Factors Affecting Refining Losses in Rice (*Oryza sativa* L.) Bran Oil

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Components of rice bran oil have been assessed for their effect on refining losses. Rice bran oil used in the study had the following (percent) analysis: free fatty acids, 6.8; phosphatides, 1.25; wax, 2.85; monoglycerides, 1.67; diglycerides, 4.84, and oryzanol, 1.85; the rest (80.74) was mostly triglycerides. The phosphatides and mono- and diglycerides had no noticeable effect on refining losses at levels of up to 2% in the oil. Waxes and oryzanol increased the refining losses substantially. In model experiments where these were incorporated into peanut oil individually and in combination, the wax at as low a level as 1% increased the refining losses by about 80% more than control and the refining losses increased with concentration of wax. Oryzanol had a similar effect. When wax and oryzanol were present together in the oil, the effect was synergistic—the refining losses were higher than the sum of their individual effects. Phosphatides, mono- and diglycerides tended to reduce the adverse effect of wax and oryzanol. The main components responsible for higher than normal refining losses in rice bran oil have been identified as wax and oryzanol.

Rice bran is a potential source of vegetable oil in rice growing countries like India. It is estimated that as much as 3.3 million ton of oil could be obtained from rice bran on an estimated world rough rice production of 411.9 million ton (1). However, problems in bran handling and processing of the oil have restricted the utilization of this source for edible oil to about 1% of the potential. The newer bran stabilization techniques, particularly the low-cost acid stabilization (1), could obviate problems of bran handling. However, problems in processing of oil, especially the high refining losses, have largely remained unsolved. Rice bran oil is difficult to refine because of its high content of free fatty acids (FFA) and its unsaponifiable matter and dark color (2). Refining losses of four to six times the FFA content have been recorded for oils with FFA of 2 to 6.3% (3). Miscella refining has been adopted in Japan to reduce refining losses and for dewaxing of the oil, but such a technology has limitations in developing countries like India. Use of several additivs (4) and progressive acetylation of hydroxylated compounds (5) have been suggested to reduce the refining losses, but these have met with little commercial success. Phosphatides (6), waxes (7), monoglycerides and other hydroxylated compounds (5) have been speculated to be the causative factors for the high refining losses in rice bran oil. However, supporting direct evidence is lacking. This paper presents data on the influence of some of the components of rice bran oil on its refining losses, using peanut oil as a model oil for the study.

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MATERIALS AND METHODS

Rice bran oil was obtained from a local solvent extraction plant. The oil had the following percentage composition: FFA, 6.8; phosphatides, 1.25; wax, 2.85; monoglycerides (MG), 1.67; diglycerides (DG), 4.84, and oryzanol, 1.85. Refined soy lecithin (ICN pharm, Cleveland, Ohio; acetone insolubles 95% and phosphorus 3.3%), distilled cottonseed monoglycerides (Myverol, Eastman Kodak Co., Rochester, New York), and glyceryl monostearate (Navsari Oil Products, Bombay, India) were used in the study. Other reagents used were of analytical grade.

Degumming of rice bran oil (RBO). Rice bran oil was heated to 75°C and 1% aqueous phosphoric acid was sprayed onto the oil (3 ml/100 g oil) while stirring the oil gently (250 rpm). The hydrated gums were allowed to settle overnight and the supernatant oil was decanted. This oil had 6.8% FFA, .06% phosphatides and 2.92% wax.

Dewaxing of rice bran oil. The degummed RBO was left at 7–8°C for 48 hr to allow the wax to crystallize and settle. The supernatant oil was decanted; it had 6.8% FFA, 0.01% phosphatides and 0.06% wax.

Purification of rice bran wax. The wax recovered from the settlings of RBO was freed from oil and phosphatides by repeated washing with methanol, acetone, ether and, finally, with chloroform (8). The wax had a m.p. of 79 to 82°C.

Model oil for assessing refining losses. Refined peanut oil (Postman brand) with FFA of 0.05% was used as the model oil for assessing the effect of various components of RBO on refining losses. The test component(s) was incorporated into the peanut oil and its FFA adjusted to 6.8% (same as RBO stock) using RBO free fatty acids prior to the refining test.

Procedure for assessing refining losses. In the initial experiments, the AOCS cup method (9) was used for assessing the refining losses. As only limited quantities of the components of RBO were available, the procedure described below was followed for studying their effect on refining losses.

The oil sample (10 g) was taken in a centrifuge tube (25 × 100 mm) and a calculated quantity of 20 Be alkali as in the standard AOCS cup method (9) was added. The mixture was stirred vigorously for three min at ambient temperature (27–30°C) with a glass rod. Then the tube was transferred to a water bath maintained at 65°C and stirred slowly for three min. After holding at 65°C for a further seven min, the tubes were taken out, cooled under running water and centrifuged in a laboratory centrifuge at 1405 g for five min. The oil was decanted and weighed. The refining losses were calculated as in the AOCS method (9). The refining losses determined by this procedure were comparable to the AOCS cup method (9) but were slightly higher than by the chromatographic method (10). Wesson losses (10) were considerably lower than the losses by any of the above three methods (Table 1). All experiments were carried out in duplicate, unless otherwise indicated.

TABLE 1

Comparison of Different Methods for Assessing Refining Losses in Rice Bran Oil

Oil sample	Free fatty acids (as oleic) %	Refining losses (%) as determined by			
		AOCS	Centrifuge[a]	Chromatographic	Wesson
RBO-1	6.8	28.5 ± 0.14	27.0 ± 1.00	25.3 ± 0.43	15.7 ± 0.45
RBO-2	10.1	32.1 ± 0.59	30.0 ± 0.79	22.7 ± 0.54	17.5 ± 0.18
Degummed RBO	6.8	26.1 ± 0.74	28.0 ± 1.30	22.2 ± 0.31	14.2 ± 0.20
Degummed & dewaxed RBO	6.8	24.8 ± 0.19	24.0 ± 0.14	19.5 ± 0.49	11.4 ± 0.29
Triglycerides of RBO	6.8	—	18.4 ± 0.12	—	—
Peanut oil (expeller)	1.5	6.0 ± 0.59	6.9 ± 0.38	2.4 ± 0.44	2.8 ± 0.35
Peanut oil (refined) as model oil	6.8[b]	17.2 ± 0.49	16.0 ± 0.32	10.5 ± 0.29	10.8 ± 0.42

Values are mean of four replicates.

[a]Method used in the present study.

[b]FFA adjusted using RBO free fatty acids.

When the effect of RBO components on refining losses had to be assessed the following correction was made for the component added:

Refining loss corrected for additives =

(Refining loss % - Additive % × 100)/(100 - Additive %)

Isolation of free fatty acids (FFA). Degummed and dewaxed RBO was saponified with 1N sodium hydroxide. The soap was extracted once with petroleum ether to free it from unsaponifiable matter, neutralized, and the FFA extracted with petroleum ether. The extract was washed with water, dried over anhydrous sodium sulphate and desolventized.

Isolation of triglycerides (TG), diglycerides (DG) and monoglycerides (MG). Degummed and dewaxed RBO (500 g) was dissolved in 500 ml hexane, and 500 g silica gel (60–120 mesh) were added. After stirring for 10 min at ambient temperature (27–30°C) the miscella was decanted. The silica gel residue was treated successively with 500 ml benzene/hexane (1:1, v/v), benzene (500 ml) and methanol (3 × 500 ml). The first two fractions consisted of triglycerides (90%) with a small quantity of FFA, whereas the methanol fraction contained partial glycerides (7.6%).

The partial glycerides fraction was subjected to column (5.8 × 70 cm) chromatography on silica gel (60–120 mesh) and MG and DG were isolated according to the AOAC procedure (11). The purity was checked by TLC.

Analytical methods. The FFA content of the oil was determined by the AOCS method (12) and expressed as percent oleic acid. Phospholipids were estimated according to the procedure of Marinetti (13), and wax as acetone insolubles by the procedure of Kumar David et al. (14) substracting the phospholipid content in the acetone insolubles. MG and DG were estimated by the AOAC procedure (11). Oryzanol was isolated from RBO soapstock and estimated by determing the optical density of the sample/oil in petroleum ether (60–80°C) at 315 nm and using specific extinction coefficent E = 358.9 (15).

Thin layer chromatography (TLC). Glass plates (20 × 20 cm) were coated with 0.25 mm silica gel G, activated at 110°C for one hr and, after spotting, were developed in a mixture of petroleum ether/diethyl ether/acetic acid (60:40:1, v/v/v). The spots were visualized by exposing the chromatoplate to iodine vapors.

RESULTS AND DISCUSSION

Refining losses in RBO were considerably higher than in peanut oil of similar FFA content (Table 1). However, when the RBO was freed from other components and the FFA of the isolated triglycerides was readjusted to the level of stock RBO, the refining losses were similar to that of the model (peanut) oil (Table 1). This indicated that rice bran oil had some constituents which enhanced its refining losses. To study the constituents responsible for the high refining losses, the known components of rice bran oil were added indi-

TABLE 2

Influence of Phosphatides on Refining Losses in a Model Oil[a]

Lecithin added (%)	Refining losses (%)	
	Observed	Corrected for lecithin content
0	16.0 ± 0.35	16.0
1	17.2 ± 0.21	16.4
2	17.8 ± 0.31	16.1
3	18.7 ± 0.49	16.2
4	19.1 ± 0.29	15.7
5	20.0 ± 0.49	15.8

[a]Refined peanut oil, FFA adjusted to 6.8% with RBO FFA. Values are mean of four replicates.

TABLE 3

Influence of Rice Wax on Refining Losses in Model and Rice Bran Oils

Rice wax %	Refining loss (%)							
	Model oil[a] having FFA (%)						Degummed and dewaxed RBO with 6.8% FFA	
	1.5		6.8		10.0			
	Observed	Corrected for wax	Observed	Corrected for wax	Observed	Corrected for wax	Observed	Corrected for wax
0.0	6.8	—	15.5	—	19.2	—	24.0	—
0.1	8.5	8.4	19.6	19.5	20.7	20.6	24.6	24.5
0.2	—	—	20.0	19.8	—	—	24.8	24.7
0.4	—	—	24.3	24.0	—	—	—	—
0.5	—	—	—	—	—	—	27.1	26.7
0.6	—	—	24.5	24.0	—	—	—	—
0.8	—	—	25.1	24.5	—	—	—	—
1.0	12.4	11.5	27.2	26.5	29.4	28.6	29.9	29.2
2.0	17.4	15.7	29.5	28.1	34.2	32.9	36.2	34.9
3.0	21.4	18.9	31.8	29.7	36.2	34.2	47.4	45.8
4.0	—	—	36.9	34.3	—	—	—	—
5.0	29.1	25.3	39.3	36.1	41.6	38.5	62.8	60.8

[a]Refined peanut oil FFA adjusted to 6.8% with RBO FFA.

vidually to peanut oil, chosen as a model oil, and the refining losses estimated.

Effect of phosphatides. The RBO after degumming showed a marginal reduction in refining losses of about 2% (Table 1). However, when the degumming losses were taken into account, there was no difference in the refining losses before and after degumming. Similarly, when 1 to 5% lecithin was incorporated into peanut oil, there was an apparent increase in the refining losses (Table 2). However, when the values were corrected for the added lecithin, the refining losses in the presence of lecithin were not noticeably higher than the oil without any phosphatides. There have been conflicting reports on the influence of phosphatides on the refining losses in vegetable oils. It has been reported that phosphatides form stable emulsions and thereby increase refining losses (6,16). Contrary to the above is the observation that oils which do not contain or have very low levels of hydratable phosphatides can be better refined by adding hydratable phosphatides (17). The present results indicate that phosphatides may not have any significant influence on the refining losses in RBO.

Effect of wax. The removal of wax from degummed RBO led to a considerable reduction in refining losses (Table 1). The effect of wax at 0.1 to 5% levels on refining losses was therefore studied in the model oil.

Data in Table 3 show the effect on refining losses of rice bran wax (Required quantity of wax was weighed into the centrifuge tube along with the oil, the tube was heated in a water bath until the wax dissolved, cooled to ambient temperature prior to addition of

TABLE 4

Effect of Partial Glycerides and Oryzanol on Refining Losses in a Model Oil[a]

A[b] level (%)	Refining loss (%)									
	Distilled cottonseed oil monoglycerides		Glycerol monostearate		RBO monoglycerides		RBO diglycerides		Oryzanol	
	Observed	Corrected[c]	Observed	Corrected[c]	Observed	Corrected[c]	Observed	Corrected[c]	Observed	Corrected[c]
0.0	16.0	—	16.1	—	16.0	—	16.0	—	16.0	—
0.1	—	—	—	—	16.3	16.2	—	—	16.8	16.7
0.5	—	—	—	—	16.6	16.2	16.8	16.4	17.8	17.4
1.0	17.1	16.3	17.7	16.9	18.4	17.6	17.4	16.9	20.4	19.6
2.0	19.1	17.4	19.7	18.0	18.3	16.6	17.1	15.4	26.3 (26.5)[d]	24.8
4.0	22.7	19.5	27.6	24.6	—	—	—	—	—	—
5.0	29.0	25.3	32.9	29.3	—	—	16.9	12.5	—	—

[a]Refined peanut oil, FFA adjusted to 6.8% with RBO FFA.

[b]Additive.

[c]Corrected for partial glycerides/oryzanol.

[d]Values are for RBO triglycerides.

TABLE 5

Effect of Combination of Different Components of RBO on Refining Losses in a Model Oil[a]

Component (%)						Refining losses (%)		
Sl. no.	Phosphatides	Wax	RBO MG	RBO DG	Oryzanol	Observed	Corrected for the additive A	Net increase A-16
1	—	—	—	—	—	16.0	—	—
2	1	—	—	—	—	17.2	16.4	0.4
3	—	3	—	—	—	31.8	29.7	13.7
4	1	3	—	—	—	25.1	22.0	6.0
5	—	—	—	—	2	26.3	24.8	8.8
6	1	—	—	—	2	22.4	20.0	4.0
7	—	3	—	—	2	46.1	43.3	27.3
8	1	3	—	—	2	39.5	35.6	19.6
9	—	—	1	—	—	18.4	17.6	1.6
10	—	—	—	2	—	17.1	15.4	-0.6
11	1	3	—	2	2	34.0	28.3	12.3
12	1	3	1	—	2	34.4	29.5	13.5
13	1	3	1	2	2	33.0	26.4	10.4

[a]Refined peanut oil FFA adjusted to 6.8% with RBO FFA.

alkali.). As little as 0.2% of rice bran wax increased the refining losses from 15.5% to 20.0%. The refining losses in the model oil doubled when the wax content was increased to 3.0% (Table 3). This increase in refining loss in the presence of wax was noticeable in both low and high FFA oils (Table 3). The adverse effect of wax on refining losses was confirmed by incorporating wax into degummed and dewaxed RBO (Table 3). RBO generaly contains from 2 to 3% wax. This underlines the importance of dewaxing prior to alkali refining.

Effect of partial glycerides and oryzanol. The refining losses were higher for RBO than the model oil of comparable FFA even after removal of phosphatides and waxes (Tables 1 and 3). Thus, it was clear that some constituent(s) besides wax influenced refining losses in RBO. Hence, the effects of other constituents, namely monoglycerides, diglycerides and oryzanol, were examined.

Commercially available distilled cottonseed oil monoglycerides and GMS, MG and DG isolated from rice bran oil were used. Monoglycerides showed no noticeable effect on refining losses up to 2%, but above 4% they increased the refining losses considerably (Table 4). Diglycerides did not have any adverse effect on refining losses at levels of up to 5%. In commercial rice bran oil levels of MG (0.4-1.5% (5) and DG (2-5%) (Prabhakar, J.V., J. Hemavathy and K.V. Lakshminvenkatesh, unpublished data) are low; hence, these partial glycerides are not likely contributors to the refining losses.

Oryzanol at 1% increased the refining losses from 16 to 20%, and the losses increased with concentration of oryzanol (Table 4). Oryzanol, generally present to the extent of 2% in RBO, could therefore contribute considerably to the increased refining losses.

Combined effect of different components. The effect of the combination of different components on refining losses is shown in Table 5. When phosphatides and wax were present together in the oil, the phosphatides exerted a beneficial effect, lowering the adverse effect of wax (Table 5). Low levels of hydratable phosphatides are known to assist in refining of oils (17). Phosphatides also reduced the adverse effect of oryzanol on refining losses. However, this cannot be taken advantage of in RBO refining, because the presence of phosphatides would make dewaxing of oil difficult (18,19).

The presence of wax and oryzanol together had a slightly synergistic effect, the refining losses being higher than those of the individual components put together.

The presence of phosphatides along with wax and oryzanol reduced the refining losses. The diglycerides and monoglycerides had similar beneficial effects. The phosphatides, monoglycerides and diglycerides together had a cumulative beneficial effect reducing the combined effect of wax and oryzanol considerably.

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