A Comparative Study Between Biorefining Combined with Other Processes and Physical Refining of High-Acid Mohua Oil

R. Sengupta and **D.K. Bhattacharyya***
Department of Chemical Technology, University Colleges of Science and Technology, Calcutta - 700 009, India

The biorefining process under optimum conditions deacidified the high-acid mohua oil by nearly 85% with considerable improvement of color. The process, in combination with alkali-refining, bleaching and deodorization, yielded excellent oil with respect to color, unsaponifiable matter content and triglyceride content. The combination of biorefining and physical refining significantly reduced the loss of oil, and the color, unsaponifiable matter and diglyceride content increased while triglyceride content decreased. The physical refining process alone, on the other hand, produced oil with considerably darker color, increased unsaponifiable matter and diglycerides, and decreased triglyceride. Biorefining followed by alkali-refining, bleaching and deodorizing steps or by physical refining can be regarded as a much better alternative refining process than the physical refining process alone for oils of high acidity.

KEY WORDS: Biorefining, free fatty acid, *Mucor miehei* lipase, physical refining.

Biorefining (1,2), which primarily involves deacidification of vegetable oils by the lipase-catalyzed esterification reaction, has been receiving considerable interest as an oil pretreatment step in the conventional chemical neutralization process or in the physical refining process for vegetable oils with high free fatty acid (FFA) contents. Chemical neutralization, such as the conventional alkali process, is unsuitable for a high-FFA oil, but such an oil can be satisfactorily deacidified by physical refining (3). This process, however, requires high temperature and high vacuum and often forms side reaction products, such as polymers and *trans* isomers (Rossell, J.B., S.P. Kochar and I.M. Jawad, private communication). In view of the need for low-energy processes, microbial lipase-catalyzed esterification (1,2) appears to be promising for deacidification in lieu of chemical esterification (4), which is invariably carried out at higher temperatures (180°-200°C) than lipase-catalyzed reactions. The microbial lipase process also is promising in terms of oil quality and refining loss.

The present investigation has been undertaken on the deacidification of high-FFA mohua oil *(Madhuca latifolia)* with the aid of a microbial lipase by varying the amount of lipase, reaction temperature and time under varying pressures and with the theoretical amount or more glycerol. The extent of FFA reduction, formation of neutral glycerides, the color change, unsaponifiable matter content and the ultimate total loss of oil when combined with either alkali neutralization or physical refining have been investigated in comparison with physical refining only.

*To whom correspondence should be addressed at Dept. of Chemical Technology, University Colleges of Science & Technology, 92, A.P.C. Road, Calcutta - 700 009, India.

EXPERIMENTAL PROCEDURES

Deacidification of oils using lipase enzyme. A high-FFA mohua oil (25.1% FFA) was supplied by M/S Asianol Lubricants (Calcutta, India). The lipase used was immobilized 1,3-specific *Mucor miehei* lipase (Lipozyme IM 20) supplied by Novo Nordisk (Bagsveard, Denmark).

Mohua oil was first degummed at 60°C with 0.1% of 85% phosphoric acid and was then bleached with 2.0% tonsil earth under vacuum at about 90°C. The oil recovered by filtration was then stirred in a reactor with lipase at various temperatures, pressures, amounts of lipase and glycerol to get the optimum conditions for biorefining. FFA was examined periodically by the standard method (5), and the reaction was stopped at the equilibrium stage. The biorefining process was combined with conventional alkali-refining, bleaching and deodorization. This process was also followed by physical refining to remove residual FFA.

Alkali-refining was done at 60°C with 20% excess of the theoretically calculated amount of alkali added as 20° Be′ caustic soda solution by stirring, followed by removal of soap stock by centrifuge. The neutral oil was washed with hot water and centrifuged. The oil was bleached again. The deodorization was done at 2 mm Hg pressure at 185° ± 5°C for 2 h (6). For physical refining, the degummed and bleached mohua oil was steam-stripped at 2 mm Hg pressure at 240°C for 2 h.

The crude mohua oil, biorefined mohua oil and other refined oils were analyzed for FFA, unsaponifiable matter (7), color by Lovibond Tintometer (ref. 8) and the percentage of monoglycerides, diglycerides and triglycerides by gas-liquid chromatography (ref. 9).

RESULTS AND DISCUSSION

From the results shown in Tables 1–6, it is noted that mohua oil can be biorefined. FFA can be reduced from 24.5% to a level of 3.8% when the degummed and bleached oil is treated continuously with 10% lipase and the stoichiometric amount of glycerol for 20 h at 60°C and 2 mm of Hg. It is interesting that in the absence of glycerol the FFA level of the oil becomes higher than in the original oil (Table 4). The use of excess glycerol over the theoretical amount results in more complete lowering of the FFA, and the color is improved also. However, the use of excess glycerol has not been examined at the optimized conditions, presumably to keep diglyceride content low in the deacidified product, even though a further decrease in FFA can be achieved.

The comparison of the refining characteristics of mohua oil by the different processes is shown in Table 6. The combination of biorefining with physical refining yielded a relatively higher-colored oil, although the oil loss was minimal. In comparison, when followed by alkali refining, bleaching and deodorization, the biorefined oil with 3.8% FFA yielded oil of excellent quality in terms of unsaponifiable matter, color and even flavor quality (bland

TABLE 1

Effect of Temperature on the Deacidification of Mohua Oil by *Mucor miehei* Lipase (10% on oil, w/w) at 2 mm Hg with a Stoichiometric Amount of Glycerol

Temperature (°C)	Reaction time (h)	% Free fatty acid in oil		Lovibond color 1/4-inch cell	
		Initial (degummed and bleached)	After reaction	Y	R
50	1	24.5	21.8	—	—
	3		16.6	—	—
	5		14.5	—	—
	9		12.8	2.5	0.3
60	1	24.5	18.4	—	—
	3		12.0	—	—
	5		9.8	—	—
	9		7.4	2.0	0.1
70	1	24.5	20.9	—	—
	3		13.3	—	—
	5		11.5	—	—
	9		9.3	2.0	0.1

TABLE 2

Effect of Pressure on the Deacidification of Mohua Oil by *Mucor miehei* Lipase (10% on oil, w/w) at 60°C with a Stoichiometric Amount of Glycerol

Pressure (mm Hg)	Reaction time (h)	% Free fatty acid in oil		Lovibond color 1/4-inch cell	
		Initial (degummed and bleached)	After reaction	Y	R
2	1	24.5	18.4	—	—
	3		12.0	—	—
	5		9.8	—	—
	9		7.4	2.0	0.1
10	1	24.5	21.5	—	—
	3		13.4	—	—
	5		11.7	—	—
	9		10.5	2.3	0.3

TABLE 3

Effect of Lipase Amount on the Deacidification of Mohua Oil at 60°C and 2 mm Hg with a Stoichiometric Amount of Glycerol

Lipase amount (% w/w on oil)	Reaction time (h)	% Free fatty acid in oil		Lovibond color 1/4-inch cell	
		Initial (degummed and bleached)	After reaction	Y	R
15	1	24.5	20.0	—	—
	3		11.1	—	—
	5		10.2	—	—
	9		8.5	2.0	0.1
10	1	24.5	18.4	—	—
	3		12.0	—	—
	5		9.8	—	—
	9		7.4	2.0	0.1
5	1	24.5	21.7	—	—
	3		12.8	—	—
	5		11.8	—	—
	9		10.2	2.3	0.3

TABLE 4

Effect of Amount of Glycerol on the Deacidification of Mohua Oil by *Mucor miehei* Lipase (10% on oil w/w) at 60°C and 2 mm Hg

Glycerol amount added	Reaction time (h)	% Free fatty acid (FFA) in oil		Lovibond color 1/4-inch cell	
		Initial (degummed and bleached)	After reaction	Y	R
Nil	1	24.5	30.7	—	—
	3		29.8	—	—
	5		29.2	—	—
	9		29.5	2.3	0.1
Stoichiometric amount (on FFA)	1	24.5	18.4	—	—
	3		12.0	—	—
	5		9.8	—	—
	9		7.4	2.0	0.1
30% Excess	1	24.5	17.2	—	—
	3		10.4	—	—
	5		7.2	—	—
	9		4.9	2.0	0.1

TABLE 5

Comparative Study of Biorefining Between Crude and Degummed/Bleached Mohua Oil[a]

Oil	% FFA in oil before and after biorefining		Reaction time (h)	Lovibond color (1/4-inch cell)		Unsaponifiable matter (%)
	Before	After		Initial	Final	
Crude	25.1	6.1	20	5Y + 0.8R	3.6Y + 0.6R	3.2
Degummed	24.5	3.8	20	2.5Y + 0.3R	2Y + 0.1R	2.1

[a]Reaction conditions: Temperature, 60°C; pressure, 2 mm Hg; enzyme, 10% on the oil (w/w); and glycerol, stoichiometric amount. FFA, free fatty acid.

TABLE 6

Comparative Refining Characteristics of Mohua Oil

	Crude oil	Biorefined oil	Biorefined, alkali-refined, bleached and deodorized oil	Biorefined, bleached and physical refined oil	Physical refined oil
Lovibond color (1/4-inch cell)	5Y + 0.8R	2Y + 0.1R	0.6Y + 0.0R	2.8Y + 0.8R	6.8Y + 1.0R
Free fatty acid (FFA) (%)	25.1	3.8	0.2	0.3	0.3
Refining factor (on FFA removal basis)	—	—	2.2	1.2	1.3
Unsaponifiable matter (%)	3.2	2.0	1.0	2.2	2.6
Triglyceride (%)	65.6	84.2	98.3	94.7	92.3
Diglyceride (%)	6.1	9.9	0.48	2.8	4.8
Monoglyceride (%)	Nil	Nil	Nil	Nil	Nil
Total process loss (%)	—	—	11.4	7.5	31.8

odor), although the oil loss was higher. The physical refining process yielded almost completely deacidified and deodorized oil, but with the highest overall process loss. The color of the oil was darker, and the unsaponifiable matter content was much higher.

Considering process losses and other refining characteristics of the oil, the biorefining process in combination with either subsequent alkali neutralization, bleaching and deodorization or with physical refining is a promising potential technology for purifying high-acid mohua oil and other fats and oils.

ACKNOWLEDGMENTS

The authors thank the Council of Scientific and Industrial Research for providing funds and Novo Nordisk (Bagsveard, Denmark) for the enzyme Lipozyme IM 20.

REFERENCES

1. Bhattacharyya, S., D.K. Bhattacharyya, A.R. Chakrabarty and R. Sengupta, *Fat Sci. Technol. 91*:27 (1989).
2. Bhattacharyya, S., and D.K. Bhattacharyya, *J. Am. Oil Chem. Soc. 66*:1469 (1989).
3. Rao, V.V., *Handbook on Rice Bran Processing and Utilisation of Products*, The Solvent Extractors' Association of India, Bombay, India, 1987, pp. 591–597.
4. Bhattacharyya, A.C., and D.K. Bhattacharyya, *J. Am. Oil Chem. Soc. 64*:128 (1987).
5. *The Official Methods and Recommended Practices of the American Oil Chemists' Society*, 3rd edn., edited by R.O. Walker, The American Oil Chemists' Society, Champaign, 1974, Method Ta-38.
6. Anderson, A.J.C., in *Refining of Oils and Fats for Edible Purposes*, 2nd edn., edited by P.N. Williams, Pergamon Press, London, 1962, p. 180.
7. *The Official Methods and Recommended Practices of the American Oil Chemists' Society*, 3rd edn., edited by R.O. Walker, The American Oil Chemists' Society, Champaign, 1974, Method 6a-40.
8. Thomson, P., *J. Am. Oil Chem. Soc. 30*:259 (1953).
9. Litchfield, C., R.D. Harlow and R. Reiser, *Ibid. 42*:849 (1965).

[Received April 3, 1992; accepted August 10, 1992]