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Lipase-Catalyzed Synthesis of Wax Ester

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Abstract. Five immobilized lipases were tested for their ability to catalyze the alcoholysis reaction of triolein and oleyl alcohol to produce oleyl oleate, a wax ester. Lipase AH, LipasePS-C, Lipozyme and Novozyme showed relatively higher specific activities. Lipozyme and Novozyme were then used to investigate the effect of continuously controlled water activity and the effect of adding silica gel on the reaction system. It was found that the reaction system using Lipozyme and Novozyme were best carried out at a_w=0.33. The amount of silica gel needed for both Lipozyme and Novozyme to reach optimal yield of ester are 0.25 g and 0.50 g; respectively. A reaction system at optimum condition was carried out and the percentage yield of oleyl oleate produced was 75.66%.

Abstrak. Lima jenis lipase sekatgerak telah diuji kebolehannya untuk memangkinkan tindakbalas alkoholisis diantara triolein dan alkohol olil untuk menghasilkan olil oleat, sejenis ester lilin. Lipase AH, Lipase PS-C, Lipozim dan Novozim telah menunjukkan aktiviti spesifik yang tinggi. Lipozim dan Novozim seterusnya telah digunakan untuk mengkaji kesan aktiviti air berterusan dan kesan penambahn gel silika keatas sistem tindakbals. Hasil yang didapati telah menunjukkan bahawa tindakbalas terbaik bagi sistem yang bermangkinkan Lipozim dan Novozim adalah pada nilai aktiviti air = 0.33. Manakala amaun gel silika yang diperlukan didalam sistem tindakbalas untuk mencapai keadaan optimum adalah 0.25g untuk Lipozim dan 0.50g untuk Novozim. Suatu sistem tindakbalas telah dijalankan pada keadaan optimum dan peratus hasil bagi olil oleat yang diperolehi adalah 75.66%.

Key words : alcoholysis, immobilized lipases, screening, water activity, silica gel

Introduction

Wax ester, long-chain esters which are derived from fatty acids and alcohols both with chain lengths of 12 carbons or more, have potential applications as lubricants, plasticizers and cosmetics [1]. These kind of esters have excellent wetting properties at interfaces. Two good examples of naturally occuring wax ester are jojoba oil and sperm whale oil. However, the supply of jojoba oil was inconsistent and sperm whale oil is depleting, so a substitute for this wax ester is desired.

Esterification and alcoholysis are the main reaction catalyzed by lipase to produce synthetic wax ester [2]. Lipases offer the advantages of catalysis under mild reaction conditions such as at atmospheric pressure, moderate pH and temperature. Lipases are also environmentally friendly, non-toxic and biodegradable. This is especially important because the mild reaction conditions reduce the side reactions and the resulting formation of by-products to a minimum.

In this paper, the alcoholysis reaction of triolein and oleyl alcohol was carried out to produce oleyl oleate, a wax ester. The first part of the work is screening of commercially available lipases for the best catalyst for the synthesis of oleyl oleate. The following part is to study the importance of water and silica gel to the reaction system. Finally, a reaction was carried out at optimal reaction condition using the best immobilized lipase.

Experimental Procedures

Materials.

Lipozyme and Novozyme were obtained from Novo Nordisk (Denmark). Lipase AK, Lipase AY-C and Lipase PS-C were obtained from Amano Int. (Japan). Oleyl alcohol and triolein were obtained from Fluka Chemika (Switzerland). All other reagents were of analytical grade.

Alcoholysis reaction.

The reaction mixture consisted of triolein (1 mmol), oleyl alcohol (3 mmol), Lipozyme (0.30 g) and hexane was added to a total volume of 10 ml. The reaction mixtures were put in vials and were incubated at 40 ^{0}C for 5 h with continuous shaking at 150 rpm in a horizontal shaker waterbath.

Screening of enzyme for alcoholysis reaction.

The alcoholysis reaction was performed as described earlier using the following immobilized lipases: Lipozyme, Novozyme, Lipase AK, Lipase AY-C and Lipase PS-C.

Effect of continuously controlled water activity on the alcoholysis reaction.

The reaction mixture of samples and controls were prepared as described earlier but 1.25 g of each hydrated salt pair (Table 1) was added into the reaction mixture.

Table 1 : List of various hydrated salt pair [3]

Hydrated salt pair	a_w
$LiSO_4.H_2O/LiSO_4$	0.128
$NaCO_3.H_2O/NaCO_3$	0.32
$Ba(OH)_2.8H_2O/Ba(OH)_2.H_2O$	0.40
$Na_4P_2O_7.10H_2O/ Na_4P_2O_7$	0.59

Effect of adding silica gel on the alcoholysis reaction.

The effect of adding silica gel on alcoholysis reaction was studied by varying the quantities of silica gel (0.00 g, 0.25 g, 0.50 g, 0.75 g, 1.00 g) and in the same condition as described earlier.

Alcoholysis reaction at optimal reaction condition.

The alcoholysis reaction was carried out using Lipozyme at the optimal conditions that is incubation period, 5 h; amount of enzyme, 0.30 g; temperature, 50 ^{0}C, molar ratio of substrates, 6 in hexane [4]. The initial water activity and continuously controlled water activity were 0.328 and 0.33; respectively with amount of silica gel, 0.50 g.

Determination Percentage Yield of Products.

The percentage yield (%) of product was calculated as below:

$$\% \text{ yield} = \frac{\text{no. of mole of ester produced}}{3 \times \text{initial no. of mole of triolein used}} \times 100\%$$

Analysis of products.

All samples were assayed in triplicate. The product of synthesis reaction (100 µl) was mixed with the same amount of internal standard (tricaprylin). The sample was then silylated using 500 µl pyridine, 200 µl hexamethyldisilazane (HDMS) and 100 µl trimethylchlorosilane (TMCS). The samples were then identified on a gas Chromatography (Shimadzu 9A, Kyoto, Japan) which was equipped with an Rtx-75TG polar column (0.32 mm internal diameter, 30 m column length, 0.10 µm film thickness) from Restek Corp. (Bellefonte, PA) and flame ionization detector (FID). Helium was used as carrier gas with flow rate 50.0 mL/min. The split ratio used was 50:1. The injector and detector temperatures were set at 385 ^{0}C. The initial column temperature was 180 ^{0}C, and the final temperature was 370 ^{0}C. The amounts of product formed were determined using the internal standard method. The mole percentage of the products formed was expressed as a percentage of number of moles of initial triolein used.

Results and Discussion

Screening of immobilized lipases for the alcoholysis reaction.

The results of the screening studies are summarized in Table 2. Lipase AH, Lipase PS-C, Lipozyme and Novozyme showed relatively higher specific activities compared to Lipase AY-C. The specific activities of the immobilized lipases could be dependent on the source of the lipases and the support used for immobilization. The relatively higher activities for Lipase PS-C, Lipozyme and Lipase AH as compared to the others are in agreement with Decagny *et al*. [5] as they are from the same source/species. The supports may also play an important role to influence the activity of the lipases. The supports used are mainly polar support such as macroporous anionic resin, macroporous acrylic resin and ceramic particles. This is because polar supports are known to give higher activity in catalyzing the enzymatic catalysis reaction [6]. Lipozyme and Novozyme were used in the subsequent studies to their availability.

Table 2: Specific activity of various immobilized lipases on the alcholysis reaction

Immobilzed lipases	Specific activity (mmol/minute/mg protein)
Lipase AH	0.2805
Lipase PS-C	0.2705
Lipozyme	0.2241
Novozyme	0.2110
Lipase AY-C	0.0774

Effect of continuously controlled water activity on the alcoholysis reaction.

Figure 1 shows the effect of continuously controlled water activity. The percentage yield of oleyl oleate increased as the a_w of the salt hydrates increased from a_w of 0.128 ($LiSO_4.1/0H_2O$) to a_w of 0.32 ($NaCO_3.1/0H_2O$) for both Lipozyme and Novozyme.

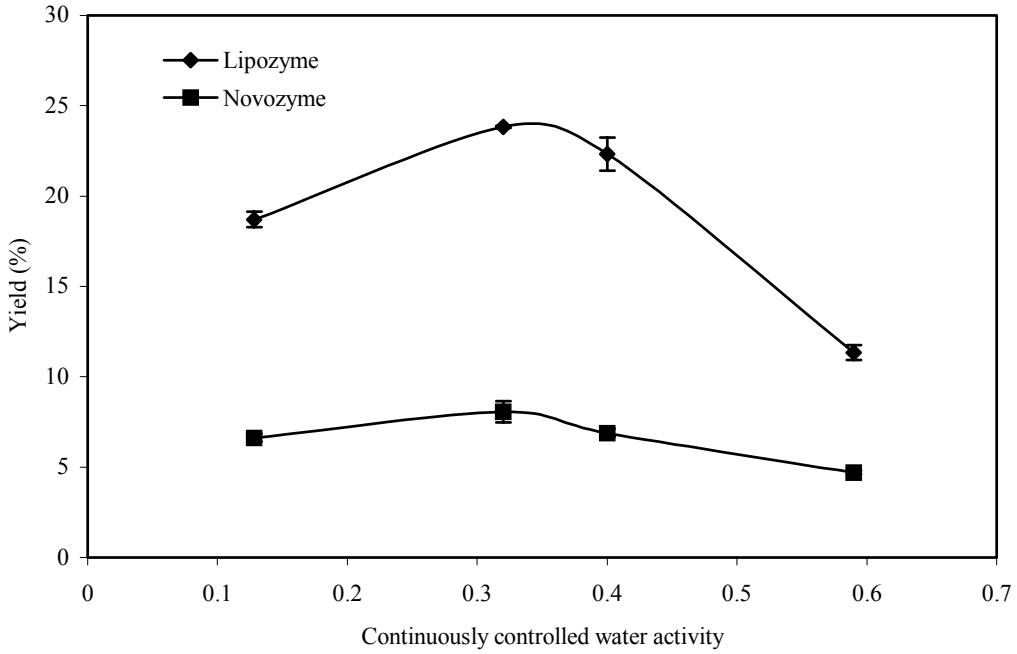

Figure 1 : Effect of continuously controlled water activity on the alcoholysis reaction of triolein and oleyl alcohol catalyzed by Lipozyme and Novozyme

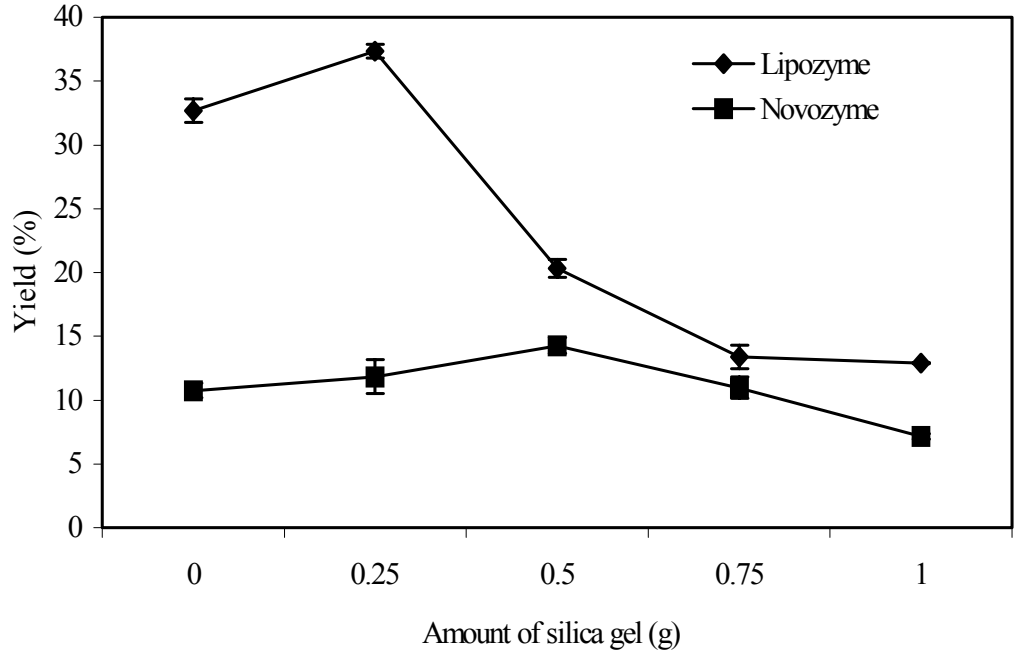

Figure 2 : Effect of amount of silica gel on the alcoholysis reaction of triolein and oleyl alcohol catalyzed by Lipozyme and Novozyme

Subsequently, the percentage yield of oleyl oleate consistently decreased as a_w of salt hydrates increased for both Lipozyme and Novozyme.

The result is in agreement with initial water activity studies [4] as Lipozyme and Novozyme tolerate low water activity probably due to the enzyme itself needs only a small amount of water to maintain its essential water layer that is important for catalytic activity [7]. Moreover, high water level could also reduced the reaction rate because the suspended catalyst particles aggregate together, leading to diffusional limitation of reaction [8].

Effect of adding silica gel on the alcoholysis reaction.

As illustrated in Figure 2, the percentage yield of ester increased as the amount of silica gel was increased from 0.00-0.25 g for Lipozyme. Subsequently, the percentage yield of ester decreased as the amount of silica gel was increased further. For Novozyme, the percentage yield of ester increased as the amount of silica gel increased up to 0.50 g and subsequently decreased as the amount of silica gel increased. In both cases, the percentage conversion of oleyl oleate increased as the amount of silica gel added increased until it reached the optimal amount. Above the optimal amount of silica gel, the percentage yield of oleyl oleate decreased.

As the alcoholysis of triglycerides produced not only wax esters, but also monoglycerides, diglycerides and glycerol; glycerol could accumulate around the enzyme and/or shift the thermodynamic equilibrium by competing with alcohol (substrate) [9]. The used of adsorbents to adsorb glycerol have been investigated by Stevenson *et al*. [10]. However, water which was bound to silica gel will be displaced by the glycerol and being used by the enzyme to hydrolyze some of the ester bonds which means that the presence of excess adsorbent will increase the level of free fatty acid in the mixture [10]. Poisson *et al*. [9] reported that addition of silica gel to adsorb glycerol did not provide a large improvement in their process to produce various wax esters from milk fat and oleyl alcohol through alcoholysis reaction.

Analysis of products.

For the analysis of the reaction mixture at optimal reaction conditions using gas chromatography, the percentage yield of oleyl oleate was 75.66 %. From the result, it shows that Lipozyme can be used to synthesize oleyl oleate with high yield in a short time even though Decagny *et al*. [5] have reported that 150 h is required to synthesize about 35% of stearyl oleate using the same enzyme. This shows that high yield of product can be achieved by studying the optimal conditions of the reaction.

Conclusion

Lipozyme can be used to synthesize oleyl oleate, a wax ester at high yield in relatively a short time at a controlled water activity and with a controlled addition of silica gel to absorbed the glycerol.

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