Study on the Composition of Rice Bran Oil and Its Higher Free Fatty Acids Value

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ABSTRACT: The compositions of rice bran oils (RBO) and three commercial vegetable oils were investigated. For refined groundnut oil, refined sunflower oil, and refined safflower oil, color values were 1.5–2.0 Lovibond units, unsaponifiable matter contents were 0.15–1.40%, tocopherol contents were 30–60 mg%, and FFA levels were 0.05–0.10%, whereas refined RBO samples showed higher values of 7.6-15.5 Lovibond units for color, 2.5–3.2% for unsaponifiable matter, 48–70 mg% for tocopherols content, and 0.14–0.55% for FFA levels. Of the four oils, only RBO contained oryzanol, ranging from 0.14 to 1.39%. High-oryzanol RBO also showed higher FFA values compared with the other vegetable oils studied. The analyses of FA and glyceride compositions showed higher palmitic, oleic, and linoleic acid contents than reported values in some cases and higher partial glycerides content in RBO than the commonly used vegetable oils. Consequently, the TG level was 79.9–92% in RBO whereas it was >95% in the other oils studied. Thus, refined RBO showed higher FFA values, variable oryzanol contents, and higher partial acylglycerol contents than commercial vegetable oils having lower FFA values and higher TG levels. The higher oryzanol levels in RBO may contribute to the higher FFA values in this oil.

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KEY WORDS: Composition, fatty acid composition, free fatty acids value, glyceride composition, groundnut oil, oryzanol, rice bran oil, safflower oil, sunflower oil, vegetable oils.

India ranks first in the annual production of crude rice bran oil (RBO) (5,00,000 tonnes) and refined RBO (>4,00,000 tonnes) (1,2). The Indian Standards specification for refined RBO allows a maximal acid value of 0.5 (equivalent to 0.25% FFA) (3,4). The FFA content of chemically refined RBO falls within the permitted levels, whereas that of physically refined RBO is always higher. Oryzanol added externally to soybean oil increases the acid value equivalent to the content of added oryzanol (5,6), but no systematic study is available to show that oryzanol naturally present in commercially available physically refined RBO interferes in the FFA determination. With this background, investigations were undertaken to assess the composition of RBO and some commonly used vegetable oils and the influence of oryzanol on FFA determinations.

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MATERIALS AND METHODS

Materials. Refined groundnut oil (Nature Fresh brand; Cargill, Gurgaon, India), refined sunflower oil (Sundrop brand; Agro Tech Foods, Secunderabad, India), and refined safflower oil (Saffola brand; Marico Industries, Mumbai, India) were procured from the local market of Mysore city. Phenolphthalein indicator was procured from M/s Ranbaxy Laboratories Ltd. (New Delhi, India). Absolute alcohol was distilled and used for FFA determination. Samples of both physically and chemically refined RBO were supplied by M/s Marico Industries Ltd. All other chemicals used were of analytical reagent grade.

Methods. The color of the oils was measured by using a Lovibond tintometer in a 1-in. (2.54 cm) cell in the transmittance mode and expressed as 5 X red + 1 X yellow Lovibond units.

Determination of FFA content. AOCS Official Method Ca 5a-40 was used for the determination of FFA content of all oils (7). Oil samples of about 5 ± 0.1 g, in triplicate, were used instead of the amounts specified in the standard method for oil having FFA < 1%, but all other conditions for this titration remained the same.

Unsaponifiable matter determination. The unsaponifiable matter contents of the oils were determined by AOCS Official Method Ca 6a-40 (7).

Total tocopherol content determination. The total tocopherol content was determined by using the Vitamin E panel method (8) through saponification of the oil followed by extraction of unsaponifiable matter and development of color using alcoholic ferric chloride and 2,2-dipyridyl and reading at 520 nm in a model UV-240 spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The concentration of total tocopherols was determined from a standard curve prepared by using α-tocopherol and the concentration expressed as mg/100 g oil.

Oryzanol content determination. Samples of accurately weighed amounts of RBO (about 10 mg each) or of the oryzanol-amended model oil in triplicate were dissolved in hexane and made up to 10 mL and mixed well. The O.D. were read in a 1-cm cell at 314 nm in a Shimadzu UV-240 double beam recording spectrophotometer (solutions having OD >1.2 were further diluted before reading OD). The oryzanol content in the oil was calculated using the formula

$$\text{Oryzanol, g\%} = \frac{\text{OD of hexane solution}}{\text{weight (g) of oil} \times 10} \times \frac{100}{358.9} \qquad [1]$$

where 358.9 is the specific extinction coefficient of oryzanol.

Determination of the glyceride composition by HPLC. The RBO and groundnut oil samples were analyzed for glycerides composition by the HPLC procedure of Foglia *et al*. (9) using a hexane/isopropanol solvent system instead of isooctane/isopropanol. The analysis was performed at 24°C (laboratory room temperature) using a hexane (100%) to hexane (90%)/isopropanol (10%) gradient for elution on a normal-phase silica column (250 ¥ 4.6 mm i.d.) and detection of components with a UV detector set at 210 nm. The flow program used was described by Foglia *et al*. (9). Standard glycerides were used for the identification and quantification of the individual components.

FA composition. The FA compositions of the oils were determined by using AOCS Official Method Ce 1-62, which constitutes a base-catalyzed methylation reaction (7). The methyl esters were analyzed by using a gas–liquid chromatograph (model GC-15A; Shimadzu Corporation), equipped with a data processor (model CR-4A; Shimadzu Corporation), an FID, and a stainless steel column (3 m × 3.3 mm i.d., packed with Chromosorb WAW 60–80 mesh, 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 based on their retention times compared with standard FAME.

RESULTS AND DISCUSSION

Physicochemical parameters of the oils used for the study are given in Tables 1–3.

Color of RBO samples. The color values are reported in Tables 1 and 2 and are within the specified limits for these oils and for the higher limit of 20 Lovibond units for RBO under the Indian standards specifications for the oil (3,4).

FFA value. The FFA contents of some of the commercial brands of vegetable oils and the samples of RBO were determined (Tables 1, 2). The data indicate that the FFA content for the vegetable oils fell below the maximum permissible limit of 0.25% FFA (0.5 acid value) under the Prevention of Food Adulteration Act of India (3,4), but most of the physically refined RBO exceeded the maximum limit of 0.25%. This has led to confusion over whether these physically refined RBO are to be permitted as refined grade for public distribution.

Tocopherols and unsaponifiable matter in RBO samples. The total tocopherols content of some samples of RBO and the common vegetable oils studied, which are shown in Tables 1 and 2, agree with literature reports (10). The levels for unsaponifiable matter in the studied oils also conform to specification (4). As tocopherols are present in very small amounts, their interference with FFA determinations may be practically zero. The unsaponifiable matter components are neutral molecules and may not contribute to acidity of the oil.

Glyceride composition of RBO in comparison with other oils. RBO differs somewhat from other oils with respect to its FA composition and contents of tocopherols, unsaponifiable matter, and especially oryzanol (1,10). The glyceride composition of groundnut oil, chemically refined RBO, and two physically refined RBO showed the following results by HPLC: TG 95.8, 92.0, 85.3, and 79.9%; DG 1.0, 7.0, 13.4, and 10.9%; and MG 3.2, 1.0, 1.3, and 1.7%, respectively. These data indicate that RBO has higher levels of partial glycerides and lower levels of the important TG compared with groundnut oil (present study) or sunflower oil (11). The oils studied did not show any peak for FFA, probably owing to the very low concentration (<1%) of FFA in these refined oils. Column chromatographic determinations of the glyceride compositions of RBO and sunflower oil

TABLE 1
Physicochemical Characteristics of Refined Rice Bran Oils (RBO) Used in the Study[a]

	Lovibond color (5R + Y units)	FFA (% as oleic)	Oryzanol (%)	Total tocopherols content (mg%)	Unsaponifiable matter (%)
Chemically refined RBO					
1	15.5	0.14	0.1436	57	2.5
2	13.8	0.16	0.1841	50	3.0
Range	13.8–15.5	0.14–0.16	0.14–0.18	50–57	2.5–3.0
Physically refined RBO					
1	9.4	0.40	1.1057	48	2.7
2	8.3	0.47	1.0875	ND	ND
3	7.6	0.48	1.0664	ND	ND
4	8.3	0.46	1.2072	ND	ND
5	8.9	0.49	1.3194	ND	ND
6	10.3	0.43	0.5567	60	2.9
7	12.4	0.35	0.6024	ND	ND
8	13.9	0.43	0.5996	ND	ND
9	13.8	0.44	0.7260	ND	ND
10	13.8	0.47	0.7529	ND	ND
11	7.6	0.29	0.6070	ND	ND
12	13.8	0.55	1.3902	70	3.2
Range	7.6–13.9	0.29–0.55	0.55–1.39	48–70	2.7–3.2

[a]All values are mean of duplicate samples. The CV was within ±2% for all studied samples. ND = values not determined.

TABLE 2
FFA Values of Selected Refined Vegetable Oils[a,b]

Oil samples used		Color (5R + Y) Lovibond units (1″ cell)	FFA (% as oleic)	Total tocopherols (mg%)	Unsaponifiable matter (%)
Refined sunflower oil	a	2.0	0.047	50	1.40
	b	2.0	0.082	52	1.40
Refined groundnut oil	a	1.5	0.065	35	0.20
	b	1.5	0.087	30	0.15
Refined safflower oil	a	1.5	0.057	60	1.30

[a]All determinations were done in triplicate. The CV is within ±2%.
[b]The letters a and b refer to different batches.

also did not show FFA in the DG + FFA fraction in our earlier studies on RBO (11).

FA composition. The oils used for the study were analyzed for FA composition for their authentication and are shown in Table 3. With the 14 RBO samples, FA composition varied with batch and type of refining process, i.e., whether physical or chemical refining was performed. The ranges of FA in RBO were C14:0, 0.1–0.3%; C16:0, 12.8–21.0%, C16:1, 0.0–0.3%; C18:0, 0.7–4.7%; C18:1, 32.4–43.4%; C18:2, 28.0–53.4%; C20:0, 0.5–1.4%; C18:3, 0.2–1.6%. The variation in the FA composition for the different RBO could also be due to varietal properties of the rice. However, the FA composition data for the common vegetable oils analyzed here and those of the RBO studied also agree with literature reports for the respective oils (10,11).

Oryzanol content of RBO samples. The results of oryzanol analyses presented in Table 1 show that the physically refined samples have medium to high oryzanol levels (0.56–1.39%) and the chemically refined samples have low to very low levels of oryzanol (0.14–0.18%). The changes in the oryzanol content are speculated to cause variation in FFA content because common vegetable oils such as groundnut oil, sunflower oil, or safflower oil that do not contain oryzanol do not show high FFA content. But no literature is available to substantiate this observation.

Oryzanol content of RBO in relation to different methods of processing. RBO is produced by either chemical or physical refining. In chemical refining, sodium hydroxide is used to remove FFA; and in physical refining dry steam stripping under vacuum is used to remove FFA. During chemical refining, the

TABLE 3
FA Composition of Different Brands of Indian Rice Bran Oils and Some Other Vegetable Oils Used in the Study

Sample[a]	FA composition[b] (relative %)							
	C14:0	C16: 0	C16:1(n-9)	C18:0	C18:1(n-9)	C18:2(n-6)	C20:0	C18:3(n-3)
Chemically refined RBO								
1	0.3	20.8	—	1.8	41.5	34.4	0.7	0.3
2	0.3	16.5	—	2.9	35.0	42.6	1.1	0.7
Range	0.3	16.5–20.8	—	1.8–2.9	35.0–41.5	34.4–42.6	0.7–1.1	0.3–0.7
Physically refined RBO								
1	0.3	21.6	0.3	4.7	42.6	28.0	1.0	0.8
2	0.3	20.5	0.1	1.8	43.4	30.9	1.3	0.8
3	0.2	21.1	—	1.4	42.8	32.8	1.0	0.4
4	0.3	20.5	—	2.3	42.8	30.3	1.2	1.2
5	0.3	20.6	—	1.5	42.4	33.8	1.1	0.4
6	0.2	15.4	—	2.9	32.4	47.0	0.6	0.6
7	0.3	15.1	—	2.7	33.6	46.2	0.9	0.7
8	0.1	12.8	0.1	3.0	29.0	53.4	0.7	0.6
9	0.3	18.7	—	2.3	40.5	34.8	1.4	1.1
10	0.3	16.4	—	3.0	37.3	41.3	0.8	0.7
11	0.3	21.3	—	0.7	39.7	36.9	0.5	0.2
12	0.3	21.1	—	1.7	43.4	32.0	—	1.6
Range	0.1–0.3	12.8–21.6	0.0–0.3	0.7–4.7	32.4–43.4	28.0–53.4	0.5–1.4	0.2–1.6
Other oils								
Groundnut oil	0.2	12.7	—	2.6	52.2	31.4	0.3	0.7
Sunflower oil	0.1	7.1	—	4.1	23.1	65.1	—	0.2
Safflower oil	0.2	9.2	—	3.2	17.7	69.7	—	—

[a]Chemically refined RBO samples 1and 2 were received from ITC Agroproducts Ltd., Hyderabad, India; physically refined RBO samples 1–12 were received from AP Solvex (pvt) Ltd., Dhuri, Punjab, India. The other oils were purchased from the local market.
[b]The values are averages of duplicate injections.

alkali added reacts with FFA and forms soap. The oryzanol present in the crude oil is carried along with the soap stock to the extent of 80–90% (2). During physical refining, the FFA present in the crude oil are removed by steam stripping (distillation). Oryzanol does not distill along with the FFA and hence is retained in the refined oil to the extent of 90%. The presence of oryzanol in high amounts in the physically refined RBO compared with chemically refined RBO has been confirmed from literature (1) as well as from the data of oryzanol shown in Table 1.

Reason for the differential behavior of RBO in comparison with other vegetable oils. In the titrimetric determination of FFA for oils such as sunflower, safflower, and groundnut, a clear end point is noticed. Only in the determination with RBO does a yellow color appear before the pink phenolphthalein end point is obtained (Table 1). The yellow is more intense when the oryzanol content is high and less for low levels of oryzanol in the titrated RBO. It is not clear from the literature how oryzanol interferes in the determination of FFA. But, from this study, it is inferred that the interfering material in refined RBO may be oryzanol.

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