Optimization Of The Enzymatic Ammonolysis Of Alkanolamide From Ketapang Kernel Oil

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Abstract. The enzymatic ammonolysis reaction of alkanolamide using Terminalia catappa L. (local language: Ketapang) kernel oil and etanolamine as substrates has been conducted. It’s commonly synthesized from derivative of petroleum and natural gas. In this research, the raw material used was ketapang seed oil, the renewable and non-edible oil. The effects of various reaction parameters such as reaction time, temperature, amount of enzyme, and molar ratio of substrates were investigated. The optimum reaction conditions obtained by a one-step lipase catalyzed reaction were ratio of ketapang seed oil and etanolamine of 1:12.5, amount of lipase (Lipozyme) of 0.1 g, temperature at 40°C for 2 h, and hexane as a solvent. The percentage yields of alkanolamide obtained at these optimum ammonolysis reaction conditions was 60.07%.

Key words: alkanolamide, ketapang kernel oil, enzymatic ammonolysis

1. Introduction
Alkanolamide or fatty acid alkanolamide are widely used as a surfactant, formulations on cosmetic and pharmaceutical ingredients. Commonly, fatty acid alkanolamides can be produced by transamidation of the fatty methyl ester with monoethanolamide at high temperature and pressure. However, it can also lead side reactions.¹,² Recent studies show that the fatty acid alkanolamides can be produced enzymatically by lipase-catalyzed transesterification.²,³ The enzymatic synthesis offers mild reaction conditions and environmentally friendly process.⁴ Usually, the basic material to synthesize alkanolamide is pure fatty acids (uneconomist price) or edible oils (unsability competition)²,³,⁵,⁶. In this research, natural non-edible oils (usability noncompetition) used to sustainable source of fatty acids, which represent raw materials that can replace pure fatty acids or edible oils. The natural non-edible oil such as Terminalia catappa L. Kernel Oil or ketapang kernel oil (local language) has been used to synthesize alkanolamide via enzymatic ammonolysis reaction.

Previous research showed that the yield of crude oil from ketapang kernel is 50.40-58.40%. Fatty acid content of Ketapang kernel oil is palmitic acid (25-30%), oleic acid (35-55%), linoleic acid (12-16%) and stearic acid (4-7%).⁷,⁸ Thus, this show that economically, ketapang kernel oil can be as a source of fatty acid and it can converted into alkanolamide. For that, it required optimization step to produce the optimal product. The effects of various parameters on ammonolysis reaction were investigated.
2. Materials and Methods

2.1 Materials
Materials that used are ketapang kernel oil, lipozyme TL.IM was produced by Novo Nordisk (Denmark), Ethanolamine, fatty acids alkanolamide standards and n-hexane were obtained from Sigma Aldrich (USA). All other chemicals were analytical grade. The reactions were analyzed by a gas chromatograph (Shimadzu GCMS-QP2010) RTx-65TG capillary column (30 m x 0.25 mm). Helium was used as the carrier gas at a flow rate 30 ml/min. The temperature was programmed at 1 min at 160°C, 10°C/min to 280°C was held for 25 min. Detector used was FID (Flame Ionization Detector) at 300°C. FT-IR Spectrophotometer from Perkin Elmer Model Frontier.

2.2 Methods

2.2.1 Synthesis and purification of alkanolamide. Ketapang kernel oil that has been extracted from the fruit is used as a source of fatty acids. The reaction mixture consisted of ketapang kernel oil, ethanolamine and lipase/lipozyme and hexane as a solvent. The reaction was incubated in a horizontal water bath shaker (150 rpm) at different variables. The product was isolated and then purified by crystallization method at 4°C. The product was collected by filtration, washed with amount volume of hexane and dried in a vacuum desicator until constant. The pure product of alkanolamide was quantitated gravimetrically.

2.2.2 Effect of Reaction Time. A series of reactions catalyzed by 0.1 g Lipozyme IM, incubated at 40°C at 150 rpm at various reactions periods (1, 2, 3, 4 and 5 h) were studied to determine the effect of reaction time. After completion of each assigned period, the alkanolamide were isolated and purified. The product was quantitated as described earlier.

2.2.3 Effect of Ratio Substrate. This effect was carried out using different mole ratios of the reactants amount of the lipase and other parameters were kept constant. The amount of ketapang kernel oil at 2 g and the amount of ethanolamine were varied from 5 to 30.0 mmol. The reaction mixtures were incubated at 40°C for 5 h at 150 rpm. The amount of the enzyme used was 0.1 g. The product was quantitated as described earlier.

2.2.4 Effect of Amount of Enzyme. The reaction mixtures containing different quantities (0.05 to 0.25 g) of enzyme were incubated at 40°C for 5 h at 150 rpm. The amount of ketapang kernel oil (2 g) and ethanolamine (10 mmol) were kept constant. The product was quantitated as described earlier.

2.2.5 Effect of Temperature. To study the effect of reaction temperature, another series of samples were incubated in a water bath shaker at 150 rpm for 5 h and different reaction temperatures (30, 40, 50, 60 and 70°C). The amount of enzyme used was 0.1 g. The product was quantitated as described earlier.
2.2.6 *Analysis of the product.* The products of the reactions were examined by thin layer chromatography (TLC) on precoated silica gels plate (60F, Merck, Darmstadt, Germany) and developed in hexane/diethyleter (87:13, v/v). Identification was made by comparison with known standards. The analysis of standards and products formed were analysed by a gas chromatograph (Shimadzu) RTx-65TG capillary column (30 m x 0,25 mm). Helium was used as the carrier gas at a flow rate 30 ml/min. The temperature was programmed at 1 min at 160°C, 10°C/min to 280°C was held for 25 min. Detector used was FID (Flame Ionization Detector) at 300°C. Infrared (IR) analysis of the products was carried out using Perkin Elmer Fourier-transform Infrared spectrophotometer. Amount of total nitrogen in alkanolamide was determined by Kjedahl method.

3. Results and Discussion

3.1 *Effect of Reaction Time*

The results (Fig. 1) show that enzymatic reaction, the reaction proceeded in 1 h. Although the maximum yield is obtained at the reaction period at around 2 h, then slows down up to 5 h. The decrease in the reaction rate may be due to some mass-transfer limitations, which inevitably arise in a reaction mixture containing a high proportion of solid product and the reaction reaches the equilibrium state where the rate of forward reaction was equal to the backward reaction.\textsuperscript{9,10} However, this result is very faster compared with the result of Rahman et al.,\textsuperscript{2} which shows that the synthesis of alkanolamide from palm kernel oil using free enzyme for *Candida rugose* and the maximum conversion at 36 h. This may be because the enzymes used in this study are immobilized enzymes. Immobilized enzymes typically have greater thermal and operational stability than free enzyme.\textsuperscript{11}

![Figure 1 Effect of Reaction Time](image)

3.2. *Effect of Ratio Substrate*
The effect of ratio substrate on the amonolysis reaction indicated competitive nature of ethanolamine and fatty acids (in ketapang kernel oil) binding. Fig. 2 shows that the highest result when the ratio of the ketapang kernel oil and the ethanolamine was at 2 and 25 mmol or 1:12.5 mmol. This result is in agreement with Wang et al.\textsuperscript{6} that shows the reaction yield increases with the increase in the concentration of ethanolamine. The high concentrations of ethanolamine will facilitate the formation bond of carbon and nitrogen.\textsuperscript{12}

![Figure 2 Effect of Ratio Substrate](image)

**Figure 2 Effect of Ratio Substrate**

### 3.3 Effect of Enzyme Amount

Fig. 3 depict the result of using different amount of enzyme. The weight of alkanolamide was increased with increasing amount of enzyme from 0.05 to 0.2 g. Further increase of lipase to substrate ratio does not significantly increase the yield. This behavior of leveling off of the reaction at higher enzyme concentrations can be explained by considering that the active sites of the enzyme molecules present in excess would not be exposed to the substrates and remain inside the bulk of enzyme particles without contributing significantly to the reaction.\textsuperscript{13}
3.4. Effect of Temperature

The ammonolysis reactions showed an increment of the product as the temperature was increased from 30°C to 40°C as shown in Fig. 4. At temperature above 40°C, the product was decreased and slightly decreased again which may cause by denaturing of the lipase. Similar result was reported by some research. García et al. reported when temperature above 50°C, the activity of energy for enzymatic reaction was low.

3.5 Characterization of Products

The enzymatic ammonolysis reaction of alkanolamide was ascertained by TLC. Rf value of the standard and the product are 0.97 and 0.95 respectively with eluent hexane/diethyleter (87:13). The characteristic peaks of IR spectrum bands at 3190 cm\(^{-1}\) and 1751-1715 cm\(^{-1}\) for stretching N-H and C=O. Similar result of characteristic peaks was reported by Adewuyi et al. Characterization of the fatty acids alkanolamide using GC-MS showed similar resemblance of the compound,
Palmitoylethanolamide and oleylethanolamide with respective retention time of the compounds was 11.9821 and 12.565 minutes.

3.6 Ammonolysis Reaction at Optimum Conditions

The enzymatic ammonolysis reaction of alkanolamide was carried out at optimum conditions (Reaction time of 2h, temperature at 40°C, amount of lipozyme of 0.1 g, and ratio of substrate, ketapang kernel oil/ethanolamine, 1:12.5). The percentage yields of alkanolamide obtained at these optimum ammonolysis reaction conditions was 60.07 %.

4. Conclusion

The optimum conditions of the enzymatic ammonolysis reaction of alkanolamide using Terminalia catappa L have been successfully investigated by a one-step reaction to produce higher yields. The GC-MS spectrum indicated that the type of alkanolamide formed is oleylethanolamide and palmitoylethanolamide

Acknowledgements

This project was financed by a grant from Directorate General of Research and Development Strengthening, The Ministry of Research Technology and Higher Education Republic of Indonesia, Scheme “Unggulan Perguruan Tinggi 2017”.

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