Addis Ababa University

Addis Ababa Institute of Technology

School of Chemical and Bio Engineering

Production of Glucose Syrup from Sweet Potato Using Acid Hydrolysis and Preservation Using Potassium Sorbate

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A thesis submitted to the school of Chemical and Bio Engineering, Addis Ababa Institute of Technology in partial fulfillment of the requirements for the Degree of Master of Science in Chemical Engineering (Process Engineering)

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Abstract

Sweet potato is one of the largest sources of starch. Due to this fact, it can be utilized to obtain value-added products. This research aims at producing glucose syrup from sweet potato using acid hydrolysis and preservation using potassium sorbate. Starch was isolated by using distilled water. The maximum yield of starch was found to be 31.59%. Work was therefore undertaken to obtain glucose syrup by hydrolyzing starch with a sulfuric acid concentration of (0.5, 1, and 1.5%) at temperatures (130, 140 and 150 °C) and times (25, 30 and 35 min). The total reducing sugar content of the hydrolysates produced was determined using the dinitro salicylic acid method and the maximum reducing sugar was found to be 241 g/L at 1 % sulfuric acid concentration, 140 °C and 30 minutes. The moisture content, dry matter, ash content, density, viscosity and pH of the product were determined and they were found to be 26%, 74% 0.26%, 1.37 g/mL, 5.63 and 4.9 respectively. Design-Expert software version 11 using Box-Behnken design was used for statistical assessment to identify the important parameters for glucose syrup production. The results of the statistical analysis showed that all the three main factors had a significant positive effect on glucose syrup yield. Since glucose syrup is perishable and degradable which has a short shelf life during storage, potassium sorbate was used to prolong its shelf-life. The effect of potassium sorbate with having concentrations of 0, 0.025, 0.05, 0.075, and 0.1% were studied for 60 days to sustain the shelf life of glucose syrup by studying its effects on the physio chemical properties. Potassium sorbate concentration 0.05% which is within permitted level, was identified as a suitable preservative to retain the quality and extend the shelf-life glucose syrup at room temperature.

Keywords: Sweet potato, Starch, Acid hydrolysis, Glucose syrup, Preservation

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Acronyms and Abbreviations

Adeq Precision	Adequacy Precision
ANOVA	Analysis of Variance
C.V	Coefficient of variance
Conc.	Concentration
CIF	Cost, Insurance and Freight
DNS	Dinitro salicylic Acid
ERCA	Ethiopian Revenues and Customs Authority
FAO	United Nation's Food and Agriculture Organization
IUPAC	International Union of Pure and Applied Chemistry
mL	Milliliter
Nm	Nanometer
R^2	correlation coefficient
μL	Microliter

Chapter 1: Introduction

1.1. Background study of the research

Sweet potato (*Ipomoea batatas)* is a dicotyledonous plant that belongs to the bindweed or morning glory family, *Convolvulaceae*. Its large, starchy, sweet-tasting and tuberous roots *(*Wolfe, 1992*).* The plant is an herbaceous perennial vine, bearing alternate heart-shaped or palmately lobed leaves and medium-sized sympetalous flowers. The edible tuberous root is long and tapered, with a smooth skin whose color ranges between yellow, orange, red, brown, purple, and beige. Its flesh ranges from beige through white, red, pink, violet, yellow, orange, and purple. Sweet potato cultivars with white or pale-yellow flesh are less sweet and moist than those with red, pink or orange flesh (Gad & George, 2009).

Sweet potato is one of the world's most important food crop in terms of both area and production (Lebot, 2010). China is the world's largest producer that accounts for approximately 70% of the global area and 80% of the global production. In 2010, approximately 81 million metric tons of the plant was produced by China (FAO, 2010).

Many villages in East Africa depend on the sweet potato for food security. In Ethiopia, the commonly found cultivars are black-skinned, cream-fleshed and called "bitatis" or "mitatis". They are cultivated in the eastern and southern lower highlands and harvested during the rainy season (June/July). In recent years, better yielding orange-fleshed cultivars were released for cultivation by Haramaya University as a less sugary sweet potato with higher vitamin A content. (Tekaligh, 2008). Sweet potatoes are widely eaten boiled as a favored snack.

Starch, the main component of sweet potato tubers, accounts for around 50-80% of their dry weight (Zhu, 2010). Starch is the most abundant reserve carbohydrates among plants and a major source of carbohydrate in the human diet, supplying mainly energy. Sweet potato starch

is widely used as a raw material for the production of low molecular weight products such as glucose, maltose which is widely applied in sugar, brewing as well as in textile industries (Cho, 2010). Thus, the conversion of starch to glucose is achieved through either chemical (acid) or enzymatic hydrolysis (Akinola & Ayanleye, 2004).

Food additives are natural or synthetic substances that can be added to foodstuff in small amounts to perform technological functions, namely color, sweetness or to extend shelf-life. Preservatives, also known as antimicrobial agents, are food additives used to extend the shelf-life of food by shielding them against deterioration caused by microorganisms (Maria & Fernando, 2016). According to European Union (2011), shelf-life is defined as the time, under defined storage conditions, during which food remains safe, retains desired sensory, chemical, physical and biological characteristics as well as complies with any label declaration. In the food industry, the shelf-life of food is the time between the production or packaging of the product and the time when it becomes unacceptable under certain environmental conditions (Ellis, 1994) and when the consumption of said food implies a risk to consumer health. There are forty-five substances used as preservatives, being their applications and purity regulated in the European Union. Although some of these substances are harmless when used in small quantities (e.g., the authorized amounts), the use of others is not without risks to human health. Among large amounts of side effects, skin rashes and itching, breathing difficulty, sneezing or gastrointestinal upsets can be found.

According to Collins & Dincer (1973) and Dow Chemical (1988), sugars at high concentrations are used as preservative agents because they bind with free water and render it unattainable for microorganisms. Determining the chemical, physical, or microbial changes in syrup during storage is important because of their effects on product quality and acceptability (palatability).

1.2. Statement of the problem

As explained in the background, sweet potato is applicable in different industries. One of the applications of sweet potato is to produce glucose syrup. Most of the time glucose syrup is produced from corn, mostly used for food consumption in Ethiopia. For this reason, searching for another cheaper and abundantly available raw material such as sweet potato is critical. Sweet potato is one of the cheapest and abundantly available materials. Thus, shifting the main raw material for glucose syrup production from a highly competitive crop like corn into a less competitive one such as sweet potato without compromising the quality and quantity of the product is crucial for a country like Ethiopia, which is still under food insecurity problem.

Moreover, most of the country`s requirement of glucose syrup is met through import. There is an existing market for glucose syrup in the food and pharmaceutical industries based in Ethiopia. According to Ethiopian revenues and customs authority (2017), in 2012, demand for glucose syrup was estimated at 5,000 tons worth 4.62 million US dollars and it was expected to reach 14,266 tons worth 13.21 million US dollars by the end of 2023. So, considering the continuous increase of population of the country and the demand for glucose syrup for the food processing industry, the manufacture of confectionery, brewing, and winemaking, paper and adhesives industry is projected to increase.

Since, glucose syrup has a short shelf-life during storage, preservative is needed to prolong its shelf life. In the food industry, unlike the most common preservatives, potassium sorbate increases shelf-life and reduces the risk of food-borne illnesses, without adversely affecting the taste, color or flavor and have the lowest allergenic potential of all food preservatives. It is preferred over the acid form because it is more soluble and can be used at a wide range of pH levels (Melissa, 2014). So, in the present study the effect of potassium sorbate was evaluated on the stability of glucose syrup to enhance its storage shelf-life.

1.3. The objective of the research

1.3.1. General objective

The general objective of this study was to evaluate the production and characterization of glucose syrup from a locally available sweet potato using acid hydrolysis method and preservation using potassium sorbate.

1.3.2. Specific objectives

The specific objectives were to:

1. Characterize sweet potato for its moisture, pH, ash, crude fat, crude fiber, and crude protein contents.
2. Isolate starch from tubers of locally available sweet potato and to determine its % yield.
3. Determine spectrophotometrically the highest possible value of glucose yield using dinitro salicylic acid method.
4. To study the main effect and interaction effect of temperature, sulfuric acid concentration and time that is involved in glucose syrup production.
5. Characterize the product for its pH, moisture, totally solid, viscosity and ash contents.
6. Evaluate the effect of preservative (potassium sorbate) to sustain the shelf-life of glucose syrup by studying its physiochemical properties (pH, moisture content and viscosity).

1.4. Significance of the study

The characteristic sweetness and its chemical, temperature and microbiological stability make glucose syrup commercially acceptable.

In general, the significance of this research can be seen from different perspectives.

- ~ The sweet potato is a hardy crop and therefore its use to manufacture glucose syrups will increase its growth in Ethiopia and therefore empower local farmers economically.
- ~ Expand further research on the utilization of sweet potato as an alternative source of starch; possibilities exist to produce bioethanol from this very same starch.
- ~ The high potential of glucose syrup made from sweet potato is expected to motivate the development of domestic industries that use glucose syrup in various food productions.
- ~ In general, the establishment of such a factory will have a foreign exchange saving effect on the country by minimizing a foreign currency by substituting the current imports if implemented. In addition, the production of glucose from starch does not use a significant amount of chemicals except sulfuric acid, which is used in a small amount. Hence, the impact on the environment due to the production of glucose is negligible.

1.5. Scope of the study

The thesis work generally covers the extraction of starch and production of glucose syrup from locally available sweet potato via acid hydrolysis process to give the desired value of glucose syrup. In addition, this work covers the characterization of used raw materials and the produced glucose syrup. Furthermore, factors (i.e. temperature, retention time, and sulfuric acid concentration) that affect the glucose syrup production were investigated. The effect of potassium sorbate for the determination of shelf life was also studied.

Chapter 2: Literature Review

The following point gives some theory for understanding this research and contains relevant background information about sweet potato starch properties and processing to produce glucose syrup.

United Nation's Food and Agriculture Organization (2011) reported that sweet potato is a very important crop in the developing world and a traditional, but less important crop in some parts of the developed world. According to FAO (2011), sweet potato is one of the seven crops in the world produce over 105 hundred million metric tons of edible food products in the world annually. The FAO statistics demonstrate the importance of sweet potato in the area where wheat production is disadvantaged due to climate restraints, wheat suitable for biscuit, noodle and bread-making cannot be grown satisfactory in many of these countries, and utilization of indigenous crops could lead to a reduction in the importation of wheat or wheaten flour. Apart from being a staple crop for some parts of the world, sweet potato can, and does, play a multitude of varied roles in the human diets being either supplemental or a luxury food (Anton, 2008). According to Moorthy (2002) fresh sweet potato contains high water content.

Green plants produce carbohydrates (sugars and starch) by photosynthesis. To do this plant needs carbon dioxide, water, light, and chlorophyll. Carbon dioxide enters the plant through small holes in the leaves; water enters through the roots. The carbon dioxide and water then produce sugars and oxygen with the help of light and chlorophyll in the leaves. Chlorophyll absorbs red light and violet light for photosynthesis and reflects green light insoluble (Raghda, 2013).

$$6CO_2 + 6H_2O \rightleftharpoons C_6H_{12}O_6 + 6O_2 \qquad 2.1$$

The sugar ($C_6H_{12}O_6$) is then changed to starch n($C_6H_{10}O_5$) and stored temporarily in the leaf or in other parts. Glucose units are linked in a linear way with α (1→4) glycosidic bonds. Branching

takes place with α (1→6) bonds occurring every 24 to 30 glucose units, resulting in a soluble molecule that can be quickly degraded as it has many endpoints for enzymes to attach onto. In contrast, amylose contains very few α (1→6) bonds or even none. This causes it to be hydrolyzed more slowly but has higher density and be insoluble (Storz & Steffens, 2004).

2.1. Starch isolation

Starch is the most abundant carbohydrate reserve in plants and is found in leaves, flowers, fruits, seeds, different types of stems and roots. Plants use starch as a source of carbon and energy. Pure starch was recovered in a 64 % yield on a dry weight basis from the sundried sweet potato flour (Smith, 2001).

Ravindra (2017) studied the isolation of starch from sweet potato by using different methods, Sodium metabisulfite, Sodium chloride, and distilled water. These were assessed for yield, functional, chemical, pasting and structural properties and showed that the starch isolated by using distilled water yielded the greatest recovery of starch, which was found 28.50% while the total starch content was found to be 41.85±1.46%. The yield of starch seems to be comparatively lower than that of the starch content of the sweet potato. Higher starch content does not necessarily mean a higher percent of extractable starch (Rahman *et al.*, 2003).

Starches were isolated according to the method of Collado and Corke (1999). The roots were washed thoroughly, immersed in ice-cold water for 1 hr., peeled, sliced, macerated and washed extensively with ice-cold water. The isolated starches were then dried in an oven at 40℃ overnight. Madzlan et al. (2012) Investigated the extraction of starch with different ratios of sweet potato and water, and 1:4 was found to be optimum with 61% extraction.

2.2. Starch composition

The natural starch is made of about 20% amylose and 80% amylopectin. Amylose is the linear polymer of glucose with α-1.4 bonds and its molecular weight is around 10^6 Dalton

whereas amylopectin has 5 % α-1.6 links in addition to mentioned bonds and its weight is about 10^8 Dalton. In this way, amylopectin has many small branches, but amylose is composed of fewer long branches. Accordingly, the structure of the starch components is shown in Figure 2-1/2. The construct, molecular weight and proportion of amylose to amylopectin are different in extracted starch from different plant sources. This leads to diverse characteristics in various types of starch (Syahariza et al., 2010). The waxy starches consist of plenty amount of amylopectin but the Hylon consists of more than 50% of amylose (Maarel & Leemhuis, 2013). At the moment, near 30% of worldwide enzyme production has allocated to starch processing enzymes.

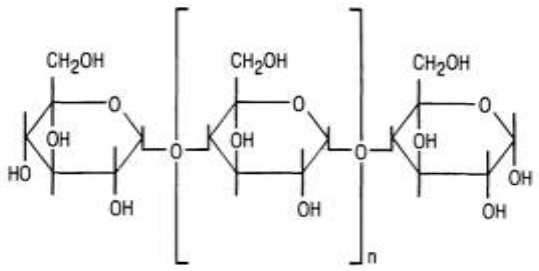

Figure 2-1: Amylose (Syaharizaet et al., 2010)

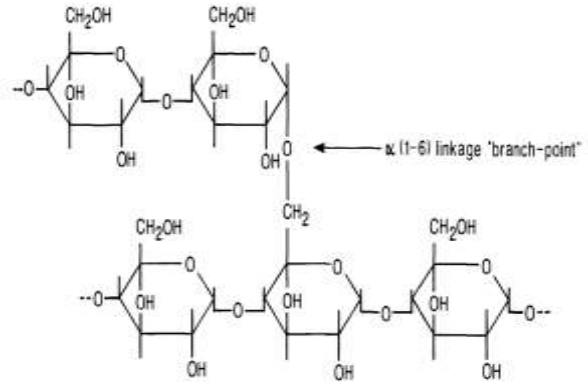

Figure 2-2: Amylopectin (Syaharizaet et al., 2010)

2.3. Glucose syrup production

Production methodology for glucose syrup derived from corn starch has been available for more than 100 years since the mid-nineteenth century. Glucose syrup processing has since moved toward highly automated continuous processes through new enzyme systems producing a variety of syrups for the food and beverage industries (Melanson et al., 2007).

The basic steps of glucose syrup production are (1) conversion, (2) purification, and (3) concentration. The starch hydrolysis defines the type of syrup produced.

Conversion is the processing step during which starch is hydrolyzed with acid–enzyme aid to result in lower molecular weight products (glucose syrups). The systems used in the conversion process are acid, acid–enzyme, and enzyme-enzyme. In the processing steps for the first two conversion systems, starch slurry is acidified and hydrolyzed with acid or acid–enzyme aid and heated in converters. Then, the liquors are cooled, neutralized, and purified to remove impurities (suspended proteins and lipids) before their concentration to a determined solid level. The purification (discoloration) step is performed using carbon treatment, and the degree of conversion is controlled by acid, temperature, and reaction time (Schenck, 2006).

2.3.1. Enzyme hydrolysis

The starch degradation process catalyzed by an enzyme is known as enzymatic hydrolysis. Liquefaction and saccharification are the main steps of this process (Sun et al., 2006).

Starch is degraded by enzymes called amylase which is derived from. α-amylase is an endo-acting enzyme that randomly hydrolyzes α-(1, 4)-glycoside bonds inside the starch structure and quickly destroys the whole starch structure (Nigam and Singh, 1995). The degradation products would be oligosaccharide fragments such as glucose, maltotetrose, maltose, maltoriose as well as oligosaccharide containing α-(1, 6)-branches. All the components are known as dextrin mixture. However, the percentage of glucose is very low and needs further enzyme treatment. The oligosaccharides formed from amylase activity are further hydrolyzed by exo-acting enzyme, glucoamylase which can cleave both α-(1, 4) and α-(1, 6)-branches from the non-reducing ends of the starch polymers and forms exclusively glucose (Linko & Wu, 1993).

2.3.2. Acid hydrolysis

Mostly, acid hydrolysis of starch was done by using sulfuric acid and hydrochloric acid. Hydrolysis of starch has commonly been carried out using hydrochloric acid at temperatures of 130-170℃ with subsequent partial neutralization. Large acid-resistant converters in batch-wise are still used for hydrolysis of starch, but there has been a strong tendency over the last two

decades to the using of continuous converters. In this process, starch slurry is acidified with hydrochloric acid and pumped through a series of steam-heated pipes where the conversion of starch into sugars occurs. (Whistler et al., 1984).

According to Wang and Copeland (2013), acid hydrolysis in combination with moist heat under pressure (Autoclave) treatment was done. so, starch stock (1 g/L) was prepared, treated with acids 1% volume per volume and autoclaved at 15 psi for 15 min. The resultant glucose concentration was determined by the 3,5-dinitrosalicylic acid method. Acid concentration 1% volume per volume was considered to avoid an extensive and un-necessary decrease in pH. So, the result of sulfuric acid hydrolysis in combination with moist heat under pressure (Autoclave) treatment was more efficient in releasing free glucose from starch and easy to remove. In this method, 1% volume per volume ratio of sulfuric acid was more efficient than 1% volume per volume ratio of hydrochloric acid. Glucose is slowly destroyed when heated in neutral solutions and the rate is accelerated by sulfuric acid but to a much greater extent by hydrochloric acid. Sulfuric acid is therefore been chosen as a suitable starch hydrolyzing agent (Pirt & Whelan, 1951).

Dilute-acid hydrolysis of lignocellulosic and starchy materials may result in sugars and other by-products in some serial and parallel reactions (Karimi et al., 2006; Gupta et al., 2009).

$$\text{Glucan(cellulose or starch)} \rightarrow \text{Oligosaccharides} \rightarrow \text{Glucose} \rightarrow 5-\text{hydroxymethylfurfural} \rightarrow \text{Levulinic acid} \quad (2.2)$$

But the acid hydrolysis of hemicellulose may lead to monomeric sugars and furans (Palmqvist & Hahn-Hagerdal, 2000; Lee et al., 1999; Zeitsch, 2000).

$$\text{Hemicellulose} \rightarrow \text{Oligosaccharides} \rightarrow \text{Sugars (xylose; arabinose; glucose; mannose; galactose)} \rightarrow \text{Furfural and } 5-\text{hydroxymethylfurfural} \rightarrow \text{Carboxylic acid} \quad (2.3)$$

2.4. The economic study of glucose syrup production

2.4.1. An economic study of acid and enzymatic hydrolysis for glucose syrup production

According to Adenise et.al. (2002) analyzing the process yields of the recovered reducing sugars from the starch present in cassava, both processes were quite efficient and similar with 94.5% for the acid hydrolysis against 97.3 % for the enzymatic hydrolysis. Looking at the time required for each process, acid hydrolysis was more advantageous than the enzymatic process. For a batch, the acid hydrolysis was completed in only 10 min plus the time to heat and cool the material. The enzymatic hydrolysis, in contrast, took 25 h and 20 min, plus the time to heat and cool the material for the whole process in a batch. Considering the chemicals necessary for a batch, acid hydrolysis was much less expensive than the enzymatic one (around 1:75 price ratio).

This study showed that both methods were almost equally efficient concerning the yield based on the reducing sugar recovered from the cassava, but economically, the acid hydrolysis was more advantageous. The only limitation with acid hydrolysis could be the issue of toxicity because the acid process increases the medium salinity, and it could be a limiting factor (Adenise et.al., 2002).

2.4.2. An evaluation of sweet potato and corn as potential sources for glucose syrup production

The recent emphasis on corn production to meet the increasing demand for glucose syrup has resulted in trepidation regarding the sustainability of the global food supply. The recent emphasis on corn production to meet the increasing demand for glucose syrup has resulted in trepidation regarding the sustainability of the global food supply. Thus, starches extracted from different sources showed different susceptibility towards acid hydrolysis. According to Johnson and Padmaja (2013) the percentage conversion to reducing sugars was computed for sweet potato and found to be 18.14g/100mL slurry (percent conversion of 95.70%). This was higher

than the conversion value obtained with corn starch 17.52g /100mL slurry (percent conversion of 94.09%). Moreover, most of the tuber starches are almost free of lipid complexation unlike in the case of cereal starches. The lipid content in starch in different cultivars of sweet potato varied from 0.11 to 0.22%, while normal corn starch contains approximately 0.87% of lipids.

In addition, according to Ziska et al. (2009) sweet potatoes are associated with low-input agricultural systems, whereas corn production per se is known to require large fertilizer input, and can be pesticide intensive (e.g. corn herbicides account for about 40% of the total pounds of herbicides, insecticides, and fungicides.

Overall, the combination of high glucose syrup yield production, combined with lower input costs makes sweet potato have greater potential as glucose syrup sources than existing corn systems.

2.5. Applications of glucose syrup

The glucose syrup industry has evolved rapidly in recent years to meet the ever-increasing demands of the food and related industries. Different types of glucose syrups, glucose/fructose syrups and a multiplicity of glucose syrup, or perhaps more correctly starch, derivatives such as polyols and cyclodextrins, have been introduced to the market. This evolution continues unabated with new products appearing continuously (Kearsley, 1995).

Glucose syrups are nutritive sweeteners made from different starch-rich materials like corn, sweet potato, sorghum, etc. Glucose syrup is mainly a solution of monosaccharides mostly glucose, maltose and other nutritive saccharides obtained from edible starch. They add structure and mouthfeel to food applications while providing sweetness and energy. Glucose syrups are used where sugar crystallization needs to be prevented, or good freeze-thaw stability is required. Glucose syrups are suitable for ice-cream, fruit preparations, brewing, baking, ketchup, sauces and hard-boiled candy, jellies, marshmallows, nougat, fondant, chewing gum,

and other confectioneries. Many manufacturers produce canned food, sweets, snacks & chips, candy and confectionery in Ethiopia. Generally, syrups are used as natural sweeteners either in food or pharmaceutical industries where syrups are utilized as sweetening agents, aimed at increasing palatability of substances by imparting the sweet flavor of sugars inherent in them, natural sweeteners are potent, safe, and low in calories (Evans, 2013).

2.6. Analysis of reducing sugar by the dinitro salicylic acid method

Most of the methods for determination of carbohydrase activity are based on the analysis of reducing sugars formed as a result of the enzymatic or acidic scission of the glycosidic bond between two carbohydrates or between a carbohydrate and a noncarbohydrate moiety. Different methods for assaying the reducing sugars have been applied in the carbohydrase activity measurements. The 3,5- dinitro salicylic acid (DNS) assay described by Miller (1959).is the most popular method used by many researchers.

3,5-dinitrosalicylic acid (DNS, IUPAC name 2-hydroxy-3,5-dinitrobenzoic acid) is an aromatic compound that reacts with reducing sugars and other reducing molecules to form 3-amino-5-nitrosalicylic acid, which absorbs light strongly at 540 nm, the wavelength of maximum absorbance. This method tests for the presence of free carbonyl group (C=O), the so-called reducing sugars. If the oxygen on the anomeric carbon(C_1) of sugar is not attached to any other structure, that sugar can act as a reducing agent and is termed a reducing sugar. The sugar act as a chemical reducer due to the free aldehyde group or ketone group presence in its molecule. In an alkaline medium, the reducing sugars are able to reduce the 3-5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid, wherever the aldehyde group is oxidized to aldonic acid (equation 2.4) and (Figure 2-7). 3-amino-5-nitrosalicylic acid is an orange color product, and the intensity of the color depends on the concentration of reducing sugar (Asheesh et al., 2016).

$$3,5\ \text{Dinitro salicylic acid} + \text{Reducing Sugar(glucose)} \longrightarrow 3-\text{Amino}-5-\text{nitrosalicylic acid} + \text{Oxidized Sugar(Gluconic acid)} \quad (2.4)$$

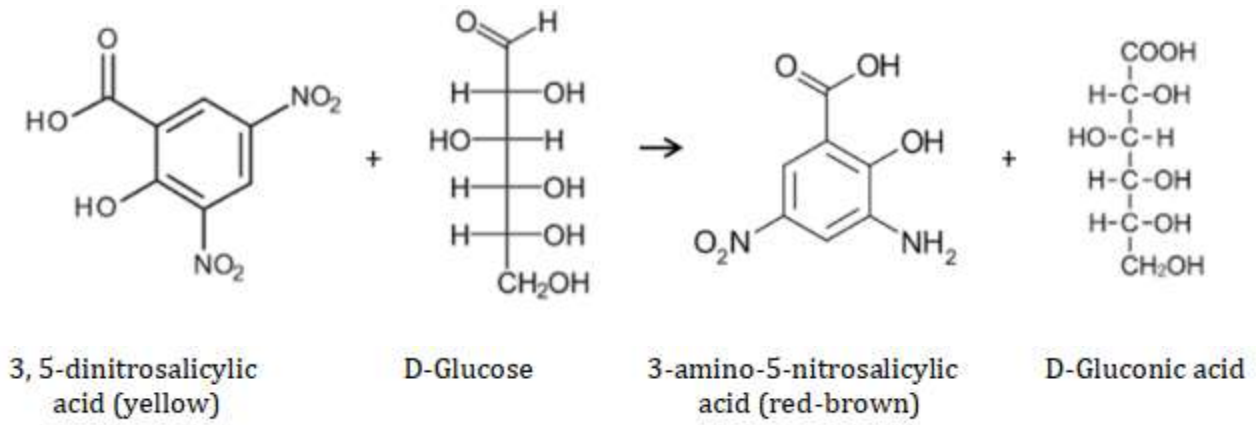

Figure 2-3: Conversion of reducing sugars by dinitro salicylic acid (Marisa et.al., 2107)

2.7. Shelf-life determination

Glucose syrup can be preserved in different ways; chemical preservation is one of these methods. Chemical preservatives are those substances, which are added to the food products to increase their shelf life. These preservatives are always food graded and these are used in the amount which is not harmful to human health. There are a number of chemical preservatives used in food for the extension of their shelf life. Some important chemical preservatives are; sodium benzoate, potassium sorbate, sodium sorbate, sorbic acid, sulfur dioxide, sodium propionate, etc. The choice of chemical preservative depends upon several factors. These include properties, safety, and cost of the compound, as well as the properties of the food and possible effect of the chemical on its quality. In addition, type and level of microorganism present, post-processing and storage conditions. The food laws must also be taken into consideration while selecting a chemical preservative (Lueck, 1980).

Sorbic acid and its salts are some of the most widely used food preservatives in the world. As food preservatives, sorbates have found wide application in various foods, especially as yeast and mold inhibitors. In high sugar products, smaller quantities of sorbic acid are adequate for preservation, because of the synergistic action of sorbet with sugar. Nevertheless, as sorbic acid is metabolized, as some fatty acids, reduces the probability of having other detrimental effects.

The use of sorbates, derivatives of boric acid, does not have more side effects that the use of sorbic acid and can be used in similar dosages. Organic acids, such as acetic acid, benzoic acid, propanoic acid, and sorbic acid are also preservatives extensively used in low pH foods. (Maria & Fernando, 2016).

Potassium sorbate is used in a wide variety of products due to its role as a preservative and its ease of manufacturing. It is a white crystalline powder that has antimicrobial properties. It is one of the safest and most commonly used preservatives today. In foods, potassium sorbate increases shelf life and reduces the risk of food-borne illnesses, without adversely affecting the taste, color or flavor. At room temperature, it looks like a white crystalline powder, but the mixture will quickly dissolve in water, which will revert it back to sorbic acid as the potassium dissolves. Many consider potassium sorbate ideal for applications in foods because it is highly soluble and can be used at a wide range of pH levels, so it can be applied to a number of products without worry that it will break down. As a food additive, potassium sorbate is used as a preservative in concentrations of 0.025 to 0.1% (Melissa, 2014).

Chapter 3: Methods and Materials

3.1. Materials and equipment

The fresh sweet potato was bought from Atklt Tera, Addis Ababa, Ethiopia. The tubers were placed at 25 °*C* in a polyethylene bag to prevent loss of moisture during storage prior to processing and analysis in the laboratory of food engineering, School of Chemical and Bio Engineering, Addis Ababa Institute of Technology, Addis Ababa University, Ethiopia.

The reagents used during this study include hexane, anhydrous D–glucose, 3,5-dinitrosalicyclic acid (DNS) were bought from Mollarie trading P.L.C., Cherkos sub city Addis Ababa, Ethiopia. Chemicals (active carbon, potassium sodium tartrate (Rochelle salt) and potassium sorbate($C_6H_7KO_2$) were bought from Neway trading P.L.C., Arada sub city Addis Ababa, Ethiopia. While chemicals (sulfuric acid solution, sodium hydroxide and distilled water) were obtained in chemistry lab, AAiT

Production and characterization of the glucose syrup involve various equipment. The major equipment used in the processing was an analytical balance (EP214C), plastic bag, rotary vacuum evaporator (Fsatom, model 801), Soxhlet extractor, UV-Visible double beam spectrophotometer (UVD-3200), filter paper, Sine-wave Vibro Viscometer (SV-10), pycnometer, autoclave (LAMCS204), pH meter, oven (DAS 42000), and other glass apparatus.

The schematic diagram for the laboratory scale extraction, enzymatic hydrolysis, and characterization of glucose syrup is given in the Figure 3-1.

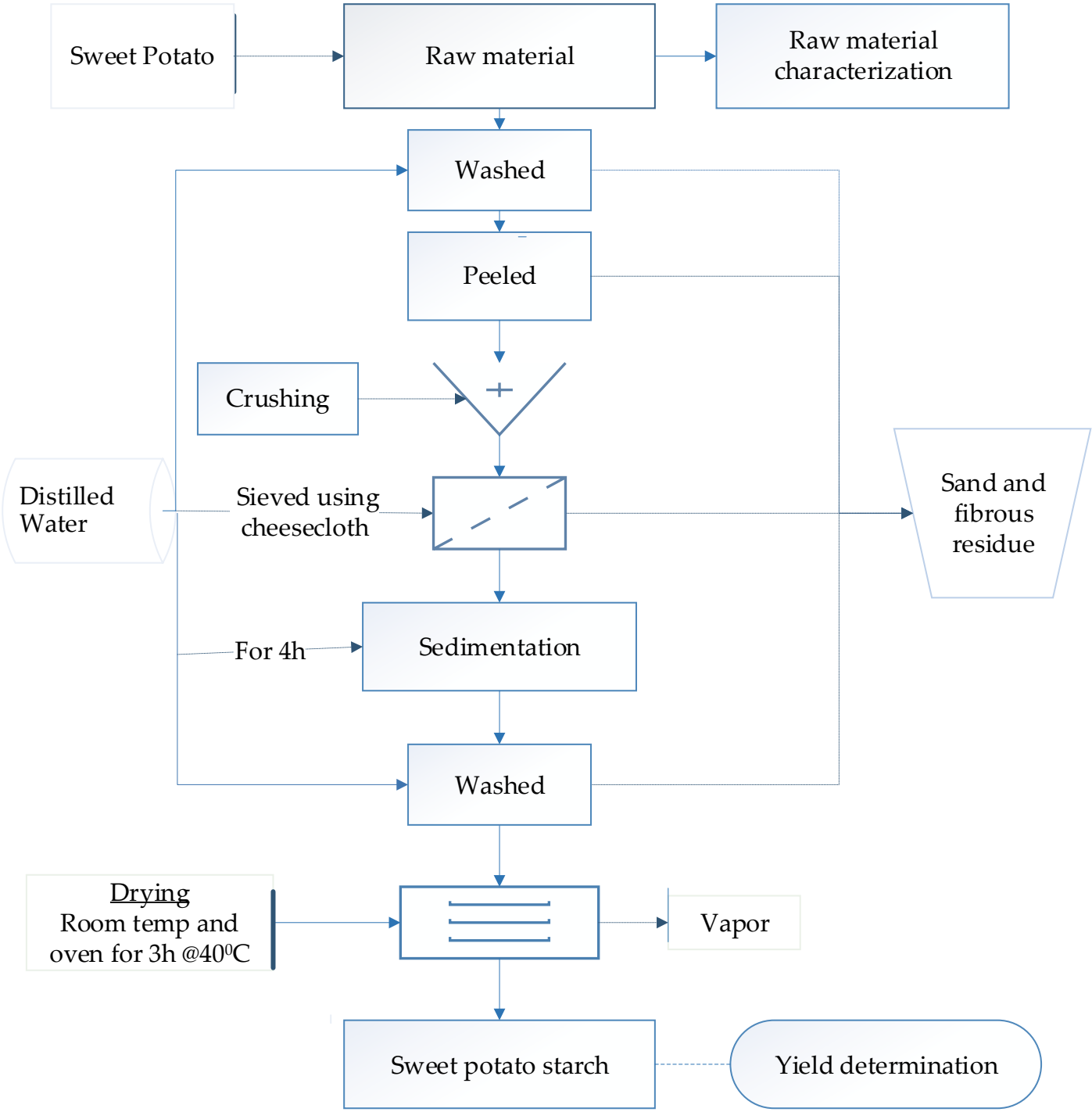

Figure 3-1: Flow chart for starch extraction and raw material characterization

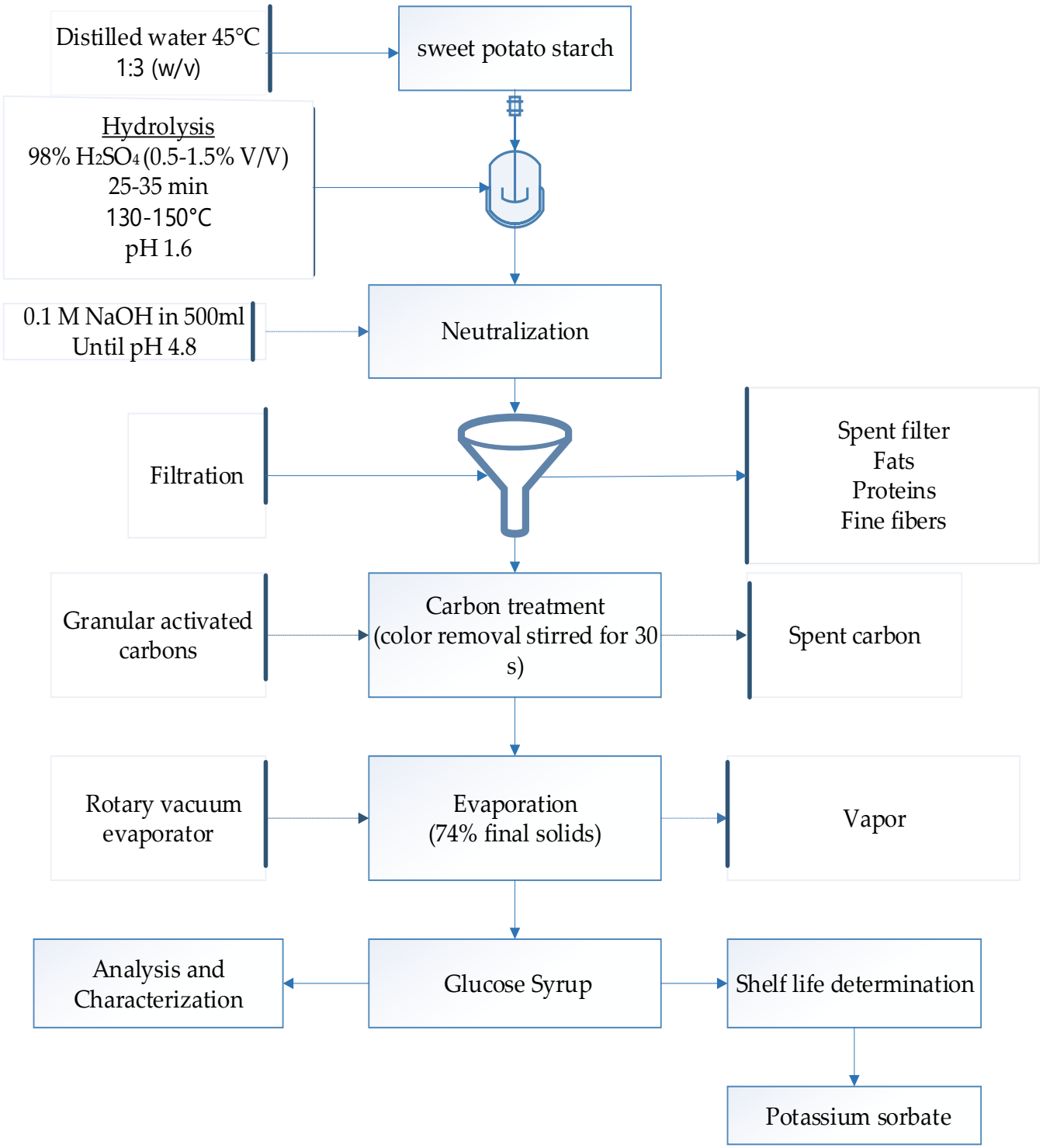

Figure 3-2: Flow chart for Production and characterization of glucose syrup

3.2. Methods

3.2.1. Raw material characterization

3.2.1.1. Moisture content determination

The moisture content was determined by taking 5 g of the sample in the tarred crucible and it was dried in a hot air oven at 100 ± 5 ℃ to a constant weight. The moisture content was calculated by the formula (AOAC, 2005):

$$\text{Moisture (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{total weight of the sample}} \times 100 \quad (3.1)$$

3.2.1.2. Ash content determination

The residue or remaining part when a carbonaceous portion of carbon is burned off is called ash content which is composed of silica, aluminum, iron, magnesium and calcium (Ahmedna et al., 2000). The ash was determined as a total inorganic matter by incineration of the samples according to the method No. 08-01 of A.A.C.C. (2000). First, an oven-dried 5 g sample was taken in a pre-weighed crucible and charred on the burner. Then it was ignited in the muffle furnace at 550 ℃ for 5 h or until the constant weight of greyish ash was obtained. The ash of the sample was calculated through the formula:

$$(\%)\text{Ash} = \frac{\text{Weight of crucible with ash} - \text{Weight of empty crucible}}{\text{Total weight of the sample}} \times 100 \quad (3.2)$$

3.2.1.3. Crude fat content determination

The crude fat was determined by Soxhlet extraction as described in the method No. 30-10 (A.A.C.C., 2000). The dried sample remained after moisture determination was taken in a thimble and placed in the extraction tube of the Soxhlet apparatus. About 250 mL of hexane(C_6H_{14}) was added in 500 mL conical flask as solvent and connected to Soxhlet apparatus. The fat was extracted by boiling flask positioned on the water bath set at 68 ℃ for 4 hrs. The

solvent was recovered, and the flask was kept in a hot air oven for 10 min at 105 ℃. The flask was cooled in a desiccator and weighed. The fat percentage was as:

$$(\%)\text{Crude fat} = \frac{\text{The final weight of flask} - \text{Empty weight of the flask}}{\text{weight of the sample}} \times 100 \quad (3.3)$$

3.2.1.4. Crude fiber content determination

Crude fiber is a nutritionally obsolete term that refers to the residue (primarily cellulose, lignin and other components of this type) remaining after food is treated with acid and alkali (Anderson, 1982). The crude fiber was determined by following the method No. 32-10 as described in A.A.C.C. (2000). Moisture and the fat-free sample were taken and placed in 1000 mL beaker. A 200 mL solution of 1.25% sulfuric acid was added in the beaker. The sample was then digested by boiling for 30 min. Then it was filtered using suction apparatus. The residue was washed with hot water until it becomes acid-free. The residue was again transferred to 1000 mL beaker and boiled with 200 mL solution of 1.2 % sulfuric acid for 30 min, filtered and the residue was transferred to the pre-weighed crucible and dried in an oven at 100 ℃ till constant weight was obtained. The dried residue was charred on a burner and ignited into muffle furnace at 550 ℃ for 5 h, cooled in a desiccator and weighed. The loss in weight during incineration represents the weight of crude fiber in the sample. The crude fiber percentage was calculated by the formula:

$$(\%)\text{Crude fiber} = \frac{\text{Weight of residue} - \text{Weight of ash}}{\text{weight of sample}} \times 100 \quad (3.4)$$

3.2.1.5. Crude protein content determination

Crude protein is a measure of how much protein is in food, based on laboratory tests studying the food's chemical composition. When you look for protein content on a food's nutrition label, the number you see refers to crude protein. Crude protein is calculated after measuring the nitrogen content of food. Because each amino acid contains nitrogen, looking at the total nitrogen content of food gives some insight into its protein content. However, because not all of

the nitrogen in food is found in protein, using crude protein as a measurement might inflate the amount of protein in a food. However, higher crude protein values are often perceived as better. In fact, crude protein does not even represent protein content directly, but rather nitrogen content. Then, a standard equation of the nitrogen percentage in the food times 6.25 is used to calculate crude protein, however, this is only an average value, and each protein has a different conversion factor depending on its amino-acid composition. (Angell et al., 2016).

The protein content was determined by the Kjeldahl method according to the method of AOAC, (1995). The Kjeldahl method described expedited by use of the kjetec digestion and Kjeldahl distillation apparatus. 10 g of the glucose syrup to be analyzed was weighed into a digestion tube. Two Kjeldahl catalyst tablets containing potassium sulfate (K_2SO_4) was added with copper selenite ($CuSeO_3 \cdot 2H_2O$). After that 30 mL of concentrated sulfuric acid was added. Then the tube was placed in the digestion rack and transferred to the block digester. The sample was heated to 480℃ slowly until foaming had ceased for 2 hours. After the digestion has been completed the digestion flask is connected to a receiving flask by a tube. The solution in the digestion flask is then made alkaline by addition of sodium hydroxide, which converts the ammonium sulfate into ammonia gas. Afterward, the steam distiller was prepared to determine the instrument blank. The steam distilled the digest into the boric acid receiving solution for about 5 min. The sampled was titrated with standardized 0.1N H_2SO_4 after distillation was completed. Finally, the amount of standardized acid needed to neutralize the boric acid was recorded. The black determination was made for all reagents and their volume was calculated.

$$\text{Nitrogen}(\%) = \left(\frac{(\text{Vol. of } H2SO4 - \text{Vol. of Blank})\text{mL x N of } H2SO4 \text{ x}1.4007}{\text{Sample weight}(g)} \right) \quad 3.5$$

$$\text{Crude Protein}(\%) = \%\ \text{Nitrogen X } 6.25 \quad 3.6$$

3.2.1.6. Total carbohydrate content determination

Total carbohydrates include all types of carbohydrates found in the food or beverage. Total carbohydrates consist of multiple nutrients, including dietary fiber, sugars, and starches. Carbohydrate is one of three macronutrients in food that provide calories, or energy, for the body (Whistler & Bemiller, 1999). Carbohydrates were calculated by the difference method as follows:

$$\text{Total carbohydrate } \% = 100 - \%(\text{Moisture} + \text{Fat} + \text{Protein} + \text{Ash} + \text{crude fiber}) \quad (3.6)$$

3.2.2. Extraction of sweet potato starch

During present investigation, sweet potato starch was extracted by using distilled water. About 3.39 kg of fresh sweet potato tubers were cleaned, peeled and cut into small pieces and milled to a fine powder. The milled mass was weighed accurately. Then the milled mass was mixed with enough water. The homogenized slurry of sweet potatoes was collected into a beaker and enough water was added in the ratio 4:1. The slurry was subsequently screened sequentially through sieve adding excess water. Filtrate could settle for 3 h at room temperature. Starch rapidly settles at the bottom. Starch free supernatant was decanted carefully. The starch was resuspended in distilled water and filtered and then left to settle in a tray for 2 h. This last step was repeated for three times using deionized water instead of tap water for the last washings. A compact mass of starch was collected and dried in hot air oven at 40 ℃ for 48 h. The final weight of isolated starch was recorded, and the starch yield was determined as:

$$\text{Starch Yield } (\%) = \frac{\text{Dry Weight of Starch Recovered from Extraction}}{\text{Milled mass of Sweet Potatoes}} \times 100 \quad (3.7)$$

$$\text{Starch Recovery } (\%) = \frac{\text{Yield of starch}}{\text{Starch content}} \times 100 \quad (3.8)$$

3.2.3. Starch hydrolysis and purification of the hydrolysate

The method of Wang and Copeland (2013) was adopted to produce glucose syrup. The dried starch was suspended in distilled water at 45 ℃ in the ratio 1:3 (starch to water) to gelatinize. Then, the solution was mixed with 98% H_2SO_4 in 0.5 up to 1.5 % (v/v) in the suspension at pH 1.6. Finally, the solution was heated by high-pressure autoclave at 130-150 ℃ for about 25-35 min. The starch molecule was converted mainly into a mixture of glucose, fructose, maltose, together with aldehydes such as 5-hydroxymethylfurfural, furfural (Judoamidjojo et al., 1989).

Thereafter, 500 mL of 0.1 M NaOH was used to neutralize the solution, until the pH value reached 4.9. Then, the diluted glucose syrup was filtered using vacuum filtration. The sample was transferred to a 250 mL beaker and 0.5 g of granular activated carbons was added and stirred for 30 seconds. Then after, the sample was filtered through Whatman paper by vacuum filtration. Finally, since the glucose syrup density is low, rotary vacuum evaporator was used to reduce the moisture or to raise the solid concentration at a temperature of 70 ℃ for 30 min. The hydrolysis runs were done by design expert software version 11 (response surface – Box-Behnken).

Table 3-1: Randomization of hydrolysis parameters

Run	Temperature(°C)	H_2SO_4 (%)	Time (min)	Glucose conc. (g/L)
1	130	0.5	30	
2	130	1	25	
3	130	1	35	
4	130	1.5	30	
5	140	0.5	35	
6	140	0.5	25	
7	140	1	30	
8	140	1	30	
9	140	1	30	
10	140	1	30	
11	140	1	30	
12	140	1.5	25	
13	140	1.5	35	
14	150	0.5	30	
15	150	1	25	
16	150	1	35	
17	150	1.5	30	

3.2.4. The reducing sugar determination by the dinitro salicylic acid method

A solution of 3,5-dinitrosalicylic acid reagent was prepared as follows: 10 g of 3,5-dinitro salicylic acid, 10 g of NaOH in 200 mL distilled water. On the other hand, Rochelle Salt was prepared by adding 200g potassium sodium tartrates in 400mL distilled water. Finally, Dinitro salicylic reagent was prepared by mixing both solutions to a final volume of 1 L with distilled water (Miller, 1959).

A standard stock solution of glucose was prepared by dissolving 100 mg of glucose in 100 mL of distilled water. The standard solutions were prepared as indicated in Table 3-2.

Table 3-2: Preparation of standard solutions

	Test tube No.					
Addition	1	2	3	4	5	6
Stand. Glucose. (mL)	0.0	0.2	0.4	0.6	0.8	1
volume of solution (mL)	1	1	1	1	1	1
H_2O (mL)	1	0.8	0.6	0.4	0.2	0.0
DNS reagent (mL)	2.0	2.0	2.0	2.0	2.0	2.0
H_2O (mL)	7.0	7.0	7.0	7.0	7.0	7.0

After replacing the above-mentioned solutions in the labeled tubes, it was shaken well, and then placed in a boiling water bath for 5 min. The tubes were cooled, and 7 mL of distilled water to each tube was added as indicated in Table 3-2. After that, some amount of the mixture from each test tube was taken to clear cuvettes and the absorbance was read at 540 nm using UV visible spectroscopy. Then, the standard curve was produced by plotting the absorbance versus glucose

concentration data. Finally, using the already prepared standard curve, the 17 unknown glucose concentrations samples were determined using the given equation (3.9).

Table 3-3: Shows the standard glucose concentration.

Tube No.	1	2	3	4	5
Glucose conc. (g/L)	0.2	0.4	0.6	0.8	1
Absorbance (A^o)					

$$\text{Glucose conc. } \left(\frac{\text{g}}{\text{L}}\right) = \left[\frac{\text{Absorbance} - \text{Y intercept}}{\text{Slope}}\right] \quad (3.9)$$

3.2.5. Experimental design

A 3-factor 3-level Box-Behnken design was established for the design of the experiment. The independent and dependent variables used in the design are listed in Table 3-4. This study design of 17 experimental runs was generated and analyzed by Design-Expert software. Using the response surface methodology, the optimum combination of the operational factors was determined.

Table 3-4: Experimental design

Study Type	**Response Surface**				Runs			**17**
Initial Design	Box-Behnken				**Blocks**			No
Design Model	Quadratic				**Levels**			3
Factor	Name	Units	Type	Low Actual	High Actual	Low Coded	High Coded	Mean
A	Temp.	℃	Numeric	130	150	-1.000	1.000	140
B	H_2SO_4 Conc.	%	Numeric	0.5	1.5	-1.000	1.000	1.0
C	Time	min.	Numeric	25	35	-1.000	1.000	30

The shelf life was also determined using preservative potassium sorbate. Each of the glucose syrup sample which contains varying amount of potassium sorbate syrup by studying its effects on the physiochemical properties (pH, moisture content and viscosity) was evaluated. To determine the effect of potassium sorbate on the glucose syrup samples, four different runs of experiments were performed. Samples void of potassium sorbate were taken as control. The experiment was carried out for 60 days at room temperature. The laboratory experiments of shelf life were run based on Microsoft Excel for the two treatment factors and five levels for each.

Table 3-5: Shelf life determination

Factor	Name	Units	Type	Levels				
A	Potassium sorbate	%	Numeric	0.0	0.025	0.05	0.075	0.1
B	Time	day	Numeric	1st	15th	30th	45th	60th

3.2.6. Characterization and analysis of glucose syrup

3.2.6.1. Total solids and moisture content determination

The moisture content of the food product affects preservation and shelf life. The dry matter of glucose syrup consists of all components on the glucose syrup except the water and volatile compounds. The components consist of glucose, maltose, and other oligosaccharides, dextrose, mineral, and non-sugar organic compounds. The moisture content was determined by taking 5 g of the sample in a tarred crucible, dried in hot air oven at 105 ℃ for four hr, removed, and cooled to room temperature in a desiccator. The samples were then placed back into an oven and dried until constant weight (± 0.1% change in the weight % solids upon one h of re-heating the sample (AOAC, 2005). The moisture content was calculated by the formula (3.10) and total solid formula (3.11).

$$\text{Moisture } (\%) = \frac{\text{Initial weight} - \text{Final weight}}{\text{Total weight of the sample}} \times 100 \quad (3.10)$$

$$\text{Total solid } (\%) = (100 - \%moisture) \quad (3.11)$$

3.2.6.2. Ash content determination

Ash content determines the quality of glucose syrup, and the lower the ash content, the better the quality of glucose syrup. Ash present in glucose syrups originates from both the starch source and also from any acid treatment of the starch, or pH adjustments, during hydrolysis and refining. The ash content of the syrup was determined according to method No. 08-01 of A.A.C.C. (2000) as follows: First 5 g of sample was placed in a clean pre-weighed crucible, and then the crucible with its content was ignited in a muffle furnace at about 550 ℃ for over 5 h until light grey ash was obtained. The crucible was removed from the furnace, cooled in a desiccator and weighed. The procedure was repeated until a constant weight was obtained and ash content was calculated as:

$$(\%)\text{Ash} = \frac{\text{Weight of crucible with ash} - \text{Weight of empty crucible}}{\text{Total weight of the sample}} \times 100 \quad (3.12)$$

3.2.6.3. pH determination

Based on the method by Meade and Chen (1977), the standard buffer solutions and samples were cooled to 25 ℃ while the electrode and receptacle were rinsed using a portion of the solution to be tested. The beaker was filled to a depth that would be covered by the bulb of the glass electrode. The temperature of the solution was recorded, the system was allowed to come to equilibrium and the pH was recorded.

3.2.6.4. Viscosity determination

The apparent viscosity of glucose syrup from sweet potato starch was carried out using a similar method as reported by Sopade and Kassum (1993) and Nsofor and Osuji (1997). A Sine-wave Vibro Viscometer SV-10 model was used for the apparent viscosity measurement. The measurement was done at room temperature. Sine-wave Vibro Viscometer measures viscosity by detecting the driving electric current necessary to resonate with the two sensor plates at constant frequency and amplitude. So, the driving electric current, which is exciting force, was detected as the magnitude of viscidity produced between the sensor plates and the sample fluid.

3.2.6.5. Density determination

Density was determined at 25 ℃ by weighing the sample in a 25 mL pycnometer. The pycnometer was filled with syrup and weighed. The mass of the glucose syrup was subtracted from the total weight of the pycnometer and glucose syrup. So, the apparent density of glucose syrup was determined from the given formula:

$$\text{Density} = \frac{\text{Mass of glucose syrup}}{\text{Volume of glucose syrup}} \tag{3.13}$$

Chapter 4: Results and Discussion

4.1. Sweet potato characterization

The samples were analyzed for proximate composition (moisture, crude protein, fat, ash, crude fiber, and carbohydrate). The chemical composition is a simple and convenient way of illustrating the purity of the starch extracts whereby lower contents of other components (protein, crude fiber, fat, and ash) were highly desirable and which could be noticed in the present study. The proximate composition moisture, protein, fat, ash, crude fiber and total carbohydrate of sweet potatoes were calculated using the formula given in equations 3.1, 3.2. 3.3 3.4, 3.5 and 3.6 and the results are shown Table 4-1 and each value is an average of three runs (Appendix A-1).

Table 4-1: Physio-chemical composition of sweet potato

Components	Average result (%)
Moisture	53.66
Protein	2.13
Fat	0.36
Ash	2.18
Crude fiber	0.31
Total carbohydrate	41.36

The results of the proximate analysis showed that the sweet potato contains moisture 53.66% on a wet weight basis. This value was found to be lower than that of values observed by Agbemafle *et al.* (2014) who indicated that fresh sweet potato had a moisture content of 59.3 %

on a wet weight basis. The variation in the moisture content of sweet potato might be due to varietal effects, stage of maturity, gaps between harvesting time and analysis, etc.

The results of fat content were higher than the literature values. Tumuhimbise et al. (2013) revealed that fat content in fresh sweet potato was 0.17 g/100 g while the recent study was 0.36%. The reason for deviation may be due to varieties used.

The protein, ash and the crude fiber content of sweet potato were found to be 2.13, 2.18 and 0.31% respectively. The results were in close agreement except for crude fiber with the results of Ahmed *et al.* (2010). According to Senanayake *et al*. (2013), the chemical composition of sweet potato varies according to genotype. As shown in Table 4-1, the carbohydrate content was 41.36%. The findings were in close agreement with Nabubuya *et al*. (2012) and Van (2000).

4.2. The isolated starch

In the present study, starch was isolated by using distilled water. Hence, starch sediment was sieved, dried in a hot air oven at 40 ℃ for 48 h and weighed from which the percent yield was calculated. Finally, the maximum extracted starch was calculated at 31.59% on a wet weight basis. The findings were relatively lower compared to that of sweet potato tuber studied by Kebede (2017) who obtained 75.1% maximum yield starch on a dry weight basis. This was due to the difference in the moisture content of milled mass. But the increased yield of starch in the present study as compared to another researcher Ravindra (2017) might be due to repeated extraction with distilled water who found 28.5% maximum starch yield.

4.3. The total reducing sugar content of the hydrolysate

The standard solutions and corresponding absorbances were recorded at a wavelength of 540 nm using a UV visible Spectrophotometer as shown in Table 4-2. The standard curve prepared by fitting the concentration vs absorbance linearly is also shown in Figure 4-1.

Table 4-2: Shows the absorbencies of the standard glucose measured at 540 nm

Tube No.	1	2	3	4	5
Glucose conc. (g/L)	0.2	0.4	0.6	0.8	1
Absorbance at 540 nm	0.0122	0.017	0.02	0.023	0.029

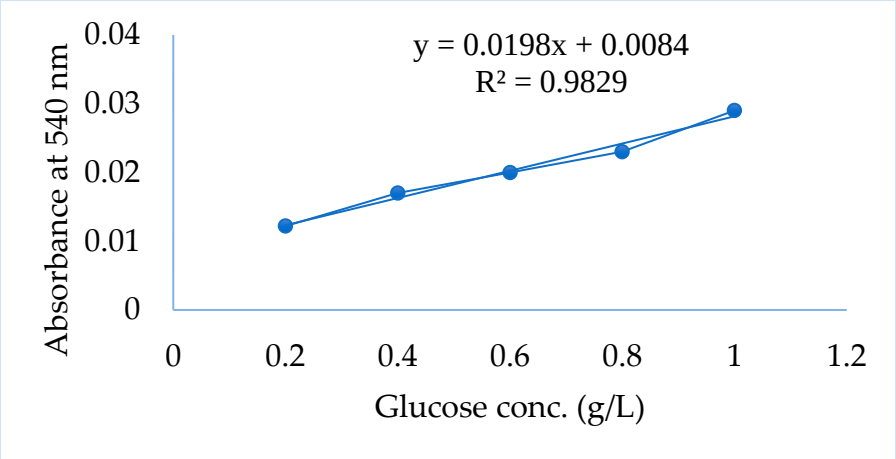

Figure 4-1: The graph of absorbance against the concentration of standard glucose

The concentrations of the samples were calculated from the equation of the standard curve.

$$X = [(Y - 0.0084)/0.0198]$$

Where x and y represent concentration and absorbance respectively.

The variation of glucose concentration yield with time, temperature, and acid concentration using 33.33% slurries are presented in Appendix A-3. The maximum reducing sugar was found to be 241 g/L at 1%, 140 ℃ and 30 min for sulfuric acid concentration, temperature and time respectively. It was observed that the glucose concentration increased with sulfuric acid concentration of 0.5 to 1%, temperature of 130 to 140 °C and time 25 to 35 min and dropped as temperature, sulfuric acid concentration and time increased.

This is due to the fact that with longer reaction times the sugars produced by hydrolysis of the starch were easily decomposed. When the heating conditions became very severe (high

temperature, high sulfuric acid concentration and long heating times), aldehyde yields increased and, correspondingly, the glucose yields decreased (Makiko & Toshitaka, 2004).

Previous researches on sweet potato starch showed a glucose yield of 81.09% (Priyanka & Majumder, 2016) while the present study obtained 69.4%. The difference could be attributed to the difference in the efficiency and sophistication of the equipment employed and the varieties of the raw material used.

4.4. Statistical analysis of experimental results

The statistical validation was entrenched by assessment of statistical parameters such as model *F*-value, lack of fit *F*-value, correlation coefficient, adjusted R-squared, predicted R-squared, predicted residual error sum of squares and adequate precision generated by ANOVA provision available in the Design-Expert software to check sufficiency and adequacy of models. Table 4-3 represents the design summary.

Table 4-3: Design summery

File Version	11.1.0.1		
Study Type	Response Surface	**Subtype**	Randomized
Design Type	Box-Behnken	**Runs**	17
Design Model	Quadratic	**Blocks**	No Blocks
Build Time (ms)	1.0000		

4.4.1. Analysis of variance (ANOVA)

Analysis of variance (ANOVA) was used to analyze the data (Table 4-4). The goodness of fit of the model was checked by the coefficient of determination (R-Squared), which was found to be 0.9968. The present R-Squared-value reflected a very good fit between the observed and predicted responses and implied that the model is reliable for glucose syrup production in the present study.

Table 4-4: Statistical analysis of variance:

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	8262.86	8	1032.86	315.38	< 0.0001	significant
A-Temp	924.50	1	924.50	282.29	< 0.0001	
B-H_2SO_4	378.12	1	378.12	115.46	< 0.0001	
C-Time	3741.13	1	3741.13	1142.33	< 0.0001	
AB	100.00	1	100.00	30.53	0.0006	
AC	100.00	1	100.00	30.53	0.0006	
A^2	502.55	1	502.55	153.45	< 0.0001	
B^2	969.60	1	969.60	296.06	< 0.0001	
C^2	1242.02	1	1242.02	379.24	< 0.0001	
Residual	26.20	8	3.28			
Lack of Fit	15.00	4	3.75	1.34	0.3920	not significant
Pure Error	11.20	4	2.80			
Cor Total	8289.06	16				

The Model F-value of 315.38 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case, A, B, C, AB, AC, A^2, B^2, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. But B × C is not significant. The P-values denote the significance of the coefficients and are also important in understanding the pattern of the mutual interactions between the variables. The above coefficients of variables show that variables named temperature, time and

sulfuric acid concentration positively affect glucose syrup production. The interactions between sulfuric acid concentration and time is not significant, which is an indication that there is no significant correlation between each of the two variables, and they did not help much in increasing the production of glucose syrup.

The Lack of Fit F-value of 1.34 implies the Lack of Fit is not significant relative to the pure error. There is a 39.20% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit indicated a good fit of the model.

4.4.2. Model adequacy measures

The R-Squared values provide a measure of how much variability in the observed or actual response values can be explained by the experimental factors. In this study, the value of the R-Squared was 0.9968 which indicates that 99.68% of the variability in the response was attributed to the given independent variables and only 0.32% of the total variations are not explained by the independent variables. The Predicted R-Squared of 0.9811 is in reasonable agreement with the Adjusted R-Squared of 0.9937; i.e. the difference is less than 0.2. The present R-Squared value reflected a very good fit between the observed and predicted responses and implied that the model is reliable for glucose production in the present study.

Table 4-5: Model adequacy measures

Std. Dev.	1.81	**R^2**	0.9968
Mean	218.24	**Adjusted R^2**	0.9937
C.V. %	0.8292	**Predicted R^2**	0.9811
		Adeq Precision	49.7248

Where R^2: correlation coefficient

Std. Dev: standard deviation

Adequate precision measures signal-to-noise ratio and detects which experimental parameters generate signals that are large in comparison to the noise. A ratio greater than 4 is desirable. In this case, the value obtained is 49.72 and is thus an indicative of an ample signal.

4.4.3. The regression model equation

The ANOVA result showed that glucose concentration was affected by temperature, time and sulfuric acid in the experiment. Therefore, the estimated model can be used for the response of glucose yield. The quadratic equation describing the relationship between predicted response (glucose concentration) in terms of coded factors of temperature, time and sulfuric acid concentration was derived as:

$$\text{Glucose conconcentraion} = +10.75 * A + 6.88 * B + 21.63 * C - 5.00 * AB - 5.00 * AC - 10.92 * A^2 - 15.17 * B^2 - 17.18 * C^2 + +238.60 \quad (4.1)$$

Where A: Temperature (℃)

B: Sulfuric acid concentration (%),

C: Time (min)

4.4.4. The diagnostics plots

Diagnostic plots such as normal probability plot, externally studentized residuals versus predicted plot, externally studentized residuals versus run plot and predicted versus actual plot were developed by Design-Expert software to investigate the goodness of fit of the proposed model.

The normal probability plot of externally studentized residuals is shown in Figure 4-2, which indicated that the maximum number of color points depicting the value of glucose concentration was located on normal probability line which proved normality of residuals and suggested that

response data provided relevant analysis. The normal probability plot indicated whether the residuals followed a normal probability distribution.

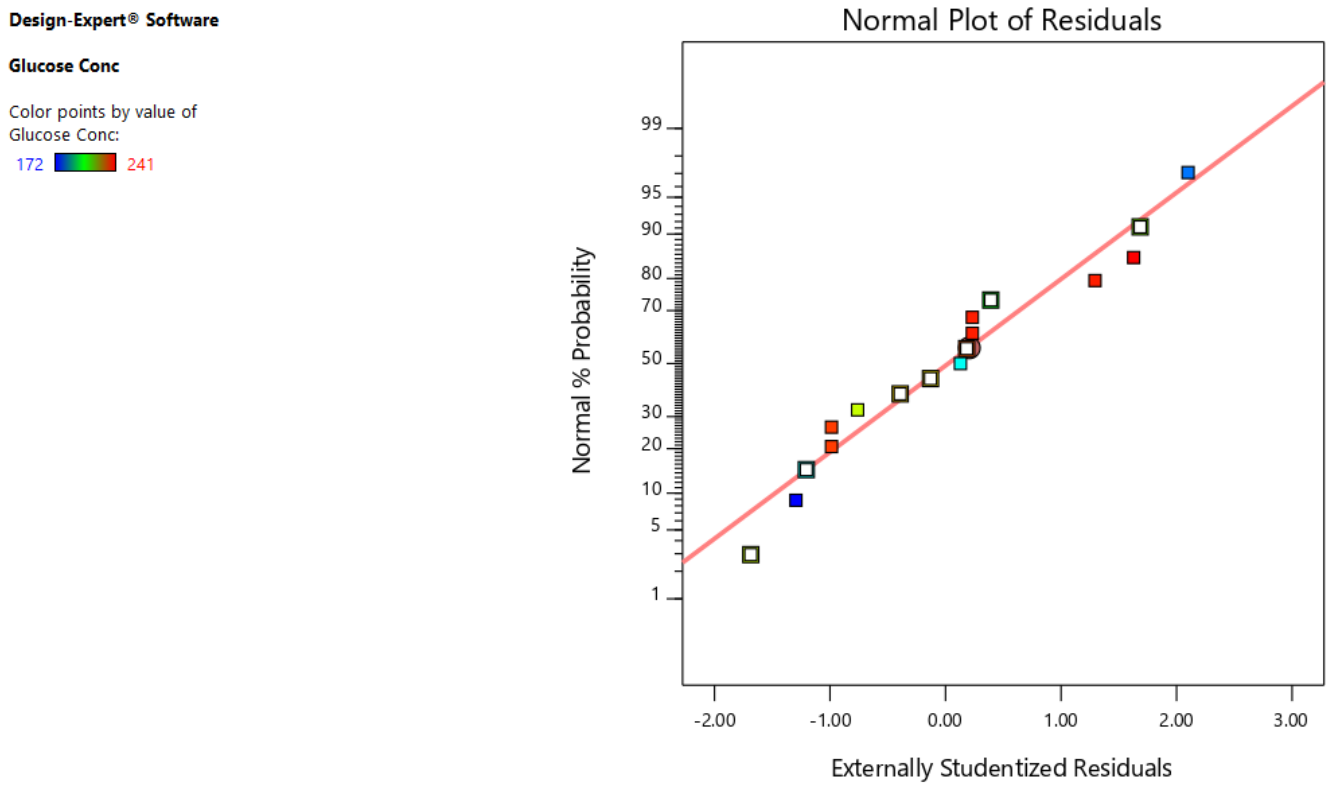

Figure 4-2: Normal plots of residuals

The graph of the predicted value versus the residuals is shown in Figure 4-3, which revealed that all color points representing mean particle size had been scattered randomly and uniformly close to zero-axis and had a constant range of residual across the graph which illustrated the absence of constant variance.

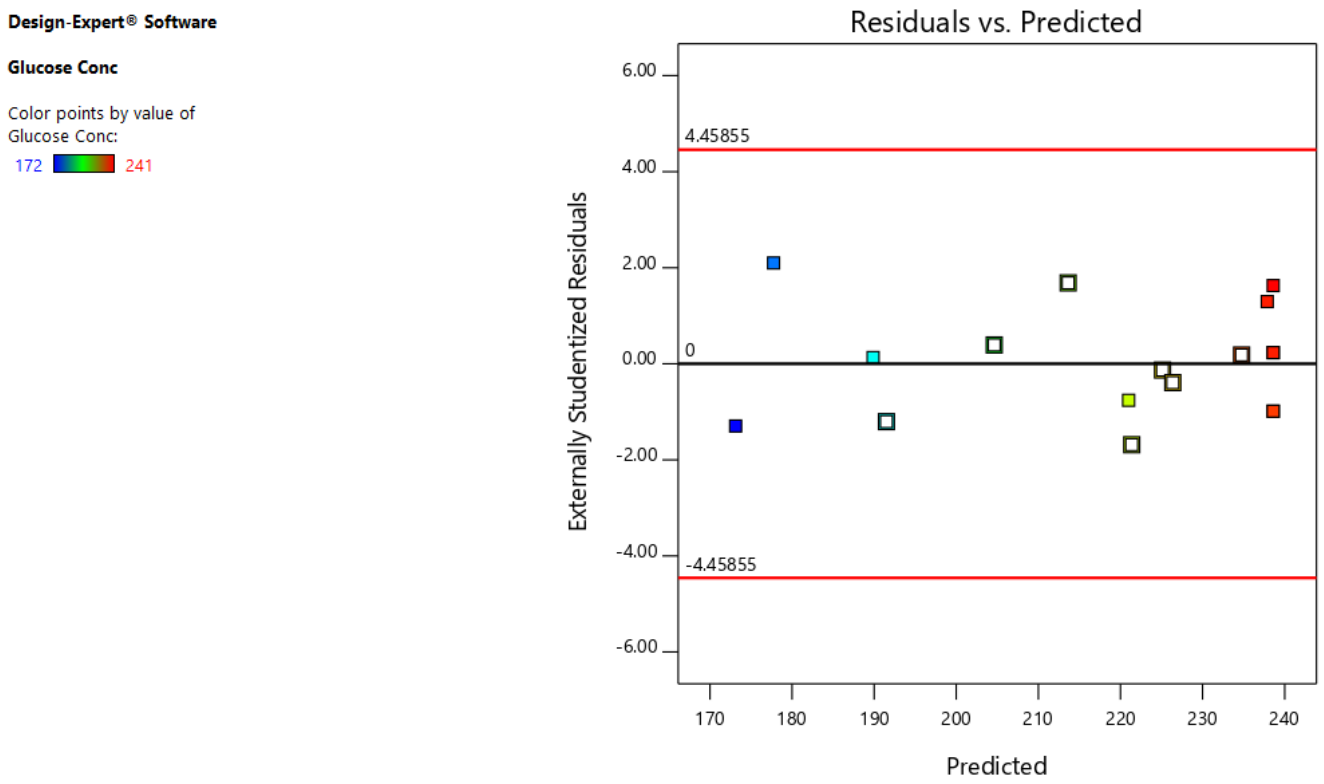

Figure 4-3: Plot of residuals versus predicted yield

Figure 4-4: explored the graph of the residuals values versus the experimental run order. It showed random and uniform scatters of color points corresponding to glucose concentration yield during the experiment. That means there is no need for improvement for minimizing personal error.

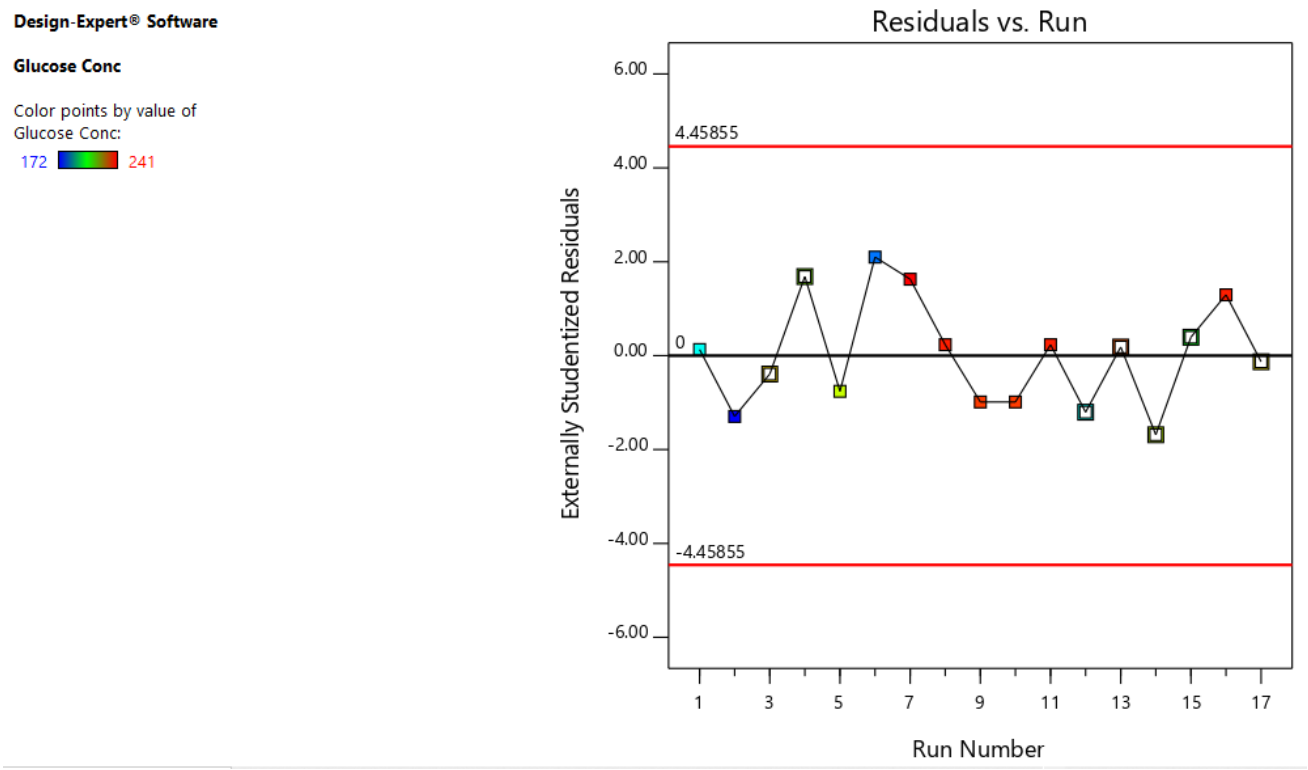

Figure 4-4: Plot of residual versus run order

The graph of the observed values versus predicted values as shown in Figure 4-5, were plotted between the actual and predicted values of mean particle size for detecting values that cannot be easily predicted by the model. Straight-line passing from origin revealed that experimentally observed values of mean particle size were analogous with predicted values.

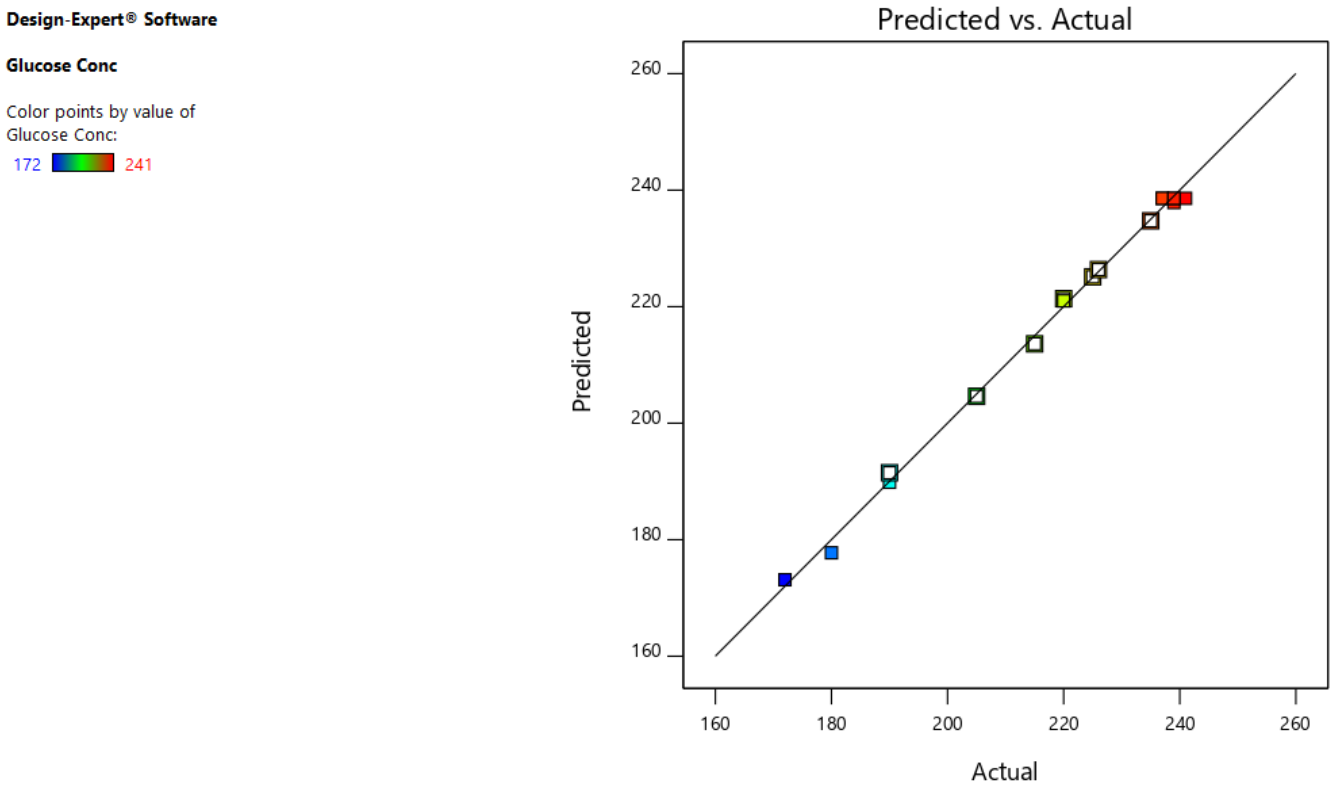

Figure 4-5: Plot of predicted versus actual

4.4.5. Effect of individual factors in product yield

The P-values denote the significance of the coefficients. The above P-value coefficients of variables show that variables named temperature ($P < 0.0001$), acid concentration ($P < 0.0001$) and time ($P < 0.0001$) showed a significant influence on glucose yield, and time was the most effective parameter. This result could also be confirmed from the regression model equation coefficients, temperature (A=10.75), sulfuric acid concentration (B=6.88) and time (C= 21.63).

Figure 4-6 shows the product yields at temperatures from 130 ℃ to 150 ℃. From the regression model equation, the coefficient of A and A^2 is positive and negative respectively. These numbers show that yield is increased with increasing temperature and decreases eventually at a very high-temperature level. On the other hand, from the figure below the maximum glucose yield ≈ 241 g/L was observed at 140 ℃ and then significantly decreased with increasing temperature.

This was in line with the findings of Makiko and Toshitaka (2004) which observed that heating increases in glucose yield until it reaches its optimum temperature.

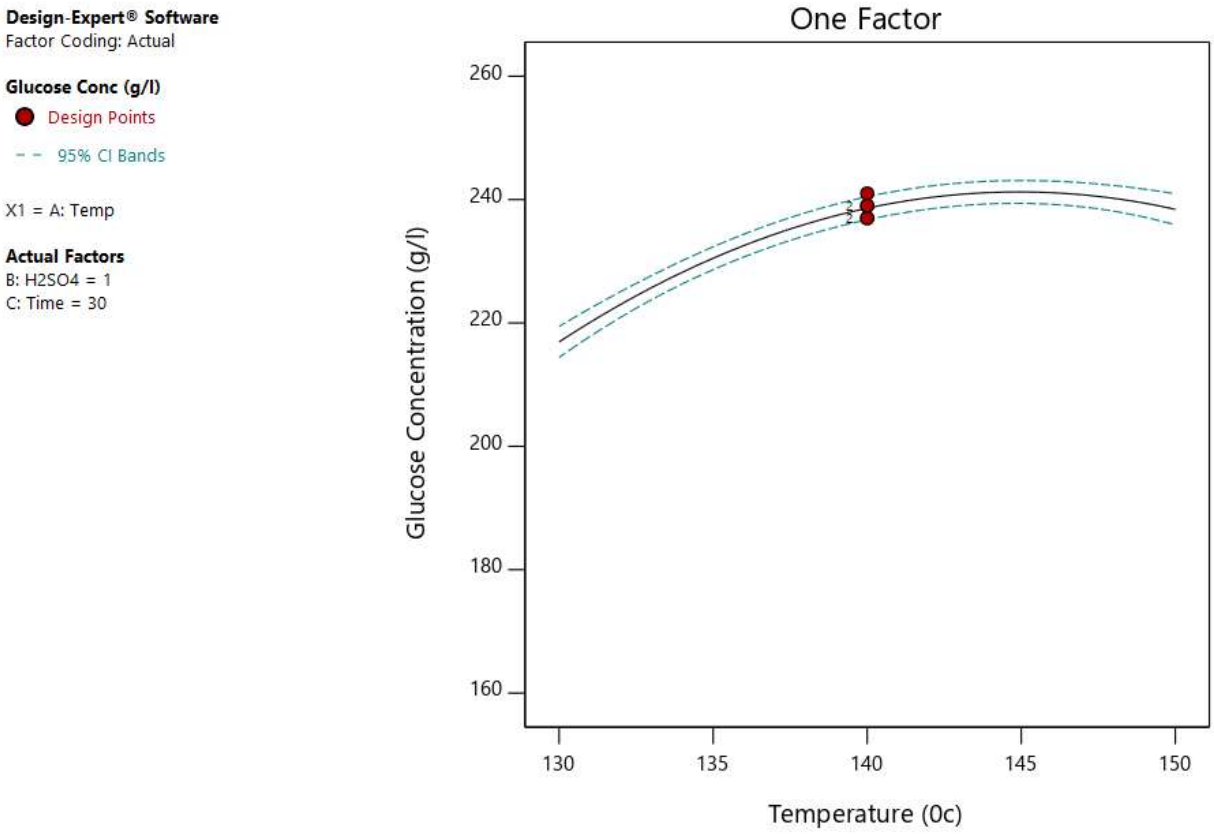

Figure 4-6: Temperature versus glucose yield

From the regression model equation, the coefficient of B and B^2 are positive and negative respectively. These numbers show that yield is increased with increasing sulfuric acid concentration and decreases eventually at extensive sulfuric acid concentration. This can be clearly seen in Figure 4.7 illustrating the production of glucose was significantly affected by the concentration of sulfuric acid. The result showed that the concentration of sulfuric acid 1% in starch hydrolysis leads to the maximum concentration of glucose released since at this range the desirability function was at the maximum value. According to Wang and Copeland (2013),

sulfuric acid concentration of 1% was favored to avoid an extensive and unnecessary decrease in pH.

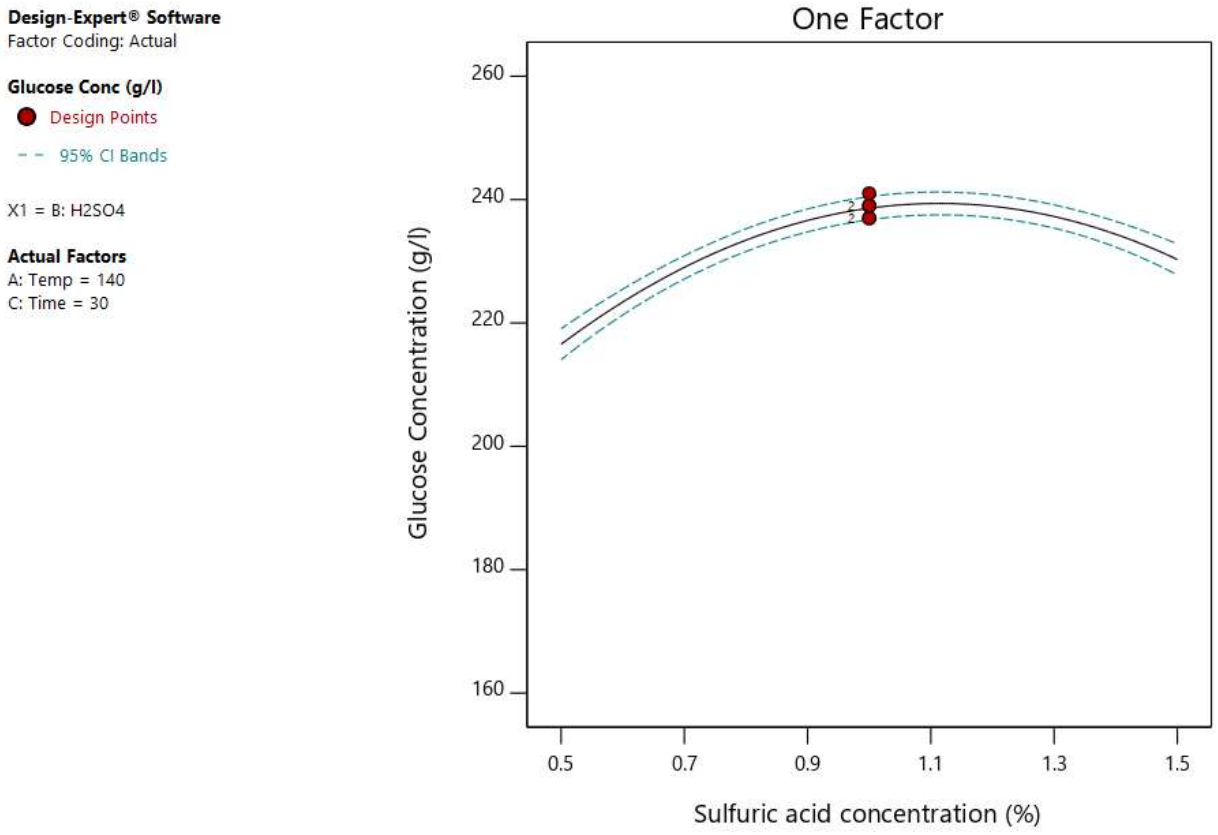

Figure 4-7: Sulfuric acid concentration versus glucose yield

From the regression model equation, the coefficient of C and C^2 are positive and negative respectively. These numbers show that yield is increased at the glance with increasing time of hydrolysis and decreases eventually as retention time increases. This can also be confirmed by Figure 4-8 which shows product yield versus time. The results showed glucose yield increased with time up to 30 min, reaching a maximum value of 241 g/L the yield decreasing thereafter. After the yields of glucose reached maximum value, it decreased with time, corresponding to the decrease of total organic carbon values. A report by Sasaki and Ara (2003) showed that as time increased the major products were monosaccharides and decomposition products such as

5-hydroxymethylfurfural and furfural, whilst polymers having a high degree of polymerization were no longer present.

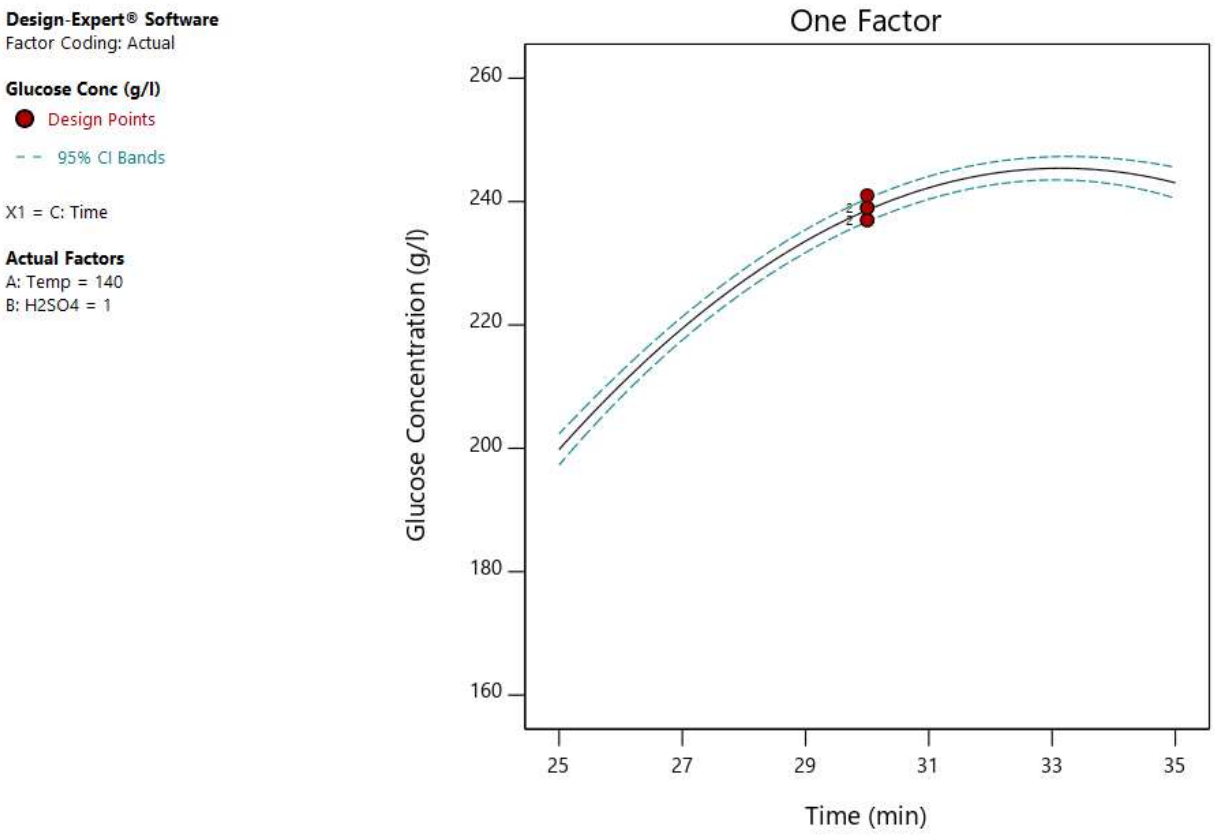

Figure 4-8: Time versus glucose yield

Generally, this could be due to the aldehyde yield increased proportionally with glucose yield up to the maximum glucose yield, and also increased significantly with decreasing glucose yield. This implies that the aldehyde was formed as a by-product, together with the production of glucose from the degradation of starch polymers. After the glucose yields reached the maximum value, heating conditions became too severe, the sugars such as glucose, maltose, and fructose might be further decomposed to produce the aldehyde (Sasaki & Arai, 2003).

4.4.6. Interaction effect of factors in product yield

The P-values denote the significance of the coefficients and are also important in understanding the pattern of the mutual interactions between the variables. Moreover, as can be observed from sulfuric acid concentration and time coefficient, the interactions between sulfuric acid concentration and time were not significant, an indication that there was no significant correlation between each of the two variables, and they did not help much in increasing the production of glucose syrup. However, there was significant interaction between temperature and sulfuric acid concentration (P =0.0006), and also between temperature and time (P =0.0006).

The interaction effects and optimal levels of the variables were determined by plotting the three-dimensional response surface curves (Figures 4-9 up to 4-10) when one of the variables is fixed at an optimum value and the other two can vary.

Figure 4-9 represents the effect of varying sulfuric acid concentration and temperature on glucose syrup production when the time was retained constant at 30 min. The increase of glucose yield occurred with an increase of sulfuric acid concentration at a temperature from 130 ℃ to 140 ℃. Further increase in temperature would decrease the glucose yield. According to these interaction effects, the maximum yield of glucose concentration activity was ≈ 241 g/L at sulfuric acid concentration ≈ 1.0% and temperature ≈ 140 ℃.

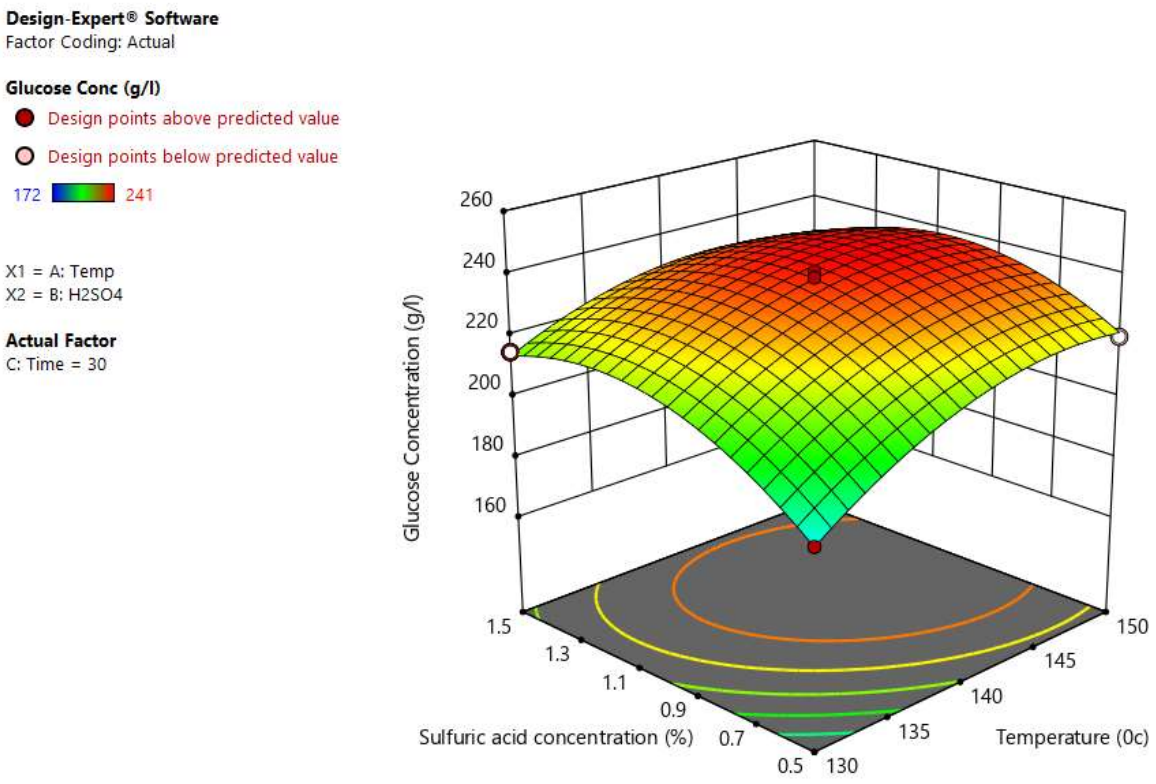

Figure 4-9: 3D response surface plots showed the effect of sulfuric acid concentration and temperature at constant time

Figure 4-10 shows the interactive effect of time and temperature at constant sulfuric acid concentration. The increase of glucose yield occurred with an increase of temperature at a time from 25 to 30 min. Further increase in time would decrease the glucose yield. According to these interaction effects, the maximum yield of glucose concentration activity was ≈ 241 g/L at time ≈ 30 min and temperature ≈ 140 ℃.

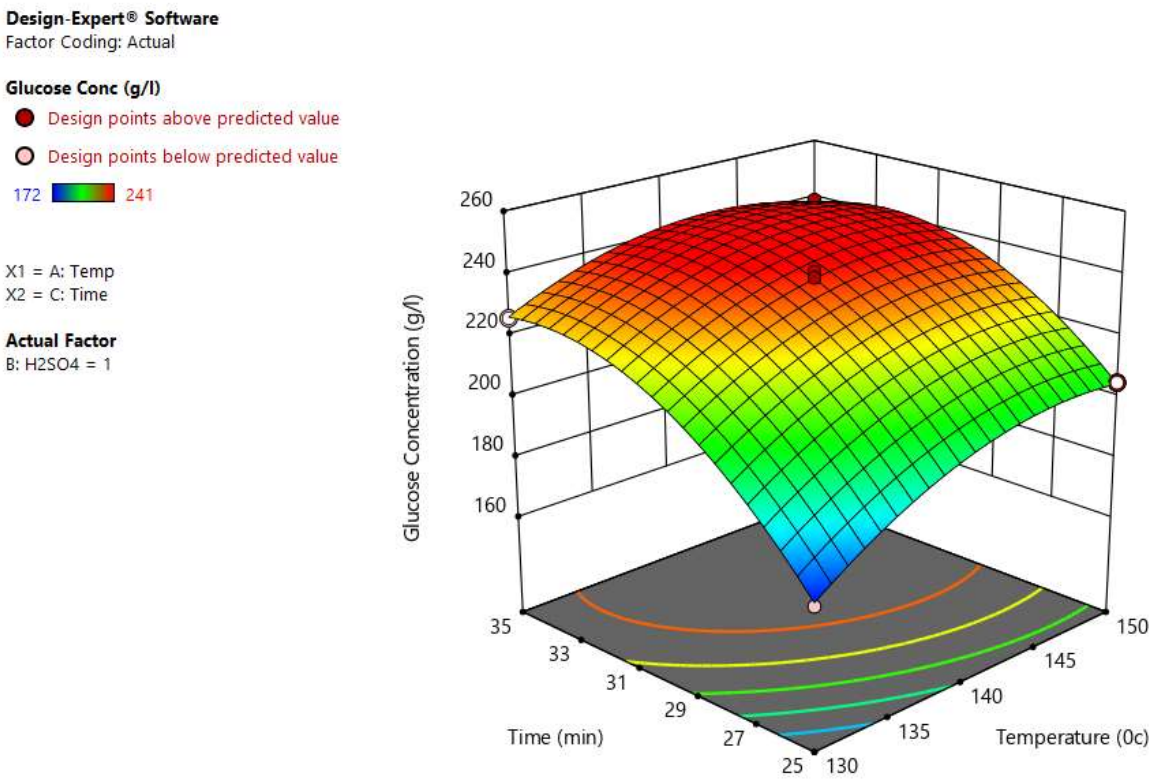

Figure 4-10 3D response surface plots showed the effect of time and temperature at constant sulfuric acid concentration

4.5. Glucose syrup characterization

The proximate composition of the product material has to be within the optimum range in order to find different industrial applications. This requires knowledge of the chemical and physical characteristics of the glucose syrup. As a general trend, it was observed that colorless, viscous liquid glucose syrup was produced by the acid hydrolysis of sweet potato (Appendix D). Thus, knowledge of moisture, ash, viscosity, density, pH, and total solids contents of the food is fundamental to the assessment of its nutritive quality. The moisture, ash, viscosity, density, pH, and total solids contents of the glucose syrup were calculated using the formula given in

equation 3.10, 3.11, 3.12 and 3.13 and the results are shown in Table 4-6 and each value is an average of three runs (Appendix A-2).

Table 4-6: Physio-chemical compositions of the product

Parameter	Content
Moisture (%)	26
Ash (%)	0.26
Glucose yield (%)	69.4
Total solid (%)	74
pH	4.9
Density g/mL	1.37
Viscosity, pa. s	5.63

4.5.1. Moisture and dry matter content

Moisture content affects the ability of syrup to flow, storage stability, processing behavior, quality and appearance of syrups (Nimbkar et al., 2006). The moisture content was determined and the dry matter was calculated as the difference from 100. Thus, the level of moisture content obtained in this research was 26% and dry matter 74%. This is relatively different from earlier studies by Sarungallo and Murtiningrum (2000) who found 71% of dry matter. This may be due to the difference in evaporation time. The lower moisture content of the glucose syrup is an indicative of a better shelf life (Eke, 2015). Because the high moisture content is an indication that the food product is prone to microbial attack in the course of storage and as such may not be stored favorably over a long period of time (Ezeama, 2007).

4.5.2. Ash content

Ash content is defined as the ratio of ash in the dry matter of glucose syrup. Data on Table 4-6 shows that the ash content of glucose syrup was 0.26%. Ash content determines the quality of glucose syrup, and the lowest the ash content, the better the quality of glucose syrup (Sarungallo & Murtiningrum, 2006). The results obtained were lower than that of Akbulut and Ozcan (2008) and Saikat et al. (2011) who reported ash content of 4.6 and 4.17% respectively.

4.5.3. Viscosity

The viscosity of glucose syrup in relation to its solids content and temperature is an important factor when the product must be pumped and stored, as heating may need to be provided for storage tanks and heavy-duty pumps required to transfer the material. From Table 4-6, a viscosity of 5.63 pa.s was obtained. This agrees with the result by Hernandez et al. (2008), who found an almost equal amount of viscosity at the same temperature. The main factors affecting the viscosity of the solutions are the nature of the continuous and the dispersed phases, particle-particle interactions, and particle-solvent, concentration, shape, particle size, and temperature (Osorio, 2001). The viscosity of glucose syrup is directly related to its moisture content and its molecular weight. According to Charley (1990), an association occurs through hydrogen bonding between them that leads to an effect of highly branched polymer that increases the resistance of the syrup to flow freely; and therefore, increases the viscosity of the system. Temperature is also very important in relation to viscosity and viscosity decreases as temperature increases. A high viscosity was believed to be essential at one time, but for fast freezing (rapid whipping) in modern equipment, a lower viscosity seems desirable. In general, as the viscosity increases, the resistance to melting and the smoothness of texture increases, but the rate of whipping decreases (Ravindra, 2017).

4.5.4. Density

The determined density value was 1.37g/mL. Density is routinely used to determine the carbohydrate concentration in syrups, juice, and beverages in the food industry (Akbulut & Ozcan, 2008). It is generally known that syrup density decreases with increasing water content, and to a lesser extent temperature.

4.5.5. pH

A widely used preservation method consists of increasing the acidity of foods either through fermentation processes or the addition of weak acids. pH is the negative log of hydrogen ion concentration (George, 2002) which is a measure of the product acidity and is a function of the hydrogen ion concentration in the food product. It is well known that groups of microorganisms have pH optimum, minimum and maximum for growing in food. Bacteria normally grow faster between pH ranges of 6.0 - 8.0, yeasts between 4.5 - 6.0 and molds between 3.5 - 4.0. An important characteristic of a food is its buffering capacity, i.e. its ability to resist changes in pH. Food with a low buffering capacity will change pH quickly in response to acidic or alkaline compounds produced by microorganisms, whereas food with high buffering capacity are more resistant to such changes. In any case, if low pH is a factor included in the preservation system of food, control of pH and the application of a margin of safety are required for these foods. From Table 4-5, the pH of the sample was 4.9, indicating acidity. Williams and Dennis (2008) explained that food at pH 4.4 - 5.0 are medium acid food and they last better than food above this range which favors microbial activity.

4.6. Shelf-life determination

In these studies, potassium sorbate was used as a preservative. For the following parameters, the best and most economic quantity of the potassium sorbate that can be used as a preservative was also determined. A higher concentration of preservatives increases the shelf life but the

higher concentration does not only lead to high production cost but can lead to serious health hazards (Nakatani & Komaki, 2002).

4.6.1. pH

Figure 4-11 shows the result obtained from the evaluation shelf life using potassium sorbate with time on the pH of glucose syrup. During storage, pH lowering was observed from 0.0 up to 0.05% of potassium sorbate concentration. This variation on the control sample or at low concentration of the potassium sorbate can be explained by the fact that carbon dioxide released during the process is converted to carbonic acid-producing carbonate ions and protons, and which increases acidity and decreases the pH of the syrup (Shen et al., 2004).

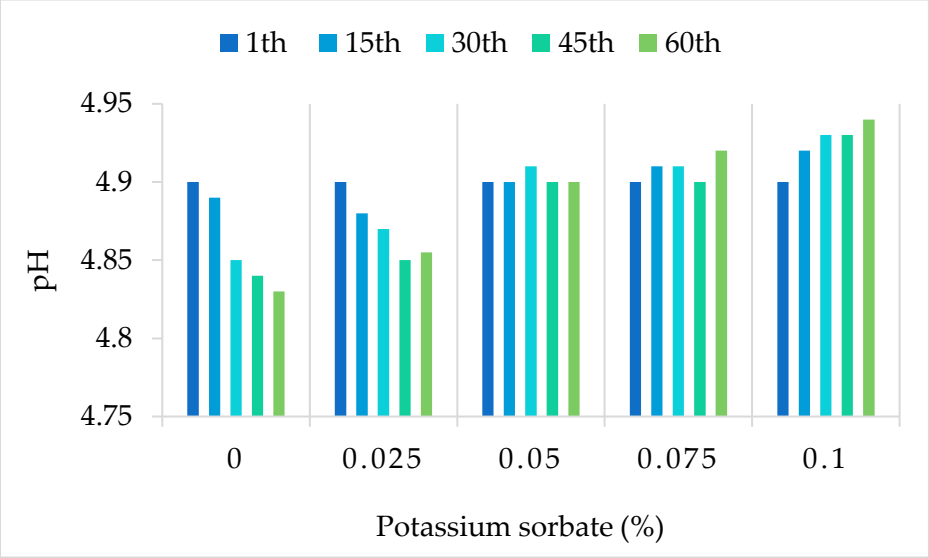

Figure 4-11: Effect of potassium sorbate with time on pH of Glucose Syrup

The variation gradually reduces to stability as the concentration of the potassium sorbate reaches 0.05%. When potassium sorbate dissolved in water, it ionizes to form sorbic acid which is effective against yeasts, molds, and selected bacteria but the addition of potassium sorbate to a glucose syrup will raise the pH depending on the amount or type of product (Melissa, 2014). So, the slight increase in pH from 0.05 to 0.1% could be due to this reason.

4.6.2. Moisture content

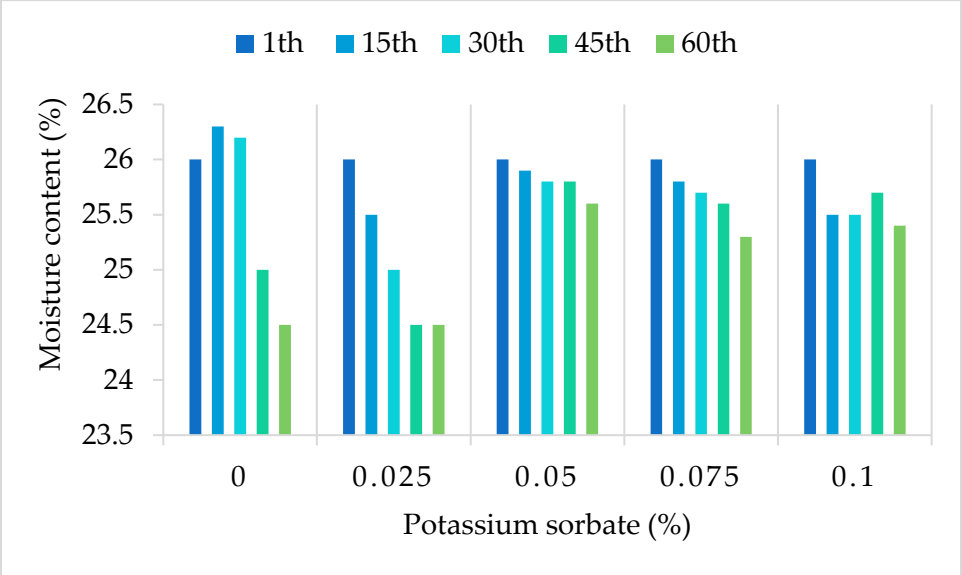

Figure 4-12: Effect of potassium sorbate with time on the moisture of glucose syrup

Figure 4-12 shows the result obtained from the evaluation shelf life using potassium sorbate with time on the moisture content of glucose syrup. During storage, moisture content variation was observed from 0.0 up to 0.05% of potassium sorbate concentration while variation gradually reduces to stability as the concentration of the potassium sorbate increases from 0.05 up to 0.1%. The slow decrease in moisture content with time can be explained by the rate of moisture migration or relative humidity of the sample and the surrounding. A product stored at elevated humidity accelerates moisture migration through the package whereas storage at dry conditions promotes drying of the syrup (Ergun et al., 2010).

4.6.3. Viscosity

The coefficient of viscosity of fluids decreases as the temperature increases. As temperature increases, the average speed of the molecules in a liquid increase and the amount of time they spend in contact with their nearest neighbors decreases. (Osorio, 2001).

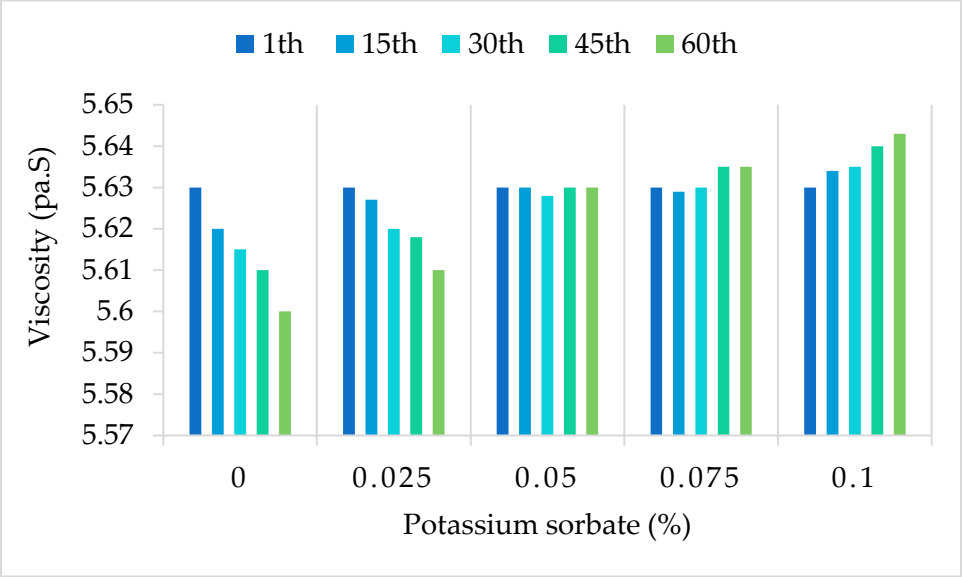

Figure 4-13: Effect of potassium sorbate with time on the viscosity of Glucose Syrup

The graph of the viscosity shown in Figure 4-13 confirms at low concentration of the preservative, there is a decrease in viscosity. This is due to a slow breakdown in the polysaccharide mixture as the product aged (Hajmeer et al., 1999). Variation reduced as the concentration of potassium sorbate reached 0.05 % and was able to maintain steady viscosity during the experiment because of the moderate amount of the preservative. However, the viscosity is strongly affected by water content (lower viscosity for higher water contents) and temperature (lower viscosity for higher temperatures (Yamamoto et al., 2006). Hence, as it can be observed from Figure 4-13, the slow increase in viscosity content or development of more viscous skin syrup surface with time can be due to loss of water to the surrounding air with the preservative ability of the potassium sorbate.

Chapter 5: Conclusions and Recommendations

5.1. Conclusions

The results of this research showed that sweet potato starch is a potential candidate to produce starch and glucose syrup by acid hydrolysis. The proximate composition of sweet potato tuber was determined before the extraction of starch. The average percent of moisture, protein, fat, ash, crude fiber, and total carbohydrate were found to be 53.66, 2.13, 0.36,0.31 and 41.36% respectively. The maximum yield of starch was found to be 31.59%.

The study revealed that glucose syrup production was significantly influenced by time, temperature and sulfuric acid concentration. When sweet potato starch was hydrolyzed at different sulfuric acid concentrations, times and temperature to give glucose syrups, the maximum glucose syrup concentration of 241g/L was found at 1% sulfuric acid concentration, 140 ℃ and 30 min. The percentage of production yield was 69.408% with moisture content 26%, dry matter 74%, ash content 0.26%, density 1.37 g/mL, viscosity 5.63 pa. s and pH 4.9.

Design-Expert software using Box-Behnken design was used for statistical assessment to identify the important parameters for glucose syrup production. Results showed that the hydrolysis time, sulfuric acid concentration, the temperature had a significant positive effect on glucose syrup yield. However, the interaction effect between temperature and sulfuric acid concentration; temperature and time had an antagonistic effect on glucose syrup production. While the interactions between sulfuric acid concentration, time was not significant, an indication that there was no significant correlation between each of the two variables, and they did not help much in increasing the production of glucose syrup.

Since glucose syrup is perishable and degradable which has a short shelf life during storage, potassium sorbate was used to prolong the shelf life of glucose syrup without jeopardizing its prevailing taste. The results observed in the present study demonstrated that potassium sorbate

played a positive role in extending the shelf life of glucose syrup within the accepted concentration range at room temperature. The concentration of potassium sorbate of 0.05% was found as optimum preservative concentration since if the optimum concentration is exceeded, it could lead to the human health hazard and also wastage of production funds. On the other hand, the control glucose syrup sample without added preservatives showed variation on pH, moisture content and in viscosity.

In conclusion, controlling hydrolysis parameters during the production of glucose syrup from acidic hydrolysis sweet potato starch is a good choice in view of increasing yields of glucose concentration and decreasing dose and cost of chemicals, and minimizing waste generation. This glucose syrup finds wide applications in brewing, baking, dairy, and confectionaries. The analysis of the chemical composition of sweet potato proves that it is a principal source of carbohydrates for the consumers.

5.2. Recommendations

This research was focused on the production and characterization of glucose syrup from sweet potato using acid hydrolysis and preservation using potassium sorbate. Further research areas are recommended to increase the yield of glucose syrup and its storage time which increases consumption for a better health benefit:

- From an economic benefit point of view from different literatures, this study used acid hydrolysis for the production of glucose syrup. A further possibility of using enzymatic hydrolysis is therefore recommended for increasing glucose syrup production and quality.
- In our country, there are no such findings in the area of glucose syrup production. So, researchers should do further research and give evidence in order to avoid the food insecurity problem of the country and to minimize a foreign currency by substituting the current imports.
- Due to shortage of time and equipment's, the proximate composition of glucose syrup for its heavy metal and sulfur dioxide contents were not determined on this study. Therefore, the proximate composition of those parameters should be done on feature work.
- The results from this study can be used by food industries, pharmaceutical industries and investors as input for their works. More specifically, the results can contribute to those who are interested in the production of both starch and glucose syrup.
- To prevent spoilage of syrup and extend its shelf life, potassium sorbate was added. But the addition of potassium sorbate to a glucose syrup will raise the pH so additional adjustment is recommended to keep the pH at a safe level.

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Appendices

Appendix A: Experimental Result

Table A-1: Physio-chemical composition of the sweet potato raw material

Particular	Run 1	Run 2	Run 3	Average result %
Moisture	52.48	53.5	55	53.66
Protein	2.09	2.10	2.20	2.13
Fat	0.39	0.34	0.36	0.36
Ash	1.95	2.1	2.5	2.18
Crude fiber	0.28	0.34	0.32	0.31
Total carbohydrate	42.81	41.62	39.62	41.36

Table A-2: Physio-chemical composition of glucose syrup

Parameter	Run 1	Run 2	Run 3	Average content (%)
Moisture	25.4	26	26.6	26
Ash	0.25	0.24	0.30	0.26
Total solid	74,6	74	73.4	74
Viscosity	2.65	2.59	2.65	20.63
Density	1.4	1.35	1.36	1.37
pH	4.95	4.75	5.0	4.9

Table A-3: Shows the absorbencies and concentrations of the unknown sample

Run	Temperature (°C)	H_2SO_4 (%)	Time(min)	Absorbance	Unknown Glucose conc. (g/l)	Vol.(l)	Glucose yield in (g)	Yield (%)
1	130	0.5	30	3.7704	190	0.072	13.68	54.72
2	**130**	**1**	**25**	**3.414**	**172**	**0.072**	**12.384**	**49.536**
3	130	1	35	4.4832	226	0.072	16.272	65.088
4	130	1.5	30	4.2654	215	0.072	15.48	61.92
5	140	0.5	35	4.3644	220	0.072	15.84	63.36
6	140	0.5	25	3.5724	180	0.072	12.96	51.84
7	**140**	**1**	**30**	**4.7802**	**241**	**0.072**	**17.352**	**69.408**
8	140	1	30	4.7406	239	0.072	17.208	68.832
9	140	1	30	4.701	237	0.072	17.064	68.256
10	140	1	30	4.701	237	0.072	17.064	68.256
11	140	1	30	4.7406	239	0.072	17.208	68.832
12	140	1.5	25	3.7704	190	0.072	13.68	54.72
13	140	1.5	35	4.6614	235	0.072	16.92	67.68
14	150	0.5	30	4.3644	220	0.072	15.84	63.36
15	150	1	25	4.0674	205	0.072	14.76	59.04
16	150	1	35	4.7406	239	0.072	17.208	68.832
17	150	1.5	30	4.4634	225	0.072	16.2	64.8

Shelf life Experimental results

Table A-4: Effect of potassium sorbate with time on pH of Glucose Syrup

	1th	15th	30th	45th	60th
0	4.9	4.89	4.85	4.84	4.83
0.025	4.9	4.88	4.87	4.85	4.855
0.050	4.9	4.9	4.91	4.9	4.9
0.075	4.9	4.91	4.91	4.9	4.92
0.10	4.9	4.92	4.93	4.93	4.94

Table A-5: Effect of potassium sorbate with time on the moisture of glucose syrup

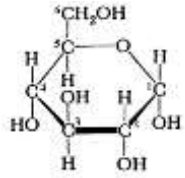
	1th	15th	30th	45th	60th
0	26	26.3	26.2	25	24.5
0.025	26	25.5	25	24.5	24.5
0.050	26	25.9	25.8	25.8	25.6
0.075	26	25.8	25.7	25.6	25.3
0.10	26	25.5	25.5	25.7	25.4

Table A-6: Effect of potassium sorbate with time on the viscosity of Glucose Syrup

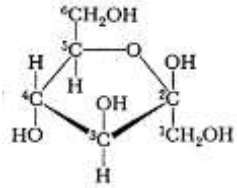
	1th	15th	30th	45th	60th
0	5.63	5.62	5.615	5.61	5.6
0.025	5.63	5.627	5.62	5.618	5.61
0.050	5.63	5.63	5.628	5.63	5.63
0.075	5.63	5.629	5.63	5.635	5.635
0.10	5.63	5.634	5.635	5.64	5.643

Appendix B: Reducing and Non-reducing sugars

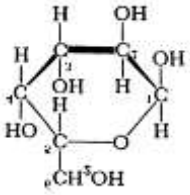
Reducing sugars



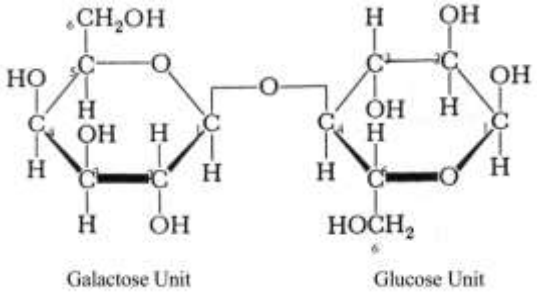
Glucose



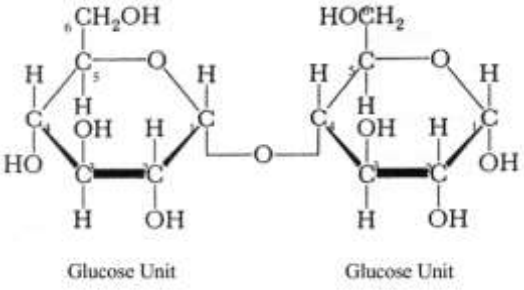
Fructose



Galactose

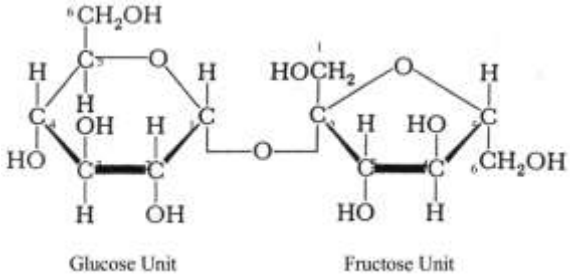


Lactose



Maltose

Non-reducing sugar



Sucrose

Appendix C: Proximate analysis

Crude fat determination

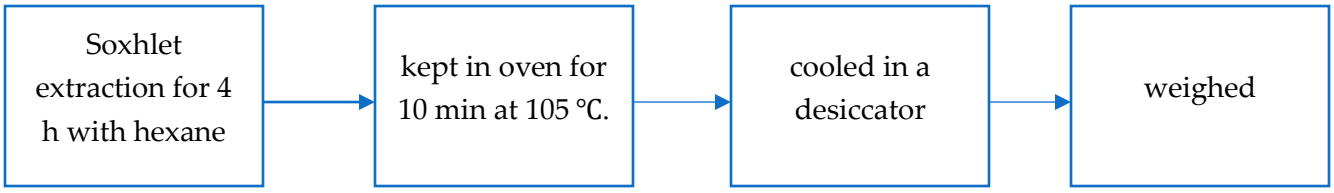


Figure C-1: Determination of fat by Soxhlet method

Crude fiber determination

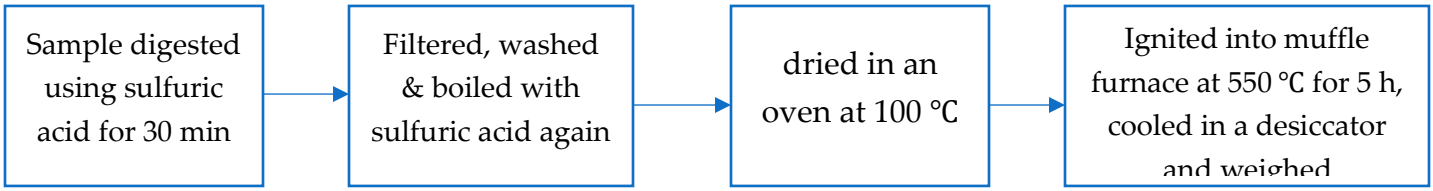


Figure C-2: Determination of crude fiber

Protein determination

Protein is determined by the analysis of the nitrogen content. From this, the protein content is calculated. Protein consists of amino acids which contain nitrogen (N) in the amino group.

Sulfuric acid(H_2SO_4) Steam H_3BO_3 in receiver vessel

Catalyst ($CuSO_4$, K_2SO_4) KOH H_2SO_4 for titration

Protein contains: 15-18% N

Average: 16% N ⇒ factor 6.25 ⇒ 16% nitrogen x6.25=100% protein

Appendix D: Images of Glucose Syrup Production

Fig D-1 Raw Sweet Potato

Fig D-2 Internal part of sweet potato

Fig D-3 Oven drying

Fig D-4 Starch packaging

Fig D-5 Gelatinization of starch

Fig D-6 Autoclave

Fig D-7 Hydrolyzed sample

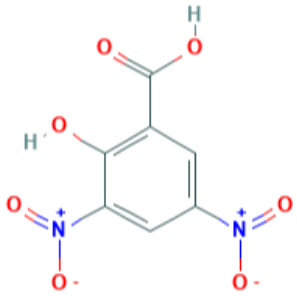
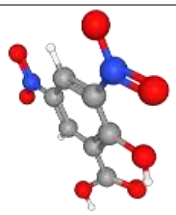
Fig D-8 Rotary vacuum Evaporation

Fig D-9 Soxhlet extraction

Fig D-10 Glucose syrup produced

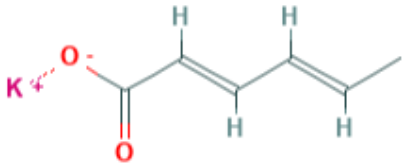
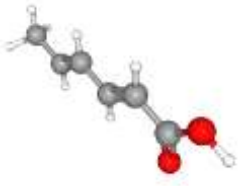
Appendix E: Chemical and Physical Property of Reagents

3,5-Dinitrosalicylic acid

Structure:  2D  3D	
Molecular Formula:	$C_7H_4N_2O_7$
IUPAC Name:	2-hydroxy-3,5-dinitrobenzoic acid
Appearance:	Yellow needles or plates
Melting point:	182°C
Solubility	Soluble in ethanol, diethyl ether, benzene and water
Chemical Names:	3,5-DINITROSALICYLIC ACID 2-Hydroxy-3,5-dinitrobenzoic acid 3,5-Dinitro-2-hydroxybenzoic acid 3,5-Dinitrosalicylate
Molecular Weight:	228.12 g/mol

3,5-dinitrosalicylic acid is a monohydroxy benzoic acid consisting of 2-hydroxybenzoic acid having nitro substituents at the 3- and 5-positions. It is used in colorimetric testing for the presence of free carbonyl groups (C=O) in reducing sugars. It has a role as a hapten. It is a C-nitro compound and a monohydroxy benzoic acid. It derives from a salicylic acid.

Potassium sorbate

Structure:		
	2D	3D
Molecular Formula:	$C_6H_7KO_2$	
IUPAC Name:	potassium;(2*E*,4*E*)-hexa-2,4-dienoate	
Appearance:	White crystals	
Melting point:	270 °C	
Odor	yes	
Density	1.363 g/cm^3	
Solubility:	Soluble in ethanol, propylene glycol and water	
Chemical Names:	POTASSIUM SORBATE Sorbic acid potassium salt Sorbistat-K Potassium 2,4-hexadienoate	
Molecular Weight:	150.22 g/mol	

Potassium sorbate is the potassium salt of sorbic acid, it is a white salt that is very soluble in water (58.2% at 20 °C). It is primarily used as a food preservative. Potassium sorbate is effective in a variety of applications including food, wine, and personal-care products.