Purification of Lipase Enzyme from Oil Palm Mesocarp by Ammonium Sulfate Fractionation: Activity and Storage Stability

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ABSTRACT

This study examines the isolation and salting-out fractionation of lipase enzyme from oil palm mesocarp as a preliminary step toward preparing a biocatalyst for enzymatic biodiesel production, aimed at overcoming the limitations of conventional acid and base catalysts such as soap formation, corrosiveness, and difficulty of separation and reuse. Lipase was isolated from oil palm mesocarp through extraction using phosphate buffer, then fractionated by salting-out with ammonium sulfate at four saturation ranges (0-20%, 20-40%, 40-60%, and 60-75%). Volumetric enzyme activity (U/mL) was determined by acid-base titration, while storage stability was evaluated by comparing the activity of fresh enzyme with that of enzyme stored for 2 weeks at 18 ºC. The crude extract obtained from isolation had a moisture content of 69.19%, indicating that the enzyme was in a fresh condition prior to fractionation. Enzyme activity increased consistently from the crude extract (4.16 U/mL) to Fraction IV (53.59 U/mL), an increase of about 12.9-fold; because protein concentration was not determined, this increase reflects volumetric activity rather than specific activity or purification fold, and is consistent with, but does not by itself confirm, separation of lipase from co-precipitating, less active proteins. After 2 weeks of storage, all fractions showed a decline in activity, but to different degrees: the crude extract lost 53.85% of its activity, whereas Fraction IV lost only 4.37%. These findings, based on single measurements, suggest that ammonium sulfate fractionation not only increases the volumetric activity of lipase but may also improve its short-term storage stability. Fraction IV is accordingly proposed as a promising biocatalyst candidate for further application in enzymatic biodiesel production from high-FFA feedstocks; confirmation through replicated measurements, protein/purity characterization, and direct catalytic testing is recommended before firmer conclusions are drawn, and further work on immobilization and stabilizing additives is suggested to extend its storage life.

Keywords: *ammonium sulphate fractionation; enzyme purification; lipase enzyme; oil palm mesocarp; storage stability*

I. INTRODUCTION

Rising national energy demand, coupled with dwindling fossil fuel reserves, is driving the development of renewable, environmentally friendly, and sustainable alternative energy sources. Biodiesel produced through esterification and/or transesterification of vegetable oil or animal fat with methanol is one of the biofuels that has been widely developed in Indonesia (Mahdi, *et al.*, 2023). Conventionally, biodiesel production uses either base or acid chemical catalysts; however, these catalysts have significant drawbacks, including soap formation when the feedstock has a high free fatty acid (FFA) content, corrosiveness, and difficulty in separation and reuse (Harmiansyah, *et al.*, 2023). This has driven the development of lipase enzyme as an environmentally friendlier alternative biocatalyst (Fan, *et al.*, 2012).

Lipase enzyme has advantages over conventional chemical catalysts, as it can directly catalyze the esterification of FFA without forming soap, operates under milder conditions, is biodegradable, and can potentially be reused (Zambare, *et al.*, 2021; Ramos, *et al.*, 2022). Lipase can be obtained from various sources, including plants, and has been widely developed as a biocatalyst in the biodiesel, food, detergent, and pharmaceutical industries (Chandra, *et al.*, 2020). Oil palm mesocarp is one potential plant-based source of lipase, given its abundant availability as a by-product of the palm oil industry in Indonesia.

However, the crude enzyme extract obtained from isolation generally still contains many contaminating proteins and exhibits low catalytic activity, so a fractionation/purification step is required to increase its catalytic activity and reduce the proportion of non-catalytic contaminating proteins. One commonly used purification method is salting-out fractionation using ammonium sulfate (Su'i & Suprihana, 2013). In addition to activity, enzyme stability during storage is also an important consideration for industrial application, since enzymes are protein biomolecules prone to a decline in activity (deactivation) during storage; a previous study reported that lipase extract remained relatively stable for only about 15 days of storage (Joshi, *et al.*, 2019). Based on the above, this study aims to (1) isolate crude lipase extract from oil palm mesocarp, (2) purify the enzyme through ammonium sulfate fractionation at various saturation ranges, and (3) evaluate the storage stability of the crude extract and each fraction over a 2-week period, in order to determine the optimum biocatalyst candidate for use in the subsequent enzymatic biodiesel production stage.

II. MATERIALS AND METHOD

2.1 Materials

The main raw material used was oil palm mesocarp as the source of lipase enzyme. The chemicals used included distilled water, 0.05 N and 0.1 N NaOH, 0.1 N phosphate buffer at pH 7.0, p.a. ammonium sulfate, acetone, methanol, and phenolphthalein (PP) indicator.

2.2 Isolation of Crude Lipase Extract

Isolation of the crude lipase extract was performed using the method presented in Figure 1. A total of 100 g of oil palm mesocarp was weighed and mixed with 100 mL of distilled water (1:1 ratio), then stirred until homogeneous. The pH of the mixture was adjusted to neutral by gradually adding 0.05 N NaOH. Subsequently, 200 mL of 0.1 N phosphate buffer at pH 7.0 was added and stirred using a digital stirrer for 30 minutes (Kimtun et al., 2015). The mixture was pressed through cloth to separate the filtrate from the residue, after which the filtrate was left to stand at 18 ^{o}C for 30 minutes and filtered through filter paper. The filtrate was centrifuged at 2,000 rpm for 5 minutes, and the resulting supernatant was the crude lipase extract, which was stored at 18 ^{o}C (Ledo, *et al.*, 2020).

2.3 Purification of Lipase Enzyme through Ammonium Sulfate Fractionation

The crude lipase extract was purified by sequential salting-out fractionation with ammonium sulfate at four increasing saturation ranges: 0-20%, 20-40%, 40-60%, and 60-75%. Solid ammonium sulfate was added stepwise to the supernatant carried over from the preceding saturation step, calculated as 11.3 g for 0-20%, 12.1 g for 20-40%, 13.0 g for 40-60%, and 10.3 g for 60-75% per 100 mL of solution. Ammonium sulfate was added stepwise with continuous stirring at a cold temperature of ±4 ^{o}C for ±30 minutes until fully dissolved, then left to stand/equilibrate for ±30 minutes before centrifugation) The precipitate at each saturation step was then separated by centrifugation at 2,000

rpm for 5 minutes (Su'i & Suprihana, 2013), collected, and redissolved in (0.1 N phosphate buffer at pH 7.0, to a final volume of ±10 mL per fraction, adjusted to the precipitate volume) prior to activity testing, while the clarified supernatant of each step was carried forward to the next, higher saturation step. All redissolved fractions were retained for activity testing and storage-stability evaluation. Because the redissolution volume can influence the apparent activity per mL, this volume is reported explicitly above so that activity values can be interpreted on a comparable basis.

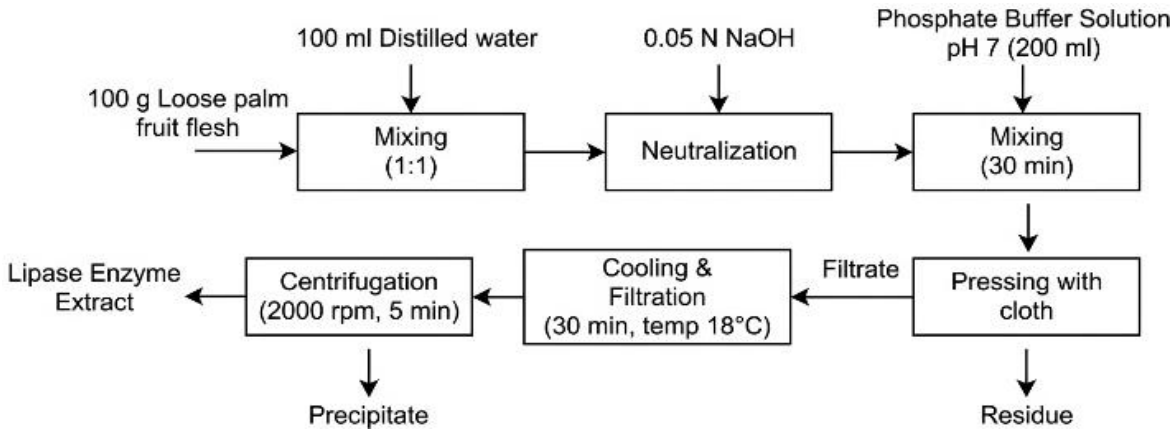

Figure 1. Isolation Scheme for Crude Lipase Extract from Oil Palm Mesocarp

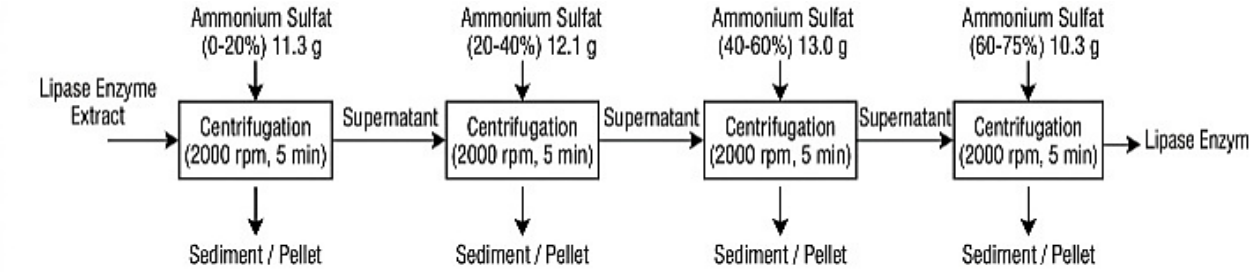

Figure 2. Purification Scheme for Lipase Enzyme via Ammonium Sulfate Fractionation

2.4 Determination of Lipase Enzyme Activity

Lipase activity was determined by titration of the free fatty acids released from crude palm oil with high FFA content, consistent with the enzyme source and the high-FFA biodiesel (Figure 3). A total of 2 g of the fatty substrate was combined with 1 mL of lipase enzyme solution and 4 mL of 0.1 N phosphate buffer at pH 7.0, then stirred for 1 hour at 35 °C. Afterward, 10 mL of acetone and 10 mL of methanol were added to stop the reaction and dissolve the fatty acids, stirred until homogeneous, followed by 4 drops of PP indicator, and titrated with 0.1 N NaOH. A blank solution was prepared in the same way but without enzyme and without incubation, so that the volume of NaOH used for the blank reflects only the background free fatty acid content of the substrate. One unit (U) of lipase activity is defined as the amount of enzyme that releases 1 mmol of free fatty acid per minute under the assay conditions described above, and activity is expressed as U per mL of enzyme solution (U/mL). Enzyme activity is determined based on Equation 1 (Istiningrum, *et al.*, 2018):

$$EA = \frac{(A - B)xN}{Vxt_i} x1000 \quad (1)$$

where:
EA = enzyme activity (U/mL)
A = volume of NaOH for sample titration (mL)
B = volume of NaOH for blank titration (mL)
N = concentration of NaOH
V = volume of enzyme (mL),
t_i = incubation time (60 minutes).

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2.5 Enzyme Storage Stability Test

The crude extract and all fractions obtained from fractionation were stored at 18 °C for 2 weeks, (represent a standard laboratory refrigerated storage condition, approximating commonly available cold-storage conditions) after which their activity was measured again using the same titration method described in Section 2.4. The percentage decrease in activity was calculated by comparing the activity after storage with the initial (fresh) activity for the same stage/fraction. Because only two time points (fresh and 2 weeks) were measured, at a single storage temperature and without replication, these results describe only the overall extent of activity loss over this specific interval and condition; they do not establish the deactivation kinetics and should not be extrapolated to other storage durations or temperatures without further testing.

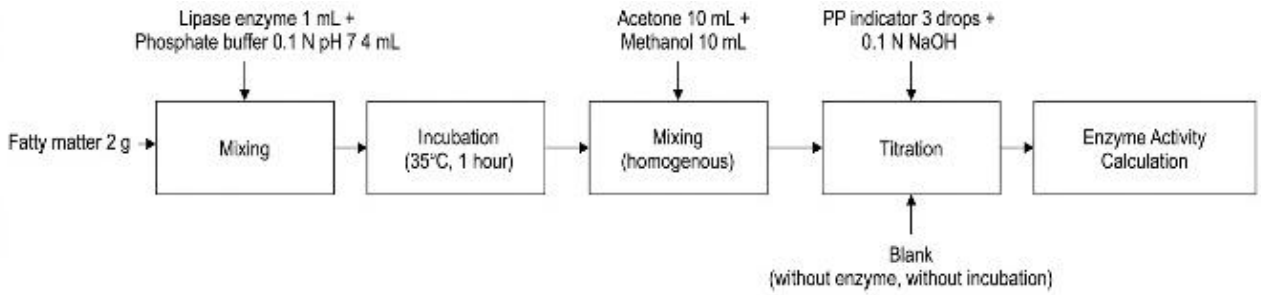

Figure 3. Scheme for Determination of Lipase Enzyme Activity

III. RESULTS AND DISCUSSION

3.1 Characteristics of the Crude Enzyme Extract

The lipase enzyme catalyst isolated from oil palm mesocarp had a moisture content of 69.19%, indicating that the enzyme was in a wet/fresh condition immediately after isolation. Moisture content plays an important role in maintaining lipase activity and stability: a minimal water layer around the enzyme molecule is required to preserve the catalytically active three-dimensional conformation, yet excessively high moisture content risks reducing storage stability and accelerating denaturation.

3.2 Increase in Enzyme Activity through Ammonium Sulfate Fractionation

The results of lipase activity measurements under fresh conditions and after 2 weeks of storage are presented in Table 1 and Figure 4. The data in Table 1 show that lipase volumetric activity increased consistently from the initial isolation stage (4.16 U/mL) to Fraction IV (53.59 U/mL), an increase of about 12.9-fold. This increase is consistent with progressive concentration and partial separation of lipase from co-precipitating, less-active proteins across the successive saturation cuts, in line with the classical salting-out mechanism in which increasing ammonium sulfate concentration reduces the hydration shell around protein surfaces, promotes hydrophobic protein-protein interactions, and drives precipitation (Su'i & Suprihana, 2013).

However, because protein content was not measured and each fraction was redissolved and assayed without normalizing for redissolution volume, part of the apparent increase in activity across fractions may also reflect differences in concentration factor rather than a true increase in specific lipase activity. Determination of protein content together with calculation of specific activity, purification fold, and recovery/yield is therefore needed in future work to confirm genuine purification. In addition, because each value in Table 1 represents a single determination rather than the mean of replicate assays, these activities should be interpreted as preliminary trends rather than statistically validated differences.

3.3 Storage Stability of the Crude Extract and Purified Fractions

After 2 weeks of storage, all enzyme fractions experienced a decline in activity, but to very different degrees. The crude extract from the isolation stage showed the largest decrease in activity (53.85%), whereas Fraction IV showed the smallest decrease (only 4.37%). The decline in enzyme activity during storage may plausibly involve mechanisms commonly reported for lipases

and other enzymes, such as partial denaturation of the protein structure, proteolytic degradation by contaminating proteases, or oxidation of amino acid residues at the active site; however, none of these mechanisms was directly examined in this study, and they should be regarded as hypotheses for future verification (e.g., via protease activity assays or protein integrity analysis) rather than established explanations. The comparatively smaller activity loss observed in the more extensively fractionated samples is consistent with, but does not directly prove, a lower content of contaminating and proteolytic proteins, since protein purity was not independently assessed in this study.

Table 1. Lipase Enzyme Activity at the Isolation and Fractionation Stages, Fresh vs. After 2 Weeks of Storage

Enzyme Stage/Fraction	Fresh Activity (U/mL)	Activity after 2 Weeks (U/mL)	Decrease (%)
Isolation	4.16	1.92	53.85
Fraction I (0-20%)	17.87	14.38	19.53
Fraction II (20-40%)	36.44	23.71	34.93
Fraction III (40-60%)	44.98	40.18	10.67
Fraction IV (60-75%)	53.59	51.25	4.37

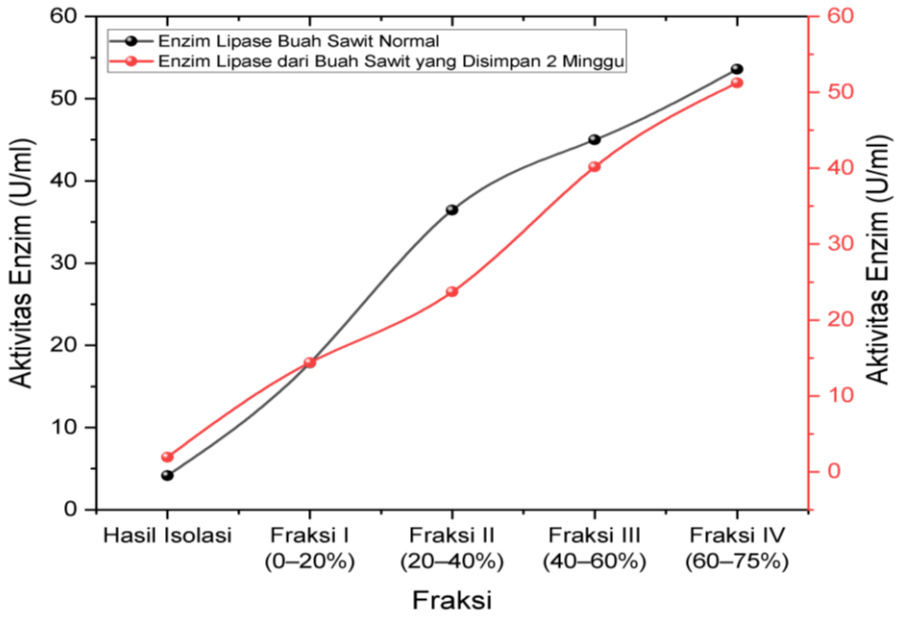

Figure 4. Activity and Stability of Lipase Enzyme at the Isolation and Purification Stages, and After 2 Weeks of Storage.

Based on these results, Fraction IV (60-75%) showed both the highest volumetric activity and the smallest relative loss of activity after 2 weeks of storage among the fractions tested, making it a promising, though still preliminary, candidate for further evaluation as a catalyst in the subsequent biodiesel production stage; confirmation through replicated assays, protein-normalized activity determination, and direct esterification/biodiesel testing is required before it can be established as the optimum biocatalyst.

IV. CONCLUSION

Isolation and salting-out fractionation of lipase enzyme from oil palm mesocarp with ammonium sulfate consistently increased the enzyme's volumetric activity from 4.16 U/mL (crude extract) to 53.59 U/mL at Fraction IV (60-75%), an increase of about 12.9-fold; because protein content was not determined, this increase should be understood as volumetric activity rather than specific activity or

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purification fold. Fraction IV also showed the highest activity retention after 2 weeks of storage, with the smallest decrease in activity (4.37%) compared with the crude extract, which lost 53.85% of its activity, suggesting that fractionation may improve both volumetric activity and short-term storage stability. Based on initial findings from this single measurement, Fraction IV (60–75%) is proposed as a potential biocatalyst candidate for the subsequent biodiesel production stage using fatty feedstocks, subject to confirmation through replicate measurements, protein characterization/purity analysis, and direct catalytic performance testing.

REFERENCES

Chandra, P., Enespa, Singh, R., & Arora, P. K. (2020). Microbial lipases and their industrial applications: A comprehensive review. *Microbial Cell Factories*, *19*, 169.

Fan, X., Niehus, X., & Sandoval, G. (2012). Lipases as biocatalyst for biodiesel production. In G. Sandoval (Ed.), *Lipases and Phospholipases: Methods and Protocols* (Methods in Molecular Biology, Vol. 861, pp. 471-483). Humana Press.

Harmiansyah, A., et al. (2023). Comparison of base and acid catalysts in biodiesel production from waste cooking oil. *Jurnal Rekayasa Kimia dan Lingkungan*, *15*(3), 234-248.

Istiningrum, R. B., Nurrokhmah, H., & Wahyuni, A. S. (2018). Compositional analysis of biodiesel converted from carica seed oil (Carica pubescens) using rice-bran lipase enzyme. *IJCA (Indonesian Journal of Chemical Analysis)*, *1*(1), 1-8.

Joshi, R., Sharma, R., Bhunia, R., Prakash, A., & Kuila, A. (2019). Lipase production from mutagenic strain of *Fusarium incarnatum* KU377454 and its immobilization using Au@Ag core shells nanoparticles for application in waste cooking oil degradation. *3 Biotech*, *9*, 411.

Kimtun, P., Choonut, O., Yunu, T., Paichid, N., Klomkloa, S., & Sangkharak, K. (2015). Biodiesel production using lipase from oil palm fruit as a catalyst. *Energy Procedia*, 79, 123-130.

Ledo, M. E. S., Nitsae, M., & Solle, H. R. L. (2020). Synthesis of biodiesel from kesambi oil using indigenous lipase. *SCISCITATIO*, *1*(1), 51-56.

Mahdi, H. I., Ramlee, N. N., Duarte, J. L. S., Cheng, Y. S., Selvasembian, R., Amir, F., de Oliveira, L. H., Wan Azelee, N. I., Meili, L., & Rangasamy, G. (2023). A comprehensive review on nanocatalysts and nanobiocatalysts for biodiesel production in Indonesia, Malaysia, Brazil and USA. *Chemosphere*, *319*, 138003.

Ramos, M. D. N., Milessi, T. S., Candido, R. G., Mendes, A. A., & Aguiar, A. (2022). Enzymatic catalysis as a tool in biofuels production in Brazil: Current status and perspectives. *Energy for Sustainable Development*, 68, 103-119.

Su'i, M., & Suprihana. (2013). Lipase fractionation of coconut endosperm by Salting out method. *Agritech*, *33*(4), 377-384.

Zambare, V., Patankar, R., Bhusare, B., & Christopher, L. (2021). Recent advances in feedstock and lipase research and development towards commercialization of enzymatic biodiesel. *Processes*, *9*(10), 1743.