Antioxidant properties of soybean with black and yellow kernel coat

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Abstract

The objectives of this study were to characterize black and yellow soybean varieties for the total phenolic, taninn, proanthocyanidin, flavonoid and tocopherol contents, and to examine antioxidant activity. Also, the content of tocopherols (α-, β-, γ-, and δ-) was detected by using HPLC method. The obtained results suggest that kernel extracts of black soybeans has considerable higher level of all the investigated phenolic classes and about double amount of the total phenolics. The total tocopherols were significantly higher in black than in yellow soybean varieties. Concentrations ranged from 264.3 to 266.7 μg/g and from 216.3 to 246.7 μg/g in black and yellow soybean varieties, respectively. β+γ-Tocopherol isomers were dominant in all samples. Correlation between total phenolics, phenolic classes, tocopherols and antioxidant activity detected by DPPH radical scavenging test, was found.

Key words: black and yellow soybean, antioxidants, phenolic compounds

Introduction

Soybean has been consumed in Asiatic countries for centuries and is now often included in Western diets, because of its beneficial nutritional effects (Sugano, 2005; Taku et al., 2007). These legumes contain complex carbohydrates, vegetable protein, dietary fiber, oligosaccharides, and minerals. Also, soybean is good source of antioxidants. Antioxidants, vitamin E and phenolic compounds, have different beneficial functions in the body, such as the ability to reduce and prevent oxidative damage associated with many diseases, and ageing (Middleton et al., 2000). Polyphenolic compounds that belong to the class of isoflavones, genistein and daidzein are unique to soybeans (Wang and Murphy, 1994). These biologically active compounds may have either weak proestrogenic (agonist) or antiestrogenic (antagonist) effects, and be related to hormone-associated diseases.

A few studies have indicated that soybean varieties with black, brown, green, and yellow seed coats might differ in their antioxidant properties, flavonoid levels, total phenolic contents, and proanthocyanidins (Takahashi et al., 2005; Xu et al., 2007; Astadi et al., 2009), indicating that this may alter their ability to affect health. Black soybeans have been widely used in traditional oriental medicine, unlike yellow soybeans, which have been used mostly as food. A case control study (Do et al., 2007) reported that black soybean consumption reduces the risk of breast cancer in women. Daily intakes of black soybean were also associated with a reduced cardiovascular disease risk (Takahashi et al., 2005). A possible explanation is the fact that black soybeans contain antioxidants, such as anthocyanins, proanthocyanidins, and flavonoids which exert a strong radical-scavenging activity (Pietta, 2000). However, proanthocyanidins, i.e. condensed tannins, as polymeric phenolic compounds form insoluble complexes with proteins (Reddy et al., 1985). These complexes are reported to be responsible for growth depression, low protein digestibility in vitro and in vivo, and decreased amino acid availability (Durigan et al., 1987).

This study aimed to characterize the antioxidant activity and phytochemical composition of soybeans with black and yellow coloured kernel coat.
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**Material and methods**

Two black (Болгария, Black Tokyo) and two yellow (Fayette, OS 101) soybean (*Glycine max* (L.) Merr.) varieties produced during the 2008 growing season at the Maize Research Institute Zemun Polje (MRIZP), Serbia, were used for experiments. The wholemeal (particle size<500 μm) was obtained by grounding soybean kernels on a Cyclotec 1093 lab mill (FOSS Tecator, Sweden).

For the DPPH test, the soybean extract was prepared by continuous shaking 0.2 g of wholemeal in 10 ml of 70% (v/v) acetone for 30 min at room temperature. After centrifugation (20 min at 20 000 g), supernatant was used for the detection of the DPPH• scavenging activity according to the Abe et al. (1998) assay. The results were expressed as an IC_{50} value that represents the amount of wholemeal (in mg d.w.) providing 50% inhibition of DPPH•. Total phenolics were determined from the same extract according to the Folin-Ciocalteu procedure (Hagerman et al., 2000). The total phenolic content was calculated as a catechin equivalent (CE) from the calibration curve of catechin standard solutions, and expressed in mg/g d.w. Total tannin content was determined by the same procedure used for total phenolics (Hagerman et al., 2000), after removal of tannins by adsorption on an insoluble matrix (polyvinylpolypyrrolidone, PVPP). Calculated values were subtracted from total phenolic contents and total tannin contents was expressed as mg catechin per g of dry weight. Proanthocyanidins were determined by a butanol-HCl assay (Hagerman et al., 2000), and their content was calculated as leucocyanidin equivalent according to the formula: (A_{550nm} x 78.26 x dilution factor) / (% dry weight) given by Porter et al. (1986). Total flavonoid content was determined using colorimetric method described previously (Eberhardt et al., 2000). The results are expressed as milligrams of catechin. HPLC method was used to detect tocopherol contents (Branković, 2006).

Significant differences between genotype means were determined by the Fisher’s least significant differences (LSD) test, after the analysis of variance (ANOVA) for trials set up according to the RCB design. Correlations between parameters were examined using the Peterson correlation.

**Results and discussion**

Total phenolic content was about two times higher (average 7.50 CE mg/g d.w.) in black soybean varieties Болгария and Black Tokyo than in yellow, Fayette and OS 101 (average 3.72 CE mg/g d.w.) (Table 1). Differences in the content of all phenolic classes were observed. The total flavonoid content was significantly higher in black soybeans than in yellow (0.280 CE mg/g d.w., and 0.064 CE mg/g d.w., respectively). By using Porter assay based on acid catalysed oxidative depolymerization of proanthocyanidins (condensed tannins) into anthocyanidins, we have detected proanthocyanidins only in soybean varieties with black kernel coat, average was about 1.89 mg leucoanthocyanidin per g of dry weight (Table 1). However, Malenčić et al., (2007) reported that the proanthocyanidin content in different soybean genotypes ranged from 1.04 to 3.31 leucoanthocyanidin mg/g dry plant material. As it is shown in Table 1, the tannins content in black soybean varieties was on average six times higher than in yellow soybean varieties.

Like cereals, soybeans and their products are relatively good sources of vitamin E (tocopherols). In addition to their function as cholesterol synthesis inhibitors (Pearce et al., 1992), tocopherols, belonging to the group of antioxidant vitamins, are also able to prevent the formation of free radicals (Frankel, 1989). According to our results, all soybean samples contained α-, β-, γ-, and δ-tocopherols (Table 2). The total tocopherols content was significantly higher (P<0.05) in black than in yellow soybean varieties. Also, soybean varieties with black kernel coat had higher content of α- and β+γ isomers than soybean varieties with yellow color of kernel coat. β+γ-Tocopherol isomers were dominant in all samples. The highest β+γ-tocopherol content was detected in the variety Болгария (179.1 μg/g d.w.), the lowest in kernels of the variety OS 101 (150.6 μg/g d.w.). The amount of δ-tocopherol was 20.92 to 29.91% of the total. The level of α-tocopherol was the lowest in all soybean samples and ranged from 5.91 to 13.58% of the total tocopherols. Although α-tocopherol has a lower antioxidant potency in biological systems than other tocopherols, it has more vitamin E activity in all cells of living tissues. Conversely, γ-tocopherol is the most powerful antioxidant in vitro but its in vivo activity is low. β- and δ-tocopherols exhibit intermediate properties (Pongracz et al., 1995). Our results are consistent with the results published by Pongracz et al. (1995).

The antioxidant capacity was measured as the DPPH• scavenging activity. Black soybean variety Black Tokyo showed the highest DPPH• scavenging activity. 1.07 mg of soybean meal was able to scavenge 50% of DPPH•. The significant differences (P<0.05) between the DPPH• scavenging activity of black soybean varieties Black Tokyo and Болгария, were not detected, but, significant differences were found between black and yellow...
varieties. Namely, both yellow soybean varieties OS 101 and Fayete had a low radical scavenging activity (IC$_{50}$ was 9.44 and 8.57, respectively) (Table 1). The radical scavenging activity and the total phenolic content were closely related (r=0.991) (Table 3). Yellow soybean varieties with the lower contents of total phenolics expressed only about 12.4% of the activity of phenolic-rich black varieties. The obtained results are in agreement with earlier findings of Takahata et al. (2001) they reported strong correlation between total phenolic content and antioxidant activity. Also, the radical scavenging activity highly correlated with the content of the total flavonoids, which exert a strong radical-scavenging activity (Pietta, 2000), as well as with other phenolic classes, judging to the calculated correlation coefficients of 0.990, 0.998 and 0.990, for total flavonoids, tannins and proanthocyanidins, respectively (Table 3). The results of Takahata et al. (2001), showed that procyanidins are quite likely to be the predominant compounds responsible for the radical-scavenging activities in the brown soybean seed coat.

Table 1. The content of total phenolics, tannins, total flavonoids, proanthocyanidins, and DPPH radical scavenging activity in black (Болгария and Black Tokyo) and yellow (Fayete and OS 101) soybean varieties

<table>
<thead>
<tr>
<th>Variety</th>
<th>Total phenolics $^1$</th>
<th>Tannins $^1$</th>
<th>Total flavonoids $^1$</th>
<th>Proanthocyanidins $^2$</th>
<th>DPPH$^*$ scavenging activity (IC$_{50}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Болгария</td>
<td>7.11$^a$</td>
<td>4.10$^a$</td>
<td>0.257$^b$</td>
<td>1.73$^b$</td>
<td>1.18$^a$</td>
</tr>
<tr>
<td>Black Tokyo</td>
<td>7.89$^b$</td>
<td>4.27$^a$</td>
<td>0.303$^a$</td>
<td>2.06$^a$</td>
<td>1.07$^a$</td>
</tr>
<tr>
<td>Fayete</td>
<td>3.96$^c$</td>
<td>0.70$^b$</td>
<td>0.076$^c$</td>
<td>n.d.</td>
<td>8.57$^b$</td>
</tr>
<tr>
<td>OS 101</td>
<td>3.48$^c$</td>
<td>0.63$^b$</td>
<td>0.053$^c$</td>
<td>n.d.</td>
<td>9.44$^c$</td>
</tr>
</tbody>
</table>

$^1$mg CE/g d.w.; $^2$mg leucoanthocyanidin/g d.w.; $^*$ values followed by the same letter within a column are not significantly different according to the list significant difference (p<0.05)

Table 2. Tocopherol content in black (Болгария and Black Tokyo) and yellow (Fayete and OS 101) soybean varieties (μg/g d.w.)

<table>
<thead>
<tr>
<th>Variety</th>
<th>α-Tocopherol</th>
<th>β+γ-Tocopherol</th>
<th>δ-Tocopherol</th>
<th>Total tocopherols</th>
</tr>
</thead>
<tbody>
<tr>
<td>Болгария</td>
<td>28.1$^b$</td>
<td>179.1$^a$</td>
<td>59.5$^b$</td>
<td>266.7$^a$</td>
</tr>
<tr>
<td>Black Tokyo</td>
<td>35.9$^a$</td>
<td>173.1$^a$</td>
<td>55.3$^b$</td>
<td>264.3$^a$</td>
</tr>
<tr>
<td>Fayete</td>
<td>14.6$^c$</td>
<td>158.3$^b$</td>
<td>73.8$^a$</td>
<td>246.7$^b$</td>
</tr>
<tr>
<td>OS 101</td>
<td>14.6$^c$</td>
<td>150.6$^b$</td>
<td>51.1$^bc$</td>
<td>216.3$^c$</td>
</tr>
</tbody>
</table>

$^a-d$ values followed by the same letter within a column are not significantly different according to the list significant difference (p<0.05)

Table 3. Correlations between the content of polyphenols, tocopherols and DPPH$^*$ scavenging activity of yellow and black soybean varieties

<table>
<thead>
<tr>
<th>DPPH$^*$ scavenging activity</th>
<th>Total phenolics</th>
<th>Tannins</th>
<th>Total flavonoids</th>
<th>Proanthocyanidins</th>
<th>α-Tocopherol</th>
<th>β+γ-Tocopherol</th>
<th>δ-Tocopherol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.991</td>
<td>0.998</td>
<td>0.990</td>
<td>0.990</td>
<td>0.953</td>
<td>0.967</td>
<td>0.223</td>
</tr>
</tbody>
</table>

Conclusions
Antioxidant activity of black soybeans was considerably higher than that of yellow varieties. Each investigated phenolic class contributed to total phenolic content. Positive correlation between content of phenolic classes and tocopherol isomers, with antioxidative activity was found. Black soybeans are better source of natural antioxidants. Also, these data suggest the possibility of developing novel soybean lines with a selected kernel coat color that could be used as bioactive ingredients in functional foods targeting different health problems.

Acknowledgements
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References


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