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WHEAT AND BUCKWHEAT MILLING FRACTIONS – INSIGHT IN THEIR FUCTIONAL CHARACTERISTICS
FRAKCIJE MLEVENJA PŠENICE I HELJDE – UVID U NJIHOVE FUNKCIONALNE KARAKTERISTIKE

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ABSTRACT

This study examined the antioxidant properties of the ethanolic extracts of wheat milling fractions (wheat flour type 400, type 500, and type 850, wholegrain wheat flour and wheat bran) and buckwheat flours (light and wholegrain buckwheat flour) and their polyphenol composition. The total phenolic content, antiradical activity on DPPH·, antioxidant activity (AOA), reducing activity and chelating activity on Fe^{2+} were tested. Buckwheat flours exhibited significantly higher ($P < 0.05$) antiradical activity, AOA and reducing activity than all investigated wheat milling fractions when their corresponding IC_{50} values were compared. Chelating activity on Fe^{2+} was the only assay in which wheat milling fractions demonstrated better potential than buckwheat flours. The antioxidant properties of wheat and buckwheat milling fractions could be attributed to the presence of polyphenols. The obtained results indicate the benefit of using buckwheat flours in wheat-based food products, i.e. their contribution in the production of new bakery functional foods.

Key words: *wheat milling fractions, buckwheat flour, antioxidant activity.*

REZIME

Upoznavanje veze između ishrane i zdravlja vodi ka narastajućem interesu za funkcionalnu hranu, te njenom konzumiranju. Obzirom da su pekarski proizvodi dominantan deo ishrane mnogih naroda, sve su učestaliji pokušaji da se osmisle nove formulacije za pekarske funkcionalne proizvode. U svrhu razvoja palete novih pekarskih proizvoda koriste se različite alternativne kulture. Heljda poseduje veliki potencijal za unapređenje funkcionalnih svojstava pekarskih proizvoda zbog njene visoke antioksidativne aktivnosti, uglavnom uslovljene prisustvom polifenolnih jedinjenja, pre svega rutina. Dokazano je da rutin ispoljava višu antioksidativnu aktivnost u poređenju sa fenolnim kiselinama, karakterističnim polifenolima mnogih žitarica. Stoga supstitucija pšeničnog brašna heljdinim brašnom u formulaciji pekarskog proizvoda rezultira njegovom povećanom funkcionalnošću, odnosno antioksidativnim kapacitetom. Usmeravajući fokus na navedene činjenice, ovaj rad obrađuje antioksidativne osobine etanolnih ekstrakata frakcija mlevenja pšenice (pšenično brašno tip 400, tip 500, tip 850, integralno pšenično brašno i pšenične mekinje) i heljdinih brašna (belo i integralno heljdino brašno) i njihov polifenolni sadržaj. U radu su određeni sadržaji ukupnih rastvorljivih fenola, antiradikalska aktivnost na DPPH·, ukupna antioksidativna aktivnost (AOA), redukciona aktivnost, kao i helataciona aktivnost na Fe^{2+}. Poredeći odgovarajuće IC_{50} vrednosti, heljdina brašna su ispoljila signifikantno višu ($P < 0,05$) antiradikalsku aktivnost na DPPH·, AOA i redukcionu aktivnost u poređenju sa svim ispitivanim frakcijama mlevenja pšenice. Određivanje helatacione aktivnosti na Fe^{2+} bilo je jedino u kome su frakcije mlevenja pšenice bile superiornije u odnosu na heljdina brašna. Antioksidativne osobine frakcija mlevenja pšenice i heljde mogle bi se pripisati prisustvu, odnosno delotvornosti njihovih polifenolnih jedinjenja. U frakcijama mlevenja pšenice dominantno polifenolno jedinjenje je ferulna kiselina, dok je u heljdinim brašnima rutin. Dobijeni rezultati ukazuju na dobrobit korišćenja heljdinih brašna u pekarskim proizvodima na bazi pšenice, odnosno njihovom doprinosu u proizvodnji novih funkcionalnih pekarskih proizvoda.

Ključne reči: *frakcije mlevenja pšenice, heljdina brašna, antioksidativna aktivnost.*

INTRODUCTION

The relationship between diet and health has led to the increased interest and consumption of functional foods (*Hasler, 2002; Jones and Jew, 2007*). Bakery products are recognized as dominant in the diet of many populations, and therefore increasing attempts to create new formulations of bakery functional foods arise. For that purpose, different alternative crops (amaranth, quinoa, buckwheat, etc.) are used for development of new bakery products. Cereals, which are the the main raw material for production of bakery products, have been known to contain high amounts of benzoic and hydroxycinnamic acid derivatives, anthocyanidins, quinones, flavonols, chalcones, flavones, flavanones, and amino phenolic compounds. that. These compounds are responsible for the functional nature of some bakery products since they render potential health benefits (*Andreasen et al., 2001*), especially when oxidative stress is incorporated in the development of some diseases (*Baublis et al., 2000*). They could act as scavengers of free radicals, reduction agents and metal chelators (*Rice-Evans et al., 1996*). *Zhou and Yu* (2004) determined that the scavenging effect and the total phenolic content of wheat were in a correlation with its ferulic acid content, predominant phenolic acid from wheat accounting for approximately 57-77% of total phenolic acids present in wheat on a dry weight basis. Buckwheat (Fagopyrum esculentum Moench) possesses a great potential for upgrading the functional properties of bakery products due to its high antioxidant activity, mainly caused by polyphenol compounds, first of all rutin (*Kreft et al., 2006*). *Zielinski and Kozlowska* (2000) have established the following hierarchy of antioxidant activity for 80% methanolic extracts originated from the whole grain: buckwheat > barley > oat > wheat = rye. *Holasova et al.* (2002) also reported that phenolic compounds in buckwheat, namely 3-flavanols, rutin, phenolic acids and their derivatives, possessed antioxidant activity stronger than antioxidant components of oat and barley. It was proved that rutin demonstrates better antioxidant activity in comparison to phenolic acids which are characteristic polyphenols of many cereals (*Yang et al., 2008*). Strong antioxidant nature of rutin is assumed as the main reason that rutin and its hemisynthetic derivatives exert different biological effects like normalization of increased vascular permeability and fragility, oedema protection (*Ihme et al., 1996*), and antihemorrhagic properties (*Hung and Morita, 2008*).

Therefore, it is expected that substitution of wheat flour with buckwheat flour in the formulation results in increased functional properties, i.e. antioxidant capacity of the produced bakery

product. There are many papers which confirm better functional properties of the products containgbuckwheat flour in their formulations (*Bojňanská et al., 2009; Fessas et al., 2008; Gawlik-Dziki et al., 2009; Lin et al., 2009*).

Being focused on these facts, this study examined the antioxidant properties of the ethanolic extracts of wheat milling fractions (wheat flour type 400, type 500, and type 850, wholegrain wheat flour and wheat bran) and buckwheat flours (light and wholegrain buckwheat flour) and their polyphenol composition. The obtained results could provide basics for upgrading of functional bakery production based on antioxidant properties of investigated milling fractions.

MATERIALS AND METHODS

Chemicals

Butylated hydroxytoluole (BHT), β-carotene, 1,1-diphenyl-2-picrylhydrazyl (DPPH), ethylene diamine tetraacetic acid disodium salt dihydrate (EDTA), 3-(2-pyridyl)-5-6-bis(4-phenylsulfonic acid)-1,2,4-triazine (ferrozine), ferrous sulfate heptahydrate, ferric chloride, Folin-Ciocalteu's reagent, ferulic acid, gallic acid, linoleic acid (99%), potassium ferricyanide, quercetin, rutin, sodium carbonate, Tween 40, and trichloracetic acid (TCA) were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). HPLC grade methanol was used for chromatography (Merck, Darmstadt, Germany). All other chemicals and solvents (ethanol, methanol, chloroform, formic acid) were of analytical grade and purchased from Merck, Darmstadt, Germany. Water was purified using Millipore Elix 10 UV water purification system, and ultrapure water used in mobile phase preparation for HPLC was obtained using Simplicity UV, Millipore.

Wheat and buckwheat milling fractions

Wheat flours (types 400, 500 and 850 with ash content < 0.45%, 0.46-0.55% and 0.80-0.90%, respectively), wholegrain wheat flour, wheat bran, light buckwheat flour and wholegrain buckwheat flour were purchased at the local market.

Proximate composition

Proximate composition of wheat and buckwheat milling fractions was analyzed using the methods of AOAC (1984) for determining the moisture, crude protein, ash, crude cellulose, crude fat and starch content.

Preparation of ethanolic extracts

Wheat or buckwheat milling fraction (10 g) was mixed with 100 mL of 96% ethanol. Extraction was carried out by shaking the mixture at room temperature for1 h. The extract was separated by filtering through the filter paper (Whatman, Grade 4 Chr, UK), and procedure was repeated twice with 100 mL of solvent. The extracts (3 x 100 mL) were combined and dried by vacuum-evaporator. The dried extract was weighed and the yield was calculated based on the wet mass of the samples. The dried extract was redissolved in 96% ethanol to 10 mL volume. The extract obtained by following this procedure was used for further investigation of antioxidant activity.

Total phenolic content

Total phenolic content of wheat or buckwheat milling fractions' extracts was determined spectrophotometrically by using Folin-Ciocalteu's reagent (*Singleton et al., 1999*). Gallic acid was used as a standard and results were expressed as gallic acid equivalents (GAE) (μg GAE/g of sample on dry mass basis). The extract (0.1 mL) of wheat or buckwheat milling fraction was diluted with pure water (7.9 mL). Folin-Ciocalteu's reagent (0.5 mL) and sodium carbonate solution (1.5 mL; concentration 20 g/100 mL) were added, and the reaction mixture was mixed thoroughly. The mixture was allowed to stand for 120 min with intermittent shaking, and the absorbance at 750 nm was measured (Jenway, 6405 UV/Vis).

DPPH radical scavenging activity

Effect of the examined extracts on the content of 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH·) was estimated according to the modified method of *Hatano et al.* (1988). The concentration of the DPPH· solution used in the assay was 90 μmol/L (22.5 mL 0.4 mmol/L DPPH· solution (0.01577 g DPPH· in 100 mL methanol) was diluted with 95% methanol to 100 mL). An aliquot (1.0 mL) of the DPPH· solution (90 μmol/L) was diluted in 2.9 mL methanol, and 0.1 mL of the extracts at various concentrations (10.0, 20.0, 30.0 and 40.0 mg/mL for wheat extracts and 1.0, 1.5, 3.0 and 5.0 mg/mL for buckwheat extracts) was added. The mixture was shaken vigorously and left to stand for 60 min in the dark, then the absorbance was measured at 517 nm (Jenway, 6405 UV/Vis) against the blank (mixture without extract). The IC_{50} value (mg/mL) was defined as the concentration of an antioxidant extract which was required to quench 50% of the initial amount of DPPH· under the experimental conditions given. It was obtained by interpolation from linear regression analysis. BHT was used as a control.

Antioxidant activity (AOA) by β-carotene bleaching method

Oxidative loss of β-carotene in a β-carotene/linoleic acid emulsion was used to assess the antioxidant activity of the examined extracts (*Moure et al., 2000*). β-carotene (2 mg) was dissolved in 10 mL of chloroform and 1 mL β-carotene solution was mixed with 20 mg of purified linoleic acid and 200 mg of Tween 40 in a round-bottom flask. Chloroform was removed by purging with nitrogen. Pure water (50 mL) was added into a β-carotene/linoleic acid emulsion and mixed well by using a vortex mixer (V1 plus BOECO, Germany). The extracts of wheat or buckwheat milling fractions at various concentrations (5.0, 10.0, 15.0 and 20.0 mg/mL for wheat extracts and 2.5, 5.0, 7.5 and 10.0 mg/mL for buckwheat extracts) (0.2 mL) and aliquots (5 mL) of the β-carotene/linoleic acid emulsion were placed in capped culture tubes and mixed thoroughly. The tubes were immediately placed in a water bath and incubated at 50 °C. Oxidation of the β-carotene/linoleic acid emulsion was monitored spectrophotometrically by measuring the absorbance at 470 nm after 120 min (Jenway, 6405 UV/Vis). A control was prepared by using 0.2 mL of 96% ethanol instead of the extract. Degradation rate of the extracts was calculated according to first order kinetics using Eq. (1) (*Al-Saikhan et al., 1995*):

$$\ln(a/b) \times 1/t = \text{sample degradation rate}, \quad (1)$$

where ln, natural log; *a*, initial absorbance (470 nm) at time zero; *b*, absorbance (470 nm) at time 120 min; t, time (min).

The antioxidant activity (AOA) was expressed as % inhibition relative to the control using Eq. (2):

$$\text{AOA} = \frac{\text{Degradation rate of control - Degradation rate of sample x 100}}{\text{Degradation rate of control}} \quad (2)$$

The IC_{50} value (mg/mL) was defined as effective concentration at which the AOA was 50% under the experimental conditions. It was obtained by interpolation from linear regression analysis. BHT was used as a control.

Reducing power

Reducing power of the ethanolic extracts was measured according to the method of *Oyaizu* (1986). Various concentrations (5.0, 10.0, 15.0 and 20.0 mg/mL for wheat extracts and 2.5, 5.0,

7.5 and 10.0 mg/mL for buckwheat extracts) of the ethanolic extracts (0.5 mL) were mixed with 2.5 mL of phosphate buffer (0.2 mol/L, pH 6.6) and 2.5 mL of potassium ferricyanide (1%). The mixtures were incubated at 50 °C for 20 min, and after that TCA (10%, 2.5 mL) was added. The mixtures were centrifuged at 650 g for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of pure water and 0.5 mL of ferric chloride and the absorbance was measured at 700 nm (Jenway, 6405 UV/Vis). Higher absorbance of the reaction mixture indicates greater reducing power. The IC_{50} value (mg/mL) was defined as an effective concentration of extract at which the absorbance of reaction mixture reach 0.5 for reducing power and was obtained by interpolation from linear regression analysis. BHT was used as a control.

Chelating activity on Fe^{2+}

Chelating activity of the ethanolic extracts on Fe^{2+} was measured according to the method of *Decker and Welch* (1990). Aliquots of 1 mL of different concentrations of ethanolic extracts of wheat or buckwheat milling fractions (0.01, 0.05, 0.1 and 0.5 mg/mL for wheat extracts and 0.1, 0.5, 1.0 and 2.0 mg/mL for buckwheat extracts) were mixed with 3.7 mL of pure water. The mixture was left to react with $FeSO_4$ (2 mmol/L, 0.1 mL) and ferrozine (5 mmol/L, 0.2 mL) for 10 min at room temperature, and then the absorbance was measured at 562 nm (Jenway, 6405 UV/Vis). A lower absorbance indicates a higher chelating power. The chelating activity on Fe^{2+} of the ethanolic extracts was compared with EDTA.

Chelating activity was calculated according to Eq. (3):

$$\text{Chelating activity}(\%) = 100 - \frac{\text{Absorbance of sample at 562 nm x 100}}{\text{Absorbance of control at 562 nm}} \quad (3)$$

The IC_{50} value (mg/mL) was defined as the concentration of an antioxidant extract which chelates 50% of present Fe^{2+} under the experimental conditions. It was obtained by interpolation from linear regression analysis.

HPLC determination of phenolic compounds

HPLC analysis was performed by using a liquid chromatograph (Agilent 1200 series), equipped with a diode array detector (DAD), on an Agilent, Eclipse XDB-C18, 1.8 μm, 4.6 x 50 mm column, at a flow-rate of 1.000 mL/min. Based on the previously used method (*Ćetković et al., 2008*), new chromatographic conditions were developed. The solvent linear gradient program was created by varying the proportion of solvent A (methanol) to solvent B (1% formic acid in water (v/v)) as follows: initial 10% A; 0-10 min, 10-25% A; 10-20 min, 25-60% A; 20-30 min, 60-70% A. The run time and post-run time were 45 and 10 min, respectively. The column was operated at 30 °C. The injected volume of samples and standards was 5 μL and it was done automatically, using autosampler. The spectra were acquired in the range 210-400 nm and chromatograms plotted at 280, 330 and 350 nm with reference wavelength set at 550/100 nm. All samples were injected as solutions in methanol: 1% acetic acid in water (1:1) with a final concentration of 8.00-15.0 mg/mL. Solutions were filtered prior to injection through Nylon membrane filters with pore size 0.20 μm (Rotilabo-Spritzenfilter 13 mm, Roth, Karlsruhe, Germany). Phenolic components in a sample extract were identified by matching the retention time and their spectral characteristics against those of the standards. The purity of the peaks was determined to ensure the identification. The external standard method was a technique used for quantification. For each component (rutin, quercetin, ferulic acid) a stock solution was made from the commercial standards which was dissolved in methanol and the obtained concentration was 1.00 mg/mL. The diluted stock solutions were used for calibration. The final concentrations were in the range of 0.005-0.20 mg/mL. The peak areas from the chromatograms were plotted against the known concentrations of the standards. The equations generated via linear regression were used to establish the concentrations of the phenolic compounds in the extracts.

Statistical analysis

All analyses were performed in triplicate, and the mean values with the standard deviations (S.D.) are reported. Analysis of variance and Duncan's multiple range test were used. Statistical data analysis software system STATISTICA (StatSoft, Inc. (2008). data analysis software system, version 8.0. www.statsoft.com) was used for analysis. P values < 0.05 were regarded as significant.

RESULTS AND DISCUSSION

Proximate composition

Proximate composition of the commercially available edible wheat products (wheat flour type 400, 500, 850, wholegrain wheat flour and wheat bran) and buckwheat flours (light buckwheat flour and wholegrain buckwheat flour) used are presented in Table 1.

Table 1. Proximate composition (% dry basis) of wheat and buckwheat milling fractions

Material	Moisture	Crude protein (Nxfactor)[a]	Ash	Crude cellulose	Crude fat	Starch
Wheat flour type 400	13.3 ± 0.18	10.5 ± 0.05	0.39 ± 0.03	n.d.	0.53 ± 0.03	72.4 ± 0.35
Wheat flour type 500	13.1 ± 0.12	11.4 ± 0.23	0.47 ± 0.03	n.d.	0.75 ± 0.04	71.3 ± 0.35
Wheat flour type 850	13.4 ± 0.11	12.6 ± 0.14	0.80 ± 0.04	0.62 ± 0.07	1.25 ± 0.05	68.4 ± 0.34
Wholegrain wheat flour	13.6 ± 0.20	13.0 ± 0.07	1.14 ± 0.02	2.57 ± 0.24	2.34 ± 0.06	60.8 ± 1.29
Wheat bran	8.15 ± 0.16	17.5 ± 0.35	0.95 ± 0.14	10.8 ± 0.41	3.28 ± 0.07	15.1 ± 0.33
Light buckwheat flour	10.1 ± 0.06	8.34 ± 0.31	0.98 ± 0.10	0.40 ± 0.11	1.95 ± 0.01	68.2 ± 0.36
Wholegrain buckwheat flour	9.76 ± 0.04	13.4 ± 0.24	1.97 ± 0.02	2.73 ± 0.14	3.08 ± 0.02	67.4 ± 0.23

[a] – Nitrogen-to-protein conversion factors are: 5.7 for wheat flour, and 6.25 for wheat bran and buckwheat flours.
n.d. – not detected;
Values are means of three determinations ± standard deviation.

Total phenolic content

Phenolic content of crude extracts obtained using 96% ethanol are presented in Table 2.

Table 2. Yield of the extract (% dry basis) and total phenolic content of wheat and buckwheat milling fractions

Extracts	Total phenolic content (μg GAE/g product)
Wheat flour type 400	19.4 ± 0.35^{a}
Wheat flour type 500	37.1 ± 0.15^{b}
Wheat flour type 850	50.8 ± 0.48^{c}
Wholegrain wheat flour	137 ± 1.69^{d}
Wheat bran	411 ± 6.98^{e}
Light buckwheat flour	1052 ± 13.1^{f}
Wholegrain buckwheat flour	1913 ± 9.76^{g}

Values are means of three determinations ± standard deviation. Values of the same column with the same superscript are not statistically different ($P < 0.05$).

The total phenolic content of wheat and buckwheat extracts differed greatly ranging from 19.4 ± 0.35 μg GAE/g in wheat flour type 400 to 1913 ± 9.76 μg GAE/g in wholegrain buckwheat flour (Table 2). The total phenolic content of buckwheat flours was significantly higher ($P < 0.05$) than for wheat milling fractions.

Amongwheat milling fractions, wholegrain wheat flour and wheat bran significantly differed in total phenolic content in comparison to wheat flours (Table 2). Their phenolic contents were significantly higher ($P < 0.05$) than those of wheat flours due to the presence of antioxidant compounds in the pericarp and aleurone layers (*Esposito et al., 2005*). During milling the bran and germ layers are removed and the remaining endosperm, which contains few antioxidant compounds, is ground into flour for making bread and other bakery products (*Decker et al., 2002*). Therefore, wholegrain wheat flour, retains the bran and germ, provides antioxidants, especially phenolic acids and phytic acid which may act synergistically to reduce the risk of a variety of diseases and to protect the final product from undesirable changes caused by lipid peroxidation (*Zhou and Yu, 2004*). Moreover, wheat bran as the separate product possesses the highest concentration of antioxidative efficient phenolic acids present in wheat grain (*Baublis et al., 2000; Yu et al., 2003*) as we also found (Table 2). The total phenolic content in buckwheat flours was found to be higher than that of all investigated wheat milling fractions (Table 2). This was in agreement with results of *Zielinski and Kozlowska* (2000) who examined total phenols of some grains and established next hierarchy order: buckwheat > barley > oat > wheat = rye. Furthermore, higher concentrations of phenolic compounds in buckwheat were found in outer layers of the grain (*Holasova et al., 2002*) and therefore the light buckwheat flour as the refined one contained less phenolic compounds in comparison to wholegrain buckwheat flour (Table 2).

Antioxidant properties

There are numerous studies showing the marked antioxidant activity of cereal products (*Adom and Liu, 2002; Miller et al., 2000*) or pseudocereals as buckwheat is (*Kreft et al., 2006*). This activity is mainly due to phenolic compounds that are presented in cereals (*Adom et al., 2003; Gallardo et al., 2006*) or in buckwheat (*Holasova et al., 2002*). Reported results of antioxidant capacity usually include the application of several tests. The most often used test for assessment of antioxidant activity is DPPH assay. The ability of wheat and buckwheat milling fractions' extracts to scavenge DPPH· was in the following order: wholegrain buckwheat flour > light buckwheat flour > wheat bran = wholegrain wheat flour = flour type 850 > flour type 500 > flour type 400 (Table 3). All extracts showed lower DPPH· scavenging activity in comparison with BHT, as indicated by their higher IC_{50} values. As DPPH assay is one of the most frequently used, the obtained results are compared with numerous published results. Regarding wheat milling fraction *Liyana-Pathirana and Shahidi* (2007) determined similar hierarchy for capabilities of similar samples to scavenge DPPH·. The results of *Hung et al.* (2009) pointed out the fact that the flours milled from the outer parts of wheat grain contained significantly higher amount of phenolics, and they exhibited significantly higher antioxidant capacity, i.e. DPPH· scavenging activity. The ability of the extracts obtained from winter wheat varieties to quench DPPH· was also confirmed by *Yu et al.* (2002a; 2002b).

The results presented in Table 3 indicate that the IC_{50} values on DPPH· for buckwheat flours are 15-fold lower than those for wheat milling fractions, probably due to the presence of much potent antioxidants in buckwheat flours in comparison to wheat. The strongest scavenging effects on DPPH· were also found for buckwheat extract compared with wheat, barley and rye extracts (*Đorđević et al., 2010*).

The DPPH· scavenging activity of buckwheat graded milling flours was investigated by *Hung and Morita* (2008). Their results indicated that the outer layer of buckwheat grains containing higher total phenolic and flavonoid contents possessed significantly higher antioxidant capacities than the inner fractions when the DPPH assay was used. This finding was in agreement with our IC_{50} values on DPPH· for buckwheat flours because the light buckwheat flour was characterized with higher IC_{50} value in comparison to wholegrain buckwheat flour, which exhibited higher antioxidant ability to scavenge DPPH· (Table 3). Strong DPPH· scavenging activity of buckwheat flour extracts was partly due to the presence of rutin which possess strong ability to scavenge DPPH· (*Hsu et al., 2008*).

Table 3. Scavenging activity on DPPH·, AOA, reducing activity and chelating activity on Fe^{2+} of wheat and buckwheat milling fractions

Extracts	DPPH· scavenging activity, IC_{50} (mg/mL)	AOA, IC_{50} (mg/mL)	Reducing activity, IC_{50} (mg/mL)	Chelating activity on Fe^{2+}, IC_{50} (mg/mL)
Wheat flour type 400	39.4 ± 0.31^{e}	16.7 ± 0.14^{e}	12.4 ± 0.48^{e}	0.06 ± 0.001^{a}
Wheat flour type 500	34.2 ± 0.26^{d}	15.9 ± 0.10^{d}	11.6 ± 0.33^{d}	0.06 ± 0.00^{a}
Wheat flour type 850	31.6 ± 0.20^{c}	15.6 ± 0.12^{cd}	10.4 ± 0.40^{c}	0.06 ± 0.00^{a}
Wholegrain wheat flour	31.3 ± 0.06^{c}	15.6 ± 0.19^{cd}	10.1 ± 0.56^{c}	0.06 ± 0.00^{a}
Wheat bran	31.6 ± 0.33^{c}	15.2 ± 0.11^{c}	8.54 ± 0.13^{b}	0.06 ± 0.00^{a}
Light buckwheat flour	1.87 ± 0.02^{b}	7.56 ± 0.06^{b}	2.58 ± 0.01^{a}	1.28 ± 0.01^{c}
Wholegrain buckwheat flour	1.49 ± 0.02^{a}	6.42 ± 0.93^{a}	2.57 ± 0.01^{a}	1.13 ± 0.02^{b}
BHT	0.57 ± 0.00	1.78 ± 0.06	0.36 ± 0.01	-
EDTA		-	-	0.05 ± 0.00

Values are means of three determinations ± standard deviation.
Values of the same column with the same superscript are not statistically different ($P < 0.05$).

Phenolic compounds, which are known as powerful chain-breaking antioxidants (Shahidi and Wanasundara, 1992), inhibit lipid peroxidation by decreasing concentration of peroxyl radicals via hydrogen atom transfer from the antioxidant to the lipid peroxyl radical, deactivating lipid peroxyl radicals by single electron transfer and chelating transition metals to suppress the initiation of radical formation during metal-catalyzed lipid peroxidation. Therefore, AOA, reducing activity and chelating activity on Fe^{2+} of the investigated extracts, expressed as IC_{50} values, were also determined (Table 3).

The AOA of wheat and buckwheat milling fractions' extracts (which only indicates their total antioxidant capacity) ranged from 6.42 ± 0.93 mg/mL for wholegrain buckwheat flour to 16.7 ± 0.14 mg/mL for wheat flour type 400, and were significantly different ($P < 0.05$) in comparison to wheat and buckwheat extracts (Table 3). IC_{50} values for AOA differed among wheat samples, but indicated that the compounds responsible for the inhibition of lipid peroxidation from different grain parts were of the similar structure. All examined extracts showed lower AOA than BHT (Table 3). This fact is in agreement with the finding obtained by *Sun and Ho* (2005) who noted that BHT exhibited better AOA than the series of investigated buckwheat extracts (acetone, butanol, ethanol, ethyl acetate and methanol). The AOA demonstrated a positive correlation with the total phenolic

content (R^2 = 0.8981) that suggested that phenolic compounds presented in both investigated series of the extracts mainly contributed to their overall antioxidant properties. Phenolic acids, including ferulic, vanillic, *p*-coumaric, caffeic and chlorogenic acid attribute to AOA of wheat milling fraction extracts (*Adom and Liu, 2002; Yu et al., 2005; Zhou et al., 2005*), while rutin, quercetin and other flavonoids which are characteristic for buckwheat grain are responsible for AOA of buckwheat flour extracts (*Dietrych-Szostak and Oleszek, 1999; Hsu et al., 2008*).

Reducing activity as an indicator of electron-donating capacity of the examined extracts showed significant differences ($P < 0.05$) between wheat and buckwheat milling fractions, while BHT exhibited the highest reduction activity compared to the extracts (Table 3). Buckwheat extract was previously noted as much more potent source of reducing agents than wheat extract by *Alvarez-Jubete et al.* (2010). The wheat bran extract had significantly stronger ($P < 0.05$) reducing activity than the extracts of wheat flours and this finding is in accordance with the result of *Liyana-Pathirana and Shahidi* (2007) who identified that the reducing power of the bran layers was better than of the endosperm.

Significant correlation was found between antioxidant activity measured using DPPH and reducing activity assays (R^2 = 0.9665). This finding can be explained by the same principles of these methods. *Alvarez-Jubete et al.* (2010) also found that there were good correlations between TPC and antioxidant activity measured by DPPH and reducing activity assays.

It is known that metal-catalyzed lipid peroxidation could be inhibited by chelating of transition metals (*Kehrer, 2000*). When there is no chelating agents, Fe^{2+} may take part in the Fenton reaction which results in forming OH· which could cause oxidative damage (*Halliwell and Chirico, 1993*). The chemicals with chelating capacity may significantly inhibit the lipid peroxidation in biological and food systems (*Yu and Zhou, 2004*). Therefore, one of the important mechanisms to protect against oxidative damage and lipid peroxidation is chelating metal ions. Significant differences ($P < 0.05$) in chelating activity were observed between wheat and buckwheat extracts (Table 3). All examined wheat extracts exhibited more potent chelating activity on Fe^{2+} than the buckwheat extracts. The potential of wheat extracts to chelate Fe^{2+} was on the same level as EDTA (Table 3). It needs to be pointed out that this observation is not in accordance with other determined parameters. All other results of antioxidant activity favoured buckwheat as much potent source of antioxidants than wheat. It could be that the extracts of wheat milling fractions contained some other potent compound(s) that were able to chelate Fe^{2+}. This assumption is in accordance with the fact that there is no correlation between chelating activity and any other tested antioxidant properties, neither with total phenolic content. The chelating properties of wheat bran extracts were also detected by *Iqbal et al.* (2007), *Zhou et al.* (2005) and *Liyana-Pathirana and Shahidi* (2007), whose results indicated the presence of Fe^{2+} chelating agents in association with bran layers.

Phenolic compounds

Phenolic acids and flavonoids are the main group of natural products commonly found in cereal and pseudocereal grains. *Zhou et al.* (2004) quantified five phenolic acids, i.e. ferulic, syringic, *p*-hydroxibenzoic, vanillic and coumaric acid. They emphasized that ferulic acid was the predominant acid in wheat and accounted for 57-78% of total identified phenolic acids. In our work ferulic acid was determined as the principal acid for wheat milling fractions. Using HPLC-DAD, ferulic acid was quantified in crude methanolic extracts of bran and wholegrain wheat flour at levels of 26.1 ± 0.80 μg/g and 17.6 ± 0.39 μg/g of product, respectively. Our results are in accordance with published data, since ferulic acid is abundant in the aleurone, pericarp, and embryo cell walls of various grains, but occurs only in trace amounts in the starchy endosperm (*Smith and Hartley, 1983*).

Phenolic compounds identified from buckwheat are flavonoids: rutin, orientin, vitexin, quercetin, isovitexin, isoorientin, kaempferol-3-rutinoside, and catechins (*Dietrych-Szostak and Oleszek, 1999*). Rutin was the major identified compound in wholegrain and light buckwheat flour presented in amount of 188 ± 0.43 μg/g and 77.3 ± 1.08 μg/g, respectively. Also, quercetin content was higher in wholegrain than in light buckwheat flour, 51.1 ± 0.66 μg/g and 48.0 ± 0.49 μg/g, respectively. Our results are in the same order of magnitude with the findings of *Quettier-Deleu et al.* (2000), although different extraction procedure was applied.

CONCLUSIONS

Buckwheat flours exhibited significantly higher ($P < 0.05$) antiradical activity on DPPH·, AOA and reducing power than wheat milling fractions when their IC_{50} values for the same assays were compared. Chelating activity was the only assay in which wheat milling fractions demonstrated better antioxidant potential than buckwheat flours. Better antioxidant activity of buckwheat flours might be due to higher content of phenolic compounds in buckwheat (rutin, quercetin) in comparison to phenolic acids in wheat milling fractions (ferulic acid). These results indicate the benefit of using buckwheat flours in wheat-based food products, i.e. their contribution in functional and tailor-made-food production.

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