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Changes in Anthocyanins in the Grains of Purple Waxy Hull-less Barley during Seed Maturation and after Harvest

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Purple waxy hull-less barley cv. Daishimochi accumulates purple pigments in the stem, awn, lemma, palea, and pericarp during seed maturation. Four major anthocyanin constituents from the grains of cv. Daishimochi were isolated and identified as cyanidin 3-*O*-(3,6-di-*O*-malonyl-β-D-glucopyranoside) (55%), cyanidin 3-*O*-(6-*O*-malonyl-β-D-glucopyranoside) (21%), cyanidin 3-*O*-(3-*O*-malonyl-β-D-glucopyranoside) (12%), and cyanidin 3-*O*-β-D-glucopyranoside (4%) by mass spectrometry and one- and two-dimensional NMR spectroscopy. These anthocyanins were observed after 28 days after flowering (DAF); they were most abundant at 35 DAF when the dry weight of grains was maximum. This accumulation time was later than that of proanthocyanidins, which are the most abundant polyphenol constituents in barley grains. These anthocyanins, especially cyanidin 3-*O*-(3,6-di-*O*-malonyl-β-D-glucopyranoside), decreased at 42 DAF and during drying preparation after harvest. Most anthocyanins are localized in the outer parts of grains and distributed into bran by the pearling process. Whole grain flour and bran of cv. Daishimochi are good sources of malonylated cyanidin derivatives.

KEYWORDS: Anthocyanins; hull-less barley; malonylated cyanidin glucoside; proanthocyanidin

INTRODUCTION

Anthocyanins are the primary pigments in black, blue, and purple cereal grains. Recently, anthocyanins have been recognized as health-enhancing substances due to their antioxidant activity (*1*–3) and anti-inflammatory (*4*), anticancer (*5, 6*), hypoglycemic (*7*), and cardioprotective effects (*8*). Barley (*Hordeum vulgare* L.) and oat grains contain more soluble dietary fiber β-glucan than wheat and rice. The U.S. Food and Drug Administration (FDA) allowed the labels of foods containing the soluble fiber from barley products to claim that the consumption of these foods may reduce the risk of coronary heart disease (*9*). Cereal grains are usually consumed as a staple food; therefore, anthocyanin-pigmented barley grains can be a stable source of anthocyanins as well as soluble β-glucan, both of which might have synergic effects to reduce the risk of coronary heart disease.

Waxy hull-less barley has been locally cultivated in the western region of Japan and used as a substitute for glutinous rice. At present, these grains are utilized as the ingredients for noodles, bread, and cakes. These landraces and cultivars accumulate purple pigments in the stem, awn, lemma, palea, and pericarp during seed maturation. However, little use is made of their characteristic purple pigment. There are numerous studies on the constituent anthocyanins of barley grains (*1, 10–12*), but these have reported different constituents, except for cyanidin 3-glucoside.

Biosynthesis of anthocyanins in barley was reported to be regulated by two genes (*13*). Biosynthesis pathways of anthocyanins and proanthocyanidins have been elucidated in *Arabidopsis* using many mutants (*14, 15*). Leucoanthocyanidins are common intermediates of anthocyanins and proanthocyanidins and are converted into anthocyanidins and subsequently into anthocyanins by leucoanthocyanidin deoxygenase and UDP-glucose transferase. They are also converted into flavan-3-ols, such as catechin, by leucoanthocyanidin reductase. The condensation mechanism of proanthocyanidins and the regulation of the leucoanthocyanidin deoxygenase and leucoanthocyanidin reductase pathways are still unsolved. In barley grains, catechin and proanthocyanidin dimers and trimers are the major phenolic constituents and are accumulated during seed maturation except for proanthocyanidin-free mutants.

The objectives of this study are to elucidate the constituent anthocyanins in purple grains of the waxy hull-less barley cv. Daishimochi and to determine the changes in their contents and composition during seed maturation in comparison with catechin and proanthocyanidins, as well as during drying and pearling after harvest, in order to explore the availabilities of their anthocyanins.

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MATERIALS AND METHODS

Plant Materials. Purple waxy hull-less barley cv. Daishimochi (Senbonhadaka//(Senbonhadaka/Mochimugi D)F_2) was grown in an experimental field at Zentsuji in 2003−2004 and at Tsukuba in 2004−2007. One hundred spikes were marked, of which the first floret was flowering on the same day, and 10 of these spikes were harvested every 7 days from the time of flowering to 42 days after flowering (DAF). Grain samples in maturing stages were manually collected from the middle portion of the spikes in order to minimize variation of their maturities, stored at −80 °C until freeze-dried, and ground in a TI-100 vibrating sample mill (CMT, Tokyo, Japan) for 90 s. Matured grain samples were harvested in bulk at 6 weeks after flowering time and dried under warm air at 40 °C until the moisture content of grains decreased to about 12%. Whole grain flour was obtained by grinding the matured grains using a Cyclotech 1093 sample mill (Techator, Höganäs, Sweden). Portions (10 g) of the matured grains were pearled from the outer layer into 90, 75, and 60% yield using a Pearlest abrasive machine (Kett, Tokyo, Japan) and ground using a TI-100 vibrating sample mill to obtain pearled grain flour.

Chemicals. Cyanidin 3-*O*-β-D-glucopyranoside, cyanidin, peonidin, pelargonidin, delphinidin, and malvidin were purchased from Extrasynthese (Genay, France) as chloride forms. Prodelphinidin B3, procyanidin B3, and (+)-catechin were from Sigma (St Louis, MO).

Analytical Chromatography. Anthocyanins were extracted from 100 mg of flour of the maturing seeds and the matured grain samples with 1 mL of 3% trifluoroacetic acid (TFA)−50% acetonitrile by shaking for 60 min and centrifuging at 12 500 rpm for 10 min. The precipitate was extracted again with the same solvent. The combined supernatant was filled up to 25 mL with 3% TFA, and 10 mL of aliquot was applied to a solid phase extraction cartridge Autoprep EDS-1 (50 mg) (Showa denko, Tokyo, Japan) equilibrated with 3% TFA and eluted with 300 μL of 3% TFA−50% acetonitrile. Samples were analyzed using an HPLC equipped with an SPD-M10AV diode array detector (Shimadzu, Kyoto, Japan). The HPLC column used was a 150 mm × 4.6 mm i.d. Zorbax SB-C18 (Agilent Technologies, Wilmington, DE). It was eluted with a gradient mobile phase consisting of 3% phosphoric acid (solvent A) and acetonitrile (solvent B) at 1.0 mL/min. The gradient was programmed as follows: 0−5 min, 10% B in A; 5−25 min, 10−20% B; 25−35 min, 20% B; 35−40 min, 20−50% B; 40−45 min, 50% B. Elution was monitored by absorbance at 520 nm, and concentrations of anthocyanins were calculated from peak areas using cyanidin 3-*O*-β-D-glucopyranoside as the standard.

Proanthocyanidins were extracted from 100 mg of flour of maturing seeds with 1 mL of 75% acetone, shaking for 60 min, and centrifuging at 12 500 rpm for 10 min. The precipitate was re-extracted with 75% acetone twice. After concentration under reduced pressure, the combined extracts were dissolved in 2.5% acetic acid and applied to an Autoprep EDS-1. Methanolic eluate of the cartridge was redissolved in 100 μL of 20% acetonitrile−20 mM ammonium phosphate buffer (pH 3.0) after solvent evaporation and centrifuged at 12 500 rpm for 10 min. Aliquots (20 μL) of the supernatants were applied on a 150 mm × 4.6 mm i.d. TSKgel ODS-100V column (Tosoh, Tokyo, Japan). The column was eluted with a gradient mobile phase consisting of 20 mM ammonium phosphate buffer (pH 3.0) (solvent A) and acetonitrile (solvent B) at 1.0 mL/min. The gradient was programmed as follows: 0−5 min, 5% B in A; 5−35 min, 5−35% B; 35−40 min, 35−50% B; 40−45 min, 50% B. The concentrations of prodelphinidin B3, procyanidin B3, and (+)-catechin were calculated from peak areas of absorbance at 280 nm using authentic compounds as standards.

Isolation and Identification of Anthocyanins. Whole grain flour of the matured grains (500 g) was extracted with 5 volumes (v/w) of 3% TFA−20% ethanol for 4.5 h at 4 °C, and the extract was centrifuged at 12 000 rpm for 20 min at 4 °C. The filtered supernatant was concentrated to 50 mL under reduced pressure, applied onto a Sephadex LH-20 column (265 mL) (GE Healthcare Bio-Sciences, Uppsala, Sweden) equilibrated with 0.4% TFA and eluted with 1 L of 3% TFA−ethanol. The obtained crude anthocyanin fraction was dissolved in 30% methanol−0.1% TFA after solvent evaporation and fractionated on a 100 mm × 25 mm i.d. μBondapak C18 column (Waters, Milford, MA) using 30−70% methanol−0.1% TFA as eluent. Four anthocyanin compounds (**1**, **2**, **3**, and **6** in **Figure 1**) were further purified by a 250 mm × 4.6 mm i.d. TSKgel ODS-80Ts QA column (Tosoh) eluted with 30−50% methanol−0.1% TFA at 1.0 mL/min by monitoring absorbance at 520 nm. Each fraction of preparative chromatography was checked for purity by analytical chromatography. One of the isolated compounds, compound **1** (**Figure 1**), was cochromatographed with authentic cyanidin 3-*O*-β-D-glucopyranoside.

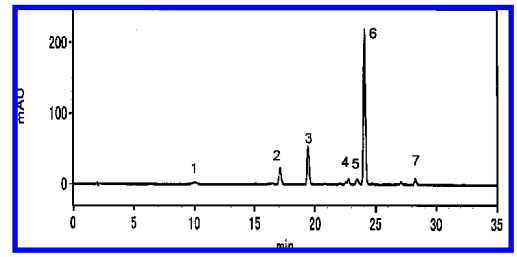


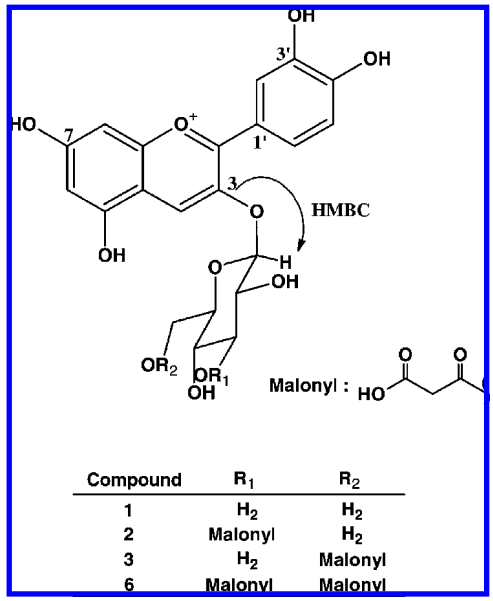
Figure 1. Chromatogram of anthocyanins extracted from the grains of waxy hull-less barley cv. Daishimochi. Peaks were detected by absorbance at 520 nm.

Electron spray ionization time-of-flight (ESI-TOF) mass spectra ranging from *m/z* 200 to 1000 were recorded on a Mariner (Applied Biosystems, Foster City, CA) in methanol under the following conditions: ESI spray tip potential, 4.0 kV (positive mode); nozzle temperature, 140 °C; nozzle potential, 230 V; sheath gas, nitrogen; Skimmer 1 potential, 10 V; and sample flow rate, 2 μL/min. High-resolution Fourier transform ion-cyclotron resonance (FT-ICR) spectra were recorded on an Apex II 70e (Bruker Daltonics Inc., Billerica, MA) by the infusion of a methanol/H_2O/acetic acid (49:49:2, v/v/v) solution with ESI positive ion mode and used to determine the composition formulas of compounds **2**, **3**, and **6**. One- and two-dimensional NMR spectra were recorded at 298 K in DMSO-d_6 containing 10% TFA-d_1 using an Avance 500 spectrometer (Bruker Biospin, Karlsruhe, Germany) equipped with a $^{13}C\{^1H\}$ CryoProbe and an Avance 800 spectrometer (Bruker Biospin, Karlsruhe, Germany) equipped with a $^1H\{^{13}C, ^{15}N\}$ TXI probehead. The linkage positions of each unit were determined by interpreting the downfield shift of ^1H signals at a glucose unit and several two-dimensional NMR measurements such as DQF-COSY, NOESY, HSQC, and HMBC.

Acid Hydrolysis of Crude Anthocyanins. The crude anthocyanin fraction (2.36 mg) prepared from the Sephadex LH-20 column was dissolved in 1 mL of 18% hydrochloride and heated for 3 min. The fraction was cooled on ice, diluted with 1 mL of distilled water, and extracted with *n*-butanol (0.5 mL) twice. The butanol fraction was concentrated under a stream of nitrogen and analyzed on a Zorbax SB-C18 column with or without authentic samples of cyanidin, peonidin, pelargonidin, delphinidin, and malvidin under the conditions used for analytical chromatography.

RESULTS AND DISCUSSION

Constituents of Anthocyanins. A typical HPLC chromatogram of anthocyanins in the matured grains of barley cv. Daishimochi is shown in **Figure 1**. Seven peaks were detected at 520 nm from the crude anthocyanin fraction of the matured grains. Compounds **1**, **2**, **3**, and **6** of the crude anthocyanins were isolated by chromatography, with yields of 0.47, 0.09, 0.20, and 0.30 mg, respectively. ESI-TOF mass spectrometry measurements of compounds **1**, **2**, **3**, and **6** showed intense ion peaks at *m/z* 449.2, 535.1, 535.1, and 621.1, corresponding to $C_{21}H_{21}O_{11}^+$, $C_{24}H_{23}O_{14}^+$, $C_{24}H_{23}O_{14}^+$, and $C_{27}H_{25}O_{17}^+$, respectively, and product ion peaks of cyanidin were observed at *m/z* 287.1. Acid hydrolysis of the crude anthocyanins gave only cyanidin as aglycone (data not shown). When compound **1** was cochromatographed with cyanidin 3-*O*-β-D-glucopyranoside, a peak overlap was observed. Thus, compound **1** was identified as cyanidin 3-*O*-β-D-glucopyranoside. High resolution FT-ICR mass spectrometry measurement of compounds **2**, **3**, and **6** gave ion peaks at *m/z* 535.1074, 535.1079, and 621.1081, respectively, that corresponded to the following theoretical values:



Compound	R_1	R_2
1	H_2	H_2
2	Malonyl	H_2
3	H_2	Malonyl
6	Malonyl	Malonyl

Figure 2. Chemical structures of cyanidin derivatives **1**−**3** and **6** isolated from the grains of waxy hull-less barley cv. Daishimochi. The arrow shows the HMBC correlation observed at the position of the glycosyl linkage.

Table 1. ^{1}H NMR Data for Compounds **2**, **3**, and **6** from Grains of Waxy Hull-less Barley cv. Daishimochi[a]

	compd **2**	compd **3**	compd **6**
position	δ (ppm), multiplicity, *J* (Hz)	δ (ppm), multiplicity, *J* (Hz)	δ (ppm), multiplicity, *J* (Hz)
		cyanidin	
4	8.923, s	8.804, s	8.833, s
6	6.702, s	6.711, d, 2.0	6.712, d, 1.9
8	6.895, s	6.887, d, 2.0	6.885, brs
2′	7.979, d, 2.4	7.978, d, 2.3	7.943, d, 2.2
5′	7.019, d, 8.7	7.017, d, 8.7	7.009, d, 8.8
6′	8.185, dd, 8.7, 2.4	8.216, dd, 8.7, 2.3	8.177, dd, 8.8, 2.2
		3-*O*-β-D-glucopyranoside	
1	5.527, d, 7.7	5.375, d, 7.7	5.554, d, 7.8
2	3.700, dd, 9.5, 7.7	3.511, dd, 9.3, 7.7	3.726, dd, 9.5, 7.8
3	5.010, dd, 9.5, 9.3	3.393, dd, 9.3, 8.8	5.028, dd, 9.5, 9.3
4	3.457, dd, 9.8, 9.3	3.231, dd, 9.9, 8.8	3.482, dd, 9.8, 9.3
5	3.615, ddd, 9.8, 5.7, 2.1	3.806, ddd, 9.9, 7.7, 1.8	3.937−3.972, m
6	3.688, dd, 12.1, 2.1	4.439, dd, 11.8, 1.8	4.394, brd, 11.1
6	3.518, dd, 12.1, 5.7	4.107, dd, 11.8, 7.9	4.158, dd, 11.8, 7.4
		3-*O*-malonyl	
CH_2	3.435[b], s		3.340[b], brs
		6-*O*-malonyl	
CH_2		3.353, d, 15.9	3.442[b], s
		3.363, d, 15.9	

[a] These data were measured in DMSO-d_6/TFA-d_1 (9/1) at 303 K with operating frequencies 800.1 MHz for compounds **2** and **3** and 500.13 MHz for compound **6**. [b] Two protons were observed equivalently.

$C_{24}H_{23}O_{14}$ = 535.1082 and $C_{27}H_{25}O_{17}$ = 621.1086. ^{1}H NMR spectra showed that the compounds consisted of cyanidin aglycone and malonylated β-glucopyranosyl residues. The linkage positions of each unit were determined by interpreting the downfield shift of ^{1}H signals at a glucose unit and several two-dimensional NMR measurements such as DQF-COSY, NOESY, HSQC, and HMBC (**Figure 2**). The β-configurations at the glycosyl linkage were confirmed by the large *J* value (7.7−7.8 Hz) at anomeric positions. The assignments of ^{1}H NMR data are shown in **Table 1**. Thus, compounds **2**, **3**, and **6** were identified as cyanidin 3-*O*-(3-*O*-malonyl-β-D-glucopyranoside), cyanidin 3-*O*-(6-*O*-malonyl-β-D-glucopyranoside, and cyanidin 3-*O*-(3,6-di-*O*-malonyl-β-D-glucopyranoside, respectively. Andersen and Fossen (*16*) isolated compounds **2**, **3**, and **6** from the stem of *Allium victorialis* and reported ^{1}H NMR data in CD_3OD/TFA-d_1 (95/5). Our results obtained in DMSO-d_6/TFA-d_1 (9/1) were in appropriate agreement with their result.

Table 2. Contents of Anthocyanins in Grains of Waxy Hull-less Barley cv. Daishimochi during Seed Maturation[a]

	dry weight	compd (nmol/seed)				
DAF	(mg/seed)	**1**	**2**	**3**	**6**	**1**−**7**
7	1.3 ± 0.1	ND	ND	ND	ND	ND
14	5.5 ± 0.1	ND	ND	ND	ND	ND
21	15.6 ± 0.2	ND	ND	ND	ND	ND
28	23.3 ± 1.0	0.05 ± 0.00 (2)	0.17 ± 0.02 (7)	0.34 ± 0.01 (13)	1.91 ± 0.09 (74)	2.58 ± 0.12 (100)
35	30.2 ± 1.0	0.35 ± 0.04 (2)	1.53 ± 0.17 (9)	2.51 ± 0.23 (15)	11.07 ± 1.17 (68)	16.34 ± 0.59 (100)
42	30.6 ± 1.3	0.42 ± 0.06 (4)	1.22 ± 0.21 (12)	2.07 ± 0.31 (21)	5.43 ± 1.05 (55)	9.95 ± 1.72 (100)

[a] Contents are expressed as mean ± standard deviation (n = 4) of cyanidin 3-*O*-β-D-glucopyranoside equivalents. Relative quantitative contents (%) are shown in parentheses. Compounds **1**−**7** are shown in **Figures 1** and **2**. ND = not detected.

Fossen et al. (*11*) reported the anthocyanins in flowering tops of *H. distichon* L. as compound **6** (63%), compound **3** (14%), peonidin 3-*O*-β-D-glucopyranoside (3%), compound **1** (2%), peonidin 3-*O*-(3,6-di-*O*-malonyl-β-D-glucopyranoside (1%), and others but did not describe compound **2**. The results reported here are the first to identify compound **2** from barley grains. Abdel-Aal et al. (*10*) reported the extraction of cyanidin 3-glucoside and petunidin 3-glucoside from the grains of blue barley with methanol acidified with hydrochloric acid. Kim et al. (*1*) reported that anthocyanins in the purple barley grains extracted with 80% methanol acidified by 0.1% hydrochloric acid were mainly cyanidin 3-glucoside, peonidin 3-glucoside, and pelargonidin 3-glucoside. In an early report of Mullick et al. (*12*), anthocyanins from pericarp and aleurone tissues of blue, purple, and black barley extracted with diluted hydrochloric acid were cyanidin, pelargonidin, and delphinidin derivatives. Andersen and Fossen (*16*) reported that the use of mineral acid such as hydrochloric acid during extraction and the chromatographic isolation procedure could destroy the linkage between an aliphatic acyl group and the anthocyanin sugar moiety in some pigments. In this study, the solvent acidified with TFA was used for extraction and chromatographic isolation of anthocyanins to prevent deacylation. Thus, anthocyanins from cv. Daishimochi were mainly considered as the malonylated cyanidin derivatives.

As shown in **Figure 1**, compound **6** was the most abundant anthocyanins in the grains of cv. Daishimochi. Malonylated cyanidin derivatives were also reported in purple corn seeds, which are used as food colorants, but the contents of compounds **3** and **6** in purple corn seeds were reported to be far smaller than that of their nonacylated form, compound **1** (*17, 18*). Therefore, the grain of purple waxy hull-less barley might be a good source for malonylated cyanidin derivatives.

Changes in Anthocyanins and Proanthocyanidins during Seed Maturation. The contents of anthocyanins in grains during maturation are shown in **Table 2**. Anthocyanins were undetectable in grains at 7, 14, and 21 DAF and accumulated after 28 DAF. Contents of the malonylated cyanidin derivatives (compounds **2**, **3**, and **6**) per seed increased up to 35 DAF and decreased at 42 DAF, while those of compound **1** increased during seed maturation. Changes in the contents of extractable anthocyanins were consistent with the appearance of the grains (i.e., the density of purple color).

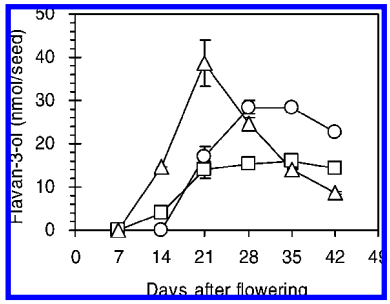


Figure 3. Contents of the major flavan-3-ols of the grains of waxy hull-less barley cv. Daishimochi during seed maturation. Flavan-3-ols were extracted from grains at 7, 14, 21, 28, 35, and 42 days after flowering. Contents of (+)-catechin (△), procyanidin B3 (□), and prodelphinidin B3 (○) were calculated with authentic samples as standards and expressed as the mean ± standard deviation ($n = 3$).

Compound **6** was the most abundant anthocyanin throughout seed maturation, although its relative content in total anthocyanins declined gradually from 28 to 42 DAF. Conversely, the relative contents of compounds **1**−**3** increased gradually from 28 to 42 DAF. Similar trends for the changes in anthocyanins during seed maturation were observed over 3 years. The dry weight of seeds increased up to 35 DAF and was almost constant from 35 to 42 DAF (**Table 2**). In general, the seed moisture of wet grains decreases gradually in the late stages of seed maturation and drastically after the dry weight of seeds is maximum. Therefore, the decrease in anthocyanins from 35 to 42 DAF was thought to have occurred with physiological dryness.

The contents of (+)-catechin and proanthocyanidin dimers in grains during seed maturation are shown in **Figure 3**. (+)-Catechin accumulated until 21 DAF and decreased drastically in the later stages, while procyanidin B3 and prodelphinidin B3 accumulated slowly compared to (+)-catechin and were relatively stable after 28 DAF. In grains of cv. Senbonhadaka, a recurrent parent of cv. Daishimochi containing no anthocyanins, the contents of (+)-catechin, procyanidin B3, and prodelphinidin B3 changed almost similarly to those in cv. Daishimochi during seed maturation (data not shown).

Proanthocyanidins accumulated at the stages in which the dry weight per seed increased exponentially and were localized in their testa. On the other hand, anthocyanins accumulated at the late stages of maturation and were localized in the pericarp. Both proanthocyanidins and anthocyanins are biosynthesized through leucocyanidin, but their accumulation time and location are different from each other in grains of cv. Daishimochi. Accumulation of proanthocyanidins prior to accumulation of anthocyanins has also been reported in bilberry fruit (*19*) and grape skin (*20, 21*). Thus, biosynthesis of anthocyanins seemed to be unaffected by that of proanthocyanidins.

Changes in Anthocyanins during Drying and Pearling Processes after Harvest. We studied the effects of drying preparation after harvest on the anthocyanin content. The total anthocyanin content in grains dried under warm air after harvest was about one-fifth of that in the freeze-dried grains (**Table 3**). In addition, the relative content of compound **6** in total anthocyanins was smaller in the warm air-dried sample (52%) than that in the freeze-dried sample (63%), while the relative content of the nonacylated form (compound **1**) was larger in the warm air-dried sample (3%) than in the freeze-dried sample (2%). In general, anthocyanin stability increases with glycosylation and acylation of the sugars (*22*). However, our results suggested that the malonylated cyanidin derivatives, rather than the nonacylated form, were unstable, especially compound **6**. Fournand et al. (*21*) reported that total anthocyanins decreased just before harvest in grape skin and that the degradation rate observed at the late stages of maturation was higher for coumaroylglucoside derivatives than for nonacylated derivatives. Furthermore, they reported that coumaroylglucoside derivatives were less extractable than nonacylated derivatives. Decreases in total anthocyanin and in the contents of compound **6** observed during seed maturation and after harvest could result from degradation, deacylation, or insolubilization. It is possible that nonacylated cyanidin is an artifact of deacylation of malonylated cyanidin derivatives during storage and extraction.

Table 3. Effects of Drying Preparations after Harvest on the Contents of Anthocyanins of Waxy Hull-less Barley cv. Daishimochi[a]

	compd (nmol/g)				
preparation	**1**	**2**	**3**	**6**	**1**−**7**
A	7.3 ± 0.3	31.4 ± 2.3	71.3 ± 5.9	239.5 ± 17.1	379.7 ± 27.6
B	2.6 ± 0.2	5.2 ± 1.0	22.2 ± 3.8	39.8 ± 6.0	77.3 ± 11.1

[a] Matured grains were freeze-dried (preparation A) or dried under warm air (preparation B). Contents were expressed as mean ± standard deviation ($n = 3$) of cyanidin 3-*O*-*β*-D-glucopyranoside equivalents. Compounds **1**−**7** are shown in **Figures 1** and **2**.

Table 4. Contents of Anthocyanins after Pearling of Grains of Waxy Hull-less Barley cv. Daishimochi[a]

	compd (nmol/g)				
pearling yield (%)	**1**	**2**	**3**	**6**	**1**−**7**
whole	9.2 ± 1.7	14.5 ± 0.7	39.0 ± 0.5	70.0 ± 3.6	157.6 ± 3.0
90	3.0 ± 0.0	5.6 ± 0.5	15.1 ± 0.6	25.7 ± 1.6	58.3 ± 3.3
75	ND	0.7 ± 0.6	2.4 ± 0.3	4.2 ± 0.5	7.3 ± 1.4
60	ND	ND	ND	1.2 ± 0.2	1.2 ± 0.2

[a] Contents were expressed as mean ± standard deviation ($n = 3$) of cyanidin 3-*O*-*β*-D-glucopyranoside equivalents. Compounds **1**−**7** are shown in **Figures 1** and **2**. ND = not detected.

The effect of pearling yield on the contents of anthocyanins was studied in the grains of cv. Daishimochi (**Table 4**). When grains were pearled to 90% yield, about two-thirds of anthocyanins in the whole grains were distributed into the bran. Furthermore, only a small amount of anthocyanins was retained in the pearled grains of 75 and 60% yield. Usually, hull-less barley grains are pearled to about 75% for fermentation materials and to about 60% for rolled barley. Purple waxy hull-less barley grains are often pearled to about 90% in order to retain purple pigments, but more than half of the pigments are wasted.

In conclusion, the present study demonstrates that malonylated cyanidin derivatives, especially cyanidin 3-*O*-(3,6-di-*O*-malonyl-*β*-D-glucopyranoside), are the principal anthocyanins of purple waxy hull-less barley cv. Daishimochi. The contents and extractabilities of these anthocyanins are affected by harvest time, drying temperature, and pearling yield. There is yet no report that suggests the superiority of malonylated cyanidin derivatives as health-enhancing substances. However, utilization of purple hull-less barley is suitable as whole grain meal in order to take advantage of the anthocyanin contents. Otherwise, the bran could be utilized as a source of natural food colorant containing malonylated cyanidin derivatives.

ABBREVIATIONS USED

DAF, days after flowering; ESI-TOF, electron spray ionization time-of-flight; FT-ICR, Fourier transform ion-cyclotron resonance; TFA, trifluoroacetic acid.

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