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Phenolic Compounds of Barley Grain and Their Implication in Food Product Discoloration

ZORY QUINDE-AXTELL AND BYUNG-KEE BAIK*

Department of Food Science and Human Nutrition, Washington State University, Pullman, Washington 99164-6376, and Department of Crop and Soil Sciences, Washington State University, Pullman, Washington 99164-6420

Barley grains contain significant amounts of phenolic compounds that may play a major role in the discoloration of food products. Phenolic acid and proanthocyanidin (PA) composition of 11 barley genotypes were determined, using high-performance liquid chromatography and liquid chromatography−mass spectrometry, and their significance on food discoloration was evaluated. Abraded grains contained 146−410 μg/g of phenolic acids (caffeic, *p*-coumaric, and ferulic) in hulled barley and 182−282 μg/g in hulless barley. Hulled PA-containing and PA-free genotypes had comparable phenolic acid contents. Catechin and six major barley PAs, including dimeric prodelphinidin B3 and procyandin B3, and four trimers were quantified. PAs were quantified as catechin equivalents (CE). The catechin content was higher in hulless (48−71 μg/g) than in hulled (32−37 μg/g) genotypes. The total PA content of abraded barley grains ranged from 169 to 395 μg CE/g in PA-containing hulled and hulless genotypes. Major PAs were prodelphinidin B3 (39−109 μg CE/g) and procyanidin B3 (40−99 μg CE/g). The contents of trimeric PAs including procyanidin C2 ranged from 53 to 151 μg CE/g. Discoloration of barley flour dough correlated with the catechin content of abraded grains ($r = -0.932$, $P < 0.001$), but not with the content of individual phenolic acids and PAs. Discoloration of barley flour dough was, however, intensified when total PA extracts and catechin or dimeric PA fractions were added into PA-free barley flour. The brightness of dough also decreased when the total PA extract or trimeric PA fraction was added into heat-treated PA-free barley flour. Despite its low concentration, catechin appears to exert the largest influence on the discoloration of barley flour dough among phenolic compounds.

KEYWORDS: Phenolic acids; proanthocyanidins; catechin; barley; discoloration

INTRODUCTION

With the recognition of the health benefits of β-glucans, such as the reduction of serum cholesterol and blood glucose (*1*, *2*), there have been numerous attempts to diversify the food uses of barley by incorporating barley flour in bread, noodles, pasta, pastries, and extruded snacks. However, the dark discoloration of barley-based food products negatively affects consumer acceptability and limits increased utilization of barley in food formulations. Retardation of barley food discoloration through genotypic selection, reduction of polyphenol content, or polyphenol oxidase (PPO) activity of grains by abrasion, heat inactivation of PPO, exclusion of oxygen, and use of a reducing agent has been reported (*3*).

The phenolic compound content of grains, expressed as catechin equivalent (CE), has been found to be 24.3 μg/mg for barley, 17.6 μg/mg for oats, 10.2 μg/mg for wheat, and 8.9 μg/mg for rye (*4*). Discoloration of barley food products during processing may occur as a result of autoxidation, metal ion-catalyzed oxidation, or enzymatic reactions, as well as further polymerization of oxidized phenolic compounds. Polyphenols that have been identified in barley grains include anthocyanins, flavonols, phenolic acids, catechins, and proanthocyanidins (PAs) and are mainly located in the outer layers (OLs) of the grain (*5*, *6*). White/yellow aleurone barley had one-fifth of the anthocyanin content of blue aleurone barley, and weakly blue aleurone barley had half the anthocyanin content of blue aleurone barley (*7*). Blue aleurone barley had an anthocyanin content of 174−291 μg/g (*8*). The flavonol content in barley ranged from 10.9 to 66 μg rutin equivalent/g (*6*). Phenolic acids, hydroxybenzoic acids (*p*-hydroxybenzoic, protocatehuic, vanillic, gallic, and syringic), and hydrocinnamic acids (*p*-coumaric, caffeic, ferulic, and sinapic) have been identified in barley grains (*9*). The phenolic acids occur in small amounts in barley grains and constitute approximately 10% of the PA and catechin content in acetone extracts (*10*).

PAs consist of oligomeric and polymeric structures of flavan-3-ol monomers: (+)-catechin (c) and (+)-gallocatechin (gc). The most abundant PAs in barley are dimeric PAs: prodelphinidin B3 (gc-[4α-8]-c) and procyanidin B3 (c-[4α-8]-c). The

* To whom correspondence should be addressed. Tel: 509-335-8230. Fax: 509-335-8674. E-mail: bbaik@wsu.edu.

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main trimers, linked together by -[4α-8]- bonds, are T1 (gc-gc-c), T2 (gc-c-c), T3 (c-gc-c), and T4 or procyanidin C2 (c-c-c) (*11*). Including these main PAs, more than 50 different PAs have been detected in barley grains (*12*).

PA-free genotypes, which have reduced contents of phenolic compounds, exhibited the lowest discoloration potential of barley in food products (*13*). The significant relationship between total polyphenol content and discoloration potentials of barley grains in foods has also been reported by the same authors (*13*). Phenolic compounds, as substrates of PPO or by autoxidation, appeared to play a major role in the discoloration of barley. Our objectives were to identify and quantify phenolic acids and PAs from various types of barley and to determine their relationship to the discoloration potential of barley.

MATERIALS AND METHODS

Materials. This study was conducted in two parts. In the first part, 11 barley genotypes of hulled PA-containing (Harrington, Baronesse, Farmington, and Steptoe), hulled PA-free (Radiant, CA803803, and WA18009-94), hulless regular starch (Bear and CDC McGwire), and hulless waxy starch (SH97110 and CDC Candle) were evaluated. The barleys were grown in Pullman, WA, in 2000 and were stored in jars at 4 °C until used. Abraded grains were prepared by removal of the OLs by 30% in hulled and by 15% in hulless barley by weight, using a tangential abrasive dehulling device (TADD, Venables Machine Works, Ltd., Saskatoon, Canada). Both whole and abraded barley (20 g) were freshly ground with a cyclone sample mill (Udy Corp., Fort Collins, CO) fitted with a 0.5 mm opening and used for phenolic compound determination. Bear, grown in Royal Slope, WA, in 2001, was used to standardize the PA extraction and chromatography procedures. In the second part of this study, we extracted PAs from barley grains of cv. Bear and determined their influence on the discoloration of dough prepared from barley flour of cv. Radiant.

Chemicals. α-Amylase from *Aspergillus oryzae* and cellulase from *Aspergillus niger*, sodium acetate, ascorbic acid, sulfuric acid, petroleum ether, methanol, acetone, dimethylformamide (DMF), acetic acid, acetonitrile, and other reagents were purchased from Sigma-Aldrich (St. Louis, MO). Solvents used for high-performance liquid chromatography (HPLC) were HPLC grade. Gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, chlorogenic acid, vanillic acid, caffeic acid, and syringic acid were obtained from Sigma-Aldrich. (+)-Catechin, *p*-coumaric acid, and ferulic acid were purchased from Indofine Chemical Co., Inc. (Somerville, NJ).

Extraction of Phenolic Acids. Phenolic acids were extracted from whole and abraded barley, as described by Yu et al. (*14*). Barley flour (0.1 g) was treated with 0.2 N H_2SO_4 (1 mL) for 1 h at 100 °C. After it was cooled, the flour was digested with α-amylase (39 units/mg solid) and cellulase (1.18 units/mg solid), followed by centrifugation. The supernatant was filtered through a 0.45 μm filter and analyzed for phenolic acids, using HPLC with an injection volume of 30 μL in triplicate.

Extraction of PAs. PAs were extracted from whole and abraded barley according to Friedrich et al. (*11*), with the additional use of ascorbic acid and petroleum ether. Barley flour (5 g) was extracted twice with 50 mL of (3:1, v/v) acetone:water at 20 ± 2 °C for 1 h. Both extracts were combined, and ascorbic acid (1 mL, 25 mg/mL) was added to prevent PA oxidation. Petroleum ether (50 mL) was then added to the extract to remove lipids. The lower extract layer was concentrated to ~5 mL in a rotary evaporator at 35 ± 2 °C. The concentrated extract was purified by elution through a polyamide column with (3:1, v/v) acetone:water (100 mL) and concentrated to about 1 mL. The PA extract was transferred to a 5 mL flask, filled with 2% acetic acid:acetonitrile (98:2, v/v), and filtered through a 0.2 μm polytetrafluoroethylene (PTFE) filter. PAs were determined in triplicate, using HPLC with an injection volume of 30 μL.

Extraction and Fractionation of PAs from OLs of Barley Grains. OLs (15%) of grains of cv. Bear were obtained by abrasion. The total PA extract from the OLs (5 g) was obtained and purified using a polyamide column, as described above. The total PA extract was further fractionated by three step elutions: first, with 30 mL of methanol for the catechin fraction; second, with 90 mL of methanol for the dimeric PA fraction; and third, with 40 mL of DMF for the trimeric PA fraction. The catechin and dimeric PA fractions were concentrated to about 1 mL. The trimeric PA fraction was concentrated to about 0.2 mL by evaporation of DMF at 35 ± 2 °C. The total PA extract was purified as described previously. Both the total PA extract and its fractions were transferred to a 5 mL flask, filled with 2% acetic acid: acetonitrile (98:2, v/v), filtered through a 0.2 μm PTEF filter, and analyzed in duplicate, using LC-MS and HPLC with an injection volume of 10 μL.

Analytical HPLC. The HPLC system consisted of an autosampler [model 1050, Hewlett-Packard (HP), Wilmington, DE], a solvent-delivering system (model 2350 HPLC pump and model 2360 gradient programmer, ISCO, Inc., Lincoln, NE), a diode array detector (model 1040A, HP), and a computer equipped with HPLC 3D ChemStation-HP software. Separations were performed using a Zorbax SB-C18 column (150 mm × 4.6 mm i.d., 5 μm) (Agilent Technologies, Wilmington, DE).

Quantification of Phenolic Acids. For separation and quantification of phenolic acids, a gradient elution with a mobile phase consisting of water:acetonitrile:acetic acid (94.9:5:0.1, solvent A) and water:acetonitrile:acetic acid (79.9:20:0.1, solvent B) was used as follows: from 0 to 50% B linear gradient in 25 min, from 50 to 80% B linear in 20 min, from 80 to 100% B linear in 5 min, back to 0% B in 15 min, and 5 min of reconditioning before the next injection. The flow rate was 0.7 mL/min, and the column was maintained at 35 °C. Phenolic acids were detected at 280 nm.

Identification and Quantification of PAs. Analysis of phenolic compounds from the total PA extract and fractions was performed using an LC-MS system. The separation of PAs was carried out in an Agilent (Palo Alto, CA) 1100 series HPLC system. A solvent gradient consisting of 0.2% acetic acid (solvent A) and acetonitrile (solvent B) and a gradient from 3 to 18% B linear in 40 min, from 18 to 95% B linear in 5 min, 95% B isocratic for 2 min, back to 3% B in 5 min, and 14 min of reconditioning before the next injection were used. The flow rate was 0.6 mL/min. The column was maintained at 30 °C, and PAs were detected at 280 nm.

For identification of PAs, the LC eluent was applied to an Applied Biosystems API-4000 triple quadruple mass spectrometer equipped with an electrospray ionization source (ESI). For quantification of PAs, the same solvent gradient and conditions as in the HPLC-UV-ESI-MS were applied to the analytical HPLC. Identification of PAs was carried out by spiking with PA fractions and (+)-catechin, by matching retention times, and comparing spectral data. Comparison of the analytical HPLC and UV-ESI-MS chromatograms, which showed a similar pattern of retention time peaks, was also used to assist in the identification of PAs. Standard curves were prepared for injection volumes of 10 and 30 μL and expressed in μg CE/g on a dry basis.

Color of Gels and Dough Sheets. Gels and dough sheets were prepared from barley flour, as detailed by Quinde et al. (*13*). The color of barley flour gels and dough sheets was determined using a Minolta CM-2002 spectrophotometer (Minolta Camera Co., Ltd., Chuo-Ku, Osaka, Japan) and expressed as CIE Lab *L** values. The lower the *L** value, the more discoloration occurred.

Effect of PAs on Color of Barley Dough Sheets. Radiant, a hulled PA-free barley, was abraded by 30% of the kernel by weight and ground into flour. A portion of the PA-free flour was autoclaved at 121 °C for 15 min to inactivate PPO. The trimeric fraction, after evaporation of DMF, was dissolved in methanol (1 mL). PA extracts were added during mixing into the dough of both unheated and heated PA-free flour. Six PA extracts, including total PA extracted from 10 g of abraded kernels (AK), total PA extracted from 5 g of OLs, total PA extracted from 10 g of OL and catechin, and dimeric PA and trimeric PA fractions from 10 g of OL, were obtained and added into dough sheets.

Dough sheets (10 g flour) were prepared with 65% water for the unheated flour and with 75% for the heated flour. Color, *L** (brightness), *a** (redness-greenness), and *b** (yellowness-blueness) were measured immediately after preparation (0 h) and at 24 h after preparation on

Table 1. Phenolic Acid and PA Content of Whole Barley

	phenolic acids (μg/g)					PAs[a] (μg CE/g)						
						dimeric		trimeric				
class/genotype	caffeic	*p*-coumaric	ferulic	total phenolic acids	catechin (μg/g)	pd B3	pc B3	t1	t2	t3	pc C2	total PA
					hulled PA (+)[b]							
Farmington	30	57	437	524	53	173	165	102	76	65	67	700
Baronesse	26	68	511	604	45	166	160	91	71	61	64	659
Harrington	16	48	456	579	50	181	184	93	88	69	71	735
Steptoe	28	74	498	600	47	127	253	64	96	95	160	842
average[c] ($n = 4$)				562 a								734 b
					PA (–)							
Radiant	20	23	567	610	8	ND[d]	ND	ND	ND	ND	ND	8
CA803803	27	39	360	427	2	ND	ND	ND	ND	ND	ND	2
WA18009-94	25	64	473	562	9	ND	ND	ND	ND	ND	ND	9
average ($n = 3$)				533 a								6 c
					hulless regular							
Bear	18	4	355	377	86	288	191	141	90	79	56	930
CDC McGwire	15	6	493	514	59	207	251	111	100	89	100	918
average ($n = 2$)				445 ab								924 a
					waxy							
SH97110	29	9	335	373	72	162	200	78	85	63	91	751
CDC Candle	36	21	301	357	80	207	255	106	99	89	101	937
average ($n = 2$)				365 b								844 ab
LSD[e]	3	5	46		4	8	11	10	6	6	9	

[a] PAs are expressed as μg CE/g. Key: pd, prodelphinidin; pc, procyanidin; and t, trimers. [b] PA (+), PA-containing; and PA (–), PA-free. [c] Averages in the same column with different letters are significantly different ($P < 0.05$). [d] Not detectable. [e] Least significant difference ($P < 0.05$). Differences between two means in each column exceeding this value are significant.

duplicate samples. The PPO activity of unheated and heated barley flours was determined spectrophotometrically (*13*).

Statistical Analysis. Statistical analysis of data was performed using SAS software (SAS Institute, Cary, NC) with generalized linear model procedure, Fisher's least significant difference (LSD), and Pearson's correlation coefficient. Differences were considered significant at $P < 0.05$, unless otherwise specified.

RESULTS AND DISCUSSION

Phenolic Acids and PAs. The phenolic acids quantified in barley grains were caffeic, *p*-coumaric, and ferulic acids. In whole barley grains, the ferulic acid content ranged from 301 to 567 μg/g (**Table 1**), which is in agreement with previous studies (*15*, *16*). The *p*-coumaric acid content was significantly lower in hulless genotypes (4−21 μg/g) as compared to hulled genotypes (23−68 μg/g), which corresponds with earlier reports that the aleurone layer is high in ferulic acid, while *p*-coumaric acid is concentrated in the hull (*17*). The caffeic acid content ranged from 15 to 36 μg/g. For feed and malting barley, Hernanz et al. (*15*) reported a caffeic acid content of 7−18 μg/g. Significant differences among genotypes were observed for all phenolic acids. Among classes, hulless barley had a lower total phenolic acid content as compared to hulled barley, mainly due to the low *p*-coumaric acid content of hulless barley. Ferulic and caffeic acid contents were similar among classes. Hulled PA-containing and PA-free barleys had similar phenolic acid contents. Phenolic acids between PA-free barley mutants and their parents (PA-containing) were similar qualitatively and quantitatively (*9*).

Figure 1 shows the chromatograms of six major PAs and catechin in barley (cv. Harrington). Several researchers have identified these phenolic compounds in barley (*6*, *11*, *12*, *18*, *19*). PAs of whole barley grains are summarized in **Table 1**. PA-free genotypes had no detectable PAs but catechin at 2−9 μg/g. The major PAs in PA-containing genotypes were dimeric prodelphinidin B3 and procyanidin B3, both representing about

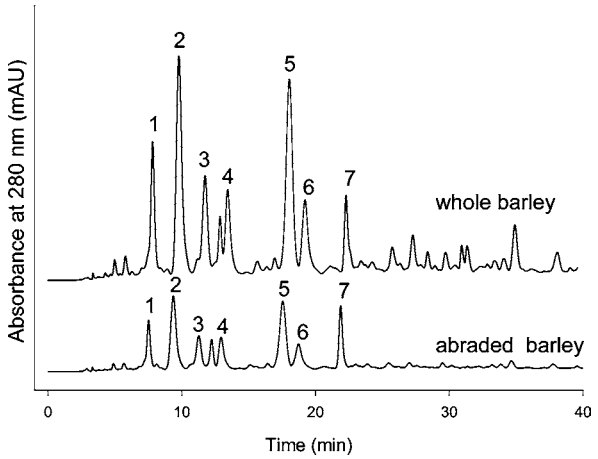

Figure 1. Chromatogram of catechin and major PAs in whole and abraded barley from cv. Harrington. Peak identification: 1, trimer 1; 2, prodelphinidin B3; 3, trimer 2; 4, trimer 3; 5, procyanidin B3; 6, procyanidin C2; and 7, catechin.

50% of the total PAs. Prodelphinidin B3 was higher than procyanidin B3 in Farmington, Baronesse, and Bear, similar in Harrington and lower in Steptoe, CDC McGwire, SH97110, and CDC Candle. Prodelphinidin B3 was higher in hulless regular genotypes than in hulled barley genotypes. The total content of four main trimeric PAs, which includes T1, T2, T3, and procyanidin C2 represented 39−49% of the total PA. Among these, trimeric PA, the T1 content was generally highest in all genotypes, except in Steptoe and SH97110. Hulless genotypes were higher in catechin content than hulled PA-containing genotypes. Significant differences in PA profiles and contents in barley whole grains among genotypes were observed, which is in agreement with previous reports (*12*, *18*).

In abraded barley, phenolic acid and PA contents were significantly lower (**Table 2** and **Figure 1**) as compared to

Table 2. Brightness (L^*) of Dough Sheets, Phenolic Acid, and PA Content of Abraded Barley

		phenolic acids (μg/g)					PAs[a] (μg CE/g) dimeric		trimeric				
class/genotype	L^* at 24 h	caffeic	*p*-coumaric	ferulic	total phenolic acids	catechin (μg/g)	pd B3	pc B3	t1	t2	t3	pc C2	total PA
						hulled PA (+)[b]							
Farmington	68.2	10	9	128	147	37	39	40	15	13	14	11	169
Baronesse	67.0	9	21	156	186	33	60	59	31	28	21	22	254
Harrington	69.5	10	9	167	187	32	60	62	31	30	25	25	265
Steptoe	67.8	10	6	195	210	33	50	99	23	37	35	56	331
average[c] ($n = 4$)					182 a								255 a
						PA (–)							
Radiant	75.0	trace	5	193	197	ND[d]	ND	ND	ND	ND	ND	ND	
CA803803	76.5	15	6	171	192	ND	ND	ND	ND	ND	ND	ND	
WA18009-94	78.1	11	20	212	244	4	ND	ND	ND	ND	ND	ND	4
average ($n = 3$)					211 a								
						hulless regular							
Bear	62.8	9	trace	173	182	71	109	75	59	30	33	18	395
CDC McGwire	63.9	11	3	190	204	48	62	76	33	34	28	32	312
average ($n = 2$)					193 a								353 a
						waxy							
SH97110	59.0	16	3	263	282	69	56	69	29	29	24	29	304
CDC Candle	60.6	12	3	184	199	69	70	92	37	32	34	35	370
average ($n = 2$)					240 a								337 a
LSD[e]		1	1	20		4	7	7	7	4	7	4	

[a] PAs are expressed as μg CE/g. Key: pd, prodelphinidin; pc, procyanidin; and t, trimers. [b] PA (+), PA-containing; and PA (–), PA-free. [c] Averages in the same column with different letters are significantly different ($P < 0.05$). [d] Not detectable. [e] Least significant difference ($P < 0.05$). Differences between two means in each column exceeding this value are significant.

whole barley (**Table 1** and **Figure 1**). Significant differences among genotypes for all phenolic acids and PAs were also observed. Similar to whole barley, ferulic acid was the most abundant in all genotypes, and the *p*-coumaric content was lower in hulless barley genotypes than in hulled genotypes. Significant differences in total phenolic acids were observed among genotypes but not among classes.

No PAs were detected in PA-free genotypes, but catechin was found in WA18009-94 at 4 μg/g. Among PA-containing genotypes, prodelphinidin B3 and procyanidin B3 were the major PAs, representing 41−47% of the total PA. The relative content of catechin increased by abrasion from 6−10 to 10−23% of the total PA. Hulless and hulled PA-containing genotypes exhibited comparable amounts of dimeric and trimeric PAs, while the catechin content was higher in hulless than in hulled PA-containing genotypes. No significant differences in total PA among classes were observed (**Table 2**).

Relationships between Phenolic Compounds and Discoloration Potential of Barley. The total polyphenol content is related to the discoloration potential of barley, playing a major role in the discoloration of barley-based food products (*13*). However, individual phenolic acids and total phenolic acids showed no relationship with brightness of gels or dough sheets. The lack of relationship between individual phenolic acids and discoloration potential of barley may be partly attributed to the narrow range of phenolic acid concentrations among classes and genotypes evaluated.

Catechin and individual PAs exhibited significant correlations with brightness of gels and dough sheets, which is mainly due to the obvious differences in PA contents between PA-containing and PA-free genotypes. Within PA-containing genotypes, the catechin content was related to the brightness (L^*) of dough sheets ($r = -0.932$, $P < 0.001$), while the content of PAs was not related to the brightness of gels or dough sheets.

Total PA Extract and Its Fractions. The fractions and total PA extract obtained from the OLs (15%) of cv. Bear are shown in **Figure 2**, and their concentrations are summarized in **Table 3**. In the catechin fraction (**Figure 2A**), catechin was the major compound, followed by a catechin glucoside with an ion $[M - H]^-$ at $m/z = 451$ (*20*). Ferulic and vanillic acids were also identified.

The major dimeric PAs were prodelphinidin B3 and procyanidin B3, followed by other dimeric PAs with molecular ions at $m/z = 593$ and $m/z = 577$ (**Figure 2A**). A previous LC-MS study of barley phenolics also detected similar phenolic compounds in the dimeric PA fraction (*11*). The major trimers were T1, T2, T3, and procyanidin C2. Trimeric PAs with ion masses at $m/z = 881$, 865, and 897 were also observed. Prodelphinidin B3, catechin, and dimeric PAs with ion masses at $m/z = 609$ and 593 were also present. Small amounts of poorly separated dimeric, trimeric, and tetrameric PAs were also detected in the trimeric fraction (**Figure 2A** and **Table 3**).

The total PA extract contained all of the PAs described above (**Figure 2B**). Other PAs, such as a dimeric PA at $m/z = 593$, a trimeric PA at $m/z = 913$, and poorly separated oligomeric PAs were also detected.

Effect of PAs on Discoloration Potential of Barley. The effect of PA extracts on discoloration of barley dough sheets is summarized in **Table 4**. For the unheated PA-free flour, the addition of PAs extracted from 10 g of AK significantly decreased the brightness (L^*) of dough sheets as compared to the control. The amount of PAs extracted from 5 g of OL was about five times the amount of PAs extracted from 10 g of AK. However, the addition of a high level of PAs did not further decrease the brightness of the dough sheets. Similarly, the addition of twice the amount of PAs extracted from 10 g of OL did not further decrease the L^* value of the dough sheets. **Figure 3** shows the decreases in brightness (ΔL^*) of dough sheets with added PAs at 24 h of storage. The addition of three different levels of total PAs, 3.9 mg of CE from AK, and 18.1 and 36.2 mg of CE from OL of barley grains resulted in similar ΔL^*. It appears that at high PA concentration, discoloration of

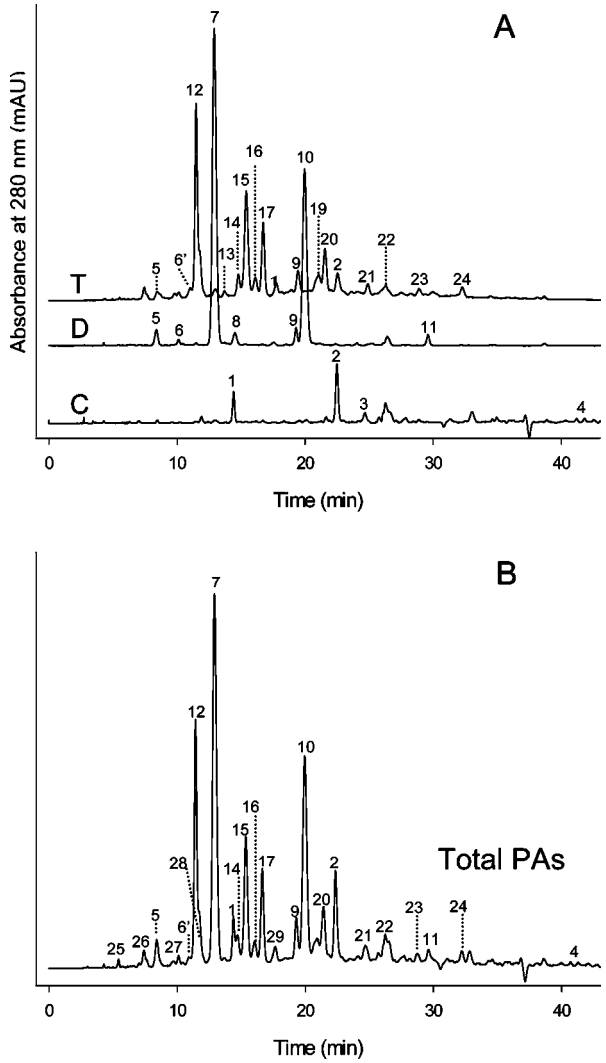

Figure 2. (**A**) Chromatograms of PA fractions from outer kernel layers of hulless cv. Bear. Corresponding PA peak assignments, mass-to-charge ratios (*m*/*z*) are in parentheses, and presumptive monomeric units are in brackets. C, catechin fraction: 1, catechin glucoside (451); 2, catechin; 3, vanillic acid; and 4, ferulic acid. D, dimeric PA fraction: 5, (609)[gc-gc]; 6, (593); 7, prodelphinidin B3; 8, (593) [c-gc]; 9, (593) [gc-c]; 10, procyanidin B3; and 11, (577) [c-c]. T, trimeric PA fraction: 5; 6′ (593); 12, trimer 1; 7; 13, (609) + (913); 14, (593) + (1185); 15, trimer 2; 16, (897); 17, trimer 3; 18, (1185); 9; 19, (881); 20, procyanidin C2; 2; 21, (881); 22, (593) + (865) + (1185); 23, (865); and 24, (881). (**B**) Chromatogram of total PA extract from outer kernel layers of hulless cv. Bear. Key: 25, (593); 26, (913); 5; 27, (881) + (1217); 6′; 12; 28, (1201) + (1796); 7; 1; 14; 15; 16; 17; 29, (1185) + (577); 9; 10; 20; 2; 21; 22; 23; 11; 24; and 4. Descriptive monomeric units: (593) [gc+c]; (609) [gc+gc]; (865) [c+c+c]; (881) [gc+c+c]; (897) [gc+c+gc]; (913) [gc+gc+gc]; (1185) [gc+c+gc+gc], (1201) [gc+gc+gc+c]; (1217) [gc+gc+gc+gc]; and (1796) [gc+gc+c+gc+gc+c].

barley flour dough is no longer controlled by PA content. Furthermore, a high concentration of phenolic compounds, as well as phenolic oxidation products, could inhibit PPO activity, thereby lowering the oxidation of phenolic compounds (*21*, *22*) and, subsequently, discoloration.

The catechin fraction induced darker dough sheets of unheated PA-free flour as compared to the dimeric and trimeric PA fractions (**Table 4**). As compared to the total PA extract (36.2 mg of CE), all PA fractions had smaller darkening effects on

Table 3. Composition of Catechin (μg/g) and PA (μg CE/g) Fractions Isolated from the OLs of Hulless Barley cv. Bear

	fractions			
	catechin	dimeric	trimeric	total PA extract
phenolic acids[a]	24			2 ± 0
		catechin		
(+)-catechin	113 ± 0		33 ± 2	134 ± 9
catechin glucoside	45 ± 0			
		dimeric		
prodelphinidin B3		769 ± 17	70 ± 13	946 ± 5
procyanidin B3		475 ± 8		605 ± 6
other dimers		95	104	220
		trimeric		
t1			450 ± 12	480 ± 3
t2			230 ± 5	297 ± 0
t3			170 ± 2	233 ± 6
procyanidin C2 (t4)			130 ± 4	187 ± 12
other trimers			147	309
tetrameric			20 ± 0	
other PAs[b]			102	206
total PAs	158	1339	1455	3618

[a] Sum of ferulic acid and vanillic acid (μg/g). [b] Coeluted PAs.

Table 4. Effect of Addition of Phenolic Compounds Extracts on Color of Barley Flour Dough Sheets[a,T][b]

		unheated			heated[d]		
extracts	extracted phenolics[c] (mg)	*L**	*a**	*b**	*L**	*a**	*b**
control		75.9 a	2.9 d	14.8 a	81.0 a	2.3 a	16.2 b
total PA from AKs (10 g)	3.9	70.0 c	4.3 b	13.7 b	80.3 bc	2.3 a	16.1 b
total PA from OLs (5 g)	18.1	69.5 cd	3.4 c	12.5 c	79.3 d	2.0 b	15.7 c
total PA from OLs (10 g)	36.2	68.8 d	3.0 d	13.0 bc	78.0 e	2.0 b	15.7 c
catechin[e]	1.8	70.1 c	4.8 a	14.7 a	79.9 cd	2.2 a	16.8 a
dimeric PA[e]	13.4	73.4 b	3.0 d	13.6 b	80.5 ab	2.0 b	15.3 cd
trimeric PA[e]	14.5	73.9 b	2.5 e	13.4 bc	78.4 e	2.0 b	15.2 d

[a] *L**, *a**, and *b** were measured in dough sheets at 24 h. [b] Two means with different letters in the same column are significantly different ($P < 0.05$). [c] Calculated based on values from **Tables 2** and **3**. [d] Radiant flour autoclaved at 121 °C × 15 min. [e] Catechin, dimeric PA, and trimeric PA fractions from 10 g of OLs.

the dough sheets (**Table 4**). The ΔL^* of the dough sheets added with PA fractions was highest with the catechin fraction, lower with the dimeric fraction, and lowest with the trimeric fraction (**Figure 3**). This result suggests that phenolics in the catechin fraction, mainly catechin, might be better substrates for PPO than dimeric or trimeric PAs. Dough sheet discoloration induced by the catechin fraction mainly occurred through the enzymatic oxidations of phenolic compounds (*23*).

Comparing the unheated and heated PA-free barley flours, the heat treatment significantly improved the brightness (*L**) of the dough sheets. The increase in *b** of dough sheets of heated flour was probably due to the formation of Maillard products (**Table 4**). Catechin and PAs are colorless unless they are oxidized via PPO catalysis or autoxidation. Heat treatment inactivated PPO. The PPO activity was 118 ± 5 units/g in the unheated flour, while in the heat-treated flour, the PPO activity

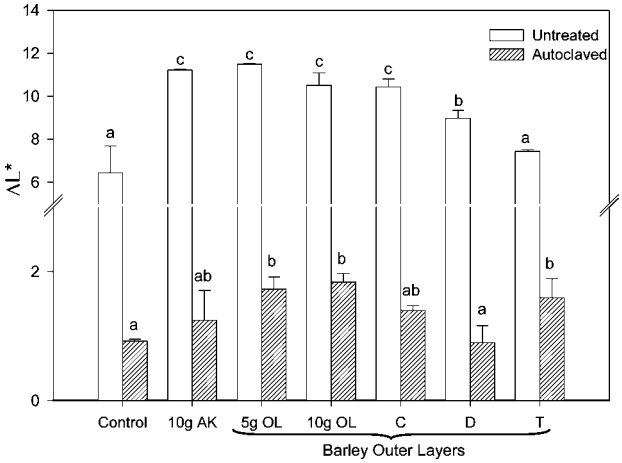

Figure 3. Effect of PAs extracts in brightness (L^* value) of barley dough sheets. ΔL^*, L^* at 0 h to L^* at 24 h of storage. Fractions: C, catechin; D, dimeric PA; and T, trimeric PA.

was 12 ± 2 units/g. Thus, the decreases in L^* observed after the addition of PA extracts in the heated PA-free flour were much smaller than those in unheated PA-free flour (**Table 4** and **Figure 3**). For the heated PA-free barley flour, the addition of total PAs from AK and OL resulted in darker dough sheets (**Table 4**), as compared to the heated control. The addition of total PAs only from the OLs produced an increased ΔL^* of dough sheets (**Figure 3**).

The addition of the catechin and trimeric fractions to the heated flour decreased the brightness of dough as compared to the control, while the dimeric PA fraction did not (**Table 4**). The ΔL^* of dough sheets with the added trimeric PA fraction was also significantly higher than the ΔL^* of the control and that of the dimeric PA fraction (**Figure 3**). The decrease in L^* value of dough sheets prepared from heated barley flour by the addition of these PAs possibly occurred because of the autoxidation of phenolic compounds. The differences in the effects of the PA fractions on discoloration of dough sheets can be attributed to differences in concentration, particularly between the catechin fraction and the trimeric PA fraction. In addition, the greater effect of the trimeric PA fraction as compared to dimeric PA fraction may suggest that dimeric PAs are less susceptible to autoxidation as well as to polymerization of oxidized PAs than trimeric and higher oligomer PAs.

In summary, phenolic acid and PA contents varied among genotypes. Among classes, PA-free barley had no detectable PAs, whereas hulless barley contained more PAs than hulled PA-containing barley. Abrasion reduced both phenolic acid and PA content. Among PA-containing abraded barley, the major PAs were dimeric PA prodelphinidin B3 and procyanindin B3. PAs appeared to affect discoloration potential of barley. However, among PA-containing barley, there was no evident relationship between the individual PAs and the discoloration potential of barley, except for catechin.

Phenolics in the catechin fraction appeared to be more readily oxidized by barley PPO than those in the dimeric or trimeric PA fractions. The catechin fraction contributed heavily to the discoloration potential of barley, although catechin was present in relatively small amounts in barley. Dimeric PAs were present in the highest amounts in barley grains, but their contribution to discoloration appeared to be smaller than catechin and trimeric PAs.

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