpad od hrane, Europska unija, predviđanje, modeliranje, umjetne neuronske mreže.

Uvod

Proizvodnja hrane jedan je od ključnih čimbenika vezanih onečišćenje okoliša na području Europe. Primarna proizvodnja hrane zahtjeva korištenje resursa kao što su gorivo, zemljište i voda (Scherhaufer i sur., 2018.). Najveći dio otpada od hrane nastaje u kućanstvima što predstavlja središnju točku problematike vezanu uz zbrinjavanje otpada od hrane, te kao važan pokazatelj održivosti predstavlja zbroj resursa koji se koriste pri proizvodnji hrane (Conrad i sur., 2018., Thi i sur., 2022). Povećanjem stanovništva neizbježna je pojava nastanka otpada od hrane, te je potrebno staviti naglasak na mehanizme rješavanja i ublažavanja nastanka istog (Jamaludin i sur., 2022.). Otpad od hrane smatra se društvenim problemom s dalekosežnim socioekonomskim i ekološkim posljedicama. Pham i sur. (2015.) navode da otpad od hrane predstavlja značajan dio krutog komunalnog otpada, te kao neiskorišteni resurs ima veliki potencijal za proizvodnju energije. Rješavanje problema otpada od hrane zahtjeva kombinaciju tehnoloških rješenja i izravne edukacije kako bi se promijenila ponašanja potrošača (Kibler i sur., 2018.). Iako je otpad od hrane nemoguće u potpunosti ukloniti, postoje mehanizmi kojima bi se omogućila pretvorba otpada od hrane u korisnu energiju. Modelima za predviđanje količine otpada od hrane olakšava se planiranje i upravljanje navedenim problemom. U posljednje vrijeme kao modeli za predviđanje sve se više koriste umjetne neuronske mreže (UNM) (Obafemi i sur., 2019.). Osnovne karakteristike UNM modela podrazumijevaju strukturu mreže, algoritam učenja i primjena prijenosne (aktivacijske) funkcije. Prednost UNM je mogućnost prilagodbe i generalizacija s obzirom na karakterističnе ulazne vrijednosti. U primjeni, kao modeli za predviđanje, najpogodnije su UNM višeslojnog perceptrona (Kartal i Özveren, 2020.; Pattanayak i sur., 2021.). UNM se sastoje od tri osnovna dijela: ulaznog, skrivenog i izlaznog sloja, te pripada kategoriji modela za „duboko učenje" (Rajković i sur., 2022.). Faezirad i sur. (2021.) proveli su istraživanje u kojem su kreirali model UNM za predviđanje količine otpada od hrane koji nastaje u studentskim restoranima, u svrhu planiranja smanjenje ukupnog otpada. Kreiranim modelom i daljnjom analizom utvrđeno je da bi se količina otpada od hrane mogla smanjiti za 79 % na promatranom području. Cilj ovog rada je kreirati model UNM za predviđanje ukupne količine otpada od hrane na području Europske unije s obzirom na specifične ulazne varijable koji obuhvaćaju socio-ekonomske i društvene čimbenike.

Materijal i metode

Podaci za izradu modela UNM prikupljeni u bazama podataka „The World Bank (za *2020. godinu*)", te su podijeljeni u smislene kategorijske varijable. Statistički obrađeni podaci prikazani su u obliku srednje vrijednosti sa standardnom devijacijom. Analizom varijance (ANOVA) i Tukey HSD post hoc testom utvrđene su varijabilnosti promatranih uzoraka (Brandić i sur., 2022.). Podaci korišteni za izradu modela UNM nasumično su podijeljeni na dio za učenje (70%), testiranje (15%) i validaciju (15 %) modela. Model je kreiran sa 100 000 ciklusa učenja s nasumičnim brojem skrivenih neurona u skrivenom sloju (1-10). UNM kreirana za predviđanje UOG (ukupna količina otpada od hrane) temelji se na ulaznih varijablama OH (otpad od hrane po stanovniku godišnje), populacije (POP), BDP (bruto domaći proizvod) i ZPO (postotak stanovništva sa završenim primarnim obrazovanjem).

Pogreška razvijenog modela izračunavat će se pomoću idućih statističkih parametara: Korijen iz prosječnog kvadrata pogreške (RMSE), prosječna pogreška pristranosti (MBE) i prosječna postotna pogreška (MPE):

$$RMSE = \left[\frac{1}{N} \cdot \sum_{i=1}^{N} (x_{pre,i} - x_{\exp,i})^2 \right]^{1/2}$$

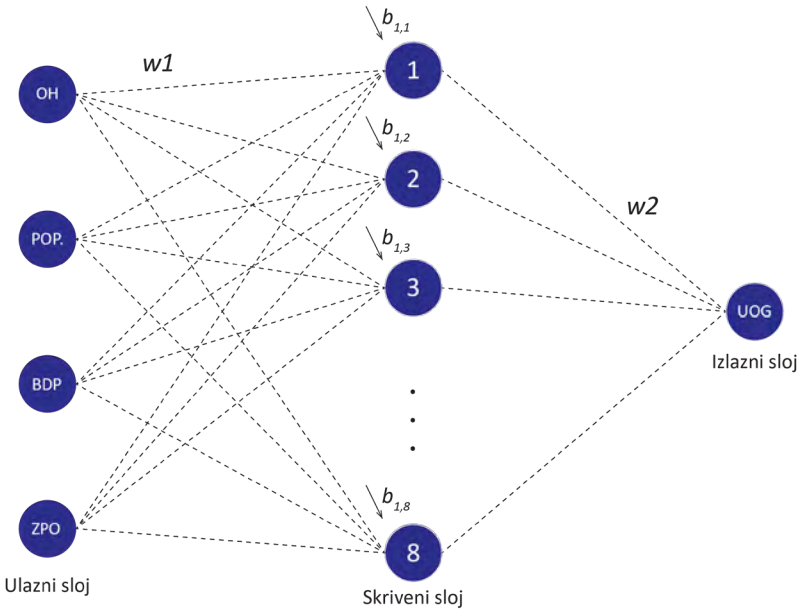
$$MBE = \frac{1}{N} \cdot \sum_{i=1}^{N} (x_{pre,i} - x_{\exp,i})$$

$$MPE = \frac{100}{N} \cdot \sum_{i=1}^{N} \left(\frac{\left| x_{pre,i} - x_{\exp,i} \right|}{x_{\exp,i}} \right)$$

Izlazne vrijednosti modela UNM računa se pomoću jednadžbe (Pezo i sur., 2013.):

$$Y = f_1(W_2 \cdot f_2(W_1 \cdot X + B_1) + B_2)$$

Y - izlazna vrijednost, X - ulazna vrijednosti, W1 - težinski koeficijent između ulaznog i skrivenog sloja, B1, B2 – vektori pristranosti skrivenog sloja, W2 – težinski koeficijent između skrivenog i izlaznog sloja.



Slika 1. Struktura razvijenog modela umjetne neuronske mreže (4-8-1)

Na slici 1. prikazana je struktura razvijenog modela UNM sa 4 umjetna neurona u ulaznom sloju, 8 umjetnih neurona u skrivenom sloju i 1 skrivenim neuronom u izlaznom sloju. Razvijeni model pokazao je najbolje performanse u predviđanju UOG

Rezultati i rasprava

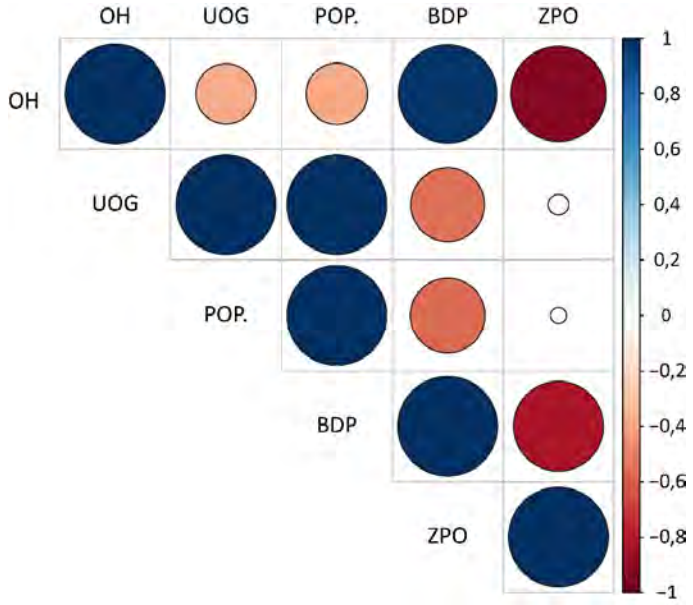
U tablici 1. prikazana je provedena statistička analiza promatranih varijabli korištenih za kreiranje modela UNM. Podaci su podijeljeni u 4 kategorije s obzirom na broj stanovnika na određenom području Europske unije; ≤ 1 mil. stanovnika, 1 - 10 mil. stanovnika, 10 - 40 mil. stanovnika, ≥ 40 mil. stanovnika.

Tablica 1. Statistička analiza promatranih varijabli

Kategorija	OH (kg)	UOG (tis. t/god.)	POP. (mil. stan.)	BDP (tis.d.stan./god.)	ZPO (%)
≤ 1 mil. stanovnika	109,5±27,58^{a}	55,97±1,19^{a}	0,58±0,09^{a}	84,47±72,43^{a}	81,55±5,16^{a}
1 - 10 mil. stanovnika	70,38±18,63^{a}	323,63±223,29^{ab}	4,67±2,79^{ab}	36,5±25,2^{a}	91,42±13,38^{a}
10 - 40 mil. stanovnika	75,38±29,92^{a}	1101,14±513,98^{b}	16,01±9,46^{b}	34,21±19,09^{a}	90,1±7,65^{a}
≥ 40 mil. stanovnika	76±7,39^{a}	4864,97±1238,57^{c}	64,26±15,06^{c}	30,00±19,7^{a}	86,23±7,01^{a}
Minimum	70,38	55,97	0,58	30,00	81,55
Maksimum	109,50	4864,97	64,26	84,47	91,42
Prosjek	82,81	1586,43	21,38	46,29	87,32

OH – Otpad od hrane po stanovniku godišnje; UOG – Ukupna količina otpada od hrane; POP. – Populacija; BDP – Bruto domaći proizvod u dolarima; ZPO – Završeno primarno obrazovanje. Srednje vrijednosti u istom stupcu, s različitim eksponentom su statistički različite (p ≤ 0,05), prema Tukeyjevom HSD testu.

Na području ≤ 1 mil. stanovnika utvrđena je najveća prosječna vrijednost OH (109,5 kg), BDP (84,47 tis. d.stan./god.) i najniža prosječna vrijednost UOG (55,97 tis.t/god.) i ZPO (81,55 %). Na području ≥ 40 mil. stanovnika utvrđena je najviša prosječna vrijednost UOG (4864,97 tis.t/god.) i BDP (30,00 tis.d.stan./god.). U kategoriji 1 - 10 mil. stanovnika utvrđena je najviša prosječna vrijednost ZPO (91,42 %).



Slika 2. Dijagram korelacije promatranih varijabli

Na slici 2. prikazan je dijagram korelacije promatranih varijabli. Pozitivne vrijednosti korelacije promatranih varijabli prikazani su plavom bojom, dok su negativne prikazane crvenom bojom. Intezitet obojenja proporcionalan je koeficijentu korelacije (Brandić i sur., 2022). UOG u pozitivnoj je korelaciji sa varijablom POP, dok je sa varijablama OH, POP i BDP i ZPO u negativnoj korelaciji.

Tablica 2. Statistička analiza pogodnosti razvijenog modela UNM

RMSE	MBE	MPE	R^2	Kurtosis
26604,62	549,23	1,80	0,99	17,30

RMSE – korijen prosječnog kvadrata pogreške; MBE – Prosječna pogreška pristranosti; MPE – Prosječna postotna pogreška; R2 – Koeficijent determinacije.

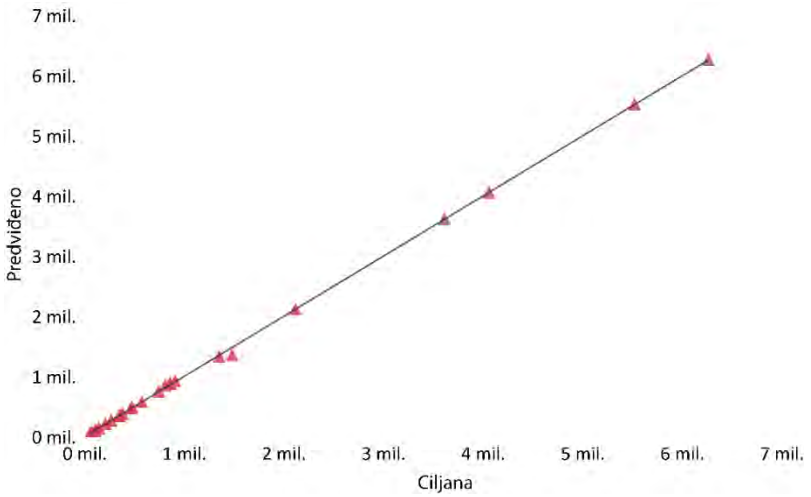
U tablici 2. prikazana je statistička analiza pogodnosti (*eng. „Goodness of fit"*) razvijene UNM u mogućnosti modela za predviđanju *UOG*. Parametrima RMSE (26604,62), MBE (549,23), MPE (1,80) i R^2 (0,99) utvrđena je sposobnost razvijenog modela u predviđanju izlazne vrijednosti *UOG* s malom razinom pogreške.

Tablica 3. Performanse razvijenog UNM modela

UNM	PU	PT	PV
MLP 4-8-1	0,99	0,99	0,99

UNM – Umjetna neuronska mreža; PU – Performanse učenja modela; PT – Performanse testiranja modela; PV – Performanse validacije modela

Tablica 3. prikazuje performanse učenja (PU), testiranja (PT) i validacije (PV) modela UNM koji su izraženi koeficijentom korelacije (0,99). Razvijeni model strukture 4-8-1 pogodan je za modeliranje *UOG* s obzirom na podatke ulaznih varijabli OH, POP, BDP i ZPO.



Grafikon 1. Odnos predviđenog i ciljanog UOG

Dijagram raspršenosti kao tehnika vizualizacije prikazuje odnos podataka na x i y osi (Keim i sur., 2010.). Na grafikonu 1. prikazan je dijagram raspršenosti predviđenog i ciljanog UOG. S obzirom na preklapanje podataka previđenih i ciljanih vrijednosti, dokazano je da je model *UNM* pogodan za predviđanje *UOG*.

Zaključak

Povećanjem stanovništva neizbježna je pojava nastanka sve većih količina otpada od hrane. Kao važan pokazatelj održivosti, otpad od hrane predstavlja zbroj resursa koji se direktno koriste u proizvodnji hrane. Kako bi se smanjila količina nastanka istog, potrebno je predvidjeti ukupnu količinu otpada od hrane čime se omogućuje prilagodba postojećih ali i stvaranje novih mehanizama za kontrolu i redukciju. Regresijskim modelima umjetnih neuronskih mreža moguće je predvidjeti ukupnu količinu otpada od hrane na području Europske unije s obzirom na karakteristične ulazne varijable koji obuhvaćaju socioekonomske i društvene čimbenike. U istraživanju predstavljen

je model umjetne neuronske mreže za predviđanje ukupne godišnje količine otpada, te je utvrđena mogućnost predviđanja s malom razinom pogreške (R^2=0,99).

Napomena

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Application of artificial neural network models to predict food waste in the European Union

Abstract

As the population grows, more and more food waste is generated. In order to plan the reduction and establish mechanisms for dealing with food waste, it is necessary to predict the amount of food waste using various models. The generation of food waste is influenced by numerous socio-economic and social factors. Models in the form of artificial neural networks are offered as tools for non-linear modelling and prediction. In this study, an artificial neural network model was built to predict the total amount of food waste in the European Union. The „Goodness of fit" analysis (R^2=0.99) showed that the developed tool is suitable for predicting the total amount of food waste.

Keywords: food waste, European union, prediction, modelling, artificial neural networks

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Energetska svojstva biomase ječma u ovisnosti o količini oborina

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Sažetak

U nadolazećim godinama proizvodnja energije zahtijevat će sve veće količine biomase te će šumski ostaci, poljoprivredna biomasa i namjenski uzgojeni usjevi biomase igrati ključnu ulogu u procesu proizvodnje goriva. U spomenutu poljoprivrednu biomasu ubraja se i biomasa ječma koji je u Hrvatskoj zastupljena u većim količinama. Stoga je cilj rada prikazati utjecaj količine oborina prilikom uzgoja na energetska svojstva biomase ječma. Istraživanje je pokazalo kako sušna godina utječe na smanjenje nepoželjne komponente pepela, dok na energetska svojstva biomase ječma nema značajan utjecaj.

Ključne riječi: proksimativna analiza, ultimativna analiza, slama ječma, sušna godina

Uvod

Prema najnovijim projekcijama američke Uprave za energetske informacije (EIA), korištenje nafte i drugih tekućih goriva porast će za 38% između 2014. i 2040., a transportni i industrijski sektor trošit će 92% svjetske potrošnje tekućeg plina u 2040. (Sieminski, 2013.; Steve, 2019; US EIA, 2019.). Ova rastuća potražnja povećava interes za proizvodnju goriva iz biomase kao zamjene za fosilna goriva.

Također, s obzirom na kontinuirano širenje svjetske populacije i posljedicu klimatskih promjena, sve je važnije razviti i primijeniti metode održive proizvodnje biomase kako bi se omogućila uspostava rastućeg i održivog biogospodarstva. Zelene tehnologije, uključujući biogoriva i bioproizvode, su među najučinkovitijim strategijama za smanjenje emisija stakleničkih plinova i globalnog zatopljenja, a istovremeno zadovoljavaju energetske potrebe čovječanstva (Antar i sur. 2021.). Biomasa trenutno daje određenu količinu energije mnogim zemljama, međutim prateće tehnologije nisu široko prihvaćene, uglavnom zbog niskih povrata za proizvođače biomase. Biomasa se ubraja u prirodni ne fosilni organski materijal koji sadrži intrinzičnu kemijsku energiju s potencijalom za kompenzaciju emisija fosilnih goriva, koja predstavlja dobru alternativu fosilnim gorivima (Rozzi i sur., 2020.). Alternativno kruto biogorivo, proizvedeno od poljoprivrednih kultura, dio je obnovljivih izvora energije sa zelenim tehnološkim pristupom (Mansora i sur., 2018.).

Pretvorbom lignoceluloznih materijala kao što su celuloza, hemiceluloza i lignin dobiva se druga generacija ili napredna biogoriva (Hayes, 2009). Lignocelulozna biomasa, iako ima nekoliko pozitivnih svojstava, povezana je s raznim nedostacima, kao što su npr. strukturna heterogenost, neujednačena fizikalna svojstva, niska gustoća energije, higroskopna priroda i niska nasipna gustoća (Medic i sur., 2010.). Sve te značajke stvaraju poteškoće u transportu, rukovanju, skladištenju i pretvorbi (Wannapeera i sur., 2011.). U lignocelulozni materijal pripada i slama ječma.

Pretvorba biomase može isporučiti različite vrste proizvoda putem različitih vrsta procesa pretvorbe uključujući toplinske, kemijske i biokemijske procese (Sengupta i Pike, 2012.). Na svaku preradu utječu vrste biomase i njezine fizikalne i kemijske karakteristike. Informacije o svojstvima biomase, uključujući ogrjevnu vrijednost, ultimativa/proksimativna svojstva i strukturne komponente, primijenjene su u nekoliko literatura za simulaciju opcija za procese pretvorbe biomase. Prema pregledu literature, proksimativna i ultimativna analiza proučavana je u pretvorbi biomase u pogledu energetske primjene kao što su izgaranje, proizvodnja energije i proizvodnja tekućeg goriva (Lan i sur., 2018.).

Proksimativna analiza provodi se kako bi se dobile informacije o sadržaju vlage, vezanog ugljika, hlapljivih tvari i pepela. Također, za elementarni sastav provodi se ultimativna analiza kako bi se dobio sadržaj ugljika, vodika, kisika, dušika i anorganskih vrsta (Nimmanterdwong i sur., 2021.). Međutim, varijacije sastava biomase vidljive su i kod istih kultura, ali u različitim literaturnim navodima, tako npr. pšenična slama u istraživanju Naik i sur. (2010.)

ima puno niži udio pepela od istraživanja provedenog od strane Raj i sur. (2015.). Navedena odstupanja u sastavu biomase moguća su zbog utjecaja vanjskih uvjeta kao što su sunčeva svjetlost, oborine, hranjive tvari u tlu, itd. (Nimmanterdwong i sur., 2021.).

Poboljšanje proizvodnje biomase ne ovisi samo o odabiru usjeva koji dobro funkcioniraju u području gdje će se proizvoditi i uz napredne poljoprivredne prakse, već se mora temeljiti i na prevladavajućim uvjetima okoliša. Klimatske promjene trenutačno su nepredvidljive, a usjevi od interesa suočavat će se s povećanim razinama i učestalostima abiotičkih i biotičkih stresova (Antar i sur., 2021.). Poboljšanje proizvodnje biomase je složeno jer na njega mogu utjecati stresni uvjeti, kao što su suša/poplave i vrućina/hladnoća, čak i oni povezani s prekomjernim unosom gnojidbe (Lyu i sur., 2020.).

Ječam dobro koristi zimsku vodu, rano počinje i ranije završava vegetaciju, pa se u tome sastoji njegova nešto veća otpornost na sušu (Sallam i sur., 2019.). Temeljem svega navedenog cilj rada je prikazati utjecaj količine oborina na energetska svojstva biomase ječma.

Materijal i metode

Sjetva četiri sorata ječma (rex, barun, lord i panonac) provedena je početkom listopada dok je žetva/uzorkovanje obavljeno krajem lipnja 2021. i 2022. godine na pokusnom polju postavljenom na površini Poljoprivrednog instituta Osijek. Rezultati količine padalina na području grada Osijeka preuzeti su od Državnog hidrometeorološkog zavoda te su prikazani u tablici 1. Tijekom vegetacije 2021./2022. bilo je sušnih razdoblja (siječanj-travanj) prilikom formiranja mase ječma u odnosu na godinu prije.

Tablica 1. Količina oborina na području Osijeka tijekom vegetacije 2020./2021. i 2021./2022.

mjesec/godina	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
2020. (mm)	-	-	-	-	-	-	-	-	-	86,5	18,0	61,4
2021. (mm)	77,5	36,3	34,4	60,7	58,9	18,4	-	-	-	72,9	71,0	75,6
2022. (mm)	7,5	28,7	6,4	35,0	66,0	77,2	-	-	-	-	-	-

Nakon prirodnog sušenja uzorci biomase 4 sorata ječma su homogenizirani i usitnjeni u laboratorijskom mlinu (IKA Analysentechnik GmbH, Njemačka). Takva mješavina uzorak je analiziran najmanje tri puta kako bi se osigurala ponovljivost analiza.

Proksimativna analiza uključivala je određivanje sadržaja vlage (HRN EN 18134-2:2015), korištenjem laboratorijske sušare (INKO, Hrvatska), pepela (HRN EN ISO 18122:2015) korištenjem muflne peći (Nabertherm, SAD) te sadržaja fiksnog ugljika i hlapljive tvari (EN 15148:2009) koji su izračunati računskim izvođenjem.

Ultimativna analiza uključivala je određivanje ukupnog ugljika, vodika, dušika i sumpora koji su određeni istovremeno metodom suhog izgaranja pomoću analizatora Vario Macro CHNS (Elementar Analysensysteme GmbH, Njemačka), prema protokolima za ugljik, vodik i dušik (HRN EN ISO 16948:2015) i sumpora (HRN EN ISO 16994:2015) dok je udio kisika izračunat iz razlike.

Ogrjevne vrijednosti određene su metodom EN 14918:2010 pomoću adijabatskog kalorimetra (C200, IKA Analysentechnik GmbH, Heitersheim, Njemačka), dok je donja ogrjevna vrijednost izračunata računskom derivacijom. Ogrjevna vrijednost izražena je u MJ/kg.

Rezultati i rasprava

U tablici 2. prikazani su rezultati proksimativne analize biomase ječma koji uključuju udio vlage, pepela, fiksiranog ugljika (Cfix) i hlapive tvari.

Tablica 2. Proksimativna analiza biomase ječma

Godina	Vlaga (%)	Pepeo (%)	Cfix (%)	Hlapive tvari (%)
2021.	8,68	10,36	6,46	75,08
2022.	9,47	7,38	8,87	75,84

Sadržaj vlage je osnovni parametar koji određuje neto energetski sadržaj biomase te istodobno ima značajan utjecaj na ogrjevnu vrijednost i proces izgaranja (Obernberger i Thek, 2004). Osim otežanog izgaranja, veći sadržaj vlage uzrokuje i povećanu emisiju štetnih plinova tijekom procesa izgaranja. Manji udio vlage u 2021. godini moguće je povezati sa manjom količinom padalina u vrijeme žetve. Osim sadržaja vlage, sadržaj pepela također je važna karakteristika čvrste biomase i odnosi se na negorivi sadržaj biomase te je nepoželjan parametar u biomasi zbog katalitičkog utjecaja na toplinskugradnju (Parmar, 2017). Značajno manji sadržaj pepela uočen je u sušnijoj vegetacijskoj godini (2021./2022.) te je iznosio 7,38 %. Dobiveni udjeli vlage i pepela u skladu su s istraživanjima Sedmihradská i sur. (2020) i Sidiras i sur. (2020).

Fiksni ugljik je količina ugljika vezanog u biomasi i predstavlja maseni sadržaj ostataka nakon otpuštanja hlapljivih tvari, isključujući pepeo i vlagu. Veći udio fiksnog ugljika dovodi do veće kvalitete biomase zbog pozitivnog utjecaja na ogrjevnu vrijednost (McKendry, 2002). Također, kao i kod sadržaja pepela bolje karakteristike temeljem udjela fiksiranog ugljika primjećene su u sušnijoj vegetacijskoj godini (2021./2022.).

Hlapljiva tvar predstavlja plinovitu fazu koja nastaje toplinskom razgradnjom biomase te omogućava lakše zapaljenje biomase (Caillat i Vakkilainen, 2013). Veći sadržaj hlapljivih tvari smanjuje energetsku učinkovitost u slučaju izravnog izgaranja biomase (Magdziarzi sur., 2011). Vegetacijska godina nije imala utjecaj na sadržaj hlapivih tvari te se udio kretao oko 75% što je u skladu s istraživanjem provedenog od Mitchell i sur. (2020).

U tablici 3. prikazani su rezultati ultimativne analize biomase ječma koji uključuju udio dušika (N), ugljika (C), sumpora (S), vodika (H) i kisika (O).

Tablica 3. Ultimativna analiza biomase ječma

Godina	N (%)	C (%)	S (%)	H (%)	O (%)
2021.	0,58	45,56	0,04	5,87	47,95
2022.	0,62	45,70	0,04	5,85	47,79

Na temelju ultimativne analize kruta biogoriva sastoje se uglavnom od C, O i H. Veći sadržaj C i H povećava ogrjevnu vrijednost, dok ga veći sadržaj kisika smanjuje (Obernberger i Thek, 2004). Sumporni oksidi (SOx) nastaju tijekom izgaranja i značajno doprinose onečišćenju česticama i kiselim kišama (Parmar, 2017). Osim emisija SO2, sumpor sadržan u krutom biogorivu također ima značajnu ulogu u procesima korozije (Obernberger i Thek, 2004). Dušik vezan za gorivo uzrokuje većinu emisija NOx proizvedenih izgaranjem biomase (Parmar, 2017). Povećani sadržaj dušika u biomasi obično rezultira povećanjem emisija NOX tijekom procesa izgaranja (Hartmann, 2012). Na osnovu rezultata našig istraživanja vegetacijske godine sa različitom raspodjelom oborina nisu imale utjecaj na ultimativna svojstva biomase te su dobivene vrijednosti u skladu sa istraživanjima Mitchell i sur. (2020). i Sidiras i sur. (2020).

U tablici 4 prikazane su ogrijevne vrijednosti biomase ječma.

Tablica 4. Ogrjevna vrijednost biomase ječma

Godina	HHV (MJ/kg)	LHV (MJ/kg)
2021.	17,43	16,14
2022.	17,41	16,13

Korištenje biomase kao goriva u toplinskim i električnim primjenama zahtijeva poznavanje njezine ogrjevne vrijednosti (Caillat i Vakkilainen, 2013). Donja ogrjevna vrijednost (LHV) je odgovarajuća vrijednost za korištenje energije koja je dostupna za kasniju upotrebu (Obernberger i Thek, 2004). Sušno razdoblje nije imalo utjecaj na ogrijevnu vrijednost biomase ječma te su dobivene vrijednosti u skladu sa istraživanjima Satpathy i sur. (2014).

Zaključak

Istraživana biomasa ječma uzgojena u različitom vegetacijskom periodu i različitim agroklimatskim uvjetima sličnih je energetskih karakteristika te se obzirom na dobivene rezultate proksimativne i ultimativne analize te ogrijevne vrijednosti može se koristiti u proizvodnji krutog goriva.

Pozitivan utjecaj sušnog razdoblja tijekom vegetacije 2021./2022. vidljiv je u smanjenju udjela pepela kao nepoželjne komponente prilikom izravnog izgaranja.

Napomena

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Energy properties of barley biomass depending on agroclimatic conditions

Abstract

In the coming years, energy production will require increasing amounts of biomass, and forest residues, agricultural biomass and purpose-grown biomass crops will play a key role in the fuel production process. The aforementioned agricultural biomass also includes the biomass of barley, which is represented in larger quantities in Croatia. Therefore, the aim of the paper is to show the influence of the amount of precipitation during cultivation on the energetic properties of barley biomass. The research showed that a dry year affects the reduction of the undesirable ash component, while it has no significant effect on the energetic properties of barley biomass.

Keywords: proximate analysis, ultimate analysis, barley straw, dry year

ORIGINAL SCIENTIFIC PAPER

Influence of a dry year on the Virginia mallow energy properties

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Abstract

This paper is based on the energy characteristics of Virginia mallow (*Sida hermaphrodita* L. Rusby) with the correlation of harvest time and dry year. Virginia mallow attracts attention as an energy crop due to the additional benefits it brings with it, such as: possibility of conversion into different forms of energy, highly attractive for pollinators due to the long flowering season, improvement of soil quality, phytoremediation and according to some research there is a small possibility of invasiveness. Since the natural habitat of Virginia mallow is wet habitats, there is possible sensitivity to drought. Virginia mallow is interesting because as a crop it can be vital from 10 to even 20 years. In addition to its versatility, it also offers valuable energy properties as a permanent crop. Average higher heating value of Virginia mallow obtained by research was 18.06 MJ/kg. There is a valuable possibility that should be taken, in form of good possibility for energy utilization combined with preservation of the ecosystem. The aim of this paper is to determine the energy properties (proximate and ultimate analysis and calorific value) of Virginia mallow in a dry year.

Keywords: *Sida hermaphrodita*, energy crop, energy utilisation, biomass, dry year

Introduction

Europe as a union and a continent depends on renewable energy development. One of the main goals of EU is to become climate neutral continent by 2050, and to cut gas emissions by 55% by 2030 (EU targets). The United Nation climate change summit in Paris 2016 set a goal to limit global warming by 2 ^{0}C, preferably 1.5 ^{0}C compared to pre-industrial levels (UN climate change). For better understanding, because of combustion of fossil fuels that accumulate greenhouse gases in atmosphere the climate change occurs (Sathre, 2014). Therefore, there are two types of energy, fossil fuels that are non-renewable and includes petroleum, coal, gas etc. and green energy that includes wind power, solar power, and biomass (Midilli et al., 2006). Renewable energy is sustainable, safe, and cost effective it encourages energy independence and diversity while reducing carbon emission (Chum and Overend, 2001). Regarding biomass for renewable energy sources, some of the main resources are wood, energy crops, forestry waste and agricultural waste. All together they can be transformed to liquid, gaseous of solid fuel. Main processes for energy utilisation of biomass are pyrolysis, gasification, and combustion. Pyrolysis converts biomass to bio-oil, gasification is used to get fuel gas and combustion is for heat (Bridgwater, 2003). Another reason why using biomass for energy production is good is its storage potential and use on demand (Jablonowski et al., 2017).

Virginia mallow is a perennial crop from the *Malvaceae* family, and its binomial name is *Sida hermaphrodita* L. Rusby. This species originates from North America, from where it was introduced to Europe primarily to Poland and Russia (Cumplindo-Marin et al., 2020). As for the physical characteristics, Virginia mallow can grow from 1 to 3 m, the leaf has 3 to 7 toothed and elongated leaflets, especially the middle one is the most elongated, and they grow alternately on the stem. Virginia mallow has a characteristic white flower (of 5 petals) that blooms from August to October or until the first frost (Kasprzyk et al., 2013; COSEWIC, 2010). The young stem of Virginia mallow has hairs that fall off completely in the mature stage of the plant. As for growth, Virginia mallow spreads by rhizomes, and in the first year only one shoot emerges, while in the following years more and more emerge, thus Virginia forms a dense assembly of 20 to 30 shoots and can last up to 20 years as a crop (COSEWIC, 2010; Franzaring, 2015).

Effects of drought on Virginia mallow

Virginia mallow tolerates winter conditions and low temperatures as low as -35 ^{0}C, but shows sensitivity to drought, some research show that in dry years the yield decreases by 30 % (School et al., 2017; Nahm and Mohart, 2018; Borkowska et al., 2016). The needs of Virginia mallow for precipitation range from a minimum of 500-600 mm, but it tolerates a minimum of 400 mm of precipitation (Borkowska et al., 2009; Borkowska and Molas, 2012; Cumplindo-Marin et al., 2020). Although it shows sensitivity to drought conditions and is more sensitive to drought conditions than other non-wood energy crops, its water requirements are still almost half that of short rotation woody crops (Borkowska and Molas, 2012; Jankowski et al., 2016). In the research conducted by Siwek et al. (2019), it was shown that the biogas yield from Virginia mallow in a dry year is almost half as much as in a year with relatively normal conditions. According to Siwek et al. (2019) biogas yield in 2017 with normal conditions was 7.246 m^3ha^{-1}, and in 2018, which was characterized as a dry year, biogas yield was 3.232 m^3ha^{-1}.

Optimal harvest time for Virginia mallow

Harvesting Virginia mallow is characteristic in that the saplings dry out at the end of the growing season, and therefore the moisture level significantly depends on the time of harvest (Borkowska and Molas, 2013; Krička et al., 2017).

Another characteristic of Virginia mallow is that small amounts of nutrients are removed from the field during harvest, because as the mallows dry, the nutrients are located in the rhizome and are returned to the soil through it, or through the fallen leaves that Virginia mallow discards as it approaches the end of its growing season (Krička et al., 2017; Pszczółkowska et al., 2012).

Conventional harvesting methods are applied for harvesting. Compared to short rotation coppice, harvesting Virginia mallow is economically more profitable, requires simpler mechanization and good weather (Vanbeveren et al., 2015; Nahm & Mohart, 2018). Also compared to short rotation coppices, Virginia mallow is dry enough for direct storage or direct combustion (Jablonowski et al., 2017).

Harvest time for Virginia mallow is correlated with calorific value, specifically crude fibers (cellulose, hemicellulose and lignin) are correlated with calorific value. Viriginia mallow has the least crude fibers in the period when it has leaves and when it flowers (Siaudinis et al., 2015; Nahm and Mohart, 2018). The amount of cellulose fibers, hemicellulose and lignin in the period of flowering is 60.2 % of a dry matter, and at the time of autumn harvest from 80 to even 88.1 % according to some studies (Pokoj et al., 2015; Slepetys et al., 2012; Michalska et al., 2015).

According to research conducted by Bury et al. (2019) for biogas production, two harvests of Virginia mallow give a higher dry mass yield than just one annual (winter) harvest. According to research, harvests are carried out in early summer and early autumn (Bury et al., 2019).

Materials and methods

Virginia mallow was harvested in November 2022 at the experimental field of University of Zagreb Faculty of Agriculture. Table 1. gives a detailed information on precipitation on monthly basis for 2021 and 2022 year. During the growing season from April until November average precipitation was 107.7 mm with average temperature 16.51 ^{0}C, and it is perceived as a drought year harvest (H 22). The average year that would be compared to drought year is 2021, with harvest also in November (H 21).

Table 1. Precipitation in Zagreb – Maksimir for 2021 and 2022 year

month/year	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
2021 (mm)	70.2	29.0	36.1	68.7	124.0	13.2	74.5	62.7	34.6	86.2	89.2	83.8
2022 (mm)	24.3	38.5	8.7	82.0	52.5	63.0	57.7	20.03	259.1	21.8	-	-

After drying, the biomass samples in a laboratory dryer at 105 ^{0}C (MEMMERT universal dryer) 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 (Cfix) and volatile matter (EN 15148:2009) which were calculated by calculation.

The ultimate analysis determined the total carbon, hydrogen, nitrogen and sulfur which were determined simultaneously by the dry combustion method using the analyser Vario Macro CHNS (Elementar Analysensysteme GmbH, Germany), according to the protocols for carbon, hydrogen and nitrogen (HRN EN ISO 16948:2015) and sulfur (HRN EN ISO 16994:2015) while the oxygen content was calculated from the difference.

Calorific values were determined using the EN 14918:2010 method using an adiabatic calorimeter (C200, IKA Analysentechnik GmbH, Heitersheim, Germany), while the lower calorific value was calculated by computational derivation. The calorific value is expressed in MJ/kg.

Results and discussion

Using various methods, the results for proximate and ultimate analysis and heating value were obtained. Proximate analysis shows values for moisture content, ash, volatile matter, fixed carbon (Cfix), and coke. Ultimate analysis shows values for carbon (C), hydrogen (H), nitrogen (N), sulphur (S) and oxygen (O).

Table 2. Proximate analysis results and higher heating value (HHV)

Sample	MC, %	Ash, % db	VM, % db	Cfix, % db	Coke, % db	HHV, MJ/kg
H 22	50.81	2.68	84.12	9.18	9.63	17.23
H 21	47.62	4.69	82.68	7.01	11.71	18.89
Average	49.21	3.68	83.40	8.09	10,67	18.06

**MC – moisture content (measured at field), VM – volatile matter, C-fix – fixed carbon, % db - % on dry basis, HHV – higher heating value*

Results from proximate analysis in Table 2. shows some differences between a dry year of 2022 and 2021. Moisture content measured directly on the field was 50.81 % which is slightly higher compared to H 21 of 47.62 %. It is visible that a drought year has lower ash content. Volatile matter is slightly higher in drought year than in average year. Fixed carbon and coke also had a bit lower value compared to average year. Considering higher heating value at drought year it was 17.23 MJ/kg which is slightly lower than 18.89 MJ/kg in 2021.

Comparing average results of H 22 and H 21 with literature findings, average ash content is 3.68 % while in research by Banks et al. (2021) it was 2.11 %, and in Sliz and Wilk (2020) ash value was 1.97 %. Volatile matter on average was 83.40 % and in Banks et al. (2021) results show volatile matter of 79.74 %, while in research obtained by Sliz and Wilk (2020) volatile matter was 85.50 %. In research by Banks et al. (2021) Cfix was 18.15 %, and in research by Sliz and Wilk (2020) Cfix was 5.63%, compared to this average result of 8.09 %, that is much lower than Banks et al. (2021) and closer to Sliz and Wilk (2020). Heating value obtained by Sliz and Wilk (2020) was 17.79 MJ/kg which compared to average value of 18.06 MJ/kg is slightly lower.

Table 3. Ultimate analysis results

Sample	Carbon, % db	Hydrogen, % db	Nitrogen, % db	Sulphur, % db	Oxygen, % db
H 22	45.75	5.64	0.22	0.03	48.01
H 21	44.93	5.59	0.18	0.02	49.09

**% db - % on dry basis*

The results obtained in Table 3. by ultimate analysis shows that drought year did not influence ultimate properties. Compared to literature findings in Sliz and Wilk (2020) values for C was 44.9 %, H 6.02 %, O 46.78 % and N was 0.33 %. In the research from Banks et al. (2021), the values of ultimate analysis were C 47.02 %, H 5.54 %, N 0.47 %, S was not detected, and O was 46.97 %. Ultimate analysis of this research compared to other shows similar results.

Conclusion

The energy analysis shows good values of proximate and ultimate analysis with satisfactory heating value, so Virginia mallow could be and should be used much more as an energy utilisation crop.

The researched biomass of Virginia mallow with a harvest date in November, in comparison with the energy characteristics from harvest year before and the literature, shows satisfactory results of proximate, ultimate analysis and calorific value. The influence of the dry year is visible in the lower ash content but generally it did not influence energy characteristics in a great proportion.

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Utjecaj hladne plazme na razvoj poljoprivrednih biljaka

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Sažetak

Primjena plazma tehnologije u poljoprivredi provodi se tretiranjem sjemena ili biljaka hladnom plazmom (NTP) ili plazmom aktivirane vode (PAW). Različit reaktivni spojevi potencirani plazmom djeluju kao signalne molekule u metabolizmu biljaka, pokreću aktivnost gena koji reagiraju na stres u biljkama ili služe kao hranjive tvari. Cilj ovoga rada je prikazati učinak tretiranja hladnom plazmom na promjene koje dovode do poboljšanja rasta poljoprivrednih kultura. Rezultati istraživanja ukazuju na različiti odgovor poljoprivrednih kultura na tretman plazmom. Zbog toga je potrebno provoditi daljnja istraživanja kako bi se utvrdio optimalan utjecaj plazme na biljke.

Ključne riječi: NTP, PAW, plazma poljoprivreda

Uvod

Suvremena poljoprivredna proizvodnja nalazi se pred brojnim izazovima od kojih su klimatske promjene jedne od najvažnijih (Pandey i sur., 2017.). Zbog utjecaja klimatskih činitelja biljke su izložene abiotičkom stresu koji je izazvan zaslanjivanjem, sušom, temperaturnim ekstremima i sl. (Malhi i sur., 2020) (Shahzad i sur., 2021.). Zbog čega dolazi do promjena u prinosu usjeva koji su ovisni o klimatskim uvjetima (Malhi i sur., 2021.). Prevladavanje navedenih poteškoća moguće je postići usvajanjem klimatski pametnog poljoprivrednog sustava koji je u skladu s FAO-ovom vizijom ciljeva održive hrane i poljoprivrede. To uključuje razvoj otpornih sorata usjeva koje mogu izdržati stres uzrokovan naglim promjenama temperatura i padalina (Arora, 2019.) ili primjenu održivih metoda za ublažavanje stresa biljaka kao što je hladna ili netermalna plazma (NTP) (Susmita i sur., 2022.). Primjena plazme može biti odgovor na probleme moderne poljoprivrede, a u skladu je s promjenama europskih strategija. Europska strategija "Od farme do stola" poziva na smanjenje upotrebe kemijskih pesticida za 50%, smanjenje gubitaka hranjivih tvari za najmanje 50% i smanjenje upotrebe gnojiva za najmanje 20% do 2030. godine. Osim toga, plan je da se 25% poljoprivrednog zemljišta stavi pod organski uzgoj (Kocira i sur., 2022.). Tretiranje sjemena s hladnom plazmom (NTP) može predstavljati ekološki prihvatljiv pristup u pripremi sjemena da izraste u biljku koja je otpornija na stresove koji se javljaju tijekom klijanja i ranog rasta. Posljedično, to može dovesti do toga da biljka može proizvesti više biomase ili plodova, čak i u teškim uvjetima, što bi moglo biti vrlo korisno obzirom na klimatske promjene (Holubová i sur., 2020.). Ukratko, promjene u sjemenu izazvane plazmom mogu utjecati na klijavost, kasniji rast i razvoj biljaka, njihovu otpornost na abiotski stres i prinos (Starič i sur., 2020.). Prema Puač i sur. (2018.) plazma poljoprivreda potencijalno nudi povećanu proizvodnju s manjim utjecajem na ekosustav, suzbijanje biljnih bolesti i povećanje prinosa usjeva. Cilj ovoga rada je prikazati promjene koje dovode do poboljšanja rasta poljoprivrednih kultura pod utjecajem tretmana hladnom plazmom (NTP) ili plazmom aktivirane vode (PAW).

Hladna plazma (NTP) i plazmom aktivirana voda (PAW)

Plazma je četvrto agregatno stanje materije. To je mješavina elektrona, pozitivno nabijenih iona, radikala, atoma plina, molekula (u pobuđenom ili osnovnom stanju) i fotona iz niza energija uključujući ultraljubičasto (UV) i vakuumsko ultraljubičasto (VUV) zračenje (Starič i sur., 2020.). Pražnjenje hladne plazme stvara reaktivne nabijene čestice kao što su elektroni i neutralne čestice, te emitira ultraljubičasto zračenje i električna polja (Schnabel i sur., 2019.). Hladna plazma (NTP) sadrži, između ostalog, djelomično ionizirane plinove sastavljene od UV fotona i visoko reaktivnih spojeva dušika, kisika i vodika (RNS, ROS i RHS) (Hertwig i sur., 2018.). Hladna plazma (NTP)

može disocirati molekule zraka i izravno ih vezati u vodu zbog čega nastaju oksidirani i reducirani oblici dušika (Puač i sur., 2018.) u vodenoj otopini odnosno plazmom aktivirana voda (PAW). U plazmom aktiviranoj vodi se nalaze reaktivni spojevi kisika (ROS) i dušika (RNS), pa se mijenja pH osnovne vodene otopine kao i električna vodljivost te njezin oksidacijsko-redukcijski potencijal (Schnabel i sur., 2019.). Općenito, pražnjenjenjem zraka u vodu pod utjecajem plazme nastaju sljedeći ROS and RNS (RONS) spojevi: vodikov peroksid (H_2O_2), nitriti (NO_2^-), nitrati (NO_3^-) i ozon (O_3), te nestabilni spojevi, kao što je hidroksilni radikal (·OH), dušikov oksid (NO·), superoksid (O_2^-), peroksinitrat ($OONO_2^-$) i peroksinitrit ($OONO^-$). Zbog nastanka različitih aktivnih spojeva, plazmom aktivirane vode se mogu razlikovati prema: pH reakciji, električnoj vodljivosti i koncentraciji otopljenog kisika (Rashid i sur., 2021.).

Uloga plazme u rastu poljoprivrednih kultura

Antistersni učinak plazme

Reaktivni spojevi stvoreni plazmom pokreću aktivnost gena koji reagiraju na stres u biljkama (Susmita i sur., 2022.). Antistresni učinak aktivnih supstanci temelji se na tome da stresor može inducirati signalne putove koji pripremaju organizam za moguće djelovanje drugog stresora. Zbog toga je odgovor na drugi, jači stres obično brži i agresivniji nego kod organizama koji nisu bili pripremljeni (Holubová i sur., 2020.). Reaktivni spojevi kisika (ROS) i dušika (RNS), uključujući H2O2 i NO biomolekule smatraju se signalnim elementima u različitim procesima staničnog metabolizma i elementima koji reguliraju reakcije biljaka na različite stresove. Dušikov oksid (NO), jedan od reaktivnih spojeva dušika kojeg stvara plazma, regulira otpornost na abiotičke i biotičke stresove te klijanje i razvoj biljaka (Sami i sur., 2018.). Dokazano je da dušikov oksid (NO) može igrati ulogu u zaštiti biljaka od oksidativnog stresa, a tretman NO-donorima zaštićuje biljke od oštećenja povećanjem aktivnosti antioksidativnih enzima. Pozitivan učinak NO na klijavost sjemena utvrđen je i u uvjetima solnog stresa. Naime, dušikov oksid može učinkovito zaštititi presadnice od oštećenja izazvanog povećanom količinom soli. Ovaj efekt je posljedica pojačane aktivnosti antioksidativnih enzima koji suzbijaju prekomjeran broj reaktivnih spojeva kisika uzrokovanih slanim stresom (Nalousi i sur., 2012.). Rezultati istraživanja Kang i sur. (2019.) sugeriraju da NO i NO3- pridonose poboljšanju rasta biljaka i otpornosti na stres, a H2O2 i NO2- imaju veću ulogu u antimikrobnom učinku. Isto tako, vodikov peroksid (H2O2) može povećati otpornost biljke na stres, npr. prema suši i toplinskom stresu (Rodríguez-Ruiz i sur., 2019.). Primjenjeni ROS-a uključuje se u mnogo različitih puteva koji su vitalni za preživljavanje biljaka. Pokazalo se da ROS ublažava biotičke i abiotičke stresove, uključujući nizak N, nizak P, nedostatak vode i napad patogena pripremanjem biljaka da se odupru stresu (Ranieri i sur., 2021.). Dodatno, mogući antistres učinak izravnog tretmana plazmom i plazmom aktivirane vode (PAW) je veća enzimska aktivnost (SOD, POD, APX i CAT) u biljci. Veća aktivnost antioksidativnih enzima smanjuje štete koje mogu nastati zbog povećane razine superoksida (Rathore i sur., 2022.). Promjena sadržaja karotenoida, cisteina i glutamilcisteina pod utjecajem PAW poboljšava otpornost biljke na niske temperature i stres izazvan hipoksijom tijekom klijanja (Kučerová i sur., 2021).

Učinak plazme na hormonski status biljke

Biljni hormoni, kao što su auksini, citokinini, etilen, giberelini (GA), apscizinska kiselina (ABA) i brasinosteroidi su molekule koje kontroliraju različite fiziološke i biokemijske procese u biljkama. Njihova aktivnost se povezuje s klijanjem, rastom i razvojem, cvjetanjem, plodonošenjem, sazrijevanjem plodova, dormantnošću sjemena i otpornosti na razne abiotičke i biotičke stresove (Starič i sur., 2020.). Vrijeme klijanja uglavnom je kontrolirano smanjenjem razine abscizinske kiseline (ABA) i povećanjem giberelinske kiseline (GA), ali uključeni su i drugi hormoni, uključujući brasinosteroide, etilen i auksin. Abscizinska kiselina inhibira, a giberelinska potiče proizvodnju ROS u sjemenu koje klija. Pretpostavka je da mehanizmi za poticanje klijanja posredovanjem ROS-a mogu uključivati oksidaciju specifičnih regulatornih proteina i RNA. To uključuje oksidaciju tiola ili karbonilaciju proteina, obje povezane s poticanjem klijanja. Budući da brzina klijanja ovisi o snazi ograničenja rasta ovojnice sjemena i njegovoj nepropusnosti za vodu i kisik, ROS iz plazme može pospješiti klijanje slabljenjem omotača sjemena modifikacijama polisaharida stanične stijenke. Spojevi dušika (NO, NO_2^- i NO_3^-) iz plazme potiču klijanje sjemena, a njihovu aktivnost posreduje dušikov oksid (NO). Razina dušikovog oksida (NO) u plazmi modulira različite hormone koji kontroliraju mirovanje sjemena, poput induciranja smanjene razine ABA i povećanja razine GA u sjemenu. Ako je sjeme izloženo vodikovom peroksidu (H_2O_2) iz PAW-a, on može izazvati promjene u razini abscizinske kiseline (ABA) i giberelinske kiseline (GA) kao hormona koji potiču klijanje sjemena (Ranieri i sur., 2021.).

Potenciranje rasta biljaka

Hladna plazma (NTP) postaje sve popularnija u poljoprivredi (Starič i sur., 2020). Međutim, iako se smatra učinkovitom tehnologijom za primjenu u poljoprivredi, studije uglavnom izvještava o utjecaju plazme na klijanje sjemena (Štepánová i sur., 2018.), rast klijanaca (Thisawech i sur., 2020.), otpornost na stres i antibakterijsku sposobnost. Isto tako, istraživanja pokazuju da primjena plazmom aktivirane vode (PAW) povećava postotak klijavosti, mijenja aktivnost enzima, a tretirano sjeme daje bolje razvijene biljke (Than i sur., 2022., Puač i sur., 2018., Puač i sur., 2018., Sivachandiran i sur., 2017.). Pozitivni učinci plazmom aktivirane vode zabilježeni su kod različitih svojstava rasta presadnica uključujući duljinu stabljike i korijena, masu lista, površinu lista i sadržaj klorofila (Kučerová, i sur., 2019.), te vigor presadnica (Sivachandiran i sur., 2017.) kao posljedicu djelovanja reaktivnih spojeva kisika i dušika koje su izazivale morfološke promjene u sjemenkama i dovele do boljeg upijanja vode i hranjivih tvari (Than i sur., 2022). Naime, tretman PAWom uklanja hidrofobnu voštanu prevlaku s površine sjemena. Prisutnost voska na površini sjemena čini ga hidrofobnim zbog čega se prolongira vrijeme potrebno da se sjeme natopi vodom. Međutim, tretman PAW-om uklanja voštanu prevlaku i čini sjeme tretirano PAW-om hidrofilnim. Prisutni RONS u PAW-u kao što su H_2O_2, otopljeni O_3, ioni NO_3^- i ioni NO_2^- oksidiraju voštanu prevlaku i time pomažu ukloniti vosak s površine sjemena (Sajib i sur., 2020.). Plazmom aktivirana voda se može učinkovito koristiti u raznim poljoprivrednim primjenama, budući da stabilni tekući RONS (H_2O_2, NO_2^-, NO_3^-) mogu djelovati kao signalne molekule u metabolizmu biljaka ili služiti kao hranjive tvari. PAW je mješavina stbilnih reaktivnih vrsta kisika i dušika (RONS), kao što su H_2O_2, NO_2^- ili NO_3^- i smatra se čistom i održivom alternativom kemijskim gnojivima. Nitrati i peroksid (NO_3^- i H_2O_2) su bez sumnje najvažniji RONS u PAW-u koji utječu na razvoj i kvalitetu biljaka (Kučerová i sur., 2021). Plazmom aktivirana voda sadrži znatno veće koncentracije RNS (NO2–, NO3⁻) u odnosu na ROS (H_2O_2, O_3). Te visoke koncentracije RNS-a vjerojatno se mogu prenijeti s PAW-a na biljke. Iz navedenih činjenica može se zaključiti da ove veće koncentracije RNS-a mogu pridonijeti pojačanom rastu biljaka. Naime, radikali povezani s dušikom (NO_2^-, NO_3^-) se apsorbiraju kao aminokiseline u korijenu i lišću biljaka kroz enzimske procese. Stoga NO_3^- igra ključnu ulogu u biokemijskim i fiziološkim procesima putem regulacije signala za metaboličku aktivnost i razvoj biljaka (Rashid i sur., 2021.). Prisutnost nitrata u PAW-u može pospješiti rast biljaka što također može djelovati kao alternativa kemijskim gnojivima. Međutim, mehanizam ovih učinaka tek treba razjasniti (Sajib i sur., 2020.). Istraživanjem utjecaja plazmom aktivirane vode uočeno je da sjeme tretirano s PAW-om pokazuje povećanje sadržaja topivog šećera (TSS) i topivih proteina (TSP). Viša koncentracija šećera znači da je dostupno više energije koja može podržati rast biljaka. Uz šećer, topljivi proteini također igraju važnu ulogu u podržavanju boljeg rasta i razvoja biljaka. Topivi proteini sadrže nekoliko enzima uključenih u stanične metaboličke procese koji na kraju pospješuju metabolizam biljaka u cjelini. Stoga, više proteina u biljkama u sjemenkama tretiranim PAW-om ukazuje na bolju kontrolu nad staničnim metabolizmom biljke (Rathore i sur., 2021). Tretiranje sjemena s PAW-om dovodi do značajnog porasta sadržaja klorofila u biljci i posljedično do bolje fotosinteze (Sajib i sur., 2019.). Isto tako, povećana količina klorofila 'a' koja je dobivena pod utjecajem tretmana PAW-om, izravno je povezana s većim usvajanjem svjetla u reakcijskom centru PS I i PS II i većom fotosintezom (Rathore i sur., 2021.).

Prema navodima Kučerová i sur. (2019.), PAW poboljšava postotak klijavosti, rast sadnica, rast cvjetova i povećava suhu masu biljke. Međutim, u istraživanjima Ahn i sur. (2019.) u poljskim uvjetima, nije dokazan statistički značajan utjecaj tretmana sjemena s PAW-om. Isto tako, u istraživanjima Shapira i sur. (2019.) nije dokazan utjecaj tretiranja sjemena plazmom na rast i prinos kod uzgoja u plasteniku.

Učinak plazme na klijavost sjemena ovisi o biljnoj vrsti i njenom prirodnom kapacitetu klijanja, veličini sjemena, tvrdoći ovojnice sjemena, debljini endosperma i morfologiji površine i sl. (Zahoranová i sur., 2016.). Zbog toga, različiti utjecaji plazme na razvoj biljaka ostaje nejasan jer vrsta odgovora može biti posljedica genetike, strukture ovojnice sjemena ili kemijskog sastava komponenti kao što su pigmenti u ovojnici sjemena koji djeluju kao antioksidansi i doprinose otvrdnjavanju ovojnice sjemena (Koga i sur., 2020.). Učinak PAW-a na klijanje također ovisi o vrsti pražnjenja, vrsti obrade plazmom (izravna ili neizravna), vrsti vode (deionizirana ili iz slavine) i eksperimentalnim uvjetima (in vitro ili in vivo), itd. (Kučerová i sur., 2019). Kako bi se dobile željene reakcije sjemena u pogledu upijanja vode, klijanja, rasta itd. treba optimizirati parametre plazme i pražnjenja (Waskow i sur., 2022., Starič i sur., 2020.). Stoga je potrebno detaljnije proučavati učinak PAW-a obzirom na pojedina svojstva pražnjenja plazme i biljne vrste, jer učinkovitost i mehanizam djelovanja mogu biti različiti (Kučerová i sur., 2019). Zajedničko djelovanje aktivnih komponenti (RONS) obično rezultira poboljšanjem rasta biljaka i njihovih fizioloških parametara, međutim njihov omjer mora biti optimiziran za daljnje poboljšanje učinka plazme na poljoprivredne biljke i eventualne primjene u poljoprivredi (Kučerová i sur., 2021).

Zaključak

Povećanje i održavanje produktivnosti biljaka je pod sve većim utjecajem promjenjivih klimatskih uvjeta, a rastući broj stanovništva na Zemlji povećava potrebe za hranom. Zbog toga, suvremena poljoprivredna proizvodnja nastoji pronaći odgovarajuće mjere za prevladavanje izazova koji su pred njom. Jedna od metoda izbora koja omogućuje uzgoj biljaka na ekološki prihvatljiv način i u skladu je s normativima prihvaćenim od strane FAO i EU je primjena hladne ili netermalne plazme (NTP). Primjena ove tehnologije u poljoprivredi provodi se direktnim tretiranjem plazmom ili plazmom aktivirane vode (PAW). PAW je mješavina dugoživućih reaktivnih vrsta kisika i dušika (RONS), kao što su H_2O_2, NO_2^- ili NO_3^- koje mogu djelovati kao signalne molekule u metabolizmu biljaka ili služiti kao hranjive tvari. Provedeno istraživanje pokazuje da se primjenom plazme može unaprijediti uzgoj poljoprivrednih kultura. Pokusima je dokazano povećanje brzine klijanja, veća klijavost i bolji porast biljaka. Ali zbog specifičnog i kompleksnog djelovanja plazme na metaboličke procese u biljkma potrebna su dodatna istraživanja vezano za biljnu vrstu, sortu i svojstva primijenjene plazme.

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Non-thermal plasma effect on growth of agricultural plants

Abstract

The application of plasma technology in agriculture is carried out by treating seeds or plants with cold plasma (NTP) or plasma activated water (PAW). Various plasma-potentiated reactive compounds act as signaling molecules in plant metabolism, increase the activity of stress-responsive genes in plants, or serve as nutrients. The aim of this study is to present effects of cold plasma treatment on the changes that lead to the improvement of the growth of agricultural plants. The research results indicate a different response of agricultural plants to plasma treatment. Further research is needed to determine the optimal effect of plasma on plants.

Keywords: NTP, PAW, plasma agriculture

Održiva proizvodnja bioplina kao izvor biogenog CO_2

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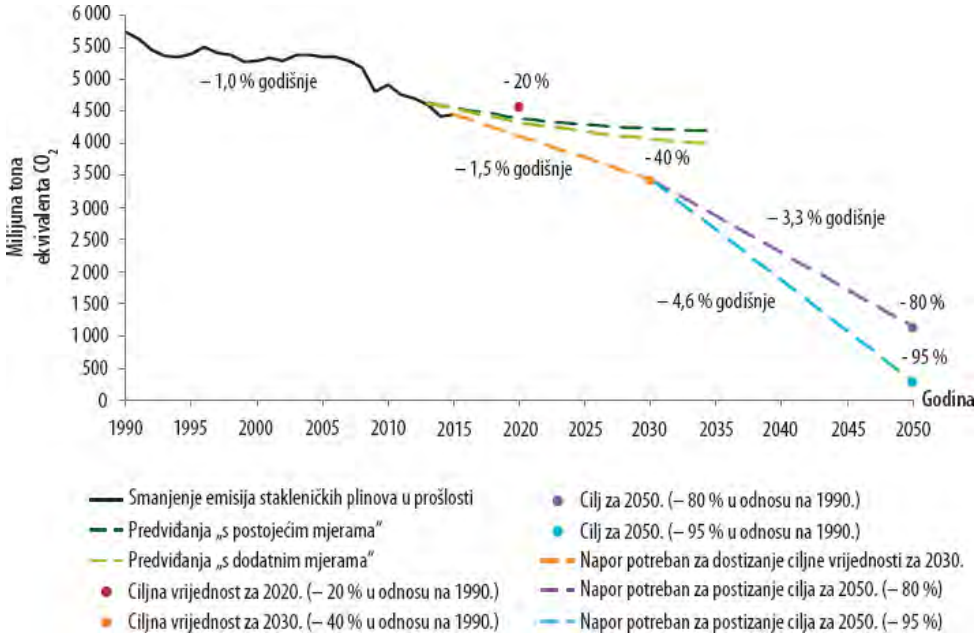
Sažetak

Održiva biomasa imat će značajnu ulogu u ispunjenju cilja smanjenja emisija stakleničkih plinova do 2030. god., obuhvaćenog EU Zelenim planom (EK, 2019). Proizvodnja i korištenje biomase u proizvodnji biogoriva i/ili bioproizvoda može biti značajno s pozicije trgovanja emisijama stakleničkim plinovima (EU ETS). Prijedlog revizije direktive 2003/87/EK o sustavu trgovanja stakleničkim plinovima po prvi puta obuhvaća i kriterije održivosti biomase, uključujući proizvodnju i korištenje biogoriva. Cilj ovog rada bio je prikazati korištenje i skladištenje biogenog CO_2, glavne načine njegova iskorištenja kao i tržišne mogućnosti. Sektor proizvodnje bioplina i biometana može osigurati tokove biogenog CO_2 koje je moguće koristiti u drugim sektorima industrije, ili za trajno ublažavanje negativnih emisija stakleničkih plinova.

Ključne riječi: biomasa, bioplin, EU ETS, održivost, klimatska neutralnost

Uvod

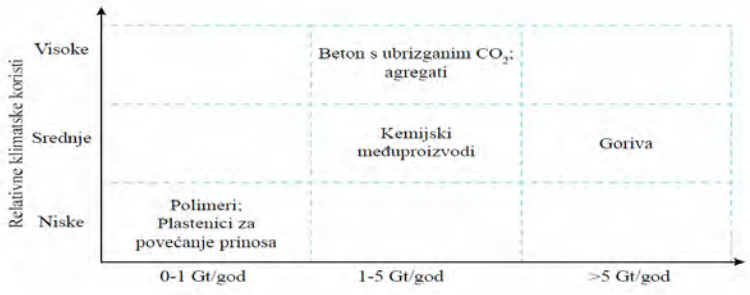
Već je dobro poznato da je jedan od načina smanjenja emisije stakleničkih plinova zamjena neobnovljivih s obnovljivim izvorima energije (OIE), budući se OIE smatraju kumulativno CO_2 neutralnima. Kumulativna CO_2 neutralnost obuhvaća ukupni vrijednosni lanac od proizvodnje sirovine i energije/biogoriva/bioproizvoda, izrade i postavljanja postrojenja pa sve do korištenja i zbrinjavanja ostataka nakon proizvodnje energije/biogoriva/bioproizvoda. Pod pojmom neutralnost, podrazumijeva se neutralnost prilikom pretvorbe OIE u iskoristiv oblik, odnosno pretpostavlja se da emisija CO_2 pri korištenju specifičnog OIE mora biti jednaka apsorpciji CO_2 prilikom procesa fotosinteze tijekom rasta biomase. Na Slici 1 grafički su prikazani trendovi, predviđanja i ciljevi u pogledu smanjenja emisija stakleničkih plinova u EU27 za razdoblje od 1990. god. do 2050. god.



Slika 1. Trendovi, predviđanja i ciljevi za smanjenje emisije stakleničkih plinova u EU do 2050. god. (Europski revizorski sud (2017)

Antropogene emisije CO_2 uglavnom su posljedica korištenja fosilnih goriva kao najvažnijeg globalnog izvora energije. Stoga bi uloga biomase u smanjenju CO_2 emisija mogla biti ključna iz razloga što je biomasa trenutno četvrti najznačajniji izvor energije u svijetu, odmah nakon ugljena, nafte i prirodnog plina (EBA, 2019). Pretpostavlja se da će povećana energetska učinkovitost i povećana proizvodnja obnovljive energije doprinijeti smanjenju emisija CO_2, no prema IEA (2019), energetska učinkovitost i obnovljiva energija nemaju potencijal smanjenja globalnih emisija CO_2 do planiranih razina, odnosno 50-80 % do 2050. god. Očekivano smanjenje postići će se isključivo ukoliko se dobro gospodari s emisijama CO_2 na način da se one hvataju i recikliraju u obliku proizvoda dodane vrijednosti. Slika 2 prikazuje predviđeni utjecaj biomase kao sirovine u proizvodnji biogoriva i/ili bioproizvoda (odnosno proizvoda dodane vrijednosti) na klimatske promjene.

Slika 2. Teoretski potencijal i klimatske koristi CO_2 derivata



Slika 2. Teoretski potencijal i klimatske koristi različitih biogoriva i/ili bioproizvoda (EBA, 2019)

Biogeni CO_2

Biogeni CO_2 je CO_2 koji nastaje razgradnjom, digestijom ili izgaranjem biomase ili proizvoda dobivenih iz biomase i dio je prirodnog ciklusa ugljika. Apsorbiran u biomasi tijekom procesa fotosinteze, atmosferski CO_2 se, ovisno o vrsti pretvorbe i konačnoj upotrebi biomase, vraća u atmosferu ili u tlo u obliku biogenog CO_2. Izvori biogenog CO_2 uključuju:

- izgaranje krutog, tekućeg i plinovitog goriva proizvedenog iz biomase,
- proces fermentacije do etanola,
- proces proizvodnje vina i piva,
- proces pročišćavanja bioplina u bioplinskoj industriji.

Kako bi se ostvarili ciljevi smanjenja emisija CO_2, potrebno je razraditi modele recikliranja antropogenog CO_2. Model hvatanja i iskorištenja ugljika (engl. *carbon capture and utilisation, CCU*) obuhvaća hvatanje emisija CO_2 za njegovo daljnje korištenje kao sirovine za proizvodnju goriva, materijala i kemikalija. Kada se u ovoj proizvodnji koristi biogeni CO_2 (bio-CCU) smanjuju se neto emisije CO_2 u atmosferu (EBA, 2019).

Trajno uskladištenje biogenog CO_2

Kada se biogeni CO_2 uhvati i trajno uskladišti u podzemnim geološkim skladištima poput iscrpljenih plinskih/naftnih polja, proces se naziva "*bio-carbon capture and storage*" (bio-CCS), i omogućuje trajno uklanjanje CO_2 iz atmosfere.

Biogeni CO_2 može se također dugotrajno uskladištiti u nekom proizvodu, poput građevinskog materijala ili plastike. Kada se ovakvo, dugotrajno skladištenje kombinira s upotrebom biogenog CO_2 za proizvodnju novih materijala kao što su bioproizvodi, proces se naziva " *bio-carbon capture, utilisation and storage*" (bio-CCUS) (EBA, 2019).

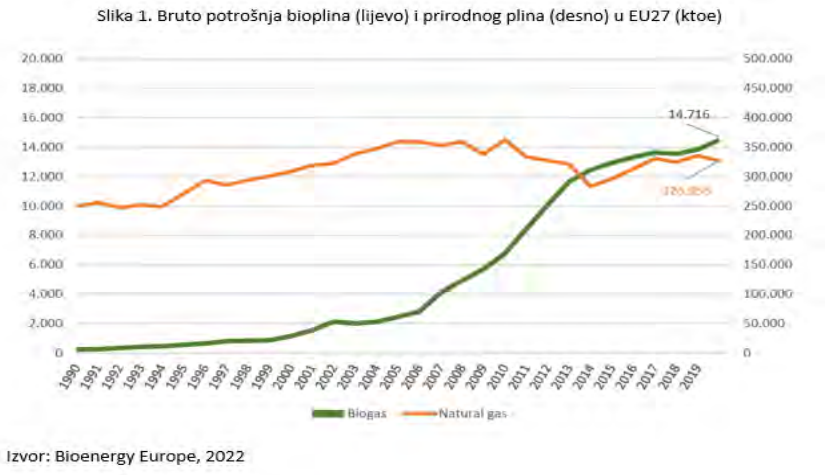
Budući da je za smanjenje globalnih emisija CO_2 do 2050. god. potrebno uključiti i druge opcije osim energetske učinkovitosti i upotrebe obnovljivih izvora energije, CCS, CCUS i njihove biološke verzije temeljene na biogenom CO_2 imaju ogroman potencijal. Strategija za smanjenje globalnih emisija CO_2 stoga bi trebala biti kombinacija:

- poboljšane energetske učinkovitosti,
- povećanja udjela OIE te
- globalne implementacije (bio)-CCS i (bio)-CCUS tehnologija.

Za razliku od fosilnog CCS-a, koji u najboljem slučaju smanjuje količinu novog CO_2 koji ulazi u atmosferu, bio-CCS ima potencijal uklanjanja CO_2 iz atmosfere, postižući negativne emisije (EBA, 2019).

Proizvodnja bioplina i CO_2

Vrijednosni lanac bioplina može proizvesti biogeni CO_2, zajedno s ostalim nusproizvodima, u dva različita područja: (i) anaerobnoj digestiji i (ii) procesu uplinjavanja. Prema EBA-i (EBA 2021), proizvodnja bioplina i biometana u EU20 iznosila je 18 mlrd m^3 u 2020. god. Trenutno taj sektor dinamično raste, a procijenjeno je da se potencijal bioplina i biometana može povećati na 35 mlrd m^3 do 2030. god., što je ekvivalent 10 % potrošnje prirodnog plina u EU. Potencijal se procjenjuje na najmanje 95 mlrd m^3 do 2050. god., što bi moglo biti dovoljno za 30-40 % trenutne potražnje za plinom (Slika 3). Potencijal je još izvjesniji nakon što je, zbog energetske krize, Europska komisija u svibnju 2022. objavila priopćenje REPowerEU (EK, 2022) kojim se potiče povećanje proizvodnje bioplina i biometana za 20 % u državama članicama do 2030. god., s ciljem povećanja njihove energetske neovisnosti.



Slika 3. Bruto potrošnja bioplina (lijevo) i prirodnog plina (desno) u EU27 (ktoe)

Kako bi modeli bio-CCS ili bio-CCUS osigurali uklanjanje ugljika iz atmosfere, proizvodnja bioplina mora biti ekološki održiva. Potencijal od 35 mlrd m^3 do 2030. i 95 mlrd m^3 do 2050. god. može se postići održivo, tj. korištenjem isključivo održivih sirovina u skladu sa zahtjevima aktualne RED II (EK, 2018). Kriteriji održivosti, prema direktivi RED II, primjenjuju se na bioplinska postrojenja kapaciteta većeg od 2 MW ukupne toplinske snage (ili 200 $Nm^3\ h^{-1}$ instaliranog kapaciteta), a uključuju značajne uštede emisija stakleničkih plinova duž lanca opskrbe uz jamstvo da izvor biomase ima minimalni utjecaj na biološku raznolikost i kvalitetu tla. Usklađenost s takvim kriterijima neophodna je da bi se energija klasificirala kao obnovljiva te kvalificirala za financijsku potporu i da bi imala odobrenu nultu ocjenu prema EU sustavu trgovanja emisijama (EU ETS).

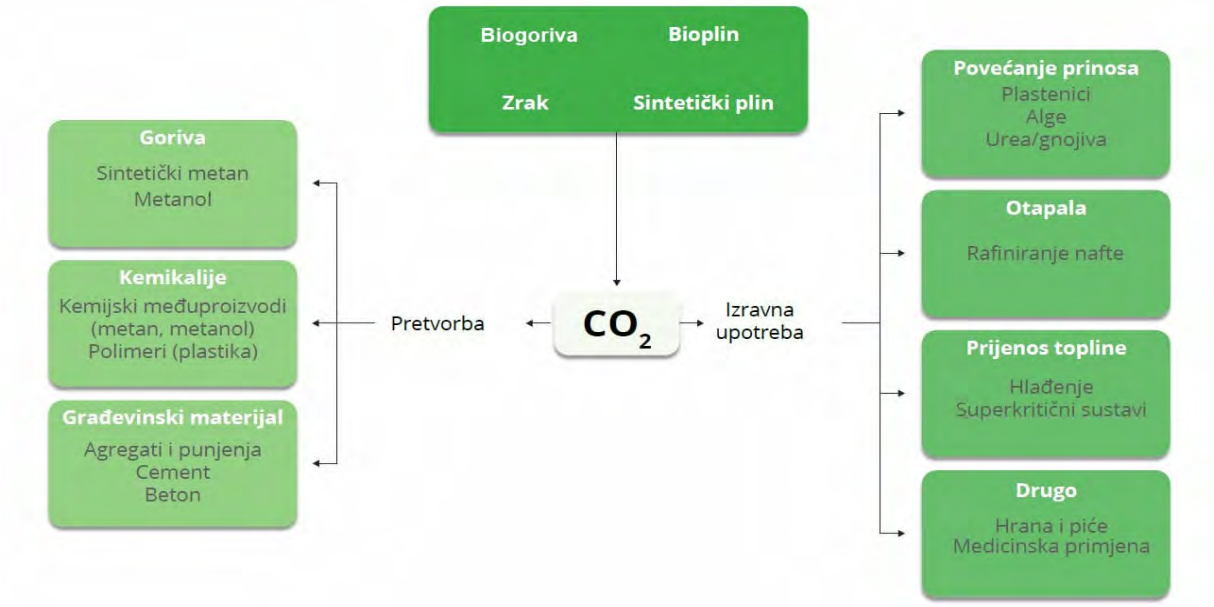
Bioplin i biometan se danas proizvode iz organskih materijala procesom anaerobne digestije. Organska tvar koja se koristi kao sirovina za bioplin i biometan izvor je organskog ugljika. Od 2013. god., broj novih bioplinskih postrojenja koja koriste prehrambene sirovine značajno se smanjio. Tržište se okrenulo prema poljoprivrednim ostacima, organskom komunalnom otpadu, industrijskim otpadnim vodama i kanalizacijskom mulju. Od 2017. godine, gotovo da nijedno novo postrojenje nije projektirano za korištenje prehrambenih usjeva (tzv. sirovine prve generacije), a zahvaljujući trenutnim regulacijama u smjeru ublažavanja emisija stakleničkih plinova, ovaj će se trend nastaviti i u budućnosti.

Hvatanje i iskorištenje biogenog CO_2

Biogeni CO_2 iz procesa anaerobne digestije može se uhvatiti na nekoliko načina:

- tijekom procesa nadogradnje bioplina u biometan, CO_2 se hvata uz relativno niske troškove zbog svoje visoke čistoće,
- u bioplinskim postrojenjima opremljenim s kogeneracijskim motorima, CO_2 se može uhvatiti iz dimnih plinova tijekom izgaranja bioplina.
- proizvodnja biovodika iz sirovog bioplina, što predstavlja mogući treći model za hvatanje biogenog CO_2 (EBA, 2019).

Danas postoje različiti putovi iskorištenja CO_2 (Slika 4). Ti se procesi mogu podijeliti na (i) putove bez pretvorbe (izravno korištenje CO_2 kao sirovine) i (ii) putove s pretvorbom (što zahtijeva specifičnu obradu CO_2 prije iskorištenja).



Slika 4. Pojednostavljena klasifikacija načina korištenja CO_2 (EBA, 2019)

EU sustav za trgovanje emisijama stakleničkih plinova (EU ETS)

Sustav Europske unije za trgovanje emisijama stakleničkih plinova, EU ETS (EK, 2003) jedan je od ključnih modela za ublažavanje klimatskih promjena i ujedno prvo tržište ugljika u svijetu. Danas sustav EU ETS obuhvaća tešku industriju i postrojenja za proizvodnju električne energije, kao i zračni prijevoz te u njemu sudjeluju sve države članice EU-a. S obzirom na dostupnu količinu biomase, sustav EU ETS bi trebao uključiti i sektor poljoprivrede i proizvodnju bioplina, budući da je poljoprivreda značajni globalni proizvođač stakleničkih plinova, a imajući na umu da je svrha ovog sustava pružiti učinkovit mehanizam za smanjenje emisija stakleničkih plinova do 2030. god., odnosno do 2050. god (EK, 2021).

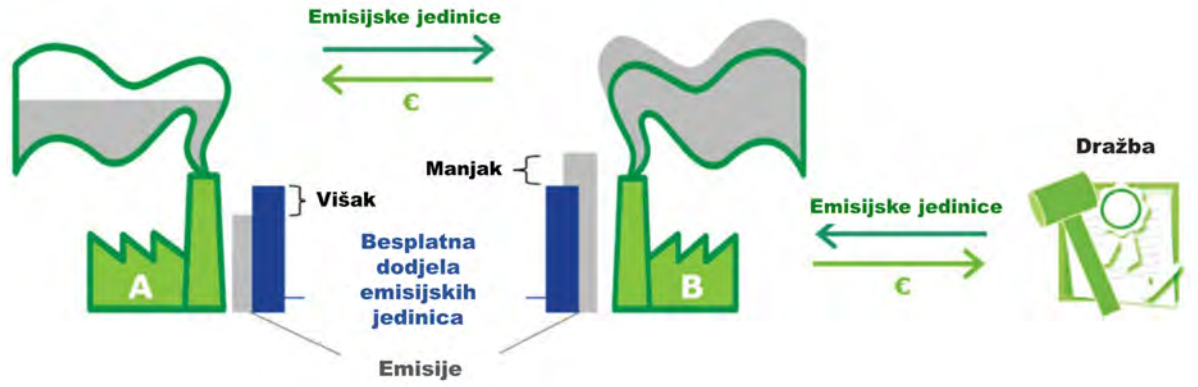
Sustav za trgovanje emisijama stakleničkih plinova je najznačajniji primjer trgovanja s emisijama CO_2 koji danas djeluje u svojoj četvrtoj fazi, obuhvaća preko 11.500 postrojenja u 30 zemalja i pokriva približno 40 % ukupnih emisija u EU27. Utjecaj EU ETS sustava na okoliš može se procijeniti u odnosu na dva specifična primarna cilja:

- učinkovito smanjenje emisija stakleničkih plinova,
- promicanje ulaganja u niskougljične tehnologije (EK, 2021).

Sustav djeluje po principu '*cap and trade*' tj. ograničenja i trgovine, na način da se postavlja ograničenje ukupne količine specifičnih stakleničkih plinova koju mogu ispustiti postrojenja obuhvaćena sustavom. Ograničenje se s vremenom smanjuje pa na taj način dolazi do smanjenja ukupnih emisija. U okviru definiranih graničnih vrijednosti, postrojenja kupuju ili primaju dozvoljene emisije kojima mogu međusobno trgovati po potrebi. Ograničenje ukupnog

broja dozvoljenih emisijskih jedinica osigurava njihovu vrijednost. Na kraju svake godine, postrojenje je u obvezi opravdati svoje emisije, u protivnom se izriču visoke kazne (EK, 2015). U Republici Hrvatskoj penalizacija je sljedeća: ukoliko operator postrojenja ne preda do 30. travnja tekuće godine količinu emisijskih jedinica u Registar EU-a u količini koja odgovara verificiranoj ukupnoj emisiji stakleničkih plinova iz postrojenja u prethodnoj kalendarskoj godini, sukladno verificiranom izvješću, dužan je platiti naknadu u iznosu od 100 EUR po toni ekvivalenta CO_2 koju postrojenje ispusti, a za koju nije predalo emisijske jedinice. Drugi oblik penalizacije se odnosi na obveze koje su operateri dužni izvršiti prema Čl. 146. Zakona o zaštiti zraka (NN 127/19).

Na Slici 5 prikazan je sustav trgovanja emisijskim jedinicama na principu dvije tvornice A i B. U ovom slučaju, tvornici A je dodijeljeno više besplatnih jedinica nego li je potrebno za pokriće njezinih emisija, stoga može prodati taj višak ili ga zadržati. Tvornici B nije dodijeljeno dovoljno besplatnih emisijskih jedinica da bi se pokrile njezine emisije, stoga tvornica B mora kupiti razliku na dražbi ili neposredno od drugih operatera, ukoliko nema zalihu emisijskih jedinica od prijašnjih godina.



Slika 5. Prikaz rada sustava ETS na principu tvornice A i tvornice B (Europski revizorski sud, 2020)

Uključivanje poljoprivrednika u ETS

Poljoprivreda je, uz promet i kućanstva, najveći proizvođač stakleničkih plinova koji nije uključen u EU ETS sustav. Postoje mnogobrojni povoljni načini kako smanjiti emisije stakleničkih plinova u poljoprivredi, a neki od njih su: poboljšano upravljanje obradivim zemljištem i pašnjacima te obnova degradiranih tala. Dodatne mogućnosti su izgradnja postrojenja za proizvodnju biogoriva, poboljšana učinkovitost poljoprivredne proizvodnje i minimiziranje udaljenosti prijevoza (Brandt i Svendsen, 2010).

Uključivanje poljoprivrednika u EU ETS sustav može također imati pozitivan učinak na ukupne proizvedene emisije. Naime, iako uključivanje poljoprivredne proizvodnje neće imati značajan utjecaj na cijenu emisijskih jedinica zbog njezine relativno male količine u usporedbi s ukupnim emisijama, ono podrazumijeva veći volumen trgovanja emisijama CO_2, što može rezultirati dobitkom u pogledu troškovne učinkovitosti. Međutim, na poticaje za razvoj i/ili implementaciju novih i čišćih tehnologija utječu buduće cijene emisijskih jedinica. Ukoliko će cijena emisijskih jedinica rasti, to će biti poticaj za tehnološka poboljšanja. Treba imati na umu da sudjelovanje u sustavu trgovanja pruža stalne poticaje da nove prakse budu financijski povoljnije i učinkovitije.

Zaključak

Korištenje biogenog CO_2 proizvedenog u bioplinskim postrojenjima će u skorije vrijeme u regulativi EU morati biti prepoznato u kalkulaciji emisija CO_2, kako bi se stvorila potražnja za istim, uz istovremeno stvaranje europskog certifikacijskog okvira koji bi trebao omogućiti pouzdano izdavanje i trgovanje kreditima za uklanjanje biogenog ugljika. Zakonodavstvo EU-a (posljedično i RH) trebat će osigurati da se operateri bioplinskih postrojenja potaknu na ko-proizvodnju biogenog CO_2 kao proizvoda.

Osim toga, imajući u vidu lanac dobave biomase, uključivanje poljoprivrednika u sustav trgovanja emisijama CO_2 imalo bi pozitivan učinak na primarnu poljoprivrednu proizvodnju, budući bi poljoprivrednici imali prije svega financijski motiv za pronalazak održivijeg i kvalitetnijeg načina zbrinjavanja ostataka biomase te bi ujedno mogli prodavati CO_2 kredite kroz proizvedenu biomasu.

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Sustainable biogas production as a source of biogenic CO_2

Abstract

Sustainable biomass will play a significant role in meeting the goal of reducing greenhouse gas emissions by 2030, included in the EU Green Deal (EC, 2019). The production and use of biomass in the production of biofuels and/or bioproducts can be significant from the point of view of greenhouse gas emissions trading (EU ETS). The proposed revision of the Directive 2003/87/EC on the greenhouse gas trading system for the first includes the time criteria for the sustainability of biomass, including the production and use of biofuels. The aim of this paper was to present the use and storage of biogenic CO_2, the main ways of its utilization as well as its market possibilities. The biogas and biomethane production sector can provide flows of the biogenic CO_2 that can be used in other sectors of industry, or for permanent mitigation of negative greenhouse gas emissions.

Keywords: biomass, biogas, EU ETS, sustainability, climate neutrality

Proces proizvodnje bioplina i električne energije u bioplinskom postrojenju Vinka u Vukovarsko-srijemskoj županiji

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Sažetak

U radu je prikazan i analiziran process proizvodnje bioplina i električne energije u bioplinskom postrojenju Vinka koje se nalazi između mjesta Jarmina i grada Vinkovaca u Vukovarsko-srijemskoj županiji. U procesu proizvodnje prikazane su glavne faze anaerobne digestije, sustav za upravljanje bioplinskog postrojenja te su dani ukupni ostvareni podaci u promatranom razdoblju za električnu i toplinsku energiju, količinu proizvedenog bioplina te digestata. Uporabom obnovljivih izvora energije, konkretnije biomase, moglo bi se u potpunosti zamijeniti tradicionalne izvore energije, čime bi se smanjila i emisija štetnih plinova u atmosferi odnosno zagađenje okoliša.

Ključne riječi: obnovljivi izvori energije, bioplin, biomasa, električna i toplinska energija

Uvod

U Europi i drugim industrijaliziranim područjima, glavni razlog za razvoj obnovljivih izvora energije je okoliš, posebice zabrinutost u vezi s globalnim klimatskim promjenama i potrebom za poboljšanjem sigurnosti i raznolikosti opskrbe energijom. U zemljama u razvoju, obnovljivi izvori primarne energije u regijama bez konvencionalne energije pružaju priliku za održivi razvoj (Ošlaj, 2010.). Prema Šikiću (2016.) u obnovljive, neiscrpne izvore energije svrstavaju se: neposredno iskorištenje sunčeva zračenja (solarna ili sunčeva energija), vodne snage (neovisno o tome o kako se velikim hidroelektranama iskorištavaju), hidroenergija, energija vjetra (eolska energija), energija morskih valova i morskih struja, unutarnja toplina vode (mora), zraka i tla, energija plime i oseke, energija biomase (uključivo ogrjevno drvo) koja se može koristiti na obnovljivi način, tako da ukupni trošak u pojedinom razdoblju bude jednak ili manji od prirasta biomase u tom razdoblju, energija sadržana u otpadu (biorazgradivom komunalnom i industrijskom te biljnom i životinjskom) koji je također obnovljiv, energija bioplina koja nastaje fermentacijom ekskrementa domaćih životinja te organskih otpadaka iz kućanstava, vrtlarstva, voćarstava, poljoprivrede i industrije. Strategija energetskog razvoja Republike Hrvatske (NN, 25/2020) temeljni je dokument kojim se utvrđuje energetska politika i planira energetski razvoj zemlje za razdoblje do 2030. godine, donesen s ciljem usklađenja sa strateškim dokumentima Europske unije i ostvarenju vizije niskougljične energije uz prijelaz na novo razdoblje energetske politike kojom se osigurava pristupačna, sigurna i kvalitetna opskrba energijom. Strategija nudi rješenja za sigurnost opskrbe energijom, konkurentnost energetskog sektora i održivost energetskog sustava. Bioplin je mješavina plinova koja nastaje fermentacijom biorazgradivog materijala u okružju bez kisika (Šljivac, 2009., Špicnagel, 2014., Šikić, 2016.). Dobiveni se bioplin najčešće koristi za dobivanje toplinske ili električne energije izgaranjem u kotlovima, plinskim motorima ili turbinama (Šljivac, 2009.). Biomasa koja se koristi za proizvodnju bioplina je raznovrsna. Kao što je vidljivo u tablici 1., kao sirovina se može koristiti razgradivi organski otpad iz poljoprivredne i prehrambene industrije biljnog i životinjskog podrijetla, otpadni muljevi, kao i energetski usjevi u koje pripadaju na primjer, kukuruz, sirak, različite vrste trava, djetelina i slično (Bilandžija i sur., 2009.). Proizvodnja bioplina može se dobro integrirati u konvencionalnu i ekološku poljoprivredu, gdje digestat zamjenjuje mineralna (umjetna) gnojiva, proizvedena uz veliki utrošak fosilnih goriva (Došen, 2017.). Proces proizvodnje bioplina i električne energije može se raščlaniti na nekoliko specifičnih koraka: prethodna obrada (mljevenje ili usitnjavanje materijala), punjenje digestora, anaerobna digestija (u trajanju od nekoliko tjedana do nekoliko mjeseci, ovisno o vrsti organskog materijala koji se koristi

i uvjetima unutar digestora), pročišćavanje bioplina (propuštanje bioplina kroz otopinu koja uklanja nečistoće), proizvodnja električne energije korištenjem plinskog motora ili plinske turbine. Električna energija se proizvodi korištenjem generatora koji pretvaraju mehaničku energiju plinskog motora ili plinske turbine u električnu energiju. Električna energija se zatim dovodi u elektroenergetsku mrežu i distribuira u domove, tvrtke i druge objekte. Osim proizvodnja električne energije proces anaerobne digestije također proizvodi nusprodukt bogat hranjivim tvarima koji se naziva digestat, a koji se može koristiti kao gnojivo. Proces proizvodnje bioplina – od proizvodnje supstrata pa do korištenja digestata kao gnojiva–čini zatvoreni ciklus hranjivih tvari. Količina ugljikovih spojeva (C) smanjuje se postupkom digestije, pri čemu se metan koristi za proizvodnju energije, a ugljikov dioksid se ispušta u atmosferu i biva ponovo vezan u biljke tijekom fotosinteze. Nešto ugljikovih spojeva ostaje u digestatu. Oni povećavaju sadržaj ugljika u tlu ako se digestat koristi u svrhu gnojidbe. Proizvodnja bioplina može se dobro integrirati u konvencionalnu i ekološku poljoprivredu, gdje digestat zamjenjuje mineralna (umjetna) gnojiva, proizvedena uz veliki utrošak fosilnih goriva (Došen, 2017.). Proces nastanka bioplina rezultat je niza povezanih procesnih koraka tijekom kojih se sirovina razlaže na sve jednostavnije spojeve, sve do nastanka bioplina. U pojedinim fazama proizvodnje djeluju specifične grupe mikroorganizma. Cilj rada je prikazati suvremeno bioplinsko postrojenje te benefit korištenja obnovljivih izvora energije koji se očituje u obliku proizvodnje električne energije.

Materijal i metode

Bioplinsko postrojenje Vinka u vlasništvu tvrtke Energija Gradec d.o.o. smješteno je u okolici grada Vinkovaca u Vukovarsko-srijemskoj županiji. Bioplinsko postrojenje sagrađeno je 2014. godine. Postrojenje se sastoji od pogona ukupne snage 2 MW. U pogonu postoje dvije mješače jame za doziranje sirovina, dva fermentora, dva postfermentora, dva bioplinska motora, dva generatora, baklja za spaljivanje viška bioplina i bazena za skladištenje tekućeg digestata (end-lagera). Cijeli process proizvodnje gotovo je u potpunosti automatiziran. Biomasa koju tvrtka koristi za proizvodnju električne energije je stajska gnojnica, stajnjak i kukuruzna silaža. Biomasu potrebnu za rad tvrtka nabavlja na farmama Pik-a, kooperanti Pik-a Vinkovci i tvornice za preradu voća i povrća Vinka d. d. Biomasa koja se koristi za proizvodnju bioplina je raznovrsna (Tablica 1.).

Tablica 1. Vrste sirovina i prinos metana t/ST različitih supstrata za proizvodnju bioplina

Vrsta supstrata	Organska tvar	C:N (omjer)	Suha tvar (ST) %	Prinos bioplina (m^3/kg)
Svinjski izmet	U, B, M	3-10	3-8	0,25-0,50
Gnoj i otpad goveda	U, B, M	6-20	5-12	0,20-0,30
Gnoj i otpad peradi	U, B, M	3-10	10-30	0,35-0,60
Iznutrice –NŽP	U, B, M	3-5	15	0,4-0,68
Slama	U, M	80-100	70-90	0,15—0,35
Ostatak nakon prerade voća		35	15-20	0,25-0,50

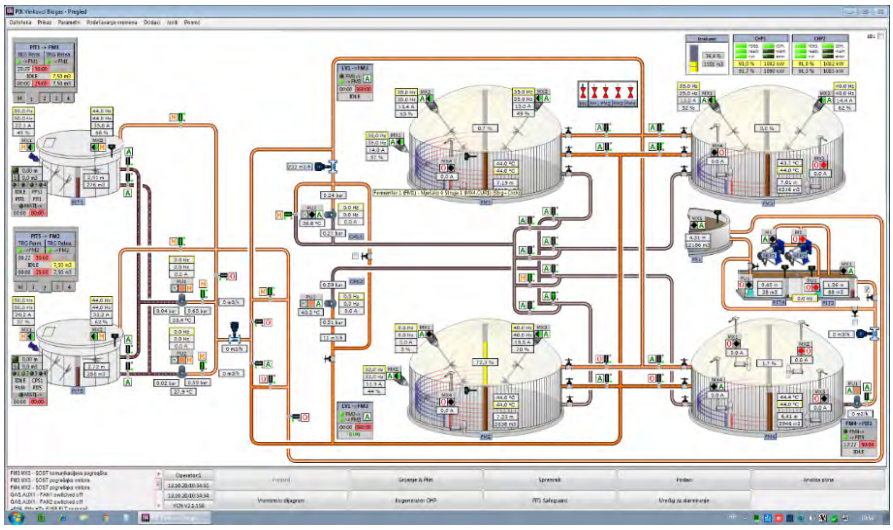
U-ugljikohidrati, B-bjelančevine, M-masti

Pojednostavljeni dijagram anaerobne digestije prikazan je na slici 1. na kojoj su naglašene četiri glavne faze u procesu nastanka bioplina: hidroliza, acidogeneza, acetogeneza i metanogeneza. Biomasa koju istraživano postrojenje koristi za proizvodnju električne energije je stajska gnojnica, stajnjak i kukuruzna silaža. Cijeli process proizvodnje gotovo je u potpunosti automatiziran koristeći sustav na kojem su vidljivi svi podatci, uslijed kojih se mogu brzo uočiti promjene te na vrijeme reagirati (Slika 2.).

Slika 1. Glavne faze anaerobne digestije

Slika 2. Podatci o radu bioplinskog motora i njegovo upravljanje

Nadzor postrojenja obavlja se putem programa (Slika 3.) koji daje uvid u osnovne podatke postrojenja kao što su trenutne količine proizvedenog plina, trenutna snaga generatora, zagrijavanje generatora, trenutna snaga motora, količine biomase u fermentorima, obavijesti i slično. Sustav u svakom trenutku dostavlja obavijesti o radu postrojenja i te se sve obavijesti spremaju u bazu podataka.



Slika 3. Sustav za upravljanje bioplinskog postrojenja Vinka

Na bioplinskom postrojenju Vinka kako bi se omogućila distribucija proizvedene električne energije postavljen je priključak na električnu mrežu. Budući da generator ne proizvodi struju dovoljno visokog napona isti se povećava uporabom transformatora, odnosno transformatorske stanice. Postrojenje ima ukupnu snagu 2 MW. Osim što služe za podizanje napona, transformatorske stanice služe Hrvatskoj elektroprivredi (HEP d.d.) za usmjeravanje struje u željenom smjeru i količini, ovisno o potrebi korisnika. Cijelokupna električna energija koja se proizvede na postrojenju se prodaje HEP-u.

Rezultati i rasprava

Tijekom analize procesa proizvodnje bioplina i električne energije u bioplinskom postrojenju Vinka u 2019. i 2020. godini prikupljeni su podaci o proizvedenoj te isporučenoj energiji. Od siječnja do rujna 2019. godine električne energije je ukupno proizvedeno 18.133,80 MWh, dok je isporučeno 16.673,31 MWh. Pri proizvodnji toplinske energije ukupno je proizvedeno 3.603,43 MWh, ukupna količina bioplina iznosila je 7.254.036,00 m^3, dok je ukupna količina digestata iznosila 54.726,68 t od čega je u tekućem obliku 52.089,88 t, a krutog 2.636,80 t (Tablica 2.)

Od siječnja do rujna 2020. godine električne energije je ukupno proizvedeno 13.647,80 MWh, dok je isporučeno 12.527,71 MWh. Pri proizvodnji toplinske energije ukupno je proizvedeno 2.039,56 MWh, ukupna količina bioplina iznosila je 5.405.895,00 m^3, dok je ukupna količina digestata iznosila 42.152,21 t od čega je u tekućem obliku 38.995,26 t, a krutog 3.156,95 t (Tablica 2.). Uspoređujući dvije istraživane godine uočava se pad proizvedene električne energije od 24,74 %, zatim 43,41 % manje proizvedene toplinske energije, dok je bioplina proizvedeno manje za 25,49 %, a digestata manje za 22,98 %.

Prema statističkom izvješću EIHP, HEP i HROTE (Energija u Hrvatskoj, 2020.) porast instaliranih kapaciteta za proizvodnju električne energije iz obnovljivih izvora prati i njezina proizvodnja pa je tako u 2020. godini proizvedeno gotovo 2.990,1 GWh električne energije iz obnovljivih izvora energije (OIE), dok je od toga iz bioplina 419,3 GWh što je 14,02 % od ukupnog iznosa. Trenutno najveći postotak proizvedene električne energije od ukupnog u iznosu od 57,54 % je dobiveno od izvora vjetra, dok je najmanji postotak u iznosu 3,13 % ostvareno koristeći izvor geotermalne energije.

Ivanković (2020.) navodi kako u Republici Hrvtatskoj se do sada bioplin uglavnom koristi za proizvodnju električne energije, dok je primjena biometana značajna u sektoru prometa. No bioplin u ukupnom elektroenergetskom sektoru zauzima samo manji udio u proizvodnji, za razliku od drugih izvora što se poklapa sa dobivenim podacima ovoga istraživanja. Pavićić (2021.) uočava kako potencijal za poticanje primjene bioplina za proizvodnju električne energije i izgradnju kogeneracijskih bioplinskih postrojenja su povlaštene tarife, a njihov iznos ovisi o nekoliko čimbenika poput veličina elektrane i količina proizvedene električne energije. Prema HGK (2021.) od ukupno 70 bioplinskih postrojenja u Republici Hrvatskoj u 2021. godini, njih 21 ima dozvolu za gospodarenjem otpadom, a njih 6 koristi biootpad iz komunalnog otpada. Na što se posebno stavlja naglasak jer se još uvijek velika količina otpada usmjerava na odlagališta. Prema Energetika (2018.) uočeno je kako na hrvatskim stočarskim farmama, točnije za njih više od 3.600 postoji mogućnost izgradnje mikro bioplinskih postrojenja (10-50 kW), na razini grada ili općine kao koncept energetske zajednice, gdje bi se također mogle uključiti i komunalne djelatnosti s ciljem ostvarivanja kružne ekonomije.

Tablica 2. Prikaz proizvodnje električne i toplinske energije, bioplina i digestata

Razdoblje	Električna energija (MWh)		Toplinska energija (MWh)	Bioplin (m^3)	Digestat (t)		
	Proizvedeno	Isporučeno	Ukupno	Ukupno	Ukupno	Kruti	Tekući
2019.	18.133,80	16.673,31	3.603,43	7.254.036,00	54.726,68	2.636,80	52.089,88
2020.	13.647,80	12.527,71	2.039,56	5.405.895,00	42.152,21	3.156,95	38.995,26

Zaključak

Bioplinsko postrojenje Vinka projektirano je kao postrojenje snage 2 MW. Proizvedena električna energija se skoro u potpunosti isporučuje Hrvatskoj elektroprivredi i to 91,79 % od ukupne proizvedene energije, a dobivena toplinska energija se ponovno vraća u process i koristi za grijanje fermentora. Značajna odlika istraživanog postrojenja je potpuna automatizacija u radu uz korištenje suvremenih sustava za upravljanje i nadzor. Proizvodnjom električne i toplinske energije putem kogeneracije bi se: umanjila energetska ovisnost o drugim državama, izravno i neizravno povećao broj zaposlenih, Republika Hrvatska bi ostvarila lakše svoju obvezu prema EU da zamjeni konvencionalna goriva s obnovljivim gorivima. Proizvodnjom bioplina putem AD rezultira se stvaranjem digestata koji zamjenjuje korištenje sirovog gnoja za gnojidbu, također ograničava korištenje dodatnih hranjivih tvari, što pomaže ispunjavanju tzv. "nitratne direktive". Bioplin ima nekoliko značajnih uloga, kao energent, gorivo u transportu, ali prvenstveno kao OIE, koji doprinosi smanjenju emisija stakleničkih plinova. Republika Hrvatska je prepoznala važnu ulogu korištenja bioplina i time brže ostvarivanje zadanih ciljeva koji su doneseni za 2030. i 2050. godinu. Uočava se na razini Republike Hrvatske porast instaliranih kapaciteta za proizvodnju električne energije iz OIE, sa ukupno proizvedene 2.990,1 GWh električne energije, od toga iz bioplina 419,3 GWh. Ostatak potencijala je na oko 3.600 stočarskih farmi na kojima je mogućnost izgradnje mikro bioplinskih postrojenja s ciljem ostvarivanja kružne ekonomije.

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Biogas and electricity production process in Vinka biogas plant in Vukovar-Srijem County

Abstract

The paper presents and analyzes the process of biogas and electricity production in the Vinka biogas plant, which is located between the town of Jarmina and the town of Vinkovci in the Vukovar-Srijem County. In the production process, the main stages of anaerobic digestion, the management system of the biogas plant are shown, and the total data achieved in the observed period for electricity and thermal energy, the amount of produced biogas and digestate are given. By using renewable energy sources, more specifically biomass, it would be possible to completely replace traditional energy sources, which would reduce the emission of harmful gases in the atmosphere, i.e. environmental pollution.

Keywords : renewable energy sources, biogas, biomass, electrical and thermal energy

Mogućnost korištenja *Arundo donax* L. kao sirovine za proizvodnju bioplina obzirom na vrijeme žetve

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Sažetak

Većina bioplinskih postrojenja u EU koristi kukuruznu silažu kao kosupstrat u proizvodnji bioplina, što predstavlja ozbiljnu konkurenciju između opskrbe energijom i proizvodnje hrane. Zbog toga su višegodišnji energetski usjevi poput *Arundo donaxa* L. idealno rješenje, kako ekološki tako i ekonomski. U ovome istraživaju uzorci su prikupljeni u ožujku (proljetna žetva) i rujnu (jesenska žetva) 2021. godine, nakon čega su određena negoriva i goriva svojstva, ogrjevna vrijednost te je proveden proces anaerobne digestije. Utvrđeno je kako vrijeme žetve utječe na energetska svojstva biomase i na konačan prinos bioplina te kako je *Arundo donax* L. pokazao uspješne performanse.

Ključne riječi: *Arundo donax* L., vrijeme žetve, energetska svojstva, bioplin

Uvod

Proizvodnja energije iz obnovljivih izvora korisna je u smanjenju globalnog zagrijavanja i dekarbonizaciji energetskih sustava samo ako procjena životnog ciklusa (LCA) učinkovito dokaže da je emitirani CO_2 manji od onoga koji emitiraju konvencionalne fosilna goriva (Hiloidhari i sur., 2017.). Naime, fosilna goriva i industrija predstavljaju najveći antropogeni izvor emisija stakleničkih plinova (GHG), te su prema podacima za 2015. godinu, odgovorni za 91 % svjetskih emisija GHG i za oko 70 % svih antropogenih emisija GHG (Šantek, 2020.). Upravo je to razlog za pronaći rješenje za zadovoljenje energetskih potreba iz obnovljive energije uz najmanju štetu za okoliš, a kao jedan od takvih nameće se bioplin. Bioplin je obnovljivo energetsko gorivo koje nastaje tijekom razgradnje složene organske tvari u atmosferi bez kisika procesom anaerobne digestije (Barbera i sur., 2019). Anaerobna digestija dovodi do mineralizacije organske tvari tijekom procesa tako da se hranjive tvari u organskim oblicima (npr. N i P) pretvaraju u mineralne i lako dostupne oblike (Tambone i sur., 2010.; Tambone i sur., 2017.). Sastav bioplina je 50-75 % metana i 25-45 % ugljičnog dioksida, s malim količinama vodene pare, kisika, dušika, amonijaka, vodika i sumporovodika (Rotunno i sur. 2017.).

U većini bioplinskih postrojenja, kukuruzna silaža je glavni supstrat za proizvodnju bioplina, što uzrokuje probleme kao iskorištavanje zemljišta namijenjenog za uzgoj hrane, rast tržišnih cijena sirovine, ugrožavanje stočarske proizvodnje, a ono što je najvažnije, kompeticija sa sirovinama za proizvodnju hrane. Iz tog razloga počinju se koristiti zamjenske kulture u proizvodnji biolina, a jedna od takvih je energetska kultura *Arundo donax* L. Korištenje energetskih usjeva za proizvodnju bioplina je efikasnije jer su oni produktivniji u smislu proizvedenog metana po kg biomase, što omogućuje uspostavu bioplinskih postrojenja srednje veličine (1 MW) (Corno i sur., 2015.).

Arundo donax L. je višegodišnja energetska kultura rasprostranjena po cijelom svijetu u sredinama s umjerenom klimom. Raste uz jezera, bare i rijeke, ali i u ekosustavima s manjom dostupnošću vode i na različitim vrstama tla (Lewandowski i sur., 2003.). Jedna od najvažnijih i prepoznatljivih karakteristika ove biljke je proizvodnja velike količine biomase po hektaru (ha) (Angelini i sur., 2009). Prednost uzgoja ove energetske kulture je mala ili nikakva upotreba gnojiva i pesticida što znači i niske troškove uzgoja i mali utjecaj na okoliš (Riffaldi i sur., 2010; Corno i sur., 2015; Soldatos i sur., 2015.).

S druge strane, veći prinos biomase po jedinici površine u usporedbi s drugim energetskim usjevima (Corno i sur., 2014.) omogućuje *Arundu donax* L. da proizvede puno više bioplina po jedinici kultivirane površine. Kako su izvijestili Corno i sur. (2014.), Ragaglini i sur. (2014.) te Schievano i sur. (2014.).dobiveni prinos metana može biti veći od 9000 Nm^3/ha.

Stoga je cilj ovog rada utvrditi mogućnost korištenja *Arundo donax* L. kao sirovine za proizvodnju bioplina s obzirom na vrijeme žetve.

Materijal i metode

Analizirani su uzorci biomase brzorastuće energetske kulture *Arundo donax* L. uzojene na pokušalištu Šašinovec Sveučilišta u Zagrebu Agronomskog fakulteta. Uzorci su prikupljeni u dvije žetve 2021. godine: proljetnoj (ožujak) te jesenskoj (rujan).

Istraživane su ulazne sirovine te su određena negoriva i goriva svojstva te ogrjevna vrijednost, kao i proizvodnja biplina. Analiziran je sadržaj vode (HRN EN 18134-2:2015) u laboratorijskoj sušnici, pepela (HRN EN ISO 18122:2015) i koksa (CEN/TS 15148:2009) u mufolnoj pečnici. Kako bi se dobio uvid u elementarni sastav određen je udio ugljika, vodika, dušika i sumpora metodom suhog izgaranja u Vario, Macro CHNS analizatoru (Elementar Analysensysteme GmbH, Njemačka), u skladu s protokolima za određivanje ugljika, vodika i dušika (HRN EN ISO 16948:2015) i sumpora (HRN EN ISO 16994:2015), dok će se sadržaj kisika izračunati iz razlike. Odrediti će se i ogrjevna vrijednost ISO metodom (HRN EN 14918:2010) pomoću IKA C200 kalorimetra (IKA Analysentechnik GmbH, Heitersheim, Njemačka). Anaerobna digestija provedena je u laboratorijskom bioreaktoru (CROTEH, Hrvatska) tijekom 26 dana pri temperaturi 39°C pri čemu je praćena produkcija bioplina i biometana (mL).

Rezultati i rasprava

Nakon provedenih analiza ulaznih sirovina u tablici 1. prikazani su rezultati negorivih svojstva, u tablici 2. rezultati sadržaja gorivih svojstava, a u tablici 3. rezultati ogrjevne vrijednosti *Arundo donaxa* L.

Tablica 1. Rezultati negorivih svojstava ulazne sirovine

Istraživani parametri (%)	*Arundo donax* L. Jesenska žetva	*Arundo donax* L. Proljetna žetva
Vlaga	55,86	40,28
Pepeo	4,84	4,06
Koks	11,40	18,26
Fiksirani ugljik	6,56	14,20
Dušik	0,39	0,32

Sadržaj vode bio je niži nakon proljetne žetve što je potvrđeno i istraživanjima Liu i sur. (2016.) koji utvrđuju da se kasnijom žetvom smanjuje sadržaj vode jer se uzorci počinju prirodno sušiti. Sadržaj pepela je jedan od glavnih čimbenika kvalitete biomase jer veće količine pepela smanjuju kvalitetu goriva, međutim, u proizvodnji bioplina sadržaj pepela nije kritičan čimbenik. Prema Krička i sur. (2017.), sadržaj pepela u *Arundo donax* L. bio je 3,56 %, dok se u ovome istraživanju sadržaj pepela kretao od 4,06 % do 4,84 % te je kao i sadržaj vode bio niži u proljetnoj žetvi. Ukoliko je sadržaj koksa i fiksiranog ugljika veći gorivo je kvalitetnije. Tako je sadržaj fiksiranog ugljika iznosio od 6,56 do 14,20 %, što je manje od 18,4 % koje navode Jegurim i Trouve (2010.). Sadržaj dušika kretao se od 0,32 do 0,39 %, ovisno o žetvi, i nešto je veći od 0,3 % koje navode Licursi i sur. (2015.).

Tablica 2. Rezultati gorivih svojstava ulazne sirovine

Istraživani parametri (%)	*Arundo donax* L. Jesenska žetva	*Arundo donax* L. Proljetna žetva
Ugljik	46,10	46,61
Sumpor	0,10	0,09
Vodik	5,54	5,55
Kisik	47,86	47,41
Hlapiva tvar	82,52	79,88

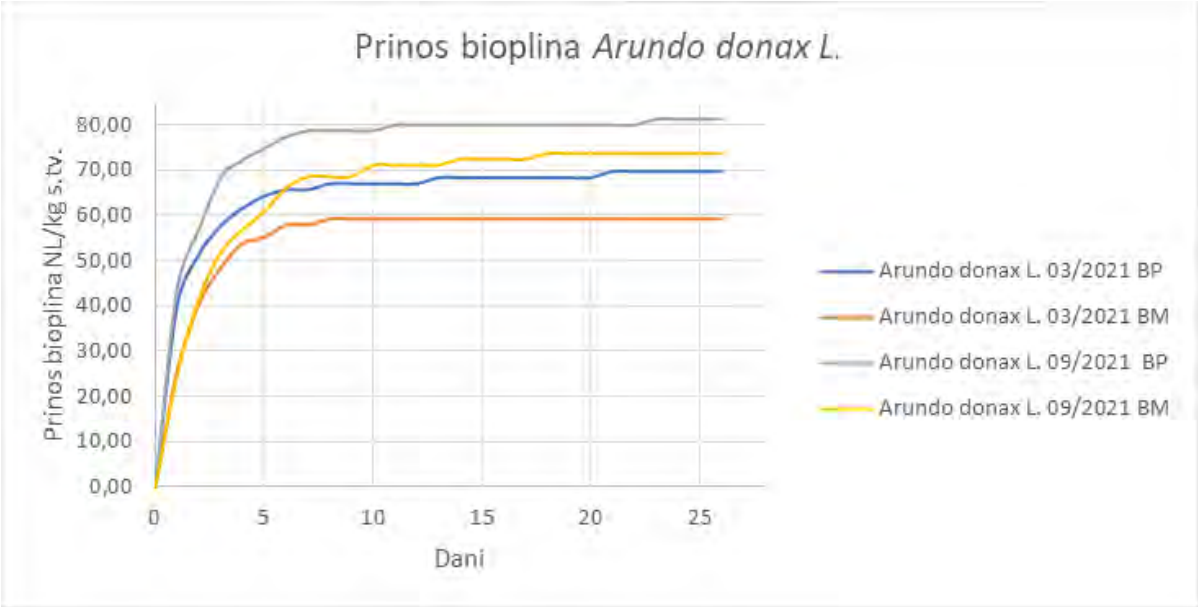
Sadržaj ugljika u biomasi je poželjno svojstvo dok je optimalni omjer s dušikom 25:1 (Al Seadi i sur., 2009.). Sadržaj ugljika kretao se oko 46 %, a vodika oko 5,55 % ukazujući da se vrijednosti nisu značajno razlikovale s obzirom na vrijeme žetve, što je u suglasju sa navodima literature Licursi i sur. (2015.) koje su iznosile 44,8 % za ugljik te 6,0 % za vodik. Hills i sur. (1979.) utvrdili su da povećanje omjera C/N utječe na smanjenje koncentracije metana u bioplinu. Sadržaj sumpora, štetnog elementa biomase, u uzorcima se kretao oko 0,10 % što je niže od 0,26 % koje su dobili Grubor i sur. (2020). Kisik koji smanjuje ogrjevnu vrijednost biomase kretao se oko 47 %, što je također usporedivo s literaturnim navodima Licursi i sur. (2015.) te Grubor i sur. (2020.). Sadržaj hlapljivih tvari bio je od 79 % do 82 %, a pokazuje da će se tijekom izgaranja najveći dio biomase ispariti i izgorjeti kao plin u sustavu (García i sur., 2012.).

Tablica 3. Rezultati ogrjevne vrijednosti ulazne sirovine

Istraživani parametri (MJ kg^{-1})	*Arundo donax* L., jesenska žetva	*Arundo donax* L., proljetna žetva
Gornja ogrjevna vrijednost, HHV	17,85	16,64
Donja ogrjevna vrijednost, LHV	17,69	16,53

Iz tablice 3. uočava se kako HHV ovisi o roku žetve te je bila 17,85 MJ kg^{-1} kod jesenske i 16,64 MJ kg^{-1} kod proljetne žetve pa je vidljivo da su rezultati u suglasju sa navodima literature. Tako Grubor i sur. (2020.) navode HHV od 17,61 MJ kg^{-1}, te LHV od 16,62 MJ kg^{-1}, dok Jurišić i sur. (2014.) navode 17,20 MJ kg^{-1} za HHV i 16,28 MJ kg^{-1} za LHV.

Na grafu 1. prikazana je količina proizvedenog bioplina po danima za proljetnu i jesensku žetvu.



Grafikon 1. Količina proizvedenog bioplina iz ulaznih sirovina

Iz Grafikona 1. vidljiva je produkcija bioplina nakon obje žetve. Biomasa iz jesenske žetve imala je najveći ukupni prinos bioplina od 81,15 NL kg^{-1}st koji je dostignut u 23. danu anaerobne digestije. Ista žetva imala je jednak intenzitet proizvodnje biometana u prvim danima fermentacije te je maksimalan prinos od 73,73 NL kg^{-1}st u 18. danu fermentacije. S druge strane, proljetna žetva imala je niži ukupni prinos bioplina od 69,55 NL kg^{-1}st koji je dostignut u 21. danu fermentacije, dok je ukupan prinos biometana bio 59,18 NL kg^{-1}st već u 8. danu. Kao što je vidljivo *Arundo donax* L. može se efikasno iskoristiti u proizvodnji bioplina kao supstitucija ili dodatak tradicionalnim energetskim kulturama u kodigestiji s životinjskim gnojem ili drugom biomasom.

Zaključak

Temeljem vlastitih istraživanja mogućnosti korištenja biomase kulture *Arundo donax* L., ovisno o roku žetve, kao sirovine za proizvodnju biolina mogu se utvrditi kvalitetna energetska svojstva s obzirom na sadržaj negorivih (niži sadržaj pepela) te gorivih tvari (visok sadržaj ugljika i vodika). HHV je ovisila o vremenu žetve i kretala se od 16,64 do 17,85 MJ kg^{-1}, dok je LHV bila između 16,53 i 17,69 MJ kg^{-1}. Ove vrijednosti pokazatelj su visokog energetskog potencijala, što je dokazano i visokom produkcijom biplina iz jesenske žetve koja je imala najveći ukupni prinos od 81,15 NL kg^{-1}st te prinos biometana od 73,73 NL kg^{-1}st. Iz svega navedenom može se zaključiti da se *Arundo donax* L. može koristiti u proizvodnji bioplina kao dodatak ili potpuna zamjena kukuruzne silaže.

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The possibility of using *Arundo donax* L. as raw material for biogas production considering the harvest time

Abstract

Most biogas plants in the EU use corn silage as a co-substrate in biogas production, which represents a serious competition between energy supply and food production. This is why perennial energy crops like *Arundo donax* L. are an ideal solution, both ecologically and economically. In this study, samples were collected in March (spring harvest) and September (autumn harvest) of 2021, after which non-fuel and fuel properties, calorific value were determined and the process of anaerobic digestion was carried out. It was determined that the time of harvest affects the energy properties of biomass and the final yield of biogas, and that *Arundo donax* L. showed successful performance.

Keywords: *Arundo donax* L., harvest time, energy properties, biogas

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Korištenje poljoprivredne biomase kao sirovine za pelete i brikete

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Sažetak

Proizvodnja peleta i briketa iz poljoprivredne biomase značajna je gospodarska djelatnost u EU zbog jednostavnosti upotrebe pred drugim čvrstim biogorivima. Međutim, problem poljoprivredne biomase kao sirovine kod korištenja je njezina velika nasipna gustoća koja može uzrokovati povećane troškova žetve, transporta i skladištenja, a istovremeno smanjujući ekološku i energetsku održivost. Najučinkovitiji način rješavanja navedenih problema je njezino zgušnjavanje peletiranjem ili briketiranjem. Iz svega navedenog, cilj ovog rada je opisati proces peletiranja i briketiranja korištenjem poljoprivredne biomase kao sirovine te kako njena fizikalno-kemijska svojstva utječu na te procese.

Ključne riječi: peleti, briketi, poljoprivredna biomasa, fizikalno-kemijska svojstva

Uvod

Klimatske promjene uzrokovane prekomjernom emisijom stakleničkih plinova (GHG) usmjerile su pažnju prema korištenju lignocelulozne biomase koja bi svojim energetskim potencijalom mogla nadomjestiti fosilna goriva (Bilandžija i sur., 2016.). Biomasa kao gorivo ima i svojih nedostataka poput nepravilnog oblika, velikog volumena, niske nasipne gustoće (poljoprivredna 80 do 100 kg m^3, drvna 150 do 200 kg m^3), nejednakog izgaranja zbog različitih udjela vlage ovisno o žetvi/berbi, kao i manju ogrjevnu vrijednost naspram fosilnih goriva (Mitchell i sur., 2007.; Vassilev i sur., 2015.). Varijabilnost sastava biomase može biti rezultat različitih faktora poput vrste ili dijela biljke, sposobnosti usvajanja hranjiva iz tla, vode i zraka tijekom rasta, te njihovog odlaganja u biljnom tkivu; agroekoloških uvjeta uzgoja; količine korištenih gnojiva i zaštitnih sredstava; načina i vremena žetve, tehnike sakupljanja i uvjeta tijekom transporta i skladištenja; razlike u udjelu pepela, te kombiniranja različitih vrsta biomasa (Vassilev i sur., 2010.). Zadnjih desetljeća istraživanja su bila usmjerena na goriva svojstva te tehnologiju prerade, dorade i korištenja poljoprivredne biomase kao krutog biogoriva, poput izravnog izgaranja za proizvodnju energije (Saleem, 2022.). Kako bi se poljoprivredna biomasa mogla pretvoriti u kruto gorivo, potrebno je osigurati dostatne količine, a navedene nedostatke moguće je znatno popraviti zgušnjavanjem (Barry i sur., 2022.).

Zgušnjavanje poljoprivredne biomase

Zgušnjavanje biomase najviše ovisi o korištenom sustavu te procesnim (tlak, temperatura) i fizikalno-kemijskim svojstvima sirovine (udio vlage i pepela, veličina čestica, ogrjevna vrijednost, lignocelulozni sastav). Zgušnjavanjem poljoprivredne biomase procesom peletiranja i briketiranja, povezuju se čestice biomase pod djelovanjem visokog tlaka i temperature uzrokujući plastificiranje i omekšavanje lignina (Tumuluru i sur., 2011a.). Rezultat je kruto gorivo visoke gustoće koje olakšava doziranje u ložišta, smanjuje troškove transporta i skladištenja, a manji udio vlage olakšava čuvanje bez kvarenja (Matin i sur., 2019.). Kaliyan i Vance Morey (2010.) navode lignin kao glavnu komponentu koja utječe na vezanje čestica u lignoceluloznoj biomasi, a onoj koja ima manji udio lignina mogu se dodati i veziva organskog podrijetla poput proteina, škroba ili melase. Poljoprivredna biomasa u prosjeku sadrži 40-50 % celuloze, 20-30 % hemiceluloze i 10-25 % lignina (Iqbal i sur., 2011.) dok šumska biomasa u prosjeku sadrži od 50-55 % celuloze, 15-25 % hemiceluloze i 20-30 % lignina (Antonović, 2007.). Lignocelulozni sastav najvažnije poljoprivredne biomase naveden je u Tablici 1.

Tablica 1. Lignocelulozni sastav poljoprivredne biomase

Vrsta	Celuloza (%)	Lignin (%)	Hemiceluloza (%)	Izvor:
Slama pšenice	30,0	15,0	50,0	Chong i sur. (2021.)
Slama ječma	40,0	15,0	30,0	Saini i sur. (2015.)
Stabljika suncokreta	32,0	22,0	18,0	Antonopoulou i sur. (2015.)
Kukuruzovina	42,2	24,6	25,7	Grubor i sur. (2021.)
Miskantus	49,3	29,3	19,3	Bilandžija i sur. (2017.)
Divlje proso	40,3	20,1	30,5	Doczekalska i sur. (2020.)

Proces peletiranja

Peletiranje je termoplastični proces zgušnjavanja biomase istiskivanjem, pri čemu se čestice sirovine pod tlakom i temperaturom oblikuju u kompaktne pelete, povećavajući nisku nasipnu gustoću biomase na gotovo 1000 kg m^{-3} (Mani i sur., 2004.). Preme normi (EN ISO 17225-6:2021) peleti su cilindričnog oblika, promjera od 6 do 25 mm i duljine od 3,15 do 50 mm. Na kvalitetu peleta najviše utječu fizikalno-kemijska svojstava korištene biomase. Veći udio elemenata poput sumpora, kalija ili klora prisutnih u poljoprivrednoj biomasi smanjuje točku taljenja pepela te predstavlja rizik od nakupljanja troske i šljake kao i pojavu korozije u kotlovima i dimnjacima (Varnero i Urrutia, 2017.). Vlaga je ključna u procesu peletiranja i zajedno s udjelom lignina jedan je od najvažnijih parametara koji određuju trajnost peleta. Ogrjevna vrijednost označava energiju oslobođenu iz određene količine biomase koja potpuno izgori uz prisutnost kisika. Gornja ogrjevna vrijednost (HHV) podrazumijeva količinu energije oslobođene izgaranjem goriva pod određenim uvjetima uz kondenzaciju vode. HHV poljoprivredne biomase je između 12 i 18 MJ kg^{-1} (Smaga i sur., 2018.). U Tablici 2. navedena su najvažnija fizikalno-kemijska svojstva poljoprivredne biomase.

Tablica 2. Fizikalno-kemijska svojstva poljoprivredne biomase

Vrsta	Vlaga (%)	Pepeo (%)	HHV (MJ kg^{-1})	Izvor:
Slama pšenice	6,7	4,2	16,7	Matin i sur. (2019.)
Slama ječma	7,1	3,7	16,4	Grubor i sur. (2017.)
Stabljika suncokreta	9,9	3,7	17,6	Grubor i sur. (2017.)
Kukuruzovina	8,2	3,3	17,9	Matin i sur. (2019.)
Miskantus	6,6	1,3	17,7	Slupska i sur. (2019.)
Divlje proso	6,2	2,1	19,6	Caraschi i sur. (2019.)

Parametri kvalitete peleta

Peleti proizvedeni od nedrvne biomase klasificiraju se prema europskoj normi (EN ISO 17225-6:2021) koja standardizira klase kvalitete goriva i specifikacije klasificiranih nedrvnih peleta. Prvi europski standard kvalitete peleta razvila je Švedska 1998. godine, ali su i drugi nacionalni standardi poput Njemačkog DINplus-a ili Austrijskog ÖNORM M 71. Međutim, kada je 2010. prihvačen europski standard (EN), nacionalni standardi su povućeni ili usklađeni s EN standardom. Ovom normom obuhvaćeni su nedrvni peleti proizvedeni od sirovina poput zeljaste, voćarske, vodene biomase te njihovih kombinacija ili smjesa. Kvaliteta peleta ovisi o fizikalno-kemijskim i mehaničkim svojstvima biomase i parametrima peletiranja poput temperature i tlaka (Garcia-Maraver i Carpio, 2015.). Udio vlage mora biti ≤ 15 %, kako se dio topline izgaranja ne bi gubio na hlapljenje vlage iz goriva, dok udio pepela mora biti ≤ 10 % (Liu i sur., 2014.). Mehanička otpornost ili otpornost na abraziju je parametar kvalitete koji se ogleda u sposobnosti zgusnutih goriva da zadrže svoju strukturu netaknutom prilikom manipulacije (HRN EN ISO 16559:2014) i kod peleta mora biti ≥ 96 % (HRN EN ISO 17225–6:2021). Temperatura je vrlo važan čimbenik u procesu zgušnjavanja biomase te Omer i sur. (2020.) navode kako bi temperatura kod proizvodnje peleta trebala biti između 90 i 95°C. Tlak peletiranja i sile koje nastaju u matrici važni su za razumijevanje i optimizaciju samog

procesa. Općenito, tlak peletiranja je između 115 i 300 M Pa, pri čemu viši tlak producira trajnije pelete smanjujući opuštanje neposredno nakon formiranja (Adapa i sur., 2011.), a što su potvrdili Mani i sur. (2004.) kod peleta slame žitarica. U Tablici 3. navedena su osnovna svojstva peleta proizvedenih od poljoprivredne biomase koja se najviše koristi u procesu peletiranja zbog dobrih fizikalno-kemijskih svojstava.

Tablica 3. Osnovna svojstva peleta iz poljoprivredne biomase

Vrsta	Vlaga (%)	Pepeo (%)	Otpornost (%)	HHV (MJ kg^{-1})	Izvor:
Slama pšenice	9,4	9,1	94,4	18,3	Miranda i sur. (2015.)
Slama ječma	9,1	10,5	95,5	17,9	Miranda i sur. (2015.)
Stabljika suncokreta	13,8	3,0	87,4	19,9	Dyjakon i Noszczyk (2019.)
Kukuruzovina	8,4	4,9	96,0	18,9	Tumuluru (2014.)
Miskantus	8,0	1,7	93,0	17,8	Styks i sur. (2020.)
Divlje proso	7,0	3,6	87,0	18,2	Yub Harun i Afzal (2015.)

Proces briketiranja

Briketiranjem dolazi do termoplastičnog povezivanja čestica biomase kontinuiranim prešanjem klipom, pokretanim hidrauličnim ili mehaničkim pogonom, rezultirajući formiranjem briketa visoke gustoće od 900 do 1300 kg m^{-3} (Tumuluru i sur., 2011b.). Sirovina veličine čestica ispod 10 mm i vlage ispod 15 % tlači se u kalupima, najčešće okruglog ili pravokutnog oblika, u briket promjera većeg od 25 mm i duljine do 400 mm (Krička i sur., 2017). Korištenje poljoprivredne biomase za proizvodnju briketa može pridonijeti uštedi resursa, smanjenju korištenja fosilnih goriva (Wang i sur., 2017.), riješiti problem odlaganja poljoprivrednih ostataka te smanjiti prekomjerno korištenje šumske biomase i emisiju CO_2 (Bhattacharya i sur., 2002.). Prednost pioljoprivrednog briketa, u odnosu na konvencionalno ogrjevno drvo, ogleda se u kompaktnosti, jednostavnosti uporabe, čistoći, konstantnosti temperature tijekom izgaranja te većoj HHV (Dinesha i sur., 2019.). Za učinkovito briketiranje udio vlage u biomasi trebao bi biti između 5 i 15 %, dok bi udio pepela trebao biti manji od 4 % jer veći udio uzrokuje manju HHV (Maia i sur. 2014.; Mopoung i Udeye 2017.). Udio vlage kao i kemijski sastav biomase ima veliki utjecaj na ogrjevnu vrijednost briketa te predstavlja količinu energije oslobođene prilikom potpunog izgaranja goriva (Demirbas, 2017.). Veličina čestica biomase tijekom proizvodnje određuje trajnost i gustoću briketa, a trebala bi biti između 1 i 10 mm (Maia i sur. 2014.; Mopoung i Udeye, 2017.). Adetogun i sur. (2014.) navode da su tijekom proizvodnje briketa od klipa kukuruza najbolju ogrjevnu vrijednost od 24.97 MJ kg^{1} dale čestice veličine 6,3 mm.

Parametri kvalitete briketa

Kvalitetan briket mora imati nizak udio vlage i pepela te zadovoljavajuću HHV (Arewa i sur., 2016.). Na postupak briketiranja utječu i parametrima poput temperature, tlaka i vremena zadržavanja (Dinesha i sur., 2019.), pri čemu je optimalni raspon temperature između 100 i 250°C, a optimalni tlak između 50 i 250 M Pa (Ibitoye i sur., 2021.). Vrijeme zadržavanja podrazumijeva isti primijenjeni pritisak kratkog trajanja na sirovinu unutar kalupa, a utječe na kompaktnost i kvalitetu briketa. Istraživanja pokazuju kako bi vrijeme zadržavanja trebalo biti između 20 i 40 sekundi (Li i Liu, 2000.). U Tablici 4. navedena su osnovna svojstva (vlaga, pepeo, trajnost i HHV) briketa proizvedenih od poljoprivredne biomase.

Tablica 4. Osnovna svojstva briketa iz poljoprivredne biomase

Vrsta	Vlaga (%)	Pepeo (%)	Trajnost (%)	HHV (MJ kg^{-1})	Izvor:
Slama pšenice	8,7	7,0	92,0	19,7	Mitchell i sur. (2020.)
Slama ječma	7,8	6,5	96,1	19,8	Mitchell i sur. (2007.)
Stabljika suncokreta	9,1	7,9	94,3	18,7	Geletukha i Zheliezna (2014.)
Kukuruzovina	6,7	3,3	88,5	18,6	Mahu i sur. (2013.)
Miskantus	7,5	3,1	91,0	17,7	Lixandru i sur. (2013.)
Divlje proso	5,9	3,7	92,8	17,2	Karunanithy i sur. (2012.)

Zaključak

Sve veća potražnja za krutim gorivom poput peleta i briketa otvara mogućnosti raznovrsnije upotrebe poljoprivredne biomase. Za razliku od šumske, poljoprivredna biomasa je godišnje dostupna u velikim količinama i cjenovno povoljnija, a može ju se učinkovito iskoristiti zgušnjavanjem čime se značajno pospješuje iskoristivost energije deponirane u biomasi. Peletiranjem i briketiranjem poljoprivredne biomase postiže se veća nasipna gustoća i bolja fizikalno-kemijska svojstva što ju čini praktičnijom za upotrebu.

Napomena

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Use of agricultural biomass as raw material for pellets and briquettes

Abstract

The production of pellets and briquettes from agricultural biomass is a significant economic activity in the EU due to its ease of use compared to other solid biofuels. However, the problem of using agricultural biomass as a raw material is its high bulk density, which can cause an increase in harvesting, transportation and storage costs, and at the same time reduce environmental and energy sustainability. The most effective way to solve the mentioned problems is its densification by pelletizing or briquetting. From all the above, the aim of this work is to describe the process of pelleting and briquetting using agricultural biomass as raw material and how its physico-chemical properties affect these processes.

Keywords: pellets, briquettes, agricultural biomass, physico-chemical properties

Impacts, threats, and potential benefits of invasive plant species

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Abstract

Vast migrations caused the first movement of plants and animals from one region to another, along with the seeds of food crops and livestock. Most of these species, especially invasive ones, have significant direct and indirect impacts on the economy, agriculture, and health. Monitoring and controlling invasive alien species is estimated to cost billions of dollars annually worldwide. After habitat destruction, climate change, and pollution, invasive alien species are one of the greatest threats to biodiversity. A fraction of the money spent on monitoring, controlling, and eradicating invasive alien species could be recouped by integrating these species into the circular economy, generating value-added products or energy, and obtaining high-value specialized metabolites.

Keywords: invasive alien species, biomass, energy crisis, circular economy

Introduction

Globalization, migration, trade, and increased transportation are all variables that contribute to the potential spread of alien species and thus the possibility of biological invasions (Hulme, 2009). These species can be spread either intentionaly or accidentaly. Unintentional introduction occurs via a vector (vehicles, ships, airplanes, clothing, shoes, feathers, hair, other species, etc.) as opposed to intentional introduction (ornamental plants, food, exotic fruits and vegetables, timber industry, research, etc.). Two-thirds of alien plant species have been intentionally introduced into Europe (Novak, 2018.). Humans have triggered the irreversible spread of species across the planet, overcoming geographic limitations. Human activities have indirectly contributed to the spread and invasion of alien species. Little space remained uninhabited by new species, some of which exhibited the characteristics of invasiveness (Jurković, 2012.). Native and alien species differ according to habitat. Most introduced species cannot survive outside their native growing conditions. For every 100 alien species introduced, an average of 10 survive in the new environment, while 1 become invasive. Whether a species becomes invasive in a new region depends on its characteristics and ecology (Keller et al., 2011.). Accordingly, an invasive alien species (IAS) would be an invader, i.e., a stranger. Although IAS are the main problem today, native species can also be harmful. Some native plants can become invasive under altered environmental conditions (e.g., soil nutrient supply, colonization by IAS) (Pyšek et al., 2004.). Species that can overcome barriers, reproduce and spread in the new habitat, have a negative impact in the new habitat, disrupt ecosystem processes, have negative impacts on humans, plants, and wildlife, and reduce biodiversity are referred to as IAS. The Convention on Biological Diversity defines an IAS as a species that has spread outside its normal range and threatens biodiversity (CBD, 2010.; CBD, 2020.). According to the EU regulation, an IAS is one whose introduction or spread threatens or negatively affects biodiversity and associated ecosystem services. After habitat destruction, climate change, and pollution, IAS are one of the greatest global threats to biodiversity (HAOP, 2020).

Because of all these invasive and aggressive capabilities, IAS are fast-growing species that continuously spread and produce a large amount of biomass while taking space from other plant and animal species. Their growing biomass and intended use are inextricably linked, which is why they are mainly considered pests. Their annual biomass production is a major problem because it must be disposed of as waste. IAS biomass is not waste, only a solution for its use must be found (Miguez et al., 2022.). Studies show that IAS biomass can be used for various purposes. It can be used to produce textiles, animal feed, medical preparations, firewood, biochar, biogas, etc (Van Meerbeek et al., 2015.). This is an interesting consideration as a major energy crisis is looming and energy sources are difficult to access. But IAS biomass can serve as a solution to this problem as it offers huge amounts of annual biomass (Ahmed et al., 2020.). Besides the ecological (pedological, climatological, botanical, zoological, etc.), health (allergies, poisoning

and diseases) and economic (agriculture, forestry, tourism, infrastructure, human health, etc.) impacts, it can also be concluded that the use of their biomass could reduce agricultural and economic losses, which are relatively high (Duncan et al., 2014.). Due to the high population growth rate, ability to spread rapidly over large areas, and invasiveness of IAS, uncontrolled production of biomass could lead to a viable solution to the energy dilemma, such as thermochemical generation of biofuels and energy from such wastes (Okoro et al., 2020.).

Impacts

Since the discovery and colonization of the New World, the problem of IAS has intensified, and in the twenty-first century they have become one of the greatest threats to biodiversity (CBD, 2020.). An increasing number of studies (Vila et al., 2011.) have analyzed the characteristics of IAS prone to invasion and have significant negative impacts on the ecosystem and human welfare. Duncan et al., (2014) stated that the impact of IAS in a given region can be observed in terms of plant community fertility and productivity, species diversity, influence of allelopathic compounds, displacement of native species, threats to certain species that enable them to reproduce, competition for food and habitat, transmission of diseases, hybridization with native species, and alteration of ecosystem structure and function. Bradley et al (2010.) emphasize that climate change may also affect the ability of IAS to survive in certain regions and their ability to compete with native species. In addition to ecological conditions (pedological, climatological, botanical, zoological, etc.), there are also health and economic impacts (allergies, poisonings, and diseases) (Duncan et al., 2014.).

Without neglecting the full spectrum of ecological problems caused by IAS, their spread into areas inhabited by native species is particularly noteworthy. In addition to displacing native species, IAS consume light, water, and nutrients; alter habitat conditions through increased soil erosion, accumulation of silt in freshwater systems, and shading of native species; alter conditions in the structure and composition of biological communities; have a predatory/parasitic relationship with native species; and their presence causes extinctions of native species, which can have cascading effects on ecosystems (Flory and Lockwood, 2020.).

Threats

There are numerous ways in which IAS cause harm to the environment in which they occur. When a new, aggressive species is introduced into an environment, it often has no natural enemies, there are no effective management or control methods, and it destabilizes the ecosystem (MINGOR, 2022.). These species enable and support rapid reproduction and spread, occupy vast areas, and smother native or indigenous vegetation. They compete with native species for nutrients and other resources, alter ecological conditions, transmit diseases to native species, interbreed with them, and hinder their reproduction by causing repeated succession of plants (Flory and Lockwood, 2020.). Consequently, IAS have a direct impact on biodiversity, the environment, and entire ecologies (Novak, 2018.). In addition to their impact on local flora, IAS can also have direct and indirect effects on human health. Various respiratory or skin allergic reactions, such as pollen-induced allergies or dermatitis triggered by phytotoxic plant sap, are considered direct impacts (MINGOR, 2022.).

In addition to environmental and health concerns, IAS also have negative economic consequences. They cause significant economic damage by reducing crop yields in agriculture, forestry, and fisheries, and it is expensive to stop their spread. Especially considering that the majority of economic, agricultural, and recreational activities depend on a healthy environment (Novak and Kravrščan, 2011.). Loss of agricultural production due to overgrowth of pastures with inedible grasses, costs of chemicals and machinery required to control IAS, the health problem caused by pathogenic and toxic alien species leading to loss of social productivity due to illness or death of affected people, and loss of tourism revenue due to disease epidemics are just some of the direct costs that affect society and the economy as a whole. Indirect costs include contamination around dams and rivers due to increased soil erosion, reduction in property values due to ecosystem alteration, damage to infrastructure due to ecosystem alteration, increased frequency of fires, floods, or landslides, and the costs of condition monitoring, surveillance, education, remediation, and control of the above activities (Charles and Dukes, 2008.). IAS also pose an indirect threat. IAS can alter the food web of an ecosystem by destroying or displacing native food sources. In addition, they may provide little or no nutritional value in the form of food for wildlife and affect the number or diversity of species important to wildlife habitat. Aggressive plant species can quickly turn a diverse environment into a monoculture, while others can alter ecosystem conditions such as soil chemistry or wildfire intensity (Edward, 2003.).

Assessments and research findings on alien IAS are critical, especially for countries at higher risk of invasion, to guide efforts to prevent the introduction and spread of alien species. In this way, it is possible to help protect human health, the economy, infrastructure, native species, and associated ecological values (Njoki, 2017.) IAS often cannot be eradicated from their native habitats except on smaller islands and other limited sites. Therefore, preventive measures through legal restrictions on the introduction of alien species into the natural environment, early detection of potentially IAS, and implementation of immediate steps to control their spread and eradication are critical. The most successful methods against IAS are the implementation of prevention and control measures at the earliest stage of invasion. According to the European Commission in 2014, Europe spends about 4.5 billion euros each year to mitigate the harmful effects of IAS, and this is only from the perspective of agriculture and human health. It is estimated that the annual damage in Europe exceeds 12.5 billion euros, while estimates in the United States are as high as 90 billion euros (Sundseth, 2014). The consequences of the spread of IAS include negative impacts on biodiversity, human life and health, disruption of ecosystem functioning, significant economic losses and damage to economic development (ecological, pedological, climatological, botanical, zoological; health impacts in the form of allergies, poisoning and diseases; economic, agricultural, forestry, tourism, infrastructure impacts, etc.). An increasing number of research is addressing the characteristics of IAS and the characteristics of communities that are sensitive to invasions and have significant impacts on the ecosystem and human well-being (Vila et al, 2011.; Moravcova et al., 2015.).

Potential benefits

Unfortunately, the strict focus on reducing the impact of IAS leads to ignoring their potential use as biomass source. IAS are not considered a nuisance and waste, but can have ecological benefits. For example, they can be used for the production of various products such as honey. Unlike other plant species, they can provide pollen and nectar to bees and insects during climatically difficult seasons because they are highly resilient to adverse environmental conditions (Aniko et al., 2022.). They can also serve as a natural source of specialized metabolites and other chemicals with antioxidant and antibacterial activity, or as an ingredient for the production of pharmaceuticals. In addition, they can be used as a raw material for firewood, biofuel, biogas, etc., and contribute to the reduction of greenhouse gas emissions, unlike fossil fuels. In addition, certain non-native plants provide ecological benefits, such as shelter and food resources for native species or timber production (Hanley and Roberts, 2019.). Although their spread and the amount of biomass they produce are among the greatest risks to biodiversity, their availability in large quantities can be beneficial in creating novel, cost-effective, and environmentally friendly solutions to future challenges (Van Meerbeek et al., 2015). Contrary to popular belief, alien IAS could be considered as ecological, economic, or even social change agents that require extensive research, continuous adaptation, and improvement of existing systems for control and management.

Conclusions

In just a few years, the claim that IAS are one of the leading drivers of biodiversity loss around the world has become the most cited source in several publications and studies dealing with biodiversity or IAS. However, in reality, IAS are mostly associated with waste and huge amounts of biomass, as well as with a problem for which there is no viable solution. In Croatia, the European Union and the rest of the world, there is insufficient research on IAS, especially plant species and biodiversity. There is a legal framework for IAS, but the implementation of agreements, policies and guidelines requires a much stronger commitment from individuals and local communities, especially from the government and legislative bodies of the Republic of Croatia, the EU and the whole world. In such a disorganized environment, it is extremely difficult to monitor IS, let alone prevent its spread. Moreover, every year the EU and the rest of the world invest more time and money to remove them from the nature and take care of them, but these are obviously only temporary measures, because it is impossible to eradicate the mentioned species permanently.

More consideration should be given to the potential use of their biomass. Just as IAS have adapted to a variety of environmental situations, we also need innovation and flexibility to effectively deal with coming disasters.

There are few IAS whose risk is recognized on a global scale. Their influence and impact will soon be felt across a wide range of sectors, including agriculture, business, and the economy. Estimates put global losses in the billions of dollars, while there is no adequate monitoring system for IAS. Therefore, it is essential to take adequate precautions and focus on the problem of IAS, as well as to find a solution that will not only keep IAS under control, but also provide a solution in the form of their further use and serve as an example of a circular economy.

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Utjecaji, prijetnje i potencijalne koristi od invazivnih biljnih vrsta

Sažetak

Masovne migracije uzrokovale su prva kretanja biljaka i životinja iz jedne regije u drugu, zajedno sa sjemenjem prehrambenih usjeva i stoke. Većina ovih vrsta, posebice invazivnih, ima značajne izravne i neizravne gospodarske, poljoprivredne i zdravstvene posljedice. Procjenjuje se da praćenje i kontrola invazivnih stranih vrsta stoji milijarde dolara diljem svijeta godišnje. Nakon uništavanja staništa, klimatskih promjena i onečišćenja, invazivne strane vrste jedna su od najvećih prijetnji bioraznolikosti. Dio novca potrošenog na praćenje, kontrolu i iskorjenjivanje stranih vrsta mogao bi se nadoknaditi integracijom tih vrsta u kružno gospodarstvo, stvaranjem proizvoda ili energije s dodanom vrijednošću i ekstrakcijom specijaliziranih metabolita visoke vrijednosti.

Ključne riječi: strane invazivne vrste, biomasa, energetska kriza, kružno gospodarstvo

ORIGINAL SCIENTIFIC PAPER

Vegetacijski indeksi kao alati za procjenu prinosa pšenice

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Sažetak

Aktualne i nadolazeće klimatske promjene odrazit će se na uzgoj pšenice u obliku smanjenog prinosa, povećanih troškova te nužnog odstupanja od tradicijskog uzgoja. Cilj ovoga rada je prikazati mogućnosti procjene prinosa za pet sorata ozime pšenice uzgojenih na tri mikroklimatski različite lokacije korištenjem vegetacijskih indeksa. Tijekom 2022. godine, praćen je rast i razvoj ozime pšenice prijenosnim spektroradiometrom te su izračunati vegetacijski indeksi NDVI (Vegetacijski indeks normalizirane razlike) i SAVI (Vegetacijski indeks prilagođen tlu) u fenofazama busanje i klasanje. Zabilježeni su prinosi zrna u kg ha^{-1}. Dobivene su korelacije vegetacijskih indeksa NDVI i SAVI i prinosa zrna te opisane linearnim modelima srednje jakosti. Dobiveni rezultati mogu se koristiti za planiranje preciznih agrotehničkih mjera.

Ključne riječi: vegetacijski indeksi, NDVI, prinos, ozima pšenica, spektroradiometar

Uvod

Pšenica (*Triticum aestivum* L.) je jedan od najrasprostranjenijih usjeva u svijetu gdje se uzgaja na više od 220 milijuna ha godišnje. Kako bi se zadovoljila svjetska sigurnost hrane, prinosi osnovnih prehrambenih usjeva kao što je pšenica morat će se povećati kako bi odgovorili na rastuću potražnju (Lin i Huybers, 2012.). Međutim, učinci klimatskih promjena mogu učiniti postizanje ovog cilja izazovnim. Klimatske promjene utječu na proizvodnju žitarica uglavnom zbog abiotskog stresa (toplinski valovi, suša, poplava i mraz) te poboljšanjem uvjeta za razvoj i napad štetnika i bolesti, odnosno biotskih čimbenika stresa (Porter i sur., 2014.). Navedeni problemi predstavljaju izazov za uzgajivače i znanstvenike koji imaju ograničeno vrijeme i resurse za prilagodbu novim uvjetima uzgoja. U današnje se vrijeme u poljoprivredi fokus stavlja na primjenu daljinskih istraživanja. Koristeći multi- i hiperspektralne senzore, koji su sposobni mjeriti spektralnu refleksiju biljaka, mogu se izračunati brojni vegetacijski indeksi korisni za procjenu biomase, prinosa i zdravstvenog stanja usjeva (Lillesand i sur., 2004.). Osim što primjena vegetacijskih indeksa pruža objektivnu osnovu za analiziranje i upravljanje poljoprivrednom površinom, njima je moguće procijeniti prinos usjeva ranije u vegetaciji te na njega konačno utjecati prezicnim agrotehničkim mjerama kao što su gnojidba, navodnjavanje i zaštita od štetnika i bolesti (Skendžić, 2022.). Cilj ovoga rada je prikazati mogućnosti procjene prinosa za pet sorata ozime pšenice uzgojenih na tri mikroklimatski različite lokacije na području Grada Zagreba i Zagrebačke županije korištenjem vegetacijskih indeksa dobivenih pomoću spektroradiometra. Hipoteza je da postoji korelacija između prinosa zrna pet sorata ozime pšenice, uzgojenih na mikroklimatski različitim lokacijama, i vegetacijskih indeksa NDVI i SAVI mjerenih u dvije fenofaze: busanje i klasanje. Dobiveni podaci mogu predstavljati preduvijet za planiranje i primjenu preciznih agrotehničkih mjera.

Materijali i metode

Istraživanje se provelo na tri mikroklimatološki i pedološki različite lokacije na području grada Zagreba i Zagrebačke županije; Rugvica (45°45'04.1"N 16°13'57.6"E), Botinec (45°44'49.1"N 15°56'13.4"E) i Odranski Obrež (45°43'58.9"N 15°57'04.6"E). Na sve tri lokacije tijekom 2022. godine praćen je rast i razvoj pet sorata ozime pšenice Bc Instituta; Bc Anica, Bc Ljepotica, Bc Vlatka, Bc Premija i Bc Opsesija, na ukupnoj površini od prosječno 0,4 ha po lokaciji. Tijekom vegetacije ozime pšenice na pokusnom polju u Rugvici vladali su optimalni uvjeti za uzgoj,

u Botincu je bio prisutan biljni stres od napada štetnika, a na polju na lokaciji Odranski Obrež, teksturno lakše tlo i slabi kapacitet zadržavanja vode u tlu induciraju jači vodni stres u uzgoju pšenice u odnosu na prethodne dvije lokacije. U fenofazama busanje (18.02.2022.) i klasanje (19.05.2022.) izvršeno je mjerenje spektralnih refleksija biljaka ozime pšenice pomoću prijenosnog spektroradiometra (Spectral Evolution® SR-2500). Instrument simultano bilježi 2151 vrpcu koje pokrivaju spektralni raspon valnih duljina od 350 do 2500 nm sa spektralnom rezolucijom od 5 nm (350-1000 nm) i 22 nm (1000 – 2500 nm).Vegetacijski indeksi su izračunati na osnovi prosijeka vrijednosti specifičnih valnih duljina spektralnih refleksija sklopa ozime pšenice, mjerenih in-situ s fiksne udaljenosti od 60 cm. Prije početnih očitanja uređaj se kalibrirao putem optički stabilne bijele kalibracijske ploče ($BaSO_4$, SphereOptics GmbH). U ovom istraživanju korišteni su indeksi (1) NDVI (Normalized Difference Vegetation Index) i (2) SAVI (Soil Adjusted Vegetation Index) izračunati prema formulama:

$$NDVI = \frac{NIR-RED}{NIR+RED} \quad \text{.....(1)}$$

$$SAVI = \frac{(NIR-RED)}{(NIR+RED+L)} * (1+L) \quad L = 0{,}5 \text{ .....(2) (Panda i sur., 2010.)}$$

Također su izračunati i zabilježeni prosječno ostvareni prinosi zrna u kg/ha za svaku sortu i lokaciju u pokusu.

Rezultati i rasprava

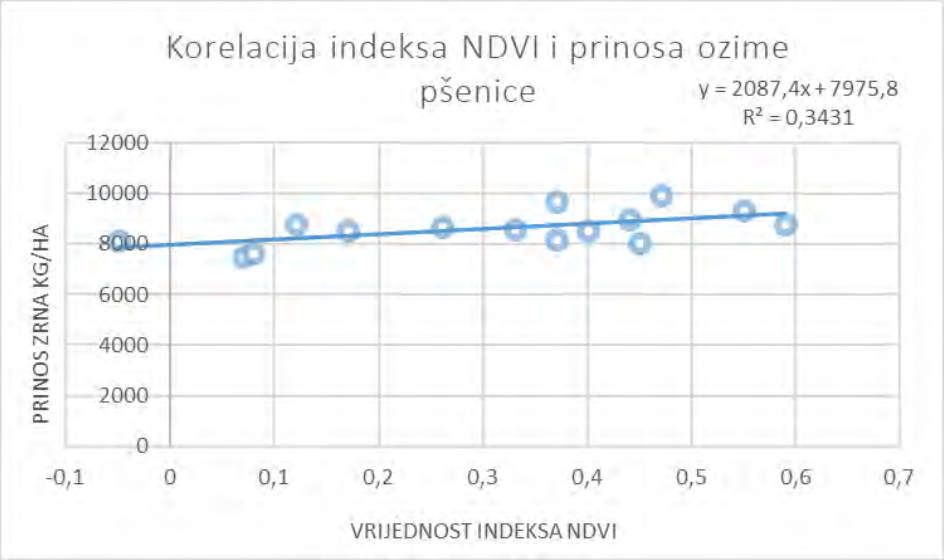
Prosječno ostvareni prinos zrna svih sorata u Rugvici iznosio je 9049 kg ha^{-1}, u Botincu 8612,5 kg ha^{-1}, a u Odranskom Obrežu 7816,5 kg ha^{-1}. Vrijednosti izračunatih indeksa NDVI i SAVI kao i prinosi zrna pojedinih sorata u pokusu prikazani su Tablicom 1.

Tablica 1. Prikaz prinosa pet sorata ozime pšenice na tri lokacije i vrijednosti izračunatih vegetacijskih indeksa NDVI i SAVI za dvije fenofaze (busanje i klasanje)

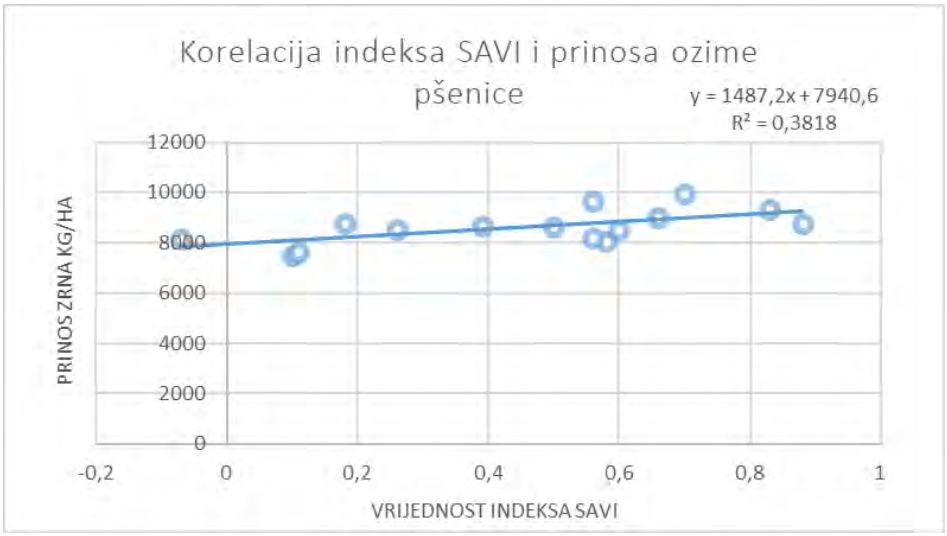
Lokacija	Sorta	Prinos zrna (kg ha^{-1})	NDVI		SAVI	
			busanje	klasanje	busanje	klasanje
Rugvica	Bc Anica	8782	0,59	0,88	0,88	1,32
	Bc Ljepotica	9654	0,37	0,88	0,56	1,31
	Bc Vlatka	9933	0,47	0,89	0,7	1,34
	Bc Premija	8050	0,45	0,88	0,58	1,32
	Bc Opsesija	9316	0,55	0,91	0,83	1,36
Botinec	Bc Anica	8554	0,17	0,67	0,26	1,00
	Bc Ljepotica	8988	0,44	0,73	0,66	0,91
	Bc Vlatka	8607	0,33	0,79	0,50	1,19
	Bc Premija	8515	0,40	0,70	0,60	1,06
	Bc Opsesija	8671	0,26	0,83	0,39	1,11
Odranski Obrež	Bc Anica	7478	0,07	0,65	0,10	0,98
	Bc Ljepotica	8197	0,37	0,61	0,56	0,91
	Bc Vlatka	8762	0,12	0,86	0,18	1,29
	Bc Premija	7619	0,08	0,72	0,11	1,07
	Bc Opsesija	8155	0,05	0,83	0,07	1,23

Dobivene su pozitivne korelacije prinosa zrna pšenice s vrijednostima vegetacijskih indeksa NDVI i SAVI izmjerenih u fenofazi busanje te su pokazale vezu srednje jakosti (R^2=0,34 za NDVI, R^2=0,38 za SAVI) (Chaddock, 1925.). U

Grafikonima 1. i 2. prikazane su analize korelacije između prinosa zrna pšenice, ostvarenih s tri lokacije, i vrijednosti indeksa NDVI i SAVI.

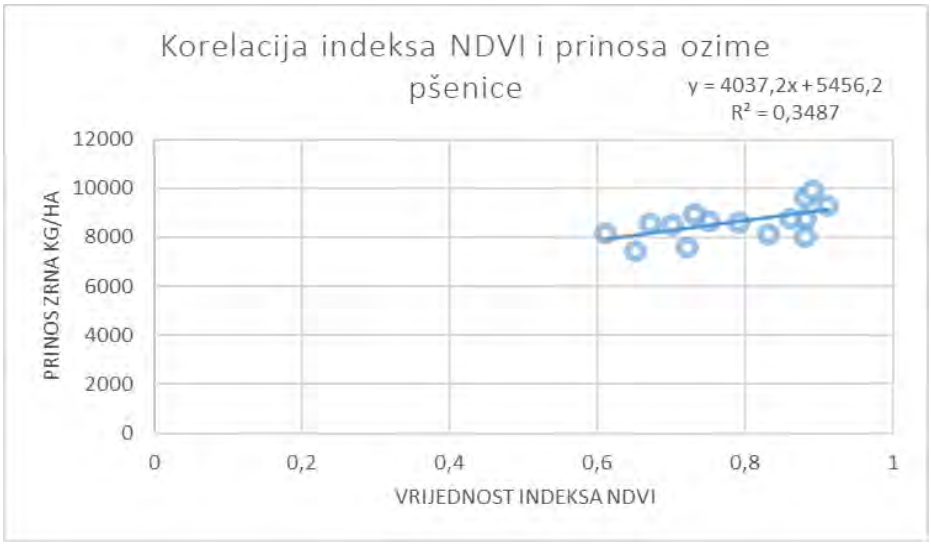


Grafikon 1. Korelacija prinosa zrna (kg ha^{-1}) i indeksa NDVI izmjerenog u fenofazi busanje

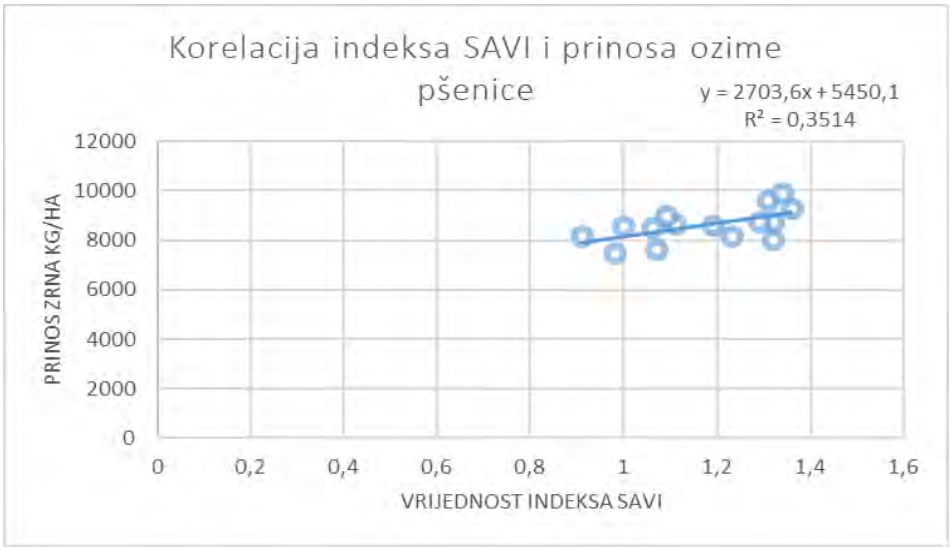


Grafikon 2. Korelacija prinosa zrna (kg ha^{-1}) i indeksa SAVI izmjerenog u fenofazi busanje

Dobivene su pozitivne korelacije prinosa zrna pšenice s vrijednostima vegetacijskih indeksa NDVI i SAVI izmjerenih u fenofazi klasanje te su pokazale vezu srednje jakosti (R^2=0,35 za NDVI, R^2=0,35 za SAVI) (Chaddock, 1925.). Grafikonom 3. i 4. prikazane su analize korelacije između prinosa zrna pšenice, ostvarenih s tri lokacije, i vrijednosti indeksa NDVI i SAVI.



Grafikon 3. Korelacija prinosa zrna (kg ha^{-1}) i indeksa NDVI izmjerenog u fenofazi klasanje



Grafikon 4. Korelacija prinosa zrna (kg ha^{-1}) i indeksa SAVI izmjerenog u fenofazi klasanje

Rezultati pokazuju porast vrijednosti vegetacijskih indeksa NDVI i SAVI mjerenih u fenofazi klasanje u odnosu na fenofazu busanje. NDVI je bitan vegetacijski indeks jer se njime mogu pratiti sezonske vegetacijske promjene, a normiranje smanjuje oblike spektralnih šumova u atmosferi (vlažnost zraka, razlike u osvjetljenosti od Sunca, i sl.). Budući da je NDVI vrlo osjetljiv na varijacije pozadine biljnog sklopa, te su njegove vrijednosti više s tamnijom pozadinom, za potrebe procjene biomase i zdravstvenog stanja usjeva često se koristi indeks SAVI koji predstavlja modifikaciju NDVI indeksa. Indeks SAVI koristi se radi otklanjanja utjecaja tla i atmosfere gdje parametar L predstavlja udio spektra koji je reflektiran od tla te na taj način eliminira njegov utjecaj (Jensen, 1996.). Vrijednosti dobivenih koeficijenata determinacije (R^2) su u rasponu od 0,34 do 0,38 za sve analizirane korelacije što ukazuje na podjednaku preciznost procjene prinosa za oba termina (fenofaze) mjerenja te za oba korištena vegetacijska indeksa. Za ostvarivanje preciznijeg modela procjene prinosa potrebno je frekventnije prikupljati podatke spektralne refleksije i vegetacijskih indeksa te uključiti i druge tehnologije daljinskih istraživanja kao što su multi- i hiperspektralne kamere i senzori nošeni satelitima ili bespilotnim letjelicama.

Zaključci

Dobivena je korelacija prinosa zrna pšenice i vegetacijskih indeksa NDVI i SAVI te su opisane linearnim modelima srednje jakosti (R^2=0,34 - 0,38). Dobiveni rezultati mogu se koristiti za planiranje i primjenu preciznih agrotehničkih mjera.

Napomena

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Vegetation indices as tools for wheat yield evaluation

Abstract

Current and upcoming climate changes will impact wheat production in the form of reduced yields, increased costs, and a necessary deviation from traditional cultivation. The aim of this paper is to show the possibilities of yield estimation for five winter wheat cultivars grown in three microclimatically different locations using vegetation indices. In 2022, the growth and development of winter wheat was observed with a portable spectroradiometer, and the vegetation indices NDVI (Normalised Difference Vegetation Index) and SAVI (Soil Adjusted Vegetation Index) were calculated at tillering and heading stages. Grain yields in kg/ha were recorded. Correlations between the vegetation indices NDVI and SAVI and grain yield were determined and described by linear models of medium strength. The obtained results can be used for planning precise agrotechnical measures.

Keywords: vegetation indices, NDVI, yield, winter wheat, spectroradiometer

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Volatile effect of essential oils on the growth of *Rhizoctonia solani* and *Pyrenophora graminea*

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Abstract

The aim of this study was to investigate *in vitro* the volatile effect of twelve essential oils on the growth of *Rhizoctonia solani* and *Pyrenophora graminea* mycelia. The essential oils were applied in a volume of 25 µl at temperatures of 15, 20, and 30 °C. The zone of inhibition was measured at 96 hours and 192 hours after inoculation. Anise and thyme oils had the strongest volatile effect on the growth of mycelia of *R. solani* and *P. graminea*, which were not affected by different incubation times and temperatures. Pine and sweet orange oils had the weakest volatile effect on the mycelial growth of *R. solani* and *P. graminea*.

Keywords: volatile effect, essential oils, *Rhizoctonia solani*, *Pyrenophora graminea*

Introduction

The genus *Rhizoctonia* contains one of the very important and economically significant species *Rhizoctonia solani* Kuehn (teleomorph of *Thanatephorus cucumeris* (Frank) Donk). *R. solani* is a soil polyphage that is distributed worldwide (Tsror, 2010). This pathogen parasitizes numerous plant species (over 200 plant species) in various agroclimatic areas. The optimal temperature for the growth of the fungus is between 23 and 28 °C (Goswami et al., 2011). Many of the world's important crops such as sugar beets, cotton, cereals, grasslands, ornamentals, fruit trees, and forest trees are hosts to various species of the genus *Rhizoctonia* (Sneh et al., 2013). The losses caused by the negative impacts of soilborne pathogens are often underestimated (Foley et al., 2016). Despite many efforts, the identification of cultivars resistant to *R. solani* appears to be difficult. One of the reasons for the difficult identification could be the necrotrophic lifestyle of *R. solani* (Kazan and Lyons, 2014).

Pyrenophora graminea S. Ito & Kurib. is the cause of the streakiness of barley leaves. It occurs only when seed previously infected with this pathogen has been sown (Ficsor et al., 2020), and in the past it was the most widespread and damaging barley disease, resulting in complete yield losses. It follows that the greatest risk for the occurrence of this disease is the sowing of the so-called "farmer's seed" that has not been previously treated with fungicides. Characteristic symptoms are visible on the leaves of infected plants in the form of chlorotic streaks along the entire length of the leaf, and all parts of the plant may become infected, including the roots of young plants. When it infects the root, it loses its ability to absorb water and nutrients, and the plants wither and die. At the end of the growing season, the leaves tear into strips and turn brown. Infected plants are retarded in growth and do not develop, while those that develop an ear contain a handful of grains (Jurković et al., 2016). Considerable efforts have been made to control this disease by breeding resistant cultivars, but any emergence of new pathotypes would negate the resistance achieved because of the marked variability of the pathogen (Arabi and Jawhar, 2012). Arabi et al. (2012) found that plants infected with the fungus *P. graminea* have a harder time withstanding high temperatures because the fungus, which requires nutrients under extreme conditions, penetrates deeper into the plant, breaks through the cell walls, and spreads further into the host's tissues.

The requirement to increase production while maintaining high quality is most easily achieved through the integrated method of agricultural production, which even today involves the repeated use of chemical agents and mineral fertilizers (Grgić et al., 2021). Because chemical fungicides are highly toxic and non-biodegradable, this method of

controlling plant diseases results in a significant environmental impact (Bajpai et al., 2010). With the emergence of increasingly common resistance to fungicides and efforts to reduce the use of chemicals in agricultural production, there is interest in alternative measures to control seed pathogens (Jevtić et al., 2022).

Numerous authors state that essential oils are alternative biological compounds due to their proven inhibitory effects on pathogens (Abdel-Kader et al. 2011; Nazzaro et al. 2017).

Various extracts and botanicals such as essential oils and their constituents offer a possible alternative to chemical pesticides (Kishore et al., 2007). According to Wilkins and Board (1989), more than 1300 plants synthesize compounds with antimicrobial activity. Essential oils are synthesized from aromatic plants and are very complex volatile compounds used to protect plants from disease and are considered safe for humans and animals (Isman, 2000). Essential oils are natural mixtures that often contain a large number of constituents (20 to 60), of which 90% of the oil itself is usually composed of three major constituents and the remainder is less than 1% (Bakkali et al., 2008, Dorman and Deans, 2000). Components of essential oils such as terpenes and terpenoids indicate rapid degradation and antifungal activity, leading to the possible future development of essential oils as natural alternatives to reduce the negative effects of chemical fungicides (Camiletti et al., 2014, Isman, 2000).

The aim of this study was to investigate *in vitro* the volatile effect of twelve essential oils at temperatures 15, 20, and 30 °C on the growth of *Rhizoctonia solani* and *Pyrenophora graminea* mycelia.

Material and methods

The test of the volatile effect of essential oils on the phytopathogenic fungi *R. solani* and *P. graminea* was carried out in the Central Agrobiotechnical Analytical Unit of the Faculty of Agrobiotechnical Sciences Osijek. The volatile effect of 12 essential oils in the amount of 25 µL was tested at different temperatures (15, 20, and 30 °C). The following oils were used: Anise (*Pimpinella anisum* L.), Pine (*Pinus sylvestris* L.), Cinnamon bark (*Cinnamomum verum* Bercht & Presl), Citronella (*Cymbopogon nardus* (L.) Rendle), tea tree (*Melaleuca alternifolia* Cheel.), cypress (*Cupressus sempervirens* L.), eucalyptus (*Eucalyptus globulus* Labill.), clove (*Syzygium aromaticum* (L.) Merr. & L. M. Perry), lavender (*Lavandula angustifolia* L.), sweet orange (*Citrus sinensis* (L.) Osbeck.), rosemary (*Rosmarinus officinalis* L.) and thyme (*Thymus vulgaris* L.). Pure cultures of the phytopathogenic fungi *R. solani* and *P. graminea* aged 7 days were used for the experiment. The experiment was performed in three replicates separately for each fungus, temperature, and essential oil according to the modified method of Edris and Farrag (2003). A circular piece of sterile filter paper with a diameter of 5 mm was placed in the center of the Petri dish and 25 µl of essential oil was applied to it; the volume fraction of oil in the air in the Petri dish was 0.04%. Circular sections of individual growing cultures were made with a sterile needle at four opposite points so that each section was 5 mm from the edge of the dish filled with potato dextrose agar. Sterile distilled water was used as a control instead of essential oil. Petri dishes were incubated in climate chambers at temperatures of 15, 20, and 30 °C, relative humidity of 70 %, and a light regime of 12 hours light/12 hours dark. The zone of inhibition (mm) was measured at 96 h and 192 h after inoculation. The effect of essential oils on the listed phytopathogenic fungi is expressed by the zone of inhibition, which is determined according to the formula of Wu et al. (2013.): $I\ (\%) = [(C\text{-}T) / (C\text{-}0, 4)] \times 100$

Where:

% I– percentage inhibition of mycelial growth in a given essential oil

C - diameter of fungal growth on pure KDA,

T - diameter of fungal growth on treated KDA

Statistical analysis of experimental results was performed with the SAS 9.2 statistical package (SAS Institute Inc, Cary, NC, USA) using analysis of variance ANOVA and the Fisher LSD test (P= 0.05 and 0.01).

Results and discussion

The results of the study on the volatile effect of essential oils applied in a volume of 25 µl at different temperatures on the growth of *R. solani* mycelia are presented in Tables 1 and 2.

Table 1. Volatile effect of essential oils and temperature on mycelial growth of *R. solani* 96 hours after inoculation (inhibition zone mm)
**The results show the inhibition zone and the mean ±standard deviation*

	Temperature			LSD	
Essential oil	15 °C	20 °C	30 °C	0.05	0.01
Anise	30.00 ± 0.00	30.00 ± 0.00	30.00 ± 0.00	0.00	0.00
Pine	16.17 ± 0.00	9.92 ± 1.70	20.67 ± 8.10	11.39	17.26
Cinnamon bark	30.00 ± 0.00	28.83 ± 1.81	30.00 ± 0.00	2.09	3.16
Citronella	30.00 ± 0.00	27.92 ± 3.61	30.00 ± 0.00	4.16	6.31
Tea tree	30.00 ± 0.00	30.00 ± 0.00	30.00 ± 0.00	0.00	0.00
Cypress	21.50 ± 5.20	19.58 ±1.46	28.33 ±2.89	7.06	10.70
Eucalyptus	24.67 ± 9.24	9.17 ± 0.88	27.08 ± 3.47	11.43	17.31
Clove	19.33 ± 2.08	19.25 ± 0.50	21.25 ± 0.75	2.62	3.96
Lavender	30.00 ± 0.00	21.75 ± 7.61	30.00 ± 0.00	8.78	13.30
Sweet orange	11.33 ± 2.16	5.00 ± 1.09	17.08 ± 1.46	3.26	4.94
Rosemary	30.00 ± 0.00	13.92 ± 3.47	30.00 ± 0.00	4.00	6.06
Thyme	30.00 ± 0.00	30.00 ± 0.00	30.00 ± 0.00	0.00	0.00
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		
LSD 0.05	5.71	4.50	4.38		
LSD 0.01	7.72	6.08	5.92		

The volatile effect of essential oils is a very suitable method to test the growth of microorganisms since there is no direct contact between essential oils and pathogens. The effect of a pair of essential oils is tested on bacteria (Inouye et al., 2001a) and fungi (Inouye et al., 2001b, Inouye et al., 2000). The volatile method of using essential oils is more effective against fungi because fungi are more accessible to the influence of oil vapors due to their surface growth (Edris and Farrag, 2003). The essential oils of anise, thyme, and tea tree completely inhibited the growth of *R. solani* mycelia 96 hours after inoculation at all temperatures. Slight growth of the fungus was observed when cinnamon bark and citronella oils were applied at a temperature of 20 °C, but no statistically significant differences were observed compared to the growth of the fungus at other temperatures. Pine and sweet orange oils showed the weakest volatile effect.

Table 2. Volatile effect of essential oils and temperature on mycelial growth of *R. solani* 192 hours after inoculation (inhibition zone mm)

	Temperature			LSD	
Essential oil	15 °C	20 °C	30 °C	0.05	0.01
Anise	30.00 ± 0.00	30.00 ± 0.00	30.00 ± 0.00	0.00	0.00
Pine	0.00 ± 0.00	0.00 ± 0.00	3.33 ± 5.77	6.66	10.09
Cinnamon bark	30.00 ± 0.00	18.33 ± 16.04	30.00 ± 0.00	18.50	28.03
Citronella	30.00 ± 0.00	20.58 ± 8.20	30.00 ± 0.00	9.46	14.33
Tea tree	30.00 ± 0.00	30.00 ± 0.00	29.25 ± 0.90	1.04	1.58
Cypress	0.00 ± 0.00	0.00 ± 0.00	13.33 ± 7.43	8.57	12.98
Eucalyptus	17.92 ± 15.52	0.00 ± 0.00	2.08 ± 3.61	18.38	27.84
Clove	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00	0.00
Lavender	30.00 ± 0.00	0.00 ± 0.00	27.00 ± 2.88	3.33	5.04

Sweet orange	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00	0.00
Rosemary	4.83 ± 8.37	0.00 ± 0.00	30.00 ± 0.00	9.66	14.63
Thyme	30.00 ± 0.00	30.00 ± 0.00	30.00 ± 0.00	0.00	0.00
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		
LSD 0.05	8.21	8.38	4.90		
LSD 0.01	11.09	11.33	6.62		

**The results show the inhibition zone and the mean ±standard deviation*

After 192 hours of inoculation, anise and tea tree oils still show complete inhibition of mycelial growth at all temperatures, while other oils such as lavender and rosemary slowly lose their volatile effect. This is especially observed at a temperature of 20 °C, which is considered by some authors to be the optimal temperature for the growth of *R. solani* mycelia (Grosch and Kofoe, 2003). Pine and sweet orange oils showed the weakest volatile effect 192 hours after inoculation.

The volatile effect of essential oils applied in equal amounts at different temperatures on the growth of *P. graminea* mycelia was tested, and the results of the research are presented in Tables 3 and 4.

Table 3. Volatile effect of essential oils and temperature on the growth of *P. graminea* mycelia 96 hours after inoculation (inhibition zone mm)

	Temperature			LSD	
Essential oil	15 °C	20 °C	30 °C	0.05	0.01
Anise	30.00 ± 0.00	30.00 ± 0.00	30.00 ± 0.00	0.00	0.00
Pine	21.92 ± 0.88	21.75 ± 0.25	20.17 ± 0.58	1.25	1.89
Cinnamon bark	29.08 ± 0.63	28.75 ± 0.50	29.42 ± 0.38	1.03	1.56
Citronella	30.00 ±0.00	29.00 ± 1.73	30.00 ± 0.00	2.00	3.03
Tea tree	30.00 ± 1.64	30.00 ± 0.00	22.25 ± 2.22	2.56	3.88
Cypress	24.00 ± 1.88	20.83 ± 1.42	28.25 ± 3.03	4.30	6.51
Eucalyptus	24.58 ± 0.88	20.58 ± 0.63	20.33 ± 1.66	2.98	4.52
Clove	26.08 ± 0.88	27.58 ± 0.58	26.17 ± 0.88	1.58	2.39
Lavender	30.00 ± 0.00	28.58 ± 1.84	30.00 ± 0.00	2.13	3.22
Sweet orange	19.67 ± 0.52	20.50 ± 0.43	20.50 ± 0.43	0.93	1.40
Rosemary	27.50 ± 1.95	26.33 ± 0.29	25.08 ± 0.29	2.30	3.49
Thyme	30.00 ± 0.00	30.00 ± 0.00	30.00 ± 0.00	0.00	0.00
Control	16.00 ± 1.00	16.00 ± 1.00	16.00 ± 1.00		
LSD 0.05	1.69	1.52	2.05		
LSD 0.01	2.29	2.06	2.77		

**The results show the inhibition zone and the mean ±standard deviation*

At all temperatures, the essential oils of anise and thyme completely inhibited the growth of *P. graminea* mycelia after 96 hours of inoculation. It is worth mentioning the excellent inhibitory effect of citronella oil, where a slight increase in mycelium was observed only at a temperature of 20 °C, but without a statistically significant difference compared to the growth of the fungus at other temperatures. Citronella and cinnamon bark oils also had a very good volatile effect on the growth of fungal mycelium. Pine and sweet orange oils had the weakest volatile effect. Terzi et al. (2007) studied the effect of tea tree essential oil (*Mealeuca alternifolia*) and its main components on the fungi *P. graminea*, *Fusarium graminearum*, *Fusarium culmorum*, and *Blumeria graminis*. Growth of *P. graminea* was reduced

by 44.13% (±5.3) and 10.33% (±0.53) when barley seeds were treated with two and one gram of tea tree essential oil per kilogram of seed, respectively.

Table 4. Volatile effect of essential oils and temperature on the growth of *P. graminea* mycelia 192 hours after inoculation (inhibition zone mm)

	Temperature			LSD	
Essential oil	15 °C	20 °C	30 °C	0.05	0.01
Anise	30.00 ± 0.00	30.00 ± 0.00	28.83 ± 2.02	2.33	3.53
Pine	5.00 ± 0.75	6.67 ± 0.58	5.92 ± 0.58	1.28	1.94
Cinnamon bark	26.08 ± 1.23	25.75 ± 2.17	27.75 ± 0.90	3.06	4.63
Citronella	30.00 ± 0.00	20.08 ± 10.55	30.00 ± 0.00	12.17	18.44
Tea tree	29.50 ± 0.87	27.42 ± 0.52	21.25 ± 12.36	14.30	21.67
Cypress	10.42 ± 0.52	7.25 ± 0.75	4.75 ± 1.25	1.79	2.71
Eucalyptus	8.58 ± 2.63	5.67 ± 2.18	3.17 ± 0.80	4.05	6.13
Clove	24.58 ± 1.81	25.17 ± 1.28	24.92 ± 1.53	3.11	4.71
Lavender	29.67 ± 0.29	15.08 ± 8.70	14.00 ± 6.06	12.23	18.54
Sweet orange	10.17 ± 0.63	8.92 ± 0.88	5.25 ± 0.25	1.28	1.94
Rosemary	13.50 ± 3.36	12.33 ± 0.88	8.67 ± 0.29	4.02	6.10
Thyme	30.00 ± 0.00	30.00 ± 0.00	30.00 ± 0.00	0.00	0.00
Control	2.42 ± 0.52	2.42 ± 0.52	2.42 ± 0.52		
LSD 0.05	2.34	6.60	6.58		
LSD 0.01	3.17	8.92	8.89		

**The results show the inhibition zone and the mean ±standard deviation*

After 192 hours of inoculation, anise and thyme oils still show the strongest volatile effects. At all temperatures, thyme oil completely inhibits the growth of mycelia of *P. graminea*, while anise oil shows a slight increase in the mycelium of the fungus at a temperature of 30°C. It is noted that the volatile effect of the essential oil of citronella is slightly weaker at a temperature of 20 °C than at 15 °C and 30 °C. Pine oil had the weakest effect on fungal growth at all temperatures, followed by sweet orange oil. While Aliaran et al (2018) investigated the inhibitory properties of cinnamon and fennel essential oils on the growth of *P. graminea* mycelium, where cinnamon oil completely stopped the growth of the fungus at

all concentrations, i.e. 100, 200, 300, and 400 ppm. The applied concentrations of 300 and 400 ppm fennel oil also inhibited the mycelium of the fungus 100%.

Conclusions

From the research results obtained and presented, it can be concluded that the essential oils of anise, citronella, tea tree, cinnamon bark, and thyme have the strongest volatile effect on the growth of the fungus *R. solani* in both measurements (96 h and 192 h after inoculation). Different temperatures and incubation times did not affect the efficacy of anise and thyme essential oils. Pine oil and sweet orange oil had the weakest volatile effect at all temperatures and incubation durations. Anise and thyme oils showed the strongest volatile effect on P. graminea mycelium growth at all temperatures in both measurements, while pine oil had the weakest effect. Studies on the volatile effect of essential oils on pathogenic fungi are rare compared to the contact effect of essential oils. With this in mind, further research is needed on this method of oil application, i.e., contact with essential oil vapors.

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Volatilni učinak eteričnih ulja na rast *Rhizoctonia solani* i *Pyrenophora graminea*

Sažetak

Cilj ovog istraživanja je u *in vitro* uvjetima ispitati volatilni učinak dvanaest eteričnih ulja na rast micelija *Rhizoctonia solani* i *Pyrenophora graminea.* Eterična ulja su primijenjena u volumenu 25 µL pri temperaturama 15, 20 i 30 °C. Mjerenje zone inhibicije provedeno je 96 h i 192 h nakon inokulacije. Najjači volatilni učinak na rasta micelija *R. solani* i *P. graminea* imala su ulja anisa i timijana na koja nisu utjecali različito vrijeme i temperature inkubacije. Najslabiji volatilni učinak na rast micelija *R. solani* i *P. graminea* imala su ulja bora i naranče slatke.

Ključne riječi: volatilni učinak, eterična ulja, *Rhizoctonia solani*, *Pyrenophora graminea*

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