

Preliminary Studies for Production of Fatty Acids from Hydrolysis of Cooking Palm Oil Using *C. rugosa* Lipase

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Abstract: *Hydrolysis of triglyceride to yield free fatty acids and glycerol from cooking palm oil have been studied for various parameters such as enzyme and oil loading, temperature, pH and agitation speed. A maximum conversion was achieved in 90 min at oil concentration of 0.1 g/ml, enzyme loading of 7.46 kLU/ml, temperature of 45°C, pH 7.5 and 200 rpm. A kinetic model based on mechanism of lipase catalyzed in oil-aqueous system using Michaelis-Menten equation was used to determine the rate constant of V_{max} and K_m , and it was found to be 370.37 mol/min mg-enzyme and 1.23 g/ml, respectively.*

Keywords: enzymatic, hydrolysis, palm oil, batch process, kinetics

Abstrak: *Hidrolisis trigliserida menghasilkan asid lemak bebas and gliserol daripada minyak masak sawit telah dikaji untuk beberapa parameter seperti beban enzim dan minyak, suhu, pH dan kelajuan pengadukan. Penukaran maksimum terhasil dalam masa 90 min pada 0.1 g/ml minyak, 7.46 kLU/ml enzim, suhu 45°C, pH 7.5 dan 200 putaran seminit. Model kinetik berdasarkan lipase dimangkirkan di dalam sistem minyak-air menggunakan persamaan Michaelis-Menten mendapati pemalar kadar untuk V_{max} dan K_m ialah masing-masing 370.37 mol/min mg-enzim dan 1.23 g/ml.*

Kata kunci: kaedah berenzim, hidrolisis, minyak kelapa sawit, proses kelompok, kinetik

1. INTRODUCTION

Production of fatty acid and glycerol from oils are important especially in oleochemical industries. Glycerol and fatty acids are widely used as raw materials in food, cosmetics and pharmaceutical industries.¹ Existing methods for production of fatty acid are based on chemical and physical methods.² The existing industrial process hydrolyzes oils to fatty acids and glycerol at temperature and pressure of 250°C and 50 bar, respectively within two hours to achieve 96%–99% conversion.^{3,4} Under these conditions, polymerization of fat and byproducts takes place resulting in extremely dark fatty acids and discolored aqueous glycerol solution. Physical refining involves subjecting the oil to steam distillation under high temperature and vacuum for removal of the free fatty

acids. Both the hydrolysis and the subsequent distillation of fatty acids to produce pure products are energy intensive process.

Nowadays, researchers have used enzyme catalyzed hydrolysis occurring at room temperature in order to reduce energy consumption and minimize thermal degradation of the products. The use of highly active lipase from *C. rugosa* has been widely studied for the purpose of fat and oil hydrolysis.^{2,5-11} The advantages of the enzyme hydrolysis technique include; the use of bio-route technology that only requires a mild temperature, simple operational procedure and low cost as well as energy consumption. Palm oil has been selected to be the main interest in this study because of Malaysia being the largest producer of oil palm plants compared to that of other vegetable oil which makes it economically intuitive to consider palm oil as the feed stock for free fatty acids production in this region.

2. MATERIALS AND METHODS

2.1 Materials

Cooking palm oil used in this study was obtained locally. Lipase (EC 3.1.1.3) from *C. rugosa* (Type VII, 746 units/mg) was purchased from Sigma-Aldrich (Japan). *Iso*-octane with 99.84% assay was purchased from Fischer Chemicals (UK). All chemicals are of analytical grade and used without further purification.

2.2 Enzymatic Hydrolysis

A stoppered 250 ml conical flask was initially filled with 3 g of cooking palm oil and 30 ml of *iso*-octane solvent. A 30 ml of phosphate buffer solution, pH 7.5 (unless otherwise stated) was added into the conical flask so that the ratio of oil to aqueous (buffer solution) is 1. The mixture formed two layers. Three other identical mixtures as above were prepared. To start the reaction, 0.3 g lipase from *C. rugosa* was added to three flasks of reaction mixtures and one left for control measurement. The mixtures were agitated in the orbital shaker (Certomat, B. Braun) at 45°C at 200 rpm. Samples were withdrawn from the oil at every 30 min from each flask.

2.3 Determination of Degree of Hydrolysis (Conversion) and Rate of Hydrolysis

The degree of hydrolysis was determined by titration of the oil phase samples with 0.1 M sodium hydroxide (NaOH). To each samples, 5 ml of the oil

phase was dissolved in 5 ml ethanol:diethyl ether (1:1% v/v). The amount of 0.1 M NaOH required to neutralize the acid was noted. A blank titration was done as control sample. Phenolphthalein was used as an indicator. The degree of hydrolysis, X is calculated as below:

$$X, \% = \frac{(\text{ml NaOH used})(\text{molarity of NaOH})(\text{average molecular weight of fatty acid})}{10(\text{weight of sample})} \quad (1)$$

The rate of hydrolysis, r was calculated as:

$$r = \frac{S_o}{W} \left(\frac{dX}{dt} \right) \quad (2)$$

where;

r = Initial rate of hydrolysis (mol/l/min)

S_o = Initial oil concentration (g/l)

W = The mean molecular weight of the fatty acid

dX/dt = Slope of degree hydrolysis versus time at $t = 0$

3. RESULTS AND DISCUSSION

3.1 Typical Hydrolysis Profile

Figure 1 shows a typical hydrolysis profile of palm oil at temperature of 45°C for an initial palm oil concentration of 0.1 g palm oil/ml *iso*-octane at agitation speed of 200 rpm and oil-aqueous ratio of 1. The enzyme used was 7.46 kLU/ml. From the graph (Fig. 1), the conversion percentage was initially increased but slowly decreased with time. The conversion of oil to the fatty acid at the interface of the oil-aqueous solution limited the surface reaction for the hydrolysis to occur. The highest percentage conversion happened after the hydrolysis reaction was carried out for 90 min. Similar hydrolysis profiles were obtained under other experimental conditions. All subsequent experiments discussed were taken at 90 min of reaction time.

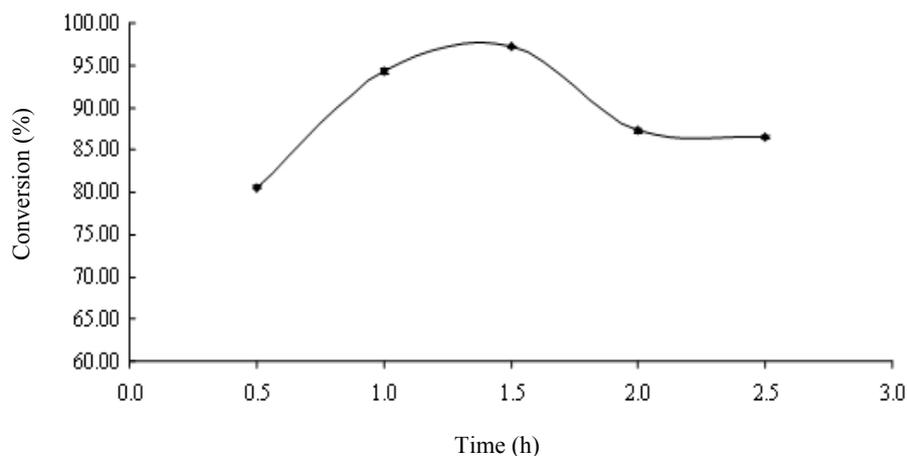


Figure 1: Typical oil hydrolysis (Temperature 45°C; E = 7.46 kLU/ml; S_0 = 0.1g/ml; pH 7.5; 200 rpm).

3.2 Effect of Enzyme Loading

The enzyme loading was varied from 3.73 kLU/ml to 14.92 kLU/ml, for cooking palm oil concentration of 0.1 g/ml and a temperature of 45°C with agitation speed of 200 rpm. Figure 2 shows the degree of hydrolysis increased as the enzyme concentration increased from 3.73 kLU/ml to 14.92 kLU/ml. However, the conversion starts to plateau with 7.46 kLU/ml enzyme loading. A similar observation was reported by other researchers for hydrolysis of various vegetable using *C. rugosa* lipase.^{3,5,8} This is due to the enzyme saturation of interface area between the oil and aqueous phase, and further increase in enzyme concentration did not give any significant changes in the reaction rate.⁸ Therefore, further increase in enzyme concentration did not give any improvement in the conversion. The optimum enzyme loading was found to be 7.46 kLU/ml.

3.3 Effect of Oil Loading

The oil loading was varied from 0.1 g/ml *iso*-octane to 0.25 g/ml *iso*-octane, for an enzyme concentration of 7.46 kLU/ml *iso*-octane and a temperature of 45°C. The degree of hydrolysis decreased as the oil concentration increased (Fig. 3). This is due to the limitation in the availability of enzyme. The active sites of lipase were saturated by the oil phase which clearly indicates the limitation of lipase has occurred. Previous researcher has reported that equilibrium conversions at oil:water ratios of 3:1 and 8:1 were 90% and 72%,

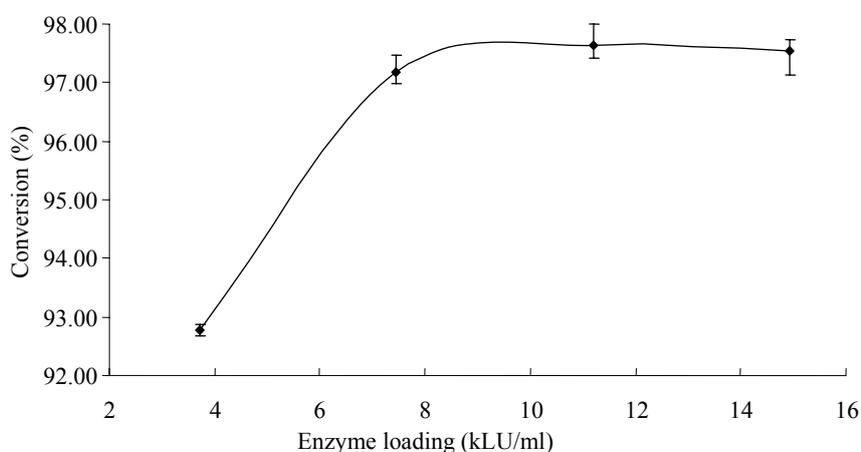


Figure 2: Effect of enzyme loading (Temperature 45°C; $S_0 = 0.1$ g/ml; pH 7.5; 200 rpm).

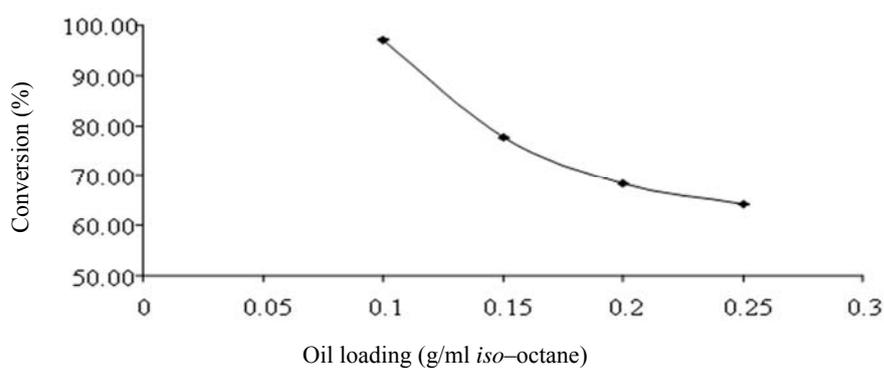


Figure 3: Effect of oil loading (Temperature 45°C; $E = 7.46$ kLU/ml; pH 7.5; 200 rpm).

respectively, and suggested that high phase ratio of oil to water will decrease the degree of hydrolysis for sunflower oil.⁸ In addition, by removing glycerol from the reaction mixture by centrifuging in between the reaction time can improve the conversion of fatty acid.⁸ The optimum oil loading is 0.1 g/ml *iso*-octane.

3.4 Effect of Temperature

The conversion of fatty acids produced from the hydrolysis of palm oil using *C. rugosa* lipase was also studied as a function of temperature (Fig. 4). Temperature was varied from 35°C to 50°C to observe the product formation.

Temperature may affect the hydrolysis reaction in a positive way or vice versa. A rise in temperature will increase the reaction rate as explained by the transition state theory. However, at a higher reaction temperature, enzyme tertiary structure may also disrupt causing it to denature.¹² This theory has been proven in Figure 4. Increasing of the reaction temperature has affected the production of fatty acids which clearly showed an increase in conversion. However, at 50°C, the conversion profile changed appreciably with low conversion values. Therefore, 45°C has been selected as an optimum temperature because after 45°C, the conversion decreased abruptly due to the enzyme denaturation process.

3.5 Effect of pH

pH plays a major role in hydrolysis reaction to achieve optimum production of fatty acids. Therefore, the effect of pH buffer used in the hydrolysis medium was investigated in the pH range of 6.5 to 8.5 with other parameters fixed. Figure 5 clearly shows that at very low pH, conversion of the hydrolysis of cooking palm oil was reduced and at a very high pH, the tendency was also give the same low conversion. The optimum pH was achieved at pH 7.5. The enzyme likely optimized its performance in an alkaline medium but nearly to neutral rather than a very acidic or alkaline medium. Enzyme is very sensitive to the operating pH medium because it might change the ionization states of the enzyme, which affect its activity and selectivity. Previously, its was also reported that the optimum pH was found at 7.5 for the hydrolysis reaction of palm oil in hexane.² About 97.4% of fatty acid was produced and pH of 7.5 was selected for hydrolysis of cooking palm oil.

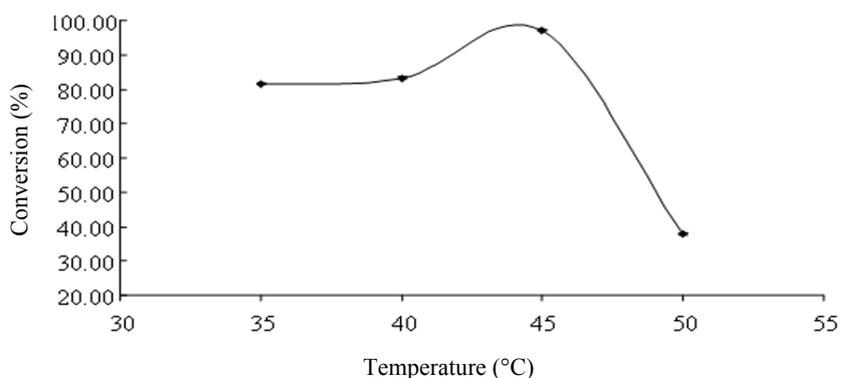


Figure 4: Effect of temperature (E = 7.46 kLU/ml; S₀ = 0.1 g/ml; pH 7.5; 200 rpm).

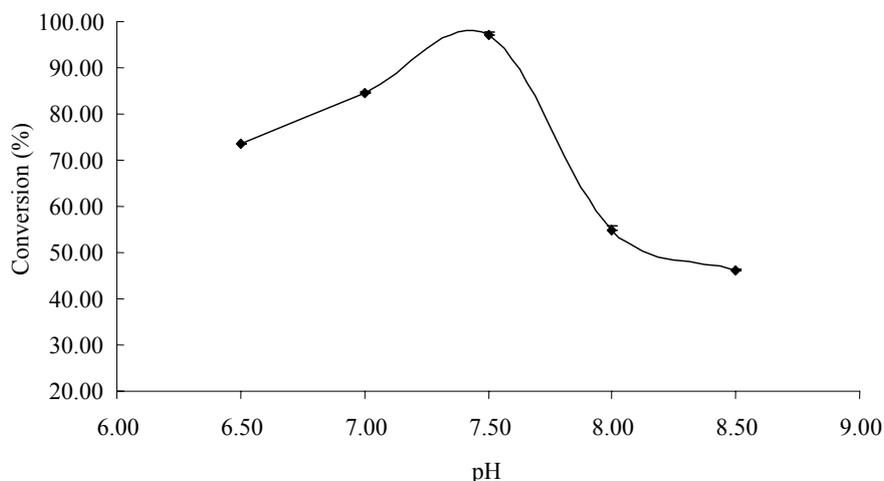


Figure 5: Effect of pH (Temperature 45°C; E = 7.46 kLU/ml; $S_0 = 0.1$ g/ml; 200 rpm).

3.6 Effect of Agitation Speed

Agitation speed actually refers to the orbital shaking rate which is rotation per minute (rpm) for hydrolysis reaction. It also has affected the degree of hydrolysis of cooking palm oil. Increasing agitation speed will increase the specific interfacial area between the oil and the enzyme present in the aqueous phase, by reducing the droplet size. Since the hydrolysis reaction takes place at the interface, the increase in the interfacial area resulted in an increase in the initial rate of hydrolysis.³ The conversion increased when the agitation speed was changed from 180 to 200 rpm, and decreased afterwards (Fig. 6). This was due to the contact surface between the aqueous and oil where the enzymes were located. If the agitation is too low or too high, the contact between the two surfaces will be less and therefore the degree of hydrolysis will be less.

3.7 Kinetics Study

The rate constants in the mathematical model were determined numerically from the experimental results. This model can be used to predict the rate of hydrolysis in a batch reactor and to determine optimal conditions. Initial rate of hydrolysis can be determined by Equation (2). The Michaelis-Menten kinetic equation [Eq. (3)] can be used to find the kinetic parameters. The double-reciprocal plot (Lineweaver-Burk plot) of reaction rate was used to evaluate the Michaelis-Menten constant, K_m and maximum velocity, V_{max} (Fig. 7). V_{max} is the maximum rate of enzyme mediated reaction where at this state, enzyme active

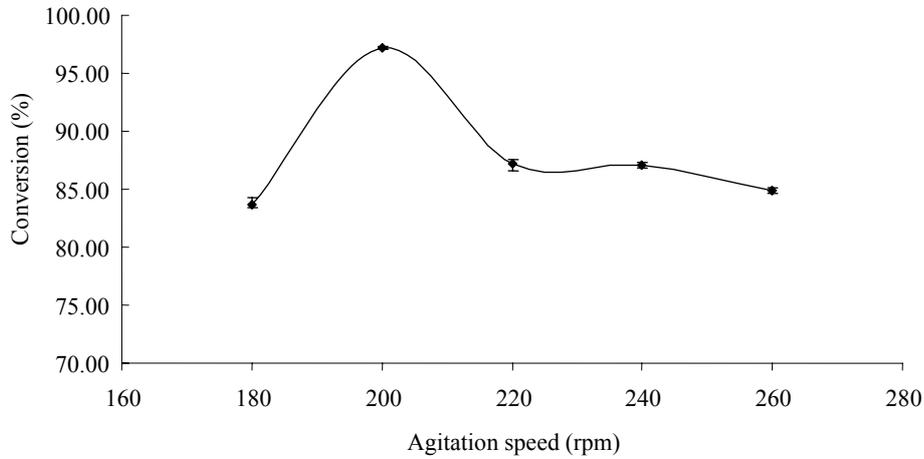


Figure 6: Effect of agitation speed (Temperature 45°C; E = 7.46 kLU/ml; $S_0 = 0.1$ g/ml; pH 7.5).

sites are saturated with substrate. Since V_{max} can never be achieved from the initial rate versus oil concentration graph, enzymes are usually characterized by the substrate concentration at which the rate of reaction is half its maximum. The hydrolysis kinetic was analyzed by finding the initial rate when varying oil concentration in the mixture. In this study, oil concentrations were varied by varying the oil loading. Oil concentrations were varied from 0.05 to 0.125 g/ml, operated at the optimum value which were obtained earlier.

$$\frac{1}{V} = \frac{K_m + [S]}{V_{max} [S]} = \frac{K_m}{V_{max}} \left[\frac{1}{[S]} \right] + \frac{1}{V_{max}} \quad (3)$$

where;

V_{max} = Initial velocity (mol/min mg-enzyme)

K_m = Michaelis-Menten constant (g/ml)

S = Substrate concentration (g/ml)

It was found that the V_{max} value equal to 370.37 mol/min mg-enzyme and K_m value equal to 1.23 g/ml.

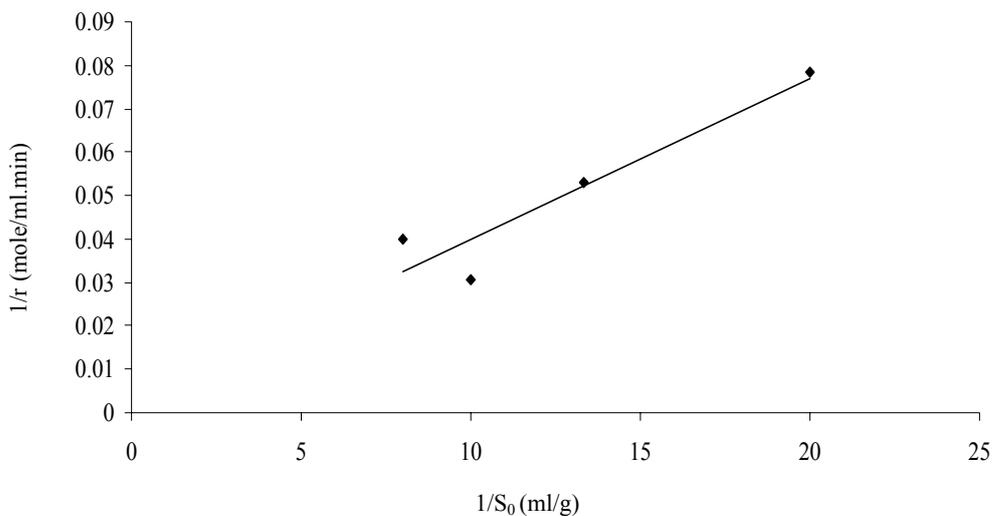


Figure 7: Lineweaver-Burk double reciprocal plot of initial rate as function of oil concentration.

4. CONCLUSION

As a conclusion, for the hydrolysis of cooking palm oil, it was found that the enzyme loading was 7.46 kLU/ ml *iso*-octane with oil loading of 0.1 g/ml to achieve highest degree of hydrolysis of 97.18%. The stability of enzyme activity decreased when the temperature went beyond enzyme functioning range (extreme condition) due to the structural deformation (denaturation) of enzyme. The optimum temperature obtained was 45°C. The enzyme was very sensitive to heat and pH which can be denatured easily if exposed at extreme stage. Decreased in enzyme activity was found at pH lower and higher than 7.5. This was also due to the enzyme denaturation. Besides, a kinetic model based on mechanism of the lipase-catalyzed hydrolysis in oil-aqueous system was described in Michaelis-Menten equation, taking account of the oil concentration. The V_{max} value was found to be 370.37 mol/min mg-enzyme and K_m value was 1.23 g/ml.

5. ACKNOWLEDGEMENT

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