Optimization of Alkyldiethanolamides Synthesis from *Terminalia catappa* L. Kernel Oil through Enzymatic Reaction

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Abstract: Alkyldiethanolamides (fatty acid diethanolamides) synthesis from *Terminalia catappa* L. kernel oil was optimized using lyposyme as a catalyst. The result showed that the optimal reaction conditions were 2 hours reaction time, with a ratio of oil mass (g) to diethanolamine (mmol) of 1:5, a ratio of oil mass to enzyme (g) of 1: 0.075, and a temperature of 40°C. The percentage of alkyldiethanolamides at optimum condition was 56-60%. The synthesis results were also analyzed by FTIR. FTIR spectra revealed specific absorption at several wave numbers (3434 cm⁻¹, 1655 cm⁻¹, 1280 cm⁻¹), indicating that amide and alcohol bonds (C=O, C-N, and O-H) were formed. GC-MS was employed to identify the types of fatty acid diethanolamides that were successfully synthesized. The fatty acid diethanolamides formed were palmitoyldiethanolamide (Rt = 32.96 min) and oleyldiethanolamide (Rt = 35.57 min). The total nitrogen content of alkyldiethanolamides was 0.26%, or 0.19 mmol of the amide group in 1 g of sample.

Key words: alkyldiethanolamide, optimization, *Terminalia catappa* L. kernel oil, enzymatic reaction

1 INTRODUCTION

Alkyldiethanolamide is a fatty acid amide compound known to have numerous benefits, especially in the pharmaceutical and surfactant industries⁴⁻¹⁰. Previous researchers have successfully synthesized this compound from relatively expensive pure fatty acids or from edible oils such as palm and coconut oil⁴⁻⁵. Therefore, it is necessary to develop a new base material that has the same content as the vegetable oils, but is not a food commodity or edible oil. One of the sources that can be utilized is *Terminalia catappa* L. (ketapang) kernel oil. Fruits of ketapang are usually produced after the tree is approximately 3 years old, and are elliptical like almonds⁷. The ketapang tree bears fruit three times a year, irrespective of the season. Therefore, the fruits are available in abundance throughout the year. A previous study has revealed that the yield of ketapang kernel oil is 54.4%⁷. The fatty acid composition of ketapang kernel oil includes 36% palmitic acid (C16:0), 5% stearic acid (18:0), 30% oleic acid (18:1), and 29% linoleic acid (18:2)⁷. This shows that ketapang kernel is economical as a raw material for the production of alkyldiethanolamides. There are two methods that can be applied to synthesize this compound: chemical and enzymatic. The chemical method, such as the one performed by Bilyk et al., requires high temperature and pressure⁹.

Other disadvantages of applying the chemical method include the presence of side reactions, high production cost, and equipment damage due to the presence of corrosive substances¹⁰,¹¹. Recently, considerable effort has been put into developing enzymatic methods to synthesize amide⁴,⁴,¹²,¹³. Such methods are very environment-friendly because they require a minimal solvent and do not yield byproducts⁸,¹¹. However, the enzymatic method is believed to be quite expensive. Regardless, in this study, the enzymatic method was chosen, but with the use of lipase enzyme that was immobilized. The immobilized enzyme confers some advantages, as the enzyme is reusable and less of it is required, overall¹⁴. Accordingly, a study to produce alkyldiethanolamides from ketapang kernel oil using an enzymatic process needs to be conducted, given the potential of the oil, the function of alkyldiethanolamides, and the environment-friendly synthesis process. This study aimed to investigate the optimum reaction conditions to produce the optimum product, which can later be applied in the industry.
2 EXPERIMENTAL PROCEDURES

2.1 Materials
The chemicals used were pro-analysis grade, including hexane, diethyl ether, chloroform and diethanolamine (Sigma Aldrich USA), silica gel, fatty acid diethanolamide standard, commercial enzyme (Lipozyme TL IM, Novo Nordisk), and Terminalia catappa L. (ketapang) kernel.

2.2 Apparatus
The apparatus used in this research were rotary evaporator, horizontal water bath shaker, FT-IR spectrophotometer from Perkin Elmer Model Frontier, GC-MS Shimadzu Model GCMS-QP2010 Ultra (Japan), and Kjeldahl Flask.

2.3 Measurements

2.3.1 Extraction and purification of ketapang kernel oil
The extraction of kernel oil (triglycerides) from ketapang kernel was performed by the soxhletation method for 6 hours, using n-hexane as solvent. Previously, the ketapang kernel was dried and mashed. The extract was evaporated to remove the solvent with a rotary evaporator at 40°C and at a speed of 125 rpm. Anhydrous sodium sulfate was added to the oil obtained to remove water. Purification of triglycerides was conducted by column chromatography. An oil extract of 50 g was placed on the top of the column chromatography containing silica gel. An eluent consisting of n-hexane and diethyl ether (87:13 v/v) was applied to the column.

2.3.2 Synthesis of alkyldiethanolamides
The synthesis of alkyldiethanolamides was carried out by reacting ketapang kernel oil containing several fatty acids with diethanolamine in 20 mL n-hexane and a number of immobilized lipase enzymes or Lipozyme TL IM. The mixture was incubated in a horizontal water bath shaker at a rate of 150 rpm. After that, the alkyldiethanolamides formed were purified and their quantity was determined by a gravimetric method.

2.3.3 Determination of the optimum conditions
In this study, the determination of the optimum condition of alkyldiethanolamides synthesis was conducted by sequentially varying variables such as reaction time, substrate ratio, enzyme amount, and reaction temperature.

2.3.4 Optimization of reaction time
The synthesis of alkyldiethanolamides was carried out by varying the reaction time in hours from 1-5, while the composition and other variables remained constant: 2 g of ketapang kernel oil, 20 mL n-hexane, 15 mmol diethanolamine, and 0.1 g enzyme. The mixture was incubated in a horizontal water bath shaker at 40°C and at a speed of 150 rpm. After that, the formed alkyldiethanolamides were purified and their quantity was determined by a gravimetric method.

2.3.5 Optimization of substrate ratio
For the substrate variable, the reaction mixture was prepared by varying the amount of diethanolamine: at 5, 10, 15, 20, or 25 mmol. Meanwhile, the amounts of ketapang kernel oil, n-hexane solvent, temperature, and enzyme were held constant, like in the previous experiment. The mixture was incubated in a horizontal water bath shaker for the identified optimum time at a rate of 150 rpm. The formed product was treated as in the previous experiment.

2.3.6 Optimization of amount of enzyme
The synthesis of alkyldiethanolamides was carried out by varying the amount of enzyme, from 0.05 to 0.1, 0.15, 0.20, or 0.25 g. The other variables were held constant. The reaction mixture and the reaction result were treated as in the previous experiments.

2.3.7 Optimization of temperature
The range of temperature optimization for the reaction between ketapang kernel oil and diethanolamine was 30, 40, 50, 60, and 70°C, while the other variables were held constant. The mixture was incubated in a horizontal water bath shaker over the temperature range as described above, at a rate of 150 rpm. The reaction result was treated as in the previous experiment.

2.3.8 Synthesis alkyldiethanolamides at optimum conditions
Alkyldiethanolamides were synthesized using the optimum condition ratios determined from the previous experiments. The reagent composition was increased five-fold. The product formed was separated from the water layer. The organic layer (n-hexane and alkyldiethanolamides) rested above the water layer. This layer was separated using a separation funnel. To obtain concentrated alkyldiethanolamides, n-hexane fraction was cooled in the freezer (≤−5°C) for 5 h and then filtered. Alkyldiethanolamides obtained on the filter paper were washed with n-hexane and dried in a desiccator that had been filled with active silica for 24 h. The resulting products were weighed gravimetrically.

2.3.9 Qualitative and quantitative test of alkyldiethanolamides
Qualitative and quantitative tests of alkyldiethanolamides were carried out by TLC, FTIR, and GC-MS. The determination of total nitrogen was performed by the Kjeldahl method. Gas chromatography was performed to analyze the product, on an RTx-65TG capillary column (30 m, Ø0,25 mm, Supelco, USA). Helium was employed as the carrier gas, with flow rate 30 ml/min. The run temperature profile was 2 min at 40°C, 8°C/min at 280°C, and 5 min at 280°C. A flame ionization detector (FID) was used at 300°C.

3 RESULTS AND DISCUSSION

3.1 Extraction and purification of ketapang kernel oil
The average kernel weight against ketapang fruit was 9.26%. The extraction of ketapang kernel oil was carried
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out by the soxhlet method with \( n \)-hexane as a solvent. \( n \)-hexane is the best solvent for ketapang kernel oil extraction, compared to other solvents (chloroform, benzene, petroleum ether and ethanol). The percentage of oil obtained after purification by column chromatography was 56.4\%. This value is slightly lower (60.45\%) than the that of a research conducted by Menkitti et al.\(^6\).

### 3.2 Determination of the optimum conditions

#### 3.2.1 Optimization of reaction time

In Fig. 1, it can be seen that the amount of the resulting product increased from 1 to 2 hours, and then from 3 hours the graph began to level off and the amount slowly decreased. Therefore, it can be said that the optimal time to obtain results is 2 hours. This is much faster than that reported in the study by Rahman et al.\(^4\), which showed an optimum time of 72 hours to synthesize alkanolamide from palm kernel oil using lipase enzyme without immobilization. This is probably due to the different types of enzymes employed. The immobilized enzyme has high operational stability. After the optimum reaction time, the amount of product decreases, according to Suhendra et al.\(^18\). This is caused by two factors; firstly, the occurrence of a mass transfer boundary that cannot be avoided, for in the reaction mixture there is quite a large proportion of solid products limiting the interaction between the substrate and the enzyme; secondly, the reaction reaches an equilibrium state where the rate of the forward reaction is equal to the rate of the backward reaction. A byproduct of this reaction is glycerol. It is possible that, over a long period, glycerol slightly dissolves alkyldiethanolamide, which has a shorter chain of fatty acids.

#### 3.2.2 Optimization of substrate ratio

The amount of alkyldiethanolamides produced increased with the addition of 0.10 mmol up to 15 mmol diethanolamine (Fig. 2). Above 15 mmol, the product reaction began to decrease with the addition of more diethanolamine. According to Worthington\(^19\), an excessive number of substrates can inhibit enzyme activities. This is due to the increasing amount of substrate. It will increase the number of substrate molecules competing to attach to the enzyme’s active surface. This can lead to blocking of the active side of the enzyme and preventing of other substrates from reacting. So, there is a decrease in the reaction rate and decline of the amount of the product. Therefore, the addition of substrate after reaching the optimum amount results in a decrease in the amounts of the resulting products. This is also supported by a study conducted by Gunawan and Suhendra\(^20\), who found that too high a substrate concentration increases the solution viscosity, causing interaction between reactants to become ineffective and potentially inhibiting the reaction or causing the substrate to act as an inhibitor.

#### 3.2.3 Optimization of enzyme amount

Enzymes accelerate the rate of reactions without altering the equilibrium position, meaning both the forward and backward reaction rates are increased by the same fold. To obtain maximum product amount, the number of substrates used should be optimized as well; however, the number of enzymes used should be minimal. The reason for this is that greater the number of enzymes used, higher are the costs required. The amount of enzyme used to produce the maximum product in this study was 0.15 g (Fig. 3).

According to Gunawan and Suhendra\(^20\), the optimum point in an enzymatic reaction is usually achieved when

![Fig. 1](image1)  The effect of reaction time.

![Fig. 2](image2)  The effect of substrate ratio.

![Fig. 3](image3)  The effect of enzyme amount.
mass of the enzyme used is 1.5% of the substrate mass. This principle can be applied when there are no limiting factors, such as low substrate concentration, presence of activator or inhibitor, or mass transfer effect. When the enzyme added is greater than 0.15 g, no significant increase in product amount is detected. This indicates that the amount of enzyme used has reached the optimum point and the amount of substrate to be changed is increasingly limited, so the increase in enzyme mass addition does not significantly affect the number of products formed, which is reflected by a more horizontal curve. Additional enzyme does not contribute to improving the product amount, due to the limitations of the substrate and mass transfer.

3.2.4 Optimization of temperature

Temperature change in reactions may affect reaction rate and enzyme activities and stability. In addition, it can also affect substrate solubility, with direct effects on the reaction and the enzyme. In this study, the optimization was carried out by varying the temperature from 30°C to 70°C at 10°C intervals. The observed data are shown in Fig. 4. Based on the data, it appears that at 30-40°C, the amount of alkyldiethanolamides was increased significantly. This is because the temperature rise caused the substrates solubility to be more compatible with the enzyme. The temperature of 40°C can therefore be selected as the optimum temperature for the synthesis of alkyldiethanolamides. At temperatures between 40 and 50°C, the product was still produced well. This is because, in this study, lipopolymerized lipozyme was used. Lipozyme enzyme is an immobilized lipase enzyme that has a more stable structure, along with other advantages; for instance, this enzyme can be reused after the washing process. At the temperature of 50 to 70°C, there was a decrease in the amount of reaction product formed. This occurred because enzymes are denatured at high temperatures. Islam et al. reported that lipase is very active at temperatures of 30-40°C, while at temperatures above 50°C, the enzyme may be deactivated.

3.3 Synthesis of alkyldiethanolamides at optimum conditions

Alkyldiethanolamides were synthesized at optimum conditions to determine the amount of product produced. The optimum conditions for the synthesis are shown in Table 1. In addition to applying the optimum conditions, synthesis of alkyldiethanolamides should also take into account pH level during the reaction, because lipase enzymes were used and they work best at neutral pH (~7). The synthesis produced two types of alkyldiethanolamides: solid and liquid. The solid phase was characterized by its white color, butter-like consistency, and greasy texture, unlike the liquid phase, which was colored yellowish-white and of quite a thick (gelatinous) consistency. The solid alkyldiethanolamides can be separated from the solvent by adjusting the sediment temperature or freezing point. Meanwhile, the liquid alkyldiethanolamide is separated from the solvent by adjusting its boiling point. The percentage of the solid and liquid phases obtained after the purification process was 27% and 29%, respectively. The total product yielded under the optimum conditions was 56%. Another researcher has synthesized monoethanolamide from palm kernel oil and palm kernel stearin with product percentage of 77% and 36%, respectively. The difference in the yield percentage is affected by the composition of fatty acids contained in oil or triglycerides and the success of purification process of the product. The reaction scheme for enzymatic synthesis of alkyldiethanolamides is shown in Fig. 5.

3.4 Qualitative and quantitative test of alkyldiethanolamides

Analysis of the reaction product was performed by thin layer chromatography. The eluent used was n-hexane:diethyl ether (87:13 v/v). The Rf value of alkyldiethanolamides obtained during the observation was similar to that of the alkyldiethanolamide standard. The next identification step was performed by FTIR and GC-MS. The FTIR analysis of alkyldiethanolamides was carried out by comparing FTIR spectra of ketapang oil (triglyceride), alkyldiethanolamide standard, and The synthesized alkyldiethanolamides. The results of the observations show that there were spectral differences between triglycerides and alkyldiethanolamides, but there were similarities between the spectra of the alkyldiethanolamide standard and the

![Fig. 4](image-url)  
**Fig. 4** The effect of temperature.

<table>
<thead>
<tr>
<th>No.</th>
<th>Optimization Parameter</th>
<th>Condition</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Reaction time</td>
<td>2 h</td>
</tr>
<tr>
<td>2</td>
<td>Oil (g) : Diethanolamine amount (mmol)</td>
<td>1 : 5</td>
</tr>
<tr>
<td>3</td>
<td>Oil (g) : Enzyme (g)</td>
<td>1 : 0.075</td>
</tr>
<tr>
<td>4</td>
<td>Reaction temperature</td>
<td>40°C</td>
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</table>
The triglyceride spectrum shows absorption for the vibration of C=O and C-O ester groups at the wave numbers of 1753 cm⁻¹ and 1164 cm⁻¹, respectively. Meanwhile, the spectrum of the synthesized alkyldiethanolamides shows that there is an absorption band for C=O groups vibration with lower intensity, but the absorption band for C-O group is absent. The success of the alkyldiethanolamide synthesis is demonstrated by the presence of absorption bands in the area of 3450 cm⁻¹, exhibiting the stretching vibrations of OH groups. Vibration of formed N-H groups usually appears at 3118 cm⁻¹. The absorption will be visible as a single peak when N-H is the secondary type formed and appears as two-peaks when N-H is the primary type. Alkyldiethanolamide, however, contains N-H tertiary type that does not provide an absorption band in the known area. This is shown by the FTIR spectrum, which does not contain any peak in the area. Spectra of the synthesized alkyldiethanolamides do not display any peak in the area, either. Thus, to prove that the synthesized product is formed, absorption at 1651 cm⁻¹ showing the stretching vibration of C=O (carbonyl) of amide is used as evidence. The presence of the amide group was also supported by the appearance of absorption bands at wavelengths around 1280 to 1246 cm⁻¹, displaying C-N stretching vibration (Fig. 6). The FTIR spectrum of the synthesized product was similar to that of the standard alkyldiethanolamides. Similar FTIR spectra were also obtained from the study of Adewuy et al.²⁶ wherein alkanolamide was synthesized from *Gliricidia sepium* oil. GC-MS analysis revealed that the types of alkyldiethanolamides formed were palmitoyldiethanolamide (Mr = 343) with retention time of 32.88 min and oleyldiethanolamide (Mr = 369) with retention time of 35.13 min. Fragmentation observed for palmitoyldiethanolamide: m/z 343[M⁺], m/z 328[M-15]⁺, m/z 252[M-91]⁺ and for oleyldiethanolamide: m/z 369[M⁺], m/z 354[M-15]⁺, m/z 298 [M-71]⁺. To determine the total amount of N contained in the alkyldiethanolamide produced, a semi-micro Kjeldahl method was applied. Based on the result of this analysis, the total amount of N contained in the dry alkyldiethanolamide sample was 0.26%, or 0.19 mmol of the amide group in a 1-g sample.

Fig. 5  Reaction scheme for enzymatic synthesis of alkyldiethanolamides.

Fig. 6  Spectrum FTIR of alkyldiethanolamides.
4 CONCLUSION

Alkyl diethanolamides were successfully synthesized from ketapang kernel oil through enzymatic reaction under optimum conditions with 56-60% yield of conversion. The reaction products were characterized by TLC, FTIR, and GC-MS. The types of alkyl diethanolamides that were successfully synthesized are palmitoyldiethanolamide and oleoyldiethanolamide.

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References


