Structural characterization and antioxidant activity of liquid sugar from Alabio potato using enzymatic hydrolysis processes

by Ahmad Saiful Haqqi

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VS Angkasawati, I Kamaliyah, MD Putra, IF Nata, and C Irawan*
Department of Chemical Engineering, Faculty of Engineering, Lambung Mangkurat University, Jl. A. Yani Km. 35.5, South Kalimantan, Indonesia 70714

Email: cirawan@ulm.ac.id

Abstract This study aims to produce functional antioxidant liquid sugar from Alabio potato (*Dioscorea alata L.*). This study focuses on the effect of the percentage of Alabio potato starch in the liquefaction process, the concentration of β-glucosidase enzymes, and the determination of the optimum concentration of cinnamon extract in its activity as an antioxidant liquid sugar. The amount of glucose/reduced sugar was analyzed by the DNS method. The optimum percentage of glucose was obtained from the liquefaction results with 10% starch and saccharification with a concentration of β-glucosidase enzyme of 1.2 mL/kg of starch with a liquid sugar concentration of 59.16 mg/mL. FTIR analysis showed that antioxidant liquid sugar had hydroxyl functional groups of C-O, C-H and O-H at wavelengths of 1560.11 cm-1, 1720.22 cm-1, and 3520 cm-1. Antioxidant liquid sugar activity showed the optimum performance at cinnamon extract concentration was 5 grams. DPPH free radicals of 56.72% were consumed for 5 minutes and reached 57.60% for 10 minutes. Liquid glucose produced could be a substitute for glucose sources with antioxidant content from the cinnamon extract.

1. Introduction

The production of sweet potato in South Kalimantan, especially in Hulu Sungai Selatan regency has an average of 19.4 ton/ha/years during 2014–2018. Most of the sweet potato is cultivated in the swampy land. One of the sweet potato types is Alabio yam *(Dioscorea alata L).* The Alabio yam could be a substitute for food because it has a fairly good nutritional composition. Purple Alabio sweet potato has a starch content of 11,07% and white sweet potato of 11,30%. The total sugar found in red Alabio yam is 4.48% and the white Alabio yam is 2,80%. Each year the production of Alabio sweet potatoes has increased in the planting area so that the number of sweet potatoes produced has also increased. In 2015 the Alabio yam production reached 17 tons.ha^{-1} [1].

The making of liquid glucose was carried out in two main stages, which are liquidation and saccharification with the help of the enzyme α-amylase and β-glucosidase. Enzymatic glucose making is an appropriate technology that can be developed directly in the community because it does not require many chemicals and comes from natural ingredients. Purple Alabio sweet potato contains anthocyanin which can be used as a natural coloring agent in food [2].

Starch is present in a variety of staple foods such as potatoes, corn, rice, wheat, and pasta [3]. Making liquid glucose is expected to be able to overcome the high domestic sugar imports. The food and beverage industry is currently growing rapidly, where a lot of food industries are starting to innovate their products. The food and beverage industry is starting to switch to using liquid sugar because it has several advantages, namely, it dissolves easily, is more practical, and has a more attractive appearance compared to crystal sugar. Liquid sugar made from the Alabio yam is expected to replace the production

of crystalline sugar and the availability of raw material sources for making liquid sugar is quite abundant and easily cultivated. This research is expected to provide benefits to the community to produce food diversity and increase the economic value of the Alabio yam by utilizing the sweet potato meat to become liquid glucose so that it can raise food as a local characteristic.

Starch is a polysaccharide found in grains and tubers and fruits. Generally, starch is used as a modified food ingredient [4], tubers, for example, become sweet after being boiled due to the starch contained in them breaks down into simple sugars such as glucose. If the starch is heated, the molecule will split into smaller molecules such as sugar called dextrin. Then dextrin decomposes again into maltose and then into glucose [5].

2. Materials and methods

2.1 Materials

The materials used in this research were Alabio sweet potato, α-amylase enzyme, β-glucosidase enzyme, distilled water, calcium carbonate ($CaCO_3$), sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), dinitro salicylic reagent (DNS), potassium sodium tartrate tetrahydrate ($H_2C_4H_4O_6$), 2,2-diphenyl-l-picrylhydrazil (DPPH), activated carbon, methanol (CH_3OH), standard glucose ($C_6H_{12}O_6$). All the chemical used is pure grade analysis.

2.2 Preparation of raw materials

The Alabio sweet potato was peeled then cleaned and soaked with water then mashed using a blender then filtered using a porous cloth. The Filtrate was added to the container and allowed to stand for 1-2 days to get starch deposits. The immersion water was released. The starch was dried using an oven at 80°C for 3 hours until it got a constant weight, then mashed and sieved with a 60 mesh sieve.

2.3 Liquidation process

The Alabio sweet potato starch was weighed and put in a three-neck flask. Added to the starch was a solution of Ca^{2+} (80 ppm) of 50 mL with starch percentages of 1%, 5%, 10%, 15% and 20% (w / v). The solution was heated to a temperature of 85°C and then the enzyme α-amylase 330 units / g was added. After the addition of the enzyme and 1 hour of the liquidation process, filtering was carried out with 100 circles of filter paper to get the filtrate free of unreacted starch residue. The filtrate obtained was then analyzed based on the content of dextrin levels formed using the DNS method.

2.4 Saccharification process

Dextrin with a pH of 3 - 3.5 was added to the enzyme β-glucosidase 293 units/kg of starch. Then the solution that had been added with the β-glucosidase enzyme was in the shaker for 60 hours at a rate of 150 rpm on temperature 60℃. Glucose content was analyzed again using DNS.

2.5 Neutralization, blanching, and evaporation processes

Liquid glucose from saccharification which was acidic was neutralized by adding 0.1 M Na_2CO_3 base to pH 6-7 then the bleaching process was carried out by mixing liquid glucose with activated carbon as much as 2% of the weight of liquid glucose. The bleaching temperature was set to 80°C for 2 hours. The last step was evaporation to get the viscosity of liquid glucose with the desired thickness.

2.6 Preparation of cinnamon extract and combined with glucose

Cinnamon was washed and oven-dried at 80°C for 2 hours. It was then mashed using a blender, sifted to get a size of 60 mesh. Cinnamon extract was made from 3, 5, and 10 grams of cinnamon powder dissolved in 100 mL water, heated at 85°C for 15 minutes. Then the cinnamon extract was mixed with liquid glucose (% v / v) in a ratio of 1: 1.

2.7 Analysis

Analysis of reduced glucose/sugar content was done using the Dinitrosalisylic (DNS) method. The antioxidant capacity used the method of 2,2-diphenyl-1-picrylhydrazil (DPPH). Scanning Electron Microscope (SEM, JEOL JSM 6500 LV) was carried out to see the morphological structure of Alabio sweet potato starch. Fourier Transform Infra-Red (FTIR) was performed to determine the functional groups of the sample [6].

3. Result and discussion

3.1 Preparation of Alabio sweet potato starch

The Alabio sweet potato starch was obtained through the process of immersion and refinement, which could be seen in figure 1. The results were obtained in the form of flour with a pink color. The yield of flour obtained from the Alabio sweet potato meat was 40%. The components contained in the Alabio sweet potato are shown in table 1, the results show that the Alabio sweet potato starch had potentials as a substrate for hydrolysis. The carbohydrate content of the Alabio sweet potato was 90.77% as a source of dextrin production.

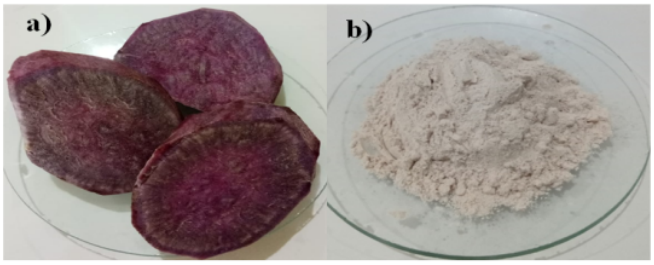

Figure 1. a) The Alabio sweet potato and b) Starch from the Alabio sweet potato

Table 1. Components of the Alabio sweet potato starch.

Components	Content (%)
Water	6,54
Ash	0,28
Protein	2,09
Fat	0,32
Carbohydrate	90,77

The results of observations with SEM of the Alabio sweet potato starch can be seen in figure 2**.** Observation of starch granules in figure a) using a magnification of 500x and in figure b) using a magnification of 1,500x shows that the starch granules of the Alabio sweet potato had a round shape with a size that was not homogeneous, about 10 - 40 µm. For other types of starch granules, such as cassava starch, it has a size of 4-35 µm [7].

3.2. Production of dextrin from Alabio flour meats by process liquidation

Liquidation is the process of hydrolysis of starch into dextrin by adding the enzyme α-amylase. The hydrolysis process melts the high viscosity starch gel. The solution used during the hydrolysis process to dissolve the starch of Alabio Sweet Potato was Ca^{2+} 80 ppm. Ca^{2+} solution was used as a cofactor to optimize the work of the α-amylase enzyme which converted the Alabio sweet potato meat starch into dextrin. The addition of enzymes to the hydrolysis reaction is beneficial for the process efficiency of the liquidation process [8]. The liquidation process is said to be good if the viscosity of the resulting dextrin is low. Based on the results of the study, the best dextrin was obtained at 10% starch concentration. This

is based on a visualization analysis of the product produced, the comparison between starch and Ca^{2+} solution that produce the most dextrin solution with low viscosity. A comparison of product results and volumes resulting from the liquidation process can be seen in table 2 and figure 3.

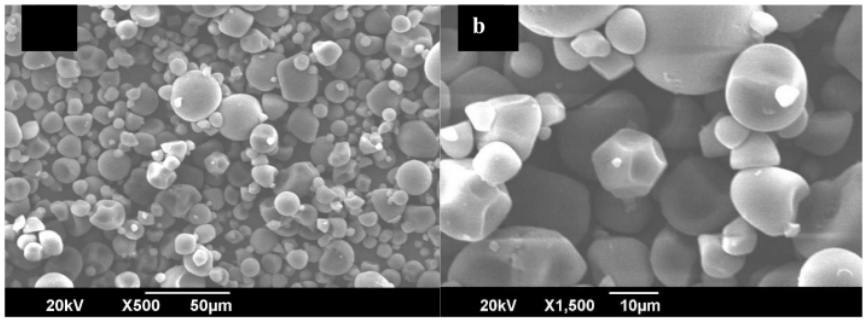

Figure 2. SEM images of a) 500x magnification of the Alabio sweet potato starch and b) 1,500xmagnification of sweet potato starch.

Table 2. Production of the liquidation process in the Alabio sweet potato starch.

The concentration of starch (%)	Result analyst	
	Production (mL)	Viscosity
1	49	Low
5	47	Low
10	46	Low
15	36	High
20	36	High

Based on the literature [6], it is calculated that the percentages of dextrins 5 and 10% have low viscosities, while the percentages of dextrins 15 and 20% have high viscosities. The dextrin content obtained from the liquidation process was analyzed by the DNS method. To get the reduced sugar concentration in the solution, a standard solution of glucose was previously made as a basic curve to determine the concentration of the sample by extracting the absorbance values obtained from the obtained line equations.

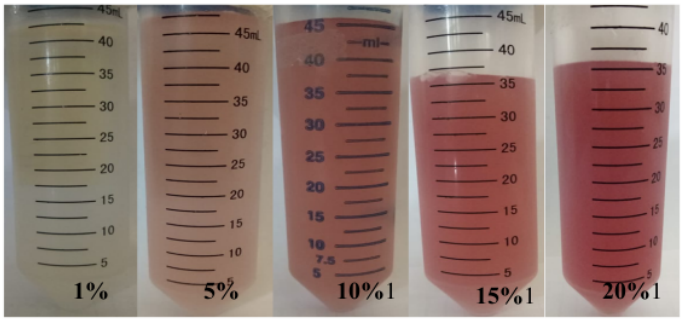

Figure 3. The liquidation process products with starch concentration of 1%, 5%, 10%, 15%, and 20% (w / v) temperature at 85 ℃ for one hour.

The higher concentration of glucose in the solution, the higher the absorbance value which is marked by the increasingly orange-brown discoloration of the final DNS analysis. After calculating and measuring with different starch concentrations, the content of dextrin at each Alabio Sweet Pot starch concentration can be seen in figure 4.

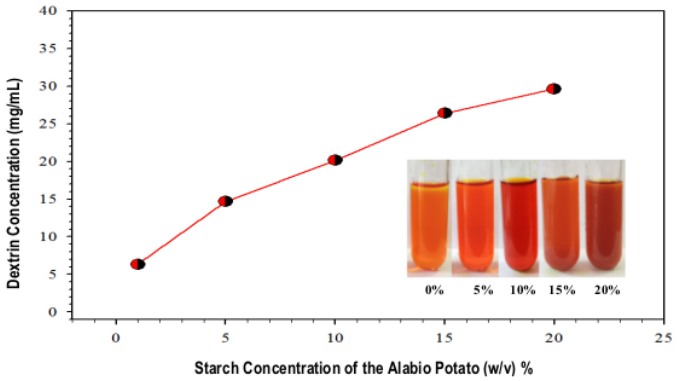

Figure 4. Relationship of dextrin concentration (mg / mL) to starch concentration (%) temperature at 85 ℃ for one hour.

The higher concentration of starch, the higher the total reducing sugar value was obtained. At starch concentrations of 10% and 15%, there was an increase in concentration from 20.11 mg / mL to 26.38 mg / mL, but the volume of the solution obtained decreased from 46 mL to 36 mL. It occurred because the starch concentration was high enough to make the enzyme unable to work optimally, so gelatinization began to increase. Based on the observations of the results of the dextrin obtained, a concentration of 10% starch was the optimum condition in the liquidation process, because it produced the largest product with low viscosity.

3.3 Conversion of dextrin to liquid glucose by the process of saccharification and addition of antioxidant sources from cinnamon extract

The saccharification process was the process of dextrins being converted into glucose after the best results from the 10% starch liquidation process. A comparison of glucose yields from products produced from the saccharification process with variations in the concentration of the β-glucosidase enzyme can be seen in figure 5. From the process of liquidation to saccharification, the reduced sugar concentration increased approximately 2 times the concentration of the liquidation product which indicated that the enzyme β-glucosidase had worked well. The enzyme concentration of β-glucosidase 1.2 mL / kg of starch was the optimum result of the saccharification process because it had the highest glucose concentration of 59.16 mg / mL. According to Ruiz [9] the saccharification process for 60 hours, there was an increase in high glucose levels of 46.9 mg / mL with a source of cassava starch. The resulting product meets the minimum requirements for reduced sugar content from liquid glucose based on the Indonesian National Standard (SNI), which is 30 mg / mL shown by the red line in figure 5.

For the liquid glucose products to be neutral, the results of liquid glucose from the saccharification process were neutralized by adding sodium carbonate (Na_2CO_3). To produce a clear product, the bleaching process was carried out by adding activated carbon. Activated carbon has a strong adhesion or absorption ability. After going through the stages of neutralization, bleaching, and evaporation, liquid glucose became more concentrated and aromatic and changed color. This happened because the impurities in liquid glucose were bound by activated carbon and some of the water content contained in liquid glucose was lost by evaporating when heating at 80 ^{0}C for 2 hours. Liquid glucose products before and after evaporation are shown in figure 6.

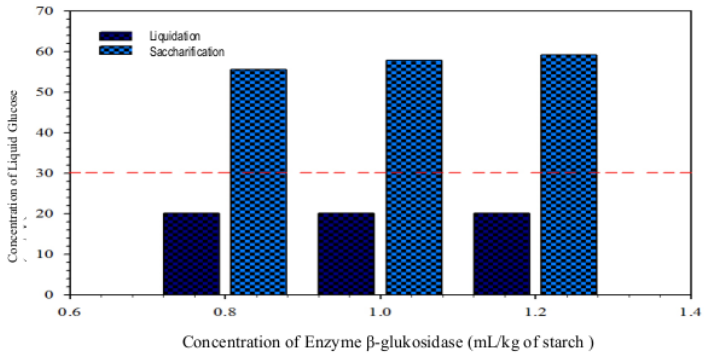

Figure 5. Relationship of dextrin concentration (mg / mL) to enzyme β-glukosidase concentration (mL/kg of starch).

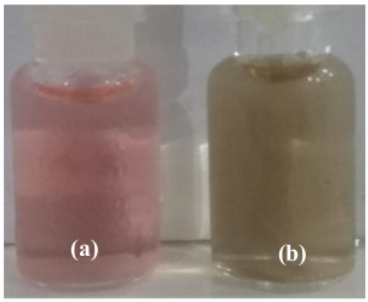

Figure 6. Liquid glucose before a) and b) After neutralization process blanching and evaporation

To produce liquid sugar with functional value, a cinnamon extract was added as a source of antioxidants with cinnamon extract concentration of 3, 5, and 10 grams. These additions gave liquid glucose antioxidant properties and created a fragrant aroma in liquid glucose. The capacity of antioxidants was measured quantitatively by measuring the capture of DPPH radicals by antioxidant compounds in cinnamon so that the value of free radical activity would be known. When the sample reacted with DPPH free radicals, there would be a transfer process of hydrogen atoms from antioxidant compounds in cinnamon to make DPPH stable and form reduced DPPH. The reaction was characterized by a change in color from purple to yellow [10]. Hydrogen/electron donated by antioxidants could prevent oxidation / neutralize oxidized compounds by binding to free radicals and reactive molecules [11]. The results of the analysis of liquid glucose with cinnamon extract on antioxidant activity can be seen in figure 7**.**

The optimum antioxidant activity of liquid glucose resulted in 5-gram cinnamon variation because 56.72% of free radicals from DPPH had been consumed for 5 minutes and reached 57.60% for 10 minutes. The red line in figure 7 shows the antioxidant activity without adding liquid glucose which was 60.3%. In 5-gram cinnamon variation was observed a high activity that almost reached the dotted line. 5-gram cinnamon variation can be said to be optimum where cinnamon extract works optimally in liquid glucose. Reduction in absorbance indicated scavenging activity (antioxidant activity) of the sample. The decrease in absorbance occurred due to the addition of electrons from antioxidant compounds to unpaired electrons in the nitrogen group in the structure of DPPH compounds. The intensity of the purple color would decrease when the DPPH radical bound with hydrogen. The stronger the antioxidant activity

in the sample, the higher decrease in purple intensity and the percentage decrease in absorbance [12]. Fourier Transform Infra-Red (FT-IR) analysis was performed to find out the functional groups of compounds with those shown by the frequency absorbed by the compound. The amount of frequency that passed through a compound was measured as a percent of transmittance [13]. FT-IR analysis on commercial liquid glucose, saccharification results, cinnamon extract, and various concentrations of the cinnamon extract can be seen in figure 8.

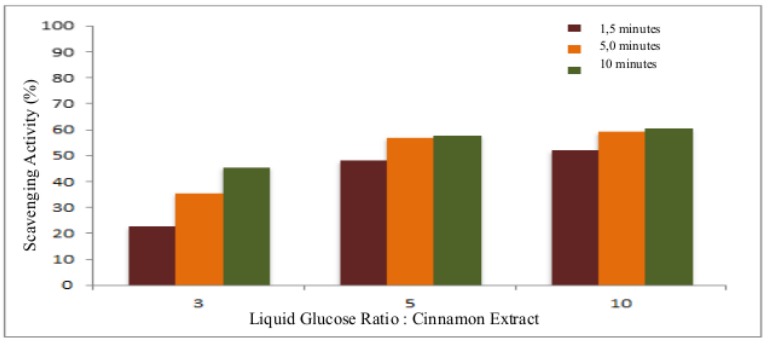

Figure 7. Relationship of scavenging activity (%) to liquid glucose ratio with cinnamon extract.

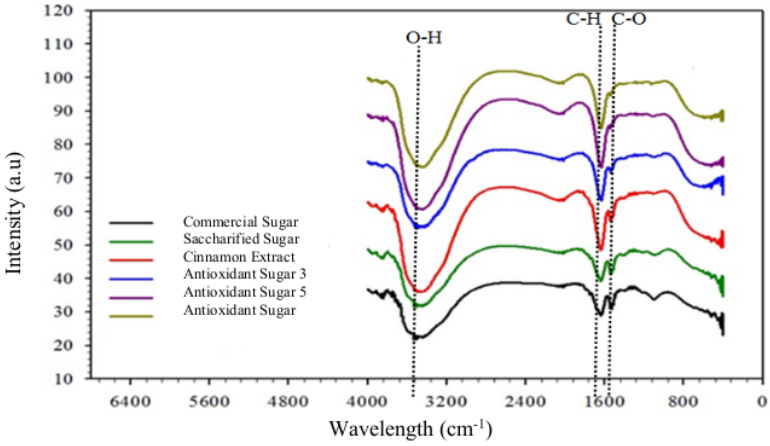

Figure 8. FT-IR for several types of samples.

Liquid glucose is a monosaccharide containing the hydroxy group -CHO aldose group [14]. The glucose function group is $C_6H_{12}O_6$. FT-IR analysis for commercial glucose and saccharification results did not show any difference, qualitatively the glucose produced was appropriate because it showed the same functional groups at each wave peak, ie CO, CH, and OH groups could be read at a wavelength of 1560.11 cm-1, 1720.22 cm-1, and 3520 cm-1. The difference between several types of samples from the FT-IR analysis occurred because the intensity of each sample was different in the O-H group. The intensity of the largest O-H group was found in the cinnamon extract samples. The cinnamon extract showed the intensity of O-H functional groups that were sharper because they contained O-H groups

which were owned by flavonoid compounds which were a source of antioxidants in the cinnamon extract [15].

4. Conclusion

The Alabio purple potato can be used as liquid glucose which is shown from the high carbohydrate content that is 90.77%. In the liquidation process, the optimum results were obtained at a starch concentration of 10% (w / v) with a TRS of 20.11 mg/mL. In the saccharification process, the optimum results were obtained at the enzyme concentration of β-glucosidase 1.2 mL/kg of starch with a TRS of 59.16 mg/mL. Based on cinnamon extract, the best results were obtained in the variation of 5 grams of cinnamon with free radical consumption of 56.72% within 5 minutes and reached 57.60% after 10 minutes.

Acknowledgments

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