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Fabrication of Bioethanol via Fermentation from Cellulose of Kepok Banana (*Musa Paradisiaca* L.) Using Bread's Yeast (*Saccharomyces cerevisiae*)

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Abstract. Bioethanol has been made by fermentation of the cellulose of Kepok banana stems, where this study went through 3 stages. First-stage isolation of cellulose from Kepok banana prevents using HNO_3 and $NaNO_2$, removal of swelling with NaOH and Na_2SO_3, and bleaching process (bleaching) using NaOCl and H_2O_2, then functional group analysis was performed with FT-IR. The second stage of cellulose obtained was hydrolysed with 1% HCl for 120 minutes of hydrolysis time. The resulting glucose was analysed by Benedict's reagent and the Luff-Schrool method. The third stage of glucose solution results from fermented hydrolysis using bread yeast (*Saccharomyces cerevisiae*) with variations in the weight of yeast 5, 6, and 7 grams, and the duration of fermentation is five days. Bioethanol obtained was tested qualitatively using K_2CrO_7 and H_2SO_4 reagents and bioethanol characterisation using GC. The study results obtained 10.18% glucose levels, and the highest levels of bioethanol were obtained at 11.27% with 88.53% purity using 7 g of yeast.

Keywords: Bioethanol, Fermentation, Kepok Banana, Cellulose, Yeast.

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Banana is a fruit that grows a lot in Indonesia. Indonesia is one of the countries known as the world's banana producer. Indonesia has produced 6.20% of the world's total production, and 50% of Asian banana production comes from Indonesia (Satuhu and Supriyadi, 2008). Besides that, banana trees can only bear fruit once. Then new shoots will grow, which will become banana trees. New. Therefore, banana trees whose fruit has been harvested will produce waste in the form of banana stems which are generally not utilised, especially in North Sumatra.

Banana stems contain more than 10-15% water and have a high cellulose and glucose content, whereas the cellulose content of banana stems is around 60-65%. Cellulose is long fibres that, together with hemicellulose, pectin, and protein, form a network structure that strengthens plant cell walls. In maturation, storage, or processing, cellulose and hemicellulose components undergo structural changes (Winarno, 1992).

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Cellulose forms the fibre component of plant cell walls. The toughness of cellulose is due to its overall structure. Cellulose molecules are chains of D-glucose up to 14,000 units as twisted, rope-like bundles held together by hydrogen bonds. A single molecule of cellulose is a linear polymer of 1,4-β-D-glucose. Complete hydrolysis of HCl, diluted with distilled water, only produces D-glucose. The disaccharide isolated from partially hydrolysed cellulose is cellobiose, which can be further hydrolysed to D-glucose with an acid catalyst or an enzyme emulsion (Fessenden, 1982).

Glucose is fermented to produce alcohol, and glucose fermentation is a biological process in which glucose is converted into cellular energy and produces bioethanol and carbon dioxide as by-products. Because this process does not require oxygen, but yeast does it, this glucose fermentation is classified as anaerobic respiration (Almatsier, 2001).

In everyday life, it takes an increase in living standards that encourages the hunt for sustainable energy to meet energy consumption worldwide. On the other hand, using fossil fuels as the primary energy source causes global problems such as environmental pollution and global warming. So that the government, industrial, and energy sectors found environmentally friendly, renewable, and sustainable energy sources. Regarding renewable energy, bioethanol is prioritised, as it represents around 40% of total energy consumption worldwide. Bioethanol contributes to reducing greenhouse gas emissions, creating job opportunities, regional development, and supply security. Bioethanol has been identified as the most widely used biofuel worldwide because it significantly contributes to reducing crude oil consumption and environmental pollution. These compounds can be produced from various raw materials such as sucrose, starch, lignocellulosic and algal biomass through a fermentation process by microorganisms. Compared to other types of microorganisms, yeast (Saccharomyces Cerevisiae) is a common microbe used in ethanol production because of its high ethanol productivity, high ethanol tolerance, and ability to ferment various sugars (Azhar, 2017)

Bioethanol (C_2H_5OH) is one of the most popular biofuels to replace petroleum. Currently, the price of crude oil is increasing; besides being less environmentally friendly, it is also a non-renewable resource. Bioethanol has advantages besides being environmentally friendly. Its use as a fuel mixture is proven to reduce carbon monoxide emissions and other smoke from vehicles. Bioethanol can be produced from various raw materials widely available in Indonesia, so it is very potential to be processed and developed because the raw material is very well known to the public. Plants have the potential to produce bioethanol. The plants with high carbohydrate content, such as sugar cane, sap, palm sugar, sorghum, cassava, cashew nuts (cashew waste), banana stems, sweet potatoes, corn, corn cobs, straw, and bagasse. (bagasse) (Komarayati, 2010).

The public benefits that can be obtained from bioethanol fuel include being used as raw material for the alcohol derivative industry, mixed liquor, and pharmaceutical industry, to raw material for

vehicle mixtures. Of course, bioethanol must be adjusted to the type of need. For example, for industrial needs, bioethanol with a content of 99.5-100% is required, or bioethanol must be completely dry and anhydrous so it is not corrosive.

Feri Susanto (2008) has conducted research on the manufacture of bioethanol from the hydrolysis of bagasse cellulose by fermentation with variations in the addition of baker's yeast and the length of fermentation time, where the highest bioethanol content is 5.12% with the addition of 2 grams of baker's yeast for six days.

Lisma Sari (2010) also conducted research on the manufacture of bioethanol from the hydrolysis of rice straw cellulose by fermentation with variations in the addition of baker's yeast and the length of fermentation time, where the highest bioethanol content was 7.43% with the addition of 6 grams of baker's yeast and a fermentation time of 6 days.

Annisa Suri (2008) conducted research on the manufacture of bioethanol from hydrolysed cellulose of empty palm oil bunches by fermentation with variations in the addition of baker's yeast and the duration of fermentation, where the highest bioethanol content was 7.59% with the addition of 6 grams of baker's yeast and six days of fermentation. This study only discussed variations in the acquisition of baker's yeast and the length of fermentation without examining the effect of the length of time hydrolysis of cellulose to produce a hydrolysed sugar solution.

Moeksin (2010) has researched the manufacture of ethanol from jicama with variations in yeast weight, time, and type of yeast with a fermentation time of 3, 5, and 7 days. In this study, the highest ethanol content from fermented yam with a weight of 6 grams of yeast and a fermentation time of 5 days was 22%.

Shokrkar Hanieh (2018) also researched the enzymatic hydrolysis of microalgae cellulose to produce bioethanol and the modelling and sensitivity analysis by hydrolysing algae cellulose to produce cellobiose and glucose. The results showed that the highest glucose yield (57%) was achieved at 50g/L microalgae biomass concentration, pH 5, and temperature of 50°C. In addition, a sensitivity analysis was performed on each kinetic model parameter. Microalgal biomass loading experiments demonstrated that cellulase could be significantly used without sacrificing glucose yield. Fermentation of the concentrated sugar medium with Saccharomyces cerevisiae produced ethanol (12.87 g/L) with a result of (0.46 g ethanol/g glucose).

Based on this background, researchers are interested in researching the weight effect of baker's yeast by hydrolysing Kepok banana stem cellulose into glucose using 1% HCl, which will produce bioethanol.

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1 Materials and Methods

1.1 Equipment

The equipment used in this study includes glassware, spatula, sieve, blender, analytical balance, oven, hot plate stirrer, mechanical stirrer, bunsen, thermometer, desiccator, incubator, stative and clamps, universal indicator, aluminum foil, pH meter, GC, autoclave, and FTIR.

1.2 Materials

The materials used in this study included: Kepok Banana Stems, Bread Yeast, $CuSO_4$.$5H_2O$, Ethanol, H_2SO_4, KH_2PO_4, $K_2Cr_2O_7$, $MgSO_4$.$7H_2O$, NaOH, Na_2SO_3, HNO_3, HCl, $NaNO_3$, Na-Hypochlorite, $C_6H_{12}O_6$, H_2O_2 and distilled water.

1.3 Preparation of Kepok Banana Stem Powder

Kepok banana stems are cleaned and washed with water. Then cut into small pieces, dried in the sun, and dried in the oven at 60°C. Then ground using a blender to form a powder. They are then sieved using a sieve measuring 80 mesh.

1.4 Isolation of Cellulose from Kepok Banana Stem

At least 75 g of finely dried Kepok banana stems are put into a 2000 mL beaker glass. Then 1000 mL of 3.5% HNO_3 and 0.01 g $NaNO_2$ were added to the beaker glass while heating in a water bath for 2 hours at 90°C. Then, filtered and washed the residue with distilled water until pH = 7. Next, 375 mL of 2% NaOH and 375 mL of 2% Na_2SO_3 were added to the beaker glass and heated for 1 hour at 50°C. After that, filter and wash the residue with distilled water until pH = 7. Then 500 mL of 1.75% Na-Hypochlorite was added to the beaker glass, heating for 30 minutes at 70°C. Then, filtered and washed the residue with distilled water until pH = 7. Then 500 mL of 17.5% NaOH was added while heating for 30 minutes at 80°C. Then filtered and washed the residue with distilled water until pH = 7. After that, H_2O_2 10% was added and heated for 15 minutes at 60°C. Then filtered and washed the residue with distilled water until pH = 7. Then the residue was dried in the oven at 60°C and put in a desiccator. (Ohwoavworhua, 2009).

1.5 Hydrolysis of Kepok Banana Stem Cellulose into Glucose and Qualitative Glucose Test

As much as 8 g of cellulose from Kepok banana stems was put into an Erlenmeyer glass. Then, added with 128 mL of 1% HCl was. After that covered with cotton and aluminium foil, heated in a thermostat at 80°C for 90 minutes, and then cooled to room temperature. Next, 0.7% NaOH was added to pH = 4 - 4.5 and filtered to get the filtrate. Then pipette 1 mL of the filtrate into a test tube and add 5 mL of Benedict's solution. After that, it is heated in a water bath until a brick-red precipitate form.

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1.6 Analysis of the Glucose Content of the Sample

As much as 2 g was put into a 50 mL measuring flask and then diluted to the marked line. Then 10 ml of the solution was taken with a volume pipette and put into the Erlenmeyer. After that, 25 ml of Luff school solution and 15 ml of distilled water were added. Then heat the mixture (try to make the solution boil for 3 minutes) and allow it to simmer for 10 minutes. They then cooled the sample with water containing ice. After cooling, they slowly added 15 ml of 20% KI solution and 25 mL of 25 % H_2SO_4. Then titrated with $Na_2S_2O_3$ 0.1N solution and added 0.5% starch indicator. Then record the volume of $Na_2S_2O_3$ 0.1 N used. After that, the same treatment was carried out for the blank book.

1.7 Glucose Fermentation Result of Hydrolysis of Kepok Banana Stem Cellulose into Bioethanol

Put 100 mL of glucose solution hydrolysed Kepok banana stems into a 250 mL Erlenmeyer glass. Then, 0.1 g of MgSO 4 .7H_2O was added, 0.1 g of KH_2PO_4, and 0.1 g of $(NH_4)_2SO_4$. Then, sterilised using an autoclave at 121°C for 1 hour and cooled. After that, 5, 6, and 7 g of Bread yeast were added and fermented for six days.

1.8 Separation of Bioethanol from Fermentation Results

The distillation apparatus was arranged for the separation of bioethanol. Then CaO was added to the sample with a ratio of 1:2 (g/mL) and distilled at 78°C for 1 hour. After that, the distillate was collected in an Erlenmeyer, which was covered with plastic and tied with rubber. Then the volume of distillate produced was measured. Then analysed by density test and tested for levels using gas chromatography.

1.9 Determination of Bioethanol Content through Density Test

The empty pycnometer was weighed to a constant three times, and then add distilled water and weighed to a continuous three times. After that, put the distillate into the empty pycnometer and consider it with an analytical balance until it is stable three times. Then the density of the distillate is calculated. The same treatment is carried out for the distillate with variations in yeast weight 5, 6, and 7, where the distillate with yeast weight variation five uses pycnometer number 588, the distillate with yeast weight variation 6 uses pycnometer number 13, and the distillate with yeast weight variation 7 using pycnometer number 616.

2 RESULT AND DISCUSSION

2.1 Results of Isolation of Kepok Banana Stem Cellulose

Isolation of cellulose from Kepok banana stem powder involved delignification, swelling, bleaching, and purification processes. The cellulose obtained consists of α, β, and γ– cellulose, so to obtain α-cellulose is separated according to its solubility in 17.5% NaOH, where α-cellulose is

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insoluble in 17.5% NaOH. In comparison, β-cellulose and γ-cellulose are soluble in 17.5% NaOH. The -cellulose precipitate αproduced at this stage is yellow. Bleaching was carried out using 10% H_2O_2 so that it turned white, then dried in an oven at 60°C. This process resulted αin white cellulose. 75 g of Kepok banana stem powder was obtained as much as 20.00 grams of pure α-cellulose (26.69% of the initial mass of Kepok banana stem fibre).

2.2 Analysis of Functional Group Using FTIR Spectroscopy

Figure 1 shows the FTIR spectra of commercial cellulose and Kepok banana steam cellulose. The –OH group appears at wave number 3417 cm^{-1}, which shows –OH stretching vibration (Pescok et al. 1976). From the FTIR spectra, there are widening bands in the 3410.15, 3448.72, and 3272.30 cm^{-1} regions which indicate the presence of OH strain vibrations from alcohols in the cellulose molecule, followed by CH strain vibrations from the alkane chain in the 2900 absorption area, 94, 2891.18 and 2915.60 cm^{-1}. In addition, vibration peaks were also seen in the absorption areas 1319.31, 1334.74, and 1334.74 cm^{-1} and 1056.99, 1018.89, and 1026.13 cm^{-1}, indicating the presence of CO strain in the cellulose ring (Nacos et al . 2006, Garside et al . 2003). Meanwhile, the CH swing vibrations of cellulose were found in the absorption areas of 894.09 and 896.10 cm^{-1}, indicating the presence of β-glycoside bonds, which are links between glucose units in cellulose structures (Alemdar et al., 2008). From the results of the FTIR analysis, it can be concluded that α-commercial cellulose and α-cellulose from Kepok banana stems are actual cellulose compounds.

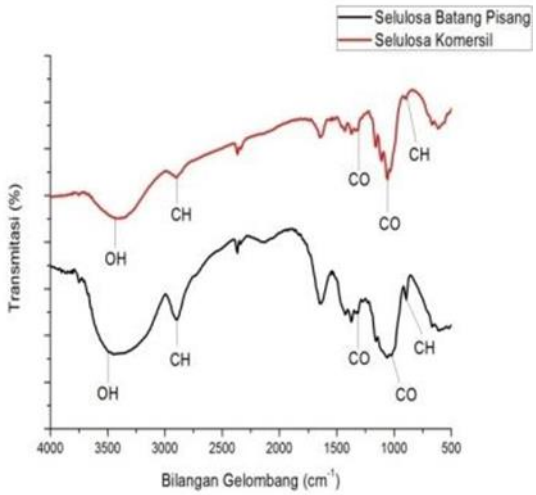

Figure 1. FTIR spectra of commercial cellulose and Kepok banana steam cellulose

2.3 Cellulose Hydrolysis Results from Kepok Banana Stems

Hydrolysis process of cellulose from Kepok banana stems with adding 1% HCl. Where cellulose bonds were broken to produce glucose, qualitative glucose testing was carried out using Benedict's reagent, while quantitative glucose tests were carried out using the Luff-Scroll method. The glucose concentration results obtained during hydrolysis with the Luff-Scroll process are shown in Table 1.

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Table 1. The results of the glucose concentration obtained during hydrolysis with the Luff-Scroll method

Hydrolysis time (minutes)	Mass sample (g)	Blank (mL)	Sample Volume (mL)	Glucose Level (%)	Yeast variation
120	2.0009	24.6	15.6	10.04	5
120	2.0011	24.6	15.3	9.83	6
120	2.0013	24.6	15.8	10.18	7

2.4 Results of Qualitative and Quantitative Analysis of Bioethanol

Glucose obtained from the hydrolysis of banana stems is then fermented with a fermentation time of 6 days, while the variations in the weight of the baker's yeast used are 5, 6, and 7 grams. After that, the distillation step was carried out by adding CaO to bind water in a ratio of 1:2 (g/mL) to obtain Bioethanol distillate whose levels were tested qualitatively with H_2SO_4 (p) + $K_2Cr_2O_7$ reagent, which would produce a solution blue. After that, quantitative testing was carried out using gas chromatography.

Table 2. Quantitative testing using gas chromatography

Hydrolysis time (minute)	Yeast mass (g)	Fermentation time (day)	H_2SO_4 + $K_2Cr_2O_7$ Addition
120	5	5	Greenish blue solution
120	6	5	Greenish blue solution
120	7	5	Greenish blue solution

Table 2 shows that all favourable variations contain bioethanol, which offers a blue-green colour change. Then, a quantitative analysis of bioethanol is carried out by converting the density of the distillate.

Table 3. Bioethanol quantitative analysis by converting the density of the distillate.

Variation of Yeast Distillate	**Empty Pycnometer (g)**	**Pycnometer + Aquadest (g)**	**Pycnometer + Distillate (g)**	**Density (g/mL)**	**Bioethanol Level (%)**
5	16.5096	26.1508	26.0505	0.9896	7.47
6	15.8320	25.5556	25.4350	0.9876	9.09
7	16.2489	26.1330	25.9818	0.9867	9.48

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Table 3 shows that the highest bioethanol content was obtained at 9.84% for the 7 g yeast variation, while the lowest was obtained at 7.47% for the 5 g yeast variation.

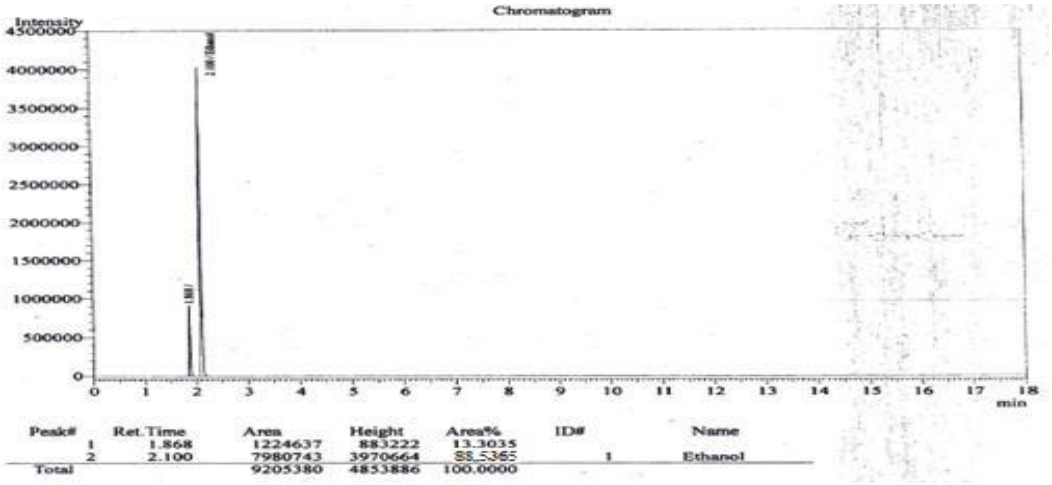

Figure 2. Chromatogram of bioethanol purity

Figure 2 shows the chromatogram, and it can be seen the purity of the bioethanol obtained was 88.53% with a variation of yeast weight of 7 g. From the distillation, the highest bioethanol content was 9.84%, and the purity of bioethanol was 88.53% for the yeast weight variation of 7 grams. Due to the glucose content variations in the weight of yeast, 7 g more than the others, with the increase in the amount of yeast, the more microbes are added, thereby increasing the alcohol content. This result follows the opinion of Khadijah (2015) that yeast (*Saccharomyces cerevisiae*) can change glucose into ethanol. If a lot of yeast is given, the ethanol produced will also increase and vice versa, lowering the density.

3 Conclusion

Isolated 21.36 g of white cellulose from kapok banana stems were obtained using delignification, swelling, and bleaching. Then the resulting distillate was then characterised using gas chromatography to determine its purity. The highest yield was received at 7 g yeast weight with 88.53% bioethanol purity. While the density is 0.9867 g/mL, it is known that the bioethanol content is 9.84%.

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