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The Antioxidant Test of Ecoenzymes from Various Fruit Peel Wastes

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Abstract

Indonesia is a country with a large population and rapid industrial growth, facing serious challenges related to waste management. Waste is the remains produced by human activities. One interesting solution to handle the problem is the production of ecoenzymes through the fermentation process of natural organic waste materials. Ecoenzymes are biotechnology products that produce various types of enzymes and organic chemicals that are useful for various applications. Apart from that, laboratory tests also revealed the presence of antioxidant activity in these ecoenzymes, for Ecoenzyme A (IC50 = 13735.9 μg/mL) about 0.4 mL of ecoenzyme solution could inhibit 50% of radical activity, and for Ecoenzyme B (IC50 = 12029.4 μg/mL) about 0.3 mL solution could inhibit 50% of radical activity, and for Ecoenzyme C (IC50 = 15765.1 μg /mL) about 0.4 mL ecoenzyme could inhibit 50% of radical activity, which, although categorized as weak, is because the volume of ecoenzyme produced is very much greater (2 L) than the volume used for antioxidant testing, this product is much more economical and has enormous potential to be developed into cosmetic ingredients and other industrial products.

Keywords: Antioxidants, Ecoenzymes, Fruit Waste

INTRODUCTION

Waste is an unavoidable problem. This is because the rate of waste production is higher than the rate of population growth which is heterogeneous with different household needs patterns (Samadikun et al., 2023). Around 80% of the total amount of waste is organic waste. Organic waste is often considered as residue that can be decomposed by the soil and has no use value whatsoever (Donde, 2017). This causes the accumulation of organic waste, causing environmental pollution, causing disease outbreaks and can disrupt the survival of other organisms. Waste organic can give more impact serious if no quick handled. Management of organic garbage / waste by the community is very minimal because public tend throw it away to land empty, water channel or burned (Eskundari et al., 2023).

Organic waste from the food processing and agricultural industries is one of the environmental challenges that requires effective handling (Loan et al., 2013). The strategy that can be adopted is the utilization of organic waste to produce high-value, environmentally friendly products. One interesting solution is the production of ecoenzymes through the fermentation process of natural organic materials (Tuhumury, Tuahatu, & Manuputty, 2024). Ecoenzymes are biotechnology products that produce various types of enzymes that are useful for various applications. The process of making ecoenzymes does not require a large area, supports the concept of *reuse*, and is relatively inexpensive, while the product is an effective cleaner (Tallei et al., 2023).

Ecoenzymes can be made from several fruit peel wastes. Cavendish banana peel waste (*Musa acuminata*), Medan orange peel (*Citrus sinensin) and Distimake vitifolius* leaves were used in this study to make ecoenzyme A**.** Papaya (*Carica papaya* L.) and pineapple (*Ananas comosus*) fruit , which are widely available and relatively inexpensive, their peels can be easily accessed and used for the production of ecoenzyme B**.** Watermelon peel (*Citrullus lanatus)* and baby java orange peel (*Citrus sinensis* L.) *and D. vitifolius* plant leaves were used to make ecoenzyme C

Ecoenzymes are made from natural ingredients and during the fermentation process contain environmentally friendly microorganisms, so ecoenzymes help reduce the use of hazardous chemicals that can pollute water, air, and soil. In addition, because they are biodegradable, ecoenzymes do not leave harmful residues, thereby reducing the burden of waste and environmental pollution (Tuhumury et al., 2024). The use of ecoenzymes also reduces exposure to hazardous chemicals that can cause skin irritation, respiratory disorders, and other health problems.

Ecoenzymes have many benefits, namely improving soil fertility and its nutrient content, accelerating the decomposition of organic matter, controlling the population of beneficial microbes, and improving overall plant growth and health (Basalingappa, Nataraj, & Thangaraj, 2018). In addition, ecoenzymes can also be used as a substitute for chemical fertilizers, helping to reduce the negative impacts of environmental pollution, creating a more sustainable agricultural environment, household stain remover, and others. This study aims to (1) test the antioxidant ability of ecoenzymes using the DPPH method and (2) carry out practical applications of e coenzymes as cleaners by comparing their performance with commercial cleaning products.

METHODOLOGY

Ecoenzyme production was conducted in Dormitory Building G, Pelita Harapan University for three months. Antioxidant test using UV-Vis spectrophotometer conducted in Chemistry Laboratory, Pelita Harapan University.

Fermenter Tool

The materials provided for designing the fermenter tool are some of the used items such as 5 liter gallons and 1.5 liter bottles. For the tools, use a knife, glue gun and 1 meter hose. To install the hose on the gallon and bottle, a knife is used to make holes in the gallon cap and bottle cap. The installed hose must be glued so that the gas flow can run properly. The gluing is done using a glue gun, and only at the hose entry. This aims to ensure that the cap of the drinking water bottle which is where the fermentation gas is can still be opened easily.

Ecoenzymes Preparation

Before making ecoenzymes, the composition of the materials to be fermented is designed to produce environmentally friendly ecoenzyme products.

Production of Ecoenzyme A

The ingredients used are 300 grams of dissolved brown sugar, Medan orange peel (*Citrus sinensin*), Cavendish banana peel (*Musa acuminata) and D. vitifolius* leaves . The composition ratio used is 1:3:10 for sugar, organic materials, and water. The ratio of fruits and vegetables that are organic materials is 7:3. Water is filled to 60% of the volume of the container used. This composition is good for providing space when microorganisms produce gas in the fermentation process so as to prevent excessive pressure. The manufacturing process carried out is (1) The brown sugar solution is put into a gallon. (2) Orange peel, banana, and *D. vitifolius leaves* are put into the gallon and mixed with the brown sugar solution. (3) After all the ingredients are put in, the gallon is closed with a tight hose condition glued with a glue gun. (4) Fermentation is carried out for 3 months.

Production of Ecoenzyme B

After the tools and materials have been prepared, wash the *D. vitifolius leaves thoroughly* , then cut them into small pieces. Next, also cut the papaya (*Carica papaya* L.) and pineapple (*Ananas comosus*) skin into small pieces. Then the brown sugar is sliced into small pieces and melted. After all the ingredients are ready, put them in a gallon. Add water, fruit skin, and brown sugar in the right ratio, which is 10 parts water, 3 parts fruit skin, and 1 part brown sugar. Next, close the gallon tightly after all the ingredients are put in. Make a hole in the gallon lid and the plastic bottle cap according to the diameter of the hose to be used. The next step is to fill the plastic bottle with water and close it tightly, also make sure the hole matches the hose. Then connect the gallon to the bottle using a hose, make sure it is tightly attached. The last step is to place the gallon and bottle in a safe place and avoid sunlight for an optimal fermentation process for 3 months.

Production of Ecoenzyme C

Prepare the tools and materials needed (Fermentor, watermelon rind (*Citrullus lanatus*) and baby java orange rind (*Citrus sinensis* L.)), *D. vitifolius* plant leaves, brown sugar, and water. Wash all raw materials: watermelon and orange rind and *D. vitifolius* leaves. Weigh the raw materials according to the specified ratio (similar to Ecoenzyme A and B) and cut them into small pieces. Put all raw materials into a container, then add water, fruit skin, and brown sugar in the right ratio, which is 10 parts water, 3 parts fruit skin, and 1 part brown sugar. Make sure all ingredients are submerged in water. Insert the hose into the fermentor, close it tightly and store in a cool and dry place. The last step is to place the gallon and bottle in a safe place and avoid sunlight for an optimal fermentation process for 3 months.

Antioxidant Test With DPPH

Preparation of DPPH Standard Solution

The preparation of DPPH Standard solution was made at a concentration of 0.15 mM and was carried out by weighing 14.79 mg of DPPH powder, then dissolved with ethanol pa (pro analysis) for a final volume of 250 ml. The work was carried out in a place protected from light.

Preparation of Ecoenzyme Solution

Ecoenzyme that has matured during 3 months of fermentation, is filtered using a clean cloth to separate the liquid from the solid. Ecoenzyme liquid is then centrifuged for 5 minutes at a speed of 5000 rpm. The supernatant (ecoenzyme liquid) is taken and then weighed to obtain the density of the ecoenzyme. The pH of the ecoenzyme solution is determined with a pH meter. Furthermore, it is diluted with a ratio of 1: 5 (5 mL ecoenzyme + 25 mL 70% ethanol) then labeled and ready for antioxidant testing.

Antioxidant Test

Ecoenzyme antioxidant test was conducted by varying the ecoenzyme concentration by taking 0.2 mL, 0.4 mL, 0.6 mL, and 0.8 mL of ecoenzyme then adding 70% ethanol to a volume of 2 mL. Next, 3 mL of 0.15 mM DPPH was added, and incubated in the dark for 30 minutes. The blank used was without ecoenzyme. After incubation, the absorbance of the solution was measured at 516 nm (Fajrin, Agustina, & Faizah, 2023).

The % inhibition formula used is:

$$\% \text{ Inhibition} = \frac{\text{Abs blank} - \text{Abs sample}}{\text{Abs blank}} \times 100\% \quad (1)$$

RESULTS AND DISCUSSION

Ecoenzyme Results

After being fermented for 3 months, the ecoenzyme liquid was filtered, density (g/mL) was determined, and the pH of the ecoenzyme was measured. The measurement results are shown in Table 1.

Table 1. pH and density of each Ecoenzyme

Ecoenzyme	Volume (mL)	pH	Density (g/mL)
A	± 2000	3.18	1,0164
B	± 1800	3.35	0.9835
C	± 1800	3.43	0.9705

Ecoenzymes generally have a pH of around 3-5 which tends to be acidic because they are produced from the fermentation of organic materials such as pineapple and papaya peels, oranges, bananas and watermelon peels. With a pH that tends to be acidic, it will support the activity of enzymes contained in ecoenzymes and can help maintain the durability of ecoenzyme products (Tahya et al., 2025). Organic acids are the most important thing in determining acidity, meaning that the higher the organic acid content, the lower the pH value. Thus, ecoenzymes that have a low pH value are caused by high organic acid content (Salsabila et al., 2023) . The results of the Ecoenzyme are shown in Figure 1. The color of the ecoenzyme product is brown with a pungent and alcoholic skin aroma.

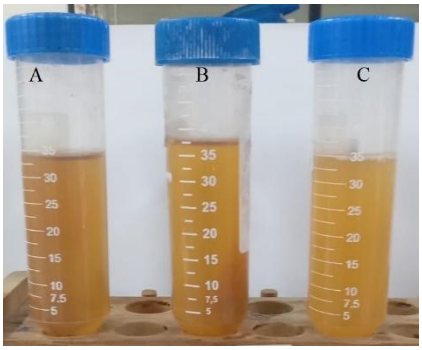

Figure 1. Product Ecoenzymes A, B and C.**2.**

Antioxidant Test Results

The results of the antioxidant test are shown in Tables 2, 3 and 4, and Figures 2, 3 and 4. The antioxidant activity of ecoenzymes produced from fruit peel waste was evaluated using the DPPH method, resulting in IC_{50} values of 13,735.9 μg/mL for Ecoenzyme A, 12,029.4 μg/mL for Ecoenzyme B, and 15,765.1 μg/mL for Ecoenzyme C. These values indicate weak antioxidant activity according to standard classifications, which typically consider IC_{50} values below 100 μg/mL as strong antioxidants (Purwaningsih et al., 2023). Among the three, Ecoenzyme B exhibited the highest antioxidant potential, likely due to the presence of papaya and pineapple peels, which are known to contain phenolic compounds and vitamin C contributing to radical scavenging (Bhowmik et al., 2013). Although the activity is relatively low, the large volume of ecoenzyme produced (approximately 2 L per batch) makes it economically viable for applications in cleaning and cosmetic formulations (Tahya et al., 2025).

Previous studies have reported similar trends in antioxidant activity for ecoenzymes derived from mixed fruit peels. Tallei et al., (2023) demonstrated that ecoenzymes from pineapple and papaya waste exhibited better antioxidant activity, supporting the superior performance of

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Ecoenzyme B. These findings suggest that the type of fruit peel used significantly influences antioxidant capacity, primarily due to variations in phenolic and flavonoid content.

When compared to conventional plant extracts, the antioxidant activity of ecoenzymes is considerably lower. For example, green tea extract and citrus peel extracts typically exhibit IC_{50} values below 50 µg/mL, indicating strong radical scavenging potential. This disparity can be attributed to the dilution effect and fermentation process in ecoenzyme production, which may degrade some bioactive compounds. However, ecoenzymes offer additional benefits such as biodegradability, cost-effectiveness, and multifunctionality, making them attractive for sustainable applications despite their weaker antioxidant performance.

Table 2. Results of antioxidant activity test using DPPH method for Ecoenzyme A

Volume Ecoenzyme (mL)	Concentration Ecoenzyme (µg /mL)	Abs1	Abs2	Abs3	Average abs	SD	% Inhibition	IC_{50} (µg /mL)
0	0	0.927	0.942	0.93	0.9330	0.0079	0	
0.2	6776	0.624	0.668	0.616	0.6360	0.0280	31.83	
0.4	13552	0.38	0.449	0.432	0.4203	0.0359	54.95	13735.9
0.6	20328	0.217	0.217	0.265	0.2330	0.0277	75.03	
0.8	27104	0.106	0.108	0.111	0.1083	0.0025	88.39	

Table 3. Results of the DPPH method of antioxidant activity testing for Ecoenzyme B

Volume Ecoenzyme (mL)	Concentration Ecoenzyme (µg /mL)	Abs1	Abs2	Abs3	Average abs	SD	% Inhibition	IC_{50} (µg /mL)
0	0	0.924	0.937	0.93	0.9303	0.0065	0	
0.2	6556.67	0.576	0.575	0.589	0.5800	0.0078	37.65	12029.4
0.4	13113.33	0.351	0.332	0.34	0.3410	0.0095	63.35	
0.6	19670.00	0.185	0.185	0.177	0.1823	0.0046	80.40	
0.8	26226.67	0.114	0.119	0.126	0.1197	0.0060	87.14	

Table 4. Results of antioxidant activity test using DPPH method for Ecoenzyme C

Volume Ecoenzyme (mL)	Concentration Ecoenzyme (µg /mL)	Abs1	Abs2	Abs3	Average abs	SD	% Inhibition	IC_{50} (µg /mL)
0	0	0.91	0.92	0.915	0.9150	0.0050	0	
0.2	6470	0.652	0.666	0.67	0.6627	0.0095	27.58	15765.1
0.4	12940	0.489	0.478	0.485	0.4840	0.0056	47.10	
0.6	19410	0.339	0.337	0.34	0.3387	0.0015	62.99	
0.8	25880	0.244	0.25	0.248	0.2473	0.0031	72.97	

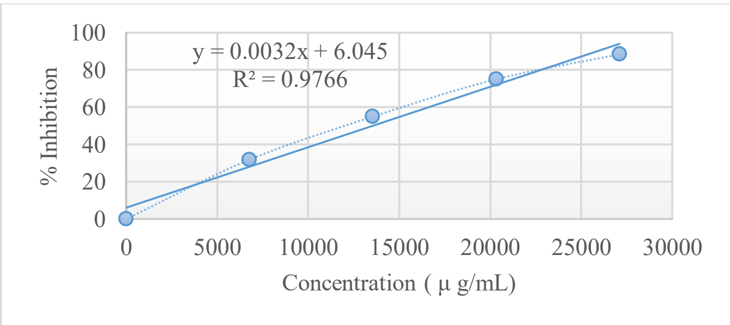

Figure 2. DPPH inhibition by Ecoenzyme A

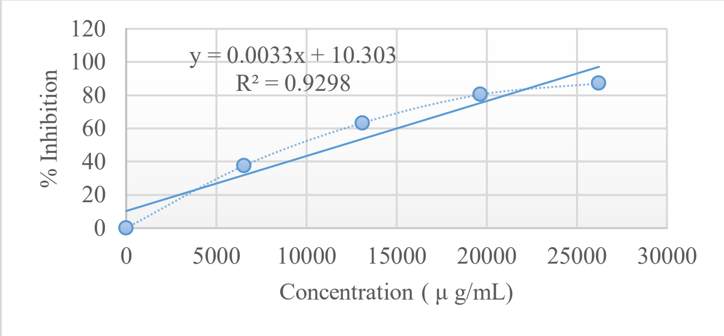

Figure 3. DPPH inhibition by Ecoenzyme B.

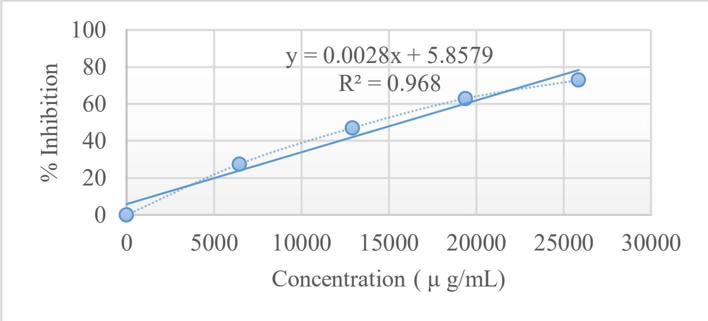

Figure 4. DPPH inhibition by Ecoenzyme C

Although our ecoenzymes product show weak antioxidant activity compared to pure extracts, their large-scale production and environmental benefits position them as promising candidates for non-critical antioxidant applications, such as household cleaners and cosmetic bases. Future research should focus on optimizing fermentation conditions to enhance phenolic retention and exploring enrichment strategies using high-antioxidant plant materials. Additionally, integrating ecoenzymes with other natural antioxidants could improve their efficacy for industrial use. This approach aligns with global efforts to reduce chemical waste and promote circular economy principles in waste management (Marzuki, Lestari, & Putra, 2022).

CONCLUSION

Ecoenzyme can be produced as a useful and environmentally friendly product. The ecoenzyme produced functions effectively as an environmentally friendly cleaner, producing a similar level of cleanliness close to that obtained from using commercial soap/detergent/cleaner. In addition, laboratory tests also revealed the presence of antioxidant activity in the ecoenzymes, namely Ecoenzyme A (IC_{50} = 13735.9 µg/mL), Ecoenzyme B (IC_{50} = 12029.4 µg/mL), and Ecoenzyme C (IC_{50} = 15765.1 µg/mL) which although categorized as weak, but because the volume of ecoenzymes produced is very large, much larger than the volume used for antioxidant tests, this product is much more economical and has great potential to be developed into cosmetic ingredients or other industrial products. The simple process of making ecoenzymes and their widespread use in everyday life show that solutions to environmental problems are easier than we think. Awareness of the importance of responsible and sustainable waste management.

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