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# Novel Organic Preservative Made from Eco-Enzyme of Tempeh Waste: The Effect of Substrate and Fermenter Types

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**ABSTRACT**

Tempeh processing produces waste in the form of liquid and solid waste that can be utilized into eco-enzyme with the addition of yeast and substrate and lemon peel as a companion raw material. This study aimed to produce eco-enzyme from tempeh waste which is then applied as a preservative for mango fruit. The fixed variables in this study are the volume of liquid waste, the amount of epidermis solid waste, the amount of lemon peel, and the amount of yeast and the length of fermentation time. The independent variables were fermenter type (1 and 2) and substrate type (brown sugar (Eco-A), granulated sugar (Eco-B), and molasses (Eco-C)). The results showed that the eco-enzyme products produced were light brown (Eco-A and Eco-C), light yellow (Eco-B) and had a fresh sour aroma typical of lemon peel. The volume of eco-enzyme products after fermentation process were Eco-A1 3.45; Eco-A2 3.27; Eco-B1 3.35; Eco-B2 3.55; Eco-C1 3.25; and Eco-C2 3.36 L. The pH value obtained ranged from 3.05-3.25. Phenol levels ranged from 0.050–0.175 mg/L. The fruit preservation process obtained with Eco-C1 is the best variable, where the mango fruit has a shelf life of up to 16 days, with commercial organic preservatives for 12 days and mango fruit without preservatives has a shelf life of 10 days. The preservative from tempeh waste is potential to develop as a commercial product.

**Keywords:** Tempe Waste, Fermentation, Eco-enzyme, Fruit Preservatives.

## INTRODUCTION

The estimated average per capita consumption of tofu and tempeh of Indonesian people according to the data from the Central Statistics Agency (BPS) in 2021 is 0.304 kg per seven days. This figure shows an increase from the previous year of 3.75% from 0.293 per seven days [BPS, 2022]. Tempeh is usually produced in small cottage industries with a production range of 10 kg-2 tons per day. There are more than 100,000 tempeh producers spread across several provinces in Indonesia [Ayuni & Putri, 2022]

Tempeh processing produces solid and liquid waste. Direct waste disposal into waters in the surrounding environment without going through the treatment process first causes blooming, deposition of organic matter in the water, decomposition and development of pathogenic microorganisms [Setiawati et al., 2019]. Most tempeh industries dispose of waste directly into the environment without any prior treatment process.

The tempeh waste that is treated properly will produce something very profitable, both in terms of environment and economic [Puger, 2018]. Tempeh waste is one of the wastes that has economic value, because it has a relatively high content of organic compounds and nutrients that are useful for fertilizing soil and plants [Sayow et al., 2020].

The high potential for tempeh waste pollution is very high, so a water treatment process is needed. The water treatment that can be applied to treat tempeh liquid waste is such as through coagulation, filtration and electrocoagulation processes [Cundari et al., 2022]. One of the steps to improve the quality of tempeh waste is processing it into eco-enzyme. Eco-enzyme is a multifunctional liquid that has a dark brown color and has a sweet-sour aroma like the characteristic smell of fermentation. The process of processing eco-enzyme through the fermentation process of organic waste with sugar which is used as an energy source by bacteria.

Eco-enzyme fermentation can be done spontaneously or non-spontaneously. One of the disadvantages of spontaneous fermentation is that it takes a long time and has the potential for product inactivity [Nuraida et al., 2022]. For this reason, in this study, the use of a starter was applied to accelerate fermentation to the formation of eco-enzymes.

The research conducted by [Nurlaela et al., 2022] with cabbage waste raw materials with variations in granulated sugar and brown sugar substrates, shows that the eco-enzymes produced from organic matter can be used as tomato fruit preservatives. Another research that made eco-enzyme which was then applied as a preservative was also carried out by [Mahmudah et al., 2021]. The study used raw materials for cucumber peel waste with variations in substrates in the form of brown sugar and granulated sugar.

The novelty of this research is in the form of the use of tempeh waste as the main raw material and lemon peel waste as a companion raw material for making eco-enzymes. The resulting Eco-enzyme will be applied as a preservative for mangoes. This research aimed to determine the characteristics of e co-enzyme from tempeh waste, determine the influence of the type of substrate and fermenter used on the eco-enzyme produced, and determine the effect of the eco-enzyme from tempeh waste as a preservative for mangoes.

## RESEARCH METHODS

The study was conducted from March to August 2023 in the Separation and Purification Laboratory of the Department of Chemical Engineering, Faculty of Engineering, University of Sriwijaya.

### Tool

The tools used in this study were gallons (fermenter), hoses, suspension, scissors, tray, basin, insulation, digital balance, filter, Funnel, pH meter, pipet volume, pipet drops, and measuring cup.

### Material

The materials used in this study were liquid waste from boiling tempeh, soybean peel solid waste, lemon peel waste, brown sugar, granulated sugar, molasses, and fermipan yeast.

### Fermentation

First, 3000 ml of tempeh liquid waste was placed into each fermenter. Soybean peel waste and lemon peel waste of 450 grams each were placed into each fermenter. The substrate (brown sugar, granulated sugar, and molasses) of 300 grams each was placed into each fermenter. The yeast (Fermipan brand) as much as 30 grams was placed into each fermenter. The schematic of the fermenter is shown at Figure 1. The fermentation process lasted 30 days. In Fermenter scheme I, there is a hose to drain the gas formed so that during the fermentation process the fermenter lid did not need to be opened. On the fermenter scheme II, in the first 1–2 days the fermenter lid was stretched to remove the gas

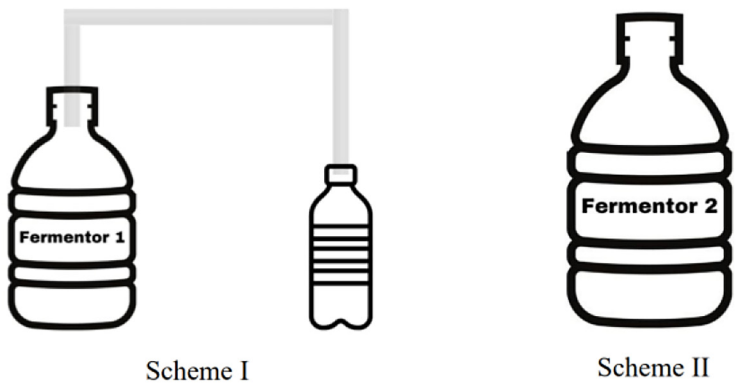


**Figure 1.** Eco-enzyme Fermenter

formed during the process. On days 3–6 the fermenter lid was opened 2–3 times a day, while in week 2nd the fermenter lid was opened every 2–3 days and in week 3rd–4th the fermenter lid was opened once a week.

### Preserving

Each eco-enzyme that has been produced is diluted using aquadest with a ratio of 1:10 v/v. The dilution process was carried out using a basin. Mango fruits were thoroughly washed and then dried. Furthermore, the mango fruit was placed into a basin containing eco-enzyme that has been diluted. The length of soaking time is 3 minutes. Furthermore, the mango was placed in an open room to analyze the level of eco-enzyme performance for 14 days.

## RESULT AND DISCUSSION

### Characteristics of eco-enzyme based on organoleptic

Figures 2 and 3 show the appearance of the eco-enzyme before and after the fermentation process. According to Table 1, the resulting eco-enzyme liquid shows different results. The results of eco-enzyme with brown sugar substrate and

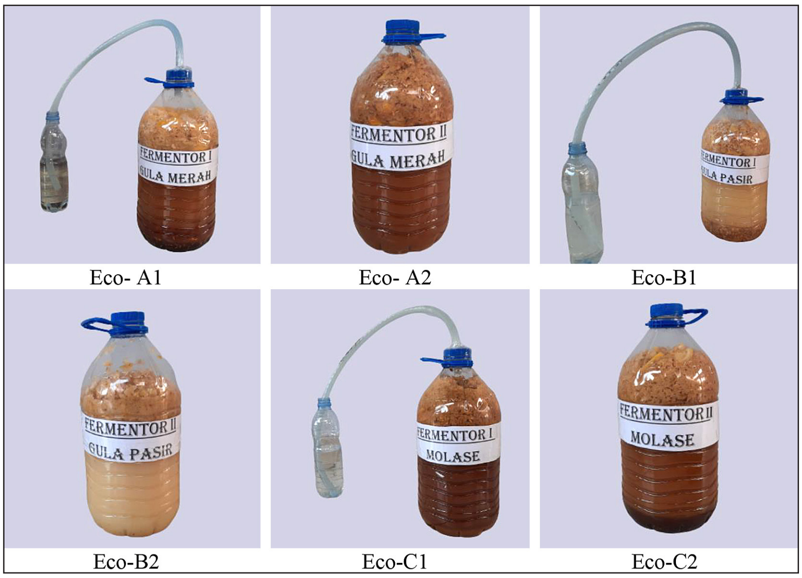


**Figure 2.** Eco-enzyme before fermentation

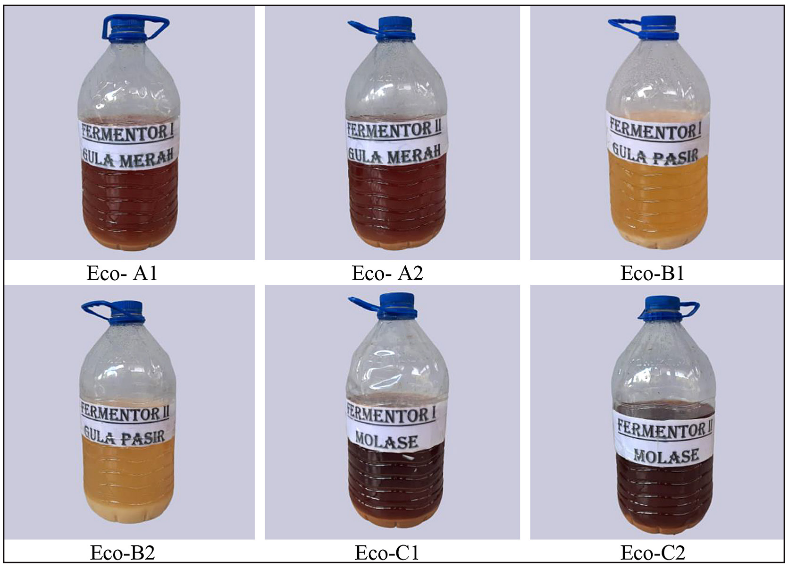


**Figure 3.** Eco-enzyme after fermentation

**Table 1.** Observation data of eco-enzyme color characteristics

| Variations | Color testing | | |
|---|---|---|---|
| | Before | After | Previous research |
| A1 | Dark brown | Light brown | Brown |
| A2 | Dark brown | Light brown | Brown |
| B1 | Deep yellow | Bright yellow | Brown |
| B2 | Deep yellow | Bright yellow | Brown |
| C1 | Dark brown | Light brown | Brown |
| C2 | Dark brown | Light brown | Brown |

molasses are in line with research [Pangemanan et al., 2022]. According to [Pangemanan et al., 2022], before the fermentation process, the eco-enzyme is dark brown, while after the fermentation process, the color change of the eco-enzyme becomes lighter. These results can be seen at Figures 2 and 3. The eco-enzyme made can be said to be successful if the results look brown and smell fresh sour like the aroma of orange peel [Dewi et al., 2021].

According to Table 2, before carrying out the fermentation process, all variations of eco-enzyme have the same smell, namely the sour aroma of tempeh waste. It can be seen that all variations produce the same aroma after going through the fermentation process. In all variations, a fresh sour aroma characteristic of lemons is produced. Eco-enzyme can be said to be good if it has a brown solution color, a distinctive fresh acidic aroma and the highest water content [Viza, 2022].

## Eco-enzyme volume

The process of making eco-enzyme uses tempeh liquid waste of 3 liters. The initial volume of eco-enzyme is 3.891 L, the measurement of eco-enzyme solution after filtering is measured with the help of a measuring cup.

On the basis of Figure 4, in terms of fermenter type, fermenter scheme I produces the volumes that are not too different from each type of substrate while fermenter scheme II produced the volumes with significant differences. This significant difference in fermenter scheme II can be caused by unstable fermenter conditions, because during fermentation the fermenter lid was often opened. The condition of the fermenter that is often opened and closed makes the activity of bacteria to decompose waste unstable.

From these data, it can also be seen that Eco-B samples or samples with granulated sugar substrates have the largest amount of volume. Different types of substrates make the content in eco-enzymes also different [Nurlaela et al., 2022]. This difference in substrate is enough to affect the process of fermentation. The substrate or sugar used is a source of energy or nutrients for *Acetobacter xylinum* bacteria [Mahmudah et al., 2021].

The increased activity of *A. Xylinum* bacteria will make the activity to convert glucose into cellulose also increased [Santosa et al., 2019]. Sucrose is one type of simple sugar [A. S. Fitri & Fitriana, 2020]. Simple sugars have small and simple molecules, these simple molecules will make the process of breakdown by enzymes faster. This allows for rapid absorption of nutrients that will allow bacteria to produce energy and decompose organic waste faster.

**Table 2.** Observation data of aroma characteristics

| Variations | Aroma testing | | |
|---|---|---|---|
| | Before | After | Previous research |
| A1 | Sour aroma of tempe waste | Fresh sour aroma characteristic of lemons | Fresh sour aroma characteristic of fermented fruit |
| A2 | Sour aroma of tempe waste | Fresh sour aroma characteristic of lemons | Fresh sour aroma characteristic of fermented fruit |
| B1 | Sour aroma of tempe waste | Fresh sour aroma characteristic of lemons | Fresh sour aroma characteristic of fermented fruit |
| B2 | Sour aroma of tempe waste | Fresh sour aroma characteristic of lemons | Fresh sour aroma characteristic of fermented fruit |
| C1 | Sour aroma of tempe waste | Fresh sour aroma characteristic of lemons | Fresh sour aroma characteristic of fermented fruit |
| C2 | Sour aroma of tempe waste | Fresh sour aroma characteristic of lemons | Fresh sour aroma characteristic of fermented fruit |

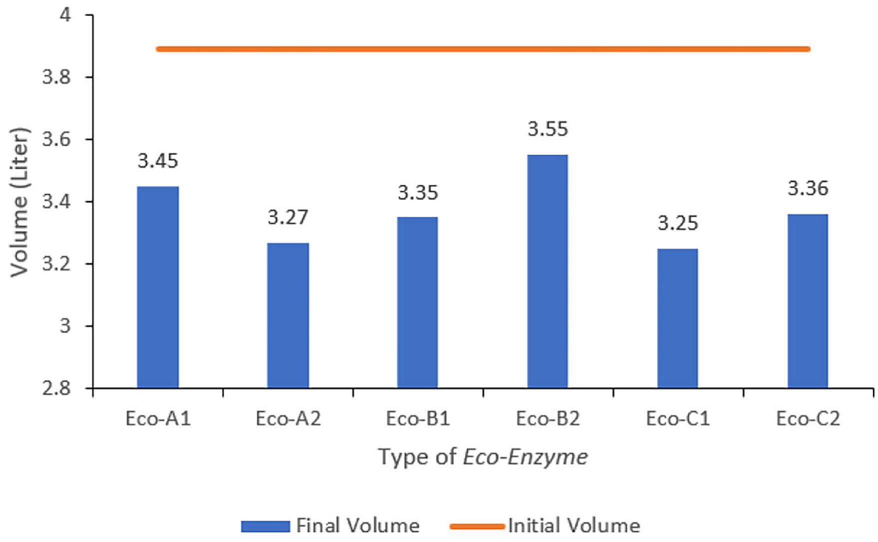


**Figure 4**. Volume of eco-enzyme

The sucrose content in granulated sugar is around 97.1% and reduction sugar is 1.24% [Rifqi et al., 2022]. The sucrose content in brown sugar is 84.31% [Afiah, 2018], while the molasses substrate has a sucrose content of 35% [Santosa et al., 2019]. On the basis of the sucrose content, it was proven that the eco-enzyme with sand substrate that has the highest sucrose content has the highest amount of volume.

## Eco-enzyme acidity degree (pH)

The results of analysis of the pH value contained in the eco-enzyme can be seen in Figure 5. The results of the pH analysis show a pH value below 4, in this case it has met the standards in the manufacture of eco-enzyme [Putra & Suyasa, 2022]. The results of this study are in line with the research conducted by [Rochyani et al., 2020], where eco-enzyme is acidic with a pH of 3–4. On the basis of the results of pH analysis, the lowest pH value is found in Eco-C1 and the highest pH in Eco-C2. The data shows that fermenter scheme I produces a lower pH of eco-enzyme than fermenter scheme II. This is due to the condition of fermenter scheme II which is often opened and closed so that the activity of bacteria to decompose waste becomes unstable considering that the fermentation of the eco-enzyme is aerobic.

Depending on the type of substrate, molasses substrates produce the eco-enzyme with the lowest pH value. Molasses is a byproduct of the process of processing sugarcane into sugar. Molasses contains many sugars and acids, organic acids [Gasperz & Fitrihidajati, 2022]. The organic acid content determines the acidity of a

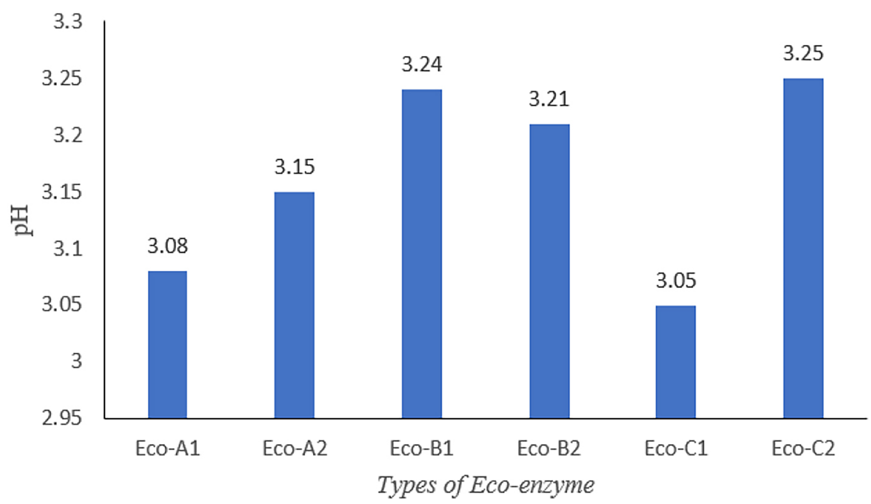


**Figure 5.** The pH value of eco-enzyme

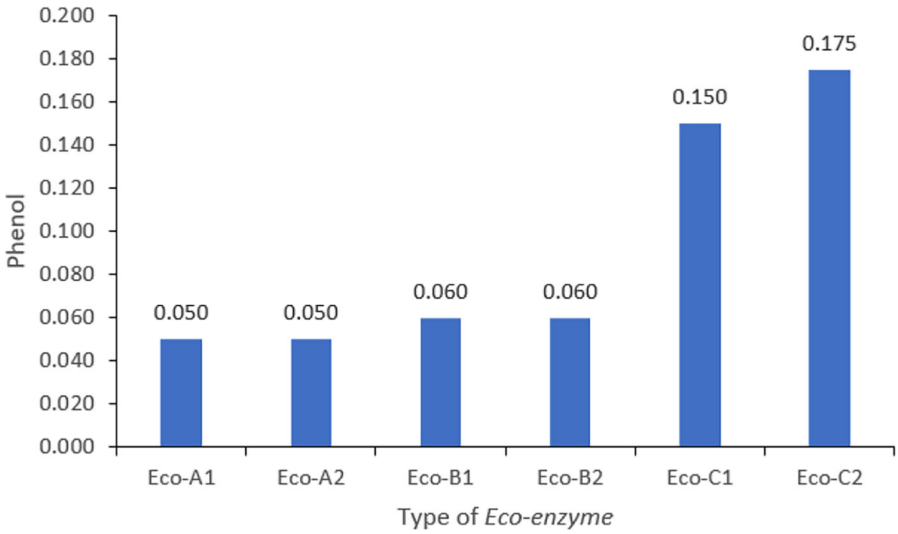


**Figure 6.** Phenol levels in eco-enzyme

compound. The greater the organic acid content, the smaller the pH of the eco-enzyme produced [Nurlaela et al., 2022].

### Eco-enzyme phenol levels

Phenol ($C_6H_6OH$) is a homologous compound in which there is a hydroxyl group directly attached to the aromatic ring [C.W. Fitri, 2017].

On the basis of Figure 6, the phenol levels produced from eco-enzyme have a significant difference between the types of Eco-C with Eco-A and Eco-B. The activity of phenol compounds will affect in inhibiting the growth of bacteria [Maharani et al., 2016]. Phenol can be said to be an antibacterial compound because phenol compounds can disrupt the function of cell membranes that will make the cell undergo lysis [Husni et al., 2020].

On the basis of the preservation process of mangoes, Eco-C1 and Eco-C2 samples with the addition of molasses substrate have the longest shelf life of 16 and 14 days when compared to other types of eco-enzymes. This long shelf life can be due to the presence of high phenol levels in the eco-enzyme sample. Phenol compounds have a habit of binding to proteins; therefore, the metabolic processes of bacteria can be disrupted [Nurliana & Musta, 2019]. Phenol levels will make a difference to antibacterial activity, which is based on the research by [Putri et al., 2014] The different phenol content of the two types of rosella petals causes differences in the antibacterial activity. The compounds that have a greater phenol content have higher bacterial activity.

### Comparison with other eco-organics

The comparison of the pH and phenol analysis of eco-organics can be seen in Table 3. This research results show the pH and phenol of eco-enzyme made from tempeh waste met the standard of manufacturing of eco-enzyme.

### Observation of preservation based on fruit texture

There were 10 samples tested, namely, Eco-A1, Eco-A2, Eco-B1, Eco-B2, Eco-C1, Eco-C2, D (Chitasil), E (Citric Acid), F ($CaCl_2$), and G (Without Preservatives). Each sample used 2 pcs of mangoes and the results in this paper show the average condition of the application. The results of observations based on the texture of mangoes can be seen in Figure 7.

Texture changes occur when the texture of the mango fruit becomes mushy and rotten. On the basis of these three graphs, it can be seen that the mango fruit used as a control experienced the longest texture change, namely in Eco-C1. Deterioration of fruit quality is characterized by an increasingly mushy texture due to tissue softening, discoloration, loss of volatile compounds that produce an unpleasant aroma (Maula et al., 2020). The process of deterioration of such quality will end with fruit rot.

On the basis of the research, it was found that eco-enzyme liquid is effective in inhibiting the decay process in mangoes. This is evidenced by the experiments where the mangoes soaked with eco-enzyme liquid change in texture more slowly experienced compared to the fruits that were not soaked with the eco-enzyme liquid.

**Table 3.** Comparison with other eco-organics

| Material | pH | Phenol (mg/L) | Reference |
|---|---|---|---|
| Pineapple peel | 3.5 | | Suprayogi dkk (2022) (Suprayogi et al., 2022) |
| Orange peel | 3.5 | | Suprayogi dkk (2022) (Suprayogi et al., 2022) |
| Pineapple peel + orange peel | 3.5 | | Suprayogi dkk (2022) (Suprayogi et al., 2022) |
| Fruit peel | 3.69 | | Gaspresz dan Fitrihidajati, 2022 (Gasperz & Fitrihidajati, 2022) |
| Pineapple peel + banana peel + mango peel | 2.4 | | Viza, 2022(Viza, 2022) |
| Pineapple peel + lime peel + watermelon peel | 2.5 | | Viza, 2022 (Viza, 2022) |
| Lime peel + mango peel + dragon fruit peel | 2.6 | | Viza, 2022 (Viza, 2022) |
| Watermelon peel + lemon peel + dragon fruit peel | 2.8 | | Viza, 2022 (Viza, 2022) |
| Tempeh waste + lemon peel (Eco-A1) | 3.08 | 0.050 | This research |
| Tempeh waste + lemon peel (Eco-A2) | 3.5 | 0.050 | This research |
| Tempeh waste + lemon peel (Eco-B1) | 3.24 | 0.060 | This research |
| Tempeh waste + lemon peel (Eco-B2) | 3.21 | 0.060 | This research |
| Tempeh waste + lemon peel (Eco-C1) | 3.05 | 0.150 | This research |
| Tempeh waste + lemon peel (Eco-C2) | 3.25 | 0.175 | This research |

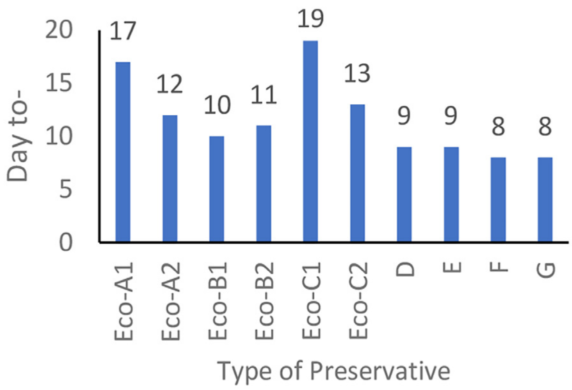


**Figure 7.** Mango fruit condition based on texture

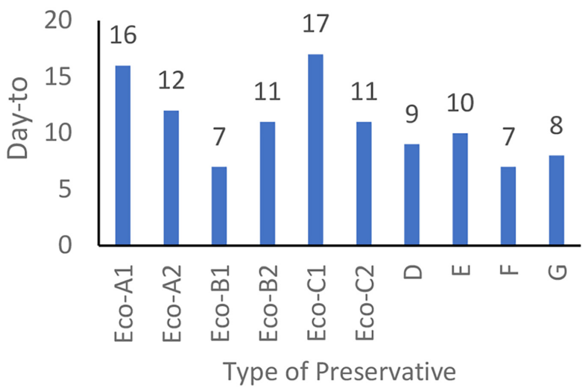


**Figure 8.** Mango fruit condition based on black spot

## Preservation observations based on fruit color

The results of observations based on the black spot of mangoes can be seen in Figure 8. Discoloration occurs when the color of the mango fruit becomes yellowish-green and black. Figure 8 shows that the mango fruit used as a control experienced the longest color change, namely at Eco-C1, seen on the 17$^{th}$ day the color of the fruit changed to black. The high quality of fruit in Eco-C1 is due to high pH and phenol levels. Phenols can help prevent the growth of bacteria and microbes on the surface of the mango fruit so that the fruit is not easily degraded and changes its color. By inhibiting microbial growth, phenol levels can help maintain fruit color longer.

## Preservation observation based on fruit skin surface

This observation is seen in terms of the surface appearance of the mango, namely the condition that has shown black spots that multiply or black spot that enlarged is clearly visible.

Figure 9 shows that the Eco-C1 sample has performed in good condition the longest until day 16. From the graph, it can also be seen that the fruit preservatives with Eco-C or the eco-enzyme with molasses substrate have a longer shelf life when compared to other types of preservatives. The high shelf life of fruit in Eco-C can be caused by the high levels of phenol in Eco-C (refer to Figure 6). It can be seen that phenol levels in Eco-C1 are 0.150 and Eco-C2 are 0.175.

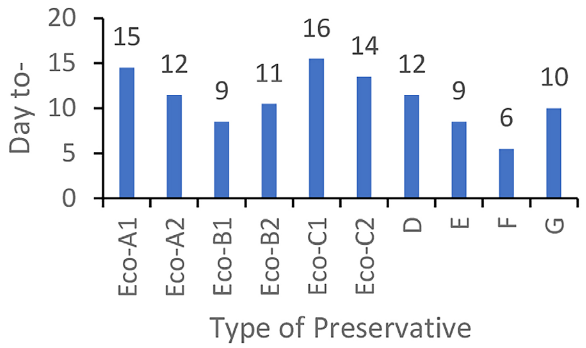


**Figure 9.** Mango fruit condition based on the surface of mango skin

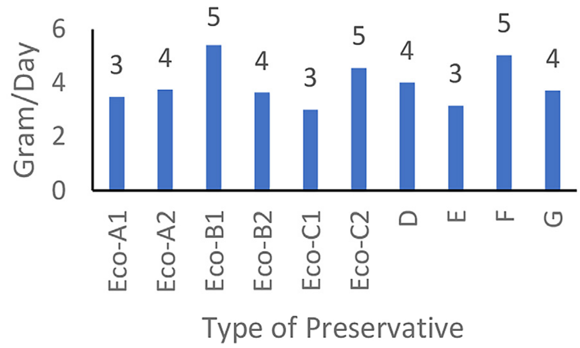


**Figure 10.** Decrease in mango mass when conditions are good

High levels of phenol in Eco-C can inhibit the growth of microbes or bacteria in the mango fruit because phenol is a compound that can disrupt the function of cell membranes that will make the cell undergo lysis [Husni et al., 2020]. Other influences can also be caused by pH on these preservatives, where according to [Sitompul et al., 2015] bacteria can grow optimally under the pH conditions of 6.5–7.5, if the pH is below 5 or above 8.5 bacteria cannot grow in good conditions.

By entering the pH conditions where the pH of each preservative, shown in Figure 5, it can be seen that the pH values of the preservatives *Eco-enzyme*, Chitasil, and Citric Acid are within the pH range that bacteria are difficult to grow. The samples with $CaCl_2$ preservatives had the fastest good condition appearance, which only lasted until day 6.

$CaCl_2$ preservatives are in the pH range at which that bacteria can still grow or live, this is what can cause $CaCl_2$ preservatives to have a fairly short shelf life when compared to other preservatives. Fruit with preservative D (Chitasil) can last up to the 12th day. This is because the addition of this chitosan preservative will make the formation of an edible layer, where this layer resists the rate of respiration of the preserved fruit so that the shelf life of the fruit becomes long [Sumarna et al., 2022].

### Observation of preservation by fruit mass

The process of preserving mangoes is also observed based on the weight reduction of the mango. The average decrease in mango mass is observed as long as the fruit is still in good condition.

Figure 10 shows that the decrease in mango mass per day, from each type of preservative is not too different, which is in the range of 3–5 grams/day. The fruits that have been released from the tree will experience a decrease in weight per day whether the fruit is coated with preservatives or not. This shrinkage can occur because the fruit will use existing food reserves to carry out its metabolism which makes the existing food reserves in the fruit will continue to decrease [Sumiasih et al., 2016]. The process of respiration and transpiration of the fruit also makes the weight of the fruit shrink, the decrease in fruit weight is caused by the dominance of the transpiration process and the respiration process when the sugar changes into $CO_2$ gas [Sukasih & Setyadjit, 2016].

On the basis of the observations that have been made on mangoes. It can be seen that the Eco-C1 sample is a sample that has a shelf life of fruit compared to others. The C1 sample has an acetic acid content of 13982.5 mg/L, pH of 3.05, phenol content of 0.150, and there are amylase, lipase, and protease enzymes of 0.7648 U/mL, respectively; 35.99 U/L; and 0.0029 mU/mL.

## CONCLUSIONS

The eco-enzyme characteristics of tempeh waste have a light brown color for brown sugar substrate type molasses and bright yellow color for granulated sugar substrate. Eco-enzyme has a fresh sour aroma typical of lemon peel fruit. The use of fermenter scheme I produces the best conditions on the volume produced, acidity (pH), and phenol content. Molasses is the best substrate that produces volume, acidity (pH), and phenol content. Eco-enzyme is effective in inhibiting the process of changing the texture, color, surface of mangoes with the best conditions of the longest change, namely on Eco-C1 on day 16.

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