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Determination of Optimum pH and Temperature for Crude Extract of Lipase Enzyme from Sprouts Palm Oil Seeds (*Elaeis guineensis Jacq*) Against Hydrolysis RBDPO (Refined Bleached Deodorized Palm Oil)

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Abstract. Determination of optimum pH and temperature for crude lipase enzyme activity from oil palm seeds germination had been done. Oil palm germination is made by separating the seed from palm coconut soaking time process, drying, heated and seed germination in temperatures 39 to 40ºC during 60 days, 30ºC during 21 days, and 25ºC during 14 days. Centrifugations obtained crude lipase enzyme during 30 minutes with the speed of rotation 5000 rpm and 10000 rpm, and addition of $(NH_4)_2SO_4$. Crude lipase enzyme diluted by buffer phosphate pH 7.0. The activity test of crude lipase enzyme done by measurement of free fatty acids levels obtained from hydrolysis process of RBDPO (Refined Bleached Deodorized Palm Oil) as substrate by titrimetric methods with temperature variations 300; 350; 400; 450; 500C and pH variations 6.0; 6.5; 7.0; 7.5 and 8.0

Keywords: Elaeis Guineensis Jacq, Enzyme Activity, Germinating Seeds, Lipase, RBDPO

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1 Introduction

Thermostable lipase or acylglycerol hydrolase (EC 3.1.1.3) is an enzyme that can hydrolyze long-chain triglycerides. This enzyme is widely used in the production of fatty acids. (Macrae. A.R, 1983). Fatty acids and glycerol are basic oleochemical products indispensable for the paint, plastic, and detergent industries. Today the process operates at a temperature of 240-2500C and a pressure of 45-50 atm. This process requires a large amount of energy to maintain its operating conditions, and the fatty acids produced are generally brown, which will cause damage to the components contained in the oil, such as -carotene. (Herawan, T., 1996).

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In the hydrolysis process using lipase enzymes, the reaction generally operates at a relatively low temperature between 30-600C and atmospheric pressure, so it is safe for the work environment and does not require large enough energy. In addition, the resulting product has relatively better quality than similar products made by chemical or physical processes because there is relatively no damage caused by heating at high temperatures. As a biocatalyst, enzymes have properties such as being active in minimal amounts and having specific catalytic activity. (Sri, WM, 2011)

Lipase enzymes can be obtained from local microbes such as Pseudomonas, Aspergillus niger, Mucor miehei, Candida rugosa (Moentamaria D., 2009), and can also be isolated from seed sprouts, such as sesame seed sprouts (*Sesamun Indicum*). Oil palm originates from West Africa, and it turns out to be suitable for development outside its native area, including Indonesia, where oil palm can grow and develop well and is most commonly found in North Sumatra and Aceh (Swadaya, P., 2001). According to Indonesian Plantation Statistics 2009-2011 data, oil palm production in North Sumatra in 2010 was 3,230,488 tons (www.regionalinvestment.bkpm.go.id)

Ejedegba et al. (2007) characterized lipase isolated from coconut (Cocos nucifera linn). Then, Arbianti et al. (2008) used sesame seeds as a source of lipase enzymes for the glycerol-lauric acid esterification reaction. Chusnul Hidayat et al. (2008) conducted a study on optimizing the lipase production of peanut seeds (*Arachis hypogaea, L*) as a biocatalyst with the Response Surface Methodology method. Enujiugha et al. (2009) reported that lipase in African peanut oil seeds (*Pentaclethra macrophylla Benth*) had more specific activity against lauric oil (short-chain fatty acid content).

Based on the description above, the researchers wanted to isolate the crude extract of the lipase enzyme from oil palm seed sprouts (*Elaeis guineensis Jacq*) and wanted to know the optimum pH and temperature for hydrolyzing RBDPO (Refined Bleached Deodorized Palm Oil).

2 Materials and Methods

2.1 Equipments

Ball-dropper, Blender, Burette, Hitachi Centrifuge, Beaker, Erlenmeyer glass, Cotton, Measuring flask, Analytical Balance, pH meter, Dropper, Volumetric Pipette, Stative and Clamp, Thermostat, Thermometer.

2.2 Materials

Oil Palm Seeds, Aquades, Ethanol, $NaH_2PO_4.H_2O$, Na_2HPO_4, Phenolphthalein Indicator, KOH(s), Oxalic Acid(s), $(NH_4)_2SO_4$(s), RBDPO (Refined Bleached Deodorized Palm Oil), Germinator, 0.4% Dithane Fungicide solution,

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2.3 Making Sprouts from Oil Palm Seeds

Oil palm seed sprouts were produced from the modified PPKS (Palm Oil Research Center) Medan, North Sumatra. The fruit bunches of the DxP variety were separated from the fruit and then peeled. Then the oil palm seeds were soaked in water for seven days, rinsed with 0.4% Dithane Fungicide solution, and dried for one day. Then put into the germinator at 40°C for 60 days, then soaked in water for three days, and rinsed with a 0.4% Fungicide solution, after that it was put in a room at 30°C for 21 days. Then put the oil palm seed sprouts into plastic and soaked in water at a temperature of 25ºC for 14 days

2.4 Preparation of Lipase Enzyme Crude Extract from Oil Palm Seed Sprouts

A total of 90 oil palm seed sprouts (416.5501 g) and separated from the shells from the inner seeds, the inner seeds (121.179 g) were added with phosphate buffer pH 7.0 and blended until smooth, then filtered. The filtrate was centrifuged at 5000 rpm for 30 min. The supernatant was added with 60% NH4(SO4)2 and then allowed to stand for one night at 40C. The suspension formed was centrifuged at 10000 rpm for 30 minutes, and the resulting precipitate was dissolved with phosphate buffer pH 7.0.

2.5 Preparation of Lipase Enzyme Crude Extract from Oil Palm Seed Sprouts

A total of 90 oil palm seed sprouts (416.5501 g) and separated from the shells from the inner seeds, the inner seeds (121.179 g) were added with phosphate buffer pH 7.0 and blended until smooth, then filtered. The filtrate was centrifuged at 5000 rpm for 30 min. The supernatant was added with 60% $NH_4(SO_4)_2$ and then allowed to stand for one night at 4°C. The suspension formed was centrifuged at 10000 rpm for 30 minutes, and the resulting precipitate was dissolved with phosphate buffer pH 7.0.

2.6 Determination of Optimum Temperature for Lipase Enzyme Crude Extract Activity on RBDPO Hydrolysis

Weighed 1 gram of RBDPO and put each into 5 Erlenmeyer glasses, added 2 mL of phosphate buffer pH 7.0; 1 mL of crude extract of lipase enzyme was added and heated in an Erlenmeyer glass at 30°C for 60 minutes. After that, 6 mL of ethanol: acetone (1:1) was added, and three drops of Phenolphthalein indicator were added, then titrated with 0.0906 N KOH until a color change occurred. Turns purple, the volume of KOH 0.0906 N used is recorded, and the % ALB and its activity are calculated. The same treatment was repeated with a temperature variation of 35°C; 40°C; 45°C; 50°C.

2.7 Determination of Optimum pH for Lipase Enzyme Crude Extract Activity on RBDPO Hydrolysis

Weighed 1 gram of RBDPO and put each into 5 Erlenmeyer glasses, added 2 mL of phosphate buffer pH 6.0 and added 1 mL of crude extract of lipase enzyme and heated at 40°C for 60

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minutes, after that 6 mL of ethanol: acetone was added. (1:1) and added 3 drops of Phenolphthalein indicator, after that it was titrated with KOH 0.0906 N until the color changed to purple, the volume of KOH 0.0906 N used was recorded, and the % ALB and its activity were calculated. The same treatment was repeated with variations of phosphate buffer pH 6.5; 7.0; 7.5;8.0.

3 RESULT AND DISCUSSION

3.1 Determination of Optimum Temperature and pH for Lipase Enzyme Crude Extract Activity from Oil Palm Sprouts Against RBDPO Hydrolysis

The levels of ALB (free fatty acids) can be determined based on the volume (mL) of KOH 0.0906 N which is used to liberate 1 mg of free fatty acids from RBDPO which is hydrolyzed by the crude extract of the lipase enzyme.

Table 1. The results of the calculation of the crude extract activity of the lipase enzyme at a temperature of 30-50°C

RBDPO Weight (g)	Volume of crude lipase enzyme (mL)	Temperature (oC)	KOH volume 0.0906 N (mL)		ALB level (%)	Activity (U/mL)
			Substrate	Blank		
1.0	1	30	0.6	5.9	13.7067	8.003
1.0	1	35	0.6	6.2	14.4036	8,456
1.0	1	40	0.7	7.1	16.4945	9,664
1.0	1	45	0.7	5.8	13.4743	7,701
1.0	1	50	0.6	5.4	12.5451	7,248

Table 2. Calculation of the activity of crude extract of lipase enzyme at pH 6.0 -8.0

RBDPO Weight (g)	Volume of crude lipase enzyme (mL)	pH	KOH volume 0.0906 N (mL)		ALB level (%)	Activity (U/mL)
			Substrate	Blank		
1.0	1	6.0	0.6	6.1	14.1713	8.3050
1.0	1	6.5	0.7	6.7	15.5652	9.0600
1.0	1	7.0	0.7	7.2	16,7268	9.815
1.0	1	7.5	0.7	5.7	13,2420	7,852
1.0	1	8.0	0.6	5.6	13.0097	7.5500

3.2 Isolation of Lipase Enzyme Crude Extract from Oil Palm Sprouts

Crude extract of lipase enzyme was obtained from oil palm (*Elaeis guineensis Jacq*) seed sprouts which were 14 days old. Then the isolation was carried out by the extraction method, namely the protein deposition method through the addition of salt $(NH_4)_2SO_4$ to a concentration of 60%. Due to the difference in density between the mineral salt $(NH_4)_2SO_4$ and the enzyme

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solution, the separation of the enzyme from $(NH_4)_2SO_4$ was carried out by centrifugation. Thus, enzymes (proteins) contained in a separate solution with non-enzyme particles and enzymes which are the heavy fraction will be deposited under the solution.

3.3 Effect of Temperature on the Activity of Lipase Enzyme Crude Extract from Oil Palm Seed Sprouts on RBDPO Hydrolysis

In Figure 1, it can be seen that the activity of the crude extract of the lipase enzyme increases with increasing temperature. The optimum temperature was reached at 40°C with an activity of 9,664 U/mL. The activity of the crude extract of the lipase enzyme began to decrease at a temperature of 45oC at 7,701 U/mL and further decreased at a temperature of 50°C with an activity of 7,248 U/mL.

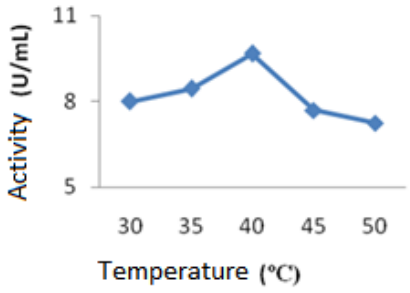

Figure 1. The curve of the effect of temperature on the activity of the crude extract of the lipase enzyme on the hydrolysis of RBDPO (Activity Vs Temperature)

This is because the lipase enzyme breaks down the protein structure at higher temperatures. Enzymes are proteins, so high temperatures can cause protein denaturation, namely damage to the protein structure that disrupts enzyme function as a catalyst, where the work of an enzyme on the substrate is analogous to a lock and key, so that the lock and key do not match, the enzyme activity on the substrate does not can occur. The analysis of copper metal ion (Cu^{2+}) adsorption by Atomic Absorption Spectrophotometer (AAS)

3.4 Effect of pH on the Crude Extract Activity of Lipase Enzyme from Oil Palm Seed Sprouts on RBDPO Hydrolysis

In Figure 2 it can be seen that the activity of the crude extract of the lipase enzyme increased with increasing pH. The figure shows that the optimum pH for crude lipase enzyme activity in hydrolyzing RBDPO is pH 7.0 with an activity of 9.815 U/mL. The activity of the crude extract of the lipase enzyme began to decrease at pH 7.5 with an activity of 7.852 U/mL and further decreased at pH 8.0 with an activity of 7.550 U/mL.

High or low pH allows protein denaturation and this will result in decreased enzyme activity. Because enzymes are proteins, changes in pH will cause the ionization of protein molecules to

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change. This change will cause the protein's three-dimensional structure to change so that the enzyme's catalytic activity is disturbed.

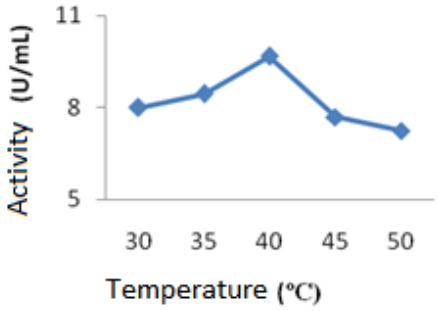

Figure 2. The curve of the effect of pH on the activity of the crude extract of the lipase enzyme on the hydrolysis of RBDPO (Activity Vs pH)

4 Conclusion

Based on the results of research that has been carried out regarding the isolation of crude extract of lipase enzyme from oil palm seed sprouts and activity test of crude extract of lipase enzyme, it can be concluded as follows: (1) Isolation of crude extract of lipase enzyme was carried out using the extraction method with the addition of salt (NH4)2SO4 to separating protein (enzyme) with non-enzyme particles and centrifugation method to separate enzyme from salt (NH4)2SO4. (2) The optimum temperature and pH of crude extract lipase enzyme activity from oil palm seed sprouts (Elaeis guineensis Jacq) on RBDPO hydrolysis were 40oC and 7.0 with the highest activity values of 9,664 U/mL and 9,815 U/mL, respectively.

References

Abigor, RD 2002. Partial Purification and Properties of Lipase from Germinating seeds of Jatropha curcas L.J. Am Oil. Soc, 79:p. 1123-1126.

Arbianti, R. 2008. Utilization of Sesame Seeds as a Source of Lipase Enzymes for Glycerol-Lauric Acid Esterification Reactions in Making Emulsifying Agents. Jakarta:: University of Indonesia.

Chusnul, H. 2008. Optimization of Lipase Production of Peanut (Arachis Hypogaea L) Sprouts as a Biocatalyst with Response Surface Methodology. Yogyakarta: Gadjahmada University.

Ejedegba, BO 2007. Characteristics of Lipase Isolated from Coconut (Cocos nucifera Linn) Seed Under Different Treatments. Nigeria: African Journal of Technology pp. 723-727.

Enujiugha, VN 2004. Lipase Activity in Dormant Seeds of The African Oil Bean (Pentaclethra macrophylla Benth). Elsevier. Food Chemistry. 88:405-410

Herawan, T. 1996. Hydrolysis of Palm Oil Using Lypozyme from Mucor meihei. Journal of Palm Oil Research, 4(2), 91-95

Macrae, AR 1983. Extracellular Microbial Lipase In Microbial Enzyme and biotechnology.England: Ed. Fogarty WM Applied Science Publisher Ltd.

Moentamaria, D. 2009. Preliminary Study of Making Immobilized Lipase Biocatalyst from Mucor Miehei for Processing Randu Oil into Biodiesel. Malang:: Department of Chemical Engineering, State Polytechnic of Malang.

Permana, M. 2012. Optimization of Indigenous Lipase Isolation of Cocoa Beans (Theobroma cacao L). Bali : Udayana University.

Siregar, NA 2011. Determination of Optimum pH and Temperature for Crude Extract Activity of Lipase Enzyme from Sprouts of Jatropha (Ricinus communis L) Seeds on Hydrolysis of Sesame Oil. Medan: University of North Sumatra.

Sri, WM 2011. Production, Characterization, and Isolation of Lipase from Aspergillus niger. Yogyakarta: National Seminar on Chemical Engineering.

Swadaya, P. 2001. Palm Oil, Cultivation, Product Utilization, and Marketing Aspects. Jakarta: Self-help Disseminator.

www.regionalinvestment.bkpm.go.id.