Determination of isoflavones in soybean meal after supercritical carbon dioxide extraction

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Abstract

Soybeans processed into oil are leaving a by-product with large amount of phenolic compounds known as isoflavones. The content of total and individual isoflavones in soybean meal after supercritical fluid extraction was determined by high performance liquid chromatography. The total isoflavone content in soybean meal ranged from 67.27 to 98.39 mg/100g of soybeans depending on the applied extraction conditions of pressure and temperature. The most abundant isoflavone was genistein. The highest content of daidzein (46.86 mg/100g) and genistein (51.54 mg/100g) was found in soybean meal after supercritical extraction at 250 bar and 50 °C. For non-treated soybeans, the total isoflavone content was 113.76 mg/100g of soybeans.

Key words: isoflavones, soybean meal, supercritical fluid extraction

Određivanje sadržaja izoflavona u sojinoj sačmi zaostaloj nakon ekstrakcije ulja iz zrna soje superkritičnim CO_2

Sažetak

Nakon ekstrakcije ulja iz zrna soje superkritičnim CO_2 zaostaje sojina sačma koja sadrži značajnu količinu fenolnih spojeva poznatih kao izoflavoni. Sadržaj ukupnih i pojedinačnih izoflavona u sojinoj sačmi određen je primjenom tekućinske kromatografije visoke djelotvornosti. Sadržaj ukupnih izoflavona u sojinoj sačmi, nakon ekstrakcije ulja iz zrna soje superkritičnim CO_2, kretao se u intervalu od 67,27 do 98,39 mg/100g soje i ovisio je o ispitanim uvjetima tlaka i temperature ekstrakcije. Najveći sadržaj daidzeina (46,86 mg/100g) i genisteina (51,54 mg/100g) dobiven je u sojinoj sačmi nakon superkritične fluidne ekstrakcije pri tlaku 250 bar i temperaturi 50 °C. U netretiranom uzorku soje, ukupni sadržaj izoflavona iznosio je 113,76 mg/100g soje.

Ključne riječi: izoflavoni, sojina sačma, superkritična fluidna ekstrakcija

Introduction

Soybean (*Glycine max* (L.) Merr.) is a legume that is consumed worldwide. Soybean meal left after the extraction of oil contains large amounts of phenolic compounds. The interest in extraction of phenolic compounds from the by-product arises from the fact that they are a source of significant amount of antioxidative compounds. Furthermore, soybean owes its recently acquired ‘functional food’ status to the presence of isoflavones (Riaz, 1999). Interest in soy isoflavones is based on the information on their potential in lowering cholesterol levels, preventing prostate and breast cancer, osteoporosis, cardiovascular disease as well as relieving menopausal symptoms (Head, 1998; Messina, 1999; Venter, 1999). The isoflavone content in soybeans comprise about 72% of the total phenols and are significantly affected by cultivar, environmental

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factors and their interactions (Wang and Murphy, 1994; Hoeck et al. 2000; Lee et al. 2003; Seguin et al. 2004).

So far, a large number of scientific papers have been published on the determination of isoflavones in soybeans (Tsukamoto et al., 2005; Hoeck et al., 2000; Lee et al., 2003; Penalvo et al., 2004; Seguin et al., 2004; Kim et al., 2005; Malenčić et al., 2007; Devi et al., 2009; Slavin et al., 2009; Kumar et al., 2010; Mujić et al., 2011), but only few papers have dealt with determination of isoflavones in soybean meal after the supercritical extraction (Rostagno et al., 2002; Zuo et al., 2008; Nakada et al., 2009; Kumhom et al., 2011).

The aim of this work was to determine the content of the isoflavones in soybean meal after the supercritical carbon dioxide extraction. Supercritical carbon dioxide extraction was performed at different conditions of temperature and pressure. The content of total and individual isoflavones was determined by high performance liquid chromatography.

Material and methods

Material. The extraction was performed on conventionally grown soybeans, variety "*Ika*," created at the Agricultural Institute of Osijek. The samples were cleaned from impurities (stick, stems, damage seeds, dirt), milled in a grinder (HR 2860, Philips), and immediately after grinding stored at +4 °C prior to extraction. The soybeans dry matter content was determined by drying the milled soybeans at 105 °C to constant weight. The analysis was done in duplicates and the average dry matter content was noted as percentage. The dry matter content was about 91.9% and was determined in all experimental runs. The average particle size (d = 0.383 mm) was determined using sieve sets (Retsch AS 200, Haan, Germany). Commercial carbon dioxide (Messer, Novi Sad, Serbia) was used. Aglycone standards of daidzein, glycitein and genistein were purchased from Acros Organics (USA). β-naftol, used as an internal standard, were purchased from Merck (Germany).

Supercritical fluid extraction. The extraction process was carried out on laboratory-scale high pressure extraction plant (HPEP, NOVA-Swiss, Effertikon, Switzerland), described previously by Pekić et al. (1995). The main plant parts and properties, by manufacturer specification, were: the diaphragm type compressor (with pressure range up to 100 MPa), extractor with internal volume 200 ml (P_{max} = 70 MPa), separator (with internal volume 200 ml, P_{max} = 25 MPa). Sample, 120 g of ground soybeans, was placed into extractor vessel. The carbon dioxide flow rate, expressed under normal conditions, was 1.629 dm^3/min. Separator conditions were 1.5 MPa and 25 °C.

Determination of isoflavone in soybean meal. In soybean meal samples after supercritical carbon dioxide extraction determination of daidzein, genistein, glycitein, and total isoflavone content were performed. Isoflavone concentrations were determined by HPLC (Perkin Elmer, SAD) as described by Vyn et al. (2002). Briefly, 0.5 g samples were mixed with 10 mL of ethanol and 2 mL of concentrated HCl. The mixtures were hydrolyzed by heating at 60 °C for 2 h in a water bath. The samples were allowed to cool down; the β-naftol, as internal standard, were added and then the mixture were centrifuged at 3000 rpm for 10 min. The clear aliquot was filtered through a 0.45 µm PTFE filter. The samples were analyzed for isoflavone content on an HPLC using the following instrumental conditions: mobile phases were solvent A (4% v/v aqueous acetic acid) and solvent B (100% methanol); solvent system (% solvent A/% solvent B), 0 min (70/30), 19.7 min (70/30), 3 min (50/50), 1.3 min (30/70), and 3 min (70/30); flow rate, 1.5 mL/min; and injection volume, 1µL. All measurements were conducted in triplicate.

Results and discussion

The total and individual isoflavone content in soybean meal after different process conditions of supercritical carbon dioxide extraction is presented in Table 1. The examined extraction temperatures were 40, 45, 50 °C, and pressures were 100, 150, 200, 250 and 300 bar at the constant CO_2 flow rate of 0.194 kg/h. The composite values for three isoflavones (aglycone), namely daidzein, genistein, and glycitein were analyzed and expressed as total isoflavone content. The total isoflavone content in soybean meal was in range from 67.26 to 98.39 mg/100g of soybean. It can be also noticed that the most abundant isoflavone in soybean meal was genistein. The genistein series has gained most attention in isoflavone research because of its potential positive effects on health (Dixon and Ferreira, 2002). In non-treated soybean sample, the total isoflavone content was 113.76 mg/100g of soybeans (daidzein 60.79 mg/100g and genistein 52.97 mg/100g). Glycitein content in soybean meals was not reported since it was below the limit of detection. The highest content of daidzein (46.86 mg/100g) and genistein (51.54 mg/100g) was found in soybean meals left after supercritical extraction at 250 bar and 50 °C. Similar results for the content of daidzein and genistein in different soybean cultivars created at Agricultural Institute Osijek were previously published by Sudar et al. (2010). They reported the daidzein content in the range from 23.05 to 38.14 mg/100 g, and genistein content in the range from 22.08 to 45.00 mg/100 g of soybeans. Mujić et al. (2011) reported that total isoflavone content in different soybean cultivars was in the range from 80.7 to 213.6 mg/100g. Several other authors considered the isoflavones as major phenolic compounds with concentration in different soybean varieties that may vary from 126.1 to 409.2 mg/100g of soybeans (Wang and Murphy, 1994; Tsukamoto et al., 1995). The total isoflavone content in five soybean cultivars published by Yamabe et al. (2007) were in range from 221 to 444 mg/100g. Furthermore, it was also reported that some soybean varieties had isoflavone content less than 100 mg/100g of soybean (Simonne et al., 2000). As can be seen from the results obtained during this investigation (Table 1) there is no regularity in the influence of extraction pressure (200 – 360 bar) and temperature (40 – 60 °C) on the content of isoflavones in soybean meal. The similar conclusions were obtained by Rostagno et al. (2002) who reported the total isoflavone content in the range from 11.65 to 86.28 μg/g, and just one sample of soybean meal that had very low total isoflavone content (1.98 μg/g) at extraction conditions of 60 °C and 300 bar .

Table 1. Isoflavone content (mg/100 g) in soybean meal after extraction of oil from soybean seeds by supercritical CO_2 at different extraction conditions

T (°C)	p (bar)	Daidzein (mg/100g)	Genistein (mg/100g)	Total isoflavone content (mg/100g)
40	100	32.14 ±2.69	35.13 ±1.62	67.27
	150	28.34 ± 3.18	58.89 ± 8.04	87.23
	200	43.19 ± 3.35	44.76 ± 5.93	87.95
	250	34.61 ± 1.08	51.25 ± 1.23	85.86
	300	34.78 ± 5.39	36.88 ± 6.49	71.66
45	100	25.95 ± 5.08	46.42 ± 5.38	72.37
	150	30.38 ± 5.94	48.24 ± 6.35	78.61
	200	30.32 ±1.72	51.17 ± 2.97	81.49
	250	31.18 ± 0.56	48.71 ± 2.09	79.89
	300	39.65 ± 1.72	45.10 ± 2.99	84.75
50	100	40.97 ± 4.05	43.53 ± 5.32	84.49
	150	42.67 ± 4.05	42.32 ± 5.32	84.99
	200	33.95 ± 0.59	36.40 ± 2.64	70.35
	250	46.86 ± 3.43	51.54 ± 1.58	98.39
	300	34.61 ± 1.78	49.58 ± 0.08	84.19
Non-treated sample		60.79 ± 4.95	52.97 ± 3.91	113.76

Conclusion

Soybean meal left after supercritical carbon dioxide extraction has a significant amount of antioxidative compounds know as isoflavones. The most abundant isoflavone in soybean meal during this investigation was genistein. The total isoflavone content in soybean seed meal was in the range from 67.27 to 98.39 mg/100g of soybeans depending on different extraction conditions. Glycitein content in soybean meal was not reported because it was below the limit of detection.

Reference

Devi M.K.A., Gondi M., Sakthivelu G., Giridhar P., Rajasekaran T., Ravishankar G.A. (2009). Functional attributes of soybean seeds and products, with reference to isoflavone content and antioxidant activity. Food Chemistry 114: 771–776.

Dixon R.A., Ferreira D. (2002). Genistein. Phytochemistry 60: 205–211

Head K.A. (1998). Isoflavones and other soy constituents in human health and disease. Alternative Medicine Review 3: 433–450.

Hoeck J.A., Fehr W.R., Murphy P.A., Welke G.A. (2000). Influence of genotype and environment on isoflavone contents of soybean. Crop Science 40: 48–51.

Kim J.J., Kim S.H., Hahn S.J., Chung I.M. (2005). Changing soybean isoflavone composition and concentrations under two different storage conditions over three years. Food Research International 38: 435–444.

Kumar V., Rani A., Dixit A.K., Pratap D., Bhatnagar D. (2010). A comparative assessment of total phenolic content, ferric reducing-anti-oxidative power, free radical-scavenging activity, vitamin C and isoflavones content in soybean with varying seed coat colour. Food Research International 43: 323–328.
Kumhom T., Elkamel A., Douglas P.L., Douglas S., Pongamphai S., Teppaitoon W. (2011). Prediction of isoflavone extraction from soybean meal using supercritical carbon dioxide with cosolvents. Chemical Engineering Journal 172: 1023– 1032
Lee S.J., Yan W., Ahn J.K., Chung I.M. (2003). Effect of year, site, genotype and their interactions on various soybean isoflavones. Field Crops Research 81: 181-192.
Malenčić D., Popović M., Miladinović J. (2007). Phenolic content and antioxidant properties of soybean (Glycine max (L.) Merr.) seeds. Molecules 12: 576-581.
Messina M.J. (1999). Legumes and soybeans grown foods: overview of their nutritional profiles and health. American Journal of Clinical Nutrition 70: 439–450.
Mujić I., Šertović E., Jokić S., Sarić Z., Alibabić V., Vidović S., Živković J. (2011). Isoflavone content and antioxidant properties of soybean seeds. Croatian Journal of Food Science and Technology 3: 9-13.
Nakada M., Imai M., Suzuki I. (2009). Impact of ethanol addition on the solubility of various soybean isoflavones in supercritical carbon dioxide and the effect of glycoside chain in isoflavones. Journal of Food Engineering 95: 564–571.
Pekić, B., Zeković, Z., Petrović, L., Lepojević, Ž., Tolić, A. (1995). Behavior of (-)-α-Bisabolol and (-)-α-Bisabololoxides A and B in cammomile flower with supercritical carbon dioxide. Separation Science and Technology 30: 3567-3576.
Penalvo J.L., Nurmi T., Adlercreutz H. (2004). A simplified HPLC method for total isoflavones in soy products. Food Chemistry 87: 297–305.
Riaz M.N. (1999). Soybeans as Functional Foods. Cereal food world 44: 88-92.
Rostagno M.A., Araújo J.M.A., Sandi D. (2002). Supercritical fluid extraction of isoflavones from soybean flour. Food Chemistry 78: 111–117.
Simonne A.H., Smith M., Weaver D.B., Vail T., Barnes S., Wei C.I. (2000). Retention and Changes of Soy Isoflavones and Carotenoids in Immature Soybean Seeds (Edamame) during Processing. Journal of Agricultural and Food Chemistry 48: 6061–6069.
Slavin M., Cheng Z., Luther M., Kenworthy W., Yu L. (2009). Antioxidant properties and phenolic, isoflavone, tocopherol and carotenoid composition of Maryland-grown soybean lines with altered fatty acid profiles. Food Chemistry 114: 20–27.
Seguin P., Zheng W., Smith D.L., Deng W. (2004). Isoflavone content of soybean cultivars grown in eastern Canada. Journal of The Science of Food and Agriculture 84: 1327–1332.
Sudar R., Sudarić A., Vratarić M., Nemet I., Jurković V. (2010). Optimizacija ekstrakcije izoflavona iz soje. In: 45rd Croatian and 5rd International Symposium on Agriculture, Opatija, HR, pp. 108-109.
Tsukamoto C., Shimada S., Igita K., Kudou S., Kokubun M., Okubo K., Kitamura K. (1995). Factors affecting isoflavone content in soybean seeds: changes in isoflavones, saponins composition of fatty acids at different temperatures during the seeds development. Journal of Agricultural and Food Chemistry 43: 1184-1192.
Venter C.S. (1999). Health benefits of soy beans and soy products: a review. Journal of Family Ecology and Consumer Sciences 27: 24-33.
Vyn T.J., Yin X., Bruulsema T.W., Jackson C.C., Rajcan I., Brouder S.M. (2002): Potassium Fertilization Effects on Isoflavone Concentrations in Soybean (*Glycine max* (*L.*) Merr.), Journal of Agricultural and Food Chemistry 50: 3501–3506.
Wang H.J., Murphy P.A. (1994). Isoflavone content in commercial soybean foods. Journal of Agricultural and Food Chemistry 42: 1666–1673.
Yamabe S., Kobayashi-Hattori K., Kaneko K., Endo H., Takita T. (2007). Effect of soybean varieties on the content and composition of isoflavone in rice-koji miso. Food Chemistry 100: 369–374.
Zuo Y.B., Zeng A.W., Yuan X.G., Yu K.T. (2008). Extraction of soybean isoflavones from soybean meal with aqueous methanol modified supercritical carbon dioxide. Journal of Food Engineering 89: 384–389.

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