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Antioxidative and Antiproliferative Properties of Selected Barley (*Hordeum vulgarae* L.) Cultivars and Their Potential for Inhibition of Low-Density Lipoprotein (LDL) Cholesterol Oxidation

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Aqueous methanolic extracts of whole kernels from six different barley cultivars, namely, Falcon, AC Metcalfe, Tyto, Tercel, Phoenix, and Peregrine, were examined for their total phenolic content (TPC), oxygen radical scavenging capacity ($ORAC_{FL}$), hydroxyl radical scavenging capacity ($HORAC_{FL}$), potency in prevention of lipid oxidation using the Rancimat method, efficacy in inhibition of Cu(II)-induced human LDL cholesterol oxidation, and antiproliferative activities using Caco-2 colorectal adenocarcinoma cell line. Total phenolic content as measured by Folin−Ciocalteu's method ranged from 0.68 to 1.19 mg of ferulic acid equiv/g of defatted material, whereas $ORAC_{FL}$ and $HORAC_{FL}$ values were 11.28−19.10 and 9.06−12.99 μmol of Trolox equiv/g of defatted material, respectively. Protection factor (PF), a measure of the effect of extracts on the prevention of oxidation of stripped corn oil as measured by Rancimat, ranged from 0.97 to 1.59. Furthermore, barley extracts showed 19.64−33.93% inhibition against Cu(II)-induced human LDL cholesterol oxidation at a final concentration of 0.02 mg/mL. The proliferation of Caco-2 colon cancer cells was significantly ($p < 0.05$) inhibited in a dose-dependent fashion in the presence of all barley extracts tested at the end of the day 4 of incubation. At the end of day 4, barley extracts rendered 29.3−51.2 and 9.3−15.9% inhibition of cell proliferation at 0.5 and 0.05 mg/mL, respectively. Phenolic extracts from whole barley kernel tested possessed high antioxidant, antiradical, and antiproliferative potentials. Therefore, inclusion of whole barley into the daily diet may render beneficial health benefits.

KEYWORDS: Antioxidants; antiproliferation; barley; Caco-2 colon cancer cell line; HORAC; LDL oxidation; ORAC; Rancimat

INTRODUCTION

Grains provide approximately two-thirds of the caloric requirement of the world, and even more in the developing countries (*1*). In addition to being the primary source of carbohydrates, cereals such as wheat, corn, and barley also provide vitamins, trace minerals, dietary fiber, and bioactive compounds. Barley is consumed around the world for bakery, brewing, and malted products. Although little barley is used for direct human consumption in Western countries, it remains a dietary staple in some Latin American and African countries. Canada produced 12.48 million metric tons (MMT) of barley in 2005 (*2*), of which a major portion was used for animal feed. In recent years barley has gained popularity due to the functional properties of its bioactives, such as β-glucan, arabinoxylan, oligosaccharides, tocols, and phenolic compounds.

With the interest in the intake of dietary antioxidants for the prevention of certain pathological conditions, cereals have also been investigated for their defense potential against reactive oxygen species (ROS) (*3*). The positive physiological roles of cereal grains have mainly been attributed to dietary fiber; however, recent research findings reveal that the contribution of nonfiber components present in cereal grains such as phenolic compounds toward mitigation of disease incidence is significant.

Independent studies indicate that a high consumption of grain products may reduce the risk of chronic diseases such as cardiovascular diseases and certain types of cancer (*4*, *5*). Dietary antioxidants are believed to play a significant role in human health by prevention of radical damage to biomolecules such as DNA, RNA, proteins, and cellular organelles. Therefore, there is increasing interest in identifying and assessing commonly consumed foods that contain bioactives with the potential to inhibit radical damage.

Cereals are reported to protect against chronic diseases by altering serum cholesterol profiles, by exerting antioxidant and antithrombic actions, and through their favorable effects on vascular reactivity as well as insulin sensitivity (*6*). The role of oxidation of LDL cholesterol in the pathogenesis of atheroscle-

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rosis is attracting increased attention. Oxidized LDL is processed by a scavenger receptor of macrophages, leading to cholesterol ester accumulation. These lipid-laden macrophages become foam cells, which in time create fatty streaks leading to atherogenesis. Many studies have attempted to determine the potential of antioxidative compounds in the prevention of LDL cholesterol oxidation. The effect of antioxidative extracts in inhibiting the oxidation of LDL cholesterol has been studied by quantifying conjugated dienes (CD), lipid peroxide formation, and the increase in negative charge of LDL cholesterol (*7*).

The imbalance between oxidants and antioxidative agents in cells may lead to tissue injuries and cancer, among many other degenerative diseases. Of the different types of cancer, colon cancer is the second most common fatal malignancy in the Western world (*8*). Therefore, it is important to investigate the potential of common food sources in mitigating colon cancer. Colon carcinoma cells (Caco-2 cell line) that are used to examine intestinal functions have also been widely adopted to mimic the small intestine in studying the effects of antioxidative extracts, toxicants, and screening drugs for intestinal absorption, among others (*9, 10*).

We have previously reported the optimization of the extraction of phenolic compounds from barley seed samples using different organic solvents (*11*). The potential of extracts so prepared in scavenging of free radicals and prevention of DNA damage was also examined. The objectives of this study were to assess the antioxidant potential and antiproliferative properties of extracts of selected barley cultivars grown in Canada and to examine the efficacy of barley extracts in inhibiting LDL cholesterol oxidation. Furthermore, the antiproliferative effects of barley kernel extracts against Caco-2 cells were tested.

MATERIALS AND METHODS

Materials. Grains of six barley cultivars from the 2002 crop year, namely, Falcon, AC Metcalfe, Tyto, Tercel, Phoenix, and Peregrine, were obtained from the Field Crop Development Center, Lacombe, AB, Canada. All cultivars examined were grown under the same climatic and agronomical conditions, in well-drained fertile loam soil under relatively cool temperatures and under full sun light conditions. Samples were prepared as described in a later section.

Sodium chloride, hexane (ACS grade), trichloroacetic acid, dimethyl sulfoxide (DMSO), and methanol were purchased from Fisher Scientific Co. (Nepean, ON, Canada). Compounds 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), ferulic acid, mono- and dibasic sodium and potassium phosphates, stripped corn oil, Trolox, human low-density lipoprotein (hLDL), copper chloride, ferric chloride, potassium ferricyanide luminol, and fluorescein were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada). An ATPlite one-step luminescence kit was purchased from Perkin-Elmer, Boston, MA. Caco-2 human colorectal adenocarcinoma cells were purchased from American Type Culture Collection (Rockville, MD). Cell culture media [McCoy's 5A medium modified with L-glutamine, antibiotic/antimycotic, and fetal bovine serum (FBS)] and 0.25% trypsin with 0.9 mM EDTA were purchased from Invitrogen (Carlsbad, CA). Disposable culture ware for cell culture assay was purchased from Corning glass works (Corning, NY).

Preparation of Crude Phenolic Extracts. Barley grains were manually hulled and ground to obtain a fine powder using a laboratory mill with a 60 mesh sieve (Tecator 3420, Tecator Inc., Boulder, CO). The so-obtained barley meals were defatted by blending with hexanes (1:5, w/v; 5 min) in a Waring blender (model 33BL73, Waring Products Division Dynamics Co. of America, New Hartford, CT) at ambient temperature. Defatted samples were vacuum packaged in polythene pouches and stored in a freezer at −20 °C until used for analysis.

Each of the defatted (with hexane) barley fractions (10 g) was separately extracted with 80% methanol under reflux conditions in a thermostated water bath at 60 °C for 40 min (*11*). 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 re-extracted with 80% methanol for another 40 min, and supernatants were combined; subsequently, 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) and stored at −20 °C until used.

Determination of Total Phenolic Content (TPC). Extracts were dissolved in methanol to obtain a concentration of 3 mg/mL. The TPC was determined according to an improved version of the procedure explained by Singleton and Rossi (*12*). Folin−Ciocalteu's reagent (1 mL) was added to centrifuge tubes containing 1 mL of methanolic extracts. Contents were mixed thoroughly, and 8 mL of sodium carbonate (75 g/L) was added to each tube. To the mixture was added 10 mL of distilled water with thorough mixing. Tubes were then allowed to stand for 2 h at ambient temperature in the dark, and subsequently the contents were centrifuged for 5 min at 4000*g*. The absorbance of the supernatant was read at 765 nm. A blank sample prepared with 1 mL of methanol in place of the sample solution was used for background subtraction. Total extracted phenolics were expressed as milligrams of ferulic acid equivalents per gram of defatted material using a standard curve prepared with 1−10 μM (final concentrations) ferulic acid.

Determination of Oxygen Radical Absorbance Capacity ($ORAC_{FL}$). The determination of $ORAC_{FL}$ was carried out using a Fluostar Optima plate reader (BMG Labtech, Durham, NC) equipped with an incubator and two injector pumps with fluorecsein as the probe and AAPH as the radical generator. The reaction was carried out in 75 mM phosphate buffer (pH 7.4) medium at a final reaction mixture of 200 μL in a 96-well Costar 2650 black plate (Nepean, ON, Canada). Fluorescein dissolved in phosphate buffer (120 μL; 64 nM, final concentration) was injected using the first injector pump into the wells containing the extract (20 μL; 20 μg/μL extract). The mixture was incubated for 20 min at 37 °C in the built-in incubator, and subsequently APPH solution (60 μL; 29 mM final concentration) equilibrated at 37 °C was rapidly injected into the wells using the second pump. The plate was shaken for 4 s after each addition at a 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 plates were used due to increased cycle time. Fluorescence was determined and recorded every minute for 60 min using a Fluostar Optima fluorometer, and the antioxidant activity of the extracts was calculated as Trolox equivalents using a standard curve prepared with 1−10 μM (final concentration) control (Trolox, buffer, fluorescein, and AAPH) and positive control (phosphate buffer and fluorescein) were used. Filters with an excitation wavelength of 485 nm and an emission wavelength of 520 nm were used (*13*).

Hydroxyl Radical Scavenging Capacity ($HORAC_{FL}$). The determination of hydroxyl radical prevention capacity was performed using fluorescein as the probe by employing the method explained by Ou and co-workers (*14*). Fluorescein (180 μL; 96 nM, final concentration) dissolved in phosphate buffer solution (0.75 mM, pH 7.0) was pipetted into the wells containing 10 μL of extract (2 mg/mL) or Trolox standard (1−10 μM final concentration). A solution of hydrogen peroxide (10 μL; 1 M) dissolved in deionized water was injected into the mixture using the first injector pump, and the mixture was incubated for 1 min before the initial reading was taken. The second injector pump was set to inject a mixture of 10 μL of cobalt fluoride (8 mM) and picolinic acid (8 mM) dissolved in deionized water into the wells in order to initiate the reaction. Fluorescence was determined and recorded every minute for 40 min using a Fluostar Optima plate reader, and the area under the decay curves (AUC) was calculated. The antioxidant activity of the extracts was expressed as ferulic acid equivalents. Control (buffer, fluorescein, H_2O_2, and CoF_2) and positive control (buffer and fluorescein) were used to compensate for the fluorogenic effect of reagents.

Measurement of Cupric Ion-Induced Human LDL Peroxidation. The method explained by Andreasen et al. (*15*) and Hu and Kitts (*16*) was used to measure LDL oxidation. Human LDL in saline phosphate buffer solution containing 0.01% ethylenediaminetetracetic acid (EDTA)

was dialyzed against 10 mM saline PBS (pH 7.4, 0.15 M NaCl) for 12 h under nitrogen at 4 °C, and EDTA-free LDL was subsequently diluted to obtain a standard protein concentration of 0.2 mg/mL with PBS. The diluted LDL solution (200 μL) was mixed with 1000 μL of PBS and 10 μL of extract (2 mg/mL) in a test tube. Oxidation of LDL was initiated by adding 5.1 mM cupric sulfate solution, resulting in a 4 μM copper concentration in the reaction mixture. The mixture was incubated at 37 °C for 100 min, and conjugated dienes (CD) of the mixture were measured (at 232 nm) at 5 min intervals over 100 min. Inhibition percentage was calculated on the basis of the CD values at 100 min of incubation.

Accelerated Oxidative Stability Test Using Rancimat. The effectiveness of barley extracts on delaying of oxidation of stripped corn oil was measured under oxidation conditions using a Rancimat apparatus (743 Rancimat, Metrohm Ion Analysis Ltd., Herisau, Switzerland). Whole barley extracts (60 mg) were added into reaction vessels of the Rancimat apparatus containing 3 g of stripped corn oil followed by sonication for 10 min. Barley extracts did not dissolve completely in the oil; however, sonication and continuous bubbling of air helped dissolution of phenolics and other antioxidative compounds in the oil. A constant dry air stream (20 L/h) obtained by passing laboratory air through a molecular sieve (0.3 nm) was blown through the samples in the reaction vessels, which were maintained at 120 °C throughout the experiment. The volatile oxidation products were collected in the measuring vessels containing 60 mL of deionized water. The conductivity of the aqueous solution was continually monitored and recorded. The inflection point (IP) was calculated by the software (743 Rancimat PC software version 1.0, 2000, Metrohm Ion Analysis Ltd.) and recorded. A blank containing pure stripped corn oil devoid of extracts was used. The protection factor (PF) of the whole barley extracts was calculated as

$$PF = IP_{extract}/IP_{control}$$

where $IP_{extract}$ is the inflection point of the corn oil sample containing extract and $IP_{control}$ is the inflection point of corn oil sample devoid of any extracts.

Measurement of the Effect of Extracts on Caco-2 Colon Cancer Cell Proliferation. Caco-2 colon cancer cell line assay was performed according to the method explained by Parry et al. (*17*) and Crouch et al. (*18*). Caco-2 human colorectal adenocarcinoma cell line was propagated in T-150T-150 flasks in McCoy's 5A medium containing 10% fetal bovine serum albumin (FBA) and 1% antibiotic/antimycotic. Flasks were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 (*19*, *20*). The media containing the cells were transferred into a 96-well microplate at a rate of 2500 cells per well and incubated overnight at 37 °C. Whole barley extracts (10 μL) dissolved in 50% DMSO were introduced separately into the wells containing 990 μL of cell medium to obtain final concentrations of 5 and 50 mg/mL. The control contained 990 μL of cell medium and 10 μL of 50% DMSO. DMSO is widely used as a solvent for dissolving phenolic extracts. The mixtures were incubated at 37 °C, and the live cells on each of the wells were counted daily for 4 days using an ATPlite one-step luminescence kit (Perkin-Elmer).

Statistical Analysis. Statistical analysis was performed using SigmaStat version 10.0 (Jandel Corp., San Raphael, CA). Results were subjected to ANOVA, and differences between means were located using Tukey's test. Correlations between various parameters were also investigated. Significance was determined at the $p < 0.05$ level. All data were reported as the mean ± SD of three determinations using the same extract.

RESULTS AND DISCUSSION

TPC. The TPC of whole barley extracts ranged from 13.6 to 22.9 mg of ferulic acid equiv/g on a the basis of lyophilisate weight. The corresponding values on the basis of defatted weight ranged from 0.68 to 1.19 mg of ferulic acid equiv/g. The order of the total phenolic content of the six barley cultivars was Peregrine > AC Metcalfe > Falcon > Tyto > Tercel > Phoenix (**Figure 1**). Zielinski and Kozlowska (*21*) reported that barley

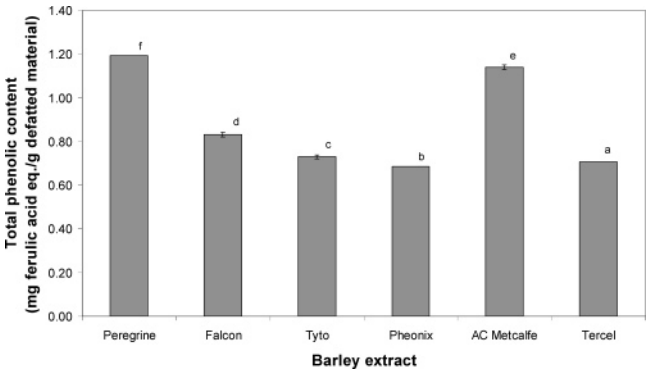

Figure 1. Total phenolic content (mean ±SD) of whole barley extracts.

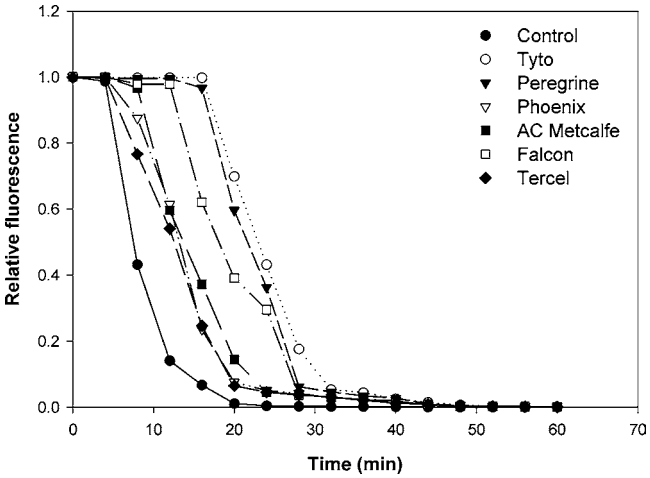

Figure 2. Fluorescence decay curves of fluorescein in the presence of whole barley kernel extracts at a concentration of 2 μg/μL in the oxygen radical absorbance capacity ($ORAC_{FL}$) assay.

cultivars Mobek and Gregor contained 26.9 and 24.3 mg of catechin equiv of total phenolics/g of lyophilisate extracted using 80% methanol. Barley cultivars tested in this study contained more total phenolics than wheat, rye, and oat as reported by Zielinski and Kozlowska (*21*).

$ORAC_{FL}$. The $ORAC_{FL}$ assay is among the standard assays accepted for measuring the antioxidative activity of botanicals, herbs, and nutraceuticals. It has been widely employed for the assessment of the free radical scavenging capacity of human plasma, proteins, DNA, pure antioxidants, and plant/food extracts (*22*). $ORAC_{FL}$ is the only assay that combines both inhibition time and degree of inhibition into a single quantity (*23*). The protective effect of an antioxidant is calculated from the net integrated area under the fluorescence decay curve (AUC) as illustrated in **Figure 2** and reported as Trolox equivalents. The oxygen radical absorbance capacity of whole barley extracts ranged from 11.28 to 19.10 μmol of Trolox equiv/g of defatted material. The order of $ORAC_{FL}$ for whole barley extracts was as follows: Tyto > Peregrine > Falcon > AC Metcalfe > Phoenix > Tercel (**Table 1**). $ORAC_{FL}$ and TPC of whole barley extracts did not correlate well ($r^2 = 0.224$).

Wu et al. (*24*) ranked foods into different categories based on the $ORAC_{FL}$/TPC ratio. This ratio provides an estimate of the effectiveness of phenolics in scavenging oxygen radicals. This ratio ranged from 1.7 in green pepper to 156.4 in garlic powder, whereas most foods fell in the range from 5 to 15. The $ORAC_{FL}$/TPC ratio for whole barley extracts ranged from 12.08 to 26.16. The order of $ORAC_{FL}$/TPC ratio was as follows: Tyto > Falcon > Phoenix > Tercel > Peregrine > AC Metcalfe (**Table 1**).

Table 1. $ORAC_{FL}$ Value and $ORAC_{FL}$/TPC Ratio of Whole Barley Extracts[a]

sample identity	$ORAC_{FL}$ value (μmol of Trolox equiv/g of defatted material)	$ORAC_{FL}$/TPC
Falcon	16.97 ± 1.8 bc	20.45 ± 2.10 b
AC Metcalfe	13.77 ± 1.1 cd	12.08 ± 0.97 d
Tyto	19.10 ± 1.0 a	26.16 ± 1.32 a
Tercel	11.28 ± 0.8 d	15.89 ± 0.98 c
Phoenix	12.15 ± 0.4 d	17.87 ± 0.52 c
Peregrine	18.43 ± 1.5 ab	15.49 ± 1.34 c

[a] Results are means of three determinations ± standard deviation. Values in each column carrying the same letter are not significantly different ($p > 0.05$).

Table 2. $HRAC_{FL}$ and Percentage Inhibition of Cu(II)-Induced Human LDL Cholesterol Oxidation by Whole Barley Extracts[a]

sample identity	$HORAC_{FL}$ value (μmol of Trolox equiv/g of defatted material)	$HORAC_{FL}$/TPC	inhibition of LDL oxidation[b] (%)
Falcon	10.39 ± 0.24 bc	12.52 ± 0.31 b	19.64 ± 2.70 d
AC Metcalfe	10.47 ± 0.53 b	9.19 ± 0.50 c	26.79 ± 3.20 bcd
Tyto	12.99 ± 0.83 a	13.68 ± 0.95 b	22.40 ± 1.20 cd
Tercel	9.06 ± 0.49 c	14.17 ± 0.62 ab	21.43 ± 0.98 cd
Phoenix	10.67 ± 0.43 b	15.70 ± 0.78 a	28.57 ± 4.20 bc
Peregrine	12.62 ± 0.01 a	8.93 ± 0.01 c	33.93 ± 2.80 b
ferulic acid			53.57 ± 3.70 a

[a] Results are means of three determinations ± standard deviation. Values in each column carrying the same letter are not significantly different ($p > 0.05$). [b] Inhibition percentage is calculated on the basis of the conjugated diene values at 100 min of incubation.

$HORAC_{FL}$. The $HORAC_{FL}$ of whole barley extracts ranged from 9.06 to 12.99 μmol of Trolox equiv/g of defatted material. The order of $HORAC_{FL}$ of the whole barley extracts was Peregrine ≈ Tyto > AC Metcalfe ≈ Phoenix ≈ Falcon > Tercel (**Table 2**). The order of $HORAC_{FL}$ values was more or less similar to that of $ORAC_{FL}$.

The hydroxyl radical is a biologically important species that can cause severe damage to biomolecules. Quantitative measurement of hydroxyl radicals has been a challenging task due to the lack of a controllable hydroxyl radical source (*25*). In the presence of low concentrations of Fe(II), H_2O_2 is converted to hydroxyl radical through the Fenton reaction. Many other transition metal ions such as Cu(II), Ti(II), Cr(II), and Co(II) also react with H_2O_2 in a similar manner as Fe(II), generating hydroxyl radical. Electron transfer between terminal metal ions and H_2O_2 does not involve an outer sphere electron-transfer mechanism. It is reported that reactions proceed through an inner sphere electron transfer, in which H_2O_2 forms a complex with transition metal ions (*26*).

Ou et al. (*14*) evaluated a number of authentic phenolic compounds for $HORAC_{FL}$ and $ORAC_{FL}$ and revealed that $HORAC_{FL}$ is consistently lower than $ORAC_{FL}$ among the phenolic compounds tested. This pattern was true for most foods tested by Ou and co-workers (*14*). A similar trend was observed for whole barley extracts as well. Ou et al. (*14*) observed that phenolic compounds with metal chelation potential show higher $HORAC_{FL}$ values, whereas the compounds with poor metal chelation activity show negligible $HORAC_{FL}$ values. Phenolics act as metal chelators by coordination to Co(II), thereby blocking the reaction sites for H_2O_2. This coordination leads to reduced concentration of Co(II) and effectively reduces the generation of hydroxyl radicals. $HORAC_{FL}$ values do not correlate with either the number of hydroxyl groups or the number of chelating sites. $HORAC_{FL}$ is mainly decided by the stability of the Co(II)−phenol complex formed (*25*). The $HORAC_{FL}$ value mainly reflects the metal chelation potential, whereas $ORAC_{FL}$ primarily indicates the peroxyl radical absorption capacity. Therefore, theoretically no correlation exists between these two parameters. Genestein, quercetin, and kaempferol with very high $ORAC_{FL}$ values have shown only modest $HORAC_{FL}$ values, whereas epigallactocatechin gallate (EGCG) and rutin have shown high values for both (*25*).

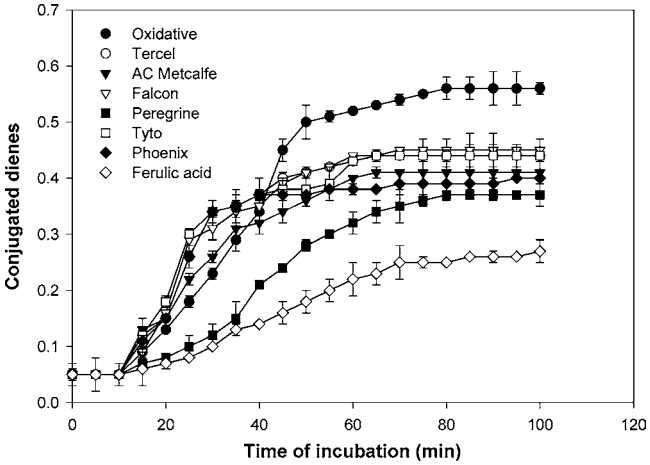

Figure 3. Development of conjugated dienes (CD) in low-density lipoprotein (LDL) cholesterol in the presence of whole barley kernel extracts at a concentration of 2 mg/mL over 100 min of incubation.

Inhibition of Cu(II)-Induced Human LDL Cholesterol Oxidation. Oxidation of LDL in vivo may contribute to the pathology of atherosclerosis (*27*, *28*). Thus, this has led to increased interest in investigating the role of natural antioxidants in preventing the oxidation of LDL and membrane lipids. There is a great body of evidence supporting the link between oxidative modification of LDL cholesterol and atherogenesis. Antioxidants are known to offer protective effects in controlling oxidative modification of LDL cholesterol, thus reducing the chances of developing atheroma in the arteries. One approach to the study of the effect of test antioxidant against LDL oxidation is by subjecting LDL to oxidation in the presence of a known concentration of the test compound and monitoring the progression of oxidation. CD are often used as an indicator of the level of peroxidation of LDL cholesterol in antioxidant studies. The inhibition of LDL cholesterol oxidation of the extracts was expressed as percentage inhibition based on the CD value after 100 min of incubation. The existing difference in the effectiveness of the samples in inhibiting the oxidation of LDL became prominent at the end of the incubation; thus, the level of oxidation at 100 min was used for estimating the antioxidant activities of the extracts. **Figure 3** illustrates the progression of oxidation of LDL in the presence of whole barley extracts over 100 min of incubation. The whole barley extracts exhibited 19.64−33.93% inhibition, with Peregrine offering the highest inhibition followed by AC Metcalfe extract. Ferulic acid exhibited the highest inhibition of 53.57% at a concentration (70 μg/mL) that corresponds to the average phenolic content of barley extracts (**Table 2**).

None of the samples showed any indication of oxidation during the first 10 min of incubation; however, all samples showed a rise in CD after 10 min of incubation. During 10−40 min of incubation, the protective effects rendered by the extracts were inconsistent; however, after 40 min, the samples containing whole barley extracts reduced the development of CD. This type

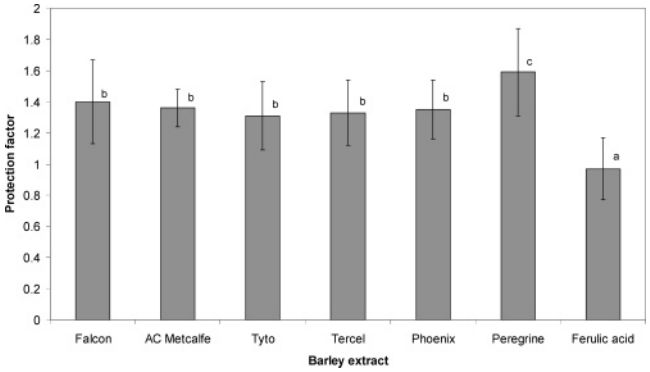

Figure 4. Effect of whole barley kernel extracts at a concentration of 20 mg/g of oil on prevention of autoxidation of stripped corn oil as measured by Rancimat.

of inconsistent behavior at the beginning of the incubation was also observed by Andreasen et al. (*15*) for authentic phenolic acids. Ferulic acid, ferulic acid dihydrodimers, sinapic acid, and *p*-coumaric acid were tested against LDL oxidation at various concentrations (10−60 μM) over 100 min. Andreasen et al. (*15*) determined the potency of phenolic extracts made from rye flour, bran, and whole grain in the prevention of LDL oxidation. Phenolic extracts obtained from rye bran, whole seeds, and flour samples behaved in a similar manner. Furthermore, it was found that extracts from bran inhibited LDL oxidation in a dose-dependent manner, whereas the extracts from whole grain showed a very weak activity. The inhibition offered by whole rye extracts (33%) was comparable to that of Peregrine (33.9%) and AC Metcalfe (26.8%). It has been reported that anthocyanins derived from grape juices (*29*), wine (*30*), and berries (*31*) are the major compounds contributing to the in vitro antioxidant activity, thus preventing LDL oxidation. The copper-induced LDL oxidation method has served as a useful model for evaluating natural antioxidants (*32*, *33*). Cupric ion and AAPH have been widely employed as agents to induce LDL oxidation in studies involving in vitro models of evaluating antioxidative extracts.

The protective effect against LDL cholesterol oxidation can be in part attributable to the Cu(II) chelation potential of the phenolic acids. Recent studies have shown that Cu(II)-mediated oxidation of LDL cholesterol can exhibit different kinetics depending on Cu(II) concentration. Propagation can proceed when antioxidants are depleted, at high Cu(II) concentrations,

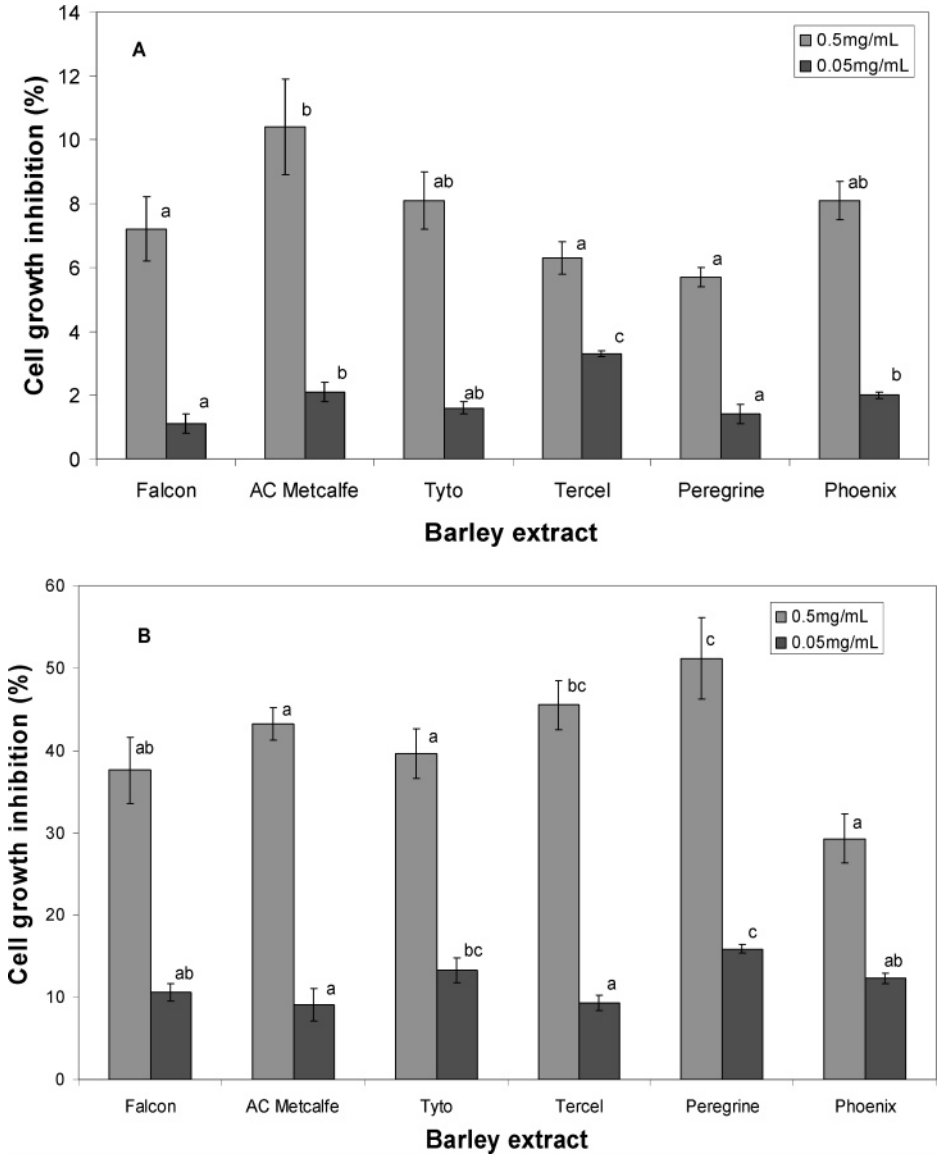

Figure 5. Percent inhibition of Caco-2 colon cancer cell proliferation by whole barley extracts at 0.5 and 0.05 mg/mL concentrations at the end of the day 1 of incubation (**A**) and at the end of the day 4 of incubation (**B**).

or when they are present at low concentrations (*34*). At high mole ratios of at least 10 Cu(II) ions per LDL molecule, oxidation continues to propagate after all of the available antioxidants are consumed (*34*). The increase in CD during the propagation phase was reported to be mainly due to the formation of cholesteryl linoleate hydroperoxides and substantial amounts of cholesteryl linoleate hydroxides (*35*).

Evaluation of Antioxidant Activity Using the Rancimat Method. The Rancimat test is an accelerated oxidation test that is used to determine the shelf life of fats and oils. Oxidation of unsaturated lipids proceeds very slowly during the initial stages, but it increases abruptly at a particular point, known as the inflection point (IP). The time from the beginning to the IP is the induction period. This IP can easily be identified by plotting conductivity data obtained through the probe. Whole barley extracts were evaluated for their potential in inhibiting accelerated autoxidation of stripped corn oil. The experiment was carried out using a Rancimat apparatus, and the protection factor was calculated for each extract. The PF is defined as the ratio of IP of the oil sample containing the test material and the IP of pure oil devoid of any additive. The PF for whole barley extracts varied between 1.31 and 1.59, whereas the PF for ferulic acid, the reference antioxidant, was 0.97 (**Figure 4**).

Oils resistant to autoxidation take a long time to reach the IP, whereas oils vulnerable to autoxidation take a short time to reach the IP. The oil used in this experiment was a commercial corn oil product stripped of its natural antioxidants. The intrinsic resistance, if any, is eliminated from PF by taking the IP of pure oil into consideration.

Inhibition of Caco-2 Colon Cancer Cell Proliferation. Colorectal cancer accounts for the second highest cancer death in North America. The American Cancer Society estimated that 56300 deaths were caused by colorectal cancer in 2005 (*8*). Thus, it is important to investigate the effect of antioxidative extracts on colorectal carcinoma cells. The Caco-2 cell line is widely used to evaluate different extracts and additives in vitro. Whole barley extracts were evaluated for their potential antiproliferative efficacy against proliferation of Caco-2 human cancer cells at 0.5 and 0.05 mg/mL levels. In this study, the antiproliferative activity was measured and reported as percent inhibition. None of the barley extracts showed significant antiproliferative activity by the end of day 1. The inhibition effect ranged from 5.7 to 10.4% and from 1.1 to 3.3% at 0.5 and 0.05 mg/mL concentrations, respectively, after 24 h of incubation. However, the effect of barley extracts on cell proliferation became evident over the incubation period and was clearly seen at the end of the day 4. The inhibition effect of whole barley extracts varied from 29.3 to 51.2% at a final concentration of 0.5 mg/mL, whereas the inhibition percentage ranged from 9.1 to 15.9% at a final concentration of 0.05 mg/mL. Although the effect of extracts was measured daily, only the contrasting results of days 1 and 4 are illustrated in **Figure 5**. Peregrine, AC Metcalfe, and Tercel rendered the highest activity against Caco-2 cell proliferation at 0.5 mg/mL concentration at the end of day 4. The higher concentration (0.5 mg/mL) used was quite effective in inhibiting cell proliferation; however, the lower concentration (0.05 mg/mL) was not effective even at the end of the day 4 in controlling the growth of Caco-2 cells. The discrepancy in inhibition of cell proliferation may be attributable to different chemical compositions of barley cultivars. Parry et al. (*17*) tested a number of fruit seed flours against Caco-2 cell proliferation and observed that the effectiveness of the extracts increased over 4 days and attained the highest level at the end of the day 4. This trend is very similar to what was observed in this study. Thus, results of the antiproliferative and other studies described above provide clear evidence that phenolic compounds present in the tested whole barley cultivars were effective in the scavenging of free radicals, controlling oxidation of LDL cholesterol, and inhibiting Caco-2 cell proliferation. Therefore, inclusion of whole barley grain and barley products in the daily diet may help mitigate oxidative stress related disease conditions, cardiovascular disease, and colon cancer, among others. However, there are many additional factors, such as bioavailability, that may affect antiproliferative action in vivo. Therefore, additional research is required to evaluate barley and its products in vivo.

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