Diversity in γ -oryzanol profiles of Japanese black-purple rice varieties

Wakako Tsuzuki¹ · Shiro Komba¹ · Eiichi Kotake-Nara¹

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Abstract The γ -oryzanol contents and the composition of steryl ferulates distributed in Japanese pigmented rice varieties were investigated using the high-performance liquid chromatography–ultraviolet detection method for the purpose of expanding their utilisation. The average γ -oryzanol content in nine black-purple, four red, four green and three brown rice varieties was 54.2, 47.3, 44.3 and 43.3 mg γ -oryzanol equivalent/100 g dried weight, respectively. Among the nine varieties of black-purple rice, five varieties showed steryl ferulate composition similar to that of brown, red and green varieties. In contrast, the composition of steryl ferulates in other four black-purple rice varieties was partially specific and was characterised by a low amount of campesteryl ferulate and high of campestanil ferulate and stigmastanyl ferulate. The latter two steryl ferulates have been recognised as minor components of γ -oryzanol in rice and as major components in wheat and corn. These results indicate that the compositions of steryl ferulates vary among Japanese black-purple rice varieties.

Keywords γ -Oryzanol · Components · Steryl ferulate · Pigmented rice

Introduction

Rice is the staple food in many Asian countries including Japan. In these countries, white rice, which is prepared from brown rice by polishing, is predominantly consumed. However, white rice has a lower nutritive value than brown rice, because many nutrients, such as proteins, calcium, magnesium, vitamins of the B complex, vitamin E and dietary fibre, are mainly located in rice bran (Orthoefer and Eastman 2004). In addition, bioactive components—ferulic acid, γ -oryzanol and γ -aminobutyric acid—are abundantly found in rice bran, but are present in considerably lower amounts in polished white rice (Yu et al. 2007). Therefore, whole grain rice is considered to be a good source material for the development of nutraceutical products and functional foods (Chotimarkorn and Silalai 2008; Min et al. 2009).

Pigmented rice varieties are distributed worldwide, and they are often consumed as whole grains, because these pigmented components are mainly located in the surface layers of grains. The pigmented rice varieties black-purple, red and green rice varieties are endowed with pigmented components anthocyanins, proanthocyanidins and chlorophyll, respectively. The antioxidant activities of these pigmented components have been reported previously (Oki et al. 2002; Min et al. 2011). In addition to the pigmented components, the pigmented rice varieties have high levels of antioxidant compounds such as polyphenols, tocopherols and γ -oryzanols (Min et al. 2011). Therefore, pigmented rice varieties have attracted increasing interest as a good source of not only various nutrients but also of bioactive compounds present in the surface of rice grains (Faiz et al. 2015).

γ -Oryzanol is a mixture of ferulic acid esters of plant sterols or triterpene alcohols (designated as steryl ferulate),

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✉ Wakako Tsuzuki
wakako@affrc.go.jp

¹ National Agriculture and Food Research Organization, Food Research Institute, 2-1-12 Kannondai, Tsukuba, Ibaraki 305-8642, Japan

and its antioxidant activity involves the ferulic acid moiety (Yu et al. 2007). There are variations in the molecular structure of sterol (or triterpene alcohol) moieties in γ -oryzanol. Moreover, the compositions of steryl ferulates of γ -oryzanol strongly depend on the cereal species, although they are also slightly influenced by environmental factors (Bergman and Xu 2003; Miller and Engel 2006). For instance, γ -oryzanol in regular brown rice varieties is mostly composed of four steryl ferulates, such as cycloartenyl ferulate, 24-methylenecycloartanyl ferulate, campesteryl ferulate and β -sitosteryl ferulate (Miller and Engel 2006). Meanwhile, the major steryl ferulates present in wheat, corn, rye and foxtail millet are β -sitosteryl ferulate, campestanol ferulate and stigmastanol ferulate (Nystrom et al. 2007; Esche et al. 2012; Tsuzuki et al. 2018). In regard to γ -oryzanol present in pigmented rice varieties, previous studies have revealed that the composition of their steryl ferulates is similar to that of regular brown rice varieties (Pereira-Caro et al. 2013a; Sing et al. 2015; Minatel et al. 2016). Furthermore, diversity in the composition of steryl ferulates present in pigmented rice varieties has not been thoroughly investigated to date.

γ -Oryzanol is one of the bioactive lipids that offers health benefits, such as prevention of obesity and cardiovascular diseases and anti-inflammatory, anticancer, as well as antioxidant properties (Akihisa et al. 2000; Iwatsuki et al. 2003; Orthoefer and Eastman 2004; Burlando and Cornara 2014; Punvittayagul et al. 2016). In previous studies, the antioxidant activity of γ -oryzanol was reported to be influenced by the composition of individual steryl ferulates (Xu et al. 2001; Kikuzaki et al. 2002; Winkler-Moser et al. 2012). Furthermore, a recent study using hypercholesterolemic rats suggested that the cholesterol-lowering activities of γ -oryzanol depended on the molecular structure of steryl ferulates (Zawistowski et al. 2009). In other in vitro studies, the hydrolysis rates of several different steryl ferulates by artificial digestive enzymes were compared (Moreau and Hicks 2004). Therefore, detailed studies on identification of the molecular structure of each steryl ferulate and its composition in pigmented rice varieties is important for understanding their physiological roles and developing therapeutic applications.

In this study, the quantitative and qualitative analyses of γ -oryzanol in various black-purple, brown, red and green rice varieties distributed in Japan were conducted using the high-performance liquid chromatography–ultraviolet detection (HPLC–UV) method and mass spectrometry. We investigated the variations in γ -oryzanol contents and in the compositions of steryl ferulates in pigmented rice varieties. The objective of this study is to characterise the γ -oryzanols present in pigmented rice varieties in order to enhance their use as sources of γ -oryzanols.

Materials and methods

Materials

Brown rice (Koshihikari) harvested in Ibaraki in the fall of 2016 was provided by the Ibaraki Agriculture Research Center (Mito, Japan). Two kinds of brown rice (Milky Queen and Akitakomachi), nine kinds of black-purple rice, four kinds of red rice and four kinds of green rice harvested from various regions in Japan in the fall of 2016 were purchased from local stores. The profiles of these pigmented rice varieties are summarised in Table 1. After purchase, all samples were stored at 4 °C prior to analysis. The γ -oryzanol standard (> 98.0%) was obtained from FUJIFILM Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All other chemical reagents of analytical grade were obtained from FUJIFILM Wako Pure Chemical Industries, Ltd. and Kanto Chemical Co., Inc. (Tokyo, Japan).

Methods

Determination of water content of rice grain

The water content of each rice variety was determined by drying 2-g samples in pre-weighed dishes at 105 °C overnight and weighing the dishes after cooling for 1 h in a desiccator.

Extraction of γ -oryzanol from rice grain

Extraction of γ -oryzanol from rice grain was performed following the method described in a previous study (Pereira-Caro et al. 2013b) with modifications in the sample size. Briefly, about 2 g of whole kernel was milled to a powder using a domestic miller (IFM-800DG; Iwatani, Osaka, Japan) and a mortar. The powdered sample (0.1 g) was precisely weighed in a glass tube, and γ -oryzanol was extracted with 2 mL of *n*-hexane/ethanol mixture (4:3, v/v) with sonication for 20 min at 0 °C (ULTRA SONIC USC-100ZA38S-22; Iwaki, Tokyo, Japan). The sample was centrifuged at 1000×*g* for 5 min and the supernatant was collected. The pellet was re-extracted twice with 1 mL *n*-hexane/ethanol solution as described above. All supernatants were combined and reduced to dryness under a gentle stream of nitrogen. The concentrates were dissolved in 1 mL ethanol and filtered through a 0.45- μ m filter (ADVANTEC, Tokyo, Japan). Next, the filtrates were subjected to HPLC–UV analysis.

Table 1 Profiles of pigmented rice varieties examined in this study and their γ -oryzanol contents

Label	Sample (district)	Harvested year	Breed name	Water content (%)	Oryzanol content (mg OE/100 g)
B1	Black-purple rice (Iwate)	2016	Unknown	12.45 $\pm$ 0.03	60.2 $\pm$ 0.8
B2	Black-purple rice (Nagasaki)	2016	Unknown	13.82 $\pm$ 0.01	59.3 $\pm$ 2.5
B3	Black-purple rice (Hiroshima)	2016	Unknown	13.82 $\pm$ 0.09	51.9 $\pm$ 1.1
B4	Black-purple rice (Kagoshima)	2016	Unknown	14.34 $\pm$ 0.08	57.6 $\pm$ 1.5
B5	Black-purple rice (Akita)	2016	Unknown	14.67 $\pm$ 0.33	53.6 $\pm$ 1.0
B6	Black-purple rice (Kumamoto)	2016	Unknown	14.26 $\pm$ 0.28	55.5 $\pm$ 1.4
B7	Black-purple rice (Hokkaido)	2016	Kitanomurasaki	14.43 $\pm$ 0.24	39.5 $\pm$ 1.8
B8	Black-purple rice (Kagoshima)	2016	Kokushien	14.53 $\pm$ 0.05	50.8 $\pm$ 1.5
B9	Black-purple rice (Fukushima)	2016	Okunomurasaki	13.02 $\pm$ 0.39	55.2 $\pm$ 1.5
G1	Green rice (Nagasaki)	2016	Unknown	14.22 $\pm$ 0.01	40.3 $\pm$ 1.9
G2	Green rice (Hiroshima)	2016	Unknown	14.27 $\pm$ 0.09	49.3 $\pm$ 1.1
G3	Green rice (Kagoshima)	2016	Unknown	14.01 $\pm$ 0.17	49.0 $\pm$ 1.6
G4	Green rice (Kumamoto)	2016	Unknown	12.54 $\pm$ 0.02	48.9 $\pm$ 1.9
R1	Red rice (Nagasaki)	2016	Unknown	13.57 $\pm$ 0.14	38.6 $\pm$ 0.6
R2	Red rice (Hiroshima)	2016	Unknown	14.05 $\pm$ 0.40	51.8 $\pm$ 1.2
R3	Red rice (Kagoshima)	2016	Unknown	14.72 $\pm$ 0.02	46.0 $\pm$ 2.0
R4	Red rice (Okayama)	2016	Unknown	11.73 $\pm$ 0.05	40.2 $\pm$ 1.5
Br1	Brown rice (Ibaraki)	2016	Koshihikari	14.21 $\pm$ 0.16	41.6 $\pm$ 0.4
Br2	Brown rice (Ibaraki)	2016	Akitakomachi	11.82 $\pm$ 0.06	47.9 $\pm$ 0.2
Br3	Brown rice (Ibaraki)	2016	Milky queen	14.30 $\pm$ 0.23	40.3 $\pm$ 1.4

HPLC analysis

γ -Oryzanol from rice grain was analysed with reverse-phase HPLC–UV using a C18 column (TSK gel ODS-100Z, 5 μ m, 4.6 mm $\times$ 150 mm; Tosoh Corp., Tokyo, Japan) with an isocratic elution of acetonitrile/methanol/2-propanol (50:45:5, v/v/v), a flow rate of 1 mL/min, a column temperature of 40 °C (CO-965; JASCO International, Tokyo, Japan), and the UV detector set at 325 nm (UV-4075; JASCO International). The γ -oryzanol amount was quantitatively determined referring to the analysis of γ -oryzanol standard reagent. γ -Oryzanol standard solutions of 10, 20, 50 and 100 ng were analysed under the same HPLC conditions. Each calibration point was run in triplicate. An aliquot of 20 μ L for each sample dissolved in ethanol was applied to the HPLC column. The amount of γ -oryzanol in the sample was estimated using the calibration curve, which was based on the total peak area of the γ -oryzanol standard. Therefore, the unit of γ -oryzanol content of the rice variety was expressed in terms of ‘mg γ -oryzanol equivalent in 100 g dried weight’ (mg OE/100 g). All extractions and HPLC analysis were performed at least in triplicates, and the data were presented as mean $\pm$ standard deviation (SD).

Identification of components of γ -oryzanol in black-purple rice B1 and regular brown rice Br1 by mass spectrometry

The components of γ -oryzanol from the black-purple rice variety B1 were characterised using HPLC retention time and negative ion spectra from atmospheric pressure chemical ionisation-mass spectrometry (APCI-MS), as described earlier by Hakala et al. (2002). The two later HPLC peaks of γ -oryzanol components in B1, which were eluted at 21.7 min and 25.3 min, were collected by fractionation. The components in the two fractions were analysed using an LTQ-Orbitrap Velos mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Mass spectrometric analysis was conducted using an APCI source in the negative ion mode, as described in a previous study (Tsuzuki et al. 2017). The fractionated samples were dissolved in 50% methanol solution (methanol/H₂O, 50:50, v/v) and applied to the ion source using a direct flow injection technique. Mass calibration was performed daily using an electrospray ionisation (ESI) negative ion calibration solution (Thermo Fisher Scientific) and polytyrosine (Pierce® Triple quadrupole calibration solution; Thermo Fisher Scientific). The vaporiser temperature and capillary gas were set at 300 °C and 250 °C, respectively. The data were acquired using a Fourier-transform full scan with a resolution power of 30,000 and the microscan count fixed to unity. The automatic gain control was set at

1×10^6 , and the mass spectrometric data were collected in the m/z range of 100–1000. Collision-induced dissociation (CID) was produced with helium (99.96%) at a pressure of 10^{-6} mTorr in the ion trap. Mass chromatograms and spectral data were acquired with Xcalibur software 2.0 (Thermo Fischer Scientific).

Statistical analysis

Extraction of γ -oryzanols and HPLC analyses were performed in triplicate, and data were presented as mean $\pm$ SD. The results were analysed using analysis of variance (ANOVA) with multiple tests to identify significant differences between samples. A p value of < 0.05 was considered statistically significant.

Results and discussion

γ -Oryzanol contents in pigmented rice varieties

The water content of each sample is presented in Table 1. The γ -oryzanol content in each rice variety was evaluated on the basis of the total HPLC peak area, and the results are summarised in Table 1. The average γ -oryzanol contents in black-purple, green, red and brown rice varieties were 54.2 ± 6.2 , 47.3 ± 4.4 , 44.6 ± 6.1 and 43.3 ± 4.1 mg OE/100 g dried weight, respectively. The γ -oryzanol contents of the regular brown rice varieties observed in this study were consistent with those reported previously (Miller and Engel 2006; Kim et al. 2015). A statistically significant difference in average γ -oryzanol content was found between black-purple rice varieties and other rice varieties ($p < 0.05$). In particular, the amounts of γ -oryzanol in B1 (60.7 ± 0.8 mg OE/100 g) and B2 (59.9 ± 2.5 mg OE/100 g) were about 1.4 times of the average amount of γ -oryzanol found in brown rice (Table 1). According to previous studies, several black-purple rice varieties contain much higher amounts of γ -oryzanol compared with those in the regular brown rice (Laokuldilok et al. 2011; Pereira-Caro et al. 2013b). However, B7 has a γ -oryzanol content of less than 40 mg OE/100 g (Table 1), suggesting the γ -oryzanol contents in black-purple rice show variations among different varieties. On the contrary, there were no statistically significant differences in the average γ -oryzanol contents among red, green and brown rice varieties ($p < 0.05$).

Composition of sterol ferulates in pigmented rice varieties

The HPLC chromatograms of γ -oryzanols extracted from nine black-purple rice varieties examined in this study were

divided into two patterns (Fig. 1). The γ -oryzanol profiles of black-purple rice varieties—B4, B6, B7, B8 and B9—were similar to that of the regular brown rice variety, as shown in Figs. 1 and 2. This was consistent with the results of previous studies (Fang et al. 2003; Cho et al. 2012). On the contrary, the HPLC chromatograms of γ -oryzanol profiles extracted from B1, B2, B3 and B5 were different from those of B4, B6, B7, B8, B9 and the brown rice variety (Figs. 1 and 2). There were indistinguishable differences among the HPLC chromatograms of γ -oryzanols extracted from three regular brown, four green and four red rice varieties. The representative HPLC chromatograms from red (R4), green (G4), black-purple (B1, B4), and brown (Br1) rice varieties are shown in Fig. 2.

The sterol ferulate composition of each HPLC peak of the γ -oryzanol standard (commercially available reagent) has been completely identified in previous studies using reverse-phase HPLC, ESI-MS (negative), nuclear magnetic resonance (NMR) or liquid chromatography-mass spectrometry (LC-MS) (Fang et al. 2003; Cho et al. 2012). In this study, the composition of sterol ferulates in the γ -oryzanol standard (Fr.G in Fig. 2) was simultaneously determined using APCI-MS. The HPLC peaks of γ -oryzanol standard were identified as cycloartenyl ferulate [molecular weight (MW) = 602.4, Component 1 in Fig. 2], 24-methylenecycloartanyl ferulate (MW = 616.4, Component 2 in Fig. 2), campesteryl ferulate (MW = 576.4, Component 3 in Fig. 2), and β -sitosterol ferulate (MW = 590.4, Component 5 in Fig. 2) (APCI-MS data are shown in Supplemental data 1). These observations are in good agreement with those reported in previous studies (Fang et al. 2003; Cho et al. 2012).

For the purpose of identifying the molecular structures of sterol ferulates extracted from the black-purple rice variety B1, five HPLC peaks eluted from 15.1 min to 23.3 min were fractionated and designated as Fr.A, Fr.B, Fr.C, Fr.D and Fr.E (Fig. 2). Sterol ferulates contained in each fraction were determined using the same APCI-MS configuration as that for the γ -oryzanol standard. As shown in Fig. 3, Fr.A, Fr.B, Fr.C and Fr.E were mainly composed of a single sterol ferulate, whose deprotonated molecules appeared at m/z 601, m/z 615, m/z 575 and m/z 591, respectively. Considering their CID species, each sterol ferulate in Fr.A, Fr.B, Fr.C and Fr.E was therefore identified as cycloartenyl ferulate (MW = 602.4), 24-methylenecycloartanyl ferulate (MW = 616.4), campesteryl ferulate (MW = 576.4) and stigmastanyl ferulate (MW = 592.4), respectively. The APCI-MS analysis of Fr.D revealed the presence of two sterol ferulates—campesteryl ferulate (MW = 578.4) and β -sitosterol ferulate (MW = 590.4)—whose deprotonated molecules appeared at m/z 577 and m/z 589 (Fig. 3), respectively. In HPLC chromatograms, Fr.F of the brown rice variety Br1 was

Fig. 1 HPLC chromatograms of γ -oryzanol extracted from the black-purple rice varieties examined in this study. Analytical conditions are described in the main text

Fig. 2 HPLC chromatograms of γ -oryzanols extracted from green rice variety (G4), red rice variety (R4), black-purple rice varieties (B1 and B4), brown rice variety (Br1), whole wheat (Whole wheat), and γ -oryzanol standard (γ -Oryzanol St.). The five fractions of black-purple rice variety (B1) for APCI-MS analyses were designated as Fr.A, Fr.B, Fr.C, Fr.D, and Fr.E. The one fraction of regular brown rice variety (Br1) for APCI-MS analyses was designated as Fr.F. The γ -oryzanol components contained in three fractions of whole wheat (Components 3, 4, 5, 6, and 7) were determined in our previous study

eluted at almost the same elution time as that of Fr.D of the black-purple rice variety B1, as shown in Fig. 2. However, the steryl ferulate in Fr.F was a single steryl ferulate, β -

(Tsuzuki et al. 2017). Each component in standard γ -oryzanol (Components 1, 2, 3, and 5) was identified in previous studies (Fang et al. 2003; Cho et al. 2012) and APCI-MS analyses of Fr.G in this study (see Supplemental data). Component 1: cycloartenyl ferulate; Component 2: 24-methylenecycloartanyl ferulate; Component 3: campesteryl ferulate; Component 4: Δ^7 -sitosteryl ferulate; Component 5: β -sitosteryl ferulate; Component 6: campestanlyl ferulate; Component 7: stigmastanyl ferulate

sitosteryl ferulate (m/z 589, MW = 590.4, Fr.F in Fig. 3). Therefore, the steryl ferulate in Fr.F was not to the same as that in Fr.D. A certain amount of campestanlyl ferulate

Fig. 3 Negative ion mode APCI-MS spectra of the components in Fr.A, Fr.B, Fr.C, Fr.D, Fr.E and Fr.F, which were prepared via fractionation of the corresponding HPLC peaks of black-purple rice variety B1 (Fr.A to Fr.E) and regular brown rice variety Br1 (Fr.F)

(MW = 578.4) was found in Fr.D but not in Fr.F, which is one of the characteristics of specific black-purple rice varieties, such as B1. The compositions of steryl ferulates in the pigmented rice varieties examined in this study were calculated on the basis of HPLC analyses, and results are summarised in Table 2. As shown in Table 2, the black-purple rice varieties B1, B2, B3 and B5 could be characterised by the presence of a high amount of stigmastanyl ferulate. Furthermore, the composition percentages of campesteryl ferulate found in B1, B2, B3 and B5 (11.1–12.8%) were significantly lower than those in the other pigmented rice varieties ($p < 0.05$). These results showed that the composition of steryl ferulate in a specific black-purple rice variety was characterised by a low

amount of campesteryl ferulate and high amounts of campestanil ferulate and stigmastanyl ferulate.

In our previous study, we investigated the composition of steryl ferulates in whole wheat (Tsuzuki et al. 2017). In this study, the last three HPLC peaks from γ -oryzanol present in specific black-purple rice varieties (Fr.C, Fr.D and Fr.E) were found to be similar to those in whole wheat, as shown in Fig. 2. Therefore, the composition of steryl ferulates in B1 could be considered as a mixture of that in regular brown rice varieties and in whole wheat, as shown in Fig. 2.

The γ -oryzanol contents of various pigmented rice varieties distributed in several regions around the world have been evaluated in previous studies (Pereira-Caro et al. 2013b; Kim et al. 2015; Sanghamitra et al. 2018).

Table 2 Composition ratio of γ -oryzanol components present in pigmented rice varieties

Variety	Cycloartenyl ferulate (%)	24-Methylene cycloartenyl ferulate (%)	Campesteryl ferulate (%)	β -Sitosteryl ferulate + campesteryl ferulate (%)	Stigmastanyl ferulate (%)	Others (%)	Total (%)
B1	27.3 $\pm$ 0.2	34.6 $\pm$ 0.4	*11.1 $\pm$ 0.2	18.1 $\pm$ 0.1	*7.8 $\pm$ 0.1	1.1 $\pm$ 0.4	100.0
B2	27.6 $\pm$ 0.1	33.4 $\pm$ 0.1	*12.8 $\pm$ 0.1	17.3 $\pm$ 0.4	*6.8 $\pm$ 0.2	2.1 $\pm$ 0.1	100.0
B3	27.0 $\pm$ 0.1	33.7 $\pm$ 0.3	*12.5 $\pm$ 0.1	18.1 $\pm$ 0.5	*7.5 $\pm$ 0.1	1.2 $\pm$ 0.1	100.0
B4	27.1 $\pm$ 0.2	32.5 $\pm$ 0.2	22.7 $\pm$ 0.4	13.9 $\pm$ 0.4	1.8 $\pm$ 0.2	2.0 $\pm$ 0.6	100.0
B5	29.2 $\pm$ 0.1	31.2 $\pm$ 0.3	*11.7 $\pm$ 0.2	17.5 $\pm$ 0.1	*8.4 $\pm$ 0.1	2.0 $\pm$ 0.3	100.0
B6	30.6 $\pm$ 0.4	30.2 $\pm$ 0.1	18.5 $\pm$ 0.3	16.5 $\pm$ 0.1	2.0 $\pm$ 0.1	2.2 $\pm$ 0.2	100.0
B7	22.6 $\pm$ 0.4	29.4 $\pm$ 0.5	23.3 $\pm$ 0.2	18.8 $\pm$ 0.2	2.7 $\pm$ 0.2	3.2 $\pm$ 0.6	100.0
B8	27.0 $\pm$ 0.5	29.4 $\pm$ 0.3	22.4 $\pm$ 0.2	15.7 $\pm$ 0.7	2.5 $\pm$ 0.5	3.0 $\pm$ 0.2	100.0
B9	27.4 $\pm$ 0.1	32.3 $\pm$ 0.3	21.9 $\pm$ 0.2	14.1 $\pm$ 0.2	2.5 $\pm$ 0.1	1.8 $\pm$ 0.1	100.0
R1	26.3 $\pm$ 0.3	33.3 $\pm$ 0.4	20.9 $\pm$ 0.9	15.8 $\pm$ 1.0	2.3 $\pm$ 0.8	1.4 $\pm$ 0.2	100.0
R2	27.6 $\pm$ 0.2	27.5 $\pm$ 0.5	23.8 $\pm$ 0.3	17.5 $\pm$ 0.1	1.7 $\pm$ 0.1	1.9 $\pm$ 0.1	100.0
R3	26.9 $\pm$ 0.2	31.4 $\pm$ 0.4	21.2 $\pm$ 0.2	15.8 $\pm$ 0.2	2.6 $\pm$ 0.3	2.1 $\pm$ 0.5	100.0
R4	20.9 $\pm$ 0.5	34.4 $\pm$ 0.3	25.1 $\pm$ 0.1	16.0 $\pm$ 0.3	2.0 $\pm$ 0.5	1.6 $\pm$ 0.2	100.0
G1	27.6 $\pm$ 0.5	27.8 $\pm$ 0.1	23.2 $\pm$ 0.4	17.6 $\pm$ 0.2	2.3 $\pm$ 0.1	1.5 $\pm$ 0.1	100.0
G2	28.2 $\pm$ 0.2	28.5 $\pm$ 0.5	22.2 $\pm$ 0.4	17.4 $\pm$ 0.1	1.8 $\pm$ 0.1	1.9 $\pm$ 0.1	100.0
G3	27.6 $\pm$ 0.1	31.2 $\pm$ 0.2	21.7 $\pm$ 0.1	16.2 $\pm$ 0.4	1.8 $\pm$ 0.4	1.5 $\pm$ 0.1	100.0
G4	29.0 $\pm$ 0.3	30.6 $\pm$ 0.1	21.4 $\pm$ 0.2	15.3 $\pm$ 0.3	1.8 $\pm$ 0.2	1.9 $\pm$ 0.1	100.0
Br1	23.3 $\pm$ 0.3	35.7 $\pm$ 0.1	24.6 $\pm$ 0.2	14.0 $\pm$ 0.1	1.4 $\pm$ 0.1	1.0 $\pm$ 0.1	100.0
Br2	24.2 $\pm$ 0.7	35.1 $\pm$ 0.1	25.1 $\pm$ 0.6	13.4 $\pm$ 0.1	1.2 $\pm$ 0.1	1.0 $\pm$ 0.1	100.0
Br3	20.1 $\pm$ 0.5	34.6 $\pm$ 0.1	28.3 $\pm$ 0.2	14.1 $\pm$ 0.1	1.8 $\pm$ 0.1	1.1 $\pm$ 0.1	100.0

The values represent the mean $\pm$ SD of three experiments

Samples were divided into 5 groups [(B1, B2, B3, and B5), (B4, B6, B7, B8, and B9), (R1, R2, R3, and R4), (G1, G2, G3, and G4), (Br1, Br2, and Br3)] for statistical processing. The values that are statistically different from those of other groups are shown using an asterisk (*) ($p < 0.05$)

However, the specific composition of steryl ferulate in B1 has not been recognised before, and it is considerably different from those of the wild rice varieties distributed in North America (Aladedunye et al. 2013). Of course, the composition of steryl ferulates is influenced by various external factors (Miller and Engel 2006). Nevertheless, the significant differences in the composition of steryl ferulates between specific black-purple rice varieties (B1, B2, B3 and B5) and other rice varieties indicate that there are at least two kinds of steryl ferulate compositions among black-purple rice varieties distributed in Japan. Previously, such variations in γ -oryzanol contents and compositions of steryl ferulates were identified in 25 corn cultivars grown at the same location (Esche et al. 2013).

According to previous studies, various environmental factors affect not only the γ -oryzanol content but also the composition of steryl ferulates in rice varieties, although the degree of maturity is not significantly related to steryl ferulate composition in rice grains (Bergman and Xu 2003; Miller and Engel 2006). Further investigation is necessary to elucidate the influence of the environmental factors as well as the origin and genotype on the γ -oryzanol profiles of pigmented rice varieties.

The health advantages of pigmented rice varieties were largely associated with their bioactive compounds found in the bran of grain kernel (Leardkamolkarn et al. 2011; Choi et al. 2013; Kaur et al. 2018). In addition to the pigmented components, γ -oryzanol in these pigmented rice varieties might simultaneously be involved in these potent physiological activities. The antioxidant activity of individual steryl ferulates was variable because of the molecular structure of their phytosterol or triterpene alcohol moieties (Zhu et al. 2016). Today, we are advancing the comparative study of physiological activities and bioavailability using γ -oryzanols extracted from two groups of black-purple rice varieties, the regular brown rice variety, and wheat varieties.

Conclusion

For the purpose of expanding the use of pigmented rice varieties, the γ -oryzanol content and composition of steryl ferulates were investigated for nine black-purple, four red, four green and three brown rice varieties distributed in Japan. The average γ -oryzanol content of black-purple rice

varieties was significantly higher than those of other rice varieties. The composition of steryl ferulates in four black-purple rice varieties among the nine varieties investigated in this study were partially specific and different from those in brown, red and green rice varieties. The results indicated diversity in the composition of steryl ferulates in Japanese black-purple rice varieties. As the physiological activities of γ -oryzanol are known to be influenced by the composition of steryl ferulates, γ -oryzanols in Japanese black-purple rice varieties would be one of the important sources of such bioactive compounds.

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