CHARACTERIZATION OF GLUCOSE SYRUP AND ETHANOL FROM STARCH EXTRACTED FROM SELECTED CASSAVA AND MAIZE VARIETIES GROWN IN UGANDA

BY

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DECLARATION

I, Lydia Bwamiki, declare that this dissertation is my original piece of work and has never been submitted to any university or higher institution of learning for any award of a degree and where other works have been included, it has been cited.

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DEDICATION

This dissertation is dedicated to my late mother, Mrs. Peninah Bwamiki for always believing in me and striving to ensure that I succeed in my education and life as a whole.

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ABSTRACT

The research aimed to extract starch from the selected cassava and maize varieties, evaluate enzymatic and acid hydrolysis processes for glucose syrup production, and assess ethanol production. Characterization of the resulting glucose syrup and ethanol was also performed. A total of four (4) cassava varieties and four (4) maize varieties were used in this study. These included two local varieties of cassava (*Mukumba* and *Mwezi mukaaga*), two improved cassava varieties (TME 14 and NAROCASS 1), two open pollinated maize varieties (Longe 4H and Longe 5H) and two hybrid maize varieties (H 5355 and H 2115). Cassava was harvested at twelve (12) months while maize was harvested at four (4) months (120 days) after planting. Cassava and maize starch were extracted in three independent replicates using the wet and alkaline extraction methods, respectively. Glucose syrup was produced using acid and enzyme hydrolysis. Glucose syrups were fermented using Young's dried active yeast (*Saccharomyces cerevisiae*) to obtain ethanol.

There was no significant difference in the starch yield of the local and improved varieties for either cassava or maize. H 2115 produced the highest yield of 50.83% among the maize varieties while *Mwezi mukaaga* had the highest yield (29.1%) among the cassava varieties. Selection of the varieties for starch characterization was based on starch yield. Swelling power and solubility for all the starch varieties increased with increasing temperatures with *Mwezi mukaaga* having the highest swelling power (18.23 g/g) at 90°C and TME 14 having the highest solubility (2.45%) at 90°C. The content of amylose of cassava starch was higher than that of maize with *Mwezi mukaaga* having highest amylose content of 24.25%. Maize had higher resistant starch content than cassava with H2115 having the highest resistant starch content of 0.82%. The gelatinization temperatures for all starch varieties were not significantly different ($p>0.05$). Cassava varieties were much lighter than the maize varieties. The cassava starch

granules were round, truncated with larger particle sizes (17.40 and 17.95 μm) as compared to the maize starch granules which were round, irregular and polygonal with smaller particle sizes (11.05 and 9.10 μm).

Acid hydrolysis significantly produced higher ($p>0.05$) sugar concentration for both crop types as compared to enzyme hydrolysis. Among maize varieties, H 2115 had highest sugar content of 46.1 g/100 g (acid hydrolysis) and H 5355, 40.3 g/100 g (enzyme hydrolysis), while among cassava varieties, TME 14 had the highest sugar content of 31.2 g/100 g (acid hydrolysis) and *Mukumba*, 12.41 g/100 g (enzyme hydrolysis).

Enzyme hydrolyzed samples showed highest sugar consumption by the yeast hence a higher alcohol (ABV) content and ultimately a better fermentation efficiency. Enzyme hydrolyzed glucose syrups had a higher ethanol content than the acid hydrolyzed syrups, with no difference between maize and cassava varieties in each treatment. These results propose that maize and cassava are good sources of starch, glucose syrup and ethanol with the maize varieties used in this study giving higher sugar concentrations than the cassava varieties.

CHAPTER ONE: INTRODUCTION

1.0 Background

Wheat, maize and rice are the world's leading staple cereals, each cultivated on some 200 million (M) ha (Erenstein et al., 2022). Maize or corn (*Zea mays* L.), is the second most produced cereal globally, after rice (Erenstein et al., 2022) and it is cultivated in every continent except Antarctica. North America cultivates more than 43% of global maize production, followed by China (18%), Africa (7.5), European Union (7%), Brazil (6%), Balkan area (3%) and Mexico (3%). The least producers are Argentina, India and South Africa (2%) (MoAF, 2019). Largest world producers of maize include the USA, China and Brazil (FAO, 2012). Maize is an important spring of nutritional fuel all over the world, and also a major raw material for livestock fodder, and for starch production and starch-based products such as ethanol (Daly et al., 2016) Maize is the main food source in several territories, especially in Africa and Latin America, where it is processed by milling, fermentation, or cooking (Erenstein et al., 2022). Maize and cassava are staple foods in Africa where per capita consumption ranges from 13.7 to 85 kg (Wallington et al., 2014). By 2014, maize per capital consumption in Uganda was reported to be 20 kg (Chune, 2022).

Uganda is ranked eighth largest maize producer in Africa and third in East Africa with its production area covering 1.15 million hectares yielding approx. 2.8 million metric tons (World Data Atlas, 2019). Uganda's maize production has steadily increased in the last 20 years from 800,000 tons in 2001 to 2,800,000 tons in 2019 (World Data Atlas, 2019).

In Uganda, maize is mainly consumed in five different forms i.e., as fresh maize (roasted or steamed), maize flour meal (posho), porridge, fried corn and animal feed (Ajambo et al., 2017). Maize is an important resource that can be transformed to several products, such as sweeteners,

beverages, starch, oil, glue as well as ethanol for fuel and other industrial applications (Ajambo et al., 2017).

Cassava (*Manihot esculenta* Crantz) is a tuber which can be preserved underground for a long period (Uarrota et al., 2016). In tropical and subtropical areas, cassava is normally harvested annually or biannually (Costa & Delgado, 2019). It is generally consumed across Sub Saharan Africa, especially in East and West Africa (Spencer & Ezedinma, 2017). Cassava is a paramount calorie spring in tropical countries. It tolerates poor soils and is resistant to diseases and drought (FAO, 2010). According to FAOSTAT (2016), global cassava production was 277 million tons in 2016; with Africa as the highest producer, contributing 57% total production. Annual global cassava production is estimated at 281 million tons. Africa contributes more than half of this production, but crop is largely used for food (Woiciechowski, et al., 2016).

In Africa, cassava is the most important (36%) starchy food followed by maize, yams, sorghum, rice, wheat, sweet potatoes, Irish potatoes, plantains and millet at 15, 14, 7, 5, 4 and 3%, respectively (FAOSTAT, 2016). Approximately 53% of the global production of cassava is found in Indonesia, Nigeria, Brazil, Ghana and Thailand (Costa & Delgado, 2019).

Cassava serves as one of the primary starch sources for production of glucose syrups and ethanol, primarily due to its high starch yield (approx. 75%, dry weight) and its ability to grow in poor soils without application of fertilizers (Abass et al., n.d.).

Starch is used in production of syrups (glucose, maltose, fructose, and glucose–fructose), amino acids and organic acids (Waterschoot et al., 2015). Starch can also be saccharified and fermented to generate ethanol that is used in formulation of beverages or biofuel (Favaro et al., 2015). Starch can be hydrolyzed into maltose, smaller oligosaccharides and dextrins by alpha-amylase which is an endo-lytic enzyme which hydrolyses α-1, 4-glycosidic bonds from inner

starch chains (Sharma & Satyanarayana, 2013). On the flip side, glucoamylase is an extracellular enzyme which is used to hydrolyze α-1, 4-glycosidic bonds from non-reducing parts of starch chains to produce glucose syrup (Marín-Navarro & Polaina, 2011).

Glucose syrup, is a clear, viscous liquid sweetener made from starch and is primarily composed of glucose molecules (Science, 2021). Glucose syrup can vary in color, from clear to light amber, depending on its production process and intended use (Hadi et al., 2021). Glucose syrup has a wide range of applications in the food and beverage industry, with some common uses including; acting like a sweetener, texture modifier, moisture retenant, a binding agent and in the brewing and fermentation process to provide fermentable sugars for alcohol and beer production (Grenby & Mistry, 2000). The refining of glucose syrup involves several steps to remove impurities and adjust its properties such as, hydrolysis of starch, which is broken down into glucose through enzymatic or acid hydrolysis. Purification of the hydrolyzed mixture to remove proteins, fats, and other impurities (Permanasari et al., 2018), concentration of the purified glucose solution to achieve the desired viscosity and sweetness level (Telis et al., 2007). Glucose syrup is known by different names in various regions. In the United States, it's commonly referred to as corn syrup. In the United Kingdom, it may be called glucose syrup or liquid glucose (Pocan et al., 2022).

Ethanol, also known as ethyl alcohol or grain alcohol, is a colourless, flammable liquid with a slightly sweet odor (International & Classification, 2010). It has a chemical formula of C2H5OH and is a type of alcohol. Ethanol production is a major industry worldwide, with significant production in countries like the United States, Brazil, and China (Taber & Participant, n.d.). Ethanol is a versatile organic compound that can be produced through fermentation and is commonly used for various purposes (Chicilo, et al., 2021).

The production of ethanol typically involves fermentation, distillation and dehydration. Ethanol is typically available in various concentrations, from beverage-grade ethanol (i.e. 5-40%) to anhydrous ethanol (over 99% pure) (Hikmawanti et al., 2021).

Ethanol is a versatile chemical compound with a wide range of applications, from fuel to pharmaceuticals, and it plays a vital role in various industries and everyday life.

In USA, about 4% of maize is used in production of glucose syrup while the largest portion is for animal feed followed by human consumption and then ethanol; only 13% is for export (Wallington et al., 2014). In developing countries, the biggest proportion of maize is used for food and animal feed (Goodla et al., 2012). In Uganda, extraction of starch from maize and cassava flour is done on a limited scale while there is almost no conversion into high value products such as glucose syrup (Kaur & Ahluwalia, 2017). Development of alternative products from maize and cassava besides the traditional ones such as maize/cassava flour meal, animal feed and porridges will increase demand and farm-gate prices for these crops in order to benefit local farmers especially the youth and women who are mostly involved at production level (Yarnell, 2008). Maize and cassava have traditionally been one of the cheaper foods and food ingredients (Bechoff, 2017). This has discouraged farmers which has resulted in decline in cassava/maize production in the county (Jackson et al., 2020). Increasing the scope of products from maize and cassava will diversify their market demand and hence price. Therefore, this study aimed to investigate production and characterization of sugar syrup and ethanol from selected Ugandan cassava and maize varieties**.**

1.1 Problem statement

Uganda is ranked as the 6th largest cassava producer in Africa and cassava is a major primary food crop in the country (USAID, 2010). Cassava and maize are Uganda's major tropical crops

which are cultivated extensively by the peasant farmers as sources of starchy food and as famine relief crops (Strategy, n.d.). These crops are grown under varying soil and climatic conditions (Adeniyan et al., 2014). Due to their high starch content, they are regarded suitable raw materials for manufacture of industrial starch (Verma et al., 2022).

Currently, there is limited value addition to cassava and maize which has negatively affected their demand on the local market and hence farm gate prices (Saediman et al., 2015). Most cassava is used in raw form as a starchy food. Less of it is processed into cassava flour and starch (Shittu et al., 2016). Cassava has a high post-harvest deterioration rate and can go to waste during harvesting seasons (Muyinza et al., 2016), necessitating an alternative form of usage for the surplus. Maize is also mainly consumed fresh as roasted, steamed and the dried corn is milled into flour of different grades that is then consumed by a substantial part of Uganda's population. Inadequate value addition is mainly due to lack of processing technology alternatives that can transform these commodities into diverse value-added products (FAO, 2014). This could have limited the scope of industrial development of cassava and maize in Uganda (Fowler, 2019). There is need to develop and diversify the product range from cassava and maize in order to increase the value of these crops and hence utilization (Okoboi, 2010).

In spite of its high starch content, cassava has not been exploited in Uganda for production of glucose syrup, ethanol and other high value products (Thomas, 2009). Glucose syrup is highly demanded sweetener in pharmaceutical, beverage and food industries (R. Johnson et al., 2009). With increase in world sugar consumption patterns coupled with fluctuations in its prices (Khalid, 2005), the need to find alternative sugar substitutes such as glucose syrup and their sources such as cassava and maize from which to derive the glucose syrup cannot be overemphasized. Glucose syrup from different sources exhibits varied properties due to differences in starch source (e.g., corn, wheat, cassava), production methods (e.g., degree of

hydrolysis, refining processes), and the presence of impurities (Science, 2021) that could affect utilization. For example; the viscosity which can vary significantly, with some syrups being thin and pourable, while others are thick and viscous. Corn-based glucose syrup, for example, tends to be thicker than cassava-based glucose syrup due to differences in starch composition (Sun et al., 2014). The sweetness of glucose syrup is similar to sucrose (table sugar), but it can vary slightly depending on the source and processing (Echeta et al., 2019). The degree of hydrolysis, which determines the ratio of glucose to other sugars, can influence sweetness as well (Luzzi et al., 2020). Highly hydrolyzed syrups may taste slightly sweeter than less hydrolyzed ones.

Glucose syrup can range in color from clear to light amber or even darker, which color depends on factors like the source of starch, the degree of refining, and the presence of impurities (Ríos et al., 2019). Cassava-based glucose syrup tends to be clear or light in color, while maize-based syrup may have a slightly darker hue. Glucose syrup is highly soluble in water, but the solubility can vary slightly based on the starch source and processing (Alves et al., 2007). Some syrups may dissolve more easily than others due to differences in starch structure and impurities. Glucose syrup is typically characterized by a mild, sweet flavor (Letviany, 2015). However, there can be subtle flavor differences based on the source of starch and any impurities or by-products from the production process.

The dextrose equivalent (DE) value is a measure of the degree of hydrolysis of glucose syrup (Montañez Soto, 2012). It represents the percentage of reducing sugars (glucose and maltose) in the syrup. DE values can vary, with higher DE values indicating a more extensively hydrolyzed syrup. Syrups with higher DE values are often sweeter and more viscous. Glucose syrup may contain impurities such as residual proteins, fats, and minerals (Al-Mhanna et al., 2018). The type and amount of impurities can vary depending on the starch source, extraction

method, and refining processes. Glucose syrup is valued for its functional properties in food processing, such as its ability to prevent crystallization, control texture, and retain moisture (Ejiofor, 2015). These functional properties can vary based on the syrup's composition and DE value.

Glucose syrup is highly valued by beverage and food manufacturing plants, because of its desirable characteristics such as sweetness, non-crystalline and ability to enhance spicy and fruit flavor (Hanover & White, 1993). Corn on the other hand, is a well-established feedstock for ethanol production (Kurambhatti et al., 2018).

Cassava and maize starch have the potential of being used for glucose production or high fructose syrup (HFS), and conditions of the process related with enzyme reactions for these products have been noted (Waliszweski et al., 2002; Morales et al., 2008). Cassava starch has higher extractability (over 70%) (Johnson et al., 2010). Cassava's ethanol yield (kg/ha/year) is estimated to be three (03) times higher than that of maize (Wang, 2002). Despite this, there is scant information on the yield and other characteristics of glucose syrup and ethanol from locally produced cassava and maize varieties in Uganda. Therefore, the current work sought to study the prospects of using local and improved varieties of maize and cassava for production of sugar syrup and ethanol. Characteristics of sugar syrup and ethanol were also studied to understand the qualitative properties of the products for potential industrial production and utilization.

Justification

Utilization of maize and cassava into the above-mentioned products is either non-existent or it is at a very small level in Uganda. Almost all the ethanol (extractional and reagent grade) and glucose syrup used in analytical laboratories and culinary and industrial food processing

activities are imported resulting in high prices. The use of Ugandan cassava and maize to produce these products using locally generated and adaptable technologies will encourage local entrepreneurs to invest in industrial production of glucose syrups and ethanol hence creating additional local market for cassava and maize. Cassava and maize could provide an alternative source to the expensive imported glucose syrups and ethanol in Uganda. It is important to validate the practical yields of starch, glucose and ethanol from local Ugandan cassava and maize varieties in order to prospect feasibility of cassava/maize as feedstocks for sugar syrup and ethanol production at industrial scale.

1.2 Objectives

1.2.1 General objective

To characterize glucose syrup and ethanol from starch extracted from selected cassava and maize varieties grown in Uganda.

1.2.2 Specific objectives

1. To determine yield and properties of starch extracted from selected cassava and maize varieties grown in Uganda.
2. To characterize the glucose syrup obtained from starch extracted from the selected cassava and maize varieties.
3. To characterize ethanol produced from glucose syrup obtained from starch extracted from the selected cassava and maize varieties.

1.3 Hypotheses

1. There is no significant difference in yield and characteristics of starch extracted from selected maize and cassava varieties grown in Uganda.

2. There is no significant difference in properties of glucose syrup produced from starch extracted from the selected cassava and maize varieties.
3. There is no significant difference in properties of the ethanol obtained from starch extracted from the selected cassava and maize varieties.

1.4 Significance of the study

The study will provide data on starch yield and characteristics of glucose syrup and ethanol from the selected Ugandan cassava and maize varieties. The results have the potential to attract more research into production of glucose and ethanol from these crops to generate more data that will encourage local investment into utilization of the crops for more industrial applications. Widening the scope of value addition alternatives for maize and cassava varieties will increase their economic and industrial value which will subsequently benefit Ugandan farmers. Research into ethanol and syrup production using maize and cassava has various socioeconomic and ecological benefits like moderation of food and oil prices. This will consequently enhance output and efficiency of these crops and thereby lead to employment creation mostly for women and youth, for socio-economic development and prosperity of the population. This is also in line with sustainable development goal (SDG 9) generating employment and income through innovation as well as Kyambogo University research agenda No 1 (agriculture production, productivity, food security and value addition) and No 9 (science technology and innovations to enhance competitiveness) and national development plan (NDP III) which emphasizes import substitution and export promotion.

CHAPTER TWO: LITERATURE REVIEW

2.1 Cassava Production

Cassava (*Manihot esculenta Crantz*), is a seasonal widely cultivated plant in equatorial nations, including Uganda (Nakabonge, 2018). Roots of cassava have abundance of starch and limited protein and lipid content and are therefore considered a reliable source of pure starch that could be utilized for a range of applications (Bayata, 2019). Cassava belongs to the family of flowering plants and is indigenous to South America though its currently cultivated in equatorial and temperate areas all over the world as a source of comestible starchy roots, which are a staple in emerging economies (R. do N. Silva et al., 2010)**.**

In Uganda, introduction of cassava was by Asian traders between 1862 and 1875 (Otim-Nape, n.d.). Cassava's propagation and farming was mainly because of its versatility to many farming environments and its resilience to aridity hence considering it a nutritional subsistence crop in Uganda and Africa (Nakabonge et al., 2018). In several areas of Uganda like West Nile, cassava is considered a major staple food for communities and for long spans has headed the group of the most weather-resistant crops though in recent years, conventional varieties have struggled with disease and climate changes which have endangered the food security of the Country (Buyinza & Kitinoja, 2018).

As for geographical distribution of cassava production, according to UBOS (2010), the Eastern region reported the highest production of cassava with a total output of 1.1 million MT (36%) followed by the northern region with 983,000 MT (34%), followed by Western region (15.2%) and Central region with 410,000 MT (14.2%) (Okello & Akullu, 2017). For the best cassava root production, 8 months of warm temperatures between (25 and 32) °C (77–90) °F, humid climates, sun-drench with no shading, clay textured loam soils in the pH spectrum

of 5.5 to 6.5 and regular rainfall are required (Buyinza & Kitinoja, 2018). Cassava is xerophilous and does not do well in flooded soils and since it is a vital crop, it is produced perennially (El-Sharkawy, 1993).

In Uganda and other regional markets, the demand for cassava as well as its products changes seasonally with its demand being very low in harvesting seasons due to higher levels of food supply and high in planting seasons (Buyinza & Kitinoja, 2018). According to FAO. (2019), the total output of cassava in Uganda was approximately 60% in relation to China, yet the farm land in Uganda on which cassava was grown was three times that of China. Cassava has latent capacity for industrial processing (Eresu & Harrison, n.d).

2.1.1 Local Varieties

Local cassava varieties are cassava cultivars or types that have been traditionally grown and adapted to specific regions or localities (Nakabonge, 2018). They are not typically the result of formal breeding programs but have evolved and been selected over time based on local farming practices and environmental conditions (Nakabonge, 2018). Selective breeding by humans has protected genetic diversity for many years (Lamprecht, 2015). However, the initiation of improved varieties for purposes of dealing with pests and diseases as well as different environmental pressures, has exposed many genetic resources to genetic degradation (Loo et al., 2011). By understanding the different local practices that determine the selection and conservation of a number of crop genetic assets, preservation of such assets is possible (Nakabonge et al., 2018).

Genetic repositories have the duty to restore crop genetic material before it vanishes and make sure it is integrated into the gene banks (FAO 2010). The role of the indigenous farmers is key in conserving the genetic diversity because they support agrobiodiversity, means of subsistence and food sufficiency (FAO, 2009).

The genetic diversity of cassava is endangered by cassava infections such as cassava brown streak disease as well as cassava mosaic disease which has yielded diminished outputs and genetic degradation (Kawuki et al. 2016). Propagation of cassava cultivars for use in industry has threatened the genetic material of cassava as farmers concentrate on cultivating cassava for economic intent (Waigumba et al., 2016). Like in the regions of Teso, improved cultivar Nase3 is chiefly grown making it difficult to trace once widespread *Jaribu* and *Ebwanatereka* (Okaasai Opolot, Personal communication, February 10 2014). In order to protect next generation breeding of cassava, backing for offsite and onsite protection of genetic resources should be encouraged (Nakabonge et al., 2018).

Studies show that cultural views and farm-based breeding could influence the distinction of local cultivars by Ugandan farmers who have different perspectives regarding the local varieties that should be comprehended and incorporated into farm level preservation policies that are adequate and authentic to the indigenous communities (Nakabonge et al., 2018). For advancement of the crop, comprehension of the social and cultural aspects that determine the choice farmers make in identifying and retaining a wide range of cassava varieties is necessary (Faizo et al., 2020). Local varieties can be stored in the soil for a number of months and can be replanted (Nakabonge et al., 2018).

Decrease in tillage of indigenous cassava varieties could be as a results of diminished harvests, proneness to diseases and pests, astringent taste, no market, the coming of improved varieties, and prolonged maturation period (Nakabonge et al., 2018).

To ensure the preservation of the local varieties, Ugandan farmer's plant in a number of plots, after harvesting, they reseed immediately, disseminate with other farmers and practice clean farming (Nakabonge, 2018). In situations where cultivation cannot be done instantly, the

cassava stalks can be stored under the shade in a standing position to maintain their viability (Robert & Brown, 2004).The release of improved varieties of cassava with all the benefits associated to them may face a challenge of poor acceptability by the local farmers due to these varieties lacking certain attributes like taste, flavour, texture, replanting possibilities that the farmers consider relevant (Kasule et al., 2020).

Nakabonge et al. (2018), found out that farmers in Uganda can cultivate up to 14 different varieties on farm, with the majority having not more than 4 local varieties with North-western and mid-western Uganda having highest numbers. According to Manano et al. (2017), some local varieties of cassava commonly grown in Uganda are; *Angaraba, Bamunanika, Empologoma, Nyaraboke* and others. In regards to preference of local cassava varieties by the Ugandan farmers, those in the Central region prefer *Kwatamumpale and Bukalasa*, those in western, *Nyaraboke and Timtim*, in the East, *Magana and Ofumbachai*, whereas in Northwest, *Gbasumenge* and *Mingoro* are preferred (Kasule et al., 2020). *Mwezi mukaaga* is a local variety commonly grown in the Central regions and was given the name by the locals because of its quick maturation, which is approximately 6 – 8 months and it can be stored longer in the soil without it spoiling (Iragaba et al., 2020). Due to the fact that this variety has been grown by the local community for a very long time, it is now considered a local variety. *Mwezi mukaaga* and *Mukumba* were the local varieties used in this study as they are fast growers, could easily be identified and were readily available.

2.1.2 Improved Varieties

As per National Crops Resources Research Institute (NaCCRI) in Namulonge, a variety can be considered improved if it has undergone a structured breeding programme, has been checked for resistance to diseases and is high yielding (Twine et al., 2007). Cassava viruses have led to

unceasing cassava genetic studies in Uganda, which has led to release of a number of improved varieties like the NASE chain also referred to as the Namulonge chain and most recently the NAROCASS chain originating from NARO (Abaca et al., 2021). CMV (Cassava Mosaic Virus) and CBSV (Cassava Brown Streak Virus) are one of the largest hindrances to cultivation of cassava in Uganda with up to Shs 81.7b being lost annually (Alicai et al., 2007). Over 86% of farmers grow NAROCASS 1 as the main cassava variety (Buyinza & Kitinoja, 2018). Other varieties are; NAROCASS 2, TME 14, NASE 3, NASE 12 and NASE 14 (Kasule et al., 2020).

Freshly harvested improved cassava varieties can have an output of 110 metric tons from a plot of 10 acres whereas the local varieties on average yield around 50 tonnes, which is not even half of what improved varieties can produce (Buyinza & Kitinoja, 2018). Therefore, improved varieties have potential to produce high level of alcohol because of their high starch content (66.72% for NASE 19 to 84.42% for NASE 3) (Manano et al., 2017)which has good properties (Komlaga et al., 2021). The ethanol produced can be used for production of drinks, solvents and fuel (Sriroth et al., 2012a). Starch from cassava can also be utilized in production of glue, feeds, textiles and plywood (Gunorubon & Kekpugile, 2012).

Advantages of improved varieties possess are; drought tolerance, high yielding, pest and disease tolerance, weed tolerance, adaptable to most areas, early maturing, easily transformed in to cash and multiply rapidly (Buyinza & Kitinoja, 2018). Top choice varieties are NASE 14 and NASE 03 despite their observed susceptibility to viral diseases. NAROCASS 1 known for its tolerance to viral diseases and high yield is the least preferred as farmers claim it is tasteless. NAROCASS 1 was more recently released in 2015 while NASE 03 in 1993 and in 2011, NASE 14 (Abaca et al., 2021).

2.1.3 Utilization of Cassava

Globally and in many areas in Uganda, cassava is grown as a staple crop and is also attracting financial importance because of utilization of its starch as food, in industry and as animal feed (Nakabonge et al., 2018). Around 88% of Uganda's cassava is ingested by humans, with 50% of this proportion being processed (Buyinza & Kitinoja, 2018). Cassava leaves are palatable as well providing a rich protein source (Ty et al., 2015). The roots can be consumed fried, roasted, raw and as a paste with the bitter roots being processed into flour and local brew using wet fermentation (Buyinza & Kitinoja, 2018). An average of 60% of the people grow cassava with almost 90% consuming it daily in a variety of forms. Freshly boiled cassava is considered the form in which it is mostly consumed, closely followed by flour and then alcoholic drinks like *enguli* (Ameny, 1995)**.**

2.2 Maize Production

Maize (*Zea mays*) is considered a financially significant crop ingested for sustenance all over the world (Ranum et al., 2014) It is grown worldwide and is made up of starch 72%, protein 10% and fat 4% (Ranum, Peña-Rosas, & Garcia-Casal, 2014). The largest percentage of starch production all over the world (80%) is from maize (Tariq & Iqbal, 2010). As regards global cultivation, maize comes before rice and wheat with an estimated production on 185 M ha of 1.02 billion tons (Wamatsembe et al., 2017). In Uganda, on 1 M ha, around 2.8 M tons is produced (Macrobert et al., 2014). Due to reduced cultivation of other local staples like bananas and cassava as a results of infestation by viral diseases, maize has gained increasing significance (Wamatsembe et al., 2017). Within Uganda and the East African region, cultivation of maize generates revenue for the farmers up to a tune of around 60 million US dollars obtained from informal and formal business (Wamatsembe et al., 2017).

In Uganda, the largest share of maize production is by the rural farmers of which 80% of these are small scale farmers (Robert & Brown, 2004). Almost every area of Uganda grows maize and is a means of subsistence to more than 2 M domestic units, more than 600 millers and 1,000 merchants with it being a significant non-traditional export cash crop fostering benefits mainly for small scale farmers (MAAIF, 2019). Due to increasing demand and a good climate that favours maize growth and enables two seasons of cropping in a year, cultivation of maize that was at 2.8 M MT in 2015 has now increased to 4 M MT as of 2017. Major importers of Ugandan maize are countries in the neighbourhood who also consider it as a staple with Kenya being the largest importer on average annually demanding over 600,000 MT (Road, 2008). Cultivation of maize is possible on a broad range of soils, though best yield is realized when planted in well aerated, properly drained and deep warm loamy soils, in temperate conditions between 30 – 34°C as temperatures lower than 10 °C and higher than 40°C lead to perishing of the plant (MAAIF, 2019)**.**

2.2.1 Hybrid Varieties of Maize Produced in Uganda

Hybrid varieties are seed types that result from the cross-pollination of two or more distinct varieties (Kutka, 2011). Through national programs for continuous research and development (R4D), the Ugandan government has given the continuous improvement of maize priority, and this has resulted in to the emergence of a number of hybrid maize varieties like Hybrid 2115, Hybrid 5355, Kawanda composite, Longe 2H, Longe 6H (MAAIF, 2015). Despite the release of these varieties, their adoption by farmers has remained poor perhaps due to not including certain crucial non-yield attributes such as taste and replanting ability (Ajambo et al., 2017). Two categories of maize varieties exist in Uganda; the Hybrids and the Open Pollinated Varieties-(OPVs) (Table 1). OPVs are characterised by high yields and the ability to be replanted for two years without a decline in the yield. It is impossible to replant hybrid varieties

(Japhether et al., 2006). A variety referred to as an open pollinated variety is one that was just selected by the breeder without the donor of the male part being known, implying that the source of pollen is ambiguous (Kutka, 2011). With proper supervision, hybrid varieties will yield more than OPVs (Abele et al., 2007).

Table 1: Comparative characteristics of open pollinated and hybrid maize varieties

Category	Variety	Grain yield (T/Ha)	Duration to maturity	Desired attributes
Open Pollinated varieties (OPV)	Longe 1	6	115 days	Resistant to MSV, drought tolerant, early maturity
	Longe 4H	4-6	105 days	Early maturation, ability to tolerate drought conditions, resistance to MSV, and other viral diseases
	Longe 5H (Nnalongo)	6	115 days	High content of quality protein, early maturation, tolerance to drought conditions and resistance to viral diseases
Hybrid Varieties	Longe 2H	8	125 days	Resistant to viral diseases and pests in storage
	Longe 6H	8	125 days	High yielding and resistant to viral diseases.
	Longe 7H	8	125 days	Resistant to viral diseases and tolerant to drought conditions.
	Longe 9H	8	120 days	Resistant to viral diseases and tolerant to drought conditions.
	Longe 10H	9	120 days	Resistant to MSV, NLB, GLS, drought tolerant

Source: MAAIF (2019) MSV: Maize Streak Virus, NLB: Narrow Leaflet Corn, GLS: Gray Leaf Spot

2.2.2 Landraces

Open pollinated varieties are seed types that come from multiplication by free pollination from a set of individuals. Landraces are traditional, locally adapted varieties of crops that have evolved over time through the natural and cultural selection of farmers (Kutka, 2011).

Maize landraces have a historical origin with a distinct identity, carry high genetic diversity, have reasonable stress resistance, fair yield and were chose by environmental factors over a number of generations as well as farmer practices like selection of seed and knowledge base (Araújo, 2002). They possess priceless genetic heritage making them suitable candidates for genetic improvement of agronomic features as well as food stability (Lima et al., 2022). Due to the ability of landraces to adjust to certain environmental conditions, and the presence of genetic variability, they have the potential to be used for crop breeding. In comparison to other commercial cultivars, they are less productive (Araújo, 2002). The genetic variability presented by landraces is considered significant in maize biodiversity, however, the limited availability of genetic and agronomic data as well as presence of improved varieties that have better genotypes having narrower genetic variability has narrowed their use, governance and preservation (Pierre et al., 2022). Studies show that landrace abandonment is common when farming passes from one generation to the next (Balirwa, 2004). Examples of landraces in Uganda include; *Obwelu, Entebbe 2, Sembule, Obusuma Bwobe, Lira maize, Iganda, Nalongo, Gulu Pink* (Iken & Amusa, 2004).

Besides the maintenance of agricultural biodiversity, growing landraces provides the population with a number of services like, soil erosion control, greenhouse gas emission and hydrological process control and nutrient cycling (Lima et al., 2022).

The presence of landraces has also been suggested by other authors to be as a result of natural selection and time with little or no human interference and where humans have played a role, it is unconsciously (FAO, 1998)

2.2.3 Utilization of Maize

In Uganda, maize provides more than 40% of the calories ingested in the country side and cityscape and for that reason, it is considered a paramount grain in the country (MAAIF, 2019). Apart from the use of maize for its dietary provisions, it can also be used in poultry farming to provide bran and in commercial applications like production of corn starch which starch can further be processed into a variety of syrups, can be used as a raw material for production of ethanol and can also act as a gelling agent and binder (Memon, et al., 2011; Kaur et al., 2014; Cook et al., 2012).

2.3 Origins and Characteristics of Starch

Starch is the prevalent carbohydrate in the wild and is also known as Amylum in Latin (Galieni & Mainz, 2015). The functional properties of starch make it compatible for a number of applications (Cornejo-Ramírez et al., 2018). Starch is made up of two homopolysaccharides, that is; amylose which has the linear α-(1 → 4) units and amylopectin consisting of short chains connected to the amylose chains by α-(1 → 6) linkages (Visakh, 2015) (Fig. 1). Amylose is made up of around 500–2000 glucose units, whereas amylopectin has over 1,000,000 units. Roughly 98 – 99% of starch content by weight is comprised of amylose and amylopectin whose ratio will fluctuate based on the phytological origin (Santana & Meireles, 2014). By mass, the starch chain is made up of 20% amylose and 80% amylopectin (Eltaboni & Alabidi, 2017). Starch can be classified based on the content of amylose present, with, <15% being considered

waxy, normal with 20–35% and those ≥40% being considered as 'high' amylose starches (MTPS, 2012).

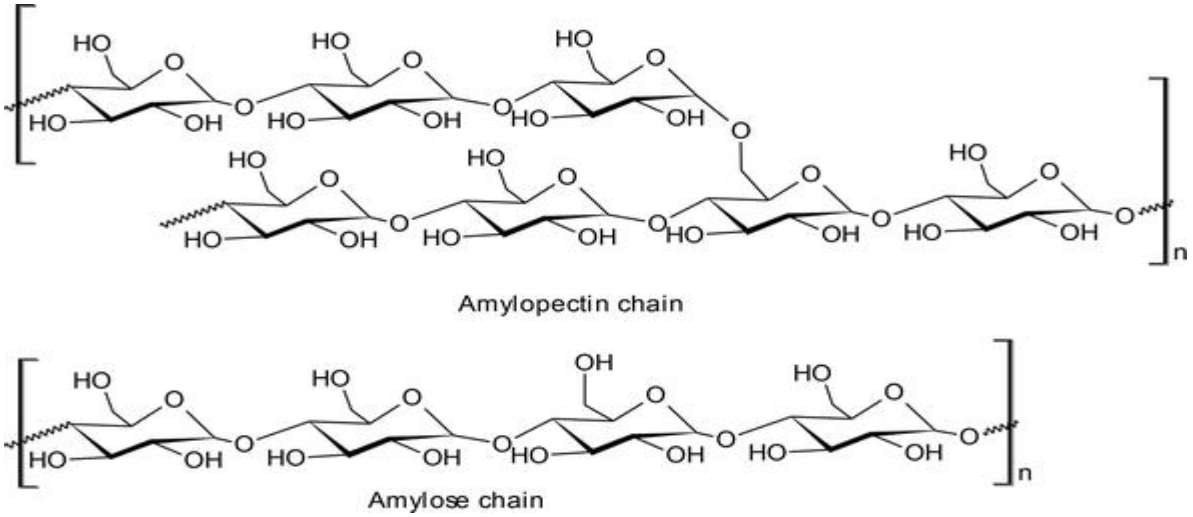

Figure 1: Starch chemical arrangement (Jiang et al., 2010a).

Traditionally, starch was used in the food industry, however, due to technological improvement, its applicability in sectors such as petroleum engineering, textile, medicine and health, agriculture and construction has been realized (Shuren, 2000).

2.3.1 Sources of Starch

In various parts of different plants, starch is found as tiny white granules and is in high concentrations in various areas of the plant like, the roots like cassava and sweet potato, stems like sago palm, seeds like rice, corn and sorghum (Pfister & Zeeman, 2016). Corn (77%), cassava (12%), wheat (7%) and potato (4%) are the dominant commercial sources of starch (Waterschoot, Gomand, Fierens & Delcour, 2015). The wheat endosperm contains the highest starch content between 80 and 85%, followed by the maize kernel at about 72%, followed by cassava tubers at 35% and potato tubers with a content between 18 – 22%. (Onipe, Jideani &

Beswa, 2015; Ranum, Peña-Rosas, & Garcia-Casal, 2014; de Bragança & Fowler, 2004). Starch from wheat and corn has approximately the same amylose and amylopectin content making it easy for the two to be used interchangeably in industrial applications and this gives them an advantage over other starches (de Bragança & Fowler, 2004).

2.3.2 Composition of Starch

Approximately 98 – 99% of the dry weight of starch is made up of two alpha-glucans whose ratio differs depending on the plant source of the starch (Kim & Kim, 2021).

Native starches have varying stabilities under diverse temperature and pH conditions which affects its functionality (Alcázar-Alay & Meireles, 2015). For example, native starch is insoluble and resistant to hydrolysis by amylase when dissolved in water at room temperature (Lehoczki et al., 2018). In order to enhance its functionality, native starch has to undergo modifications (Albano et al., 2014). The correlation between the functional and physical characteristics of the starch from different sources can inform industry on the appropriate modifications (Zou et al., 2023; Cornejo-Ramírez et al., 2018; Ogunmolasuyi et al., 2016).

Starch alteration can be attained by chemical, physical, enzymatic and gene modification processes (Neelam et al., 2010). Modification using physical means is simple and cheap and involves processes such as deep freezing and thawing, osmotic pressure treatment, super heating, pulsed electric field treatment, corona electrical discharges, controlled pressure-drop process and others (Neelam et al., 2012). Chemical modification on the other hand is considered more effective and results in marked physicochemical changes due to the initiation of new functional moieties to the starch molecules via hydroxyl groups (Nawaz et al., 2020). Variables like the degree of substitution, botanical origin of the starch, the reagents used and their concentration, reaction time, pH, presence of catalysts, spatial distribution of alterations within

the molecule and others will affect the functional characteristics of the chemically modified starch (Chen et al., 2017). Processes such as acetylation, cationization, oxidation, hydrolysis, cross-linking, and others are some of the processes through which chemical modification can be achieved (Eltaboni & Alabidi, 2017).

2.3.2.1 Amylose and Amylopectin

The ratio of amylose to amylopectin and their individual properties play a role in determining the characteristics of starch, such as its gelatinization behavior, viscosity, and functional properties (Cornejo-Ramírez et al., 2018). The presence of amylose affects the texture, retrogradation, and digestibility of starch-containing foods (Kong, 2020). The varying percent composition and arrangement of amylose / amylopectin, the molecular and structural composition as well as physicochemical properties, often influence the starch properties and for this reason, determining the content of each component and their overall concentration in starch is essential (McClements, 2019).

Amylose is a straight-chain polymer composed of glucose units connected by α-1,4-glycosidic bonds and chains can vary in length, typically ranging from several hundred to several thousand glucose units (Eltaboni & Alabidi, 2017). The unique structure of amylose gives it several important characteristics such as; the tendency to form helical structures due to the arrangement of glucose units (Khatami et al., 2021). This helical conformation is stabilized by hydrogen bonding between adjacent glucose residues (Hancock & Tarbet, 2000). The helical structure of amylose allows the chains to pack tightly, resulting in a compact arrangement (Seung, 2020). This compact packing is responsible for the semi-crystalline nature of starch granules (Bertoft, 2017). Amylose is less soluble in water compared to amylopectin (Cuevas et al., 2010). The linear structure of amylose limits its interaction with water molecules, reducing its solubility (Seung, 2020). However, amylose can form colloidal suspensions known as "iodine-starch

complexes" due to its ability to interact with iodine molecules (Yu et al., 1996). Heating of amylose in the presence of water and then cooling it can lead to gelation. The helical chains align and associate with each other, forming a gel matrix that entraps water. This property is important in various food and industrial applications (Tako et al., 2014).

Energy in plants is stored by amylose (Zhong et al., 2022). The functionality of starch varies between different species of plants and within the same cultivars cultivated in varying environments (Copeland et al., 2009). This variability produces starches with varying properties, which will ultimately cause challenges during processing arising from inconsistent raw materials (Copeland et al., 2009). The amylose chain is depicted by resistance to amylase, high susceptibility to retrogradation, high thermostability and high crystallinity (Hoover et al., 2010; Wang et al., 2011). Amylose has intact granules that have a highly ordered molecular structure and this makes digestibility by enzymes slow. In order to increase the vulnerability of starch to digestion by enzymes, processing such as cooking that can disrupt the ordered structure is required (Wang & Copeland, 2013).

Two methods can be employed for assessing amylose levels in a starch sample (Subroto, 2020). That is; gravimetry and spectrophotometry (Bahdanovich et al., 2022) . The principle behind the spectrophotometric method is the measurement of the absorbance of the sample which necessitates the sample to have a chromophore group (Dadi & Yasir, 2022). Chromophore groups are typically conjugated systems of double bonds or certain functional groups that are responsible for the absorption of light and the resulting colour (Rajesh et al., 2017). Starch itself does not possess a chromophore group and this therefore necessitates the use of reactants such as iodine solution that will bind to the amylose straight chain hence giving the starch solution a coloured absorption spectrum (Subroto, 2020). The principle of gravimetry is based on the fact that iodine forms a complex with amylose but not with amylopectin. By selectively

precipitating amylose and then measuring its mass, one can determine the amylose content in the starch sample (Hoover & Ratnayake, 2005).

Amylopectin is a cross-linked polymer of glucose units also connected by α-1,4-glycosidic bonds, but it contains additional α-1,6-glycosidic bonds. It has a highly ramified structure with both linear and branched regions (Nakamura, 2015) ref. The α-1,6-glycosidic connections create divergences in the amylopectin molecule, leading to a tree-like structure (Bello-Pérez et al., 1996). The amylopectin molecules are typically larger than amylose, with a higher molecular weight (White & Chiaramonte, 2021), with chains that are relatively short having distribution profiles that are broad and on average, 18–25 units long (Tester at al., 2004). Amylopectin exhibits greater solubility in water compared to amylose because of the branching, which increases the interaction with water molecules (Green, 1975). The major component of the non-crystalline regions in starch granules is composed of amylopectin, contributing to the overall structure of starch (Bertoft, 2017). The crystal structure of starch is decided by the amylopectin content and more amylopectin will result in a glassy or waxy texture (Santana & Meireles, 2014). The amylopectin chain has a low repasting temperature and is easily attacked by enzymes such as amylase (Cheung & Mehta, 2015). The starch of most agricultural products contains higher amounts of amylopectin reaching to levels between 70 and 85% (Bello-Pérez et al., 1996).

2.3.3 Starch Properties

Starch has chemical and physical properties (Omoregie, 2020). The physical properties of starch refer to the characteristics that can be observed or measured without altering the chemical makeup of the starch (Gallant et al., 1992). These characteristics include; appearance, particle size, texture, water Absorption, solubility, viscosity, retrogradation and shear-thinning behavior (Marquezi et al., 2016).

The chemical properties of starch describe its behaviour and reactions at the molecular level (Cornejo-Ramírez et al., 2018). These properties are determined by its chemical composition and the functional groups present (Omoregie Egharevba, 2020b). These properties include; composition, of which starch is composed of two glucose polymers (Tester et al., 2004). Hydrolysis, which is a chemical reaction in which the starch molecule is broken down into its constituent glucose units, occurring through enzymatic or acid-catalyzed processes (Azmi et al., 2017). Iodine reaction, where starch forms a blue or bluish-black colour complex with iodine resulting from establishment of an inclusion complex between iodine molecules and the helical structure of amylose in starch (Moulay, 2013). Chemical modification, which introduces specific functionalities like crosslinking, esterification, etherification, oxidation and enzymatic modifications, which modifications can affect the solubility, stability, gelation properties and other characteristics of starch (Introduction, 2009). Gelatinization, is another chemical property of starch and is a procedure in which the starch granules hydrate and expand upon heating, leading to the disorder of the crystalline structure. Retrogradation, which occurs after gelatinization has taken place (Tako et al., 2014).

2.3.3.1 Gelatinization and Solubility of Starch

Solubility and gelatinization are two important starch properties playing a significant part in its functionality and applications (Santana & Meireles, 2014).

Starch has limited solubility in chilled water (Sarifudin et al., 2021). When starch granules are dispersed in water, they do not dissolve completely but rather form a suspension (Bertoft, 2017). However, as the temperature increases during heating, starch undergoes a process called gelatinization, which leads to increased solubility (Crochet et al., 2005). Gelatinization involves the swelling and bursting of starch granules, resulting in the release of starch molecules into

the surrounding liquid (Tako et al., 2014). During gelatinization, the interaction between starch molecules and water molecules disrupts the ordered structure of the starch granules, allowing for greater water penetration (Chakraborty et al., 2022). The hydrogen bonds between starch molecules and water molecules are formed, causing the starch granules to swell and eventually rupture (Cornejo-Ramírez et al., 2018). This process enhances the solubility of starch, leading to the formation of a viscous paste or solution (Ratnayake & Jackson, 2006). The solubility of starch can also be affected by variables like temperature, pH, and the presence of other ingredients (Kumoro et al., 2021). Higher temperatures generally promote greater starch solubility, while low pH (acidic conditions) can increase the solubility as well (Sakkara et al., 2020). However, excessive heat or prolonged exposure to acidic conditions can lead to degradation of starch molecules (J. Singh et al., 2007).

Gelatinization is the mechanism by which starch granules absorb water and undergo structural changes when heated (Tako et al., 2014). It is a crucial step in cooking and processing starch-based foods and serves as the basis for the formation of gels, pastes, and thickening properties (Ratnayake & Jackson, 2008). During gelatinization, starch granules swell as water is absorbed, causing them to lose their crystalline structure (Jackson & Ratnayake, 2006). The interaction between starch molecules and water disrupts the hydrogen bonds within the granules, leading to a rise in molecular mobility (Scott & Awika, 2023). Due to this, the starch molecules become more dispersed in the aqueous medium (Xie et al., 2006). The gelatinization temperature of starch changes depending on its source and composition (Li et al., 2015). Various types of starch have varying gelatinization temperature ranges (Ubwa et al., 2012). For example, corn starch typically gelatinizes between 60-70°C, while potato starch has a gelatinization range of 55-65°C (Ubwa et al., 2012). Gelatinized starch exhibits unique properties such as thickening ability, viscosity, and gel formation upon cooling (Sandhu & Singh, 2007). The gel formed by

gelatinized starch is often referred to as a starch gel or starch paste (Bemiller, 2011). It can provide structure, stability, and desirable texture in a number of food products, like; sauces, puddings, fillings, and bakery items (raj et al., 2020). The gel strength and viscosity of the starch gel rely on factors like starch concentration, heating time, cooling rate, as well as other ingredients in the system (Schirmer et al., 2015).

2.3.3.2 Retrogradation and Shear Properties

Retrogradation refers to the way in which gelatinized starch undergoes structural reorganization and recrystallization upon cooling (Wang et al., 2015). After the gelatinization of starch, the disrupted molecular structure begins to reassociate and form ordered regions, leading to the retrogradation phenomenon (Wang et al., 2015). During retrogradation, the amylose and amylopectin molecules in the starch gel rearrange and form intermolecular hydrogen bonds, resulting in the development of a more rigid and crystalline structure (Biduski, et al., 2018). This reorganization can lead to changes in the texture and stability of starch-based products over time, often resulting in the development of a firm or gritty texture (Ottenhof & Farhat, 2004). The retrogradation process is affected by various factors such as, temperature, time, starch type, and storage conditions (Chang et al., 2021). Starches with a higher amylose content tend to exhibit a more pronounced retrogradation tendency compared to those with a higher amylopectin content (Chang et al., 2021). Retrogradation can impact the quality and shelf life of starch-containing products (Okpani & Kelechi, 2019). It can cause firmness, syneresis (the release of water), and staling in baked goods, as well as undesirable changes in the texture and mouthfeel of starch-based sauces, puddings, and gels (Denchai et al., 2019). Proper storage conditions, such as refrigeration or freezing, can slow down retrogradation and extend the shelf life of starch-based products (Denchai et al., 2019).

Shear of starch refers to the force or stress applied to a material when it undergoes deformation, particularly in response to a sliding or flowing motion (Xie et al., 2006). Shear forces can affect the properties and behaviour of starch-based systems, particularly their viscosity and flow characteristics (Z. Ji et al., 2017). When a shear force is applied to a starch-containing system, such as stirring or mixing, it can cause changes in the arrangement and alignment of starch molecules (J. A. Han & Lim, 2004). This reorientation of starch molecules leads to a decrease in viscosity, resulting in the thinning or reduction of the system's resistance to flow (Chen et al., 2014). This property is known as shear-thinning or pseudoplastic behaviour (M. A. Rao et al., 1997). The shear-thinning behaviour of starch is highly desirable in various food applications (Sana et al., 2020). It allows for easy pouring, spreading, and coating of starch-based products (Mohamed et al., 2021). For example, in sauces and dressings, shear thinning allows the product to flow smoothly when poured or drizzled but regain viscosity and cling to the food surface once the shear force is removed (Li et al., 2012). Starches with a higher amylose content generally exhibit more shear-thinning behaviour compared to those with a higher amylopectin content (Ansharullah et al., 2020). The extent of shear-thinning can also be influenced by factors like, starch concentration, temperature, pH, and the presence of other ingredients (V. Subramanian, Hoseney & Bramel-cox, 1994).

2.3.3.3 Shape and Size

Starch granules come in a diverse array of sizes, varying from a few micrometers to several tens of micrometers in diameter (Li et al., 2001). The starch granule size can vary depending on the botanical origin and the specific type of starch (Pérez et al., 2009a). Starch granules from different sources can exhibit distinct size distributions (Guo et al., 2023). For instance, potato starch granules range from 5 to 100 micrometers, while corn granules typically range from 10 to 35 micrometers in diameter and cassava starch granules range from 5 to 40 micrometers (Cui

et al., 2014). The size of starch granules can impact various properties, including solubility, gelatinization behavior, and textural attributes (Narváez-González et al., 2007). Smaller granules tend to have a greater surface-to-volume ratio, which can enhance their solubility and susceptibility to gelatinization compared to larger granules (Narváez-González et al., 2007).

The shape of starch granules is highly subject to botanical source and starch type (Pérez et al., 2009). Starch granules can be classified into various shapes, including spherical, elliptical, ovoid, polygonal, or irregular shapes (Pérez et al., 2009). For example, granules of potato starch are typically oval or elliptical, while granules of corn starch are polygonal with rounded edges (Chung & Lai, 2006). Wheat starch granules are more irregular in shape, exhibiting a combination of elongated and rounded forms (Zhang et al., 2021). The shape of starch granules can influence their packing and interactions within starch-based systems (Smits, 2001). It can affect factors such as sedimentation, flow behavior, and gelation properties (Ee et al., 2020). The shape can also impact the textural attributes of starch-containing products (Cornejo-Ramírez et al., 2018).

Granules of starch have a unique microstructure consisting of amorphous and crystalline regions (Pérez et al., 2009). Amorphous regions contain ramified amylopectin molecules, while the crystalline regions consist of unbranched amylose molecules (Martens et al., 2018). The arrangement and organization of these amorphous and crystalline regions contribute to the overall structure and functional properties of starch granules (Cornejo-Ramírez et al., 2018). The crystalline regions form ordered structures, while the amorphous regions allow for swelling and interactions with water and other molecules (Chuang et al., 2017). The microstructure of starch granules can be observed and analyzed using various techniques, such as microscopy (e.g., optical microscopy, electron microscopy), X-ray diffraction, and spectroscopic methods

(Ohtani et al., 2000). These techniques provide insights into the internal structure, organization, and composition of starch granules (Błaszczak et al., 2006).

2.3.3.4 Swelling and Viscosity

Swelling power of starch refers to its ability to absorb and retain water when exposed to moisture or heated in an aqueous medium (Kaur et al., 2011). Starch granules have a unique structure that allows them to undergo swelling, resulting in an increase in their volume and a change in their physical properties (Syafutri et al., 2018). When starch granules come into contact with water, they absorb water molecules through the amorphous regions, leading to their hydration and swelling (Wang et al., 2014). The water enters the granules, causing them to expand and increase in size (Moran, 2019). The extent of swelling relies on factors such as starch type, concentration, temperature, and time (Debet & Gidley, 2006) . The swelling power of starch is affected by the presence of both amylose and amylopectin components (Vamadevan & Bertoft, 2020). Starches with a higher amylose content tend to have a higher swelling power in relation to those with a higher amylopectin content (Sumardiono et al., 2019). This is because amylose has a more linear structure, allowing for easier water penetration and swelling (Debet & Gidley, 2006). The swelling power of starch is a significant property in various applications, particularly in food and industrial processes (Compart et al., 2023). In food applications, the swelling power of starch affects characteristics such as texture, gel formation, and moisture retention (Gani et al., 2010). It is crucial in processes like thickening, gelling, and binding. In industrial applications, such as papermaking and adhesives, the swelling power of starch influences factors like viscosity and binding properties (Kusumayanti et al., 2015).

Viscosity indicates the resistance of a fluid to flow. In the case of starch, viscosity plays a vital role in determining its thickening and gelling properties (Megusar et al., 2022). Starch-based

systems can exhibit different viscosity profiles depending on factors like starch concentration, temperature, shear rate, and time (Acosta-Osorio et al., 2011). Viscosity is strongly associated with the gelatinization process of starch (Hubacz & Buczyńska, 2011). When starch granules are heated in presence of water, they undergo gelatinization, which involves the swelling and bursting of the granules, leading to the release of starch molecules into the surrounding liquid (Tako et al., 2014). This increase in starch concentration contributes to the viscosity of the system (Reddy & Bhotmange, 2014). The viscosity of starch is influenced by the existence of both amylose and amylopectin (Karakelle et al., 2020). Amylose molecules have a more linear structure and can form more organized regions, resulting in higher viscosity (Cornejo-Ramírez et al., 2018). Amylopectin, on the other hand, with its branched structure, contributes to lower viscosity due to decreased intermolecular interactions (Charles et al., 2005). The consistency of starch-based systems can also exhibit shear-thinning behavior (Mohamed et al., 2021). Shear-thinning indicates a reduction in viscosity with a rise in shear rate or applied force (Rosti & Takagi, 2021). Starch-based solutions or pastes tend to exhibit higher viscosity at rest but reduce in viscosity when subjected to shear forces, allowing for easy flow and processing (Ansharullah et al., 2020). The viscosity of starch-based solutions and pastes is critical in various applications, including food processing, pharmaceuticals, and industrial processes (Iida et al., 2008). It influences factors such as texture, mouthfeel, stability, and flow characteristics of the final product (Yazid et al., 2018).

2.3.3.5 Digestion-Resistant Starch

It is a unique component present in starch granules that resists breakdown in the small bowel and behaves like dietary fibre (Raigond et al., 2019). It goes through the digestive system largely intact until it gets to the large intestine, where it undergoes fermentation by gut microflora (Sajilata et al., 2017). It can also refer to the fraction of starch that evades digestion

in the upper gastrointestinal tract due to its structural properties (Bojarczuk et al., 2022). Unlike other forms of starch, this starch remains undigested by human enzymes in the small intestine (Bojarczuk et al., 2022). Digestion-resistant starch has several notable characteristics such as; resistance to digestion by amylases, fermentability (Yi et al., 2021): where once it reaches the large intestine, it serves as a material for fermentation by colonic bacteria, resulting in the production of short-chain fatty acids (SCFAs) and other metabolites, has a low glycemic response implying that it has a reduced impact on blood glucose levels compared to rapidly digestible carbohydrates, potential health benefits like improved gut health, satiety, enhanced insulin sensitivity, and potential cancer-protective effects (Raigond et al., 2019).

This starch type is categorized into several types based on its structural characteristics and how it resists digestion (Arp et al., 2020). The classification includes: RS1: Type 1 Resistant Starch: refers to starch that is inaccessible physically due to its association with other food components (Han et al., 2023). Examples include starch in whole or partially milled grains, seeds, or legumes (Sajilata et al., 2017). The starch is trapped within a complex matrix and resists digestion until it is released during processing or cooking (Leszczynski & Technology, 2004). RS2: Resistant Starch Type 2 - Resistant Granular Starch: it is native, undamaged starch granules that resist digestion due to their compact structure and crystalline organization (Harris, 2019) ref. Foods rich in RS2 include uncooked potatoes, green bananas, and high-amylose corn starch (Raigond et al., 2019). Heat or mechanical processing can partially break down the crystalline structure, increasing its digestibility (Raigond et al., 2019). RS3: Resistant Starch Type 3 - Retrograded Starch: RS3 is formed when cooked starch is cooled, causing the reassociation of starch molecules into an ordered, crystalline structure. Foods such as cooked and cooled potatoes, pasta, and bread crusts contain RS3 (Sajilata et al., 2006). The retrograded starch resists digestion and behaves as resistant starch (Sajilata et al., 2017). RS4: Resistant

Starch Type 4 - Chemically Modified Starch: RS4 refers to chemically modified starches that have been specifically designed to resist digestion (Nurmilah & Subroto, 2021). These starches are typically used in processed foods as functional ingredients. Examples include chemically cross-linked starches, acetylated starches, and enzyme-resistant starches (Kasote et al., 2014).

The content and formation of resistant starch can be influenced by various factors like; processing and cooking such as milling, grinding, and heat treatment that affect the accessibility and digestibility of starch (Pereira et al., 2014). Cooking and cooling processes can lead to the formation of retrograded resistant starch (Thuy & Tai, 2022). Different types and sources of starch vary in the content and type of resistant starch (Raigond et al., 2019) . The amylose content equally affects the resistant starch content with starches that have higher amylose content tending to have higher levels of resistant starch (S. Tian & Sun, 2020).

2.3.4 Applications of Starch

Around 80% of the world market production of starch is from maize (Ranum et al., 2014). The physicochemical properties of maize starch make it a useful ingredient in the production of number of food products where it acts as a water retention agent, an adhesive, a stabilizer and a thickener (Zhu & Wang, 2013). The starch sources that are regarded as non-conventional such as that from root tubers like potatoes are equally extending the range of preferred functional properties that are desired in food product development (Phukan & Nongkhlaw, n.d.). This starch can be used in food manufacturing as preservation and quality enhancers of foods that are baked, mayonnaises, soups and sauces and as additives that facilitate thickening (Tagliapietra et al., 2021). Starch differs based on the botanical origin from which it is derived with each plant having different compositions and structures like the ratio of the amylose / amylopectin chains, the fat and protein content, the organ reserves and others (Kovrlija et al.,

2020). Due to these differences, starch has diverse functional properties that expand its scope for industrial applications (Butt et al., 2018). These differences also affect its interaction with various constituents in the food that ultimately give the desired texture and taste to the final product (Santana & Meireles, 2014; Agrosynergie, 2010). Starch can help raise the product lifespan of food products by resisting the gel breakdown during processing (Jiang et al., 2010a). Commercial production of starch is relatively cheap given that it can be obtained from a number of cereal grains and tubers, is easy to extract and its purification process is not complicated (Daudt et al., 2014).

2.3.5 Starch Extraction Methods

Starch extraction refers to the process of separating starch from its source, typically plants like corn, wheat, potatoes, or cassava (Alavitalab, 2022). There are several methods used for starch extraction, and the selection of the method relies on the specific plant type and preferred quality of the extracted starch (Muslim et al., 2011).

2.3.5.1 Wet Milling

Wet milling is a common method used for starch extraction from corn (maize) (Deepak & Jayadeep, 2022). The process begins by soaking the corn kernels in water containing sodium hydroxide, which helps to loosen the starch from other components (Ramirez et al., 2008). The softened kernels are then mechanically ground to break them into smaller pieces (Deepak & Jayadeep, 2022). The resulting slurry, called masa, consists of starch, protein, fibre, oil, and other components (Jan et al., 2013). The masa is subjected to a series of steps to separate the starch (Ratnayake et al., 2007). First, the slurry goes through a number of screens or sieves to remove coarse particles (Kakhia, n.d.). Then, the finer fraction is processed through a centrifuge or hydrocyclone to separate the starch from protein and fibre (Afam-Mbah, 2019). This step

produces a starch milk, which is a suspension of starch particles in water (Saengchan et al., 2015). The milk undergoes further refining, including washing and settling processes, to remove impurities (Hernández-Carmona et al., 2017). To recover the starch from the milk, it is dewatered through processes such as centrifugation or vacuum filtration (Ratnayake et al., 2007). The resulting wet starch is then dried using methods like flash drying or drum drying. The dried starch is subsequently milled into a fine powder for further use (Thanh et al., 2020).

2.3.5.2 Dry Milling

Dry milling is another method commonly used for corn starch extraction, but it differs from wet milling in that it does not involve soaking the corn kernels (Suksomboon & Naivikul, 2006). Instead, the dry kernels are cleaned to remove impurities and then milled into a fine powder (Ray & Ramachandran, 2018). The powder is sifted through screens to separate the starch from other components such as protein, fiber, and germ (Steeneken & Helmens, 2009). After separation, the starch undergoes further processing steps to refine and purify it (Leewatchararongjaroen & Anuntagool, 2016) . This can include air classification, where the starch particles are sorted based on size, as well as gravity separation, which uses the difference in density between starch and other components to further purify the starch fraction (De Schepper et al., 2021). The resulting starch is then dried using methods such as flash drying or fluidized bed drying (Steeneken & Helmens, 2009).

2.3.5.3 Tuber Starch Extraction

Starch extraction from potatoes or cassava can also be called the tuber starch extraction method or wet method for starch extraction (Kaur et al., 2016). It involves a different approach compared to corn (Kringel, 2020). The tubers are first cleaned, washed, and then peeled (Sukhija et al., 2016). They are then grated or chopped into small pieces or a pulp-like

consistency (Sukhija et al., 2016). The pulp is blended with water to form a slurry, and the slurry is typically left to settle for a period of time (Patkar et al., 2020). During settling, the heavier starch particles sink to the bottom, while the lighter impurities and fibres float to the top (Pei lang, 2004). The supernatant, containing impurities, is drained off, and the settled starch is collected (Huang et al., 2022). The collected starch undergoes a washing process to remove residual impurities and is then dewatered through processes like centrifugation or filtration. The wet starch is finally dried using methods like hot air drying or drum drying (Fakir et al., 2013).

2.3.6 Modification of Starch

Modification of starch refers to the process of altering the physical, chemical, or enzymatic properties of starch to enhance its functionality for specific applications (FAO, 2018). Starch, being a naturally occurring polysaccharide, possesses unique characteristics such as thickening, gelling, and binding properties (Qi et al., 2023). However, native starch may not always meet the requirements of certain industries due to limitations in solubility, stability, or compatibility (Zarski et al., 2021) ref. Starch modification techniques aim to overcome these limitations and expand the range of applications by modifying its structure and properties (Neelam et al., 2012). Some common methods of starch modification are;

Physical modification: Examples of this type of alteration include; Gelatinization, which improves starch solubility and viscosity (BeMiller, 2017). Retrogradation, which results in a more rigid gel with increased stability (Liu et al., 2021). Annealing and Heat-Moisture Treatment, where controlled heating and moisture treatments can modify starch structure, leading to altered gel properties and improved stability (Fonseca et al., 2021). High-Pressure Treatment and Extrusion, which involves applying high pressure or extrusion forces to starch

to disrupt its granular structure, resulting in modified properties such as increased solubility and improved film-forming ability (BeMiller, 2017).

Chemical modification: Involves processes such as; Etherification and esterification reactions, where starch is altered by incorporating functional groups such as hydroxyethyl, hydroxypropyl, or carboxymethyl through etherification or esterification reactions (Neelam et al., 2012). These modifications enhance starch solubility, stability, and resistance to shear and heat (He et al., 2023). Cross-linking, where agents like phosphoryl chloride, epichlorohydrin, or divinyl sulfone can be used to create covalent bonds between starch molecules, resulting in improved stability, resistance to shear, and increased viscosity (Jyothi et al., 2006). Oxidation, this happens with oxidizers like sodium hypochlorite or hydrogen peroxide introducing carbonyl and carboxyl groups, leading to enhanced thickening, robustness, and film-forming properties (Chen et al., 2017).

Enzymatic modification: It involves processes such as; enzymatic hydrolysis, which involves using enzymes like α-amylase and glucoamylase to hydrolyze starch, breaking it down into smaller molecules (Vitolo, 2020). The process results in modified starches with different properties such as reduced viscosity and improved solubility (Author & Daniel, n.d.). Starch phosphorylation and branching is another way of modifying starch enzymatically where enzymes like phosphorylase or branching enzymes can introduce phosphate groups or create branch points within the starch molecule, altering its functional properties such as gel strength and stability (Blennow et al., 2000).

Starch modification finds application in different industries like food, pharmaceuticals, textiles, papermaking, and adhesives (Chiu & Solarek, 2009). Altered starches are used as thickeners, stabilizers, gelling agents, film-forming agents, and encapsulation materials (Imeson, 2010). Starch modification contributes to sustainability efforts by utilizing renewable resources and

reducing waste in industries (Halysh et al., 2022). Ongoing research focuses on developing environmentally friendly modification methods and exploring novel applications for modified starches (Saboonchia et al., 2021).

2.4 Description of Glucose Syrup

Glucose syrup, also known as corn syrup or liquid glucose, is a thick, viscous syrup that is primarily composed of glucose molecules (Ünlü & Soysal, 2017). It is produced through the hydrolysis of starch, typically derived from corn or other starch sources like wheat, potatoes, cassava or rice (Zia, 2010). Glucose syrup is characterized by its high solubility in water and its ability to provide sweetness, texture, and moisture retention in various food applications (Science, 2021).

The chronical of glucose syrup traces its roots to the early 19th century when it was first unearthed and developed as a food ingredient (Hobbs, 2009). It was discovered as a simple sugar and a key energy source for living organisms (De, 2010). In the late 18th century, chemist Carl Wilhelm Scheele identified and isolated glucose by hydrolyzing starch with sulfuric acid (Hull, 2010). In the early 19th century, German chemist Andreas Sigismund Marggraf expounded on the process of hydrolyzing starch into glucose using diluted sulfuric acid. This breakthrough opened the doors to the commercial production of glucose syrup (Hull, 2010).

Industrial manufacture of glucose syrup began around the mid-19th century, primarily in Germany and France (Zainab et al., 2011). The process involved treating starch with acid or enzymes to break it down into glucose molecules, resulting in a thick syrup known as glucose syrup or corn syrup (Rosida & Amelia, 2022). In the United States, the production of glucose syrup gained momentum in the late 19th century. The availability of corn as a raw material and

the development of industrial processing techniques led to the prevalent use of corn syrup in the American food industry (Zainab et al., 2011).

Glucose syrup is created by breaking down the starch chains into shorter glucose chains or individual glucose units through enzymatic or acid hydrolysis (Arif et al., 2019). This process converts the starch into glucose, resulting in a syrupy liquid (Turini et al., 2021). The resulting syrup can be further processed to adjust its concentration, remove impurities, and achieve desired properties (Lambri et al., 2014). Over time, refining techniques have been developed to produce different grades of glucose syrup, such as light, medium, and dark syrups, hinging on the degree of starch hydrolysis (Hull, 2010). Each grade offers specific characteristics and uses in the food industry (Science, 2021).

In the 20th century, advancements in enzymatic hydrolysis technology replaced the use of acids in glucose syrup production (Pavas et al., 2020). Enzymes, particularly amylases, became the preferred method for breaking down starch into glucose, resulting in a more controlled and efficient process (Parker et al., 2010).

Glucose syrup can be categorized based on various factors such as its composition, viscosity, and dextrose equivalent (DE) (Ejiofor, 2015). This type of categorization allows for a better understanding of its characteristics and enables its selection and usage in various applications within the food industry (Reichenbach et al., 2019).

Based on composition, it is the most basic form of syrups, consisting primarily of glucose molecules and does not contain any other significant components or additives (Emerton et al., 2008). High-Fructose Corn Syrup (HFCS): it’s another type of glucose syrup that has undergone additional processing to convert a portion of the glucose into fructose (Parker et al., 2010). It is typically used as a sweetener in food and beverage manufacture (R. do N. Silva et

al., 2010). Based on viscosity, thin or low-viscosity syrup is a variety of glucose syrup which has a low viscosity and flows more easily (Kognou et al., 2022). It is often used in applications where a thin, pourable consistency is desired, such as in beverages, sauces, or glazes (Ershadi et al., 2021). Thick or high-viscosity syrup on the other hand has a higher viscosity and is more syrupy in texture (Darras et al., 2015). It is commonly used in confectionery, baking, and other applications where a thicker consistency is required (Schellart, 2011). The last categorization is based on the Dextrose Equivalent (DE), which is a measure of the degree of starch hydrolysis in glucose syrup (Montañez, 2012). It represents the percentage of reducing sugars (primarily glucose) present in the syrup (Rong et al., 2009). The higher the DE value, the more hydrolyzed the starch and the sweeter the syrup (Radeloff & Beck, 2014). Low DE syrups have a low DE value, indicating that the starch has been minimally hydrolyzed (Montañez, 2012). They are less sweet and often used in applications where a milder sweetness is desired, such as in baked goods or dairy products (Science, 2021). High DE Syrup have a high DE value and have undergone more extensive starch hydrolysis therefore have a higher glucose content (Shariffa et al., 2009). They are sweeter in taste and commonly used as sweeteners in confectionery, beverages, and other food products (Sarungallo & Murtiningrum, 2005).

The categorization of glucose syrup may vary across regions and manufacturers, and different countries may have specific regulations or labelling requirements for glucose syrup products (Hafner et al., 2022).

2.4.1 Glucose Syrup Production

The manufacture of glucose syrup begins with starch hydrolysis, which starch is obtained from plant sources and is made up of long chains of glucose molecules (Hii et al., 2012). The starch

is first broken down into shorter chains or individual glucose units through an enzymatic or acidic hydrolysis process (Miao & Bemiller, 2023).

2.4.1.1 Acid Hydrolysis

Acid hydrolysis of starch is a chemical process that involves breaking down the starch molecules into smaller units, primarily glucose, using acid as a catalyst (Azmi et al., 2017). This method is one of the traditional approaches for starch hydrolysis and glucose syrup production (Wang & Copeland, 2015). The acid used in the hydrolysis process is typically an inorganic acid, with hydrochloric acid (HCl) and sulfuric acid (H_2SO_4) being the most commonly employed (Priyanka & Majumder, 2017). These acids are chosen for their strong acidic properties and ability to catalyze the hydrolysis reaction effectively (Winarti et al., 2019). The concentration of the acid is an essential factor that influences the reaction rate and extent of hydrolysis (Grandgirard et al., 2002). Generally, a high concentration of acid is used to accelerate the hydrolysis process (Singh & Ali, 2008). Concentrations between 1% and 4% are commonly employed, although the exact concentration may vary depending on the desired reaction conditions and specific requirements (Chen et al., 2017). The acid hydrolysis of starch is typically performed under controlled reaction conditions, including temperature, time, and pH (da Silveira et al., 2019). The process is usually conducted at raised temperatures, ranging from 100°C to 150°C, to accelerate reaction kinetics (Hargono et al., 2018). The reaction time can vary subject to the desired degree of hydrolysis and the specific reaction circumstances (Kolusheva & Marinova, 2007).

The acid hydrolysis process involves the cleavage of glycosidic bonds present in the starch molecules (Shi et al., 2020). The acid catalyst offers a proton (H+) to the glycosidic bond, resulting in the formation of a positively charged intermediate (Romero-Téllez et al., 2019).

This intermediate subsequently undergoes hydrolysis, breaking the glycosidic bond and yielding glucose molecules (Ayoola et al., 2013). The hydrolysis reaction breaks down the starch polymer chains, yielding a mixture of glucose chains of varying lengths (Pratiwi et al., 2018) . The resulting glucose syrup contains a range of glucose molecules, including glucose monomers, dimers, and higher oligomers (Azmi et al., 2017). After the acid hydrolysis process, the glucose syrup is typically neutralized to adjust its pH and remove excess acid (Fontana et al., 2008). Neutralization is achieved by adding a base, such as calcium hydroxide (lime) or sodium carbonate, to neutralize the residual acidity (Simpson et al., 2022). The syrup is then further processed through filtration, evaporation, and purification steps to remove impurities and achieve the desired concentration and quality (Rahman et al., 2022). Acid hydrolysis has certain drawbacks, such as the need for careful control of reaction conditions, the potential for degradation of glucose molecules due to the harsh acidic environment, and the formation of unwanted by-products (Wang & Copeland, 2015). These limitations have led to the development of alternative enzymatic hydrolysis methods, which offer milder reaction conditions and higher selectivity in producing glucose syrup (Wang et al., 2017).

2.4.1.2 Enzyme Hydrolysis

Enzyme hydrolysis of starch is a process that utilizes enzymes to break down the starch molecules into smaller units, primarily glucose (Bednarska, 2015). This method is largely used in the manufacture of glucose syrup and other starch-derived products (Marchal & Tramper, 1999). Enzymes used for starch hydrolysis are typically derived from microbial sources, like bacteria or fungi (Souza & Magalhães, 2010). The most commonly employed enzymes for starch hydrolysis are amylases, which specifically target the α-1,4-glycosidic bonds in starch (Wang et al., 2022). Amylases are of two types; Alpha-Amylase, which is responsible for randomly cleaving the internal α-1,4-glycosidic bonds in starch, resulting in the production of

shorter chains called dextrins and Glucoamylase (Amyloglucosidase) which acts on the non-reducing ends of starch and dextrin molecules, progressively hydrolyzing the α-1,4-glycosidic bonds to release glucose molecules (Ayoola et al., 2013).

In some cases, a combination of different amylases is used to achieve optimal hydrolysis (Zhu et al., 2017). The combination of alpha-amylase and glucoamylase enzymes can work synergistically to achieve a more complete breakdown of starch into glucose (Presecki et al., 2013). Enzyme hydrolysis is typically carried out under controlled conditions to ensure maximum efficiency and activity of the enzymes (Bautista-expósito et al., 2020). The ideal temperature for amylases lies between 50°C to 70°C, while the pH range is typically between 4.5 and 6.5. These conditions can vary depending on the specific enzymes used (Y. Li et al., 2012). Prior to enzyme hydrolysis, the starch is normally gelatinized by heating it in the presence of water (Uthumporn et al., 2012). Gelatinization disorganizes the starch granules, resulting in starch being readily available to the enzymes hence facilitating the hydrolysis process (Hall, 2019). The starch slurry is mixed with the selected amylase enzyme(s), and the hydrolysis reaction is allowed to proceed under controlled conditions (Jung et al., 2017). Alpha-amylase initially breaks down the starch into dextrins of various lengths (Bijttebier et al., 2008). Glucoamylase then acts on these dextrins, progressively hydrolyzing them into glucose molecules (Marchal & Tramper, 1999).

The degree of hydrolysis refers to the extent to which the starch is broken down into glucose molecules (Lee et al., 2012). It can be controlled by adjusting factors like the reaction time, enzyme concentration, and temperature (Salwanee et al., 2013). The desired degree of hydrolysis depends on the intended application and the desired sweetness level of the resulting glucose syrup (Bednarska, 2015). Enzyme hydrolysis of starch primarily yields glucose molecules (Pavas et al., 2020). The resulting glucose syrup contains a mixture of glucose

monomers, as well as shorter chains of glucose called dextrins (Brouns, 2020). The composition of the syrup can be adjusted by controlling the reaction conditions and enzyme selection (Robinson, 2015). Once the desired hydrolysis is achieved, the enzyme activity is typically stopped by raising the temperature or adjusting the pH (Roman, 2021). The resulting glucose syrup is then subjected to purification processes, such as filtration, activated carbon treatment, and evaporation, to remove impurities and concentrate the syrup (Taher et al., 2017).

Enzyme hydrolysis of starch offers several advantages over acid hydrolysis, including milder reaction conditions, higher selectivity, and the production of a more controlled composition of glucose syrup (Wahlström & Suurnäkki, 2015). It is widely used in the food industry to produce glucose syrup, sweeteners, and other starch-derived products (I. A. de Souza et al., 2019).

2.4.1.3 Amylolytic Enzymes

Amylolytic enzymes are a class of enzymes that specifically target starch and its breakdown into smaller carbohydrate molecules (Negi & Vibha, 2016). These enzymes are produced by various organisms, including plants, animals, and microorganisms (Yadav et al., 2021). They play a crucial role in the hydrolysis of starch, converting it into simpler sugars like glucose, maltose, and dextrins (Guzmàn-Maldonado et al., 1995). Amylolytic enzymes can be organized into different groups based on their specific mode of action and substrate specificity (A. Sharma & Satyanarayana, 2013).

2.4.1.3.1 Endo-Acting Amylases

Endo amylases are a type of amylolytic enzymes that cleave the inner α-1,4-glycosidic bonds within starch molecules, resulting in the production of shorter fragments (Shukla et al., 2015). Each enzyme exhibits unique properties and substrate specificities, facilitating efficient hydrolysis of starch and the production of desired carbohydrate products (Bijttebier et al.,

2008). The choice of a certain endo amylase depends on the specific application requirements and desired end product (Saini et al., 2017). Examples include; fungal alpha-amylase: produced by fungi such as *Aspergillus* or *Bacillus* species (Shukla et al., 2015). It is widely used in various industries, including starch processing, baking, brewing, and detergent manufacturing (Bijttebier et al., 2008). Examples include *Aspergillus oryzae* alpha-amylase and *Bacillus licheniformis* alpha-amylase (Saranraj & Stella, 2013).

Bacterial alpha-amylase: it is commonly used in industrial applications for starch hydrolysis, textile processing, and detergent manufacturing. Examples include *Bacillus subtilis* alpha-amylase and *Bacillus amyloliquefaciens* alpha-amylase (Al-Bedak et al., 2022). Plant alpha-amylase: found in various plant sources, particularly in seeds, like barley, wheat, and rice (Zhang et al., 2021). Plant alpha-amylases play a vital role in the germination process and starch degradation during seed development (Zhang et al., 2021). Examples include barley alpha-amylase and wheat alpha-amylase (Stanley et al., 2005). Bacterial isoamylase: they specifically cleave the α-1,6-glycosidic bonds at the branch points in amylopectin. Examples include *Pseudomonas amyloderamosa* isoamylase and *Neisseria polysaccharea* isoamylase (Olempska; 2007).

2.4.1.3.2 Exo-Acting Amylases

Exo amylases are a type of amylolytic enzymes that cleave the α-1,4-glycosidic bonds at the ends of starch molecules, releasing smaller carbohydrate units (Bijttebier et al., 2008). Examples of exo amylases include; glucoamylase (amyloglucosidase): glucoamylase is an exo-acting enzyme that specifically hydrolyzes the α-1,4-glycosidic bonds at the non-reducing ends of starch, dextrins, and oligosaccharides (Slivinski et al., 2011). It releases glucose units, leading to the complete breakdown of starch into glucose. Examples include *Aspergillus niger* glucoamylase and *Rhizopus oryzae* glucoamylase (Kumar & Satyanarayana, 2009).

Beta-amylase: although primarily considered an endo-acting enzyme, it also exhibits exo-acting properties at the non-reducing ends of starch (Husain & Ullah, 2019). It specifically cleaves the α-1,4-glycosidic bonds, releasing maltose units. Examples include barley beta-amylase and *Bacillus cereus* beta-amylase (Husain & Ullah, 2019). Alpha-glucosidase: it is an exo-acting enzyme that acts on the non-reducing ends of starch, dextrins, and oligosaccharides (Y. Feng et al., 2022). It hydrolyzes the α-1,4-glycosidic bonds, releasing glucose units. Examples include yeast alpha-glucosidase and bacterial alpha-glucosidase (Smita, 2018).

2.4.1.3.3 Limit-Dextrinase Enzymes

Debranching amylolytic enzymes, also known as debranching enzymes, limit-dextrinase enzymes or pullulanases, specifically target the α-1,6-glycosidic linkages in branched starch molecules (Møller et al., 2016). They hydrolyze these linkages, breaking down the branching structure and generating linear fragments (Møller et al., 2016). Examples of debranching amylolytic enzymes include; pullulanase: it is a debranching enzyme that hydrolyzes the α-1,6-glycosidic bonds in starch, amylopectin, and related substrates (Malakar & Tiwari et al., 2011). It releases linear fragments and facilitates further hydrolysis by other amylolytic enzymes (Naik et al., 2023). Examples include bacterial pullulanase and fungal pullulanase (Kylä-Puhju et al., 2005). Isoamylase: it specifically cleaves the α-1,6-glycosidic bonds at the branch points in amylopectin (Lin et al., 2013). It plays a role in the degradation of glycogen, a highly branched storage polysaccharide (Cenci et al., 2013). Examples include bacterial isoamylase and plant isoamylase (Kubo et al., 1999). Limit dextrinase: it is an enzyme that acts on the limit dextrins, which are branched dextrins formed by the action of other amylolytic enzymes like alpha-amylase (Møller et al., 2015). It hydrolyzes the α-1,6-glycosidic bonds, releasing glucose units. Examples include microbial limit dextrinase (Yang et al., 2009).

2.4.1.3.4 Malt Enzymes

Malt enzymes, also known as maltogenic amylases or maltogenic enzymes, are a specific group of enzymes involved in the production of malt, which is an important ingredient in brewing and distilling processes (Egi, 2014). Malt enzymes play a critical role in the transformation of starch into fermentables, mainly maltose (Egi, 2014). Malt enzymes, specifically alpha-amylase and beta-amylase, are responsible for converting starches into fermentable sugars, particularly maltose, during the malting process and are essential enzymes in brewing and distilling industries, providing the necessary sugars for yeast fermentation and contributing to the flavors and characteristics of the final beverages (Evans et al., 2010).

There are two main types of malt enzymes: alpha-amylase: it randomly cleaves internal α-1,4-glycosidic bonds in starch, producing dextrins, maltotriose, and other short-chain polysaccharides (Viader et al., 2021). It is produced naturally during the germination of grains and seeds, such as barley, and it is also commercially available for various applications (Guzmán-Ortiz et al., 2019). Beta-amylase: it releases maltose units from the starch chain, resulting in the production of maltose and some residual maltotriose (Henson et al., 2020). It is mainly present in germinating barley and other grains, and it plays a significant role in the production of fermentable sugars during the mashing process (Henson et al., 2020).

The combination of alpha-amylase and beta-amylase activities is crucial for production of malt (Duke & Henson, 2009). During the malting process, barley grains are soaked, germinated, and then kiln-dried (Soares et al., 2016). This stimulates the production and activation of malt enzymes (Neylon et al., 2020). The malted barley is then ground and hydrated in a process called mashing (Rani & Bhardwaj, 2021). The malt enzymes, particularly alpha-amylase and beta-amylase, transform the starches in the barley into maltose and other fermentable sugars, creating the necessary sugars for yeast fermentation during brewing or distilling (Beans &

Grind, n.d.). The activity of malt enzymes can be affected by factors like temperature, pH, and the specific characteristics of the malt (Rani & Bhardwaj, 2021). Brewers and distillers often optimize these conditions to achieve the desired sugar profiles and flavours in their final products (Taylor, 1993).

2.4.2 Uses of Glucose Syrup

Glucose syrup is a versatile sweetener and ingredient with a broad range of applications in the food industry (Science, 2021). Different grades of glucose syrup with varying properties and characteristics are available, allowing for tailored applications across different industries (Ejiofor, 2015). It can be put to use in the following areas; as a sweetener: it is primarily used as a sweetener in a many food and beverage products (Simpson et al., 2022). It provides a source of sweetness and can be used as a substitute for sucrose (table sugar) in many applications (Mohan & Singh, 2020). Glucose syrup has a similar sweet taste but with a higher level of sweetness than sucrose (Letviany, 2015).

Bakery and confectionery: glucose syrup plays a vital role in bakery and confectionery products (Zargaraanet al., 2016). It enhances moisture retention, provides a smooth texture, and prevents crystallization. It is commonly used in the production of candies, chocolates, caramels, fudges, frostings, and icings (Nwalo & Cynthia, 2014). Glucose syrup helps maintain a soft and chewy texture in confectionery items (Wilek & Michel, n.d.).

In ice-cream and frozen desserts: it is used in the production of ice cream and other frozen desserts (Zeng et al., 2022). It helps control crystallization, improves texture, and contributes to a smoother mouthfeel (Zeng et al., 2022). Glucose syrup also prevents the formation of ice crystals, resulting in a creamier and more stable frozen product (Science, 2021).

Beverages: glucose syrup is added to beverages to provide sweetness and enhance flavour (Hull, 2010). It is commonly used in carbonated soft drinks, fruit juices, sports drinks, energy

drinks, and flavoured water (Spinosa et al., 2016). Glucose syrup is highly soluble and mixes well with liquids, making it an ideal sweetening agent for beverages (Science, 2021). Canned and processed foods: it is used as a sweetener and thickening agent (Burey et al., 2009). It provides viscosity, improves texture, and helps in maintaining the desired consistency of products such as sauces, dressings, gravies, and canned fruits (Nwalo & Cynthia, 2014). Medical and pharmaceutical applications: glucose syrup finds uses in the medical and pharmaceutical industries (Simpson et al., 2022). It is utilized as an energy source in intravenous (IV) fluids, providing glucose to patients who are unable to consume food orally (Simpson et al., 2022). Glucose syrup is also utilized in the production of oral rehydration solutions and various pharmaceutical formulations (Simpson et al., 2022). It is employed in fermentation processes for the production of alcohol, ethanol, and other biofuels (Lin et al., 2014). Glucose syrup is also used in the production of adhesives, paper and textile sizing, and as a component in cosmetic and personal grooming items (Abiola et al., 2019).

2.4.3 Attributes of Glucose Syrup

Glucose syrup possesses various physical and functional properties that make it a versatile ingredient in the food industry (R. do N. Silva et al., 2010). Its attributes are directly related to its dextrose equivalent (Wilek & Michel, n.d.). Key physical and functional properties of glucose syrup are;

Viscosity: glucose syrup exhibits different viscosities depending on its concentration and degree of starch hydrolysis (Chetana et al., 2004). It can range from thin, low-viscosity syrups to thick, high-viscosity syrups (Deumier & Bohuon, 2005). The viscosity of glucose syrup affects its flow, spreadability, and texture in food applications (Schellart, 2011). Sweetness: glucose syrup provides sweetness to food products, although it is less sweet than sucrose (table sugar) (Saulo, 2005). The degree of sweetness can vary based on factors such as the dextrose

equivalent (DE) value and the presence of other sugars like fructose in high-fructose corn syrup (HFCS) (Starkey et al., 2022). Hygroscopicity: glucose syrup has high hygroscopic properties, meaning it readily absorbs and retains moisture from the environment (Okafor, 2019). This property helps prevent crystallization, improves texture, and enhances the shelf life of products by maintaining moisture content (Farahnaky et al., 2010).

Solubility: glucose syrup is highly soluble in water and other aqueous solutions. Its solubility allows for easy incorporation into various food systems and facilitates uniform distribution throughout the product matrix (Alves et al., 2007). Maillard reaction: glucose syrup has the ability to undergo the Maillard reaction, a chemical reaction among reducing sugars (such as glucose) and amino acids or proteins (Feng et al., 2022). This reaction contributes to browning, flavor development, and aroma enhancement in cooked or baked food products (Xing, 2002)

Bulking Agent: Glucose syrup acts as a bulking agent, providing volume and body to food products (Auerbach et al., 2007). It contributes to the overall texture and mouthfeel by improving viscosity and enhancing the sensory experience (Wilek & Michel, n.d.).

Humectant: The hygroscopic nature of glucose syrup enables it to retain moisture, prevent drying out, and maintain freshness in baked goods and other products (Naknaen et al., 2016). It helps extend shelf life by reducing water activity and inhibiting microbial growth (Zainab et al., 2011).

Texture modifier: glucose syrup can act as a texture modifier in various food products (Zeng et al., 2022). It influences factors such as chewiness, softness, and elasticity in confectionery items like candies, gummies, and marshmallows (Eke-Ejiofor, 2015).

Emulsifying and stabilizing agent: glucose syrup can serve as an emulsifying and stabilizing agent, helping to create and maintain stable emulsions in products like dressings, sauces, and

ice creams (Rahmani-Manglano et al., 2022). It aids in preventing phase separation and improves product consistency (Wilek & Michel, n.d.).

Anti-crystallizing agent: The hygroscopic nature and high molecular weight of glucose syrup inhibit the crystallization of sugars, such as sucrose, in food products. It helps maintain a smooth texture and prevents the formation of unwanted crystals (Science, 2021; Lynn, 2012).

2.5 Description and attributes of ethanol

Ethanol, also known as ethyl alcohol or grain alcohol, is a volatile and flammable organic compound with the chemical formula C_2H_5OH (Cheng et al., 2016). It is one of the most widely used alcohols and has a wide range of uses in industries ranging from food and beverage to pharmaceuticals and fuel (Edeh, 2021). Physically, ethanol is a colourless liquid with a characteristic odour and a slightly sweet taste (Akpan et al., 2015).

It has a density of approximately 0.79 g/cm³, making it less dense than water, a boiling point of 78.37°C (173.1°F) and a melting point of -114.14°C (Ademiluyi & Mepba, 2013). It is highly soluble in water, forming a homogeneous mixture in all proportions. Ethanol is flammable and forms a flammable vapor-air mixture (Ncodi, 2014). Chemically, ethanol is a primary alcohol, meaning it has the hydroxyl (-OH) functional group attached to a primary carbon atom and because of this hydroxyl group, it undergoes various chemical reactions (Dagle et al., 2020). Ethanol can act as a weak acid and a weak base, participating in acid-base reactions (Lopez-Olmos et al., 2020). It readily reacts with many organic and inorganic compounds, including acids, aldehydes, and halogens (Tangka et al., 2011). Regarding ethanol's solvent properties, it is an excellent solvent and is used in various industries (Hikmawanti et al., 2021). It can dissolve a wide range of substances, including polar and nonpolar compounds (Permadi et al., 2021).

Ethanol's solvent properties make it effective in extracting and dissolving various substances, such as flavors, fragrances, and active compounds in pharmaceutical preparations (Hikmawanti et al., 2021b).

Ethanol has hygroscopic properties, meaning it has a tendency to absorb moisture from the environment (Saharin et al., 2011). It can mix with water in any proportion, and this property is crucial in many applications where water content needs to be controlled (Suiter & Widegren, 2021). Ethanol is psychoactive substance and is the active ingredient in alcoholic beverages (Almeida-González et al., 2023). It has both short-term and long-term effects on the central nervous system and can cause intoxication, impaired judgment, and addiction. In high concentrations, ethanol can be toxic and even fatal (Warrell et al., 2016). Ethanol is widely used as a biofuel and fuel additive (Wu et al., 2021). It has a high octane rating, making it suitable for use in gasoline blends (Johnson et al., 2015). It burns relatively cleanly, producing carbon dioxide and water as byproducts (Oliveira et al., 2005). It is considered a renewable and eco-friendly fuel substitute to fossil fuels (Kheiralla et al., 2012). Medically, ethanol has antiseptic and disinfectant properties and is commonly used as a topical antiseptic to clean wounds and sterilize medical equipment (Kramer et al., 2022). It is also used as a solvent for pharmaceutical preparations and as a preservative in some medications (Yavuz, 2022).

2.5.1 Fermentation

Fermentation is a natural metabolic process involving the conversion of organic compounds, typically carbohydrates, into simpler compounds through the action of microorganisms (Paulová et al., 2013). It is an extensively used process in various industries, including food and beverage production, biofuel manufacturing, and pharmaceuticals (Pasteur & Adp, n.d.). Fermentation is an anaerobic process, primarily carried out by microorganisms such as yeast, bacteria, and some fungi (Sharma et al., 2020). During fermentation, microorganisms break

down complex organic molecules, particularly carbohydrates, into simpler compounds like ethanol, lactic acid, carbon dioxide, and various other by-products (Smith.Joseph & Hugetr, 2015).

The process involves a series of biochemical reactions, including glycolysis, where carbohydrates are transformed into pyruvate, and subsequent fermentation pathways, which further convert pyruvate into end products (Paulová et al., 2013). Various types of fermentation exist like; alcoholic fermentation that is performed by yeast and some bacteria (Ezemba & Ezemba, 2022). It involves the conversion of sugars, such as glucose, into ethanol and carbon dioxide (Taveira et al., 2021). Alcoholic fermentation is used in the production of alcoholic beverages like beer, wine, and spirits (Ezemba & Ezemba, 2022).

Lactic acid fermentation: this fermentation route occurs in certain bacteria, including Lactobacillus and Streptococcus (Narayanan et al., 2004). It converts sugars into lactic acid, which is used in the manufacture of fermented dairy products like yogurt and cheese, as well as in pickling and sauerkraut production (Juodeikiene et al., 2012).

Acetic acid fermentation: this type of fermentation entails the conversion of ethanol into acetic acid by acetic acid bacteria. It is utilized in the production of vinegar (Kanchanarach et al., 2010). There are many other fermentation processes, such as propionic acid fermentation, butyric acid fermentation, and citric acid fermentation, which have specific uses in the food, pharmaceutical, and chemical industries (Ezemba & Ezemba, 2022).

Saccharomyces cerevisiae is the most common yeast used in alcoholic fermentation. It is a single-celled fungus that is naturally present on fruits, grains, and in the environment (Booklet, n.d.). Certain bacteria and fungi, such as *Zymomonas mobilis* and *Kluyveromyces marxianus*, are also capable of carrying out alcoholic fermentation, although they are less commonly used in industrial processes (Maicas, 2020).

Alcoholic fermentation begins when yeast or other fermentative microorganisms are provided with a suitable nutrient-rich environment containing fermentable sugars (Walker & Stewart, 2016). Yeast cells metabolize glucose and other sugars through a series of enzymatic reactions, primarily in the absence of oxygen (anaerobic conditions) (Feldmann, 2012). The first step is glycolysis, where glucose is broken down into two molecules of pyruvate, generating a small amount of ATP (adenosine triphosphate) and NADH (nicotinamide adenine dinucleotide) (Vishali & Kavitha, 2021). In the absence of oxygen, pyruvate is converted into ethanol and carbon dioxide through the process of fermentation (Pillay, 2023). This conversion involves the action of enzymes, including pyruvate decarboxylase, which removes a carbon atom from pyruvate, forming acetaldehyde, and alcohol dehydrogenase, which reduces acetaldehyde to ethanol, with the simultaneous production of carbon dioxide (Walker & Stewart, 2016).

The major end products of alcoholic fermentation are ethanol and carbon dioxide (Mutton et al., 2019). Ethanol is the desired product in alcoholic fermentation and contributes to the intoxicating effects and flavors of alcoholic beverages (Dragone et al., 2009). Carbon dioxide gas is a by-product of alcoholic fermentation and is responsible for the carbonation and effervescence observed in many fermented beverages (Pongsub et al., 2022). Alcoholic fermentation is employed in the manufacture of various alcoholic drinks such as; beer, where malted grains (usually barley) are enzymatically converted to fermentable sugars, followed by the addition of hops and yeast (Aroh, 2019). The yeast ferments the sugars, resulting in the production of alcohol and carbon dioxide, providing the desired characteristics of beer (Willaert, 2006). Wine, the natural sugars present in grapes or other fruits are converted to alcohol by yeast during fermentation (Mathew et al., 2017). The process involves crushing the grapes, extracting the juice, and allowing the yeast to ferment the sugars present in the juice.

Spirits like rum, whiskey, vodka, and gin (Letaief, 2016). Fermentation provides the alcohol content necessary for subsequent distillation processes (Palo, 2022).

2.5.1.1 Factors that affect fermentation

Alcoholic fermentation is a complex process influenced by several factors that can impact its efficiency, rate, and final product quality (Bonassa et al., 2015). It is vital to note that various fermentation applications may have specific requirements and considerations, and adjustments to these factors may vary accordingly (Sitanggang et al., 2010). Key factors affecting alcoholic fermentation are: Temperature: it plays a critical role in alcoholic fermentation as it affects the activity and growth of yeast cells (Zohri et al., 2015). Yeast has an optimal temperature range for fermentation, typically between 20°C and 30°C, depending on the yeast strain (Slaa., Gnode., & Else, 2009). Lower temperatures slow down fermentation, while higher temperatures can cause yeast stress, leading to undesirable flavors and aromas (Zohri et al., 2015).

Nutrient availability: yeast requires essential nutrients to carry out fermentation effectively (Maicas, 2020). These include nitrogen sources, vitamins, minerals, and trace elements (Kampen, 2014). Adequate levels of nitrogen, such as amino acids and ammonia, are particularly important for yeast growth and fermentation efficiency (Roca-mesa et al., 2020). Insufficient nutrient availability can lead to sluggish fermentation, stuck fermentation, or the production of off-flavors (Y. Lin et al., 2014).

Sugar concentration: the concentration of fermentable sugars in the fermentation substrate affects the fermentation process and the final alcohol content (Mengesha et al., 2022). Yeast can ferment a range of sugars, such as glucose, fructose, and sucrose. High sugar concentrations can be inhibitory to yeast growth, while low sugar concentrations may lead to incomplete fermentation (Zohri et al., 2015). pH level: the pH level of the fermentation environment affects

yeast metabolism and enzymatic activity. Yeast generally prefers a slightly acidic pH range of around 4 to 6 for optimal fermentation (Liu et al., 2015). Extreme pH levels can inhibit yeast growth and fermentation, affecting the final product quality (Y. Lin et al., 2014).

Oxygen availability: alcoholic fermentation occurs under anaerobic conditions, meaning the absence of oxygen is essential for the process (Dashko et al., 2014). Oxygen exposure during fermentation can lead to the growth of spoilage microorganisms or result in the production of undesirable flavors (Rao et al., 2019).

Proper handling and management of fermentation vessels, such as using airlocks or closed systems, help maintain anaerobic conditions (Mengesha et al., 2022). Yeast strain selection: The choice of yeast strain for fermentation greatly impacts the process of fermentation and the characteristics of the final product (Dimopoulou et al., 2022). Different yeast strains exhibit variations in their fermentation capacity, temperature tolerance, alcohol tolerance, and flavor profiles. Yeast selection should consider the specific requirements and objectives of the fermentation process, such as alcohol content, flavor profiles, and production scale (Jasman et al., 2015).

Time: fermentation time can vary depending on the fermentation conditions, yeast strain, sugar concentration, and desired product characteristics (Amadi & Ifeanacho, 2016). Longer fermentation periods allow for more complete sugar conversion and flavor development, but excessive fermentation time can lead to over-fermentation and off-flavors (Amadi & Ifeanacho, 2016).

2.5.2 Uses of ethanol

Ethanol is extensively used as a renewable biofuel additive in gasoline, commonly known as ethanol fuel or E10 (10% ethanol, 90% gasoline) (Schnepf, 2014). It helps reduce greenhouse gas emissions, enhances octane ratings, and improves engine performance (Smith & Sun,

2019). Ethanol can also be used as a standalone fuel in flexible-fuel vehicles (FFVs) that can run on blends of up to 85% ethanol (E85) (Eriksson, 2008).

Ethanol is the main component responsible for the intoxicating effects in alcoholic beverages (Vonghia et al., 2008). It is used in the production of various alcoholic beverages such as beer, wine, spirits, liqueurs, and cocktails (Tomassetti et al., 2018). Different fermentation processes and distillation techniques result in a wide range of flavors, strengths, and characteristics in alcoholic beverages (Wang et al., 2003).

Ethanol is a highly effective solvent that can dissolve many organic compounds (Baldosano et al., 2015). It is widely used as a solvent in industries like pharmaceuticals, cosmetics, perfumes, and personal care products (Grodowska & Parczewski, 2010). Ethanol is used to extract and concentrate active compounds from plants for medicinal purposes (Bekers & Vigants, n.d.).

Ethanol's antimicrobial properties make it an effective disinfectant and antiseptic (Sauerbrei, 2020). It is commonly used as a disinfectant in healthcare settings, laboratories, and household cleaning products (Mazzola et al., 2009). Ethanol-based hand sanitizers and surface disinfectants are widely used to kill bacteria, viruses, and other pathogens (Onuki et al., 2008).

In pharmaceutical and medical applications, ethanol is used as a solvent and carrier in the pharmaceutical industry for the production of drugs, tinctures, and extracts (Jiang et al., 2010). It is used in compounding medications, preparations of herbal medicines, and as a preservative. In addition, ethanol is also used in some medical procedures and formulations, such as in antiseptic solutions and medications for topical applications (Note, 2019).

Chemical intermediate: ethanol serves as a starting material or intermediate compound in the production of various chemicals (Yavuzy, 2022). These chemicals find applications in industries such as plastics, textiles, paints, coatings, and adhesives (Xu et al., 2010).

Food and favouring: ethanol is used in the food industry for various purposes (Alzeer & Abou Hadeed, 2016). It is used as a solvent for flavour extraction, particularly in the production of vanilla extract, almond extract, and other food flavourings (Alañón et al., 2017). Ethanol is also used as a carrier for food colourings and as a preservative in certain food products.

Renewable energy and sustainable products: ethanol can be produced from renewable sources such as sugarcane, corn, wheat, and cellulosic biomass, making it a viable alternative to fossil fuels (Prasad et al., 2007). It is a key component in the production of bioethanol, a renewable energy source that can be used to generate heat and electricity (Tse, Wiens, & Reaney, 2021). Ethanol can also be converted into other sustainable products such as bioplastics, bio-based solvents, and bio-based chemicals (Kumar et al., 2010).

2.5.3 Production of ethanol

The production process of ethanol (Fig. 2) can vary slightly based on the type of raw material used, like sugarcane-based ethanol or cellulosic ethanol (Leal et al., 2014). However, the fundamental steps of milling, saccharification, fermentation, distillation, dehydration, and post-processing are common to most ethanol production processes (Lopes et al., 2016).

Raw material selection: ethanol can be produced from various raw materials, including sugarcane, corn, wheat, barley, potatoes, cassava and cellulosic biomass (such as agricultural residues and energy crops) (Li et al., 2022). The selection of raw material relies on factors such as availability, cost, regional suitability, and the desired end product (Ranum et al., 2014).

Milling and Pre-treatment: For starchy raw materials (e.g., corn, wheat, and barley), the first step is milling to break down the grains into a fine powder (Dabija et al., 2022). In the case of cellulosic biomass, pre-treatment processes such as physical or chemical treatments

are employed to break down the complex cellulose structure into simpler sugars (Adesanya et al., 2007).

Saccharification: in this step, enzymes are added to the milled or pre-treated raw material to convert complex carbohydrates into simple sugars (Alrumman, 2016). For starchy raw materials, amylases are used to break down starch into glucose (Magallanes-Cruz et al., 2017). Cellulosic biomass requires a combination of cellulases and hemicellulases to break down cellulose and hemicellulose into fermentable sugars (Ferreira et al., 2010).

Fermentation: the saccharified material is then mixed with water and yeast or other microorganisms capable of fermenting sugars into ethanol (Azhar et al., 2017). Yeast, typically *Saccharomyces cerevisiae*, is the most commonly used microbe in ethanol fermentation because of its efficiency and tolerance to alcohol (Ruchala et al., 2020). The fermentation process occurs in large fermentation vessels (fermenters) under controlled conditions of temperature, pH, and oxygen levels (Ezemba & Ezemba, 2022). During fermentation, yeast metabolizes the sugars, converting them into ethanol and carbon dioxide (Varize et al., 2022).

Distillation: after fermentation, the resulting mixture, known as the "beer," contains ethanol, water, residual sugars, and other impurities (Thamilvanan & Selvi, 2013). Distillation is employed to separate ethanol from the impurities and concentrate it to the desired strength (Kiss, 2022). Distillation is typically carried out using a continuous or batch distillation process, involving heating the beer and collecting the evaporated ethanol vapor, which is then condensed into liquid form (Silva et al., 2011).

Dehydration: The ethanol obtained from distillation is typically in the form of a water-ethanol mixture, known as "hydrous ethanol" (Xu et al., 2010). To obtain anhydrous ethanol (pure ethanol), a dehydration process is carried out to remove the remaining water (Mekala

et al., 2022) Various methods can be used for dehydration, including molecular sieves, membranes, or azeotropic distillation (Gomis et al., 2015).

Denaturing (optional): in some cases, ethanol intended for industrial or fuel use is denatured to render it unsuitable for consumption (Pinnacle Engineering, 2013). Denaturing involves adding small amounts of specific chemicals to ethanol, making it unfit for human consumption but retaining its usefulness for industrial applications or fuel (Silva et al., 2011). Post-processing and refining: the final step involves post-processing and refining of ethanol to meet the required specifications and quality standards (Gomis et al., 2015). This may include processes such as filtration, carbon treatment, and rectification to remove any remaining impurities and ensure the desired purity and clarity of the ethanol (Offeman et al., 2008).

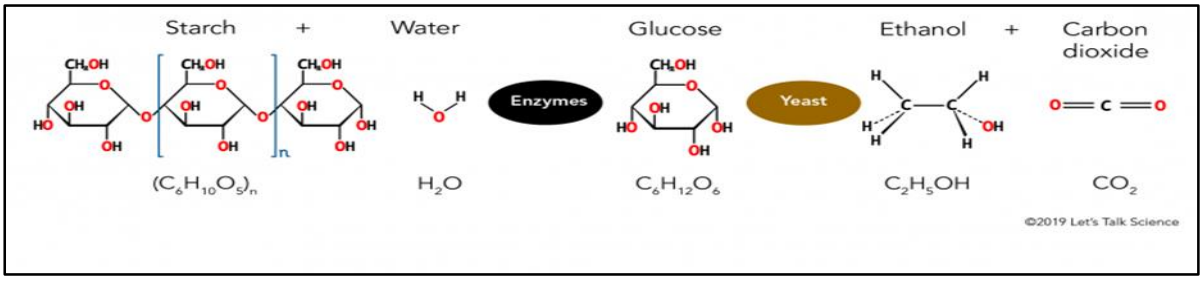

Figure 2: Production of ethanol (Sriroth et al., 2012)

CHAPTER THREE: METHODOLOGY

3.1 Source and Description of Study Materials

Selected varieties of cassava and maize were the key feedstocks; alpha and beta amylase (Sigma-Aldrich) were used for starch saccharification. Hydrochloric acid (Thermo Fisher Scientific Inc.) was also used for starch hydrolysis; while brewer's yeast (*Saccharomyces cerevisiae*) (Young's) was used for fermentation of the saccharified mass to produce a brew that was distilled to obtain ethanol. Other materials included analytical grade reagents used for starch extraction and chemical characterization.

3.2 Sample Selection

Samples were obtained from the same geographical area (NACCRI) to minimize variations due to soil type.

Two local cassava varieties (*Mwezi mukaaga* and *Mukumba*) and two improved varieties developed at National Crops Resources Research Institute, Namulonge (TME 14 & NAROCASS 1) were used at a maturity stage of 12 months. Two high yielding hybrid maize varieties (H 5355 and H 2115) and two high yielding open pollinated maize varieties (Longe 4H and Longe 5H) at a maturity stage of 120 days were used in this research. NAROCASS 1 is the main improved variety grown at Namulonge and within the community as well and is high yielding and resistant to the cassava mosaic virus disease and cassava brown streak virus disease. TME 14 (Tropical *Manihot esculenta* 14) is an improved variety with good starch quality properties, is high yielding and disease resistant. Maize was obtained from NaCRRI. The maize was certified seed (seed that was derived from foundation seed and was planted by farmers for commercial use). The varieties used in this study were chosen because they were

readily available and could easily and accurately be identified by National crops Resources Research Institute contact staff.

3.3 Study Design

Purposive sampling of the crop varieties was used to allow for a more targeted and efficient study. The selection criteria for the cassava varieties were local and improved varieties at a maturity stage of 12 months. For the maize varieties, open pollinated and hybrid maize varieties at 120 days maturity were selected with the help of experts in the field of crop science from NaCCRI.

Starch extraction from cassava samples was achieved by wet method with slight modifications and from the maize samples using the wet alkaline method with slight modifications. Starch samples of each of the selected varieties of cassava and maize were separately prepared and subjected to liquefaction and saccharification. The extracted starch was kept at -18°C before analysis. Starch was saccharified using enzymes (α-amylase and β-amylase) and acid hydrolysis (HCl) to produce the sugar syrups that were kept at -70°C before analysis. The extracted glucose syrup was characterized and the remaining part of the glucose syrup was fermented to produce alcohol. The alcohol was then distilled using the simple distillation method to obtain a relatively pure ethanol which was also characterized.

3.4 Cassava Starch Extraction

Starch was extracted from the selected cassava varieties by employing the wet method of Noorfarahzilah et al. (2020) with some modifications as follows; the freshly harvested cassava root was peeled using a stainless knife, washed with tap water and sliced. Then, 800 g of the sliced cassava was weighed using a weighing balance (Shimadzu, ATY 224R) and 5 g of sodium metabisulphite (7681-57-4, Thermofisher Scientific, United Kingdom) was added to

prevent discoloration of the starch during extraction and storage. The cassava was then crushed in a blender (4696, Yutai, China) at a high speed of 20,000 RPM until smooth slurry was formed. The slurry was washed in a nylon cloth with 8 litres of clean water to get the starch suspension which was left to stand overnight (16 h) for starch sedimentation. Clear supernatant was decanted and white starch re-washed with another 8 L of clean water as before. This was repeated four times until clean starch sediment was obtained. A clean starch sediment is indicated by a clear or translucent supernatant. Starch sediment was then put on a tray and oven-dried (M120, Macadams, South Africa) for 12 h at 55°C. Dried starch was weighed using a weighing balance (Shimadzu, ATY 224R), ground, using a blender (4696, Yutai, China) put into stomacher bags and sealed using an impulse sealer (IS400, Packer, China). The starch was put in a black polythene bag and kept at -18°C until analysis.

The extraction was carried out in three independent replicates.

Percent extraction yield was calculated as follows:

$$\%Extraction\ yield = \frac{Starch\ weight\ (g)}{Cassava\ weight\ (g)} \times 100$$

3.5 Maize Starch Extraction

Maize starch was extracted by wet alkaline method of Sandhu, Singh & Malhi (2005) and Permanasari et al. (2018) with some modifications as follows; the maize grains were milled using a milling machine (TX2235, Henan taixing, China) to remove the husk and the germ. Then, 200 g of maize flour obtained was combined with 500 ml of water containing 0.1% sodium metabisulphite (Thermofisher Scientific) and 0.05N NaOH (Thermo Fisher Scientific Inc.) solution and soaked in that solution at room temperature for 18 h. The suspension was crushed in a high speed blender (4696, Yutai, China) until a smooth slurry was formed. Then, the slurry was washed on a nylon cloth using 8 L of clean water. Suspension was left

undisturbed for 16 h to allow starch to settle in same way cassava starch was treated (section 3.4). Supernatant was decanted and sediment washed again with 8 L of clean water. The process was repeated four times until clean starch sediment was attained. A clean starch sediment is indicated by a clear or translucent supernatant. Starch was oven-dried (M120, Macadams, South Africa) at 55^{o}C for 12 h. Resulting starch was weighed using a weighing balance (Shimadzu, ATY 224R), milled using a blender (4696, Yutai, China), put in a stomacher bag and sealed using an impulse sealer and finally put in a black polythene bag and kept at -18°C until use. Percentage extraction yield was calculated as in section 3.4 for cassava starch. Extraction was done in triplicate.

3.6 Characterization of the Starch

3.6.1 Amylose Content

Amylose content was determined using the method of Ronoubigoura & Avaro (2011) with slight modifications. Starch sample was oven-dried (M120, Macadams, South Africa) for 60 min at 135°C. Then, 100±0.001 mg of the starch sample was weighed using a weighing balance (Shimadzu, ATY 224R) in 50-ml centrifuge tube, 1 ml of ethanol (95%,v/v) and 9 ml of 1 M sodium hydroxide (Thermo Fisher Scientific Inc.) solution were added. Suspension was put in a boiling water bath (6032011, J.P. Selecta, Spain) at 95°C for 15 min (5 min were first allowed for the plastic container to equilibrate with heat energy from the bath). The contents were cooled for 10 min at ambient temperature. The volume was topped to 50 ml of the extract using distilled water. Two and a half milliliter aliquot volume was put in a clean tube and 1 ml of 1 N acetic acid (Hanghai Huayi Holding Group Co. Ltd.) added. Then, 1 ml of 0.2% iodine solution (iodine: 2 g/KI: 20 gL^{-1}) (Junsei Chemical Co. Ltd.) was added. The total volume was brought to 50 ml by addition of distilled water. Contents were incubated for 30 min at room

temperature. A standard calibration curve was prepared using Amylose Megazyme kit (Nexcelom Bioscience) in concentration ranges of 0.05-0.4 mg/ml. The spectrophotometric (Jenway 7315, Cole-Parmer, India) absorbance readings for Optical density values was taken at 620 nm.

3.6.2 Determination of Resistant Starch

One hundred milligrams of dry milled starch was weighed using a weighing balance (Shimadzu, ATY 224R) in 50 ml centrifuge tube. Then, KCl-HCl buffer, pH 1.5 (10 ml) and 0.2 ml pepsin solution (1 g pepsin/l0 ml KCl-HCl buffer) was added, mixed well and incubated for 60 min at 40°C in a shaking water bath (11L, YCW-04M). Samples were removed, left to cool to ambient temperature, 9 ml of 0.1 M Trismaleate buffer (pH 6.9), KCl-HCl buffer (pH 1.5) and 1 ml of α-amylase (Sigma-Aldrich, 30.6 U/mg) solution (40 mg of 0.1 N Tris-maleate buffer, pH 6.9, and 4 mM amylase/ml of Tris-maleate buffer) added and mixed well. $CaC1_2$ (Thermo Fisher Scientific Inc.) and 0.1 M KOH (Thermo Fisher Scientific Inc.) were then added and incubated for 16 h in a shaking water bath (11L, YCW-04M). Samples were spinned using a centrifuge for 15 min at 4941 RPM; supernatant was discarded. The pellet was washed with l0 m1 distilled water, spinned using a centrifuge as before and supernatant discarded. Then, 3 ml distilled water was mixed with the residue, carefully moistening the sample. Thereafter, 3 ml of 4 N potassium hydroxide solution (Thermo Fisher Scientific Inc.) was added, mixed and left at ambient temperature for 30 min with periodic shaking. Approx. 5.5 ml 2 N hydrochloric acid (Thermo Fisher Scientific Inc.), 3 ml of 0.4 N sodium acetate buffer (pH 4.75) (Thomas Scientific) and 80 ml of amyloglucosidase (Megazyme)were added, mixed well and left at 60°C for 45min in a shaking water bath (11L, YCW-04M). The sample was spinned at 4941 RPM for 15 min and supernatant collected in a volumetric flask. Residue was washed with 10 ml distilled water and spinned. The supernatant was combined with one previously obtained. The solution was topped

to 1000 ml with distilled water. Standard glucose solutions (1-60 ppm) were also prepared. Water/blank (0.5 ml), the sample and standard glucose solutions were added in separate test tubes; 1 ml reagent from the glucose determination kit (GOD-PAP) (Megazyme) was added, mixed well and left at 37°C for 30 min in a water bath. Absorbance values for sample and standards were recorded at 500 nm against the blank. Sample glucose concentration was extrapolated from the standard curve and used to calculate resistant starch content as mg of glucose x 0.9.

3.6.3 Determination of Starch Solubility and Swelling Power

Swelling power was determined on a 2% aqueous starch suspension using the protocol of Nuwamanya et al. (2010) with minor modifications. The dry starch (1 g) was put in a pre-weighed 50- ml centrifuge tube, mixed with 45 ml distilled water and heated for 30 min at 95°C in a water bath (11L, YCW-04M), with periodic shaking. The tube was cooled for 30 min at room temperature, spinned for 10 min at 6000 RPM, supernatant removed and the residue weighed. Swelling power was then determined as ratio of wet mass to dry mass of the sample.

$$Swelling\ power = \frac{Sediment\ weight}{Amount\ of\ dry\ starch}$$

Solubility of starch was estimated from the results of swelling power. Supernatant from above was transferred into a clean pre-weighed can. The weight of the can and the sample was noted. The contents were oven-dried (M120, Macadams, South Africa) at 105°C overnight and final weight taken.

The weight of dried residue was determined and expressed as a percentage of the initial weight.

$$\%Solubility = \frac{Weight\ of\ soluble\ starch\ (dried\ residue)(g)}{Weight\ of\ sample\ (dry\ basis)} \times 100$$

Note that the temperature was adjusted to 60^{o}C, 70^{o}C, 80^{o}C and 90°C accordingly. A centrifugal force 1600 RPM was used.

3.6.4 Determination of Gelatinisation Properties

Rapid Visco-Analyzer (RVA4500, Perten Instruments, Australia) was used to analyze gelatinization profiles of individual starches. Using the Thermocline software, total running time was set at 13 min and viscosity values were recorded after every 4 sec as temperature was increased from 50°C to 95°C before re-cooling to 50^{o}C. Rotation speed was set at 960 RPM for the first 10 sec and 160 RPM up to the end. Three grams of the sample and 25 ml of distilled water were transferred in the canister, a paddle inserted and thoroughly mixed before the canister was transferred in the Analyzer. Results of set gelation, peak gelatinization and end set gelatinization were obtained.

3.6.5 Colour Measurement

Starch colour was determined by a Minolta colorimeter (CR-310, Japan), using L *a*b* notation as described by (Paraginski et al., 2014). To perform colour measurement, the sample was placed onto a 6.4 mm diaphragm optical glass. The parameters determined were black, white, greenness, redness, blueness and yellowness. L* denotes lightness of the sample; a* determines redness-greenness of the sample, where +a* is redder and -a* is greener; and b* determines the yellowness-blueness of the sample, where +b* is yellower and -b* is bluer.

The colour parameters used were L* (100, white and 0, black), a* (red to green) and b* (blue to yellow).

3.6.6 Morphology and Size of Starch Granules

Size and morphology of starch granules was established according to protocol of Wilson et al. (2006). To obtain purified granules that did not contain surface protein contaminants, starch

was washed in distilled water for three times; washed in gelatinization buffer (62.5 mM Tris-HCl (pH 6.8); 2% sodium dodecyl sulphate (Sigma-Aldrich) and β-mercaptoethanol (5%, v/v) (Sigma-Aldrich) for three times; washed in ethanol (95% v/v) (Merck) for three times; and then lyophilized in a vacuum drier (276 DigiVac, United States). Dried starch granules were kept at -80˚C for optical analysis and protein extraction.

The starch pellet was mixed with 1 ml distilled water to form a slurry, and stained by mixing two drops of the slurry with two drops of 1% periodic acid in a micro centrifuge tube. The mixture was briefly vortexed and left for 15 min undisturbed. Saturated iodine/potassium iodide (Junsei Chemical Co. Ltd.) solution (2 drops) and 1 ml of distilled water were added and mixed by vortex (Fisher Scientific, USA). Then, one drop of the mixture was transferred on 1 × 25 × 75 mm glass slide and a cover slip added. The cover slip was ringed with mounting medium (Cytoseal 60, Stephens Scientific, USA) to minimize sample dry-out during image analysis. The slip was gently tapped to ensure even starch distribution without air bubbles. Three different slides were prepared for each sample in order to obtain granule counts ranging from 1,000 to 5,000 particles per analysis in order to attain total count effects after analysis and correction. All slides were examined in a bright-field microscope coupled to a nine-colour monitor (Javelin CVM, USA) and an imaging system (PGT Imagist II, v.7.1, Princeton Gamma-Tech, USA) operated on a SUN SPARC station (USA), with a 32-bit processor for image acquisition and analysis concomitantly performed. Starch granules were visualized with a X25 objective lens which enabled analysis of particles less than 0.84 µm.

3.7 Production of Glucose Syrup

3.7.1 Enzymatic Hydrolysis

Enzymatic hydrolysis was performed according to the protocol of Permanasari et al. (2018). Cassava and maize starch were the substrate. Starch (60 g) was mixed in 300 ml distilled water. Alpha and β-amylase (Sigma-Aldrich) were added at concentrations based on the enzyme activity values provided by the manufacturer. pH of starch suspension was adjusted to 6.9 (optimum for alpha amylase activity) using NaOH. The starch suspension was heated and constantly stirred until 80°C. Then, 20 mg of α-amylase (Sigma-Aldrich) was added and suspension held at that 80^{o}C for 10 min. Temperature was increased to 95°C and held for another 10 min until complete gelatinization. The brix value was determined using a handheld refractometer (LHRF-A10, Labtron, United Kingdom), temperature decreased to 63°C and pH adjusted to 4.9 using a HACH pH meter (HQ 40d, Hach, USA) which is ideal for the activity of β-amylase (Sigma-Aldrich, 35 U/mg). Then, 50 mg of β-amylase (Sigma-Aldrich) was added and temperature maintained at 63°C. A sample was taken every 30 min for determination of ^{o}brix using a handheld refractometer (LHRF-A10, Labtron, United Kingdom) until there was no change in the brix readings over three readings. The saccharified mixture was filtered through a nylon cloth, pasteurized at 95°C using a heating mantle (HB-1000T) for 10 min (to stop enzyme activity) in order to obtain the sugar syrup, which was then cooled, the pH measured and then bottled and kept at -70°C for further use. This preparation was done in duplicate.

3.7.2 Acid Starch Hydrolysis

Acid starch hydrolysis was performed using the methods of Woiciechoroski et al. (2002) and Widodo et al. (2016). Sixty grams of starch sample was mixed with 300 ml 0.3 M hydrochloric

acid solution (Thermo Fisher Scientific Inc.), to make a 20% starch mixture. Brix was taken using a handheld refractometer (LHRF-A10, Labtron, United Kingdom) immediately after mixing the solution. Iodine test was employed to ascertain presence of starch in the solution. The mixture was brought to boil at 95°C using a heating mantle (HB-1000T) and held for 30 min. Brix was taken using a handheld refractometer (LHRF-A10, Labtron, United Kingdom) at the start of boiling and after every 10 min by withdrawing a small portion of the sample, cooling it to 20°C and taking the reading using a handheld refractometer (LHRF-A10, Labtron, United Kingdom). After 30 min, iodine test was conducted to establish presence of starch. The hydrolysate was cooled to ambient temperature, and pH reading taken using a HACH pH meter (HQ 40d, Hach, USA). pH was adjusted to 4.5 for optimal activity of *Saccharomyces cerevisiae* (Young's); the mixture was filtered and pasteurized for 10 min at 95°C using a heating mantle (HB-1000T) to kill any pathogenic microorganisms. The resulting solution was then bottled and stored at -70°C for further use. The preparation was conducted in duplicate.

3.8 Characterisation of Glucose Syrup

3.8.1 Determination of pH

The syrup sample (10 ml) was taken in a 100 ml beaker. pH of the sample was determined using a HACH pH meter (HQ 40d, Hach, USA).

3.8.2 Determination of Glucose Concentration

The sugar content of syrups was determined using high performance liquid chromatography with RI-detection based on the protocol of Bogdanov & Baumann (1988). Retention times were used for identification of peaks. External standards (Merck) were used for quantification of glucose on the basis of peak areas or peak heights. A syringe with a pre-mounted membrane filter of 0.45 µm (HiMedia) was used to filter the sample solution into

the vial. The eluent solution (mobile phase) was prepared by mixing 20 volumes of water with 80 volumes of acetonitrile (Merck) and degassed before use.

A Shimadzu (Kyoto, Japan) HPLC system (Model: NEXERA XR 20A) equipped with a communication bus module (CMB-20A), degassing unit (DGU 20A 5R), liquid chromatograph (LC 20 AD XR), autosampler (SIL-20AC XR) and an RI detector (20A) thermostated at 30^{o}C was used. An analytical stainless-steel column with 4.6 mm diameter, 250 mm length, containing amine modified silica gel with 5-7 µm particle size was employed for HPLC. The sample (10 µl) was injected into the vial and flow rate of 1.3 ml/min was set while maintaining column and detector temperature at 30^{o}C.

Identification and quantification of moieties in glucose syrup were performed by comparison of the retention times and the peak area of the sugar syrup (glucose) with those of standard sugar (glucose).

Percentage of glucose (g/100g) was calculated using the following formula;

$G = A_l \times V_1 \times m_1 \times 100 / A2x\ V_2 \times m_o$

Where

A_l = Peak area/peak height of sugar compound in sample solution, expressed as length or integration or units of area.

A_2 = Peak height sugar compound in standard solution, expressed as length or integration or units of area.

V_1 = Total volume (ml) of sample solution

V_2 = Total volume (ml) of standard solution

M_1 = Mass (g) of sugar in total volume of the standard (V_2)

m_o = sample weight (g)

Results were rounded to the nearest one decimal place.

3.8.3 Determination of Soluble Solids

Soluble sugars were determined using a handheld refractometer (LHRF-A10, Labtron, United Kingdom).The refractometer prism was wiped and rinsed using the glucose syrup solution. Then one drop of the glucose syrup was placed on refractometer prism and the reading taken in ^{o}Brix (Zainab et al., 2011).

3.8.4 Determination of Syrup Density

Density of syrup was established using the density bottle method ("II.6: Density, Specific Gravity, and Specific Volume," 2020), at 20^{o}C (room temperature). A density bottle (pycnometer) of known volume was weighed when empty (w_1), the bottle was then filled with glucose syrup (50 ml) and the weight of the bottle containing the syrup was taken (w_2). The weight of the syrup was then determined as $w_2 - w_1$. The density of the syrup was calculated from the following formula; density =mass / volume.

3.8.5 Determination of Viscosity

Viscosity of the sugar syrup was determined using the protocol of Hamilton & Quail (2011). Sugar syrup sample at 20^{o}C (room temperature) was introduced into a U-shaped capillary tube (Fig 3) mounted onto a stand in a vertical position at 90°C which was moved by suction in such a way that the volume on either side of the bend was not even (i.e. upper mark of the tube). Suction was released to allow the sample flow back to the lower mark of the tube and the minutes taken for this to happen was recorded. The viscosity of water was also determined in the same way as described above.

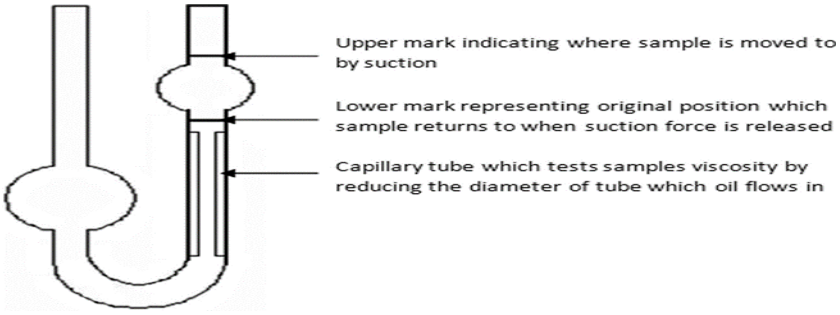

Figure 3: Diagram of capillary tube viscometer

Viscosity of the syrup was calculated from the following formula:

$$\eta_s = \left(\frac{ts \cdot \rho_s}{t_w \cdot \rho_w}\right) \cdot \eta_w$$

Where; η_s is the viscosity of the sample
ts is the time taken for the sample to move between the upper and lower
ρ_s is the density of the sample
t_w is the time taken for the water to move between the upper and lower
ρ_w is the density of water
η_w is the viscosity of water

3.8.6 Determination of Specific Gravity

A graduated hydrometer (M100, Brannan, England) was filled with the glucose syrup at 20^{o} C. The hydrometer was then placed into the jar and twirled to dislodge any air bubbles on its surface. Once the hydrometer had settled, the specific gravity reading was taken from the scale.

3.9 Fermentation of the Glucose Syrup

The syrup was boiled for 10 min at 95°C using a heating mantle (HB-1000T) so as to kill pathogenic micro-organisms, cooled to about 25°C and checked for ^{o}Brix using a handheld refractometer (LHRF-A10, Labtron, United Kingdom) and specific gravity using a graduated hydrometer (M100, Brannan, England). Then, yeast (0.1 g) was added and gently mixed with 50 ml of boiled syrup. The process was conducted aseptically to avoid microbial contamination into the solution. The samples were put in an anaerobic jar fitted with a gas pack to scavenge

oxygen and an indicator strip which changes colour in presence of oxygen. Fermentation was conducted for 3 days at 20°C to 22°C before measuring the specific gravity using a graduated hydrometer (M100, Brannan, England) and °Brix using a handheld refractometer (LHRF-A10, Labtron, United Kingdom). Fermentation was extended for another 48 h and the °Brix and specific gravity were checked again. After fermentation, the product was filtered using nylon cloth and kept at -70°C prior to distillation.

3.10 Determination of Fermentation Parameters

3.10.1 Sugar Consumption

Sugar consumed was measured after fermentation using AOAC (1990) protocols. Initial sugar concentration (or °Brix before fermentation) was measured using a handheld refractometer (LHRF-A10, Labtron, United Kingdom), and final sugar concentration was determined after five (05) days of fermentation. This was used to calculate the sugar consumption according to the following formula

Sugar consumption = °Brix before fermentation - °Brix after fermentation

3.10.2 Percent Alcohol Content

Percentage alcohol content produced during fermentation was estimated using the formula given by Winemakersdepot (2014) by deducting the final °Brix from the initial °Brix and multiplying the difference by 0.55

Alcohol (ABV) = (Original Brix – Final Brix) X 0.55

3.10.3 Fermentation Efficiency

Fermentation efficiency was established using AOAC (1990) protocols according to the expression below.

Fermentation efficiency = (Sugar consumed / Initial sugar content) X 100

3.11 Distillation of Alcohol to Ethanol

The laboratory distillation setup used for distillation of ethanol from the fermented sugar syrup is illustrated in Fig 4.

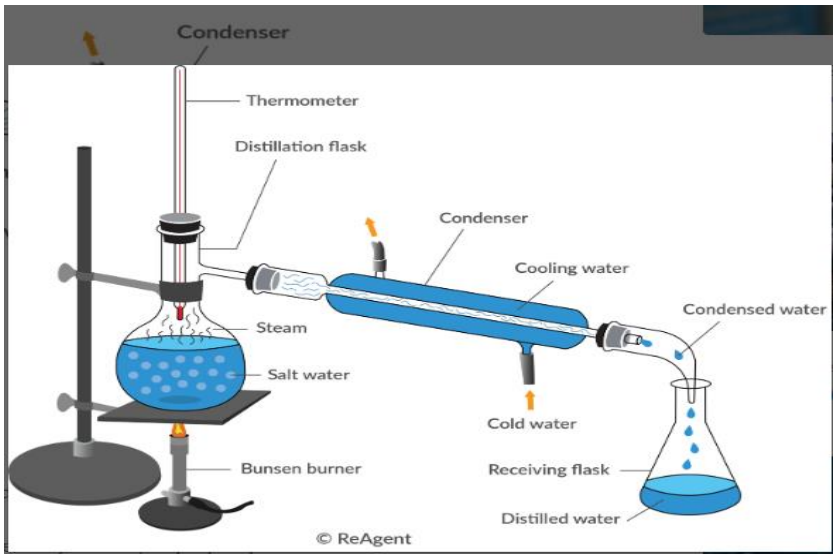

Figure 4: Distillation set of fractional separation of ethanol from the fermented sugar syrup

Distillation begun with heating the fermented product to 78°C ±2°C which is the boiling point of ethanol. The ethanol evaporated to form a vapour which was then cooled using cold water from the tap making it condense to form a distillate that was collected in the receiving flask.

3.12 Characterisation of Distilled Ethanol

3.12.1 Determination of Ethanol Content

The content of ethanol produced after distillation was measured using gas chromatography/ mass spectrometry (GC-MS) (8890, Agilent, India). The apparatus consisted of a GC fitted with an FID detector and an oven that had capability to operate isothermally at 115°C, chromatography column, auto-sampler and auto-diluter. Sample temperature was adjusted to

20°C, and filtered 50 ml aliquot into a 100 ml conical flask. The first portion (25 ml) of the filtrate were discarded and filter funnel was covered with a glass clock to minimize ethanol loss. The conical flask was placed in ultrasonic bath (B200, Thomas scientific, USA) and sonicated to remove any carbondioxide. The flask was sealed until it was required for analysis.

The gas chromatograph was set to the following programme; oven temperature, 115°C; injector temperature, 150°C; detector temperature, 200°C; carrier gas (Nitrogen) at flow rate of 45 ml/min. Each of ethanol standards (2 ml) was diluted in 20 ml n-butanol (Merck) (internal standard) using an auto-diluter. Temperature was similarly maintained at 20°C ±0.1°C before diluting and mixed thoroughly. Then, 1 µl of the first diluted ethanol standard was injected into the GC and peak areas of ethanol and n-butanol internal standards were determined. The procedure was repeated for the other ethanol standard. Then, 2 ml of prepared sample was diluted in 20 ml n-butanol internal standard as described above. The diluted sample (1 µl) was injected into the GC. Peak areas due to ethanol in the sample and n-butanol internal standard were calculated. A linear graph passing through the origin was plotted for ethanol peak area/internal standard peak area against ethanol concentration (v/v) from the results obtained for each of the calibration standards (Appendices I-II). The corresponding concentration of ethanol from the sample was read off from the standard curve. The results were expressed as % ethanol (v/v) at 20°C, and corrected to two decimal places.

3.12.2 Determination of Boiling point of Ethanol

The boiling point of ethanol was established using the distillation method as described in 3.11. As bulk of the material was undergoing distillation, the temperature at which the distillate started collecting in the condenser was noted on the thermometer; this was recorded as the boiling point of ethanol.

3.13 Data Analysis

All data were analyzed for statistical significance using Statistical Package for Social Sciences (SPSS), v. 20 to generate means ±standard deviations. Means were analysed for significant difference using the Tukey test and differences were considered significant at $p<0.05$. Data were tested for normality using the Shapiro-Wilk test. Correlation analysis between dependent and independent variables was performed using principal component analysis. Two-way analysis of variance (2 way ANOVA) was used to test for differences in the brix readings of the different varieties as the number of fermentation days increased.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Preamble

The study aimed to produce and characterize glucose syrup and ethanol from selected Ugandan cassava and maize varieties that is local cassava varieties (*Mwezi mukaaga* and *Mukumba*) and improved varieties (TME 14 and NAROCASS 1) at 12 months maturity; as well as hybrid maize varieties (H 5355 and H 2115) and open pollinated varieties (Longe 4H and Longe 5H) at 120 days maturity. Cassava starch was extracted by wet extraction method whereas the wet alkaline method was used for starch extraction from maize varieties. Starch yield was determined and starch was characterized for amylose content, solubility, resistant starch, swelling power, gelatinization properties, colour and size and morphology of the granules. Enzyme and acid hydrolysis were employed to produce glucose syrup from the starch. Glucose syrup was characterized for pH, glucose concentration, soluble solids, density, viscosity and specific gravity, and was then subjected to fermentation by brewer's yeast (*Saccharomyces cerevisae*) to produce alcohol which was distilled to produce ethanol. Ethanol was characterized for ethanol content and boiling point determination.

4.2 Yield of Starch Extracted from Selected cassava and Maize Varieties

Starch yield from local and improved cassava and maize varieties is shown in Table 2. Starch yield from maize varieties ranged from 43.33 in Longe 5H to 50.83% in Hybrid 2115, while that of fresh cassava roots ranged from 19.85 in NAROCASS 1 to 29.17% in *Mwezi mukaaga*.

Table 2: Starch yield of the selected cassava and maize varieties

Crop	Variety	Starch yield (%)
Cassava	NAROCASS 1	19.85±1.91[c]
	TME 14	26.60±4.42[bc]
	Mwezi mukaaga	29.17±3.23[b]
	Mukumba	25.60±1.04[bc]
Maize	Hybrid 2115	50.83±1.44[a]
	Hybrid 5355	44.90±2.51[a]
	Longe 4H	43.90±0.87[a]
	Longe 5H	43.33±5.77[a]

Values in the column with same superscript letters are not significantly different ($p>0.05$).

Values are means of two independent replicates ± standard errors of means

There was no significant difference in starch yield for all maize varieties ($p>0.05$). There was significant difference in starch yield of NAROCASS 1 and *Mwezi mukaaga* ($p<0.05$) but yield from both varieties was not different from that of TME 14 and *Mukumba*. NAROCASS 1 had the lowest yield (19.85%) whereas the local cassava variety *Mwezi mukaaga* had the highest starch yield (29.17%) amongst cassava varieties.

Cassava starch is typically extracted using the wet extraction method, while maize starch is often extracted using the wet alkaline method, primarily due to the differences in the composition and characteristics of these two types of starches (Muslim et al., 2011). Cassava starch has relatively low levels of impurities like proteins and fibers (1-2% protein by dry weight and fiber around 1-2% by dry weight) (Morgan & Choct, 2016), whereas, maize has a higher protein and fiber content (around 8-12% protein by dry weight and dietary fiber typically ranging from 2% to 3% by dry weight) (Adeniyi & Ariwoola, 2019). The wet alkaline method

for maize starch extraction involves treating the maize kernels with an alkaline solution (usually sodium hydroxide). This alkaline treatment helps break down the protein matrix and allows for better separation of starch from other components (Scott & Awika, 2023). Cassava starch granules are generally large and easily separated from the other components of the cassava root (Nuwamanya et al., 2010). This makes them readily accessible for extraction with water, whereas maize starch granules are smaller and more tightly bound to the protein matrix making them less readily accessible for simple mechanical extraction (Scott & Awika, 2023).

The findings of starch yield for cassava were congruent with those of Okello and Akullu (2017); these authors reported cassava starch yield between 15% and 32% for mature cassava roots of 10 – 12 months. Kaur et al. (2016) also revealed starch extraction yield of 25% for cassava roots produced in Ludhiana, India. Azizur et al. (2011) reported a yield of 29.41% for cassava from Gazipur, India.

The starch yield from maize varieties examined in this work was harmonious with those reported by Paraginski et al. (2014), who reported yield between 45.99% and 66.94% for maize from Santo Augusto, Brazil. Azizur et al. (2011) also reported starch yield of 48.85% from two varieties of maize (Barimaize-5 and Shuvra from Bangladesh Agriculture Research Institute (BARI), Gazipur.

Generally, the yield of cassava starch was high in the samples under study. This could be attributed to right maturity of samples and the fact that starch extraction was performed from freshly harvested cassava tubers (Hasmadi et al., 2021). It has been reported that long periods of storage before extraction reduces starch yield from cassava especially when wet-milling is used (Abera & Sudip, 2004). This is attributed to starch degradation due to respiration and interactions between starch and other substances (Abera & Sudip, 2004). In this study, all cassava varieties were harvested at one (01) year of maturity to ensure uniformity and

maximum yield devoid of fibre that reduces recoverable starch (Okello & Akullu, 2017). Starch content of the substrate is an important consideration together with process parameters during ethanol production (Aithal, 2023). Azmi et al. (2016) attempted to optimize hydrolysis of starch from cassava, mixed with cassava leaves and nitric acid. These authors reported that the concentration of starch is more important compared to that of acid and temperature at which hydrolysis is conducted (Azmi et al., 2017).

Other factors that affect yield of starch include root storage, conditions for drying raw materials, status of roots used for extraction, environmental conditions for crop growth and time of harvest (Zhu, 2015).

Starch extraction from maize was conducted after steeping to reduce the germ density and soften the kernel for easy crushing and to enhance separation of hull, gluten, germ and fiber from the starch granules. Sodium metabisulphite was added during steeping to inhibit enzymatic and bacterial activity and production of acids hence minimizing thinning of the starch (Nyakabau et al., 2013). The use of alkaline wet extraction to aid in binding and precipitation of proteins could have improved extraction and starch purity (Ratnayake et al., 2007).

The starch yield obtained from cassava and maize grown in different regions of Uganda can vary due to a combination of factors related to climate, soil conditions, agricultural practices, and crop genetics.

4.3 Characterisation of Starch Extracted from Selected Cassava and Maize Varieties

Starch extracted from two of each of cassava and maize varieties was characterised for resistant starch, solubility, amylose content, swelling power, gelatinization properties, colour, size and morphology of the granules. Selection of the samples was based on the starch yield since sugar

syrup production is based on available starch (Nwalo & Cynthia, 2014). Improved cassava variety TME 14 was selected because it had the highest starch yield (26.60%) among improved cassava varieties. Among local varieties, *Mwezi mukaaga* was also selected because it had the highest starch yield (29.17%). H 2115 was the hybrid maize variety selected because it had the

Table 3: Properties of starch isolated from the selected cassava and maize varieties

Crop type	Varieties	Swelling power 80 (g/g)	Swelling power 90 (g/g)	Solubility 80 (%)	Solubility 90 (%)	Amylose content (%)	Resistant starch (%)
Cassava	TME 14	12.62±2.14^{a}	12.74±0.94^{a}	1.69±0.05^{a}	2.45±0.05^{a}	22.05±0.28^{a}	0.55±0.04^{bc}
	Mwezi mukaaga	14.41±0.16^{a}	18.23±3.74^{a}	1.30±0.03^{b}	1.77±0.06^{b}	24.25±0.14^{a}	0.43±0.05^{c}
Maize	H 2115	10.66±1.72^{a}	15.91±0.28^{a}	1.08±0.02^{c}	1.15±0.03^{c}	15.33±1.45^{b}	0.82±0.04^{a}
	Longe 4H	10.89±1.04^{a}	14.62±0.62^{a}	0.91±0.02^{d}	0.96±0.01^{d}	17.58±0.18^{b}	0.65±0.42^{ab}

Values in columns with similar superscript letters are not significantly different ($p>0.05$). Values are means of two independent replicates ± standard errors of means

highest starch yield (50.83%). Longe 4H an open pollinated maize variety was also selected because it had the highest starch yield (43.90%). Table 3 shows the functional characteristics of starch isolated from the selected maize and cassava varieties.

4.3.1 Solubility and Swelling Power

For all varieties, solubility and swelling power increased as temperature was increased. Solubility and swelling power at 80°C and 90°C for cassava and maize varieties did not differ significantly ($p>0.05$). Solubility for cassava and maize varieties is significantly ($p<0.05$) different. The highest swelling power at 80°C and 90°C among all varieties was recorded by *Mwezi mukaaga*, 14.41 g/g and 18.23 g/g respectively. The highest solubility among the varieties was by TME 14 of 1.69% at 80°C and 2.45% at 90°C.

The results of the maize varieties were in agreement with data by Sandhu & Singh (2007), who revealed that swelling power of starch from nine maize varieties in USA at 90°C ranges from 13 to 20.7 g of water per gram of starch. Charles et al. (2005) showed that the swelling power of cassava starch ranges from 27.2 g/g to 42.3 g/g. Onitilo et al. (2007) showed swelling power of cassava to be between 9.0 g/g to 16.9 g/g 80°C. This study had results that were in agreement with Onitilo et al. (2007) for the cassava varieties. At ambient conditions, starch granules absorb water in excess of 30% of their weight before they start to swell (Waterschoot et al., 2015). During heating, starch absorbs more water and swells. At higher temperatures (80°C), some polysaccharides dissolve and leach out of starch granules (Waterschoot et al., 2015).

Swelling power refers to the capability of starch to hydrate and increase in size when there is excess water. Swelling power and solubility of starch correlate directly with relative contents amylopectin and amylose (Ratnayake, Hoover & Warkentin, 2002). Low swelling power at temperatures lower than 70°C is indicative of high stability of starch granules at temperatures lower than the gelatinization temperature range (Ssonko & Muranga, 2017). Increase in

swelling power at temperatures of 80^{o}C, could be due to the fact that towards gelatinization temperature, there is high energy to cause unwinding of the starch helical structures or breaking up of intermolecular hydrogen linkages in amorphous areas to enable more water to penetrate and sink into it hence causing swelling and pasting of the starch (Sasaki & Matsuki, 1998). Different scholars indicated that swelling power correlates with amylose together with its properties (Ritika et al., 2010; Sasaki & Matsuki, 1998; Lii et al., 1996). However, it has been postulated by Nuwamanya et al. (2011) that the extent of lipid-amylose complexation, phosphate content and leached amylose, have profound impact on starch swelling power. Lipid-amylose complexes lower swelling power, whereas presence of phosphates in starch increases water binding capacity of starch and thereby its swelling power (Nuwamanya et al., 2011). Swelling power can also be influenced by granule size whereby large granules result in high swelling power (Wickramashinge et al., 2009). Solubility represents the amount of starch which leaches into solution during swelling power determination. Solubility and swelling power represent evidence of interaction between crystalline and amorphous areas of starch (Gafuma, 2019).

The strength of internal bonds in starch granules affects their swelling power (Leach et al., 1959). When starch is hydrated and subjected to elevated temperature, hydrogen linkages break and become replaced with water. Then, the capacity of starch to hydrate and swell depends on its water-holding capacity through hydrogen bonding which is affected by its amylopectin and amylose content, presence of phospholipids, presence of channels and holes, and granule size (Uthumporn et al., 2010). Ability to hydrate and swell leads to changes in viscosity of starch. As observed in this study, *Mwezi mukaaga* had the highest swelling power among all the cassava and maize varieties with a value of 18.23 g/g at 90°C, and consequently, it had the highest viscosity values in both the acid (2.66 η) and enzyme (2.47 η) treatments among.

Channels and holes in starch granules cause structural weakness to allow intake of enzymes and reagents which disrupt their amorphous region, hence enhancing starch modifications for example hydrolysis (Cornejo-Ramírez et al., 2018). Starch swelling power shows a negative correlation with resistant starch meaning that high swelling power starches have lower resistant starch content (Cornejo-Ramírez et al., 2018). This is congruent with this work where by starch from *Mwezi mukaaga* that had the highest swelling power (18.23 g/g) at 90°C also had the lowest resistant starch content (0.43%, Table 3). Swelling distorts double helix of starch and increases interaction of water and hydroxyl molecules increasing susceptibility of swollen granules to digestive enzymes (Chisenga et al., 2019); and in this study, this would mean higher sugar concentrations after amylase treatment as was evident with all the varieties selected for starch characterization. When starch is heated in presence of water, its crystalline structure is destroyed and water binds to free hydroxyl groups of amylopectin and amylose by hydrogen bonds, which leads to increase starch solubility (Muhardina et al., 2016) as seen for all starches in Table 3.

High solubility coupled with low swelling power of starch indicate weak interactions in starch granules which is desirable in sugar syrup processing because it enables hydrolysis (Aryee et al., 2006). Starch damage which occurs during extraction is one of the causes of weak interactions between starch granules. Starch solubility is also dependent on swelling power, leaching of amylose, and source of starch (Hasmadi et al., 2021). Chemical treatment and heating increase solubility, whereas processes which promote the crystalline structure reduce starch solubility (Nuwamanya et al., 2011).

4.3.2 Amylose Content

Starch properties and amylose content relates to majority of its applications uses and in most cases determine its behaviour (Nuwamanya et al., 2011).

In the current work, amylose content of cassava starch differed significantly from that of maize varieties ($p<0.05$). Amylose content of cassava varieties; *Mwezi mukaaga* and TME 14 were not significantly different ($p>0.05$), with *Mwezi mukaaga* having the highest amylose content of 24.25% among all varieties. Amylose content of cassava starch was in agreement with the findings of Subroto (2020), who indicated that cassava starch has amylose ranging from 16.04 to 26.95%. According to Science (2020), amylose content for majority of maize starches ranges between 15.2 and 26.5% and this is still in agreement with the values obtained in this study that ranged from 15.33 to 24.25% (Table 3). Nuwamanya et al. (2011) observed high amylose content for cassava starch and this is concordant with data from the current work.

Amylose content is used to classify starch into high-amylose (>40%), semi-waxy (3-15%), waxy (0-2%) and normal/regular (15-35%) starches (Subroto, 2020). Based on this criterion, cassava and maize starch varieties obtained in this study could be classified as normal or regular starches. Maize starch which is normal contains 70% highly-branched amylopectin and 30% linear amylose, which are arranged in granules that have double helix semi-crystalline structure (Jiang et al., 2010). Generally, the higher the amylose content, the higher the gelatinization temperature (Science, 2020). In this study, the amylose content of maize starch was lower than that of cassava starch. However, gelatinization temperatures of both starches were not statistically different ($p>0.05$; Table 4).

High amylose promotes retrogradation to form an organized rigid crystalline structure, whereas low amylose results in an open structure which readily breaks down in water (Biduski et al., 2018). Amylose content has an effect on chemical modification of starch (Le Thanh-Blicharz et al., 2022; Nurmilah & Subroto, 2021; Nawaz et al., 2020). Generally, low amylose starches are more susceptible to physical and/or chemical modification than higher amylose counterparts (Biduski et al., 2018). This was also observed in the current work whereby maize

starch that had less amylose than cassava starch (Table 3) gave higher sugar concentrations (Tables 7 and 8).

High amylose starches such as cassava have higher water absorption capacity, low viscoelastic properties and high tendency to retrograde, limiting their application in confectionery, due to failure to develop an extendable dough leading to drier dough (Nuwamanya et al., 2011). During heating, starch structure disruption leads to dissolution of amylose (leaching) and highlights starch behaviour during heating. Tuber and root crops characteristically leach high amylose than cereals due to differences in granule size, crystalline nature and structure of starch (Zuluaga et al., 2007). Given that starch properties are dependent on its crystalline structure, heat readily destroys weaker amylopectin structures in cassava starch to release amylose (Nakamura, 2015b). This has an effect on its gelatinization properties; thereby influencing its applications in industry particularly in binders, adhesives and other products (Nuwamanya et al., 2011).

4.3.3 Resistant Starch

Resistant starch is the proportion of starch which resists hydrolysis by pancreatic α- amylase in the ileum and thus reaches the large bowel undigested, where it is fermented by the gut microflora (Maki et al., 2012). From this work, there was significant difference in resistant starch content between cassava and maize starch varieties ($p<0.05$) but no significant difference within varieties. H2115 had the highest resistant starch content (0.82%), when compared with the other varieties (Table 3). Resistant starch promotes human health (Ashwar et al., 2015) and contributes to quality attributes such as crunchiness, mouth-feel and palatability in the final food product. (Hasmadi et al., 2021).

Levels of resistant starch (RS) in cassava starch samples were 0.55% and 0.43% for TME 14 and *Mwezi mukaaga* respectively. These levels were similar to those in cassava flour reported by Pereira & Leonel (2014) that ranged between 0.19 and 2.21% on dry basis.

Amorphous areas of enzyme-resistant starches are highly ordered which implies that starch resistance is not only influenced by crystalline amylopectin. Many starches with higher amylose content are more resistant to enzyme activity than those with low content (Gallant et al., 1997). High-amylose small starches are more resistant to amylase action than large starches with low amylose (Hoover & Zhou, 2003). In this study, *Mwezi mukaaga* had the highest amylose content of 24.25 and consequently had the lowest sugar concentration by enzyme hydrolysis of 10.00 g/100g.

4.3.4 Gelatinization Properties

Table 4 indicates results of gelatinization attributes of starch extracted from selected cassava and maize varieties. Peak (T_p), onset (T_o) and end (T_e) temperatures of gelatinization were measured.

Table 4: Thermal attributes (gelatinization) of different starches extracted from selected cassava and maize varieties

Varieties	T_o (°C)	T_p (°C)	T_e (°C)
TME 14	65.50 ± 0.28^{c}	74.05 ± 0.35^{b}	82.20 ± 0.14^{ab}
Mwezi mukaaga	66.60 ± 0.28^{b}	73.90 ± 0.42^{b}	81.10 ± 0.28^{b}
Hybrid 2115	69.60 ± 0.14^{a}	76.60 ± 0.14^{a}	83.60 ± 0.28^{a}
Longe 4H	67.60 ± 0.28^{b}	75.05 ± 0.21^{b}	81.60 ± 0.57^{b}

Values in columns with similar superscript letters are not significantly different ($p>0.05$). Values are means of two independent replicates ± standard errors of means. T_p: peak temperature, T_o: on set temperature, T_e: end temperature

For the cassava varieties; TME 14 and *Mwezi mukaaga*, the T_o was significantly different ($p<0.05$), however, T_p and T_e were not significantly different ($p>0.05$). For maize varieties, T_p, T_o, T_e were all significantly different ($p<0.05$) for both varieties (Table 4). Gelatinization temperature refers to the temperature range at which starch absorbs water, swells and undergoes structural changes which lead to thickening and viscosity of starch paste. It is an important parameter to consider in various food and industrial starch applications. Findings from this work suggested that starch from the different cassava and maize varieties generally had similar thermal properties in terms of gelatinization. This implies that these starch varieties have comparable characteristics, and their behaviour during processing and utilization might be similar in terms of gelatinization. However, the higher values observed for gelatinization temperatures of starch from hybrid maize H2115, could indicate a potential difference in the thermal behaviour of this starch compared to the other varieties. The higher onset, peak, and end temperatures of gelatinization observed for in H2115 starch suggested that this starch may require slightly higher temperatures to undergo gelatinization compared to the other varieties. This difference could have practical implications in processing and utilizing starch from maize hybrid H2115 in specific applications. These parameters affect the functionality and application of starch in different industries, including food, pharmaceuticals, and textiles (Zainab et al., 2011).

T_o is associated with the start of gelatinization and relates with crystallite melting. T_p occurs at total loss of birefringence. T_e is the final temperature required for completion of gelatinization. As temperature increases, starch granules swell in the sol-to-gel dispersion. Larger starch granules attain higher gelatinization temperature and take much time to form a gel, compared with smaller granules (Koch & Jane, 2000). This is because the latter hydrate and swell more efficiently than larger ones (Moita et al., 2008).

Amylose and amylopectin side chains form crystalline structures, that are categorised as A, B and C. In type A-, glucose helixes are densely packed; for type B-, crystalline starch is less packed, allowing water molecules between branches; whereas for type C-, crystalline starch is composed of a combination of types A- and B- (Martens et al., 2018). Types A- and B- starches have the same T_o values (Geera et al., 2006).

T_e is higher for smaller granules due to variations in amylopectin chain length distribution (Vermeylen et al., 2005). Granules with less branched amylopectin have broader gelatinization temperature range (Ao & Jane, 2007). High amylose starches need higher energy and temperatures for gelatinization (Shi, Capitani, Trzasko, & Jeffcoat, 1998). In this study, cassava starch varieties presented with higher amylose content (Table 3), however, their gelatinization temperatures did not differ significantly ($p>0.05$) compared with maize starch varieties that had slightly lower amylose content.

Milling decreases particle size and damages starch granules which leads to increased gelatinization capability and reduced time for gelatinization (Cornejo-Ramírez et al., 2018). Consequently, smaller starch granules exhibit higher capacity for gelatinization (Chen et al., 2010). Therefore, variation in levels of starch granules with different sizes together with levels of amylose are the major factors that influence their gelatinization attributes (Asare et al., 2011; Koch & Jane, 2000). Presence of channels and pores also promote starch hydration, swelling and gelatinization (Nadiha et al., 2010).

4.3.5 Starch Colour

Colour indices of the selected cassava and maize starch varieties are shown in Table 5. Results indicated that cassava starch had L* values were 77.23 and 78.55, a* values 1.26 and 1.50, and b* values 20.19 and 22.06. Maize starch had L* values of 72.57 and 76.10, a* values of 1.68 and 2.02, and b* values of 23.23 and 25.28.

Among cassava starches, TME 14 had the highest L* value (78.55), indicating that it had the lightest colour. Conversely, Longe 4H exhibited the darkest colour among maize starches, with an L* value of 72.57. For both cassava and maize varieties, the *b values did not differ significantly ($p>0.05$) and were positive implying that starch colour was more of yellow than blue. Similarly, the *a values for all starch varieties was positive implying that the starch had more of a reddish colour than green.

Table 5: Colour indices of starch extracted from selected cassava and maize varieties

Crop type	Varieties	L*	a*	b*
Cassava	TME 14	78.55 ± 0.30^{a}	1.26 ± 0.05^{b}	20.19 ± 0.24^{c}
	Mwezi mukaaga	77.23 ± 0.44^{ab}	1.50 ± 0.11^{b}	22.06 ± 0.11^{b}
Maize	H 2115	76.10 ± 0.30^{b}	1.68 ± 0.06^{ab}	23.23 ± 0.56^{b}
	Longe 4H	72.57 ± 0.35^{c}	2.02 ± 0.21^{a}	25.28 ± 0.08^{a}

Values in rows with similar superscript letters are not significantly different ($p>0.05$). Values are means of two independent replicates ± standard errors of means

Colour provides information on the visual characteristics of starch, which has implications for its application in sugar syrup production. Colour parameters, such as L*, a*, b* are widely used for quantification and colour description of various substances. L* denotes lightness, with higher values indicating lighter colours (closer to white), and lower values representing darker colours (closer to black). The a* index represents colour ranges from red to green; positive indices indicate more redness whereas negative indices imply greenness. The b* index denotes colour range from blue to yellow; positive indices indicate more yellowness while negative indices highlight blueness.

The colour variations observed among starches in the current work could be explained by differences in their chemical composition, processing methods, and inherent genetic variations in the selected crop varieties. Starch colour depends on particle size, presence of pigments and

degree of gelatinization (Subramanian et al., 1994). In the context of sugar syrup production, starch colour can have implications for the final product quality attributes (Osuji et al., 2020). Lighter-coloured starches such as that obtained from cassava variety TME 14 in this study, are generally preferred for sugar syrup production as they result in a clearer and more visually appealing syrup. Darker-coloured starches such as that obtained from maize variety Longe 4H may impart a yellowish or brownish tint to the syrup, which is sometimes undesirable. None-the-less colour is just one aspect of syrup quality, and other parameters such as taste, viscosity, and purity also need to be considered.

The correlation between starch colour and syrup quality is usually examined from several perspectives: (i) visual appeal: The colour of sugar syrup plays a significant role in its visual appeal. Lighter-coloured syrups are often preferred in applications where clarity and transparency are desired, such as in the production of clear beverages, confectionery, and light-coloured sauces (Takano, 2005). Starches with lighter colours tend to produce syrups with a clearer appearance. Therefore, crop varieties that yield lighter-coloured starches are generally preferred for applications where visual appeal is crucial. (ii) Flavour and taste: While the colour of starch may not directly affect the flavour or taste of sugar syrup, it indirectly influences sensory attributes. Consumers normally associate darker-coloured syrups with stronger flavours or certain taste characteristics (Matta et al., 2005). Thus, starches that produce lighter-coloured syrups are preferred in applications where a neutral flavour profile is desired, allowing natural or added flavours to come through without interference from the syrup's colour (Athan & Anestis, 2019). (iii) Purity: Starch colour can indicate presence of impurities or undesirable components in the resulting syrup. Darker-coloured starches usually contain more impurities, such as pigments or non-starch components, which contribute to darker colour in the syrup (Aït-Aissa et al., 2020). Results from this study therefore suggested that cassava starch

generally contained fewer impurities than maize starch. Starch varieties with lower levels of impurities and lighter colours, such as from improved cassava variety TME 14, are generally favoured to produce syrups with higher purity levels.

4.3.6 Starch Granule Size and Morphology

There were statistical differences in particle size ranges among starch granules for each of the selected cassava and maize varieties. The cassava starch had greater particle sizes (16.85-16.95 μm) than maize starch varieties (9.10-11.05 μm). Overall, Mwezi *mukaaga* had the highest particle size range of 17.40 to 17.95 μm (Table 6). Determining particle size of starch granules multiple times helps to capture the complete granule size distribution, and account for sample heterogeneity, with variations in granule size distribution across different regions or particles within the sample being put into consideration (Tanaka et al., 2017). From the current work, particle size was determined twice, i.e. particle size 1 and 2.

Granule size affects solubility, swelling and water absorption whereby small granules have high surface area which increases their solubility (Agnes et al., 2017) and enhances their water absorption capacity (Cornejo-Ramírez et al., 2018). Cassava starch in this work had truncated or round granules whereas maize starch granules were polygonal. These structures were observed under bright-field illumination.

Table 6: Morphological properties of granules of the different starches

Crop type	Varieties	Particle size 1 (μm)	Particle size 2 (μm)	Shape
Cassava	TME 14	16.85 ± 0.49^{a}	16.95 ± 0.21^{a}	Round, truncated
	Mwezi mukaaga	17.40 ± 0.14^{a}	17.95 ± 0.35^{a}	
Maize	H 2115	11.05 ± 0.49^{b}	11.00 ± 0.28^{b}	Round, irregular, polygonal
	Longe 4H	9.10 ± 0.14^{c}	9.20 ± 0.14^{c}	

Values in rows with similar superscript letters are not significantly different ($p>0.05$). Values are means of two independent replicates ± standard errors of means

Granular size and surface morphology influence its properties and yield of glucose obtained by starch hydrolysis (Subramanian & Vediappan, 2018). Starch shape and size have varying degrees of saccharification due to variations in starch content, purity and morphology (Subramanian & Vediappan, 2018).

Particle size of starch granules affects their functional characteristics and applications. Cassava starch granules typically exhibit a wide range of particle sizes (Abdullah et al., 2018). Cassava starch has round or truncated granules. On average, cassava starch granules are 3-32 µm (Eke et al., 2009) of which cassava starches in this study were within this range. Maize starch granules generally have a more uniform size distribution compared to cassava ones. Average maize starch granules range between 5 and 25 µm (Chisenga et al., 2019), of which the maize starch granules in this study were within the range, i.e., 9.10 to 11.05 µm. Maize starch granules are typically smaller than cassava starch granules and have a relatively uniform shape. The granules are often angular, irregular or polygonal, with a characteristic central hilum or pore. Size and shape of maize starch granules contribute to their unique properties, such as improved thickening and gelling capabilities (Waterschoot et al., 2015). In both cassava and maize starches, the granule size affects their functional characteristics, including gelatinization temperature, viscosity, swelling capacity, and film-forming ability (Lindeboom et al., 2004). Starch particle size distribution depends on variety of the crop, processing techniques, and conditions during starch extraction and drying (DAI et al., 2008). Different granule shapes and sizes and their distribution influence their gelatinization and enzyme/ water penetrability (Subramanian & Vediappan, 2018).

Size and morphology of starch granules are also important in sugar syrup production. The relationship between granule size, morphology, and sugar syrup production is described as

follows: Smaller starch granules typically have higher surface area-to-mass ratio compared to larger granules. Increased surface area allows for more efficient enzymatic or acid hydrolysis of starch, as the enzymes or acids access a larger portion of the granule surface. Therefore, smaller starch granules tend to have higher reactivity and hydrolyzed more rapidly, leading to increased glucose production during the syrup production process (Subramanian & Vediappan, 2018). This was evident in this study where maize granules had smaller sizes and also gave higher sugar concentrations after hydrolysis compared to starch from cassava varieties. Morphology of starch granules influences enzyme access to the internal molecules of starch (Sangwongchai et al., 2023). Granules with irregular shapes or pores provide more pathways for enzyme penetration and diffusion, enhancing enzymatic hydrolysis. In contrast, granules with smoother surfaces and compact structures have lower accessibility to enzymes, resulting in slower hydrolysis rates (Arantes & Saddler, 2011). Maize starch granules have irregular shape and this could explain the high sugar concentrations derived from maize starch in this study as compared to cassava varieties.

Gelatinization and swelling properties of starch also impact sugar syrup production (Sopade et al., 2004). During gelatinization, starch absorbs water which leads to changes in its structure, leading to increased porosity and easier access for enzymes or acids. Smaller granules, as is the case with starch granules from maize, which typically have larger surface areas, tend to gelatinize more rapidly and swell to a greater extent, facilitating the penetration of hydrolyzing agents and increasing the efficiency of glucose production (Waterschoot et al., 2015).

4.4 Production of glucose syrup

The starch extracted from cassava and maize varieties was used to produce glucose syrup using acid and enzyme hydrolysis. Figures 5 and 6 represent results of maize and cassava starch hydrolysis by acid and enzyme treatments.

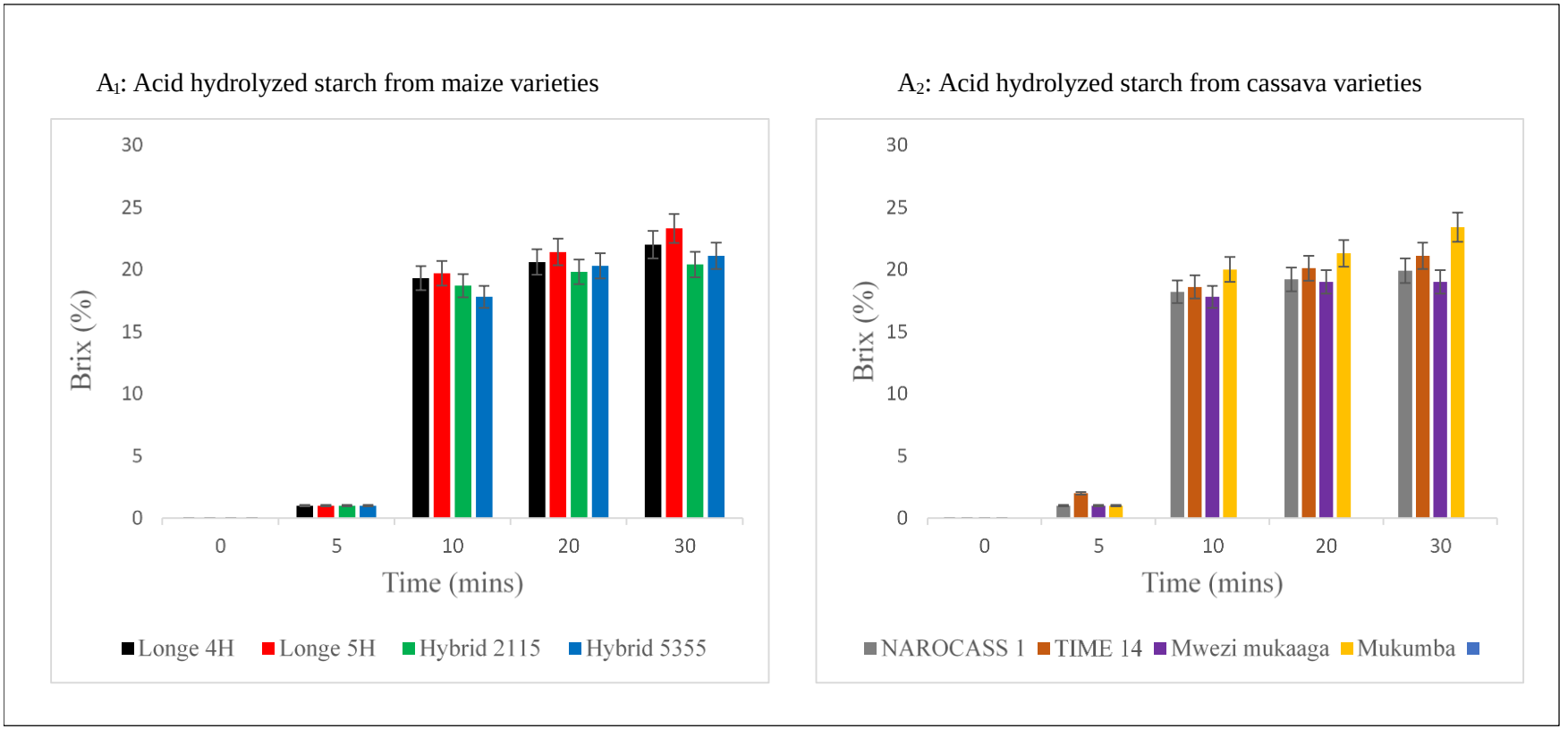

Figure 5: Hydrolysis of maize (A_1) and cassava (A_2) starch using 0.3M hydrochloric acid. Maize starch: Open pollinated varieties; Longe 4H (black line) and Longe 5H (red line); Hybrids, 2115 (green line) and 5355 (blue line). Cassava starch: improved varieties; NAROCASS 1 (grey line), TME 14 (brown line), local varieties; *Mwezi mukaaga* (purple line) and *Mukumba* (yellow line). Points are means of two independent experiments and error bars are ± standard errors of means ($p < 0.05$).

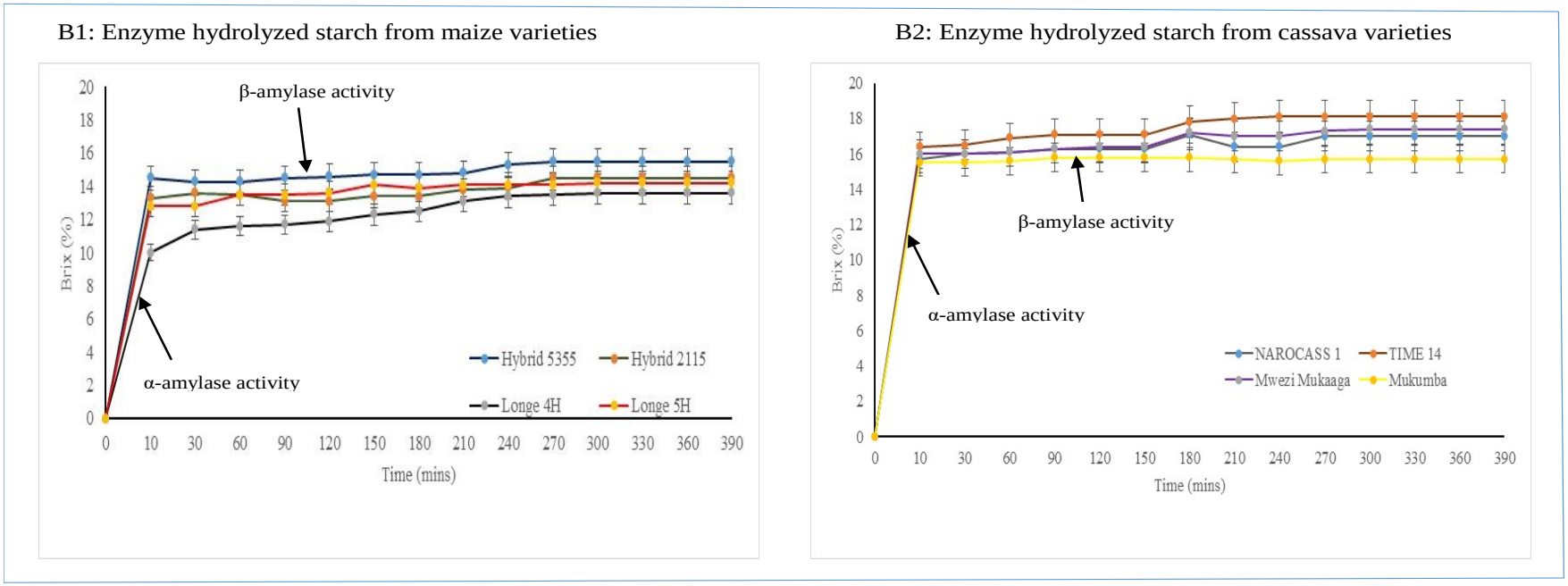

Figure 6: Hydrolysis of maize (B_1) and cassava (B_2) starch using α- and β-amylase enzymes. Maize starch: Open pollinated varieties (Longe 4H (black line) and Longe 5H (red line); Hybrids, 2115 (green line) and 5355 (blue line). Cassava starch: improved varieties NAROCASS 1 (grey line), TME 14 (brown line) and local varieties; *Mwezi mukaaga* (purple line) and *Mukumba* (yellow line). Points are means of two independent experiments and error bars are ± standard errors of means ($p < 0.05$).

Results of acid hydrolysis showed that brix level increased rapidly between 5 to 10 minutes of the experiment and did not significantly change thereafter (Fig 5). For cassava and maize varieties, after 5 minutes of acid treatment, brix only increased slightly among the varieties. After 10 minutes, there was no significant increase in brix for all varieties. The final brix values of glucose syrup from cassava starch were as follows; NAROCASS 1 (19.9%), TME 14 (21.1%), Mwezi mukaaga (19%) and Mukumba (23.4%). For maize starch, brix values were; Longe 5H (23.3%), Longe 4H (22%), Hybrid 2115 (20.4%) and hybrid 5355 (21.1%). An iodine test performed after the 30 minutes indicated a negative result implying complete starch hydrolysis for all samples. Acid hydrolysis is simple, cheap, and is readily available. However, limitations such as requirement for high temperatures and formation of toxic by-products has promoted use of enzyme hydrolysis, which is highly specific and selective producing less toxic by-products (Azmi et al., 2017), and gives higher glucose yields (Tunde, 2020). Enzymatic starch hydrolysis is also a milder process, which requires less energy (Pavas et al., 2020). However, enzymes give low reaction rate, they are expensive and give different sugar monosaccharides that are difficult to separate (Azmi et al., 2017a). Acid hydrolysis is a chemical process which changes starch's functional and structural attributes without disruption of its granular morphology (Wang & Copeland, 2015).

Results of enzyme hydrolysis showed that the ^{o}brix (sugar content) rapidly increased in the first 10 minutes (Fig 6) and thereafter levelled off at 15-18^{o}brix implying that α-amylase hydrolysed cassava and maize starch rapidly before introduction of β-amylase. Introduction of β-amylase at 10 min caused a small increase in ^{o}Brix. For each of cassava and maize varieties, there was no significant difference in brix between local and hybrid varieties ($p>0.05$). The final results of enzyme hydrolysis for cassava starch indicated a °brix value of 17.0% and 18.1% for NAROCASS 1 and TME 14 (improved varieties) and 17.3% and 15.7% for *mwezi mukaaga*

and *mukumba* (local varieties). The final results of enzyme hydrolysis for maize starch indicated no significant difference between hybrid 5355 (15.5%) and hybrid 2115 (14.5%) ($p>0.05$) whereas for open pollinated varieties, the syrup from Longe 5H had significantly higher brix (14.2%) than that of Longe 4H (13.6%) ($p<0.05$). Overall, cassava starch generated higher brix (sugar) (15.7-18.1%) than maize starch (13.6-15.5%).

Acid hydrolyzed syrups had higher glucose syrup yields compared to the enzyme hydrolyzed syrups and this could be attributed to the fact that acid hydrolysis went to completion as shown by the iodine test whereas enzyme hydrolysis did not go to completion despite prolonged periods of hydrolysis with the brix value not changing after three consecutive readings of 30 minute intervals. The iodine test was carried out at the end of enzyme hydrolysis and it indicated a positive reaction implying that starch was still present. If the starch sample contains complex structures or branching that these enzymes cannot efficiently break down, some portions may remain unhydrolyzed (Streb et al., 2012). Another factor could be the complex starch structure, where some starch samples may have highly branched amylopectin molecules, making it hard for enzymes to access and break down these complex structures, leading to incomplete hydrolysis (Bertoft, 2017). Presence of inhibitors or interference; some substances in the starch sample or reaction mixture such as heavy metals and inhibitors produced by microorganisms in the starch mixture can act as inhibitors or interfere with enzyme activity, reducing their effectiveness. In such cases, acid hydrolysis may be less affected by these inhibitors (Oboh et al., 2012).

To address incomplete hydrolysis in enzyme-based processes, researchers may experiment with different enzyme combinations, optimize reaction conditions, or employ additional enzymes or treatments to target specific starch structures (Bednarska, 2015). Acid hydrolysis, on the other hand, is a more indiscriminate process that can break down starch into simpler sugars more

completely but may also result in degradation products that are not desirable in some applications (Pervez et al., 2014).

The enzyme α-amylase caused rapid starch hydrolysis while β-amylase had minimal effect. This could be attributed to high α-amylase activity relative to β-amylase. Alpha-amylase and beta-amylase are added at different times during the hydrolysis process for several reasons such as; Specificity of action; where α-amylase hydrolyzes the internal alpha-1,4-glycosidic bonds in starch molecules, cleaving the large, branched amylopectin molecules found in starch, whereas on the other hand, β-amylase specifically cleaves the non-reducing ends of starch molecules, acting on linear portions of the starch, such as amylose (de Souza & Magalhães, 2010). Temperature and pH sensitivity; α-amylase is typically more active at higher temperatures and a wider pH range, whereas β-amylase is more sensitive to temperature and pH, and is often added at a later stage when the conditions are more favourable for its activity (Ullah et al., 2021).

Alpha-amylase and beta-amylase are added at different times during enzyme hydrolysis to take advantage of their specific enzymatic activities and to control the composition of the hydrolysis products. This sequential addition allows for the production of a wide range of starch-derived products with varying properties to meet specific industrial and food processing needs (Özcan & Sipahioğlu, 2020).

The slowing down of enzyme activity when beta amylase was added in both treatments shows that the enzyme activity could have been low or may be due to exhaustion of substrate (starch) by the first enzyme treatment.

Production of sugar syrups is mainly influenced by substrate and enzyme concentration (Spinosa et al., 2016). Sugar concentration is enhanced through increase in substrate concentration (Permanasari et al., 2018b). However, increasing the substrate concentration

from 30-40% during feasibility trials caused the solution to become too viscous and this could have hindered enzyme activity (Uribe & Sampedro, 2003). Knowing the physicochemical attributes of starch enables its effective processing (Alcázar-Alay & Meireles, 2015). High porosity of granule surface of different starches makes it susceptible to enzyme action (Azmi et al., 2017).

The dynamics of starch hydrolysis is mainly evaluated in two ways: (i) soluble sugar concentration, (ii) and insoluble residues recovered from the process. Most starches possess two-stage hydrolysis process whereby the initial rate is fast; this is followed by slower rate second stage (Kim et al., 2012). The initial rate corresponds to hydrolysis of amorphous areas in the starch granule, whereas the second stage is due to hydrolysis of amorphous and crystalline regions (Wang et al., 2012). Starch's amorphous regions are more accessible to acid action due to less compaction of starch chains compared to crystalline areas (Gaenssle et al., 2021). The first stage of hydrolysis is influenced by surface porosity, level of lipid-amylose complex, granule size and amylose content (Suntharamoorthy, 2017). The second stage, happens after action on amorphous and crystalline areas; it is affected by degree of packing of the double helices within the crystallites, distribution of α (1→6) branches between amorphous and crystalline lamellae and amylopectin content (Le Corre et al., 2010). Two hypotheses explain low rate of hydrolysis crystalline starch granule regions; (i) high level of packing of starch chains within crystallites which does not allow quick intake of hydrogen ions into these areas (Mendez-Montealvo et al., 2022), (ii) slow alteration of glucopyranose rings from chair to half-chair conformation due to sugar immobilization in starch crystallites (Jayakody & Hoover, 2002). Most of glucosidic oxygens are concealed in inner sections of the double helix making them less accessible to acid action (Wang & Copeland, 2015).

Enzyme adsorption onto the surface is necessary for starch hydrolysis (Tian et al., 2023). The extent of this depends on surface structure and size of starch granule (Sujka & Jamroz, 2007). The surface area of cassava starch is generally less than that of maize starch (Abdullah et al., 2018). Results from this study therefore suggested that there was lower level of enzyme adsorption on cassava starch surface than on maize starch; this could account for the difference observed in susceptibility of these starches to amylase action. None-the-less, there are no reports relating the degree of starch hydrolysis and extent of enzyme adsorption on granules of different botanical sources (Leszczynski & Technology, 2004). Therefore, specific reasons for higher enzyme hydrolysis of cassava compared to maize starch as observed in this study, require further investigation.

Electron microscopy was employed to examine starch granules from different botanical sources and subjected to amylase treatment (Chakraborty et al., 2020). These authors reported that type A (cereal starch) undergoes extensive enzymatic hydrolysis because it is centrally degraded by α-amylase; this leads to formation of tunnels that are directed to interior of the granule, followed by solubilisation of amorphous parts of the granule rim. As starch crystallinity reduces, and double helices are disrupted in amorphous parts, this leads to decrease in granule swelling, increase in gelatinization temperature, and increased susceptibility to enzyme action (Gunaratne & Hoover, 2002). It may therefore be concluded that resistance of raw starch from some botanical sources to amylolytic enzyme action is largely determined by its structure, particularly its β-type crystallinity (Leszczynski & Technology, 2004), which may partly explain the difference in results obtained in this study.

4.5 Characterization of glucose syrups produced from cassava and maize starch

Tables 7 and 8 show the physico-chemical profiles of sugar syrup produced from the selected maize and cassava starch using acid and enzyme hydrolysis respectively.

Table 7: Physico-chemical profiles of glucose syrup produced by acid hydrolysis of starch obtained from selected cassava and maize varieties

Crop type	Varieties	Specific gravity (g/m3)	Density (g/m^3)	Viscosity (η)	Degrees Brix	pH	Sugar (g/100g)
Cassava	NAROCASS 1	1.080 ± 0.00^{bc}	1.06 ± 0.03^{a}	2.09 ± 0.13^{ab}	19.90 ± 0.14^{bc}	5.60 ± 0.11^{a}	21.90 ± 0.16^{c}
	TME 14	1.085 ± 0.00^{b}	1.12 ± 0.11^{a}	2.16 ± 0.13^{ab}	21.10 ± 0.42^{b}	5.50 ± 0.21^{a}	31.20 ± 0.31^{a}
	Mukumba	1.094 ± 0.00^{a}	1.03 ± 0.00^{a}	1.63 ± 0.10^{b}	23.40 ± 0.57^{a}	5.60 ± 0.05^{a}	20.20 ± 0.28^{c}
	Mwezi mukaaga	1.076 ± 0.00^{c}	1.05 ± 0.00^{a}	2.66 ± 0.27^{a}	19.00 ± 0.01^{c}	5.70 ± 0.21^{a}	26.00 ± 0.26^{b}
Maize	H 2115	1.081 ± 0.00^{c}	1.04 ± 0.02^{a}	2.60 ± 0.14^{a}	20.40 ± 0.57^{c}	5.60 ± 0.07^{a}	46.10 ± 0.11^{a}
	H 5355	1.084 ± 0.00^{bc}	1.03 ± 0.00^{a}	1.97 ± 0.36^{a}	21.10 ± 0.14^{bc}	5.60 ± 0.14^{a}	36.90 ± 0.44^{b}
	Longe 4H	1.087 ± 0.00^{b}	1.05 ± 0.00^{a}	2.13 ± 0.40^{a}	22.00 ± 0.28^{ab}	5.70 ± 0.21^{a}	35.00 ± 0.18^{c}
	Longe 5H	1.092 ± 0.00^{a}	1.05 ± 0.01^{a}	2.74 ± 0.22^{a}	23.30 ± 0.14^{a}	5.50 ± 0.14^{a}	31.00 ± 0.33^{d}

Values for cassava in columns with similar superscript letters are not significantly different ($p>0.05$). Values for maize in columns with similar superscript letters are not significantly different ($p>0.05$).Values are means of two independent replicates ± standard errors of means.

Table 8: Physico-chemical properties of glucose syrup produced by enzyme hydrolysis of starch obtained from selected cassava and maize varieties

Crop type	Varieties	Specific gravity (g/m3)	Density (g/m^3)	Viscosity (η)	Degrees Brix	pH	Sugar (g/100g)
Cassava	NAROCASS 1	1.067 ± 0.00^{ab}	1.04 ± 0.01^{a}	2.16 ± 0.55^{a}	17.00 ± 0.28^{ab}	4.50 ± 0.00^{a}	10.10 ± 0.28^{b}
	TME 14	1.072 ± 0.00^{a}	1.05 ± 0.01^{a}	2.15 ± 0.15^{a}	18.10 ± 0.14^{a}	5.05 ± 0.64^{a}	10.23 ± 0.57^{b}
	Mukumba	1.062 ± 0.00^{b}	1.04 ± 0.01^{a}	1.90 ± 0.09^{a}	15.70 ± 0.71^{b}	4.95 ± 0.07^{a}	12.41 ± 0.14^{a}
	Mwezi mukaaga	1.068 ± 0.00^{ab}	1.04 ± 0.01^{a}	2.47 ± 0.58^{a}	17.30 ± 0.14^{ab}	4.90 ± 0.14^{a}	10.00 ± 0.28^{b}
Maize	H 2115	1.058 ± 0.00^{a}	1.02 ± 0.01^{a}	2.57 ± 0.88^{b}	14.50 ± 0.71^{a}	5.20 ± 0.14^{a}	37.10 ± 0.71^{b}
	H 5355	1.062 ± 0.00^{a}	1.01 ± 0.01^{a}	3.06 ± 0.16^{b}	15.50 ± 0.71^{a}	5.10 ± 0.14^{a}	40.30 ± 1.13^{a}
	Longe 4H	1.054 ± 0.00^{a}	1.03 ± 0.02^{a}	5.30 ± 0.20^{a}	13.60 ± 0.57^{a}	5.30 ± 0.00^{a}	20.00 ± 0.28^{c}
	Longe 5H	1.056 ± 0.00^{a}	1.06 ± 0.01^{a}	2.71 ± 0.13^{b}	14.20 ± 0.28^{a}	5.15 ± 0.07^{a}	15.30 ± 0.42^{d}

Values for cassava in columns with similar superscript letters are not significantly different ($p>0.05$). Values for maize in columns with similar superscript letters are not significantly different ($p>0.05$).Values are means of two independent replicates ± standard errors of means.

4.5.1 Specific gravity

Specific Gravity (SG) or relative density represents the density of the syrup relative to that of equal quantity of water at specified temperature.

Among the glucose syrups produced by acid hydrolysis (Table 7), for cassava varieties, there was no significant difference in specific gravity (SG) of syrups made from improved cassava varieties ($p>0.05$). *Mwezi Mukaaga* and *Mukumba* were significantly different from each other. For the maize varieties, SG of syrups made from the hybrid varieties were not significantly different ($p>0.05$). The open pollinated varieties were significantly different from each other ($p<0.05$). Among the glucose syrups produced by enzyme hydrolysis (Table 8), SG of syrups made from all cassava varieties were not significantly ($p>0.05$) different from each other. Equally, there was no significant ($p>0.05$) difference among all maize varieties.

Specific gravity of glucose syrup produced by acid hydrolysis ranged from 1.080 to 1.094 g/m^3 and that of enzyme hydrolysis ranged from 1.05 to 1.07 g/m^3. In general, the syrups were denser than water (S.G of 1.00). Values of SG of glucose syrup have been reported to range from 1.31 to 1.39 (Osuji, 2018). These values are higher than those obtained in the current study implying that the concentration of sugars in the syrups obtained from this study was much lower than reported. Specific gravity varies for a number of reasons including amount of solids (sugars) as well as temperature of the syrup (Modupe et al., 2018).

4.5.2 Density

There was no significant difference in density of glucose syrup produced by means of acid and enzyme hydrolysis for both cassava and maize varieties ($p>0.05$). The density of sugar syrup produced by means of acid hydrolysis ranged from 1.03 g/m^3 to 1.12 g/m^3 with the syrup from TME 14 cassava starch having the highest (1.12g/m^3). Syrup from Longe 5H maize starch had the highest density for the maize varieties of 1.05g/m^3. The density of

enzyme hydrolyzed starch ranged from 1.01 g/m^3 to 1.06 g/m^3 with sugar syrup from TME 14 cassava starch having the highest (1.05g/m^3) among cassava varieties, and Longe 5H recording the highest (1.06g/m^3) among maize starch varieties. At room temperature (20°C), the density of pure glucose syrup and honey range from 1.427 to1.431 g/m^3. The density of syrups from this study was below the above range by a factor of 0.3 g/m^3, which could be attributed to lower sugar concentration in the glucose syrups examined in this study. Density is used to quantify dissolved solids in liquids. For example, high sugar concentration syrup increases syrup density (Walker, 2010).

4.5.3 Viscosity

Viscosity indicates density and hence thickness or lightness of the syrup. In the case of acid hydrolysis, for the cassava varieties, there was no significant ($p>0.05$) difference in viscosity of glucose syrup obtained from improved cassava varieties NAROCASS 1 and TME 14, which were also not significantly ($p>0.05$) different from the local varieties. *Mwezi mukaaga* and *Mukumba* were significantly different from each other. For the maize varieties, there was no significant ($p>0.05$) difference between the OPV and the hybrid varieties (Table 7).

A high viscosity syrup adds volume and chewiness in sweets (Modupe Elizabeth et al., 2018). Syrups with high viscosity are recommended for use in confectionary and beverages production (R. Johnson et al., 2009). Therefore, sugar syrup from Longe 5H acid hydrolyzed starch could find application in confectionery and beverage industries owing to its high viscosity. In the case of enzyme hydrolysis, there was no significant difference in viscosity of syrups obtained from all cassava varieties ($p>0.05$). For maize starch varieties, there was no significant difference in viscosity of syrups obtained from hybrid varieties and Longe 5H ($p>0.05$). However, there was a significant ($p<0.05$) difference between the OPV varieties (Table 8).

Glucose syrups are Newtonian fluids implying that shear rate does not affect their viscosity but water content and temperature have an effect on their viscosity (E. B. Jackson, 1995).

Viscosity and concentration of substrate relate to each other. Substrate concentration refers to quantity of substrate in total volume whereas viscosity is indicative of resistance to flow (Uribe & Sampedro, 2003). Elevated concentration of dissolved solutes leads to increase in viscosity (Abbott et al., 2005). Starch is used to thicken other food products and increases their viscosity without generally changing their other attributes (Calmarza-chueca et al., 2022). Wee et al. (2011) evaluated the effect of high concentration of substrate on yield of reducing sugars after glucoamylase hydrolysis. These authors concluded that reducing sugar yield decreased as substrate concentration increased, due to increased viscosity of the matrix which led to inefficient mixing of samples (Azmi et al., 2017).

Thus, the higher the concentration of sugars, the higher the viscosity. For this study, it was observed that acid hydrolyzed samples had a higher sugar concentration but viscosity was lower than the enzyme hydrolyzed samples. This could be attributed to sugar dilution due to the water added during pH adjustment after hydrolysis from 4.5 – 6.5 which is the ideal pH for enzyme action hence lowering viscosity of the syrup. Enzyme hydrolysis did not go to completion as confirmed by iodine test, implying that not all the starch was converted to simple sugars and presence of starch could have contributed to increased viscosity as was observed from the results of enzyme hydrolyzed samples. This has an advantage of enhancing texture and body to the syrups as well as improved stability. The reported viscosity of glucose syrup at 20°C is 1.95η (Science, 2021), which is close to some of the values obtained from this study (Tables 7 and 8).

4.5.4 Degrees Brix

Brix (°brix) refers to grams of sucrose in 100 grams of liquid. For acid hydrolyzed cassava starch samples, there was no significant ($p>0.05$) difference between the degrees brix of the improved cassava varieties, as well as between the local cassava varieties. For the maize varieties, there was no significant ($p>0.05$) difference between the degrees brix of the hybrid varieties, as well as between the OPV varieties. Longe 4H was equally not significantly ($p>0.05$) different from H5355. (Table 7). In enzyme hydrolyzed cassava starch samples, there was no significant ($p>0.05$) difference between the degrees brix of the improved varieties and local variety *Mwezi mukaaga*. Local cassava varieties were equally not significantly ($p>0.05$) different from each other. There was no significant ($p>0.05$) difference between the hybrid and OPV maize varieties (Table 8).

It was therefore evident that acid hydrolyzed starch had higher brix irrespectively of source, compared to enzyme hydrolyzed samples. This could be due to differences in hydrolytic potential of the two approaches where acid has higher hydrolytic power than the enzymes. Hence, the acid hydrolyzed samples were hydrolyzed to completion and all starch was converted to simple sugars. This may not have been the case with the enzyme hydrolyzed samples as hydrolysis for both the cassava and maize samples was stopped before completion as there was no further change in °brix despite starch still being present as verified by the iodine test. This implies that some starch was still present and unconverted to sugars hence the lower °Brix readings in the enzyme hydrolyzed samples. Beta-amylase has limitations in its action. It cannot break down longer starch fragments or dextrins effectively because it acts specifically on maltose and maltotriose (Bijttebier et al., 2008). This means that if there are significant amounts of longer starch fragments or dextrins remaining after alpha-amylase treatment, beta-amylase may not efficiently convert them to glucose (Park et al., 2018). To achieve complete

starch hydrolysis and maximize glucose yield, amyloglucosidase (glucoamylase) is typically used after alpha-amylase (Oliveira et al., 2019). Amyloglucosidase is highly efficient at cleaving the alpha-1,4-glycosidic bonds, including those in longer starch fragments and dextrins, releasing individual glucose molecules (Jiang et al., 2010). This enzyme complements the action of alpha-amylase and ensures a more thorough conversion of starch into glucose.

The process of enzyme hydrolysis involved holding starch paste for 10 minutes at 95°C to attain uniformity. Gelatinization involves heating starch granules in liquid which makes them to swell and burst, leading to thickening of gel (Ji et al., 2022). Gelatinization improves accessibility of starch for amylase hydrolysis. Temperature of gelatinization for cassava starch (68°C) is slightly higher than the one of maize starch (66°C) (Abdullah et al., 2018). The smaller the starch granule, the higher the gelatinization temperature (Langenaeken et al., 2019). The reported mean granule size of starch from maize is 10.1 µm, while that for cassava is 7.3 µm (Q. Ji et al., 2004). In this study, the peak gelatinization temperatures (section 4.3.4) were not significantly different for cassava and maize varieties ($p>0.05$). In situations where the maize kernel is not milled to disrupt the endosperm as was the case in this study, it may necessitate heating the starch slurry to temperatures approaching or exceeding 100°C to cause disruption of the endosperm so that gelatinization of starch can take place (Ilori *et al.,* 1991).

The amount of resistant starch in maize samples could be another factor that could contribute to lower brix readings. Maize starch has higher resistant starch content than cassava (Remya & Jyothi, 2015) and hence the lower sugar levels in the maize syrups in comparison to cassava syrup samples. In the current work, maize varieties had higher resistant starch content than the cassava varieties (section 4.3.3). That is; H 2115 (0.82%), Longe 4H (0.65%) as compared to *Mwezi mukaaga* (0.43%) and TME 14 (0.55%).

4.5.5 pH

From the results in Tables 7 and 8, pH of acid hydrolyzed sugar syrup ranged from 5.50 to 5.70 and for enzyme hydrolyzed samples, the pH ranged from 4.50 to 5.30. Typical glucose syrup will have a pH in the range of 4.5 to 6.0 (Chemical, 2012). The glucose syrup obtained by acid and enzyme hydrolysis in this study was within the reported range. There was no statistical difference in pH across all syrups from cassava and maize starches produced by either acid or enzyme hydrolysis ($p>0.05$). The final syrup pH could be attributed to the standardized pH of the starch slurry required for proper functioning of the enzymes. Syrup pH affects yeast cell development and function hence ethanol yield (Liu et al., 2015). Maintaining pH in range 4 to 5 is critical, but if this is exceeded, by-products like butyric and acetic acid could consume some of the substrates to reduce efficiency of ethanol fermentation (Mengesha et al., 2022).

Once extracellular pH varies much from optimal (5.0 to 5.5), yeast would require more energy to pump-in and pump-out protons so as to sustain optimal intracellular pH (Narendranath & Power, 2005). If this pH varies a lot from optimal range, cells find it hard to maintain constant intracellular pH, and enzymes may fail to function normally leading to yeast death which reduces ethanol production. With the e efficiently (Narendranath & Power, 2005)xception of cassava varieties *Mwezi mukaaga* and NAROCASS 1 whose pH were lower than 5.0, the results from this study suggested that all sugar syrups were suitable for yeast fermentation to produce ethanol based on their pH values. This could explain the observed low fermentation efficiency for enzyme hydrolyzed cassava hybrid variety; NAROCASS 1 which had a pH of 4.50 and a fermentation efficiency of 68.94% (section 4.3.3).

4.5.6 Sugar concentration

From the results of acid hydrolysis (Table 7), sugar concentration for cassava varieties, there was a significant ($p<0.05$) difference between the improved varieties as well as between the

local varieties. NAROCASS 1 and *Mukumba* were not significantly ($p>0.05$) from each other. For maize varieties, there was a significant ($p<0.05$) difference between all varieties. For enzyme hydrolyzed cassava starch samples, there was no significant ($p>0.05$) difference between the improved varieties and *Mwezi mukaaga*. The local varieties were significantly ($p<0.05$) different from each other. For the maize varieties, there was a significant ($p<0.05$) difference between all varieties. Maize varieties for both acid and enzyme treatments had higher sugar concentration compared to cassava varieties.

Maize starch has channels which connect internal parts with outside environment (Huber & BeMiller, 1997). Thus, hydrolytic enzymes can reach the interior of starch granules through channels, which increases its digestibility. Channels, cavities and pores that characterise maize starch increase its effective surface area (Dhital et al., 2010; Adejumo et al., 2013) and this could explain the higher sugar concentrations obtained in maize syrups compared to cassava syrups.

Sugar analysis was conducted by HPLC which is more sensitive compared to refractometry that was used for brix determination. HPLC is more accurate which could explain the variation between results of sugar concentration (by HPLC) and brix measurement by refractometry that gave lower sugar readings for all samples analysed. For instance, enzyme hydrolyzed sugar syrup for NAROCASS 1, had 19.9 ^{o}brix but mean sugar concentration by HPLC was 21.9 g/100g. Maize variety H 2115 had 20.4 ^{o}brix but sugar concentration of the sample by HPLC was 46.1 g/100g. Maize hybrid variety H 2115 had 14.5 ^{o}brix and sugar concentration of 37.1 g/100g by HPLC. Brix is determined by measuring the refractive index of the solution. Viscosity can affect the way light travels through the syrup. In highly viscous syrups, the movement of light may be slower, and this can influence the refractive index and result in inaccurate Brix readings (Pongsawatmanit et al., 2011). This could explain the large variations

between the brix readings and sugar concentrations for some varieties. For example, in the acid hydrolyzed syrups, NAROCASS 1 with a viscosity reading of 2.09 η had a brix of 19.90°brix and a sugar concentration of 21.90 g/100g, which values are close, however, H 2115 with a higher viscosity reading of 2.60 η had a brix of 20.40°brix and a sugar concentration of 46.10 g/100g, which values are very widely spread. Looking at enzyme hydrolyzed syrups, the same can be observed. For example, *Mukumba* with a viscosity of 1.90 η had a brix reading of 15.70°brix and a sugar concentration of 12.41 g/100g, which values are relatively close, however, H 2115 with a viscosity of 2.57 η had a brix of 14.50°brix and a sugar concentration of 37.10 g/100g, which values are very widely spread.

4.6 Fermentation

Tables 9 and 10 show the average °Brix changes during fermentation of the glucose syrup from starch extracted from the selected cassava and maize varieties. Brix readings were taken at 0 days immediately after boiling (at 95°C) and cooling the syrup; at 3 days and at 5 days of fermentation. Initial brix readings in Tables 9 and 10 are different from those reported in Tables 7 and 8 because before fermentation begun, the syrup was boiled at 95°C for 10 minutes to kill any possible microbial contaminants which could be present. The process could have concentrated sugars in the matrix hence increasing the brix values.

Table 9: Changes in brix during fermentation of glucose syrup produced by acid hydrolysis of starch extracted from the selected maize and cassava varieties

Crop type	Varieties	Days of fermentation / changes in °Brix		
		0	3	5
Cassava	NAROCASS 1	21.25±0.35	12.25±0.35	10.75±0.35
	TME 14	23.75±1.06	15.25±0.35	13.50±0.71
	Mukumba	24.00±0.71	15.25±1.77	12.00±0.00
	Mwezi mukaaga	16.75±0.35	10.75±1.06	9.00±0.00
Maize	H 2115	23.50±0.71	16.50±0.71	12.00±0.00
	H 5355	19.50±0.71	16.25±0.35	10.25±0.35
	Longe 4H	22.95±0.64	18.10±0.14	11.00±0.71
	Longe 5H	25.40±0.14	21.50±0.71	16.50±0.71

Table 10: Changes in brix during fermentation of glucose syrup produced by enzyme hydrolysis of starch extracted from the selected maize and cassava varieties

Crop type	Varieties	Days of fermentation / changes in °Brix		
		0	3	5
Cassava	NAROCASS 1	20.25±1.77	9.25±1.06	6.25±0.35
	TME 14	22.25±1.06	10.50±1.41	5.75±0.35
	Mukumba	21.50±0.71	8.50±0.71	3.25±0.35
	Mwezi mukaaga	17.25±0.35	8.25±1.06	2.25±0.00
Maize	H 2115	18.50±0.71	3.50±0.71	2.00±0.00
	H 5355	14.75±0.35	2.25±0.35	0.0±0.00
	Longe 4H	20.25±0.35	3.75±1.06	0.0±0.00
	Longe 5H	13.25±0.35	0.00±0.00	0.00±0.00

Two way ANOVA was used to compare the change in brix readings for the acid and enzyme hydrolyzed glucose syrups for the different varieties as fermentation progressed over 5 days.

For acid and enzyme hydrolysis, It was observed from the tests of between-subjects effects table (Appendix IV and Appendix V, respectively), that the variety / number of fermentation days interaction was significant at the set p value ($p<0.05$). This implies that the effect of the variety is modified by the number of fermentation days, meaning that the effect of the fermentation days is not uniform across varieties. In such a situation, it is not possible to explain the effect of either variety or number of fermentation days individually.

The initial brix readings for acid hydrolyzed cassava and maize glucose syrup were higher than those of enzyme hydrolyzed syrup. After 3 days of fermentation, brix readings for both acid and enzyme hydrolyzed syrups decreased significantly ($p<0.05$) with enzyme hydrolyzed syrups showing the highest decrease. After 5 days of fermentation, no syrups from acid hydrolysis had sugars completely utilized by the yeast. This could be due to the presence of sugar degradation products such as furfurals and 5-hydroxymethyl furfurals which are yeast inhibitors and are normally found in high concentrations in the acid hydrolysates (Taherzadeh et al., 2000). These aldehydes have an effect on rates of yeast growth and fermentation (Nilsson & Gorwa-grauslund, 2005). *Mukumba* had the lowest brix reading of 9.00 after 5 days of fermentation.

After 5 days of fermentation, enzyme hydrolyzed syrups showed significant decrease in sugar concentration as compared to their acid hydrolyzed counter parts with some varieties such as; H 5355, Longe 4H and Longe 5H showing zero brix readings after 5 days. The syrups from maize varieties generally showed more efficient sugar utilization compared to those from cassava varieties. As per the manufacturer instructions of young's dried active yeast, the recommended duration of fermentation is 4 days. In this study, the fermentation was carried

out for 5 days to ensure total sugar utilization. Fermentation involves conversion of sugars to organic acids, alcohol and gases using yeast (Maicas, 2020). The following equation shows alcoholic/ethanol fermentation of glucose, which has the chemical formula C6H12O6. A molecule of glucose is converted to two molecules of each of ethanol and carbon dioxide:

$C6H12O6 \rightarrow 2\ C2H5OH + 2\ CO2$.

Primary fermentation is brief and yeast begins to work on raw ingredients converting sugars into alcohol. Secondary fermentation is the longer stage and lasts for 4-5 days. In this stage, levels of alcohol increase which kills off yeasts as their substrate becomes scarce (Martens et al., 1997). The pH of the ferment differs from the beginning, and this affects the chemical reactions that take place between yeasts and the fermenting medium. Majority of yeasts survive alcohol levels up to 8–10% and then die off which prevents further fermentation (Ezemba & Ezemba, 2022). The highest alcohol content of 11.14 % (v/v) as obtained in this study (Table 12) was from enzyme hydrolyzed syrup of the maize variety Longe 4H. This means that the effect of increased alcohol content killing the yeast hence affecting fermentation could have not been a factor that affected fermentation in this study. The decreasing food source is evident from the decrease in the sugar concentrations with progressing fermentation. After the fermentation process, distillation is required to evaporate water in order to obtain higher alcohol concentration (Ezemba & Ezemba, 2022)

4.7 Fermentation parameters

Tables 11 and 12 show the average fermentation parameters of glucose syrups from starch of the selected cassava and maize varieties. These include Alcohol by Volume (ABV) (%, v/v), sugar consumption and fermentation efficiency (%). These parameters were measured at the end fermentation (after 5 days). Alcohol by volume was the alcohol content obtained after

fermentation before distillation and concentration of ethanol (%) was the ethanol concentration after distillation, determined using GC-MS.

Table 11: Average fermentation parameters of glucose syrup produced by acid hydrolysis from starch of the selected cassava and maize varieties

Crop type	Varieties	Alcohol (ABV) (%, v/v)	Sugar consumption (°Brix)	Fermentation efficiency (%)	Concentration of ethanol (%)
Cassava	NAROCASS 1	5.78±0.00^{ab}	10.50±0.00^{ab}	49.50±0.71^{a}	31.05±0.03^{c}
	TME 14	5.64±0.97^{ab}	10.25±1.77^{ab}	43.00±5.65^{a}	31.69±0.03^{b}
	Mwezi mukaaga	6.60±0.71^{a}	12.00±0.71^{a}	50.00±1.41^{a}	34.39±0.27^{a}
	Mukumba	4.27±0.19^{b}	7.75±0.35^{b}	46.00±1.41^{a}	29.35±0.07^{d}
Maize	H 2115	6.33±0.39^{a}	11.50±0.71^{a}	49.00±1.41^{a}	31.70±0.04^{b}
	H 5355	5.09±0.58^{a}	9.25±1.06^{a}	47.50±3.54^{a}	29.82±0.04^{c}
	Longe 4H	6.58±0.04^{a}	11.95±0.07^{a}	52.00±1.41^{a}	34.47±0.30^{a}
	Longe 5H	4.90±0.47^{a}	8.90±0.85^{a}	35.00±2.83^{b}	29.70±0.16^{c}

Values for cassava in columns with similar superscript letters are not significantly different ($p>0.05$). Values for maize in columns with similar superscript letters are not significantly different ($p>0.05$).Values are means of two independent replicates ± standard errors of means.

Table 12: Average fermentation parameters of glucose syrup produced by enzyme hydrolysis from starch of the selected cassava and maize varieties

Crop types	Varieties	Alcohol (ABV) (%, v/v)	Sugar consumption (°Brix)	Fermentation efficiency (%)	Concentration of ethanol (%)
Cassava	NAROCASS 1	7.71±1.17^{a}	14.00±2.12^{a}	69.00±4.24^{b}	33.48±0.13^{c}
	TME 14	8.81±0.39^{a}	16.00±0.71^{a}	72.00±0.00^{b}	36.97±0.14^{b}
	Mwezi mukaaga	10.04±0.20^{a}	18.25±0.35^{a}	85.00±1.41^{a}	36.39±0.27^{b}
	Mukumba	8.26±0.39^{a}	15.00±0.71^{a}	87.00±2.83^{a}	37.89±0.03^{a}
Maize	H 2115	9.08±0.39^{b}	16.50±0.71^{b}	89.00±0.00^{b}	40.95±0.07^{a}
	H 5355	8.12±0.19^{bc}	14.75±0.35^{bc}	100.00±0.00^{a}	35.26±0.04^{c}
	Longe 4H	11.14±0.20^{a}	20.25±0.35^{a}	100.00±0.00^{a}	34.34±0.06^{d}
	Longe 5H	7.29±0.20^{c}	13.25±0.35^{c}	100.00±0.00^{a}	39.56±0.04^{b}

Values for cassava in columns with similar superscript letters are not significantly different ($p>0.05$). Values for maize in columns with similar superscript letters are not significantly different ($p>0.05$).Values are means of two independent replicates ± standard errors of means.

4.7.1 Alcohol by Volume

Alcohol by volume (ABV, expressed as a volume percent) is defined as the amount of alcohol (ethanol) contained in a particular volume of a beverage. Among the glucose syrups produced by acid hydrolysis (Table 11), for cassava, there was no significant ($p>0.05$) difference between improved and local varieties. For maize, there was no significant difference between the hybrid and OPV varieties ($p>0.05$). Among glucose syrups produced by enzyme hydrolysis, (Table 12), for cassava, ABV values were not significantly different among all varieties ($p>0.05$). For maize varieties, there was no significant ($p>0.05$) difference between the hybrid varieties. The OPV's were significantly ($p<0.05$) different from each other. Longe 5H was not significantly ($p>0.05$) different from H5355.

Alcoholic fermentation involves anaerobic conversion of glucose and fructose into carbon dioxide and ethanol by yeast (*Saccharomyces cerevisiae*). Towards the end of fermentation, yeast activity drastically decreases due to decrease in sugar consumption which leads to cessation of fermentation before the fermentable sugars are completely metabolised. Some of the factors that affect the process and hence impact on alcohol production / ethanol yield include aeration, temperature, ethanol content and pH of the sugar syrup (Mengesha et al., 2022). These factors must be controlled to avoid variations. Oxygenation / aeration has several functions in metabolism of yeast, with the main function being enhancing ergosterol and unsaturated fatty acid biosynthesis; these are needed for anaerobic growth during fermentation (Verbelen, Saerens, et al., 2009). Yeast cells begin to grow when the level of these compounds is adequate, for which oxygen is required. Yeast also take up oxygen and use it in development of mitochondria (Malina et al., 2018). It is therefore important to optimize oxygen supply because too much of it leads to degeneration of yeast, due to formation of reactive oxygen species which are toxic, while under aeration leads to reduced growth of yeast, stuck

fermentations, less lipid synthesis, reduced viability and changes in beer flavour (Verbelen, Depraetere, et al., 2009). In this study, oxygen was introduced into the ferment by dissolving the yeast in the glucose syrup which involved constant mixing for approximately 5 minutes. Temperature affects fermentation in two ways; very high temperature of fermentation leads to detrimental intracellular changes, high levels of volatile compounds especially esters and increase in dead yeast, whereas very low temperature of fermentation leads to reduced fermentation rates (Şener et al., 2007). Normal temperature of fermentation ranges from 20 to 22°C (Verbelen, Depraetere, et al., 2009). Fermentation temperature in this study was maintained at (20 to 22) °C.

Alcohol also affects yeast reproduction and growth. Every strain has a limit, known as yeast's "alcohol tolerance" which determines the level at which yeast becomes dormant and does not ferment any longer (Kumar et al., 2011). As yeast growth and sugar metabolism continues, there is alcohol accumulation to toxic levels which eventually kill cells. Majority of yeast strains are tolerant to 8–10% alcohol before dying off (Varize et al., 2022). In general, wine yeasts have higher tolerance to alcohol between 12-18% (Osho, 2005). Alcohol tolerance varies depending on nutrients, yeast health, yeast strain and available sugars (Carlsen et al., 1991). In the current study the final alcohol content was in range 4.26 to 6.60 % (v/v) for acid hydrolyzed syrups and 7.29 to 11.14 % (v/v) for the enzyme hydrolyzed syrups. The values were within the alcohol tolerance range of yeast and therefore, it was concluded that alcohol produced during fermentation did not affect the yeast activity and fermentation efficiency.

4.7.2 Sugar consumption

Sugar consumption refers to the sugar consumed by yeast during fermentation. This is the difference between the initial brix and brix measured after fermentation. Among the glucose syrups produced by acid hydrolysis (Table 11), for the cassava varieties, there was no

significant ($p>0.05$) difference between the local and improved varieties. The same was observed for maize, with no significant ($p>0.05$) difference between the hybrid and OPV varieties. For the glucose syrups produced by enzyme hydrolysis, for cassava, there was no significant ($p>0.05$) difference between the local and improved varieties. For the maize varieties, there was no significant ($p>0.05$) difference between the hybrid varieties. OPV's were significantly ($p<0.05$) different from each other. Longe 5H was no significantly ($p>0.05$) different from H5355 (Table 12). Syrup from maize hybrid H 2115 acid hydrolyzed starch had the highest sugar concentration of 46.1 g/100g and the corresponding sugar consumption value was 11.50 °Brix. Syrup produced from the same starch variety (H 2115) by enzyme hydrolysis had a sugar concentration value of 37.1 g/100g and correspondingly higher sugar consumption value of 16.5 °Brix. Similarly for cassava, acid hydrolyzed TME 14 starch whose sugar concentration was 31.2 g/100g registered sugar consumption of 10.25 ^{o}brix. The enzyme hydrolyzed starch of the same variety had sugar concentration of 10.23 g/100g and sugar consumption of 16.0 ^{o}brix which was higher than that of the acid hydrolysis counterpart.

Variation in sugar consumption by yeast could be attributed to possible presence of toxic by-products of acid hydrolysis for example furans that interfere with yeast activity (Azmi et al., 2017). Therefore, decreased fermentation rate in acid-treated starches could possibly be attributed to inhibition by furans which is a common challenge for commercial production of ethanol from acid-treated starch hydrolysates (Wang et al., 2017). Sugar degradation by-products such as 5-hydroxymethyl furfurals and furfurals are also known yeast inhibitors and are usually found in high concentrations in acid-hydrolysates (Taherzadeh et al., 2000). These carbonyls are known to affect growth rate and rate of yeast fermentation (Nilsson & Gorwa-grauslund, 2005).

4.7.3 Fermentation efficiency

Fermentation efficiency of the process evaluated the efficiency of utilization of available sugars to produce alcohol. The ratio of sugar consumed, which was the difference between initial and final brix and the initial sugar concentration present in the syrup were used to calculate fermentation efficiency. Enzyme hydrolyzed starch had higher fermentation efficiency compared to the acid hydrolyzed syrups for both the cassava and maize starch varieties. Among the glucose syrups produced by acid hydrolysis (Table 11), for cassava, there was no significant difference among all varieties ($p>0.05$). For maize varieties, fermentation efficiency of H 2115, H 5355 and Longe 4H were not significantly different ($P>0.05$); but all were significantly different from Longe 5H. Longe 5H had the highest brix reading after 5 days of fermentation (16.50°brix) implying that the sugars were not properly utilized by the yeast possibly due to presence of toxic by-products of acid hydrolysis that interfere with yeast activity (Azmi et al., 2017) and consequently a low fermentation efficiency. Fermentation efficiency of 52.1% obtained from starch of Longe 4H had low sugar consumption of 11.95 °Brix, and was the highest efficiency recorded from acid hydrolyzed starch. Among the glucose syrups produced by enzyme hydrolysis (Table 12), for the cassava varieties, NAROCASS 1 and TME 14 were not significantly different ($p>0.05$); but both were significantly different from *Mwezi mukaaga* and *Mukumba* which were not different from each other. For the maize varieties, H 5355, Longe 4H and Longe 5H were not significantly different ($p>0.05$); but these were different from that of H 2115. Syrup from enzyme hydrolyzed maize H 5355, Longe 4H and Longe 5H starches which had sugar consumption values of 14.75 °Brix, 20.25 °Brix and 13.25 °Brix respectively registered the highest fermentation efficiency of 100%.

4.7.4 Concentration of ethanol in the distillate

Sugar syrups were fermented and distillation was carried out to obtain ethanol. Boiling point of ethanol obtained from this work ranged between 76°C and 78°C. This is the temperature at which the distillate started to collect in the condenser. Among the sugar syrups produced by acid hydrolysis (Table 11), there was significant difference in ethanol concentration among all cassava variety distillates ($p<0.05$) with *Mwezi mukaaga* having the highest ethanol concentration of 34.39%. For the maize varieties, the ethanol concentration of H 5355 and Longe 5H distillates was not significantly different ($p>0.05$); but both significantly differed ($p<0.05$) from those of H 2115 and Longe 4H which were also different from each other. Longe 4H had the highest ethanol concentration of 34.47%. Among the syrups produced by enzyme hydrolysis (Table 12), ethanol concentration significantly differed among all cassava and maize variety distillates ($p<0.05$). *Mukumba* had the highest ethanol concentration among the cassava varieties (37.89%) and H 2115 had the highest ethanol concentration among all the varieties (40.95).

The ethanol concentration of 49.76% was reported from cassava starch by Zamora et al. (2010). This value obtained was generally higher than values obtained in this study. Alcohol obtained from enzyme hydrolyzed fermented syrup in this study generally had similar ethanol concentration to that of acid hydrolyzed counterparts despite marked differences in sugar concentration between acid and enzyme hydrolyzed maize and cassava starches.

Ethanol obtained in this study had a concentration between 29.35% and 40.95% and is typically referred to as a "weak ethanol solution" (Nose & Hojo, 2009) and can be used in various industrial applications, for example; can be used as cleaning agents in various industries, such as electronics manufacturing, pharmaceuticals, and healthcare as they are effective in removing contaminants, sterilizing surfaces, and cleaning sensitive equipment without leaving residue

(Ribeiro et al., 2015). Ethanol solutions in this range are employed in the production of pharmaceuticals, cosmetics, and personal care products. They can be used as solvents, preservatives, and in the formulation of tinctures, ointments, and lotions (Lachenmeier, 2008). Can be used in food and beverage processing for purposes such as extracting flavors from natural ingredients, producing extracts and tinctures, and as a food-grade solvent for certain formulations.

4.8 Correlation Analysis

Principal component analysis (PCA) was performed using SPSS v. 20 software to determine the correlation between the starch properties that were characterized (particle size, amylose content and resistant starch content), the sugar syrup viscosity, density, specific gravity and concentration obtained after subjecting the starch to enzyme and acid hydrolysis as well as the ethanol content of the fermented sugar syrup. The varieties whose starch was characterized (TME 14, *Mwezi mukaaga*, H 2115 and Longe 4H) are the varieties that were subjected to the PCA analysis. The starch characterization parameters were not varied as the same starch sample was used for both enzyme and acid hydrolysis. Therefore, it is only the sugar concentration and ethanol content that changed. The correlation matrix indicated direction and strength of linear correlations between variables. Positive values indicated a positive correlation, meaning that as one variable increased, the other also increased. Negative ones indicated negative correlation, indicating that as one variable increased, the other one decreased.

4.8.1 Enzyme hydrolyzed starch

Correlations were determined between the different variables of enzyme hydrolyzed cassava and maize starch varieties (Fig. 7). The first two principal components PC 1 and PC 2 explained

86.57% of cumulative variance, with F1 accounting for 62.548% and F2 accounting for 24.022% of the total variance.

According to the correlation matrix, sugar concentration of the enzyme hydrolyzed starch correlated positively with ethanol concentration (r = +0.698), resistant starch content (r = +0.924), viscosity (r = +0.112), starch yield (r = +0.924), pH (r = +0.322) and variety (r = +0.563) and correlated negatively with level of amylose (r = -0.924), specific gravity (r = -0.683), density (r = -0.644), sugar consumption (r = -0.115), particle size 1 (r = -0.715), and particle size 2 (r = -0.728). From the Pearson correlation data, positively correlated factors directly affected the sugar concentration of the enzyme hydrolyzed starch. This implies that resistant starch content, the different variety types, starch yield, pH and syrup viscosity directly impacted on sugar concentration of syrup derived from enzyme hydrolysis of cassava varieties (TME 14 and *Mwezi mukaaga*) as well as maize varieties (Longe 4H and H 2115). The negative factors did not influence the sugar concentration.

The ethanol content of the fermented enzyme hydrolyzed starch was positively correlated to sugar concentration (r = +0.698), resistant starch (r = +0.596), starch yield (r = +0.394), specific gravity (r = +0.010) and pH (r = +0.018) and negatively correlated to variety (r = -0.191), sugar consumption (r = -0.721), amylose content (r = -0.422), particle size 1 (r = -0.015), particle size 2 (r = -0.039), density (r = -0.268) and viscosity (r = -0.600). Positively correlated factors such as the sugar concentration, resistant starch content, specific gravity and pH directly impacted the ethanol concentration.

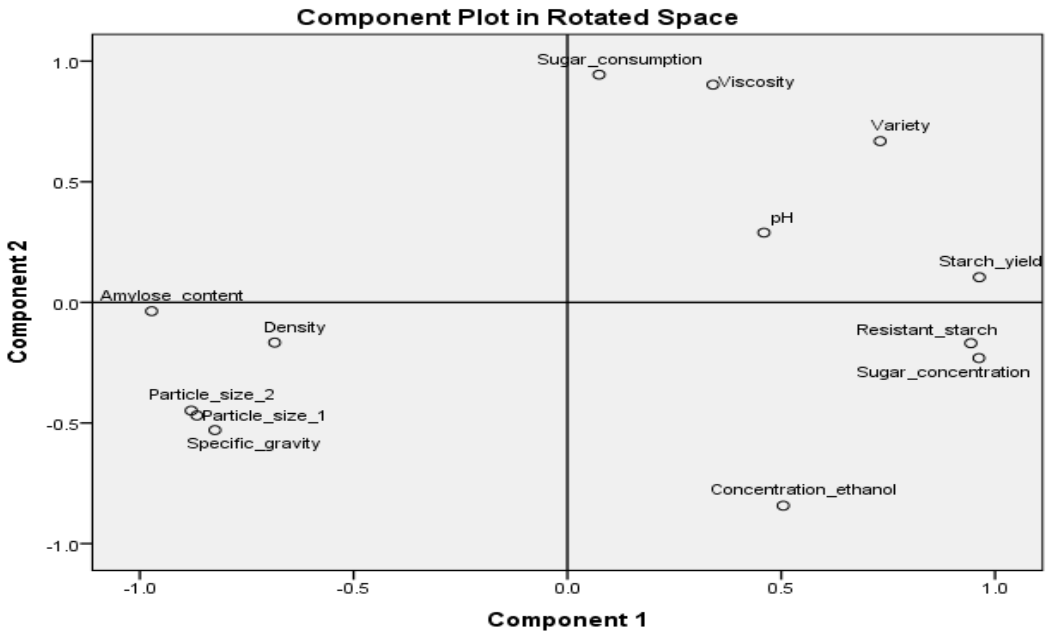

Figure 7: Principal Component Analysis of the enzyme hydrolyzed starch. (IBM SPSS ver. 20).

4.8.2 Acid hydrolyzed starch

Correlations were determined between the different variables of acid hydrolyzed cassava and maize starch varieties (Fig. 8). As per the Eigen values, four PCs F1, F2, F3 and F4 explained 96.93% of cumulative variance, with F1 accounting for 49.514% , F2 accounting for 21.46%, F3 accounting for 14.230% and F4 accounting for 11.72% of the total variance.

According to the correlation matrix, sugar concentration of the acid hydrolyzed starch was positively correlated to the variety (r = + 0.496), resistant starch content (r = +0.969), starch yield (r = +0.872), specific gravity (r = +0.270), viscosity (r = +0.077) and sugar consumption (r = +0.006), and correlated negatively against level of amylose (r = -0.930), particle size 1 (r = -0.703), particle size 2 (r = -0.727), density (r = -0.2020) and pH (r = -0.121).

The ethanol content of the fermented acid hydrolyzed starch was correlated positively with variety (r = +0.405) and level of amylose (r = +0.296), sugar consumption (r = +0.565) and pH (r = +0.473) and negatively correlated to resistant starch (r = -0.472), starch yield (r = -0.100),

particle size 1 (r = -0.117), particle size 2 (r = -0.071), specific gravity (r = -0.138), density (r = -0.331) and viscosity (r = -0.005).

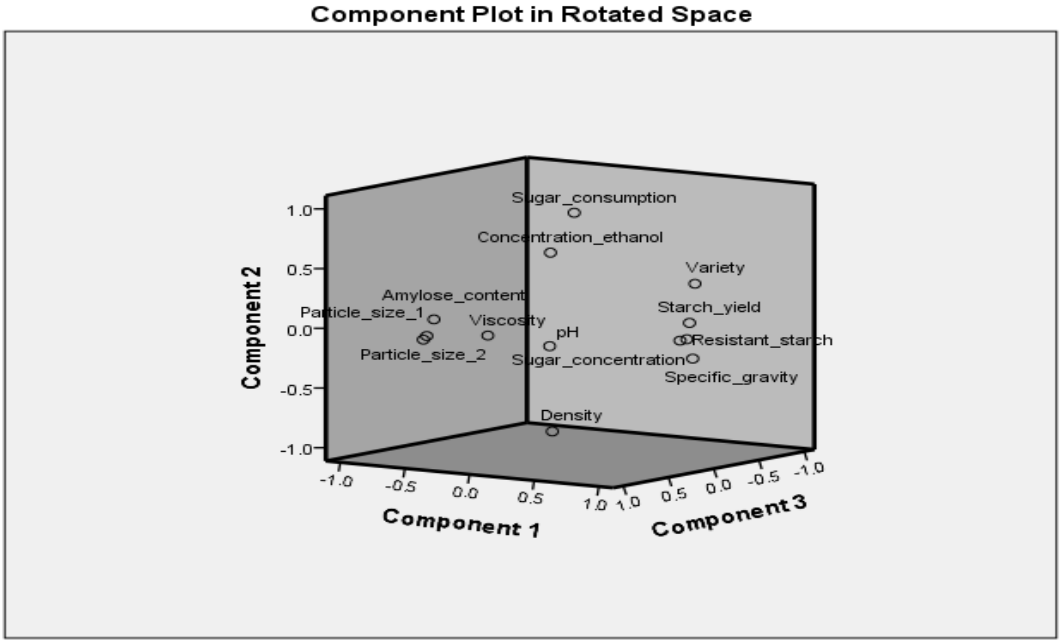

Figure 8: Principal component analysis of the acid hydrolyzed starch. (IBM SPSS ver. 20).

The variety, resistant starch content and viscosity positively impacted sugar concentration in the enzyme or acid hydrolysed syrup whereas amylose content, density and particle size 1 and 2 negatively impacted / had no impact on the sugar concentration irrespective of the treatment. pH is the only variable that positively affected the ethanol concentration produced from the syrup derived from either enzyme or acid hydrolysis of starch. Particle size, density and viscosity negatively impacted the ethanol concentration cutting across both treatments.

Type of crop variety had a positive correlation of (r = +0.803) with the yield of starch.

CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

5.0 Conclusions

This research aimed to produce and characterize glucose syrup and ethanol from selected Ugandan cassava and maize varieties.

There was no difference in starch yield among local and/or improved cassava and maize varieties. The best yield from the improved cassava varieties was from TME 14 (26.60%) and for local varieties, *Mwezi mukaaga* (29.17%) at 12 months maturity. From the open pollinated maize varieties, Longe 4H (43.90%) and the hybrids; H 2115 (50.83%) at 120 days maturity.

For enzyme hydrolysis, the sugar concentration was directly affected by the variety used (whether cassava or maize), the starch yield from the variety, the resistant starch content, pH of the syrup as well as its viscosity. *Mukumba* had the highest sugar concentration (12.41 g/100g) among the cassava varieties and among the maize varieties, H 5355 (40.30 g/100g). The ethanol produced from the enzyme hydrolysis process was directly affected by the starch yield, its resistant starch content, the sugar concentration of the fermented glucose syrup, its specific gravity and pH. *Mukumba* had the highest ethanol yield (37.89%) for the cassava varieties and H 2115 (40.95%) for the maize varieties.

For acid hydrolysis, the sugar concentration was directly affected by the variety, the starch yield, the resistant starch content, specific gravity of the syrup, the sugar consumption as well as the pH of the syrup. Among the cassava varieties, TME 14 had the highest sugar concentration (31.20 g/100g) and among the maize, H 2115 (46.10 g/100g).

The ethanol produced from the acid hydrolysis process was affected by the crop variety, amount of amylose, sugar consumption during fermentation and pH of the syrup that was fermented.

The highest ethanol content from the cassava varieties was from *Mwezi mukaaga* (34.39%) and from maize; Longe 4H (34.47%).

Acid starch hydrolysis generated higher sugar syrup concentration than enzyme hydrolysis.

Maize based syrups produced higher sugar concentration than the cassava counterparts. The ethanol content was relatively the same for the maize and cassava varieties. Therefore, if the objective is production of glucose syrup, maize varieties offer better prospects in terms of starch yield and, sugar concentration. However, if the end goal is ethanol production, either crop type, whether cassava or maize will relatively give a good yield.

A major challenge arising from this study is that the profiling of the glucose syrup in terms of nutritional composition was not carried out and hence a comparison to that on the market is not very possible.

5.1 Recommendations

For cassava, whether it is an improved or local variety, there was no significant difference in the starch yield and the same applied to the maize varieties. Therefore, any of the four cassava or four maize varieties can be used as a source for commercial starch production.

The starch extracted from cassava and maize irrespective of the extraction method can have numerous industrial uses in the food industry as a thickening agent, in the paper industry as a binder and coating agent, in the textile industry to size and finish fabrics, in pharmaceuticals as an excipient.

When producing sugar syrup either by acid or enzyme hydrolysis, factors that will directly impact the sugar concentration and cut across both treatments and hence should be controlled include; crop variety, starch yield and content of resistant starch in starch material to be hydrolyzed. Other factors such as the pH, viscosity, specific gravity etc. are treatment specific.

If the goal is to produce glucose syrup, improved maize varieties show better prospects in terms of yield, while both treatments are acceptable in terms of quality attributes.

For production of ethanol, the only variable that cuts across both treatments is the pH of the glucose syrup produced after hydrolysis that is subjected to fermentation. The rest of the variables are dependent on the type of hydrolysis to be carried out.

If the end goal is ethanol production, glucose syrup derived from enzyme hydrolysis of maize varieties gives the best yield.

Further research should evaluate the saccharification potential of different malted grains using various local starch sources such as maize and cassava to create a local enzyme source for the cheap production of sugar syrups as commercial enzymes are costly.

There is also a need to evaluate the presence, effect and safety aspects of inhibitory substances such as furans that could possibly be present in acid hydrolyzed sugar syrups and could affect fermentation efficiency as well as being harmful to human health.

To address the challenge of food security arising from competition with glucose syrup and ethanol using cassava and maize as feedstocks and yet they are the staple foods in a number of communities, it's important for industries that use these food crops for non-food purposes, such as syrup production, to adopt responsible and sustainable practices. That can include:

a) Promoting efficient resource utilization to minimize waste and maximize food production.

b) Collaborating with the agricultural and food sectors to ensure a balance between food and non-food crop production.

c) Investing in research and development to improve crop yields and agricultural sustainability.

Ultimately, it is essential for policymakers, industry stakeholders, and agricultural experts to work together to strike a balance between the needs of non-food industries and the imperative

of maintaining food security for all. Responsible resource management and sustainable practices can help mitigate potential conflicts between food and non-food production.

Other products that could have been generated with the ethanol produced in this study were not tested for. This is an area for further research.

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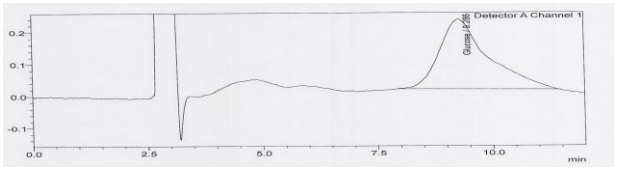
APPENDICES

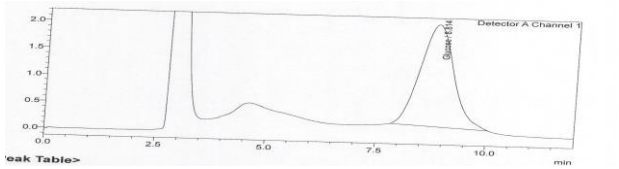
Appendix I: HPLC spectrum showing detection of glucose in acid hydrolyzed starch samples. A_1&A_2: local cassava varieties; A_3&A_4: hybrid cassava varieties; B_1&B_2: local maize varieties; B_3&B_4: hybrid maize varieties.

CASSAVA VARIETIES

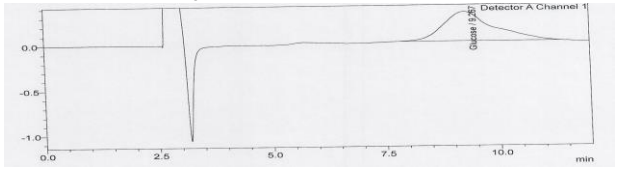
A_1: Mukumba



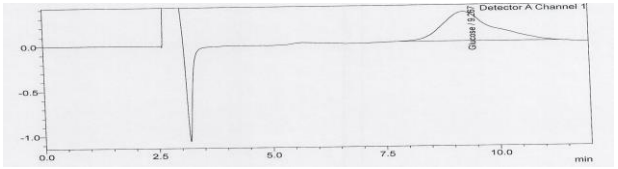
A_2: Mwezi mukaaga



A_3: NAROCASS 1

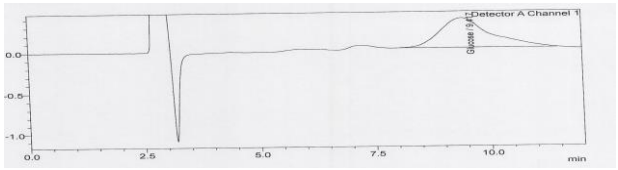


A_4: TIME 14

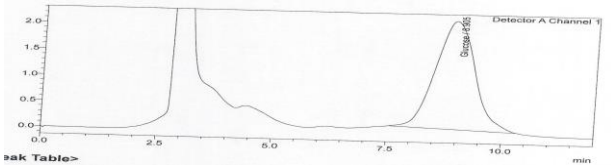


MAIZE VARIETIES

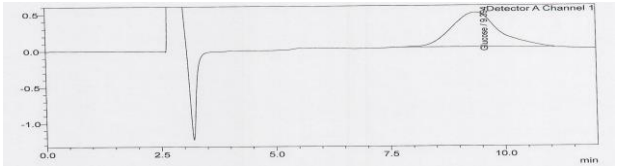
B_1: Longe 4H



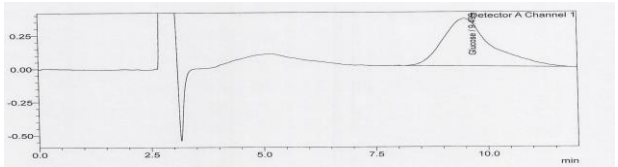
B_2: Longe 5H



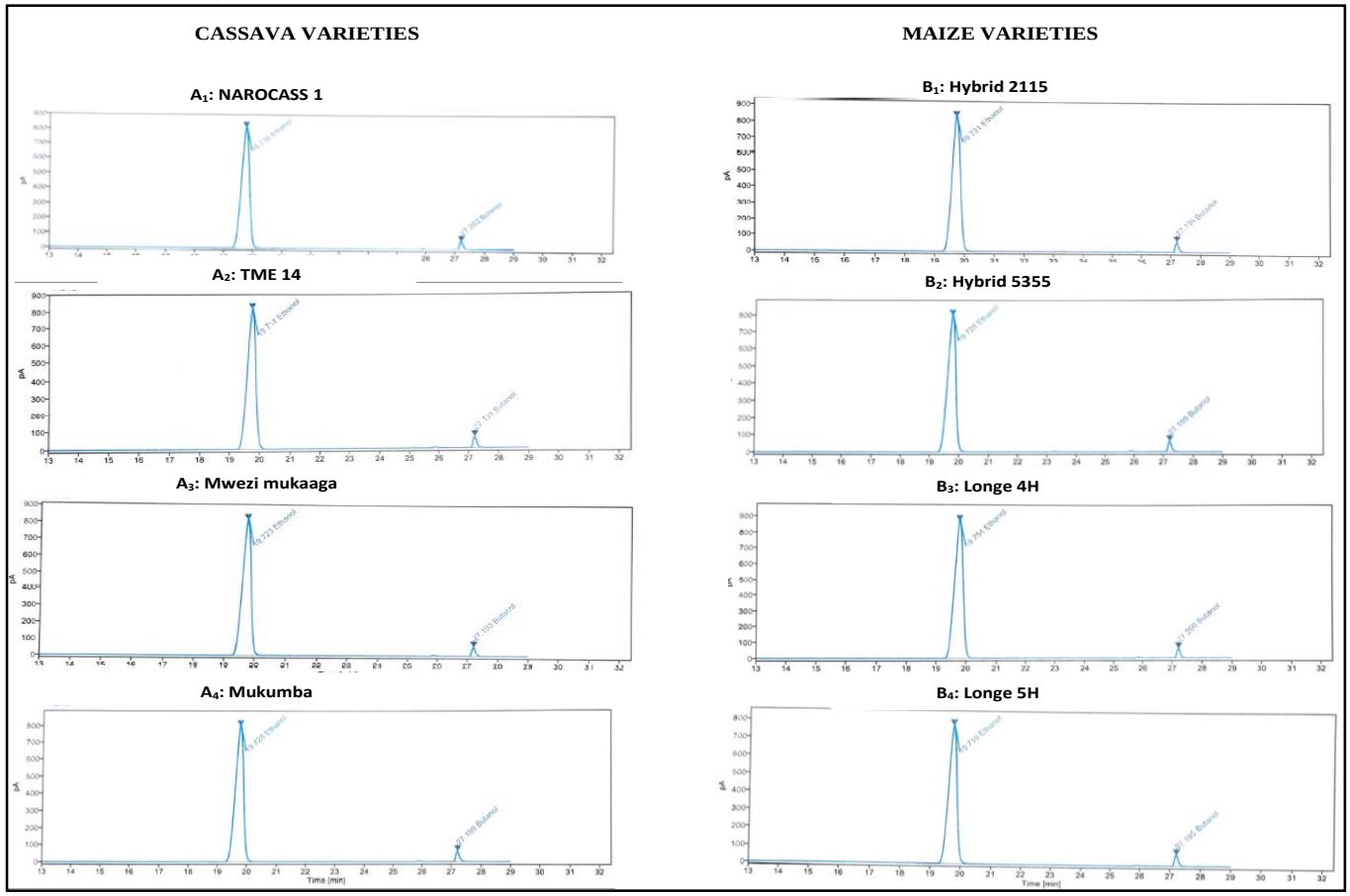
B_3: Hybrid 2115



B_4: Hybrid 5355



Appendix II: GC-MS spectrum showing the detection of ethanol (Rt, 19.7 min) and butanol (control, Rt, 27.2 min) in acid (0.3M HCl) hydrolysate of starch (20% w/v, pH, 4.5) obtained from selected cassava and maize varieties. A_1&A_2: hybrid cassava varieties; A_3&A_4: local cassava varieties; B_1&B_2: hybrid maize varieties; B_3&B_4: local maize varieties.



Appendix III: Photos of the cassava and maize varieties used in the study. A_1&A_4: hybrid cassava variety, $A_{2\&}A_3$: local cassava varieties, $B_{1\&}B_2$: Hybrid maize varieties, $B_{3\&}B_4$: open pollinated maize varieties

Appendix IV: 2 way ANOVA (Tests of between-subjects effects table) for changes in brix during fermentation of glucose syrup produced by acid hydrolysis

Tests of Between-Subjects Effects

Dependent Variable: Brix_changes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1200.178[a]	23	52.182	117.098	.000
Intercept	13197.017	1	13197.017	29614.624	.000
Variety	284.241	7	40.606	91.121	.000
Number_of_days	859.891	2	429.946	964.815	.000
Variety * Number_of_days	56.045	14	4.003	8.983	.000
Error	10.695	24	.446		
Total	14407.890	48			
Corrected Total	1210.873	47			

a. R Squared = .991 (Adjusted R Squared = .983)

Appendix V: 2 way ANOVA (Tests of between-subjects effects table) for changes in brix during fermentation of glucose syrup produced by enzyme hydrolysis

Tests of Between-Subjects Effects

Dependent Variable: Brix_changes

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2740.729[a]	23	119.162	243.395	.000
Intercept	3798.521	1	3798.521	7758.681	.000
Variety	369.813	7	52.830	107.909	.000
Number_of_days	2301.542	2	1150.771	2350.511	.000
Variety * Number_of_days	69.375	14	4.955	10.122	.000
Error	11.750	24	.490		
Total	6551.000	48			
Corrected Total	2752.479	47			

a. R Squared = .996 (Adjusted R Squared = .992)