Utilization of sesame seed sprout biomass as lipase source to hydrolyse palm oil

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Abstract. Lipase is known enzyme for its ability to hydrolyse triglycerides into free fatty acid. There are so much sources of lipase such as plants, microbes, and animal. But, lipase from plants seems interesting owing to its huge amount than microbes' lipase and less harm than animal’s lipase. Many experts have proved that vegetable lipase is a potential biocatalyst so that lipase from sesame seed and sesame sprout was investigated in this research. Lipase which was obtained from each sources were then poured with 0.3 g PVA and 40% palm oil in demineral water. The lipase-palm oil solutions were then titrated with 0.05 M NaOH obtaining hydrolysis percentage. Based on the research, sesame seed supernatant produces 1.36 mmol FFA with 39% hydrolysis. Sesame sprout extract produces 1.37 mmol FFA with 39.14% hydrolysis. Those samples also contain lauric acid, miristic acid, palmitic acid, oleic acid and stearic acid which detected by GC instrument.

Keywords: Hydrolysis, Lipase Biocatalyst, Palm oil, Sesame Seed Sprout, Triglycerides

1. Introduction
Lipase is an enzyme which takes important group of enzymes in biodiesel production (processing fatty acid alkyl esters) [5]. It has different characteristic from chemical catalyst such as eco-friendly, high specificity, and stereoselective. In addition, can be found in many living creatures such as animals, plants, and microbes [2]. So there is high potency of replacing the usage of chemical catalyst with biocatalyst since they both have good performance.

Commercial lipase is now available but the price is way more expensive than recoverable chemical catalyst [8]. This research comes up with that problem and attempt to find economic method of producing lipase. Previous experiments focus on extracting lipase from sap papaya, beans sprout, and rice peel waste. In this research, sesame sprout was used to find its hydrolysing performance towards palm oil. Sesame seed (Sesamum indicum L.) known for its nutritious content and great source of essential oil (51.1%).

One benefit of utilising catalyst is adding palm oil value. Nowadays, palm oil is still restricted as a raw material of cooking oil or oleochemical product. Meanwhile, palm oil contains fatty acid that can be hydrolysed into energy. This research tried to find another biocatalyst to help maximizing the esterification process.

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2. Material and Methods
The research was conducted at Bioprocess Laboratory, Chemical Engineering Department, Engineering Faculty, University of Indonesia, Depok. Analysing sesame sprout content with HPLC in Balai Besar Pascapanen, Cimanggu, Bogor. Fresh sesame sprout and palm oil were purchased from local market around Jakarta. Polyvinyl alcohol (PVA) was purchased from Bratachem.

2.1 Preparation sesame seed into sprout
Washed sesame seed was divided into two group, the one which directly used and the other one to be cultivated into sprout. Sesame seed which prepared to be cultivated was then soaked into fungicide solution (1ml/l demineral water) for 10 minutes. Soaked sesame seed was removed from the fungicide solution, sowed onto sterile sand at 30°C for six days [1].

2.2 Obtaining sesame extract
Both germinated and ungerminated seed were then washed with demineral water and homogenized for 10 minutes in 0.15 M phosphate buffer (5 ml/g fresh weight) containing 0.6 M sucrose, 1 mM EDTA, 10 mM KCl, 1 mM MgCl₂. Phosphate buffer was adjusted to pH 7.5 with KOH. Those solutions were centrifuged for 30 minutes at 5,000 rpm. Supernatant from centrifugation process and sesame sprout extract were used in hydrolysis activity test.

2.3 Hydrolysis reaction
Supernatant from sesame seed and sesame sprout extract were soluted into 4 ml phosphate buffer (0.1 M, pH 7.5) separately. Each solution was then stirred at 800 rpm for 30 minutes to dissolve enzyme content. After that, poured 10 ml palm oil in 15 ml demineral water and 0.3 g PVA into each enzyme solution and shook in a shakerbath at 150 rpm, 37°C. Hydrolysed solutions were then filtered by Whatmann paper filter then continued by centrifugation to separated oil and water content. Lipase activity was determined by continuous titration of FFA released by lipolysis of emulsified oils. 2 ml centrifuged oil content was tested with 3 droplets of pp indicator and titrated with 0.05 M NaOH.

2.4 Free fatty acid analysis
Oil sample was weighed 0.1-0.5 g to be poured 2 ml NaOH-methanol 50% and heated up at 80°C for 15 minutes. When the solution cool enough, added 2 ml BF₃ solution and heated up again at 80°C for 45 minutes. Let the solution cool before added 1-2 ml Hexane and shook gently until homogen. Replaced the hexane solution before injected into GC instrument.

3. Results and Discussion
3.1 Hydrolysis reaction using sesame sprout’s enzyme
The experiment shows that hydrolysis result from sesame seed and sesame sprout has only slight different. It means that both sesame seed and sesame sprout are potential as lipase source to hydrolize palm oil. Compared to candida rugosa and rhizopus oryzae, hydrolysation percent were not the highest, but sesame is the best lipase source from an economic consideration. Beside that, both sesame seed and sesame sprout does not need long preparation to obtain its lipase. Unlike obtaining lipase from fungus, its process is relatively simple and easy. One of advantages to be considered to produce a commercial lipase from sesame seed or sesame sprout. Here is the data result from 8 hours hydrolysis in pH 7, 37°C and 150 rpm.
After 8 hours of hydrolysing, sesame seed could hydrolyse 39% palm oil and sesame sprout could hydrolyse 39.14% palm oil. Candida rugosa as the highest hydrolysis agent could hydrolyse 52% olive oil and Rhizopus oryzae could only hydrolyse 30% palm oil.

3.2 Content analysis using GC instrument

3.2.1 Pure Palm Oil. The data below shows that palm oil contains free fatty acid naturally. Those fatty acids were detected by GC instrument with various concentration for each fatty acid as seen below.

| Pure Oil | Oil Concentration (mmol/100 gr) | 0 hour | 8 hour |
|----------|---------------------------------|--------|--------|
| Lauric Acid | 0 | 5,580 |
| Miristic Acid | 0 | 0,644 |
| Palmitic Acid | 0 | 5,074 |
| Oleic Acid | 0 | 1,816 |
| Stearic Acid | 0 | 7,253 |

3.2.2 Sesame Sprout and Sesame Seed Supernatant. The data below shows free fatty acid concentration in sesame sprout and sesame supernatant. Those fatty acids were detected by GC instrument with various concentration for each fatty acid as seen below.

| Sesame sprout | Concentration (mmol/100 gr) | 0 hour | 8 hour |
|---------------|-------------------------------|--------|--------|
| Lauric Acid | 0 | 2,575 |
| Miristic Acid | 0 | 3,399 |
| Palmitic Acid | 0 | 3,066 |
| Oleic Acid | 0 | 3,362 |
| Stearic Acid | 0 | 4,032 |
Table 3. Free fatty acids content in Sesame supernatant

| Supernatant | Concentration (mmol/100 gr) |
|-------------|----------------------------|
|             | 0 hour | 8 hour |
| Lauric Acid | 0      | 3,515  |
| Miristic Acid| 0     | 1,407  |
| Palmitic Acid| 0    | 0,000  |
| Oleic Acid | 0      | 8,606  |
| Stearic Acid| 0    | 5,419  |

The tables above show different contents of free fatty acids in sesame sprout and sesame supernatant after hydrolysis reaction was done. Sesame sprout seems having the average concentration for each fatty acids. In the other hand, sesame supernatant could only produce lauric acid, a little miristic acid, high oleic acid, and stearic acid while palmitic acid were not detected by GC instrument.

4. Conclusion
Enzyme activity during hydrolysis reaction showed good result. Both sesame seed and sesame sprout broke triglycerides into free fatty acid with relatively same amount (1.36 mmol and 1.37 mmol). Prosentase hydrolysis for each method is as follows 39% and 39.14%. From GC analysis, sesame seed formed oleic acid as the highest fatty acid continued by stearic acid, lauric acid, miristic acid, and the absence of palmitic acid (8.606, 5.419, 3.515, and 1.407 mmol/gr). On the contrary sesame sprout formed stearic acid, oleic acid, lauric acid, miristic acid, palmitic acid as muc as 4.032, 3.362, 2.575, 3.399, 3.066 mmol/100gr. From the data, lipase content in plants is relatively same with lipase content in microbes. But the interesting point in producing lipase from plant is its huge source in nature than microbes.

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