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Esterification of Fatty Acid Using Porcine Pancreas Lipase

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The esterification of a long-chain fatty acid was conducted using porcine pancrease lipase in a mainly anhydrous non-polar organic medium, heptane. Propyl laurate was produced from lauric acid and *n*-propanol. The present study was confined to optimize the effect of the parameters, temperature, substrate concentration and the enzyme concentration on the esterification process. It was found that maximum yields were possible at 35°C, 0.75 M substrate concentration (the substrates being taken at equimolar quantities) and at 40 mg/mL enzyme concentration. Specific studies with these parameters show a maximum conversion with respect to increase in temperature and substrate concentration. As regards the effect of enzyme concentration on percentage conversion beyond 40 mg/mL concentration, the increase in per cent conversion was not very significant. Kinetic data were collected with optimum temperature and optimum enzyme concentration for each substrate concentration and it was found that the data obey Michaelis-Menton kinetics. The K_m and V_{max} values were found to be 4.185 g mol/L and 625 mmol/h-L respectively.

Key Words: Lipase, Porcine pancreas, Lauric acid.

INTRODUCTION

During the last decade, interest in biotechnology of fats and oils has increased considerably. Before that time, the study of biological catalysts and their use in technical applications was almost restricted to purely aqueous system. The growing need for highly specific chemicals and biochemicals which are only slightly soluble or completely insoluble in water and sometimes which cannot be synthesized in a purely chemical way has been a challenge and a driving force for the fundamental study of the behaviour of the enzymes in organic media and of the possible applications of these biocatalysts in technological processes.

Esters made from alcohols and fatty acids have many applications, and those prepared from long-chain acids (12–20 carbon atoms) and short-chain alcohols (3–8 carbon atoms) have been used increasingly in the food, detergent, cosmetic and pharmaceutical industries. Esters resulting from the reaction of long-chain acids with long-chain alcohols (12–20 carbon atoms) also have important applications as plasticizers and lubricants.

Ester formation by lipases is a well-know reaction. Two-phase aqueous systems have been used for the synthesis of glycerides[1, 2] or other fatty acid esters[1, 2–6]. More recently ester synthesis using an organic solvent is reported[7–10]. Many organic esters, which are important precursors in chemical synthesis, are optically active and therefore, difficult to synthesize chemically in pure form. Based on the specificity of biocatalysts such as esterases and hydrolases, however, a racemic mixture of chiral compounds can be separated and one enantiomeric ester or alcohol can be stereospecifically synthesized[5, 11].

Both lipase and carboxyl esterase normally catalyze the hydrolysis of glycerides[12] and carboxylic ester[13] respectively and the equilibrium of these processes is far in the direction of hydrolysis products since water is present in excess. When the concentration of water in the reaction reduced, *e.g.*, by working in an organic solvent containing a limited amount of water, a drastic shift of the chemical equilibrium can be obtained, so that reagents and products are present in comparable concentrations. Mechanistically, this means that the reverse reaction of the esterification takes place at a rate comparable to that of the hydrolysis reaction.

An important point of consideration when working with enzymes in organic solvent is the stability and preservation of the activity and specificity of the biocatalyst[14]. Traditionally enzymes have been confined to aqueous environments and their use in a non-aqueous system involves the need of stabilization and protection against the denaturing action of the most organic solvents. Besides the direct synthesis, esters can also be obtained by transesterification reactions[4, 5, 18].

EXPERIMENTAL

Lipase enzyme from porcine pancreas (EC 3.1.1.3) obtained from Sigma Chemical Co., St. Louis, M.O. The crude enzyme preparation purchased (Sigma type II) had a nominal specific lipolytic activity as 220 units per unit and contained *ca.* 25 per cent protein based on the pierce BCA protein assay.

n-Heptane (99% pure) obtained from Merck, Bombay was used as the reaction medium. Lauric acid supplied by Loba Chemie Pvt. Ltd., India and *n*-propanol, 99% purity, supplied by E. Merck Ltd., India were used.

Esterification Method

The esterification was carried out in ground glass stoppered conical flask containing 100 mL of *n*-heptane. The reaction mixture was agitated on a rotary shaker at around 100 rpm. The shaker was placed in an incubator for the higher reaction temperatures. Reaction conditions for the synthesis of propyl laurate from lauric acid and *n*-propanol are as shown in Table-1.

TABLE-1
REACTION CONDITIONS CONSIDERED FOR THE PRESENT STUDY

Substrate concentration (g mol/L) equimolar mixtures	0.25, 0.5, 0.75, 1.0, 1.25
Temperature (°C)	25, 35, 45, 55
Enzyme concentration (mg/mL)	10, 20, 30, 40, 50, 60

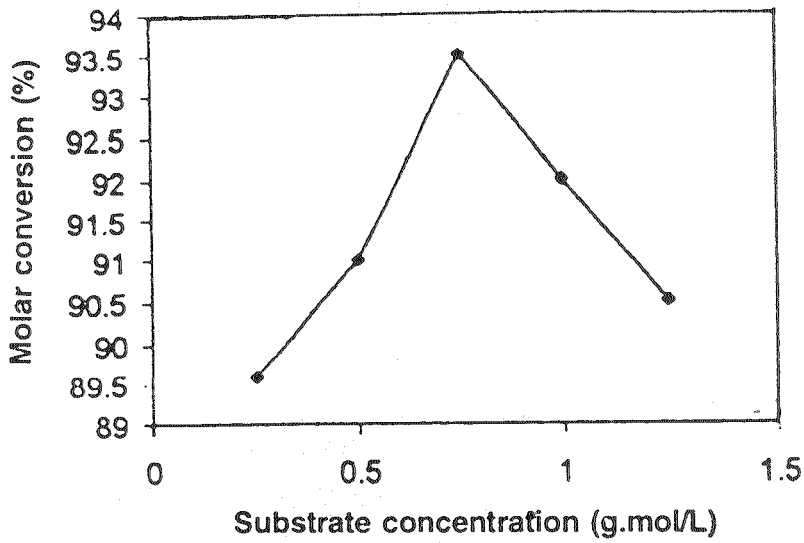

Fig. 2. Effect of substrate concentration on % molar conversion of lauric acid in *n*-heptane. Enzyme concentration 40 mg/mL. Substrates are in equimolar amounts (T = 35°C)

by direct esterification is presented in Fig. 3. It appears that when the enzyme concentration increases, the percentage conversion of lauric acid increases. However, over and above 40 mg/mL of lipase, the increase in percentage molar conversion of lauric acid is not significant. The minimum concentration necessary to achieve maximum yield (89.6%) was found to be 40 mg/mL.

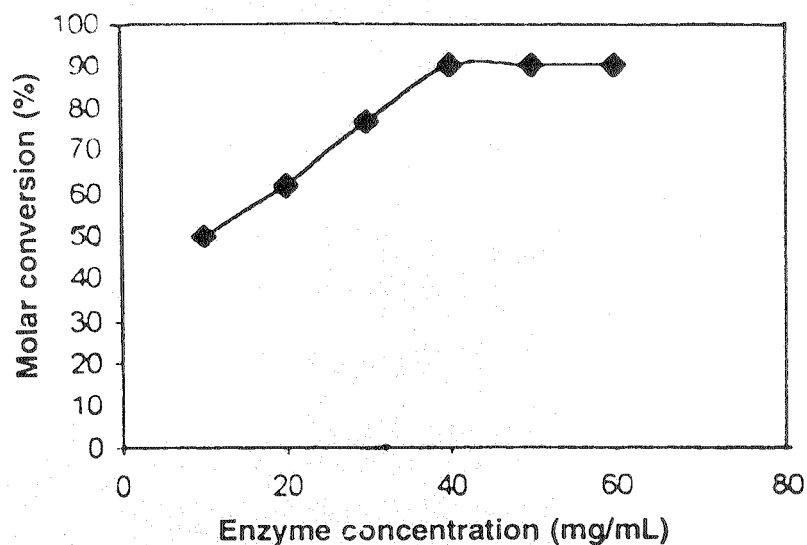

Fig. 3. Effect of enzyme concentration on % molar conversion of lauric acid in *n*-heptane. Substrates are in equimolar amounts at 0.25 g.mol/L (T = 35°C)

Kinetic studies were carried out at 35°C and at an enzyme concentration of 40 mg/mL, using different substrate concentrations. Initial reaction rate of lauric acid esterification at various equimolar amounts of substrate concentrations was calculated using the time dependent results for both lauric acid consumption and propyl laurate formation by determining the slope of a straight line fitted to the initial linear portion of the data. Table-2 shows the results obtained at 35°C and the enzyme concentration was 40 mg/mL.

Analysis

Samples of the reaction media were periodically withdrawn and analyzed by titrimetric analysis. The amount of lauric acid consumed was determined by titrating the reaction mixture with 0.1 N NaOH using phenolphthalein as indicator. From the decrease in their value the extent of ester synthesis was calculated.

RESULTS AND DISCUSSION

The optimum conditions observed for the substrate concentration, temperature and the enzyme concentration are 0.75 mol/L, 35°C and 40 mg/mL, respectively.

To determine the thermal characteristics of the esterification reaction, a series of experiments were carried out at 25, 35, 45 and 55°C, showing the influence of reaction temperature on percentage conversion of lauric acid (Fig. 1). The percentage molar conversion *vs.* temperature shows a typical profile with optimum temperature at 35°C after 48 h reaction; for these reactions the substrates are in equimolar amounts of 0.25 mol/L and the concentration of enzyme is 30 mg/L. At higher temperatures, percentage conversion decreased may be due to thermo inactivation.

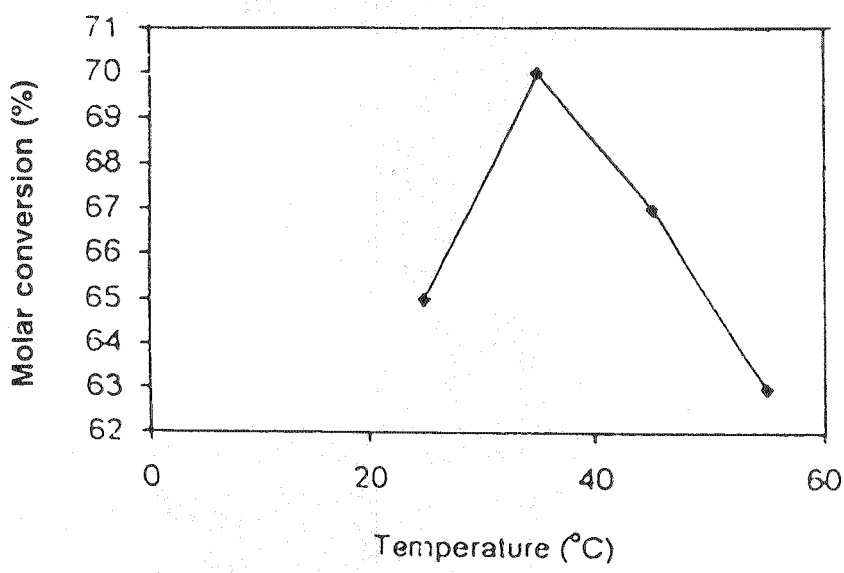

Fig. 1. Effect of temperature on % molar conversion of lauric acid in *n*-heptane. Substrates are in equimolar amounts at 0.25 gm-mol/L, T = 35°C

The substrate concentrations were found to have a significant effect on the percentage molar conversion of lauric acid, as depicted in Fig. 2; for the reaction shown in this figure, the substrates were always present in equimolar amounts. As seen, the percentage conversion of lauric acid increases with increase in substrate concentration reaching a maximum at about 0.75 mol/L and then decreasing as the substrate concentrations are further increased.

The amount of enzyme added for volume of reaction mixture in a given bioprocess is a crucial economic factor. The amount of enzyme added per volume of reaction mixture reported is often too high for any industrial applications. The effect of enzyme concentration on the percentage molar conversion of lauric acid

TABLE-2
DATA OF INITIAL RATE AND SUBSTRATE CONCENTRATION

Substrate concentration (S) (gmol/L)	Initial reaction rate (v) (mol/L hr)	1/S	1/v
0.250	0.550	4	18.18
0.500	0.072	2	13.79
0.625	0.081	1.6	12.3
0.750	0.097	1.33	10.3
1.000	0.012	1	8.33
1.250	0.142	0.8	7.04

System: Lauric acid-*n*-propanol; Temperature: 35°C; Enzyme concentration: 40 mg/mL

A typical Lineweaver-Burk plot showing 1/v *vs.* 1/s for propyl laurate synthesis at 35°C and using lipase concentration of 40 mg/mL is given in Fig. 4.

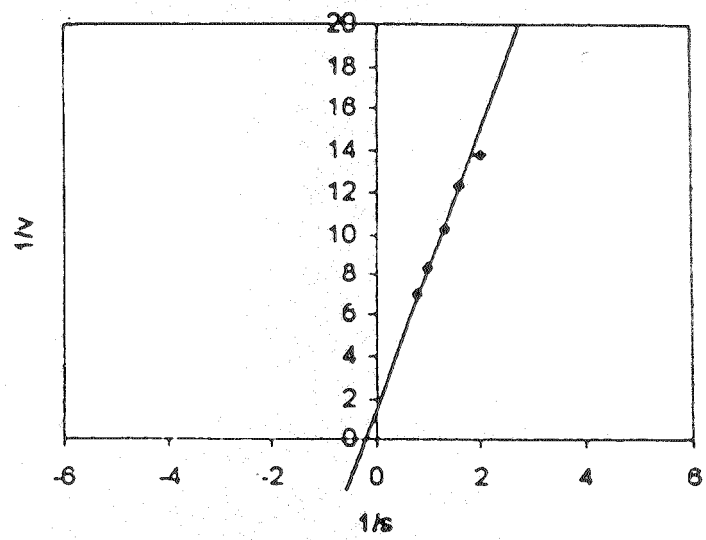

Fig. 4. Lineweaver-burk plot for propyl laurate synthesis at 35°C and using lipase concentration of 40 mg/mL

The apparent K_m and V_{max} were calculated by Lineweaver-Burk method. The values of K_m and V_{max} obtained are 4.185 g mol/L and 625 mmol/h L, respectively.

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