Utilization of Acid Oils in Making Valuable Fatty Products by Microbial Lipase Technology

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ABSTRACT: Microbial lipase-catalyzed hydrolysis, esterification, and alcoholysis reactions were carried out on acid oils of commerce such as coconut, soybean, mustard, sunflower, and rice bran for the purpose of making fatty acids and various monohydric alcohol esters of fatty acids of the acid oils. Neutral glycerides of the acid oils were hydrolyzed by *Candida cylindracea* lipase almost completely within 48 h. Acid oils were converted into fatty acid esters of short- and long-chain alcohols like C_4, C_8, C_{10}, C_{12}, C_{16}, and C_{18} in high yields by simultaneous esterification and alcoholysis reactions with *Mucor miehei* lipase as catalyst. Acid oils of commerce can be utilized as raw materials in making fatty acids and fatty acid esters using lipase-catalyzed methodologies.
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KEY WORDS: Acid oil, alcoholysis, *Candida cylindracea* lipase, column chromatography, *Mucor miehei* lipase, short- and long-chain alcohols.

Acid oils constitute a major by-product in the alkali refining process. Some of the major acid oils of commerce are soybean, sunflower, cottonseed, rice bran, rape-mustard, peanut, and coconut. Acid oils are generally dark colored and contain about 40–80% free fatty acids, 20–50% neutral glycerides, and unsaponifiables of the oils and other impurities. To date, acid oils of commerce have been used mostly in producing cheap-grade soaps. Considering the chemical composition, however, each acid oil has enormous potential as a raw material in producing fatty acid esters to meet the requirements of pharmaceuticals, cosmetics, and other chemical industries.

The importance of fatty acids and their various esters is well documented in the literature. It is known that short-chain fatty acid esters are important in the food industries as flavor and aroma constituents (1–3). Methyl and ethyl esters of long-chain fatty acids can be properly utilized for fatty alcohol production and for diesel fuel substitutes (4).

The production of long-chain alcohol esters of fatty acids by esterification and alcoholysis reaction with chemical catalysts is no doubt well established, but there are certain limitations to the chemical process. Unless due precautions are taken, acids of the more unsaturated types undergo polymeric or other changes during esterification. Also, fatty acids having functional groups like epoxy and hydroxy are difficult to esterify without deterioration of the functional groups. With lipase catalysis it may be possible to make esters, which are difficult to obtain by chemical methods. Another advantage of lipase technology is that lipases are active under mild reaction conditions.

In recent years, microbial lipase technology has shown enormous potential for making ester derivatives for various specific applications (5,6). But no effort has been made to utilize acid oils as cheap raw materials for the isolation of fatty acids or the synthesis of fatty acid esters of short- and long-chain fatty alcohols for various applications. The present paper endeavors to use some acid oils of commerce as sources of fatty acids and feedstocks for the synthesis of esters of fatty acids of different alcohols by microbial lipase-catalyzed hydrolysis, esterification, and alcoholysis reactions using random or regioselective lipases.

MATERIALS AND METHODS

Substrate and enzyme. Sunflower acid oil was supplied by I.T.C. (Agro-Tech.) Ltd. (Hyderabad, India). Soybean and rice bran acid oils were supplied by K.N. Oil Industries Ltd. (Raipur, M.P., India); coconut and mustard acid oils were obtained from Edible Products (I) Pvt. Ltd. (Calcutta, India). The immobilized lipase *Mucor miehei* (Lipozyme IM-20) was a gift of NOVO A/S (Copenhagen, Denmark). *Candida cylindracea* lipase was purchased from Sigma Chemical Co. (St. Louis, MO). Butanol and other fatty alcohols were purchased from E. Merck (India) Limited, Worli (Bombay, India). Unless otherwise specified, all other chemicals are reagent-grade.

Enzymatic hydrolysis. The substrate (20 g acid oil) was placed in a 100-mL glass stoppered Erlenmeyer flask. Water (60% by weight of the neutral glycerides present) containing 0.08 g (0.4% of the weight of acid oil) of *C. cylindracea* lipase powder was added. The reaction mixture was stirred with a one-inch Teflon-coated magnetic stir bar in a 35 ± 2°C controlled bath. The hydrolysis reaction was followed by estimating the free fatty acid content in samples periodically withdrawn. Each sample was taken into a centrifuge tube directly, and the emulsion was broken by heating at 80°C. The

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fatty layer containing enzyme and glycerol was separated by centrifugation. The fatty layer (hydrolyzed product) was transferred to a tared flask, dried, and the free fatty acid content in the hydrolyzed product was determined by a standard method (7).

Simultaneous enzymatic esterification and alcoholysis reaction. Acid oils (about 1.0 g accurately weighed) and different alcohols in 1:1 molar ratio were taken in a 10-mL round-bottom flask and stirred by a magnetic stirrer at 60 ± 2°C for 4 h using 10% (by weight of the acid oils) of *M. miehei* lipase. The lipase had 2% water in it to maintain its activity. After the reaction, the product mixture was filtered to remove the enzyme. The molar concentration of acid oils was calculated from their total fatty acid equivalents and average molecular weights of the fatty acids (coconut—218.4, soybean—264.1, mustard—295.5, sunflower—263.6, and rice bran—260) based on total fatty acid composition. A typical example of proportion of the reactants used in the preparation of esters is 0.9713 g (3736 μmole) rice bran acid oil and 0.5890 g (3735 μmole) C_{10}-alcohol.

Analysis of fatty acids, unsaponifiable matter, and mono-, di-, and triglycerides. The fatty acid composition of each acid oil was determined by gas chromatography (GC) of the methyl esters of the total fatty acids of each individual acid oil. The methyl esters were prepared with BF_3–methanol as methylating agent according to standard procedures (8). The fatty acid composition was then analyzed by GC following standard procedures (9). The unsaponifiable matter content of each acid oil was determined by standard methods (10). Each acid oil (about 1.0 g) was just neutralized by 0.1 (N) sodium hydroxide solution in 90% alcohol medium, and the neutral glycerides were extracted with diethyl ether, from which the mono-, di-, and triglycerides were determined by standard methods (11).

Estimation of percent yield of esters. The yield of esters in percent by weight from each acid oil could be estimated by standard column chromatographic methods (12). Silica gel (mesh size 120–130) was used, and about 170 mg of accurately weighed product mixture from each acid oil was separated. The flow rate of eluting solvent was maintained at 2.5 mL/min. An eluate volume of 100 mL hexane/diethyl ether (99:1, vol/vol) for the ester fraction, 90 mL hexane/diethyl ether (92:8, vol/vol) for the alcohol fraction, and 60 mL diethyl ether for the monoglyceride fraction were collected.

RESULTS AND DISCUSSION

The compositions of the acid oils as shown in Table 1 point out that they are characterized by high free fatty acid content and neutral glycerides and low unsaponifiable matter. Fatty acid composition of the acid oils as shown in Table 2 indicates that each acid oil has identical composition to that of the original oil.

Enzymatic hydrolysis of acid oils using *C. cylindracea* lipase indicates that each acid oil can be nearly completely hydrolyzed to fatty acids in 48 h (Fig. 1). Each acid oil is therefore a potential raw material for fatty acid production. The extent of hydrolysis, taking coconut oil as an example, com-

TABLE 1
Composition of Acid Oils

Acid oils	Free fatty acid (w/w %)	Unsaponifiable matter (w/w %)	Neutral glycerides (w/w %)		
			Monoglycerides	Diglycerides	Triglycerides
Coconut	69.2 (as lauric)	0.4	5.1	4.2	21.1
Soybean	79.2 (as oleic)	2.9	2.8	3.9	11.2
Mustard	76.5 (as oleic)	1.6	3.1	2.0	16.8
Sunflower	38.8 (as oleic)	2.8	4.4	6.2	47.8
Rice bran	56.2 (as oleic)	4.2	4.6	3.2	31.8

TABLE 2
Composition of Fatty Acids of Total Acid Oils

Acid oils	Fatty acids (wt%)									
	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:1}$	$C_{22:1}$
Coconut[a]	5.1	44.8	18.4	11.5	3.7	10.1	3.4	—	—	—
Soybean	—	—	—	22.3	3.6	18.1	51.2	4.8	—	—
Mustard	—	—	—	6.1	3.2	20.2	18.3	6.6	7.5	38.1
Sunflower	—	—	—	11.0	4.6	33.1	50.9	0.4	—	—
Rice bran	—	—	—	20.6	5.6	42.6	31.2	—	—	—

[a]Coconut acid oil also contains $C_{6:0}$—1.2% and $C_{8:0}$—1.8%.

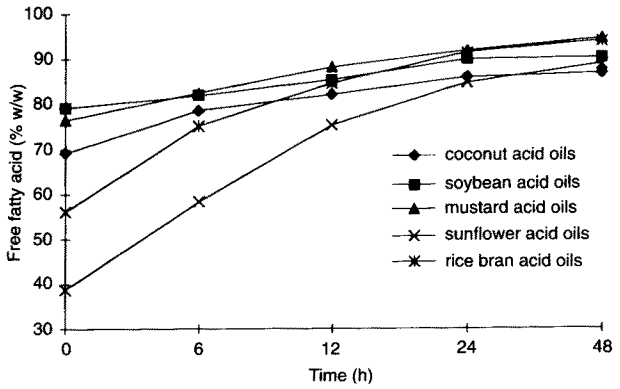

FIG. 1. Free fatty acid production by the hydrolysis of acid oils with *Candida cylindracea* lipase at 35 ± 2°C.

pares well with results reported previously using *Aspergillus niger* lipase (13).

The conversion of acid oils into fatty acid esters of C_4–C_{18} saturated alcohols in very high yield has been achieved by simultaneous esterification of the free fatty acid part and alcoholysis of the neutral glyceride fraction with the aid of *M. miehei* lipase. It can be noted from Table 3 that the yield (in wt%) of esters is very high.

On the basis of the fatty acid content of each acid oil added, the percent conversion of each acid oil into alcohol esters is shown in Table 4. In the simultaneous esterification and alcoholysis reaction with a 1,3-regiospecific lipase (*M. miehei*), the free fatty acids are converted to esters, and only 1,3-fatty acids of neutral glycerides are converted into alcohol esters. Therefore, the percent conversion of acid oils into esters is directly related to the free fatty acid content and neutral glyceride content. For example, with sunflower acid oil (free fatty acid—38%), the percent conversion is about 76–77% for C_8–C_{18} alcohols and with mustard acid oil (free fatty acid—76.5%), the percent conversion is in the range of 89–90% for C_8–C_{18} alcohols.

The percent conversion of alcohols into esters by weight is shown in Table 5. From Tables 4 and 5 it can be stated that the percent conversion on the basis of alcohol and acid oil is comparable. This is due to the fact that the acid oils and alcohols are taken in 1:1 molar ratio for the synthesis of esters.

The results demonstrate that microbial lipase technology offers excellent means of producing fatty acids and various esters of fatty acids from relatively cheap raw materials such

TABLE 3
Yield[a] (wt%) of Esters of Different Alcohols

Acid oils	Yield of alcohol esters (wt%)					
	C_4	C_8	C_{10}	C_{12}	C_{16}	C_{18}
Coconut	94.3	136.5	148.7	158.2	184.8	195.6
Soybean	105.4	125.4	134.4	143.3	161.8	168.1
Mustard	99.2	124.0	132.8	141.7	158.8	167.0
Sunflower	85.4	109.8	117.5	125.4	139.3	145.8
Rice bran	89.2	111.9	119.2	127.6	143.5	151.5

[a]Ester yield (wt%) = weight of ester obtained/weight of acid oil taken × 100.

TABLE 4
Percent Conversion[a] (wt%) of Acid Oils into Alcohol Esters

Acid oils	Alcohol esters (w/w %)					
	C_4	C_8	C_{10}	C_{12}	C_{16}	C_{18}
Coconut	75.1	90.2	90.6	89.4	91.2	90.8
Soybean	86.9	88.1	87.8	87.6	87.5	86.0
Mustard	83.4	89.9	90.1	90.3	90.4	90.2
Sunflower	70.4	77.1	76.7	76.6	75.3	74.5
Rice bran	73.4	78.2	77.5	77.5	77.1	76.9

[a]Percent conversion of acid oil into alcohol ester = total equivalent fatty acids – equivalent free fatty acid in the product/total equivalent fatty acids × 100.

TABLE 5
Percent Conversion[a] (wt%) of Alcohols into Alcohol Esters (on the basis of alcohol added)

Acid oils	Alcohol esters (w/w %)					
	C_4	C_8	C_{10}	C_{12}	C_{16}	C_{18}
Coconut	73.8	90.1	89.4	88.2	90.1	89.7
Soybean	85.8	87.8	87.1	86.8	87.1	85.4
Mustard	82.6	89.1	89.8	89.2	90.1	88.8
Sunflower	69.1	77.0	74.9	75.4	75.1	72.9
Rice bran	71.8	77.1	76.5	77.3	76.0	76.6

[a]Percent conversion of acid oil into alcohol esters = weight of alcohol taken for reaction – unreacted alcohol/weight of alcohol taken for reaction × 100.

as acid oils. This may prove to be the preferred catalytic route for the production of high-quality acids and esters in high yield from such inferior-grade feedstocks.

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