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Note

Correlations between Barley Constituents and the Browning Reaction in Heat-Treated Barley Pastes

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The browning reaction in barley products after boiling or steaming is undesirable in human food. We estimated the degree of browning reaction—the browning index (*BI*)—by the difference in the Hunter's whiteness values of barley pastes before and after heat treatment (90°C, 2 h). The apparent polyphenol content of barley flours, as determined by the Prussian blue method, was significantly correlated with *BI* in 42 barley varieties (r=0.766, p<0.001). Proanthocyanidin-free mutant lines showed very low *BI*s. Of the constituents of the crude polyphenol fraction, levels of prodelphinidin B3, procyanidin B3, an unidentified compound ('compound C') and (+)-catechin were correlated with *BI* (r=0.595, p<0.001; r=0.599, p<0.001; r=0.400, p<0.01; and r=0.384, p<0.05, respectively). These 4 constituents may influence the heat-induced browning reaction in barley products.

Keywords: hull-less barley, browning reaction, proanthocyanidin, (+)-catechin, polyphenol

Barley grain has been used by humans as a staple food since ancient times, although since the beginning of the 20th century it has been replaced by wheat and rice as living standards have improved (Bhatty, 1992; Fujita, 1998). Recent studies have indicated that a barley-based diet can reduce serum lipids in hypercholesterolemic people (McIntosh *et al*., 1991; Ikegami *et al*., 1996). Therefore, barley grain has been reevaluated as a human food. Barley grain is often processed into pearl barley and rolled-barley, which are generally cooked with rice as rice extenders in Japan and Korea. These barley products, when boiled or steamed, easily undergo a browning reaction, which can result in an undesirable taste. It is therefore important to breed a barley variety that does not show a severe browning reaction, and to reduce the browning reaction in barley products during processing. For these purposes, it is important to elucidate the main constituents involved in the browning reaction.

From a previous study (Fujita *et al*., 2000), we know the following things about the color of barley products after heating. In 31 hull-less barley varieties, the Hunter's whiteness (*W*) values of pearled barley grains after boiling showed little correlation with *W* values of pearled barley flours. However, *W* values of barley pastes after heating showed a high correlation with *W* values of pearled barley grains after boiling (r=0.875, p<0.001), and also showed a significant negative correlation with the polyphenol content of the barley grains (r=–0.768, p<0.001), as determined by the Prussian blue method. The Prussian blue method is often used for polyphenol determination in grains (Price & Butler, 1977; Yanagisawa & Amano, 1995), but the determination is probably affected by substances that may be extracted with the polyphenols, such as cysteine, tryptophan, ascorbic acid, and indole compounds (Graham, 1992). Therefore, barley polyphenols—or some constituents that react with the Prussian blue reagent—are likely to influence the color of cooked barley products.

Barley grain contains more simple flavanols than do rice and wheat (McMurrough *et al*., 1983). (+)-Catechin and the following proanthocyanidins have been identified in barley: prodelphinidin B3 (PDB3), procyanidin B3 (PCB3), prodelphinidin T1, prodelphinidin T2, prodelphinidin T3, and procyanidin T4 (Outtrup & Schaumburg, 1981). Since barley proanthocyanidins cause undesirable haze formation during beer brewing (Gramshaw, 1969), breeders have isolated many mutants of malting barley in which the biosynthesis of proanthocyanidins is genetically blocked (Jende-Strid, 1993).

We analyzed the correlations between the degree of browning reaction and the polyphenol content in 42 barley varieties, including proanthocyanidin-free mutant lines. We also analyzed the relationships between the major constituents of the crude polyphenol fraction and the degree of browning reaction.

Materials and Methods

Materials Twenty varieties from the standard local variety barley (*Hordeum vulgare*) collection at Okayama University were harvested from the university's experimental plots at Kurashiki, Okayama, in 1998. Nineteen improved cultivars and 3 proanthocyanidin-free mutant lines (ant 13-152, ant 17-148 [Jende-Strid and Møller, 1981], and Daikei HJ-2) were harvested from the experimental plots at Zentsuji, Kagawa, in 1997 and 1998. The varieties tested were either 6-rowed (25) or 2-rowed (17), and were either hulled (26) or hull-less (16). As the weight of the hull is about 10% of that of the grain, grains were pearled

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to 55% yield in the case of hulled varieties or to 60% yield in the case of hull-less varieties, and milled with a Cyclotec 1093 sample mill (Tecator, Höganäs, Sweden). (+)-Catechin, PDB3, PCB3 and kojic acid were purchased from Sigma (St. Louis, MO).

Measurement of browning reaction Each barley paste was prepared by mixing 5 g of the flour with 10 ml of distilled water in a petri dish. The L^* (lightness), a^* (redness), and b^* (yellowness) values of the paste were then measured with a Model CR310 colorimeter (Minolta Co., Osaka) before and after heat treatment (90°C, 2 h). W value was calculated from the equation $W=100-((100-L^*)^2+a^{*2}+b^{*2})^{1/2}$. The browning index ($BI$) was defined as the difference in W values before ($W1$) and after ($W2$) heat treatment: $BI=W1-W2$. Browning tests were performed in duplicate and the values used were averages of each measurement.

Determination of apparent polyphenol contents The apparent polyphenol content of the barley flours was determined by the Prussian blue method according to the procedure of Yanagisawa and Amano (1995), and expressed as milligrams (+)-catechin equivalent per gram of flour.

Analysis of crude polyphenol fraction The crude polyphenol fractions were extracted from 1.0 g of barley flour with 5.0 ml of 75% acetone by vigorous shaking for 1 h at 30°C, in the presence of 20 μg of kojic acid as an internal standard. The extracts were applied on Sep-pak C18 cartridges (Waters, Milford, MA), and the unabsorbed fractions were evaporated and redissolved in 1.0 ml of 75% methanol. The samples obtained were separated by HPLC in a TSK gel ODS-80Ts QA column, (4.6×250 mm; Tosoh, Tokyo), at 30°C, using a linear gradient of solvent B (2.5% acetic acid in methanol) from 0% to 30% (v/v) in solvent A (2.5% acetic acid in water) for 30 min at a flow rate of 1 ml/min. The elution was monitored at 280 nm using a Model L-7450 diode array detector (Hitachi, Tokyo). Known amounts of kojic acid were used for calibration. The most popular hull-less barley variety in Japan 'Ichibanboshi' was used as a standard. A working standard sample of 'Ichibanboshi' was analyzed every 11 injections to facilitate comparison and identification of each peak by its retention time and spectrum. The levels of the compounds in peaks A to H (Fig. 1) were calculated from the areas under their peaks and estimated as amounts per gram of flour by the average of 3 determinations. Aliquots (10 μl) of authentic samples of (+)-catechin, PDB3 and PCB3 (0.1 mg/ml in methanol) were analyzed under the same conditions with and without the sample of 'Ichibanboshi' (50 μl).

Statistical analysis Correlation analysis, regression analysis and analysis of variance (ANOVA) were performed with a statistics software package, JMP version 3.2.2, developed by SAS Institute Inc. (Cary, NC).

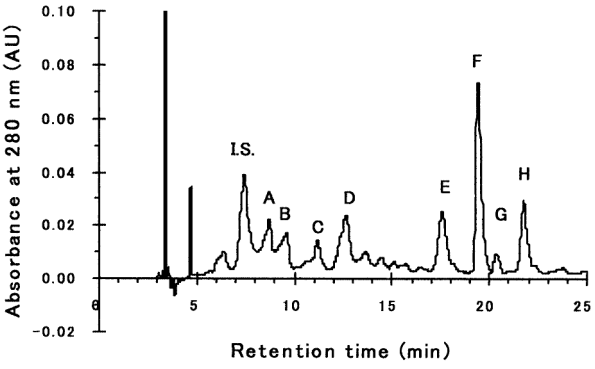

Fig. 1. Chromatogram of the crude polyphenol fraction of 'Ichibanboshi'. The crude polyphenol fraction was extracted from barley flour with 75% acetone in the presence of kojic acid as an internal standard (I.S.) and separated by HPLC on a reversed-phase column. The average retention times of the 8 peaks in the 42 varieties was as follows: 9.09±0.26 min (A), 9.99±0.28 min (B), 11.81±0.41 min (C), 13.27±0.42 min (D), 18.25±0.47 min (E), 19.62±0.30 min (F), 20.41±0.38 min (G), 22.43±0.46 min (H).

Results and Discussion

$W1$ values were positively correlated with $W2$ values (Table 1). $W1$ value is likely to be influenced by both the color of the flour and the early color change after the addition of water. To exclude the influence of color before heat treatment, BI was used as an indicator of the degree of the browning reaction during heat treatment. BI of the 42 varieties ranged from 18.3 to 28.5 (average 24.5). The apparent polyphenol content of the 42 varieties was between 0.14 to 0.69 mg/g of flour (average 0.50 mg/g). No significant differences ($p>0.05$) were observed in BI or in the apparent polyphenol content between 2- and 6-rowed varieties or between hulled and hull-less varieties. Proanthocyanidin-free mutant lines showed very low BI values (18.3 to 21.6) as well as low apparent polyphenol content (0.14 to 0.33 m/g). BI was significantly and positively correlated with the apparent polyphenol content (Table 1). When a regression analysis was performed with BI as the dependent variable and the apparent polyphenol content as the independent variable, the contribution ratio due to

Table 1. Correlation coefficients among whiteness values of barley pastes before and after heat treatment, browning indexes, and apparent polyphenol content.

	$W1^{a)}$	$W2^{b)}$	$BI^{c)}$	$PP^{d)}$
$W1$	—	0.669***	–0.145	–0.178
$W2$		—	–0.832***	–0.672***
BI			—	0.766***
PP				—

[a)]Whiteness value before heat treatment.
[b)]Whiteness value after heat treatment.
[c)]Browning index.
[d)]Apparent polyphenol content, as determined by the Prussian blue method.
***significant at $p<0.001$ (n=42).

Table 2. Correlation coefficients between the constituents of the crude polyphenol fractions and the browning index or apparent polyphenol content.

	A	B	C	PDB3	PCB3	F	G	(+)-Catechin
$BI^{a)}$	0.231	0.122	0.400**	0.595***	0.599***	0.108	0.248	0.384*
$PP^{b)}$	0.232	0.206	0.676***	0.713***	0.686***	0.357	0.321	0.445**

[a)]Browning index.
[b)]Apparent polyphenol content, as determined by the Prussian blue method.
*, ** and ***: significant at $p<0.05$, 0.01, and 0.001, respectively (n=42).

Table 3. Correlation coefficients among the contents of compound C, PDB3, PCB3, and (+)-catechin.

	C	PDB3	PCB3	(+)-Catechin
C	—	0.850***	0.570***	0.247
PDB3		—	0.738***	0.444**
PCB3			—	0.537***
(+)-Catechin				—

** and ***: significant at $p< 0.01$ and 0.001, respectively (n=42).

regression was calculated as 0.587. Therefore, *BI* cannot be predicted from the apparent polyphenol content alone, although this content would strongly influence *BI*.

To search for the constituents that might influence *BI*, we separated and analyzed the crude polyphenol fractions of the 42 varieties. A typical chromatogram of the crude polyphenol fraction is shown in Fig. 1. Eight peaks (A to H) were commonly detected at 280 nm in the crude polyphenol fractions of all varieties, except in those of 3 proanthocyanidin-free mutant lines, where no peaks of C, D, or E, and only a trace of H, were observed. The commercially available authentic samples of PDB3, PCB3 and (+)-catechin were consistent with the peaks D, E and H, respectively, in the crude polyphenol fraction from 'Ichibanboshi'; the compounds in peaks D, E and H were therefore identified as PDB3, PCB3, and (+)-catechin. We then analyzed the correlations between the amounts of the constituents of the 8 peaks and either the apparent polyphenol content or *BI* (Table 2). The PDB3 content and the PCB3 content were significantly and positively correlated with both factors ($p<0.001$). The contents of compound C and (+)-catechin were also positively correlated with both factors, although their correlation coefficients were smaller than those for PDB3 and PCB3. For none of these 4 compounds was the correlation with *BI* higher than that with apparent polyphenol content. This is probably because the browning reaction in barley paste is caused by the interaction of many constituents, not by the action of a single compound.

In general, positive or negative correlations between 2 constituents can reflect a cooperative or competitive response to the regulation of biosynthesis. Pairwise correlations were analyzed among the contents of the above 4 compounds (Table 3). The content of compound C was highly positively correlated with the PDB3 content, and compound C was not detected in proanthocyanidin-free mutant lines; thus, compound C is most probably a kind of proanthocyanidin. Since positive correlations were observed among the contents of compound C, PDB, PCB3, and (+)-catechin (Table 3), it may be possible to regulate the contents of all of these constituents simultaneously.

From the above results, it appears that the heat-induced browning reaction in barley products is greatly influenced by the compounds that react with the Prussian blue reagent, probably endogenous polyphenol constituents. Moreover, PDB3, PCB3, compound C, and (+)-catechin are likely to be the constituents responsible for the browning reaction. It may be possible to reduce the browning reaction in barley products by using a variety with low levels of these components, for example, a variety that has a deficiency in the biosynthetic pathways of these components.

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