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SCHOOL OF CHEMICAL AND BIO ENGINEERING

Production of Antioxidant Rich Glucose Syrup from Ensete Ventricosum Starch Through Enzymatic Hydrolysis and Preservation Using Green Tea Extract

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This is to certify that the thesis prepared by Tigist Abebe, entitled: '**Production of Antioxidant Rich Glucose Syrup from Ensete Ventricosum Starch Through Enzymatic Hydrolysis and Preservation Using Green Tea Extract'**, submitted in partial fulfillment of the requirement for the degree of Master of Science in Chemical and Bio Engineering (Biochemical Engineering), complies with the regulation of the university and meets the accepted standards with respect to originality and quality.

Signed by the Examining committee:	**Signature**	**Date**
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Declaration

I, the undersigned, declare the thesis work entitled '**Production of Antioxidant Rich Glucose Syrup from Ensete Ventricosum Starch Through Enzymatic Hydrolysis and Preservation Using Green Tea Extract**' is my original work and has not been presented in any form for another degree, diploma or an award at any university or other institution. I confirm that appropriate credit has been given within this thesis where reference has been made to the work of others and has been duly acknowledged. The work was under the guidance of Dr. Shimeles Shumi, Assistant Professor of Chemical Engineering at Addis Ababa University, School of Chemical and Bio Engineering**.**

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Abstract

Ensete ventricosum is a plant native to Ethiopia with a high content of starch. This study aimed to produce antioxidant rich glucose syrup from enset starch by using alpha amylase and glucoamylase enzymes and preservatives using green tea polyphenols. The starch was isolated from the corm and pseudostem parts of Enset plant and characterized. The main process steps for enzymatic production of glucose syrup from enset starch were: gelatinization, liquefaction, saccharification, centrifugation followed by treatment with activated charcoal and evaporation to final solids (>70%). The preservative effect of green tea extract on the shelf life of the syrup was also examined for 30days, by studying its effect on pH, moisture content and viscosity of the glucose syrup. Samples were treated with 0, 100, 200, and 300ppm of green tea polyphenols. The study showed that the moisture content, ash content, crude fat content, protein content, and amylose content of enset starch were: 8.98%, 0.15%, 0.38%, 0.1%, and 29.01%, respectively. Response Surface Methodology Design (RSMD), particularly, Box Behnken Design (BBD) was applied to investigate the effect of temperature (50, 60 and 70℃), time (12, 24 and 36hr), and glucoamylase concentration (0.1, 0.15 and 0.2wt.%), on the process of saccharification using Design expert® 12 software, which shows the significant influence on the value of dextrose equivalent (DE). The maximum concentration of glucose (95.64%) in terms of DE was found at the parameter interaction of 0.1wt.% (glucoamylase concentration), 60℃, and 36hr. The result showed that the optimum condition for the saccharification process was 62℃, 12hr and 0.2wt.% (glucoamylase dosage) which resulted in 91.79±0.5% of DE. The moisture content, total soluble solid, ash content, density, specific gravity, viscosity and pH of the product were: 24.01%, 75.32°Brix, 0.35%, 1380.8 kg/m^3, 1.3833, 940 mPa.s, and 5.5, respectively. Generally, it was observed that green tea extracts with a concentration of 100ppm of TPC was found to be suitable as preservative to keep the quality and extend the shelf-life of glucose syrup at room temperature. Hence, Enset starch is a promised raw material to develop glucose syrup enriched with extracts of green tea to elevate its shelf life.

Keywords: Ensete ventricosum starch, glucoamylase concentration, glucose syrup, green tea polyphenols, saccharification temperature, time.

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Acronyms

AARC	Areka Agriculture Research Center
AMG	Amyloglucosidase
AOAC	Association of Official Analytical Chemists
AGU	Amyloglucosidase unit
BBD	Box Behnken Design
BU	Brabender units
CSA	Central Statistics Agency
DE	Dextrose equivalent
DNS	3,5-dinitrosalicylic acid
DPPH	2,2-diphenyl-1-picrylhydrazyls
GAE	Gallic acid equivalents
GTE	Green tea extract
HFGS	High-fructose glucose syrup
mPa.s	Millipascal second
SNI	Indonesian National Standard
TPC	Total phenol content

1. Introduction

1.1. Background

In 1811 with Napoleon Bonaparte wagging war all over Europe, both honey and sugar became very scarce [1]. A shortage of sugar from the West Indies inspired many chemists to look for ways of producing a sweet alternative. A breakthrough came in 1811 when a German scientist, Gottlieb Kirchhoff obtained a sweet syrup by boiling wheat starch with dilute sulfuric acid. Afterward, Potato starch was used as source of starch and sulfuric acid was replaced by hydrochloric acid and indirect heating of the reaction vessel was commonplace [2].

In 1812, Kirchoff was elected as an Extraordinary Academician in Chemistry. This encouraged him to continue his study on starch, which resulted in 1816, with the discovery that enzymes were also capable of breaking down starch [1]. Nowadays, the process of making glucose syrup can be carried out by acid and/or enzymatic hydrolysis. Acid hydrolysis breaks the chain of starch at random parts, while enzymatic hydrolysis breaks at certain branching specifically, which results in a more specific processes and expected products. Moreover, enzymatic hydrolysis has better advantages such as controlled manufacturing process, cheaper purification costs, minimized color damage, and fewer by products [3].

Development of science and technology has successfully revealed the importance and potential materials containing starch as a raw material in industry [4]. The most representative biological starchy raw materials that are commonly used in glucose syrup industry are rice, corn, wheat, barley, potatoes, cassavas and sweet potatoes [5]. One of the crops that has great potential to be developed into glucose syrup is also Ensete ventricosum, because it has high content of soluble carbohydrates, mainly starch. Enset is highly resistant to adverse environmental conditions such as excessive rain, flooding, extended drought and other stress factors [6]. Among the starch crops in Ethiopia, Enset has been reported to produce the highest yield per hectare, with relatively low inputs and management requirements [7]. Accordingly, this paper aimed to produce glucose syrup from enset starch, which is inexpensive and abundantly available in Ethiopia, through the process of enzymatic hydrolysis using alpha amylase and glucoamylase enzymes as a catalyst.

Due to their high osmotic pressure, low water activity and high temperature processing, glucose syrups are usually resistant to bacterial spoilage. However, some spoilage microorganisms, such as yeast and mold spores, can survive in the syrup and still grow under storage conditions [8]. Due

to this reason preservative is needed to prolong its shelf life during storage. Food products can be preserved in different ways by using synthetic or natural additives [9]. Mostly, glucose syrup is preserved using chemical additives such as sulfur dioxide, sodium sulfite, potassium metabisulfite, potassium sorbate, sodium sorbate, sorbic acid, sodium bisulfite and among others [10]–[12].

Scientists found that some chemical food additives have positive impacts on processed food flavor and storage time, but some have turned out to have negative effects on human health, especially if they are used above optimum concentration. For instance, Butylated hydroxyl anisole, amaranth, triphenylmethane dyes, cyclamates and possibly saccharin (suspected carcinogen) [9]. Likewise, high concentration of sodium bisulfite intake could lead to serious health hazards [10]. Therefore, adequate measures must be put in place to select the right preservatives.

Some natural products, especially natural antioxidants, and antimicrobial agents, could be safe alternatives to synthetic compounds [9]. Many research has been undertaken on plant extracts and spices such as grape seeds, rosemary, thyme, and sage, among others to provide natural and safe antioxidant options in order to replace synthetic antioxidants [13], [14]. Green tea extracts are very promising for this purpose due to their ubiquity, abundance, and low cost [14].

Green tea extract mainly contains polyphenols know as catechin that possessing high antioxidant activity [15]. These extracts not only serve as antioxidants, but also antimicrobial agents, and have numerous health benefits [14], [16]. Nowadays, catechins have been widely applied in the cosmetics, pharmaceutical, beverage and food industry [13], [14], [16], [17]. In this study it is also aimed to determine the effectiveness of using green tea extract as preservative, to extend the shelf life of the glucose syrup rather than using synthetic additives which have adverse health effects.

1.2. Statement of the Problem

Food, beverage and pharmaceutical industries in Ethiopia is relying on imported glucose syrups from other countries. According to Ethiopian revenues and customs authority (2017) reports, in 2012, demand for glucose syrup was estimated at 5,000 tons worth 4.62 million US dollars and expected to reach 14,266 tons worth 13.21 million US dollars by the end of 2023 [12]. Owing to increase in demand for glucose syrup in the food processing, brewing and winemaking, textile and adhesives industry, technologies should have developed to provide a local supply of 'glucose syrup' to substitute for the imported product, with cheap and locally available raw materials. One of the potential alternative sources of starch found in Ethiopia is Ensete ventricosum. The corm and pseudostem of enset contain the high concentration of starch, which makes the corm resemble a very large potato and the fraction of starch in pseudostem is similar to sorghum which has the highest concentration found among cereals.

Analysis of data from Ethiopia's Central Statistics Agency, shows the area of land under enset production has increased approximately 46% over the past twenty years, whilst yield has been reported to increase twelve-fold, implying substantial productivity improvements [18]. Ethiopian Government has launched a project on enset plant familiarization to non-enset growing regions of the country [19], [20]. Studies have brought for the possibility of using enset starch as pharmaceutical excipients. However, in my knowledge, there was no study conducted on the utilization of enset starch for sugar and glucose syrup production. As aforementioned, Enset ventricosum has the potential to be used as a raw material for making sweetener due to its high content of starch, its availability throughout the year, and low-cost when compared to starch crops such as corn, potato, sweet potato, and wheat.

After glucose syrups is produced, preservation is needed, because yeast and mold spores can survive in the syrup and still grow under storage conditions. Mostly, it has been preserved using chemical additives. Whilst chemical additives have positive impacts on processed food flavor and storage time, but some have turned out to have negative effects on human health. Plant extracts, including those from green tea, provide natural and safe antioxidant options to replace synthetic antioxidants. Green tea extract contains several polyphenolic components mainly catechins which have antioxidant, antimicrobial properties and several health benefits. These catechins showed comparable antioxidant performance to conventional synthetic antioxidant tert-butylhydroquinone

and may be more cost-effective than other natural sources of antioxidants [17]. In addition to its significant health benefits, using green tea polyphenols in the produced glucose syrup perhaps improve the quality and shelf life of the syrup.

1.3. Objectives of the Research

1.3.1. General Objective

The general objective of this study was to produce antioxidant rich glucose syrup from locally available Ensete ventricosum starch through enzymatic hydrolysis and preservation using green tea extracts.

1.3.2. Specific Objectives

The specific objectives were:

1. To evaluate the proximate composition of Ensete ventricosum starch.
2. To investigate the effects of temperature, glucoamylase concentration, and time on the process of saccharification.
3. To characterize and evaluate the quality of produced glucose syrup.
4. To evaluate the preservative effects of green tea extract (antioxidants) on the shelf life of glucose syrup.

1.4. Research Questions

- Does the starch of Enset potentially suitable for glucose syrup production?
- Does the green tea extract able to extend the shelf life of produced glucose syrup?

1.5. Significance of the Study

Cultivation of Enset contributes to the local environment by improving the nutrient balance in soil, providing shadow, thus moderating temperature, and being part of farming systems with high biodiversity. In addition, it provides food and fodder, fibers, traditional medicine and it can be used as a partial substitution of barley malt in beer production [21].

- This study shows direction for the others to expand further research on the utilization of enset as an alternative source of industrial raw material.

The demand for glucose syrup in the food processing, confectionery, pharmaceuticals, brewing and winemaking, textile and adhesives industry increases.

- Consequently, the establishment of such a factory using cheaper and locally available raw materials such as enset will have a foreign exchange saving effect for the country by minimizing a foreign currency by substituting the current import, improvement in income levels, employment creation, efficient use of natural resources and easy access to an industrial raw material among others.

1.6. Scope of the Study

This study generally covers the isolation of starch and production of glucose syrup from indigenous plant (Ensete ventricosum) available only in Ethiopia through the process of enzymatic hydrolysis to get the desired value of glucose syrup. Moreover, this work covered the characterization of the raw materials (starch) and the produced end product. Furthermore, the effect of saccharification variables such as temperature, time and concentration of glucoamylase that affect the hydrolysis process was investigated. It also includes the extraction of green tea polyphenols and evaluate the effect of polyphenols on the shelf life of glucose syrup.

2. Literature Review

2.1. Overview of Enset Plant

Enset (Ensete ventricosum (Welw.) Cheesman) or false banana is an indigenous, herbaceous, monocarpic evergreen perennial root crop belonging to the family Musaceae that resembles a banana plant but bananas belong to the related genus Musa [19], [22]. Both Enset and Musa have large paddle-shaped leaves, a bundle of leaf sheaths (pseudostem), and large underground corm. Enset plant reaches 1 m in diameter and 4-8 m or even up to 11 m in height [22]. It is cultivated in Ethiopia for more than 10,000 years and considered as food source consumed by about 20% of the population mainly in the highlands of central, south and southwestern Ethiopia [19], [23].

Agriculture of Enset is one of the four major agricultural systems in Ethiopia which represents 65% of the total crop production in the southern region and grown within a wide range of climates, altitudes and soil type [24]. Due to its deep roots systems, it has a greater resilience to drought than other cereal crops, thus the plant is available throughout the year [19]. Compared to other crops, the productivity of enset is substantial, particularly which has reached full maturity, its cultivation is not labor intensive and not commercial fertilizer demanding in relative terms [23], [24]. The plant takes 3 to 5 years to reach full maturity, but the food need not be harvested immediately [24].

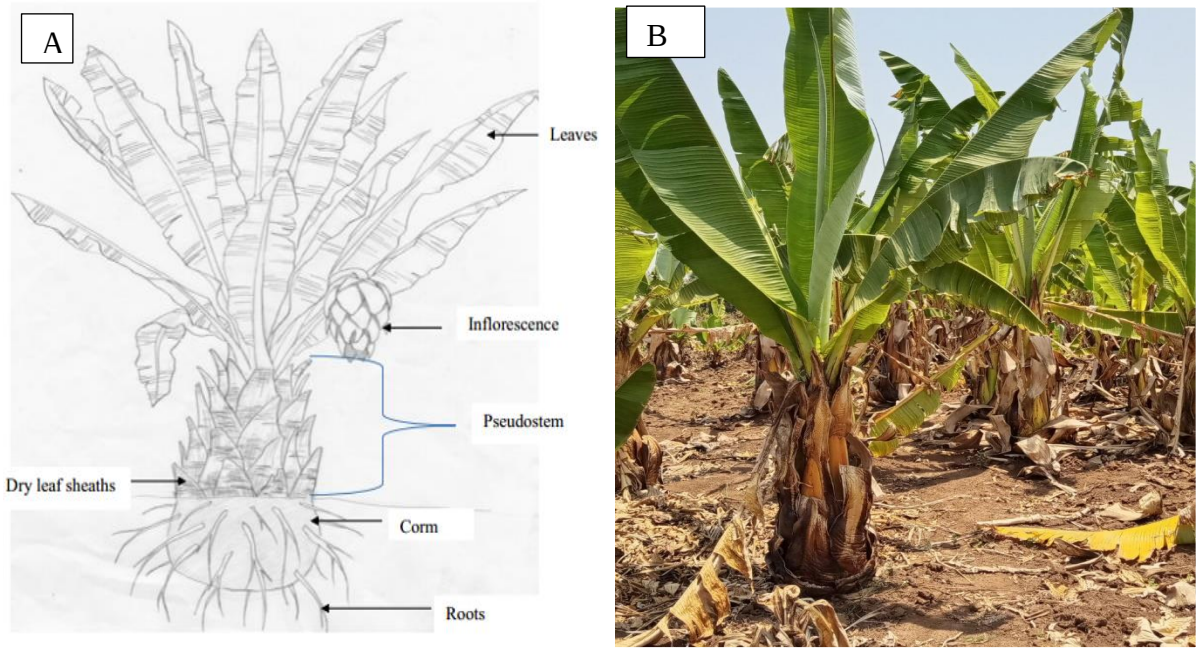


Figure 2.1. Enset plant (A) parts of Enset plant (B) Enset plant picture

Enset is a classic multipurpose crop that every botanical part is used for numerous material cultures such as, binding material in fences and house-building, mattresses and seats, packaging material, human food, cattle feed, industrial fiber, and substitute for plates or umbrellas [19], [23]. Studies reported as the corm and pseudostem of enset are rich in carbohydrate. The fraction of starch in pseudostem of Enset is comparable to sorghum which has the highest concentration found among cereals, however, enset has low protein and lignin content [25]. Indeed, the starch yield and nutrient compositions vary among enset clones or varieties [23]. An average yield of the pseudostem and corm was found to be 26-42 kg of the food per plant, but varies depending on endemic factors, altitude, cultural practices and varietal differences [24].

2.2. Varieties and Composition of Enset

Ensete ventricosum belongs to order Schistaminae and family Musaceae. The Musaceae family has two genera, Ensete and Musa [23]. Currently, AARC reported as 720 clones of Enset were collected and six varieties were identified and released with merits of best 'kocho' yield and quality, for early and late set maturity varieties. According to AARC, Yanbule, Gewada and Endale variety from early maturing (3 to 4 years) and Kelisa, Zerita and Mesena variety from late maturing (4 to 5 years) are preferred for their processed starch, kocho quality and productivity. Whereas, Arke, sweete and Neqaqa are cultivated exclusively for their corm [23].

Daba [23] also categorizes Enset varieties into two main groups namely the kocho or pseudostem types and the corm types, Gena and Neqaqa which were considered to represent the kocho/bulla and corm types, respectively. The study reported, the nutritive value of bulla (Gena) and corm (Neqaqa) on the basis of air dried enset flour. Bulla is supposed to be the best quality product obtained from the juice of decorticated leaf sheath or pseudostem. According to the study, the dry matter contents of the air dried enset flour samples were 92% in Gena and 94% in corm (Neqaqa), and the organic matter fraction on dry basis was around 99%. As fractions of dry matter, the carbohydrate contents were 97.18% in bulla and 68.72% in corm. Accordingly, bulla has higher carbohydrate (starch) contents, though the corm has better composition of other nutrients [23].

There are differences in the nutritive values among enset cultivars and different botanical parts used as food. Mohammed *et al.* [25] compared the relative compositions of the corm, above ground, pseudostem and leaves of the same enset. The article illustrated as the dry matter content of enset was 11 to 15% of fresh weight and the organic matter fraction of dry matter was around

90%. As fractions of dry matter, crude protein content was 3 to 13%, crude fat 0.4 to 5%, crude fibre 6 to 24% and soluble carbohydrates was 50 to 80% for different parts of enset. The fraction of starch on dry matter basis were 1.21 to 2.45% for leaves, 71.19% for corm and 64.93% for pseudostem. In their study, the corm and pseudostem are higher in starch content and the above ground is better in fiber and the leaf was the best in protein content.

Table 2.1. Relative composition of Ensete ventricosum [25]

Components (%)	Corm	Pseudostem	Leaves	Whole plant
Dry matter	14.08	14.59	10.88 - 12.90	14.34
Organic matter	91.83	92.54	83.23 - 88.34	90.87
Ash	8.17	7.47	11.70 - 16.80	9.13
Crude protein	3.33	3.65	12.41 - 13.15	5.98
Crude fat	0.41	0.36	2.49 - 5.23	0.84
Crude fiber	5.65	7.51	20.38 - 24.13	9.48
Soluble carbohydrates	82.44	81.02	44.47 - 49.31	74.57
Cellulose	8.75	10.81	25.94 - 32.96	14.95
Hemicellulose	5.93	8.61	20.69 - 23.54	9.39
Lignin	2.11	0.79	3.26 - 4.24	1.97
Starch	71.19	64.93	1.21 - 2.45	60.62
Sugar	-	2.28	8.44 - 11.14	0.85

Note: - Dry matter as percentage of fresh weight; other components as percentage of dry weight.

2.3. Starch

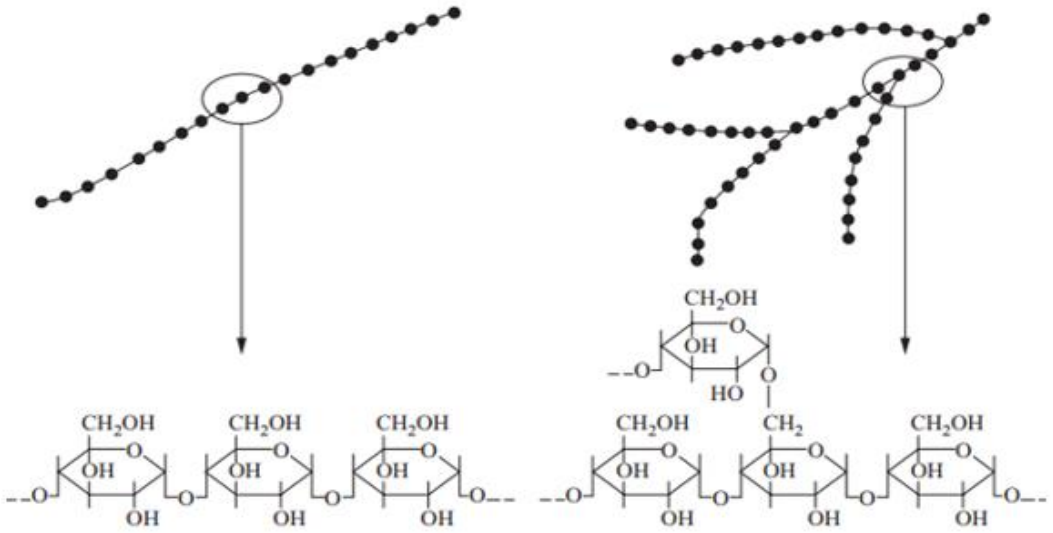
Although the development of science and technology has successfully revealed the importance and potential materials containing starch, the main commercial sources used as a raw material in the glucose syrup industry are maize, wheat, rice, barley, potato, sweet potato, cassava and arrow roots [26], [27]. In the United States, glucose syrups are made from maize, because it is so readily available. Both Holland and Poland make glucose syrups from potatoes, which again are a major agricultural crop. In Scandinavia, barley is the raw material, whilst in Australia and Northern Europe, wheat is the preferred starch, because it grows a lot of wheat. Cassava (also known as tapioca) and rice are commonly used in Asia [1].

Whilst in theory any starch can be used, in practice, it is a combination of the availability of the starch at a commercially acceptable price and politics, together with the cost and technology involved in converting the starch to a syrup [1]. One of the starch rich crops which has great potential to be developed into glucose syrup is Ensete ventricosum, since it contains up to 80% of soluble carbohydrates and 65% of starch, on a dry basis [25]. On the other hand, enset ventricosum is available throughout the year, harvested at any time and gives the highest yield per hectare relative to other starch crops in Ethiopia, with relatively low inputs and management requirements [7]. However, it should be processed as soon as it has been harvested, with the resultant starch being dried, and then stored or converted into a glucose syrup.

When extracting starch, several by-products/co-products are produced, and these by-products can then be sold to help offset the cost of producing the starch. A good example is wheat, where the by-product is vital wheat gluten which is sold to flour millers who use it to increase the protein content of flour, that has been made from low-protein wheat. In the case of maize, both the fibre and the gluten are sold for cattle feed [1]. Parenthetically, enset fibre is one such byproduct traditionally used to make sacks, bags, ropes, cordage, mats, and sieves in the rural areas [28]. This fibre can be vended for paper pulp production, cordage and fibre craft industries and the leaves can be used as a cattle feed.

2.3.1. Structure and Composition of Starch

Whilst starch is considered to be a polymer of dextrose, it is in fact made up of two distinct polymers called amylose and amylopectin ranged between 5-25 μm in size. Depending upon the botanical source of starch, the relative proportions of amylose to amylopectin will vary, but generally will be in the range of 20–25% amylose and 75–80% amylopectin. The amylose fraction consists of dextrose molecules (100-1000 dextrose) joined to each other at the C1 and C4 positions to form a long, spiral chain. The sites where the C1 and C4 are join together are referred to as a 1–4 linkages (shown in Figure 2.3). In the amylopectin fraction, there is also a chain of dextrose molecules joined at the C1 and C4 positions, but with an additional straight chain attached at the C6 position and this site at the C6 position is referred to as a 1–6 linkage (shown in Figure 2.3). Finally, both the amylose and amylopectin spiral chains in turn form spiral chains with each other [1], [29].



A) Amylose Structure

B) Amylopectin Structure

Molecular weight typically up to 500,000

Molecular weights typically up to 250,000,000

Figure 2.2. Structural Differences between Amylose and Amylopectin

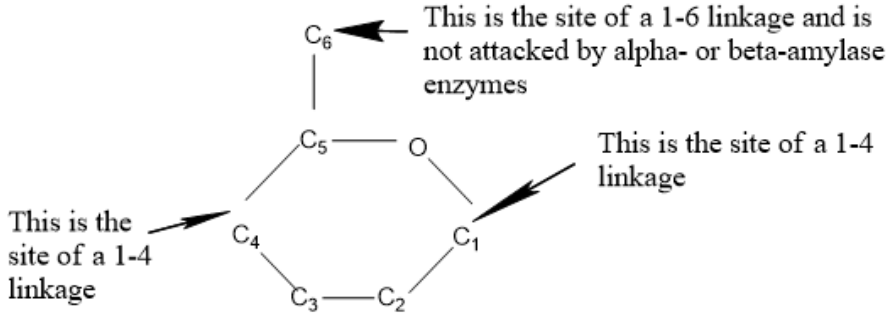


Figure 2.3. Position of 1-4 and 1-6 linkages

The starch used for manufacturing glucose syrup must be of the highest possible quality, particularly, it must have a low protein content, ideally less than 0.3%, with no metallic or microbial contamination. Both protein and metal ions have been implicated as causes for poor colour and ageing characteristics in syrups. Whilst starch of a lower quality can be used to make glucose syrup, the resulting syrup will have a bad colour, poor storage properties, and possibly off flavours, and it will also be difficult and expensive to improve the quality of the syrup, if at all. The starch should also not have any flavor, or colour and be free from any substance which could be harmful [1].

In addition to these, starch susceptibility to enzymatic hydrolysis is affected by several factors, such as amylose and amylopectin content, crystalline structure, surface porosity, extent of

molecular association between starch components, particle size, and the presence of enzyme inhibitors [30], [31]. Thus, the knowledge of those physical and chemical characteristics allows the selection of starch with the most appropriate properties for particular application.

2.3.2. Physicochemical Properties of Enset Starch

Enset starch is obtained from Ethiopian indigenous crop known as Ensete ventricosum which is highly resistant to adverse environmental conditions such as excessive rain, flooding, extended drought and other stress factors [6].

A. Proximate Composition

The study conducted by Awol *et al.* [32] on the proximate compositions of enset starch stated that the starch content was found to be 95.6%. The analytical data for amylose, moisture, total ash, protein and fat contents of enset starch were 28.3, 14.53, 0.2, 0.4, and 0.33%, respectively. The previous study carried out by Gebre-Mariam and Schmidt [26] has been also reported as the proximate composition of enset starch on dry weight basis contains, 14.0% of moisture, 0.16% of ash, 0.25% of fat, 0.35% of protein, and 99.24% starch and 29.0% of amylose. The slight variations are shown between the two studies and the reasons for variations in chemical composition of enset starch might be attributed to differences in cultivar, part of the enset plant which the starch extracted, age of the plant, handling and storage systems, physical and chemical nature of the soil, method of cultivation and climatic conditions [28], [32].

B. Physicochemical Properties

A scanning electron micrograph exhibited as the starch of enset composed of entirely single entities of small and large grains with characteristic shape that is somewhat angular and elliptical; Although the granule surfaces appeared quite smooth under lower magnification, a few granules showed some signs of fissures under high magnification [26], [32]. According to Gebre-Mariam and Schmidt [26], the differential scanning calorimetric analysis of enset starch at a heating rate of 10 °C/min and starch to water ratio of 1:2, the onset (To), peak (Tp), and end set (Te) temperatures of gelatinization were 61.8, 65.2 and 71.7°C, respectively. Awol *et al.* [32] also reported as the differential scanning calorimetric analysis of enset starch at the same heating rate (10 °C/min) and moisture level of 1:2 (~67%), (To), (Tp), and (Te) of gelatinization were 64.37, 68.77, and 73.37 °C, respectively. The results are slightly higher than the results of previous study. The variation might be due to the sample preparation method, age, variety and part of the enset

plant at which the starch obtained, amylose to amylopectin ratio, granular architecture (crystalline to amorphous ratio), and molecular structure of amylopectin may be attributed to the results obtained [32].

C. Comparison of Enset starch with Potato and Maize starch

Gebre-Mariam and Schmidt also compared the similarities and differences of enset starch among the well utilized starches, potato and maize (Table 2.2 and Table 2. 3). The fat and protein content of enset starch was greater than potato starch and lower than maize starch. The ash content of Enset starch was higher than maize starch but lower than potato starch. Enset starch showed normal particle size distribution similar to that of potato starch with the average volumetric particle diameters being about 46 and 44pm respectively, and X-ray powder diffraction patterns indicate that enset starch is of B-type. The properties of swelling and solubility of enset starch is lower than potato starch and it has higher property than that of maize [26]. The Swelling capacity and solubility of enset starch increase with increasing temperature, making it suitable in products of higher temperature processing. Increase in temperature resulted in higher leaching of amylose from the starch, as granules gelatinize at higher temperature resulting in higher swelling power and swelling of starch granules also affects their solubility [32].

Table 2.2. Comparison on the proximate composition of Enset, Potato and maize starches

Starch component	Enset	Potato	Maize
Moisture	14.0	13.50	13.0
Ash	0.16	0.20	0.10
Fat	0.25	0.05	0.50
Protein	0.35	0.20	0.40
Amylose	29.0	29.3	29.7

Table 2.3. Comparison on swelling power and solubility of enset, potato and maize starches

Temperature	Swelling power			Solubility (%)		
	Enset	Potato	Maize	Enset	Potato	Maize
65℃	20	78	9	17	19	3
75℃	45	150	10	26	28	5
85℃	80	340	13	37	45	10

According to Gebre-Mariam and Schmidt, the To, Tp, and Te, of enset starch (61.8, 65.2 and 71.7℃, respectively) are higher than potato starch (58.7, 62.6 and 68.1℃) but lower than maize starch (63.2, 69 and 75.2℃). As the gelatinization temperature reflects the degree of orderly arrangement of the molecules in the starch granules, it may be assumed that potato starch is more fragile than enset or maize starch. The enthalpy (ΔH) value of gelatinization of enset starch (21.6 J/g) is also higher than potato starch (19.8 J/g) and maize starch (16.2 J/g). After gelatinization, the viscosity of each starch increases markedly, mainly because of the lack of water to act as a lubricant between the swollen granules [26].

The peak viscosity (Pv) at any concentration is an important distinguishing feature of a starch from other species. Enset starch shows a peak viscosity value (884 BU) which is between potato starch (1668 BU) and maize starch (302 BU). Potato starch exhibits unrestricted swelling showing maximum viscosity at a relatively shorter period of heating (14.3 minutes). But the granules being fragile, the viscosity falls precipitously with time of heating. Enset starch attains its maximum viscosity after a heating period of 33 minutes which was slightly longer than for maize starch (30 minutes). It is also noteworthy that the maximum viscosities of enset and maize starch are attained at higher temperature (95℃) as compared to potato starch (72℃). During the isothermal holding at 95℃, the viscosity of all the starches goes down, but at a relatively lower rate for enset and maize starches as compared to potato starch [26].

2.3.3. Industrial Applications of Enset Starch in Ethiopia

- Enset starch has a significant and wider application in the pharmaceutical industries. Since native enset starch has similar binding and disintegrating properties with potato starch in granulated tablet formulations, it is used in pharmaceutical gelling, drug loading and release processes, and as a tablet binder and disintegrant [28].
- The squeezed and dehydrated product of enset (Bulla) is used as a gelling agent, capable of producing an equivalent number of shoots, roots, leaves, shoot height, and associated fresh weight of plantlets in pineapple by replacing agar invitro propagation [28].
- Enset fibre which produced mainly from decorticated pseudostem has an excellent structure and a strength equivalent to the fibre of abaca. Around 600 tons of enset fibers have been sent to factories each year. This infers that enset fibre has a significant application in paper

and pulp industries and also in construction as reinforcement in gypsum room decorations and panels [28]. Even though, enset starch and fibre have better qualities and potential, there is a limited use of enset plant in industrial applications.

2.4. Overview of Glucose Syrup

A glucose syrup is defined as 'A purified and concentrated aqueous solution of nutritive saccharides derived from starch', and having the following characteristics [1]:

- Dry matter of not less than 70%.
- A dextrose equivalent (DE), expressed as D-glucose, of not less than 20% based on dry matter.
- A sulphated ash content of not more than 1% on a dry basis.

Glucose sugar is available in liquid, solid and transparent form (similar to honey) and used in large quantities in fruits, crystallized fruits, bakery products, pharmaceuticals, and brewery products [27]. Glucose or dextrose sugar is also found in nature in sweet fruits such as grapes or honey [33].

2.4.1. Types of Glucose Syrups

Depending on the method used to hydrolyze starch and on the extent to which the hydrolysis reaction has been allowed to proceed, different grades of glucose syrup are produced, which have different characteristics and uses. The syrups are generally categorized according to their dextrose equivalent [8]. Most glucose-bearing syrups are sold in liquid form usually containing between 70 and 85% dry substance, but they can also be dried to form granular, semicrystalline, or amorphous products. These solid syrups range in degree of conversion from low-DE maltodextrins to highly converted (>96 % DE) total sugars [34]. Schenk [34] divided glucose syrups into five groups based on the basis of their dextrose equivalent (DE) as shown in Table 2.4.

Table 2.4. Classification of glucose containing syrups according to DE

Degree of hydrolysis	Accepted terminology
< 20 DE	Maltodextrins
20 - 38 DE	Type I
38 - 58 DE	Type II
58 - 73 DE	Type III
> 73 DE	Type IV

Dextrose equivalent

The degree of hydrolysis is commonly defined as the dextrose equivalent (DE), which is a measure of the total reducing sugars present in a glucose syrup [2]. Starch has a DE value of zero, whilst dextrose, the final end product of starch hydrolysis, has a DE value of 100. DE does not tell you how much dextrose is present in the syrup. The amount of dextrose in a syrup, together with other sugars, can be determined using high pressure liquid chromatography. The DE of a glucose syrup can be measured using the Lane and Eynon Fehling's Titration, DNS method, or by using a cryoscope [1].

2.5. Glucose Syrup Production Techniques

As mentioned previously, starch is a polymer of dextrose units, all joined together. The initial process of converting starch to a glucose syrup is usually referred to as either 'hydrolysis' or 'conversion', which means breaking down the chain of starch into dextrose units, either individual units or several units joined together, through a chemical (acid) or an enzymatic process. Originally, there was only one type of glucose syrup-42 DE glucose syrup, also known as 'Confectioners Glucose', and was made by the hydrolysis of a starch slurry with acid, under pressure in a batch process. Today, with the availability of both acids and enzymes, there are now several different techniques such as acid hydrolysis, acid enzyme hydrolysis, acid enzyme-enzyme hydrolysis, and enzyme-enzyme hydrolysis, resulting in syrups of any DE or sugar spectrum [1]. The most commonly used technique is acid hydrolysis and enzyme hydrolysis.

2.5.1. Acid Hydrolysis

In the past, hydrolysis of starch was widely carried out using acids such as hydrochloric acid and sulfuric acid as catalyst [35]. In the acid conversion process, a starch slurry usually having a dry substance content between 30 and 40 wt. % prepared at a temperature of about 20–30℃ is acidified to pH 2 or lower and subjected to a combination of time, temperature, and pressure to effect hydrolysis [1], [34]. Higher temperature, permitted by operation under pressure, reduces the reaction time considerably. For example, whereas KIRCHOFF's conversion at atmospheric pressure, lasted 36hr, early starch converters that were limited to pressures of 35 kPa took 6hr to complete the reaction. Moreover, modern converters accomplish the conversion within minutes, at 415 – 620 kPa and up to 160℃ [34].

Typically, modern processes use a two-stage plate and frame heat exchanger to obtain the required residence time for the hydrolysis reaction. The acidified starch slurry is pumped into the first heat exchanger, which is heated, indirectly to a temperature of 143℃. At this temperature, the starch slurry is instantly gelled to form a very viscous paste. As the paste leaves the heat exchanger, about 90% of it is recycled and mixed with about 10% of the ungelled acidified starch slurry which is feeding the heat exchanger, and is again heated to 143℃. The reason for recycling part of the gelled starch is to ensure that the starch is properly gelled, and to reduce the initial viscosity of the freshly gelled starch to a pumpable paste. It also helps to keep the ungelled starch granules in suspension during the initial conversion stage. The gelled starch now passes to the second heat exchanger, which has a temperature of 135℃ and residence time of about twenty minutes. This ensures that all of the starch has been fully gelled and converted into a 42 DE syrup [1].

After hydrolysis, When the desired degree of conversion is achieved, temperature and pressure are reduced and the reaction is stopped by addition of a neutralizing agent (usually sodium carbonate), raising the pH to 4 – 5.5 [34]. This pH is also the isoelectric point for the proteins present in the starch, which are now precipitated out of solution [1]. The precipitated impurities, together with any fine fibers, fats and oil from the original starch are removed by centrifugation or by skimming operations; solid impurities can be removed by passing the liquor through deep tanks with a weir that separate the fat and protein flocks from the liquor [1], [34]. The remaining impurities are color precursors, flavor and odour contaminants, proteins or protein hydrolyze products, peptides, amino acids and are refined using powdered or granular activated carbon techniques or ion-exchange resins [35]. After refining, the syrup liquor is now evaporated under vacuum up to 70% solids to prevent microbial growth, and either stored at these solids, prior to further processing or evaporated up to a final solid of about 80-82%, based upon the syrup [1].

Several disadvantages attach to the aforedescribed process.

- While acid conversion is a random-action mechanism, it results in remarkably reproducible saccharide composition for any given degree of hydrolysis. This reproducible composition is actually a limitation of the acid process because the manufacturer cannot influence the saccharide distribution for a given degree of conversion, which is important in some applications of glucose syrups.

- Acid syrups having a DE value below 30 will tend to cloud upon standing due to precipitation (retrogradation) of the longest chain linear polymers.
- Also, even if acid hydrolysis to DE values in the high 80s is possible, the extreme conditions required to produce DE values of 50, or higher, promote the formation of excessive amounts of glucose degradation products; typically, the production of hydroxymethylfufural and other bitter compounds, due to side reactions which are difficult to remove during refining. These color precursors cause the product to yellow eventually [34]. The most common acid glucose product has a DE value of about 40 – 45 % because of these upper and lower conversion limitations.
- Acid hydrolysis is energy intensive, require the use of materials that do not corrode easily, relatively difficult to control, give rise to high colour and salt-ash content (after neutralization). For this reason, enzymes are now used for producing high DE syrups. As a general rule, acids are cheaper than enzymes, but enzymes are more specific [1].

2.5.2. Enzyme Hydrolysis

Currently, Enzymatic hydrolysis is largely substituting the acid hydrolysis because it is easy to control, effective even in mild ambient conditions and do not give rise to any by-product. Enzymatic hydrolysis of starch is done with the use of amylase enzymes from microbial and plant sources [35]. The industrial processing of starch through enzymatic hydrolysis involves gelatinization, hydrolysis (liquefaction and saccharification), carbon treatment, ion exchange treatment, and evaporation to final solids [1], [27]. However, before conversion of starch to glucose started, the starch must be separated from the plant material. This includes removing fiber and protein, since protein produces off-flavours and colours due to the Maillard reaction, and fiber is insoluble and has to be removed to allow the starch to become hydrated. The enzymatic production process is described below.

a) Gelatinization

For an enzyme to be effective in converting starch, the starch must be made into a slurry, which is then cooked to form a gel or paste. Next, both the temperature and pH have to be adjusted to working range for that particular enzyme [1]. Gelatinization is merely a process of breaking inter- and intra-molecular hydrogen bonds and hydrophobic interactions within the starch granule, which

results in granular swelling, crystallite melting, loss of birefringence, viscosity development, and solubilization [36], [37]. The gelatinization temperature is different for different starches. It depends on the moisture content, degree of crystallinity within the granule, granule size, and amylose to amylopectin ratio [4]. The solids of the starch slurry should be about 30–35%. The reason for the starch slurry having lower solids than for acid hydrolysis is because the initial viscosity of the starch paste is very high, therefore by using lower starch solids, the viscosity of the paste is reduced to more manageable proportions. If the viscosity of the starch paste is reduced by shearing, then there is the possibility that the enzyme could be destroyed [1]. Generally, gelatinization process results in the preparation of starch for hydrolysis.

b) Liquefaction

Conventionally, liquefaction is the process of melting starch gel to obtain lower viscosity, by reducing the length of amylose and amylopectin/hydrolyzing starch into simpler molecules of oligosaccharides or dextrin through using the α-amylase enzyme [38]. In such an enzymatic process, the pH and temperature of the process are determined by the conditions suitable for use of the enzyme [4], [39]. During this process thermostable α-amylase is added and hydrolyses the α-1,4-glycosidic bonds in pregelatinized starch, producing water soluble maltose, and higher maltodextrin oligomers [40]. Enzymatic liquefaction requires careful control of reaction parameters such as percentage of solids, temperature, time, pH, and calcium level to ensure efficient hydrolysis, minimize enzyme cost, and reduce the potential of downstream processing problems. Typical operational ranges for the enzymatic hydrolysis are discussed as follow:

1. **Starch Solids:** The maximum obtainable dextrose level is mainly a function of the nature of the enzyme used and the dry substance [41]. For instance, reducing solids from 30 to 20 or to 10%, increases maximum attainable dextrose level from 96 to 97.5 and nearly 98%, respectively. However, operating at lower solids concentration require larger tanks, increase cost of water removal from final product, and increase risk of microbial infection in a dilute system. Whereas at higher solids concentrations, available water content is not sufficient to achieve complete starch gelatinization, and the re-polymerization (reversion) is also favored and consequently, the maximum obtainable dextrose is reduced. Normally, an initial dry substance level of around 30-35% w/w is preferred as an economic compromise between too low final dextrose and too high evaporation cost [2], [29].

2. pH: Starch slurry pH normally is controlled at 5.5-6.5 during liquefaction process. At a higher pH, by product formation is increased, resulting in loss of product yield and increased refining costs. If pH is decreased below the optimum range, enzyme becomes more subject to inactivation if upsets in process control occur [29].

3. Calcium: Some α-amylase enzymes require calcium ions to act as a stabilizer/activator. Thus, calcium is added in the chloride or oxide form as an enzyme cofactor to enhance α-amylase thermostability. Generally, a calcium level of 100-200 ppm (starch dry basis) is sufficient for B. stearothermophilus and B. licheniformis enzymes. A higher calcium level of about 300ppm is often used with B. subtilis enzymes [29]. However, calcium salts could cause a haze in the final syrup [1].

4. Time/Temperature: Reaction time and temperature should be balanced to provide the optimum conditions for enzyme activity and rapid starch gelatinization. Normally, temperature is controlled to be as high as possible, while still permitting sufficient enzyme activity to achieve the desired degree of hydrolysis. For the typical commercial thermal stable bacterial α-amylase enzyme, the optimal temperature range is 90-110℃.

c) Saccharification

The Second enzymatic process is the conversion of the liquefied starch to a sugar syrup (saccharification). In saccharification, liquid dextrin further hydrolyzed into glucose by a single enzyme (glucoamylase) or mixed enzyme, glucoamylase and pullulanase [36]. At this stage, liquefied hydrolysate of 30-35% (w/w) solids is adjusted to pH 4-4.5 and 55-65℃ based on working condition of saccharifying enzyme(s) [4], [41]. The breakdown product formed in the addition of glucoamylase during saccharification is glucose, which has been split off from the non-reducing end of the substrate molecule. Maltotriose and in particular maltose are hydrolysed at a lower rate than higher saccharides, and α-l,6-linkages are broken down more slowly than α-1,4-linkages. Eventually, a practically complete conversion of starch into glucose is obtained [2].

Alternatively, α-1,6-linkages of the branched dextrins are rapidly hydrolysed by using Promozyme together with an amyloglucosidase at the start of the saccharification. Promozyme® is a pullulanase, which hydrolyses the α-1,6-glycosidic bonds in starch which has been partly hydrolysed by α-amylase, provided that there are at least two glucose units in the side chain [2].

Consequently, fewer branched oligosaccharides accumulate towards the end of the saccharification. The point at which reversion outbalances dextrose formation is thus shifted towards a higher dextrose level. The maximum dextrose obtainable with AMG and Promozyme mainly depends on the enzyme dosage ratio and dry substance level. The action of amylases and debranching enzymes is shown in Figure 2.4.

Glucoamylase also catalyzes reversion, in which dextrose molecules are combined to form maltose and isomaltose. The time necessary to reach the maximum obtainable dextrose level depends on the enzyme dosage. If a given dose of glucoamylase yields maximum dextrose in 4 days, doubling the dose will reduce reaction time in to 2 days, so the final hydrolysate can be processed rapidly to prevent excessive reversion. Consequently, over dosing with glucoamylase or extending reaction time beyond the maximum dextrose level will result in a decrease in dextrose yield because of continued formation of isomaltose via reversion [29]. In order to prevent excessive reversion of glucose to isomaltose, it is necessary to inactivate the glucoamylase within a few hours after maximum dextrose has been obtained. When certain enzymes like promozyme are used in combination with glucoamylase, the dosage of glucoamylase and rate of reversion will normally be relatively low, as a result the risk of losing dextrose by reversion is reduced. [29], [41].

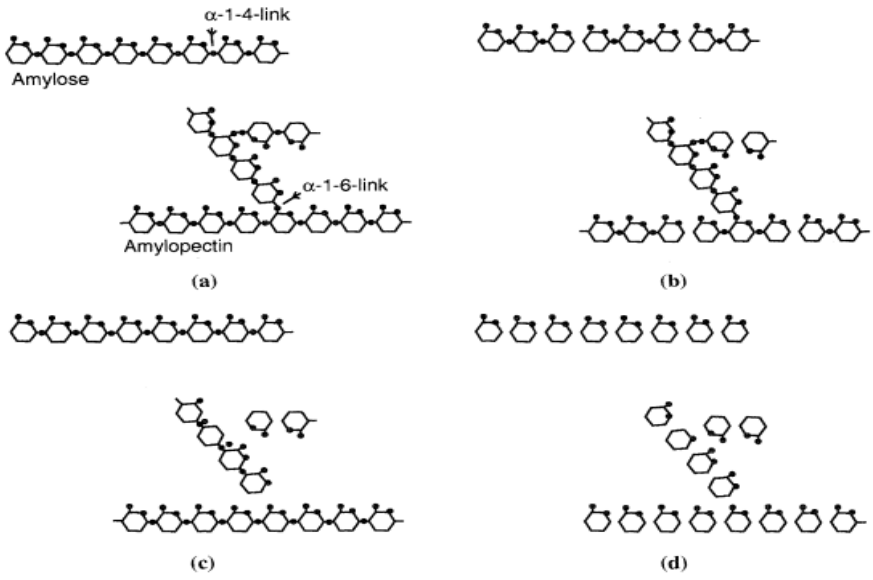


Figure 2.4. Effect of the action of starch degrading enzymes (A) Amylose and amylopectin structures (B) Effect of fungal a-amylase on amylose and amylopectin (C) Effect of a debranching enzyme on amylose and amylopectin (D) Effect of amyloglucosidase and debranching enzyme on amylose and amylopectin [41]

d) Filtration and Refining

The saccharified liquid is then filtered to remove insoluble impurities such as denatured protein, fat to improve its color and stability. In most cases filtration is followed by carbon treatment and ion exchange [41]. Activated carbon is used to remove the organic based impurities, such as proteins, and other colour precursors, while ion exchange resins are used to remove metallic ions (salts and minerals) [1].

e) Evaporation

After refining, the dilute glucose syrup is evaporated under vacuum to raise the solids concentration more than 70% [40]. The reason why syrups are evaporated under vacuum is because the evaporation temperature of the syrup is lower, which means that there is less risk of colour formation due to excessive heat. By boiling at as lower a temperature as possible, there is a cost saving, because a lower steam pressure can be used, and therefore less energy is required [1].

The exact final solids will depend upon the DE of the syrup its viscosity and osmotic pressure. As a general rule, the lower the DE, the lower the solids of the syrup. This is because low DE syrups are viscous, and at higher solids, they would be impossible to handle. This is one of the reasons why low DE syrups and maltodextrins are usually spray dried. The other reason for spray drying is that low DE syrups, because of their high molecular weight, have a low osmotic pressure, and hence are more susceptible to microbiological growth. However, high DE syrups, such as 95 DE, can also be a problem not due to osmotic pressure, but to dextrose crystallisation [1].

Generally, the use of enzymes is preferred to acid, since it produces high yields of desired products, less formation of undesired products such as toxics compounds and ash, the process is more specific, the conditions can be controlled, less cost for purification, and the color damage can be minimized [42].

2.6. Enzymes

Enzymes are biological catalysts, derived from living organisms, such as yeast, bacteria and plants, and are very specific in their reactions. Both the 1–4 and 1–6 linkages in starch are very critical when using enzymes for making glucose syrups, because some enzymes can attack these sites, whilst other enzymes cannot. Therefore, the proper choice of enzyme becomes crucial for a successful process. The starch industry uses four main types of enzymes to convert starch to syrup, namely α-amylase, β-amylase, glucoamylase and pullulanase, and then there is one particular

enzyme, isomerase, which converts dextrose syrup into fructose syrup [1], [35]. Since isomerase is a very expensive enzyme, it is immobilized on a porous carrier. The syrup passes over the immobilized enzyme, as opposed to being mixed with the syrup, and being an immobilized enzyme, it is being continually used, making it operationally more cost effective. The operating pH and temperature of isomerase is ranged between 7.5–8.0, and 60–65℃, respectively [1].

Both Alpha-amylase and β-amylase attacks the gelled starch at the 1–4 linkages to produce dextrose and maltose, but they are unable to hydrolyze the 1–6 linkages. The operating pH and temperature of β-amylase is ranged between 4.5–5.0 and 55–60℃. Most α-amylases work in the pH range of 5.0–7.0, and at a temperature of 65–70℃. However, the glucose industry now uses high temperature stable α-amylases which can typically withstand up to 120℃ for 20 min [1].

Glucoamylase/Amyloglucosidase is capable of hydrolyzing both the 1–4 and 1–6 linkages to produce dextrose, and therefore it is sometimes referred to as a 'saccharifying' enzyme. It hydrolyzes maltotriose and in particular maltose at a lower rate than higher saccharides, and also 1-6 linkages are broken down more slowly than 1-4 linkages [2]. Its operating pH and temperature are ranged between 4.5–5.0 and 55–60℃. A debranching enzyme that can hydrolyse the 1–6 linkages, in which other enzymes cannot attack, thereby making more of the higher sugars available for hydrolysis to maltose and dextrose is referred as Pullulanase. It is for this reason that pullulanase is often used in conjunction with other enzymes like β-amylase and glucoamylase [1].

2.7. Applications of Glucose Syrups

The major applications of glucose syrup in commercially prepared food products are as a thickener, sweetener, humectant (an ingredient that retains moisture and thus maintains a food's freshness), to soften texture, add volume, prevent crystallization of sugar and enhance flavor. They are also used in large quantities in fruits, liquors, crystallized fruits, bakery products, pharmaceuticals and brewery products. Dextrose is used as a raw material for production of sorbitol and polydextrose [29]. Some of the major applications of glucose syrup are discussed below:

a) In fermented products and non-fermented goods

In baking fermented products, it is used as a fermentable carbohydrate source for yeast to provide the proper degree of carbon dioxide production and product volume. In addition, it is important in improving bread strength for better handling and slicing, for producing desired color and flavor

via the Maillard reaction with protein, and for providing good texture and crumb and crust characteristics [29].

In non-fermented goods such as sponges, cakes and pastry, starch-based sweeteners can be used, where they can replace some of the sucrose or reduce the overall sweetness of the cake [1]. Dextrose exhibits about 75% of the sweetness of sucrose and, therefore, is used in applications in which less sweetness is desired [29]. They can also increase the shelf life of the product with their humectant properties and enhance the colour and improve the volume [1].

b) Beverage

Glucose syrups are used in beer, wine, beverage powders and soft drink for their fermentable properties, flavor enhancement, and to reduce excessive sweetness [29]. In a soft drink the use of glucose syrups, particularly HFGS are ideally suited since the sugar profile of glucose syrups is totally stable being unaffected by pH or temperature, and therefore will not change during storage resulting in both the sweetness and flavor profiles remaining unchanged [1].

c) Confectionery

The sugar confectionery companies, which make hard boiled sweets, toffees, caramels, fudge, fruit gums, etc., are possibly one of the largest users of glucose syrup. In confections, dextrose is used in candies to control crystallization and provide sweetness and softness. It is also used for coating, strength, hardness, color, and gloss in candy and gum [29].

d) Medical applications

Dextrose is used in a purified anhydrous form in medical applications such as intravenous solution production and tableting. Additionally, it is added to pharmaceutical products to protect their shelf life, usually for a period of up to 12 months and used as a raw material in fermentations for the production of antibiotics, citric acid, amino acids, enzymes, lactic acid and ethanol [29].

e) Miscellaneous

Additionally, Glucose syrup can be used:

- in ice creams and jams extensively, to tailor the sweetness profile of products, to enhance both texture and flavor, to control crystallisation, to protect their shelf life of jams, due to their high osmotic pressure, glucose syrup have excellent preserving properties and etc. [1].

- in both tomato based products (ketchup, sauce and puree) and salad type dressings (salad cream, mayonnaise, salad dressings and vegetable mixes such as sandwich spread), by replacing sucrose wholly or partly, they offer both a cost saving and a product enhancement by improving both the texture and viscosity of these products [1].
- as a health or sports drink is to supply the body with a ready source of energy (since they are easily broken down by the body), to replace electrolytes, for example sodium chloride and water, lost through perspiration and to maintain or enhance an athlete's performance [1].
- as an additive in adhesives to control flow, in library paste to increase bonding time, in wall board to prevent brittle edges, in concrete to retard setting, in resins as a binder, in dyes as a diluent, in metal treatment as a reducing agent, in leather tanning for pliability, and as part of the mixture that goes into creating fake blood for films and television [29].

2.8. Effects of Green Tea Extract on The Shelf Life of Glucose Syrup

Because of high osmotic pressure, low water activity and high temperature processing of glucose syrups, they are usually resistant to bacterial spoilage. However, some spoilage microorganisms, such as yeast and mold spores, can survive in the syrup and still grow under storage conditions [8]. Due to this reason preservative is needed to prolong shelf life of the syrup during storage. Preservation of food products can be performed by using either chemical (synthetic) or natural additives [9]. Usually, glucose syrup is preserved using chemical additives such as sulfur dioxide, sodium sulfite, sodium hydrogen sulfite, potassium metabisulfite, potassium sorbate, sodium sorbate, sorbic acid, sodium bisulfite, and among others [10]–[12].

Scientists found that some chemical additives have positive impacts on processed food flavor and storage time, but some have turned out to have negative effects on human health, such as Butylated hydroxyl anisole, triphenylmethane dyes, amaranth, cyclamates and possibly saccharin (suspected carcinogen). Additionally, a high intake of benzoic acid could cause some adverse health effects, such as metabolic acidosis, convulsions, and hyperpnea [9]. High concentration of sodium bisulfite intake could also lead to serious health hazards [10]. Therefore, adequate measures must be put in place to check the shelf life of glucose syrup.

Some natural products, especially natural antioxidants, and antimicrobial agents, could be used as safe alternatives to synthetic compounds. For instance, polyphenols (natural antioxidants) which

are commonly extracted from vegetables, fruits, beverages (juices tea, wine,), plants, seaweeds, and some herbs have significant antioxidant and antimicrobial abilities. Furthermore, natural food additives, such as tasmannia lanceolate solvent extracts, eucalyptus and tarragon essential oil have antioxidant, and antibacterial properties to fight bacteria and fungi growth, and are not harmful to human health [9].

Green tea extracts provide natural and safe antioxidant options to replace synthetic antioxidants [14]. It contains numerous polyphenolic components with antioxidant properties, but the main active components are the flavanol monomers known as catechins, where epigallocatechin-3-gallate and epicatechin-3-gallate are the most effective antioxidant compounds [17]. Additional active components of green tea extract include the other catechins such as epicatechin and epigallocatechin [14]. Tea polyphenols have been reported to have strong antioxidant property and free radical scavenging activity due to possession of a phenolic hydroxyl group attached to theflavan-3-ol structure [43]. Free radicals are closely related with oxidative damage, while antioxidants are reducing agents, which reduce oxidative damage to biological structures by donating electrons to free radicals and passivating them. [44].

In addition to their antioxidant activity phenolic compounds have been associated with properties such as anticancer, antidiabetic, antimicrobial, [13], [45]–[47]. Green tea extract exhibited similar antioxidant performance to conventional synthetic antioxidant tert-butylhydroquinone and perhaps more cost-effective than other natural sources of antioxidants [17]. Accordingly, green tea extracts may act as a promising antibacterial agent, antifungal agent as well as a natural antioxidant substitute and thereby contribute to human health. Therefore, the present study was undertaken also to study the effects of green tea extracts on the quality and shelf life of the produced glucose syrup.

3. Materials and Methods

3.1. Experimental Framework

This research was conducted according to the Figure 3.1 which shows the main activities performed, starting from sample collection/preparation to the end product preservation.

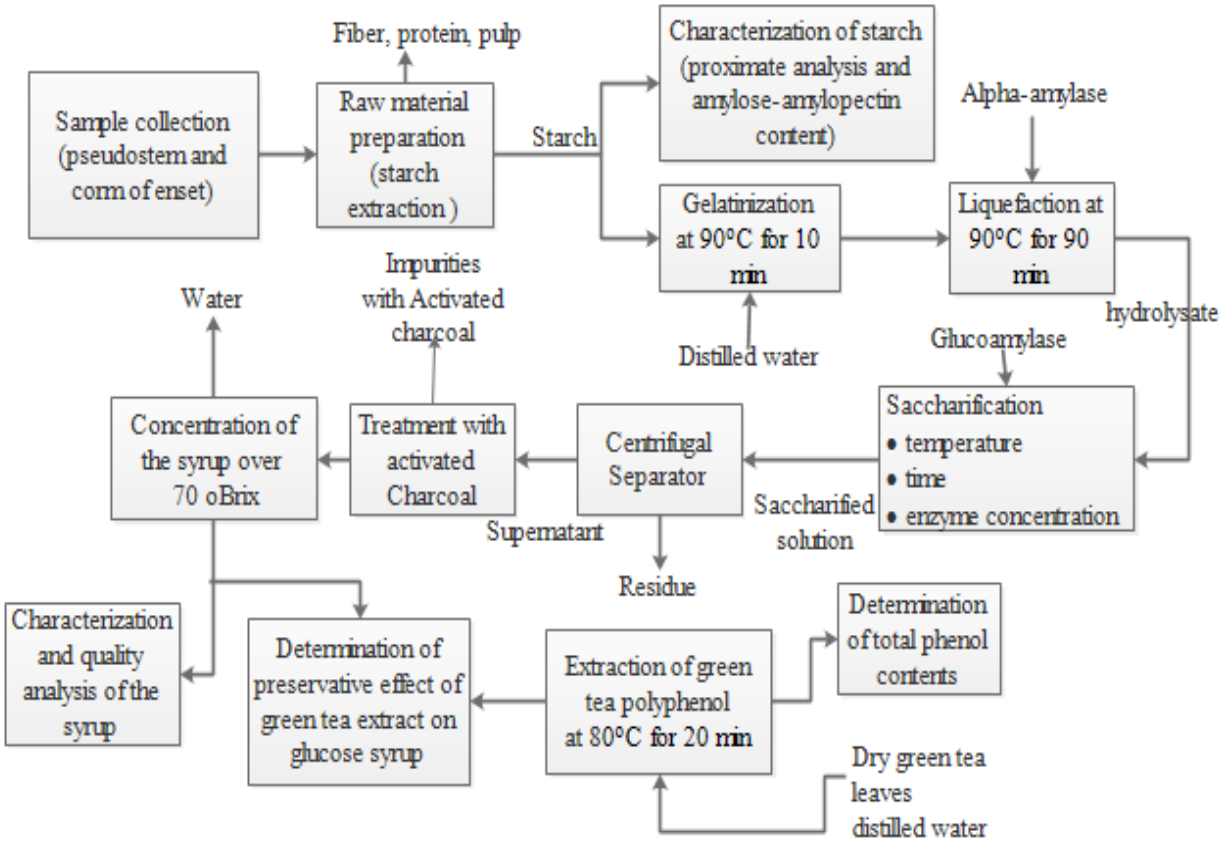


Figure 3.1. Experimental Framework

3.2. Materials

3.2.1. Sample Collection, Preparation and Experimental Site

Sample collection and Experimental sites: The raw material used in this study was the starch extracted from the corm and pseudostem of Ensete ventricosum (Gewada variety), which reaches maturity stage. The sample (plant) was obtained from Areka Agriculture Research Center (AARC) and transported to AAIT, School of Chemical and Bio Engineering laboratory wherein the experimental work was carried out. It was stored at room temperature in an open air before use. Dry green tea leaves processed in Ethiopia were purchased from local supermarket.

Preparation: First, the sample taken from the corm and pseudostem was chopped with the help of knife and soaked in water. Then it was pulverized using heavy duty onion chopper machine and squeezed in order to extract the starch from fibre, pulp and etc. With the aim of extracting the remaining starch, the sample was soaked with water and squeezed again. The liquid/starch slurry was allowed to settle and the supernatant was decanted. Water was added to form homogenized slurry and the slurry was filtered using a muslin cloth to remove the remaining particles with several washes until the wash water became clear. The starch slurry was allowed to settle and the supernatant was drained. Compact mass of starch was collected, dried on the sun and packed with polyethylene bag to be used as raw material for glucose syrup production.

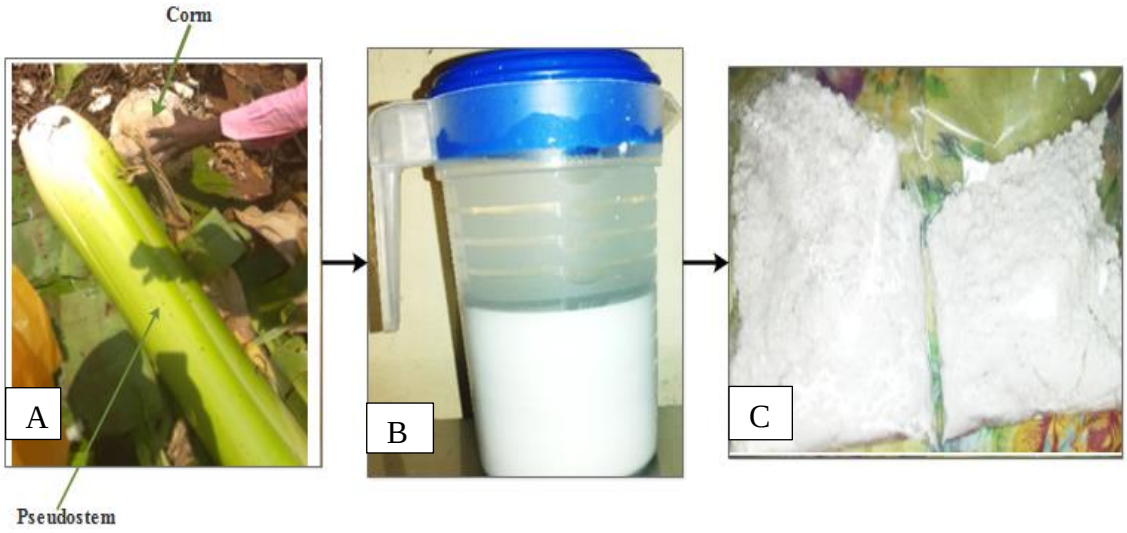


Figure 3.2. Raw material preparation

Whereas,

- A represents harvested Enset plant
- B represents isolated starch from Enset after several wash
- C represents enset starch dried on sun

3.2.2. Chemicals and Reagents

Table 3.1. List of chemicals and reagents

Chemicals and Reagents	**Purpose**
Alpha amylase (Hitempase STXL)	To hydrolyze the starch (liquefaction)
Glucoamylase (Attenuzyme® Core)	For hydrolysis (saccharification process)
Sodium hydroxide Hydrochloric acid	For pH adjustment during production of syrups
Dextrose anhydrous DNS Reagent • 3,5-Dinitrosalicylic acid • Rochelle salt • NaOH	For determination of reducing sugar contents of glucose syrup
Calcium chloride	For stabilization of alpha amylase
Diethyl ether	For determination of crude fat content
Iodine solution • Potassium iodide • Iodine Ethanol, Acetic acid	For iodine-starch test For determination of amylose-amylopectin contents of enset starch
Folin- Ciocaltu reagent, sodium carbonate, Gallic acid, Methanol	For determination of total phenol content in green tea extract
Sulfuric acid, H_2O_2, Hydrochloric acid boric acid, potassium sulphate and copper sulphate	For determination of crude protein content

3.2.3. Equipment and Instruments

The equipment and instruments such as onion chopper machine, Water bath (Optima TXF200), Overhead stirrer (IKA EUROSTAR 40 digital), Spectrophotometers (X - ma 1200 and UVD-3200), Drying oven (Memmert 100-800), Incubator (Memmert,), Orbital shaker (GFL 3017), Centrifuge (Universal 320 R), pH meter (Jenway 3505), Moisture analyzer (Ohaus MB45), Rotary viscometer (Elcometer 2300 RV), Density meter (Anton Paar, DMA 4100 M), Analytical balance (FA2004), Kjeldhal analyzer (kjelteck 2300), Soxhlet apparatus (Soxtec 2055), Rotary evaporator, and Muffle furnace (stuart) were used in the isolation and characterization of Enset starch, and in the production and characterization of glucose syrup and green tea extracts.

3.3. Methods

3.3.1. Characterization of Ensete Ventricosum Starch

Proximate composition of the raw materials such as moisture content, total ash, crude fat, and crude protein of the sample (Ensete ventricosum starch) were evaluated according to the standard methods of the Association of Official Analytical Chemists (AOAC). Amylose content was also assayed using method used by R N Ramli *et al.* [48].

3.3.1.1. Determination of Moisture Content

Moisture content of the starch sample was determined according to the standard method of AOAC 930.15. A clean box on its inverted lid was dried in the drying oven for 1hr at 130±3℃, the box covered with the lid, cooled in the desiccators for 30 minutes, and weighed. From the prepared sample 2g was weighed and shaken until contents evenly distributed. Then the box with the sample was placed in a drying oven and dried for 1hr±5 min at 130±3℃. Afterward, the box was taken out, cooled in a desiccator for 30 minutes and then weighed. The amount of water present in sample is considered to be the loss of weight after drying in oven at 135℃. Finally, the moisture content was calculated as the loss in weight on drying as estimate of water as follow:

$$\text{Moisture (\%)} = \frac{W_1 - W_2}{\text{SW}} \times 100 \quad (1)$$

Whereas, W_1: Weight of the box and fresh sample

W_2: Weight of dry sample and cap

SW: Sample weight

3.3.1.2. Determination of Ash Content

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. Ash content determination was performed following the standard method of the AOAC 942.05. The crucible was washed with 6N HCl by heating on a hot plate, rinsed 3 times with deionized water, dried in an oven at 100℃ for 30 minutes and then cooled in a desiccator for 30 minutes and weighed (W_1). Then, 2.0 g of sample (W_3) was weighed, transferred to the dried crucible and ignited in a muffle furnace at 600℃ for 2hr until light gray ash results. Thereafter, the crucible was cooled in a desiccator for 45-60 minutes and weighed (W_2). The ash content was calculated as:

$$\text{(\%) Ash} = \frac{W_1 - W_2}{W_3} \times 100 \quad (2)$$

3.3.1.3. Determination of Crude Fat Content

The crude fat was determined using a standard method of AOAC official method 2003.06. First aluminum cup with boiling chips was dried in drying oven 30 minutes at 102±2℃, cooled in the desiccator to room temperature and weighed. Therefore, about 5 g of the starch sample was transferred to extraction flask and 90 mL of solvent (diethyl ether) was added in the cup using the connecter on the top of extraction unit. The ethyl ether was evaporated from the extraction flask. After finishing all the process, the extraction cup was removed from the extractor, dried in an oven for 30 min at 102±2℃ to remove moisture, cooled in desiccator for 30 minutes and weighed. The amount of fat/fat percentage in the sample was calculated using the following equation:

$$(\%)\ \text{Crude fat} = \frac{\text{Weight of aluminum cup after extraction} - \text{Weight of aluminum cup}}{\text{weight of the sample}} \times 100 \qquad (3)$$

3.3.1.4. Determination of Crude Protein Content

Crude protein is a measure of how much protein is in food. 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. 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.

Protein content was determined by the Kjeldahl method according to the official method 2001.11 of AOAC. The general procedure included the following steps of digestion, distillation and titration. One g of the starch sample was added in Kjeldahl tube and digested with the addition of concentrated sulfuric acid and then H_2O_2 step by step. When violent reaction stopped, 3g of catalyst (mixture of potassium sulphate and copper sulphate) was added and left for 15 minutes. Then the mixture was digested in digester for 1hr at 420℃. After cooling, it was distilled by steam distillation with 40% of sodium hydroxide and the ammonium (NH_4^+) was released as a form of ammonia (NH_3). Finally, the condensed NH_3 was trapped by 1% boric acid and titrated by 0.1M standard HCl and the analyte was referred to as a crude protein, because the method is determining the nitrogen in a component of all protein.

$$\text{Kjeldahl nitrogen (\%)} = \frac{(V_S - V_B) \times M \times 14.01}{W \times 10} \quad (4)$$

Total crude protein of starch sample was calculated as total nitrogen.

$$\text{Crude Protein (\%)} = \%N \text{ x } CF = 6.25 \times \%N \quad (5)$$

Whereas, Vs= volume (ml) of standard acid used to titrate a test;

V_B= volume (ml) of standard acid used to titrate a blank;

M= Molarity of standard HCl;

14.01 = atomic weight of nitrogen;

W= weight (g) of test portion or standard;

10= factor to convert mg/g to percent;

CF= convert ion factor to convert nitrogen to protein;

Factors are 5.70 for wheat, 6.38 for dairy product and 6.25 for other feed material.

3.3.1.5. Determination of Amylose and Amylopectin Content

Amylose content was assayed using method used by R N Ramli *et al.* [48]. The starch sample of 100mg was weighted and added into 100 mL volumetric flask and 1 mL of 99% ethanol with 9 mL of 1M sodium hydroxide were added. The solution was mixed thoroughly and heated in boiling water for 10 minutes, cooled, and diluted with distilled water until the mark on volumetric flask. Then, from the prepared sample, 5mL of aliquot was pipette out and transferred into new 100 mL volumetric flask and 1mL of acetic acid and 2 mL of iodine solution were added to the new flask. Distilled water was added to the mark and shaken to mix well. The absorbance of the sample was then measured using spectrophotometer at a wavelength of 620 nm. The amylose and amylopectin content were calculated as below:

$$\text{Amylose content (\%)} = 3.06 \text{ x absorbance x } 20 \quad (6)$$

$$\text{Amylopectin (\%)} = 100 - \% \text{ amylose content} \quad (7)$$

3.3.2. Production of Glucose Syrup

For the production of glucose syrup, a suspension of 35% (w/v) of isolated starch from Enset (30g) was prepared using distilled water in 250 mL Erlenmeyer flask. The resulting slurry was homogenized and the pH was adjusted to 5.5 with the addition of either 1M HCl or NaOH. Then the suspension was maintained under heating, gelatinized at 90℃ for 10 minutes in a water bath with overhead stirrer at 200 rpm. Thermostable α-amylase (Hitempase STXL) was added in

pregelatinized starch with a concentration of 0.05% w/w (on dry weight of starch) and calcium ion (150 ppm) was also added in the form of calcium chloride, to keep the catalytic activity of the α-enzyme as stabilizer/protectant and activator for enzyme. Liquefaction was started immediately after the addition of thermostable α-amylase. It was conducted at 90℃, 200 rpm and pH of 5.5, in a water bath with an overhead stirrer. After 90 minutes of hydrolysis, the resulting maltodextrin syrup was boiled for 10 minutes to destroy the activity of enzyme and for sterilization.

Following liquefaction, the resulting syrup was cooled to 60℃ and the pH was adjusted to 4.8 with the addition of HCl. Glucoamylase (Attenuzyme® Core, Novozyme, Denmark, with declared activity of 1600 AGU/g) was added into the suspension with the concentration of 0.1, 0.15 and 0.2 wt.%, and incubated in a shaker incubator. Saccharification was conducted at 50, 60 and 70℃, 100 rpm for 12, 24 and 36 hours. After this process, the hydrolysis reaction was stopped by boiling for 5 min and cooled to room temperature. Afterwards, the hydrolyzate was centrifuged at 6000 rpm for 30 minutes, the supernatant was clarified by heating with activated charcoal 2% (w/v) at 80℃ for 30 minutes and then filtered through filter paper. Finally, the purified syrup was concentrated using vacuum evaporator at 65℃ with a rotational speed of 50 rpm for around 15 minutes to over 70°Brix in a rotary evaporator and DE of final syrup was calculated.

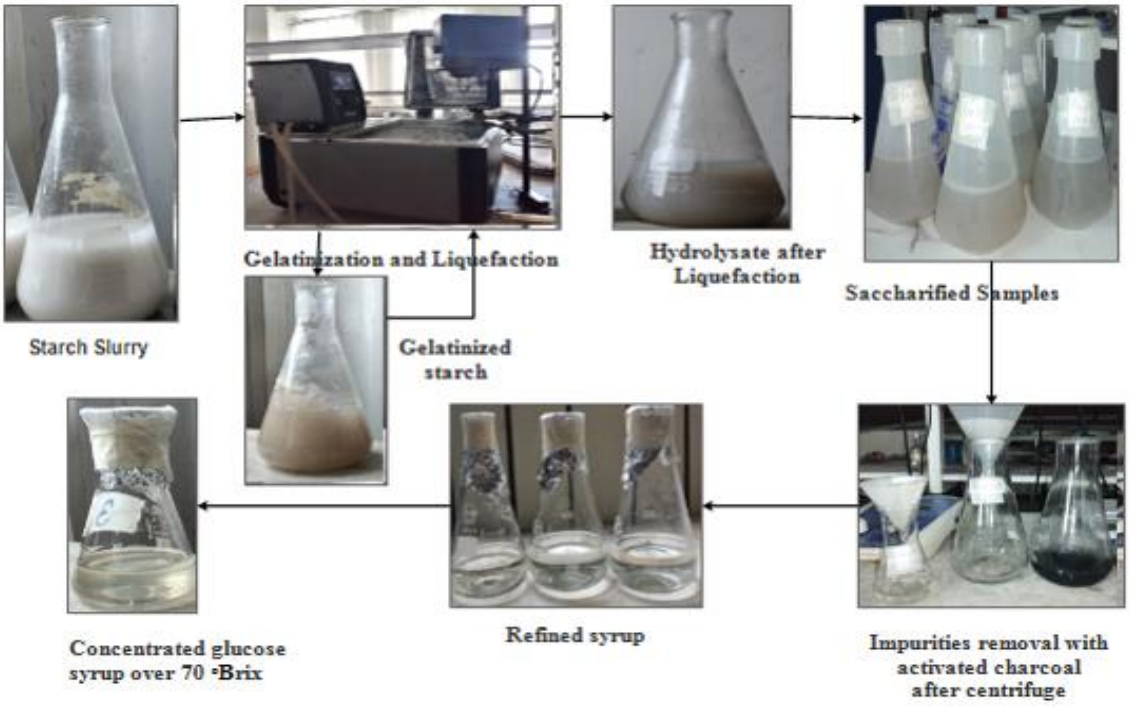


Figure 3.3. Production process of enset glucose syrup via enzymatic hydrolysis

3.3.3. Determination of Reducing Sugar and Dextrose Equivalent

Concentration of Reducing sugar was determined according to DNS method [49], with a little modification and the samples absorbance were analyzed using X - ma 1200 spectrophotometer at a wavelength of 540 nm. The DNS method is a colorimetric technique which consists of a redox reaction between the 3,5-dinitro salicylic acid (DNS, IUPAC name 2-hydroxy-3,5-dinitrobenzoic acid) and the reducing sugars present in the sample. 3,5-dinitrosalicylic acid is an aromatic compound which reacts with reducing sugars and other reducing molecules to form 3-amino-5-nitrosalicylic acid, which absorbs light strongly at maximum wavelength of 540 nm. This method tests for the presence of free carbonyl group (C=O), the so-called reducing sugars. If the oxygen on the anomeric carbon (C1) 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 (shown in Appendix C). 3-amino-5-nitrosalicylic acid is an orange color product, and the intensity of the color depends on the concentration of reducing sugar [12]. Dextrose equivalent is the total reducing sugars expressed as dextrose and calculated as a percentage of overall dry matter.

Preparation steps of 3,5-dinitrosalicylic acid solution 100mL: - Solution of 3,5-dinitrosalicylic acid reagent was prepared by dissolving 1g of 3,5-dinitrosalicylic in 30 mL of distilled water in 100 ml volumetric flask; 30g of Potassium sodium tartrate tetrahydrate (Rochelle salt) in 20mL of distilled water; and by preparing 2N of Sodium hydroxide in 20mL of distilled water. Then the prepared solutions of potassium sodium tartrate tetrahydrate and NaOH were added gradually in to volumetric flask containing the solution of 3,5-dinitrosalicylic acid, mixed and diluted to the mark with distilled water. After the components were completely dissolved, the solution was filtered by filter paper and stored in dark glass bottles at ambient temperature.

Preparation of standard graph: - At first, standard stock solution of glucose was prepared by dissolving 100 mg of anhydrous dextrose with distilled water in 100 mL volumetric flask. Different aliquots of Standard glucose ranging from 0.2 - 1mL were pipette out in to different dry test tubes. The volume in each tube was made up to 1mL with distilled water including blank (distilled water). One mL of DNS reagent was added to all tubes. The tubes are then incubated in a boiling water bath for 5 minutes, cooled to room temperature, and 8ml of distilled water was added to all the

tubes. The absorbance was measured at 540nm and plotted against the concentration of sugar (along X axis) to obtain a standard curve. This curve has to pass through a maximum number of points plotted in line with origin.

Preparation and determination of reducing sugar of the unknown samples: - The unknown samples of glucose syrup were diluted with a dilution factor of 1000 (1g/L). From each of the diluted samples of glucose syrup, 1mL were pipette out in to different dry test tubes and 1mL of DNS reagent was added to all tubes. The tubes are then incubated in a boiling water bath for 5 minutes, cooled to room temperature, and 8mL of distilled water was added to all the tubes. The absorbance was measured at 540nm. The concentration of the samples in terms of reducing sugar were calculated from the equation of the standard curve.

3.4. Extraction of Green Tea Catechins

Studies reported that the optimal conditions to attain the maximum extraction efficiency of green tea catechins with water was obtained at 80℃, and 20 minutes by using 40mL of water for 1g of green tea [15], [50]. Accordingly, the method of extracting green tea catechins was performed at a temperature of 80℃ for 20 minutes, with 1:40 green tea to distilled water ratio in a water bath. The extraction mixture was constantly stirred with the help of overhead stirrer at 300 rpm. After 20 minutes of extraction, the extraction mixture was cooled, vacuum filtered (0.45μm filter paper) and the solvent evaporated under vacuum at 65℃ with the aid of rotary evaporator. Total phenolic contents of the extract were evaluated and finally, its effect on the shelf life of the syrup was determined.

3.5. Characterization and Analysis of Quality of Produced Glucose Syrup

The produced syrup was characterized with the following properties: - Moisture content, Ash content, pH values, Viscosity, Density/specific gravity, total dissolved solids/total sugar levels (°Brix), taste, odor, and color.

3.5.1. Determination of Density, Specific Gravity and Sugar Concentration

Density, sugar concentration and specific gravity were measured by Anton Paar digital density and concentration meter (DMA 4100 M). The measurement is based on the oscillating U-tube method (patented Pulsed Excitation Method) which was invented by Dr. Hans Stabinger and Dr. Hans Leopold at the Joanneum Research Institute (Graz, Austria) and first introduced onto the market by Anton Paar [51].

In order to determine density, specific gravity, and sugar concentration, around 2mL of glucose syrup was introduced into prewashed and dried U-shaped borosilicate glass tube that is being excited to vibrate at its characteristic frequency which is directly related to the density of the sample. The characteristic frequency changes depending on the density of the sample. Through a precise determination of the characteristic frequency and a mathematical conversion, the density of the sample can be measured. The density meter calculated the density from the quotient of the period of oscillations of the U tube and the reference oscillator and displayed density, specific gravity and sugar concentration on its screen [51].

3.5.2. Rheological Properties of Glucose Syrup

Viscosity is a measure of thickness of a fluid and the way which the fluid flow. The produced glucose syrup was determined for its viscosity with the aid of an Elcometer Rotary Viscometer (2300 RV) using Spindle type L4, at a shear rate of 100 rpm, in accordance with ISO 2555 standards. The glucose syrup was transferred into sample bottles and the content in the sample bottle was introduced directly into the rotating spindle and values of the viscosity displayed on the screen of viscometer in mPa.s.

3.5.3. Determination of pH Value

pH is the scale of measurement of acidity or alkalinity in aqueous solutions. The pH meter (3505, Jenway) was used to measure the pH value of the various glucose syrup samples. To make a pH measurement, the calibrated electrode was immersed into the sample solution until a steady reading was observed. Finally, the reading was taken from the display.

3.5.4. Determination of Moisture Content

The moisture content affects preservation and shelf life of the food product. The dry matter of glucose syrup consists of all components (glucose, maltose, and other oligosaccharides, mineral, and non-sugar organic compounds) on the glucose syrup except the water and volatile compounds. The moisture contents of the glucose syrup were determined with the aid of moisture analyzer (Ohaus MB45) according to the thermo-gravimetric or loss on drying principle.

At first, two filter papers were placed on aluminum pan and dried out in a moisture analyzer to remove its moisture content and tared after drying. Afterwards, the glucose syrup was distributed evenly on a dry filter paper with the help of pipette, covered immediately with another dry filter paper and placed on pan. At the start of the measurement, the moisture Analyzer determines the

weight of the sample; the sample was then quickly heated by the integral halogen/infrared dryer unit and the moisture vaporizes at 100℃ for 15-20 minutes. During the drying operation, the instrument continuously determines the weight of the sample and displayed the result as % moisture content until the result become constant.

3.5.5. Determination of Ash content

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 pH adjustments, during hydrolysis and refining. Ash content testing carried out using gravimetric methods (AOAC 1990). Porcelain dish was dried in a 105°C oven for about 1 hour, cooled in a desiccator for 20 - 30 minutes then weighed. A total of 2 g of the sample was weighed and put in a dry porcelain cup. Then the sample was burned on the flame of the burner until it was no longer smoky, then carried out ignition in an electric furnace at a temperature of 550°C for 5hr until white ash was formed. The sample cooled in a desiccator, then weighed, and repeated until a constant weight obtained. Then ash content was calculated by using Equation 11.

$$(\%)\ \text{Ash Content} = \frac{\text{weight of Porcelain dish with ash} - \text{weight of dry Porcelain dish}}{\text{weight of sample}} \times 100 \quad (8)$$

3.6. Determination of Total Polyphenol Content of Green Tea Extract

The green tea extract was evaluated for its total phenol content by the Folin-Ciocalteau method. 40 µL aliquot of a sample (1g of green tea extract per 10mL of methanol) was taken in a test tube and diluted with 3.16 mL of distilled water. Then 200 µL Folin- Ciocaltu reagent was added in a test tube, shaken gently and allowed to incubate for 8 minutes. Sodium carbonate solution (600 µL) was added and the mixture was incubated at 40 ℃ for 30 min. The absorbance of the sample was recorded via UV-vis spectrophotometer at 765 nm against the blank. The assay was conducted in triplicate. The total phenolic contents were calculated on the basis of the calibration curve of gallic acid and expressed as gallic acid equivalents (GAE), in milligrams per gram of the sample. Gallic acid solutions with concentrations of 25, 50, 100, 150 and 200 mg/L were used for calibration. GAE of the total phenolic compounds in the extract was calculated as:

$$\text{Total phenolic content (TPC)} = \text{GAEc} \times \text{V/W} \quad (9)$$

Whereas, GAEc = Concentration of gallic acid equivalent (mg/mL) from standard curve

V= Total volume of the extract (mL)

W= Sample weight (gm)

After determination of TPC, the effect of green tea extract on the shelf life of glucose syrup was evaluated by measuring moisture content, pH, and viscosity of glucose syrup, which contain varying amount of green tea polyphenol, 100ppm, 200ppm, and 300ppm for 30 days within a regular interval of 10 days.

3.7. Experimental design

In this study, Response Surface methodology, particularly, a 3-factor 3-level Box-Behnken design (Design-Expert 12 software) was used for determining the number of experimental runs and the center point for independent variables, resulting in a total of 17 runs. The effect of variables (temperature, time, and concentration of glucoamylase) on the process of saccharification were scrutinized and continued with the optimization of the operational factors using response surface methodology. The design matrix for three variables is varied at three levels (+1, 0 and -1). The results of the response (glucose level) were in the form of dextrose equivalent (%).

Table 3.2. Design summary

File Version	12.0.3.0		
Study Type	Response Surface	**Subtype**	Randomized
Design Type	Box-Behnken	**Runs**	17
Design Model	Quadratic	**Blocks**	No Blocks
Build Time (ms)	484.00		

Table 3.3. Experimental factors, code and the actual value of the research variables

Factor	Name	Unit	Type	Min.	Max.	Coded Low	Coded High	Mean
A	Saccharification Temperature	℃	Numeric	50.00	70	-1 ↔ 50	+1 ↔ 70	60.00
B	Saccharification Time	hr	Numeric	12.00	36.00	-1 ↔ 12	+1 ↔ 36	24.00
C	Glucoamylase Concentration	% w/w	Numeric	0.100	0.200	-1 ↔ 0.10	+1 ↔ 0.20	0.150

4. Results and Discussion

In this study, proximate analysis and amylose-amylopectin contents of the raw material (isolated starch from Ensete ventricosum, Gewada variety) were determined. The concentration of the produced glucose syrup from enset starch was measured in terms of DE and effects of saccharification factors (temperature, time and glucoamylase concentration) on the level of DE were evaluated using RSMD. Then the product was characterized and assayed against standards. Afterwards, total phenolic content of green tea extracts was evaluated and its preservative effects on the shelf life of glucose syrup was examined.

4.1. Characterization of Ensete Ventricosum Starch

Since, the chemical and physical characteristics of the granule starches involve in their properties and functionality, the knowledge of those characteristics allows the selection of starch with the most appropriate properties for particular application and the proper method of modification to obtain the required functional characteristics for a particular end use. The moisture content, total ash, crude fat, crude protein and amylose-amylopectin contents of the raw material (Ensete ventricosum starch) were evaluated.

Table 4.1. Proximate analysis and amylose-amylopectin contents of Enset starch

Component	**Contents (wt.%)**
Moisture	8.98
Ash	0.15
Crude fat	0.38
Protein	0.1
Amylose	29.01
Amylopectin	70.99

Table 4.1 presents the data on proximate composition of starch obtained from Ensete ventricosum plant (Gewada variety). Moisture content influences the storage stability of starches. The recommended moisture level for storing commercial starches is 10–12%, since the moisture contents >12%, encourages microbial contamination and induces degradative biochemical reactions leading to spoilage of starches during storage [52]. As shown in the Table 4.1, the moisture content of the enset starch was found 8.98%, which is lower than the values observed by

Gebre-Mariam and Schmidt that indicates 14%, and Awol *et al.* showed 14.53%, on a dry weight basis. The main reason for the variation in the moisture content of enset starch perhaps attributed to the method of drying applied (sun drying was used in this study).

The result for protein content (0.1%) shows variance with previously studies [26], [32]. The crude ash content complies with the result found by Awol et al. and slightly lower than the result found by Gebre-Mariam and Schmidt. The fat contents of the enset starch obtained in this study was higher than the previous reported studies [26], [32]. The reasons for the variations in chemical composition of enset starch might be attributed to differences in cultivar, part of the enset plant which the starch extracted, age of the plant, handling and storage systems, physical and chemical nature of the soil, method of cultivation, climatic conditions and the method in which the analysis was performed [32].

Native starches usually contain around 20–30% of amylose, and 70–80% of amylopectin [53], which indicates that the amylose contents of enset starch (29.01%) is within the normal range. The amylose-to-amylopectin ratio of starches significantly affects gelatinization and retrogradation properties, swelling power, and the enzymatic susceptibility of starches [54]. High-amylose starches had higher gelatinization temperatures and formed stronger gels [55]. The hydrolysis of the native starches by α-amylase is inversely related to the amylose content, i.e., the starch susceptibility to the hydrolysis was higher for granules with low levels of amylose, indicating that the enzymatic hydrolysis occurred in the branched starch fraction [37].

For instance, one study reported that a higher percentage of hydrolysis for waxy maize starch (56%) was obtained when compared with normal/native starch (39%) [31]. In contrast, Adejumo *et al.* [30] reported that the maize starch with the higher amylose content yields the higher value of DE. Thus, it is not clear whether amylose or amylopectin fraction is more affected by enzymatic hydrolysis. Wu *et al.* [55] investigated the effects of amylose contents on ethanol fermentation efficiency, and reported that the cereals starches which contain <30% of amylose shows insignificant effect on ethanol fermentation efficiency, which may indicate the same effects on the enzymatic hydrolysis of native starch.

4.2. Determination of Reducing Sugar and Dextrose Equivalent (DE)

Concentration of reducing sugar was determined through colorimetric technique that consists of a redox reaction between the 3,5-dinitro salicylic acid and the reducing sugars present in the sample.

The reducing sugar present in the samples were obtained from the calibration curve (shown in Figure 4.1) of known concentration of glucose versus absorbance at 540 nm. Then, DE (the total reducing sugars expressed as dextrose) was calculated from equation (12).

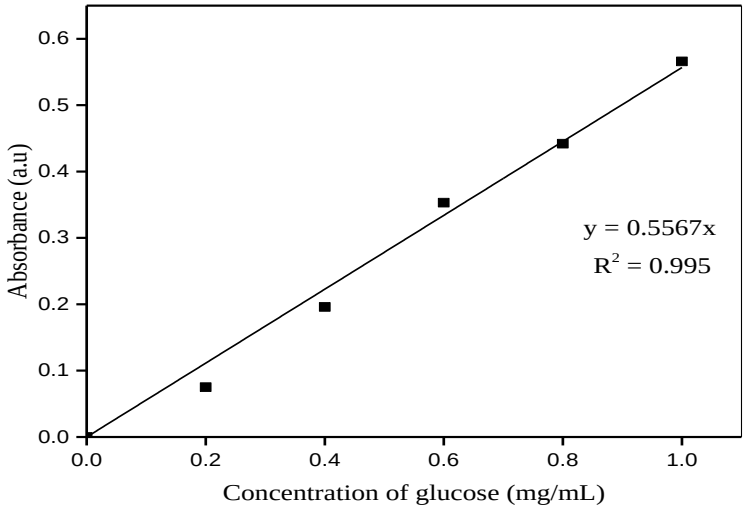


Figure 4.1. The standard graph of known concentration of glucose versus absorbance

The glucose concentrations of the samples were calculated from the equation of the standard curve y = mx + b. The intercept (b) is zero because the line should pass through the origin.

Thus, y = 0.5567x, $R^2 = 0.995$.

From the above straight-line equation, $x = \frac{y}{m} = \frac{y}{0.5567}$ (10)

Reducing sugar (%) = x*DF*100% (11)

Concentration of glucose in terms of DE was calculated as:

$$DE\ (\%) = \frac{\text{Reducing sugar } (\%)}{Total\ soluble\ solid\ (°Brix)} \times 100 \quad (12)$$

Whereas, y = Absorbance at 540 nm, x = Concentration of glucose (mg/mL)

DF = Dilution factor, which was 1000 for the produced syrup.

The effect of temperature, time, and dosage of glucoamylase on the level of dextrose equivalent were studied. Table 4.2 shows, the experimental results obtained within the selected operating range of temperature, time, and dosage of glucoamylase. Based on the result of ANOVA analysis, saccharification temperature ($p = 0.0340$), time ($p < 0.0001$), and dosage of glucoamylase ($p < 0.0001$) has a significant effect on DE. The value of DE increase with increase of reaction time, glucoamylase dosage and temperature. However, the rise of temperature above 60℃, shows a significant decrease of DE, due to thermal deactivation of glucoamylase at high temperature. As it

is observed on the Table 4.2, the lowest DE (72.52%) was obtained at 12hr, 0.1wt.% (dosage of glucoamylase), and 60℃, for saccharification process. Similarly, the highest yield of DE was obtained at 60℃, with lower concentration of enzyme (0.1wt.%), and higher reaction time of 36hr, which resulted in 95.64% of DE. The obtained result in this study was higher when compared with the results of a study conducted by Yunianta *et al.* [4], where they were used sabrang potato starch and the obtained DE was 88.82%, at 60℃, 24hr and saccharification enzyme concentration of 4.50 mg/mL. The variation might be attributed to the variety of starch (amylose-amylopectin ratio) used, the activity of enzyme used and reaction time.

Table 4.2. A dependent variable response against experimental factors

	Factors			**Response**	
Run	A: Saccharification Temperature (℃)	B: Saccharification Time (hr.)	C: Concentration of Glucoamylase (wt.%)	Reducing sugar (%)	Dextrose equivalent (%)
1	60	24	0.15	59.28	90.7
2	60	24	0.15	60.53	91.44
3	70	24	0.1	56.22	83.55
4	70	24	0.2	63.59	89.86
5	50	36	0.15	59.46	86.69
6	50	12	0.15	51.55	77.65
7	50	24	0.1	55.86	82.06
8	70	36	0.15	57.84	87.29
9	60	24	0.15	65.38	92.09
10	60	12	0.2	65.38	90.83
11	60	12	0.1	48.50	72.52
12	60	24	0.15	67.18	92.79
13	70	12	0.15	57.48	79.9
14	60	36	0.2	61.25	87.53
15	60	24	0.15	62	92.5
16	50	24	0.2	59.27	88.02
17	60	36	0.1	65.21	95.64

4.3. Characterization and Analysis of Glucose Syrup

In this study, moisture content, ash content, total soluble solid/sugar, pH, density/specific gravity, viscosity, DE, color, taste, and odor of the product were determined. The proximate composition of the product must be within the specified standard range in order to find different industrial

applications. Glucose syrup should have a dextrose equivalent content of not less than 20.0%, and a total solids content of not less than 70.0% [1], [56]. Table 4.3 shows the physicochemical properties of glucose syrup made from Enset starch against standard quality requirements of glucose syrup according to East African [57] and Indonesian Standards (SNI 01-2985-1992) [58].

The obtained results show that the produced glucose syrup has a high DE content, which fulfills the requirements and included within type-IV glucose syrup (DE >73%) according to Schenk [34] in the world of commerce. However, the moisture content of the syrup was slightly higher than the standards set by Indonesian National Standard (SNI 01-2985-1992) [58], [57]. The increase in the moisture content perhaps due to high water content, volatile solids and the method used to determine the moisture content. Whilst the conventional color of glucose syrups is a 'watery like' or colorless, the color of the produced syrup was found to be light yellowish, which shows similar result with Putri *et al.* [3]; after they treated liquid sugar from sago starch with ion exchange resins. This color formation may occur as a result of Maillard browning reactions between reducing sugars and proteins or amino acids and caramelization due to the effect of heat on carbohydrates [1].

Table 4.3. Quality of produced glucose syrup according to East African/Uganda Standards [52] and SNI 01-2985-1992 [53]

Parameter	**Enset glucose syrup**	**Requirements**
Dextrose equivalent	91.79%	>20%
Moisture	24.01%	Max. 20%
Ash	0.35%	Max. 1%
pH	5.5	4.5-7
Total soluble solid	75.32 ^{o}Brix	>70%
Taste	Sweet	Sweet
Odor	Odorless	Odorless
Color	Light yellow	Colorless

Viscosity: - The produced glucose syrup had a viscosity of 940 mPa.s at 21℃. The viscosity of a glucose syrup is directly related to its moisture content and its molecular weight, as a result viscosity is inversely related to DE. It is also related to the carbohydrate composition of the syrup and this effect is particularly significant when selecting a glucose syrup for confectionery manufacture. Temperature is significant in relation to viscosity and viscosity decreases as temperature increases [2].

Density/Specific gravity: - The determined density and the specific gravity value of the syrup was 1380.8 kg/m^3 and 1.3833, respectively. Density is routinely used to determine the carbohydrate concentration in syrups, juice, and beverages in the food industry. In binary mixtures, the density of the mixture is a function of its composition. Thus, by using density/concentration tables, the density value of a binary mixture can be used to calculate its composition. Accordingly, the sugar concentration can be measured with a density meter. It is generally known that glucose syrup density decreases with increasing water content, and to a lesser extent temperature.

Figure 4.2. (A) Produced glucose syrup (B) Crystalized syrup at room temperature

4.4. Statistical Analysis of the Experimental Results

4.4.1. Experimental Design Analysis

In order to determine the effect of each parameter on the process of saccharification (concentration of glucose), Response surface modeling based on the Box-Behnken design was used. Temperature (A), saccharification time (B), and concentration of glucoamylase (C) were chosen as independent variables to model and optimize according to the conducted experiments. The dependent variable (glucose concentration) was used as a response parameter and express in terms of dextrose equivalent. All experiments were carried out in a randomized order to minimize the effect of unexpected variability in the observed response due to extraneous factors. The experimental results (shown in Table 4.2) were analyzed using Design-Expert v12 software to develop a single model equation that can describe the significance of hydrolysis (saccharification) parameters.

4.4.2. Fit Summary Statistics

As the Sequential Model Sum of Squares, Lack of Fit Tests and Model Summary statistics revealed, the best model for the response surface dextrose equivalent that fits the data is quadratic model.

The fitness of the model equation was expressed by the coefficient of determination, R^2. The coefficient of determination is a statistical measure in a regression model that determines the proportion of variance in the response that can be explained by the independent variables (factors). In other words, R-squared shows how well the observed data fit the regression model (the goodness of fit). Higher R-squared values represent smaller differences between the observed data and the fitted values. Baskar *et al.* [59] have suggested for a good fit of a model, the minimum value of regression coefficient R^2 should be at least 80%. In this case, the regression model was found to be highly significant with the correlation coefficient of determination of R^2 having a value of 0.9920. It implies that 99.20% of the total variation in dextrose equivalent is attributed to the factors and indicating a good fit between the model and experimental data.

Table 4.4. Model Summary Statistics

Source	Std. Dev.	R^2	Adjusted R^2	Predicted R^2	PRESS	
Linear	5.36	0.3833	0.2410	-0.1043	668.79	
2FI	4.45	0.6726	0.4762	0.0938	548.84	
Quadratic	**0.8316**	**0.9920**	**0.9817**	**0.9398**	**36.45**	**Suggested**
Cubic	0.8426	0.9953	0.9812		*	Aliased

4.4.3. Analysis of Variance (ANOVA)

The adequacy of the model was tested by analysis of variance. The P values were used as a tool to check the significance of each of the coefficients, which in turn are necessary to understand the pattern of the mutual interactions between the test variables. The statistical significance of the model was also determined by F-test for analysis of variance. The larger the magnitude of F-test value and the smaller the magnitude of P-values, the higher the significance of corresponding coefficient. P-values less than 0.0500 indicate model terms are significant. Values greater than 0.1000 indicate the model terms are not significant. In this case, Quadratic model has a smaller p-value of < 0.0001 which is less than 0.05 and the Model F-value of 96.54 implies the model is

significant. There is only a 0.01% chance that an F-value this large could occur due to noise. Thus, the model fitted well with the response of dextrose equivalent.

As the results of analysis of variance of the quadratic model on the Table 4.5 revealed A, B, C, BC, A^2, B^2, C^2 are significant model terms while AB, and AC are not significant model terms, i.e., the individual effects of saccharification temperature (A), time (B), glucoamylase concentration (C) and the interaction effects between saccharification time and glucoamylase concentration shows a significant influence on the response of dextrose equivalent value while the interaction effects between temperature and time, and temperature and glucoamylase concentration was not significant.

Table 4.5. ANOVA for Quadratic model

Response: Dextrose equivalent

Source	**Sum of Squares**	**Df**	**Mean Square**	**F-value**	**p-value**	
Model	600.80	9	66.76	96.54	< 0.0001	significant
A-Saccharification Temperature	4.77	1	4.77	6.90	0.0340	
B-Saccharification Time	164.26	1	164.26	237.53	< 0.0001	
C- Glucoamylase Concentration	63.11	1	63.11	91.27	< 0.0001	
AB	0.6806	1	0.6806	0.9842	0.3542	
AC	0.0306	1	0.0306	0.0443	0.8393	
BC	174.50	1	174.50	252.35	< 0.0001	
A^2	100.66	1	100.66	145.57	< 0.0001	
B^2	71.89	1	71.89	103.96	< 0.0001	
C^2	5.49	1	5.49	7.94	0.0258	
Residual	4.84	7	0.6915			
Lack of Fit	2.00	3	0.6670	0.9395	0.5004	not significant
Pure Error	2.84	4	0.7099			
Cor Total	605.64	16				

Factor coding is Coded. Sum of squares is Type III – Partial

The Lack of Fit F-value of 0.94 implies the Lack of Fit is not significant relative to the pure error. There is a 50.04% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good.

Table 4.6. Model statics of the design

Std. Dev.	**0.8316**	**R^2**	**0.9920**
Mean	87.12	**Adjusted R^2**	0.9817
C.V. %	0.9545	**Predicted R^2**	0.9398
		Adeq Precision	34.9213

The Predicted R^2 of 0.9398 is in reasonable agreement with the Adjusted R^2 of 0.9817; i.e., the difference is less than 0.2. This indicates that the predicted values are closer to experimental data and the quadratic polynomial could represent the system for the given experimental domain. The closer the R^2 value to unity, the stronger the model and the better it predicts the response. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 34.921 indicates an adequate signal. Therefore, this model can be used to navigate the design space. A lower value of coefficient of variation, CV = 0.9545% indicates the precision with which the experiments were conducted.

Final Equation in Terms of Coded Factors: - The model equation was developed to show the correlation between the saccharification parameters and percentage of dextrose equivalent.

$$\text{Dextrose equivalent} = + 91.90 + 0.7725A + 4.53B + 2.81C - 0.4125AB + 0.0875AC - 6.61BC - 4.89A^2 - 4.13B^2 - 1.14C^2 \quad (13)$$

Whereas: A is the saccharification Temperature (℃),

B is the saccharification time (min) and

C is the concentration of glucoamylase (wt.%)

The equation in terms of coded factors can be used to make predictions about the dextrose equivalent for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Final Equation in Terms of Actual Factors

$$\text{Dextrose equivalent} = - 176.05275 + 6.00090A + 3.61244B + 446.91500C - 0.003437AB + 0.175000AC - 11.00833BC - 0.048895A^2 - 0.028694^2 - 456.80000C^2 \quad (14)$$

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.

4.4.4. Diagnostic Plots

From the normal probability plot as shown below, the normal probability plot indicates that the residuals following by the normal % probability distribution. Residual is the difference between the value of actual results from experiments and the value of predicted results from the software. In the case of this experimental data the points in the plots show fitted to the straight line in the Figure 4.3, this shows that the quadratic polynomial model satisfies the analysis of the assumptions of variance.

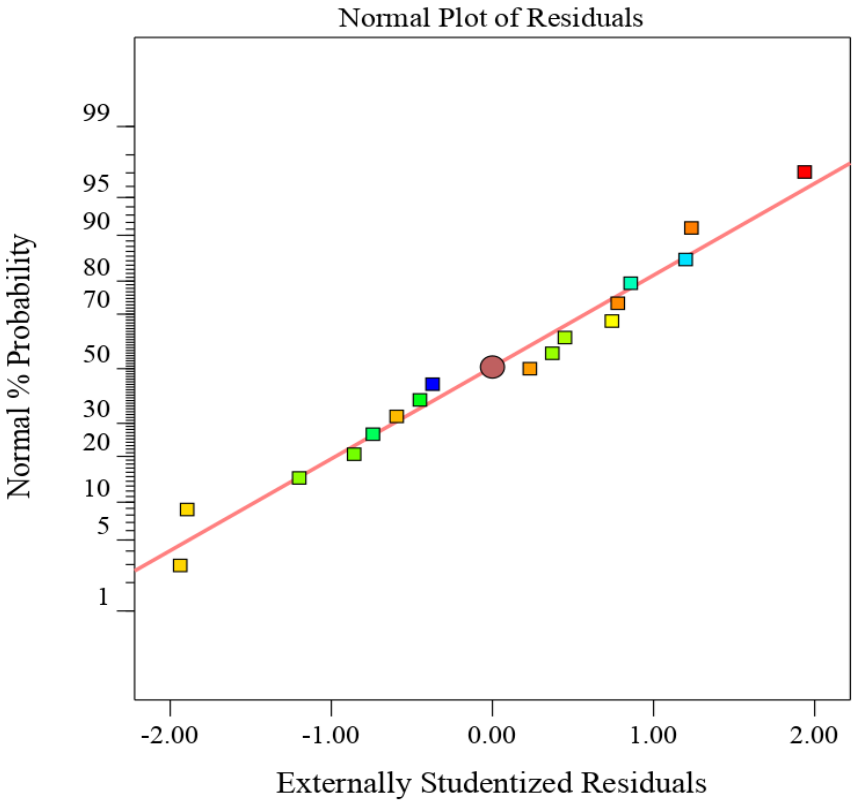


Figure 4.3. Normal probability plot for experimental values

The graph of the observed values versus predicted values as shown in Figure 4.4 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.

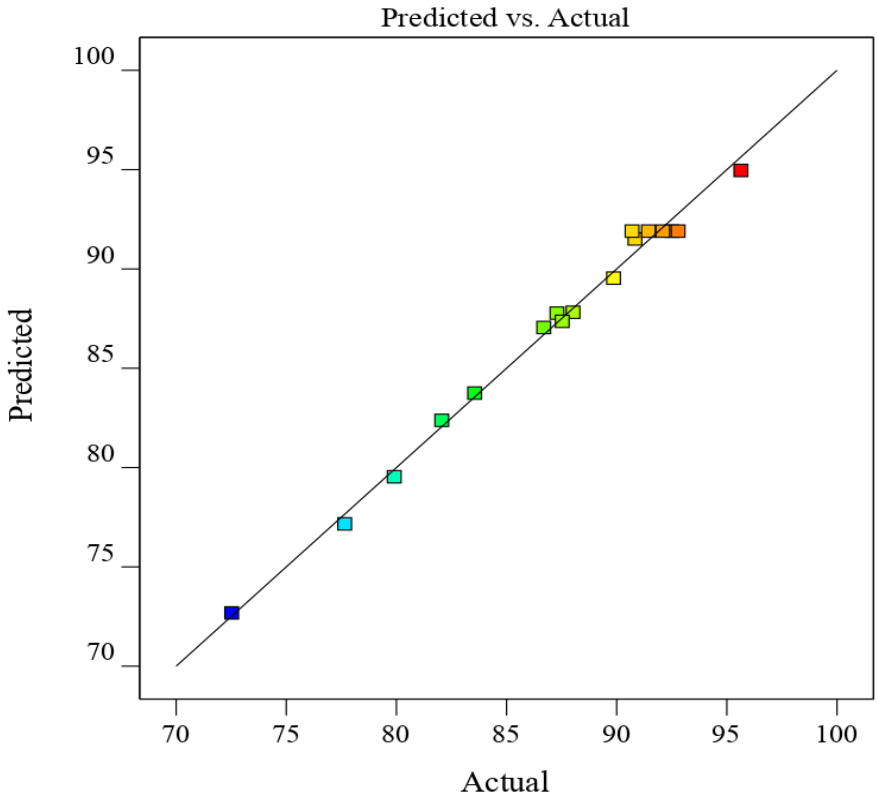


Figure 4.4. Actual versus predicted data plot of response values in the regression model

4.4.5. Effect of Individual Variables on The Level of Dextrose Equivalent

The Individual effect of temperature, time, and dosage of glucoamylase on the level of dextrose equivalent were evaluated to select the best operation conditions for saccharification process using model graphs of RSMD. The range of tested process variables were: Temperature (50, 60, and 70 ℃), Time (12, 24 and 36hr) and Concentration of glucoamylase (0.1, 0.15, and 0.2wt.%).

4.4.5.1. Effect of Temperature

Figure 4.5 represents the effect of saccharification temperature on the level of dextrose equivalent at 24hr, and 0.15wt.% (glucoamylase concentration). As shown in the Figure 4.5, when reaction temperature starts to rise from 50℃, DE increase with the rise of reaction temperature, since the increment of temperature enhance the rate of the hydrolysis reaction i.e. the number of the enzymatic activated molecule goes up with the rise of temperature, so it makes for the process of catalyzing hydrolysis reaction, the yield of reducing sugar shows trend of increase rapidly; when

reaction temperature reaches around 60℃, the yield of reducing sugar turns up the maximum value, temperature continues to rise this moment, however, a significant decrease in the yield of reducing sugar was observed, as a result of the reduction of stability of glucoamylase at high temperature. Thus, as different studies suggest the suitable operating temperature for glucoamylase to get the maximum yield of dextrose equivalent is 60℃ [34], [60].

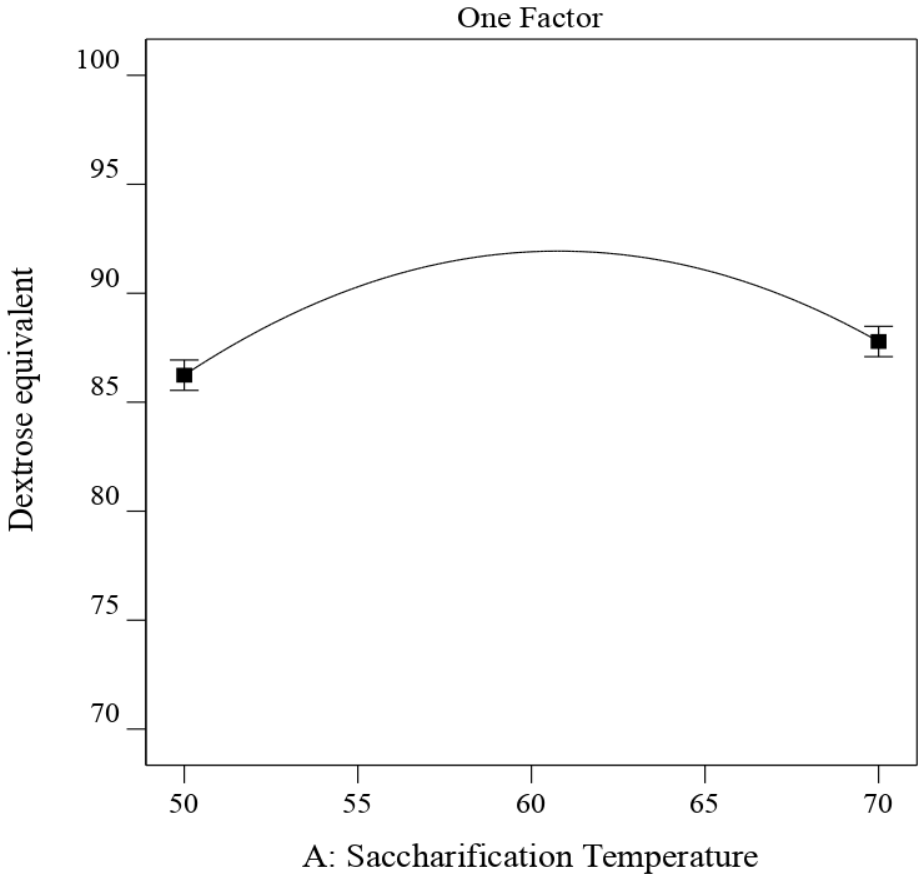


Figure 4.5. Effect of saccharification temperature on DE

4.4.5.2. Effects of Saccharification Time

The time necessary to reach the maximum obtainable yield of glucose depends on the enzyme dosage/nature of the enzyme used, and reaction temperature. The effect of the duration of saccharification time on the yield of glucose at a constant temperature (60℃) and concentration of glucoamylase (0.15wt.%) is shown in Figure 4.6.

At the initial stage of the saccharification process, the level of dextrose equivalent (DE) continually increases and grows rapidly, it means in the stage of reaction time from 12 - 24hr, the value of DE

increases continuosly; yet when reaction time reach to 30hr, value of DE increases slowly, after 30hr the rate of enzyme hydrolysis reaction tends to be gentle. This is due to, when the enzyme hydrolysis product increases and accumulates to a certain degree, inhibitory action enhance obviously, saccharication enzyme deactivated gradually, so hydrolysis rate becomes slow down. However, increasing of the incubation time beyond 32hr decreases the value of the DE, as a result of the reverse reaction and/or the efficiency of enzyme catalytic reaction has deprived by means of the prolonged incubation time, i.e., the enzyme has achieved its maximum enzyme thermal deactivation process after being exposed at high temperature for a long time. So the suitable reaction time with the maximum yield of dextrose equivalent at 60℃, pH of 4.8, and 0.15wt.% (dosage of glucoamylase) was 30hr which results in 93.19% of DE.

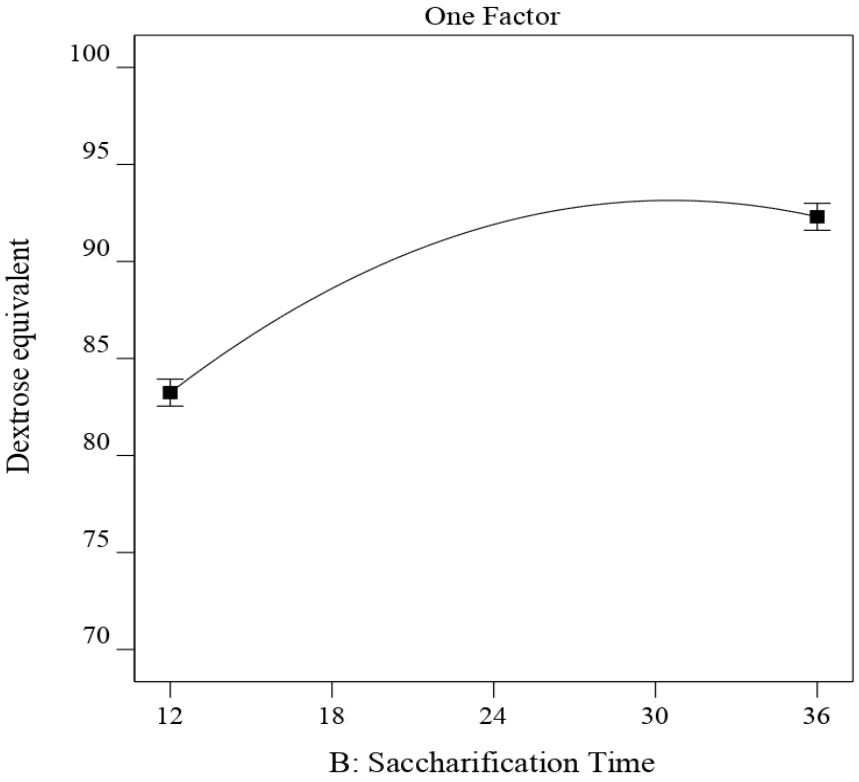


Figure 4.6. Effect of duration of saccharification time on DE

4.4.5.3. Effects of Glucoamylase Concentration

Figure 4.7 illustrates the influence of enzyme dosage on the amount of glucose formed during saccharification process at constant temperature (60℃) and time (24hr). As shown in the Figure 4.7, with the increasing of glucoamylase concentration, the yield of reducing sugar increases when the dosage of glucoamylase increases under low concentration from 0.1-0.18wt.%. After the

dosage of glucoamylase reaches 0.18wt.%, the yield increases gently, which means the increase of enzyme has little contribution to the yield of dextrose equivalent. The higher enzymes concentration in the solution, the higher the binding of substrate to the active side of the enzyme; however, when the substrate was entirely converted into the product, the increase of enzyme concentration showed no increase of product amount [42]. Glucoamylase also catalyzes the reverse reaction, in which dextrose molecules are combined to form maltose and isomaltose. As a result, over dosing with glucoamylase will result in a decrease in dextrose yield because of continued formation of isomaltose via reversion [29]. In this study, the dosage of glucoamylase with the maximum yield of DE at 60℃, and 24hr was 0.2wt.%, which results in 93.6% of DE.

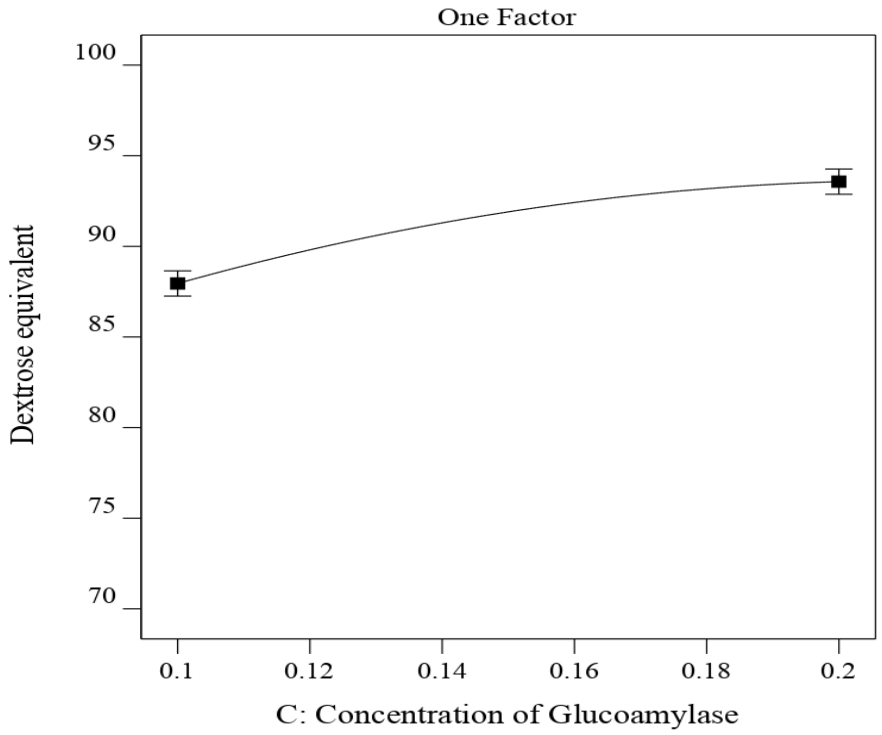


Figure 4.7. Effects of Glucoamylase Concentration on DE

4.4.6. The Interaction Effects of Process Variables

The response surface plots describing the interaction effect between pair of factors on hydrolysis of enset starch at a saccharification stage were given in Figure 4.8, 4.9 and 4.10, by keeping the remaining one factor constant at the middle level.

4.4.6.1. Interaction Effect of Saccharification Temperature and Time

Figure 4.8 shows the interaction effect of saccharification temperature and time on the response of DE while glucoamylase dosage was kept constant at 0.15wt.%. It was observed that at both lower

and higher level of incubation time (12 and 36hr), the value of dextrose was enhanced with rising temperature from 50 to 60℃, reaches maximum at 60℃ and decreased with the rise of temperature from 60 to 70℃. As shown in the Figure 4.8, at the middle to higher level of saccharification time and around the middle level of saccharification temperature around 60℃ the yield of DE was high.

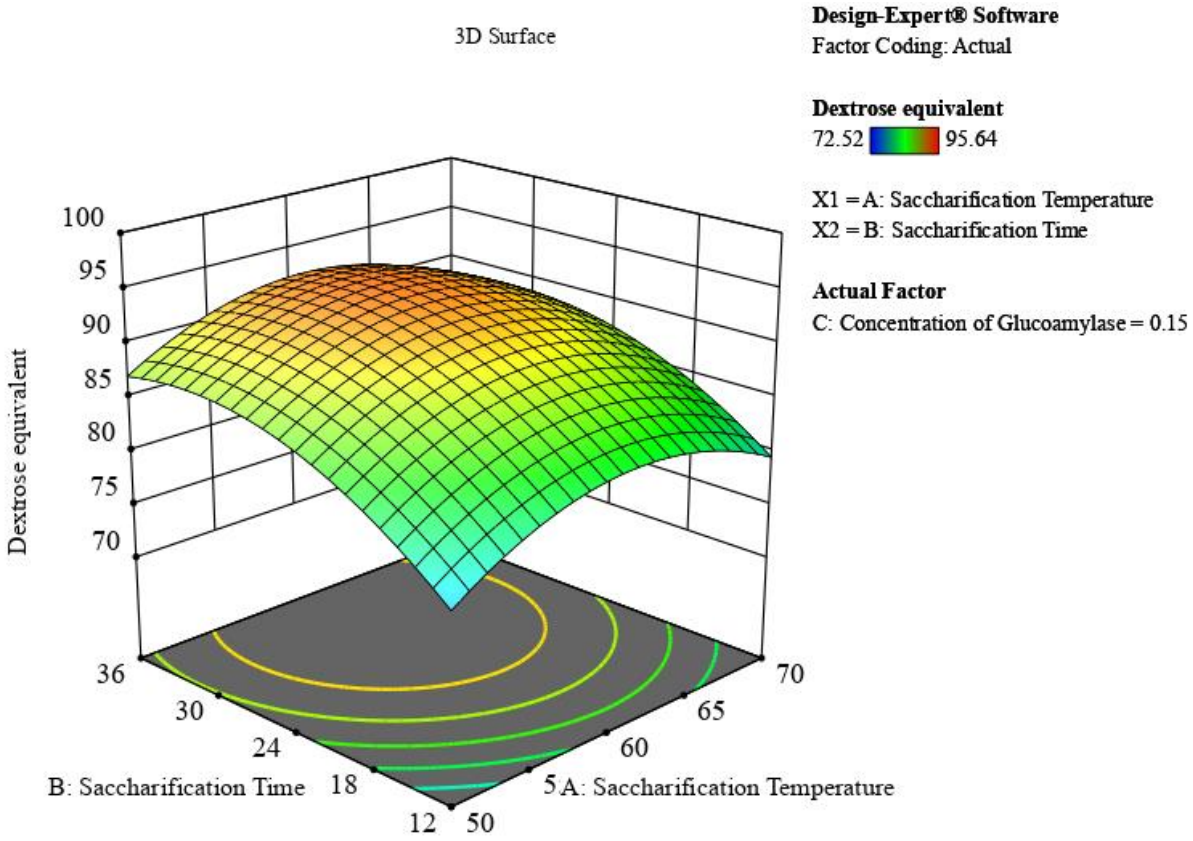


Figure 4.8. The interaction effect of saccharification temperature and time on the response of DE

4.4.6.2. The Interaction Effects of Temperature and Glucoamylase Concentration

Figure 4.9 shows the interaction effect of saccharification temperature and glucoamylase concentration on the response of dextrose equivalent while the saccharification time was kept constant 24hr. It was observed that at middle to higher level of glucoamylase concentration (0.15 to 0.2 wt.%) and at the middle level of temperature around 60℃ the value of DE yield was high.

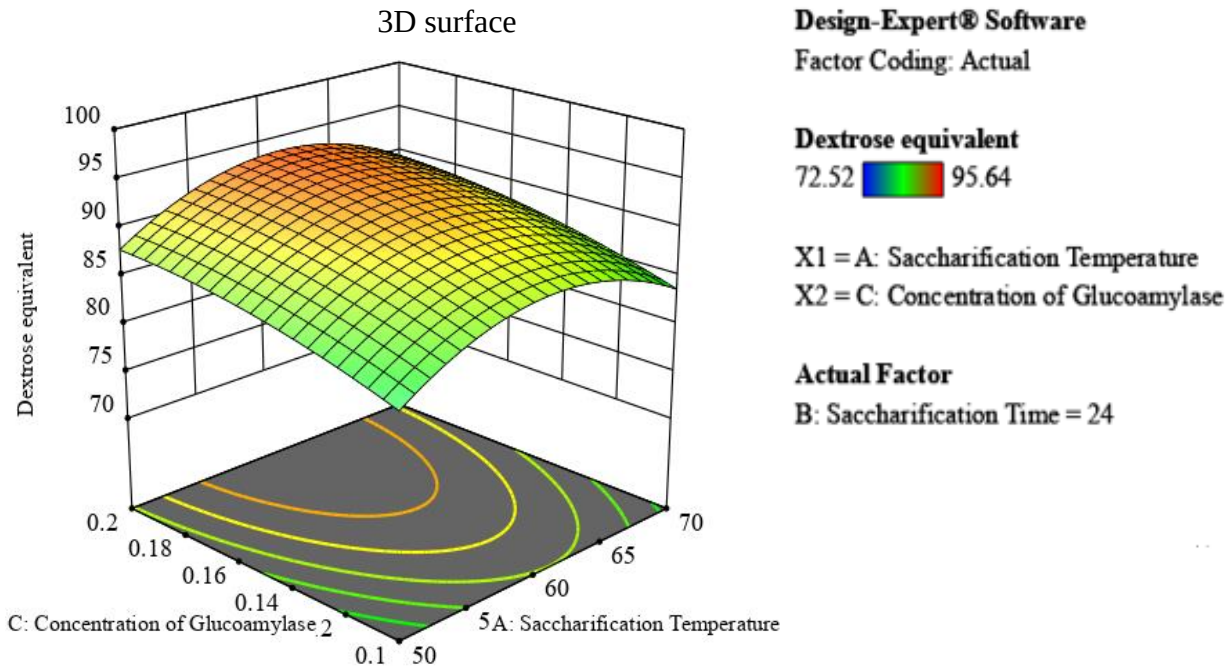


Figure 4.9. The interaction effect of temperature and glucoamylase on the response of DE.

4.3.6.3 The Interaction Effects of Saccharification Time and Glucoamylase Dosage

Figure 4.10. shows the interaction effect of saccharification time and glucoamylase dosage on the response of DE, while the saccharification temperature was kept constant at 60℃. At lower level of glucoamylase concentration (0.1wt.%), the value of DE increased with the rise of time from 12-36hr. However, at the higher level of glucoamylase concentration (0.2wt.%), DE was increased when the reaction time increased from 12 to 20hr, afterward DE was highly decreased with the rise of reaction time. This is due to the reverse reaction, which is a condensation of glucose molecules to mainly isomaltose at higher level of glucoamylase and/or extending reaction time beyond the maximum dextrose level. This indicates that interaction of reaction time and dosage of glucoamylase has more significant effect on the response of DE from others.

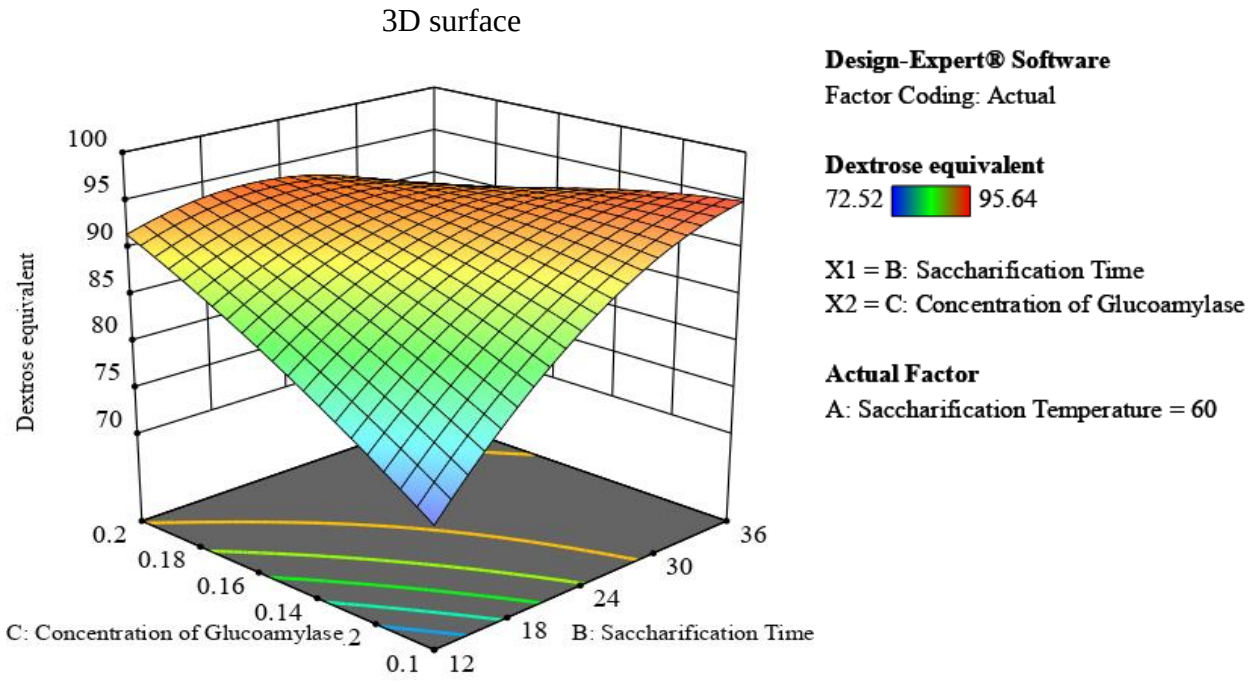


Figure 4.10. The interaction effect of saccharification time and glucoamylase dosage on DE

Saccharification process takes long reaction time period up to 72hr depending on the slurry concentration, reaction temperature, enzyme activity and dosage, which leads to the high cost [60]. The optimum reaction time reported by many studies for the production of industrial glucose syrup from corn, sago, cassava, and palm starch was ranged between 48 and 72hr [61], [2]. Minimizing of the reaction time will benefitted to the cost reduction, energy saving and the economical profit to the producers.

One study used a reduced saccharification times less than 2hr for the production of glucose syrup from cassava starch, to obtain glucose syrups with 38 to 73 DE values by using high concentration of glucoamylase [60]. However, high dosage of enzymes may leads to the high cost in the saccharification process and increase in color formation due Maillard reaction. Therefore, determining optimum operating parameter is neccesary in order to establish an economically feasible process. In comparision to the previous reported studies, the reaction time used in this study was short, ranged from 12 to 36 hr which results in maximum value of 95.64% DE, at 60℃, 36hr and 0.1wt.% of glucoamylase concentration.

4.4.7. Optimization of the Saccharification Parameters

The optimum process parameters of saccharification (temperature, time and glucoamylase concentration) were obtained by response surface methodology using Box Behnken Design optimizer. Optimization was performed to determine the optimum/minimum time points saccharification is needed and the amount of the enzymes needed to produce high DE glucose syrup at suitable operating temperature of glucoamylase as exhibited in the Table 4.7. Thirty solutions were found and the first solution with the highest desirability was chosen. In order to verify the optimization results, confirmation experiment was conducted with triplicates for the predicted optimum conditions. The predicted optimal conditions of saccharification process occurs at a time of 12hr, with a glucoamylase concentration of 0.2wt.%, and temperature of 61.3℃, which may result in 91.58% of DE. As shown in the Table 4.8, the result from the experiments were confirmed, and the predicted value was close enough to the experimental results, which reveals a high accuracy of the model.

Table 4.7. Optimization constraints of saccharification variables using design expert program

Name	Goal	Lower Limit	Upper Limit
Saccharification Temperature	is in range	50	70
Saccharification Time	minimize	12	36
Concentration of Glucoamylase	is in range	0.1	0.2
Dextrose equivalent	maximize	72.52	95.64

Confirmation

Table 4.8. Result of optimization and model validation

Parameter	Model predict	Experimental result
Saccharification Temperature (°C)	61.308	62
Saccharification Time (hr)	12	12
Concentration of Glucoamylase (wt.%)	0.2	0.2
Dextrose equivalent (%)	91.5829	91.79 $\pm$0.5

4.5. Determination of Total Polyphenol Content of Green Tea Extract

Total polyphenol content (TPC) was quantified using calibration curve of gallic acid (0.025 - 0.2 mg of gallic acid/mL); the equation of curve is shown in Equation (15). The total polyphenol content of green tea extract was obtained, 1.87$\pm$0.034 mg GAE/g of GTE. Ramírez-A. *et al.* [15]

reported as the TPC obtained for different brands of green tea extracted with similar solvent (water) and temperature (80℃) was ranged between 29.34 ± 0.66 – 55.06 ± 1.03 mg GAE/g per sample, which is higher than the result obtained in the present study. The value obtained in this study was the total polyphenol contents of concentrated extract of green tea, without considering the water content, since the objective was to determine the TPC in the green tea extract (which was then used to determine its preservative effect on produced glucose syrup), but not the yield.

Phenolic compounds are responsible for the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity. Studies reported that the positive correlation between DPPH activity and phenolic content are shown, an increase in TPC resulted in the increase of DPPH radical scavenging in tea infusions [15], [62].

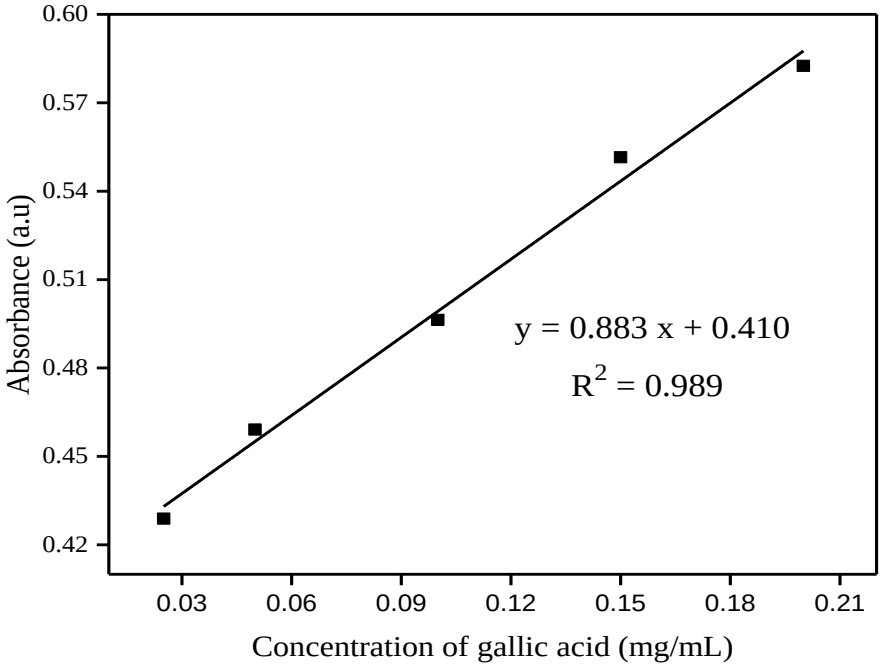


Figure 4.11. Standard curve for determination of total phenolic content in green tea extract

$$y = 0.883x + 0.41,\ R^2 = 0.989 \quad (15)$$

4.6. Preservative Effects of Green Tea Extract on Shelf Life of Glucose Syrup

In this study, green tea polyphenols/extract was used as a preservative. The effect of the extract on the shelf life of glucose syrup was evaluated. Moisture content, pH, and viscosity of glucose syrup, which contain varying amount of green tea extract, 100ppm, 200ppm, and 300ppm of TPC was

evaluated for 30 days, within a regular interval of 10 days. To determine the effect of the preservative on the glucose syrup, a sample void of green tea extract (0.0ppm) was served as control.

4.6.1. Moisture content

Figure 4.12 shows the result obtained from the evaluation of the effect of green tea extract on the moisture content of glucose syrup. Generally, the moisture contents of all the samples were decreased within the given storage time period as the concentrations of extract increased. During the storage time of 30 days, the moisture contents of the glucose syrup sample which contain 0ppm (zero concentration of preservative), 100ppm, 200ppm, 300ppm of green tea polyphenol shows a variation of about 0.96%, 0.36%, 3.98%, and 1.19%, respectively. The 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 [12].

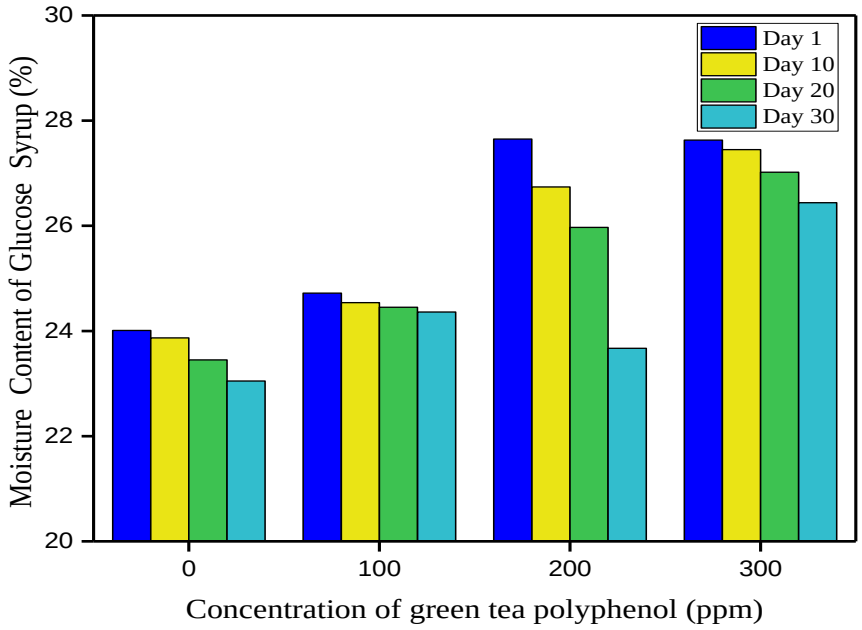


Figure 4.12. Effect of green tea extract with time on the moisture content of Glucose Syrup

4.6.2. pH

As it is observed on Figure 4.13, the pH of glucose syrup with 0.0ppm/control sample show an increment of 0.04, as the experiment progresses from day 1 - day 30, while the pH value of the glucose syrups which contain the extract was decreased within the given storage time period as the concentrations of extract increased. During the storage time of 30 days, the pH of glucose syrup

which contain 100ppm, 200ppm, 300ppm of TPC of green tea extract shows a variation of about 0.03, 0.05, and 0.08, respectively. This variation might be explained due to 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 [12]. Green tea catechins are more stable at low pH and temperature values during storage [63].

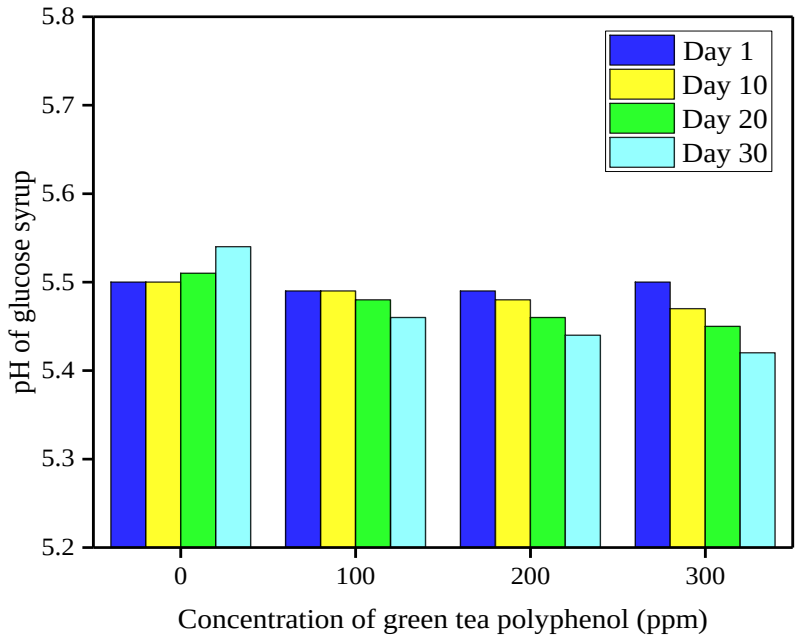


Figure 4.13. Effect of green tea extract with time on the pH of Glucose Syrup

4.6.3. Viscosity

The viscosity of a glucose syrup is related to the moisture content and carbohydrate composition of the syrup [2]. Figure 4.14 shows the result obtained from the evaluation of the effect of green tea extract on the viscosity of glucose syrup. During the storage time of 30 days, all the samples of glucose syrup with 0ppm, 100ppm, 200ppm, 300ppm TPC of green tea extracts shows a gradual fall in the viscosity. This explains that, as there was the possibility of the glucose syrup to be hygroscopic i.e. gradual absorption of moisture from the atmosphere affected viscosity [10], as a result the decrease in the viscosity value observed. As shown in Figure 4.14, the syrup with the low concentration of preservative (100ppm), shows gentle reduction in viscosity relatively to others.

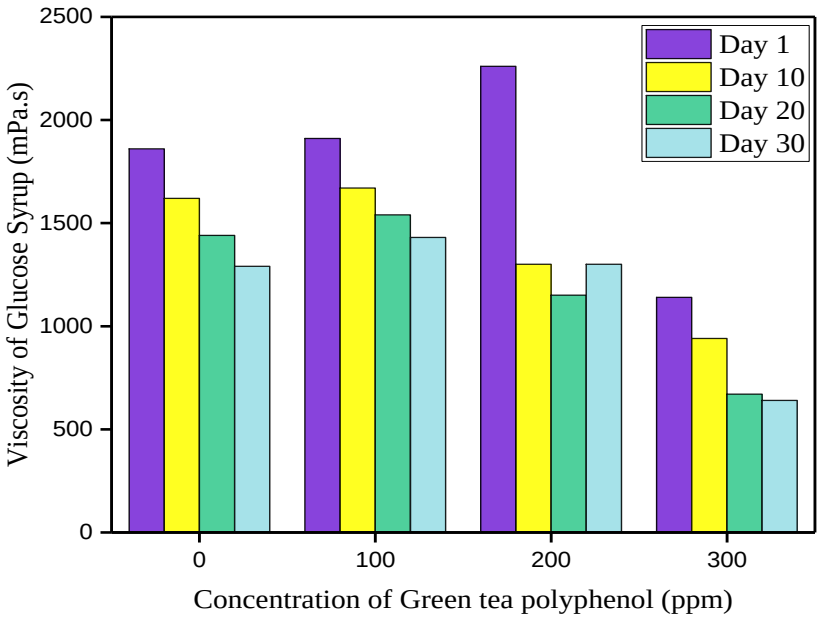


Figure 4.14. Effect of green tea extract with time on the viscosity of Glucose Syrup

4.6.4. Color

The addition of green tea extracts onto the syrup resulted change in colour of glucose syrup from light yellowish to yellow and brown, proportional to their concentration (shown in Figure 4.15). It is known that the Maillard reaction plays an important role in colour formation.

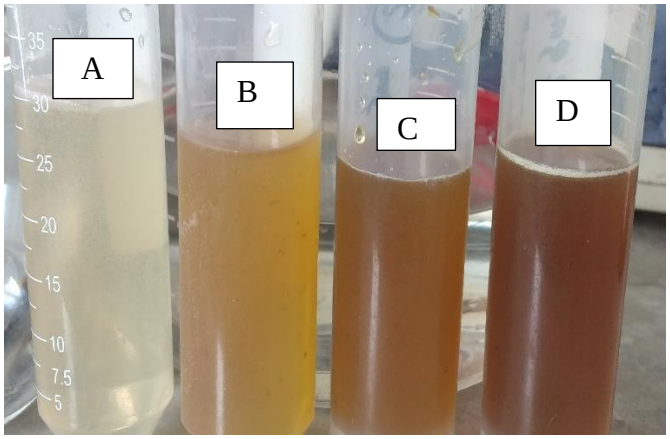


Figure 4.15. Effects of green tea extract on the color of glucose syrup

Whereas,

- A represent void glucose syrup sample
- B represent glucose syrup samples with 100ppm TPC of green tea extract
- C represent glucose syrup samples with 200ppm TPC of green tea extract

- D represent glucose syrup samples with 300ppm TPC of green tea extract

When glucose interacts with the gallic acid in green tea, the glucose hydroxyl groups are serially substituted by gallic acid complex compounds, such as pentagalloylglucose, tetragalloylglucose and trigalloylglucose, are likely to be formed. In addition, tea polyhydric phenols, resorcinol, pyrogallol are capable of condensing with reducing monosaccharides [64]. These phenomena and other possible explanation may result in variation of pH, moisture content, viscosity values and colour formation. Therefore, green tea extract with low concentration (100ppm of TPC), show better result according to the above measured parameters. Apart from extending shelf life of the syrup, the extract addition into glucose syrup has an effect on health, as it would increase the antioxidant intake.

5. Conclusions and Recommendation

5.1. Conclusions

The results of this research showed that Ensete ventricosum starch has a big potential to be the raw material in the making of glucose syrup. The proximate composition and amylose-amylopectin ratio of enset starch was determined. The study shows as moisture content, ash content, crude fat content, protein content, and amylose content of enset starch were: 8.98%, 0.15%, 0.38%, 0.1%, and 29.01%, respectively.

The enzymatic production process of glucose syrup was carried out in five main stages such as gelatinization, liquefaction, saccharification, filtration/refining, and evaporation. The result presented that temperature, time, and glucoamylase concentration had a significant positive effect on saccharification process. The maximum DE (95.64%) was found at the parameter interaction of 0.1wt.% (glucoamylase concentration), 60℃, and 36hr. The optimum process condition for saccharification temperature, time and glucoamylase concentration were: 62℃, 12hr and 0.2wt.%, respectively which resulted in 91.79±0.5% of DE.

Characterization and quality analysis of the produced glucose syrup was performed. The moisture content, total soluble solid, ash content, density, specific gravity, viscosity, and pH of the product were: 24.01%, 75.32°Brix, 0.35%, 1380.8 kg/m^3, 1.3833, 940 mPa.s, and 5.5, respectively. The syrup was yellowish in color, sweet and odorless. Accordingly, the product has fulfilled the SNI 01-2985-1992 requirement except in moisture content and color, and categorized in the type-IV glucose syrups based on its percentage of dextrose equivalent.

Whilst chemical additives have positive impacts on processed food flavor and storage time, but some have turned out to have negative effects on human health. In order to substitute these chemical additives with natural antioxidant from plant extract, the preservative effect of green tea extract on the shelf life of the syrup was examined for 30 days, by studying its effect on pH, moisture content and viscosity of the glucose syrup. Samples were treated with 0, 100, 200, and 300ppm TPC of green tea extract. Green tea extract with a concentration of 100ppm TPC was identified as a suitable preservative to retain the quality and extend the shelf-life of glucose syrup at room temperature.

From the results obtained in this study, it is concluded that Enset starch is a promised raw material to develop glucose syrup enriched with extracts of green tea to elevate its shelf life and health benefits. Hence, glucose syrup can be preserved by green tea extracts, which provide natural and safe antioxidant options to replace synthetic antioxidants.

5.2. Recommendation

The following recommendations were made for further study on the utilization of Enset, isolation of enset starch, enzymatic production of glucose syrup, characterization and preservation of the product, and green tea extracts.

- There is a large production capacity of Ensete ventricosum plant in Ethiopia. It is a potential source of soluble carbohydrate (mainly starch), fibers (cellulose), and used as traditional medicine. However, industrial usage of this large cultivation of the Enset plant is limited. So, it is recommended to expand further research on the utilization of enset as an alternative source of industrial raw material.
- The extraction of Enset starch used for this research was made in home due to lack of experience and equipment in laboratories. As a result, it is recommended to accomplish the isolation of starch as soon as the plant is root out (harvested), to minimize loss of starch during transportation.
- In this study activated carbon was used for refining, which resulted in incomplete removal of impurities such as salts and minerals. These impurities were resulted in color formation and increase the ash content of the syrup. So, it is suggested to use ion exchange resins for further removal of metallic ions to upgrade the quality of the syrup in the future.
- Due to shortage of time and equipment's, the proximate composition of glucose syrup for its metallic ions (minerals salts) and sulfur dioxide contents, and microbial analysis were not determined on this study. It is recommended that other researchers should be done further characterization on those parameters.
- In order to increase the health benefits, quality and extend the shelf life of syrups, the effect of green tea extract on the shelf life of glucose syrup was studied for 30 days. However, the addition of the green tea extract to the syrup raises the color formation which reduce the requirement and it is not convincing due shortage of days. As a result, it is recommended:
    - to remove the chlorophyl found in green tea to minimize color formation

- to use pure phenolic compounds of green tea after complete removal of moisture and byproducts
- to study preservative effect of the extract with low DE syrup, since the produced syrup in this study was high DE syrup which resulted in criticization during storage at room temperature, thus it needs exposure to high temperature during examination, and this reduces the antioxidant activity of the extracts.

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Appendices

Appendix A: Experimental Result

Data for determination of dextrose equivalent

Table A. 1. Absorbance versus glucose concentration data for construction of standard curve

Concentration of glucose in mg/mL	Absorbance
0.2	0.075
0.4	0.196
0.6	0.353
0.8	0.442
1	0.566

Table A. 2. Absorbance, Total soluble solid, Reducing sugar and Dextrose equivalent of the produced glucose syrup

	Factor						**Response**
Run	A: Sac. Temp.	B: Sac. Time	C: Glucoamylase Conc.	Total soluble solid	Absorbance	Reducing sugar	Glucose Concentration in terms of DE
	℃	Hr	wt.%	°Brix	a.u	%	%
1	60	24	0.15	65.36	0.330	59.28	91.75
2	60	24	0.15	66.2	0.337	60.53	91.44
3	70	24	0.1	67.29	0.313	56.22	82.62
4	70	24	0.2	70.76	0.354	63.59	84.86
5	50	36	0.15	69	0.333	59.46	85.49
6	50	12	0.15	66.39	0.287	51.55	75.2
7	50	24	0.1	68.08	0.311	55.86	79.16
8	70	36	0.15	66.26	0.322	57.84	86.49
9	60	24	0.15	71.0	0.364	65.38	92.09
10	60	12	0.2	72	0.364	65.38	87.88
11	60	12	0.1	66.88	0.270	48.5	77.4
12	60	24	0.15	72.4	0.374	67.18	92.79
13	70	12	0.15	72	0.320	57.48	79.3
14	60	36	0.2	70	0.341	61.25	85.6
15	60	24	0.15	67	0.345	62	89.95
16	50	24	0.2	67.34	0.330	59.27	81.43
17	60	36	0.1	68.18	0.363	65.21	95.64

Determination of Total Phenolic Content of Green Tea Extract

Table A. 3. Concentration of gallic acid vs. Absorbance data for construction of standard curve

Concentration of gallic acid in mg/mL	**Absorbance**
0.025	0.4289
0.05	0.4591
0.1	0.4963
0.15	0.5515
0.2	0.5825

Table A. 4. Absorbance of green tea extract at 765 nm

Absorbance of green tea extract at 765 nm			
Trial 1	Trial 2	Trial 3	Mean
0.5733	0.5801	0.5743	0.5759

- 1gm of green tea extract was dissolved in 10mL of methanol. Thus V=10mL, m = 1gm.

Table A. 5. Amylose-amylopectin data for Enset starch

Absorbance at 620nm			
Trial 1	Trial 2	Trial 3	Mean
0.473	0.474	0.476	0.474

Amylose-amylopectin contents of enset starch was calculated using the following equations:

Amylose content (%) = 3.06 x absorbance x 20

Amylopectin (%) = 100 – % amylose contents

Then amylose and amylopectin contents of enset starch:

Amylose content (%) = 3.06 x 0.474 x 20 = 29.01%

Amylopectin content (%) = 100 – 29.01% = 70.99%

Experimental results for Shelf-life determination

Table A. 6. Effect of green tea polyphenol with time on Moisture Content of Glucose Syrup

Concentration of green tea polyphenol (ppm)	**Moisture Content (%)**			
	Day 1	Day 10	Day 20	Day 30
0	24.01	23.87	23.45	23.05
100	24.72	24.54	24.45	24.36
200	27.65	26.74	25.97	23.67
300	27.63	27.45	27.02	26.44

Table A. 7. Effect of green tea polyphenol with time on pH of Glucose Syrup

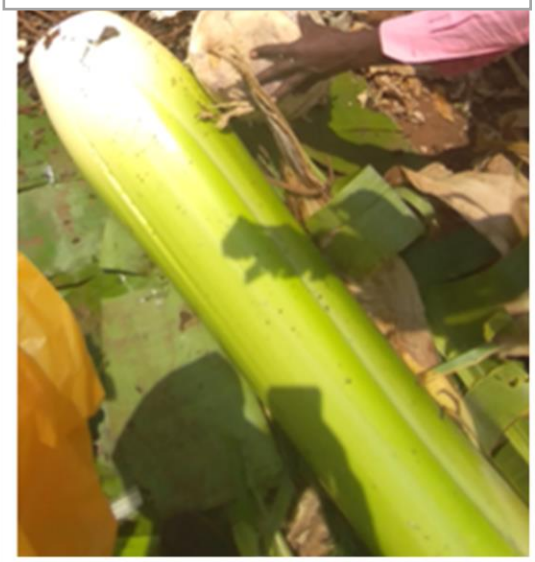
Concentration of green tea polyphenol (ppm)	**pH**			
	Day 1	Day 10	Day 20	Day 30
0	5.5	5.5	5.51	5.54
100	5.49	5.49	5.48	5.46
200	5.49	5.48	5.46	5.44
300	5.5	5.47	5.45	5.42

Table A. 8. Effect of green tea polyphenol with time on viscosity of Glucose Syrup

Concentration of Green tea polyphenol (ppm)	**Viscosity (mPa.s)**			
	Day 1	Day 10	Day 20	Day 30
0	1860	1620	1440	1290
100	1910	1670	1540	1430
200	2260	1300	1150	1300
300	1140	940	670	640

Appendix B: Selected Picture of Common Procedures in Laboratory Work

A) Root out of Enset plant



B) Settled starch after isolation

C) Enset starch after several wash

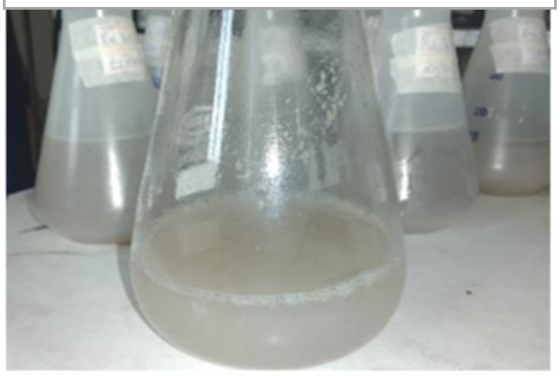
D) Sun dried Enset starch

E) Starch slury

F) Geletanized starch

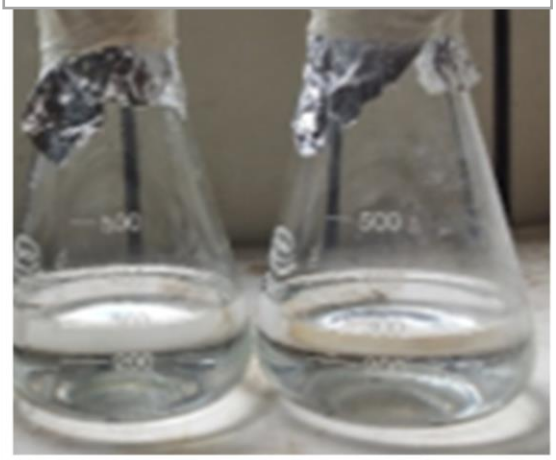
G) Hydrolysate after liquefaction

H) Saccharify



I) Treatment with activated charcoal

J) Clarified glucose syrup



K) Water removal using rotary vacuum evaporator

L) Concentrated glucose syrup

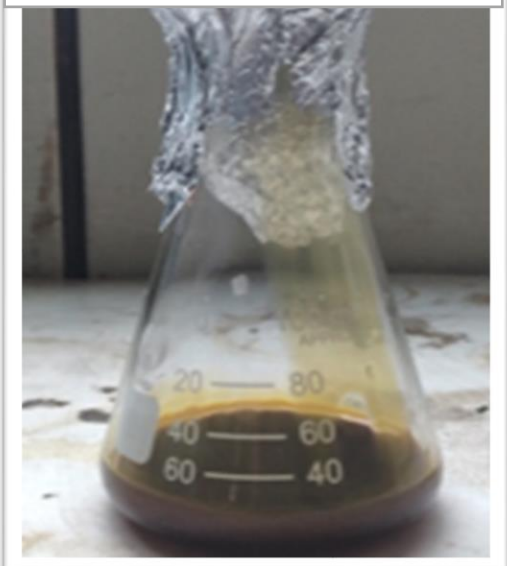
M) Crystalized glucose syrup

N) Determination of reducing sugar using

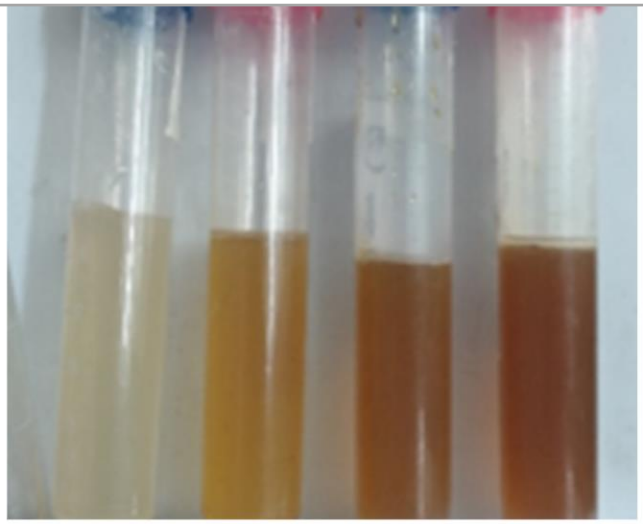
O) Extraction of green tea

P) Vacuum filtration of extract

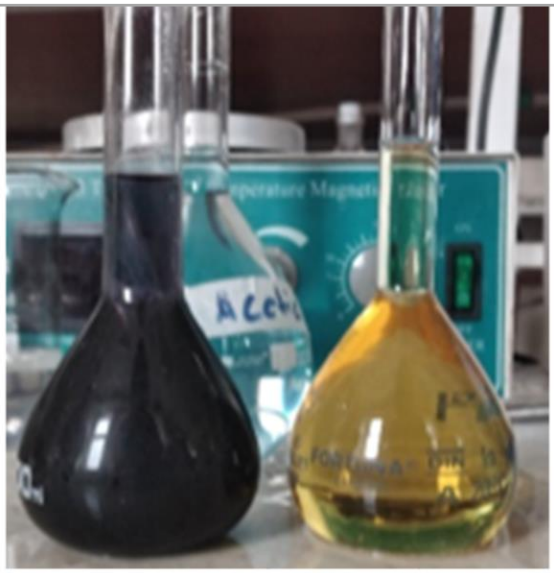
Q) Concentrated green tea



R) Preservation of glucose syrup with 0, 100, 200, 300ppm of green tea polyphenol

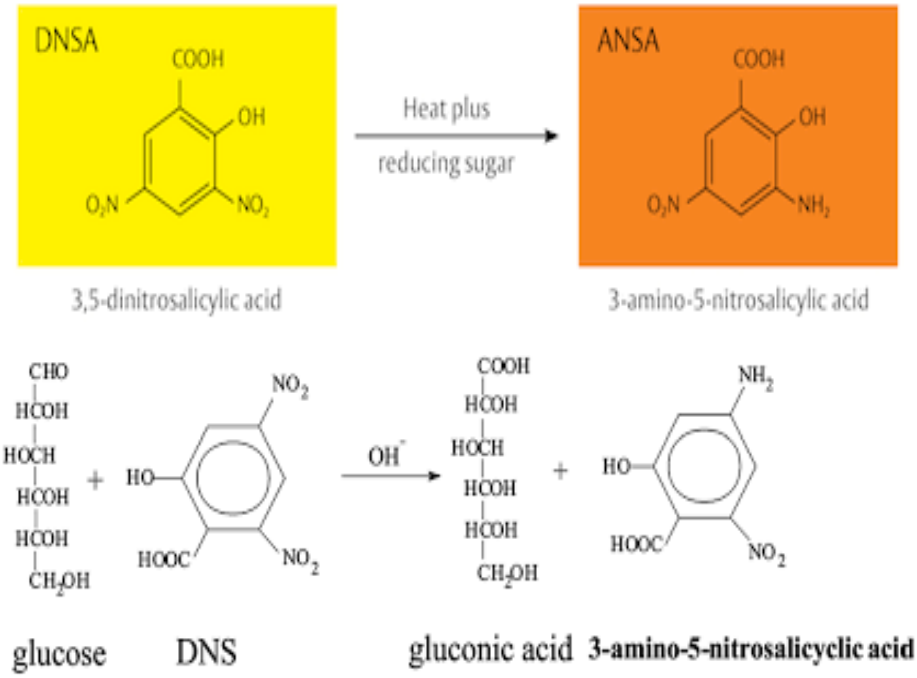


S) Amylose-Amylopectin determination



Appendix C. Chemical Reaction Between DNS and Reducing Sugar

3, 5 Dinitro salicylic acid + Reducing Sugar (glucose) ⟶ 3 - Amino - 5 - nitrosalicylic acid + Oxidized Sugar (Gluconic acid)



Appendix D. Iodine Test for Enset Starch

Preparation of 'Lugol's solution'
10 g of potassium iodide and 5 g of iodine crystals was dissolved in 100 mL of distilled water

For starch sample

1gm of Enset starch wash dissolved in 5mL of distilled water → 2-3 drops of Lugol's solution was added and vortexed → The test tube with the sample was heated in water bath until the color disappears and cooled → The color changed to blue black due to the presence of starch

For control

5 mL of distilled water was taken in dry test tube → 2-3 drops of Lugol's solution was added and vortexed → The test tube was heated in water bath and cooled → The color remain yellow