JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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pubs.acs.org/JAFC

# Phytochemical Composition, Anti-inflammatory, and Antiproliferative Activity of Whole Wheat Flour

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**ABSTRACT:** Whole wheat flour from five wheat cultivars was evaluated for phenolic, carotenoid, and tocopherol compositions as well as anti-inflammatory and antiproliferative activities against HT-29 cells. The total ferulic acid content ranged from 452 to 731 $\mu$g/g among the five cultivars and was primarily present in the insoluble-bound form. Lutein was the only carotenoid detected and ranged from 1.5 to 4.0 $\mu$g/g, and $\alpha$-tocopherol levels ranged from 12 to 61 $\mu$g/g. Extracts of four cultivars demonstrated significant anti-inflammatory activity, measured as inhibition of interleukin-1$\beta$ (IL-1$\beta$) mRNA expression; however, none of the extracts inhibited tumor necrosis factor-$\alpha$ (TNF-$\alpha$) mRNA expression, a second indicator of anti-inflammatory activity. Proliferation of HT-29 adenocarcinoma cells was inhibited by extracts from all cultivars at the dose of 100 mg botanical equivalent/mL. The cultivar WestBred 936 had the greatest antiproliferative activity at lower concentrations (20 and 50 mg botanical equivalent/mL), had the greatest anti-inflammatory effect against IL-1$\beta$, and also had the highest levels of ferulic acid and $\alpha$-tocopherol. This research shows that whole wheat flours of these five cultivars varied significantly in their contents of phenolics, carotenoids, and $\alpha$-tocopherol as well as in their anti-inflammatory and antiproliferative potentials, suggesting the possibility that wheat varieties can be selected based on potential health benefits.

**KEYWORDS:** *wheat, anti-inflammatory, antiproliferative, HT-29 cells, phenolic acids, lutein, α-tocopherol*

## INTRODUCTION

Consumers have shown increasing interest in reducing disease risk or managing chronic disease through the use of health-enhancing dietary ingredients. Wheat-based products have been widely consumed by humans for thousands of years, and in the past decades, whole wheat has been studied for its health beneficial properties. The wheat bran and germ in particular have been found to contain high levels of phytochemicals that may have bioactive effects.[1] Identifying and characterizing these specific factors in wheat may promote the development of healthier, more nutrient-dense wheat cultivars and wheat foods. The development of nutraceuticals or functional foods from wheat may help to promote the consumption of wheat products and enhance overall public health.

Chronic inflammation has been identified as a cause of various diseases, including coronary heart disease (CHD).[2] Dietary antioxidants have been linked to reductions in inflammation and also reduced risk of cancer and cardiovascular diseases.[3] The possible mechanism by which antioxidants exert these benefits may involve termination of free radical-mediated oxidative chain reactions, stimulation of antioxidant enzymes, reduction of peroxides, and chelation of transition metals.[4,5] Numerous studies have shown that wheat and wheat-based food products contain significant amounts of natural antioxidants, including phenolic acids, carotenoids, and tocopherols.[4,6,7] The consumption of whole wheat has also been shown to reduce markers of inflammation.[8]

In addition to its overall antioxidant capacity, several studies have identified the specific effects of wheat components in reducing risk of disease. In 1997, Kiss et al.[9] found that wheat germ reduced human colonic epithelial cell proliferation, suggesting a reduced risk of colon cancer. Jenab and Thompson[10] reported that phytic acid from wheat bran could affect colon morphology, cell differentiation, and apoptosis of cancer in a rat model. Ferulic acid, a prominent phenolic acid in wheat, has shown an inhibitory effect on macrophage inflammatory protein-2 (MIP-2), an inflammatory cytokine.[11] More recently, it was shown that diets containing wheat bran reduced oxidative stress and inflammation in Zucker rats.[12]

It is well accepted that the phytochemical composition and biological activities of botanicals may vary due to varieties, genotypes, and environments, as well as interactions among them.[4,13] Therefore, it is important to investigate wheat cultivars to determine if there is a difference in their chemical composition and health-beneficial effects and to select those with the most favorable profile for use in foods.

This study was conducted to investigate and compare phenolic, carotenoid, and tocopherol compositions of whole wheat flours from white and red wheat cultivars. Cultivar differences

**Received:** September 19, 2011
**Revised:** February 2, 2012
**Accepted:** February 9, 2012
**Published:** February 9, 2012





dx.doi.org/10.1021/jf203807w | *J. Agric. Food Chem.* 2012, 60, 2129–2135

in antiproliferative activity in HT-29 human colon cancer cells and anti-inflammatory effects against interleukin-1$\beta$ (IL-1$\beta$) and tumor necrosis factor-$\alpha$ (TNF-$\alpha$) were also evaluated. The results from this study will be used to promote the utilization of the studied wheat varieties for improving human health.

## MATERIALS AND METHODS

**Wheat Samples.** Five spring wheat cultivars, Alpowa, Blanca Grande, Louise, Macon, and WestBred 936, were field-grown in eastern Washington in 2009. Alpowa and Louise are soft white cultivars having soft grains (soft kernel texture) and carry the Purodinolines at the *Hardness* locus,[14,15] Blanca Grande and Macon are hard white cultivars, and WestBred 936 is a hard red cultivar. The hard wheats carry a mutation in either *Puroindoline a* or *Puroindoline b*.[14,15] Wheat grain was stored at 4 °C and ground in a Udy Cyclone mill (Udy Corp., Fort Collins, CO, model 3010-030) equipped with a 0.8 mm sieve.

**Materials.** Antibiotic/antimycotic, phosphate-buffered saline (PBS), and TRIzol were purchased from Invitrogen (Carlsbad, CA). A StrataScript First Strand cDNA Synthesis kit was obtained from Stratagene (Santa Clara, CA). ABI Prism 7000 Sequence Detection System and TaqMan Universal PCR Master Mix for Real-time PCR was developed by Applied Biosystems (Carlsbad, CA). The TaqMan Assay-On-Demand Gene Expression Assay was purchased from Applied Biosystems: Tnf (Mm00443258_m1), Il1b (Mm01336189_m1), and Il6 (Mm00446190_m1). An ATP-Lite firstep Luminescence Assay System was obtained from Perkin-Elmer (Waltham, MA). All cell culture media components were purchased from Invitrogen. Phenolic standards, $\alpha$-, $\gamma$-, and $\delta$-tocopherol, and lutein were obtained from Sigma-Aldrich (St. Louis, MO). All other chemicals and solvents were of the highest commercial grade and used without further purification.

**Phenolic Acid Composition.** The wheat flours were analyzed for their phenolic composition. Soluble free, soluble conjugated, and insoluble bound phenolics were extracted according to the method described by Lutterodt et al.[16] Soluble free phenolics were extracted with ethyl acetate and dried, and soluble conjugated phenolics were extracted from the flour residue with 62.5% aqueous methanol. The methanol extract was hydrolyzed under acidic conditions to release conjugated phenolics, which were extracted with ethyl acetate. Insoluble bound phenolics in the flour residue were first released by NaOH hydrolysis before extraction with ethyl acetate. Ethyl acetate extracts were dried, then redissolved in dimethylsulfoxide (DMSO), filtered through a 0.45 $\mu$m Iso-Disc filter, and analyzed by high-performance liquid chromatography (HPLC) according to a previously described protocol.[17] A Shimadzu LC-20AD with autosampler and UV detector was used. The flow rate was 1.0 mL/min, on a linear gradient elution program as follows: (solvent A, 50 mM pH 3.3 sodium phosphate and 10% methanol; solvent B, 70% methanol). Elution was initially 100−70% A in 15 min, then 70−65% A in 30 min, 65−60% A in 20 min, 60−50% A in 5 min, and finally 50−0% A in 25 min. The injection volume was 10 $\mu$L. The area under the peak of each phenolic standard was used to plot standard curves to estimate the respective phenolic content in the extracts. All samples were extracted in triplicate, and duplicate analysis of each extract was carried out.

**Tocopherol and Lutein Content.** The tocopherol composition and lutein content were analyzed by HPLC. Briefly, 0.10 g of wheat flour was combined in a tube with 3.0 mL of 0.1% TBHQ (*tert*-butylhydroquinone) in ethanol, vortexed for 10 s, and heated at 85 °C for 5 min. KOH (10 M, 0.19 mL) was added, and tubes remained in a heated water bath for 10 min. Tubes were removed and placed on ice. A 3.0 mL amount of 1 M NaCl was added, and the tubes gently inverted to mix. A 3.0 mL amount of hexane was added, and the tubes were vortexed and centrifuged. Hexane was transferred to a new tube, and the extraction was repeated twice more. The combined hexane supernatants were washed with sodium carbonate, and then, pure water and hexane were evaporated under nitrogen. Samples were redissolved in 0.25 mL of isopropyl alcohol and filtered through a 0.2 $\mu$m syringe filter for HPLC analysis. HPLC analysis was conducted with a Shimadzu LC-20AD. The column was a Waters Xterra phenyl column (3.9 mm × 150 mm × 3.5 $\mu$m). The injection volume was 20 $\mu$L. Isocratic elution was conducted with MeOH:ACN:water (22.5:48:29.5) from 0 to 40 min, then a linear gradient to 100% MeOH in 4 min, followed by 100% MeOH for 7 min, a linear gradient to the initial conditions for 4 min, and re-equilibration at the initial conditions for 7 min. The column temperature was maintained at 30 °C. The UV detector was set at 450 nm for lutein. The evaporative light scattering detector (ELSD) was set with a frequency of 10 Hz, nitrogen pressure of 350 kPa, and drift tube temperature of 40 °C for the detection of tocopherols.

**Anti-inflammation Activity of Wheat Extracts in J774A.1 Mouse Macrophage.** To determine the anti-inflammatory activity of the five wheat cultivars, mouse J774A.1 macrophages were cultured in six-well plates overnight and reached 80% confluence. Approximately 8 g of wheat flour was extracted in 100% ethanol by Soxhlet, then evaporated under nitrogen, and redissolved in DMSO. The cells were first treated with wheat extract (10 mg botanical equivalent/mL) for 2 h. Then, lipopolysaccharide (LPS) was added into the media at a final concentration of 0.5 $\mu$g/mL. The treatment was performed in triplicate at a final concentration of 10 mg botanical equiv/mL in the media with 0.1% DMSO. Cells were incubated at 37 °C and 5% $CO_2$ for 24 h. After 24 h of incubation, cells were washed with PBS, and TRIzol reagent was added for total RNA isolation. StrataScript First Strand cDNA Synthesis kit was used to reverse transcribe cDNA. Real-time PCR was performed on ABI Prism 7000 Sequence Detection System using TaqMan Universal PCR Master Mix. The TaqMan Assay-On-Demand Il1b and Tnf were used for gene detection of IL-1$\beta$ and TNF-$\alpha$ gene expression, respectively. mRNA quantities were normalized to the internal control, Tbp mRNA. The following amplification parameters were used for PCR: 50 °C for 2 min, 95 °C for 10 min, and 46 cycles of amplification at 95 °C for 15 s and 60 °C for 1 min.

**Antiproliferative Effects of Wheat Extracts in HT-29 Colon Cancer Cells.** Approximately 8 g of wheat flour was extracted in ethanol, evaporated under nitrogen, and redissolved in DMSO as previously described. The final concentrations of extracts in cell media were 0, 20, 50, and 100 mg botanical equivalent/mL. HT-29 human colorectal adenocarcinoma cell proliferation inhibition was investigated according to a previously reported protocol.[18] HT-29 cells were grown at 37 °C and 5% carbon dioxide in McCoy's 5A medium supplemented with 10% fetal bovine serum and 1% antibiotic/antimycotic. The treatment was performed at a final concentration of 1% DMSO in triplicate. An ATP-Lite 1-step kit (Perkin- Elmer Life and Analytical Sciences, Shelton, CT) was used to determine cell proliferation. The emitted luminescence was determined using a Victor3 multiwell plate reader (Perkin-Elmer, Turku, Finland) immediately prior to treatment and at 0, 4, 24, 48, 72, and 96 h after initial treatment. The first reading was taken immediately after treatments were added to media, and after 4 h, another reading was taken, and then, treatment media were replaced every 24 h until a reading was taken on the plate.

**Statistical Analysis.** Data were analyzed using SPSS for Windows (version rel. 10.0.5, 1999, SPSS Inc., Chicago, IL). Differences in means were analyzed using one-way analysis of variance and Tukey's HSD posthoc test. Correlation analysis was analyzed with Pearson's correlation. Statistical significance was declared at $P < 0.05$. Data are reported as means ± standard deviations.

## RESULTS AND DISCUSSION

**Phenolic Acid Composition.** Phenolic acids in foods are a source of antioxidant and anti-inflammatory activity. Ferulic acid in particular has recently been shown to have antioxidant activity in vivo as measured by glutathione-*S*-transferase in colon tissue.[19] Ferulic acid also decreased radiation-induced damage to lymphocytes in vitro.[20] Other phenolic acids also have protective effects. For example, vanillic acid has demonstrated activity against the inflammatory markers COX-2 and NF-$\kappa$B p 65 in colon tissue of mice.[21]

It has previously been demonstrated that the majority of phenolic acids in wheat are bound in the cell wall and only available upon hydrolysis.[22,23] In the current study, wheat flour

Table 1. Ferulic and *p*-Coumaric Acid Contents of Whole Wheat Flour from Five Wheat Cultivars[a]

| | μg/g | | | | |
|---|---|---|---|---|---|
| wheat cultivar | soluble free | soluble conjugated | insoluble bound | total soluble | total |
| | | | ferulic acid content | | |
| Blanca Grande | 2.41 c ± 0.04 | 28.11 c ± 0.10 | 659.01 d ± 1.22 | 30.52 c ± 0.14 | 689.53 d ± 1.12 |
| Alpowa | 2.39 c ± 0.01 | 21.82 a ± 0.02 | 427.44 a ± 0.99 | 24.21 a ± 0.03 | 451.65 a ± 1.01 |
| Louise | 1.88 a ± 0.10 | 28.91 d ± 0.04 | 587.70 c ± 0.82 | 30.79 c ± 0.14 | 618.49 c ± 1.10 |
| WestBred 936 | 2.13 b ± 0.10 | 30.03 e ± 0.10 | 699.09 e ± 2.30 | 32.16 d ± 0.20 | 731.25 e ± 2.22 |
| Macon | 1.91 a ± 0.04 | 24.77 b ± 0.10 | 523.12 b ± 1.41 | 26.68 b ± 0.14 | 549.80 b ± 1.12 |
| | | | *p*-coumaric acid content | | |
| Blanca Grande | 0.36 c ± 0.01 | 1.89 e ± 0.02 | 4.35 d ± 0.09 | 2.25 d ± 0.03 | 6.60 e ± 0.10 |
| Alpowa | 0.11 a ± 0.01 | 0.46 a ± 0.00 | 2.16 a ± 0.01 | 0.57 a ± 0.01 | 2.73 a ± 0.02 |
| Louise | 0.27 b ± 0.00 | 1.31 c ± 0.00 | 3.38 b ± 0.02 | 1.58 b ± 0.00 | 4.96 b ± 0.02 |
| WestBred 936 | 0.34 c ± 0.01 | 1.19 b ± 0.01 | 4.73 e ± 0.04 | 1.53 b ± 0.02 | 6.26 d ± 0.12 |
| Macon | 0.25 b ± 0.01 | 1.41 d ± 0.02 | 4.10 c ± 0.02 | 1.66 c ± 0.03 | 5.76 c ± 0.04 |

[a]Results are presented as the mean of two replicates ± SDs. Different letters in the same column under the same phenolic acid indicate a significant difference ($P \leq 0.05$) by Tukey's HSD test.

was evaluated for soluble, soluble-conjugated, and insoluble-bound phenolic acid composition, and here, we report on the two most abundant phenolic acids, ferulic acid and *p*-coumaric acid (Table 1). WestBred 936 flour contained the overall highest quantity of ferulic acid, and Blanca Grande flour contained the highest level of *p*-coumaric acid. Moore et al.[4] reported that total phenolic content in wheat flour was predominantly influenced by growing environment rather than genotype. The present cultivars were grown in the same geographical location, and we expect that differences related to growing conditions are limited. Additionally, differences in particle size are known to influence extraction efficiency of bioactive components from grain.[24] While these cultivar samples were ground with the same size sieve, differences in hardness may account for variation in extraction efficiency and therefore the measured phytochemical content.

Ferulic acid was the predominant phenolic in all wheat samples (451.6−731.3 μg/g flour). Moore et al.[23] reported that total ferulic acid was 456−621 μg/g flour among eight Maryland soft red winter wheat cultivars, which is consistent with the current study. *p*-Coumaric acid was present at levels ranging from 2.7 to 6.6 μg/g flour and was somewhat lower than previously reported (10.4−14.1 μg/g flour).[23] Ferulic and *p*-coumaric acids were found primarily in the insoluble bound form. These results agree with previous studies of wheat flour.[22,23] Although insoluble-bound phenolics have been shown to have poor bioavailability after consumption in foods,[25] the mechanisms by which they protect from disease may be more complex than simple absorption.[26] In addition, processing methods such as yeast fermentation and enzyme treatment may increase the bioavailability of these compounds from wheat grain.[8]

**Tocopherol and Lutein Content.** Tocopherols and carotenoids are lipophilic antioxidants and have bioactive effects. Lutein is present in eye tissue and is believed to play a role in prevention of age-related macular degeneration.[27] Lutein has been identified as the major carotenoid present in wheat, although the levels can differ by variety.[22,23] In the current study, lutein was identified in wheat flour extracts, but no other carotenoids were detected. The lutein content ranged from 1.5 and 4.0 μg/g (Table 2), depending on cultivar type. The Alpowa cultivar had the highest lutein levels, followed by Macon. Lutein in wheat flour was previously reported at somewhat lower levels than we report here, ranging from 0.26 to 1.43 μg/g[22] and from 0.82 to 1.14 μg/g.[23] The lutein level of

Table 2. α-Tocopherol and Lutein Content of Whole Wheat Flour[a]

| wheat cultivar | α-tocopherol (μg/g) | lutein (μg/g) |
|---|---|---|
| Blanca Grande | 46.93 d ± 1.63 | 1.98 b ± 0.01 |
| Alpowa | 12.21 a ± 0.02 | 3.98 e ± 0.03 |
| Louise | 17.32 b ± 0.10 | 2.07 c ± 0.03 |
| WestBred 936 | 61.03 e ± 1.04 | 1.53 a ± 0.07 |
| Macon | 20.89 c ± 1.69 | 2.70 d ± 0.08 |

[a]Data represent the means of three replicate extractions ± SDs. Different letters in the same column indicate a significant difference ($P \leq 0.05$) by Tukey's HSD test.

WestBred 936 (1.5 μg/g) in this study was the lowest of the five cultivars and is close to the previously reported levels in other wheat varieties. Differences in cultivars and/or environmental conditions may partly explain the higher lutein levels found in the current study. Tocopherols are used as antioxidants for the preservation of food products, and α-tocopherol has vitamin E activity. α-Tocopherol has been studied for health effects on cardiovascular disease, but there is still not a clear conclusion as to whether it is beneficial beyond dietary levels.[28] However, persons with higher serum levels of tocopherols were found to have an overall lower risk of mortality.[29] This study identified α-tocopherol as the only tocopherol detected in the wheat flours, which is consistent with results previously reported by Moore et al.[23] Others have reported β-tocopherol and small amounts of γ-tocopherol in some wheat varieties.[30] α-Tocopherol of the wheat flours in the current study ranged from 12.2 to 61.0 μg/g (Table 2), with the WestBred 936 variety containing the highest level ($P \leq 0.05$). This result is somewhat higher than that previously found in spring wheat (11.3−16.0 μg/g)[30] and Maryland soft wheat (3.4−10.1 μg/g).[23]

**Anti-inflammation Activity of Wheat Extracts in J774A.1 Mouse Macrophage.** The links between inflammation and cancer have been extensively studied and discussed over the past decade. Chronic inflammation has been linked to the initiation and progression of cardiovascular disease, diabetes, arthritis, pulmonary disease, Alzheimer's, and autoimmune disease.[31,32] IL-1β and TNF-α are two of the most important inflammation mediators, which substantially affect cardiovascular function and enhance local inflammation through macrophage regulated cytokine expressions.[33] In this study, the anti-inflammatory effect of ethanol flour extracts

from five wheat cultivars was studied using J774A.1 mouse macrophage cells. Significant inflammatory responses were triggered by LPS treatment (Figures 1 and 2). Treatment with

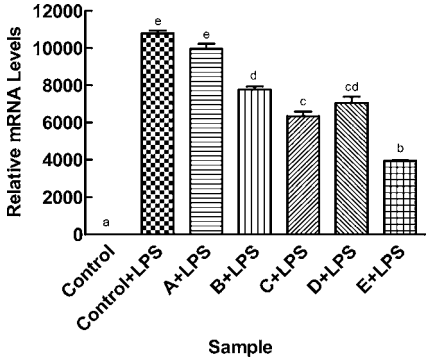


**Figure 1.** Effects of whole wheat flour extracts on mRNA levels of IL-1$\beta$ in mouse J774A.1 macrophage cells. J774A.1 cells ($1.5 \times 10^5$/mL) were incubated overnight prior to treatment with extract and LPS. A, Alpowa; B, Blanca Grande; C, Louise; D, Macon; and E, WestBred 936. Each column represents the mean ± SD ($n$ = 3). Columns marked by different letters are significantly different from each other ($P \leq 0.05$) by Tukey's HSD test.

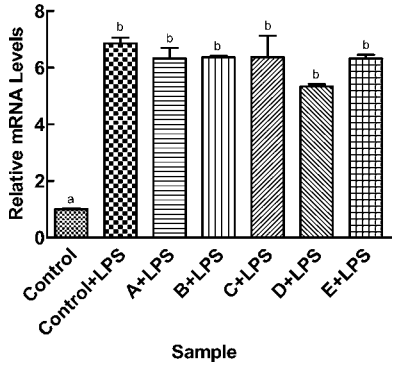


**Figure 2.** Effects of whole wheat flour extracts on mRNA levels of TNF-$\alpha$ in mouse J774A.1 macrophage cells. J774A.1 cells ($1.5 \times 10^5$/mL) were incubated overnight prior to treatment with extract and LPS. A, Alpowa; B, Blanca Grande; C, Louise; D, Macon; and E, WestBred 936. Each column represents the mean ± SD ($n$ = 3). Columns marked by different letters are significantly different from each other ($P \leq 0.05$) by Tukey's HSD test.

10 mg of botanical equiv/mL significantly inhibited IL-1$\beta$ mRNA expression in all of the cultivars except Alpowa (Figure 1). WestBred 936 extract showed the strongest anti-inflammatory activity, which inhibited about 60% of IL-1$\beta$ mRNA expression at 24 h, while Blanca Grande, Louise, and Macon showed weaker but significant inhibition (Figure 1). Another inflammatory cytokine, TNF-$\alpha$, was also tested; however, flour extracts did not significantly inhibit TNF-$\alpha$ mRNA (Figure 2). There was significant negative correlation found between the levels of measured IL-1$\beta$ mRNA expression and $p$-coumaric acid ($R = -0.707$, $P \leq 0.01$) (Table 3) as well as $\alpha$-tocopherol ($R = -0.674$, $P \leq 0.01$). However, given the variety of bioactive chemicals contained in wheat, it is not possible to attribute the observed anti-inflammatory activity entirely to the chemicals measured in this study. It is further noted that a correlation was seen between $p$-coumaric acid and IL-1$\beta$ levels; yet, a similar correlation was not seen with ferulic acid. Lutein levels showed a significant positive correlation with IL-1$\beta$ mRNA expression ($R = 0.858$, $P \leq 0.01$), which may suggest that lutein had a pro-inflammatory effect. However, different extraction methods were required for optimal phytochemical extraction and the anti-inflammatory assays; therefore, correlation between these two measurements may not necessarily indicate a link between them.

**Antiproliferative Activity against HT-29 Cells.** In recent years, colorectal cancer has been the second most fatal and the third most diagnosed type of cancer worldwide.[34] One strategy that is commonly used to fight against cancers involves prevention through dietary interventions.[35] In this study, 20, 50, and 100 mg of botanical equiv/mL wheat extracts were used to treat HT-29 colorectal cancer cells, which were monitored over a 96 h time period (Figure 3). All of the extracts showed a strong inhibitory effect at 100 mg botanical equiv/mL, with the cultivar Louise showing the greatest efficacy. At lower concentration, WestBred 936 extract exhibited a moderate inhibitory effect at both 20 and 50 mg/mL. At these lower concentrations, Alpowa, Blanca Grande, and Macon showed slight bioactivity, while Louise had no effect on HT-29 cell proliferation. Our results indicated that wheat cultivars vary in their impact on HT-29 colon cancer cell growth, and the effect was highly dose-dependent in all cultivars. Previous studies have shown that ferulic acid and $p$-coumaric acid have biological effects on HT-29 and Caco-2 colon cancer cells.[36,37] In the current study, the % inhibition by the 20 mg botanical equiv/mL extract was positively correlated with soluble ferulic acid levels ($R = 0.614$, $P \leq 0.05$) (Table 3), while % inhibition at 50 mg botanical equiv/mL was positively correlated with soluble $p$-coumaric acid levels ($R = 0.608$, $P \leq 0.05$). Additionally, the $\alpha$-tocopherol levels were positively correlated with % inhibition at 50 mg botanical equiv/mL ($R = 0.954$, $P \leq 0.01$). While tocotrienols were not measured in this study,

**Table 3. Correlations between Phytochemicals, Anti-inflammatory, and Antiproliferative Activity**[a]

| | ferulic | $p$-coumaric | $\alpha$-toco | lutein | IL-1$\beta$ | TNF- $\alpha$ | HT-29 (20) | HT-29 (50) |
|---|---|---|---|---|---|---|---|---|
| $p$-coumaric | −0.117 | | | | | | | |
| $\alpha$-toco | 0.241 | 0.808** | | | | | | |
| l | 0.318 | −0.950** | −0.760** | | | | | |
| IL-1$\beta$ | 0.506 | −0.707** | −0.674** | 0.858** | | | | |
| TNF- $\alpha$ | 0.299 | 0.066 | 0.163 | −0.098 | 0.072 | | | |
| HT-29 (20) | 0.614* | −0.369 | 0.222 | 0.437 | 0.205 | 0.021 | | |
| HT-29 (50) | 0.346 | 0.608* | 0.954** | −0.595* | −0.609* | 0.229 | 0.440 | |
| HT-29 (100) | −0.149 | 0.104 | −0.184 | −0.236 | −0.040 | 0.402 | −0.668* | −0.214 |

[a]Data represent Pearson's correlation coefficient ($R$). Ferulic, ferulic acid; $p$-coumaric, $p$-coumaric acid; and $\alpha$-toco, $\alpha$-tocopherol. IL-1$\beta$ and TNF-$\alpha$ represent mRNA levels of inflammatory markers after treatment with wheat extract. HT-29 (20, 50, 100) = % inhibition of HT-29 cells at 20, 50, or 100 mg botanical equiv/mL wheat extract, respectively. *Significant at $P \leq 0.05$. **Significant at $P \leq 0.01$.

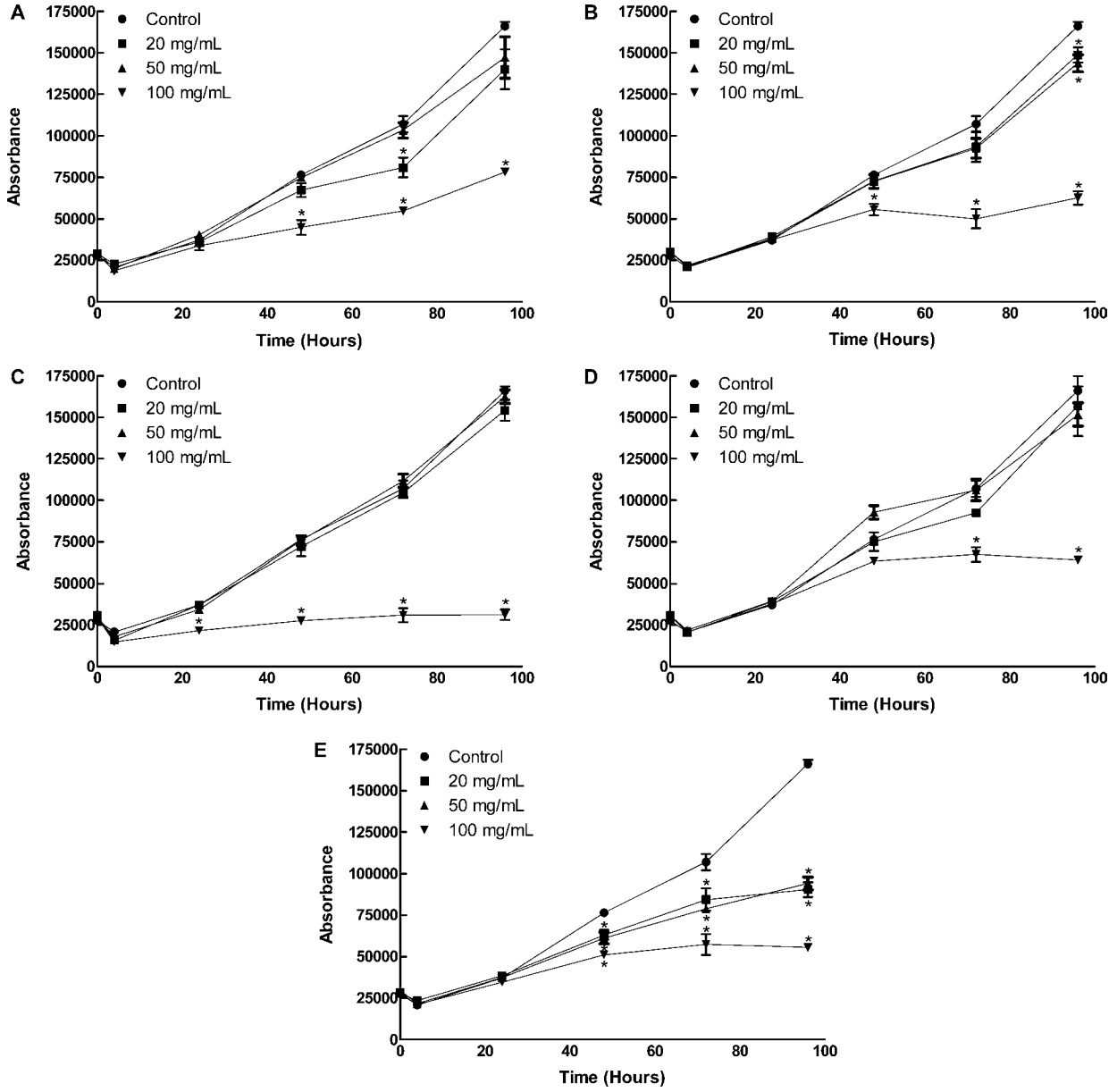


**Figure 3.** Time- and dose-dependent effects of whole wheat flour extracts on human HT-29 colon cancer cell growth. HT-29 cells ($2.5 \times 10^4$/mL) were incubated overnight prior to treatments. Extracts were then added to media at the indicated concentrations. Relative luminescence is proportional to the number of viable cells. A, Alpowa; B, Blanca Grande; C, Louise; D, Macon; and E, WestBred 936. Values are based on triplicate tests, with means ± SDs shown ($n$ = 3). Values marked with * are significantly different than the control ($P \leq 0.05$).

they are present in wheat varieties at levels 2−3 times greater than tocopherols.[30,38,39] They have also been associated with antiproliferative activity against multiple types of cancer cells, including colorectal adenocarcinoma.[40,41] It would therefore be of interest to measure tocotrienol levels in these wheat varieties in a future study. As was noted for the anti-inflammatory assays, extraction conditions were different between the phytochemical measurement assays and the antiproliferative assay; therefore, we cannot draw definitive conclusions about the effects of the phytochemicals on antiproliferative activity.

In summary, the whole wheat flours of five cultivars in the current study are sources of phenolics, lutein, and $\alpha$-tocopherol and possess anti-inflammatory and antiproliferative activities. Ferulic acid was the major phenolic acid found in wheat flour, although the majority was contained in the insoluble-bound form. WestBred 936 and Blanca Grande cultivars contained the highest levels of total ferulic and $p$-coumaric acids. The WestBred 936 cultivar also contained the highest $\alpha$-tocopherol level. In addition, WestBred 936 showed the greatest anti-inflammatory effect against IL-1$\beta$ and had greater antiproliferative effects at moderate concentration than other cultivars. Further research seems warranted on the health-related potential of the WestBred 936 cultivar and whether this potential is directly or indirectly associated with the higher levels of ferulic acid and $\alpha$-tocopherol, and possibly with red pigments, since this was the only red cultivar studied. However, the sample size in the current study limits the interpretation of results regarding the properties of each specific cultivar. On the basis of the overall results, whole wheat flours of all of the studied cultivars appear to have multiple potential health-enhancing properties, all of which varied depending upon the cultivar. The results suggest that in the future, wheat varieties might be selected based on potential health benefits.

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### Funding

This research was supported, in part, by NIFA, AFRI Grant #2009-02347.


### Notes

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The authors declare no competing financial interest.

## REFERENCES


(1) Okarter, N.; Liu, R. Health benefits of whole grain phytochemicals. *Crit. Rev. Food Sci. Nutr.* **2010**, *50*, 193−208.

(2) Libby, P.; Crea, F. Clinical implications of inflammation for cardiovascular primary prevention. *Eur. Heart J.* **2010**, *31*, 777−783.

(3) Wilcox, J. K.; Ash, S. L.; Catignani, G. L. Antioxidants and prevention of chronic disease. *Crit. Rev. Food Sci. Nutr.* **2004**, *44*, 275−295.

(4) Moore, J.; Liu, J. G.; Zhou, K.; Yu, L. L. Effects of genotype and environment on the antioxidant properties of hard winter wheat bran. *J. Agric. Food Chem.* **2006**, *54*, 5313−5322.

(5) Xie, Z.; Liu, W.; Huang, H.; Slavin, M.; Zhao, Y.; Whent, M.; Blackford, J.; Lutterodt, H.; Zhou, H.; Chen, P.; Wang, T. T.; Wang, S.; Yu, L. L. Chemical composition of five commercial *gynostemma pentaphyllum* samples and their radical scavenging, antiproliferative, and anti-inflammatory properties. *J. Agric. Food Chem.* **2010**, *58*, 11243−11249.

(6) Zhou, K.; Su, L.; Yu, L. L. Phytochemicals and antioxidant properties in wheat bran. *J. Agric. Food Chem.* **2004**, *52*, 6108−6114.

(7) Zhou, K.; Yin, J. J.; Yu, L. L. Phenolic acid, tocopherol and carotenoid compositions, and antioxidant functions of hard red winter wheat bran. *J. Agric. Food Chem.* **2005**, *53*, 3916−3922.

(8) Anson, N.; Aura, A. M.; Selinheimo, E.; Mattila, I.; Poutanen, K.; van den Berg, R.; Havenaar, R.; Bast, A.; Haenen, G. Bioprocessing of wheat bran in whole wheat bread increases bioavailability of phenolic acids in men and exerts anti-inflammatory effects ex-vivo. *Nutrition* **2011**, *141*, 137−143.

(9) Kiss, R.; Camby, I.; Duckworth, C.; De Decker, R.; Salmon, I.; Pasteels, J. L.; Danguy, A.; Yeaton, P. In vitro influence of Phaseolus vulgaris, Griffonia simplicifolia, concanavalin A, wheat germ, and peanut agglutinins on HCT-15, LoVo, and SW837 human colorectal cancer cell growth. *Gut* **1997**, *40*, 253−261.

(10) Jenab, M.; Thompson, L. U. Phytic acid in wheat bran affects colon morphology, cell differentiation and apoptosis. *Carcinogenesis* **2000**, *21*, 1547−1552.

(11) Sakai, S.; Ochiai, H.; Nakajima, K.; Terasawa, K. Inhibitory effect of ferulic acid on macrophage inflammatory protein-2 production in a murine macrophage cell line, RAW264.7. *Cytokine* **1997**, *9*, 242−248.

(12) Belobrajdic, D. P.; Lam, Y. Y.; Mano, M.; Wittert, G. A.; Bird, A. R. Cereal based diets modulate some markers of oxidative stress and inflammation in lean and obese Zucker rats. *Nutr. Metab. (Lond.)* **2011**, *8*, 27.

(13) Whent, M.; Hao, J.; Slavin, M.; Zhou, M.; Song, J.; Kenworthy, W.; Yu, L. L. Effect of genotype, environment, and their interaction on chemical composition and antioxidant properties of low-linolenic soybeans grown in Maryland. *J. Agric. Food Chem.* **2009**, *57*, 10163−10174.

(14) Morris, C. F. Puroindolines: The molecular genetic basis of wheat grain hardness. *Plant Mol. Biol.* **2002**, *48*, 633−647.

(15) Bhave, M.; Morris, C. F. Molecular genetics of puroindolines and realted genes: Allelic diversity in wheat and other grasses. *Plant Mol. Biol.* **2008**, *66*, 205−219.

(16) Lutterodt, H.; Slavin, M.; Whent, M.; Turner, E.; Yu, L. Fatty acid composition, oxidative stability, antioxidant and antiproliferative properties of selected cold-pressed grape seed oils and flours. *Food Chem.* **2011**, *128*, 391−399.

(17) Sakakibara, H.; Honda, Y.; Nakagawa, S.; Ashida, H.; Kanazawa, K. Simultaneous determination of all polyphenols in vegetables, fruits, and teas. *J. Agric. Food Chem.* **2003**, *51*, 571−581.

(18) Slavin, M.; Kenworthy, W.; Yu, L. Antioxidant properties, phytochemical composition, and antiproliferative activity of Maryland-grown soybeans with Colored Seed Coats. *J. Agric. Food Chem.* **2009**, *57*, 11174−11185.

(19) Bolling, B.; Ji, L.; Lee, C. H.; Parkin, K. Dietary supplementation of ferulic acid and ferulic acid ethyl ester induces quinone reductase and glutathione-S-transferase in rats. *Food Chem.* **2011**, *124*, 1−6.

(20) Ma, Z. C.; Hong, Q.; Wang, Y. G.; Tan, H. L.; Xiao, C. R.; Liang, Q. D.; Wang, D. G.; Gao, Y. Ferulic acid protects lymphocytes from radiation-predisposed oxidative stress through extracellular regulated kinase. *Int. J. Radiat. Biol.* **2011**, *87*, 130−140.

(21) Kim, S. J.; Kim, M. C.; Um, J. Y.; Hong, S. H. The beneficial effect of vanillic acid on ulcerative colitis. *Molecules* **2010**, *15*, 7208−7217.

(22) Adom, K.; Sorrells, M.; Liu, R. Phytochemical profiles and antioxidant activity of wheat varieties. *J. Agric. Food Chem.* **2003**, *51*, 7825−7834.

(23) Moore, J.; Hao, Z.; Zhou, K.; Luther, M.; Costa, J; Yu, L. Carotenoid, tocopherol, phenolic acid, and antioxidant properties of Maryland-grown soft wheat. *J. Agric. Food Chem.* **2005**, *53*, 6649−6657.

(24) Zhou, K.; Laux, J.; Yu, L. Comparison of Swiss red wheat grain and fractions for their antioxidant properties. *J. Agric. Food Chem.* **2004**, *52*, 1118−1123.

(25) Kern, S.; Bennett, R.; Mellon, F.; Kroon, P.; Garcia-Conesa, M. T. Absorption of hydroxycinnamates in humans after high-bran cereal consumption. *J. Agric. Food Chem.* **2003**, *51*, 6050−6055.

(26) Manach, C.; Scalbert, A.; Morand, C.; Remesy, C.; Jimenez, L. Polyphenols: Food sources and bioavailability. *Am. J. Clin. Nutr.* **2004**, *79*, 727−747.

(27) Carpentier, S.; Knaus, M.; Suh, M. Associations between lutein, zeaxanthin, and age-related macular degeneration: An overview. *Crit. Rev. Food Sci. Nutr.* **2009**, *49*, 313−326.

(28) Clarke, M.; Burnett, J. Vitamin E in health and disease. *Crit. Rev. Clin. Lab. Sci.* **2008**, *45*, 417−450.

(29) Wright, M.; Lawson, K.; Weinstein, S.; Pietinen, P.; Taylor, P.; Virtamo, J.; Albanes, D. Higher serum concentrations of Vitamin E are associated with lower total and cause-specific mortality in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. *Am. J. Clin. Nutr.* **2006**, *84*, 1200−1207.

(30) Lampi, A.; Nurmi, T.; Ollilainen, V.; Piironen, V. Tocopherols and tocotrienols in wheat genotypes in the HEALTHGRAIN diversity screen. *J. Agric. Food Chem.* **2008**, *56*, 9716−9721.

(31) Aggarwal, B. B. Nuclear factor-kappa-B: The enemy within. *Cancer Cell* **2004**, *6*, 203−208.

(32) Reuter, S.; Gupta, S. C.; Chaturvedi, M. M.; Aggarwal, B. B. Oxidative stress, inflammation, and cancer. How are they linked? *Free Radical Biol. Med.* **2010**, *49*, 1603−1616.

(33) Gustafson, B. Adipose tissue, inflammation and atherosclerosis. *J. Atheroscler. Thromb.* **2010**, *17*, 332−341.

(34) Araujo, J. R. A. J. R.; Goncalves, P.; Martel, F. Chemopreventive effect of dietary polyphenols in colorectal cancer cell lines. *Nutr. Res. (N.Y.)* **2011**, *31*, 77−87.

(35) Brown, J.; Byers, T.; Thompson, K.; Eldridge, B.; Doyle, C.; Williams, A. M. Nutrition during and after cancer treatment: A guide for informed choices by cancer survivors—American Cancer Society Workgroup on nutrition and physical activity for cancer survivors. *CA: Cancer J. Clin.* **2001**, *51*, 153−181.

(36) Janicke, B.; Hegardt, C.; Krogh, M.; Onning, G.; Akesson, B.; Cirenajwis, H.; Oredsson, S. The antiproliferative effect of dietary fiber phenolic compounds ferulic acid and p-coumaric acid on the cell cycle of Caco-2 cells. *Nutr. Cancer* **2011**, *63*, 611−622.
(37) Ferguson, L.; Zhu, S.; Harris, P. Antioxidant and antigenotoxic effects of plant cell wall hydroxycinnamic acids in cultured HT-29 cells. *Mol. Nutr. Food Res.* **2005**, *49*, 585−593.
(38) Panfili, G.; Fratianni, A.; Irano, M. Normal phase high-performance liquid chromatography method for determination of tocopherols and tocotrienols in cereals. *J. Agric. Food Chem.* **2003**, *51*, 3940−3944.
(39) Okarter, N.; Liu, C.; Sorrells, M. E.; Rui, H. L. Phytochemical content and antioxidant activity of six diverse varieties of whole wheat. *Food Chem.* **2010**, *119*, 249−257.
(40) Miyazawa, T.; Nakagawa, K.; Sookwong, P. Health benefits of vitamin E in grains, cereals, and green vegetables. *Trends Food Sci. Technol.* **2011**, *22*, 651−654.
(41) Eitsuka, T.; Nakagawa, K.; Miyazawa, T. Down-regulation of telomerase activity in DLD-1 human colorectal adenocarcinoma cells by tocotrienol. *Biochem. Biophys. Res. Commun.* **2006**, *348*, 170−175.