Research Article

Distribution of Phenolic Acids and Antioxidant Activities of Different Bran Fractions from Three Pigmented Wheat Varieties

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Phenolic acid profiles and antioxidant activities of outer bran, coarse bran, and shorts from blue, black, and purple wheat were analyzed. Phenolic acids were mainly in the bound form in pigmented wheat bran fractions. Phenolic acid content decreased in the order of outer bran, coarse bran, and shorts for the three pigmented wheat varieties. HPLC analysis of phenolic extracts demonstrated that the bound form of phenolic acids contained more ferulic, isoferulic, and p-coumaric acids compared to their free counterparts. Among the three pigmented wheat varieties, the bran fractions from blue wheat contained higher bound phenolic acids than the other two pigmented wheat bran fractions, except for purple coarse bran. The blue wheat outer bran had the highest total bound phenolic acid of 3458.71 $\mu\text{g/g}$ while the purple wheat shorts had the lowest of 1730.71 $\mu\text{g/g}$. The contribution of bound phenolic acids to the total phenolic content and antioxidant activity was significantly higher than that of free phenolic acids. Blue wheat bran fractions had the highest radical scavenging activity against DPPH• while those of purple wheat gained the highest ABTS^{•+} scavenging activity. High correlations were observed between TPC and radical scavenging capacities for DPPH and ABTS ($R^2 > 0.85$, $P < 0.05$).

1. Introduction

Oxidative damage to important biomolecules such as DNA, proteins, and membrane lipids has been considered as causative of carcinogenesis, coronary heart disease, and many other age-related health problems [1–3]. Antioxidants can modulate cellular oxidative status and reduce the risk of these diseases and health problems [4–6]. Many previous researches demonstrated that wheat grain contained significant level of natural antioxidants [3, 7–12].

Wheat contains a wide range of chemical substances with antioxidant property [13]. Growing evidence indicated that a significant portion of the antioxidant property of wheat is attributed to phytochemicals instead of traditional vitamins [3, 14, 15]. Genotypes and culturing environment of wheat had significant influences on the phytochemical compositions and antioxidant activities [3, 16–18]. The antioxidant mechanisms may include acting as free radicals scavengers to end the radical chain reaction, transition metals chelators to inhibit the initiation of radical formation, reducing agents,

the antioxidative enzyme systems stimulators, or quenchers of singlet oxygen [19]. Phenolic acids in wheat are supposed to significantly contribute to the antioxidant abilities and health benefits of whole wheat ingestion observed in many epidemiological studies [8, 16, 20]. There are mainly two groups of phenolic acids in wheat bran: hydroxybenzoic and hydroxycinnamic acid derivatives. Vanillic and salicylic acids are mainly hydroxybenzoic acid derivatives while ferulic acids and p-coumaric are the most common derivatives of hydroxycinnamic acid [21]. Phenolic acids in cereal grains are in free, soluble conjugates, and bound forms. The majority of them existed in bound form, esterified to cell wall material in bran [16, 22].

Phenolics were not equally distributed in the wheat grains but concentrated in the outer layers [10]. Wheat bran fractions enriched in phenolics and antioxidants can be obtained by debranning and used as functional food ingredients or nutrient supplement [8, 14]. Pigmented wheat, such as blue, black, and purple wheat, is currently gained increasing attention due to their anthocyanin pigments [23–26]. However,

most of these studies focused on the anthocyanin and their properties. Distribution of phenolic acids and antioxidant activities of bran fractions from different pigmented wheat has not been reported.

Pigmented wheat has great potential as functional foods (such as whole grain products) or functional food ingredients (such as anthocyanin-rich fractions) for disease prevention and health promotion [24]. However, too much bran in flour may lead to bad influences on food processing performance and acceptability by consumers [27]. Debranning of wheat before milling has been widely accepted to improve roller-milling property and obtain ingredients rich in special nutrients [8]. The present study obtained three bran fractions (outer bran, coarse bran, and shorts) by combining debranning and milling of three pigmented wheat varieties. Phenolic acid profiles and antioxidant activities of both free and bound phenolic acids in pigmented bran fractions were analyzed.

2. Materials and Methods

2.1. Materials. Three pigmented wheat varieties (blue, black, and purple wheat) were harvested from demonstration field of Tai'an Subcenter of National Wheat Improvement Center, Shandong province, China, during 2010 season. The demonstration field is located at 36.18°N latitude and 117.13°E longitude. The purple wheat was ShanNong Zimai 1. The blue and black wheat were one of breeding lines. Gallic, protocatechuic, p-hydroxybenzoic, chlorogenic, vanillic, caffeic, syringic, p-coumaric, ferulic, salicylic, and trans-cinnamic acids were purchased from Sigma-Aldrich (Shanghai, China). All other chemicals and solvents were of analytical or HPLC-grade purity.

Three pigmented wheat varieties were debranned by a friction debranning machine (6MT-5, Shandong Agriculture Machinery Institute, China) to obtain 8% outer bran. The debranned wheat were milled by a laboratory mill (JNFB70×30, Chengdu Grain Storage Research Institute, China) and yielded coarse bran (10% based on raw wheat), shorts (6% based on raw wheat), and flour (76% based on raw wheat). The wheat bran fractions were further milled with addition of liquid nitrogen and sieved through a 40-mesh screen and then stored at -20°C before analysis.

2.2. Extraction of Free Phenolic Acids. Free phenolic acids were extracted according to the procedure of Kim et al. [16] with some modification. The pigmented wheat bran fractions were defatted twice with hexane at a 10:1 ratio (v/w) in an ultrasonic cleaner for 50 min at room temperature. The mixture was centrifuged at 4000 rpm for 15 min to recover the bran fractions. The defatted samples were dried in vacuum drying oven at room temperature. The defatted bran fractions were extracted twice with 80% methanol at a 10:1 ratio (v/w) for 50 min at room temperature and then centrifuged at 4000 rpm for 15 min. The supernatants were transferred to pear shape flasks and rotary evaporated to dryness at 40°C. The extracts were redissolved in 4 mL of HCl solution (pH 2.0) and extracted with 4 mL of ethyl acetate/ethyl ether (1:1, v/v) three times. The organic layers were combined and

rotary evaporated to dryness at 40°C. The solid extracts were reconstituted to 10 mL with methanol and frozen at -20°C before further analysis.

2.3. Extraction of Bound Phenolic Acids. The residues (1 g) after methanol extraction were dispersed with 40 mL NaOH solution (2.0 mol/L) in a 150 mL conical flask. The conical flask was purged with nitrogen to reduce the oxidation of phenolic compounds. The mixture was hydrolyzed in a mechanical shaker for 4 h at room temperature. The suspension was adjusted to pH 7.0 with 4.0 mol/L HCl and centrifuged at 4000 rpm for 15 min. The supernatant was adjusted to pH 2.0 and reextracted with ethyl acetate/ethyl ether (1:1, v/v). After evaporation of ethyl acetate and ethyl ether, the phenolic acid extract was reconstituted to 10 mL with methanol and frozen at -20°C until further analysis within a three-month period.

2.4. HPLC Analysis. The samples prepared above were filtered through a 0.45 μ m syringe filter before HPLC analysis. HPLC analysis was carried out using a Shimadzu LC-20AT liquid chromatograph equipped with an autosampler and a diode-array detector. The phenolic acids were separated on a reverse phase C18 column Inertsil ODS-SP (250 mm $\times$ 4.6 mm, 5 μ m). The temperature of column oven was 40°C. The mobile phase consisted of water (solvent A) and acetonitrile (solvent B), both containing 0.1% formic acid (v/v). The flow rate was kept at 1.0 mL/min and the gradient elution was employed as follows: 0% B to 10% B in 20 min, 10% B to 50% B in 10 min, 50% B to 100% B in 10 min, 100% B to 10% B in 5 min, and 10% B in 15 min. The injection volume was 10 μ L. Identification of phenolic acids was accomplished by comparing the retention time of peaks in the samples to that of the standards under the same HPLC conditions. Phenolic acids were quantified by comparing the peak area of the samples with the peak area of the calibration curves for all of the phenolic acids (all $R^2 > 0.999$).

2.5. Determination of Total Phenolic Content. Total phenolic content (TPC) of defatted wheat bran fractions was determined using the Folin-Ciocalteu reagent according to a modified procedure [16]. An aliquot of the methanolic extract (0.1 mL) was added to 0.5 mL Folin-Ciocalteu reagent. After equilibration for 5 min, 1.5 mL sodium carbonate solution was added to the mixture and mixed and made up to a final volume of 10 mL with distilled water. The mixture was allowed to stand at room temperature for 120 min, and then the absorbance was measured at 765 nm against a blank of distilled water. The total phenolic content in each extract was determined using a standard curve prepared using gallic acid and expressed as gallic acid equivalents (μ g GAE/g defatted bran).

2.6. Determination of Antioxidant Activity

2.6.1. DPPH Radical Scavenging Capacity. The DPPH• scavenging capacity of the bran extracts was determined according to Verma et al. [28] with some modifications. The initial concentration was 50 μ M for DPPH in all antioxidant-radical

reactions, freshly prepared in methanol before measurement. The bran extracts (0.1 mL) were reacted with 3.9 mL DPPH• solution to initiate antioxidant-radical reaction. After 60 min of reaction, the absorbance of the reaction mixture was measured at 517 nm against a blank of methanol. Control group was prepared in a similar way as for the experimental group except for the replacement of the testing sample solution with the corresponding extraction solvent. DPPH radical scavenging activity (%) = $[1 - \text{absorbance of sample} / \text{absorbance of control}] \times 100$.

2.6.2. ABTS^{•+} Scavenging Activity. The ABTS^{•+} scavenging activity of the bran extracts was determined using a commercial kit from Beyotime Institute of Biotechnology (Shanghai, China). Trolox (6-hydroxy-2,5,7,8-tetra-methyl-chroman-2-carboxylic acid) was used as an antioxidant standard. Eighty percent ethanol was used to prepare the solutions of Trolox and to determine the reagent blank. The Trolox equivalent antioxidant capacity (TEAC) values of the extracts were calculated using a Trolox standard curve on the basis of the absorbance at 734 nm after 30 min of reaction and expressed in $\mu\text{mol Trolox/g}$ defatted sample. The range of concentration of Trolox used for standard curve was 0.15–1.5 mmol/L. The tests were conducted in triplicate for each sample extract.

2.7. Statistical Analysis. Data were reported as mean $\pm$ standard deviation for triplicate determinations. ANOVA and Tukey's tests were performed with SPSS18.0 for Windows to identify differences among means. Statistical significance was declared at $P < 0.05$.

3. Results

The content of lipids in wheat bran fractions ranged from 2.9% to 3.8% at 12% moisture level. Samples were defatted to remove lipids and lipid-soluble ingredients which may influence phenolic acids and their antioxidant activities.

3.1. Distribution of Phenolic Acids in the Bran Fractions. Previous studies demonstrated that the widespread phenolic acids in wheat are gallic, protocatechuic, p-hydroxybenzoic, chlorogenic, vanillic, caffeic, syringic, p-coumaric, ferulic, and salicylic acids [29]. Gallic, protocatechuic, p-hydroxybenzoic, vanillic, syringic, p-coumaric, ferulic, isoferulic, salicylic acids were detected in this study (Table 1).

The content of free phenolic acids in the bran fractions was shown in Table 1. Gallic acid, ferulic acid, and salicylic acid were the major free phenolic acids in all bran fractions. Gallic acid and protocatechuic acid were only identified in the free form. On the whole, the content of phenolic acids presented a descending trend in the order of outer bran, coarse bran, and shorts, with the exception of p-coumaric acid and ferulic acid, which had the highest concentration in shorts fractions. Ferulic acid was the majority and it accounted for 16.81–23.01% of the total detectable free phenolic acids. In the outer bran of three pigmented wheat varieties, the concentration of salicylic acid was higher than that of ferulic acid, respectively. The content of free phenolic acids in purple wheat bran fractions was higher than blue and black wheat.

Table 2 showed the concentration of bound phenolic acids which were released during the alkaline hydrolysis process. Cereals were rich in substituted cinnamic acids such as ferulic acid which was esterified to arabinoxylan and arabinogalactan in the aleurone layer and pericarp [30]. Alkaline hydrolysis was often used to cleave the ester bond to separate and identify particular phenolic compounds [31]. Different wheat bran fractions contained different phenolic acid profiles. Gallic acid was not detectable in bound phenolic acids but was in free phenolic acids, while protocatechuic acid was only found in purple wheat shorts fraction in bound phenolic acids but was in all free phenolic acids, suggesting that the optimum method to obtain these phenolics is by 80% aqueous methanol extraction. Similarly, salicylic acid, which was found in free phenolic acids, was only detected in outer bran fractions in bound phenolic acids. The other phenolic acids were prevalent in the bound form. However, only ferulic, isoferulic, p-coumaric acids were predominant in the various fractions of wheat bran. The results demonstrated that the hydroxycinnamic acid derivatives can be easily liberated from the bound form by alkaline hydrolysis. They are the most common phenolic acids in wheat and hold promise as antioxidants, whereas only ferulic acid was released in significantly higher amount than any others in all bran fractions and accounted for about 49–89% of the total identified phenolic acids. From outer bran, coarse bran to shorts, amount of ferulic acid decreased progressively in blue wheat. Nevertheless, the coarse bran of black wheat and purple wheat had larger amount of ferulic acid than the other fractions. The difference in the distribution of ferulic acid might be due to the different colors and varieties. The outer bran of blue wheat had the greatest concentration of total phenolic acids (3459 $\mu\text{g/g}$), while the shorts of purple wheat had the least amount of total phenolic acids (1731 $\mu\text{g/g}$). There was a significant increase of phenolic acids on the basis of alkaline hydrolysis, 16-fold for blue wheat outer bran, 13-fold for black wheat outer bran, 8-fold for purple wheat outer bran.

3.2. Folin-Ciocalteu (FC) Determination of Total Phenolic Content. Total phenolic content of free and bound phenolic acids of the bran fractions, expressed as microgram gallic acid equivalents per gram defatted bran, was presented in Table 3. The antioxidant extracts differed significantly in their total phenolics contents. The total phenolic contents of both free and bound phenolic acids gradually decreased from the outer to the inner fractions, ranged from 7737.17 to 4387.99 $\mu\text{g GAE/g}$ defatted bran for blue wheat, from 8269.97 to 4647.64 $\mu\text{g GAE/g}$ defatted bran for black wheat, and from 8012.64 to 3942.18 $\mu\text{g GAE/g}$ defatted bran for purple wheat. These results showed that the phenolics of wheat were mostly concentrated in the outer layer of wheat grains. Blue wheat outer bran contained the greatest level of total phenolics, whereas purple wheat shorts contained the least level of total phenolics. In the present study, black wheat and purple wheat bran exhibited larger amounts of phenolic acids than blue wheat. In the three pigmented wheat varieties, the bound phenolic concentration was significantly higher than free phenolic. It appeared that the dominating phenolic acids in

TABLE 1: The content ($\mu\text{g/g}$ defatted bran) of free phenolic acids in the bran fractions of three pigmented wheat varieties.

Phenolic acids	Blue wheat			Black wheat			Purple wheat		
	Outer bran	Coarse bran	Shorts	Outer bran	Coarse bran	Shorts	Outer bran	Coarse bran	Shorts
Gallic acid	32.77 $\pm$ 0.37	31.99 $\pm$ 0.41	29.70 $\pm$ 0.17	29.07 $\pm$ 0.34	32.97 $\pm$ 1.33	29.08 $\pm$ 0.18	30.87 $\pm$ 0.22	29.88 $\pm$ 0.97	29.30 $\pm$ 0.15
Protocatechuic acid	3.03 $\pm$ 0.06	1.43 $\pm$ 0.12	0.90 $\pm$ 0.08	2.09 $\pm$ 0.14	2.91 $\pm$ 0.14	2.32 $\pm$ 0.10	17.18 $\pm$ 0.94	5.69 $\pm$ 0.21	2.82 $\pm$ 0.11
p-Hydroxybenzoic acid	11.17 $\pm$ 0.31	8.40 $\pm$ 0.06	8.38 $\pm$ 0.08	9.78 $\pm$ 0.07	8.60 $\pm$ 0.05	8.52 $\pm$ 0.04	13.83 $\pm$ 0.20	9.96 $\pm$ 0.07	8.99 $\pm$ 0.09
Vanillic acid	11.82 $\pm$ 0.14	5.73 $\pm$ 0.09	5.40 $\pm$ 0.38	15.88 $\pm$ 0.66	6.03 $\pm$ 0.20	4.59 $\pm$ 0.09	34.98 $\pm$ 2.20	9.94 $\pm$ 0.59	4.11 $\pm$ 0.40
Syringic acid	18.24 $\pm$ 0.01	13.92 $\pm$ 0.08	13.44 $\pm$ 0.15	20.93 $\pm$ 0.49	15.19 $\pm$ 0.34	14.01 $\pm$ 0.04	31.59 $\pm$ 0.63	14.03 $\pm$ 0.53	12.37 $\pm$ 0.00
p-Coumaric acid	24.66 $\pm$ 0.45	25.84 $\pm$ 0.03	25.88 $\pm$ 0.14	24.89 $\pm$ 0.82	23.67 $\pm$ 0.06	24.93 $\pm$ 0.19	26.53 $\pm$ 0.73	24.34 $\pm$ 0.14	28.44 $\pm$ 0.02
Ferulic acid	40.99 $\pm$ 1.14	40.37 $\pm$ 0.45	42.46 $\pm$ 0.81	32.45 $\pm$ 0.86	34.84 $\pm$ 0.37	35.88 $\pm$ 0.60	33.23 $\pm$ 0.46	38.11 $\pm$ 1.59	39.72 $\pm$ 0.21
Isoferulic acid	26.61 $\pm$ 0.27	24.85 $\pm$ 0.17	24.92 $\pm$ 0.16	25.34 $\pm$ 0.08	24.70 $\pm$ 0.05	24.66 $\pm$ 0.06	28.23 $\pm$ 0.36	25.10 $\pm$ 0.01	24.70 $\pm$ 0.05
Salicylic acid	48.20 $\pm$ 7.62	33.14 $\pm$ 0.67	33.42 $\pm$ 0.98	32.59 $\pm$ 0.25	29.97 $\pm$ 0.19	30.48 $\pm$ 0.82	65.01 $\pm$ 2.12	37.70 $\pm$ 2.00	31.99 $\pm$ 0.13
Total phenolics (HPLC)	217.49	185.67	184.49	193.01	178.89	174.48	281.45	194.75	182.44

TABLE 2: The content ($\mu\text{g/g}$ defatted bran) of bound phenolic acids in the bran fractions of three pigmented wheat varieties.

[illegible]

TABLE 3: Total phenolic content ($\mu\text{g GAE/g}$ defatted bran) of free and bound phenolic acids in the bran fractions of three pigmented wheat varieties.

Samples	FPA	AHPA	Total
Blue wheat outer bran	874.49 ± 21.73	6862.68 ± 93.41	7737.17
Blue wheat coarse bran	476.41 ± 23.50	4522.18 ± 84.82	4998.59
Blue wheat shorts	450.15 ± 10.69	3937.84 ± 44.30	4387.99
Black wheat outer bran	590.38 ± 26.33	7679.60 ± 127.91	8269.97
Black wheat coarse bran	495.25 ± 14.52	5179.15 ± 105.51	5674.40
Black wheat shorts	452.25 ± 11.01	4195.39 ± 91.88	4647.64
Purple wheat outer bran	1139.79 ± 25.11	6872.68 ± 71.99	8012.46
Purple wheat coarse bran	636.35 ± 22.53	5257.32 ± 150.55	5893.67
Purple wheat shorts	433.49 ± 18.15	3508.70 ± 267.75	3942.18

FPA, free phenolic acid; AHPA, alkaline-hydrolysable phenolic acids.

FIGURE 1: DPPH radical scavenging of the free and bound phenolic acids in the bran fractions of three pigmented wheat varieties. ^aValues are means of three determinations $\pm$ standard deviation, determined by HPLC analysis. ^bThe same letters are not significantly different ($P < 0.05$). Blue: blue wheat; Black: black wheat; Purple: purple wheat; 1: outer bran; 2: coarse bran; 3: shorts; FPA, free phenolic acid; AHPA, alkaline-hydrolysable phenolic acids.

the pigmented wheat bran fractions were not extractable by aqueous methanol but released upon alkaline hydrolysis.

3.3. Antioxidant Capacity

3.3.1. DPPH Radical Scavenging Capacity. The scavenging capacity of the stable organic DPPH radical was used in the evaluation of antioxidant potential of free and bound phenolics of the bran fractions. The DPPH radical, with a dark purple color, has absorbance at 517 nm. It loses this absorption when accepting an electron or a free radical species and it is reduced to its nonradical form by antioxidants, which results in a colorless. It can detect numerous samples in a short period and is sensitive enough to measure active constituents at low contents [32]. The DPPH radical scavenging property of each wheat bran sample was reported as the percent of DPPH radical scavenging, with a lower value of percent of DPPH scavenging associated with a stronger DPPH scavenging ability [33]. Figure 1 showed the percentage of DPPH radical scavenging of the free and bound phenolics in the bran fractions. In the present study, remarkable DPPH radical scavenging capacity was obtained in all wheat bran

extracts although the scavenging activities of phenolics were different among various wheat bran fractions. The bound phenolic acids were more effective than free phenolic acids in terms of DPPH radical scavenging activity. This indicated that the bound phenolics of all fractions had higher antioxidant activity than that of free phenolics. These results were derived from the higher total phenolic contents in the bound phenolics than those in the free phenolics. In agreement with that observed in TPC, the antioxidant activities of pigmented wheat bran were significantly decreased from outside to inside. The outer bran of the three wheat varieties exhibited the highest DPPH radical scavenging activity while the shorts fractions showed lowest scavenging efficacies. The result may be due to the fact that the endosperm diluted the antioxidant substances present in shorts resulting in lower antioxidant capacity compared to the outer bran and coarse bran. In general, DPPH radical scavenging activity of bound phenolic acids in blue wheat bran fractions was higher than black and purple wheat. However, blue wheat shorts possessed the lowest DPPH radical scavenging efficiency of free phenolic acids. The TPC and DPPH radical scavenging ability of pigmented wheat bran samples of both free and bound phenolics displayed a strong correlation ($R^2 = 0.91$ for blue wheat, $R^2 = 0.95$ for black wheat, and $R^2 = 0.94$ for purple wheat, $P < 0.05$).

3.3.2. ABTS^{•+} Scavenging Capacity. To better understand the radical scavenging properties, the antioxidant activity of the pigmented wheat bran fraction extracts was also detected using the TEAC (Trolox equivalent antioxidant capacity) method. The ABTS^{•+} scavenging capacity assay is a decolorization assay that examines the capacity of antioxidant substances to directly interact with (scavenge) ABTS^{•+}, generated by a chemical way. ABTS^{•+} is a nitrogen centered radical having a typical blue-green color. It becomes colorless when reduced by antioxidant ingredients to its nonradical (ABTS) form [13]. The TEAC value of a compound corresponds to the content of Trolox (a water-soluble vitamin E analogue without the side-chain moiety) which is an antioxidant standard substance [34]. Wheat bran extracts were measured and compared for their radical scavenging activities against ABTS^{•+}. Free and bound phenolics in all three pigmented

FIGURE 2: ABTS^{•+} scavenging capacity of the free and bound phenolic acids in the bran fractions of three pigmented wheat varieties. ^aValues are means of three determinations $\pm$ standard deviation, determined by HPLC analysis. ^bThe same letters are not significantly different ($P < 0.05$). Blue: blue wheat; Black: black wheat; Purple: purple wheat; 1: outer bran; 2: coarse bran; 3: shorts; FPA, free phenolic acid; AHPA, alkaline-hydrolysable phenolic acids.

wheat varieties showed significant ABTS^{•+} scavenging capacity (Figure 2), although some differences among samples were noted. The TEAC values ranged from 0.81 to 6.01 and from 6.46 to 12.81 $\mu\text{mol TE/g}$ of defatted bran for free and bound phenolics, respectively. Similar to DPPH radical scavenging, the TEAC values of bound phenolics were higher than those of the free phenolics, suggesting that the contribution of bound phenolics to total antioxidant activity was significantly higher than that of free phenolics in all wheat bran samples. TEAC values gradually decreased from the outer bran to shorts fractions in both black and purple wheat, with the exception of blue wheat bran fractions. The coarse bran of blue wheat gained the lowest antioxidant activity in all fractions. No difference in ABTS^{•+} scavenging activity of bound phenolics was observed among the fractions obtained from outer bran of three pigmented wheat varieties while significant difference was found in other fractions. These data indicated that color might be related to ABTS^{•+} scavenging activity of wheat. The highest TEAC value might be attributed to their highest total phenolic content. Both free and bound phenolics exhibited a strong correlation between TPC and TEAC ($R^2 = 0.85$ for blue wheat, $R^2 = 0.97$ for black wheat, and $R^2 = 0.85$ for purple wheat, $P < 0.05$).

4. Discussion

Phenolic acid compositions and concentration in the pigmented wheat bran fractions varied significantly. Liyana-Pathirana and Shahidi [12] have suggested that the majority of the phenolic acids were ferulic acid, p-coumaric acid, vanillic acid, and sinapic acid in whole wheat and all fractions examined. In this study, gallic, protocatechuic, p-hydroxybenzoic, vanillic, syringic, p-coumaric, ferulic, isoferulic, salicylic acids were present in free phenolic acids, but no gallic, protocatechuic acids were detected in bound phenolic acids. In this study, the majority of phenolic acids existed in bound form in the pigmented wheat bran and ferulic acid was the primary individual phenolic acids in bound form. The

bound phenolics were supposed to improve health more effectively as they may avoid upper gastrointestinal digestion along with cell wall components and are absorbed into blood plasma during digestion of intestinal microorganism [35]. Meanwhile, a decreasing trend in phenolic acids content was found from outer bran to shorts. This observation is supported by other studies [29]. In wheat grains, phenolic acids are mainly found in the cell walls of their outer layers, mainly esterified to the arabinose side groups of arabinoxylans [36]. By debranning and milling, bran-rich fraction can be obtained and used to a source of natural antioxidant substance.

In agreement with other studies [16, 37], the effect of pigment on the concentration of phenolic acids was observed in this research. Kim et al. [16] reported that the total phenolic content of the red wheat bran was higher than that of the white wheat. According to Chandrasekara and Shahidi [38], millets with dark brown pigment had a higher phenolic content of soluble phenolic parts than those with white or yellow testa and pericarp.

According to Zhou and Yu [3], the TEAC ranged from 3.09 to 15.26 $\mu\text{mol TE/g}$ for Akron wheat bran and from 2.74 to 12.04 $\mu\text{mol TE/g}$ for Trego wheat bran. In present study, the consequences of the scavenging activity of wheat bran extracts against ABTS^{•+} were lower (0.81–12.81 $\mu\text{mol TE/g}$ defatted bran) than the previous reports, which could be explained by different wheat varieties, locations, extraction methods, and the way used to prepare ABTS^{•+}. Widespread used approaches for producing ABTS^{•+} include oxidation with potassium persulfate [29] or MnO_2 [39] and attenuation in ethanol or phosphate buffer. In this study, a commercial kit was used to determine the TEAC.

Total phenolic content has been found to be significantly associated with the antioxidant activity [8]. In this paper, significant correlations ($R^2 > 0.85$, $P < 0.05$) were observed between TPC and the antioxidant activity. Beta et al. [8] reported a strong correlation of total phenolic content and antioxidant activity for pearled wheat and milled fractions. However, Zhu et al. [40] reported that the relationship between TPC and free radical scavenging activities against both DPPH[•] and ABTS^{•+} was complex. In this study, all fractions of three pigmented wheat varieties had effective radical scavenging properties. Blue wheat bran fractions had the strongest radical scavenging activity against DPPH[•] while those of purple wheat gained the strongest ABTS^{•+} scavenging activity. The scavenging activity of the same sample of DPPH radical was inconsistent with that of the ABTS^{•+} radical. The 70% ethanol extract showed the best DPPH radical scavenging activity but did not necessarily have the highest activity to quench ABTS^{•+} [40]. Several reasons could be provided for this observation: (1) DPPH and ABTS assays carried out in methanol and aqueous ethanol media, respectively; (2) different mechanisms related to the radical-antioxidant reactions; ABTS radical scavenging activity is a single electron transfer reaction, while the DPPH radical scavenging assay transfers hydrogen atom or electrons [41]; (3) stereoselectivity of the radicals or the solubility of wheat extracts in different estimating systems [42].

The bound phenolics exhibited significantly greater antioxidant activity than that of free phenolics in all samples. This may be due to the higher total phenolic content in the bound phenolic extracts than those in the free phenolic extracts. Consequently, the bound phenolic acids were the major antioxidant components of wheat bran and existed abundantly. These components were not extractable by aqueous methanol but released upon alkaline hydrolysis.

5. Conclusions

Three pigmented wheat varieties were debranned and milled to produce three bran fractions (outer bran, coarse bran, and shorts). The phenolic acids content and antioxidant capacity were investigated. In general, the content of phenolic acids and antioxidant activities both in free and bound forms gradually decreased in the order of outer bran > coarse bran > shorts. The content of bound phenolic acids and antioxidant capacities were significantly higher than free phenolic acids. The multiple differences existing in different fractions may be attributed to the differences in the composition and amount of phenolic acids. Ferulic acid was the predominant phenolic acid in the bound form. The total phenolics content and antioxidant capacity of both free and bound phenolics showed quite a strong correlation ($R^2 > 0.85$, $P < 0.05$). Pigmented wheat varieties have a considerable promise as a source of bioactive material for potential use in the functional food industry.

Abbreviations

TPC:	Total phenolic content
GAE:	Gallic acid equivalents
FC:	Folin-Ciocalteu
HPLC:	High performance liquid chromatography
DPPH:	2,2-Diphenyl-1-picrylhydrazyl
ABTS ⁺ :	(2,2'-Azino-bis[3-ethylbenzthiazoline-6-sulfonic acid])
TEAC:	Trolox equivalent antioxidant capacity; Trolox (6-hydroxy-2,5,7,8-tetra-methyl-chroman-2-carboxylic acid)
FPA:	Free phenolic acid
AHPA:	Alkaline-hydrolysable phenolic acids
AAPH:	2,2'-Azobis-(2-methylpropionamidine) dihydrochloride.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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