Enzymatic Synthesis of Fatty Amides from Palm Olein

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Abstract: Fatty amides have been successfully synthesized from palm olein and urea by a one-step lipase catalyzed reaction. The use of immobilized lipase as the catalyst for the preparation reaction provides an easy isolation of the enzyme from the products and other components in the reaction mixture. The fatty amides were characterized using Fourier transform infrared (FTIR) spectroscopy, proton nuclear magnetic resonance (^1H NMR) technique and elemental analysis. The highest conversion percentage (96%) was obtained when the process was carried out for 36 hours using urea to palm oil ratio of 5.2: 1.0 at 40°C. The method employed offers several advantages such as renewable and abundant of the raw material, simple reaction procedure, environmentally friendly process and high yield of the product.

Key words: palm olein, urea, fatty amides, Lipozyme

1 INTRODUCTION

Oleochemicals are important for many chemical industries as they are derived from renewable, biodegradable, environmental friendly, easily available and low toxicity raw materials. They are analogous to petrochemicals which are chemicals derived from petroleum.

Among of important oleochemicals are fatty amides and their derivatives. They have attracted much attention due to their biological activities¹⁾ and potential industrial applications such as surfactants, lubricants, cosmetics, detergents and anti-blocking agents in the plastics processing industry²⁻⁴⁾. Fatty amides are produced in thousands of metric tons a year from fatty acids by reaction with anhydrous ammonia at approximately 200°C and 345–690 kPa⁵⁾.

Fatty amides can be synthesized from fatty acids or their esters through treatments with amine compounds^{6,7)}. Enzymatic synthesis of these compounds from the same starting material has also been reported⁸⁻¹⁰⁾.

Triglycerides can be converted into their fatty amides via amidation using ammonia or amine compounds^{11,12)}. Fatty amides were synthesized from fatty acids with urea instead of ammonia or other amidation reactant using *Candida antartica* lipase (Novozyme 435) as a catalyst at 60°C for 48 h and atmospheric pressure¹³⁾. It was reported

in our previous paper¹⁴⁾ that N, N' carbonyl difatty amides were synthesized by refluxing palm oil and urea using sodium ethoxide as a catalyst in the presence of ethanol at 78°C for 8 h. Our literature search shows that there is no information regarding enzymatic synthesis of fatty amides directly from palm olein and urea.

In the present study, fatty amides are synthesized from palm olein and urea by a one-step lipase catalyzed reaction. The presence of long chains fatty acids from palm olein (mainly 16 and 18 carbon atoms) containing O and N atoms suggests fatty amides should be very useful as organic reagents for extraction and separation of metal ions from aqueous solution¹⁵⁾. Additionally, it is predicted that fatty amides will offer the potential application as surfactants for many applications including clay modification to produce polymer nanocomposites.

2 EXPERIMENTAL

2.1 Materials

Palm olein was obtained from Ngo Chew Hong Oils and Fats (M) Sdn. Bhd., Malaysia (The saponification value (mg KOH/g oil) of palm olein: 198 and the fatty acids composition: C12:0, C14:0, C16:0, C16:1, C18:0, C18:2 and C18:3).

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Lipozyme was obtained from Novo Nordisk, Denmark. Urea, sodium hydroxide and hexane were purchased through local suppliers from Merck, Germany.

2.2 Synthesis of fatty amides

Fatty amides were synthesized by reacting 3.84 g of commercial palm olein in 17 mL of hexane as a solvent with 4.20 g of urea. The pH was adjusted to 7 by adding of about 5 mL of 0.5 M NaOH. The reaction was carried out in the presence of lipase catalyst in a 100 mL stoppered flask. The mixture was incubated in water shaker batch (125 rpm) at 40°C for 36 h. Hot hexane was added to the reaction to dissolve the product. The organic phase was then separated from water phase using a separation funnel. To obtain the solid fatty amides, the hexane fraction was cooled in a refrigerator at 5°C for 5 h, filtered and then reprecipitated by hexane. The product was then dried in an oven at 50°C. The preparation reaction is shown in the **Scheme 1**.

2.3 Measurement of conversion

The conversion of palm olein into fatty amides carried out by using the optimum reaction conditions of amidolysis as presented in **Table 1**. The conversion percentage of palm olein amidolysis was calculated using the following equation (equation 1).

$$\text{Conversion(\%)} = \frac{\text{mmol FATTY AMIDES}}{\text{mmol fatty acid in palm OLEIN}} \times 100 \quad (1)$$

Depend on nitrogen content of dry fatty amides analyzed by the elemental analysis; the amount of mmol product was determined. The amount of mmol fatty acid in the palm olein was calculated from mmol of potassium hydroxide required to saponify 1 g of palm olein¹⁶.

2.4 Characterization

Qualitative analysis of amide groups of fatty amides was carried out by observing the colored complex formed after the ethanolic solution of the fatty amides reacts with copper(II). The presence of amide in fatty amides was also determined by FTIR spectra. FTIR spectra of the products were recorded by the FTIR spectrophotometer

(Perkin Elmer FT-IR-Spectrum BX, USA) using KBr disc technique

Elemental analyzer (LECO CHNS-932) was used for quantitative analysis of nitrogen contents. The determination was carried out under N₂ atmospheric conditions using sulfamethazine as the standard.

The presence of amide (CO NH) proton in fatty amides was determined by ¹H nuclear magnetic resonance (Joel, Japan) using CD₃ COOD as a solvent.

3 RESULTS AND DISCUSSION

The proposed mechanism of palm olein into fatty amides is shown in the **Scheme 2**. Using of the lipase catalyst leads to create active position at carbonyl group of triglyceride in substrate-lipase complex and allows to attack first or second the carbonyl by -NH₂ of urea molecule to form alkoxy ion, as proposed in the **Scheme 2(A)**. Alkoxy ion is less acidity than water molecule. Therefore, alkoxy ion withdraws proton from H₂O to form the alcohol and allows attacking carbonyl group of urea by hydroxyl of water forming 2- hydroxypropane-1,3-difatty ester or 3-hydroxypropane-1,2-difatty ester, carbamic acid (amino formic acid) and one molecule of fatty amide. Carbamic acid is a compound that is unstable under normal circumstances. It is rapid decomposition to form ammonia and carbon dioxide¹⁷ as illustrated in the **Scheme 2(B)**. After two consecu-

Table 1 Optimum Reaction Conditions of Conversion Palm Olein into Fatty Amides.

Parameter	Condition
Lipase	Lipozyme
Solvent	Hexane
Reaction time	36 h
Temperature	40°C
Ratio Lipozyme (mg): Palm olein (mmol)	36.7:1
Ratio urea (mmol): Palm olein (mmol)	05.2:1

R = C12: 0, C14: 0, C16: 0, C16:1, C18: 0, C18: 2, or C18: 3

Scheme 1 Fatty Amides Synthesis from Palm Olein.

Scheme 2 Proposed Mechanism of Synthesis Fatty Amides from Palm Oleic: (A) Amidation, (B) Decomposition of Carbamic Acid and (C) Amidation.

tive reactions with same manner, 2 molecules of fatty amide, glycerol, ammonia and carbon dioxide are formed (the Scheme 2(C)).

3.1 Enzyme screening

Two commercial enzymes were screened to select the most efficient enzyme for the reaction. Effect of temperature on conversion palm olein into fatty amides was studied using Lipozyme and Novozyme. In the reaction temperature range from 30°C to 60°C, reaction catalyzed by Lipozyme gave the highest percentage of conversion as shown in Fig. 1 indicating that the immobilized lipase (Lipozyme) was active for the reaction than Novozyme. Therefore, Lipozyme was used as the lipase in the subsequent experiments. It was also observed that Lipozyme is most efficiently at 40°C. The percentage of conversion of fatty amides decreases above 40°C due to destroy its catalytic activity by thermal energy¹⁸. Therefore, a reaction temperature of 40°C was used for further studies.

3.2 Effect amount of enzyme

Figure 2 illustrates the results of using different amount of Lipozyme. The percentage of conversion of fatty amides increased as the ratio of lipase: palm olein was increased. The highest conversion percentage of fatty amides was obtained when the ratio of lipase: palm olein was 36.7 mg to 1.0 mmol. Further increase of lipase to palm olein ratio did not significantly increase the percentage of conversion, as the active sites of the enzyme molecules present are not fully exposed to the substrate and remain inside the bulk of enzyme particles with out contributing significantly to the reaction¹⁹.

Fig. 1 Effect of Temperature on Conversion Palm Olein into Fatty Amides Enzymes: (a) Lipozyme and (b) Novozyme.

Other reaction conditions: urea/palm oil molar ratio = 5.2:1, hexane = 17 mL, reaction time = 36 h, pH = 7 and reaction temperature = 40°C.

3.3 Effect of organic solvents

The polarity of different solvents in terms of their log-P values played crucial role. In the course of the amidolysis reaction, various organic solvents were used for the study of the effect of organic solvent on the amidolysis of palm olein. Figure 3 shows that hexane (log-P = 3.5) was the best solvent for amidolysis of palm olein due to the reaction medium was changed from hydrophilic (log-P < 3) to hydrophobic (log-P > 3) organic solvents, the overall efficiency of the enzyme changed²⁰. Therefore, hexane was choose the solvent in the subsequent experiments.

Fig. 2 Effect of Catalyst Loading on Conversion of Palm Olein into Fatty Amides.

Other reaction conditions: urea/palm oil molar ratio = 5.2:1, hexane = 17 mL, pH = 7 and reaction temperature = 40°C.

Fig. 3 Effect of Catalyst Loading on Conversion of Palm Olein into Fatty Amides.

Other reaction conditions: urea/palm oil molar ratio = 5.2:1, hexane = 17 mL and reaction temperature = 40°C, pH = 7 and lipozyme = 0.05 mg.

3.4 Effect of reaction time

The time course of enzymatic amidolysis was shown in Fig. 4. The percentage of conversion into fatty amides increased with increasing reaction time. The highest conversion percentage was obtained when the process was carried for 36 h. The reaction rate change after 36 h was small as the reaction reached the equilibrium state. This result is in agreement with Hacking *et al.*²¹⁾

Fig. 4 Effect of Reaction Period on Conversion Palm Olein into Fatty Amides.

Other reaction conditions: urea/palm oil molar ratio = 5.2:1, hexane = 17 mL, lipozyme = 0.05 g, pH = 7 and reaction temperature = 40°C.

3.5 Effect of molar ratio of urea to palm olein

The conversion percentage of palm olein into fatty amides was highest when the ration of urea to palm olein was 5.2 mmol: 1.0 mmol as illustrated in Fig. 5. This molar ratio was lower compared to the results of Sharifah *et al.*¹⁶⁾

Fig.5 Effect of Molar Ratio of Urea to Palm Olein on Conversion Palm Olein into Fatty Amides.

Other reaction conditions: reaction temperature = 40°C, hexane = 17 ml, lipozyme = 0.05 g, pH = 7 and reaction time = 36 h.

They stated that the percentage of biosynthesis of fatty hydrazide was highest when the molar ratio of the hydrazine hydrate to palm oil was 13.4:1. A similar phenomenon was observed by Gunawan *et al.*²⁰⁾ that shows high concentration of oleyl alcohol inhibited the reaction.

Under the reaction conditions stated above, the highest conversion percentage of palm olein into fatty amides was around of 96%. The acid value of the final product was 0.41 (mg KOH/g sample). Based on a report by Osman and Nor²²⁾, the fatty acids composition of the final product is thus C12:0, C14:0, C16:0, C16:1, C18:0, C18:2 and C18:3.

3.6 Characterizations of fatty amides

3.6.1 FTIR Spectra

Characteristic bands of palm olein were observed at, 2910, 2852, 1747, cm^{-1} resulting from, C-H asymmetric stretching of CH_2 , C-H symmetric stretching of CH_2 , C=O stretching of ester (glyceride), respectively¹⁶⁾. The fatty amides spectra show absorption bands at 3312, 1624 and 1045 cm^{-1} attributed to $-\text{NH}_2$ group stretching, C=O stretching and C-N stretching of amide, respectively. The disappearance of the peak at 1747 cm^{-1} and presence of the peaks at 3312, 1626 and 1045 cm^{-1} indicate that fatty amides have been formed¹³⁾.

3.6.2 Complex formation test

Complexes of fatty amides with copper(II) is green which is a common color of the complex observed when this metal ion is reacted with amide group²³⁾.

3.6.3 ^1H NMR spectra of Fatty Amides

(400 MHz) (CD_3COOD): δ 0.88 (t, $J = 8.2$ Hz, 3H, CH_3), 1.19 (m, H, CH_2), 1.61 (2H, 2 x $\text{CH}_2\text{CH}_2\text{CO NH}_2$), 2.01 (4H, 2 x $\text{CH}_2\text{CH}=\text{CH}$), 2.42 (t, $J = 10.3$ Hz, 2H, $\text{CH}_2\text{CO NH}_2$), 5.32 (2H, $\text{CH}=\text{CH}$), 6.01 (br, s, 2H, CO NH_2).

4 CONCLUSION

This paper describes a novel method for synthesis of fatty amides from palm olein and urea by immobilized lipase in presence of hexane as an organic solvent. The method employed offers technical simplicity, high percentage of conversion, easy isolation of enzyme from the products and other components in the reaction mixture. The optimum reaction conditions were as follows: temperature 40°C, molar ratio of urea to palm olein 5.2: 1.0, amount of Lpozyme (mg) to amount of palm olein (mmol) 36.7: 1.0 and reaction time 36 h. Results of qualitative analyses of complex formation, FTIR spectroscopy and ^1H NMR technique confirm the formation of fatty amides.

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