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OPTIMIZING THE CONDITIONS OF ETHANOL PRODUCTION FROM CASSAVA AND SWEETPOTATO

By

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DECLARATION

I hereby declare that this submission is my own work towards the PhD and that, to the best of my knowledge, it contains no material previously published by another person, nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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ABSTRACT

Cassava and sweetpotato are root and tuber crops cultivated in Ghana that could serve as industrial raw material for ethanol production. Investigations were conducted to optimize the yield of ethanol from two (2) varieties each of Cassava and Sweetpotato. Response surface methodology was used to model the optimum liquefaction, saccharification and fermentation conditions for ethanol production from the cassava and sweetpotato varieties. Three starch hydrolytic enzymes (*Liquozyme SC DS, Spirizyme Fuel, Viscozyme L*) and two strains of yeast (*Bio-Ferm XR*, Baker's yeast) were used for fermentation. The best liquefaction, saccharification and fermentation times established were 2.1 hours, 4 hours and 57.4 hours at 34^{o}C respectively with *Liquozyme SC DS, Spirizyme Fuel/Viscozyme L* and *Bio-Ferm XR* (Lallemand) yeast. The combination of *Viscozyme L and Spirizyme Fuel* enzymes in a ratio of 1:1 was the best enzyme mix for saccharification for a duration of 4 hours. The yeast to employ for best fermentation was Bio-Ferm XR at temperature of 34^{o}C for 57.4 hours duration. The results also indicated that 10 months matured Sika bankye and 3 months old Apomuden were the best cassava and sweetpotato varieties respectively for ethanol production. The best ethanol yield established from the study was 16.2% v/v from a 50:50 cassava:sweetpotato flour combination. Ethanol production with Sika bankye and Apomuden in a 50:50 ratio with GH¢ 119.00 as cost of fresh roots and tubers, ethanol yield of 15.5% v/v, selling price of GH¢ 4.7 per litre of ethanol and the use of a 10,000 litres per day capacity ethanol distilling plant generates net profit of between 11% and 31% over a period of

five years. Ethanol production with cassava and sweetpotato is therefore a profitable venture.

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LIST OF ABBREVIATIONS

CSIR-FRI – Council for Scientific and Industrial Research – Food Research Institute

DE – Dextrose equivalence

DOE – United States Department of Energy

EC number – Enzyme commission number

GCDS - Global Cassava Development Strategy

HQCF – High Quality Cassava Flour

HQSPF – High Quality Sweetpotato Flour

MoFA-SRID – Ministry of Food and Agriculture (Ghana)-Statistics, Research and Information Directorate

SSA - Sub-Saharan Africa

USA – United States of America

DEDICATION

To the memories of my late Father, Michael Jerome Kosi Komlaga and Aunt, Francisca Afua Komlaga, for the immense sacrifices you made in contributing to the success of my education. May your gentle souls rest in perfect peace.

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To all my good and lovely friends, colleagues and family members, your contributions to the success of this work are valuable and appreciated. I salute you all.

CHAPTER ONE

INTRODUCTION

1.1 Background

Ethanol, also called ethyl alcohol, is a volatile, colourless, flammable liquid which belongs to a class of organic compounds that are given the general name alcohols (Vollhardt and Schore, 2014; McMurry, 2015). Ethanol for industrial use as a solvent or chemical intermediate is largely obtained by acid-catalyzed (H_3PO_4) hydration of ethylene at a high temperature of 250^oC (McMurry, 2015). Ethanol is also produced via biological processes by fermenting sugars with yeasts and bacteria, the method used for alcoholic beverages (Cardona *et al*, 2010; Roehr, 2001; Nelson and Cox, 2008). Ethanol is the most widely used biofuel today with production and consumption over 40 billion litres based primarily on corn (Boundy *et al*, 2011; Vollhardt and Schore, 2014; McMurry, 2015). Ethanol is also used as a solvent, extractant, antifreeze, fuel supplement and an intermediate feedstock in the synthesis of innumerable organic chemicals (Roehr, 2001). Bimolecular dehydration of ethanol gives diethyl ether, which is employed as a solvent, extractant and anesthetic. These and other ethanol-derived chemicals are used in dyes, drugs, synthetic rubber, adhesives, explosives and pesticides (Roehr, 2001). Ethanol is the principal psychoactive constituent in alcoholic beverages, with depressant effects on the central nervous system (McMurry, 2015). Ethanol is used in medical wipes and in most common antibacterial hand sanitizer gels because it kills micro-organisms by denaturing their proteins and dissolving their lipids.

Biochemical ethanol production has some advantages over thermochemical ethanol production as the ethanol is produced from a renewable resource, having

economic relevance, and that starchy crops can readily grow in poorer hotter climates (Get Rivising, 2017). Relatively less amounts of energy is required during bioethanol production since the saccharification and fermentation temperatures are relatively low. Environmental management, capital investment and sourcing of raw materials for thermochemical ethanol production may be challenging in developing countries. Biochemical method of ethanol production could therefore be considered as the best to employ in developing countries like Ghana where starchy crops such as cassava and sweetpotato abound as raw material.

Corn, sugar cane and wheat are major crops that are used globally to produce ethanol (Boundy *et al,* 2011; Vollhardt *et al,* 2014; McMurry, 2015). Cassava and sweetpotato are ideal for ethanol production because of their high yields and characteristic high starch content, therefore producing high amounts of ethanol per tonne of roots and tubers (Lareo *et al,* 2013). Cassava and sweetpotato grow well in many farming conditions and have few diseases. They can be grown in poor soils with little fertilizer. Cassava gives yields of up to about 200 liters ethanol per tonne of wet roots weight whereas a tonne of sweetpotato yields up to 182 Kg of ethanol (Lareo *et al*, 2013). In a report by Ministry of Food and Agriculture, (MoFA Ghana Statistics, 2016), Ghana produced 17,213,000 tonnes of cassava in 2015. Out of this production figure, 60 to 70% was used to meet subsistence needs leaving a surplus production of 30 to 40%. This suggests a large opportunity for industrial growth (Grow Africa, 2015). Much of this excess cassava is either wasted or remains un-harvested, and can be captured for industrial use without any effect on food security (Grow Africa, 2015). Evolving numbers of investments in ethanol and starch industries suggest the growing potential to add value to cassava. These investments

are likely to catalyze increased interest in improving the value chain to promote growth.

Several studies have been carried out in the recent past to demonstrate the use of cassava and sweetpotato as raw material for ethanol production (Archibong *et al*, 2016; Swain *et al*, 2013; Oyeleke *et al*, 2012; Ademiluyi *et al*, 2013; Ocloo and Ayernor, 2010). The search for the optimum processing conditions to hydrolyse and ferment sugars from the starches in cassava and sweetpotato had been the major focus of most of the investigations. The effects of substrate, temperature, enzyme types and concentrations, the reaction times of saccharification and fermentation had all been investigated on ethanol yield. The various investigations identified some processing conditions which could optimize the yield of ethanol from cassava and sweetpotato. Nonetheless, varietal selection of cassava and sweetpotato which is a key factor to consider when processing ethanol have not been investigated in Ghana for optimum ethanol production. Much of the investigations regarding use of cassava and sweetpotato also rely on yeast strains (*Saccharomyces cerevisiae*) that could not stand high alcohol levels during fermentation. Another important issue was about dextrinization of starch molecules during starch hydrolysis. Generally, amylases cannot survive the high gelatinization temperatures of starches in most crops. There is therefore the need to explore dextrinization enzymes at the cooking stage to effect thinning of the mash to enable saccharifying enzymes to break up dextrins into simple sugars. Successful optimization of the conditions of ethanol production from cassava and sweetpotato would make ethanol production from these crops a viable venture.

1.2 Problem statement

Ghana virtually imports all of its ethanol requirements. The volume of ethanol imports into Ghana for direct consumption (after blending with herbs) and use in other industries over the years has been high. For instance, over seventy (70) million litres of ethanol was imported into Ghana for the various industries in 2016 (Ghanabusinessnews.com, 2017). The trend had not been so different from the past decade and beyond. The current cost of one litre of ethanol on the international market is US$0.44 (Trading Economics, 2017). This means Ghana used approximately US$30,800,000.00 to import ethanol into the country in 2016 (Trading Economics, 2017). There are few registered distilleries in Ghana but they are actually not distilling ethanol but rather import ethanol from other countries and blend or repackage for distribution in Ghana. The only large scale distillery currently running trials on ethanol production in Ghana is Caltech Ventures Ltd, located in Hodzo in the Ho Municipality of Volta Region. Though there are no available statistics on how much imported ethanol the registered distilleries in Ghana blend or distribute, estimated figures suggest that these companies together use about 70% of the imported ethanol into Ghana (Ghanabusinessnews.com, 2017).

1.3 Justification

Successful identification of the right varieties of cassava and sweetpotato, right maturity periods of the crops and further optimisation of the conditions for processing ethanol from cassava and sweetpotato in commercial quantities in Ghana would absorb the surplus fresh cassava and sweetpotato roots and tubers from farmers. This would create employment opportunities and cut down on or eliminate importation of ethanol which subsequently could save the country some foreign exchange on ethanol imports.

1.4 Objective

The main objective of the work was to develop economically viable optimized standard methods for producing ethanol from cassava and sweetpotato varieties in Ghana and identify suitable cassava and sweetpotato varieties that could be used as raw materials for ethanol production.

1.5 Specific objectives

The specific objectives of the work were;

- To optimize the conditions of ethanol production from cassava and sweetpotato.
- To determine the optimum maturity periods and best variety of cassava and sweetpotato for higher ethanol yields.
- To establish the yield of ethanol from composite flour of cassava and sweetpotato.
- To determine the profitability of ethanol production from cassava and sweetpotato.

CHAPTER TWO

LITERATURE REVIEW

2.1 Ethanol

Ethanol, also called ethyl alcohol, pure alcohol, grain alcohol, or drinking alcohol, is a volatile, colourless, flammable liquid (boiling point 78.5 °C) with an agreeable ethereal odour and a burning taste (Vollhardt *et al*, 2014; McMurry, 2015). Ethanol is a member of a class of organic compounds that are given the general name alcohols. The chemical formula for ethanol is CH_3CH_2OH. The chemical structure is made up of a methyl group (CH_3-), a methylene group (-CH_2-), and a hydroxyl group (-OH). Essentially, ethanol is ethane with a hydrogen molecule replaced by a hydroxyl radical, -OH, which is bonded to a carbon atom. The hydroxyl group is a functional group consisting of a hydrogen atom covalently bonded to an oxygen atom. The hydroxyl group is denoted by -OH in chemical structures and has a valence charge of -1 (Vollhardt *et al*, 2014; McMurry, 2015).

Methyl group -CH3-, Methylene group –CH2- and Hydroxyl group –OH.

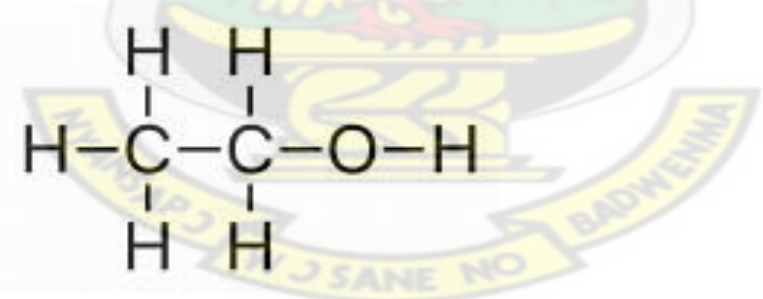


Figure 2.1: Structure of ethanol.

Source (McMurry, 2015)

2.1.1 Ethanol production process

Ethanol is produced in two different ways; as a fermentation product (bioethanol) by fermenting sugars with yeast or from the petroleum by-product

ethylene (**s**ynthetic ethanol), through the hydration of ethylene (Vollhardt *et al*, 2014; McMurry, 2015; Oak Ridge National Laboratory, 2010). Hydration of ethylene is achieved by passing a mixture of ethylene and a large excess of steam at high temperature (250^{o}C) and pressure (68Atm) over an acidic catalyst, H_3PO_4 (McMurry, 2015). Synthetic ethanol accounts for less than 10% of ethanol production and is used almost exclusively in industrial applications (Oak Ridge National Laboratory, 2010).

Bioethanol accounts for the vast majority of non-petroleum based ethanol production. Specifically, ethanol formed by fermentation of starch-based or cellulosic-based feedstock accounts for nearly all of today's ethanol market, with only minimal amounts currently manufactured via thermochemical production (McMurry, 2015). In the fermentation process, simple sugar molecules are metabolized by yeast to produce alcohol and carbon dioxide. The ethanol is separated from water through fractional distillation in the distillation process, therefore increasing the purity of ethanol (Cardona *et al*, 2010; Roehr, 2001). The following general steps are followed for the production of bioethanol;

- Milling and liquefaction: The high starch source (raw material) is first passed through hammer mills, which grind it into fine powder called "meal". The meal is then mixed with water and α-amylase (thermostable), and will pass through cookers where the starch is liquefied. Heat is applied at this stage to enable liquefaction. Cookers with high temperature (120-150^{0}C) and lower temperature holding period (95^{0}C) are used. These high temperatures reduce bacteria levels in the mash.

- Saccharification: The mash from the cookers is then cooled and the secondary enzyme (gluco-amylase) is added to convert the liquefied starch to fermentable sugars (dextrose).
- Fermentation: Yeast (S*accharomyces cerevisiae*) is then added to the mash to ferment the sugar to ethanol and carbon dioxide. Using a continuous process, the fermenting mash is allowed to flow, or cascade, through several fermenters until the mash is fully fermented and then leaves the final tank.
- Distillation: The fermented mash, now called "beer", will contain about 10% alcohol, as well as all the non-fermentable solids from the carbohydrate source and the yeast cells. The mash is then pumped to the continuous flow, multi-column distillation system where the alcohol is removed from the solids and the water. The alcohol leaves the top of the final column at about 96% strength, and the residue mash, called stillage, is transferred from the base of the column to the co-product processing area.
- Dehydration: The alcohol from the top column is then passed through a dehydration system where the remaining water is removed. Most ethanol plants use a molecular sieve to capture the last bit of water in the ethanol. The alcohol product at this stage is called anhydrous (pure, without water) ethanol and is approximately 100% proof.
- Denaturing: Ethanol that will be used for fuel is then denatured with 2-5% of gasoline.

The chief raw materials fermented for the production of industrial alcohol are sugar crops such as beets and sugarcane and grain crops such as corn (Roehr, 2001). Current commercial production of ethanol is based almost exclusively on starch and sugar-based feedstocks. In the United States, the ethanol industry is dominated by

corn, with 91.5% of production capacity from facilities using corn alone and another 7.9% of capacity from facilities using a blend of corn and other grain, with corn as the primary feedstock (Environmental Protection Agency, 2010). Facilities using other grains without corn make up an additional 0.4% of capacity. The remaining U.S. production capacity (0.3%) comes from facilities processing other feedstocks, such as cheese whey (lactose fermentation), potato waste, and beverage or brewery waste. Brazil, the world's second-largest producer of ethanol, used sugarcane to produce 6.9 billion gallons of ethanol in 2010 (Renewable Fuels Association, 2011). In Europe, the most common feedstock is wheat, although other cereal based grains can be used (barley, maize, rye); and two-thirds of all raw materials used are cereal grains, while the rest of the feedstock is mainly derived from sugar beets (Occupational Safety and Health Administration, 2017).

The preference for the choice of the feedstock in the various regions depend on various factors. In the United States, much corn is used as feedstock for ethanol production because it is a productive and versatile crop. Corn has incredible high yield compared with most other US crops and it grows nearly everywhere in the country especially thriving in the Midwest and Great plains (Foley, 2013). Advanced agricultural and processing systems have been well developed and promoted leading to its use as a cheap feedstock in ethanol production in the US. In Brazil, the use of sugarcane as feedstock for ethanol production is as a result of the advanced agro-industrial technology and its enormous amount of arable land. Brazil's ethanol fuel program is based on the most efficient agricultural technology for sugarcane cultivation in the world which makes the feedstock cheap.

2.1.2 Uses of ethanol

The largest single use of ethanol is as a motor fuel and fuel additive which constitutes 79.6% or 51,044 million litres of the global ethanol usage (Piyachomkwan, 2011). All cars used to run on the same type of fuel, but fuel stations now offer not only unleaded gasoline but alternative fuels, such as ethanol, diesel, biodiesel and others. Alcohol fuels have been around for years, typically mixed with gasoline in a blend also known as gasohol E10, with a ratio of 10% ethanol to 90% gasoline. E85, a mixture of 85% ethanol to 15% gasoline, can be used in flex-fuel vehicles, and car enthusiasts have modified their vehicles to run on ethanol or methanol alone. Because ethanol is biodegradable, nontoxic and dissolves in water, E85 has been applauded by the United States Department of Energy (DOE) as producing emissions that contain less carbon dioxide and carbon monoxide than emissions from vehicles that run on gasoline (Environmental Protection Agency, 2010).

The major controversy concerning ethanol fuel relates to the raw materials used to produce the fuel. Corn-based ethanol could lead to food insecurity due to competition with humans. Fortunately, cellulosic ethanol does not have those drawbacks because it is derived from the cellulose found in non-food agricultural and waste products (Cutzu and Bardi, 2017). For instance, switch grass is a fast-growing plant that has a high yield of energy and requires little in terms of fertilization and other high-energy production costs. Fuel from switch grass can compete with gasoline for fuel efficiency and not affect the price and supply of grains and other vital vegetation (Greenthefuture.com, 2017).

Ethanol is the principal psychoactive constituent in alcoholic beverages, with depressant effects on the central nervous system (McMurry, 2015). Moderate amounts of ethanol relax the muscles and produce an apparent stimulating effect by depressing the inhibitory activities of the brain, but larger amounts impair coordination and judgment. Ethanol is a depressant, and depending on dose, can be a mild tranquilizer or a general anesthetic. At very low doses, ethanol appears to be a stimulant by suppressing certain inhibitory brain functions (McMurry, 2015). However, as concentration increases, further suppression of brain functions produce the classic symptoms of intoxication: slurred speech, unsteady walk, disturbed sensory perceptions, and inability to react quickly. At very high concentrations, ethanol produces general anesthesia; a highly intoxicated person may sleep and may be very difficult to wake, and if awakened, unable to move voluntarily (Science is fun, 2009).

Ethanol is also an important industrial chemical used as a solvent in the synthesis of other organic chemicals and has widespread use as a base chemical for other organic compounds because it is easily mixed with water and many other organic compounds (Roehr, 2001). It is a common solvent in personal care products like deodorants, hairsprays and astringents. It is also found in food additives like food colouring and flavouring. Paints, lacquers, and varnish also have ethanol as one of their components (Garcia, 2016).

Ethanol is used extensively as a solvent in the manufacture of perfumes, as a preservative for biological specimens, in the preparation of essences and flavourings and in many medicines and drugs. Ethanol is used as disinfectant in medical wipes and in most common antibacterial hand sanitizer gels at a concentration of about

62% v/v as an antiseptic. Ethanol kills organisms by denaturing their proteins and dissolving their lipids and is effective against most bacteria and fungi, and many viruses (McMurry, 2015).

2.1.3 Marketing of ethanol

Over the last 10 years, the ethanol market has grown rapidly, mainly due to the support of national governments for biofuels as an environmentally-friendly gasoline substitute. Out of a global ethanol consumption of about 110 billion litres, approximately 87% is fuel ethanol while the rest is non-fuel ethanol- beverages, industrial use, cosmetics and others. (European Union Biofuels Annual, 2016; Sucden.com, 2017). The main benefit of ethanol when used as transport fuel is the reduction of greenhouse gas emissions. United States of America, Brazil and the European Union have indeed made a choice in favour of using biofuels. Together, they represent about 80% of world ethanol consumers, account for 80% of worldwide ethanol production and they are committed to using ethanol as fuel substitute to gasoline (International Energy Agency, 2017). New areas, such as the Asia-Pacific region, are gradually increasing biofuel consumption. The International Energy Agency forecasts worldwide biofuel blending in road-transport gasoline at 5.0% by 2020 (International Energy Agency, 2017)

2.2 Starch crops for ethanol production

Starch-based feedstocks include grains, such as corn and wheat, and tubers such as sweetpotatoes and cassava. These feedstocks contain long complex chains of sugar molecules that can easily be converted to fermentable sugars. The sugar can then be converted to ethanol drop-in fuels. In Europe, wheat is currently the

main starch crop for bioethanol production. About 0.7% of European Union (EU) agricultural land and 2% of Europe's grain supply is used for production of renewable ethanol (EU Biofuels Annual, 2016). After corn and rice, wheat is the most produced crop worldwide (Food and Agriculture Organisation, 2016) with a starch content of about 70%. Wheat is a major food crop but can also be converted to bioethanol. About 2.8 million tonnes of wheat were processed to ethanol in 2014 (European Union Biofuels, 2016).

Corn is the feedstock for more than 90% of ethanol production in the United States of America (USA) due to its abundance and low price with most ethanol produced in the corn-growing states of the Midwest (National Corn Growers Association, 2014). The economic output of the renewable fuels industry in the USA is $184 billion. Ethanol production in the USA supports over 852,000 jobs and $56 billion in wages and generates about $14.5 billion in local and state tax revenue every year (National Corn Growers Association, 2014).

Barley is a winter crop that is planted in rotation with crops such as corn and soybean. It shows potential as a biofuel feedstock, particularly in regions where the market for barley is not so big. 541,000 tonnnes of barley was used as feedstock for ethanol production in Europe in 2014 (European Union Biofuels Annual, 2016). Rye is a rather robust grain that also grows on poorer soils. Its starch content is about 60%. In 2014, 846,000 tonnes served as biofuel feedstock in Europe (European Union Biofuels Annual 2016).

Millet and sorghum species, cassava and sweetpotato can all be used as feedstock for ethanol production. These crops grow well on marginal soils, need

low inputs and improved soil productivity, and can be used in crop rotation systems as an ameliorant. The starch content of these crops are relatively high to produce substantial amounts of ethanol. Ethanol production from millet cultivars and corn was investigated by Wu *et al* (2006). Results showed that the fermentation efficiencies of pearl millet on starch basis were comparable to those of corn and grain sorghum. China is a big promoter of cassava as a biofuel feedstock whilst Thailand uses substantial amounts of cassava for ethanol production (Grow Africa, 2015). The potential of sweetpotato as a feedstock has been investigated for many decades. A study by Duvernay *et al*, (2013) and United States Department of Agriculture (2008), suggested sweet potatoes may offer three times the yield of corn in terms of ethanol per hectare.

2.3 Cassava

Cassava (*Manihot esculenta*) is a perennial woody shrub with an edible root which grows in tropical and subtropical areas of the world. Cassava is consumed as a staple crop in many regions of the developing world. It has become the most important root crop in Ghana and is becoming an increasingly important staple food. Cassava's combined abilities to produce high yields under poor conditions and store its harvestable portion underground until needed makes it a classic "food security crop" (Amarachi *et al*, 2015). Cassava production is increasing due to its ease of cultivation, low maintenance requirement, drought tolerance and ability to provide a root yield over an extended harvest period. The potential of the crop is large because it offers the cheapest source of food calories and the highest yield per unit area (Duvernay *et al*, 2013; Lee *et al*, 2012). It also has multiple roles as a famine reserve, food and cash crop, industrial raw material and livestock feed (Amarachi *et al*, 2015;

Hillocks *et al*, 2002). Cassava roots can be harvested between 6 months and 3 years after planting. It is a root crop of choice to subsistence farmers because it provides food security and cash income when required. It is rich in carbohydrates, calcium, thiamine, riboflavin, niacin, vitamin C and essential minerals. The protein content of cassava is however low compared to the other nutrients (Amarachi *et al*, 2015)

The diversity of secondary products cassava offers, makes it a very useful root crop. However, once harvested, cassava roots are highly perishable and signs of deterioration begin to appear. Cassava is a perishable commodity with a shelf life of less than 3 days after harvest. Due to the high perishability of cassava, early processing of the roots is an inevitable option once they are harvested. Processing involves different combinations of grating, dewatering, drying, soaking, boiling and fermentation of whole or fragmented roots to remove cyanogenic compounds which impart toxicity to the roots. More than 40% of cassava is currently processed, mainly into traditional food products (Amarachi *et al*, 2015). Processing provides a means of producing shelf stable products (thereby reducing losses), adding value and reducing the bulk to be marketed. Apart from its use as food, cassava is very versatile and its derivatives and starch are applicable in many types of products such as foods, confectionery, sweeteners, glues, plywood, textiles, paper, biodegradable products, monosodium glutamate and drugs (Amarachi *et al*, 2015; Hillocks *et al*, 2002). Cassava chips and pellets are used in animal feed and alcohol production (Kleih *et al*, 2013).

Nigeria is the highest cassava producer in the world, producing a third more than Brazil and almost double the production capacity of Thailand and Indonesia (Amarachi *et al*, 2015; Food and Agriculture Organization Statistics, 2016). Ghana

produces about 17 million metric tons of cassava annually (Ministry of Food and Agriculture Statistics, 2016) and ranked the 7th leading producer of cassava in the world (worldlistmania.com, 2016). Cassava is suitable for cultivation in Ghana in large quantities because it is able to grow almost anywhere. Until about a decade ago, cassava was mainly cultivated in Ghana as a subsistent crop. The crop is usually eaten at household levels without much use in industries. The bakery, brewery and plywood industries had used cassava in the past decade but not in significant quantities.

Fresh cassava roots contain about 30% starch and the starch is one of the best fermentable substances for the production of ethanol (Kuiper *et al*, 2007). One litre of ethanol can be produced from 5-6 Kg of fresh roots (containing 30% starch) and 3 Kg of cassava chips (14% moisture content). On a per tonne basis, 1 tonne of fresh cassava roots yield 150 litres of ethanol and 1 tonne of dry cassava chips yields 333 litres of ethanol (Kuiper *et al*, 2007). Under optimal conditions ethanol yield from cassava is the highest of all the main crops for ethanol production (up to 6 t/ha). Moreover, a cassava ethanol plant requires less complex processing equipment resulting in lower investments (Nguyen *et al*., 2007). Cassava roots represent an alternate source of starch that could reduce production costs of ethanol. Low technology is required to produce cassava with higher starch content (85-90% of dry matter), lower protein and mineral content than maize or potato. Some additional advantages of using cassava are that, it is one of the ten most important tropical crops with a high potential yield, lower starch gelatinization temperature and higher amylose solubility compared to maize starch (Lopez-Ulibarri and Hall, 1997).

2.4 Sweetpotato

Sweetpotato (*Ipomoea batatas*) is a dicotyledonous plant that belongs to the morning glory family Convolvulaceae. The large, starchy, sweet-tasting, tuberous root of sweetpotato is a root vegetable. The edible tuberous root is long and tapered, with a smooth skin whose colour ranges between yellow, orange, red, brown, purple and beige. The flesh of sweetpotato ranges from beige through white, red, pink, violet, yellow, orange and purple. Sweetpotato is a native American plant that was the main source of nourishment for early homesteaders and for soldiers during the revolutionary war (International Potato Center, 2017). Of the approximately 50 genera and more than 1,000 species of Convolvulaceae, *Impomoea batatas* is the only crop plant of major importance.

Sweetpotato is cultivated throughout the tropical and warm temperate regions wherever there is sufficient water to support growth. The plant does not tolerate frost. It grows best at an average temperature of 24 °C, abundant sunshine and warm nights. Annual rainfalls of 750–1,000 mm are considered most suitable, with a minimum of 500 mm in the growing season. The crop is sensitive to drought at the tuber initiation stage (50–60 days after planting) and it is not tolerant to water-logging, as it may cause tuber rots and reduce growth of storage roots if aeration is poor. Depending on the cultivar and conditions, tuberous roots mature in 2 to 9 months (International Potato Center, 2017). Sweetpotato has several agronomic characteristics such as drought resistant, high multiplication rate and low degeneration of the propagation material, short growth cycle, low illness incidence and plagues, cover rapidly the soil and therefore protect it from the erosive rains and controlling the weed problem (Cao *et al*, 2011; Duvernay *et al*, 2013). These characteristics account for its wide adaptation to marginal lands.

Sweetpotato is among the most nutritious foods in the vegetable kingdom. Besides simple starches, raw sweet potatoes are rich in complex carbohydrates, dietary fibre and beta-carotene (a provitamin A carotenoid), while having moderate contents of other micronutrients, including vitamin B_5, vitamin B_6 and manganese (International potato center, 2017; Bouvell-Benjamin, 2007). They are packed with calcium, potassium, and vitamins A and C. This is why one colonial physician called them the ″vegetable indispensable″. Nutritionally, sweetpotato greatly outweigh yam. Sweetpotato contains amylases that convert most of its starches into sugars as the potato matures (Dziedzoave *et al*, 2010). This sweetness continues to increase during storage and when they are cooked (Louisiana Sweetpotato Commission, 2014).

Sweetpotato is the 6th principal world food crop (International Potato Center, 2017). Approximately 90% of the world's crop is grown in Asia. China is by far, the world's leading producer of sweetpotatoes and accounted for 81% of global sweetpotato production in 2007 (International Potato Center, 2017). The United States produced less than 1% of the total 2.8 billion tonnes harvested around the world that year (Agricultural marketing resource center, 2013). Sweetpotato is a favourite staple of many cultures and is an ingredient in many ethnic cuisines. In northern United States, sweetpotato is used only as human food. In southern United States, it is far more extensively used in regional cuisines, and a large part of the crop is fed to livestock. Efforts are being made to breed varieties that will inexpensively produce large yields, so that they can be grown specifically for feed or industrial applications (Agricultural marketing resource center, 2013). In developing countries, sweetpotatoes are grown mainly as a substitute for rice and corn (International Potato Center, 2017). Sweetpotato ranks as the fifth most important food crop on a fresh-

weight basis, after rice, wheat, corn and cassava (International Potato Center, 2017). Sweetpotato use has diversified considerably over the last four decades, having great potential as source of local value-added products and ingredients. Some examples include food products like noodles and desserts, animal feed, and some industrial products such as flour, starch and pectin for local and export markets (Agricultural marketing resource center, 2013). In Africa and Asia, dried sweetpotato is used as a substitute for yellow corn animal feeds (Agricultural marketing resource center, 2013). The steady increase in the use of sweetpotato roots and vines for pigs and other livestock in China during the last 30 years means that 30 to 50 million tonnes or more are used annually as animal feed. Sweetpotato flour can be fermented to make products like soy sauce and alcohol or if immediately cooked, it can be further processed into wine, vinegar and "nata de coco", or "on-the-go," a dessert popular in the Philippines and in Japan (Agricultural marketing resource center, 2013).

Sweetpotato is an attractive raw material for fuel ethanol, since up to 4800L ethanol per hectare can be obtained (Lareo *et al*, 2013). Sweetpotato has been considered a promising substrate for alcohol fermentation since it has a higher starch yield per unit land cultivated than grains (Duvernay et al. 2013; Lee *et al*, 2012; Srichuwong *et al*, 2009; Ziska *et al*, 2009). Industrial sweetpotato are not intended for use as a food crop. They are bred to increase its starch content, significantly reducing its attractiveness as a food crop when compared to other conventional food cultivars. They offer potentially greater fermentable sugar yields from a sweetpotato crop for industrial conversion processes and the opportunity to increase planted acreage (even on marginal lands) beyond what is in place for food. It has been reported that some industrial sweetpotato breeding lines developed could produce

ethanol yields of 4500–6500 L/ha compared to 2800–3800 L/ha for corn (Duvernay *et al*, 2013; Ziska *et al*, 2009).

2.5 Starch hydrolysis, fermentation and distillation.

2.5.1 Starch

Starch is a polymeric carbohydrate consisting of a large number of glucose units joined by glycosidic bonds. This polysaccharide is produced by most green plants as an energy store. It is the most common carbohydrate in human diets and it is found in large amounts in staple foods such as potatoes, wheat, maize (corn), rice and cassava (Nelson and Cox, 2008). Most naturally occurring starch granules, regardless of the plant source, occur in two forms; amylose and amylopectin (Perez *et al*, 2009; Bertoft, 2004). The amylose fraction consists of either a single or few chains in which all the D-glucose units are bound by α-(1-4) linkages, thus making the molecule linear or slightly branched. The amylose content of most starches is 20–30% (Bertoft, 2004). The amylose chains are poly-disperse and vary in molecular weight from few thousands to five hundred thousand. Amylose is considered not to be truly soluble in water but forms hydrated micelles that give a blue colour with iodine (Nelson and Cox, 2008). In cassava starch the amylose content is approximately 30-35% of the total starch content (Rolland-Sabate and Dufour, 2012).

Amylopectin, the major component of most starches, consist of large number of short chain carbohydrates that are bond together at their reducing end side by α(1-6)-linkage, which makes this very large polysaccharide extremely branched molecule. The average length of the branches vary from 24 to 30 glucose residues,

depending on the source (Bertoft, 2004; Nelson and Cox, 2008). The backbone α(1-4) glycosidic linkage constitutes about 94-96% of the bonds whilst the α(1-6) branch point linkages constitute only 4-6%. The majority of amylopectin molecules have an external chain length of 10 to 18 and an interior chain length of 5 to 9. Amylopectin yields colloidal or micellar solutions that give a red-violet colour with iodine. Its molecular weight may be as high as 100 million (Nelson and Cox, 2008).

Cellulose is the most abundant cell-wall and structural polysaccharide in the plant world. It has a linear polymer of glucose in β(1-4) linkage. The only chemical difference between starch and cellulose is that starch has α(1-4) linkages whilst cellulose has β(1-4) linkages (Nelson and Cox, 2008). The β-linkages are more stable than α-linkages hence starch tends to be more labile than cellulose (Nelson and Cox, 2008).

2.5.2 Starch Hydrolysis

Starch is the commonest storage carbohydrate in plants. It is the major source of nutrition for the plants themselves, microbes and higher organisms so there is a great diversity of enzymes able to catalyse its hydrolysis. The utilisation of starch and starch derivatives in many industrial processes, particularly food processing, has led to the development of numerous methods of starch processing and breakdown (Butler *et al*, 2004). The use of enzymatic methods in the recent past to hydrolyse starch have largely replaced the use of chemicals (acid hydrolysis). This change is partly due to enzymes being safer and healthier for both the environment and consumers of starch containing products. Enzymes are also better when used for hydrolysis as they perform more specific hydrolysis reactions, give higher yields and also create fewer by-products and consequently require less purification. Acid

hydrolysis is now largely replaced by enzymic processes, as it required the use of corrosion resistant materials, gives rise to high colour and salt content (after neutralisation), needed more energy for heating and was relatively difficult to control (Butler *et al*, 2004; Chaplin, 2014).

The enzymes involved in the breakdown of starch chains are primarily of four types;

- enzymes that hydrolyse (1-4) α-D-glucosidic bonds are referred to as amylases.
- those that hydrolyse (1-6) α-D-glucosidic bonds are called isoamylases.
- those that transfer (1-4) α-D-glucosidic bonds are referred to as glucanosyltransferases.
- branching enzymes called α-(1-4) α-(1-6) transferases.

Amylases can further be divided into three classes as endo-acting α-amylases, the exo-acting β-amylases and isoamylases (Robyt, 2009). The α-amylases are made up of different enzymes from different biological sources such as bacteria, fungi, plants and animals. The α-amylases are not identical, having different product specificities and producing specific kinds of malto-oligosaccharide products (Robyt, 2009).

Amylopectin presents the greatest challenge to hydrolytic enzyme systems between the two components of starch (Bertoft, 2004; Robyt, 2009; Chaplin, 2014). This is due to the residues involved in α-1,6-glycosidic branch points which constitute about 4-6% of the glucose present. Most hydrolytic enzymes are specific for α-1,4-glucosidic links yet the α-1,6-glucosidic links must also be cleaved for complete hydrolysis of amylopectin to glucose. Some of the most impressive recent exercises in the development of new enzymes have concerned debranching enzymes.

The processes involved in converting starch to dextrose are gelatinization, liquefaction and saccharification. Starch is found in nature as insoluble, non-dispersible granules resistant to enzymic breakdown. Starch-bearing grains such as corn, wheat, rye and sorghum must be ground to a fine meal to expose the starch granules to the slurrying water. Gelatinization is the swelling of the starch granule in the presence of heat and excess water. It occurs necessarily and naturally when starchy foods are cooked (Masakuni *et al*, 2014). The starch loses its crystallinity and becomes an amorphous gel that can be attacked by enzymes. At this point, the starch or ground grain slurry thickens considerably and would be difficult to process if an alpha-amylase were not added to partially hydrolyze the starch to dextrins. The dextrin solution is much more fluid and said to be liquefied. The alpha-amylase serves to reduce the viscosity of the solution and also to produce a lower molecular size substrate. This smaller substrate molecule is needed for the efficient action of glucoamylase which hydrolyzes the dextrins to glucose.

The liquefied starch is subsequently saccharified. Saccharification is the hydrolysis of oligosaccharides or dextrins to low molecular weight sugars like glucose and maltose or mixtures of these and their by-products (Kunze, 2004). The hydrolysis is catalysed either by exo-amylases or thermolabile endo-amylases. Unlike liquefaction, saccharification is usually a lengthy process and hence requires the use of large vessels when carried out in a batch-wise process in order to maintain optimum continuous output.

The starch and glucose syrup industry uses the expression, dextrose equivalent (DE), to describe the level of products from starch hydrolysis, where:

$$DE = 100 \times \left(\frac{\text{Number of glycosidic bonds cleaved}}{\text{Initial number of glycosidic bonds present}} \right)$$

In practice, this is usually determined analytically by use of the closely related, but not identical, expression:

$$DE = 100 \times \left(\frac{\text{Reducing sugar, expressed as glucose}}{\text{Total carbohydrate}} \right)$$

Dextrose equivalent (DE) is a measure of the amount of reducing sugars present in a hydrolysate, expressed as a percentage on a dry basis relative to dextrose. The dextrose equivalent gives an indication of the average degree of polymerisation (DP) of starch structure. As a rule of thumb, DE × DP = 120 (Rong *et al*, 2009, Chaplin, 2014). In all glucose polymers, from the native starch to glucose syrup, the molecular chain begins with a reducing sugar, containing a free aldehyde. As the starch is hydrolysed, the polymeric chain become shorter and more reducing sugars are present. Therefore, the DE describes the degree of conversion of starch to dextrose. Thus, DE represents the percentage hydrolysis of the glycosidic linkages present. Pure glucose has a DE of 100, pure maltose has a DE of about 50 (depending upon the analytical methods used) and starch has a DE of zero (Rong *et al*, 2009, Chaplin, 2014)

Commercial enzymes (Table 2.1) used for the industrial hydrolysis of starch are mostly produced by *Bacillus amyloliquefaciens* and *B. licheniformis* (Chaplin, 2014). They differ principally in their tolerance of high temperatures. The maximum DE obtainable using bacterial α-amylases is around 40 but prolonged treatment leads to the formation of maltulose (4-α-D-glucopyranosyl-D-fructose), which is resistant to hydrolysis by glucoamylase and α-amylases. DE values of 8-12 are used in most commercial processes where further saccharification is to occur. The principal

requirement for liquefaction to this extent is to reduce the viscosity of the gelatinised starch to ease subsequent processing.

Various manufacturers use different approaches to starch liquefaction using α-amylases but the principles are the same. Granular starch is slurried at 30-40% (w/w) with cold water, at pH 6.0-6.5, containing 20-80 ppm Ca^{2+} (which stabilises and activates the enzyme) and the enzyme is added (via a metering pump). The α-amylase is usually supplied at high activities so that the enzyme dose is 0.5-0.6 kg $tonne^{-1}$ (about 1500 U kg^{-1} dry matter) of starch. When Termamyl is used, the slurry of starch plus enzyme is pumped continuously through a jet cooker, which is heated to 105ºC using steam. Gelatinisation occurs very rapidly and the enzymatic activity, combined with the significant shear forces, begin the hydrolysis. The residence time in the jet cooker is very short. The partly gelatinised starch is passed into a series of holding tubes maintained at 100-105ºC and held for 5 min to complete the gelatinisation process. Hydrolysis to the required DE is completed in holding tanks at 90-100ºC for 1 to 2 hours. These tanks contain baffles to prevent back-mixing. Similar processes may be used with *B. amyloliquefaciens* α-amylase but the maximum temperature of 95ºC must not be exceeded. This has the drawback that a final 'cooking' stage must be introduced when the required DE has been attained in order to gelatinise the residual starch grains present in some types of starch which would otherwise cause cloudiness in solutions of the final product (Chaplin, 2014).

Table 2.1 Enzymes used in starch hydrolysis.

Enzyme	EC number	Source	Action
α-Amylase	3.2.1.1	*Bacillus amyloliquefaciens*	Only α-1,4-oligosaccharide links are cleaved to give α-dextrins and predominantly maltose (G2), G3, G6 and G7 oligosaccharides
		B. licheniformis	Only α-1,4-oligosaccharide links are cleaved to give α-dextrins and predominantly maltose, G3, G4 and G5 oligosaccharides
		Aspergillus oryzae, *A. niger*	Only α-1,4 oligosaccharide links are cleaved to give α-dextrins and predominantly maltose and G3 oligosaccharides
Saccharifying α-amylase	3.2.1.1	*B. subtilis* (*amylosacchariticus*)	Only α-1,4-oligosaccharide links are cleaved to give α-dextrins with maltose, G3, G4 and up to 50% (w/w) glucose
β-Amylase	3.2.1.2	Malted barley	Only a α-1,4-links are cleaved, from non-reducing ends, to give limit dextrins and β-maltose
Glucoamylase	3.2.1.3	*A. niger*	α-1,4 and α-1,6-links are cleaved, from the non-reducing ends, to give β-glucose
Pullulanase	3.2.1.4	*B. acidopullulyticus*	Only α-1,6-links are cleaved to give straight-chain maltodextrins

Source: Chaplin, 2014.

2.5.3 Fermentation

Fermented foods are those foods that have been subjected to the action of microorganisms or enzymes so that desirable biochemical changes cause significant modification in the food. The fermentation process may make the food more nutritious or digestible or may make them safer or tastier (Bamforth, 2005). Fermented foods have been produced for many centuries with the basic aim of increasing the storage stability of processed foods and modifying the organoleptic and textural properties of food materials. Fermented foods are very popular as substantial percentage of foods consumed daily are fermented. Notable examples of fermented foods include yoghurt, cheese, wine, beer, cider and pickles. The main

reason for their popularity, however, is their specific organoleptic properties rather than their preservation stability (Soccol *et al*, 2013).

Fermentation is in fact a complex series of conversions that brings about the conversion of sugar to CO_2 and alcohol (Nelson and Cox, 2008; Yobrew.co.uk, 2016). Fermentation is a metabolic process that is facilitated by yeasts and bacteria. Fermentation is used more broadly to refer to the bulk growth of microorganisms on a growth medium, often with the goal of producing a specific chemical product. Alcoholic fermentation is the conversion of sugar into carbon dioxide gas (CO_2) and ethyl alcohol. Fermentation is carried out by yeast cells using a range of enzymes. The reactions within the yeast cells which make this happen are very complex but the overall process is as follows:

$C_6H_{12}O_6$ (glucose) $\rightarrow$ 2 C_2H_5OH (ethyl alcohol) + $2CO_2$ (carbon dioxide gas) + Energy.

From the above, one mole of glucose is converted into two moles of ethanol and two moles of carbon dioxide but in reality it is far from this simple. In addition to CO_2 and alcohol, the sugar is incorporated into other by-products such as yeast biomass, acids (pyruvic, acetaldehyde, ketoglutaric, lactic) and glycerol. The efficiency of the yeast and fermentation conditions such as temperature alters the proportions of various by-products (Kunze, 2004; Nelson and Cox, 2008). This knowledge is used by wine makers to get fuller bodied wines by brewing in conditions that causes fermentation to produce more of the by-product glycerol (Yobrew.co.uk, 2016).

Several enzymes, produced by yeast cells, are involved in the process of converting sugar into carbon dioxide and ethanol. The final step is an amylase reduction reaction which takes the end product of the other enzymes

(acetaldehyde/glycerol), and turns this into ethyl alcohol. High concentrations of ethanol actually inactivate enzymes and kill the yeast cell. Different strains of yeast can tolerate different concentrations of alcohol. Brewer's yeast cannot tolerate ethanol beyond 5 or 6% by volume (Nelson and Cox, 2008). Wine yeast is more tolerant at a range of 10-15%. Distiller's yeast can tolerate ethanol levels up to 21% (Nelson and Cox, 2008; Yobrew.co.uk, 2016).

2.5.4 Distillation

Distillation is a process of separating a liquid component of interest from a liquid mixture by selective evaporation and condensation. Distillation is a widely used method for separating liquid mixtures based on differences in the boiling point temperatures. To separate a mixture of liquids with different boiling temperature, the liquid can be heated to evaporate components, at their boiling points, into the vapour phase. The vapour is then condensed back into liquid form and collected (Freudenberger, 2009). Repeating the process on the recovered liquid to improve the purity of the product is called double distillation. Distillation may result in essentially complete separation (nearly pure components), or it may be a partial separation that increases the concentration of selected components of the mixture. In either case the process exploits differences in the volatility of the mixture's components. Distillation is a unit operation of universal importance, but it is a physical separation process and not a chemical reaction (Vollhardt and Schore, 2014; McMurry, 2015).

Commercially, distillation has many applications. For example:

- In the fossil fuel industry distillation is a major class of operation in obtaining materials from crude oil for fuels and for chemical feed stocks.

- In the field of industrial chemistry, large ranges of crude liquid products of chemical synthesis are distilled to separate them, either from other products, or from impurities, or from unreacted starting materials.

- Distillation of fermented products produces distilled beverages with a high alcohol content, or separates out other fermentation products of commercial value.

2.6 Developments in ethanol production from cassava roots and sweetpotato tubers.

Major food crops that are currently used globally for bioethanol production are corn, sugar cane and wheat (Boundy *et al*, 2011; Vollhardt and Schore, 2014; McMurry, 2015). It is, however, reported that cassava and sweetpotato are ideal for ethanol production because of their high yields and characteristic high starch content, therefore producing high amounts of ethanol per tonne of roots and tubers (Lareo *et al*, 2013). Cassava roots and sweetpotato tubers have higher starch yield per unit land cultivated than grains (Duvernay *et al*, 2013; Lee *et al*, 2012; Srichuwong *et al*, 2009; Ziska *et al*, 2009). Relatively low technology is required to produce cassava with higher starch content (85-90% of dry matter), lower protein and mineral content than maize or potato (Lopez-Ulibarri *et al*, 1997). Some additional advantages of using cassava as raw material for ethanol production are that, it is one of the ten most important tropical crops with a high potential yield, lower starch gelatinization temperature and higher amylose solubility compared to maize starch (Lopez-Ulibarri *et al*, 1997). Above all these, cassava ethanol processing plant requires less complex processing equipment resulting in lower investments (Lopez-Ulibarri *et al*, 1997).

Several investigations in the recent past using cassava and sweetpotato as raw materials for bioethanol production suggests that the crops could be used to produce commercially viable ethanol (Ocloo and Ayernor, 2010; Swain *et al*, 2013). Oyeleke *et al* (2012) reported that ethanol could be profitably produced from cassava and sweetpotato peels. Studies have also revealed that fermentation time, co-culturing of yeast strains and pH of the fermentation medium could all have effect on ethanol yield with cassava and sweetpotato as raw material for bioethanol production process (Ocloo and Ayernor, 2010; Swain *et al*, 2013). High yields of ethanol could be achieved from cassava varieties with high starch content, high dry matter, low fiber and low protein content (Ademiluyi *et al*, 2013).

Though Foust *et al*, (2009) reported that there is no distinct difference between the economic and environmental impact, biochemical and thermochemical gasification process for second generation ethanol production, the best option for processing ethanol in developing countries is by biochemical method. The selection of this method is based on the relatively easy technologies available to produce high starch content cassava and sweetpotato as raw materials for the ethanol production. The relatively low cost of investments of the ethanol processing plant and low energy requirements for running the processing plant are other factors which make the biochemical method a better option compared to the thermochemical method.

The starch content of starchy raw materials for ethanol production, conditions of starch hydrolysis, amount of fermentable materials in the wort during mashing and the fermentation conditions determine the yield of ethanol (Kunze, 2004). The various investigations on the use of cassava and sweetpotato identified some factors which could optimize the yield of ethanol from cassava and sweetpotato (Archibong

et al, 2016; Swain *et al*, 2013; Oyeleke *et al*, 2012; Ademiluyi *et al*, 2013; Ocloo and Ayernor, 2010). Nonetheless, varietal selection of cassava and sweetpotato which is a key factor to consider when processing ethanol have not been investigated in Ghana for optimum ethanol production. Movement of crop varieties from one geographical location (country) to another could be disastrous as the crops in most of the cases do not survive the new environments due to diseases and other factors. Much of the investigations regarding use of cassava and sweetpotato also rely on yeast strains (*Saccharomyces cerevisiae*) that could not tolerate high alcohol levels during fermentation. Another important issue is about dextrinization of starch molecules during starch hydrolysis. Generally, amylases cannot survive the high gelatinization temperatures of starches in most crops. There is therefore the need to explore dextrinization enzymes at the cooking stage to effect thinning of the mash to enable saccharifying enzymes to break up dextrins into simple sugars. The current work therefore is aimed at investigating suitable cassava and sweetpotato varieties, use of improved and thermostable amylases and high ethanol tolerant yeast strains for the fermentation process. Successful optimization of the conditions of ethanol production from cassava and sweetpotato would make ethanol production from these crops a viable venture.

CHAPTER THREE

OPTIMISATION OF THE CONDITIONS FOR ETHANOL PRODUCTION FROM CASSAVA AND SWEETPOTATO

3.1 Introduction

Cassava and sweetpotato have great potential as raw materials for the production of ethanol. However, there is the need to establish the right conditions for production. Ethanol quality and yield depends on saccharification and fermentation conditions (Kunze, 2004). The proportion of fermentable sugars is determined by the variable activity of enzymes, the mashing time and temperature and the mashing pH. Fermentation factors to consider are the type of yeast, the temperature and the duration for fermentation (Kunze, 2004).

Most of the world's ethanol production is based on production from corn (Boundy *et al*, 2011; Vollhardt *et al*, 2014; McMurry, 2015). The systems for this production have been designed and improved upon over the years with Brazil, United States of America (USA) and European countries as the leaders of this industry. Several studies in the recent past had proven that ethanol could be produced from cassava and sweetpotato. For instance, investigations by Lareo *et al*, (2013) and Ziska *et al*, (2009), show that cassava and sweetpotato yield two to three times as much carbohydrate for ethanol production as field corn. The search, however, for systems to adequately control the conversion process of starches in cassava and sweetpotato to obtain maximum yields of desirable products has been the focus of most research activities (Dziedzoave, 2004). The effects of temperature, substrate and enzyme concentrations and types, saccharification and fermentation durations have been reported by Ocloo and Ayernor (2010); Swain *et al*, (2013); Oyeleke *et al*, (2012); Archibong *et al*, (2016) and Chutima *et al*, (2014).

The various investigations identified some production conditions which when addressed could optimize the yield of ethanol from cassava and sweetpotato. Most of the investigations rely on yeast strains, *Saccharomyces cerevisiae*, that could not tolerate high alcohol levels during fermentation. Another important issue was about dextrinization of starch molecules during starch hydrolysis. Since most amylases cannot survive high cooking temperatures of starch, there is need to explore dextrinization enzymes which can tolerate the cooking stage to allow for thinning of the mash which would enable the hydrolytic enzymes flow easily in solution to break up starch molecules for easy saccharification. Thus the objectives of this study were;

- to determine the optimum time regime for starch degradation and fermentation of cassava and sweetpotato wort to produce ethanol.
- to determine the best yeast strain for optimum fermentation and subsequent ethanol yield.
- to assess the synergic effect of mono and mixed enzyme on starch hydrolysis and subsequent ethanol yield from cassava and sweetpotato.

3.2 Materials and Methods

3.2.1 Materials

Processed Sika Bankye, Ampong, Apomuden and Tuskiki flour samples were used for the study. Enzymes used for the work were starch degrading enzymes; *Liquozyme SC DS, Viscozyme L and Spirizyme Fuel* supplied by Novozymes, Denmark. Yeast types used for the work were Bio-Ferm XR (unique yeast strain of *Saccharomyces cerevisiae)* produced by Lallemand, Georgia, USA and Baker's Yeast (*Saccharomyces cerevisiae*) bought from the grocery shop in Accra.

3.2.2 Experimental design

The Response Surface Methodology model was used to study the conditions for the optimization of the saccharification and fermentation processes (Liquefaction time, Saccharification time, Saccharification Enzyme type, Fermentation time, Fermentation temperature, Yeast type). The detailed model designs are shown in Tables 3.1 and 3.2. The liquefaction, saccharification and fermentation times were selected based on preliminary work. The fermentation temperatures were selected based on the yeast manufacturer's instruction for optimum activity.

Table 3.1: Response surface model design for liquefaction and sacharification.

Run order	Liquefaction time (hours)	Saccharification time (hours)	Saccharifying enzyme*
1	2	3	Viscozyme
2	2	3	Viscozyme
3	2	5	Viscozyme
4	2	1	Viscozyme
5	3	3	Viscozyme
6	2	3	Viscozyme
7	1	3	Viscozyme
8	3	5	Viscozyme
9	1	5	Viscozyme
10	2	3	Viscozyme
11	2	3	Viscozyme
12	3	1	Viscozyme
13	1	1	Viscozyme

*The same design was used to run the experiment for two other saccharifying enzymes; spirizyme and combination of spirizyme and viscozyme in 1:1 ratio.

Table 3.2: Response surface model design for fermentation.

Run order	Fermentation time (hour)	Fermentation temperature (°C)	Yeast type*
1	60	34	Bio-Ferm XR
2	72	34	Bio-Ferm XR
3	72	30	Bio-Ferm XR
4	60	32	Bio-Ferm XR
5	72	32	Bio-Ferm XR
6	60	32	Bio-Ferm XR
7	48	34	Bio-Ferm XR
8	48	32	Bio-Ferm XR
9	60	32	Bio-Ferm XR
10	48	30	Bio-Ferm XR
11	60	30	Bio-Ferm XR
12	60	32	Bio-Ferm XR
13	60	32	Bio-Ferm XR

*The same design was used to run the experiment for Baker's Yeast.

3.2.3 Preparation and analysis of samples

Fifty grams (50 g) of cassava/sweetpotato flour sample was weighed and transferred into 500 mL Erlenmeyer flask. Distilled water was added (about 150 mL) to the flour and mixed, after which more distilled water was added to make a slurry of 250 mL. The pH of the sample was taken and recorded. *Liquozyme SC DS* enzyme (3.0 mL) was then added to the mixture, stirred gently and heated on water bath, Grant OLS 200, (Grant Instruments, Cambridge, UK) at speed of 100 strokes/min at 85°C for various times (1, 2, 3 hours). The mixture was then cooled to a temperature of 48°C. *Viscozyme L and Spirizyme Fuel* enzymes (3.0 ml) were then added separately as well as addition of *Viscozyme L and Spirizyme Fuel* together (1.5 ml each) and the mixtures maintained on the water bath for various times (1, 3, 5 hours). The Brix of the sample extract was determined using a refractometer at the end of the saccharification period. Iodine test was also carried out after every 30 min from the start of the saccharification process. The sample was then cooled to various

temperatures (30°C, 32°C, 34°C) and 0.1 g yeast (Bio-Ferm XR, Baker's Yeast) added to the mixture and fermented for various times (48, 60, 72 hours) in an Erlenmeyer flask. The brix of the fermented sample was taken after the fermentation period. The efficiency of the various fermentation (Attenuation) regimes was determined using the formula;

$$A = \left(\frac{OE - AE}{OE}\right) x\ 100$$

where A = Attenuation or Efficiency of fermentation, OE is original extract (extract before fermentation) and AE is the apparent extract (extract after fermentation). The Minitab version 17 software was used to carry out regression analysis of the experimental data and contour graphs were plotted. The Contour Plots were used to determine optimal conditions of the variables.The best predicted extract values from the contour plots were then validated with test runs using the predicted parameters.

3.3 Results and Discussion

Figures 3.1 to 3.3 show the contour plots of extracts from flour samples after saccharification when viscozyme and spirizyme were used as saccharifying enzymes individually and used together. Figures 3.1 to 3.3 show the optimum extract yields observed after liquefaction and saccharification for the various enzymes used. The optimum extract yields observed for Viscozyme was 15.0 °Bx obtained for liquefaction time of 2.5 hours and saccharification time of 4.3 hours. The optimum extract yield observed for Spirizyme was 15.0 °Bx for liquefaction time of 2.5 hours and saccharification time of 4.7 hours. The optimum extract yield observed for the combination of Viscozyme and Spirizyme was 15.4 °Bx for liquefaction time of 2.1 hours and saccharification time of 4 hours. The amount of extract in a solution indicates how efficient the hydrolytic enzymes are able to break down starch into

simple sugars. The amount of extract in a mash or solution gives an idea about how much fermentable sugars are in the solution which eventually relates to amount of ethanol yield (Kunze, 2004). The combination of viscozyme and spirizyme for saccharification resulted in higher extract yield production in less time compared to using Viscozyme and Spirizyme individually.

Confirmatory tests were run on the best saccharifying times for the various enzymes to validate the results. The results as presented in Table 3.5 indicate that the observed extracts were comparable to those predicted by the contour plots. It could be said therefore that Viscozyme/spirizyme for saccharification was the best enzyme to use in this study. The best enzyme selected for the saccharification process in subsequent experiments in this study was therefore combination of Viscozyme and Spirizyme (1:1) for duration of 4 hours after liquefaction time of 2.1 hours using liquozyme.

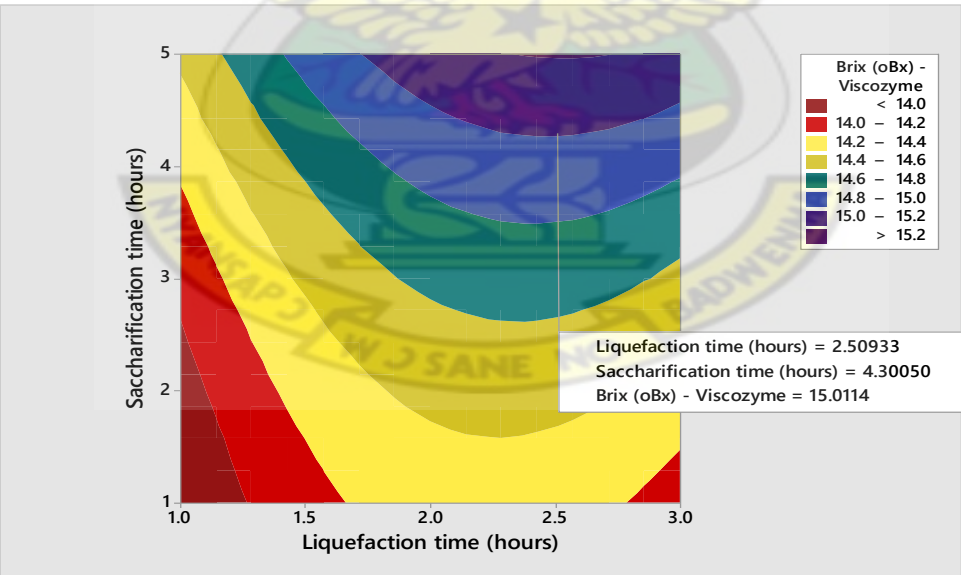


Figure 3.1: Extract (^{o}Bx) after liquefaction and saccharification with Viscozyme.

The regression equation; Brix (^{o}Bx) = 12.633 + 1.431 (Lt) + 0.007 (St) – 0.333 $(Lt)^2$ + 0.0168 $(St)^2$ + 0.05 (Lt*St).

where Lt = Liquefaction time and St = Saccharification time.

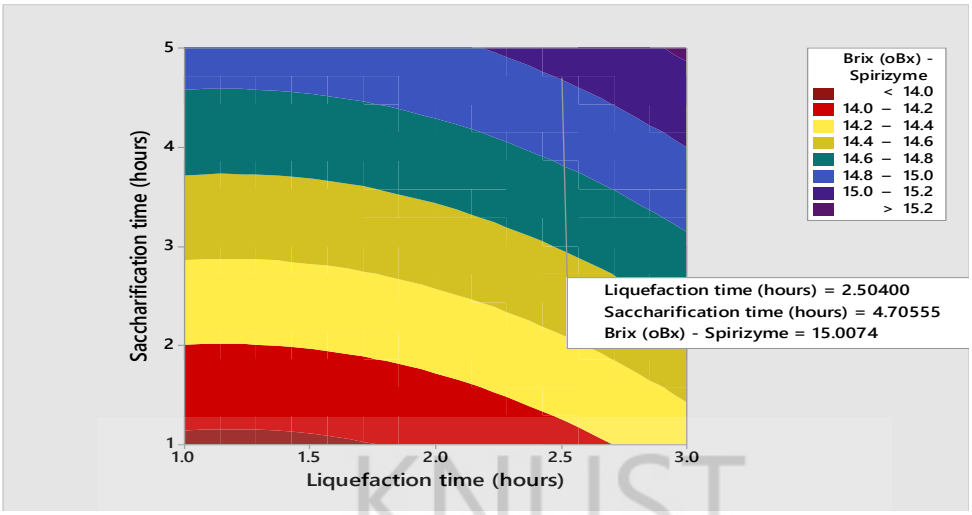


Figure 3.2: Extract (°Bx) after liquefaction and saccharification with Spirizyme.

The regression equation; Brix (°Bx) = 13.867 - 0.233 (Lt) + 0.2333 (St) + 0.1 $(Lt)^2$ - 0.00 $(St)^2$ - 0.00 (Lt*St).

where Lt = Liquefaction time and St = Saccharification time

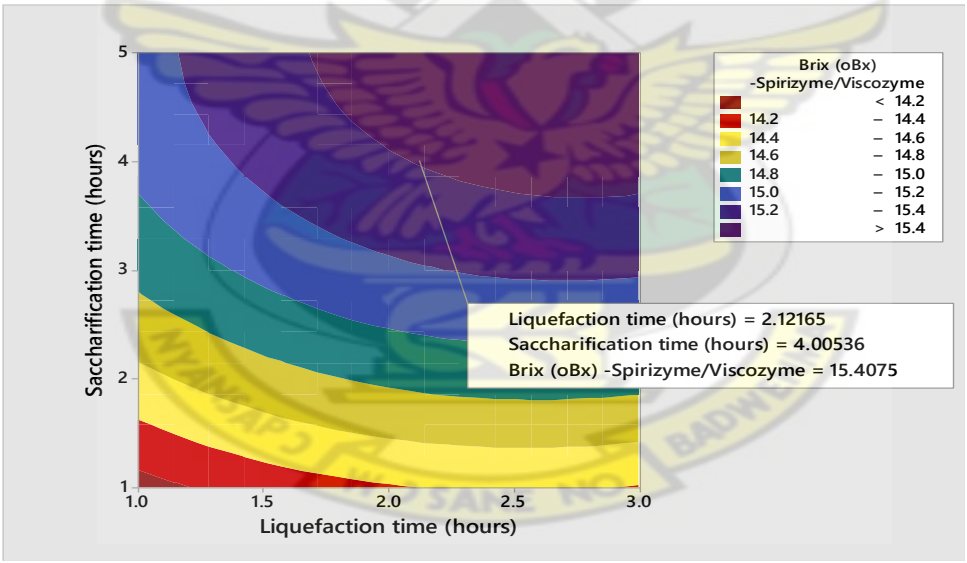


Figure 3.3: Extract (°Bx) after liquefaction and saccharification with mixture of Viscozyme and Spirizyme.

The regression equation; Brix (°Bx) = 13.091 + 0.626 (Lt) + 0.569 (St) - 0.129 $(Lt)^2$ - 0.0573 $(St)^2$ + 0.025 (Lt*St).

where Lt = Liquefaction time and St = Saccharification time

Table 3.5 Extract yield confirmatory test compared with predicted extract.

	Parameter			
Saccharifying enzyme	Liquefaction time (hours)	Saccharification time (hours)	Predicted Brix (^oBx)	Actual Brix (^oBx)
Viscozyme	2.5	4.3	15.0	14.90 ± 0.12
Spirizyme	2.5	4.7	15.0	14.91 ± 0.08
Viscozyme/Spirizyme	2.1	4.0	15.4	15.20 ± 0.10

Fermentation optimisation with Bio-Ferm XY and Baker's yeast results are as presented in Figures 3.4 and 3.5. The optimum fermentation efficiency observed for Bio-Ferm XY from the contour plot was 80% at 34^{o}C for duration of 57.4 hours and that for Baker's yeast was 75% at 31.3^{o}C for a duration of 62.8 hours. Confirmatory tests were conducted with the best temperature and time to validate the predicted fermentation efficiencies for the yeast samples employed. The observed fermentation efficiencies of the yeast were comparable to those predicted from the contour plots (Table 3.6). Alcoholic fermentation is the conversion of sugar into ethanol and carbon dioxide gas. The reaction is carried out using a range of enzymes. The efficiency of the yeast and fermentation conditions alter the proportions of the various products (Kunze, 2004; Nelson and cox, 2008). The most efficient yeast for fermentation from the results is therefore Bio-Ferm XY at temperature of 34^{o}C and fermentation time of 57.4 hours.

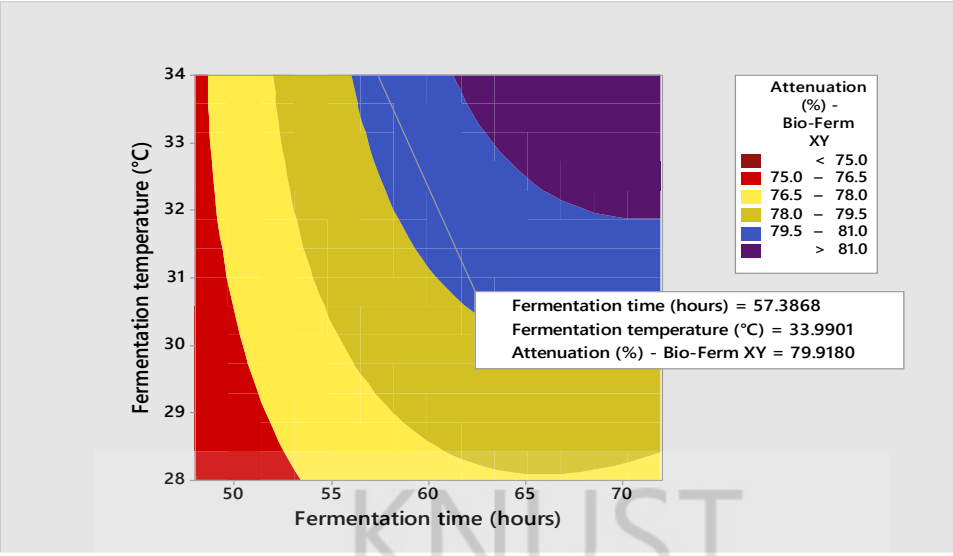


Figure 3.4: Fermentation efficiency (%) as a function of fermentation time and temperature with Bio-Ferm XY yeast.

The regression equation; Attenuation (%) = 37 + 0.51 (Fh) + 0.88 (Fc) - 0.00929 $(Fh)^2$ - 0.031 $(Fc)^2$ + 0.0254 (Fh*Fc).

where Ft = Fermentation time and Fc = Fermentation temperature

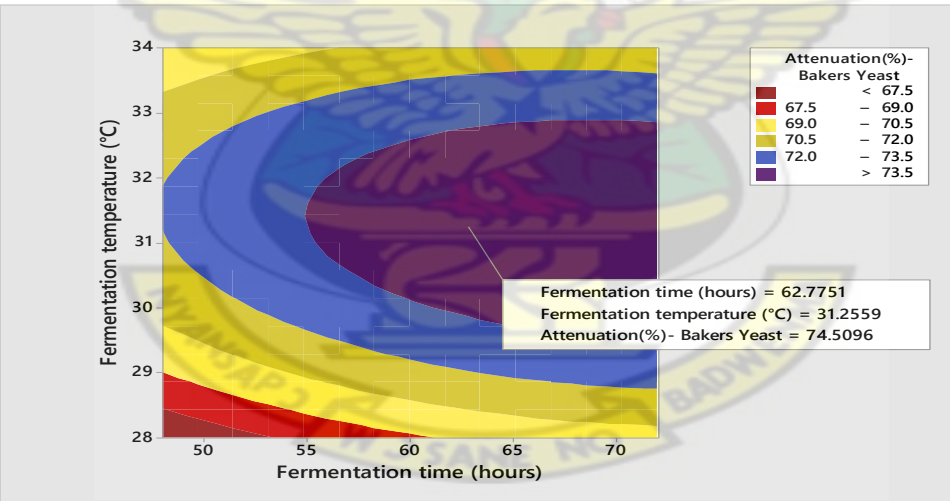


Figure 3.5: Fermentation efficiency (%) as a function of fermentation time and temperature with Baker's yeast.

The regression equation; Attenuation (%) = -452.9 + 1.206 (Fh) + 31.1 (Fc) - 0.00552 $(Fh)^2$ - 0.4830 $(Fc)^2$ - 0.0136 (Fh*Fc).

where Fh = Fermentation time and Fc = Fermentation temperature

Table 3.6 Fermentation efficiency confirmatory test compared with predicted efficiency.

	Parameter			
Yeast type	Fermentation time (hours)	Fermentation temperature (^oC)	Predicted fermentation efficiency (%)	Actual fermentation efficiency (%)
Bio-Ferm XR	57.4	34.0	80	79 ± 0.6
Baker's yeast	62.8	31.3	75	74 ± 0.8

3.4 Conclusion

The results of the study indicate that the best liquefaction time is 2.1 hours using *Liquozyme SC DS* enzyme. The combination of *Viscozyme L and Spirizyme* in a ratio of 1:1 was the best enzyme for saccharification for a duration of 4 hours. The best yeast to employ for best fermentation efficiency was Bio-Ferm XR at a temperature of 34 ^oC for 57.4 hours duration.

CHAPTER FOUR

EFFECT OF MATURITY OF CASSAVA AND SWEETPOTATO ON ETHANOL YIELD

4.1 Introduction

Cassava and sweetpotato are considered industrial crops because they could be used as raw materials for commercial ethanol production (Grow Africa, 2015; Papong and Malakul, 2010; Roehr, 2001; Chutima *et al*, 2014; Ocloo and Ayenor, 2010; Lareo *et al*, 2013; Sanette and Tando, 2013). It is also reported that cassava and sweetpotato have higher starch yield per unit land cultivated than grains (Duvernay *et al*, 2013; Lee *et al*, 2012; Srichuwong *et al*, 2009; Ziska *et al*, 2009). Fresh cassava roots contain about 30% starch and 1 L of ethanol can be made from 5-6 Kg of fresh roots (containing 30% starch) and 3 Kg of cassava chips (14% moisture content). On a per tonne cassava basis, 1 tonne of fresh cassava roots yields 150 litres of ethanol and 1 tonne of dry cassava chips yields 333 litres of ethanol (Kuiper *et al*, 2007). Sweetpotato has been considered as a promising substrate for alcohol fermentation since it has a higher starch yield per unit land cultivation than grains (Duvernay *et al*, 2013; Lee *et al*, 2012; Lareo *et al*, 2013).

Cassava roots can be harvested between 6 months and 3 years after planting. It is a root crop of choice to subsistence farmers because it provides food security and cash income when required (Amarachi *et al*, 2015). Cassava is rich in carbohydrates and the content ranges from 32-35 % in fresh weight and about 80-90 % in dry matter, making it a good source of energy (Amarachi *et al*, 2015). Depending on the cultivar and conditions, tuberous roots of sweetpotato mature in 2 to 9 months. Most of the macronutrients such as carbohydrates, fat and protein are higher in unpeeled cassava roots than in peeled roots (Amarachi *et al*, 2015). The amount of fermentable

carbohydrates available in cassava root and sweetpotato tuber depends on the variety and growth conditions of the crops (Teerawanichpan et al, 2008). The maturity periods could therefore have some effects on the fermentable carbohydrates in the cassava and sweetpotato varieties which could have direct relation with amount of ethanol yield. The studies in this chapter therefore aimed specifically at:

- evaluating the physico-chemical composition of the cassava and sweetpotato varieties harvested at different maturity periods.
- determining the ethanol yields from the two varieties of cassava and sweetpotato harvested at various maturity periods.
- establishing the best variety of cassava and sweetpotato for processing ethanol.
- establishing the best maturity periods of cassava and sweetpotato for ethanol processing.

4.2 Materials and Methods

4.2.1 Materials

Two varieties of cassava (Sika bankye and Ampong) and two varieties of sweetpotato (Apomuden and Tuskiki) cultivated at Caltech Ventures Ltd farms, Ho, in the Volta region and Mantsi, in Greater Accra region, respectively, were used for the study. The cassava roots were harvested at 8, 10 and 12 months and the sweetpotato was harvested at 3, 4 and 5 months and processed for the study. The enzyme samples *Spirizyme Fuel, Liquozyme SC DS and Viscozyme L* were supplied by Novozymes, Denmark for the study. The yeast strains used for the work were Bio-Ferm XY (Lallemand) and Baker's Yeast.

4.2.2 Methods

4.2.2.1 Processing of cassava after harvest

The cassava varieties cultivated were harvested at 8, 10 and 12 months maturity dates and processed using three (3) different methods and two (2) different treatments.

Method 1: The freshly harvested cassava was washed and weighed, moisture content determined, peeled or unpeeled, sliced (average of 2 mm thick), blanched for 10 min (using steam), dried at 62 ^{o}C in a forced air oven dryer for 6 hours, milled, starch content determined, proximate analysis carried out, visco-amylograph analysis and other physicochemical properties determined. Two sets of blanched cassava flour samples (peeled and unpeeled) were obtained for ethanol production.

Method 2: The freshly harvested cassava was washed and weighed, moisture content determined, peeled or unpeeled, sliced (average of 2 mm thick), dried in a forced air oven tray dryer for 6 hours at 62 ^{o}C, milled, starch content determined, proximate analysis done, visco-amylograph analysis and other physicochemical properties determined. Two sets of High Quality Cassava Flour samples (peeled and unpeeled) were obtained for ethanol production.

Method 3: The freshly harvested cassava was washed and weighed, moisture content determined, peeled or unpeeled, sliced (average of 2 mm thick), dried in a solar tent dryer for four (4) days at an average temperature of 50 ^{o}C, milled, starch content determined, proximate analysis carried out, visco-amylograph analysis and other physicochemical properties determined. Two sets of slightly fermented cassava flour samples were obtained for ethanol production.

4.2.2.2 Processing of sweetpotato after harvest

The two (2) sweetpotato varieties were harvested at 3, 4 and 5 months maturity and processed using three (3) different methods.

Method 1: The freshly harvested sweetpotato was washed and weighed, moisture content determined, sliced (average of 2 mm thick), blanched for 10 minutes (using steam), dried at 62 ^{o}C in a forced air oven tray dryer for 6 hours, milled, starch content determined, proximate analysis done, visco-amylograph analysis and other physicochemical properties determined. Blanched sweetpotato flour samples were obtained for ethanol production.

Method 2: The freshly harvested sweetpotato was washed and weighed, moisture content determined, sliced (average of 2 mm thick), dried in a forced air oven tray dryer for 6 hours at 62^{o}C, milled, starch content determined, proximate analysis done, visco-amylograph analysis and other physicochemical properties determined. High Quality Sweetpotato Flour (HQSF) samples were obtained for ethanol production.

Method 3: The freshly harvested sweetpotato was washed and weighed, moisture content determined, sliced (average of 2 mm thick), dried in a solar tent dryer for 4 days at an average temperature of 50^{o}C, milled, starch content determined, proximate analysis done, visco-amylograph analysis and other physicochemical properties determined. Slightly fermented sweetpotato flour samples were obtained for ethanol production.

4.2.2.3 Starch content determination (Litner's method)

Five grams (5 g) of cassava flour was triturated with 10 mL of water, and 20 mL hydrochloric acid (sp.gr.1.15) added in small portions. The mixture was washed into a 100 mL volumetric flask with hydrochloric acid (12% w/w HCl) and 5 mL of

5% phosphotungstic acid added to precipitate proteins and the volume made up to 100 mL with 12% hydrochloric acid. The mixture was shaken, filtered and the optical rotation of the filtrate was measured in a 200 mm tube. The mean specific rotation of starch was taken as +200.

$$\text{Starch (\%)} = \frac{2000 \text{ x optical rotation}}{\text{Specific rotation}}$$

4.2.2.4 Moisture content determination

The moisture content of the samples were determined using AOAC, 1990 method (Helrich, 1990). The freshly harvested cassava/sweetpotato roots/tubers were randomly selected, washed and sliced into 2 mm thick pieces after which 5 g sample was weighed and placed in pre-heated metal dish. The dish with the contents was dried for 4 hours in an oven (Nickel-Electro Ltd, North Somerset, England) provided with opening for ventilation and maintained at 101°C. The samples after 4 hours of drying were transferred into a desiccator. The samples were then weighed after cooling to room temperature (31^{o}C). The loss in weight was recorded as moisture. The moisture was calculated using the formula;

$$\text{Moisture (\%)} = \frac{(\text{weight of dish + fresh sample}) - (\text{weight of dish + dried sample})}{(\text{weight of dish + fresh sample}) - (\text{weight of dish})} x100$$

4.2.2.5 Protein Content determination

The protein content of the samples were determined using AOAC, 1990 method (Helrich, 1990). The method follows the application note for the determination of nitrogen using the Kjeltec 8400 Analyser Unit, Foss Analytical Co. Ltd, Sweden. 5.0 g of the cassava/sweetpotato flour sample was weighed unto a piece of filter paper and placed in 250 ml digestion tube. A controlled sample was

prepared by weighing 0.20 g Lysine dihydrochloride into a weighing boat and transferred into a digestion tube as done for the samples. A blank sample was prepared by weighing 0.1 g ammonium sulphate into a weighing boat, transferred into a digestion tube and 80 ml of water added. One Kjeltab Cu 3.5 and 15 ml concentrated H_2SO_4 were added to the blank, control sample and test sample and shook gently to "wet" the sample with the acid. The aspirator was turned on and the samples digested until they turned greenish-blue. The digested samples were then left to cool to room temperature (31^oC). The cooled digested sample was diluted with 80 ml distilled water followed by addition of 80 ml NaOH to neutralize the solution. The sample is then placed in the Kjedal distillation apparatus for distillation. Boric acid (4%) is placed in a receiver flask with 2 drops of methyl indicator to receive the distillate. 150 ml of the distillate was collected. The distillate was titrated with 0.1 M HCl until a pink end-point was achieved. The volume of acid consumed (titre) in the titration was recorded. The Nitrogen (%) was calculated using the formula;

$$\text{Nitrogen (\% w/w)} = \frac{[(T - B)x\ N \times 14.007]}{10 \times \text{Weight of sample}}$$

where T = Titration volume for sample, B = titration volume for blank and N = Normality of acid.

Crude protein was calculated using the formula,

Crude protein (% w/w) = Nitrogen (%) x 6.25.

4.2.2.6 Fat content determination

The fat was determined in accordance with AOAC 920.39C, 2000 method (Horwitz, 2000) using Soxtherm equipment by Gerhardt GmbH & Co. KG, Konigswinter, Germany. 5.0 g of the cassava/sweetpotato flour sample was weighed

unto a filter paper, folded and placed into a thimble. The thimble was placed in the extractor and 240 ml of petroleum ether added to a pre-weighed flask. The sample was extracted for 12 hours at a condensation rate of 8 drops per second. The solvent was evaporated and distilled. The sample was then dried in the oven (Nickel-Electro Ltd, North Somerset, England) at 101^{o}C for 1 hour. The sample was cooled in the desiccator and weighed. Blank determinations were done by evaporating 240 ml petroleum ether and drying as in actual determination of samples. The Fat (%) was calculated using the formula;

$$\text{Fat (\%)} = \frac{[\{(\text{weight of flask} + \text{fat}) - (\text{weight of empty flask})\} - \text{Blank}]}{\text{weight of sample}} \text{ x } 100$$

where Blank = weight of flask without sample after extraction – weight of empty flask

4.2.2.7 Ash content determination

The ash content of the samples were determined using AOAC, 2000 method of analysis with Thermconcept furnace, Thermconcept GmbH, Bremen, Germany. Crucibles were placed in the furnace ignited at 550°C for 20 minutes and cooled in a desiccator to room temperature. 5 g of the cassava/sweetpotato flour sample was placed in the pre-heated crucibles. The furnace was ignited at 550°C for 8 hours. The temperature of the furnace was allowed to drop to 250°C by stopping it after 8 hours. The crucible was then transferred to a desiccator. The crucible was weighed soon after reaching room temperature (31^{o}C). The ash content was calculated using the formula;

$$\text{Ash (\%)} = \frac{(\text{weight of crucible} + \text{ash}) - (\text{weight of empty crucible})}{(\text{Weight of crucible} + \text{sample}) - (\text{weight of empty crucible})} x100$$

4.2.2.8 Crude fibre determination

Three grams (3.0 g) of cassava/sweetpotato flour was weighed and transferred into Erlenmeyer flask. Petroleum ether (15 ml) was added, the solution stirred, allowed to settle for 15 min and decanted. The washing procedure was repeated three times. The extracted sample was air dried and transferred into a 1000 ml conical flask. 200 ml H_2SO_4 (0.1275M) was added and the solution brought to boiling for 30 min by heating. The boiling sample was poured into a prepared buckner funnel after allowing to stand for 1 min. Insoluble matter was washed with hot water until the washing was free from acid. The insoluble matter was washed back into the 1000 ml conical flask with 200 ml of 0.313M NaOH solution by means of a wash bottle. The solution was boiled for 30 min, allowed to stand for 1 min and filtered through a filter paper. The insoluble material was transferred to the filter paper with boiling water. The material was washed with 1% HCl and finally with boiling water until free from acid. The sample was washed twice with 20 ml ethanol and 3 times with 20 ml of petroleum ether. The insoluble material was then transferred to a dry pre-weighed filter paper, dried at 100^{o}C to constant weight. The paper and contents was incinerated in the Thermconcept furnace, Thermconcept GmbH, Bremen, Germany, at 550^{o}C for 8hrs to ash. The weight of the ash was subtracted from the increase of weight on the filter paper due to insoluble material and the difference reported as crude fibre.

$$\text{Crude fibre (\%)} = \frac{\text{Weight of insoluble material} - \text{Weight of ash}}{\text{Weight of sample}} \text{ x } 100$$

4.2.2.9 Water absorption capacity determination

Water absorption capacity of the cassava/sweetpotato flour samples were determined based on a modification of the centrifugation method of American Association for Clinical Chemistry methods (AACC), 8th edition. Two grams (2 g) of the flour sample was mixed with 20 ml distilled water. Samples were then allowed to stand at 30°C for 30 min, then centrifuged at 3500 rpm for 30 min. The reduction in the volume of the supernatant in a graduated cylinder was noted and recorded as water absorption capacity. Means of triplicate determinations were recorded.

Water absorption capacity (%) = $V_1 - V_2$,

where V_1 is the initial level (volume) of supernatant and V_2 is the final level (volume) of supernatant.

4.2.2.10 Swelling power determination

Swelling power was determined using the method described by Afoakwa *et al*, (2012). One gram (1 g) of flour was transferred into a weighed graduated centrifuge tube (50 mL). Deionized water was added to give a total volume of 40 mL. The sample in the centrifuge tube was heated at 85 ^{o}C in a thermostatically controlled temperature water bath (Grant OLS 200, Keison products, Chelmsford, UK) for 30 min with constant shaking (80 strokes/min). The tube was then removed, wiped dry on the outside and cooled to room temperature. It was centrifuged (Hermle Z 206 A, Hermle Labortechnik GmbH, Germany) for 15 min at 2200 rpm. The swelling power was determined by evaporating the supernatant in a hot air oven (Gallenkamp Oven, England, UK) and weighing the sediment paste and supernatant residue. The swelling power was then calculated using the formula;

$$\text{Swelling power} = \left(\frac{\text{Weight of precipitated paste}}{\text{Weight of sample}}\right) - \text{Weight of residue in supernatant}$$

4.2.2.11 pH determination

The pH of the samples was measured with a Metler Toledo (Seven Compact) pH meter, Metler Toledo group, Switzerland. Ten grams of each flour sample were homogenized in 50 ml of distilled water. The pH of the resulting suspensions were determined using the calibrated pH meter.

4.2.2.12 Pasting characteristics determination

The pasting characteristics of the cassava/sweetpotato flour sample was determined using Visco-Amylograph (Viscograph-E), Brabender GmbH & Co, KG, Illinois, USA. The moisture content of the flour sample was determined using Sartorius MA 45 moisture analyzer, Sartorius AG, Goettingen, Germany, and the value fed into the software of the Viscograph-E (the software calculates the quantities of flour sample and distilled water to mix for the test). The sample was then weighed, distilled water added and mixed well to form a consistent slurry with no lumps. The sample was transferred into the reaction chamber of the Viscograph-E machine and the head of the lever carefully lowered into the sample. The machine was started by clicking the start button on the software to start the analysis. The water pump was turned on at the end of the heating and holding period (after 45 mins) to begin the cooling period which lasted for 30 mins. The data generated at the end of the analysis were recorded.

4.2.2.13 Ethanol determination

Fifty grams (50 g) of cassava/sweetpotato flour sample was transferred into a 500 mL Erlenmeyer flask. Distilled water (250 mL) was added in bits to mix the flour into a slurry. The pH of the sample was taken and recorded. *Liquozyme SC DS* enzyme (3 mL) was then added to the mixture, stirred gently and heated on a water

bath (Grant OLS 200) set at speed of 100 strokes/min at 85ºC for 2.1 hours. The mixture was then cooled to a temperature of 48 ºC and the pH recorded. *Viscozyme L and Spirizyme fuel* enzymes (1.5 mL each) were then added. The sample was stirred gently and maintained on the water bath set at speed of 100 strokes/min and 48 ºC for 4 hours. The Brix of the mixture was determined after 4 hours of mashing. Iodine test was also carried out after every 30 min from the start of the saccharification process. The sample was then cooled to 34 ºC and 0.1 g of Bio-Ferm XR yeast added to the mixture and fermented for 57.4 hours at temperature of 34ºC with constant shaking of the Erlenmeyer flask. The brix of the fermented sample was taken after 57.4 hours. The ethanol yield by weight after 57.4 hours was determined using the Cutaia *et al*, 2009 formula;

$A_{w/w} = 0.38726 * (OE – AE) + 0.00307 * (OE – AE)^2$

where $A_{w/w}$ is ethanol content by weight, OE is original extract and AE is the apparent extract. The ethanol content by volume conversion was done using the Probrewer conversion table and reported.

4.2.2.14 Data analysis

Analysis of variance (ANOVA) was carried out on the ethanol yields from the five samples at 95% confidence level using Minitab version 17.1 (Kutner *et al*, 2005).

4.3 Results and Discussion

4.3.1 Assessment of cassava varieties

The moisture content of the cassava varieties studied ranged between 56% and 67% as presented in Figure 4.1. The moisture contents of the roots studied were

comparable with the 68.1% value reported by Amarachi *et al*, 2015. The moisture content of Ampong roots was significantly higher ($p < 0.05$) compared to Sika bankye for all three maturity levels. The amount of moisture is related to dry matter content of root crops. The higher the moisture content, the lower the dry matter content. It therefore implies that, Sika bankye has relatively higher dry matter content than Ampong at the same maturity levels. It was also observed from the two varieties that, the more matured the cassava roots, the less moisture content. The cassava roots were harvested in June, August and October, 2015 with highest root moisture content registered in June and the least moisture realised in October. There was much rains in June at the time of the 8 months harvest than in August and October in the location of the cultivation. The moisture in the soil at the time of harvest could make the roots absorb more water which could lead to a higher moisture content in the roots in the 8 months matured roots than the 10 and 12 months matured roots.

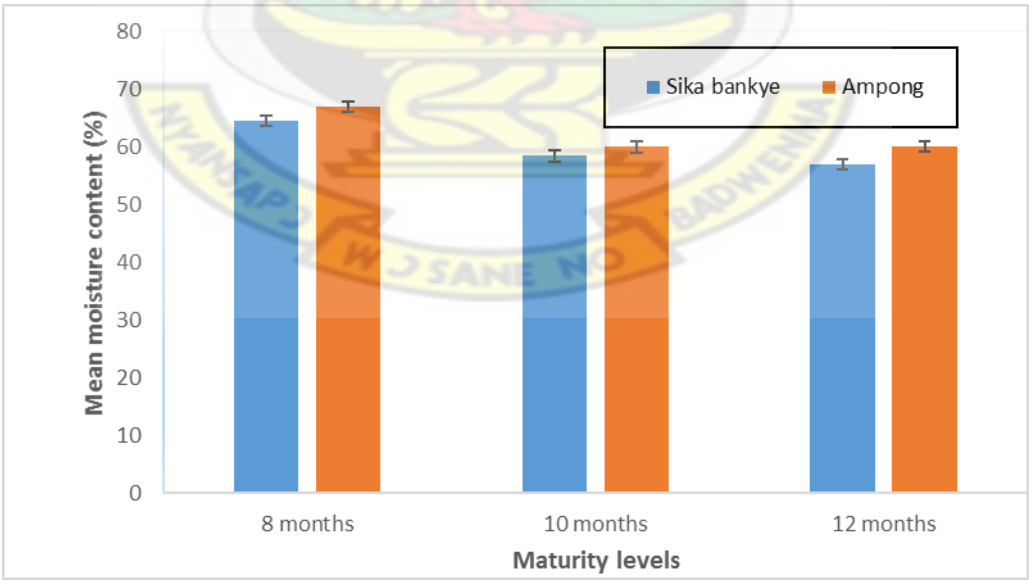


Figure 4.1: Mean moisture contents of Sika bankye and Ampong

The starch content of Sika bankye and Ampong from the study is presented in Figure 4.2. It was observed that the starch content of Sika bankye was significantly higher at all levels of maturity than in the Ampong variety. The yield of ethanol produced from a starchy raw material is largely dependent on the starch content of the raw material (Li *et al*, 2015). Ademiluyi *et al*. (2013) reported that high yields of ethanol could be achieved from cassava varieties with high starch content, high dry matter, low fibre and low protein content. It was also reported by Teerawanichpan *et al*. (2008) that the amount of hydrolysable carbohydrates available in cassava root and sweetpotato tuber depends on the variety and growth conditions of the crops. Apart from Otuhia cassava variety, Sika bankye has higher starch and dry matter contents than all cassava varieties in Ghana (Agrihome expressions, 2016). Since one of the aims of the study was to identify a suitable cassava variety that could give higher or optimum ethanol yields, it could be said that, Sika bankye is a better variety for ethanol production compared to Ampong based on the fact that it has higher dry matter and higher starch contents.

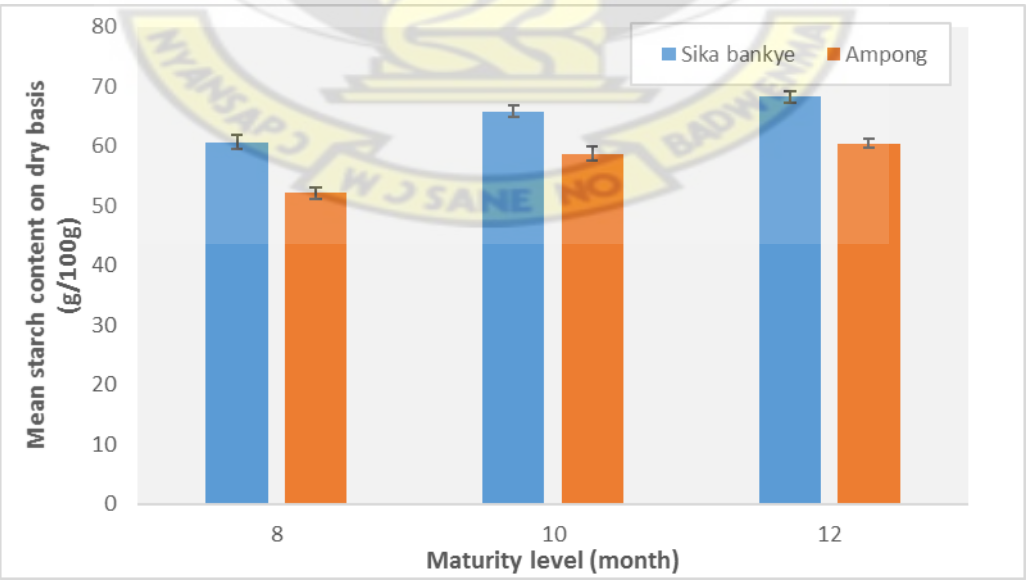
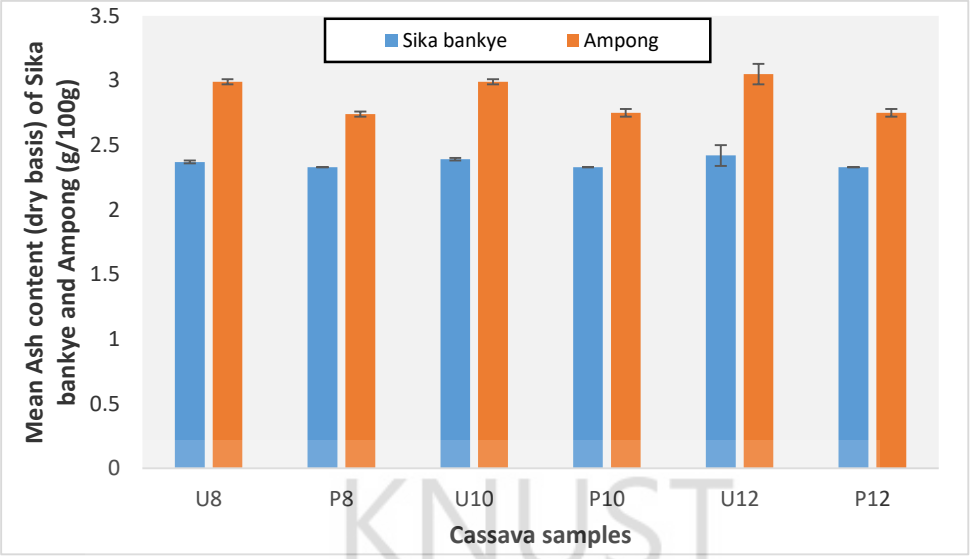


Figure 4.2: Mean starch contents of Sika bankye and Ampong

The proximate analysis results of Sika bankye and Ampong cassava varieties determined during the study are in Figures 4.3 to 4.7. The nutrients (ash, fat, protein, carbohydrates, crude fibre) are generally higher in Sika bankye than in Ampong except for ash content. Nutrients in a wort during brewing (fermentation) are key to how well the sugar is fermented into ethanol (Kunze, 2004). The yeast for brewing needs amino acids to build proteins and new cells, they need vitamins and minerals to make enzymes work correctly and they need phosphorous to create new DNA. Nitrogen is a key factor in determining the ethanol yield in brewing (Agu *et al*, 2009). Nitrogen makes approximately 10% of the dry weight of yeast cells. Since the nutrients are relatively higher in Sika bankye than in Ampong especially that of protein, it suggests that Sika bankye could supply, to a large extent, the needed nutrients to yeast during fermentation than Ampong. Sika bankye could therefore be the best variety in terms of nutrients supply for ethanol production than Ampong. It was also observed that, the protein content of the cassava varieties was relatively higher but not significantly different ($p > 0.05$) in the unpeeled samples than peeled samples (Figure 4.4). This is in line with reports by Amarachi *et al*, 2015. It will therefore be prudent to produce ethanol using the unpeeled roots for maximum use of the proteins that could be in the peels of the root.



U= Unpeeled cassava, P= Peeled cassava, 8, 10, 12 are maturity dates in months.

Figure 4.3: Mean ash contents of 8, 10 and 12 months old Sika bankye and Ampong flours.

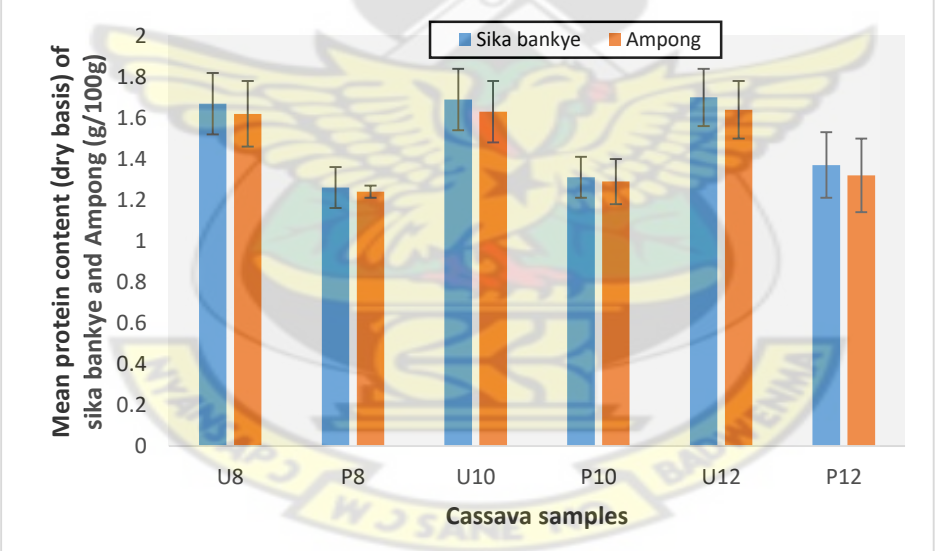


Figure 4.4: Mean protein contents of 8, 10 and 12 months old Sika bankye and Ampong.

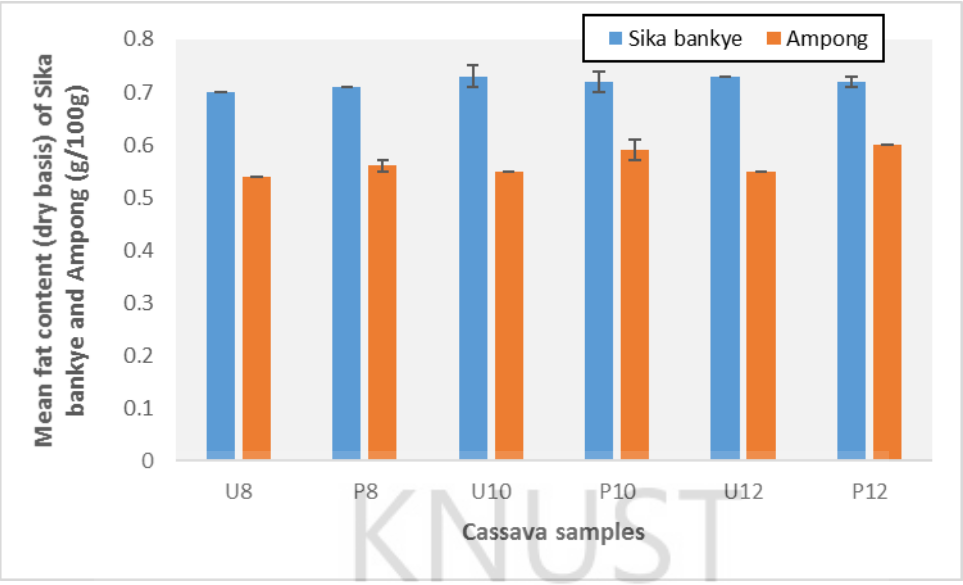


Figure 4.5: Mean fat contents of 8, 10 and 12 months old Sika bankye and Ampong.

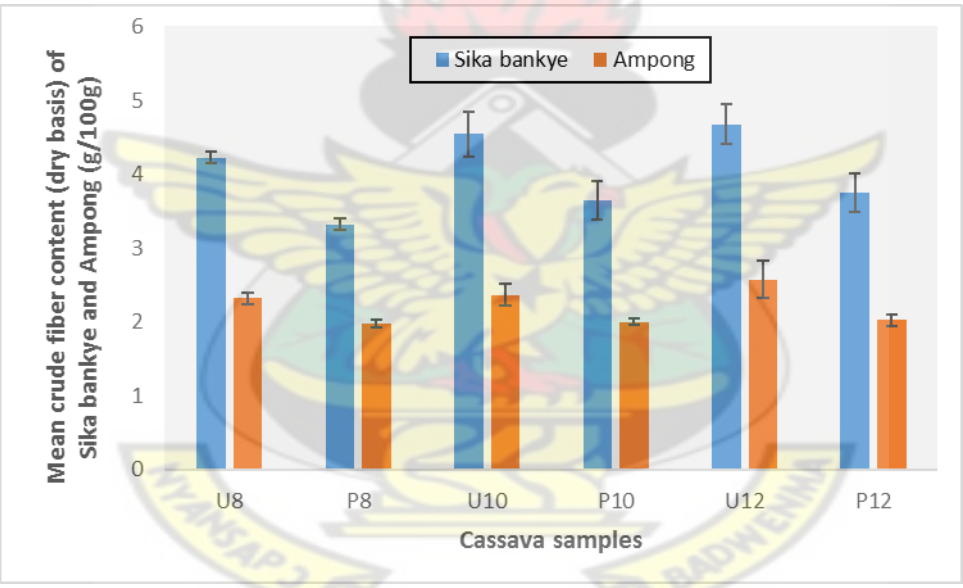


Figure 4.6: Mean crude fiber contents of 8, 10 and 12 months old Sika bankye and Ampong.

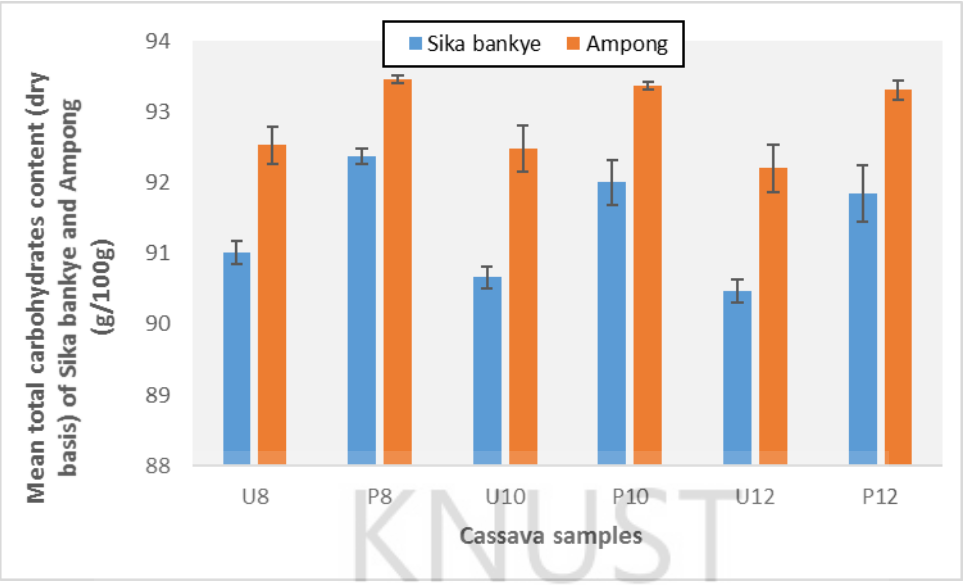


Figure 4.7: Mean total carbohydrates contents of 8, 10 and 12 months old Sika bankye and Ampong.

The gelatinisation temperature, pH, water absorption capacity and swelling capacity of the cassava samples are presented in Table 4.1. The gelatinisation temperatures of the cassava samples ranged between 68^{o}C and 71^{o}C. Gelatinisation of starch is a thermal point at which the intermolecular bonds of starch molecules are broken down in the presence of water and heat. The process irreversibly dissolves the starch granule in water. Gelatinisation improves the availability of starch for amylase hydrolysis. Lopez-Ulibarri *et al*, (1997) reported that the advantage of using cassava as raw material for ethanol production are that, it is one of the ten most important tropical crops with a high potential yield, lower starch gelatinization temperature and higher amylose solubility compared to maize starch. The gelatinisation temperatures observed in the study fall far below the optimum temperature (85^{o}C) of the *Liquozyme SC DS* enzyme (Table 4.1) used for dextrinization in this study. This was an assurance that all the starch present in the cassava samples gelatinised so as to be broken down into short chain carbohydrates for subsequent hydrolysis by saccharifying enzymes.

The pH values obtained during the study for all cassava samples were between 5 and 6. The pH values observed were ideal for the dextrinization, saccharification and fermentation of the cassava samples worked on. This is because all the enzymes and yeast used for the study work at their optimum between pH values of 5 and 6. There was no need therefore to adjust the pH of the medium used which could have economic implication on the production.

The swelling capacities of the cassava flour samples studied ranged from 8.7 to 10 with Sika bankye samples generally having relatively low swelling capacities compared to Ampong samples. Swelling capacity is a measure of the ability of starch to imbibe water and expand in volume at a particular temperature (Amarachi *et al*, 2015). Low swelling capacity of flour suggests that the starch granules have strong binding force and low amylose content. Low-amylose starch has an excellent functionality of easy digestibility when compared with high-amylose starch (Amarachi *et al*, 2015). In addition, low swelling power in cassava flour is a clear indication of restricted starch which shows a high resistance to breaking during cooking. Since both varieties have relatively low swelling capacities, they could all be digested easily hence ideal for ethanol production.

The water absorption capacity values of Sika bankye and Ampong ranged between 0.29 and 0.51. Sika bankye had relatively higher water absorption capacity than Ampong. Water binding capacity or water absorption capacity is the ability to take up and retain water either by adsorption or absorption. It is influenced by the extent of starch disintegration. Low water absorption capacity could be attributed to the protein content in a product because protein has been reported to limit the ability of water uptake in food (Amarachi *et al*, 2015).

Table 4.1: Mean physico-chemical properties of Sika bankye and Ampong.

Sample/Parameter	Gelatinisation temperature (°C)	pH	Swelling power	Water absorption capacity
Sika bankye (B)	$55.2^{a*} \pm 0.3$	$5.9^{c} \pm 0.0$	$9.3^{d} \pm 0.1$	$0.4^{f} \pm 0.1$
Sika bankye (H)	$68.8^{b} \pm 0.3$	$5.8^{c} \pm 0.2$	$9.4^{d} \pm 0.3$	$0.5^{f} \pm 0.1$
Sika bankye (K)	$69.1^{b} \pm 0.5$	$5.6^{c} \pm 0.1$	$9.3^{d} \pm 0.2$	$0.5^{f} \pm 0.2$
Ampong (B)	$55.1^{a} \pm 0.3$	$6.0^{c} \pm 0.0$	$9.8^{e} \pm 0.1$	$0.3^{f} \pm 0.2$
Ampong (H)	$69.1^{b} \pm 0.3$	$6.0^{c} \pm 0.0$	$10.0^{e} \pm 0.3$	$0.4^{f} \pm 0.1$
Ampong (K)	$71.1^{b} \pm 0.1$	$5.6^{c} \pm 0.1$	$9.7^{e} \pm 0.2$	$0.3^{f} \pm 0.1$

B = Blanched and oven dried sample, **H** = Oven dried, **K** = Solar tent dried.
*Means in the same column with different letters are significantly different ($p<0.05$)

Table 4.2 shows the mean ethanol content of 8, 10 and 12 months old Sika bankye and Ampong samples. The ethanol values observed in the study ranged between 10.5 and 14.9% v/v. Ocloo and Ayernor (2010); Cutzu and Bardi (2017) and Begea *et al*, (2010) reported maximum ethanol yields of 8.3% v/v, 10.22% v/v and 15.18% v/v respectively from cassava and agro waste bio-fermentation. The ethanol contents of all the 10 months old Sika bankye and Ampong samples observed were generally higher and significantly different from the values of 8 months old Sika bankye and Ampong. The higher ethanol contents of the 10 months old samples could be attributed to the higher dry matter, starch and carbohydrates content compared to the 8 months old samples as reported by Li *et al*, (2015); Ademiluyi *et al*, (2013) and Teerawanichpan *et al*, (2008). The ethanol content of Sika bankye samples are higher and significantly different ($p < 0.05$) compared to those of Ampong variety of same maturity level. Again, this could be attributed to the higher dry matter, starch, carbohydrate and other nutrients like protein and fat which are relatively higher in Sika bankye than Ampong. There was no significant differences between the ethanol yield of cassava samples of 10 and 12 months old maturity. There is therefore no economic value according to the findings of this work to keep

cassava roots after 10 months on the field if they are meant for processing ethanol. The economic maturity period for cassava meant for processing ethanol, according to this study, is therefore ten (10) months.

Table 4.2: Mean alcohol content of 8, 10 and 12 months old cassava varieties.

Sample	Attenuation (%)	Ethanol (%v/v)	Attenuation (%)	Ethanol (%v/v)	Attenuation (%)	Ethanol (%v/v)
	8 months maturity level		10 months maturity level		12 months maturity level	
Sika bankye (U, B)*	81.3 ± 0.5	13.3^{a}** ± 0.2	81.9 ± 0.2	14.8^{f} ± 0.4	81.3 ± 0.3	14.7^{f} ± 0.1
Sika bankye (P, B)	81.0 ± 0.2	12.4^{b} ± 0.4	81.5 ± 0.3	14.3^{g} ± 0.3	81.3 ± 0.5	14.1^{g} ± 0.2
Sika bankye (U, H)	80.3 ± 0.5	12.3^{b} ± 0.2	81.2 ± 0.4	13.7^{h} ± 0.1	80.0 ± 0.2	13.7^{h} ± 0.4
Sika bankye (P, H)	79.4 ± 0.4	12.0^{c} ± 0.0	80.2 ± 0.5	13.4^{h} ± 0.2	79.8 ± 0.6	13.3^{h} ± 0.2
Sika bankye (U, K)	80.0 ± 0.3	11.2^{d} ± 0.2	80.5 ± 0.4	12.4^{i} ± 0.2	80.2 ± 0.4	12.5^{i} ± 0.1
Sika bankye (P, K)	80.0 ± 0.6	10.8^{e} ± 0.7	80.3 ± 0.8	11.9^{j} ± 0.3	79.3 ± 0.2	12.0^{j} ± 0.3
Ampong (U, B)	79.3 ± 0.2	11.7^{c} ± 0.2	81.3 ± 0.2	13.2^{h} ± 0.3	82.3 ± 0.2	13.3^{h} ± 0.2
Ampong (P, B)	80.0 ± 0.6	11.5^{c} ± 0.0	80.5 ± 0.6	12.7^{i} ± 0.2	81.3 ± 0.4	12.7^{i} ± 0.0
Ampong (U, H)	80.3 ± 0.5	11.6^{c} ± 0.1	80.3 ± 0.2	12.4^{i} ± 0.2	80.5 ± 0.6	12.7^{i} ± 0.3
Ampong (P, H)	79.4 ± 0.6	11.3^{d} ± 0.2	80.4 ± 0.2	12.2^{i} ± 0.3	80.2 ± 0.7	12.3^{i} ± 0.2
Ampong (U, K)	79.5 ± 0.3	10.7^{e} ± 0.1	80.2 ± 0.6	11.7^{j} ± 0.3	79.2 ± 0.3	11.8^{j} ± 0.1
Ampong (P, K)	80.0 ± 0.6	10.6^{e} ± 0.1	79.6 ± 0.3	11.0^{k} ± 0.3	79.7 ± 0.4	10.8^{k} ± 0.0

***U** = Unpeeled sample**, P** = Peeled sample, **B** = Blanched sample, **H** = Oven dried sample, **K** = Solar tent dried sample.
**Means in the same column with different letters are significantly different ($p<0.05$) and means in the same row with different letters are significantly different ($P<0.05$).

4.3.2 Assessment of sweetpotato varieties

Figure 4.8 shows the moisture content of Apomuden and Tuskiki sweetpotato varieties harvested at 3, 4 and 5 months. The moisture contents of the tubers ranged between 65% and 72%. The moisture content of Tuskiki tubers was significantly higher compared to Apomuden for all three harvesting periods. The amount of moisture is related to dry matter content of root crops. The higher the moisture content, the lower the dry matter content. It implies that, Apomuden has relatively higher dry matter content than Tuskiki at the same maturity levels. It was also

observed from the study that the more matured the sweetpotato, the less moisture content it has. This could be due to the time of the harvest or genetic constitution of the roots.

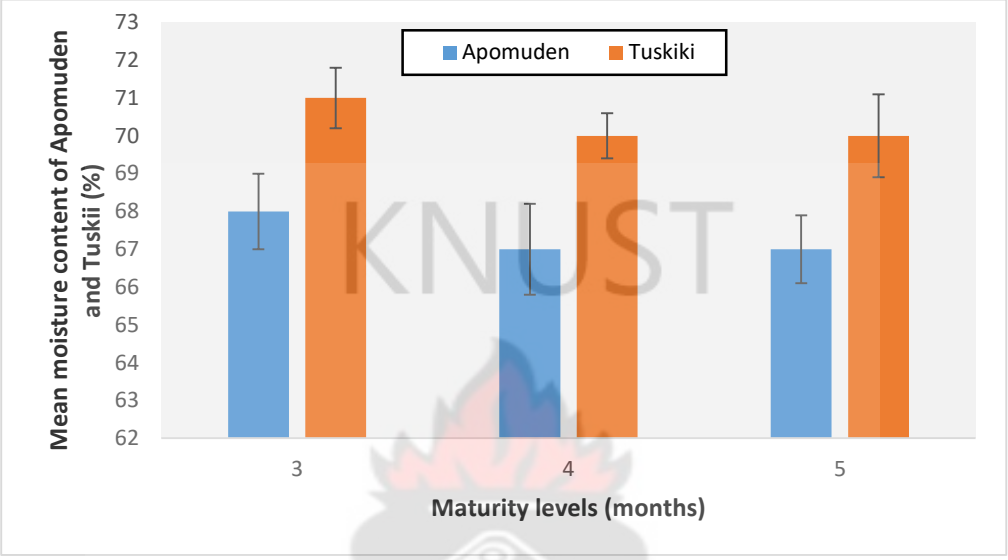


Figure 4.8: Mean moisture contents of Apomuden and Tuskiki.

The starch contents of Apomuden and Tuskiki from the study is presented in Figure 4.9. The starch content of Apomuden was significantly higher at all levels of maturity than in the Tuskiki variety. The ethanol yield from a starchy raw material is largely dependent on the starch content of the raw material (Li *et al*, 2015; Ademiluyi *et al*, 2013; Teerawanichpan *et al*, 2008). Since one of the aims of the study was to establish a suitable sweetpotato variety that could give higher or optimum ethanol yields, it could be said that of the two sweetpotato varieties, Apomuden is a better variety for ethanol production compared to Tuskiki based on the fact that it has higher dry matter and starch contents (Figure 4.9).

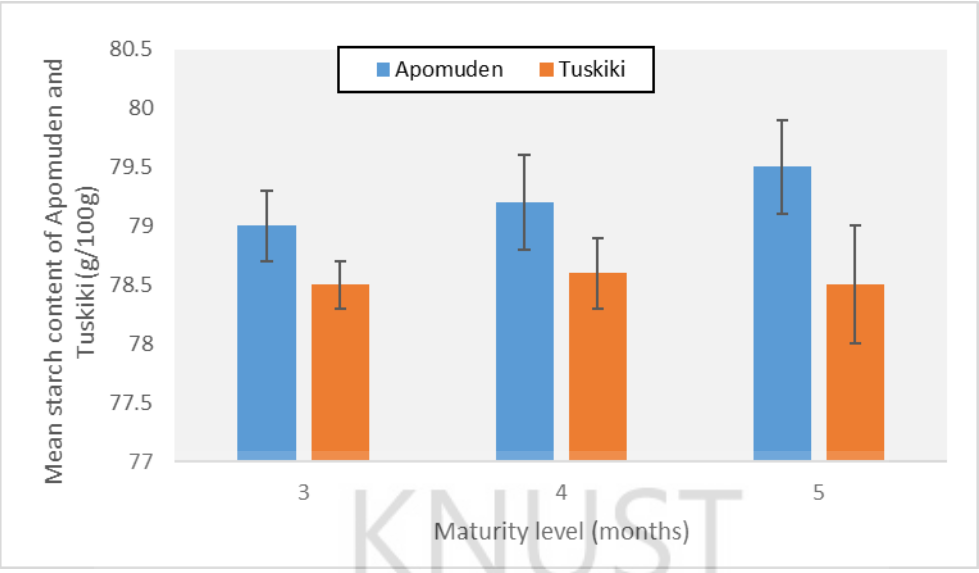


Figure 4.9: Mean starch contents of Apomuden and Tuskiki.

The proximate composition of Apomuden and Tuskiki sweetpotato varieties observed are presented in Figures 4.10 to 4.14. The nutrients determined (ash, fat, protein, carbohydrates, crude fibre) were generally higher in Apomuden than in Tuskiki except for fat content. The fat content of Tuskiki was relatively higher for all levels of maturity than that of Apomuden (Figure 4.11). The nutrients in a wort during brewing (fermentation) are key to how well the sugar is fermented into ethanol (Kunze, 2004). The yeast for brewing needs amino acids to build proteins and new cells, they need vitamins and minerals to make enzymes work correctly and they need phosphorous to create new DNA (Agu *et al*, 2009). Nitrogen is a factor in determining the ethanol yield in brewing (Agu *et al*, 2009). Since the nutritional value is higher in Apomuden than in Tuskiki, it suggests that Apomuden may have the needed nutrients to support yeast growth during fermentation than Tuskiki. Apomuden could therefore be the best variety in terms of nutrients supply for ethanol production than Tuskiki.

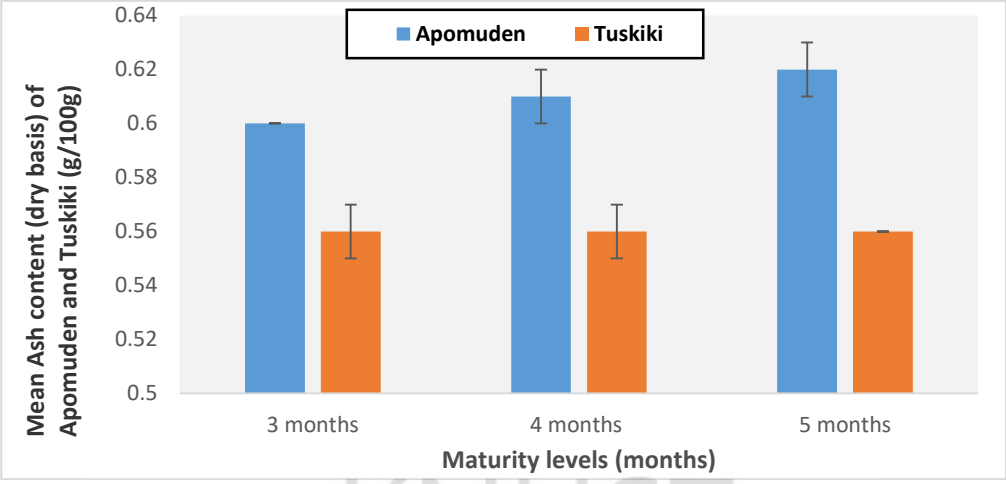


Figure 4.10: Mean ash contents of Apomuden and Tuskiki against maturity levels.

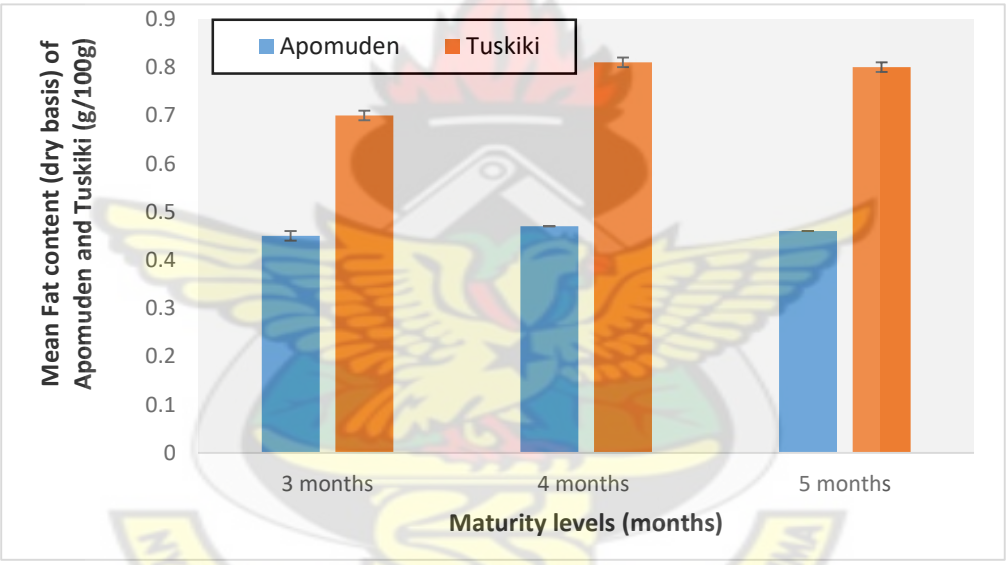


Figure 4.11: Mean fat contents of Apomuden and Tuskiki against maturity levels.

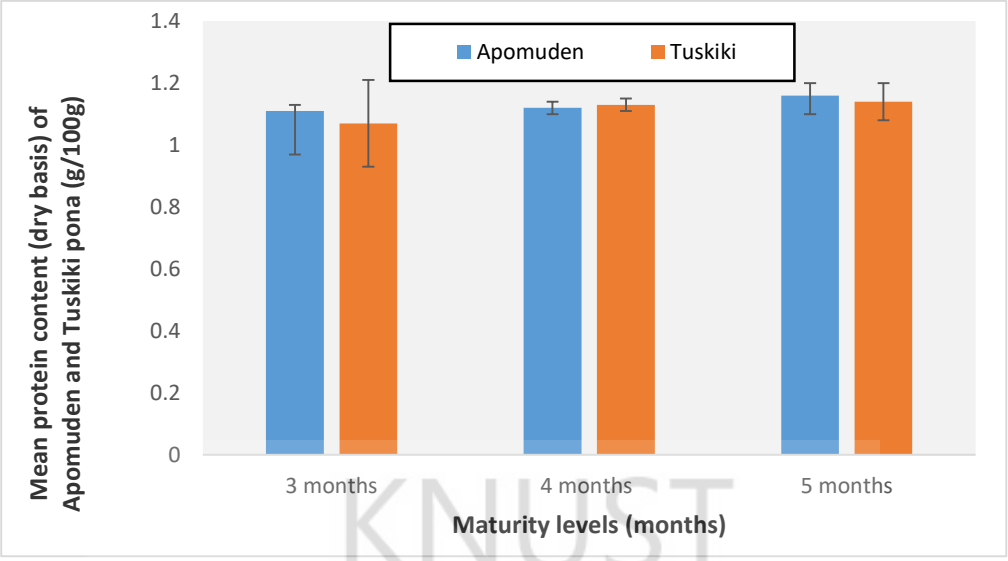


Figure 4.12: Mean protein contents of Apomuden and Tuskiki against maturity levels.

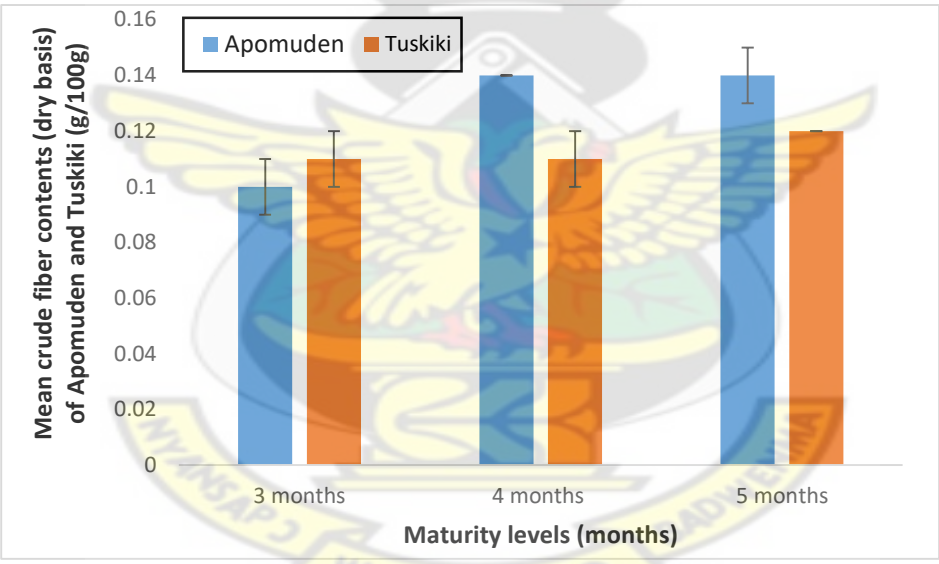


Figure 4.13: Mean crude fibre contents of Apomuden and Tuskiki against maturity levels.

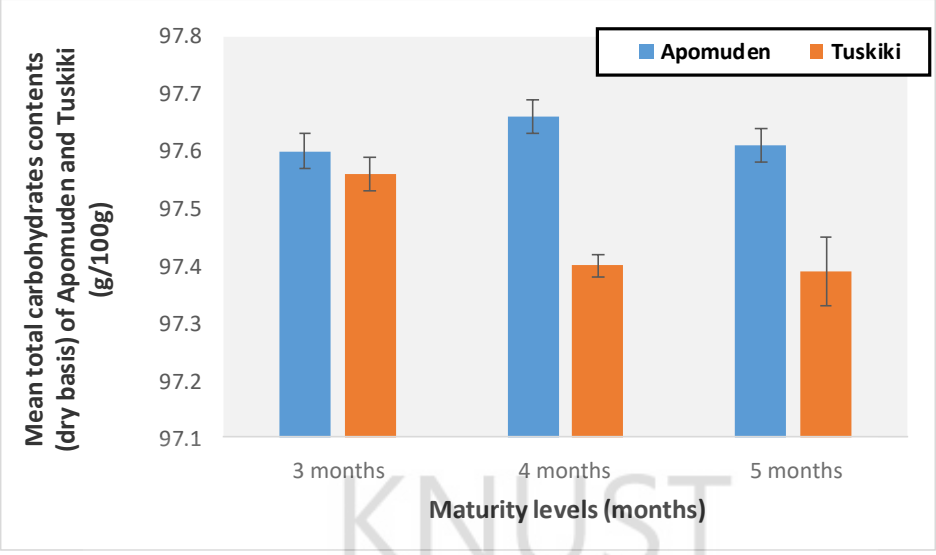


Figure 4.14: Mean total carbohydrate contents of Apomuden and Tuskiki against maturity levels.

Table 4.3 shows the gelatinisation temperature, pH, water absorption capacity and swelling capacity of sweetpotato samples studied. The gelatinisation temperatures of the sweetpotato samples ranged between 58°C and 73°C. The gelatinisation temperatures of 59°C observed for two samples {Apomuden (B) and Tuskiki (B)} were lower compared to the others because the samples were blanched before the pasting characteristics analysis. Gelatinisation of starch is a thermal point at which the intermolecular bonds of starch molecules are broken down in the presence of water and heat. The process irreversibly dissolves the starch granule in water. Gelatinisation improves the availability of starch for amylase hydrolysis. The gelatinisation temperatures observed in the study (Table 4.3) fall far below the optimum temperature (85°C) of the *Liquozyme SC DS* enzyme used for dextrinization in this study. This was ideal because all the starches present in the sweetpotato samples may all have gelatinised to be broken down into short chain carbohydrates for subsequent hydrolysis by saccharifying enzymes.

The pH values obtained during the study for all sweetpotato samples fell between 5 and 6. The pH values observed were ideal for the dextrinization, saccharification and fermentation processes of the sweetpotato samples studied. This is because all the enzymes used for the study have optimum activity between pH 5 and 6 according to the producers (Novozymes, Denmark). There was no need therefore to adjust the pH of the medium used which could have economic implications on the production process of ethanol from sweetpotato.

The swelling capacities of the sweetpotato flour samples studied ranged between 5.3 and 5.9. Swelling capacity is a measure of the ability of starch to imbibe water and expand in volume at a particular temperature (Amarachi *et al*, 2015). Low swelling capacity of flour suggests that the starch granules have strong binding force and low amylose content. Low-amylose starch has an excellent functionality of easy digestibility when compared with high-amylose starch (Amarachi *et al*, 2015). In addition, low swelling power in sweetpotato flour is a clear indication of restricted starch which shows a high resistance to breaking during cooking. Since both Apomuden and Tuskiki varieties have relatively low swelling capacities, they could all be digested easily hence ideal for ethanol production.

The water absorption capacity values of Apomuden and Tuskiki under investigation ranged between 2.7 and 3.2. There was no significant difference between Apomuden and Tuskiki in relation to water absorption capacity. Water binding capacity or water absorption capacity is the ability to take up and retain water either by adsorption or absorption. It is influenced by the extent of starch disintegration. Low water absorption capacity could be attributed to the protein content in a product because protein has been seen to limit the ability of water uptake

in food (Amarachi *et al*, 2015). Since there was no significant difference between the protein content of Apomuden and Tuskiki (Figure 4.12), there was not much difference between the water absorption capacities. The results in this work confirms the fact that protein content of food could have some relationship with water uptake.

Table 4.3: Mean physico-chemical properties of Apomuden and Tuskiki**.**

Sample/Parameter	Gelatinisation temperature (°C)	pH	Swelling power	Water absorption capacity
Apomuden (B)	$59.0^{a*} \pm 1.0$	$5.8^{c} \pm 0.0$	$5.7^{d} \pm 0.2$	$2.9^{e} \pm 0.1$
Apomuden (H)	$72.2^{b} \pm 0.6$	$5.8^{c} \pm 0.0$	$5.8^{d} \pm 0.0$	$3.1^{e} \pm 0.1$
Apomuden (K)	$72.6^{b} \pm 0.4$	$5.5^{c} \pm 0.3$	$5.5^{d} \pm 0.3$	$3.1^{e} \pm 0.1$
Tuskiki (B)	$59.0^{a} \pm 1.0$	$5.7^{c} \pm 0.2$	$5.4^{d} \pm 0.2$	$2.8^{e} \pm 0.2$
Tuskiki (H)	$72.3^{b} \pm 0.6$	$5.8^{c} \pm 0.2$	$5.6^{d} \pm 0.1$	$2.9^{e} \pm 0.2$
Tuskiki (K)	$71.9^{b} \pm 0.9$	$5.6^{c} \pm 0.1$	$5.6^{d} \pm 0.2$	$3.0^{e} \pm 0.1$

B = Blanched and oven dried sample, **H** = Oven dried, **K** = Solar tent dried.

* Means in the same column with different letters are significantly different ($p<0.05$).

Table 4.4 shows the mean ethanol content values of 3, 4 and 5 months old Apomuden and Tuskiki samples. The ethanol contents of all the 3 months old Apomuden and Tuskiki are not significantly different from the values of 4 and 5 months old Apomuden and Tuskiki. The high ethanol contents of all samples could be attributed to the higher dry matter, starch and carbohydrates content as reported by Li *et al*, (2015). The higher ethanol yields realised for all the sweetpotato varieties could also be due to the β-amylase activity of the sweetpotato varieties reported by Dziedzoave *et al*, (2010).

Table 4.4: Mean ethanol content of 3, 4 and 5 months old sweetpotato varieties.

Sample	Attenuation (%)	Ethanol (%v/v)	Attenuation (%)	Ethanol (%v/v)	Attenuation (%)	Ethanol (%v/v)
	3 months maturity level		4 months maturity level		5 months maturity level	
Apomuden (B)	83.3 ± 0.5	15.7^{a} ± 0.2	82.7 ± 0.4	15.1^{b} ± 0.1	83.7 ± 0.2	15.1^{b} ± 0.1
Apomuden (H)	83.5 ± 0.6	14.9^{b} ± 0.5	84.2 ± 0.2	15.0^{b} ± 0.2	85.3 ± 0.2	14.9^{b} ± 0.3
Apomuden (K)	82.3 ± 0.5	14.1^{c} ± 0.2	81.9 ± 0.2	14.2^{c} ± 0.4	81.4 ± 0.3	14.2^{c} ± 0.2
Tuskiki (B)	82.4 ± 1.0	14.8^{b} ± 0.3	82.5 ± 0.1	14.9^{b} ± 0.2	81.9 ± 0.2	14.8^{b} ± 0.1
Tuskiki (H)	81.2 ± 0.4	14.6^{b} ± 0.2	81.8 ± 0.1	14.8^{b} ± 0.2	81.7 ± 0.1	14.6^{b} ± 0.0
Tuskiki (K)	81.3 ± 0.6	14.2^{c} ± 0.1	83.6 ± 0.0	14.3^{c} ± 0.3	82.5 ± 0.2	14.3^{c} ± 0.1

B = Blanched and oven dried sample, **H** = Oven dried sample, **K** = Solar tent dried sample.

*Means in the same column with different letters (a-c) are significantly different ($p<0.05$) and means in the same row with different letters are significantly different ($P<0.05$).

4.4 Conclusion

The nutrients in Sika bankye at the same level of maturity are generally higher than in Ampong except for ash. Sika bankye has more dry matter and higher starch content at the same level of maturity which gave higher ethanol yield than Ampong. The best maturity period for ethanol production from the cassava varieties is 10 months. Sika bankye had the highest ethanol yield (14.8% v/v) between the two cassava varieties at maturity date of 10 months. Apomuden has relatively higher nutrients than Tuskiki at all levels of maturity except for fat. Apomuden has more starch and produced much ethanol than Tuskiki at the same levels of maturity. Apomuden had the highest ethanol yield (15.7% v/v) between the two sweetpotato varieties at maturity date of 3 months. The best economical maturity date for processing sweetpotato into ethanol according to the research is 3 months.

CHAPTER FIVE

ETHANOL YIELD FROM COMBINATIONS OF CASSAVA AND SWEETPOTATO

5.1 Introduction

This chapter seeks to explore the possibility or otherwise of improved ethanol yield from the combinations of cassava and sweetpotato in various proportions as raw material for ethanol production.

Investigations by Dziedzoave *et al*, (2010) and Dziedzoave (2004) show that there is significant β-amylase activity in sweetpotato which could aid the degradation of starch during mashing to produce simple sugars. The presence of β-amylase in sweetpotato could have potential benefits on ethanol production. The combination of cassava and sweetpotato together with the support of external commercial saccharifying enzymes could have synergetic effects on ethanol yield. The main objectives of this study were therefore to;

- Establish whether or not the combination of cassava and sweetpotato has any potential effect on ethanol yield.
- Establish the best combination ratios of cassava and sweetpotato that result in optimum ethanol yield.

5.2 Materials and Methods

5.2.1 Materials

Flour samples processed from 10 months old Sika bankye and 3 months old Apomuden were used for this work. *Liquozyme SC DS, Viscozyme L and spirizyme* were supplied by Novozymes, Denmark. The yeast used for fermentation was Bio-Ferm XR (Lallemand).

5.2.2 Methods

Ten months old unpeeled Sika bankye flour (processed as described in method 1 of section 4.2.2.1) and three months old Apomuden flour (processed as described in method 1 of section 4.2.2.2) were mixed in the ratios of 70:30, 50:50 and 30:70, respectively. The ratios were selected based on preliminary studies. Fifty grams (50 g) each of the composite flour was mixed with 250 mL of distilled water in an Erlenmeyer flask. The pH of the sample was taken. *Liquozyme SC DS* enzyme (3 ml) was then added to the mixture, stirred gently and heated on water bath (Grant OLS 200) at speed of 100 strokes/min at 85^{o}C for 2.1 hours. The mixture was then cooled to a temperature of 48 ^{o}C and the pH recorded. *Viscozyme L and Spirizyme fuel* enzymes (1.5 ml each) was then added and maintained on the water bath for 4 hours at 48 ^{o}C. The Brix of the sample was determined at the end of the 4 hours. Iodine test was also carried out after every 30 min from the start of the saccharification process. The sample was then cooled to 34^{o}C and 0.1g Bio-Ferm XR yeast added to the mixture and fermented for 57.4 hours in an Erlenmeyer flask. The brix of the fermented sample was taken after the 57.4 hours. The ethanol yield by weight after 57.4 hours was determined using the Cutaia *et al,* (2009) formula;

$A_{w/w} = 0.38726 * (OE – AE) + 0.00307 * (OE – AE)^2$,

where $A_{w/w}$ is Alcohol content by weight, OE is original extract and AE is the apparent extract. The alcohol by volume conversion was done using the Probrewer conversion table (software) and recorded.

5.2.3 Data analysis

Analysis of variance (ANOVA) was carried out on the ethanol yields from the five samples at 95% confidence level using Minitab version 17.1 (Kutner *et al,* 2005).

5.3 Results and Discussions

Table 5.1 shows results of mean ethanol yields from different combinations and separately processed Sika bankye and Apomuden flours. The flour mixture from ten (10) months old Sika bankye and three (3) months old Apomuden in the ratio of 50:50 respectively showed the highest yield of 16.2% v/v ethanol on laboratory scale. The average recovery rate of ethanol from 1 tonne of Sika bankye and Apomuden (50:50) from pilot scale trials on Caltech Ventures Ltd distillation plant at Ho in the Volta Region of Ghana was 155 litres of ethanol at 95% purity. Analysis of variance (ANOVA) on the results at 95% confidence level using Minitab version 17.1 software showed significant differences among four out of the five samples analysed. This means that the different cassava and sweetpotato samples evaluated yielded different ethanol values when they were treated with the same processing conditions. The results also indicated that ethanol yields were higher when Sika bankye and Apomuden flour combinations were processed together compared to processing Sika bankye and Apomuden separately. The relative higher ethanol values obtained for processing the various flour mixtures of Sika bankye and Apomuden could be due to the chemical composition and possible biochemical interactions between the two crops during processing. The starch content of the sweetpotato varieties studied are relatively higher than the cassava varieties studied (Figures 4.2 and 4.9). The amount of ethanol produced from a starchy raw material is largely dependent on the starch content of the raw material (Li *et al*, 2015). It could therefore be deduced that the higher ethanol yields from the separately processed Apomuden and mixtures of Sika bankye and Apomuden could be due to the relatively higher starch content of the Apomuden.

It was reported by Dziedzoave *et al* (2010) that there is significant β-amylase activity in sweetpotato which could aid the degradation of starch during mashing to produce simple sugars. This fact could also be a contributing factor to the higher ethanol values recorded for processing Apomuden separately and the mixtures of Sika bankye and Apomuden compared to processing Sika bankye separately.

Table 5.1: Mean ethanol yields from Sika bankye and Apomuden composite flour samples.

Sample	Attenuation (%)	Ethanol yield (%$_{v/v}$)
Sika bankye:Apomuden flour mix (7:3)	83.9 ± 0.3	15.5^{a}* ± 0.2
Sika bankye:Apomuden flour mix (1:1)	84.7 ± 0.2	16.2^{b} ± 0.1
Sika bankye:Apomuden flour mix (3:7)	84.5 ± 0.2	15.9^{b} ± 0.3
Sika bankye flour	82.5 ± 0.3	14.9^{c} ± 0.1
Apomuden flour	83.5 ± 0.3	15.2^{d} ± 0.1

*Means in the same column with different letters (a-d) are significantly different ($p<0.05$).

5.4 Conclusion

The results from the study indicate that combinations of cassava and sweetpotato varieties for ethanol production yield more ethanol than processing cassava and sweetpotato separately. The best combination ratio of cassava and sweetpotato for optimum ethanol yield from the results is 50:50 which gave the highest yield of 16.2% v/v ethanol in the laboratory but 155 litres of ethanol at 95% purity on pilot scale trial.

CHAPTER SIX

GENERAL ECONOMICS OF ETHANOL PROCESSING FROM CASSAVA AND SWEETPOTATO

6.1 Introduction

Ethanol imports into Ghana over the past decade has been quite high. The over seventy (70) million litres of ethanol imported into Ghana for the various industries in 2016 (ghanabusinessnews.com, 2017) could have been produced in Ghana using cassava and sweetpotato as raw materials. This may only be made possible if the cost of production is competitive compared to the cost of importing ethanol into Ghana. Ethanol produced from cassava and sweetpotato would therefore need to be priced competitively in order to compete favourably with ethanol produced globally from corn and other raw materials. The current cost of one litre of ethanol on the international market is US$0.44 (Trading Economics, 2017).

According to Ministry of Food and Agriculture (MoFA), Ghana, 2015 agricultural data, the average yield of cassava per hectare in Ghana on farmer fields is 18.78 tonnes. Studies have, however, proved that cassava yields could reach 45 tonnes per hectare in Ghana. The yield per hectare for sweetpotato in Ghana has also been reported to be an average of 15 tonnes per hectare but potential yields of 55 tonnes per hectare have been reported (MoFA, 2016).

The general objective of this chapter was to analyse the cost of ethanol production from sweetpotato and cassava. The specific objectives were to evaluate;

- the yield and cost of producing cassava and sweetpotato from one hectare of land.

- the energy, labour and consumable cost for producing ethanol from cassava and sweetpotato.
- the cost benefit analysis of producing ethanol from cassava and sweetpotato.

6.2 Materials and Methods

6.2.1 Methods.

6.2.1.1. Cassava and sweetpotato yield determination

Sika bankye and Ampong cassava varieties were planted in alternate rows (1m by 1m) on one (1) hectare of land. The total cassava farm cultivated was divided into three portions before the 8 months maturity harvest. One portion of the farm was harvested at 8 months maturity. The area and number of the Ampong and Sika bankye cassava plants harvested at 8, 10 and 12 months maturity was weighed and counted respectively. The weight of roots harvested and the number of plant stands harvested were noted. The total weight of roots obtained for the entire 1 hectare farm was determined by adding the weights of roots obtained at 8, 10 and 12 months harvest. The total number of plant stands harvested from the entire 1 hectare farm was determined by adding the plants harvested at 8, 10 and 12 months maturity periods.

The total sweetpotato farm cultivated (1 hectare) was divided into three portions before the 3 months maturity harvest. One portion of the farm was harvested at 3 months maturity. The total tubers of the Apomuden and Tuskiki harvested at 3, 4 and 5 months maturity was weighed and recorded. The total weight of roots obtained for the entire 1 hectare farm was determined by adding the weights of roots obtained at harvest of 3, 4 and 5 months.

6.2.1.2 Cost of production of one hectare of cassava and sweetpotato

The cost of production of one (1) hectare of cassava was determined by noting all the expenditure from land renting to harvesting of the crop. The cost elements included hiring of the land, land clearing, ploughing, composting, ridging, cost of planting material, manual planting labour, cost of pre-emergence herbicide application, labour for manual weeding, cost for post-emergence herbicide application, and labour for harvesting. The cost of production of one (1) hectare of sweetpotato was determined by noting all the expenditure from land acquisition to harvesting of the crop. The cost elements included hiring of the land, land clearing, ploughing, composting, ridging, cost of planting material, manual planting fee, cost of pre-emergence weedicide application, fee for manual weeding, and labour for harvesting.

6.2.1.3 Cost of producing 1 litre of ethanol from cassava and sweetpotato

The best result from the studies in terms of raw material for ethanol production was used for the economics of scale studies. A combination of cassava and sweetpotato in the ratio of 50:50 was used as the raw material base for this analysis. The amount of ethanol obtained from 50 g of Sika bankye and Apomuden (50:50 combination) according to the findings was 16.2% (v/v) which translates to 162 litres of ethanol per tonne of raw material. Pilot scale ethanol production trials with Sika bankye and Apomuden (50:50 ratio combination) was done on Caltech Ventures Ltd distillation plant at Ho in the Volta Region of Ghana with average yield of 155 litres of ethanol at 95% purity. A financial plan and analysis for large scale ethanol production was performed using the results from the research to ascertain the profitability or otherwise of an ethanol distilling plant of ten thousand (10,000) litres

per day capacity. The cost of the 10,000 litres per day capacity plant, the production cost of 1 tonne of cassava and sweetpotato, total labour costs of staff operating the ethanol plant, the utilities cost (energy and water), Consumables (Enzymes, Yeast, Cleaning agents), Communication, Tax and other incidental costs were used to determine the cost of processing fresh cassava and sweetpotato roots.

6.3 Results and Discussion

The production cost of one (1) tonne of fresh Sika bankye roots is GH¢100 and the production cost of one (1) tonne of fresh Apomuden tubers is GH¢138 (Table 6.1). The cost of production of one (1) tonne of 50:50 combination of Sika bankye and Apomuden is therefore Gh¢119 (GH¢50 + GH¢69). Table 6.6 shows the information on the income and expenditure of ethanol production over five (5) years period using Sika bankye and Apomuden. The laboratory recovery rate of ethanol from Sika bankye and Apomuden according to the study was 16.2% (v/v) which translates to 162 litres of ethanol per one tonne of raw material. Pilot scale ethanol production trials with Sika bankye and Apomuden (50:50) was done on Caltech Ventures Ltd distillation plant at Ho in the Volta Region of Ghana with yields of 155 litres of ethanol at 95% purity.

Table 6.1: Production cost and yield of fresh Sika bankye and Apomuden

	Sika Bankye	Apomuden
Production cost of roots/tubers per hectare (GH¢)	3,300	2,900
Yield of roots/tubers per hectare (tonne)	33	21
Production cost of roots/tubers per tonne (GH¢)	100	138

Table 6.2: Expenditure for cultivating 1 hectare of fresh cassava roots and sweetpotato tubers

	Expenditure (GH¢)	
Activity	**Cassava**	**Sweetpotato**
Land rent	200.00	200
Land preparation (Clearing, Ploughing, Composting, Ridging)	750.00	700
Planting material	300.00	200
Labour for planting	150.00	200
Pre-emergent herbicide	100.00	100
Manual weeding	250.00	300
Post-emergent herbicide	250.00	0
Harvesting	500.00	400
	800.00	800
TOTAL	**3,300.00**	**2,900.00**

The financial performance of the ethanol production from Sika bankye and Apomuden for 5 years with some underlying assumptions was carried out (Alexander, 2008; Brigham and Houston, 2005). Production of ethanol is expected to start from 70% capacity in year one to peak at about 85% capacity by year 5. The total capital required for this project was in the sum of GH¢15,234,900 which will cover the cost and installation of the ethanol plant and will be funded by debt (loan). It is envisaged that the enterprise can borrow this amount at an estimated cost of capital of 10% per year over a five (5) year period which is possible in the business environment in Ghana. At this cost of borrowing, the cash flows generated (Table 6.7) are adequate to service both principal and interest payments.

The key revenue line shall be sale of ethanol. The revenue assumptions include average price increases of 10% year on year. Average price per litre of ethanol in Ghana was GH¢4.7 (Caltech Ventures Ltd., 2017). Installed production capacity of 10,000 litres ethanol per day with one (1) set of equipment each operating at eight (8) hour shift and one shift a day. Optimal product mix is 100% ethanol and base case assumption is expected to remain unchanged. The cost assumptions include the cost of raw material in year one at GH¢ 119 per tonne with an average increase after each year at 10%. The primary cost element in the production cycle is the cost of fresh cassava which can be sourced either from the enterprises own farm or from out-grower or block farmers. Direct cost for the analysis comprises of raw material, labour and fuel cost as in Table 6.5.

Table 6.3: Details of the ethanol processing plant.

Cost of ethanol plant (GH¢)	15,234,900*
Capacity of plant (Litres per day)	10,000
Debt (10% per anum dollar rate)	15,234,900
Raw material (fresh cassava and sweetpotato) intake (tonne/day)	50
Days of processing (Per month)	20
Energy consumption at full load (Kw/hr)	225
Personnel (Number)	19
Price of ethanol (GH¢/litre)	4.7
Inflation (%)	10

*__Exchange rate of GH¢: USD is 1: 0.23__

Table 6.4: Monthly wages of staff working on the ethanol plant.

Personnel	Gross wage per month (GH¢)
Chief Executive Officer	6,500.00
Production Manager	4,500.00
Assistant Production Officer	3,300.00
Administrator	4,000.00
Laboratory Technician	2,500.00
Marketing Officer	3,500.00
Accountant	4,000.00
Estates Officer	1,800.00
Purchasing Officer	1,800.00
Factory hands (6)	7,200.00
Security (4)	3,600.00
TOTAL	**42,700.00**

Table 6.5: Ethanol direct production cost per month.

Cost Item	Cost per month (GH¢)
Fresh Cassava	119,000
Personnel cost	42,700
Electricity cost	58,128
Water cost	200
Administrative	10,000
Consumables	60,000
Repairs	5,000
TOTAL	**295,028**

Table 6.6: Income and expenditure on production of ethanol for five (5) years

Income/Expenditure item	AMOUNT (GH₵)				
	YEAR 1	YEAR 2	YEAR 3	YEAR 4	YEAR 5
Revenue from Ethanol	7896000	9306000	11601480	12761628	14037791
Direct material cost	-1289806	-1520129	-1895094	-2084604	-2293064
Staff cost	-512400	-614880	-737856	-885427	-1062513
Direct Expenses	-1419935	-1631921	-1879106	-2167807	-2505536
Repairs and Maintenance	-60000	-66000	-72600	-79860	-87846
Cost of sales	-3282141	-3832931	-4584656	-5217698	-5948959
Gross Profit	4613859	5473069	7016824	7543930	8088832
Sales and Marketing Expenses	-157920	-186120	-232030	-255233	-280756
Administration costs	-120000	-144000	-172800	-207360	-248832
Depreciation	-1523490	-1371141	-1234027	-1110624	-999562
Earnings before interest and tax	2534529	3441688	4973138	5508121	6030094
Interest charges on loan	-1409228	-1104530	-799832	-495134	-190436
Profit before tax	1125301	2337158	4173306	5012987	5839658
Tax (25%)	-281325	-584290	-1043326	-1253247	-1459915
Profit after tax	843976	1752869	3129979	3759740	4379744
Net Profit margin	**11**	**19**	**27**	**29**	**31**

Table 6.7: Cash flow of loan payment over three (3) years.

Year	Quarter	Opening balance (GH¢)	Principal repayment (GH¢)	Interest repayment (GH¢)	Closing balance (GH¢)
	1	15,234,900	761,745	380,873	**14,473,155**
	2	14,473,155	761,745	361,829	**13,711,410**
	3	13,711,410	761,745	342,785	**12,949,665**
One	4	12,949,665	761,745	323,742	**12,187,920**
	1	12,187,920	761,745	304,698	**11,426,175**
	2	11,426,175	761,745	285,654	**10,664,430**
	3	10,664,430	761,745	266,611	**9,902,685**
Two	4	9,902,685	761,745	247,567	**9,140,940**
	1	9,140,940	761,745	228,524	**8,379,195**
	2	8,379,195	761,745	209,480	**7,617,450**
	3	7,617,450	761,745	190,436	**6,855,705**
Three	4	6,855,705	761,745	171,393	**6,093,960**

Considering the field data collated on production of roots and tubers, the staff wages, details of the ethanol plant, (Tables 6.2 to 6.4) and other assumptions made, the profit to be generated in the first year of operations based on the model is Gh¢843,976 (Table 6.6). This is derived from gross sales revenue of Gh¢7,896,000. For subsequent years, the business operation would generate more profit after tax in all years increasing from an initial profit after tax of Gh¢1,752,869 in year two to Gh¢3,129,979 in year three and peaking at approximately Gh¢4,379,744 at the end of the fifth year (Table 6.6). The net profit margin is 11% in year one and increases to 31% at the end of year five (5). This is because the percentage increase in revenue

is more than the percentage increase in total cost. Producing ethanol using combination of Sika bankye and Apomuden in the ratio of 50:50 is therefore a profitable venture.

6.4 Conclusion

Ethanol production from Sika bankye and Apomuden in a mixture of 50:50 ratio for ethanol with 15.5% v/v ethanol yield, GH¢ 119.00 cost of fresh roots/tubers per tonne, selling price of GH¢ 4.7 per litre of ethanol and using a 10,000 litres per day capacity ethanol distilling plant generates net profit of between 11% and 31% over a period of five years.

CHAPTER SEVEN

GENERAL CONCLUSIONS AND RECOMMENDATIONS

The principal objectives of the investigations were;

- To optimize the conditions of ethanol production from cassava and sweetpotato.
- To determine the optimum maturity of cassava and sweetpotato for higher ethanol yields.
- To establish the yield of ethanol from different proportions of cassava and sweetpotato.
- To determine the profitability of ethanol production from cassava and sweetpotato

7.1 General conclusions

The results of the studies indicated that the optimum conditions for ethanol production from cassava and sweetpotato were liquefaction time of 2.1 hours using *Liquozyme SC DS* enzyme at 85^{o}C, combination of *Viscozyme L and Spirizyme Fuel* enzymes in a ratio of 1:1 for saccharification at 48^{o}C for 4 hours and the use of Bio-Ferm XR yeast for fermentation at temperature of 34^{o}C for 57.4 hours duration.

The nutrients evaluated in the cassava varieties at the same level of maturity were generally higher in Sika bankye than in Ampong except for ash. Sika bankye had more dry matter and higher starch content at the same level of maturity which eventually gave higher ethanol yield than Ampong. The best maturity date of cassava varieties evaluated for ethanol processing was 10 months. Sika bankye had the highest ethanol yield (14.8% v/v) between the two cassava varieties at maturity

period of ten (10) months. Sika bankye was therefore the best cassava variety for ethanol production. For sweetpotato, Apomuden had relatively higher nutrients than Tuskiki at all levels of maturity except for fat. Apomuden had more starch and produced more ethanol than Tuskiki at the same level of maturity. Apomuden had the highest ethanol yield (15.7% v/v) between the two sweetpotato varieties evaluated at maturity period of three (3) months. The best economical maturity date for processing Apomuden into ethanol according to the findings was therefore three (3) months.

Combining cassava and sweetpotato varieties (flours) for ethanol production yields more ethanol than processing cassava and sweetpotato separately according to the results. The best combination ratio of cassava and sweetpotato for optimum ethanol yield from the results is 50:50 which gave the highest ethanol yield of 16.2% v/v at laboratory scale but 155 litres of ethanol per tonne of raw material at 95% purity on pilot scale trial.

Processing Sika bankye and Apomuden in a mixture of 50:50 ratio for ethanol with 15.5% v/v ethanol yield, GH¢ 119.00 cost of fresh roots/tubers per tonne, selling price of GH¢ 4.7 per litre of ethanol and using a 10,000 litres per day capacity ethanol distilling plant generates net profit of between 11% and 31% over a period of five years. Production of ethanol from cassava and sweetpotato is therefore a profitable venture.

7.2 Contribution to scientific knowledge

- Information has been provided on optimising the yield of ethanol from cassava and sweetpotato varieties from the studies.
- The use of composite flour from cassava and sweetpotato for ethanol production has synergetic activity resulting in higher ethanol yields.
- Economic maturity dates for cassava and sweetpotato for ethanol production are 10 and 3 months, respectively.

7.3 Recommendations

- Synergetic effects of saccharifying enzymes was reported for the work. Time restraints could not allow this to be extended to the fermentation yeasts used for the work. Further studies is therefore recommended on combination of fermentation yeasts to evaluate for synergetic effect on ethanol production.
- Some improved sweetpotato varieties were released in Ghana. Further pilot ethanol production trials should be carried out with the sweetpotato varieties and Sika bankye to obtain the best sweetpotato variety for ethanol production.

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APPENDIX

Raw data.

Table 3.3: Extract yield (ºB) by starch hydrolysing enzymes.

Run order	Liquefaction time (hours)	Saccharification time (hours)	Brix (°B) from Viscozyme	Brix (°B)from Spirizyme	Brix (°B) from Spirizyme/Viscozyme
1	2	3	14.4	14.5	15.0
2	2	3	14.8	14.5	15.1
3	2	5	15.3	15.0	15.5
4	2	1	14.4	14.0	14.6
5	3	3	14.8	14.8	15.5
6	2	3	14.6	14.6	15.1
7	1	3	14.1	14.4	14.8
8	3	5	15.0	15.2	15.5
9	1	5	14.4	14.9	15.2
10	2	3	14.6	14.5	15.2
11	2	3	14.5	14.4	15.2
12	3	1	14.0	14.3	14.2
13	1	1	13.8	14.0	14.1

Table 3.4: Fermentation efficiency of Bio-Ferm XR and Baker's yeast.

Run order	Fermentation time (hours)	Fermentation temperature (ºC)	Bio-Ferm XR Attenuation (%)	Baker's Yeast Attenuation (%)
1	72	30	80	73.3
2	60	30	75.3	66.7
3	60	34	78.7	72.3
4	60	32	80.1	73.3
5	60	32	80	72.7
6	60	32	80.1	72.7
7	48	32	75.3	69.3
8	60	32	81.3	73.5
9	72	34	83.3	74.6
10	48	34	74.7	70
11	60	32	80.7	73.3
12	48	30	76.7	68.7
13	72	32	81.3	74.6