

Enzymatic assay of coffee peel and papaya peel waste eco enzyme

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Abstract. Coffee is one of Indonesia's main products with the consumption rate reach 10 million tons in 2020-2021 globally. This high productivity raises the problem of coffee peel waste which has been utilized as animal feed or fertilizer. Eco enzyme, a complex solution formed by the fermentation of organic waste, sugar, and water can be used as an alternative for utilizing coffee peel waste. This research was conducted to test the enzymatic activity of eco enzyme from coffee peel and papaya peel that fermented for 2, 3, and 4 months. The enzymes tested were protease, amylase, and lipase using selective agar media 1% skim milk, 1% starch, and 5% olive oil respectively. The results obtained was the eco enzyme contains protease as indicated by the formation of a clear zone around the sample. Meanwhile, the negative results of amylase activity were thought to be due to the presence of caffeine from coffee peel and certain compounds from papaya peel which can inhibit α -amylase activity. The results of the lipase activity test could not be observed due to the large range of pH value of methyl red so that the reddish zone was not formed by the hydrolyzation of triglycerides into fatty acids that cause the pH value decrease.

1 Introduction

Coffee is one of the most popular drinks in the world with consumption rate reached 10 million tons in 2020-2021, while Indonesia become the fourth biggest coffee exporter. Based on data from the Central Bureau of Statistics (BPS), about 774.6 thousand tons coffee has been produced in 2021 [1, 2]. This high productivity caused problems when the waste is not managed properly, especially coffee peels that the proportion reach 40-50% of a whole coffee cherry [3]. Cases of irresponsible coffee waste disposal have reported in Rejang Lebong, Bengkulu and Tangerang, Banten [4, 5]. To prevent and overcome this issue, several policies, innovation, and research have been conducted. People usually utilize coffee peel waste as

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animal feed or processed into fertilizer. Some people also make innovative products from coffee peel waste such as flour, jam, textile dyes [6-8].

One of the simple methods to utilize organic waste like coffee peels is by producing eco enzyme, a complex solution from organic waste fermentation that has been mixed with sugar and water before. Eco enzymes produced by using spontaneous fermentation or no additional microbial culture required, hence the indigenous and environmental microbes take the important role to generate fermentation process. Various compounds such as acids, enzymes, and secondary metabolites produced during fermentation have a role in providing an antimicrobial effect on eco enzymes so that eco enzymes can be used as pesticide, disinfectant, soap and shampoo, or other cleaning products [9-11].

In order to increase eco enzyme properties, types of organic waste, sugar, and fermentation time need to be considered well. This research combines coffee peel and papaya peel waste as material to make eco enzyme, considering its high productivity number in Indonesia. Just like the case with coffee, papaya has a high consumption rate that reached 79 g/capita/week, along with its production number that reached 1 million Tons in 2021 [12, 13]. This matter caused the accumulation of organic waste. Besides, papaya contains papain, a protease enzyme that can disrupt the physical integrity of bacterial extracellular polymeric substance (EPS), causing cell death [14]. Therefore, this study conducted to find out enzyme content in coffee peel and papaya peel waste eco enzyme through enzymatic assay.

2 Experimental detail

Materials used in this research are coffee peel, papaya peel, molasses, distilled water, agar powder bacteriological, skim milk powder, soluble starch, iodine, olive oil, Tween-80, methyl red, Enzyplex, and blank paper disc. As for the tools used are spatula, tweezers, digital balance, autoclave, laminar air flow, and some glassware such as Erlenmeyer, petri dish, and measuring cylinder.

Eco enzyme produced by mixing 75 g coffee peel, 75 g papaya peel, 50 g molasses, and 500 mL distilled water into a container or fermenter that can be closed tightly so that no oxygen can enter. Then, left the mixture to stand for 2, 3, and 4 months before the filtration is done. The filtrate of the eco enzyme is then used for enzymatic activity test using selective media. The media used for protease activity test is 1% skim milk agar with 24 hours incubation in room temperature [15]. The positive result is indicated by the formation of clear zone around the disc. The media used for amylase activity test is 1% starch agar with 48 hours incubation in room temperature. the positive result is indicated by the formation of clear zone around the disc after the addition of iodine solution. The media used for lipase activity test is 5% olive oil agar mixed with Tween-80 and methyl red with 24 hours incubation in room temperature [16]. The positive result is indicated by the formation of a reddish zone around the disc.

3 Results and Discussion

Enzymatic assay of coffee peel and papaya peel waste eco enzyme was conducted using selective media and observed by the formation of zone around the sample disc. Skim milk powder used as substrate in protease activity test due to the protein content such as casein that can be hydrolyzed into polypeptides or amino acids. It is characterized by the formation of a clear zone around the disc. The same thing happened to amylase activity test that used starch as the substrate. However, visualization using iodine solution is required because starch agar media is colorless. The I₂ molecule will interact non-covalently with the hydrophobic interior of the amylose helical structure to form a blue I₂-starch complex [17].

Meanwhile, olive oil is used as a substrate in the lipase activity test because it contains a number of triglycerides which can be hydrolyzed into fatty acids and glycerol. In this test, Tween-80, which has an amphipathic structure, functions as an emulsifier, while methyl red is useful for visualization based on pH changes in the media caused by the formation of fatty acids. As for the positive control, Enzyplex is used, due to its composition that contains certain amount of protease, amylase, and lipase.

Table 1. Enzymatic Assay Result

Enzyme	Sample fermentation time (month)			
	0	2	3	4
Protease	-	+	+	+
Amilase	+	-	-	-
Lipase	×	×	×	×

Note: (-) negative result; (+) positive result; (×) no data obtained

Table 1 showed the overall enzymatic assay result. The protease activity test as shown in Fig. 1, clear zones were formed around the sample of 2nd, 3rd, and 4th months fermentation due to the hydrolysis of proteins. It means that there is production of extracellular proteases by the microbes contained in eco enzyme during fermentation process. This result is in accordance with the previous study conducted by Neupade and Khadka (2019) about papaya peel eco enzyme that also reveal the presence of protease activity [15].

The result of amylase activity test shown in the Fig. 2 indicate that there is no amylase activity found in the sample of 2nd, 3rd, and 4th months fermentation. This is suspected due to the α -amylase inhibitor compounds contained in the coffee peel and papaya peel. Caffeine contained in coffee peel can inactivate α -amylase by binding to apoenzyme and causing metal dissociation in the active enzyme, while papaya peel extract has antidiabetic activity which is related to the inhibition of the α -amylase enzyme in the body [18, 19]. This could be the reason why amylase activity is not detected in the sample. After the fermentation process, the compounds were extracted into eco enzyme solution so that it could interfere with the amylase activity during the test.

The lipase activity test result in Fig. 3 does not show any reddish zone around the disc sample nor in the positive control. So, the result could not be determined. This is suspected due to the wide range of pH value of methyl red, that is between 4.4-6.2. In addition, based on the research conducted by Rasit et.al (2018 & 2019), the optimum pH of lipase is around 7-8 [20, 21]. Meanwhile, the medium used in the test had pH value around 4.4-6.2 due to the orange color, so that the lipase activity is considered too low and cannot significantly lower the pH which can change the color of the medium.

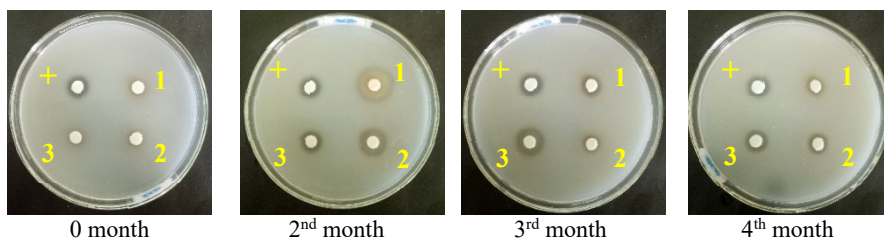


Fig. 1. Protease activity test result: (+) positive control (Enzyplex); (1) first repetition; (2) second repetition; (3) third repetition

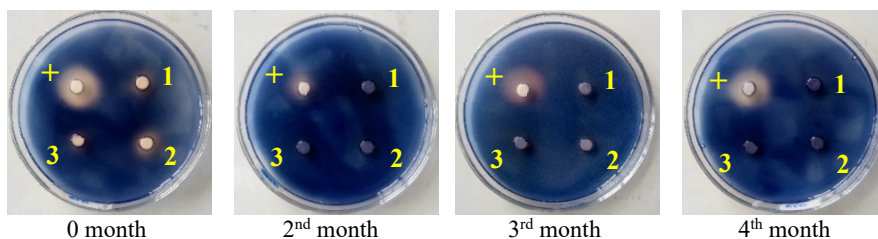


Fig. 2. Amylase activity test result: (+) positive control (Enzyplex); (1) first repetition; (2) second repetition; (3) third repetition

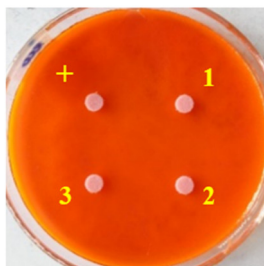


Fig. 3. Lipase activity test result: (+) positive control (Enzyplex); (1) first repetition; (2) second repetition; (3) third repetition

4 Summary

In summary, enzymatic assay of eco enzyme fermented for 2, 3, and 4 months showed positive result toward protease and negative result toward amylase, while lipase activity test did not show any change in the sample disc nor in the positive control.

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