Fat-Soluble Nutraceuticals and Fatty Acid Composition of Selected Indian Rice Varieties

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ABSTRACT: The fat-soluble nutraceuticals—oryzanol, tocopherols, and tocotrienols—and FA composition of three varieties of rice—Basmati, Jaya, and parboiled (brown rice is defined as dehulled rice, and brown rice after polishing is known as milled rice)—were investigated. Lipid content ranges were 2.75–4.49% for brown rice and 0.7–1.2% for milled rice. The range in oryzanol content was 500–720 ppm for brown rice, 10,700–14,300 to ppm for brown rice lipids, 70–120 ppm for milled rice, and 4500–6300 ppm for milled rice lipids. Tocopherol content ranged from 22 to 31 ppm for brown rice and 79 to 951 ppm for brown rice lipids while the tocotrienol content ranged from 0 to 26 ppm for brown rice and 0 to 792 ppm for brown rice lipids. Parboiling of paddy rice affected the tocopherol and tocotrienol content adversely, but the oryzanol content remained unchanged. Basmati variety brown rice had the highest tocopherol and tocotrienol contents, although the oryzanol content was lower than that of the Jaya variety brown rice. The FA composition of brown rice differed from that of milled rice for each variety; higher amounts of saturated FA were extracted from the oil of milled rice than from brown rice for all three varieties studied. Brown rice varieties in this study contained all of the fat-soluble nutraceuticals, compared with milled rice.

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KEY WORDS: Nutraceuticals, oryzanol, rice lipids, tocopherols, tocotrienols.

Rice (*Oryza sativa* L.) is an important cereal in many Asian countries. India produces about 92 million tonnes of paddy rice annually (1). The rice grain is encased in an easily removable protective hull and covered by a second region of the bran layer. The lipid content (hexane-extractable oil) of rice ranges from 0.38 to 2.98%, and a value as high as 3.5%, obtained by extraction with ethanol, also has been reported (2). The hulls also contain 1% of lipids (2). The rice grain contains about 5% bran, of which 12–18.5% is oil; the current production of rice bran oil in the world is about 705,000 tonnes, out of which 500,000 tonnes is produced in India (3,4). The bran contains fat-soluble nutraceuticals, *viz*., oryzanol, tocopherols, tocotrienols, phytosterols, and phospholipids (3,4). Oryzanol is the name given to a mixture of ferulic acid esters found in major amounts only in rice bran oil. Ferulic acid is esterified to 24-methylene cycloartanol, cycloartanol, cycloartenol, campesterol, and β-sitosterol in oryzanol and exists at levels of 1.0 to 3.0% in rice bran oil. These are known to reduce cholesterol in the blood of experimental animals and human volunteers; therefore, rice bran oil is in great demand in Japan, India, European countries, and the United States (4). Tocopherols and tocotrienols are found in rice bran oil in quantities equal to or even higher than what is present in palm oil. Tocotrienols are also known to reduce cholesterol and are valued as antioxidants as well (5). Phytosterols are found in rice bran oil at levels up to 1.5% including β-sitosterol, campesterol, and stigmasterol (3). Rice bran oil, having a balanced FA composition, is quite stable and resistant to oxidation.

Parboiling is a hydrothermal process in which the crystalline form of starch in grain is changed into an amorphous one through soaking, steaming, drying, and milling. After parboiling, the milling yield is higher and the nutritional quality improves as there is a higher content of vitamins and mineral salts due to migration of nutrients from the bran to the starchy endosperm layer. Parboiled rice after milling keeps longer and better than nonparboiled rice before and after milling (6).

In commercial rice milling, rice is often polished to achieve a white appearance (7) and to enhance its stability (8). Milling removes the bran layer of the rice grain, leaving a core composed of mostly carbohydrates, and thus removes nutrients of vital importance in the diet. The degree of milling determines the extent of bran retention on the germ surface. The effect of the degree of milling of four Indian varieties of rice on the total tocopherol and α-tocopherol contents of rice bran has been reported (9). But the individual tocopherols and tocotrienols and oryzanol content in relation to milling for the Indian rice varieties has not been examined. The aim of this research was to determine the fat-soluble nutraceutical content of commercially available milled and brown rice of three selected varieties.

MATERIALS AND METHODS

Three varieties of rice—Basmati (brown and milled), Jaya (brown and milled), and commercial variety parboiled rice (brown and milled) were procured from a local rice mill. Standard α-, β-, γ-, and δ-tocopherols and FAME were purchased from Sigma Chemical Co. (St. Louis, MO). Tocotrienols (α-, β + γ, and δ) were isolated from palm oil by semipreparative HPLC (10), and their retention times were compared with that of tocopherols and tocotrienols of palm oil and also standard tocopherols. All solvents and reagents used were of analytical grade.

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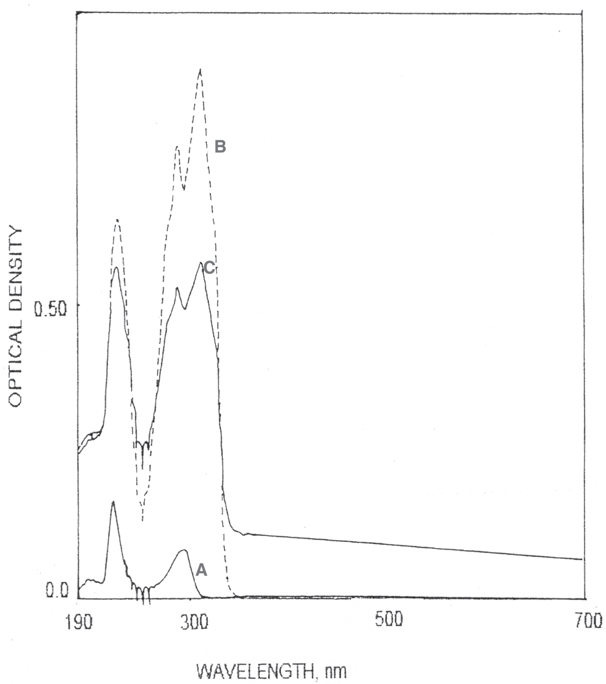

FIG. 1. UV absorption spectra of (A) α-tocopherol, (B) oryzanol, and (C) oil extracted from brown rice.

The moisture and fat contents of the rice flours were determined by AOCS (11) methods (Ca 2c-25 and Bc 3-49, respectively). The oryzanol content of the oil and flour was determined spectrophotometrically by determining the absorption at 314 nm in hexane using the specific extinction coefficient 358.9 (12). Tocopherols and tocotrienols, which absorb at 292–298 nm (13), did not interfere during the direct spectrophotometric analysis of oryzanol as shown in Figure 1. The oryzanol content of rice bran oil is about 10–20 times the tocopherol content in the same oil, and the specific extinction coefficient of tocopherols is lower than that of oryzanol by 4.5 times; hence, the interference, even if it exists, would be negligible.

Contents of tocopherols and tocotrienols were determined by HPLC using model LC-10 AVP (Shimadzu Corp., Tokyo, Japan) fitted with C18 (ODS) column [250 × 4.6 mm i.d. (SGE, Melbourne, Australia] and fluorescence detector. The mobile phase consisted of acetonitrile/methanol/isopropanol/water (45:45:5:5 by vol), which was programmed to acetonitrile/methanol/isopropanol (50:45:5 by vol) from 6 to 10 min according to Rogers *et al.* (10). As the fluorescence spectra of each tocopherol and the corresponding tocotrienol are similar, individual tocopherols and tocotrienols were quantified as α-tocopherol [as suggested by AOCS Official Method Ce 8-89 (11)].

A table (Table 1) of typical retention times has been included as a reference when standard tocopherols and tocotrienols are not available. The relative retention time (RRT) for tocopherols with respect to α-tocopherol and the RRT for tocotrienols with respect to α-tocotrienol are similar, as observed here and elsewhere [AOCS Official Method Ce 8-89 (11)].

Oils were subjected to methylation using boron trifluoride/methanol according to AOCS Official Method Ce 1-62 (11). The methyl esters were analyzed by using a gas–liquid chromatograph (GC-15A; Shimadzu Corp.) equipped with a data processor (model CR-4A; Shimadzu Corp.), an FID, and a stainless steel column [3 m × 3.3 mm i.d., packed with Chromosorb WAW 60–80 mesh (Shimadzu Corp.), precoated with 15% diethylene glycol succinate]. The gas chromatograph was operated under the following conditions: nitrogen flow 40 mL/min, hydrogen flow 40 mL/min, air flow 300 mL/min, column temperature 180°C, injector temperature 200°C, and FID temperature 220°C. The FA were identified with the help of standard FAME.

TABLE 1
Retention Times for Tocopherols and Tocotrienols Observed in the Study

Tocopherol	Retention time (min)	Relative retention time with respect to α-tocopherol
α-Tocopherol	24.658	1.0000
β- + γ-Tocopherol	22.367	0.9071
δ-Tocopherol	20.758	0.8418
α-Tocotrienol	16.325	0.6621
β- + γ-Tocotrienol	15.000	0.6083
δ-Tocotrienol	13.583	0.5509

From one representative sample of each selected rice variety, three subsamples were taken and used in the determinations of moisture and fat contents. For each rice variety, the fat obtained was analyzed in triplicate for oryzanol content, and the fat samples were then pooled into one sample; from it duplicate samples were prepared for determinations of tocopherols, tototrienols, and FA composition by using HPLC and GC. Duplicate injections in succession were carried out on these samples, and concentrations were averaged. SD values were computed.

RESULTS AND DISCUSSION

The moisture content of brown rice was slightly lower than that of milled rice (Table 2) probably because the bran in brown rice is a water-resistant material containing fat, fiber, protein, and ash. Oil was extracted from rice flour by Soxhlet extraction using hexane. The oil content of Basmati brown rice was 4.7-fold greater than in Basmati milled rice (Table 2). Similarly, Jaya (5.7-fold) and parboiled rice varieties (2.3-fold) had a higher oil content in brown rice than in milled rice. Compared with Basmati and Jaya brown rice, the oil content of parboiled brown rice was lower, which may be due to differences in the variety of rice used for the study.

The oryzanol contents of oil extracted from brown and milled rice flours ranged from 10,700 to 14,300 ppm for brown rice lipids (Table 2), whereas milled rice lipids contained 4,500–6,300 ppm. Brown rice flour contained a higher amount of oryzanol (500–720 ppm) than milled rice flour (70–120 ppm). The oryzanol content of brown rice lipids was about 1.7–3.0 times greater than in milled rice lipids, and brown rice flour contained 5.0–10.3 times more oryzanol than milled rice flour.

TABLE 2
Moisture, Oil, and Oryzanol Content[a] of Three Varieties of Rice

	Moisture %		Oil[b] (%)		Oryzanol[b] (ppm)			
					Oil		Flour	
Sample	Brown	Milled	Brown	Milled	Brown	Milled	Brown	Milled
Basmati	9.28 ± 0.11	9.50 ± 0.06	3.28 ± 0.04 (4.7×)	0.70 ± 0.01 (1×)	10,700 ± 300 (1.7×)	6,300 ± 100 (1×)	620 ± 120 (5.2×)	120 ± 10 (1×)
Jaya	9.64 ± 0.09	10.07 ± 0.02	4.49 ± 0.03 (5.7×)	0.79 ± 0.01 (1×)	14,300 ± 170 (2.51×)	5,700 ± 200 (1×)	720 ± 10 (10.3×)	70 ± 10 (1×)
Parboiled	8.33 ± 0.04	8.72 ± 0.01	2.75 ± 0.01 (2.3×)	1.20 ± 0.01 (1×)	13,300 ± 600 (3×)	4,500 ± 400 (1×)	500 ± 30 (5×)	100 ± 10 (1×)

[a]The values are mean ± SD from three determinations.
[b]Values for heading "Milled" are the basis of comparison (i.e., 1×). Values in parenthesess under the column heading of "Brown" represent fold differences.

The oryzanol content of oil extracted from 18 varieties of Indian rice varieties was reported earlier (14) and ranged between 1.63 and 2.72% (16,300 and 27,200 ppm). Values of 17,800 and 20,600 ppm oryzanol have been reported (14) for oil extracted from the bran of Basmati 370 and Punjab Basmati 1 varieties of rice. The value of 10,700 ppm oryzanol for the oil extracted from a Basmati rice variety in the present study was lower than the literature report (14). The variety used in the present study may be a local variety, and it may have been grown under different environmental conditions.

The tocopherol and tocotrienol contents of brown rice and the lipid fraction are given in Table 3, and the HPLC patterns are given in Figures 2 and 3. The total tocopherol contents of Basmati brown rice (31.19 ppm) and the lipid fraction (951 ppm) were highest, compared with Jaya brown rice (26.23 ppm) and the lipid fraction (585 ppm) and parboiled brown rice (2.18 ppm) and the lipid fraction (79 ppm). Gopala Krishna *et al.* (9) reported a total tocopherol content of 31.30–48.70 ppm for four varieties of brown rice, Madhu (improved), Jaya, Pushpa, and IR-20. The total tocopherols content for Jaya variety brown rice reported earlier was 36.30 ppm, and the results of the present study [total tocopherols (26.23 ppm) plus tocotrienols (3.86 ppm) equals 30.09 ppm] agree with those findings. Similarly, the total tocotrienol contents of Basmati brown rice (25.99 ppm) and Basmati brown rice lipids (792 ppm) were higher than for Jaya brown rice (3.86 ppm) and its lipid fraction (86 ppm), and the parboiled brown rice showed an absence of tocotrienols (0–100 ppm) and traces of tocopherols (2.18 ppm for brown rice and 79 ppm for the lipid fraction). Parboiling of paddy rice adversely affected the tocopherol and tocotrienol contents in the rice; therefore, the oil extracted from parboiled brown rice may contain little or no tocopherols and tocotrienols. In the case of Basmati rice lipids, β + γ-tocopherol was higher (439 ppm), followed by α-tocopherol (369 ppm) and δ-tocopherol (143 ppm), and α-tocotrienol was higher (652 ppm) compared with β + γ-tocotrienols (60 ppm) and δ-tocotrienol (81 ppm). The tocopherol content trend for Jaya variety rice was similar to Basmati variety rice β- + γ-tocopherol (270 ppm), α-tocopherol (221 ppm), and δ-tocopherol (94 ppm), whereas β- + γ-tocotrienols (340 ppm) and δ-tocotrienol (35 ppm) were slightly greater than α-tocotrienol (17 ppm). The commercial rice bran oils contained 71.34% total tocotrienols, whereas the varieties tested here contained 45.44, 12.82, and 0.0% tocotrienols of the total tocols. This could be due to a loss of tocopherols and tocotrienols during parboiling of the rice (in the case of parboiled rice), whereas for the other two varieties, it could be a varietal difference in the tocopherol and tocotrienol contents.

The FA compositions of brown and milled rice of the Basmati, Jaya, and parboiled rice studied are measurably different

TABLE 3
Tocopherol and Tocotrienol (tocol) Contents[a] and Composition of Brown Rice Flour and Its Lipid Fraction

	Tocopherols (ppm)				Tocotrienols (ppm)			
Rice flour/lipid fraction	α	β + γ	δ	Total	α	β + γ	δ	Total
Basmati brown rice	12.09	14.41	4.69	31.19	21.38	1.96	2.65	25.99
Lipid fraction	369	439	143	951	652	60	81	792
% of total tocols	21.2	25.2	8.2	54.5	37.4	3.4	4.7	45.5
Jaya brown rice	9.92	12.11	4.20	26.23	0.77	1.52	1.57	3.86
Lipid fraction	221	270	94	585	17	34	35	86
% of total tocols	32.9	40.2	14.0	87.2	2.5	5.1	5.2	12.8
Parboiled brown rice	0.74	0.99	0.45	2.18	ND	ND	ND	ND
Lipid fraction	27	36	16	79	ND	ND	ND	ND
% of total tocols	34.2	45.6	20.2	100.0	—	—	—	—
Rice bran oil	97	342	29	468	22	1117	48	1188
% of total tocols	5.9	20.7	1.7	28.3	1.3	67.5	2.9	71.7

[a]Values are average of two injections from duplicate samples. ND, not detected/absent.

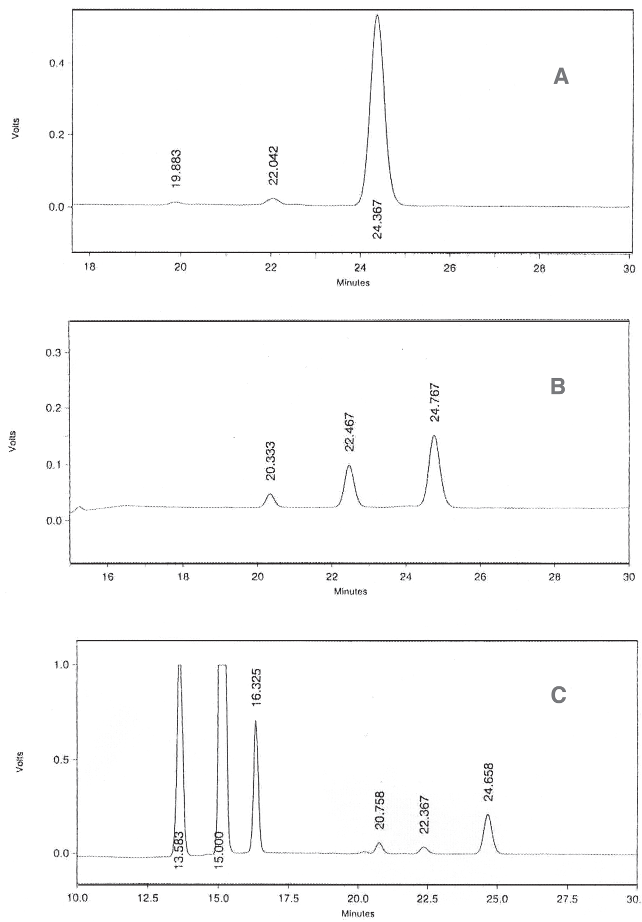

FIG. 2. HPLC analysis of standard (A) α-tocopherol, (B) tocopherol mixture, and (C) tocopherol and tocotrienol composition of palm oil.

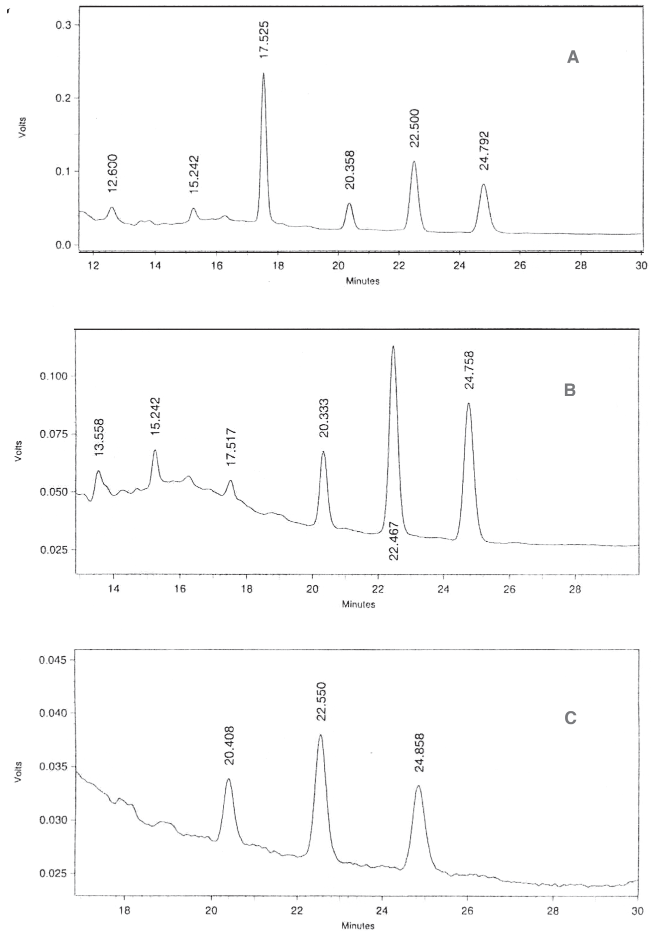

FIG. 3. HPLC analysis of (A) Basmati brown rice oil, (B) Jaya brown rice oil, and (C) parboiled brown rice oil.

(Table 4). The saturated FA content of milled rice is greater than that of brown rice in all three varieties, which agrees in general with literature reports for the Indian, Japanese, and Malagasy varieties of rice (16–20). The palmitic acid (48.9%) content of parboiled milled rice in the present study was highest and was notably higher compared with the palmitic acid content of parboiled brown rice (23.0%). Similarly, the stearic acid content was slightly greater for all three varieties of milled rice. In the case of Basmati milled rice, the oleic acid content was slightly greater than for brown rice, but the Jaya and parboiled milled rice contained slightly less oleic acid than brown rice. Commercially produced rice bran oil showed similar amounts of oleic acid compared with all three varieties of brown rice. The linoleic acid content for all three varieties of milled rice was appreciably less than for brown rice. Jaya variety milled rice had a linoleic acid content of 2.8%, which is about 11–12 times less than its brown rice (32.4%), probably due to milling. Arachidic acid was not present in any variety of milled rice but was found in the brown rice; and the linolenic acid contents of brown and milled rice were not appreciably

TABLE 4
FA Composition[a] of Oil from Brown and Milled Rice Used in the Study

Oil	FA[b] (relative %)							
	$C_{12:0}+C_{14:0}$	$C_{16:0}$	$C_{16:1n-7}$	$C_{18:0}$	$C_{18:1n-9}$	$C_{18:2n-6}$	$C_{20:0}$	$C_{18:3n-3}$
Basmati brown rice flour	0.7	21.4	—	2.8	46.4	26.6	1.0	1.1
Basmati milled rice flour	1.6	32.7	—	5.0	50.9	8.7	—	1.1
Jaya brown rice flour	1.1	19.6	1.3	3.5	41.3	32.4	0.8	—
Jaya milled rice flour	1.0	39.5	—	7.0	47.3	2.8	—	2.4
Parboiled brown rice flour	0.5	23.0	—	2.7	45.4	26.3	0.9	1.2
Parboiled milled rice flour	0.9	48.9	—	6.1	36.2	7.1	—	0.8
Rice bran oil	0.6	19.0	0.4	4.6	42.0	31.2	1.1	1.1

[a]The values are average of two injections from duplicate samples.
[b]Values are expressed relative to total FA.

TABLE 5
FA Ratio of Oil from Brown and Milled Rice Used in the Study

Sample	Saturated FA (%)	Monounsaturated FA (M)	PUFA (%)	S:M:P ratio[a]
Basmati brown rice flour	25.5	46.3	27.7	1.02:1.85:1.11
Basmati milled rice flour	39.3	50.9	9.9	1.18:1.53:0.30
Jaya brown rice flour	25.0	42.6	33.4	1.00:1.70:1.34
Jaya milled rice flour	34.9	34.9	3.9	1.05:1.05:0.12
Parboiled brown rice flour	26.7	45.0	27.3	1.07:1.80:1.09
Parboiled milled rice flour	55.8	36.1	7.9	1.12:0.72:0.16
Rice bran oil (commercial)	24.8	42.2	31.7	0.99:1.69:1.27

[a]S:M:P, saturated FA:monounsaturated FA:PUFA.

different in any of the three varieties studied. Some rice varieties have been shown to contain trace amounts of palmitoleic (C16:1n-7), *cis*-vaccenic (C18:1n-7), and other acids in the oil extracted from them, but these FA were not found in our study. The change in FA composition of the lipid fraction between brown (unmilled) and milled rice caused a lowering of the ratio of saturated FA to monounsaturated FA to PUFA (S:M:P ratio) in milled rice (Table 5).

Other sources of ferulates besides rice are limited. However, oat bran, corn bran, and wheat germ contain ferulic acid esters of phytostanols (21). The tocopherol contents of cereals and foods are listed in the literature, and they are known to decline during processing (13,21). Indeed, the tocopherol contents of milled rice are lower by 50% than brown rice (22). It may be concluded from this study that brown rice contains a higher amount of the lipid fraction having a balanced FA composition, and higher amounts of oryzanol compared with milled rice, irrespective of variety.

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