Study of the Composition and Radical Scavenging Capacity of Buckwheat Seed and Buckwheat Leaf Flour of Two Cultivars Grown in Hungary

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ABSTRACT

Because of its regular consumption, buckwheat is an excellent raw material for functional food products enhancing the human immune system, contributing to the prevention of certain diseases and accelerating recovery after illness. Buckwheat seeds as well as buckwheat leaves contain considerable quantities of antioxidant compounds. In this work the composition of buckwheat seeds and buckwheat leaf samples from common buckwheat species (Fagopyrum esculentum Möench) was examined. Two buckwheat varieties ‘Hajnalka’ and ‘Oberon’ grown in Hungary were found to be gluten-free, with high content of protein and dietary fibre; as well as a good source of vitamin B. Compared to whole meal wheat flour, whole meal flour of hulled buckwheat seeds contains higher K, Mg and Fe, but lower Na content. Buckwheat leaf flour is rich in proteins and minerals, such as K, Mg, Ca, Cu, Mn and Fe. Dehulled groats and buckwheat leaves did not contain vitamin C in detectable quantity while the average tocopherol content of groats was 136 mg/kg DM (dry matter) and that of buckwheat leaf flour samples was 490 mg/kg DM. The rutin content of leaf flour was 28565 and 88400 mg/kg DM, respectively. The total phenolic content of the groats and leaf flour was also considerable. Radical scavenging capacity of dehulled buckwheat and buckwheat leaf flour was 272 and 492 TE/g (troclox equivalent/g) and 4216 and 5543 TE/g in the varieties tested.

Keywords: antioxidant capacity, chemical composition, fagopyrin, functional food, nutraceutical, rutin, tocopherol

INTRODUCTION

Generally, two Fagopyrum species are produced all over the world, common buckwheat (Fagopyrum esculentum Möench) and tartary buckwheat (Fagopyrum tataricum Gärtn). Common buckwheat, the most widely consumed variety, has a sweeter taste, larger seeds and is easier to dehull than tartary buckwheat. Common buckwheat was grown as early as the 15th century in Hungary. Currently, buckwheat production is on a small scale, but its use shows an increasing trend especially in reform diets. Buckwheat is suitable for organic farming. By dehulling, the microbiological state of the seed will be favourable as well. Buckwheat seed flour is used for bread and pasta making while groats are used for replacing rice e.g. as filler in black and liver pudding. In addition, dehulled seeds and flour from common buckwheat are also raw materials for new products, buckwheat flakes and extruded buckwheat are also becoming available.

It has become repeatedly evident that adequate nutrition (balanced daily diets) correlates positively to the reduced risk of some serious diseases (Bíró 1996; Ilme et al. 1996; Kawa et al. 1996; Pados 1996; Zajkás 1996; Nestler et al. 1999; Krkoskova and Mrazova 2005; Shen et al. 2008). Recently, cereal-based foodstuffs have come to play a significant role in daily diets. However, foods which are prepared exclusively from cereals (wheat, rye, barley, oat) are only foodstuffs of medium biological value (Pomerantz 1982). The nutritive value and protective effect against diseases of cereal-based foodstuffs can be considerably increased by natural ways, for example by the utilization and addition of buckwheat, having high nutritive value and higher level of protective substances (vitamins, minerals, dietary fibre, polyphenolics and antioxidants).

The pseudo-cereal buckwheat is an important food with functional properties since it contains proteins of high biological value and balanced amino-acid composition. It also contains essential amino acids such as lysine, methionine and threonine (Eggum 1980; Williams 1995; Biasc et al. 2002). Its mineral and microelement (K, Mg, Fe, Se) as well as vitamin B content is significant but its sodium content is low (Léder 2000; Ikeda 2002). Minerals and vitamin B are especially enriched in the bran fraction of buckwheat (Bonafaccia et al. 2003a). Buckwheat has relatively high dietary fibre content (Bonafaccia et al. 2003b). The high fibre content is believed to be the most probable reason for low protein digestibility.

The content of gluten in buckwheat is less than the allergy level (ALINORM 08/31/26 2008), so coeliac people can consume buckwheat products. However, because of the missing gluten-producing protein component the bread prepared with conventional procedures will be denser and has a tightly packed crumb and lower loaf volume (Kadan et al. 2001; Arendt et al. 2004). Buckwheat has a medium glycemic index of 50-59% (Bíró and Lindner 1995). After consumption of buckwheat meals, the absorption of carbohydrates takes place slowly, so blood sugar level rises gradually in comparison with eating wheat-based products. Fat content of buckwheat is 1.7-2.6%, from which unsaturated fatty acid is 80% and polyunsaturated essential fatty acid, as linoleic acid, is more than 40% (Krkoskova and Mrazova 2005).

Apart from its high nutritive value, buckwheat also contains dietary antioxidants (vitamin C, tocopherols, flavonoids and non-flavonoid phenolics) which can scavenge free radicals emerging in the human body and thereby strengthening the body's antioxidant defensive potential (Bíró 2003). The tocopherol content of Korean buckwheat seeds was between 5.9-9.8 mg/100 g DM (Shim et al. 1998), while the tocopherol content of dehulled seeds was 2.0
mg/100 g DM (Holasa et al. 2002). As the human body is unable to synthesize flavonoids with an antioxidant effect, such a nutrient should be taken from the diet. The recommended daily intake of flavonoids is 180-350 mg (Schilcher et al. 1990) to achieve the adequate clinical effects. The flavonoid content of buckwheat is reported to be higher than that of apples, red wine or tea (Hertog et al. 1992, 1993). Ascorbic acid enhances considerably the antioxidant effects of rutin (Askari et al. 2003). Attempts to improve the rutin content of common buckwheat (Zhanaeva 1996) resulted in content being 20% higher than in the mature or already senescent leaves. According to Hirni et al. (1998), the vitamin C content of the buckwheat seeds is 5.44 mg/100 g, while cereals do not contain vitamin C at all.

Buckwheat plant contains other substances of antioxidant effect, such as phenolic type compounds and flavonoids, mainly rutin, in large quantities. The content of total flavonoids in leaves was 7.8-15.9% and in stems 1.4-4.1% g, while cereals do not contain vitamin C at all. Rutin content depends on the buckwheat species, such as leaves. As Jiang et al. (2009) have reported that fagopyrin is present mainly in the flowers and hull and not in the leaves, stems and groats. Eguchi et al. (2009) reported that fagopyrin is present mainly in the leaves and flowers and only slightly in the stems, hulls and groats. In spite of the different opinions authors agree that fagopyrin is not present or exists only at very low concentrations, in buckwheat groats. In common buckwheat leaves 44.5-63.6 mg/100 g DM of fagopyrin, and in stems 14.3-26.4 mg/100 g DM were found (Ozbolt et al. 2008). For human consumption, especially for children, milling of the dehulled seeds is suggested because of the high fagopyrin content of the hull.

Besides its favourable properties, buckwheat also has some antinutritional factors. Buckwheat dust and proteins may cause allergic reactions, asthma and asthmatic attacks, urticaria and gastrointestinal disorders (Wieslander and Norback 2001). The rutin content of foodstuffs prepared from buckwheat extract or leaf flour can be significantly increased, improving their dietary physiological effects. The addition of 2.5% green part extract of tartary buckwheat to wheat flour breads of good quality, lead to adequate volume and porosity (Gawlik-Dziki et al. 2009). The authors stated that the addition of plant extracts in higher proportion decreased bread quality significantly. Similarly, the favourable nutritional, physiological and green colouring effects of plant extracts can also be utilized for ice cream manufacturing (Kreft et al. 2006).

The purpose of our work was to determine the main components and biologically active compounds of two Hungarian buckwheat varieties, to compare these with the values for common cereals, and to confirm the role of buckwheat in health and nutrition.

MATERIALS AND METHODS

Plant materials

All our measurements were carried out with two common buckwheat (F. esculentum) varieties. The seed and leaf samples of two Hungarian buckwheat varieties (‘Hajnalka’ and ‘Oberon’) grown in two different sites in Hungary in 2007 and 2008 were examined. ‘Hajnalka’ was grown in East Hungary near Nyíregyháza in acidic sandy soils (low in organic matter) while ‘Oberon’ was grown in West Hungary near Szombathely in somewhat acidic soils. In both 2007 and 2008, two buckwheat seed and two leaf samples from both territories were tested.

After preliminary cold-water treatment (setting 15.5% moisture content) buckwheat seeds were dehulled in a laboratory-scale huller machine and the hulls were separated by a laboratory-scale air flow separator.
In both years the leaf samples (young expanding leaves, mature and senescent leaves) of both buckwheat varieties were collected at the same time after sowing. Leaves were dried carefully at 50°C to decrease the moisture content to a level lower than 10%. Groats and dried leaf samples were milled in a Laboratory Mill 3100 (Perten) to produce flour of 315 μm particle size.

Results obtained for the buckwheat seeds and leaf samples (‘Hajnalka’ and ‘Oberon’) were compared with those of common wheat (Triticum aestivum). Average values were used for each parameter determined by chemical analysis in wheat varieties grown in Hungary (Bíró and Lindner 1995).

Specifying main component values

Moisture content was measured by drying at 105°C till constant weight was obtained; oil content was measured by petrol ether extraction in Soxhlet equipment. Ash content was determined by ashing at 550°C until a constant weight was obtained. Crude protein content was measured with an Elementar Rapid N Cube. Fibre content was determined by the AOAC Method No. 991.431 (2003).

Macro- and microelements were determined by atomic absorption spectroscopy (Solaar M5 Thermo Elemental, Waltham, MA, USA) in line with official AOAC Methods No. 986.15 (2003), 965.09 (2003), 983.02 (2003).

Following acidic and enzymatic extraction, the vitamin content of the buckwheat samples was determined by an Alliance Waters HPLC system consisting of a Model 2695 separation module with Zorbax eclipse XDB column (C-18, 3.5 μm), a Model 2996 photodiode array detector and a Model 2475 fluorescent detector. The system was operated by Empower software. The standard ergosterol, α-tocopherol and β-carotene were purchased from Sigma-Aldrich. Authentic materials for other carotenoids were supplied by the Department of Bio- and Medical Chemistry, Medical School, University of Pécs (Pécs, Hungary, Daood et al. 2008).

Determination of rutin content

The rutin content was determined by HPLC. Circumstances of the extraction and HPLC separation were arranged according to the modified method of Kim et al. (2008).

Buckwheat samples (5-20 mg) were extracted with 1 ml of 10% methanolic phosphoric acid and vortexed for 5 min under an infra-red lamp (about 35°C). After centrifugation at 10,000 rpm, the supernatant was pooled and filtered. The rutin content was determined by HPLC. Circumstances of the extraction and HPLC separation were arranged according to the modified method of Kim et al. (2008).

Determination of total phenolic content

Total phenolic content was determined with Folin-Ciocalteu’s photometric method according to Singleton (1969). 1 g of buckwheat samples was extracted in 50 ml 80% methanol. 0.3-0.5 ml of the filtrate was pipetted into a test tube and the volume was adjusted to 4.25 ml with distilled water. Then 0.25 ml Folin-Ciocalteu reagent and 0.5 ml saturated Na2CO3 solution were added, and the samples were left in the dark for 30 min. The absorbance was measured by a spectrophotometer (UV-160 A, Shimadzu, Japan) at 750 nm using blank samples. Total phenolic content was calculated according to a calibration curve prepared with gallic acid (Sigma-Aldrich).

Determination of the free radical scavenging capacity

Scavenging capacity of free radicals was determined by Yamauchi’s method (1998) in a spectrophotometric manner presenting the result in Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Fluka) units. The reagent was 1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl, Sigma-Aldrich) in methanol solution (freshly diluted 10 folds with 90% methanol). Extraction of the buckwheat samples (1 g + 50 ml methanol) was made with 80% methanol; after an adequate dilution 0.05 ml filtrate was pipetted into a test-tube adding 2 ml reagent. Samples were incubated at 37°C for 30 min and measured by a spectrophotometer (UV-160 A, Shimadzu, Japan) at 517 nm against 80% methanol. The absorbance value of the sample containing antioxidant was subtracted from that of the blind sample not containing antioxidant. Calibration was performed from a Trolox stock solution (1 mmol/L). Free radical scavenging capacity was presented in Trolox unit as calculated from the difference.

All reagents were of analytical or HPLC grade as required.

RESULTS AND DISCUSSION

Results from three replicate samples are summarized in Table 1, such as protein, fat, carbohydrate, ash, fibre, energy and minerals content of dehulled seeds and leaf flour of the two buckwheat varieties.

Table 1 Comparison of Hungarian buckwheat (Fagopyrum esculentum) varieties, buckwheat groat and buckwheat leaf flour with the adequate values of wheat.

<table>
<thead>
<tr>
<th>Components</th>
<th>Wheat* (whole grain)</th>
<th>Buckwheat groat</th>
<th>Buckwheat leaf flour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>'Hajnalka'</td>
<td>'Oberon'</td>
<td>'Hajnalka'</td>
</tr>
<tr>
<td>Nutritional value</td>
<td>g/100 g DM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>16.76</td>
<td>15.10</td>
<td>17.12</td>
</tr>
<tr>
<td>Fat</td>
<td>2.10</td>
<td>2.84</td>
<td>3.51</td>
</tr>
<tr>
<td>Fibre</td>
<td>1.76</td>
<td>1.34</td>
<td>1.50</td>
</tr>
<tr>
<td>Ash</td>
<td>2.09</td>
<td>2.20</td>
<td>2.46</td>
</tr>
<tr>
<td>N-free Extract</td>
<td>77.29</td>
<td>78.52</td>
<td>75.40</td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>1457</td>
<td>1565</td>
<td>1563</td>
</tr>
<tr>
<td>Dietary fibre</td>
<td>11.71</td>
<td>13.05</td>
<td>12.89</td>
</tr>
<tr>
<td>Mineral content</td>
<td>mg/100 g DM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>434.10**</td>
<td>554.00</td>
<td>592.00</td>
</tr>
<tr>
<td>Na</td>
<td>2.01**</td>
<td>2.31</td>
<td>2.48</td>
</tr>
<tr>
<td>Mg</td>
<td>130.50**</td>
<td>284.00</td>
<td>300.00</td>
</tr>
<tr>
<td>Ca</td>
<td>45.40</td>
<td>22.00</td>
<td>24.32</td>
</tr>
<tr>
<td>Cu</td>
<td>0.47</td>
<td>0.85</td>
<td>0.81</td>
</tr>
<tr>
<td>Zn</td>
<td>3.60</td>
<td>3.12</td>
<td>3.15</td>
</tr>
<tr>
<td>Mn</td>
<td>3.48</td>
<td>1.51</td>
<td>1.31</td>
</tr>
<tr>
<td>Fe</td>
<td>1.98</td>
<td>3.80</td>
<td>4.05</td>
</tr>
<tr>
<td>Se (μg/100 g DM)</td>
<td>4.46</td>
<td>2.37</td>
<td>2.37</td>
</tr>
</tbody>
</table>

* Bíró and Lindner 1995; **Léder et al. (2001); - not measured; DM: dry matter; Each sample was analysed in triplicate.
The obtained results were compared to the corresponding values of wheat. Results are calculated as the average values of buckwheat samples grown in two years (2007 and 2008) under different soil and climatic conditions. Despite the different growing conditions there were no significant differences between the corresponding main components values of the two buckwheat varieties and of the two years studied.

The 1000-grain weight (TGW) of ‘Hajnalka’ and ‘Oberon’ with dark-brown hulls was different, 22-28 and 33-37 g, respectively. Both varieties are good sources of protein. The average protein content of dehulled groat was 12.5-13.1 and 14.1-16.0 g/100 g, respectively. Both dehulled seeds and leaf flour are good resources of tocopherol. Leaf flour was rich in α-tocopherol, exceeding 80%, while buckwheat groat contained γ-tocopherol, accounting for up to 90% of total tocopherol content (Table 2). Distribution of tocopherols in dehulled buckwheat and buckwheat leaf flour is demonstrated in Fig. 1. Leaf flour contains higher α-tocopherol level than groats. α-Tocopherol is the most important biologically active component of vitamin E (Kent 1975; Rodler 2005).

As a comparison: total tocopherol contents of wheat (32 mg/kg DM), oat (41 mg/kg DM) and barley (47 mg/kg DM; Bíró and Lindner 1995) are significantly lower than the value of 131 and 141 mg/kg DM in buckwheat groats.

The total phenolic and rutin content as well as the radical scavenging capacities of the dehulled buckwheat and leaf flour samples are higher than those of dehulled barley and dehulled oat (Table 2). The two varieties with different growing conditions (soil, weather) differed significantly in their phenolic and rutin content and the values of the radical scavenging capacities as well. According to the HPLC results, 96-98% of the total phenolic compounds of both buckwheat leaf samples was rutin, while total phenolic compounds in the groat samples contained only 1-2% rutin. Table 2 concerns rutin content the HPLC analysis gave the values of 88400 and 28565 mg/kg DM in the leaf flour of ‘Oberon’ and ‘Hajnalka’, respectively. The obtained values are in the ranges reported in the literature (Table 2). This significant

![Fig. 1 Distribution of tocopherols of dehulled seeds and leaf flour of buckwheat varieties.](image)

### Table 2 Total phenolics, rutin and tocopherols as well as radical scavenging capacity of the hulled seeds, leaf flour and hulls of buckwheat varieties

<table>
<thead>
<tr>
<th>Tested material</th>
<th>Total phenolics mg GA/kg DM</th>
<th>Rutin mg/kg DM</th>
<th>Tocopherols mg/kg DM</th>
<th>Radical scavenging capacity TE/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oat</td>
<td>1200</td>
<td>&lt;0.1</td>
<td>41</td>
<td>11</td>
</tr>
<tr>
<td>Barley</td>
<td>2500</td>
<td>&lt;0.1</td>
<td>47</td>
<td>27</td>
</tr>
<tr>
<td>Buckwheat groat ‘Hajnalka’</td>
<td>3241</td>
<td>46</td>
<td>131</td>
<td>272</td>
</tr>
<tr>
<td>Buckwheat groat ‘Oberon’</td>
<td>5124</td>
<td>64</td>
<td>141</td>
<td>492</td>
</tr>
<tr>
<td>Buckwheat groat ‘Oberon’</td>
<td>3903</td>
<td>134-230</td>
<td>203.3-30.7</td>
<td>-</td>
</tr>
<tr>
<td>Buckwheat groat seed ‘Oberon’</td>
<td>-</td>
<td>126-359</td>
<td>59-98</td>
<td>-</td>
</tr>
<tr>
<td>Buckwheat leaf flour ‘Hajnalka’</td>
<td>29086</td>
<td>28565</td>
<td>432</td>
<td>4216</td>
</tr>
<tr>
<td>Buckwheat leaf flour ‘Oberon’</td>
<td>91232</td>
<td>88400</td>
<td>549</td>
<td>5543</td>
</tr>
<tr>
<td>Buckwheat leaf flour seed ‘Oberon’</td>
<td>39514</td>
<td>2700-23443</td>
<td>104.7</td>
<td>-</td>
</tr>
<tr>
<td>Buckwheat hull ‘Hajnalka’</td>
<td>5286</td>
<td>60</td>
<td>-</td>
<td>681</td>
</tr>
<tr>
<td>Buckwheat hull ‘Oberon’</td>
<td>5631</td>
<td>126</td>
<td>-</td>
<td>789</td>
</tr>
</tbody>
</table>

GA: gallic acid; DM: dry matter; TE: trolox equivalent; Each sample was analysed in triplicate.


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difference can be explained not only by the difference between cultivars, but also by the different microclimate of the regions. ‘Oberon’ was grown in the southern part of Hungary, where the sum of sunny days is the highest. On the other hand, the rate of young and senescent leaves could not be determined exactly, although the leaf samples were picked at the same ripening period.

CONCLUDING REMARKS

The results confirm that buckwheat is an excellent raw material for functional food and its consumption may contribute to the prevention and cure of diseases.

According to our results, the dehulled seeds and leaf flour of buckwheat with varieties are valuable raw materials for functional food with high biological value. This statement is based on the fact that dehulled seeds and leaf flour samples have high protein content and they are rich in minerals and compounds of antioxidant effect as well. In the leaf flour more than 80% of the total tocopherol content was α-tocopherol, the biologically active homologue of vitamin E. Significant difference between the two buckwheat varieties found only in the total phenolic compounds and rutin content of the leaf flour samples. The ratio of the total phenolic compounds and the rutin content was the same in both varieties, so both varieties can be used as a functional raw material. We argued that in addition to the environmental effects (sunshine period, soil etc.), the rate of the ripe, senescent and young leaves also made an impact on the different polyphenolic and rutin contents of the two leaf flour samples.

The high biological value and disease preventive effects of the buckwheat products such as extruded products, flakes and puffed products can be consciously utilized in novel foods. Further possibilities include the development of new normal and functional foods with leaf flour of high rutin content, use of the extract from the complete green plant as well as the direct use of rutin extracted from the green plant.

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