Lipase-Catalyzed Synthesis of Hydroxy Stearates and Their Properties

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ABSTRACT: Hydroxy fatty acid (HFA) esters of long-chain alcohols, such as hydroxy stearates, have potential applications from lubricants to cosmetics. These esters were synthesized enzymatically to overcome the problems associated with chemical processes. An immobilized lipase, *Rhizomucor miehei,* was employed as catalyst in the esterification reaction between hydroxy-stearic acid as a source of HFA and monohydric fatty alcohols (C_8–C_{18}). The yields of esters were in the range of 82–90% by conducting the reactions at 65 ± 2°C, 2–5 mm Hg pressure, and 10% lipase concentration. The products were analyzed by infrared spectroscopy, and some of their analytical characteristics were determined.

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KEY WORDS: Enzymatic esterification, hydroxy fatty acid, hydroxy stearates, lipase, *Rhizomucor miehei,* wax esters.

Hydroxy fatty acids (HFA), derived from several natural and synthetic sources, have many applications. HFA and their derivatives are used in cosmetics, in paints and coatings, in lubricants, and in the food industry. They are useful intermediates in the synthesis of fine chemicals and pharmaceuticals (1).

HFA are found in many natural sources. For example, castor oil contains 12-hydroxy oleic acid, or ricinoleic acid, which incorporates a hydroxyl group at the C_{12}-atom. When subjected to hydrogenation, this castor oil fatty acid gives 12-hydroxy stearic acid. Hydroxy stearates are usually known in the form of wax esters. These wax esters form gelatinous crystalline matrixes; by contrast, with the nonhydroxy derivatives, either no crystals at all are formed, or the crystals are not held in suspension. The hydroxy derivatives have wide application in leather coatings, for oil resistance and water imperviousness, and in roll leaf foils, because of their alcohol solubility and excellent wetting and adhesion to metallic particles (2). These wax esters can be produced either by hydrogenation of castor oil fatty acid esters or directly by reacting the fatty acid and an alcohol at high temperature in the presence of tin, titanium, or sulfuric acid catalyst for up to 20 h. This high-temperature process can lead to degradation of the ester and undesired side reactions; additionally, the resulting energy costs are high. Recently, the possibility of carrying out reactions with microbial lipases has been explored because of regio- and stereospecificity of the enzymes and because of the greater degree of purity that can be obtained for the desired reaction products.

Application of enzymes in various biochemical modifications of fats and oils is well established, and the catalytic activity of lipases toward hydroxy fatty acids is also well studied by various workers (3–11). According to this information, 1,2-specific lipases cannot act upon the hydroxyl group of HFA; this property has been taken advantage of in the esterification of HFA and alcohol so that estolide or lactone formation is avoided. For example, *Rhizomucor miehei* lipase has been employed in the esterification of several different HFA (3,5,11) and free estolide (9). This 1,3-specific lipase is important because, unlike random lipases, it cannot catalyze ester formation at the hydroxyl group. Ghosh Ray and Bhattacharyya (3) and Mukesh *et al.* (4) have produced ricinoleic acid wax esters by *R. miehei* lipase-catalyzed esterification of ricinoleic acid or alcoholysis of castor oil. Wagner *et al.* (8) synthesized wax esters of aleuritic (C_{16} trihydroxy) acid by a similar reaction scheme and achieved moderate yields. Similarly, Lang *et al.* (6), Steffen *et al.* (7), and Hayes (10) also employed *R. miehei* to prepare wax esters by esterification or alcoholysis reactions.

Taking these aspects into consideration, the synthesis of hydroxy stearates by lipase-catalyzed esterification of hydroxy stearic acid and different monohydric alcohols was done under solvent-free anhydrous conditions. The reaction medium was composed solely of the substrates and enzyme and offers an advantage in reducing downstream processing. The effect of alkanol chainlength on the esterification reaction and some physical properties of the esters have been studied.

MATERIALS AND METHODS

Substrate and enzyme. 12-Hydroxy stearic acid was supplied by Jayant Oil Mills (Mumbai, India). Fatty alcohols, such as octanol, decanol, dodecanol and 9-octadecene-1-ol (oleyl alcohol), were purchased from E. Merck (India) Ltd. (Mumbai, India). Unless otherwise specified, all other reagents were of analytical grade. *Rhizomucor miehei* lipase (Lipozyme IM 20) was a generous gift of Novo Nordisk (Copenhagen, Denmark).

Esterification reaction. Pure 12-hydroxy stearic acid was mixed with pure fatty alcohol in 1:1 or 1:2 molar ratio. *Mucor miehei* lipase was added to the mixture at varying levels

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(2–12% of the total charge, w/w) with vigorous stirring. The temperature was kept constant at 65 ± 2°C because maximal effectiveness of the enzyme lies in the 60–65°C temperature range. Several sets of experiments were carried out at different pressures to standardize the process variables. Free fatty acids in the reaction mixture were determined (12) at half-hourly intervals until they reached a constant value, to ensure maximal esterification. After completion of the reaction, the product was dissolved in diethyl ether, and the enzyme was filtered off. The unreacted acid was neutralized by sodium bicarbonate solution, and the ester was dried *in vacuo* (1 mm Hg) at high temperature. Formation of the ester was confirmed by infrared (IR) spectrophotometry.

Analytical methods. The esters obtained were tested for different physical properties. Determinations of slip melting point (13), saponification value (14), and iodine value (15) were carried out by standard methods.

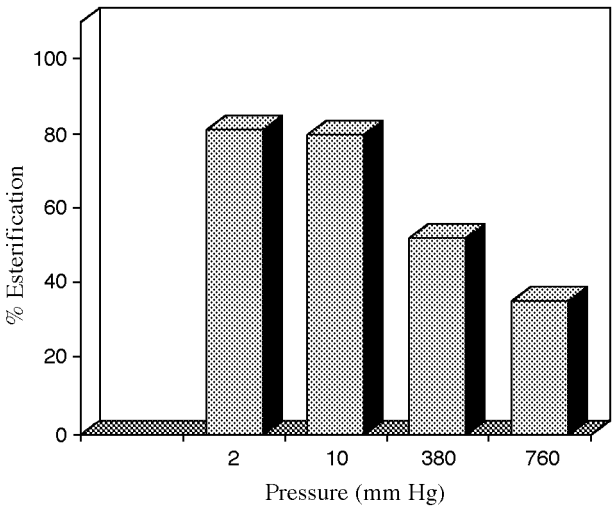

FIG. 2. Percentage esterification of 12-hydroxy stearic acid with fatty alcohol (octanol) at different pressures.

RESULTS AND DISCUSSION

In the IR spectral data of hydroxy-stearates, a strong O–H absorption band appears at 3477 ± 5 cm^{-1}, which indicates that the hydroxy group of the parent acid remains unaffected by action of the enzyme. The carboxylic carbonyl absorption shifts from 1712 cm^{-1} due to the change from acid to ester group, which actually proves the formation of ester and is typical for esterification reactions. Other unaltered chromophoric absorptions point out that the original skeleton of hydroxy stearic acid does not change upon enzymatic esterification except for the change from carboxyl group to ester group. For unsaturated alcohol (oleyl alcohol), the absorption at 720 cm^{-1} proves that the double bond of the alcohol is also unchanged by the esterification reaction. Hydrolysis of all esters yields the corresponding alcohols and acids, which have the same IR spectra as the original compounds.

To standardize the process for lipase-catalyzed esterification, several sets of experiments were carried out to optimize the extent of esterification at varying process variables (Figs. 1–4). The yield was maximal at 65°C, 2–5 mm Hg pressure and 10% lipase concentration (w/w, of the total charge) after 360 min of reaction time. It is also clear from Figures 3 and 4 that there is an effect of substrate (acid and alcohol) ratio on the early esterification rates of hydroxy stearic acid and fatty alcohols, but the ultimate rates of reaction are more or less the same for all fatty alcohols. Unsaturation (present in the oleyl alcohol) has no effect on the rate of esterification. Another significant result of this work is documentation of the lack of selectivity of the lipase with respect to fatty alcohol chainlength.

Some analytical characteristics of the esters are presented in Table 1. The slip melting points show a gradual increase

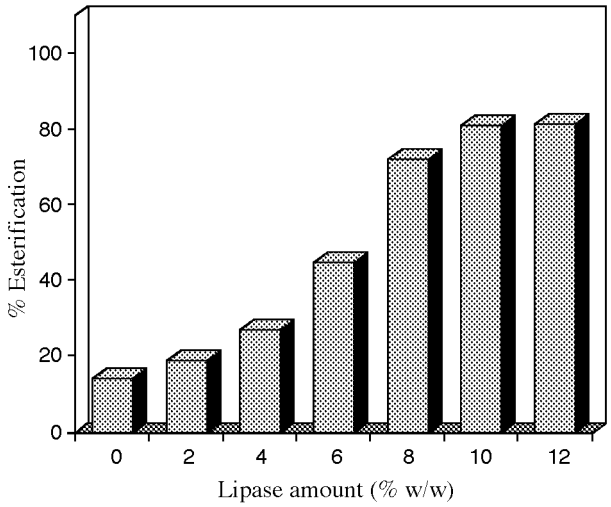

FIG. 1. Percentage esterification of 12-hydroxy stearic acid with fatty alcohol (octanol) at different lipase (*Mucor miehei*) concentrations.

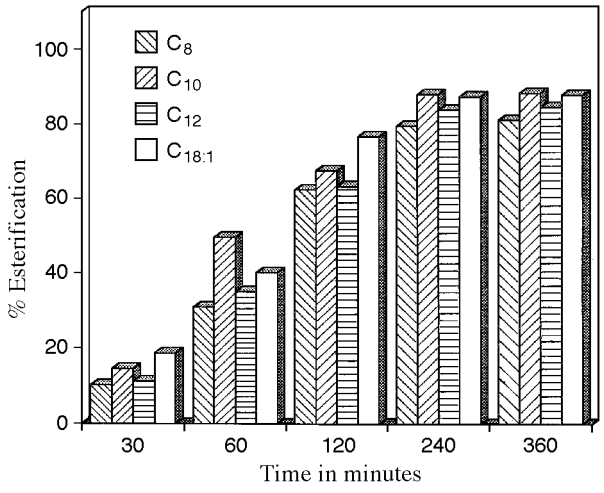

FIG. 3. Percentage esterification of 12-hydroxy stearic acid with different fatty alcohols as function of time (1:1 molar ratio of acid and alcohol).

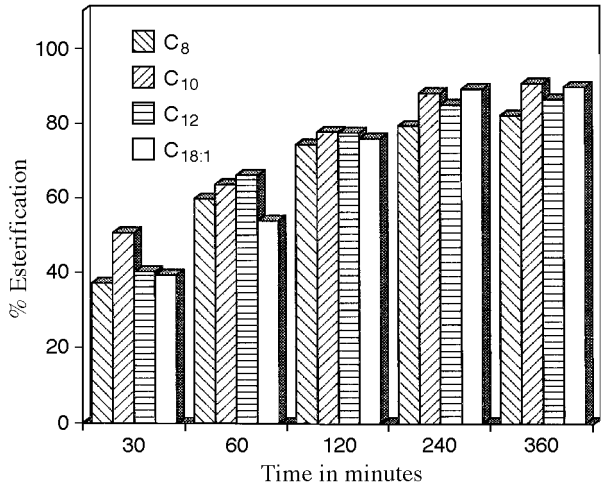

FIG. 4. Percentage esterification of 12-hydroxy stearic acid with different fatty alcohols as function of time (1:2 molar ratio of acid and alcohol).

by 6–8 degrees due to insertion of an additional $–CH_2–CH_2–$ group in the alkyl hydroxy-stearate molecule with $C_8–C_{12}$ saturated alcohols. For oleyl hydroxy stearate, however, the slip melting point is not as high as expected, because of the presence of unsaturation in the alcohol molecule. The saponification value decreases with increasing chainlength in the alcohol part of the ester molecule. Iodine value also shows an inverse relationship with increasing molecular weight of the $C_8–C_{12}$ saturated alcohol, again with the exception of the $C_{18:1}$ unsaturated alcohol, which shows a higher value of 62.7 because of the double bond in the alcohol part. This iodine value also proves that the unsaturation present in the alcohol molecule is unaffected by the lipase action.

These experimental findings have shown that 12-hydroxy stearic acid can be esterified quite readily with long-chain aliphatic alcohols by an enzyme without deforming the basic structure of the parent hydroxy acid, and the final esterified products show a large depression in slip melting points compared with their parent blends. The results confirm the findings of previous workers that the lipase does not catalyze esterification of the functional group in the ester molecule.

TABLE 1
Analytical Characteristics of Alkyl-Hydroxy-Stearates

	Hydroxy stearates			
Characteristics	Octyl	Decyl	Dodecyl	Oleyl
Slip point (°C)	38.1 (66.5)[a]	44.2 (66)	49.8 (65.5)	42 (63)
Saponification number	114.2	110.9	106.6	87.7
Iodine number	3.7	3.0	2.8	62.7

[a]Parentheses represent the values of the corresponding blends before esterification.

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