Conversion of Cassava Starch to Produce Glucose and Fructose by Enzymatic Process Using Microwave Heating

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Abstract. In this study, variation of glycosidase enzyme concentration and saccharification time on enzymatic hydrolysis using microwave have been investigated. Concentration and kinetic parameters rate of glucose and fructose were analyzed. Cassava starch was liquefied and gelatinized by microwave at 80°C. The gelatinized starch was saccharified at 60°C using (0.2;0.4;0.6;0.8;1% (w/v) ) glycosidase enzyme for 24, 48 and 72 hours. The glucose which has been saccharified with 1% glycosidase enzyme for 72 hours gave highest conversion 66.23 %. The optimization process by multilevel reaction gave the highest conversion at enzyme concentrations 0.88 % and saccharification time 29 hours that 68.82%. The highest conversion of glucose was isomerized to fructose. The fructose which has been isomerized for 180 minutes gave highest conversion 20.05 %. The kinetics enzymatic reaction was approached and determined by Michaelis - Menten equation, Km and Vmax of reaction for glucose 22.94 g/L; 2.70 g/L hours and for fructose 3.39 g/L; 0.38 g/L. min respectively.

1 Introduction

Sugar is one of basic needs for the Indonesian people, especially for consumption and food processing. Sugar demand in Indonesia is still dominated by sucrose. Sugar demand in Indonesia is still dominated by sugar (sucrose). Glucose is a monosaccharide with molecular formula C₆H₁₂O₆. Glucose is widely used in the food and pharmaceutical industries. Enzymatic hydrolysis produces glucose concentrations higher than the acid hydrolysis [1]. Fructose is the sugar isomer of glucose that is mostly used to produce drinks [2-6].

Raw material for producing glucose is material containing polysaccharide, obtained from starch. Starch is a polymer with the chemical formula anhydrous monosaccharide (C₆H₁₀O₅) and with the main constituent amylose and amylopectin [7-10]. Starch for producing glucose and fructose are corn starch, cassava starch, sweet potato starch, and sorghum. The conversion of glucose from cassava starch is greater than corn starch that is equal to 93.56% while the corn starch is only 91.8% [7].

Cassava starch is widely used in the food sector, paper industry, textile industry and chemical industry as a producer of bioethanol [1, 9, 11-13]. Today, the conventional method of acid hydrolysis has been replaced by enzymatic hydrolysis. The enzyme concentration is one that affects the yield of glucose and fructose. Enzymes contribute 23-40% of the total cost of the production process or in other words the amount of enzyme has a significant impact on the economic viability of the process [11]. This can be overcome by optimizing operating conditions of enzymatic hydrolysis [8-9, 12-13]. Gelatinization heating conventional takes a long time and a lot of energy to reach a temperature of gelatin. Microwave is used to accelerate warming and reduce energy required [7, 14-17]. This study will be focused on the conversion of glucose and fructose from cassava starch by enzymatic process using microwaves. Afterwards, determine the optimal concentration of enzyme saccharification time and kinetics parameter of enzymatic reaction is calculated of each process.

Fructose is a natural sugar that serves as a substitute for cane sugar or sucrose. Advantage fructose is containing triglycerides-resistant, which can reduces cholesterol. Fructose is commonly produced by fruits. Difference fructose and glucose are the optical rotation. Glucose has the optical rotation to the right, while the fructose to the left. Fructose round to the left is what causes the bitter taste of fructose compared to glucose [10].

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2 Materials and method

2.1 Materials

Commercial cassava starch was purchased from a cassava industry in Margoyoso, Pati (Indonesia). Other materials used included α-amylase enzyme, glycosidase enzyme with activities of each 4,000 u/g and 150,000 u/g from Sqzyme, glycoisomerase enzyme from Novozyme and other chemicals agents were analytical grade from Merck (Germany).

2.2 Liquification

In this stage, suspension of starch was prepared by mixing of 200 g starch and aquadest to 500 ml and CaCl2.2H2O 40 ppm. Furthermore, the pH of suspension was adjusted to 5.3 or 6 with addition of HCl 5% or 5% NaOH. Then, α-amylase enzyme with concentration of 0.1% (w/v) was added into starch suspension. Starch suspension was heated in a microwave at 80°C for 10 minutes. This stage finish was characterized by a change of the thick white suspension starch solution to brown (caramel) dilute. After the liquefaction process, the enzyme was inactivated with addition of 5% HCl to pH 4.5 [1].

2.3 Saccharification

The results of the previous stages, maltodextrin was divided into five sections. Each of these sections would be used as an ingredient saccharification at 60°C and pH 4.5. Glucosidase enzyme was added into suspension starch with various concentrations (0.2%; 0.4%; 0.6%; 0.8%; 1% (w/v)). The suspension starch was saccharified for 24, 48, 72 hours [11]. The saccharification product was filtered. Filtrate was analyzed dextrose equivalent value using HPLC. The scheme of experiment can be see in Figure. 1.

2.4 Fructose Isomerization

50 mL of the best glucose product was mixed with 16 mg MgSO4.7H2O. The mixture pH was measured to 7.5. The mixture was added with 50 mg glucoisomerase enzyme. The mixture was incubated in shaking incubator at 60°C for 3 hours. Samples was taken 10 ml every 30 minutes for analysis of enzymatic kinetic reactions [11]. Fructose concentrations were estimated by HPLC.

2.5 Determination of Concentration of Glucose and Fructose by HPLC

Samples was filtered and then was injected into the injector of 20 μL to a passed by port injection which pressurized. Furthermore, by pass tap was opened, so that the sample flowed into the column pressure, from HPLC equipment which using mobile phase aquabidest and detector refraction index [11]. The chromatogram described a retention time of each component in the sample was observed on the printer. Further, interpreted the proportions of the components were compared in the sample. The sample before it was injected into the column, glucose standard solution was injected as the standard comparator. HPLC method was used for quantitative analysis and qualitative at the same time. For qualitative analysed by comparing the sample chromatogram with the chromatogram reference standard based on their retention times. As for the quantitative analysed can be used in equation 1.

\[ C_x = \frac{A_x}{A_p} \times C_p \]  

Where \( C_x \) is the concentration of the sample, \( C_p \) is the standard concentration, \( A_x \) is the peak area of the sample and the \( A_p \) is the peak area of the standard.

2.6 Enzymatic Reaction Kinetics

Kinetics of enzymatic reactions in this study approached by Michaelis-Menten equation. Substrate concentration at time t was obtained from the equation stoichiometry calculations hydrolysis and isomerization reactions. The data obtained by experiments conducted initial substrate \([S_o]\), the substrate at time t \([S_t]\) and reaction time (t). The data was estimated by Equation 2 [4].

\[ \frac{1}{S} \ln \frac{S_o}{S} = \frac{V_{max}}{K_m} \frac{S_o - S}{K_m t} \]  

With \( t \) is the reaction time (h), \( V_{max} \) is the maximum reaction rate (mole/L.h) and \( S \) is the substrate concentration (mole/L). The equation was linearized to evaluating the value of \( K_m \) and \( V_{max} \).

\[ \frac{1}{S} \ln \frac{S_o}{S} = \frac{V_{max}}{K_m t} \]  

\( S_o - S \) is the y-axis, \( K_m t \) is ax and \( V_{max} \) is b.

3 Results and Discussion

3.1 Liquifaction

The liquefaction process produces maltodextrin, which has a dextrose equivalent (DE) of less than 20. In this study, liquefaction time 10 minutes using a microwave with a temperature of 80°C. The household microwave does not have temperature indicator, so
controlling the temperature was done by power settings. The power is used 100% to get a temperature 80°C. The principle of microwave heating is the collision solvent and governed by two phenomena that ionic conduction and dipole rotation. Microwave radiation can reduce the solubility and break up the crystalline structure of starch. Heating with microwaves can also affect the concentration of amylase and amylopectin better than conventional heating. The advantage of using microwaves are the liquefaction process becomes faster and more efficient because can reduce heat loss [5].

### 3.2 Saccharification

Saccharification is a fermentation process which convert maltodextrin into glucose. At this stage it is a glycosidase enzyme used. Mechanism of action of the enzyme α-glycoside is break-1.6 glycoside bond and presented in the Figure 2. Conditions operating specific of glycosidase enzyme are pH 4.5 and a temperature of 60°C. Factors that affect the reaction of glucose are glycosidase enzyme concentration and incubation time [7]. Result from experimental determination of enzyme concentration and time can be seen in Figure 3.

![Reaction of Starch hydrolysis](image2)

**Fig 2.** Starch Hydrolysis Reaction

![Graph of Glucose Conversion](image3)

**Fig 3.** Relation of The Hydrolysis Incubation Time and Conversion Glucose by Various Glucosidase Enzyme Concentration

Table 1. shows that at 24 to 29 hours the reaction increased, but the reaction 36 hours decreased conversion from 68.82% to 64.65%. This is due to the enzyme has reached the maximum conversion so that the addition of substrate. This process is more efficient in terms of cost because it requires little glycosidase enzyme and incubation times are also shorter. Enzymatic hydrolysis using microwaves could be considered for the production of glucose in the future because it can accelerate the process of gelatinization. Glucose conversion obtained from previous research is not reaching the standards in the industrial standard at least 95-96% [21].

### 3.3 Isomerization of Fructose

Isomerization is reaction of formation fructose from glucose [3, 9, 13, 21]. Isomerization was using enzymes glycoisomerase as biocatalyst. Glycoisomerase was obtained from commercial enzyme artificial Sweetzyme or was isolated from *Lactobacillus brevis*, *Pseudomonas hydrophila*, *Streptomyces phalochromogenes* as activators in the form of compounds MgSO_4·7H_2O. Glycoisomerase enzyme optimal conditions was obtained at pH 7.5 at 60°C. The incubation time should not be too long to avoid arising the presence of dyes [7]. Isomerization fructose reaction conversion data was presented in Figure 4 and Figure 5 respectively.
The highest conversion of fructose 20.05% was reached at 180 minutes. The reaction times affect conversion increase. If the enzyme reaches saturated condition, reaction time and concentration of the substrate does not affect the conversion of fructose. Even the addition of substrate and reaction time can reduce the yield of fructose [20]. Conversion of fructose does not depend on the content of starch and glucose content, but the raw materials used [7].

3.4 Kinetics Enzymatic Reactions

This study obtained kinetic enzymatic hydrolysis graph that shows in Figure 6. From this figure the equation can be calculated Michaelis-Menten constant (K_M) and maximum speed (V_max). Line equation y = -0.0436x + 0.1177, so the value of K_M is 22.94 g/L and V_max was 2.70 g/L.h. The result has been tested with a total sum square regression, a significant value of the coefficient of 99.14%. It shows that kinetic coefficient is significant.

Previous researcher conducted a study and calculation of the reaction kinetics of enzymatic hydrolysis of starch to glucose [8]. The calculated result of K_M value was 194.5 g/L and V_max was 50.8 g/L.min. Differences constant value and the maximum speed is obtained due to the starch substrate and reaction temperatures used. To fructose chart is presented in Figure 7.

4 Conclusions

Based on research that has been done can be concluded as follows: i) glucosidase enzyme concentration affects the conversion of glucose, the greater the concentration of the enzyme, the greater the yield of glucose obtained, ii) incubation time affects the yield of glucose, the longer the incubation time.The higher the yield of glucose produced, iii) glucosidase enzyme concentration was 1% and the optimal incubation time of 72 hours, the yield obtained under these conditions is 66.23%, iv) in order to more optimally use glucosidase enzyme reaction multilevel, optimal glucosidase enzyme concentration of 0.88 % with a reaction time of 29 hours, the resulting conversion of 68.82%, v) the best conversion of fructose 20.05 % was obtained at the incubation time 180 minutes, vi) coefficient of enzymatic reaction kinetics K_M glucose is 22.94 g/L and V_max is 2.70 g/L.h, and vii) coefficient of enzymatic reaction kinetics fructose K_M and V_max are 3.39 g/L and 0.38 g/L.min.
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