Enzymatic hydrolysis of coconut oil using free and immobilized porcine pancreas lipases

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ABSTRACT

The aim of this study is to evaluate the effect of some factors on the hydrolysis of coconut oil (CO) in the present of two kind of enzymes, the free lipases and immobilized lipases porcine pancreas. The activities of these two lipases under the optimal hydrolysis conditions was determined.

The effects of factors on hydrolysis degree of coconut oil was investigated: the ratio of enzyme to substrate, the pH condition, and the temperature. The best conditions for the high hydrolysis degree in case of using lipase from porcine pancreas ascatalyst included: the ratio of the enzyme to substrate of 90(U/mL), and the pH condition of 8.5 at the temperature of 40°C. The best reaction condition the case of using immobilized porcine pancreas lipase as the catalyst was determined, including: the ratio enzyme to substrate of 393U/g, the pH condition of 7.5 and the temperature of 35°C. The hydrolysis degree of CO by immobilized porcine pancreas lipase was increased slower than free lipase at the first time. The highest hydrolysis degree achieved with immobilized porcine pancreas and free porcine pancreas lipase was 72.26% and 68.61%, respectively.

***Keywords**: coconut oil, hydrolysis, immobilized porcine pancreas lipase, free porcine pancreas lipase*

1. INTRODUCTION

Coconut oil (CO) is a popular agricultural product in Vietnam as well as in other tropical countries such as Philippines, Thailand, India, Indonesia, Sri Lanka, Malaysia and New Guinea. CO is composed of short and medium chain fatty acids. Coconut oil has been used for health promotion, aliment prevention and for medication [6]. Hydrolysis products from

coconut oil such as: glycerol and saturated fatty acids are mainly used in food industry, pharmaceutical and cosmetics [5].

Depending on the hydrolysis conditions, the hydrolysis products have different features. In which, there are some compounds having bioactive. Lauric acid is a medium chain fatty acid with high antibacterial characteristic [4]. The applications of lauric acid on food industry has been reported by researchers and nutrition experts [1,3].

The products from the enzymatic hydrolysis of coconut oil have higher bioactive activity than the hydrolysis products from chemical hydrolysis [2]. Enzymatic hydrolysis is an advantageous method because it can be performed at lower temperature, leading to products with fewer side product. However, lipases as well as other enzymes has low thermal stability and high cost, which limits its potential applications in industrial hydrolytic reactions. To increase the stable and lower the cost of lipase, using of immobilized lipase on several supports has been reported for oil hydrolysis reactions [7]. The aim of this study was to compare the effect of lipases from porcine pancreas and immobilized porcine pancreas on the hydrolysis of coconut oil

2. MATERIALS AND METHODS

Refined coconut oil used in this study was purchased from Tin Vui company, Vietnam. Lipase from porcine pancreas (type II, L3126, 60 U/mg) were supplied by Sigma-Aldrich Co. (USA). Carriers hydrotalcite was prepared at the Institute of Chemical Technology - Vietnam Academy of Science and Technology. Acasia gum (Instantgum^{TM}BA) (Nexira, France) was purchased from Asian Shine company. Reagents used in this study were NaOH solution of 0.1M, KOH solution of 0.1M, H_3PO_4 85%, phenolphthalein 1% in ethanol as the pH indicator, H_2SO_4 solution of 0.1N, borate buffer. These reagents were at analytical standard and these solutions were prepared as procedures described in Vietnamese standard No 4320-86.

2.1 Hydrolysis of coconut oil using free porcine pancreas lipase (PPL)

Coconut oil emulsion was prepared in borate buffer, pH from 7.5 to 9.0

A mixture of acasia gum (3g/100ml) and 30 ml of CO and buffer solution (the volume of buffer were changed respectively 15, 30, 60, 90 ml) were placed in an 250ml –Erlenmeyer flask. Emulsifying the mixture was carried out using an homogenizer in 20 minutes. To start the reaction, 1% solution of lipase porcine pancreas in borate buffer were added slowly. This mixture was stirred using magnetic stirrer for 10 minutes. The hydrolysis reaction was performed in an appropriate duration. The range of reaction duration was 5 hours at temperature of (30 - 60^{o}C). The mixture was shaken for 10 minutes in every one hour. To stop the reaction, 3ml of ethanol 99.5% were added in the reaction mixture. The effect of tree factors including the pH condition, the temperature and the concentration of enzyme in the CO hydrolysis were studied. From the output data, the change of hydrolysis degree versus the reaction time was determined.

Hydrolysis rate was calculated as the amount in milliliter of KOH 0.1M needed per a minute:

Hydrolysis rate,

$$r(\mu mol/minute) = \frac{(a-b)*0.1*1.000.000}{1000*60}$$

a: The amount of ml KOH 0.1M in sample

b: The amount of ml KOH 0.1M in blank

Hydrolysis degree (DH)%

$$DH(\%) = \frac{(a-b) \times 0.1 \times \bar{M}}{m \times 10}$$

a: The amount of ml KOH 0.1M insample

b: The amount of ml KOH 0.1M in blank

$\bar{M}$:The average molecular weight of fatty acids in coconut oil

m: The volume of coconut oil was used (g).

2.2 Hydrolysis of CO using immobilized porcine pancreas lipase on HT ace 0.15M- 500

2.2.1 Lipase immobilization

The porcine pancreas lipase was immobilized on the hydrotalcite carrier by co-precipitation method using two kinds of salt $Al(NO_3)_3$ and $Mg(NO_3)_2$ with the ratio of Mg/Al was 2/1 and intermixing acetate ion concentration of 0.15M. Then the hydrotalcite was baked at 500^{o}C for 2 hours. The lipase was mixed with hydrotalcite carrier in the borate buffer solution at pH 7.5. The mixture was stirred at the temperature of 32^{o}C and the speed of 300rpm in 5 hours. After that, the immobilized porcine pancreas lipase was separated from the solution by a centrifuge. The activity of immobilized enzyme was 1965.4U/ g.

2.2.2 Hydrolysis of coconut oil with immobilized lipase:

Hydrolysis reaction was carried out the same way with free lipase. To stop the reaction, immobilized enzyme was centrifuged at 6,000rpm for 10 minutes at the room temperature in order to separate the solution and the immobilized enzyme. Immobilized PPL was rinsed with borate buffer and reused.

2.3 Analytical methods

Acid, peroxide and iodine value of coconut oil were determined according to Vietnamese standard No 6127:2010; 6121:2010; 6122:2010

Composition and concentration of fatty acid were determined by gas chromatography according to AOAC standard No 969.33. A GC 2010 gas chromatograph (Shimadzu, Japan) equipped with FID detector was used. Separation was carried out in a TR-Fame column with dimension of 60mx 0.25mm i.d.x 0.25µm. The oven temperature profile was: 150^{o}C (3 min), 20^{o}C/min to 220^{o}C (7min), 5^{o}C/min to 240^{o}C (5min). Helium was used as a gas carrier. Samples and standard were injected at the volume of 1µl.

Analysis of variance (ANOVA) with Statgraphic Centurion XV.I software was employed to analyze the differences among group means ($P \leq 0.05$)

3. RESULTS AND DISCUSSION

3.1 Characteristics of coconut oil

Total lipid content in coconut oil was 99.25% in which free fatty acids was 0.06%. Acid, iodine, and peroxide value was 0.13mg KOH/g, 9.85g/100g, and 2.0 meq/kg, respectively.

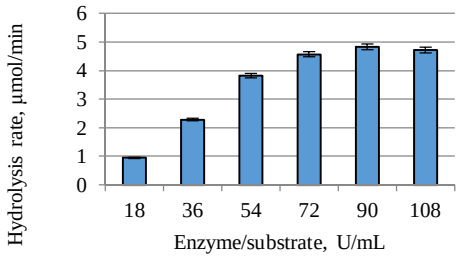
Table 1 showed that the major fatty acid in coconut oil was acid lauric (49.2%), a kind of saturated fatty acid. From the content of fatty acids, average molecular weight of coconut fatty acids was 206.99g.

Table 1. Composition and content of fatty acids in coconut oil, %

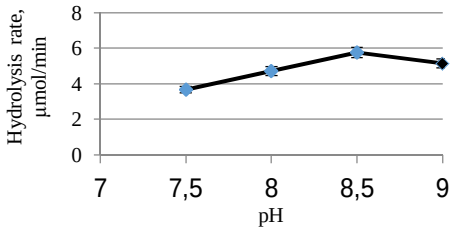
Fatty Acid	Common name	Content (%)
Octanoic acid, C8:0	Caprylic acid	10,9
Decanoic acid, C10:0	Capric acid	7,56
Dodecanoic acid, C12:0	Lauric acid	49,2
Tetradecanoic acid, C14:0	Myristic acid	17,4
Hexadecanoic acid, C16:0	Palmitic acid	7,29
Octadecanoic acid, C18:0	Steric acid	1,98
Cis-9-Octadecenoic acid, C18:1	Oleic acid	4,38
Cis- 9,12-Octadecadienoic acid, C18:2	Linoleic acid	1,28

3.2 Investigation of oil hydrolysis conditions using free PPL

a) Enzyme/substrate



b) pH



c) Temperature

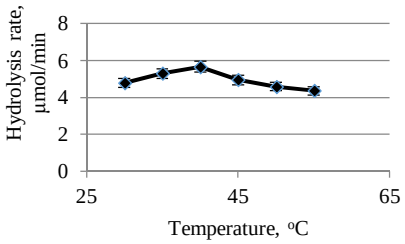
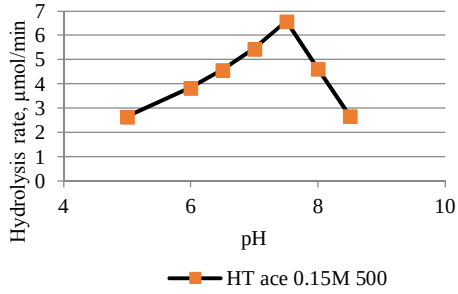


Figure 1. The ratio of enzyme/substrate (a), pH (b) and temperature (c) effect on hydrolysis of coconut oil by free PPL

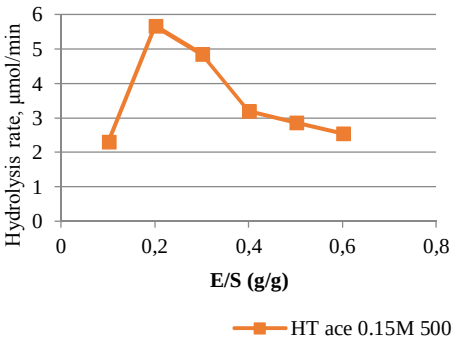
The effect of factors on the hydrolysis coconut oil using PPL as a catalyst were shown in Figure 1. Accordingly, the appropriate parameters were selected as the ratio of the enzyme to the substrate of 90U/mL, the suitable pH condition was 8.5 and the temperature was 40°C.

3.3 Investigation of oil hydrolysis conditions using PPL immobilized on HT ace- 0.15M – 500

a) Enzyme/substrate



b) pH



c) Temperature

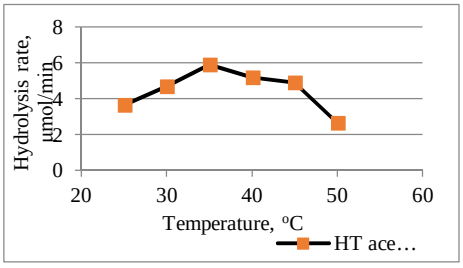


Figure 2. The ratio of enzyme/substrate (a), pH (b) and temperature (c) effect on hydrolysis of coconut oil by HT ace 0.15M 500

The effect of factors on the hydrolysis coconut oil using immobilized LPP as a catalyst were shown in Figure 2. The results showed that appropriate ratio of the immobilized enzyme to the substrate of 0.2g/g (392U/g), the suitable pH condition was 7.5 and the temperature of 35°C.

As well as free enzyme, immobilized enzyme has a pH value appropriate for the reaction. However, optimum pH for immobilized lipase was 7.5 while the free enzyme has optimum pH was 8.5. Thus, it can be said that the support has a pH condition affected on enzyme activity. The effects of the support on optimum pH of the enzyme was studied by Lee Dong-Geun *et al* (2009) with the enzyme pocine pancreas lipase immobilized on hydrophobic nano-sized magnetic particles, when examining the influence of pH on the activity of the free and immobilized lipase enzyme, resulting in optimum pH of 6.7 for PPL free while immobilized was 7.7 [8]. In our case, the optimum of pH condition of immobilized lipase was moved to rather acidify area. Thus, under the influence of the support, the nature of the enzyme also varies as the optimal parameters change as well.

3.4 Hydrolysis of coconut oil with free PPL and immobilized PPL on HT ace 0.15M -500

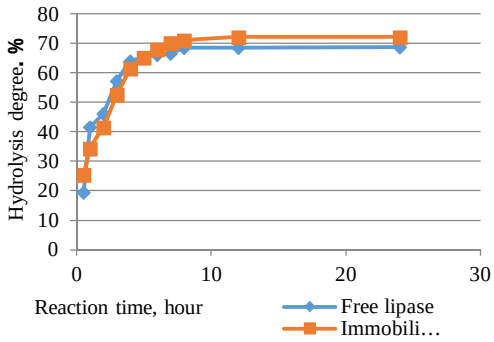


Figure 3. Hydrolysis degree (%) of CO according to the reaction time of free and immobilized PPL

Figure 3 shown that, the hydrolysis degree of CO by free PPL was higher than the one from immobilized at the first time of the reaction. However, after 8 hours of hydrolysis, the hydrolysis degree of CO by PPL was increased slowly and reached to the highest of 68.49% while the highest hydrolysis degree of the HT ace 0.15M- 500 was 72.12% after 12 hours. And after 24 hours the hydrolysis degree of these two enzyme were slightly reduced.

3.5 Reuse times of the immobilized lipase

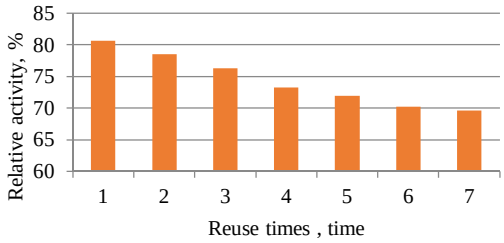


Figure 4. The reusability of immobilized lipase on the HT ace 0.15M-500

Reusability is a major advantage of using immobilized lipase. The activity of immobilized porcine pancreas lipase maintained 69.6% after being reused 7 times. This result can be compared with some other authors, Knezevic *et al.*, immobilized lipase from *Candida cylindracea* on zeolite to hydrolyze palm oil, after 7 times the reused relative activity was 13.2% [9], Li *et al.*, used lipase from *Candida rugosa* immobilized on polyacrylonitril to hydrolyze soybean oil, after 7 times of use, the activity was 62.7% [7]

4. CONCLUSION

The hydrolysis of coconut oil by PPL and HT ace 0.15M 500 had the appropriate temperature were 40°C and 35°C, respectively. The pH condition for the PPL was more alkaline (8.5) than the immobilized enzyme, which was 7.5. The ratio of PPL to substrate was 90U/mL while the ratio of immobilized lipase to substrate was 385U/g. The hydrolysis degree of CO by the free PPL was higher than that by the immobilized lipase around five hours at the first time of the reaction. The hydrolysis degree of CO by immobilized lipase was slightly raised to 72.12% after 12 hours. The activity of immobilized porcine pancreas lipase maintained 69.6% after being reused 7 times.

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Thủy phân dầu dừa bằng enzyme porcine pancreas tự do và cố định

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TÓM TẮT

Mục đích của nghiên cứu này là đánh giá ảnh hưởng của một số yếu tố đến quá trình thủy phân dầu dừa bằng hai loại enzyme porcine pancreas tự do và cố định, đồng thời xác định được điều kiện thủy phân tối ưu của hai loại enzyme này.

Một số yếu tố ảnh hưởng đến quá trình thủy phân dầu dừa bởi hai loại enzyme porcine pancreas tự do và cố định được khảo sát là: tỉ lệ enzyme đối với cơ chất, điều kiện pH và nhiệt độ. Điều kiện thủy phân dầu dừa tối ưu với xúc tác enzyme porcine pancreas tự do là: tỉ lệ enzyme/ cơ chất: 90 (U/mL), pH = 8.5, nhiệt độ 40°C. Trong khi đó, điều kiện thủy phân dầu dừa tối ưu với xúc tác là porcine pancreas cố định trên chất mang hydrotalcite là: tỉ lệ enzyme/ cơ chất: 393U/g, pH = 7.5 và nhiệt độ 35°C. Mức độ thủy phân dầu dừa bằng enzyme cố định ở giai đoạn đầu tăng chậm hơn so với enzyme porcine pancreas tự do. Hiệu suất thủy phân dầu dừa cao nhất đạt được với enzyme porcine pancreas cố định và tự do lần lượt là: 72.26% và 68.61%

***Từ khóa**: dầu dừa, enzyme porcine pancreas cố định, enzyme porcine pancreas tự do, thủy phân.*

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