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Comparison of Bioethanol Production Efficiency from Aren Nira (*Arenga pinnata Merr.)* Using Tempe Yeast and Bread Yeast through Fermentation Process in Region X

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ABSTRACT

Bioethanol production from palm sap (*Arenga pinnata* Merr.) represents a promising renewable energy alternative due to the high sugar content that facilitates efficient fermentation. This study compares the efficiency of bioethanol production using tempe yeast (*Rhizopus oligosporus*) and baker's yeast (*Saccharomyces cerevisiae*) at different concentrations during palm sap fermentation. Fermentation was conducted for five days, followed by distillation at 80–85 °C. Bioethanol yield and alcohol content were subsequently analyzed. The results indicate that tempe yeast produced a higher average bioethanol yield (45.59%) than baker's yeast (41.40%), with corresponding alcohol contents of 16.20% and 14.13%. The optimal yeast concentrations were 2 g/200 mL for tempe yeast and 10 g/200 mL for baker's yeast. Although the differences were not statistically significant, tempe yeast demonstrated more consistent fermentation performance. These findings suggest that tempe yeast is a promising alternative for enhancing bioethanol production from palm sap.

Keywords: bioethanol; palm sap; fermentation; tempeh yeast; baker's yeast

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1. Introduction

The aren tree, which belongs to the *Aracaceae* (*areca palm*) family, has a thornless and unbranched trunk, with a height of up to 25 meters and a diameter of up to 0.5 meters. The leaves are long-stemmed, reaching 1.5 meters, with leaflets 1.45 meters long and about 7 cm wide, and there is a waxy coating on the underside of the leaves. For a long time, people have recognized this tree as a source of raw materials for

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various handicraft industries. Almost all parts of the aren palm plant can be utilized and have a high economic value [1].

Palm sap is one of the non-timber forest products obtained from palm trees through the tapping process. The processing of palm sap has been widely developed to produce various products, including bioethanol. The high sugar content in palm sap allows the fermentation process to convert sugar into alcohol. Fresh palm sap is known to have a sugar content of 13.9-14.9% [2]. However, the natural fermentation process takes a long time to convert all the sugar into alcohol. A number of studies have been directed to accelerate this fermentation process, among others by utilizing bacteria, fungi, or enzymes produced by fungi. Some previous studies have used EM4 bacteria and enzymes from *Saccharomyces cerevisiae* fungi to increase bioethanol production. One other type of fungus that has been developed and is commonly used in the food industry is *Rhizopus oligosporus*, which is known as the main component in making tempeh yeast [3], [4].

The high sugar content of the sugar palm plant makes it have great potential as a raw material for ethanol production. Because this raw material contains sugar in solution form, the ethanol production process can be carried out more quickly, namely directly through the fermentation stage of palm sap (saguer). Sweet nira is obtained from tapping palm trees, but if left unprocessed, the nira will undergo natural fermentation and turn into tuak with low ethanol content. Under these conditions, the utilization of nira is usually limited to alcoholic beverages and raw materials for making palm sugar [5].

Bioethanol is an alternative fuel derived from plants and has the advantage of reducing CO_2 emissions by up to 18%. There are three main groups of bioethanol-producing plants, namely starchy plants (such as cassava, oil palm, tengkawang, coconut, kapok, jatropha, rambutan, soursop, malapari, and nyamplung), sugary plants (such as molasses, palm juice, sugarcane juice, and sweet sorghum juice), and cellulosic fiber plants (such as sorghum stalks, banana stalks, straw, wood, and bagasse) [6]. Ethanol or ethyl alcohol (C_2H_5OH) itself is a polar liquid solvent that is widely used to dissolve various substances and also in the sterilization process, especially in the medical and pharmaceutical fields. The increasing need for ethanol causes demand to continue to rise, so the price has also soared [7]. The limited supply occurs because most ethanol is still dependent on petroleum, which is finite and nonrenewable. To overcome this, various efforts have been made in developing ethanol from natural materials, known as bioethanol. One promising source of natural materials for bioethanol development is palm sap.

Yeast is an enzyme produced by a type of fungus and has the ability to break down sugar into alcohol. Yeast is often used in the fermentation process of various food products. One of the important stages in bioethanol production is fermentation, where sugar is converted into alcohol. This process can be done by utilizing the natural properties of yeast [8]. The use of yeast in bioethanol production from palm sap has been proven to accelerate the formation of alcohol, resulting in a shorter fermentation duration.

Therefore, a strategy is needed to switch to new and renewable energy sources that have great potential in Indonesia. One of the natural materials that can be utilized as raw material for bioethanol is the sugar palm plant. Bioethanol itself is a type of alternative energy that has similar characteristics to gasoline. Besides being used as fuel, bioethanol also functions as a solvent in various chemical processes and as an industrial raw material, such as in the production of formaldehyde and methyl esters [9].

However, despite the considerable potential of palm sap as a bioethanol feedstock, most previous studies have primarily focused on the use of *Saccharomyces cerevisiae* as the fermentation agent. Comparative investigations involving alternative yeast, such as *Rhizopus oligosporus*, particularly with respect to fermentation efficiency and yeast concentration, remain limited. Therefore, this study aims to compare the performance of *Rhizopus oligosporus* and *Saccharomyces cerevisiae* at different concentrations in the fermentation of palm sap for bioethanol production.

Based on the description above, this research intends to conduct research on bioethanol production from palm sap through a fermentation process using baker's yeast.

2. Materials and Method

This research was conducted over a two-month period, from January to May 2025. Palm sap sampling was carried out in Region X, while the fermentation and distillation processes were performed in a laboratory equipped with fermentation units, distillation apparatus, and alcohol content measurement instruments.

A total of 3 L of fresh palm sap was used as the main raw material. Tempe yeast (*Rhizopus oligosporus*), commercial baker's yeast (*Saccharomyces cerevisiae*, Fermipan), granulated sugar for starter preparation, and distilled water were used as additional materials. The equipment included Erlenmeyer flasks, measuring cylinders, a distillation flask with condenser, an electric heater, a thermometer, an alcohol meter, a pH meter, a digital balance, filter paper, test tubes, and aluminum foil.

Fermentation was performed using 200 mL of palm sap per fermentation vessel with yeast concentrations of 2, 6, 10, and 14 g per 200 mL, along with a control treatment without yeast. The treatments were grouped into three categories: fermentation using *R. oligosporus*, fermentation using *S. cerevisiae*, and a control without yeast addition. All samples were fermented for five days at room temperature under anaerobic conditions and protected from direct sunlight.

After fermentation, the samples were filtered to remove solid residues and subsequently distilled at 80–85 °C for 1–2 h to separate bioethanol. The distillates were collected and used for further analysis.

Some parameters observed in this study include the volume of bioethanol produced, alcohol content in bioethanol, and pH value before and after fermentation. Bioethanol volume was measured using a measuring cup, while alcohol content was measured with an alcohol meter. The pH value was measured using a pH meter. Parameters measured include the amount of bioethanol and alcohol content of bioethanol obtained. The amount of bioethanol obtained from the distillation process is measured with a glass measuring instrument (beaker) with units of mL. Measurement of alcohol content in the results of bioethanol is done using an alcohol meter. Bioethanol is put into a small and long tube container which is filled to the brim. The alcohol meter is inserted into the container containing bioethanol until the tool floats in the bioethanol liquid. The measuring scale of the alcohol meter parallel to the surface of the bioethanol liquid is the value of the alcohol content in bioethanol.

This research was analyzed by analysis of variance (ANOVA) with RAL pattern where the variable is the concentration of yeast in the palm at the level of 2 g/200 mL, 6 g/200 mL, 10 g/200 mL, 14 g/200 mL, and control (without yeast mixture). Parameters observed included pH level, amount of bioethanol, and alcohol content of bioethanol. Correlation analysis was used to see the relationship between the percentage of bioethanol amount and bioethanol alcohol content.

3. Result

pH value

Observation of pH value is one of the important parameters that need to be considered in the sugar fermentation process. The change of sugar structure into alcohol structure will be followed by an increase in the acidity of the material.

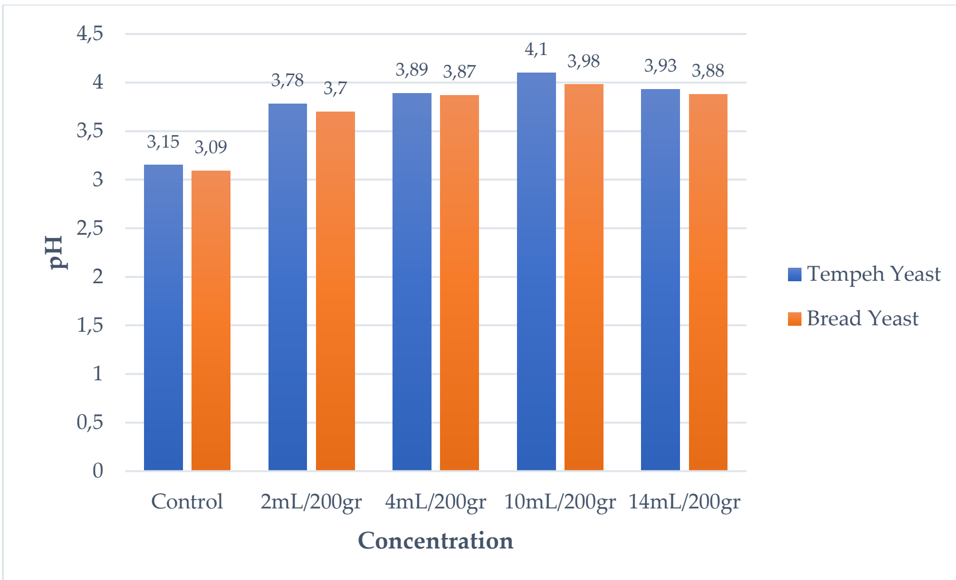

Figure 1. pH value of sugar palm before distillation process

Based on Figure 1, which shows the pH value of the arenes before the distillation process at various yeast concentrations, showed an increase in pH up to an optimum yeast concentration, followed by a slight decrease at higher concentrations. In the control sample without yeast addition, the lowest pH value was recorded, which was 3.15 for tempeh yeast and 3.09 for baker's yeast. An increase in yeast concentration from 2 mL to 10 mL per 200 grams of material showed a significant increase in pH, with the highest value reached at a concentration of 10 mL/200 g, which was 4.10 for tempeh yeast and 3.98 for baker's yeast. However, at a concentration of 14 mL/200 g, the pH decreased slightly, to 3.93 for tempeh yeast and 3.88 for baker's yeast, respectively. This indicates that a concentration of 10 mL/200 g is the optimum point in increasing the pH before distillation. After passing this point, the addition of more yeast does not have a significant effect on increasing pH, and even tends to decrease, possibly due to saturation of microorganism activity or accumulation of metabolites that inhibit the fermentation process. In addition, in general, baker's yeast showed a slightly higher ability to increase pH compared to tempe yeast at most concentrations tested.

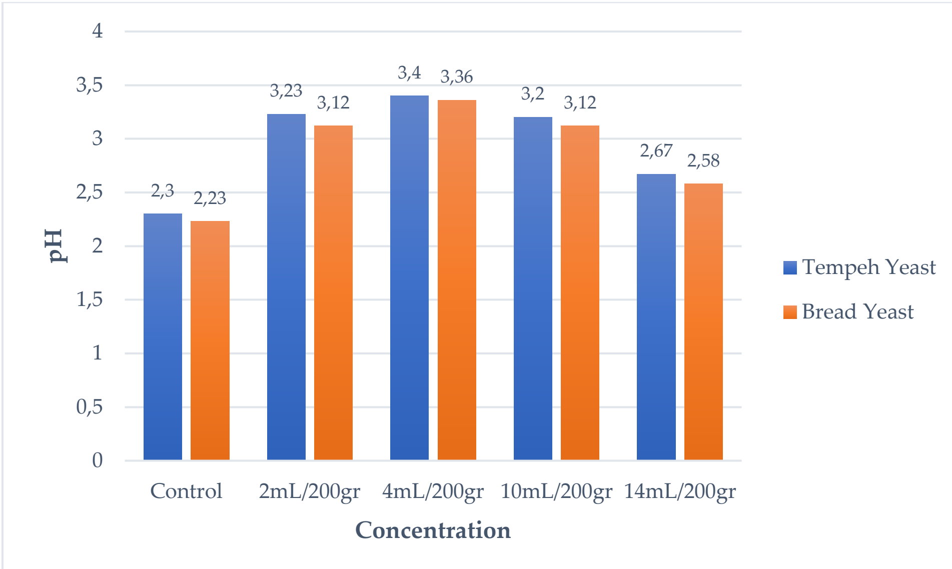

Figure 2. pH value of sugar palm after distillation process

Based on Figure 2, which shows the pH values of the arenes after the distillation process at various yeast concentrations, it can be seen that the distillation process caused a decrease in pH compared to the values before distillation. In the control sample without yeast addition, the lowest pH value was recorded, which was 2.30 for tempeh yeast and 2.23 for baker's yeast. As the yeast concentration increased up to 4 mL/200 g, there was an increase in pH value, where the highest pH was reached at that concentration, which was 3.40 for tempeh yeast and 3.36 for baker's yeast.

However, after passing the concentration of 4 mL/200 g, the pH value tends to decrease. At a concentration of 10 mL/200 g, the pH value decreased to 3.20 (tempeh yeast) and 3.12 (baker's yeast), and continued to decrease until the concentration of 14 mL/200 g became 2.67 and 2.58. This pattern shows that too high yeast concentration does not always have a positive effect on pH stability after distillation.

In general, tempe yeast and baker's yeast showed similar pH change patterns, with tempe yeast tending to produce a slightly higher pH than baker's yeast after distillation. This indicates that tempeh yeast may have a better influence in maintaining the pH stability of the arenes post-distillation. The concentration of 4 mL/200 g can be considered as the optimum point to maintain a more stable pH after the distillation process.

Palm sap bioethanol

Bioethanol measurement is done after fermentation of palm and separation by distillation technique. This process then produces a number of bioethanol as well as variations in the alcohol content contained in bioethanol which is a parameter of the size of the variables and methods used. The results of the amount and alcohol content of bioethanol are presented in Table 1.

Table 1. Percentage amount and alcohol content of bioethanol

Parameters	Control	2 g/200 mL	6 g/200 mL	10 g/200 mL	14 g/200 mL	Average
Bread Yeast						
Bioethanol Quantity (%)	37,36 ± 3,03	40,00 ± 2,00	38,33 ± 1,15	49,33 ± 5,33	41,00 ± 2,00	41,40^{tn}
Alcohol Content (%)	14,66 ± 2,00	13,33 ± 1,15	13,00 ± 1,00	15,66 ± 2,67	13,00 ± 1,00	14,13^{tn}
Tempeh Yeast						
Bioethanol Quantity (%)	38,33 ± 3,33	53,28 ± 16,57	45,00 ± 0,00	48,33 ± 3,33	43,00 ± 2,00	45,59^{tn}
Alcohol Content (%)	15,66 ± 2,00	14,33 ± 4,00	17,67 ± 0,67	14,00 ± 3,33	19,33 ± 2,30	16,20^{tn}

Description: tn = no effect; * = real effect (P<0,05); ** = very significant effect (P<0,01)

Based on Table 1 which shows the percentage of the amount and alcohol content of bioethanol in palm sap using baker's yeast and tempe yeast with various concentrations, it can be concluded that both the type of yeast and the concentration used have an influence on the yield of bioethanol. In general, tempe yeast produced higher bioethanol (average 45.59%) than bread yeast (average 41.40%). This was also reflected in the alcohol content, where tempe yeast produced higher alcohol content (16.20%) than baker's yeast (14.13%). Peak bioethanol production for baker's yeast occurred at a concentration of 10 g/200 mL, which was 49.33 ± 5.33%, while for tempe yeast, peak production occurred at a concentration of 2 g/200 mL, which was 53.28 ± 16.57%. Bioethanol production tends to decrease again at a concentration of 14 g/200 mL for both types of yeast. This is in line with the decrease in pH observed in the pH graph after previous distillation in Figure 2 which shows that at high concentrations, the pH decreases below the optimal range of fermentation thus decreasing fermentation efficiency and ethanol production.

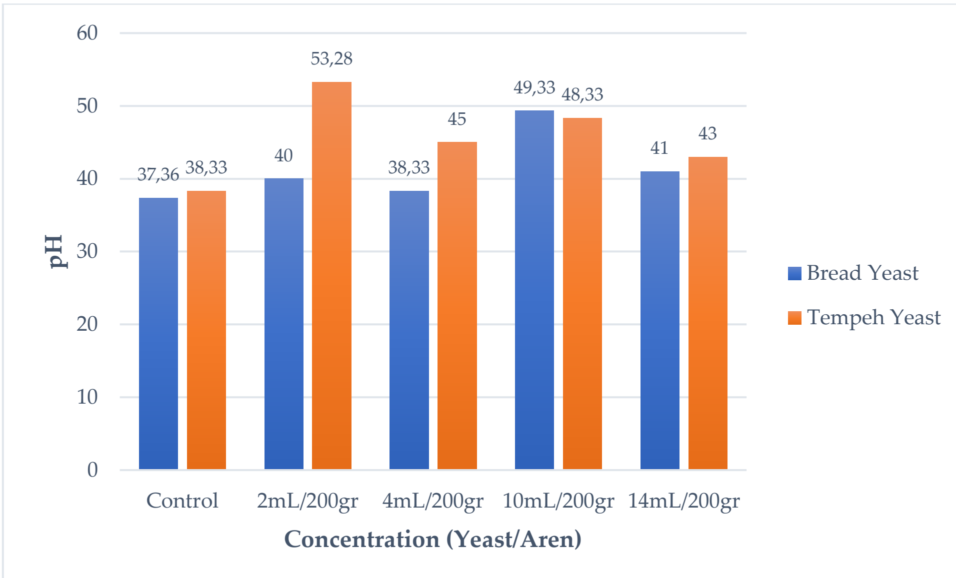

Figure 3. Amount of Bioethanol after fermentation with baker's yeast and tempeh

Figure 3 shows the amount of bioethanol produced after the fermentation process using two types of yeast, namely baker's yeast and tempe yeast, with variations in yeast concentration to the volume of palm solution (g/200 mL). In general, it can be seen that yeast concentration affects the amount of bioethanol produced, and there are differences in effectiveness between baker's yeast and tempeh yeast. In the control condition (without the addition of yeast), the bioethanol produced was quite low, about 37.36% with baker's yeast and 38.33% with tempe yeast. When 2 g of yeast per 200 mL of palm solution was added, there was a significant increase, especially in tempe yeast which produced the highest bioethanol of 53.28%, while baker's yeast produced 40.00%.

At a concentration of 6 g/200 mL, the amount of bioethanol decreased to 38.33% for baker's yeast and 45.00% for tempeh yeast. Then, at a concentration of 10 g/200 mL, both types of yeast showed increased results, with baker's yeast reaching 49.33% and tempeh yeast at 48.33%. However, at the highest concentration (14 g/200 mL), there was a decrease in the amount of bioethanol, which was 41.00% for baker's yeast and 43.00% for tempeh yeast. It can be concluded that the optimal yeast concentration to produce the highest bioethanol differs depending on the type of yeast. Tempe yeast produced the highest bioethanol at a concentration of 2 g/200 mL, while baker's yeast showed the best results at a concentration of 10 g/200 mL. However, overall, tempe yeast tends to produce higher amounts of bioethanol than baker's yeast at most concentrations.

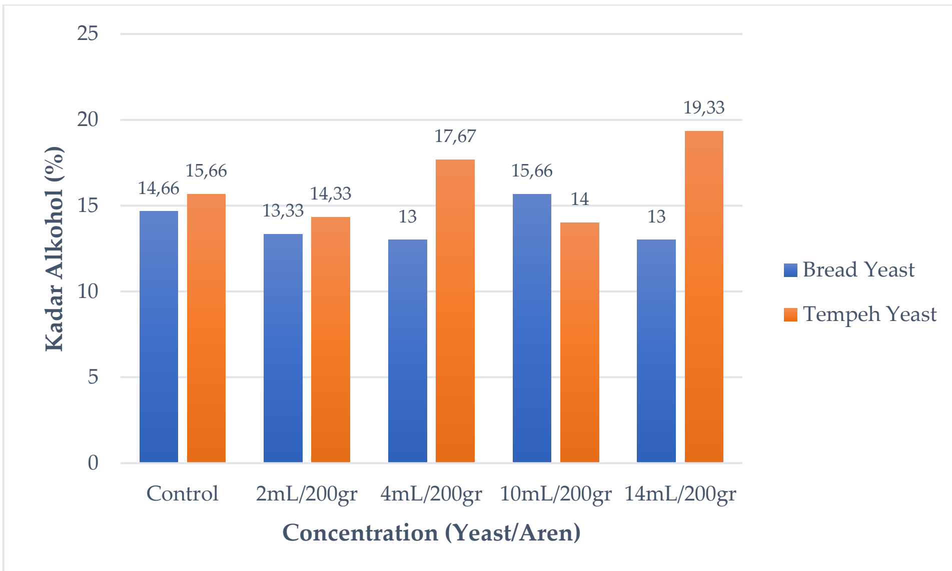

Figure 4. Alcohol content after fermentation with baker's yeast and tempeh

Figure 4 shows the alcohol content (%) produced after the fermentation process using two types of yeast, namely baker's yeast and tempe yeast, at various concentrations (g/200 mL) of palm wine solution. In general, it can be seen that tempe yeast tends to produce higher alcohol content than baker's yeast at most concentrations. In the control condition (without yeast addition), the alcohol content was 14.66% for baker's yeast and 15.66% for tempeh yeast. When 2 g of yeast was added, the alcohol content decreased to 13.33% for baker's yeast and 13.33% for tempeh yeast. A decrease also occurred in baker's yeast at a concentration of 6 g/200 mL (13%), while tempeh yeast actually experienced a significant increase to reach 17.67%, which is the highest alcohol content in tempeh yeast.

At a concentration of 10 g/200 mL, the alcohol content rose again to 15.66% for baker's yeast, while tempeh yeast decreased to 14%. At the highest concentration of 14 g/200 mL, baker's yeast produced 13% alcohol, while tempeh yeast again showed a drastic increase until it reached the highest alcohol content of all data, which was 19.33%.

It can be concluded that tempe yeast is more effective in producing high alcohol content than baker's yeast, especially at concentrations of 6 g and 14 g per 200 mL. In contrast, the effectiveness of baker's yeast was less stable and tended to produce lower alcohol content than tempe yeast, except at a concentration of 10 g/200 mL where the result was quite high. This shows that the type and concentration of yeast greatly influenced the alcohol fermentation results.

4. Discussion

The pH values obtained from the results of this study showed that in general there was a decrease in pH after the distillation process was carried out in all variations of yeast concentration, both tempeh yeast and baker's yeast. This indicates an increase in acidity which is an indication of increased fermentation activity. The highest pH decrease was recorded at a yeast concentration of 14 g/200 mL, where the pH dropped to 2.67 for tempe yeast and 2.58 for baker's yeast. This finding is in line with Mussa's opinion that a decrease in pH after fermentation reflects changes in taste and odor due to an increase in compounds from microbial metabolism [10]. In addition, this also supports the results of Yunus' research which states that the addition of tape yeast can cause palm juice to become more acidic [11]. On the other hand, these results are in contrast to Sinaga's findings who conducted fermentation without microbial assistance and produced a relatively neutral final pH (pH 6.8-6.9) [12]. The decrease in pH value in this study can be explained as part of the fermentation process where the sugar in the juice is converted into alcohol by microbes, which is generally accompanied by the formation of acidic compounds as by-products [10].

The results showed a variation in the percentage of bioethanol produced from palm sap depending on the type and concentration of yeast used. In general, the average amount of bioethanol produced reached 45.59% for tempe yeast and 41.40% for baker's yeast, with alcohol content of 16.20% and 14.13%, respectively. The highest percentage was obtained from fermentation using 2 g tempe yeast in 200 mL of nira solution, which amounted to 53.28%, while baker's yeast showed maximum production at a concentration of 10 g/200 mL at 49.33%. This value is higher than those reported by Riza and Adrianto, who reported bioethanol yields of 2.6-7.3% and 20-24%, respectively [13], [14].

Although the amount of bioethanol obtained is relatively high, the alcohol content produced in this study is still lower than the alcohol content of palm nira bioethanol in Luntungan and Ibrahim's research which reached 77.6-83.8% and 87.5%, respectively. This lower alcohol content may be associated with the distillation temperature applied, as temperatures exceeding the optimal boiling point of ethanol can promote water co-distillation [5], [15].

In addition, the provision of yeast did not have a significant effect on the percentage of bioethanol and alcohol content statistically. This assumption is strengthened by the presence of Rhizopus sp. fungus in tempeh yeast, which is known to be more active in breaking down fat into amino acids than converting sugar into alcohol efficiently [16]. This result is also consistent with Ardian's findings that tempeh yeast contains various types of Rhizopus, not all of which are effective in the alcohol fermentation process [17]. Bioethanol produced from the cooking process at high temperatures also tends to have high water content, as stated by Letelay who stated that heating near 100°C causes water to be distilled with ethanol, while temperatures of 75-85°C are more optimal in separating alcohol from water [18].

5. Conclusions

Based on the results of the research conducted, it can be concluded that the use of different types of yeast showed differing trends in bioethanol production efficiency, although the statistical analysis indicated no significant differences. Tempe yeast (Rhizopus oligosporus) generally showed superior results compared to baker's yeast (Saccharomyces cerevisiae) in terms of the amount of bioethanol produced and the alcohol content contained therein. The average amount of bioethanol produced with tempe yeast was 45.59% with 16.20% alcohol content, while baker's yeast produced an average of 41.40% bioethanol with 14.13% alcohol content. Peak bioethanol production for tempeh yeast occurred at a concentration of 2 g/200 mL, while baker's yeast achieved the highest results at a concentration of 10 g/200 mL. This shows that the effectiveness of fermentation is strongly influenced by the type of yeast and the dosage used. However, the use of tempe yeast proved to be more consistent in producing bioethanol with relatively higher alcohol content at most concentrations tested, so it can be concluded that tempe yeast is more efficient than baker's yeast in the fermentation process of palm sap for bioethanol production.

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