Extracting Glucose from Tapioca Flour Enzymatically Using Saccaromyces Cereviceae (YEAST)

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Abstract. Indonesia is located at the equator line with its tropical climate which allows many species of plants to grow lushly. One of these species of plants is cassava which can be found from the west to the east of Indonesia. Cassava can produce amylum which can be turned into glucose through hydrolysis process. This research aims at figuring out the comparison of reaction times, i.e.: 12, 24, 36, 48, and 60 hours, comparison of substrate concentration, i.e.: 20:120, 20:140, 20:160, 20:180, and 20:200 gr/ml and comparison of yeast content: 5%, 10%, 15%, 20%, and 25%. The hydrolysis is performed by mixing the cassava starch pulp at 20 grams and yeast at 11.6 grams. Later, it is fermented for variable times. After obtaining the optimal hydrolysis time, i.e. 12 hours, a hydrolysis is done at variable substrate concentrations for 12 hours and an optimum sugar level is obtained at 20:120 gr/ml ratio. It is then hydrolyzed for 12 hours at substrate concentration 20:120 gr/ml ratio, hence the optimum reduction sugar level is obtained when the yeast content is 10% of the tapioca flour pulp weight.

Keywords: glucose, enzymatic, yeast

1. Introduction
Indonesia is a country gifted with abundant amount of natural resources. These resources play an important role in fulfilling the need of Indonesian people. However, in fulfilling this needed, these natural resources should be exploited reasonably (as needed) and wisely based on environmental ethics (for example, when exploiting plants, new plants should be planted for regeneration purpose to allow the fulfillment of need through natural resources in the future).

The natural resources that Indonesia has in its territory include fuel and oil (petroleum, diesel, gasoline, and so forth), coal, various species of plants and trees, etc. The plants growing in Indonesia can grow lushly for its soil is highly good for farming. The plants frequently found in Indonesia are generally those which contain great extent of carbohydrate, such as paddy, corn, cassava, and so on.

Cassava is one of the most frequently cultivated plants in Indonesia. It can be a substitute to staple foods (rice). It can even be processed in many ways. One can directly boil it and immediately eat it or processed first into semi-finished goods in the form of flour, the tapioca flour. This tapioca flour can last for a very long time and it is usually used to make cakes, snacks, etc.

Based on its carbohydrate content, cassava has the greatest one after water. Hence, it is reasonable to assume that tapioca flour has the same amount of carbohydrate content as cassava or in a lesser
amount due to the process it undergoes to turn into flour. Carbohydrate is the raw material to make glucose, thus it is possible to make glucose from tapioca flour. Glucose can be used as sweetener. From the natural resources potential of this cassava plant, it is expected that the business opportunity in the field of cassava utilization and processing can increase.

2. Theoretical Basis
Cassava is an annual plant from Tropical America. The nutrient content in each 100 gram cassava is as follows (Table 1). Hydrolysis is a process of breaking bonds of a compound, be it organic or inorganic compound, into another compound by water. Hydrolysis can be done using enzyme catalyst and every type of starch such as potato, yam, and corn, which will produce glucose when hydrolyzed. The factors which influence the hydrolysis process are time, reactor ratio, and the type of enzyme used.

Table 1. Nutrient content in each 100 gram cassava

| Composition       | Nourishment Content of 100 gr of Ingredients |
|-------------------|---------------------------------------------|
| Energy (kal)      | 158                                         |
| Water (gram)      | 60                                          |
| Protein (gram)    | 0.7                                         |
| Fat (gram)        | 0.7                                         |
| Carbohydrate (gram) | 37.9                                     |
| Fiber (gram)      | -                                           |
| Ash Content (gram) | -                                           |
| Ca (mg)           | 33                                          |
| Fe (mg)           | 0.7                                         |
| P (mg)            | 40                                          |
| Vitamin B4 (mg)   | 230                                         |
| Vitamin B2 (mg)   | 0.06                                        |
| Vitamin C (mg)    | 0                                           |

3. Research Method
The raw materials used consist of main and auxiliary ingredients. The main ingredients are tapioca flour (starch from cassava) and yeast (Saccharomyces Cereviceae). The auxiliary ingredients used include distilled water, standard glucose solution, Nelson A and Nelson B reagents, Arsenomolybdate reagent, HCL 37%, Alcohol 30%, and NaOH solution 45%.

The research steps taken in this research are raw material preparation, equipment preparation, water content analysis, starch analysis, and output analysis. To analyze the output, the glucose in tapioca flour (sample solution) is compared to the Optical Density (OD) value in standard curve. The standard curve itself is made using the following steps:

1. Make a standard glucose solution (10 mg glucose anhydrite/100 ml).
2. From this standard glucose solution, perform 6 dilutions to obtain glucose solution at 2, 4, 6, 8, and 10 mgs/100 ml concentrations.
3. Prepare 6 clean test tubes, each is filled with 1 ml standard solution above. 1 tube is filled with 1 ml distilled water as a blank.
4. Add into each test tube 1 ml Nelson reagent, and heat all tubes into boiling water bath for 20 minutes.
5. Take all tubes and cool them down together in a glass cup containing cold water until the tube temperature reaches 25°C.
6. After it is cold, add 1 ml Arsenomolybdate reagent and shake it until all existing Cu$_2$O deposits are dissolved again.
7. After all Cu$_2$O deposits are completely dissolved, add 7 ml distilled water and shake it until it is homogeneous.
8. Shake it until it is homogeneous and cool it down until it reaches room temperature.
9. See the “Optical Density” (OD) of these solutions using cuvette tube at 540 nm wavelength.
10. Make a standard curve which shows the correlation between glucose concentration and OD. To measure the Optical Density (OD) of sample solutions, the following steps are taken:
   1. Prepare the sample solutions which have glucose content of around 2-8/100 ml. Please note that this sample solutions should be clear, thus when turbid or colored sample solutions are found, a cleansing needs to be done first using Pb-Acetate or Aluminium hydroxide pulp.
   2. Pipette 1 ml clear sample solution into a clean test tube.
   3. Add 1 ml Nelson reagent, and then treat it as in the preparation of standard curve above.
   4. Determine the amount of glucose based on the OD of sample solutions and the standard curve of glucose solution.

4. Result and Discussion
   4.1. Standard Glucose Solution
   This standard glucose solution is obtained from glucose anhydrite at 10 mg which is dissolved in 100 ml. From this standard glucose solution, glucose solutions are made at 2, 4, 6, 8, and 10 mg/100 ml concentrations.

| No. Tube | 1  | 2  | 3  | 4  | 5  | 6  |
|----------|----|----|----|----|----|----|
| Aquadest, ml | 1.0 | 0.8 | 0.6 | 0.4 | 0.2 | 0  |
| Standart glucose Solution, ml | 0  | 0.2 | 0.4 | 0.6 | 0.8 | 1.0|
| Glucose Concentration, mg/100ml | 0  | 2  | 4  | 6  | 8  | 10 |

From the analysis of standard glucose solution, the correlation between glucose concentration solution and Optical Density (OD) of experiment output is obtained and using the Linear Regression Equation the Optical Density (OD) of calculation result.

| Glucose Concentration (mg/100 ml) | Optical Density Experimental (nm) | Optical Density Calculation (nm) |
|-----------------------------------|----------------------------------|----------------------------------|
| 1                                 | 0                                | 0.04145                          |
| 2                                 | 0.23                             | 0.18887                          |
| 3                                 | 0.34                             | 0.33629                          |
| 4                                 | 0.51                             | 0.48371                          |
| 5                                 | 0.61                             | 0.63113                          |
| 6                                 | 0.77                             | 0.77855                          |

1. Result of Water Content Analysis
   Original crucible weight : 46.51 grams
   Original tapioca flour weight : 10 grams
   Original (tapioca flour+crucible) weight : 56.51 gram
Then, it is ovened for 1.5 hours and put in an exicator gradually until the water content becomes 7.7%.

4.2. Result of Starch Analysis

Tapioca flour sample: 3.5 gram
Water volume (filtrate): 250 ml
HCl 25% volume: 20 ml
Dilution volume: 500 ml
Volume sample: 1 ml

Then, the sample is analyzed using spectrophotometer and the Optical Density (OD) is found to be 0.063 nm. Based on the standard curved glucose solution, the glucose concentration can be figured out to be 0.4 mg/100ml. From this calculation of glucose concentration and dilution volume, a glucose weight at 1 gram can be found. Thus, the starch content produced is 25.71%.

4.3. Reaction Time Variable

The hydrolysis is performed by reacting substrate (20 gr starch and 120 ml distilled water / 1 : 6) at 116 gr with yeast at 11.6 gr and added with distilled water 20 ml. To figure out the influence of comparison of time to the glucose solution produced, an experiment is performed for variable times between 12 and 60 hours. From the experiment, the following data are obtained:

| No | Time (Hour) | Volume of glucose Solution (ml) | Weight of glucose Solution (gram) |
|----|-------------|---------------------------------|----------------------------------|
| 1  | 12          | 13                              | 10.89                            |
| 2  | 24          | 18                              | 15.86                            |
| 3  | 36          | 25                              | 22.54                            |
| 4  | 48          | 26.5                            | 23.77                            |
| 5  | 60          | 27                              | 23.94                            |

The result above is analyzed with sample 1 ml using spectrophotometer, and a correlation between reaction time and Optical Density (OD) is obtained.

Dilution factor: 1000x

| No | Time (Hour) | Optical Density (nm) |
|----|-------------|-----------------------|
| 1  | 12          | 0.301                 |
| 2  | 24          | 0.271                 |
| 3  | 36          | 0.255                 |
| 4  | 48          | 0.245                 |
| 5  | 60          | 0.211                 |

Using the formula from the equation in standard glucose solution, a reduction sugar content can be found:
Table 6. Correlation between reaction time and reduction sugar content

| No | Time (Hour) | Glucose Levels (mg/ml) | Weight Glucose (mg) |
|----|-------------|------------------------|---------------------|
| 1  | 12          | 3.521                  | 45.773              |
| 2  | 24          | 3.114                  | 56.052              |
| 3  | 36          | 2.897                  | 72.425              |
| 4  | 48          | 2.761                  | 73.1665             |
| 5  | 60          | 2.3                    | 62.1                |

From the data above on the enzymatic time and output reduction sugar content, it can be seen that the longer the enzymatic reaction time the lesser the reduction sugar content obtained between 12 and 60 hours reaction time would be. This is because the ability of glucoamylase enzyme to turn starch into glucose is decreasing and it can be said that the glucose breaks into alcohol. Thus, it can be concluded that the 12-hours enzymatic reaction time is the most optimal (best) time.

The produced glucose solution will be increasingly greater. This is because of the ability of glucoamylase enzyme in changing starch into glucose.

From the graphic, i.e. between the duration of enzymatic reaction time and the reduction sugar content produced, it can be approached using the equation:

\[ y = 3.8689e^{-0.0081x} \]

Where,

- \( y \) = the produced reduction sugar content
- \( x \) = duration of enzymatic reaction time

The equation above is applicable to 12-60 hours enzymatic reactions. The mean uncertainty = 95.62%.

4.4. Substrate Concentration Variable

After the optimum hydrolysis time is obtained, i.e. 12 hours, a hydrolysis is then performed at variable substrate concentration for 12 hours. To figure out the substrate concentration to produced reduction sugar, an experiment is made with variable substrate concentrations between tapioca flour and distilled water. The tapioca flour weight is 20 gr and the distilled water starts from 120 ml (1:6), 140 ml (1:7), 160 ml (1:8), 180 ml (1:9), and 200 ml (1:10). The hydrolysis is performed by reacting the substrate, 11.6 gr yeast and added with 20 ml distilled water, for 12-hours fermentation time.

From the experiment, the following results are obtained:

Table 7. Correlation between substrate concentration and glucose solution output

| No | Substrate concentration (gr/ml) | Volume of glucose solution (ml) | Weight of glucose solution (gr) |
|----|---------------------------------|---------------------------------|---------------------------------|
| 1  | 20:120                          | 7                               | 4                               |
| 2  | 20:140                          | 9                               | 6.23                            |
| 3  | 20:160                          | 15                              | 11.87                           |
| 4  | 20:180                          | 40                              | 36.45                           |
| 5  | 20:200                          | 22                              | 18.99                           |

The result above is analyzed using 1 ml sample using spectrophotometer, and a correlation between reaction time and Optical Density (OD) is obtained.

Dilution factor : 1000x
Table 8. Correlation between reaction time and Optical Density (OD)

| No | Time (Hour) | Optical Density (nm) |
|----|-------------|----------------------|
| 1  | 12          | 0.240                |
| 2  | 24          | 0.195                |
| 3  | 36          | 0.182                |
| 4  | 48          | 0.178                |
| 5  | 60          | 0.192                |

Using the formula from the equation in standard glucose solution, the following reduction sugar content is obtained:

Table 9. Correlation between substrate concentration and glucose content

| No | Substrate concentration (gr/ml) | Glucose levels (mg/ml) | Weight glucose (mg) |
|----|---------------------------------|------------------------|---------------------|
| 1  | 20:120                          | 2.694                  | 18.858              |
| 2  | 20:140                          | 2.083                  | 18.747              |
| 3  | 20:160                          | 1.907                  | 28.065              |
| 4  | 20:180                          | 1.853                  | 74.12               |
| 5  | 20:200                          | 2.042                  | 44.924              |

From the table above, it is found that the optimal substrate concentration to obtain glucose is 20 gr tapioca flour with 120 ml distilled water.

From the data, between substrate concentration and produced reduction sugar content can be approached using the equation:

\[ y = 201.21e^{-96.653x} \]

where:

- \( y \) = produced reduction sugar content
- \( x \) = substrate concentration

If the equation above is used again to calculate the produced sugar content, a mean uncertainty at 51.29% is obtained.

4.5. Yeast Content Variable

To figure out the influence of yeast content on produced reduction sugar, an experiment is made with variable yeast contents between tapioca flour pulp weight and yeast. The tapioca flour weight is 20 gr, and the yeast contents are 5%, 10%, 15%, 20%, and 25%. The experiment is performed for the same duration, i.e. 12 hours with substrate concentration 20:120 gr/ml and added with distilled water 20 ml.

From the experiment which has been conducted, the following data are obtained:

Table 10. Correlation between yeast content and produced glucose solution

| No | Yeast levels (%) | Volume of glucose solution (ml) | Weight of glucose solution (gram) |
|----|------------------|---------------------------------|----------------------------------|
| 1  | 5 %              | 15                              | 12.81                            |
| 2  | 10 %             | 14                              | 11.53                            |
| 3  | 15 %             | 13                              | 10.49                            |
| 4  | 20 %             | 13.5                            | 11.35                            |
| 5  | 25 %             | 5                               | 4.01                             |

The result above is analyzed with 1 ml sample using spectrophotometer and the correlation between reaction time and Optical Density (OD) is obtained.
Using the formula from equation in standard glucose solution, the following reduction sugar content is obtained:

\[
y = 3.0682e^{-0.076x}
\]

where

- \( y \) = produced reduction sugar content
- \( x \) = yeast content

If the equation above is used again to calculate the produced sugar content, a mean uncertainty of 33.52% is obtained.

5. Conclusion
1. Extracting glucose solution from tapioca flour can be done enzymatically using *Saccaromyces Cereviceae*. The glucose content obtained is influenced by reaction time, substrate concentration, and yeast content.
2. In the enzymatic hydrolysis reaction, distilled water is needed as an addition to allow distilled water molecules collide well.
3. From the research result, the optimal sugar content is obtained when it is done for 12 hours with the substrate concentration for tapioca flour and distilled at 20:120 gr/ml, and the yeast content at 10% of the tapioca flour pulp.

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