



EXTRACTION AND IMMOBILIZATION OF LIPASE FROM AVOCADO (*Persea americana* Mill.) AND COCONUT (*Cocos nucifera* L.) SEEDS USING NA-ALGINATE

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ABSTRAK

Enzim digunakan secara luas dalam industri karena fungsinya sebagai katalisator alami yang efisien, terutama lipase. Biji alpukat (*Persea americana* Mill.) dan kelapa (*Cocos nucifera* L.) merupakan sumber potensial penghasil lipase. Seiring dengan semakin luasnya penggunaan enzim, metode imobilisasi enzim sedang dikembangkan untuk meningkatkan stabilitas dan aktivitas enzim. Na-alginat adalah polimer yang terbentuk dari asam D-mannuronat dan L-guluronat yang dikenal dapat meningkatkan ketahanan enzim terhadap suhu dan pH, mengurangi hambatan, serta menjaga kestabilan enzim. Tujuan dari penelitian ini adalah ekstraksi lipase dari biji alpukat dan kelapa serta imobilisasi lipase dengan Na-Alginat. Metode yang dilakukan dalam penelitian ini yaitu ekstraksi lipase dari biji alpukat dan kelapa, uji aktivitas lipase, dan imobilisasi lipase dengan Na-alginat. Hasil penelitian menunjukkan bahwa lipase telah berhasil diekstrak dari biji alpukat dan kelapa. Uji aktivitas menunjukkan bahwa aktivitas ekstrak kasar lipase pada biji alpukat (0,870 mg/mL) lebih tinggi dibandingkan kelapa (0,626 mg/mL). Keberhasilan proses imobilisasi lipase dengan Na-alginat ditunjukkan dengan adanya butiran yang stabil yang ditetesi ke dalam larutan CuCl_2 dan FeCl_2 . Penelitian ini menemukan sumber lipase yang ramah lingkungan, sehingga perlu dilakukan pengembangan lebih lanjut untuk aplikasi industri.

ABSTRACT

Enzymes are widely used in industry due to their function as efficient natural catalysts, especially lipase. Avocado (*Persea americana* Mill.) and coconut (*Cocos nucifera* L.) seeds are potential sources of lipase. Along with the increasing use of enzymes, enzyme immobilization methods are being developed to improve enzyme stability and activity. Na-alginate is a polymer formed from D-mannuronic acid and L-guluronic acid, which is known to increase enzyme resistance to temperature and pH, reduce inhibition, and maintain enzyme stability. The purpose of this study was the extraction of lipase from avocado and coconut seeds and lipase immobilization with Na-Alginate. The methods used in this study were lipase extraction from avocado and coconut seeds, lipase activity test, and lipase immobilization with Na-alginate. The results showed that lipase had been successfully extracted from avocado and coconut seeds. The activity test showed that the activity of the crude lipase extract in avocado seeds (0.870 mg/mL) was higher than that in coconut (0.626 mg/mL). The success of the lipase immobilization process with Na-alginate was demonstrated by the presence of stable granules dropped into CuCl_2 and FeCl_2 solutions. This research identified an environmentally friendly source of lipase, necessitating further development for industrial applications.

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INTRODUCTION

Enzymes are widely used in biotechnology processes due to their safe and sustainable properties and specific functions. Among the various enzymes, lipase holds significant potential due to its crucial role in various industries (Angelotti et al., 2022). Lipase (EC 3.1.1.3), also known as triacylglycerolase, catalyzes the hydrolysis of triglycerides into glycerol and free fatty acids (Chandra et al., 2020). This reaction catalyzes the release of fatty acids, mono- and diglycerides, glycerol, and the synthesis of various esters.

Lipases are found in almost all types of organisms, including animals, plants, and microbes (Vidal et al., 2023). Avocado (*Persea americana* Mill.) and coconut (*Cocos nucifera* L.) seeds are known as natural sources of lipase enzymes and can be utilized for the production of Virgin Coconut Oil (VCO) (Sedijani & Rasmi, 2023). Virgin Coconut Oil (VCO) can be converted into diacylglycerol (DAG) through hydrolysis or transesterification processes involving enzymatic methods (Fatimah, 2021). Lipases are typically used to adapt natural lipids to meet specific properties for various applications, such as food, nutrition, industry, and cosmetics.

Applications in various industrial fields require enzymes to be able to withstand various conditions to maintain their durability and stability. One approach is immobilization. The lipase immobilization process aims to increase enzyme stability, improve selectivity, and strengthen its resistance to changing environmental conditions, allowing for repeated use (Ismail & Baek, 2020). One factor influencing lipase activity is pH. This enzyme exhibits peak performance within a specific optimal pH range; excessively acidic conditions can cause protein denaturation, which reduces enzyme activity. Generally, the optimal pH for some types of lipase is between 5.1 and 5.6. The optimum pH value is crucial for identifying enzyme characteristics, as differences in substrates can lead to differences

in the enzyme's optimum pH value (Nugroho et al., 2022). Temperature also increases the rate of immobilization. Higher optimum temperatures and activity are associated with higher enzyme stiffness after immobilization, which can maintain a higher percentage of catalytic activity (Zhou et al., 2021). The percentage of lipase in monomeric and open forms determines the success of the immobilization method (Moentamaria et al., 2022).

Sodium alginate is known as a polymer that functions as a lipase immobilizer because it contains 30-60% alginic acid, which increases water solubility and has gel-like properties in aqueous solutions, thus maintaining enzyme stability. Previous research has investigated the enzymatic activity of lipase from avocado seeds by Kupnik et al. (2023), but has not yet reached the immobilization stage. Therefore, this study opens new perspectives on lipase extraction from avocado and coconut seeds and lipase immobilization using sodium alginate polymer. Therefore, this research can provide future prospects for improving the stability of plant-derived lipases for their use in various industrial fields. By exploring local potential, such as avocado and coconut seeds, as environmentally friendly sources of lipase enzymes, and applying immobilization technology using Na-alginate, this research directly contributes to solving the problem of sustainable biocatalyst availability for industry.

METHOD

Tools and Materials

The equipment used in this study included an analytical balance, blender, sieve, clonical tubes, centrifuge, micropipette, syringe, vial, UV spectrophotometer, magnetic stirrer, and various glassware used in both the preparation and analysis stages.

The main materials used in this study were avocado seeds and coconut flesh. The

avocado seeds used were dried and germinated, while the coconut flesh was fresh. The chemicals used included acetone (Merck), ethanol (Merck), phosphate buffer with pH 7.0 and 8.0, biuret reagent, sodium alginate (Na-alginate), bivalent salt solutions such as calcium chloride (CaCl_2), copper(II) chloride (CuCl_2), and iron(II) chloride (FeCl_2), virgin coconut oil (VCO), gum arabic, phenolphthalein (PP) indicator, sodium hydroxide (NaOH), and sodium chloride (NaCl).

Research Procedure

Lipase Extraction from Avocado and Coconut Seeds

A total of 25 grams of avocado and coconut seed samples were weighed and ground. 100 mL of 0.005 M phosphate buffer, pH 7, was added to the avocado and coconut seed samples, followed by filtration. The samples were then placed in a 15 mL conical flask. The avocado and coconut seed solution was centrifuged at 3000 rpm at 4°C for 30 minutes. The solution was then decanted, and the cream fraction of the avocado and coconut seed solution was collected after centrifugation. The supernatant was then decanted into a 50 mL conical flask. The avocado and coconut seed solution was separated from the residue and stored at -20°C in a freezer.

Protein Determination

Biuret Test Preparation

0.375 grams of CuSO_4 and 1.15 grams of $\text{NaKC}_4\text{H}_6\text{O}_6$ were each added to 50 mL of distilled water, and then poured into a beaker. Then, 7.5 grams of NaOH was mixed with 100 mL of distilled water. The mixed solution was transferred to a 250 mL volumetric flask and distilled water was added to the mark.

Preparation of a 10,000 ppm stock solution

0.1 grams of casein was transferred to a beaker, followed by 50 mL of 0.1 M phosphate buffer solution with a pH of 8. The solution was then stirred at 50°C for approximately 30 minutes.

Determining the maximum wavelength

1.5 mL of the stock solution was transferred to a 10 mL volumetric flask, followed by 6 mL of Biuret reagent. The solution was then diluted with distilled water to reach the final volume. The maximum wavelength was measured using a UV-Vis spectrophotometer in the range of 450-800 nm.

Calibration curve

The stock solutions were diluted to 5000, 4000, 3000, 2000, and 1000 ppm in 10 mL volumetric flasks. Then, 1.5 mL of casein standard, 6 mL of Biuret reagent, and distilled water were added to each. The maximum wavelength was then determined using a UV-Vis spectrophotometer.

Protein content measurement

The enzymes from avocado and coconut seeds were each diluted with 8 mL of 0.05 M buffer, pH 7. 1.5 mL of the extract was added to a 10 mL volumetric flask. Then, 6 mL of Biuret reagent was added and diluted to the mark. Analysis was performed using a UV-Vis spectrophotometer at the maximum wavelength.

Lipase activity test from avocado and coconut seeds

A blank solution was prepared by adding 4 mL of phosphate buffer solution at pH 7 to a conical flask, adding 500 mL of VCO and 0.20 g of gum arabic, and mixing thoroughly. A sample solution was prepared using the same steps as the blank solution, adding 1 mL of enzyme and incubating for 45 minutes at 35°C. 10 mL of acetone:ethanol (1:1) and 3 drops of PP indicator were added, followed by titration with NaOH.

Immobilization of lipase from avocado and coconut seeds

Avocado and coconut seed lipase extracts were prepared using the modified method (Bie et al., 2022), and 25 mL of each was added to a beaker. Next, 0.5 g of 2% Na-alginate was added little by little. Stirred with a magnetic stirrer until homogeneous. The

homogeneous solution was transferred to a syringe. A drop of bivalent chlorine solution (CaCl_2 , CuCl_2 , FeCl_3 , MgCl_2 , and ZnCl_2) was added to a 50 mL petri dish. The immobilized product was stored in a refrigerator for 24 hours. After storage, the enzyme beads were washed with distilled water until the pH was neutral, placed in a vial, weighed, and then stored back in the refrigerator.

RESULT AND DISCUSSION

Lipase Extraction from Avocado and Coconut Seeds

Extraction is a separation method that utilizes the differences in solubility in two immiscible liquids, such as water and an organic solvent, to obtain specific compounds from a mixture. In this process, 25 grams of avocado and coconut seeds are first ground,

then 0.05 M phosphate buffer with a pH of 7 is added to maintain stable reaction conditions. The addition of phosphate buffer prevents enzyme inactivation caused by pH fluctuations and maintains a neutral pH, allowing the extraction process to proceed optimally (Warninghiyun et al., 2023). The next step is filtration, which aims to separate the solid particles from the crude lipase extract. The filtered extract undergoes centrifugation at 3,000 rpm for 30 minutes. This centrifugation separates the supernatant, which is the crude lipase extract, from the cream fraction that forms during the centrifugation process (Nenobahan et al., 2020). The resulting extract was then stored in a refrigerator to maintain the enzyme's integrity and prevent damage. The extraction results are shown in Figure 1.

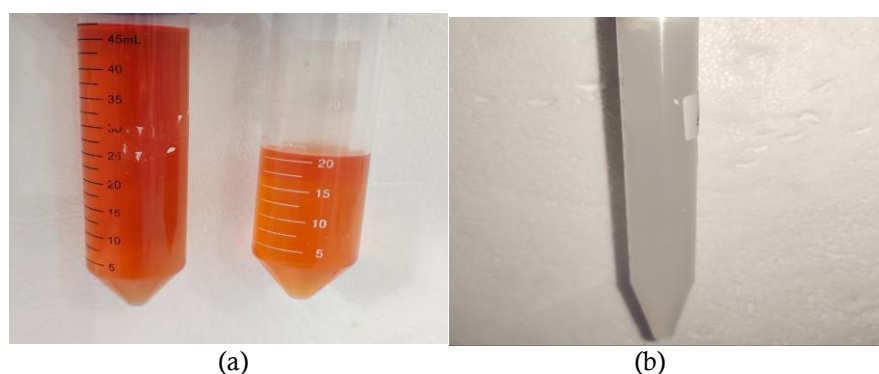


Figure 1. a) Crude lipase extract from avocado seeds b) Crude lipase extract from coconut

Determination of Protein Content in Avocado and Coconut Seeds

Research conducted by Nasution et al. (2022) determined protein content using UV-Vis spectrophotometry with Biuret reagent using color criteria analysis. In this method, purple color is used as an indicator of the presence of protein. This color is formed when a protein solution reacts with copper ions (Cu^{2+} from CuSO_4) under alkaline conditions provided by NaOH. This reaction produces a purple protein solution at each concentration tested (Nasution et al., 2022). Protein content determination using the Biuret method is based on measuring the absorption of purple light by protein complexes formed from the reaction between protein and Cu^{2+} ions in Biuret

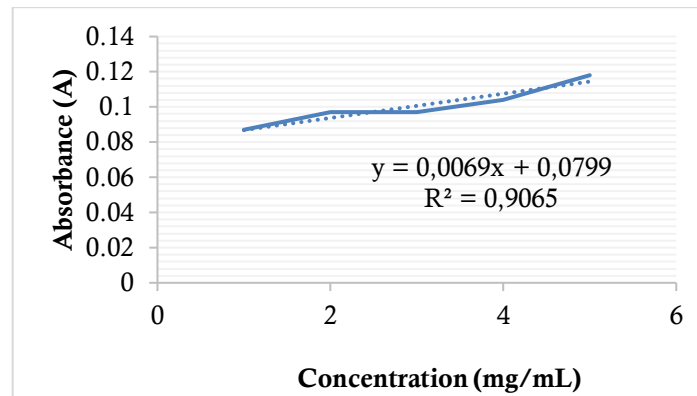
reagent under alkaline conditions. The greater the intensity of light absorbed by the spectrophotometer, the greater the number of Cu^{2+} complexes formed, indicating a higher protein content in the sample (Porodjia & Nurkamiden, 2021). The Biuret method is used to measure protein levels in samples by determining the maximum absorbance value using a UV-Vis spectrophotometer. An increase in absorbance indicates an increase in protein concentration in the sample. Before absorbance measurements are performed, the maximum wavelength is determined to maximize sensitivity while minimizing errors (Khatmizarullah et al., 2021). The wavelength used in this study was 590 nm.

Table 1. Comparison of Concentration with Absorbance

Standart	Absorbance (a)	Concentration (mg/mL)
1	0,077	1
2	0,087	2
3	0,97	3
4	0,104	4
5	0,118	5

Based on Table 1, a calibration curve graph of the relationship between absorbance

and concentration values is obtained, shown in Figure 2.


Figure 2. Calibration curve graph of the relationship between absorbance value and concentration

Based on Figure 2, the calibration curve yields a linear regression equation relating concentration to absorbance. The linear equation obtained is $y = 0.0069x + 0.0799$, where y is absorbance and x is concentration, with a correlation coefficient of 0.9065. This equation shows that concentration is directly proportional to absorbance, meaning that the higher the protein concentration, the greater the measured absorbance. The absorbance of the coconut sample was 0.158, and that of the avocado seed sample was 0.126. Protein content was also determined in the samples. Protein concentration was calculated using the calibration curve formula, yielding values of 9.1304 mg/mL in the coconut sample and 7.285 mg/mL in the avocado seed sample. Research conducted by Permana and Suhendra (2021) used the Response Surface Methodology (RSM) approach to identify the optimum temperature and pH for lipase enzyme activity produced by microorganisms in coconut pulp-based media. The results of the study showed that the optimum temperature was obtained at an enzyme content of 3.41 mg/mL. Meanwhile, according to research by

Kupnik et al. (2023), the total protein content in avocado seeds varies with the extraction method used. In the study, several extraction techniques were used, including Ultrasonic Extraction (UE) with water as a solvent which produced a protein content of 1.243 mg/mL of extract, Soxhlet Extraction (SE) using ethanol as a solvent producing 1.174 mg/mL of extract and Supercritical Fluid Extraction (SFE) with CO_2 as a solvent and ethanol as a co-solvent producing the highest protein content of 1.610 mg/mL of extract. Coconuts, especially mature coconuts, naturally contain active lipase in their flesh. Lipase levels in coconut flesh tend to be quite high, especially if the coconut is allowed to germinate. In general, and from a biological perspective, avocado seeds are not a primary fat reserve like coconut flesh, so their lipase levels tend to be lower. In comparison, avocado seeds exhibited higher specific lipase activity, indicating that the lipase enzyme in avocado seeds is more active per mg of protein.

Lipase Activity Test

Lipase activity testing on avocado and

coconut seeds was conducted to assess the enzyme's ability to catalyze the hydrolysis of triglycerides into fatty acids and glycerol. Lipase acts as a biocatalyst with a crucial function in lipid metabolism, particularly in the digestion of fats. In this study, using the method (Reynaldi et al., 2025), enzyme extracts were obtained from avocado and coconut seeds through an extraction method using a phosphate buffer at a specific pH to maintain enzyme stability. Lipase activity was measured based on the amount of free fatty

acids formed during the enzymatic reaction, expressed in units of enzyme activity (U/mL) or by measuring lipase in solution by weight (mg/mL). Research conducted by Reynaldi et al., (2025) stated that varying conditions such as temperature, pH, and substrate concentration in milk significantly influence enzyme activity. In avocado seeds, lipase activity tends to be higher than in coconut, which may be due to differences in nutrient content and environmental conditions of the enzyme's origin.

Table 2. Results of titration of crude lipase extraction from avocado and coconut seeds

No.	Treat	Volume (mL)
1.	Blanko	Coconut: 3,5 Avocado:3,5
2.	Sample 1	Coconut: 6,5 Avocado:7,4
3.	Sample 2	Coconut: 7,1 Avocado: 7,3
4.	Sample 3	Coconut: 5,4 Avocado: 7,6

Based on the titration results for crude lipase extracts from avocado and coconut seeds, the volume of extract obtained varied with the treatment applied. In the blank treatment, the volume obtained during titration was 3.5 mL. For sample 1, the titration volume of the crude extract from coconut was 6.5 mL, while that from avocado was 7.4 mL. This indicates that lipase from avocado seeds was extracted more effectively than from coconut in this treatment, possibly due to differences in seed structure or enzymatic protein content. In sample 2, the titration volume of the crude extract from coconut increased to 7.1 mL, while that from

avocado increased to 7.3 mL. This increase indicates that certain treatments can improve the efficiency of enzyme extraction from both seed types. Conversely, for sample 3, the titration volume of the crude extract from coconut decreased to 5.4 mL, whereas that from avocado remained relatively high at 7.6 mL. This decrease could be due to enzymatic degradation or changes in pH during the extraction process. These results indicate that avocado seeds consistently produced a higher volume of crude extract than coconut seeds, suggesting a more potent lipase content or activity in avocado seeds compared to coconut seeds.

Table 3. Results of calculation of lipase activity of immobilized avocado and coconut seeds

Sample	Titration Volume (mL)				Enzyme activity mg/mL
	Blanko	P1	P2	P3	
Avocado seed extract	3,5	7,4	7,3	7,6	0,870
Coconut extract	3,5	6,5	7,1	5,4	0,626

Based on Table 3, the lipase activity of avocado and coconut seeds measured by the titration method showed significant differences between the two samples. In avocado seed extract, lipase activity was recorded at 0.870 mg/mL. This value indicates that lipase from avocado seeds exhibits relatively good catalytic activity under specific test conditions. The titration volume obtained from avocado samples (P1, P2, and P3) showed better consistency than coconut, with values of 7.4 mL, 7.3 mL, and 7.6 mL, respectively. In contrast, the lipase activity of coconut extract was 0.626 mg/mL. This value may indicate the influence of inhibitory factors during testing, such as a mismatch in pH or reaction temperature relative to the optimal conditions for coconut lipase. The titration volume in coconut samples also varied more widely, namely 6.5 mL, 7.1 mL, and 5.4 mL for each treatment, indicating variability in enzyme activity. These results indicate that lipase from avocado seeds has higher stability and activity than that from coconut in this test.

Immobilization of Lipase from Avocado and Coconut Seeds

Enzyme immobilization is a method used to restrict an enzyme's mobility by binding it to a specific matrix or surface, so that the enzyme remains active but insoluble in the reaction medium. This technique offers several advantages, including increased enzyme stability, repeated use, and simplified product separation from the reaction mixture. Lipase immobilization is specifically carried out to increase the enzyme's stability, selectivity, and resistance to changing environmental conditions, allowing for repeated application (Ismail & Baek, 2020). Some advantages of this enzyme immobilization include greater resistance to environmental changes and easier

recovery/recycling compared to the free form. The main benefit of this technique is the enzyme's protection against extreme conditions such as high temperatures and unstable pH. Furthermore, immobilized enzymes are widely used in various large-scale industrial sectors, including the food, medical, detergent, textile, and pharmaceutical industries, as well as wastewater treatment plants (Maghraby et al., 2023). Previous research by Malik (2024) identified lipase enzymes in avocado seeds, but it had not yet reached the stage of implementing enzyme immobilization technology. Therefore, this study provides a new contribution by not only exploring the extraction of lipase from avocado and coconut seeds but also examining the application of immobilization techniques using sodium alginate polymers. The immobilization method used is cross-linking, which increases enzyme stability and forms strong chemical bonds, thereby reducing the risk of enzyme leakage. In this process, the crude lipase extract is mixed with a 2% Na-alginate solution and homogenized. The use of alginate was chosen because it is safe, biocompatible, readily available, and allows for simple and economical application of the technique. Alginate concentration affects the matrix's resistance to leakage, its ability to maintain cell viability (the ability of cells to survive), and its ability to maintain cell biomass (Summiati & Irdawati, 2024). A homogenized mixture of lipase and Na-alginate is dripped into a bivalent chlorine solution (CaCl_2 , CuCl_2 , and FeCl_2). This process aims to create chelation bonds through the chain mechanism in sodium alginate. Increasing the Na-alginate concentration increases cross-links, leading to tighter pores. Strengthening the alginate gel indicates the successful formation of cross-links between alginate molecules and ions in the solution.

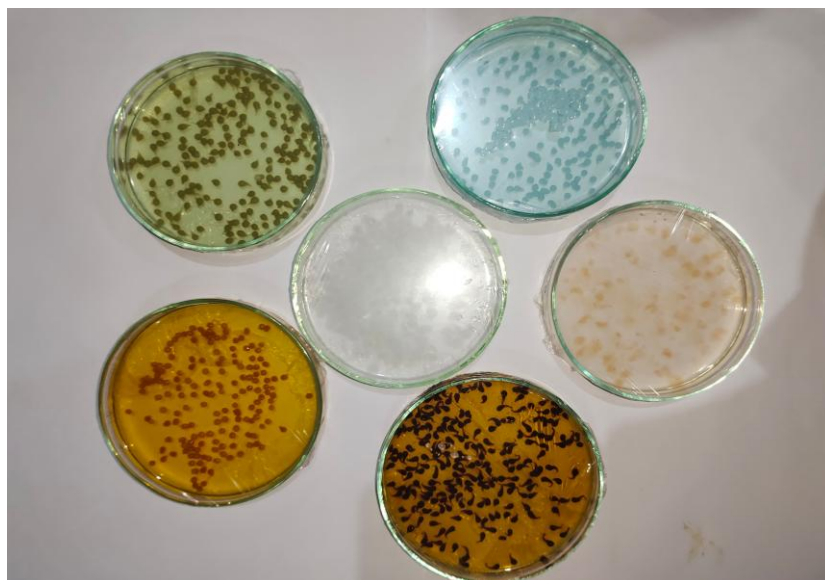
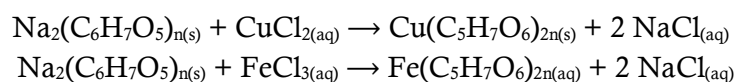


Figure 3. Formation of Enzyme Beads from Avocado and Coconut Seeds. Blue color with blue granules indicates coconut enzyme beads in CuCl_2 solution. Orange color with orange granules indicates coconut enzyme beads in FeCl_3 solution. White color with white granules indicates coconut beads in CaCl_2 solution. Green color with dark green granules indicates avocado enzyme beads in CuCl_2 . Dark orange color with brown granules indicates avocado enzyme beads in FeCl_3 solution. Cream color with yellow granules indicates avocado enzyme beads in CaCl_2 solution.

Figure 3 shows that the color differences that appear in enzyme beads extracted from avocado and coconut seeds when soaked in CuCl_2 , FeCl_3 , and CaCl_2 solutions are caused by chemical interactions between metal ions and bioactive compounds contained in each seed. Compounds such as tannins, flavonoids, and polyphenols are known to have the ability to form colored complexes with certain metal ions. In beads from coconut seeds, immersion in CuCl_2 solution produces a distinctive blue color from the complex of Cu^{2+} ions with phenolic compounds. Meanwhile, FeCl_3 solution gives an orange color as a result of the interaction between Fe^{3+} ions and phenolic compounds such as tannins. In CaCl_2 solution, beads appear white or transparent because Ca^{2+} ions do not form colored complexes. In contrast, beads from avocado seeds show a dark green color when soaked in CuCl_2 , which is most likely the result of a combination of the blue Cu^{2+} complex and the yellow pigment from avocado seeds.

Soaking in FeCl_3 produces a deep brown color due to the formation of a Fe^{3+} complex with tannin, while in CaCl_2 solution, a creamy color with yellow grains appears as a result of natural pigments trapped in the bead matrix. This color difference indicates the differences in chemical composition between avocado and coconut seeds, as well as the ability of each bioactive compound to interact with specific metal ions.

Using sodium alginate concentrations below 4% results in low viscosity, and the resulting beads are easily destroyed or have low density and non-uniform shape. Therefore, it is not recommended for use to maintain the viability of fixed cells during storage. Conversely, sodium alginate has a high viscosity at concentrations above 8%, making bead formation difficult (Summiati & Irdawati, 2024). In previous research, beads were formed on enzymes and Na-alginate dropped into CuCl_2 and FeCl_3 solutions. The reaction that occurs is as follows:



Ions such as Ca^{2+} , Sn^{2+} , and Mg^{2+} function to form cross-links with alginate. According to research conducted by Bie et al. (2022), enzyme immobilization using sodium alginate can be associated with the cross-linking method, as both aim to increase enzyme stability and efficiency in industrial applications. The main link between these two methods is increased enzyme stability, as both alginate and cross-linking help maintain enzyme activity across a wide range of pH and temperature conditions. Thus, this research has the potential to be an initial step in efforts to improve the stability of plant-based lipases for broader applications in various industrial sectors.

CONCLUSION

Lipase extraction from avocado and coconut seeds has been successfully carried out. Activity tests showed that the activity of Na-alginate-immobilized lipase in avocado seeds was 0.870 mg/mL higher than in coconut seeds (0.626 mg/mL). The success of the lipase immobilization process with Na-alginate was indicated by the presence of stable granules dropped into CuCl_2 and FeCl_2 solutions. This study found an environmentally friendly source of lipase and needs further development for industrial applications.

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