

Vitamin E Profiles and Triacylglycerol Molecular Species of Colored Rice Bran Cultivars at Different Degree of Milling

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Abstract

The objective of this study was to evaluate the tocopherol distributions, lipid components and molecular species of triacylglycerols (TAG) in three colored rice bran cultivars. The dominant tocopherol were γ -tocotrienol, α -tocopherol and α -tocotrienol with smaller amounts of γ -tocopherol, δ -tocopherol and δ -tocotrienol. These lipids comprised mainly TAG (78.0-81.6 wt%), free fatty acids (FFA: 5.6-8.8 wt%), and phospholipids (PL: 6.3-7.0 wt%), while other components were present in minor proportions (0.4-2.3 wt%). Sixteen different TAG molecular species were detected and quantified by successive applications of AgNO₃-TLC and GC. The major TAG molecular species were SM₂ (6.1-9.8%), S₂D (4.8-7.3%), M₃ (16.4-18.7%), SMD (6.2-9.2%), SD₂ (6.5-9.5%), SMT (6.3-7.7%), M₂D (12.3-15.5%), MD₂ (8.4-10.4%), SDT (4.3-5.4%) and D₃ (10.2-15.2%) (where S, M, D, and T denote saturated FA, monoene, diene, and triene, respectively). The results showed that colored rice bran lipids contain large amounts of nutraceutical with proven positive health effects.

Keywords: bran lipids, colored rice, molecular species, tocopherol homologues, triacylglycerols

Abbreviations: FFA, free fatty acids; GC, gas chromatography; HMRB, half-milled rice bran; HPLC, high-performance liquid chromatography; PL, phospholipids; TAG, triacylglycerols; TLC, thin-layer chromatography; WMRB, well-milled rice bran

1. Introduction

Rice (*Oryza sativa* L.) constitutes the world's principal source of food (Yadav & Jindal, 2001). Rice is the staple food of Asia, where 90% of the world's rice crop is produced and consumed. Rice is commonly consumed as milled or white rice, which is produced by removing the hulled and bran layers of the rough rice kernel in dehulling and milling processes, respectively. The colored grain are caused by anthocyanins pigment that gives the milled rice a red and brownish red color in red rice or a dark purple color in black rice (Oki et al., 2002). These two types of rice were gaining popularity in Japan as functional food and often mixed with rice to enhance the color, flavor, and nutritional value (Itani & Ogawa, 2004). Moreover, rice grain quality is an important economic trait that influences rice production in many rice-producing areas. Although the fat or oil in rice grain is low (i.e., 2-3%) and is concentrated in the germ and bran, it is a key determinant of the processing and cooking quality of rice (Zhou et al., 2002a). For instance, the surface lipid content has been thought to be an indication of the degree of milling (Siebenmorgan, Matsler, & Earp, 2006). The milling efficacy is measured by degree of milling. The amount of bran remaining on the rice kernel after milling affects rice quality, appearance, and texture and rice is thus milled to the end-use preference of different consumers (Perdon et al., 2001; Saleh & Meullenet, 2007). In addition, rice lipid, which frequently forms complexes with starch granules, was shown to affect starch gelatinization, water availability to starch, and rice swelling and thus influences the eating and cooking qualities of rice (Champagne, Marshall, & Goynes, 1990; Marshall, Norma, & Goynes, 1990). Besides dietary consumption, the unique health benefits of rice fat, which includes many unsaturated fatty acids, has drawn much attention (Jennings & Akoh, 2009). A number of studies have shown that rice bran oil reduces the harmful cholesterol (low-density lipoprotein) without affecting the good cholesterol (high-density lipoprotein) in

plasma (Sugano & Tuji, 1997; Wilson et al., 2007). In addition, rice bran oil, which is rich in tocotrienol (Vitamin E), has anti-cancer and anti-radiation effects (Wilson et al., 2007). A combination of tocopherols and tocotrienols, preferably from natural sources, has been suggested to be an essential part of a diet preventing Alzheimer disease (Morris et al., 2005) or to be aiding in the treatment of Parkinson's syndrome (Itoh et al., 2006). On the other hand, some reports have shown that the hydrolysis and oxidation of rice fat are responsible for rice aging and deterioration of grain flavor during storage, and low-oil rice cultivars are more suitable for storage (Zhou et al., 2002b).

Several studies on lipid fractions of rice brans have been published (Taira et al., 1988; Jennings & Akoh, 2009; Przybylski et al., 2009). However, to the best of our knowledge, no data have been reported on the vitamin E homologues, lipid components and fatty acid compositions of colored rice bran cultivars at different degrees of milling. Therefore, the aim of this study was to investigate the tocochromanol distributions and the molecular species of triacylglycerols (TAG) obtained from the three colored rice bran cultivars, based on differences in the degrees of milling.

2. Materials and Methods

2.1 Raw Materials

Commercially mature rice seeds used were from the three different colored Japanese cultivars: black (*Okunomurasaki*), red (*Beniroman*) and green (*Midorimochi*) harvested in September 2012 at the same district (Takaichigun, Nara Prefecture) of Japan. These colored rices were purchased from regional alliance center for industrial technology at Asuka district of Nara prefecture. Black and red rices were nonglutinous rice, and green rice was a glutinous rice. For analysis, seeds were selected for uniformity based on seed weight of 19.8-21.1 mg for black, 20.6-21.7 mg for red and 17.2-18.4 mg for green, respectively. Rices of each cultivar were packed in polyethylene bags under nitrogen gas and placed in a stainless-steel container at -35 °C until analysis.

2.2 Chemicals and Reagents

All chemicals were of analytical grade (Wako Pure Chemical Inc., Osaka, Japan). TLC-plates were purchased from Merck (Darmstadt, Germany). Standard mixture for TLC, containing monoacylglycerols, diacylglycerols, free fatty acids (FFA), triacylglycerols (TAG), steryl esters and hydrocarbons, was purchased from Wako Pure Chemical. Standard TAG (glyceryl trimyristate, glyceryl tripalmitate, glyceryl tristearate, glyceryl trioleate, glyceryl trilinoleate and glyceryl trilinolenate) was procured from Sigma-Aldrich Co. (St. Louis, Mo, USA). Vitamin E homologues (α , β , γ and δ) were acquired from Sigma-Aldrich Co. All tocopherols were of the D-form (*RRR*-), and their purities were better than 98.8%. Fatty acid methyl esters (FAME) standards (F & OR mixture #3) were obtained from Altech-Applied Science (State College, PA, USA). The internal standards, pentadecane and methyl pentadecanoate, were purchased from Merck, and then 100 mg of each was dissolved in *n*-hexane (20 ml). Boron trifluoride (BF_3) in methanol (14%; Wako Pure Chemical Inc., Osaka, Japan) was used to prepare the FAME.

2.3 Lipid Extractions

Rice bran of each cultivar (500 g) was milled using a domestic miller (BR-CA25, Zojirushi Ltd., Osaka, Japan). Before extraction, each bran prepared from half-milled rice and well-milled rice was defined as HMRB and WMRB, respectively. The degree of milling was 5% milling for HMRB and 10% for WMRB. Total lipids were extracted from 20 g of bran in 300 ml chloroform/methanol (2:1, v/v) with vigorous shaking for 15 min at 0 °C three times, following the Folch procedure (Folch et al., 1957). The extracted lipids were weighed to determine the lipid content of the rice brans and then transferred to a 25-ml brown-glass volumetric flask with chloroform/methanol (2:1, v/v), flushed by nitrogen, and stored in the dark at -35 °C until further use.

2.4 Tocochromanol Analysis

Determinations of tocochromanols were performed by HPLC according to the methods reported previously (Yoshida, Tomiyama, & Mizushima, 2010). An aliquot (10 μl) from this sample solution was subjected to HPLC analysis, and the amount of each tocochromanol was determined with a fluorescence detector (Shimadzu RF-10 AXL, Kyoto, Japan) as previously reported (Yoshida, Tomiyama, & Mizushima, 2010).

2.5 Lipid Analysis

According to the previous procedure (Yoshida et al., 2013), total lipids were fractionated by TLC into nine fractions using a solvent system of *n*-hexane/diethyl ether/acetic acid (80:20:1, v/v/v). Bands corresponding to hydrocarbons, steryl esters, TAG, unknown, FFA, 1,3-diacylglycerols, 1,2-diacylglycerols, monoacylglycerols and PL were scraped into separate test-tubes. Methyl pentadecanoate (10-100 μg) from a standard solution (5

mg/ml) was added to each tube as the internal standard, except that pentadecane (10 µg) was used as the internal standard for hydrocarbons analysis. FAME was prepared by heating with silica gel for 30 min at 80 °C in BF₃/methanol (Kitts et al., 2004). The FAME was quantified by gas chromatography (GC, 14B GC Shimadzu) equipped with a hydrogen flame ionization detector (FID) at 350 °C and a polar capillary column (ULBO HE-SS-10 for FAME, fused silica WCOT [no. PSC5481], cyanopropyl silicone, 30 m x 0.32 mm i.d.; Shinwa Chem. Ind., Ltd., Kyoto, Japan) at a column temperature of 180 °C. The component peaks were identified and compared against that of the standard FAME as a previously described method (Yoshida et al., 2013). The detection limit was 0.05 wt% of total fatty acids for each FAME in the FAME mixture, and the results are expressed as wt% of total FAME. The other GC conditions were as in the previously described (Yoshida et al., 2010).

2.6 TAG Analysis

The TAG isolated by TLC was directly analyzed by GC according to a previously described method (Molkentin, 2007), using the Shimadzu Model-14C GC equipped with a hydrogen FID. Helium was used as the carrier gas at a flow rate of 50 ml/min. The injection port and the FID were set at 320 and 350 °C, respectively. The oven temperature was programmed to increase from an initial temperature of 285 °C (5 min hold), rising to 320 °C at a rate of 2 °C/min, which was held isothermally (320 °C) for 20 min. TAG peaks were identified by co-chromatography with known standards. Peak areas were calculated by the addition of a known weight (50 µg) of glyceryl trimyristate as the internal standard, using an electronic integrator (Shimadzu C-R6A).

2.7 TAG Species Composition

Molecular species separation from total TAG was carried out by AgNO₃-TLC (Bilyk et al., 1991). TAG classes differing in unsaturation were isolated by AgNO₃-TLC using 1.2-2.8% (v/v) methanol in chloroform, depending on the degree of unsaturation (Jham et al., 2005). The plates were streaked with 10-15 mg TAG using the microsyringe, developed with 1.2% (v/v) methanol in chloroform, and S₃, S₂M, SM₂, S₂D, M₃, SMD and SD₂ were easily separated. Furthermore, TAG molecular species such as SMT, M₂D, MD₂, SDT, D₃, MDT, M₂T, MT₂, and D₂T (where S, M, D and T denote saturated FA, monoene, diene, and triene, respectively) were separated by developing the plate with 2.8% (v/v) methanol in chloroform. Each TAG subfraction was identified by comparison with the R_f-values of the TAG standard. Each band was recovered from the plate by extraction with 3.0% aqueous HCl in the purified diethyl ether, and then the solvent was then vaporized under a gentle stream of nitrogen. Methyl pentadecanoate (10-50 µg) of the standard solution (2-10 µl; 5 mg/ml) was added to each tube as the internal standard. After methylation of all TAG sufractions, the relative amount of each TAG fraction was quantified by GC as described in the preceding paragraphs. The other conditions were as previously described (Yoshida et al., 2010).

2.8 Data Analysis

Data in this study were expressed as means ± SD for at least three independent experiments. Differences between the means of individual groups were assessed by one-way analysis of variance and Tukey's multiple range test using the SAS statistical software package (SAS, Cary, NC, USA). Differences were considered significance at $P < 0.05$.

3. Results and Discussion

3.1 The Content of Brans and Their Lipids in Colored Rices

The bran content of the rice black, red and green cultivars (500 g) were 41.9 g (8.4%), 29.6 g (5.9%) and 19.4 g (3.9%) from the HMRB, and then 76.7 g (15.3%), 59.2 g (11.8%) and 37.5 g (7.5%) from the WMRB for black, red and green cultivars, respectively. The bran contents of the HMRB and WMRB were significantly ($P < 0.05$) different among the three cultivars. On the other hand, the lipid contents obtained from these rice brans was 7.4 g (17.7%), 5.4 g (18.2%) and 4.8 g (24.7%) from the HMRB and then 11.0 g (14.4%), 8.9 g (15.1%) and 7.9 g (21.1%) from the WMRB, for black, red and green cultivars. The percentage of lipid contents was significantly ($P < 0.05$) higher in the HMRB than in the WMRB, and was in the rank order: green > red > black in both brans. Therefore, the lipids may be higher in the outer bran layer than in the interior bran layer.

3.2 Tocochromanol Contents in the Rice Bran Cultivars

The lipid content of the rice samples analyzed ranged from 2.2 to 3.7% (data not shown). When comparing tocochromanols among the three cultivars as shown in Table 1, the individual amounts was significantly ($P < 0.05$) lower in the green brans than in the black or red brans. With a few exceptions, the predominant tocochromanol forms in rice bran cultivars were γ -tocotrienol, α -tocotrienol, and α -tocopherol. The content of γ -tocotrienol ranged from 325 to 593 mg/kg, α -tocotrienol was found between 93.6 and 293 mg/kg and

α -tocopherol varied in the range of 78.4-452 mg/kg. In addition to these forms, δ -tocopherol, γ -tocopherol and δ -tocotrienol were also detected in the range of 27.2-56.2 mg/kg, 5.8-89.3 mg/kg and 12.3-25.8 mg/kg, respectively, in all samples. Among individual vitamin E homologues (Heinemann et al., 2008), α -tocopherol, α -tocotrienol, and γ -tocotrienol were the most abundant components in *japonica* rice, while in *indica* rice, the highest mean level was for γ -tocotrienol, followed by α -tocopherol and α -tocotrienol. Tocochromanol composition was in good agreement with the tocopherol composition of several grape seed oils reported in the literature (Wie et al., 2009; Demirtas et al., 2013). With a few exceptions, the percentage of tocotrienols was significantly ($P < 0.05$) higher in the HMRB than in the WMRB. Therefore, tocotrienols could be present more in the outer bran layer, while tocopherols would be distributed more in the interior layer. The main vitamin E homologue was the α - or γ -isomer among tocopherols or tocotrienols.

Table 1. Tocol composition of colored rice bran cultivars (mg/kg lipid)*

Bran	Lipid class	Cultivar											
		Black			Red			Green					
HMRB	α -Tocopherol	174	\pm	9.2 ^c	(15.2)	168	\pm	6 ^c	(16.3)	78.4	\pm	2.0 ^f	(14.3)
	α -Tocotrienol	252	\pm	8 ^b	(22.0)	235	\pm	6 ^c	(22.7)	98	\pm	2.3 ^d	(17.9)
	β -Tocopherol	nd				nd				nd			
	β -Tocotrienol	nd				nd				nd			
	γ -Tocopherol	89.3	\pm	2.4 ^a	(7.8)	39.4	\pm	0.6 ^b	(3.8)	5.8	\pm	0.2 ^e	(1.1)
	γ -Tocotrienol	563	\pm	14 ^b	(49.2)	538	\pm	12 ^c	(52.1)	325	\pm	8 ^e	(59.4)
	δ -Tocopherol	46.8	\pm	1.5 ^b	(4.1)	35.8	\pm	0.4 ^c	(3.5)	27.2	\pm	0.7 ^d	(5.0)
	δ -Tocotrienol	18.5	\pm	0.7 ^b	(1.6)	16.8	\pm	0.4 ^b	(1.6)	12.8	\pm	0.3 ^c	(2.3)
	Σ Tocopherol	310.1	\pm	7.5 ^c	(27.1)	243.2	\pm	5.1 ^d	(23.5)	111.4	\pm	2.0 ^f	(20.4)
	Σ Tocotrienol	833.5	\pm	10.2 ^c	(72.9)	789.8	\pm	16.3 ^d	(76.5)	435.8	\pm	9.37 ^f	(79.6)
	Σ Tocol	1143.6	\pm	21.4 ^c		1033.0	\pm	20.1 ^d		547.2	\pm	11.6 ^f	
WMRB	α -Tocopherol	452	\pm	12 ^a	(31.4)	437	\pm	8 ^b	(31.6)	113	\pm	4.2 ^e	(17.8)
	α -Tocotrienol	284	\pm	2.8 ^a	(19.7)	293	\pm	7.3 ^a	(21.2)	93.6	\pm	2.5 ^d	(14.8)
	β -Tocopherol	nd				nd				nd			
	β -Tocotrienol	nd				nd				nd			
	γ -Tocopherol	27.0	\pm	2.9 ^c	(1.9)	23.5	\pm	0.7 ^d	(1.7)	7.3	\pm	0.2 ^e	(1.2)
	γ -Tocotrienol	593	\pm	17 ^a	(41.3)	557	\pm	13 ^c	(40.3)	372	\pm	10 ^d	(58.6)
	δ -Tocopherol	56.2	\pm	1.8 ^a	(3.9)	47.6	\pm	0.8 ^b	(3.4)	32.6	\pm	0.8 ^c	(5.1)
	δ -Tocotrienol	25.8	\pm	0.7 ^a	(1.8)	24.6	\pm	0.7 ^a	(1.8)	16.3	\pm	0.5 ^b	(2.5)
	Σ Tocopherol	535.2	\pm	9.8 ^a	(37.2)	508.1	\pm	8.9 ^b	(36.7)	152.6	\pm	2.9 ^e	(24.1)
	Σ Tocotrienol	902.8	\pm	12.2 ^a	(62.8)	874.6	\pm	12.3 ^b	(63.3)	481.9	\pm	8.3 ^e	(75.9)
	Σ Tocol	1438.0	\pm	26.0 ^a		1382.7	\pm	23.0 ^b		634.5	\pm	12.4 ^e	

HMRB: half-milled rice brans. WMRB: well-milled rice brans. nd: (not detectable) < 0.01 mg/kg.

*Mean values \pm standard error. Each value represents the average of three determinations, and is expressed as mg/kg lipid. Values in parentheses are relative wt% contents of the individual tocopherols in HMRB or WMRB. Values in the same row with different superscripts are significantly different between the individual cultivars ($P < 0.05$).

Vitamin E is represented by α -, β -, γ -, and δ -tocopherols, and α -, β -, γ - and δ -tocotrienols, all of which occur in nature, and 14 vitamins are theoretically possible (Bramley et al., 2000). Vitamin E is a term frequently used to designate a family of related compounds, namely, tocopherols and tocotrienols (Amaral et al., 2005), which are important lipophilic antioxidants with essential effects in living system against aging (Agostinucci et al., 2002)

and reducing the risk of cancer (Lee et al., 2000). However, α -tocopherol is regarded as the most active and predominant form (Bender & Mayes, 2003). Tocotrienols have been indicated to suppress the effects of reactive oxygen species more effectively than tocopherols, and different studies of *in vitro* and *in vivo* effects suggest that tocotrienols may lower cholesterol levels and suppress tumor growth (Schaffer, Muller, & Eckert, 2005). Published data related to the tocopherol and tocotrienol content of these colored rice bran lipids is lacking and comparison is not currently possible.

3.3 Lipid Compositions in the Rice Bran Cultivars

Profiles of lipid components were compared among the HMRB and WMRB from the three cultivars (Table 2). Dominant components were TAG (HMRB: 78.4-81.2%; WMRB: 78.0-81.6%), followed by FFA (HMRB: 6.7-7.6%; WMRB: 5.6-8.8%) and PL (HMRB: 6.3-6.8%; WMRB: 6.5-7.0%), accompanied by very small amounts (0.4-2.3%) of other lipid components. When comparing the nine lipid components of the HMRB and WMRB among all three cultivars, the percentage of TAG was significantly ($P < 0.05$) lower in the green cultivar than that in the black or red cultivar, while the percentage of FFA was significantly ($P < 0.05$) higher in the green cultivar than that in the black cultivar. However, with a few exceptions, no substantial differences ($P > 0.05$) in the contents of the lipid components were observed between the values estimated by a combination analysis of TLC and GC using the internal standard (C15:0).

Presumably, the minor components, such as FFA, 1,3- and 1,2-diacylglycerols or monoacylglycerols, may be formed by the partial enzymatic hydrolysis of reserve TAG during the storage of the rice seeds (Aboul-Nasr, Ramadan, & El-Dengawy, 1997). The lipid components resulting from 'fat by hydrolysis' in starch granules were determined, showing the presence of FFA with lysolecithin and lysoglycolipids (Okunishi & Ohtsubo, 2008).

Table 2. Lipid components obtained from colored rice bran cultivars*

Bran	Lipid class	Cultivar											
		Black			Red			Green					
HMRB	Hydrocarbons	29.7	±	0.7 ^c	(0.4)	21.5	±	0.5 ^d	(0.4)	19.2	±	0.4 ^d	(0.4)
	Steryl esters	74.2	±	1.8 ^c	(1.0)	64.6	±	1.6 ^d	(1.2)	52.7	±	1.3 ^e	(1.1)
	Triacylglycerols	5999	±	40 ^d	(80.7)	4374	±	39 ^e	(81.2)	3752	±	38 ^f	(78.4)
	Unknown	59.3	±	1.4 ^b	(0.8)	26.9	±	0.7 ^d	(0.5)	24.0	±	0.5 ^e	(0.5)
	Free fatty acids	497	±	12 ^c	(6.7)	399	±	10 ^d	(7.4)	364	±	9 ^e	(7.6)
	1, 3-Diacylglycerols	118.7	±	2.9 ^b	(1.6)	70.0	±	1.7 ^c	(1.3)	110.2	±	2.8 ^b	(2.3)
	1, 2-Diacylglycerols	111.2	±	2.7 ^c	(1.5)	64.6	±	1.6 ^e	(1.2)	63.9	±	1.5 ^e	(1.3)
	Monoacylglycerols	66.7	±	1.6 ^d	(0.9)	26.9	±	0.7 ^e	(0.5)	76.7	±	1.8 ^c	(1.6)
	Phospholipids	475	±	11 ^d	(6.4)	339	±	8.5 ^e	(6.3)	326	±	8.2 ^e	(6.8)
WMRB	Hydrocarbons	77.3	±	1.9 ^a	(0.7)	71.5	±	1.7 ^a	(0.8)	39.4	±	1.0 ^b	(0.5)
	Steryl esters	154.6	±	3.8 ^a	(1.4)	89.4	±	2.3 ^b	(1.0)	141.7	±	3.5 ^a	(1.8)
	Triacylglycerols	8968	±	65 ^a	(81.2)	7276	±	48 ^b	(81.6)	6124	±	42 ^c	(78.0)
	Unknown	44.2	±	1.1 ^c	(0.4)	71.5	±	1.8 ^a	(0.8)	23.6	±	0.6 ^f	(0.3)
	Free fatty acids	619	±	15 ^b	(5.6)	500	±	12 ^c	(5.6)	693	±	16 ^a	(8.8)
	1, 3-Diacylglycerols	176.7	±	4.3 ^a	(1.5)	98.3	±	2.4 ^c	(1.1)	78.7	±	1.9 ^d	(1.0)
	1, 2-Diacylglycerols	154.6	±	3.8 ^a	(1.4)	134.1	±	3.4 ^b	(1.5)	78.7	±	2.0 ^d	(1.0)
	Monoacylglycerols	88.4	±	2.2 ^b	(0.8)	98.3	±	2.5 ^b	(1.1)	141.7	±	3.5 ^a	(1.8)
	Phospholipids	773	±	19 ^a	(7.0)	581	±	14 ^b	(6.5)	535	±	13 ^c	(6.8)

HMRB: half-milled rice brans. WMRB: well-milled rice brans.

*Mean values ± standard error. Each value represents the average of three determinations, and is expressed as mg lipid class per 500 g of rice. Values in the same row with different superscripts are significantly different between the individual cultivars ($P < 0.05$). Values in parentheses are relative wt% contents of the individual lipids in the total lipids.

3.4 Fatty Acid Composition of Major Lipids in the Rice Bran Cultivars

Fatty acid compositions of total lipids, TAG, FFA and PL in the rice bran lipids were compared among the HMRB and WMRB of the three cultivars (Table 3). The distribution of total unsaturated fatty acids, particularly linoleic (18:2n-6) and oleic (18:1n-9) acids, which accounted for 77.2-79.3% (total lipids), 77.9-79.9% (TAG), 70.8-73.5% (FFA) and 71.6-76.1% (PL), respectively. These patterns were very similar within total lipids, TAG, FFA or PL among the HMRB and WMRB from all three cultivars. However, some differences ($P < 0.05$) in fatty acid composition were noted when comparing the four lipid classes (total lipids, TAG, FFA and PL) as shown in Table 3. The percentage of palmitic (16:0) acid was significantly ($P < 0.05$) higher in the FFA (24.1-26.2%) and PL (21.8-25.4%) than that in the total lipids (18.3-19.3%) and TAG (17.1-19.5%) among all three cultivars. With a few exceptions for the green rice cultivar (total, TAG and PL) in both brans, the percentage of oleic (18:1n-9) acid was significantly ($P < 0.05$) higher in the black and red bran lipids than in the green bran lipids for the four lipid classes. On the other hand, with a few exceptions of FFA for the HMRB and WMRB, the percentage of linoleic (18:2n-6) acid was significantly ($P < 0.05$) higher in the green rice bran than in the black or red rice bran for total lipids, TAG and PL fractions. It has been demonstrated that there exists distinct differences between nonglutinous and glutinous types of cereals in lipid content and fatty acid composition (Fujino & Mano, 1972; Taira, 1984; Taira & Lee, 1988). These fatty acid composition for the black and red rice brans (nonglutinous) are very similar to the results observed for rice bran lipids in the cultivars: *Koshihikari*, *Haenuki*, *Akitakomachi*, *Hitomibore* and *Sasanishiki* reported in a previous paper (Yoshida et al., 2011). The data for fatty acid distribution of minor lipid components (steryl esters, 1,3- and 1,2-diacylglycerols or monoacylglycerols) in Table 2, were not included in Table 3 because these lipid components were present in too low concentrations to provide reliable results for their fatty acid compositions.

Table 3. Fatty acid distribution of major lipid components obtained from colored rice bran cultivars*

Bran	Lipid class	Cultivar	Fatty acid (wt%)					Total USFA	
			16:0	18:0	18:1	18:2	18:3		Others
HMRB	Total	Black	19.4±1.0 ^a	1.6±0.1 ^b	43.2±1.3 ^a	33.2±1.1 ^b	1.4±0.1 ^a	1.2±0.1 ^b	78.0 ^a
		Red	19.3±1.0 ^a	2.4±0.1 ^a	40.1±1.2 ^b	35.2±1.2 ^b	1.5±0.1 ^a	1.5±0.1 ^a	77.2 ^a
		Green	19.3±1.0 ^a	1.6±0.1 ^b	36.1±1.2 ^c	40.5±1.3 ^a	1.5±0.1 ^a	1.0±0.1 ^b	78.3 ^a
	TAG	Black	18.3±0.8 ^b	1.7±0.1 ^d	46.1±2.1 ^a	31.5±1.2 ^d	1.2±0.1 ^b	1.0±0.1 ^b	79.0 ^a
		Red	17.3±0.7 ^b	1.8±0.1 ^b	43.5±2.0 ^b	34.9±1.3 ^c	1.3±0.1 ^b	1.2±0.1 ^a	79.8 ^a
		Green	19.3±0.8 ^a	1.6±0.1 ^b	35.1±1.2 ^c	41.5±1.5 ^a	1.5±0.1 ^a	1.0±0.1 ^b	79.0 ^a
	FFA	Black	25.2±1.0 ^b	2.7±0.1 ^a	41.5±2.1 ^c	28.7±1.0 ^a	1.2±0.1 ^c	0.7±0.1 ^c	71.6 ^b
		Red	24.1±1.0 ^b	1.9±0.1 ^a	44.9±2.2 ^a	27.8±1.1 ^a	0.6±0.1 ^d	0.7±0.1 ^c	73.5 ^a
		Green	26.1±1.1 ^a	1.8±0.1 ^c	43.8±2.1 ^a	25.4±1.3 ^c	1.6±0.1 ^b	1.3±0.1 ^b	70.9 ^b
	PL	Black	22.3±1.0 ^b	1.3±0.1 ^a	38.6±1.3 ^b	34.6±1.3 ^c	1.5±0.1 ^a	1.7±0.1 ^a	74.9 ^b
		Red	23.1±1.0 ^b	1.1±0.1 ^a	38.9±1.2 ^b	34.7±1.3 ^c	1.5±0.1 ^a	0.7±0.1 ^c	75.4 ^a
		Green	25.4±1.1 ^a	0.8±0.1 ^c	29.9±1.2 ^d	41.8±1.8 ^a	1.3±0.1 ^b	0.8±0.1 ^c	73.2 ^b
WMRB	Total	Black	18.4±0.8 ^a	1.6±0.1 ^b	43.3±1.7 ^a	34.2±1.2 ^b	1.3±0.1 ^b	1.2±0.1 ^b	78.8 ^a
		Red	18.3±0.8 ^a	1.4±0.1 ^c	42.5±2.0 ^a	35.3±1.2 ^b	1.2±0.1 ^b	1.3±0.1 ^b	79.3 ^a
		Green	19.3±1.0 ^a	1.6±0.1 ^b	36.1±1.2 ^c	40.5±2.0 ^a	1.5±0.1 ^a	1.0±0.1 ^b	78.3 ^a
	TAG	Black	18.5±0.8 ^b	1.7±0.1 ^b	46.1±2.0 ^a	31.5±1.2 ^d	1.2±0.1 ^b	1.0±0.1 ^b	79.0 ^a
		Red	17.1±0.8 ^b	2.0±0.1 ^a	43.5±2.0 ^b	34.9±1.2 ^c	1.3±0.1 ^b	1.2±0.1 ^a	79.9 ^a
		Green	19.5±1.2 ^a	1.6±0.1 ^b	37.0±1.3 ^c	39.3±1.4 ^b	1.4±0.1 ^a	1.2±0.1 ^a	77.9 ^b
	FFA	Black	25.3±1.0 ^b	2.2±0.1 ^b	42.4±1.5 ^b	26.7±1.2 ^b	1.8±0.1 ^a	1.6±0.1 ^a	71.4 ^b
		Red	25.5±1.0 ^b	1.6±0.1 ^c	43.6±1.5 ^a	26.8±1.2 ^b	1.1±0.1 ^c	1.4±0.1 ^b	71.8 ^b
		Green	26.2±1.0 ^a	2.1±0.1 ^b	40.9±1.3 ^c	28.6±1.2 ^a	1.0±0.1 ^c	1.2±0.1 ^b	70.8 ^b
	PL	Black	21.8±1.0 ^c	1.3±0.1 ^a	40.7±1.3 ^a	33.7±1.2 ^c	1.2±0.1 ^b	1.3±0.1 ^b	76.1 ^a
		Red	23.3±1.1 ^b	1.3±0.1 ^a	39.2±1.3 ^b	34.0±1.3 ^c	1.2±0.1 ^b	1.0±0.1 ^b	74.7 ^b
		Green	25.4±1.1 ^a	1.3±0.1 ^b	32.1±1.2 ^c	38.2±1.4 ^b	1.3±0.1 ^b	1.7±0.1 ^a	71.6 ^c

HMRB: half-milled rice brans. WMRB: well-milled rice brans. USFA: Unsaturated fatty acids.

*Mean values ± standard error. Each value represents the average of three determinations, and is expressed the relative wt% contents of the individual fatty acids. Values in the same column with different superscript are significantly different between the individual cultivars ($P < 0.05$). "Others" include minor fatty acids such as C14:0, C16:1, C20:0 and C22:0.

3.5 Distribution of TAG Molecular Species

The carbon number (TCN) denotes the total length of the three acyl-chain present in the TAG. For example, 54 are predominantly composed of 18:0, 18:1, 18:2 and 18:3. These TCN within TAG obtained from the three rice bran cultivars ranged from 48 to 56 as listed in Figure 1. Each value is the mean of triplicate determinations and is expressed as milligram lipid per 20 g brans. Dominant components were 52 (41.3-42.6%) and 54 (47.5-48.6%) TAG, followed by small amounts of 50 (9.5-10.2%), 56 (0.2-0.3%) and 48 (0.2-0.3%) TAG, respectively.

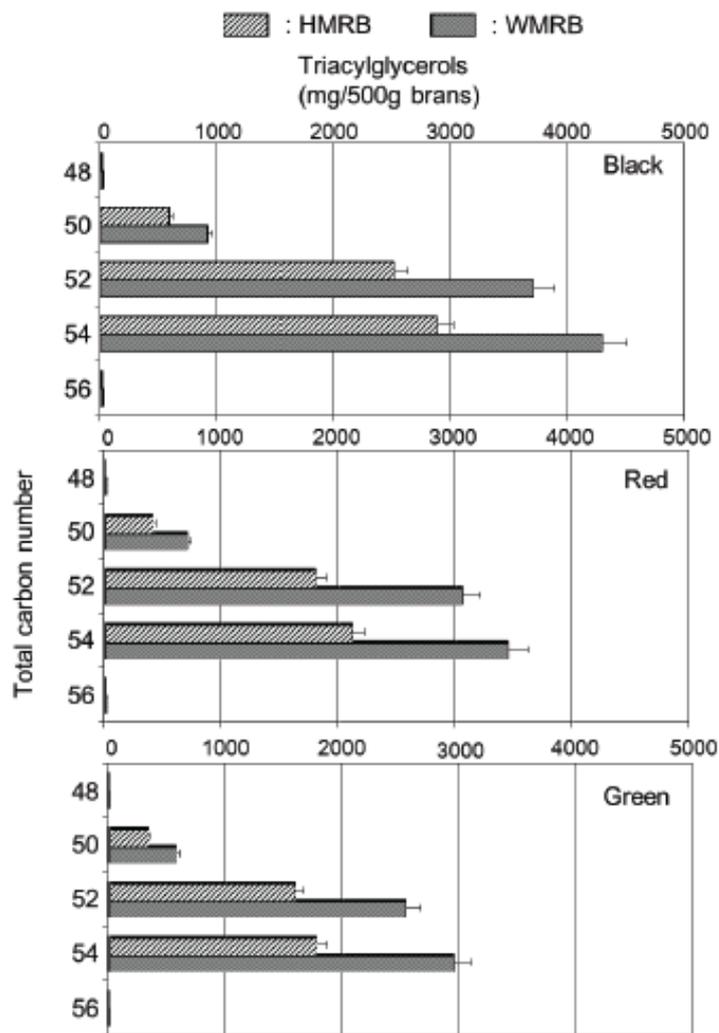


Figure 1. Content of TAG prepared from colored rice bran cultivars. Each value represents the average of three replicates. *Horizontal bars* depict the mean and standard deviation of three determinations

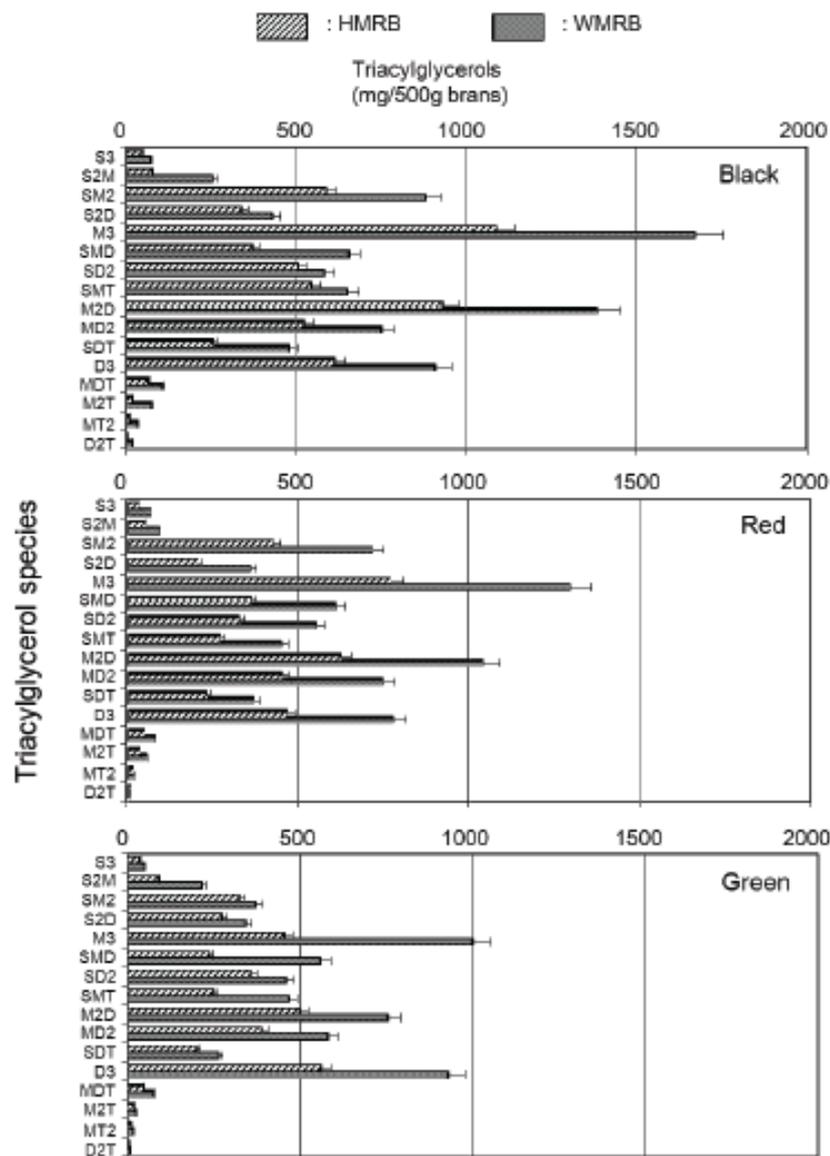


Figure 2. Characteristics of the major molecular species of TAG isolated from colored rice bran cultivars

Saturated FA (S) consists of myristic (14:0), palmitic (16:0), stearic (18:0) and arachidic (20:0) acids. Unsaturated FA, palmitoleic (16:1), oleic (18:1*n*-9), linoleic (18:2*n*-6) and α -linolenic (18:3*n*-3), are denoted as monoene (M), diene (D) and triene (T), respectively. *Horizontal bars* depict the mean and standard deviation of three determinations.

The distribution patterns of the individual TAG molecular species are shown in Figure 2. Sixteen different molecular species were detected among the TAG isolated from these rice bran lipids. These species were arranged according to the degree of unsaturation on the acyl-chain length of TAG (from top to bottom in Figure 2, respectively). In the three cultivars, the major TAG molecular species were SM₂ (POO or StOO), S₂D (PPL or PStL or StStL), M₃ (OOO), SMD (POL or StOL), SD₂ (PLL or StLL), SMT (POL or StOL), M₂D (OOL), MD₂ (OLL), SDT (PLL*n* or StLL*n*) and D₃ (LLL) in the three cultivars. On the other hand, the other species (S₃; PPP or PStSt or StStSt, S₂M; PPO or PStO or StStO, MDT; OLL*n*, M₂T; OOL*n*, MT₂; OLnLn and D₂T; LLL*n*) were minor components (less than 3.5%). However, the three-letter designation does not demonstrate regioselective positional isomers of fatty acyl in the TAG: P, palmitic (16:0), St, stearic (18:0), O, oleic (18:1*n*-9); L, linoleic (18:2*n*-6); Ln, α -linolenic (18:3*n*-3) fatty acid moieties. Thus, these distribution patterns in the molecular species of TAG were very similar to each other among the HMRB and WMRB for from the three cultivars.

4. Conclusions

The predominant components were γ -tocotrienol, α -tocopherol, and α -tocotrienol with much smaller amounts of γ - and δ -tocopherols, and δ -tocotrienol. Major lipid components in three different Japanese colored rice bran cultivars were TAG, FFA, and PL, while other components were also present in minor proportions. Sixteen molecular species of TAG were identified in these rice brans. The main components were palmitodiolein or stearodiolein (6.1-9.8%), triolein (16.4-18.7%), palmitoleolinolein or stearoleolinolein (6.3-9.2%), palmitodilinolein or stearodilinolein (6.5-9.5%), palmitoleolinolenin or stearoleolinolenin (6.2-9.2%), dioleolinolein (12.3-15.5%), oleodilinolein (8.4-10.4%) and trilinolein (10.2-15.2%). To the best of our knowledge this is the first report of the TAG composition of colored rice bran cultivars. In general, the distribution patterns were not significantly different ($P > 0.05$) among the HMRB and WMRB from the three cultivars, suggesting similar TAG molecular species and tocochromanol compositions. Currently, the consumer awareness of health food products is increasing and food scientists have been searching for interesting sources of healthful natural components. The results showed that rice bran extracts contain large amounts of nutraceuticals with proven positive health effects (Ha et al., 2006).

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