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Antioxidant Properties of Pearled Barley Fractions

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Two barley varieties (Falcon and AC Metcalfe) were separated by pearling into seven fractions and subsequently extracted with 80% methanol. The extracts, after solvent removal, were evaluated for their radical scavenging efficacy using Trolox equivalent antioxidant capacity (TEAC). The radical scavenging capacity of the extracts was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, oxygen radical absorbance capacity ($ORAC_{FL}$), and superoxide radical assays and a photoinduced chemiluminescence technique. In both barley varieties the outermost fraction (F1) yielded the highest phenolic content. In general, Falcon had a significantly higher total phenolic content than AC Metcalfe. A similar trend was observed for TEAC, DPPH, and superoxide radical scavenging capacities of the extracts. The contents of water-soluble antioxidants of Falcon and AC Metcalfe were 1.15−12.98 and 2.20−12.25 μmol of Trolox equiv/(g of defatted material), while the corresponding lipid-soluble counterparts varied from 1.44 to 4.70 μmol of α-tocopherol equiv/(g of defatted material). Phenolic acids, namely, vanillic, caffeic, *p*-coumaric, ferulic, and sinapic acids, were identified by HPLC in barley fractions.

KEYWORDS: Antioxidants; barley; $ORAC_{FL}$; photochemiluminescence; HPLC

INTRODUCTION

The prevalence of diabetes, obesity, cardiovascular diseases, and cancer is on the increase, especially in the developed countries, including USA and Canada. Dietary habits and nutrition are believed to play a key role in the prevention or attenuation of these disease conditions (*1*). The data from both experimental and epidemiological studies have shown that grains, vegetables, and fruits contain a myriad of phytochemicals. Major phytochemicals include phenolic acids, phytosterols, saponins, phytoestrogens, flavonoids, and coumarin derivatives, among others (*2*). These phytochemicals are known to combat oxidative stress in the human body by helping to maintain a balance between oxidants and antioxidants (*3*). An imbalance caused by overproduction of oxidants leads to oxidative stress, resulting in damage to biomolecules such as lipids, DNA, RNA, and proteins (*3*). Recent research has shown that the complex mixture of phytochemicals in food provides better protective health benefits than single phytochemicals through a combination of additive and/or synergistic effects (*4*). Plants have different compositions of phytochemicals with varied structures and thus offer a wide range of protective functions. Therefore, a variety of phytochemicals from a mixture of sources such as fruits, vegetables, and whole-grain-based foods are recommended.

While some of the antioxidants in grain and grain-based products have been identified, their potential contribution to health has not been fully explored (*5*). Phenolic acids are the common antioxidants that are ubiquitous in grains and, to a larger extent, in vegetables and fruits (*5*). Phenolic acids exist primarily as benzoic and cinnamic acid derivatives in cereals (*6*). It is widely accepted that phenolic compounds contribute significantly to the overall antioxidant properties of cereals. Besides, a large number of potentially anticarcinogenic agents are founds in cereals and cereal-based products, including carotenoids, tocopherols, tocotrienols, and selenium and phenolics such as flavonoids and lignans (*7*).

Antioxidants are not evenly distributed in the cereal grains. Salomonsson et al. (*8*) indicated that *p*-coumaric acid was present in the lowest amount in the center of the barley kernel and rapidly increased toward the outer layers, such as lignified husk. Some researchers have reported that phenolic acids are concentrated in the cell walls of outer layers (*9*), while others have indicated that phenolic acids were mainly present in aleurone layer and endosperm (*10*). The content of ferulic acid was highest in the cell walls of the aleurone layer, rich in arabinoxylans (*11*). Maillard and Berset (*9*) have separated and identified *trans*-ferulic acid, *trans*-*p*-coumaric acid, and *cis*-ferulic acid from barley and malt. In plants, phenolic acids are esterified with other smaller molecules of aliphatic alcohols, phenols, and alkaloids (*12*).

The natural antioxidants in cereals may act as free radical scavengers, reducing agents, potential complexers of prooxidant metals, and singlet oxygen quenchers (*13*). Moreover, many of the natural antioxidants exhibit a wide range of biological effects, including antibacterial, antiviral, antiinflammatory, antiallergic, and antithrombotic effects, and may also be involved in vasodilatory actions (*14*). Moreover, cereals are one of the

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richest sources of dietary fiber, which help in maintaining the health of the digestive system, lowering plasma cholesterol, and reducing colon cancer.

A close scrutiny of the literature over the past decade reveals that most of the work on cereal antioxidants has been directed toward wheat, buckwheat, and oat. However, little work has been done on antioxidative components of barley. The objective of this study was to determine antioxidative efficacy of milling fractions of two barley varieties, namely, Falcon and AC Metcalfe.

MATERIALS AND METHODS

Reagents. Sodium carbonate, sodium chloride, methanol, and hexane were purchased from Fisher Scientific Co. (Nepean, ON, Canada). Compounds 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), tetrazolium, 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO), xanthine oxidase, hypoxanthine, ferulic acid, Folin−Ciocalteu's reagent, mono- and dibasic sodium and potassium phosphates, ethylenediaminetetraacetic acid (EDTA), Trolox, fluorescein, phenolic acid standards, and diethylenetriaminepentaacetic acid were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada).

Preparation of Barley Samples. Two barley cultivars, the hulless variety Falcon and hulled variety AC Metcalfe, were obtained from Field Crop Development Center, Lacombe, Alberta, Canada, during the 2002 crop year. The samples were tempered to 14% moisture over a 65 h period and dehulled prior to further processing. The dehulled barley samples were pearled with Satake type TM pearler, and the pearling byproducts (pearling fines) were collected at ∼5−10% weight intervals by successively abrading the kernels up to 50% of their original weight.

Analytical Methods. Preparation of Crude Phenolic Extracts. Ten grams of each of the defatted barley fractions (with hexanes) was separately extracted with 80% methanol under reflux conditions in a thermostated water bath at 60 °C for 30 min. The resulting slurries were centrifuged for 5 min at 4000*g* (ICE Centra M; International Equipment Co., Needham Heights, MA), and supernatants were collected. The residue was reextracted with 80% methanol for another 30 min, supernatants were combined with those from the first extraction, and the mixture was desolventized in vacuo at 40 °C. The resulting concentrated solutions were lyophilized for 72 h at −49 °C and 25 × 10^{-3} mbar (Freezone, Model 77530, Labconco Co., Kansas City, MO).

Determination of Total Phenolic Content. Extracts were dissolved in methanol to obtain a concentration of 3 mg of extract/(mL of solution). Because fraction 1 (F1) of both cultivars yielded absorbance values exceeding the appropriate range, they were further diluted appropriately with methanol and used for the assay. The total content of phenolics was determined according to the procedure explained by Singleton and Rossi (*15*). Folin-Ciocalteu's reagent (1 mL) was added to each centrifuge tube containing 1 mL of methanolic extract. Contents were mixed thoroughly, and 8.0 mL of sodium carbonate (75 g/L) was added to each tube. To the mixture, 10 mL of distilled water was added and mixed thoroughly. Tubes were then allowed to stand for 2 h at ambient temperature. Contents were centrifuged for 5 min at 4000*g* (ICE Centra M5; International Equipment), and absorbance of the supernatant was read at 765 nm. A blank sample for each extract was used for background subtraction. The content of total phenolics in each extract was determined using a standard curve prepared for ferulic acid. Total phenolics were expressed as milligrams of ferulic acid equivalents per gram of defatted material.

Total Antioxidant Capacity by Trolox Equivalent Antioxidant Capacity Assay. The Trolox equivalent antioxidant capacity (TEAC) assay is based on scavenging of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) radical anion ($ABTS^{\bullet-}$). A solution of $ABTS^{\bullet-}$ was prepared in 100 mM saline phosphate buffer (pH 7.4, 0.15 M sodium chloride) (PBS) by mixing 2.5 mM 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH) with 2.0 mM $ABTS^{2-}$. The solution was heated for 16 min at 60 °C, protected from light by covering in a tin foil, and stored at ambient temperature until used. The $ABTS^{\bullet-}$ solution was used within 2 h because the absorbance of the radical itself depletes with time. Barley extracts were dissolved in PBS at a concentration of 3 mg/mL and diluted accordingly to have them fit in the range of values in the standard curve (drop of 0−0.3 of optical density). For measuring antioxidant capacity, 40 μL of the sample was mixed with 1.96 mL of $ABTS^{\bullet-}$ solution. Absorbance of the above mixture was measured at 734 nm after 6 min because the extracts needed a minimum of 6 min in order to complete the reaction. The decrease in absorption at 734 nm after 6 min of addition of a test compound was used for calculating TEAC values. A standard curve was prepared by measuring the reduction in absorbance of $ABTS^{\bullet-}$ solution at different concentrations of Trolox. Appropriate blank measurements (decrease in absorption at 734 nm due to solvent without any compound added) were carried out and the values recorded (*16*). TEAC values were expressed as micromoles of Trolox equivalents per gram of defatted material.

Superoxide Radical Scavenging Assay. Superoxide radicals were generated via an enzymatic reaction. The reaction mixture contained 1 mL of 3 mM hypoxanthine, 1 mL of 100 milli-international units (mIU) of xanthine oxidase, 1 mL of 12 mM diethylenetriaminepentaacetic acid, 1 mL of 186 μM nitro blue tetrazolium, and 1 mL of the extracts (final concentration of the extracts in the assay medium was 0.1 mg/mL). Ferulic acid was used as the reference antioxidant. All solutions were prepared in a 100 mM phosphate buffer (pH 7.4) solution. The absorbance values of the mixtures were read at 560 nm up to 60 min. Readings at 10 min were used to calculate superoxide radical scavenging capacities. The following equation was used to calculate superoxide radical scavenging capacity (*17*).

$$\text{superoxide radical scavenging capacity, \%} = 100 - \{\text{absorbance of medium containing the additive of concern/absorbance of the control medium}\} \times 100$$

DPPH Radical Scavenging Assay. The effect of extracts on the DPPH radical was monitored according to the method of Hatano et al. (*18*). The extracts (100 μL at a concentration of 3 mg/mL) were added to a methanolic solution (1.9 mL) of DPPH radical (final concentration of DPPH radical was 5.7 μM). The mixture was shaken vigorously and left standing at room temperature for 20 min; the absorbance was measured spectrophotometrically at 517 nm. DPPH radical scavenging capacity was expressed as micromoles of ferulic acid equivalents per gram of defatted material based on the depletion of absorbance after 20 min of the assay.

Photoinduced Chemiluminescent Detection of Water and Lipid Soluble Antioxidants. The measurement of the photoinduced chemiluminescence (PCL) was performed using the Photochem apparatus (Analytik Jena, Shelton, CT) according to the method explained by Madhujith et al. (*19*). An aliquot of 20 μL of the sample was mixed with 1480 μL of diluent (water) and 1 mL of buffer (0.1 M, pH 10.5 containing 0.1 mM EDTA). The contents were mixed vigorously, and 25 μL of luminol (1 mM) was added to the mixture. Immediately after the addition of luminol, the contents were mixed briefly and introduced to the sample intake port of the Photochem apparatus. The luminescence generated by the reaction between the remaining radicals and the detection chemical was monitored and registered by the instrument for 80−160 s on water soluble (ACW) mode. Trolox (0.1 nM; 5−30 μL) was used as the standard. The lipid-soluble antioxidant content was measured as follows; an aliquot of 20 μL of the sample was mixed with 2.3 mL of diluent (methanol) and 200 μL of buffer (0.1 M, pH 10.5 containing 0.1 mM EDTA). The contents were mixed vigorously, and 25 μL of luminol (1 mM) was added to the mixture. Immediately after the addition of luminol, the contents were mixed briefly and introduced to the sample intake port of the Photochem apparatus. The luminescence generated by the reaction between the remaining radicals and the detection chemical was registered by the instrument exactly for 140 s on lipid soluble (ACL) mode. α-Tocopherol (0.275 nM; 5−30 μL) was used as a standard.

Determination of Oxygen Radical Absorbance Capacity. The determination of oxygen radical absorbance capacity ($ORAC_{FL}$) was carried out by using a Fluostar Optima plate reader (BMG Labtech, Durham, NC) equipped with an incubator and two injector pumps with

Table 1. Yield of 80% Methanolic Extracts Obtained from Two Barley Cultivars and Their Total Phenolic Contents[a]

cultivar/pearling fraction	% kernel weight range	% yield of pearling	% yield of extraction	total phenolics (mg of ferulic acid equiv/(g of defatted material))
Falcon				
F1	0–9.7	9.7	13.9 ± 0.6	6.26 ± 0.11a
F2	9.8–18.8	9.1	9.8 ± 0.7	2.35 ± 0.00b
F3	18.9–26.7	7.9	7.0 ± 0.7	1.18 ± 0.02c
F4	26.8–33.3	6.6	8.4 ± 1.2	0.58 ± 0.01d
F5	33.4–39.8	6.5	6.3 ± 1.3	0.99 ± 0.03e
F6	39.9–45.3	5.5	3.1 ± 0.5	0.36 ± 0.01f
F7	45.4–49.9	4.6	3.5 ± 0.8	0.51 ± 0.00d
AC Metcalfe				
F1	0–9.4	9.4	14.0 ± 1.2	4.16 ± 0.08a
F2	9.5–17.7	8.3	10.9 ± 0.9	2.42 ± 0.08b
F3	17.8–25.5	7.8	9.6 ± 0.8	1.38 ± 0.04c
F4	25.6–32.4	6.9	7.4 ± 0.6	1.26 ± 0.23c
F5	32.5–38.0	5.6	6.8 ± 0.5	1.24 ± 0.00c
F6	38.1–43.1	5.1	4.6 ± 0.6	0.48 ± 0.00d
F7	43.2–47.7	4.6	3.3 ± 0.9	0.17 ± 0.00e

[a] Results are means of three determinations ± standard deviation. Values in each row (within a single cultivar category) having the same letter are not significantly different ($p > 0.05$).

fluorecsein as the probe and AAPH as the radical generator. Fluorescein was dissolved in phosphate buffer (0.75 M, pH 7.0) to obtain a final concentration of 32 nM and injected into the wells containing 20 μL of extract or standards using the first pump in the first cycle. The mixture was incubated for 20 min at 37 °C in the built-in incubator, and subsequently 60 μL of APPH solution was injected into the wells using the second pump. The plate was shaken for 4 s after each addition at 4 mm shaking width. To optimize the signal amplification in order to obtain maximum sensitivity, a gain adjustment was performed at the beginning by manually pipetting 200 μL of fluorescein into a designated well. No more than 35 wells of the 96-well plate (Costar 2650 black plate) were used due to increased cycle time. Fluorescence was determined and recorded every minute for 60 min, and the antioxidant activities of the extracts were calculated as Trolox equivalents using a standard curve prepared with 1−10 μM (final concentration) Trolox. Fluorescein filters with an excitation wavelength of 485 nm and an emission wavelength of 520 nm were used (*20*).

HPLC Analysis of Phenolic Acids. Separation of phenolic acids (i.e., free and those liberated from soluble esters and soluble glycosides) was achieved from the extract according to Amarowicz and Weidner (*21*). An aqueous suspension of extract (500 mg in 20 mL) was adjusted to pH 2 (6 M HCl), and free phenolic acids were extracted 5 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 2 M NaOH and then 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 hydrolysates 5 times into 30 mL of diethyl ether using a separatory funnel. The samples obtained in this way were injected onto an HPLC column. Phenolic acids were analyzed using a Shimadzu HPLC system (Shimadzu Corp., Kyoto, Japan) consisting of a LC-10AD pump, SCTL 10A system controller, and SPD-M 10A photodiode array detector. Phenolic acids separation was done by a prepacked LiChrospher 100 RP-18 column (4 × 250 mm, 5 μm; Merck, Darmstad, Germany). The mobile phase water−acetonitrile−acetic acid (88:10:2; v/v/v) (*21*) was delivered at a rate of 1 mL/min. The detection was monitored at 320 and 260 nm. Phenolic acids were identified by DAD profiles and using authentic standards based on the retention time.

RESULTS AND DISCUSSION

Yield and Phenolic Contents of the Extracts. The successive abrasion of the grains resulted in seven fractions designated as fractions (F1−F7) with F1 and F7 representing the outermost and innermost fractions, respectively. **Table 1** lists the percentage weights of each fraction of the Falcon and AC Metcalfe cultivars. Nearly 50% of the grain was obtained as fractions, while the rest of the grain was not subjected to further pearling.

In this study, preliminary experiments carried out with whole barley seed extracts revealed that extraction with 80% methanol at 60 °C for 30 min yields a maximum antioxidant activity as reflected in TEAC values (10.12 μmol of Trolox equiv/(g of defatted material)). Collins and co-workers (*22*) used aqueous methanol to extract phenolics from oat groats and hulls, while Zielinski and Kozlowska (*23*) reported that 80% methanol was the most efficient solvent for extraction of phenolics from barley. The extraction yields obtained varied from 9.7 to 4.6% in Falcon and 29.7 to 10.75% in AC Metcalfe. When moving from F1 to F7, the recovery of antioxidative compounds declined gradually in both cultivars (**Table 1**). Outer fractions, especially F1 and F2, contain pericarp and aleurone layers that are rich in phenolic compounds, leading to a higher recovery.

The total phenolic content (TPC) of Falcon barley fractions, reported as ferulic acid equivalents (**Table 1**), ranged from 0.36 to 6.26 mg/(g of defatted material), while that of AC Metcalfe ranged from 0.17 to 4.16 mg/(g of defatted material). A general trend of decreasing TPC was observed when moving from the outermost layer to the center of the barley grain in both cultivars. The average TPC of Falcon was significantly higher than that of AC Metcalfe (data not shown). The outermost layer (F1), which is basically the bran, contained the highest TPC. F2 also showed a significantly higher TPC compared to the rest of the inner fractions.

Trolox Equivalent Antioxidant Capacity. The TEAC value of a compound represents the concentration of Trolox (a water-soluble vitamin E analogue without the side-chain moiety) that has the same antioxidant capacity as the compound or a mixture of compounds of interest (*16*). Thus, the TEAC value may be considered as a stoichiometric number related to TEAC, for Trolox of 1. In the original TEAC assay, $ABTS^{\bullet-}$ is generated through the peroxidase activity of metmyoglobin in the presence of hydrogen peroxide. The assay described in this paper uses pregenerated $ABTS^{\bullet-}$ by reacting $ABTS^{2-}$ with AAPH. The generation of the radical before the antioxidative compound is added prevents interference of compounds which affect the radical formation, hence improving the accuracy of the test (*16*). The $ABTS^{\bullet-}$ produced as a result of reaction between AAPH and $ABTS^{2-}$ reacts instantly with Trolox, and the reaction is completed within 1 min; however, the extracts may take up to 6 min to complete the reaction, indicating a biphasic pattern of reaction. Therefore a 6 min period was used to read the final absorbance values. The TEAC for Falcon and AC Metcalfe ranged from 0.45 to 59.71 and from 0.69 to 56.09 μmol of Trolox equiv/(g of defatted material), respectively, on the basis of scavenging during 6 min (**Table 2**). As expected, TEAC values gradually decreased from the outermost layer to inner fractions in both varieties. The high TEAC of F1 might be attributed to its highest phenolic content. Both cultivars exhibited a strong correlation between TPC and TEAC (Falcon, $r^2 = 0.97$, and AC Metcalfe, $r^2 = 0.91$). The TEAC value of fraction 1 of Falcon was approximately 131 times higher than that of fraction 7, while the corresponding value for AC Metcalfe was 81.

DPPH Radical Scavenging Activity. The reduction of DPPH radical in the presence of extracts and standards was monitored spectrophotometrically at 515 nm after 20 min of mixing. The DDPH radical scavenging capacity of Falcon fractions varied from 2.18 to 69.3 μmol of ferulic acid equiv/(g of defatted material), while the corresponding values ranged from 0.8 to 46.59 μmol of ferulic acid equiv/(g of defatted material) in AC

Table 2. Trolox Equivalent Antioxidant Capacity (TEAC) of Barley Fractions[a]

fraction	dilution factor used for TEAC	TEAC[b]	DPPH radical scavenging capacity[c]
Falcon			
F1	5	59.71 ± 3.1c	69.30 ± 0.1c
F2	3	17.72 ± 1.3d	36.53 ± 1.33d
F3	1	10.99 ± 0.3e	9.11 ± 0.05e
F4	1	3.83 ± 0.2f	6.72 ± 0.48f
F5	1	2.72 ± 0.2g	3.55 ± 0.49g
F6	1	1.19 ± 0.3h	1.57 ± 0.41g
F7	1	0.45 ± 0.0i	2.18 ± 0.11g
AC Metcalfe			
F1	5	56.09 ± 3.2c	46.59 ± 0.00c
F2	3	25.15 ± 0.0d	14.84 ± 0.19d
F3	1	11.99 ± 0.4e	9.68 ± 0.74e
F4	1	7.85 ± 0.3f	6.59 ± 0.35f
F5	1	6.31 ± 0.3g	5.10 ± 0.55g
F6	1	5.2 ± 0.2h	2.58 ± 0.43h
F7	1	0.69 ± 0.0i	0.80 ± 0.00i

[a] Results are means of three determinations ± standard deviation. Values in each row (within a single cultivar category) having the same letter are not significantly different ($p > 0.05$). [b] Expressed as micromoles of trolox equivalents per gram of defatted material. [c] Expressed as micromoles of ferulic acid equivalents per gram of defatted material.

Metcalfe (**Table 2**). The average DPPH radical scavenging capacity of Falcon was superior to that of the AC Metcalfe. F1 and F2 of Falcon cultivars were more efficient in scavenging DPPH radical (Falcon F1 is 32 times, Falcon F2 is 16 times, AC Metcalfe F1 is 59 times, and AC Metcalfe-F2 is 19 times the efficiency of the F7 fraction), while the inner fractions exhibited low scavenging efficacies. DPPH radical scavenging capacity displayed a strong correlation with TEAC in both cultivars (Falcon, $r^2 = 0.93$, and AC Metcalfe, $r^2 = 0.98$).

Superoxide Radical Scavenging Capacity. In this study, superoxide radical was enzymatically produced using a hypoxanthine/xanthine oxidase (X/XO) system. The generation of superoxide radical was characterized by the development of an ink-blue color in the assay medium due to the reduced nitro blue tetrazolium (*17*). The generation of the blue color was used to monitor the synthesis of superoxide radical in the assay medium. In the control, the color developed continuously and leveled off after 1 h. Superoxide radical scavenging capacity of Falcon ranged from 82 to 94%, while that of AC Metcalfe was from 73 to 87%. In both cultivars the F1 fraction exhibited the highest activity, which gradually decreased toward F7. In general, all the extracts were quite capable of scavenging enzymatically generated superoxide radical (**Figure 1**).

Photoinduced Chemiluminescent Detection of Antiradical Activity. Measurement of antioxidant activity of a substance can be carried out in a system containing a free radical generator and a detector which indicates changes of the measuring signal in the presence of an antioxidant (*24*). Photochem photochemiluminometer, a newly developed device, generates free radicals photochemically by UV radiation of a photosensitizer solution.

During UV radiation, the assay mixture is continuously pumped through the measuring cell of the chemiluminometer which registers the light being produced by the photochemically generated free radicals in reactions illustrated below

$$S + h\nu_1 \rightarrow R^{\bullet -}$$

$$R^{\bullet -} + AH \rightarrow \text{nonradical products}$$

$$R^{\bullet -} + D \rightarrow h\nu_2$$

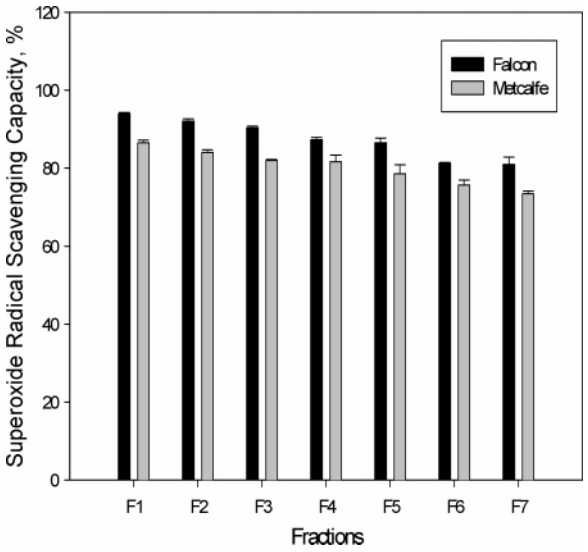

Figure 1. Superoxide radical scavenging capacity of Falcon and AC Metcalfe barley fractions.

where S = photosensitizer, R = free radical, AH = antioxidant, and D = free radical detecting compound (*24*).

UV radiation ($h\nu_1$) of the assay mixture containing the photosensitizer (luminol) generates superoxide radicals. These radicals are scavenged, to different extents, by the antioxidant compounds present in the extract/standard. After a certain period of time the remaining radicals are quantified by reacting with luminol, which generates UV light ($h\nu_2$). Depending upon the measuring mode (ACW, water soluble, or ACL, lipid soluble), the process is recorded as a decreased rise of the emission signal (*24*). Antioxidants in the sample were quantified by comparing their inhibitory effect on luminescence generation against that of a standard. The standards used for water-soluble and lipid-soluble antioxidants were Trolox and α-tocopherol, respectively, and the antioxidative capacities were expressed as equivalent units of the corresponding standards. **Figure 2** illustrates the on-line plot of change of luminescence for water-soluble antioxidative components from Falcon cultivar, while **Figure 3** illustrates the change of luminescence of lipid-soluble counterparts.

Antioxidant activity of water-soluble and lipid-soluble fractions from two barley varieties are depicted in **Table 3**. Water-soluble compounds were expressed as Trolox equivalents, while the lipid-soluble counterparts were expressed as α-tocopherol equivalents. The water-soluble antioxidant amounts of Falcon fractions ranged from 1.07 to 179 μmol of Trolox equiv/(g of defatted material), while those of AC Metcalfe ranged from 4.92 to 80.50 μmol/(g of defatted material) as Trolox equivalents. The corresponding lipid-soluble counterparts ranged from 1.3 to 93.5 and from 0.5 to 65.8 μmol of α-tocopherol equiv/(g of defatted material). The water-soluble antioxidant activity was significantly higher in F1 and F2 of both cultivars, while this pattern was not consistent in the rest of the fractions. The contents of water-soluble and lipid-soluble antioxidants were fairly correlated ($r^2 = 0.79$) with each other, while TEAC and water-soluble antioxidant activity were strongly correlated ($r^2 = 0.99$) in the Falcon cultivar. However, the lipid-soluble antioxidant content was not well correlated with TEAC in both cultivars.

Oxygen Radical Absorbance Capacity. Several methods available for measuring antioxidant activity in vitro have been developed and reviewed. However, the $ORAC_{FL}$ method is becoming a widely used method for assessing antioxidant capacity in biological and food samples. The $ORAC_{FL}$ value is

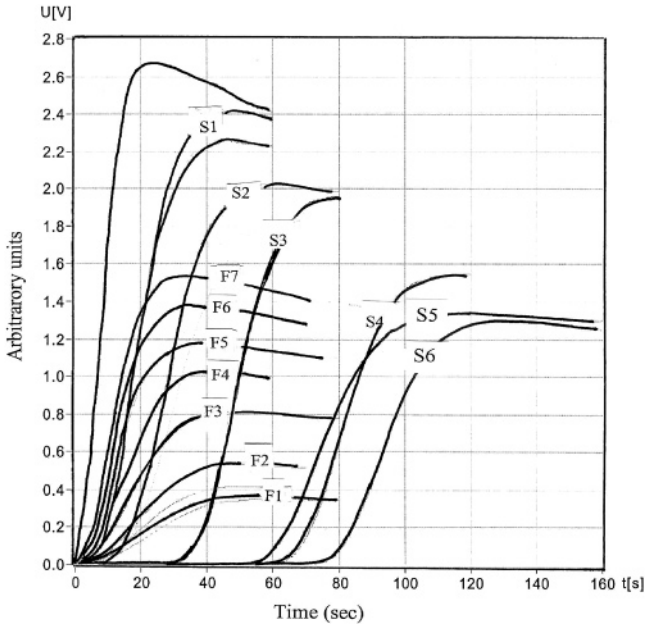

Figure 2. Antioxidant activity of water-soluble components of Falcon barley cultivars as measured by photochemiluminescence. S1–S5 are Trolox standards: S1, 0.3 nmol; S2, 0.6 nmol; S3, 0.9 nmol; S4, 1.2 nmol; S5, 1.6 nmol; S6, 2.0 nmol. F1–F5, 10 μg of Falcon fractions 1–5; F6–F7, 40 μg of Falcon fractions 6 and 7.

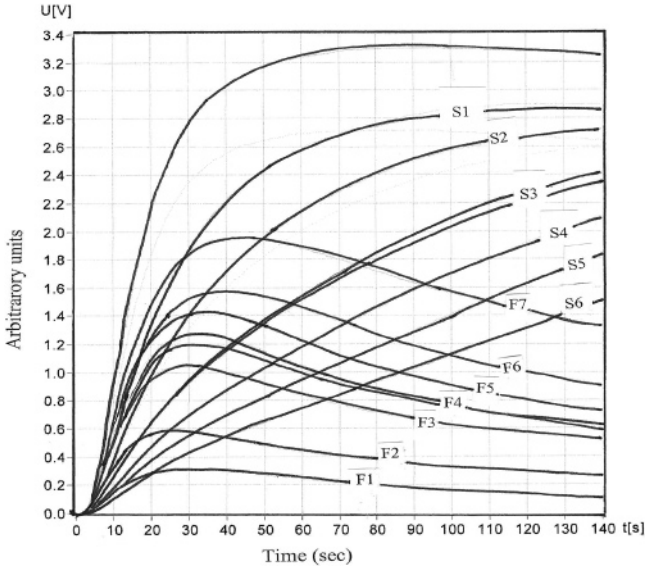

Figure 3. Antioxidant activity of lipid-soluble components of Falcon barley cultivars as measured by photochemiluminescence. S1–S6 are α-tocopherol standards: S1, 0.3 nmol; S2, 0.6 nmol; S3, 0.9 nmol; S4, 1.2 nmol; S5, 1.6 nmol; S6, 2.0 nmol. F1–F5, 10 μg of Falcon fractions 1–5; F6–F7, 40 μg of Falcon fractions 6 and 7.

based on the inhibition of the peroxyl radical-induced oxidation initiated by thermal decomposition of azo compounds such as APPH. $ORAC_{FL}$ is the only assay that combines both the inhibition time and the degree of inhibition into a single quantity (*25*). The $ORAC_{FL}$ values of Flacon and AC Metcalfe cultivars ranged from 8.8 to 188.1 and from 4.2 to 123.5 μmol of Trolox equiv/(g of defatted material), respectively (**Table 4**). Fractions F1 and F2 of the Falcon cultivar exhibited significantly ($p < 0.05$) higher antioxidant activity compared to that of AC Metcalfe. However, the rest of the fractions did not show any consistent pattern. The $ORAC_{FL}$ value of F7 of the Falcon cultivar was 21-fold lower than that of F1, while the corresponding value for AC Metcalfe was 29. The overall average $ORAC_{FL}$ value of Falcon stood significantly ($p < 0.05$) higher than that of AC Metcalfe (data not shown). $ORAC_{FL}$ values and antioxidant activity as measured by photochemiluminesence were strongly correlated with each other ($ORAC_{FL}$ and water-soluble antioxidant activity, $r^2 = 0.98$; $ORAC_{FL}$ and lipid-soluble counterparts, $r^2 = 0.95$ for the Falcon cultivar). Moreover, $ORAC_{FL}$ values were strongly correlated ($r^2 = 0.92$) with TEAC values in Falcon cultivars; however, a poor correlation was observed in AC Metcalfe ($r^2 = 0.57$). **Figure 4** illustrates the time course of the reaction of fluorescein with AAPH (decay curves) for Falcon fractions.

Table 3. Inhibition of Photoinduced Chemiluminescence by Water-Soluble and Lipid-Soluble Components of Antioxidants from Barley Fractions[a]

	water soluble[b]		lipid soluble[c]	
fraction	Falcon	AC Metcalfe	Falcon	AC Metcalfe
F1	179.12 ± 1.6c	80.50 ± 5.9c	93.5 ± 0.5c	65.8 ± 5.7c
F2	55.48 ± 0.3d	49.05 ± 1.5d	59.5 ± 1.2d	44.4 ± 6.6d
F3	29.82 ± 0.3e	39.7 ± 0.1e	34.0 ± 2.3e	31.6 ± 0.1e
F4	13.87 ± 0.0f	30.50 ± 0.2f	14.9 ± 0.8f	4.3 ± 0.1f
F5	1.42 ± 0.0g	26.48 ± 0.8f	22.2 ± 0.0g	2.7 ± 0.1f
F6	0.91 ± 0.0g	17.56 ± 0.1g	1.1 ± 0.0h	1.7 ± 0.1f
F7	1.07 ± 0.1g	4.92 ± 0.0h	1.3 ± 0.0h	0.5 ± 0.0f

[a] Results are means of three determinations ± standard deviation. Values in each row having the same letter are not significantly different ($p > 0.05$). [b] Expressed as micromoles of Trolox equivalents per gram of defatted material. [c] Expressed as micromoles of α-tocopherol equivalents per gram of defatted material.

Table 4. Oxygen Radical Absorbance Capacity ($ORAC_{FL}$) of Falcon and AC Metcalfe Fractions[a]

	$ORAC_{FL}$ value (μmol of Trolox equiv/(g of defatted material))	
fraction	Falcon	AC Metcalfe
F1	188.08 ± 8.7a	123.46 ± 5.9a
F2	89.17 ± 1.8b	72.02 ± 10.7b
F3	49.13 ± 2.1c	60.77 ± 8.9bc
F4	35.14 ± 5.4d	55.54 ± 0.9bc
F5	17.65 ± 0.0e	45.68 ± 5.6c
F6	14.18 ± 1.7e	23.31 ± 2.1d
F7	8.77 ± 0.9e	4.23 ± 0.2e

[a] Results are means of three determinations ± standard deviation. Values in each row (within a single cultivar category) having the same letter are not significantly different ($p > 0.05$).

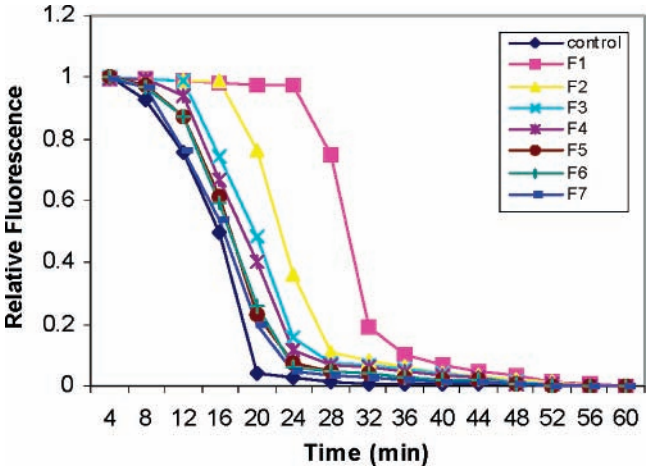

Figure 4. Fluorescent decay curves of fluorescein in the presence of Falcon barley fractions. F1– F7 are Falcon fractions (0.5 μg).

Table 5. Content of Free Phenolic Acids in Falcon and AC Metcalfe Barley Cultivars[a]

fraction	vanillic	caffeic	*p*-coumaric	ferulic	sinapic
Falcon					
F1	5.86 ± 0.01	3.86 ± 0.26	1.71 ± 0.36	3.57 ± 0.35	nd
F2	5.71 ± 0.12	0.71 ± 0.11	1.14 ± 0.02	4.86 ± 0.24	nd
F3	4.29 ± 0.01	nd	1.14 ± 0.11	4.71 ± 0.05	nd
F4	2.86 ± 0.02	nd	0.57 ± 0.01	2.71 ± 0.01	nd
F5	2.00 ± 0.04	nd	0.57 ± 0.00	2.00 ± 0.00	nd
F6	1.29 ± 0.02	nd	0.57 ± 0.00	1.71 ± 0.01	nd
F7	0.86 ± 0.00	nd	0.23 ± 0.02	1.14 ± 0.01	nd
AC Metcalfe					
F1	3.43 ± 0.01	1.14 ± 0.01	1.14 ± 0.01	2.29 ± 0.25	nd
F2	3.43 ± 0.10	0.71 ± 0.02	0.86 ± 0.01	2.14 ± 0.22	nd
F3	2.14 ± 0.05	0.29 ± 0.17	0.86 ± 0.00	1.71 ± 0.26	nd
F4	1.86 ± 0.00	0.29 ± 0.11	0.71 ± 0.01	1.57 ± 0.01	nd
F5	1.43 ± 0.11	nd	0.43 ± 0.04	1.43 ± 0.07	nd
F6	1.14 ± 0.00	nd	0.42 ± 0.01	1.14 ± 0.01	nd
F7	0.23 ± 0.00	nd	nd	0.86 ± 0.00	nd

[a] Free phenolic acids content is expressed in micrograms per gram of defatted material basis; nd, not detected.

Table 6. Content of Esterified Phenolic Acids in Falcon and AC Metcalfe Barley Cultivars[a]

fraction	vanillic	caffeic	*p*-coumaric	ferulic	sinapic
Falcon					
F1	21.44 ± 0.01	17.9 ± 0.26	8.27 ± 0.36	62.69 ± 0.35	36.59 ± 1.25
F2	8.46 ± 0.12	11.5 ± 0.11	4.35 ± 0.02	34.14 ± 0.24	30.81 ± 1.11
F3	6.00 ± 0.01	7.7 ± 0.05	1.45 ± 0.11	16.5 ± 0.05	10.57 ± 0.68
F4	8.00 ± 0.02	2.11 ± 0.02	4.65 ± 0.01	5.21 ± 0.01	3.57 ± 0.09
F5	6.00 ± 0.04	2.58 ± 0.04	0.88 ± 0.00	8.51± 0.00	4.78 ± 0.56
F6	5.00 ± 0.02	1.13 ± 0.00	0.08 ± 0.00	2.64 ± 0.01	1.94 ± 0.09
F7	4.14 ± 0.00	1.12 ± 0.00	0.23 ± 0.02	2.42 ± 0.01	1.79 ± 0.69
AC Metcalfe					
F1	24.01 ± 0.01	21.82 ± 0.01	6.98 ± 0.01	89.83 ± 0.25	70.00 ± 3.21
F2	12.27 ± 0.10	14.66 ± 0.02	4.48 ± 0.01	43.97 ± 0.22	29.21 ± 1.22
F3	7.17 ± 0.05	8.05 ± 0.17	2.12 ± 0.00	35.02 ± 0.26	16.49 ± 0.87
F4	5.04 ± 0.00	4.09 ± 0.11	1.74 ± 0.01	25.67 ± 0.01	10.31 ± 0.18
F5	2.85 ± 0.11	2.18 ± 0.00	1.2 ± 0.04	15.10 ± 0.07	4.55 ± 0.22
F6	1.31 ± 0.00	1.25 ± 0.03	0.52 ± 0.01	6.75 ± 0.01	1.94 ± 0.09
F7	0.43 ± 0.00	0.29 ± 0.00	0.20 ± 0.02	0.14 ± 0.00	0.48 ± 0.02

[a] Esterified phenolic acid content is expressed in micrograms per gram of defatted material basis.

HPLC Analysis of Phenolic Acids. Table 5 lists the contents of free phenolic acids of the Falcon and AC Metcalfe barley cultivar fractions. Vanillic, caffeic, *p*-coumaric, and ferulic acids were the major phenolic acids detected in the barley fractions. Ferulic acid and vanillic acid were the major free phenolic acids detected in both cultivars. The content of free phenolic acids was significantly ($p < 0.05$) higher in Falcon compared to AC Metcalfe, and this correlates with the higher antioxidant activities observed in TEAC, $ORAC_{FL}$, DPPH, and PCL tests. Fractions 1−3 contained higher amounts of free phenolic acids, while the rest of the fractions contained substantially low amounts. **Table 6** lists the content of esterfied phenolic acids that were liberated during the alkaline hydrolysis process. Sinapic acid which was not identified in the free phenolic acid fraction was identified in the soluble esters after alkaline hydrolysis. Phenolic acids that are present esterified with alcohols; phenols and alkaloids are released into soluble fraction upon hydrolysis of the material. Generally, alkaline hydrolysis is the method mostly used for extracting esterified or bound phenolics at room temperature. In F1 of the Falcon cultivar, there was an increase of phenolic acids upon hydrolysis, 4.8-fold for vanillic acid, 5.8-fold for caffeic acid, 6.1-fold for *p*-coumaric acid, and 19-fold for ferulic acid, while the corresponding values for F1 of AC Metcalfe were 8.0-, 20.4-, 4.8-, and 42-fold, respectively. There is an increased awareness and interest in the antioxidant behavior and potential health benefits associated with phenolic acids. Humans consume an estimated range of 25 mg to 1 g of phenolic acids on a daily basis from fruits, vegetables, grains, tea, coffee, and spices, among others (*26*). Besides the protective behavior of phenolic acids, they possess other biological activities. Caffeic acid that was detected in substantial amounts in barley fractions has been reported to possess antitumor activity against colon cancer (*27*), selectively blocking biosynthesis of leukotrienes that are directly involved in allergic reactions, asthma, and other immunoregulation disease (*28*).

In summary, the antioxidant and antiradical activities are mainly concentrated in the outer fractions, F1−F3 (approximately up to 25% of the grain on weight basis). In general, Falcon cultivar exhibited a higher antioxidant activity compared to AC Metcalfe in TEAC and DPPH, superoxide radical assays. PCL and $ORAC_{FL}$ exhibited a similar trend. The barley fractions contained phenolic acids, most of which are present in the esterified form. Barley, a cereal that is currently not substantially utilized for human consumption, carries antioxidative constituents. It is important not to remove the outer layers from the grain during processing steps as this may lead to substantial loss of bioactives.

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