Enzymatic Synthesis of Fatty Alcohol Esters by Alcoholysis

B.K. De, D.K. Bhattacharyya*, and C. Bandhu

Department of Chemical Technology, Oil Technology Division, University of Calcutta, Calcutta 700 009, W.B. India

ABSTRACT: Lipase-catalyzed conversions of some minor oils and fats like mowrah *(Madhuca latifolia)*, mango *(Mangifera indica)* kernel, and sal *(Shorea robusta)* fats into low, medium, and high molecular weight alcohol esters have been investigated. In solvent-free medium, alcoholysis of the above-mentioned fats with 10% (w/w) *Mucor miehei* lipase produced alcohol esters in good yield. The percentage molar conversions of C_4, C_8, C_{10}, C_{12}, C_{14}, C_{16}, C_{18}, and $C_{18:1}$ alcohols into corresponding alcohol esters ranged from 86.8 to 99.2, while the percentage molar conversions on the basis of oil were in the range of 108.0 to 123.5.

Paper no. J8770 in *JAOCS 76,* 451–453 (April 1999).

KEY WORDS: Alcohol esters, fatty alcohol, mango kernel fat, mowrah fat, *Mucor miehei,* sal fat.

Long-, medium-, and short-chain alcohol esters of fatty acids find specific applications in the food, pharmaceutical, and chemical industries. Long-chain alcohol esters are a natural substitute for waxes and lubricants. Depending upon the consistency (from liquid to wax), the product may be used in the textile industry also (1). Esters of short-chain fatty acids are important as flavor and aroma constituents in the food industry (2). Methyl and ethyl esters of long-chain fatty acids are useful as valuable oleochemical species and have gained importance as biodiesel fuels (3).

Alcoholysis is the exchange of an alcohol moiety of an ester molecule by another alcohol. This can be used as a tool for converting cheap fatty raw materials to fatty acid esters of fatty alcohols by an enzyme-catalyzed process.

The synthesis of esters of long-chain fatty acids by means of chemical alcoholysis (4–6) of oils and fats and by the esterification of fatty acids with alcohols is well established. Bryant *et al.* (4) have described the acid-catalyzed alcoholysis of *Vernonia galamensis* oil with methanol, ethanol, 1-propanol, 2-propanol, and 1-butanol. Kildiran *et al.* (5) have proposed *in-situ* alcoholysis of soybean oil, and Ozgul and Turkay (6) have described *in-situ* esterification of rice bran oil with different monohydric alcohols. They have exploited the advantage of simultaneous easy extraction of neutral lipids from the seed and bran when *in-situ* alcoholysis is carried out. However, Ghosh and Bhattacharyya (7) have taken advantage of the lipase-catalyzed alcoholysis process for better utilization of acid oils of commerce to produce fatty esters.

The main current use of sal, mowrah, and mango kernel fats is in the manufacture of cocoa butter substitutes (8). To divert the utilization pattern of these above-mentioned fats and many others having similar characteristics, the present study aims at investigating the possibility of synthesizing fatty alcohol esters.

MATERIALS AND METHODS

Substrate and enzyme. Sal and mango kernel fats were supplied by K.N. Oil Industries Ltd. (Raipur, M.P., India). Mowrah fat was obtained from Kanchan Oil Mills (Jhargram, W.B., India). All the fatty alcohols (C_4–$C_{18:1}$) used for the alcoholysis were purchased from E. Merck (Darmstadt, Germany). Silicic acid, used for the column chromatogra- phic separation of product esters, was purchased from LOBA Chemicals (Bombay, India). All the chemicals used were of reagent grade.

All the reactions under investigation were catalyzed by Lipozyme IM-20 (immobilized lipase), a 1,3-specific lipase derived from *Mucor miehei*. Lipozyme IM-20 was a gift of Novo A/S (Copenhagen, Denmark).

Alcoholysis. Approximately 2.1 g (2.43 mmol) of accurately weighed fat and the alcohol under reaction (20% excess over the stoichiometric ratio of oil/alcohol = 1:2 for 1,3-specific lipase) were placed in a 25 mL conical flask. This reactant mixture was stirred with a 3/4-in. Teflon-coated magnetic stir bar at 60 ± 2°C, using 10% w/w Lipozyme IM-20. The lipase contained 10% w/w water (as claimed by Novo A/S). The reaction was carried out in a stoppered flask for 6 h.

In a preliminary experiment, the sample was drawn at 1 h intervals and the conversion of oil into ester was monitored by thin-layer chromatographic (TLC) analysis of the product mixtures. From TLC analysis the reaction was found to be completed at 6 h.

TLC. The reactant mixture, along with the samples drawn at each 1 h interval and a standard triglyceride (TG), from Ranbaxy Laboratories (New Delhi, India), was spotted on TLC plates coated with a 0.2 mm thick layer of silica gel G. The plate was developed with a solvent system of hexane/diethyl ether (90:10, vol/vol). After development of the plate,

---

*To whom correspondence should be addressed at Department of Chemical Technology, University of Calcutta, 92, A.P.C. Road, Calcutta 700 009, W.B. India. E-mail: dkb@cucc.ernet.in the separated spots were visualized by strong iodine absorption. Completion of the reaction was indicated by the disappearance of the TG spot with simultaneous appearance of a strong ester spot (with nearly same R_f as that of standard methyl stearate) in the product mixture drawn after 6 h of reaction. A reaction period of 6 h was therefore decided upon for completion of reaction.

Determination of fatty acid composition. Methyl esters of the fatty acids were made by shaking one drop of oil with 1 mL of N/2 methanolic KOH solution for 10 min followed by its acidification and extraction with *n*-hexane (9).

Fatty acid composition of the mowrah, mango kernel, and sal fat were determined by gas–liquid chromatography of the methyl esters. The gas chromatograph was a Hewlett-Packard 5890 model equipped with a flame-ionization detector. The oven, injection port, and detector block temperatures were maintained at 190, 230, and 240°C, respectively, and no oven temperature programming was needed. Nitrogen was carrier gas at a rate of 30 mL/min.

Estimation of alcohol ester and TG content. To determine the ester and TG fractions, 225 to 250 mg (accurately weighed) of completely neutralized fat was subjected to column chromatographic separation (11). A glass column (1.5 cm internal diameter) was packed with 8.5 g silicic acid (120–130 mesh). The product mixture was then added and the ester and TG fractions were separated by eluting with 110 mL hexane/diethyl ether (99:1, vol/vol) and then 75 mL hexane/diethyl ether (95:5, vol/vol), respectively. The eluted volume of each solvent system was collected in a tared flask. The major volume of solvent was distilled off first and later removed completely under vacuum (10 mm Hg) at 90°C. The quantity of each fraction was then determined gravimetrically, and the purity of each fraction was confirmed by TLC on silica gel plate (developed by hexane/diethyl ether 90:10, vol/vol). The alcohol-ester spot was identified by comparing with standard oleyl-oleate (S.D. Fine Chem., Mumbai, India).

Content of unsaponifiable matter and the slip point of the fats were determined by following the standard AOCS methods (10).

RESULTS AND DISCUSSION

Fatty raw materials for the alcoholysis reactions, such as mowrah, mango kernel, and sal fats, are characterized by high TG content (96.3 to 97.5% w/w), and the component fatty acids are rich in saturated fatty acids ($C_{16:0}$, $C_{18:0}$, and $C_{20:0}$). The slip melting points range from 30.9 to 35.1°C and content of unsaponifiables ranges from 1.2 to 2.3% (w/w), depending upon the fat used. These analytical characteristics clearly indicate the authenticity of the samples (8).

In Table 1 the percentage conversion into ester (molar basis) from mowrah, mango kernel, and sal fats is presented. The results indicate that high conversion of esters of C_4, C_8, C_{10}, C_{12}, C_{14}, C_{16}, C_{18}, and $C_{18:1}$ alcohols have been achieved from all three fats. One explanation for higher percentage conversions (108.0 to 123.5) into alcohol ester may be by acyl migration of fatty acids from the 2-position to the 1- and 3-positions in a batch reaction, followed by its alcoholysis to form alcohol ester. The ability of lipozyme to act on secondary glyceride esters may also account for the higher yield.

The percentage molar conversion of alcohols into their alcohol esters, 86.8 to 99.2 (as shown in Table 1), was also found to be satisfactorily high with respect to the theoretically expected 100% (molar basis) conversion, considering one mole alcohol is converted into one mole ester.

TABLE 1
Conversion (% molar basis) of Oils and Alcohols into Alcohol Esters

	Percent conversion[a,b] (molar basis)							
Oils used	C_4	C_8	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	$C_{18:1}$
Mowrah								
a	122.2	118.9	122.4	116.3	118.1	121.2	108.0	117.1
b	(98.3)	(95.6)	(98.3)	(93.4)	(97.3)	(92.7)	(86.8)	(94.1)
Mango kernel								
a	112.3	123.5	114.8	121.2	123.0	116.7	111.7	116.9
b	(90.3)	(99.2)	(92.2)	(97.3)	(98.8)	(93.7)	(89.8)	(92.2)
Sal								
a	121.2	116.3	121.4	111.1	119.3	115.6	114.8	117.9
b	(92.7)	(93.4)	(97.6)	(89.3)	(95.9)	(92.9)	(92.2)	(94.7)

[a] Percent conversion into ester (on oil) =

$$\frac{\text{mmol of ester formed}}{\begin{array}{c}\text{theoretical maximum possible number of mmol of ester}\\ \text{from oil assuming the synthesis of two moles of ester}\\ \text{per mol of triglyceride by a 1,3 - specific lipase}\end{array}} \times 100$$

[b] Parentheses indicate percent conversion into ester on alcohol basis =

$$\frac{\text{mmol of ester formed}}{\text{theoretical maximum possible number of mmol of ester formed}} \times 100$$

TABLE 2
Slip Melting Point of the Different Alcohol Esters

	Slip melting point of alcohol esters (°C)							
Oils used	C_4	C_8	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	$C_{18:1}$
Mowrah	14.0	17.3	24.5	31.1	41.2	52.3	58.1	31.7
Mango kernel	15.6	18.5	26.0	33.3	43.1	54.4	58.0	33.8
Sal	16.2	19.3	26.8	35.1	44.6	56.0	59.8	35.5

Determination of slip melting point is worthy to characterize the wax esters and to have an idea of their consistency. Low-melting wax esters are widely used as thin film lubricating agents, pan release agents (12), and food lubricants. Slip melting point is an important parameter in choosing the optimal wax ester for these applications. Slip melting points of the esters (as depicted in Table 2) are found to increase steadily with increase in alcohol chain length (from C_4 to C_{18}), and declined with the incorporation of unsaturation for the same chain length (as from C_{18} to $C_{18:1}$).

From these results, it is evident that the microbial lipase-catalyzed process is an excellent means for the synthesis of low-, medium-, and long-chain fatty alcohol esters. This study will definitely broaden the narrow field of utilization of lesser known fats like mowrah, mango kernel, sal, and many others like these which are available in other countries.

REFERENCE

1. Meffert, A., Technical Uses of Fatty Acid Esters, *J. Am. Oil Chem. Soc. 61*:255–258 (1984).
2. Trani, M., F. Ergan, and G. Andre, Lipase-Catalyzed Production of Wax Esters, *Ibid. 68*:20–22 (1991).
3. Mittelbach, M., Lipase-Catalyzed Alcoholysis of Sunflower Oil, *Ibid. 67*:168–170 (1990).
4. Bryant, K.A.A., C.P. Nwaonicha, M.A. Anderson, and F.O. Ayorinde, Acid-Catalyzed Alcoholysis of *Vernonia galamensis* Oil, *Ibid. 69*:1023–1026 (1992).
5. Kildiran, G., S.O. Yucel, and S. Turkay, *In-situ* Alcoholysis of Soybean Oil, *Ibid. 73*:225–228 (1996).
6. Ozgul, S., and S. Turkay, *In-situ* Esterification of Rice Bran Oil with Methanol and Ethanol, *Ibid. 70*:145–147 (1993).
7. Ghosh, S., and D.K. Bhattacharyya, Utilization of Acid Oils in Making Valuable Fatty Products by Microbial Lipase Technology, *Ibid. 72*:1541–1544 (1995).
8. *Nontraditional Oil Seeds and Oils in India,* edited by N.V. Bringi, Oxford IBH Publishing Co. Pvt. Ltd., New Delhi, 1987.
9. *Analysis of Triglycerides,* edited by C. Litchfield, Academic Press, New York, 1972, p. 32.
10. *Official and Tentative Methods of the American Oil Chemists' Society,* edited by W.E. Link, American Oil Chemists' Society, Champaign, 1974, Methods Ca 6a-40 and Cc 4-25.
11. *Techniques of Lipidology,* edited by M.Kates, North Holland Publishing Company, Amsterdam, 1972, p. 402.
12. *Bailey's Industrial Oil and Fat Products,* 4th edn., edited by D. Swern, Wiley-Interscience Publication, New York, 1981, Vol. 2, p. 391.

[Received January 26, 1998; accepted December 2, 1998]