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Antioxidant properties of bran extracts from 'Platte' wheat grown at different locations

Liangli Yu*, Kequan Zhou

Department of Nutrition and Food Science, Room 0112, Skinner Building, University of Maryland, College Park, Maryland 20742, US

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Abstract

The effects of environmental factors including total solar radiation, average daily solar radiation, and number of hours exceeding 32 °C on the antioxidant properties and total phenolic contents (TPC) of Platte wheat were evaluated. Bran extracts of Platte wheat grown at one irrigated and four nonirrigated testing locations in Colorado were examined for their radical scavenging activities against $ABTS^{\cdot+}$ and DPPH·, Fe^{2+} chelating capacity, and TPC. Bran samples from Fort Collins, the irrigated location, had the greatest $ABTS^{\cdot+}$ scavenging activity of 35.8 μmole trolox equivalent/g bran, and the strongest DPPH· scavenging capacity. Differences in chelating activities and TPC were also detected among bran samples from individual growing locations. Total phenolic contents of Platte bran grown at five different locations were correlated with the number of hours exceeding 32 °C ($r = -0.86$, $P = 0.06$). No correlation between solar radiations and any of the tested antioxidant properties was detected in this study. These data indicated the potential influence of growing conditions on the antioxidant properties and TPC of wheat. Furthermore, individual antioxidant property of a selected wheat genotype may respond to the environmental changes differently.

Keywords: Wheat; Radical scavenging; Antioxidant; Phenolic; Chelating; $ABTS^{\cdot+}$; DPPH·

1. Introduction

Diet can significantly alter the overall health and quality of life (Dreher, 1997; Simopoulos, 1997). Dietary treatment plays an important role in preventing the diseases that have no medicines to cure once the disease is established. Cancer, the leading cause of death for people less than 65 years old in Western countries (Lavillonniere & Bougnoux, 1999), and coronary heart disease (CHD), estimated with an annual cost of $50–100 billion in the United States (Aygustin & Dwyer, 1999) are two such diseases. Growing evidence suggests that the reactive oxygen species (ROS) generated during cellular metabolism or lipid peroxidation play a causative role in the pathogenesis of cancer and CHD (Frenkel, 1992; Marnett, 2000; Slaga et al., 1987; Zhao, Lahiri-Chatterjee, Sharma, & Agarwal, 2000). Antioxidant treatments may terminate ROS attack and reduce the risk of coronary heart diseases, cancers, and other aging associated diseases including Parkinson's disease (Espin, Soler-Rivas, & Wichers, 2000; Merken & Beecher, 2000; Neff, 1997; Wong, Li, & Stadlin, 1999). In addition, antioxidants are important food additives to enhance the quality, stability, and safety of food products (Yu, Scanlin, Wilson, & Schemidt, 2002b). Novel natural antioxidants with desired physicochemical properties are in high demand for their applications as nutraceuticals, as well as food additives because of their consumer preference.

Hard winter wheat (*Triticum aestivum*) is an important commodity in Colorado and the Great Plains of the United States. The average annual farm gate value of the hard winter wheat is over $300 million in Colorado alone (Yu et al., 2002a). Recently, the wheat producers have had to search for new value-added marketing opportunities for the wheat because the farmers have suffered from the record low price of the wheat market. Identification of the health promoting factors and novel

* Corresponding author. Tel.: +1-301-405-0761; fax: +1-301-314-3313.
E-mail address: ly46@umail.umd.edu (L. Yu).

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value-added utilizations of wheat will enhance their marketing potentials, and benefit the agricultural economy. It has been well accepted that natural antioxidants may inhibit lipid peroxidation in food products and improve food quality and safety (Yu et al., 2002b). Natural antioxidants may also improve the redox status in biological systems and reduce the risk of aging associated health problems including cancers and heart diseases (Halliwell, 1996; Wang & Zheng, 2001; Yu et al., 2002a; Yu, Haley, Perret, & Harris, 2002c). Therefore, investigating antioxidant properties of wheat may lead to the production of novel wheat antioxidants, wheat based food products containing enhanced levels of natural antioxidants and new wheat varieties rich in antioxidants, and consequently may improve the agricultural economy, the quality and safety of food products, and human health.

The proposed antioxidative mechanisms include suppressing the generation of the first few radicals which initiate the oxidative chain reactions, directly reacting with and quenching free radicals in the system to terminate radical attacks on biological and food components, scavenging singlet oxygen molecules, acting as reducing agents, and inducing the activity of the antioxidative defense system in biological systems (Yu, Perret, Davy, Wilson, & Melby, 2002d; Zielinski & Kozlowska, 2000). Significant antioxidant activities have been detected in wheat (Onyeneho & Hettiarachchy, 1992; Yu et al., 2002a; Yu et al., 2002b; Zielinski & Kozlowska, 2000) and wheat-based cereal products (Baublis, Clydesdale, & Decker, 2000a; Baublis, Lu, Clydesdale, & Decker, 2000b; Yu et al., 2002d). In 1992, Onyeneho and Hettiarachchy reported that extracts of Durum wheat (*Triticum durum*) suppressed lipid peroxidation in oils. Later in 2000, extracts of Almari and Henika wheat were shown to inhibit radical-induced lipid peroxidation in liposomes, and quench radical cations (Zielinski & Kozlowska, 2000). Our previous studies showed that the grains of the hard winter wheat Akron, Trego and Platte (*Triticum aestivum*) contained significant antioxidant activities (Yu et al., 2002a, 2002c). The grain extracts prepared from the three wheat varieties directly reacted with and quenched stable 2,2-diphenyl-1-picryhydrazyl radicals (DPPH$^{\cdot}$) and the 2,2′-azino-di[3-ethylbenzthiazoline sulfonate] radical cation (ABTS$^{\cdot+}$). In addition, significant Fe^{2+} chelating activities and inhibitory effects of lipid oxidation in fish oils were detected in wheat grain extracts (Yu et al., 2002a, 2002c). Furthermore, the three varieties of wheat differed in their antioxidant activities. These results showed the possibility of developing natural antioxidants from hard winter wheat, as well as the potential influence of genotype on the antioxidant properties.

It is well known that environmental factors, such as the number of hours exceeding 32 °C during the grain filling period, may significantly influence the baking quality of hard winter wheat (Blumenthal, Barlow, & Wrigley, 1993; Peterson, Graybosch, Shelton, & Baenziger, 1998). Recently, the effects of growing conditions on the antioxidant properties of strawberry were demonstrated (Wang & Zheng, 2001). In that study, the effects of growing temperature on phenolic acid, flavonol, and anthycyanin contents and antioxidant activities of two strawberry cultivars were evaluated. Strawberrys grown at higher temperature conditions exhibited greater antioxidant activities and higher phenolic contents. Recently, a preliminary study conducted in our laboratory showed that growing conditions may influence the antioxidant properties of Akron wheat, a hard red winter wheat variety (Yu, Perret, Harris, Wilson, & Haley, 2003). This study was conducted as part of our series investigation to determine whether and how environmental factors may influence the antioxidant properties of hard winter wheat. This information will be used to identify the optimum conditions to produce a selected wheat variety rich in natural antioxidants. In the present study, we examined antioxidant properties of bran extracts from Platte wheat, a hard white winter variety, grown at five different locations, and determined the potential correlation between each antioxidant property and each selected environmental factor. This information is needed for production of value-added wheat grain high in natural antioxidants.

2. Materials and methods

2.1. General

Grain samples of 'Platte', a hard white winter wheat variety, adapted for production in Colorado and the west central Great Plains, were used in this study. Wheat grain samples were provided by Dr. Scott Haley in the Soil and Crop Science Department at Colorado State University (Fort Collins, Colorado). These grains were obtained at harvest from breeding trials conducted at four nonirrigated testing sites located throughout eastern Colorado (Akron, Burlington, Julesburg, and Walsh) and 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-diphenyl-1-picryhydrazyl radical (DPPH$^{\cdot}$), 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid, disodium ethylenediaminetetraacetate, and 2,2′-bipyridyl were purchased from Sigma–Aldrich (St. Louis, MO), while 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.

2.2. Extraction and testing sample preparation

Platte grain from each location was ground and separated into bran and flour fractions using a Quadromat Junior experimental mill. 10 g of bran was extracted with 100 ml of ethanol for 15 h under nitrogen at ambient temperature (Yu et al., 2003). The ethanol extracts were kept under nitrogen until further analysis. The dimethyl sulfoxide (DMSO) solution of each bran sample was prepared from the ethanol extract. Ethanol was removed under vacuum from 20 ml of the ethanol extract, and the solid residue was quantitatively re-dissolved in 10 ml of DMSO. The resulting DMSO solution was also kept in dark under nitrogen until further analysis.

2.3. Radical cation $ABTS^{\cdot+}$ scavenging activity

The $ABTS^{\cdot+}$ scavenging activity of Platte bran extract was determined following a laboratory procedure using a commercial kit from Randox Laboratories Ltd. (San Francisco, CA). Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) was used as an antioxidant standard to prepare the standard curve and calculate the trolox equivalent for each sample. The radical cation scavenging activity of bran extract was expressed as trolox equivalent (Yu et al., 2002a).

2.4. Radical DPPH scavenging activity

The free radical scavenging capacity of each bran extract was estimated following a previously reported procedure using the stable 2,2-diphenyl-1-picryhydrazyl radical ($DPPH^{\cdot}$) (Yu et al., 2002a). Briefly, freshly prepared $DPPH^{\cdot}$ solution was added into a bran extract to start the radical-antioxidant reaction. 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. The final concentration was 100 μM for $DPPH^{\cdot}$.

2.5. Chelating activity

Fe^{2+} chelating capacity was determined using a 2,2′-bipyridyl competition assay (Yu et al., 2002b). The reaction mixture contained 200 μl of antioxidant solution, 100 μl 2% SDS, 100 μl of 1.8 mM $FeSO_4$ solution, 800 μl of 2,2′-bipyridyl solution (0.1% in 0.2 M HCl), 320 μl of 10% hydroxylamine-HCl, and 800 μl of Tris–HCl buffer (pH 7.4). The absorbance at 522 nm was measured and used to evaluate Fe^{2+} chelating capacity according to a standard curve. The standard curve was prepared using several known concentrations of disodium ethylenediaminetetraacetate (EDTA).

2.6. Total phenolic contents

The total phenolic content of each bran extract was measured using Folin–Ciocalteu reagent according to a laboratory procedure (Yu et al., 2002a). Briefly, the reaction mixture contained 100 μl of bran extract, 500 μl of the Folin–Ciocalteu reagent and 1.5 ml of 20% sodium carbonate. The final volume was made up to 10 ml with water. After two hours of reaction at ambient temperature, the absorbance at 765 nm was determined and used to calculate the phenolic contents using gallic acid as a standard.

2.7. Statistical analysis

Data were reported as means of 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, whereas a Pearson Correlation test was carried out to identify the correlations among means. Statistical significance was declared at $P < 0.1$.

3. Results and discussion

Bran extracts of Platte wheat grown at Akron, Burlington, Fort Collins, Julesburg and Walsh were examined and compared for their free radical scavenging activities against radical cation $ABTS^{\cdot+}$. All extracts showed significant $ABTS^{\cdot+}$ scavenging capacity (Fig. 1). The bran obtained from Fort Collins had the greatest activity to quench $ABTS^{\cdot+}$, followed by that from Burlington, Walsh, Julesburg, and Akron. The trolox equivalents were 35.8, 30.4, 30.4, 30.3, and 25.8 μmole per gram bran for Platte wheat at Fort Collins, Walsh, Julesburg, Burlington, and Akron, respectively. The $ABTS^{\cdot+}$ scavenging activity of Platte bran from Fort Collins was significantly higher than that from the other locations ($P < 0.05$), while the bran from Akron had a significantly lower $ABTS^{\cdot+}$ scavenging activity as compared to that from the other four locations ($P < 0.05$). No difference in $ABTS^{\cdot+}$ scavenging activity was found among the bran samples obtained from the locations of Burlington, Julesburg and Walsh. These data indicated that the growing conditions might alter the $ABTS^{\cdot+}$ scavenging activity of hard white winter wheat. This observation was supported by our study of the $ABTS^{\cdot+}$ scavenging activity in the bran extracts of Akron wheat (Yu et al., 2003). In that study, bran extracts prepared from Akron wheat grown at the five locations in Colorado differed in their $ABTS^{\cdot+}$ scavenging activities. No correlation was detected between $ABTS^{\cdot+}$ scavenging activity and any environmental factors including total

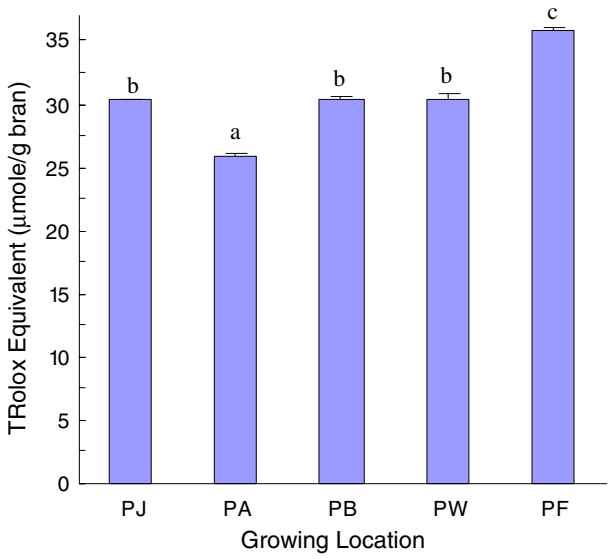

Fig. 1. Radical cation scavenging capacity of Platte bran. The radical cation scavenging capacity of the five bran extracts of Platte wheat was expressed as trolox equivalent. PJ, PA, PB, PW, and PF represent the Platte bran at the growing locations of Julesburg, Akron, Burlington, Walsh, and Fort Collins in Colorado, respectively. Vertical bars represent the standard deviation of each data point ($n = 3$). Values marked by the same latter are not significantly different ($P < 0.05$).

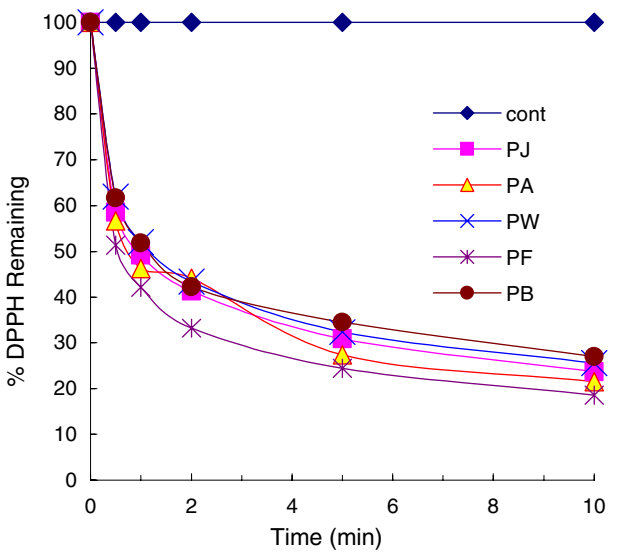

Fig. 2. Reaction kinetics of Platte bran extracts with DPPH radical. PJ, PA, PB, PW, and PF represent Platte bran at growing locations of Julesburg, Akron, Burlington, Walsh, and Fort Collins in Colorado, respectively, while the cont represents the control containing no antioxidant.The DPPH radical concentration was 100 μM in all reaction mixtures. All tests were conducted in triplicate and the means are used.

solar radiation, daily average solar radiation and number of total hours exceeding 32 °C (Table 1).

It has been widely accepted that the radical system used for the antioxidant evaluation may influence the experimental results, and two or more radical systems are required to investigate the radical scavenging capacities of a selected antioxidant (Yu et al., 2002d). To better understand the radical scavenging properties of Platte extracts, stable DPPH radicals were also employed in this research. The bran extracts from Platte wheat grown at all locations showed free radical scavenging activity against DPPH· (Figs. 2 and 3). Similar dose and time effects were observed for all Platte bran extracts (Fig. 2), suggesting similar reaction kinetics were followed in the Platte antioxidant-DPPH· reactions. Furthermore, significant differences ($P < 0.05$) in their capacities to react with and quench DPPH radicals were exhibited among bran samples obtained from different locations (Fig. 3). The bran from Fort Collins had the strongest scavenging activity, which corresponded to the lowest level of % DPPH· remaining, and was followed by the bran from Akron, Julesburg, Walsh, and Burlington. These data also suggest potential effects of growing conditions on the antioxidant properties of wheat. However, none of the three growing factors, including total solar radiation, average daily solar radiation and number of total hours exceeding 32 °C, was significantly correlated with the DPPH radical scavenging activity of Platte bran extracts. This observation was similar to that detected in Akron bran extracts but differed to that detected in Trego bran extracts obtained from different growing locations (Zhou & Yu, 2004). Positive correlation was observed between the DPPH· scavenging activity of Trego bran samples from different growing locations and both total solar radiation ($r = 0.97$, $P = 0.03$) and daily average solar radiation ($r = 0.97$, $P = 0.03$), while the DPPH· scavenging activity of Akron bran extracts was not correlated to solar radiation or temperature stress (Yu et al., 2003). These observations suggest that individual wheat genotypes may respond to environmental changes differently for a

Table 1
Total solar radiation (SR), average daily SR, and number of hours exceeding 32 °C during the six-week grain filling period at five wheat growing locations in Colorado (Yu et al., 2003)

Growing location	Total SR (MJ m^{-2})	Daily average SR (MJ m^{-2})	Hours exceeding 32 °C	Location type
Akron	1140	26.5	124	Nonirrigated
Burlington	624	14.5	113	Nonirrigated
Julesburg	1173	27.3	133	Nonirrigated
Walsh	1007	23.4	176	Nonirrigated
Fort Collins	994	23.1	57	Irrigated

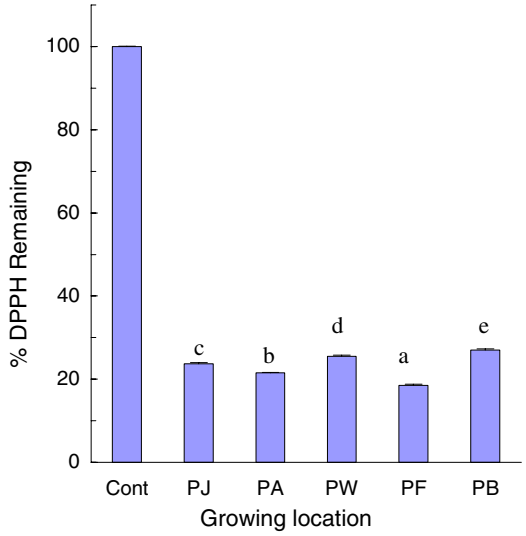

Fig. 3. Radical DPPH scavenging activity of Platte bran extracts. PJ, PA, PW, PF, and PB represent Platte bran at growing locations of Julesburg, Akron, Walsh, Fort Collins, and Burlington in Colorado, respectively, while the cont represents the control containing no antioxidant. The DPPH radical concentration was 100 μM in all reaction mixtures. The concentrations of the five Platte bran samples are on a 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 ($n = 3$). Values marked by the same letter are not significantly different ($P < 0.05$).

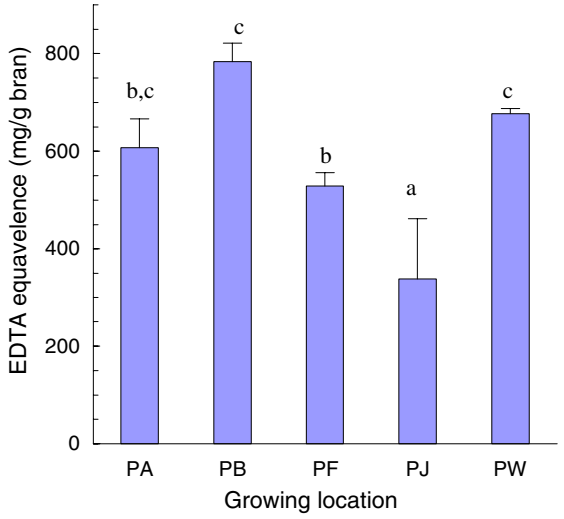

Fig. 4. Chelating capacity of Platte bran. The chelating activities of the bran extracts were expressed as the EDTA equivalent. PA, PB, PF, PJ, and PW represent Platte bran at the growing locations of Akron, Burlington, Fort Collins, Julesburg, and Walsh in Colorado, respectively. The vertical bars represent the standard deviation ($n = 3$). Values marked by the same letter are not significantly different ($P < 0.05$).

particular antioxidant property. Interactions between genotype and environmental factors may also contribute to the antioxidant properties of wheat.

It has been well recognized that chelating agents may stabilize transition metals and reduce their availability as catalysts, to inhibit the production of the first few free radicals and consequently suppress lipid peroxidation in biological and food systems (Nawar, 1996; Yu et al., 2002d). The chelating activities of Platte bran extracts were estimated against Fe^{+2} and reported as EDTA equivalents (Fig. 4). The greatest chelating activity was detected in bran samples obtained from Burlington, followed by those from Walsh, Akron, Fort Collins, and Julesburg. The bran extracts significantly differed in their chelating activities ($P < 0.05$), except the bran samples collected at Akron and Walsh, demonstrating the effects of environmental factors on the antioxidant properties. No correlation was detected between the chelating activity of Platte bran extracts and any of the tested growing conditions including total or daily average solar radiation, and number of hours exceeding 32 °C. This observation was similar to that detected in the bran extracts of Trego wheat, but differed to that observed in bran extracts of Akron wheat grown at different locations in Colorado (Yu et al., 2003; Zhou & Yu, 2004). The chelating activity of Akron bran samples from the nonirrigated locations of Burlington, Walsh, Akron, and Julesburg was significantly correlated to total solar radiation or daily average solar radiation ($r = -1.00$, $P = 0.001$). These observations indicate that environmental factors and interactions between the environmental factors and genotypes may modulate antioxidant properties of wheat.

The five bran samples were analyzed for their total phenolic contents (TPC) and these expressed as gallic acid equivalents (GE) (Fig. 5), since phenolic compounds may contribute to the antioxidant capacities of wheat (Yu et al., 2002a). The greatest GE of 3.4 mg GE/g bran was detected in Platte wheat grown at Fort Collins, and followed by that grown at Akron, Burlington, Walsh, and Julesburg. A negative correlation between the TPC values of Platte grown at the five testing locations in Colorado and the total hours exceeding 32 °C was observed ($r = -0.86$, $P = 0.06$), while no correlation was detected between the TPC and solar radiations.

In conclusion, bran extracts from the Platte wheat grown at five testing locations in Colorado might significantly differ in their radical scavenging activities against $DPPH^{\cdot}$ and $ABTS^{\cdot+}$, chelating capacity, and TPC. The total phenolic content of Platte bran was correlated with the number of hours exceeding 32 °C, a measurement of temperature stress. These data indicated the potential effects of environmental factors on the antioxidant properties of hard winter wheat. Furthermore, individual antioxidant properties may respond to the environmental changes differently. Additional research is needed to adequately map the effects of growing conditions and the interaction

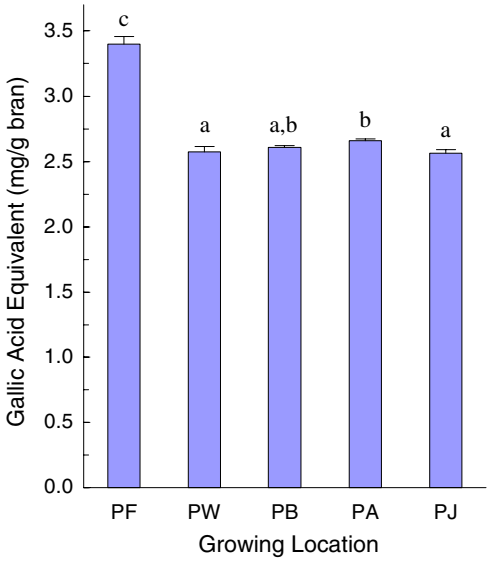

Fig. 5. TPC of Platte bran. PF, PW, PB, PA, and PJ represent Platte bran at the growing locations of Fort Collins, Walsh, Burlington, Akron, and Julesburg in Colorado, respectively. The vertical bars represent the standard deviation ($n = 3$). Values marked by the same letter are not significantly different ($P < 0.05$).

between the environmental factors and genotypes on the antioxidant production in hard winter wheat, using a greater sample of both genotypes and locations.

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