LIPASE ACTIVITIY OF MIXTURE OF FERMENTED AVOCADO (Persea americana), BANANA (Moses paradisiaca) AND SNAKEFRUIT (Salacca zalacca)

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ABSTRACT

Fermentation is one of the bioconversion processes to produce anaerobic microbes which are beneficial and can produce enzymes. One of the enzymes that can be produced is lipase. Mixed fruit fermentation is an effort to produce lipase that can be done simply. The purpose of this research is to know the activity of lipase enzyme from mixture of fermented avocado fruit (Persea americana), bananas (Musa paradisiaca) and snakefruit (Salacca zalacca). Lipase enzyme activity is tested by measuring of free fatty acid (FFA) content obtained from olive oil hydrolysis process by lipase enzyme. The results showed the highest activity obtained 17.425 U/ml and the percentage of FFA produced was 12.94%. The highest activity of lipase enzyme was obtained at 45^0C and pH 6.5. From this research can be concluded that the fermentation of the mixture of avocado, bananas and snakefruit can produce lipase enzyme that has relatively high activity.

Keywords: *Enzyme Activity, Fruits, Fermentation, Lipase.*

INTRODUCTION

Fresh fruits and vegetables have great benefits as a source of vitamins and minerals. Besides, fruits also contain many non-nutritional substances that are not widely known but are needed by the body in the process of digestion, such as carotenoid pigment, flavonoids, provitamin and enzymes.

Carotenoids are pigments that act as antioxidants and can protect cells (Wirakusumah, 2006), flavonoids can prevent various cancers caused by exposure to free radicals (Ismayanti, et al, 2013). In fruits and vegetables there are also enzymes that contribute to digestion and absorption (Wirakusumah, 2006). Enzyme is a product that has high economic value and has become an important practical tool because it is necessary to support various processes in the food industry and non food (Darwis and Sukara, 1990)

Enzymes along with vitamins work to accelerate the biochemical reactions in the body. Enzymes in large quantities found in fresh foods such as fruit and vegetables. The production of enzymes is mostly done by submerged fermentation fermentation (Riwayati, et al, 2012).

Fermented fruits can be utilized to produce enzymes as a result of excretion and metabolism from microbes. This situation can occur because fermentation is a bioconversion process for anaerobic microbial growth that is beneficial and can produce enzymes. Among the enzymes that can be produced is lipase (Sumarlin, et al, 2013).

Lipase (E.C. 3.1.1.3) is an enzyme that catalyzes the hydrolysis of triglycerides into glycerol and free fatty acids in the oil-water phase (Kamini & Iefuji 2001; Gupta et al., 2004; Feng et al., 2013). One type of enzyme that has an important and incomparable role in the development of biotechnology is lipase (Sumarsih, 2004). Lipase is known for its high activity in hydrolysis reaction and in chemical synthesis. Lipases may act as biocatalysts for esterification reactions, alkoholysis, acidification and aminolysis (Gunasekaran dan Das, 2005).

The biocatalytic properties of this lipase allow its use for various purposes such as detergent formulation (Thirunavukarasu et al., 2008), biosensors (Kartal et al., 2007), food industry (Ferrer et al., 2005), dairy industry and oleo industry Chemistry (Sana et al., 2004) ester synthesis (Jin et al., 2013), pharmaceutical and waste treatment (Savendsen, 2000). Lipase is also widely used in the processing of fats or oils, chemical synthesis, paper synthesis, cosmetic production and also biodiesel industry (Sari, 2012).

According to Euromonitor International, Company Report (2009), world production volume of enzymes has continued to rise since 2002. Lipase production is only about 21% of the total volume of enzymes produced worldwide in 2008 (Riwayati, et al, 2012). Increase in lipase demand every year is still filled with imported roads and the price is relatively expensive, so it is not profitable in terms of foreign exchange and biotechnology development in Indonesia. This encourages the research to obtain the source of lipase enzyme (Permana, 2009).

Production of enzymes in large quantities and have a high activity need to be considered important factors such as growth conditions, how to isolate, and the type of substrate used. The growth conditions that support maximal enzyme production are pH, incubation temperature, incubation time, and growth media composition must contain energy source, carbon source, nitrogen source and mineral.

Given the easyness of fruits obtained and cultivated in Indonesia, research needs to be done on the potential lipase enzymes contained in the fruit. In this study used a mixture of avocado fruit, bananas and fruits because they contain many lipase enzymes (Salas et al., 2000; Jadhav et al., 2013).

MATERIALS AND METHODS

Materials

All reagents and chemicals used were of analytical grade and all solutions were prepared from distilled water. C_2H_5OH, NaOH, $NaH_2PO_4.H_2O$, Na_2HPO_4, $H_2C_2O_4.2H_2O$, Acetone, Phenolphthalein, olive oil, avocado (Persea americana), banana (Musa Paradisiaca), snakefruit (Salacca Zalacca), brown Sugar.

Instruments

Analytical balance, centrifuge type of Hitachi, waterbath, vortex mixer, micro Burettes, Volumetric flask, measuring cylinder, Graduated Pipettes, Volumetric Pipette, beaker glass, Erlenmeyer Flask, sealed container made of glass, knife of stainless steel, filter cloth, pH measurements performed on pH Meter type HANNA.

PROCEDURE

Preparation of solutions

Fenolftalein 1%. The solution was prepared by dissolving 1 g of phenolftalein in 100 mL etanol 50% in volumetric flask.

NaOH 0,1 N solution. This solution was prepared by dissolving 4 g of sodium hydroxide in distilled water and adding the distilled water to 1000 mL in a volumetric flask.

$H_2C_2O_4.H_2O$ 0,1 N soltion. This solution was prepared by dissolving exactly 1.5757 g of oxalic acid in distilled water and adding the distilled water to 250 mL in a volumetric flask.

Standardization of NaOH with H_2C_2O4 . 10 ml of oxalic acid 0,1000 N solution was titrated with a standardized NaOH 0.1 N solution using phenolptalien indicator. The color change occurs from colorless to pink.

Preparation

Prepared containers of clean and dry glass covered bottles. Fruits consist of avocado, bananas and snakefruits are not fully ripened skin and cut thinly without washing. Prepared also red sugar that has been weighed as much as 100 g and distilled water of 1000 ml.

Fruit Fermentation. Inserted 1/3 part pieces of avocado, banana and salak alternately into a container of glass bottle. In each layer of fruit added brown sugar that has been cut into small pieces and filled with distilled water as much as 1 liter or up to 80% container glass bottle section. Contain contained fruit mixture then incubated for fermentation process by being kept closed at room temperature for 7 days (Macedo et al., 1997). On the third day, the bottle is opened to release the gas produced during the fermentation process. Bottle containers need to be re-closed to avoid contamination. After 7 days, the resulting fermentation fluid is filtered for testing.

Lipase Activity Test (Lindfield, et al., 1984; Jadhav et al., 2013)

Enzymes Isolation. The fermented liquids are saturated with 70% acetone and cooled overnight at temperatures below 10 ° C. The suspension formed was centrifuged at 15,000 rpm for 30 min and the resulting precipitate was dissolved with a phosphate buffer of pH 7.0 for the lipase activity test and free fatty acid content resulting from the hydrolysis of the olive oil substrate.

Effect of Temperature. Weighed 1 g of olive oil was added to the erlenmeyer flask and then added 4 ml of phosphate buffer pH 7.0 and 1 ml of lipase enzyme. The mixture in the vortex until homogeneous and heated at 30, 35, 40, 45 and 50 ^oC in waterbath for 10 minutes. Furthermore, 10 ml solution of ethanol solution was added: Acetone (1: 1) and 3 drops of phenolptalien were then titrated with NaOH 0.1 N until pink appears. The resulting volume of NaOH was used to calculate lipase enzyme activity and free fatty acid content at each temperature.

Effect of pH. Weighed 1 g of olive oil was added to the erlenmeyer flask and then added 4 ml of phosphate buffer each at pH 5.0, 6.0, 6.5, 7.0, 7.5, 8.0 and 1 ml lipase enzymes. The mixture in the vortex until homogeneous and heated in a waterbath for 10 minutes at the optimum temperature obtained previously. Furthermore, 10 ml solution of ethanol solution was added: Acetone (1: 1) and 3 drops of phenolptalien were then titrated with NaOH 0.1 N until pink. The resulting volume of NaOH was used to calculate lipase enzyme activity and free fatty acid content at each pH.

Blank Titration. Blank titrations should be performed without using enzyme solution by using the optimum pH already obtained in the previous procedure. Based on data of titration volume of balnko solution and sample solution on temperature and pH variation, lipase enzyme activity and free fatty acid (FFA) content were calculated by the formula:

$$\text{Lipase activity (U/ml)} = \frac{(V_s - V_b) \times \text{N NaOH} \times 1000}{V_E \times t}$$

$$\text{\% Free Fatty Acid (FFA)} = \frac{\text{mL NaOH} \times \text{N NaOH} \times 256}{\text{weight of olive oil (g)} \times 1000} \times 100$$

With: Vs = ml NaOH sample titration; Vb = ml NaOH blank titration; V_E = volume of enzimyme (ml); t = incubation time (min); N NaOH = Normality of NaOH; 1000 = conversion factor of mmol to μmol, and 256 = Mr Palmitic Acid.

RESULTS AND DISCUSSION

Lipase Enzyme Isolation

Isolation of lipase enzyme was done to concentrate lipase concentration from fermentation of mixture of avocado, banana and snakefruit. Isolation was performed for 7 days to obtain better lipase activity. This is according to the results of research Liman et. al., (2010), which fermented from seeds for 7 days. The production of enzymes with the highest enzyme activity is largely determined by the length of fermentation time and several other factors such as aeration rate, stirring rate, and initial pH.

Effect of temperature

The effect of temperature was carried out with five different temperature variations, namely 30 0C, 35 ^{0}C, 40 ^{o}C, 45 ^{o}C, and 50 ^{o}C to obtain optimum lipase activity. Temperature optimization is important because temperature changes have the potential to alter the enzyme charge on the active side. According Bisswanger (2013), the influence of temperature caused by the existence of three-dimensional structure of the enzyme is very sensitive to temperature. From the test results, the optimum temperature of 45 ^{o}C with lipase activity of 11,102 U / ml and FFA content of 4,438%, as shown in table 1.

Table 1. The value of lipase activity and % FFA with different temperature variations

Temperature (^{o}C)	Volume of NaOH 0,1 N (ml)				Activity (U/ml)	% FFA
	Blank	Average	Lipase	Average		
30	0,9 1,0	0,95	1,6 1,6	1,6	5,551	3,47
35	0,9 0,9	0,9	1,9 1,6	1,75	7,259	3,792
40	0,9 0,8	0,85	1,9 1,9	1,9	8,967	4,12
45	0,8 0,7	0,75	2,0 2,1	2,05	11,102	4,438
50	0,8 0,8	0,8	1,5 1,5	1,5	5,978	3,241

At temperatures below the optimum (45 °C) lipase activity was relatively stable with an increased tendency (Figure 1), but the ability to hydrolyze the olive oil substrate was not maximized. As the temperature increases, the frequency of collisions between the enzyme and the substrate molecule increases, so the resulting product gets bigger. At temperatures above 45 ^{o}C there is a dramatic decrease in lipase activity. According to Yuneta et al., (2009), above the optimum temperature of enzyme activity decreases sharply due to denaturation of proteins that can change the conformation of the molecular structure so that the enzyme loses its catalytic properties. Prior research conducted by Pratiwi et al ., (2013), has isolated lipase from Pseudomonas aeruginosa obtained optimum temperature 40 ^{0}C. While Yuneta (2009), successfully isolated the lipase from Bacillus subtilis bacteria with olive oil substrate obtained optimum temperature of 45 ^{0}C. This optimum temperature difference is possible because of the influence of temperature associated with different types of microbes and substrates (Bisswanger, 2013).

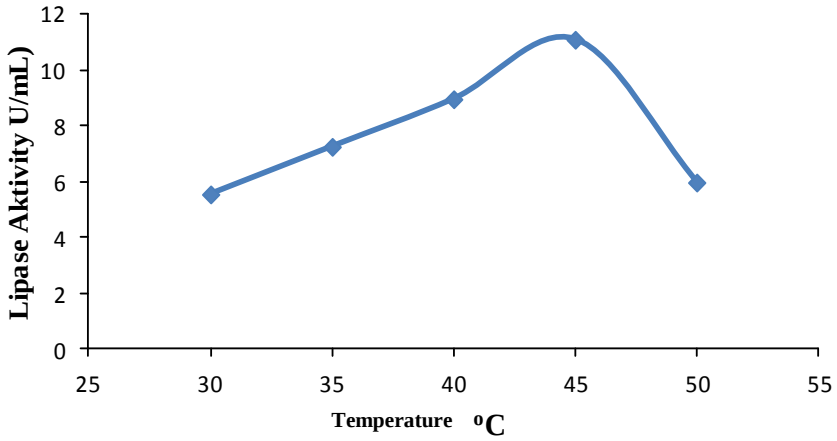

Figure 1. Graph of effect of temperature on lipase activity

Effect of pH

The effect of acidity degree (pH) was done with six variations of pH, ie 5.0; 6.0; 6.5; 7.0; 7.5 and 8.0 to obtain the optimum lipase activity using the optimum temperature obtained from the previous procedure. PH greatly influences the activity of enzymes in catalyzing its substrate (Sumarlin, et al, 2013). This is because the hydrogen ion concentration affects the dimensional structure of the enzyme and its activity. Each enzyme has an optimum pH wherein the pH is three-dimensional structure most conducive to binding to the substrate (Lehninger, 1998). At the optimum pH the amino acid group side load is in a suitable state so that the enzyme is very efficient in accelerating the reaction (Pratiwi, et al, 2013). From the test results obtained optimum lipase activity at pH 6.5 with the activity of 17.425 U / ml and FFA levels was 12.94%, as shown in table 2.

As shown in Figure 2, with increasing pH, lipase activity also increases. The increase in lipase activity lasted until it reached the optimum pH of 6.5 and increased sharply at pH 6.0. At a pH above 6.5 starts to decrease drastically and begin to tend to be constant at pH above 7.5. At low pH the acidic atmosphere causes decreased activity, whereas with the increase of pH into base can cause the structure of the enzyme is damaged. If an enzyme works at an extreme pH, then the enzyme will be denatured.

Research Liman et al., (2010), found that lipase from Africa locust bean, Castor seed, and Africa oil bean has an optimum pH 7. Similarly, Pratiwi et al., (2013) explained that

Pseudomonas aeruginosa bacteria obtained The optimum lipase activity at pH 7. While in the Yuzo and Sakaya (2003) studies, it was explained that lipase bacteria would be stable at optimum pH of 6-8, and would decrease if the pH was above 8. This optimum pH difference was an indication that the source of lipase- Type of substrate will affect the optimum pH of lipase activity.

Table 2. The value of lipase activity and % FFA with different temperature variations

pH	Volume of NaOH 0,1 N (ml)				Activity (U/ml)	% FFA
	Blank	Average	Lipase	Average		
5,0	2,2 2,1	2,05	2,8 2,8	2,8	6,375	6,080
6,0	3,2 3,2	3,2	4,0 4,0	4,0	6,8	8,643
6,5	4,0 4,0	4,0	6,1 6,0	6,05	17,425	12,94
7,0	1,0 1,1	1,05	1,9 1,9	1,9	7,225	4,085
7,5	1,0 1,0	1,0	1,7 1,7	1,7	5,95	3,669
8,0	0,9 0,9	0,9	1,4 1,5	1,45	4,675	3,117

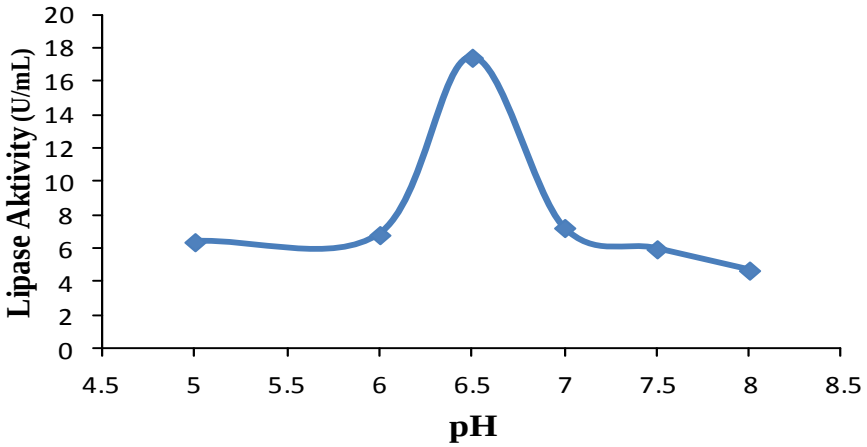

Figure 2. Graph of effect of pH on lipase activity

The utilization of fermented lipase so far has not been used as medicine or health drink that is consumed directly. Therefore, lipase enzyme produced from fermented mixture of avocado, banana and snakefruit is expected to be consumed directly as a health drink.

High lipase activity is very good for the health of the body, because lipase functions as a break down of fat into fatty acids and glycerol. Deficiency of this enzyme can be an indication of the occurrence of damage to the pancreas and trigger the onset of various diseases, such as cholesterol disease (Wirakusumah, 2006). Fat that should be broken down into simpler molecules and then absorbed by the body becomes inhibited due to lack of lipase enzymes so that fat will accumulate and clog the blood vessels. This will lead to more severe disease such as stroke. Adequacy of lipase in the body is very good to facilitate bowel work, treatment for constipation and as an enhancer of stamina (Dewi, 2013).

CONCLUSION

Production of lipase enzymes can be obtained from the fermentation of a mixture of avocado, banana and snakefruit. The test results obtained optimum activity at 45 ^{o}C and at pH 6.5 each with lipase activity of 11.102 U/ml and 17.425 U/ml. The existence of relatively high lipase activity from fermented mixture of fruit can be used for consumption as a health drink for the body.

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