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Characteristic Test of Bioethanol Made from Leftover Fruit (Food Material After Ceremony) Using Yeast (Saccharomyces Cerevisiae)

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A B S T R A C T

Bioethanol can be produced from plants that contain a lot of starch and cellulose compounds by using the help of yeast activity from fruit skin waste. Sugar compounds in fruits are usually a mixture of glucose and fructose. Fruits contain carbohydrates, glucose, and fructose, so fruits have great potential to be raw materials for bioethanol. The study results obtained bioethanol with a long fermentation time of up to the fifth day with different yeast concentrations, namely 2%, 4%, 6%, 8%, and 10%. The maximum bioethanol concentration results were produced by a concentration of 6% S. cerevisiae, namely 8.17%, the content because the addition of yeast is by the amount of nutrients in the sample.

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INTRODUCTION

One solution to reduce waste is to convert organic solid waste into alternative energy, namely bioethanol. The raw materials for bioethanol can come from plants containing starch, sugar, and cellulose fiber. Alternative sources of raw materials that can be used are agricultural and organic waste. One of the agricultural wastes that is less useful and abundant is the remaining fruit (Food Ingredients After the Ceremony) (Illiya N. Gafiera, Fara P. Swetachattra, 2019).

Bioethanol can be produced from plants that contain a lot of starch and cellulose compounds with the help of yeast activity. Bioethanol is one of the biofuels that is present as an alternative fuel that is more environmentally friendly and renewable. Bioethanol is a renewable bioenergy, has little pollution, and can be produced from materials containing sugar and starch such as corn, potatoes, wheat, sugar cane, molasses, and others (Susmiati, 2018). Meanwhile, the use of agricultural land to produce bioenergy crops will compete with the cultivation of food crops (Dwi Novelia, Arief Yandra Putra, 2022).

Bioethanol (alcohol) production with plant raw materials containing starch or carbohydrates, is carried out through the process of converting carbohydrates into water-soluble sugar (glucose). As an alternative, a mixture of bioethanol and gasoline is used. Before being mixed, bioethanol must be purified to 100%. This mixture is known as gasohol (Gautama, 2009).

Various studies on the production of bioethanol by fermentation of yeast or enzymes from fruit peel waste have been widely conducted. The maximum bioethanol concentration is produced by a concentration of 2% S. cerevisiae, which is 5.44% because the addition of yeast is by the amount of nutrients in the sample. The order of concentration from highest to lowest is 2%> 4%> 3%> 5%> 1%. While the maximum pH value of 4.9 with a concentration of 2% is obtained in ethanol production by Saccharomyces cerevisiae (Muliarta et al., 2023). The use of yeast Saccharomyces cerevisiae in the fermentation of bioethanol from pineapple peel material obtained an optimum time of 3 days and a bioethanol content of 15.45% (Firmanto et al., 2014). The addition of 20–40 grams of Saccharomyces cerevisiae and a fermentation time of 2–10 days. The best results with the highest bioethanol content of 3.965% were obtained by adding 30 grams of yeast and a fermentation time of 10 days (Harimbi Setyawati, 2020).

The increase in bioethanol levels from whey with pineapple skin substitution was not affected by the fermentation time. The highest bioethanol levels in the range of 1.21–2.25% were obtained at 60 hours. Yeast for fermentation of Saccharomyces cerevisiae was used as much as 50 grams within 12-60 hours (Nulhakim et al., 2019). Bioethanol production from banana and pineapple peels by hydrolysis and anaerobic fermentation methods using Saccharomyces cerevisiae and Aspergillus niger. The results of the reduction in sugar concentration in pineapple and banana peels were respectively between 0.27 and 0.94 mg/cm3 and 0.20-0.82 mg/cm3 after 7 days of fermentation (Casabar et al., 2019). Bioethanol of 5.98% v/v ± 1.01 g/L can be produced in a

48-hour fermentation process using yeast and pineapple skin as raw materials (Itelima, J.U., et al., 2015).

Generally, the method of waste disposal is done by landfilling techniques. The main purpose of landfilling is to convert waste into soil and change it into the natural metabolic cycle. In terms of technical aspects, this process is filling the land using waste (Hidayati et al., 2018).

Vegetable and fruit waste has a high percentage. Sugar compounds in fruits are usually a mixture of glucose and fructose. Fruits also contain various organic acids, especially citric, malic, and tartaric acids. These acids play a role in the sour taste of young fruit. During ripening, the concentration of these acids will decrease, while the sugar concentration will increase. Because fruits contain carbohydrates, glucose, and fructose, fruits have great potential to be raw materials for bioethanol (Hidayati et al., 2018).

THEORETICAL REVIEW

Bioethanol (C2H5OH) is a biochemical liquid from the fermentation process of sugar from carbohydrates using the help of microorganisms. Bioethanol is also defined as a chemical produced from food ingredients containing starch, such as cassava, sweet potatoes, corn, and sago. Bioethanol is a fuel from vegetable oil that has properties similar to premium oil (Seftian et al., 2012).

Bioethanol is an alternative fuel derived from plants that have the advantage of reducing CO2 emissions by up to 18%. According to the Center for Starch Technology (B2TP) in Wusnah et al. (2016), three groups of plants can be used as bioethanol, namely plants containing starch (cassava, coconut, oil palm, rengkawang, jarak pagar, rambutan, soursop, nyamplung, and malapari), sugar (molasses or sugar cane drops, sugar cane sap, sweet sorghum sap, and aren sap), and cellulose fibers such as wood, banana stems, sorghum stems, straw, and bagasse) (Pangestu, 2022).

Rotten orange fruit waste consists of organic materials such as glucose (6.84%), fructose (5.12%), and sucrose (1.05%). Based on this, this study was conducted by utilizing orange peel and guava leaf waste to be converted into an alternative energy source, namely ethanol. The cell walls contain 25-35% cellulose, 50-60% hemicellulose, pectin, protein, and fat (Badger, 2002). Glucose can be further fermented into organic acids and ethanol. This study was conducted by utilizing orange fruit waste to be converted into an alternative energy source, namely bioethanol (Sutrisno et al., 2021).

Mango is a fruit plant that provides the third largest contribution to national fruit production after bananas and oranges. Indramayu mango has a carbohydrate content of 19.53%, fat of 3.80%, and protein of 3.78% (Matsuri, N. Istiana, A. Cristina, P. Dwijananti, 2015). Bioethanol is often written with the formula EtOH. The molecular formula of ethanol is COH, while the empirical formula is C2HO, and the structural formula is CH3-CH26-OH. Bioethanol is part of the methyl group (CH3-), which is linked to the methylene group (-CH2-) and linked to the hydroxyl group (-OH). In general, the acronym for bioethanol is EtOH (Ethyl-(OH)). 2H.5.

Hydrolysis is a chemical reaction between water and another substance that produces one or more new substances and also the decomposition of a solution using water. The addition of this catalyst serves to increase the activity of water so that the hydrolysis reaction runs faster. Catalysts that are often used are sulfuric acid, nitric acid, and hydrochloric acid (Retno & Nuri, 2011). In this reaction, a hydrochloric acid catalyst is used so that the reaction equation formed is as follows:

$$(C_6H_{10}O_5)_n + nH_2O \longrightarrow n(C_6H_{12}O_6)$$

Ethanol fermentation is the process of glucose metabolism in certain yeasts that occurs anaerobically so that it can convert glucose into ethanol. The use of Saccharomyces cerevisiae yeast is widely used to increase bioethanol production from sugar because it does not require sunlight in growth (Ismawadi, 2012). Saccharomyces cerevisiae in the form of yeast can be used directly as an inoculum in ethanol cultivation, so no special inoculum preparation is required (Ratnaningsih et al., 2009).

METHODOLOGY

Preparation of Materials

The first research procedure is to pretreat the remaining fruit waste (food ingredients after the ceremony), namely: bananas, oranges, and mangoes, namely by cutting the papaya into small pieces and then adding distilled water, then blending and taking the filtrate. Then take a sample with a weight of 100 grams of papaya pulp each and put it into a 500-ml Erlenmeyer flask.

Hydrolysis Process With H2SO4 With Various Temperature Changes

Hydrolysis of H2SO4 with a ratio of 1:6 based on volume/weight (v/b). Furthermore, 100 grams of fruit samples were added to the H2SO4 solution. Then the fruit sample was hydrolyzed at a temperature of 195oC and 210oC for 10 minutes. The hydrolysate was filtered with filter paper and cooled to room temperature (27oC). The concentration of H2SO4 was 0.5%. The ratio of biomass weight to H2SO4 volume was 1:6 (Nurkholis et al., 2019).

Treatment of Influence of Fermentation Time

After that, the fermentation process is carried out. Put yeast into the remaining fruit pulp (Food Ingredients After the Ceremony), namely: bananas, oranges, and mangoes with a concentration of 1, 2, 3, 4, and 5% of the weight of the papaya pulp. Then stir for 5 minutes until homogeneous. The liquid containing ethanol is separated by filtering using filter paper. Purification of the results is carried out using an evaporator to obtain bioethanol at a temperature of 79oC.

pH analysis

The pH value was obtained by utilizing an electric pH meter. Before use, the pH meter was calibrated first at pH 4 and pH 7 (Purbasari et al., 2014).

RESULTS

Results of pH Analysis Before and After Fruit Bioethanol Fermentation

The results of the differences in pH concentration before and after fruit bioethanol fermentation can be seen in table 1 and figure 1.

Table 1. Differences in pH concentration before and after fermentation of bioethanol from fruits

No	Sample	pH before fermentation	pH after fermentation process
1	2%	4.0	6.1
2	4%	4.7	6.4
3	6%	5.1	6.9
4	8%	5.8	7.3
5	10%	6.3	7.1

The results of pH testing after fermentation varied. This is shown in Figure 1.

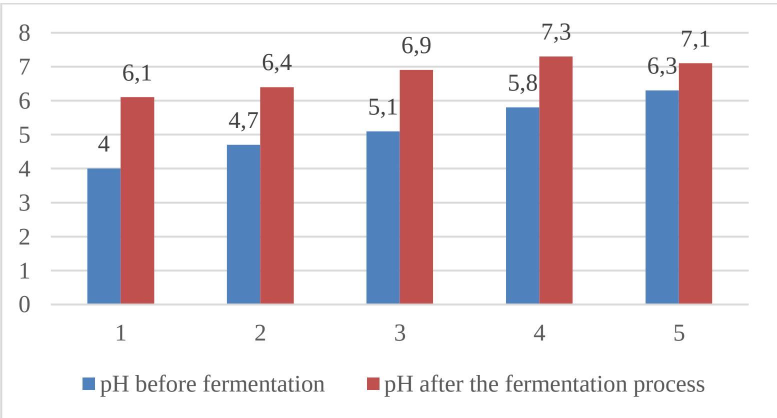

Figure 1. Results of Differences in pH Concentration Before and After Fermentation of Bioethanol from Fruits

The Effect of Fermentation Time on the Bioethanol Produced

Fermentation on the fifth day shows a comparison of bioethanol production produced by S. cerevisae in Table 5.1. and Figure 5.1.

Table 2. Amount of bioethanol content produced by S. cerevisiae

Concentration of S. cerevisiae	Ethanol Content
2%	4.28%
4%	5.88%
6%	8.17%
8%	7.85%
10%	5,83%

The highest bioethanol yield was produced by a concentration of 5% S. cerevisiae, which was 8.78%. The order of concentration from highest to lowest was 2% > 10% > 4% > 6% > 8%.

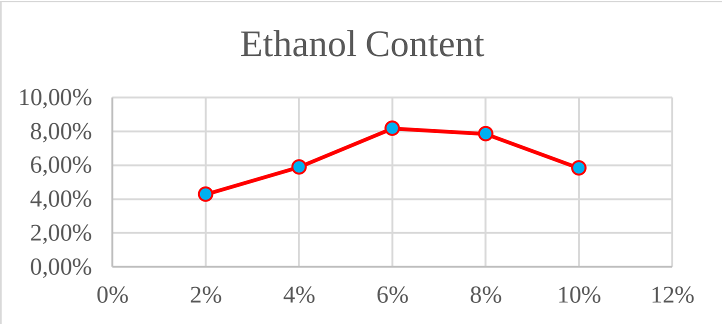

Figure 2. Amount of bioethanol produced

Effect of Reducing Sugar Levels

Table 3. Absorbance Value of Glucose Bioethanol from Fruits

Concentration (ppm)	Absorbance
0	0.000
20	0.119
40	0.2490
60	0.3960
80	0.5150
100	0.6470

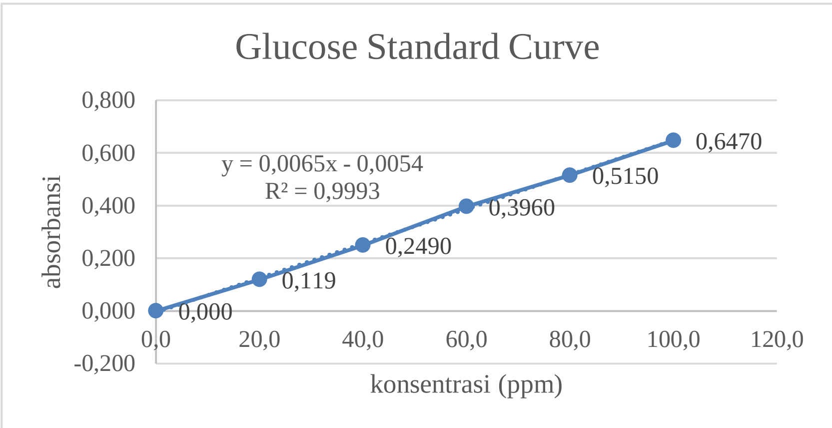

Figure 3. Standard Curve of Bioethanol Glucose in Fruits

Table 4. Initial Slurry Glucose before Hydrolysis

No	Sample Code	Weight		Concentration	Absorbance	Concentration		Dilution Factor	Reducing Sugar (Glucose) Content
		g	mg	mg/mL		ppm	mg/ml		%
1	U1	200	200000	500	0.1286	20.6154	0.0206	100	0,412
2	U2	200	200000	500	0.1288	20.6462	0.0206	100	0,413
3	U3	200	200000	500	0.1282	20.5538	0.0205	100	0,411

Effect of Glucose Hydrolysis of Fruit Bioethanol with 0.5% H2SO4 at a Temperature of 195oC

Table 5. Glucose Hydrolysis of Fruit Bioethanol with 0.5% H2SO4 at a Temperature of 195oC

No	Sample Code	Weight		Concentration	Absorbance	Concentration		Dilution Factor	Reducing Sugar (Glucose) Content
		g	mg	mg/mL		ppm	mg/ml		%
1	U1	10	10000	100	0.8216	127.2308	0,1272	10	1.272
2	U2	10	10000	100	0.8218	127.2615	0,1272	10	1.273
3	U3	10	10000	100	0.8215	127.2154	0,1272	10	1.272

Effect of Glucose Hydrolysis of Fruit Bioethanol with 0.5% H2SO4 at a Temperature of 210oC

Table 6. Glucose Hydrolysis of Fruit Bioethanol with 0.5% H2SO4 at a Temperature of 210oC

No	Sample Code	Weight		Concentration	Absorbance	Concentration		Dilution Factor	Reducing Sugar (Glucose) Content
		g	mg	mg/mL		ppm	mg/ml		%
1	U1	100	100000	1000	0.2324	36.5846	0.036585	500	1.829
2	U2	100	100000	1000	0.2323	36.5692	0.036569	500	1.828
3	U3	100	100000	1000	0.2322	36.5538	0.036554	500	1.828

DISCUSSION

Results of pH Analysis Before and After Fruit Bioethanol Fermentation

The change and decrease in the initial pH of the substrate during the fermentation process is caused by the formation of secondary yeast metabolism by-products in the form of organic acids that dissolve together with ethanol products. This shows that the pH required for yeast growth is still within the range of previous research results by Budianti (2016), namely between pH 3-5.

In fermentation, pH is used to create environmental conditions as a place for Saccharomyces cerevisiae to grow in converting glucose into ethanol. Incompatibility of pH with the fermentation environment can be detrimental to yeast growth (Rosdee et al., 2020). Low pH can slow down the fermentation process, while higher pH will increase fermentation activity but can produce by-products in the form of glycerin (Roukas, 1996). This indicates the optimum time in the fermentation process. The condition that occurs after the optimum time is that the ethanol content decreases because the nutrients in the solution are increasingly depleted, so many microbes die (Kurniati et al., 2021).

Acidity indicated by pH value in the fermentation process greatly affects the metabolism of microbes growing in the fermentation medium. To grow optimally, microbes are generally conditioned at a pH range of 3–8. The microbes used in this study were the Saccharomyces cerevisiae type. Based on the pH zone, this type of microbe is included in the acidophilic microbe group, namely microbes that can grow well at a pH ranging from 2.0 to 5.0 (Mudya, 2019). Observations were made for two different pH conditions, namely 4 and 5. This pH range provides the maximum microbial growth so that enzyme activity is also at its maximum point [8]. The results of the analysis at the end of the fermentation reaction showed that slightly higher ethanol levels were produced at pH 4 compared to pH 5, indicating that the enzyme works most optimally at that pH. The maximum ethanol content produced was 6.73%. (Herliati et al., 2019).

The most optimal fermentation time for the bioethanol production process is 4 days. If fermentation is carried out for more than 4 days, the alcohol content can decrease (Minarni et al., 2013). The decrease in ethanol content is caused by the alcohol being converted into other compounds, such as esters. The longer the fermentation time, the number of microorganisms will decrease and will enter the death phase because the alcohol produced is increasing and the nutrients available as microbial food are decreasing (Uun Kunaepah, 2008). The accumulation of high concentrations of Saccharomyces cerevisiae metabolism inhibits growth and causes death in Saccharomyces cerevisiae cells (Wignyanto et al., 2001).

The Effect of Fermentation Time on the Bioethanol Produced

From the analysis results, it can be concluded that with a yeast concentration of 6%, the maximum results are obtained because the addition of yeast is by the amount of nutrients in the sample. With the addition of yeast doses of 8% and 10%, the bioethanol produced decreased because the productivity of microorganisms decreased due to the lack of nutrients needed by the microorganisms. If too much yeast is added, it will also produce a lot of acid, and the bioethanol produced will also be small.

Rahayu's findings (2019) state that Saccharomyces cerevisiae can oxidize sugar into CO2 and water with the help of oxygen. S. cerevisiae yeast converts 70% of glucose in the substrate into CO2 and alcohol. According to Andriani's data (2013), there is a glucose content in papaya of 8.23%. So it can be stated that the glucose content of the fruit has great potential to be reused as a raw material for bioethanol fermentation.

The bioethanol process using S. cerevisiae in the process requires simple and more complex sugar substrates in the form of starch, starch, and lignocellulose (Da Silva Fernandes et al., 2022; Nurjanah et al., 2020). The composition of this substrate affects the fermentation of several carbohydrates into ethanol. Lignocellulose, in this case, pentose, namely xylose, cannot be fermented by S. cerevisiae (Matsushika et al., 2009). In addition, the number of S. cerevisiae yeast cells affects bioethanol production, where 5% of S. cerevisiae shows a decrease in the production of bioethanol. This decrease is due to the excessive amount causing competition for nutrients. This is also evidenced by the research of Nasrun et al. (2017), where the amount of 20 grams of S. cerevisiae showed a decrease in alcohol content and 15 grams of S. cerevisiae showed the best results.

Hydrolysis is carried out using chemicals, namely sulfuric acid, to obtain glucose, which will be used as a source of substrate in fermentation media that can be converted into bioethanol by microorganisms under appropriate conditions. The results of fiber hydrolysis are in the form of a glucose solution whose concentration is analyzed using an anthrone reagent with a spectrophotometer through visible light, namely by measuring glucose absorbance at λ = 545 nm, which has been previously determined. The results of the initial glucose concentration of the hydrolysis process can be seen in the table below.

The glucose obtained is the result of the reaction between sulfuric acid and cellulose. The mechanism that occurs is that the protons from the acid will interact quickly with the glycosidic bonds of oxygen in the two sugar units so that they will form conjugate acids. The presence of conjugate acids causes an unstable conformation so that the C-O bond is broken and the conjugate acid is released in an unstable conformation. The presence of water in the system will cause OH- from water to bind to the carbonium ion, thus releasing sugar and protons. The protons formed will interact quickly with the glycosidic bonds of oxygen in the other two sugar units. This process occurs continuously until all cellulose molecules are hydrolyzed into glucose (Minarni et al., 2013).

Effect of Reducing Sugar Levels

Reducing sugar is one of the determinants of honey quality according to SNI; reducing sugar is also one of the main components of honey, which consists of 2 groups of monosaccharides consisting of glucose and fructose. By the fruit quality standards that have been set by SNI 3545:2013, the reducing sugar content is set at a minimum of 65% b/b. The reduced sugar content calculated is the glucose content. Based on the results of the analysis of the reducing sugar content test, the results of storing kelulut honey for a long time have a high reduced sugar content.

This is influenced by the high activity of the diastase enzyme that hydrolyzes sucrose into glucose and fructose. The diastase enzyme in fruits can convert starch into glucose with the help of iodine, which will change the color of the solution. Hydrolysis of starch in acidic fruit conditions will break down into simple molecules, and the result is glucose. Low activity of the diastase enzyme can cause the reduced sugar content of honey to not meet SNI standards.

The decrease in reduced sugar levels can also be caused by a reversal in acidic conditions that increase the levels of long-chain sugars in the form of oligosaccharides and polysaccharides (Crane et al., 1979). Glucose greatly influences the rate of formation and speed of crystallization that occurs in honey. The rate of crystallization of honey depends on the ratio of glucose to water and glucose to fructose.

Effect of Glucose Hydrolysis of Fruit Bioethanol with 0.5% H2SO4 at Temperatures of 195oC and 210oC

This can be seen from the changes in reducing sugar values before and after inversion. From the table results and diagram curves above, another factor that affects changes in reducing sugar values in fruits is the acid content. The sucrose content is non-reducing because it does not have a reactive OH group, but with the presence of an acid, sucrose will be hydrolyzed (decomposition of substances) with the help of heat into inverted sugars, namely glucose and fructose, which are reducing sugars. The breakdown of glycosidic bonds due to heating will allow non-reducing sugars (sucrose) to be broken down into reducing sugars such as glucose and fructose (Seal et al., 2020).

The reducing sugar content also plays a role in the browning process. The brownish color of fruits is caused by the Maillard browning reaction and caramelization, which produce melanoidin pigments (brown pigments). The heating process can cause a Maillard reaction between sugar and amino acids contained in the sap liquid, resulting in a brown color. The color of palm sugar that is too pale is caused by a browning reaction that occurs and is not perfect (Crysse et al., 2013).

The heating temperature of an oven affects the caramelization reaction that occurs during fruit cooking. The caramelization reaction occurs because the fruit (glucose, fructose, sucrose, etc.) is heated to its melting point, so the higher the cooking temperature, the higher the color intensity of the resulting fruit. In addition, inverted fruits can undergo oxidation into acids (aldonic acid and ketonic acid) (Adna Ridhani & Aini, 2021).

The process known as sucrose inversion is the hydrolysis of sucrose into glucose and fructose. Fruits with high glucose content or inversion fruits will be difficult to harden and have a short shelf life (Ellya Indahyanti, Budi Kamulyan, 2014). The higher the concentration, the lower the level of reducing sugar produced, and for natural preservatives, the level of reducing sugar is higher than the level of reducing sugar produced.

Reducing sugar is a sugar that has a free aldehyde group in its chemical structure. The content of reducing sugar plays a role in the browning process of fruits. Reducing sugar also affects the level of sweetness because glucose and fructose have low levels of sweetness (Kusuma et al., 2017). The results of the analysis of variance showed that the calculated F value > F table at the concentration of natural preservatives had a significant effect on the level of reducing sugar produced. High levels of reduced sugar cause sugar to become more hygroscopic (easily absorb water) so that it melts easily during storage (Fitriyanti et al., 2020). Fruits also affect the quality of fruits; the lower the sugar value, the higher the quality of the fruits produced, because it affects the hardness, color, and taste of the fruits (Naufalin et al., 2013). Reducing sugar according to SNI-01-7343-95 is 10% (National, 2015).

In all treatments, the reduced sugar content was low, which was below the maximum limit of the SNI standard of 10% bb. The lower the reduced sugar value, the higher the quality of the fruit, which will affect the level of hardness, color, and taste of the fruit (Naufalin et al., 2013). The color produced is influenced by the reduced sugar content produced; the higher the reduced sugar content, the more brownish the fruit produced, in contrast to the color produced by fruits with low reduced sugar content, which have a more reddish brown color. The reduced sugar content plays a role in the browning process of fruit; inverted sugar contains a lot of reduced sugar, which will experience more browning so that the color is browner (Zuhrotun et al., 2023). Reducing sugar also affects the level of sweetness because glucose and fructose have low levels of sweetness. In addition, the color of the fruit is also caused by cooking; this is due to the statement that heating for too long will cause caramelization and changes in the color of the fruit (Kurniati et al., 2021). The process and results of the bioethanol analysis test can be seen in Figure 4.

Samples from Fruits

Bioethanol sample preparation process from Fruits

Bioethanol Testing Process from Fruits

Bioethanol Results

Figure 4. Process and results of bioethanol analysis tests

CONCLUSIONS AND RECOMMENDATIONS

1. The study results obtained bioethanol fermentation time that was carried out until the fifth day with different yeast concentrations, namely 2%, 4%, 6%, 8%, and 10%. This is because microorganisms have adapted to the environment and available nutrients so that microorganisms grow and divide a lot and their numbers increase rapidly compared to other days.
2. The maximum bioethanol concentration results were produced by a concentration of 6% S. cerevisiae, which was 8.17% because the addition of yeast was by the amount of nutrients in the sample. With the order of concentration from highest to lowest being 2%> 10%> 4%> 6%> 8%. While the maximum pH value of 7.3 with a concentration of 8% was obtained in ethanol production by Saccharomyces cerevisiae.

FURTHER STUDY

The results of this study are expected to provide real contributions to efforts to utilize organic waste, especially leftover fruits after religious ceremonies. By knowing the characteristics of the bioethanol produced, its potential use as an alternative fuel or industrial raw material can be explored further. In addition, this study can also be a reference for further, more in-depth research on bioethanol production from various types of organic waste.

For the development of the bioethanol production process from fruit waste, it is recommended to optimize several process parameters, such as fermentation temperature, fermentation time, substrate concentration, and yeast type. In addition, further research is needed on effective pretreatment methods to increase the efficiency of starch and cellulose hydrolysis processes in fruit waste. Thus, higher bioethanol yield and lower production cost can be obtained.

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