Fructose Syrup Production from Tapioca Solid Waste

(Onggok) By Using Enzymatic Hydrolysis in Various pH And Isomerization Process

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Abstract: Tapioca solid waste (onggok) from cassava contains high carbohydrate (about 76%). The carbohydrate contains amylase and amylopectin which are the raw material of glucose syrup. Fructose is isomer of glucose which has higher dextrose equivalent. It is potential to produce fructose syrup from onggok by using enzymatic hydrolysis and through isomerization process. This research aimed to produce fructose syrup from cassava solid waste in various pH of enzymatic hydrolysis (liquefaction and saccharification) and isomerizing the glucose to be fructose syrup. Onggok in particle size 63 µm was dissolved in distilled water to make substrate concentration of 25%, 30%, and 35% (w/v). Each of the substrate concentration was hydrolyzed with α-amylase as the liquefaction process and was continued with addition of glucoamylase in saccharification process. The hydrolysis process was set in various pH, 5.5, 6, and 6.5. In liquefaction, the substrate suspension was added by 0.067% α-amylase (v/v) with heating temperature 90°C for 60 min. Then, it was continued for saccharification by adding glucoamylase in the same ratio with α-amylase for 120 minutes in 55°C. During the hydrolysis process, the sample was taken from each solution in 10 minutes to analyze the reducing sugar concentration by DNS reagent. After the hydrolysis process, the suspension was filtered and evaporated to reduce the water content. Filtrate with the highest reducing sugar concentration was isomerized by adding 1% of glucoamylase and heated in 60°C, pH 8.2 for 43 hours. The fructose syrup concentration was then analyzed by resorcinol reagent and the result was 229.3 g/l, yield 9.298%, and conversion 24.587%.

Keywords: onggok, enzymatic hydrolysis, isomerization, fructose syrup

1. Introduction

Rapid increase of sugar cane consumption in food and beverage industry becomes a business opportunity to artificial sweetener production. Glucose and fructose syrup are kind of liquid sugar that can substitute half of sugar cane consumption. Liquid sugar has less impurities and it does not require solubilisation. This will become a better alternative substitution for crystal sugar cane. Fructose syrup is healthy liquid sugar which has highest sweetness degree than glucose and sucrose. The sweetness comparison of glucose, sucrose, and fructose are 74, 100, 180, respectively [1]. Fructose is sugar which is found naturally in fruits and some vegetables. Artificial fructose worldwide known is High Fructose Corn Syrup, made from corn starch. Fructose syrup is made from glucose by enzymatic isomerization using glucose isomerase. Starch can be efficiently converted to glucose and then to
various amounts of fructose by using glucose isomerase [2]. Fructose syrup can be made from starch which contains amylose and amylopectin. Some researcher made fructose syrup from cassava starch [3], Jerusalem Artichoke Extracts [4], cashew apple juice derivatives [5], etc. The important thing in the production of HFS is we need to produce high glucose concentration syrup first, before it was converted to HFS. Glucose can be made from enzymatic hydrolysis by α-amylase and glucoamylase [6],[7], or using the combination of acid hydrolysis and enzymatic hydrolysis [8]. Hydrolysis is a chemical decomposition using water to separate the chemical bonds of the substance. While the hydrolysis of starch is a breaking process of starch molecule become the constituent parts of starch, like dextrin, isomaltose, maltose, and glucose [9].

Indonesia is one of the largest cassava producers in the world. Cassava production in 2015 reached 21.801.415 tons (Indonesia Central Bureau of Statistics) [10]. It is usually processed into tapioca flour. In this process, 2/3 part of cassava turn into solid waste called “Onggok”. It still contains carbohydrate at about 72.49%-85.99% and it contains amylose and amylopectin [11]. It makes ongkok very potential to be converted to fructose syrup. The objective of this study was to produce fructose syrup from tapioca solid waste (onggok) by enzymatic hydrolysis in various pH and isomerization process.

2. Experimental details

2.1. Material

Onggok from Sumedang (West Java) was used in this study. It was grinded and sifted in particle size of 63 µm. The components of ongkok were listed in Table 1. Amylose and amylopectin in ongkok starch could be converted into glucose syrup or liquid sugar. While table 2 showed the characteristic of enzyme used in this experiment.

Table 1. Red sorghum starch content

| Content       | Amount (%) |
|---------------|------------|
| Starch        | 76.055     |
| - Amylose     | 15.843     |
| - Amylopectin | 60.212     |
| Protein       | 2.086      |
| Fiber         | 9.786      |
| Fat           | 1.187      |

Table 2. Enzyme Characteristic

| No | Enzyme Characteristic | α-amylase | Glucoamylase | Glucose isomerase |
|----|-----------------------|-----------|--------------|------------------|
| 1  | Form                  | liquid    | liquid       | Powder           |
| 2  | Enzyme activity (U/ml)| 2.029     | 2.968        | 2.358            |
| 3  | Density (g/ml)        | 1.244     | 1.155        | -                |
| 4  | Total viable count (/g)|< 100     |< 200         | -                |
| 5  | Coliform bacteria (/g) |< 4        |< 4           | -                |
| 6  | pH                    | 5.5-8     | 4.5-5        | 8.2              |
| 7  | Temperature (°C)      | 90        | 55           | 60               |

2.2. Experimental
2.2.1. Enzymatic Hydrolysis

1) Substrate was made in three concentrations (w/v), 25%, 30%, and 35% for 300ml solution by dissolve ongkok in distilled water. α-amylase and glucoamylase used in hydrolysis process were 0.067% (v/v) against the substrate solution. 2) In Liquefaction, gelatinization became the first process. Substrate solution was stirred and heated until 50°C then α-amylase was added and continued for liquefaction by increase the heating temperature until 90°C for 60 min. 3) Saccharification was carried out the temperature to 60°C by adding glucoamylase with the ratio of 1:1 to α-amylase after liquefaction. The heating process was set in a constant temperature at 55°C for 120 min. 4) The sample was taken every 10 min for reducing sugar concentration determination. All steps of the experiment were observed in various pH 5, 5.5, and 6.

2.2.2. Isomerization Process

6) Isomerization was proceeded to convert glucose to become fructose. It was made only for the highest reducing sugar concentration from the enzymatic hydrolysis process before. There were 3 stages of purifying hydrolysate called bleaching, filtering, and evaporation. The bleaching process was carried out with a solution from saccharification process mixed with a 0,2% w/v activated charcoal powder and heated to 80°C for 15 minutes. The solution was filtered and obtained light yellow filtrate. The filtrate was evaporated in 70°C to reduce the water content (up to 37.5% brix) to make the viscous solution. This solution was isomerized by adding 1% (b/v) glucose isomerase for 43 hours in 60°C, pH 8.2. The samples were taken from the solution for 0 hour and 43 hours.

2.2.3. Determination of Reducing Sugar and Fructose Concentration

Reducing sugar concentration of each sample (from hydrolysis and isomerization process) was determined by DNS method. 2 ml of each sample was added by 3 ml 3,5-dinitrosalicylic acid (DNS) solution, then it was heated in boiling water for 15 min and fast cooled in icy water. The sample of absorbance in room temperature was analyzed by spectrophotometer (Labo) with visible detector in wavelength of 540nm [12].

Fructose concentration from isomerization samples were determined by resorcinol method [11]. 1 ml of sample was added by 0,5 ml resorcinol reagent and shaken, then 3,5 ml hydrochloride acid (5 ml HCl and 1 ml distilled water) was added and shaken again. The samples solution were heated in water bath at 80 °C for 10 min, and then read the absorbance in spectrophotometer visible detector (Cecil CE 1011) at 520 nm. [13]

3. Result and Discussion

3.1. Enzymatic hydrolysis

Enzymatic hydrolysis was carried out in three steps called gelatinization, liquefaction, and saccharification.. Gelatinization is a process to increase amylopectin amorphous region and enzyme accessibility by heating the starch grains with excess of water. 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 which is carried out without gelatinization will take longer time than the gelatinized substrate [14].

The gelatinization was continued by liquefaction. The addition of α-amylase in solution after gelatinization intend to break the chemical bond α-(1-4) of starch, producing dextrin, maltose, malt triose and malt pentoses with a dextrose equivalent (DE) below 30 at high temperature (80-110°C.
In this study, the ratio of α-amylase to substrate solution was 0.067%. Liquefaction was carried out in 90 °C because α-amylase has optimum temperature process on 90°C–105°C. Too high temperatures process will disrupt and damage the enzyme performance, whereas too low temperature will cause in imperfect starch gelatinization [16]. (Richardson (2012)). Saccharification became the next process of liquefaction. In this process the heating temperature of solution was decrease until 55 °C, the optimum temperature for glucoamylase [17] and it was added with the ratio of 1:1 to the α-amylase. Saccharification used glucoamylase (amyl glucosidase) hydrolyze the chemical bonds α-(1-4) and α-(1-6), to obtain products such as maltose or D-glucose syrups with DE around 40 and 96, respectively [15].
The hydrolysis process of amylose and amylopectin on *onggok* starch become monosaccharide was shown in Fig. 1 (a) - (c). Reducing sugar concentration was represented as the glucose concentration. In Fig. 1 (a) - (c) liquefaction was carried out in 0 – 60 min and saccharification was held in 60 – 180 min. It showed from all figures that reducing sugar concentration increases rapidly in first 10 min after the addition of each enzyme in each process and increase slowly for the next runtime. Increasing of reducing sugar concentration indicated that the product was formed as a function of time. 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. Michaelis-Menten Model showed that the enzyme was still actively converting substrate into product and vice versa, but the reaction of equilibrium has been attained [18]. Fig.1 (a) - (c) gave that the highest reducing sugar concentration was obtained from hydrolysis in pH 5.5 at all substrate concentration. It showed that the optimum pH for liquefaction and saccharification was 5.5. Other researchers explained that optimum pH in glucoamylase was in range of 5-7.5 based on some starch source [17] and pH 5-6 for α-amylase give the higher enzyme activity than pH 4-7 [19]. pH 5.5 in this study was in the range of both optimum pH enzyme (α-amylase and glucoamylase). The structure of the enzyme ions depended on the pH of the environment. Enzymes could form positive ions, negative or charged ions (zwitter ion). The change of pH in environment would affect the effectiveness of the active side of the enzyme in forming the substrates. Low pH or high pH could also cause the denaturation and caused decreased enzyme activity. Therefore, enzymes had different optimum pH. Enzyme showed maximum activity in the pH range between 4.5-8.0. The change in enzyme reactivity was thought to be a result of changes in the pH of the environment [20].

The end product of hydrolysis process in various substrate concentration and pH was shown in Fig. 1 for 180 min hydrolysis time. The highest reducing sugar concentration was obtained in 35% substrate in pH 5.5, 408.55g/l. The substrate concentration in 35% gave the highest reducing sugar concentration. It was caused by higher substrate concentration would give higher source of starch (amylose and amylopectin). Here it was still possible to enhance the reducing sugar concentration by increasing the substrate concentration, but it made the solution too viscous [21].
3.2. Isomerization

Isomerization is a process to convert glucose become fructose using glucose isomerase. It is reversible reaction. It begins with protonation of C2-OH and continued furanose aldehyde intermediate. Fructose is produced from hydride transfer from C2 to C1 in furanos aldehyde branch followed by re-dehydration of carbon C2 [22]. In this study, only on the highest reducing sugar concentration was isomerized. It needed high substrate concentration because the isomerizing reaction was reversible. Liquid sugar (hydrolysate) from enzymatic hydrolysis process was isomerized and gave the result in Table Table 3.

| Time  | Fructose conc. (g/l) | Reducing sugar conc. (g/l) | Yield (%) | Conversion (%) |
|-------|----------------------|-----------------------------|-----------|---------------|
| 0     | 191.335              | 408.355                     | 9.298     | 24.587        |
| 43    | 229.303              | 307.952                     |           |               |

Fructose concentration in Table 3 showed that it had been formed during the enzymatic hydrolysis of ongkok starch become glucose syrup. It could not be specifically identified by DNS reagent because DNS just reacted with reducing group of sugar that had it. In isomerization process, glucose in reducing sugar of hydrolysis process was converted to fructose. Fructose concentration was specifically identified by resorcinol reagent. The yield on this reaction was calculated by determining the fructose formation during the reaction and compared with the reducing sugar as the reactants. While conversion was determined by moles reaction of the process compared to moles initial of reducing sugar. From thus result, yield was 9.298% while the conversion was 24.587%. It indicated that 37.82% of glucose was converted to fructose and the other was side product. Another researcher got the conversion and yield from the isomerization process of cassava and sweet potato as the substrate 42%–43% and 28%-29%, [23]. The conversion was higher because it was compared to the glucose concentration, while in this research was compared to reducing sugar.

4. Conclusion

Substrate concentration and pH played a role in hydrolysis process to produce glucose syrup as the reducing sugar and raw material of fructose syrup. High glucose concentration from hydrolysis process obtained the high fructose concentration by isomerizing process. The best result of hydrolysis process was obtained for 35% substrate and pH 5.5 with 408.55 g/l reducing sugar, while the fructose syrup concentration result was 229.3 g/l, yield 9.298%, and conversion 24,587%.

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