Biorefining of High Acid Rice Bran Oil

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Rice bran oil with a high free fatty acid content (FFA) after degumming and dewaxing can be converted into edible quality oil of satisfactory refining characteristics by first adopting the biorefining process to reduce the major portion of the FFA by converting them into neutral glycerides with the aid of 1,3-specific lipase under optimum conditions and later deacidifying the residual FFA of the biorefined oil by alkali neutralization.

Rice bran oil, as obtained in commerce, varies in its free fatty acid content depending on the quality of bran from which the oil is extracted. Generally, the free fatty acid level varies from about 2 to 5 per cent but oils having high free fatty acid content—from about 15 per cent to as high as 40 per cent—are also commercially produced. The refining of high FFA rice bran oil has been accomplished by miscella refining (1) with single solvent-like hexane or with double solvent consisting of hexane and alcohol (both ethanol [2] and isopropanol [3]), by physical refining (4) and by their combination (4). Efforts have also been made to deacidify high FFA rice bran oil by reesterification using a chemical catalyst (5). The refining of high FFA rice bran oil by isopropanol extraction and alkali neutralization has also been reported (6).

The unique properties of some microbial lipases to synthesize triglyceride from a fatty acid and glycerol can be conceived for utilization to develop an alternative process for deacidifying a vegetable oil. The process, in particular, may be useful in the deacidification of high FFA vegetable oils. The present study makes an effort to investigate the potential of the enzymatic deacidification process for refining high FFA rice bran oil by examination of the enzymatic esterification reaction variables like enzyme concentration, reaction temperature and reaction time, glycerol concentration and amount of moisture in the reaction mixture.

This study involves the determination of free fatty acid with the progress of esterification reaction under different conditions. The efficacy of the method has been tested in terms of the refining factor when ultimately the alkali refining has been combined to remove residual FFA.

MATERIALS AND METHODS

Rice bran oil was supplied by K. N. Oil Industries, Raipur, Madhya Pradesh. The oil was degummed (7) by 0.1 per cent phosphoric acid of 85 per cent strength as 10 per cent aqueous solution at 60°C for 30 minutes and the degummed oil was then dewaxed by treatment with 0.2 per cent $CaCl_2$ in the form of 10 per cent aqueous solution at 15°C for 4 hr (8).

The degummed and dewaxed rice bran oil was enzymatically deacidified by *Mucor miehei* lipase (Lipozyme TM) supplied by Novo Industry, Denmark.

Refining was conducted with a different percentage of the enzyme at different temperatures and pressures with or without adding glycerol and with or without adding water. The reaction was also carried out at first at atmospheric pressure and later under vacuum. The amount of glycerol added was stoichiometric and a 30 per cent excess of the stoichiometric amount. The FFA percentage was examined (9) at different intervals of the reaction by recovering the oil from enzyme by extraction with normal hexane and desolventizing the oil.

The biorefined oil from the optimum conditions was further refined by adding a theoretical quantity of dilute 12° Be′ caustic soda solution at ca. 60°C.

The degummed, dewaxed rice bran oil and biorefined oils were analyzed for FFA (9), unsaponifiable matter (10) and color (11). The percentage of monoglcerides, diglycerides and triglycerides was determined by gas liquid chromatography (12).

RESULTS AND DISCUSSION

The results obtained indicated that the extent of deacidification depends mainly on the amount of glycerol, enzyme, water and also on temperature and pressure employed during the deacidification reactions (Tables 1-5). The data in Table 1 shows that by using stoichiometric amounts of glycerol, the FFA level decreases significantly compared to not using glycerol during the reesterification reaction. The use of excess amounts of glycerol over the theoretical amount does not show any improvement in the rate and degree of deacidification.

The amount of enzyme used in the reaction (Table 2) appears to be satisfactory at the 10 per cent level as can be seen in terms of the reduction in FFA level when compared with the amount of enzyme used at 5 per cent and 15 per cent levels. However, a drop in FFA level by about 80 per cent by using 5 per cent enzyme by weight in about 10 hr can be achieved. By increasing the enzyme to 10 per cent, the rate of deacidification improves by another 8 per cent. A further increase in the enzyme amount does not show any improvement in the rate and degree of deacidification.

It is important to maintain water at 10 per cent of the weight of enzyme in order to achieve a better deacidification rate (Table 3).

Among the variables, the pressure maintained during the reaction appears to greatly influence the rate of the deacidification reaction (Table 4). It is necessary that the reaction is conducted at low pressure at 10 mm Hg. The rate of FFA drop is enhanced when the reaction is conducted at 30 mm Hg instead of at atmospheric pressure. The rate is further enhanced when the reaction is conducted at 10 mm Hg. However, the difference between the working pressures of 30 mm and 10 mm Hg is no doubt significant but not as significant as it is when the reaction is conducted at atmospheric pressure.

As expected, the temperature during the reaction has an influence on the rate of deacidification (Table 5). Thus at 50°C the FFA is lowered to about 8 per cent from 30 per cent in 10 hr. While at 70°C, the FFA drops to 3.5 per cent. When the reaction is conducted at 80°C, the

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drop in the FFA is less due to the expected enzyme deactivation at that temperature.

Under the optimum conditions of esterification, the deacidified oil still contains 3.6 per cent FFA, 1.3 per cent monoglycerides (MG), 12.8 per cent diglycerides (DG) and 80.3 per cent triglycerides (TG) (Table 6). The original crude oil contained 30 per cent FFA, 4 percent unsaponifiable matter and nearly the same amount of MG and DG but only about 53 per cent TG, which is significantly less than the biorefined sample. This observation that the FFA has been converted into mostly TG is highly

TABLE 1

Effect of Glycerol on the Extent of Deacidification of Degummed and Dewaxed Rice Bran Oil in Biorefining Process

Reaction condition:
i) Enzyme used—10% on the wt. of oil
ii) Pressure—10 mm Hg
iii) Temperature—70°C
iv) Water-10%

Glycerol used	Reaction time in hr	% FFA in oil	Lovibond color (1 cm cell) Y	R
Without glycerol	1	28.8	—	—
	2	25.4	—	—
	5	18.0	—	—
	7	18.7	—	—
	10	19.8	14.2	2.8
Theoretical amount	1	10.8	—	—
	2	8.5	—	—
	5	4.7	—	—
	7	3.6	—	—
	10	3.6	9.0	2.2
30% excess on theoretical	1	10.7	—	—
	2	8.1	—	—
	5	4.1	—	—
	7	3.8	—	—
	10	3.6	12.0	2.0

TABLE 2

Effect of Enzyme on the Extent of Deacidification of Degummed and Dewaxed Rice Bran Oil

Condition:
i) Glycerol—theoretical amount
ii) Temperature—70°C
iii) Pressure—10 mm Hg
iv) Water—10% on the wt of enzyme

Amount of enzyme used	Reaction time in hr	% FFA in oil	Lovibond color (1 cm cell) Y	R
5%	1	12.6	—	—
	2	9.2	—	—
	5	6.4	—	—
	7	5.8	—	—
	10	5.4	12.2	2.6
10%	1	10.8	—	—
	2	8.5	—	—
	5	4.7	—	—
	7	3.6	—	—
	10	3.5	9.0	2.2
15%	1	10.7	—	—
	2	8.2	—	—
	5	4.8	—	—
	7	3.8	—	—
	10	3.6	10.0	2.2

TABLE 3

Effect of Water on the Extent of Deacidification of Degummed and Dewaxed Rice Bran Oil

Condition:
i) Glycerol—theoretical amount
ii) Enzyme—10% on the wt of oil
iii) Pressure—10 mm Hg
iv) Temperature—70°C

Water used	Reaction time in hr	% FFA in oil	Lovibond color (1 cm cell) Y	R
Without water	1	12.0	—	—
	2	11.6	—	—
	5	6.9	—	—
	7	4.5	—	—
	10	4.6	12.0	2.4
10% on the wt of enzyme	1	10.8	—	—
	2	8.5	—	—
	5	4.7	—	—
	7	3.6	—	—
	10	3.5	9.0	2.2

TABLE 4

Effect of Pressure on the Extent of Deacidification of Degummed and Dewaxed Rice Bran Oil

Condition:
i) Glycerol—theoretical amount
ii) Enzyme—10% on the wt of oil
iii) Temperature—70°C
iv) Water—10% on the wt of enzyme

Pressure	Reaction time in hr	% FFA in oil	Lovibond color (1 cm cell) Y	R
Atmospheric	1	18.9	—	—
	2	15.6	—	—
	5	10.7	—	—
	7	8.4	—	—
	10	8.2	12.8	2.5
30 mm Hg	1	15.1	—	—
	2	10.8	—	—
	5	6.2	—	—
	7	5.2	—	—
	10	4.7	10.8	2.2
10 mm Hg	1	10.8	—	—
	2	8.5	—	—
	5	4.7	—	—
	7	3.6	—	—
	10	3.5	9.0	2.2

TABLE 5

Effect of Temperature on the Extent of Deacidification of Degummed and Dewaxed Rice Bran Oil in Biorefining Process

Condition:
i) Glycerol—theoretical amount
ii) Enzyme—10% on the wt of oil
iii) Pressure—10 mm Hg
iv) Water—10% on the wt of enzyme

Temperature in C	Reaction time in hr	% FFA in oil	Lovibond color (1 cm cell) Y	R
50	1	14.7	—	—
	2	12.0	—	—
	5	8.8	—	—
	7	8.2	—	—
	10	7.9	13.4	2.5
70	1	10.8	—	—
	2	8.5	—	—
	5	4.7	—	—
	7	3.6	—	—
	10	3.5	9.0	2.2
80	1	15.1	—	—
	2	14.3	—	—
	5	8.8	—	—
	7	6.9	—	—
	10	5.0	10.0	2.3

TABLE 6

Characteristics of Crude and Refined Rice Bran Oil Samples

	FFA %	Unsap. %	MG %	DG %	TG %	Lovibond color (1 cm cell) Y	R
Crude rice bran oil	30.0	4.0	1.2	12.5	53.3	27.0	4.0
Biorefined oil	3.6	2.0	1.3	12.8	80.3	9.0	2.2
Biorefined and alkali refined oil[a]	0.1	2.0	1.0	12.5	84.0	5.0	0.6

[a]Refining factor of the oil is 1.2.

encouraging. The reaction has occurred either between the glycerol used and the FFA or between the DG and the FFA. Quite likely both of the two esterification reactions have taken place simultaneously because the DG content in the crude and the biorefined sample is nearly the same and the TG content in the refined sample is significantly increased.

The combined biorefining and alkali refining process compares well in terms of refining factor and color with the miscella refining process regarding hexane (1) and a hexane-alcohol mixture (2) as shown previously in our laboratory and is by far superior to the combined physical refining and alkali neutralization process investigated by the authors in respect to the refining characteristics. The refining factor includes the total per cent loss of oil from the stages of biorefining and alkali refining divided by the FFA of the crude oil.

The overall results obtained in the present study and the fact that the energy required is much lower compared to other processes can suggest that high FFA rice bran oil can be refined with a high degree of economy by a combination of enzymatic deacidification and alkali neutralization.

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ERRATUM

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