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Effect of Sago Starch Concentration on Characteristic of Sago Glucose Syrup

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ABSTRACT

Opportunity to use sago as a basic ingredient for syrup glucose is very large because of the high carbohydrate content reached 75.88% - 85.08%. Sago starch contains 27% amylose and 73% amylopectin. This study aims to determine the effect of sago starch substrate concentration on reducing sugar, total dissolved solids, dextrose equivalent, and sweetness level from the glucose syrup produced. This research was conducted in 3 stages, namely gelatinization, liquefaction, and saccharification. The use of sago substrate concentrations were 25%, 30%, and 35%. The use of amylase enzyme is 0.1% dry weight and glucoamylase enzyme is 0.008 g/g dry weight. This study used a completely randomized design with a factorial pattern with two replications and data analysis using Duncan test. The use of α-amylase and glucoamylase enzymes in the manufacture of glucose syrup from sago starch affects the glucose syrup produced. The best result is obtained from 30% substrate concentration with reducing sugar value is 186.07 g/L, total dissolved solid is 36.13%, dextrose equivalent value is 62.02%, and sweetness level value is 33.92 ºbrix.

Keywords: glucose syrup, liquefaction, saccharification, sago

INTRODUCTION

The need of liquid sugar for industries tends to increase every year. Food, beverages, and pharmaceutical industries currently have a tendency to use glucose syrup (Rika et al., 2020). Glucose syrup is a clear and thick liquid containing D-glucose, maltose, and D-glucose polymer obtained from the hydrolysis of starches, such as tapioca, sago, corn starch, and tuber starch (Suripto et al., 2013). The food and beverage industry are starting to use glucose syrup a lot because has several advantages, including not crystallizing, being easier to process because it is more dissolved, more practical, and has a more attractive appearance when compared to granulated sugar in general (Permanasari & Yulistiani, 2017). In addition, glucose syrup has several advantages when applied to food products such as glucose ice cream products can suppress the freezing point and increase the smoothness of texture, in cake products it can keep cakes fresh and not easy to crack. Whereas in candy products, glucose can prevent microbiological damage and improve texture (Suripto et al., 2013).

The manufacture of glucose syrup consists of two hydrolysis methods, namely the enzymatic and non-enzymatic or its

combination (Betiku et al., 2013). Enzymatic hydrolysis has fundamental differences with acid hydrolysis. Hydrolysis of starch is a breaking process of starch molecule to become constituent parts of the starch like dextrin, isomaltose, maltose, and glucose (Terahara et al., 2004). The enzymatic hydrolysis process is more effective than acid hydrolysis because the enzyme breaks the glycosidic bond specifically, leaving no residue and minimum color damage (Azmi et al., 2017). Glucose can be made from enzymatic hydrolysis by α-amylase and glucoamylase (Permanasari & Yulistiani, 2017).

The manufacture of glucose syrup by enzymatic hydrolysis consists of two stages, namely the liquefaction stage using the α-amylase enzyme and the saccharification stage using a mixture of glucoamylase and pullulanase enzymes. The α-amylase enzyme will cut the α-1,4-glycosidic bonds on the inside of the starch (amylose and amylopectin chains), while the glucoamylase and pullulanase enzymes will break the α-1,6 glycosidic bonds in the amylopectin polymer which is not able to be done by the α-amylase at liquefaction stage (Mardawati et al., 2019).

Sago stalks can be processed into sago starch. However, the use of natural starch (native) directly causes several problems, namely retrogradation, syneresis, low stability, and low resistance of pasta to pH and temperature. Therefore, it is necessary to modify starch physically, chemically, and enzymatically. The use of starch as a pharmaceutical product and fermentation medium is carried out through bioconversion, one of which is hydrolysis. Opportunity to use sago as a basic ingredient for syrup glucose is very large because the starch content is between 72% - 94% (Azmi et al., 2017). Sago starch contains 27% amylose and 73% amylopectin (Soraya et al., 2019). The purpose of this research is to determine the effect of sago concentration on the glucose syrup produced using α-amylase and glucoamylase enzymes.

MATERIALS AND METHODS

Tools and Materials

Sago flour obtained from North Luwu regency, South Sulawesi province, Indonesia. The other materials such as distilled water, α-amylase enzyme, glucoamylase enzyme, standard glucose DNSA (3,5-dinitrosalicylic acid) natrium sodium tartrate ($KNaC_4H_4O_6$-$4H_2O$), and NaOH were available from Hasanuddin University.

The tools in this research are spectrophotometer, oven, hotplate, autoclave, thermometer, pH meter, analytic measurer, desiccator, refrigerator, vortex, stopwatch, stirring rod, measuring glass, Erlenmeyer, reaction tube, micro pipette, and magnetic stirrer.

Methods

Raw Material Preparation

Sago flour dried by using blower then tested the water content. Sago concentration used in this research were 25%, 30%, and 35% w/v.

Gelatinization Process

Sago flour made in suspension by added distilled water. CaCl2 cofactor added, and the pH of suspension adjusted to 6.0-6.5 by added acid or base solution. The suspension heated until 121^0C and added α-amylase enzyme (0.1% dw). When the gelatinization process reached, the temperature maintained for 15 minutes (Megavitry, 2019).

Liquefaction Process

Suspension temperature lowered until 80^0C and the suspension added by the α-amylase enzyme (0.1% dw). The stirring process carried out for 90 minutes. At this

stage, the result obtained was maltodextrin (Megavitry, 2019).

Saccharification Process

After 90 minutes of the liquefaction process, the suspension temperature lowered until 60^0C and pH set to 4.5 for the saccharification stage. Glucoamylase enzyme added into the suspension and stirred for 5 minutes. The suspension inserted into the Erlenmeyer 250 ml to incubated in a water bath shaker for 72 hours. Sampling was done in every 6 hours (Megavitry, 2019) (Figure 1).

Analysis

Reducing Sugar Analysis

The DNS method is a colorimetric technique that consists of a redox reaction between the 3,5-dinitro salicylic acid and the reducing sugars present in the sample. The reagent is a solution formed by the following compounds: 3,5-Dinitrosalicylic acid (2-hydroxy-3,5-dinitrobenzoic acid), which acts as an oxidant, Rochelle salt (sodium potassium tartrate), which prevents the dissolution of oxygen in the reagent and sodium hydroxide to provide the medium required for the redox reaction to occur (Garriga et al., 2017).

1 ml of clarified sample and 3 ml of DNS was pipetted into a test tube. The solution heated in boiling water for 5 minutes and immediately cooled in running water. Analysis of reducing sugar content was carried out using a spectrophotometer at wavelength of 550 nm.

Total Dissolved Solid Analysis

Determination of total dissolved solid was calculated using the water content method (Andarwulan et al., 2011). Around 5 g of sample dried using oven with temperature 105^0C for 6 hours. The weight was considered constant if the difference in weighing did not exceed 0.5 mg.

Dextrose Equivalent (DE) Analysis

According to (Yunianta et al., 2015), the value of DE can be determined by using the formula:

$$\text{DE} = \frac{\sum \text{reducing sugar } (\frac{\text{w}}{\text{v}})}{\sum \text{total dissolved solid } (\frac{\text{w}}{\text{v}})} \text{ x } 100\%$$

Sweetness Level Analysis

The level of sweetness was tested using a hand refractometer (Apriyantono et al., 1989).

Data Analysis

Data processing was carried out using Completely Randomized Design (CRD) method with 2 replications. If the results are significantly different, Duncan's real distance difference test will be carried out as a further test.

RESULTS AND DISCUSSION

Reducing Sugar Level

Reducing sugars are sugars that have the ability to reduce electron-accepting compounds, this is due to the presence of free aldehyde and ketone groups. Glucose is a type of reducing sugar.

The results of the reducing sugars analysis showed that the use of 25% sago concentration resulted in a reducing sugar value of 143.93 g/L experienced a significant increase to the use of 30% sago concentration resulted in a reducing sugar value of 186.07 g/L, then the use of 35% sago concentration the reducing sugar value increased significantly of 211.94 g/L (Table 1).

Hydrolysis of starch by α-amylase enzyme produces glucose, maltose, maltotriose, and various types of α-limit dextrin, namely oligosaccharides consisting of 4 or more sugar residues containing many α-1,6 glycosidic bonds. Then the starch chain pieces that have been hydrolyzed by α-amylase enzyme will be further hydrolyzed

into glucose by the glucoamylase enzyme, so that more glucose is produced. Each hydrolyzed sugar chain has one reducing sugar group so that the more starch hydrolyzed into simple chain sugars, the higher the amount of reducing sugar.

Increasing substrate concentration increases the rate of reaction. This is because more substrate molecules will be colliding with enzyme molecules, so more product will be formed. However, after a certain concentration, any increase will have no effect on the rate of reaction, since substrate concentration will no longer be the limiting factor. The enzymes will effectively become saturated, and will be working at their maximum possible rate (Istia'nah et al., 2020). The high concentration of sago starch allows the bond between the sago starch as substrate and enzymes to increase so that the resulting product in the form of simple chain sugars is also higher.

Increasing the substrate concentration can increase the enzyme reaction. The rate of reaction (V) catalyzed by the enzyme increases with increasing substrate concentration [S], until a state is reached where the addition of substrate concentration [S] no longer increases the initial rate of reaction and when all enzymes are saturated by substrate [ES], the reaction rate will reach maximum state (Mardawati et al., 2019). High concentration of the enzyme will affect the speed of the reaction. Furthermore, the substrate concentration is low, the rate of enzyme action is low. On the other hand, if the substrate concentration is high, the enzyme work will be fast and if the substrate is in excess, the enzyme will not decrease but remain constant (Budiyanto et al., 2019).

Several studies have been conducted and show that increasing the substrate concentration can increase the yield of reducing sugars, such as the study conducted by Ticoalu et al., (2016), regarding the utilization of purple sweet potatoes into anthocyanin drinks through enzyme hydrolysis, it was stated that the higher the concentration of purple sweet potatoes used, the higher the reducing sugar produced because the starch substrate that could be hydrolyzed by α-amylase and glucoamylase enzymes was also getting bigger.

Total Dissolved Solid Analysis

Total dissolved solid is the amount of solid contained in a material containing water. The results of total dissolved solids will be used to calculate the dextrose equivalent (DE) value of glucose syrup produced together with reducing sugar.

The results of total solid analysis showed that the use of 25% sago concentration resulted in total solids value of 31.09% and increased significantly to the use of 30% sago concentration with total solids value of 36.13%, then the use of 35% sago yielded total solids value of 38.83% (Table 2).

The significant increase in total solids with increasing concentration of sago was caused by the increase in the amount of starch in the suspension. Starch is composed of amylose and amylopectin, therefore the increasing concentration of substrate used means that the quantity of amylose and amylopectin also increases so that more water is bound to the substrate and causes an increase in total solids although at 35% sago starch concentration the increase in total dissolved solids was not very significant. The increase in total dissolved solids was caused by the breaking of long chains of carbohydrate compounds into soluble sugar compounds. The increase in total dissolved solids which is in line with the increase in temperature and cooking time is due to the higher the temperature causing the breaking of long chains of carbohydrate compounds into soluble sugar compounds to be faster, so that the sugar content in the suspension will

dissolve more (Meikapasa & Seventilofa, 2016). Basically, the total dissolved solids of a material include reducing sugars, non-reducing sugars, organic acids, pectin, salts, and proteins which greatly affect the brix (Megavitry et al., 2019). The increase in reducing sugar resulted in the total soluble solids value of sago glucose syrup to increase.

Several studies have been conducted and show that increasing the substrate concentration can increase the total solids gain, such as the study conducted by Yunianta et al., (2015) regarding the production of glucose syrup made from canna starch, that the addition of canna starch substrate concentration causes a decrease in the water content in glucose syrup so that the total solids produced as a result of the saccharification process increase due to the binding of water by canna starch substrate. Similar results were obtained from a study conducted by (Mardawati et al., 2019), that the production of glucose syrup from corn starch, that the dissolved solids content of glucose syrup tends to increase with increasing substrate concentration.

Dextrose Equivalent Analysis

DE shows the amount of starch polymer that has been cut into simple sugar molecules, namely glucose, maltose, and dextrin. The DE value is the main parameter that describes how much starch is converted to glucose due to enzyme hydrolysis (Ni'maturohmah & Yunianta, 2015). Commercially the use of starch is influenced by the value of DE. The higher the DE solution, the higher the glucose level and the lower the dextrin level.

The results of dextrose equivalent analysis showed that the use of 25% sago concentration resulted in dextrose equivalent value of 57.57% and increased significantly to the use of 30% sago concentration with dextrose equivalent value of 62.02%. However, at 35% sago concentration, the dextrose equivalent value decreased to 60.55% (Table 3).

The higher the concentration of sago used the value of dextrose equivalent tends to decrease, because at higher starch concentration the time required for the enzyme to convert starch into dextrin takes longer so that increasing the concentration at the same time causes a decrease in product DE, while in this research the time used for each concentration was the same, namely 72 hours. Dextrose equivalent is a measure of the percentage of glycosidic bonds in starch that has been hydrolyzed, referring to the reducing sugar content, an indication of the large number of dextrose (glucose) molecules released during starch hydrolysis, on dry mass basis. So even though the value of reducing sugar produced increases, if the concentration of sago used also increases without the addition of enzyme and hydrolysis time, the dextrose equivalent produced can't decrease. Adrian et al., (2020) stated that DE decreased because the excess of the given substrate was not converted into reducing sugars because the enzymes used remained constant. Enzyme activity will decrease if the substrate concentration exceeds the optimum concentration, because excess substrate can be an inhibitor for enzyme activity.

The degree of hydrolysis is generally expressed as dextrose equivalent, the quantity indicates the dextrose (glucose) molecules released during hydrolysis of starch, on dry mass basis. The dextrose equivalent value is inversely proportional to the molecular weight, namely the degree of polymerization and as an indicator of the degree of hydrolysis, so glucose has dextrose equivalent of 100 while starch has a dextrose equivalent of zero (Sun et al., 2010).

Several studies have been conducted and show that the dextrose equivalent value can be influenced by the

concentration of the substrate used, such as the research of Zadha & Raharjo (2013), regarding the isolation of dextrin from sorghum starch that the increase in substrate concentration is inversely proportional to the equivalent dextrose value obtained so that the addition of starch substrate concentration sorghum causes a decrease in the value of DE in the resulting dextrin. The smaller the starch concentration used and the longer the hydrolysis time used, the greater the DE value obtained until it reaches the optimal value, and inversely, the greater the starch concentration used and the shorter the hydrolysis time used, the smaller the DE value obtained.

Sweetness Level Analysis

The sweetness level is one parameter of how much simple sugars are formed in a product or food ingredient. The level of sweetness (°brix) can also determine the number of solids dissolved in a solution. The total value of dissolved solids was measured using a hand refractometer. The value measured on the °brix scale or hydrometer scale can indicate the percent by weight of sugar present in the solution.

The results of sweetness level analysis showed that the use of 25% sago concentration resulted in sweetness level value of 28.88°brix and increased significantly to the use of 30% sago concentration with sweetness level value of 33.92°brix, then the use of 35% sago yielded sweetness level value of 36.65°brix (Table 4).

The increase in the value of the sweetness level correlated with the increase in the total dissolved solids obtained. Basically, the total dissolved solids of a material include reducing sugars, non-reducing sugars, organic acids, pectin, salts, and proteins which greatly affect the brix. The higher the concentration of sago used, the more substrate that can bind to the enzyme so that more substrate can be converted by the enzyme. Sago contains amylose and amylopectin which can be converted into simple sugars by α-amylase and glucoamylase enzymes, so that with increasing concentrations of sago used, more amylose and amylopectin can react with enzymes and produce glucose which results in an increase in total dissolved solids and affect the sweetness level of glucose syrup. Hadiwijaya et al., (2020) stated that total dissolved solids are the content of water-soluble materials such as glucose, sucrose, fructose, and pectin. Total dissolved solids are often used as an indicator of sweetness. Kalsum & Surfiana (2013) stated DE value also affects the level of sweetness. The higher the DE syrup, the higher the sweetness level. The higher the DE, the easier the syrup to absorb and retain water, so syrup with a high DE is more hygroscopic, so it has a high level of sweetness

Measurement of total dissolved solids value using a refractometer aims to measure total sugar roughly. With the assumption that the higher the total dissolved solids value, the higher the sweetness of the glucose syrup produced. Basically, the total dissolved solids are sugars and various other compounds such as organic acids, soluble amino acids, fats, minerals, and others. The refractometer measures the total dissolved solids based on their refractive index. The refractive index value is obtained from the speed of light in a vacuum compared to when light penetrates the sample. When light penetrates the sample, its speed will decrease. This is due to the presence of dissolved solids in the sample. The higher the concentration of dissolved solids in the sample, the higher the refractive index. This also applies the other way around.

Several studies have been conducted and show that the sweetness level value can be influenced by the concentration of the substrate used, such as the research of

Adrian et al., (2020), regarding the saccharification of white sweet potato into sugar dextrose enzymatically the addition of white sweet potato substrate concentration causes increase in sweetness level. This is possible because the total solids other than reducing sugars are more due to the use of a larger substrate concentration than the others. The level of sweetness is the same as the number of monosaccharides formed, especially glucose which is the final product. The higher the glucose formed, the higher the level of sweetness.

CONCLUSION

The use of α-amylase and glucoamylase enzymes in the manufacture of glucose syrup from sago starch affects the glucose syrup produced. The treatment of sago starch concentration affects the value of reducing sugar and total dissolved solids. The best glucose syrup was obtained from the treatment of 30% sago starch concentration with reducing sugar value of 186.07 g/L, total dissolved solid value 36.13%, DE value of 62.02%, and sweetness level of 33.92ºbrix.

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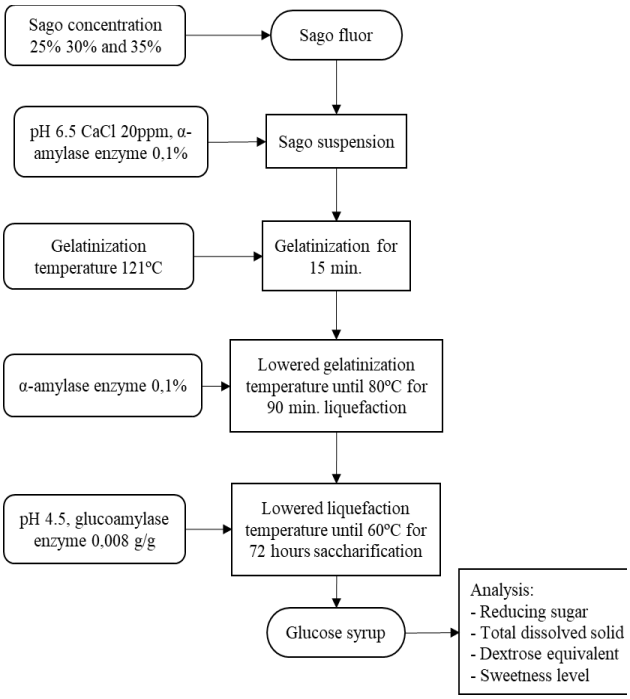

Figure 1. The research process of sago glucose syrup with variations in substrate concentration

Table 1. The Relationship of Variations in Substrate Concentration to Reducing Sugar (g/L)

Treatment	Average	Notation
25%	143.93	a
30%	186.07	b
35%	211.94	c

Table 2. The Relationship of Variations in Substrate Concentration to Total Dissolved Solid (%)

Treatment	Average	Notation
25%	31.09	a
30%	36.13	b
35%	38.83	c

Table 3. The Relationship of Variations in Substrate Concentration to Dextrose Equivalent (%)

Treatment	Average	Notation
25%	57.57	a
30%	62.02	b
35%	60.55	c

Table 4. The Relationship of Variations in Substrate Concentration to Sweetness Level (°brix)

Treatment	Average	Notation
25%	28.88	a
30%	33.92	b
35%	36.65	c