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Antioxidant Properties of Bran Extracts from "Akron" Wheat Grown at Different Locations

LIANGLI YU,*,† JONATHAN PERRET,† MARY HARRIS,† JOHN WILSON,† AND SCOTT HALEY‡

Department of Food Science and Human Nutrition and Department of Soil and Crop Sciences, Colorado State University, Fort Collins, Colorado 80523

Bran extracts of Akron wheat grown at four nonirrigated and one irrigated testing locations were examined and compared for their free radical scavenging properties against the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) and the radical cation ABTS•+, chelating capacities, and total phenolic content (TPC) to determine the potential effects of environmental factors on the antioxidant properties of hard winter wheat. The environmental factors included total solar radiation, average daily solar radiation, and number of hours exceeding 32 °C. The results showed that bran samples from different growing locations may significantly differ in their radical scavenging activities against both DPPH• and ABTS•+, chelating capacities, and TPC. A significant negative correlation was detected between the chelating activities of the bran samples from the four nonirrigated locations and total solar or daily average solar radiation ($r = -0.999$ and $P = 0.001$). These data suggest potential influences of growing conditions on the antioxidant properties of hard winter wheat and the possibility of producing wheat that is strong in a selected antioxidant property by optimizing the growing conditions of a selected wheat variety. More research is required to further investigate the relationship among antioxidant properties and environmental factors using different wheat varieties and larger sample sizes.

KEYWORDS: Wheat; barn; radical scavenging; antioxidant; phenolic; chelating; ABTS•+; DPPH

INTRODUCTION

Recently, antioxidants in grains, vegetables, and fruits have obtained great attention for their potential applications in improving the quality and safety of food products, as well as in preventing chronic diseases and promoting general human health (*1*−*4*). Antioxidant activities have been detected in wheat [*Triticum aestivum*; winter cultivar Almari, spring cultivar Henika, *T. durum* (macaroni) wheat, and hard winter wheat cultivars Akron, Trego, and Platte] and wheat-based food products (*1*, *2*, *5*−*7*). Our previous studies have examined and compared the antioxidant properties of the grain extracts of three hard winter wheat varieties, including Akron, Trego, and Platte (*1*, *2*). Ethanol was shown to be an effective solvent to extract wheat antioxidants for value-added utilization of wheat grain (*1*). Free radical scavenging capacities of the wheat grain extracts were determined against stable 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH•) and 2,2′-azino-di[3-ethylbenzthiazoline sulfonate] radical cation (ABTS•+) using spectrophotometric and electron spin resonance (ESR) spectrometry methods, while the total phenolic content was determined using the Folin−Ciocalteu reagent (*1*). Significant levels of total phenolic content and free radical scavenging capacities were detected in all three wheat grain extracts. On a dry weight basis, Akron seed showed the strongest radical-quenching capacity against DPPH• and the greatest total phenolic content while the strongest radical cation scavenging activity was observed in Platte grain extracts against ABTS•+ (*1*). The inhibitory effect of the three wheat grain extracts on lipid peroxidation was also investigated by measuring the oil stability index using a Rancimat instrument (model 679, Metrohm Ltd., Switzerland) (*2*). Trego extracts had the strongest inhibitory activity against lipid peroxidation in fish oils. In addition, the chelating activities of the three wheat grain extracts were determined because the chelating agents may stabilize the transition metals and reduce their catalytic activity in lipid peroxidation reactions (*2*, *8*). Akron and Trego extracts had similar levels of chelating activity, but Platte had a lower activity (*2*). These previous studies showed that antioxidative properties might vary among wheat cultivars. However, no study has been performed to examine the effects of environmental conditions on the antioxidant properties of wheat. It is widely accepted that phenolic compounds, including ferulic, vanillic, *p*-coumaric, caffeic, and chlorogenic acids, are concentrated in the bran portion of cereal kernels and may contribute to the total antioxidant activities of wheat (*5*). Our recent studies of antioxidants in Akron, Trego, and Platte wheat varieties also showed that antioxidant activities are mainly distributed in the

* Corresponding author (telephone (970)-491-5676; fax (970)-491-7252; e-mail yu@cahs.colostate.edu; address 230 Gifford Building, Colorado State University, Fort Collins, CO 80523-1571).
† Department of Food Science and Human Nutrition.
‡ Department of Soil and Crop Sciences.

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bran fractions (data not been published), suggesting that bran may be a good source of wheat antioxidants.

It has been noted that the growing conditions might alter the functionalities of hard winter wheat produced in the U.S. Great Plains (*12*, *13*). The environmental factors, including the hours exceeding 32 °C, have shown a greater influence on the baking quality of wheat than genotype (*12*). Recently, Wang and Zheng (*4*) reported that day/night growing temperature combinations might significantly influence the antioxidant properties and antioxidant content of strawberries, suggesting the potential effects of growing conditions on the antioxidant properties of a horticultural crop.

This study was conducted as a part of our series of investigations to determine the potential effects of environmental conditions including solar radiation and high-temperature stress on the antioxidant properties of hard winter wheat varieties. This information will be useful in determining the potential influence of growing conditions and potential interactions between genotype and growing conditions on wheat antioxidant properties and in identifying the optimum conditions to produce a selected wheat variety containing high levels of natural antioxidants. These wheat and wheat-based products may be used to prepare natural antioxidants or functional food products for improving the quality and safety of food products, as well as for enhancing human health.

MATERIALS AND METHODS

Materials. Grain samples of "Akron", a hard red winter wheat variety, adapted for production in Colorado were used for this study. Samples were obtained at harvest from breeding trials conducted at four nonirrigated testing sites located throughout eastern Colorado (Akron, Burlington, Julesburg, Walsh) and at one irrigated testing location along the Front Range of Colorado (Fort Collins). Agronomic practices at each location were considered to be representative of typical wheat production conditions in eastern Colorado. 2,2′-Bipyridyl and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$) were purchased from Sigma-Aldrich (St. Louis, MO). A total antioxidant status kit was purchased from Randox Laboratories Ltd. (San Francisco, CA). All other chemicals and solvents were of the highest commercial grade and used without further purification.

Extraction and Testing Sample Preparation. Grain samples were cleaned using air seed cleaners to remove all nongrain debris present following harvest. Wheat grain from each location was milled on a Brabender Quadromat Jr. experimental mill for separation into bran and flour fractions. An amount of 10 g of each bran sample was extracted for 15 h with 100 mL of absolute ethanol under nitrogen at ambient temperature (*1*). The ethanol extracts were kept in darkness under nitrogen until further analysis. To prepare dimethyl sulfoxide (DMSO) solution, ethanol was removed under vacuum from a known volume of the ethanol extract, and the solid residue was quantitatively redissolved in DMSO. The resulting DMSO solution was also kept in darkness under nitrogen until further analysis.

Radical Cation ABTS$^{\bullet+}$ Scavenging Activity. The radical cation ABTS$^{\bullet+}$ scavenging activity was estimated using a commercial kit from Randox Laboratories Ltd. (San Francisco, CA) (*1*). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as an antioxidant standard. The trolox equivalent was calculated and used to compare the radical cation scavenging activity of each antioxidant. The tests were conducted in triplicate for each bran extract.

Radical DPPH Scavenging Activity. Free radical scavenging capacity of bran extracts from five growing locations were determined according to the previously reported procedure using the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$) (*1*). Briefly, freshly made DPPH$^{\bullet}$ solution was added to a bran extract to start the radical−antioxidant reaction at a final concentration of 100 μM DPPH$^{\bullet}$. The absorbance at 517 nm was measured against a blank of pure ethanol at 0, 0.5, 1, 2, 5, and 10 min and used to estimate the remaining radical levels according to a standard curve. The absorbance measured at 5 min of the antioxidant−DPPH radical reactions was used to compare the DPPH radical scavenging capacity of each bran extract.

Table 1. Total Solar Radiation (SR), Average Daily Solar Radiation, and Number of Hours Exceeding 32 °C during the 6 Week Grain Filling Period at Five Wheat Growing Locations in Colorado (2001)

growing location	location type	total SR, MJ m^{-2}	daily average SR, MJ m^{-2}	hours exceeding 32 °C
Akron	nonirrigated	1140	26.5	124
Burlington	nonirrigated	624	14.5	113
Julesburg	nonirrigated	1173	27.3	133
Walsh	nonirrigated	1007	23.4	176
Fort Collins	irrigated	994	23.1	57

Chelating Activity. Fe^{2+} chelating activity was measured by a 2,2′-bipyridyl competition assay (*2*, *9*). The reaction mixture contained 0.25 mL of 1 mM $FeSO_4$ solution, 1 mL of Tris-HCl buffer (pH 7.4), 0.25 mL of antioxidant solution, 0.4 mL of 10% hydroxylamine·HCl, 1 mL of 2,2′-bipyridyl solution (0.1% in 0.2 M HCl), and 2.5 mL of ethanol. The final volume was made up to 5 mL with water. The absorbance at 522 nm was measured and used to evaluate Fe^{2+} chelating activity using disodium ethylenediaminetetracetate (EDTA) as a standard.

Total Phenolic Content. The total phenolic content of wheat extracts was determined using the Folin−Ciocalteu reagent (*1*, *10*, *11*). The reaction mixture contained 100 μL of bran extracts, 500 μL of freshly prepared Folin−Ciocalteu reagent, and 1.5 mL of 20% sodium carbonate. The final volume was brought up to 10 mL with pure water. After 2 h of reaction at ambient temperature, the absorbance at 765 nm was measured and used to calculate the phenolic content using gallic acid as a standard. Triplicate reactions were conducted.

Statistical Analysis. Data were reported as the mean ± SD of 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 the mean values, while a two-tailed Pearson correlation test was conducted to determine the correlations among the mean values. Statistical significance was declared at $P \leq 0.05$.

RESULTS

Field Testing Locations. The nonirrigated testing locations (Akron, Burlington, Julesburg, and Walsh) in our study were characterized by below-normal precipitation and drought stress during vegetative development (prior to heading stage), above-normal precipitation near flowering, and higher than normal temperatures during the grain filling period (e.g., the 6 week period preceding wheat harvest). At the irrigated testing location (Fort Collins), moderate air temperatures and plentiful supplemental irrigation throughout the growing season resulted in excellent plant growth and very high yields (~6100 kg ha^{-1}, more than twice that realized at any of the nonirrigated locations). Solar radiation, influenced by both altitude and the degree of daily cloud cover, differed markedly among the wheat-growing locations in our study (**Table 1**). As with solar radiation, marked differences were noted among testing locations for the occurrence of high-temperature conditions during the grain filling period (**Table 1**).

Radical Cation Scavenging Activity. Bran extracts of Akron wheat grown at five locations were measured and compared for their free radical scavenging activities against the radical cation ABTS$^{\bullet+}$. All five bran extracts showed ABTS$^{\bullet+}$ scavenging capacity (**Figure 1**). The bran obtained from Akron had the greatest activity for quenching ABTS$^{\bullet+}$, followed by Burlington, Walsh, Fort Collins, and Julesburg. The trolox equivalents were 28.7−33.0 μmol/g for the Akron bran samples grown at the five locations. No significant difference in their

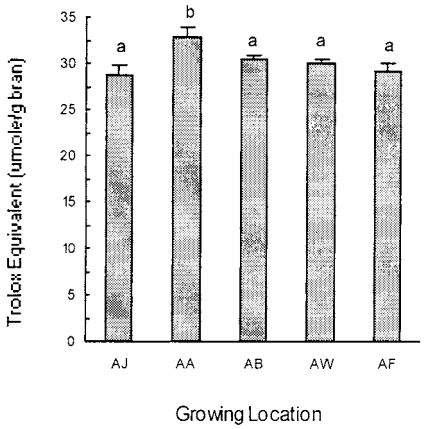

Figure 1. Radical cation scavenging capacity of bran extracts. The radical cation scavenging capacity of the five bran extracts was expressed as trolox equivalent. AJ, AA, AB, AW, AF represent Akron wheat at growing locations of Julesburg, Akron, Burlington, Walsh, and Fort Collins, respectively. Vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ($P < 0.05$).

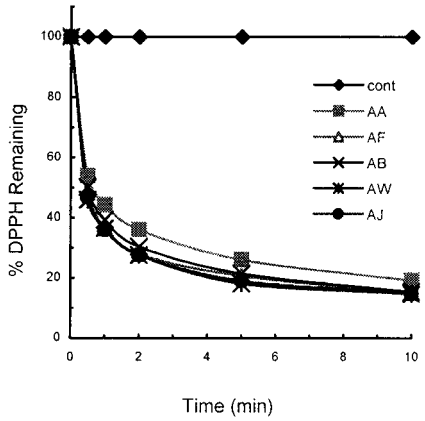

Figure 2. Reaction kinetics of bran extracts with DPPH radical. AA, AF, AB, AW, and AJ represent Akron wheat at growing locations of Akron, Fort Collins, Burlington, Walsh, and Julesburg, respectively, while cont represents the control containing no antioxidant. The final DPPH radical concentration was 100 μM in all reaction mixtures. The concentrations of bran extracts were on the same dry bran weight basis. All tests were conducted in triplicate, and the mean values are used.

radical cation scavenging activities was detected among bran samples from Julesburg, Walsh, Burlington, and Fort Collins, while bran samples from Akron had $ABTS^{\bullet+}$ scavenging capacity that was significantly higher than that from the other four locations (**Figure 1**). The $ABTS^{\bullet+}$ scavenging capacity was not correlated to total solar radiation, daily average solar radiation, or number of hours exceeding 32 °C.

Radical DPPH Scavenging Activity. The bran extracts from each location showed free radical scavenging activity against $DPPH^{\bullet}$ (**Figures 2** and **3**). Similar dose and time effects were observed for all bran extracts (**Figure 2**), suggesting similar reaction kinetics was followed in the bran antioxidant−$DPPH^{\bullet}$ reactions. Furthermore, some differences in their capacities to react with and quench DPPH radicals were detected among bran samples obtained from different locations (**Figure 3**). The bran from Akron had the strongest $DPPH^{\bullet}$ scavenging activity, followed by the bran from Burlington, Fort Collins, Walsh, and Julesburg. This is in the same order as the radical cation scavenging activity for the bran samples from the four nonirrigated locations of Akron, Burlington, Walsh, and Julesburg.

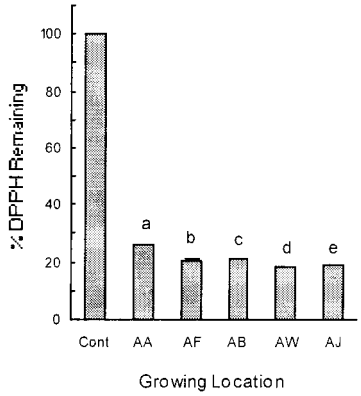

Figure 3. Radical DPPH scavenging activity. AA, AF, AB, AW, and AJ represent Akron wheat at growing locations of Akron, Fort Collins, Burlington, Walsh, and Julesburg, respectively, while cont represents the control containing no antioxidant. The final DPPH radical concentration was 100 μM in all reaction mixtures. The concentrations of bran extracts were on the same dry bran weight basis. All tests were conducted in triplicate, and the mean values are used. The vertical bars represent the standard deviation of each data point. Values marked by the same letter are not significantly different ($P < 0.05$).

Table 2. Chelating Properties and Phenolic Content of Bran Extracts[a]

growing location	location type	equiv of EDTA (μg/g of bran)	equiv of gallic acid (mg/g of bran)
Akron	nonirrigated	566 ± 33a	2.87 ± 0.02a
Burlington	nonirrigated	666 ± 25b	2.29 ± 0.03b
Julesburg	nonirrigated	557 ± 25a	2.55 ± 0.03c
Walsh	nonirrigated	596 ± 23a	2.85 ± 0.04a
Fort Collins	irrigated	565 ± 79c	3.24 ± 0.02d

[a] Data are expressed as the mean ± standard deviation ($n = 3$). Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

Bran samples from the five locations significantly differed in their DPPH radical scavenging activities. The $DPPH^{\bullet}$ scavenging capacity was not correlated with total solar radiation, daily average solar radiation, or number of hours exceeding 32 °C.

Chelating Activity of Akron Bran Extracts. The chelating properties of bran extracts were examined against Fe^{2+} and reported as EDTA equivalents (**Table 2**). The greatest chelating activity was detected in bran extract from Burlington, followed by locations Walsh, Akron, Julesburg, and Fort Collins. The EDTA equivalent was 557−665 μg/g of bran for bran samples from the nonirrigated testing locations and was 440 μg/g of bran for bran obtained from the Fort Collins irrigated location. The bran extracts differed significantly in their chelating activities, except among the bran samples obtained from Julesburg, Akron, and Walsh. The chelating activities of the bran samples from the four nonirrigated locations were significantly correlated with both total solar radiation and the daily average solar radiation. The correlation coefficient (r) was −0.999 ($P = 0.001$) between the EDTA level and the total solar or daily average solar radiation for the four nonirrigated testing locations. No correlation between EDTA content and number of hours exceeding 32 °C was observed.

Total Phenolic Content. The bran samples from the five locations were examined and compared for their total phenolic content (TPC) expressed as gallic acid equivalents (**Table 2**). Significant difference in TPC was observed among bran extracts except between bran samples from Akron and Walsh. The gallic acid equivalent was 3.24 mg/g of bran for wheat grown at Fort Collins, which is significantly higher than that detected in the

Table 3. Antioxidant Properties of Bran Extracts on a per Unit of TPC Basis[a]

growing location	location type	(EDTA Eq)/(mg of TPC) (μg)	(TE Eq)/(mg of TPC) (μmol)	(% DPPH remaining)/(mg of TPC)
Akron	nonirrigated	197.3	11.5	9.1
Burlington	nonirrigated	291.2	13.3	9.3
Julesburg	nonirrigated	218.1	11.2	7.4
Walsh	nonirrigated	209.2	10.6	6.3
Fort Collins	irrigated	136.1	9.0	6.4

[a] TPC stands for total phenolic content, while EDTA Eq stands for EDTA equivalent. TE Eq is a measurement of ABTS$^{\bullet+}$scavenging capacity and is expressed as the trolox equivalent. The percent DPPH remaining was determined at 5 min of the antioxidant–DPPH radical reaction.

bran samples from the four nonirrigated testing locations (2.26−2.87 mg/g of bran). No correlation was detected among the TPC and DPPH radical scavenging activity, radical cation scavenging activity, or the chelating activity under the experimental conditions. The TPC was not significantly correlated with total solar radiation, daily average solar radiation, or number of hours exceeding 32 °C.

Antioxidant Capacities on a per Milligram of TPC Basis. Chelating ability and radical scavenging capacities against both DPPH$^{\bullet}$ and ABTS$^{\bullet+}$, on a per milligram of TPC basis, were calculated and expressed as (EDTA Eq)/(mg of TPC), (% DPPH remaining)/(mg of TPC), and (TE Eq)/(mg of TPC) (**Table 3**; see table footnote for definitions). The same hierarchy of (TE Eq)/(mg of TPC) and (%DPPH remaining)/(mg of TPC) was noted for bran samples from the four nonirrigated testing locations, although no significant correlation ($r = 0.85$, $P = 0.07$) between them was detected (**Table 3**). Interestingly, the (EDTA Eq)/(mg of TPC) was significantly correlated to the (TE Eq)/(mg of TPC) for the bran samples from the five growing locations ($r = 0.96$, $P = 0.01$). No correlation between (EDTA Eq)/(mg of TPC) and (% DPPH remaining)/(mg of TPC) was detected.

DISCUSSION

In this study, the bran extracts prepared from Akron wheat grown at different testing locations in Colorado significantly differed in their radical scavenging activities against DPPH$^{\bullet}$ and ABTS$^{\bullet+}$, indicating that the environmental factors may significantly alter the antioxidant properties of a particular wheat variety. This was further supported by the observation in this study that chelating activities and total phenolic content of the tested bran extracts significantly differed from each other. Chelating agents may inhibit the initiation of the free radical mediated peroxidation reactions, and phenolics are considered as a major group of compounds that contribute to the antioxidant activities. The variation of antioxidant activity among the bran samples of Akron wheat grown at the five testing locations is comparable to that among the three varieties (*1*, *2*). For instance, a 31% difference in the TPC was noted for the bran samples from the five locations in this study, while a 47% difference in the TPC was observed for the three hard winter wheat grains (Akron, Trego, and Platte) (*1*). These results strongly suggest that environmental conditions influence the antioxidant properties of hard winter wheat. This conclusion is supported by the observation that the growth temperature alters the antioxidant properties of strawberry (*4*) and by the observations that environmental conditions influence the baking quality and gluten protein quality of wheat (*12*, *13*). In addition, it was noted in this study that the orders of % DPPH$^{\bullet}$ remaining and ABTS$^{\bullet+}$ scavenging activity of the tested bran extracts are same for the four unirrigated locations. This order was different from the order of the chelating capacities of the bran extracts from the four locations, suggesting that growing conditions may have different influences on individual antioxidant activity of Akron wheat.

The correlations of selected environmental factors and individual antioxidant measurement were determined in this study. The selected environmental factors included the total solar radiation, average daily radiation, and number of hours over 32 °C during the grain filling period. Solar radiation reflects the UV exposure, which may be associated with free radical formation and singlet oxygen production. Elevated levels of free radicals and singlet oxygen may increase the oxidative stress in wheat and resulted in an increased production of antioxidants for self-defense against environmental stress. The number of hours over 32 °C during the grain filling period is considered as an indicator of high-temperature stress for hard winter wheat and has previously been shown to adversely affect the baking quality of hard winter wheat (*12*). In this study, no significant correlation between any antioxidant measurement and number of hours exceeding 32 °C was noted, suggesting that changes in the environmental conditions may have different influence on a selected physicochemical or functionality of wheat. Interestingly, a strong correlation was observed between the chelating activity and total solar radiation or average daily solar radiation for the bran obtained from the four nonirrigated locations, suggesting the potential use of solar radiation as a predictor for the chelating activity of Akron bran. This also indicated the possibility of producing a selected wheat variety with a desired antioxidant property under optimized growing conditions. Additional studies are required to evaluate other cultural conditions on the antioxidant properties of wheat using a greater sample of both field locations and varieties. Ongoing research is being conducted to investigate environmental effects, including solar radiation and heat stress, on wheat antioxidant properties using other hard winter wheat varieties.

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