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Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions

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Abstract

Four different types of wheat bran were extracted and analyzed for phenolic acids using the Folin–Ciocalteu method and HPLC. The extracts and their hydrolysis products were also evaluated for their antioxidant activities. The total phenolic content of the red wheat bran was higher than that of the white wheat. We found that the majority of the phenolic acids existed in a bound form in wheat bran. These phenolic acids can be released by hydrolyzing the bran under alkaline or acidic conditions; however, the former was more efficient in the release of free phenolic acids than the latter. Ferulic, vanillic, and syringic acids were the major individual phenolic acids in the studied wheat bran. The main portion of the total ferulic acid was from alkaline hydrolysis. The alkaline hydrolysable fractions had greater antioxidant activities, while the acid hydrolysable fractions showed lower activities in both the red and white bran. The antioxidant activity of bran extract was stronger than that of free phenolic acids.
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Keywords: Wheat bran; Phenolic acids; Antioxidant activity; Hydrolysis; Bond phenolics; Hydrolysable phenolics

1. Introduction

Phenolic acids are a group of natural products commonly found in many cereal grains. Higher concentrations of these compounds are found in the outer layers of the kernel which constitute the bran (Baublis, Clydesdale, & Decker, 2000; Baublis, Lu, Clydesdale, & Decker, 2002; Onyeneho & Hettiarachchy, 1992; Saadi, Lempereur, Sharonov, Autran, & Manfait, 1998). These phenolic acids may vary in structure due to difference in number and position of the hydroxyl groups on the aromatic ring. As a group, these naturally occurring compounds have been found to be strong antioxidants against free radicals and other reactive oxygen species (ROS), the major cause of many chronic human diseases such as cancer and cardiovascular diseases (Andreasen, Kroon, Williamson, & Garcia-Conesa, 2001a; Yu et al., 2002; Yu, Perret, Harris, Wilson, & Haley, 2003). The health benefits of cereal grains have significant implications for the improvement of food quality, particularly through applications in functional foods and nutraceuticals (Abdul-Hamid & Luan, 2000; Truswell, 2003). There are mainly two groups of phenolic acids in cereal bran: benzoic and cinnamic acid derivatives. Ferulic acid and other hydroxycinnamic acids (caffeic and *p*-coumaric acid derivatives) have been found to have good antioxidant activities (Andreasen, Kroon, Williamson, & Garcia-Conesa, 2001b; Emmons, Peterson, & Paul, 1999). The presence of the CH=CH–COOH group in the hydroxycinnamic acids is considered to be key for the significantly higher antioxidative efficiency than the COOH in the hydroxybenzoic acids (White & Xing, 1997).

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Wheat is one of the popular cereal grains, and its bran represents not only a good source of dietary fibres (Alabaster, Tang, & Shivapurkar, 1997; Møller, Dahl, & Bøckman, 1988), but also of phenolic acids, and the latter are known to contribute significantly to the total antioxidant activity of wheat (Baublis, Decker, & Clydesdale, 2000; Onyeneho & Hettiarachchy, 1992; Yu et al., 2003). Wheat bran extracts contain several phenolic acids, including vanillic, *p*-coumaric and, largely, ferulic acid (Kähkönen et al., 1999). These compounds, particularly ferulic acid, are not evenly distributed in the wheat; most are found in the bran (Baublis et al., 2002). Extract of wheat bran, having high concentration of phenolic acids, was shown to have stronger antioxidant activity than other fractions of wheat (Onyeneho & Hettiarachchy, 1992). In addition, wheat bran has been reported to be able to inhibit lipid oxidation catalyzed by either iron or peroxyl radicals (Baublis, Decker, et al., 2000). Most recently, Zhou, Laux, and Yu (2004) reported that wheat grain, bran and fractions had different antioxidant activities and total phenolic contents (TPC). Their study also showed that ferulic acid was a major contributor to the antioxidant activity. In a phosphatidylcholine liposome system, the percentage of liposome oxidation is reduced by increasing the concentration of isolated phenolic acids from whole-grain breakfast cereal (Baublis, Clydesdale, et al., 2000). Analytical procedures can significantly affect the antioxidant activity of phenolic acids because of the variable contents and types of phenolic acids through different sample preparations (Lehtinen & Laakso, 1997; Onyeneho & Hettiarachchy, 1992), extraction (Zieliński & Kozłowska, 2000) and hydrolysis procedures (Kader, Rovel, Girardin, & Metche, 1996; Nuutila, Kammiovirta, & Oksman-Caldentey, 2002). The hydrolysis method, in particular, can affect the yield and profile of phenolic acids in wheat bran because phenolic acids exist in esterified forms in the cell walls (Saadi et al., 1998). These bound phenolic acids can be hydrolyzed using an acid or an alkali to release so-called hydrolysable phenolic acids. Although the antioxidant activity of phenolic acids from other cereals has been intensively investigated, information on that in wheat bran is scarce (Baublis, Clydesdale, et al., 2000, Baublis, Decker, et al., 2000; Onyeneho & Hettiarachchy, 1992; Yu et al., 2003). Studies on the phenolic acid profile (both quantity and identity) in different varieties of wheat, and the effect of processing, e.g., hydrolysis and extraction methods, are particularly lacking.

The objectives of this study were, therefore, to qualify and quantify phenolic acids in wheat bran, to investigate the effect of hydrolysis and extraction conditions on the yield and profile of phenolic acids and to measure antioxidative activities of the extracts and the hydrolysis products.

2. Materials and methods

2.1. Materials

Hard red and soft white wheat bran samples were received from Hayhoe Mills Ltd. (ON, Canada). The same types of wheat bran were also obtained from AACC (American Association of Cereal Chemist) for comparison. Gallic, protocatechuic, *p*-hydroxybenzoic, gentisic, chlorogenic, vanillic, caffeic, syringic, *p*-coumaric, ferulic, salicylic and *trans*-cinnamic acids were purchased from Sigma–Aldrich (Oakville, ON, Canada). Butylated hydroxytoluene (BHT), β-carotene (type I; synthetic 95%), linoleic acid and Tween 40 (polyoxyethylenesorbitan monopalmitate) were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals and solvents were of analytical or HPLC-grade purity from Caledon Laboratories Ltd. (Georgetown, ON, Canada).

2.2. Extraction

Each of the four wheat bran samples was milled using a laboratory miller (A-10 S2 Kika-Labortechnik, Germany) and sieved through a 32-mesh screen. The fine flour (200 g) was transferred to an Erlenmeyer flask, defatted twice with hexane at a 4:1 ratio (v/w), and kept on a mechanical shaker for 1 h at room temperature. Each time, the mixture was filtered through a Whatman No. 1 filter paper, and the final defatted bran was dried in a hood at room temperature. The filtrate was evaporated to dryness and the residue was weighed as the total lipid content. The defatted bran was then extracted twice with 80% methanol at a 5:1 ratio (v/w) for 1 h at room temperature. The mixture was filtered through a Whatman No. 1 filter paper, and the combined supernatant was concentrated to dryness using a rotary evaporator at 40 °C. The methanolic extract was further freeze-dried and stored in a sealed container at 5 °C prior to being analyzed. Aliquots of the residue and the methanolic extract were also subjected to experiments related to hydrolysable phenolic acids.

2.3. Extractable phenolic acids

An aliquot of the methanolic extract, equivalent to 1 g bran, of each sample was re-dissolved in 4 ml of acidified water (pH 2 with HCl) and partitioned with 4 ml of ethyl ether, three times. The combined ether layer contained free phenolic acids (FPA). The water phase was neutralized to pH 7 with 2 M NaOH and dried using a vacuum evaporator. The residue was dissolved in 4 ml of 2 M NaOH and stirred for 4 h at room temperature. The solution was then acidified to pH 2, and extracted with ethyl ether as mentioned above. The resulting ether layer contained alkaline-hydrolysable phenolic acids (BHPA). The remaining water phase was treated with

3 ml of 6 M HCl and heated at 95 °C for 20 min. The solution was again partitioned with ethyl ether, which contains acid-hydrolysable phenolic acids (AHPA). All fractions were reconstituted in methanol, filtered through a 0.45 μm syringe filter (Acrodisc, Gelman Laboratory, Ann Arbor, MI) and subsequently subjected to total phenolic determination, HPLC analysis and antioxidant activity measurement.

2.4. Bound phenolic acids

The residue, after the methanol extraction, was hydrolyzed by two different protocols to determine the effect of hydrolysis conditions on the yield and profile of hydrolysable phenolic acids in the bran that were not extractable by aqueous methanol. Method A started with alkaline hydrolysis (1 g residue in 40 ml 2 M NaOH, 4 h, at room temperature) and was subsequently followed by acid hydrolysis of bran residue (6 M HCl, 1 h at 95 °C), resulting in two fractions A1 and A2, respectively. In method B, hydrolysis by acid was done first, and subsequently followed by alkaline hydrolysis, resulting in fractions B1 and B2. All fractions were extracted at pH 2 with ethyl ether (40 ml × 3). The same analyses were done for all these fractions.

2.5. Determination of total phenolic content

Each fraction (0.2 ml) was mixed with 1 ml of the Folin–Ciocalteu reagent and 0.8 ml of saturated sodium carbonate (Na_2CO_3) solution. The mixture was allowed to stand at room temperature for 30 min and then the absorbance was measured at 765 nm in a Varian Cary 3C spectrophotometer (Varian analytical instruments, Harbor City, CA). The total phenolic content was expressed as microgrammes of gallic acid equivalent (GAE) per millilitre of solution. The total phenolic contents in wheat bran were subsequently calculated from these data.

2.6. HPLC analysis

HPLC analysis was carried out using an Agilent Technologies 1100 series liquid chromatograph equipped with an auto sampler and a diode-array detector. The analytical column was a Phenomenex Luna C18 (2) (250 × 4.6 μm; 5 μm) with a C18 guard column (Phenomenex, Torrance, CA). The mobile phase consisted of acetonitrile (solvent A) and 2% acetic acid in water (v/v) (solvent B). The flow rate was kept at 1.0 ml min^{-1} for a total run time of 70 min and the gradient programme was as follows: 100% B to 85% B in 30 min, 85% B to 50% B in 20 min, 50% B to 0% B in 5 min and 0% B to 100% B in 5 min. There was 10 min of post-run for reconditioning. The injection volume was 10 μl and peaks were monitored simultaneously at 280 and 320 nm for the benzoic acid and cinnamic acid derivatives, respectively. All samples were filtered through a 0.45 μm Acrodisc syringe filter (Gelman Laboratory, MI) before injection. Peaks were identified by congruent retention times and UV spectra and compared with those of the standards. Detection limits, for all compounds, were from 0.1 to 0.5 ppm, and the correlation coefficient R^2 was >0.9993 from 0.5 to 200 ppm. The detection limit was defined as the concentration at which the signal to noise ratio (S/N) was equal to or greater than three. All samples were prepared and analyzed in duplicate.

2.7. β-Carotene-linoleic acid model system (β-CLAMS)

The β-CLAMS method is based on the de-coloration of β-carotene by the peroxides generated during the oxidation of linoleic acid (a free radical chain reaction) at elevated temperature (Miller, 1971). In this study, the β-CLAMS was modified for the 96-well microplate reader. In brief, β-carotene (0.5 mg) was dissolved in ca. 2 ml of $CHCl_3$ in a 200 ml round-bottom flask, to which 25 μl of linoleic acid and 200 mg of Tween 40 were added. $CHCl_3$ was removed using a rotary evaporator. Oxygenated HPLC-grade water (100 ml) was added, and the flask was shaken vigorously until all material dissolved. The oxygenated water was obtained by bubbling water with compressed oxygen gas for at least 2 h at room temperature. This test mixture was prepared fresh and used immediately. To each well, 250 μl of the reagent mixture and 35 μl sample or standard solution or water (blank), were added. The plate was incubated at 45 °C. Readings were taken at 490 nm, immediately after and every 15 minutes, for 300 min, using a visible/UV microplate kinetics reader (EL 340, Bio-Tek Instruments, Inc., Winooski, VT). All phenolic acid standards were prepared at 200 ppm, and run in triplicate.

3. Results and discussion

3.1. Total phenolic contents

Eighty percent aqueous methanol gave the highest yield of extractables of the bran (8–9%). The total phenolic contents (TPC) of different fractions of the wheat bran are shown in Table 1. TPCs of the commercial wheat bran were similar to those of the AACC standards, although at individual fraction levels, they were significantly different. All fractions of the red wheat bran, except for the AHPA of the AACC red wheat bran, showed higher TPC than their corresponding white wheat bran. This result is consistent with what was reported by Maziya-dixon, Klopfenstein, and Leipold (1994). TPCs of wheat bran in our study ranged from 3.3 mg of gallic acid equivalents per gramme (GAE/g) of bran to 3.9 mg of GAE/g. The level of TPCs

Table 1
Total phenolic contents in different wheat bran fractions (μg GAE/g)[a]

Bran	Extractable phenolic acids (μg GAE/g[a])			Bound phenolic acids (μg GAE/g[a])		Total[e](μg/g)
	FPA[b]	BHPA[c]	AHPA[d]	BHPA[c]	AHPA[d]	
Red, AACC	336 ± 2.4	146 ± 11.4	151 ± 8.3	2312 ± 4.2	889 ± 14.6	3834 ± 6.8
White, AACC	281 ± 0.2	116 ± 2.5	165 ± 1.0	2145 ± 7.6	654 ± 13.3	3362 ± 24.3
Red, commercial	186 ± 1.4	148 ± 8.1	236 ± 6.1	2326 ± 4.1	1070 ± 6.2	3967 ± 5.8
White, commercial	185 ± 1.4	103 ± 1.4	170 ± 4.4	2266 ± 10.5	1060 ± 7.8	3784 ± 22.9

[a] Microgrammes gallic acid equivalents per gramme of bran.
[b] Free phenolic acid.
[c] Alkaline-hydrolysable phenolic acids.
[d] Acid-hydrolysable phenolic acids.
[e] Sum of all fractions of bran.

was close to, but slightly higher than, what was reported by Zhou and Yu (2004). The bound phenolic contents were significantly higher than the extractable phenolic contents in all varieties, indicating that the major phenolic acids in wheat bran were not extractable by aqueous methanol but released upon alkaline or acid hydrolysis. Adom, Sorrells, and Liu (2003) also found that phenolic content of wheat occurred mostly in the bound form and

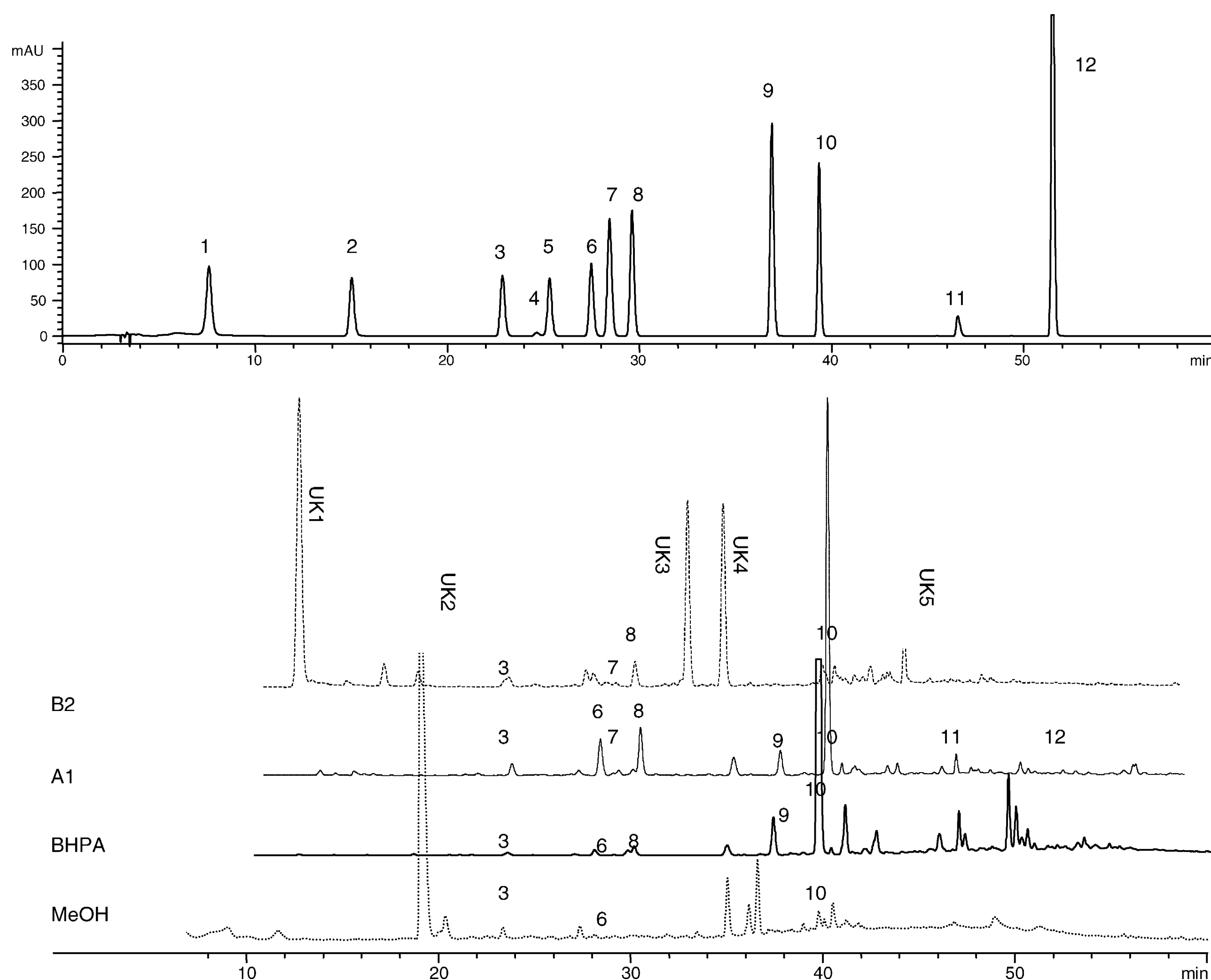

Fig. 1. HPLC chromatograms of a standard mixture of phenolic acids (top) and profiles of typical fractions of wheat bran extracts. Peak 1, gallic acid; 2, protocatechuic acid; 3, *p*-hydroxybenzoic acid; 4, gentistic acid; 5, chlorogenic acid; 6, vanillic acid; 7, caffeic acid; 8 syringic acid; 9, *p*-coumaric acid; 10, ferulic acid; 11, salicylic acid; 12, *trans*-cinnamic acid. A1, bound phenolic acids hydrolyzed by alkaline in method A; B2, bound phenolic acids hydrolyzed by alkaline in method B; MeOH, aqueous methanol (80%)-extractable phenolic acids; BHPA, alkaline-hydrolysable phenolic acids in the methanol-extractable fraction (see Section 2). All chromatograms were from the commercial red wheat bran.

the bound phenolic content was 2.5–5.4-fold higher than the free phenolic content in wheat grains. Our data confirmed their finding (Table 1).

3.2. Individual phenolic acids

The 12 standard phenolic acids shown in Fig. 1 have been reported in wheat (Onyeneho & Hettiarachchy, 1992; Sosulski, Krygier, & Hogge, 1982; Wu, Haig, Pratley, Lemerle, & An, 1999); however, only ferulic, vanillic, caffeic, syringic, *p*-hydroxybenzoic, *p*-coumaric, salicylic and *trans*-cinnamic acids were detected in the various fractions of bran analyzed in this study. Different fractions contained different phenolic acid profiles (Fig. 1). The crude methanolic extract of the commercial red wheat bran contained few FPAs, but it was the unknown compound (UK2) at near 19 min that dominated the HPLC profile. Concentrations of individual phenolic acids in different fractions of wheat bran are shown in Table 2 and 3. Ferulic acid, *p*-hydroxybenzoic acid

Table 2
Phenolic acids in different fractions of AACC wheat bran

Phenolics	Extractable phenolic acids (μg/g)			Bound phenolic acids (μg/g)		Total (μg/g)[d]
	FPA[a]	BHPA[b]	AHPA[c]	A1[b]	A2[c]	
Red wheat bran						
3. *p*-Hydroxybenzoic acid	3.41 ± 0.06	2.11 ± 0.11		12.9 ± 1.37	20.8 ± 0.29	39.2 ± 0.62
6. Vanillic acid	7.92 ± 0.46	4.78 ± 0.04	10.7 ± 0.02	19.8 ± 0.30	39.4 ± 0.31	82.6 ± 0.19
7. Caffeic acid	0.80 ± 0.00					0.80 ± 0.00
8. Syringic acid	10.5 ± 0.28	20.53 ± 0.84	14.2 ± 0.04	61.7 ± 0.00	17.9 ± 0.14	125 ± 0.34
9. *p*-Coumaric acid	0.84 ± 0.02	1.01 ± 0.00		33.1 ± 0.06		35.0 ± 0.03
10. Ferulic acid	7.66 ± 0.28	5.71 ± 0.17		1905 ± 2.97		1918 ± 1.59
11. Salicylic acid				89.0 ± 0.00		89.0 ± 0.00
White wheat bran						
3. *p*-Hydroxybenzoic acid		1.40 ± 0.00	2.55 ± 0.02	8.14 ± 0.08	12.5 ± 0.08	24.6 ± 0.04
6. Vanillic acid	5.62 ± 0.10	3.20 ± 0.01		20.3 ± 1.15	16.5 ± 0.23	45.5 ± 0.53
8. Syringic acid	4.16 ± 0.00	10.5 ± 0.02	9.82 ± 0.21	40.0 ± 0.40	7.14 ± 0.20	71.5 ± 0.16
9. *p*-Coumaric acid		1.30 ± 0.01		36.8 ± 0.08		38.1 ± 0.05
10. Ferulic acid	4.19 ± 0.45	10.4 ± 0.04	2.44 ± 0.06	1359 ± 8.18		1376 ± 4.00
12. *trans*-Cinnamic acid				2.70 ± 0.04		2.70 ± 0.04

Data are means ± standard deviation ($n = 3$) determined by HPLC analysis.
[a] Free phenolic acid.
[b] Alkaline hydrolysables.
[c] Acid hydrolysables.
[d] Sum of all fractions.

Table 3
Phenolic acids in different fractions of commercial wheat bran

Phenolics	Extractable phenolic acids (μg/g)			Bound phenolic acids (μg/g)		Total (μg/g)[d]
	FPA[a]	BHPA[b]	AHPA[c]	A1[b]	A2[c]	
Red wheat bran						
3. *p*-Hydroxybenzoic acid		2.68 ± 0.12	4.55 ± 0.06	7.46 ± 1.98	13.6 ± 0.00	28.3 ± 0.96
6. Vanillic acid	1.41 ± 0.01	8.18 ± 0.20		13.4 ± 0.56	55.0 ± 0.79	78.0 ± 0.35
7. Caffeic acid				1.78 ± 0.00		1.78 ± 0.00
8. Syringic acid		23.8 ± 0.60	24.1 ± 0.42	26.6 ± 0.82	108 ± 0.94	182 ± 0.23
9. *p*-Coumaric acid		1.60 ± 0.03	0.25 ± 0.00	41.7 ± 0.47	2.98 ± 0.06	46.6 ± 0.22
10. Ferulic acid	2.46 ± 0.08	37.5 ± 0.52	4.76 ± 0.05	1934 ± 2.31	43.1 ± 0.33	2020 ± 0.94
11. Salicylic acid				1.88 ± 0.00		1.88 ± 0.00
White wheat bran						
3. *p*-Hydroxybenzoic acid		2.54 ± 0.03	4.32 ± 0.18	10.4 ± 1.30	28.3 ± 0.96	45.5 ± 0.61
6. Vanillic acid	2.21 ± 0.00	1.90 ± 0.00	5.88 ± 0.04	18.1 ± 0.00	52.7 ± 3.31	80.8 ± 1.48
8. Syringic acid		8.04 ± 0.15	8.16 ± 0.25	42.5 ± 0.00	86.4 ± 0.37	145 ± 0.16
9. *p*-Coumaric acid		1.38 ± 0.00	0.39 ± 0.01	34.9 ± 0.00	2.08 ± 0.06	38.8 ± 0.03
10. Ferulic acid	4.13 ± 0.00	40.0 ± 0.13	2.86 ± 0.00	1904 ± 13.08	40.8 ± 0.18	1992 ± 5.81

Data are means ± standard deviation ($n = 3$) determined by HPLC analysis.
[a] Free phenolic acid.
[b] Alkaline hydrolysables.
[c] Acid hydrolysables.
[d] Sum of all fractions.

and vanillic acid were the main FPAs extracted into the crude methanol extract; however, only ferulic acid was released in significantly higher amount upon alkaline hydrolysis of the residue (Table 3, A1). The concentrations of individual phenolic acids of the commercial wheat bran were higher than those of the AACC samples. Also, the concentrations of individual phenolic acids in red bran were higher than those in white bran (Tables 2 and 3). These results were consistent with the TPC data.

3.3. Hydrolysis of wheat bran

Most phenolic acids in cereals primarily occur in the bound form as conjugates with sugars, fatty acids, or proteins (White & Xing, 1997); therefore, it is important that a hydrolysis process is adopted in order to obtain maximum yield of the phenolic acids of cereal grains such as wheat and wheat bran.

HPLC profiles of bound phenolic acids obtained by two hydrolysis methods are shown in Table 4. Using method A, vanillic, caffeic, syringic, *p*-hydroxybenzoic, *p*-coumaric, salicylic, *trans*-cinnamic acids and ferulic acid were released from the bound form and detected by HPLC. Ferulic acid was the predominant phenolic acid in fraction A1, whereas an unknown peak (UK 1), at 11 min, dominated the profile of fraction A2. This peak was also observed in fractions B1 and B2, obtained using method B (Fig. 1). The HPLC profile of B1 was similar to A2; however, in B2, in addition to the aforementioned unknown peak, three other unknown products (UK 3–5) were observed (Fig. 1, Table 4). The different hydrolysis protocols clearly affected the concentrations of phenolic acids. In general, method A was more efficient in releasing the phenolic acids, particularly ferulic acid (Fig. 1, Table 4). However, salicylic acid was an exception; it was mainly released by method B after acid hydrolysis. Acidic and alkaline hydrolyses are often used to cleave the ester bond in separation and characterization of specific phenolic compounds (Nuutila et al., 2002) but, as our results show (Table 4), the hydrolysis conditions, acid or alkaline only, or in different sequence, can significantly affect the total yield and profile of phenolic acids. Cinnamic acid derivatives, *p*-coumaric, caffeic and ferulic acids, were found to degrade under hot acidic conditions (Gao & Mazza, 1994; Robbins, 2003). This may partially explain why acid hydrolysis gave a low yield of hydrolysable phenolic acids.

3.4. Antioxidant activities of wheat bran extracts

Percent original absorbance was used as an endpoint for the evaluation of the antioxidant activity measured using the β-CLAMS method. The higher the percentage, the stronger was the antioxidant activity at a certain time interval. Although different methods have been used to interpret the data (Tsao & Yang, 2003), we have chosen to use the data at 180 min in this discussion. As shown in Fig. 2A, the alkaline-extractable fractions had greater antioxidant activities, while the acid-extractable fractions, in general, showed the lowest in both of red and white bran. Methanol-extractable fractions of wheat bran also showed strong antioxidant activities (approximately 67% on average). During the first 60 min, the antioxidant activity of the methanol-extractable fraction of the commercial white bran inhibited the peroxidation slightly better than did BHT.

The antioxidant activity of wheat bran extracts only indicates the total antioxidant capacity of the mixture. The role of individual phenolic acids and their contribution to the total antioxidant activity cannot be deciphered from the mixtures. To find the antioxidant capacities of the phenolic acids identified in the bran, individual compounds were subjected to the same evaluation using the β-CLAMS test. As shown in Fig. 2B, although the concentrations were the same, the antioxidant activities of these compounds were significantly different. Caffeic, syringic, and ferulic acids were the strongest antioxidants, whereas *trans*-cinnamic and vanillic acids were the weakest. This may explain why extracts with high contents of ferulic acid, such as those

Table 4
Effect of different hydrolysis conditions on the individual phenolic acids (μg/g defatted bran)[a]

Phenolic acids	Method A[b]			Method B[b]		
	A1	A2	Total	B1	B2	Total
p-Hydroxybenzoic acid	8.92	11.04	20.0	17.5	5.24	22.8
Vanillic acid	13.0	60.8	73.8	30.0	14.5	44.4
Syringic acid	27.5	109	137	81.7	35.7	117
trans-Cinnamic acid	0.88		0.88			
Caffeic acid	2.28		2.28			
p-Coumaric acid	41.4	2.9	44.3	1.66		1.66
Ferulic acid	1932		1932	34.4	10.3	44.7
Salicylic acid	1.88		1.88	158		158

Data are the average of duplicates determined by HPLC analysis. Data were from the commercial red wheat bran.
[a] All concentrations were on dry matter basis.
[b] See Fig. 1 caption.

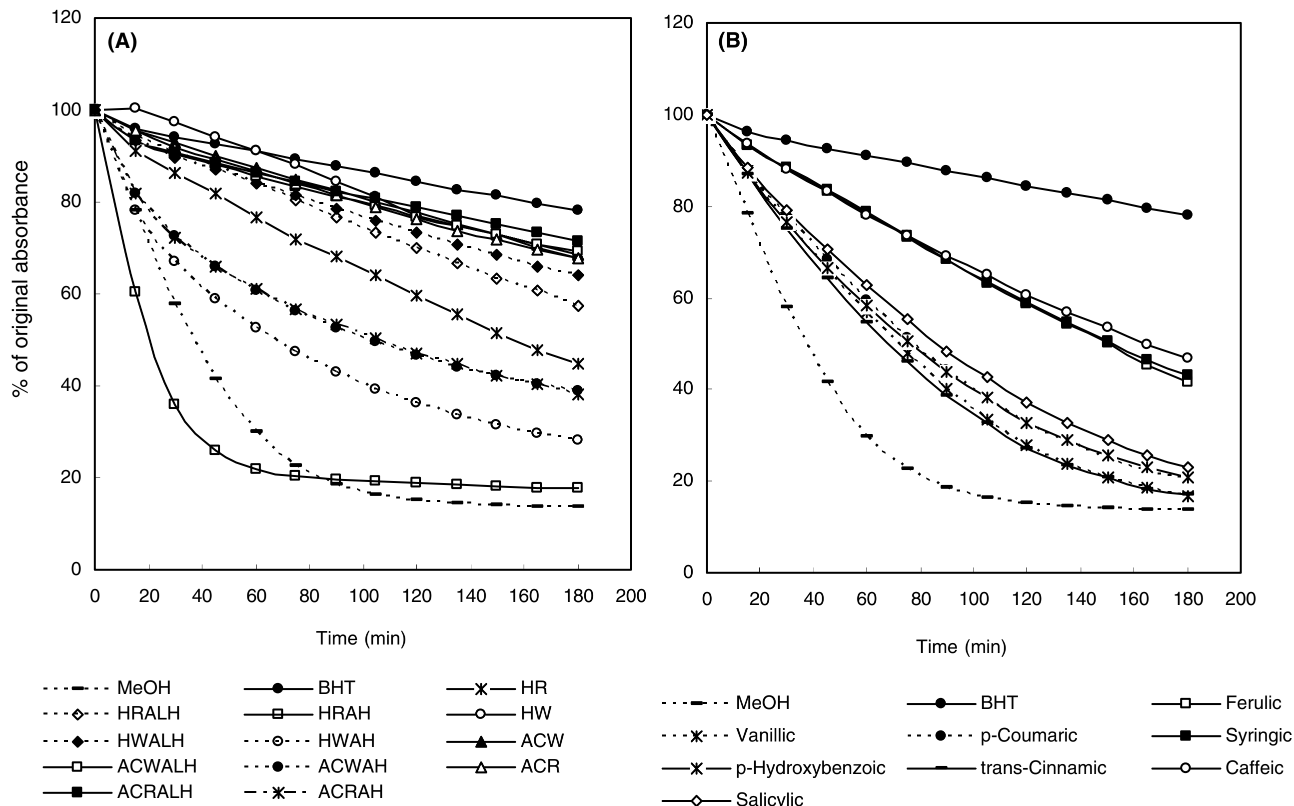

Fig. 2. Antioxidant activities of wheat bran extracts (A) and typical wheat phenolic acids (B). HR, aqueous methanol-extractable fraction from the commercial red wheat bran; HW, aqueous methanol-extractable fraction from the commercial white wheat bran; ACR, aqueous methanol-extractable fraction from the AACC red wheat bran; ACW, aqueous methanol-extractable fraction from the AACC white wheat bran; HRALH, alkaline-hydrolysable bound phenolic acids in the commercial red wheat bran; HRAH, acid-hydrolysable bound phenolic acids in the commercial red wheat bran; HWALH, alkaline-hydrolysable bound phenolic acids in the commercial white wheat bran; HWAH, acid-hydrolysable bound phenolic acids in the commercial white wheat bran; ACRALH, alkaline-hydrolysable bound phenolic acids in the AACC red wheat bran; ACRAH, acid-hydrolysable bound phenolic acids in AACC red wheat bran; ACWALH, alkaline-hydrolysable bound phenolic acids in the AACC white wheat bran; ACWAH, acid-hydrolysable bound phenolic acids in AACC white wheat bran.

by alkaline hydrolysis had higher antioxidant activity. We also found that the methanolic extracts had strong antioxidant activity despite having a low concentration of phenolic acids. Other antioxidant phytochemicals may have contributed to the activity. The unknown peak in the extract (Fig. 1) is considered one such compound producing strong antioxidant activity. Its identity is currently being investigated.

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References

Abdul-Hamid, A., & Luan, Y. S. (2000). Functional properties of dietary fibre prepared from defatted rice bran. *Food Chemistry, 68*, 15–19.

Adom, K. K., Sorrells, M. E., & Liu, R. H. (2003). Phytochemical profiles and antioxidant activity of wheat varieties. *Journal of Agricultural and Food Chemistry, 51*, 7825–7834.

Alabaster, O., Tang, Z., & Shivapurkar, N. (1997). Inhibition by wheat bran cereals of the development of aberrant crypt foci and colon tumours. *Food and Chemical Toxicology, 35*, 517–522.

Andreasen, M. F., Kroon, P. A., Williamson, G., & Garcia-Conesa, M.-T. (2001a). Intestinal release and uptake of phenolic antioxidant diferulic acids. *Free Radical Biology and Medicine, 31*, 304–314.

Andreasen, A. F., Kroon, P. A., Williamson, G., & Garcia-Conesa, M.-T. (2001b). Esterase activity able to hydrolyze dietary antioxidant hydroxycinnamates is distributed along the intestine of mammals. *Journal of Agricultural and Food Chemistry, 49*, 5679–5684.

Baublis, A. J., Clydesdale, F. M., & Decker, E. A. (2000). Antioxidants in wheat-based breakfast cereals. *Cereal Food World, 45*, 71–74.

Baublis, A. J., Decker, E. A., & Clydesdale, F. M. (2000). Antioxidant effect of aqueous extracts from wheat based ready-to-eat breakfast cereals. *Food Chemistry, 68*, 1–6.

Baublis, A. J., Lu, C., Clydesdale, F. M., & Decker, E. A. (2002). Potential of wheat-based breakfast cereals as a source of dietary antioxidants. *J. Am. Coll. Nutr., 19*, 308S–311S.

Emmons, C. L., Peterson, D. M., & Paul, G. L. (1999). Antioxidant capacity of oat (*avena sativa* L.) extracts. 2. In vitro antioxidant

activity and contents of phenolic and tocol antioxidants. *Journal of Agricultural and Food Chemistry, 47*, 4894–4898.

Gao, L., & Mazza, M. (1994). Rapid method for complete chemical characterization of simple and acylated anthocyanins by high-performance liquid chromatograph and capillary gas–liquid chromatography. *Journal of Agricultural and Food Chemistry, 42*, 118–125.

Kader, F., Rovel, B., Girardin, M., & Metche, M. (1996). Fractionation and identification of the phenolic compounds of highbush blueberries (*Vaccinium corymbosum*, L). *Food chemistry, 55*, 35–40.

Kähkönen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J.-P., Pihlaja, K., Kujala, T. S., et al. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry, 47*, 3954–3962.

Lehtinen, P., & Laakso, S. (1997). Antioxidative-like effect of different cereals and cereal fractions in aqueous suspension. *Journal of Agricultural and Food Chemistry, 45*, 4606–4611.

Maziya-dixon, B. B., Klopfenstein, C. F., & Leipold, H. W. (1994). Protective effects of hard red versus hard white winter wheats in chemically-induced colon-cancer in CFI mice. *Cereal Chemistry, 71*, 359–363.

Miller, H. E. (1971). A simplified method for the evaluation of antioxidants. *Journal of the Americal Oil Chemists Society, 48*, 91–95.

Møller, M. E., Dahl, R., & Bøckman, O. C. (1988). A possible role of the dietary fibre product, wheat bran, as a nitrite scavenger. *Food Chemistry and Toxicology, 26*, 841–845.

Nuutila, A. M., Kammiovirta, K., & Oksman-Caldentey, K.-M. (2002). Comparison of methods for the hydrolysis of flavonoids and phenolic acids from onion and spinach for HPLC analysis. *Food chemistry, 76*, 519–525.

Onyeneho, S. N., & Hettiarachchy, N. S. (1992). Antioxidant activity of durum wheat bran. *Journal of Agricultural and Food Chemistry, 40*, 1496–1500.

Robbins, R. J. (2003). Phenolic acids in foods: an overview of analytical methodology. *Journal of Agricultural and Food Chemistry, 51*, 2866–2887.

Saadi, A, Lempereur, I., Sharonov, S., Autran, J. C., & Manfait, M. (1998). Spatial distribution of phenolic materials in durum wheat grain as probed by confocal fluorescence spectral imaging. *Journal of cereal science, 28*, 107–114.

Sosulski, F., Krygier, K., & Hogge, L. (1982). Free, esterified, and insoluble-bound phenolic acids. 3. Composition of phenolic acids in cereal and potato flours. *Journal of Agricultural and Food Chemistry, 30*, 337–340.

Truswell, A. S. (2003). Cereal grains and coronary heart disease. *European Journal of Clinical Nutrition, 56*, 1–14.

Tsao, R., Yang, R., & Young, J. C. (2003). Antioxidant iIsoflavones in Osage Orange, Maclura pomifera (Raf.) Schneid. *Journal of Agricultural and Food Chemistry, 51*, 6445–6451.

White, P. J., & Xing, Y. (1997). Antioxidants from cereals and legumes. In F. Shahidi (Ed.), *Natural antioxidants, chemistry, health effects, and application* (pp. 25–63). Champaign IL: AOCC press.

Wu, H., Haig, T., Pratley, J., Lemerle, D., & An, M. (1999). Simultaneous determination of phenolic acids and 2,4-dihydroxy-7-methoxy-3-one in wheat (*Triticum aestivum* L.) by gas chromatography-tandem mass spectrometry. *Journal of Chromatography A, 864*, 315–321.

Yu, L., Haley, S., Perret, J., Harris, M., Wilson, J., & Qian, M. (2002). Free radical scavenging properties of wheat extracts. *Journal of Agricultural and Food Chemistry, 50*, 1619–1624.

Yu, L., Perret, J., Harris, M., Wilson, J., & Haley, S. (2003). Antioxidant properties of bran extracts from "Akron" wheat grown at different locations. *Journal of Agricultural and Food Chemistry, 51*, 1566–1570.

Zhou, K., & Yu, L. (2004). Antioxidant properties of bran extracts from Trego wheat grown at different locations. *Journal of Agricultural and Food Chemistry, 52*, 1112–1117.

Zhou, K., Laux, J. J., & Yu, L. (2004). Comparison of Swiss red wheat grain and fractions for their antioxidant properties. *Journal of Agricultural and Food Chemistry, 52*, 1118–1123.

Zieliński, H., & Kozłowska, H. (2000). Antioxidant activity and total phenolics in selected cereal grains and their different morphological fractions. *Journal of Agricultural and Food Chemistry, 48*, 2008–2016.