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Analysis of Amylase, Protease, and Peroxidase Enzymes in Eco-Enzymes Based on Mango Peel, Pineapple, and Cabbage Using UV-Vis Spectrophotometry

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ABSTRACT
Waste accumulation is dominated by organic waste, originating from food scraps, household waste, and commercial activities. This study aimed to examined the relationship between raw material composition and enzyme activity in eco-enzyme products using UV-Vis spectrophotometry. Eco-enzyme was produced from the fermentation of organic materials (pineapple peel, mango peel, and cabbage), molasses, and water with a mass ratio of 3:1:10, then fermented for 5 months. Qualitative tests to determine the presence or absence of amylase, protease, and peroxidase enzymes were carried out using the Benedict, Ninhydrin, and Addy Goodman methods. Quantitative tests were carried out using UV-Vis spectrophotometry to determine enzyme activity.The results showed that the eco-enzyme had an acidic pH (2.51-2.35) with a brown color and a sour aroma. The highest enzyme activities for amylase, protease, and peroxidase were found in composition 4 (3.86384 U/mL); composition 4 (3.27301 U/mL); and composition 2 (1,554.48 U/mL), respectively. Thus, the resulting eco-enzyme has potential as an enzyme source and can be applied in several fields.

Keywords: eco-enzyme, UV-Vis spectrophotometry, amylase, peroxidase, protease

1. INTRODUCTION

Open dumping is the open disposal of waste without any treatment. Open waste accumulation increases air pollution, marked by increased concentrations of NO_2, O_3, SO_2, dust, and NH_3 (Sukarmawati, et al., 2023). In Indonesia, the waste problem remains unresolved to this day (Larasati, et al., 2020). One way to address the negative impacts of household waste pollution, particularly organic waste, is to process it into eco-enzyme. Eco-enzyme is a product made from fermented kitchen waste using cane molasses or molasses made from brown sugar (Novianti & Muliarta, 2021). Through this fermentation process, fruit peels and vegetable scraps can be converted into a multipurpose liquid with economic value (Setyawati, 2022). Eco-enzyme was originally used to improve agricultural land as an organic fertilizer. It has since also been used as a floor cleaner, for washing vegetables and fruit, for pest and insect control, and as a plant fertilizer (Larasati, et al., 2020). However, despite the many benefits produced by eco-enzyme, scientific research further examining its chemical content remains limited. Chemical compounds found in eco-enzyme include

–OH and –COOH groups, as well as amylase, protease, and lipase. Incubation results have also revealed metabolite compounds such as flavonoids, alkaloids, quinones, and saponins in eco-enzyme liquid (Vama & Cherekar, 2020). An in-depth chemical analysis is needed regarding the effect of variations in the mass composition ratio of raw materials used to produce eco-enzyme on enzyme content and activity. This would allow further exploration of the previously unrevealed potential of eco-enzyme products and serve as a reference for future research.

2. RESEARCH METHOD

This study examined the relationship between material composition and the enzyme activity produced in eco-enzyme products, beginning with a qualitative test to determine the presence or absence of amylase, protease, and peroxidase enzymes using the Benedict, Ninhydrin, and Addy Goodman methods. This was followed by an activity test using UV-Vis spectrophotometry to determine concentration through the absorbance produced at the maximum wavelength based on the Lambert-Beer law. Characterization was carried out by observing pH, color, and odor.

Eco-enzyme Preparation

Eco-enzyme was prepared by mixing all ingredients into a clean, dry 5-liter jar. The material composition used a molasses:water:organic material ratio of 1:10:3. Based on the capacity of the jar used (80% of the container volume), the materials required were 0.280 kg molasses, 0.85 kg organic material, and 2.830 liters of water. The fermentation process lasted 5 months (September 8, 2025 – January 21, 2026) in a tightly sealed (airtight) plastic container. The organic material ratios are presented in Table 1.

Table 1. Comparison of Organic Material Composition

Composition	Comparison		
	Pineapple	Mango	Cabbage
1	1	1	1
2	2	0	1
3	0	1	2
4	1	2	0

Qualitative Analysis of Amylase Enzyme

A total of 0.125 mL of eco-enzyme sample was placed in a test tube, then 750 mL of 1% starch solution was added to each tube. 0.125 mL of Benedict's reagent was then added to each tube. The solution was heated for approximately 3 minutes until a color change or precipitate formed. The presence of amylase was indicated by a color change from yellow to brick-red; the more intense the color, the stronger the enzyme activity (Muliasari & Permatasari, 2022). The same procedure was carried out for a standard 1% amylase enzyme as a positive control and a blank containing distilled water as a negative control, to compare qualitative results.

Preparation of Maltose Standard Solution

Standard solutions were prepared with volume variations as presented in Table 2. The mixture was homogenized by shaking the bottom of the test tube. The container was then closed with a ventilated cap and placed in a boiling water bath for exactly 5 minutes. Afterward, the container was removed from the boiling water bath and the solution was cooled in ice water to room temperature. The solution was then transferred to a 10 mL volumetric flask and distilled water was added to the calibration mark, yielding the maltose standard solution.

Table 2. Composition of the Maltose Standard Solution Series

Solution	Blank	Standard					
		1	2	3	4	5	6
1.95 mM standard maltose (μL)	0	250	300	350	400	600	800
DNS (μL)	1000	1000	1000	1000	1000	1000	1000
Distilled water (μL)	1000	750	700	650	600	400	200
Total volume (μL)	2000	2000	2000	2000	2000	2000	2000

Determination of the Maximum Wavelength for the Maltose Standard

The absorption spectrum of a 0.35 mL maltose standard solution was measured using a UV-Vis spectrophotometer over a wavelength range of 400–600 nm, yielding a maximum wavelength of 484.2 nm. This wavelength was used to measure the other standard solutions (standards 1–6).

Measurement of Maltose Concentration in Samples

A total of 0.5 mL of diluted eco-enzyme was pipetted into a test tube, and 0.5 mL of 1% starch solution was added. The solution was then incubated for 10 minutes at an optimum temperature of 25°C so that the amylase enzyme in the sample could hydrolyze the starch into monosaccharides. The reaction was stopped by adding 1 mL of DNS reagent. The solution was then incubated in a boiling water bath for 5 minutes to accelerate the reaction between DNS and the reducing sugars from hydrolysis, producing a color change from yellow to light brown. Afterward, the solution was cooled with ice to room temperature. The solution was then transferred to a 10 mL volumetric flask and distilled water was added to the calibration mark [8]. The sample was then measured using a UV-Vis spectrophotometer at a wavelength of 484.2 nm, corresponding to the maximum wavelength obtained from the maltose standard curve.

Qualitative Analysis of Protease Enzyme

Testing was carried out by pipetting 62.5 μL of eco-enzyme into a test tube, then adding 625 μL of ninhydrin solution using a micropipette. The solution was heated in a water bath at 80–100°C for 1–3 minutes until a purple color appeared, indicating a reaction with the ninhydrin solution. Bromelain was considered present if the mixture turned purple [8]. The same testing procedure was carried out on a standard protease enzyme sample as a positive control and phosphate buffer pH 7.0 as a negative control.

Preparation of Tyrosine Standard Solution

Standard solutions were prepared by pipetting volume variations as shown in Table 3. 5.5 mL of solution (50 parts 2% Na_2CO_3 in 0.1 N NaOH and 1 part 0.5% $CuSO_4$) was added to each test tube and homogenized. 0.5 mL of Folin–Ciocalteu reagent was then added, mixed, and left to stand for 30 minutes (the reaction was carried out in test tubes covered with aluminum foil).

Table 3. Composition of the Tyrosine Standard Solution Series

Solution	Blank	Standard								
		1	2	3	4	5	6	7	8	9
1.0 mM tyrosine std (μL)	0	250	300	325	350	375	400	450	550	600
Phosphate buffer pH 7 (μL)	1000	750	700	675	650	625	600	550	450	400
Total volume (μL)	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000

Determination of the Maximum Wavelength for the Tyrosine Standard

A 375 µL tyrosine standard solution was measured using a UV-Vis spectrophotometer over a wavelength range of 200–800 nm, yielding a maximum wavelength of 763.5 nm. This wavelength was used for measuring the standard solutions.

Measurement of Tyrosine Concentration in Samples

A total of 15 mL of eco-enzyme was centrifuged at 3000 rpm for 10 minutes. The centrifugation product was then separated by decantation to separate the supernatant from the suspension. The resulting supernatant was filtered, and 100 µL was taken into a test tube and mixed with 900 µL of phosphate buffer pH 7.0. 1 mL of 1% casein solution was then added to the eco-enzyme sample, which was incubated at 37°C and allowed to react for 10 minutes. The reaction was stopped by adding 5 mL of 5% trichloroacetic acid (TCA).

The solution was centrifuged again at 3000 rpm for 10 minutes. 1 mL of the resulting supernatant was separated by decantation, then filtered and placed in a separate test tube. 5.5 mL of solution (50 parts 2% Na_2CO_3 in 0.1 N NaOH and 1 part 0.5% $CuSO_4$) was added to the test tube. 0.5 mL of Folin–Ciocalteu reagent was then added, mixed, and left to stand for 30 minutes (Gumilar, et al., 2025). Measurement was carried out at the wavelength corresponding to the standard solution measurement, 763.5 nm.

Qualitative Analysis and Activity of Peroxidase Enzyme

Peroxidase enzyme analysis was carried out using the Addy and Goodman method. Qualitative analysis was performed by reacting a solution consisting of 3 mL of pyrogallol in phosphate buffer solution (0.05 M pyrogallol in 0.1 M phosphate buffer, pH 7.0) with 0.5 mL of 1% H_2O_2. 0.1 mL of enzyme extract was then added to the test tube. A positive result for peroxidase enzyme activity was indicated by the formation of purpurogallin, causing the solution to turn brown. Phosphate buffer pH 7 was used as a negative control.

Quantitative analysis used the same reagents as the qualitative test. The change in absorbance per unit time ($\Delta A/\Delta t$) was then rapidly measured using a UV-Vis spectrophotometer at a wavelength of 430 nm. The change in peroxidase activity was calculated using a formula based on the extinction coefficient of oxidized pyrogallol (4.5 liters/mol) (Nemade, et al., 2011).

3. RESULTS AND ANALYSIS

Characteristics of Eco-enzyme from Fruit and Vegetables

The characteristics of the eco-enzyme product derived from the fermentation of fruit and vegetables are presented in Table 4. The eco-enzyme shown in Figure 1 has a distinctive, fairly sharp sour aroma resembling vinegar, indicating the formation of organic acids during the fermentation process. Volatile ester compounds in fruit are reported to contribute to sweet, fruity aromas, while cruciferous vegetables such as cabbage produce volatile sulfur compounds such as isothiocyanates that give a pungent aroma (Zhang, et al., 2023). Composition 3 had a more pungent aroma due to its higher vegetable (cabbage) content.

Figure 1. Fermentation Results of Eco-enzyme Compositions 1–4

The resulting eco-enzyme showed physical characteristics of a dark brown color tending toward cloudiness. This color forms as a result of the fermentation of organic materials, which produces colored compounds from substrate degradation, as can be seen in Figure 1. In addition to aroma and color, acidity/alkalinity was also measured. The pH results for the four compositions, as shown in Table 4, ranged from 2.35–2.51, meeting the standard for fully fermented eco-enzyme, which has a pH below 4.0; this typically occurs after three months of fermentation (Sari, et al., 2025).

Table 4. Characteristics of Eco-enzyme

Composition	pH	Color	Aroma
1	2.43	Dark brown	Sour, molasses-like aroma
2	2.35	Dark brown	Sour, pungent aroma
3	2.51	Dark brown	Sour, very pungent aroma
4	2.50	Dark brown	Sour, molasses-like aroma

During eco-enzyme fermentation, a colorless-to-yellowish mold layer appeared, presumed to be pitera, along with a jelly-like layer ("mother of enzyme") that grew on top of the eco-enzyme liquid. Mother of enzyme is a colony of bacteria and yeast living symbiotically in the fermentation solution, forming a thick biofilm/colony on the surface (Titiaryanti, et al., 2022).

Composition 1 Composition 2 Composition 3 Composition 4

Figure 2. Appearance of Mold on the Surface of the Eco-enzyme

Qualitative Analysis and Activity of Amylase Enzyme

Qualitative Testing of Amylase Enzyme

The Benedict test is based on the reduction of Cu^{2+} to Cu^{+} by free aldehyde or ketone groups under alkaline conditions; complexing agents such as citrate or tartrate are usually added to prevent $CuCO_3$ precipitation (Dasyanti, 2013). For samples containing reducing sugars, the sample color changes during heating from blue (no reducing sugar) to green, yellow, orange, red, and brick-red or brown (high concentration) (Tiwari, 2015). The results of the qualitative amylase enzyme test using the Benedict method are presented in Figure 3 and Table 5.

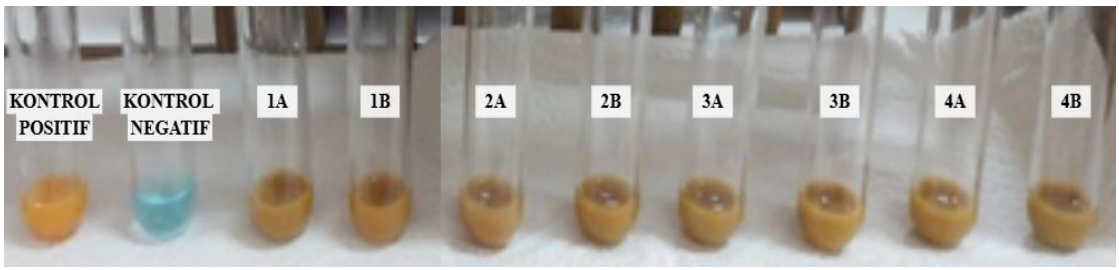

Figure 3. Qualitative Test Results for Amylase Enzyme

Table 5. Qualitative Test Results for Amylase Enzyme

Composition	Sample	Observation Result	
		Before Heating	After Heating
Positive Control		Blue	Yellow
Negative Control		Blue	Blue
1	1A	Green	Light brown
	1B	Green	Light brown
2	2A	Green	Light brown
	2B	Green	Light brown
3	3A	Green	Light brown
	3B	Green	Light brown
4	4A	Green	Light brown
	4B	Green	Light brown

Amylase Enzyme Activity Testing

Quantitative analysis was carried out using UV-Vis spectrophotometry by measuring the absorbance of the sample solution after reacting with the reagent. The method used was the DNS (dinitrosalicylic acid) method. The principle of this amylase enzyme test is a colorimetric reaction between DNS and the alpha-amylase enzyme, forming a yellow-to-light-brown colored complex. Amylase enzyme activity is presented in Table 6. This data is consistent with the carbohydrate content of mango, which is higher than that of pineapple and cabbage. Carbohydrate content, based on USDA (2023) food composition data, is presented in Table 7. High sugar content can serve as a substrate for microorganisms during fermentation, thereby increasing metabolic activity and enzyme production, including amylase.

Table 6. Absorbance, Maltose Concentration, and Amylase Enzyme Activity Data

Composition	Sample	Corrected Absorbance	Maltose Concentration (mM)	Amylase Activity (U/mL)	Mean Amylase Activity (U/mL)
1	1A	0.41732	0.08722	3.48873	3.40668
	1B	0.41216	0.08644	3.45752	
	1C	0.38178	0.08184	3.27379	
2	2A	0.40354	0.08513	3.40539	3.48278
	2B	0.40906	0.08597	3.43877	
	2C	0.43641	0.09010	3.60417	
3	3A	0.40972	0.08607	3.44276	3.36937
	3B	0.41135	0.08632	3.45262	
	3C	0.37168	0.08032	3.21271	
4	4A	0.48414	0.09732	3.89282	3.86384
	4B	0.48824	0.09794	3.91762	
	4C	0.46566	0.09453	3.78107	

Table 7. Carbohydrate Content in Fruit and Vegetables

No.	Fruit/Vegetable	Carbohydrate per 100 g
1.	Pineapple	11.82
2.	Mango	14.98
3.	Cabbage	5.80

Qualitative Analysis and Activity of Protease Enzyme

Qualitative Testing of Protease Enzyme

The results of the qualitative protease enzyme test using the Lowry method can be seen in Figure 6 and Table 8. Qualitative analysis of the protease enzyme used the Ninhydrin test, in which the protease enzyme contained in the eco-enzyme sample hydrolyzes protein to produce primary amino acids, forming Ruhemann's Purple after the ninhydrin-treated sample solution is heated (Stauß, et al., 2024).

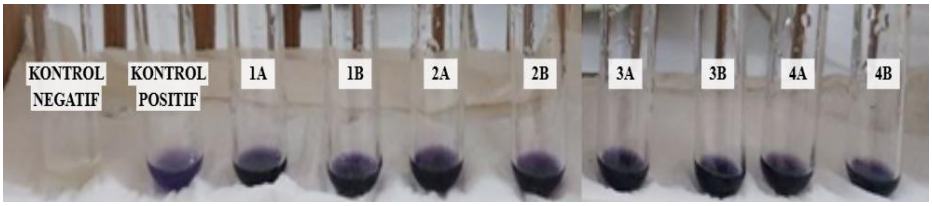

Figure 6. Qualitative Test Results for Protease Enzyme

Table 8. Qualitative Test Results for Protease Enzyme

Composition	Sample	Observation Result	
		Before Heating	After Heating
Negative Control		Colorless	Colorless
Positive Control		Colorless	Purple
1	1A	Yellow	Purple
	1B	Yellow	Purple
2	2A	Yellow	Purple
	2B	Yellow	Purple
3	3A	Yellow	Purple
	3B	Yellow	Purple
4	4A	Yellow	Purple
	4B	Yellow	Purple

Protease Enzyme Activity Testing

Quantitative analysis was carried out using UV-Vis spectrophotometry by measuring the absorbance of the sample solution after reacting with the reagent. The method used was the Lowry method. Protease enzyme activity data is presented in Table 9. Pineapple is known to contain the protease enzyme bromelain, which exhibits various proteolytic activities. This indicates that pineapple has high proteolytic activity. Therefore, protease activity was higher in composition 4, due to its more dominant content of active protease enzyme.

Table 9. Absorbance, Concentration, and Protease Enzyme Activity Data

Composition	Sample	Corrected Absorbance	Maltose Concentration (mM)	Amylase Activity (U/mL)	Mean Amylase Activity (U/mL)
1	1A	0.56189	0.46592	3.26147	3.22263
	1B	0.56177	0.46583	3.26078	
	1C	0.54173	0.44938	3.14566	
2	2A	0.54178	0.44942	3.14595	3.14275
	2B	0.52867	0.43866	3.07064	
	2C	0.55322	0.45881	3.21166	

Cont.

	3A	0.50552	0.41967	2.93766	
3	3B	0.49088	0.40765	2.85356	2.89772
	3C	0.49930	0.41456	2.90193	
	4A	0.57443	0.47621	3.33350	
4	4B	0.56212	0.46611	3.26279	3.27301
	4C	0.55515	0.46039	3.22275	

Furthermore, the second-highest protease activity, found in composition 1, indicates a balance between enzyme sources, microorganisms, and nutrients during the fermentation process. Pineapple (Ananas comosus) is known to contain the main protease enzyme, bromelain. Cabbage, meanwhile, acts as a source of fermentative microorganisms. Mango, together with molasses, provides a carbon source that supports microbial growth. This combination allows both the natural enzymes from the ingredients and the enzymes produced during fermentation to combine, maximizing protease activity. Composition 2 showed lower activity than composition 4 despite containing more pineapple. This is presumably because the absence of mango as an additional carbon source meant that microbial activity from the cabbage did not develop optimally, even with molasses present. As a result, the enzyme contribution produced during fermentation was lower, and total protease activity was not maximized. The lowest activity was obtained in composition 3. This is because pineapple, the main source of protease enzyme, was absent, so the measured enzyme activity came only from microorganisms present during fermentation.

Qualitative Analysis and Activity of Peroxidase Enzyme

The results of the qualitative peroxidase enzyme test using the Addy and Goodman method are presented in Figure 7 and Table 10. A color change resulting from the reaction of two substrates – pyrogallol and H_2O_2 – in the presence of peroxidase can be observed as a qualitative test result, namely the formation of orange-colored purpurogallin (Ghadiri, et al., 2013)

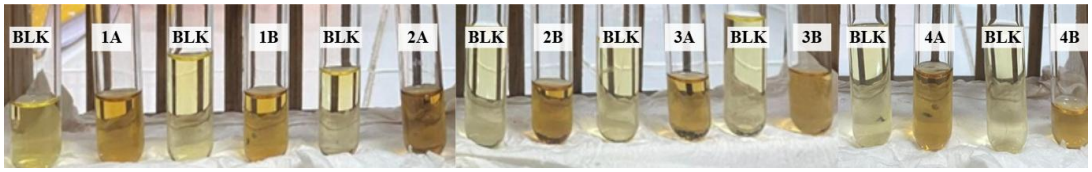

Figure 7. Qualitative Test Results for Peroxidase Enzyme

Table 10. Qualitative Test Results for Peroxidase Enzyme

Material	Observation Result	
	Before Oxidation	After Oxidation
Blank	Yellow	Yellow
Sample EE 1A	Yellowish brown	Light brown
Blank	Yellow	Yellow
Sample EE 1B	Yellowish brown	Light brown
Blank	Yellow	Yellow
Sample EE 2A	Yellowish brown	Light brown
Blank	Yellow	Yellow
Sample EE 2B	Yellowish brown	Light brown
Blank	Yellow	Yellow
Sample EE 3A	Yellowish brown	Light brown
Blank	Yellow	Yellow

Cont.

Sample EE 3B	Yellowish brown	Light brown
Blank	Yellow	Yellow
Sample EE 4A	Yellowish brown	Light brown
Blank	Yellow	Yellow
Sample EE 4B	Yellowish brown	Light brown

The peroxidase enzyme activity test is presented in Table 11; the calculated peroxidase enzyme activity in the eco-enzyme, from highest to lowest, was composition 2, 4, 1, and 3, respectively; the corresponding organic material compositions are presented in Table 12. This data is consistent with the phenolic content reported by Fauzi, et al. (2023), Safiitri, et al. (2023) and Liang, et al. (2019).

Table 11. Reaction Rate and Peroxidase Enzyme Activity

Composition	Reaction Rate (ΔA/Δt (min))		Enzyme Activity (U/mL)		Mean (U/mL)
	A	B	A	B	
1	0.0634	0.0637	507.3600	509.2800	508.3200
2	0.1966	0.1920	1.572.9600	1.536.0000	1.554.4800
3	0.0037	0.0037	29.7600	29.7600	2.3776
4	0.0643	0.0649	514.5600	518.8800	516.7200

Table 12. Phenolic Content in Fruit and Vegetables

No.	Fruit/Vegetable	Phenol (mg GAE/g)
1.	Pineapple	63.4400
2.	Mango	53.4182
3.	Cabbage	1.2554

In this study, an impure enzyme extract (eco-enzyme) was used, so the measured peroxidase activity value may be influenced by the presence of other compounds, not just the peroxidase enzyme. Given the relatively high results, it is possible that not only peroxidase reacted, but also phenolic compounds and metal ions from the ingredients that were oxidized, potentially increasing the measured absorbance. The enzyme activity obtained therefore represents total oxidative activity rather than specific peroxidase activity, because not only the pyrogallol from the reagent reacted, but natural phenolics were also oxidized.

4. CONCLUSION

Protease, amylase, and peroxidase enzymes were found to be present in eco-enzyme made from pineapple peel, mango peel, and cabbage, based on qualitative testing. The differences in the activity of the three enzymes indicate that each enzyme has different controlling factors. Protease activity was influenced by the presence of natural enzymes from pineapple, amylase activity was influenced by the availability of carbon sources for microorganisms, while peroxidase activity was influenced by the phenolic compound content and oxidative components of the materials. The highest enzyme activities obtained were, respectively, amylase (composition 4; 3.86384 U/mL), protease (composition 4; 3.27301 U/mL), and peroxidase (composition 2; 1,554.48 U/mL).

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