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Antioxidant Properties of Wheat As Affected by Pearling

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The effects of pearling on the content of phenolics and antioxidant capacity of two Canadian wheat classes, namely, Canada Western Amber Durum; *Triticum turgidum* L. var. *durum*; CWAD) and Canada Western Red Spring; *Triticum aestivum* L.; CWRS) were examined. The antioxidant activity of wheat phenolics was evaluated using oxygen radical absorbance capacity (ORAC), inhibition of photochemiluminescence (PCL), Rancimat method, inhibition of oxidation of low-density lipoprotein, and DNA. The phenolic composition of wheat extracts was determined using high-performance liquid chromatography. The antioxidant capacity of both pearled grains and byproducts significantly decreased as the degree of pearling increased. Among grains, the unprocessed whole grains demonstrated the highest antioxidant capacity. The byproducts always demonstrated higher antioxidant capacity compared to the pearled grains, regardless of the wheat class. The resultant byproducts from 10−20% pearling possessed the highest antioxidant capacity. Processing of cereals may thus exert a significant effect on their antioxidant activity. The concentration of grain antioxidants is drastically reduced during the refining process. As phenolic compounds are concentrated in the outermost layers, the bran fractions resulting from pearling may be used as a natural source of antioxidants and as value-added products in the preparation of functional food ingredients or for enrichment of certain products.

KEYWORDS: Phenolic acids; ORAC; Rancimat; photochemiluminescence; oxidative stability; LDL and DNA oxidation; HPLC analysis; sinapic acid; pearling

INTRODUCTION

The growing concern for general health, amelioration of chronic diseases, and prevention of the effects of aging has intensified consumers' interest in phytonutrients, which are plant-derived, naturally occurring compounds with curative, preventive, or nutritive value (*1*). In general, plant foods such as fruits, vegetables, and grains are known to contain compounds that contribute to health and wellness both by their traditional nutritive value and through enhancing the body's defense against chronic diseases (*2*). Physiological functionality of plant chemicals has received much attention in association with human health promotion (*3*). Antioxidative action, one of the important physiological functions of plant chemicals, is supposed to protect living organisms from oxidative damage and thus deleterious effects arising from various diseases such as cancer, cardiovascular diseases, and diabetes (*4*).

Oxidative stress may result from an imbalance in the body between oxidants and antioxidants due to a decrease in natural cell antioxidant capacity or an increase in the amount or overproduction of reactive oxygen species (ROS) in organisms (*5*). Consumption of foods rich in antioxidants may lead to scavenging of free radicals and ROS that could otherwise cause oxidative damage to biomolecules such as lipids, proteins, and nucleic acids (*6*). Increased consumption of plant-derived phenolics has been associated with a reduced risk of degenerative diseases such as cardiovascular diseases, cancer, and other chronic diseases (*7*). Grains are a major source of phenolic compounds such as hydroxycinnamic acids (HCA) and hydroxybenzoic acids (HBA) known for their antioxidant activity (*8*−*10*). In general, phenolic acids have been recognized as potent antioxidants (*11*, *12*). Several ferulic acid dehydrodimers have been shown to possess antioxidant activity in different in vitro models (*13*−*15*). The HCA derivatives are the most notable phenolic acids, strengthening their potential role as nutritional antioxidants (*12*). Phenolic acids, which are known to influence the flavor, taste, and color of foods, have gained much attention due to their antioxidative, anti-inflammatory, antimutagenic, and anticancer properties as well as the ability to modulate some key enzyme functions in the cell (*16*).

The addition of antioxidants to food systems may increase their shelf life, thereby reducing waste and nutritional loss by inhibiting and delaying oxidation (*17*). Recently, much attention has been paid to replacing synthetic antioxidants with natural alternatives, primarily plant phenolics (*18*). This has led to the isolation and characterization of effective natural antioxidants (*19*). In fact, plant phenolics, present in fruits and vegetables,

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are of much interest because of their antioxidant properties and health benefits (*20*).

According to Dietrych-Szostak and Oleszek (*21*), buckwheat grains may be stored for a long time without any symptoms of chemical changes owing to the presence of several natural antioxidants that stabilize them during storage. However, the concentration of natural antioxidants may vary considerably depending on many factors, including variety, location, and environmental conditions, among others. Processing of cereals may also have a significant effect on their antioxidant activity because the bran fraction alone exhibits the highest antioxidant activity. This may be explained by localization of phenolics in grains; the outer layers such as the husk, pericarp, testa, and aleurone layers contain the highest concentrations of total phenolics (*22*). Phenolic compounds are primarily concentrated in the bran fraction (*23*−*26*). According to Xing and White (*22*), ≈80% of the *trans*-ferulic acid in both rye and wheat grains is found in the bran. Moreover, according to Onyeneho and Hettiarachchy (*27*), the freeze-dried bran fraction of durum wheat exhibited a stronger antioxidant activity than extracts of other milling fractions.

The U.S. Department of Agriculture (USDA) has recommended daily consumption of 6−11 servings of grain products that form the base of the USDA food guide pyramid. Although many of the protective bioactive compounds present in whole grains are also found in fruits and vegetables, some of the compounds, including phenolic ferulates, are unique to whole grains (*28*). Therefore, the effect of sequential removal of the outermost layers (pearling) on the antioxidant activity and phenolic composition of wheat grains was studied using in vitro models. The antioxidant activity and phenolic constituents of wheat byproducts were also determined to evaluate their potential for possible use as value-added nutraceutical components.

MATERIALS AND METHODS

Materials. The compounds 2,2′-azobis-(2-methylpropionamidine) dihydrochloride (AAPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin−Ciocalteu phenol reagent, sodium carbonate, monobasic potassium phosphate, dibasic potassium phosphate, ferulic acid, human low-density lipoprotein (LDL) cholesterol, ethylenediaminetetraacetic acid (EDTA), l-ascorbic acid, ferric chloride, fluorescein, ferrous chloride, sodium bicarbonate, deoxyribonucleic acid (DNA, pBR 322 plasmid, *Escherichia coli* strain RRI), Trizma base, boric acid, ethidium bromide, agarose, ferrous sulfate, copper sulfate, bromophenol blue, xylene cyanol, and glycerol were purchased from Sigma-Aldrich Chemical Co. (Oakville, ON, Canada). All other solvents were purchased from Fisher Scientific (Nepean, ON, Canada) and were of ACS grade or better.

Samples of the two predominant wheat classes grown in western Canada, Canada Western Amber Durum; *Triticum turgidum* L. var. *durum*; CWAD) and Canada Western Red Spring; *Triticum aestivum* L.; CWRS), were composites of commercial production (crop years 2002 and 2001, respectively). The CWRS wheat class is composed of high-protein hard red spring varieties and is primarily used in the manufacture of high-quality bread products, whereas the CWAD wheat class is mainly used for the production of high-quality pasta products and couscous (*29*).

Sample Preparation. Wheat grains were "debranned" to various levels using a model TM05C pearler (Satake, Missisauga, ON, Canada). The pearling process involved sequential removal of bran layers from wheat kernels by abrasion. Initially, 500 g portions of each unprocessed wheat were subsampled from 5 kg samples, and the remaining 4.5 kg was pearled. Starting from unprocessed grain, kernels were pearled from 10 to 50% in 10% increments, and pearled wheat and corresponding byproducts equivalent in weight to products derived from 500 g of initial unprocessed grain were collected separately at each level. Pearled grains and their byproducts were ground, if necessary, in a coffee grinder (model CBG5 series, Black and Decker Canada Inc., Brockville, ON, Canada) and passed through a sieve of 1000 μm aperture (Tylor 16 test sieve, Mentor, OH). All samples were defatted by blending the ground material with hexane (1:5, w/v, 5 min, ×3) in a Waring blender (model 33BL73, Waring Products Dividion, Dynamics Corp. of America, New Hartford, CT) at ambient temperature. Defatted wheat samples were air-dried for 12 h and stored in vacuum-packaged polyethylene pouches at −20 °C until used for further analysis within a week.

Extraction of Crude Phenolics of Wheat. The crude phenolic compounds present in whole wheat and its milling fractions were extracted into 80% aqueous ethanol (1:10, w/v) at 4 °C for 16 h. The resulting slurries were centrifuged at 4000*g* for 5 min, and the supernatants were collected. The residues were re-extracted (1:5, w/v; 80% ethanol) under the same conditions, and supernatants from both extractions were combined. The solvent was removed under vacuum at 40 °C, and the resulting concentrated slurries were lyophilized for 72 h at −47 °C and 69 × 10^{-3} mbar. Yields of the crude phenolic extracts were reported as a percentage of defatted material.

Determination of Oxygen Radical Absorbance Capacity (ORAC). The ORAC of wheat was determined according to the method of Dávalos et al. (*30*) using 75 mM phosphate buffer (pH 7.4) at 37 °C. A FLUOstar Optima microplate reader (BMG Labtechnologies GmbH, Offenberg, Germany) equipped with FLUOstar OPTIMA evaluation software version (1.30-0) and black, polystyrene, nontreated 96-well microplates (Costar Corning Inc., Corning, NY) were used. The outer wells of the microplate were not used in the analysis. The excitation and emission filters were 485-P and 520-P, respectively. The final reaction mixture (200 μL) consisted of the extract/antioxidant (20 μL, 6 mg/mL, dilution factor 15−20), florescein (120 μL, 70 nM final concentration), and AAPH (60 μL, 12 mM final concentration). The samples were loaded into designated wells in the microplate. A gain adjustment was performed by pipetting 200 μL of florescein into a designated well before starting the program in order to optimize signal amplification. In cycle 1, pump 1 was programmed to inject florescein, after which the samples and solutions were incubated at 37 °C for 15 min followed by the addition of AAPH using the second pump in cycle 2. The instrument read the fluorescence of each well after the addition of AAPH over 100 cycles. All of the measurements were expressed relative to the initial reading. The results were calculated using the differences in area under the florescein decay curve between the blank and the sample and expressed as micromoles of Trolox equivalents per gram of defatted material. The standard curve was prepared using 1−25 M Trolox (final concentration).

Evaluation of Antioxidant Activity Using Photochemiluminescence (PCL). The antioxidant capacity of hydrophilic (ACW) and hydrophobic (ACL) compounds was assessed using a Photochem (Analytik Jena USA, Delaware, OH). The extracts were dissolved in distilled water and methanol, respectively, for ACW and ACL systems followed by centrifugation (4000*g*, 5 min). The supernatants were used in the determination of antioxidant activity with further dilution using respective solvents, if necessary. Both ACW and ACL analyses were carried out in 0.1 M carbonate buffer (pH 10.5) (*31*−*33*). The photosensitizer luminol (reagent 3) was procured from Analytik Jena USA. The compositions of reaction mixtures for ACW and ACL were as follows: the ACW reaction mixture contained buffer (1000 μL), sample (1−20 μL), water (1500 μL sample volume), and luminol (25 μL); the ACL system was buffer (200 μL), sample (1−20 μL), methanol (2300 μL sample volume), and luminol (25 μL). The antioxidant activity of hydrophilic compounds is expressed as micromoles of ascorbic acid equivalents per gram of defatted material, whereas that of hydrophorbic compounds is expressed as micromoles of α-tocopherol equivalents per gram of defatted material.

Measurement of Antioxidant Activity Using Rancimat Method. The oxidative stability of fats and oils in the presence or absence of wheat extracts was determined using an automated Metrohm Rancimat apparatus (model 743, Herisen, Switzerland) capable of operating over a temperature range of 50−200 °C. The oxidative stability of seal blubber oil (SBO) and stripped corn oil (SCO) was determined at 100 °C, whereas that of shortening was determined at 120 °C. The glassware

was thoroughly cleaned and dried prior to each use. Samples of oil or fat were weighed directly into the reaction vessels. Wheat extracts were added at 25 mg/g of oil or fat concentration. The air flow rate through the sample was adjusted to 20 L/h. The volatile reaction products released during the oxidation of oil or fat sample were collected in 60 mL of distilled water in the collection vessel. The change in the conductivity was plotted automatically until the end point was reached. With each oil or fat a control test (with no additives) was included and subjected to the same experimental conditions. The oxidative stability was measured in duplicate for each sample and the IP (h) was recorded. The relative activity on the antioxidant compounds was expressed as the protection factor (PF) where PF = IP of oil with extracts/IP of the control (no extract) (*34*).

Inhibition of Copper-Induced Oxidation of Human Low-Density Lipoprotein (LDL) Cholesterol. The procedure described by Hu and Kitts (*35*, *36*) was employed in this study with slight modifications. LDL was dialyzed in 10 mM PBS (pH 7.4) at 4 °C in the dark for 24 h. LDL (0.2 mg of LDL/mL) was mixed with different amounts of wheat extracts dissolved in 10 mM PBS. Ferulic acid was used as the reference antioxidant. Reaction was initiated by adding a solution of cuprous sulfate (10 μM), and samples were incubated for 22 h at 37 °C. The formation of conjugated dienes was measured at 234 nm using a diode array spectrophotometer (Agilent Technologies Canada Inc., Mississauga, ON, Canada). A separate blank, containing all reagents except LDL, was used for each extract. The inhibitory effect of wheat extracts on the formation of conjugated dienes (% inhibition$_{CD}$) was calculated using the following equation:

$$\%\ \text{inhibition}_{CD} = (\text{Abs}_{\text{oxidative}} - \text{Abs}_{\text{sample}}/\text{Abs}_{\text{oxidative}} - \text{Abs}_{\text{native}}) \times 100$$

where

$$\text{Abs}_{\text{sample}} = \text{absorbance of LDL} + \text{CuSO}_4 + \text{wheat extract or standard}$$

$$\text{Abs}_{\text{native}} = \text{absorbance of LDL} + \text{PBS}$$

$$\text{Abs}_{\text{oxidative}} = \text{absorbance of LDL} + \text{CuSO}_4 + \text{PBS}$$

Using percentage values, the amount of protein (μg) that can be protected against copper-mediated oxidation by 1 g of defatted wheat samples was obtained.

Inhibition of Strand Breaking of Supercoiled DNA. DNA strand breaking by hydroxyl radical was performed according to the method described by Johnson and Grossman (*37*) and Hiramoto et al. (*38*) with slight modifications. The reaction was carried out in 1 M phosphate buffer (pH 7.4). The reaction mixture contained 2 μL of phosphate buffer, 4 μL of a solution of extract at the indicated final concentration (3 or 6 mg of extract/mL), 2 μL of a solution of supercoiled plasmid pBR 322 DNA (4300 base pairs) at 100 μg/mL, 6 μL of 0.33 mM H_2O_2, and 6 μL of 0.33 mM $FeSO_4$ added in the order stated. The reaction was carried out in an Eppendorf tube (1 mL) and incubated at 37 °C for 1 h. Simultaneously, the plasmid DNA was also incubated with the restriction endonuclease *Hin*dIII (*Hin*dIII has one restriction site on the pBR 322 plasmid DNA, thus producing one fragment having the original number of base pairs. The reaction mixture contained 8 μL of DNA (100 μg/mL), 2 μL of *Hin*dIII restriction enzyme, 2 μL of restriction buffer (×10), and 8 μL of distilled water. For identification, the base pair ladder DRIgest III was run along with the extracts. After incubation, 2 μL of the loading dye (0.25% bromophenol blue/0.25% xylene cyanol/50% glycerol) were added, and the whole mixture was loaded onto an 0.8% (w/v) agarose gel prepared in Tris/borate/EDTA (TBE) electrophoresis buffer (pH 8.3). Agarose gel electrophoresis was performed using TBE electrophoresis buffer at 116 V for 75 min. The gel was stained with 0.5 μg/mL ethidium bromide, and bands were visualized under ultraviolet light. The images were analyzed using AlphaEase Stand Alone software (Alpha Innotech Corp., San Leandro, CA). The protective effects of the crude extracts were measured using the retention percentage of supercoiled DNA.

Table 1. Oxygen Radical Absorbance Capacity (ORAC) of Pearled Grains and Byproducts of Two Wheat Cultivars, Canada Western Amber Durum (CWAD) and Canada Western Red Spring (CWRS)[a]

	ORAC (μmol/g of defatted material)			
	CWAD		CWRS	
degree of pearling (%)	pearled wheat	byproduct	pearled wheat	byproduct
0[b]	100 ± 1f		95 ± 5f	
10	86 ± 3e	195 ± 5e	87 ± 3e	207 ± 4e
20	76 ± 2d	173 ± 3d	79 ± 2d	189 ± 3d
30	67 ± 3c	146 ± 3c	73 ± 1c	163 ± 1c
40	62 ± 1b	137 ± 2b	58 ± 2b	138 ± 4b
50	47 ± 1a	101 ± 2a	41 ± 1a	115 ± 1a

[a] Values are means of three determinations ± standard deviation. Values in each column with the same letter are not different ($P > 0.05$). [b] Whole grain.

Analysis of Phenolics Composition Using High-Performance Liquid Chromatography (HPLC). The HPLC procedure described by Amarowicz and Weidner (*39*) was used for the determination of phenolic acids. A Shimadzu (Kyoto, Japan) HPLC system equipped with an LC-AD pump, an SPD-M10A diode array detector, and an SCL-10A system controller was used for analytical and preparative HPLC of wheat crude extracts. Conditions for preparative HPLC were as follows: prepacked LiChrospher 100 RP-18 column (5 μm, 4 × 250 mm, Merck, Darmstadt, Germany); water/acetonitrile/acetic acid (88:10:2, v/v/v) as the mobile phase; flow rate of 1 mL/min; injection volume of 20 μL. The content of vanillic acid was calculated from chromatograms that were recorded at 260 nm, whereas other phenolics were identified at 320 nm. Free phenolics and those liberated from soluble esters were isolated from the extract according to a previously described method (*40*) with some modifications. An aqueous suspension of the extract (800 mg/20 mL) was adjusted to pH 2 using 6 M HCl, and free phenolic acids were extracted five times into 20 mL of diethyl ether using a separatory funnel. The extract was evaporated to dryness under vacuum at room temperature. The aqueous solution was neutralized to pH 7 with NaOH and lyophilized. The residue was dissolved in 20 mL of 2 M NaOH and hydrolyzed for 4 h at room temperature under a nitrogen atmosphere. After acidification to pH 2 using 6 M HCl, phenolic acids released from soluble esters were extracted from the hydrolysate five times into 30 mL of diethyl ether using a separatory funnel. The total free and esterified phenolic acid content was expressed as micrograms per gram of defatted material.

Statistical Analysis. All analyses were performed in triplicate, and data are reported as mean ± standard deviation, unless otherwise stated. Analyses of variance were performed using the General Linear Model of Minitab release 14 Xtra for Windows (Minitab Inc., State College, PA). Significant differences ($P < 0.05$) among means were determined using Tukey's multiple-range test at a fixed level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

Several antioxidant mechanisms such as hydrogen or electron donation, metal chelation, and protein binding may explain the antioxidant activity of phenolic compounds (*41*). This study was designed to evaluate the ability of wheat phenolics to act as antioxidants using different methods. It was apparent that wheat phenolics demonstrated antioxidant properties that can be attributed to one or more of the antioxidant mechanisms described above.

ORAC of Phenolics of Pearled Wheat Grains and Their Byproducts. The antioxidant activity of wheat fractions, measured by the ORAC procedure, showed effective scavenging of peroxyl radical especially by the byproducts at 10−20% pearling (**Table 1**). Zhou et al. (*42*) determined the antioxidant activity of the bran and aleurone layer of a Swiss red wheat variety and found that the aleurone layer had a higher antioxidant

Table 2. Oxidative Stability of Seal Blubber Oil (SBO) and Stripped Corn Oil (SCO) in the Presence of Extracts of Pearled Grain and Byproducts of Two Wheat Cultivars, CWAD and CWRS, As Evaluated by Rancimat

	oxidative stability (protection factor)			
	pearled grains		byproducts	
degree of pearling (%)	SBO	SCO	SBO	SCO
		CWAD		
0[b]	1.67 ± 0.1b	1.47 ± 0.05b		
10	1.49 ± 0.09a	1.33 ± 0.08a	1.93 ± 0.06c	1.66 ± 0.06b
20	1.51 ± 0.1b	1.27 ± 0.1a	1.64 ± 0.05b	1.53 ± 0.08ab
30	1.46 ± 0.04a	1.29 ± 0.06a	1.58 ± 0.06a	1.43 ± 0.05a
40	1.4 ± 0.03a	1.31 ± 0.02a	1.59 ± 0.08a	1.38 ± 0.03a
50	1.43 ± 0.03a	1.29 ± 0.05a	1.49 ± 0.03a	1.41 ± 0.04a
		CWRS		
0[b]	1.81 ± 0.04b	1.66 ± 0.15b		
10	1.73 ± 0.08ab	1.46 ± 0.1ab	2.48 ± 0.03c	1.93 ± 0.05b
20	1.66 ± 0.1a	1.38 ± 0.05a	1.91 ± 0.06b	1.61 ± 0.05a
30	1.63 ± 0.04a	1.37 ± 0.08a	1.74 ± 0.1a	1.54 ± 0.03a
40	1.54 ± 0.09a	1.36 ± 0.03a	1.7 ± 0.1a	1.47 ± 0.05a
50	1.58 ± 0.08a	1.38 ± 0.06a	1.72 ± 0.07a	1.47 ± 0.7a

[a] Values are means of three determinations ± standard deviation. [b] Whole grain.

activity than the bran. The aleurone layer alone exhibited 7−8-fold higher activity than the bran.

According to Hendelman et al. (*9*) the ORAC of oat varied from 2.08 to 8.13 μmol/g. These authors found that the bran and flour had similar antioxidant activities, due to the mixing of the bran with the starchy endosperm. The aleurone layer possessed the highest ORAC value. In contrast, this study demonstrated that the byproducts at 10 and 20% pearling, containing mainly bran and aleurone portions, possessed a much higher ORAC value than all pearled grains and byproducts at 30−50% pearling; wheat had a superior antioxidant activity than oat in the ORAC assay (*9*). However, ORAC was determined using two different methods in the two studies, and hence comparison becomes rather difficult. The antioxidant capacity of wheat can primarily be attributed to the constituents present in the bran layers.

Effects of Wheat Phenolics Extracts on Oxidative Stability of Oils and Fats. Because fats and oils are susceptible to oxidative deterioration, addition of antioxidants may prevent the development of off-flavors and undesirable compounds. Because the use of synthetic antioxidants has been questioned due to associated toxic and possible carcinogenic effects (*43*), there is considerable interest in developing plant-derived natural antioxidants (*35*). The results of the Rancimat method suggest that wheat phenolics enhanced oxidative stability of SBO and SCO compared to the control that had no additives (**Table 2**). The byproducts at 10−20% pearling level produced the most noticeable effect, especially in the SBO system. The PF of byproducts of the CWRS cultivar varied from 1.72 to 2.48 in the SBO system. Phenolic compounds are effective antioxidants for polyunsaturated fatty acids (PUFA) as they can transfer a hydrogen atom to lipid peroxyl radicals with ease. The stable aryloxyl radical formed does not act as a chain initiater; hence, lipid peroxidation is interrupted (*44*).

Wheat phenolics may prevent the propagation of peroxidation by scavenging free radicals and function as antioxidants. Wheat extracts, especially those of byproducts, enhanced the oxidative stability of SCO and SBO. One of the most important parameters that influence lipid oxidation is the degree of unsaturation of its fatty acid constituents (*34*). The major fatty acids in SCO were palmitic, oleic, and linoleic acids; the latter accounted for ≈59% of the total fatty acids.

The total saturated (SFA) and monounsaturated fatty acids (MUFA) made up about 13 and 26% of the SCO, respectively, whereas PUFA content was approximately 60%. Although in SBO PUFA content was much less than that of SCO, >90% of SBO PUFA was made up of highly unsaturated fatty acids such as eicosapentaenoic, docosahexaenoic, and docosapentaenoic acids, which are extremely susceptible to oxidation. The Rancimat method is commonly used to evaluate the antioxidative efficiency of natural and synthetic antioxidants (*45*). In general, oxidation proceeds slowly under normal conditions until it reaches a point after which the rate starts to increase sharply (*46*). In the Rancimat method, the elevated temperatures and the presence of excess oxygen may allow one to obtain results within a relatively short period. The differences observed for 30−50% pearled samples were not quite significant. In the presence of chain-breaking antioxidants such as phenolic compounds in wheat extracts, the antioxidant will donate a

Table 3. Antioxidant Activity of Pearled Grains and Byproducts of Two Wheat Cultivars, CWAD and CWRS, As Evaluated by Photochemiluminescence in Antioxidant Capacity of Hydrophilic (ACW) and Antioxidant Capacity of Hydrophobic (ACL) Systems[a]

	antioxidant activity			
	pearled grains		byproducts	
degree of pearling (%)	ACW (μmol of ascorbic acid equiv/ g of defatted material)	ACL (μmol of tocopherol equiv/ g of defatted material)	ACW (μmol of ascorbic acid equiv/ g of defatted material)	ACL (μmol of tocopherol equiv/ g of defatted material)
		CWAD		
0[b]	5.0 ± 0.02f	6.1 ± 0.06f		
10	3.77 ± 0.03e	4.09 ± 0.07e	11.1 ± 0.09e	13.71 ± 0.07e
20	3.09 ± 0.04d	3.35 ± 0.03d	9.03 ± 0.06d	8.47 ± 0.06d
30	2.52 ± 0.04c	2.71 ± 0.04c	6.56 ± 0.03c	6.74 ± 0.05c
40	2.39 ± 0.02b	2.22 ± 0.03b	5.92 ± 0.03b	6.26 ± 0.04b
50	1.88 ± 0.04a	1.78 ± 0.02a	4.54 ± 0.04a	5.05 ± 0.02a
		CWRS		
0[b]	5.2 ± 0.05f	6.8 ± 0.04f		
10	4.82 ± 0.06e	4.47 ± 0.04e	12.6 ± 0.06e	15.49 ± 0.08e
20	4.14 ± 0.05d	3.64 ± 0.04d	10.4 ± 0.02d	10.44 ± 0.06d
30	2.75 ± 0.04c	3.0 ± 0.03c	8.28 ± 0.03c	9.14 ± 0.02c
40	2.05 ± 0.07b	2.16 ± 0.05b	5.66 ± 0.05b	7.05 ± 0.03b
50	1.63 ± 0.05a	1.81 ± 0.03a	4.58 ± 0.04a	2.09 ± 0.03a

[a] Values are means of three determinations ± standard deviation. Values in each column with the same letter are not different ($P > 0.05$) [b] Whole grain.

hydrogen atom and, consequently, the free radical chain reaction will be terminated.

Inhibition of PCL by Wheat Phenolics. The antioxidative activity of the pearled grains and their byproducts was measured by the PCL method, and results are shown in **Table 3**. In this method, the radical scavenging capacity is evaluated by measuring the inhibition of the photoinduced chemiluminescent autoxidation of luminol (*32*).

The antioxidant activity was highest for the byproducts resulting from 10% pearling of both CWAD and CWRS. In the ACW system, the pearled grains of CWAD and CWRS at 10% pearling demonstrated 2 and 2.9 times stronger antioxidant activity than those pearled to 50%, respectively. The corresponding values for the ACL system were 2.3 and 2.5 times. Moreover, the byproducts were much stronger than the pearled grains. Whole grains of both CWAD and CWRS exhibited higher inhibitory activity against PCL compared to pearled wheat grains.

Superoxide radical anions, produced by means of a photosensitizer, are detected by their reaction with a chemiluminogenic substance and by measuring the chemiluminescence produced. Luminol acts as the radical generator and detector (*32*). In the presence of an antioxidant compound the intensity of PCL is attenuated due to scavenging of $O_2^{\bullet -}$. The antiradical properties of the additive may be quantified and expressed in equivalent concentration units of a reference compound, for instance, ascorbic acid or tocopherol equivalents in water- and lipid-based systems, respectively. The PCL method allows determination of the effects of both hydrophilic and hydrophobic compounds using the same system (*33*). The PCL method has been used to assess the antioxidant activity of plant extracts and blood plasma (*33*, *47*−*51*). This study is the first report on antioxidant activity of wheat pearlings using the PCL method. With many fractions the hydrophobic antioxidants demonstrated greater activity than hydrophilic constituents, and hence values of ACL analysis were higher than those of ACW results. Thus, the protective capacity of both hydrophilic and hydrophobic constituents present in wheat phenolics and their action against ROS can be measured by this method.

Effect of Pearling on Inhibition of LDL Oxidation of Phenolics of Pearled Wheat Grains and Byproducts Thereof. Dietary antioxidants that prevent LDL from oxidizing are of great importance in protection against atherosclerosis (*51*). Cereals such as wheat (*53*, *54*), rye (*55*−*57*), and barley (*58*) have been shown to be rich sources of phenolic acids. Hydroxycinnamic acids and ferulic acid dehydrodimers are most common among cereal phenolic acids (*53*−*56*, *58*).

The activity of wheat samples in preventing the oxidation of human LDL was greater for byproducts at 10−20% pearling than higher pearling levels. The inhibition of oxidation of human LDL by wheat decreased with sequential removal of the bran layers (**Table 4**). There was a significant difference in the inhibition of LDL oxidation by the extracts with an increase in pearling degree. The highest activity of 10−20% byproducts may be attributed to the presence of a greater proportion of bran and/or aleurone layer in these fractions than in the pearlings from 30−50%. Extracts of whole wheat and their milling fractions in 80% aqueous ethanol were quite efficient in inhibiting copper-induced oxidation of LDL (*59*). LDL is known to contain endogenous antioxidants such as α-tocopherol that may contribute to antioxidative activity in copper-induced LDL oxidation assay. There may be synergism between the well-known chain-breaking antioxidant α-tocopherol and the hydrogen donors (*60*) in the wheat samples. Thus, the phenolic hydrogen donors in the wheat extracts may spare tocopherols from consumption and revert the tocopheryl radical directly to tocopherol. Moreover, phenolic antioxidants may scavenge other radicals, thereby preventing them from attacking tocopherol (*60*). Another factor that may bring about antioxidant action in the LDL system is the interaction of wheat phenolics with LDL. Phenolic antioxidants may bind with apo-lipoprotein B, thus preventing the copper catalyst from binding to LDL. Moreover, the phenolic−protein binding may promote the access of phenolics to lipids (*41*). Several antioxidant mechanisms such as hydrogen donation, metal chelation, and protein binding may explain the antioxidant activity of phenolics against in vitro LDL oxidation (*41*). The ability of an antioxidant to inhibit copper-induced LDL oxidation may also be attributed to efficient removal of copper from the surface of LDL (*61*). Thus, dietary antioxidants may play a significant role in retarding the development of atherosclerosis that may otherwise lead to coronary heart disease. The presence of wheat antioxidants in the assay medium reduced the susceptibility of LDL to copper-induced oxidation.

Table 4. Inhibition of Oxidation of Low-Density Lipoprotein by Pearled Grains and Byproducts of Two Wheat Cultivars, CWAD and CWRS[a]

	inhibition of oxidation (μg of protein/g of defatted material)			
	CWAD		CWRS	
degree of pearling (%)	pearled wheat	byproduct	pearled wheat	byproduct
0[b]	834 ± 5f		866 ± 10f	
10	680 ± 5e	1524 ± 7e	843 ± 6e	1587 ± 9e
20	619 ± 6d	1378 ± 9d	727 ± 8d	1510 ± 8d
30	538 ± 5c	1168 ± 8c	658 ± 3c	1359 ± 11c
40	551 ± 3b	969 ± 4b	588 ± 2b	1057 ± 4b
50	441 ± 5a	778 ± 5a	517 ± 5a	898 ± 5a

[a] Values are means of three determinations ± standard deviation. Values in each column with the same letter are not different ($P > 0.05$). [b] Whole grain.

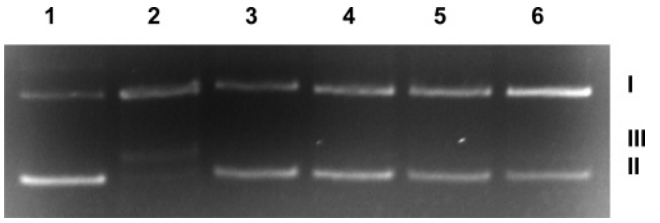

Figure 1. Agarose gel electrophoresis of supercoiled DNA treated with hydroxyl radical in the presence of extract of byproduct at 10% pearling of grains of CWRS cultivar (lane 1, supercoiled DNA; lane 2, supercoiled DNA + $OH^{\bullet}$; lane 3, supercoiled DNA + $OH^{\bullet}$ + 6 mg/mL extract; lane 4, supercoiled DNA + $OH^{\bullet}$ + 3 mg/mL extract; lane 5, supercoiled DNA + $OH^{\bullet}$ + 2 mg/mL extract; lane 6, supercoiled DNA + $OH^{\bullet}$ + 1 mg/mL extract): form I, supercoiled DNA; form II, nicked open circular DNA; form III, linear DNA.

Influence of Pearling of Wheat on $HO^{\bullet}$-Mediated Supercoiled DNA Scission. The supercoiled pBR DNA (form I) is converted into a nicked open circular form (form II) and a linear form (form III) upon strand breakage. It has been shown that $HO^{\bullet}$ can effectively induce single-strand breaks in DNA (*62*). Thus, monitoring of single-strand breaks in DNA induced by $HO^{\bullet}$ may be used in the evaluation of antioxidant properties of a compound (*63*). Wheat antioxidants present in pearled fractions differed in their ability to protect DNA from nicking by the Fenton reaction mediated $HO^{\bullet}$. The $HO^{\bullet}$ cleaved supercoiled plasmid pBR 322 DNA completely into nicked circular and

Table 5. Retention Capacity of pBR 322 Supercoiled DNA against Hydroxyl Radical Mediated Scission by Pearled Grains and Byproducts of Two Wheat Cultivars, CWAD and CWRS[a]

	retention capacity (μg of DNA/g of defatted material)			
	CWAD		CWRS	
degree of pearling (%)	pearled wheat	byproduct	pearled wheat	byproduct
0[b]	395		351	
10	327	742	312	819
20	288	617	285	699
30	242	537	248	583
40	238	509	190	481
50	182	407	156	391

[a] Values are averages of two determinations. [b] Whole grain.

Table 6. Inhibition of Hydroxyl Radical Mediated pBR 322 Supercoiled DNA Scission by Extracts of Pearled Grains and Byproducts over a 1–6 mg/mL Concentration Range[a]

	inhibition (%)							
	pearled grains (mg/ mL)				byproducts (mg/ mL)			
degree of pearling (%)	1 mg/mL	2 mg/mL	3 mg/mL	6 mg/mL	1 mg/mL	2 mg/mL	3 mg/mL	6 mg/mL
				CWAD				
0[b]	42	49	56	74				
10	40	48	52	70	61	69	77	84
20	35	42	50	64	58	63	68	74
30	33	40	48	58	51	57	63	70
40	30	41	47	55	48	50	56	71
50	31	37	42	52	42	48	55	66
				CWRS				
0[b]	46	53	62	78				
10	44	50	56	72	66	73	82	91
20	38	51	53	67	61	67	72	83
30	39	46	53	62	50	60	70	76
40	36	43	48	60	49	54	63	74
50	33	42	50	55	43	49	59	69

[a] Values are averages of two determinations. [b] Whole grain.

linear DNA in the absence of any protection (**Figure 1**, lane 2). Wheat antioxidants demonstrated a dose-dependent activity against HO•-mediated cleavage of DNA (**Figure 1**, lanes 3−6). The byproducts of wheat pearling fractions were more effective than pearled grain products in preventing HO• damage. In the presence of wheat antioxidants DNA was converted mostly to nicked circular DNA and rarely to linear DNA and fragments.

Table 5 shows the dose-dependent effects of wheat antioxidants on inhibiting DNA strand breakage by Fenton reaction mediated ROS, whereas **Table 6** presents the amount of DNA retained by pearled grains and byproducts of wheat. Thus, byproducts of pearling were more efficient than pearled grains in retaining DNA against HO•-mediated oxidation. Moreover, the effects were significantly reduced with increased degree of pearling of the grains and resultant byproducts. The extracts derived from byproducts at 10% pearling of CWRS at a 6 mg/mL concentration rendered >90% protection against DNA scission (**Table 7**). The inhibitory activity of wheat antioxidants may be attributed to their scavenging of HO• or chelation of iron(II). The oxidizing agents can damage deoxyribose as well as DNA. When deoxyribose is attacked by HO•, it leads to the production of malonaldehyde, which is highly reactive and leads to cross-linking with DNA and proteins (*64*).

Phenolic Acid Composition of Pearled Wheat Grains and Byproducts. The HPLC analysis of 80% aqueous ethanolic extracts of wheat grains and byproducts thereof revealed that vanillic, *p*-coumaric, ferulic, and sinapic acids were the major phenolic acids present in both pearled grains and their byproducts (**Table 7**). The content of phenolic acids liberated from soluble esters was significantly higher than that of free phenolics in the wheat fractions. In the current study most of the pearled grains and byproducts possessed sinapic acid as the major phenolic compound. However, sinapic acid was not always present in the free phenolic acid fraction. The byproducts contained higher amounts of phenolic acids than the pearled grain products. In fact, the byproduct resulting from 10% pearling possessed the highest amount of phenolics in both cultivars.

Although HCA were more prevalent than HBA in the wheat extracts studied, ferulic acid was not the main phenolic present in total phenolics as shown in several studies (*53*, *58*). Meanwhile, vanillic acid was the only benzoic acid derivative present in wheat samples tested. The bran that was represented by the byproduct at 10% pearling possessed the highest content of phenolic acids in both cultivars. Andreasen et al. (*55*, *56*) have shown that rye bran is a rich source of phenolics and that the concentration of ferulic acid and its dehydrodimers was ≈10−20 times higher in the bran than in the endosperm.

Table 7. Phenolic Acid Content of Selected Pearled Grains and Byproducts of Two Wheat Cultivars, CWAD and CWRS[a]

	content (μg/g of crude extract)							
	vanillic		*p*-coumaric		ferulic		sinapic	
wheat fraction	free	ester	free	ester	free	ester	free	ester
				CWAD				
whole grain	1.24 ± 0.06	5.39 ± 0.19	0.12 ± 0.001	0.74 ± 0.06	0.43 ± 0.01	4.46 ± 0.12	0.19 ± 0.02	21.1 ± 1.61
10% pearled grain	1.01 ± 0.06	3.7 ± 0.11	0	1.06 ± 0.06	1.12 ± 0.06	4.42 ± 0.17	0	4.59 ± 0.34
30% pearled grain	0.65 ± 0.02	1.81 ± 0.04	0.17 ± 0.01	0.7 ± 0.04	1.18 ± 0.04	2.23 ± 0.08	0	2.52 ± 0.21
flour	0.34 ± 0.01	1.5 ± 0.05	0.1 ± 0.005	0.38 ± 0.02	0.75 ± 0.03	2.2 ± 0.1	0	1.25 ± 0.1
10% byproduct	9.75 ± 0.32	25.44 ± 0.85	0.95 ± 0.04	8.9 ± 0.42	4.98 ± 0.21	27.24 ± 0.95	0.74 ± 0.05	16.54 ± 1.27
30% byproduct	2.3 ± 0.09	10.12 ± 0.37	0.28 ± 0.01	1.66 ± 0.09	2.85 ± 0.09	11.68 ± 0.37	0	2.48 ± 0.18
				CWRS				
whole grain	0.16 ± 0.01	5.56 ± 0.16	0.11 ± 0.05	1.46 ± 0.05	0.54 ± 0.05	5.72 ± 0.22	0.05 ± 0.005	18.36 ± 1.4
10% pearled grain	1.25 ± 0.05	4.99 ± 0.16	0.21 ± 0.01	0.99 ± 0.05	1.77 ± 0.05	6.81 ± 0.21	0.1 ± 0.01	11.49 ± 0.88
30% pearled grain	0.77 ± 0.05	3.07 ± 0.1	0.14 ± 0.005	0.43 ± 0.02	1.68 ± 0.05	3.74 ± 0.14	0	8.54 ± 0.62
flour	0.28 ± 0.08	1.36 ± 0.04	0.08 ± 0.004	0.2 ± 0.01	0.44 ± 0.02	1.56 ± 0.04	0	3.2 ± 0.02
10% byproduct	5.29 ± 0.22	24.62 ± 0.86	0.54 ± 0.02	5.4 ± 0.22	2.05 ± 0.11	21.6 ± 0.76	0.43 ± 0.04	48.06 ± 3.56
30% byproduct	tr[b]	8.28 ± 0.28	tr	1.29 ± 0.09	tr	16.83 ± 0.55	0	6.9 ± 0.55

[a] Values are means of three determinations ± standard deviation. [b] Trace.

Conclusions. Ethanolic extracts of pearled wheat grains and their byproducts exhibited multiple antioxidant effects in in vitro assays. The 10% byproduct from the pearling of wheat demonstrated the highest antioxidant activity. Subsequent removal of external layers resulted in a decrease in phenolic content with concurrent lower antioxidant activity values for higher degrees of pearling. Both pearled grains and byproducts of wheat followed a similar trend. Among grain products, the whole unprocessed grain possessed the highest antioxidant capacity. There was a dilution of antioxidative constituents by endosperm, as pearling removed the external layers, including the bran and aleurone layers, the latter being the outermost layer of the endosperm. The antioxidant activity of wheat phenolics may be attributed to different possible antioxidant mechanisms. In the methods of ORAC, PCL, and Rancimat, wheat phenolics may act as free radical scavengers by donating a hydrogen atom. In assays that determine the ability to inhibit LDL and DNA oxidation by wheat phenolics, the antioxidative properties may be rendered by both hydrogen donation and/or metal chelation. Wheat phenolics appeared to serve as powerful antioxidants by radical scavenging and/or metal chelation in a consistent manner. However, further investigations in in vivo experiments and the absorption and metabolism of wheat bioactives are still necessary to further shed light on their efficacy in disease risk reduction.

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