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Synthesis of wax ester using lipase as catalyst

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ABSTRACT

Wax esters are long chain esters that derived from long chain fatty acids and long chain alcohols with chain lengths of 12 carbons or more. The compounds have many potential applications. The present work focuses on the synthesis of wax ester by esterification of palmitic acid and oleyl alcohol using Novozyme lipase. The effects of various reaction parameters such as reaction time, temperature and amount of lipase were investigated. The optimum condition to produce oleyl palmitate ester were respectively, incubation time, 1h – 2h, temperature 40°C - 50°C and amount of enzyme of 100 mg. Analysis of the yield of the product at optimum condition showed that 80% of wax ester produced.

Key words: Esterification, lipase, wax ester, catalyst.

INTRODUCTION

Wax esters are important ingredients in the cosmetic formulations (cleansers, conditioners and moisturizers) [1], in pharmaceuticals (as an anti foaming agent in the production of penicillin and timed release in the production of pharmaceutical tablet) [2], lubricants, plasticizers and polishes [3]. This is due to their unique property of having excellent wetting behavior without the oily feeling. Wax ester can be extracted from animals and plant materials such as beeswax, sperm whale and jojoba oil. However, they are often either too scarce or expensive for commercial use and the main obstacles to large-scale use them are its availability[4].

Wax ester can be synthesized using chemical and enzymatic methods. The use of homogeneous chemical catalyst may lead to several problems such as corrosion of equipment, hazards of handling of the corrosive acids, high energy consumption and degradation of esters [5, 6], where enzymatic synthesis offers mild reaction conditions and environmentally friendly process. In this study, the esterification reaction of palmitic acid and oleyl alcohol, catalyzed by Novozyme to produce oleyl palmitate was carried out. The effects of various parameters on the reaction were investigated.

MATERIALS AND METHODS

Materials

Immobilized lipase from Candida antartica (Novozyme) was purchased from Novo Nordisk (Denmark). Palmitic acid and oleyl alcohol were obtained from Fluka Chemika, AGCH, Switzerland. All other chemicals and reagent were of analytical grade.

Synthesis and analysis of wax ester

The mixture of palmitic acid, oleyl alcohol and enzyme were incubated in a horizontal water bath shaker at 40°C and 150 rpm for an hour. All experiments were assayed in triplicate. The reaction was terminated by dilution with 3.0 ml of ethanol/

acetone (1:1 v/v). The remaining free palmitic acid in the reaction mixture was determined by titration with automatic titrator (ABU 90, Radiometer Copenhagen) to an end point of pH 9.5. The percentage of conversion (%) for each reaction was expressed as number of moles palmitic acid consumed as a percentage of number of moles of initial palmitic acid used. The reaction was analyzed by a gas chromatograph (Hitachi model G-3000, Tokyo, Japan), using Rtx-65TG capillary column (30 x 0.25 mm). Helium was used as the carrier gas at a flow rate of 30 mL/min. The temperature was programmed at 2 min at 150°C, 20°C/min to 300°C and 10 min at 300°C.

Optimization studies

The reaction mixtures were incubated at the above-mentioned conditions but at different (i) reaction times (0, 5 minutes, 15 minutes, 30 minutes, 1h, 2h, 3h, 4h, 5h, 6h and 8h); (ii) temperature (30ºC, 40ºC, 50ºC, 60ºC and 70ºC) and (iii) amount of lipase (2.5, 5.0, 10.0, 15.0, 25.0, 50.0, 100.0, 200.0 and 300.0 mg). The percentage conversion of the product was determined as described earlier.

RESULTS AND DISCUSSIONS

Analysis of the Products

The product of the reaction after incubation was ascertained by thin layer chromatography and the ester spot showed a retention time of 16.290 min for wax ester of oleyl palmitate when detected with gas chromatography. Comparison with the known standard showed that the ester was oleyl palmitate. A typical gas chromatogram of the products of the esterification reaction is shown in Figure 1. Based on Figure, Peak A represents the solvent (hexane), peaks B, C and D are oleyl alcohol, palmitic acid and oleyl palmitate at 4.868 min, 6.756 min and 16.290 minutes respectively.

Infrared Spectroscopy

Product formation and reactant disappearance for esterification of oleyl palmitate ester was monitored by Infrared spectroscopy. Figure 3 shows the spectrum of reaction mixture alter incubation for oleyl palmitate ester. They were 3 major peaks observed for oleyl palmitate ester which are O-H stretching, C=O stretching and C-O stretching. O-H stretching was found at 3352 cm^{-1},

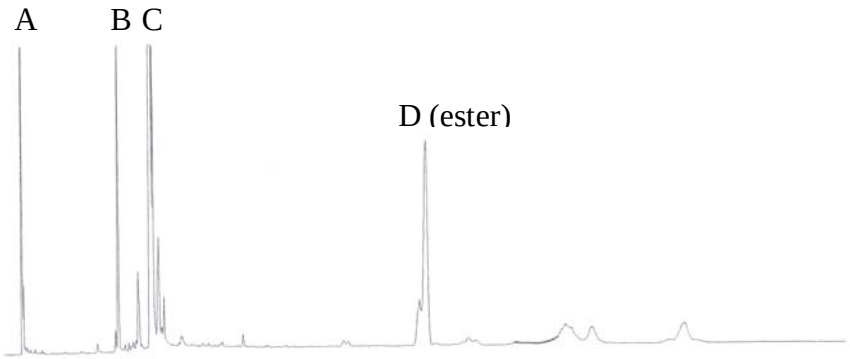

Fig. - 1: Gas chromatogram of oleyl palmitate ester A = Solvent (hexane), B = Oleyl alcohol, C = Palmitic acid, D = Ester of Oleyl Palmitate

C=O stretching at 1738 cm^{-1} and C-O stretching at 1774 cm^{-1}. The existence of ester was shown by absorption peaks of C-O and C=O.

Optimization studies

Effect of reaction time

Time course study provides insight into performance of an enzyme as the reaction progress. Such progress curves help determine the shortest time necessary for obtaining good yields and so enhance cost effectiveness of the process. Fig. - 3 shows the time course for enzymatic synthesis of oleyl palmitate ester in hexane catalyzed by Novozyme. The percentage conversion was

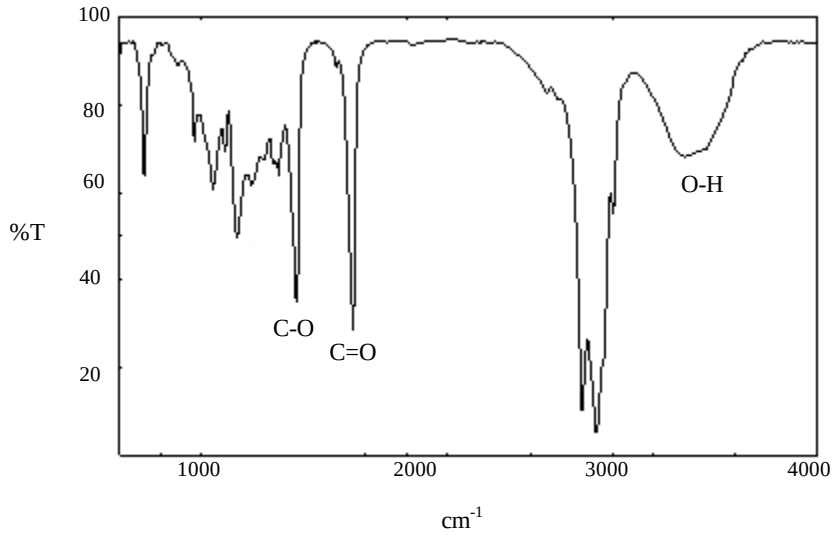

Fig. - 2: Fourier transform infrared spectrum of oleyl palmitate ester

increased with increasing reaction time. The rate of esterification reaction increased rapidly within the first 1h. After which, not much difference in percentage conversion was observed. The maximum conversion was achieved after 1 h reaction, which 80% conversion using Novozyme. However, the yield could not be improved when the incubation time was above 1h. An increased in incubation time to 3h slightly decreased the percentage conversion of the product. This may be due to the reaction has achieved the equilibrium state where the rate of forward reaction is equal to the rate of backward reaction; hence the concentration of the product was unchanged. As the reaction progressed, substrate concentration decreased which led to a fall in the degree of saturation of the enzyme with substrate.

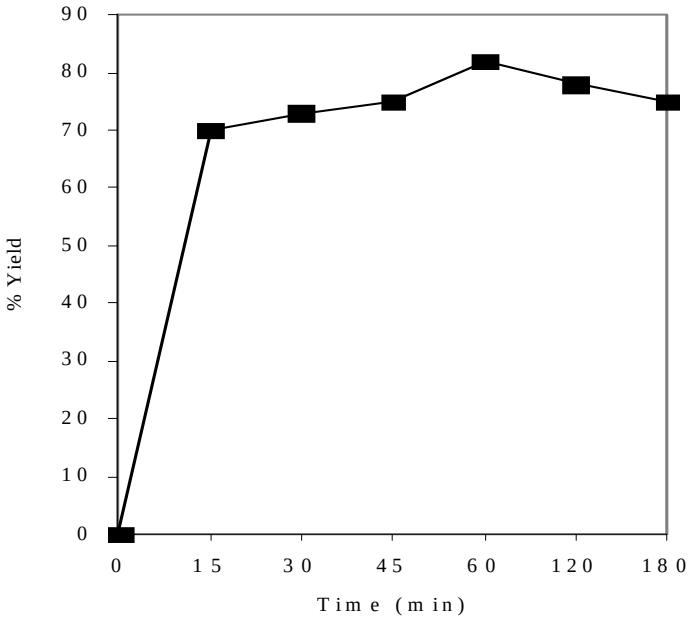

Fig. - 3: Effect of contact time on the esterification of oleyl palmitate ester

Effect of temperature

The effects of temperature can be apportioned to its effect on substrate solubility as well as its direct influences on the reaction and the enzyme. On increasing reaction temperature, substrate solubility is improved by reducing mass transfer limitations and making the substrate more available to the enzyme. Higher reaction temperature also promotes collisions between enzyme and substrate molecules to result in accelerated rates of reaction. In the present study, the influence of temperature was investigated at the temperature range from 30 to 70°C. Fig.-4 illustrates the effect of temperature on the esterification process. The percentage of conversion increased with increasing temperature and reached the maximum percentage conversion at 50°C as energy from the heat was used to increase the frequency of interaction of lipase to substrate. The conversion then was slightly reduced when the reaction temperature was increased to 60°C. This is probably because beyond a critical temperature the lipase may have been deactivated [7]. For a reaction using Novozyme as catalyst, 80% conversion was detained at 50°C.

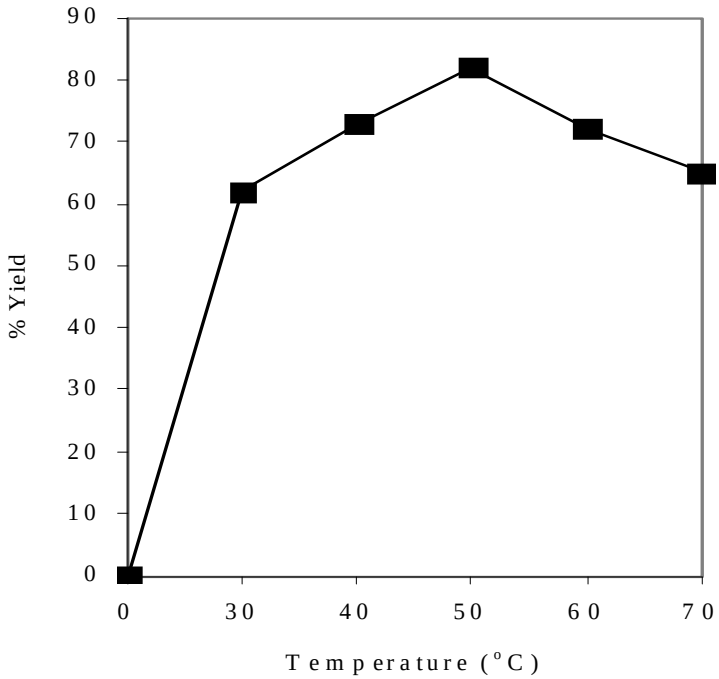

Fig. - 4: Effect of temperature on the esterification of oleyl palmitate ester

Effect of amount of lipase

From an applied point of reaction, the substrate concentration should be as high as possible to obtain a higher degree of esterification. Simultaneously, the amount of immobilized enzyme used should be as low as necessary to obtain the desired result. In term of production cost, the impact of the amount of enzyme is crucial. The effect of amount of enzyme was studied by varying the amount of the enzyme added to the reaction mixture. Figure 5 shows the effect of amount of enzyme on esterification of oleyl alcohol and palmitic acid. In this study, the percentage conversion of ester increased rapidly and reached maximum conversion at 100 mg of enzyme. However, in addition of enzyme, the conversions remain constant thereafter. This may be due to the limiting effect of the substrate. The findings of Basri *et al*.[8] indicated that, very little increased in yield were observed for enzyme loading higher than 100 mg. The excess of enzyme did not contribute to the increase in percentage conversion.

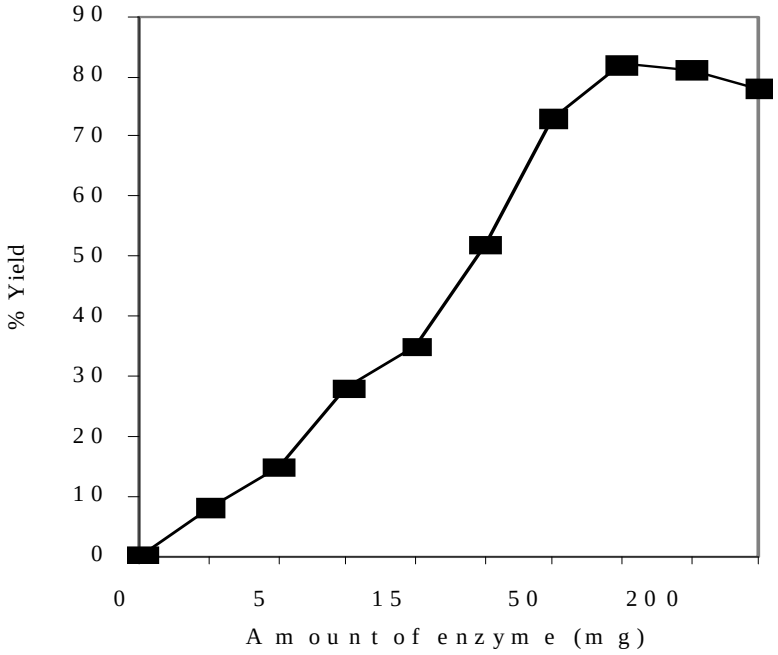

Fig. - 5: Effect of amount of enzyme on the esterification of oleyl palmitate ester

CONCLUSIONS

This work suggests that wax esters can be synthesized from oleyl alcohol and palmitic acid by Novozyme with high percentage of yield. Application of this process will produce high value-added product.

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