RESEARCH ARTICLE

Phenolic Compounds from the Insoluble-Bound Fraction of Whole Grains Do Not Have Any Cellular Antioxidant Activity
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Abstract
The present study reports the total phenolic content, phenolic composition, and antioxidant activity of eight grains and the cellular antioxidant activity of phenolic compounds previously reported to be found in the insoluble-bound fraction of whole grains. Total phenolic content of the insoluble-bound fraction of grains ranged from 24 (amaranth and buckwheat) to 255 (corn) mg GAE/100 g. Ferulic acid and p-coumaric acid were found in the insoluble-bound fraction of all grains. Caffeic acid was only detected in the insoluble-bound fraction of barley and corn. p-Hydroxybenzoic acid was only detected in the insoluble-bound fraction of quinoa and amaranth and vanillic acid was only detected in the insoluble-bound fraction of quinoa. Flavonoids (quercetin, kaempferol, catechin, and rutin) were not detected in the insoluble-bound fraction of any grain. None of the phenolic compounds in the present study had any cellular antioxidant activity, most likely because these phenolic compounds do not have the structure necessary to impart cellular antioxidant activity. The data from the present study suggest that the potential health benefit of whole grain consumption in the lower gastrointestinal tract is independent of the cellular antioxidant activity of the phenolic compounds found in the insoluble-bound fraction of whole grains.

Keywords: Whole grains; antioxidant activity; flavonoids; phenolic acids; colon cancer.

1. Introduction
A whole grain consists of the intact, ground, cracked or flaked caryopsis, whose principal anatomical components - the starchy endosperm, germ and bran - are present in the same relative proportions as they exist in the intact caryopsis [1]. Whole grain products can be intact, having the original composition of bran, germ, and endosperm throughout the entire lifetime of the product from field to consumption, or reconstituted having the original components of a whole grain recombined to the relative proportion naturally occurring in the grain kernel [1]. Pseudocereals, plants with seeds that can be milled and used much the same way as cereal flours, have cereal-like properties and for that reason are considered to be grains. Consumption of pseudocereals is becoming increasingly popular as the seeds from these plants do not contain the protein gluten, intolerance to which is known as Celiac disease [2]. A summary of common grains and food products is provided in Table 1.

<table>
<thead>
<tr>
<th>Species</th>
<th>Common Name</th>
<th>Common Food Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zea mays</td>
<td>Corn</td>
<td>Corn cakes, tortilla, popcorn, hominy</td>
</tr>
<tr>
<td>Orize sativa</td>
<td>Rice</td>
<td>White rice, brown rice, parboiled rice</td>
</tr>
<tr>
<td>Triticum aestivium</td>
<td>Wheat</td>
<td>Breads, flours, pasta, baked goods</td>
</tr>
<tr>
<td>Avena sativa</td>
<td>Oats</td>
<td>Oatmeal, flour</td>
</tr>
<tr>
<td>Hordeum vulgare</td>
<td>Barley</td>
<td>Hull barley</td>
</tr>
<tr>
<td>Chenopodium quinoa</td>
<td>Quinoa</td>
<td>Cooked quinoa, pasta,</td>
</tr>
<tr>
<td>Amaranthus caudatus</td>
<td>Amaranth</td>
<td>Breads, pasta</td>
</tr>
<tr>
<td>Fagopyrum esculentum</td>
<td>Buckwheat</td>
<td>Porridge, pasta, pancakes, breads</td>
</tr>
</tbody>
</table>
Phytochemicals are naturally-occurring, non-nutrient plant compounds in fruits, vegetables, grains, and other plant foods. A major class of phytochemicals is phenolics, compounds containing one or more aromatic ring and one or more hydroxyl group. These compounds include phenolic acids and flavonoids (Figure 1). Phenolic acids are generally found esterified or bound to cell wall polymers and are therefore insoluble when extraction solvents are used to extract phenolic compounds from whole or refined grains. Andreasen et al. (2001) proposed a mechanism by which insoluble-bound phenolic compounds may impart their potential health benefits in the lower gastrointestinal tract [3], most likely by imparting their antioxidant activity (transferring a hydrogen atom to a free radical and delocalizing the resulting lone electron throughout the aromatic ring).

Figure 1: Structure of phenolic acids and flavonoids.

![Figure 1: Structure of phenolic acids and flavonoids.](image)

<table>
<thead>
<tr>
<th>a. Flavonoids</th>
<th>b. Hydroxycinnamic Acids</th>
<th>c. Hydroxybenzoic Acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin</td>
<td>OH</td>
<td>OH</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>H</td>
<td>OH</td>
</tr>
<tr>
<td>Rutin</td>
<td>OH</td>
<td>Glu-Glu</td>
</tr>
<tr>
<td>Apigenin</td>
<td>OH</td>
<td>H</td>
</tr>
</tbody>
</table>

b. Hydroxycinnamic Acids

<table>
<thead>
<tr>
<th>Ferulic Acid</th>
<th>H</th>
<th>OMe</th>
<th>OH</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-Coumaric Acid</td>
<td>H</td>
<td>H</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>Caffeic Acid</td>
<td>H</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
</tr>
</tbody>
</table>

c. Hydroxybenzoic Acids

| p-Hydroxybenzoic Acid | H | H | OH | H |
| Syringic Acid        | H | OMe | OH | OMe |
| Vanillic Acid        | H | OMe | OH | H |

Generally, chemistry assays have been used to determine the antioxidant activity of grains [4, 5]. Fardet et al. questioned whether or not findings from these in vitro chemical antioxidant activity assays were reflected in vivo as chemical antioxidant activity assays, generally, use non-physiological temperature and/or pH, and do not account for bioavailability, uptake, or metabolism of compounds of interest [6, 7]. Wang et al. (1999) developed a cell-based antioxidant activity assay, in which 2',7'-dichlorofluorosceine diacetate (DCFH-DA) is used to measure the loss of fluorescence upon quenching of the AAPH-induced reactive oxygen species by the pure compound or sample extract in HepG2 cell cultures [8]. This assay has been further improved and used to determine the cellular antioxidant activity of a wide range of phenolic compounds and phenolics extracts from fruits and vegetables [7, 9, 10].

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Though the cellular antioxidant activity of some common phenolic compounds found in the insoluble-bound fraction of whole grains (ferulic acid and caffeic acid) has been reported [7], the cellular antioxidant activity of other common phenolic compounds found in whole grains has not been determined. The use of a cellular antioxidant activity assay to determine the antioxidant activity of whole grain phenolic compounds in a more relevant biological system may be more valuable towards understanding the potential health benefit of whole grain consumption. The objectives of this study were to determine the total phenolic content, antioxidant activity, and phenolic composition of the insoluble-bound eight grains, and to complete the data regarding the cellular antioxidant activity of the phenolic compounds from the insoluble-bound fraction of the whole grains.

2. Methods

2.1. Grain samples
The grain samples used in this study were purchased at a local organic food store. Each sample was milled to a fine powder using 20, 40, and 60 mesh size screens successively and stored at -20°C until extraction.

2.2. Extraction of insoluble-bound phenolics
Insoluble-bound phenolic compounds were extracted from the residue resulting from the free phenolic extraction according to a previously described method [11]. The residue was first digested with 2 M sodium hydroxide at room temperature for 1 h whilst shaking under nitrogen. The mixture was then neutralized to pH 2 with an appropriate amount of hydrochloric acid. Hexanes were used to extract lipids from the mixture. The remaining mixture was then extracted with ethyl acetate five times. The ethyl acetate fractions were pooled and evaporated to dryness. The phenolics were reconstituted in methanol:hydrochloric acid (1 M, 85:15 v/v) and stored at -40°C.

2.3. Total phenolic content determination
Total phenolic content was determined using the previously described Folin Ciocalteu Reagent colorimetric method [11, 12]. Briefly, extracts were reacted with FolinCiocalteu Reagent and then neutralized with sodium carbonate. After 90 minutes, the absorbance of the resulting solution was measured at 760 nm. Gallic acid was used as the standard and total phenolic content was expressed as mg GAE/100 g sample.

2.4. Phenolic composition determination
The phenolic composition of the insoluble-bound fraction was determined using a previously described rp-HPLC-DAD [11] with slight modification that allowed for the simultaneous detection and quantification of phenolic acids and flavonoids. Samples were filtered through a 0.45 μm filter prior to analysis. Briefly, the mobile phase [water at pH 2.8 with acetic acid (A) and acetonitrile/water 70:30 v/v to pH 2.8 with acetic acid (B)] was delivered using a Waters 600E quaternary pump at a flow of 1.5 mL/min using the following gradient: 0 – 10% B for 2.5 minutes, 10 – 12% B for 2.5 minutes, 12 – 23% B for 10 minutes, 23 – 95% B for 4 minutes, and 95 – 0% B for 4 minutes. Total run time for each injection was 25 minutes. Seventy five microliters of sample were injected using a Water 717 Autosampler. Separation of phenolic compounds was done using a C18 column (5 μm, 250 mm x 4.6 mm column; Grace Vydac, Baltimore, MD). Phenolic compounds were detected using a Waters 996 Photodiode Array Detector. Each injection was monitored at 315 nm. p-Hydroxybenzoic acid and vanillic acid were quantified at 254 nm. Catechin and syringic acid were quantified at 275 nm. Ferulic acid, p-coumaric acid, salicylic acid, and caffeic acid were quantified at 315 nm. Quercetin, rutin, and kaempferol were quantified at 365 nm. Data signals were acquired and processed using Waters Empower software (2002) (Waters Corp., Milford, MA).

2.5. Antioxidant activity
The antioxidant activity was determined using the previously described Oxygen Radical Absorbance Capacity (ORAC) assay [11, 13]. The assay was performed in black-walled 96-well plates (Corning Scientific, Corning, NY). The outside wells of the plate were not used as there was much more variation from them than from the inner wells. Each well contained 20 μL extracts or 20 μL Trolox standard (range 6.25 – 50 μM), and 200 μL fluoroscein (final concentration 0.96 μM), which were incubated at 37 °C for 20 minutes. After incubation, 20 μL of 119 mM AAPH was added to each well. Fluorescence intensity was measured using Fluoroskan Ascent FL plate-reader (Thermo Labsystems, Franklin, MA) at excitation of 485 nm and emission of 520 nm for 35 cycles every 5 min. Phenolic extract dilutions were prepared with 75 mM phosphate buffer (pH 7.4).
2.6. Cell culture
HepG2 cells were cultured using a previously described cell culture method [7]. HepG2 cells were grown in William's Medium E (WME) containing 5% FBS, 10 mM HEPES, 2 mM L-glutamine, 5 μg/mL insulin, 0.05 μg/mL hydrocortisone, 50 units/mL penicillin, 50 μg/mL streptomycin, and 100 μg/mL gentamicin. Cells were maintained at 37°C and 5% CO₂.

2.7. Cell number determination
Cell number was determined using a previously described methylene blue stain method with modification that allowed for cell counting in a 96-well plate [14, 15]. HepG2 cells were seeded at 4 x 10⁴ cells per well on a 96-well plate in 100 μL growth medium and incubated for 4 hours at 37°C. The medium was removed and the cells were treated with varying concentrations of p-coumaric acid, vanillic acid, p-hydroxybenzoic acid, or syringic acid prepared in 2% DMSO in growth medium. The plate was incubated at 37°C for 24 h. After 24 h, the treatment medium was removed and the cells were washed with PBS. A volume of 50 μL/well methylene blue fixing/staining solution (98% HBSS, 0.67% glutaraldehyde, 0.6% methylene blue) was applied to each well and the plate was incubated at 37°C for 1 h. The dye was removed and the plate was immersed in fresh deionized water three times, or until the water was clear. The water was tapped out of the wells and the plate was allowed to air-dry before 100 μL elution solution (49% PBS, 50% ethanol, 1% acetic acid) was added to each well. The plate was placed on a bench-top shaker for 20 minutes to allow uniform elution. The absorbance was read at 570 nm with blank subtraction using the Dynex Technologies MRX II spectrophotometer (Dynex Technologies Inc., Chantilly, VA).

2.8. Cellular antioxidant activity
The cellular antioxidant activity of each pure phenolic compounds was determined using a previously described method [7, 8]. HepG2 cells were seeded at a density of 6 x 10⁶ cells/well on a 96-well plate in 100 μL growth medium/well. Twenty-four hours after seeding, the growth medium was removed. Triplicate wells were treated for 1 h with 100 μL treatment medium containing various concentrations of pure phenolic compound plus 25 μM DCFH-DA. Then, 600 μM AAPH was applied to the cells in 100 μL Hank’s Balanced Salt Solution (HBSS). Emission at 538 nm was measured with excitation at 485 nm every 5 min for 1 h with a Fluoroskan Ascent FL plate-reader (Thermo Labsystems, Franklin, MA) at 37°C. Each plate included triplicate control and blank wells: control wells contained cells treated with DCFH-DA and oxidant; blank wells contained cells treated with DCFH-DA and HBSS without oxidant.

2.9. Statistical analysis
Data were reported as mean ± standard deviation for three replicates. ANOVA and Tukey’s comparison test were performed using Minitab Statistical Software v. 15 (State College, PA).

3. Results and Discussion
3.1. Total phenolic content, phenolic composition, and antioxidant activity of the insoluble-bound fraction of whole grains
The total phenolic content of the insoluble-bound fraction of whole grains was assessed using the previously reported Folin-Ciocalteu Reagent method. The phenolic composition of the insoluble-bound fraction of whole grains was determined using a modified version of the previously reported rp-HPLC-DAD assay, which allowed for the simultaneous detection of phenolic acids and flavonoids. The antioxidant activity was determined using the ORAC assay. The total phenolic content, phenolic composition and antioxidant activity of the insoluble-bound fraction of whole grains are summarized in Table 2. The total phenolic content of the insoluble-bound fraction of whole grains ranged from 24 (amaranth and buckwheat) to 255 (corn) mg GAE/100 g. Ferulic acid and p-coumaric acid were found in the insoluble-bound fraction of all grain samples. The ferulic acid content ranged from 5 (buckwheat) to 558 (corn) μmol/100 g grain. The p-coumaric acid content ranged from roughly 6 (amaranth and buckwheat) to 70 (corn) μmol/100 g grain. Caffeic acid was only detected in the insoluble-bound fraction of barley and corn (4.2 and 1.8 μmol/100 g, respectively). Vanillic acid was only detected in the insoluble-bound fraction of quinoa. The insoluble-bound p-hydroxybenzoic acid content of amaranth and quinoa was 11.2 and 15.2 μmol/100 g, respectively. No flavonoids (quercetin, kaempferol, catechin, or rutin) or syringic acid were detected in the
insoluble-bound fraction of whole grains. The ORAC of the insoluble-bound fraction of whole grains ranged from 748 (amaranth) to 10089 (corn) μmol TE/100 g grain. The ORAC of the insoluble-bound fraction of the pseudocereals (quinoa, amaranth, and buckwheat) was statistically similar. The ORAC of the insoluble-bound fraction of whole grains was correlated with the total phenolic content of the insoluble-bound fraction of whole grains \( (R^2 = 0.779, p < 0.01) \).

### Table 2: The total phenolic content, Oxygen Radical Absorbance Capacity, and phenolic acid and flavonoid contents of the insoluble-bound fraction of whole grains.

<table>
<thead>
<tr>
<th></th>
<th>TPC†</th>
<th>ORAC‡</th>
<th>FA*</th>
<th>p-CA*</th>
<th>p-HBA*</th>
<th>VA*</th>
<th>QUE*</th>
<th>RUT*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>255 ± 5.6 a</td>
<td>10089 ± 803 a</td>
<td>558 ± 8.8 a</td>
<td>70.2 ± 2.4 a</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Wheat</td>
<td>122 ± 0.9 b</td>
<td>2730 ± 554 c</td>
<td>192 ± 15.7 b</td>
<td>12.1 ± 0.3 d</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Barley</td>
<td>94.1 ± 4.7 c</td>
<td>7081 ± 427 b</td>
<td>133 ± 12.2 c</td>
<td>19.0 ± 1.3 c</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Oats</td>
<td>79.5 ± 3.9 d</td>
<td>2891 ± 227 c</td>
<td>70.2 ± 7.4 d</td>
<td>26.4 ± 3.0 b</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Brown Rice</td>
<td>65.8 ± 0.7 e</td>
<td>2516 ± 122 cd</td>
<td>88.6 ± 6.5 d</td>
<td>32.7 ± 0.8 b</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Quinoa</td>
<td>39.4 ± 2.9 f</td>
<td>1641 ± 217 de</td>
<td>35.5 ± 5.1 e</td>
<td>13.1 ± 1.0 d</td>
<td>15.2 ± 1.2 a</td>
<td>19.2 ± 11.2</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Amaranth</td>
<td>23.8 ± 0.3 g</td>
<td>748 ± 36 e</td>
<td>23.3 ± 1.7 e</td>
<td>6.8 ± 0.2 e</td>
<td>11.2 ± 0.9 b</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>23.5 ± 0.8 g</td>
<td>921 ± 54 e</td>
<td>5.3 ± 0.2 f</td>
<td>6.3 ± 0.4 e</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

†Total phenolic content values expressed as mg GAE/100 g grain
‡Oxygen Radical Absorbance Capacity expressed as μmol TE/100 g grain
*Phenolic acid and flavonoid contents reported as μmol/100 g grain
nd – not detected

The phenolic content of grains has been reported previously [4, 16, 17]. Adom and Liu reported that the total phenolic content of the insoluble-bound fraction of corn, wheat, oats, and rice was 228, 104, 81, and 59 mg GAE/100g of grain, respectively [4]. Gorstein et al. reported that the total phenolic content of buckwheat and quinoa was 91 and 60 mg GAE/100g of grain, respectively [16]. Ward et al. also reported that the total phenolic content of the insoluble-bound fraction of barley ranged from 10 to 55 mg GAE/100g of grain [17]. The total phenolic content values reported in the present study are similar to previously-reported values. The phenolic composition of grains has also been previously-reported [18, 19]. Hung reported ferulic acid and rutin contents as high as 314 and 64 μmol/100 g grain, respectively in the phenolics rich fractions that were analyzed [18]. Mattila et al. reported that the total ferulic acid content of grains ranged from 458 (whole wheat) to 129 (oats and barley) μmol/100g grain, the total p-coumaric acid content ranged from 24 (barley) to 9 (buckwheat) μmol/100g grain, and the total p-hydroxybenzoic acid content ranged from 80 (buckwheat) to 4 (corn) μmol/100g grain [19]. The high total p-hydroxybenzoic acid content in buckwheat is most likely due to the contribution of the free fraction. The phenolic composition of the grains reported in the present study is similar to previously-reported data. The total flavonoid content of wheat, buckwheat, quinoa, and amaranth has been reported previously [16, 20]. However, the flavonoid composition of each wheat variety was not determined [20]. It is more important to determine the flavonoid composition of grains because structural differences, degrees of glycosylation, and position of the C-ring can have a significant effect on the cellular antioxidant activity of flavonoids [21]. Though the flavonoids rutin and quercetin have been detected in the insoluble-bound fraction of buckwheat [18], these compounds were not detected in the present study. This is most likely due to the low phenolic content of the buckwheat sample used in this study and not due to the ability of the rp-HPLC-DAD assay to detect phenolic compounds. Studies have reported the antioxidant activity of grains using a variety of different antioxidant activity assays [4, 22]. The results from these different antioxidant activity assays are difficult to compare because these methods have not been standardized to one another [6]. Flavonoids also have varying antioxidant activities. Of the flavonoids from the present study, rutin had the highest ORAC followed by apigenin, quercetin, and kaempferol [21]. None of these flavonoids were detected in the insoluble-bound fraction of any grain from the present study.

### 3.2. Cellular antioxidant activity of select pure compounds

The cellular antioxidant activity of p-coumaric acid, p-hydroxybenzoic acid, syringic acid, and vanillic acid was assessed using a modification of the DCFH-DA assay. The concentrations used to determine the cellular
antioxidant activity did not reduce the number of HepG2 cells by more than 10% compared to the medium control after 24 hours as determined using the modified methylene blue stain assay [7, 14]. None of the phenolic acids tested inhibited the increase in fluorescence due to DCFH oxidation, though quercetin inhibited the increase in fluorescence due to DCFH oxidation in a dose-dependent manner (Figure 2).

3.3. Structure activity relationships of flavonoids and phenolic acids

One study determined the Oxygen Radical Absorbance Capacity and cellular antioxidant activity of flavonoids. The Oxygen Radical Absorbance Capacity of flavonoids was not correlated with cellular antioxidant activity ($R^2 = 0.214$, $p > 0.05$) [21]. Upon further analysis, the researchers concluded that cellular antioxidant activity depends on the structure and degree of glycosylation of the flavonoid [21]. Quercetin and kaempferol have the highest cellular antioxidant activity, most likely due to presence of a hydroxyl group at the 3 position of the C-ring and a keto group and the 4 position of the C-ring (Figure 1). Because quercetin has the highest cellular antioxidant activity, quercetin is used as a standard in the cellular antioxidant activity assay. No flavonoids were found in the insoluble-bound fraction of any of the grains analyzed in the present study (Table 2). Phenolic acids have relatively low cellular antioxidant activity compared to flavonoids (Figure 3). Of the phenolic acids found in the insoluble-bound fraction of whole grains, only caffeic acid had any cellular antioxidant activity. All other phenolic acids had no cellular antioxidant activity (Figure 3). This may be due to the structure of caffeic acid. Caffeic acid is the only phenolic acid that has two hydroxy groups located next to each other on the aromatic ring.

Hydroxylation of the B-ring (Figure 1) of flavonoids was an important factor affecting the cellular antioxidant activity of flavonoids [21]. The phenolic acids used in this study and found in the insoluble-bound fraction of whole grains have either one hydroxyl group (p-coumaric acid and $p$-hydroxybenzoic acid) or a combination of hydroxyl and methoxy groups (ferulic acid, syringic acid, and vanillic acid) (Figure 1). Quercetin, the flavonoid with the highest cellular antioxidant activity, has two hydroxy groups located next to each other on the B-ring [21]. The structural motif found in caffeic acid is also similar to that found in the C-ring of quercetin, kaempferol, and galangin, another flavonoid with high cellular antioxidant activity [21].

3.4. Epidemiological data regarding grains and antioxidant activity

Studies reporting the effects of whole and refined grain consumption in humans are rare. Enright and Slavin reported that there were no effects on antioxidant measures (Oxygen Radical Absorbance Capacity in blood, and isoprostane and thiobarbituric acid reactive substances in urine) after 14 days of consumption of whole grain or refined grain food products in healthy subjects (mean age, 27.1 years; mean BMI, 23.9 kg/m$^2$) [23]. Whole grains, generally have higher total phenolic contents, higher in vitro antioxidant activities, and more phenolic compounds than refined grains [19]. The results from study suggest that higher total phenolic content, higher in vitro antioxidant activity, and more phenolic compounds do not correlate with increased antioxidant activity in vivo.

3.5. Challenges in determining the cellular antioxidant activity of whole grains

The insoluble-bound fraction of whole grains contains phenolic compounds that have relatively low or no cellular antioxidant activity (Figure 3). Therefore, it is highly unlikely that phenolics extracts from whole grains will have any cellular antioxidant activity, particularly in the insoluble-bound fraction which consists of entirely phenolic acids. Phenolics extracts from the insoluble-bound fraction of both whole wheat and refined wheat reduce the number of HepG2 cells by greater than 10% compared to HepG2 cells grown under normal growth conditions at a concentration of 10 mg/mL [24]. For comparison, concentrations of greater than 60 mg/mL of blueberry phenolics extracts, consisting of a wide range of flavonoids [25] with relatively high cellular antioxidant activity [10], elicited the same response in HepG2 cells [7]. Further, phenolic extracts from the insoluble-bound fraction of whole wheat were shown to inhibit the proliferation of colon cancer cells, in vitro [26]. These observations support the claim that the potential health benefit of phenolic compounds from the insoluble-bound fraction of whole grains may be independent of any cellular antioxidant activity.
Figure 2: Peroxyl radical-induced oxidation of DCFH to DCF in HepG2 cells and the inhibition of oxidation by (A) quercetin, (B) p-coumaric acid, (C) p-hydroxybenzoic acid, (D) vanillic acid, and (E) syringic acid, over time. The curves shown in each graph are from a single experiment (mean ± standard deviation, n = 3).

4. Conclusion
The insoluble-bound fraction of whole grains consists mainly of the phenolic acids. No flavonoids were found in the insoluble-bound fraction of whole grains. It was hypothesized that phenolic compounds from the insoluble-bound fraction of whole grains can protect against colon cancer by scavenging free radicals in the lower gastrointestinal tract, preventing the initiation of normal cells [4]. With the exception of caffeic acid, none of the phenolic compounds found in the insoluble-bound fraction of whole grains had any cellular antioxidant activity (Figure 3). This finding suggests that the potential health benefit of whole grain consumption in the lower gastrointestinal tract is independent of the cellular antioxidant activity of phenolic compounds from the insoluble-bound fraction of whole grains.
Figure 3: Cellular antioxidant activity of phenolic compounds found (mean ± standard deviation, n = 3). References are in brackets.

Abbreviations
AAPH, 2,2'-Azobis (2-amidinopropane) dihydrochloride; BMI, body mass index; DCFH-DA, 2',7'-dichlorofluorosceine diacetate; DMSO, dimethyl sulfoxide; FA, ferulic acid; FBS, fetal bovine serum; GAE, gallic acid equivalents; Glu, glucoside; HBSS, Hank’s Balance Salt Solution; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; p-CA, p-coumaric acid; p-HBA, p-hydroxybenzoic acid; PBS, phosphate buffered saline; QUE, quercetin; rp-HPLC-DAD, reversed phase high-performance liquid chromatography-diode array detection; RUT, rutin; TE, Trolox equivalents; TPC, total phenolic content; VA, vanillic acid.

Competing Interests
The author has no competing interests. This research was conducted in partial fulfillment of the Doctor of Philosophy degree from Cornell University.

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