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WAX ESTERS PRODUCTION BY ALCOHOLYSIS OF PALM OIL FRACTIONS

Erin Ryantin Gunawan* and Dedy Suhendra

Chemistry Department FMIPA Universitas Mataram
Jl. Majapahit No. 62 Mataram 83125

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ABSTRACT

The lipase synthesis of wax esters using palm oil fractions (palm oil and palm kernel oil) and long chain alcohol as substrates was carried out. The present work focuses on the synthesis of wax esters using Lipozyme. Five parameters such as reaction time, temperature, amount of enzyme, molar ratio of substrates and various organic solvents of the reaction system were investigated. The optimum yields were achieved at the reaction temperature of 40 – 50 °C for palm oil (PO) and 40 °C *for palm kernel oil (PKO) alcoholysis, a reaction time of 5 – 7 h for PO and 7 – 10 h for PKO alcoholysis, 0.15 g of enzyme for both PO and PKO alcoholysis, molar ratio at 3:1 (alcohol: PO or PKO), and the best solvent for the reactions was hexane. Percentage yields of esters obtained at these optimum reaction conditions was 83% refined, bleached and deodorized (RBD) palm oil alkoholysis and 87% for RBD palm kernel oil alcoholysis respectively*

Keywords: *palm oil, palm kernel oil, enzymatic, alcoholysis, wax ester, oleyl alcohol.*

INTRODUCTION

Palm oil is a good substrate for esters synthesis to produce palm-based esters, a type of wax ester. Like other vegetable oils, triacylglycerol (TAG) is the major constituents of palm oil. Over 95% of palm oil consists of TAG. Palm oil (PO) and palm kernel oil (PKO) are two types of oils/fats that can be extracted from oil palm fruit. Semi solid at room temperature, these oils/fats can be fractionated into solid and liquid fractions to yield stearin and olein, respectively. They can also be processed through physical and chemical refining, to yield either refined, bleached and deodorized (RBD) or neutralized, bleached and deodorized (NBD) oils. Combination of these processes lead to various types of palm oil/ palm kernel oil products (POP) [1].

Most of the fatty acids of palm oil are present as TAGs. The different placement of fatty acids and fatty acid types on the glycerol molecule produces a number of different TAGs. The proximate concentration of fatty acids (FAs) in palm oil is as follows; Saturated: lauric C12:0 (0.1-0.3%), myristic C14:0 (0.9-1.5%), palmitic C16:0 (39.2-45.2%), stearic C18:0 (3.7-5.1%), Monounsaturated: oleic C18:1 (37.5-44.1%), Polyunsaturated: linoleic C18:2 (8.7-12.5%). For palm kernel oil the fatty acid content is; Saturated: caproic C6:0 (0.3%), caprylic C8:0 (4.4%), capric C10:0 (3.7%), lauric C12:0 (48.3%), myristic C14:0 (15.6%), palmitic C16:0 (7.8%), stearic C18:0 (2.0%), Monounsaturated: oleic C18:1 (15.1%), Polyunsaturated: linoleic C18:2 (2.7%) [2].

Wax esters are long chain esters that are derived from fatty acids and alcohols with chain lengths of 12 carbons or more [3]. The cosmetic industry appears to be the principal market for wax ester products as around 2,000 tones per year are thought to be utilized by this industry. This equates to almost 80% of total market share. The other major industry is pharmaceutical sector. Lubricant applications provide a market for around 100 tones of wax ester annually.

Wax esters can be extracted from animals and plant materials such as beeswax [4], sperm whale and jojoba oil [5]. However, they are often either too scarce or expensive for commercial use and the main obstacles to large-scale use them are its availability [6-7].

Wax esters can be synthesized chemically [8-9] and enzymatic methods [10-12]. However, 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 [7]. Meanwhile, the enzymatic synthesis has attracted attention because of the mild reaction conditions under which an environmentally friendly process. Furthermore, the use of immobilized enzymes can withstand high temperature and organic solvents that are normally used in industrial process.

In this work, the substrates used on the synthesis of wax esters were palm oil or palm kernel oil and long chain alcohols catalyzed by lipase. The effects of various parameters on alcoholysis reactions were investigated.

EXPERIMENTAL SECTION

Material

Immobilized lipase from Mucor miehei (Lipozyme) were purchased from Novo Nordisk (Denmark). Palm

* Corresponding author.
Email address : erinryantin@yahoo.com

oil and palm kernel oil were obtained from Southern Edible Oil Sdn. Bhd. (Malaysia). Oleyl alcohol was obtained from Fluka Chemika (Switzerland). Oleyl laurate, oleyl myristate, oleyl palmitate, oleyl caproate, oleyl caprylate, oleyl caprate, oleyl stearate, oleyl oleate, oleyl linoleate and methyl linoleate were obtained from Sigma Aldrich (USA). Hexane, ethyl acetate, chloroform, heptane, nonane and isooctane were obtained from J.T. Baker (USA). All other chemicals were of analytical grade.

Procedure

Alcoholysis reaction

The reaction mixture consisted of PO or PKO (1 mmol), oleyl alcohol (3 mmol) and Lipozyme (0.15 g), unless otherwise stated. Hexane was added to a total volume of 10 mL. The reaction mixture was incubated in a horizontal shaker water bath with a speed of 150 rpm at a desire temperature for 24 h unless otherwise stated. The control experiments were carried out without enzyme. The reaction was terminated, by separating the enzyme from the mixture (using Whatman no.1 filter paper for about 2 min). The preparation of reaction series for the optimization studies follows the above method unless otherwise indicated. All experiments were carried out in triplicates. The control experiments were carried out without enzyme.

Product Isolation and Purification

After the enzyme was removed by filtration, the solvent was removed by rotary evaporation. The crude product was then subjected to column chromatography.

Identification of the reaction mixtures

TLC is a preliminary method to identify the ester. Each isolated fraction from column chromatography was spotted on thin layer chromatography (TLC) sheet, coated with silica gel (silica gel 60 $_{FTLC}$ plastic sheet, Merck Germany). The plate was then developed with solvent system of hexane – dry ether (8:1.5, v/v) the esters were detected as brown spots when visualized by iodine.

Reaction mixture analysis

The esters were analyzed using a gas chromatograph (Hitachi model G-3000, Tokyo, Japan). Separations were performed on an Rtx-65TG capillary column (30m x 0.25 mm, Suppelco, USA). Helium was used as the carrier gas at a flow rate of 30 mL/min. The temperature was programmed at 2 min at 150 ºC, 20 ^{0}C/min to 300 ºC and 10 min at 300 ºC. The product composition was quantitated by an internal standard method with methyl linoleate as the internal standard. The concentration of esters was calculated by the equation:

$$C_X = \left(\frac{A_x}{A_{IS}}\right) x \left(\frac{D_{Rf\,IS}}{D_{Rfx}} C_{IS}\right)$$

where C is the amount of component *X* or internal standard, A is ×response factor for component *X* or internal standard ($D_{Rf\,x} = A_x/C_x$ and $D_{Rf\,IS} = A_{IS}/C_{IS}$). The relative response of component $D_{Rf} = D_{Rfx}/D_{Rf\,IS}$

Effect of reaction time

A series of reactions catalyzed by 0.15 g Lipozyme IM, incubated at 40 ºC with the shaking speed of 150 rpm at various reactions periods (0, 1, 2, 3, 4, 5, 7, 10, 15, 18, 21 and 24 h) were carried out to find the effect of reaction period on the percentage of conversion. After completion of each assigned period, the percentage yield was determined using GC.

Effect of reaction temperature

To study the effect of reaction temperature, another series of samples were incubated in a water bath shaker at 150 rpm for 24 h and at different reaction temperatures (30, 40, 50, 60 and 70 ºC). The amount of enzyme used was 0.15 g.

Effect of amount of enzyme

The reaction mixtures containing different quantities (0.02, 0.05, 0.10, 0.15, and 0.20) of enzyme were incubated at 40 ºC for 24 h with shaking speed of 150 rpm. The concentration of palm oil (1 mmol) and oleyl alcohol (3 mmol) were kept constant.

Effect of molar ratio of substrate

The percentage yield was carried out using different mole ratios of the reactants but the concentration of the lipase and other parameters were kept constant. The concentration of substrate (1 mmol) and the amount of oleyl alcohol were varied from 1 – 10 mmol. The reaction mixtures were incubated at 40 ºC for 24 h with the shaking speed of 150 rpm. The amount of the enzyme used was 0.15 g.

Effect of various organic solvents

The effect of various organic solvents in the reaction mixtures was studied. The solvents used were acetonitrile (log P = 0.33), ethyl acetate (log P = 0.68), chloroform (log P = 2.00), hexane (log P = 3.50), heptane (log P =4.00), isooctane (log P = 4.50) and nonane (log P = 5.00).

RESULT AND DISCUSSION

Analysis of Products

The alcoholysis of PO or PKO was carried out by a one-step lipase catalyzed reaction. The oil was reacted rapidly with oleyl alcohol to give esters as the

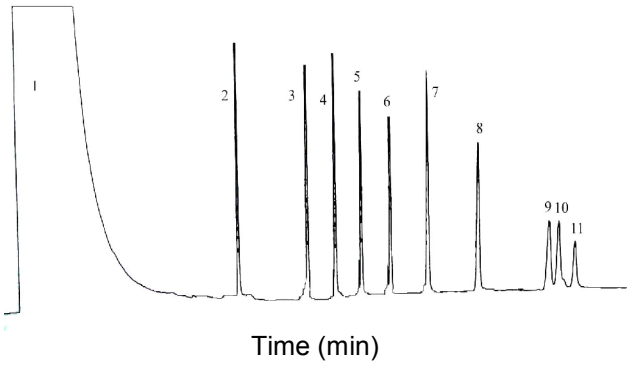

Fig 1. Chromatogram of ester standards: 1 solvent; 2 internal standard; 3 oleyl caproate (Rt = 8.1 min); 4 oleyl caprylate (Rt = 8.8 min); 5 oleyl caprate (Rt = 9.5 min); 6 oleyl laurate (Rt = 10.5 min); 7 oleyl myristate (Rt = 11.2 min); 8. oleyl palmitate (Rt = 12.6 min); 9 oleyl stearate (Rt = 14.5 min); 10 oleyl oleate (Rt = 14.7 min); 11 oleyl linoleate (Rt = 15.1 min)

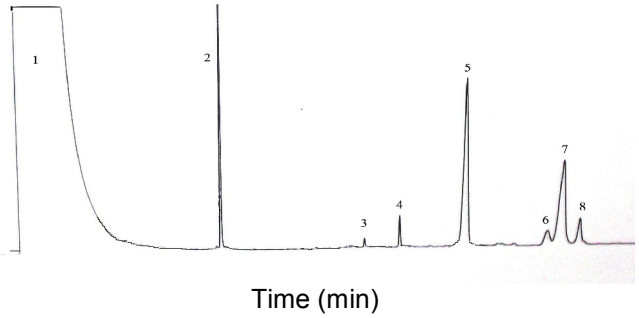

Fig 2. Chromatogram of esters from alcoholysis of palm oil: 1 solvent; 2 internal standard (Rt = 6.6 min); 3 oleyl laurate (Rt=10.7 min); 4 oleyl myristate (Rt = 11.8 min); 5 oleyl palmitate (Rt = 13.8 min); 6 oleyl stearate (Rt = 16.1 min); 7 oleyl oleate (Rt = 16.6 min); 8 oleyl linoleate (Rt = 17.0 min)

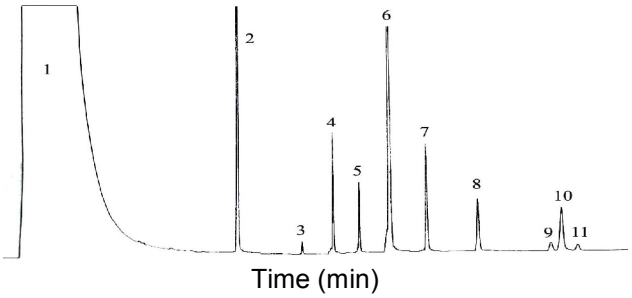

Fig 3. Chromatogram of esters from alcoholysis of palm kernel olein: 1 solvent; 2 internal standard (Rt = 6.3 min); 3 oleyl caproate (Rt = 8.0 min); 4 oleyl caprylate (Rt = 8.8 min); 5 oleyl caprate (Rt = 9.5 min); 6. oleyl laurate (Rt = 10.3 min); 7 oleyl myristate (Rt = 11.2 min); 8 oleyl palmitate (Rt = 12.5 min); 9 oleyl stearate (Rt = 14.4 min); 10 oleyl oleate (Rt = 14.7 min); 11 oleyl linoleate (Rt = 15.1 min)

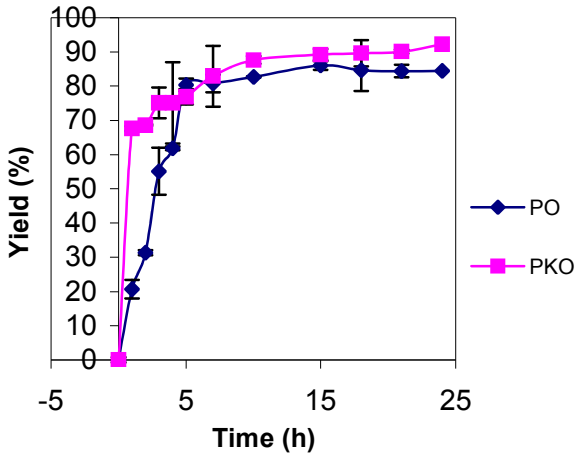

Fig 4. Effect of reaction time on the total yield of PO and PKO alcoholysis. The reaction mixture consisted of palm oil/palm kernel oil (1 mmol), oleyl alcohol (3 mmol), hexane (to a total volume of 10 cm^3) and Lipozyme (0.15 g) at 40 ºC and 150 rpm.

major product. The products were identified using GC by comparing the esters with the known standard. Quantitative analysis of the product was carried out by using methyl linoleate as internal standard. The GC chromatograms of esters standard and the products are presented in Figures 1, 2 and 3.

Effect of reaction time

The time course is a good indicator for enzyme performance and reaction progress. It can pinpoint the shortest or the adequate time necessary to obtain good yields and minimize process expenses. The effect of reaction time is presented in Figure 4. The percentage yield for both alcoholysis reactions increased with increasing reaction time and gave high percentage yield within a reaction period of 5h (80.3%) for PO and 9h (87.6%) for PKO alcoholysis. This may be due to the equilibrium being achieved in that time. Alcoholysis of PKO is slower than that of PO. This is in agreement with Vaysse *et al.* [13] that show esterification of short-chain fatty acids (major component in palm kernel oil) were slower than that of long-chain fatty acids (major component in palm oil). Thus, they explained that this may be due to the higher solvation of short-chain fatty acid compared to long-chain fatty acid, that would selectivity shift the reaction equilibrium towards alcoholysis for short-chains [14] and reduce their thermodynamic activity and thus their reactivity in the competition with long chains in the reaction mixture [13].

The product yield, however, does not increase beyond that time. This may be due to (i) some mass-transfer limitations, which inevitably arise in a reaction mixture containing a high proportion of product, and (ii) the reactions achieved the equilibrium state where the rate of forward reaction was equal to the reverse reaction. Hence, the concentration of the product was

unchanged. However, there was no alcoholysis reaction activity found in the control system until 24 h. In the alcoholysis reaction between palm oil or palm kernel oil with oleyl alcohol, the products are not only esters but also glycerol. Glycerol, soluble in water, will strip the essential water of enzyme [15] and disrupt enzyme conformation causing inactivation [16]. Thus, this may inhibit the reaction by limiting the interaction of the substrate and the enzyme.

Effect of reaction temperature

Changes in the reaction temperature can affect the activity and stability of the enzymes and thus the rate of reaction. And the effect of temperature can be apportioned to its effect on substrate solubility as well as its direct influences on the reaction and the enzyme. The reaction of palm oil/palm kernel oil with oleyl alcohol was performed at five different reaction temperatures using Lipozyme as biocatalyst (Figure 5).

For palm oil alcoholysis, the enzymatic reaction showed increment of percentage yield of esters, as the temperature was increased from 30 ºC to 50 ºC. While, for palm kernel oil alcoholysis, the percentage yield of enzymatic reactions was increased with the increasing temperature (30 ºC to 40 ºC). Faciolli and Barrera-Arrelano [17] suggested that on increasing reaction temperature, substrate solubility improved reducing mass transfer limitation and making the substrate more available to the enzyme. In this research, the optimal temperature for enzymatic reactions of palm oil and palm kernel oil alcoholysis were at 50 and 40 ºC, respectively. Different optimum condition for PO alcoholysis (50 ºC) and PKO alcoholysis (40 ºC) was observed which may be due to the high content of palmitic acid (melting point at 63.1 ºC) in palm oil that require higher temperature to react.

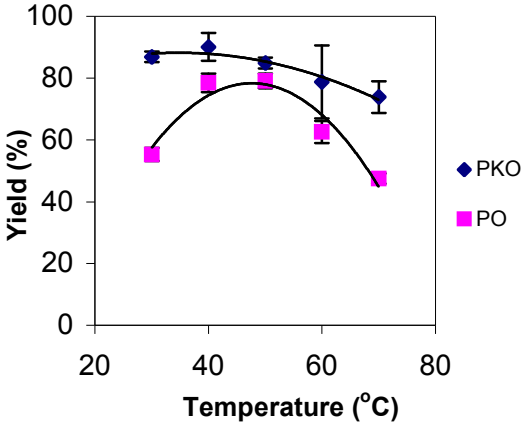

Fig 5. Effect of reaction temperature on the total yield of palm oil and palm kernel oil alcoholysis. The reaction mixture consisted of palm oil/palm kernel oil (1 mmol), oleyl alcohol (3 mmol), hexane (to a total volume of 10 cm^3) and lipozyme (0.15 g), 150 rpm for 5 h (PO) and 10 h (PKO)

At temperatures above 50 and 40 ºC, the percentage yield was decreased. This may be due to the denaturation of the enzyme at relatively higher temperature. Garcia *et al*. [18] reported that the thermal deactivation of the enzyme occurred when the temperature was higher than 50 ºC. However, Trani *et al*. [19] reported that Lipozyme showed optimum activitiy at 60 ºC. The recommended thermal stability of Lipozyme is at 50-70 ºC as suggested by the manufacturer (Novo Nordisk A/S Product Sheet B347c-GB 200, 1992). However, its action is also simultaneously influenced by other factors such as solvent hydrophobicity, type and relative amounts of reactants, enzyme amount and moisture content of reaction medium. Athawale and co-workers [20] found that at 50 ºC, the rate of transesterification of soybean and linseed oil with n-butanol increased with time up to 6 h and then slightly decreased in the next 2 h.

Effect of amount of lipase

From an applied point of view, the substrate concentration should be as high as possible to obtain a higher degree of yield. Simultaneously, the amount of enzyme used should be as low as possible to obtain the desired result. In terms of production cost, the impact of the amount of lipase used is crucial.

Figure 6 depicts the result of using different amount of lipase. The percentage yield of total esters for palm oil and palm kernel oil alcoholysis was increased with increasing the amount of enzyme used up to 1.5%. According to Rammamuthi and McCurdy [21], increased amount of lipase will lead to an increase in the esterification rate. This relationship holds when there are no limiting factors such as low substrate concentration, presence of activators, inhibitors or mass transfer effect. Further increase in the lipase to

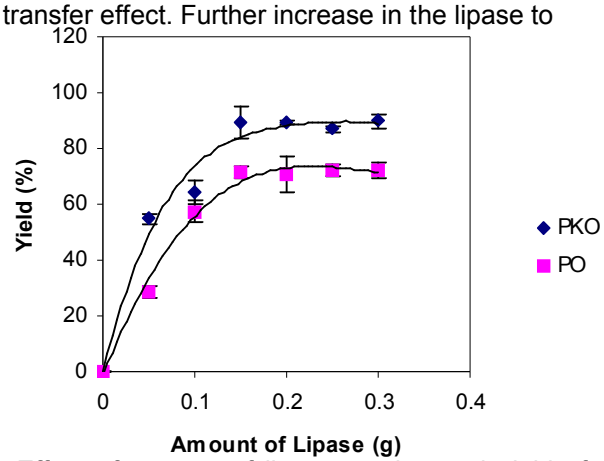

Fig 6. Effect of amount of lipase on the total yield of palm oil and palm kernel oil alcoholysis. The reaction mixture consisted of palm oil/palm kernel oil (1 mmol), oleyl alcohol (3 mmol), hexane (to a total volume of 10 cm^3) and Lipozyme at 50 ^{0}C and 150 rpm for 5 h (PO) and 10 h (PKO)

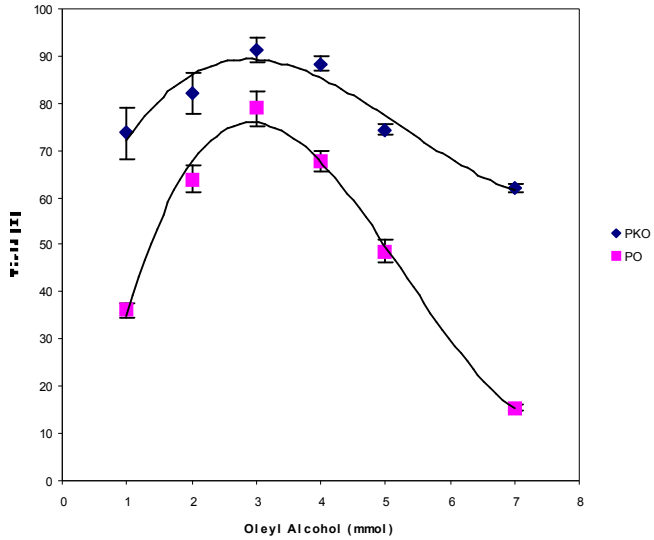

Fig 7. Effect of molar ratio of substrate on the total yield of palm oil and palm kernel oil alcoholysis. The reaction mixture consisted of palm oil or palm kernel oil (1 mmol), oleyl alcohol, hexane (to a total volume of 10 cm^3) and Lipozyme (0.15 g) and 150 rpm for 5 h (PO) and 10 h (PKO)

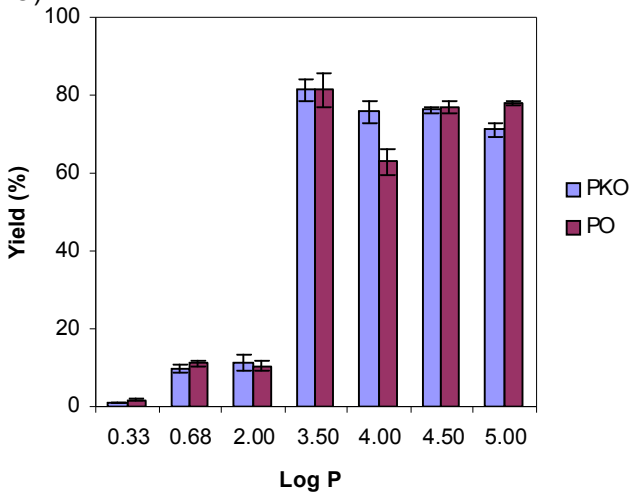

Fig 8. Effect of various organic solvents on the total yield of palm oil and palm kernel oil alcoholysis. The reaction mixture consisted of palm oil/palm kernel oil (1 mmol), oleyl alcohol (3 mmol) , solvents (to a total volume of 10 mL) and Lipozyme (0.15 g) at 50 ºC and 150 rpm for 5 h (PO) and 10 h (PKO). The solvents used were acetonitrile (log P=0.33), ethyl acetate (log P=0.68), chloroform (log P= 2.00), hexane (log P= 3.50), heptane (log P=4.00), isooctane (log P=4.50) and nonane (log P=5.00).

substrate ratio reaction does not significantly increase the yield. This can be explained by considering that the active sites of the enzyme molecules present in excess would not be exposed to the substrates and remain inside the bulk of enzyme particles without contributing significantly to the reaction [22].

Effect of molar ratio

The maximum mole ratio of substrates used is a challenge to be met in industrial implementation of any reaction. The effect of oleyl alcohol to palm oil/palm kernel oil molar ratio on the alcoholysis indicated the competitive nature of oleyl alcohol and fatty acid (in palm oil/palm kernel oil) binding (Figure 7). The molar ratio at 3:1 (oleyl alcohol: palm oil) produced the highest total percentage yield (78.9%) and decrease thereafter. However, a similar molar ratio on palm kernel oil alcoholysis produced 86.4% of total esters.

The decrease in percentage yield of esters at high oleyl alcohol concentration (4:1, 5:1, 6:1 and 7:1) may reflect the ability of the excess oleyl alcohol to distort the essential water layer that stabilizes the immobilized enzyme which could inhibit the activity of the enzyme. Steinke et al. [23] reported that lipase-catalyzed esterification of stoichiometric mixtures of long-chain and very long-chain fatty acids with the corresponding mixture of alcohols gave quantitative yields of esters. On the other hand, Salis et al. [24] found that the highest conversions (94,1%) was achieved at a 3:1 molar ratio on transesterification of sheep milk fat oil with cetyl alcohol by Lipozyme RMIM.

The result also showed that the percentage yield of palm oil ester is lower than palm kernel oil ester. This indicates that there is some effect of the length (palm kernel oil has high lauric acid/short chain content) and structure of the acid molecule (palm oil has high unsaturated fatty acids content) on the activity of the enzyme. Zaidi et al. [25] had reported that the esterification rates of oleic acid (unsaturated fatty acid) with oleyl alcohol were lower than those with butanoic acid (short chain fatty acid).

Effect of organic solvent

Two important properties of a solvent to be used in biocatalysis are its ability to solubilize the substrate and the products of the reaction, and its influence on enzyme activity and stability. A solvent must also have the ability of portioning the substrates and products into different phases. Solvent also changes the enzyme specificity, including substrate specificity, enantioselectivity, prochiral selectivity, regioselectivity and chemoselectivity.

The polarity of the various solvents in terms of their log-P values played a crucial role in the course of the alcoholysis reaction. Log P values is defined as the logarithm of the solvent partition coefficient between water and octanol. The Log P values reflect solvent hydrophobicity and the extent to which it can dissolve in water to enter the relatively polar phase around the enzyme. Solvents such as acetonitrile (log P=0.33), ethyl acetate (log P=0.68), chloroform (log P= 2.00), hexane (log P= 3.50), heptane (log P=4.00), isooctane

(log P=4.50) and nonane (log P=5.00) were chosen as solvent based on their different polarity and solubility on the substrate. The effect of various organic solvents on the synthesis of ester from palm and palm kernel oil are presented in Figures 8.

Generally, the percentage yield increases with the increase in log-P value of the solvents. The lower percentage of product formed in solvents with higher polarity was due to the ability of the more polar solvent to strip off the water layer around the enzyme molecules [26], which is essential to preserve the active spatial conformation of the enzyme [27].

When a polar organic solvent, such as acetone, used in synthesis of fatty acid methyl ester from sunflower oil by lipozyme, significantly lower conversion (20%) was detected [28]. They also suggested that in such medium, the solvent might alter the native conformation of the enzyme by disrupting hydrogen bonding and hydrophobic interactions, thereby leading to very low alcoholysis rate. Yadav and Lathi, [7] explained that lipases function better in the more hydrophobic solvents.

Hexane (log P=3.5), in this research, was found to be the best solvent in the synthesis of esters with total percentage yield from palm oil ester and palm kernel oil ester, to be 81.3 and 81.4%, respectively. This finding was in agreement with those found by Laane et al. [26]. More specifically, hexane has been reported to be the optimal solvent for lipase-catalyzed reaction. This is because nonpolar solvent (log P between 2 and 4) is weak water distorters and so distorts the enzymes active conformation only weakly [26].

Slight differences of percentage yield in solvents with log P>4 such as isooctane and nonane is probably due to the relatively high viscosity of the solvent, which hinders efficient interaction between the catalyst and substrate [23]. Chen and Wang [3] reported in their study that hexane and heptane were found to be the best solvents to synthesize ester as these solvents can eliminate the diffusional effects contributed by the substrates and the enzyme.

Alcoholysis reaction at optimum condition

The alcoholysis reaction of palm oil was carried out using the optimum condition of palm oil alcoholysis. This optimum reaction conditions involves incubation period of 5h, temperature at 50 ºC, amount of Lipozyme of 0.15 g, molar ratio of substrates 1:3 (palm oil/oleyl alcohol) and hexane as organic solvent. Meanwhile, the alcoholysis reactions of palm kernel were carried out using the optimum condition of palm kernel oil alcoholysis. Which involve incubation period of 9h, temperature at 40 ºC, amount of Lipozyme of 0.15 g, molar ratio of substrates 1:3 (palm kernel oil/oleyl alcohol) and hexane as organic solvent.

The percentage yield of palm oil esters are lower (80.7%) than palm kernel oil (84.4%). As mentioned above, palm kernels consist of the high content of lauric acid (short chain fatty acid). Basri et al. [29] explained that immobilized lipase favored the esterification of fatty acids with short chain (C_8-C_{12}). This could be due to the fact that the more rigid nature of the immobilized enzyme might restrict the longer substrates from reaching the active site.

CONCLUSION

The enzymatic alcoholysis of palm-based esters catalyzed by Lipozyme is possible with high percentage yield (more than 85%) and could be applicable to a large-scale production of esters. The influence of various reaction conditions on the alcoholysis of palm oil and palm kernel oil fractions has been described.

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