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ANALYSIS OF ECO ENZYME CHARACTERISTICS WITH VARIATION OF "TAPE" YEAST CONCENTRATIONS

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ABSTRACT

Eco enzyme is a multipurpose liquid which produced from organic waste. The liquid made with the ratio of raw materials in the form of brown sugar: organic waste: water is 1:3:10 which is processed using a fermentation process for 90 days. One method to shorten the fermentation time is adding yeast as catalyst throughout the fermentation process. This research aims to analyze the characteristics of the eco enzyme with variations in "tape" yeast concentrations. Raw material used in this research included organic waste (orange and melon peels with a ratio of 1:1), brown sugar, water, and "tape" yeast (yeast concentrations of 1, 2, 3, 4 and 5%w/v) through fermentation process for ten days. The eco enzyme products were tested to determine the pH value, TDS value, acetic acid content, aroma, color and protease enzyme activity. The analysis results showed that the pH value of eco enzyme products was in the range of 3.9 to 4.1, TDS (Total Dissolved Solids) value of 1339 to 1405 ppm, acetic acid content of 0.81 to 1.08%w/w, had bright yellow and deep yellow color with a fermented aroma. The selected eco enzyme product in this research was on eco enzyme with yeast concentration of 3 %w/v which had pH value of 3.9 TDS value 1403 ppm, acetic acid content of 0.81%w/w, and the color was bright yellow with fermented aroma. However, there was no clear zone around the colony in the test indicating that there was no protease enzyme activity.

Keywords: acetic acid, eco enzyme, pH, "tape" yeast, TDS

1. INTRODUCTION

Required to resist waste accumulation, waste management is a systematic, comprehensive, and continuous activity that includes waste reduction and disposal. Currently, community waste management relies on the end-of-pipe approach, in which waste is collected, transported, and disposed of at the final waste processing site. In fact, large amounts of waste piles at the final waste processing site have the potential to generate methane gas, which could contribute to greenhouse gas emissions and global warming [1]. Organic waste is one of the causes of waste accumulation produced from various sources (such as agricultural waste, market waste, kitchen waste, and municipal waste) that could be decomposed by microorganism naturally. If the waste is not adequately processed, it might cause numerous environmental issues [2]. The waste could be classified into two types, including wet and dry wastes. Wet organic wastes include leftover vegetables, fruit peels, rotten fruit, onion skins and others. Meanwhile, dry organic wastes are organic wastes that contain little water such as wood, tree branches, wood, and dry leaves. Organic waste

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management could include the use of landfill, burning, and biological decomposition of organic waste [3].

Rosukon Poompanvong from Thailand, who found the Thai Organic Agriculture Association, had been involved in eco enzyme research since 1980. Eco enzyme is a fermented liquid made from organic waste such as fruit, vegetables, vegetable stems and other organic wastes which has numerous benefits [4]. The advantages of eco enzyme are classified into three categories: agricultural (as liquid organic fertilizer and vegetable pesticides), health (as a disinfectant and cleaning fluid), and household (as a substitute for soap, floor cleaners, and mouthwash) [5]. Eco enzyme comprises protease, amylase, and lipase enzymes, all of which could be employed as cleaning agents [6]. Eco enzyme is produced using a 1:3:10 ratio of raw materials brown sugar, organic waste, and water [7]. The fermentation process for making environmental enzyme takes three months [8]. In general, many factors could affect the fermentation process, including fermentation time, fermentation temperature, and the type of yeast [9]. The following Equation (1) occur during the production of eco enzyme [10].

$$\underset{\text{(Cellulose)}}{C_6H_{10}O_5} + \underset{\text{(Water)}}{H_2O} \xrightarrow{\text{“tape” yeast}} \underset{\text{(Acetic acid)}}{3CH_3COOH} \quad (1)$$

There are also researches being conducted on the production of eco enzyme from various raw materials such as orange peels, tomato peels, pineapple peels, and various vegetable wastes [11-12]. These studies resulted in eco enzyme products with pH values ranging from 3.5 to 4, TDS values ranging from 1000 to 2500 ppm, acetic acid content ranging from 3.32 to 5%, a liquid phase, brownish yellow color, and a fruity and fermented aroma [12-15]. However, in those researches, the fermentation process for producing eco enzyme still took three months. Rahayu et al (2020) researched the making of eco enzyme using baker’s yeast, with the product tested after eight to ten days. The research produced a faster eco enzyme fermentation process time than earlier studies. According to the research, the best time to produce eco enzyme for disinfection purposes is eight to ten days. The product of fermentation had an alcohol content of 60 to 70% and the pH value of eco enzyme of less than 4.0 [16].

According to prior researches, the organic waste could be converted into eco enzyme. In this study, it could be produced by using organic waste from orange and melon peels. Aside from its widespread availability, this substance has a pleasant odor when employed as a cleaning agent. Orange and melons fruits are popular fruits, either directly or indirectly. Orange fruits and melons are used as raw materials in the production of juices, jams, flavors, cosmetics, medications, and other products. As a result, eating of these fruits, both of which are relatively large, will generate waste in the form of skins.

In this research, the process for producing eco enzymes was carried out using organic waste added with brown sugar as nutrition and “tape” yeast as a catalyst to accelerate the fermentation process. The yeast grows on mold microorganisms *Amylomyce rouxii*, *Mucor sp*, and *Rhizopus sp*, yeast *Pichia burtonii*, *Saccharomyces cerevisiae*, and *Candida utilis*, and bacteria *Pediococcus sp* and *Bacillus sp*. “Tape” yeast is widely used in fermentation to obtain bioethanol, and is usually shaped like a flat round, white in color with

a smooth texture [17]. This research aims to analyze the characteristics of the eco enzyme with variations in “tape” yeast concentrations. Eco enzyme characteristics were determined using pH value, TDS value, acetic acid content, aroma, color and protease enzyme activity.

2. RESEARCH METHOD

This research was conducted experimentally to analyze the characteristics of the eco enzyme with variations in “tape” yeast concentrations. The experimental step consists of raw material preparation, fermenter preparation, fermentation process, and product analysis.

The materials used in the production of eco enzyme included orange and melon peels, “tape” yeast from NKL brand (containing mold microorganisms *Amylomyce rouxii*, *mucor sp*, and *Rhizopus sp*, yeast *Pichia burtonii*, *Saccharomyces cerevisiae* and *Candida utilis*, and bacteria *Pediococcus sp*, and *Bacillus sp*), coconut brown sugar, water, and hot glue. While the materials for the analysis of eco enzyme products were 0.1 N NaOH solution, phenolphthalein indicator, aquadest, skimmed milk agar, 70% alcohol, matches, gauze, cotton, and thread.

The tools used for making eco enzyme included a 1.5 liter mineral water bottle, a ¼ inch waterpass hose, knife, cutting board, measuring cup, and analytical scale. While the tools used for product analysis were filters, containers, pH meters, TDS meters, dropper pipettes, ball pipettes, 10 mL measuring pipettes, burettes, clamps and statives, 250 mL erlenmeyer, glass funnels, glass beakers, sterile petri dishes, test tubes, spirit burner, sterile micro pipette tips, vortex mixer, heater, incubator oven, autoclave, watch glass, and micro pipette 100 to 1000 microliters.

2.1. Raw Material Preparation

Brown sugar, orange and melon peels, and water in a 1:3:10 ratio, and varying concentrations of "tape" yeast are utilized as raw ingredients in this research. Orange and melon peels were weighed at 150 grams each and then sliced into little pieces to make fruit peels. Furthermore, the brown sugar was weighed up to 100 grams and chopped into little pieces. The "tape" yeast was made by weighing 10, 20, 30, 40, and 50 grams (for 1% w/v yeast concentration indicates 10 grams of yeast with 1 L of water). While the water was prepared by leaving it in the container for 24 hours.

2.2. Fermenter Preparation

Preparation of the fermenter was carried out by preparing 10 bottles of 1.5 L mineral water and then washing them using warm water. The bottle cap was perforated with one hole, the size of which was set according to the diameter of the waterpass hose (¼ inch). A 50 cm long hose was fitted to a perforated bottle top to allow air bubbles to escape. The hose was hot glued to the bottle cap, and it was hoped that there will be no holes in the bottle cap.

2.3. Fermentation Process

The fermentation process was started with the addition of 100 grams of brown sugar and 1 liter of water to the fermenter, then was shaken until homogenous. The fruit peels combination were added to the sugar solution and agitated until homogenous. Orange peels weighed 150 grams and melon peels weighed 150 grams (1:1) before being mixed with 10 grams of "tape" yeast. The fermenter containing the mixture was sealed with a

bottle cap with a hose on one side, and the other side of the hose was inserted into an air bubble bottle filled with water to allow air bubbles to escape throughout the fermentation process. The fermenter that has been filled was labeled with the number, date of production, ingredients used, type of yeast, concentration of yeast, and harvest time of the eco enzyme. Then the fermentation process (anaerobic) waited for ten days.

During the fermentation process, observations were conducted to see if there were any air bubbles in the water bottle. The fermentation process was repeated with the same treatment for different yeast concentrations. The "tape" yeast concentration of 1, 2, 3, 4, and 5% w/v was made by diluting 10, 20, 30, 40, and 50 grams of the yeast into 1000 mL of water. All eco enzyme products were filtered and tested after ten days of fermentation process.

2.4. Product Analysis

a. pH and TDS values

A pH value test is to determine the acidity of eco enzyme product while the TDS value test is performed to determine the amount of dissolved solids present in eco enzyme product. The pH and TDS values were measured through placing the sensor of pH meter and TDS meter into 40 mL of sample.

b. Acetic Acid Content

Acetic acid content test is to determine the acetic acid content formed in eco enzyme product. The acetic acid content was determined using acid-base titration which was carried out by mixing 2 mL of sample with 8 mL of aquadest (for dilution factor of 5). Three drops of phenolphthalein indicator were added into the sample, then was titrated with 0.1 N NaOH until it turned pink. Acetic acid content could be determined using the Equation (2) [18].

$$\text{acetic acid content } (\%) = \frac{V_{NaOH} \times N_{NaOH} \times Mr_{\text{Acetic Acid}} \times \text{Dilution factor}}{M_{sample} \times 1000} \times 100\% \qquad (2)$$

Note:

V_{NaOH} = Volume of NaOH (mL)

N_{NaOH} = Normality of NaOH (N)

$Mr_{\text{Acetic Acid}}$ = Relative molecular mass of acetic acid (g/gmol)

M_{sample} = Molarity of sample (M)

Dilution factor = 5

c. Organoleptic Properties

Organoleptic tests, including color and aroma tests, were conducted by 25 respondents with an age range of 20 to 22 years using a Google Form.

d. Protease Enzyme Activity

Eco enzyme contains protease, amylase, and lipase enzymes. In previous research of the protease enzyme test, the formation of a clear zone surrounding the colony indicates the activity of the protease enzyme [6]. The pour plate method was utilized to determine protease enzyme activity before diluting the selected eco enzyme samples from 10^{-1} to 10^{-4}. In the first test tube, a 10^{-1} dilution was obtained by mixing 1 ml of eco enzyme sample with 9 ml of sterile distilled water and homogenizing it with a vortex mixer. Then, 1 ml of sample from the first test tube was transferred to the second test tube with 9 ml

of sterile distilled water for a 10^{-2} dilution. This dilution method was repeated until a dilution of 10^{-4} was obtained. A diluted sample of up to 0.1 mL was transferred aseptically to a sterile petri dish. Skim milk agar was applied to samples in a sterile petri dish (skim milk powder 7 g/250 mL, yeast extract 0.625 g/250 mL, sabouraud dextrose agar from Himedia brand 0.25 g/250 mL, soyabean casein digest agar (tryptone soya agar) from Himedia brand 1.25 g/250 mL, agar 15 g/L). Sample in the dish was incubated at 37°C for 48 hours [6].

3. RESULTS AND DISCUSSIONS

3.1. Results

Table 1 shows the results of observations and data analysis of eco enzyme products in this study.

Table 1. The results of the analysis of eco enzyme products using "tape" yeast with the fermentation process

"Tape" Yeast Concentrations (% w/v)	pH	TDS (ppm)	Acetic Acid Content (%w/w)	Color	Aroma
1	3.9	1339	1.08	Deep Yellow	Fermented Aroma
2	4.1	1338	0.945	Bright Yellow	Fermented Aroma
3	3.9	1403	0.81	Bright Yellow	Fermented Aroma
4	4.0	1392	0.81	Bright Yellow	Fermented Aroma
5	4.0	1405	0.945	Deep Yellow	Fermented Aroma

3.2. Discussions

a. Production of Eco Enzyme

Eco enzymes are generated from organic waste and have a raw material ratio of 10: 3: 1 (water: fruit peels: brown sugar) [16]. Because the density of water is 1 g/mL, the weight of water could be assumed to be equal to its volume, so the mass of water is equal to the volume of water. Organic waste was employed in this research in the form of 150 gram orange peels and melon peels which widely available in Indonesia and also 100 grams of brown sugar which provided nutrients for microorganisms during the fermentation process. The third ingredient is water, which could be in the form of ground water, AC (air conditioner) waste water, PAM (*Perusahaan Air Minum*) water left for 24 hours, rainwater collected directly, or gallons of water [19]. PAM water (1000 mL) was used in this study since it was easily available in Indonesia.

a b c d e

Figure 1. Eco enzyme products by fermentation process for ten days with each concentration of “tape” yeast (a) 1%, (b) 2%, (c) 3%, (d) 4%, and (e) 5%w/v

In this fermentation process, "tape" yeast is added to act as a catalyst, especially to speed up the fermentation process. The process was conducted for ten days with the condition shown in Figure 1.

b. The Impact of “Tape” Yeast Concentrations on pH value of Eco Enzyme Products

pH measures how acidic or alkaline a substance is. The pH scale is in the range 1 to 14. A pH level of 7 denotes neutral pH, a value greater than 7 indicates an alkaline condition, and a value less than 7 suggests an acidic condition. In this study, the pH test took place to measure the degree of acidity in the eco enzyme products. Figure 2 depicts the results of the eco enzyme pH test at each concentration.

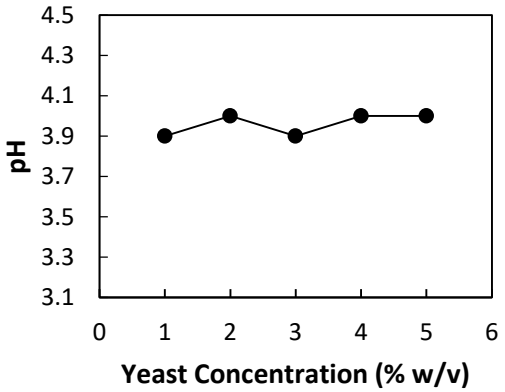


Figure 2. pH value of eco enzyme products by the fermentation process for ten days at each concentration of “tape” yeast (a) 1%, (b) 2%, (c) 3%, (d) 4%, and (e) 5%w/v

Figure 2 illustrates that the pH rises with increasing yeast concentration. The increase in pH was not significant enough that the addition of yeast had no influence on the resultant pH value. In this research, the pH value of the eco enzyme products was 3.5 to 4.1. These results show the range of pH value eco enzyme which is in accordance with the research of Suprayogi et al (2022) and Rasit et al (2019) which stated that the pH value of eco enzyme was around 3.5 to 4 [11-12]. Eco enzyme could be produced by organic matter, such as fruit peels, produce acidic chemical parameters with low pH values. Organic acids have an important role in acidity determination. The lower the pH value, the higher the organic acid content. Eco enzymes have a low pH value due to the high presence of organic acids (acetic acid or citric acid) [20]. According to research of Sambaraju and Lakshmi (2020), eco enzyme fermentation could produce a variety of essential organic acids such as acetic acid, lactic acid, malic acid, oxalic acid, and citric acid [21]. Furthermore, fruit peel wastes contain citric acid, which contributes to the low pH value [22]. The existence of a high acid product, as demonstrated by the low pH value of the eco enzyme product, indicates that the fermentation process is proceeding well.

c. The Impact of “tape” Yeast Concentrations on TDS value of Eco Enzyme Products

The TDS test is to measure the dissolved solids content of eco enzyme products. The TDS test results for eco enzyme at each concentration could be seen in Figure 3.

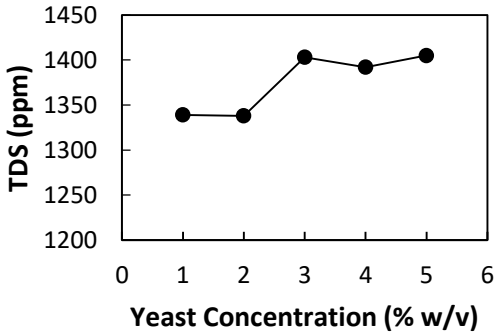


Figure 3. TDS value of eco enzyme products by the fermentation process for ten days at each concentration of “tape” yeast (a) 1%, (b) 2%, (c) 3%, (d) 4%, and (e) 5%w/v

TDS value of eco enzyme from previous studies were in the range of 1000 to 2500 ppm [12-15]. According to Figure 3, the TDS value of eco enzyme using "tape" yeast increases with increasing concentration. The lower TDS value at 4% concentration could be caused by less-than-optimal bacteria in the yeast that decompose organic waste. The presence of TDS in eco enzyme suggests that organic materials are dissolved in the enzyme. Organic matter in brown sugar, orange peel, yeast, and organic matter generated after fermentation, notably acetic acid, are examples of solids in eco enzyme. The higher the TDS value in eco enzyme, the more organic materials will dissolve in the eco enzyme product.

d. The Impact of “tape” Yeast Concentrations on Acetic Acid Content of Eco Enzyme Products

Acetic acid (CH_3COOH) is well known as one of the constituents of eco enzyme. Acetic acid in eco enzyme could decompose germs, viruses, and bacteria that used as cleaning agents [14]. Figure 4 depicts the acetic acid content of eco enzyme products at each "tape" yeast concentration.

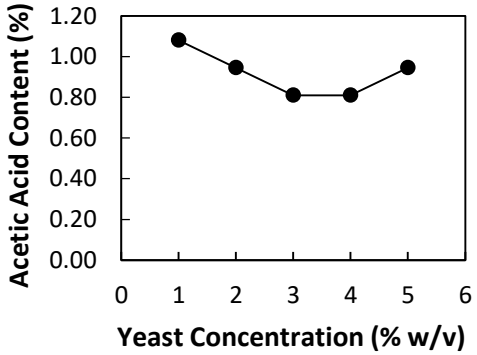


Figure 4. The acetic acid content of eco enzyme products by the fermentation process for ten days at each concentration of “tape” yeast (a) 1%, (b) 2%, (c) 3%, (d) 4%, and (e) 5%w/v

The decreasing value of acetic acid content suggested that the activity of bacteria in degrading organic waste decreased as the concentration of "tape" yeast increased. Thus,

the decomposition reaction of organic materials such as cellulose in orange peels and melon peels could only be broken down to reach these concentrations.

Due to the presence of acetic acid during the fermentation process, eco enzyme could be categorized in the acidic solution. Acetic acid is generated by the metabolic processes of bacteria found naturally in fruit and vegetable remains. The process of anaerobic metabolism, also known as the fermentation process, is an attempt by microorganism to get energy from carbohydrates under anaerobic conditions (without oxygen) and with by-products in the form of alcohol or acetic acid (depending on the kind of microorganism).

According to prior research, the eco enzyme from sour oranges (kaffir lime) had the greatest acetic acid content of 5.53%, while the eco enzyme from a blend of sweet and sour oranges had the lowest acetic acid content of 3.32% [18]. In this study, the highest acetic acid content was eco-enzyme with a yeast concentration of 1%w/v, which was 1.08%, while the lowest acetic acid content was an eco-enzyme with a yeast concentration of 3% and 4%w/v, which was 0.81%. The raw materials utilized could be the reason for the decreasing acetic acid content in the literature. When high acid raw materials are used, enzyme eco products will contain high acetic acid.

e. Organoleptic Properties of Eco Enzyme Products

The organoleptic properties test is a way of identifying changes in a product by employing the five senses [23]. Color and aroma were evaluated as organoleptic properties. According to some research, eco enzyme fermentation is successful if a brownish solution with an orange-like or fruit-like odor and a pH less than 4 or an acidic pH is produced [24]. Originally, eco enzyme products had an opaque brown appearance because the brown sugar utilized was brown, which was then blended with the pulp/residue of orange peels [25].

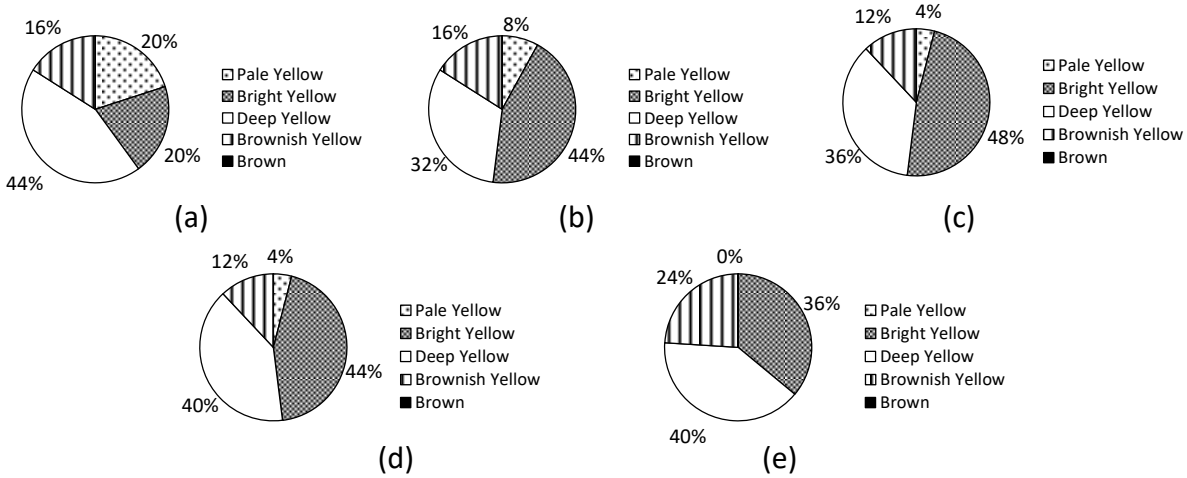


Figure 5. The results of the color test of eco enzyme products by the fermentation process for ten days at each concentration of “tape” yeast (a) 1%, (b) 2%, (c) 3%, (d) 4%, and (e) 5%w/v

The results of the organoleptic properties test in the form of the color of eco enzyme at each concentration in this study could be shown in Figure 5. The color test on eco enzyme using “tape” yeast in Figure 5 shows that the results at a concentration of 1% and

5% produce a deep yellow color while at a concentration of 2%, 3% and 4% they have a bright yellow color. The difference in the color of eco enzyme could be caused by the type of raw material used [26]. In this study, the raw materials used were orange peels and melon peels which produced bright yellow and deep yellow colors whereas organic matter could cause a deep yellow color in products. This is demonstrated by the high TDS value of eco enzyme products.

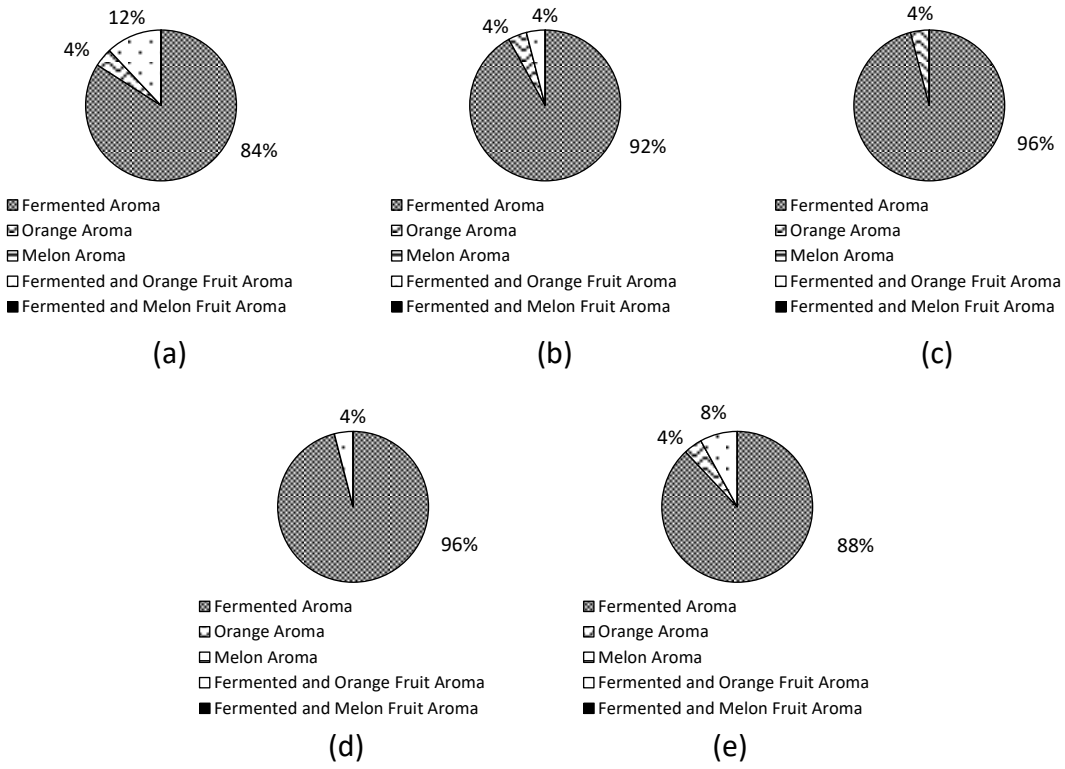


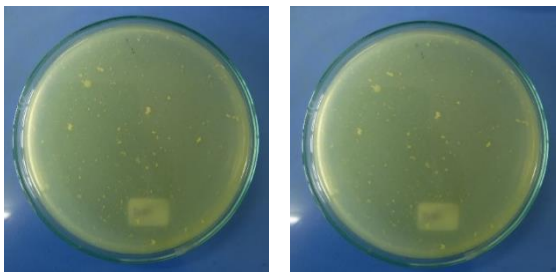
Figure 6. The results of the aroma test of eco enzyme products by the fermentation process for ten days at each concentration of “tape” yeast (a) 1%, (b) 2%, (c) 3%, (d) 4%, and (e) 5%w/v

In addition, an aroma test in each yeast concentration based on observations from 25 respondents was performed with the results could be seen in Figure 6. Eco enzyme aroma test took place to determine whether or not eco enzyme products containing "tape" yeast have the desired aroma. Eco enzyme with a sour aroma is generated from the fermentation of organic matter using a solution of brown sugar as the mole. The sour fragrance is caused by the fermentation process decomposing alcohol into acetic acid. Acetic acid in eco enzyme produces an acidic aroma. Aside from the sour aroma, the main ingredients used have other aromas [26]. The aroma of the eco enzymes produced during this research is consistent with the literature, having a characteristic fermented aroma and the aroma of orange fruit as a raw material.

f. Protease Enzyme Activity in Eco Enzyme Product

Protease enzyme activity test was carried out on selected eco-enzyme products, namely eco-enzyme with a yeast concentration of 3% w/v. The protease enzyme activity

in eco enzyme products in each “tape” yeast concentration could be presented in Figure 7.



(a) (b)

Figure 7. Observation of protease enzyme activity in eco enzyme products using the fermentation process for ten days with incubation time for (a) three days and (b) six days

According to Cherekar and Lapsia (2020), the presence of a clear zone indicates the activity of the protease enzyme [6]. However, in this study there was no clear zone around the colonies on the third or sixth day. As a result, it could not be determined whether a protease enzyme is present. Inappropriate results might be induced by a lack of specificity in the materials utilized as media.

4. CONCLUSIONS AND RECOMMENDATIONS

Based on the research, the concentration of “tape” yeast impacts the properties of eco enzyme on numerous parameters, including pH value, TDS value, acetic acid content, color, aroma, and protease enzyme activity. The addition of "tape" yeast concentration resulted in a pH value of 3.9 to 4.1 with no significant difference. The pH value of eco enzyme is affected by the organic acid content in it. While the TDS value rises because the more the yeast concentration added, the more organic matter produced. The results of acetic acid content analysis showed a reduction with increasing concentration of "tape" yeast. The color test produced a bright yellow and deep yellow color, while the aroma test produced a fermented aroma. The selected eco enzyme product with a concentration of “tape” yeast at 3%w/v had a pH value of 3.9, TDS value of 1403 ppm, acetic acid content of 0.81%w/w and had a bright yellow color with fermented aroma. However, the protease enzyme activity tests did not reveal a clear zone in the colony.

For the further research, it could be recommended to prepare skim milk agar with different compositions, incubate at the appropriate temperature, to observe in liquid culture. This process must be carried out properly because it greatly affects the colonies to be observed.

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