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Antioxidant Properties of Bran Extracts from Trego Wheat Grown at Different Locations

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The effects of growing conditions during the grain-filling period, including high temperature stress, total solar radiation, and average daily solar radiation, on the antioxidant properties of Trego wheat were evaluated. Bran extracts were prepared from Trego wheat, grown at four nonirrigated and one irrigated location in Colorado, and compared for their radical scavenging activities against $ABTS^{\bullet+}$ and $DPPH^{\bullet}$, Fe^{2+} chelating capacities, and total phenolic contents. Significant differences in radical scavenging activities, chelating capacities, and total phenolic contents were detected among Trego bran samples grown at different locations, suggesting that growing conditions may influence the antioxidant properties of wheat. The bran sample obtained from Fort Collins had the strongest scavenging activity against either $ABTS^{\bullet+}$ or $DPPH^{\bullet}$ radicals and the greatest chelating activity, whereas the highest total phenolic content was detected in bran samples from Walsh, indicating that each antioxidant activity may respond to the environmental changes differently. Positive correlations were detected between the $DPPH^{\bullet}$ scavenging activity and either total solar radiation ($r = 0.97$, $p = 0.03$) or average daily solar radiation ($r = 0.97$, $p = 0.03$). In addition, HPLC analysis detected the presence of ferulic, syringic, vanillic, *p*-hydroxybenzoic, and coumaric acids in wheat bran. Additional research is needed to further investigate the effects of environmental conditions and the interactions between genotype and environmental factors on the antioxidant properties of wheat to promote the production of wheat with improved antioxidant properties by optimizing the growing conditions for a selected genotype.

KEYWORDS: Wheat; radical scavenging; antioxidant; phenolic; chelating; $ABTS^{\bullet+}$; DPPH

INTRODUCTION

Free radical mediated lipid peroxidation develops rancidity, forms toxic chemicals in food products, and is a major problem for food quality and safety. Antioxidants may suppress the formation of free radicals, react with and quench the existing radicals, and reduce the availability of oxygen in the system to suppress the lipid peroxidation in food products, consequently improving food quality and safety. Free radicals may also attack and damage important biological components, including DNA and membrane lipids, in biological systems and lead to illnesses, including cancers, heart diseases, and other aging-associated health problems. Antioxidant treatments have shown the potential to prevent these illnesses. It has been a continuous effort to develop natural antioxidants for their potential applications in improving the quality and safety of food products and preventing human illnesses (*1*−*10*), because of consumer concerns about the safety of the synthetic antioxidants. For this same reason, consumers prefer natural antioxidants from edible materials, such as spices and wheat.

Significant antioxidant activities have been detected in wheat and wheat-based food products (*1*, *2*, *11*−*17*). Our previous studies detected significant antioxidant activities in hard winter wheat varieties of Akron, Trego, and Platte (*Triticum aestivum*) (*1*, *2*). Grain extracts prepared from the three wheat varieties showed significant free radical scavenging capacities against stable 2,2-diphenyl-1-picrylhydrazyl radicals ($DPPH^{\bullet}$) and 2,2′-azinodi[3-ethylbenzthiazoline sulfonate] radical cation ($ABTS^{\bullet+}$), Fe^{2+} chelating activities, and significant levels of phenolic components (*1*, *2*). It was also noted that the three varieties of wheat might significantly differ in their antioxidant activities. In addition, the three wheat grain extracts were evaluated for their capacity in inhibiting lipid oxidation in fish oils. Trego extracts had strongest inhibitory activity against lipid peroxidation in fish oils, on a grain weight basis (*2*). These results showed the potential of developing natural antioxidants from hard winter wheat, as well as the potential influence of genotype on the antioxidant properties. Recent study in our laboratory (*17*) showed that the growing conditions, such as solar radiation and temperature stress, might influence the antioxidant properties of Akron wheat, a hard red winter wheat variety (*T. aestivum*).

The present study was conducted as a part of our continuing efforts to investigate the potential effects of growing conditions

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and the interactions between growing conditions and genotype on the antioxidant properties of hard winter wheat, which is an important component of both dryland and irrigated production systems in Colorado and the west central Great Plains. The antioxidant properties of bran extracts from Trego wheat grown at five different locations and the potential correlations between antioxidant activities and growing conditions including solar radiation were examined to identify the influence of environmental factors on the antioxidant properties of hard white winter wheat. The results from this study will be used to promote the production of wheat rich in natural antioxidants.

MATERIALS AND METHODS

Materials. Grain samples of Trego, a hard white winter wheat variety adapted for production in Colorado and the west central Great Plains, were used for this study. Samples were obtained at harvest from breeding trials conducted at four nonirrigated testing locations in eastern Colorado, including Akron (A), Burlington (B), Julesburg (J), and Walsh (W), and an irrigated testing location at Fort Collins (F). Grain samples were cleaned using seed cleaners to remove all nongrain debris present following harvest. DPPH$^{\bullet}$ and 2,2′-bipyridyl were purchased from Sigma-Aldrich (St. Louis, MO). A total antioxidant status kit was purchased from Randox Laboratories Ltd. (San Francisco, CA).

Extraction and Testing Sample Preparation. Wheat grain of each location was milled on a Quadromat Junior experimental mill and separated into bran and flour fractions. Ten grams of the bran fraction was extracted with 100 mL of absolute ethanol for 15 h under nitrogen at ambient temperature. The ethanol extracts were kept in the dark under nitrogen until further analysis. To prepare a dimethyl sulfoxide (DMSO) solution of each bran sample, 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 the dark under nitrogen until further analysis.

Radical Cation ABTS$^{\bullet+}$ Scavenging Activity. The ABTS$^{\bullet+}$ scavenging activity was determined using a commercial kit from Randox Laboratories Ltd. 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 bran sample (*1*). The tests were conducted in triplicate for each extract.

Radical DPPH$^{\bullet}$ Scavenging Activity. The total free radical scavenging capacity of each bran extract was estimated according to the previously reported procedure using the stable DPPH$^{\bullet}$ (*1*). Briefly, freshly made DPPH$^{\bullet}$ solution was mixed into a bran extract to start the radical−antioxidant reaction. The final concentration was 100 mM for DPPH$^{\bullet}$. The absorbance at 517 nm was determined against a blank of pure ethanol at 0, 0.5, 1, 2, 5, and 10 min of reaction and used to estimate the remaining radical levels according to a standard curve, which was prepared by plotting the absorbance at 517 nm of standard DPPH radical solution against the concentration.

Chelating Activity. Fe^{2+} chelating capacity was measured using a 2,2′-bipyridyl competition assay (*2*, *18*). The reaction mixture contained 0.25 mL of 1 mM $FeSO_4$ solution, 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), 1 mL of Tris-HCl buffer (pH 7.4), and 1.5 mL of EtOH. The final volume was made up to 5 mL with ethanol. The absorbance at 522 nm was determined against a solvent blank and used to evaluate Fe^{2+} chelating capacity using disodium ethylenediaminetetraacetate (EDTA) as a standard.

Total Phenolic Contents. The total phenolic content of each bran extract was determined using the Folin−Ciocalteu reagent following a previously described procedure (*1*, *19*, *20*). The reaction mixture contained 100 μL of bran extract, 500 μL of 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 contents using gallic acid as a standard. Reactions were performed in triplicate.

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

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

Phenolic Acid Composition. After the removal of acetone, the wheat 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 (*21*). The ethyl ether/ethyl acetate was evaporated at 25 °C using a nitrogen evaporator, and the solid residue was redissolved in methanol, filtered through a 0.45 μm membrane filter, and analyzed by HPLC using a Phenomenex C18 column (250 mm × 4.6 mm). The mobile phase contained 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 (*22*). Identification of phenolic acids was accomplished by HPLC-MS and comparing the retention of peaks in wheat samples to that of the standard compounds.

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

RESULTS

Field Testing Locations. The year 2001 wheat-growing season in Colorado was characterized by below normal precipitation and drought stress prior to heading, above normal precipitation near flowering, and higher than normal temperatures during the grain-filling period (e.g., the 6-week period preceding wheat harvest). Solar radiation, influenced both by altitude and by 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 Trego wheat grown at Akron, Burlington, Fort Collins, Julesburg, and Walsh were examined and compared for their free radical scavenging activities against radical cation ABTS$^{\bullet+}$. All five Trego bran extracts showed significant ABTS$^{\bullet+}$scavenging capacity (**Figure 1**). The bran obtained from Fort Collins had the greatest activity to quench ABTS$^{\bullet+}$, followed by Walsh, Julesburg, Burlington, and Akron. The Trolox equivalents were 35.3, 31.4, 28.2, 26.7, and 24.2 μmol/g of bran for locations Fort Collins, Walsh, Julesburg, Burlington, and Akron, respectively. The five bran samples differed significantly in their radical cation scavenging activities, but no significant correlation was detected between the cation ABTS$^{\bullet+}$scavenging capacity of Trego bran extracts and the total solar radiation, or average daily solar radiation, or the total hours exceeding 32 °C.

Radical DPPH Scavenging Activity. The bran extracts from Trego wheat grown at all locations showed free radical scavenging activity against DPPH$^{\bullet}$ (**Figures 2** and **3**). Similar dose and time effects were observed for all Trego bran extracts (**Figure 2**), suggesting a similar reaction kinetics was followed

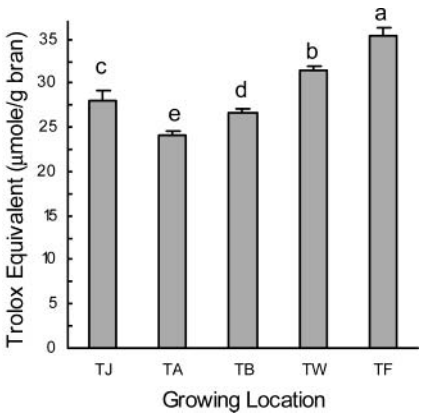

Figure 1. Radical cation scavenging capacity of Trego bran. The radical cation scavenging capacity of the five Trego bran extracts was expressed as Trolox equivalent. TJ, TA, TB, TW, and TF represent Trego wheat grown at Julesburg, Akron, Burlington, Walsh, and Fort Collins in Colorado, respectively. Vertical bars represent the standard deviation of each data point. Locations marked by the same letter are not significantly different ($n = 3$, $p < 0.05$).

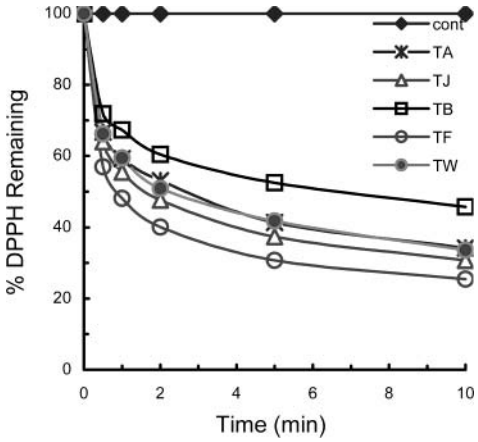

Figure 2. Reaction kinetics of Trego bran extracts with DPPH radical. TA, TJ, TB, TF, and TW represent Trego wheat grown at Akron, Julesburg, Burlington, Fort Collins, and Walsh in Colorado, respectively, whereas cont represents the control containing no antioxidant. The final DPPH radical concentration was 100 μM in all reaction mixtures ($n = 3$, $p < 0.05$).

in the Trego bran antioxidant−DPPH$^{\bullet}$ reactions. The bran from Fort Collins had the strongest DPPH$^{\bullet}$ scavenging activity, followed by the bran from Julesburg, Walsh, Akron, and Burlington. The bran extracts differed significantly in their capacities to react with and quench DPPH radicals, except between samples collected from Akron and Walsh. The DPPH radical scavenging activity of the bran from the four nonirrigated locations was significantly correlated to both total solar radiation, with a correlation coefficient of 0.97 ($p = 0.03$), and the daily average solar radiation, with a correlation coefficient of 0.97 ($p = 0.03$).

Chelating Activity. The chelating activities of bran extracts were estimated against Fe^{2+} and reported as EDTA equivalents (**Figure 4**). The greatest chelating activity was detected in the bran sample obtained from the Fort Collins testing location, followed by Walsh, Burlington, Akron, and Julesburg. The EDTA equivalents were 889, 759, 752, 701, and 629 μg/g of bran for samples obtained from Fort Collins, Walsh, Burlington, Akron, and Julesburg, respectively. The bran extracts significantly differed in their chelating activities, except among the bran samples collected at Akron, Burlington, and Walsh.

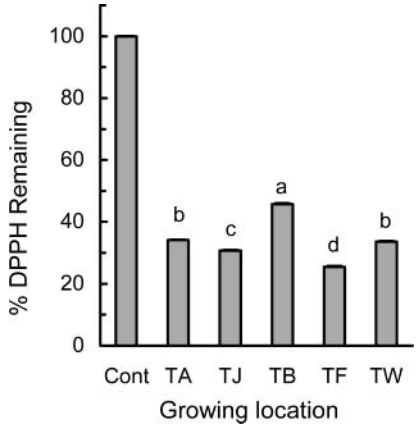

Figure 3. Radical DPPH scavenging activity of Trego bran extracts. TA, TJ, TB, TF, and TW represent Trego wheat grown at Akron, Julesburg, Burlington, Fort Collins, and Walsh in Colorado, respectively, whereas Cont represents the control containing no antioxidant. The final concentration of DPPH radical was 100 μM in all reaction mixtures. The concentrations of the five Trego bran samples were on the same dry weight basis. The % DPPH remaining was determined at 10 min of each reaction. The vertical bars represent the standard deviation of each data point. Locations marked by the same letter are not significantly different ($n = 3$, $p < 0.05$).

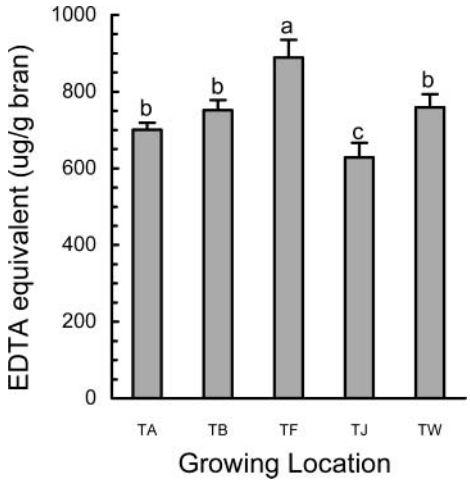

Figure 4. Chelating property of Trego bran. The chelating activities of the bran extracts were expressed as the EDTA equivalent. TA, TB, TF, TJ, and TW represent Trego wheat grown at Akron, Burlington, Fort Collins, Julesburg, and Walsh, respectively. The vertical bars represent the standard deviation ($n = 3$). Locations marked by the same letter are not significantly different ($p < 0.05$).

Furthermore, the chelating activity of the Trego bran extracts was not significantly correlated with any recorded growing conditions including the hours exceeding 32 °C, total solar radiation, or daily average radiation.

Total Phenolic Content. The total phenolic contents (TPC) of the five bran samples were reported as gallic acid equivalents (**Figure 5**). Bran obtained from Walsh contained the greatest level of TPC (3.05 mg/g of bran), followed by that from Fort Collins, Burlington, Julesburg, and Akron. The gallic acid equivalents were 2.97, 2.49, 2.46, and 2.29 mg/g of bran for the samples from Fort Collins, Burlington, Julesburg, and Akron, respectively. A significant difference in TPC was detected among the bran samples except the samples from Walsh and Fort Collins or from Burlington and Julesburg. No significant correlation was observed between the TPC values of Trego bran

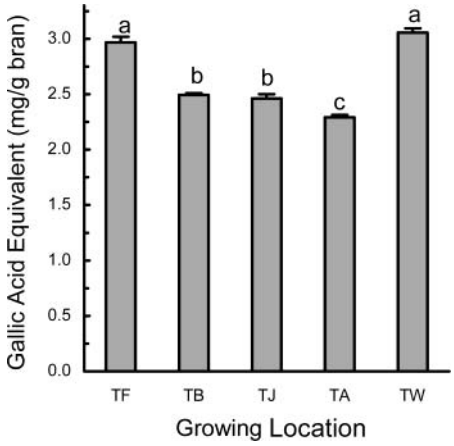

Figure 5. Total phenolic contents of Trego bran. TF, TB, TJ, TA, and TW represent Trego wheat grown at Fort Collins, Burlington, Julesburg, Akron, and Walsh, respectively. The vertical bars represent the standard deviation ($n = 3$). Locations marked by the same latter are not significantly different ($p < 0.05$).

Table 2. Antioxidant Properties of Trego Bran Extracts on per Unit of TPC Basis[a]

growing location	location type	EDTA equiv/ mg of TPC (μg)	TE equiv/ mg of TPC (μmol)	% DPPH quenched/ mg of TPC
Akron	nonirrigated	305.9a	10.6a	85.1b
Burlington	nonirrigated	302.1a	10.7ab	81.6a
Julesburg	nonirrigated	255.6b	11.4bc	87.5c
Walsh	nonirrigated	248.0b	10.3a	89.0d
Fort Collins	irrigated	299.3a	11.9c	91.4e

[a] TPC, total phenolic contents; EDTA equiv, EDTA equivalent; TE equiv, measurement of $ABTS^{\bullet+}$ scavenging capacity, expressed as the Trolox equivalent. % DPPH quenched was determined at 10 min of the antioxidant–DPPH radical reaction. Values within the same column marked by the same latter are not significantly different ($p < 0.05$)

and any tested growing conditions including total or daily average solar radiation or the hours exceeding 32 °C.

Antioxidant Capacities on a per Milligram of TPC Basis. Fe^{2+} chelating capacity and radical scavenging activities against both $DPPH^{\bullet}$ and $ABTS^{\bullet+}$, on a per milligram of TPC basis, were calculated and expressed as EDTA equivalents per milligram of TPC, percent DPPH remaining per milligram of TPC, and TE equivalents per milligram of TPC, respectively (**Table 2**). Interestingly, the bran sample from Fort Collins, an irrigated location, showed the greatest radical scavenging activities against both $DPPH^{\bullet}$ and $ABTS^{\bullet+}$, but the lowest chelating capacity against Fe^{2+}, on a per milligram of TPC basis. No correlation was observed among these antioxidant activities on a per TPC basis.

Phenolic Acid Composition. The phenolic acid composition was examined only for bran samples collected from Burlington and Walsh due to the sample availability. Ferulic acid was the predominant phenolic acid present in wheat bran, along with syringic, vanillic, *p*-hydroxybenzoic, and coumaric acids (**Table 3**). Trego bran samples from different growing locations differed in all phenolic acids content except that of syringic acid.

DISCUSSION

Diet can significantly alter the overall health and quality of life (*1*). Dietary treatment is of paramount importance for the prevention of diseases that have no medicines to affect a cure once the disease is established. Cancer, the leading cause of death for people less than 65 years old in Western countries (*23*), and coronary heart disease (CHD), estimated as having an annual cost of $50−100 billion in the United States (*24*), are two such diseases. 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 CHD (*7*−*9*, *25*). Antioxidant treatments may terminate ROS attacks and reduce the risk of CHD, cancer, and other aging-associated diseases including Parkinson's disease (*10*, *17*, *26*−*28*). In addition, antioxidants are important food additives to enhance the quality, stability, and safety of food products. Novel natural antioxidants with desired physicochemical properties are in high demand for their applications as nutraceuticals in disease prevention and health promotion, as well as food additives to improve the quality, stability, and safety of food products. This study was conducted as part of our series of investigations to determine whether and how environmental conditions may affect the antioxidant properties of hard winter wheat. This information will be used to identify optimum conditions to produce the selected wheat variety rich in natural antioxidants.

It was observed that environmental conditions, such as temperature stress (total hours exceeding 32 °C), might alter the baking quality of hard winter wheat grown in Nebraska (*29*). In 2001, Wang and Zheng (*4*) reported that the growing temperature influenced the antioxidant capacity of strawberry (*Fragaria* × *ananassa* Duch.). Wang and Zheng (*4*) evaluated four day/night temperature conditions, including 18/12, 25/12, 25/22, and 30/22 °C, for their effects on the productions of phenolic acid, flavonol, and anthocyanin and on antioxidant capacities against peroxyl radicals, superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen, in Earliglow and Kent strawberry cultivars. The highest day/night temperature condition (30/22 °C) resulted in the greatest production of phenolics and antioxidant activities in strawberry fruits. Therefore, it was interesting to know whether temperature stress, the total hours exceeding 32 °C, might influence the antioxidant properties of hard winter wheat. No correlations between the temperature stress and any tested antioxidant properties of Trego bran samples were detected in this study, suggesting that the antioxidant production in hard winter wheat may not be sensitive to temperature changes. This was supported by our previous observation that none of the tested antioxidant properties of Akron bran samples obtained from the five locations was correlated with temperature stress (*17*).

In addition to the temperature stress, total and daily average solar radiations were investigated for their potential effects on

Table 3. Phenolic Acid Composition of Trego Bran Samples[a]

growing location	*p*-hydroxybenzoic acid (μg/g of bran)	vanillic acid (μg/g of bran)	syringic acid (μg/g of bran)	coumaric acid (μg/g of bran)	ferulic acid (μg/g of bran)
Burlington	11.1a ± 0.1	15.2a ± 0.4	33.3a ± 0.3	3.7a ± 0.0	90.9a ± 3.1
Walsh	21.9b ± 1.3	13.1b ± 0.2	32.5a ± 0.5	6.4b ± 0.2	111.4b ± 2.0

[a] Means ($n = 3$) within a column followed by different letters are significantly different ($p < 0.05$).

the antioxidant properties of Trego wheat, because the solar radiation reflects the UV light exposure that may alter the oxidative stress and consequently modify the production of antioxidants in plants for self-defense. The results from this research showed significant positive correlation between DPPH radical scavenging activity of the bran samples grown at the four nonirrigated locations and total or daily average solar radiation, suggesting that UV stress may alter the antioxidant properties of wheat. In contrast to that observed with the DPPH scavenging activity, chelating activity and radical cation $ABTS^{\bullet+}$ scavenging ability of Trego bran were not correlated with either total solar or average daily solar radiation. These observations are different from what we have detected in bran extracts of Akron wheat grown at the same locations in the same year (*17*). The chelating activity of Akron bran was negatively correlated to total or average daily solar radiation, but no correlation was detected between solar radiation and radical scavenging activities of Akron bran extracts against either $DPPH^{\bullet}$ or $ABTS^{\bullet+}$, suggesting that wheat varieties may differ in how their antioxidant properties respond to environmental changes. These data also suggest the possibility to produce wheat and wheat-based products rich in antioxidants by optimizing the growing conditions for a selected wheat variety. More research is necessary to further investigate the environmental conditions and the interactions between genotype and environmental conditions for their effects on the antioxidant properties of wheat.

It has been well recognized that individual genotypes of an agricultural crop may not exhibit the same relative antioxidant activities in different testing systems, such as radical scavenging activities against $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ (*1*, *2*, *30*, *31*). Yu and others (*1*) detected greatest $DPPH^{\bullet}$ scavenging activity in the Akron extract, followed by Platte and Trego, but observed the strongest $ABTS^{\bullet+}$ scavenging activity in the Platte extract under the same experimental conditions. In this study, bran extracts prepared from Trego wheat grown at different locations showed different relative antioxidant activities in different testing systems, suggesting that environmental conditions may differentially influence the production of individual antioxidant compounds and antioxidative activities. The bran extracts from wheat grown at Fort Collins, an irrigated location with lower stress levels overall, had the greatest radical scavenging activity against $ABTS^{\bullet+}$ followed by that grown at Walsh, Julesburg, Burlington, and Akron, whereas the lowest $DPPH^{\bullet}$ scavenging capacity was detected in bran extracts prepared from wheat grown at Burlington. These results suggest that Trego bran that has a greater scavenging activity against $DPPH^{\bullet}$ does not necessarily have a higher activity to quench $ABTS^{\bullet+}$, indicating that individual antioxidant properties of wheat may respond differently to the environmental changes.

In conclusion, bran extracts from Trego wheat grown at five locations in Colorado might significantly differ in their radical scavenging capacities against $ABTS^{\bullet+}$ and $DPPH^{\bullet}$, Fe^{2+} chelating activities, and total phenolic contents, indicating the potential effects of growing conditions on the antioxidant properties of wheat. The DPPH radical scavenging activity of Trego bran from the four nonirrigated locations was positively correlated with total or daily average solar radiation, whereas no correlation was observed between high-temperature stress and any antioxidant activities of Trego wheat, suggesting that individual antioxidant properties may respond to environmental changes differently. Further research is required to investigate individual environmental factors and the interactions between the genotype and environments on each antioxidant property of wheat, to promote the production of wheat rich in natural antioxidants.

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