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Relationship between Phenolic Compounds, Anthocyanins Content and Antioxidant Activity in Colored Barley Germplasm

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Barley and its products are good sources of antioxidants. This experiment was conducted to examine the classification and concentration of phenolic compounds, proanthocyanidins, and anthocyanins in 127 lines of colored barley. Their relationship with 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was also examined. Barley was placed into seven groups using the colorimeter: hulled (black 1, black 2, black 3, and purple) and unhulled (black, blue, and purple). The contents of phenolic compounds and anthocyanins were analyzed by using HPLC. The average content of phenolic compounds in unhulled barley groups (268.6 μg/g) was higher than that in hulled (207.0 μg/g) ($P > 0.05$). The proanthocyanidins content was determined by modified vanillin assay. The average content of proanthocyanidins was significantly higher in purple and blue barley groups compared with black ($P < 0.05$). The content of anthocyanins varied from 13.0 to 1037.8 μg/g. Purple and blue barley groups contained higher average contents of anthocyanins than black ($P < 0.05$). The most common anthocyanin in the purple barley groups was cyanidin 3-glucoside, whereas delphinidin 3-glucoside was the most abundant anthocyanin in the blue and black groups. In colored barley, DPPH radical scavenging activity had high positive correlation to the content of phenolic compounds and proanthocyanidins.

KEYWORDS: Barley; phenolic compounds; anthocyanins; antioxidant activity; DPPH

INTRODUCTION

Barley (*Hordeum vulgare* L.) is a widely consumed cereal because of its dietary health benefits, ready availability, reasonable cost, and processing properties for products such as beer, barley teas, soup, and baked products (*1*). Barley grains differ considerably from those of other cereals in regard to chemical constitution. Grains containing protein of high biological value are characterized by high levels of dietary fiber, which include the valuable β-glucans and arabinoxylans. They also contain tocotrienols and tocopherols (*2*). In recent years, the demand for natural, functional, and healthy foods has tended to increase. For this reason, many researchers have studied the phytochemicals in fruits, vegetables, and cereals. Several papers have reinforced the case for whole grains as health-promoting components in the diet (*3*−*5*). Recent analyses of whole grains and their products demonstrate that they are good sources of antioxidants (*6*). Compared with refined grains, whole grains are richer in insoluble fibers and several nutrients such as antioxidants, indoles, and phenolic compounds, which are generally recommended as being an important part of a healthy human diet (*7*).

Barley grains also contain various phytochemicals such as phenolics and flavonoids. They have a wide range of phenolic acids, in particular, derivatives of benzoic acid (C_6−C_1) and cinnamic acid (C_6−C_3), with ferulic acid being the most abundant (*8*, *9*). Various classes of phenolic compounds in grains contain proanthocyanidins, anthocyanidins, quinones, flavanones, flavones, flavonols, chalcones, and amino phenolic compounds (*10*, *11*). Phenolic compounds are antioxidants, and natural antioxidants may exhibit one or more of the following roles: free radical scavenger, reducing agent, potential producer of prooxidant metals, and quencher of singlet oxygen formation (*12*). Their chemical actions are related to a number of hydroxyl groups in phenolic compounds. For this reason, the phenolic compounds present in grains as either antioxidant materials or the agents of other mechanisms contribute to anticarcinogenic or cardioprotective action. Phenolic acids scavenge the free radicals responsible for promoting lipid oxidation at biologically significant rates (*13*). Several studies have been published on the antioxidant activity of phenolic compounds such as *p*-coumaric acid, ferulic acid, and vanillic acid, as well as on the

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mutagenicity of some major flavonoids such as myricetin, kaempferol, and quercetin (*14*, *15*). Moreover, proanthocyanidins also have potent antioxidant capacity and possible protective effects on human health (*16*).

Anthocyanins constitute one of the major groups of natural pigments, are responsible for the colors of many fruits and flowers, and possess antioxidant activity *in vitro*. They have greater antioxidant activity than either vitamin C or E (*17*). Anthocyanins are glycosides of polyhydroxy-2-phenylbenzopyrylium or flavylium salts, which are widely distributed in fruits and vegetables (*18*). Barley is an anthocyanin-colored cereal and exists in black, blue, red, and purple varieties due to the presence of melanins and anthocyanins (*19*).

The aim of this study was to screen hulled and unhulled colored barley varieties with respect to their contents of phenolic compounds and anthocyanins and to investigate their 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities. Hence, this study also examined the relationship between the concentration of phenolic compounds, anthocyanins, and antioxidant activity in colored barley.

MATERIALS AND METHODS

Grain Samples and Sample Preparation. Colored barley genetic resources were supplied from the National Gene Bank, Rural Development Administration, Korea. Barley was propagated during the 2004−2005 season at Iksan (Honam Agricultural Research Institute, NICS), Korea. The following 127 barley lines were used: 96 lines of hulled black barley (including Spratt), 3 lines of hulled purple barley (including D.S.B. 10-2), 7 lines of unhulled black barley (including A-35), 3 lines of unhulled blue barley (including Ab 2231), and 18 lines of unhulled purple barley (including Chan Tung) (Supporting Information, Table 1).

Whole grains were ground using a roter mill (Fritsch, Idar-Oberstein, Germany) equipped with a 0.2 mm screen. Grains and grain flour samples were put into plastic bags and stored at −20 °C under vacuum until used.

Color Characteristics of Colored Barley. Instrumental colorimetric analyses were performed with a Spectro Colorimeter (model JS-555, Color Techno System Co. Ltd., Japan). A glass cell containing the grain was placed above the light source, covered with a white plate, and *L*, *a*, and *b* values were then recorded. *L* values indicate lightness, *a* values indicate redness, and *b* values indicate yellowness of the samples. The presence of anthocyanins was investigated (data not shown), One gram of powdered whole grain sample was extracted with 20 mL of 80% methanol containing 0.1% HCl in a conical flask (wrapped in aluminum foil) for 24 h at 4 °C. After 24 h, the extract was centrifuged at 3000*g* for 20 min. The supernatant was scanned the whole wavelength range of 200−700 nm with a UV−vis spectrophotometer (Hitachi Ltd., Tokyo, Japan).

Quantitative Analysis of Phenolic Compounds. Analysis of phenolic compounds was conducted according to the method of Chung et al. (*20*). The 30 phenolic compound standards, benzoic acid, biochanin A, caffeic acid, chlorogenic acid, *trans*-cinnamic acid, *m*-coumaric acid, *p*-coumaric acid, *o*-coumaric acid, 3,4-dimethoxybenzoic acid, ferulic acid, formononetin, gallic acid, homogentisic acid, *o*-hydroxyphenylacetic acid, phloroglucinol, protocatechuic acid, pyrogallol, β-resorcylic acid, salicylic acid, syringic acid, vanillic acid, (+)-catechin, hesperetin, hesperidin, kaempferol, myricetin, naringenin, naringin, quercetin, and rutin, were purchased from Sigma-Aldrich (St. Louis, MO) and Extrasynthese (Gernay, France) and used for calibration curves. The standard stock solutions (100 ppm) were made with dimethyl sulfoxide (DMSO). All standard calibration curves showed high degrees of linearity ($r^2 > 0.99$) (data not shown). Sample compounds were identified on the basis of the retention times of standard materials and were quantified by comparing their peak areas with those of standard curves. HPLC diluents, sample solvents, and 21 standard materials used were of HPLC grade.

Figure 1. Normal barley and seven groups of colored barley germplasms.

Two grams of ground whole grain sample was mixed with 10 mL of acetonitrile and 2 mL of 0.1 N HCl in a 125 mL Erlenmeyer flask. It was then stirred for 2 h at room temperature and filtered through a Whatman no. 42 filter paper (Whatman, Maidstone, U.K.). The extracts were freeze-dried under −50 °C, and the residue was redissolved in 10 mL of 80% high-performance liquid chromatography (HPLC) grade methanol. Methanolic extracts were filtered through a 0.45 μm membrane filter (nylon, Titan) and transferred to 1.0 mL vials.

A 20 μL sample of each filtrate was analyzed using an HPLC system equipped with a Young-Lin M930 pump, an M720 UV−vis detector at 280 nm, and a Marathon autoinjector with a 20 μL sample loop. Separation was achieved with a YMC-Pack ODS AM-303 (5 μm, 250 mm × 4.6 mm i.d.) column. The mobile phase was distilled water with 0.1% glacial acetic acid (solvent A) and acetonitrile with 0.1% glacial acetic acid (solvent B). The following gradient was used: 0 min, 8% B in A; 2 min, 10% B in A; 27 min, 30% B in A; 50 min, 90% B in A; 52−56 min, 100% B in A; 56−60 min, 8% B in A. Run time was 60 min, and the flow rate was maintained at 0.8 mL/min.

Determination of Proanthocyanidins Content. The proanthocyanidins content of the barley was determined according to the modified vanillin assay of Sun et al. (*21*). Powdered whole grain sample (0.2 g) was extracted with 10 mL of methanol containing 1% HCl in a 50 mL conical flask. The mixture was stirred for 2 h at room temperature and centrifuged at 3000*g* for 10 min. The supernatant was filtered through a 0.45 μm membrane filter (nylon, Titan) and used to determine the content of proanthocyanidins. Two milliliters of methanolic extract solution, 2.5 mL of 1% (w/v) of vanillin in methanol, and 2.5 mL of 25% (v/v) of H_2SO_4 in methanol were mixed. The absorbance was measured 20 min later at 500 nm. The results were expressed in

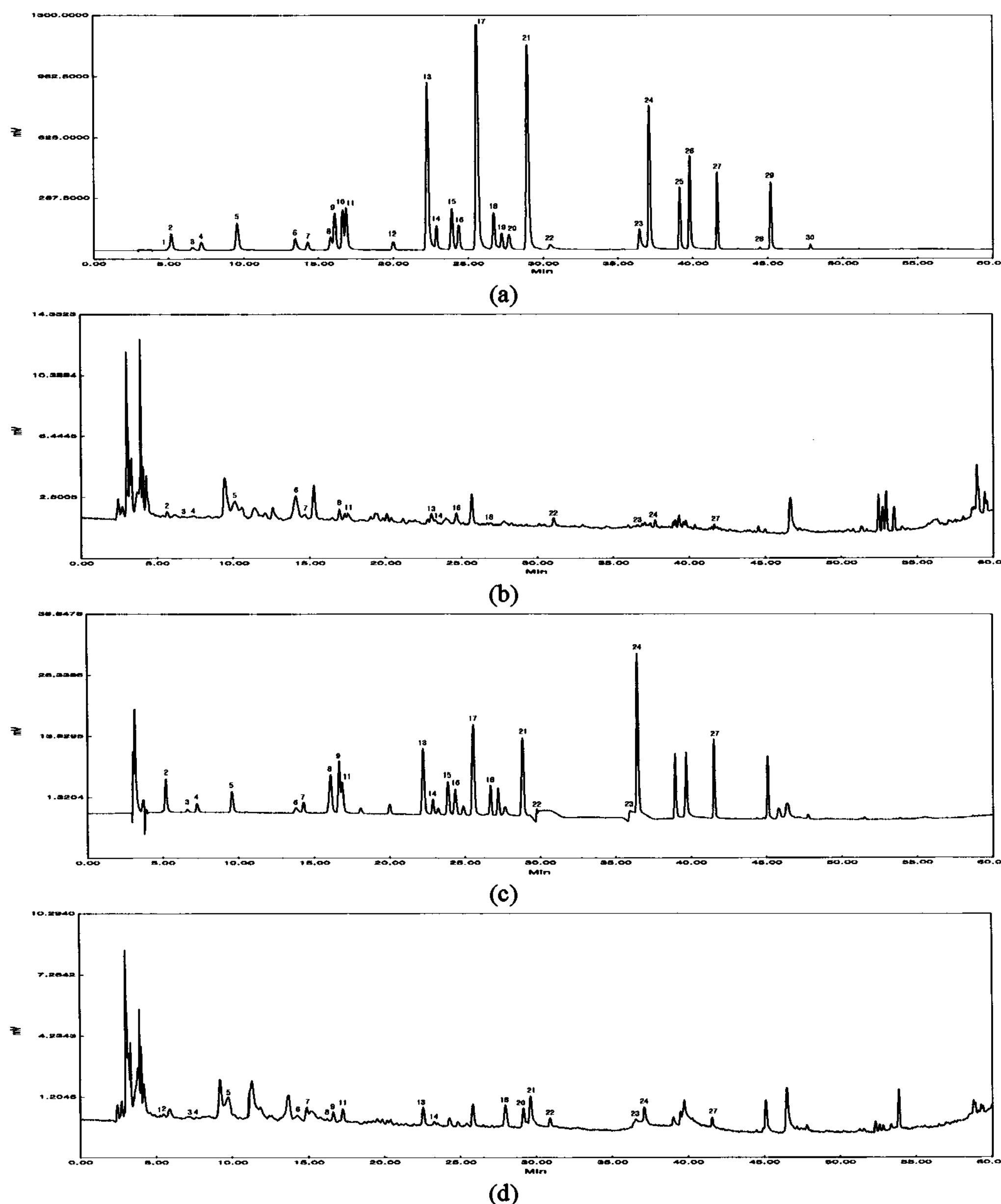

Figure 2. Chromatograms of phenolic compounds in colored barley: (**a**) standards of phenolic compounds; (**b**) black; (**c**) blue; (**d**) purple. Peaks: (1) phloroglucinol; (2) gallic acid; (3) pyrogallol; (4) homogentisic acid; (5) protocatechuic acid; (6) chlorogenic acid; (7) catechin; (8) β-resorcylic acid; (9) vanillic acid; (10) syringic acid; (11) caffeic acid; (12) *o*-hydroxyphenylacetic acid; (13) *p*-coumaric acid; (14) rutin; (15) ferulic acid; (16) 3,4-dimethoxybenzoic acid; (17) *m*-coumaric acid; (18) salicylic acid; (19) naringin; (20) hesperidin; (21) benzoic acid; (22) *o*-coumaric acid; (23) myricetin; (24) quercetin; (25) *trans*-cinnamic acid; (26) naringenin; (27) kaempferol; (28) hesperetin; (29) formononetin; (30) biochanin A.

Table 1. Correlation[a] among Antioxidant Activity, Phenolics, and Anthocyanins in Colored Barley

	DPPH	total phenolic acids	total flavon-oids	total phenol-ics	pro-anthocy anidins	total antho-cyanins
DPPH		0.38***	0.21*	0.37**	0.56***	
total phenolic acids			0.47***	0.92***	0.52***	0.41***
total flavonoids				0.77***	0.28**	0.32***
total phenolics					0.50***	0.43***
proanthocyanidins						0.58***
total anthocyanins						

[a] *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

micrograms per gram of catechin in comparison with standard (+)-catechin treated in the same conditions.

Quantitative Analysis of Anthocyanins. Analysis of anthocyanins was conducted according to the modified method of Kim et al. (*22*). The 11 anthocyanin standards, cyanidin, cyanidin-3-glucoside, delphinidin, delphinidin-3-glucoside, malvidin, malvidin-3-glucoside, pelargonidin, pelargonidin-3-glucoside, peonidin, peonidin-3-glucoside, and petunidin, were purchased from Extrasynthese and used for calibration curves. The standard stock solutions (100 ppm) were made with 80% MeOH containing 0.1% HCl in a dark cold room. The other procedures were the same as for the analysis of phenolic compounds.

Each 0.2 g powdered whole grain sample was added to 2 mL of 80% methanol containing 0.1% HCl in a conical flask (wrapped with aluminum foil) for 24 h at 4 °C. The mixture was centrifuged at 10000*g* for 10 min, and the supernatant was filtered through a 0.45 μm membrane filter (nylon, Titan) and transferred to an amber glass vial.

A 20 μL sample of each filtrate was analyzed using a Waters 2695 Separations Module system equipped with a Waters 2487 dual λ absorbance detector at 520 nm with an injection volume was 20 μL. Separation was achieved with an XTerra RP18 (5 μm, 250 mm × 4.6 mm i.d.) column. The mobile phase was 5% formic acid in water (solvent A) and water/acetonitrile/formic acid (50:45:5, v/v/v; solvent

Table 2. Total Concentrations (Micrograms per Gram) of Phenolic Compounds in Colored Barley Groups

	hulled				unhulled			
	black 1	black 2	black 3	purple	black	blue	purple	LSD (0.05)
phenolic acids								
Phl[a]	2.4 ± 7.0d[b]	0.8 ± 2.8d	3.6 ± 10.4d	19.3 ± 27.3c	46.3 ± 21.4ab	52.8 ± 59.6a	34.2 ± 16.5bc	15.41
Gal	14.6 ± 0.5a	15.2 ± 0.9a	15.0 ± 1.0a	14.6 ± 0.5a	15.6 ± 0.9a	15.7 ± 13.3a	15.7 ± 1.6a	2.42
Pyr	15.7 ± 8.0a	19.7 ± 4.4a	23.3 ± 11.0a	15.2 ± 0.4a	21.8 ± 11.0a	22.2 ± 3.1a	22.5 ± 9.0a	8.96
Hom	5.5 ± 3.3c	7.8 ± 4.3abc	10.3 ± 4.5ab	7.2 ± 1.4ab	11.3 ± 5.1ab	11.9 ± 0.4a	9.8 ± 2.7ab	4.24
Pro	8.1 ± 6.6a	7.7 ± 5.4a	13.2 ± 5.7a	8.6 ± 1.8a	9.3 ± 1.7a	12.2 ± 7.4a	10.7 ± 7.0a	6.28
Chl	30.0 ± 7.1ab	33.3 ± 9.4ab	38.8 ± 12.7a	25.0 ± 2.6b	29.2 ± 6.2ab	35.6 ± 6.1a	30.6 ± 6.1ab	10.16
Res	4.5 ± 1.8b	4.0 ± 1.0b	5.1 ± 4.3b	6.3 ± 2.0b	4.0 ± 1.3b	19.5 ± 18.7a	4.1 ± 2.0b	4.18
Van	0.5 ± 0.8b	0.4 ± 0.5b	0.3 ± 0.8b	0.4 ± 0.6b	0.2 ± 0.3b	9.3 ± 9.0a	1.1 ± 1.4b	1.71
Syr		0.0 ± 0.1b				1.4 ± 1.1a	0.0 ± 0.2b	0.20
Caf	10.3 ± 2.3a	10.3 ± 2.5a	8.4 ± 4.8a	11.1 ± 0.5a	11.6 ± 1.1a	11.0 ± 0.4a	8.1 ± 5.1a	3.86
Hyd		0.1 ± 0.3a	0.1 ± 0.5a			0.1 ± 0.1a	0.2 ± 0.7a	0.43
pCo	3.4 ± 0.5ab	3.0 ± 0.4bc	2.9 ± 0.3cd	3.3 ± 0.3ab	2.6 ± 0.1d	3.5 ± 0.9a	2.7 ± 0.3cd	0.41
Fer	0.2 ± 0.3b	0.2 ± 0.2b	0.1 ± 0.2b			2.6 ± 3.5a		0.62
Dim	0.1 ± 0.3c	0.1 ± 0.3c	0.2 ± 0.4c	1.9 ± 2.6a		0.9 ± 1.3b	0.2 ± 0.4c	0.60
Sal	10.9 ± 6.9b	11.0 ± 7.0b	14.2 ± 4.3b	15.8 ± 0.7b	16.4 ± 0.6b	28.1 ± 15.3a	15.5 ± 4.1b	6.43
Ben	2.0 ± 3.5c	4.4 ± 7.6c	1.9 ± 1.6c	12.4 ± 9.5a	4.6 ± 6.3bc	9.2 ± 13.1ab	2.3 ± 5.4c	6.11
Co	4.1 ± 1.0c	3.8 ± 1.3c	4.5 ± 1.6c	10.4 ± 5.7a	4.3 ± 0.2bc	5.2 ± 1.2bc	7.5 ± 4.7b	2.46
total	112.3 ± 23.6e	121.8 ± 17.3cd	142.0 ± 30.6cd	151.5 ± 20.0bc	177.2 ± 24.4b	241.3 ± 62.2a	165.2 ± 30.6bc	28.74
flavonoids								
Cat	17.7 ± 6.0ab	17.6 ± 6.7ab	16.7 ± 6.9ab	13.1 ± 3.3b	16.8 ± 6.1ab	23.8 ± 13.3a	20.0 ± 10.3ab	7.90
Rut	3.2 ± 1.7a	3.0 ± 1.4a	3.7 ± 2.2a	4.6 ± 1.2a	0.7 ± 1.1b	4.6 ± 2.9a	3.4 ± 2.7a	2.10
Nar	0.0 ± 0.0a	0.1 ± 0.2a	0.1 ± 0.2a				0.1 ± 0.2a	0.17
Hes	0.4 ± 1.3b	0.2 ± 0.7b	0.2 ± 0.8b	2.4 ± 3.4a		1.0 ± 1.5b	0.4 ± 1.0b	1.13
Myr	28.0 ± 1.1c	27.7 ± 1.0c	27.7 ± 1.1c	39.1 ± 13.6a	33.4 ± 8.6b	42.6 ± 16.0a	31.4 ± 2.2bc	4.29
Que	17.4 ± 1.1b	17.1 ± 3.0b	17.4 ± 0.7b	19.3 ± 2.0b	14.8 ± 6.0b	66.6 ± 69.8a	17.9 ± 1.6b	11.76
Kae	12.7 ± 2.0b	12.3 ± 0.6b	12.2 ± 0.9b	13.4 ± 2.1b	11.9 ± 0.2b	23.9 ± 16.9a	13.1 ± 2.3b	3.14
total	79.3 ± 8.4b	78.1 ± 7.9b	77.9 ± 7.9b	91.9 ± 19.9b	77.6 ± 10.2b	162.5 ± 98.3a	86.3 ± 12.8b	18.99
total phenolic compounds	191.6 ± 26.2c	199.9 ± 20.2c	220.0 ± 34.7bc	243.4 ± 15.2b	254.7 ± 26.1b	403.8 ± 144.9a	251.4 ± 38.0b	39.02

[a] Phl, phloroglucinol; Gal, gallic acid; Pyr, pyrogallol; Hom, homogentisic acid; Pro, protocatechuic acid; Chl, chlorogenic acid; Res, β-resorcylic acid; Van, vanillic acid; Syr, syringic acid; Caf, caffeic acid; Hyd, *o*-hydroxyphenylacetic acid; pCo, *p*-coumaric acid; Fer, ferulic acid; Dim, 3,4-dimethoxybenzoic acid; Sal, salicylic acid; Ben, benzoic acid; oCo, *o*-coumaric acid; Cat, catechin; Rut, rutin; Nar, naringin; Hes, hesperidin; Myr, myricetin; Que, quercetin; Kae, kaempferol. [b] Means in the same column with different letters are significantly different ($p < 0.05$).

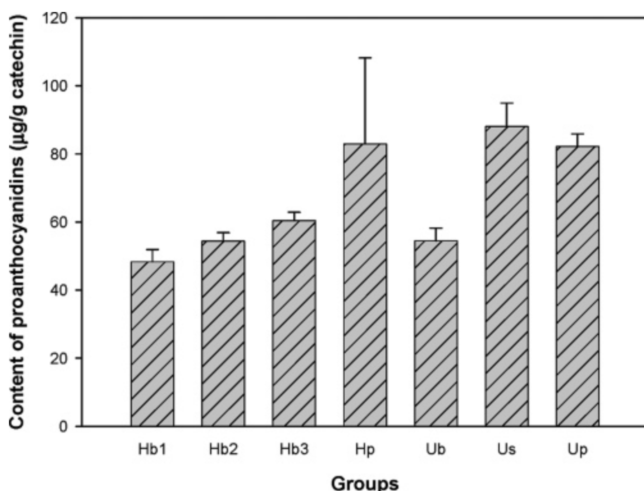

Figure 3. Content of proanthocyanidins in colored barley groups: Hb1, hulled black 1; Hb2, hulled black 2; Hb3, hulled black 3; Hp, hulled purple; Ub, unhulled black; Us, unhulled blue; Up, unhulled purple.

B). The following gradient was used: 0 min, 25% B in A; 10 min, 27% B in A; 44 min, 33% B in A; 50 min, 100% B in A. Run time was 50 min, and the flow rate was maintained at 0.9 mL/min.

Measurements of Antioxidant Activity. Extracts for the determination of DPPH radical scavenging activity were prepared by mixing 5 g of ground whole grain samples with 100 mL of 80% methanol. The mixture was stirred at room temperature for 24 h and then filtered through a Whatman no. 42 filter paper. The filtrate was evaporated at below 30 °C and concentrated to a powder using a freeze-dryer. Residues were dissolved in 80% methanol to make a 1% solution (w/v) and then kept in a refrigerator until required for analysis.

The free radical scavenging capacity of grain extracts was measured using stable DPPH radicals according to the method of Yoshida et al. (*23*). Whole grain flour extracts (0.25 mL) were added to 2.5 mL of 0.35 mM DPPH in 50% ethanol. The mixture was gently homogenized and left at room temperature for 1 min in the dark. Absorbance of the reaction solution was measured spectrophotometrically at 517 nm. The percentage of DPPH inhibition of the sample was calculated according to the equation

$$\text{DPPH radical scavenging activity (\%)} = [(\text{ABS}_{\text{control}} - \text{ABS}_{\text{sample}})/\text{ABS}_{\text{control}}] \times 100$$

Statistical Analyses. Analyses of variance of results were performed using both the general linear model and the cluster procedure using the Ward method from SAS statistical software, version 9.1 (*24*). Results reported in this study are averages of three replications. Comparisons of anthocyanins, proanthocyanidins, phenolic acids, and flavonoids contents and DPPH radical scavenging activity were carried out with least significant difference (LSD) tests at a 0.05 probability level.

RESULTS AND DISCUSSION

Classification of Colored Barley. Barley can be classified as black, blue, and purple on the basis of lemma, pericarp, and aleurone color. The results of colored barley classifications are presented in **Figure 1** and Supporting Information Table 1. First, barley was divided into black, blue, and purple groups by macroscopic classifications. Following this, instrumental color measurements of colored barley were performed for classifications within the hulled black group. The *L* values had a high

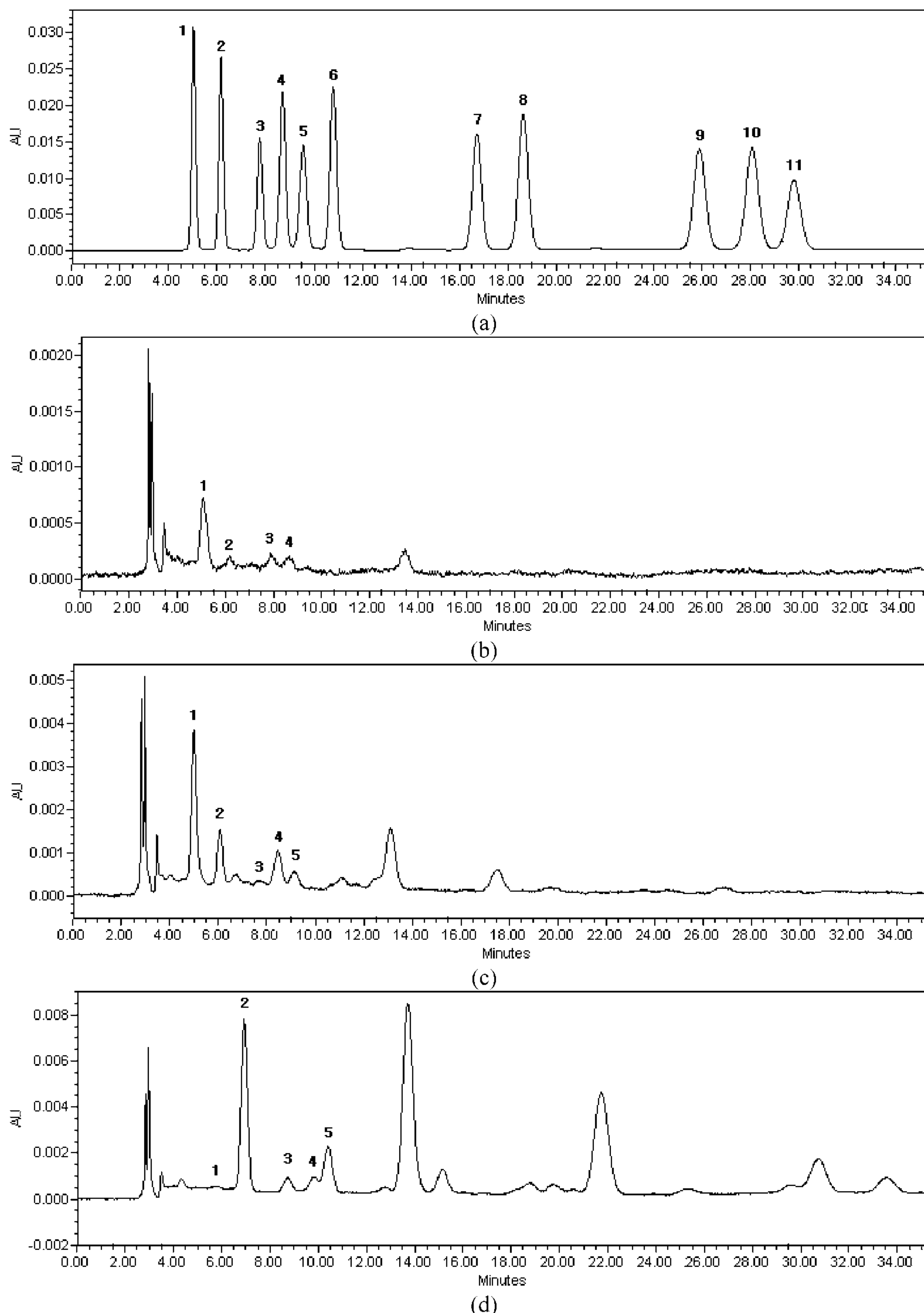

Figure 4. Chromatograms of anthocyanins in colored barley: (**a**) standards of anthocyanins; (**b**) black; (**c**) blue; (**d**) purple. Peaks: (1) delphinidin-3-glucoside; (2) cyanidin-3-glucoside; (3) pelargonidin-3-glucoside; (4) peonidin-3-glucoside; (5) malvidin-3-glucoside; (6) delphinidin; (7) cyanidin; (8) petunidin; (9) pelargonidin; (10) peonidin; (11) malvidin.

positive correlation with the *b* values (0.84***). The *L* and *b* values, which indicate the relative lightness and yellowness of the sample, respectively, were standard classifications in hulled black barley. These lines were divided into three groups using the Proc Ward minimum variance method in the SAS system because they were virtually identical under macroscopic classification. The *a* values (redness) were highest in the purple barley group. This result was similar to that observed with colored rice (*22*). Barley was placed into seven groups: hulled black 1 (Hb1), 2 (Hb2), and 3 (Hb3); hulled purple (Hp); unhulled black (Ub); unhulled blue (Us); and unhulled purple (Up).

Composition of Phenolic Compounds in Colored Barley. Phenolic compound profiles were analyzed in 127 colored barley lines (**Figure 2**). Total concentrations of phenolic compounds in colored barley varied from 191.6 to 403.8 μg/g (**Table 2**). The total average contents of phenolic compounds in the unhulled groups (268.6 μg/g) were significantly higher than that in the hulled groups (207.0 μg/g). Total average contents of phenolic compounds in the blue and purple groups (269.5 μg/g) were significantly higher than that of the black groups (209.1 μg/g). In the Hb groups, total average contents of phenolic compounds of the anthocyanin-containing lines were significantly higher than that in the non-anthocyanin-containing lines.

Table 3. Total Average Concentrations (Micrograms per Gram) of Anthocyanins[a] in Colored Barley Groups

group	Delg	Cyag	Pelg	Peog	Malg	total
hulled black 1	42.4 ± 23.9b[b]	8.6 ± 11.3b	20.3 ± 13.0bc	4.9 ± 10.4b	0.8 ± 3.4c	77.0 ± 51.9b
hulled black 2	39.0 ± 15.3b	12.3 ± 9.4b	16.5 ± 11.5b	5.6 ± 12.5b	0.6 ± 3.5c	74.0 ± 36.1b
hulled black 3	22.3 ± 14.5bc	21.2 ± 19.9b	12.1 ± 10.1c	3.3 ± 6.1b	1.0 ± 3.6c	59.8 ± 31.0b
hulled purple	22.5 ± 10.8bc	239.3 ± 153.4a	42.2 ± 24.0a	32.7 ± 19.3a	13.7 ± 11.6b	350.3 ± 216.7a
unhulled black	31.6 ± 24.7bc	24.1 ± 6.6b	17.8 ± 12.2bc	8.0 ± 9.6b	3.0 ± 7.4bc	84.5 ± 37.2b
unhulled blue	167.6 ± 90.5a	54.8 ± 25.8b	41.4 ± 29.2a	35.2 ± 27.0a	38.6 ± 28.6a	337.6 ± 152.4a
unhulled purple	14.7 ± 11.4c	210.7 ± 172.9a	35.6 ± 24.9ab	37.5 ± 32.0a	14.3 ± 19.3b	312.7 ± 244.3a
LSD (0.05)	26.45	91.64	18.36	20.04	12.01	137.39

[a] Delg, delphinidin-3-glucoside; Cyng, cyanidin-3-glucoside; Pelg, pelargonidin-3-glucoside; Peog, peonidin-3-glucoside; Malg, malvidin-3-glucoside. [b] Means in the same column with different letters are significantly different ($p < 0.05$).

In this experiment, Ub groups had the highest concentration of phenolic compounds.

Contents of phenolic compounds differed in each group: these are presented in **Table 2**. Among the 30 phenolic compound standards, 17 phenolic acids and 7 flavonoids were detected in this study (**Table 2**). Although biochanin A, *trans*-cinnamic acid, *m*-coumaric acid, formononetin, hesperetin, and naringenin were detected in all barley groups, they were not quantified because of poor resolution or low levels.

The total content of phenolic acids varied from 90.2 to 287.4 μg/g. Total average phenolic acids content in the Ub group was significantly different from that of other groups at the 0.05 probability level. Chlorogenic acid and phloroglucinol were present in hulled and unhulled groups as the major compounds, respectively. All barley groups had very low concentrations of syringic acid, *o*-hydroxyphenylacetic acid, ferulic acid, and 3,4-dimethoxybenzoic acid. However, Zieliński et al. (*25*) reported that ferulic acid is the dominant phenolic acid found in wheat, barley, and rye. Ferulic acid is a major low molecular weight phenolic in barley (*26*), and genetic or cultivar differences in its content could result from differences in caryopsis structure (*27*). In the Hb groups, grain color was darker and they contained higher concentrations of phenolic acids ($P > 0.05$). Compared to unhulled, hulled groups contained significantly lower concentrations of phloroglucinol, homogentisic acid, vanillic acid, syringic acid, salicylic acid, and *o*-coumaric acid. In contrast, *p*-coumaric acid was high. The concentrations of phloroglucinol acid, vanillic acid, syringic acid, *o*-hydroxyphenylacetic acid, 3,4-dimethoxybenzoic acid, salicylic acid, and *o*-coumaric acid in the blue and purple groups were significantly higher than those in the black groups.

The total content of flavonoids varied from 62.0 to 300.8 μg/g. Total average flavonoid content in the Us group was significantly different from that of other groups. However, there is no significant difference among the Hb groups. In all groups, there was no significant difference in rutin and naringin contents.

In colored barley, myricetin was the main flavonoid, followed by (+)-catechin and quercetin. In contrast, the colored barleys contained only traces of naringin and hesperidin. Flavan-3-ols constitute the major class of phenolics in barley and appear in both monomer [(+)-catechin and (−)-epicatechin] and polymer forms [unit of (+)-catechin and (+)-gallocatechin]. They are considered to be primary antioxidants that react with lipid radicals to convert them to more stable products (*28*). The myricetin and kaempferol contents in the unhulled groups were higher than that of the hulled groups. In comparison with other groups, the concentrations of quercetin and kaempferol in the Us group and that of hesperidin in Hp groups were significantly high. Compared to the black groups, the concentrations of naringin, quercetin, and kaempferol in the blue and purple groups were significantly different.

Content of Proanthocyanidins in Colored Barley. The content of proanthocyanidins varied from 15.8 to 131.8 μg/g of catechin. The highest level occurred in Shiras in the Up group, whereas the lowest was SLB34-65 in the Hb2 group. The content of proanthocyanidins in the unhulled barley groups (75.9 μg/g of catechin) was higher than that in the hulled barley groups (56.2 μg/g of catechin). The blue and purple groups (83.0 μg/g of catechin) contained significantly higher amounts than those in the black groups (55.3 μg/g of catechin) (**Figure 3**). In the Hb groups, contents of proanthocyanidins of the anthocyanin-containing lines (52.6 μg/g catechin) were significantly lower than those in the non-anthocyanin-containing lines (60.5 μg/g of catechin). In comparison among hulled black groups, group 3 had a significantly higher level than group 1. The content of proanthocyanidins had a high positive correlation with the content of total phenolic acid (0.52***), flavonoids (0.28**), and anthocyanins (0.58***) (**Table 1**).

Composition of Anthocyanins in Colored Barley. The highest efficiency in extracting pigments from colored barley was in a solution of 80% MeOH that contained 0.1% HCl. As such, colored barley was extracted with this method to detect the UV−vis absorption spectrum of pigment compounds. The acidic alcohol extract was determined at an absorbance of 200−700 nm. Colored barley lines possessed maximum absorption near 520 nm except for some of the black lines. Therefore, the total anthocyanin composition in anthocyanin-containing lines was determined using HPLC (**Figure 4**). Results of anthocyanin concentrations of different barley groups are presented in **Table 3**. The aglycon forms of anthocyanins, cyanidin, delphinidin, pelargonidin, peonidin, malvidin, and petunidin, were not detected in colored barley.

The total concentration of anthocyanins in the colored barley varied from 13.0 to 1037.8 μg/g. The highest concentration was detected Shiras in the Up group, whereas the lowest was SLB34-65 in the Hb2 group. Total average concentrations of anthocyanins in the blue and purple groups (320.5 μg/g) were significantly higher than those in the black group (49.0 μg/g). Jende-Strid (*29*) reported that yellow, blue, and black barley varieties contain delphinidin and cyanidin and that purple varieties also contain pelargonidin. Abdel-Aal et al. (*30*) reported that only two anthocyanins, cyanidin 3-glucoside (1.2 μg/g) and petunidin 3-glucoside (2.9 μg/g), were detected in the blue barley (cv. Tankard). In the present study, delphinidin and pelargonidin were detected and petunidin was not detected in the blue and black groups. The most common anthocyanin in the purple group was cyanidin 3-glucoside (214.8 μg/g), followed by peonidin 3-glucoside and pelargonidin 3-glucoside, being about 50−79% of the total anthocyanins, whereas delphinidin 3-glucoside was the most abundant anthocyanin in the blue (167.6 μg/g) and black (36.0 μg/g) barley groups. In all black groups,

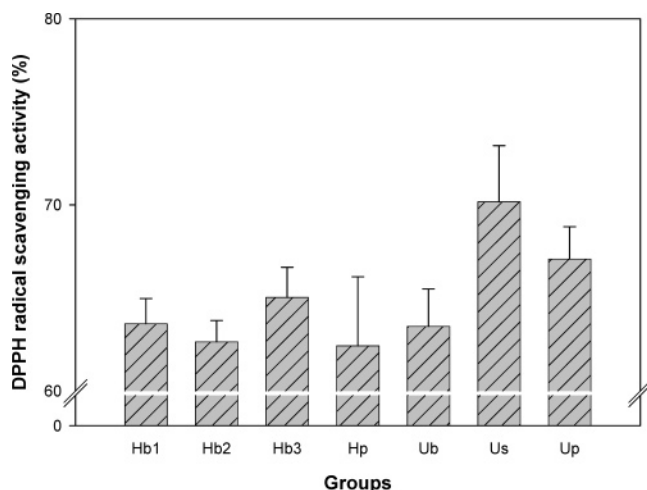

Figure 5. DPPH radical scavenging activities in colored barley groups Hb1: hulled black 1, Hb2: hulled black 2, Hb3: hulled black 3, Hp: hulled purple, Ub: unhulled black, Us: unhulled blue, Up: unhulled purple.

peonidin 3-glucoside and malvidin 3-glucoside were detected, but they were not quantified because of poor resolution or low levels.

Antioxidant Activity in Colored Barley. The antioxidant properties of phenolic compounds are somewhat associated with the number of OH groups found in their molecular structures. Phenolic acids have been shown to possess high antioxidant activity. Flavonoids and anthocyanins also have antioxidant activity. The DPPH radical scavenging activities of the colored barley groups are shown in **Figure 5**. In colored barley, the DPPH radical scavenging activities varied from 46.4 to 86.3%. The highest radical scavenging activity of varieties and lines was found in D.S.B-547 (86.3%, in the Hb3 group), and the lowest activity was found in D.S.B-61 (46.4%, in the Hb3 group). Average radical scavenging activity in the unhulled barley groups (66.5%) was higher than that of hulled barley (63.5%). Highest DPPH activity was in the Us group (68.5%), and the lowest activity was in the Hp group (62.4%). The radical scavenging activity among colored barley groups was not significantly different. However, in unhulled barley, the purple groups contained higher total average contents of anthocyanins than did that of the black groups, and the DPPH radical scavenging activity of purple (67.4%) groups was higher than that of the black (63.5%) groups. This result indicates that antioxidant capacity is related to the concentration of phenolic compounds such as chlorogenic acid, 3,4-dimethoxybenzoic acid, homogentisic acid, protocatechuic acid, and rutin. DPPH radical scavenging activity had high positive correlation with the content of phenolic compounds and proanthocyanidins (**Table 1**).

Barley grains are used as components in various foods, and barley meals and fractions are now gaining renewed interest as ingredients in the production of functional foods such as in pasta and baked products (*31*). Devreux (*32*) reported that the presence of anthocyanins may be an important characteristic in the barley varieties used for beer production. In fact, when colored barley is fermented into beer, the resulting product has a pinkish hue that has to be removed by a clarification treatment. Although anthocyanins have an adverse effect in some foods, they can still be used as functional and healthy food materials. At present, other colored wheat, rice, and corn are used as various functional foods or functional food colorants. Moreover, phenolic compounds and proanthocyanidins also have potent antioxidant capacity and possible protective effects on human health. These results suggest that phenolic compounds, proanthocyanidins, and anthocyanins could be used as important components of foods produced by colored barley, and these colored barley germplasms could be good resources in breeding of functional cereals.

Supporting Information Available: List and characteristics of the barley germplams used in this study. This material is available free of charge via the Internet at http://pubs.acs.org.

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