

# **Fatty acid production from avocado seed by activating Lipase Enzyme in the seed**

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**Abstract.** During this time avocado seeds are not utilized optimally because they are considered as waste. The amount of avocado seeds from day to day is increasing, along with the increasing production of avocados. This avocado seed has many benefits including having oil and lipase enzymes that can be used to produce fatty acids. This study aims to produce fatty acids directly from avocado seeds by activating the lipase enzyme found in the seeds of the avocado. This research is carried out by destroying the avocado seeds using a blender, then adding water with variations between 0% - 40% to the mass of avocado seeds. This reaction is carried out at temperatures of 30 °C and 35 °C and is carried out by stirring and without stirring. Analysis of acid levels is carried out from 0 hours to 48 hours. From the research conducted, the highest fatty acid content is 11.67%. This result is obtained after 12 hours of reaction at temperatures of 35 °C with the addition of water by 40% and is carried out by stirring.

## **1. Introduction**

Avocados are very easy to get in Indonesia, and many people like this fruit because it tastes delicious. However, most people only eat the fruit while avocado seeds are thrown away and become waste. Based on FAO data in 2016, it is known that Indonesia is the fifth largest avocado producer in the world (after Mexico, the Dominican Republic, Colombia and Peru) with a production of 300,000 tons/year [1]. As avocado production increases, the avocado seed produced also increases. Percentage of seed mass to total fruit weight is 17-31% [2], so avocado waste produced by Indonesia is about 51,000-93,000 tons/year. Avocado seeds are rich in polyphenols with antioxidants and antimicrobials, and are also rich in triglycerides, but low percentage of fatty acids [3]. The amount of oil content in avocado seeds makes avocado seeds can be utilized by turning them into oleofood and oleochemical. Oleofood and oleochemicals from vegetable materials are more favored by consumers compared to oleochemicals derived from animals or from synthetic materials, because they are biodegradable and the price is cheaper [4].

One of the oleochemical products that can be obtained from avocado seeds is fatty acid. Fatty acids are widely used in various industries such as the tire, cosmetics, plastic, paint, pharmaceutical, detergent, and soap industries [4]. Based on the many benefits of this fatty acid and the availability of abundant avocado seeds, it is necessary to make fatty acids derived from avocado seeds. The process of making fatty acids is carried out enzymatically by utilizing the lipase enzyme found in avocado seeds.

## **The oil content in avocado seeds**

Generally, every oil is formed in plants, so fatty acids will also be formed. Fatty acids are

obtained from animals and plants such as oil palm, coconut, corn, soybeans, castor seeds, sunflower seeds, and avocado seeds. While synthetic fatty acids can be obtained from the petrochemical industry. In its use, fatty acids play an important role in the oleochemical industry, such as in the tire, soap, detergent, fatty alcohol, polymer, fat amine, cosmetics and pharmaceutical industries [4]. Based on the study of Prasetyowati, et al., (2010), they found that avocado seeds contained 25.15 of oil [5]. Pramudono, et al., (2008) also examined that avocado seeds contained 18.69% of oil, which was obtained by extraction using n-hexane solvents for 2 hours [6].

### **Lipase Enzyme in Avocado Fruit Seeds**

The enzyme that is very influential in the formation of fatty acids and glycerol is the lipase enzyme. Lipase enzymes are abundant in oil-containing grains, such as soybeans, castor seeds, oil palm, coconut, sunflower seeds, corn seeds, and also found in animal flesh and in several types of bacteria [4]. Olaeta (2007) reported the presence of enzymes in avocado seeds [7], and Sya'bani, et al. (2017) were successful in isolating lipase enzyme from avocado seeds [8]. Sya'bani reported that the lipase enzyme activity of isolated Avocado seeds had an activity of 2 U/ml for normal conditions and 6.6 U/ml for optimum conditions.

### **Hydrolysis of Oil with H<sub>2</sub>O**

Fat or oil can be hydrolyzed into fatty acids and glycerol at high temperatures and high pressures. Hydrolysis is a reversible reaction in which fatty acids are released from triglycerides, and produce free fatty acids and glycerol. The hydrolysis reaction is the same as the saponification reaction, but oil and fat react with water to obtain the yield of fatty acids and glycerol. During the hydrolysis reaction, a light phase containing fatty acids and heavy phases containing glycerol, and each fat or oil molecule will produce one mole of glycerol and three moles of fatty acid [10].

### **Hydrolysis of Oil with Enzymes**

Besides at high temperatures and pressures, fats and oils are also enzymatically hydrolyzed at low temperatures. The enzyme that plays a role in this hydrolysis is the lipase enzyme [11]. At this time the enzyme lipase which can already be used commercially include *Immobilizes lipase* derived from *Candida antartica* (Novozyme 435), *Mucormiehe* (Lipozyme IM), and *Candida cilindracea* (Sigma) [4].

### **Enzymatic direct hydrolysis of avocado seeds**

Olie(1974) had investigated that hydrolysis of triglycerides directly by activating the lipase enzyme as a biocatalyst found in palm oil can produce fatty acids and glycerol [12]. Tambun (2007) also investigated that direct hydrolysis of oil palm by activating the lipase enzyme as a biocatalyst contained in oil palm is an alternative process that can be done to obtain fatty acids, and the enzymatic hydrolysis directly reduce the cost of producing fatty acids because it only requires low energy consumption and also do not need to buy lipase enzymes that are very expensive [4].

## **2. Methods**

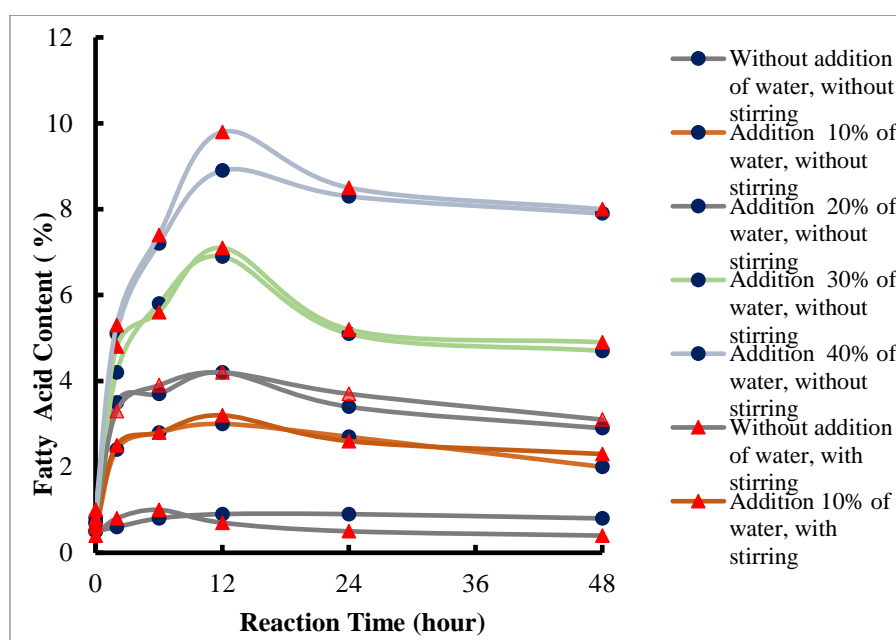
In this experiment, the avocado seeds are cleaned from the skin, washed, and cut into pieces. Water is added in varying amounts, water is added with varying amounts, ranging from 0%, 20%, 30% and 40% of the weight of the seeds, then stirred using a blender. The mixture of water and avocado seeds are then allowed to react naturally with lipase enzyme catalysis in

avocado seeds. This reaction is carried out at temperatures of 30 °C and 35 °C, with stirring and without stirring. The stirring in this experiment is done for 10 minutes every 2 hours. FFA contents are tested from the beginning then after 2 hours, 6 hours, 12 hours, 24 hours and 48 hours, and before tested, the water content is reduced by heating in an oven with at temperature of 105 °C [13]. The oil and water is separated from the seed by using hand press. Then the density, viscosity and fatty acid contents of the oil are analyzed. In this experiment, the composition of fatty acid content is analyzed by using gas chromatography.

### 3. Results and Discussion

#### 3.1 Effect of water addition and reaction time on fatty acid content of Avocado seed at 30 °C

The fatty acid content formed is influenced by many things, such as reaction time, the amount of water added, stirring, and the reaction temperature. In Figure 1, we can see that at the reaction temperature of 30 °C, the levels of fatty acids increase with increasing water content and reaction time, both with stirring and without stirring. The highest fatty acid content at the reaction temperature of 30 °C is 9.8%, which is obtained by adding 40% of water and reaction time of 12 hours, and by stirring. While if without stirring, the highest fatty acid content is 8.9%. From the results obtained, it is known that the reaction carries out by stirring gives higher content of fatty acids than without stirring. This is because the stirring process helps reduce saturation due to the product being formed, so that the reaction can continue.

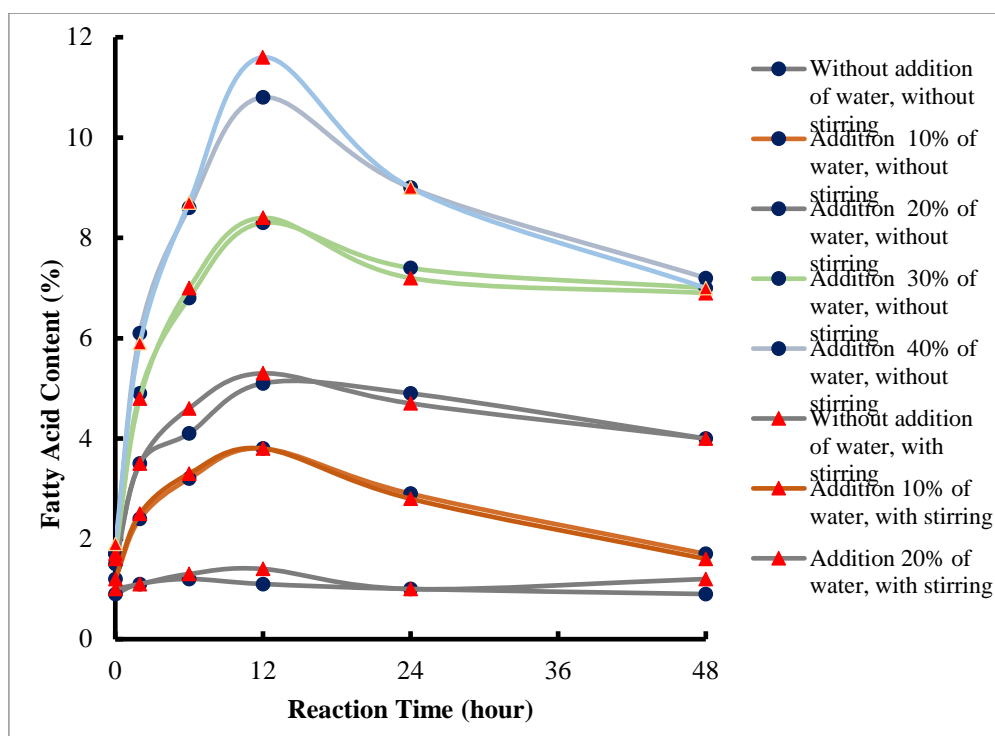


**Figure 1.** Effect of water addition and reaction time on fatty acid content of Avocado seed at 30 °C

#### 3.2 Effect of water addition and reaction time on fatty acid content of Avocado seed at 35 °C

The lipase enzyme that acts as a biocatalyst in the hydrolysis reaction has an optimum temperature of around 35 °C. The fatty acid levels obtained at temperatures of 35 °C are

higher than at room temperature because at a temperature of 35 °C the lipase enzyme has optimal activity. Figure 2 illustrates the hydrolysis process at 35 °C with a variation of the addition of water up to 40%, carried out by the stirring process and without stirring. As time increases and the number of water increases, fatty acid content increase to reach the highest level of 11.67%, which is achieved at 12 hours and the addition of water is 40%, with a stirring treatment. While if without stirring, the highest fatty acid content is 10.8%.



**Figure 2.** Effect of water addition and reaction time on fatty acid content of Avocado seed at 35 °C

From the figure above it can be concluded that the reaction time affects the amount of fatty acids formed. It can be seen that at temperatures of 30 °C with the addition 0%, 10%, 20%, 30%, and 40% of water, both by stirring and without by stirring, the fatty acid content increase until the reaction time reach 12 hours, and after that the fatty acid content decrease. Likewise at 35 °C with the addition 10%, 20%, 30%, and 40% of water, the fatty acid content increase until the reaction time reach 12 hours, and after that the fatty acid content decrease. According to Li D et al. (2015) the hydrolysis reaction is a reversible reaction [14]. The reactions until a certain time can increase the activity of the lipase enzyme, but if the reaction is continued the activity of the lipase enzyme can be reduced so that the fatty acid as a product by hydrolysis will decrease [4, 12, 15]. The decrease of fatty acid content because fatty acids as a product can be as inhibitor for the enzymatic hydrolysis reaction or because the fatty acid content is too high causing reversible reaction, so the fatty acid decomposes again into triglycerides.

#### Effect of adding water to fatty acid levels

Water content is one of the important parameters in an enzymatic hydrolysis reaction. In an enzymatic hydrolysis reaction, water acts as a reactant and also as a modifier for lipase

enzymes during the reaction. The water content in the reaction affects the reversibility of the hydrolysis reaction. Addition of water content increases the balance of the reaction in a positive direction, which means increasing the level of hydrolysis so that the reaction can produce high levels of fatty acids [13, 16-17]. If the water content is low in the hydrolysis reaction, the reaction will be difficult, because the lipase enzyme catalyzes the esterification reaction requires enough water to reach equilibrium [18]. But if the hydrolysis reaction has too much water content, the hydrolysis level will decrease too. This happens because the water content that is too high will cause the rate of hydrolysis to slow down due to reduced contact between lipase enzyme and oil. Excess water will make a layer thick enough on the surface of the enzyme and cause diffusivity problems from the substrate and the product from the active side of the enzyme. Excess of water can also cause enzyme denaturation [16].

### Composition of Fatty Acids from Avocado Seed Oil

The fatty acid composition of Avocado Seeds as a result of Gas Chromatography analysis can be seen in Table 1. Based on result obtained by using Gas Chromatography, it is known that the most fatty acid content in avocado seeds is linoleic acid, which is as much as 37.98%. This is close to the research of fatty acid content of avocado seeds conducted by Bora et al, where the highest fatty acid content produced from avocado seeds is linoleic acid, with a content of 38.89% [9].

**Table 1.** Fatty Acid Composition of Avocado Seed by Using Gas Chromatography Analysis

Fatty Acid Composition	Results (%)
Heptadecanoic acid	7.42
Oleic acid	30.43
Linoleic acid	37.98
Linolenic acid	24.17

### Density and Viscosity Analysis

In the tests carried out, it was found that the density of avocado seed oil ranged from 0.79 g/cm<sup>3</sup> (at 0 hour, without adding water, without stirring) to 0.9377 g/cm<sup>3</sup> (at 12 hours, adding water as much as 40% of the mass sample, and with stirring), while the viscosity of avocado seed oil in this study ranged from 7,771 cSt (at 0 hour, without adding water, without stirring) to 13,925 cSt (at 12 hours, adding water as much as 40% of the mass sample, and with stirring). Based on Risyad's analysis (2016) by using Gas Chromatography, it was found that linoleic acid was the most abundant fatty acid obtained in avocado seeds, so the density obtained was compared with the density of linoleic acid [19]. Theoretically, linoleic acid has a density of 0.901 g/cm<sup>3</sup>, and viscosity of 17.452 cSt [20].

### 4. Conclusion

Fatty acids can be made directly from avocado seeds by activating lipase enzyme that is in avocado seeds. The fatty acid content will be higher with the addition of more water. Increasing fatty acids content is also influenced by stirring and the length of reaction time. The highest fatty acid content about 11.67% is obtained after 12 hours of reaction at temperatures of 30 °C with the addition of water by 40% and is carried out by stirring.

### 5. References

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