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Free Radical Scavenging Properties and Phenolic Content of Chinese Black-Grained Wheat

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Free radical scavenging properties and phenolic content of extracts from a novel Chinese black-grained wheat were evaluated for comparison with selected wheat controls. Extracts of bran and whole meal were compared for their scavenging activities against the 2,2-diphenyl-1-picryhydrazyl (DPPH) free radical. The total phenolic content and phenolic acid levels were determined using colorimetric and high-performance liquid chromatography (HPLC) methods, respectively. There were significant differences in radical scavenging activities and phenolic contents among bran or whole meal samples of Chinese black-grained wheat and selected wheat controls. Chinese black-grained wheat had the strongest scavenging activity and the highest total phenolic content among the wheat samples. The scavenging activity and total phenolic content of wheat bran was generally twice as high as that of whole meal. A positive correlation was found between DPPH radical scavenging activity and total phenolic content of bran ($R = 0.86$) and whole meal ($R = 0.96$). In addition, HPLC analysis detected the presence of gallic, *p*-hydroxybenzoic, caffeic, syringic, *p*-coumaric, vanillic, gentisic, *o*-coumaric acid, and ferulic acids in wheat bran. Ferulic acid content was highest among the phenolic acids. Chinese black-grained wheat may be considered as a potential source of natural antioxidants given its high free radical scavenging ability and phenolic content. Additional research is needed to further investigate other phenolic compounds and evaluate their contribution to the antioxidant activity in order to understand the nutraceutical value of the novel black-grained wheat genotype.

KEYWORDS: Black-grained wheat; radical scavenging; antioxidant; phenolic; DPPH free radical

INTRODUCTON

Recently, studies on antioxidants have strongly indicated that consumption of grains, vegetables, and fruits may prevent many diseases and promote good health (*1*, *2*). Grains are a major source of antioxidants in our daily diets. The main antioxidative components in grains are classified as phytoestrogens, phenolic compounds, and other substances such as lignans, phytic acid, tannins, sterols, vanillin, ferulic acid (FA), caffeic acid (CA), *p*-hydroxybenzoic acid (*p*-HA), protocatechuic acid, *p*-coumaric acid (*p*-CA), gentistic acid (GEA), sinapic acid, isoferulic acid, cholorogenic acid, vanillic acid (VA), *p*-hydroxy-phenylacetic acid, and syringic acid (SA) (*3*). Beneficial effects of antioxidants on promoting health are believed to be achieved through several possible mechanisms, such as directly reacting with and quenching free radicals, chelating transition metals, reducing peroxides, and stimulating the antioxidative defense enzyme system (*4*). Wheat is one of the most important grains with phytochemical substances that display in vitro antioxidant activity (*5*−*7*). Wheat with a high level of antioxidant activity should have potential for use as an excellent dietary source of antioxidants for disease prevention and health promotion.

However, many factors such as genotype (*8*) and growing conditions (*6*, *9*) have been reported to have a significant effect on the antioxidant properties of wheat. In this study, free radical scavenging properties and phenolic content of a novel Chinese black-grained wheat (BGW) variety were evaluated. The black-grained genotype was developed from previously existing blue and purple lines (*10*, *11*). It is now available for utilization as a new raw food material for value-added products (*12*−*15*). The elemental Se content of BGW is about 1.04 mg/kg in comparison with 0.26 mg/kg of common wheat (*12*). The grain of this particular wheat is black, hence the term Chinese BGW, and the grain size is comparable to that of common wheats. The color of wheat, usually white or red (although purple is known),

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is related to pigments in the seed coat. Basic wheat pigments include carotenes, xanthophylls, and phenolic compounds (*16*, *17*). The main pigment component of BGW seed was an anthocyanin phenolic compound (*18*). Anthocyanins are known to exhibit good antioxidant activity (*19*). The objectives of the present study were to evaluate the free radical scavenging ability and phenolic content of BGW and therefore understand its value as a potential source of natural antioxidants.

MATERIALS AND METHODS

Materials. Grain samples of Chinese BGW, Dongjian purple-grained wheat (DPGW), Wu blue-grained wheat (WBGW), and Dongjian white-grained wheat (DWGW) were supplied from the Wheat Biotechnology Laboratory of the Institute of Crop Genetics, Shanxi Academy of Agricultural Science. Anthograin wheat bran was supplied from InfraReady Products Limited (Saskatoon, SK). 2,2-Diphenyl-1-picryhydrazyl (DPPH•) free radical and 12 kinds of phenolic acid standard compounds were purchased from Sigma-Aldrich (St. Louis, MO). Phenolic acid standard compounds were as follows: gallic, gentistic, *p*-coumaric, *m*-coumaric, caffeic, sinapinic, ferulic, syringic, *o*-coumaric, vanillic, protocatechuic, and *p*-HAs. All other chemicals and solvents were of the highest commercial grade and used without further purification.

Sample Extraction. Wheat grain of each genotype was milled and separated into bran and flour fractions using a Quadrumat Junior laboratory mill (Brabender OHG, Duisberg, Germany). Whole meal was prepared by grinding wheat grain with a sample mill containing a 1 mm mesh sieve (Krups 50, Germany). The rough bran obtained from Brabender mill was further ground into fine bran using the sample mill. Methanol (100%) was used as a solvent for extraction of finely ground samples. The extraction procedure for DPPH assays involved addition of 20 mL of solvent to 2.0 g samples each in 50 mL brown bottles and shaking of the samples at 300 rpm for 16 h at ambient temperature in a rotary shaker (Permentation Design Inc., Allentown, PA). Samples in 50 mL tubes were then centrifuged at 5 °C at 10000 rpm for 15 min (SS-34 Rotors, RC5C Sorvall Instruments). The methanol extracts of supernatant fluid were kept at −20 °C in the dark until further analysis for free radical scavenging activity and total phenolic content (TPC).

DPPH Radical Scavenging Activity. The DPPH method of Brand-William et al. (*20*) was modified for this assay. The method involves the reaction of the antioxidants with the stable DPPH radical in a methanol solution. Briefly, a 60 μm DPPH radical solution was freshly made in methanol. Sample extracts (200 μL) were reacted with 3.8 mL of the DPPH radical solution for 60 min. The absorbance at 515 nm was measured against a blank of pure methanol at $t = 0$, 5, 10, 20, 30, 40, 50, and 60 min and used to estimate the level of free radical scavenging ability. The chemical kinetics of antioxidant activity of wheat samples was also recorded. Antioxidant activity was calculated as followed: % DPPH radical scavenging activity $= (1 - [A_{\text{sample}t}/A_{\text{control}t=0}]) \times 100$.

TPC. The TPC of extracts was determined using the Folin−Ciocalteau reagent (*17*, *21*). An extract (200 μL) was added to 1.8 mL of freshly diluted 10-fold Folin−Ciocalteau reagent (BDH Inc., Toronto, ON). Sodium carbonate solution (1.8 mL) (60 g/L) was then added to the mixture. After 120 min of reaction at ambient temperature, the absorbance of the contents was measured at 725 nm against a blank of methanol. FA was used as a standard.

Phenolic Acid Composition. The hydrolysis method of Krygier et al. (*22*) was modified for HPLC assay. The sample (2 g) was placed into a 250 mL dark Erlenmeyer flask, and 60 mL of 4 M NaOH solution was added. The flask was immediately filled with nitrogen and sealed. The mixture was magnetically stirred during a hydrolysis period of 4 h at ambient temperature. The hydrolyzed sample was adjusted to pH 1.5−2.5 using ice-cold 6 M HCl solution and then centrifuged at 10000 rpm for 20 min. The supernatant was extracted three times each with 70 mL of ethyl acetate. After it was dried with 2 g of anhydrous Na_2SO_4, the combined organic phase was evaporated to dryness using a rotary vacuum evaporator (RE III Rotavapor, Büchi, Switzerland) set at 35 °C. The residue was dissolved in 4 mL of 50% methanol, filtered through a 0.45 μm nylon filter, and analyzed by HPLC method (*23*). A Waters HPLC 2695 separations module (Waters, Mississauga, ON) equipped with a Waters μBondapak RP-C18 column (300 mm × 3.9 mm) and a Waters 2996 photodiode array detector were used. The mobile phase contained solvent A (water containing 1% (v/v) HAc) and solvent B (100% methanol). The solvent gradient was programmed as follows: at 0 min 15% B, 10 min 20% B, 16 min 23% B, 24−28 min 27% B, 30−33 min 15% B in 33 min with a flow rate of 1.5 mL/min. Phenolic acids in samples were identified by comparing their retention times with those of the standard compounds.

Statistical Analysis. Data were reported as means of triplicate measurements and subjected to analysis of variance. Means were separated using Fisher's protected least significant difference (LSD) test at $P = 0.05$. Quantitative results were generally expressed on a dry weight basis (dwb).

Table 1. Free Radical Scavenging Activity of Wheat Bran and Whole Meal Extracts (at 60 min)[a]

wheat genotype	DPPH• scavenging (%)	wheat genotype	DPPH• scavenging (%)
BGW bran	70.87 a	BGW whole meal	33.51 a
DPGW bran	67.59 b	DPGW whole meal	25.57 b
anthograin bran	56.60 c	NA	NA
DWGW bran	56.04 c	DWGW whole meal	25.40 b
WBGW bran	49.64 d	WBGW whole meal	23.66 c
LSD	3.26	LSD	1.51

[a] LSD, least significance difference at $P = 0.05$ level of probability. Mean values for samples having similar letters in the same row are not significantly different; NA, not available.

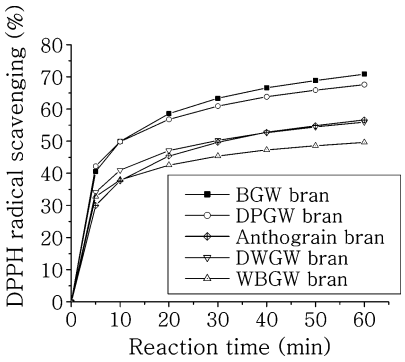

Figure 1. Antioxidant activity kinetics of wheat bran extracts with DPPH radical.

RESULTS AND DISCUSSION

DPPH Radical Scavenging Activity. Free radical scavenging activities of bran and whole meal extracts are shown in **Table 1**. The bran from Chinese BGW had the strongest DPPH radical scavenging activity, followed by the bran from DPGW, anthograin, DWGW, and WBGW. Bran extracts from anthograin and DWGW had similar free radical scavenging activity; however, there were significant differences ($P < 0.05$) among bran extracts from other wheats. The scavenging activity of bran extract from BGW was higher than that of WBGW, DWGW, anthograin, and DPGW by 21.3, 14.8, 14.3, and 3.3%, respectively (**Table 1**). All bran extracts displayed superior antioxidant activity (50−71%) when compared to values (10−23%) reported on wheat bran obtained by pearling (*17*). Reaction kinetics of bran extracts with DPPH radical are shown in **Figure 1**. The reaction rate was rapid in the first 20 min, but afterward, it became progressively slow and stable enabling measurement of scavenging capacity after 20 min for bran extracts. Regarding reaction time with DPPH radical, Awika et al. (*24*) reported

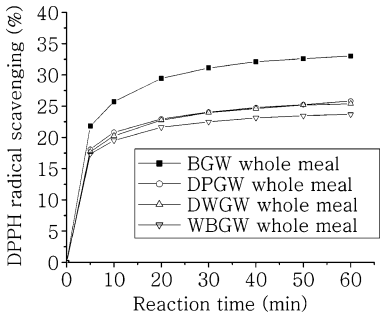

Figure 2. Antioxidant activity kinetics of wheat whole meal extracts with DPPH radical.

that the reaction time was 8 h, because there was very minimal change in activity for most of the sorghum extracts after 8 h.

The whole meal extract from Chinese BGW also showed the strongest DPPH radical scavenging activity as compared to the other extracts (**Table 1**). There was similar scavenging activity between whole meal extracts from DPGW and DWGW although significant differences were observed among their bran extracts. Whole meal extract from BGW had a higher scavenging activity than that from WBGW, DWGW, and DPGW by 9.85, 8.11, and 7.94%, respectively (**Table 1**). The increase (9.85%) in free radical scavenging activity of whole meal extracts from BGW over WBGW was obviously lower as compared to the 21.33% increase observed among their bran extracts. Reaction kinetics of whole meal extracts are also shown in **Figure 2**. The same dose and time effects were observed for all bran (**Figure 1**) and whole meal (**Figure 2**) extracts suggesting that there was similar kinetic behavior during the process of radical scavenging of DPPH by antioxidants.

The scavenging ability of the bran extract was twice as high as compared to the whole meal extract for each wheat genotype (**Table 1**). The DPPH method is a very common and low cost assay for the prediction of antioxidant activity. It has a high correlation ($R^2 = 0.97$) with the oxygen radical absorbance capacity (ORAC) method (*24*). Prooxidants (e.g., ROO$^\bullet$, OH$^\bullet$) used in the ORAC method are considered to have significance for studying pathological process (*25*). Growing evidence suggested that the reactive oxygen species (ROS) including free radicals generated during cellular metabolism or oxidation of lipids and proteins play a causative role in the pathogenesis of cancer and coronary heart disease (*26*). Enhancing food antioxidants that scavenge ROS may be a good approach for reducing the risk of cancer and coronary heart disease. Chinese BGW, with its high free radical scavenging activity, may be a potential candidate for developing novel foods containing natural antioxidants.

TPC. The TPCs, expressed as FA equivalents, of wheat bran and whole meal extracts are shown in **Table 2**. Bran from BGW contained the greatest amount of TPC, followed by the bran from DPGW, DWGW, anthograin, and WBGW. For bran extracts, TPC from BGW was higher than that from WBGW, anthograin, DWGW, and DPGW by 999, 672, 199, and 125 mg/kg, respectively (**Table 2**). There were significant differences in TPC among all of the five wheat bran samples. A positive correlation ($R = 0.86$) was observed between DPPH radical scavenging and TPC of bran extracts as previously reported (*17*).

Whole meal from BGW also contained the greatest amount of TPC followed by DPGW, DWGW, and WBGW. TPC of whole meal from BGW was higher by 402, 291, and 179 mg/kg in comparison with the whole meal from WBGW, DWGW, and DPGW, respectively (**Table 2**). Significant differences in TPC were detected among all of the four wheat samples. A high correlation ($R = 0.96$) was observed between DPPH radical scavenging and TPC of whole meal extracts.

Table 2. TPCs of Wheat Bran and Whole Meal Extracts[a]

wheat genotype	equiv of ferulic acid (mg/kg)	wheat genotype	equiv of ferulic acid (mg/kg)
BGW bran	2415 a	BGW whole meal	1108 a
DPGW bran	2290 b	DPGW whole meal	929 b
anthograin bran	1742 d	NA	NA
DWGW bran	2215 c	DWGW whole meal	817 c
WBGW bran	1416 e	WBGW whole meal	706 d
LSD	66	LSD	94

[a] LSD, least significance difference at $P = 0.05$ level of probability. Mean values for samples having similar letters in the same row are not significantly different. Equiv, equivalent; NA, not analyzed due to unavailability.

Table 3. Phenolic Acid Composition in Wheat Bran Hydrolysates[a]

	mg/kg					
phenolic acids	BGW bran	DPGW bran	anthograin bran	DWGW bran	WBGW bran	LSD
GAA	13.5 a	8.0 b	15.3 a	3.5 c	4.5 cb	3.7
p-HA	19.8 a	20.4 a	18.9 a	17.6 a	17.4 a	8.4
CA	48.4 a	35.3 b	31.4 b	28.4 b	28.0 b	7.9
SA	57.6 a	46.8 b	44.4 cb	36.2 cb	34.9 c	10.6
p-CA	104.7 b	109.1 b	104.2 b	88.8 b	145.1 a	29.0
VA	117.1 a	65.7 b	119.0 a	27.5 c	23.8 c	8.7
GEA	159.9 a	127.4 a		44.5 b	153.3 a	48.6
o-CA	453.6 a	551.4 a	94.6 b	71.9 b	598.2 a	153.4
FA	1849.2 ba	2119.4 a	1550.1 b	1665.6 b	1837.2 ba	436.7

[a] LSD, least significance difference at $P = 0.05$ level of probability. Mean values for samples having similar letters in the same row are not significantly different.

The TPC of bran was two times higher than TPC of whole meal (**Table 2**). The results indicated that phenolic compounds were mainly concentrated in the bran portion of wheal kernel and TPC varied among wheat genotypes. Phenolic compounds contribute to the in vitro total antioxidant activities of wheat (*5*). Extracts from Trego wheat had strong inhibitory activity against lipid peroxidation in fish oil (*9*). The high TPC in bran and whole meal from BGW may be very useful for its value-added utilization, suggesting that its bran may be a good source of wheat antioxidant.

Phenolic Acid Composition. The phenolic acid composition after hydrolysis of wheat bran and HPLC analysis is shown in **Table 3**. Nine types of phenolic acids were detected in bran hydrolysate from all wheat samples except for anthograin. The phenolic acids included gallic acid (GAA), *p*-HA, CA, SA, *p*-CA, VA, GEA, *o*-coumaric acid (*o*-CA), and FA. GEA was not discovered in bran hydrolysate from anthograin. FA was the predominant phenolic acid present in all five wheat bran samples. Other main phenolic acids (over 100 mg/kg) were *o*-CA, GEA, VA, and *p*-CA in the bran sample from BGW. GAA content was the lowest among the identified phenolic acids. Two other phenolic acid compounds, protocatechuric acid (PA) and chlorogenic acid (CHA), were detected in durum wheat bran (*5*). The highest level of CAs and SAs were found in the bran hydrolysate from BGW in comparison to the other four wheats. Significant differences in CAs and SAs were found between the bran hydrolyte from Chinese BGW and the other wheats.

The antioxidant capacity of some free phenolic acids in soy oil was reported in the order PA > CHA > CA > *p*-HA > GEA > FA > VA > SA > *p*-CA (*5*). Antiradical efficiencies of CA, GAA, and FA were 2.75, 2.62, and 0.12, respectively

(*27*). Pharmacokinetics of free FA and bound FA (such as FA bound to arabinoxylans in wheat bran) indicated that plasmas of rats fed with wheat bran show a better antioxidant activity and bioavailability than the pure free FA (*28*). The results indicated that the phenolic acid components, acting together, play a major role in antioxidant activity in wheat bran and whole meal extracts. Anthocyanins present in BGW (*18*) may also act as antioxidants (*19*). It is possible that they may also enhance the antioxidant activity of Chinese BGW bran and whole meal extracts.

In conclusion, bran and whole meal extracts from different wheat genotypes differed significantly in their radical scavenging capacities against DPPH and in TPC, indicating the potential effect of genotype on the antioxidant properties of wheat. These studies provide further evidence that clearly demonstrates that wheat bran is a richer source of antioxidant activity and phenolic compounds than its whole meal. Strong antioxidant activity and high TPC were detected in the bran from Chinese BGW, suggesting that it may have potential for utilization as a novel cereal, rich in natural antioxidants. Further research is required to investigate the types of anthocyanins present in the bran of Chinese BGW and evaluate their contribution to the antioxidant activity for further enhancement in breeding programs. An important extension of this work in the future will be to evaluate the survival of the free radical scavenging activity after thermal processing of wheat through baking, boiling, or steaming prior to consumption.

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