

The effect of substrate and enzyme concentration on the glucose syrup production from red sorghum starch by enzymatic hydrolysis

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Abstract. Red sorghum starch contains 19% amylose and 34% amylopectin but low gluten, therefore, it is not suitable for bakery production. On the other hand, red sorghum is potential for glucose syrup production. Glucose syrup can be produced by enzymatic hydrolysis from starch by liquefaction process with α -amylase and saccharification process with glucoamylase. Enzyme concentration plays an important role in the yield of hydrolysis process. This experiment aimed to produced glucose syrup from red sorghum starch by hydrolysis enzymatic method. Liquid α -amylase and glucoamylase were applied for red sorghum starch with particle size 125 μ m. Concentrations of α -amylase and glucoamylase were 0.2 and 0.27% (v/v), respectively. Substrate concentration was varied at 10, 20, 30, and 40% (w/v) for each 300 ml substrate solution. Liquefaction process was carried out at 90°C for 60 min whereas saccharification process was carried out at 60°C for 120 min with constant pH 6 for each process. The substrate was then strained as the hydrolysate. The hydrolysate was analyzed for its reducing sugar content by dinitrosalicylic acid (DNS) reagent. The glucose concentration was analyzed by HPLC. The highest reducing sugar concentration was 191.60 g/l, 17.44% glucose observed for 40% substrate and 0.27% enzyme concentration, respectively.

1. Introduction

Nowadays consumption of sugar cane in food and beverage industry is increasing rapidly. To fulfil its high consumption, artificial sweetener is applied recently. Liquid sugar which has less impurities and does not require solubilization is a better alternative for sugar cane crystal substitution. Liquid sugar includes glucose and fructose was produced from some biomass source such as leucalyptus, mahogany sawdust, rice husk [1], water hyacinth [2], starch from rotten potato [3], and cassava (*Manihot esculenta* Crantz) [4].

Sorghum is a staple food crop in some semi-arid tropics region. It has a good potential for commercial exploitation [5]. Starch content of sorghum grain is up to 72% [6] and it is potential for liquid sugar such as glucose or fructose syrup application [6].

Hydrolysis of starch to produce glucose syrup can be obtained with acid hydrolysis e.g. sulfuric acid or hydrochloric acid, enzymatic hydrolysis, or its combination. Enzymatic hydrolysis has fundamental differences with acid hydrolysis. Acid hydrolysis breaks the chain of starch at random parts, whereas enzymatic hydrolysis breaks the chain of starch at certain branching specifically. Enzymatic hydrolysis has some advantages i.e. the process is more specific, the conditions can be controlled, less cost for purification, less ash and by-products, and the color damage can be minimized [7].



Glucose can be made from enzymatic hydrolysis by α -amylase and glucoamylase [8,9], or by combination of acid hydrolysis and enzymatic hydrolysis [7]. Hydrolysis is a chemical decomposition using water to separate the chemical bonds of the substance. Hydrolysis of starch is a breaking process of starch molecule to become constituent parts of the starch like dextrin, isomaltose, maltose, and glucose [10].

This experiment aimed to produced glucose syrup from red sorghum starch by hydrolysis enzymatic method process in various enzyme and substrate concentration.

2. Experimental details

2.1. Material

Red sorghum starch from Bandung was used in this study. It was grinded and sieved to obtain particle size of 125 μm . The component of red sorghum starch is listed in Table 1. Amylose and amylopectin in red sorghum starch can be converted into glucose syrup or liquid sugar.

Table 1. Red sorghum starch content.

Component	Amount (%)
Starch	52.7
- Amylose	18.6
- Amylopectin	34.1
Protein	8.9
Fiber	9.8

Table 2 show the characteristics of enzymes used in this experiment.

Table 2. Enzyme characteristics.

No	Enzyme characteristics	Enzyme	
		α -amylase	Glucoamylase
1	Form	liquid	liquid
2	Enzyme activity	148,39(KNU/g)	293(AGU/g)
3	Density (g/ml)	1,244 g	1,155
4	Total viable count (/g)	< 100	< 200
5	Coliform bacteria (/g)	< 4	< 4

2.2. Experimental

2.2.1. Enzymatic hydrolysis. Red sorghum starch as the substrate was diluted at 10, 20, 30, and 40% for each 300 ml substrate solution (w/v), while the enzyme concentration was varied at 0.2, 0.27, and 0.30% (v/v) for each substrate concentration. In gelatinization, substrate solution was stirred and heated until 60°C then α -amylase was added and continued for liquefaction. The temperature of the solution was increased until 90°C for 60 min. Samples were taken every 10 min for brix value determination. Saccharification was carried out by decreasing the temperature to 60°C. Glucoamylase was added at the ratio 1:1 to α -amylase after liquefaction. The heating process was set in a constant temperature at 60°C for 120 min with sampling rate every 10 min for brix value as the total sugar concentration. All steps of the experiments were observed in constant pH 6.

2.2.2. Reducing sugar and glucose content. Reducing sugar concentration was determined A 3,5-dinitrosalicylic acid (DNS) solution and the samples absorbance were analyzed by spectrophotometer (Labo) with visible detector and wavelength of 540 nm [11].

High liquid performance chromatography (HPLC) was used to observed the glucose and fructose contents. HPLC detector used was Agilent 1260 refractive Index Detector with positive polarity; Agilent Zorbax Carbohydrate 4.6 x 150 mm, 5 μ column was used in this observation.

3. Result and Discussion

Enzymatic hydrolysis was carried out in three steps namely gelatinization, liquefaction, and saccharification. Gelatinization is an important step where starch grains are heated with excess of water to increase amylopectin amorphous region and enzyme accessibility. Gelatinization is the prefix process before liquefaction. It is a process of swelling of starch granules due to heating which breaks the hydrogen bond on the starch glycoside bond. The swelling of the granule is irreversible or cannot be back to its original shape. Liquefaction carried out without gelatinization will take longer time than the gelatinized substrate [12].

3.1. Liquefaction Process

Liquefaction was carried out by amylases that hydrolyzed the chemical bond α -(1-4) of starch, producing dextrin, maltose, maltotriose and maltopentoses with a dextrose equivalent (DE) below 30 at high temperature [13]. The result of liquefaction is shown in Figure 1a-d. The total sugar concentration was measured in terms of brix value for each process.

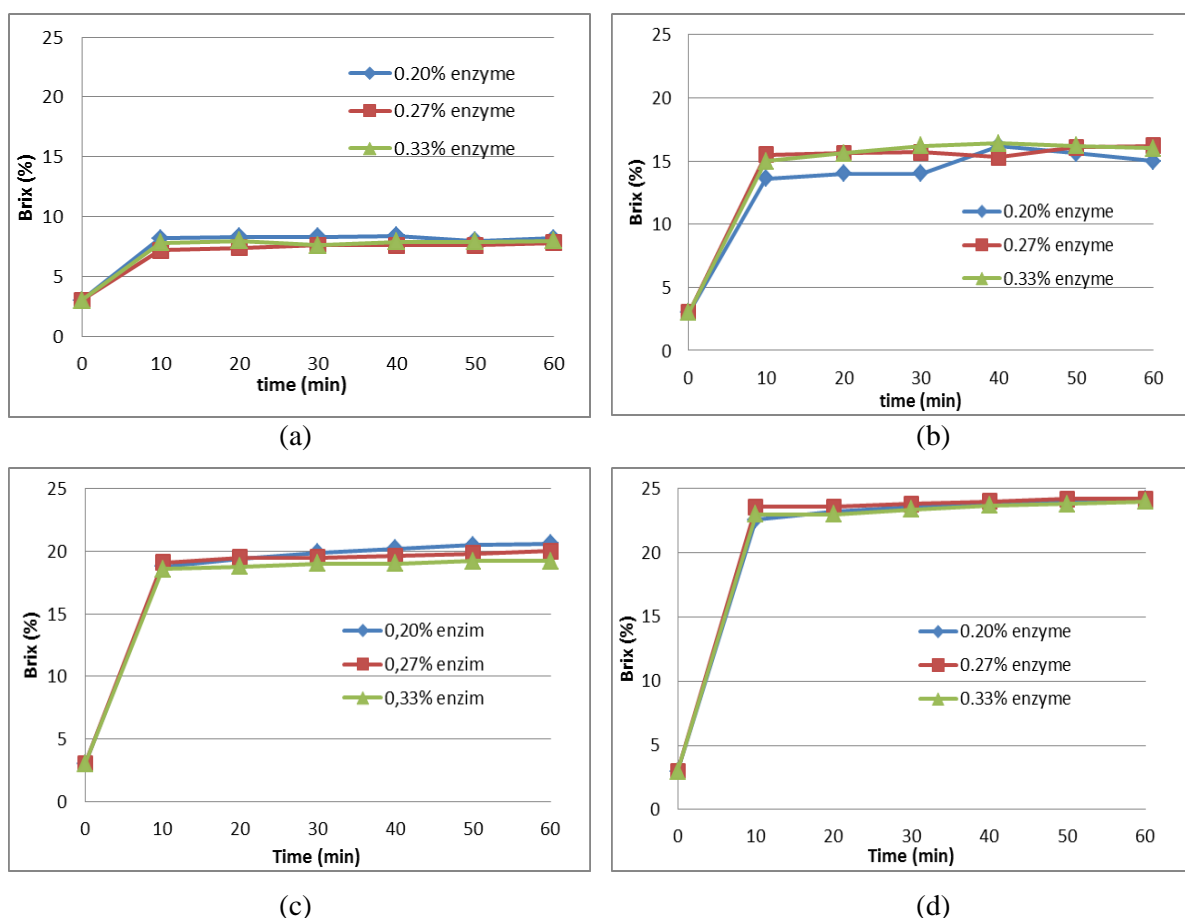


Figure 1. Total sugar level in liquefaction process with varied substrate and enzyme concentration (a) 10% substrate, (b) 20% substrate, (c) 30% substrate, (d) 40% substrate.

All figures show that total sugar increased rapidly in the first 10 min and slowly for the next run time. It indicated that α -amylase could convert the red sorghum starch rapidly because of the high enzyme activity. Increasing of total sugar concentration that showed in % brix was similar with increasing of reducing sugar concentration. Increasing of reducing sugar concentration indicated that the product was formed as a function of time for a series of substrate concentrations. As expected, the amount of product formed increased with time, although eventually a time was reached when there was no net change in the concentration of substrate or product. By the Michaelis-Menten Model, the enzyme was still actively converting substrate into product and vice versa, but the reaction equilibrium had been attained [14]. In various concentration of additional enzyme, the brix value increased rapidly in the first 10 min then it rose up slowly until constant brix value above 23%. It indicated that the amylose and amylopectin were running out at those substrate solutions become the product. For the 10% substrate concentration showed the maximum total sugar formed until brix 8.2%. It was the lowest substrate concentration so the amylose and amylopectin which were converted to glucose were lowest than the other concentration of substrate.

3.2. Saccharification

Saccharification process of partially hydrolyzed starch was carried out at lower temperatures. Saccharification by glucoamylase to hydrolyze the chemical bonds α -(1-4) and α -(1-6), produces maltose or D-glucose syrups with DE around 40 and 96, respectively [13].

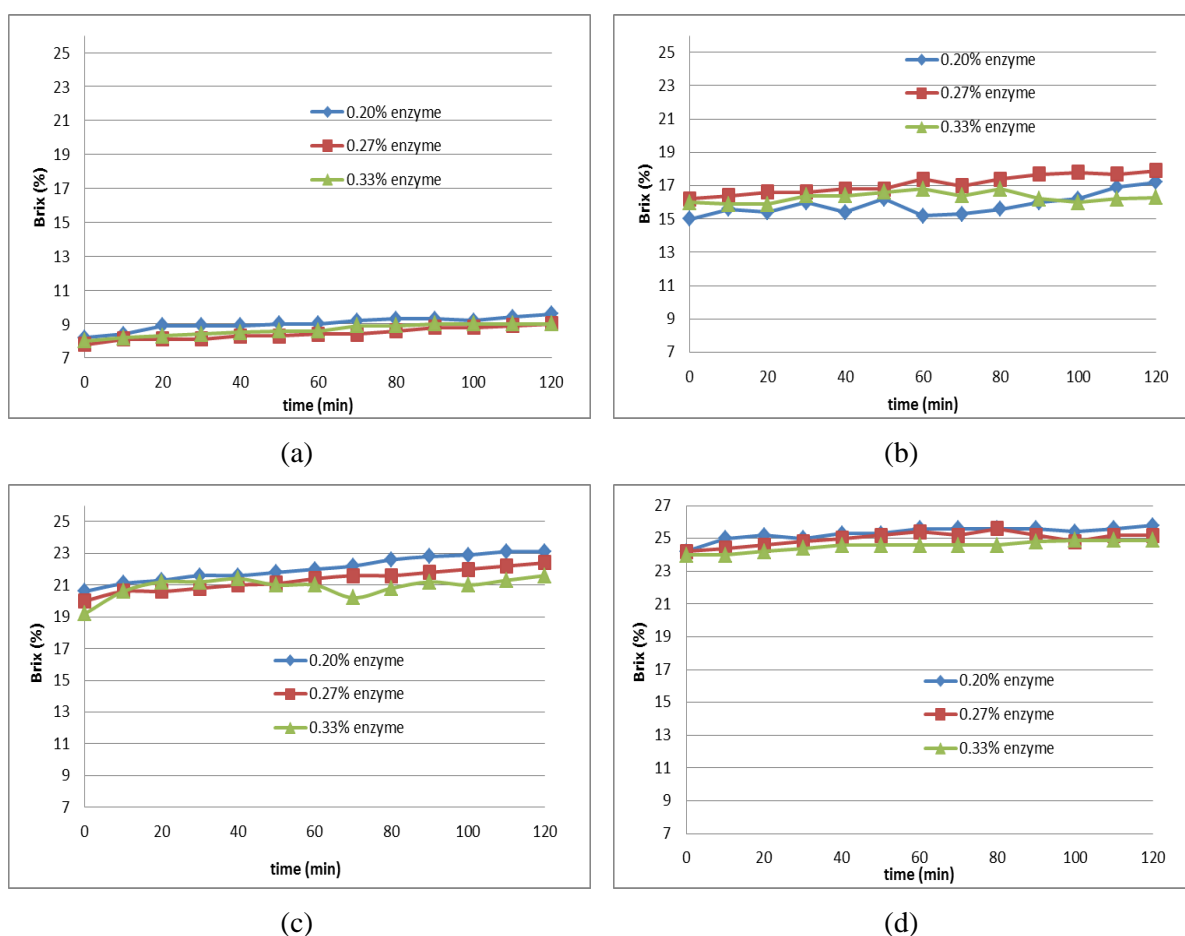


Figure 2. Total sugar level in saccharification process with varied substrate and enzyme concentration (a) 10% substrate, (b) 20% substrate, (c) 30% substrate, (d) 40% substrate.

The result of saccharification is shown in Figure 2a-d. The brix value in those figures increased slowly and became constant. It means that the total sugar production had almost reached the maximum value in the end of liquefaction process for each substrate concentration. The addition of glucoamylase in the solution did not provide a significant improvement to the brix value. The products were then analyzed for the reducing sugar as the glucose concentration. Glucoamylase converted the dextrin to produce D-Glucose or maltose [15]. It would appear if the solution was analyzed with DNS reagent as the reducing sugar concentration.

3.3. Identification of hydrolysis end product

The end product of each variation of condition is shown in Figure 3. The reducing sugars increased slowly as substrate concentration increasing at every enzyme concentration. This means that the reaction rate did not depend on the substrate concentration [16]. The addition of enzymes decreased starch in the substrate. The higher enzymes concentration in the solution, the higher the binding of substrate to the active side of the enzyme. When the substrate was entirely converted into the product, the increase of enzyme concentration showed no increase of product amount [17]. The reducing sugar as the end product become the accumulation of sugar formed as long as the liquefaction and saccharification occurred.

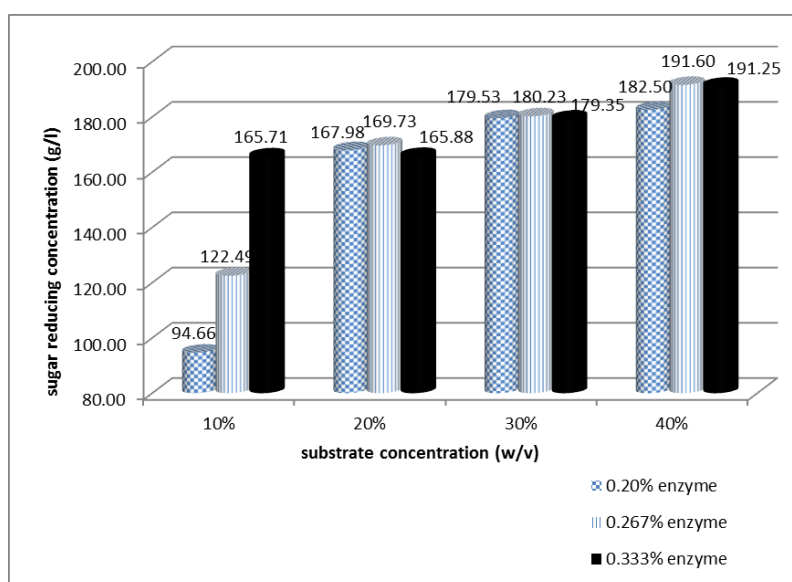


Figure 3. Concentration of hydrolysis product for varied substrate and enzyme concentration.

Figure 3 shows that the highest reducing sugar concentration was made from 40% substrate at any various enzyme concentration. The reducing sugar concentration were 182.50 g/l, 191.60 g/l, and 191.25 g/l respectively for each enzyme concentration. It is still possible to enhance the reducing sugar concentration by increasing the substrate concentration. However, this addition caused the solution too viscous [15]. Therefore, it was difficult to hydrolyze by acidic or enzymatic treatment.

The glucose formed was of 17.44% for the highest end product concentration as of 40% substrate concentration, 0.27% enzyme concentration.

4. Conclusion

Enzyme and substrate concentration play a role in the production of glucose syrup from red sorghum starch. The best result was obtained for 40% substrate and 0.267% enzyme concentration with 191.60 g/l reducing sugar and 17.44% glucose.

Acknowledgments

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