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EFFECT OF BAKER'S YEAST CONCENTRATIONS ON ECO ENZYME PRODUCTS BY THE FERMENTATION PROCESS

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ABSTRACT

Eco enzyme is a liquid fermented from organic waste such as vegetable or fruit peels, brown sugar or molasses, water, and yeast. In the production of eco enzyme, yeast is used as a catalyst to accelerate the fermentation process, such as baker's yeast, one type of yeast that could be used. The purpose of this research is to determine how the concentration of baker's yeast affects the eco enzyme products. Citrus peels, melon peels, coconut brown sugar, water, and baker's yeast were ingredients used in this research. The eco enzyme fermentation process lasted ten days, after which the eco enzyme product was examined for pH value, acetic acid content, TDS (Total Dissolved Solids) value, organoleptic properties such as color and scent, and protease enzyme activity. The results showed that the value of pH value, acetic acid content, and TDS value increased as the concentration of baker's yeast was added in the production of eco enzyme. In this research, the range of pH values was 4.15-4.2, acetic acid content was 1.35-2.03% v/v, TDS values was 1475-2056. Data from 25 panelists was used to generate color and scent from eco enzyme products. The test results showed that eco enzyme products had a fermented and citrus fruit scent and a pale yellow to deep yellow tint. Protease enzyme activity tests were performed on samples containing 5% % w/v baker's yeast, and it was discovered that there was one colony incubating for six days, but the results were not highly significant.

Keywords: acetic acid, eco enzyme, organic waste, organoleptic properties, TDS

1. INTRODUCTION

The amount of garbage generated by various activities increased in Indonesia along with population growth. Waste is any unusable substance that remains after a production process or activity, whether on a small scale in a home, an industrial environment, or a mine. Waste is categorized into two categories, organic waste and inorganic waste, depending on its composition. One kind of garbage that is mostly produced by human activity is organic waste. Organic waste is defined as any waste that can completely decompose through biological processes, both aerobic and anaerobic. Food scraps, vegetables, wood chips, dry leaves, and other organic trash could all decompose biologically through a process known as biodegradation. Organic waste may continue to decompose, emitting an unpleasant odor [1].

Organic waste is frequently disposed of directly into the environment without treatment, causing in negative environmental effects such as pollution, disruption of environmental aesthetics, and disruption of public health. One method of dealing with this

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waste is to create useful products such as eco enzyme. Eco enzyme is a fermented liquid generated from organic waste such as fruit pulp, peels, and vegetables, as well as sugar (cane sugar or brown sugar) and water. The fermentation process used in the production of eco enzymes is anaerobic fermentation, which is a fermentation process that does not require oxygen [2]. Eco enzyme solution has a dark brown color and a fermented scent similar to a strong, fresh sour [3]. According to Sulaeman et al. (2005), the solution could reduce the need for liquid floor cleaners and insect repellents [4]. The advantages of eco enzymes include floor cleaning fluids, air purifiers, pesticides, and bathroom and toilet cleaners [5].

Eco enzymes were first discovered in 1980 by Rosukon Poompoanvong, founder of the Association of Organic Agriculture in Thailand, who has been active in research on eco enzymes for 30 years. Based on the results of his research regarding the processing of eco enzymes from organic waste, they could be processed as organic cleaners or household cleaning agents [6]. The research produced eco enzymes after a 90 day or three month fermentation procedure, and according to numerous reference sources, the ideal raw material ratio for eco enzymes was the ratio of brown sugar to fruit peels to water of 1:3:10 [7].

The length of time for eco enzyme fermentation of approximately three months has resulted in the emergence of several studies on ways to speed up the fermentation process in the production of eco enzyme. One of them was a research by Rahayu et al. (2021) that used 1% b/v yeast from total water in the production of an eco enzyme. The research used various organic wastes as raw materials, such as rambutan peels, corn cobs, and chayote skins. The fermentation time for the research was only ten days due to the addition of yeast to the fermentation process, which the microorganisms in the yeast might speed up [8]. Types of yeast that could be used in the fermentation process for making eco enzymes include "tape" yeast, "tempe" yeast, and baker's yeast. Baker's yeast is one of the yeasts that could be used as a catalyst to accelerate the reaction rate in the fermentation process because it contains the microorganism *Saccharomyces cerevisiae*, which has been developed in the fields of conventional biotechnology and genetic engineering [9]. *Saccharomyces cerevisiae* has several advantages, including the ability to multiply quickly, resistance to high alcohol content, stable properties, and rapid adaptation [10]. Furthermore, under ideal conditions, the microorganism could produce large amounts of alcohol with an alcohol content of 8–20% [11].

Based on the previous research, there has not been any information provided about yeast concentration, which could be added to the production of eco enzymes to reduce the fermentation process time. As a result, this research is to investigate the concentration of baker's yeast in the production of eco enzyme in a shorter fermentation time. Despite the fact that there is no standard for eco enzyme, according to Rosukon Poompoanvong's research and several other studies, eco enzyme products have a yellow to brown color, a fermented scent, a pH value of 3–4, a high content of acetic acid, TDS, and the activity of the enzymes in them can be known [12]. The purpose of this research is to determine how the concentration of baker's yeast affects the eco enzyme products produced.

2. RESEARCH METHOD

This research was conducted based on experimental research, a type of research carried out under controlled conditions, to determine the effect of a certain variable on others. In this research, variations in the concentration of baker's yeast (1, 2, 3, 4, and 5% w/v) in the total water for ten days of fermentation time and the ratio of raw materials (coconut brown sugar: fruit peels: water) were 1:3:10. The product obtained was tested for pH value, acetic acid content, TDS value, organoleptic properties in the form of color and scent, and the activity of the enzymes produced.

Making eco enzyme with a fermentation process consists of equipment and material preparation, production of eco enzyme, and analysis of eco enzyme products, which could be done as follows:

2.1. Equipment and Material Preparation

Equipment used in this research includes experimental and analytical equipment. The experimental equipment used was a fermenter and an air bubble container made from a 1.5 L mineral water bottle, a water hose, a knife, a swallow, a 1000 mL measuring cup, and an analytical balance. The manufacturing process was that a 1.5-liter mineral water bottle was perforated, connected to a waterpass hose, and glued together. After that, the other side of the water-pass hose was connected to a fermenter bottle as a container for eco enzyme fermentation. While the equipment used for product analysis was a pH meter, TDS meter, dropper pipette, 250 mL Erlenmeyer, burette, clamps and statives, autoclave, vortex mixer, incubator oven, glass catcher, glass funnel, micropipette measuring 100–1000 microliters, cup petri, test tube, and spirit burner.

Furthermore, the materials used in this research consisted of experimental and analytical equipment. The materials used in this research for each sample consisted of 1000 mL of water that had been allowed to stand for 24 hours, 150 g of citrus peel, 150 g of melon peel, 100 g of coconut brown sugar, and fermipan brand yeast (10, 20, 30, 40, and 50 g). While the materials for product analysis consisted of eco enzyme samples, distilled water, 0.1 N NaOH solution, and phenolphthalein indicator, the materials for analysis of protease enzyme activity were in the form of agar media in 250 mL of distilled water consisting of 7 g of skim milk powder, 0.625 g of yeast extract, 0.25 g of dextrose from Himedia brand, 1.25 g of tripton from Himedia brand, 3.75 g of plain agar from Swallow Globe brand, and other ingredients that included 70% alcohol, matches, fat cotton, gauze, and thread.

2.2. Production of Eco Enzyme

The first step to producing eco enzyme was to mix 100 grams of brown sugar and 1000 mL of water in a fermenter and shake until homogenous. The sugar solution was then added to the fermenter and stirred until homogenous. Furthermore, each variation of baker's yeast concentration (10, 20, 30, 40, or 50 g) was added to the fermenter for each sample. The fermenter containing the mixture was sealed with a bottle cap connected to a water passage hose and then inserted into the bottle, where air bubbles escaped during the hardening process. It was labeled with the number, date of production, materials used, yeast type, yeast concentration, and harvest time for the eco enzyme. To ensure proper breastfeeding, wait ten days for the process of suckling (anaerobic) and observe whether there are air bubbles in the water bottle where air bubbles come out of the boiling water pass hose. After a ten-day fermentation process, all eco enzyme products were filtered, analyzed, and packed in prepared bottles.

2.3. Analysis of Eco Enzyme Products

The analysis of eco enzyme products includes the following parameters:

a. pH and TDS values

The pH test is to measure the degree of acidity of an eco enzyme products. It was conducted using universal pH paper dipped in 40 mL of eco enzyme product until the pH value was read.

Aside from the pH test, a TDS test was performed to measure the presence of dissolved organic matter in an eco enzyme solution using a TDS meter. TDS meter was dipped in 40 mL of eco enzyme product until the TDS value was read.

b. Acetic acid content

The acetic acid content test is used to determine the acetic acid content of the eco enzyme products. The test was carried out with 2 mL of the sample diluted with 8 mL of distilled water, and then 3–4 drops of the PP indicator were added to it. After that, it was titrated with acid-base using a standard 0.1 N NaOH solution until the eco enzyme sample turned pink, and then the required volume of NaOH solution was recorded.

Before calculating acetic acid content, it was necessary to calculate the molarity of the sample using Equation (1) with the mass ratio of coconut brown sugar (1): fruit peels (3): water (10) = 100 g: 300 g: 1000 mL, and the relative molecular mass of glucose is 180 g/gmol.

$$\mathrm{M} = \frac{\text{fruit peels substrate mass+brown sugar mass}}{\text{Mr Glucose}} \mathrm{x} \frac{1000\ \mathrm{mL}}{\text{Water volume}} \quad (1)$$

After the molarity of the sample was determined, the acetic acid content could be calculated using Equation (2) with the normality of NaOH of 0.1 N, the relative molecular mass of acetic acid of 60 g/gmol, and the dilution factor of 5 (obtained from the volume of the sample after being diluted compared to the volume of the sample before being diluted).

$$\text{Acetic Acid Content (\%)} = \frac{\mathrm{V} \times \mathrm{N} \times \text{Mr Asam Asetat} \times \text{Dilution Factor}}{(\mathrm{M} \times 1000)} \mathrm{x}\ 100\% \quad (2)$$

Note:
M = Molarity of Sample (gmol)
V = Volume of NaOH (mL)
N = Normality of NaOH (N)
Mr = Relative Molecular Mass (g/gmol)

c. Organoleptic properties of eco enzyme product

Organoleptic tests are used to determine certain physical characteristics of eco enzyme products in the form of color and scent. Testing was carried out by evaluating 25 panelists in the age range of 20 to 22 years via Google Form.

d. Activity of protease enzyme

A protease enzyme activity test is used to determine the content of the protease enzyme in the resulting eco enzyme product. This test was conducted by observing the protease enzyme activity in the selected sample based on the results of other analyses (pH value, acetic acid content, TDS value, and organoleptic properties). The protease enzyme activity in eco enzyme products was tested by serially diluting the sample from 10^{-1} to 10^{-4}. For 10^{-1} dilution, it was made by mixing 1 mL of the enzyme sample with 9 mL of sterile distilled water and then homogenizing. Next, 1 mL of the sample was taken from the first test tube and put into the next test tube containing 9 mL of sterile distilled water for a 10^{-2} dilution. This dilution method was continued until a 10^{-4} dilution was obtained.

The next step was the preparation of agar media from a mixture of 250 mL of distilled water, 7 g of skim milk powder, 0.625 g of yeast extract, 0.25 g of dextrose, 1.25 g of tryptone, and 3.75 g of plain agar, which was heated with a heater. After the media had adequately changed color (clear yellow), it was allowed to stand at roughly 25°C before placing 1/3 of the agar medium into the petri dish. The material was subsequently moved aseptically to a sterile petri dish, skim milk agar was added, and it was incubated for six days at 37°C. After incubation, the appearance of the sample was examined for the presence of the protease enzyme in the eco enzyme, which was indicated by the formation of a clear zone in the middle of the petri dish.

3. RESULTS AND DISCUSSION

Anaerobic fermentation was used to produce eco enzymes by reacting organic waste in the form of citrus and melon peels, coconut brown sugar, water, and baker's yeast in a batch system. The fermentation reaction is a reaction between cellulose in citrus and melon peels and water with baker's yeast as a catalyst. The yeast has an active microorganism (*Saccharomyces cerevisiae),* which could speed up the fermentation time, and it requires nutrients to provide energy when it is growing during the fermentation process for producing eco enzyme [2]. The energy is generally obtained from glucose contained in brown sugar [13].

$$\underset{\text{(Cellulose)}}{C_6H_{10}O_5} + \underset{\text{(Water)}}{H_2O} \xrightarrow{\textit{Saccharomyces cerevisiae}} \underset{\text{(Acetic Acid)}}{3CH_3COOH} \quad (1)$$

In this research, eco enzyme production was accomplished by varying the concentration of baker's yeast as a raw material. Figure 1 shows the results of observing the eco enzyme fermentation process for ten days at a baker's yeast concentration of 1 to 5% w/v.

Following the ten day fermentation process, eco enzyme product characteristic tests were performed at various baker's yeast concentrations (% w/v), including pH, TDS, acetic acid content, organoleptic properties (color and scent), and protease enzyme activity tests on the yield. Table 1 summarizes the results of this research.

(a) (b) (c) (d) (e)

Figure 1. The results of observing the eco enzyme fermentation process for ten days at a baker's yeast concentration of (a) 1% w/v, (b) 2% w/v, (c) 3% w/v, (d) 4% w/v, and (e) 5% w/v.

Table 1. The results of pH value, acetic acid content, TDS value, and organoleptic properties of eco enzyme products fermented with baker's yeast for ten days at a concentration of 1 to 5% w/v

No	Baker's Yeast Concentration (% w/v)	pH	Acetic Acid Content (% v/v)	TDS (ppm)	Organoleptic Properties	
					Color	Scent
1	1	4.2	1.35	1475	Pale Yellow	Fermented and Citrus Fruit Scent
2	2	4.2	1.35	1562	Pale Yellow	Fermented and Citrus Fruit Scent
3	3	4.1	1.62	1763	Pale Yellow	Fermented and Citrus Fruit Scent
4	4	4.2	2.03	2056	Dark Yellow	Fermented and Citrus Fruit Scent
5	5	4.2	2.03	2056	Dark Yellow	Fermented and Citrus Fruit Scent

3.1. The Effect of Baker's Yeast Concentration on The pH Value of Eco Enzyme Products

Figure 2 depicts the effect of baker's yeast concentration on the pH value of eco enzyme products.

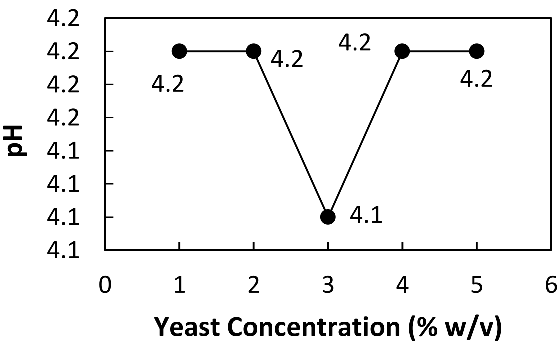


Figure 2. The pH value of eco enzyme products with the concentration of baker's yeast by fermentation process for ten days.

Based on the eco enzyme pH tests with baker's yeast in Figure 2, which were tested using a pH meter, the pH values of the five eco enzyme samples at a concentration of 1 to 5% w/v had pH values in the range of 4.1 to 4.2. The pH value is inversely related to the concentration of baker's yeast because adding more baker's yeast when making eco enzyme causes more acid to be produced during the fermentation process, leading the pH value to decrease. In this research, the pH value of the eco enzyme was in the pH range of 4. The eco enzyme pH value obtained in this research was the same as the eco enzyme pH value found by Rochyani et al. (2020), which was less than 4 to 4 [5]. This pH is classified as acidic (pH value less than 7). The acid solution in eco enzyme was formed due to the presence of acetic acid (CH_3COOH), which was produced during the fermentation process. The fermentation process, also known as anaerobic metabolism, is an attempt by bacteria to get energy from carbohydrates while remaining anaerobic (without oxygen) and producing by-products such as alcohol or acetic [14]. Acetic acid generally gives a sour smell to liquids or food, resulting from the metabolic processes of bacteria that are naturally present in fruit and vegetable waste [15]. As a result of the high content of organic acids such as acetic acid, the pH value of eco enzymes is low [16]. The pH value decreases over time due to the degradation of organic matter by microorganisms present in the eco enzyme solution [6].

3.2. The Effect of Baker's Yeast Concentration on The Acetic Acid Content of Eco Enzyme Products

Acid-base titration testing is used to determine the effect of baker's yeast concentration on the acetic acid content of eco enzyme products, as shown in Figure 3.

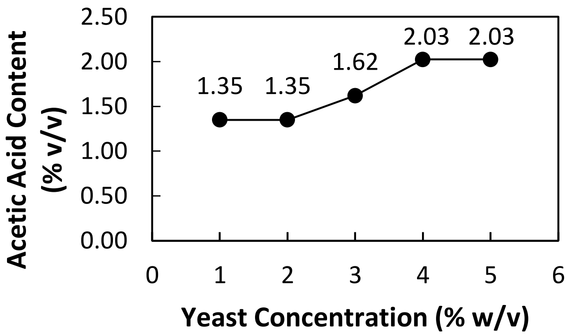


Figure 3. The acetic acid content of eco enzyme products with the concentration of baker's yeast by fermentation process for ten days.

Apart from pH and TDS tests, the acetic acid content of eco enzyme products could also be tested, as shown in Figure 3. Based on the result, it shows that the concentration of baker's yeast is directly proportional to the acetic acid content. When the baker's yeast concentration increases from 1 to 5% w/v, the acetic acid content also increases from 1.35% v/v to 2.03% v/v. According to Arun and Sivashanmugam's research (2015), eco enzyme fermentation could generate a variety of important organic acids, including acetic acid, lactic acid, malic acid, oxalic acid, and citric acid [17]. One of the organic acids, acetic acid, which is produced through the fermentation process, is obtained when the sucrose contained in the fermentation solution turns into alcohol and continues to become acetic acid [18]. The acetic acid contained in eco enzyme is capable of destroying germs, viruses and bacteria [12]. The acetic acid content test can be carried out by the acid-base titration method using a standard solution of 0.1 N NaOH.

Based on the results of this research, the acetic acid content increases with increasing fermentation time as well as the yeast concentration added in the production of eco enzyme. The higher the yeast concentration used, the more organic substances in the fermentation solution turn into alcohol and continue to become acetic acid, resulting in a higher acetic acid content. In addition, the pH value and acetic acid content show an inverse relationship, with the higher the acetic acid content in the enzyme, the lower the pH value.

3.3. The Effect of Baker's Yeast Concentration on the Total Dissolved Solid (TDS) Value of Eco Enzyme Products

Figure 4 shows the influence of baker's yeast concentration on the TDS value of eco enzyme products.

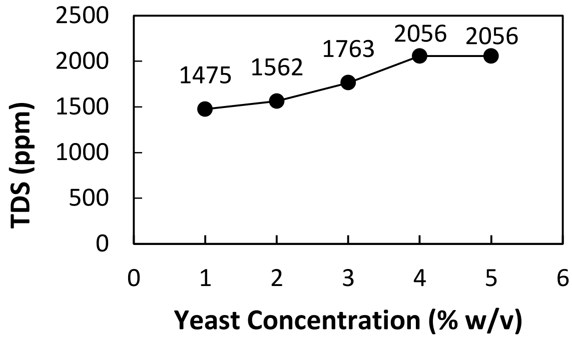


Figure 4. The TDS value of eco enzyme products with the concentration of baker's yeast by fermentation process for ten days.

TDS is total dissolved solids, which are generated from organic and inorganic compounds dissolved in a liquid solution. TDS in eco enzyme indicates that organic solids are dissolved in the eco enzyme solution. According to Figure 4, the concentration of baker's yeast is directly proportional to the TDS value, with a significant increase in the TDS value from a yeast concentration of 1 to 5% w/v, that is, from 1475 ppm to 2056 ppm. Based on Muliarta and Darmawan's research (2021), the range of eco enzyme TDS values ranges from 1000 to 2000 ppm [19]. The highest TDS value (2056 ppm) was obtained in eco enzyme products containing 4 and 5% w/v baker's yeast. The higher the TDS value in the eco enzyme, the more organic solids are dissolved in the product. It shows that eco enzyme fermentation could run optimally because organic waste raw materials could be degraded into eco enzyme products with the help of microorganisms. Furthermore, the raw materials used in the production of eco enzymes affect the pH and TDS values tested. According to Rochyani et al. (2020), the findings of eco enzyme pH and TDS testing using molasses differed from brown sugar [5]. When molasses is used instead of brown sugar, the value of these characteristics is reduced [17].

3.4. The Effect of Baker's Yeast Concentration on The Organoleptic Properties (Color and Scent) of Eco Enzyme Products

The effect of baker's yeast concentration on the organoleptic properties color of eco enzyme products could be shown in Figure 5.

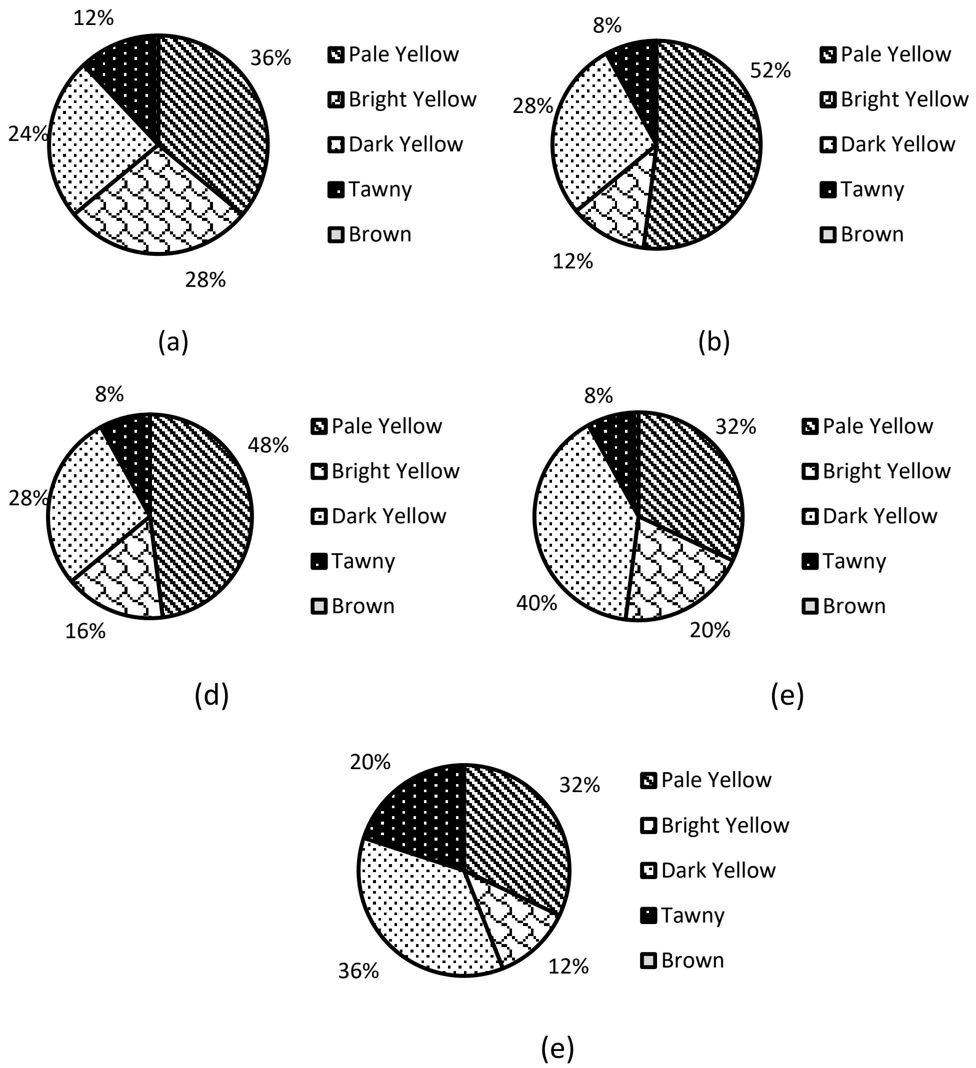
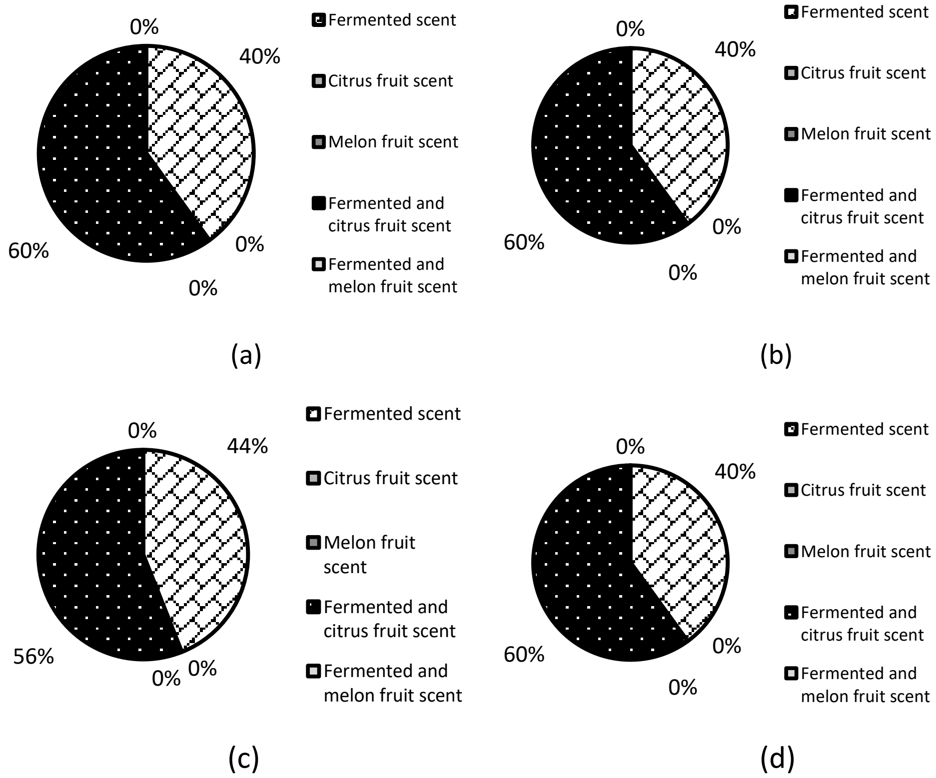


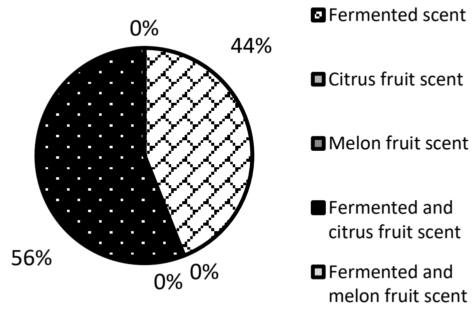
Figure 5. The results of observing and color tests for eco enzyme products using baker's yeast at concentrations of (a) 1% w/v, (b) 2% w/v, (c) 3% w/v, (d) 4% w/v, and (e) 5% w/v with a fermentation process for ten days.

Figure 5 shows that of the 25 panelists, the majority chose or rated pale yellow eco enzyme products at eco enzyme concentrations of 1 to 3% w/v. At eco enzyme concentrations of 4 to 5% w/v, the most common choice is dark yellow. Based on the research conducted, it shows that the higher the yeast concentration used in the production of eco enzyme, the more concentrated the color of the eco enzyme becomes. According to Hemalatha (2020), eco enzyme liquid had a dark brown color and a strong sour or fresh scent [3]. According to Larasati et al. (2020), the diverse colors of eco enzymes were caused by the raw ingredients used. The raw ingredients utilized in this research (citrus and melon peels, as well as brown sugar) cause the color of eco enzyme products to be yellow (pale yellow to brownish yellow). According to Larasati et al. (2020), molasses as a raw material for producing eco enzyme produces eco enzyme with a color ranging from brown to blackish brown [14].

Besides color tests, the organoleptic properties of eco enzyme products could be examined using scent tests. Figure 6 shows the results of the scent of eco enzyme products at a baker's yeast concentration of 1 to 5% w/v.

Figure 6 demonstrates that the majority of panelists chose the scents of fermentation and citrus fruits. The fermentation process in eco enzyme production is considered successful when a brownish solution appears with a citrus like or fruity odor and a pH less than 4 or an acidic pH value [12]. According to the results, all eco enzyme samples in this research have a fermented scent reminiscent of citrus fruits. It indicates that there is an alcoholic and sour scent because the eco enzyme produces alcohol and acids such as acetic acid during the fermentation process [14]. Aside from color, differences in raw materials influence the scents of the eco enzymes generated; for example, citrus peels utilized as key ingredients could enhance the scent of citrus in the eco enzyme products, as observed in this research.





(e)

Figure 6. The results of observing and scent tests for eco enzyme products using baker's yeast at concentrations of (a) 1% w/v, (b) 2% w/v, (c) 3% w/v, (d) 4% w/v, and (e) 5% w/v with a fermentation process for ten days.

3.5. The Effect of 5% Baker's Yeast Concentration on Protease Enzyme Activity of Eco Enzyme Products

The parameter test of pH value, acetic acid content, TDS value, and organoleptic properties of eco enzyme, the selected product could be obtained from the concentration of baker's yeast added at a concentration of 5% w/v. Figure 7 shows the test results for the protease eco enzyme activity at a concentration of baker's yeast of 5% w/v.

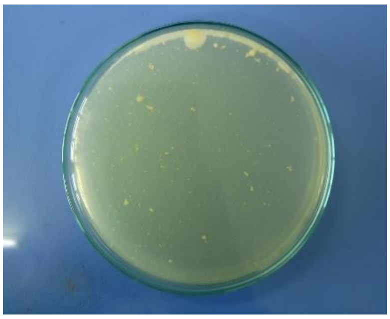


Figure 7. The result of observing for protease enzyme activity in eco enzyme products using baker's yeast at a concentration of 5% w/v with a fermentation process for ten days.

Some enzymes found in eco enzymes, including lipase, protease, and amylase, have the ability to destroy or prevent harmful germs. Protease is a proteolytic enzyme that converts protein molecules into short chains of oligopeptides or amino acids, which are subsequently utilized to enhance the potency of cleaning chemicals in stain cleaning operations. However, from the research conducted, the colonies formed were not translucent, whereas according to research from Vama and Cherekar (2020), the protease enzyme in eco enzyme products could be seen from the presence of a clear zone in the middle of the cup. It implies that it was not certain that the colonies generated were caused by a protease enzyme [20]. This disparity could be caused by an incompatible material composition and operating conditions.

4. CONCLUSIONS AND SUGGESTIONS

Based on the results of this research, the concentration of baker's yeast affects the end result of eco enzyme products such as pH value, acetic acid content, TDS value, organoleptic properties of color and scent, and enzyme activity such as protease enzymes. The higher the percentage of baker's yeast, the higher the pH, acetic acid content, and TDS value of the eco enzyme produced. The selected eco enzyme sample at a concentration of baker's yeast of 5% w/v has a pH value of 4.2, an acetic acid content of 2.03% v/v, and a TDS value of 2056 ppm, respectively. Increasing the concentration of baker's yeast in the production of eco enzyme results in a rise in acetic acid content. It influences the pH value, which falls as more acetic acid is produced, and subsequently influences the growing TDS value when producing eco enzyme. The characteristics of the selected eco enzyme products produced are that they have a deep yellow color and a fermented and citrus fruit scent. The protease enzyme activity test of eco enzyme products with a 5% w/v baker's yeast concentration showed the formation of colonies, but it was not consistent with previous research that stated protease enzyme activity could be seen by the formation of a clear zone in the middle of the pour cup method. Therefore, based on the test parameters of pH value, TDS value, acetic acid content, and organoleptic properties, it was possible to conclude that the higher the concentration of baker's yeast added in the production of eco enzyme, the better the properties of the products.

It could be suggested for future research to prepare skim milk agar with different compositions, incubate at the appropriate temperature, and observe in liquid culture. This procedure must be followed precisely because it has a significant impact on the colonies being observed.

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