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# Anthocyanin Composition and Oxygen Radical Scavenging Capacity (ORAC) of Milled and Pearled Purple, Black, and Common Barley

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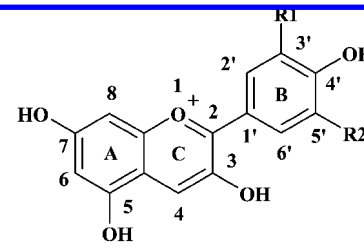
The importance of anthocyanins to the total antioxidant capacity of various fruits and vegetables has been well established, but less attention has been focused on cereal grains. This study investigated the antioxidant capacity and anthocyanin composition of a bran-rich pearling fraction (10% outer kernel layers) and whole kernel flour of purple (CI-1248), black (PERU-35), and yellow (EX-83) barley genotypes. HPLC analysis showed that as much as 6 times more anthocyanin per unit weight ($\mu$g/g) was present in the bran-rich fractions of yellow and purple barley (1587 and 3534, respectively) than in their corresponding whole kernel flours (210 and 573, respectively). Delphinidin 3-glucoside, delphinidin 3-rutinoside, cyanidin 3-glucoside, petunidin 3-glucoside, and cyanidin chloride were positively identified in barley, with as many as 9 and 15 anthocyanins being detected in yellow and purple barley, respectively. Antioxidant activity analysis showed that the ORAC values for the bran-rich fractions were significantly ($p < 0.05$) higher than for the whole kernel flour.

**KEYWORDS: Barley; pearling; anthocyanins; ORAC; antioxidant activity**

## INTRODUCTION

Anthocyanins are a group of water-soluble flavonoids responsible for the attractive red, violet, and blue colors of most fruits, vegetables, and cereal grains. In plants, anthocyanins are believed to have important physiological functions in pollination, seed dispersal, and photoprotective activity against harmful UV-B radiation (*1*, *2*). Anthocyanins are glycosides of anthocyanidins, the basic structure of which is presented in **Figure 1**. With an estimated average daily intake of about 13 mg in the United States (*1*), anthocyanins are considered to be the most important source of dietary flavonoids in the North American diet (*3*, *4*). Consumption of foods rich in anthocyanins has been linked with a lower risk of chronic diseases including hypercholesterolemia (*5*), hyperglycemia (*6*), and cancer (*7*). These possible health benefits appear to be related to the strong antioxidant capacity of anthocyanins (*8*), which can protect humans from the damaging effects of chemical oxidative stressors (*3*).

Various foods found in North America can be considered good sources of anthocyanins, including blueberry (3650 $\mu$g/g), blackberry (2450 $\mu$g/g), raspberry (100−3650 $\mu$g/g), strawberry (210−975 $\mu$g/g), grapes (367 $\mu$g/g), red cabbage (3220 $\mu$g/g), red radish (1000 $\mu$g/g), black beans (445 $\mu$g/g), eggplant (857 $\mu$g/g), and onions (121 $\mu$g/g) (*1*, *9*, *10*). However, all of these foods are not as commonly consumed by North Americans as are cereal products. This reality underscores ongoing collaborative research efforts among Canadian plant breeders and food scientists aimed at developing grain varieties that can be used for the production of cereal-based foods rich in anthocyanin compounds.




| Anthocyanidin | R1 | R2 |
|---|---|---|
| Cyanidin | OH | H |
| Peonidin | OCH3 | H |
| Pelargonidin | H | H |
| Malvidin | OCH3 | OCH3 |
| Dephinidin | OH | OH |
| Petunidin | OCH3 | OH |

**Figure 1.** Basic structure of the anthocyanidin standards.

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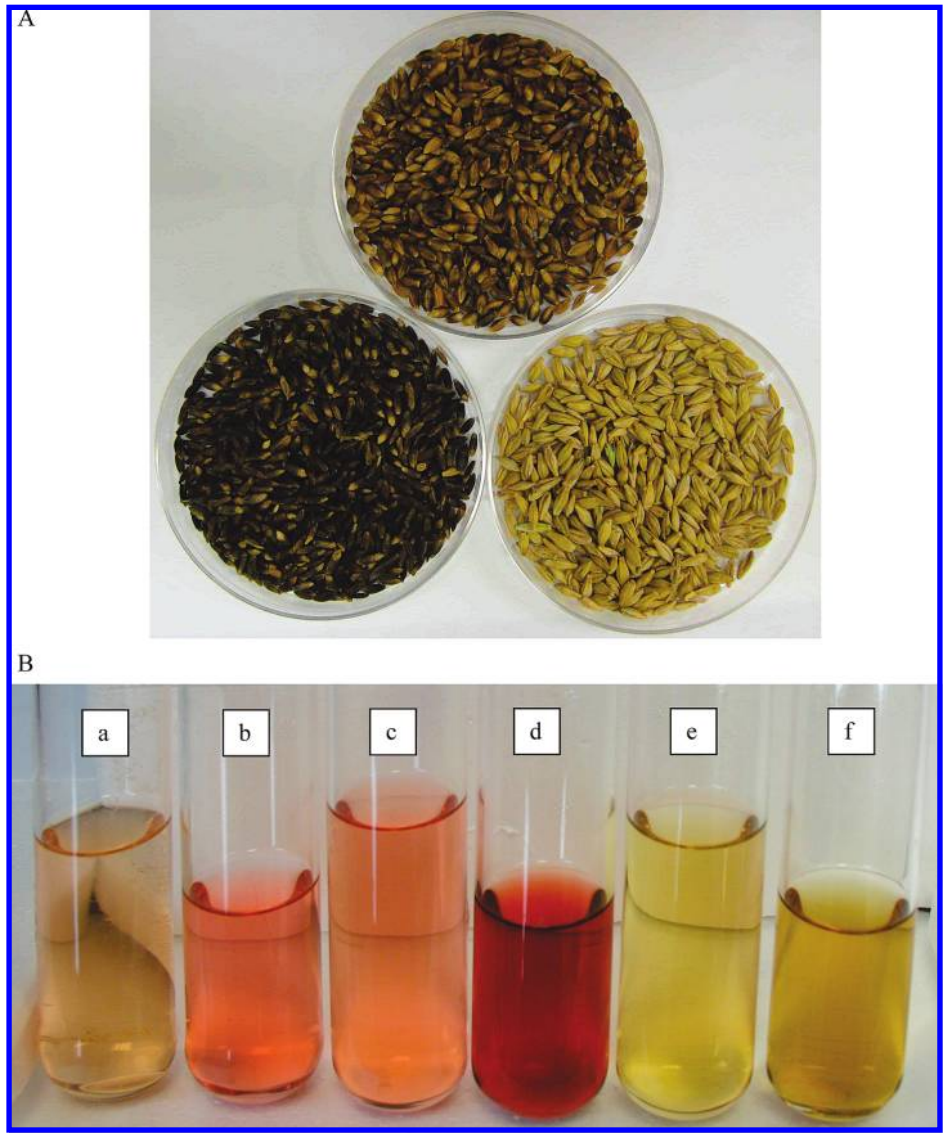


**Figure 2.** (**A**) Experimental yellow (right), purple (top), and black (left) barley grains. (**B**) Color of the concentrated methanolic extracts (~10-fold) obtained from 100% kernel flour (a, c, e) and the bran (b, d, f) of yellow, purple, and black barley, respectively.

Black, blue, and purple grains rich in anthocyanins have been identified as promising ingredients for the development of cereal-based functional foods as they are a source of natural antioxidants (*11*−*14*). However, besides anthocyanins, a large number of chemical compounds with free radical scavenging capacity have been identified in cereal grains, including barley. These bioactive compounds include phytoestrogens, sterols, lignans, phytic acid, proanthocyanidins, vanillin, ferulic acid, caffeic acid, *p*-coumaric acid, gentisic acid, sinapic acid, isoferulic acid, chlorogenic acid, vanillic acid, *p*-hydroxyphenylacetic acid, and syringic acid (*15*). Most of the papers examining the antioxidant capacity of barley extracts have focused their attention on elucidating the role played by phenolic acids and proanthocyanidins, as they are found in significant concentrations in barley (*16*−*18*). It is widely accepted that the antioxidant capacity of a phenolic compound arises due to the presence of an −OH group in its molecular structure, with antioxidant capacity varying in relation to substitution patterns (i.e., in relation to the location of the −OH groups relative to each other on the aromatic ring) (*19*).

Similarly, anthocyanin compounds varying in substitution patterns possess different antioxidant capacities. Hence, precise characterization of the composition of anthocyanin compounds in barley varieties is an important consideration in the selection of anthocyanin-rich barley breeding lines. An additional reason for studying anthocyanin composition in barley lines is related to the notion that food consumption of barley could be increased if barley fractions rich in antioxidant properties could be developed and incorporated into mainstream foods, such as muffins, breads, noodles, and pasta (i.e., functional foods) (*17*). A strategy that has been useful to concentrate the bioactive compounds present in the kernel layers of barley (*17*, *20*, *21*) and other grains (*22*, *23*) is pearling. This unit process operation applies abrading forces to separate outer from inner layers of the kernel, which results in gentler and more effective fractionation of the barley kernel layers relative to roller milling, which is the standard particle size reduction operation used by the grain industry. For example, using pearling fractionation, Madhujith et al. (*17*) obtained seven pearling fractions from two varieties of barley corresponding to 0−9.8% (F1), 9.8−18.8% (F2), 18.9−26.7% (F3), 26.8−33.3% (F4), 33.4−39.8% (F5), 39.9−45.5% (F6), and 45.4−49.9% (F7) of the outer kernel layers. By contrast, as illustrated for wheat, Beta et al. (*23*), using roller milling, were able to separate the outer layers of the wheat kernel into shorts, bran, and bran flour, although none of these fractions could be confidently related to any specific

proportion of the outer layer of the kernels. Roller milling uses corrugated break rolls to break open the kernel endosperm while attempting to maintain the outer kernel pericarp layers as intact as possible. One objective of this work was to quantify the ORAC antioxidant capacity and to characterize the anthocyanin composition of acidified methanolic extracts (pH 1) obtained from the bran (10% outer kernel layers) and whole ground kernels of three barley genotypes (PERU-35, EX-83, and CI-1248). Phenotypically, EX-83 represented a yellowish grain with a hulled caryopsis, CI-1248 a hull-less purplish grain, and PERU-35 a hull-less black grain. Another objective of this study was to assess the contribution of the outer bran layer of the experimental samples to their total antioxidant capacity.

## MATERIALS AND METHODS

**Barley Samples.** The three barley genotypes used in the present study, EX-83 (yellow), CI-1248 (purple), and PERU-35 (black), were obtained from Agriculture and Agri-Food Canada's Brandon Research Station located within the Barley Breeding and Genetics program, Brandon, Manitoba (**Figure 2A**). Experimental samples were prepared as follows. The barley samples were first cleaned from extraneous matter and then either ground into 100% barley flour in a SmartGrind coffee mill (Black & Decker, Miramar, FL) or pearled to remove 10% of their outer kernel layers in a TM-5 laboratory scale (200 g capacity) pearler (Satake Corp., Hiroshima, Japan). The pearler was equipped with a 40 grit carborandum stone and a 1.0 × 16 mm slit-type steel mesh to separate fine from coarse debranned particles. To carry out the grinding operation, barley grain (60 g) was ground at maximum speed for 60 s. The grinding operation was repeated until enough ground barley was obtained. Conversely, two different protocols were adopted to pearl the barley depending on whether the barley grains possessed a hull or not. For hulled barley (EX-83), hulls were first removed from the kernels (and discarded from the experiment) by abrading the samples in the Satake pearler at a speed of 1150 rpm for 1 min. The outer layers (10%) of dehulled (EX-83) barley were then removed by pearling the samples at 1150 rpm for 4 min. To pearl the hulless barley samples, the protocol for the dehulled barley was followed, except that removal of outer layers, or more strictly debranning, was accomplished by pearling the barley sample at a speed of 1150 rpm for 2.5 min (CI-1248) or 3 min (PERU-35). The kernel outer layers or bran fractions were then sifted over an 18 mesh to remove small flour particles. The percent of outer layer removal of the samples was obtained from the difference between the initial sample weight and pearled kernel weight, divided over the initial sample weight. Because 10% of the outer layers of barley kernels are rich in bran components (*17, 24*), this pearling fraction will be referred to throughout this paper as the "bran pearling fraction" or "bran fraction", although clearly it is virtually impossible to obtain a kernel fraction composed of 100% barley bran (i.e., free of embryo constituents) using pearling. Whereas the total bran of hull-less barley is constituted by as much as 30% of the outer layers of its kernels (*24*), this study abraded off only 10% of the whole kernel to minimize the presence of endosperm constituents, because it has been observed that a greater degree of abrading (e.g., ≥20%) causes complete removal of the aleurone layer and part of the subaleurone layer, which is already in direct contact with the starchy endosperm (*24*). Individual batches of ground or pearled barley were pooled, thoroughly mixed, and kept under refrigeration in sealed double polyethylene bags until a bulk sample was equilibrated overnight to room temperature prior to experimentation.

**Color.** A Hunterlab spectrophotometer CM-3500d colorimeter (Minolta Co., Ltd., Osaka, Japan) with SpectraMagic version 3.6 software was used to measure the color of the ground and pearled barley samples. The color was expressed using the *L*, *a*, and *b* color space coordinates, where *L* represents lightness, $+a^*$ redness, $-a^*$ greenness, $+b^*$ yellowness, and $-b^*$ blueness.

**Chemicals.** The solvents for the high-performance liquid chromatography assay, methanol, acetonitrile, and acetic acid, were of HPLC grade (Fisher Scientific Co., Ottawa, ON, Canada). The anthocyanin monomeric standards used to identify the anthocyanin profile of the barley extracts, delphinidin 3-glucoside, cyanidin 3-galactoside, delphinidin 3-rutinoside, cyanidin 3-glucoside, cyanidin 3-rutinoside, petunidin 3-glucoside, peonidin 3-glucoside, malvidin 3-glucoside, and cyanidin chloride, were purchased from Polyphenols Laboratories AS (Sandnes, Norway). Each anthocyanin standard was dissolved in acidified methanol (1 N HCl, 85:15, v/v) in a dark cold room to obtain anthocyanin stock solutions with a concentration of 1 mg/mL.

For the oxygen radical absorbance capacity (ORAC) assay, 2,2′-azobis(2-methylpropionamide) dihydrochloride (AAPH), catechin, monobasic potassium phosphate ($KH_2PO_4$), and dibasic potassium phosphate ($K_2HPO_4$) were purchased from Sigma-Aldrich (St. Louis, MO), whereas Trolox and fluorescein reagents were purchased from Fisher Acros Organics (Morris Plains, NJ).

**Extraction and Purification.** Anthocyanin extracts (**Figure 2B**) from barley samples were prepared and purified according to the method of Hosseinian and Beta (*10*) with some modifications. The barley sample (2.5 g) was suspended in 25 mL of acidified methanol (1 N HCL, 85:15, v/v) and its pH adjusted to 1.0 using 1 N HCl. The suspension was shaken for 2.5 h at room temperature (25 °C) and its pH corrected to 1.0, if necessary, 15 min after shaking had begun. The suspension was then sonicated for 30 min (room temperature) and centrifuged at 10000 rpm for 30 min at 15 °C. The supernatant was retained, filtered through a 0.45 μm nylon Acrodisc syringe filter, and concentrated ∼10-fold under a constant stream of nitrogen in a nitrogen evaporator set at 40 °C. The pH of the crude extracts was then adjusted to 1.0 (25 °C) using 1 N HCl. Preparation of spiked samples was also conducted by adding a known amount of the standards to one of the samples and measuring the recovery. Recovery was found to be >92%.

The extraction equipment included a G-25 shaker (Eberbach, Ann Arbor, MI), a Bransonic B-3200R-2 sonicator (Branson, Shelton, CT), an SLA-3000 centrifuge fitted with a GS-3 rotor (Sorvall Instruments, ON, Canada), and an N-EVAP 112 nitrogen evaporator (Organomation Associates, Inc., Berlin, MA).

**HPLC Analysis.** HPLC analysis was conducted according to the method of Hosseinian and Beta (*10*) with some modifications. A 10 μL sample of each experimental filtrate was analyzed with a 2695 HPLC system (Waters Corp., Milford, MA) equipped with a model 996 photodiode array detector and model 717 plus autosampler (Waters). Empower 2 software was used to acquire and analyze experimental chromatographic data. Separation of anthocyanins was accomplished on a 150 mm × 3 mm i.d., 3 μm, Luna 3u C18 column fitted with a guard column (Phenomenex, Torrance, CA). The column temperature was maintained at 35 °C by a Waters temperature control module (*10*). The mobile phase consisted of 4.5% formic acid in double-deionized water (solvent A) and 100% methanol (solvent B). The following gradient was used: solvent B, 0 min, 10%; 30 min, 25%; 34 min, 33%; 42 min, 90%; 45−50, 10%. Other chromatographic conditions included a constant flow rate of 0.4 mL/min, an injection volume of 10 μL, and a run time of 50 min. The relative retention time (RT), percentage peak area under the curve, and spectroscopic data of the anthocyanin standards were used to identify the type and quantity of anthocyanins present in the samples. Cyanidin 3-glucoside was used as an external standard to quantify the amount of anthocyanins present in the sample but for which the identity could not be established using the standards available. On the basis of the experimental chromatographic conditions, the limit of detection (LOD) and limit of quantification (LOQ) were found to be 200 ng/mL (S/N > 5) and 400 ng/mL (S/N > 10), respectively. Both LOD and LOQ were determined using the method of Skoog and Leary (*25*) by assessing the chromatogram for the standard at various concentrations (0, 100, 200, 400, 1000, 2000, 11000 and 111000 ng/mL).

**Antioxidant Activity Determination.** Antioxidant activity in the barley samples was measured using the ORAC assay according to the method of Huang et al. (*26*) and the minor modifications made by Li et al. (*27*). The assay is based on the principle that antioxidant compounds present in the sample (e.g., anthocyanins) will inhibit the decay in fluorescence intensity of a fluorescent probe, fluorescein, after it had been combined with AAPH, a free radical generator that acts as an oxidizing agent. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as the standard, with one ORAC unit being equal to the antioxidant protection given by 1 μM Trolox. Antioxidant

**Table 1.** Hunterlab Color *L*, *a**, and *b** Values for Six Experimental Barley Samples and Their Methanolic Extracts Obtained from Three Barley Genotypes That Had Been Ground into Flour (Yellow, Purple, and Black) or Pearled To Remove 10% of Their Outer Bran Layers (Yellow Bran, Purple Bran, and Black Bran)

| | mean[a,b] ($n$ = 3) color value for barley samples | | | |
|---|---|---|---|---|
| | *L* (0.82) | *a** (0.24) | *b** (1.07) | color of extract |
| kernel | | | | |
| yellow | 58.59 f | 4.05 c | 25.42 a | |
| purple | 41.38 h | 7.2 a | 19.81 b | |
| black | 32.61 i | 3.12 d | 10.29 f | |
| whole ground | | | | |
| yellow | 81.11 b | 1.90 f | 13.95 d | light pink |
| purple | 76.96 c | 2.40 e | 12.04 e | light red |
| black | 66.68 d | 1.49 g | 8.85 g | yellow-green |
| 10% outer bran | | | | |
| yellow bran | 84.47 a | 1.11 h | 13.79 d | light red |
| purple bran | 65.20 e | 4.63 b | 16.22 c | dark red |
| black bran | 54.89 g | 3.02 d | 11.87 e | yellow-brown |

[a] Number in parentheses next to color Hunterlab values denotes Fisher's least significant difference (LSD). [b] Means within each column followed by the same letter are not significantly ($p < 0.05$) different.

activity in the samples was expressed as micromoles of Trolox equivalents per 100 g of sample, on a dry weight basis.

Antioxidant activity was measured and calculated using a Precision 2000 well automated microplate pipetting system (Bio-Tek Instruments Inc., Winooski, VT) that automatically transferred ORAC reagents into a 96-well flat-bottom polystyrene microplate (Corning Inc., Corning, NY), and an FLx800 microplate fluorescence reader (Bio-Tek Instruments Inc.) controlled by KC4 3.0 software. The latter instrument was equipped with fluorescence filters with an excitation wavelength of 485 nm and an emission wavelength of 520 nm in order to measure changes in the fluorescence of fluorescein under controlled temperature conditions (37 °C).

**Statistical Analysis.** One-way analysis of variance of results (i.e., color, anthocyanin content, and ORAC values) was performed using SAS statistical software version 9.1. Results reported in this work are averages of three replications. Significant differences among sample means were tested using Fisher's least significant difference (LSD) test. Comparisons of anthocyanin composition, color, and ORAC antioxidant activity within each experimental flour and bran stream were carried out using Pearson's correlation coefficient. The use of significance was based on $p < 0.05$ unless stated otherwise (* denotes $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.001$).

## RESULTS AND DISCUSSION

**Color Measurements. Figure 2A** shows the color characteristics of the experimental samples. On the basis of their appearance, PERU-35 is classified as a hull-less black grain, EX-83 as a hulled light yellow grain (or normal barley), and CI-1248 as a hull-less purple grain. Information on the type of caryopsis (hulled or hull-less) and on the color characteristics of colored barley in terms of Hunterlab color values has proven useful to phenotypically segregate colored barley grains into black, blue, and purple groups (*28*). **Table 1** shows that the *L* value, which measures lightness, was lowest for the black barley ($L = 32.61$), highest for the light yellow barley ($L = 58.59$), and of intermediate value for the purple barley ($L = 41.38$), regardless of whether color measurements were made in the whole kernel, bran fraction, or ground whole kernel flour (100% kernel flour). Results also showed that the *a* value (redness) was highest for the purple grain, its bran, and its ground kernel flour. The *b* value (yellowness) was highest for the yellow grain and its ground kernel but not for its bran. Rather, the purple grain gave the bran with the highest yellowness values. Kim et al. (*28*) showed that the *L* and *b* values of a total of 127 lines

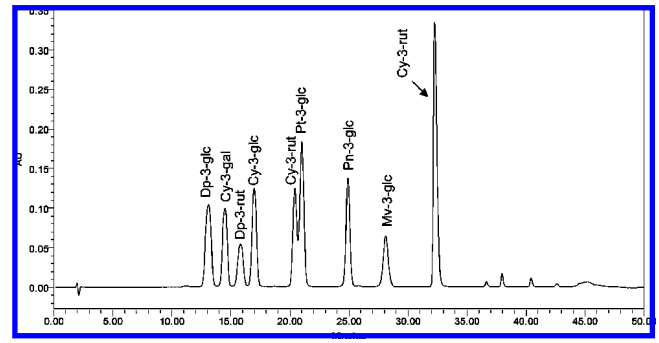


**Figure 3.** Typical illustration for the HPLC chromatogram (520 nm) of nine anthocyanin standards.

of barley, namely, black, purple, and blue barley, were positively correlated ($r = 0.84$***), regardless of whether their caryopsis was hulled or hull-less. Correlation analysis of the *L* and *b* color values of the kernels of black, yellow, and purple barley revealed similar relationships ($r = 0.94$***), suggesting that the biosyntheses of blackish and bluish compounds were events that had occurred concomitantly in the seed coat of colored and normal barley grains.

**Composition of Colored Barley.** The chromatograms of the anthocyanin standards and the anthocyanin compounds detected in the experimental samples can be seen in **Figure 4A**. **Table 2** summarizes the anthocyanin composition of the experimental barley samples using our current extraction methods, except for black barley, for which no anthocyanin compounds were detected chemically or visually from the color of the methanolic extracts (**Figure 2B**).

The total anthocyanin contents (TAC) in the purple and normal barley were 573 and 210 μg/g (**Table 2**). TAC for the purple barley was 1.6−1.8 times higher than for the two purple barley varieties (hulled and hull-less) reported by Abdel-Aal et al. (*13*). For the anthocyanin-containing samples, removal of 10% of the outer layers of the kernel (a bran-rich fraction) by pearling was a useful strategy to concentrate anthocyanins, as the TAC increased significantly ($p < 0.05$) for the purple and normal barley fractions to 3534 and 1587 μg/g, respectively. Using this information it can be found that 10% of the kernel outer layers of purple and normal barley contained about 61.7% [= (3533 μg × 0.1)/573 μg × 100%] and 75.6% [= (1587 μg × 0.1)/210 × 100%] of the total anthocyanin content in the kernel. TAC in the bran-rich fractions was significantly higher than that reported (*13*) for the whole ground kernel of blue barley (4 μg/g), blue and purple wheat (153 and 13 μg/g), black rice (2284 μg/g), red rice (22 μg/g), and blue, pink, purple, red, and Fiesta corn (225, 93, 965, 559, and 100 μg/g, respectively).

In terms of the anthocyanin composition of the experimental samples, results showed that of the 17 anthocyanin compounds detected in the samples, standards for only 5 of them were available. The compounds that could not be labeled due to the lack of standard were considered to be anthocyanins because they displayed a maximum absorption band in the 505−530 nm region (*29*). Delphinidin 3-glucoside, cyanidin 3-glucoside, petunidin 3-glucoside, and nine unidentified anthocyanins were detected in purple barley. The most abundant anthocyanins in the purple barley were cyanidin 3-glucoside (99 μg/g), delphinidin 3-glucoside (93 μg/g), and an unidentified anthocyanin compound with a retention time of 31.2 min (79 μg/g). In the bran fraction of purple barley, however, an additional anthocyanin (cyanidin chloride) and three unidentified anthocyanins were detected. The most dominant anthocyanin compounds were two unidentified anthocyanins with concentrations of 607 and

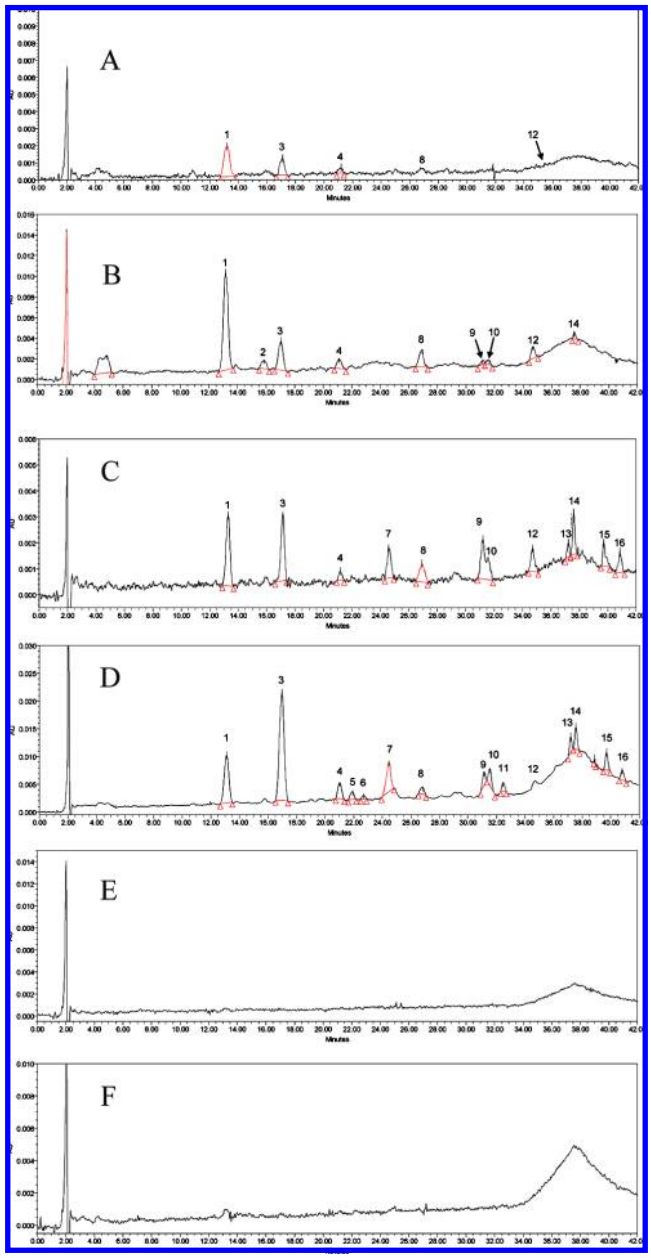


**Figure 4.** Typical illustration of HPLC chromatograms (520 nm) of anthocyanins obtained from 100% kernel flour (**A**, **C**, **E**) and the bran (**B**, **D**, **F**) of yellow, purple, and black barley, respectively. The number above each peak corresponds to the anthocyanin compound numbers in **Table 2**.

521 $\mu$g/g and retention times of 31.6 and 37.2 min, respectively. The third most dominant bran anthocyanin was cyanidin 3-glucoside with an anthocyanin content of 505 $\mu$g/g.

Using five anthocyanin standards (cyanidin 3-glucoside, delphinidin 3-glucoside, pelargonidin 3-glucoside, peonidin 3-glucoside, and malvidin 3-gluoside) and their aglycones (including petunidin), Kim et al. (*28*) also studied anthocyanin composition in black, blue, and purple barley. They detected no significant differences between the anthocyanin composition of hulled and hull-less purple barley and found that for purple barley, cyanidin 3-glucoside was the most abundant anthocyanin, accounting for 67−68% of the total anthocyanin content of hulled and hull-less purple barley (313−350 $\mu$g/g). The compounds peonidin 3-glucoside and malvidin 3-glucoside, which were not detected in our (hull-less) purple barley sample, accounted for 9−12 and 4−5% of the total anthocyanin concentration. The absence of these compounds in our purple barley sample may be due to genotypic differences. The remainder of the anthocyanin detected (*28*) was pelargonidin 3-glucoside (11−12%), for which a standard was not available in the present study and, hence, could not be detected.

Conversely, the anthocyanin composition for the normal barley was simpler. In the normal barley sample (104 $\mu$g/g) and its bran fraction (737 $\mu$g/g), the anthocyanin present in the highest concentration was delphinidin 3-glucoside, accounting for nearly 50% of the total anthocyanin concentration (**Table 2**). A total of five anthocyanins were detected in the normal barley, and this number increased to nine for its yellow (normal) bran fraction (**Figure 4B**).

**Antioxidant Activity.** The ORAC results for the experimental samples are shown in **Table 3**. The ORAC values ($\mu$mol of Trolox equivalents per 100 g, on a dry weight basis) for the black and yellow (normal) barley (whole grains) were not significantly different from one another, but they were significantly higher (5430 and 5601, respectively) than the ORAC values found in the purple barley (3937). When the barley bran fraction was examined, ORAC values were significantly different for the three samples, with the purple barley fraction exhibiting the highest ORAC values (11157), followed by the black barley fraction (10254) and, last, the yellow (normal) barley fractions (9004).

The contribution of the outer layers of the kernel to the total antioxidant capacity of cereal grains can be considered a useful parameter as it will aid in determining suitable extraction rates for the development of pearling fractions with functional properties. However, despite concerted efforts to find a suitable indicator of antioxidant capacity, such as the ORAC value (*30*), other indices of antioxidant capacity continue to be explored and introduced in the literature in an attempt to better relate functional compounds found in foods to their free radical scavenging capacity in an in vivo system. Often, more than one antioxidant capacity index is measured for the same food or a bioactive compound extracted thereof. Because the underlying mechanism for detecting free radical scavenging capacity for these assays is not the same, interpretation of data is not as transparent. One way to consolidate the vast amount of antioxidant capacity data available in the literature for the same pearling fraction of a cereal grain is to express the antioxidant capacity of the pearling fraction relative to the antioxidant capacity for the whole grain. This strategy permits comparison of free radical scavenging capacity for any given pearling fraction (e.g., 10% outermost layers) of cereal grains when measured by different types of antioxidant capacity assays. Analysis of experimental data from various reports (*17*, *20*, *22*, *23*) shows that depending on the antioxidant capacity assay, the bran fraction of a barley cultivar can exhibit a wide range of free radical scavenging capacity. For example, the bran fraction obtained from the Falcon barley cultivar possessed as little as 15.4% and as much as 64.2% of the total free radical scavenging capacity of the kernel, depending on whether the capacity of the extract to scavenge ABTS [2,2′-azinobis(3-ethylbenzothiazoline-5-sulfonate)] radical anions is measured spectrophotometrically in terms of Trolox equivalents using the Trolox equivalent antioxidant assay (TEAC) or whether the capacity of the extract to protect against peroxyl radical-induced DNA breakage is measured in ferulic acid equivalents by the DNA protection against peroxyl radicals (DNA-PAPR) assay (*20*). In general DNA-PAPR and DNA-PAHR (DNA protection against hydroxyl radicals) assays allocated the lowest antioxidant capacity scores to the bran fractions of Falcon and AC Metcalfe

**Table 2.** Anthocyanin Composition[a,b] (Micrograms per Gram of Dry Weight) of Two Barley Genotypes Ground into Flour (Yellow and Purple) or Pearled To Remove 10% of Their Outer Bran Layers (Yellow Bran and Black Bran)

| compd | RT (min) | anthocyanin[c] | yellow | yellow bran | purple | purple bran | LSD[d] |
|---|---|---|---|---|---|---|---|
| **1** | 13.2 | Dp-3-glc | 104 ± 71 b | 737 ± 232 a | 93 ± 60 b | 290 ± 252 b | 335 |
| **2** | 15.9 | Dp-3-rut | ND | 87 ± 10 | ND | ND | |
| **3** | 17.1 | Cy-3-glc | 30 ± 15 c | 177 ± 97 b | 99 ± 42 bc | 505 ± 174 a | 141 |
| **4** | 21.1 | Pt-3-glc | 20 ± 9 c | 73 ± 11 b | 37 ± 24 c | 118 ± 17 a | 25 |
| **5** | 22.0 | unknown | ND | ND | ND | 40 ± 14 | |
| **6** | 22.8 | unknown | ND | ND | ND | 28 ± 16 | |
| **8** | 24.6 | unknown | ND | ND | 50 ± 17 b | 261 ± 88 a | 119 |
| **9** | 26.9 | unknown | 47 ± 23 b | 320 ± 280 a | 44 ± 16 b | 405 ± 204 a | 328 |
| **10** | 31.2 | unknown | ND | 16 ± 7 b | 79 ± 59 ab | 100 ± 42 a | 79 |
| **11** | 31.6 | unknown | ND | 52 ± 34 b | 26 ± 12 b | 607 ± 303 a | 332 |
| **12** | 32.5 | Cy-Cl | ND | ND | ND | 9 ± 3 | |
| **13** | 34.7 | unknown | 9 ± 6 b | 99 ± 80 ab | 32 ± 17 ab | 102 ± 49 a | 90 |
| **14** | 37.2 | unknown | ND | ND | 19 ± 2 b | 521 ± 263 a | 351 |
| **15** | 37.6 | unknown | ND | 26 ± 4 b | 37 ± 19 b | 83 ± 27 a | 30 |
| **16** | 39.7 | unknown | ND | ND | 30 ± 13 b | 305 ± 147 a | 197 |
| **17** | 40.8 | unknown | ND | ND | 27 ± 11 b | 160 ± 76 a | 102 |
| | | total | 210 ± 21 c | 1587 ± 85 b | 573 ± 264 bc | 3534 ± 1244 a | 1202 |

[a] Mean ± SD. [b] Moisture content of samples was as follows: 8.36% (yellow), 7.27% (yellow bran), 8.92% (purple), and 7.24% (purple bran). [c] Cy, cyanidin; Dp, delphinidin; Pt, petunidin; glc, glucoside; rut, rutinoside. Unknown anthocyanins were quantified in terms of cyanidin 3-glucoside equivalents. [d] LSD denotes Fisher's least significant difference. Means within each row followed by the same letter are not significantly ($P < 0.05$) different.

**Table 3.** ORAC[a] Values for the Methanolic Extracts Obtained from the Experimental Barley Samples

| | mean[b] ($n = 3$) ORAC value (LSD = 753) |
|---|---|
| whole ground | |
| yellow | 5601 ± 329 d |
| purple | 3937 ± 223 e |
| black | 5430 ± 438 d |
| 10% outer bran | |
| yellow bran | 9004 ± 411 c |
| purple bran | 11157 ± 375 a |
| black bran | 10254 ± 643 b |

[a] Expressed as micromoles of Trolox equivalents per 100 g of sample, on a dry weight basis. [b] Means within each column followed by the same letter are not significantly ($p < 0.05$) different.

barley (*20*), whereas other assays allocated higher antioxidant capacity scores to the same barley fractions (*17, 20*). More specifically, the TEAC and $IC_{50}$-DPPH assays allocated the highest antioxidant capacity scores to the bran fraction, suggesting that these two assays are more sensitive to the presence of bioactive compounds located in the pericarp and aleurone layers (bran layers) of the kernels. $IC_{50}$-DPPH measures the capacity of the extract to decrease the initial concentration of the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical by 50% as measured by electron paramagnetic resonance spectrometry. The ORAC, DPPH, and $IC_{50}$-OH (like $IC_{50}$-DPPH but using hydroxyl radicals instead) assays showed good sensitivity to the bioactive compounds in the bran fraction of the Falcon barley cultivar, but not as good as the TEAC and $IC_{50}$-DPPH assays. The DNA-PAHR and DNA-PAPR assays were most sensitive to the bioactive compounds located within the inner layers of the kernel, as nearly 40% of the DNA-protecting compounds were within 50% of the inner kernel (*20*). The DNA-PAHR and DNA-PAPR assays were equally as sensitive to the bioactive compounds located in the outer 10% layers of the barley kernel as to those located within 10−20% of the outer kernel layers. The DPPH assay revealed that the bran of barley compared to the bran of wheat possessed a greater proportion of the total antioxidant capacity of their kernels.

Using the same analysis for the experimental black barley samples, it can be demonstrated that 10% of the outer kernel layer contained 18.9% (= [10254 × 0.1]/[5430 × 1] × 100%) of the total antioxidant capacity of the kernel. For the experimental purple and normal barley, the same barley bran fraction represented 16.1 and 28.3% of the TAC of the whole grain. Analysis of the free radical scavenging capacity of barley bran indicates that the experimental colored barley varieties had a greater concentration of antioxidant compounds, as measured by ORAC, remaining in the inner layers of their kernel (>10%) compared to those remaining in the experimental yellow (normal) barley variety (EX-83) or compared to those remaining in the yellow (normal) barley varieties reported in ref *17*, the Falcon hulless barley variety, and the AC Metcalfe hulled barley variety. Because a greater proportion of the bioactive compounds with antioxidant capacity present in colored barley remained in inner layers of their kernels [relative to the proportions found in yellow (normal) barley genotypes], our study suggests that higher extraction pearling fractions with higher antioxidant capacity could be obtained from colored barley compared to yellow (normal) barley varieties, regardless of whether the former are hulled or hull-less.

The present study showed that the antioxidant capacity of the purple and normal barley genotypes, CI-1248 and EX-83, respectively, as measured by the ORAC assay, was highly correlated to the total anthocyanin content detected in their whole kernels and bran (10% outer layers) fraction. Although its whole kernel yielded a milled fraction with higher antioxidant activity than CI-1248 and EX-83, the so-called antholine PERU-35 had no detectable anthocyanin compounds, suggesting that phytochemicals other than anthocyanins were responsible for the observed antioxidant capacity of this black hull-less grain. Results from the present study indicated that barley fractions containing as much as 6 times more anthocyanin per unit weight than in the whole grain can be obtained by removing outer tissue layers from the kernel through an abrasive reduction process, such as pearling. Analysis of experimental results in light of previous studies on the anthocyanin content of barley grains indicates that colored barley varieties are more suitable than the yellow (normal) barley variety to produce higher extraction pearling fractions rich in bioactive compounds (i.e., antioxidants).

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