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# Fermented Sugar From Ultrasound-Assisted Acid Hydrolysis Berenuk Fruit (Crecentia Cujete L)

(Gula Fermentasi Dari Hidrolisis Asam Berbantu Gelombang Ultrasonik Buah Berenuk (Crecentia Cujete L))

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## *ABSTRACT*

Berenuk fruit (Crecentia Cujete L) is a non-food plant often found in tropical areas. Berenuk has quite a high content of carbohydrates and its derivatives. Among them are 18.61% carbohydrates, 59.86% sucrose, 25.09% fructose, and 18.24% galactose, so berenuk fruit has the potential to be a source or ingredient for bioethanol. The bioethanol process from biomass goes through several steps: raw material preparation, hydrolysis process and fermentation. The hydrolysis process is the process of breaking down carbohydrate into glucose units which are connected by β-1,4 glycosidic bonds. Another method of breaking sugar polymers can be done using alternative energy such as ultrasonic waves (50Hz, 50W) or microwaves. In this research, an ultrasonic-assisted acid hydrolysis process was carried out. The type of acid used is concentrated sulfuric acid ($H_2SO_4$). Ingredients ratio 1:20 (w/v) berenuk powder sample with sulfuric acid. The sulfuric acid concentrations used were 1 M, 2 M, and 3 M. Hydrolysis times were 40 minutes, 60 minutes, and 80 minutes at a temperature of $100^0$C. The results of research that has been carried out on the preparation process of bereneuk fruit have an average water content of 97.44%, so treatment is required before proceeding to the hydrolysis and fermentation process, namely by drying the drying process is carried out directly in sunlight for seven days, then continued by heating the oven for 8 hours at $100^0$C. In the ultrasonic wave assisted hydrolysis process carried out at a $H_2SO_4$ concentration of 3 M, a hydrolysis time of 80 minutes, and a temperature of $100^0$C produces 22.20% glucose.

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## 1. INTRODUCING

The use of new renewable energy that provides abundant, sustainable, cheap, clean and environmentally friendly energy has attracted interest over the past few decades and is very important in addressing global energy demand and environmental problems [1]. To realize the sustainable development goals (SDGs) of accessibility to modern, affordable, reliable and sustainable energy, biofuels are expected to play an important role as a viable advanced alternative energy source. To meet the requirements of the sustainability and economic criteria of alternative energy sources, the use of biofuel derived from biomass raw materials is very important [2]. Biomass energy sources have been identified for bioenergy production and can be lignocellulosic or non-lignocellulosic, very abundant in nature, cheap, and widely available [3]. Bioethanol can be produced from various raw materials in the form of sucrose, starch or lignocellulose-based biomass [4]. Berenuk fruit (Crecentia Cujete L) is a non-food plant that is often found in tropical areas. The availability of this plant in Indonesia is quite large. In West Papua, specifically in Sorong, berenuk has not been utilized optimally because people think that this plant is poisonous. Berenuk has quite a high content of carbohydrates and its derivatives. Among them are 18.61% carbohydrates, 59.86% sucrose, 25.09% fructose, and 18.24% galactose [5]. Based on this data, berenuk fruit has the potential to be a source or raw material for bioethanol.

The bioethanol process from biomass goes through several steps, namely raw material preparation, hydrolysis process and fermentation. The hydrolysis process is the process of breaking down cellulose into glucose units which are connected by β-1,4 glycosidic bonds [6]. The hydrolysis process can use two methods, namely acid and enzymatic hydrolysis. The use of strong or weak acid compounds in acid hydrolysis can cut carbohydrate polysaccharides better than using bases. The use of bases for hydrolysis provides a caramelization effect on the polysaccharide structure. Apart from that, the addition of an acid catalyst aims to speed up the reaction because the hydrolysis reaction using water is slow [7]. Cellulose, which is converted into reducing sugar (glucose). has high economic value. The conversion of cellulose into glucose is a strategic stage because glucose is needed for various uses.

Another method of breaking sugar polymers can be done using alternative energy such as ultrasonic waves or microwaves. Utilization of ultrasonic waves can increase mass transfer and shorten reaction time [8]. Furthermore, ultrasonic waves can extract and hydrolyze natural materials, which have been carried out in several studies, such as the hydrolysis of pineapple peel waste [7], acid hydrolysis and ultrasonic waves on bamboo stems [9], carrageenan depolymerization process [10], Ant nest flavonoid extraction [11], and acid hydrolysis of palm leaves [12]. Cavitation bubbles are created from ultrasonic waves in solution material. When the bubble bursts close to the cell wall, a shock wave and liquid jets will form which will rupture the cell wall. The rupture of the cell wall will cause the components inside the cell to come out and mix with the solution [7], [11], [13].

Based on the description above and previous research, the use of berenuk fruit as raw material for making ethanol produces levels of 12%-16% using conventional methods [14]. Therefore, this research will carry out ultrasonic-assisted acid hydrolysis of berenuk fruit for the production of fermentable sugar.

## 2. RESEARCH METHOD

The materials used in this research were berenuk (Crecentia Cujete L), $H_2SO_4$, yeast (Saccharomyces cerevisiae), NPK, distilled water, and $K_2Cr_2O_7$. The equipment used is a series of hydrolysis equipment, digital ultrasonic sonicator CD-2840A krisbow (50 Hz, 50 W, 240V), glass beaker, an analytical balance, a measuring flask, and a refractometer.

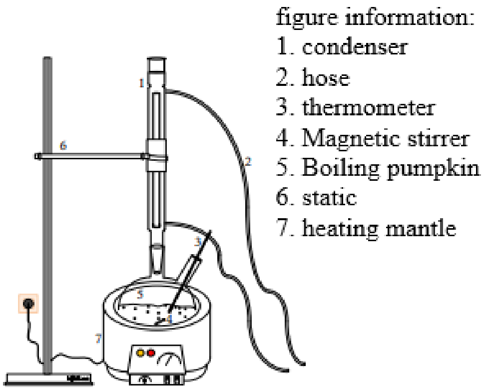


Figure 1. Hydrolysis Equipment

Figure 2. Digital Ultrasonic

Figure. 3 Refractometer

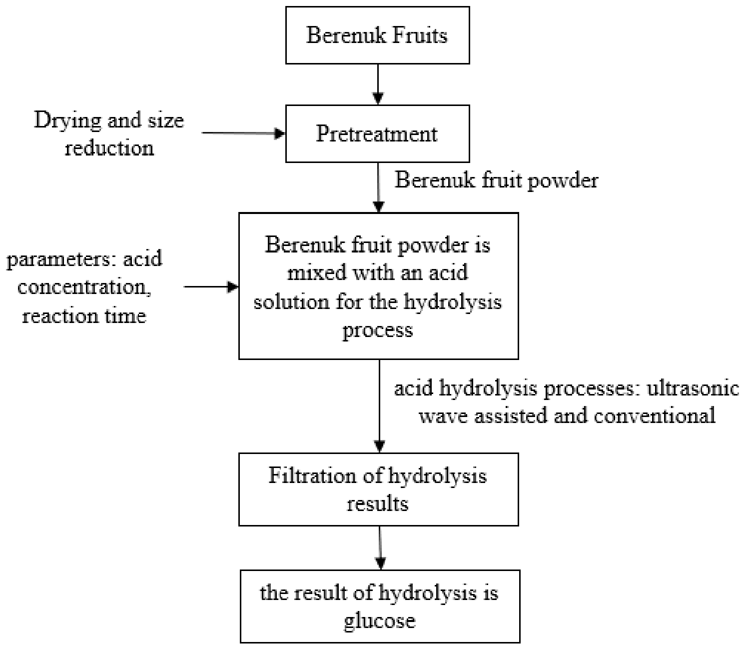


Figure 4. Process Flow Diagram

### Material Preparation

Separation of berenuk fruit fiber from the skin. The fruit fiber is then blended and then filtered from the water produced by the fruit fiber. After the fibers are separated, they are dried in the sun for seven days followed by oven for 8 hours at a temperature of $100^0$C. The resulting dry fiber is then blended to obtain berenuk fruit powder.

### Ultrasonic-Assisted Acid Hydrolysis (50 Hz, 50 W)

In this research, an ultrasonic-assisted acid hydrolysis process was used. The type of acid used is concentrated sulfuric acid ($H_2SO_4$). Ingredients ratio 1:20 (w/v) berenuk powder sample with sulfuric acid. The sulfuric acid concentrations used were 1 M, 2 M, and 3 M. Hydrolysis times were 40 minutes, 60 minutes, and 80 minutes at a temperature of $100^0$C.

**Analysis**

a. A refractometer is a tool for measuring the concentration of dissolved substance discovered by Dr.Ernest Abbe, a scientist from Germany, around 2010. Brix (%) is used to express the concentration of dissolved materials which is the percentage of dissolved materials in water solution [15].

b. FTIR (Fourier Transform Infrared) is a tool that decomposes (disperses) infrared radiation into frequency components. Infrared spectroscopy is useful for determining the functional groups contained in organic compounds. If a compound is irradiated using infrared, some of the light will be absorbed by the compound while others will be transmitted, this absorption is due to Because organic compound molecules are some that cannot be detected by infrared, each bond has unique properties [16].

## 3. RESULTS AND DISCUSSION

### 3.1 Water Content in Berenuk Fruit

The water content of berenuk is shown in Table 1 and Figure 5.

Table 1. Water content of berenuk fruit

| Before (gram) | After (gram) | Content (%) |
|---|---|---|
| 3.300 | 80,00 | 97,57 |
| 2.700 | 72,33 | 97,32 |
| 3.100 | 79,90 | 97,42 |

Based on the data above, the water content in berenuk fruit is an average of 97.44%. Therefore, pretreatment is necessary before getting berenuk fruit powder. Water content is one of the inhibiting factors in the reaction rate process, including the hydrolysis process. The less water content in liquid glucose, the better the quality of the liquid glucose [17]. The pretreatment process that has been carried out is by drying and blending to obtain berenuk fruit powder with low water content. The drying process is carried out directly in sunlight for 7 days, then followed by heating in the oven for 8 hours at a temperature of $100^0$C.

a b

Figure 5. a. water content of berenuk fruit; b. Berenuk Fruit Powder

### 3.2 Characteristics of Berenuk Fruit Powder

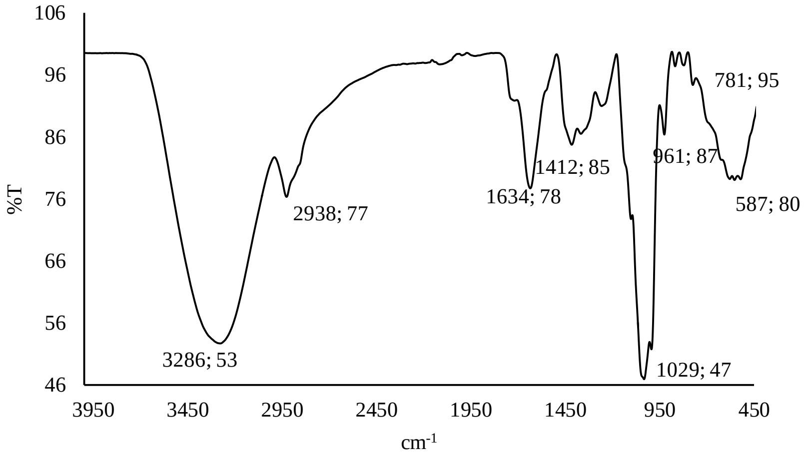


Figure 6**.** FTIR Characterization of Berenuk Fruit Powder

Berenuk fruit powder has a high content of carbohydrates and its derivatives. Among them are 18.61% carbohydrates, 59.86% sucrose, 25.09% fructose, and 18.24% galactose [5]. Figure 6 shows the peak at wave number 3286.53 $cm^{-1}$ which is the absorption of the O-H functional group with a wide and sharp intensity. The O-H functional group indicates that the sample contains carbohydrate compounds and their derivatives. The wave number 2938.77 cm-1 with moderate intensity is the C-H stretching absorption of carboxylic acids [18]. The wavelength is 1634.78 $cm^{-1}$ with a sharp intensity indicating C=O absorption. The C=O functional group is found in galactose. Sucrose, glucose and fructose were detected at wavelengths between 1500-900 $cm^{-1}$. The spectral region found at 900-750 $cm^{-1}$ is characteristic of several types of saccharides [19]. In this study, the wavelength obtained was 1412.85 $cm^{-1}$; 961.27 $cm^{-1}$ and 781.95 $cm^{-1}$. The anhydride functional group (CO-O-CO) is shown at a wavelength of 1029.47 $cm^{-1}$ [20].

### 3.3 Effect of Sulfuric Acid Concentration

The effect of acid concentration on the hydrolysis process is presented in Figure 7.

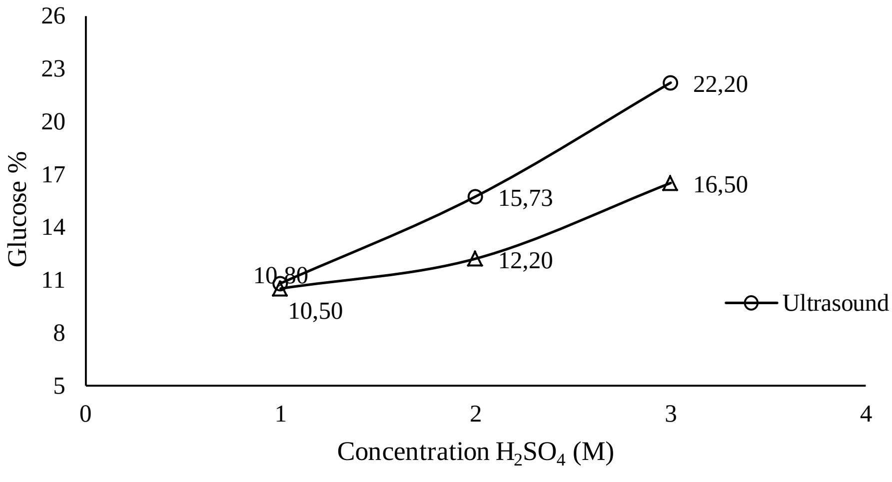


Figure 7 Graph of the relationship between Glucose and $H_2SO_4$ concentration

Figure 7 shows that the higher the acid concentration, the more glucose levels are produced in both, ultrasonic wave-assisted and conventional processes carried out at a processes time of 80 minute. The ultrasonic wave-assisted process with a sulfuric acid concentration of 3M, the glucose content was 22.20% higher compared to the conventional process. This result is the same as previous research, which reported that sulfuric acid acts as a catalyst, namely, accelerating the hydrolysis reaction [21]. The acid will cause a decrease in activation energy so that the reaction runs quickly and the glucose produced will be greater [6], [22]. In addition, the concentration of the acid catalyst is one of the factors that can influence the binding rate of amylopectin molecules that have absorbed water to be broken down into reducing sugars [23].

### 3.4 Effect of Hydrolysis Time

Time is another factor that influences the hydrolysis process. The effect of time in this research is presented in Figure 8.

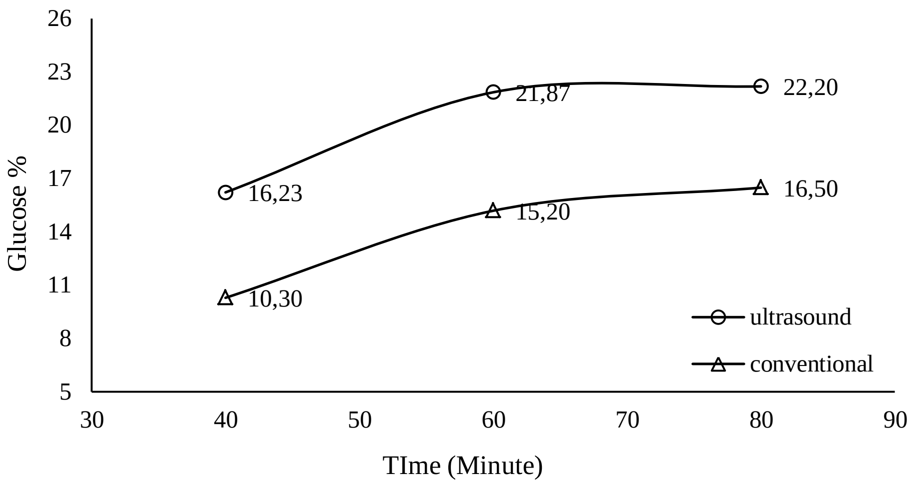


Figure 8. Graph of the Relationship between Glucose Levels and Hydrolysis Time

Figure 8 shows that the longer the hydrolysis time, the more glucose levels are produced. At 80 minute with a concentration 3M, the glucose produced was 22.20%. The same results were also obtained in previous research that glucose levels increased with increasing hydrolysis time [24]. The increase in reducing sugar levels is caused by a longer hydrolysis process, causing more $H^+$ ions to break the cellulose polymer chain, there by producing free radicals which will bind with $OH^-$ ions to form glucose monomers [25]. The longer the hydrolysis time increase the number of collisions of reacting substances, the more molecules that react produce more products [17], [26].

### 3.5 Fermentation of Reducing Sugar to Bioethanol

The fermentation process is carried out anaerobically or without oxygen because oxygen can cause the oxidation of ethanol which is formed into acetic acid [21]. Glucose fermentation of berenuk fruit is carried out using baker's yeast as a source of saccharomyces cerevisiae [25]. The fermented sample must have a pH of 4-5 because yeast can breed in this condition. In this research, the sample was conditioned to pH 5, the hydrolysis sample had a pH of 1, then the pH value was increased using a 2 M NaOH solution. The fermentation ratio used reducing sugar by adding 3 grams of NPK and 5 grams of baker's yeast. Analysis of ethanol content was carried out using a qualitative test using the potassium dichromate ($K_2Cr2O_7$) oxidation method. The presence of ethanol produced during the fermentation process can be seen in the resulting color change from orange to bluish-green. Ethanol is a primary alcohol compound where the C, atom which is bound to the OH group, binds 2 H atoms so that $K_2Cr_2O_7$ will oxidize it to acetic acid in a relatively short time, resulting in a color change in 2% $K_2Cr_2O_7$ from orange to green when the ethanol is dropped. The green color comes from $Cr^{3+}$ which is caused by a decrease in the $Cr^{6+}$ oxidation state to $Cr^{3+}$. $H_2SO_4$ functions as a catalyst to speed up this reaction [14]. The oxidation reaction that occurs is as follows:

$CH_3CH_2OH + 2\ K_2Cr_2O_7 + 8\ H_2SO_4 \rightarrow 3\ CH_3COOH + 2\ Cr(SO_4)_3 + 2\ K_2SO_4 + 11\ H_2O$

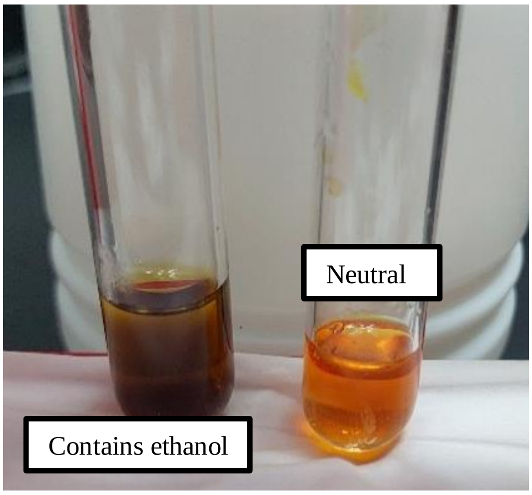


Figure 9. Qualitative Test of Ethanol Content in Samples After Fermentation

In Figure 9, it can be seen that there is a color change, but the bluish-green color is not very visible because it is possible that there are still other compounds besides ethanol contained in the $K_2Cr_2O_7$ sample after being dropped on the distilled sample that experiences a color change. During the distillation process, the temperature cannot be conditioned to remain at $78^0C$, so it is possible that there is still water contained in the distillation results.

## 4. CONCLUSION

Furthermore, the research results it can be concluded that the process of preparing bereneuk fruit has an average water content of 97.44%, so treatment is required before proceeding to the hydrolysis and fermentation process, namely by drying the drying process is carried out directly in sunlight for seven days, then continued by heating the oven for 8 hours at $100^0C$. The hydrolysis process was carried out at an $H_2SO_4$ concentration of 3 M, a hydrolysis time of 80 minutes, and a temperature of $100^0C$ to produce 22.20% glucose. The results of the FTIR analysis showed that berenuk fruit powder contains carbohydrate compounds and their derivatives, such as sucrose, fructose, and galactose. Berenuk fruit has the potential to be a raw material for bioethanol.

## 5. ACKNOWLEDGMENTS

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