Deacidification of High-Acid Rice Bran Oil by Reesterification with Monoglyceride

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ABSTRACT: Autocatalytic esterification of free fatty acids (FFA) in rice bran oil (RBO) containing high FFA (9.5 to 35.0% w/w) was examined at a high temperature (210°C) and under low pressure (10 mm Hg). The study was conducted to determine the effectiveness of monoglyceride in esterifying the FFA of RBO. The study showed that monoglycerides can reduce the FFA level of degummed, dewaxed, and bleached RBO to an acceptable level (0.5 ± 0.10 to 3.5 ± 0.19% w/w) depending on the FFA content of the crude oil. This allows RBO to be alkali refined, bleached, and deodorized or simply deodorized after monoglyceride treatment to obtain a good quality oil. The color of the refined oil is dependent upon the color of the crude oil used.

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Rice bran (*Oryza sativa* L.) oil (RBO) may contain as much as 30 to 40% free fatty acid (FFA), if the bran is not processed properly prior to the extraction of oil. This occurs because of the lipase activity present in the bran. Higher FFA content, however, is one of the main drawbacks to refining RBO, as high FFA content is responsible for greater oil refining loss and the darker color of processed oil (1) produced from the conventional refining process.

Conventional approaches to refining RBO include degumming, dewaxing, alkali refining, bleaching, and deodorization, and are best used with low-FFA oils.These approaches, however, lead to higher refining loss several authors when applied to high FFA oils. Therefore, several authors have proposed physical refining for high-FFA oils (1–4). This process removes FFA along with odoriferous compounds by purging saturated steam through the oil at a high temperature under high vacuum. Physical refining, however, results in large quantities of distilled fatty acids with concomitant reduction in the quantity of the original oil, especially if the oil being processed is high in FFA content.

Alternative approaches have been proposed by workers to reduce neutral oil loss during physical refining. Liquid–liquid extraction with polar solvents (e.g., azeotropic isopropanol) is a better process for extracting FFA along with color and peroxide bodies. Bhattacharyya *et al.* (5) took advantage of this process in refining high FFA (20.5 to 56.0%) RBO and showed that a good quality edible-grade RBO could be obtained by this process.

Chemical or biochemical reesterification of FFA is another approach for the deacidification of high-FFA RBO. Enzymatic deacidification (6–10) of vegetable oils by microbial lipases (i.e., biorefining) has recently received much attention. Lipases can esterify the FFA to hydroxyl groups containing compounds already present in the oil or to the hydroxyl groups of added glycerol. Bhattacharyya and Bhattacharyya (7) successfully brought down the FFA content of RBO from 30 to 3.6%. This process produced an excellent quality RBO by subsequent alkali refining, bleaching, and deodorization.

Bhattacharyya and Bhattacharyya (11) studied chemical esterification of high FFA (15–30%) RBO using an acid catalyst and metal salts. With the acid catalyst they were able to reduce the FFA content from 15–30% to 2–6%, which, on further alkali refining, bleaching, and deodorization, yielded an oil of acceptable color.

Kurashige (12) used diglycerides (DG) for the esterification of crude palm olein by using a lipase (*Pseudomonas fluorescens*)-catalyzed process. It was shown that the extent of esterification is high if DG are used in place of glycerol. This was attributed to the better solubility of DG in the oil.

It was previously reported (13) that, like glycerol, monoglycerides (MG) can esterify FFA. Applying this concept, Sengupta and Bhattacharyya (14) proposed enzymatic esterification of RBO FFA (8.6 to 16.9%) with commercial MG and *Mucor miehei* lipase. They reported that FFA can be reduced to 2 to 4%, depending on the amount of MG used. According to these authors, MG could be used effectively instead of glycerol to reduce FFA in the oils, producing oils of better quality with respect to triglyceride (TG) content.

The main advantage of the esterification process for deacidifying vegetable oils with high FFA contents is the increase in the content of neutral glycerides, especially TG. However, the chief barrier to enzymatic deacidification using MG (in which TG content increases notably) is the high cost of enzymes. The present study was therefore aimed at investigating an autocatalytic high-temperature, low-pressure direct esterification process for the deacidification of RBO using MG.

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MATERIALS AND METHODS

Crude RBO samples of varying acidity were supplied by Sethia Oils Ltd. (Burdwan, WB, India). Commercial MG samples were a gift from Fine Organics (Mumbai, India). Hexane (bp 65–70°C) and diethyl ether (bp 35–40°C) were purchased from S.D. Fine Chem. (Boiser, India). Silica gel G (thin-layer chromatographic grade) was purchased from Tara Chemicals (Calcutta, India).

RBO samples were degummed and dewaxed using a combined single step water degumming-dewaxing process (4) and bleached prior to esterification.

Esterification. Degummed, dewaxed, and bleached RBO (*ca.* 100 g) was placed into a 250-mL conical flask with a ground-glass B-24 joint. A predetermined amount (either stoichiometric or excess) of MG was added to the oil. The oil was then slowly heated to 210°C (at 10 mm Hg) and stirred with a magnetic stir bar (2.54 cm). Samples were drawn at 2-h intervals until no further reduction of FFA occurred.

Deodorization/physical refining. Deodorization and physical refining were both carried out conventionally by steam stripping method. Deodorization was done at 180°C at 10 mm Hg, whereas physical refining was carried out at 235 ± 5°C at 6 mm Hg.

Quantitave determination of MG, DG, and TG. The MG, DG, and TG contents of the crude and refined oil samples were estimated by preparative thin-layer chromatography (PTLC). A 0.5-mm-thick layer of silica gel G (110–120 mesh) was applied to a 20 × 20-cm glass plate using 14 g silica gel and 28 mL distilled water. Plates were activated at 110°C for 70 min. Approximately 0.1 g (accurately weighed) of oil was applied onto each plate and the plate was then developed in 100 mL *n*-hexane/diethyl ether (80:20, vol/vol). Three bands (for MG, DG, and TG) were visualized by iodine absorption and identified by the R_f values (15) specified for each of the components in that particular solvent system. Each band was scraped from the plate and the lipid fraction was extracted with chloroform. Quantitation of each fraction was done gravimetrically by evaporating the solvent under vacuum (30 mm Hg, 90°C). Results are expressed as weight percentage of the oil.

Alkali refining and deodorization were carried out according to the method described in our previous publication (4). The quality of the crude and refined oil samples was assessed through the measurement of color, FFA, peroxide value, total gum and wax, and unsaponifiable matter content (16). Total gum and wax content was expressed as percentage acetone-insoluble matter in the oil at 4°C. The data were obtained by holding a solution of the oil in acetone (1 g oil/5 mL acetone) at this temperature for 4 h, then filtering and weighing the resultant precipitate.

The fatty acid composition of the commercial MG and the oil samples was determined by gas–liquid chromatography of the methyl esters prepared from these samples. Methyl esters were prepared following the method of Litchfield (17). Fatty acid methyl esters (FAME) were analyzed on a Hewlett-Packard gas chromatograph (HP 5890A, Palo Alto, CA), equipped with a flame-ionization detector (FID). The column was packed with Chromosorb-WHP and coated with DEGS (6 × 1/8 in i.d.). The column temperature, injection port temperature, and detector block temperature were maintained at 190, 230, and 240°C, respectively. N_2 was the carrier gas used at a flow rate of 30 mL/min. The peaks were identified by using standard FAME.

The smoke point of a MG-treated RBO sample and that of a caustic refined sample was determined with a Cleveland Open Cup instrument [Petroleum Instruments (India) Pvt. Ltd., Calcutta, India] following a standard procedure (18).

RESULTS AND DISCUSSION

Table 1 shows the physical and chemical characteristics of crude RBO samples with FFA contents ranging from 9.5 to 35.0% (w/w) and the composition of a commercial MG sample. The oils are not suitable for an alkali refining process owing to the high refining loss (1) that invariably results from carrying out the complete refining process with degumming, dewaxing, alkali neutralization, bleaching, and deodorization. The MG sample used in the investigation contained 89.9% MG (by wt) and the required amount of MG (calculated on the basis of 89.9% w/w MG content) was added during the

TABLE 1
Characteristics of Crude RBO and Commercial MG Used for Esterification[a]

Characteristics	Samples used					
	RBO-I	RBO-II	RBO-III	RBO-IV	RBO-V	MG
Color (Lovibond, 1 in cell)	38Y + 5.2R	30Y + 5.7R	32Y + 4.2R	35Y + 5.1R	40Y + 5.1R + 1.1B	—
FFA	9.5	12.5	17.6	21.0	35.0	—
PV	27.3	18.4	20.1	26.7	23.5	—
Unsaponifiable matter	4.7	4.2	4.4	4.0	4.0	—
Total gum and wax	3.8	3.7	3.8	3.5	3.4	—
Neutral glyceride composition						
TG	80.1	75.2	68.6	63.0	48.2	0.8
DG	3.4	5.3	7.2	8.4	8.9	9.3
MG	2.3	2.8	3.2	3.6	3.9	89.9

[a]Values for FFA, total gum and wax, and neutral glyceride composition are expressed as % w/w. PV is expressed as meq/kg.
Abbreviations: FFA, free fatty acid; PV, peroxide value; RBO, rice bran oil; TG, triglyceride; DG, diglyceride; MG, monoglyceride; Y, yellow; R, red; B, blue; —, not detected.

TABLE 2
Effect of Temperature on the Esterification of RBO-I FFA by MG[a]

Molar ratio of FFA/MG	Temperature (°C)	FFA (% w/w) at different reaction times (h)				
		0	2	4	6	8
	170		7.31	6.63	5.10	4.62
2.5:1	190		5.26	4.32	3.29	2.15
	210		4.31	2.69	2.47	2.00
2:1	190	9.5	7.52	5.23	4.46	3.27
	210		5.90	4.36	3.20	1.74
2:1.25	210		3.85	2.36	1.06	0.42

[a]All reactions were carried out 10 mm Hg. For abbreviations see Table 1.

esterification reaction. The fatty acid composition (% w/w) of the MG sample was 1.4 $C_{16:0}$, 47.5 $C_{18:0}$, and 51.1 $C_{18:1}$. The average molecular weight of the fatty acids of the commercial MG sample (and therefore that of MG molecules) was calculated from the fatty acid composition of MG. The fatty acid composition shows that the MG was derived from an oil other than the RBO and this MG was used in the present study. The amount of MG used was not expected to affect the fatty acid composition of RBO to any significant extent as gleaned from the fatty acid profile of RBO (19).

The effect of temperature and MG concentration in the reaction mixture on the progress of neutralization is shown in Table 2. The results show that the use of an operating temperature below 200°C does not reduce the FFA content to a sufficiently low level. However, at the reaction temperature of 210°C, esterification proceeds satisfactorily, as is evident from the reduction of FFA content. It also appears that the rate of esterification increases (as expected) if a higher operating temperature (210°C) is used. If the FFA/ MG ratio is varied from 2.5:1 to 2:1.25 (molar basis) at 210°C and 10 mm Hg, it is found that the use of MG over its stoichiometric ratio gives better results in terms of the reduction of FFA. However, use of excess MG results in a product having more MG than when either a stoichiometric or lower quantity of MG is used. The higher residual MG content in the product when excess MG is used suggests that the use of a stoichiometric amount of MG is desired to control the amount of MG in the esterified product.

In Table 3, the characteristics of completely refined RBO, achieved by esterification using MG, followed by either alkali refining, bleaching and deodorization, or bleaching and physical refining, are shown. The reactions have been carried out in triplicate and the results have been expressed as mean ± SE. The results depict that acceptable quality RBO may be obtained following the aforementioned combined processes for complete refining. The FFA in the final product ranged from 0.10 ± 0.04 to 0.20 ± 0.03% w/w. However, the content of MG and DG in the completely refined oils is high to a certain extent. The increased DG level may be due to the fact that DG have not reacted sufficiently with FFA to form TG in view of its relatively lower reactivity compared to MG. The MG remained in the refined oils almost in the same proportion as the crude oils before refining.

One RBO sample (RBO-II) was also refined conventionally following single-stage degumming, dewaxing, caustic refining, bleaching, and deodorization. The glyceride composition (Table 3) indicates that this sample contained much more TG (90.1 ± 1.4% w/w) than the refined sample (80.7 ± 2.1% w/w) obtained by esterification.

Since the glyceride compositions of the refined RBO samples were broadly similar, the smoke point of one esterified and completely refined sample (RBO-III) was checked to de-

TABLE 3
Characteristics of Completely Refined RBO Through Esterification Using MG[a]

Oil sample used	Characteristics of refined RBO								
	FFA[b] (% w/w)	Color[c] (Lovibond 1 in cell)	FFA[c] (% w/w)	PV[c] (meq/kg)	UM[c] (% w/w)	MG[c] (% w/w)	DG[c] (% w/w)	TG[c] (% w/w)	Smoke point (°C)
RBO-I[d]	0.5 ± 0.10	(16 ± 1)Y, (1.8 ± 0.2)R	0.16 ± 0.03	2.1 ± 0.2	2.3 ± 0.1	2.8 ± 0.2	10.1 ± 0.5	84.6 ± 1.3	—
RBO-II[d]	0.6 ± 0.09	(16 ± 1.3)Y, (2.0 ± 0.3)R	0.10 ± 0.04	1.9 ± 0.2	2.2 ± 0.2	3.0 ± 0.1	14.0 ± 0.4	80.7 ± 2.1	—
RBO-III[d]	0.9 ± 0.12	(12 ± 1.2)Y, (1.8 ± 0.2)R	0.18 ± 0.06	1.9 ± 0.3	2.4 ± 0.3	3.5 ± 0.2	15.6 ± 0.5	78.3 ± 1.9	250
RBO-IV[e]	2.9 ± 0.22	(18 ± 2.3)Y, (2.3 ± 0.3)R	0.19 ± 0.04	1.7 ± 0.2	2.4 ± 0.1	2.9 ± 0.1	15.0 ± 0.5	79.5 ± 2.0	—
RBO-V[e]	3.5 ± 0.19	(20 ± 2.1)Y, (2.6 ± 0.5)R	0.20 ± 0.03	2.3 ± 0.4	2.6 ± 0.1	3.2 ± 0.3	15.8 ± 0.4	78.1 ± 1.9	—
RBO-II[f]	—	—	0.18 ± 0.06	1.8 ± 0.3	1.7 ± 0.2	1.5 ± 0.2	7.2 ± 0.3	90.1 ± 1.4	258

[a]Esterification was carried out for 8 h at 210°C and at 10 mm Hg. FFA/MG ratio was 2:1 by mole for all oil samples except RBO-II.
[b]After water degumming and dewaxing, bleaching, and esterification.
[c]After physical refining or alkali refining, bleaching, and deodorization after esterification.
[d]Alkali refined, bleached, and deodorized after esterification.
[e]Physical refining after esterification.
[f]Degummed, dewaxed, alkali refined, bleached, and deodorized. UM, unsaponifiable matter; see Table 1 for other abbreviations.

termine its cooking quality. The smoke point of the MG-refined sample was found to be quite good (250°C) when compared to the oil (258°C) obtained after single-stage degumming, dewaxing, caustic refining, bleaching, and deodorization.

The content of TG in the refined oil samples compared to that in the original crude oils always increased, but the increase was not as extensive as expected from the conversion of FFA to neutral glycerides. This shows that there is relatively less TG formation in the finished oils than expected from the esterification reaction.

From a cost point of view, the present process of refining high-FFA content RBO appears to be competitive with miscella refining with a single or mixed solvent, biorefining, or alkali refining procedures. However, taking into consideration the current cost of high-acid crude RBO, commercial pure MG, the quantity of refined oil obtained, the average utility expenditure, and the price of refined oils, the process appears to be a promising one which is not competitive with physical refining processes.

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