Activating Lipase Enzyme in the Candlenut Seed to Produce Fatty Acid Directly from Candlenut Seed

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Abstract. Candlenut seed contains a minimum of 60% of oil. Candlenut oil in the seeds can be used to turn it into oleochemicals such as paint, varnish, soap, medicines, cosmetics, and fuel. This study aims to produce fatty acids directly from the Candlenut by activating the lipase enzyme found in Candlenut. This study is done by varying the immersion time, the addition of water and stirring. The method of this research is by manually breaking the Candlenut, then blended with water and soaked with a certain time variation at temperatures of 30 °C and 35 °C. During the immersion, a pH test is carried out to determine the reaction pH. The Candlenut oil produced is tested for free fatty acid content according to AOCS Official Method Ca 5a-40, and then the composition of fatty acid is determined by using gas chromatography. From this study, it can be concluded that the lipase enzyme in candlenut can be activated directly to produce fatty acids. The highest fatty acid content in this study is 9.5%, achieved at a reaction time of 24 hours by stirring and adding 40% of water.

1. Introduction
Candlenut crop, known by the Latin name Aleurites moluccana (L.) Willd is a plant that has many benefits. Almost every piece of Candlenut trees can be used, including leaves, bark, wood, roots, gums, and flowers can be used for medicine, fuel torches, building materials, dyes, food, and other uses. Pure Candlenut seed oil is widely sold into the cosmetic industry and the slag can also be used as fertilizer after oil removed [1].

Candlenut seeds have a minimum oil content of 60% [2]. Based on research from Rashmi and Bhardwaj, oil content in the seed of Candlenut is 58.15% on the air-dried basis and 60.68% on the moisture-free basis [3]. The oil content in Candlenut seed can be processed into oleochemicals such as paint, varnish, soap, medicines, cosmetics, and fuel. Oleo food and oleochemicals from vegetable material more favorable to consumers than the oleo food and oleochemicals derived from animal or synthetic material, because it is biodegradable and it is cheaper [4].

One of the utilization of oleochemicals which can be obtained from the Candlenut seeds is a fatty acid. For Indonesia, the fatty acid needs will increase in coming years, because these fatty acids are widely used in various industries such as industrial tires, cosmetics, plastics, paints, pharmaceuticals, detergents, and soaps [4]. Therefore, it is necessary to take a step in fulfilling fatty acids in Indonesia, because so far the main cause of low fatty acid production is due to the uneconomical manufacturing process. In this study, the production of fatty acids directly from candlenut seed has been studied by activating the lipase enzyme contained in the seeds.
1.1. Candlenut oil content in seeds
Candlenut seeds contain high levels of unsaturated fats such as linolenic acid, linoleic acid, and oleic acid. Candlenut seeds also contain saturated fats such as palmitic acid, stearic acid, and icosanoic acid [3]. Table 1 shows the composition of fatty acid in Candlenut oil [5].

| Fatty acid     | Lipid numbers | Composition (%) |
|----------------|---------------|-----------------|
| palmitic       | C16: 0        | 5.0 – 9.0       |
| stearic        | C18: 0        | 2.0 – 5.0       |
| oleic          | C18: 1        | 20.0 – 30.0     |
| linoleic       | C18: 2        | 32.0 – 42.0     |
| alpha-linolenic| C18: 3        | 20.0 – 30.0     |
| icosanoic      | C20: 0        | ≤ 0.5           |

1.2. Candlenut Oil Quality
Factors that can cause an increase in the levels of free fatty acids in Candlenut oil are the water content in Candlenut oil and enzymes that function as catalysts in Candlenut oil. According to SNI 01-4462-1998, the chemical and physical properties of Candlenut oil are shown in Table 2 [2].

| No   | Parameter                   | Requirements       |
|------|-----------------------------|--------------------|
| 1    | FFA (%)                     | 0.10 to 1.50       |
| 2    | Iodine numbers (G 12/100 g sample) | 136-167           |
| 3    | Saponification (Mg KOH / g sample) | 184-202     |
| 4    | Color                       | Normal             |
| 5    | Density (g/cm³)             | 0.9240 to 0.290    |
| 6    | The refractive index        | 1.4730 to 1.4790   |

1.3. Enzymes in Candlenut Seeds
Lipase is the enzyme catalyzing the hydrolysis of the ester bonds of triglycerides to produce free fatty acids, diglycerides, monoglycerides and glycerides [6]. An enzyme that plays a role in the formation of fatty acids and glycerol in candlenut seeds is lipase enzyme. Indications of lipase enzyme activity can be identified by measuring the increase in acid numbers. The lipase enzyme will be damaged at temperatures above 60 °C, and the activity of this enzyme is slow in the newly harvested, but its activity will rapidly increase if injured.

1.4. Fatty Acid Production Process
Fats and oils can be hydrolyzed into fatty acids and glycerol. In this case, the hydrolysis reaction of triglyceride is equal to the saponification reaction, but the oils and fats react with water to obtain fatty acids and glycerol. During the hydrolysis reaction, a light phase containing fatty acids, and a heavy phase containing glycerol and impurity. During the hydrolysis reaction, oil or fat molecules will produce one mole of glycerol and three moles of fatty acid [7]. The hydrolysis reaction can be seen in figure 1 [4].
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\begin{align*}
\text{CH}_2\text{RCOO} & \quad \text{CH}_2\text{OH} \\
\frac{1}{\text{CH}_3\text{RCOO}} + 3 \text{H}_2\text{O} & \rightarrow \frac{1}{\text{CH}_3\text{OH}} + 3 \text{RCOOH} \\
\text{triglyceride} & \quad \text{water} \quad \text{glycerol} \quad \text{fatty acid}
\end{align*}
\]

Figure 1. Oil hydrolysis reaction with water

The process carried out on hydrolysis can be done in three ways, namely hydrolysis at high temperatures and high pressures, enzymatic hydrolysis with the addition of enzymes, and by activating the lipase enzyme in the fruit. In this study, hydrolysis is carried out by activating the lipase enzyme found in Candlenut seeds.

2. Methods

2.1. Materials and Equipment

The material used in this study is the Candlenut seed and Aquadest (H\textsubscript{2}O). The equipment used is Buret, Funnels separator, electric oven, stative and clamps, glass beaker, blender, erlenmeyer, centrifuges, drop pipette, pycnometer, Ostwald viscometer, hand press, pH meter, and gas chromatography.

In this study, the first thing to do is to peel the candlenut seeds from their shells, then the candlenut seeds are cut into thin pieces with a thickness of ± 1 cm. Water is added to the pieces of candlenut seeds, then blended. The amount of water added is 0%, 10%, 20%, 30%, and 40% of the weight of the candlenut seeds. The mixture of candlenut seeds and water is soaked with a time variation of 2 hours, 6 hours, 12 hours, 24 hours, and 48 hours at temperatures of 25 \textdegree C and 35 \textdegree C. During the immersion process, a mixture of candlenut seeds and water is given treatment without stirring, and stirring with a stirrer for 10 minutes every 2 hours. After the specified time, mix the candlenut seeds and water then heated to remove water using the oven. Oil and solids from the oven are then separated using a hand press. After the oil and water are separated, the pure oil produced is put into a centrifuge so that the oil and the remaining deposits can be separated. The candlenut oil obtained is then tested for its density, viscosity, fatty acid level and its composition.

3. Results and Discussion

3.1 The influence of reaction time and water addition on fatty acids content

From the results obtained in Figure 2 and Figure 3, it can be seen that at a temperature of 30 \textdegree C, the effect of stirring is not significant in increasing the content of fatty acids. The effect of adding water is also not very influential for raising fatty acid content at 30 \textdegree C. In general, it can be seen that the increase in fatty acids from 2 hours to 48 hours of reaction duration only ranges from 1-1.5% for the addition of 10% - 40% of water. From Figure 2 and Figure 3, it can be seen that the highest increase is obtained by adding 10% water, but the highest level of fatty acid is obtained by adding 40% water. The highest fatty acid level at 30 \textdegree C is obtained after the reaction run for 48 hours, which is done by stirring.
Figure 2. Effect of reaction time and water addition on the fatty acid formed at a temperature of 30 °C with stirring

Figure 3. Effect of reaction time and water addition on the fatty acid formed at a temperature of 30 °C without stirring

In contrast to figure 4 and figure 5, it can be seen that at an operating temperature of 35 °C, the effect of stirring is very influential in increasing the content of fatty acids. At each addition of water, these fatty acids increase drastically at a temperature of 35 °C. The highest increase is
obtained by adding 40% of water, and the highest fatty acid level is also obtained by adding 40% of water, which is 9.5%. These fatty acid levels are obtained after the reaction runs for 24 hours. The results obtained are in accordance with the results of a study conducted by Sharma, et al., which states that with stirring, the chance of enzyme interactions with the substrate becomes greater so that the reaction speed is higher. Thus, the fatty acids produced will also be higher. Sharma, et al. also concluded that the amount of water has an important role in the hydrolysis reaction. In the case of oil hydrolysis, water molecules react with the ester bonds in triglycerides and increase fatty acid and glycerol as the product. Similarly, the excess amount of water may also inhibit the hydrolysis reaction through inhibition of substrate to bind the enzyme [8].

![Figure 4](image-url)

**Figure 4.** Effect of reaction time and water addition on the fatty acid formed at a temperature of 35 °C with stirring
3.2 Characteristics of the Candlenut fatty acid seeds
The characteristics of the Candlenut seed fatty acids consist of density, viscosity and pH. Density, viscosity, and pH value cannot be concluded whether it meets SNI or not because until now there has not been a density, viscosity and pH standard requirement for Candlenut oil fatty acid. From the results obtained, it is obtained that the density of Candlenut seeds fatty acid range from 0.917 g/cm$^3$ to 0.92 g/cm$^3$. This density is compared with linoleic acid density because linoleic acid is the largest component in the Candlenut seeds fatty acid. In theory, linoleic acid has a density of 0.901 g/ cm$^3$.

In testing the viscosity of the Candlenut seeds which is carried out at room temperature of 30 $^\circ$C, it is found that the viscosity of candlenut seeds fatty acid range from 17,192 cSt - 18,66 cSt. For comparison, Rabelo et al., in predicting viscosity for fatty acid studies, reported that their results show that the kinematic viscosity of linoleic acid is 17.452 cSt [9].

In this study, the pH obtained is 6, both at 30 $^\circ$C and at 35 $^\circ$C. This result is different from several other studies. Fu, et al., report that lipase proved to be a more effective catalyst with optimal activity at pH 6.5-7.0 [10], while Khor, et al., report that the lipase from C. rugosa exhibits an optimal activity at pH 7.5 [11].

3.3 Fatty Acid Composition of Candlenut Seed Oil
Based on the analysis of Gas Chromatography, the fatty acid content in Candlenut Seed oil can be seen in Table 3. The results of the analysis using Gas Chromatography show that the highest fatty acid content in the Candlenut Seed oil is linoleic acid, which is 38.25%. This is in accordance with the results of testing the content of fatty acid Candlenut Seed oil from Akoma International (UK) LTD, where the highest fatty acid content produced from Candlenut Seed oil is linoleic acid with a content of 38.6% [6].

**Figure 5.** Effect of reaction time and water addition on the fatty acid formed at a temperature of 35 $^\circ$C without stirring
Table 3. Fatty Acid Composition in Candlenut Seed Oil

| Fatty acid     | Composition (%) |
|---------------|-----------------|
| heptadecanoic | 4.6             |
| stearic       | 2.65            |
| oleic         | 29.05           |
| linoleic      | 38.25           |
| linolenic     | 23.01           |
| tetradecanoic | 2.44            |

4. Conclusion
The lipase enzyme in Candlenut seed can be activated directly to produce fatty acids by injuring the seeds and adding water. The fatty acid content also increase according to the length of reaction time and influenced by stirring. The highest fatty acid content of 9.5% is achieved at a reaction time of 24 hours, adding 40% of water and by stirring.

5. References
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