

## Enzymatic Synthesis of Epoxidized Fatty Hydrazides from Epoxidized Palm Oil Using Native *Muchor meihei* Lipase in *n*-Hexane Solvent

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Epoxidized fatty hydrazides have been synthesized through an enzymatic catalytic synthesis reaction of epoxidized palm oil with hydrazine monohydrate by native *Muchor meihei* lipase enzyme in *n*-hexane solvent. Optimum reaction conditions were studied by observing the yield of epoxidized fatty hydrazides production using several parameters. The highest production of epoxidized fatty hydrazides was obtained when reaction temperature was 50 °C with an addition of 5 % of enzyme to catalyze the reaction. This were accompanied using stirring speed of 165 rpm under 24 h of reaction. The epoxidized fatty hydrazides was further characterized using Fourier transform infrared spectroscopy, nuclear magnetic resonance spectrometry and CHN elemental analyzer.

**Keywords:** Enzymatic synthesis, Epoxidized palm oil, Fatty hydrazides.

### INTRODUCTION

Hydrazides have received a lot of attention due to their unique properties. They are valuable starting materials and intermediates to produce anionic surfactants [1], ionic surfactants [2,3], ligands [4], plastic additive [5,6] and also as a corrosion inhibitors [7]. In addition, due to their suitability on the biological and environmental activities, hydrazides can also be used as an antimicrobial agent [8] and also as an antituberculosis agent [9,10]. Furthermore, the structure of the hydrazides itself, which consists of multifunctional group such as an amide, an amine and a long alkyl chain in the molecular structure, leads to a chemically reactive molecule that can favour many organic synthesis reactions.

There are various methods that have been developed to synthesize hydrazides, either by using chemical methods [11-13] or enzymatic methods [14-17]. Chemical method generally requires multiple steps that start with heating of corresponding oils or esters with hydrazine hydrate in a basic condition for a few hours. Through this chemical synthesis of reaction process, it is found to be costly, requiring multiple steps, requiring high pressure and temperature, time consuming and also producing unwanted byproduct [18]. Enzymatic synthesis of hydrazides has become a good alternative way to produce hydrazides. This is because enzymatic synthesis offers many advantages such as having no by-products produced at the end of reaction, the

potential of being done in milder condition compared to using chemical method and also being more reaction selective [18]. Furthermore, this kind of technique is also known as less intensive of energy usage, as it does not require high temperature and pressure and also avoids the formation of byproducts [18].

In enzymatic synthesis reaction, many types of enzyme have been studied. The most common type of enzyme used for organic reaction is lipases (triacylglycerol hydrolases, EC.3.1.1.3). Lipases have good properties to be used in organics synthesis reaction such as high stability in organic solvent, can be cofactor-independent and insoluble in the organic media as well [19] and also demonstrating high enzymatic activity at organic media [20]. Other than that, lipases also have properties such as stereo-selectivity, chemoselectivity and regio-selectivity so that it is widely used for biocatalyst in various reactions [21].

Based on the literature review, no studies have been carried out to synthesize epoxidized fatty hydrazides directly from epoxidized palm oil using enzymatic method. Therefore, in this study, fatty hydrazides will be synthesized directly from epoxidized palm oil with hydrazine monohydrate using native *Muchor meihei* lipase as an enzyme. The use of native *Muchor meihei* lipase in this study is due to its good performance under mild reaction condition. Various conditions parameters during catalytic synthesis of epoxidized fatty hydrazides will be monitor such as temperature, dosage of enzyme used, stirring speed and the reaction time. Characterization of the synthesized product will

be characterized using Fourier transform infrared spectroscopy, nuclear magnetic resonance and CHN elemental analyzer.

## EXPERIMENTAL

Epoxidized palm oil was supplied by Advance Oleochemical Technology Division (AOTD) of Malaysian Palm Oil Board. Hydrazine monohydrate of reagent grade (65-64 % purity) and phosphorus pentoxide was supplied by Sigma Aldrich and used as received. Analytical grade *n*-hexane was purchased from Fisher Scientific and ethanol from Merck Company whereas, native *Muchor meihei* lipase was supplied by Creative Enzymes (New York, USA).

The product was analyzed by using FTIR spectrometry, in the range of 4000-300  $\text{cm}^{-1}$  using (Perkin Elmer-Spectrum BX Fourier transform infrared) spectrometer instrument equipped with an attenuated total reflectance. The structure of the product was reconfirmed using  $^1\text{H}$  NMR spectrometry (Bruker 600 MHz). Meanwhile, nitrogen content in epoxidized fatty hydrazides was analyzed by using a LECO Elemental Analyzer 932).

**Synthesis of epoxidized fatty hydrazides:** The synthesis reaction was carried out by mixing 1 mmol epoxidized palm oil with 12 mmol of hydrazine monohydrate in a 100 mL conical flask containing *n*-hexane in the presence of enzyme. The mixture was then transferred into water bath at different conditions based on the parameters of the study. The parameters involve were (a) temperature, (b) stirring speed, (c) dosage of the enzyme and (d) reaction time. Once the reaction period was completed, the white paste formed was transferred into a bigger conical flask, mixed with ethanol and heated at 60-70 °C for 15 min. This is to ensure that the enzyme was separated from the clear solution. The free enzyme solution was left for 3 h at 28 °C to let it precipitate. The white precipitate was further filtered using a Whatman 4B filter paper in Buchner funnel under a reduced pressure and dried under reduced pressure in the presence of phosphorus pentoxide. This method was a modified procedure as described earlier [11,14,15].

## RESULTS AND DISCUSSION

Variation of reaction conditions were found to have significant effect on the production of epoxidized fatty hydrazides. Determination of optimum reaction condition will help us to understand the system.

**Effect of temperature:** In order to identify the optimum temperature for enzymatic synthesis of epoxidized fatty hydrazides, the enzyme activity of native *Muchor meihei* lipase was screened over the reaction temperature ranging from 30 to 60 °C. The yield of epoxidized fatty hydrazide increase linearly as the temperature increase from 30 to 50 °C (Fig. 1). At 30 °C, the yield of epoxidized fatty hydrazide was 233.9 mg, 455.5 mg was achieved at 40 °C while 609.2 mg of epoxidized fatty hydrazide was produced at 50 °C. However, as the temperature increase above 50 °C, the yield drops slightly to 519.2 mg. Therefore, the optimum temperature for hydrazides production is 50 °C. These trends of optimum temperatures for the lipase catalyzed reactions were usually observed using enzyme which is isolated from the fungus family. It is already reported that the optimum temperature for synthesis of organic compounds using lipases from fungus *Mucor* family was between 45 and

55 °C [22], 40 °C [14,15], 50 to 60 °C [23] and 60 °C [24]. The differences of optimum reaction temperature also mainly depend on the type of immobilization nature of the enzyme itself and where they fixed on. Furthermore, from Fig. 1, the yield of epoxidized fatty hydrazides decreased at higher temperature, possibly due to the nature of enzyme itself. Structure of the enzyme will begin to disrupt at higher thermal energy, causing low enzymatic catalyzed activity. Thus, resulting to the decreased amount of epoxidized fatty hydrazides yield [25].

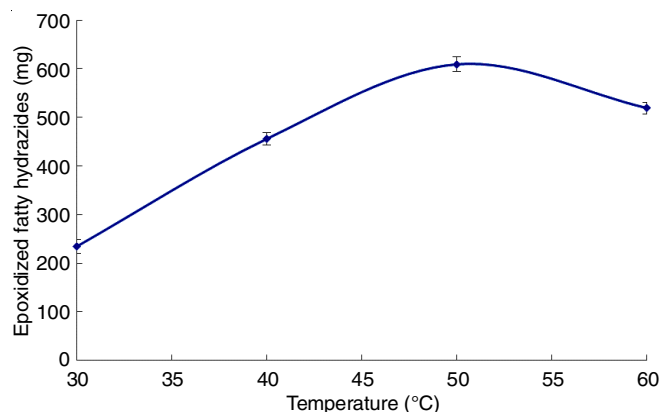


Fig. 1. Effect of temperature on the yield of epoxidized fatty hydrazides production (condition of reaction: 24 h, 165 rpm, 5 % of enzyme used, *n*-hexane as a solvent)

**Effect of amount of enzyme:** Amount of enzyme used to catalyze reaction is one of the main factors to get the highest yield of hydrazides with the usage of minimal amount of catalyst. Interesting trend was observed from Fig. 2. Production of epoxidized fatty hydrazide can still occur without addition of enzyme to catalyze the reaction. With the use of enzyme, we found that the yield of epoxidized fatty hydrazides increased significantly from 262.4 to 609.2 mg with the increasing amount of enzyme 1 up to 5 %, respectively. However, further increase on the amount of enzyme into the system, yields of epoxidized fatty hydrazide started to decrease. Similar findings were observed by Ismail *et al.* [18]. This information indicates that the increased amount of catalyst load will lead to higher catalytic activity results in formation of acyl-enzyme complexes [26] and further increase the formation of hydrazides. Hereby, the yield started to decrease because the huge amount of active site of the enzyme was not fully exposed and remained inside the bulk enzyme

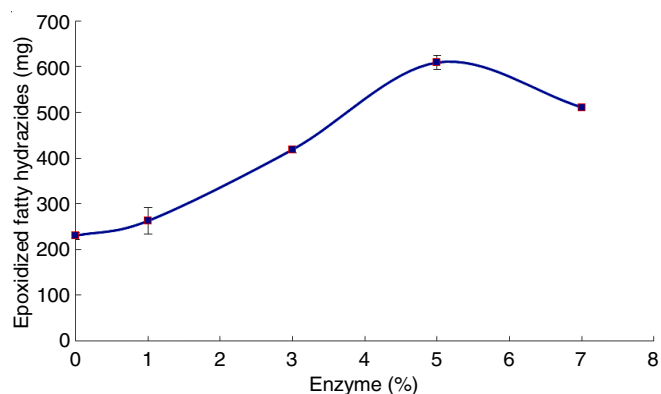


Fig. 2. Effect of enzyme on the yield of epoxidized fatty hydrazides production (condition of reaction: 24 h, 50 °C, 165 rpm, *n*-hexane as a solvent)

and did not contribute to any other reactions that can cause to the increased amount of the yield of hydrazides [27]. This implies that the presence of excess enzyme in the system affect the catalytic activity of reaction.

**Effect of reaction time:** Fig. 3 shows the effect of reaction time on the yield of hydrazides. The yields rapidly increased from 98.9 mg for 3 h to 609.2 mg for 24 h reaction time and then, become constant virtually after 24 h of reaction time. At the early stage of reaction, the collision between substrate and enzyme was done in a limited time or in the kinetic regime. The chances for the conversion of epoxidized palm oil to epoxidized fatty hydrazides were not highly significant. However, when the reaction period remained longer and up until 24 h, the reaction was done in optimum period or in diffusion limiting regime and it reached their equilibrium state. As a result, further increase of reaction time did not affect the product yield.

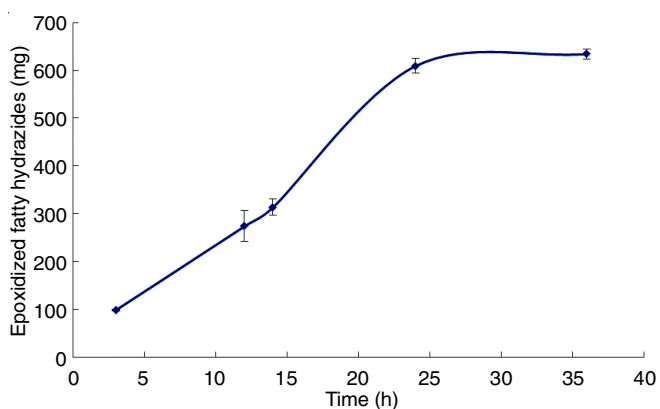


Fig. 3. Effect of reaction time on the yield of epoxidized fatty hydrazides production: (Reaction condition :165 rpm, *n*-hexane as solvent, 5 % of enzyme, 50 °C

**Effect of stirring speed:** Stirring speed of waterbath shaker was also taken into consideration, because it plays the major role that can boost up the reaction. The speed was set between 130 to 200 rpm based on the operation limit of the shaker itself. Fig. 4 shows the effect of reaction speed on the yield of hydrazides. It was found that the yield was increased from 561.4 to 613.3 mg with the increasing of speed from 130 to 200 rpm, respectively. However from 165 to 200 rpm, the increment of the yield was not clearly significant which is 609.2 mg at the stirring speed of 165 rpm and 613.3 mg at stirring speed of 200 rpm. This observation showed that the reaction at 165 rpm was already enough to get a high yield of epoxidized fatty hydrazides.

**Characterization of epoxidized fatty hydrazides:** The functional groups of epoxidized palm oil and epoxidized fatty hydrazides synthesis at highest yield (609.2 mg) with reaction condition (50 °C reaction temperature, 5 % amount of enzyme used, 165 rpm stirring speed and at 24 h reaction time) were determined using FTIR spectrometry (Fig. 5). FTIR spectra of epoxidized palm oil showed C-O-C epoxide group stretching vibration at 839 cm<sup>-1</sup>. This observation is almost similar with Cheing *et al.* [28]. They found C-O-C stretching from oxirane vibration of epoxidized palm oil in the range of 950-850 cm<sup>-1</sup> and at 1250 cm<sup>-1</sup> which is usually weak and overlays with other peaks [28]. Other than that the strong absorption peak

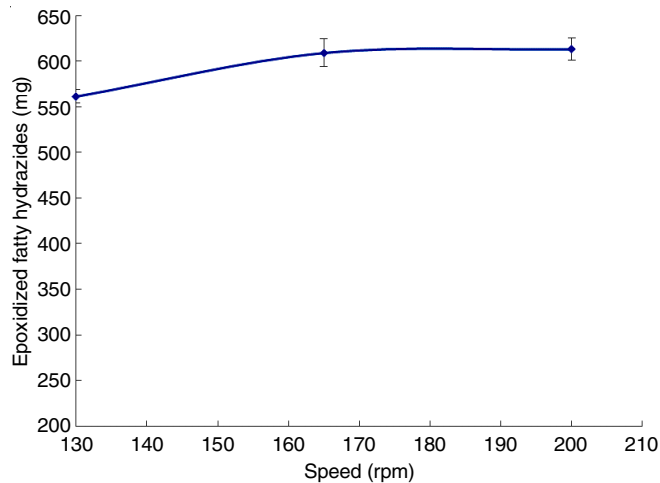


Fig. 4. Effect stirring speed on the yield of epoxidized fatty hydrazide production (condition of reaction: 24 h, *n*-hexane as solvent, 5 % of enzyme, 50 °C)

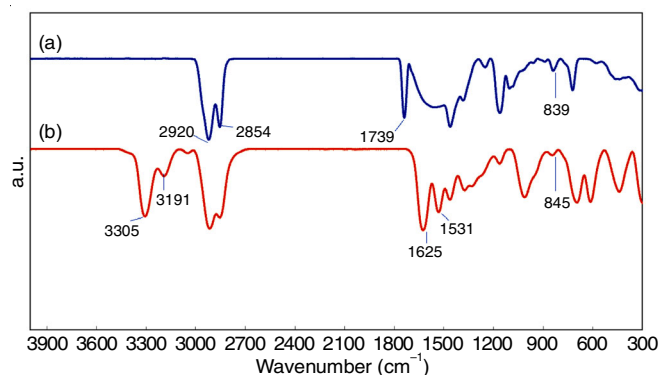
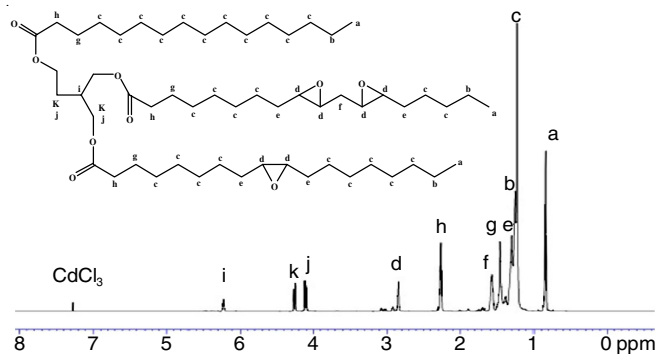
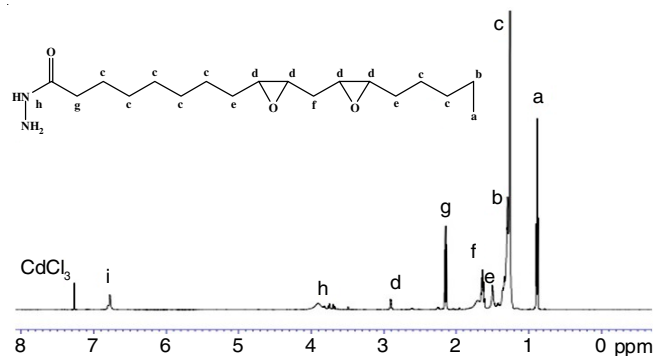


Fig. 5. FTIR spectra of (a) epoxidized palm oil and (b) epoxidized fatty hydrazides at optimum reaction condition

for C=O of carbonyl group attached at epoxidized palm oil structure was spotted at 1739 cm<sup>-1</sup>. Then, a strong absorption band at 2920 cm<sup>-1</sup> belongs to the C-H stretching of terminal methyl group of the triglyceride chains, while peak at 2854 cm<sup>-1</sup> was due to the C-H symmetric stretching in the saturated fatty acid backbone.

The resulted epoxidized fatty hydrazides were analyzed and monitored by the presence of few important peaks. Firstly, the peak at the region of 3305 and 3191 cm<sup>-1</sup> was due to asymmetrical and symmetrical N-H of primary amine. On the other hand, the stretching of C=O of amide group was observed at 1625 cm<sup>-1</sup> and N-H bending of the primary amine appeared at the frequency of 1531 cm<sup>-1</sup>. The peak of C-O-C of epoxide group was observed to remain after 24 h of reaction with lower intensity at 845 cm<sup>-1</sup> compared to the original epoxidized palm oil. Overall, the important peaks were appeared at epoxidized fatty hydrazides spectrum indicates the hydrazinolysis reaction taken place and the product was successfully synthesized.

**NMR analysis:** The presence of epoxy group, proton N-H and NH<sub>2</sub> was reconfirmed by comparing the chemical shift for epoxidized palm oil and epoxidized fatty hydrazides spectra as shown in Fig. 6a-b. From these figures, proton NH<sub>2</sub> in the epoxidized fatty hydrazides structure was present in the region of 3.70 ppm, while the N-H of amide group was observed at the region of 6.81 ppm. This peak was observed at low ppm

Fig. 6a. <sup>1</sup>H NMR spectrum for epoxidized palm oilFig. 6b. <sup>1</sup>H NMR spectrum for epoxidized fatty hydrazides at optimum reaction condition

compared to the study conducted by other researchers [11]. This might be due to the difference in solvents used, temperature, concentration and other reasons which can lead to the difference in the actual ppm value but still in the range of 5.00–9.00 ppm [29]. Other than that, peak at 2.90 ppm was attributed for proton attached at the epoxy group. The observation from the spectrum suggests that the epoxy group from epoxidized palm oil was still being preserved after the hydrazinolysis reaction was completed and the addition of amide and amine group was successfully introduced in the structure.

In addition, CHN elemental analyzer also used to analyze the presence of the nitrogen contents in the products, which is one of the signs of the formation of the epoxidized fatty hydrazides. The content of the nitrogen was found to be 9.92 % indicated that the epoxidized fatty hydrazide was successfully synthesized during the reaction process.

## Conclusion

This study shows that epoxidized fatty hydrazides has been successfully synthesized directly through enzymatic method. The product was further characterized using FTIR spectroscopy, NMR spectrometry and CHN elemental analyzer. From overall data analysis, it was found that the optimum condition for preparing the epoxidized fatty hydrazides at 50 °C, with 5 % of enzyme, 165 rpm speed of reaction and 24 h of reaction period.

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