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Phytochemicals and Antioxidant Properties in Wheat Bran

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Bran samples of seven wheat varieties from four different countries were examined and compared for their phytochemical compositions and antioxidant activities. Phenolic acid composition, tocopherol content, carotenoid profile, and total phenolic content were examined for the phytochemical composition of wheat bran, whereas the measured antioxidant activities were free radical scavenging properties against 2,2-diphenyl-1-picrylhydrazyl radical, radical cation 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, peroxide radical anion $O_2^{\bullet -}$, and oxygen radical and chelating capacities. The results showed that the tested wheat bran samples differed in their phytochemical compositions and antioxidant properties. Ferulic acid, with a concentration range of 99−231 μg/g, was the predominant phenolic acid in all of the tested bran samples and accounted for about 46−67% of total phenolic acids on a weight basis. The concentrations for α-, δ-, and γ- tocopherols were 1.28−21.29, 0.23−7.0, and 0.92−6.90 μg/g, respectively. In addition, lutein and cryptoxanthin were detected in all of the tested bran samples with levels of 0.50−1.80 and 0.18−0.64 μg/g, respectively. Zeaxanthin was detected in the six bran samples, and the greatest zeaxanthin concentration of 2.19 μg/g was observed in the Australian general purpose wheat bran. β-Carotene was detected in four of the tested bran samples at a range of 0.09−0.40 μg/g. These data suggest that wheat and wheat bran from different countries may differ in their potentials for serving as dietary sources of natural antioxidants.

KEYWORDS: Antioxidant; wheat; bran; phenolic; phenolic acid; tocopherol; carotenoid; chelating; radical

INTRODUCTION

It has been postulated that antioxidants may modulate cellular oxidative status and prevent biologically important molecules such as DNA, proteins, and membrane lipids from oxidative damage and consequently reduce the risk of several chronic diseases including cancer and cardiovascular disease (*1*−*3*). Increasing evidence indicates that wheat and wheat-based food products contain significant levels of natural antioxidants, which may provide health benefits to consumers in addition to general nutrients and energy (*3*−*12*). Wheat is an important agricultural commodity and dietary component across the world. Previous studies showed that wheat varieties vary in their antioxidant properties, total phenolic contents (TPCs), phenolic acid compositions, and carotenoid profiles (*3*−*5*, *13*). It was also noted that growing conditions and the interaction between environmental factors and genotype altered the antioxidant properties and phytochemical compositions of wheat grain and bran (*6*, *13*). A recent study of Swiss red wheat grain and fractions (*10*) showed that phenolic antioxidants are concentrated in the aleurone fraction of wheat bran, and further micronization increased the availability of antioxidants in the aleurone samples. Several phenolic acids have been detected in wheat and wheat fractions. Ferulic acid was the predominate phenolic acid in Trego wheat bran produced in Colorado and accounted for 59−60% of the total phenolic acids on a per weight basis, along with significant levels of syringic, *p*-hydroxybenzoic, vanillic, and coumaric acids at a concentration range of 4−33 μg/g bran (*13*). Earlier in 1992, Onyeneho and Hettiarachchy (*4*) 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. Later in 2003, Adom and others (*8*) detected ferulic acid in grain samples of 11 wheat varieties and experimental lines.

In addition to the phenolic acids, carotenoids including lutein, zeaxanthin, and β-cryptoxanthin were detected in grain samples of wheat varieties and experimental lines at concentration ranges of 25−145, 8.5−27, and 1−13.5 μg/100 g grain, respectively (*8*, *14*, *15*). Also noted was the presence of β-carotene in wheat (*15*). Carotenoids are considered as a group of radical scavengers through two proposed mechanisms (*16*). Tocopherols are another group of well-recognized natural antioxidants with potential health benefits. To date, little research has been performed to investigate the carotenoid or tocopherol profiles in wheat bran.

The previous studies of wheat antioxidants generally involved locally produced wheat varieties using different assays or testing conditions. This made it very hard to compare the data from different studies. No study has been conducted to compare wheat varieties grown in different countries under the same analytical

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conditions. Therefore, the present study aimed to determine the profiles of tocopherols, carotenoids, and phenolic acids in the bran fractions of the selected seven wheat varieties from four different countries. The present research also aimed to examine the antioxidant properties and TPCs of the bran samples. In addition, the potential correlations among individual antioxidant properties and antioxidant components were determined. This research is part of our continuous efforts to promote the improved production and utilization of value-added wheat for disease prevention and health promotion.

MATERIALS AND METHODS

Materials. One bran sample of Swiss red, Canadian hard white, Canadian durum, Illinois soft red, and Australian general purpose wheat was provided by the Buhler AG (Uzwil, Switzerland), whereas bran samples of wheat varieties Akron and Avalanche, representing hard winter red and white wheat, were obtained from Dr. Scott Haley in the Department of Soil and Crop Sciences, Colorado State University (Fort Collins, CO). Disodium ethylenediaminetetraacetate (EDTA), 2,2′-bipyridyl, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), fluorescein (FL), lauryl sulfate sodium salt, hypoxanthine (HPX), xanthine oxidase (XOD), nitro blue tetrazolium (NBT), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), tocopherols (α-, δ-, and γ-), and β-carotene were purchased from Sigma-Aldrich (St. Louis, MO), while 2,2′-azobis(2-amino-propane) dihydrochloride (AAPH) was obtained from Wako Chemicals U.S.A. (Richmond, VA). β-Cyclodextrin was purchased from Cyclolab R & D Ltd. (Budapest, Hungary). Lutein, zeaxanthin, and β-cryptoxanthin were purchased from Indofine Chemical Co. Inc. (Hillsborough, NJ). All other chemicals and solvents were of the highest commercial grade and used without further purification.

Extraction and Testing Sample Preparation. Four grams of each wheat bran sample was ground to fine powder (100 mesh) using a micro-mill manufactured by Bel Art Products (Pequannock, NJ) and extracted for 15 h with 40 mL of 50% acetone under nitrogen at ambient temperature. The 50% acetone extracts were kept in the dark under nitrogen at room temperature until further evaluation of TPCs and antioxidant properties except the DPPH• scavenging capacity, because the 50% acetone extracts may form precipitates during the assay. The 50% acetone extracts were also subjected to additional treatment for phenolic acid analyses.

Superoxide Anion Radical $O_2^{\bullet-}$ Scavenging Activity. The $O_2^{\bullet-}$ scavenging activity was determined using HPX/XOD system following a procedure described previously (*10*). NBT solution (0.34 mM), HPX (2 mM), and XOD solution (0.56 unit/mL) were prepared in a sodium phosphate buffer (0.05 M; pH 7.4). The reaction mixture contained 0.2 mL of 0.34 mM NBT solution, 0.7 mL of 2 mM HPX solution, 0.1 mL of wheat bran antioxidant in 50% acetone, and 0.2 mL of 0.56 units/mL XOD solution. The concentration of wheat bran antioxidant in test solution was 8.3 mg bran equivalent/mL.

The decrease in absorbance was measured at 560 nm every minute for a 7 min period, and the % $O_2^{\bullet-}$ remaining was calculated to evaluate the $O_2^{\bullet-}$ scavenging capacity of each bran extract according to the following equation:

$$\%\ O_2^{\bullet-}\ \text{remaining} = \text{slope of Abs}_{\text{sample}}/\text{slope of Abs}_{\text{control}} \times 100$$

The slope of $\text{Abs}_{\text{sample}}$ was obtained by plotting the $A_{560\ \text{nm}}$ of the bran antioxidant−radical reaction against the corresponding reaction time, while the slope of $\text{Abs}_{\text{control}}$ was determined by plotting the $A_{560\ \text{nm}}$ of the control radical reaction containing no antioxidant against the reaction time.

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 (*10*, *17*). Fifty microliters of bran extracts was diluted with 450 μL of 50% acetone to obtain the testing samples. $ABTS^{\bullet+}$ was prepared by oxidizing 5 mM aqueous solution of ABTS′ 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 50% acetone solution for the control. The absorbance at 734 nm was measured at 1 min of the reaction, and the Trolox equivalent (TE) was calculated using a standard curve prepared with Trolox.

Oxygen Radical Absorbance Capacity (ORAC) Assay. The ORAC assay was conducted using FL as the fluorescent probe using a Turner Quantech digital fluorometer (Barnstead International, Dubuque, IA) according to a protocol described by Huang and others (*10*, *18*). The final assay mixture contained 0.067 μM FL, 60 mM AAPH, and 300 μL of wheat bran antioxidants or 50% acetone for a reagent blank. The fluorescence of an assay mixture was determined at ambient temperature and recorded every min for the duration of about 2 h. The excitation wavelength was 490 nm, whereas the emission filter has a wavelength of 515 nm. The TE was calculated using a standard curve prepared with Trolox and used to compare ORAC of wheat bran samples.

Radical DPPH Scavenging Activity. Five grams of each ground wheat bran sample was extracted for 2 h with absolute ethanol using a Soxhlet extractor, and the final volume of the extract was brought to 200 mL. The radical DPPH scavenging capacities of wheat bran extracts were determined according to the previously reported procedure using the stable DPPH• (*3*). The initial concentration was 100 μM for DPPH• in all antioxidant−radical reactions. 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 kinetics of antioxidant−radical reactions was compared for wheat bran antioxidants at a final concentration of 12.5 mg bran equivalent per mL. To determine the ED_{50} value of the wheat antioxidants against DPPH radicals, seven levels of each bran extract were employed. The $A_{517\ \text{nm}}$ value at 80 min of reaction was used to establish the ED_{50} value under the experimental conditions. The ED_{50} value is the concentration of an antioxidant to quench 50% radicals in the reaction mixture under the assay condition.

Chelating Activity. The 2,2′-bipyridyl competition assay was conducted to measure the Fe^{2+} chelating activity of bran extracts (*11*). The reaction mixture contained 0.1 mL of 1 mM $FeSO_4$ solution, 50 μL of wheat bran extract in 50% acetone, 0.3 mL of 10% hydroxylamine−HCl, 0.4 mL of 2,2′-bipyridyl solution (0.1% in 0.2 M HCl), and 0.8 mL of Tris-HCl buffer (pH 7.4). The absorbance at 522 nm was measured and used to determine the Fe^{2+} chelating activity with EDTA as a standard.

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

Phenolic Acid Composition. The 50% acetone extract of each wheat bran was evaluated for the phenolic acid profile. After acetone was removed, the wheat bran antioxidants were hydrolyzed with 4 N NaOH, acidified using 6 N HCl, and extracted with ethyl ether−ethyl acetate (1:1, v/v) according to the procedure described previously (*10*, *13*). The ethyl ether−ethyl acetate was evaporated at 30 °C using a nitrogen evaporator, and the solid residue was redissolved in methanol, filtered through a 0.20 μm membrane filter, and kept in dark under nitrogen until high-performance liquid chromatography (HPLC) analysis. The phenolic acid composition in the methanol solution was analyzed by HPLC using a Phenomenex C18 column (250 mm × 4.6 mm) according

to an established protocol (*10*, *13*). The 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 (*10*, *13*). Identification of phenolic acids was accomplished by comparing the retention time of peaks in wheat bran samples to that of the standard compounds. Quantification of an individual phenolic acid was conducted using the total area under each peak with external standards.

Carotenoid Composition. Carotenoids were extracted and analyzed using HPLC-diode array detection-electrospray ionization (ESI)-tandem mass spectrometry method (*14*, *15*). Briefly, 200 mg of the ground wheat bran sample was extracted with 10 mL of methanol/tetrahydrofuran (1:1, v/v) at ambient temperature for 15 h and then sonicated for another 10 min. The resulting extraction mixture was subjected to centrifugation at 600 rpm for 5 min at ambient temperature. After centrifugation, the supernatant was filtered through a 0.20 μm membrane filter and kept in the dark under nitrogen until HPLC analysis of carotenoids, as well as for tocopherol analysis. HPLC analysis was performed using a TSQ Quantum tandem mass spectrometer (Thermo-Finnigan, San Jose, CA) equipped with an ESI interface and Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA). HPLC separation was accomplished according to a previously described protocol with modifications (*15*). The HPLC was performed using a Zorbax SB C18 column (Agilent Technologies), 1.0 mm i.d. × 50 mm, 3.5 μm particle size, at room temperature. The carotenoids were eluted using a mobile phase of water as solvent A and methanol:acetonitrile: 2-propanol (54:44:2; v/v/v) as solvent B. The gradient procedure was as follows: (i) the gradient was linear from 50 to 99% of solvent B, and the flow rate was increased from 0.20 to 0.27 mL/min in the first 10 min; and (ii) 99% of solvent B and flow rate of 0.27 mL/min for 10 min. The HPLC column was reequilibrated for another 10 min with 50% solvent B, prior to injection of the next sample. The wavelength of UV detection was set at 440 nm. The TSQ Quantum was operated in the positive ion mode under the following conditions: nitrogen (>99.7%) was used for sheath gas and auxiliary gas at a pressure of 30 psi and 5 units, respectively. The temperature of the heated capillary was maintained at 300 °C, and the spray voltage of ESI was set at 4.5 kV. A collision-induced dissociation was achieved using argon as the collision gas at the pressure adjusted to more than 1.0 mTorr above the normal, and the applied collision offset energy was set to −45 eV. Identification of carotenoids was accomplished by comparing the HPLC retention time and selected reactant monitoring (SRM) analysis of the sample peaks with that of the authorized pure commercial carotenoid compounds. The *m*/*z* from 568.6 (molecular ion) to 157.3 (major fragment) was set for lutein and zeaxanthin, and *m*/*z* 552.6 → 145.3, 536.6 → 119.3 were set for cryptoxanthin and β-carotene, respectively. Data were acquired with Xcalibur software system (Thermo-Finnigan). The quantification for each carotenoid compound was conducted using the total ion counts with an external standard.

Tocopherol Composition. The methanol/tetrahydrofuran extracts of the bran samples prepared for carotenoid analysis were used to evaluate the α-, δ-, and γ-tocopherol concentrations in wheat bran samples. HPLC separation was performed using a Zorbax SB C18 column (Agilent Technologies), 1.0 mm i.d. × 30 mm, 3.5 μm particle size, at room temperature. The tocopherols were eluted using a mobile phase of water as solvent A and acetonitrile as solvent B. The gradient procedure was as follows: (i) the gradient was linear from 80 to 99% of solvent B, and the flow rate was 0.3 mL; and (ii) 99% of solvent B was kept for 10 min. The HPLC column was reequilibrated for another 10 min with 50% of solvent B, prior to the injection of the next sample. The identification of tocopherols was conducted by comparing the HPLC retention time and SRM analysis of the sample peaks with that of the authorized pure commercial tocopherol compounds. The *m*/*z* from 430.6 (molecular ion) to 165.3 (major fragment) was set for α-tocopherol, and *m*/*z* 416.6 → 151.3 and 402.6 → 137.3 were set for γ-tocopherol and δ-tocopherol, respectively. The quantification for each tocopherol was accomplished using the total ion counts with external standards.

Statistical Analysis. Data were reported as means ± standard deviations (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, while a Pearson correlation test was conducted to determine the correlations among means (*6*, *11*). Statistical significance was declared at $P < 0.05$.

Table 1. Antioxidant Properties of Wheat Bran[a]

wheat bran	% $O_2^{\bullet-}$ remaining	$ABTS^{\bullet+}$ (TE μmol/g bran)	ORAC (TE μmol/g bran)
Swiss red	59.19b ± 0.13	19.74d ± 0.17	107.53d ± 4.1
Canadian hard white	67.07d ± 0.08	18.59c ± 0.68	124.29e ± 6.70
Canadian durum	57.40a ± 0.15	18.46b,c ± 0.48	94.89c ± 10.90
U.S. soft red	60.19c ± 0.14	17.78a,b ± 0.53	89.56c ± 4.54
Australian general purpose	67.21d ± 0.15	17.45a ± 0.24	62.32b ± 8.94
U.S. Akron	59.33b ± 0.26	18.99c,d ± 0.29	72.55b ± 2.05
U.S. Avalanche	59.22b ± 0.08	18.85c ± 0.50	45.02a ± 8.32

[a] Free radical scavenging activities of the 50% acetone extracts were evaluated against radical anion $O_2^{\bullet-}$ and cation $ABTS^{\bullet+}$ and expressed as means ($n = 3$) ± SD. The final concentration of wheat bran antioxidant was 8.3 mg/mL in test solution for radical anion $O_2^{\bullet-}$ scavenging capacity determination. Within each column, means with the same letter are not significantly different ($P < 0.05$).

RESULTS

Superoxide Anion Radical ($O_2^{\bullet-}$) Scavenging Activity. The $O_2^{\bullet-}$ scavenging activity of wheat bran samples was evaluated using the HPX/XOD system and expressed as % $O_2^{\bullet-}$ remaining. Significant $O_2^{\bullet-}$ scavenging activity was detected in all tested bran samples, while the bran of Canadian durum wheat had the greatest capacity to react with and quench $O_2^{\bullet-}$ on a per weight basis, under the experimental conditions (**Table 1**). The bran extract of Canadian durum wheat quenched about 10% more anion radicals in the radical−antioxidant reaction mixture than that scavenged by Australian general purpose wheat bran. Bran extracts of different wheat samples might significantly differ in their $O_2^{\bullet-}$ scavenging activities (**Table 1**). The correlation was detected between $O_2^{\bullet-}$ scavenging activity and TPC ($r = 0.83$ and $P = 0.02$).

Radical Cation Scavenging Activity. The $ABTS^{\bullet+}$scavenging capacity of the bran extracts ranged from 17.5 to 19.7 μmol TE per gram of bran (**Table 1**). Wheat bran samples might significantly differ in their radical cation scavenging activities. The greatest $ABTS^{\bullet+}$ scavenging capacity was detected in the Swiss red wheat bran, whereas the bran of Australian general purpose wheat was least effective to directly react with and quench $ABTS^{\bullet+}$ in the reaction mixture, under the experimental condition. The $ABTS^{\bullet+}$ scavenging capacity was not correlated to any other tested antioxidant activities or phytochemical levels.

ORAC Assay. The ORAC values were determined using the 50% acetone extracts of bran samples and expressed as μmol of TE per gram of bran. All bran extracts exhibited significant ORAC values (**Table 1**). The greatest ORAC value of 124 μmol TE/g was observed in the bran extract of the Canadian hard white wheat and followed by that of the Swiss red wheat. The lowest ORAC value of 45 μmol TE/g, which was about 36% of that in the Canadian hard white wheat bran, was detected in the bran extract of Avalanche wheat collected from Colorado in the United States. No correlation between ORAC value and any of the tested antioxidant activity or phytochemical concentration was observed.

Radical DPPH Scavenging Activity. The ethanol extracts of wheat bran samples were analyzed and compared for their ED_{50} values against $DPPH^{\bullet}$ (**Figure 1**). ED_{50} is the required concentration of wheat bran antioxidants to scavenge 50% DPPH radicals in the reaction mixtures under the experimental

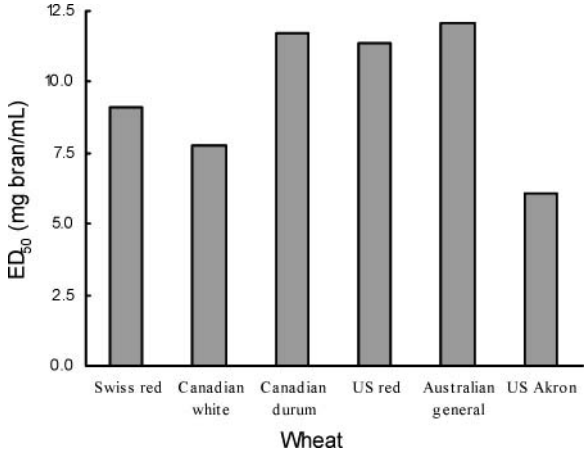

Figure 1. ED_{50} of wheat bran extracts against DPPH radicals: Swiss red, Canadian white, Canadian durum, U.S. red, Australian general, U.S. Akron, and U.S. Avalanche stand for Swiss red wheat bran, Canadian hard white wheat bran, Canadian durum wheat bran, soft red wheat bran from Illinois, bran of Australia general purpose wheat, Akron red wheat bran from Walsh (Colorado), and Avalanche wheat bran from Burlington (Colorado), respectively. All tests were conducted using the ethanol extracts. The initial DPPH• concentration was 100 μM in all reaction mixtures. ED_{50} is the concentration of wheat bran extracts to quench 50% of DPPH radicals in the reaction mixture within 80 min under the experimental conditions.

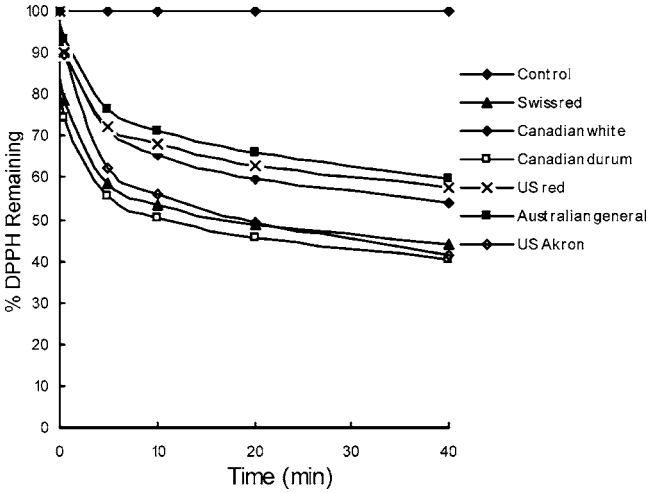

Figure 2. Radical DPPH scavenging activities of wheat bran extracts: Swiss red, Canadian white, Canadian durum, U.S. red, Australian general, and U.S. Avalanche stand for Swiss red wheat bran, Canadian hard white wheat bran, Canadian durum wheat bran, soft red wheat bran from Illinois, bran of Australia general purpose wheat, and Avalanche wheat bran from Burlington (Colorado), respectively. All tests were conducted using the ethanol extracts. The initial DPPH• radical concentration was 100 μM in all reaction mixtures, while the final concentration of wheat extracts was 12.5 mg wheat equivalent per mL.

conditions, with the ED_{50} value negatively associated the DPPH• scavenging activity. The ED_{50} values ranged from 6.1 mg bran equivalent per mL for Akron wheat bran to 12.1 mg bran equivalent per mL for Australian general purpose wheat bran, indicating that individual wheat bran samples may significantly differ in their DPPH radical scavenging capacities. The kinetics of each wheat antioxidant−DPPH radical reaction was determined and reported in **Figure 2**. The bran extract of Canadian durum wheat had the greatest initial rate in reacting with DPPH• and quenched the largest quantity of radicals in the system when the antioxidant−DPPH• reaction reached equilibrium. Interestingly, the bran extract of Swiss red wheat had the greater initial reaction rate than that of Akron wheat, but the bran extract of Akron wheat quenched more radicals in the testing system when the radical−antioxidant reaction reached equilibrium (**Figure 2**). These data indicated that the bran extract from Swiss red wheat is a kinetically more active scavenger against DPPH• than that from Akron wheat, whereas the bran extract of Akron wheat is a thermodynamically stronger radical inhibitor under the testing conditions. The DPPH• scavenging capacity was positively correlated with TPCs under the experimental condition ($r = 0.91$, $P = 0.01$).

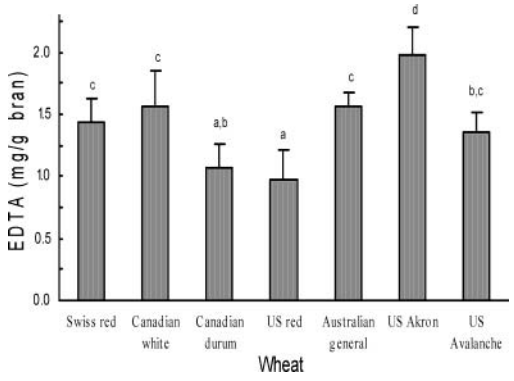

Figure 3. Chelating capacity of wheat bran samples: Swiss red, Canadian white, Canadian durum, U.S. red, Australian general, U.S. Akron, and U.S. Avalanche stand for Swiss red wheat bran, Canadian hard white wheat bran, Canadian durum wheat bran, soft red wheat bran from Illinois, bran of Australia general purpose wheat, Akron red wheat bran from Walsh (Colorado), and Avalanche wheat bran from Burlington (Colorado), respectively. The vertical bars represent the SD ($n = 3$), and values marked by the same letter are not significantly different ($P < 0.05$).

Chelating Activity. The chelating properties of the 50% acetone extracts of wheat bran samples were expressed as EDTA equivalents per gram of bran (**Figure 3**). The Fe^{2+} chelating activity of the bran samples ranged from 1 to 1.9 mg EDTA equivalent per gram of bran under the experimental conditions. A significant difference in chelating activity was observed among some of the wheat bran samples (**Figure 3**). The bran extract of Akron wheat from Colorado in the United States showed the strongest chelating capacity, whereas the bran extracts of soft red wheat from Illinois had the lowest chelating activity. The chelating activity was not correlated with any tested antioxidant activity or antioxidant concentration under the experimental conditions.

TPC. The wheat bran samples were examined and compared for their TPCs expressed as gallic acid equivalents (GE). The seven bran samples might differ from each other in their TPCs (**Figure 4**). The greatest TPC of 2.9 mg GE/g bran was detected in the Avalanche wheat from Colorado, while bran of the general purpose wheat from Australia had the lowest TPC value of 2.2 mg GE/g bran. The TPC was correlated with the $O_2^{\bullet -}$ scavenging capacity of the 50% acetone extracts ($r = 0.83$, $P = 0.02$) and the DPPH scavenging capacity of the ethanol extracts ($r = 0.91$, $P = 0.01$).

Phenolic Acid Composition. Ferulic, syringic, *p*-OH benzoic, vanillic, and coumaric acids were detected in all seven tested wheat bran samples (**Table 2**). Ferulic acid was the predominant phenolic acid in all tested bran samples and accounted for about 46.1−67.2% of the total identified phenolic acids on a per weight basis. The bran of Akron wheat had the greatest concentration of total phenolic acids (359 μg/g) and ferulic acid (230.5 μg/g). Bran samples of Swiss red and U.S. soft red wheat also had total phenolic acids above 300 μg/g with ferulic acid

Table 2. Phenolic Acid Composition of Wheat Bran[a]

	p-OH benzoic (μg/g bran)	vanillic (μg/g bran)	syringic (μg/g bran)	coumaric (μg/g bran)	ferulic (μg/g bran)
Swiss red	19.65d ± 0.35	16.55b ± 0.27	57.15c ± 0.47	9.01e ± 0.02	209.3e ± 0.33
Canadian white	29.50f ± 0.03	19.31e ± 0.38	60.62d ± 0.37	8.00d ± 0.09	100.5b ± 0.54
Canadian durum	20.79e ± 0.34	26.45g ± 0.13	29.27a ± 0.36	16.21g ± 0.08	146.9c ± 0.11
U.S. red	18.01c ± 0.33	15.01a ± 0.32	60.38d ± 0.77	10.22f ± 0.07	207.9e ± 1.19
Australian general	19.85d,e ± 0.52	17.48d ± 0.08	74.63e ± 0.16	5.49c ± 0.08	163.1d ± 0.77
U.S. Akron	14.00b ± 1.24	25.52f ± 0.40	85.01f ± 0.44	4.38b ± 0.36	230.5f ± 1.38
U.S. Avalanche	10.53a ± 0.10	17.11c ± 0.01	30.79b ± 0.11	3.70a ± 0.16	98.54a ± 0.39

[a] Swiss red, Canadian white, Canadian durum, U.S. red, Australian general, U.S. Akron, and U.S. Avalanche stand for Swiss red wheat bran, Canadian hard white wheat bran, Canadian durum wheat bran, soft red wheat bran from Illinois, general purpose wheat bran from Australia, Akron wheat bran from Walsh (Colorado), and Avalanche wheat bran from Burlington (Colorado), respectively. *p*-OH benzoic, vanillic, syringic, coumaric, and ferulic stand for *p*-OH benzoic, vanillic, syringic, coumaric, and ferulic acids, respectively. Within each column, means with the same letter are not significantly different ($P < 0.05$; $n = 3$).

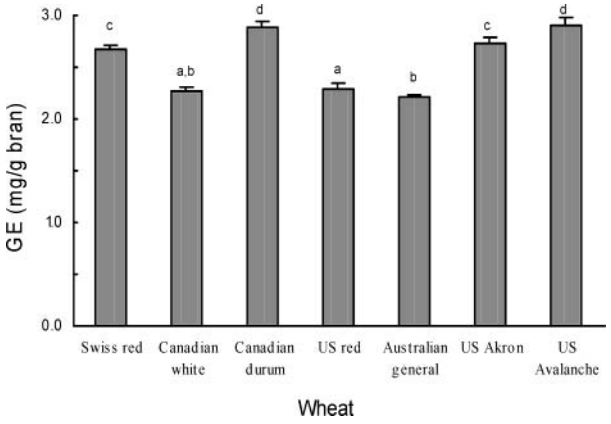

Figure 4. TPCs of wheat bran samples: Swiss red, Canadian white, Canadian durum, U.S. red, Australian general, U.S. Akron, and U.S. Avalanche stand for Swiss red wheat bran, Canadian hard white wheat bran, Canadian durum wheat bran, soft red wheat bran from Illinois, bran of Australia general purpose wheat, Akron red wheat bran from Walsh (Colorado), and Avalanche wheat bran from Burlington (Colorado), respectively. The vertical bars represent the SD ($n = 3$). Values marked by the same letter are not significantly different ($P < 0.05$).

Table 3. Tocopherol Profiles of Wheat Bran[a]

	α-tocopherol (μg/g)	δ-tocopherol (μg/g)	γ-tocopherol (μg/g)	total tocopherols (μmol/100 g)
Swiss red	6.55c ± 0.67	5.48c ± 0.22	6.77c ± 0.38	4.51c ± 0.28
Canadian white	7.86d ± 0.95	6.41d ± 0.42	2.87a,b ± 2.11	4.10c ± 0.74
Canadian durum	21.29f ± 1.74	2.03b ± 0.39	6.06c ± 0.46	6.90e ± 0.44
U.S. red	3.29b ± 0.10	5.96c,d ± 0.36	2.98b ± 0.12	2.96b ± 0.13
Australian general	2.29a,b ± 0.09	7.04e ± 0.39	2.23a ± 0.08	2.81b ± 0.09
U.S. Akron	14.17e ± 1.03	0.23a ± 0.04	8.30d ± 0.45	5.34d ± 0.14
U.S. Avalanche	1.28a ± 0.06	0.25a ± 0.05	2.32a ± 0.10	0.92a ± 0. 22

[a] Swiss red, Canadian white, Canadian durum, U.S. red, Australian general, U.S. Akron, and U.S. Avalanche stand for Swiss red wheat bran, Canadian hard white wheat bran, Canadian durum wheat bran, soft red wheat bran from Illinois, general purpose wheat bran from Australia, Akron wheat bran from Walsh (Colorado), and Avalanche wheat bran from Burlington (Colorado), respectively. Within each column, means with the same letter are not significantly different ($P < 0.05$; $n = 3$).

levels over 200 μg/g (**Table 2**). The ferulic acid concentration was not correlated with any other identified phenolic acids or antioxidant properties in the wheat bran extracts.

Tocopherol Profile. α-, δ- and γ-tocopherols were detected in all tested wheat bran samples (**Table 3**). Total tocopherols ranged 0.92−6.90 μmol per 100 g of bran. Canadian durum wheat bran contained the greatest level of total (6.90 μmol/100 g) and α-tocopherol (21.29 μg/g) among all tested wheat bran samples (**Table 3**). The highest δ-tocopherol level of 7.04 μg/g was detected in the bran of Australian general purpose wheat, and the greatest γ-tocopherol at a level of 8.30 μg/g was observed in the U.S. Akron wheat bran. Bran samples might significantly differ in their tocopherol compositions. Interestingly, α-tocopherol was the primary tocopherol in the bran samples of Canadian white, Canadian durum, and U.S. Akron wheat; δ-tocopherol was the major isomer in the bran samples of U.S. soft red and Australian general purpose wheat; and γ-tocopherol was the primary form for bran samples of Swiss red and U.S. Avalanche wheat, suggesting that bran samples differed in both the total tocopherol concentrations and the tocopherol profiles. The seven tested wheat bran samples exhibited 16.6-, 30.6-, and 3.7-fold differences in their α-, δ-, and γ-tocopherol contents, respectively. A significant correlation was detected between total and α-tocopherol contents ($r = 0.93$, $P = 0.002$). In addition, the δ-tocopherol concentration was correlated with the TPC ($r = 0.88$, $P = 0.01$), whereas the γ-tocopherol level was correlated with DPPH radical scavenging capacity ($r = 0.96$, $P = 0.02$).

Carotenoid Profile. The carotenoid composition including β-carotene, zeaxanthin, lutein, and cryptoxanthin was examined for all seven wheat bran samples (**Table 4**). Lutein and cryptoxanthin were detected in all tested bran samples. Zeaxanthin was detected in six of the seven tested bran samples under the experimental conditions, whereas β-carotene was only present in four of the tested wheat bran samples. Bran samples might significantly differ in their carotenoid profiles. The greatest total carotenoid level of 0.68 μmol/100 g bran was observed in bran of Canadian durum or Australian general purpose wheat. Avalanche wheat bran had the lowest level of total carotenoids among all tested bran samples. Interestingly, β-cryptoxanthin presented in all tested bran samples but was not the major carotenoid in any individual wheat bran sample (**Table 4**). The concentration of β-cryptoxanthin was 0.18−0.64 μg/g in the tested wheat bran samples under the experimental conditions. Zeaxanthin was the primary carotenoid in three of the tested wheat bran samples, and lutein was the major carotenoid for the other four wheat bran samples including Swiss red, Canadian durum, Akron, and Avalanche wheat. The greatest zeaxanthin level of 2.19 μg/g was detected in Australian general purpose wheat bran, while the highest lutein content of 1.80 μg/g was observed in Akron wheat bran. The total carotenoid content among the seven bran samples exhibited a 5.7-fold difference, while lutein and β-cryptoxanthin concentrations had 3.6- and 3.5-fold differences, respectively.

Antioxidant Properties of Bran Extracts on a Per Unit of TPC Basis. The chelating activity, radical cation $ABTS^{\bullet+}$

Table 4. Carotenoids Profile of Wheat Bran[a]

	β-carotene (μg/g)	zeaxanthin (μg/g)	lutein (μg/g)	cryptoxanthin (μg/g)	total carotenoids (μmol/100 g)
Swiss red	ND	0.48b ± 0.02	0.71b ± 0.01	0.35b ± 0.02	0.27b ± 0.01
Canadian white	0.09a ± 0.08	1.99d ± 0.06	0.77b ± 0.02	0.42b ± 0.02	0.59d ± 0.03
Canadian durum	0.40c ± 0.01	1.23b ± 0.03	1.58c ± 0.03	0.59d ± 0.01	0.68e ± 0.02
U.S. red	ND	1.34c ± 0.02	0.74b ± 0.02	0.50c ± 0.02	0.46c ± 0.01
Australian general	0.18b ± 0.01	2.19d ± 0.03	0.79b ± 0.03	0.64d ± 0.02	0.68e ± 0.01
U.S. Akron	0.11a ± 0.02	0.25a ± 0.01	1.80d ± 0.03	0.44b ± 0.02	0.46c ± 0.02
U.S. Avalanche	ND	ND	0.50a ± 0.02	0.18a ± 0.01	0.12a ± 0.02

[a] Swiss red, Canadian white, Canadian durum, U.S. red, Australian general, U.S. Akron, and U.S. Avalanche stand for Swiss red wheat bran, Canadian hard white wheat bran, Canadian durum wheat bran, soft red wheat bran from Illinois, general purpose wheat bran from Australia, Akron wheat bran from Walsh (Colorado), and Avalanche wheat bran from Burlington (Colorado), respectively. Within each column, means with the same letter are not significantly different ($P < 0.05$; $n = 3$); ND, not detected.

Table 5. Antioxidant Properties of Bran Extracts on a Per Unit of TPC Basis[a]

wheat bran	EDTA Eq/mg of TPC (μg)	TE/mg of TPC (μmol)	ORAC/mg of TPC (μmol TE)
Swiss red	0.54b ± 0.07	7.39c ± 0.06	40.27d ± 1.54
Canadian hard white	0.69c ± 0.12	8.19e ± 0.30	54.74e ± 2.95
Canadian durum	0.37a ± 0.07	6.39a ± 0.17	32.83c ± 3.77
U.S. soft red	0.43a,b ± 0.10	7.74d ± 0.23	38.98d ± 1.97
Australian general purpose	0.71c ± 0.05	7.9d,e ± 0.11	28.22b,c ± 4.05
U.S. Akron	0.72c ± 0.08	6.94b ± 0.11	26.51b ± 0.75
U.S. Avalanche	0.47a,b ± 0.05	6.49a ± 0.17	15.5a ± 2.86

[a] EDTA Eq stands for EDTA equivalent, a measurement of the chelating capacity. TE/mg of TPC is the ABTS scavenging capacity on per unit of TPC basis. ORAC represents the oxygen radical absorbing capacity. Within each column, means with the same letter are not significantly different ($P < 0.05$; $n = 3$).

scavenging property, and ORAC, on a per milligram of TPC basis, were calculated and expressed as EDTA eq/mg of TPC, TE/mg of TPC, and ORAC/mg of TPC (**Table 5**; see table footnote for details). The bran of Canadian white wheat had the greatest $ABTS^{\bullet+}$ scavenging property, the strongest chelating activity against Fe^{2+}, and the highest ORAC value on a per unit of TPC basis. The same hierarchy of TE/mg of TPC and ORAC/mg of TPC was observed in the seven wheat bran samples from four different countries, although no correlation was detected ($r = 0.70$, $P = 0.08$).

DISCUSSION

In the present study, bran samples of seven wheat varieties produced in four different countries were evaluated and compared for their carotenoid contents including β-carotene, zeaxanthin, lutein, cryptoxanthin, and total carotenoid concentrations. Among the seven tested wheat bran samples, lutein ranged from 0.5 to 1.8 μg per gram of bran, which equals to 50−180 μg/100 g. This range is comparable to that of about 25−145 μg per 100 g of grain detected in the whole grains of the 11 wheat varieties (*8*). These data indicate that a significant level of lutein is presented in the bran fraction of wheat, but lutein may not be concentrated in the bran. This conclusion supported the previous observations that all inner layers of the durum wheat kernel and the bran had a significant amount of carotenoids measured as lutein and zeaxanthin, and bran might contain less carotenoids than the inner fractions (*15*, *19*). In the present study, we detected significant amounts of zeaxanthin (123 μg/100 g) in the Canadian durum wheat bran, along with lutein at a concentration of 158 μg/100 g. This makes a total of 281 μg/100 g for lutein and zeaxanthin. This total level is greater than that of 125−220 μg/100 g detected by Hentschel and others (*15*) in the bran samples of eight durum wheat varieties obtained from Germany and France. However, the present study detected similar amounts of lutein and zeaxanthin in the Canadian durum wheat bran, whereas Hentschel and others (*15*) observed only trace levels of zeaxanthin in the grain and bran samples of the eight durum wheat varieties. Furthermore, we determined in this study that lutein was the primary carotenoid in four wheat bran samples, and zeaxanthin was the major carotenoid in the other three wheat bran samples. This was in contrast to the observation that lutein was the predominant carotenoid in wheat grain of the 11 wheat varieties (*8*) and in the eight durum wheat varieties (*15*). This may be partially due to the fractions of wheat used in the studies and the difference in wheat variety and growing environmental conditions. This result also suggests that the total of lutein and zeaxanthin may be a preferred measurement of carotenoid contents in wheat and wheat bran. Interestingly, lutein and zeaxanthin are positional isomers, which differ from each other only in the position of one double bond. According to the chemical structures, zeaxanthin has a longer conjugated system than lutein and is more stable than lutein. Therefore, lutein may be isomerized to zeaxanthin under certain conditions such as storage at ambient temperature or heat generated during the milling process. It has been demonstrated that the lutein content rapidly decreased during seed aging or storage (*14*), but the authors did not simultaneously measure the zeaxanthin contents. In 1997, Mortensen and Skibsted (*16*) showed that both zeaxanthin and lutein could react with phenoxyl radicals, and zeaxanthin is a slightly kinetic preferred reagent and has a greater relative first-order rate constant. These data and information suggest the possibility that lutein may be converted to zeaxanthin during bran preparation and storage, and this conversion may increase the antioxidant activity of bran carotenoid. Future research is required to test this hypothesis.

Cereal grains are important dietary sources of tocopherols (*20*−*22*). Among tocopherol isomers, α-tocopherol exhibits the strongest vitamin E activity and has the greatest reactivity against singlet oxygen (*20*, *22*). The δ-tocopherol has the strongest antioxidative potency among all tocopherol isomers, followed by the γ-, β-, and α-isomers, respectively. In the present study, significant levels of α-, δ-, and γ-tocopherols were detected in the seven wheat bran samples. The bran samples differed in their α-, δ-, γ-, and total tocopherols, suggesting the potential influence of wheat variety and growing condition on tocopherol production in wheat bran and grain. The α-tocopherol level ranged from 1.28 to 21.29 μg/g in the seven bran samples tested in the present study, which is comparable to the level of about 16 μg/g in the wheat bran sample from Finland (*21*), 8.2 μg/g detected in wheat flour (*22*), and the level of 9.9 μg/g observed in freshly milled whole meal wheat flour (*20*), and 9.5−10.4 μg/g in wheat meal (*21*). This

range is much lower than that of 200−240 μg/g in wheat germ (*21*) or the levels of 90, 180, and 150 μg/g in the olive, soybean, and peanut oils (*22*). The α-tocopherol detected in this study is the (*R*,*R*,*R*)-α-tocopherol, which is the natural form of α-tocopherol and is preferentially retained and distributed throughout the body, although all tocopherols are absorbed equally after ingestion (*23*). The most recent U.S. recommended daily allowance suggests that healthy adults need 11−15 mg of (*R*,*R*,*R*)-α-tocopherol to meet the vitamin E requirement (*24*). Wheat bran and whole grain may significantly contribute to the daily dietary intake of vitamin E to meet life requirements, prevent deficiency symptoms in normal humans, and prevent several chronic diseases.

In agreement with the previous observation (*4*, *10*, *13*, *25*), ferulic acid was the predominant phenolic acid detected in the bran extracts of the seven wheat samples and accounted for 46−70% of the total phenolic acids on a per weight basis. Ferulic acid ranged from 99 to 231 μg per gram of bran among the seven bran samples. This range is comparable to that of 91−111 μg/g detected in the bran extracts of Trego wheat grown at different locations (*13*). Ferulic acid has been evaluated for its antioxidant properties (*25*) and potential application as an analytical parameter in rapid determination of bran carryover in flour during milling (*26*). In addition, the bran samples of the seven selected wheat varieties differed significantly in their total carotenoid contents, TPCs, phenolic acid compositions, and antioxidant properties, confirming the previous observation that genotype and growing conditions may influence the production of phytochemicals including antioxidants in wheat (*3*, *6*, *8*, *13*, *15*).

In summary, this research suggests that bran samples of wheat from different sources may significantly differ in their antioxidant properties and phytochemical compositions. Wheat bran is an excellent source of dietary natural antioxidants and phenolic acids, and may contribute to total dietary carotenoids and tocopherols.

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