Simple Phenolic Acids in Flours Prepared from Canadian Wheat: Relationship to Ash Content, Color, and Polyphenol Oxidase Activity[1]

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ABSTRACT

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Simple phenolic acid levels were determined on pooled millstreams of five different classes of Canadian wheat milled to ~75, 80, and 85% extraction. Pooled flours and whole grain were analyzed by reversed-phase high-performance liquid chromatography (RP-HPLC) to establish endogenous levels of insoluble bound, soluble esterified, and free phenolic acids. Only ferulic acid was detected in the insoluble bound category, which accounted for >80% of the total phenolic acids present in every flour. The soluble esterified phenolic acids accounted for up to 17% of the overall total phenolic acid content within a flour. The major constituents were sinapic, ferulic, and vanillic acids, with minor amounts of coumaric, caffeic, and syringic acids. Free phenolic acids accounted for a maximum of 6% of the total phenolic content of any prepared flour. Ferulic acid was the major free phenolic acid, while sinapic acid was not detected in any flour. Significant correlations (r = 0.64–0.97, $P < 0.05$) were observed between insoluble bound ferulic acid, individual soluble esterified acids, and most free acids with polyphenol oxidase activity, as well as color and ash content for each class.

Processing of end-products such as Middle Eastern flat breads, Chinese steamed bread, and Indian *chapattis*, which use high extraction flours, have the associated problem of undesirable dough and product discoloration that may be caused by enzymatic browning (Abrol et al 1971, Dexter 1984, and Faridi 1988). Previous work by Hatcher and Kruger (1993) has shown that polyphenol oxidase (PPO) distribution in millstreams closely parallels the efficiency of the milling process. Milling to elevated extraction yield (>70%) causes the enzyme levels to rise dramatically. Phenolic acids, potential substrates of this enzyme, are endogenous to the wheat plant (Bose 1972), grain (Gallus and Jenning 1971, Maga and Lorenz 1974, Kuninori and Nishiyama 1986, Pussayawanin et al 1989) and wheat flour (Sosulski et al 1982, Jackson and Hoseney 1986). PPO is believed to be involved in the oxidation of such endogenous wheat phenolics, resulting in the production of labile quinones. The quinones produced can react with a number of compounds; amines and thiols or undergo self-polymerization to produce highly colored products (Pierpoint 1969, Taylor and Clydesdale 1987).

The primary objective of this study was to determine how individual phenolic acids, potential substrates of PPO, varied in flours composited at different levels of purity from different classes of wheat milled to varying extraction rates. McCallum and Walker (1990), using the Folin-Denis assay as a means to estimate total soluble phenolics in wheat flour, observed a correlation with PPO levels in New Zealand wheat millstreams. A secondary objective, therefore, was to ascertain whether a similar relationship existed between PPO activity and the individual simple phenolic acids present in Canadian wheat flours.

MATERIAL AND METHODS

Milling

Wheat samples, obtained from certified seed growers to ensure varietal purity, were representative of the five classes of Canadian wheat grown in western Canada: Canadian Western Soft White Spring (CWSWS *var*. Fielder), Canadian Prairie Spring Red (CPSR *var*.HY320), Canadian Western Red Winter (CWRW *var*. Norstar), Canadian Western Red Spring (CWRS, *var*. Katepwa), and Canadian Western Extra Strong (CWES *var*. Glenlea). On a class basis, CWSWS is considered a soft wheat with a particle size index (PSI) of ~65–68, CPSR a medium wheat (PSI 55–60), while CWRW, CWRS, and CWES are considered hard wheats (PSI 48–55). Wheat analytical properties are shown in Table I. Wheats were cleaned, tempered, and milled in the Grain Research Laboratory's pilot mill by three protocols. One was a typical North American mill flow that yields flour extraction rates near 75% (Black 1980). The second was a higher extraction mill flow of ~80% flour extraction (Dexter et al 1987). The third mill flow simulated a typical Chinese standard milling extraction of 85% (Dexter et al 1984). Pooled flours were prepared; first patent flours represented the initial 45% of the flour yield, second patent the next 22.5%, while first and second clears represented the remaining flour yield split equally into two parts. The Chinese standard flour was prepared by adding additional low refinement millstreams from the 85% extraction milling to yield a cumulative ash content of 1.20%. Analytical properties of the flours produced for each wheat class can be found in Table I.

Phenolic Acid Extraction

The phenolic acid composition was determined by a method similar to that outlined by Krygier et al (1982) in which the phenolic acids were separated into three categories: soluble free phenolic acids, soluble esterified acids, and insoluble bound acids.

Wholemeal or flour (15 g) was mixed with 100 mL of nitrogen-saturated acetone and water (80:20) in a 250-mL centrifuge bottle. The sample was extracted for 15 min, with constant agitation, at room temperature before centrifugation (15,000 × *g* for 10 min). The supernatant was drawn off and placed in a sealed nitrogen environment. The process was repeated using 75 mL of the 80% acetone, and the supernatants were pooled.

The residual pellet was extracted by the same two-step process using nitrogen-saturated methanol and water (80:20). The resulting grist was frozen, lyophilized, and stored under nitrogen in a freezer at –20°C before subsequent analysis of the insoluble bound phenolic acids.

The acetone fraction was rotary evaporated under reduced pressure at 37°C until all the acetone was removed. The methanol extract was added and the process repeated. The resulting aqueous solution, containing both free and soluble esterified phenolic acids, was acidified to pH 2.0, with 5M HCl. The aqueous solu-

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tion was extracted with four volumes (100, 75, 75, and 50 mL) of an ethyl ether and ethyl acetate (1:1) mixture. The organic phase drawn off at each stage was pooled in a closed flask under nitrogen and dried over sodium sulfate. This extract, containing free phenolic acids, was filtered to remove sodium sulfate granules, concentrated by rotary evaporation, and solubilized in 5 mL of 100% methanol.

The remaining aqueous phase was rotary evaporated to remove any residual organic solvent before hydrolysis in $2N$ NaOH, under nitrogen, for 4 hr at room temperature. The solution was brought to pH 2.0 with $5M$ HCl, extracted with four volumes of the ethyl ether and ethyl acetate mixture, stored, and dried as described above. This fraction contained the soluble esterified phenolic acids.

The lyophilized grist containing the insoluble bound phenolic acids was ground to a fine powder in a coffee grinder. A sample (2.0 g) was hydrolyzed under nitrogen in 100 mL of $2N$ NaOH, for 4 hr with continuous stirring at 20°C. Water (80 mL) was added to the hydrolyzate, and the solution was brought to pH 5.0 with $5M$ HCl. The solution was centrifuged (15,000 × g for 15 min) and the supernatant was removed. The pellet was resuspended in 20 mL of water, centrifuged, and the combined supernatants brought to 250 mL in a volumetric flask.

A 100-mL aliquot of this fraction was adjusted to pH 2.0 with $5M$ HCl, applied to a 1.0 g Supelcoclean (Supelco Inc. Bellefonte, PA.) C-18 solid phase extraction column (Seo and Morr 1984, Jaworski and Lee 1987) previously wetted with methanol and washed extensively with $0.05N$ HCl. After the addition of the sample, the column was washed with 25 mL of $0.05N$ HCL. The phenolic acids were eluted from the column with 4 mL of 100% methanol. The eluant was made up to 5 mL in a volumetric flask and passed through a Millipore 0.45 μM filter before high-performance liquid chromatography (HPLC) analysis. All samples were analyzed in duplicate with the average coefficient of variation for the determination of each phenolic acid, by category, found in Table II.

HPLC System and Conditions

Analysis was done on a Waters (Milford, MA.) 860 chromatography data system consisting of two M510 pumps, a WISP autoinjector, and a M490 multiple wavelength detector under computer control. The analytical column, at room temperature, was a reverse-phase Supelco (Bellefonte, PA) LC-18 (3.3 cm × 4.6 mm) column preceded by a 2-cm Supelguard column of the same material. The elution profile was a 1.0 mL/min linear gradient from 100% A:0% B to 82%A:18% B, over 30 min. Solvent A consisted of 0.1% (v/v) trifluoracetic acid (Pierce, IL) in water; solvent B was 0.1% (v/v) trifluoracetic acid in HPLC grade acetonitrile (Fisher, Rockford, NJ). Detection was done simultaneously at 280, 254, and 320 nm. Calibration standards consisting of protocatechuic, vanillic, caffeic, syringic, coumaric, ferulic, and sinapic acids were run with each series of samples. Absorbance peak ratios were calculated and used to confirm subsequent peak purity and identification in subsequent flour samples. Each acid displayed a linear response, $r > 0.98$ ($P < 0.05$) over the 0.02–0.40, μg calibration series.

Flour Characterization

Flour color was determined by two different methods to select different aspects of the color components. The Kent Jones (KJ) color was determined using a Simon series IV (Henry Simon, Stockport, UK) flour color grader with the results converted to Kent-Jones color units. Agtron flour color was analyzed using the AACC method 14-30 (AACC 1995). Protein content was determined by the modified Kjeldhal method of Williams (1973). Ash content was obtained by AACC method 08-01 (AACC 1995) on a 4-g sample in a silica dish incinerated overnight at 600°C.

PPO Analysis

Analysis of PPO activity was made using a YSI model 5300 biological oxygen monitor (Yellow Spring Instrument Co., Yellow Spring, OH) by the method of Marsh and Galliard (1986) as modified by Hatcher and Kruger (1993). Ground wheat or flour samples, 10–200 mg, were assayed in 4 mL of $0.01M$ McIlvaines buffer (pH 6.8) maintained at 37°C using 0.1 mL of $0.8M$ catechol as the substrate. Results, average of triplicate analyses, are expressed in nanomoles of O_2 consumed per minute per gram.

Statistical Analysis

Regression and analysis of variance (ANOVA) were performed using SAS software (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Insoluble Bound Phenolic Acids

The insoluble bound phenolic acids were by far the largest contributor to the total phenolic acid content in all five wheat classes, contributing over 80% of the total phenolic content in each flour. Without exception, ferulic acid, was the only simple phenolic component detected at measurable levels (Table III). These results are in agreement with that reported by Sosulski et al (1982), where the authors found that *trans*-ferulic acid accounted for up to 90% of the total phenolic acids in wheat flour.

TABLE I

Analytical Properties of Wheats and Flours[a] used for the Determination of Phenolic Acid Components and Polyphenol Oxidase Activity

	CWSWS		CPSR		CWRW		CWRS		CWES	
	Ash[b]	Protein[b]	Ash	Protein	Ash	Protein	Ash	Protein	Ash	Protein
Wheat	1.36	8.9	1.54	11.1	1.18	11.4	1.75	15.5	1.69	12.8
Flours										
75% str. grade	0.43	7.7	0.57	10.5	0.42	11.0	0.54	15.3	0.59	12.5
1st patent	0.35	7.4	0.48	10.0	0.32	10.2	0.40	13.9	0.41	11.7
2nd patent	0.51	8.1	0.63	10.5	0.45	11.9	0.58	16.9	0.68	13.1
1st clear	0.88	10.1	0.99	12.8	0.56	11.3	0.98	16.3	0.97	13.4
2nd clear	0.90	9.2	1.16	12.8	1.13	14.1	1.62	19.5	1.90	16.2
80% str. grade	0.53	8.2	0.66	10.5	0.48	11.3	0.66	15.6	0.70	12.3
1st patent	0.36	7.2	0.48	9.8	0.34	10.2	0.43	13.7	0.42	11.3
2nd patent	0.57	8.5	0.74	11.2	0.51	12.1	0.63	17.6	0.78	13.3
1st clear	0.95	10.7	1.06	12.9	0.68	11.6	1.06	15.6	1.15	14.2
2nd clear	1.40	9.3	1.54	13.5	1.31	14.1	1.86	19.9	2.12	16.0
85% str. grade	0.70	8.7	0.80	11.3	0.66	11.8	0.99	16.4	1.00	13.3
Chinese standard	1.18	10.2	1.21	12.8	1.08	12.7	1.22	18.0	1.24	14.0

[a] Canadian Western Soft White Spring (CWSWS), Canadian Prairie Spring Red (CPSR), Canadian Western Red Winter (CWRW), Canadian Western Red Spring (CWRS), and Canadian Western Extra Strong (CWES).

[b] Ash and protein are expressed on a % basis with wheat and flour at 13.5 and 14.0 % mb, respectively.

Comparatively, there were minimal differences in the amounts of insoluble ferulic acid found in the whole wheat samples among classes. Only CWRW, at 274.8 ppm was found to be significantly different (P <0.05) from the other four classes, which ranged from 305.6 to 337.6 ppm. The uniqueness of the CWRW sample was reflected in the lower levels of ferulic acid detected in the better quality (low ash) flours. For example, the 75% extraction low ash first patent flour, had 22.5 ppm ferulic acid, which was significantly lower than the remaining four classes tightly grouped between 34.4 to 40.0 ppm.

In general, the variation in levels of insoluble bound phenolic acids within a particular blended flour, at each extraction rate, were similar. The first patent flours contained only 8 to 12.5% of the insoluble bound ferulic acid levels found in the corresponding whole wheat samples. These results are in agreement with those reported by Jackson and Hoseney (1986), who found 36.1 ppm ferulic acid in their patent flour. Examination of the 80% extraction first patent flours also indicated that the concentration of the insoluble ferulic acid remained relatively unchanged (30.0–48.8 ppm) when compared to their 75% extraction counterparts. Analyses of the 75% extraction straight-grade flours yielded insoluble bound ferulic acid values (57.5–75.7 ppm), which were consistent with those reported by Sosulski et al (1982) for a straight grade Neepawa (CWRS) wheat flour (63.6 ppm).

Maximum insoluble ferulic acid levels were found in the 80% extraction high ash second clear flours, with values ranging from 369.4 to 449.4 ppm. These levels exceed those found in the whole wheat, as this prepared flour was primarily composed of bran material. Differences in ferulic acid content were detected between classes based on wheat hardness. The softest wheat, CWSWS, followed by the medium CPSR, had significantly higher ferulic acid content than did the harder classes.

TABLE II
Average Coefficient of Variation (%) for High-Performance Liquid Chromatography (HPLC) Analysis of Individual Phenolic Acids

Phenolic Acid	Free Acid[a]	Soluble Bound	Insoluble Bound
Ferulic	4.31	2.53	2.83
Vanillic	4.08[b]	3.74	nd[c]
Sinapic	nd	5.27	nd
Caffeic	2.59[d]	2.83	nd
Coumaric	4.47[e]	4.97	nd
Syringic	6.93[d]	3.66	nd

[a] N = 65, unless otherwise specified.
[b] N = 64.
[c] Not detected.
[d] N = 55.
[e] N = 46.

TABLE III
Mean Insoluble Bound Ferulic Acid Content (ppm) in Prepared Flours[a]

	CWSWS	CPSR	CWRW	CWRS	CWES
Wheat	321.4	334.4	274.8	337.6	305.6
Flour					
75% str. grade	57.5	63.8	57.5	65.7	75.7
1st patent	40.0	37.5	22.5	34.4	38.8
2nd patent	63.8	61.3	50.0	66.4	76.3
1st clear	147.5	141.3	110.0	153.8	123.8
2nd clear	334.0	285.7	281.9	267.8	289.4
80% str. grade	79.5	93.8	46.3	95.4	100.0
1st patent	48.8	37.5	30.0	45.7	39.0
2nd patent	105.0	105.0	78.8	81.3	122.5
1st clear	206.3	217.5	208.8	268.2	190.0
2nd clear	449.4	438.8	369.4	372.5	405.0
85% str. grade	173.7	178.7	160.2	179.2	161.8
Chinese standard	273.1	312.4	256.6	199.5	161.0

[a] Canadian Western Soft White Spring (CWSWS), Canadian Prairie Spring Red (CPSR), Canadian Western Red Winter (CWRW), Canadian Western Red Spring (CWRS), and Canadian Western Extra Strong (CWES).

Examination of the Chinese standard flours highlighted the differences in the quality of the 85% extraction mill streams which were used to prepare this flour. Since the criteria for preparing this flour was based solely on a final cumulative ash content of 1.20%, direct comparison among classes was limited due to their different stream composition. The harder wheats, CWES and CWRS, displayed significantly less insoluble ferulic acid as the contribution of the later streams was limited by their generally higher ash content.

All of the results described above can be explained by the presence of insoluble bound phenolic acids in the bran. This is apparent by the strong relationship that existed between the amount of insoluble bound ferulic acid and ash content ($r > 0.93$, $P < 0.05$) as shown in Fig. 1. Note that the relationship is dependent on the wheat class.

The relationship between insoluble bound ferulic acid with KJ flour color values was found to be not as high ($r > 0.87$, $P < 0.05$). An inflection point was observed in the relationship between color and phenolic acid content between KJ values of 0.0–2.0, depending on the wheat class (Fig. 2). This is consistent with the breakpoint relationship between color and bran (Hook 1985), where flour color changes rapidly due to a marked increase in bran content accumulation. Agtron readings, which in the green mode measures via reflectance the presence of noncarotenoid pigments, had a weaker but inverse ($r > -0.80$, $P < 0.05$) relationship with insoluble ferulic acid (results not shown).

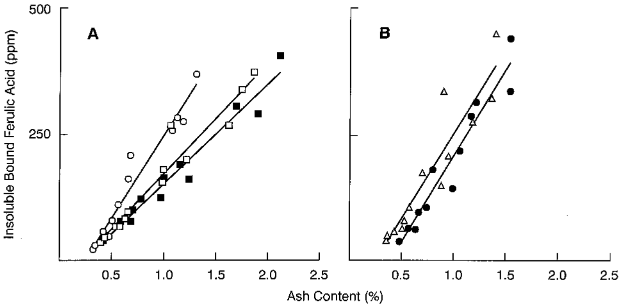

Fig. 1. Relationship between insoluble bound ferulic acid levels and the ash content of blended flours from different classes of wheat. **A.** ○ = CWRW (Canadian Western Red Winter), □ = CWRS (Canadian Western Red Spring), and ■ = CWES (Canadian Western Extra Strong). **B.** Δ = CWSWS (Canadian Western Soft White Spring) and CPSR (Canadian Prairie Spring Red).

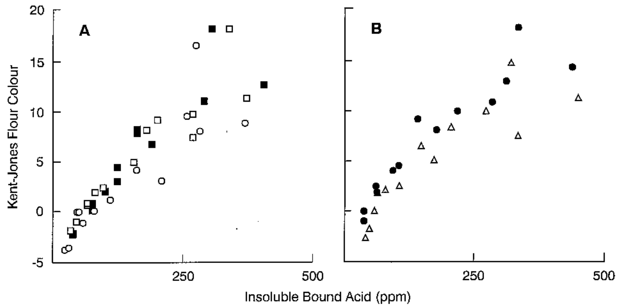

Fig. 2. Relationship between insoluble bound ferulic acid levels and the flour Kent-Jones flour color of blended flours from different classes of wheat. **A.** ○ = CWRW (Canadian Western Red Winter), □ = CWRS (Canadian Western Red Spring), and ■ = CWES (Canadian Western Extra Strong). **B.** Δ = CWSWS (Canadian Western Soft White Spring) and ● = CPSR (Canadian Prairie Spring Red).

TABLE IV

Individual and Total Soluble Esterified Phenolic Acids (ppm) in Wheat and Flours[a]

	Sinapic	Ferulic	Vanillic	Syringic	Caffeic	Coumaric	Total
CWSWS							
Wheat	25.6	13.0	4.6	1.7	3.2	1.8	49.9
75% str. grade	0.9	2.0	1.1	0.4	0.4	0.2	5.0
1st patent	2.1	1.4	0.5	nd[b]	0.2	nd	4.2
2nd patent	1.1	2.9	1.5	0.6	0.6	0.4	7.1
1st clear	7.6	8.0	4.1	1.5	1.6	0.7	23.5
2nd clear	11.4	9.0	6.3	1.6	1.8	0.8	30.9
80% str. grade	5.5	4.7	2.5	0.6	0.7	0.3	14.3
1st patent	2.3	2.0	1.1	0.3	0.3	0.2	6.2
2nd patent	5.9	5.0	2.7	0.7	0.6	0.4	15.3
1st clear	19.9	11.4	4.6	1.6	2.3	1.0	40.8
2nd clear	44.7	25.6	16.1	3.5	2.6	1.4	93.9
85% str. grade	9.2	7.4	3.1	0.9	1.0	0.8	22.4
Chinese Standard	20.6	11.3	6.2	1.8	2.9	1.7	44.5
CPSR							
Wheat	29.9	17.6	4.0	4.0	2.1	nd	57.6
75% str. grade	1.0	2.0	1.2	0.5	0.1	nd	4.8
1st patent	0.2	1.3	0.5	nd	nd	nd	2.0
2nd patent	1.0	2.1	1.2	0.8	nd	0.5	5.6
1st clear	3.4	6.7	2.9	2.3	0.5	0.5	16.3
2nd clear	4.6	11.1	4.8	3.3	0.7	0.2	24.7
80% str. grade	1.3	3.5	1.7	1.1	0.2	0.1	7.9
1st patent	0.3	1.6	0.7	nd	nd	0.3	2.9
2nd patent	0.8	4.1	1.8	1.4	0.2	0.5	8.8
1st clear	17.7	10.3	3.2	2.5	0.6	1.4	35.7
2nd clear	27.2	18.3	9.4	5.8	1.4	0.2	62.3
85% str. grade	2.7	4.8	3.6	1.5	0.3	0.7	13.6
Chinese Standard	23.1	9.8	4.7	3.6	1.6	0.9	43.7
CWRW							
Wheat	16.9	8.6	4.1	3.8	4.0	0.8	38.2
75% str. grade	0.9	1.7	1.3	0.6	0.1	0.1	4.7
1st patent	0.3	0.3	0.3	nd	0.1	nd	1.0
2nd patent	0.9	1.8	1.0	0.6	0.1	0.1	4.5
1st clear	1.9	3.1	2.0	0.9	0.3	0.2	8.4
2nd clear	5.9	7.7	4.5	2.7	3.8	0.6	25.2
80% str. grade	1.1	2.1	1.1	0.7	0.2	0.1	5.3
1st patent	0.3	0.8	0.4	0.3	0.1	0.1	2.0
2nd patent	2.1	2.2	1.2	0.8	0.3	0.2	6.8
1st clear	7.8	5.0	2.9	1.6	0.6	0.3	18.2
2nd clear	16.3	10.5	7.8	4.0	4.1	0.7	43.4
85% str. grade	5.2	4.6	2.4	1.8	0.9	0.4	15.3
Chinese Standard	19.2	9.0	4.7	3.5	1.6	0.9	38.9
CWRS							
Wheat	30.5	10.7	7.2	6.5	2.9	0.8	58.6
75% str. grade	2.0	1.4	0.9	0.6	nd	nd	4.9
1st patent	0.8	0.7	0.3	0.2	nd	nd	2.0
2nd patent	1.8	1.6	0.8	0.6	nd	nd	4.8
1st clear	8.4	3.7	2.8	1.6	0.3	0.2	17.0
2nd clear	17.2	7.3	4.2	3.2	0.3	0.5	32.7
80% str. grade	3.7	2.6	2.1	1.3	nd	0.1	9.8
1st patent	1.9	1.2	0.6	1.0	nd	nd	4.7
2nd patent	2.7	2.4	1.3	1.2	nd	0.1	7.7
1st clear	12.0	4.8	4.6	2.8	nd	0.2	24.4
2nd clear	26.3	10.6	8.9	5.9	1.4	0.8	53.9
85% str. grade	8.4	5.0	4.3	2.8	0.7	0.5	21.7
Chinese Standard	15.4	6.5	5.7	3.4	0.9	0.6	32.5
CWES							
Wheat	30.3	13.7	5.3	4.5	2.0	1.4	57.2
75% str. grade	4.4	2.0	0.7	0.5	nd	nd	7.6
1st patent	1.4	1.0	0.3	0.3	nd	nd	3.0
2nd patent	5.7	2.6	0.8	0.5	nd	0.1	9.7
1st clear	8.5	3.3	1.1	0.8	0.2	0.2	14.1
2nd clear	21.7	9.8	3.9	2.6	0.6	0.7	39.3
80% str. grade	7.2	5.2	2.7	1.7	0.5	0.4	17.7
1st patent	2.1	1.9	1.1	0.7	0.2	0.2	6.2
2nd patent	4.1	5.2	2.5	1.9	0.5	0.5	14.7
1st clear	17.7	9.8	4.9	3.6	1.2	1.0	38.2
2nd clear	39.3	22.5	9.8	8.2	2.0	2.3	84.1
85% str. grade	6.4	7.8	3.4	2.5	0.9	0.8	21.8
Chinese Standard	17.2	9.5	4.2	3.1	1.3	1.0	36.3

[a] Canadian Western Soft White Spring (CWSWS), Canadian Prairie Spring Red (CPSR), Canadian Western Red Winter (CWRW), Canadian Western Red Spring (CWRS), and Canadian Western Extra Strong (CWES).

[b] Not detected.

Soluble Esterified Phenolic Acids

The individual soluble phenolic acids detected in the five wheat classes fell into two broad categories: dominant components (sinapic, ferulic, and vanillic acids) and minor components (syringic, caffeic, and *p*-coumaric acids). Sinapic acid was the major phenolic acid in most flours, followed by ferulic acid, while the vanillic acid content was approximately half that of ferulic acid. These three acids accounted for over 75% of the total soluble esterified phenolic acid content of any flour.

Total soluble esterified phenolic acids for each wheat and composited flour are shown in Table IV. Soluble esterified acids were no more than 17% of the overall total phenolic acid content of any composited flour. Within the whole wheat samples of all classes, with the exception of CWRW, sinapic acid contributed 51–52% of the total 49.9–58.6 ppm of soluble esterified phenolic acids. The CWRW sample, at 38.2 ppm, had a sinapic acid contribution of only 44% of the total soluble esterified acids. The high contribution of sinapic acid may be due to the large involvement of sinapic acid in the lignin core of the bran suggested by Schwartz et al (1989). Ferulic acid did not show this same consistency, ranging in contribution from 18 to 30% of the total soluble esterified acids, while vanillic varied from 7 to 15%.

Minimum soluble esterified phenolic levels were detected in the first patent (75%) flours, which varied significantly ($P < 0.05$) between the classes, from 1.0 to 4.2 ppm. With the exception of CWSWS at 8.25%, these values did not exceed 5.0% of the total soluble esterified phenolic acids present in the whole wheat.

TABLE V
Total Free Phenolic Acids (ppm) in Prepared Flours[a]

	CWSWS	CPSR	CWRW	CWRS	CWES
Wheat	10.8	9.5	11.0	12.2	9.2
Flour					
75% str. grade	3.1	2.0	1.3	1.2	0.9
1st patent	2.8	1.3	0.9	0.7	0.2
2nd patent	3.2	1.9	1.5	1.1	0.9
1st clear	5.4	3.8	2.2	2.5	2.0
2nd clear	7.7	6.2	6.0	4.2	3.7
80% str. grade	4.0	2.4	1.8	1.9	2.1
1st patent	2.4	1.5	0.9	1.0	0.8
2nd patent	3.3	2.7	1.9	1.6	2.6
1st clear	6.3	4.5	4.3	3.4	4.6
2nd clear	12.6	11.6	9.2	7.6	9.5
85% str. grade	5.1	5.3	3.3	4.6	3.9
Chinese Standard	9.2	10.9	7.2	6.0	5.0

[a] Canadian Western Soft White Spring (CWSWS), Canadian Prairie Spring Red (CPSR), Canadian Western Red Winter (CWRW), Canadian Western Red Spring (CWRS), and Canadian Western Extra Strong (CWES).

TABLE VI
Polyphenol Oxidase Levels (nmoles O_2/g/min) in Prepared Flours[a]

	CWSWS	CPSR	CWRW	CWRS	CWES
Wheat	864	1,369	1,011	1,266	1,767
Flour					
75% str. grade	74	103	125	92	99
1st patent	18	44	17	20	18
2nd patent	130	110	76	94	118
1st clear	423	440	162	282	289
2nd clear	463	541	646	670	812
80% str. grade	228	197	109	152	175
1st patent	32	32	20	45	30
2nd patent	161	191	104	113	193
1st clear	358	404	239	375	289
2nd clear	913	1108	622	697	767
85% str. grade	238	248	191	199	246
Chinese Standard	536	478	488	412	436

[a] Canadian Western Soft White Spring (CWSWS), Canadian Prairie Spring Red (CPSR), Canadian Western Red Winter (CWRW), Canadian Western Red Spring (CWRS), and Canadian Western Extra Strong (CWES).

Significant increases in soluble esterified phenolics were observed within all classes with increasing ash content, within an extraction level, or with progressively higher extraction rates. These large increases were primarily the result of changes in the sinapic, ferulic, and vanillic acid levels.

Examination of the straight-grade flours from the 75% mill extraction indicated that all classes, with the exception of CWES, were similar in total soluble phenolic acid content (4.7–5.1 ppm). The CWES sample was significantly higher at 7.6 ppm due mainly to a larger contribution (4.4 ppm) of sinapic acid. All classes, except CWRW, underwent approximately a twofold or greater increase in total soluble esterified phenolic acid content when the 80% extraction straight-grade flours were prepared. Further significant increases were also observed in the prepared 85% straight-grade flours for each class, although the harder wheats had considerably greater values than the softer CWSWS and CPSR samples.

The individual esterified phenolic acid contents mirrored the milling process with each acid displaying a significant linear relationship ($r = 0.76–0.99$, $P < 0.05$) with ash content (Fig. 3). The 75% first clear flours with their elevated bran component represented the first flour in which all six phenolic acids were detected in every class.

Each of the six individual soluble esterified phenolic acids also displayed a significant relationship ($r = 0.68–0.97$, $P < 0.05$) with KJ flour color (Table IV and data not shown). McCallum and Walker (1990) had found a relationship between total soluble phenolics and KJ flour color. Caffeic acid displayed the highest correlations with all classes ($r = 0.87–0.97$), while vanillic acid showed the least ($r = 0.68–0.92$).

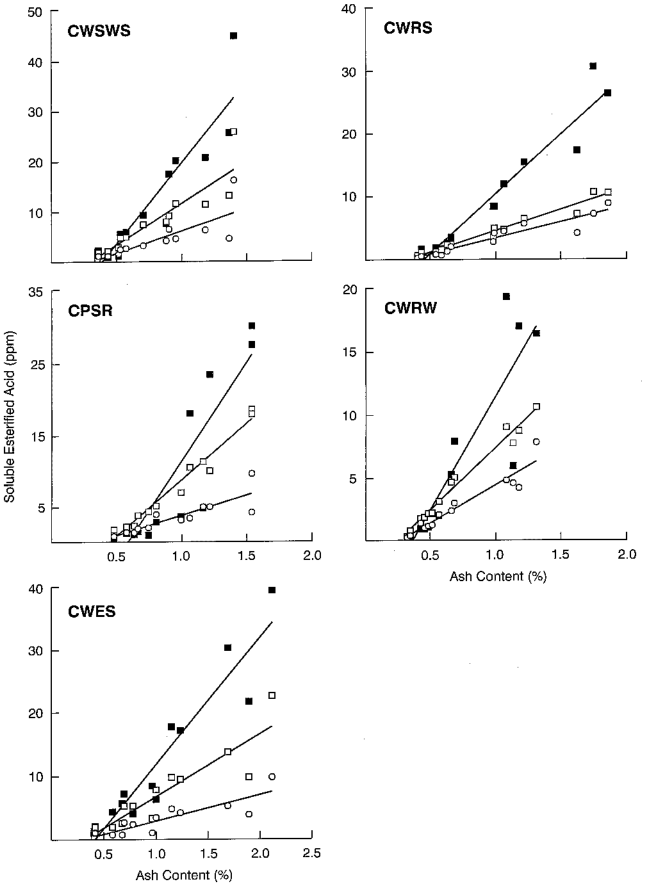

Fig. 3. Relationship between soluble esterified phenolic acids and ash content of blended flours from different classes of wheat. ■ = Sinapic acid, □ = ferulic acid, ○ = vanillic acid.

Free Phenolic Acid Composition

Placed in perspective, the total free phenolic acids (Table V) accounted for only 2–3.5% of the total phenolic acid content in the ground wheat sample and did not exceed 6% of the total phenolic content of any prepared flour. The most notable observation in the analyses of the free phenolic acids was the lack of sinapic acid, the dominant soluble esterified component, in any of the flour samples. Ferulic acid was the predominant component (0.2–6.3 ppm) found in all classes accounting for 32–67% of the total free acid content for any individual flour (results not shown). Vanillic acid was the second most common free acid detected in all composited flours. The presence of free coumaric or caffeic acid was not detected in many classes of low ash flours, and only minor amounts of coumaric acid were detected in the higher bran content flours.

In general, the softer wheats had slightly higher free phenolic acid content in all of the flours examined (Table V). Analysis of the 75% extraction first patent flours determined that the softest wheat, CWSWS, followed by the medium CPSR, had the highest content of free phenolic acids at 2.8 and 1.3 ppm, respectively. The harder wheats, CWRW, CWRS, and CWES each displayed levels <1.0 ppm. The influence of milling yield on the various straight-grade flours indicated only minimal differences in free acids between either the 75 or 80% extraction millings, although large increases were observed in all classes (except CWSWS) upon achieving a 85% yield. Unlike the insoluble bound or soluble esterified acids, the second clear flours displayed free acid content of the same magnitude as the whole wheat. This would be in agreement with the concept that ferulic acid is primarily esterified to hemicellulosic components of cell walls and little of the free form exists (Schwartz et al 1989).

A high correlation ($r > 0.87$ and $r > 0.92$ ($P < 0.05$) between ash content and both ferulic and vanillic acids, respectively (Fig. 4) was found for each wheat class. There were also corresponding high correlations ($P < 0.05$) between the free ferulic acid content and both the soluble esterified ($r > 0.88$) and insoluble bound ($r > 0.92$) ferulic acid levels. The levels of free coumaric, syringic, and caffeic acids were low, <1, 1.5, and 4 ppm, respectively, in any sample (data not shown).

A significant relationship between total free phenolic acids and KJ flour color ($r > 0.90$, $P < 0.05$) was evident for every class. Closer examination of each of the individual free acids revealed that all exhibited a similar, significant correlation ($r > 0.83$, $P < 0.05$) (data not shown).

PPO Activity in Pooled Flours

The PPO levels, expressed on a per gram basis, for the prepared flours representing increasing extraction rate can be found in Table VI. The first patent flours at both the 75 and 80% extraction rates, representing the initial 45% of the cumulative mill yield, displayed minimal amounts of enzyme activity. Values ranged from 17 to 45 nmoles of O_2/g/min, representing <3.7% of the activity in the ground grain. These values agreed with those reported by Marsh and Galliard (1986) of <30 nmoles O_2/g/min in a 75% extraction first patent flour.

A significant relationship between PPO activity and ash content ($r > 0.90$, $P < 0.05$) was detected for all classes except CWES where $r = 0.78$. However, unlike the phenolic acid components, all prepared flours, with the exception of one CWSWS sample, displayed lower PPO values than those found in the whole wheat sample. It is believed that this difference represents the localization of the enzyme in the bran layer (Marsh and Galliard 1986).

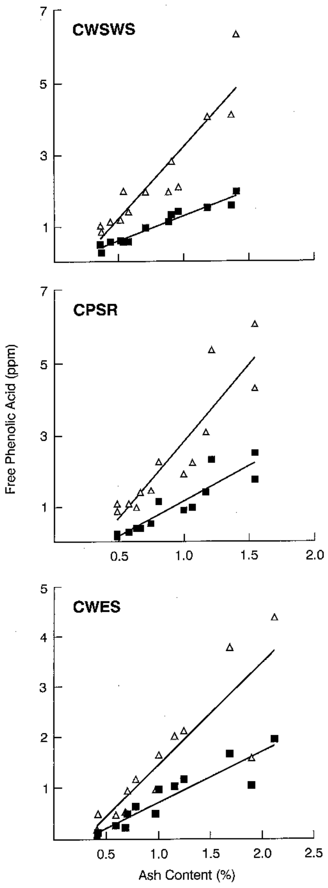

Fig. 4. Relationship between free phenolic acids and ash content of blended flours from different classes of wheat. Δ = Ferulic acid, ■ = vanillic acid.

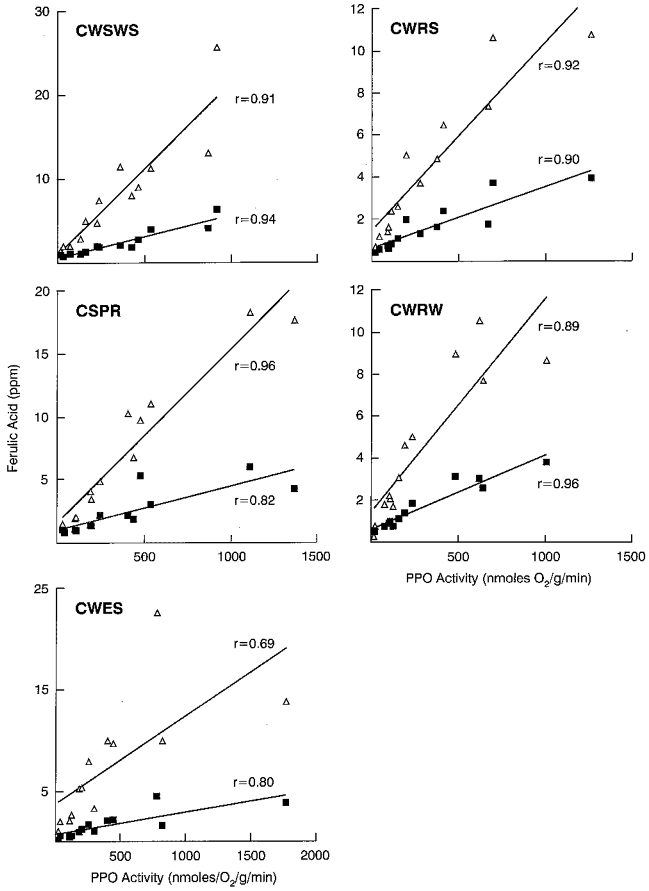

Fig. 5. Relationship between ferulic acid and polyphenol oxidase (PPO) activity in different classes of wheat. Δ = Soluble esterified ferulic acid, ■ = free ferulic acid.

TABLE VII
Correlation Coefficients ($P < 0.001$) Among Polyphenol Oxidase and Flour Phenolic Acid Components in Prepared Flours[a]

Acids	CWSWS	CPSR	CWRW	CWRS	CWES
Insoluble bound	0.93	0.88	0.86	0.88	0.79
Soluble esterified	0.92	0.93	0.89	0.94	0.76
Free phenolic	0.98	0.84	0.96	0.94	0.84
Total phenolic	0.94	0.89	0.88	0.89	0.79

[a] Canadian Western Soft White Spring (CWSWS), Canadian Prairie Spring Red (CPSR), Canadian Western Red Winter (CWRW), Canadian Western Red Spring (CWRS), and Canadian Western Extra Strong (CWES).

There were highly significant correlations between the PPO activity of individual class flours and their total phenolic acid, insoluble ferulic acid, total soluble esterified phenolics, and total free phenolic acid content as shown in Table VII. There was greater variability in PPO activity contrasted to phenolic acid content, which is in agreement with McCallum and Walker (1990) for New Zealand wheats. Significant correlations were also found between the enzyme and both the soluble esterified and free ferulic acids as represented in Fig. 5. The strong correlation between flour color and PPO activity ($r > 0.91$, $P < 0.05$) reinforced the previous findings on individual mill streams (Hatcher and Kruger 1993).

CONCLUSIONS

The objective of this research was to establish the simple phenolic acid content (divided into three categories: insoluble bound, soluble esterified, and free acids) of flours representative Canadian wheat classes, milled at varying extraction levels. Phenolic acid components in different classes of Canadian flours were generally higher than those reported in the literature. Unfortunately there are only a limited number of references to wheat flour phenolic levels for comparison. Ferulic acid dominated the overall phenolic composition as it was the only acid detected in the major phenolic acid class, the insoluble bound acids. Agreement was noted with Sosulski et al (1982) and Jackson and Hoseney (1986) for this component and to the comparable work on individual mill streams by Pussayanawin et al (1988). However, the work of Rybka et al (1993), using ultraviolet absorption spectrum of wheat extracts to estimate ferulic acid content in Polish wheats, reported values approximately twice that found in this study. This difference may be attributable to widely different genetic and environmental factors or to the methodology employed.

Comparison with levels reported by Sosulski et al (1982) or Jackson and Hoseney (1986) in terms of soluble bound acids indicated that their values (3.8 ppm each) were considerably lower than those detected in the present study. This difference was primarily due to the lack of sinapic or any other acid detected in their patent flours. Agreement was found however in the present study, in terms of free soluble ferulic acid content, which in 75% extraction of first patent flours ranged from 0.21 to 0.98 ppm. Differences in the total amount of free acids can be attributed to the quantitation of the other acids that Jackson and Hoseney (1986) reported as being present only in trace amounts.

Significant ($P < 0.05$) correlations were observed between PPO activity and either individual or total phenolic acids within each category for each class. The close relationship between the enzyme activity and its potential substrates suggests a role for such simple phenolic acids in the complex process of enzymatic darkening in end-products. Further research is anticipated to elucidate their role as well as to investigate the involvement of other phenolic components in the process.

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