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Antioxidant Activity of Grains

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Epidemiological studies have shown that consumption of whole grains and grain-based products is associated with reduced risk of chronic diseases. The health benefits of whole grains are attributed in part to their unique phytochemical composition. However, the phytochemical contents in grains have been commonly underestimated in the literature, because bound phytochemicals were not included. This study was designed to investigate the complete phytochemical profiles in free, soluble conjugated, and insoluble bound forms, as well as their antioxidant activities in uncooked whole grains. Corn had the highest total phenolic content (15.55 ± 0.60 μmol of gallic acid equiv/g of grain) of the grains tested, followed by wheat (7.99 ± 0.39 μmol of gallic acid equiv/g of grain), oats (6.53 ± 0.19 μmol of gallic acid equiv/g of grain), and rice (5.56 ± 0.17 μmol of gallic acid equiv/g of grain). The major portion of phenolics in grains existed in the bound form (85% in corn, 75% in oats and wheat, and 62% in rice), although free phenolics were frequently reported in the literature. Ferulic acid was the major phenolic compound in grains tested, with free, soluble-conjugated, and bound ferulic acids present in the ratio 0.1:1:100. Corn had the highest total antioxidant activity (181.42 ± 0.86 μmol of vitamin C equiv/g of grain), followed by wheat (76.70 ± 1.38 μmol of vitamin C equiv/g of grain), oats (74.67 ± 1.49 μmol of vitamin C equiv/g of grain), and rice (55.77 ± 1.62 μmol of vitamin C equiv/g of grain). Bound phytochemicals were the major contributors to the total antioxidant activity: 90% in wheat, 87% in corn, 71% in rice, and 58% in oats. Bound phytochemicals could survive stomach and intestinal digestion to reach the colon. This may partly explain the mechanism of grain consumption in the prevention of colon cancer, other digestive cancers, breast cancer, and prostate cancer, which is supported by epidemiological studies.

KEYWORDS: Phytochemicals; phenolics; grains; ferulic acid; antioxidant activity

INTRODUCTION

Epidemiological studies have strongly suggested that diets play a crucial role in the prevention of chronic diseases such as heart disease, cancer, diabetes, and Alzheimers's disease (*1, 2*). Consumption of fruits and vegetables, as well as grains, has been associated with reduced risk of chronic diseases (*1*−*3*). This has been hypothesized to be because they contain phytochemicals that combat oxidative stress in the body by helping to maintain a balance between oxidants and antioxidants. An imbalance caused by overproduction of oxidants leads to oxidative stress, resulting in damage to large biomolecules such as lipids, DNA, and proteins. Oxidative damage increases the risk of degenerative diseases such as cancer and cardiovascular diseases (*1, 4, 5*). Antioxidants reduce oxidative damage to biomolecules by modulating the effects of reactive oxidants (*6, 7*). Therefore, increased consumption of fruits and vegetables containing high levels of antioxidants has been recommended. The importance and health benefits of grain consumption in the prevention of chronic diseases such as cancers and heart disease have also been documented (*8*−*16*). However, the attention paid to grain consumption has been little compared to that for fruits and vegetables, although nutritional guidelines put grains and grain products at the base of the food guide pyramid to emphasize their importance for optimal health (*17*).

Recent research has shown that the complex mixture of phytochemicals in foods provides better protective health benefits than single phytochemicals through a combination of additive and/or synergistic effects (*18*). This has been supported by results from previous studies on health benefits of single antioxidants that gave inconsistent results in human clinical trials (*19, 20*). About 5000 of phytochemicals present in plants have been identified, and still a large percentage remains unknown (*21*). Different plants have different compositions of phytochemicals with different structures and thus offer different protective functions with different extents. Hence, for the maximum health benefits, sufficient amounts of phytochemicals from a variety of sources such as fruits, vegetables, and whole grain-based foods are recommended.

Grains contain unique phytochemicals that complement those in fruits and vegetables when consumed together. For instance,

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various classes of phenolic compounds in grains include derivatives of benzoic and cinnamic acids, anthocyanidins, quinones, flavonols, chalcones, flavones, flavanones, and amino phenolic compounds (*9*, *21*, *22*, *23*). Grains contain tocotrienols and tocopherol (*9*), and rice contains oryzanols (*23*). Some of these phytochemicals such as ferulic acid and diferulates are predominantly found in grains but are not present in significant quantities in some fruits and vegetables (*21*, *24*). Phenolic compounds present in grains have antioxidant properties associated with the health benefits of grains and grain products. Flavonoids have potent antioxidant and anticancer activities. Fruits and vegetables have most of their phytochemicals in free or soluble conjugate forms as glycosides (*25*, *26*). On the other hand, grain phytochemicals may exist in free, soluble conjugate, and insoluble bound forms. Most are in the insoluble bound forms, bound to cell wall materials (*23*, *24*, *27*). About 74% and 69% of the total phenolics present in rice and corn, respectively, are in the insoluble bound forms, with ferulic acid being the major phenolic compound present. Cell wall materials are difficult to digest and may survive gastrointestinal digestion to reach the colon. Colonic digestion of such materials would release the bulk of bound phytochemicals. Andreasen et al. (*28*) showed that human and rat colonic microflora can release diferulic acids from dietary cereal brans. Thus, nondigested or bound phytochemicals may have their unique health benefits in the colon and beyond after absorption.

Most previous studies in the literature reported the phenolic levels of grains using various aqueous solutions of methanol, ethanol, and acetone to extract soluble phenolics (*29*−*33*). These studies assumed long extraction times and/or use of finely powdered samples would ensure maximum extraction of phenolic compounds from grains. Therefore, the total phenolic contents of grains were underestimated in the literature without determining the content of bound phenolics. Maillard and Berset (*22*) reported total antioxidant activity of free and bound extracts of barley and malt, as well as bound ferulic acid and *p*-coumaric acid. However, they did not report free and soluble conjugate ferulic acids or free and bound total phenols and total flavonoids. Sosulski et al. (*27*) reported free, soluble conjugate, and bound phenolic acid contents of rice, oats, wheat, and corn flours, but antioxidant activity, total phenols, and total flavonoids were not investigated.

To our knowledge, there is still limited literature on the complete profile (free, soluble conjugate, insoluble bound) of phenolic compounds and total antioxidant activity of grains. Additionally, the total phenolic contents of grains have been underestimated in the literature. The objective of this study was to investigate the complete phytochemical profiles that exist in the free, soluble conjugate, and insoluble bound forms, as well as their antioxidant activity in corn, wheat, oats, and rice.

MATERIALS AND METHODS

Chemicals and Reagents. Folin−Ciocalteu reagent, sodium nitrite, catechin, and gallic acid were purchased from Sigma (St. Louis, MO). Sodium hydroxide, hexane, aluminum chloride, and acetonitrile were obtained from Fisher Scientific (Pittsburgh, PA), while ethyl acetate, triflouroacetic acid, and ethanol were purchased from Mallinckrodt (Paris, KN).

Grain Samples and Sample Preparation. Samples of oats, corn, wheat, and rice were obtained from General Mills (Golden Valley, MN). Whole oats and whole brown rice were received as flours. Whole grain wheat and Sunlite whole yellow corn were received as dehulled kernels. The wheat and corn samples were milled in a coffee grinder to a fine powder. All samples were individually mixed thoroughly and divided using the quartering system. Each sample was divided into two portions and stored at −20 and −80 °C. The −20 °C samples were used for routine analysis within 2 weeks.

Extraction of Free Phenolic Compounds. Free phenolic compounds in grains were extracted by blending 25 g of whole grain flour with 50 mL of 80% chilled ethanol for 10 min. After centrifugation at 2500 g for 10 min, the supernatant was removed and extraction was repeated one more time. Supernatants were pooled, evaporated at 45 °C to 10 mL, and reconstituted with water to a final volume of 25 mL. The extracts were stored at −40 °C until use (*18*).

Extraction of Bound Phenolic Compounds. One gram of whole grain flour was extracted twice with 80% chilled ethanol with centrifugation at 2500*g* for 10 min, and the supernatant was discarded after each extraction. The residues were then digested with 2 M sodium hydroxide at room temperature for 1 h with shaking under nitrogen gas. The mixture was neutralized with an appropriate amount of hydrochloric acid and extracted with hexane to remove lipids. The final solution was extracted five times with ethyl acetate. The ethyl acetate fraction was evaporated to dryness. Phenolic compounds were reconstituted in 10 mL of water and stored at −40 °C until use (*27*).

Extraction of Soluble Conjugated Ferulic Acid. Extracts from the free phenolic extractions above were used for soluble conjugate extractions. The extract (0.5 mL) was digested with 2 M NaOH for 1 h under nitrogen gas, and the solution was neutralized with an appropriate amount of HCl. The mixture was extracted five times with ethyl acetate, and the ethyl acetate fraction was evaporated to dryness at 35 °C under nitrogen gas. Phenolics were recovered for analysis in 2 mL of water (*22*, *27*).

Determination of Ferulic Acid Content. Ferulic acid in sample extracts was quantified using a RP-HPLC procedure employing a Supelcosil LC-18-DB, 150 mm × 4.6 mm, 3 mm column. Isocratic elution was conducted with 20% acetonitrile in water adjusted to pH 2 with triflouroacetic acid, at a flow rate of 0.6 mL/min. This was delivered using a Waters 515 HPLC pump (Waters Corp., Milford, MA). A Waters 2487 dual wavelength absorbance detector (Waters Corp.) was used for UV detection of analytes at 280 nm. Data signals were acquired and processed on a PC running the Waters Millennium software, version 3.2 (1999) (Waters Corp.). The ferulic acid concentration of sample extracts was extrapolated from the pure *trans*-ferulic acid standard curve. Ten microliter injections were made in each run, and peak heights were used for all calculations. The recoveries for free ferulic acid and bound ferulic acid analyses were 105.13 ± 5.23% ($n = 3$) and 89.30 ± 1.01% ($n = 3$), respectively.

Determination of Total Phenolic Content. The total phenolic content of each extract was determined using methods previously described by Singleton et al. (*34*). Briefly, the appropriate dilutions of extracts were oxidized with Folin−Ciocalteu reagent, and the reaction was neutralized with sodium carbonate. The absorbance of the resulting blue color was measured at 760 nm after 90 min. Using gallic acid as standard, total phenolic content was expressed as micromoles of gallic acid equivalent per gram of grain. Data are reported as mean ± SD for at least three replications.

Determination of Total Flavonoid Content. Total flavonoid content was determined by a colorimetric method described previously (*35*). Appropriate dilutions of sample extracts were reacted with sodium nitrite, followed by a flavonoid−aluminum complex formation using aluminum chloride. Solution absorbance at 510 nm was immediately measured and compared to that of catechin standards. Flavonoid content was expressed as micromoles of catechin equivalent per gram of grain. Data are reported as mean ± SD for at least three replications.

Determination of Total Antioxidant Activity. A modified total oxyradical scavenging capacity (TOSC) assay (*18*, *36*) was used for determining the total antioxidant capacity of extracts. In this assay, peroxy radicals formed from 2,2′-azobis-amidinopropane (ABAP) oxidize α-keto-γ-methiolbutyric acid (KMBA) to form ethylene gas, which was measured by gas chromatographic headspace analysis. The degree of inhibition of ethylene gas formation by sample extracts was used as the basis for calculating the total antioxidant capacity. The dose required to cause a 50% inhibition (EC_{50}) for each sample was used to calculate the total antioxidant activity, which was expressed as micromoles of vitamin C equivalent per gram of grain.

Table 1. Ferulic Acid Contents of Grains and the Percentage Contribution of Each Fraction to the Total (μmol of ferulic acid/100 g of grain)

	free	soluble conjugate	bound	total
corn	0.92 ± 0.02 (0.1%)[a]	8.95 ± 0.11 (1%)	896.27 ± 9.09 (98.9%)	906.13 ± 9.09
wheat	0.57 ± 0.02 (0.2%)	3.27 ± 0.27 (1%)	329.60 ± 16.20 (98.8%)	333.44 ± 16.20
oats	0.65 ± 0.04 (0.4%)	3.4 ± 0.56 (1.84%)	180.61 ± 4.57 (97.8%)	184.66 ± 4.61
rice	0.7 ± 0.05 (0.5%)	9.9 ± 0.34 (6.5%)	142.80 ± 8.68 (93%)	153.39 ± 8.68

[a] Mean ± SD (% contribution to total).

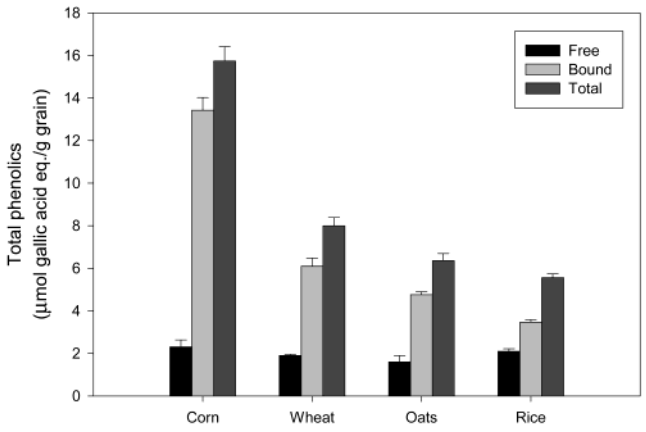

Figure 1. Phenolic content of grains.

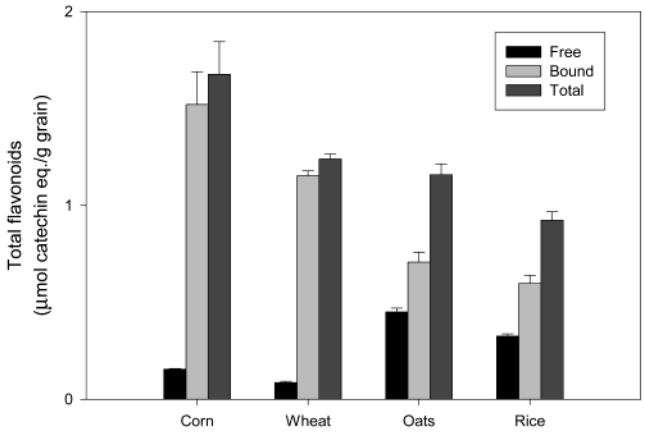

Figure 2. Flavonoid content of grains.

Statistical Analysis. Data were reported as mean ± SD for at least three analyses for each type of extraction and parameter. Results were subjected to ANOVA, and differences between means were located using Tukey's multiple comparison test run on Minitab Release 12 software (State College, PA). Correlations between various parameters were also investigated.

RESULTS

Phenolic Contents of Grains. The phenolic contents of the four grains were expressed as micromoles of gallic acid equivalent per gram of grain (**Figure 1**). Corn had the highest free phenolic content (2.12 ± 0.09 μmol/g of grain), followed by rice (2.10 ± 0.12 μmol/g of grain) and then wheat (1.90 ± 0.06 μmol/g of grain). Oats had the lowest free phenolic content (1.77 ± 0.12 μmol/g of grain). In statistical testing, $p < 0.05$ for corn versus oats, and $p > 0.05$ for all other comparisons. The bound phenolic content was highest for corn (13.43 ± 0.59 μmol/g of grain), followed by wheat (6.10 ± 0.39 μmol/g of grain) and then oats (4.76 ± 0.14 μmol/g of grain). Rice had the lowest bound phenolic content (3.46 ± 0.13 μmol/g of grain). There were significant differences ($p < 0.05$) in the bound phenolic contents of all grains. Also bound phenolics were significantly higher than free phenolics. The total phenolic content was highest in corn (15.55 ± 0.60 μmol/g of grain), followed by wheat (7.99 ± 0.39 μmol/g of grain) and then oats (6.53 ± 0.19 μmol/g of grain). Rice (5.56 ± 0.17 μmol/g of grain) had the lowest total phenolic content. There were significant differences ($p < 0.05$) in total phenolic contents among the grains.

Ferulic Acid Contents of Grains. Ferulic acid contents of grains were expressed as micromoles of ferulic acid per 100 g of grain (**Table 1**). Free ferulic acid was highest in corn (0.92 ± 0.02 μmol/100 g of grain), followed by rice (0.7 ± 0.05 μmol/100 g of grain) and oats (0.65 ± 0.04 μmol/100 g of grain), and was lowest in wheat (0.57 ± 0.02 μmol/100 g of grain). Statistical analyses show $p < 0.05$ for corn versus other grains and for rice versus wheat, and they show $p > 0.05$ for all other comparisons. Soluble conjugated ferulic acid contents were similar ($p > 0.05$) in corn (8.95 ± 0.11 μmol/100 g of grain) and rice (9.9 ± 0.34 μmol/100 g of grain), and both grains had higher ($p < 0.01$) conjugated ferulic acid contents than wheat (3.27 ± 0.27 μmol/100 g of grain) and oats (3.4 ± 0.56 μmol/100 g of grain). There were no differences in soluble conjugated ferulic acid levels between wheat and oats ($p > 0.05$). The bound ferulic acid content was highest in corn (896.27 ± 9.09 μmol/100 g of grain), followed by wheat (329.60 ± 16.20 μmol/100 g of grain) and then oats (180.61 ± 4.57 μmol/100 g of grain). Rice had the lowest bound ferulic acid content (142.80 ± 8.68 μmol/100 g of grain). The bound ferulic acid contents in grains were all significantly different ($p < 0.05$) from each other (**Table 1**). The bound ferulic acid contents were significantly higher ($p < 0.01$) than both free and soluble conjugate ferulic acid contents in all grains tested. The total ferulic acid content was highest in corn (906.13 ± 9.09 μmol/100 g of grain) followed by wheat (333.44 ± 16.20 μmol/100 g of grain) and then oats (184.66 ± 4.61 μmol/100 g of grain), with rice having the lowest total ferulic acid content (153.39 ± 8.68 μmol/100 g of grain).

Flavonoid Contents of Grains. Flavonoid contents of grains were expressed as micromoles of catechin equivalent per gram of grain (**Figure 2**). The free flavonoid content was highest in oats (0.45 ± 0.02 μmol/g of grain), followed by rice (0.33 ± 0.01 μmol/g of grain) and then corn (0.16 ± 0.004 μmol/g of grain). Wheat (0.09 ± 0.01 μmol/g of grain) had the lowest flavonoid content. There were significant differences ($p < 0.05$) in free flavonoid contents among the grains. For bound flavonoid content, the level in corn (1.52 ± 0.17 μmol/g of grain) was higher ($p < 0.01$) than that in wheat (1.15 ± 0.03 μmol/g of grain). Both corn and wheat had higher flavonoid contents ($p < 0.01$) than rice (0.60 ± 0.04 μmol/g of grain) and oats (0.71 ± 0.05 μmol/g of grain). There was no difference in bound flavonoid contents between rice and oats ($p > 0.05$). The total flavonoid content of corn (1.68 ± 0.17 μmol/g) was higher ($p < 0.01$) than those of other grains. The total flavonoid contents of wheat (1.24 ± 0.03 μmol/g of grain) and oats (1.16 ± 0.06 μmol/g of grain) were similar ($p > 0.05$), and both had levels higher ($p < 0.05$) than that of rice (0.92 ± 0.04 μmol/g of grain).

Total Antioxidant Activities of Grains. The total antioxidant activities of grains were expressed as micromoles of vitamin C

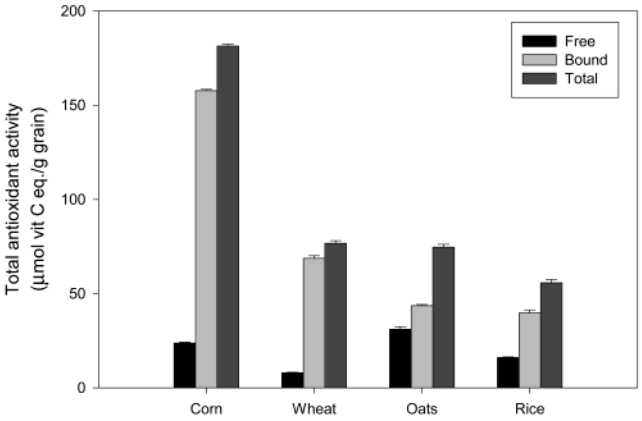

Figure 3. Total antioxidant activity of grains.

Table 2. Percentage Contributions of Free and Bound Fractions of Grains to Total Phenolics, Flavonoids, and Total Antioxidant Activity

	phenolic content (%)		flavonoid content (%)		total antioxidant activity (%)	
	free	bound	free	bound	free	bound
corn	15	85	9	91	13	87
wheat	25	75	7	93	10	90
oats	25	75	39	61	42	58
rice	38	62	35	65	29	71

equivalent per gram of grain (**Figure 3**). Free phytochemical extracts of oats had the highest antioxidant activity (31.07 ± 1.37 μmol/g of grain), followed by corn (24.00 ± 0.43 μmol/g of grain) and then rice (16.02 ± 0.37 μmol/g of grain). Free phytochemical extracts in wheat had the lowest antioxidant activity (8.00 ± 0.30 μmol/g of grain). The antioxidant activities of free phytochemical extracts were different ($p < 0.05$) among the grains. The antioxidant activity of bound phytochemicals in corn (157.68 ± 0.75 μmol/g of grain) was higher ($p < 0.01$) than those in other grains. There was a significant difference ($p < 0.05$) among the antioxidant activities of bound phytochemicals in wheat (68.74 ± 1.35 μmol/g of grain), oats (43.60 ± 0.59 μmol/g of grain), and rice (39.76 ± 1.58 μmol/g of grain). The total antioxidant activity of corn (free + bound) was 181.42 ± 0.86 μmol/g of grain and was the highest ($p < 0.01$) of those of the grains tested. The total antioxidant activities of wheat (76.70 ± 1.38 μmol/g of grain) and oats (74.67 ± 1.49 μmol/g of grain) were similar ($p > 0.05$) but higher ($p < 0.05$) than the total antioxidant activity in rice (55.77 ± 1.62 μmol/g of grain).

There was a low correlation between parameters measured for free extracts (**Table 3**). For bound extractions, however, the total antioxidant activity highly correlated with phenolic content ($R^2 = 0.991$, $p < 0.01$) and ferulic acid content ($R^2 = 0.999$, $p < 0.01$) (**Table 3**). There was a high correlation between phenolics and ferulic acid content for bound extracts ($R^2 = 0.994$, $p < 0.01$). Total values for phenolics, ferulic acid, and flavonoids were highly correlated to the total antioxidant activity. Also total ferulic acid and flavonoids correlated with total phenolic content.

DISCUSSION

Oxidative damage to biomolecules in the body has been associated with certain disease conditions. Grains contain a wide array of phytochemicals that exert health benefits in humans through various mechanisms including antioxidant properties and mediation of hormones. Several studies involving whole grain intakes have shown a consistent protective role of whole grain consumption in reduced risk of colorectal cancer, breast cancer, coronary heart disease, and diabetes, and reduced total mortality (*8*, *9*, *12*−*14*, *16*, *37*, *38*). Thompson (*9*) suggests that lignans and phytoestrogens in grains may reduce the risk of hormone-related diseases such as prostate and breast cancers. Since most grain phenolics such as phenolic acids occur in the outer layers of grains, whole grains (compared to refined grains) are recommended for optimal health. Phytochemical contents of grains have been underestimated in the literature without determining the content of bound phytochemicals (*29*−*32*). This study was designed to determine the complete profile of phenolic phytochemicals in grains, and their relationship and contribution to the total antioxidant activities.

The total phenolic contents were analyzed using the method by Singleton et al. (*34*), without distinguishing specific structures. The free phenolic content represents contributions from free and soluble conjugated phenolics. Conjugated phenolics may still be oxidized and contribute toward total phenolic content and antioxidant activity. Our results clearly showed that most grain phenolics were in the bound fraction (**Figure 1** and **Table 2**). The percentage contributions of free and bound phenolics to the total are shown in **Table 2**. The free phenolic contribution ranged from 15% in corn to 38% in rice. The bound phenolic contribution ranged from 62% in rice to 85% in corn. Therefore, the total phenolic contents of grains were clearly underestimated in the literature without including the bound phenolics. The total phenolic content among grains followed the same concentration trend as the bound phenolics because of the larger contribution from bound phenolics (**Figure 1**). Corn had the highest total phenolic content while rice had the lowest content. Bound phenolics in grains are associated with cell wall materials that may survive upper gastrointestinal digestion conditions and may finally reach the colon. Colonic digestion of such materials by intestinal microflora may release the bulk of bound phytochemicals. On the basis of our results, most of the grain phenolic compounds may be released in the colon to exert their health benefits locally and beyond after absorption. Andreasen et al. (*28*) reported that both human and rat gastrointestinal esterase (from intestinal mucosa and microflora) can release ferulic acid and diferulic acids from cereal bran. These compounds have potent antioxidant properties, and their absorption into the blood plasma has been reported (*28*). Therefore, our results suggest that bound phytochemicals in whole grains may have a more profound effect on health benefits. We believe this could partly explain the inverse association between increased whole grain and whole grain-

Table 3. Correlation Analysis of Phenolics and Total Antioxidant Activity

	free extracts		bound extracts		total	
	total antioxidant activity	phenolics	total antioxidant activity	phenolics	total antioxidant activity	phenolics
phenolics	0.076[a]		0.991**[b]		0.983**	
ferulic acid	0.0003[c]	0.695[c]	0.999**	0.994**	0.974*	0.998**
flavonoids	0.517	0.324	0.872	0.865	0.925*	0.933*

[a] Correlation coefficient R^2. [b] Significantly different: *, $p < 0.05$; **, $p < 0.01$; all others, $p > 0.05$. [c] Total of free and soluble conjugate ferulic acid.

based products consumption and reduced incidence of colon cancer, breast cancer, prostate cancer, heart disease, and diabetes, and reduced total mortality (*8*, *9*, *11*−*16*, *37*−*40*).

The reverse phase HPLC method used for ferulic acid analysis achieved complete resolution of the ferulic acid peak (data not shown), especially for bound extracts, allowing for accurate quantification. High recoveries for both free (105.13 ± 5.23%) and bound (89.28 ± 1.01%) ferulic acid analysis were obtained. The results showed that bound ferulic acid was significantly higher than free and soluble conjugate ferulic acid in corn, wheat, oats, and rice (**Table 1**). The ratio of free, soluble conjugated, and bound ferulic acid in corn and wheat was 0.1:1:100. Free and soluble conjugated ferulic acids made very small contributions (<0.6% and <7.0%, respectively), while bound ferulic acid was the prevalent form of ferulic acid present in the grains (>93%). Thus, total ferulic acid followed the same concentration trend as bound ferulic acid (**Table 1**). The occurrence of bound ferulic acid in such relatively high concentrations also strongly supports our hypothesis that phytochemicals in grains have been underestimated in the literature by excluding the bound fractions. Maillard and Berset (*22*) estimated that free phenolic acids in methanol extracts of barley were 100-fold lower than bound phenolic acids, which was consistent with our findings. Although our extraction and analysis procedures were similar to those used by Maillard and Berset, our percentage recoveries for ferulic acid were much higher. Preliminary work in our laboratory showed that 2 N NaOH digestion for 1−4 h produced similar percentage recoveries with or without rice samples. On the basis of these findings, we used a 1 h alkaline digestion combined with subsequent five times ethyl acetate extractions for extracting bound phenolics. Maillard and Berset used a 4 h alkaline digestion followed by three times ethyl acetate extraction, giving a 63% ferulic acid recovery. It is possible that phenolic acids might be partially destroyed during prolonged alkaline hydrolysis. Our preliminary work showed that about 10% loss of bound ferulic acid occurred during alkaline hydrolysis for 1−4 h (unpublished results). This may account for differences in the results obtained, as well as possible varietal differences between wheat samples used. The ferulic acid value reported in this work for wheat (648 μg ferulic acid/g of grain) was slightly higher than that obtained (590 μg ferulic acid/g of grain) by Yang et al. (*41*), who used the method of Maillard and Berset. Ferulic acid and its conjugates have antioxidant properties (*15*), and they are present in high concentrations in grains.

The free flavonoid contents in grains were low compared to the bound flavonoid content, and they were all significantly different ($p < 0.05$) from each other (**Figure 2**). Total flavonoid contents followed a similar pattern as bound flavonoid contents in all grains, because of the large contribution from bound flavonoids. The percentage contributions of free and bound flavonoids to the total are shown in **Table 2**. The free flavonoid contribution to the total ranged from 7% in wheat to 39% in oats. The bound flavonoid contribution ranged from 61% in oats to 93% in wheat (**Table 2**). The total flavonoid contents of wheat and oats were similar, although their free and bound flavonoid contents were different. This could be attributed to the relatively higher free flavonoids in oats. Thus, whole wheat may deliver more flavonoids to the colon compared to whole oats. Flavonoids have potent antioxidant and anticancer activity.

The total antioxidant activities of grains were different between grains and among different fractions (free and bound) of the same grain. Free extracts of corn, wheat, oats, and rice had significantly lower antioxidant activity ($p < 0.01$; **Figure 3**) compared to those of the bound extracts, as was also observed by Maillard and Berset (*22*). This is attributable to higher phenolic content in bound extracts compared to free extracts (**Table 2**). Although the free and bound antioxidant activities of wheat and oats (**Figure 3**) were significantly different ($p < 0.05$), their total antioxidant activities were similar ($p > 0.05$). This could be attributed to the relatively higher antioxidant activity of free oat extracts or bound wheat extracts. The major contribution to total antioxidant activity of whole grains was from bound extracts and ranged from 58% to 90%. The TOSC assay measures the overall antioxidant activity of extracts including both additive and/or synergistic effects of phytochemicals. This gives a more accurate representation of antioxidant capacity of extracts. Free antioxidant activities represent contributions from both free and soluble conjugate fractions of grains. Some derivatives of phenolic compounds (conjugates) are known to have antioxidant properties. Avenanthramides are cinnamoyl conjugates that occur in oats with higher antioxidant activity (*33*, *42*). Long chain mono- and di-alcohol esters of ferulic and caffeic acids had potent antioxidant activity (*43*). Garcia-Conesa et al. (*15*) measured the antioxidant activity of 8,8′-diferulic acid and compared that to the antioxidant activities of ferulic acid and other diferulates. The Trolox equivalent antioxidant capacity (TEAC) for ferulic acid was 1.96. The TEAC values for various diferulic acid conjugates ranged from 1.49 to 4.00. Generally, diferulic conjugates were reported to be more potent antioxidants than ferulic acid in both aqueous and lipid phases. Among the diferulates tested, 8,8′-diferulic acid was the most potent antioxidant in the aqueous phase.

The total phenolic content has been directly related to the total antioxidant activity and may explain the high correlation observed between these parameters (**Table 3**). Flavonoids and ferulic acid contribute to total phenolics in corn, wheat, oats, and rice. The high correlation between phenolics and ferulic acid content for bound extracts reflects the major contribution of ferulic acid to total phenolic content. The free ferulic acid contribution to free phenolics was <0.5% in all grains, implying the contributions of other compounds to total phenolics in the free extracts were more important. On the other hand, the contribution of bound ferulic acid content to bound phenolics was 76% in corn, 61% in wheat, 43% in oats, and 47% in rice. Velioglu et al. (*29*) reported a significant relation ($R^2 = 0.905$) between total phenolics and antioxidant activity of grain products.

Our results have shown that phytochemical contents of grains have been underestimated in the literature without including the bound phytochemicals. Among the grains we tested, corn had the highest content of phenolic compounds followed by wheat and oats, while rice had the lowest phenolic content. Ferulic acid was the major phenolic compound in grains and was mainly present in the bound form. Free, soluble conjugated, and bound ferulic acids were present in the ratio 0.1:1:100. Corn had the highest total antioxidant activity followed by wheat and oats, and then rice. Our results also show the major portions of phytochemicals in the grains are present in the bound form and may survive stomach and intestinal digestion to reach the colon. This may partially explain the mechanism of grain consumption in the prevention of colon cancer, other digestive cancers, breast cancer, and prostate cancer. Further studies of the unique grain phytochemicals and their mechanism of action are warranted.

LITERATURE CITED

(1) Temple, N. J. Antioxidants and disease: More questions than answers. *Nutr. Res.* **2000**, *20*, 449−459.

(2) Willet, W. C. Diet and Health: what should we eat. *Science* **1994**, *254*, 532−537.
(3) Willet, W. C. Diet, Nutrition, and avoidable cancer. *Environ. Health Perspect.* **1995**, *103* (suppl 8), 165−170.
(4) Wagner, J. R.; Hu, C.; Ames, B. N. Endogenous oxidative damage of deoxycytidine in DNA. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 3380−3384.
(5) Ames, B. N. Identifying environmental chemicals causing mutations and cancer. *Science* **1979**, *204*, 587−593.
(6) Fraga, C. G.; Motchnik, P. A.; Shigenaga, M. K.; Helbock, H. J.; Jacob, R. A.; Ames, B. N. Ascorbic acid protects against endogenous oxidative DNA damage in human sperm. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 11003−11006.
(7) Duthie, S. J.; Ma, A.; Ross, M. A.; Collins, A. R. Antioxidant supplementation decreases oxidative DNA damage in human lymphocytes. *Cancer Res.* **1996**, *56*, 1291−1295.
(8) Smigel, K. Fewer colon polyps found in men with high-fiber, low fat diets. *Natl. Cancer Inst.* **1992**, *84*, 80−81.
(9) Thompson, L. U. Antioxidant and hormone-mediated health benefits of whole grains. *Crit. Rev. Food Sci. Nutr.* **1994**, *34*, 473−497.
(10) Soler, M.; Bosetti, C.; Franceschi, S.; Negri, E.; Zambon, P.; Talamini, R.; Conti, E.; La Vecchia, C. Fiber intake and the risk of oral, pharyngeal and esophageal cancer. *Int. J. Cancer* **2001**, *91*, 283−287.
(11) Kasum, C. M.; Jacobs, D. R., Jr.; Nicodemus, K.; Folsom, A. R. Dietary risk factors for upper aerodigestive tract cancers. *Int, J, Cancer* **2002**, *99* (2), 267−272.
(12) Jacobs, D. R.; Meyer, H. E.; Solvoll, K. Reduced mortality among whole grain bread eaters in men and women in the Norwegian County Study. *Eur. J. Clin. Nutr.* **2001**, *55*, 137−143.
(13) Jacobs, D. R.; Slavin, J.; Marquart, L. Whole grain intake and cancer: A review of literature. *Nutr. Cancer* **1995**, *22*, 221−229.
(14) Jacobs, D. R., Jr.; Meyer, K. A.; Kushi, L. H.; Folsom, A. R. Whole grain intake may reduce risk of coronary heart disease death in postmenopausal women: The Iowa Women's Health Study. *Am. J. Clin. Nutr.* **1998**, *68*, 248−257.
(15) Garcia-Conesa, M. T.; Wilson, P. D.; Plumb, G. W.; Ralph, J.; Williams, G. Antioxidant properties of 4,4′-dihydroxy-3,3′-dimethoxy-b,b′-bicinnamic acid (8−8-diferulic acid, noncyclic form). *J. Sci. Food Agric.* **1999**, *79*, 379−384.
(16) Meyer, K. A.; Kushi, L. H.; Jacob, D. R., Jr.; Slavin, J.; Sellers, T. A.; Folsom, A. R. Carbohydrates, dietary fiber, incident type 2 diabetes mellitus in older women. *Am. J. Clin. Nutr.* **2000**, *71*, 921−930.
(17) National Research Council, Food and Nutrition Board. *Recommended Dietary Allowance*, 10th ed.; National Academy Press: Washington, DC, 1989.
(18) Eberhardt, M. V.; Lee, C. Y.; Liu, R. H. Antioxidant activity of fresh apples. *Nature* **2000**, *405*, 903−904.
(19) Rapola, J. M.; Virtamo, J.; Ripatti, S.; Huttunen, J. K.; Albanes, D.; Taylor, P. R.; Heinonen, O. P. Randomised trial of alpha-tocopherol and beta-carotene supplements on incidence of major coronary events in men with previous myocardial infaction. *Lancet* **1997**, *349*, 1715−1720.
(20) The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Group Study Group. The effect of vitamin E and b-carotene on the incidence of lung cancer and other cancers in male smokers. *New Engl. J. Med.* **1994**, *330*, 1029−1035.
(21) Shahidi, F.; Naczk, M. Phenolic Compounds in grains. In *Food Phenolics: Sources, chemistry, effects, applications*; Technomic Publishing Company Inc: Lancaster, PA, 1995; pp 3−39.
(22) Maillard, M. N.; Berset, C. Evolution of antioxidant activity during kilning: Role of insoluble bound phenolic acids of barley and malt. *J. Agric. Food Chem.* **1995**, *43*, 1789−1793.
(23) Lloyd, B. J.; Siebenmorgen, T. J.; Beers, K. W. Effect of commercial processing on antioxidants in rice bran. *Cereal Chem.* **2000**, *77*, 551−555.
(24) Bunzel, M.; Ralph, J.; Martia, J. M.; Hatfield, R. D.; Steinhart, H.; Diferulates as structural components in soluble and insoluble cereal dietary fibre. *J. Sci. Food Agric.* **2001**, *81* (7), 653−660.
(25) Vinson, J. A.; Hao, Y.; Su, X.; Zubik, L. Phenol antioxidant quantity and quality in foods: vegetables. *J. Agric. Food Chem.* **1998**, *46*, 3630−3634.
(26) Vinson, J. A.; Hao, Y.; Su, X.; Zubik, L. Phenol antioxidant quantity and quality in foods: Fruits. *J. Agric. Food Chem.* **2001**, *49*, 5315−5321.
(27) Sosulski, F.; Krygier, K.; Hogge, L. Free, esterified, and insoluble-bound phenolic acids. 3. Composition of phenolic acids in cereal and potato flours. *J. Agric. Food Chem.* **1982**, *30*, 337−340.
(28) Andreasen, M. F.; Kroon, P. A.; Williamson, G.; Garcia-Conesa, M. T. Intestinal release and uptake of phenolic antioxidant diferulic acids. *Free Radical Biol. Med.* **2001**, *31*, 304−314.
(29) Velioglu, Y. S.; Mazza, G.; Gao, L.; Oomah, B. D. Antioxidants activity and total phenolics in selected fruits, vegetables, and grain products. *J. Agric. Food Chem.* **1998**, *46*, 4113−4117.
(30) Dietrych-Szostak, D.; Oleszek, W. Effect of processing on the flavonoid content in buckwheat (*Fagopyrum esculentum* Moench) grain. *J. Agric. Food Chem.* **1999**, *47*, 4384−4387.
(31) Miller, H. E.; Rigelhof, F.; Marquart, L.; Prakash, A.; Kanter, M. Antioxidant content of whole grain breakfast cereals, fruits and vegetables. *J. Am. Coll. Nutr.* **2000**, *19*, 312s−319s.
(32) Zielinski, H.; Kozlowska, H. Antioxidant activity and total phenolics in selected cereal grains and their different morphological fractions. *J. Agric. Food Chem.* **2000**, *48*, 2008−2016.
(33) Bryngelsson, S.; Dimberg, L. H.; Kamal-Eldin, A. Effects of commercial processing on levels of antioxidants in oats (Avena sativa L.). *J. Agric. Food Chem.* **2002**, *50*, 1890−1896.
(34) Singleton, V. L.; Orthofer, R.; Lamuela-Raventos, R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.* **1999**, *299*, 152−178.
(35) Jia, Z.; Mengcheng, T.; Wu, J. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.* **1999**, *64*, 555−559.
(36) Winston, G. W.; Regoli, F.; Duga, A. J.; Fong, J. H.; Blanchard, K. A. A rapid gas chromatographic assay for determining oxyradical scavenging capacity of antioxidants and biological fluids. *Free Radical Biol. Med.* **1998**, *24*, 480−493.
(37) Jacobs, D. R., Jr.; Meyer, K. A.; Kushi, L. H.; Folsom, A. R. Is whole grain intake associated with reduced total and cause-specific death rates in older women: The Iowa Women's Health Study. *Am. J. Public Health* **1999**, *89*, 322−329.
(38) Liu, S. M.; Stampfer, M. J.; Hu, F. B.; Giovannucci, E.; Rimm, E.; Manson, J. E.; Hennekens, C. H.; Willett, W. C. Whole grain consumption and risk of coronary heart disease: results from the Nurses' Health Study. *Am. J. Clin. Nutr.* **1999**, *70*, 412−419.
(39) Jain, M. G.; Hislop, G. T.; Howe, G. R.; Ghadirian, P. Plant foods, antioxidants, and prostate cancer risk: findings from case-control studies in Canada. *Nutr. Cancer* **1999**, *34* (2), 173−184.
(40) Nicodemus, K. K.; Jacobs, D. R., Jr.; Folsom, A. R. Whole and refined grain intake and risk of incident postmenopausal breast cancer. *Cancer, Causes Control* **2001**, *12*, 917−925.
(41) Yang, F.; Basu, T. K.; Ooraikul, B. Studies on germination conditions and antioxidant content of wheat grain. *Int. J. Food Sci. Nutr.* **2001**, *52*, 319−330.
(42) Collins, F. W. Oats phenolics: Avenanthramides, novel substituted N-cinnamoylanthranilate alkaloids from oats groats and hull. *J. Agric. Food Chem.* **1989**, *37*, 60−66.
(43) Daniels, D. G. H.; Martin, H. F. Antioxidant in oats: Mono-esters of caffeic and ferulic acids. *J. Sci. Food Agric.* **1967**, *18*, 589−595.

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