Published Online First: December, 2024
https://doi.org/10.21123/bsj.2024.10707
P-ISSN: 2078-8665 - E-ISSN: 2411-7986

Baghdad Science Journal

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

***Gun Gun Gumilar**[*1,2], **Asep Kadarohman**[2,3], **Muhammad Fauzan Fakhrurozi**[3], **Nahadi**[1,2], **Florentina Maria Titin Supriyanti**[3], **Heli Siti Halimatul Munawaroh**[3]*

[1] Study Program of Chemistry Education, Faculty of Mathematics and Science Education, Universitas of Pendidikan Indonesia, Bandung, Indonesia.
[2] Study Program of Science Education, Faculty of Mathematics and Science Education, Universitas of Pendidikan Indonesia, Bandung, Indonesia.
[3] Study Program of Chemistry, Faculty of Mathematics and Science Education, Universitas of Pendidikan Indonesia, Bandung, Indonesia.
*Corresponding Author.

Received 16/01/2024, Revised 16/07/2024, Accepted 18/07/2024, Published Online First 20/12/2024

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

Published Online First: December, 2024
https://doi.org/10.21123/bsj.2024.10707
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

and waste (FLW) in the world. The study reveals that the dominant domestic waste in Indonesia is contributed by food waste. Due to this 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 biogas[6], creating liquid organic fertilizer[7,8], utilizing waste as feed for larva black soldier fly[9], 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-acne[17], microbial fuel cell as a new potential of renewable energy[18], treatment of greywater[13], treatment of dairy wastewater[19] 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

Published Online First: December, 2024
https://doi.org/10.21123/bsj.2024.10707
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

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^{0}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. The values are expressed as the arithmetic means ± 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 Sivashanugam[25] with some modifications. The ecoenzyme was centrifugated 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 continuesly at 70^{0}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 Na_2CO_3 2% in 0.1 N NaOH and 1 part 0.5% $CuSO_4$) 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 ± 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 ºC 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 ± 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 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

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 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 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.

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

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.

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

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

Published Online First: December, 2024
https://doi.org/10.21123/bsj.2024.10707
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

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].

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

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 activitie 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 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.

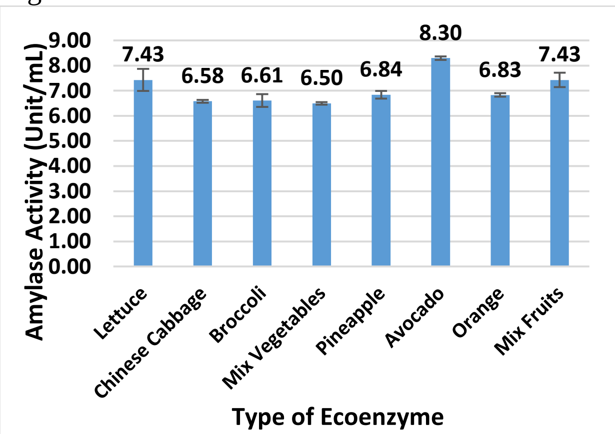

Figure 1. Amylase activity of ecoenzyme

Published Online First: December, 2024
https://doi.org/10.21123/bsj.2024.10707
P-ISSN: 2078-8665 - E-ISSN: 2411-7986

Figure 2. Black Fungi

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

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 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 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.

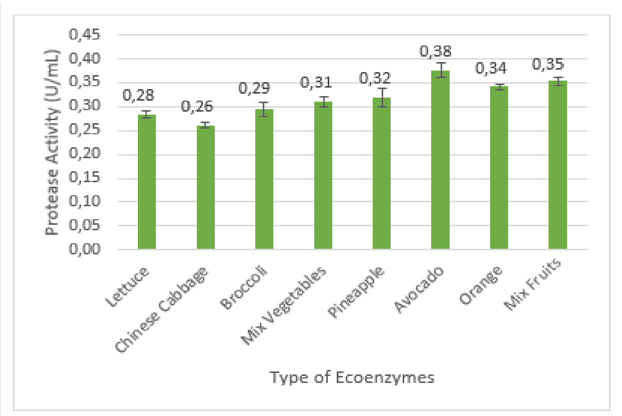

Figure 3. Protease activity of ecoenzyme

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

Published Online First: December, 2024
https://doi.org/10.21123/bsj.2024.10707
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

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 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.

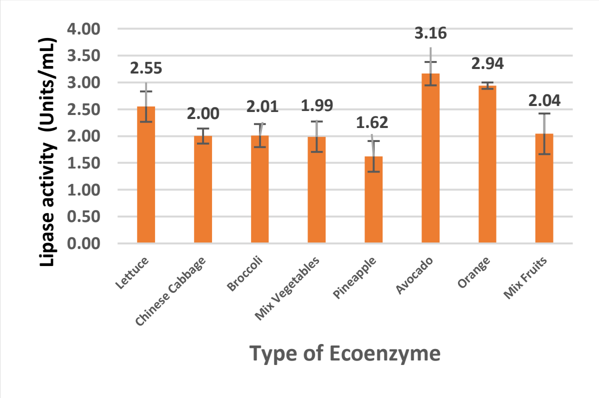

Figure 4. Lipase activity of ecoenzyme

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

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.

Acknowledgment

This research was supported by Universitas Pendidikan Indonesia.

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 re-publication, 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 of 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.

References

1. Ominski K, Mcallister T, Stanford K, Mengistu G, Kebebe EG, Omonijo F, et al. Utilization of by-products and food waste in livestock production systems: A Canadian perspective. Anim Front. 2021; 11(2): 55–63. https://doi.org/10.1093/af/vfab004
2. Farahdiba AU, Warmadewanthi IDAA, Fransiscus Y, Rosyidah E, Hermana J, Yuniarto A. The present and proposed sustainable food waste treatment technology in Indonesia: A review. Environ Technol Innov .2023; 32: 103256. https://doi.org/10.1016/j.eti.2023.103256
3. Abadi B, Mahdavian S, Fattahi F. The waste management of fruit and vegetable in wholesale markets: Intention and behavior analysis using path analysis. J Clean Prod .2021; 279: 123802. https://doi.org/10.1016/j.jclepro.2020.123802
4. Meng X, Zhang Y, Sui Q, Zhang J, Wang R, Yu D, et al. Biochemical conversion and microbial community in response to ternary ph buffer system during anaerobic digestion of swine manure. Energies. 2018; 11(11): 1–17. https://doi.org/10.3390/en11112991
5. Liang Z, Liao W, Yu Y, Li G, An T. How Does Vegetable Waste Decomposition Influence the Antibiotic Resistome and the Human Bacterial Pathogen Structure in Leachates? ACS Environ Sci Technol Water. 2022; 2(1): 226–36. https://doi.org/10.1021/acsestwater.1c00392
6. Masebinu SO, Akinlabi ET, Muzenda E, Aboyade AO, Mbohwa C. Experimental and feasibility assessment of biogas production by anaerobic digestion of fruit and vegetable waste from Joburg Market. Waste Manag. 2018 May;75: 236–50. https://doi.org/10.1016/j.wasman.2018.02.011
7. Walunguru L, Kaka Mone MM. The effect of N, P and K organic substances enhancers on fruit waste liquid organic fertilizer on tomato plants. IOP Conf Ser Earth Environ Sci. 2023 Oct; 1255(1): 012004. https://doi.org/10.1088/1755-1315/1255/1/012004
8. Ginandjar S, Frasetya B, Nugraha W, Subandi M. The Effect of Liquid Organic Fertilizer of Vegetable Waste and Planting Media on Growth and Yield of Strawberry (Fragaria spp.) Earlibrite Cultivar. IOP Conf Ser Earth Environ Sci. 2019 Oct; 334(1): 012033. https://doi.org/10.1088/1755-1315/334/1/012033
9. Romano N, Webster C, Datta SN, Pande GSJ, Fischer H, Sinha AK, et al. Black Soldier Fly (Hermetia illucens) Frass on Sweet-Potato (Ipomea batatas) Slip Production with Aquaponics. Horticulturae. 2023 Sep; 9(10): 1088-page??. https://doi.org/10.3390/horticulturae9101088
10. Tian H, Wang X, Lim EY, Lee JTE, Ee AWL, Zhang J, et al. Life cycle assessment of food waste to energy and resources: Centralized and decentralized anaerobic digestion with different downstream biogas utilization. Renew Sustain Energy Rev . 2021; 150(December 2020): 111489. https://doi.org/10.1016/j.rser.2021.111489
11. Alsalim HAA. Assessment of Azotobacter chroococcum's ability to produce hydrolytic enzymes and enhance growth of Vicia faba. Baghdad Sci J. 2019; 16(1): 34–9. https://doi.org/10.21123/bsj.2019.16.1.0034
12. Galintin O, Rasit N, Hamzah S. Production and characterization of eco enzyme produced from fruit and vegetable wastes and its influence on the aquaculture sludge. Biointerface Res Appl Chem. 2021; 11(3): 10205–14. https://doi.org/10.33263/BRIAC113.1020510214
13. Rasit N, Fern LH, Ghani WWK. Production and characterization of eco enzyme produced from tomatoand orange wastes and its influence on the aquaculture sludge. Int J Civ Eng Technol. 2019; 10(3): 967–80.
14. Arifin LW, Syambarkah A, Purbasari HS, Ria R, Puspita VA. Introduction of eco-enzyme to support organic farming in Indonesia. As J Food Ag-Ind. 2009; 356–9.
15. Tong Y, Liu B. Test research of different material made garbage enzyme's effect to soil total nitrogen and organic matter. IOP Conf Ser Earth Environ Sci. 2020 Jun; 510(4): 042015. https://doi.org/10.1088/1755-1315/510/4/042015
16. Sahar IH, Zaid AH, Milad A. Purification and characterization of L-asparaginase produced from Bacillus sp. Baghdad Sci J. 2023; 20: 2342–54. https://doi.org/10.21123/bsj.2023.9231
17. Ramadani AH, Karima R, Ningrum RS. Antibacterial Activity of Pineapple Peel (Ananas comosus) Eco-enzyme Against Acne Bacteria (Staphylococcus aureus and Prapionibacterium acnes). Indones J Chem Res. 2022; 9(3): 201–207. http://dx.doi.org/10.30598//ijcr.2022.9-nin
18. Dewi I, Ambarsari L, Maddu A. Utilization of Ecoenzyme Citrus reticulata in a microbial fuel cell as a new potential of renewable energy. J Kim Sains dan Apl. 2020 Feb; 23(2): 61–7. https://doi.org/10.14710/jksa.23.2.61-67
19. Srimathi N, Subiksha M, Abarna J, Niranjana T. Biological Treatment of Dairy Wastewater using Bio Enzyme from Citrus Fruit Peels. Int J Recent Technol Eng. 2020 May; 9(1): 292–295. https://doi.org/10.35940/ijrte.A1530.059120

20. Muliarta IN, Darmawan IK. Processing Household Organic Waste into Eco-Enzyme as an Effort to Realize Zero Waste. Agriwar J . 2021;1(1):6–11. https://doi.org/10.22225/aj.1.1.3658.6-11
21. Vidalia C, Angelina E, Hans J, Field LH, Santo NC, Rukmini E. Eco-enzyme as disinfectant: a systematic literature review. Int J Public Heal Sci. 2023; 12(3):1171–1180. https://doi.org/10.11591/ijphs.v12i3.22131
22. Maryanti A, Wulandari F. Production and Organoleptic Test of Onion Peel Eco enzyme. J Biol Trop. 2023; 23(2): 311–318. https://doi.org/10.29303/jbt.v23i2.4708
23. Vama L, Cherekar MN. Production, extraction and uses of eco-enzyme using citrus fruit waste: wealth from waste. Asian J Microbiol Biotechnol Environ Sci. 2020; 22(2): 346-351.
24. Arun C, Sivashanmugam P. Solubilization of waste activated sludge using a garbage enzyme produced from different pre-consumer organic waste. RSC Adv. 2015; 5(63): 51421–51427. https://doi.org/10.1039/C5RA07959D
25. Arun C, Sivashanmugam P. Investigation of biocatalytic potential of garbage enzyme and its influence on stabilization of industrial waste activated sludge. Process Saf Environ Prot. 2015 Mar; 94: 471–478. https://doi.org/10.1016/j.psep.2014.10.008
26. Mardina V, Harmawan T, Fitriani, Sufriadi E, Febriani, Yusof F. Euthynnus affinis viscera-an alternative source for protease and lipase enzymes: Characteristic and potential application as destainer agent. Biodiversitas. 2020; 21(12): 5858–5864. https://doi.org/10.13057/biodiv/d211250
27. Nazim F. Treatment of Synthetic Greywater Using 5% and 10% Garbage Enzyme Solution. Bonfring Int J Ind Eng Manag Sci. 2013 Dec; 3(4): 111–117. https://doi.org/10.9756/BIJIEMS.4733
28. Tang FE, Tong CW. A Study of the Garbage Enzyme's Effects in Domestic Wastewater. World Acad Sci Eng Technol. 2011; 5(12): 887-892. https://doi.org/10.5281/zenodo.1332982
29. Hemalatha M, Visantini P. Potential use of eco-enzyme for the treatment of metal based effluent. IOP Conf Ser Mater Sci Eng. 2020 Jan; 716(1): 012016. https://doi.org/10.1088/1757-899X/716/1/012016
30. Hanifah IA, Primarista NPV, Prasetyawan S, Safitri A, Adyati T, Srihadyastutie A. The Effect of Variations in Sugar Types and Fermentation Time on Enzyme Activity and Total Titrated Acid on Eco-Enzyme Results of Fermentation. Proceedings of the 7th International Conference on Biological Science (ICBS 2021). Atlantis Press; 2022. https://doi.org/10.2991/absr.k.220406.084
31. Indraloka AB, Istanti A, Utami SW. The physical and chemical characteristics of eco-enzyme fermentation liquids from several compositions of local fruits and vegetables in banyuwangi. IOP Conf Ser Earth Environ Sci. 2023 Apr; 1168(1): 012018. https://doi.org/10.1088/1755-1315/1168/1/012018
32. Rusdianasari, Syakdani A, Zaman M, Sari FF, Nasyta NP, Amalia R. Production of Disinfectant by Utilizing Eco-enzyme from Fruit Peels Waste. Int J Res Vocat Stud. 2021 Dec; 1(3): 01–7. https://doi.org/10.53893/ijrvocas.v1i3.53
33. Rijal M. Application of Eco-enzymes from Nutmeg, Clove, and Eucalyptus Plant Waste in Inhibiting the Growth of E. coli and S. aureus In Vitro. Biosel. 2022; 11(1): 31-44. https://doi.org/10.33477/bs.v11i1.4194
34. Ningsih AP, Glorya A, Samosir M, Hidayatullah BS. Production of Eco-Enzymes from Fruit Peel Waste as an Effort to Address Organic Waste Management Issues in Indonesia. JBIO J biosains . 2023; 9(2): 93-100. https://doi.org/10.24114/jbio.v9i2.49179
35. USDA. Food and Nutrient Database for Dietary Studies. 2023.
36. Bangar SP, Dunno K, Dhull SB, Kumar Siroha A, Changan S, Maqsood S, et al. Avocado seed discoveries: Chemical composition, biological properties, and industrial food applications. Food Chem X. 2022 Dec; 16: 100507. https://doi.org/10.1016/j.fochx.2022.100507

Published Online First: December, 2024
https://doi.org/10.21123/bsj.2024.10707
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

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

غون غون غوميلار[1,2]، أسيب كادار وهمان[2,3]، محمد فوزان فخرروزي[3]، نهادي[1,2]، فلورنتينا ماريا تيتين سوبريانتي[3]، هيلي سيتي حليماتول مناوروه[3]

[1] برنامج دراسة تعليم الكيمياء، كلية تعليم الرياضيات والعلوم، جامعة بنديديكان إندونيسيا، باندونج، إندونيسيا.
[2] برنامج دراسة تعليم العلوم، كلية الرياضيات وتعليم العلوم، جامعة بنديديكان إندونيسيا، باندونج، إندونيسيا.
[3] برنامج دراسة الكيمياء، كلية تعليم الرياضيات والعلوم، جامعة بنديديكان إندونيسيا، باندونج، إندونيسيا.

الخلاصة

يمثل هدر الطعام 44% من إجمالي كمية النفايات المنتجة في إندونيسيا. ومن هذا المجموع، تشكل نفايات الفواكه والخضروات 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 والخضروات. 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 بتركيبة 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 والخضروات قد انخفض في درجة الحموضة الحمضية مقارنة بالتخمر 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 والخضروات 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 الانزيمية ، التخمر، مخلفات الفاكهة، مخلفات الخضروات.