Effect of Refining of Crude Rice Bran Oil on the Retention of Oryzanol in the Refined Oil

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ABSTRACT: The effect of different processing steps of refining on retention or the availability of oryzanol in refined oil and the oryzanol composition of Indian paddy cultivars and commercial products of the rice bran oil (RBO) industry were investigated. Degumming and dewaxing of crude RBO removed only 1.1 and 5.9% of oryzanol while the alkali treatment removed 93.0 to 94.6% of oryzanol from the original crude oil. Irrespective of the strength of alkali (12 to 20° Be studied), retention of oryzanol in the refined RBO was only 5.4–17.2% for crude oil, 5.9–15.0% for degummed oil, and 7.0 to 9.7% for degummed and dewaxed oil. The oryzanol content of oil extracted from the bran of 18 Indian paddy cultivars ranged from 1.63 to 2.72%, which is the first report of its kind in the literature on oryzanol content. The oryzanol content ranged from 1.1 to 1.74% for physically refined RBO while for alkali-refined oil it was 0.19–0.20%. The oil subjected to physical refining (commercial sample) retained the original amount of oryzanol after refining (1.60 and 1.74%), whereas the chemically refined oil showed a considerably lower amount (0.19%). Thus, the oryzanol, which is lost during the chemical refining process, has been carried into the soapstock. The content of oryzanol of the commercial RBO, soapstock, acid oil, and deodorizer distillate were in the range: 1.7–2.1, 6.3–6.9, 3.3–7.4, and 0.79%, respectively. These results showed that the processing steps—viz., degumming (1.1%), dewaxing (5.9%), physical refining (0%), bleaching and deodorization of the oil—did not affect the content of oryzanol appreciably, while 83–95% of it was lost during alkali refining. The oryzanol composition of crude oil and soapstock as determined by high-performance liquid chromatography indicated 24-methylene cycloartanyl ferulate (30–38%) and campesteryl ferulate (24.4–26.9%) as the major ferulates. The results presented here are probably the first systematic report on oryzanol availability in differently processed RBO, soapstocks, acid oils, and for oils of Indian paddy cultivars.

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KEY WORDS: Indian paddy cultivars, oryzanol, processing of RBO, rice bran oil.

Oryzanol is an important component of crude rice bran oil (RBO), and its content ranges from 1.5 to 2.9% in RBO (1). It has many beneficial biological effects. Some of the biological effects of oryzanol and RBO are: (i) reduction of cholesterol in the blood of experimental rats and human volunteers (2,3), (ii) improvement of capillary action of blood vessels (4), (iii) anti-aging effect similar to tocopherols (5), and (iv) anti-dandruff and anti-itching properties (6). Many product patents based on oryzanol are available in the literature (5–9).

The oryzanol was first extracted from RBO and presumed to be a single component (10). Later it was found to be a mixture containing ferulic acid (4-hydroxy-3-methoxy cinnamic acid) esters of triterpene alcohols and plant sterols. Individual components were identified as ferulic acid esters of cycloartanol, 24-methylene cycloartanol, campesterol, β-sitosterol, and cycloartenol. Physiological effects that have been shown to be associated with oryzanol intake are decreasing plasma cholesterol (11,12), decreasing platelet aggregation (13), decreasing hepatic cholesterol biosynthesis (14), increasing fecal bile acid excretion (14), and decreasing cholesterol absorption and aortic fatty streaks formation (15). Oryzanol has also been used to treat nerve imbalance and disorders of menopause (16). The industrial problem associated with oryzanol is that it increases the loss of neutral oil during refining of RBO (17). Recently the literature has been reviewed by Kaimal (9). Previous studies did not indicate how much oryzanols are removed/retained during processing of RBO. Therefore, an attempt has been made to understand the availability of oryzanol in differently processed RBO. The content and composition of a number of commercial RBO samples along with the bran oil extracted from 18 Indian paddy cultivars and the effect of processing on the retention of oryzanol in the refined oil have been examined in this investigation.

MATERIALS AND METHODS

RBO (crude, degummed, dewaxed, refined, bleached, and deodorized) and soapstocks/acid oils, and deodorizer distillate were obtained from M/s M.K. Agro Industries (Srirangapatna, near Mysore, India). Physically refined RBO were obtained from M/s Eastman Agro Mills Ltd. (New Delhi) and AP Solvex Ltd. (Dhuri, Punjab, India). RBO soapstocks were also obtained from M/s Sri Muruga Rajendra Oil Industries Ltd. (Chitradurga, Karnataka, India).

The moisture content of the soapstock was determined by the AOCS method O.M. No. Da 2a-48 (18). Degumming and dewaxing of the crude oil were done as reported earlier (17). Refining of crude RBO was done in the laboratory on 500-g batch sizes using AOCS cup method (Ce 9a-52) on quadru-

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plicate samples (18). Oryzanol content in the oil was determined by spectrophotometric method by determining ultraviolet (UV) absorption at 314 nm in a 1-cm cell of the solution in hexane followed by calculation using the specific extinction coefficient 358.9 (1). The composition of oryzanol was determined by high-performance liquid chromatography (HPLC) by using acetonitrile/methanol/isopropanol (10:9:1) solvent system of Rogers *et al*. (19) as the mobile phase and C18 Shimpak (Shimadzu Corporation, Tokyo, Japan) HPLC column (150 mm × 4.6 mm i.d.). Oryzanol was isolated from the soapstock of crude RBO for use as a standard by the method covered by the Indian patent (20). The pattern of ferulic acid sterol esters was the same as reported in literature (19,21,22).

RESULTS AND DISCUSSION

Oryzanol content of bran oil from different Indian paddy cultivars. The brans of 18 varieties of paddy rice were prepared in the laboratory, and the oil was extracted with chloroform/methanol as the solvent (23). The oil was analyzed for oryzanol content, which is given in Table 1. The oryzanol content ranged from 1.63 to 2.72%. The data agreed well with the reports for Indian (1) and Japanese RBO (24) This is the first report for oil extracted from different Indian paddy cultivars.

Effect of refining of RBO on oryzanol retention in refined oil. Yoon and Kim (25) found that during refining of high free fatty acid crude RBO 12–59% of oryzanol is lost during the caustic refining process. Kim *et al*. (26) observed 41.7, 7.3, and 8.6% loss of oryzanol during caustic, solvent, and steam refining of high free fatty acid crude RBO. However, the investigations of Rogers *et al*. (19) showed that the refined RBO has oryzanol content of 100–800 ppm in commercial refined RBO. De and Bhattacharyya (27) found a marginal loss of 4.1–11.3% during caustic refining and no loss of oryzanol during physical refining. Data on the effect of refining steps on retention of oryzanol in refined RBO during processing of low free fatty acid crude RBO are fewer and contradictory. Therefore, a systematic study on the effect of the different refining steps such as degumming, dewaxing and alkali refining, physical refining, bleaching and deodorization of a low free fatty acid crude RBO has been conducted to find the retention or loss of oryzanol in the processed oil. The results in Table 2 show that alkali treatment removes a considerable amount (93–94.6%) of the oryzanols while degumming and dewaxing do not affect the content of oryzanol in the oil considerably (1.1 and 5.9%, respectively). Some of the commercial products of the RBO industry analyzed for oryzanol content are shown in Table 3. Also, some of the soapstocks and acid oils analyzed showed that 2.2 to 7.4% of oryzanol was found in the soapstock-acid oils (expressed on dry basis). With this background, the crude RBO was degummed in the laboratory on 500-g batches, which was later dewaxed. The refined oil obtained from crude, degummed, or dewaxed RBO showed 82.8–94.6% reduction in the oryzanol content confirming the data obtained on commercial samples (Table 4) which is probably the first systematic report of its kind on oryzanol losses at different stages of processing of crude RBO.

Effects of strength of alkali on retention of oryzanol in refined RBO. It was of interest to note that irrespective of strength of alkali used, the loss of oryzanol in the refined oil was >85% of the starting material (Table 4). Strength of alkali used during the refining of crude/degummed/dewaxed oil showed the same effect in carrying over of oryzanol to the soapstock (loss of oryzanol in the refined oil 83–95%). However, only degumming before refining gave 85–94% reduction of oryzanol in the oil while degumming and dewaxing

TABLE 1
Oryzanol Content of Oil of Rice Bran Extracted from Different Paddy Cultivars

Name of the variety	Oryzanol[a] (%)	Name of the variety	Oryzanol (%)
1. Basmati 370	1.78	10. Garima	1.77
2. Punjab Basmati 1	2.06	11. Srinivas	1.63
3. Anupama	2.54	12. Rajendra	2.51
4. Sonasali	2.72	13. MR	2.38
5. Purva	1.85	14. JR 75	2.46
6. Sheshu 572	1.85	15. Vikramarya	1.97
7. Vikas	2.29	16. Swarnadhan	1.83
8. Soma Mansarowar	2.35	17. IR 20	1.74
9. Tela Harmsa	1.93	18. HLR	2.55

[a]Values are mean of duplicate determinations.

TABLE 2
Effect of Processing of Crude Rice Bran Oil (RBO) on Oryzanol Content in the Refined Oil[a]

	Oryzanol content in refined oil (%)	Loss (%)
Control RBO (FFA, 6.8%)	1.86	—
Degumming	1.84	1.10
Dewaxing	1.75	5.90
Control RBO + alkali treatment	0.10	94.60
Degummed RBO + alkali treatment	0.11	94.10
Dewaxed RBO + alkali treatment	0.13	93.00

[a]Values are averages of samples from four determinations, and the coefficient of variation (CV) is <2% for all processing steps. FFA, free fatty acid.

TABLE 3
Oryzanol Content of Some Commercial Products of RBO Industry

Product	Oryzanol content (%)	Loss from oil (%)
Crude RBO	1.81	—
Degummed RBO	1.71	2.80
Refined RBO	0.19, 0.20	89.50
Physically refined RBO	1.10, 1.74[a], 1.10	
Deodorizer distillate	0.79	
Soapstock—soft (DB)	6.71	[b]
Soapstock—hard (DB)	2.21	
Acid oil	3.28–7.36	

[a]The crude oil had 1.6% oryzanol for the 1.74% oryzanol containing physically refined RBO (CV < 2%).
[b]Concentrated by 1.6 to 4.1 times the initial value in the oil. DB, dry basis; see Table 2 for other abbreviations.

TABLE 4
Effect of Strength of Alkali on Loss of Oryzanol in Refined Oil

	Alkali strength	Oryzanol content in refined oil (%)[a]	% Loss
Crude RBO (FFA, 6.8%)[b]	12°Be	0.10	94.6
	16°Be	0.17	90.9
	18°Be	0.20	89.2
	20°Be	0.32	82.8
Degummed RBO (FFA, 6.8%)	12°Be	0.11	94.1
	16°Be	0.17	90.9
	18°Be	0.28	85.0
	20°Be	0.26	86.0
Degummed and dewaxed RBO (FFA, 6.8%)	12°Be	0.13	93.0
	16°Be	0.26	91.4
	18°Be	0.13	93.0
	20°Be	0.18	90.3

[a]Values are averages from four refining tests and the CV < 2%.
[b]Starting RBO had a oryzanol content of 1.86%. See Table 2 for abbreviations.

followed by refining also produced 90.3–93% loss of oryzanol in the refined oil. The crude oil subjected to refining without degumming and dewaxing yielded a refined oil with 4–8% higher oryzanol contents which is not appreciably high.

Oryzanol content of commercial products of RBO industry. The commercial soapstocks and other products from the RBO industry were obtained from two manufacturers and analyzed for their chemical composition. The data show that the soapstocks contain 6.3–6.9% of oryzanol expressed on dry weight basis (Table 5). Therefore, the soapstock acid oil/soapstock is the actual raw material which is to be processed further to recover the important constituent oryzanol. Many methods are available to recover oryzanol from RBO, soapstocks (1,9,10,20,28), and acid oils (22) which are covered under patents. The molecular distillation method not only is expensive but also may not give pure product in the opinion of the authors. Solvent partition/extraction methods, although involving large volumes of solvents, can give high-purity oryzanol (1,20,21,23,24,28).

Effect of physical refining and deodorization on retention of oryzanol in the refined oil. The data on oryzanol content of commercial samples of physically refined RBO, alkali-refined RBO, and deodorizer distillate of alkali refined RBO are given in Table 3. Removal of fatty acids by vacuum distillation (physical refining) retained the oryzanol content in the refined oil to the extent of 100% of the original oil, indicating that physical refining only can retain most of the oryzanols in the refined oil compared to the alkali treatment refining process (compare data in Tables 3–5). The data of oryzanol content of the deodorizer distillate of 0.79% in Table 3 (obtained during alkali refining process) indicate little loss of oryzanol during deodorization of refined RBO, in agreement with literature report of De and Bhattacharyya (27). However, due to the retention of >100% of oryzanol during physical refining of RBO (starting oil 1.6%, and after physical refining 1.74% oryzanol), it may be argued that there is no loss of oryzanol during the deodorization step. The data of De and Bhattacharya (27) also indicate a slight increase in oryzanol content during the deodorization step.

TABLE 5
Composition of Commercial Soapstocks of RBO[a]

Sample no.[b]	Content (% WB)		Oryzanol (%) in	
	Moisture	Dry matter	Soap	Acid oil
1	58.8	41.2	2.8	6.9
2	46.2	53.8	3.6	6.8
3	66.8	33.0	2.1	6.5
4	59.8	43.2	2.5	6.3

[a]Values are averages of three replicates for each sample and the CV < 4%.
[b]Samples were obtained from two manufacturers: M/s M.K. Agro Industries (sample 1) and M/s Sri Muruga Rajendra Oil Industries Ltd. (samples 2, 3, 4). WB, wet basis; see Table 2 for other abbreviations.

Composition of oryzanol. The oryzanol compositions of crude RBO and of the soapstocks were investigated by HPLC method, and chromatograms are shown in Figure 1. The composition given in Table 6 shows that the soapstock-acid oil contained methyl ferulate (7.2–8.5%), cycloartanyl ferulate (12.8–14.7%), and β-sitosteryl ferulate (9.3–15.8%) as the minor ferulates and 24-methylene cycloartanyl ferulate (34.6–38.0%) and campesteryl ferulate (24.4–26.9%) as the major ferulates present in RBO and its soapstock-acid oil. The crude RBO contained methyl ferulate (0.3%), cycloartanyl ferulate (0.14%), 24-methylene cycloartanyl ferulate (0.56%), campesteryl ferulate (0.49%), and β-sitosteryl ferulate (0.24%). While the soapstock had more concentrations of ferulate than the original crude oil, nevertheless the relative percentages did not vary appreciably.

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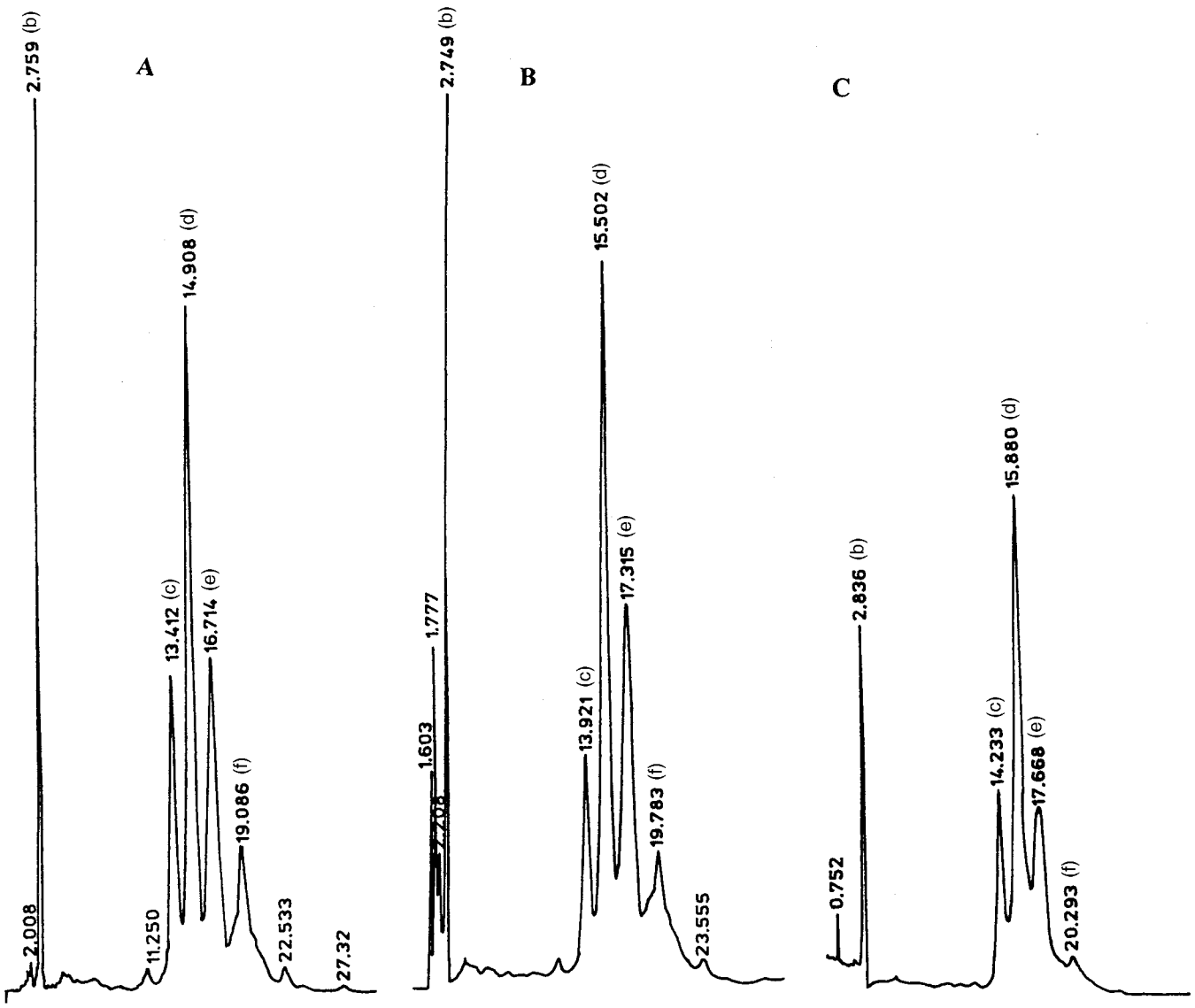

FIG. 1. High-performance liquid chromatography pattern of oryzanol components of (A) crude rice bran oil, (B) soapstock-acid oil, and (C) pure oryzanol. Peak identities: (b) methyl ferulate, (c) cycloartanyl ferulate, (d) 24-methylene cycloartanyl ferulate, (e) campesteryl ferulate, and (f) β-sitosteryl ferulate/cycloartenyl ferulate. Other peaks not identified. Numbers on the peaks represent elution times, in minutes.

TABLE 6
Composition of Oryzanol of Soapstock-Acid Oil and RBO

	Oryzanol composition (g/100 g relative %)									
	1[b]		2[c]		3[d]		4[e]		5[f]	
Sample[a]	A	B	A	B	A	B	A	B	A	B
Crude RBO	0.30	16.30	0.14	7.60	0.56	30.30	0.49	26.30	0.24	13.00
Soapstock (acid oil)	0.54	7.9	0.95	13.8	2.49	36.3	1.76	25.7	0.86	12.6
Oryzanol isolated	9.3	9.3	24.7	24.7	36.5	36.5	16.2	16.2	8.2	8.2

[a]The oryzanol contents of crude RBO, soapstock-acid oil, and pure oryzanol were 1.86, 6.85, and 100%, respectively, and are the average of three determinations. A—content; B—% of total.
[b]Methyl ferulate.
[c]Cycloartanyl ferulate.
[d]24-Methylene cycloartanyl ferulate.
[e]Campesteryl ferulate.
[f]β-Sitosteryl ferulate/cycloartenyl ferulate. See Table 2 for abbreviation.

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