Technical Analysis of Liquid Sugar Production Process of Raw Sago Starch Using the Enzymatic Hydrolisis Method of Pilot Plant Scale

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Abstract. Indonesia has the potential for sago around 50% of the world sago production but has not been used optimally. Technologically, the process of making liquid sugar from sago starch has been developed. Therefore, further research to improve the color quality of liquid sugar is needed. The research objective is to conduct a technical study of liquid sugar production process made from raw sago starch by doing purification treatment using activated charcoal and ion-exchange resin. The liquid sugar production process of raw sago starch using hydrolysis method is in the condition of the ratio of starch to water is 1:4, the liquidation process is at the temperature of 90°C for 90 minutes with the addition of enzyme α-amylase 1.2 mL kg⁻¹ of starch with pH of 5. Saccharification process is at the temperature of 50 °C for 72 hours with the addition of enzyme glucoamylase 1.2 mL kg⁻¹ starch with pH of 4.5. Purification by ion-exchange resin and evaporation by a vacuum evaporator are at 80° C for 90 minutes with pressure of 0.4 atm. Technically, the technology of liquid sugar production process using the enzymatic hydrolysis method could be developed for the pilot plant and industrial scale.

Keywords: Liquid Sugar, Sago Starch, Enzyme

1. Introduction

Liquid sugar (Glucose Syrup) is the innovative results of sugar development process. Liquid sugar is a type of monosaccharide with the molecular formula C₆H₁₂O₆. This product can function as a substitute for sugar and is economically feasible to develop [1].

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. The use of liquid sugar in food and beverage industries have some more advantages than other sugars, such as improving the taste and appearance of the final products, improving the consistency of final product, improving the durability of the product, and having the highest level of safety.

It shows the opportunity in developing glucose syrup industries. The aim is to overcome the balance of trade deficit which shows the consumption of glucose syrup in this country is quite high. This opportunity is even greater if glucose syrup can be accepted by the market as the substitute of rafination sugar or to cover the deficiency of rafination sugar needs.

Technologically, the process of making glucose syrup has been done by several previous researchers. The process of making glucose syrup can be carried out using acid hydrolysis and
enzimatic hydrolysis processes. Enzymatic hydrolysis has some advantages, such as; more specific processes and expected products, controlled manufacturing process, cheaper purification costs, minimized color damage, and fewer by-products [2]. The hydrolysis process of starch into glucose molecules can be seen in Figure 1.

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\begin{array}{cc}
\text{Starch} & \text{water} \\
\text{catalyst and heat} & \text{glucose} \\
\end{array}
\]

Figure 1. The hydrolysis process of starch to glucose

Suba Indah, LLC (Cilegon), BAJ, LLC (East Java), and Associated British, LLC (West Java) are industries that manufacture liquid sugar or glucose syrup. The raw material that has been developed in liquid sugar industries is tapioca. It is quite interesting opportunity to offer sago starch as raw material for liquid sugar industries. All this time, sago starch is still seen as “marginalized commodity”.

The disadvantages of previous studies are that the result of liquid sugar is yellow or reddish yellow so it does not meet the SNI 01-2978-1992 on liquid sugar. The researches are also still being developed on a laboratory scale. Further research is indispensible in order to improve the color quality of liquid sugar made from sago. The development of purification methods by using an ion exchange resin (anion cation) and activated charcoal are expected to increase the color clarity of the liquid sugar.

Furthermore, in-depth technical studies are needed on the production of liquid sugar made from sago starch in plant scale as an illustration in industrial scale development. The needs of liquid sugar will be fulfilled from this processing industry of sago starch. The objective of this research is to determine the optimization of liquid sugar production process made from sago starch by using treatment in the purifying process. The research results are expected as an effort to improve the added value of sago starch and to reduce dependence on imported sugar.

The production process of glucose through enzymatic hydrolysis consists of the liquidation stage and the saccharification stage [3]. The liquidation process is the process of melting starch gels using the α-amylase enzyme that hydrolyzes starch into simpler molecules than oligosaccharides or dextrins [4]. The α-amylase is an enzyme that hydrolyzes typically through the interior (endo-hydrolase) by producing oligosaccharides from alpha configurations that break the α- (1,4) glycosidic bond in amylose, amyllopectin and glycogen. The α- (1,6) glycosidic bond cannot be broken by α-amylase, but can be made into shorter branches. Starch separation by α-amylase is conducted randomly as seen in Figure 2.

![Figure 2. The hydrolysis mechanism of starch by α-amylase](image)

The glucoamylase enzyme can inverse the configuration of the broken chain and can break the α- (1,6), α- (1,3), α- (1,2) and α- (1,1) glycosidic bonds, although the breaking speed is lower compared to the cut of α- (1,4) glycosidic bond as seen in Figure 3.
Figure 3. The hydraulic mechanism of starch by glucoamylase

Meanwhile, similar research results from Murtias et.al (2016) [5] showed that sago starch from Southeast Sulawesi has physical characteristics of fine powder, white color, typical sago flavor, normal sago aroma, water content of 7.21%, ash 0.11%, fat 0.56%, protein 0.36%, crude fiber 0.37%, carbohydrates 91.76%, starch 80.69%, copper metal 1.28 ppm and no tin, mercury and arsenic contained, with numbers the total plate is 4.5x10^1 colonies / g and contains no mold. The optimum condition of liquid sugar production with sago starch derived from Southeast Sulawesi obtained a starch ratio 1: 4 of water with the 1.2 mL kg^-1 starch of α-amylase enzyme and 1.2 mL kg^-1 starch of amiloglucosidase enzyme. The resulting liquid sugar has physical characteristics with a total dissolved solid of 60 °Brix, sweet taste, sweet aroma of sugar, a reddish yellow color with the content of water 35.26%, ash 0.06%, reducing sugar agent 50.46%, copper metal 1, 24 ppm, zinc metal 3.59 ppm and no tin and arsenic contained. The results of microbiological analysis for total plate, mold, and yeast meet the required standards.

2. Methods

2.1 Materials
The materials used in this research were sago starch (Metroxylon sp.) originating from the Koto Marapak region, Pariaman, West Sumatra, aquadest, α-amylase enzyme, glucoamylase enzyme, activated charcoal, filter paper and Lewatit type of ion-cation resin, acetic acid 30%, 30% of NaOH solution, 5% of CaCO₃ solution and 15% of H₃PO₄ solution. The instruments used were liquid sugar reactors, magnetic stirrer hotplates, stoves, pans, evaporators, shaker baths, glassware, analytical scales, vacuum pumps, pH meters and thermometers.

2.2 Methods
The optimization stages of production process of liquid sugar [6] in the modification:

a. Material preparation
   Sago starch is washed with water, stirred thoroughly then filtered so that all impurities are filtered out. Dry it in the oven at a temperature of 45-50° C for 5-6 hours, then the dried sago is mashed and sieved.

b. Liquidation Process
   Amount of sago starch 100 g and 400 mL of water equivalent to 1: 4 are mixed then heated in a hotplate to a temperature of 90 °C for 90 minutes, at pH 5 the addition of the α-amylase enzyme (1.2 mL / kg starch) is mixed into the reactor. During the liquidation process the stirring is carried out, so that the starch does not clot. Then it is cooled to a temperature of 50 °C.

c. Saccharification process
   Cold starch is added to the glucoamylase enzyme (1.2 mL / kg starch) at pH 4.5 and temperature of 50°C for 72 hours.

d. The bleaching and filtering stages are intended to remove impurities and to stop enzyme activity, so that clear liquid sugar is obtained.
The first treatment is 100 ml of a hydrolyzate solution mixed with 0.8 g of activated charcoal and heated to a temperature of 80 °C for 1 hour. The second stage is filtering. The mixture of active substrate and charcoal is then filtered until a light yellow filtrate is obtained. The second treatment is done by passing the hydrolyzate into a column containing an ion exchange resin to reduce color. The sample is first purified through the carbonation process. A total of 100 ml of hydrolyzate is heated at 55 °C. The 10 ml of 5% CaCO$_3$ solution is added gradually to pH 10.5. Temperature and pH are maintained during the carbonation process. 15% of H$_3$PO$_4$ solution is added to reduce the pH to 7. Leave for ± 3 hours until the precipitate decreases and then filtered. Then pass it into the ion exchange resin column.

3. Results and Discussion

3.1 Characteristics of Sago Starch

The first step in this research is to characterize the raw materials of sago starch used. This is important to be conducted because sago starch from a certain location has different characteristics. The results of the characterization of sago starch from Koto Marapak, Pariaman can be seen in Table 1.

There are two kinds of liquid sugar (glucose syrup) production process technology, namely acid hydrolysis technology and enzymatic technology. The main difference between the acid hydrolysis process and the enzymatic process lies in the substances used to break down starch. In the acid hydrolysis process, the breakdown of starch is carried out with acids. In the enzymatic process, the breakdown of starch is carried out with enzymes. The process used in this research is the enzymatic process. The enzymatic process technology was chosen because it has several advantages over the technology of acid hydrolysis process. According to Norman (1981) [2], enzymatic hydrolysis has a fundamental difference compared to chemical and physical hydrolysis in terms of the specificity of breaking the starch polymer chain. Chemical and physical hydrolysis will randomly break the polymer chains, whereas enzymatic hydrolysis will break the polymer chains specifically at certain branches. Enzymatic hydrolysis has several advantages, such as the process is more specific and the product is produced as expected. In addition, the process conditions can be controlled, fewer purification costs, fewer byproducts and ash produced, less energy used and minimized color damage. These are a number of advantages of the enzymatic process.

| Parameter                  | Sago  |
|----------------------------|-------|
| Water content (%)          | 13.75 |
| Ash content (%)            | 0.39  |
| Fiber content (%)          | 1.03  |
| Protein content (%)        | 0.7   |
| Starch Content (%)         | 85.16 |
|   · Amylose (%)            | 28.93 |
|   · Amylopectin (%)        | 71.07 |
| Fractination Passed 100 mesh (%) | 68.65 |
| Gelatinization Temperature (°C) | 85    |
| White degree (%)           | 71.22 |
| Solubility (%)             | 5.36  |
| Water absorption (%)       | 60.38 |

3.2 Production Process in Pilot Plant Scale

The production process is selected producing the best liquid sugar characteristics. The process stages are done by modifying some previous research [6]. Optimization of the production process of liquid
sugar made from selected Sago starch, which is the process of hydrolysis enzymatic with the comparison condition of starch and water is 1:4, the process of liquidation at 90°C for 90 minutes with the addition of amylase enzyme 1.2 mL kg⁻¹ condition pH 5. The process of saccharification with the addition of glucoamylase enzyme 1.2 mL kg⁻¹ starch at pH 4.5 and temperature 50°C for 72 hours, purification with ion exchange resin and evaporation using vacuum evaporator at pressure 0.4 ATM temperature 80°C for 90 minutes. The process flow of liquid sugar production in this research is in line with the established methods which can be seen in Figure 4.

**Figure 4. Process Flow of Liquid Sugar Production**

3.3 Characteristics of Liquid Sugar
The resulting sugar product is tested for its quality through organoleptic tests including flavor and aroma, as well as a physical characteristic test that includes total dissolved/° Brix solids (refractometers), pH values, turbidity, total sugar levels and color measurements (chromameter). Chemical characteristics are with the parameters of water content test (SNI 01-2891-1992), ash
content (SNI 01-2891-1992), reducing sugar levels (SNI 01-2891-1992) and metal content (Pb, Cu, Zn, As) (SNI 2354.5:2011). Microbiological characteristics include total plate number (ALT) test (ISO 4833:2003), mold and yeast (FDA-BAM 2001). The test results of physical characteristic of liquid sugar produced can be seen in Table 2.

| No  | Treatment                             | Thickness (°Brix) | Turbidity NTU | Color (°h) | pH  |
|-----|---------------------------------------|-------------------|---------------|------------|-----|
| 1   | Reference liquid sugar               | 80.00             | 1.09          | 98a        | 6.8 |
| 2   | Liquid sugar before purification     | 30.55             | 1.61          | 73a        | 5.0 |
| 3   | Purification with activated charcoal  | 60.82             | 9.28          | 62a        | 7.2 |
| 4   | Purification with resin              | 65.75             | 1.30          | 93a        | 7.0 |

Brix is a dry solid matter dissolved in solution (g / 100 g solution) which is calculated as sucrose [7]. Brix can also be defined as the percentage of the mass of sucrose contained in the mass of the sucrose solution. Whereas the sucrose solution mass is the sucrose mass contents the mass of its solvent. The highest brix value is found in the purifying treatment with resin which is 65.75°brix, but the value is still far from the thickness/brix of reference liquid sugar. The reference liquid sugar used is liquid sugar from tapioca starch that is exist in the market today. Lower brix values due to sago starch are not completely hydrolyzed. It happens because enzyme activity that does not optimally work. The process of breaking down sago starch to glucose is assisted by enzyme activity. Enzyme concentration is also considered to affect the brix value of liquid sugar which is characterized by reduced substrate. The enzyme concentration of 1.2 mL / kg is the optimum concentration [5] which can increase the sugar reducing agent because of the large ability or activity of enzyme to conduct catalytic reactions [4]. High enzyme concentrations with longer saccharification time will increase the product (reducing sugars) [8]. Saccharification time in liquid sugar production with an optimum temperature of 50° C will affect the brix value [9]; [8]; [10]. This research was conducted for 72 hours. Enzyme activity during saccharification time is greatly influenced by environmental factors such as acidity and temperature which are considered to change during the saccharification process, so that enzyme performance is not maximum.

Turbidity shows the level of muddiness of a solution caused by the formed colloids. The turbidity level decreased with the purification process using resin, this can be seen from the turbidity value which decreased from 1.61 to 1.30. It does not occur in the purification treatment using activated charcoal. The turbidity level increased due to the color of activated charcoal dissolve in sugar. Purification using activated charcoal with simple pilot plant scale technology produces turbid liquid sugar so high technology is needed in order to the absorption of color can be effective.

The pH value of the liquid sugar product purified with resin is 7 (neutral), it is because neutralization is conducted before the purification process at the end of the carbonation process. The pH value of the liquid sugar without purifying process is lower than the pH value of the reference liquid sugar or the purified product. The range of this pH value is the optimum condition for the saccharification process by the glucoamylase enzyme, which is pH 5. Before the purification process with activated charcoal, neutralization is carried out to stop the glucoamylase enzyme working. The pH value of the liquid sugar filtered by activated charcoal is higher, which is 7.2. The pH difference is not significant and is still considered neutral. The condition is possibly caused by the active charcoal contaminated by the alkaline environment due to previous uptake.

Based on the glucose syrup quality standards issued by the Indonesian National Standard (SNI), the quality of glucose syrup is colorless. To fulfill SNI standard color, purification process is needed. The research results showed that the liquid sugar from sago starch from purification with activated charcoal showed a reddish yellow color, while the liquid sugar with purification resin had a more yellowish color (yellowish white). The color value (°h) represents the dominant wavelength that will determine whether the color tends to be red, green or yellow. The highest color value (°h) in liquid sugar from sago starch lies in the purification treatment with an ion exchange resin. The liquid sugar
The color of liquid sugar from sago starch is influenced by several factors. The protein content of sago starch will affect the color of liquid sugar from sago starch [3]. The protein content of sago starch is higher when compared to tapioca starch [11]. The higher the protein content, the darker the color of the liquid sugar. The protein contained in sago starch will react to the reduced sugar through the Maillard reaction which causes non-enzymatic browning. Another factor that causes differences in the color of liquid sugar is the difference in sago growing region. Research conducted by Fridayani (2006) [3] stated that liquid sugar from sago starch from North Sulawesi and Irian Jaya has a darker color than sago starch from West Java, Riau and South Kalimantan.

Based on the analysis results of physical characteristics of liquid sugar in Table 2, the optimal process is obtained in the production process of liquid sugar by purification using an ion exchange resin. The yield produced is 80%. The product results from the optimal process are continued by testing the characteristics based on SNI liquid sugar. The results can be seen in Table 3. Liquid sugar products produced from sago starch generally meet the SNI 01-2978-1992 requirements, only the color parameters do not yet meet the standard (yellowish). However, the result is better than the results of a similar study from Murtias et.al (2016) [5] which shows that liquid sugar from sago starch from Southeast Sulawesi is reddish yellow. According to Tjokroadikoesoemo (1986) [12], the produced glucose syrup is included in the type III category (sugar reducing agent 58-73) in the world of commerce.
Table 3. The test results of liquid sugar products according to SNI 01-2978-1992 [13]

| No. | Test Criteria                  | Unit   | Requirement   | Product  |
|-----|--------------------------------|--------|---------------|----------|
| 1   | Situation                      |        | Odorless      | Odorless |
| 1.1 | Smell                          |        | Sweet         | Sweet    |
| 1.2 | Taste                          |        | Colorless     | Yellowish|
| 2   | Water                          | % b/b  | Max. 20       | 19.16    |
| 3   | Ash                            | % b/b  | Max. 1        | 0.85     |
| 4   | Reducing sugar is calculated   | % b/b  | Min. 30       | 60.4     |
|     | as D-glucose                   |        |               |          |
| 5   | Starch                         |        | None          | None     |
| 6   | Metal contaminants:            | ppm    | Max. 1        | -        |
| 6.1 | Lead                           |        | Max. 10       | 1,14     |
| 6.2 | Copper                         |        | Max. 1        | -        |
| 6.3 | Zinc                           |        | Max. 25       | 2.59     |
| 7   | Arsenic                        | ppm    | Max. 0.5      | -        |
| 8   | Microbial contamination:       | Colony | Max. 5 x 10^{1} | 3,5x10^{1} |
| 8.1 | Total plate figures            | Colony | Max. 20       | -        |
| 8.2 | Coliform bacteria              | APM    | Less than 3   | -        |
| 8.3 | E. coli                        | APM    | Max. 50       | -        |
| 8.4 | Mold                           | Colony | Max. 50       | -        |
| 8.5 | Yeast                          | Colony | Max. 50       | -        |

4. Conclusion
The optimal processes of liquid sugar production from sago starch are the enzymatic hydrolysis process with the conditions of the ratio of starch to water is 1:4, the process of liquidation at 90°C for 90 minutes with the addition of the α-amylase enzyme 1.2 mL/kg at pH 5, saccharification process by adding 1.2 mL/kg of glucoamylase enzyme starch at pH 4.5 and temperature of 50°C for 72 hours, purification with ion exchange resin and evaporation with a vacuum evaporator at a pressure of 0.4
atm at 80 °C for 90 minutes. The yield produced is 80%. The resulting liquid sugar produced meets SNI 01-2978-1992, except color parameters.

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