Antioxidant activity of faba bean extract and fractions thereof

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Abstract

Phenolic compounds were extracted into 80% (v/v) acetone. The crude extract so obtained was separated into fraction of low-molecular-weight compounds (Fraction I) and a fraction of condensed tannins (Fraction II) using Sephadex LH-20 column chromatography with ethanol and acetone/water (1:1, v/v) as respective mobile phases. The antioxidants activities of the crude extract and fractions thereof was investigated in an emulsion system and using ABTS, DPPH, and reducing power assays. Fraction II exhibited a higher antioxidant efficacy than those of the crude extract and Fraction I. In the crude extract, 21 compounds, namely phenolic acids (gallic, p-hydroxybenzoic, protocatechuic, p-coumaric, ferulic), flavonoids (catechin, epigallocatechin, kaempferol, apigenin, luteolin, and derivatives), gallate procyanidins, vanillin, protocatechuic aldehyde, and tryptophan were identified by HPLC-DAD-MS. Gallate procyanadin dimer, gallate procyanidins, and acetylated kaempferol hexose were the major phenolics present in the extract.

Keywords: Broad bean; Antioxidant activity; Phenolic compounds.

1. Introduction

Phenolic compounds of plant origin play important role in food technology as well in human nutrition. As the natural antioxidants they can inhibit or delay the oxidation of nutrients present in food products. In the human organism phenolics can act as protectors of lipids, proteins and DNA against reactive oxygen and nitrogen species (RONS) (Halliwell and Gutteridge, 1992; Willett, 1994). The protective effect of consumption of phenolic-rich grains, legumes, berries, nuts, and oilseeds against several chronic diseases was demonstrated by several researcher groups (Zgang et al., 2018; Chang et al., 2016; Shahidi and Ambigaipalan, 2015; Alshikh et al., 2015; Shahidi and Chandrasekara, 2013).

Legumes are an important source of proteins, starch, oligosaccharides (prebiotics), dietary fibers, vitamins, and minerals in the human diet (Vaz Patto et al., 2015; Flight and Clifton, 2006). The can also serve as a rich source of natural antioxidants and thus can play an important role in caridio- and cancer-protection (Shahidi and Ambigaipalan, 2015; Bouchenak and Lamri-Senhadji, 2013; Aparicio-Fernandez et al., 2008; Madhujith and Shahidi, 2005; Peeters et al., 2003; Kolonel et al., 2000; Kushi et al., 1999).

Faba bean (Vicia faba) is a species of Fabaceae family. It is native to North Africa, South America, southwest and south Asia, and is extensively cultivated elsewhere. Like other legumes, faba bean seeds contain phenolic compounds (Turco et al., 2016). The presence of condensed tannins in faba bean was confirmed in the research of Zdunczyk et al. (2018), Jin et al. (2012), Makkar et al. (1997), and Khalil et al. (1995). Antioxidant potential of faba bean was also reported using several assays, including the DPPH (Zdunczyk et al., 2018), FRAP (Jin et al., 2012), and ORAC (Zdunczyk et al., 2018). Very high antioxidant capacity of faba bean sprouts was reported by Okumura et al. (2016).

The present study was aimed to determine the polyphenolic profiles and antioxidant properties of faba bean extract and its low-molecular-weight as well as tannin fractions.
2. Materials and methods

2.1. Materials

Faba bean (*Vicia faba*) seeds of the Polis cultivar were bought from the Plant Breeding Station in Olsztyn (Poland).

2.2. Chemicals and standards

Organic solvents, trichloroacetic acid, potassium ferricyanide, and ferric chloride were purchased from the POCh Company (Gliwice, Poland). Linoleic acid, β-carotene, polyoxyethylene sorbitan mono-palmitate (Twee 40), polyoxyethylene sorbitan monopalmitate (Twee 40), butylated hydroxyanisole (BHA), 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox), Folin-Ciocalteu’s phenol reagent, Sephadex LH-20, 2,2'-diphenyl-1-picrylhydrazyl (DPPH), vanillin, gallic acid, p-hydroxybenzoic acid, trans-p-coumaric acid, (+)-catechin, (−)-epicatechin, and tryptophan were obtained from Sigma (Poznań, Poland). Luteolin, apigenin, kaempferol, epigallocatechin gallate, catechin gallate, and epicatechin gallate were purchased from Extrasynthèse (Genay Cedex, France).

2.3. Extraction

Phenolic compounds were extracted from defatted seed samples with hexanes into 80% acetone (v/v). Condition of extractions were as follows: 3 × 30 min, 50 °C, a solid-to-solvent ratio of 1:10 (w/v) (Amarowicz and Shahidi, 2018). Extraction was carried out using a Büchi rotary evaporator. The sample was then freeze-dried.

2.4. Column chromatography

The separation of the low-molecular-weight phenolic and condensed tannin fractions from the crude acetonic extract was carried out using a Sephadex LH-20 column chromatography (Strumeyer and Malin, 1975). A portion of 800 mg of the extract was loaded onto the column (5 × 50 cm) packed with gel suspended in 95% (v/v) ethanol. Fraction I (low-molecular weight phenolic compounds) was eluted from the gel by using 1 L of ethanol. For elution of the Fraction II (condensed tannin) 600 mL of 50% (v/v) methanol was used. The organic solvents were then evaporated using a rotary evaporator and any remaining water from Fraction II was removed by lyophilisation.

2.5. Total phenolic compounds

The content of total phenolics in the crude extract and Fractions I and II was determined using Folin-Ciocalteu’s phenol reagent (Naczk and Shahidi, 1989). The results were expressed as (+)-catechin equivalents per g of the extract or fractions thereof.

2.6. Condensed tannins

Condensed tannins were determined using a vanillin/HCl colorimetric method (Price et al., 1978). The results obtained were reported as absorbance units at 500 nm per 1 g extract or its fractions (Å500/g).

2.7. UV spectra

UV spectra of the extract and its fractions dissolved in 80% (v/v) methanol was recorded using a Beckman DU 7500 diode array spectrophotometer (Beckman Coulter, Inc., Brea, CA, USA).

2.8. Determination of total antioxidant activity (TAA)

The total antioxidant activity was assayed using a Randox kit (Randox Laboratories Ltd., Crumlin, UK) and the analytical procedure outlined by the supplier. Results were expressed as mmol Trolox equivalents (TE)/g extract or fractions thereof.

2.9. Antioxidant activity in β-carotene-linoleate model system

The method of Miller (1970) was carried out for measuring the inhibition of the linoleic acid autoxidation in emulsion system. In this assay, 2 mg of crude extract or Fraction I, or 1 mg of Fraction II dissolved in 0.2 mL of methanol were added to 5 mL of a prepared emulsion of linoleic acid and β-carotene stabilized with Tween 40. The samples were incubated for 2 h at 50 °C and their absorbance was read at 15 min intervals.

2.10. Reducing power

Reducing power of the extracts and fraction thereof, was investigated using the method of Oyaizu (1978). The results were expressed by the increase of the sample absorbance read at 700 nm.

2.11. Antiradical activity against DPPH*

The method described by Yen and Chen (1995) was used for determination of the antiradical activity of the crude extracts and its fractions against DPPH radical. The sample was composed of 2 mL of methanol, 0.25 mL of 1 mM solution of DPPH radical, and 0.1 mL of methanolic solution containing between 0.05 and 2.5 mg of the crude extract or low-molecular-weight fraction—and between 0.1 and 0.5 mg of fraction tannin fraction. After 20 min of scavenging reaction at room temperature, the absorbance of the sample was read at 517 nm.

2.12. HPLC-DAD

For the HPLC analysis, a Water 2001 system (a quaternary pump, an autoinjector, a diode-array detector, Millennium software; Waters Corp., Milford, MA, USA) was used. Phenolic compounds were separated on a Nova-Pak C18 column (300 × 3.9 mm, 4 μm; Waters, Warsaw, Poland), using a gradient of acetonitrile in water according to Dueñas et al. (2004). All analyses of the samples were triplicated. The results were reported as mean value standard deviation.

2.13. HPLC-ESI-MS

Mass spectra were recorded using a Hewlett Packard 1100 mass selective detector (MSD, Palo Alto, CA, USA) equipped with an atmospheric pressure chemical ionization (APCI) source and an electrospray
2.14. Identification and quantification of phenolic compounds

The UV spectra and MS data of the compounds were compared with standards. Some unknown compounds with UV spectra similar to those of flavonoids and gallates, were identified as their derivatives. Quantitative analysis was made by using an external standard method based on calibration curves for each compound; apigenin, luteolin, and kaempferol were used as standards for flavonoids. The contents of proanthocyanidins were expressed as (+)-catechin equivalents.

3. Results and discussion

3.1. Content of total phenolics and condensed tannins

Fraction II obtained using a Sephadex LH-20 column was characterized by the content of total phenolics compounds (285 mg/g and 116 A500/g) condensed tannins (116 A500/g) was much higher than that was determined in the crude extract and in Fraction I (Table 1). The reason for the relatively low content of total phenolic in fraction I is the presence of sugars in this fraction. The solvent system used in this research was able to also extract sugars/oligosaccharides from faba bean seeds.

The results obtained in this research show that faba bean is a rich source of phenolic compounds as well as condensed tannins. A lower content of total phenolic compounds was previously reported for extracts of pea (Amarowicz and Troszyńska, 2003), white bean (Orak et al., 2016), red bean (Orak et al., 2015), broad bean (Amarowicz and Shahidi, 2017; Boudjou et al., 2013).

The high content of condensed tannins in the fractions eluted with the mobile phase of acetone-water (1:1; v/v) from the column packed with Sephadex LH-20 gel was reported before for adzuki bean, broad bean, pea, lentil (Amarowicz et al., 2010, 2009, 2008; Amarowicz and Troszyńska, 2003). The presence of condensed tannins in faba bean seeds determined by vanillin method was reported by Amarowicz et al. (2004) and Baginsky et al. (2013). According to Luo et al. (2015), content of condensed tannins in faba bean extracts ranged from 0.9 to 1.9 g of gallic acid equivalent.

### Table 1. Total phenolics, tannins content, total antioxidant activity in faba bean extract and its fractions and UV spectral data

<table>
<thead>
<tr>
<th>Analyzed material</th>
<th>Total phenolics (mg/g)</th>
<th>Tannins (Absorbance A500/g)</th>
<th>Total antioxidant activity (mmol trolox eq./g)</th>
<th>λmax (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>56 ± 3</td>
<td>5.96 ± 0.25</td>
<td>0.88 ± 0.04</td>
<td>276</td>
</tr>
<tr>
<td>Fraction I</td>
<td>33 ± 2</td>
<td>3.92 ± 0.18</td>
<td>0.66 ± 0.03</td>
<td>276</td>
</tr>
<tr>
<td>Fraction II</td>
<td>285 ± 14</td>
<td>116 ± 35</td>
<td>4.21 ± 0.21</td>
<td>276</td>
</tr>
</tbody>
</table>

### Table 2. Phenolic compounds identified by HPLC-DAD and HPLC-ESI-MS in a faba bean acetonic extract

<table>
<thead>
<tr>
<th>Peak/compound number</th>
<th>λmax (nm)</th>
<th>[M–H]− (m/z)</th>
<th>Ion fragments (m/z)</th>
<th>Compounds</th>
<th>Content (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>260</td>
<td>169</td>
<td></td>
<td>Gallic acid</td>
<td>26.9 ± 2.3</td>
</tr>
<tr>
<td>2</td>
<td>255</td>
<td>153</td>
<td></td>
<td>Protocatechuic acid</td>
<td>18.3 ± 1.1</td>
</tr>
<tr>
<td>3</td>
<td>280, 311</td>
<td>137</td>
<td></td>
<td>Protocatechuic aldehyde</td>
<td>6.4 ± 0.7</td>
</tr>
<tr>
<td>4</td>
<td>255</td>
<td>137</td>
<td>577, 169</td>
<td>p-Hydroxybenzoic acid</td>
<td>4.7 ± 0.5</td>
</tr>
<tr>
<td>5</td>
<td>277</td>
<td>745</td>
<td></td>
<td>Gallate proanthocyanidin dimer</td>
<td>689 ± 33</td>
</tr>
<tr>
<td>6</td>
<td>277</td>
<td>203</td>
<td></td>
<td>Tryptophan</td>
<td>12.4 ± 1.8</td>
</tr>
<tr>
<td>7</td>
<td>279</td>
<td>289</td>
<td></td>
<td>Catechin</td>
<td>9.4 ± 0.9</td>
</tr>
<tr>
<td>8</td>
<td>265, 348</td>
<td>447</td>
<td></td>
<td>Kaempferol hexose</td>
<td>18.8 ± 1.3</td>
</tr>
<tr>
<td>9</td>
<td>278</td>
<td>289</td>
<td></td>
<td>Gallate proanthocyanidin</td>
<td>89.8±5.4</td>
</tr>
<tr>
<td>10</td>
<td>278</td>
<td>289, 169</td>
<td></td>
<td>Gallate proanthocyanidin</td>
<td>18.3 ± 1.1</td>
</tr>
<tr>
<td>11</td>
<td>275</td>
<td>457</td>
<td></td>
<td>Epigallocatechin gallate</td>
<td>18.3 ± 1.1</td>
</tr>
<tr>
<td>12</td>
<td>274</td>
<td>442</td>
<td>169</td>
<td>Catechin gallate</td>
<td>203 ± 1.3</td>
</tr>
<tr>
<td>13</td>
<td>274</td>
<td>442</td>
<td>169</td>
<td>Epicatechin gallate</td>
<td>363 ± 16</td>
</tr>
<tr>
<td>14</td>
<td>314</td>
<td>163</td>
<td></td>
<td>p-Coumaric acid</td>
<td>25.8 ± 31</td>
</tr>
<tr>
<td>15</td>
<td>276, 308</td>
<td>151</td>
<td></td>
<td>Vanillin</td>
<td>3.1 ± 0.8</td>
</tr>
<tr>
<td>16</td>
<td>268, 345</td>
<td>510</td>
<td>447</td>
<td>Acetylated luteolin hexose</td>
<td>56.8 ± 6.3</td>
</tr>
<tr>
<td>17</td>
<td>278</td>
<td>169</td>
<td></td>
<td>Gallate proanthocyanidin</td>
<td>30.8 ± 1.5</td>
</tr>
<tr>
<td>18</td>
<td>268, 335</td>
<td>593</td>
<td>269</td>
<td>Apigenin hexose</td>
<td>32.9 ± 2.6</td>
</tr>
<tr>
<td>19</td>
<td>265, 348</td>
<td>447</td>
<td>285</td>
<td>Kaempferol hexose</td>
<td>19.9 ± 1.0</td>
</tr>
<tr>
<td>20</td>
<td>278</td>
<td>289, 169</td>
<td></td>
<td>Gallate proanthocyanidin</td>
<td>28.6 ± 1.5</td>
</tr>
<tr>
<td>21</td>
<td>265, 338</td>
<td>489</td>
<td>447</td>
<td>Acetylated kaempferol hexose</td>
<td>76.8 ± 4.6</td>
</tr>
</tbody>
</table>
lents/100 g extract. In this research the authors precipitated tannins by using polypyrrolidone (PVPP) and used Folin-Ciocalteu phenol reagent for determination of phenolic compounds.

Low values of absorbance obtained for fraction after colour reaction with vanillin (Table 1) were caused by the presence catechins (flavan-3-ols) in this fraction which agrees with the results of HPLC-DAD-MS analysis (Table 2).

3.2. UV spectra

The UV spectra recorded for the crude extract of faba bean, and its low-molecular-weight and tannin fractions exhibited maxima at the same wavelength of 276 nm (Figure 1). The same wavelengths was obtained for the UV spectra of extract of broad bean crude extract and its two fractions (Amarowicz and Shahidi, 2017). The UV spectra depicted in Fig. 1 are agreement with the results from HPLC-DAD analysis reported in Table 2 where several separated phenolic compounds showed UV spectra with a maximum close to 276 nm.

3.3. Antioxidant activity

The total antioxidant activity (TAA) of the Fractions II (4.21 mmol...
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The crude extract of phenolic compounds of faba bean and its two fractions exhibited antioxidant activity in a β-carotene-linoleate model system (Figure 2). The effect of extract on the coupled oxidation of linoleic acid and β-carotene was the highest. However, the amounts of tannins fraction added to the emulsion system was a half of that of the crude extract and Fraction I. The results obtained are important because they prove the fact that condensed tannins also exhibited their antioxidant activity in the emulsion system. Our previous studies in a β-carotene-linoleate model system found lower antioxidant activity for extracts of leguminous seeds such as broad bean, lentil, adzuki bean, and effectively (Amarowicz and Shahidi, 2017; Amarowicz et al., 2010, 2009, 2008).

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Figure 3 depicts the effect of phenolic compounds present in the extract of faba bean and its two fractions (Fraction I and II) on the formation of Fe²⁺ from Fe³⁺/ferricyanide. This effect was monitored by the measuring optical density at 700 nm. The tannin fraction exhibited much stronger reducing activity than those of the crude extract and Fraction I. The strong reducing power obtained for tannin fraction separated from faba bean crude extract (Figure 3) was observed by several authors (Orak et al., 2015, 2016; Alshikh et al., 2015; Ramírez-Jiménez et al., 2014; Huber et al., 2014). In relation to cited authors, the results of the faba bean obtained in this study are considered to be high.

Figure 3. HPLC chromatogram of a faba bean acetonic extract.

3.4. Content of individual phenolic compounds

Figure 5 shows an HPLC chromatogram of a faba bean acetonic extract. The results of HPLC-DAD and HPLC-ESI-MS are given in Table 2. Based on the retention times and UV spectra of commercial standards, the presence of p-hydroxybenzoic aldehyde, gallic, protocatechuic, p-hydroxybenzoic, trans-p-coumaric acids, (−)-epicatechin, catechin gallate, epicatechin gallate, epigallocatechin gallate, vanillin, and tryptophan in the crude extract of broad bean was confirmed.

Compound 5 showed a negative molecular ion [M–H]⁻ at m/z of 745 and two fragment ions [F–H]⁻ at m/z of 577 and 169, corresponding to a procyanidin dimer and gallic acid, respectively. This compound was identified as gallate procyanidin dimer. Compounds 9, 10, 17 and 20 with a maximum at 278 nm according to fragment ions [F–H]⁻ at m/z of 289 and 169 corresponding to epicatechin or catechin and gallic acid, respectively, were identified as procyanidin gallates. Compounds 8 and 19 with a negative
molecular ion [M–H]− at m/z of 447 and compounds 18 with a negative molecular ion [M–H]− at m/z of 442 were identified as kaempferol and apigenin hexoses, respectively. Based on the UV and MS spectra compounds 16 and 21 were identified as acetylated luteolin and acetylated kaempferol hexoses.

Gallate procyanidin dimer, gallate procyanidins, and acetylated kaempferol hexose were the major phenolics present in the extract of faba bean (Table 2). The presence of catechins is very typical for legume seeds (Yeo, J., and Shahidi, 2017; Limón et al., 2015; Alshikhet et al., 2005). The presence of caffeic, p-coumric, ferulic, and sinapic acids was confirmed by Yao et al (2011) in such legumes as lima bean, broad bean, coman bean, pea, jack bean, adzuki bean, rise bean, mung bean. Gallate procyanidin dimer, gallate procyanidins were previously reported in the extracts of broad bean and lentils (Amarowicz and Shahidi, 2017; Amarowicz et al., 2010, 2009). Procyanidin in faba bean were also determined in faba bean by Abu-Reidah et al. (2014) using UHPLC-ESI-QTOF-MS method.

4. Conclusion

Faba bean is a potentially valuable legume crops with high antioxidant potential. The tannin fraction separated from the crude extracts using a Sephadex LH-20 column chromatography displayed a stronger antioxidants activity than the low-molecular-weight phenolic fraction. Faba bean seeds may serve as an important can be a valuable component in the diet of the vegetarian and the general populations.

References


