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Phenolic Acid, Tocopherol and Carotenoid Compositions, and Antioxidant Functions of Hard Red Winter Wheat Bran

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An electron spin resonance (ESR) spectrometry study was conducted to examine the free radical scavenging properties of bran extracts of Alliance and Wichita wheat using hydroxyl radical ($HO^{\bullet}$), 2,2-diphenyl-1-picryhydrazyl radical ($DPPH^{\bullet}$), and superoxide radical anion ($O_2^{\bullet-}$) and their chelating capacities against Cu^{2+}. Also reported is the radical cation 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) scavenging activity, oxygen radical absorbing capacity (ORAC), and chelating property against Fe^{2+} of the bran extracts measured by the spectrophotometric methods. Significant radical scavenging and chelating capacities were detected in the bran extracts, along with significant levels of phenolic acids, tocopherols, and carotenoids. Ferulic acid, with a concentration range of 130.60−146.38 μg/g, was the predominant phenolic acid in all of the tested bran samples and accounted for ∼53−67% of total phenolic acids on a weight basis. Total tocopherol concentration ranged from 1.87 to 2.95 μmol/100 g of bran, whereas total carotenoid level was 0.20−0.33 μmol/100 g of bran. In addition, both wheat variety and growing conditions might significantly alter antioxidant properties and concentrations of beneficial components in wheat bran.

KEYWORDS: ESR; wheat; bran; antioxidants; phenolic acid; tocopherol; carotenoid; chelating; radical

INTRODUCTION

Wheat is an important agricultural commodity and food ingredient across the world. Winter wheat (*Triticum aestivum*) is an important component of both irrigated and dryland production systems in Colorado and the west central Great Plains in the United States. Because of the recent depressions in export markets and increased productions in other wheat-producing areas of the world, the market prices for wheat have fallen to record lows and the farm economy has suffered tremendously. Wheat producers are looking for value-added opportunities for marketing wheat grain to overcome some of the inherent difficulties in these volatile commodity markets. Identification of components in the wheat grain and fractions that would contribute to enhanced value would significantly promote the utilization of Colorado wheat and improve the profitability and sustainability of the farm economy.

Growing evidence suggests that wheat and wheat-based food products contain significant levels of beneficial components including natural antioxidants, which may provide health benefits to consumers in addition to general nutrition (*1*). Previous studies showed that the extracts of wheat fractions and wheat-based food products exhibited radical scavenging capacities, showed chelating activity against Fe^{2+}, and suppressed lipid peroxidation in oils, liposome, and human low-density lipoprotein (LDL) (*2*−*11*). It was also noted that genotype, growing conditions, and the interaction between growing condition and genotype altered the antioxidant properties of wheat samples and their levels of beneficial components, including total phenolics, phenolic acids, carotenoids, and tocopherols (*4*, *9*, *12*−*15*). These data suggested the possibility of the production of a wheat variety rich in antioxidants and other beneficial components for food utilization while enhancing the agricultural economy. More research is required to investigate additional wheat varieties/experimental lines to identify such value-added wheat varieties rich in antioxidants and/or beneficial components for individual wheat-growing locations using additional assays.

In the present study, bran extracts of hard red winter wheat varieties (Alliance and Wichita) grown at two testing locations in Colorado were examined for their antioxidant properties including radical scavenging capacities against hydroxyl radical ($HO^{\bullet}$), 2,2-diphenyl-1-picryhydrazyl radical ($DPPH^{\bullet}$), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\bullet+}$), and superoxide radical anion ($O_2^{\bullet-}$), their oxygen radical absorbing capacity (ORAC), and chelating capacities against Fe^{2+} and Cu^{2+}, as well as their profiles of tocopherols, carotenoids, and phenolic acids. The present study is part of our continuous effort in promoting the production and utilization of hard wheat varieties rich in selected antioxidant activity and

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beneficial components for improving human nutrition while enhancing the U.S. agricultural economy.

MATERIALS AND METHODS

Materials. 5-*tert*-Butoxycarbonyl 5-methyl-1-pyrroline *N*-oxide (BMPO) was a gift from Prof. B. Kalyanaraman of the Biophysics Research Institute and Free Radical Research Center at the Medical College of Wisconsin (Milwaukee, WI), whereas bran samples of Alliance and Wichita wheat, both hard red winter wheat varieties, were provided by Dr. Scott Haley of the Department of Soil and Crop Science, Colorado State University, Fort Collins, CO. Grain samples of both wheat varieties were obtained at harvest from breeding trials conducted at two nonirrigated testing sites located at Walsh and Burlington in eastern Colorado. Agronomic practices at each location were considered to be representative of typical wheat production conditions in eastern Colorado. Disodium ethylenediaminetetraacetate (EDTA), hydroxylamine hydrochloride, 2,2′-bipyridyl, DPPH•, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), fluorescein (FL), diethylenetriaminepentaacetic acid (DTPA), hypoxanthine (HPX), nitro blue tetrazolium (NBT), 5,5-dimethyl *N*-oxide pyrroline (DMPO), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), tocopherols (α-, δ-, and γ-), β-carotene, and superoxide dismutase (SOD) were obtained 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). Xanthine oxidase (XOD) was obtained from Roche Applied Science (Indianapolis, IN). 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. Five grams of each bran sample was ground to fine powder using a micromill manufactured by Bel Art Products (Pequannock, NJ) and extracted for 15 h with 50 mL of 50% acetone at ambient temperature. The 50% acetone extracts were kept in the dark at room temperature until further evaluation of antioxidant properties and subjected to additional treatment for phenolic acid analyses.

Superoxide Anion Radical $O_2^{\bullet-}$ Scavenging Activity. $O_2^{\bullet-}$ scavenging activity was determined by an electron spin resonance (ESR) method using the $O_2^{\bullet-}$ generated through the xanthine/xanthine oxidase system (*16*). The reaction mixture contained 2 mM xanthine, 100 mM BMPO, 0.2 mM DTPA, and 0.08 unit/mL of xanthine oxidase solution (XOD). The total volume of the reaction mixture was 100 μL, and the final concentration of wheat antioxidants was 20 mg of bran equiv/mL of reaction mixture. XOD solution was added to initiate the antioxidant−radical reaction, and BMPO was used as the trapping agent (*17*). The ESR spectra were recorded at 2 min of reaction with 10 mW incident microwave power and 100 kHz field modulation of 1 G at ambient temperature, using a Varian E-109X-Band ESR spectrometer (Varian, Inc., Palo Alto, CA) in the Center for Food Safety and Applied Nutrition at the U.S. Food and Drug Administration (College Park, MD). The strength of the $O_2^{\bullet-}$ signal is proportional to its concentration in the testing system. The $O_2^{\bullet-}$ radical concentration in the reaction mixture was quantified by measuring the amplitude of the ESR spectra and expressed as SOD equivalents. SOD is a well-known antioxidative enzyme and used as an antioxidant standard in this study.

HO• Scavenging Activity. HO• scavenging capacities of the wheat bran extracts were examined by an ESR method. HO• was generated by Fenton reaction, whereas DMPO was used as the trapping agent (*18*). The reaction mixture contained 10 μL of 3 mM freshly prepared $FeSO_4$, 80 μL of 0.75 mM EDTA, 15 μL of 0.5 mM H_2O_2, 15 μL of 1 M DMPO, and 30 μL of bran extracts or solvent for the blank. The final concentration was 20 mg of bran equiv/mL for all bran extracts in the antioxidant−radical reaction mixtures. The ESR measurements were conducted at 1 and 20 min of each reaction at ambient temperature with the following spectrometer settings: microwave power of 10 mW, field modulation frequency of 100 kHz, and a modulation amplitude of 1 G. The strength of the HO• signal is proportional to its concentration in the testing system. Under the same testing conditions, the greater amplitude of the ESR peak is associated with a higher concentration of HO• in the reaction mixture.

Radical DPPH Scavenging Activity. Radical DPPH scavenging capacities of wheat bran extracts were determined by an ESR spectrometry method using the stable DPPH• (*4*). Each bran extract was mixed with DPPH• stock solution to initiate the antioxidant−radical reaction. The final concentration was 250 μM for DPPH• in all reaction mixtures. The final concentration was 50 mg of bran equiv/mL of reaction mixture for all bran samples, and the control reaction contained no antioxidant. ESR signals were recorded at 1, 25, and 50 min of the reaction, with 20 mW incident microwave power and 100 kHz field modulation of 2 G (*4*, *19*). The strength of the DPPH• signal is proportional to its concentration in the testing system. Under the same testing conditions, the greater amplitude of the ESR peak is associated with a higher concentration of DPPH• in the reaction mixture. The scavenging activity of each wheat extract was estimated by comparing the DPPH• in the antioxidant−radical reaction mixture with that in the control reaction at the same period of reaction time and expressed as percent DPPH• quenched.

Radical Cation $ABTS^{\bullet+}$ Scavenging Activity. Radical scavenging capacity of bran extracts was evaluated against $ABTS^{\bullet+}$ generated by the chemical method according to a previously reported protocol (*9*, *20*). Briefly, $ABTS^{\bullet+}$ 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 bran extracts or 50% acetone 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.

ORAC Assay. The ORAC assay was conducted using FL as the fluorescent probe according to a previously described procedure (*9*, *21*). The final assay mixture contained 0.067 μM FL, 60 mM AAPH, and 300 μL of bran extracts or 50% acetone for a reagent blank. The fluorescence of an assay mixture was determined and recorded every minute at the emission of 515 nm and the excitation of 490 nm for a duration of 60 min. The Trolox equivalent was calculated using a standard curve prepared with Trolox and used to compare ORAC of wheat bran samples.

Chelating Activity against Fe^{2+} and Cu^{2+}. 2,2′-Bipyridyl competition assay was conducted to measure the Fe^{2+} chelating activity of wheat bran extracts (*6*). The reaction mixture contained 0.1 mL of 1 mM $FeSO_4$ solution, 50 μL of bran extracts, 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 Fe^{2+} chelating activity using EDTA as a standard.

ESR measurements were carried out to determine the potential chelating capacity of the wheat extracts against Cu^{2+} according to previously described conditions with slight modification (*22*). Briefly, 150 μL of bran extracts was mixed with 150 μL of copper chloride ($CuCl_2$) solution. ESR spectra were recorded with 40 MW incident microwave power and 100 kHz field modulation of 5 G at 77 K.

Phenolic Acid Composition. After the removal of acetone, the wheat bran isolates 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 (*9*). The ethyl ether−ethyl acetate was removed using a nitrogen evaporator, and the solid residue was dissolved in methanol, filtered through a 0.20 μm membrane filter, and subjected to high-performance liquid chromatography (HPLC) analysis using a Phenomenex C18 column (250 mm × 4.6 mm) according to a previously described protocol (*9*). The presence of phenolic acids was detected at 280 nm. 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 (*9*). Identification of phenolic acids was accomplished by comparing the retention time of peaks in wheat bran extracts to that of the standard compounds.

Carotenoid Composition. Carotenoids were extracted and analyzed using a high-performance liquid chromatography−diode array detector−electrospray ionization tandem mass spectrometry (HPLC-DAD-ESI-

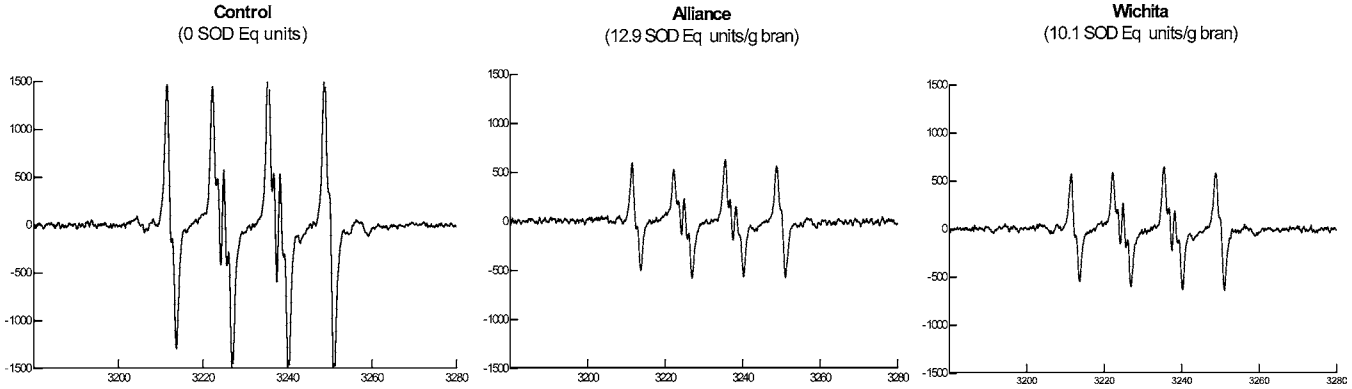

Figure 1. $O_2^{\bullet-}$ scavenging activities of wheat bran extracts determined by ESR. Alliance and Wichita stand for bran samples of Alliance and Wichita wheat grown at Walsh in Colorado, respectively, whereas Control represents the control reaction containing no antioxidant. The final reaction mixture contained 2 mM xanthine, 100 mM BMPO, 0.2 mM DTPA, bran extracts at a concentration of 20 mg of bran equiv/mL, and XOD at a level of 0.08 unit/mL. The total volume was 100 μL for all reaction mixtures. The ESR spectra were recorded at 2 min of the reaction at ambient temperature.

MSMS) method (*12*, *13*, *23*). Briefly, 200 mg of ground bran was extracted with 10 mL of methanol/tetrahydrofuran (1:1, v/v) for 15 h at ambient temperature and then sonicated for another 10 min. The resulting extraction mixture was centrifuged at 6000 rpm, followed by a filtration through a 0.20 μm membrane filter, and subjected to HPLC analysis of carotenoids, as well as for tocopherol analysis. A TSQ Quantum tandem mass spectrometry (Thermo-Finnigan, San Jose, CA) equipped with an ESI interface and an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA) was employed in the HPLC analysis. HPLC separation was accomplished using a Zorbax SB C18 column (1.0 mm i.d. × 50 mm, 3.5 μm particle size) at room temperature according to a previously described protocol with slight modification (*13*). The carotenoids were eluted using a mobile phase of water as solvent A and methanol/acetonitrile/2-propanol (54:44:2) as solvent B. The gradient procedure was as follows: (1) the gradient was linear from 50 to 99% of solvent B, and the flow rate was increased from 0.2 to 0.27 mL/min in the first 10 min, and (2) 99% of solvent B and a flow rate of 0.27 mL/min for 10 min. 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 pressures 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 (CID) was achieved using argon as the collision gas at the pressure adjusted to >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 those 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, whereas *m*/*z* 552.6 → 145.3 and 536.6 → 119.3 were set for β-cryptoxanthin and β-carotene, respectively. Data were acquired with an Xcalibur software system (Thermo-Finnigan, San Jose, CA). The quantification for each carotenoid compound was conducted using the total ion counts with an external standard.

Tocopherol Composition. HPLC analysis was performed to evaluate the α-, δ-, and γ-tocopherol concentrations in wheat bran samples using a Zorbax SB C18 column (Agilent Technologies), 1.0 mm i.d. × 30 mm, 3.5 μm particle size, at ambient 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: (1) the gradient was linear from 80 to 99% of solvent B and the flow rate was 0.3 mL/min, and (2) 99% of solvent B was kept for 10 min. Identification of tocopherols was conducted by comparing the HPLC retention time and SRM analysis of the sample peaks with those of the authorized pure commercial tocopherol compounds. LC-MS conditions were set the same as described above for carotenoid composition. 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 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. A Pearson correlation test was also conducted to determine the correlations among means. Statistical significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

$O_2^{\bullet-}$ Radical Scavenging Activity. The ESR spectrometry method is considered to be a reliable measurement of the presence of free radicals and has been used to investigate the radical scavenging properties of antioxidants (*16*, *18*, *19*). In the present study, ESR measurements showed that bran extracts of both Alliance and Wichita wheat grown at Walsh had strong $O_2^{\bullet-}$ scavenging activity (**Figure 1**). Alliance bran had a greater $O_2^{\bullet-}$ scavenging activity at a level of 12.96 units SOD equiv/g of bran, whereas that of Wichita bran was 10.06 units SOD equiv/g of bran, under the same experimental conditions. However, the scavenging capacities of the two bran extracts were not significantly different.

The $O_2^{\bullet-}$ scavenging capacities of Alliance and Wichita wheat bran extracts detected by ESR in the present study were supported by our previous findings that Swiss red wheat grain fractions and bran samples of seven wheat from four different countries exhibited $O_2^{\bullet-}$ scavenging activity, as evaluated by a spectrophotometric method using the hypoxanthine/xanthine oxidase system (*9*, *23*).

$HO^{\bullet}$ Scavenging Activity. The present study investigated the $HO^{\bullet}$ scavenging capacity of wheat bran for the first time. ESR measurements indicated that both Alliance and Wichita bran extracts exhibited significant $HO^{\bullet}$ scavenging capacities under the experimental conditions (**Figure 2**). Alliance bran had a stronger $HO^{\bullet}$ scavenging capacity than that of Wichita bran at both 1 and 20 min of the reaction on the same bran weight basis, suggesting the two hard red winter wheat varieties may differ in their $HO^{\bullet}$ scavenging compounds. Also noted was that the $HO^{\bullet}$ scavenging activities of both bran extracts were time dependent, with a greater $HO^{\bullet}$ scavenging activity associated with a longer reaction time between bran antioxidantsand $HO^{\bullet}$ (**Figure 2**).

Radical DPPH Scavenging Activity. Both Alliance and Wichita wheat bran extracts had capacity to directly react with and quench $DPPH^{\bullet}$ in the reaction mixtures under the ESR

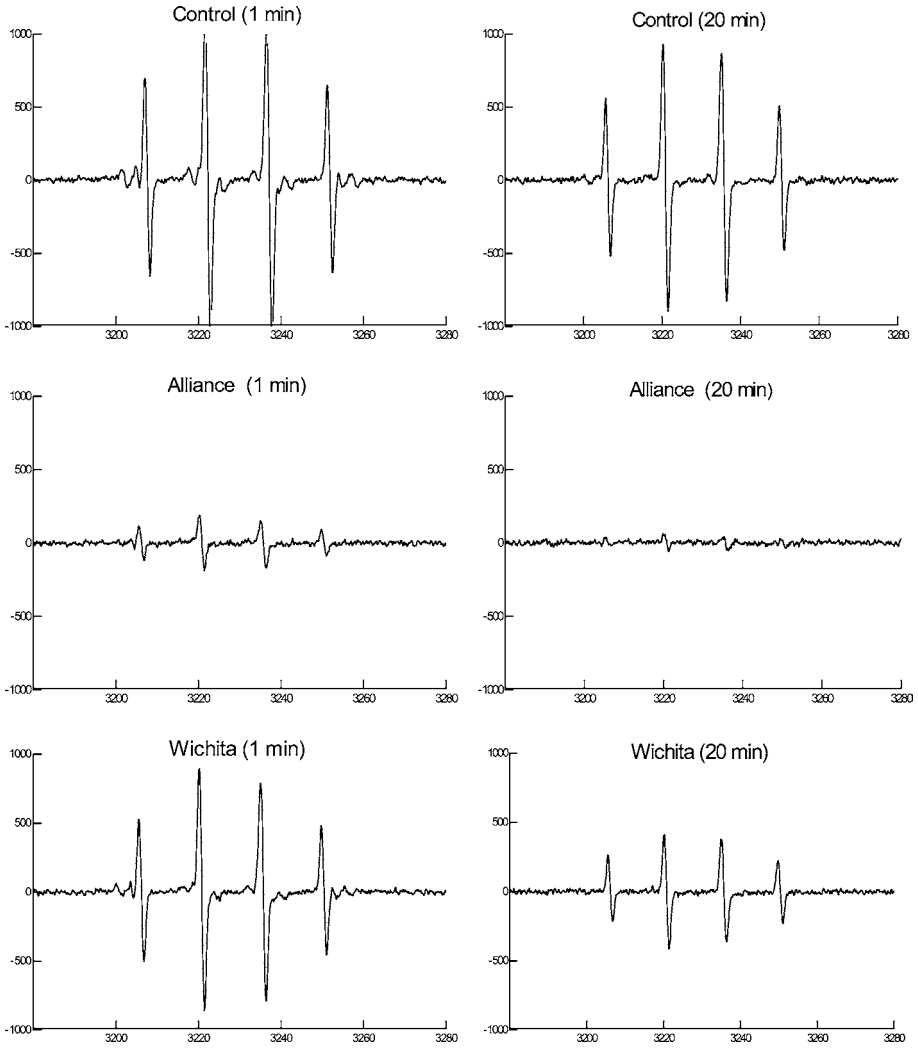

Figure 2. Hydroxyl radical scavenging activities of wheat bran extracts determined by ESR. Alliance and Wichita stand for bran samples of Alliance and Wichita wheat, respectively, whereas Control represents the control reaction containing no antioxidant. Each reaction mixture contained 10 μL of freshly prepared 3 mM $FeSO_4$, 80 μL of 0.75 mM EDTA, 15 μL of 1 M DMPO, 15 μL of 0.5 mM H_2O_2, and 30 μL of wheat bran extract at a concentration of 100 mg of bran equiv/mL. ESR signals were recorded at 1 and 20 min of the reaction at ambient temperature.

determination conditions, and the ESR result of the DPPH$^{\bullet}$−bran extract of Alliance is reported in **Figure 3**. Also noted from **Figure 3** was the time dependence of the DPPH$^{\bullet}$ scavenging capacity of wheat bran. The bran extract quenched more free radical in longer reaction time. Furthermore, the DPPH$^{\bullet}$ scavenging capacity of the bran extracts was quantified by ESR. The bran extract of Alliance wheat exhibited a stronger DPPH$^{\bullet}$ quenching activity ($P = 0.009$) than did that of Wichita at 1 min of the radical−antioxidant reaction, suggesting that the two hard red winter wheat varieties differed in their free radical scavenging properties against DPPH$^{\bullet}$ (**Figure 4**).

Radical Cation Scavenging Activity. Bran extracts of Alliance and Wichita wheat grown at Walsh and Burlington in Colorado were evaluated for their ABTS$^{\bullet+}$ scavenging capacities using a spectrophotometric method. ABTS$^{\bullet+}$ scavenging capacity of the bran extracts ranged from 17.99 to 18.85 μmol of TE/g of bran (**Table 1**). TE is the Trolox equivalent. The cation radical scavenging capacity of both wheat varieties from the two locations was comparable to that previously detected in wheat grain and bran (*8*−*10*). No difference in their ABTS$^{\bullet+}$ scavenging capacities was observed among the four bran extracts prepared from Alliance and Wichita wheat grown at two locations (**Table 1**). Wheat bran samples had no difference in their radical cation scavenging activities, suggesting that both genotype and growing conditions had less influence on the ABTS$^{\bullet+}$ scavenging capacity of hard red winter wheat bran. No correlation was observed between the ABTS$^{\bullet+}$ scavenging capacity and ORAC, Fe^{2+} chelating activity, or any measured chemical components in the bran.

ORAC Assay. ORAC values were determined for bran extracts of Alliance and Wichita wheat grown at Walsh and Burlington, CO, and expressed as micromoles of TE per gram of bran. All bran extracts exhibited strong ORAC, with an ORAC value ranging from 50.59 to 65.94 μmol of TE/g of bran (**Table 1**). The ranges are comparable to that of 45.02−124.29 μmol of TE/g of bran detected in 50% acetone extracts of seven wheat bran samples from four different countries (*9*, *23*). The range is greater than those of 3.41−6.25 and 4.46−5.60 μmol of TE/g of bran detected in ethanol extracts of Trego and Akron wheat bran, respectively (*24*). This may be explained by the solvent effect on antioxidant activity estimation (*25*). Zhou and Yu (*25*) discussed the solvent effect on hard wheat antioxidant activity estimation in detail and demonstrated that 50% acetone extracts of the hard wheat bran exhibited greater ORAC values than those of the ethanol extracts. Bran extracts of Wichita wheat had greater ORAC value than that of Alliance wheat grown at the same location, suggesting the possible influence of wheat genotype on ORAC. Also noted was that bran extracts prepared

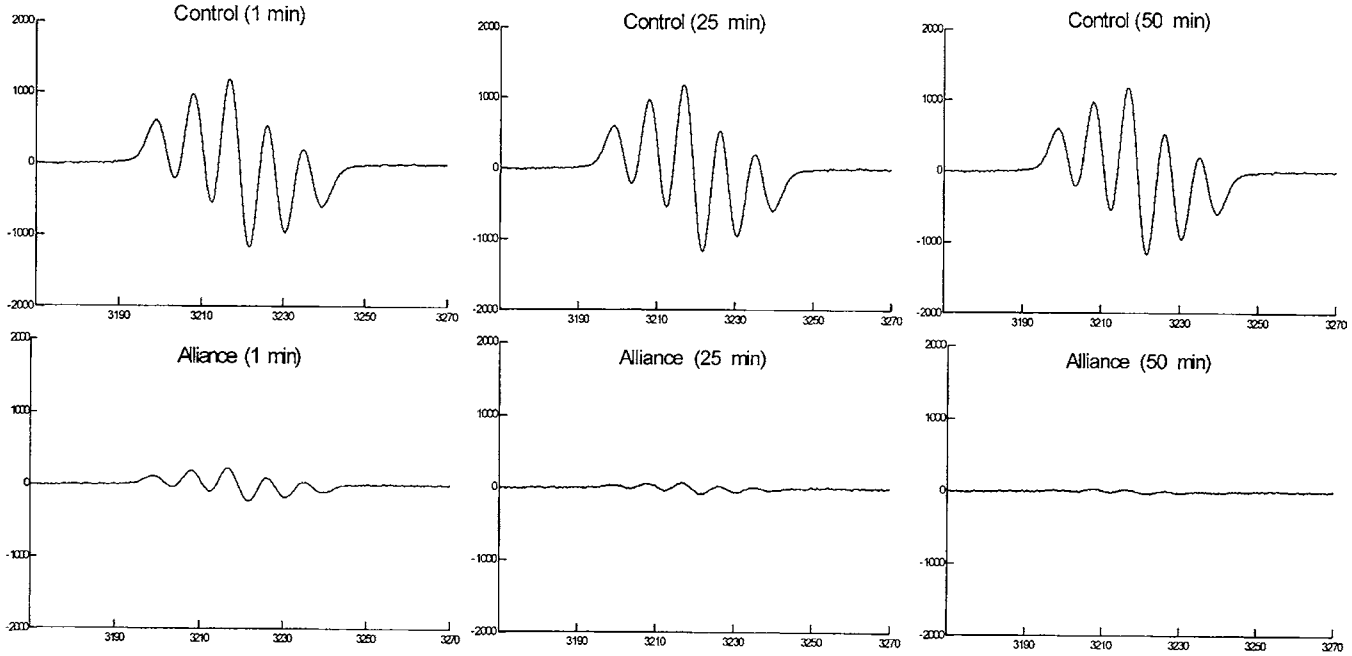

Figure 3. DPPH• scavenging activities of wheat bran extracts determined by ESR. Alliance stands for bran samples of Alliance wheat, whereas Control represents the control reaction containing no antioxidant. The final concentrations were 250 μM for DPPH• and 50 mg of bran equiv/mL for wheat bran in all reaction mixtures. ESR signals were recorded at 1, 25, and 50 min of each reaction at ambient temperature.

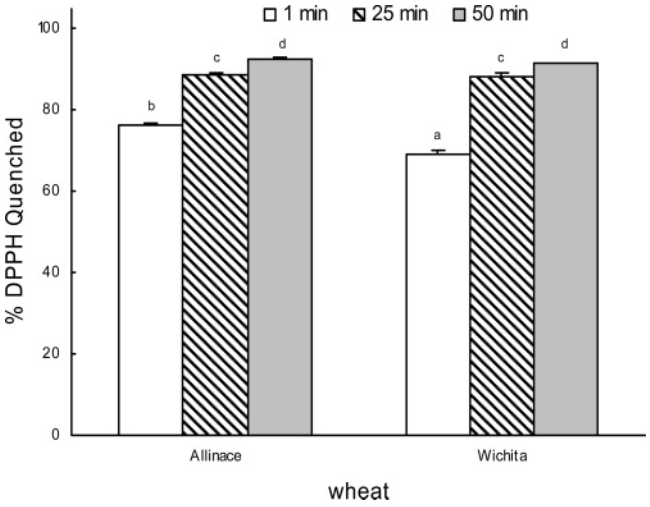

Figure 4. DPPH• scavenging activities of wheat bran extracts at different reaction times. Alliance and Wichita stand for bran samples of Alliance and Wichita wheat, respectively, whereas Control represents the control reaction containing no antioxidant. The DPPH• scavenging activities of wheat bran extracts are expressed as percent DPPH• quenched. The vertical bars represent the standard deviation of each data point ($n = 3$). For the same wheat variety, values marked by the same letter are not significantly different ($P < 0.05$).

from the same wheat variety grown at Burlington had a greater ORAC value than that grown at Walsh, indicating the possible influence of growing conditions on the ORAC value of wheat bran. Future study involving a larger number of wheat varieties from multiple growing locations is required to further investigate the potential effects of variety and growing conditions on antioxidant properties of hard wheat bran. However, the difference of ORAC values among the four bran extracts was not significant. ORAC values were not correlated to any examined chemical components of wheat bran or Fe^{2+} chelating activity.

Table 1. Antioxidant Properties of Hard Red Winter Wheat Bran[a]

wheat variety	location	ABTS•+ (TE μmol/g)	ORAC (TE μmol/g)	Fe^{2+} chelating activity (EDTA equiv mg/g)
Alliance	Walsh	18.05a ± 0.50	50.59a ± 8.57	1.04b ± 0.12
Alliance	Burlington	18.85a ± 0.48	57.37a ± 4.44	1.18b ± 0.09
Wichita	Walsh	17.99a ± 0.42	60.29a ± 0.23	0.73a ± 0.12
Wichita	Burlington	18.79a ± 0.51	65.94a ± 9.13	0.56a ± 0.08

[a] Both Alliance and Wichita are hard red winter wheat varieties. The Fe^{2+} chelating activities of the 50% acetone extracts were examined by a spectrophotometric method and reported as EDTA equivalents (EDTA equiv). ABTS•+ was generated by the chemical method. ORAC measures the prevention of bran antioxidants against free radical induced oxidative damage. The results are expressed as mean value ± standard deviation. Within each column, means with the same letter are not significantly different ($p < 0.05$).

Chelating Activity against Fe^{2+} and Cu^{2+}. Chelating activity against Fe^{2+} was examined and expressed as EDTA equivalents per gram of bran (**Table 1**). The Fe^{2+} chelating activity ranged from 0.56 to 0.73 mg of EDTA equiv/g of bran and from 1.04 to 1.18 mg of EDTA equiv/g of bran for Wichita and Alliance wheat, respectively. Both Alliance and Wichita bran samples from different locations differed in their Fe^{2+} chelating activities, suggesting growing conditions might alter the chelating properties of hard red winter wheat. Interestingly, Alliance bran grown at both locations had significantly stronger Fe^{2+} chelating activity than that of Wichita wheat from the same locations, indicating the possible effect of wheat variety. The chelating activity of both wheat varieties from the two locations was also comparable to that previously detected in wheat grain and bran (*8*−*10*). Fe^{2+} chelating activities of wheat extracts were not correlated with their radical cation scavenging activities, ORAC, or any chemical components measured under the experimental conditions.

The chelating capacity against Cu^{2+} was evaluated by ESR measurements. Bran extracts of both Alliance and Wichita grown at Walsh formed strong chelating complexes with Cu^{2+} under the experimental conditions (**Figure 5**). It needs to be

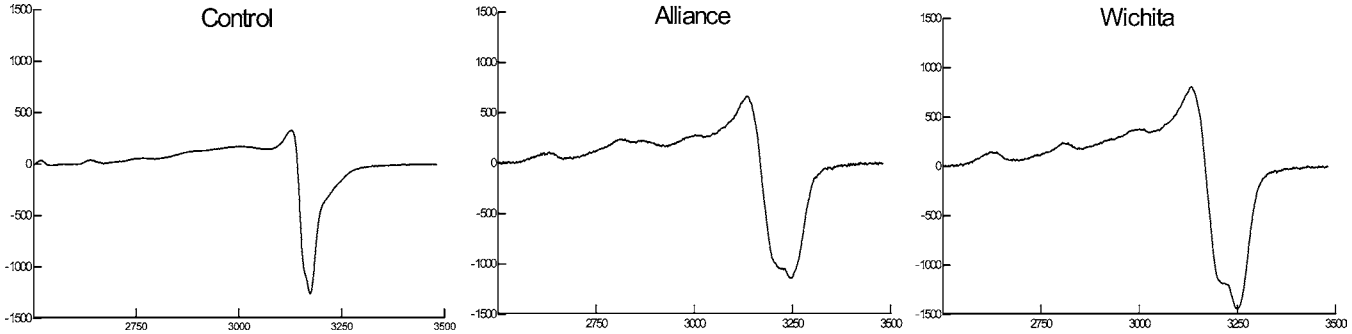

Figure 5. Interaction between Cu^{2+} and individual wheat bran extracts measured by ESR. The final concentrations were 50 mg of bran equiv/mL for bran antioxidants and 1 mM for copper chloride ($CuCl_2$). The ESR spectrum was recorded at 1 min of reaction at 77 K.

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

wheat variety	location	*p*-hydroxybenzoic acid (μg/g)	vanillic acid (μg/g)	syringic acid (μg/g)	coumaric acid (μg/g)	ferulic acid (μg/g)
Alliance	Walsh	17.88b ± 5.69	14.45a ± 0.73	39.30b ± 0.50	5.84a ± 0.00	142.37b ± 0.52
Alliance	Burlington	8.89a ± 0.20	16.48b ± 1.13	39.18b ± 0.27	8.60b ± 1.74	146.38b ± 1.86
Wichita	Walsh	18.98b ±1.78	33.11c ± 0.31	55.70c ± 0.57	5.81a ± 0.09	130.54a ± 2.55
Wichita	Burlington	11.49ab ± 0.59	17.59b ± 0.04	36.45a ± 0.52	7.02ab ± 0.08	130.06a ± 0.65

[a] Both Alliance and Wichita are hard red winter wheat varieties. The results are expressed as mean value ± standard deviation. Within each column, means with the same letter are not significantly different ($P < 0.05$, $n = 3$).

Table 3. Carotenoids Profile of Hard Red Winter Wheat Bran[a]

wheat variety	location	β-carotene (μg/g)	zeaxanthin (μg/g)	lutein (μg/g)	β-cryptoxanthin (μg/g)	total carotenoids (μmol/100 g)
Alliance	Walsh	0.10d ± 0.00	0.40b ± 0.00	1.43d ± 0.01	0.25b ± 0.00	0.33d ± 0.00
Alliance	Burlington	0.06c ± 0.00	0.25a ± 0.00	1.11b ± 0.00	nd	0.22b ± 0.00
Wichita	Walsh	0.03a ± 0.00	0.29a ± 0.01	0.97a ± 0.02	0.12a ± 0.08	0.20a ± 0.02
Wichita	Burlington	0.05b ± 0.00	0.40b ± 0.00	1.25c ± 0.05	0.33b ± 0.0	0.30c ± 0.01

[a] Both Alliance and Wichita are hard red winter wheat varieties. The total carotenoids were calculated by summing the micromoles of individual carotenoid in 100 g of bran. The results are expressed as mean value ± standard deviation. Within each column, means with the same letter are not significantly different ($P < 0.05$, $n = 3$). nd, not detected.

pointed out that ESR measurements presented in **Figure 5** are not quantitative, so it is not known which wheat variety bran extract had the stronger Cu^{2+} chelating capacity.

Phenolic Acid Composition. Ferulic, *p*-coumaric, *p*-hydroxybenzoic, vanillic, and syringic acids were detected in all tested wheat bran extracts (**Table 2**). Similar to other wheat varieties and commercial wheat bran samples, ferulic acid was the predominant phenolic acid in all bran samples and accounted for ~53.47−66.68% of the total identified phenolic acids on a per weight basis (*2*, *9*, *10*, *14*, *23*). Bran of Wichita wheat grown at Walsh had the greatest total phenolic acid level of 244.14 μg/g. Ferulic acid contents in bran samples of Alliance grown at Walsh or Burlington were significantly greater than that of Wichita wheat from the corresponding locations, respectively, suggesting the effect of growing conditions on ferulic acid concentration in wheat bran.

Carotenoid Profile. Carotenoid composition including β-carotene, zeaxanthin, lutein, and β-cryptoxanthin was analyzed for bran samples of Alliance and Wichita wheat grown at Walsh and Burlington, CO. Total carotenoids ranged from 0.20 to 0.33 μmol/100 g of bran (**Table 3**). β-Cryptoxanthin was detected in three of the four tested wheat bran samples, but not in Alliance bran from Walsh. The concentration ranges of β-carotene and zeaxanthin were 0.03−0.10 and 0.25−0.40 μg/g, respectively, for the tested bran samples. Among the four tested wheat bran samples, lutein was the primary carotenoid and ranged from 0.97 to 1.43 μg/g of bran, which equals 97−143 μg/100 g. This range is comparable to that of ~25−145 μg/100 g of grain detected in the whole grains of the 11 wheat varieties (*14*), and 50−180 μg/g of bran in the 7 wheat bran samples from four different countries (*23*). In the present study, zeaxanthin levels ranged from 0.25 to 40 μg/g in the Alliance and Wichita brans, which is similar to that in Akron bran from Colorado and the commercial Swiss red wheat bran (*23*).

Tocopherol Profile. Cereal grains serve as important dietary sources of tocopherols (*26*−*28*). In the present study, significant levels of α-, δ-, and γ-tocopherols were detected in the bran samples of Alliance and Wichita wheat grown at Walsh and Burlington in Colorado (**Table 4**). Total tocopherols ranged from 1.87 to 2.95 μmol/100 g of bran. This level is comparable to that of 0.92−6.90 μmol/100 g of bran detected in the seven wheat bran samples from four different countries (*23*). The α-tocopherol concentration ranged from 4.10 to 6.51 μg/g in the four bran samples tested in this study, which is comparable to the levels of 1.28−21.29 μg/g in the seven bran samples (*23*), 9.5−10.4 μg/g in the wheat meal (*26*), ~16 μg/g in the wheat bran sample from Finland (*26*), 8.2 μg/g observed in wheat flour (*28*), and 9.9 μg/g detected in freshly milled whole-meal wheat flour (*27*). This range is lower than that of 200−240 μg/g in wheat germ (*26*) or the level of 90−150 μg/g in olive, soybean, and peanut oils (*28*). The greatest α-tocopherol concentration of 6.51 μg/g of bran was detected in Alliance wheat grown at Walsh, followed by that of Alliance and Wichita wheat bran grown at Burlington and that Wichita bran from Walsh. Alliance

Table 4. Tocopherol Profiles of Hard Red Winter Wheat Bran[a]

wheat variety	location	α-tocopherol (μg/g)	δ- tocopherol (μg/g)	γ- tocopherol (μg/g)	total tocopherols (μmol/100 g)
Alliance	Walsh	6.51c ± 0.09	0.38d ± 0.00	5.59c ± 0.04	2.95d ± 0.01
Alliance	Burlington	5.13b ± 0.29	0.23c ± 0.00	5.75d ± 0.04	2.63c ± 0.07
Wichita	Walsh	4.10a ± 0.04	0.16a ± 0.00	3.68a ± 0.00	1.87a ± 0.00
Wichita	Burlington	5.07b ± 0.17	0.20b ± 0.00	5.19b ± 0.02	2.47b ± 0.04

[a] Both Alliance and Wichita are hard red winter wheat varieties. The total tocopherols were calculated by summing the micromoles of individual tocopherol compound in 100 g of bran. The results are expressed as mean value ± standard deviation. Within each column, means with the same letter are not significantly different ($P < 0.05$, $n = 3$).

wheat bran from Walsh also had the greatest level of γ-tocopherol at 5.59 μg/g of bran, whereas the lowest level was found in Wichita bran grown at Walsh. The tested wheat bran samples might differ in their individual and total tocopherol compositions (**Table 4**), suggesting the possible effect of wheat variety and growing conditions on tocopherol composition in wheat bran.

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