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Effect of liquefaction time and enzyme addition on liquid sugar production from sweet sorghum starch by enzymatic hydrolysis

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Abstract. The development of liquid sugar production is being widely discussed both in the aspects of materials and methods. Sweet sorghum is one of the potential raw material for liquid sugar production. In this study, liquid sugar from sorghum was produced by enzymatic hydrolysis of two stages, namely liquefaction process using α -amylase and saccharification process using amyl-glucosidase. The purpose of this study is to determine the effect of liquefaction time and enzyme addition (α -amylase and amyl-glucosidase) to produce liquid sugar from sorghum by enzymatic hydrolysis. Raw material used in this study was sorghum starch. The experiment was arranged in complete randomized design with liquefaction time (10, 20, and 30 minutes), enzyme addition consist of α -amylase addition (5 and 3 mL/Kg) and amyl glucosidase-addition (5 and 3 mL/Kg), repeated two times. Results showed that ANOVA between liquefaction time and enzyme addition have significantly different of their characteristics ($p < 0.05$). The optimum condition of liquid sugar production was 5 mL/Kg for α -amylase addition, 3 mL/Kg for amyl-glucosidase addition, and 10 minutes for liquefaction time. Characteristics of liquid sugar from this condition are yellow colour, total soluble sugar of 22° Brix, total sugar of 32.96%, pH of 4.98, and yield of 82.99%.

1. Introduction

Sugar is a strategic sweetener commodity which has become the necessity for food, beverage, and pharmaceuticals in both domestic and industries levels [1]. People generally consume crystal sugar for daily needs. However, crystallization process in sugar production is the most energy-intensives stages in crystal sugar industries [2]. Energy dissolution can be avoided by the production of liquid sugar. No crystallization process is used in production of liquid sugar. Liquid sugar processing saves energy and is being considered as crystal sugar product replacement. Moreover, in the food and/or beverage industries, the utilization of crystal sugar requires dissolving in water before use.

Starch is the primary raw material for liquid sugar production. It is the carbohydrate reserve of plants like corn, potato, rice, cassava, wheat, and sorghum. Sorghum is an important cereal crop which is ranked as the fifth largest produced cereal in the world after wheat, rice, barley, and maize [3]. It is a crop with high biomass and sugar yield, contains glucose, sucrose, cellulose, and hemicellulose in almost equal quantity [4]. Rather than producing starch, sweet sorghum carbohydrates are stored in the stalk as sugar with concentration of 12-20% [5]. So, sweet sorghum is thus considered as a good raw material for liquid sugar production.



Liquid sugar is known as glucose syrup or fructose syrup, which is a concentrated solution of monosaccharide group obtained from starch by acid hydrolysis, enzyme hydrolysis, or combination of both. The enzymatic process of hydrolyzing starch into glucose syrup is considered more effective and efficient than acid hydrolyzing process. Regassa and Wortmann [5] stated that enzymatic hydrolysis can produce greater conversion with a more specific and simple process compared to acid hydrolysis. Generally, α -amylase and amyl-glucosidase are used for enzymatic hydrolysis from starch to glucose. The process of enzymatic starch hydrolysis is divided into two steps, i.e. liquefaction and saccharification. In liquefaction process, starch is gelatinized and treated by α -amylase, which fragments the starch into regularly sized chains, resulting in dextrin, maltose, malt-triose and malt-pentose [6]. The second step is saccharification process, which are dextrin, maltose, malt-triose and malt-pentose hydrolyzed to glucose by amyl-glucosidase [7, 8].

Researches of liquid sugar from cassava [9], sweet potato [9], cane sugar [1], beet [10], yam [11], cashew apple [12] and sago [13] have been conducted. But no studies have examined liquid sugar production from sweet sorghum. This research studied the effect of liquefaction time and enzyme addition on liquid sugar production from sorghum. The purpose of this study was to determine the effect of liquefaction time and enzyme addition (α -amylase and glucoamylase) to produce liquid sugar from sorghum by enzymatic hydrolysis to physicochemical characteristics, such as color, total soluble solids, total sugar content, yields, and acidity.

2. Methods

2.1. Raw materials

Raw material used in this research was sweet sorghum starch obtained from Parung, Bogor, West Java. Enzymes used in this research were α -amylase and amyl-glucosidase. Chemical used for characterization of liquid sugar. Equipment used included oven, analytical balance, filter cloth, stirrers, spoons, pH-meter (HI-2011), thermometer, refractometer, chromameter (Minolta 300), stoves, buckets, wooden stirrers, blender, spectrophotometer UV 6500, cuvettes, and glassware for analyses.

2.2. Research methods

Liquid sugar prepared following the methods of Parwiyanti et al. [11] with some modification. It was produced from a conventional process consisting of soaking in water, precipitation, liquefaction, saccharification, and evaporation. Sweet sorghum starch was soaked in 1000 ml water (1:5) at a temperature of 35° C. A mixture of starch in water was allowed to settle for 2 hours. Then, α -amylase enzyme was added according the treatment (3 mL/kg and 5 mL/kg) and heated until temperature of 90-95° C. Liquefaction process has been done according the treatment (10, 20, and 30 minutes). Then, it was cooled down until temperature of 60° C. Saccharification process has been done by the addition of amyl-glucosidase enzyme according to the treatment (3 mL/kg and 5 mL/kg). Starch mixture was precipitated for 24 hours and heated to obtain TPT of 60-70° Brix

2.3. Research analysis

Sample analysis was conducted for sweet sorghum starch as raw material and liquid sugar as a product. Raw material analysis included proximate analysis and starch content [14]. Moreover, liquid sugar was analyzed their performance such as color, total soluble solid, total sugar content, yields, and pH [14].

2.4. Statistical analysis

This research was conducted by Completely Random Design (CRD). All data were subjected to the analysis of variance (ANOVA) using SAS 9.1.3 version. Differences between mean values were estimated using Duncan's multiple range tests at a confidence level of 95%. All experiments were performed in duplicates.

3. Results and discussion

3.1. Raw materials characterization

Proximate analysis of sweet sorghum starch was carried out to find out the contents of raw materials (**Table 1**). Based on **Table 1**, sweet sorghum starch can be used as raw materials of liquid sorghum productions.

Table 1. Raw materials characterization of sweet sorghum

Parameters	Results
Water (%)	4.73
Ash (%)	0.13
Protein (%)	5.87
Fat (%)	0.56
Carbohydrate (%)	88.71
Starch (%)	75.52

Starch ($C_{12}H_{22}O_{11}$)_n is one of the most ancient and distributed biopolymers in world. This biopolymer is hydrolyzed by α -amylase and amylo-glucosidase: starch-degrading enzymes [15]. They degraded starch to diverse products as dextrin, and progressively smaller polymers to finally obtained glucose unit ($C^6H^{12}O^6$).

3.2. Color

Color is the most significant quality determinant. All of the liquid sugar produced resulted in a yellow color (**Table 2**). It is caused by some factors, such as heating process, reducing sugars content, and protein content [16]. Sweet sorghum starch which is used as raw material has high protein content (**Table 1**). This colorization increases during processing as a result of enzymatic reactions and heat induced procedures involved. There was no difference in liquid sugar color between treatment of liquefaction time and enzymes addition. The yellow color of liquid sugar from sweet sorghum starch was caused by their high protein content. Protein contained in starch reacts with reducing sugars through Maillard reaction, allowing occurrence of non-enzymatic browning reaction [17].

Table 2. Color of liquid sugar from sweet sorghum starch

Volume of α -Amylase (mL/kg)	Volume of Amyl Glucosidase (mL/kg)	Color		
		Liquefaction Time (Minutes)		
		10	20	30
3	3	Yellow	Yellow	Yellow
	5	Yellow	Yellow	Yellow
5	3	Yellow	Yellow	Yellow
	5	Yellow	Yellow	Yellow

3.3. Total soluble solids

Total soluble solids were starches which were not hydrolyzed during the process. Statistical analysis showed that enzymes addition, liquefaction time, and interaction between both of them were significantly different for total soluble solids.

Leaes et al. [18] stated that the addition of enzymes causes other components in the material to be hydrolyzed. α -amylase enzyme hydrolyzed starch substrates which have more open granules and then produce amylose and amylopectin components with shorter chains. When the α -amylase enzyme is added to the substrate which has undergone gelatinization, the enzyme quickly hydrolyses the bonds in starch which have weakened hydrogen bonds accompanied by a rapid decrease in viscosity due to

depolymerization [19]. This resulted in a decrease of viscosity produced total soluble solids is also low.

Liquefaction process affects the total soluble solids produced by liquid sugar from sweet sorghum starch. A decrease in the total soluble solids occurs along with the duration of the liquefaction time. In the liquefaction process, starch is broken down into dextrin, maltose and glucose. Dextrin is a result of imperfect starch hydrolysis. The process also involves alkali and oxidizing agents. Reduction of the chain length will cause the properties changing in starch. It is not soluble in water easily but converted into soluble dextrin which very soluble in hot or cold water with a relatively low viscosity [20, 21]. Based on **Table 3**, total soluble solids have decreased in the 30th minute of liquefaction time.

After the liquefaction process end, then saccharification process started. The addition of amylglucosidase in variation volume to the solution does provide a significant improvement to the brix value as total soluble sugar. This amyl-glucosidase converted the dextrin into D-Glucose or maltose. Table 3 showed that from liquefaction process there was still some amount of starch that can be converted by amyl-glucosidase. The brix value increased until the end of enzyme hydrolysis.

Table 3. Total soluble solids of liquid sugar from sweet sorghum starch

Treatments	Volume of α -amylase (mL/kg)	Volume of Amyloglucosidase (mL/kg)	Total Soluble Solids ($^{\circ}$ Brix)		
			Liquefaction Time (Minutes)		
			10	20	30
Before	3	3	19.00 ^{b(A)}	21.80 ^{a(A)}	22.00 ^{a(A)}
		5	19.55 ^{b(A)}	21.00 ^{a(A)}	21.50 ^{a(A)}
	5	3	22.00 ^{a(A)}	19.40 ^{c(A)}	21.00 ^{b(A)}
		5	22.00 ^{a(A)}	20.00 ^{b(A)}	20.50 ^{b(A)}
After	3	3	64.50 ^{a(A)}	61.00 ^{b(A)}	61.00 ^{b(B)}
		5	63.00 ^{b(A)}	61.00 ^{c(A)}	68.00 ^{a(B)}
	5	3	64.50 ^{a(A)}	60.00 ^{c(A)}	62.50 ^{b(A)}
		5	68.00 ^{a(A)}	60.00 ^{b(A)}	61.50 ^{b(A)}

Different letters on the same lines indicate significantly different

3.4. Total sugar content

Generally, starch consisted of two fractions: soluble fraction (amylose) and insoluble fraction (amylopectin). It can be separated with hot water. Amylose is an unbranched single chain polymer, formed from monomers 500-20000 α -D-glucose linked by α (1-4) glycosidic bond. While amylopectin is a branched chain polymer, formed from 100.000 glucose monomers linked by α -1,4 glycosidic bond in the main chain and α (1-6) glycosidic in the branch [22]. High content of amylose and amylopectin in sorghum starch makes this plant's starch potential to be converted into glucose syrup or liquid sugar.

In this research, hydrolysis of starch to glucose was using α -amylase and amyl-glucosidase. α -amylase is endohydrolase which hydrolyses α (1-4) glycosidic bond of starch. Amyl-glucosidase hydrolyses α (1-4) and α (1-6) glycosidic bonds of starch from the non-reducing ends [8]. Generally, amyl-glucosidase is slower in hydrolyzing α (1-6) branch links.

Statistical analysis showed that enzymes addition, liquefaction time, and interaction between both of them were significantly different for total sugar content. Enzyme addition can increase the glucose content of liquid sugar. But, if too many enzymes are added, the sugar produced will decrease. This condition is in line with the results of research by Parwiyanti et al. [11] which this states that sugar content will decrease with the addition of too many enzymes.

Table 4. Total sugar content of liquid sugar from sweet sorghum starch

Treatments	Volume of α -amylase (mL/kg)	Volume of Amyl Glucosidase (mL/kg)	Total Sugar Content (%)		
			Liquefaction Time (Minutes)		
			10	20	30
Before	3	3	22.43 ^{b(B)}	29.15 ^{a(A)}	22.54 ^{b(A)}
		5	29.08 ^{a(A)}	22.15 ^{b(B)}	15.34 ^{c(B)}
	5	3	32.96 ^{a(A)}	29.68 ^{b(A)}	31.44 ^{a(A)}
		5	23.46 ^{b(B)}	28.37 ^{a(A)}	27.19 ^{a(B)}
After	3	3	88.10 ^{a(A)}	68.98 ^{b(B)}	37.39 ^{c(A)}
		5	72.04 ^{b(B)}	77.32 ^{a(A)}	24.28 ^{c(B)}
	5	3	77.48 ^{a(A)}	45.76 ^{b(B)}	44.94 ^{b(B)}
		5	39.52 ^{c(B)}	86.15 ^{a(A)}	65.86 ^{b(A)}

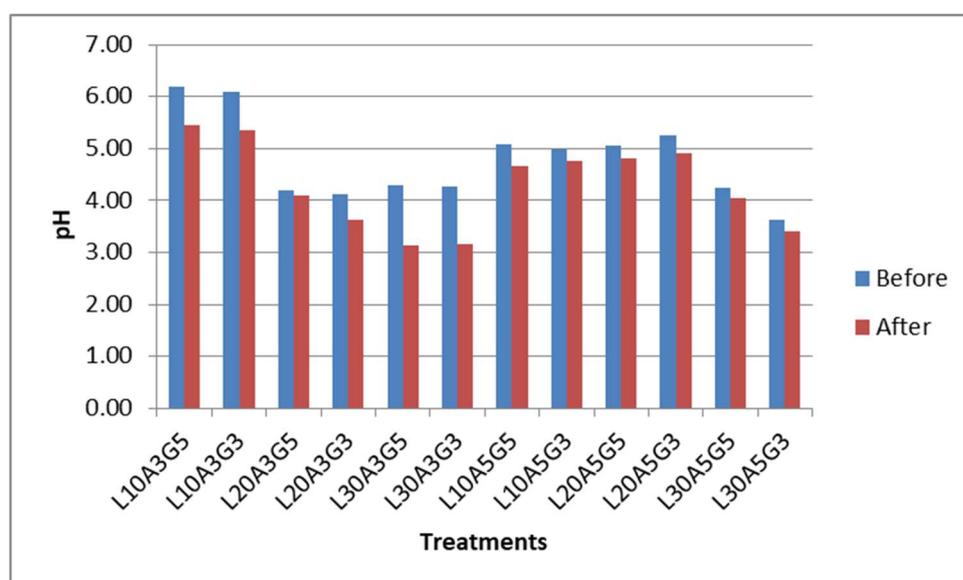
Different letters on the same lines indicate significantly different

Based on **Table 4**, total sugar content has decreased along with the liquefaction time. This is caused by inhibition of enzyme activity by reducing sugar compounds resulting from the starch breakdown. Reduced sugar compounds react with enzymes, enzymes-substrates, or both which cause inhibition of the enzyme's activity.

The longer the hydrolysis process, the greater the sugar produced. This increase in glucose content is caused by the longer of liquefaction times. Longer of liquefaction times will produce better starch breakdown into glucose. So that higher glucose content can be obtained. But if the process is too long and more enzymes were added, there will not be a significant result to increase the glucose content. This can be caused by the activity of α -amylase and amyl-glucosidase enzymes that specifically in hydrolyzing substrate (sweet sorghum starch).

3.5. pH

The pH value of a substrate greatly determines the catalytic activity of an enzyme and affects the quality of the liquid sugar produced. The results of pH measurements on liquid sugar resulting from enzymatic hydrolysis of sweet sorghum starch showed that the pH produced was in the range of 3.14-6.20 (acidic conditions).

**Figure 1.** pH of liquid sugar from sweet sorghum starch

Generally, the enzyme has optimum condition at pH 5-6. Optimum pH condition of α -amylase enzyme was 5.5, while optimum pH condition of amyl-glucosidase enzyme was 4-6. Furthermore, Ruiz et al. [23] stated that the enzyme kinetics in the liquefaction process was optimum at pH 7.0, while saccharification was completed at pH 4.0–4.5. Based on Table 5, pH of liquid sugar produced still in the range of optimum conditions (acidic conditions). Statistical analysis results showed that liquefaction time and interaction between liquefaction time and enzymes addition were significantly different for pH of liquid sugar. Enzymes addition did not affect for pH of liquid sugar.

Liquefaction time is very influential on pH. A long liquefaction time will produce low pH value. This is because the longer time for enzymatic hydrolysis, the higher content of reduction sugars resulting from the hydrolysis process of starch. The enzymatic hydrolysis process can increase the acid content in the liquid sugar produced. Sugars which are formed from enzymatic hydrolysis processes including from aldehyde groups which can affect the acidity of the material.

3.6. Yields

Yields of liquid sugar can be affected by liquefaction and saccharification process. It was calculated based on the ratio of liquid sugar produced at the end of the enzyme's hydrolysis process with the weight of the raw material multiplied by one hundred percent. Yields of liquid sugar is a result of enzyme hydrolysis on starch glycosidic bonds, which produce glucose, maltose, and malt-triose. Statistical analysis results showed that enzymes addition, liquefaction time and interaction between liquefaction time and enzymes addition were significantly affecting the yields of liquid sugar.

The longer of liquefaction time, the higher yield will be (**Table 5**). A long hydrolysis time cause's enzymes hydrolysis of starch was even greater. Furthermore, α -amylase and amyl-glucosidase enzymes hydrolyzed specifically on α -(1-4) and α -(1-6) glycosidic bonds. Hydrolysis will stop when all the bonds have been hydrolyzed. Although the highest yield was obtained in the treatment with 30 minutes liquefaction time, 3 mL / kg α -amylase addition, and 5 mL/kg amyl-glucosidase addition, but the sugar content produced in this treatment was low (**Table 4**). This is due to the evaporation process which is not yet perfect. So that the yield of liquid sugar produced still contains water.

Table 5. Yields of liquid sugar from sweet sorghum starch

Volume of α -amylase (mL/kg)	Volume of Amyl Glucosidase (mL/kg)	Yields (%)		
		Liquefaction Time (Minutes)		
		10	20	30
3	3	74.1 ^{a(A)}	69.95 ^{a(A)}	72.24 ^{a(A)}
	5	72.16 ^{a(A)}	74.83 ^{a(A)}	95.2 ^{b(B)}
5	3	82.99 ^{b(A)}	91.2 ^{a(A)}	94.58 ^{b(A)}
	5	73.04 ^{c(B)}	81.8 ^{a(A)}	77.49 ^{b(A)}

Different letters on the same lines indicate significantly different

3.7. Determination of the selected freezing condition

The main parameter in determining the best process of producing liquid sugar is the total soluble solids (TSS) and total sugar content. Based on the results, liquid sugar with high total soluble content and total sugar content was liquefaction time of 10 minutes, enzymes addition of 5 mL/kg α -amylase and 3 mL/kg amyl-glucosidase. In this condition, liquid sugar characteristics before evaporation are TSS of 22oBrix, total sugar of 32.96%, and pH of 4.98. While their characteristics after evaporation process are TSS of 64.50oBrix, total sugar of 77.48%, pH of 4.77, yields of 82.99%, and yellow color.

4. Conclusions

Enzymes addition and liquefaction time had significantly different of their physicochemical characteristics. The best enzymes addition and liquefaction for liquid sugar production are 5 ml/Kg for α -amylase addition, 3 ml/Kg for amyl-glucosidase addition, and 10 minutes for liquefaction time.

Liquid sugar characteristics before evaporation are TSS of 22°Brix, total sugar of 32.96%, and pH of 4.98. While their characteristics after evaporation process are TSS of 64.50°Brix, total sugar of 77.48%, pH of 4.77, yields of 82.99%, and yellow color.

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