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Distribution of phenolic compounds in the graded flours milled from whole buckwheat grains and their antioxidant capacities

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Abstract

Whole buckwheat grains were milled into 16 flour fractions using the gradual milling system and the phenolic compounds and the antioxidant capacity of each flour fraction were investigated. The phenolic and flavonoid contents of both free and bound phenolic extracts of buckwheat flour fractions significantly increased in the order from the fraction number 1 (phenolics less rich fraction) to the fraction number 16 (phenolics rich fraction). The phenolic compounds in buckwheat existed primarily in free form, whereas the flavonoids existed in grain in insoluble bound forms, bound to cell wall materials. The amounts of ferulic acid and rutin increased from 2.5 and 2.5 μg/g flour of the phenolics less rich fraction to 609.5 and 389.9 μg/g flour of the phenolics rich fraction of grain, respectively. The higher phenolic contents in the phenolics rich fractions exhibited the stronger antioxidant capacity than the phenolics less rich fractions. As a result, the flour milled from the outer layers of buckwheat grains with large amount of phenolic compounds and antioxidant capacity are considered to have significant health benefits.

Keywords: Buckwheat; Phenolics; Flavonoids; Gradual milling; Antioxidant; Rutin

1. Introduction

Buckwheat (*Fagopyrum esculentum* Moench) is an alternative crop belonging to the Polygonaceae family and is usually grouped with cereals because of similarity in cultivation and utilization though it is not cereal grain. Buckwheat grains contain large amounts of protein, starch and vitamins. The protein of buckwheat consists of well-balanced amino acids with a high biological value (Pomeranz, 1983) and is excellent supplement for cereal grains (Maeda, Miyake, Tahara, & Morita, 2004). In addition, buckwheat grains have been well known as a plant source of rutin, quercetin, kaempferol-3-rutinoside, and a trace quantity of a flavonol triglycoside (Holasova et al., 2002). Buckwheat contains more rutin than most of the other plants, which exhibits antioxidative, antihemorrhagic and blood vessel protecting properties (Baumgertel, Grimm, Eisenbeib, & Kreis, 2003). Dietrych-Szostak and Oleszek (1999) reported that the total flavonoid concentrations of buckwheat seed and hull are 18.8 and 74 mg/100 g flour, respectively. The flavonoids in buckwheat seed are only rutin and isovitexin, whereas buckwheat hull contains rutin, orientin, vitexin, quercetin, isovitexin and isoorientin. Phenolic compounds in buckwheat have been reported to possess antioxidant activity (Holasova et al., 2002) and higher concentrations of these compounds are found in the outer layers of the grain containing bran. Therefore, consumption of large amounts of buckwheat bran is considered to have significant nutritional or medicinal benefits. However, refined buckwheat flour is milled by removing

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most portions of fiber, mineral and phenolic compounds in bran and germ. Therefore, a gradual reduction milling system has been developed to improve the nutritional constitution in flour. This method separates whole wheat grains into various fractions by weight from outer to inner parts (Maeda, Ohkura, & Morita, 1999). For the gradual milling of buckwheat, the grains were first broken and the husks were removed by a dehulling apparatus with disks. Then the groats were cut into 7–8 pieces and the cut germ is removed. The remaining groats with endosperm and bran were milled to various flour fractions by their weight. The quality of these flour fractions improved by the increase of nutritive components from bran and germ which are rich in dietary fiber, resistant starch, vitamins, minerals and microconstituents. The previous studies reported that the outer layers are rich in protein, lipid, dietary fiber and ash contents, whereas the inner layers exhibit lower allergenic activity (Morita et al., 2006; Skrabanja et al., 2004). In this study, phenolic compounds in the graded buckwheat flour fractions were extracted and evaluated for their antioxidant capacity.

2. Materials and methods

2.1. Materials

Mankan buckwheat grains imported from China were used to mill into 16 fractions using the gradual milling system as previously reported by Morita et al. (2006). Fractions were named by FS, F1, F2 and F3 corresponding to the fractions graded from the innermost layers to the outermost layers of whole buckwheat grain, respectively. This means FS was the central part of grain and the other fractions were the parts approaching to the outer layer of the bran, respectively. Furthermore, FS, F1, F2 and F3 fractions were separated into various smaller fractions. Total fractions were 16 including FS-1, -2, -3, -4, -5, F1-1, -2, F2-1, -2, -3, -4, -5 and F3-1, -2, -3, -4 and abbreviated by fraction 1 to fraction 16 in the order from the innermost part to the outermost part of grain as shown in Table 1. The milling procedure was carried out at Miyake Flour Milling Co., Ltd. (Osaka, Japan).

The compounds 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS), 2,2′-azobis-(2-amidinopropane)dihydrochloride (ABAP), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin–Ciocalteu phenol reagent, sodium carbonate, were purchased from Wako Chemical Co. (Osaka, Japan).

2.2. Extraction of free and bound phenolic compounds

Phenolic compounds of buckwheat fractions were extracted into free and bound phenolics according to the methods of Adom and Liu (2002) and Sosulski, Krygier, and Hogge (1982), respectively, with a slight modification. Free phenolic compounds of flours (1 g) were extracted with 10 ml of 80% chilled ethanol for 20 min with continuous shaking. After centrifugation at 2500*g* for 10 min, the supernatant was collected. The residue was re-extracted twice with 10 ml of 80% chilled ethanol under the same conditions. All supernatants were combined and evaporated to dryness under reduced pressure. Then the concentrated slurry was dissolved with methanol to a final volume of 10 ml. The free phenolic compounds were then stored at −40 °C until use.The residue from the extraction of free phenolic compound was hydrolyzed directly with 20 ml of 2 N NaOH for 90 min with continuous shaking at 60 °C (Yeh, Hoseney, & Lineback, 1980). The hydrolysate was acidified to pH 2 (6 N HCl) and centrifuged to separate cloudy precipitate. The clear supernatant was extracted five times with hexane at a hexane to water phase ratio of 1:1 to

Table 1
Ferulic and rutin contents of buckwheat graded milling flours[a]

Fraction number	Fraction name	Ferulic acid contents (μg/g flour)			Rutin contents (μg/g flour)		
		Free	Bound	Total	Free	Bound	Total
1	FS-1	1.7 ± 0.3	0.8 ± 0.2	2.5	2.5 ± 0.1	nd	2.5
2	FS-2	1.8 ± 0.2	nd	1.8	3.0 ± 0.4	nd	3.0
3	FS-3	3.2 ± 0.5	2.0 ± 0.3	5.2	3.0 ± 0.5	nd	3.0
4	FS-4	3.2 ± 0.4	2.4 ± 0.2	5.6	5.8 ± 0.0	nd	5.8
5	F1-1	5.8 ± 0.6	2.5 ± 0.4	8.3	5.3 ± 0.3	nd	5.3
6	F1-2	12.1 ± 0.8	2.8 ± 0.2	14.9	9.5 ± 1.0	1.0 ± 0.3	10.5
7	FS-5	12.7 ± 0.8	4.0 ± 0.5	16.7	23.1 ± 0.3	1.0 ± 0.4	24.1
8	F2-1	42.2 ± 1.3	8.9 ± 0.4	51.1	50.4 ± 0.6	1.9 ± 0.2	52.2
9	F2-2	73.3 ± 1.5	19.7 ± 0.5	93.0	79.3 ± 6.1	4.4 ± 0.4	83.7
10	F2-3	95.2 ± 1.8	56.5 ± 0.8	151.7	96.5 ± 9.1	8.7 ± 0.5	100.2
11	F2-4	102.5 ± 2.0	77.0 ± 1.5	179.5	158.2 ± 6.3	14.8 ± 0.5	173.0
12	F2-5	207.2 ± 4.3	76.8 ± 2.3	284.0	219.8 ± 7.2	22.1 ± 0.2	241.9
13	F3-1	207.3 ± 5.0	105.3 ± 3.2	312.6	274.0 ± 7.8	34.9 ± 0.4	308.9
14	F3-2	345.5 ± 7.0	116.6 ± 3.5	462.1	324.4 ± 9.0	47.2 ± 0.8	371.6
15	F3-3	350.9 ± 8.5	179.5 ± 4.1	530.4	337.8 ± 5.3	65.7 ± 1.0	403.5
16	F3-4	429.0 ± 9.0	180.5 ± 5.2	609.5	330.6 ± 9.2	59.3 ± 1.2	389.9

[a] Values are means of duplicate measurements.

remove free fatty acids and other lipid contaminants. The liberated phenolic acids were then extracted six times with ethyl acetate at a solvent to water phase ratio of 1:1. The ethyl acetate extracts were evaporated to dryness and then bound phenolic compounds were dissolved and filled up to 10 ml of methanol and stored at −40 °C until use.

2.3. Determination of total phenolic content

The contents of free and bound phenolics in buckwheat fractions were determined according to the method of Liyana-Pathirana and Shahidi (2007) based on the procedure described by Singleton and Rossi (1965). The appropriate dilutions of free and bound phenolic extracts (0.5 ml) were oxidized with Folin–Ciocalteu's reagent (0.5 ml) in a centrifuge tube (50 ml). The reaction was neutralized with saturated sodium carbonate solution (1 ml), followed by adjusting the volume to 10 ml with distilled water. The contents in the tubes were thoroughly mixed and allowed to stand at ambient temperature for 45 min until the characteristic blue color developed. Then the tubes were centrifuged for 5 min at 4000*g*. Absorbance of the clear supernatants was measured at 725 nm using a spectrophotometer (UV-160A, Shimadzu, Kyoto, Japan). The content of total phenolics in each extract was calculated based on a standard curve prepared using ferulic acid and expressed as milligrams of ferulic acid equivalent (FAE) per gram of sample. Standard calibration was made from 0, 20, 40, 60, 80 and 100 μg/ml.

2.4. Determination of total flavonoid content

Flavonoid contents of free and bound phenolics in buckwheat fractions were determined using the aluminum chloride colorimetric method of Chang, Yang, Wen, and Chern (2002) based on the method of Woisky and Salatino (1998). The appropriate dilution of extracts (0.5 ml) were mixed with 1.5 ml of 95% ethanol, followed by 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with a Shimadzu UV-160A spectrophotometer (Kyoto, Japan). The flavonoid content was calculated using a standard calibration of rutin solution and expressed as micrograms of rutin equivalent (RE) per gram of sample.

2.5. HPLC analysis

Phenolic compounds in buckwheat extracts were determined using a high-performance liquid chromatography system (L-6200 intelligent pump, Hitachi, Japan) equipped with a L-4200 UV–Vis detector and an auto sampler.

For analysis of phenolics, the analytical column used was a Wakosil-II 5C18 HG (150 × 4.6 mm, 5 μm). The mobile phase and gradient program were used as previously described by Kim, Tsao, Yang, and Cui (2006). The mobile phase consisted of 2% acetic acid in water (v/v) (solvent A) and acetonitrile (solvent B). The gradient program was as follows: 100% A to 85% A in 30 min, 85% A to 50% A in 20 min, 50% A to 0% A in 5 min and 0% A to 100% A in 5 min. The flow rate was 1.0 ml/min and injection volume was 10 μl. The peaks of all components were detected at 280 nm.

For analysis of flavonoids, a Shiseido capcell pak C18 column (150 × 2.1 mm) was used. Rutin and quercetin in buckwheat extracts were detected at 350 nm with the mobile phase consisted of 2% acetic acid (solvent A) and of 0.5% acetic acid in water and acetonitrile (50:50, v/v; solvent B). The gradient program was as follows: 90% A to 45% A in 50 min, 45% A to 0% A in 10 min, 0% A to 90% A in 5 min. The flow rate was 0.5 ml/min and injection volume was 20 μl.

2.6. DPPH radical scavenging assay

DPPH radical scavenging capacities of buckwheat extracts were determined by the reduction of the reaction color between DPPH solution and sample extracts as previously described by Huang, Ou, and Prior (2005). A final concentration of DPPH solution used was 0.15 mM for buckwheat phenolic extracts instead of 0.075 mM for wheat extracts. DPPH solution (3.9 ml) was mixed with sample solution (0.1 ml). The mixture was kept in the dark at ambient temperature. The absorbance of the mixtures was recorded at 515 nm for exactly 30 min. Blank was made from 3.9 ml of DPPH and 0.1 ml methanol and measured absorbance at $t = 0$. The scavenging of DPPH was calculated according to the following equation (Liyana-Pathirana & Shahidi, 2007):

$$\%\ \text{DPPH scavenging} = \left\{\left(\text{Abs}_{(t=0)} - \text{Abs}_{(t=30)}\right)/\text{Abs}_{(t=0)}\right\} \times 100,$$

where $\text{Abs}_{(t=0)}$ = absorbance of DPPH radical + methanol at $t = 0$ min; $\text{Abs}_{(t=30)}$ = absorbance of DPPH radical + phenolic extracts at $t = 30$ min.

2.7. Measurement of total antioxidant capacity

Total antioxidant activities of the buckwheat extracts were determined according to the method described by Liyana-Pathirana and Shahidi (2007). ABAP (2.5 mM) and $ABTS^{2-}$ (2.0 mM) were prepared in a 0.1 M phosphate buffer (pH 7.4) containing 0.15 M sodium chloride (PBS) and a solution of ABTS radical anion ($ABTS^{\cdot-}$) was formed by mixing 2.5 mM ABAP with 2.0 mM $ABTS^{2-}$ at a 1:1 (v/v) ratio. The mixture was heated at 60 °C for 12 min and stored in dark at room temperature. A standard calibration was prepared using different concentrations of Trolox and the TEAC values of buckwheat extracts were expressed as μmol Trolox equivalents/g of flour. The procedure for determination of TEAC as follows: Trolox or sample extract (20 μl) was mixed with the

$ABTS^{\bullet-}$ solution (980 μl) over a period of 6 min and the reduction of absorbance was measured. A blank was used for each measurement that measured the reduction of absorbance of solvent without Trolox/sample addition.

TEAC values were calculated as follows:

$$\Delta Abs_{Trolox} = (Abs_{t=0\,Trolox} - Abs_{t=6\,Trolox}) - \Delta Abs_{solvent(0\text{–}6\ min)},$$

$$\Delta Abs_{Trolox} = m \times [Trolox],$$

$$\Delta Abs_{Extract} = (Abs_{t=0\,Extract} - Abs_{t=6\,Extract}) - \Delta Abs_{solvent(0\text{–}6\ min)},$$

$$TEAC_{Extract} = (\Delta Abs_{Extract}/m) \times \text{dilution factor},$$

where ΔAbs is the reduction of absorbance, $Abs_{t=0\ Trolox/extract}$ is the absorbance of $ABTS^{\bullet-}$ solution + Trolox or phelonic extract at $t = 0$ min, $Abs_{t=6\ Trolox/extract}$ is the absorbance of $ABTS^{\bullet-}$ solution + Trolox or phelonic extract at $t = 6$ min, [Trolox] is the concentration of Trolox.

3. Results and discussion

3.1. Total phenolic content (TPC)

The phenolic contents of free and bound phenolic extracts of buckwheat flour fractions are shown in Fig. 1. Phenolic content of the free phenolic extract of each flour fraction was significantly higher than that of the bound phenolic extract. In cereals, phenolic acids may exist in free, soluble conjugate or insoluble bound forms. The previous studies reported that the phenolic compounds existed primarily in bound forms associated with cell wall materials in wheat, rice, corn, rye, oat (Adom & Liu, 2002; Sosulski et al., 1982). Thus, the results of this study show that the phenolic compounds in buckwheat existed primarily in free form contrary to other cereals investigated. The phenolic contents of both free and bound phenolic extracts of buckwheat flour fractions significantly increased in the order from the fraction number 1 (phenolics less rich fraction) to the fraction number 16 (phenolics rich fraction). Total phenolic contents of the F3-3 and F3-4 fractions, which were the phenolics rich fraction, were 30-fold higher than those of the FS-1 and FS-2 fractions, which were the phenolics less rich fraction. The previous studies reported that the outer layers of buckwheat grain also contained higher amounts of protein, lipid, ash and dietary fiber than the inner fractions (Morita et al., 2006; Skrabanja et al., 2004). As a result, the outer layers of buckwheat grains with higher amount of phenolic compounds along with higher amounts of protein, lipid, ash and dietary fiber are considered to be good materials for cereal-based food processing with the significant health benefits.

3.2. Total flavonoid content (TFC)

The flavonoid contents of free and bound phenolic extracts of buckwheat flour fractions are shown in Fig. 2. Contrary to the phenolic contents, the flavonoid content of the bound phenolic extracts was higher than that of the free phenolic extracts. The low amount of free flavonoid compared to the bound flavonoid was also investigated in other grains such as rice, wheat, corn, and oat (Adom & Liu, 2002). Thus, the flavonoids existed in grain in insoluble bound forms, bound to cell wall materials, rather than in free forms. The total flavonoid concentrations in buckwheat seed and hull were 18.8 and 74 mg/100 g, respectively (Dietrych-Szostak & Oleszek, 1999). However, the quantity of flavonoid was varied depending on the cultivars and environment effects (Oomah & Mazza, 1996). In this study, the results also show that the flavonoid contents of both free and bound phenolic extracts significantly increased in the order from the fraction number 1 (phenolics less rich fraction) to the fraction number 16 (phenolics rich fraction). The total flavonoid content of the flour fraction gradually increased from FS-1 which was the phenolics less rich fraction (23.5 μg rutin equivalent/g of flour) to F3-4 which was the phenolics rich fraction (1354.1 μg rutin equivalent/g of flour). These results indicate that the remained flavonoid in the commercial buckwheat flour was low because most of the outer part

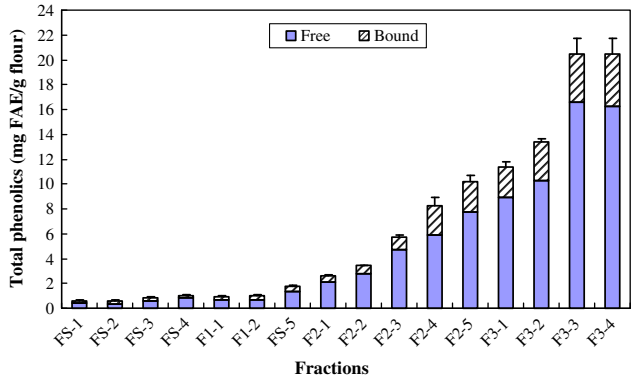

Fig. 1. Free and bound phenolic contents of buckwheat graded milling flours. Values are means of triplicate measurements.

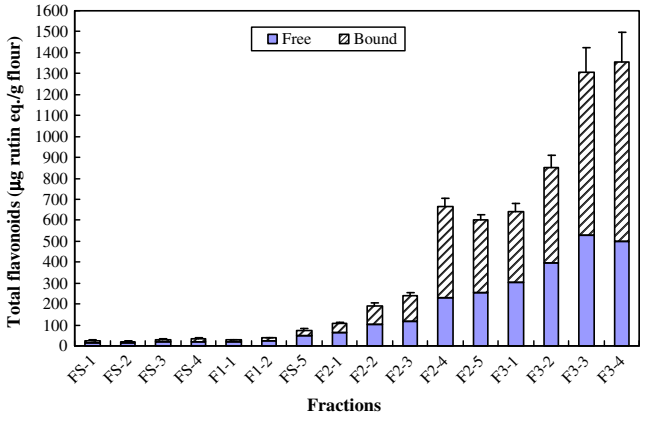

Fig. 2. Free and bound flavonoid contents of buckwheat graded milling flours. Values are means of triplicate measurements.

of grain is usually removed during milling. Therefore, it is necessary to consume all parts of grain for its healthy nutritional values.

3.3. Phenolic compound profiles

Phenolic acids are a group of natural products commonly found in many cereal grains. Ferulic, vanillic and syringic acids were found as the major individual phenolic acids in wheat (Kim et al., 2006). Buckwheat seed were found to contain rutin, tocopherols and phenolic acids, which possess antioxidant activity (Holasova et al., 2002; Oomah & Mazza, 1996; Watanabe, 1998; Watanabe, Ohshita, & Tsushida, 1997). In this study, the main phenolic acids such as gallic, *p*-hydroxybenzoic, *p*-coumaric and ferulic acids and the main flavonoids such as rutin and quercetin were also found in the free and bound phenolic extracts by using the above-mentioned HPLC system. Table 1 shows the concentrations of ferulic acid and rutin which were the highest concentration among the compounds detected in buckwheat flour fractions. The total concentrations of phenolic acids and flavonoids in the free phenolic extracts were significantly higher than that in the bound phenolic extracts in each flour fraction (data not shown). These results agreed with the results of total phenolic content determined by Folin–Ciocalteu method as above. However, the amounts of rutin in the free phenolic extracts were larger than that in the bound phenolic extracts though the total flavonoid content of the bound phenolic extracts were higher as described above. This result indicates that the rutin existed in buckwheat grain was mostly in free forms and easily extracted by using ethanol or methanol compared with other flavonoids which existed in bound forms associated with cell wall materials. The total amounts of ferulic acid and rutin gradually increased in the order from the fraction number 1 (phenolics less rich fraction) to the fraction number 16 (phenolics rich fraction). The phenolics less rich fraction (FS-1) contained only 2.5 and 2.5 μg/g flour of ferulic acid and rutin, respectively, whereas the amounts of these compounds in the phenolics rich fraction (F3-4) were 609.5 and 389.9 μg/g flour, respectively. As a result, the outer layers of buckwheat grains are considered to have high nutritional values because they are rich in the phenolic acids and rutin, which exhibited the antioxidative, anti-inflammatory and anticarcinogenic effects.

3.4. DPPH radical scavenging of free and bound phelonic compounds

DPPH is one of a few stable and commercially available organic nitrogen radicals and has a maximum UV–Vis absorbance at 515 nm. Scavenging of DPPH radical is based on the measurement of reducing ability of antioxidants toward DPPH· (Huang et al., 2005; Prior, Wu, & Schaich, 2005). The scavenging of the stable DPPH radical was widely used to evaluate antioxidant activity of phenolic compounds extracted from fruit and vegetable, cereal grain, wine, etc. In this study, the antioxidant activities of the free and bound phenolic extracts in buckwheat flour fraction were evaluated using the DPPH assay. The concentration of DPPH was adjusted to 0.15 μM (final concentration), which was 2-fold higher than that used for wheat phenolic extracts as reported by Liyana-Pathirana and Shahidi (2007). The scavenging capacity of DPPH radical gradually increased in the order from the fraction number 1 (phenolics less rich fraction) to the fraction number 16 (phenolics rich fraction) for both free and bound phenolic extracts (Fig. 3). After 30 min, the DPPH scavenging of FS-1 (phenolics less rich fraction) were 5.5% and 1.1%, whereas the scavenging of F3-4 (phenolics rich fraction) were 94.4% and 34.2% for the free and bound phenolic extracts, respectively. These results illustrated that the outer layers of buckwheat grains containing higher total phenolic and flavonoid contents possessed significantly higher antioxidant capacities than the inner fractions. In the DPPH assay, the bound phenolic extracts exhibited significantly lower scavenging than the free phenolic extracts, which is contrary to the other cereal grain extracts (Adom & Liu, 2002; Liyana-Pathirana, & Shahidi, 2007). These results indicate that the antioxidant capacity of the phenolic extracts was caused by higher total phenolic and rutin contents in the free phenolic extracts than the bound phenolic extracts of buckwheat flour fractions.

3.5. Total antioxidant capacity of free and bound phelonic compounds

Total antioxidant capacity of the free and bound phenolic extracts was also determined using the Trolox equivalent antioxidant capacity (TEAC) method and the results are shown in Fig. 4. The TEAC values also gradually increased in the order from the fraction number 1 (phenolics less rich fraction) to the fraction number 16 (phenolics rich fraction). TEAC values of the free phenolic extracts

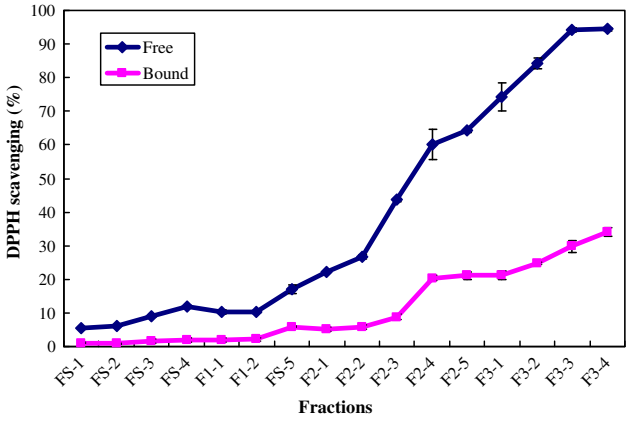

Fig. 3. DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging capacity of free and bound phenolic extracts from buckwheat graded milling flours. Concentration of DPPH = 0.15 mM. Values are means of triplicate measurements.

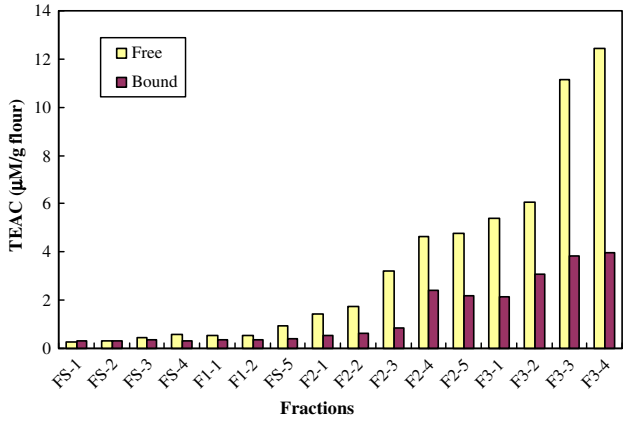

Fig. 4. Total antioxidant capacity (TEAC, μM Trolox equivalent/g of flour) of free and bound phenolic extracts from buckwheat graded milling flours. Values are means of triplicate measurements.

increased from 0.28 μM TE/g flour for FS-1 (phenolics less rich fraction) to 12.45 μM TE/g flour for F3-4 (phenolics rich fraction), whereas the TEAC values of the bound phenolic extracts increased from 0.32 μM TE/g flour for FS-1 (phenolics less rich fraction) to 3.99 μM TE/g flour for F3-4 (phenolics rich fraction). Thus, the antioxidant activity of the free and bound phenolic extracts in the phenolics less rich fraction were not significantly different as determined by TEAC method, whereas the TEAC of the free phenolic extracts in the phenolics rich fraction were significantly higher than that of the bound phenolic extracts. As a result, antioxidant compounds in buckwheat grain existed in free forms and were located in the outer layers of grain which contain mostly bran. These results are contrary to wheat fraction, in which the antioxidant compound existed mostly in bound forms and the endosperm of grain also exhibited considerable antioxidant activity (Liyana-Pathirana, & Shahidi, 2005, 2007).

3.6. Relationship between TPC, TFC, FAC, RC and antioxidant activities

The relationship between total phenolic compounds, total flavonoid, ferulic and rutin contents and antioxidant capacity are given in Table 2. The antioxidant capacities determined both in DPPH radical scavenging and TEAC methods exhibited highly positive correlation with the free and bound phenolics and flavonoids. These results indicate that the outer layers of buckwheat grain contained higher amounts of antioxidant compounds than the endosperm.

Table 2
Correlations between antioxidant capacities and phenolic compounds

	Phenolics		Flavonoids		Ferulic acid	Rutin
	Free	Bound	Free	Bound		
TEAC	0.993**	0.984**	0.984**	0.994**	0.980**	0.953**
DPPH	0.968**	0.989**	0.981**	0.955**	0.970**	0.985**

** Correlation is significant at the 0.01 level.

In addition, both ferulic acid and rutin showed highly positive correlation with the antioxidant activity indicating the important role of these compounds to antioxidant effects.

4. Conclusion

The results of this study show that the total phenolics and flavonoids in buckwheat significantly increased in the order from the fraction number 1 (phenolics less rich fraction) to the fraction number 16 (phenolics rich fraction). The phenolic acids are mainly located in the outer layers of grain in free forms, which can be easily extracted using ethanol or methanol solutions. In contrast, the flavonoids existed in the buckwheat grain in the bound forms, which were bound to cell wall materials and needed further alkaline, acid or enzyme treatments to extract. However, rutin, one of main flavonoid in buckwheat grain, existed mostly in the free phenolic extracts using ethanol solution. The higher phenolic contents in the phenolics rich fractions exhibited the stronger antioxidant capacity than the phenolics less rich fraction. Ferulic acid and rutin were the major antioxidant compounds of buckwheat and existed mostly in the outer layers of grain. As a result, the outer layers of buckwheat grains with higher amount of phenolic compounds along with higher amounts of protein, lipid, ash and dietary fiber are considered to be good materials for cereal-based food processing with significant health benefits.

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