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Bread Yeast Fermentation Method Production of Papaya (Carica Papaya L.) Fruit Bioethanol

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A B S T R A C T

Making bioethanol from papaya fruit is done through a fermentation process. Yield is influenced by the duration of fermentation and the mass variation of the bread yeast Saccharomyces cerevisiae. The pH treatment and initial sugar content of the substrate were also tested before the addition of glucose. This study aims to examine the effect of yeast concentration and fermentation time on the amount of bioethanol produced from papaya fruit in the manufacture of bioethanol. The maximum concentration of bioethanol produced by a concentration of 2% S. cerevisiae is 5.44% due to the addition of yeast, according to the number of nutrients in the sample. In order of concentration from high to low, it is 2% > 4% > 3% > 5% > 1%. While the maximum pH value of 4.9 with a concentration of 2% was obtained in ethanol production by Saccharomyces cerevisiae.

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INTRODUCTION

Indonesia is an archipelagic country producing various types of fresh fruits in Southeast Asia, such as papayas, oranges, rambutans, pineapples, etc. According to data from the Central Statistics Agency (2020), pineapple production in Indonesia from 2017–2020 decreased from 1,795,986 tons to 1,641,087 tons. Meanwhile, papaya production in 2017–2020 increased from 875.112 metric tons to 937.639 metric tons. Bener Meriah District in Aceh is known as a papaya-producing area in Aceh (Statistics, 2020a). According to Aceh BPS data (2020), papaya production increased from 106,847 quintals to 137,913 quintals. Most of the fruits produced in several provinces are not yet processed into canned fruit products or other economically valuable products, so they are only used as table fruit, and the contents and skins are taken as waste (Statistics, 2020b). This is the background for researching the effect of adding bread yeast mass variations on the production of bioethanol by fermentation from papaya fruit (Salian et al., 2022).

Various studies on the manufacture of bioethanol by yeast or enzyme fermentation from fruit waste materials have been carried out. Fermentation of sugars and starch components of plants can be a source of bioethanol in the manufacture of renewable energy. Bioethanol comes from corn, sugarcane, potatoes, rice, beetroot, and waste from the processing of agricultural products (R. Nurjanah et al., 2020). The conversion of bioethanol can be processed directly from sugar, starch, and cellulose lignin. One source of bioethanol that is easy to use is papaya fruit. According to Nurhakim (2019), 100 grams of papaya fruit contain sugar, 86.7 grams of water, 12.2 grams of carbohydrates, and 46 calories. Based on this, papaya fruit has the potential to be used as a raw material for bioethanol because there are still high levels of carbohydrates and sugar (Nulhakim et al., 2019).

Setyawati's research (2017) states that adding 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 with the addition of 30 grams of yeast and a fermentation time of 10 days (Harimbi Setyawati, 2020). In addition, according to research conducted by Itelima (2015) using Saccharomyces cerevisiae and Aspergillus niger in the production of bioethanol from banana and pineapple peels by hydrolysis and anaerobic fermentation methods, The results of reducing the concentration of sugar in pineapple and banana peels were between 0.27 and 0.94 mg/cm3 and 0.20 and 0.82 mg/cm3 after 7 days of fermentation (Itelima, 2015). Meanwhile, Casabar et al. (2019) concluded that bioethanol of 5.98% v/v ± 1.01 g/L could be produced in a 48-hour fermentation process using yeast and raw pineapple skin.

Papaya (Carica papaya L.) is a tropical plant from southern Mexico that is now widespread throughout the tropics. Papaya has a fairly high nutritional value; the nutrients needed by humans are carbohydrates, protein, vitamins, and minerals. Papaya protein content is only 4-6 grams per kilogram of fruit weight. This low amount can all be absorbed and digested by the body (Novelina & Amelia, 2023). Papaya also contains pectin and papain, which have the ability to eliminate hunger for a full day. papain enzyme, which can speed up the digestive process (Jusniati, Patang, 2017).

One type of organic waste that has not been utilized is papaya fruit waste. Seeing the amount of papaya fruit that has experienced quite a lot of decay can certainly cause problems in the environment. Some communities have used rotten papaya fruit, which has become waste that is managed to be used as material in the manufacture of bioethanol (Ramadhany et al., 2023). Bioethanol is a chemical that can be used as a solvent, an antiseptic, and an alternative fuel (Hartantio et al., 2018). Raw materials for ethanol production consist of three types: raw materials containing sucrose, starch, and lignocellulosic Raw materials containing sucrose, which are first-generation raw materials, are widely used because the conversion process into bioethanol is simpler and does not need to go through hydrolysis as is done for raw materials containing starch and lignocellulosic (Thamrin, 2022).

Making bioethanol from papaya fruit is done through a fermentation process. Yield is influenced by the duration of fermentation and mass variation of the yeast Rotisacharomyces crevice. The pH treatment and initial sugar content of the substrate were also tested before the addition of glucose. The process of adding glucose is carried out to increase the yield of bioethanol and is useful as an additional source of carbohydrates. This study aims to examine the effect of yeast concentration and fermentation time on the amount of bioethanol produced from papaya fruit in the manufacture of bioethanol. It is hoped that the results of this bioethanol research can provide an alternative technology for engineering agricultural waste into a potential renewable energy source from biomass.

THEORETICAL REVIEW

Bioethanol is an alcohol consisting of hydrogen and carbon. Ethanol can be made from the cooking, fermentation, and distillation processes of several types of plants. So that this bioethanol has the potential to be quite bright as a substitute for gasoline because the material and manufacture are fairly easy (Shodiqin, 2013). Bioethanol is a fuel made from plants that contain high levels of carbohydrates, such as sugarcane, sap, palm sugar, sorghum, cassava, cashew nuts (cashew waste), arrowroot, banana stems, sweet potatoes, corn, corn cobs, and straw. The raw materials for the production of bioethanol are sugar, starch, and cellulose. 20).

Bioethanol is part of a metal group (CH3-), which is linked to a methylene group (-CH2-) and linked to a hydroxyl group (-OH) so that the molecular formula of bioethanol can also be written as EtOH (Ethyl-OH) (Mukhlsi, Fakhri Yacob, 2023). Then materials containing glucose can be fermented directly into ethanol; however, starch disaccharides and complex carbohydrates must be hydrolyzed first using acid or enzymatic hydrolysis to become components that are simple to produce ethanol during the fermentation process (Nur Alam et al., 2022).

Papaya fruit waste contains nutrients, namely 1.87% nitrogen, 3.13% phosphorus, and 3.28% potassium, which are needed by plants so that they can be absorbed into the soil (topsoil), because the process of stimulating the decomposition of microorganisms can procure nutrients, namely papaya fruit, and can expand absorption for plants (Ramadhany et al., 2023). In line with that, Susi et al. (2018) said that organic waste contains nitrogen, phosphorus, and potassium nutrients that spur plant growth. Nitrogen functions as a constituent of chlorophyll, increasing growth and protein synthesis. Phosphorus functions to form ATP and coenzymes for energy storage. Potassium functions in enzyme activation, stomata activity, and the water balance system (Susi, 2019).

The fermentation process in this study used baker's yeast and tape yeast with a concentration of 0.1 g to produce a high bioethanol content. Factors that affect the fermentation process, namely Acidity (pH), Microbes, Temperature, and fermentation temperature, determine the dominant microbial type, and at a temperature of 10–30 OC, more alcohol is formed because yeast works optimally at that temperature (Bahri et al., 2018).

Fermentation generally uses the yeast Saccharomyces cerevisiae (Anggreni, 2013). Saccharomyces cerevisiae has been used for thousands of years in food and beverage production and is by far the most studied yeast species and the most widely used microorganism in first-generation bioethanol production from sugar or starch crops (Radecka et al., 2015). Saccharomyces cerevisiae is a species of yeast, better known in Indonesia as yeast, that has had an extraordinary history in the fermentation industry because of its ability to produce alcohol. Saccharomyces cerevisiae has long been used in the alcohol and alcoholic beverage industries because it can ferment glucose into ethanol. Some of the advantages of Saccharomyces in fermentation are fast breeding, resistance to high alcohol levels, resistance to high temperatures, stable properties, and quick adaptation (Kerina et al., 2022).

Treatment of the pH of the fermentation medium will affect the production of bioethanol. pH is the acid-base condition of a microorganism's medium, which can affect the growth (cell division activity) of a particular microorganism. pH is the acid-base condition of the fermentation medium, which is related to the activity of the growth of microorganisms. A pH that is too low (acid) or too high (alkaline) can trigger the death rate of microbial cells. The high death rate of microorganisms will affect the speed of fermentation because the number of microbes will decrease when reducing glucose to ethanol (Taslim et al., 2017).

METHODOLOGY

Material Preparation

The first research procedure was to pretreat the papaya fruit by cutting the papaya into small pieces and then giving it aqua dest with a ratio of 1:1 to the feed weight, then blending it and taking the filtrate. Then take a sample weighing 200 grams of papaya pulp and put it in a 500-ml Erlenmeyer.

Treatment Effect of Papaya Bioethanol Fermentation Time

After that, the fermentation process is carried out. Enter the yeast into the papaya fruit with a concentration of 1, 2, 3, 4, and 5% by weight of the papaya pulp. After that, a 500-ml Erlenmeyer containing papaya fruit pulp was connected to a rubber hose, and the end of the hose was put into the water to prevent direct contact with air. Then the solution is fermented for 5 days. Then the solution and the papaya fruit pulp are separated to obtain an alcohol-water liquid. Liquid containing ethanol is separated from the papaya fruit by filtering it using filter paper. Purification of the results is carried out using an evaporator to obtain bioethanol at a temperature of 80^{o}C.

pH Analysis of Papaya Fruit Bioethanol

The pH value is obtained by using an electric pH meter. Before use, the pH meter is calibrated first (Purbasari et al., 2014).

Analysis of Bioethanol with the Gas-Chromatography (GC) Method

The chemical composition of the constituents in bioethanol from papaya fruit was carried out using the GG-MS tool at the Laboratory of the Faculty of Mathematics and Natural Sciences, Udayana University, Bukit Jimbaran. The principle of gas chromatography is that by injecting the sample into the end of the chromatogram column, the sample will evaporate and elute the inert gas as the mobile phase.

RESULTS

Bioethanol Manufacturing Process

The sample uses the amount of raw material, namely 200 grams, mashed into a slurry, the water filtrate, and the slurry as a starter for fermentation. According to Salsabila (2013), the addition of powdered yeast, Saccharomyces cerevisiae, to the substrate functions as a microbial seed for the conversion of carbohydrates into ethanol. The success of the fermentation process was observed by changes in the color and smell of the papaya fruit filtrate. In this study, the starter starch filtrate, which was originally yellow in color, turned frothy and whitish in color at the top, and the smell changed to fragrant tape or sweet alcohol (Salsabila et al., 2013).

Good Concentration of Yeast in the Process of Making Papaya Bioethanol

Fermentation on the fifth day shows a comparison of bioethanol production produced by S. cerevisiae in Table 1. and Figure 1.

Table 1. Total Levels of Bioethanol Produced by S. cerevisiae

S. Cerevisiae Concentration	Ethanol Content
1%	3.89%
2%	5.44%
3%	5.14%
4%	5.34%
5%	4.24%

The highest bioethanol yield was produced by a concentration of 5% S. cerevisiae, which was 5.44% at a concentration of 2% bioethanol, followed by a concentration of 4% S. cerevisiae bioethanol, which was produced as much as 5.34%, and a concentration of 3% S. cerevisiae bioethanol, which was produced as much as 5.14%. The lowest yield of bioethanol content was produced by treatment with a concentration of 1%. S. cerevisiae produced 3.89% bioethanol. At a concentration of 5%, the resulting bioethanol decreased to 4.24%. In order of concentration from high to low, it is 2% > 4% > 3% > 5% > 1%.

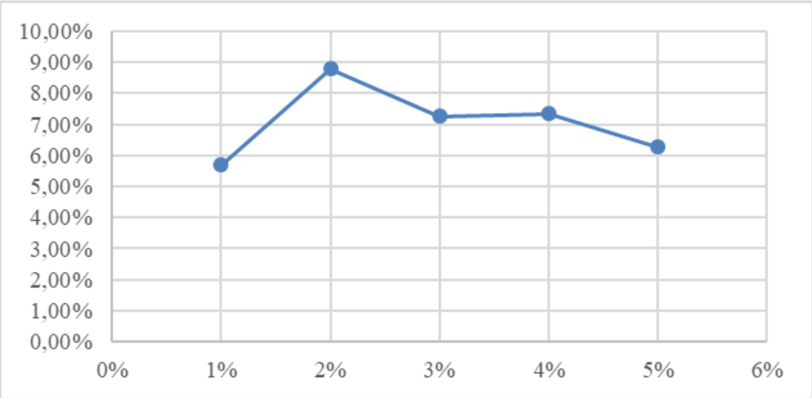

Figure 1. Total Levels of Bioethanol Produced by S. cerevisiae on the Fifth Day

Results of pH Analysis Before and After Papaya Bioethanol Fermentation

Results Differences in pH concentrations before and after papaya bioethanol fermentation can be seen in table 2 and figure 2.

Table 2. Differences in pH Concentrations Before and After Papaya Bioethanol Fermentation

No.	**S. cerevisiae concentration**	**pH before fermentation**	**pH after the fermentation process**
1	1%	5.5	6.6
2	2%	6.1	7.4
3	3%	5.6	6.9
4	4%	5.4	6.9
5	5%	5.3	6.8

Bioethanol fermentation is affected by pH. In this research, pH measurements were carried out before and after papaya fruit fermentation. The results of testing the pH before fermentation were varied, namely at 1% yeast mass, pH 5.5; at 2% yeast, pH 6.1; at 3% yeast, pH 5.6; and 4% yeast, pH 5.6.pH 5.4 obtained; and yeast 5%, pH 5.3. This is shown in Figure 5.2, with a maximum pH value of 6.1 obtained in ethanol production by Saccharomyces cerevisiae. Fluctuations in pH values are shown in Figure 5.2. This is influenced by the formation of CO2 and organic acids during the yeast fermentation process.

The results of testing the pH after fermentation were varied, namely at 1% yeast mass, pH 3.5; at 2% yeast, pH 4.9; at 3% yeast, pH 3.7; at 4% yeast, pH 3.6; and yeast 5%, pH 3.1. This is shown in Figure 5.2, with a maximum pH value of 4.9 and a concentration of 2% obtained in ethanol production by Saccharomyces cerevisiae.

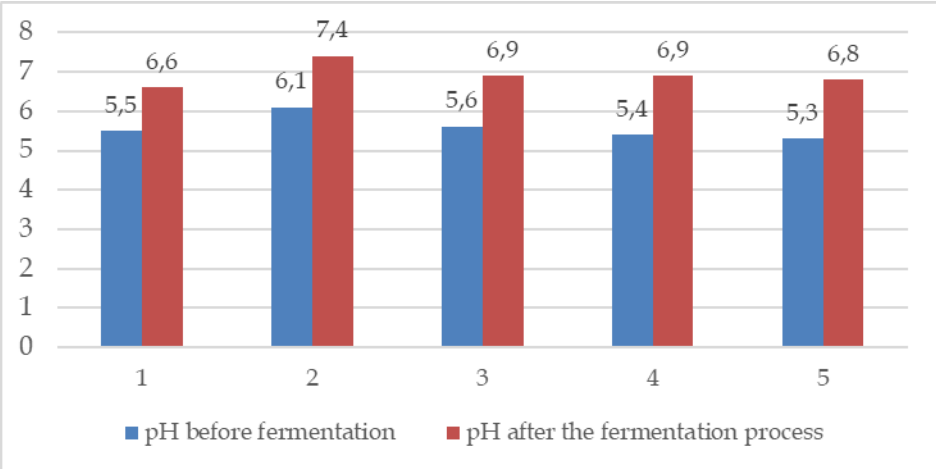

Figure 2. Results Differences in pH Concentrations Before and After Papaya Bioethanol Fermentation

Results of Gas-Chromatography (GC) Analysis of Papaya Bioethanol

Table 3 describes the results of the GC analysis. The highest bioethanol content was obtained at 2% yeast mass with GC test results of 5,44 %. There are differences in the results of the levels of bioethanol obtained, but the levels of bioethanol obtained are still in the range of 3-5%.

Table 3. Bioethanol levels using the GC-MS tool

S. cerevisiae concentration	**Gas-Chromatography (GC)**
1%	3.89%
2%	5.44%
3%	5.14%
4%	5.34%
5%	4.24%

The highest bioethanol yield was produced by a concentration of 5% S. cerevisiae, which was 5.44% at a concentration of 2% bioethanol, followed by a concentration of 4% S. cerevisiae bioethanol, which was produced as much as 5.34%, and a concentration of 3% S. cerevisiae bioethanol, which was produced as much as 5.14%. The lowest yield of bioethanol content was produced by treatment with a concentration of 1%. S. cerevisiae produced 3.89% bioethanol. At a concentration of 5%, the resulting bioethanol decreased to 4.24%. In order of concentration from high to low, it is 2% > 4% > 3% > 5% > 1%.

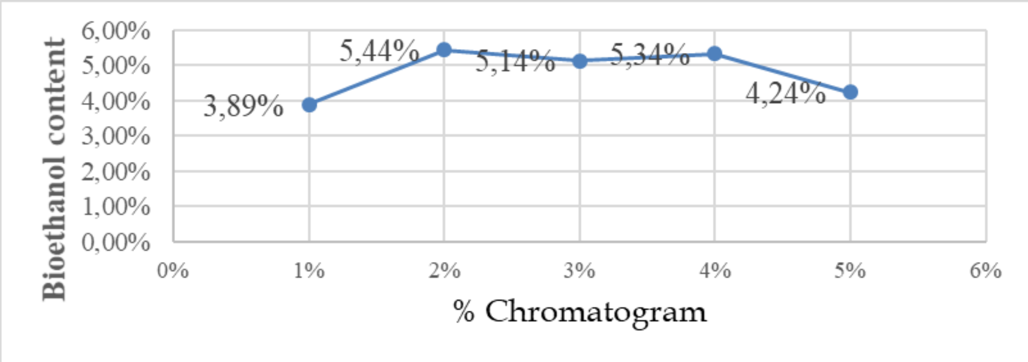

Figure 3. Papaya Bioethanol GC Results

DISCUSSION

Bioethanol Manufacturing Process

From the research results, it was determined that bioethanol took a long time to ferment, namely until the fifth day with different yeast concentrations. This is because microorganisms have adapted to the environment and available nutrients, so many microorganisms grow and divide, and their numbers increase rapidly compared to other days. On the third, fourth, and fifth days, the growth of microorganisms is not matched by sufficient nutrition, so the bioethanol produced decreases. Besides that, the bioethanol formed will be hydrolyzed to become acid if it is fermented too long.

Good Concentration of Yeast in the Process of Making Papaya Bioethanol

From the results of the analysis, it can be concluded that with a yeast concentration of 2%, you get maximum results because the addition of yeast increases the amount of nutrients in the sample. With the addition of yeast doses of 3% and 5%, the resulting bioethanol decreased because the productivity of microorganisms decreased due to a lack of nutrients needed by these microorganisms. If you add too much yeast, it will also produce a lot of acid, and little bioethanol will be produced.

The yeast Saccharomyces cerevisiae can oxidize sugar to CO2 and water with the help of oxygen. The yeast S. cerevisiae converts 70% of the glucose in the substrate into CO2 and alcohol (Sally Mandari1, Elvi Yenie2, 2016). According to data from Andriani (2013), there is a glucose content in papaya of 8.23%. So it can be stated that papaya glucose levels have great potential to be reused as raw material for bioethanol fermentation (Andriani et al., 2013).

The bioethanol process using S. cerevisiae requires simple sugar substrates and more complex ones in the form of starch, starch, and lignocellulose (N. Nurjanah et al., 2020). The composition of this substrate is influential in the fermentation of some carbohydrates into ethanol. Lignocellulose, in this case, pentose, namely xylose, cannot be fermented by S. cerevisiae. In addition, the number of S. cerevisiae yeast cells affects bioethanol production, whereas 5% of S. cerevisiae shows a decrease in the amount of bioethanol production. This decrease was caused by too much food, causing competition in the fight for nutrients. This is also proven by the research by

Nasrun et al. (2017), where an amount of 20 grams of S. cerevisiae showed a decrease in alcohol content, and 15 grams of S. cerevisiae showed the best results (Nasrun et al., 2017).

Results of pH Analysis Before and After Papaya Bioethanol Fermentation

Changes and decreases in the initial pH of the substrate during the fermentation process are caused by the formation of by-products from the secondary metabolism of yeast in the form of organic acids, which dissolve together with the ethanol product. The results of testing the pH during the fermentation took place between 3 and 4. This shows that the pH required for yeast growth is still within the range of the results of previous studies by Budianti (2016), namely between pH 3 and 5.

Results of Gas-Chromatography (GC) Analysis of Papaya Bioethanol

Based on the research data, it can be concluded that the increase in the bioethanol production process from carbohydrate starch as a raw material for renewable energy is efficient. Important requirements are needed in the form of the right type of microbes that can convert various types of sugar into raw materials and can survive stressful environmental conditions. The best types of bacteria and yeast seeds that are effective for sugar metabolism are Saccharomyces cerevisiae, Aspergillus niger, and Escherichia coli, which can be selected for bioethanol production by anaerobic fermentation by observing the mass factor of yeast and the optimum time of fermentation.

Based on the results of research by Thamrin (2022), it shows that the use of yeast (Saccharomyces cerevisiae) in the manufacture of bioethanol from various types of fruit does not have a standard, so the ethanol yield is still very variable. So it can be seen that the factors of inoculum volume, type of microbial strain, yeast mass, and fermentation time greatly determine the effectiveness of the fermentation process from glucose originating from fruit peel waste (Thamrin, 2022).

The output of this study can be a reference that papaya fruit still contains sugar content as an alternative source of glucose nutrition in bioethanol conversion by anaerobic fermentation. The results of the flame characteristic test carried out on the five bioethanol treatment samples showed the nature of a blue flame at 100°C that burned quickly and disappeared. The level of bioethanol using the GC-MS test is 5,44%.

CONCLUSION AND RECOMMENDATIONS

The results of the study showed that bioethanol took a long time to ferment until the fifth day with different yeast concentrations, namely 1%–5%. This is because microorganisms have adapted to the environment and available nutrients, so many microorganisms grow and divide, and their numbers increase rapidly compared to other days.

The maximum concentration of bioethanol produced by a concentration of 2% S. cerevisiae is 5.44% due to the addition of yeast, according to the number of nutrients in the sample. In order of concentration from high to low, it is 2% >

4% > 3% > 5% > 1%. While the maximum pH value of 4.9 with a concentration of 2% was obtained in ethanol production by Saccharomyces cerevisiae.

The suggestions that need to be carried out in this research are that it is necessary to carry out research using various types of enzymes or other yeasts to obtain higher levels of bioethanol and that the characterization of the bioethanol obtained meets SNI and international bioethanol quality standards.

FURTHER STUDY

The results of this study can provide answers regarding the effect of yeast concentration and fermentation time on the amount of bioethanol produced from papaya so that we can get more complete information about the activity and efficacy of papaya fruit as a result of processed bioethanol and produce products that can be sold in the market.

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