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Production of Glucose from Hydrolysis of Potato Starch

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ABSTRACT

Glucose is a very important raw material for the pharmaceutical and beverage industries. Currently industrial production of glucose involves enzyme hydrolysis of potato starch. Potato tuber is carbohydrate rich crop, but less exploited for glucose production. In this project we attempt to produce glucose from potato tuber the process involve extracting extract followed by acid hydrolysis. We also produce a sulphonated salicylic acid (SSA). The SSA was explored as a solid acid for the potato starch hydrolysis. The SSA was converted into polymeric frame work using Bakelite reaction. The rate of glucose formation using HCl was compared to the polymeric SSA.

Keywords: Glucose, Potato, Starch; Enzyme, Hydrolysis, Sulphonated Salicylic Acid

1. INTRODUCTION

Root and tuber crops supply most of starch in Africa markets. The major raw materials for production of starch are cassava, wheat, corn and potato. In developing countries such as Nigeria, root crops are relatively more important sources of starch than cereals crops (Regy and Padmaja, 2013). Root crops account for 60 % of starch production in Asian and Africa (Regy and Padmaja, 2013). Sweet potato is a true root that is rich in starch (25-40%). This starch can be hydrolyzed to glucose syrups, which are employed by the food industry to make sweets,

drinks, juices and for fermentation into products such as citric acid, gluconic acid and ethanol as well as in paper and textile industry (Crabb and Mitchinson, 2007).

Sweet potato (*Ipomoea batatas*) belongs to the *Convolvulaceae* (morning glory) family. It is a perennial plant that is widely cultivated in the tropics and sub tropics, where it serves as a major food source. Africa accounts for 75-80% of worldwide sweet potato production with an annual production of 78.8×10^9 kg followed by Nigeria with about 3.3×10^9 kg (Mkumbira *et al.* 2005). One of the challenges faced by developing countries such as Nigeria is the lack of storage facilities, some million tons of the tubers are destroyed due to poor storage management. In order to proffer solution to this wastage, value addition to these tubers to produce other useful products is imperative. The processing of starch to glucose can be carried out either by acid or enzymatic hydrolysis. However, the use of enzyme is preferred to acid, because it produces high yields of desired products and less formation of undesired products such as toxic compounds (Sanjust *et al.* 2004).

Campbell *et al.* (2014) reported the use of native sweet potato starch and their blends with rice flour and wheat flour, as the raw material for glucose production. The saccharified slurry from sweet potato-rice flour blends contained 70-72 g reducing sugars/100 g, which was higher than that released from native sweet potato starch (~69%). Although the percentage conversion to glucose was similar for sweet potato or their blends with cereal flours (42-43 %), glucose yield was higher in native potato starch and potato-rice blends than the other flour blends.

Lucyna *et al.* (2013) reported on a single stage hydrolysis of potato starch. They carried out the enzymatic depolymerisation of potato starch to glucose by starch-degrading amylase during a two-stage hydrolysis: liquefaction using bacterial α -amylase followed by saccharification with glucogenic (fungal amylase). Hydrolysis was run by Liquozyme Supra, Maltogenase 4000L and San Super 360L enzymes (Novozymes) at different temperatures. During the single-stage method of starch hydrolysate production the most desirable results was obtained for the maltose hydrolysate at 80 °C (51.6 DE) and for the glucose hydrolysate at 60 °C (96 DE). It was concluded that the unconventional single-stage method for starch hydrolysis leads to results comparable to those obtained with the two-stage method. Thus, in the production of glucose hydrolysates, the single stage method yields the most desirable results at 60 °C.

2. AIM AND OBJECTIVES

The overall aim of this research is to produce glucose from potato starch using acid hydrolysis. The specific objectives to be achieved include:

- i. Extract starch from potato tuber.
- ii. Carry out hydrolysis of the potato starch in Hydrochloric acid.
- iii. Prepare a solid acid and to evaluate the solid acid for hydrolysis of the potato starch.

3. MATERIALS AND METHODS

3.1. Materials

The apparatus used in this research include UV- Visible Spectrometer, thermometer, Conical flask, Beaker, Standard flask, pipette, Burette, pipette filler, test tube, test tube rack, oven, filter paper, thermometer, Aluminium foil, magnetic stirrer and bar.

The reagents used are hydrochloric acid, NaOH, formaldehyde, distilled water, sweet potato, DNS (dinitrosalicylic acid), sodium sulphite, salicylic acid, teralpthaldehyde and potassium tartate.

3. 2. Methods

3. 2. 1. Sweet Potato Starch Preparation

Sweet Potatoes were obtained from Ganmo market, Ilorin East Local Government Area of Kwara State, Nigeria. The tubers were washed to remove dirt, the washed potato tuber were peeled and crushed using grinding machine. The crushed pulp was sieved using a cloth. The slurry is then allowed to stand for 12 hours. The granule settled at the bottom. The super formed liquid was then decanted to obtain the starch cake. The starch cake was sun dried. The dried starch was then packed in a container for storage.

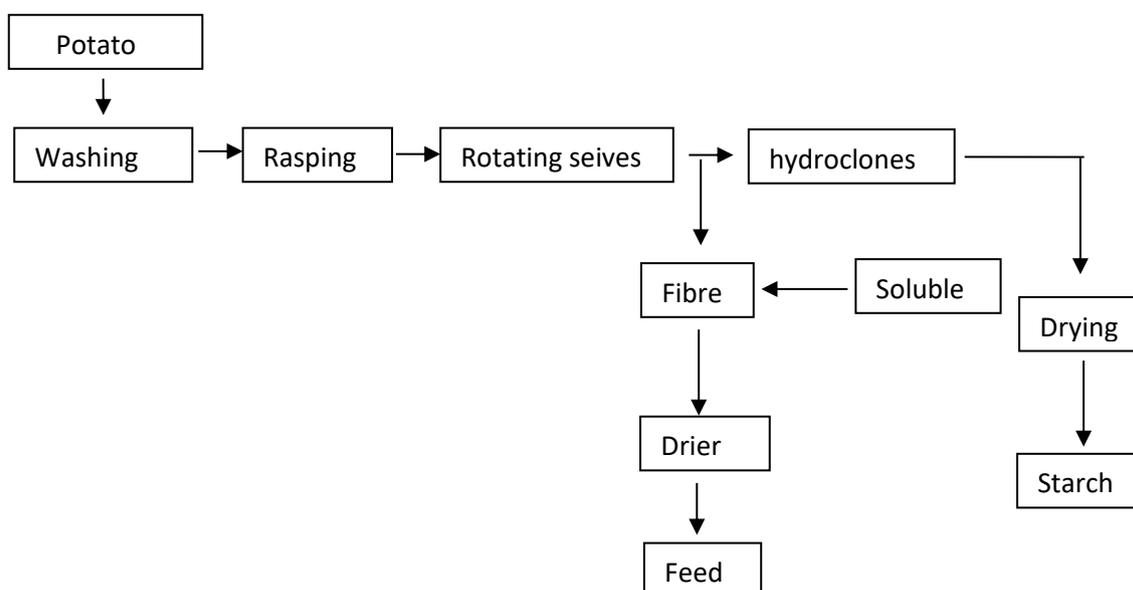


Figure 1. Manufacture of potato Starch (Mitchinson 1997).

3. 3. Preparation of Solutions

a) **Preparation of 0.1M Hydrochloric acid Solution**

A known quantity of distilled water was poured into 250 ml standard flask after which 2.10 ml of concentrated HCl was introduced to the flask using pipette. The flask was made up to mark with distilled water.

b) **Preparation of 0.1M Sodium Hydroxide Solution**

0.4 g of sodium hydroxide was dissolved in a little quantity of distilled water and the solution was poured into 100 ml standard flask. The flask was made up to mark with distilled water.

c) **Preparation of Dinitrosalicylic Acid (DNS) Solution**

1 g of DNS and 4 g of Potassium tartate were dissolved in 40 ml of distilled water in a beaker. 1.6 g of sodium hydroxide pellet was weighed and dissolves in 20 ml of distilled

water in another beaker. The content of the two beakers were quantitatively transfer into 100 ml standard flask and was make to mark with distilled water.

3. 4. Glucose calibration curve

Glucose Solution

0.2 g of glucose was dissolved in 40 ml of distilled water in a small beaker. The solution was poured in 100 ml standard flask and was makes up to the mark with distilled water.

Procedure

The glucose solution was placed on the magnetic stirrer at 50 °C temperature for 6 hours. 1 ml of the solution was pipette in a test tube. 3 ml of DNS solution was added boils in water bath for 5 minutes and cooled. The absorbance was taken at 540 nm.

Note: the sample is taken and the same procedure is repeated every one hour of stirring for the 6 hours.

3. 5. Hydrolysis of Potato Starch using hydrochloric acid

Starch Solution

0.2 g of the potato starch was dissolved in 40 ml of 0.1M HCl in a small beaker. The solution was poured in 100 ml standard flask and was makes up to the mark 0.1M HCl.

Procedure

Starch Solution was placed on magnetic stirrer at 50 °C temperature for 6 hours. 2 ml of the solution was neutralized with 2 ml of 0.1M NaOH. The solution was filtered. 1 ml of the filtrate was poured in a test tube and 3 ml DNS solution was added to the filtrate. The solution was boils in water bath for 5 minutes and cooled. The absorbance was taken at 540 nm.

Note: the sample is taken and the same procedure is repeated every one hour of stirring for the 6 hours.

Preparation of Solid Acid A

14 g of salicylic acid and 4 g of sodium hydroxide were dissolved in 100 ml distilled water in a beaker. The solution was placed on magnetic stirrer at temperature of 90 °C to dissolve completely. 8 ml of formaldehyde was added to the solution and leave for 30 minutes. 13 g of sodium sulphite Na₂SO₃ was added to the solution and leave for 2 hrs. 17 ml of concentrated HCl was placed in a burette and was put drop-wise in the solution over 2 hours. 10 ml of concentrated HCl was added and leave for 30 minutes. The solution was removed, filtered and filtrate was oven dry at 80 °C for 4 hours.

Calculation

$$\text{Percentage yield of the sample} = \frac{\text{Actual yield}}{\text{Theoretical yield}} \times 100\%$$

3. 6. Starch Solution

0.2 g of the potato starch and 0.2g of solid acid were dissolved in 40 ml distilled water in a small beaker. The solution was poured in 100 ml standard flask and was makes up to the mark distilled water.

Hydrolysis of Potato Starch using solid acid A.

Starch Solution was placed on magnetic stirrer at 50 °C temperature for 6 hours. 2 ml of the solution was filtered. 1 ml of the filtrate was poured in a test tube and 3 ml DNS solution was added to the filtrate. The solution was boils in water bath for 5 minutes and cooled. The absorbance was taken at 540 nm.

Note: the sample is taken and the same procedure is repeated every one hour of stirring for the 6 hours.

Preparation of Solid Acid B.

14 g of salicylic acid and 4 g of sodium hydroxide were dissolved in 100 ml distilled water in a beaker. The solution was placed on magnetic stirrer at temperature of 90 °C to dissolve completely. 8 ml of formaldehyde was added to the solution and leave for 30 minutes. 13 g of sodium sulphite Na₂SO₃ and 13 g of teralphtaldehyde were added to the solution and leave for 2 hrs. 17 ml of concentrated HCl was placed in a burette and was put drop-wise in the solution over 2 hours. 10 ml of concentrated HCl was added and leave for 30 minutes. The solution was removed, filtered and filtrate was oven dry at 80 °C for 4 hours.

Calculation:

$$\text{Percentage yield of the sample} = \frac{\text{Actual yield}}{\text{Theoretical yield}} \times 100\%$$

3. 7. Starch Solution

0.2g of the potato starch and 0.2g of solid acid with teralphtaldehyde were dissolved in 40 ml distilled water in a small beaker. The solution was poured in 100 ml standard flask and was makes up to the mark distilled water.

Hydrolysis of potato Starch using solid acid B.

Starch Solution was placed on magnetic stirrer at 50 °C temperature for 6 hours. 2 ml of the solution was filtered. 1 ml of the filtrate was poured in a test tube and 3 ml DNS solution was added to the filtrate. The solution was boils in water bath for 5 minutes and cooled. The absorbance was taken at 540 nm.

Note: the sample is taken and the same procedure is repeated every one hour of stirring for the 6 hours.

Hydrolysis of potato starch using distilled water

Starch solution

0.2 g of the potato starch was dissolved in 40 ml distilled water in a small beaker. The solution was poured in 100 ml standard flask and was makes up to the mark distilled water.

Procedure

Starch Solution was placed on magnetic stirrer at 50 °C temperature for 6 hours. 1 ml of the filtrate and 3 ml DNS solution was put in a test tube, boils for 5 minutes and cooled. The absorbance was taken at 540 nm.

Note: The sample is taken and the same procedure is repeated every one hour of stirring for the 6 hours.

4. RESULT AND DISSCUSSION

4. 1. Extraction of potato starch

The raw material for glucose production is starch. Corn is most common source of industrial starch. However, tubers are less exploited but important sources of starch. Potato is rich in starch and is important carbohydrate diet in Asia and African countries. Starch was extracted from potato tubers. Figure 1 shows the picture of the extracted potato starch.



Figure 2. Potato starch extract.

4. 2. Glucose calibration curve

Glucose is usually obtained by hydrolysis of starch. Starch is a polysaccharide; the hydrolysis involves breaking starch from polysaccharide → oligosaccharide → disaccharide → monosaccharide (glucose).

The extent hydrolysis process is normally monitored by determination of the dextrose equivalent (DE). DE is a measure of the amount of reducing sugar groups in the reaction mixture. However, since the starting material is starch and the end product is glucose, the hydrolysis reaction can also be monitored by determination concentration of starch or glucose in the reaction mixture as a function of time. Dinitrosalicylic acid (DNS) is one of the methods for determining glucose concentration. So DNS determination of glucose was used to monitor the rate of glucose formation. Figure 2 shows the calibration curve of glucose using DNS method.

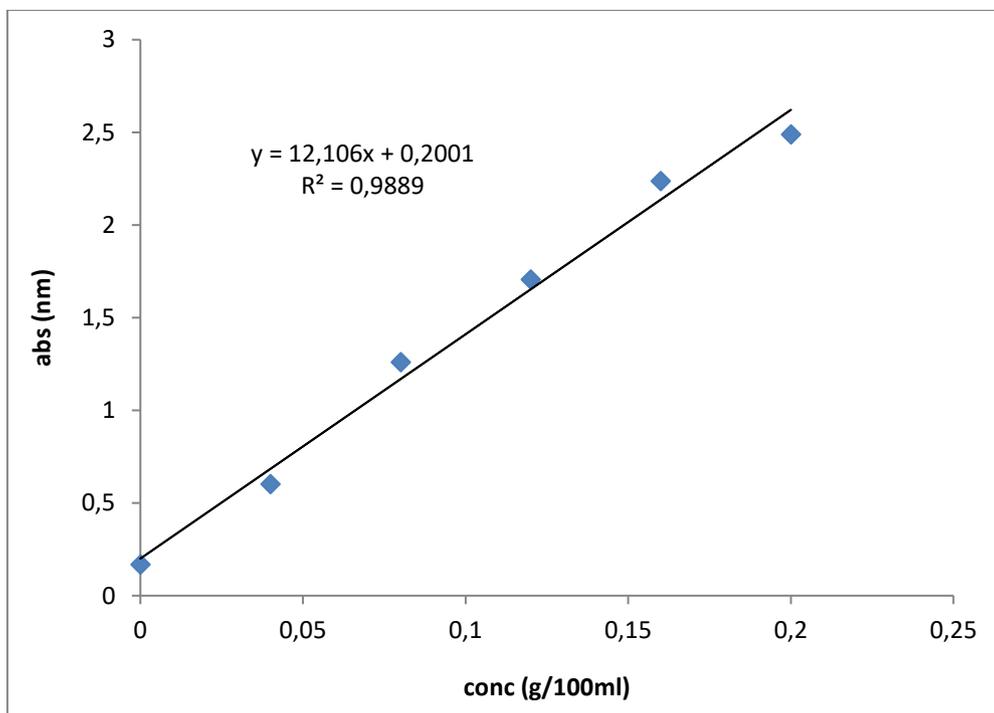


Figure 3. Glucose calibration curve

4. 3. Solid Acid

Generally there are two types hydrolysis process for production of glucose from starch. Namely: mineral acid hydrolysis and enzymatic hydrolysis. The enzymatic hydrolysis is the most popular industrial practice which can be a two step- two enzyme process i.e. liquefaction using amylase followed by scarification using gluco-amylase, or a one step process using a corktail of enzymes. However the enzymes are expensive and can be easily denatured at high temperature.

Mineral acid is cheaper and the reaction can be carried out at high temperature but the use of mineral acid is less popular in the industry because higher tendency of formation of by-products and difficulty of removal of mineral acid catalyst from the product.

In this research, we seek to design acid functionalised polymeric frame work. We attempt to prepare co-polymers of acidic monomers and determine their potential for hydrolysis of potato starch. Figure 4 and 5 shows the equation for the reaction process for the formation of sulphonated solid acid.

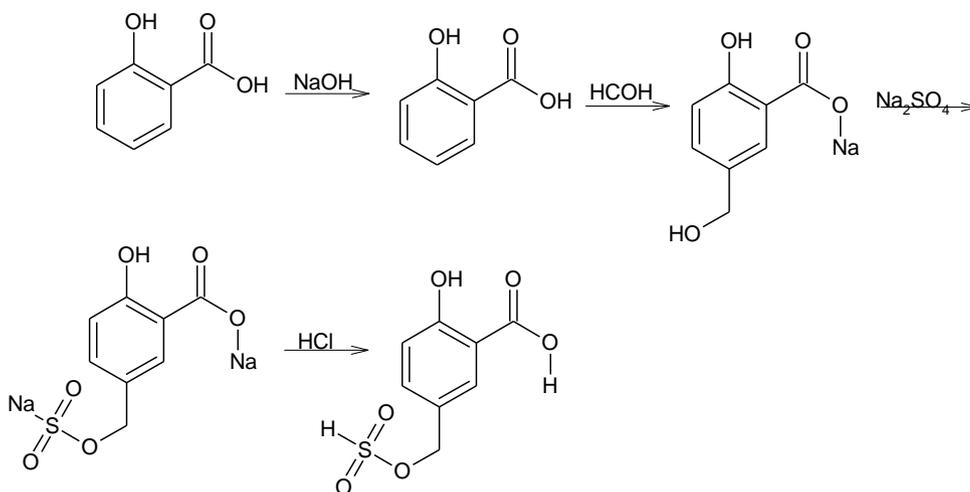


Figure 4. Equation for the formation of solid acid A.

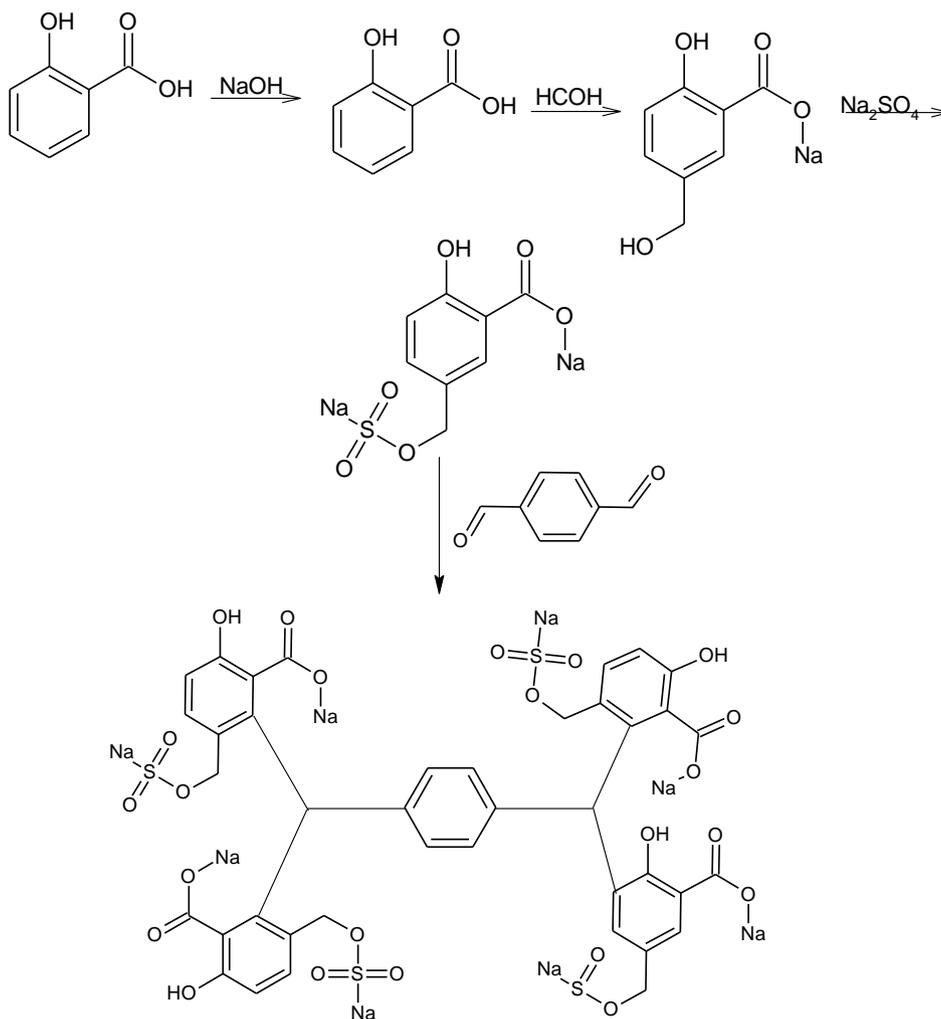


Figure 5. Equation for the formation of solid acid B.

4. 4. Hydrolysis of potato Starch using 0.1M HCl

For comparism we first carry out hydrolysis of the potato starch using HCl. Glucose formation as a function of time from HCl hydrolysis of potato starch is shown in Figure 6 below.

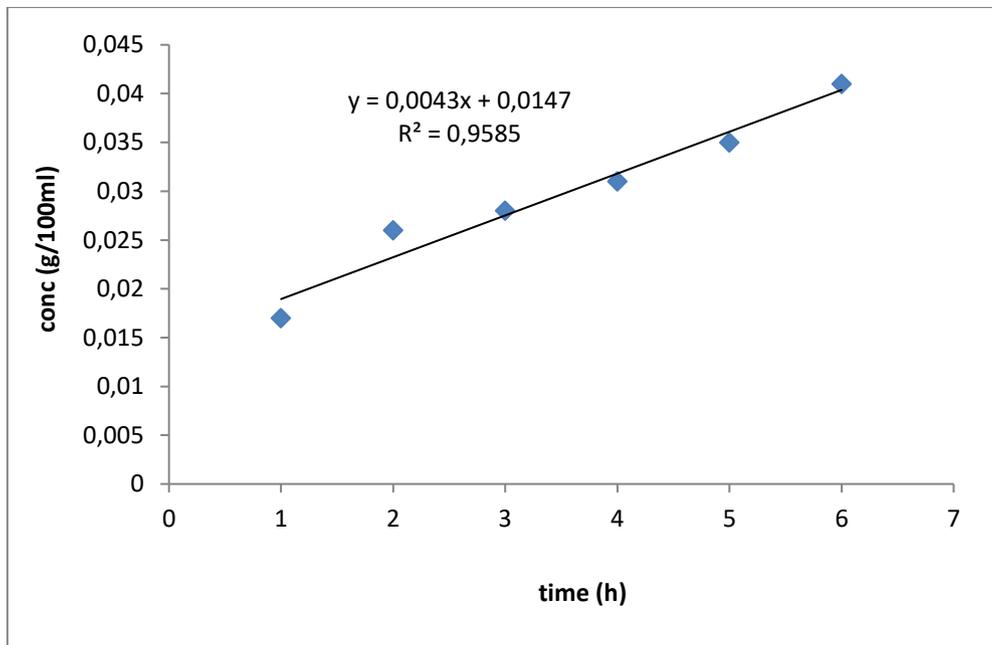


Figure 6: Hydrolysis of potato Starch using 0.1M HCl

4. 5. Hydrolysis of potato Starch using solid acids

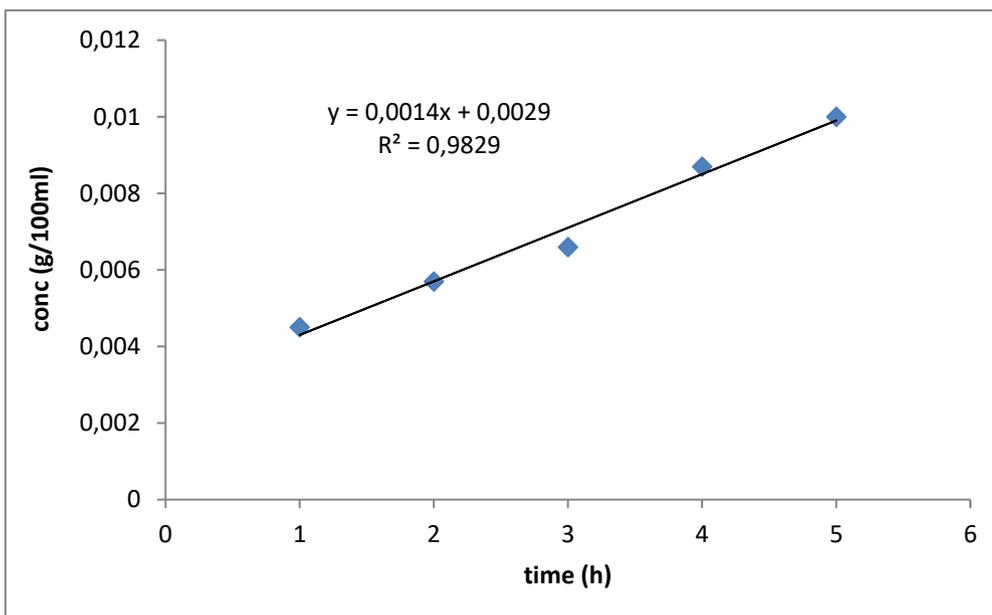


Figure 7. Hydrolysis of potato Starch using solid acid A.

For comparison we also carry out hydrolysis of the potato starch using sulphonated solid acid A and B which reveal that solid acid B give higher glucose formation than A due to presence of more or bulky acidic group. Figure 7 and 8 shows the hydrolysis of potato starch with Sulphonated solid acid A and B respectively.

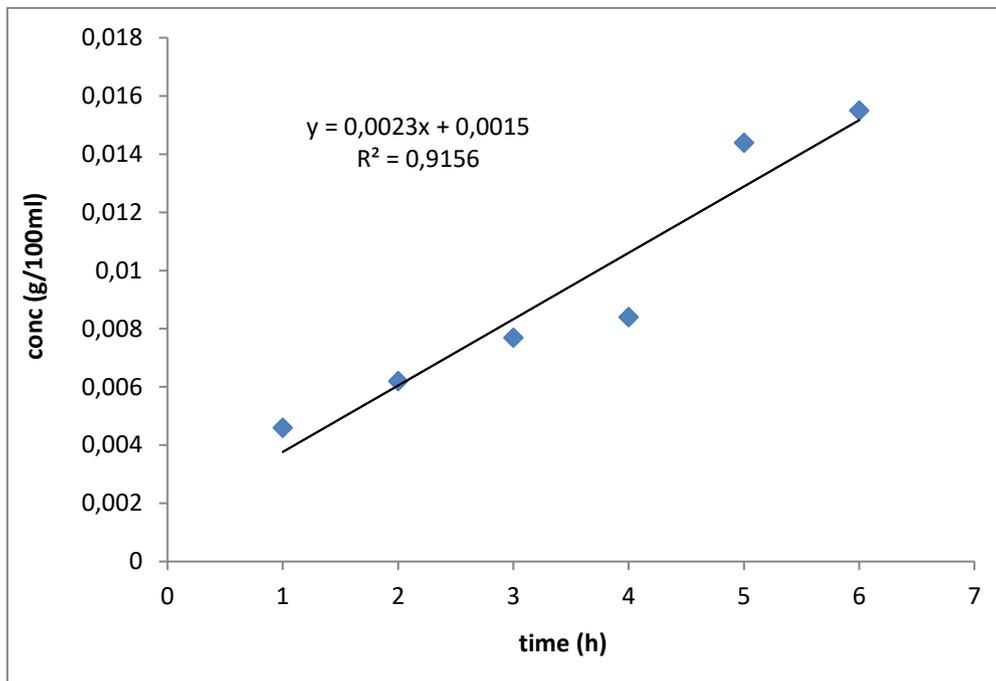


Figure 8. Hydrolysis of potato starch using solid acid B

4. 6. Fourier transform infrared spectroscopy (FTIR) of solid acids

The solid acids produced was further characterised using FTIR. This is done to identify the functional group of the acids produced. Figure 9, 10, and 11 shows the FTIR of salicylic acid, solid acid A and solid acid B respectively. The Table 1 below shows the interpretation of Fourier transform infrared spectroscopy (FTIR) of the solid acid.

Table 1. FTRIR Interpretation

FUNCTIONAL GROUP	PEAK (cm ⁻¹)
O-H stretching	3238.12
C-H SP ³ stretching	2856.49
C=O carboxylic acid stretching	1659.02
C=C aromatic stretching	1613.03
C-O bending	1295.48, 1249.08, 1210.54

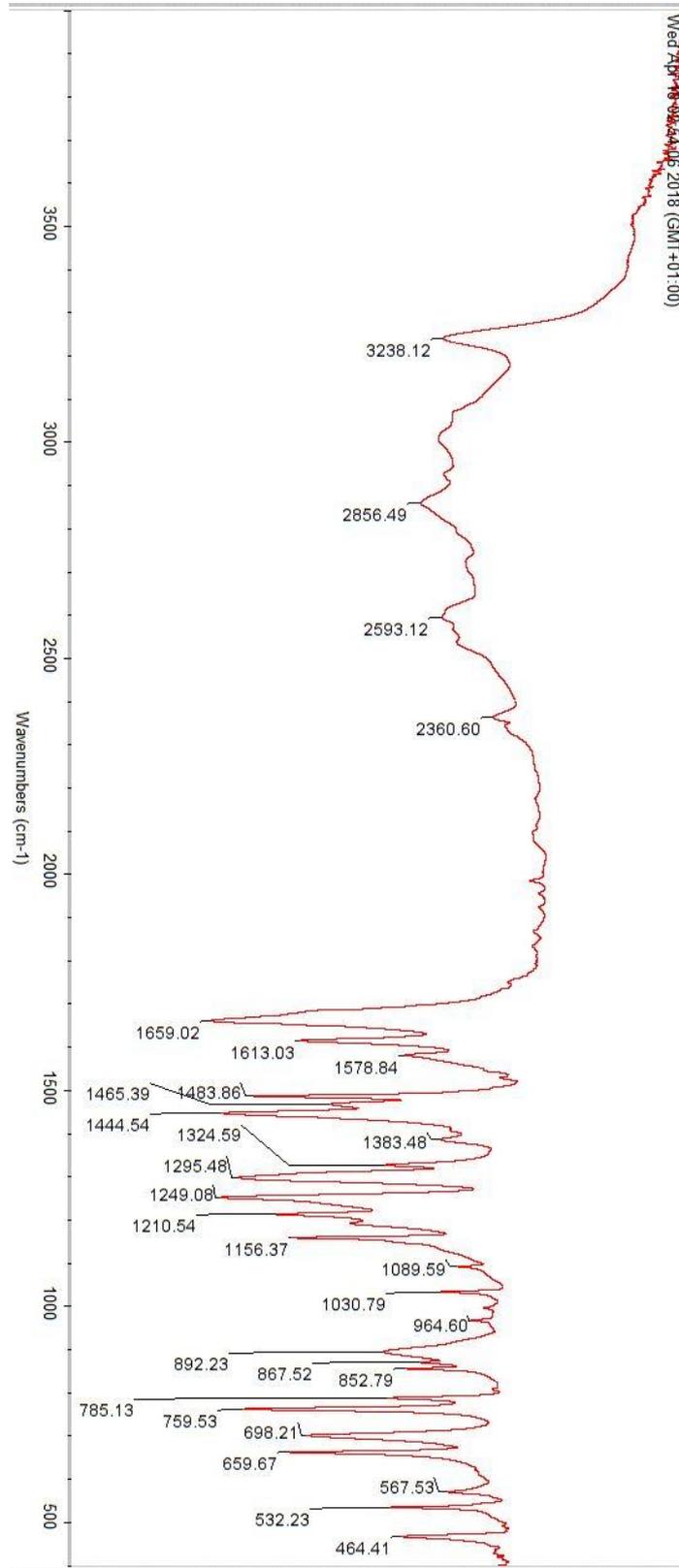


Figure 9. FTIR of salicylic acid.

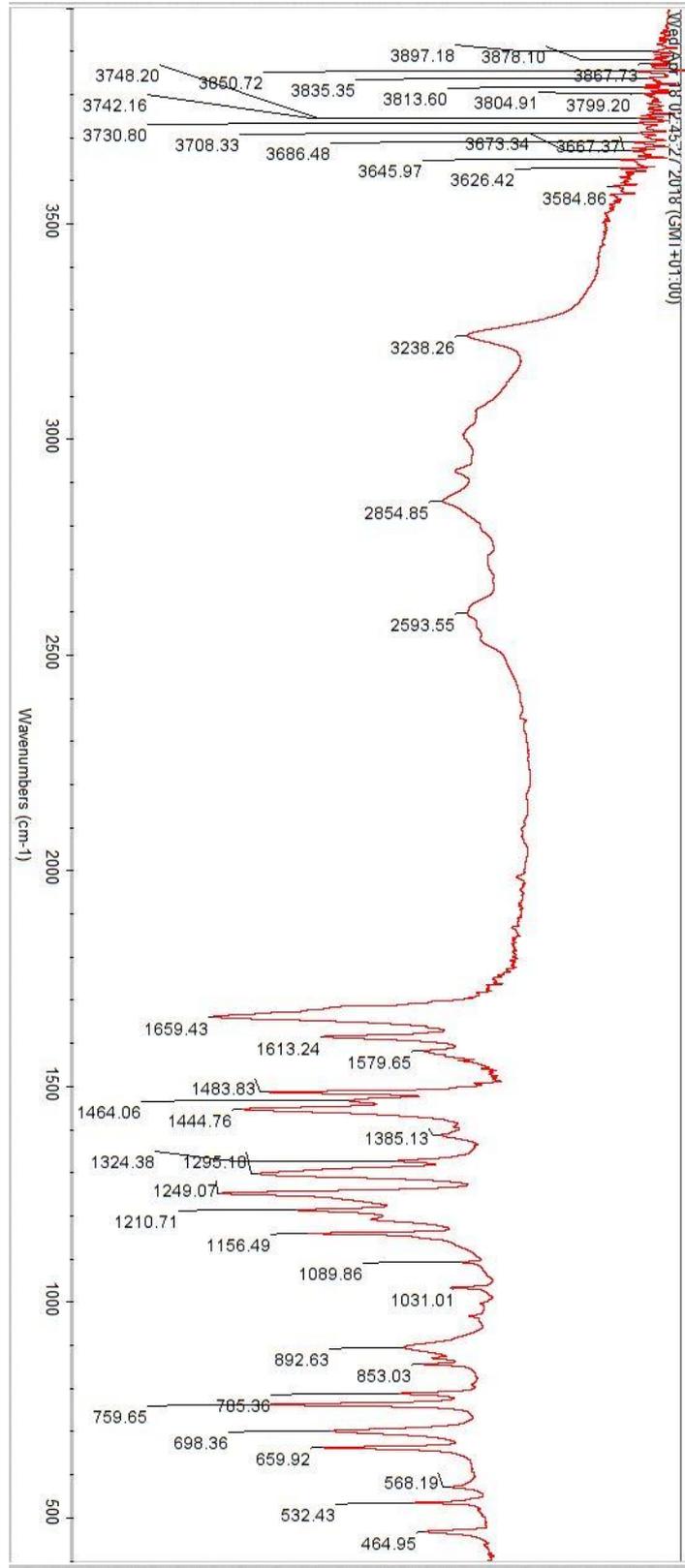


Figure 10. FTIR of solid acid A.

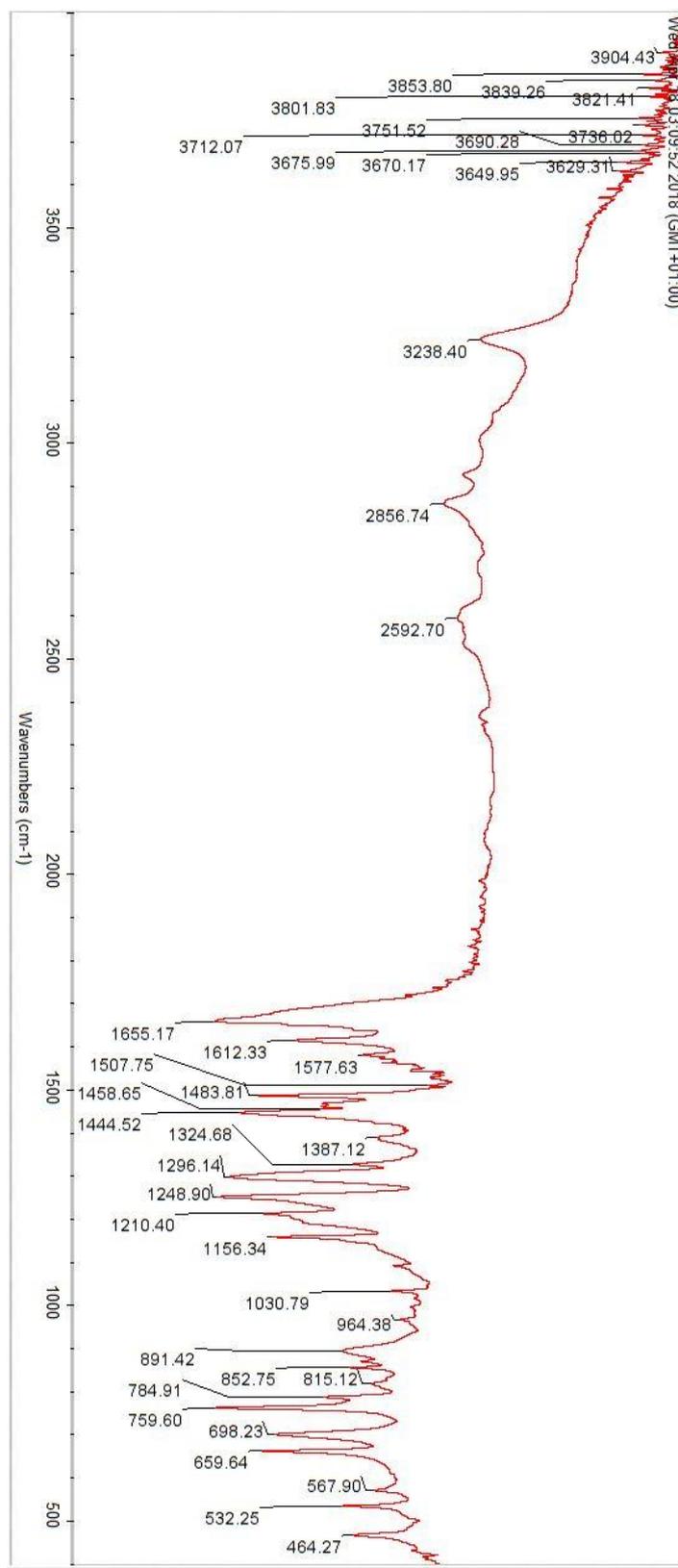


Figure 11. FTIR of solid acid B.

4. 7. Hydrolysis of potato starch using distilled H₂O

Since glucose is formed from both mineral and solid acids, hydrolysis of potato starch was also carried out using distilled H₂O to confirm if glucose can be formed in the absence of acid. The result shows that hydrolysis of starch using distilled H₂O gives no glucose formation. Figure shows the hydrolysis of potato starch with distilled H₂O.

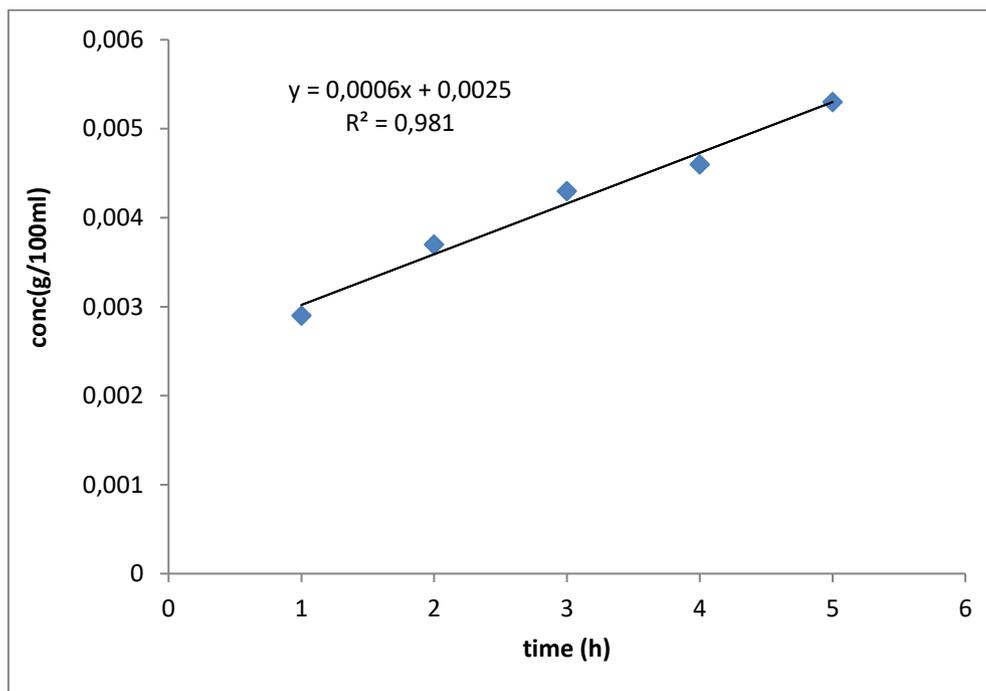


Figure 12. Hydrolysis of potato starch using distilled H₂O.

5. CONCLUSIONS

Although corn starch appears to be the industrial standard raw material for glucose production, carbohydrate rich tubers such as cassava, potato etc, are increasingly becoming important raw material for glucose production. This research explored glucose production from potato tuber. This entails starch extraction followed by acid hydrolysis. For easy separation of the acid catalyst from the glucose product, a polymeric frame work from co-polymerisation of sulphonated salicylic acid and terephthaldehyde via bakelite reaction. Although hydrolysis with mineral acid (hydrochloric acid) gives higher glucose yield than the solid acid, it is a homogeneous catalyst has disadvantages of difficulty of it removal from reaction mixture but the solid can easily be removed by filtration and reuse multiples of times.

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