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Hydrolysis profile of gadung *(dioscorea hispida dennst)* starch to glucose using alpha amylase enzyme

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Abstract

Indonesia is an agrarian country that has abundant natural resources. It has potential to be used as industrial raw materials such as sugar reduction from starch. Gadung, which is abundant in Gandus and it can be monitored for its presence in the dry season, is one source of starch. The aims of this research is to obtain glucose with a high yield percentage, to know the optimum concentration of α-amylase enzyme to hydrolysis gadung starch into glucose, and to know the relationship between hydrolysis time and concentration the glucose formed by the addition of the enzyme variation. The implementation of the research is carried out in three stages, first is preparation of raw materials, hydrolysis of gadung starch into glucose enzymatically, and analysis of glucose level using the UV-Vis spectrophotometry with Nelson reaction. The free variables used are the time variation of hydrolysis 0, 10, 20, 30, 40, 50 minutes and alpha amylase enzyme 1 %, 2 %, 3 % b/v. The optimum hydrolysis results on this study were at 1 % b/v enzyme variation, 10 minutes hydrolysis time, and a temperature of 90 ^{o}C. The yield of glucose concentration is 255.35 ppm. From this reseach, it is known that the longer the starch hydrolysis takes place, the observed glucose tends to experience in fluctuative consentration caused by the increase of α- amylase enzyme variations.

Keywords: α-amilase enzym, enzym hydrolysis, gadung, glucose, starch.

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1. INTRODUCTION

Indonesia is an agricultural country that has abundant natural resources and has the potential to be used as industrial raw materials. One of them is sugar reduction from starch (Koswara, 2006).

Glucose is an attractive raw material for chemical, pharmaceutical, and other agro-industries. The selection of gadung as a raw material for making alternative sugar is because gadung has a fairly high carbohydrate composition, which is 23.3 grams per 100 grams of dry matter (Koswara, 2015). In addition, gadung does not affect food stability in Indonesia and is not a staple food in Indonesia, especially in Palembang city, South Sumatra, although it can be used as a substitute for rice but people are not familiar with gadung food. The production of gadung tubers in

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Indonesia is quite high, which at peak harvest time can reach up to 19.7 tonnes/ha. (Deptan, 2005).

In the process, gadung starch goes through a hydrolysis stage first to break down the starch into glucose using a variety of α-amylase enzymes as biocatalysts. In the starch processing industry, enzymatic hydrolysis has the advantages of being more specific, producing more stable products in general, requiring less energy, and not requiring a neutralisation stage (Satyanarayana et al., 2005). Hydrolysis of starch by α-amylase will produce dextrin as the main product, where complete hydrolysis will produce glucose as the final product. Dextrin is a carbohydrate formed during the hydrolysis of starch into sugars by heat, acid or enzymes (Miftah, 2017).

Based on information from several articles, the reaction of glucose formation from gadung starch is still little found, so researchers are interested in observing the hydrolysis reaction of glucose formation from gadung starch using variations of α-amylase enzyme as a biocatalyst.

1.1 Gadung

Gadung is a climbing shrub that can reach 5-10 m in height. Gadung contains chemical composition that has the potential to produce glucose. In terms of chemical composition, gadung has a carbohydrate content of 23.3 grams. The chemical composition of gadung per 100 grams of dry matter can be seen in Table 1 below.

Table 1. Gadung Chemical Composition (per 100 grams of dry matter)

Parameters	Quantity
Moisture	63 % a
Carbohydrate	23,3 gram a
Protein	2,0 gram a
Starch	32 % a
Fat	0,98 % a
Ash	1,2 % a
Cyanide	50 - 400 ppm c
Calorie	102 kal

Remarks: Sibuea (2002), Koswara (2015)

1.2 Glucose Formation Reaction

The reaction that occurs in the starch hydrolysis process is simply shown in the following reaction.

$$(C_6H_{10}O_5)n + H_2O \xrightarrow{\text{Alpha amylase enzyme}} n(C_6H_{12}O_6) \quad .....(1)$$

Starch water Glukose

1.3 α-Amilase Enzyme

Amylase is classified as a saccharidase (an enzyme that cleaves polysaccharides). Amylase is a digestive enzyme, mainly performed by the pancreas and salivary glands. The main function of the enzyme amylase is to break down starch into glucose (Ariandi, 2016).

The α-amylase enzyme hydrolyses the α-1,4 glucosidic bonds of amylose, amylopectin, and glycogen. α-amylase is an endoamylase, which is an enzyme that

breaks down starch randomly from the centre or inside of the molecule. The presence of calcium that binds to the enzyme protein molecule makes the α-amylase enzyme relatively resistant to temperature, pH, and compounds such as urea (Suhartono, 1989). The structure of α-amylase can be seen in Figure 1.

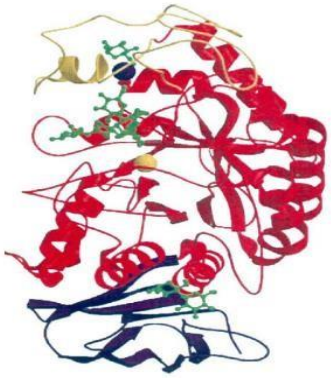


Figure 1. α-amylase Structure (Wahyuni, 2015)

Hydrolysis of amylose by α-amylase occurs in two stages, the first being degradation to dextrin which occurs randomly, very rapidly, and is followed by a rapid decrease in viscosity. The second stage is relatively very slow with the formation of glucose and maltose as the final products (Suhartono, 1989).

The results of decomposition by α-amylase are dextrin, limit dextrin, oligosaccharides, and cyclodextrin derivatives. Dextrin is a complex mixture of oligosaccharides that has the molecular formula $C_6H_{10}O_3$. Dextrin is a product between starch and dextrose/glucose. The mechanism of starch hydrolysis using α-amylase enzyme catalyst can be seen in Figures 2, and 3.

1.4 Enzymatic Hydrolysis of Starch

Enzyme hydrolysis is carried out using α-amylase and glucoamylase (*amyloglucoosidase*) enzymes. The α-amylase enzyme is used in the liquification process, while glucoamylase is used in the saccharification process. Enzyme hydrolysis results in greater conversion of starch to glucose than acid hydrolysis. Enzyme hydrolysis can prevent side effect reactions due to the specific nature of the enzyme catalyst (Winarno, 1995).

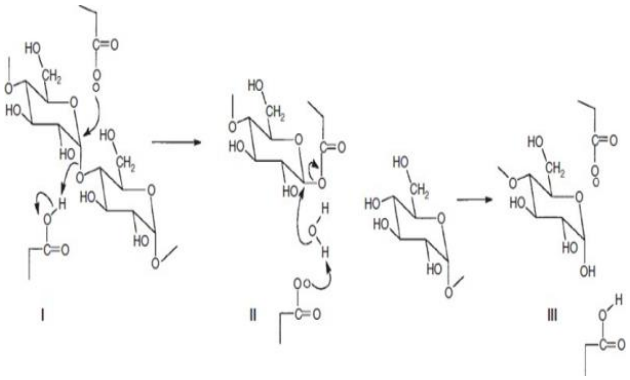


Figure 2. SN2 mechanism of α-1,4-glycosidic bond cleavage by the enzyme α-amylase
Source: Wahyuni, 2015

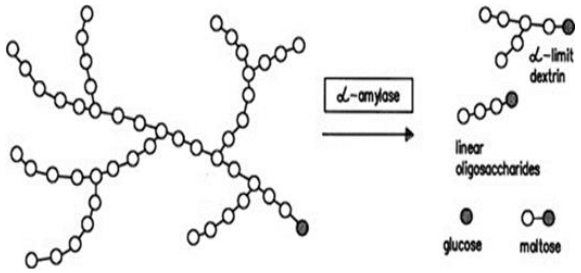


Figure 3. Hydrolysis of starch by α-amylase enzyme
Note: (-) reducing α-D-glucose residue; (○) non-reducing α-D-glucose residue
Source: Wahyuni, 2015

2. MATERIALS AND METHODS

This research was conducted at the Laboratory of Industrial Chemical Technology Study Programme of State Polytechnicof Sriwijaya Palembang in 2018. The raw material used in this research is gadung tuber. Gadung used comes from wild plants in Gandus sub-district Palembang. Gadung that has been taken, then cleaned and made into gadung flour. In this study, the tool used for glucose analysis was UV-VIS spectrophotometry. The process of hydrolysis of gadung starch can be seen in Figure 4.

2.1 Glucose Analysis

The method used to calculate glucose levels is the UV-VIS Spectrophotometer method with nelson and phosphomolybdate reagents. The steps taken in analysing glucose are as follows:

a. Standard curve generation

Prepare 9 clean test tubes. Each 1 ml of standard glucose was put into a test tube, and prepared 1 tube filled with blank distilled water. Then added 1 ml of Nelson's reagent to each test tube. Heating all tubes using a boiling water bath for 20 minutes. Then cooled all the tubes to a temperature of 25 ^{o}C. When all tubes had cooled, added 1 ml of phosphomolybdate reagent and dissolved it until the precipitate formed dissolved again. Then measure the absorbance of each solution and scan the wavelength from 20 nm to 600 nm, and make a standard solution curve showing the relationship between glucose concentration and absorbance.

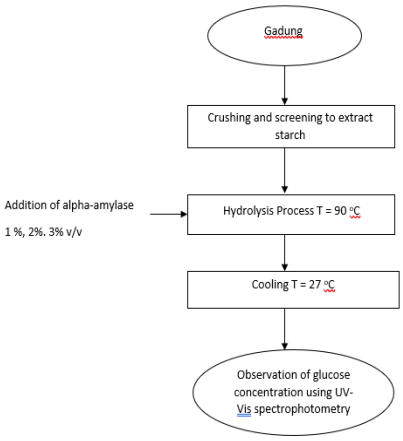


Figure 4. Hydrolysis Process of Gadung Starch

b. Determination of sugar in the sample

Add 1 ml of Nelson reagent to 1 ml of the sample solution. Next, treat it in the same way as the solution used to create the standard curve. Then determine the amount of reducing sugar based on the absorbance of the sample solution and the standard curve.

c. Preparation of Nelson reagent solutions A, B, and C

Nelson A

Anhydrous Na_2CO_3 12.5 grams, Rochelle salt (Na-K-Tartrate) 12.5 grams, $NaHCO_3$ 10 grams, and anhydrous Na_2SO_4 100 grams were dissolved into 350 ml of distilled water. Then, dilute it to a volume of 500 ml.

Nelson B

Dissolve 7.5 grams $CuSO_4$ and 5 ml H_2O into 50 ml distilled water. Then add 1 to 2 drops of concentrated H_2SO_4.

Nelson's reagent

Nelson A:Nelson B = 25:1 (made fresh each time).

d. Preparation of Ammonium Molybdate solution ($(NH_4)_6MO_7O_24.4H_2O$)

Weighed 4 grams of solid ammonium molybdate and put it in a glass beaker. Then dissolve with 50 ml of distilled water and dilute the solution into a 100 ml volumetric flask.

Analysis of glucose content was carried out to determine the level of glucose concentration obtained in the hydrolysis of gadung starch, so that the resulting glucose content is the glucose content after the hydrolysis process of gadung starch. The glucose flow chart can be seen in Figure 5.

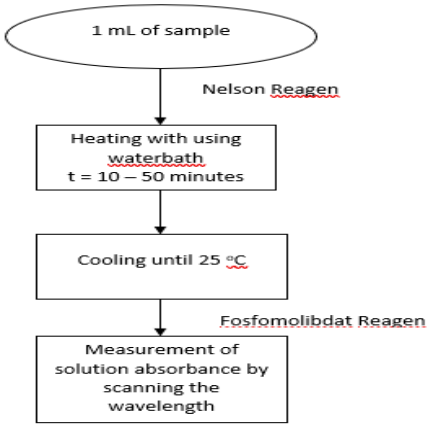


Figure 5. Glucose Analysis Diagram

3. RESULTS AND DISCUSSION

The following is the observation data of glucose concentration using UV-VIS spectrophotometry

3.1 The Observation of glucose concentration formed

From the results of the hydrolysis of starch using α-amylase enzyme, data on glucose concentration with the addition of 1 % α-amylase enzyme can be seen in Figure 6.

In Figure 6, the addition of 1 % b/v α-amylase enzyme showed an initial glucose concentration value of 174.98 ppm. At 10 minutes, the hydrolysis stage began, the glucose concentration increased to 255.35 ppm. The temperature used is 90 ^{o}C, the reactivity of α-amylase to convert the substrate into glucose is very high.

Based on Risnoyatiningsih's research, (2011) stated that the longer the reaction time, the greater the glucose content produced. The length of the reaction is also influenced by the amount of substrate hydrolysed and the amount of enzyme added. However, the data obtained from this study showed different results. With the addition of 1 %, 2 %, and 3 % enzyme, it is known that the longer the hydrolysis time, the glucose observed fluctuates and tends to decrease. It is possible due to the factor of enzyme addition both the amount and type of enzyme that determines the result of glucose content contained in gadung starch.

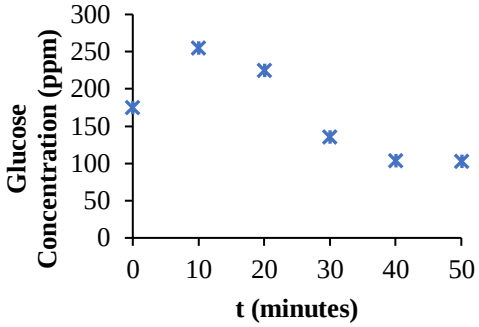


Figure 6. Glucose Concentration Vs Hydrolysis Time at 1 % enzyme variation

Based on the research of Risnoyatiningsih, (2011) it is explained that the more the amount of enzyme added to starch, the more glucose content will be produced but the ratio used is 1:500 kg, where the enzyme used is 2 kg in each tonne of starch. Whereas in this study, the enzyme used was 0.5 ml in 50 ml of starch solution. This means that it is possible that the level of enzyme added as much as 1 % b/v in 50 ml of starch solution is enough to work optimally for 1 gram of starch and at a time of 10 minutes, but at a time of 20 minutes the enzyme reactivity is quite high not proportional to the amount of substrate that will be converted into glucose so that the glucose reading decreases.

At the 20th minute there was a decrease in glucose concentration, the glucose concentration formed at the 20th minute was 224.70 ppm. at the 30th minute there was a decrease in glucose concentration of 135.05 ppm. Based on Figure 6, it can be concluded that the glucose produced from the hydrolysis of gadung starch with the addition of 1 % b/v α-amylase enzyme is the longer the hydrolysis time, the more volatile and tends to decrease the glucose concentration value produced. In Figure 6, the optimum hydrolysis time is at the 10th minute. Where the highest glucose concentration was obtained at 255.35 ppm. Furthermore, the addition of enzyme as much as 2 % b/v can be seen in Figure 7.

In Figure 7, there was a decrease in glucose concentration to 121.84 ppm at 10 minutes. The next increase and decrease in glucose concentration fluctuated with

the addition of 2 % b/v α-amylase enzyme. decrease and increase in glucose concentration occurred every 10 minutes. The highest glucose concentration at 10 minutes was 164.38 ppm.

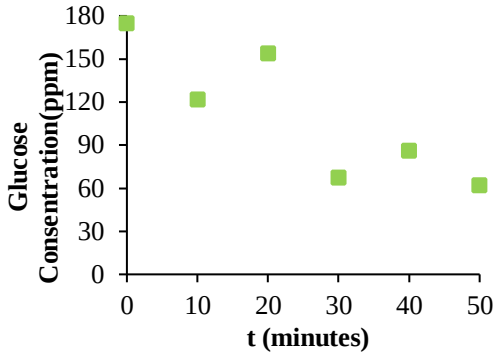


Figure 7. Glucose Concentration Vs Hydrolysis Time at 2 % enzyme variation

From Figure 7, it is known that the relationship between hydrolysis time and glucose concentration at 2 % enzyme variation is that the longer the hydrolysis time, the glucose concentration contained in the starch tends to decrease. This is due to the nature of the enzyme which functions as a biocatalyst making the reaction run very fast, the activeness of the α-amylase enzyme to convert the substrate into glucose is very high. In Figure 7, it is known that the highest glucose content is at time zero, which is 174.98 ppm. In the hydrolysis reaction, the amount of glucose decreased due to the nature of the alpha amylase enzyme that breaks down starch occurs through two stages, in previous research (Suhartono, 1989) explained that the hydrolysis of amylose by alpha amylase in the first stage degraded into dextrin first randomly, very quickly, and followed by a rapid decrease in viscosity. This is evidenced by the non-linear and fluctuating hydrolysis graph. In a fairly short time, up to 50 minutes. Then from previous research it is explained that in the second stage of hydrolysis takes place relatively very slowly with the formation of glucose and maltose as the final result. So it can be concluded that if the hydrolysis time is extended, there is a possibility of an increase in glucose. In this study, the reactivity and stability of the enzyme were unstable in the addition of 2 % and 3 % enzyme. While the addition of 1 % b/v enzyme decreased the glucose level quite stable. Furthermore, the addition of 3 % enzyme can be seen in Figure 8.

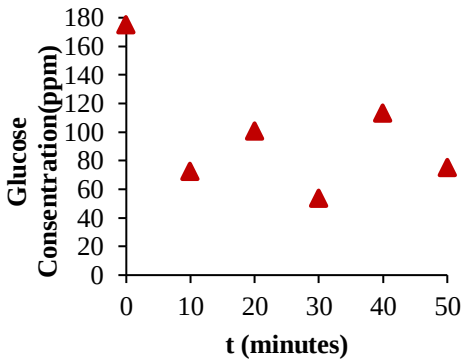


Figure 8. Glucose Concentration Vs Hydrolysis Time at 3 % enzyme variation

In Figure 8, the addition of 3 % enzyme showed almost the same result as the addition of 2 % b/v enzyme. There was a decrease in glucose concentration from zero minute 174.98 ppm to 72.18 ppm at 10 minutes. Then followed by an increase and decrease in glucose concentration fluctuated. The highest glucose concentration was located at 40 minutes. There was a change in concentration every 10 minutes.

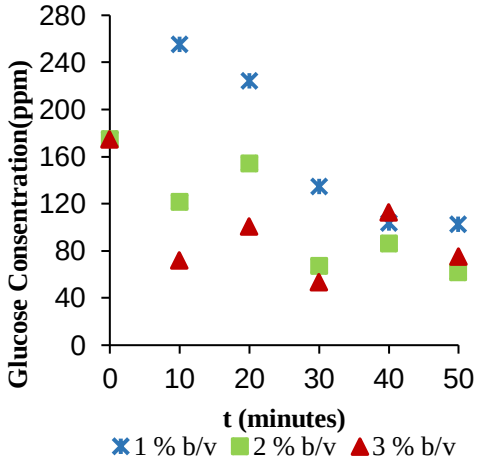
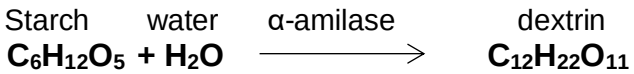


Figure 9. Glucose Concentration Vs Hydrolysis Time at 1%, 2%, and 3% enzyme variation

Based on the glucose concentration data formed in Figure 9, it is known that each addition of α-amylase enzyme variation shows fluctuating glucose concentration. There was a change in the average glucose concentration every 10 minutes. At 50 minutes, the glucose concentration decreased but the enzyme performance was not yet inactive, indicated by the glucose concentration value which still increased. Based on theory, the longer the reaction time, the higher the glucose content produced due to the contact time between the enzyme and the substrate lasts long with the appropriate pH. however, in this case based on the opinion of (Suhartono, 1989) the performance of the α-amylase enzyme only breaks the alpha 1,4 bond inside the molecule on amylose and amylopectin randomly, seen in the fluctuating graph. according to Winarno, (1995) the way α-amylase works on amylose molecules occurs in the first stage, the degradation of amylose into maltose and maltotriose which occurs randomly. This degradation occurs very quickly and is followed by a rapid decrease in viscosity as well. the second is relatively very slow, namely the formation of glucose and maltose as the final result which occurs non-randomly. while the workings of α-amylase on amylopectin molecules will produce glucose, maltose, and alpha-limit dextrin. the type of alpha-limit dextrin is an oligosaccharide consisting of 4 or more sugar residues containing alpha-1,6 bonds.

In this study, it can be concluded that the concentration of glucose formed is fluctuated by the amount of substrate, hydrolysis time, and the addition of the amount of α-amylase enzyme as an enzyme that functions to break down starch into glucose. The effect of adding a certain amount of α-amylase enzyme in this study is to break down starch randomly. In some research journals, it is mentioned that the initial stage of α-amylase enzyme only lasts until the liquefaction stage, which is the process of liquefying the starch gel. The next hydrolysis result is dextrin (Othmer, 1976). The reaction formed is:



To produce a stable glucose concentration, it is necessary to carry out a saccharification process, which is the process of hydrolysis of dextrin into glucose with the help of the enzyme amyloglucosidase.

4. CONCLUSION

The conclusion of the research is the addition of the percentage of α- amylase enzyme that produces the highest glucose concentration in this study is as much as 1 % b/v. The relationship between the concentration of glucose formed and the hydrolysis time at each enzyme addition is the longer the hydrolysis time, the glucose observed fluctuates and tends to decrease. This research needs to be further reviewed with a longer hydrolysis process followed by a liquefaction process and the addition of glucoamylase enzyme to get a linear glucose graph, hopefully it can be fermented to become bioethanol so that the reaction kinetics can be calculated, and the final product can be used as alternative energy in the form of bioethanol.

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