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ARTICLE

Effects of Phenolic Compounds on the Browning of Cooked Barley

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Barley grain products undergo browning when cooked. To evaluate effects of phenolic compounds on browning, various amounts of (+)-catechin, proanthocyanidins, or related phenolic compounds were added to aqueous barley extracts or barley pastes, which were heated at 90 °C for 1 or 2 h, respectively. In barley extract, (+)-catechin, procyanidin B3 (PCB3), prodelphinidin B3 (PDB3), and a trimer of gallocatechin−gallocatechin−catechin (PDT1) dose-dependently elevated absorbance at 420 nm after heating. PDB3 caused browning faster than PCB3 and (+)-catechin. In barley paste, PDB3 and PDT1 decreased the L^* value and increased the a^* and b^* values of the paste dose-dependently after heating and PCB3 and (+)-catechin did so to a lesser extent. Caffeic acid promoted the browning in both of the extract and paste, while protocatechuic acid, eriodictyol, and (+)-taxifolin promoted it in the extract and myricetin and quercetin promoted it in the paste. Compounds promoting browning have catechol or pyrogallol structures in common.

KEYWORDS: Barley; browning; proanthocyanidin; (+)-catechin; polyphenol

INTRODUCTION

Barley has been used as a staple food grain for millennia. In Japan and Korea, pearled barley and rolled barley are the most commonly used forms. Pearled barley is obtained by removing the outer layers of barley grain by abrasive action, and rolled barley is made from pearled barley that has been steamed and rolled flat. The removed outer layers of the grains constitute the bran. The percentage of pearled grain weight compared to the initial whole grain weight represents the pearling yield; "60% pearled" means that 60% of the weight remains as pearled grain and 40% is removed as bran. Pearled or rolled barley is usually cooked with rice as a rice extender or boiled as risotto or gruel. Barley products readily undergo browning, which is accompanied by an unwanted taste when the barley is cooked and kept warm. Consumers prefer white cereals; therefore, regulating the extent of browning is required to increase the consumption of barley products. It is therefore necessary to elucidate the constituents responsible for the browning of barley grains.

There are many studies on the discoloration of heated barley products, such as pastes (*1*−*3*), gels (*4*−*7*), and porridges (*8*). The major differences among these barley products are in the ratio of barley flour/water and heating conditions. The pearling yield of barley grains affects the whiteness of pastes (*1*) and the brightness of gels (*4*, *7*) after heat treatment; therefore, it is suggested that the outer layers promote browning. Total polyphenol contents also correlate negatively with the whiteness of heated pastes (*2*) and the brightness of heated gels (*6*). Barley grains contain large amounts of flavanols, such as (+)-catechin and proanthocyanidins. The major proanthocyanidins are two dimers [prodelphinidin B3 (PDB3) and procyanidin B3 (PCB3)] and four trimers (*9*−*11*), whose structures were elucidated by ^{1}H nuclear magnetic resonance (NMR) (*10*). Proanthocyanidin-free mutants of barley, in which the biosynthesis of proanthocyanidins is genetically blocked, are bred (*12*, *13*), and barley products from proanthocyanidin-free mutants are less discolored by heating compared to those from proanthocyanidin-containing varieties (*2*, *5*−*7*). Furthermore, the contents of PDB3, PCB3, and (+)-catechin correlated with the degree of browning in 42 varieties of barley pastes (*2*). These results strongly suggest that proanthocyanidins and (+)-catechin cause the browning of cooked barley products. However, because the contents of these compounds are correlated with each other in barley grains (*2*), it is difficult to determine which compound is the main contributor to browning when barley grains are used in experiments. Additionally, barley grains contain several phenolic acids, such as *p*-coumaric, ferulic, and diferulic acids (*14*), as well as *C*-glycosyl flavones and *O*-glycosyl-*C*-glycosyl flavones (*15*). The effects of these phenolic acids and glycosyl flavones on the browning of cooked barley products have not been examined.

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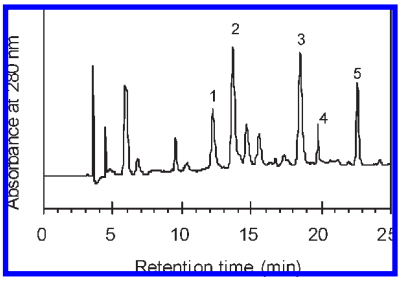

Figure 1. HPLC profile of the crude flavanol fraction. Identified peaks are numbered: **1**, PDT1; **2**, PDB3; **3**, PCB3; **4**, L-tryptophane; and **5**, (+)-catechin.

The objective of this study was to verify the effect of phenolic compounds, including flavonoids and phenolic acids, on the browning of heat-treated barley products. We examined the browning of aqueous barley extracts and barley pastes, to which each proanthocyanidin or related phenolic compound was added in various amounts, by heating.

MATERIALS AND METHODS

Materials. Grains of barley (*Hordeum vulgare* L.) cv. Ichibanboshi were harvested in an experimental field at Zentsuji (Kagawa, Japan) in 1998 and pearled from the outer layer to a 60 or 40% yield using a TM05 abrasive debranner (Satake, Higashi-Hiroshima, Japan). Bran of 60% pearling yield was manually sieved with a 1 mm screen and stored at −30 °C until a crude flavanol fraction was prepared from it. We ground pearled grains using a Cyclotec 1093 Sample Mill (Tecator, Höganäs, Sweden) with a 1 mm screen and stored them at −30 °C until use in experiments.

3-Indoleacrylic acid, (+)-catechin, PDB3, PCB3, *p*-hydroxybenzoic acid, vanillic acid, protocatechuic acid, *p*-coumaric acid, ferulic acid, caffeic acid, naringenin, apigenin, quercetin, and myricetin were purchased from Sigma-Aldrich (St. Louis, MO). Homoeriodictyol, eriodictyol, (+)-taxifolin, chrysoeriol, luteolin, isovitexin, homoorientin, and kaempferol were obtained from Extrasynthèse (Genay, France). Other chemicals were obtained from Wako Pure Chemical Industries (Osaka, Japan).

Preparation of a Crude Flavanol Fraction and Purification of Endogenous Compounds. Sieved bran (1 kg) was defatted with hexane and then extracted twice with 75% acetone−water (4 L). The combined extract, concentrated under reduced pressure, was used as a crude flavanol fraction. Major constituents of the crude flavanol fraction were isolated by a modified method based on that of McMurrough et al. (*11*). A total of 10 g of the crude flavanol fraction was suspended in 40 mL of distilled water and centrifuged at 3000 rpm for 10 min. The supernatant was applied to a 350 × 32 mm inner diameter Sephadex LH-20 column (GE Healthcare Bio-Sciences, Uppsala, Sweden) equilibrated with distilled water, and the column was washed with distilled water (950 mL) and 20% ethanol (950 mL) at a flow rate of 5.0 mL/min. The column was then eluted with 80% ethanol at a flow rate of 5.0 mL/min; fraction I (200−800 mL) and fraction II (800−1500 mL) were collected and concentrated. Fraction I was further purified using a 250 × 20 mm inner diameter TSK-gel ODS-80 Ts column (Tosoh, Tokyo, Japan) with an isocratic elution of 5% (v/v) methanol in 2.5% (v/v) acetic acid at a flow rate of 5.0 mL/min to obtain three major compounds **2**, **3**, and **5**. Fraction II was also purified using the same column with an isocratic elution of 2% (v/v) methanol in 2.5% (v/v) acetic acid at a flow rate of 5.0 mL/min to obtain the major compound **1**. The crude flavanol fraction (4.5 g) dissolved in methanol was applied to a 283 × 15 mm inner diameter Sephadex LH-20 column equilibrated with methanol. The fraction eluted with methanol (40−100 mL) was collected and further purified using a 300 × 7.8 mm inner diameter μBondapak C18 column (Waters, Milford, MA) with a linear gradient of 0−20% methanol in water for 30 min at a flow rate of 3.0 mL/min. The major compound was rechromatographed with the same column to obtain compound **4**.

The crude flavanol fraction and the obtained compounds **1**−**5** (**Figure 1**) were analyzed using a 250 × 4.6 mm inner diameter TSK-gel ODS-80 Ts QA column (Tosoh, Inc.) at 30 °C, with a linear gradient of 0−30% (v/v) solvent B (2.5% acetic acid in methanol) in solvent A (2.5% acetic acid in water) by monitoring the UV−vis absorption in the range of 240−400 nm on a L-7450 diode array detector (Hitachi, Tokyo, Japan). The purity of each compound was determined from the ratio of the peak area to the total peaks on the chromatogram at 280 nm.

Instrumental Analyses. We recorded electron spray ionization time-of-flight (ESI−TOF) mass spectra ranging from *m*/*z* 200 to 1000 on a Mariner (Applied Biosystems, Foster City, CA) in methanol. Matrix-assisted laser desorption/ionization time-of-flight (MALDI−TOF) mass spectra were obtained using a Reflex II instrument (Bruker Daltonics, Bremen, Germany) with 3-indoleacrylic acid as the matrix. ^{1}H NMR spectra were obtained in CD_3OD containing trifluoroacetic acid-*d* with a Bruker Avance 500 spectrometer (Karlsruhe, Germany) operating at 500.13 MHz.

Browning Test of an Aqueous Barley Extract. A total of 20 g of barley flour from 40% pearled grains was mixed with 200 mL of 50 mM sodium phosphate buffer (pH 7.0) and stirred for 1 h at room temperature. After centrifugation at 10 000 rpm for 10 min, the supernatant was used as the aqueous barley extract. A total of 1 mL of the aqueous barley extract was mixed with 100 μL of sample solution (0−100 μg in methanol) in a snap-cap microcentrifuge tube and heated at 90 °C using a dry block bath (Taitec, Koshigaya, Japan). After incubation for 1 h, the tube was transferred on ice and centrifuged at 10 000 rpm for 10 min at 4 °C to remove colloidal precipitates, and the absorbance at 420 nm of the supernatant was measured using a Shimadzu UV-2400PC spectrophotometer (Kyoto, Japan) with a path length of 10 mm.

Measurement of Absorption Spectra. A total of 100 μL of sample solution (20 μg in methanol) was mixed with 1 mL of 50 mM sodium phosphate buffer (pH 7.0) in snap-cap microcentrifuge tubes, which were heated at 90 °C for 0, 10, 20, 30, 45, 60, 120, or 180 min and then kept on ice. The absorbance of each solution was scanned between 780 and 250 at 1 nm intervals using a Shimadzu UV-2400PC spectrophotometer with a path length of 10 mm.

Browning Test of Barley Paste. A test compound was dissolved in methanol (20 mg/mL), and 5, 15, or 50 μL (0.1, 0.3, or 1.0 mg) of the solution was pipetted into glass Petri dishes (16 mm inner diameter). After drying under a nitrogen stream, we added 400 mg of 60% pearled barley flour and 1 mL of 50 mM sodium phosphate buffer (pH 7.0) to each dish and gently stirred to make a paste. Paste without a test sample was used as a control, and paste containing 1.0 mg of (+)-catechin was used as a positive control. Pastes with glass covers were heated at 90 °C for 2 h in a conventional oven and cooled at room temperature. The color of the paste before heating was measured through the bottom glass of the dish, and the color of the top surface of the paste after heating was measured using a CM-3500d spectrophotometric colorimeter (Konica Minolta) after removing the paste from the dish. Tests were performed in triplicates.

Paste color was expressed using the CIELAB color system, in which L^*, a^*, and b^* values represent brightness, redness, and yellowness, respectively. The total color difference (ΔE) caused by heat treatment was calculated as

$$\Delta E = [(L^*_{\text{after}} - L^*_{\text{before}})^2 + (a^*_{\text{after}} - a^*_{\text{before}})^2 + (b^*_{\text{after}} - b^*_{\text{before}})^2]^{1/2}$$

Statistics. Analyses of variance and significant differences among means were tested by one-way ANOVA and Dunnett's test, using a JMP version 6 software (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Purification and Identification of the Major Compounds in the Crude Flavanol Fraction. A total of 1 kg of sieved bran yielded 33.5 g of crude flavanol fraction by extraction with acetone−water. We analyzed the crude flavanol fraction by high-performance liquid chromatography (HPLC) and identified several components with absorption maxima around 280 nm. Compounds **1**−**5** shown in **Figure 1** were purified by a combination of column chromatographies. ESI−TOF mass spectra of compounds **2** and **3** showed intense ion peaks at *m*/*z* 617.2 and 601.3, corresponding to sodium additive ions of PDB3 and PCB3, respectively. A comparison of retention times and UV−vis spectra between purified compounds **1**−**5** and the authentic

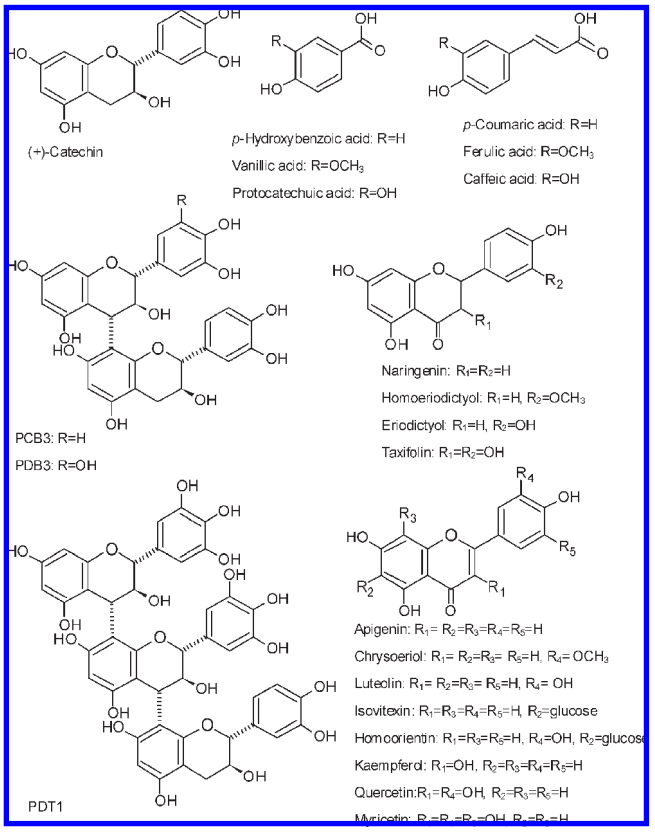

Figure 2. Chemical structures of the tested compounds.

samples of PDB3, PCB3, and (+)-catechin showed that the compounds **2**, **3**, and **5** were PDB3, PCB3, and (+)-catechin, respectively, which was confirmed by each co-elution analysis.

The MALDI−TOF mass spectrometry of compound **1** indicated that the most intense ion peak at m/z 921.5 was a sodium additive ion of a trimer comprising one (epi)catechin unit and two (epi)gallocatechin units. The postsource decay fragment analysis gave ions at m/z 617.6 ($[M+Na-304]^+$) and 327.3 ($[M + Na - 304 - 290]^+$) corresponding to the central-terminal unit and the top unit of the trimer after quinone-methide fission of the interflavanoid bond, respectively (*16*). This indicated that the top unit was (epi)gallocatechin and the central-terminal unit was (epi)catechin−(epi)gallocatechin or (epi)gallocatechin−(epi)catechin. Furthermore, the fragment ion at m/z 465.5 ($[M + Na - 304-152]^+$) corresponded to a retro-Diels−Alder fission product of the ion at m/z 617.6, which suggested that the terminal unit was (epi)catechin. Therefore, we identified the compound **1** as a trimer of gallocatechin−gallocatechin−catechin (PDT1), present in barley grains as a major constituent (*9*−*11*). The chemical structures of these compounds are shown in **Figure 2**.

The ^{1}H NMR spectrum of compound **4** was consistent with the authentic sample of L-tryptophan. A comparison of retention times and spectra between L-tryptophan and compound **4** indicated that compound **4** was L-tryptophan, and this was confirmed by co-elution analysis.

The obtained PCB3, PDB3, and PDT1 whose purities were 97, 95, and 93%, respectively, according to absorbance at 280 nm, were used in the following browning tests.

Effects of Barley Flavanols on Browning by Heating. To examine the dose effect of the major flavanols in barley on browning, various amounts of (+)-catechin, PCB3, PDB3, PDT1, and L-tryptophan were mixed with the aqueous barley extract and heated. We used 40% pearled grain flour for preparing the aqueous barley extract to minimize endogenous phenolic compounds. **Figure 3** shows the effects of these five compounds on the browning of the aqueous barley extracts. Increasing amounts of (+)-catechin, PCB3, PDB3, and PDT1 increased the absorbance of the barley extract at 420 nm in an almost linear fashion. On the other hand, L-tryptophan produced no change in the absorbance of the barley extract at 420 nm. Although these five compounds dissolved in a sodium phosphate buffer (pH 7.0) were colorless before heating, (+)-catechin and three proanthocyanidin solutions turned pink or orange after heating at 90 °C for 1 h. On the other hand, a L-tryptophan solution remained colorless upon heat treatment. These results suggest that (+)-catechin and proanthocyanidins readily cause browning in solutions with or without other barley constituents after heating.

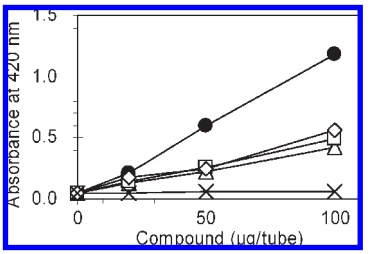

Figure 3. Dose-dependent color deepening of aqueous barley extracts after heating at 90 °C for 1 h with (+)-catechin (●), PCB3 (◇), PDB3 (□), PDT1 (△), or L-tryptophane (×).

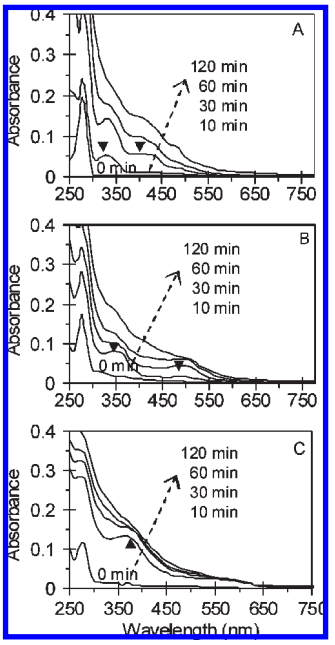

Figure 4. Changes in UV−vis absorption spectra of (A) (+)-catechin, (B) PCB3, or (C) PDB3 in phosphate-buffered solutions (pH 7.0, 20 μg/1.1 mL) after heating at 90 °C for 0, 10, 30, 60, or 120 min. Triangles denote absorption maxima.

Upon monitoring the color of the solution by absorbance at s 420 nm, we observed that the browning caused by (+)-catechin was greater than that caused by proanthocyanidins (**Figure 3**). We measured absorbance at 420 nm because this value is often used to measure the enzymatic browning reaction of phenolic compounds with polyphenol oxidase (*17*). To compare the browning reactivities of (+)-catechin, PCB3, and PDB3, the solutions containing 20 μg of each compound were heated for various times and their absorption spectra were measured. The (+)-catechin solution had absorption maxima at 330 and 400 nm after heating for 10 min, but these disappeared after heating for 120 min (**Figure 4A**).

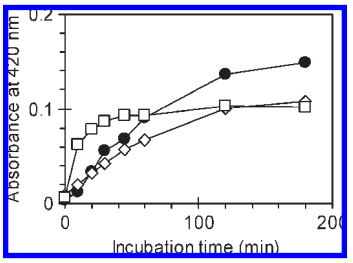

Figure 5. Browning of PDB3 (□), PCB3 (◇), and (+)-catechin (●) in phosphate-buffered solutions (pH 7.0, 20 μg/1.1 mL) after heating at 90 °C for 0−180 min.

Table 1. Effect of the Flavanols on the Color of Pearled Barley Pastes[a]

		before heating			after heating			
compound (mg)		*L**	*a**	*b**	*L**	*a**	*b**	Δ*E*
control		72.92	0.45	7.68	49.91	2.07	7.40	23.07
PDT1	0.1	73.09	0.48	7.48	47.45	3.40[b]	9.43	25.88
	0.3	73.48	0.59	7.53	44.75[b]	4.98[b]	12.10[b]	29.42[b]
	1.0	72.74	0.80	7.38	38.83[b]	9.28[b]	16.66[b]	36.17[b]
PDB3	0.1	73.05	0.54	7.34	46.45	3.79[b]	10.12[b]	26.94
	0.3	73.01	0.57	7.33	44.51[b]	5.22[b]	12.38[b]	29.32[b]
	1.0	72.34	0.57	7.48	38.40[b]	10.59[b]	16.21[b]	36.45[b]
PCB3	0.1	72.51	0.58	7.54	48.57	3.50[b]	7.89	24.13
	0.3	72.55	0.63	7.54	48.03	4.82[b]	8.84	24.91
	1.0	73.13	0.62	7.51	47.57	7.29[b]	9.45	26.48[c]
(+)-catechin	0.1	73.02	0.46	7.40	49.61	2.62	7.88	23.52
	0.3	73.22	0.54	7.77	48.96	3.85[b]	9.44[c]	24.54
	1.0	72.36	0.69	7.53	48.08	5.30[b]	12.11[b]	25.14[c]

[a] Pearled barley pastes added with each compound were heated at 90 °C for 2 h. *L**, *a**, and *b** values were expressed as the average of triplicate determinations. [b] Significantly different from the control by Dunnett's test, $p < 0.01$. [c] Significantly different from the control by Dunnett's test, $p < 0.05$.

Absorption maxima of the solution containing PCB3 were observed at 350 and 500 nm after heating for 10 min and disappeared after heating for 120 min (**Figure 4B**). Absorption maximum of the PDB3 solution was observed at 362 nm after heating for 10 min and disappeared after heating for 60 min (**Figure 4C**). These results suggest that the browning effect of (+)-catechin may be overestimated in comparison to those of PCB3 and PDB3 when monitoring at a wavelength near the absorption maximum. Of the three compounds, PDB3 elevated absorbance at 420 nm first and reached a plateau at 45 min (**Figure 5**). Initial velocities of the increase in absorbance at 420 nm were estimated to be 5.62, 1.20, and 0.85 mAU/min for PDB3, PCB3, and (+)-catechin, respectively. Therefore, PDB3 was more susceptible to heat-induced browning than PCB3 and (+)-catechin.

Next, the browning of barley pastes by heating in the presence of (+)-catechin and proanthocyanidins was evaluated. **Table 1** shows the color of the barley pastes before and after heating and their color differences (Δ*E*). Although (+)-catechin and three proanthocyanidins (PCB3, PDB3, and PDT1) did not affect the *L**, *a**, and *b** values of the pastes before heating compared to the control, the color of the pastes was clearly changed after heating. The *a**, *b**, and Δ*E* values increased with increasing amounts of these compounds, while the *L** values decreased. The effects of PDB3 and PDT1 were greater than those of (+)-catechin and PCB3.

Quinde-Axtell and Baik (*18*) reported that the *L** value of barley flour dough sheets stored at 20 °C for 24 h decreased with the addition of proanthocyanidin extract but that the discoloration was not dependent upon the amount of proanthocyanidin extract. In our experimental conditions, (+)-catechin and three

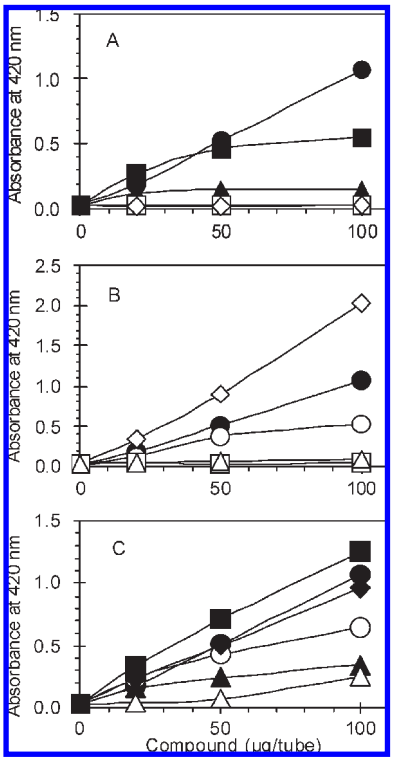

Figure 6. Effects of phenolic compounds on the color of aqueous barley extract after heating at 90 °C for 1 h. Symbols represent (A) (+)-catechin (●), protocatechuic acid (▲), *p*-coumaric acid (□), ferulic acid (◇), and caffeic acid (■); (B) (+)-catechin (●), (+)-taxifolin (○), eriodictyol (◇), homoeriodictyol (△), and naringenin (□); and (C) (+)-catechin (●), myricetin (○), quercetin (▲), kaempferol (△), homoorientin (■), and isovitexin (◆).

proanthocyanidins facilitated the browning dose-dependently in both the aqueous barley extracts and barley pastes. Therefore, the degree of browning in barley products upon heating may be regulated by the amounts of (+)-catechin and proanthocyanidins.

Effects of Proanthocyanidin Precursors and Related Compounds on the Browning by Heating. Several precursors and related compounds of proanthocyanidins were mixed with the aqueous barley extract, and the effects of these compounds on browning by heating were evaluated by absorbance at 420 nm. **Figure 6** shows the effects of phenolic acids (**Figure 6A**), flavanones and flavanonols (**Figure 6B**), and flavones and flavonols (**Figure 6C**) on the browning of barley extract heated at 90 °C for 1 h. (+)-Catechin was used as a positive control in each assay.

Solutions containing phenolic acids showed low absorbance at 420 nm (<0.1) before heating. Increasing amouts of caffeic acid increased absorbance at 420 nm after heating, although the effect reached a plateau at high concentrations (**Figure 6A**). Protocatechuic acid showed the same trend as caffeic acid, but its effect was smaller than that of caffeic acid. On the other hand, *p*-coumaric and ferulic acids had negligible effects on browning after heating (**Figure 6A**), and *p*-hydroxybenzoic and vanillic acids had no effect on browning (data not shown). **Table 2** shows the effects of phenolic acids on the color of barley pastes before and after heating. Phenolic acids did not affect the color of pastes before heating. After heating, increasing amounts of caffeic acid produced a significant decrease in the *L** value and increases in the *b** and Δ*E* values. Protocatechuic acid showed the same trend as caffeic acid, but the effect was not significant (data not shown). On the other hand, ferulic acid significantly suppressed the *a** and *b** values and increased the *L** value of the barley pastes after

Table 2. Effect of the Phenolic Acids on the Color of Pearled Barley Pastes[a]

		before heating			after heating			
compound (mg)		L^*	a^*	b^*	L^*	a^*	b^*	ΔE
control		72.82	0.40	7.43	46.62	2.59	8.84	26.33
(+)-catechin	1.0	72.60	0.62	7.56	45.94	4.74[b]	12.47[b]	27.43
p-coumaricacid	0.1	72.52	0.44	7.35	47.03	2.00	8.03	25.54
	0.3	73.15	0.50	7.46	47.10	2.21	8.42	26.12
	1.0	73.14	0.52	7.31	47.50	2.05	8.09	25.70
ferulic acid	0.1	73.14	0.42	7.43	47.86	1.74[b]	7.89	25.31
	0.3	73.05	0.41	7.39	48.54[b]	1.44[b]	7.56[c]	24.54[c]
	1.0	73.22	0.41	7.38	47.72	1.72[b]	8.85	25.58
caffeic acid	0.1	72.79	0.50	7.47	46.52	2.18	9.06	26.37
	0.3	72.64	0.45	7.37	45.30	2.38	9.59	27.51
	1.0	72.37	0.50	7.44	43.81[b]	2.73	11.29[b]	28.90[b]

[a] Pearled barley pastes added with each compound were heated at 90 °C for 2 h. L^*, a^*, and b^* values were expressed as the average of triplicate determinations. [b] Significantly different from the control by Dunnett's test, $p < 0.01$. [c] Significantly different from the control by Dunnett's test, $p < 0.05$.

Table 3. Effect of the Flavanones and Flavanonols on the Color of Pearled Barley Pastes[a]

		before heating			after heating			
compound (mg)		L^*	a^*	b^*	L^*	a^*	b^*	ΔE
control		73.03	0.58	7.60	46.87	2.59	8.90	26.36
(+)-catechin	1.0	73.03	0.58	7.59	45.80	5.08[b]	11.37[b]	27.87
naringenin	0.1	72.58	0.46	7.51	46.23	2.58	9.00	26.48
	0.3	73.09	0.44	7.69	46.97	2.53	9.37	26.27
	1.0	72.70	0.34	8.04	46.64	2.55	9.84	26.22
homoeriodictyol	0.1	72.76	0.49	7.71	46.81	2.39	8.89	26.05
	0.3	72.97	0.46	7.63	46.74	2.46	9.38	26.38
	1.0	73.33	0.47	7.69	47.06	2.46	9.74	26.42
eriodictyol	0.1	73.27	0.42	7.70	47.29	2.38	8.96	26.08
	0.3	73.00	0.37	8.03	46.82	2.68	10.15[c]	26.37
	1.0	73.16	0.30	9.30[b]	47.85	2.13	12.54[b]	25.59
(+)-taxifolin	0.1	73.55	0.35	7.64	46.26	2.71	9.85	27.48
	0.3	73.24	0.47	8.15[c]	46.97	2.56	10.51[b]	26.46
	1.0	72.84	0.36	8.84[c]	46.30	2.95	14.26[b]	27.22

[a] Pearled barley pastes added with each compound were heated at 90 °C for 2 h. L^*, a^*, and b^* values were expressed as the average of triplicate determinations. [b] Significantly different from the control by Dunnett's test, $p < 0.01$. [c] Significantly different from the control by Dunnett's test, $p < 0.05$.

heating, although the dose−effect relationship was not clear. *p*-Coumaric acid showed the same trend as ferulic acid. Thus, caffeic acid facilitated the browning of aqueous barley extracts and barley pastes by heating dose-dependently, but ferulic acid reduced the browning of barley pastes. Because ferulic and *p*-coumaric acids are the most and second most abundant phenolic acids in grains of different barley varieties, respectively (*14*), the effects of endogeneous phenolic acids on the browning should be smaller than those of (+)-catechin and proanthocyanidins.

Among flavanones and flavanonols, eriodictyol and (+)-taxifolin in increased amounts significantly elevated the absorbance of aqueous barley extract at 420 nm after heating (**Figure 6B**), although both compounds also slightly increased absorbance before heating (data not shown). On the other hand, homoeriodictyol and naringenin had little effect on the absorbance of the extract after heating (**Figure 6B**). **Table 3** shows the effects of flavanones and flavanonols on the color of barley pastes before and after heating. Flavanones and flavanonols tested here did not affect the L^* and a^* values before or after heating. Increasing amounts of eriodictyol and (+)-taxifolin significantly increased the b^* value after heating. Naringenin and homoeriodictyol showed the same trend, but the effect was not significant. Thus, eriodictyol and (+)-taxifolin facilitated the browning of aqueous barley extracts by heating dose-dependently, but their effects on the browning of barley pastes were not clear based on the ΔE value.

Table 4. Effect of the Flavones and Flavonols on the Color of Pearled Barley Pastes[a]

		before heating			after heating			
compound (mg)		L^*	a^*	b^*	L^*	a^*	b^*	ΔE
control		73.02	0.51	7.57	47.27	2.19	8.28	25.82
(+)-catechin	1.0	73.04	0.67	7.43	46.19	4.38[b]	11.44[b]	27.41
apigenin	0.1	73.07	0.28	7.75	47.90	1.90	9.01	25.25
	0.3	73.53	−0.23	8.88	48.25	1.68	10.00[b]	25.38
	1.0	73.56	−0.84[b]	10.75[b]	50.46[b]	1.19[c]	10.51[c]	23.20[b]
chrysoeriol	0.1	72.77	−0.35[b]	8.98	46.96	1.72	10.11[b]	25.92
	0.3	73.41	−1.20[b]	11.16[b]	48.22	0.92[b]	10.95[b]	25.28
	1.0	73.62	−3.73[b]	17.00[b]	51.25[b]	−0.48[b]	14.68[b]	22.73[b]
luteolin	0.1	73.24	−0.08	8.79	46.88	1.65	11.36[b]	26.55
	0.3	72.90	−0.52[b]	10.70[b]	45.45[c]	1.99	14.39[b]	27.85[c]
	1.0	71.89[b]	−0.93[b]	13.79[b]	46.20	1.32	18.80[b]	26.28
quercetin	0.1	73.10	−0.81[b]	10.49[b]	45.90	2.76	11.70[b]	27.46
	0.3	73.09	−2.61[b]	15.64[b]	44.68[b]	3.65[b]	15.97[b]	29.11[b]
	1.0	72.67	−4.53[b]	22.01[b]	42.77[b]	6.15[b]	23.01[b]	31.76[b]
myricetin	0.1	72.54	−2.36[b]	15.84[b]	45.30[c]	2.83	11.10[b]	28.14[b]
	0.3	72.29	−2.88[b]	18.28[b]	39.84[b]	6.67[b]	15.91[b]	34.06[b]
	1.0	72.28	−2.74[b]	17.80[b]	33.42[b]	13.51[b]	23.04[b]	42.45[b]

[a] Pearled barley pastes added with each compound were heated at 90 °C for 2 h. L^*, a^*, and b^* values were expressed as the average of triplicate determinations. [b] Significantly different from the control by Dunnett's test, $p < 0.01$. [c] Significantly different from the control by Dunnett's test, $p < 0.05$.

When methanolic solutions of flavone aglycones (apigenin, chrysoeriol, and luteolin) were mixed with a phosphate buffer, yellow precipitates were formed. Consequently, the glycosylated compounds (isovitexin and homoorientin) were tested. Increasing amounts of homoorientin, isovitexin, myricetin, quercetin, and kaempferol increased absorbance at 420 nm after heating (**Figure 6C**). However, solutions containing these compounds were pale yellow before heating. After heating, the solutions produced dark yellow precipitates, and absorbances of the supernatants at 420 nm decreased during heating. Therefore, the effects of flavones and flavonols on browning were not evident in the aqueous barley extracts. **Table 4** shows the effects of flavones and flavonols on the color of barley pastes before and after heating. Being yellow in color, flavones and quercetin in increasing amounts reduced the a^* value and increased the b^* value before heating. Increasing amounts of apigenin and chrysoeriol inhibited the browning of barley pastes after heating, because they significantly increased the L^* values after heating and reduced the ΔE values. Luteolin promoted an increase in the b^* value after heating, but its effect on browning was not clear based on its ΔE value. On the other hand, increasing amounts of quercetin and myricetin significantly decreased the L^* value and increased the a^* value after heating. Therefore, both compounds facilitated the browning of barley pastes significantly compared to (+)-catechin. The effect of myricetin on the browning was greater than that of quercetin based on the ΔE value.

The browning of barley pastes from proanthocyanidin-free mutants is less than that of pastes from wild-type varieties (*2*). Some proantocyanidin-free mutants are also reported to accumulate proanthocyanidin precurcers, such as homoeriodictyol and chrysoeriol, in their grains (*12*, *13*). Our results are consistent with the above observation, because homoeriodictyol and chrysoeriol did not facilitate browning in barley pastes, whereas proanthocyanidins did promote it. Barley grains also contain

many glycosylated apigenin and luteolin derivatives (*15*). Because apigenin and luteolin showed no promoting effect on the browning of barley pastes, endogenous flavones would not be responsible for the browning of barley products by heating.

Relationships between Structures of Phenolic Compounds and the Browning Potency of Heating. Proanthocyanidins and (+)-catechin significantly facilitated the browning by heating in aqueous barley extracts and barley pastes. Caffeic acid also facilitated it in both conditions. Protocatechuic acid, eriodictyol, and (+)-taxifolin promoted it significantly in aqueous barley extracts but not in barley pastes. Quercetin and myricetin facilitated browning significantly in barley pastes but ambiguously in aqueous extracts. These results suggest that compounds with catechol or pyrogallol structures, except for luteolin, trigger the browning by heating in the presence of other barley constituents (**Figure 2**). Furthermore, compounds with a pyrogallol residue on the B ring (PDB3 and myricetin) facilitated the browning of barley pastes more effectively than corresponding compounds with a catechol residue on the B ring (PCB3 and quercetin). On the other hand, compounds including *p*-hydroxy or *m*-methoxy-*p*-hydroxy structures showed no effect or inhibitory effects on browning by heating. These structure−activity relationships are similar to the structure−antioxidant activity relationships reported for flavonoids and phenolic acids (*19*−*24*). Flavonoids and phenolic acids act as antioxidants against free radicals but demonstrate prooxidant activity when a transition metal is available (*21*, *23*, *24*).

In previous reports, the addition of transition metals accelerated the browning of barley products, whereas the addition of chelating agents suppressed it (*3*, *6*, *8*). The prooxidant characteristics of flavonoids and phenolic acids might be related to their browning potency by heating. The discoloration of barley dough sheets stored at 20 °C for 24 h was reduced by inactivation of endogenous polyphenol oxidase, and discoloration produced by catechin was greater than that produced by proanthocyanidins because catechin might be a better substrate for polyphenol oxidase than proanthocyanidins (*18*). In our experimental conditions, in which aqueous barley extracts and barley pastes were heated at 90 °C, endogenous polyphenol oxidase would be inactivated during heat treatment. Additionally, PDB3 and PDT1 caused the discoloration of barley pastes to a greater extent than (+)-catechin. These results suggest that the browning of barley products after heating is caused by the autooxidation of phenolic compounds and not by endogenous polyphenol oxidase.

In conclusion, (+)-catechin and proanthocyanidins, which are easily auto-oxidized during heating, were the main contributors to the browning of heated barley products. Use of varieties including no or few (+)-catechin and proanthocyanidins should be considered as a means of preventing the unwanted browning of barley products.

ABBREVIATIONS USED

PDB3, prodelphinidin B3; PCB3, procyanidin B3; PDT1, trimer of gallocatechin−gallocatechin−catechin; ESI−TOF, electron spray ionization time-of-flight; MALDI−TOF, matrix-assisted laser desorption/ionization time-of-flight.

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