Determination of isoflavones in soybean meal after supercritical carbon dioxide extraction

Rezica SUDAR1, Stela JOKIĆ2, Darko VELIĆ2, Mate BILIĆ2, Aleksandra SUDARIĆ1, Snježana KELEKOVIĆ3, Sanja ZEC ZRINUŠIĆ2
1Agricultural Institute Osijek, Južno predgrađe, 17, 31000 Osijek, Croatia
2Faculty of Food Technology Osijek, F. Kuhača 20, 31000 Osijek, Croatia
(e-mail: darko.velic@ptfos.hr)

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

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...
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; Malečić et al., 2007; Devi et al., 2009; Slavin et al., 2009; Kumar et al., 2010; Mujčić 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 \(_{\text{max}}\) = 70 MPa), separator (with
internal volume 200 ml, P \(_{\text{max}}\) = 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.
Isolevone 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. Muccić 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 CO2 at different extraction conditions

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>p (bar)</th>
<th>Daidzein (mg/100g)</th>
<th>Genistein (mg/100g)</th>
<th>Total isoflavone content (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>100</td>
<td>32.14 ± 2.69</td>
<td>35.13 ± 1.62</td>
<td>67.27</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>28.34 ± 3.18</td>
<td>58.89 ± 8.04</td>
<td>87.23</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>43.19 ± 3.35</td>
<td>44.76 ± 5.93</td>
<td>88.95</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>34.61 ± 1.08</td>
<td>51.25 ± 1.23</td>
<td>85.86</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>34.78 ± 5.39</td>
<td>36.88 ± 6.49</td>
<td>71.66</td>
</tr>
<tr>
<td>45</td>
<td>100</td>
<td>25.95 ± 5.08</td>
<td>46.42 ± 5.38</td>
<td>72.37</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>30.32 ± 5.94</td>
<td>48.24 ± 6.35</td>
<td>78.61</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>31.18 ± 0.06</td>
<td>48.71 ± 2.09</td>
<td>79.89</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>39.55 ± 1.72</td>
<td>45.10 ± 2.99</td>
<td>84.75</td>
</tr>
<tr>
<td>50</td>
<td>100</td>
<td>40.97 ± 4.05</td>
<td>43.53 ± 5.32</td>
<td>84.49</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>42.67 ± 4.05</td>
<td>42.32 ± 5.32</td>
<td>84.99</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>33.95 ± 0.59</td>
<td>36.40 ± 2.64</td>
<td>70.35</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>46.86 ± 3.43</td>
<td>51.54 ± 1.58</td>
<td>98.39</td>
</tr>
<tr>
<td>Non-treated sample</td>
<td>60.79 ± 4.95</td>
<td>52.97 ± 3.91</td>
<td>113.76</td>
<td></td>
</tr>
</tbody>
</table>

Conclusion

Soybean meal left after supercritical carbon dioxide extraction has a significant amount of antioxidative compounds known 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


Acknowledgement

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