ANTIOXIDANT PROPERTIES OF EXTRACTS FROM BUCKWHEAT BY-PRODUCTS

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ABSTRACT

Background. In the course of production of buckwheat groats by-products are produced, such as bran and hull, which apart from high content of dietary fiber, may also constitute valuable sources of antioxidants. The aim of these investigations was to determine the antioxidant activity of extracts from by-products produced during processing of buckwheat for groats.

Material and method. Analyses were conducted on bran and hull of buckwheat cv. Kora. Extraction was run using acetone, methanol and water at room temperature for 24 h. The level of phenolics was determined spectrophotometrically with the Folin-Ciocalteau reagent, using (+) catechin as a standard. Antioxidant activity of extracts was analysed in relation to linoleic acid, running incubation for 19 h, by scavenging of stable radicals of DPPH (2,2-diphenyl-1-picrylhydrazyl) and on the basis of metal chelating ability. Recorded results were compared with the activity of BHT (butylated hydroxytoluene).

Results. The highest content of polyphenols was found in the methanol extract of hull (168.5 mg/g d.m.), which was also characterised by the best antiradical properties. The lowest content of total phenols was found for water extracts of bran after grinding and final bran, at 20.3 mg/g d.m. and 10.2 mg/g d.m. In the emulsion system the highest activity was found for methanol extracts of hull and bran after grinding ($W_o = 0.89$), as well as the extract of final bran ($W_o = 0.85$). A higher chelating ability in relation to Fe (II) ions was observed for bran extracts (after grinding – 76.1%, final bran – 62.2%) than for hull extracts (26%).

Conclusions. Extracts obtained from by-products of buckwheat were characterised by high antioxidant activity in the applied model systems.

Key words: buckwheat, hull, bran, polyphenols, antioxidant activity

INTRODUCTION

Buckwheat is considered to be an increasingly interesting material on the world market of probiotic foodstuffs. It is a source of protein of high biological value, lipids rich in unsaturated fatty acids, as well as vitamins B1, B2 and B6 [Bonafaccia et al. 2003, Alvarez-Jubete et al. 2010]. Buckwheat constitutes also a valuable source of biologically active compounds, e.g. polyphenols with high antioxidant activity [Holasova et al. 2002, Bonafaccia et al. 2003]. These

compounds protect the human organism against oxidative stress and prevent the development of chronic disease, e.g. atherosclerosis and neoplastic lesions [Kaliora and Dedoussis 2007, Hollman 2001]. They also indicate antibacterial, antiviral, anti-inflammatory and anti-allergenic action [Kishore et al. 2010, Kim et al. 2003].

Phenolic compounds are found mainly in outer layers of buckwheat grains and their fraction comprises

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flavonoids and phenolic acids found in the free form and in ester and complex combinations. The primary flavonoid in buckwheat is rutin found at 4-6% [Chłopicka 2008]. Its content changes depending on the parameters of the technological processes applied in seed processing [Zadernowski et al. 1992], e.g. seed roasting, causes an approx. 4-fold decrease in flavonoid contents. In the coats of buckwheat grains we may find rutin, orientin, isoorientin, vitexin and quercetin in amounts ranging from 60 mg to 74 mg/100 g coat, while buckwheat groats contain mainly rutin and isovitexin [Chłopicka 2008].

Buckwheat is processed mainly to groats, it is also used to produce flour for the production of bread, pasta, biscuits and pancakes and even buckwheat-fruit mixtures for drinks are produced [Kreft et al. 2006, Chłopicka 2008].

The technological production process of buck-wheat groats includes such stages as cleaning and thermal conditioning (roasting) of grains, size storing, hulling, sorting after hulling and sorting of groats connected with the separation of waste and by-products. Similarly as in case of flours, the nutritive value of groats, i.e. their chemical composition, is dependent on the degree of comminution and hulling of grain. During the production of buckwheat groats by-products such as bran and hull are produced, which due to their high content of dietary fiber may be used in the production of high-fiber preparations [Dziedzic et al. 2010]. At the same time they may constitute a valuable source of antioxidants.

Due to the high content of biologically active compounds in buckwheat and the positive effect of polyphenols on the human organism, it deserves to be focused on as a valuable raw material for functional food. There are few studies describing the antioxidant action of polyphenols contained in buckwheat byproducts, which may successfully be used in the production of preparations utilized in the development of new probiotic products. Thus the aim of the conducted investigations was to determine the antioxidant activity of extracts from buckwheat bran and hull.

MATERIAL AND METHODS

Reagents. All chemicals used were analytical grade: 2,2-diphenyl-1-picrylhydrazyl (DPPH),

6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin-Ciocalteau reagent, (+) catechin, ferrozine, linoleic acid, Tween (Sigma-Aldrich, Poznań, Poland); ddH₂O, KOH, K₂HPO₄, methanol, acetone (POCh, Gliwice, Poland); BHT (Merck, Germany).

Grain samples. The experimental material comprised grains of buckwheat cv. Kora and products coming from individual stages of production of buckwheat groats, i.e. unhulled buckwheat grain (UBG), hulled buckwheat grain (HBG), buckwheat hull (BH), final bran (FB) and bran after grinding (GB). The raw materials were obtained from a cereal processing plant Zakład Zbożowo-Młynarski in Białystok.

Preparation of extracts. Tested material was comminuted in a Cyclotec mill. Extraction of phenolics was run twice using acetone, methanol and water (material-to-solvent ratio of 1:15, w/v) at room temperature for 24 h.

Total phenolic. The content of total phenolic compounds in extracts was determined by colorimetry at a wavelength of 750 nm according to the Folin-Ciocalteau method [Horwitz 1970]. Results were expressed as mg of (+) catechin equivalents g⁻¹ d.m. of extract.

Antioxidant activity. Antioxidant activity of extracts was estimated on the basis of the capacity of the extracts to scavenge DPPH (2,2-diphenyl-1-picryl-hydrazyl) radicals in relation to linoleic acid and on the basis of metal chelating capacity. Recorded results were compared with the activity of BHT (butylated hydroxytoluene).

Scavenging of DPPH radical. The DPPH radical scavenging capacity was determined on the basis of colorimetrically determined ($\lambda = 517$ nm) changes in the concentration of stable DPPH radicals in relation to the blank sample [Sanchez-Moreno et al. 1998, Mensor et al. 2001]. Results were expressed as mM Trolox equivalents per g^{-1} d.m. of extract.

Emulsion system. The capacity of inhibit autooxidation of linoleic acid was determined according to Lingnert et al. [1979]. The method consists in the spectrophotometric determination ($\lambda = 234$ nm) of an increase in contents of conjugated dienes in the emulsion of linoleic acid at a concentration of 10 mM at pH 7.2, after 19 h incubation in the dark at a temperature of 37°C. Antioxidant efficiency (Wo) was expressed

by a ratio of an increment in absorbance of the control and the sample tested to the increase in absorbance of the control:

$$Wo = rAbs_{234K} - rAbs_{234} / rAbs_{234K}$$

where: Wo > 0 antioxidative properties, Wo < 0 prooxidative properties of the additive.

Metal chelating activity. Antioxidant properties of extracts were also estimated on the basis of metal chelating properties [Tang et al. 2002]. The assay consisted in the colorimetric measurements of the degree of discolouration of iron (II) chloride complexes with ferrozine caused by the extracts. The applied wavelength was 562 nm. The chelating activity of metals (Ach) was determined on the basis of the following equation:

$$Ach [\%] = \{1 - Abs_{sample} / Abs_{control}\} \times 100$$

Statistical analysis. Results presented in the study constitute an arithmetic mean of independent series of measurements conducted in three replications. Least significant differences were analysed using a one-way analysis of variance and the analysis of the linear correlation coefficient in the Statistica 9 software. Statistical inference was conducted at the significance level $\alpha = 0.05$.

RESULTS

Obtained extracts differed in terms of total contents of phenolics. The highest contents were found for methanol extracts, followed by acetone extracts, while the lowest was recorded in water extracts (Table 1).

The highest content of polyphenols was recorded in the methanol extract of hull (168.5 mg/g d.m.). Methanol extracts from unhulled grains and hulled grains in comparison to extracts from hull were characterised by approx. 2-fold lower contents of phenolics (79.3) and 70.7 mg/g d.m., respectively). Contents of polyphenolics in extracts of bran after grinding and final bran were 2.5- and 3-fold lower than in the extract from hull. Acetone and water applied as extractants exhibited a lower capacity to extract phenolics than methanol. In their extracts from hull a 2- and 7-fold lower content of phenolics was recorded than in case of methanol extracts. The lowest contents of total phenolics were found for water extracts of bran after grinding and final bran (20.3 mg/g d.m. and 10.2 mg/g d.m., respectively). In both cases this content was lower than contents of polyphenolics determined in both types of buckwheat grains.

The highest radical scavenging capacity was found for extracts from buckwheat hull, with methanol extract (165.8 mmol Trolox/g d.m.) exhibiting a 2 times higher activity than the acetone extract (73.5 mmol Trolox/g d.m.) and 8 times higher activity than the water extract (19.9 mmol Trolox/g d.m.; Fig. 1).

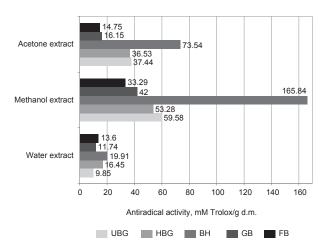
A significantly lower capacity to scavenge DPPH radicals was observed for bran extracts, which activity was always lower than that of extracts from unhulled and hulled buckwheat grains. Acetone extracts of bran after grinding exhibited approx. 1.5-fold higher radical scavenging capacity than water extracts, while the activity of final bran for these extracts was similar, although significantly different, at 13.6 and 14.8 mM Trolox/g d.m.

Table 1. Total phenolic content of buckwheat extracts

Sample	Acetone extract mg (+) catechin/g d.m.	Methanol extract mg (+) catechin/g d.m.	Water extract mg (+) catechin/g d.m.
UBG	49.04 ^d ±1.56	$79.73^{h} \pm 1.56$	29.75° ±0.73
HBG	$58.64^{e} \pm 1.68$	$70.68^g \pm 1,55$	$65.22^{\rm f} \pm 0.90$
ВН	$77.43^{\rm h}\pm 1,29$	$168.47^{i}\pm1.53$	$23.25^{\rm b}{\pm}0.79$
GB	$25.17^{bc} \pm 0.79$	$65.63^{\text{f}} \pm 1.30$	$20.34^{\rm b}{\pm}0.35$
FB	$29.85^{\circ} \pm 1.29$	$52.89^d \pm 1.17$	$10.29^a \pm 0.15$

abc... – the same letters mean no statistically significant differences (p < 0.05).

Methanol extract



40.87 Water extract BHT 0.02% 10 20 30 50 90 Ach, % UBG

■36.63

95.87

97 45

Fig. 1. The DPPH scavenging effect of buckwheat extracts

Fig. 3. The chelating activity of buckwheat extracts

■ HBG

■ BH

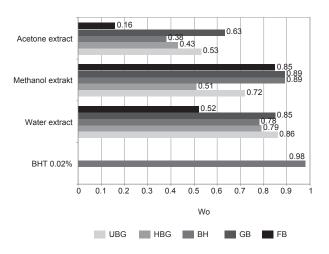


Fig. 2. The antioxidants activity of buckwheat extracts in emulsion system

Statistical analysis showed a very strong positive correlation between total contents of phenolics (r = 0.948, p < 0.05) and DPPH radical scavenging capacity.

Figure 2 shows the effect of 0.01 ml extract on the stability of linoleic acid in the emulsion. Calculated protection factors (W_a) showed that the highest capacity to inhibit autooxidation of linoleic acid was found for methanol extracts from hull and bran after grinding ($W_0 = 0.89$). A slightly lower activity ($W_0 = 0.85$) was found for extract of final bran. The capacity of methanol extracts from buckwheat by-products to inhibit oxidative changes in linoleic acid was significantly higher than that of extracts of unhulled and hulled grains ($W_0 = 0.72$ and $W_0 = 0.51$, respectively). Water extracts exhibited a greater protective effect in relation to emulsified linoleic acid than acetone extracts. Among extracts from buckwheat by-products, only bran after grinding showed a better protective effect in relation to extracts of buckwheat grains. BHT most actively inhibited oxidative changes in the emul $sion (W_0 = 0.98).$

Statistical analysis did not show a correlation between antioxidant efficiency and total content of polyphenolics.

The capacity of the tested extracts to chelate iron ions was presented in Figure 3. Chelating activity of 1 ml extract was analysed in each sample. A significant effect was found of the type of extract on the capacity to form iron ion complexes. The chelating activity of the methanol extract from unhulled buckwheat grains was high, amounting to 97.5%. An almost 4-fold lower activity was recorded for the extract from hulled buckwheat grains (25%). Among buckwheat by-products a higher capacity of Fe(II) ion chelation was found for bran extracts (after grinding - 76.1%, final bran -62.2%) than hull extracts (26%). A high complexing capacity in relation to iron ions was observed for the acetone extract of bran after grinding, the water extract of hulled grains, as well as the water extract from hull, i.e. 95.9%, 74.5% and 61.1%, respectively. The other

extracts exhibited a lower chelating capacity, ranging from 21.6% to 40.9%. A slight activity was also shown by BHT (19.1%).

It was shown that the chelating capacity of extracts of buckwheat grains, both before and after hulling, was correlated with total contents of phenolics (r = 0.73, p < 0.05). In case of buckwheat by-products such correlation was not found.

DISCUSSION OF RESULTS

Analysed extracts were characterised by a higher content of phenolics than ethanol extracts of wheat germs, lentil, sunflower and mung beans [Samotyja et al. 2007] and wheat bran extracts [Klepacka and Fornal 2006]. Studies conducted by Zieliński and Troszyńska [2000] showed that the content of phenolics differentiates the species of plant raw material. The authors reported that the total content of phenolics in hulled buckwheat grains was 4082 µg/g, in barley grain it was 1118 µg/g, in oat and rye grain it ranged from 656 to 669 μ g/g, while in wheat it was 548 μ g/g. The antioxidant activity of methanol cereal extracts was ordered similarly, i.e. buckwheat > barley > oat > wheat = rye [Zieliński and Kozłowska 2000]. Whole buckwheat contains 2-5 times more phenolic compounds than oats and barley, while buckwheat bran and hulls have 2-7 times higher antioxidant activity than barley, triticale and oats [Zduńczyk et al. 2006, Holasova et al. 2002]. In the examined extracts contents of polyphenolics and antioxidant activity were dependent on the applied extractant. The highest activity was found for methanol. In general, polar organic solvents were most effective for producing higher level of phenolic compounds and antioxidant activity [Przybylski et al. 1998]. It was found that in 80% methanol extract of buckwheat was 64 times more phenolic compounds than in water extract. Methanol extract also showed 4 times higher antioxidant activity [Zieliński and Kozłowska 2000]. Other studies investigated extraction capacity of methanol, ethanol, butanol, acetone and ethyl acetate in relation to phenolic compounds of buckwheat flour [Sun and Ho 2005]. Despite the fact that the greatest amounts of polyphenolics were extracted using acetone, the highest antioxidant activity was recorded for methanol extracts. In turn, Gallardo et al. [2006] showed a similar level of phenolics, but

a higher antioxidant activity of water extracts from buckwheat flour in comparison to 80% methanol extracts. Inglett et al. [2011] reported that 50% ethanol extraction achieved significantly higher free phenolic content compared with water and absolute alcohol. The absolute ethanol extraction contained considerable more bound phenolic and flavonoid compounds.

The capacity of scavenging DPPH radicals was observed earlier in case of phenolics extracts obtained from rye, wheat, barley and oats grain, as well as their processed products [Ragaee et al. 2006, Yu et al. 2002].

The effect of scavenging the DPPH radical observed in this study for extracts from different buckwheat products is higher than that observed in case of ethanol extracts from buckwheat flour [Inglett et al. 2011]. Alvarez-Jubete et al. [2010] showed a higher activity in scavenging DPPH radicals by extracts of buckwheat grains in comparison to amaranth, quinoa and wheat. In turn, phenolic compounds extracted with different solvents from buckwheat seeds by Sun and Ho [2005], at a dose of 0.1 mg/ml, yielded a scavenging effect ranging from 40% to 80%, while in case of acetone and methanol extracts this effect was recorded at approx. 80% and 60%, respectively. A similarly high scavenging capacity of the DPPH radical was shown for ethanol extracts of buckwheat groats [Heś et al. 2006].

In the conducted investigations a strong positive correlation was found between total contents of phenolics and scavenging capacity of DPPH radicals. However, not always a strong relationship may be found between contents of polyphenolics and antioxidant activity. A confirmation may be found in studies by Oomah and Mazza [1996], who showed a weak correlation between contents of flavonoids in buckwheat and antioxidant activity. The authors assumed that the antioxidant activity may also be influenced by other components of buckwheat. In contrast to Velioglu et al. [1998], they did not record any relationship between contents of rutin and antioxidant activity of buckwheat.

Analysed extracts were characterised by a high inhibitory activity in relation to autooxidation processes of linoleic acid in the emulsion. Burda et al. [2001] assessed the activity of antioxidants contained in wheat, rye, barley and oat grain on the basis of a comparison of the capacity of the extracts to inhibit oxidation

of β -carotene and linoleic acid. It was found that the highest activity was exhibited by oat extract, slightly lower for those of rye and barley, while the lowest in that of wheat.

The metal chelating capacity may significantly influence the course of oxidative reactions, thus metal-binding compounds are included to the class of oxidation inhibitors. They comprise e.g. polyphenols, which – as it was reported by Miura et al. [1994, 1995] – may cause a reduced production of intermediate products in the cycle of free radical reactions in which metal ions participate and as a consequence also reduce amounts of produced radicals.

In studies conducted by Baraniak et al. [2006] the chelating activity of the extract from buckwheat at 0.6 mg amounted to 38.4% and it was almost three times higher than the activity of synthetic BHT. It was shown that the chelating capacity was dependent on the concentration of the extract. The greatest differences were observed within the range from 0.1 to 0.3 mg. A further increase in the concentration of the extract did not cause a significant increase in the activity of the tested samples. Moreover, a higher chelating capacity was found in the extract from cooked buckwheat groats rather than that of raw buckwheat groats [Hes et al. 2006]. In turn, ethanol extract of green tea at 1000 ppm showed the chelating activity at 27%, while for the water extract of black tea it was 17% [Gramza et al. 2004]. It seems that in view of the above, the chelating activity of extracts from grains and by-products of buckwheat is high.

Recorded results indicate a diversity of mechanisms of the action of antioxidants contained in the tested raw material. However, the analyses proved a considerable potential of buckwheat as a source of antioxidants with a high activity. According to Sun and Ho [2005], natural antioxidants of buckwheat may replace synthetic ones. Since they are safer, they may be added at higher doses and the antioxidant effect will be comparable.

CONCLUSIONS

- 1. Buckwheat extracts were characterised by a high antioxidant activity in the applied model systems.
- 2. The capacity scavenging DPPH radicals by extracts from buckwheat hull was higher than that of

extracts from unhulled and hulled buckwheat grains. In turn, bran extracts exhibited a lower activity in comparison to both types of grains.

- 3. The capacity of methanol extracts from buckwheat by-products to inhibit oxidative changes in linoleic acid was significantly higher than that of extracts from buckwheat grains.
- 4. Extracts exhibited a diverse capacity to chelate metals. Methanol and acetone bran extracts to a higher degree formed complexes with iron ions than hull extracts. An opposite dependence was observed in case of water extracts.

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PRZECIWUTLENIAJĄCE WŁAŚCIWOŚCI EKSTRAKTÓW Z UBOCZNYCH PRODUKTÓW GRYKI

STRESZCZENIE

Wprowadzenie. Podczas produkcji kaszy gryczanej powstają produkty uboczne, takie jak otręby i łuska. Oprócz dużej zawartości błonnika, mogą one być cennym źródłem związków przeciwutleniających. Celem przeprowadzonych badań było określenie aktywności przeciwutleniającej ekstraktów z produktów ubocznych, powstałych podczas przerobu gryki na kasze.

Materiał i metody. Badaniom poddano otręby oraz łuskę gryki odmiany Kora. Ekstrakcję prowadzono z użyciem acetonu, metanolu oraz wody w temperaturze pokojowej, przez 24 h. Poziom związków fenolowych oznaczono spektrofotometrycznie z odczynnikiem Folina-Ciocalteau, stosując jako wzorzec (+) katechinę. Aktywność antyoksydacyjną ekstraktów badano wobec kwasu linolowego, prowadząc inkubację przez 19 h, metodą zmiatania trwałych rodników DPPH (2,2-difenylo-1-pikrylhydrazyl) oraz na podstawie zdolności chelatowania metali. Uzyskane wyniki porównano z aktywnością BHT.

Wyniki. Największą zawartość polifenoli stwierdzono w metanolowym ekstrakcie łuski (168,5 mg/g s.m.), który charakteryzował się też najlepszymi właściwościami przeciwrodnikowymi. Najmniejszą zawartością związków fenolowych ogółem charakteryzowały się ekstrakty wodne otrąb po śrutowaniu oraz otrąb końcowych, odpowiednio 20,3 mg/g s.m. i 10,2 mg/g s.m. W układzie emulsyjnym największą aktywnością cechowały się metanolowe ekstrakty łuski i otrąb po śrutowaniu (W_o = 0,89) oraz ekstrakt otrąb końcowych (W_o = 0,85). Stwierdzono większą zdolność chelatowania jonów Fe (II) przez ekstrakty otrąb (po śrutowaniu – 76,1%, końcowe – 62,2%) niż ekstrakty łuski (26%).

Wnioski. Ekstrakty otrzymane z ubocznych produktów gryki cechowały się dużą aktywnością przeciwutleniającą w zastosowanych układach modelowych.

Słowa kluczowe: gryka, łuska, otręby, polifenole, aktywność przeciwutleniająca

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