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Kinetics of Glucose Synthesis from the Peel of the King Banana (*Musa textilia*) by Enzymatic Hydrolysis

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A B S T R A C T

Indonesia is one of the agricultural countries with an abundance of farm products, including bananas. The utilisation of banana peel waste has a very high value if used as a raw material for glucose production. One method used for glucose production is enzymatic hydrolysis. The objective of this study is to investigate the effect of liquefaction time and saccharification time on the rate of enzymatic hydrolysis reaction, as well as to determine the kinetic equation for the enzymatic hydrolysis synthesis of banana peel glucose. The stages of this study include the raw material preparation stage, the enzymatic hydrolysis stage, and the final content analysis stage. Glucose content analysis was performed using a portable refractometer. The Michaelis-Menten method was used to determine the reaction constant. This study was conducted with an initial substrate concentration of 30% (w/v) at liquefaction times of 80, 100, 120, 140, and 160 minutes.

Additionally, saccharification times of 12, 16, 20, 24, and 28 hours were employed. The dextrin formed during liquefaction was 29.6%, 29.2%, 28.8%, 28.5%, and 27.8%, respectively. These dextrin levels were used as reactants in the saccharification process to produce glucose. The glucose formed from the hydrolysis of banana peel carbohydrates with a dextrin content of 29.6% and a saccharification time of 12 hours was 4.2%. The reaction rate equation formed from this process is $-r_A = r_R = 0.0341 \frac{C_{E0} C_A}{0.7789 + C_A}$ $mol/L.hour$

Contribution to Sustainable Development Goals (SDGs):
SDG 2: Zero Hunger
SDG 9: Industry, Innovation, and Infrastructure
SDG 12: Responsible Consumption and Production
SDG 13: Climate Action

1. INTRODUCTION

1.1. Research Background

Indonesia is one of the agricultural countries with an abundance of agricultural products, including bananas. According to the Directorate General of Horticulture, in 2020, banana production in Indonesia was substantial, reaching 7.26 million tonnes in 2018. Bananas are primarily produced in East Java Province (29%), Lampung Province (15%), and Central Java Province (9%), while other provinces account for less than 5%. This has led to the development of many home-based industries that typically process bananas into snacks such as chips, chips, dodol, and others. One type of banana commonly processed into banana chips is the raja banana [1]. It is known that the nutritional content of banana peels varies depending on the type. The carbohydrate content of Ambon banana peels, kepok banana peels, and raja banana peels is 25.09%, 25.35%, and 27.64%, respectively [2]. The utilisation of banana peel waste has significant value when

used as raw material for glucose production. One method used for enzymatic hydrolysis is the process of breaking down molecules with the aid of enzymes [4]. Enzymatic hydrolysis can be influenced by glucose production, which is a key factor in enzymatic hydrolysis [3]. Substrate, pH, time, temperature, and granule size [5].

1.2. Literature Review

Banana peels are one of the most common types of fruit waste. Generally, this waste is not utilised optimally and is often treated as organic waste or used as feed for livestock such as buffaloes, goats, cows, and others. Banana peels from 4 fruits in large quantities have greater value if they can be utilised as the main raw material for ethanol and biogas production [3]. The chemical composition of banana peels is shown in Table 1 [6].

Table 1. Nutritional Content of Banana Peel per 100 grams

No	Nutrient	Component
1	Water	69,8 g
2	Carbohydrates	27,64 g
3	Energy	120 Kkal
4	Protein	1,2 g
5	Fibre	5,3 g
6	Iron	0,8 mg
7	Zinc	0,7 mg
8	Vitamin C	10 mg
9	Calcium	10 mg
10	Phosporus	22 mg
11	Ash	1,0 g
12	Beta-Carotene	53 mcg
13	Thiamine	0,06 mg
14	Riboflavin	0,14 mg
15	Niacin	1,2 mg

From Table 1, the largest components in banana peel are water and carbohydrates, which have the potential to be utilised as the main raw materials for ethanol and biogas production through hydrolysis [3].

Enzymatic hydrolysis is the process of breaking down complex compounds such as carbohydrates into simpler compounds using enzymes and water, which act as reaction catalysts to break bonds. There are several advantages to the enzymatic hydrolysis process. The benefits of enzymatic hydrolysis include its ability to be controlled with greater precision, relatively lower purification costs, and reduced production of ash and by-products. Additionally, colour damage to the final product can be minimised. In the starch hydrolysis process to produce glucose, the enzymes commonly used include amylase and glucoamylase, which work synergistically to break down polysaccharide chains into free glucose units. The enzymes that can be used for the enzymatic hydrolysis process are amylase and glucoamylase [7].

1.3. Research Objective

The purpose of this study was to examine the effect of liquefaction time and saccharification time on the rate of enzymatic hydrolysis reaction, as well as to determine the kinetic equation of the enzymatic hydrolysis reaction for banana peel glucose synthesis.

2. MATERIALS AND METHODS

2.1. Materials and Tools

The materials used in this study were distilled water, hydrochloric acid, and sodium hydroxide, which were purchased from UD. Nirwana; banana peels obtained from a home-based banana chip industry located at Jalan Setro Baru 11, Surabaya; and α-amylase and glucoamylase enzymes obtained from the Microbiology Laboratory of Airlangga University.

The equipment used in the hydrolysis process included a 1 L beaker glass equipped with a magnetic stirrer, a thermocouple, a stand, and a clamp.

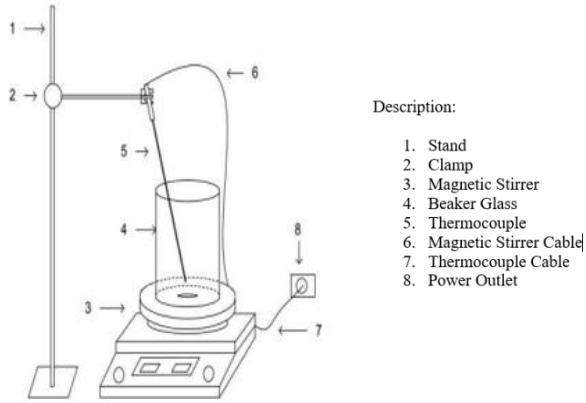

Figure 1. Enzyme Hydrolysis Equipment

2.2. Research Procedure

2.2.1. Raw Material Preparation Process

The banana peel is cut into smaller pieces. The chopped banana peel is dried in an oven at 100°C for 4 hours. The dried banana peel is then ground using a blender, and the particle size is standardised using a 60-mesh screen.

2.2.2. Enzyme Hydrolysis Process

A. Liquefaction Process
Prepare banana peel flour as a substrate at a concentration of 30% (w/v) and add 1000 ml of distilled water. The liquefaction process is carried out at a temperature of 90°C. Then add 0.7 ml of α-amylase enzyme. The process lasts for 80, 100, 120, 140, and 160 minutes.

B. Saccharification Process
The saccharification process is carried out for 16, 20, 24, 28, and 32 hours at a temperature of 60°C and a pH of 5-6, then 0.7 ml of glucoamylase enzyme is added. Next, the filtration process is carried out, and the glucose content of the filtrate obtained is analysed.

2.2.3. Final Product Content Analysis

Analysis of the final product content in the form of filtrate resulting from filtration using refractometer analysis. Where the reaction occurs is:

$$\underset{\text{Dextrin}}{C_6H_{12}O_5} \xrightarrow{\text{glucoamylase}} \underset{\text{Glucose}}{C_6H_{12}O_6}$$

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The kinetic reaction in the hydrolysis of carbohydrates using glucoamylase enzymes can be calculated using the Michaelis-Menten equation [12].

$$-r_A = r_R = k\frac{C_{E0}C_A}{C_M+C_A} \quad \text{.....[1]}$$

Where :

-rA = reaction rate of reactant A (mol/L.s)
rR = reaction rate of product R (mol/L.s)
k = reaction rate constant
CE0 = total enzyme requirement (mol/L)
CM = Michaelis-Menten constant
CA = final concentration of reactant (mol/m3)

3. RESULT AND DISCUSSION

3.1. Raw Material Analysis

Banana peel was used as a raw material in this study, undergoing a preparation stage that involved drying and grinding into a powder to increase its surface area and enhance the efficiency of the hydrolysis process. The content was analysed using the Luff-Schoorl method at the BSPJI Surabaya Testing and Calibration Laboratory. The initial analysis results of the carbohydrate content in banana peel powder are shown in Table 2

Table 2. Preparation of banana peel powder

Test Parameters	Unit	Test Result	Test Method
Carbohydrate	Percent (%)	25.4 %	Luff Schrool

3.2. Analysis of Reducing Sugar Content in the Liquefaction Process

After obtaining the carbohydrate test results, a pretreatment process was carried out on the material before undergoing hydrolysis. The pretreatment process was carried out to turn the banana peel into banana peel powder. The banana peel powder is then subjected to liquefaction using 0.7 ml of alpha-amylase enzyme at a substrate concentration of 30% and liquefaction times of 80, 100, 120, 140, and 160 minutes. The effect of liquefaction time on product concentration is shown in Figure 2 as follows:

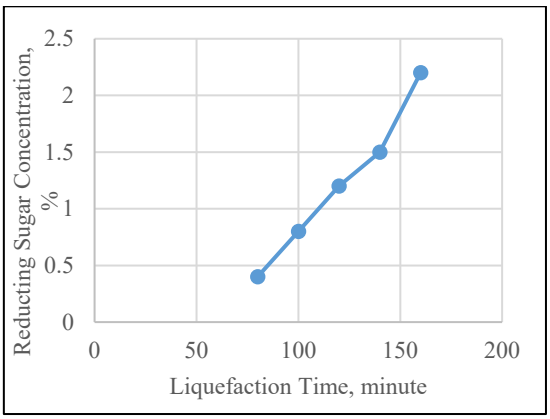

Figure 2. Correlation between liquefaction time and reducing sugar concentration

Figure 2 shows that the longer the liquefaction time, the higher the reducing sugar content obtained. The highest reducing sugar concentration was achieved at a substrate concentration of 30% with a liquefaction time of 180 minutes, resulting in 2.8% yield.

3.3. Analysis of Glucose Levels in the Saccharification Process

After reducing sugar is obtained in the liquefaction process, it can be stated that the reducing sugar content increases while the dextrin content decreases as the liquefaction process time increases, thereby increasing the efficiency of converting dextrin into glucose in the saccharification stage. The effect of dextrin concentration and saccharification time on glucose product concentration can be seen in Figure 3 as follows:

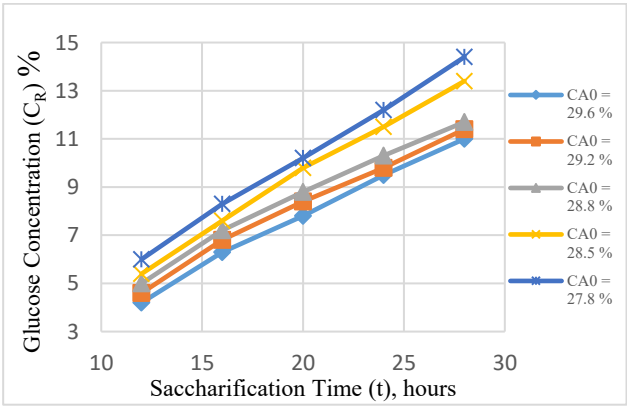

Figure 3. Effect of Saccharification Time vs. Glucose Concentration

Figure 3 shows that the longer the saccharification time, the higher the glucose content obtained. The highest glucose concentration was observed at a dextrin concentration of 27.8% with a saccharification time of 28 hours, corresponding to 14.4% of the total glucose.

Based on the reaction that occurred,

$$\underset{\text{Dextrin}}{C_6H_{12}O_5} \xrightarrow{\text{glucoamylase}} \underset{\text{Glucose}}{C_6H_{12}O_6}$$

The equation indicates that the reaction occurs at first order.

$$-r_A = -\frac{dC_A}{dt} \quad \text{.....[2]}$$

In the batch system, equation 1 is substituted with equation 2 to obtain the following line equation.

$$\frac{(C_A-C_{A0})}{ln\frac{C_{A0}}{C_A}} = -C_M + k_3C_{E0}\frac{t}{ln\frac{C_{A0}}{C_A}} \quad \text{.....[3]}$$

In this reaction, the stoichiometric coefficients for dextrin, water, and glucose are the same, so it can be concluded that the formation of 1 mole of glucose indicates that 1 mole of dextrin has reacted.

3.4. The Correlation between C_{A0}-C_A/ln(C_{A0}/C_A) vs C_{E0}t/ln(C_{A0}/C_A)

Following the acquisition of the above data, a graph was plotted showing the relationship between C_{A0}-C_A/ln(C_{A0}/C_A) as the Y-axis and C_{E0}t/ln(C_{A0}/C_A) as the X-axis at 12, 16, 20, 24, and 28

hours with initial dextrin concentrations of 29.6, 29.2, 28. 8, 28.5, and 27.8% to calculate the reaction rate constant and the *Michaelis-Menten* constant, as shown in Figure 4 below:

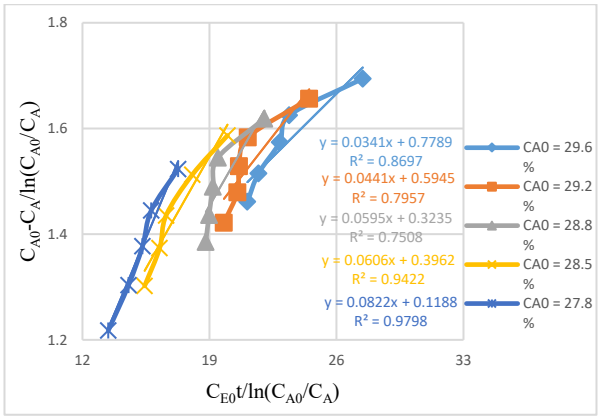

Figure 4. Relationship between CA0-CA/ln(CA0/CA) vs CE0t/ln(CA0/CA)

It is known that the graph is a linear line, so the equation of the line y=ax + b can be made, where the value of a is the slope, which is the reaction rate constant (k), and the value of b is the intercept, which is the Michaelis-Menten constant (CM) at each time.

From Figure 4, which shows the relationship between CA0-CA/ln(CA0/CA) vs CE0t/ln(CA0/CA), the highest reaction equation at CA0 is 27.8% with the line equation y = 0.0822x + 0.1188, yielding a slope value of 0.0822, which indicates the value of k, and an intercept value of 0.1188 which is the value of CM. Thus, the Michaelis-Menten equation is obtained as:

$$-r_A = r_R = 0.0822 \frac{C_{E0} C_A}{0.1188 + C_A} \; mol/L.hour$$

4. CONCLUSION

There is a relationship between liquefaction time and dextrin content, whereby the longer the liquefaction time, the lower the dextrin content produced, thereby increasing the efficiency of dextrin conversion to glucose during the saccharification stage. The Michaelis-Menten constant value obtained was 0.0341-0.7789. The rate equation follows the equation:

$$-r_A = r_R = 0.0341 \frac{C_{E0} C_A}{0.7789 + C_A} mol/L.hour\,.$$

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