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FE(II), CU(II) AND ZN(II) CHELATING ACTIVITY OF BUCKWHEAT AND BUCKWHEAT GROATS TANNIN FRACTIONS

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Key words: tannins, buckwheat, chelation of Fe(II), Zn(II), Cu(II), tetramethylmurexide (TMM), ferrozine, SE-HPLC

Phenolic compounds were extracted from buckwheat and buckwheat groats into 80% acetone (v/v). The crude extracts were separated with Sephadex LH-20 column chromatography into low molecular weight phenolic compounds and tannin fractions. The tannin fractions were characterised by colour reaction with vanillin/HCl reagent, ability for protein precipitation as well as SE-HPLC method. Chelation of Cu(II) and Zn(II) by tannin fractions was determined by reaction with tetramethylmurexide (TMM) whereas Fe(II) chelation was investigated by forming complexes with ferrozine.

The tannin fractions obtained from buckwheat and buckwheat groats gave similar results in vanillin test; A_{500}/mg was 1.04. Also results obtained using precipitation method were comparable. SE-HPLC chromatograms showed that in tannin fraction of buckwheat groats polymers with molecular weight ≥ tannic acid were present. On the chromatogram of buckwheat tannins peaks were shifted to lower molecular weight compounds.

Tannins of buckwheat were stronger chelators of all metal ions tested (Fe(II), Cu(II) and Zn(II)) than tannins of buckwheat groats. Ions of copper were chelated the most effectively; 1 mg of buckwheat and buckwheat groats tannin fractions added per assay bound 86.0% and 72.9% of Cu(II), respectively. Buckwheat tannins used in the test at a level of 2.5 mg/assay bound approximately twice more Fe(II) than the same addition of buckwheat groats tannins. Zn(II) was chelated to the lowest extent, 17.2% was bound by buckwheat tannins and 13.5% by buckwheat groats tannins (5 mg of tannin/assay).

INTRODUCTION

Buckwheat seems to be a valuable component of food products for the reason of its high nutritional quality as well as antioxidant properties. Buckwheat is rich in vitamins B_1, B_2, and C, mineral compounds, dietary fibre, polyunsaturated fatty acids and has balanced amino acid composition with high lysine content [Eggum *et al*., 1981; Steadman *et al*., 2001; Wijngaard & Arendt, 2006]. Phenolic compounds present in buckwheat seeds, *i.e.* phenolic acids and their derivatives, catechins, flavonols (rutin, quercetin, hyperin) and condensed tannins, are responsible for their antioxidant properties [Oomah & Mazza, 1996; Watanabe, 1998; Watanabe *et al*., 1997; Steadman *et al*., 2001]. Antioxidant activity of whole buckwheat seeds, as well as dehulled seeds (buckwheat groat), hulls, straws and leaves have already been tested [Holasova *et al*., 2002].

Buckwheat tannins comprise approximately 1.5–1.8% of dry matter depending on variety [Eggum *et al*., 1981]. Most of them accumulate in hulls. Steadman *et al*. [2001] noted that concentration of condensed tannins (proanthocyanidins) in bran of buckwheat, containing hull fragments, was 11–15 g/kg. Watanabe *et al*. [1997] separated buckwheat hulls extract using Sephadex LH-20 column chromatography and stated that proanthocyanidins were present in fractions which exhibited antioxidant activity. Also fractions of condensed tannins isolated from buckwheat seeds and buckwheat groats showed strong antioxidant properties [Amarowicz *et al*., 2005].

Antioxidant activity of tannins can result from their ability to chelate bivalent transition metal ions, *i.e.* iron, copper and zinc. These ions can generate highly reactive $^{\bullet}OH$ radicals by Fenton reactions [Borg, 1993]. Chelating agents, which stabilize prooxidative transition metal ions by complexing them, are regarded as secondary antioxidants. Until now studies were conducted on the metal ions chelating activity of pure tannins (commercial products) of known structure. Lopes *et al.* [1999] reported antioxidant properties of tannic acid to result from forming stable complexes with Fe(II). Mila *et al.* [1996] noted that polyphenols (tannins) remove Fe(III) from other iron/ligand complexes. However data considering how metal ions are chelated by tannin fraction separated from plant extracts, is scarce.

The aim of the study was to characterise tannin fractions obtained from buckwheat and buckwheat groats and to investigate their Fe(II), Cu(II) and Zn(II) chelating ability.

MATERIALS AND METHODS

Materials. The seeds of buckwheat were obtained from Plant Breading Station in Olsztyn. Roasted buckwheat groats was purchased at local market in Olsztyn.

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Chemicals. Sephadex LH-20, vanillin, bovine serum albumin (BSA), hexamethylenetetramine (hexamine), tetramethylmurexide ammonium salt (TMM), ferrozine (3-(2--Pyridyl)-5,6-diphenyl-1,2,4-triazine-4',4"-disulfonic acid), tannic acid, gallic acid, were obtained from Sigma-Aldrich Co. Ltd. (Poznań, Poland). Acetonitrile and trifluoracetic acid of HPLC grade were obtained from Merck (Darmstad, Germany). Proanthocyanidin B_2 was acquired from Extrasynthese S.A. (Genay Cedex, France). Potassium chloride (KCl), ferrous chloride ($FeCl_2 \cdot 4H_2O$), cupric sulfate ($CuSO_4 \cdot 5H_2O$), zinc chloride ($ZnCl_2$), acetone, ethanol and other reagents, all analytical grade, were purchased from P.O.Ch. Company (Gliwice, Poland).

Extraction of phenolic compounds. Ground buckwheat and buckwheat groats (20 g) were weighed out into sealable flasks and 160 mL of 80% acetone (v/v) was poured into [Amarowicz *et al.,* 1995]. Flasks were placed in a water bath at 70°C and shaken for 15 min. After cooling, supernatant was decanted carefully. Extraction was repeated twice more. Supernatants were combined, acetone was evaporated using a rotary evaporator at 40°C, and aqueous residue was lyophilised.

Isolation of tannins. Buckwheat and buckwheat groats extracts (2 g) dissolved in 20 mL of ethanol were applied into a column (5 × 40 cm) packed with Sephadex LH-20 gel. Ethanol (1 L), used as the first eluent, allowed removing low molecular weight phenolic compounds. Then 600 mL of 50% acetone (v/v) was used to elute tannins. Solvent from tannin fractions was removed using a rotary evaporator, and water was removed during lyophilisation.

Content of condensed tannins. The content of condensed tannins in such obtained materials was determined according to a modified vanillin assay [Price *et al.,* 1978]. Tannin fractions of buckwheat and buckwheat groats were dissolved in methanol (0.5 mg/mL). To 1 mL of prepared solution, 5 mL of vanillin/HCl reagent (0.5 g vanillin in 4% hydrochloric acid in methanol (v/v)) was added. Samples and controls (without vanillin) were allowed to stand for 20 min in darkness and then absorbance was read at 500 nm. Results were expressed as absorbance units per 1 mg of tannin fraction (A_{500}/mg).

BSA precipitation method. To 1 mL of tannin solution in water (1 mg/mL), 2 mL of bovine serum albumin solution in 0.2 mol/L acetic buffer, pH 5.0 with 0.17 mol/L NaCl (1 mg/mL) was added and mixed carefully [Hagerman & Butler, 1978]. After 15 min the samples were centrifuged at 5000 g for 15 min. The supernatant was removed, and pellet was dissolved in 4 mL of aqueous solution containing 1% SDS and 4% triethanolamine. Then 1 mL of 0.01 mol/L $FeCl_3$ in 0.01 mol/L HCl was added. After 30 min the absorbance was recorded at 510 nm. Results were expressed as absorbance units per 1 mg of tannin fraction (A_{510}/mg).

SE-HPLC. Tannin fractions from buckwheat and buckwheat groats were dissolved in 45% acetonitrile (v/v) with 0.1% TFA (v/v) (2 mg/mL) and analysed using a Shimadzu HPLC system consisting of LC-10AD_{Vp} pumps, UV-VIS SPD--M10A_{Vp} photo-diode array detector, SCL-10A_{Vp} system controller. The samples were injected into TSK Gel G2000SW_{XL} column (5 μm, 7.86 × 300 mm, TosoHaas) and eluted with 45% acetonitrile containing 0.1% TFA, with a flow rate of 0.2 mL/min. Injection volume was 20 μL. Gallic acid, proanthocyanidin B_2 and tannic acid were used as standards.

Zn(II) and Cu(II) chelating activity. The examined tannin fractions were dissolved in 0.01 mol/L hexamine/HCl buffer with addition of 0.01 mol/L KCl, pH 5.0 [Asakura *et al*., 1990]. $CuSO_4 \cdot 5H_2O$ or $ZnCl_2$ were dissolved in the same buffer at concentration of 0.25 mol/L and 0.8 mol/L, respectively. Tannin solution (1 mL) at concentration in the range of 0.2–1.0 mg/mL for testing copper ions chelating activity or 1–5 mg/mL in the case of zinc ions was mixed with 1 mL of salt solution, then 0.1 mL of an aqueous solution of tetramethylmurexide at a concentration of 1 mmol/L was added. Absorbance was recorded at 482 nm (Cu(II)) or 462 nm (Zn(II)) and 530 nm (Cu(II) and Zn(II)) using Beckman DU 7500 diode array spectrophotometer and the ratio of A_{482}/A_{530} (Cu(II)) or A_{462}/A_{530} (Zn(II)) was calculated. Control samples were prepared in the same way as proper samples, 1 mL of the examined tannin fraction solution was mixed with 1 mL of adequate salt solution; 0.1 mL of redistilled water was added instead of TMM reagents.

A standard curve of absorbance ratio *vs*. metal ions concentration was prepared; Cu(II) in the range of 0.025 - 0.125 mmol/L and Zn(II) from 0.2 to 2.0 mmol/L. The percentage of bound metal ions Me(II) was calculated using the following equation:

$$Bound\ Me(II)\,(\%) = \left(1 - \frac{Concentration\ of\ free\ Me(II)}{Concentration\ of\ total\ Me(II)}\right) \times 100$$

Fe(II) chelating activity. Aqueous solutions of buckwheat and buckwheat groats tannin fractions at a concentration in the range of 0.2–1.0 mg/mL were prepared. This solution (2.5 mL) was mixed well with 0.25 mL of 0.4 mmol/L $FeCl_2 \cdot 4H_2O$ and 0.5 mL of 5 mmol/L ferrozine [Boyer & McCleary, 1987]. Then the reaction mixture was allowed to stand for 10 min at ambient temperature, and absorbance at 562 nm was measured. Control samples were prepared in the same way, but water was added instead of ferrozine solution. The percentage of Fe(II) bound was calculated similarly to the TMM method.

RESULTS AND DISCUSSION

Buckwheat and buckwheat groats tannin fractions were obtained from the acetonic crude extracts using Sephadex LH-20 column chromatography. In order to characterise fractions, reaction of tannins with vanillin/HCl reagent and their ability to precipitate bovine serum albumin (BSA)

TABLE 1. Condensed tannin content and protein precipitation capacity of buckwheat and buckwheat groats tannin fractions. The results are expressed as absorbance per mg of tannin fractions.

Tannin	Condensed tannins (A_{500}/mg)	Protein precipitation capacity (A_{510}/mg)
Buckwheat	1.04±0.012	0.37±0.011
Buckwheat groats	1.04±0.010	0.39±0.009

were examined. The results of assays are reported in Table 1. There were no differences between condensed tannin content of buckwheat and buckwheat groats fractions. The results expressed as A_{500}/mg for both samples were 1.04. Also protein precipitation capacity of buckwheat and buckwheat groats tannins expressed as A_{510}/mg was similar for both fractions: 0.37 and 0.39, respectively. Naczk *et al.* [2001] reported comparable results of BSA test for beach pea, canola hulls and faba bean tannins (A_{510}/mg 0.2–0.4) and much higher for evening primrose tannins. In the cited study tannins after reaction with vanillin gave A_{500}/g between 394 (evening primrose) and 1694 (beach pea). On the other hand, Troszyńska *et al.* [2006] analysing crude extracts of buckwheat and buckwheat groats obtained 10 times lower values of A_{510}/g.

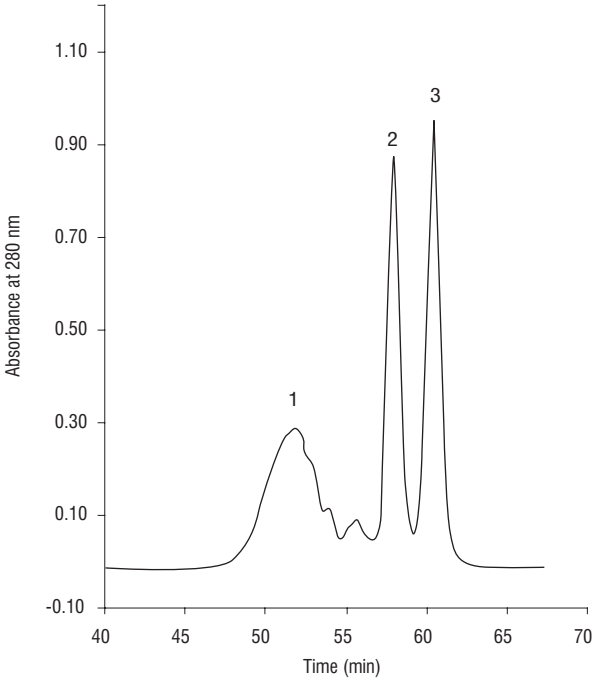

FIGURE 1. SH-HPLC chromatogram of molecular weight standards; 1 – tannic acid, 2 – proanthocyanidin B_2, 3 – gallic acid.

Tannins separated from plant materials are the mixture of polymeric compounds with different degree of polymerization. Therefore in this study SE-HPLC analysis was carried out to characterise molecular weight distribution of buckwheat and buckwheat groats tannin fractions. Gallic acid (molecular weight – 170), proanthocyanidin B_2 (578) and tannic acid were used as standard for TSK Gel G2000SW_{XL} column calibration (Figure 1). Tannic acid is heterogeneous and variable mixture of galloyl esters and its molecular weight can be estimated only approximately; usually the value of 1290 is given. Figure 2A depicts SE-HPLC separation of buckwheat tannins. In this fraction polymers of molecular weight equal tannic acid and proanthocyanidin B_2 are present, but polymers of intermediate molecular weights are dominant. Two weakly separated peaks with retention times of 52.34 and 53.62 min are shown in the chromatogram. The UV spectra of molecules giving those peaks are similar, and their maxima were recorded at 281 and 284 nm, respectively (Figure 2B, 2C). SE-HPLC chromatogram of buckwheat

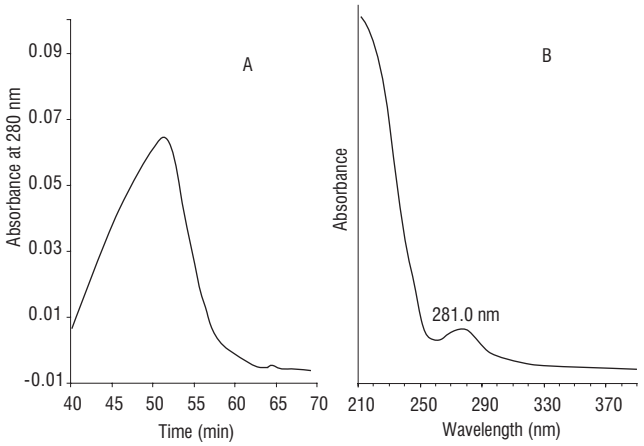

FIGURE 3. SE-HPLC chromatogram of buckwheat groats tannin fraction (A) and UV-spectrum of compound related to peak with t_r 51.30 min (B).

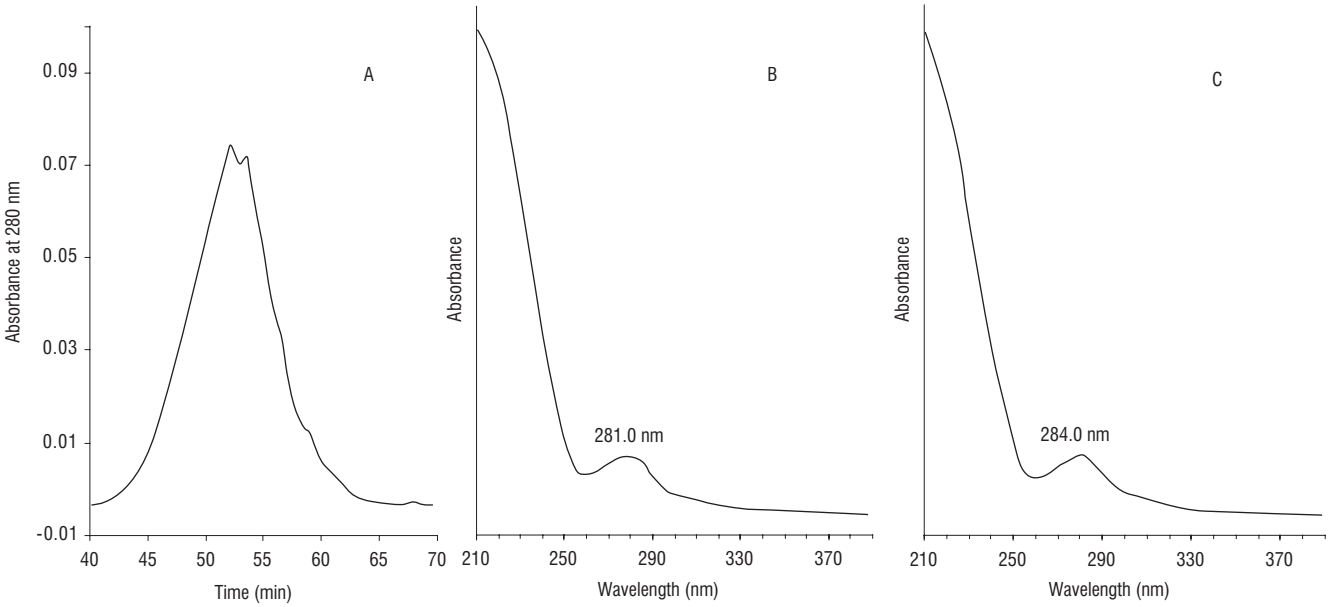

FIGURE 2. SE-HPLC chromatogram of buckwheat tannin fraction (A) and UV-spectra of compounds related to the peaks with t_r 52.34 min (B) and t_r 53.62 min (C).

tannins did not reveal the presence of compounds of low molecular weight comparable to gallic acid FW. Distribution of molecular weight of buckwheat groats tannins was moderately different (Figure 3A). Polymers characterised by molecular weight similar to tannic acid FW were dominant, but larger compounds, with longer or more branched polymers were also present. Absorbance response of molecules adequate to proanthocyanidin B_2 was much weaker than in the case of buckwheat tannins. Low molecular weights compounds were detected neither in buckwheat nor in buckwheat groats. The peak of t_r 51.30 min characterised by maximum of UV at 281 nm (Figure 3B) was dominant in the SE-HPLC chromatogram of buckwheat groats tannin fractions.

Cu(II) and Zn(II) chelating activity of buckwheat and buckwheat groats tannin fractions was determined by the method applying tetramethylmurexide (TMM). In the first step, the absorption spectra of TMM and complexes of metal ions and TMM were recorded. The absorption maxima allow to set wavelengths at which absorbance should be recorded. The ratio of absorbance of TMM to absorbance of TMM--metal ions complexes was plotted *vs*. various amounts of metal ions. On the basis of this standard curve the amount of metal ions bound by the compound tested was evaluated. Therefore absorption spectra of reaction products of ions of copper and zinc with TMM were recorded (Figure 4). The wavelengths for measurement were set at 482 nm for copper ions and 462 nm for zinc ions. The TMM method cannot be used to evaluate Fe(II) chelating activity of buckwheat and buckwheat groats tannins because this kind of samples (high tannin content) cause high absorbance reading for the control samples [Karamać & Pegg, 2007]. Therefore the method with ferrozine was applied examine iron ions chelating activity of the tannins tested.

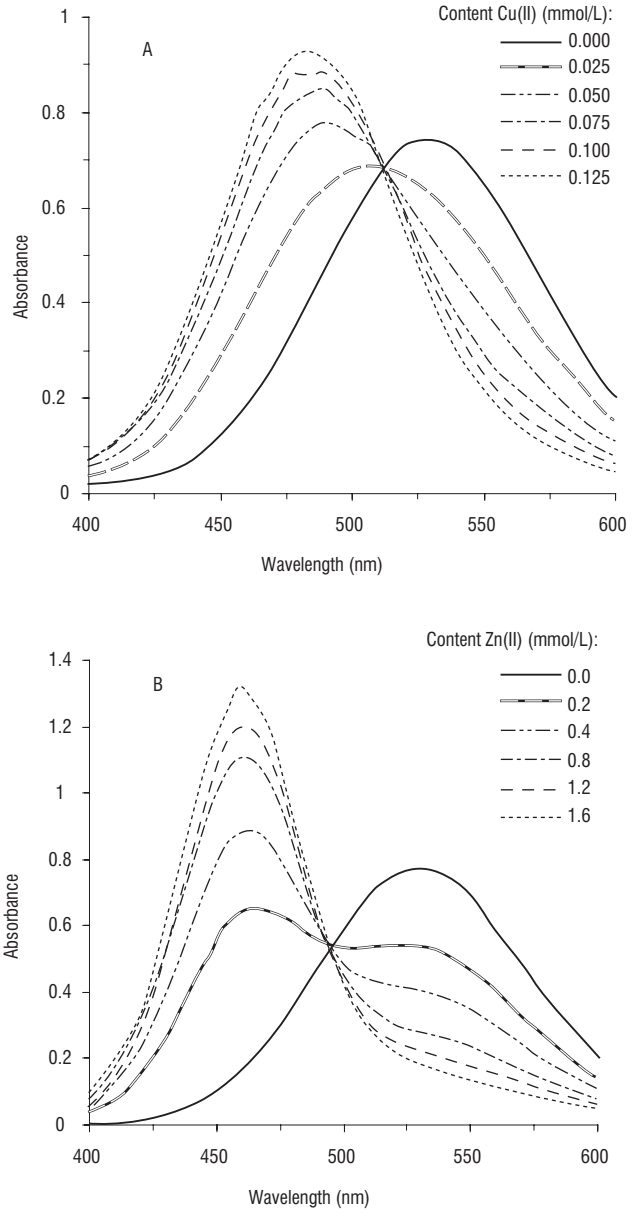

FIGURE 4. Absorption spectra of TMM at various concentrations of Cu(II) (A) and Zn(II) (B).

Figure 5 depicts ferrous ions chelating activity of buckwheat and buckwheat groats tannin fractions. The reactions mixture containing 2.5 mg of buckwheat tannin fraction was able to bound half (50.3%) of Fe(II) added, whereas the same amount of buckwheat groats tannin fraction bound only 24%. Also smaller addition of buckwheat tannins caused twice higher chelation of ferrous ions than buckwheat groats. Copper ions chelating activity of buckwheat and buckwheat groats tannin fractions (Figure 6) was much higher: 1 mg of fractions added, bound 86.0% and 72.9% Cu(II), respectively. The greatest increment in chelating copper ions was caused by the addition of 0.4 mg of buckwheat fraction and 0.2 mg of buckwheat groats tannin. Above this values the rate of Cu(II) binding reduced, especially for buckwheat fraction. Among all metal ions tested, Zn(II) chelating activity was the lowest (Figure 7). Also in this case, buckwheat tannins were more effective chelators. Addition of 5 mg buckwheat tannin fraction bound 17.2% of zinc ions, whereas the same amount of buckwheat groats tannins chelated only 13.5%. The increment of zinc ions bound was comparable for both samples tested when 1 mg of tannins was added.

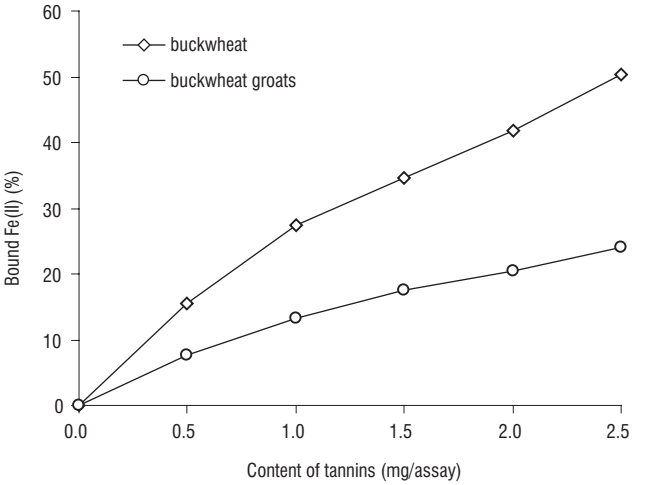

FIGURE 5. Fe(II) chelating activity of buckwheat and buckwheat groats tannin fractions.

It is known from literature data that phenolic compounds can complex metal ions only when their have suitably oriented functional groups in their structure [van Acker *et al*., 1996]. The presence of 3'-4' and/or 7-8 *o*-dihydroxyphenyl

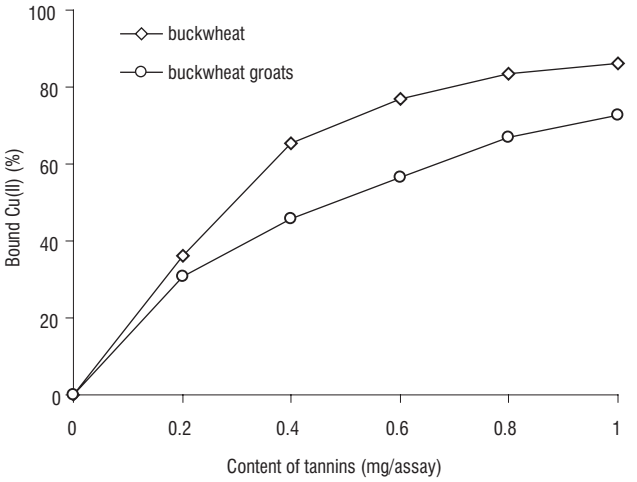

FIGURE 6. Cu(II) chelating activity of buckwheat and buckwheat groats tannin fractions.

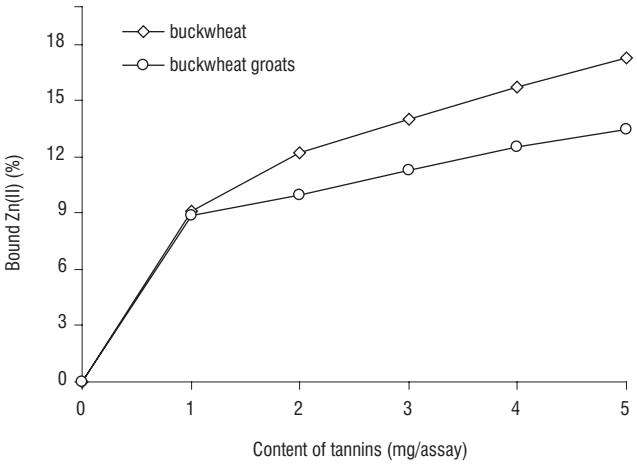

FIGURE 7. Zn(II) chelating activity of buckwheat and buckwheat groats tannin fractions.

(catechol) groups or 5-OH and/or 3-OH in conjugation with a C4 keto group in phenolic compounds structure is important for iron ions binding [Khokhar & Apenten, 2003]. The chelating activity increases when galloyl moiety (3',4',5'-OH trihydroxybenzene) is present in phenolic compound molecule. The compounds with galloyl group precipitate copper more efficiently than compounds with catechol [McDonald *et al*., 1996]. Nevertheless glycosylation of phenolic groups makes metal ions binding impossible [Hider *et al*., 2001]. When chemical structure of tannins is considered, it could be presumed that condensed tannins, which are catechin polymers, bind metal ions mainly to catechol groups, whereas hydrosylable tannins (derivatives of gallic acid) to galloyl groups. In our study, buckwheat and buckwheat groats tannin fractions revealed similar results in the vanillin test and protein precipitation method, but metal ions chelating activity of buckwheat tannins was stronger. The difference could be explained by the diversity of their structure, which is confirmed by SE-HPLC results (Figures 2 and 3). Probably, buckwheat tannins consist of slightly shorter or less branched polymers, but their molecules could be more esterified with gallic acid. On the other hand, the ratio of hydrolysable to condensed tannins in buckweat groats tannin fraction could be lower. The third possibility is that a large amount of carbohydrate molecules bind to buckwheat polyphenols.

It is difficult to compare the results of Cu(II), Fe(II) and Zn(II) chelated obtained in the present study with literature data. The authors express chelating activity of a specific amount of ligand in different ways. Fe(II) was bound by 100 ppm of extracts of hulls and whole sesame in 62% and 50% (black sesame) and 25.8% and 17.1% (white sesame) [Wettasinghe & Shahidi, 2002]. The same amount of crude extracts of borage and evening primrose bound ferrous ions in 43% and 63%, respectively [Shahidi *et al*., 2006]. On the other hand, 250 μg of nettle extract bound 92% of Fe (II) added [Gulcin *et al*., 2004]. Zn(II) was chelated by 0.1% instant coffee in over 90% [Asakura *et al*., 1990], and Cu(II) by extracts of 25 edible tropical plants in approximately from 40% to 95% [Wong *et al*., 2006].

CONCLUSIONS

Buckwheat and buckwheat groats tannin fractions react with vanillin and precipitate BSA to a similar extent. Their molecular weight distribution analysed by SE-HPLC method differs slightly. All ion metals tested (Fe(II), Cu(II) and Zn(II)) were chelated stronger by buckwheat than buckwheat groats tannin fractions. Copper ions chelating activity of the investigated tannins was higher than that of ferrous and zinc ions.

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REFERENCES

1. Amarowicz R., Piskuła M., Honke J., Rudnicka B., Troszyńska A., Kozłowska H., Extraction of phenolic compounds from lentil seeds (*Lens culinaris*) with various solvents. Pol. J. Food Nutr. Sci., 1995, 45, 53–62.
2. Amarowicz R., Troszyńska A., Karamać M., Antioxidant activity of plant extracts with high tannin content. Bromat. Chem, Toksykol. – Supl., 2005, 38, 193–195 (in Polish).
3. Asakura T., Nakamura Y., Inoue N., Murata M., Homma S., Characterization of zinc chelating compounds in instant coffee. Agric. Biol. Chem., 1990, 54, 855–862.
4. Borg D.C., Oxygen free radicals and tissue injury. 1993, *in*: Oxygen Free Radicals in Tissue Damage (eds. M. Tarr, F. Samson). Birkhauser, Boston, pp. 12–53.
5. Boyer R.F., McCleary, C.J., Superoxide ion as a primary reductant in ascorbate-mediated ferritin iron release. Free Rad. Biol. Med., 1987, 3, 389–395.
6. Eggum B.O., Kreft I., Javornik B., Chemical composition and protein quality of buckwheat (*Fagopyrum eseulentum*). Qual. Plant - Plant Foods Hum. Nutr., 1981, 30, 175–179.
7. Gulcin I., Kufrevioglu O.I., Oktay M., Buyukokuroglu M.E., Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.). J. Ethnophar., 2004, 90, 205–215.
8. Hagerman A.E., Butler L.G., Protein precipitation method for the quantitative determination of tannins. J. Agric. Food Chem., 1978, 26, 809–812.
9. Hider R.C., Liu Z.D., Khodr H.H., Metal chelation of polyphenols. Methods Enzymol., 2001, 335, 190–203.
10. Holasova M., Fiedlerova V., Smrcinova H., Orsak M., Lachman

J., Vavreinova S., Buckwheat - the source of antioxidant activity in functional foods. Food Res. Inter., 2002, 35, 207–211.
11. Karamać M., Pegg R.B., The limitation of the tetrametylmurexide method for investigation of Fe(II) chelation activity of phenolic compounds. J. Agric. Food Chem., 2007, submitted.
12. Khokhar S., Apenten R.K.O., Iron binding characteristics of phenolic compounds: some tentative structure – activity relations. Food Chem., 2003, 81, 133–140.
13. Lopes G.K.B., Schulman H.M., Hermes-Lima M., Polyphenol tannic acid inhibits hydroxyl radical formation from Fenton reaction by complexing ferrous ions. Bioch. Bioph. Acta., 1999, 1472, 142–152.
14. McDonald M., Mila I., Scalbert A., Precipitation of metal ions by plant polyphenols: optimal conditions and oridin of precipitation. J. Agric. Food Chem., 1996, 44, 599–606.
15. Mila I., Scalbert A., Expert, D., Iron withholding by plant polyphenols and resistance to pathogens and rots. Phytochemistry, 1996, 42, 1551–1555.
16. Naczk M., Amarowicz R., Zadernowski R., Shahidi F., Protein precipitating capacity of condensed tannins of beach pea, canola hulls, evening primrose and faba bean. Food Chem., 2001, 73, 467–471.
17. Oomah B.D., Mazza G., Flavonoids and antioxidative activities in buckwheat. J. Agric. Food Chem., 1996, 44, 1746–1750.
18. Price M.L, Van Scoyoc S., Butler L.G., A critical evaluation of the vanillic reaction as an assay for tannin in sorghum grain. J. Agric. Food Chem., 1978, 26, 1214–1218.
19. Shahidi F., Liyana-Pathirana C.M., Wall D.S., Antioxidant activity of white and black sesame seeds and their hull fractions. Food Chem., 2006, 99, 478–483.
20. Steadman K.J., Burgoon M.S., Lewis B.A., Edwardson S.E., Obendorf R.L., Minerals, phytic acid, tannin and rutin in buckwheat seed milling fractions. J. Sci. Food Agric., 2001, 81, 1094–1100.
21. Troszyńska A., Amarowicz R., Wołejszo A., Sensory and chemical approach to astringency of extracts from selected tannin – rich foods. Pol. J. Food Nutr. Sci., 2006, 56, 291–295.
22. van Acker S.A.B.E., van den Berg D.J., Tromb M.N.J.L., Griffioen D.H., van Benekom W.P., van der Vijgh W.J.F., Bast A., Structural aspects of antioxidant activity of flavonoids. Free Rad. Biol. Med., 1996, 20, 331–342.
23. Watanabe M., Ohshita Y., Tsushida T., Antioxidant compounds from buckwheat (*Fagopyrum esculentum Moench*) hulls. J. Agric. Food Chem., 1997, 45 1039–1044.
24. Watanabe M., Catechins as antioxidants from buckwheat (*Fagopyrum esculentum Moench*) groats. J Agric. Food Chem., 1998, 46 839–845.
25. Wettasinghe M., Shahidi F., Iron (II) chelation activity of extracts of borage and evening primrose meals. Food Res. Int., 2002, 35, 65–71.
26. Wijngaard H.H., Arendt E.K., Buckwheat. Cereal Chem., 2006, 83, 391–401.
27. Wong S.P., Leong L.P., Koh J.H.W., Antioxidant activities of aqueous extracts of selected plants. Food Chem., 2006, 99, 775 –783.

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CHELATOWANIE FE(II), CU(II) I ZN(II) PRZEZ FRAKCJE TANINOWE Z GRYKI I KASZY GRYCZANEJ

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Ekstrakty acetonowe z gryki i kaszy gryczanej rozdzielano na kolumnie wypełnionej żelem Sephadex LH-20 na frakcję niskocząsteczkowych związków fenolowych i taninową. Frakcje taninowe scharakteryzowano za pomocą reakcji z waniliną, zdolności do strącania białka oraz metodą SE-HPLC z zastosowaniem kolumny TSK Gel G2000SW$_{XL}$ (TosoHaas). Kompleksowanie jonów Cu(II) i Zn(II) przez taniny oznaczano w reakcji z tetrametylomureksydem (TMM). Chelatowanie Fe(II) badano wykorzystując reakcję barwną kompleksowania ferrozyny.

Wyniki testu z waniliną przeprowadzonego z frakcjami taninowymi z gryki i kaszy gryczanej były podobne; wartość A_{500}/mg wynosiła 1,04. Również w metodzie strąceniowej obie próby wykazały zbliżone do siebie rezultaty. Rozdział SE-HPLC tanin z kaszy gryczanej charakteryzował się obecnością polimerów o masach cząsteczkowych większych lub równych masie cząsteczkowej kwasu taninowego. Na chromatogramie frakcji taninowej z gryki piki były przesunięte w stronę mniejszych mas cząsteczkowych.

Frakcja tanin z gryki wiązała więcej wszystkich testowanych jonów metali w porównaniu z frakcją tanin z kaszy gryczanej. Obie frakcje tanin najefektywniej chelatowały jony Cu(II). Frakcja tanin gryki dodana do próby w ilości 1 mg związała 86,0% jonów miedzi, natomiast frakcja z kaszy gryczanej – 72,9%. Dodatek 2,5 mg/próbę tanin gryki wiązał ponad dwa razy więcej jonów Fe(II), niż taka sama ilość tanin z kaszy gryczanej. Jony cynku były najsłabiej chelatowane. Frakcja tanin gryki (5 mg) kompleksowała 17,2% a tanin kaszy gryczanej – 13,5% jonów Zn(II).