Hydrolysis of sorghum starch to glucose using organic acid catalyst from rosella flower extract (*Hibiscus sabdariffa* L.)

Riniati1,*, S H Abdulloh1, R H Fauziyah1 and Istiqomurohmah1

1Chemical Analysis Study Program, Chemical Engineering Department, Politeknik Negeri Bandung, Indonesia

*E-mail: riniati@polban.ac.id*

Abstract. Highly starch content in sorghum seed has a high potential raw material for the sorghum flour to be a raw material of liquid sugar known as glucose syrup. The syrup is usually produced through enzymatically or chemically hydrolysis using a strong acid. In this study, sorghum starch is hydrolyzed chemically using a catalyst of organic acids extracted from rosella flower (*Hibiscus sabdariffa* L.). The goal of the study is to produce glucose syrup free from chemical agents so as generally recognized as safe (GRAS). Rosella flowers are known to contain high amounts of organic acids such as citric acid, malic acid, tartaric acid, oxalic acid, and hibiscus acid. Apart from having natural acid content, rosella flowers also contain vitamins (ascorbic acid) and are rich in natural red pigment dyes from the anthocyanins that can be an added value for the glucose syrup products. Organic acid extraction of dried rosella flowers was carried out at a temperature of 100°C for 30 minutes in a water bath and filtered. The resulting acid extract then determined the value of the total titrated acid (TTA) by the acid-base titration method. Sorghum starch is prepared by mixing sorghum flour with distilled water and then cooled at 4°C for 12 hours. Starch is obtained from the dried filtrate to obtain a water content of <14%. The starch hydrolysis process with rosella flower extract was carried out in several variations, namely the hydrolysis time of 1 - 4 hours, the TTA concentration of rosella 4 - 16%. Meanwhile, the temperature and stirring speed were fixed at 96°C and 200 rpm. The resulting glucose levels were analyzed by the spectrophotometric method. From this research, the result shows that the best sorghum starch hydrolysis condition was obtained at the addition of 16% acid extract for 180 minutes which gave 13.83% of glucose yield

1. Introduction
Sorghum (*Sorghum bicolor* L. Moench) is a cereal crop that has an average starch content of 69.5%. Sorghum starch consists of amylose (20–30%) and amylopectin (70–80%), depending on genetic and environmental factors [1]. Based on these data, sorghum has excellent potential to be processed into other, more useful products based on its nutritional content. The high starch content in sorghum plants makes sorghum widely used as a raw material in various industries such as the beer starch industry, liquid sugar (syrup), jaggery, ethanol, glue, paint, paper, degradable plastics and others[2]. Sorghum seeds can be made into sugar, liquid glucose, or fructose syrup according to the sugar content of the seeds [3].

Several studies of hydrolysis with synthetic organic acids have been carried out. Organic acids, such as citric acid and malic acid, are able to interact with the hydroxyl groups of cassava starch and act in the acid hydrolysis of starch molecules, reducing the starch chain connections [4]. Hydrolysis of durian...
rind to produce reducing sugar used 0.68 N maleic acid catalyst for 120 minutes at 95 °C to produce reducing sugar levels of 0.119 mg/mL [5]. Meanwhile, in the study of hydrolysis of sweet potato starch with a 5% citric acid catalyst for 1 hour at 45 °C with stirring which still produced a dextrose equivalent value of 2.21%, the DE value also showed an increase along with the increase in acid concentration and hydrolysis time [6].

Modification of starch using citric and stearic acids can reduce the value of swelling power as well as increase the solubility of starch which is thought to be due to the formation of short chains in acid hydrolysis and the effect of increasing the hydration capacity of starch chain hydrolysis[2]. The addition of citric acid to rice flour causes partial hydrolysis, which results in linear and short branched chains of crystalline and amorphous sections [7]. Heating at a higher temperature (> 80 °C) in the presence of citric acid causes hydrolysis of the amylose and amylopectin chain acids, and which affects the gel strength and retrogradation of the corn starch gel [8]. Citric acid is a naturally occurring polycarboxylic acid that can be extracted from various fruits and is generally considered safe [9]. Organic acids have high potential in the production of modified starch for the food and processing industries. Since starch and organic acids are readily available and low-cost materials, modification of starch with organic acids and their derivatives allows much room for further research on the reaction of starch with organic acids [10].

Rosella extract contains a high presentation of organic acids, namely 13–24% hibiscus acid, 12–20% citric acid, 2–9% malic acid, 8% tartaric acid, and 0.02–0.05% ascorbic acid [11]. Fresh roselle petals contain ascorbic acid from 6.7 to 14 mg/100 g while dried petals are 260–280 mg/100 g [12]. The presence of organic acids with a high presentation is expected to increase the DE value from hydrolysis. Variables that can affect the hydrolysis process using an acid catalyst include the size of the material to be hydrolyzed, the stirring speed during hydrolysis, the acid concentration, the time and temperature of the hydrolysis [13]. The operating temperature used for the hydrolysis of cellulose can increase glucose levels to the optimum level [14].

2. Material and methods
In general, the research begins with the preparation of sorghum starch and roselle extract, then performs hydrolysis by mixing starch and roselle extract under specific ratios and conditions. Glucose resulting from hydrolysis was analyzed qualitatively using Benedict reagent. To determine glucose levels, quantitatively analyzed using a refractometer and spectrophotometer.

2.1. Making sorghum starch
The sorghum flour is added with distilled water with a ratio of flour: distilled water = 1: 2 (1 kg flour: 2 L distilled water), then the mixture is filtered using a filter cloth. The dregs from the filtering are extracted again with the ratio of dregs: distilled water = 1: 1. Then the starch suspension was stored in the refrigerator at 4 °C for 12 hours. The topwater is removed, and wet starch is obtained. Wet starch is dried to obtain a water content of <14%.

2.2. Rosella extraction
For the manufacture of natural acid catalysts, dried roselle flowers are added with distilled water with a ratio of roselle flowers: aqua dest = 1:10 (100 g of dried roselle flowers: 1 L of distilled water) then the sample is extracted at 100 °C for 30 minutes and filtered until no solids are seen. On the filtrate. The extract obtained was measured the total titrated acid (TTA) by the acid-base titration method using the 848 Titrino Plus auto titrator. In the TTA measurement, a 10 g sample was put into a 100 ml measuring flask and diluted to the mark with distilled water. The 10 mL diluted sample is transferred to a small beaker, and distilled water is added until the electrode is immersed. Titration is carried out using a 0.1 N NaOH solution, which has been standardized until the endpoint of the titration is reached. The titration is done in duplicate. The total titrated acid is calculated using the equation:

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\% \text{ total titrated acid} = \frac{\text{mL NaOH x N NaOH x Dilution Factor}}{\text{gram of extract}} \times 100.
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2.3. Hydrolysis of sorghum starch with acid catalyst from roselle extract

One of gram sorghum starch is added with distilled water as much as 50 mL then heated until it dissolves and thickens. The solution was added to the acid extract with a volume variation of 4; 8; and 16 % TTA. Then refluxed at 95°C and stirred 200 rpm for 1, 2, 3 and 4 hours.

2.4. Analysis of glucose result of hydrolysis of starch sorghum

2.4.1. Glucose qualitative test with Benedict's reagent

One without of mL hydrolysis sample was put in a test tube, then added 1 mL of Benedict's reagent and stirred until blended. The test tube is then put into a beaker filled with boiling water, and boil for 5 minutes. After 5 minutes, the test tube and beaker are removed from the hotplate and cooled. The color change of the solution was then observed.

2.4.2. Analysis of glucose levels by Somogyi-Nelson method

One without of mL solution sample was added with 1 mL of Nelson's reagent and heated in a water bath for ± 20 minutes and immediately cooled down by putting it in a beaker filled with cold water so that the tube temperature reached 25°C then added to each tube 1 mL of Arsenomolybdate reagent, shaken until all the Cu₂O precipitates dissolved again. After all Cu₂O precipitates were completely dissolved, 7 mL of distilled water was added and shaken until homogeneous and measured the absorbance (A) of each solution using a Vis spectrophotometer with a wavelength of 540 nm. A standard curve was made showing the relationship between standard glucose concentration and absorbance.

3. Result and Discussion

3.1. The total value of titrated acid (TTA) of roselle extract

To determine the TTA value, a number of roselle extract samples titrated with a standardized NaOH solution. From the calculation results based on the volume of the equivalence point on the titration curve in figure 1, the dry roselle extract with a concentration of 10% (w/v) has a % TTA of 31.46%. The total acid in the sample will correlate with the pH of the sample. The dried roselle extract solution is purple-red and has a pH of 2.25.

![Figure 1. TTA analysis titration curve of rosella extract.](image)

3.2. Glucose qualitative test with benedict's reagent

After hydrolyzing sample for 1 hour, using reagent benedict qualitatively showed positive glucose. The presence of glucose ranging from 0.5% to 1% is indicated by the appearance of yellowish-green colour and cloudy. The other blue colour one of starch sorghum is the solution which no glucose contained as figure 2.
3.3. Effect of TTA concentration and hydrolysis time on glucose levels from hydrolysis

The acid concentration of the roselle extract was varied based on % TTA, by diluting the roselle extract to 4; 8 and 16% were added to the preheated starch (final starch concentration 1 g/100 mL). In the preliminary test to determine glucose levels by measuring % Brix using a refractometer, it appears that the higher the % TTA of organic acids from the roselle extract added, the higher the % Brix, this indicates the higher glucose levels as figure 3. From the time variation of 1–4 hours, it appears that the hydrolysis process decreases after the 3 hours.

Figure 3. Effect of % TTA and hydrolysis time on the value of % brix.

The results of glucose concentration from absorbance measurements by spectrophotometry which have been converted into % yield of glucose can be seen in figure 4. With an increase in glucose levels in hydrolyzed starch, it can be said that organic acids in roselle extract can affect the starch hydrolysis process. When water-insoluble starch is heated in water, the starch granules expand and burst. At this time, a gelatinization process occurs in which amylose and amylopectin can be removed from the granules, and the starch suspension becomes a thick paste. It is thought that organic acids such as citric acid enter the starch granules, causing hydrolysis of the glucose chains in the starch granules, then causing many of the glucose chains to dissolve into the starch paste.

4. Conclusion

This research can be concluded that the hydrolysis process of sorghum starch using an acid catalyst from rosella flower extract is influenced by the TTA concentration value. With 16% concentration of TTA against 1 gram of starch, it can produce 13.8% glucose at the optimum time for hydrolysis for 3 hours. This study recommends further research in the manufacture of glucose syrup using acids from roselle flower extracts.
Figure 4. The effect of TTA concentration of rosella flower extract on glucose levels.

Acknowledgment
We gratefully acknowledge support from the POLBAN Research and Community Service Center (PPPM) grant No. B/249.41/PL1.R7/PG.00.03/2020 which has provided funding for this research.

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