PROCESSING RUMBIA STARCH INTO GLUCOSE POWDER BY ENZYMATIC HYDROLYSIS WITH OPTIMUM SACCHARIFICATION TEMPERATURE

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Abstract

Glucose powder is glucose in solid form which has the main component of glucose syrup obtained from starch hydrolysis, including sago from rumbia starch. As the population increases, it increases consumption of sugar as a sweetener for basic necessities such as food and drinks, the purposes of this research is identifying the optimum temperature for saccharification of the glucose powder produced from the enzyme hydrolysis process. This research used an enzyme hydrolysis process with the stages of gelatinization, liquification and saccharification for 72 hours with variations in temperature of 40°C, 50°C, 60°C and 70°C and obtained the optimum temperature results from these four temperatures. The optimal saccharification temperature is achieved by incubating at 50°C and 60°C with an incubation time of 72 hours or 3 days.

***Keywords**: glucose powder, rumbia starch, saccharification, sweetener, optimum temperature.*

INTRODUCTION

Glucose powder is a form of glucose that has been dried into fine powder. Glucose is also known as glucose sugar or dextrose, where this sugar has a simple structure (monosaccharide) that serves as the main source of energy for the human body and many other organisms. Glucose powder is commonly used in the food, beverage, and pharmaceutical industries. Glucose is one of the significant types of monosaccharides. Sometimes, it is known as blood sugar due to its presence in the blood, also referred to as grape sugar because it's found in grapes, or known as dextrose because it has the ability to rotate the polarization of light to the right. Around 14% of glucose is found in molasses [1].

Sago palm, scientifically known as Metroxylon sagu Rottb, naturally grows along riverbanks, peat swamps, freshwater swamps, and areas near water sources. Sago palm starch is a component with great potential to meet the increasing demands of the food industry cause the world's population is growing and more and more artificial sweeteners are needed that are safe for food and drinks [2, 3]. Glucose powder production research has been carried out on a laboratory scale in research on making glucose powder by Kartika [4], and pilot-scale research on making glucose powder by Suharno [5].

Making liquid glucose using enzyme hydrolysis has been widely used. There has been no research about making glucose powder which compares four different temperatures to find out the optimum temperature used to make glucose powder with the best quality. Enzymatic hydrolysis is a process in which cellulose and hemicellulose are converted into reducing sugars with the help of enzymes. Xylanase enzyme is used to convert hemicellulose into glucose, while cellulose enzyme is used to convert cellulose into glucose. In the hydrolysis process, α-amylase and glucoamylase enzymes are used. α-Amylase enzyme plays a role in breaking the 1,4-glycosidic bond in amylose and amylopectin, converting them into dextrins which are oligosaccharides. This combination of enzymes is used to break the 1,4-glycosidic and 1,6-glycosidic bonds in amylose, amylopectin, and dextrins, resulting in monosaccharides that form glucose syrup [6]. In the process of making glucose syrup at the enzyme breakdown stage, the gelatinization and liquefaction process is better at temperatures of 50°C and 90°C [7].

Glucose syrup is processed to form glucose powder through filtration, evaporation, crystallization, and drying processes. During the filtration process, filter paper is used to separate glucose syrup from its extract and to change the color of the originally brown glucose syrup from the saccharification process to a bright yellow color. Evaporation is employed to remove the contained water until the brix value reaches 68-75% [6]. Crystallization aims to form crystals from liquid glucose using a cooling crystallization method at room temperature, controlled by the amount of seed crystals used. Drying the glucose powder reduces the moisture content in the oven and cools it to a desiccator [8].

Research on glucose powder often involves different saccharification temperatures such as 50°C [9], 60°C, and 55°C - 65°C [8], thus this study aims to find the optimal saccharification temperature for producing the best glucose powder, along with a comparison of seed crystallization at room temperature and drying temperature. The resulting glucose powder product is expected to meet Indonesian National Standards (SNI) [10].

METHODE

This research was conducted for approximately 2 months at the Bioprocess Engineering Laboratory, Department of Chemical Engineering, Sriwijaya State Polytechnic. The materials used include 500 grams of sago palm starch, 0.6 mL of α-amylase enzyme, 0.6 mL of glucoamylase enzyme, 1N HCl (hydrochloric acid), 1N NaOH (sodium hydroxide), and glucose powder seed.

The equipment used comprises glassware (beakers, test tubes, Erlenmeyer flasks, measuring cylinders, volumetric pipettes, droppers), a hot plate, analytical balance, pH meter, oven, thermometer, filter paper, stirrer, spatula, aluminum foil sheets, water bath, and a refractometer.

Sample preparation for rumbia sago starch involves weighing 500 grams, and then adding 1 liter of water in a 1:4 ratio. Stir until the water and sago palm starch are thoroughly mixed. Next, add NaOH to adjust the pH of the sago solution to 5. For the gelatinization process, heat the sago solution to 90°C while stirring continuously until it thickens and changes from white to clear. Subsequently, in the liquefaction process at a constant temperature of 90°C, add 0.6 mL of α-amylase enzyme and stir until the gelatin turns back into a liquid without any lumps. Proceed to the saccharification stage where the liquefied sago solution is cooled to 50°C, and then add 0.6 mL of glucoamylase enzyme. Adjust the pH to 4.5 using an HCl solution [6]. The sago solution is incubated at various temperatures of 40°C, 50°C, 60°C, and 70°C over 72 hours to determine the optimal saccharification temperature and produce glucose syrup.

The resulting glucose syrup is filtered using a filter cloth or filter paper to separate the syrup from the extract, yielding a clearer color. The filtered glucose syrup is then evaporated at 100°C using atmospheric evaporation. This process aims to evaporate as much water as possible until the brix level reaches 68-75%. As this study involves room temperature crystallization, the %brix is increased to 80-85%. In the crystallization process of this study, a comparison is made between glucose seed, both without using seed and with 5% seed. After adding the seed, the crystallization process is carried out through cooling, conducted at room temperature. The crystallized glucose syrup is broken down using a chopper and then dried to reduce moisture content. This drying process is conducted at temperatures of 50°C and 60°C using an oven [4]. The resulting glucose powder is analyzed for moisture content, ash content, reducing sugar content, and total sugar content in accordance with Indonesian National Standards (SNI).

Table 1. Quality standards for glucose powder

No.	Test Criteria	Units	Requirements
1	Organoleptic		
	Color	-	White
	Odor	-	Odorless
	Tase	-	Sweet
2	Reducing sugar	% (b/b)	Min. 99,5
3	Moisture content	% (b/b)	Max. 9
4	Ash content	% (b/b)	Max. 0,1
5	Sulfate	mg/kg	Max. 250
6	Chloride	mg/kg	Max. 180
7	Starch	-	Negative
8	pH (50% in water)	-	5,0 - 7,0
9	Specific Rotation		(+)52,6 s.d.(+) 53,2
10	Sulfur Dioxide	mg/kg	Max. 20
11	Heavy Metal Contamination		
	Plumbum (Pb)	mg/kg	Max. 0,5
	Copper (Cu)	mg/kg	Max. 10
	Zinc (Zn)	mg/kg	Max. 250
12	Microbial Contamination		
	Total plate count	colony/g	Max. 100
	Yeast and Mold	colony/g	Max. 10
	Escherichia coli	colony/g	Negative
	Salmonella	colony/g	Negative
13	Genetically Modified Organism (GMO) status	-	Negative

RESULTS AND DISCUSSION

The Effect of Saccharification Temperature on %Brix

The process of making glucose powder involves several stages, one of which is the saccharification process. In this process, enzymatic hydrolysis occurs, where the glucoamylase enzyme breaks down into oligosaccharides, maltotriose converts to maltose, and maltose converts to glucose. Saccharification is the final stage in the production of glucose syrup, resulting in glucose syrup with a minimum %brix of 25% [6]. The higher the resulting brix, the sweeter the taste and the higher the concentration of dissolved sugar in the water.

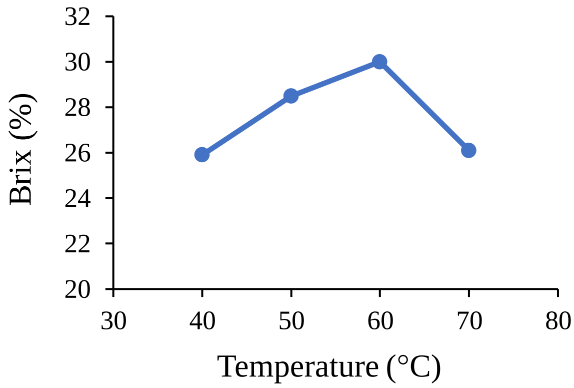

Figure 1. The effect of saccharification temperature on %brix

In this case, experiments were conducted with four different temperature variations in the saccharification process to determine the optimal temperature that yields the best results. As shown in Figure 1, it is evident that a temperature of 60°C resulted in the highest %Brix, reaching 30%. On the other hand, the lowest %Brix was obtained at 40°C, measuring 25.9%, and at 70°C, the %Brix obtained was lower than that at 60°C, at 26.1%. All four temperature variations in the saccharification process can still proceed to the next stage of glucose powder production because the %Brix produced is not less than 25%, indicating that they still possess sufficient sugar content for further processing.

The Effect of Inoculation on Time at Saccharification Temperature

The crystallization method used in this study is the cooling method, where supersaturation is achieved by cooling the solution to room temperature. The cooling of the solution occurs through a decrease in pressure at equilibrium. According to Sudarsono, the more seed crystals are added, the faster the crystallization process occurs [5].

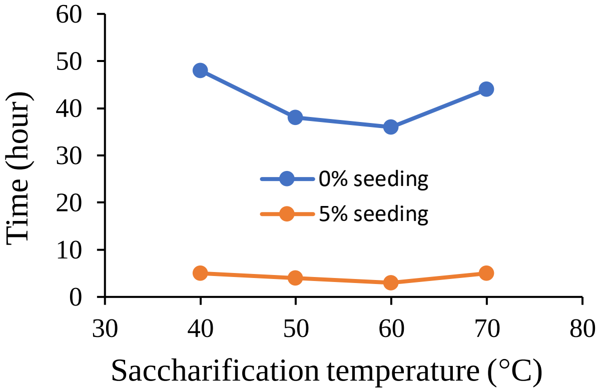

Figure 2. The effect of seeding on saccharification temperature

The inoculation using glucose powder significantly affects the crystallization rate in the glucose powder production process. As shown in Figure 2, samples with a 5% inoculation freeze approximately 9-10 times faster compared to samples without any inoculation. Samples without inoculation take up to 2 days to crystallize, while samples with inoculation only require 3-5 hours.

In this case, across both the non-inoculated samples and the samples with a 5% inoculation, the temperature variation applied during saccharification remains consistent. The sample with saccharification temperature of 60°C consistently outperforms in terms of crystallization speed, followed by the 50°C sample. The 70°C sample follows suit, while the slowest crystallization occurs with the 40°C sample.

Moisture Content Analysis

After going through the step-by-step processes to obtain glucose powder with variations in saccharification temperature, seed inoculation for room temperature crystallization, and drying temperature, an analysis is conducted on the moisture content, ash content, reducing sugars, and total sugars of the glucose powder. This research aims to produce glucose powder with suitable variations to achieve analytical results in accordance with Indonesian National Standards (SNI).

Moisture content refers to the amount of water contained within a substance or material. According to the Indonesian National Standards (SNI), glucose powder has a maximum allowable moisture content of 9%.

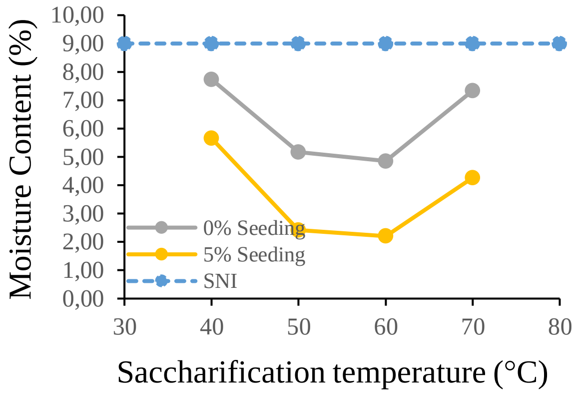

Figure 3. Moisture content levels of glucose powder product

In the examined sample variations, the analytical results indicate that the produced glucose powder in this research complies with the Indonesian National Standards (SNI) due to the moisture content being within the acceptable range. The highest moisture content obtained in this study was 7.74%, which is from the saccharification temperature variation of 50°C, crystallized without seed inoculation. The lowest moisture content obtained was 2.20%, which is from the saccharification temperature variation of 60°C, crystallized with 5% seed inoculation.

Ash Content Analysis

Ash content is the percentage of remaining minerals or inorganic compounds after a substance is burned at a high temperature. Organic materials like food and biomass generally consist of a mixture of carbon, hydrogen, oxygen, nitrogen, along with some minerals and inorganic compounds.

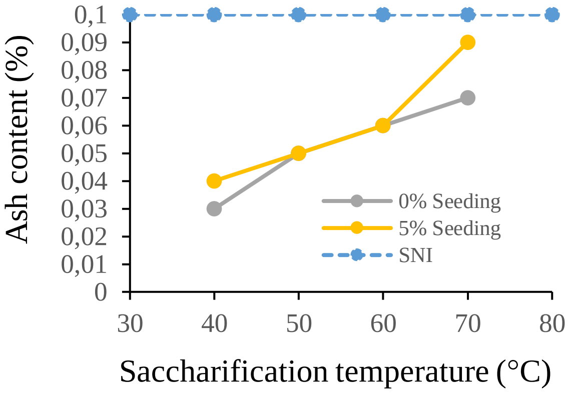

Figure 4. Amount of ash content in glucose powder product

According to the Indonesian National Standards (SNI), glucose powder has a maximum allowable ash content of 0.1% [10]. In the examined sample variations, the analytical results indicate that the produced glucose powder in this research complies with the Indonesian National Standards (SNI) due to the ash content being within the acceptable range. The highest ash content obtained in this study was 0.09%, which is from the saccharification temperature variation of 70°C, crystallized without seed inoculation. The lowest ash content obtained was 0.03%, which is from the saccharification temperature variation of 40°C, crystallized without seed inoculation.

Figure 4 illustrates that samples subjected to higher temperatures have higher ash content compared to samples subjected to lower temperatures. Additionally, the seed inoculation variation also influences the ash content, as samples with seed inoculation tend to have higher ash content.

Reducing Sugar Analysis

Reducing sugar content refers to the amount of reducing sugars present in a food substance. Reducing sugars are a type of sugar that has the ability to reduce or convert metal ions such as copper ions (Cu^{2+}) to copper ions (Cu^{+}), resulting in the formation of colored compounds. Common examples of reducing sugars include glucose and fructose.

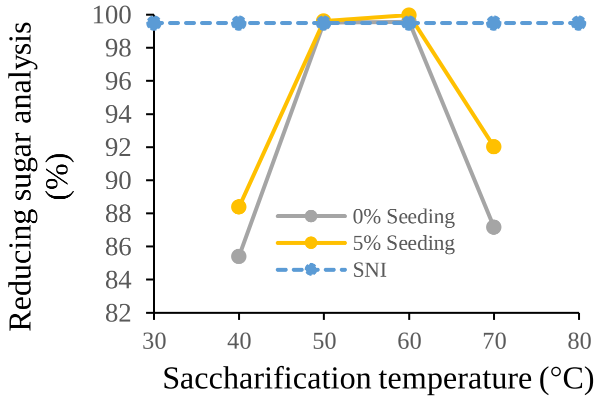

Figure 5. Amount of reducing sugar content in glucose powder

According to the Indonesian National Standards (SNI), glucose powder should have a minimum reducing sugar content of 99.5% [10]. Figure 5 demonstrates that not all glucose powder produced in this study complies with the Indonesian National Standards (SNI). None of the samples from the 40°C and 70°C variations meet the Indonesian National Standards (SNI), regardless of seed inoculation and drying temperature variations. In fact, their values are significantly distant from the Indonesian National Standards.

From the above research data, it can be concluded that saccharification temperature variations affect the amount of reduced sugar content. The glucoamylase enzyme doesn't work optimally in breaking down starch into glucose at temperatures of 40°C and 70°C. On the other hand, at temperatures of 50°C and 60°C, the glucoamylase enzyme successfully works at its maximum efficiency in converting starch into glucose, as indicated by the reducing sugar content reaching 99.5%.

Total Sugar Analysis

Total sugar content refers to the overall amount of sugar present in a solution, food, beverage, or food material. Total sugar includes all types of sugars present in the sample, whether they are reducing sugars or non-reducing sugars. Some examples of sugars included in total sugar content are glucose, fructose, sucrose, lactose, and maltose.

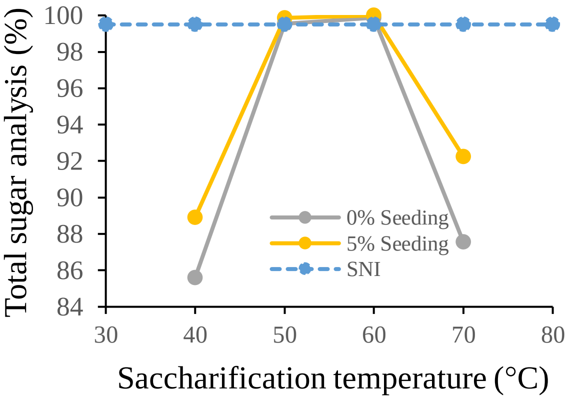

Figure 6. Amount of total sugar content in glucose powder

Measurement of total sugar content is carried out using chemical analysis methods that identify and quantify the total amount of sugar in a sample. Typically, in the food industry, methods like spectrophotometry, high-performance liquid chromatography (*HPLC*), and Luff schrool are used to measure total sugar content.

According to Kartika, glucose powder has a total sugar content standard equal to or greater than its reducing sugar content [4]. Figure 6 illustrates that there are no anomalies in the calculation of total sugar content resulting from this research.

Total sugar content is closely related to reducing sugar content, where the minimum standard for reducing sugar content is 99.5%. In the case of the 40°C and 70°C samples with seed inoculation and drying variations, none of them meet the minimum standard for total sugar content. Therefore, the total sugar content meeting the Indonesian National Standards (SNI) is only observed in the 50°C and 60°C samples, along with their respective seed inoculation variations.

CONCLUSION

From the above research, it can be concluded that:

1. The optimal saccharification temperature is achieved by incubating at 50°C and 60°C with an incubation time of 72 hours or 3 days.
2. The addition of seed inoculation in the crystallization process affects key parameters such as the speed of crystallization, moisture content, reduced sugar content, and total sugar content.

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