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NOTE

Lipase catalyzed transesterification of castor oil by straight chain higher alcohols

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Biolubricants from Castor oil were produced enzymatically by transesterification with higher alcohols using a lipase mixture of immobilized *Mucor miehei* (RMIM) and immobilized *Candida antarctica lipase B* (Novozym 435) under low water conditions. The conversions were in the range of 80–95% under the optimized conditions.

[**Key words:** Low water enzymology; Enzyme immobilization; Valorization of castor oil; Lipases; Transesterification; Non edible oils]

Esters of the medium chain and long chain alcohols find a wide variety of applications in industrial sectors like textiles, plastics, cosmetics and as industrial lubricants (1). The replacement of petroleum based lubricants with biodegradable synthetic lubricants is considered a desirable goal (2). The oils from the vegetable sources can be converted into esters of alcohols by the transesterification reaction (3) (Fig. S1). While the esters of short chain alcohols such as methanol and ethanol are used as biodiesel, the esters of higher chain alcohols function as lubricants (4,5). Castor oil, the oil derived from castor bean seeds is considered a non edible oil in view of its toxic nature. Its use as a starting material for synthesis of biolubricants has started attracting attention (6). India is the major producer of castor oil in the world, producing about 65% of total castor seed production (0.8 million tons) and 51% of castor oil (0.3 million tons) (7). The transesterification reaction with vegetable oils can be catalyzed either by chemical catalysts or by enzymes (8). The enzyme catalyzed transesterification for synthesis of biolubricants has however attracted less attention. The esters of long straight chain alcohols as biolubricants have also attracted very little attention (3).

The present work describes the use of commercially available lipases for carrying out transesterification of castor oil with 1-hexanol, 1-octanol and 1-dodecanol. So far most of the work on transesterification with medium chain/long chain alcohols, even with chemical catalysts has been carried out by first converting the oil into a methyl or ethyl ester. That involves an additional step. The present study involves direct enzyme catalyzed transesterification of castor oil with the above mentioned alcohols.

Castor oil (0.5 g) and alcohol (1-hexanol, 1-octanol, 1-dodecanol) were taken in molar ratio in the range of 1:3–1:8 with *n*-hexane (5 mL) or without *n*-hexane (solvent free) in a screw-capped vial. Lipase (5%–7.5% w/w of oil) was added to this reaction mixture and incubated at different temperature (30°C–60°C) with a constant shaking at 200 rpm. Aliquots (20 μL in case of solvent free and 200 μL in case of hexane) were taken at different time intervals and the percentage conversion to alkyl ester was determined by carrying out the GC analysis of the samples (9).

RMIM is the commercially available immobilized preparation of *Mucor miehei* lipase which has been used fairly extensively in low water media (9,10). With castor oil, *M. miehei* lipase has been frequently employed (11). This was chosen for the preparation of the fatty acid esters. Solvent free synthesis utilizes substrates as such, as the reaction media. It allows working with higher concentration of the substrates, is less expensive and avoids the use of volatile organic solvents (12–14). Hence, initially transesterification of the castor oil with three different long chain alcohols 1-hexanol, 1-octanol and 1-dodecanol at different ratios of oil: alcohol was tried (Fig. 1). Theoretically three moles of alcohol are needed for complete transesterification of the triglyceride. In the present case, three times molar excess of the alcohol was found to give the best results for all the three alcohols. The conversion after 24 h was found to be 64%, 60% and 47% for 1-hexanol, 1-octanol and 1-dodecanol, respectively, using 3 M excess of the alcohols (Fig. 1).

RMIM has been used up to 50°C in low water media (9,10). Hence, the above reaction with the three alcohols was performed at 30°C, 40°C and 50°C. The highest temperature of 50°C gave the best performance with all the three alcohols (69%, 63% and 56% for 1-hexanol, 1-octanol and 1-dodecanol, respectively) and was chosen for all further reactions (Fig. S2). It is noteworthy that conversions obtained in 24 h with the different alcohols followed the trend 1-hexanol > 1-octanol > 1-dodecanol. The specificity of a lipase preparation is known to depend upon the chain length of the alcohol (15).

Castor oil has considerably high viscosity as compared to other vegetable oils (16). While solvent free synthesis do have the

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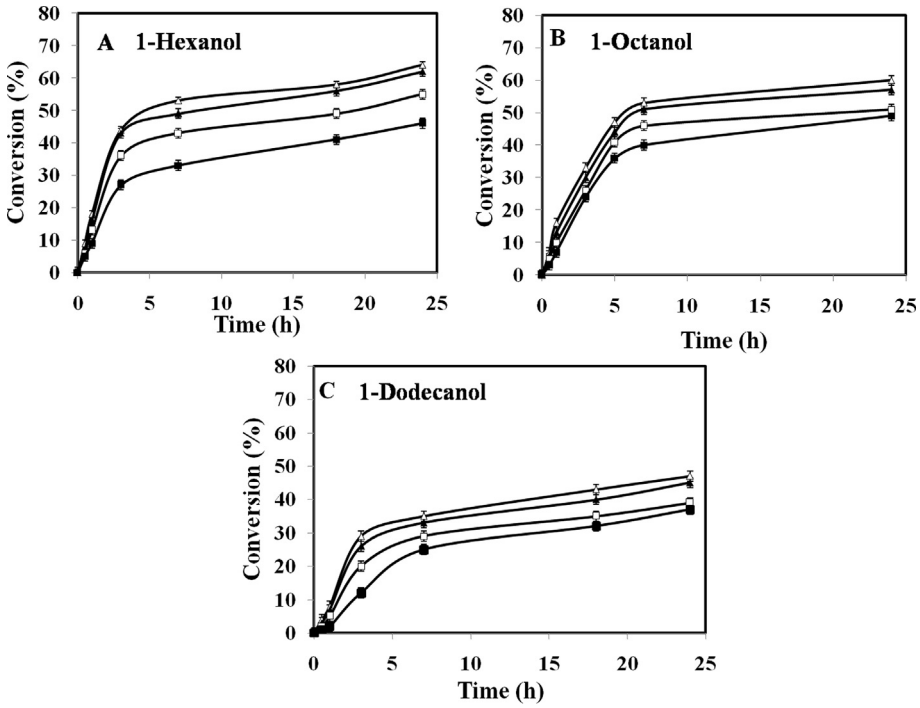

FIG. 1. Effect of molar ratio of castor oil: alcohol on solvent free synthesis of the fatty acid esters. (A) 1-Hexanol, (B) 1-octanol, and (C) 1-dodecanol were taken in molar ratio of 1:3 (open triangles), 1:4 (closed triangles), 1:6 (open squares), and 1:8 (closed squares). Reaction was carried out at 40°C at 200 rpm using RMIM (5% w/w of oil).

advantages like greenness, high molarity of substrates can be dissolved and cost reduction, it was decided to examine the effect of reducing the medium viscosity by adding hexane. Moreover, higher conversions are reported when transesterification of triglycerides with alcohols are carried out in hexane as solvent as compared to solvent free conditions (17). So, it was necessary to examine whether the use of solvent outweighed the advantages of solvent free system in the present instance. The choice of hexane as the solvent was made because transesterification of oils/fatty acids has frequently been carried out using hexane as a reaction medium (5). Table S1 shows the results of the effect of adding hexane. With all the three alcohols, both initial rates and % conversion in 24 h were

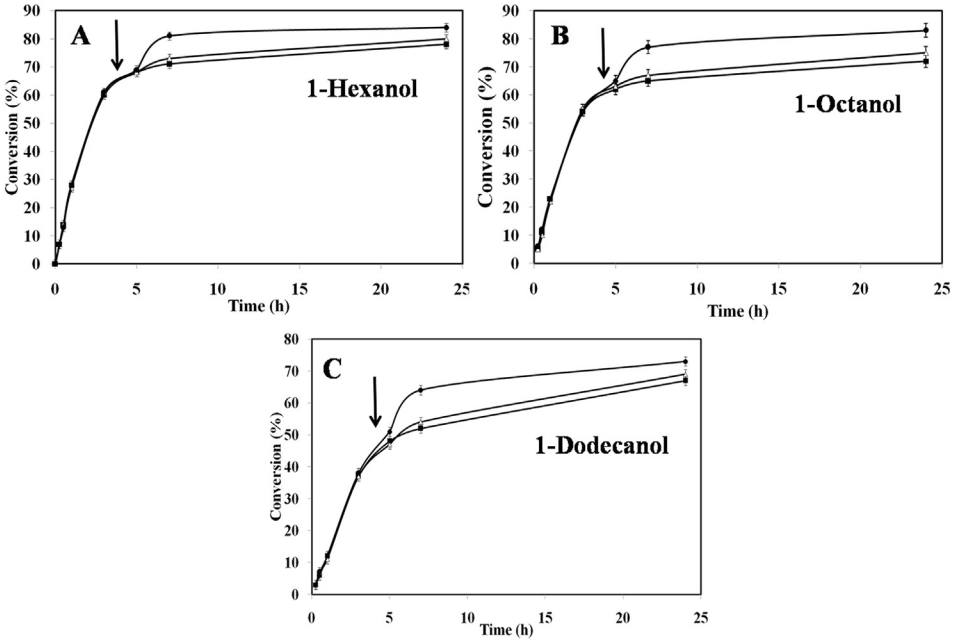

FIG. 2. Effect of additional RMIM/Novozym 435 on synthesis of the fatty acid esters. Synthesis of the fatty acid esters was carried out at 50°C, 200 rpm with castor oil and (A) 1-hexanol (B), 1-octanol, and (C) 1-dodecanol in molar ratio of 1:3 in the presence of hexane as the solvent. Additional RMIM/Novozym 435 (2.5% w/w of oil) was added to the reaction (containing RMIM- 5% w/w of oil) at 4th hour. Symbols are represented as follows: closed squares, only RMIM (5% w/w of oil); open triangles, RMIM (5% w/w of oil) + RMIM (2.5% w/w of oil); closed circles, RMIM (5% w/w of oil) + Novozym 435 (2.5% w/w of oil).