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# Comparison of wheat flours grown at different locations for their antioxidant properties

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**Abstract**

Flour extracts from three winter wheat varieties ('Trego', 'Akron' and 'Platte') grown at five testing locations were evaluated for their free radical scavenging abilities against the stable 2,2-diphenyl-1-picryhydrazyl radical (DPPH·) and 2, 2′-azino-di[3-ethylbenzthiazoline sulfonate] radical cation ($ABTS^{\cdot+}$), and for $Fe^{2+}$ chelating capacities and total phenolic contents. All flour samples showed significant DPPH· scavenging activities and chelating capacities, and contained significant levels of phenolic compounds, but no flour contained detectable amount of $ABTS^{\cdot+}$ scavengers. Both variety and growing location may have significant influence on the DPPH radical scavenging and chelating properties, as well as the phenolic contents of the flour samples. Pearson Correlation tests did not detect any significant correlation between a single antioxidant property of each wheat variety and a selected environmental factor, including total solar radiation, daily average solar radiation, or the hours exceeding 32 °C. The results from this study indicate the potential to produce a wheat flour rich in natural antioxidant for improving human nutrition by optimizing the growing conditions of a selected variety.


*Keywords:* Wheat; Flour; Antioxidant; Radical scavenging; $Fe^{2+}$-chelating; Phenolics

## 1. Introduction

Dietary antioxidants may play a paramount role in the prevention of chronic diseases. Cancer and coronary heart disease (CHD), the two leading causes of death for people in the United States, are two such diseases (Aygustin & Dwyer, 1999; Lavillonniere & Bougnoux, 1999). Growing evidence suggests that the reactive oxygen species (ROS) generated during cellular metabolism or peroxidation of lipids and proteins 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 attacks and reduce the risks of CHD and cancer, as well as other ROS-related diseases such as Parkinson's disease (Chung et al., 1999; Espin, Soler-Rivas, & Wichers, 2000; Merken & Beecher, 2000; Neff, 1997; Wong, Li, & Stadlin, 1999). Recently, health benefits of food products become critical to market competitiveness because consumers are seeking an improved life through diets, which provide health-promoting and disease-preventing activities beyond traditional nutrients. Two-thirds of grocery shoppers reported that their purchase decisions are driven by their desire to either reduce the risk of, or manage, a specific health condition (Sloan, 2000). Developing functional foods rich in natural antioxidants may improve human nutrition and reduce the risks of ROS-associated health problems. The key for such food production is to identify the value-added ingredients rich in natural antioxidants.

Antioxidant activities were detected in wheat and wheat-based food products (Onyeneho & Hettiarachchy, 1992; Yu, Haley, Perret, & Harris, 2002b; Yu et al.,

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2002a; Zielinski & Kozlowska, 2000). Our previous studies showed that ethanol extracts prepared from three hard winter wheat varieties (*Triticum aestivum*) directly reacted with and quenched free 2,2-diphenyl-1-picryhydrazyl radicals (DPPH·) and 2,2′-azino-di[3-ethylbenzthiazoline sulfonate] radical cation ($ABTS^{\cdot+}$), as determined by spectrophotometric and electron spin resonance (ESR) spectrometry methods (Yu et al., 2002b, 2002a). The wheat extracts also suppressed lipid peroxidation in fish oils (Yu et al., 2002b). Significant levels of phenolics, which are considered the major contributors of the total antioxidant capacities, were determined in all three hard winter wheat varieties, including Akron, Trego and Platte. In addition, the three wheat varieties also differed in their radical scavenging capacity, inhibitory effect against lipid peroxidation and total phenolic contents. Recently, our laboratory observed the effects of growing conditions, including solar radiation and number of hours exceeding 32 °C during the 6-week grain filling period, on antioxidant properties in Akron wheat bran (Yu, Perret, Harris, Wilson, & Haley, 2003). To our knowledge, no study has been performed to evaluate wheat flour, a commonly used primary food ingredient, for its potential beneficial factors. In the present study, flour fractions of the three hard winter wheat varieties (Akron, Trego and Platte) grown at five different Colorado locations were examined and compared for their radical scavenging properties, chelating capacities and total phenolic contents. Results from this study may contribute to a better understanding of the effects of growing condition and variety on the antioxidant properties of wheat flour. This information will be used to promote the production and utilization of wheat flour with a high level of natural antioxidants for improving human health.

## 2. Materials and methods

### 2.1. Materials

Grain samples of 'Trego', 'Akron' and 'Platte' were obtained at harvest from breeding trials conducted at five testing locations, including Akron (A), Burlington (B), Julesburg (J), Walsh (W) and Fort Collins (F) in eastern Colorado. Akron is a hard red winter wheat, and Trego and Platte are two hard white winter wheat varieties. Fort Collins is an irrigated location, and all others are non-irrigated locations. Grain samples were cleaned using seed cleaners to remove all non-grain debris present following harvest. 2,2-Diphenyl-1-picryhydrazyl radical (DPPH·), 2,2′-bipyridyl and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma–Aldrich (St. Louis, MO). A total antioxidant status kit was purchased from Randox Laboratories Ltd. (San Francisco, CA) for the radical cation scavenging activity assay. All other chemicals and solvents were of highest commercial grade and used without further purification.

### 2.2. Extraction of antioxidants

Wheat grain of each variety grown at each testing location was milled on a Brabender Quadromat Jr. experimental mill and separated into flour and bran fractions. Twenty grams of each flour sample was extracted with 100 ml of ethanol for 24 h under nitrogen at ambient temperature (Yu et al., 2002a). The ethanol extracts were kept in the dark under nitrogen until further analyses. In order to prepare dimethyl sulfoxide (DMSO) solution, ethanol was removed from a measured aliquot of each sample using a rotary evaporator, and the solid residue was quantitatively re-dissolved in DMSO. The resulting DMSO solution was kept under nitrogen in the dark until further analysis.

### 2.3. Free radical scavenging activity

Free radical scavenging capacity of each flour extract was estimated according to a previously reported procedure, using the stable 2,2-diphenyl-1-picryhydrazyl radical (DPPH·) (Yu et al., 2002a). Briefly, freshly made DPPH· solution was mixed into the flour extract to start the radical–antioxidant reaction. The final concentration was 100 μM for DPPH·. The absorbance at 517 nm was measured against a blank of pure ethanol at 10 min of each reaction and used to estimate the percent remaining radical levels according to a standard curve.

### 2.4. Radical cation $ABTS^{+}$ scavenging activity

The radical cation $ABTS^{\cdot+}$ scavenging activity was measured 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. DMSO was used to prepare the solutions of trolox and flour extracts, and to determine the reagent blank, because ethanol would result in precipitation and disrupt the assay. The trolox equivalent was calculated and used to compare the radical cation scavenging activity of each flour extract (Yu et al., 2002a).

### 2.5. Chelating activity

$Fe^{2+}$ chelating activity was measured by 2,2′-bipyridyl competition assay (Yu et al., 2002b). The reaction mixture contained 0.25 ml of 1 mM $FeSO_4$ solution, 0.25 ml of flour extract, 1 ml of Tris–HCl buffer (pH 7.4), 1 ml of 2,2′-bipyridyl solution (0.1% in 0.2 M HCl), 0.4 ml of 10% hydroxylamine–HCl and 2.5 ml of ethanol. The final volume was made up to 5 ml with water. The absorbance at 522 nm was determined and used to calculate

$Fe^{2+}$ chelating activity using ethylenediaminetetraacetic acid (EDTA) as a standard.

### 2.6. Total phenolic contents

The total phenolic contents of flour extracts were determined using Folin–Ciocalteu reagent (Yu et al., 2002a). The reaction mixture contained 100 μl of flour extracts and 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 2 h of reaction, the absorbance at 765 nm was determined and used to estimate the phenolic contents using a standard curve prepared using gallic acid.

### 2.7. Statistical analysis

All tests were conducted in triplicate. Data are reported as means ± SD. Analysis of variance and least significant difference tests were conducted to identify differences among means, while a Pearson Correlation test was conducted to determine the correlations among means. Statistical significance was declared at $P < 0.05$.

## 3. Results and discussion

Free radical chain reaction is a common mechanism that may explain the direct attack of radicals on physiologically important components, including DNA, protein and membrane lipid, as well as other oxidative damage in the biological systems mediated by the radicals. Radical scavengers may react with and quench free radicals in the system and terminate their potentially hazardous effects. On the other hand, chelating agents reduce the availability of transition metals. This reduction may suppress the formation of free radicals and inhibit the initiation of the free radical chain reaction. Therefore, both radical scavenging and chelating activities are beneficial antioxidative effects against the radical-associated health problems, such as cancer and coronary heart disease. In the present study, selected wheat flour samples were evaluated and compared for their free radical scavenging properties and $Fe^{2+}$ chelating capacities. In addition, total phenolic contents of the flour samples were determined since phenolic compounds, universal in plant materials, including wheat, have been shown to be a group of chemicals that may exhibit radical scavenging and chelating capacities.

All flour samples prepared from the three hard winter wheat varieties (Akron, Trego, and Platte) grown at the five locations in Colorado showed significant free radical scavenging capacity against DPPH radicals (Fig. 1), but no radical cation $ABTS^{\cdot+}$ scavenging capacity was detected in any flour samples under the experimental conditions. Variety and growing conditions might significantly influence the DPPH radical scavenging capacities of wheat flours. Akron flour had the strongest scavenging activity against DPPH radicals among the three wheat varieties grown at the same location, except at location Burlington (Figs. 1(A) and (B)), followed by Trego flour and Platte flour, respectively (Figs. 1(A)–(C)). This is different from our previous observation that the Akron grain had the greatest DPPH radical scavenging activity, followed by Platte and Trego grains, respectively (Yu et al., 2002a). The different observations from the two studies may be due to differential distribution of antioxidants in bran and endosperm or to the fact that the wheat grain samples used in the earlier study were collected in a different year. The flours from the same wheat variety grown at different locations might differ significantly in their radical DPPH scavenging activity (Figs. 1(A)–(C)), suggesting that growing

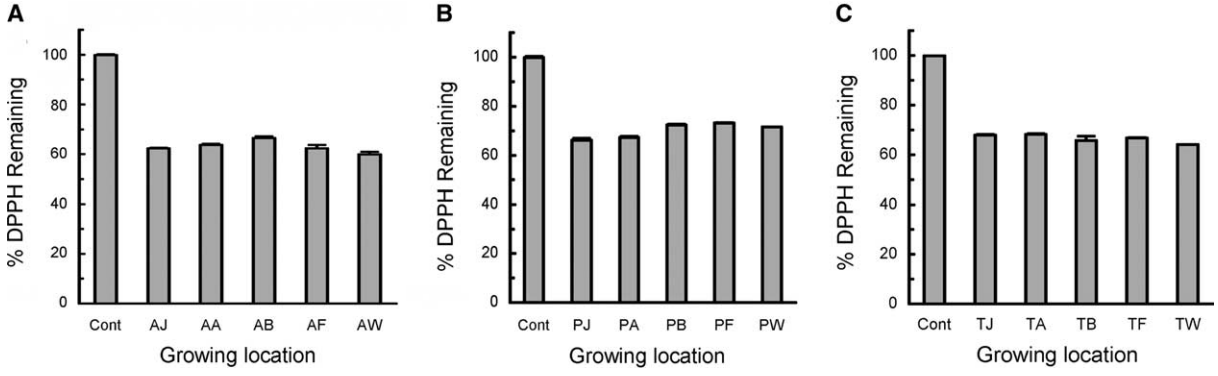


Fig. 1. Radical DPPH scavenging capacity of flour extracts. The DPPH radical concentration was 100 μM in all reaction mixtures. Cont represents the control containing no antioxidant. (A) AJ, AA, AB, AF and AW represent the flours of Akron wheat grown at Julesburg, Akron, Burlington, Fort Collins and Walsh, respectively; (B) PJ, PA, PB, PF and PW represent the flours of Platte wheat grown at Julesburg, Akron, Burlington, Fort Collins and Walsh, respectively; and (C) TJ, TA, TB, TF and TW represent the flours of Trego wheat grown at Julesburg, Akron, Burlington, Fort Collins and Walsh, respectively. The concentrations of all flour samples are on a same dry weight basis. All tests were conducted in triplicate and the means are used. The vertical bars represent the standard deviation of each data point.

conditions might have significant influence on the antioxidant properties of flours. This finding was supported by our previous observation that Akron wheat bran samples, obtained from different growing locations, differed significantly in their antioxidant properties (Yu et al., 2003). This finding was also supported by Wang and Zheng's observation (2001). Wang and Zheng (2001) examined the effect of a group of day/night temperature combinations on antioxidant activities and phenolic contents in the fruit juice of Earliglow and Kent strawberry cultivars. The highest day/night temperature resulted in fruits with the greatest phenolic content as well as antioxidant activities (Wang & Zheng, 2001), suggesting that the growing conditions may alter the antioxidant properties of plant materials. In the present study, the potential correlations between the DPPH radical scavenging activity of the flour sample and the total solar radiation, daily solar radiation, or the hours at the locations exceeding 32 °C, were analyzed, to determine the potential influence of growing conditions on the antioxidant activities of wheat flour. No significant correlation was detected between the DPPH radical scavenging activity of flour and the total solar radiation, daily solar radiation, or the hours exceeding 32 °C. This might be partially due to the relatively small sample size used in this study. These data suggest that both variety and growing location may have some influence on the DPPH radical scavenging activity of wheat flour. More research is required to evaluate the influences of growing factors on the free radical scavenging activities of wheat flour against DPPH radicals. Value-added wheat flour, rich in radical scavengers, may be produced by optimizing growing conditions for a selected variety.

It has been recognized that chelating agents may inhibit radical mediated oxidative chain reactions by stabilizing transition metals, which are required to catalyze the formation of the first few radicals to initiate the radical reactions (Nawar, 1996). The effect of growing locations on the chelating activities of the flour samples is shown in Fig. 2. The flour samples prepared from the same wheat variety grown at different locations might significantly differ in their chelating activities. Akron flours grown at the five locations differed significantly in their chelating activities, except between the flours from Fort Collins and Julesburg. Platte flours from different locations also differed significantly in their chelating properties against $Fe^{2+}$, except between the flours from Burlington and Walsh. In addition, significant differences in chelating activities were observed among Trego flours from the five locations, except between Burlington and Julesburg, as well as between Burlington and Walsh. These results suggest potential effects of environmental factors on the chelating activities of wheat flour. The correlations between the chelating activity and total solar radiation, daily average solar radiation, or total hours exceeding 32 °C, were determined. No significant correlation was found for any variety of wheat under the experimental conditions. However, a correlation coefficient of 0.890 ($P = 0.110$) was detected for the chelating activity of Akron flour and the total hours of the grown location exceeding 32 °C during the 6-week grain-filling period. Further studies with a larger sample size are needed to re-test the correlations between the chelating activities of wheat and selected growing factors. Platte and Trego flours from Fort Collins, an irrigated testing location, showed greater chelating activities than the flours of both varieties from other testing locations (Figs. 2(B) and (C)), suggesting that the irrigation may alter the chelating property of white hard winter wheat. Compared to the wheat grains (Yu et al., 2002b), the wheat flour showed much less chelating activity, suggesting that the bran may contain more chelating agents than the

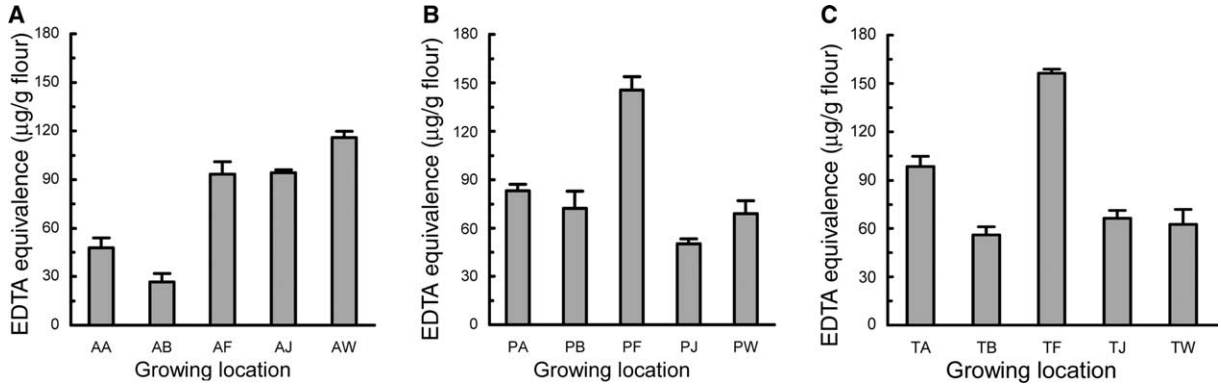


Fig. 2. Chelating capacities of flour extracts. The chelating activities of the flour extracts are expressed as the EDTA equivalent. (A) AJ, AA, AB, AF and AW represent the flours of Akron wheat grown at Julesburg, Akron, Burlington, Fort Collins and Walsh, respectively; (B) PJ, PA, PB, PF and PW represent the flours of Platte wheat grown at Julesburg, Akron, Burlington, Fort Collins and Walsh, respectively; and (C) TJ, TA, TB, TF and TW represent the flours of Trego wheat grown at Julesburg, Akron, Burlington, Fort Collins and Walsh, respectively. All tests were conducted in triplicate and the means are used. The vertical bars represent the standard deviation ($n = 3$).

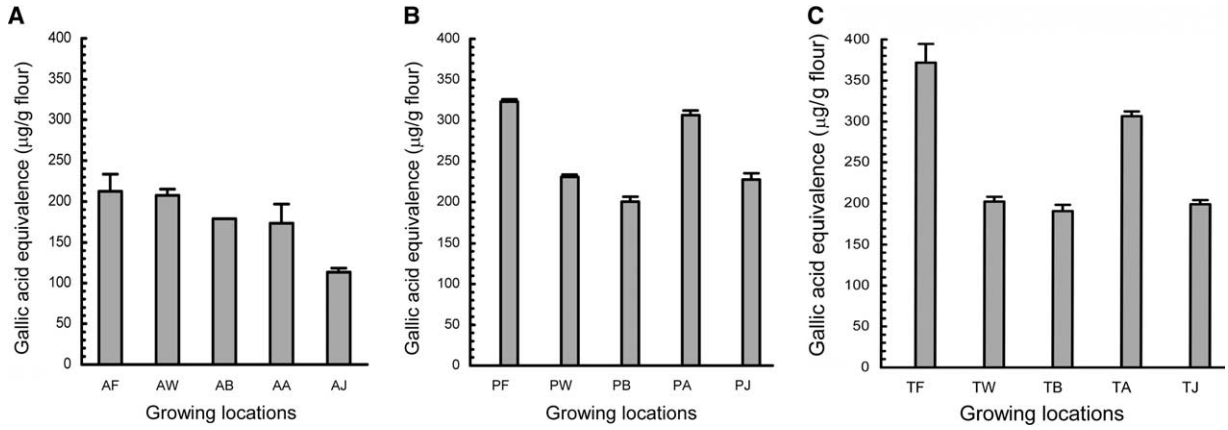


Fig. 3. Total phenolic contents of the flour extracts. (A) AJ, AA, AB, AF and AW represent the flours of Akron wheat grown at Julesburg, Akron, Burlington, Fort Collins and Walsh, respectively; (B) PJ, PA, PB, PF and PW represent the flours of Platte wheat grown at Julesburg, Akron, Burlington, Fort Collins and Walsh, respectively; and (C) TJ, TA, TB, TF and TW represent the flours of Trego wheat grown at Julesburg, Akron, Burlington, Fort Collins and Walsh, respectively. All tests were conducted in triplicate and the means are used. Vertical bars represent the standard deviations of each data point ($n = 3$).

flour fraction, although significant levels of chelating activities were detected in the flours.

The total phenolic content in each flour sample was examined, since the phenolics might be the major contributor of the antioxidant activities. Significant amounts of total phenolics were detected in all wheat flours (Figs. 3(A)–(C)). The potential effects of both variety and growing conditions were noted. The phenolic content in Akron flour was 113–212 μg/g of flour, which was less than that detected in Trego and Platte flours (190–371 and 200–306 μg/g flour, respectively). In our previous study, greatest phenolic content was detected in Akron grain (Yu et al., 2002a). These results suggest that each wheat variety may have a different distribution of phenolics in flour and bran fractions. Similar trends of the total phenolic contents were observed in both Platte and Trego flours from the five testing locations (Figs. 3(B) and (C)), suggesting that a similar influence of growing conditions on the total phenolic contents may exist for the hard winter white wheat varieties.

In summary, the present study detected significant levels of free radical scavenging activities against stable DPPH radicals, $Fe^{2+}$ chelating capacities, and total phenolics in the flour fractions of the Akron, Trego and Platte wheat grown at the five testing locations in Colorado. Both variety and growing location might significantly alter the antioxidant properties and the total phenolic contents of wheat flour. These data demonstrated the possibility of producing wheat flour rich in natural antioxidants by optimizing the growing conditions of a carefully selected wheat variety. The flour high in antioxidants may be used in food products to promote human health and prevent ROS-associated diseases. More research is needed to clarify how varieties and growing conditions alter the antioxidant properties of wheat, wheat flour and bran.

## Acknowledgements

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