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Comparison of Swiss Red Wheat Grain and Fractions for Their Antioxidant Properties

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Swiss red wheat grain, bran, aleurone, and micronized aleurone were examined and compared for their free radical scavenging properties against 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), radical cation $ABTS^{\bullet+}$ and peroxide radical anion $O_2^{\bullet-}$, oxygen radical absorbance capacity (ORAC), chelating capacity, total phenolic content (TPC), and phenolic acid composition. The results showed that micronized aleurone, aleurone, bran, and grain may significantly differ in their antioxidant properties, TPC, and phenolic acid composition. Micronized aleurone had the greatest antioxidant activities, TPC, and concentrations of all identified phenolic acids, suggesting the potential of postharvesting treatment on antioxidant activities and availability of TPC and phenolic acids. Ferulic acid was the predominant phenolic acid in Swiss red wheat and accounted for ~57−77% of total phenolic acids on a weight basis. Ferulic acid concentration was well correlated with scavenging activities against radical cation and superoxide anion, TPC, and other phenolic acid concentrations, suggesting the potential use of ferulic acid as a marker of wheat antioxidants. In addition, 50% acetone and ethanol were compared for their effects on wheat ORAC values. The ORAC value of 50% acetone extracts was 3−20-fold greater than that of the ethanol extracts, indicating that 50% acetone may be a better solvent system for monitoring antioxidant properties of wheat. These data suggest the possibility to improve the antioxidant release from wheat-based food ingredients through postharvesting treatment or processing.

KEYWORDS: Wheat; bran; aleurone; radical scavenging; antioxidant; phenolic; phenolic acid, chelating; $ABTS^{\bullet+}$; DPPH•

INTRODUCTION

Severe oxidative stress, a result of imbalance between the antioxidant defense system and the formation of reactive oxygen species, may damage life-important membrane lipids, proteins, DNA, and carbohydrates (*1*, *2*). The damage may cause cell injury and death and exacerbate the development of several aging-related chronic diseases including cancer and heart disease (*1*). It has been well accepted that dietary antioxidants may prevent these physiologically important molecules from oxidative damage and consequently reduce the risk of aging-related diseases and/or promote general human health (*2*−*4*). Significant antioxidative activities have been detected in several wheat samples, including spring cultivar Henika (macaroni) wheat (*Triticum durum*) (*5*), winter cultivar Almari and spring cultivar Henika (*Triticum aestivum*) (*6*), hard winter wheat cultivars Akron, Trego, and Platte (*T. aestivum*) (*3*, *7*, *8*), and blue aleurone spring wheat (*T. aestivum*) (*9*). Wheat antioxidants were capable of directly reacting with and quenching free radicals, reducing the availability of transition medals (chelating activity), and suppressing lipid peroxidation in fish oil and liposome (*3*, *5*−*8*). It is widely accepted that phenolic compounds significantly contribute to the overall antioxidant properties of wheat. Onyeneho and Hettiarachchy (*5*) reported that ferulic, vanillic, and *p*-coumaric acids were major phenolics in wheat bran extracts, along with other free phenolics including caffeic, chlorogenic, gentisic, syringic, and *p*-hydroxybenzoic acids. It was also noted that phenolic acids are concentrated in the bran and aleurone fractions of wheat (*10*). Phenolic acids in wheat flour and grain have been investigated, because the phenolic acid content in flour was considered as a potential parameter to monitor the carry-over of bran in white flour. The previous studies also showed that the antioxidative properties of wheat might vary among wheat cultivars and may be significantly altered by growing conditions (*3*, *7*, *8*).

However, no research has been performed to compare the antioxidant properties of wheat aleurone and bran. It is also interesting how postharvesting treatment or processing may influence the antioxidant properties of wheat-based products. Therefore, the present study was conducted to examine and compare the antioxidant properties of aleurone extracts from

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Swiss red wheat with that of grain, bran, and micronized aleurone. This research is part of our continuous efforts to promote the improved production and utilization of value-added wheat for health promotion and disease prevention.

MATERIALS AND METHODS

Materials. Swiss red wheat gain, bran, aleurone, and micronized aleurone were provided by Bühler AG (Uzwil, Switzerland). 2,2′-Bipyridyl, disodium ethylenediaminetetraacetate (EDTA), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, fluorescein (FL), lauryl sulfate sodium salt, hypoxanthine (HPX), xanthine oxidase (XOD), nitro blue tetrazolium solution (NBT), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), α-tocopherol (vitamin E), ascorbic acid (vitamin C), and butylated hydroxytoluene (BHT) were purchased from Sigma-Aldrich (St. Louis, MO), whereas 2,2′-azobis(2-aminopropane) dihydrochloride (AAPH) was obtained from Wako Chemicals USA (Richmond, VA). β-Cyclodextrin (RMCD) was purchased from Cyclolab R&D Ltd. (Budapest, Hungary). All other chemicals and solvents were of the highest commercial grade and used without further purification.

Extraction and Testing of Sample Preparation. Four grams of each wheat sample was ground to fine powder using a micromill manufactured by Bel-Art Products (Pequannock, NJ) and extracted for 15 h with 40 mL of 50% acetone under nitrogen at ambient temperature or extracted for 2 h with absolute ethanol using a Soxhlet extractor. The ethanol was evaporated at 30 °C under reduced pressure, and the residue was redissolved in 7% RMCD solution. The 7% RMCD solutions from the ethanol extracts and the 50% acetone extracts were kept in the dark under nitrogen at room temperature until further analysis.

Radical Cation $ABTS^{\bullet+}$ Scavenging Activity. The radical scavenging capacity of wheat antioxidant was evaluated against $ABTS^{\bullet+}$ generated by the chemical method according to a previously reported protocol (*11*). Fifty microliters of wheat antioxidants in 50% acetone was diluted with 450 μL of 7% RMCD to obtain the testing samples. $ABTS^{\bullet+}$ was prepared by oxidizing a 5 mM aqueous solution of ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, with manganese dioxide at ambient temperature for 30 min. The $ABTS^{\bullet+}$−antioxidant reaction mixture contained 1.0 mL of $ABTS^{\bullet+}$ with an absorbance of 0.7 at 734 nm and 80 μL of antioxidant testing sample or 80 μL of 7% RMCD solution for the control. The absorbance at 734 nm was measured at 1 min of the reaction, and the Trolox equivalent was calculated using a standard curve prepared with Trolox.

Radical DPPH Scavenging Activity. The free radical scavenging capacity of wheat extracts was determined according to the previously reported procedure using the stable DPPH• (*3*). The final concentration was 100 mM for DPPH•, and the final reaction volume was 2.0 mL. The absorbance at 517 nm was measured against a blank of pure ethanol at 0, 1, 5, 10, 20, 40, 80, and 1400 min and used to estimate the remaining radical levels according to a standard curve. The absorbance measured at 40 min of the antioxidant−DPPH radical reactions was used to compare the DPPH radical scavenging capacity of wheat extracts to that of 50 mM ascorbic acid, BHT, and α-tocopherol. To determine the ED_{50} value of the wheat antioxidants against DPPH radicals, seven levels of each wheat extract were employed. A_{517nm} at 80 min of reaction was used to establish the ED_{50} value. The ED_{50} value is the concentration of antioxidant required to quench 50% radicals in the reaction mixture under the experimental condition.

Superoxide Anion Radical $O_2^{\bullet-}$ Scavenging Activity. $O_2^{\bullet-}$ scavenging activity was determined using an HPX/XOD system following a procedure previously described (*12*). NBT, HPX, and XOD solutions were prepared with 50 mM phosphate buffer (pH 7.4). The reaction mixture contained 0.2 mL of 0.34 mM NBT, 0.7 mL of 2 mM HPX, 0.1 mL of wheat antioxidant in 50% acetone, and 0.2 mL of 0.56 unit/mL XOD. The decrease in absorbance at 560 nm was measured at 7 min of the reaction. The $O_2^{\bullet-}$ scavenging activity was expressed as $O_2^{\bullet-}$ remaining.

Oxygen Radical Absorbance Capacity (ORAC) Assay. The ORAC assay was conducted by using FL as the fluorescent probe according to a protocol described by Huang and others (*13*). The final assay mixture contained 0.067 μM FL, 60 mM AAPH, 300 μL of wheat antioxidants, or 7% RMCD for a reagent blank. The fluorescence of an assay mixture was determined and recorded every minute for 60 min. The Trolox equivalent was calculated using a standard curve prepared with Trolox and used to compare ORAC of each antioxidant. All tests were conducted in triplicate.

Chelating Activity. Fe^{2+} chelating activity was measured by 2,2′-bipyridyl competition assay (*8*). The reaction mixture contained 0.1 mL of 1 mM $FeSO_4$ solution, 50 μL of wheat antioxidant in 50% acetone, 0.3 mL of 10% hydroxylamine−HCl, 0.4 mL of 2,2′-bipyridyl solution, and 0.8 mL of Tris-HCl buffer (pH 7.4). The absorbance at 522 nm was measured and used to evaluate Fe^{2+} chelating activity using EDTA as a standard. All tests were conducted in triplicate.

Total Phenolic Contents. The total phenolic contents of wheat extracts were determined using Folin−Ciocalteu reagent (*3*). The Folin−Ciocalteu reagent was prepared by refluxing a mixture of sodium tungstate, sodium molybdate, 85% phosphoric acid, and concentrated hydrochloric acid for 10 h, followed by reaction with lithium sulfate and oxidation by a few drops of bromine. The resulting solution was filtered and ready for testing. In brief, the reaction mixture contained 50 μL of wheat extracts, 250 μL of the Folin−Ciocalteu reagent freshly prepared in our laboratory, 0.75 mL of 20% sodium carbonate, and 3 mL of pure water. After 2 h of reaction at ambient temperature, the absorbance at 765 nm was measured and used to calculate the phenolic contents using gallic acid as a standard. All tests were conducted in triplicate.

Phenolic Acid Composition. After removal of the acetone, the wheat antioxidants were hydrolyzed with 4 N NaOH for 4 h at 55 °C under nitrogen, acidified using 6 N HCl, and extracted with ethyl ether/ethyl acetate (1:1, v/v) according to the procedure described previously (*14*). The ethyl ether/ethyl acetate was evaporated at 25 °C using a nitrogen evaporator, and the solid residue was redissolved in methanol, filtered through a 0.45 μm membrane filter, and kept in the dark under nitrogen until HPLC analysis. The phenolic acid composition in the methanol solution was analyzed by reverse-phase HPLC with a Phenomenex C18 column (250 mm × 4.6 mm). Phenolic acids were separated using a linear gradient elution program with a mobile phase containing solvent A (acetic acid/H_2O, 2:98, v/v) and solvent B (acetic acid/acetonitrile/H_2O, 2:30:68, v/v/v). The solvent gradient was programmed from 10 to 100% B in 42 min with a flow rate of 1.5 mL/min (*15*). Identification of phenolic acids was accomplished by HPLC-MS and comparing the retention of peaks in wheat samples to that of the standard compounds.

Statistical Analysis. Data were reported as mean ± SD for triplicate determinations. Analysis of variance and least significant difference tests (SPSS for Windows, version rel. 10.0.5, 1999, SPSS Inc., Chicago, IL) were conducted to identify differences among means, whereas a Pearson correlation test was conducted to determine the correlations among means. Statistical significance was declared at $p < 0.05$.

RESULTS

Radical Cation Scavenging Activity. Fifty percent acetone extracts of Swiss red wheat grain, bran, aleurone and micronized aleurone were examined and compared for their free radical scavenging activities against radical cation $ABTS^{\bullet+}$. All extracts showed $ABTS^{\bullet+}$ scavenging capacity (**Table 1**). The greatest $ABTS^{\bullet+}$scavenging capacity was detected in micronized aleurone extract, followed by aleurone, bran, and grain, respectively (**Table 1**). Aleurone (Asp1 and Asp2), isolated from the bran, had greater $ABTS^{\bullet+}$ scavenging capacity than bran, suggesting that antioxidants are concentrated in the aleurone. Significant difference in their radical cation scavenging activities was detected among wheat samples, except between aleurone sample 2 (Asp2) and the micronized aleurone prepared from the aleurone sample 1 (Asp1). Micronized aleurone (mAsp1) exhibited a significantly stronger $ABTS^{\bullet+}$ scavenging capacity than did the aleurone (Asp1), suggesting that postharvesting treatment may alter the antioxidant activities of wheat. For all

Table 1. Free Radical Scavenging Properties of Swiss Red Wheat[a]

	% $O_2^{\bullet-}$ remaining	$ABTS^{\bullet+}$ (TE μmol/g of wheat)	ED_{50}[b] for $DDPH^{\bullet}$ (mg/mL)
grain	77.56a ± 3.17	14.67a ± 0.48	20
bran	59.19b ± 0.13	19.74b ± 0.17	9.1
Asp1	58.62b ± 1.77	22.56c ± 0.22	8.8
Asp2	44.31c ± 0.08	24.29d ± 0.50	8
mAsp1	43.13c ± 0.37	24.47d ± 0.24	6.2

[a] Grain, Swiss red wheat grain; bran, bran; Asp1, aleurone sample 1; Asp2, aleurone sample 2; mAsp1, micronized aleurone prepared from aleurone sample 1; TE, Trolox equivalent. Free radical scavenging activities against radical anion $O_2^{\bullet-}$ and cation $ABTS^{\bullet+}$ were examined using 50% acetone extracts, whereas that against stable $DDPH^{\bullet}$ was evaluated using the ethanol extracts. Within each column, means with the same letter are not significantly different ($n = 3$, $p \leq 0.05$). [b] ED_{50} is the concentration of wheat extracts to quench 50% of DPPH radicals in the reaction mixture under the experimental conditions.

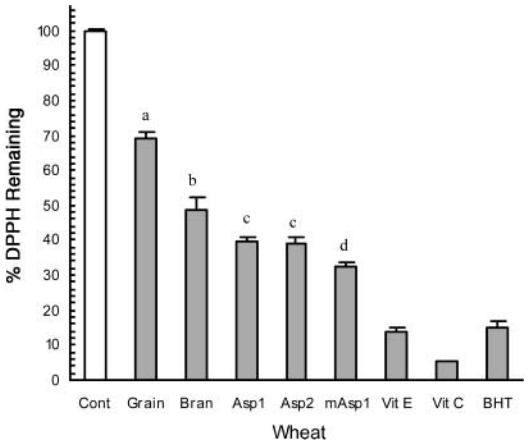

Figure 1. Radical DPPH scavenging activity. Grain, Bran, Asp1, Asp2, and mAsp1 represent Swiss red wheat grain, bran, aleurone sample 1, aleurone sample 2, and micronized aleurone prepared from the aleurone sample 1, respectively, whereas Cont represents the control containing no antioxidant. All tests were conducted using the ethanol extracts. The final DPPH radical concentration was 100 μM in all reaction mixtures, whereas the final concentration of wheat extracts was 12.5 mg of wheat equivalent/mL. Vit E, Vit C, and BHT represent α-tocopherol, ascorbic acid, and butylated hydroxytoluene, respectively, at a final concentration of 50 mM. All tests were conducted in triplicate, and the means are used. The vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ($p < 0.05$).

of the tested 50% acetone extracts, $ABTS^{\bullet+}$ scavenging capacity was correlated to $O_2^{\bullet-}$ scavenging activity ($r = 0.96$, $p = 0.009$), ORAC ($r = 0.97$, $p = 0.05$), chelating capacity against Fe^{2+} ($r = 0.92$, $p = 0.03$), and the total phenolic contents ($r = 0.95$, $p = 0.014$). In addition, the $ABTS^{\bullet+}$ scavenging capacity was also correlated with the DPPH radical scavenging capacity of the ethanol extract ($r = 0.94$, $p = 0.02$).

Radical DPPH Scavenging Activity. The ethanol extracts were analyzed and compared with α-tocopherol, BHT, and ascorbic acid for free radical scavenging activity against stable $DPPH^{\bullet}$. All wheat extracts, at a concentration of 12.5 mg of wheat equivalent/mL, were capable of directly reacting with and quenching $DPPH^{\bullet}$ (**Figure 1**; **Table 1**), although their $DPPH^{\bullet}$ scavenging activities were weaker than that of 50 mM α-tocopherol, BHT, or ascorbic acid. At 40 min of reaction, micronized aleurone quenched the greatest amount of $DPPH^{\bullet}$ in the system, followed by aleurone, bran, and grain, respectively (**Figure 1**), on a per weight basis. The concentration required to scavenge 50% of the free radicals in the reaction mixture,

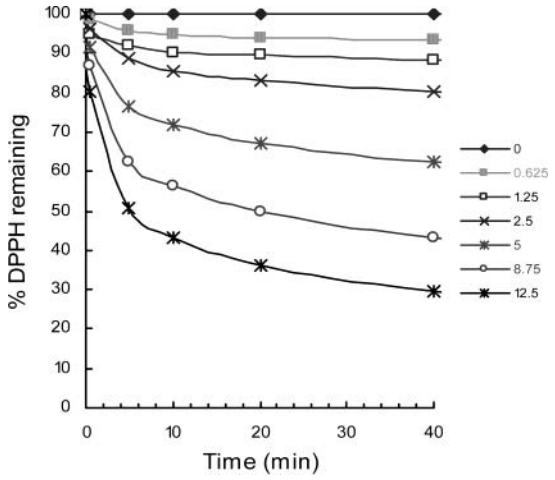

Figure 2. Reaction kinetics of wheat extracts with DPPH radical. 0, 0.625, 1.25, 5, 8.75, and 12.5 represent the final antioxidant concentration of 0, 0.625, 1.25, 5, 8.75, or 12.5 mg of wheat equivalent/mL in the reaction mixtures. All tests were conducted using the ethanol extracts. The final DPPH radical concentration was 100 μM in all reaction mixtures.

the ED_{50} value, was also determined. The order of the ED_{50} values for the wheat samples was micronized aleurone < aleurone < bran < grain (**Table 1**). A smaller ED_{50} value corresponds to a greater $DPPH^{\bullet}$ scavenging activity. These data indicated that radical scavengers are concentrated in the aleurone fraction of wheat bran, and reduction of particle size might increase the availability of wheat antioxidants. Similar dose and time effects were observed in all wheat antioxidant−DPPH radical reactions, and the dose and time effects of micronized aleurone extract are reported in **Figure 2** as an example. The DPPH radical scavenging activity of the ethanol extracts was not correlated with the ORAC of ethanol extracts. Interestingly, the DPPH radical scavenging activity of the ethanol extracts was correlated with the $ABTS^{\bullet+}$ scavenging capacity ($r = 0.94$, $p = 0.02$), $O_2^{\bullet-}$ scavenging activity ($r = 0.92$, $p = 0.03$), ORAC ($r = 0.97$, $p = 0.05$), chelating capacity against Fe^{2+} ($r = 0.94$, $p = 0.01$), and the total phenolic contents ($r = 0.90$, $p = 0.04$) of the 50% acetone extracts.

Superoxide Anion Radical $O_2^{\bullet-}$ Scavenging Activity. The $O_2^{\bullet-}$ scavenging activity of wheat samples was evaluated using the 50% acetone extracts and expressed as percent $O_2^{\bullet-}$ remaining. Micronized aleurone showed the greatest $O_2^{\bullet-}$ scavenging activity, whereas grain had the lowest activity (**Table 1**). The relative $O_2^{\bullet-}$ scavenging activity of the 50% acetone extracts was micronized aleurone > aleurone > bran > grain, on a per weight basis. The $O_2^{\bullet-}$ scavenging activity of aleurone was significantly greater than that of the bran used to isolate the aleurone, suggesting the potential effect of postharvest processing on the antioxidant property of wheat. Correlation was detected between $O_2^{\bullet-}$ scavenging activity and ORAC ($r = 0.94$, $p = 0.027$), $ABTS^{\bullet+}$ scavenging capacity, chelating activity ($r = 0.96$, $p = 0.01$), $DPPH^{\bullet}$, and TPC ($r = 0.97$, $p = 0.01$).

ORAC Assay. ORAC values were determined for both 50% acetone and ethanol extracts and expressed as Trolox equivalent (TE). Both ethanol extracts and 50% acetone extracts exhibited significant ORAC (**Table 2**). The greatest ORAC value was observed in Asp1 among ethanol extracts, followed by that of Asp2, micronized Asp1, and bran. This order differed from that detected among the 50% acetone extracts. Furthermore, no correlation in the ORAC values was detected between ethanol extracts and 50% acetone extracts. These data suggested the

Table 2. ORAC of Swiss Red Wheat[a]

	ORAC (TE μmol/g of wheat)	
	ethanol extract	50% acetone extract
grain	N/A	51.46a ± 6.54
bran	5.068au ± 1.73	107.53bv ± 4.1
Asp1	40.48bx ± 2.27	125.18cy ± 13.4
Asp2	37.08bcw ± 1.95	136.42cz ± 9.53
mAsp1	34.47cs ± 2.33	126.48ct ± 6.89

[a] Grain, Swiss red wheat grain; bran, bran; Asp1, aleurone sample 1; Asp2, aleurone sample 2; mAsp1, micronized aleurone prepared from aleurone sample 1; TE, Trolox equivalent; ORAC, oxygen radical absorbance capacity. The same letter (a–c) indicates the means within a column are not significantly different ($p \leq 0.05$), whereas the same letter (u–z) indicates the means within the same row are not different at $p \leq 0.05$ ($n = 3$).

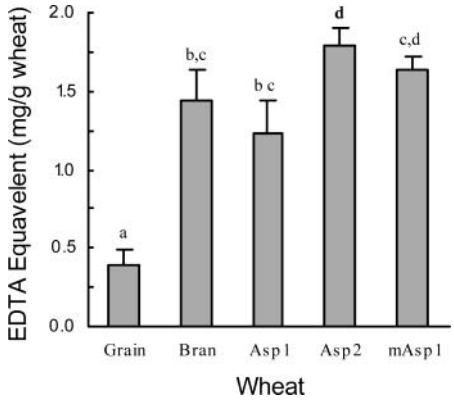

Figure 3. Chelating capacity of wheat extracts. The chelating activities of wheat extracts were expressed as EDTA equivalent. Grain, Bran, Asp1, Asp2, and mAsp1 represent Swiss red wheat grain, bran, aleurone sample 1, aleurone sample 2, and micronized aleurone prepared from the aleurone sample 1, respectively. All tests were conducted using the 50% acetone extracts. The vertical bars represent the standard deviation ($n = 3$). Values marked by the same letter are not significantly different ($p < 0.05$).

potential effects of extraction solvent on antioxidant activity estimation. The TE value of 50% acetone extract was 3−20-fold greater than that of the corresponding ethanol extract (**Table 2**), suggesting that a larger portion of wheat antioxidants is bound and has greater polarity. Wheat fractions differed in their ORAC values regardless of extraction solvent, indicating that antioxidants are mainly distributed in the aleurone fraction of wheat bran. The ORAC value of 50% acetone extracts was correlated with $O_2^{\bullet -}$ scavenging activity, $ABTS^{\bullet +}$ scavenging capacity, $DPPH^{\bullet}$ scavenging capacity, and chelating activity ($r = 0.94$, $p = 0.02$). No correlation between ORAC value and TPC was observed.

Chelating Activity. The chelating properties of the 50% acetone extracts were examined against Fe^{2+} and reported as EDTA equivalents (**Figure 3**). The grain had significantly lower chelating activity than bran and aleurone fractions, but bran and aleurone isolated from the bran showed similar chelating activities (**Figure 3**). Reduction in particle size did not result in a further increase in the chelating activity of aleurone. The chelating activity was correlated with $O_2^{\bullet -}$ scavenging activity, $ABTS^{\bullet +}$ scavenging capacity, $DPPH^{\bullet}$ scavenging capacity, and ORAC value, but not with total phenolic contents under the experimental condition.

Total Phenolic Content. The Swiss red wheat samples were examined and compared for their total phenolic contents (TPC) expressed as gallic acid equivalent (GE). The five wheat samples differed from one another in their TPC (**Figure 4**). The greatest

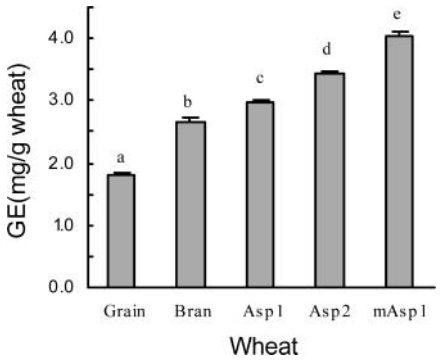

Figure 4. Total phenolic contents of wheat extracts. Grain, Bran, Asp1, Asp2, and mAsp1 represent Swiss red wheat grain, bran, aleurone sample 1, aleurone sample 2, and micronized aleurone prepared from the aleurone sample 1, respectively. All tests were conducted using the 50% acetone extracts. The vertical bars represent the standard deviation ($n = 3$). Values marked by the same letter are not significantly different ($p < 0.05$).

TPC of 4.04 mg of GE/g of wheat was detected in the micronized aleurone, whereas grain had the lowest TPC value of 1.8 mg of GE/g of wheat. TPC was correlated with $O_2^{\bullet -}$, $DPPH^{\bullet}$ scavenging capacity, and $ABTS^{\bullet +}$ scavenging capacity, but not ORAC ($r = 0.87$, $p = 0.055$) or chelating activity ($r = 0.87$, $p = 0.053$).

Phenolic Acid Composition. Five phenolic acids, including ferulic, syringic, *p*-hydroxybenzoic, vanillic, and coumaric acids, were detected in the wheat extracts (**Table 3**). Ferulic acid was the predominant acid in all extracts and accounted for ∼57−78% of the total identified phenolic acids on a per weight basis. Aleurone samples had the greatest concentration of each detected phenolic acids, followed by that of bran and grain, suggesting the phenolic acids are concentrated in the aleurone fraction of Swiss red wheat bran (**Table 3**). The two aleurone samples isolated from the same bran contained different amounts of phenolic acids, indicating the potential influence of the aleurone isolation procedure on the antioxidant properties of aleurone. Micronized aleurone (mAsp1) had a significantly greater concentration of each detected phenolic acid than the aleurone (Asp1) (**Table 3**), although similar ratios of ferulic, syringic, *p*-hydroxybenzoic, vanillic, and coumaric acids were observed in both extracts. These results suggested that micronization increased the availability of phenolic acids under the experimental conditions.

In addition, the ferulic acid concentration in wheat samples was correlated with scavenging capacities against $ABTS^{\bullet +}$ and $O_2^{\bullet -}$, as well as total phenolic contents. The correlation coefficients (r) were 0.88 ($p = 0.05$), 0.91 ($p = 0.03$), and 0.98 ($p = 0.003$) between ferulic acid concentration on a per weight basis and $ABTS^{\bullet +}$ scavenging activity, $O_2^{\bullet -}$ quenching capacity, and total phenolic content of the 50% acetone extracts, respectively. Ferulic acid concentration was also highly correlated with all other identified phenolic acids in the wheat extracts. The correlation coefficient was 0.92−0.98 ($p < 0.01$) between ferulic acid concentration and the concentration of *p*-hydroxybenzoic, vanillic, or coumaric acids and was 0.97 between ferulic acid and syringic acid levels ($p = 0.02$).

DISCUSSION

It has been widely accepted that diet may significantly influence human health and life quality. Recently, more consumers are interested in food products that either reduce the risk of, or manage, a specific health condition (*16*). To achieve the maximum benefits from diet, it is critical to understand the

Table 3. Phenolic Acid Composition of Swiss Red Wheat[a]

	p-hydroxybenzoic acid (μg/g of wheat)	vanillic acid (μg/g of wheat)	syringic acid (μg/g of wheat)	coumaric acid (μg/g of wheat)	ferulic acid (μg/g of wheat)
grain	5.0a ± 0.2	4.9a ± 0.2	13.7a ± 0.3	1.9a ± 0.1	33.7a ± 1.3
bran	19.7b ± 0.3	16.5b ± 0.3	57.2b ± 0.5	9.0b ± 0.0	209.3b ± 0.3
Asp1	24.2c ± 4.1	19.7c ± 0.7	69.3c ± 0.6	8.2c ± 0.0	279.7c ± 0.4
Asp2	28.4c ± 0.1	20.0c ± 0.4	90.3d ± 0.1	10.6d ± 0.0	373.6d ± 0.7
mAsp1	38.8d ± 3.5	29.6d ± 0.6	97.7e ± 0.8	14.7e ± 0.1	625.7e ± 3.7

[a] Grain, Swiss red wheat grain; bran, bran; Asp1, aleurone sample 1; Asp2, aleurone sample 2; mAsp1, micronized aleurone prepared from aleurone sample 1. The analysis was conducted using the 50% acetone extracts. Within each column, means with the same letter are not significantly different ($p \leq 0.05$, $n = 3$).

bioactive factors and their distributions in food ingredients, as well as the effects of food formula, food processing, and storage on the availability of these beneficial components. This understanding is also important for improving the safety and quality of consuming functional foods, because of the interactions among food components and foods, as well as the potential interactions between foods and supplements or medicines. In addition, this understanding may lead to an improved utilization and application of agricultural products including wheat and enhance the agricultural economy. For instance, wheat grain may be further processed into flour, bran, aleurone, and micronized aleurone and used to prepare food products, which may have different requirements in sensory properties, quality, and stability and different health benefits for different groups of consumers. This research is part of our continuous effort to promote the value-added production and utilization of wheat in improving human nutrition for disease prevention and health promotion.

In this study, aleurone isolated from bran, bran, and grain were compared for their antioxidant properties, total phenolic contents, and phenolic acid composition. Aleurone had the greatest radical scavenging activities, total phenolic contents, and phenolic acid concentration on a per weight basis under the experimental conditions, followed by bran and grain, respectively. These results, in agreement with the previous studies (*5*, *17*, *18*), demonstrate that antioxidants including phenolics are concentrated in the aleurone fraction of wheat bran, suggesting the potential to further isolate the aleurone fraction from bran and use it as a concentrated dietary source of natural wheat antioxidants; the remaining bran low in phenolics may be used as a food ingredient for dietary insoluble fiber. This bran ingredient may be used in making some food products (including Chinese noodles) rich in fiber with less concern about color instability, which is caused by the enzymatic browning reaction of phenolics. In other words, understanding of the distribution of bioactive components in wheat may lead to a value-added production and consumption of wheat and wheat-based products. This will benefit both consumers and wheat producers.

It was noted in this research that micronization, a postharvest treatment, increased antioxidant activities, total phenolic content, and phenolic acid concentration of aleurone. This may be explained by the increased surface area through micronization. Micronization reduced the particle size of aleuroen and consequently increased the total particle surface area on a per weight basis. Enlarged surface area increased the amount of extractable antioxidants. In other words, micronization improved the availability of wheat antioxidants. This observation suggests potential effects of postharvest treatment and processing on the overall antioxidant properties of wheat products. This observation was supported by an earlier research observation that pearling time may alter the antioxidant and phenolic content in the pearling fractions of oat groats (*19*). It is also noted that only one wheat variety was employed in this research. More research is required to further investigate postharvest treatment and processing on the antioxidant capacity of wheat products.

In addition, it was noted that the ORAC value of 50% acetone extracts was 3−20-fold greater and better correlated with other antioxidant activities, total phenolic contents, and phenolic acid concentrations than that of the ethanol extracts. This may be partially explained by the procedure used to prepare the ethanol extracts for the ORAC test. The ethanol was removed from the ethanol extracts, and the residue was redissolved in 7% RMCD. The resulting 7% RMCD solution was centrifuged, and the clear supernatant was used for the ORAC assay. Low-polarity compounds and the compounds hard to redissolve were lost in the solid precipitates. This may result in the reduction in ORAC values. Furthermore, these data also indicate the potential effects of solvent used in antioxidant extraction on results of antioxidant activity tests. Several solvent systems have been used to extract wheat antioxidants for evaluations of different antioxidative activities. These included ethanol (*3*, *7*, *8*), pure water (*6*, *20*), acetone/water (4:1, v/v), ethanol/water (4:1, v/v), methanol/water (4:1, v/v) (*6*), 95% ethanol (*5*), and 50% acetone water in this study. Recently, methanol and 1.0 N HCl (85:15, v/v) were also used to extract total anthocyanin from blue-grained wheat (*9*). It is important when the results obtained from different laboratories are compared to examine and compare the extraction efficacy of each commonly used solvent system. Ongoing study is being conducted in our laboratory to evaluate a group of solvent systems for their efficacies in wheat antioxidant extraction.

In agreement with the previous observation (*5*, *10*), ferulic acid was the predominant phenolic acid detected in Swiss red wheat antioxidant extracts and accounted for 57−78% of the total phenolic acids on a per weight basis. The grain of Swiss red wheat contained 33.71 μg of extractable ferulic acid/g of seeds, which is >5 ppm (μg/g) of free and soluble bound ferulic acid (*21*) but is much lower than the reported typical level of 500 μg of ferulic acid/g of ground whole wheat (*10*, *21*). The extractable phenolic acid is a portion of, and may account for <10% of, the total phenolic acid presented in wheat (*21*). Ferulic acid has been evaluated for its potential application as an analytical parameter in the rapid determination of bran carryover in flour during milling (*22*). Antioxidant properties of ferulic acid have been evaluated and reviewed by Graf (*10*). In this study, ferulic acid content was well correlated with antioxidant activities, total phenolic content, and concentrations of other identified individual phenolic acids. Therefore, ferulic acid may serve as a marker for quality control of wheat antioxidants or may be used to monitor wheat antioxidant processing.

In conclusion, this study indicates that antioxidants including phenolic acids are concentrated in the aleurone fraction of wheat bran. Further micronization may increase the antioxidant activities, total extractable phenolic content, and extractable phenolic

acid of wheat aleurone. Fifty percent acetone may be a better solvent system than ethanol for monitoring antioxidants in wheat and wheat-based food ingredients. Ferulic acid is the predominant phenolic acid in Swiss red wheat and accounts for 57−78% of the total phenolic acids on a weight basis. The ferulic acid concentration of all Swiss red wheat samples tested in this study was well correlated with $ABTS^{\bullet+}$ and $O_2^{\bullet-}$ quenching abilities, total phenolic content, and other phenolic acid concentrations, suggesting that ferulic acid may be a potential marker for quality control of wheat antioxidants.

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