Hydrolysis of Rubber Seed Oil

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Abstract: Rubber seed from the rubber tree are in abundance in most of country which non–edible oil could be obtained. However, the seeds are wasted in the rubber plantations annually event though the extractable oil has potential technical applications. If the full potentials of an oil are to be realized, there is no need to have a data base oil information on the oil extraction process and its properties. The objective of this research is to produce fatty acid from rubber seed oil through hydrolysis. Existing methods of fatty acids production are based on chemical methods. In this study Thermomyces lanuginosus lipase reactions were utilized to hydrolysis rubber seed oil. Besides, the effect of different parameters, such as pH value, temperature and moisture contain on the fatty acids had been studied using hydrolysis. The optimum conversion was found 0.68% fatty acids, 1:3 molar ratio oil to water at 30^{0}C, pH 8, 200rpm for 120 minutes reaction of time. The studied also found that the enzyme was very sensitive to heat and pH which can be denatured easily if exposed at extreme stage. Therefore, this research conducted to solve the agriculture waste problem by producing fatty acids from non-edible oil which is rubber seed oil.

Keywords: Rubber seed oil, hydrolysis, temperature, moisture content, pH

I. INTRODUCTION

Rubber tree or Heavea Brasiliensis belong to the family Euphorbiaceous (Kittigowittana et al., 2013). Rubber tree of Heavea Brasiliensis originally from South America (Amazon) and cultivated as an industrial crop since its introduction South East Asian (Thailand, Malaysia and Indonesia). Thailand are one of the countries that produced the largest nature rubber followed by Indonesia, Malaysia, India and China. (Reshad, Tiwari, & Goud, 2015). There are 40% to 60 % of kernel by weight provided from rubber seed (Zamberi & Ani, 2016). Example of rubber seed that collected from Segamat Johor showed at figure 1below.

Fig 1: Rubber seed from Batu Anam Sime Darby Rubber Plantation, Segamat, Johor.

Rubber seed is a waste product from rubber plantations which is agro waste contains a nutritive value that can be used as food for animals or as an energy which is biodiesel and several other products. Research shown that seed of rubber tree is a rich source of oil which is 40 -60% wt (Reshad et al., 2015). Meanwhile inside the rubber seed oil contain 17-82 % fatty acids (Ebewele et al., 2010). Fats and oils are esters of triglycerides and fatty acids.

Hydrolysis can break down a fat or oil and release the triglycerides and fatty acids. Hydrolysis is the chemical breakdown of substance by water. Hydrolysis of fat using water produced two-phase system which is an oil phase containing glyceride, fatty acid, glycerol and water and the other phase is a water phase containing glycerol and water (Antia et al., 2012). The example of usually used during the hydrolysis reaction of oil using water is generally showed as Figure 2 below. Where R, R1, R2, are alkyl groups.

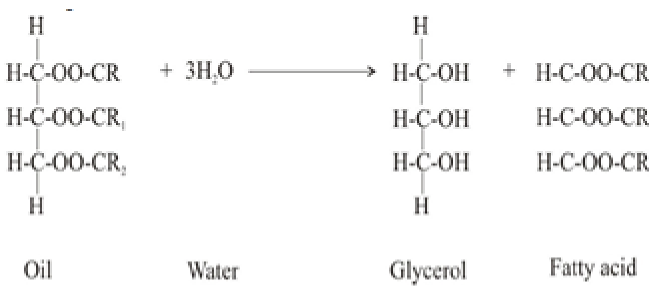

Fig 2 : Hydrolysis of oil to glycerol and fatty acids(Antia et al., 2012)

Fatty acids are known as a carboxylic acids and can be classified into three type which is saturated, monosaturated and polyunsaturated fatty acids (Zolkarnain & Yusof, 2007). Fatty acids are widely used as a raw material in biotechnology industry such as food, cosmetic, pharmaceutical, detergent, greases and other type of products. Besides that, biodiesel also can be produce from fatty acid by esterification with methanol (Satyarthi et al., 2011).

There are lot of research done to produce fatty acid such as enzymatic hydrolysis using lipase from Aspergillus niger, C. rugosa, Rhizopus javanicus, Penicillium solitum , and subcritical water (Salimon et al., 2011). However, this study focus on production of fatty acids from rubber seed oil by using hydrolysis and to study the effect of operating condition such as temperature, water content and pH on the fatty acid. This is because, Rubber seeds are considered as agro waste, meaning that they do not sit for a significant purpose. At the same time, rubber seed also contributes to global warming since it can cause air, water and land pollution. Malaysia, just like other developing countries, is facing the problem with the increase in the multiple type of waste and accompanying problems associated with waste disposal (Lau, 2004).

In 2007 there are around 1,229,940 hectares of rubber plantation was estimated by Association of Natural Rubber Producing Countries, Kuala Lumpur, Malaysia. Based on the value the production of rubber seed would be around of 1000 kg seeds per ha/ yr, meanwhile the annual production in Malaysia around 1.2 million metric tons. Moreover, Malaysia was one of the biggest rubber plantations growing country in the world. Thus, it is estimated that Malaysia wastes of rubber seed is about 355,200,000 kg fat and around 136,800,000 kg protein per year. Besides, in some country such as Vietnam there are 420,000 hectares of rubber trees with density of 500 tree/ha. Based on an estimated production of approximately 300 kg rubber seed /ha, it is then possible to collect nearly 130,000 metric tons rubber seed equivalent to 65,000 metric tons of rubber seed meal without hulls every year from this level of rubber production (Eka et al., 2010). Based on the the information given, if Malaysia managed to produced fatty acid from the rubber seed , its will make Malaysia one of the biggest country produce fatty acids.

II. METHODOLOGY

A. *Materials*

The main raw material that used in this research project is rubber seed oil. Rubber seed were collected from Batu Anam Sime Darby Rubber Plantation, Segamat, Johor. The parts of seeds were separated into shell and kernel and they were crushed and sieved to a particle size. All chemical are of analytical grade and used without further purification.

B. *Solvent Extraction Process*

A 10g of 0.5 mm rubber seeds powder was transferred into a thimble. Next, a thimble was placed into the Soxhlet extractor and the ground seeds were added into the extractor. Ensure that the sample and thimble were under the siphon side arm of the extractor. Other than using a thimble, cotton balls are also acceptable. 150 ml of n-hexane was added into a 250 ml round-bottomed flask, and was fitted to the bottom of the extractor. Then, the condenser was attached to the extractor with water supply tubes to complete the extraction apparatus. Then, the heating mantle was switched on after water was supplied to the condenser. The extraction time started when the first drop of solvent enters the thimble stuffed with the sample. Note to never leave the experiment unattended and ensure that the water bath never runs dry.

C. *Determination of Degree of Hydrolysis*

The hydrolysis of rubber seed oil 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 is defined as the percentage weight of fatty acids, X is calculated as below:

$$X,\% = \frac{V_{NaOH} \; X \; M_{NaOH} \; X \; MM}{Wt \; X \; 100} \text{ x } 100\%$$

where:

V = Volume of sodium hydroxide solution (NaOH) required during titration
M = NaOH concentration
MM = Average molecular mass of fatty
Wt = Weight of the sample taken.

III. RESULTS AND DISCUSSION

A. *Study of Hydrolysis Profile*

Figure 3 shows a typical hydrolysis profile of rubber seed oil at temperature of 30ºC for an at agitation speed of 200 rpm and moisture content ratio of 1:3. From the graph (Fig. 3), the conversion percentage was initially increased but slowly decreased with time. The conversion of oil to the fatty acid at the interface of the moisture content solution limited the surface reaction for the hydrolysis to occur. The highest percentage conversion happened after the hydrolysis reaction was carried out for 180 min. Similar hydrolysis profiles were obtained under other experimental conditions. All subsequent experiments discussed were taken at 180 min of reaction time.

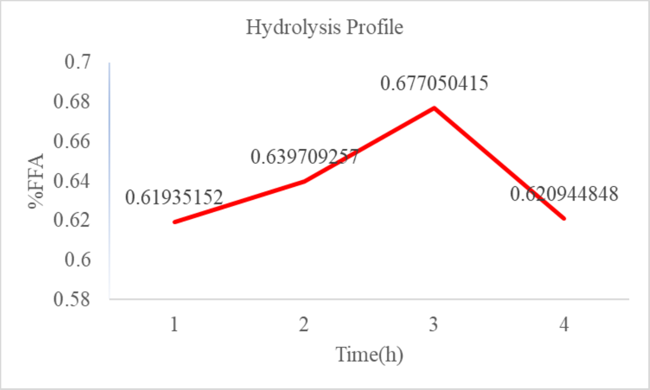

Fig 3 : Study of oil hydrolysis (Temperature 45 ºC ; pH 8; 200 rpm)

B. *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 5, 6, 7, 8 and 9 with other parameters fixed. Figure 4 clearly shows that at very low pH, conversion of the hydrolysis of rubber seed 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 8. The enzyme likely optimized its performance in an alkaline medium but nearly to neutral rather than a very acidic or alkaline medium. Most enzymes work at neutral pH 7.4(Serri et al., 2008). The optimum pH for the most studied seed lipase; castor bean lipase is in the range of between pH 4 to 4.2 (Widayat et al., 2013).

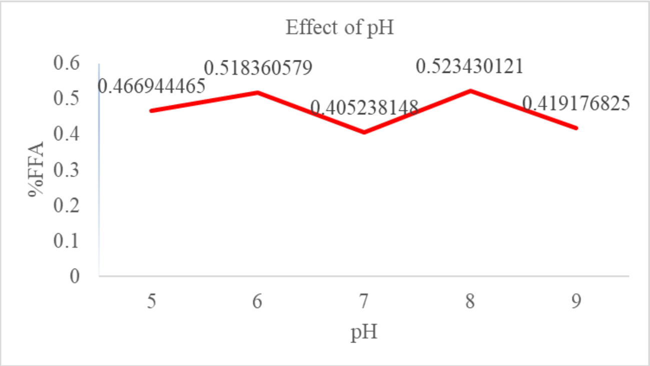

Fig 4: Effect of pH (Temperature 30 ºC;200 rpm)

C. *Effect of Temperature*

The conversion of fatty acids produced from the hydrolysis of rubber seed oil was also studied as a function of temperature (Fig. 4). Temperature was varied from 30ºC, 40ºC, 50ºC, 60ºC and 70ºC 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. This theory has been proven in Figure 5. Increasing of the reaction temperature has affected the production of fatty acids which clearly showed an increase in conversion. However, at 50ºC, the conversion profile changed appreciably with low conversion values. Therefore, 30ºC has been selected as an optimum temperature because after 30ºC, the conversion decreased abruptly due to the enzyme denaturation process. The conversion decreased due to the enzyme denaturation process (Serri et al., 2008)

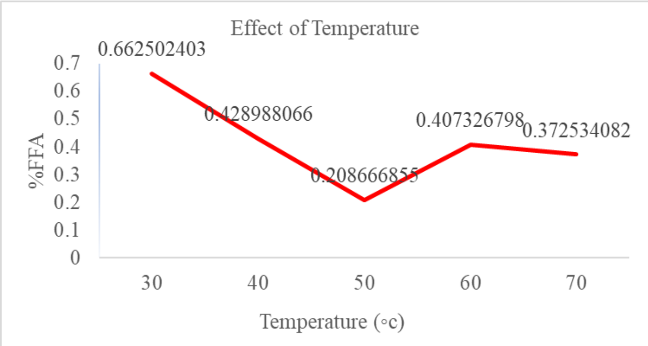

Fig 5 : Effect of temperature (pH 8.0; 200 rpm)

D. *Effect of Moisture Content*

The moisture content was varied from 1:1, 1:2, 1:3, 1:4 and 1:5 with the other parameters are fixed. The degree of hydrolysis decreased as the oil concentration increased (Fig. 6). This is due to the limitation in the reactant. However, at 1:1, 1:2,1:4 and 1:5, the conversion profile changed appreciably with low conversion values. Therefore, 1:3 has been selected as an optimum moisture content because before and after 1:3 the conversion was decreased.

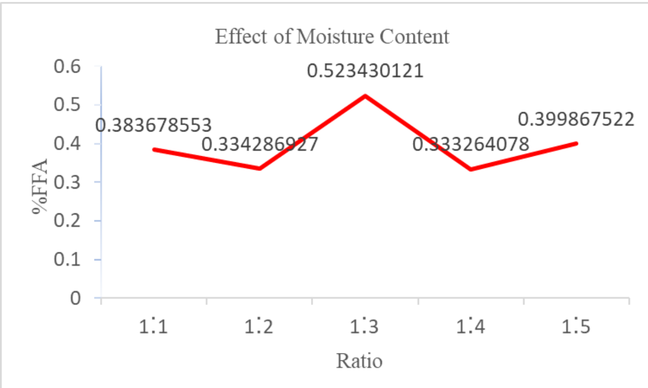

Fig 6 : Effect of Moisture Content (Temperature 30 ºC; pH 8; 200 rpm)

E. *Comparison study hydrolysis of fatty acids from edible and non-edible oil*

Table 1: Comparison study hydrolysis of fatty acids from edible and non-edible oil

Oil/fat	Fatty Acid content (wt%)
Edible Oil:	
Coconut oil	0.2
Palm olein oil	0.3
Soybean oil	0.3
Non-Edible Oil:	
Castor oil	3.1
Jatropha oil	3.7
Karanja oil	5.8
Palm oil	6.6
Rubber seed oil	17.0
Rubber seed oil,Johor	0.68

Table 1 shows the hydrolysis of edible and non-edible oil. Non-edible oil is low quality fatty acids feedstock compared to edible oil. This is because non edible oil contain phospholipids, sterol, water, odorants and other impurities in very small amount (less than 2 wt%)(Satyarthi et al., 2011)

IV. Conclusion

As a conclusion, Rubber seed content high quantity of oil. For the hydrolysis of rubber seed oil, it was found that the maximum conversion 0.6 % was achieved in 120 min at pH 8 and 200 rpm compared to 90 min at pH 7.5 and 200 rpm for the studies hydrolysis of cooking palm oil. 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 30ºC compared to 45◦C for the studies before. The enzyme was very sensitive to heat and pH which can be denatured easily if exposed at extreme stage. Besides, decreased in enzyme activity was found at pH lower and higher than 8. This was also due to the enzyme denaturation.

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