Enzymatic Preparation of Ricinoleic Acid Esters of Long-Chain Monohydric Alcohols and Properties of the Esters

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Apart from the conventional chemical esterification process, long-chain alkyl ricinoleates also can be prepared by enzymatic esterification or by enzymatic alcoholysis with high yield and without undesirable side reactions. On sulfonation to the hydroxyl group, the alkyl ricinoleates produce surface-active compounds. The tetradecyl ricinoleate shows the best surface-active behavior and seems to be much better than that of sulfonated castor oil commonly known as "Turkey Red Oil".

KEY WORDS: Alkyl ricinoleates, enzymatic alcoholysis, enzymatic esterification, hydroxy acid esters, monoglycerides, *Mucor miehei* lipase, ricinoleic acid, sulfonated alkyl ricinoleates.

Due to its high content of 12-hydroxy oleic acid (ricinoleic acid), nonedible castor oil has been extensively studied by chemical transformation reactions for its utilization in lubricant and paint industries and also for production of surface-active compounds for fatliquoring in the leather industry. However, biotechnological transformation of castor oil has not been extensively studied. Recently, the possibility of carrying out reactions with microbial lipases has been explored because of the regiospecificity and stereospecificity of the lipases and because of the greater degree of purity that can be obtained for the desired reaction products.

The aims of the present study are: i) to investigate the possibilities of carrying out specific enzymatic esterification of the carboxyl group of the ricinoleic acid as occurring in castor oil with different long-chain aliphatic monohydric alcohols; ii) to prepare monoglycerides and monohydric alcohol esters of castor oil fatty acid by enzymatic alcoholysis of castor oil with long-chain aliphatic monohydric alcohols; and iii) to examine the effect of alkanol chainlength on the physical and surface-active properties of the prepared esters as such and after sulfonation.

EXPERIMENTAL PROCEDURES

Castor oil fatty acids were isolated by the conventional fat splitting method with ethanolic caustic soda followed by dilute hydrochloric acid. Castor oil fatty acids containing 88% ricinioleic acid were mixed with pure fatty alcohol (E. Merck, Darmstadt, Germany) in 1:1.1 molar ratio. *Mucor miehei* lipase (Lipozyme TM, supplied by NOVO Industri A/S, Denmark) was added to the mixture at varying levels (2–12% of the total charge, w/w) with vigorous stirring. The temperature was kept constant at 60°C, since maximum effectiveness of the enzyme could be obtained at that temperature. Several sets of experiments were carried out at varying atmospheric pressures to standardize the process variables. The free fatty acid of the reaction mixture was determined from time to time at half-hourly intervals until it reached a constant value to ensure complete esterification. The enzyme was then recovered by filtration, washed with solvent, dried and stored at 4°C for reuse.

The unreacted acid was removed in the form of soap by treating with aqueous ethanolic caustic soda. The unreacted alcohol was removed by fractional crystallization from hexane at lower temperatures (10–15°C), followed by filtration. Finally, the ester was dried *in vacuo* and formation of the ester was confirmed by infrared (IR) spectrophotometry.

For alcoholysis reactions, refined and bleached castor oil was mixed with pure fatty alcohol at 1:2.2 molar ratio and the standardized procedure described above was followed. Vacuum was employed for alcoholysis reaction, presumably to avoid the possible hydrolysis of the ricinoleates by the enzyme in the presence of moisture. Completeness of ester formation was determined by preparative thin-layer chromatography (TLC) (1) with the solvent system n-hexane/diethyl ether/acetic acid (90:10:1, v/v/v), followed by scraping of the required bands, extraction with chloroform and finally weighing from time to time at half-hourly intervals. Quantitation was continued until a constant amount of product was observed for two consecutive withdrawals of aliquots. The monoglyceride produced by the reaction and the unreacted alcohol were removed from the main product mass by the same procedure of fractional crystallization as described earlier.

The esters obtained by alcoholysis were compared with those obtained by direct enzymatic esterification and tested for different physical properties. Determinations of slip melting point (2), saponification value (3), specific gravity (4), refractive index (5), iodine value (6) and kinematic viscosity (7) were carried out by the standard methods. Different esters were sulfonated by the chlorosulfonic acid below 30°C as per the standard procedure (8). Measurements of surface tension (9) and foam height (10) were also carried out by standard methods.

RESULTS AND DISCUSSION

In the IR spectral data of alkyl ricinioleates a strong O-H absorption band appears at 3477 ± 5 cm^{-1}, indicating that the hydroxyl group of the parent acid remains unaffected by the action of enzyme. The carboxylic carbonyl absorption shifts from 1712 cm^{-1} to 1779 ± 5 cm^{-1} due to the change from acid to ester group, which actually ensures the formation of ester and is typical for esterification reactions. Other unaltered chromophoric absorptions point out that the original skeleton of ricinioleic acid does not change upon enzymatic esterification except for the change from carboxyl group to ester group. Hydrolysis of all esters yield corresponding alcohols and acids having the same IR spectra as the original compounds.

In order to standardize the process for lipase-catalyzed esterification, several sets of experiments were carried out to optimize the extent of esterification as shown in Figures

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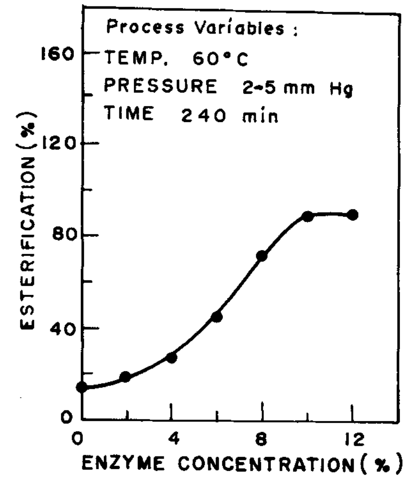

FIG. 1. Effect of enzyme concentration on esterification.

1-3 at varying process variables. The maximum yield was found to be *ca.* 90% at 60°C, 2-5 mm pressure of Hg and 10% lipase concentration (w/w, of the total charge) after 240 min of reaction time. Under the same standardized reaction conditions, an average yield of 65% (theoretical yield 67%) of the desired ester has been found for alcoholysis reaction, due to the selectivity of the *Mucor miehei* lipase to react only at the 1,3 positions of a triglyceride molecule. It is also important to note that the average yield of the by-product monoglyceride has been found to be 32% of the castor oil taken, as expected from the theoretical yield (33%).

Certain physical and chemical properties are presented in Table 1. The slip melting points show a gradual increase by an average 10 units with insertion of each -CH_2-CH_2- group in the alkyl ricinoleate molecule. The saponification value decreases with increasing chainlength in the alcohol part of the ester molecule. Specific gravity does not show any significant change beyond a small increase in the third decimal place. Refractive index, which is a function of molecular volume, increases in the fourth decimal place, showing good agreement with the change in specific gravity values of the prepared alkyl ricinoleates. Iodine value also shows an inverse relationship with increasing molecular weight.

The temperature relationship of the kinematic viscosity values, as evident from the curves shown in Figure 4, shows whether these esters could be used in high-temperature lubrication for metal working. The kinematic viscosity of octadecyl ricinoleate was not determined because this ester is solid at room temperature (30°C).

In Figure 5, lowering of surface tension is prominent for all compounds, considering that distilled water has a surface tension of 71 dynes/cm at 30°C. Surface tension lowering is comparable to, and even better than, that of sulfonated castor oil. The surface tension values decrease with an increase in the chainlength of the alcohol part of the ester molecules up to tetradecyl ricinoleate, but an increasing trend is observed for higher ricinoleates. Maximum lowering has been observed for sulfonated tetradecyl ricinoleate at all concentrations.

TABLE 1

Physical and Chemical Characteristics of Alkyl Ricinoleates

Characteristics	Alkyl ricinoleates				
	Decyl	Dodecyl	Tetradecyl	Hexadecyl	Octadecyl
Slip melting point (°C)	2	12	23	31	40
Saponification value	110.9	106.0	96.6	90.4	87.7
Specific gravity (at 30°C)	0.880	0.884	0.886	0.887	0.890
Refractive index (N_d^{30})	1.4635	1.4641	1.4646	1.4652	1.4659
Iodine value	50.3	45.3	44.4	42.4	40.3

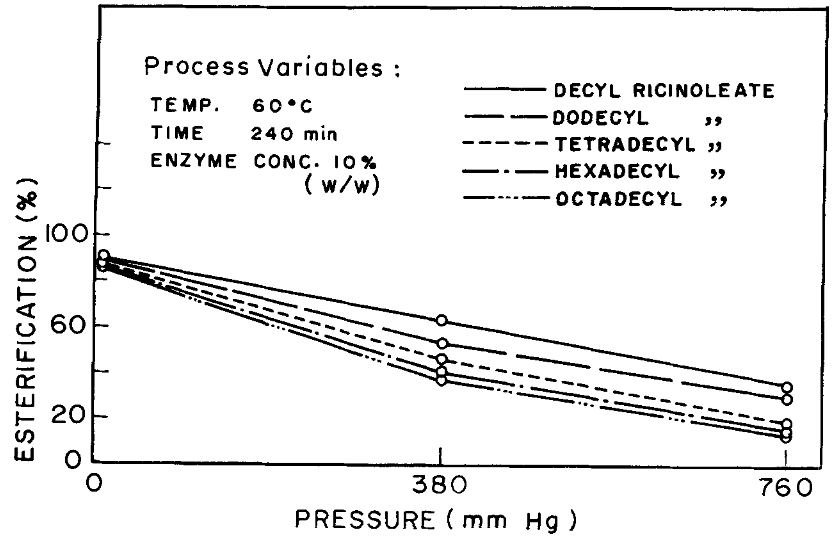

FIG. 2. Effect of reaction pressure on esterification.

The relative foaming powers of different sulfonated products are reflected in Table 2. The foam height characteristics of sulfonated alkyl ricinoleates seem to be better than that of sulfonated castor oil. Here also, a consistent trend of lowering of foam height is observed from lower to higher ricinoleates, due to consecutive insertion of two -CH_2- groups in the alcohol part of the hydroxy ester molecules.

Considering all the aspects regarding esterification, physical, chemical and surface-active properties of the prepared hydroxy esters, we have shown that:

i) Castor oil fatty acids containing 88% ricinoleic acid can be esterified quite readily by long-chain aliphatic alcohols (even C_{10}–C_{18}) without deforming the basic structure of the parent hydroxy acid, by enzymatic esterification or by direct enzymatic alcoholysis reaction.

ii) Alcoholysis of castor oil fatty acids by a specific lipase enzyme, such as *Mucor miehei*, produces monoglycerides as a by-product, which has various industrial applications.

iii) Due to low reaction temperature and selectivity of the enzyme, this process has no undesirable side reactions. Moreover, this process shows no interference upon the structural configurations of the reactant molecules.

iv) The hydroxy ester obtained from tetradecyl alcohol has the best surface activity upon sulfonation among all

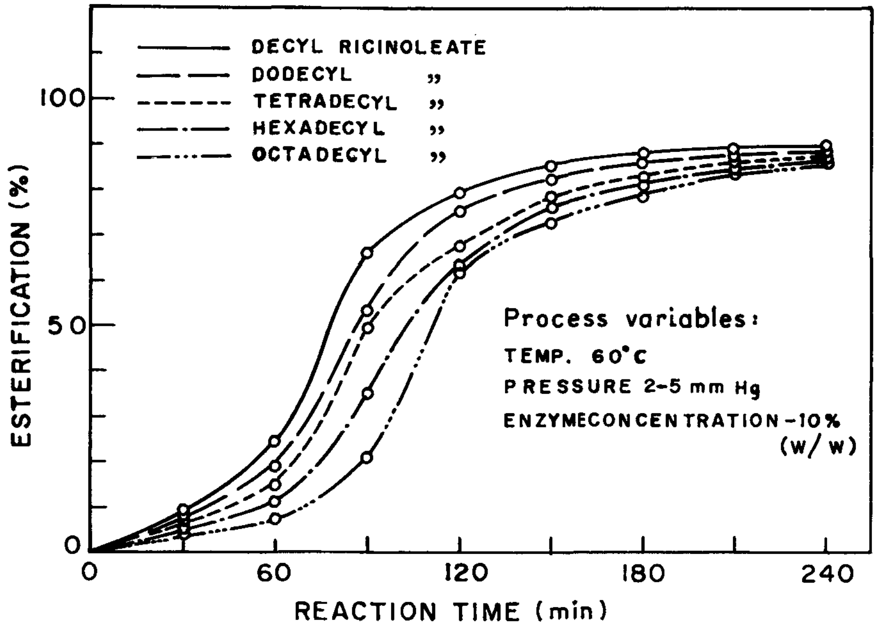

FIG. 3. Esterification plotted against reaction time.

TABLE 2

Relative Foaming Power of Sulfonated Surface-Active Products at 30°C

Sulphonated compounds	0.25 Initial	0.25 Final	0.50 Initial	0.50 Final	1.0 Initial	1.0 Final	2.0 Initial	2.0 Final
	Foam heights in cm at concentrations (%)							
Castor oil	3.1	1.5	3.9	1.9	4.4	2.4	5.0	2.5
Decyl ricinoleate	4.7	2.3	5.8	2.8	6.4	3.5	6.9	4.0
Dodecyl ricinoleate	4.1	2.1	4.8	2.5	5.7	3.0	6.3	3.6
Tetradecyl ricinoleate	4.0	1.9	4.5	2.3	5.1	2.9	5.8	3.2
Hexadecyl ricinoleate	3.3	1.9	3.9	1.8	4.2	2.2	4.8	2.6
Octadecyl ricinoleate	2.9	1.2	3.4	1.2	3.0	0.9	2.1	0.6

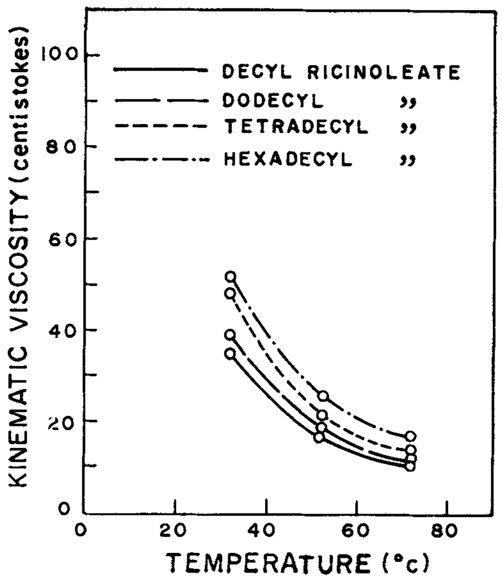

FIG. 4. Effect of temperature on kinematic viscosity.

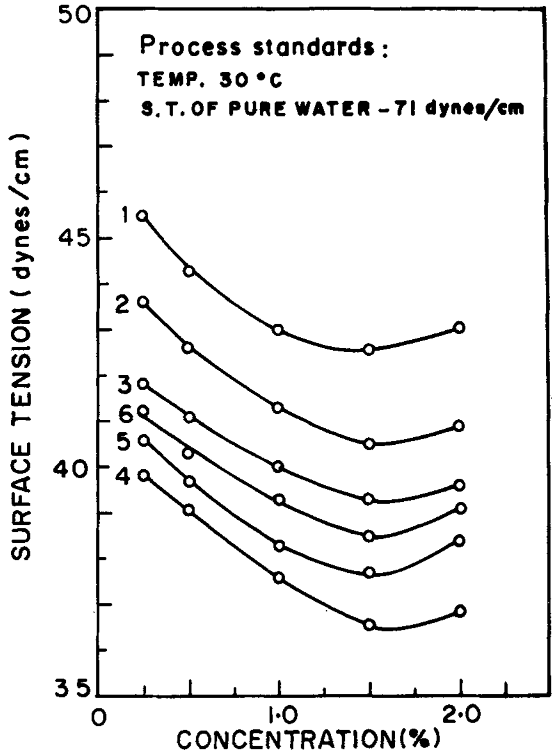

FIG. 5. Surface tension plotted against concentration. 1, Sulfonated castor oil; 2, sulfonated decyl ricinoleate; 3, sulfonated dodecyl ricinoleate; 4, sulfonated tetradecyl ricinoleate; 5, sulfonated hexadecyl ricinoleate; and 6, sulfonated octadecyl ricinoleate.

the hydroxy esters. From the surface tension and foam height data it may be inferred that the surface-active properties for alkyl ricinoleates up to tetradecyl are better than those of sulfonated castor oil, but they are considerably inferior for esters beyond tetradecyl ricinoleate.

Long-chain monohydric alcohol esters of castor oil fatty acids can be considered an important class of potential raw material for preparing surface-active molecules not only by sulfonation, which has been investigated in the present study, but also by other kinds of derivatives, to cater to textile and other applications where wetting properties of the surface-active molecules are important.

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