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Ivana J. Sedej, Marijana B. Sakač, Aleksandra Č. Mišan, Anamarija I. Mandić

Institute for Food Technology, University of Novi Sad,
Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

ANTIOXIDANT ACTIVITY OF WHEAT AND BUCKWHEAT FLOURS

ABSTRACT: Antioxidative activities of wheat flours (type 500 and wholegrain) and buckwheat flours (light and wholegrain) were tested using 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$)-scavenging activity, reducing power and chelating activity on Fe^{2+}. Also, the content of the total phenolics of ethanolic extracts was estimated.

Polyphenolics content (expressed as gallic acid equivalent, GAE) in wheat flours varied between 37.1 and 137.2 μg GAE/g extract, while its content in buckwheat flour were at least four time higher and ranged between 476.3 and 618.9 μg GAE/g extract.

Ethanolic extracts of buckwheat flours exhibited higher antioxidant activities in all the assays, except for chelating activity.

Regarding all the obtained results, it can be concluded that bakery products produced with buckwheat flour could be regarded as potential functional foods.

KEY WORDS: antioxidant activity, polyphenolics, wheat and buckwheat flours

INTRODUCTION

Buckwheat, unlike most cereals, is an alternative crop belonging to the Polygonaceae family. The increasing attention for buckwheat cultivation and utilisation of buckwheat products is due to rising number of data focused on its functional characteristics, which can provide many health benefits based on buckwheat products consummation, first of all during prevention and healing chronic diseases (Li and Zhang, 2001).

Functional properties of buckwheat based foods are due to proteins and many rare components with healing effects. Among them, the most attractive ones are flavones, flavonoids, phytosterols, fagophyrins, and thiamin-binding proteins found in buckwheat seed.

Cody and co-workers (1986) reviewed the biological and pharmaceutical effects of plant flavonoids, including buckwheat flavonoids, on human beings and test animals. Many of the biological functions, such as antimutacigenity, anticarcinogenicity, and antiaging, originate from antioxidant activity

of antioxidative enzymes and nonenzymic antioxidants (Cook and Samman, 1996). Cody and co-workers (1986) cited the medical effects of plant flavonoids; they are known for their effectiveness in reducing cholesterol levels in the blood, keeping capillaries and arteries strong and flexible, and assisting as a preventative measure against blood pressure, as well as many other cardiovascular diseases.

Oomah and Mazza (1996) reported that flavonoids in buckwheat can perform high antioxidative activity that may have the potential to show pharmaceutical effects from this characteristic.

Six flavonoids (rutin, orientin, vitexin, quercetin, isovitexin and isoorientin) have been isolated and identified in buckwheat, but in buckwheat seed only rutin and isovitexin were found, and rutin attributed most of the flavonoid content in buckwheat seed (Dietrych-Szostak and Oleszek, 1999). Rutin and its hemisynthetic derivatives exert different medical effects like normalisation of increased vascular permeability and fragility, oedema protection (Ihme et al., 1996), antioxidant (Wojcicki et al., 1995), hypotensive (Evans, 1996), and antiinflammatory effects.

Flavonoids isolated from buckwheat hulls showed radical scavenging activity (Watanabe et al., 1997; Watanabe, 1998), which is important in suppressing radical damage in lipid peroxidation processes involved in food deterioration or some diseases. Holasova and co-workers (2002) also reported that phenolic compounds in buckwheat, namely 3-flavanols, rutin, phenolic acids and their derivatives, possessed antioxidative activity stronger than antioxidative components of oats and barley.

Based on the fact that antioxidative components from buckwheat flour significantly contribute to its functionality, the aim of this work was to investigate antioxidative properties of the commercially accessible buckwheat flours in comparison to the wheat flour type 500 and wholegrain wheat flour, the most frequently used wheat products for bakery industry, by measuring DPPH radical scavenging activity, reducing power, chelating effect on Fe^{2+} and total phenolics content.

MATERIALS AND METHODS

Materials

Buckwheat flours (light and wholegrain) and wheat flours (type 500 and wholegrain) were provided by local market.

Extraction

Buckwheat or wheat flour (10 g) was mixed with 100 mL of 96% ethanol. Extraction was carried out with shaking at room temperature during 1 h. Extract was separated by filtering through the filter paper (Whatman, Grade 4 Chr, UK), and procedure was repeated with 100 mL of solvent two times. The

extraction solutions (3 x 100 mL) were combined and dried by vacuum-evaporator. The dried extract was weight and the yield was calculated based on the wet weight of the sample. The dried extract was resolved in 96% ethanol to obtained 10 mL volume. The extract obtained by this procedure was used for further investigations of antioxidant activity.

Determination of total phenolics content

The total phenolics content in investigated extracts of buckwheat and wheat flours, measured as gallic acid equivalents, were determined spectrophotometrically using Folin-Ciocalteau's reagent (Singleton et al., 1999). The extract (0.1 mL) of buckwheat or wheat flours was diluted with distilled water (7.9 mL). Folin-Ciocalteau'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. The absorbance was determined in a spectrophotometer Jenway (6405 UV/Vis) at 750 nm.

DPPH free radical-scavenging activity test

The effect of the examined extracts on the content of 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$) radicals was estimated according to the modified method of Hatano and co-workers (1988). The concentration of the $DPPH^{\cdot}$ solution which was used in the assay was 90 μM (22.5 mL 0.4 mM $DPPH^{\cdot}$ solution (0.01577 g $DPPH^{\cdot}$ in 100 mL methanol) was diluted with 95% methanol to 100 mL). An aliquot (1.0 mL) of the $DPPH^{\cdot}$ solution (90 μM) was diluted in 2.9 mL methanol, and 0.1 mL of the examined extracts at various concentrations (0.1, 0.5, 1.0 and 2.0 mg/mL for buckwheat flour extracts and 10, 20, 30 and 40 mg/mL for wheat flour 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 against the blank (without extract) in a Jenway (6405 UV/Vis) spectrophotometer.

IC_{50} (mg/mL) was defined as the concentration of an antioxidant extract which was required to quench 50% of the initial $DPPH^{\cdot}$ under the experimental conditions given. It was obtained by interpolation from linear regression analysis.

BHT and α-tocopherol were used as controls.

Reducing power

The reducing power of the ethanolic extracts was measured according to the method of Oyaizu (1986). Various concentrations (0.1, 0.5, 1.0 and 2.0 mg/mL for buckwheat flour extracts and 10, 20, 30 and 40 mg/mL for wheat flour extracts) of the ethanolic extracts (0.5 mL) were mixed with 2.5 mL of

phosphate buffer (0.2 M, 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 distilled water and 0.5 mL of ferric chloride and the absorbance was measured at 700 nm in a Jenwey (6405 UV/Vis) spectrophotometer. Higher absorbance of the reaction mixture indicates greater reducing power.

IC_{50} value (mg/mL) is the effective concentration at which the absorbance was 0.5 for reducing power and was obtained by interpolation from linear regression analysis.

BHT was used as control.

Chelating activity on Fe^{2+}

The 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 buckwheat and wheat flours (0.1, 0.5, 1.0 and 2.0 mg/mL and 0.01, 0.05, 0.1 and 0.5 mg/mL, respectively) were mixed with 3.7 mL deionized water. The mixture was left for reaction with $FeSO_4$ (2 mM, 0.1 mL) and ferrozine (5 mM, 0.2 mL) for 10 min at room temperature, and then the absorbance was measured at 562 nm in a Jenway (6405 UV/Vis) spectrophotometer. A lower absorbance indicates a higher chelating power.

The chelating activity on Fe^{2+} of the ethanolic extracts was compared with that of EDTA at a level of 0.036 mM.

Statistical analysis

Experimental results were given as mean ± SD of three parallel trials and measurements. P values < 0.05 were regarded as significant.

RESULTS AND DISCUSSION

The total phenolic content of each flour extract was estimated, since phenolics may significantly contribute to its overall antioxidant activity. The amount of total phenolics in wheat and buckwheat flours expressed as μg of gallic acid equivalents (GAE) per 1 g of extract was presented in Figure 1. Phenolics content in wheat flours varied between 37.1 and 137.2 μg GAE/g extract, while its content in buckwheat flours was at least forty-five time higher and ranged between 476.3 and 618.9 μg GAE/g extract.

Comparing the results among flour types, higher content of phenolics was found in wholegrain flour in both, wheat and buckwheat. Results with similar trend of increased content of phenolics in the flours containing more outer

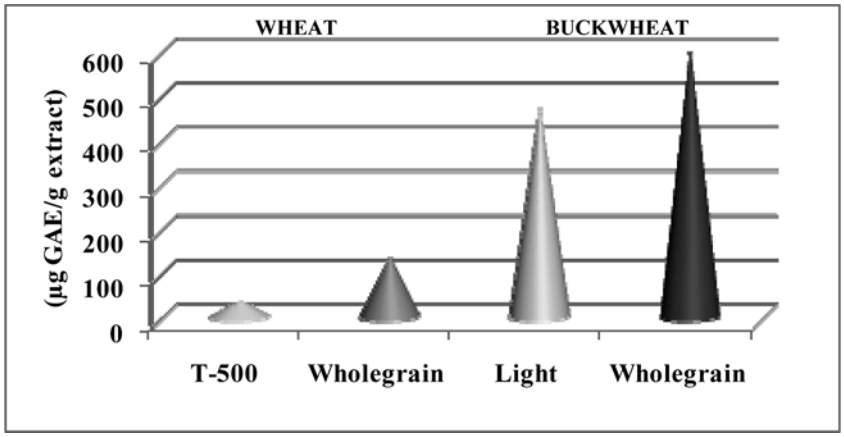

Fig. 1 — Total phenolic content of flours

layers of grain and bran were obtained in the study of Hung and Morita (2008).

The investigated extracts differed significantly ($P < 0.05$) in their total phenolics content that is contributed to the different abilities to inhibit lipid peroxidation (Fig. 2—4), i.e. to exhibited differences in antioxidative activities (AOA). Zielinski and Kozlowska (2000) have the statistically significant correlation between antioxidative activities and total phenolics of cereals and their fractions. A correlation between antioxidative activity and rutin content or total flavonoids content in buckwheat cultivars has been shown (Jiang et al., 2006).

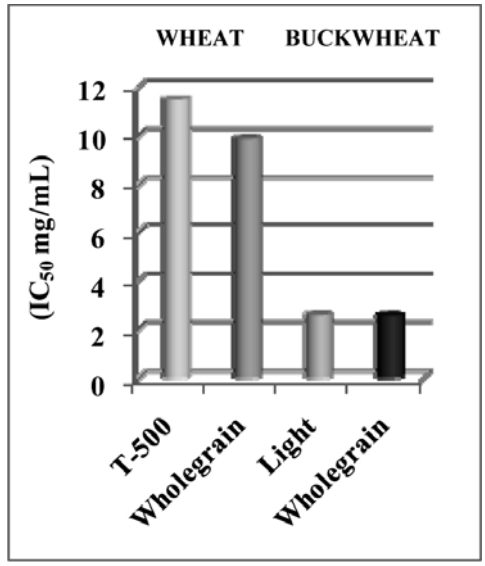

Fig. 2 — Reducing power

High differences in AOA comparing wheat and buckwheat flour extracts were showed in reducing power assay (Figure 2). Better antioxidant activity was found in buckwheat than in wheat flours indicated with lower IC_{50} values.

Strong antioxidative activity of buckwheat flour extracts might be attributed to the presence of polyphenols, especially rutin, as the main antioxidative component in buckwheat (Dietrych-Szostak and Oleszek, 1999). Rutin possesses all structural features which have been demonstrated to increase antioxidative activity of flavonoids and their *O*-glycosides (Afanas'ev et al., 1989).

Wheat, as other cereals, has been known to contain hydroxycinnamic acid derivatives, which demonstrated antioxidative activities (Andreasen et al., 2001). Ferulic acid was reported to be the predominant phenolics acid accounting for approximately 57—77% of total phenolic acids in wheat (Zhou at al., 2004). This acid possesses lower antioxidative capacity than rutin, according to the structural characteristics of these components (Cook and Samman, 1996). This fact could explain the higher AOA of ethanolic extract of buckwheat flours in comparison to the extracts of wheat flours. In addition, Liyana and Shahidi (2007) found that wheat flour possessed the lowest amount of ferulic acid among the different milling fractions of wheat, so this was reflected in its relatively low antioxidative activity.

Ethanolic extracts of buckwheat and wheat flours showed significant ($P < 0.05$) difference in their ability to reduce concentration of DPPH radicals by donating H-atoms from the OH-groups of polyphenols (Brand-Williams et al., 1995), which was confirmed by their IC_{50} values (Figure 3).

DPPH· activities were higher in buckwheat than in wheat flours, indicated by lower IC_{50} values, as the consequence of higher polyphenolics content in

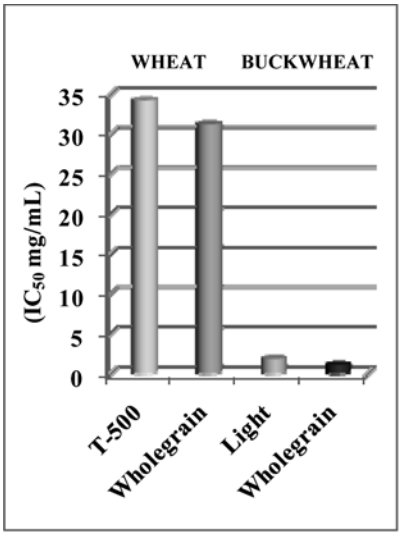

Fig. 3 — Scavenging activity on DPPH·

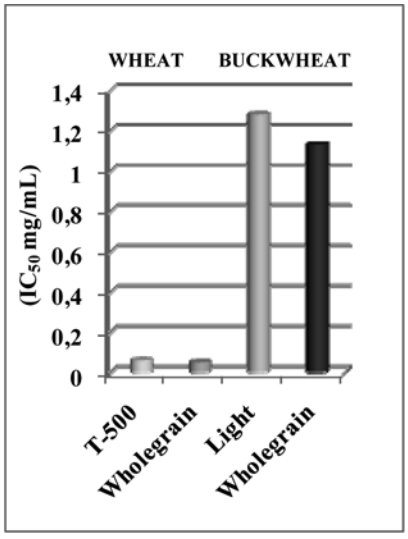

Fig. 4 — Chelating activity on Fe^{2+}

buckwheat flours. Those values were 34.24 and 31.26 mg/mL, and 1.87 and 1.49 mg/mL for wheat and buckwheat flours, respectively.

Reduction of DPPH radicals reveals that examined extracts possess radical inhibitors or scavengers with possibility to act as primary antioxidants. They might react with free radicals, particularly with the peroxy radicals, which are the major propagators of the auto-oxidation chain of fat, thereby terminating the chain reaction (Shahidi and Wanasundara, 1992). Based on the results obtained, the antioxidative activity of investigated extracts, particularly buckwheat extracts, could in part be markedly caused by their radical scavenging properties. Watanabe (1997) and Watanabe and co-workers (1998) reported that flavonoids, first of all rutin, isolated from buckwheat hulls showed radical scavenging activity. Also, Sun and Ho (2005) reported powerful antiradical activity on DPPH radicals of ethanolic extract of whole buckwheat grain, but our results are not completely comparable because of differences in applied methods.

The chelating activity on Fe^{2+} were significantly ($P < 0.05$) higher for wheat flours extracts than for buckwheat flours extracts (Figure 4).

Since ferrous and cupric ions are the most effective pro-oxidant in food systems (Yamaguchi et al., 1988), and ferrous ions are commonly found in food systems, high chelating activity of investigated extracts would be beneficial in retarding metal-catalyzed oxidation (Kehrer, 2000). The possibility of complexing metal ions, as the antioxidant properties themselves, depends on structure of compound, that is in the case of polyphenols (predominant flavonoids from buckwheat and phenolic acids from wheat) implies the number and position of OH- and CH_3O-groups.

Although rutin possesses more structural features than ferulic acid for complexing metal ions, the ethanolic extracts of wheat flours exhibited more potent chelating activity on Fe^{2+} than the buckwheat extracts. Lower antioxidative capacity of rutin as metal chelator in comparison to ferulic acid (and other hydroxycinnamic acids from wheat flour) might be due to steric hindrance of the sugar moiety of rutin. The presence of some more potent chelating component(s) in wheat flour extract might be responsible for its higher chelating capacity. Significant Fe^{2+} chelating activities and inhibitory effects were detected in wheat grain extracts (Yu et al., 2002) which is in correlation with our results presented in the Figure 4. Furthermore, in the Fe^{2+} chelating assay, wheat bran demonstrated superior chelating properties over the other milling fractions, while wheat flour had the lowest chelating capacity (Liyana and Shahidi, 2007).

CONCLUSION

The obtained results clearly indicated that ethanolic extracts of buckwheat flours (light and wholegrain) possessed good antioxidant properties, including reducing power, scavenging abilities on DPPH radicals and chelating abilities on Fe^{2+} ions. Those extracts showed better antioxidative properties than the ethanolic extracts of wheat flours (type 500 and wholegrain) except when the

chelating activity on Fe^{2+} ions was taken into consideration. Also, wholegrain flours, both wheat and buckwheat, exhibited better antioxidant properties in comparison with light ones.

Concerning all the results, and previously emphasized, buckwheat flours with their antioxidant characteristic as quality criteria, could be used in wheat--based food products and would contribute to their added-value for functional and tailor-made-food production. Besides, buckwheat extract could be used in food as an additive, i.e as a source of natural antioxidants in order to replace the synthetic ones.

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АНТИОКСИДАТИВНА АКТИВНОСТ БРАШНА ОД ПШЕНИЦЕ И ХЕЉДЕ

Ивана Ј. Седеј, Маријана Б. Сакач, Александра Ч. Мишан, Анамарија И. Мандић

Институт за прехрамбене технологије Нови Сад, Универзитет у Новом Саду, Булевар цара Лазара, 1, 21000 Нови Сад, Србија

Резиме

Антиоксидативна активност пшеничног брашна (тип 500 и интегрално) и хељдиног брашна (бело и интегрално) одређена је применом 1,1-дифенил-2-пикрилхидразил (DPPH·) теста, на основу мерења редукционе активности и хелатационе активности на Fe^{2+}. Такође, одређен је и садржај укупних фенола у испитиваним етанолним екстрактима.

Садржај полифенола (изражен као еквивалент галне киселине, GAE) у пшеничним брашнима је износио 37,1 и 137,2 μg GAE/g екстракта, док је садржај у брашнима од хељде био најмање четири пута виши и варирао од 476,3 и 618.9 μg GAE/g екстракта. Етанолни екстракти брашна од хељде су показали вишу антиоксидативну активност у свим тестовима, осим за хелатациону активност.

На основу свих добијених резултата може се закључити да обогаћење пекарских производа хељдиним брашном може допринети функционалности производа.