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Research Article

Optimization of pH on Enzymatic Activity of Eco-Enzyme *Averrhoa bilimbi* L. in Plaju District, South Sumatra

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ABSTRACT

Enzymes are biocatalysts that have many benefits in the industry and the environment. Eco-enzymes are reported to have amylase, lipase, and protease activities. Enzymatic activity is strongly influenced by several factors, one of which is pH. This study aims to determine the optimum enzymatic activity (amylase, lipase and protease) of Averrhoa bilimbi L. fruit eco-enzymes with various pH treatments (5, 6, 7, 8). Enzymatic activity was measured using a spectrophotometric method. Amylase activity assay using starch as a substrate. Casein and para nitrophenol palmitate were used as substrates. Casein was used as a substrate in the protease activity assays. In the lipase activity assays, para nitrophenol palmitate was used as substrate. Data analysis results are presented in graphical and descriptive. Averrhoa bilimbi L. fruit eco-enzyme has a protein concentration of 0.459 mg/ml. The optimum activity of amylase at pH 5 was 11,713.871 U/mg. Optimum activity and protease activity occurred at pH 8 and 6 of 3.667 U/mg and 13,400.77 U/mg, respectively.

Key words: *Averrhoa bilimbi L; Eco-enzyme: Enzymatic activity; pH..*

Introduction

The use of eco-enzymes has been widely carried out in various sectors such as agriculture, industry and waste management. Eco-enzymes have many advantages, namely being more economical and environmentally friendly (Nazim & Meera, 2015; Jadid et al., 2022). Eco-enzyme is a fermented liquid from organic matter, sugar and water. Those materials form a multifunctional complex organic substance. The raw materials used come from organic waste, such as leftover fruits and vegetable scraps, which are added with other ingredients and then fermented. The results of this fermentation cause eco-enzymes to be acidic (Rochyani et al., 2020).

The fruit of *Averrhoa bilimbi* L. (starfruit) is often found in South Sumatra and can be used as a raw material for making eco-enzymes. *A. bilimbi* L. has a sour taste and is high in folic acid. This fruit is rarely in demand by the public, so it is often found

to fall (Engka et al., 2016). Nonetheless, *A. bilimbi* L. is one of the important medicinal plants from many tropical and subtropical countries in the world which has been widely used in traditional medicine systems for the treatment of various diseases, especially as an antidiabetic, antihypertensive and antimicrobial agent (Alhassan & Ahmed, 2016).

Hemalatha & Visantini Research (2020); Zainudin & Kesumaningwati (2022); Arun & Sivashanmugam (2015), stated that eco-enzymes produced from fruit waste contain protease, lipase and amylase enzymes. These content make eco-enzymes widely used to treat waste containing carbohydrates, lipids and proteins. Eco-enzymes are also widely used as disinfectants, hand sanitizers (Nurdin et al., 2021), toilet cleaners, detergent substitutes, floor cleaning fluids, organic fertilizers for plants (Nurhamidah et al., 2021), natural pesticides, fruit and vegetable washes (Larasati et al., 2020), dishwashing liquid, insect and pest repellant (Astra et al., 2021), and a wound drying agent (Budiyanto et al., 2022). This eco-enzyme is also environmentally friendly. Making eco-enzyme produces O_3 (ozone) to finally produce H_3COOH (acetic acid) and other enzymes that can be used as killers of bacteria and viruses, as well as producing NO_3 and CO_3 which are very useful for soil nutrition (Rochyani et al., 2020).

Enzymes are proteins composed of amino acids in a fixed composition (Wahjuni et al., 2017). Enzymes have been developed in industrial biotechnology, medicine, and analysis. The use of enzymes in biotechnology has advantages, enzymes are natural ingredients and are non-toxic, enzymes are active at low concentrations, can accelerate reaction results without causing unwanted results, enzymes are environmentally friendly compounds, the reaction speed can be adjusted by adjusting the pH, temperature, and the amount of enzyme needed (Sumarlin, 2008). Some enzymes that are often used in biotechnology are lipase, amylase, and protease enzymes (Supriyatna et al., 2015). Rasit et al. (2019) reported that eco-enzymes from tomato and orange waste have amylase, lipase and protease activities. The optimum pH for amylase activity from tomato and orange waste eco-enzyme is 6.5 and 7. The optimum pH of lipase and protease is at pH 8 and 6.5-7.5.

Enzyme activity is strongly influenced by various factors, one of which is pH. Enzymes that are below or above the optimum pH will decrease enzyme activity. The three-dimensional enzyme structure is denatured, and the enzyme's active site cannot bind to the substrate (Nurkhotimah et al., 2017). Based on this, it is necessary to carry out research to determine the optimum amylase, lipase and protease activity of the eco-enzyme *A. bilimbi* L. with different pH treatments.

Materials and Methods

The sample used is an eco-enzyme sample from the fruit of *A. bilimbi* L. Eco-enzyme was made with a ratio of 10 (water): 3 (*A. bilimbi* L.): 1 brown sugar. The enzymatic activity of eco-enzyme *A. bilimbi* L. begins with measuring the protein concentration. Furthermore, the enzymatic activity assay (amylase, lipase, protease) of the eco-enzyme *A. bilimbi* L.

Measurement of Protein Concentration

Protein concentration was measured using the Bradford method with Bovine Serum Albumin (BSA) as a substrate for making a standard curve. Standard curves were prepared by dissolving BSA in several concentrations (0 mg/ml; 0.25 mg/ml; 0.5 mg/ml; 0.75 mg/ml; 1 mg/ml; 1.25 mg/ml; 1, 5 mg/ml) and reacted with Bradford reagent. Then each was vortexed and incubated for 10 minutes at room temperature. Measure the absorbance with a wavelength of 595 nm. The measurement results are made in a regression equation. The protein concentration in *A. bilimbi* L. eco-enzyme was measured by preparing a

reaction mixture consist of 800 µL of Bradford reagent and 200 µL of *A. bilimbi* L. eco-enzyme added. The mixture was then vortexed until homogeneous. Incubate for 10 minutes at room temperature. Measure the absorbance with a wavelength of 595 nm. The absorbance value is substituted into the standard curve equation (Sari & Fadhilah, 2021).

Amylase activity assay

Amylase activity assay was performed using the DNS (3,5-dinitro salicylic acid) method and glucose as a standard curve (0 µg/ml, 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml, 500 µg/ml, 600 µg/ml, 700 µg/ml, 800 µg/ml). Amylase activity assay was carried out with different pH variations, potassium phosphate buffer (pH 5, pH 6) and tris-HCl buffer (pH 7, pH 8). Amylase activity was measured at a wavelength of 540 nm (Fitriani et al., 2013).

Lipase activity assay

In the lipase activity assay, a standard curve was prepared using 4-nitrophenol at several concentrations (0 mg/ml; 0.25 mg/ml; 0.5 mg/ml; 0.75 mg/ml; 1 mg/ml; 1.25 mg/ml; 1.5 mg/ml). Absorbance was measured at a wavelength of 410 nm. The measurement results are made in a regression equation. Lipase activity in the eco-enzyme *A. bilimbi* L. was measured by preparing a reaction mixture consist of 1 mL para nitrophenol palmitate, 1 mL potassium phosphate buffer (pH 5, pH 6) or tris-HCl buffer (pH 7, pH 8) and 1 mL eco- enzymes. Vortex the reaction mixture until homogeneous. Incubate the reaction mixture at 37°C for 10 minutes. Then add 0.1M Na_2CO_3 as much as 0.25ml. For blanks, 1 mL of eco-enzyme is replaced with 1 mL of distilled water. Measure the absorbance with a wavelength of 410 nm. The absorbance value is substituted into the 4-nitrophenol standard curve equation. One unit of lipase activity is defined as the ability of several enzymes to liberate 1 µmol para nitrophenol from para nitrophenyl palmitate (Soleha & Retnaningrum, 2020).

Protease activity assay

The protease activity assay begins by making a standard curve for tyrosine at several concentrations (0 ppm, 50 ppm, 100 ppm, 150 ppm, 200 ppm, 250 ppm, 300 ppm). Then 1 ml of each concentration was taken, then added with 0.5 mL of 0.1M Na_2CO_3 and 1 mL of folin reagent. Measure the absorbance with a wavelength of 660 nm. The measurement results are made in a regression equation. Lipase activity in *A. bilimbi* L. eco-enzyme was measured by preparing a reaction mixture consist of 1 mL eco-enzyme plus casein in 0.5 mL potassium phosphate buffer (pH 5, pH 6) or tris-HCl buffer (pH 7, pH 8). Incubate at 37°C for 10 minutes. Add trichloro acetate (TCA) to the reaction mixture. Centrifuge the reaction mixture and collect the supernatant. Add 1.25 mL of 0.1M Na_2CO_3 and 0.25mL of folin's reagent to the supernatant. Incubate for 20 minutes at 37°C. For blanks, 1 mL of eco-enzyme is replaced with 1 mL of distilled water. Measure the absorbance at a wavelength of 660 nm. The absorbance value is substituted into the standard tyrosine curve equation (Pagarra et al., 2021).

Results and Discussion

Measurement of Protein Concentration

Measurement of the eco-enzyme *A. bilimbi* L. protein concentration with three repetitions showed consistent results. Eco-enzyme *A. bilimbi* L. has a protein concentration of 0.459 mg/ml. Measurement of the protein concentration of the eco-enzyme of *A. bilimbi* L. fruit was carried out to determine the specific enzymatic activity (amylase, lipase, protease). Several studies have reported that eco-enzymes consist of complex components, one of which is an enzyme. According to Kusumaningrum et al. (2019), enzymes are protein biomolecules that act as catalysts in a biochemical reaction. Arun & Sivashanmugam (2015), stated that eco-enzymes contain enzymes (proteins) that have optimum amylolytic, lipolytic and

proteolytic activities at a given pH treatment.

Protein measurements were carried out using the Bradford method and BSA as standard. According to Purwanto (2014), the Bradford method is the most stable method for measuring protein concentration. Proteins can be identified using the Bradford method. Amino acids resulting from protein hydrolysis will bind to the sulfonate groups of Coomassie Brillant Blue.

Amylase activity assay

The enzymatic activity assay showed that the eco-enzyme of *A. bilimbi* L. fruit contained amylase (Figure 1). The existence of amylase is proven by the presence of activity formed from the enzymatic activity assays that have been conducted. The pH treatment caused a difference in amylase activity. The optimum amylase activity was at pH 5. Amylase activity decreased significantly from pH 6 to 9. According to Nangin & Sutrisno (2015), pH is a factor that influences enzyme activity. Enzyme conformation can change according to the environmental pH. An inappropriate pH will cause the complex between the substrate and the enzyme not to form.

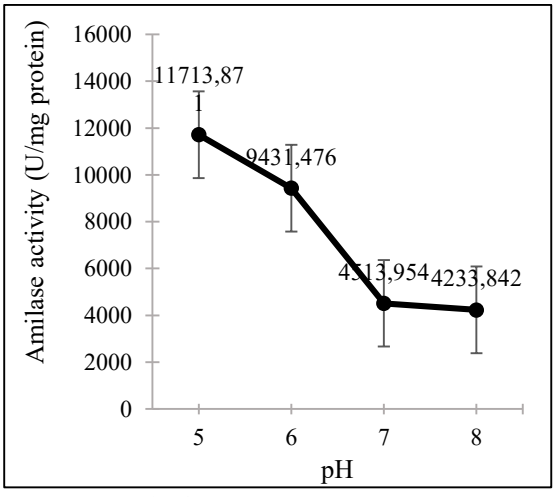

Figure 1. Optimum pH on amylase activity of the eco-enzyme *A. bilimbi* L.

Amylase from the fruit eco-enzyme *A. bilimbi* L. has optimum activity at pH 5 of 11713.871 U/mg protein. At a variation of pH 6, the amylase activity was 9431.476 U/mg protein. Amylase activity decreased at pH 7 and 8 at 4513.954 U/mg protein and 4233.842 U/mg protein, respectively. Amylase is an enzyme whose role is to catalyze starch into simple molecules such as dextrin, glucose, and maltose (Nangin & Sutrisno, 2015), which are used in the bakery, pharmaceutical, and textile industries (Tazkiah et al., 2017). Amylase is an enzyme that catalyzes the hydrolysis of alpha-1,4-glycosidic polysaccharides to produce D-glucose, dextrin, maltose, and oligosaccharides (Ariandi, 2016).

Lipase activity assay

A lipase activity assay was carried out using a variation of the pH of two types of buffer, potassium phosphate buffer (pH 5, pH 6) and tris-HCl buffer (pH 7, pH 8). Lipase activity is influenced by pH. According to Soleha & Retnaningrum (2020), lipase has an optimum pH for its activity and is very sensitive to changes in pH. At pH 5, lipase activity was 3.056 U/mg protein. Lipase activity decreased at pH 6 (1.722 U/mg protein) and 7 (2.013 U/mg protein. The optimum lipase activity from the eco-enzyme *A. bilimbi* L. was at pH 8, at 3.667 U/mg protein (Figure 2) Lestari et al. (2016), stated that most of the optimum activity of lipases was at pH 8 with maximum activity in the range of pH 6 – 8.

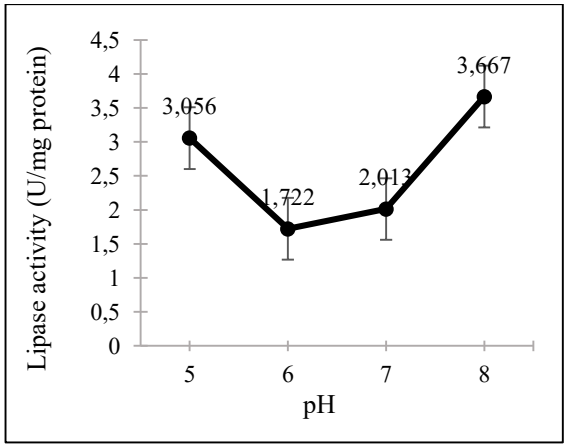

Figure 2. Optimum pH on lipase activity of the eco-enzyme *A. bilimbi* L.

The results showed that lipase of eco-enzyme *A. bilimbi* L. had optimum activity at pH 8. Lipase activity was unstable at pH 5 – 6 (Figure 2). Soleha & Retnaningrum (2020), stated that the catalytic lipase decreases when it is above or below the optimum pH. Lipase is a group of enzymes that catalyze the hydrolysis of long-chain triglycerides. This enzyme is often used as a detergent formulation because it can catalyze oil and fat and is soluble in water (Layly & Wiguna, 2016). The degradation process begins with an oxidation reaction in the alkyl group, which requires a coenzyme that causes the alkyl chain to shorten. After the shortening of the alkyl chain occurs, desulphonation will occur and the disappearance of the sulfonate catalyzed by enzymes, coenzymes, and oxygen (Pratamadina & Wikaningrum, 2022).

Protease activity assay

The protease activity assay showed that the optimum protease activity (Figure 3) was at pH 6 of 13400.77 U/mg protein. Protease activity was unstable at pH 5 and decreased at pH 7 and 8. Protease activity at pH 5 was 12882.04 U/mg protein. At pH 7 and 8, the protease activity decreased to 12010 U/mg protein and 6346.094 U/mg protein, respectively. The decrease in activity is due to the three-dimensional structure of the enzyme denatured by extreme changes in pH. According to Sumardi et al. (2019), disruption of interactions between non-covalent bonds causes the three-dimensional structure of the enzyme to become unstable and experience denaturation. Noncovalent bond interactions become unstable due to extreme changes in pH.

Proteases the eco-enzyme *A. bilimbi* L. can optimally hydrolyze proteins at pH 6. Proteases hydrolyze proteins into amino acids and peptides. Proteases are used in the food, textile and pharmaceutical industries (Soeka & Sulistiani, 2017). In addition, protease enzymes can be used as beer clarifiers, meat tenderizers, and protein hydrolysates (Tondais et al., 2020). Penitobe (2021), states that eco-enzymes that contain proteases can be used as cleaners. Proteases will break peptide chains in high-molecular proteins into simple molecules so that the attached amino acids and peptides will separate.

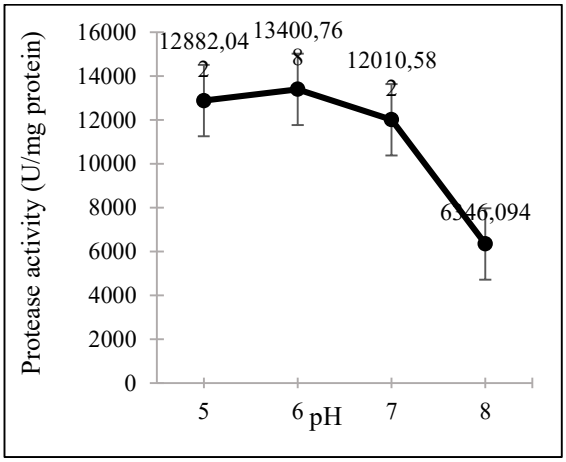

Figure 3. Optimum pH on protease activity of the eco-enzyme *A. bilimbi* L.

The difference in pH of each enzyme activity is due to the differences in the characteristics of each enzyme. Enzyme activity is influenced by pH. Differences in pH in its activity can result in changes in the charge. It can affect enzyme activity. A pH that does not match the enzyme characterization can cause enzyme denaturation. An enzyme can decrease activity or damage the enzyme structure(Istia'nah et al., 2020).

Conclusion

Eco-enzyme from *A. bilimbi* L. fruit has a protein concentration of 0.459 mg/ml. Eco-enzyme from *A. bilimbi* L. fruit contains amylase, lipase and protease. The optimum activity of amylase was 11,713.871 U/mg protein at pH 6. The lipase and protease optimum activity was at pH 8 (3.67 U/mg protein) and pH 6 (13,400.77 U/mg protein).

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