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Characterization and Enzymatic Evaluation of Ecoenzyme Derived from Fruit and Vegetable Waste: An Effort to Achieve Zero Waste Concept

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RESEARCH ARTICLE

Characterization and Enzymatic Evaluation of Ecoenzyme Derived from Fruit and Vegetable Waste: An Effort to Achieve Zero Waste Concept

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ABSTRACT

Food waste has accounted for 44% of the total amount of waste produced in Indonesia. From that total, fruits and vegetables waste constitute the main dominant contributors. Production of ecoenzyme has the potential to reduce the environmental waste problem and convert as well the waste into valuable products. This study was employed to characterize and evaluate the enzymatic activity of ecoenzymes produced from the fruit and vegetable waste. The fruits used to produce these ecoenzymes were pineapple, avocado, and orange, while the vegetables-ecoenzymes were generated from lettuce, Chinese cabbage, and broccoli. The ecoenzyme was produced through a fermentation process for up to 3 months with the formulation of the ecoenzyme is molasses, fruit or vegetable waste, and water in a ratio of 1:3:10. Characteristics of the ecoenzyme products were determined by observing the pH, color, odor, and enzymatic activities. The results showed both fruit- and vegetable-ecoenzyme has decreasing acidic pH compared to the initial fermentation, except for avocado-ecoenzyme. The color of ecoenzymes was turned to reddish-brown after 6–12 weeks of fermentations. The sour and fresh smells were detected for ecoenzyme derived from vegetables and fruit, respectively. Enzyme activity assay revealed that all generated ecoenzymes contain protease, amylase, and lipase. The highest enzymatic activity was displayed for avocado-ecoenzyme with protease 0.38 U/mL, amylase activity of 8.30 U/mL, and lipase 3.16 U/mL. The results indicated that the fermentation process from fruit and vegetables was successful. Recycle and reusing natural waste for ecoenzyme productions provide eco-friendly and economical multi-purpose application products.

Keywords: Ecoenzyme, Enzymatic activity, Fermentation, Fruit waste, Vegetable waste

Introduction

The increase in world population in the era of global industrialization has led to a crisis of environmental pollution.¹ The growing world population will eventually need more food, including fruits, vegetables, and grains, to meet demand. Total fruit and vegetable production in the world is on an increasing

trend, at the same time many types of vegetables and fruits are wasted and become waste. This is also true in Indonesia, according to the Indonesian Ministry of Environment and Forestry (KLHK), in 2018, up to 44% of Indonesia was considered the second largest producer of food loss and waste (FLW) in the world. The study reveals that the dominant domestic waste in Indonesia is contributed by food waste. Due to this

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reason, Indonesia has committed to handling 70% of this waste by 2025.² Inadequate storage facilities cause the accumulation and sometimes ineffective transportation, fruit and vegetable waste is produced in large quantities daily. The high production of fruit and vegetable wastes becomes a major threat to the environment and ecosystem.³ In addition, the waste also can be generated from many parts of fruits and vegetables that are not used such as leaves, stems, seeds, and skin. Much of this waste is dumped on the ground to rot outdoors. The smell and grime of rotting waste also disturb the area. Improper disposal of fruit and vegetable waste will negatively affect the environment. The decomposition of this waste is a big problem due to the production of greenhouse gases such as methane and nitrous oxide.⁴ In addition, landfills with large amounts of food waste have generated leachates and released antibiotic resistance bacteria (ARB) that are harmful to land.⁵

Many studies have been carried out to overcome the issue of fruit and vegetable waste. The effort was explored to realized the zero waste by making the waste of fruits and vegetables into biogas⁶, creating liquid organic fertilizer^{7,8}, utilizing waste as feed for larva black soldier fly⁹, and the most recent effort is converting those waste into ecoenzymes.¹⁰ Ecoenzyme is expected containing many biological catalytic molecules that can be further utilize to produce different essential products.¹¹ Production ecoenzymes become one of the best approaches to helping the natural cycle. The procedure for making ecoenzymes is also simple and easy to do because is not require to add starter bacteria to facilitate the fermentation process, but release the multi-purpose products. Amylase, protease, and lipase enzymes available in the ecoenzyme can be used in the processing of mud aquaculture^{12,13}, and as fertilizer in plants.^{14,15} Those enzymes are examples of the hydrolytic enzymes that are capable to recycle nutrients as well as degrading organic compounds and minerals.¹⁶

Furthermore, ecoenzymes can be used as anti-acne¹⁷, microbial fuel cell as a new potential of renewable energy¹⁸, treatment of greywater¹³, treatment of dairy wastewater¹⁹ etc. Ecoenzymes have many advantages and are one of the alternatives to produce zero waste.²⁰ Not only that, ecological enzymes can be used as an alternative source of enzymes that are potential to be used for many applications.²¹ Therefore, this current research aims to produce the ecoenzyme from fruit and vegetables waste as an effort to reduce the waste in the environment and evaluate its characteristics for further applications. Vegetables and fruits are the best sources to produce ecoenzyme since they contain rich of organic acids.

Materials and methods

Production of ecoenzyme

The production of ecoenzyme was carried out by referring to previous study of Galintin et al.¹² with some modifications. Ecoenzymes were produced with 8 variations from different sources of the raw materials both single and mixture. Part of vegetables used in this current work is varied: the lettuce waste derived from the stems and leaves; white cabbage waste generated from outer leaves and stems, broccoli waste used in the form of leaves and stems, and vegetable mix (a mixture of lettuce, white cabbage, and broccoli waste). Production of fruits-ecoenzyme utilized meat scraps, skin, stems, and leaves of pineapple; using seeds and skin for avocado and oranges; and fruit mix (a mixture of pineapple, avocado, and orange waste). The initial step is cutting the fruit and vegetable waste into smaller pieces with the total weighing of 3 kg and putting the smaller pieces into a container. Into the container was added one kg of molasses and 10 kg of water and homogenized the mixture by stirring. The fermentation process was running by tightly closed the container and stored it a room temperature and without any light. The lid of the container was opened every day in the first month of the fermentation process. After 3 months, the ecoenzyme was harvested and filtered to obtain the ecoenzyme liquid.

Characterization of ecoenzyme

Characteristics of the ecoenzyme products were analyzed by measuring the pH value, color, and aroma. The pH of the ecoenzymes products was measured using a pH indicator strip and pH meter.¹² The color and aroma of ecoenzymes were evaluating by conducting an organoleptic test.^{22,23}

Amylase activity

The amylase activity was determined by referring to the method described by Arun et al.²⁴, with slight modification. The ecoenzyme was centrifugated at 10,000 rpm prior to use in the amylase activity assay. A 0.5 mL of 1% starch solution was added to 0.5 mL of ecoenzyme extract and the mixture was allowed to incubate at 25°C for 10 minutes. After that, the mixture was added to a flask containing 1 mL of DNS and was allow to incubate in the boiling water in a water bath for 5 minutes. The mixture was then allowed to cool at room temperature and continued for amylase activity by observing its absorbance at 540 nm using UV-Vis spectrophotometer. All assays were carried out in three replicates.

Table 1. pH of ecoenzymes from vegetable waste.

No	Vegetable type	pH week 5–12	Final pH
1	Lettuce	4	3.62
2	Chinese cabbage	4	3.58
3	Broccoli	4	3.58
4	Mix Vegetables	4	3.94

The values are expressed as the arithmetic means \pm SD. The significance differences among various groups were evaluated by one-way analysis variance (ANOVA) followed by the Turkey HSD test. The statistical significances were considered to be significant when $P < 0.05$. All statistics data were analyzed using the SPSS version.

Protease activity

The protease activity was evaluated following the method described by Arun and Sivashanugam²⁵ with some modifications. The ecoenzyme was centrifuged at 10,000 rpm before use in the protease activity assay. A 1 mL of 1% casein solution is mixed with 1 ml of ecoenzyme and continued to incubate at 37°C for 10 minutes in water bath. The reaction was terminated by adding 5 ml of 5% trichloroacetic acid (TCA) and heating continuously at 70°C for additional 30 minutes. The mixture was centrifuged at 3000 rpm for 10 minutes and supernatant was collected for further analysis. A1 mL of supernatant is added to 5.5 mL solution (50 parts Na₂CO₃ 2% in 0.1 N NaOH and 1 part 0.5% CuSO₄) and 0.5 mL of Folin–Ciocalteu reagent. The mixture was incubated for 30 minutes. The protease activity was observed at a wavelength of 660 nm. All assays were performed in three replicates. The values are expressed as the arithmetic means \pm SD. The significance differences among various groups were evaluated by one-way analysis variance (ANOVA) followed by the Turkey HSD test. The statistical significances were considered to be significant when $P < 0.05$. All statistics data were analyzed using SPSS version 25.

Lipase activity

The lipase activity was tested by referring the method described by Mardina et al.²⁶ with slight modifications. The ecoenzyme was centrifuged at 10,000 rpm prior to use in the enzyme activity test. A 9.5 mL substrate solution (1 part 3.0 mM p-NPP in 2 propanols with 9 parts 0.4% Triton X100 and 0.1% Arabic gum) was added to 0.5 mL ecoenzyme solution and the mixture was then incubated at 37°C for 20 minutes. The lipase activity was assessed by

observing its absorbance at 410 nm (26). All assays were carried out in three replicates. The values are expressed as the arithmetic means \pm SD. The significance differences among various groups were evaluated by one-way analysis variance (ANOVA) followed by the Turkey HSD test. The statistical significances were considered to be significant when $P < 0.05$. All statistics data were analyzed using SPSS version 25.

Results and discussion

Characteristics of ecoenzyme from fruit and vegetable waste

The characteristics of ecoenzymes were evaluated by analyzing physicochemical parameters of pH, Chemical Oxygen Demand (COD), Biological oxygen demand (BOD),^{27,28} Total Dissolve Solid (TDS), Total Soluble Solid (TSS),²⁹ Total Acid,³⁰ Aroma, and Color.³¹

The pH is the most common parameter used in characterizing ecoenzymes because a good ecoenzyme has a lower-power of hydrogen (pH) of 4 or less.³² Table 1 showed that the production of ecoenzymes derived from vegetable waste have acidic pH. The results of this study were in line with previous study conducted by Rusdianasari, et al.³² which found that the pH value of the ecoenzyme changed to acidic in the week of 5–12 and reached to their final pH of 3.58–3.94. It means that starting from week of 5, the alcohol generated from the fermentation process has converted into acetic acids. The lower pH of the single vegetables-ecoenzymes indicates that the higher conversion of alcohol in the single-vegetables than those of mix-vegetables.

Table 2 showed the pH measurements of the ecoenzymes products derived from fruit waste. The results showed that almost all of the single fruit-based ecoenzymes and mixfruits-based have acidic pH value, except avocado-based ecoenzymes. The ecoenzymes of avocado waste have a higher pH than those of pineapple and orange waste because probably containing more organic acids. Organic acids derived from pineapple and orange waste will be extracted during the fermentation process. One such organic acid is ascorbic acid. Pineapples and oranges have been known to be fruits high in ascorbic acid.²³ That's what makes the ecoenzymes of pineapple and orange waste have a more acidic pH. This is also due to the contribution of the organic acid content of pineapples and oranges. The results of this study are consistent with the research from Rusdianasari, et al.³² that the pH produced is acidic.

Table 2. pH of ecoenzymes from fruit waste.

No	Fruit type	pH week 5-9	pH week 10-12	Final pH
1	Pineapple	4	3-4	3.54
2	Avocado	4	3-4	4.13
3	Orange	4	3-4	3.59
4	Mix Fruits	4	3-4	3.52

Color characteristics of ecoenzyme from fruit and vegetable waste

Color is a parameter commonly used to characterize ecoenzymes. Ecoenzyme has a reddish-brown color. The reddish brown comes from the sugar used. In this research, the ecoenzyme was made using molasses so that the resulting color was reddish brown. The dark brown in this molasses comes from caramelized sucrose so it has a blackish brown color.

Table 3 shows that ecoenzymes derived from vegetable waste all have a reddish-brown color. All of the ecoenzymes made from vegetable waste are black when they first start to produce. The color then turns brown and finally reddish brown. This result is in line with a study conducted by Rijal (2022)³³ which found that ecoenzymes produced are a reddish-brown liquid. The color shift to reddish brown happened more quickly in the ecoenzyme of Chinese cabbage and broccoli waste, happening in the sixth week, followed by the ecoenzyme of mixed vegetables in the seventh week and the lettuce ecoenzyme in the eighth week. Vegetable waste's extracted tannin content may also be the reason for the brown color.

Table 4 shows the color changes of the ecoenzyme products produced from fruits. Ecoenzymes from fruit waste all have a reddish-brown color. This is in accordance with studies done by Rijal.³³ The reddish-brown color can come from the tannins contained in fruit waste. The ecoenzymes derived from fruit waste make a transformation from black to brown to reddish brown within a week. The ecoenzymes of pineapple, avocado and mixed fruit waste experienced a change from brown to reddish brown in the 6th week, while the ecoenzymes of orange waste changed in the 7th week.

Aroma characteristics of ecoenzyme from fruit and vegetable waste

One of the factors that's frequently utilized to characterization an ecoenzyme's quality is aroma. Good ecoenzymes will have a sour aroma.³¹ The sour aroma is obtained from fermentation in the form of acetic acid and organic acids extracted from fruit and vegetable waste.

Table 5 resumes the aroma of the vegetable-ecoenzymes products. Ecoenzymes from vegetable

waste have a sour aroma. This indicates that the ecoenzyme produced from vegetable waste is a good ecoenzyme.³¹ In the ecoenzymes from broccoli waste and mixed vegetables, a sour aroma was obtained in the 9th week faster than in the ecoenzymes from lettuce and chinese cabbage waste. This indicates that the fermentation results from the ecoenzymes of broccoli and mixed vegetables are formed more quickly than the ecoenzymes from lettuce and chinese cabbage.

Ecoenzymes from fruit waste have a fresh sour aroma as displayed in Table 6. The fresh aroma comes from the esters found in fruit which are used as a source of ecoenzymes. This indicates that the ecoenzyme produced from fruit waste is good. In the ecoenzyme from avocado waste, a sour aroma was obtained in the 6th week faster than in the ecoenzyme from pineapple, orange, and mixed fruit waste. This indicates that the fermentation results in avocado ecoenzymes are formed more quickly than in pineapple, orange, and mixed fruit ecoenzymes. The fresh sour aroma of fruit waste ecoenzymes is following research on the aroma of ecoenzymes from fruit peels.³⁴

Enzymatic activity of ecoenzyme from fruits and vegetables waste

Amylase activity of ecoenzyme from fruits and vegetables waste

The activity of amylase was assessed for both ecoenzyme products from vegetables and fruits. The activities of amylases of vegetables waste-ecoenzymes were shown in Fig. 1. The Chinese cabbage waste ecoenzymes has the lowest amylase activity (6.58 U/mL) compared to broccoli waste ecoenzymes (6.61 U/mL) and lettuce waste ecoenzymes (7.43 U/mL), but higher than that of mix vegetables-ecoenzyme. Table 7 shows the comparable results of the carbohydrate content in each vegetable or fruits. Data shown in Table 7 shows that the amylase activity comparable to the lower content of carbohydrate in the Chinese cabbage waste than those of broccoli waste and lettuce. However, an interesting thing was found that the lettuce waste ecoenzyme had higher activity compared to the broccoli waste ecoenzyme even though the carbohydrate content in lettuce was lower. This can be caused by the presence of contaminants during the ecoenzyme production process. During the production of ecoenzymes from lettuce waste, black fungi was found to grow as can be seen in Fig. 1. Contaminants in the form of fungi can also secrete amylase enzymes into the ecoenzyme fluid so that the activity of the lettuce waste ecoenzyme is greater than that of the broccoli waste ecoenzyme.

The results of one-way ANOVA showed that the amylase activity was significantly different between

Table 3. Color of ecoenzymes from vegetables waste.

Weeks	Vegetable's type			
	Lettuce	Chinese cabbage	Broccoli	Mix Vegetables
1	Black	Black	Black	Black
2	Brownish	Brownish	Brownish	Brownish
3	Brownish	Brownish	Brownish	Brownish
4	Brownish	Brownish	Brownish	Brownish
5	Brownish	Brownish	Brownish	Brownish
6	Brownish	Reddish brown	Reddish brown	Brownish
7	Brownish	Reddish brown	Reddish brown	Reddish brown
8	Reddish brown	Reddish brown	Reddish brown	Reddish brown
9	Reddish brown	Reddish brown	Reddish brown	Reddish brown
10	Reddish brown	Reddish brown	Reddish brown	Reddish brown
11	Reddish brown	Reddish brown	Reddish brown	Reddish brown
12	Reddish brown	Reddish brown	Reddish brown	Reddish brown

Table 4. Color of ecoenzymes from fruits waste.

Weeks	Fruits type			
	Pineapple	Avocado	Orange	Mix fruit
1	Black	Black	Black	Black
2	Brownish	Brownish	Brownish	Brownish
3	Brownish	Brownish	Brownish	Brownish
4	Brownish	Brownish	Brownish	Brownish
5	Brownish	Brownish	Brownish	Brownish
6	Reddish brown	Reddish brown	Brownish	Reddish brown
7	Reddish brown	Reddish brown	Reddish brown	Reddish brown
8	Reddish brown	Reddish brown	Reddish brown	Reddish brown
9	Reddish brown	Reddish brown	Reddish brown	Reddish brown
10	Reddish brown	Reddish brown	Reddish brown	Reddish brown
11	Reddish brown	Reddish brown	Reddish brown	Reddish brown
12	Reddish brown	Reddish brown	Reddish brown	Reddish brown

Table 5. The aroma of ecoenzymes from vegetable waste.

Weeks	Vegetable's type			
	Lettuce	Chinese cabbage	Broccoli	Mix Vegetables
1	Molasses	Aroma of chinese cabbage and molasses	Aroma of broccoli and molasses	Molasses
2	Aroma of vegetables and molasses	Aroma of chinese cabbage and molasses	Aroma of broccoli and molasses	Aroma of vegetables and molasses
3	Aroma of vegetables and molasses	Aroma of chinese cabbage and molasses	Aroma of broccoli and molasses	Aroma of vegetables and molasses
4	Aroma of vegetables and molasses	Aroma of chinese cabbage and molasses	Aroma of broccoli and molasses	Aroma of vegetables and molasses
5	Aroma of vegetables	Aroma of chinese cabbage	Aroma of broccoli	Aroma of vegetables
6	Aroma of vegetables	Aroma of chinese cabbage	Aroma of broccoli	Aroma of vegetables
7	Aroma of vegetables	Aroma of chinese cabbage	Aroma of broccoli	Aroma of vegetables
8	Aroma of vegetables	Aroma of chinese cabbage	Aroma of broccoli	Aroma of vegetables and sour
9	Aroma of vegetables and acid	Aroma of chinese cabbage and sour	Sour aroma	Sour aroma
10	Aroma of vegetables and sour	Aroma of chinese cabbage and sour	Sour aroma	Sour aroma
11	Aroma of vegetables and acid	Aroma of chinese cabbage and sour	Sour aroma	Sour aroma
12	Sour aroma	Sour aroma	Sour aroma	Sour aroma

Table 6. Aroma of ecoenzymes fruits waste.

Weeks	Vegetable's type			
	Pineapple	Avocado	Orange	Mix Fruits
1	Aroma of pineapple and molasses	Aroma of molasses	Aroma of orange and molasses	Aroma of Mix fruits and molasses
2	Aroma of pineapple and molasses	Aroma of molasses	Aroma of orange and molasses	Aroma of Mix fruits and molasses
3	Aroma of pineapple and molasses	Aroma of molasses	Aroma of orange and molasses	Aroma of Mix fruits and molasses
4	Aroma of pineapple and molasses	Aroma of molasses	Aroma of orange and molasses	Aroma of Mix fruits and molasses
5	Aroma of pineapple and sour	Slightly sour aroma	Aroma of orange and sour	Aroma of Mix fruits and sour
6	Aroma of pineapple and sour	Sour aroma	Aroma of orange and sour	Aroma of Mix fruits and sour
7	Aroma of pineapple and sour	Sour aroma	Aroma of orange and sour	Aroma of Mix fruits and sour
8	Aroma of pineapple and sour	Sour aroma	Aroma of orange and sour	Aroma of Mix fruits and sour
9	Sour aroma	Sour aroma	Sour aroma	Sour aroma
10	Sour aroma	Sour aroma	Sour aroma	Sour aroma
11	Sour aroma	Sour aroma	Sour aroma	Sour aroma
12	Sour aroma	Sour aroma	Sour aroma	Sour aroma

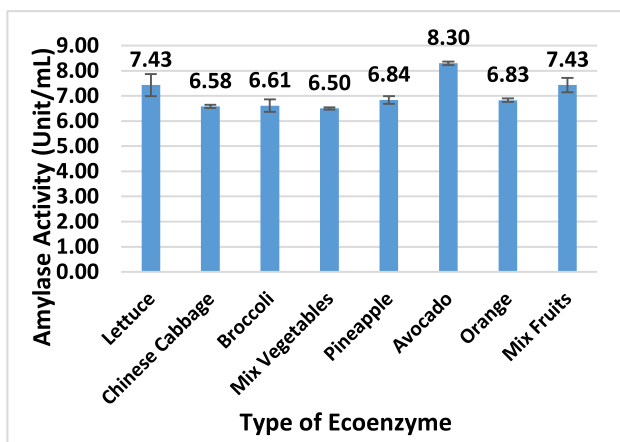


Fig. 1. Amylase activity of ecoenzyme.



Fig. 2. Black fungi.

the eight ecoenzymes-product ($P < 0.05$). The Turkey HSD test showed that the amylase activity of lettuce waste-ecoenzyme was significantly different with other three ecoenzymes derived from Chinese cabbage, broccoli, and mix-vegetables.

The amylase activity from fruits-based were also compared. The results displayed in Fig. 1 shows that avocado waste ecoenzymes have the highest amylase activity (8.30 U/mL) compared to pineapple waste ecoenzymes (6.84 U/mL) and orange waste ecoenzymes (6.83 U/mL), This is comparable if we

Table 7. Carbohydrates content in fruits and vegetables.

No	Vegetable type	Carbohydrates (/100 g)
1	Lettuce ³⁵	2.87 g
2	Chinese cabbage ³⁵	2.18 g
3	Broccoli ³⁵	6.27 g
4	Pineapple ³⁵	13.1 g
5	Avocado ³⁶	64.9 g
6	Orange ³⁵	11.75 g

look at the carbohydrate content in avocado waste which is higher than in pineapple and orange waste. These results correlated with a higher carbohydrate content that can cause bacteria to secrete more amylase enzymes so that the activity of the amylase enzyme will also increase. In the mix fruit ecoenzyme, the activity value obtained was (7.43 U/mL), which means the value is relatively between the avocado waste ecoenzyme and the pineapple and orange waste ecoenzyme. The result of Turkey HSD test showed that the amylase activity of avocado waste-ecoenzyme was significantly different from other three ecoenzymes derived from pineapple, orange, and mix-fruits waste.

Protease activity of ecoenzyme from fruits and vegetables waste

The results of one-way ANOVA showed that on average the protease activity was significantly different between the eight ecoenzymes-products ($P < 0.05$). The Turkey HSD test showed that the protease activity of Chinese cabbage waste-ecoenzyme was not significantly lettuce-ecoenzyme, but significantly different with broccoli, and mix-vegetables as shown in Fig. 3. In contrast, ecoenzymes from vegetable waste, chinese cabbage waste ecoenzymes have the lowest protease activity (0.26 U/mL) compared to broccoli waste ecoenzymes (0.29 U/mL) and mix vegetable-waste ecoenzymes (0.31 U/mL). This is comparable if we look at the protein content of Chinese cabbage

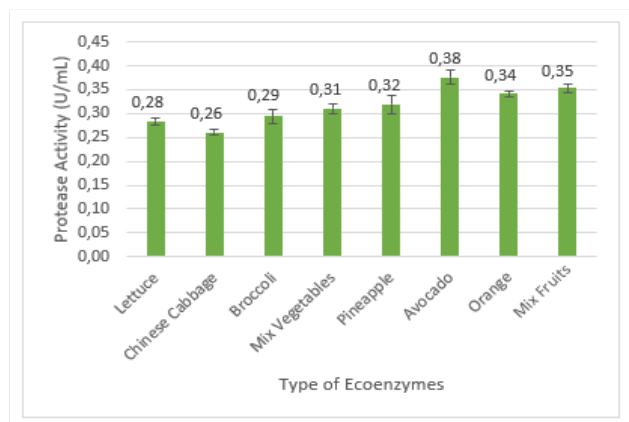


Fig. 3. Protease activity of ecoenzyme.

Table 8. Proteins content in fruits and vegetables.

No	Vegetable type	Proteins (/100 g)
1	Lettuce ³⁵	1.36 g
2	Chinese cabbage ³⁵	1.50 g
3	Broccoli ³⁵	2.57 g
4	Pineapple ³⁵	0.54 g
5	Avocado ³⁶	7.75 g
6	Orange ³⁵	1.50 G

waste as shown in Table 8, which is lower than broccoli waste. Lower protein content will result in lower secretion of protease enzymes by bacteria as well.

In ecoenzymes from fruit waste, the Turkey HSD test performed that avocado waste ecoenzymes have the highest protease activity (0.38 U/mL) compared to pineapple waste ecoenzymes (0.32 U/mL) and orange waste ecoenzymes (0.34 U/mL), but not significantly different with ecoenzyme derived from mix-fruits. This is comparable if we look at the protein content in avocado waste as has been resumed in Table 8, which is higher than in pineapple waste and oranges. Higher protein content will cause bacteria to secrete more protease enzymes so that protease enzyme activity will also increase. The pineapple waste enzyme ecoenzyme has the lowest protease activity value among the ecoenzymes originating from fruit waste because the protein content of pineapple is the lowest among other fruit waste even though pineapple is known to contain the enzyme bromelain. In the mix fruit ecoenzyme, the activity value obtained was (0.35 U/mL), which means the value is relatively between the avocado waste ecoenzyme and the pineapple and orange waste ecoenzyme.

Lipase activity of ecoenzyme from fruits and vegetables waste

The results of one-way ANOVA showed that the lipase activity was significantly different between the

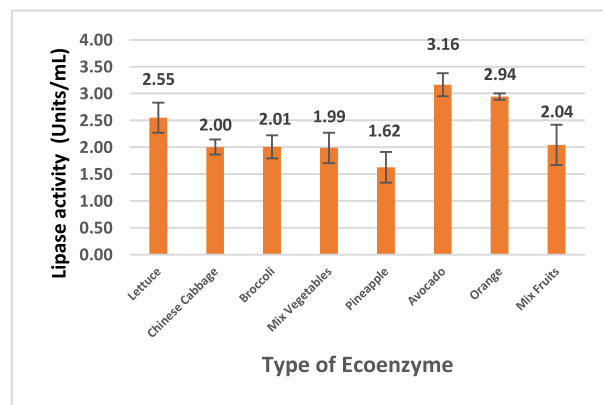


Fig. 4. Lipase activity of ecoenzyme.

Table 9. Lipids content in fruits and vegetables.

No	Vegetable type	Lipids (/100 g)
1	Lettuce ³⁵	0.15 g
2	Chinese cabbage ³⁵	0.20 g
3	Broccoli ³⁵	0.34 g
4	Pineapple ³⁵	0.12 g
5	Avocado ³⁶	3.90 g
6	Orange ³⁵	0.31 g

eight ecoenzymes-product ($P < 0.05$). The Turkey HSD output showed no significantly different of the lipase activities among four vegetables-based ecoenzymes products. The significant different of lipase activity was found in ecoenzymes produced from fruit waste and figured out in Fig. 4 The avocado waste ecoenzymes have the highest lipase activity (3.16 U/mL) compared to pineapple waste ecoenzymes (1.62 U/mL) and fruit mix (2.04 U/mL), but was not significant with orange waste ecoenzymes (2.94 U/mL). This is comparable if we look at the lipid content in avocado waste as has been resumed in Table 9, which has a higher content compared to pineapple waste and oranges. A higher lipid content will cause bacteria to secrete more lipase enzymes so that lipase enzyme activity will also increase. This is also proven by the lowest lipase activity in pineapple because the lipid content in pineapple is the lowest.

Conclusion

Ecoenzymes are known as magic liquid since it has multifunctional in diverse applications. Ecoenzyme from fruits waste and vegetables waste has the characteristics of a reddish-brown color, acidic pH, sour aroma for vegetable waste ecoenzymes, and fresh sour aroma for fruit waste. The highest enzymatic activity was found in avocado waste ecoenzymes, with activities: Amylase (8.30 U/mL), Protease

(0.38 U/mL), and Lipase (3.16 U/mL). Ecoenzyme production provides alternative approach for achieving the zero-waste framework by converting waste material to valuable things. The multi-purposes of ecoenzymes give economic benefit. The success of the ecoenzymes production is significantly impacted by raw material for the fermentation process, sugar sources, and water.

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Authors' declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for republication, which is attached to the manuscript.
- No animal studies are present in the manuscript.
- No human studies are present in the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at Universitas Pendidikan Indonesia, Bandung, Indonesia.

Authors' contribution statement

G.G.: supervision, methodology, writing – original draft. A.K.: project administration, supervision, validation. M.F.F.: investigation, formal analysis, writing – original draft. N.: resources, supervision, data curation. F.M.T.S.: supervision, resources. H.S.H.M.: funding acquisition, conceptualization, writing – original draft.

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توصيف والتقييم الانزيمي للانزيمات الصديقة للبيئة المشتقة من مخلفات الفاكه والخضراوات : لتحقيق توازن بيئي مع صفر للنفايات

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الخلاصة

يمثل هدر الطعام 44% من إجمالي كمية النفايات المنتجة في إندونيسيا. ومن هذا المجموع، تشكل نفايات الفواكه والخضراوات المساهم الرئيسي المهيمن. إن إنتاج الإنزيم البيئي لديه القدرة على تقليل مشكلة النفايات البيئية وتحويل النفايات أيضًا إلى منتجات ذات قيمة. استخدمت هذه الدراسة لتوصيف وتقييم النشاط الأنزيمي للإنزيمات البيئية المنتجة من مخلفات الفواكه والخضراوات. وكانت الفواكه المستخدمة لإنتاج هذه الإنزيمات البيئية هي الأناناس والأفوكادو والبرتقال، في حين تم إنتاج الإنزيمات البيئية النباتية من الخس والملفوف الصيني والقرنبيط. تم إنتاج الإنزيم البيئي من خلال عملية تخمير لمدة تصل إلى 3 أشهر بتركيب الإنزيم البيئي عبارة عن دبس السكر ومخلفات الفاكهة أو الخضار والماء بنسبة 1:3:10. تم تحديد خصائص منتجات الإنزيم البيئي من خلال مراقبة درجة الحموضة واللون والرائحة والأنشطة الأنزيمية. أظهرت النتائج أن الإنزيم البيئي للفواكه والخضراوات قد انخفض في درجة الحموضة الحمضية مقارنة بالتخمير الأولي، باستثناء الإنزيم البيئي للأفوكادو. تحول لون الإنزيمات البيئية إلى اللون البني المحمر بعد 6-12 أسبوع من التخمير. تم الكشف عن الروائح الحامضة والطازجة للإنزيم البيئي المشتق من الخضار والفاكهة، على التوالي. كشف اختبار نشاط الإنزيم أن جميع الإنزيمات البيئية المولدة تحتوي على البروتياز، الأميليز، والليباز. تم عرض أعلى نشاط إنزيمي للإنزيم البيئي الأفوكادو مع البروتياز 0.38 وحدة / مل، ونشاط الأميليز 8.30 وحدة / مل، والليباز 3.16 وحدة / مل. أشارت النتائج إلى أن عملية التخمير من الفواكه والخضراوات كانت ناجحة. توفر إعادة تدوير النفايات الطبيعية وإعادة استخدامها لإنتاج الإنزيمات البيئية منتجات تطبيقية متعددة الأغراض صديقة للبيئة واقتصادية.

الكلمات المفتاحية: الإنزيمات الصديقة للبيئة، الفعالية الأنزيمية، التخمير، مخلفات الفاكهة، مخلفات الخضراوات.