International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2017): 7.296

Determination of Lipase Enzyme Activity from Rambutan Seed Germination

Meilynda Pomeistia

Mataram University, Department of Science Education, Indonesia

Abstract: ***The purpose of this research is to know lipase enzyme activity isolated from rambutan seed germinated. This research method includes activity test using titration method with olive oil substrate. The results of this study note that lipase enzyme activity tested using olive oil as much as 0.708 U / mL is proven in the rambutan seed germinated contain lipase enzymes.***

Keywords: Enzymes, Lipase, Activity, Germination, Rambutan seed.

1. Introduction

Lipase is the trivial name of the glycerol-ester hydrolase enzyme that catalyzes the reaction of fat and oil hydrolysis. In the field of fat and oil industry, lipase enzymes are also important because of their role in controlling the process of producing oils and fats; for example on cooking oil and margarine in the process of removing unwanted flavors and odors. By using lipase enzyme, some desired flavors can be arranged as desired[1]. Lipase enzymes are widely used in the fields of industry, cosmetics, and even pharmaceuticals. However, lipase in Indonesia is still imported from European countries, so much research is done to find the source of lipase enzymes which obtained the highest lipase enzyme activity on citrus fruits[2] and watermelon fruits of 1.36 U / mL[3].

Lipases have specific properties depending on their origin and substrate, the optimum lipase activity is highly dependent on pH and temperature. Under normal circumstances the pH should be stable because of the changes will cause a shift in enzyme activity[4]. The lipase enzyme requires a special substrate. This particularity becomes a major consideration factor in its analysis and application. Based on the substrate type, lipase is classified into several types, namely specificity in fatty acids, position, alcohol, acylglycerol, stereo and chiral.

The optimum pH of the enzyme to react is 4.5 to 8[1]. The activity of the lipase depends also of the emulsifying agent used and the presence or absence of salt in the substrate. Optimal lipase temperature generally ranges between 35oC and 45oC. Enzymes are a class of proteins, so they have physical and chemical properties similar to proteins. In doing its activity, enzyme is influenced by environment. Such influence can disrupt the stability of the enzyme so that it becomes a problem that is often encountered in the industry. The stability of the enzyme can be defined as the stability of enzyme activity during storage and use of the enzyme, and the stability of the damaging compounds such as certain solvents (acid, base) and by the influence of temperature and extreme pH [5].

Lipase is widespread in animals, plants and microorganisms, although lipases from many different sources have been widely described, relatively few have been studied to the detail [6]. Research on the spread of lipase in plants is still very little except in the seeds and fruits. Germinating seeds have high lipolytic activity in order to meet energy needs. Lipase is present in the seeds or fruit of coconut plants[7], distance [8], and sunflower [9]

2. Material and methods

Materials used olive oil, rambutan seed germinated, 0.05 M phosphate buffer, acetone, alcohol, phenolphthalein (PP) indicator, NaOH 0.05 M. The tools used in this research are pH meter, water bath, measuring pipette, test tube, erlenmeyer, a set of titration tools.

Procedure methodology

Enzyme assay

A total of 2.5 mL of aquabides was added 1 mL Tris-HCl buffer pH 7.3 and then added 2 ml of olive oil, shaker and put in oven at 27 ° C. The incubation was carried out for 5 minutes, after the sample was removed and then 1 ml of lipase enzyme was added, shaken and reincubated for 30 min, after incubation was finished the sample added 3 mL 95% ethanol was shaken and then immediately straightened. Lipase enzyme activity can be calculated as follows:

$$Enzyme\ assay\ (U/mL) = \frac{(A-B)\times[NaOH]\times 1000}{30}$$

Description: A = mL NaOH for the titration of the sample, B = mL NaOH for the blank titration, factor 1000 for conversion from mmol to µmol, and 30 = reaction time (30 min).

3. Results

The results of the crude extract activity test, the result showed that the samples incubated for 30 minutes at 27 ° C were hydrolyzed by lipase enzymes, this can be seen in the difference in titration volume performed, as in Table 1

Table 1: Volume of titration results

sample	Volume (mL)
Blank	4
Crude	4,425

The resulting volume is then incorporated into the equation to determine the enzyme activity with the lipase enzyme activity value of rambutan seed germinate 0.708 U / mL.

4. Discussion

Lipase activity has unit unit (U). One unit of lipzone activity is equivalent to 1 µmol of free fatty acid produced from the hydrolysis of the substrate catalyzed by lipase per minute[5]. Lipase enzyme activity shows the quality of the enzyme, where if the activity is high then when the enzyme used will produce good quality and vice versa. The more substrates hydrolyzed by the enzyme the higher the activity, but at a certain point when the enzyme has been reacted so much any substrate concentration added enzyme activity will not increase. The activity of lipase is obtained by hydrolysis reaction, because this reaction is most easily observed and performed. The principle of activity test with this method is that when the oil in this case olive oil consisting of triglyceride hydrolysis reaction which is catalyzed by lipase enzyme, then triglyceride will decompose into glycerol and free fatty acid. The occurrence of this chemical reaction is where the substrate is bound to the active side of the enzyme. Here is a general reaction of triglyceride hydrolysis which is catalyzed by lipase enzyme.

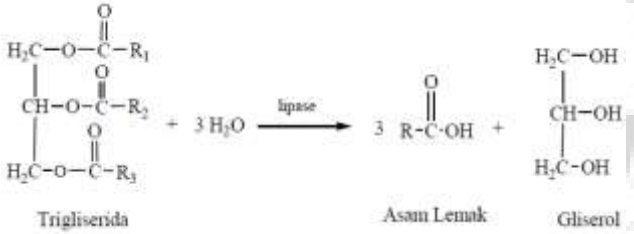

Figure 4.1: hydrolysis reaction

Table 1 shows that the difference in volume to the blank (without lipase enzyme) with the sample (with lipase enzyme) indicates that the hydrolysis process takes place and produces free fatty acids, free fatty acids released in this hydrolysis reaction which will then be titrated with NaOH. The more free fatty acids are produced, the required volumes of NaOH for the titration will increase the more meaningful the enzyme activity will be. Enzyme activity describes the ability of an enzyme to trigger a reaction or convert a substrate into a product of a particular reaction. The value of lipase enzyme activity on rambutan seed germinate shows that there is lipase enzyme that hydrolyzes olive oil substrate to free fatty acid.

5. Conclusion

The results of this study can be concluded that the presence of lipase enzyme contained in rambutan seed germinate with enzyme activity of 0.708 U / mL.

References

[1] Winarno, F.G. 1995. Enzim Pangan. Penerbit PT. Gramedia Pustaka Utama. Jakarta. 113 Hlm.
[2] Hermanto, S., Sumarlin L. O., Fatimah W. 2013. Diverentiation of Bovine and Porcine Gelatine Based on Spectroscopic and Electrophoretic Analysis. Journal Food Pharmaceutical Sciences. 68-73.
[3] Selvamohan, T., Ramadas, V., & Sathya, T. a. 2012. Optimization of Lipase Enzyme Activity Produced By Bacillus
[4] Girindra, A. 1993. Biokimia 1. Jakarta: Penerbit PT. Gramedia Pustaka Utama
[5] Akoh,C.C. and D.B.Min. 2002. Food Lipid : Chemistry, Nutrition and Biotechnology. 2nd edition, revised and expanded. New York : Marcel Dekker Inc
[6] Su'i, M., Harijono, & Aulani'am. 2010. Hidrolysis Activity of Lipase Enzyme from Coconut Houstorium for Coconut Oil. *AGRITECH*, *30*(3), 164–167.
[7] Abigor, R. D., Uadia, P. O., Foglia, T. A., Haas, M. J., Scott, K., & Savary, B. J. 2002. Partial Purification and Properties of Lipase from Germinating Seeds of Jatropha curcas L . *JAOCS*, *79*(11), 1123–1126.
[8] Sagiroglu, A., & Arabaci, N. 2010. Sunflower Seed Lipase : Extraction , Purification , and Sunflower Seed Lipase : Extraction , Purification , and Characterization. *Preparative Biochemistry and Biotechnology*, *35*(December 2012), 37–41.