Isoflavone content and antioxidant properties of soybean seeds

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Summary

The isoflavone content and antioxidant properties of five Croatian soybean seed cultivars from two locations were analysed. The content of total and individual isofavones was determined by high performance liquid chromatography. For determination of antioxidant properties scavenging capacity on DPPH$^{\cdot}$ radicals has been applied. The total phenolic content, oil and protein content in soybean cultivars were also determined. Significant differences in the content of individual isoflavones were observed within the soybean cultivars. The total phenol content in soybean cultivars ranged from 87.2 to 216.3 mg GAE/100g of soybean. The total isoflavone content in soybean seeds ranged from 80.7 to 213.6 mg/100g of soybean. The most abundant isoflavone in soybean seeds was genistein. There was statistically significant difference ($p < 0.05$) among two locations in total and individual isoflavone contents. The highest contents of total isoflavones were found in cultivar "os55-95". Conversely, cultivars poor in isoflavones also showed low levels of DPPH-radical scavenging activity.

Keywords: isoflavone, soybean seeds, antioxidant activity, total phenolic content

Introduction

Soybean (*Glycine max* L. Merrill) is a legume that is consumed worldwide. Soybean is a complex food matrix containing low or no starch, about 20 % oil and 40 % high-quality protein in addition to several important bioactive compounds, including lunasin, trypsin inhibitors, isoflavones, and saponins (Božanić, 2006). Furthermore, soybean owes its recently acquired 'functional food' status to the presence of isoflavones (Riaz, 1999), the phenolic compounds, concentration of which ranges from 1–3 mg/g in the mature seeds. The isoflavone content in soybeans comprise about 72 % of the total phenols (Seo and Morr, 1984). Total phenolic content is a potential candidate as a selection criterion for antioxidant activity in soybeans (Malenčić et al., 2007). Interest in soy isoflavones is based on data suggesting potential in lowering cholesterol levels, preventing prostate and breast cancers, osteoporosis, cardiovascular disease as well as relieving menopausal symptoms (Head, 1998; Messina, 1999; Venter, 1999). The isoflavones are included into the so called phytoestrogens. Genistein and daidzein are the isoflavones that can be found in the highest percentage in food, in the form of conjugated glycosides (Vacek et al., 2008). Bioactive compounds present in soybeans vary greatly with the cultivar, weather and geographical sowing location (Hoeck et al., 2000; Seguin et al., 2004). Devi et al. (2009) observed that Indian cultivars are rich in genistein content compared to the European and American soybean cultivars.

The objective of this study was to compare the content of isoflavones in five soybean cultivars from two different locations and to determine their antioxidant activity. Furthermore, the total phenolic content, protein and oil content of soybean cultivars was determined as well.

Materials and methods

Material

The analysis was performed on soybean cultivars: "Hrvatica", "Lea", "Danica" supplied from Bc Institut d.d. Zagreb, and two soybean cultivars "os55-95" and "os64-07", from Agriculture Institut Osijek in 2008. The soybeans were hand-selected to eliminate those that were cracked or otherwise damaged. The soybeans were analyzed in triplicate for oil and protein content according to the standard methods.

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Oil was determined by solvent extraction (AOAC 991.36, 1997) and protein by the Kjeldahl method (AOAC 976.05, 2000).

Chemicals

Glycoside standards of daidzin, genistin, glycitin, aglycone standards of daidzein and glycitein, and rutin, used as an internal standard, were purchased from Sigma-Aldrich (Steinheim, Germany). Standard of genistein was supplied from Riedel de Haen® (Castle Hill, N.S.W., Australia).

Determination of isoflavone in soybeans

The soybean samples (15 g) were powdered, 5 mL of internal standard (rutin in MeOH), and 50 mL of 80 % MeOH were added. The mixtures were sonicated on an ultrasonic bath for 3h at room temperature. The mixture was then filtered through a Whatman No. 1 filter paper, and the volume was reduced to 5 mL under a stream of nitrogen. This was filtered over 0.45 μm syringe cellulose filter, and transferred into HPLC vials. HPLC analysis of the extracts was performed using Agilent 1200 series HPLC with RR Zorbax SB-C18 column (3.5 μm, 30 x 2.1 mm). Mobile phase A was 0.2 % formic acid in water and mobile phase B was acetonitrile. The injection volume was 1 μL, and elution at 0.45 mL/min with gradient program (0-1.24 min 2 % B, 1.24-3.70 min 2-29 % B, 3.70-8.00 min 29-30 % B, 8.00-9.00 min 30-98 % B, 9.00-10.00 min 2 % B). UV detection was carried out at 260 nm. Four mixed standards containing all six analyzed isoflavones were used for quantification. Rutin (internal standard) was added to each isoflavone standard at a concentration of 25 μg/mL. Single standards were also prepared for peak identification. Isoflavone concentrations were calculated as mg of isoflavones per 100 g of soybean. All measurements were conducted in triplicate (Tsangalis et al., 2002).

Total phenolic content in soybeans (TPC)

The soybean samples (about 15 g) were powdered, and 1 g of powder was added in 10 ml of 80 % MeOH. The mixtures were sonicated on an ultrasonic bath for 3h at room temperature. This was filtered over 0.45 μm syringe cellulose filter. Total phenolic content of the soybean extracts was determined using Folin-Ciocalteau reagent. An aliquot (200 μl) of extract was mixed with 1.6 ml of Folin-Ciocalteau reagent and 0.8 ml of 7.5 % sodium carbonate. The absorbance was measured at 740 nm against distilled water as blank after incubation for 120 min at room temperature. Total phenolic content was expressed as gallic acid equivalents (mg GAE/100g of soybean) through standard calibration curve of freshly prepared gallic acid. All measurements were conducted in triplicate (Singleton et al., 1999).

DPPH radical-scavenging activity

Five different concentration were used for each soybean extract for estimating the EC_{50} value against DPPH. The DPPH solution (0.9 ml, 0.04 mg/ml in MeOH) was added into 0.1 ml of soybean extract. The absorption at 515 nm was measured after 30 min of initation of teh reaction. Absorbance of a blank with 1 ml of MeOH, and a control with the mixture of 0.1 ml of MeOH and 0.9 ml of DPPH solution were also measured. The percentage of DPPH radical remaining after 30 min is determinated according to the following equation:

$$\% \text{ DPPH remaining} = \left[(A_{sample}) / (A_{control} \times A_{blank}) \right] \times 100 \qquad (1)$$

where A_{sample}, A_{blank}, and $A_{control}$ are the absorbance of sample, blank, and control reactions (after 30 min), respectively. Based on the values of % DPPH remaining, the EC_{50} of each sample was obtained by plotting the % DPPH remaining against antioxidant concentration. The EC_{50} value is the concentration of an antioxidant to quench 50 % radicals in the reaction mixture under the assay condition. The results are expressed as mg of soybean equivalent/ml testing solution. All measurements were conducted in triplicate (Blois, 1958).

Statistical analysis

One-way analysis of variance (ANOVA) and multiple comparisons (Duncan's *post-hoc* test) were used to evaluate the significant difference of the data at $p < 0.05$. Data were expressed as means ± standard deviation.

Results and Discussion

As a chemical structure of phenolic compounds is responsible for their antioxidant activity, measurement of total phenolic content could be related to antioxidant properties of investigated material. Total phenolics content and antioxidant activity of five soybean cultivars is presented in Table 1. There were statistically significant differences ($p < 0.05$) in the total phenolic content related to soybean cultivars. Among all the cultivar analysed, the highest value of TPC was observed in

cultivar „os55-95“ (216.3 mg GAE/100g of soybean) from Agriculture Institut Osijek and the lowest in cultivar „Danica“ (87.2 mg GAE/100g of soybean) from Bc Institut d.d. Zagreb. Similar results were published for Indian yellow soybean varieties where total phenol content ranged from 104 to 154 mg GAE/100g of soybean (Kumar et al., 2010).

Table 1. Total phenolic content and antioxidation activity of soybean cultivars

Soybean cultivar	Location	Total phenolic content (mg GAE/100g of soybean)	EC_{50} (mg of soybean/ ml testing solution)
Hrvatica	Rugvica	115.2 ± 0.3 [a]	19.4 ± 0.3 [a]
Lea	Rugvica	128.7 ± 0.9 [b]	11.1 ± 0.2 [b]
Danica	Rugvica	87.2 ± 0.6 [c]	22.1 ± 0.3 [c]
os55-95	Osijek	216.3 ± 1.9 [d]	7.3 ± 0.2 [d]
os64-07	Osijek	121.1 ± 0.9 [e]	12.6 ± 0.2 [e]

Data are expressed as mean value of replication (n) ± SD (standard deviation);
The same letter in the same column indicates no significant differences
(Duncan's test, $p < 0.05$)

The EC_{50} values were used to report the $DPPH^{\cdot}$ scavenging capacity of soybean cultivars. The EC_{50} is the required initial concentration of a selected antioxidant sample to quench 50 % of the free radicals initially in the reaction system; therefore, a higher EC_{50} value corresponds to a lower antioxidant activity in the sample (Slavin et al., 2009).
There were statistically significant differences ($p < 0.05$) in obtained EC_{50} value according to soybean cultivars. EC_{50} value, expressed in mg of soybean/ml testing solution, ranged from 7.3 to 22.1 for soybean cultivars. Among all the cultivars analyzed, „Danica“ exhibited the highest while a „os55-95“ cultivar, the lowest EC_{50} value. The similar range of EC_{50} values were published by Prakash et al. (2007) where 30 varieties of soybean seeds were investigated. The antioxidant activity of soybeans was in correlation with total phenols content. With increasing of total phenols content EC_{50} value decrease, what indicate higher antioxidant activity. Soybean cultivars with highest content of total phenolic compounds, has a lowest EC_{50} value and because of that highest antioxidant activity. The antioxidant activity correlated well with TPC ($R^2 = 0.839$) in soybean cultivars. The similar results were published by Kumar et al. (2010) where antioxidant activity of soybeans also correlated well with TPC ($R^2 = 0.660$).
Flavonoids are considering as phenol compounds with highest antioxidant activity due to their chemical structure. Among dietary flavonoids, isoflavones, especially genistein, shows one of the highest antioxidant activities (Heim et al., 2002). The total and individual isoflavone content of different soybean cultivars is presented in Table 2.

Table 2. Isoflavone content of soybean cultivars

Soybean cultivar	Location	Content of isoflavone (mg/100g of soybean)					
		Daidzin	Glycitin	Genistin	Daidzein	Genistein	Total
Hrvatica	Rugvica	18.8 ± 0.4 [a]	7.0 ± 0.1 [a]	28.7 ± 0.6 [a]	25.6 ± 0.5 [a]	32.9 ± 0.6 [a]	112.9
Lea	Rugvica	20.6 ± 0.4 [b]	7.0 ±0.1 [a]	37.1 ±0.7 [b]	25.1 ± 0.5 [a]	37.0 ±0.6 [b]	126.8
Danica	Rugvica	13.3 ±0.3 [c]	6.6 ± 0.1 [b]	24.5 ±0.5 [c]	15.0 ± 0.3 [b]	21.4 ± 0.4 [c]	80.7
os55-95	Osijek	24.4 ± 0.5 [d]	11.4 ±0.2 [c]	32.2 ±0.6 [d]	67.4 ± 1.3 [c]	78.3 ±1.2 [d]	213.6
os64-07	Osijek	12.4 ± 0.2 [c]	5.9 ± 0.1 [d]	18.6 ±0.4 [e]	34.7 ±0.7 [d]	46.5 ±0.9 [e]	118.1

Data are expressed as mean value of replication (n) ± SD (standard deviation);
The same letter in the same column indicates no significant differences
(Duncan's test, $p < 0.05$)

The composite values for six isoflavones, namely daidzin, genistin, glycitin, daidzein, genistein, and glycitein were analyzed and expressed as total isoflavone content. The total isoflavone content in soybean cultivars was in range from 80.7 to 213.6 mg/100g of soybean. Several authors considered the isoflavones as major phenolic compounds and their concentration in different soybean varieties may varied from 126.1 to 409.2 mg/100g of soybeans (Wang and Murphy, 1994; Tsukamoto et al., 1995; Carrão-Panizzi et al., 1999). The total isoflavone content in five soybean varieties published by Yamabe et al. (2007) were in range from 221 to 444 mg/100g. Furhermore, it is also published that the

isoflavone content is significantly lower in soybean seeds that are exposed to higher temperatures during sowing compared to the seeds exposed to a low temperature (Tsukamoto et al., 1995). However, some soybean varieties contained less isoflavone than 100 mg/100g of soybean (Simonne et al., 2000). In this study there were statistically significant differences among the five soybean cultivars. „os55-95" cultivar had the highest isoflavone content (213.6 mg/100g of soybean), while „Danica" had the lowest (80.7 mg/100g of soybean). Glycitein content in soybean seeds was not reported because it was below limit of detection. Similar results for soybean cultivars created at Agricultural Institute Osijek have been published by Sudar et al. (2010) where daidzein content was in a range from 23.05 to 38.14 mg/100g and genistein was in a range from 22.08 to 45.00 mg/100g. There was significant difference among two locations in total and individual isoflavone contents. This was also well documented by Wang and Murphy (1994) and Hoeck et al. (2000). The content of individual isoflavones varied depending on the varieties of soybeans, genotype, growing conditions, growing area, year and temperature (Wang and Murphy, 1994; Lee et al., 2003; Seguin et al., 2004). It can be also noticed that the most abundant isoflavone in soybean cultivars was genistein. The genistein series has gained most attention in isoflavone research because of its potential positive effects on health (Dixon and Ferreira, 2002). Highly significant positive correlations were observed between genistein content and total isoflavone content (R^2 = 0.973). Seguin et al. (2004) also observed significant positive correlation between individual and total isoflavone. TPC correlated extremely well with total isoflavone content (R^2 = 0.999) as expected (Devi et al., 2009).
Table 3 presents the maturity group of each soybean cultivar as well as the protein and oil content. There were significant differences between locations for protein and oil contents of soybeans. Soybean cultivars „os55-95" and „os64-07" had the highest oil content, as well as the protein content. The similar results for oil content in soybean cultivars from Osijek have been published by Sudar et al. (2003) where the oil content in soybean was determinate by nondestructive nucleic magnetic resonance method. Variation in both oil and protein content were relatively negligible in soybean cultivars from Agriculture Institut Osijek. It has been well documented (Wilcox and Shibles, 2001; Poysa and Woodrow, 2002) that seed protein content negatively correlated with oil. This was confirmed in the case of soybean cultivars from Bc INSTITUT d.d. Zagreb, where protein content is highly negatively correlated to seed oil (R^2 = 0.984).

Table 3. Oil and protein content in soybean cultivars

Soybean cultivar	Location	Maturity group	Oil content (%)	Protein (%)
Hrvatica	Rugvica	0	18.06 ± 0.5 [a]	35.04± 0.7 [a]
Lea	Rugvica	0/1	16.06 ± 0.3 [b]	36.05 ± 0.9 [b]
Danica	Rugvica	00	15.06 ± 0.2 [b]	37.03 ± 0.2 [c]
os55-95	Osijek	1	21.67 ± 0.4 [c]	40.21 ± 0.3 [d]
os64-07	Osijek	0/1	21.00 ± 0.8 [d]	39.8 ± 0.5 [d]

Data are expressed as mean value of replication (n) ± SD (standard deviation);
The same letter in the same column indicates no significant differences
(Duncan's test, $p < 0.05$)

Conclusions

Significant differences in the content of individual isoflavones were observed within the soybean cultivars. The total phenolic content in soybean cultivars ranged from 87.2 to 216.3 mg GAE/100g of soybean. The total isoflavone content in soybean seeds ranged from 80.7 to 213.6 mg/100g of soybean. The most abundant isoflavone in soybean seeds was genistein. There was statistically significant difference ($p < 0.05$) among two locations in total and individual isoflavone contents. The highest contents of total isoflavones were found in cultivar "os55-95". Conversely, cultivars poor in isoflavones also showed low levels of DPPH-radical scavenging activity.

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