UTILIZATION OF AGRICULTURAL FOOD WASTE PRODUCTS FOR BIOETHANOL GENERATION, KIAMBU COUNTY, KENYA

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DECLARATION

DECLARATION BY CANDIDATE

This thesis is my original research work and has not been submitted for the award of any degree or diploma in any University.

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DEDICATION

To my parents Mr and Mrs Osei and my siblings Adwoa Achiaah and Salomey Serwaa for their love, encouragement, inspiration and support towards my education.

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LIST OF ACRONYMS / ABBREVIATIONS

E85	85% Ethanol
Mph	Miles per hour
OD	Optical Density
pH	Negative logarithm of Hydrogen ion concentration
ppm	Parts per million
Rpm	Revolutions per minute
UV	Ultra-violent
YPD	Yeast extract, Peptone and Dextrose

ABSTRACT

Increase in food supply has led to the generation of high quantities of wastes from agricultural products. Globally, 40-50% of food wastes arise from fruits, cereals and vegetables. Wastes from agricultural food products are easily degraded and difficult to separate from the whole quantity of wastes. There are also difficulties in handling and storage because of their high water and rich organic compositions. The study employed rare incubation techniques which are not yet utilized by researchers to produce bioethanol. The main objective was to utilize agricultural food wastes to generate bioethanol. The specific objectives were: i) To determine the amount of bioethanol that can be generated from different types of selected agricultural waste products (cereals, vegetables, fruits) under different incubation conditions and durations. ii) To optimise different incubation techniques at different temperatures for bioethanol production from selected agricultural food waste products. iii)To optimise different fermentation durations for bioethanol production from the selected agricultural food waste products. Food wastes were collected and subjected to physical pre-treatment and then enzymatic hydrolysis by the use of commercial enzyme cellulase from *Aspergillus niger* and finally anaerobic fermentation to convert the produced sugars into ethanol. The different incubation techniques used for fermentation took place in an incubator set at 30^{o}C, in the dark room at temperature 21^{o}C and under the soil at temperature 19^{o}C with different fermentation durations of 48 hours, 96 hours and 144 hours. All collected data were entered in excel before being subjected to analysis of variance using Genstat statistical package (Discovery version 4) at 5% level of probability ($P<0.05$). Ethanol productivity, concentration and yield were the three parameters used to determine the amount of bioethanol produced from each wastes. The highest bioethanol yield of 240.6 ml/kg was recorded from cereal wastes followed by fruits (156.0 ml/kg) and vegetables (63.5 ml/kg). There was decrease in bioethanol concentration and yield with increasing fermentation time in fruits and vegetables but for the cereals, there were gradual increases. In all the substrates used, there were decreases in bioethanol productivity with time. There were slight decreases in bioethanol concentration and yield from 19^{o}C to 21^{o}C and sharp increase from 21^{o}C to 30^{o}C in cereal substrates. In vegetable substrate, there were slight increases in bioethanol concentration and yield from 19^{o}C to 21^{o}C but sharp decrease at temperature 30^{o}C whilst in fruit substrates, there were slight decreases from 19^{o}C to 21^{o}C but sharp decrease at temperature 30^{o}C. Results showed that the optimum temperatures for cereals, fruits and vegetables were 30^{o}C, 19^{o}C and 21^{o}C respectively. The findings of this work suggest that agricultural food waste products are rich in fermentable sugars and can be used effectively for bioethanol production. This will be beneficial to the Ministry of Environment in Kenya in solving waste management issues confronting the nation. Also the use of incubators for fermentation especially in vegetable and fruit substrates can be replaced with dark room and under soil which are more economically feasible.

CHAPTER ONE: INTRODUCTION

1.1. Background Information

Agricultural wastes are residues obtained from raw products of agriculture such as fruits, vegetables, cereals, dairy products and crops (Obi *et al*., 2016). High amount of agricultural wastes are produced worldwide each year. The average rates of annual increases are 5%-10% (Wang *et al*., 2016). About 40-50% of global food wastes arise from fruits, vegetables and cereal crops (Jahid *et al*., 2018).

The disposal of wastes in early times was easy because of less waste that was generated as a result of low population and large land availability for their disposal (Thapa *et al*., 2017). Githurai is a rapidly growing town because of its proximity to Nairobi city and Thika town. The relative ease of transportation and its affordable housing makes it a preferred place for most people working in Nairobi city and Thika town. The increase in population has led to increased demands in food consumption resulting in the generation of high quantities of wastes in the area (Njoku, 2010).

The uncontrolled fermentation of these wastes in landfills causes emission of greenhouse gases. High quantity of water and the possibility of dioxin generation make incineration of food waste impractical. The friendliest method of managing food waste is by converting to ethanol fuel. Food wastes are potential source of fermentative substrates because of their rich organic content due to the presence of soluble sugar, starch, lipids, proteins, cellulose and other compounds (Moukamnerd *et al*., 2013).

Utilization of agricultural wastes into energy has gained worldwide recognition in recent years. The Intergovernmental Panel on Climate Change (IPCC) has included the role of agriculture in mitigating climate change at the top of the agenda, hence the conversion of agricultural wastes into energy

(example bioethanol, biogas) is one of the most feasible ways for agricultural waste pollution control. Agricultural wastes are now been considered as the main tool to achieve sustainable development in the economy and the environment when wisely utilized in energy production (Wei *et al*.,2020).

Kenya has recently identified energy as one of the key enablers on which the economic pillar of the Kenya's vision 2030 development blue print is built on under the Third Medium Term Plan 2018-2022 of the vision. The government has established regulations Energy Act 2019 in pursuit to improve the energy sector. The act created the Rural Electrification and Renewable Energy Corporation agency which is mandated to ensure the use of renewable energy technologies like bioethanol, biodiesel, biogas, biomass, etc. The Ministry of Energy will formulate a national strategy to coordinate research in renewable energy; promote the use of renewable sources of energy for either power generation or transportation and also promote the use of municipal waste for energy production (Otieno and Ogutu, 2020; Mugo and Gathui, 2010).

Ethanol is a universal organic dissolving agent that is produced in most countries. In 2001, 24.8 million tonnes of ethanol was produced out of which 92% was utilized as fuels. The rate of ethanol production in 2009 was 74 billion tonnes which later rose to 85 billion tonnes in 2011 (Khraisheh and Li, 2010; Oleskowiczn-Popiel *et al*., 2010; Saini *et al*., 2015). Brazil and USA are the leading producers of ethanol in the world with a total contribution of 85%. The main feed stocks utilized in Brazil and USA for ethanol production are sugarcane and corn respectively (Kim and Dale 2004; Azhar *et al.*, 2017).

Bioethanol production in Kenya started in 1977 with the construction of the Kenya Chemical and Food Corp (KCFC) to produce ethanol for blending. In 1983, another power alcohol plant known as Agro Chemical and Food Corp (ACFC) was also constructed to support the national blending programme. The fuel blending programme was abandoned in 1995 after the liberalization

of the industry due to insufficient policy framework and unsustainable commercial arrangements (Otieno and Ogutu, 2020; Ndegwa *et al*., 2011).

Currently, petroleum is the main source of commercial energy in Kenya and it accounts for about 80% of the energy requirements in the country. Fast growth in the transport and industrial sectors has resulted in increased energy demand in Kenya. Non-commercial sources of energy mostly used in Kenya are biomass whilst solar energy, wind power and biogas are less utilized (Wepukhulu, 2011). Bioethanol is environmentally and economically friendly as compared to fossil fuels. This makes it attractive to be used as a fuel either alone or in combination with other fuels (Thapa *et al*., 2017).

Bioethanol production which requires the utilization of feed stocks rich in sucrose and starch is termed as first generation bioethanol whilst feed stocks rich in lignocellulose biomass and algal biomass are termed as second generation and third generation bioethanol respectively (Azhar *et al*., 2017). Lignocellulosic biomass is the biodegradable portion of organic products, waste and residues which are normally obtained from agriculture, forestry and industries (Muktham *et al*., 2016). Wastes from agricultural food products contain lignocellulose which consists mainly of two structural polysaccharides termed as cellulose and hemicellulose which easily undergo hydrolysis to generate fermentable sugars glucose and xylose respectively (Tropea *et al.,* 2014).

1.2 Problem Statement and Justification

Observations from visits to Githurai market revealed the largest proportion of wastes to be from food products like oranges, pineapples, bananas, corn wastes etc. They are disposed off haphazardly and inappropriately to the extent of blocking trenches meant for drainage. These wastes decompose rapidly because of their high water content and rich compositions causing irritating odours in the market. Rodents, insects and scavengers are common in the market because of the heaps of garbage creating unfavourable atmosphere

in the market. There is high risk of health hazards like cholera, typhoid etc. in the area.

The major issues confronting developing countries are improper management of waste and energy insufficiency (Miezah *et al*., 2007). The increased population has led to the decline in the production of oil globally from 25 billion barrels to roughly 5 billion barrels which in effect cause deficiency in energy (Byadgi and Kalburgi, 2016). In Kenya, a strategy was developed to introduce bioethanol blends in the market but the programme has not yet begun to date (Otieno and Ogutu, 2020). There is increased demand for petroleum which is the main source of commercial energy in Kenya recently by the transport and industrial sectors (Wepukhulu, 2011). According to a report by Solomon *et al*. (2007), the use of fossil fuels has resulted in rise in global temperature from 1.4 to 5.8ºC. Fossil fuels have significant impacts on human health due to the various forms of diseases such as heart disorders, cancers etc. associated with their emissions (Hossain and Fazliny, 2010).

Conversion of these wastes to bioethanol will reduce their quantities in the market in addition to fuel generation. It will also add values to them instead of allowing them to cause nuisance in the environment. Utilization of wastes in bioethanol production is now highly considered rather than energy crops due to the rise in food prices (Nwosu-Obieogu *et al*., 2016). Agricultural food waste products are cost effective, renewable and abundant in quantities which make them suitable substrates to be used in the generation of bioethanol (Shrivastava *et al*., 2014; Nwosu-Obieogu *et al.*, 2016; Khamala and Alex, 2013).

Bioethanol causes less effect on the environment making it environmentally friendly (Byadgi and Kalburgi, 2016). It is more advantageous to use as fuels as compared to petroleum due to its low toxicity, easily degradability, low emissions of air-borne pollutants and also higher latent heat of vaporization (Azhar *et al.*, 2017). It also has higher energy content making it a potential

transportation fuel and a fuel oxygenate that can successfully replace gasoline (Kim and Dale, 2004). The replacement will cope with fossil fuel resources depletion and also minimise the emission of CO_2 into the atmosphere which in effect will reduce the risks of global warming (Ali *et al*., 2016).The implementation of bioenergy production in developing countries will boost their growth and development (Miezah *et al*., 2007).

1.3 Research Questions

a) What is the amount of bioethanol that can be produced from each type of agricultural food waste products under different incubation conditions and durations?
b) Which incubation techniques generated the maximum bioethanol from each agricultural food waste products?
c) What fermentation duration was optimum for bioethanol production in each agricultural food waste products?

1.4 Research Objectives:

1.4.1 Main Objective

- To utilize agricultural food waste products to generate bioethanol.

1.4.2. Specific objectives

i. To determine the amount of bioethanol that can be generated from different types of selected agricultural food waste products (cereals, vegetables, fruits) under different incubation conditions and durations.
ii. To optimise different incubation techniques at different temperatures for bioethanol production from selected agricultural food waste products.
iii. To optimise different fermentation durations for bioethanol production from the selected agricultural food waste products.

1.5 Hypothesis

- The amount of bioethanol produced from each agricultural food wastes is equal.

- There is a significant difference in the amount of bioethanol produced from each agricultural food wastes at different incubation temperature conditions.
- There is a significant difference in the quantity of bioethanol produced from each agricultural food wastes at different fermentation durations.

1.6 Significance of the study

The study applied simple, reliable, effective and economical incubation methods for the fermentation of bioethanol. It has proved the feasibility of using traditional methods in place of incubators for bioethanol fermentation process. These rare incubation methods can be utilized by other researchers especially in developing countries for bioethanol production. Researchers who cannot afford incubators or research conducted in remote areas where there is no electricity, these conventional methods can be applied to achieve good results. The results from the study has proved the efficiency of waste conversion into bioethanol and this idea can be employed by the Ministry of Environment in Kenya to solve waste management issues confronting the nation. It can also be beneficial to the Kenya energy sector in reforming their policies to include organic wastes as feedstock for ethanol production in Kenya instead of relying only on molasses in order to reduce pressures on the sugar industries.

1.7 Conceptual framework

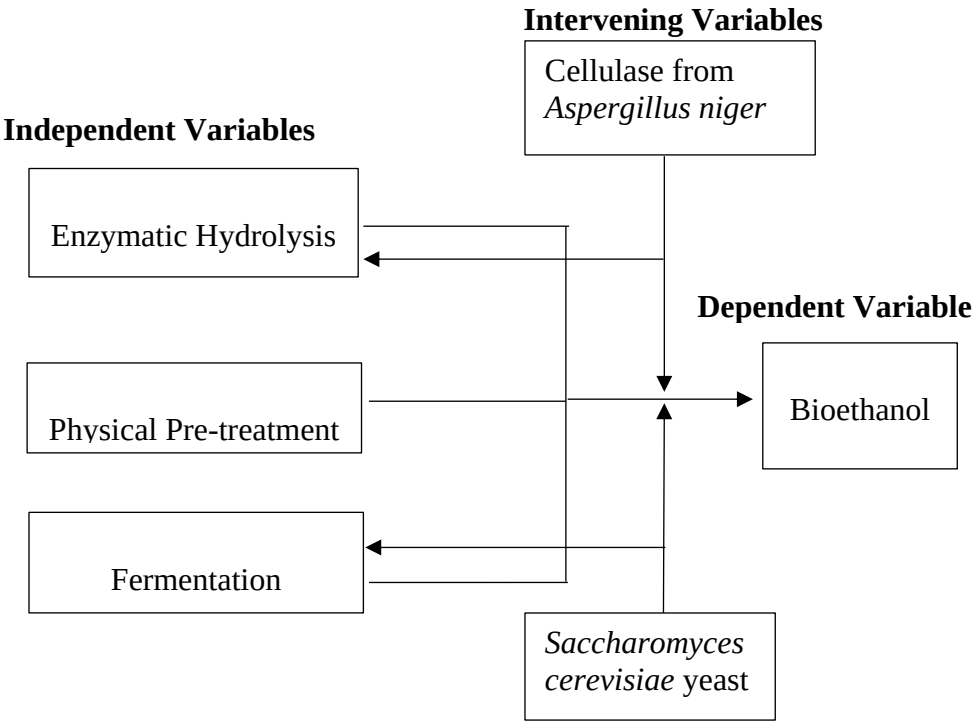

Fig 1.1: Conceptual framework shows the dependent, intervening and independent variables for bioethanol production from agricultural food wastes.

Bioethanol production depends on the independent variables (enzymatic hydrolysis, physical pre-treatment and fermentation) and intervening variables (cellulase from *Aspergillus niger* and *Saccharomyces cerevisiae*). Wastes were physically pre-treated in order to increase their surface area for hydrolysis. Enzymatic hydrolysis was carried out by the use of cellulase from *Aspergillus niger* whilst fermentation into bioethanol was done by using *Saccharomyces cerevisiae.*

1.8 Definition of Terms

Physical pre-treatment: It is a type of pre-treatment that requires the breaking of the structure of biomass to enhance their hydrolysis rate by maximising the surface area for chemical and enzymatic reactions (Moukamnerd *et al*., 2013).

Hydrolysis: It is the second stage in bioethanol production where the feedstock is converted into sugars that are easily fermented into ethanol (Azhar *et al*., 2017).

Fermentation: It is anaerobic metabolic activity that converts hydrolysates into ethanol (Triana, 2016).

Biomass: It is a term for all organic materials which are produced through the process of photosynthesis (McKendry, 2002).

Enzymes: Biological catalysts that increase the rate of biological and chemical reactions in living organisms (Robinson, 2015).

Cellulase: It is a set of enzymes that is used in the hydrolysis of cellulose (Saini *et al*., 2015).

Anaerobic metabolism: It is the biochemical decomposition of organic materials by bacterial activities without the use of oxygen (Lohri *et al.*, 2017).

Hydrolysates: These are the substances produced from the process of hydrolysis**.**

Supernatant: It is a clear liquid of lower destiny and free of precipitate that lies on top of the solid residue after centrifuging, crystallizing or settling.

Centrifugation: It is a process that uses centrifuge to separate the components of complex mixtures.

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

This chapter highlights the importance of reviewing literature and how it can be useful to lay the groundwork for the methods used to produce bioethanol from agricultural waste products. Present design and methods used to convert agricultural waste products to ethanol are discussed in this session. The chapter reviews the concept of bioethanol production from agricultural waste products in order to address the problem of waste management and fuel crisis. Previous works under these concepts are also reviewed.

2.2 Biofuels

Biofuels are energy sources or carriers that are obtained from living materials. They are produced through thermo-chemical processes like liquefaction, pyrolysis, gasification etc. from bio-based materials (Woldesenbet *et al*., 2016). Biofuel production is a reliable substitute for the generation of energy in order to minimize the use of fossil fuels which emissions cause an increase in global warming. Biofuels are classified as primary and secondary biofuels based on their source and type. Primary biofuels are in an unrefined form and normally used for cooking, heating and the generation of electricity. They are obtained from by- products in processes like municipal wastes, food industries, agriculture, and deforestation. Secondary biofuels are produced from biopolymers that are found in raw materials through biological ways like anaerobic metabolism or fermentation (Triana, 2016).

Secondary biofuels can be divided further into first, second and third generation based on the type of raw materials and technology used. The first generation is the biofuels that are derived from seeds, grains and sugars through fermentation. The second generation is the biofuels that are derived from lignocellulose biomass to either generate ethanol through hydrolysis and fermentation or methane through anaerobic digestion. The third generation biofuels are obtained from algae and sea weeds. Biofuels can be solids like fuel wood, charcoal and wood pallets; or liquids like ethanol, biodiesel and

pyrolysis oils; or gaseous like biogas and hydrogen (Triana, 2016). Biofuels are environmentally friendly energy sources and also provide economic benefits to the agriculture industry worldwide (Bawa, 2008).

2.3 Bioethanol

Ethanol is derived from sugars through hydrolysis and fermentation. Any feedstock containing sufficient quantity of sugar or materials which can easily be converted into sugar is suitable for ethanol production. Ethanol is a volatile, flammable and colourless chemical compound. It has unique chemical characteristics as a solvent, beverage, antifreeze, fuel, sedative, disinfectant and it is also versatile when used as a chemical intermediate for other organic chemicals. Ethanol has a molecular weight of 46.07g/mol, boils at 78.5°C and has the molecular formula C_2H_5OH. It easily mixes with water in all dimensions but when is completely free of water is known as absolute ethanol. Bioethanol is an ethanol obtained from biomass (Gerlach, 2012; Endalew, 2015).

Bioethanol was first utilized as a motor fuel in the internal combustion engine that was invented by Nikolas Otto in 1897. The oil crises in the 1970s led to the establishment of ethanol as an alternative fuel. It is predominantly generated currently from corn and sugarcane derived feed stocks. These feed stocks are used to produce first generation bioethanol (Lohri *et al*., 2017). The drawbacks in the use of first generation bioethanol is that the raw materials involved are inadequate in meeting the higher fuel demands and may eventually lead to deforestation in order to obtain enough farmland. These negative impacts have led to the evolvement of second-generation bioethanol. The raw materials involved in the second-generation bioethanol are from agricultural residues, wood, paper and municipal solid waste (Saini *et al*., 2015).

Bioethanol is known globally as a transportation fuel with economic and environmental merits. It is mainly used in inks and coatings as carrier solvents; in cosmetic preparations like hair setting sprays; in pharmaceutical

and personal care products like mouthwashes and also in detergent preparations (Gashaw and Getachew, 2014). The carbon dioxide that is released after burning ethanol is recycled back into plant materials which they use during photosynthesis to synthesize cellulose. This makes ethanol an environmentally friendly energy resource because the processes in their production use energy from energy resources that are renewable hence causing a closed carbon system where there is no net carbon dioxide addition to the atmosphere (Li and Khraisheh, 2008). Ethanol is made up of 35% oxygen that enhances complete fuel combustion and hence minimises the emission of particulate materials that poses health problems to living beings (Saini *et al*, 2015). Ethanol has an octane number of 107 and an energy density of 29.6kJ/g which implies that it can be a potential source of fuel for engines and also in the generation of electricity (Triana, 2016). Ethanol can be used to replace petrol and also as a blend of ethanol/hydrocarbon. It is used effectively in the production of Ethyl Tertiary Butyl Ether (bioETBE) by its chemical reaction to isobutylene-A, a by-product of the petroleum refining process (Awole, 2014).

2.4 Bioethanol Production from Agricultural Waste Products

Bioethanol generated from agricultural waste is known as second generation bioethanol. Agricultural waste products which include corn cobs, banana peels, orange peels, pineapple peels and so on are examples of second-generation feed stocks (Priyanka *et al*., 2019). Agricultural waste products consist of lignocellulose which is considered as an adequate raw material for the production of ethanol (Wyman, 1996). Globally, lignocellulose is known as the preferred biomass for fuels and chemicals production. It is the most common and widespread carbon source in nature and is known to be the only source capable of providing enough feedstock to meet the world's energy and chemical needs in a renewable manner. Lignocellulose materials can generate up to 442 billion litres of bioethanol per year (Woldesenbet *et al*., 2016).

Lignocellulose biomass is made up of raw materials which comprise primarily of cellulose, hemicellulose and lignin. Cellulose $(C_6H_{10}O_5)n$ is a linear

homopolysaccharide composed of β-D glucopyranose units, bound by β-(1-4)-glycosidic bonds, forming cellobiose molecules linked in long chains. The long chain polymers connected by bonds of hydrogen and Van der Waals result in a packed micro fibril covered with hemicelluloses and lignin. Native cellulose's degree of polymerization is in the range of 7,000-15,000. Cellulose consists of sheets that form a flat surface of glucopyranose rings. The replication on top of each other of these flat sheets shapes the three-dimensional cellulose structure. Cellulose is converted to 5-hydroxymethylfurfural (HMF) glucose in a harsh environment. The HMF is further decomposed to levulinic acid and formic acid. Hemicellulose $(C_5H_8O_4)n$ is a complex polymer of carbohydrates that acts as a linking agent between lignin and cellulose. Hemicellulose's composition is spontaneous and amorphous, with much less power than cellulose. They are partly soluble in water due to their amorphous nature. They are often called pentosans or polyoses because they consist of different units of sugar such as D-xylose, D-mannose, D- galactose, D- glucose, L-arabinose and glucuronic acids. At example β-(1,4) and sometimes β-(1,3)-glycosidic bonds, the backbone of hemicellulose can be either a homo-polymer or hetero-polymer with short branches. There is also some degree of heteroxylan acetylation in hemicelluloses (Erdei *et al*., 2013; Purwadi, 2006; Johansson, 2013; Taymaz, 2013).

The distinction between hemicelluloses and celluloses is that hemicelluloses consist of different sugar units and have shorter and branched molecular chains, they also have random, amorphous structure with low strength that make them easily hydrolysed by dilute acid or base and hemicellulase enzymes but celluloses are crystalline, strong and hydrolysis resistant (Talebnia, 2008). Lignin ($C_6H_{11}O_2$) is a biopolymer that connects the material's cellular walls with cellulose and hemicellulose (Traina, 2016). Lignin's structure has a complicated three dimensional and highly cross-connected amorphous heteropolymer that is made up of three central phenyl-propane units that are connected by various forms of linkages. The degree of

polymerization of lignin is difficult to define because its structure is split during extraction and its substructures also have irregular repeating units (Erdei *et al*., 2013; Taymaz, 2013). Lignin is a complex natural polymer that is it extremely resistant to chemical and biological degradation due to its structure and heterogeneity (Talebnia, 2008). During hydrolysis, lignin is partially decomposed to phenolic compounds of high and low molecular weight. The small molecular weight compounds are highly inhibitory to *Saccharomyces cerevisiae* (Johansson, 2013).

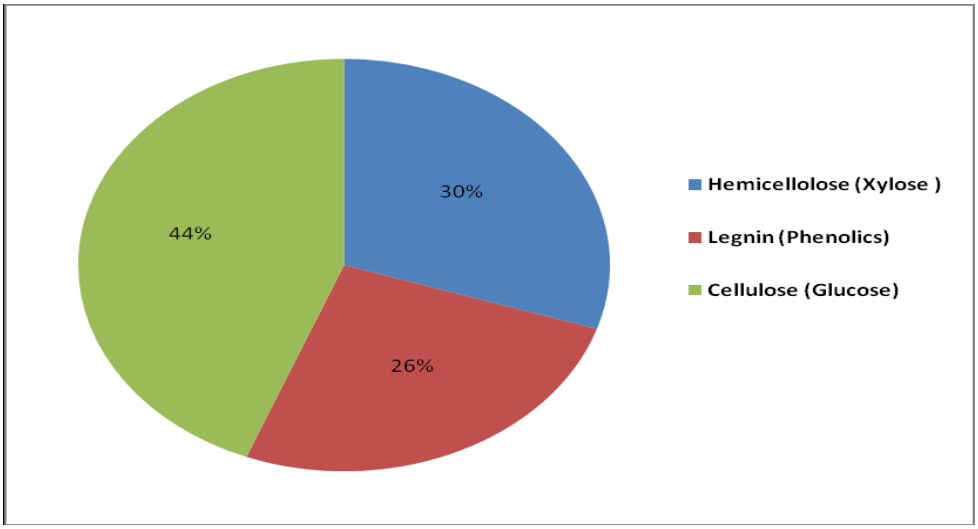

Fig 2. 1: Constituents of lignocellulosic materials (Woldesenbet *et al.*, 2016).

Bioethanol production from lignocellulose materials involves three main stages. They are: pre-treatment stage for delignification to release cellulose and hemicellulose prior hydrolysis; the hydrolysis of cellulose and hemicellulose to produce fermentable sugars and the fermentation of the reduced sugars into bioethanol (Sarkar *et al*., 2012).

2.4.1 Pre-treatment Stage

Pre-treatment is used to change the size and structure of the macroscopic and microscopic biomass and also the chemical composition and structure of the sub microscopic biomass (Chandel *et al*., 2007). The pre-treatment stage requires high temperatures and pressures to separate the bonds and release

hemicellulose and part of cellulose to make them more susceptible to the activity of chemical agents (Triana, 2016). The purpose of this stage is to improve the substratum's surface area and porosity, minimise cellulose crystallinity and break up cellulosic materials' heterogeneous structure. Pre-treatment facilitates hydrolysis to produce higher fermentable sugars. Pre-treatment together with hydrolysis lead to the production of fermentable pentose and hexose sugars leaving lignin as a by-product that can successfully be utilized as a fuel for the development of heat or electricity (Talebnia, 2008).

Pre-treatment is the most important factor for successful cellulosic bioethanol technology because it determines the degree of conversion of cellulose and hemicellulose carbohydrates into bioethanol. A successful pre-treatment must conform to the following demands: enhance sugar formation or the capability to form sugars subsequently by hydrolysis; avoid carbohydrate degradation or loss; prevent the formation of by-products that form inhibitors in the subsequent processes of hydrolysis and fermentation; and be less expensive (Li *et al*., 2007).

Techniques used in pre-treatment require the solubilisation and removal of one or more biomass constituents to render the remaining solid biomass more available for subsequent treatments by chemicals or biological agents. The methods used for pre-treatments are physical, chemical and biological (Hafid *et al*., 2017; Sarkar *et al.,* 2012). Inhibitors like furfural, carboxylic acid and phenolic compounds result in a decline in fermentation yield and therefore it is important to select the right pre-treatment method in order to reduce undesirable effects on the final yields of ethanol (Taymaz, 2013).

2.4.1.1 Physical pre-treatment methods

Physical pre-treatment requires the breaking of biomass structure to improve hydrolysis rate in order to increase available surface area for chemical and enzymatic reactions. The use of chemical agents is not required in physical pre-treatment. It includes un-catalyzed steam explosion, liquid hot water pre-treatment, mechanical comminution and radiation from high energy. The

steam explosion and liquid hot water methods are commonly used (Hafid *et al.*, 2017; Sarkar *et al.*, 2012).

2.4.1.1.1 Un-catalyzed Steam Explosion (Auto-hydrolysis)

It is a pre-treatment technique in which high pressure steam heats lignocellulosic biomass rapidly without adding any chemicals for an amount of time to facilitate hemicellulose hydrolysis (Li *et al*., 2007). Treatment time, temperature, particle size and moisture content are the key factors involved in un-catalyzed steam explosion. It is normally conducted for several seconds to a few minutes at a temperature of 160^{o}C-270^{o}C before the pre-treated contents are discharged into a cooling vessel. Longer temperatures and longer periods of residence time are better than higher temperatures and shorter periods. Utilization of very small particles is not beneficial in optimising the process efficiency (Sarkar *et al.*, 2012).

Un-catalyzed steam explosion requires low amount of energy and no reuse or environmental costs, but the main drawbacks associated with this method include: destroying a portion of the xylan fraction; partial breakdown of the lignin- carbohydrate matrix and the production of compounds that may inhibit microorganisms used in hydrolysis and fermentation processes (Gashaw and Getachew, 2014).

2.4.1.1.2 Liquid Hot Water Pre-treatment

This method is also referred to as hydrothermal pre-treatment. It alters the structure of the insoluble fractions to make them more easily biodegradable. This method does not require any acid or chemical and is carried out at temperatures ranging from 60^{o}C to 170^{o}C, therefore it is cost effective and environmentally friendly (Hafid *et al*., 2017). Under this method pressure is used to keep water at high temperatures in the liquid state. The biomass goes through cooking at high pressure in water at high temperatures. This approach improves the digestibility of cellulose, sugar extraction and pentose recovery by generating pre-hydrolysates with little or no sugar fermentation inhibitors (Sarkar *et al.,* 2012). It solubilises mainly the hemicellulose followed by lignin

which is partially depolymerized to make the cellulose easily accessible and reduce inhibitor formation. This method has high water input which causes a lower amount of solubilized hemicelluloses and lignin products hence reducing the concentration of degradation products. Inhibitory products are not formed in the various fractions so that higher yields are possible under specific conditions (Zheng, 2014; Bhatia *et al*., 2012).

2.4.1.1.3 Mechanical comminution

It is a sequence of activities including chipping, grinding, milling and /or combination of the activities that are used to minimize biomass particle size. This method primarily destroys the crystallinity of cellulose, reduces the degree of polymerization and maximises the specific surface area of cellulosic biomass by disrupting the biomass into smaller particles. Surface width is usually 10-30 mm after chipping and 0.2-2 mm after milling or grinding. Milling can be divided into different types such as hammer and ball milling (wet, dry, vibratory rod/ball milling), compression milling, ball milling / beating, agitation bead milling, pan milling and other forms of milling (including fluid energy milling, colloid milling, two roll milling). Vibratory ball milling is more efficient than the ordinary ball milling in terms of disrupting the cellulose crystallinity of spruce and aspen chips and enhancing the digestibility of biomass. Compression milling is the only process that has been validated and widely used on a production scale. Mechanical comminution involves reducing the particle size to less than 12mm in order to attain efficiency in production yield. The power required in the mechanical comminution process involving the use of agricultural materials depends on the properties of the waste biomass and the final particle size (Li *et al*., 2007; Sarkar *et al*., 2012; Bhatia *et al*., 2012).

2.4.1.1.4 High energy radiation

This involves the use of radiation, ultrasound, electron beam, pulsed electrical field, UV and microwave heating. The mode of action in high energy radiation methods could be one or more changes in the characteristics of cellulosic

biomass including increased specific surface area, decreased polymerization and crystallinity of cellulose, hemicellulose hydrolysis and incomplete depolymerisation of lignin. These methods are slow, require high amount of energy, expensive and strongly substrate specific (Sarkar *et al*., 2012).

2.4.1.2 Chemical pre-treatment

The main objective of chemical pre- treatment is to increase cellulose's biodegradability by eliminating lignin and/or hemicellulose and reducing the degree of polymerization and crystallinity of the cellulose constituents. It increases the solubility of the cellulose by adding chemical substitutes or hydration in order to swell the cellulosic fibres (Sarkar *et al*., 2012). Chemical pre-treatment results are dependent on the methods used and the substrate properties (Hafid *et al*., 2017). The commonly used chemical pre-treatments methods are acid, alkaline, catalysed steam explosion, ammonia fibre/freeze explosion, organosolv, pH-controlled liquid hot water and ionic liquids pre-treatments.

2.4.1.2.1 Catalyzed steam explosion

This method is almost equal to un-catalyzed steam explosion method depending on their mode of action. The only difference is that this method involves the use of acidic chemicals (gases and liquids) which includes SO_2, H_2SO_4, CO_2, oxalic acid etc. as catalysts used to infuse the biomass before steam-explosion. Catalyzed steam explosion has more complete removal of hemicellulose resulting in improved enzymatic digestibility of biomass with less inhibitory compounds compared to un-catalyzed steam explosion (Sarkar *et al*., 2012).

2.4.1.2.2 Acid pre-treatment

The method was derived from hydrolysis involving the use of concentrated acids. It utilizes acids like dilute sulphuric acid, dilute nitric acid, dilute hydrochloric acid, and dilute phosphoric acid, per acetic acid etc. This method solubilizes hemicellulose and remains intact with lignin and cellulose in order to improve cellulose's enzymatic digestibility. The most widely used acid-

based pre-treatment method is sulphuric acid because it is less expensive and effective. Acid pre-treatment provides a suitable environment for micro-organisms growth in the biomass hydrolysate aside breaking down the lignin component. The biomass portion of hemicellulose is hydrolysed into monosaccharides; xylose, mannose, glucose and galactose (Sarkar *et al.,* 2012; Hafid *et al*., 2017).

Dilute acid pre-treatment shows higher xylose yield as compared to steam explosion. Dilute acid pre-treatment is frequently used industrially because of its relatively cheap operational and process maintenance with low fermentation inhibitors produced as well as their capability of recovering up to 80% of the initial amount of hemicellulose sugars. The use of concentrated acids cause the formation of high amount of degraded by-products and unwanted inhibitory compounds, loss of fermentable sugars due to increased sugar degradation at extreme temperatures and additional costs to neutralize acidic conditions before fermentation to bioethanol (Sarkar *et al.,* 2012; Hafid *et al*., 2017). It also has environmental problems as a result of the use of strong acids which make the process corrosive and not safe. These disadvantages make the application of concentrated acid in pre-treatment less attractive (Taymaz, 2013).

2.4.1.2.3 Alkaline pre-treatment

It involves the combination of hydrogen peroxide etc. with sodium hydroxide, calcium hydroxide (lime), potassium hydroxide, aqueous ammonia, ammonia hydroxide and sodium hydroxide. The mostly used chemicals in this method are calcium hydroxide (lime) or sodium hydroxide. They form salts that are incorporated into the biomass or that need to be extracted. The conditions of reaction used in this method are moderate, but longer reaction time is required. The moderate conditions contribute to better lignin solubilisation particularly in plant materials with low lignin content. This also results in less degraded product formation. The action mechanism involves in alkaline pre-treatment is saponification of intermolecular ester bonds between xylan hemicelluloses and other components. Alkaline pre-treatments on lignocellulosic materials cause

swelling which results in decreased polymerization and crystallinity, increased internal surface area, breaking of lignin structure and the division of structural connections between lignin and carbohydrates (Sarkar *et al.*, 2012; Taymaz, 2013).

The main effect of this form of pre-treatment is the release of lignin from the lignocellulose feed stocks to improve the digestibility of cellulose. Compared to the acid cycle, they also exhibit slight cellulose and hemicellulose solubilisation. Alkaline pre-treatment performance changes with factors like substrate and treatment conditions. The use of alkaline pre-treatment on agricultural residues and herbaceous crops is more efficient than on wood materials. Ammonia pre-treatment comprises the ammonia fibre explosion method, ammonia recycle percolation and soaking in aqueous ammonia. The ammonia fibre explosion process exposes the biomass to hot liquid ammonia for a specific duration under high pressure after which the pressure is instantly released. The rapid pressure reduction opens up the lignocellulosic biomass structure leading to increased biomass. This method de-lignifies and solubilizes some hemicellulose simultaneously and at the same time recrystallizes cellulose (Zheng, 2014; Li *et al*., 2007).

Ammonia fibre explosion method needs effective ammonia recovery to be cost effective due to high ammonia cost. The advantages associated with this process are lower moisture content, lower sugar degradation product formation as a result of mild conditions, 100% solid material recovery and the capability of ammonia to reduce the effect of lignin on enzymatic hydrolysis. The major parameters that influence this method are ammonia loading, temperature, high pressure, biomass moisture content and residence time (Gashaw and Getachew, 2014; Bhatia *et al*., 2012).

2.4.1.2.4 Organosolv

This process requires delignification with different simultaneous hemicellulose solubilisation. Under this method, the bonds of the internal lignin and hemicellulose are broken by an organic or aqueous organic solvent

mixture with or without an acid or alkali catalyst. Methanol, ethanol, acetone, glycerol etc. are examples of organic solvents used (Sarkar *et al*., 2012). In this method, organic acids such as oxalic, acetylsalicylic and salicylic acid can be used as a catalyst. Adding catalysts at high temperatures (above 185^{o}C) are unnecessary for satisfactory delignification (Sun, 2002). This process produces comparatively high quality lignin as a by-product that can be used for applications with higher value of lignin. The solvent must however, be extracted and recovered to reduce the cost and the environmental impact that can hinder enzymatic and fermentation processes (Zheng, 2014).

2.4.1.2.5 pH- Controlled liquid hot water

In this process, water is used to improve the disintegration and separation of the lignocellulosic constituents. The time for processing changes from a few minutes to hours depending on the pre-treatment temperature which is commonly in the range of 160^{o}C-240^{o}C (Taymaz, 2013). During the process of liquid hot water pre-treatment method, pH value usually drops to below 4 which lead to inhibitor formation as a result of carbohydrate degradation. Through incorporating bases such as KOH, the pH value of liquid hot water can be regulated between 5 and 7. The main function of the base is to keep the pH level above 5 and below 7 to limit monosaccharides hydrolysis (Sarkar *et al*., 2012).

2.4.1.2.6 Ionic liquids pre-treatment

Ionic liquids pre-treatment break up large quantities of cellulose under relatively moderate conditions and about 100% of ionic liquids used are restored to their original purity. The process of cellulose dissolution in ionic liquids requires oxygen and hydrogen atoms of cellulose hydroxyl groups to form electron donor-electron acceptor complexes that react with the ionic liquids. Cellulose-OH interaction with ionic liquids cause the hydrogen bonds to be disrupted resulting in the exposure of hydrogen bonds between cellulose molecular chains. This interaction finally leads to cellulose dissolution. Fast precipitation with anti-solvents like water, ethanol, methanol or acetone

recovers the solubilized cellulose. This method is expensive, lack toxicological data and produce inhibitors that influence enzymatic and fermentation processes (Sarkar *et al*., 2012; Zheng, 2014).

2.4.1.3 Biological pre-treatment

This method makes use of wood decomposing microorganisms which include white, brown, soft-rot fungi and bacteria to change the chemical composition and /or lignocellulosic biomass structure to make it easier for enzyme digestion. They are mostly used in waste materials to degrade lignin and hemicellulose. Cellulose is mainly destroyed by brown rots while white and soft rots destroy both cellulose and lignin. The most active basidiomycetes for biological pre-treatment of lignocellulosic materials are the white rot fungi (Li *et al*., 2007). The advantages in the use of this method include no chemical demand, low energy requirements and environmentally sustainable working conditions. The method takes a very long time and need cautious monitoring of growth conditions as well as large space for treatment quality. These drawbacks make them commercially less attractive (Sarkar *et al*., 2012).

2.4.2 Hydrolysis Stage

Hydrolysis is the second stage in bioethanol production that converts feedstock into fermentable sugars (Azhar *et al*., 2017).

The equation for the process of hydrolysis

$(C_6H_{10}O_5)_n + n\ H_2O \longrightarrow nC_6H_{12}O_6$ (Sarkar *et al*., 2012).

The hydrolysis process is affected by factors including porosity or available surface area, crystallinity of cellulose fibre, and lignin and hemicellulose content. Acids and enzymes are mostly used in this approach. Acid hydrolysis involves a complicated heterogeneous reaction between solid and liquid. Typically this process is applied after pre-treatment with acid. Acids normally used are sulphuric, sulphurous, phosphoric, nitric, hydrochloric acids etc. Acid hydrolysis is sub divided into concentrated and dilute acid hydrolysis. The concentrated acid method involves the treatment of biomass with high acid

concentration at near environmental temperatures and the use of minimal pressure created by moving the materials from vessel to vessel. This method has a very high yield of sugar, relatively cheap with little degradation but it takes time and requires an acid recovery system to prevent excess concentrated acid from killing yeast that are introduced into the glucose product. Dilute acid hydrolysis makes use of acids with low concentration and is carried out under high temperature and pressure with a reaction time within seconds or minutes. In this process, the use of high temperatures increases the rate of decomposition of sugar and corrosion of equipment. The process is costly to run and sugar decomposition lowers the overall sugar yields and also produces a variety of by-products with significant inhibitory effects on the fermentation process (Li *et al*., 2007; Talebnia, 2008; Maurice, 2011; Chandel *et al.,* 2007). Acid hydrolysis drawbacks include by-product inhibition of yeast growth (such as 5-hydroxymethylfurfural (5-HMF)), neutralization prior to fermentation and expensive materials for construction due to the corrosion hazards (Woldesenbet *et al*., 2016).

Enzymatic hydrolysis is the use of enzymes to disrupt cellulose into sugars. Cellulose enzymatic hydrolysis is performed using cellulase enzymes. Three steps are involved: adsorption of cellulase enzymes to the cellulose substrate, biodegradation of cellulose to fermentable sugars and cellulose desorption (Li *et al*., 2007). Different factors related to operating conditions and cellulase inhibition influence enzymatic hydrolysis. It is possible to optimize operations such as temperature, time, pH, enzyme dosage and solid loading for maximum yield. The inhibition factor of the cellulase is associated with the slow -down of cellulase activity during enzymatic hydrolysis due to the cellulose/lignin irreversible adsorption of cellulase. High dosage of enzymes is therefore necessary for acceptable hydrolysis of cellulose (Benjamin, 2014). Enzyme hydrolysis has high glucose conversion yield but enzymes costs are high (Woldesenbet *et al*., 2016). There is low toxicity and corrosion when compared with acid hydrolysis. No inhibitory by-products are formed when enzymatic hydrolysis is used (Gashaw and Getachew, 2014).

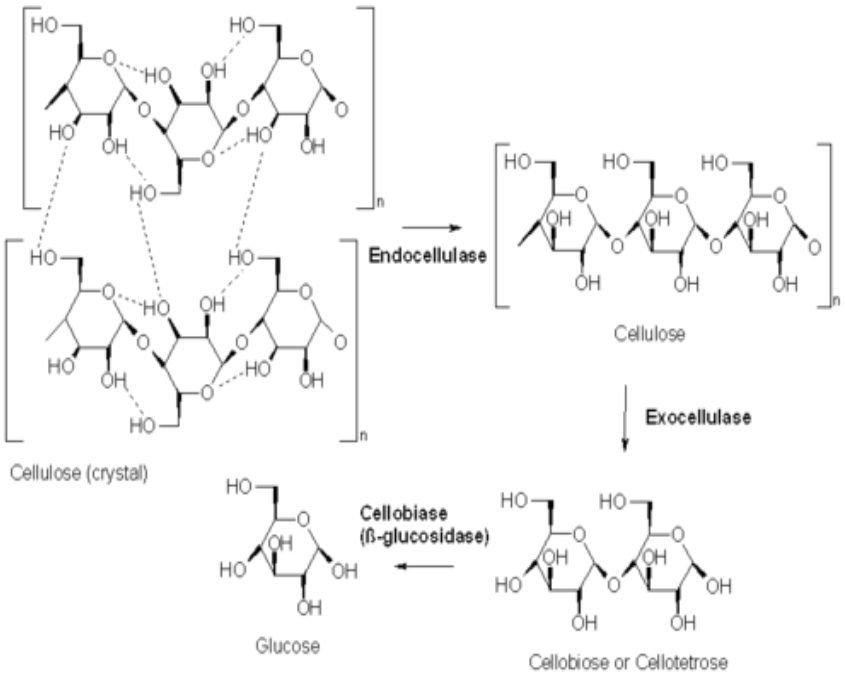

Fig 2. 2: Reaction path of cellulose to glucose (Maurice, 2011)

2.4.3 Fermentation Stage

Fermentation is the most utilized method in converting sugars present in lignocellulose feed stocks into fuels like bioethanol (Woldesenbet *et al.*, 2016). This requires the use of microorganisms either bacterium, yeast or fungi under anaerobic conditions (Li *et al*., 2007). Through the glycolysis pathway, a sugar such as glucose is directly metabolized by the yeast cells to obtain energy for biosynthesis. The overall reactions of the fermentation process produce two moles of ethanol and carbon dioxide per mole of glucose consumed (Talebnia, 2008). The overall chemical formula for the fermentation of ethanol is:

$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$

There are several processes used in the fermentation stage for bioethanol production. Separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF) and simultaneous saccharification and co-fermentation (SSCF) are the three most common ones. Bioethanol

fermentation can be performed in batch, fed-batch, repeated batch or continuous modes (Azhar *et al*., 2017).

Batch fermentation is the pitching of microorganisms particularly yeast into a highly concentrated substrate which results in a higher ethanol production. Substrate is provided at the start of the batch process without the medium being added or removed. The yeast is added at the start of the fermentation process and after the fermentation, the yeast is separated by centrifugation. Initially, the microorganisms work in highly concentrated substrates but in the final stage, they operate in highly concentrated products. The fermentation process is conducted at the beginning in a closed-loop system with high concentration of sugars and inhibitors and finishes with high concentration of the product. There are three distinct phases characterizing the growth in batch mode: lag phase, log phase and stationary phase. During the lag phase, cells produce enzymes and proteins which are important for growth under the available conditions. When adequate amount of desired proteins and enzymes are formed, cells begin to grow and enter the log phase. Metabolic products like ethanol, acetate and glycerol compile and contribute to an unvarying change through the entire fermentation as growth proceeds. Growth stops and cells enter the stationary phase in response to a lower concentration of sugar and nutrients. The benefits of batch system are complete sterilization, easy to manage the feedstock, low investment costs are needed, process control requirements are lower, labour skills are not necessary, product parameters can be managed easily and flexibly (Azhar *et al*., 2017; Talebnia, 2008; Deenanath, 2014; Chandel *et al*., 2007; Johansson, 2013).

Cell-recycle batch fermentation also known as repeated batch process minimises inoculum preparation time and cost, easy selection of cells, stable activity and long-term productivity. To promote the division of cells for cell recycling, sugar materials and immobilized yeast cells are used. This method is more effective when using immobilized yeast cells compared to free yeast cells (Azhar *et al*., 2017).

Fed-batch fermentation is a mixture of batch and continuous mode which involves adding substrate into the fermenter without the removal of the medium (Azhar *et al*., 2017). The feed is added at constant intervals in this process while the effluent is discontinuously removed (Talebnia, 2008). In this method, the microorganism operates at low concentration of substrates with an increase in ethanol concentration during the fermentation process (Chandel *et al*., 2007). Yeast is pitched into substrates with a low concentration and the substrate is fed into the fermenting vessel for yeast proliferation which results in a gradual increase of the ethanol concentration (Deenanath, 2014). It is used in batch operation to overcome substrate inhibition problems. The productivity of fed-batch fermentation can be enhanced by maintaining a low concentration of substrate that enables the conversion of adequate quantities of fermentable sugars to ethanol. This method is advantageous when substrate has inhibitory effects because of the exposure of the microorganisms to low substrate concentration. This type of fermentation has higher productivity and oxygen dissolved in medium, shorter fermentation periods and lower toxicity effect of the medium components compared to other fermentation types (Azhar *et al*., 2017).

Continuous mode is conducted by the constant supply of substrates, culture medium and nutrients into a bioreactor that contains active microorganisms. The volume of the culture must be constant in continuous operation and the fermentation products must be collected from the media continuously. In this method, yeast at high cell densities is added in stirred fermentation tanks with intermittent aeration and the addition of fresh substrates with the removal of yeast biomass at equal rates of substrates addition. This results in ethanol being produced rapidly. The advantages in this mechanism are higher productivity, offers ease of control, smaller quantities of bioreactors and lower investment and operating costs but the main drawback to be considered is the issue of contamination (Azhar *et al*., 2017; Talebnia, 2008; Deenanath, 2014).

2.4.3.1 Separate Hydrolysis and Fermentation

Under this method, the hydrolysis of the lignocellulosic materials is isolated from the fermentation of the ethanol. The isolation enables enzymes to function at their required temperature for better performance while fermentation organisms can be operated at optimum temperature to optimize the use of sugar (Azhar *et al*., 2017). SHF occurs in two stages, the first stage is carbohydrate hydrolysis to fermentable sugars, and the second is sugar fermentation to ethanol. During hydrolysis, the glucose produced inhibits the components of β-glucosidae. Monomeric and dimeric sugars that inhibit enzymes increase production costs as higher dosage of enzymes are needed. SHF can operate at different optimal conditions for hydrolysis and fermentation (Williams, 2017).

2.4.3.2 Simultaneous Saccharification and Fermentation

SSF combines both enzymatic hydrolysis and fermentation processes in a single vessel, which will intend minimise equipment cost. This method minimizes cellulase inhibition by avoiding sugar accumulation which happens during separate hydrolysis and fermentation, since the sugar is used in fermentation simultaneously with hydrolysis. This will then reduce the amount of cellulase needed for hydrolysis, thus causing a reduction in the cost involved in ethanol production. The glucose that is formed by hydrolysis and fermentation in one vessel is quickly converted by yeast to ethanol which eliminates inhibitors from the broth thereby preventing drawbacks associated with sugar accumulation, enzyme inhibition and contamination. The main disadvantage in this method is that hydrolysis and fermentation require different optimum temperatures (Williams, 2017; Talebnia, 2008; Hafid *et al*., 2017).

2.4.3.3 Simultaneous Saccharification and Co-fermentation

This method involves the use of yeast that is able to ferment both glucose and xylose. One way to minimise the bioethanol's production cost is to increase the yield of ethanol per tonne of feedstock as well as the fermentation broth's final concentration of ethanol. This is enabled by glucose and xylose co-

fermentation. A genetically engineered yeast strain is required to ferment the hexose and pentose sugars released during hydrolysis to allow the use of all the available sugars. In this method, the micro-organisms involved in the co-fermentation process must be compatible with the operating pH and temperature (Williams, 2017; Saini *et al*., 2015).

The following biochemical reactions occur during the production of bioethanol by hydrolysis and fermentation. Equation (2-4) only applies to co-fermentation, where a xylose utilizing yeast strain is used.

$C_6H_{10}O_5$ (cellulose) + $H_2O \longrightarrow C_6H_{12}O_6$ (glucose) (2-1)

$C_5H_8O_4$ (hemicellulose) + $H_2O \longrightarrow C_5H_{10}O_5$ (xylose) (2-2)

$C_6H_{12}O_6 \longrightarrow 2CH_3CH_2OH$ (ethanol)+ $2CO_2$ (2-3)

$3C_5H_{10}O_5 \longrightarrow 5CH_3CH_2OH$ (ethanol) + $5CO_2$ (2-4)

(Williams, 2017).

2.4.4 Factors Affecting Bioethanol Production

2.4.4.1 Temperature

The optimum hydrolysis and enzyme temperature is around 50^{o}C, whereas the optimum fermentation temperature varies from 28^{o}C to 37^{o}C, depending on the yeast strain that is used (Williams, 2017). The temperature range for the production of cellulase is within 25-35^{o}C for various types of microbial strains example *Trichoderma reesei*, *Aspergillus niger*, *Aspergillus ornatus*, *Aspergillus wentii, Penicillum rubrum*, *Thielavia terrestris* (Saini *et al*., 2015). Temperature directly influence growth rate of microorganisms. Most enzymes that control the activities of the microbes and the fermentation process are sensitive to high temperatures that can easily destroy their structure and inactivate them (Azhar *et al*., 2017).

2.4.4.2 Sugar concentration

The increase in the concentration of sugar to a certain amount will cause an increase in the fermentation rate. High sugar concentration will result in a

steady rate of fermentation because the concentration of sugar consumption is beyond the microbial cells uptake capacity. When the used sugar concentration is 150 g/L, the maximum rate of ethanol production is achieved. The initial sugar use concentration is also a significant factor in bioethanol generation. High initial sugar concentration will cause high bioethanol yield in batch fermentation (Azhar *et al.*, 2017).

2.4.4.3 Enzymes

Enzymes improve the efficiency of hydrolysis by means of synergistic advantages. They are not directly involved in the cellulose hydrolysis and hence boost the liberation of fermentable sugars from lignocellulosic biomass (Williams, 2017). Reese first discovered the cellulose-degrading enzymes. Cellulase complex are set of enzymes involved in the complete hydrolysis of cellulose and are made from organisms that reside on cellulosic materials which are either generated in a separate reactor or purchased from suppliers from industries. These enzymes are normally produced from bacteria or fungi. The preparations of cellulase enzyme involve enzyme cocktails that are made up of endoglucanase, β-glucosidase and small amount of hemicellulose. Hemicellulase or xylanase is used to hydrolyse hemicellulose polysaccharide covering the cellulose fibres to boosts cellulose hydrolysis. Cellulose and the modified cellulose –degrading enzymes are grouped into three main types of enzymes namely endo-glucanases, exoglucanases and β-glucosidae (Saini *et al.*, 2015; Li *et al.*, 2007).

Endo-β-(1,4) or 1,4-β-α-glucan-4-glucanohydrolases commonly known as endoglucanases are distinguished by their random hydrolysis of the linkages of β-(1,4)-glucosidic. Endoglucanases cleave cellulose chains and reduce degree of polymerisation. There is quick reduction in chain length which leads to changes in viscosity which is relative to the removal of reducing end groups as a result of their random cleavage. Exoglucanases Exo-β-(1-4)-glucanase or 1,4-β-α-glucan cellobiohydrolases commonly referred to as exoglucanases split the units of cellobiose from non-reducing ends of cellulose molecules. Exoglucanase releases the units of cellobiose from the cellulose chains ends.

β-glucosidae hydrolyses cellobiose to glucose by reducing the formation of inhibitors by cellobiose during enzymatic hydrolysis using cellulase. Most of them are productive on a range of glucose β-dimers (Williams, 2017; Saini *et al*., 2015). Each enzymes present in cellulase acts in an interconnected way to obtain an effective cellulose hydrolysis from different substrates. Cellulases can be differentiated from other glycoside hydrolases because of their capability in hydrolysing specifically the bonds of β-1,4- glucosidic (Neagu *et al*., 2012).

2.4.4.4 pH

pH affects enzyme and cells metabolism, contamination of bacteria, the growth of yeast, the rate of fermentation and the formation of by-product. The concentration of H^+ in fermentation broth affects the entry of some essential nutrients into the cells. Yeasts survival and growth are influenced by pH from 2.75 to 4.25. The best pH range of *Saccharomyces cerevisiae* for ethanol fermentation is 4.0-5.0. Intracellular pH and the activity of enzymes are greatly affected when the pH of the environment is over the operational pH (pH 2.0-pH 7.0). A method for cycling pH was utilized in the past using 3% cellulose and *Trichoderma reesei* QM 9414 in order to attain high productivity of cellulase from 25 to 38.75 IU/I/h (Saini *et al*., 2015; Azhar *et al*., 2017).

2.4.4.5 Fermentation time

Fermentation time influences growth of microorganisms. Shorter fermentation duration leads to ineffective fermentation because of inadequate growth of microbes. Longer fermentation time causes toxicity on microbial growth especially in batch mode because of high concentration of ethanol in the fermented broth (Azhar *et al*., 2017).

2.4.4.6 Agitation rate

Agitation rate regulates the entry of nutrients from the fermentation broth to the cells and the release of ethanol from the cell to the fermentation broth. Greater agitation rate results in higher ethanol production. It enhances sugar consumption and minimizes ethanol inhibition on cells. It also helps in the

dissolution and improvement of the transfer of oxygen and maintains homogenous physical and chemical conditions in the fermentation medium. At a perpetual rate of oxygen supply, agitation rate enhances homogenous cell distribution and also uniformly stir the media to remove extra heat to maintain a constant temperature. Commonest agitation rate for yeast cells fermentation is 150-200 rpm. Excessive agitation rate causes limitations in cell's metabolic activities resulting in low yield of ethanol (Azhar *et al*., 2017; Aziz, 2010; Rosada, 2019).

2.4.4.7 Inoculum concentration

Inoculum concentration highly influences sugar consumption rate and the productivity of ethanol as compared to the concentration of ethanol. The production of ethanol increases with increase in number of cells but remains constant as cell numbers increase within a certain range leading to reduction in fermentation time because of rapid cell growth and high sugar consumption to ethanol (Azhar *et al*., 2017).

2.4.4.8 Oxygen concentration

Ethanol production is an anaerobic fermentation process but requires oxygen supply to yeast culture in order to enhance an initial quantity of cell mass for higher production of ethanol (Aziz, 2010). Oxygen is required for cells growth and cells will die when oxygen is not enough. Dissolved oxygen percentage for cellulase production is kept above 30% (Saini *et al*., 2014).

2.4.4.9 Yeast (*Saccharomyces cerevisiae*)

Yeasts are unicellular microbes that are members of the Kingdom Ascomycotina. They break down their sources of energy externally and assimilate the nutrient into cellular molecules. Budding is the main type of asexual reproduction used by yeast and in some few cases binary fission is used. Yeasts that reproduce through budding are called 'true yeasts' and are categorised in the order *Saccharomycetales* (Arshad, 2011; Promon, 2015). *Saccharomyces cerevisiae* is yeast in the order *Saccharomycetales* that produces maximum ethanol productivity, yields and has high ethanol

inhibition resistance making it more desirable to use in ethanol production. This yeast is resistance to low pH and inhibitors derived from lignocellulose biomass and has optimal growth between 5.0 and 5.5 (Williams, 2017).

The production of ethanol using yeasts depends on factors such as strains, environmental conditions and macro and micro nutrients. The predominant environmental factor that affects the growth of yeast and the efficiency of ethanol production is temperature. Temperature affects yeasts growth rate and viability, fermentation rate, duration of lag phase, the activity and membrane function of enzyme. The growth rate and metabolism of yeast increase with increase in temperature until an optimum value is reached. Increased ethanol concentration during fermentation can inhibit the growth and viability of microorganism. The efficiency of fermentation using *Saccharomyces cerevisiae* at high temperatures (above 35^{o}C) is low due to increase in membrane fluids causing a change in the composition of fatty acids. High growth temperatures also result in biosynthesis of heat shock proteins that are required to confer thermal and bioethanol cross-tolerance in different organisms. Nitrogen is an important nutrient for the growth of yeast and it also has an influence on the rate of ethanol production and tolerance. Yeast extract is widely utilized as a source of nitrogen for yeast growth and also as nutrient additive in the production of bioethanol (Woldesenbet *et al*., 2016; Azhar *et al.*, 2017).

Baker's yeast is made from *Saccharomyces cerevisiae* strains which consist of roughly 30-33% dry materials, 6.5-9.3% nitrogen, 40.6-58.0 % lipids, 5.0-7.5% minerals and various quantities of vitamins depending on its type and conditions required for its growth. Active dry yeast comprises of live dried yeast cells grains with leavening powder whereas instant dry yeast are made up of fine particles which do not need to be rehydrated before use (Bekatorou *et al*., 2006). Wild type *Saccharomyces cerevisiae* can effectively ferment glucose, mannose fructose, disaccharides, sucrose and maltose. *Saccharomyces cerevisiae* is a facultative anaerobe meaning it has the ability to survive and multiply under both aerobic and anaerobic environments. It can

undergo Crabtree effect (respiratory and respiro-fermentative metabolism) in the presence of oxygen. The aerobic growth is controlled by available conditions like the kind and amount of carbon source; the availability of oxygen etc.(Erdei *et al*., 2013; Johansson, 2013).

The disadvantage in the use of this yeast is that it can only ferment hexose but not pentose. This problem can be resolved by the use of hybrid, genetically engineered or co-culture of two strains of yeast. The hybrid strain has been produced by joining protoplast of *Saccharomyces cerevisiae* with xylose-fermenting yeast such as *Pachysolen tannophilus*, *Candida shehatae* and *Pichia stipitis* (Azhar *et al*., 2017). During fermentation, the *Saccharomyces cerevisiae* produces ethanol by the Embden-Meyerhof-Parnas (EMP) pathway or glycolysis. Glucose is changed into pyruvate by series of reactions and each reaction is controlled by specific enzymes. The pyruvate is decarboxylated to acetaldehyde by pyruvate decarboxylase enzyme which is later reduced to ethanol by alcohol dehydrogenase in the presence of the electron donor NADH (Deenanath, 2014; Biradar *et al*., 2016).

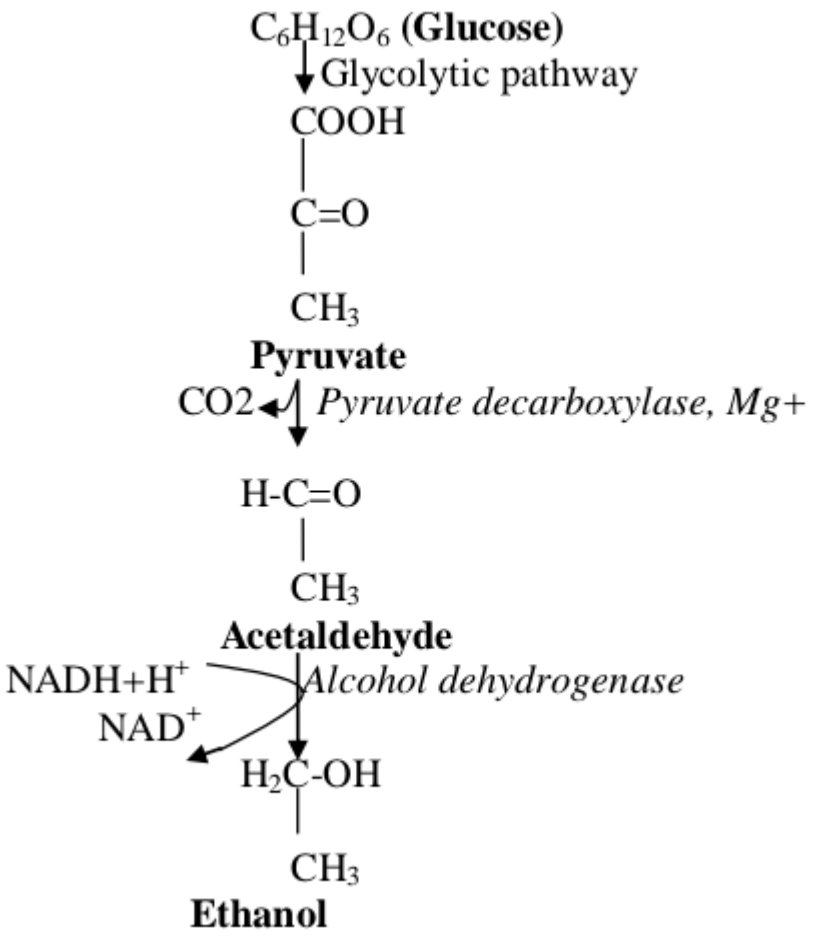

Fig 2.3: Yeast fermentation of glucose into ethanol (Biradar *et al.,* 2016).

2.5 Bioethanol Analysis Methods

The colorimetric method is one of the methods commonly used in determining the concentration of bioethanol. This is normally done by the use of potassium dichromate and spectrophotometer to read the absorbance. Potassium dichromate crystals are monoclinic plate-shaped crystals which are orange-red in colour (Liu *et al.,* 2017). They oxidise primary alcohols to their corresponding carboxylic acids. The intermediate product formed is aldehyde. Chromium from dichromate reagent gets reduced and the reduced form of the reagent changes colour from orange to dark brown or blue–green. The colour intensity is proportional to the concentration of alcohol present. The reaction

relies on the concentration of hydrogen ions for complete oxidation instead of a mixture of aldehyde and acid. The redox reactions in case of ethanol are

$3CH_3CH_2OH + CrO_7^{2-} + 8H^+ \longrightarrow 3CH_3CHO + 2Cr^{3+} + 7H_2O$

Ethanol Acetaldehyde

$3CH_3CHO + Cr_2O_7^{2-} + 8H^+ \longrightarrow 3CH_3COOH + 4H_2O$

Acetaldehyde Acetic acid

The overall equation for the reaction is

$2Cr_2O_7^{2-} + 3CH_3CH_2OH + 16H^+ \longrightarrow 4Cr^{3+} + 3CH_3COOH + 11H_2O$

ethanol acetic acid

(Sirromet, 2007; Singh and Singh, 2015).

The dichromate oxidation method can be directly used to assess the concentration of ethanol in yeast culture broths such as *Saccharomyces cerevisiae*, *Candida shehatae, Candida tropicalis*, and *Pichia kudriavzevii*. Chromium ions oxidize ethanol present in aqueous solution by reducing from +6 oxidation state to +3 causing a change of colour from orange to blue-green (Miah *et al*., 2017).

Gas chromatography is also an analytical tool used in the determination of the amount of ethanol present. Under this method the sample is vaporized by heat at an injection port, the sample vapour is sent to an adsorbent or absorbent column. Depending on the physical and chemical property, each component in the sample is separated within the column. The concentration of each compound is measured at the end of the column by a detector (Onuki *et al*., 2008).

The High Performance Liquid Chromatography is an analytical tool used in determining the concentration of bioethanol. Liquids are used as mobile phase and the port does not heat up the samples. This method is costly and less sensitive as compared to gas chromatography. Infrared spectroscopy can also be used in estimating the amount of ethanol present in a sample. This process uses infrared adsorption where the sample is passed through infrared with

different wavelengths. Infrared absorbability varies between different compounds and infrared wavelengths. The samples are defined by comparing infrared absorbability (Onuki *et al.,* 2008).

2.6 Current status of bioethanol production and utilization in Kenya

Bioethanol is produced currently in Kenya by Agro-chemical and Food Company Limited and Kibos sugar and allied industries. The two companies have a combined ethanol production capacity of 125,000 litres per day. Mumias Sugar Company has built a bio refinery plant with the capacity to produce 22 million litres of ethanol per year. Kwale international sugar company is also planning to build a 30,000 litre per day ethanol plant. The ethanol industry in Kenya is expected to grow rapidly in the next decade. The main feedstock used for ethanol production in Kenya is molasses, making the ethanol industry solely dependent on the sugar sector. There is enough sugarcane currently produced for the generation of 49 million litres of ethanol if only molasses are utilized and 345 million litres if the whole sugar cane is used in ethanol production (Otieno and Ogutu, 2020; Ndegwa *et al*., 2011).

Agro-chemical and Food Company Limited and Kibos sugar and allied industries produce approximately 1.2 million litres of ethanol annually to be used as cooking fuel. The Kenyan ethanol cooking fuel master plan has highlighted the use of molasses, sugarcane juice and cassava as the feedstocks for ethanol cooking fuel in Kenya to reduce the challenges from the use of only molasses (Dalberg, 2020). There is an on-going project in Kisumu in the Western part of Kenya which focuses on piloting ethanol stove and fuel market system. The goals of the pilot project are to test the viability of ethanol as a clean, affordable and easily accessible household fuel and also to stimulate demands for it in households in the target area and ultimately other places in Kenya (“Piloting Bioethanol’’, 2021).

2.7 Agricultural food products utilized in the study

2.7.1 Maize

Maize (*Zea mays*) belongs to the tribe Maydae of the family *Poaceae*. It has a genome size of 2.3 gigabase and contains over 32,000 genes on ten chromosomes. It was first grown in a form of wild grass called *teosinte* in Central America. The term 'maize' was derived from a word 'mahiz' meaning 'source of life' of Tano language of the people of the Caribbean islands and it later became 'maiz' in Spanish. It is commonly called 'corn' in English speaking countries (Singh and Kumar, 2016). The use of corn rather than maize in United States is due to the referral of maize as *Indian corn* during the arrival of early European settlers in the New World. Corn originated from the Germanic word 'korn' which referred to any edible grass. The spread of maize to Africa and East Asia was due to colonial conquests and trade (Abbassian, 2006).

Maize plant prefers deep, well drained, rich soils and areas with average daily temperatures of 18-21^{o}C and annual rainfall in the range of 230 to 4,100 mm for growth. The annual global production of corn is about 520 Tera gram. Major production regions are North America (42%), Asia (26%), Europe (12%) and South America (9%). Leading producers are USA with about 40% of global production followed by China with 20%. Corn is made up of 73% carbohydrate, 9% protein, 4% oil and 14% other components like fibres and also has energy density of (365 Kcal/100) (VIB, 2017).

2.7.2 Millet

Millet (*Panicum miliaceum*) is a cereal crop plant which belongs to the grass family *Graminae*. The term millet is used to refer to several types of small seeded annual grasses which belong to species under the five genera in the tribe *Paniceae* (*Panicum, Setaria, Echinochloa, Pennisetum* and *Paspalum*) and one genus *Eleusine* in the tribe *Chlorideae*. The most important millets are pearl millet (*Pennisetum glaucum*), finger millet (*Eleusine coracona*), proso millet (*Panicum miliaceum*) and foxtail millet (*Stalia italica*). Pearl millet

originated from West Africa and later spread to India through trade. Finger millet known as African millet in Africa and ragi or madua millet in India is mostly grown in eastern and southern Africa especially in Uganda, Kenya, Tanzania, Malawi, Zaire, Zambia and Zimbabwe and in southern peninsular regions in the states of Karnataka, Tamil Nadu, Andhra Pradesh and Maharashtra in India (Prasad and Staggenborg, 2009; FAO, 2001).

It is believed to be originated from East Africa and India. Proso millet originated from Central and Eastern Asia. It has been grown in China since 3000 BC. It is the milium of the Romans and the true millet of history in the Old testament. Foxtail millet also known as Italian or German- Hungarian or Siberian millet originated in the highlands of central China. World production of millets is about 29 million tonnes of which about 95% is produced in Asia and Africa. Asia accounts for 40% which is mainly contributed by India and China. Africa also accounts for 55% of the total millet production with its main contributors concentrated in Nigeria, Niger, Burkina Faso, Mali and Kenya. Millets contain starch, soluble and insoluble dietary fibres, minerals, crude protein and crude fat. They are grown in areas with low rainfall, high temperatures and poor soils with pH ranging from 4.5 – 8.0 (Kumar *et al*., 2018; Saleh *et al*., 2013).

2.7.3 Rice

Rice (*Oryza sativa*) belongs to the *Poaceae* family. It is believed to have been originated from southern India, then spread to the north of the country and then to China. It is a staple food for over half of the people in the world and is grown on approximately 146 million hectares of land. The total world production is about 535 million tons of which 91% is produced in Asia principally in China, India, Indonesia and Bangladesh with smaller amounts grown in Japan, Pakistan and various Southeast Asian nations. It is also cultivated in parts of Europe, in North and South America and in Australia. They prefer regions with high humidity, prolonged sunshine and assured water supply. Rice plant is normally grown on submerged land in the coastal plains, tidal deltas and river basins of tropical, semitropical and temperate regions

with the exception of the type called upland rice which is grown on dry soil. The main constituent of rice is carbohydrate (starch) (Britannica, 2020; Lantin, 1999).

2.7.4 Pineapple

Pineapple (*Ananas comosus*) is one of the most vital fruits globally and is the leading edible member of the family *Bromeliaceae* and has about 2000 species with annual global production of over 14 million tons. It is the second most traded product after bananas and the eighth abundantly produced fruit in the world. It was first a native plant in Brazil and later spread to other parts of America during the time of Columbus who took it to Europe. The plant is known as pineapple because its fruit look like pine cone. The native word in Tupi for the fruit was *anana* which meant excellent fruit and it later originated into *ananas*. They prefer warm and humid climate for their cultivation that is why they are now widely grown in the tropical and sub-tropical regions of the world in over 82 countries on over 2.1 million acres of land with a global production of 15,287metric tonnes (Upadhyay *et al.*,2010; Asim *et al.,* 2015; Ndungu 2014). The top ten countries in pineapple production according to the 2013 statistics are Thailand, Philippines, Brazil, China, India, Costa Rica, Nigeria, Mexico, Columbia and Indonesia (Das and Sahoo 2017). Pineapple peels contain 19.8% cellulose whilst the fresh fruit contains 11.2% cellulose, 25.8% reducing sugar and 5.7% non-reducing sugar (Upadhyay et al.2010). Bromelain which is used in food industries, cosmetics and dietary supplements is an enzyme extracted from pineapple leaves (Hemalatha and Anbuselvi, 2013).

2.7.5 Banana

Banana (*Musa acuminate*) belongs to the genus *Musa* of the *Musaceae* family. The family includes only two genera; *Musa* and *Ensete*. It originated from Malaysia peninsula, New Guinea and South-East Asia. The generic name *Musa* is rooted in the Sanskrit word Moca. They are broadly classified into two types namely dessert and cooking. Dessert types are eaten raw when ripe

whilst the cooking types are boiled, fried, roasted before consumption. Plantains are the best known among the cooking bananas and they form about one third of the total banana production. Banana is the second largest produced fruit after citrus and contributes about 16% of the world's total fruit production. They are grown in the tropics and to a limited extent in the sub-tropics with 37% in south and southeast Asia and the Pacific, 30% in tropical Africa, 26% in Central and South America and the Caribbean and about 7% elsewhere. The top producing countries in the world are India with 15% of the total banana production, China, Ecuador, Brazil, Philippines contributing 5-6 % each. It is mostly grown in the tropical rain forest. They prefer deep well drained and fertile soils rich in humus with pH ranging from 6-7.5. They contain high carbohydrate content, dietary fibres and minerals like magnesium, phosphorus and potassium with energy density of 371 KJ (Augstburger *et al*., 2002; Nayar, 2010; Sidhu and Zafar, 2018).

2.7.6 Orange

Orange (*Citrus sinenesis*) belongs to the genus *Citrus* in the family *Rutaceae*. Other members include tangerine, lemon, limes and grapefruits. It originated from southern China where it was referred to as 'Chinese apple' but is today grown commercially worldwide in the tropical, semi-tropical and some warm temperate regions. They are eaten fresh with about one third being processed into orange juice. They prefer fertile and well aerated soils with pH between 6 and 6.5 for their growth. They contain carbohydrate in the form of sugars: sucrose, glucose and fructose and also good source of fibres with very low protein and fat content. The estimated global production is 60 million metric tonnes as of 2005 with half coming from Brazil and United States of America. The fruit consists of two distinct regions: the pericarp which is also called skin, peel or rind and the endocarp or pulp and juice sacs (Etebu and Nwauzoma, 2014; Liu *et al*., 2012).

2.7.7 Tomato

Tomato (*Solanum lycopersicum*) is in the *Solanacea* family which also include species like nightshade (*Solanum nigrum)* brinjals (*Solanu, melongena*), potatoes (*Solanum tuberosum*). Its origin is South America and later spread to Europe in the 16th century and East Africa in 1900. Common names for tomato are tomate (Spain, France), tomat (Indonesia), faan ke'e (China), tomato (West Africa), tomatl (Nahuatl), jitomate (Mexico), pomodoro (Italy), nyanya (Swahili). It is the second most important vegetable after potatoes in most parts of the world. They are rich sources of minerals, vitamins, essential amino acids, sugars and dietary fibres, calories, phosphorus, calcium. They are mostly cultivated on deep well drained loam with pH ranging from 6-7.5 (Kithinji, 2016; Dam *et al*., 2005). Worldwide production of tomato is estimated to be 162 million tonnes in an area of 4.8 million hectares. The leading producers are China with 50 million tonnes followed by India with 17.5 million tonnes. The total tomato production in Africa for 2012 was 17,938 million tonnes with Egypt leading with 8,625 million tonnes. Leading producers of tomato in Africa are Egypt, Nigeria, Morocco, Tunisia, Cameroon, Algeria, South Africa, Sudan, Kenya, Ghana, Tanzania, Mozambique, Benin, Libya and Niger (Arah *et al*., 2015).

2.7.8 Spinach

Spinach (*Spinacia oleracea*) is in the family of *Chenopodiaceae* which also include vegetable crops like Swiss chard and Beets. It is an annual low growing fleshly leaves that forms a heavy rosette of broad, crinkly tender leaves. Their classification is based on seed for cultivation (round or prickly), leaf texture (smooth or crinkled), leaf colour, shape and pose and petiole length. It was first cultivated in Iran in 400 AD before it was introduced in Spain in 1100 AD and later spread to the rest of Europe by 1400 AD. It is closely related to species *S. tetranda*, *S. spinose*, *S. inermis* and *S. turkestanica* with *S. tetranda* been considered as its ancestor. They prefer cool climate for their cultivation and can be grown on variety of soils. The minimum temperature required for seed germination is 2^{o}C with a maximum

germination temperature of 30^{o}C and an optimum range of 7 to 24^{o}C. Regular supply of moisture is needed because it is a shallow rooted crop. Spinach is a rich source of fibre, vitamins A, C, E, K, B6, B2 and also magnesium, manganese, iron, calcium, potassium, copper, phosphorus, zinc, selenium, folate, betaine, folic acid, protein, niacin, omega-3 fatty acids, carotenoids beta-carotene and lutein (Nonnecke,1989; Roughani and Miri, 2019).

2.7.9 Collard greens

Collard green (*Brassica oleracea*) is biennial leafy vegetable in *Brassicaceae* family which also include cabbage and broccoli and is closely related to kale. It has large, broad, flat, smooth leaves with smooth leaf margin. It is widely cultivated in North America and Europe. It is one of the oldest members in the family *Brassicaceae*. Ancient Greeks cultivated kale and collards without making distinction between them. Popular cultivars include 'Georgia Southern', 'Morris Heading', 'Butter Collard' or (couve-manteiga) and couve tronchuda. Couve-manteiga and couve tronchuda are highly cultivated in Brazil and Portugal. They are grown on fertile and well-drained soils with pH of 6.5 – 6.8. Collard greens contain high content of protein, carbohydrates, fibre, vitamin A, vitamin C and minerals like iron, calcium, copper, manganese, selenium and zinc. They are also good sources of flavonoid poly-phenolic antioxidants like lutein, carotenes, zeaxanthin, crypto-xanthin (Steiner *et al*.,2019; Chen *et al*.,2014).

2.8 Agricultural Food Wastes

Wastes are materials that have not been fully utilized. They include plant materials, agricultural, industrial, municipal wastes and residues (Okonko *et al*., 2009). Food wastes are materials that are intended for human consumption but are discharged, lost, degraded or contaminated (Girotto *et al*., 2015). Food is a basic need for human survival. The global per-person food supply as measured in calories per person has increased in recent years. This increase in food supply has resulted in the generation of high quantities of wastes from agricultural food products. Food waste carbon footprint is estimated to be 3.3

billion tons of carbon dioxide which is equivalent to the amount of greenhouse gas emitted into the atmosphere per year. Approximately, 1.4 billion hectares of land which represents 28% of the world's agricultural area are used annually to produce food that is lost or wasted (Nair *et al*., 2017).

Agricultural food wastes go through life cycle which begins from the farm and progress through processing, distribution, retail and lastly consumption and/or dumping. The degree of food waste depends on factors like: consumer acceptance of produce; market demand and visual appearance of produce; storage, transport facilities and processing efficiency etc. (Ghosh *et al*., 2016). The generation of food wastes produce an impact at the environmental, social and economic level. Food wastes impact the environment by contributing to the emission of greenhouse gases during final disposal in landfills (methane release) and natural resource depletion in terms of water, soil etc. The social impacts are the ethical and moral dimension within the general concept of global food security whilst the economic impacts are due to the costs associated with food wastage and their effects on farmers and consumers incomes (Girotto *et al*., 2015).

2.9 Agricultural Waste Management

Agricultural waste management system is a structured process that involves the installation and management of all necessary components to regulate and use agricultural production by- products in a way that sustain and improve the value of air, water, soil, plant and animal resources. This system comprises of six basic functions: which are production, collection, storage, treatment, transfer and utilization. Production depends on the quantity and nature of the waste generated from agricultural materials. Collection involves the capturing and putting together the generated wastes from where they were deposited. The storage function deals with temporary containment or holding of the waste. Treatment includes physical, biological and chemical mechanisms aimed at minimizing the contamination and the toxicity of the waste in order to increase its potential beneficial use (Obi *et al*., 2016). Biological treatment

is the controlled conversion of waste by living organisms. It is mostly used in the treatment of agricultural waste products. One of the methods used in biological treatment of waste is composting. Composting is the decomposition of organic matter in the presence of oxygen to produce humus. Under this method, microorganisms decompose organic matter to generate carbon dioxide, water and heat (Lohri *et al.,* 2017).

According to Khamala and Alex (2013), a number of community-based organizations and private holdings in Nairobi are involved in the composting of organic waste for sale. About 1.2 tons/day of combined compost production are produced in the city by community help groups and private companies. Anaerobic digestion also known as bio-methanization or bio-methanation is the biochemical decomposition of organic matter by the activities of bacteria in the absence of oxygen. Biogas and digestate are the main products of anaerobic digestion (Lohri *et al*., 2017). Biogas is generated by bacteria under anaerobic conditions through the bio-degradation of organic material. Biogas consists of roughly 55- 60% methane, 35-40% carbon dioxide, 2-7% water, 2% hydrogen sulphide, 0-0.05% ammonia, 0-2% nitrogen, 0-2% oxygen and 0-1% hydrogen (Williams, 2017).

Fermentation is a biological treatment that is employed in agricultural waste management. It is a series of chemical reactions performed by microorganisms and requires the partial oxidation of organic compounds without the use of oxygen to obtain chemical energy (Marin *et al*., 2017). Fermentation is the most important process used in bioethanol production (Lohri *et al*., 2017). Bioethanol as a biofuel is normally preferred to biogas due to the high concentration of methane which is a greenhouse gas present in biogas. Transfer as a fifth function is the movement and transport of waste across the system from collection to utilization stage. Utilization is the beneficial use of the waste which includes recycling of reusable waste products and the reintroduction of non-reusable waste materials into the environment (Obi *et al*., 2016).

Santi (2012) researched on bioethanol production using agro food waste in Italy. The agro wastes that were used are orange peel waste, olive pomace and grape pomace. The orange peel waste was the best substrate for bioethanol production. The total fermentable sugars were up to 48% of the dry matter and the production of lignin, phenols and xylose were low. Overall ethanol yield by the orange peel waste was 41.5%.

Braide *et al*. (2016) utilized agro wastes for bioethanol production using *Saccharomyces cerevisiae* in Nigeria. The agro wastes that were used are sugarcane bagasse, sugarcane bark, corn cob, corn stalk and corn husk. The incubation time for the fermentation process was 5 days and the ethanol yield, specific gravity, pH and total reducing sugar were determined. The specific gravity, pH and sugar content decreased over time. The highest percentage ethanol yields of the sugarcane bagasse, sugarcane bark, cornstalk, corncob and cornhusk were 6.72, 6.23, 6.17, 4.17 and 3.45 respectively at 72 hours of fermentation and pH of 3.60, 3.82, 4.00, 3.64 and 3.65.

Cutzu and Bardi (2017) studied on the production of bioethanol from agricultural wastes in Italy. The wastes used were apple, kiwifruit, peaches and corn threshing residue with *Saccharomyces bayamus* as starter yeast. Fermentations took place at 28^{o}C and 35^{o}C using raw fruits, blanched fruits and corn threshing residue. The corn threshing residue gave the maximum ethanol yield of 10.22% (v/v) followed by apple fruits with 8.71% (v/v) then kiwi fruits with 7.97% (v/v) and peaches with 4.26% (v/v).

Patel *et al*. (2007) studied on ethanol production from microbial pre-treated agricultural residues in India. The agricultural wastes that were used are wheat straw, rice straw, rice husk and bagasse. Six different fungi obtained as a result of screening were combined and used for the pre-treatment process and the yeast *Saccharomyces cerevisiae* was utilized in the fermentation process. The pre-treatment of the wheat straw and rice straw with *Aspergillus niger* and *Aspergillus awamori* gave the maximum ethanol yield of 2.5 g/l and 2.2 g/l respectively. The pre-treatment of the rice husk and bagasse with *Aspergillus*

awamori and *Pleurotus sajor-caju* recorded an ethanol yield of 8.5 g/l and 9.8 g/l respectively.

Mushimiyimana and Tallapragada (2016) generated bioethanol from agro waste (carrot peel, onion peel, potato peel and sugar beet) by using *Saccharomyces cerevisiae* in India. Fermentation was performed at durations of 14, 21 and 28 days at 30^{o}C. The highest ethanol concentration was attained at 28 days incubation period. Sugar beet peel recorded the highest ethanol percentage of 14.52 followed by potato peel, onion peel and carrot peel with 11.06, 8.50 and 2.78 respectively.

Gnanambal *et al*. (2014) used agricultural wastes from sugarcane bagasse, sweet sorghum, maize stover, groundnut shell, paddy straw and forestry waste, wood chips as substrates to produce bioethanol in India. The substrates were treated by solid state fermentation process using *Pleurotus florida*. Simultaneous saccharification and fermentation process was used and sweet sorghum substrate of 20.9g/kg generated the highest quantity of ethanol after an incubation period of 72 hours.

Khandaker *et al*. (2018) studied bioethanol production in Malaysia by using *Saccharomyces cerevisiae* for fermentation and fruit and vegetable wastes as substrates. The fruit wastes used were pineapple, orange, watermelon and the vegetable waste used were potato, tomato and other leafy vegetable wastes. The maximum ethanol was produced by the pineapple wastes with orange waste recording the highest glucose content and elemental concentration in the bioethanol produced.

Utama *et al*. (2019) used indigenous yeasts consortium in bioethanol production from fruit and vegetable wastes in Indonesia. The substrates used (banana, papaya and napa cabbage) were pre-treated by mashing them with a blender. The indigenous yeast used were *Candida krusei* and *Hanseniaspora guilliermondii* which were cultured using modified nutrient broth with 3% yeast extract and 10 ppm amoxicillin for 48 hours and at room temperatures (23-28^{o}C). Each of the substrates was inoculated with 3% of *Candida krusei*

and *Hanseniaspora guillermondii* (1:1) followed by fermentation for 72 hours at room temperatures (23-28^{o}C). Banana substrate recorded the highest ethanol concentration of 7.38% at 24 hours incubation time but there was a decrease in their ethanol production after the 24 hours. Napa cabbage recorded the lowest amount of bioethanol in the study.

Gosavi *et al*. (2017) determined the amount of bioethanol produced from fruits and vegetable wastes in India through the use of acid hydrolysis and a pure *Saccharomyces cerevisiae* yeast culture. The fruit wastes that were used are Indian water chestnut, sweet potato, jackfruit and pineapple. The pineapple waste produced the highest ethanol concentration of 0.090%, followed by sweet potato, Indian water chestnut and jack fruit waste of 0.079%, 0.045%, 0.045% respectively.

Promon *et al*. (2018) utilized vegetable peels wastes from potato, papaya, pumpkin and cucumber as substrates to produce bioethanol in Bangladesh. Temperatures (25^{o}C, 30^{o}C, 37^{o}C, 40^{o}C, 44^{o}C), reducing sugar concentration and pH (2-10) were optimized for maximum ethanol production in the substrates. The best conditions for maximum ethanol concentration were at temperature 30^{o}C and pH 6.0 within 48 hours of incubation.

Ahamad (2016) used *Saccharomyces cerevisiae* for the fermentation of kokum and butter fruit substrates in order to produce bioethanol in India. Fermentation was performed for 7 days and the ethanol content was calculated for every 24 hours till 7 days for both fruit resources. The maximum amount of ethanol for both fruit samples were achieved on 4, 5, 6 and 7 days and at pH and temperature of 6.7 and 30^{o}C for the kokum fruit and 5.8 and 30^{o}C for the butter fruit.

Hossain *et al*. (2015) utilized rotten fruits for the production of bioethanol in Saudi Arabia. The data regarding the fermentation of rambutan, mango, banana and pineapple ethanol production was compared with that of the spoilt fruits. The highest bioethanol concentration of 9.4 (%v/v) was achieved from the fermentation of pulp fruit part at pH 5 for 2 days.

Jahid *et al*. (2018) produced bioethanol under milder condition from fruit wastes in India. The study focussed on determining the feasibility of producing reduced and pentose sugars, and bioethanol from fruit wastes. The free sugars were recovered by submerging in water and steaming. Enzymatic hydrolysis process that made use of cellulase and xylanase enzymes gave the maximum yields of total reduced and pentose sugars. The enzymatic hydrolysis process was the best option for bioethanol production when compared to acid hydrolysis method used.

Babu *et al*. (2014) used isolated microbial strains for the production of bioethanol from fruit wastes (grapes, sugarcane, mosambi and water melon) in Egypt. Fermentation parameters like temperature, pH and sugar concentration were optimised. Fermentation was performed for 48 hours (with samples been withdrawn after every 12 hours) using *Saccharomyces cerevisiae* yeast. The highest amount of ethanol was generated in sugarcane at a pH 6, 20% sugar concentration and 30^{o}C temperature.

Benjamin *et al*. (2014) utilized banana peel in the production of bioethanol by Simultaneous Saccharification and Fermentation process which involved the use of co-cultures of *Aspergillus niger* and *Saccharomyces cerevisiae* in India. Different temperatures (20^{o}C to 50^{o}C) and pH (4-7) were optimised for fermentation and ethanol content was determined after every 24 hours for 7 days. Yeast concentrations of 3% to 12% were used in the fermentation process. The highest amount of bioethanol was achieved by using 12%, 9%, 6%, 3% yeast inoculum in 2, 3, 5, 7 days respectively at pH 6 and temperature 30^{o}C.

Waghmare and Arya (2016) used unripe banana peel to produce ethanol in India. The study involved the use of chemical characterization, optimization of acid hydrolysis and fermentation conditions and yeast strain selection. The chemical characterization of the unripe banana peel powder revealed 41% w/w of starch, 9.3% w/w of cellulose and protein to be 8.4% w/w. 35.5 g/l of

ethanol was generated when the selected strain of *Saccharomyces cerevisiae* NCIM 3095 was used at optimized fermentation conditions.

Chongkhong (2015) studied on the production of bioethanol from banana peels in Thailand. The main objective was to determine the hydrolysis of banana peels using vinegar solution, an organic acid that does not inhibit yeast growth. Microwave heating that is energy efficient was applied to help the hydrolysis in order to reduce energy consumption. The fermentation step was evaluated using commercial baker's yeast. The hydrolysate sugar content was maximised over ranges of vinegar concentration, microwave power and hydrolysis time. The results obtained after the study suggested that combining microwave application together with hydrolysis using organic acid is cost efficient for ethanol production using organic waste.

Shinde and Patil (2016) utilized orange and banana peels to produce bioethanol using *Saccharomyces cerevisiae* in India. Fermentation was done in 7 days (with samples been withdrawn after every 24 hours) using Simultaneous Saccharification and fermentation technique. The maximum ethanol yields from banana peels were 8.34% v/v, 7.45% v/v and that of orange peels were also 3.98% v/v and 2.58% v/v. The results obtained showed that the banana peels produced higher quantity of ethanol than the orange peels.

Sharma *et al*. (2007) used kinnow waste and banana peels for bioethanol production in India. The study assessed the effects of temperature, inoculum concentration, incubation and agitation times on the generation of bioethanol. Temperature 30^{o}C, inoculum size of *Saccharomyces cerevisiae* 6%, incubation duration of 48 hours and the first 24 hours agitation were the best conditions for the production of bioethanol from the two substrates.

Tropea *et al.* (2014) utilized pineapple wastes for bioethanol production in United Kingdom. The study involved the use of direct fermentation and addition of enzymes to determine the ability of the yeast to ferment the untreated substrate; separate hydrolysis and fermentation in the same reactor;

simultaneous saccharification and fermentation. Glucose, uronic acid, xylose, galactose, arabinose and mannose were the main sugars that were obtained from the pineapple wastes. The highest amount of bioethanol (3.9%) was achieved using simultaneous saccharification and fermentation after incubation period of 30 hours.

Oiwoh *et al*. (2018) also utilized pineapple wastes to generate bioethanol from Simultaneous Saccharification and fermentation using enzyme cellulase and yeast *Saccharomyces cerevisiae* in Nigeria. The influence of the broth pH (2-6), yeast loading (2-10g/l) and ammonium sulphate concentration (1-5g/l) on bioethanol generation were studied using a three-factor Box-behnken design and response surface methodology. The highest concentration of bioethanol 5.82%v/v was achieved at a pH of 6, 8ml yeast loading and 5g/l ammonium sulphate concentration.

Itelima *et al.* (2013) utilized banana, plantain and pineapple peels in bioethanol production by Simultaneous Saccharification and Fermentation process. Maximum ethanol yield of 1.89 (OD) was produced by pineapple substrate with banana producing 1.60 (OD) and plantain 0.98 (OD). They concluded that the use of fruit wastes for bioethanol production is feasible and therefore they should not be discarded into the environment to cause pollution.

Udegbunam and Mike-Anosike (2018) studied on the feasibility of producing bioethanol from pineapple fruit skin by the use of *Zymomonas mobilis* and *Saccharomyces cerevisiae*. Acid dichromate solution together with a spectrophotometer was used to determine the concentration of bioethanol in the pineapple fruit skin. Temperature, pH, reducing sugar and specific gravity of the substrates decreased with increasing fermentation times. The use of sugar was faster in the *Zymomonas mobilis* as compared to the *Saccharomyces cerevisiae*. The maximum ethanol yield using the *Saccharomyces cerevisiae* and *Zymomonas mobilis* were 51% and 78% respectively.

A research by (Zain *et al*., 2012) utilized liquid pineapple wastes to generate bioethanol by the use of bakers' yeast that is immobilized in modified PVA-

alginate beads in Malaysia. Temperature was set at 30^{o}C, pH 5, agitation speed 200 rpm and fermentation was performed for 72 hours. The maximum productivity of ethanol from the use of immobilized yeast was 0.0752g/l/h which was lower as compared to the amount obtained from the free cells.

Taymaz (2013) utilized orange waste for bioethanol production in Cyprus. The research focussed on cellulosic fragments of the orange waste. A multi-step pre-treatment strategy was followed by enzymatic hydrolysis using the wild type yeast *Saccharomyces cerevisiae* NRRLY-132 and a 91% glucose yield was attained taken into consideration the theoretical maximum glucose content. The treated orange waste had an overall ethanol of 4.5, the untreated orange waste and partially treated had an overall ethanol yield of 10.5 and 7.8 respectively.

Zhou *et al.* (2008) generated bioethanol from citrus peel waste by using *Saccharomyces cerevisiae* yeast and Simultaneous Saccharification and fermentation technique at a pH of 4.2-4.8 for 48 hours. The ethanol yield was determined by the use of High Performance Liquid Chromatography. The obtained result showed the maximum ethanol yield to be 4.05% and was achieved at 18 hours incubation time.

Talebnia (2008) used encapsulated *Saccharomyces cerevisiae* to generate bioethanol from orange peel wastes in Sweden. The essence of using encapsulated *Saccharomyces cerevisiae* was to remove the inhibition by D-limonene in orange wastes during fermentation. The yeast cells were encapsulated using alginate membranes which are normally used to overcome the side effects of limonene. Enzymatic hydrolysis was done using cellulase and pectinase. The ethanol yield produced at the end of the experiment was 0.44g/g at 7 hours incubation time.

Ali and Kemat (2017) utilized *moringa oleifera* seed husk which is an agro waste to generate bioethanol in Malaysia. Batch fermentation was performed using *Saccharomyces cerevisiae* at different dosages of 1,3 and 5 g/l, pH values of 4.5, 5.0 and 5.5 , incubation periods of 3,6,9 and 12 hours with

temperature at 32ºC. The maximum ethanol yield of 29.69 g/l was achieved at 3 hours incubation time, pH of 4.5 and yeast dosage of 1g/l.

Gutierrez *et al*. (2015) studied on the use of ripe carabao mango peelings for the generation of bioethanol in Philippines. The study emphasised on the effect of using different incubation times and yeast loading on the quantity of bioethanol produced at the end of the fermentation process. The results obtained showed that the maximum ethanol percentage of 0.33% was achieved at the lowest incubation time and highest yeast loading.

Gawande and Patil (2017) used damaged corn grains for the production of bioethanol in India. Damaged blackened corn grain was compared with fine corn grain in terms of sugar and bioethanol production. There was 40% and 17% decrease in starch and protein in the damaged grains. Damaged and fine corn contained 35 g/L and 48 g/L of reducing sugar respectively at the end of liquefaction with α amylase. The ethanol yield from the damaged and fine corn grains at the end of the experiment were 0.17 and 0.22 g ethanol/g respectively. The research demonstrated the feasibility of using damaged corn grains for bioethanol production.

Agrawal *et al.* (2019) utilized de-oiled rice bran which is an agro waste to produce bioethanol by *Saccharomyces cerevisiae* MTCC 4780 under optimized fermentation conditions in India. The parameters were fermentation durations (24-96 hours), pH (4.0-7.0), temperatures (20-35ºC). The maximum ethanol production of 9.68% was obtained at temperature 30ºC, pH 6 and fermentation duration of 48 hours.

Walker *et al*. (2013) studied on bioethanol production in United States of America using food processed waste from corn, potato and pasta. Enzymatic hydrolysis was done by the use of the enzymes alpha-amylase and glucoamylase whilst fermentation was done by *Saccharomyces cerevisiae* yeast. The early stage of the experiment recorded low amount of bioethanol production due to the low amount of starch present initially in the waste

products. The concentrations of ethanol increased with increasing the dosage of the enzyme levels.

Shrivastava *et al*. (2014) utilized sugarcane bagasse, corn cob, wheat bran in bioethanol production by the use of Simultaneous Saccharification and Fermentation method using *Thermomyces lanuginosus* SS-8 and *Pichia stipis* NCIM 3497 and NCIM 3498 in India. Maximum amount of ethanol was observed under the fermentation of sugars derived from the saccharification of sugar cane bagasse followed by corn cob and wheat straw.

Singh and Singh (2015) used organic residues of agro waste materials to produce bioethanol in India. The agro waste materials that were used are rice straw, corn cob, potato peels and banana peels. The maximum carbohydrate of 92.5% was found in potato peels, followed by banana peels, corn cob and rice straw which were 88.2%, 85.6% and 82% respectively. Potato peels produced the highest ethanol percentage of 4.3% and corn cob, banana peels and rice cob produced 3.3%, 3.18% and 2.48% respectively at 96 hours fermentation time.

Rakin *et al.* (2009) used corn meal to produce bioethanol in Serbia. The effects of inoculum concentration and stability of the immobilized particles were studied in repeated batch fermentation process using immobilized *Saccharomyces cerevisiae* var. ellipsoideus yeast cells. The immobilization was done by the use of either polyvinyl alcohol (PVA) or Ca- alginate. The highest concentration of bioethanol 10.05% (w/w) was obtained at an inoculum concentration of 5% (v/v) of yeast cells that was immobilized in Ca-alginate. Low amount of bioethanol was produced from the use of yeast cells immobilized in PVA carrier but in terms of stability, there were better as compared to those immobilized in Ca- alginate.

De Souza Schneider *et al*. (2018) used broken rice grains to produce ethanol. The concentration of starch in the substrates was quantified. Enzymatic hydrolysis was done using α-amylase and glucoamylase whilst yeast *Saccharomyces cerevisiae* was used in the fermentation process. Samples were

analysed by the use of High Performance Liquid Chromatography and an average starch concentration of 80.1% and ethanol of 29.2g per 100g of broken rice grain were obtained.

Tiwari *et al*. (2015) studied on the generation of bioethanol from rice bran by the use of *Bacillus cereus* strain McR-3 to optimize fermentation parameters in India. Specific gravity method was applied for the quantitative estimation of bioethanol. The parameters that were considered were temperature and pH. *Bacillus cereus* strain McR-3 was used to inoculate the substrate at different incubation temperatures of 19, 22, 25, 28, 31, 34, 37, 40, 43, 46, 49, 52, 55, 58 and 61^{o}C and pH of 2, 3, 4, 5, 6, 7 and 8. The maximum bioethanol was produced at temperature 37^{o}C and pH 5.

Chaijamrus and Mouthung (2011) utilized malted rice from waste paddy for bioethanol production in Thailand using submerged fermentation. The highest ethanol productivity 4.08g/kg waste paddy/hr ; concentration 149+/-7.0 g/kg waste paddy and yield of 48.38g/100g of sugar consumed were achieved after 48 hours incubation time with an ethanol efficiency of 67% which is almost the same as that of malted rice from normal paddy which is 68%.

Gohel and Duan (2012) utilized Indian broken rice and pearl millet to generate bioethanol in India by using a no-cook process. Granular starch hydrolysing enzyme containing fungal alpha amylase and a glucoamylase was used for the no-cook process. The chemical composition of the broken rice was found out to be made up of 68.45 starch whilst that of the pearl millet was 60.00 starch. The amount of ethanol produced by broken rice was higher than that of the pearl millet due to the higher starch content of 68.45% in the broken rice as compared to the pearl millet of 60%.

Wilson *et al.* (2007) studied on the feasibility of using pearl millet to generate bioethanol in Puerto Rico. The amount of ethanol produced by the pearl millet was compared with that of maize by the use of Superpro Designer Dry Grind model and it was observed that the yield of pearl millet was 8% less than that of maize. In terms of the rate of fermentation, the pearl millet was faster and

reached 85% fermentation approximately 12 hours earlier than that of the maize.

Elkahlout *et al*. (2017) used tomato waste and wheat straw as substrates in bioethanol production by using immobilized *Saccharomyces cerevisiae* in Palestine. The efficiency of free and immobilized yeast cells in calcium alginate beads with microwave-assisted acidic pre-treatment were compared by using the wheat and tomato substrates. The immobilization technique gave higher ethanol yield compared with the free system for tomato waste but lower yield with wheat straw. The maximum amount of ethanol produced for wheat straw using free cells was 641mg/g and that for tomato waste using immobilized cells was 543.5mg/g.

Sarkar *et al*. (2012) studied on the production of bioethanol from rice straw, wheat straw, corn straw and bagasse in India using available and accessible technologies. Various pre-treatment, hydrolysis and fermentation methods were discussed under this study. The chemical composition of rice straw, wheat straw and corn straw were estimated to be 32-47%, 35-45%, 42.6 cellulose, 19-27%, 20-30%, 21.3 % hemicellulose and 5-24%, 8-15% , 21.3% lignin respectively.

Wu *et al.* **(**2006) studied on the generation of bioethanol from pearl millet using *Saccharomyces cerevisiae* yeast. The fermentation process was conducted using an incubator shaker at 30^{o}C for 72 hours with an agitation rate of 150 rpm. The pearl millet mashes with 20, 25, 30 and 35% dry mass produced ethanol yields of 9, 11, 13-14 and 16-17% (v/v) respectively. The results showed that the quantity of ethanol generated was proportional to the quantity of starch present in the pearl millet samples and were comparable to those of corn and oat.

Barcelos *et al*. (2011) used sorghum grains to produce bioethanol in Brazil. Enzymatic hydrolysis was performed by using the enzymes α-amylase and glucoamylase and fermentation was done by the yeast *Saccharomyces cerevisiae* for 24 hours. The starch content in the grains was 79% making it a

good substrate for ethanol production. The highest ethanol concentration was 106 g/l, productivity 4.4 g/l/hr and the yield based on the substrate used was 0.499 g/g.

Erdei *et al*. (2013) utilized wheat straw for the generation of bioethanol. A part of the study focussed on integrating steam pre-treated wheat straw substrate with pre-hydrolysed, fully hydrolysed or fermented wheat meal by *Saccharomyces cerevisiae* using simultaneous saccharification and fermentation method. Ethanol yield above 80% of the theoretical and ethanol concentration of 6% were obtained from the batch SSF process. Second part of the work focussed on development and investigation of integrated process configurations in order to improve the utilization of xylose by the use of genetically modified xylose-fermenting yeast strain, *Saccharomyces cerevisiae* TMB3400. 92% ethanol yield was achieved after both glucose and xylose fermentation with an almost complete uptake of xylose.

Braide *et al*. (2018) used agricultural wastes from cassava, yam and potato peels to produce bioethanol in Nigeria. Fermentation was done using *Saccharomyces cerevisiae* and *Zymomonas mobilis* for 120 hours with the quantity of ethanol determined at 24 hours interval. There was an increase in ethanol production in all the substrates from 24 to 72 hours but declined after the 72 hours. The highest ethanol was achieved at pH of 4.1 and 4.2 for *Zymomonas mobilis* and *Saccharomyces cerevisiae* respectively at 72 hours incubation. The maximum ethanol production using *Saccharomyces cerevisiae* for fermentation was 6.77% v/v for yam peels, 7.39% v/v for potato peels, 5.51% v/v for cassava peels and that of the combined substrates was 6.64% v/v.

A research by (Sheikh *et al*., 2016) focussed on the effects of using different fermentation periods in bioethanol production from potato peels wastes in Egypt. The fermentation was performed at 30^{o}C using *Saccharomyces cerevisiae*. The ethanol content was determined after every 24 hours of

incubation for five days. Fermentation periods of three and four days recorded the highest amount of bioethanol.

Sahu (2016) used co-culture of *Saccharomyces cerevisiae* and *Aspergillus awamori* to generate bioethanol from waste potatoes in India. The fermentation methods used under this investigation to produce bioethanol were Solid State Fermentation (SSF) and Simultaneous Saccharification and Fermentation (SiSF). The maximum bioethanol yield of 6.7% was obtained at incubation temperature and time of 30^{o}C and 168 hours respectively using the SSF method. The highest yield of ethanol 5.2% in the case of SiSF was achieved at pH of 6 with fermentation temperature and time of 30^{o}C and 168 hours.

Bekele *et al*. (2015) utilized potato peel waste for bioethanol production in Ethiopia. *Aspergillus niger* and Bacillus species were used for hydrolysis and *Saccharomyces cerevisiae* of bakery and brewery yeast was used for fermentation of the potato peel flour with 87% moisture content. After distillation of 150 ml broth, maximum ethanol distillate was obtained from sequential culture of *Aspergillus niger* and bakery yeast without autoclaving while the minimum was from bacillus and brewery yeast.

Matsakas and Christakopoulos (2015) used food wastes from households to generate bioethanol at high dry material content in Greece. Liquefaction/saccharification process was used in the fermentation process to cause a reduction in the viscosity of the high solid content substrate. Remaining solids after fermentation were subjected to hydrothermal pre-treatment in order for them to be used as raw materials in further fermentation process. The soluble glucose, cellulose and hemicellulose of the household food wastes were 33.81+/- 0.42 (%w/w), 18.30+/-0.19 (%w/w) and 7.55+/-0.39 respectively. The maximum ethanol production of 34.85g/l (using 35% w/v of dry matter) and 42.78g/l (using 45% w/v of dry matter) were obtained after 15 hours of incubation. In terms of the ethanol yields the highest of 0.443g/g was obtained from 35% dry matter whilst 0.423g/g was from 45%

dry matter. The use of separate liquefaction /saccharification and subsequent fermentation resulted in high ethanol production from the household food wastes.

A study by (Akpan *et al*., 2008) focussed on bioethanol generation from old newspapers and maize wastes in Nigeria. Acid and enzymatic hydrolysis methods were used and 42% and 63% fermentable sugars were achieved respectively and converted into bioethanol by the use of *Saccharomyces cerevisiae*. The amount of ethanol generated at the end of the experiment from 2,500 grams of wastes used was 0.86 litres.

Bekmuradov (2015) generated bioethanol using source-separated organic waste in Canada. Pre-treatment was done using modified cellulose- organic-solvent -based lignocellulosic fractionation (COSLIF). Fermentation was carried out with recombinant strains *Zymomonas mobilis* 8b and *Saccharomyces cerevisiae* DA2416. 90% yield of glucose was obtained from the pre-treated substrates whilst 10% was obtained from the untreated samples. *Saccharomyces cerevisiae* DA2416 gave maximum bioethanol yield as compared to *Zymomonas mobilis* 8b.

Thapa *et al.* (2017) generated bioethanol in Nepal and India from food waste materials. Acid hydrolysis and fermentation using *Saccharomyces cerevisiae* was utilized in the fermentation process. Varying acid concentration and temperature were considered for optimization in hydrolysis. The maximum reducing sugar yield obtained was 32.63g/100g of dry food waste at 7.5% acid concentration at 135^{o}C. The yield of ethanol was 13.78g/100g of dry food waste.

Kim *et al.* (2011) researched on the feasibility of generating bioethanol from food waste in South Korea. Enzymatic hydrolysis and fermentation were done in batch mode and continuous mode using carbohydrase and *Saccharomyces cerevisiae* respectively. Ethanol yields of 0.31g/g and 0.43g/g were obtained from Simultaneous Saccharification and Fermentation (SSF) and Separate Hydrolysis and Fermentation methods respectively in batch mode. For the

continuous mode, ethanol yield of 0.3g/g and productivity of 1.18g/l/hr were obtained from Separate Hydrolysis and Fermentation method whilst that of the Simultaneous Saccharification and Fermentation was 0.2g/g ethanol yield and productivity of 0.8 g/l/hr.

A study by (Miezah *et al*., 2007) focussed on biogas and bioethanol production from agricultural food waste products in Ghana. Some of the wastes samples were sorted whilst the others remained unsorted. The unsorted biodegradable solid waste materials were made up of 38.7%, 8.3%, 10.1 % and 7.6% dry matter glucan, hemicellulose, lignin and ash respectively with an ethanol yield of 0.17l/kg dry matter. Cassava, yam and plantain peeling wastes obtained the highest ethanol yield when the sorted fractions were taken into consideration.

Moukamnerd *et al.* (2013) researched on feasibility of ethanol production from food wastes obtained from bread crust, potato chips and rice grain in Japan. This research used an integrated continuous solid-state fermentation consisting of a revolving drum reactor, a humidifier and a condenser. This system incorporates enzymatic hydrolysis, fermentation of ethanol and ethanol recovery in one operation. Ethanol yield was highest in bread crust (100.9% +/-5.1%) followed by rice grain (108.0%+/-7.9%) and then potato chips 80.7% (+/-4.7%). High salt content of potato chips negatively affected yeast activity resulting in low ethanol production.

Le Man *et al*. (2010) optimized fermentation parameters for the generation of bioethanol from leachate obtained from food wastes in South Korea. The parameters were optimised by using response surface method that was based on 2^3- full factorial central composite design. Dinitrosalicylic acid method was used to determine the food waste leachate reduced sugar concentration and was obtained as 75g/L. In determining the optimal condition for the generation of ethanol from the waste substrates, a model referred to as second order polynomial was invented. The highest concentration of ethanol 24.17g/L was

achieved at optimum temperature (38^{o}C), pH (5.45) and reduced sugar concentration of 75g/L.

Zelelew *et al.* (2018) researched on the feasibility of using lignocellulosic waste biomass in tvhe production of bioethanol in Ethiopia. Acid hydrolysis using diluted sulphuric acid and distilled water was used and the effects of the acid concentration, temperature and retention times were determined. Under optimum conditions with 12 hours distilled water hydrolysis, the maximum bioethanol concentrations of 4.4, 5.5 and 5.1g/ml were observed by maintaining boiling temperature with reflux and 120^{o}C hydrolysis temperature kept at 30^{o}C with baker's yeast.

Li *et al*. (2011) utilized food wastes in Korea to generate bioethanol continuously by synchronous saccharification and fermentation method. The continuous production of ethanol was carried out by the process of filling and drawing. The synchronous saccharification and fermentation method was done by using a continuous decompression and distillation process of ethanol at an initial optimal saccharification temperature of (50^{o}C) and fermentation temperature of (30^{o}C) which was later changed to 35^{o}C. The level of reduced sugar intake was 3.86 g/L/hr when the temperature was held at 35^{o}C. The maximum ethanol production rate for the continuous ethanol fermentation at 352 hours was 12.4 g/L/hr.

Koshy *et al.* (2014) quantitatively estimated the amount of bioethanol produced from lignocellulosic and household wastes in India. Both chemical and enzymatic hydrolysis was performed in this experiment and extracted sugar molecules were converted into ethanol by submerged batch fermentation. Quantitative estimation of bioethanol produced was carried out by a biochemical method and a gas chromatography method. The maximum amount of ethanol produced using enzymatic hydrolysis after the third day was 15.43%, 9.85 and 22.31% for pea wastes, orange pulps and molasses respectively. Estimation done by using potassium dichromate yielded higher amount of ethanol than the use of gas chromatography.

Hosny *et al*. (2016) researched on parameters that influence the production of bioethanol in Egypt using hydrolysed bagasse. The factors studied were fermentation time, temperature, pH, concentrations of carbon sources, nitrogen sources and size of the inoculum. Conventional optimization approach (which is one factor at a time) was used. At 12 hours of incubation; 30^{o}C temperature; pH 5; carbon source 75%; peptone as the source of nitrogen and inoculum size of 5%, maximum ethanol production of (61ml/l) was achieved.

Nyachaka *et al.* (2013) in Nigeria researched on bioethanol fuel generation and quality evaluation from groundnuts shell waste. Pure cultures of *Aspergillus niger* and *Saccharomyces cerevisiae* were used in the enzymatic hydrolysis and fermentation processes respectively. The experiment was performed in five batches. In all the five batches, there was a reduction in reducing sugar concentration as fermentation time was increasing. The total ethanol yields by using 420 g groundnut shell substrates were 55.8 ml, 45.60 ml and 43.76 ml in batches one, two and three respectively. The results obtained in batches four and five were almost the same to that of batch three.

Buttowski *et al.* (2014) studied bioethanol production from food waste in Thailand. Response surface method was used to determine the effects of nitrogen source $(NH_4)2SO_4$, phosphorus source (KH_2PO_4), yeast extract and inoculum size on the production of ethanol from food waste using co-cultures of *Zymomonas mobilis* and *Candida shehatae* under non-sterile conditions. From the results, the most important parameter for the production of ethanol was yeast extract. The ethanol yield of food waste hydrolysate in co-culture batch fermentation was 0.15 g/ethanol/g-food waste.

Su *et al.* (2010) conducted a research in China on the generation of biodiesel and ethanol from food waste. Open fermentation (without autoclaved) was used in this study. The use of open fermentation resulted in the production of high quantity of bioethanol due to the fact that, the microorganism's native to the garbage stimulated the hydrolysis process and prevented the side-effect of

the autoclave. The concentration of bioethanol generated from the food wastes was 44 g/L.

Rahman *et al.* (2018) studied on the use of kitchen waste in bioethanol generation. A fermenting isolate of *Saccharomyces cerevisiae* derived from date juice was cultured in Yeast extract, peptone and dextrose (YPD) medium of an earlier published research work. Kitchen waste was made up of plant organelles and was highly rich in cellulose and monomers of starch and glucose. 7.3% (v/v) bioethanol was obtained after 48 hours fermentation in a shaking incubator.

Triana (2016) researched on bioethanol production from lignocellulose biomass specifically corn stover in London, United Kingdom. The method utilized a dynamic approach to enhance the process effectiveness by considering new technologies and models that would minimise the costs of operations, the use of utilities, and the size of units. The study considered the utilization of heat storage units to minimize the usage of utilities such as steam and cooling water by reducing overall annual costs. The results from the experiment showed that the utilization of heat integration caused an overall annualised costs to be decreased by 7% and the total energy consumption by 10%.

Saini *et al.* (2015) from India elaborated the feasibility of using agricultural wastes in the production of bioethanol. They emphasized on the main concepts and current advancements in pre-treatment, enzymatic hydrolysis and fermentation processes in the production of bioethanol from agricultural wastes in environmentally sustainable and cost-effective manner. They also enlightened on the different steps involved in second-generation bioethanol production. They later concluded that the production of bioethanol from lignocellulosic agricultural wastes is a practicable technology and should be enhanced in order to solve current and future energy issues.

Oleskowicz-Popiel *et al.* (2010) researched on the potential of generating biogas and bioethanol from organic farming in Denmark. This study focussed

on inventing new methods for the co-generation of bioethanol, biogas and animal feed depending on agricultural resources. The raw materials that were selected for this experiment were fresh maize, ensiled maize, fresh rye, dry rye, fresh clover, clover silage dry clover, dried vetch, whey permeate and cattle manure. The ability to produce bioethanol from the selected materials was determined on the basis of the amount of sugar present in each substrate. Sugars were present in higher quantities in dry rye and fresh maize as compared to the other substrates. Ensiling used as a wet-storage and a biological pre-treatment method for the generation of bioethanol was examined and the results achieved proved their capability in keeping crop's freshness and avoiding the decay of wet-biomass whiles at the same time having a positive effect on fermentation process.

Luque-Moreno (2015) used a pyrolysis-based bio refinery method to produce bioethanol from corn cobs and switch grass in Canada. The effects of the removal of alkaline ion from the biomass on the yield of ethanol were determined by assessing two demineralization steps. HPLC assay was used to assess the sugar to inhibitor ratio which was later utilized as an independent index for the substrate during fermentation. The biomass ash content reduced significantly by 82% and 90% in corn cobs when they were demineralized using acetic or nitric acid respectively whilst in switch grass only a decrease of 50% for both acetic and nitric acids were obtained. The yields of ethanol that were obtained based on the initial fraction of the cellulose were 27.8% and 27.0% in switch grass and corn cobs respectively.

Kim and Dale (2004) studied on the generation of bioethanol from wasted crops in USA. This study determined the amount of bioethanol that can potentially be generated from starch, sugar crops and agricultural residues. Results showed that about 491 GL of ethanol can be generated using wasted crops and other raw materials containing lignocellulose which is about 16 times more than the recent world ethanol production of 31 GL. They further stated that bioethanol can be a substitute for 353 GL of gasoline when utilize in E85 fuel for midsize passenger car.

2.10 Literature Gap

Incubator is the only equipment utilized by researchers for ethanol fermentation. This study for the first time has employed under soil and dark room as means of incubation in ethanol fermentation process. These traditional methods were utilized in combination with an incubator and the best condition was determined at the end of the experiment. The study has initiated these techniques which can be applied by other researchers.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study Area

Waste samples were collected from Githurai 45 market. The market is about 12 km from the centre of Nairobi city and found between latitudes 36º54'49.77''E, 1º12'10.07''S and longitudes 36º55'10.62'' E, 1º12'10.21''S. Githurai 45 is in Juja constituency of Kiambu County (Njoku, 2010).

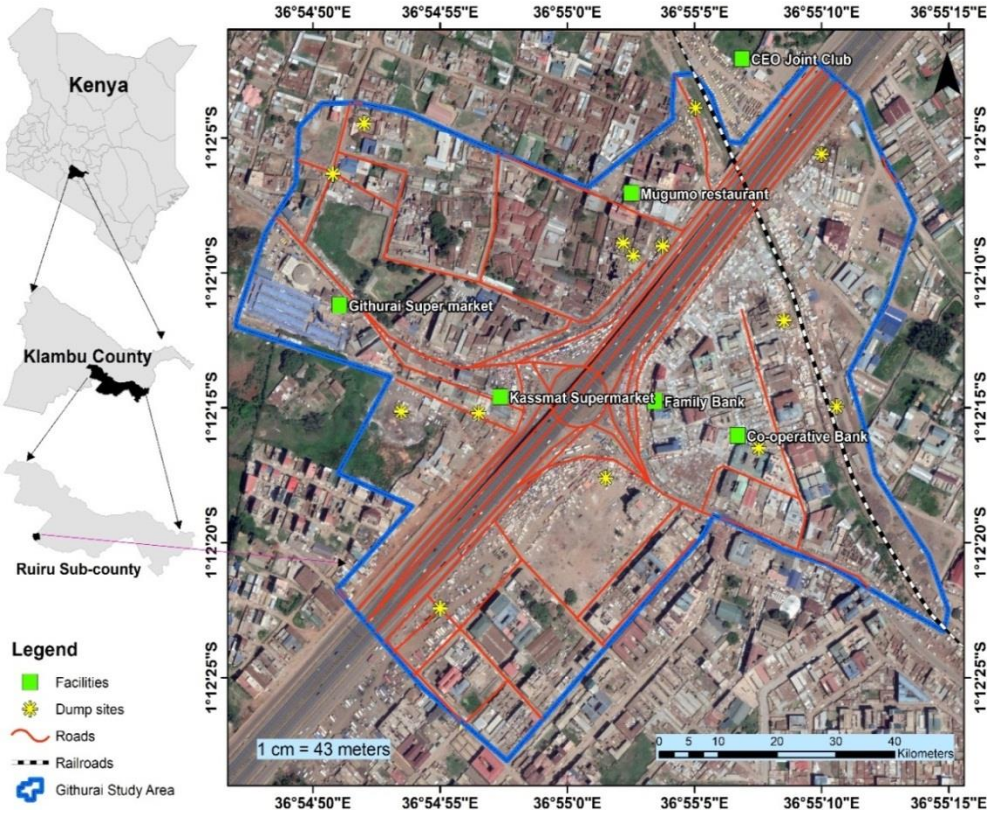

Fig 3.1: Map of Kiambu county showing Githurai market the study area. (Source: ArcGIS Platform, 2019).

3.1.1 Climate

3.1.1.1 Temperature

Githurai has a temperate climate due to its altitude. The average daily temperatures range from 29°C in dry season to 24°C for the rest of the year however temperature becomes very low at night at 9ºC (Kenya Meteorological Department, 2019).

3.1.1.2 Rainfall

Rainfall in Githurai is divided between two rainy seasons with the short rains falling in November and early December whilst the long rains fall between April and mid-June. The amount of rainfall in a year varies from 500 mm to 1500 mm with an average of 1000 mm (Kenya Meteorological Department, 2019).

3.1.1.3 Wind velocity

Strong winds occur at mid- morning to early afternoon during the dry season at velocities of 20 to 25 m.p.h in Githurai. At other times of the year, the speed of the wind are normally 10 to 15 m.p.h. Light wind normally occurs at night (Kenya Meteorological Department, 2019).

3.1.1.4 Sunshine hours

Githurai has twelve hours constant daylight throughout the year due to its proximity to the equator. The sun normally rises at 6:30-7:00 am and sets at 6:30-7:00 pm (Kenya Meteorological Department, 2019).

3.1.2 Geology and Soils

Kiambu County is divided into four topographical zones. Githurai lies in the upper midland zone with the landscape comprising of volcanic middle level uplands. The main types of soils present in the area are sandy and clay which supports drought resistant crops like soya beans and the vegetation is grassland savannah (Njoku, 2010).

3.1.3 Socio-economic Activities

The total human population in Githurai is approximately 600,000 with the largest part as children and young people. The area is characterised by the availability of cheap housing, transport and market where food is affordable. Majority of the population are literates and trading is the major occupation in the area. The people are accessible to electricity and technologies (television, smart phones etc.) (Njoku, 2010).

3.2 Research Design

Simple random sampling was used to select the sample by using random number method to assign each individual within the population a number using random number generator. Agricultural food wastes were collected from the selected sample twice every month for four months. Experimental research design was employed to determine the set objectives and test the hypothesis. Treatments utilized in this research were fermentation durations at (48, 96 and 144 hours) and incubation temperature conditions (under soil, dark room and incubator) at 19^{o}C, 21^{o}C and 30^{o}C respectively. Different incubation temperature conditions were selected as treatment in the study because no studies have been conducted on bioethanol using such conditions whilst the fermentation durations were chosen as means to determine bioethanol production at short and long hours. Statistical analysis was done to determine the effects of the treatments on each substrate. Conclusions and recommendations were developed based on the research objectives.

3.3 Sample Size and Sampling Procedure

Wastes from collard greens (*Brassica oleracea*), spinach (*Spinacia oleracea*), tomatoes (*Solanum lycopersicum*), pineapples (*Ananas comosus*), bananas (*Musa acuminate*), oranges (*Citrus sinensis*), maize (*Zea mays*), rice (*Oryza sativa*) and millet (*Panicum miliaceum*) were obtained from Githurai market. Sampling was done by defining the sample of interest which was tomatoes, collard greens, spinach, pineapples, bananas, oranges, maize, rice and millet sellers from the whole population in the market. Sample frame was obtained by finding the complete list of the population in the market. The population size of the market was estimated to be 2500 people. The sample size was determined using the confidence interval method where confidence level is taken as 95% and margin of error as 5%. With the help of a sample size calculator, the sample size was determined as 333 based on the estimated population size of 2500 people.

Simple random sampling was used to select the sample to ensure that each respondent has equal chance of being selected into the sample. This was done by using random number method to assign each individual within the population a number using random number generator where subset of the population was randomly picked. Food wastes were collected from the selected sample twice every month for four months (Research Lifeline, 2012). Wastes from vegetables (tomatoes, spinach and collard greens) were collected together in a single sack; that of fruits (pineapples, bananas and oranges) were also collected together in another different sack with cereals (rice, millet and corn cob, husks and kernels) also in another sack.

3.4 Data Collection

Wastes were analysed in the laboratory after each time of collection to determine the amount of bioethanol produced. The main processes involved in the analysis were pre-treatment, enzymatic hydrolysis and fermentation. The experimental procedures for the analysis were carried out in Kenyatta University's Environmental science, Biochemistry and Chemistry laboratories.

3.4.1 Determination of Quantities of Types of Collected Agricultural Waste Products

Each sorted wastes (vegetables, fruits and cereals) were weighed using a weighing balance and quantities of each were determined at the particular time of collection. Total weight of whole waste collected at a time was determined by addition of all weighed wastes quantities collected at that period. The percentage composition of collected waste fractions was determined by the formula

$$Percentage\ composition\ of\ waste\ fraction = \frac{\text{Weight of waste fraction}}{\text{Weight of total agricultural food wastes}} \times 100$$

(Miezah *et al.*, 2015).

3.4.2 Bioethanol Production from Selected Agricultural Food Waste Products

3.4.2.1 Pre -treatment Process

Fruit and vegetable wastes were blended separately using a blender because of their high moisture content. Corn kernels, husks and cobs were kept in an oven overnight at 110ºC and grinded together with rice and millet using a grinder. The pre-treated wastes were stored in the refrigerator at 2ºC until further use. Experiments were performed in duplicates. 80 g of each pre-treated wastes was mixed with 400 ml distilled water and pH adjusted to 4.5 using HCl and NaOH. Substrates were sterilized at 120ºC for 15 minutes using an autoclave to avoid any microbial contamination.

Fig 3. 2: Pre-treated tomatoes, spinach and collard greens mixture

Fig 3. 3: Pre-treated pineapples, bananas and oranges wastes mixture

Fig 3. 4: Mixture of pre-treated corn, millet and rice wastes

3.4.2.2 Enzymatic Hydrolysis

Enzyme cellulase from *Aspergillus niger* purchased from Kobian Scientific Limited was used in the hydrolysis process. A 0.05 M citrate buffer solution with pH of 4.8 was made by dissolving 12.04 g sodium citrate dehydrate in 800 ml distilled water. 1.74 g citric acid was added and the solution was topped up with distilled water until a volume of 1 litre was reached. pH was adjusted to 4.8 using hydrochloric acid and sodium hydroxide with the aid of a pH meter. An enzyme solution was prepared for each substrate by dissolving 1g of cellulase in 10 ml citrate buffer solution for 15 minutes. The enzyme

solution was added to each sterilized pre-treated substrate with each mixture shaken at 150 rpm for 15 minutes and then incubated at 35ºC for 18 hours. Clear liquid hydrolysates of each substrate were obtained by centrifugation using a centrifuge set at 5000 rpm for 15 minutes followed by filtration by the use of filter papers (Kim *et al*., 2011).

Fig 3. 5: Cereals, vegetables and fruits hydrolysates produced after enzymatic hydrolysis

3.4.2.3 Fermentation Process

Anaerobic batch fermentation was carried out to convert the released sugars into bioethanol. pH of each hydrolysates was adjusted to 4.5 and sterilized at 120ºC for 15 minutes before fermentation. 1 ml of cultured yeast suspension containing approximately 8.4×10^7 yeast cells was added to 10 ml of each sterilized hydrolysates and then shaken with the aid of a shaker set at 150 rpm for 10 minutes. Fermentation was carried out in 100 ml cotton- plugged conical flasks covered with aluminium foils (Walker *et al*., 2013).

3.4.2.3.1 Yeast Culture Media Preparation

Baker's yeast (*Saccharomyces cerevisiae*) obtained from the supermarket was cultured in YPD broth before inoculating into the hydrolysates. The YPD broth contained 20 g/l dextrose, 4 g/l yeast extract and 3 g/l peptone. pH of broth was adjusted to 6.5 and sterilized at 120ºC for 15 minutes using an autoclave. 0.6 g of baker's yeast was added to the sterilized YPD broth and

media was shaken for 15 minutes at 150 rpm before being kept in an incubator set at 30^{o}C for 42 hours (de Albuquerque *et al.*, 2014).

The number of cells present in the culture media was determined by using haemocytometer together with microscope. In order to determine the number of viable cells present, 0.4% trypan blue solution was prepared using 4 g/l trypan blue together with 8 g/l NaCl, 11.2 g/l KCl, 1.44 g/l Na_2HPO_4 and 0.24 g/l KH_2PO_4 (Kay, 2009). 50 ul of trypan blue solution was mixed with 50 ul culture media to a dilution of 1:2. 5 ul of mixture was put on haemocytometer, covered with slide and viewed under the microscope. Non-stained cells which are viable cells were counted on one chamber of the haemocytometer containing 4 corner squares. With 5 ul of mixture viewed under the microscope, approximately 84 cells were counted viable.

The formula for calculating the number of cells per ml is

Cells/ml=average number of cells per corner square ×dilution factor $\times 10^4$

$84/4 \times 2 \times 10^4$

42×10^4 cells in 5 ul of solution

100 ml culture media contains approximately 8.4×10^9cells per ml.

Fig 3. 6: Sterilized Yeast extract, Peptone and Dextrose broth with *Saccharomyces cerevisiae* cells

3.4.2.3.2 Optimization of fermentation times and temperatures

90 ml hydrolysates from each wastes was divided into 10 ml in nine conical flasks and labelled appropriately. Optimization was done by using different incubation techniques at different temperatures and different fermentation durations to determine the best condition and duration for bioethanol production in each substrate. Different incubation techniques at different temperatures for fermentation took place in an incubator set at 30^{o}C, in the dark room at temperature 21^{o}C and under soil inside a carton placed in the laboratory at temperature 19^{o}C with different fermentation durations of 48 hours, 96 hours and 144 hours. Temperatures in the dark room and under soil were determined by the use of a thermometer.

3.4.3 Bioethanol Analysis

Analysis of bioethanol was carried out using potassium dichromate and sulphuric acid together with UV-vis spectrophotometer. Prepared standards were used to estimate the concentration of bioethanol produced from each substrate. Aqueous phase samples obtained after 48, 96 and 144 hours were centrifuged at 4000 rpm for 5 minutes. 1 ml of each supernatant was pipetted into test tubes and the same amount of distilled water, potassium dichromate solution and sulphuric acid used in the preparation of standards were added. Vortex was used to homogenize the prepared solutions. Spectrophotometer at 620 nm wavelength was used to determine the absorbance of each prepared solution. Standard curve was drawn from known concentrations of standard ethanol solutions and their corresponding absorbance values. Bioethanol concentration at different incubation temperature and fermentation duration was determined from the curve and expressed as %vol/vol (Endalew, 2015; Singh and Singh, 2015).

Bioethanol yield was determined from the concentration divided by utilized substrates and expressed as ml of ethanol per kg substrates (ml/kg) according to the formula $Bioethanol\ yield\ \left(\frac{ml}{kg}\right) = \frac{\text{Bioethanol conc}(\%)\times 400\ (ml)}{80(\text{g})\times 10\ (ml)} \times 1000$ where 10 ml is the amount of hydrolysate used for fermentation, 80 g is the quantity of substrate, 400 ml is the volume of distilled water used to dissolve the substrates.

Bioethanol productivity was also derived from the concentration and divided by fermentation time and expressed as percentage ethanol per time in hours (%/ hr) (Mayzuhroh *et al*., 2016).

3.4.3.1 Standard Preparation

Six clean test tubes were collected and five were filled with 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1 ml of 2% absolute ethanol. The remaining one was left empty and labelled as blank (0.0). All the six test tubes were filled with distilled water to make a total volume of 5 ml. 1 ml of 10% potassium dichromate solution was added to each tube followed by 2 ml concentrated sulphuric acid.

A UV-vis spectrophotometer at 620 nm wavelength was used to determine the absorbance of each prepared standards.

Fig 3.7: Prepared Standard Ethanol Solutions

3.4.3.2 Ethanol Standard Curve

Standard concentrations were determined by the formula

$C_1V_1=C_2V_2$ where C_1 is the initial concentration of the absolute ethanol; V_1 is the initial volume of the absolute ethanol; C_2 is the final concentration to be determined and V_2 is the final volume of the prepared solution. C_1 = 2% absolute ethanol used; V_2= the final prepared standard volume which was 8 ml

For 0.2 initial standard volume

$$C1V1 = C2V2$$

$$2\times0.2/8=C2$$

$$C2= 0.05\%$$

For 0.4 initial standard volume

$$C1V1 = C2V2$$

$$2\times4/8=C2$$

$$C2=0.1\%$$

For 0.6 initial standard volume

$$C1V1 = C2V2$$

$$2\times0.6/8=C2$$

$$C2 =0.15\%$$

For 0.8 initial standard volume

$$C1V1 = C2V2$$

$$2\times0.8/8=C2$$

$$C2=0.2\%$$

For 1ml initial standard volume

$$C1V1 = C2V2$$

$$2\times1/8=C2$$

$$C2 =0.25\%$$

The standard concentrations were 0.00%, 0.05%, 0.1%, 0.15%, 0.2% and 0.25%. Their corresponding absorbance values were 0.00, 0.127, 0.219, 0.300, 0.377 and 0.450 respectively.

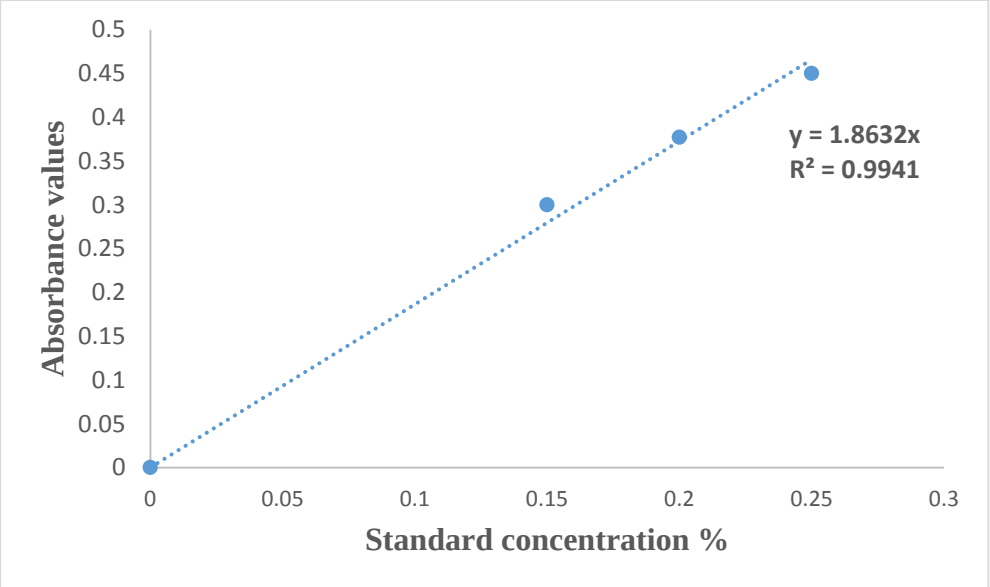

Fig 3.8: Ethanol standard curve

The concentrations in %v/v of each of the substrates at different fermentation durations and temperatures were determined by dividing their absorbance values by 1.8632.

3.5 Data Analysis

Collected data were entered in excel 2013 before statistical analysis was done using Genstat statistical package (Discovery version 4). One way ANOVA with no blocking was used in the analysis to determine the amount of bioethanol produced from each wastes under different incubation conditions and durations; the effects of different fermentation durations on bioethanol production and; the effects of different incubation techniques at different temperatures on bioethanol production. Two way ANOVA with no blocking was used in the analysis to determine the effects of increasing fermentation durations at different temperatures for bioethanol production. Significant differences among means were separated by using least significant difference method at 5% level of probability ($P<0.05$). Ethanol standard curve was drawn by using Excel 2013.

CHAPTER FOUR: RESULTS AND DISCUSSION

This chapter highlights the results and discussion of the study and reveals the feasibility of using agricultural waste products in bioethanol production. It reports results on the quantities of agricultural food waste products randomly collected from Githurai market, the amount of bioethanol produced from each agricultural food waste products and the effects of different fermentation durations and incubation techniques at different temperatures for bioethanol production in cereals, fruits and vegetables wastes.

4.1 Determination of quantities of agricultural food waste products collected from Githurai market

The table below shows the percentage composition and the total weight of the sorted agricultural food wastes collected from Githurai market twice every month for four months.

Table 4. 1: Total weight and percentage composition of collected agricultural food waste products

Agricultural waste products	Total weight of each collected samples (kg)	Composition of collected waste samples (%)
Cereals	14.419	19
Fruits	36.837	49
Vegetables	24.078	32

Fruit wastes comprising of bananas, pineapples and oranges had the highest percentage composition followed by vegetables and cereals wastes respectively based on the quantities collected for this study. These selected wastes were utilized into beneficial end products in the study.

4.2 Determination of amount of bioethanol produced from cereals, fruits and vegetables wastes under different incubation conditions and durations

Bioethanol productivity, concentration and yield were the three parameters used to determine the amount of bioethanol produced from the three different

agricultural food wastes. According to Mayzuhroh *et al*. (2016), the efficiency in bioethanol production can be evaluated by these three parameters. Table 4.2 shows means of quantities of bioethanol produced from each agricultural food wastes. From the table, bioethanol productivity, concentration and yield of cereals (maize, rice and millet) gave the highest results followed by fruits (bananas, pineapples and oranges) and then vegetables (spinach, tomatoes and collard greens).

There was highly significant difference between each wastes in all the different parameters used to determine the amount of bioethanol produced. The highly significant difference is due to highly unequal amount of bioethanol produced from each wastes due to differences in their chemical constituents necessary for bioethanol generation. Examples of these chemical constituents are cellulose, hemicellulose, lignin, starch, sugars, and so on. According to Nuwamanya *et al*. (2012), differences in characteristics of constituents of feed stocks highly affect their hydrolysis process which in effect influence the kind of sugars produced and thus control the metabolic activities carried out by yeast under such conditions.

Table 4. 2: Quantities of bioethanol produced from vegetables, fruits and cereals wastes

Types of agricultural wastes	**Bioethanol productivity (%/hr)**	**Bioethanol concentration (% v/v)**	**Bioethanol yield (ml/kg)**
Vegetables	0.001772a	0.1270a	63.5a
Fruits	0.004159b	0.3120b	156.0b
Cereals	0.006084c	0.4812c	240.6c
P value	***	***	***
l.s.d (p<0.05)	0.000753	0.01455	7.28

NS= not significant (P>0.05), *= significant at P<0.05, **= moderately significant at P<0.01, ***= highly significant at P<0.001. l.sd = least significant difference among all the different substrates used. Different letters represent mean values that are significantly different but same letters represent no significant difference

High bioethanol production in cereals is due to high cellulose content in corn cobs and husks and high starch content in wasted rice grain, millet and corn kernels. Starch is a name given to saccharides that consist of complicated molecules comprising of thousands of glucose molecule chains which are easily hydrolysed to produce glucose (Virginie *et al.,* 2018). Due to their high starch and cellulose content, high amount of sugar hydrolysate was produced after enzymatic hydrolysis hence higher bioethanol production.

According to Schwietzke *et al*. (2009), corn kernels are made up of 71.7% starch, 2.4% cellulose whilst corn cobs are made up of 42% cellulose. Sun (2002); Santi (2012) and Muktham *et al*. (2016) reported the cellulose composition of corn cobs to be 45%. High amount of bioethanol produced from cereal substrates can also be attributed to the lower moisture content present in them as compared to fruits and vegetables substrates. According to Swain *et al*. (2013), high moisture levels increase the chances of contamination during fermentation leading to high rate of by- products formation. In accordance to this, the quality of bioethanol produced from cereal substrates is higher than fruits and vegetables because of the possibilities of low by- products formation during fermentation. By- products introduce impurities into bioethanol content and thus decrease their quality (Onuki *et al*., 2016).

A study by (Gohel and Duan, 2012) used pearl millet and broken rice as substrates in bioethanol production. They reported high yields of bioethanol in both substrates and attributed the bioethanol yields to the high starch content of 68.45% in broken rice and 60% in pearl millet. Wu *et al*. (2006) also reported the bioethanol yields of pearl millet to be proportional to its starch content. This shows that the amount of starch available in a substrate is a contributing factor in ethanol production.

Fruit wastes gave reasonable amount of bioethanol due to the presence of free sugars and cellulose contents. According to Jahid *et al*. (2018) fruit wastes

contain hemicellulose and cellulosic biopolymers besides their free available sugars. They reported the cellulose content of banana peels to be 34.8% and pineapple peels 22.4% making them good substrates for ethanol production. Orozco *et al*. (2014) also reported orange bagasse, orange peel and banana peels to consist of 9.93, 11.93 and 11.45 % cellulose respectively.

The low bioethanol production in vegetables wastes is due to their low cellulose content and the presence of high levels of inhibitory compounds. Leafy vegetables have high amount of antioxidants like phenolic components, vitamins and chemo preventive components that inhibit yeast activity to produce higher amount of bioethanol. Tomato wastes are made up of lycopene and carotenoids which also acts as inhibitors during fermentation (Utama *et al*., 2019; Elkahlout *et al*., 2017). According to Szymanska-Chargot *et al*. (2017**)** tomato is made up of 8.6% cellulose. Besides the low cellulose contents and inhibitors, they also have low starch content and free available sugars to enhance high ethanol production (Health line, 2019). The presence of high levels of inhibitors, low cellulose and starch contents and low amount of free available sugars in tomato and leafy vegetable wastes accounted for their extremely low amount of bioethanol.

Utama *et al*. (2019) reported low amount of bioethanol in napa cabbage as compared to bananas and papayas. Elkahlout *et al*. (2017) also reported low bioethanol yield in tomato waste when wheat straw and tomato wastes were used in ethanol production. Cutzu and Bardi (2017) reported maximum ethanol production from corn threshing residue as compared to apple and kiwi fruits. Miezah *et al.* (2007) reported low glucan (starch and cellulose) content in vegetable waste as compared to fruit wastes hence lower bioethanol yield in vegetable waste at the end of the experiment.

The amount of bioethanol from fruit wastes was comparatively less when compared to works carried out by (Benjamin *et al.*, 2014) who reported 0.944% of bioethanol from banana peels using 4% *Aspergillus niger* and 3%

inoculum of *Saccharomyces cerevisiae* in simultaneous saccharification and fermentation method and (Hossain *et al*., 2015) with 5.09% bioethanol from banana wastes and 7.46% bioethanol from pineapples wastes using 4 g/l yeast suspension for fermentation. The study gave higher results when compared to (Gosavi *et al*., 2017) that produced 0.090% (v/v) of bioethanol from pineapple wastes. For vegetable substrates, lesser amount of bioethanol was produced when compared to studies conducted by (Khandaker *et al*., 2018; Elkahlout *et al*., 2017 and Utama *et al*., 2019). Khandaker *et al*. (2018) and Elkahlout *et al*. (2017) reported 5.067% and 0.543% bioethanol concentration from tomato wastes respectively whilst (Utama *et al*., 2019) reported 0.38% bioethanol from napa cabbage.

In terms of cereal wastes, the study produced less bioethanol percentage as compared to studies of (Akpan *et al*., 2008; Rakin *et al*., 2009; Braide *et al*., 2016 and Chaijamrus and Mouthung, 2011). Braide *et al.* (2016) reported 6.17% and 3.45% bioethanol from corn cob and corn husk respectively using 2 g of *Saccharomyces cerevisiae* grown on YPD agar plate. Chaijamrus and Mouthung (2011) also reported 14.9% bioethanol in malted rice from waste paddy using 3 litres hydrolysate. Akpan *et al.* (2008) produced 0.86 litres of bioethanol from 1200 g of maize and 1300 g of old waste newspapers and (Rakin *et al*., 2009) also produced 10.05% ethanol from the fermentation of 150 ml corn meal hydrolysate using 5% (v/v) inoculum concentration of yeast immobilized in calcium alginate.

According to Mardawati *et al*. (2019), utilization of higher amount of substrate speed up hydrolysis rate because of the binding of more compounds to the active side of enzymes leading to higher sugar production and hence higher bioethanol yield. Stengberg *et al*. (2000) reported higher bioethanol yield when high concentrations of enzyme was used. Benjamin *et al.* (2014) also reported high bioethanol percentage with higher yeast concentrations. The low bioethanol production in this study when three different substrates were combined is due to low amount of fermentable sugars produced as a result of

low quantities of wastes substrate and hydrolysates used thereby leading to additions of lower concentrations of enzymes and yeast suspensions. Therefore use of high quantities of substrates and hydrolysates would have resulted in high bioethanol production.

4.3 Optimization of different fermentation durations for bioethanol production in vegetables, cereals and fruits wastes

There was an overall highly significant difference between the different agricultural food wastes products used in terms of bioethanol concentration, productivity and yield with incubation time as seen from table 4.3.

Table 4. 3: Differences in bioethanol production from cereals, fruits and vegetables wastes at different fermentation durations

Substrates	Fermentation duration (hours)	Bioethanol productivity (%/hr)	Bioethanol concentration (% v/v)	Bioethanol yield (ml/kg)
Vegetables	48	0.003375d	0.1620 c	81.0c
Vegetables	96	0.001258b	0.1208b	60.4 b
Vegetables	144	0.000682a	0.0982a	49.1 a
Fruits	48	0.007320f	0.3514f	175.7f
Fruits	96	0.003285d	0.3153e	157.7e
Fruits	144	0.001871c	0.2694d	134.7d
Cereals	48	0.009816g	0.4712 g	235.6g
Cereals	96	0.005056e	0.4854 g	242.7g
Cereals	144	0.003381d	0.4869 g	243.5g
P value		***	***	***
l.s.d(P<0.05)		0.0003619	0.02210	11.05

NS= not significant (P>0.05), *= significant at P<0.05, **= moderately significant at P<0.01, ***= highly significant at P<0.001. l.sd = least significant difference among all the different substrates used. Different letters represent mean values that are significantly different but same letters represent no significant difference

The table also shows the significant differences between each of the agricultural food waste products with increasing fermentation time when compared alone. There was no significant difference in bioethanol

concentrations and yields with increasing fermentation time in cereals but there was highly significant difference in fruits and vegetables with increasing time. There was highly significant difference in each of the agricultural food waste products in terms of bioethanol productivity. Significant differences among each substrate with increasing time were determined from the alphabets as seen from the table. Same alphabets denote no significant difference whilst different alphabets denote highly significant difference.

These effects with regards to bioethanol concentration and yield are clearly seen in figures 4.1 and 4.2 where there are slight increases in bioethanol concentration and yield of cereals with increasing fermentation times but sharp decreases in concentration and yield of both fruits and vegetables with increasing fermentation time. With regards to bioethanol productivity, figure 4.3 shows sharp decreases in all the agricultural food waste products used.

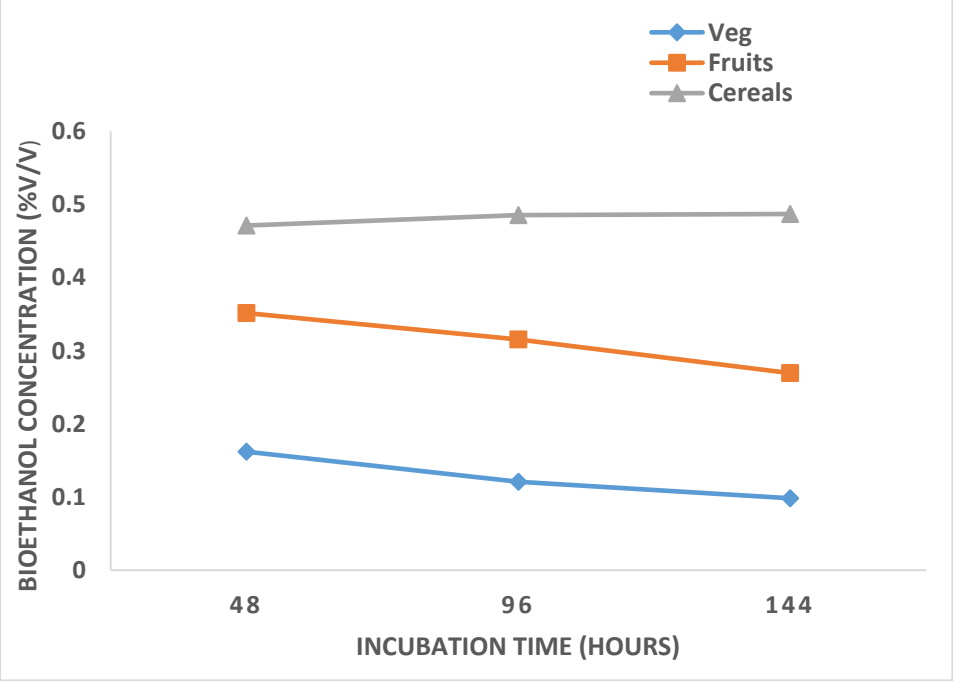

Fig 4. 1: Effects of different fermentation durations on bioethanol concentration

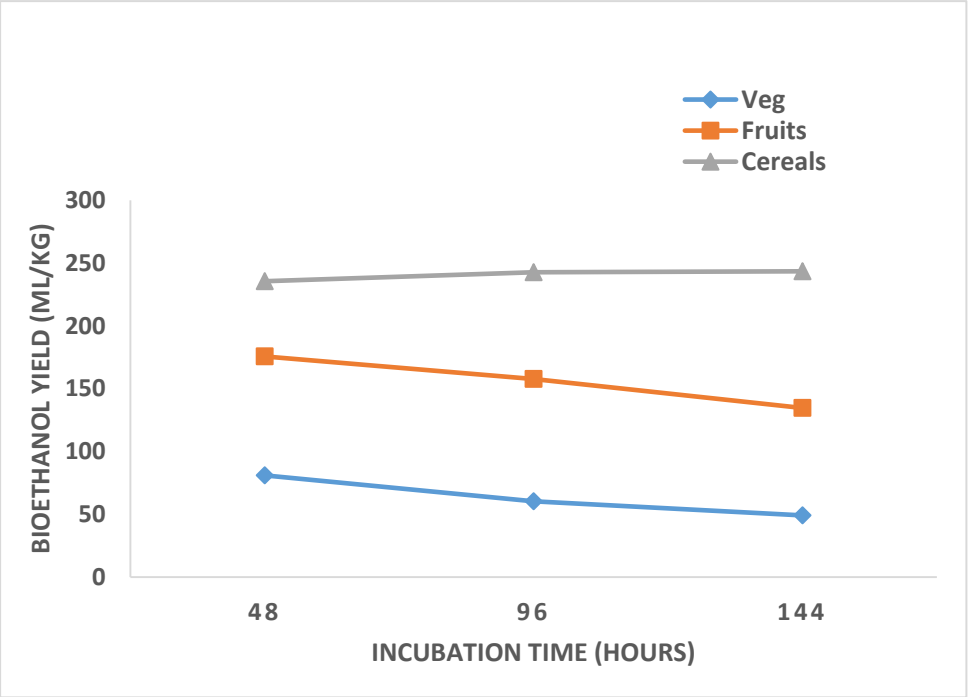

Fig 4. 2: Effects of different fermentation durations on bioethanol yield

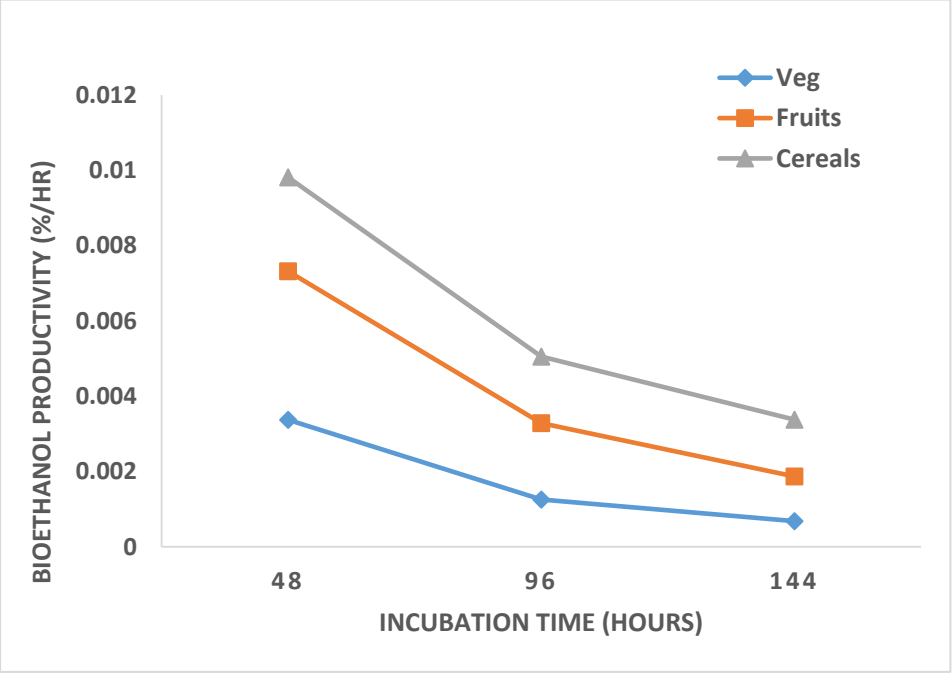

Fig 4. 3: Effects of different fermentation durations on bioethanol productivity

Maximum bioethanol concentration and yield in fruits and vegetables wastes at 48 hours fermentation duration is due to yeast cells consuming all dissolved oxygen from the medium within the first hours of fermentation, thus causing rapid production of ethanol within the 48 hours. This initial aerobic condition called the glycolytic pathway or Embden-Meyerhof-Parnas route catabolizes the six carbon sugars (glucose) to pyruvic acid. The synthesized pyruvate primarily by way of acetaldehyde is converted into bioethanol and carbon dioxide in the absence of oxygen (Amadi and Ifeanacho, 2016). According to Gawande *et al*. (2017), increase in bioethanol concentration and yield at 48 hours in fruits and vegetables substrates might also be as a result of early entry of cells into the log phase because of high initial inoculum of yeast suspension. They further also stated that, decrease in yield and concentration with time is due to accumulation of inhibitors and toxic metabolic by-products which affect yeast growth. These toxic metabolites destroy viable yeast cells leading to declination of yeast cell biomass (Zain *et al.,* 2012).

Reduction in their hydrolysates sugar level as fermentation time increases also accounted for the decrease in bioethanol yield and concentration in fruits and vegetables. According to (Gibson *et al.,* 2007), yeast cells experience diauxic shift when there is depletion in sugar level. Diauxic shift is reduction in growth as cells metabolism is modified to use non-fermentable carbon sources which in effect cause reduction in the amount of bioethanol produced. Another factor for the decrease may also be due to yeast cells progressing to the stationary phase and could no longer utilize the limited sugar available in the samples (Braide *et al*., 2018). According to Mayzuhroh *et al*. (2016) *Saccharomyces cerevisiae* can form by-products such as organic acids as fermentation time increases leading to decrease in bioethanol content. Organic acids interact with alcohol accumulated in samples to form ester compounds which intend decrease bioethanol content.

A similar finding was reported by (Sharma *et al.,* 2007) in their study where there was decrease in ethanol production from 48 to 72 hours using mixture of

citrus and banana wastes substrates. The highest bioethanol production was achieved at 48 hours of incubation. Arumugam and Manikandan, (2011**)** also reported decrease in ethanol content after 48 hours of incubation using banana and mango mixture. They attributed the decline to be as a result of reduced substrate concentration and decrease in the number of viable yeast cells as fermentation time increases. A study by (Woldesenbet *et al*., 2016) reported decrease in bioethanol yield as fermentation time increased using raw coffee wet processing wastes. Gawande and Patil (2017) also reported decrease in ethanol production after 48 hours fermentation time. They attributed the decrease to the possibility of yeast cells reaching stationary phase after 48 hours.

Ali and Kemat (2017) reported decrease in bioethanol yield with increased fermentation time from *Moringa oleifera* seeds husk substrate. Gutierrez *et al.* (2015) reported similar work where there was decrease in ethanol percentage as fermentation time increased using ripe carabao mango peelings. They accounted the decrease to be as a result of stress from yeast cells leading to reduction in their viability and vigour thus lowering ethanol yield.

Udegbunam and Mike-Anosike (2018) studied separate hydrolysis and fermentation to produce bioethanol using pineapple fruit skin and reported decrease in bioethanol concentration as fermentation duration increased. They attributed the decrease to be as a result of the build-up of inhibitory toxins that were produced in the fermentation medium as incubation time increased. Hossain *et al.* (2015) reported decrease in bioethanol production with increase in fermentation time using pineapple wastes. Shahzad *et al.* (2019) also reported decrease in bioethanol concentration from 48 to 96 hours using cotton stalk.

The slight increases in bioethanol concentration and yield in cereal hydrolysates with increased fermentation time is as a result of gradual increase in the number of yeast cells as incubation time increases (Tahir *et al*., 2010).

Increase in the number of yeast cells can be attributed to the resistant of yeast cells in cereal hydrolysate to toxic metabolites and unfavourable conditions during fermentation process. The slight increase can also be due to the fact that the sugar level in cereal hydrolysate was maintained at a constant rate as fermentation time increased and yeast cells were still in the logarithm growth phase to utilize available sugars to produce bioethanol. The velocity of logarithm growth phase of yeast is influenced by the availability of nutrients in media (Fahrizal *et al.*, 2013).

A **s**tudy done by (Akpan *et al*., 2008) reported increase in bioethanol concentration from 12 to 144 hours in maize and old waste papers. There was a sharp increase in bioethanol concentration from 12 to 96 hours but slight increase from 96 hours to 144 hours. Pothiraj *et al.* (2015) also reported increase in bioethanol concentration in cassava waste as fermentation time increased from 24 to 60 hours using *Aspergillus terreus* inoculated with *Zymomonas mobilis* in simultaneous saccharification and fermentation method. Muchtaridi *et al*. (2012) reported increase in bioethanol concentration from 0.1078 to 0.2067, 0.5284, 0.8715 and 2.6653 (%v/v) with increase in fermentation time using black sticky rice. Mushimiyimana and Tallapragada (2016) used dichromate method to determine the amount of bioethanol present in sugar beet peels, carrot peels, onion peels and potato peels. They reported increase in bioethanol yields from 14 to 28 days in each substrates used.

Braide *et al*. (2018) reported increase in bioethanol concentration in all substrates used (yam peels, cassava peels, potato peels and combined potato, cassava and yam peels) with increase in fermentation time from 24 to 72 hours. Fahrizal *et al*. (2013) reported increase in ethanol content with increase in fermentation time from 72 to 168 hours using arenga plant. Hemavathy *et al*. (2014) also reported increase in bioethanol concentration with increase in fermentation time using *Saccharomyces cerevisiae*. Siddesh *et al*. (2019) reported increase in bioethanol concentration as fermentation time increased using corn and sugarcane bagasse feedstock. Sahu (2016) also reported

increase in bioethanol production as fermentation time increased from 72 to 144 hours.

A similar study by (Gerchman *et al*., 2012) reported increase in bioethanol concentration with increase in fermentation time from 2 to 10 days. Sakharkar (2018) reported increase in bioethanol concentration in food waste with increase in incubation time from 24 to 90 hours. Chethana *et al.* (2011) reported increase in bioethanol concentration as incubation time increased from 12 to 48 hours using rice water waste. Irfan *et al*. (2014) reported increases in bioethanol production in sugarcane bagasse, rice straw and wheat straw with increase in incubation time from 24 to 72 hours. A study by (Thapa *et al*., 2017) reported increase in bioethanol concentration with increase in incubation time from 2 days to 5 days using food waste collected from houses.

The decrease in bioethanol productivity with increasing time in cereals which recorded slight increases in their bioethanol concentration and yield is as a result of decrease in the amount of substrate and increasing the amount of products which act as inhibitory agent (Thapa *et al*., 2017). The bioethanol content acts as inhibitors when their concentration is high. According to Tiukova (2014) bioethanol is toxic and sometimes represents a limiting factor in ethanol production. Thapa *et al.* (2017) reported increase in bioethanol concentration with time but decrease in productivity. Ingale *et al.* (2014) also reported increase in bioethanol concentration and yield but decrease in bioethanol productivity as incubation time increased from 36 hours to 60 hours using agricultural wastes.

The decrease in bioethanol productivity in both fruits and vegetables is as a result of low substrate and product formation with increasing time. There was decrease in bioethanol concentration and yield in both substrates and these decreases automatically accounted for decrease in bioethanol productivity with time. Zain *et al.* (2012) reported decrease in bioethanol concentration and productivity from 48 to 72 hours using free *Saccharomyces cerevisiae* yeast cells.

4.4 Optimization of different incubation techniques at different temperatures for bioethanol production in vegetables, cereals and fruits wastes

There was slight increase in bioethanol yield and concentration in vegetables from 19^{o}C to 21^{o}C but sharp decrease at temperature 30^{o}C but in fruit substrates, there was slight decrease from 19^{o}C to 21^{o}C and sharp decrease at temperature 30^{o}C. There were slight increases in bioethanol yield and concentration from 19^{o}C to 30^{o}C in cereal hydrolysates. This shows the optimum temperatures for cereals, fruits and vegetables to be 30^{o}C, 19^{o}C and 21^{o}C respectively.

Table 4. 4: Differences in bioethanol production from cereals, fruits and vegetables wastes at different incubation temperatures

Substrates	**Temperature (^{o}C)**	**Bioethanol concentration (%v/v)**	**Bioethanol yield (ml/kg)**
Vegetables	19	0.1325ab	66.3ab
Vegetables	21	0.1399b	70.0b
Vegetables	30	0.1086a	54.3a
Fruits	19	0.3270d	163.5d
Fruits	21`	0.3239d	162.0d
Fruits	30	0.2852c	142.6c
Cereals	19	0.4781e	239.0e
Cereals	21	0.4768e	238.4e
Cereals	30	0.4886e	244.3e
P value		*	*
l.s.d(P<0.05)		0.02521	12.60

NS= not significant (P>0.05), *= significant at P<0.05, **= moderately significant at P<0.01, ***= highly significant at P<0.001. l.sd = least significant difference among all the different substrates used. Different letters represent mean values that are significantly different but same letters represent no significant difference

*19^{o}C= under soil conditions; 21^{o}C= dark room conditions; 30^{o}C= incubator conditions.

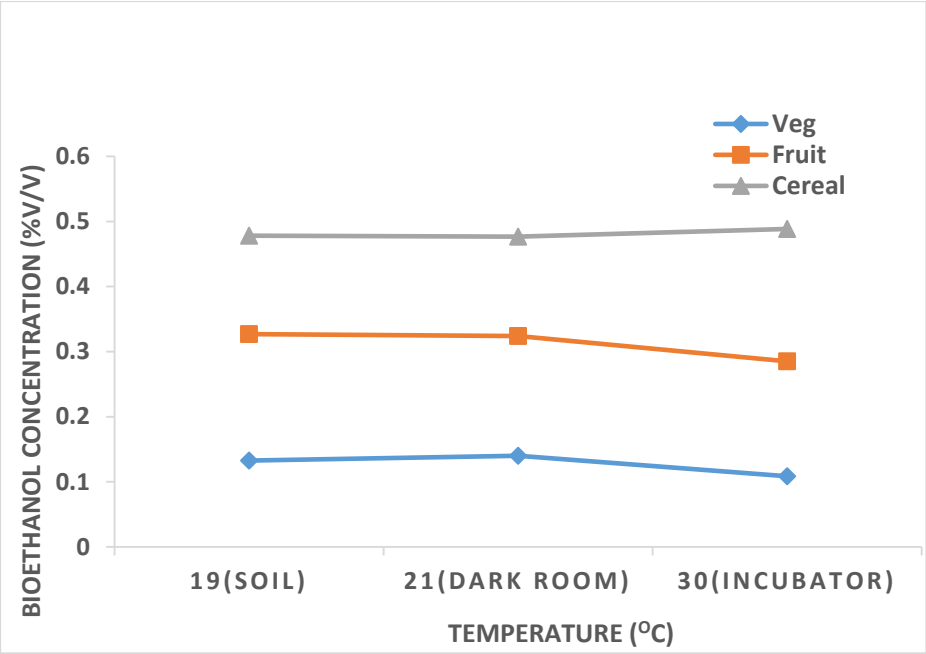

Fig 4. 4: Effects of different incubation temperatures on bioethanol concentration

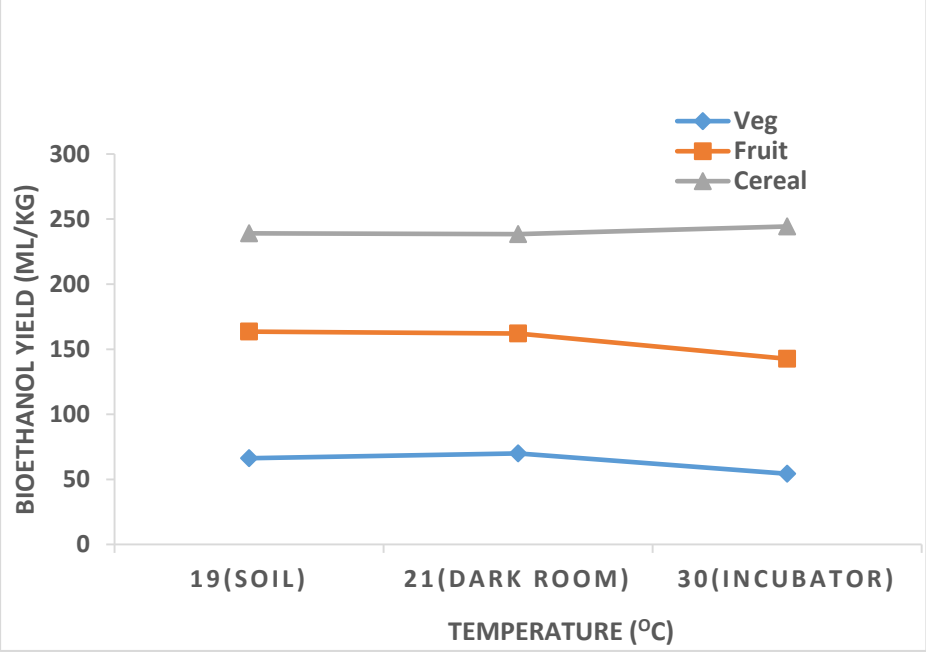

Fig 4. 5: Effects of different incubation temperatures on bioethanol yield

According to Raines-Casselman (2005) the media composition determines the growth of yeast cells and fermentation performance under different temperature conditions. In accordance to this, bioethanol production from different substrates at different temperatures depends on their hydrolysate compositions. The slight decreases in bioethanol production from cereal hydrolysates under soil and dark room is as a result of those temperatures being too low for more effective yeast growth or other conditions present under soil and in dark room aside temperature were not favourable for high ethanol production in their media composition as compared to the use of incubator. According to Gibson *et al.* (2007), low temperature conditions lead to reduction in membrane fluidity of yeast cells causing stress in yeast growth.

Temperature 30^{o}C was conducive for proper yeast growth and fermentation performance in cereal hydrolysate hence high bioethanol production. High bioethanol content at temperature 30^{o}C in cereal substrate is consistent with the findings of (Agrawal *et al.,* 2019) who reported 30^{o}C as the optimum temperature for bioethanol production when different temperatures (20^{o}C-35^{o}C) were optimised for bioethanol production in de-oiled rice bran. Kumar *et al.* (2019) also reported maximum bioethanol yield at temperature 30^{o}C from corn wastes under simultaneous saccharification and fermentation method. Tahir *et al.* (2010) reported maximum bioethanol at temperature 30^{o}C when different temperatures (10^{o}C-40^{o}C) were optimised for bioethanol production using *Saccharomyces cerevisiae* Bio-07. Mutreja *et al.* (2011); Hosny *et al.* (2016); Hossain *et al.* (2015) and Benjamin *et al.* (2014) all reported increases in bioethanol production at temperature 30^{o}C using different agricultural wastes.

Many researchers have proven temperature 30^{o}C to be the optimum temperature for maximum bioethanol production. Therefore, decrease in bioethanol production in fruits and vegetables under incubation temperature of 30^{o}C according to Tiukova (2014) might be as a result of the glucose catabolism to bioethanol in their hydrolysates in the incubator released high

amount of heat that caused further increase in the 30^{o}C temperature. Aside the possibility of heat formation leading to increase in the set temperature, the low bioethanol yield in fruits and vegetables substrates may also be due to the fact that, the temperature 30^{o}C was higher for successful yeast growth and metabolism. Maurice (2011) stated that if temperature is high for a particular substrate during fermentation, yeast cells undergo stresses as they reproduce leading to low bioethanol production. Examples of these stresses on yeast cells are increase in membrane permeability, reduced proton motive force, reduced intracellular pH and inhibition of glycolysis (Gibson *et al.*, 2007).

According to Gibson *et al*. (2007) toxic effect of bioethanol are influenced by high temperatures. Ethanol produced at high temperatures reduces yeast viability which eventually leads to low fermentation rates. This can also be the reason behind the low bioethanol production in fruit and vegetable hydrolysates at temperature 30^{o}C. They further reported that, the rapid fermentation at 15^{o}C brix honey solutions resulted in loss of yeast cell viability at temperature 30^{o}C but no loss of yeast cells were recorded at temperature 15^{o}C. Sahu (2016) optimised different temperatures (25^{o}C, 30^{o}C and 35^{o}C) at pH 4.5 for bioethanol production using potato wastes. When simultaneous saccharification and fermentation method was used, he recorded the maximum bioethanol yield at the lowest temperature which is similar to this study where the highest bioethanol yield in fruits and vegetables were achieved at the lowest temperatures.

The findings suggest that low temperatures were conducive for maximum bioethanol production in fruits and vegetables hydrolysates. It can also be due to the fact that dark room and biological conditions under soil were favourable for fermentation processes in their respective hydrolysates. Aside temperature, abiotic factors like soil organic matter, pH, conductivity, availability of water and macronutrients like nitrogen, phosphorus, potassium, sodium and magnesium present in soils influence yeast growth according to Yurkov (2017). The presence of these factors in soil may have caused proper yeast

growth and metabolism leading to high bioethanol production in fruits and vegetable hydrolysates.

4.5 Determination of effects of increasing fermentation durations at different incubation temperatures for bioethanol production from vegetables, fruits and cereals wastes

Increase in fermentation durations at different temperatures in vegetables was significant at $p<0.05$ for bioethanol concentration and yield but highly significant at $p<0.001$ in terms of bioethanol productivity. At temperature 21^{o}C, all fermentation durations recorded the highest bioethanol productivity, concentration and yield followed by 19^{o}C and then 30^{o}C.

Table 4.5: Effects of increasing fermentation durations at different incubation temperatures for bioethanol production in vegetables, fruits and cereals wastes

Temperature (^{o}C)	**Fermentation duration (hrs)**	**Bioethanol concentration (%v/v)**	**Bioethanol productivity (%/hr)**	**Bioethanol yield (ml/kg)**
		Vegetables		
19	48	0.1797b	0.003743e	89.83b
21	48	0.1928b	0.004017e	96.41b
30	48	0.1135a	0.002365 d	56.76a
19	96	0.1171a	0.001219abc	58.54a
21	96	0.1244a	0.001296c	62.22a
30	96	0.1209a	0.001259bc	60.45a
19	144	0.1008a	0.000700ab	50.38a
21	144	0.1026a	0.000712abc	51.29a
30	144	0.0914a	0.000635a	45.69a
P value		*	***	*
l.s.d (P<0.05)		0.03407	0.0005920	17.04

		Fruits		
19	48	0.3831c	0.007981d	191.5c
21	48	0.3778c	0.007870d	188.9c
30	48	0.2932ab	0.006109c	146.6ab
19	96	0.3206b	0.003339b	160.3b
21	96	0.3139b	0.003270b	157.0b
30	96	0.3116b	0.003245b	155.8b
19	144	0.2773ab	0.001926a	138.6ab
21	144	0.2800ab	0.001945a	140.0ab
30	144	0.2508a	0.001742a	125.4a
P value		NS	***	NS
l.s.d (P<0.05)		0.04842	0.0006520	24.21
		Cereals		
19	48	0.4655a	0.009697c	232.7a
21	48	0.4688ab	0.009767cd	234.4ab
30	48	0.4792abc	0.009984d	239.6abc
19	96	0.4817bc	0.005018b	240.8bc
21	96	0.4849cd	0.005051b	242.5cd
30	96	0.4895cd	0.005099b	244.8cd
19	144	0.4871cd	0.003383a	243.6cd
21	144	0.4767abc	0.003310a	238.3abc
30	144	0.4969d	0.003451a	248.5d
P value		NS	NS	NS
l.s.d (P<0.05)		0.01450	0.0002454	7.250

NS= not significant (P>0.05), *= significant at P<0.05, **= moderately significant at P<0.01, ***= highly significant at P<0.001. l.sd = least significant difference among all the different substrates used. Different alphabets denote significant difference whilst same alphabets denote no significant difference

*19^{o}C= under soil conditions; 21^{o}C= dark room conditions; 30^{o}C= incubator conditions.

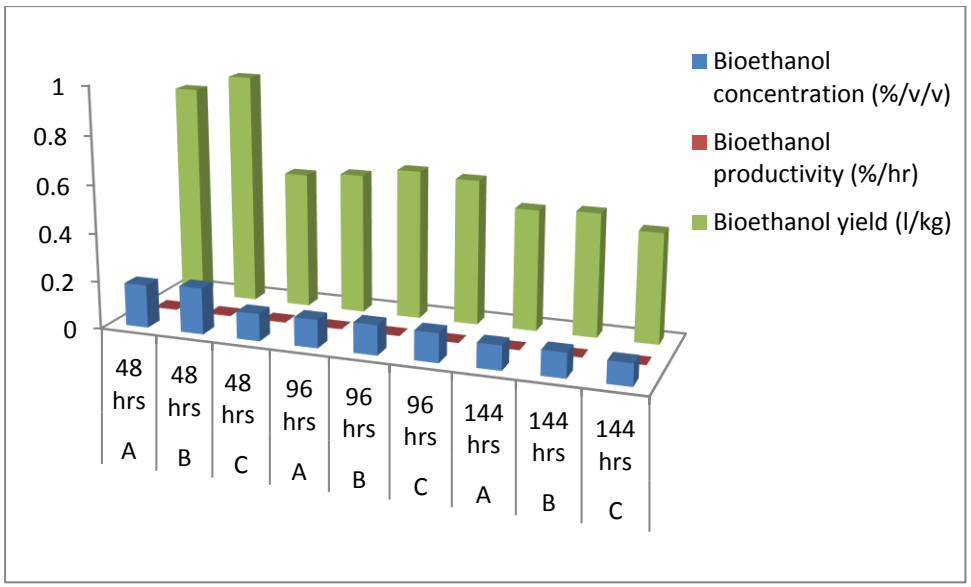

Fig 4.6: Effects of different fermentation times and incubation temperatures on bioethanol production of vegetable wastes
*A= Temperature 19^{o}C; B= Temperature 21^{o}C and C = Temperature 30^{o}C

There was decrease in bioethanol productivity with increasing fermentation time in all incubation temperatures as seen from figure 4.6 and table 4.5. At temperature 19^{o}C and 21^{o}C, there was decrease in bioethanol concentration and yield with increasing incubation time but at temperature 30^{o}C there was increase from 48 to 96 hours but decrease from 96 to 144 hours. The increase is due to the fact that the yeast cells at that temperature were still in their exponential phase from 48 to 96 hours but approached the stationary phase from 96 to 144 hours causing the decrease. Dark room and under soil incubation techniques gave the best results in bioethanol production using vegetable substrate in this study. Sahu (2016) also reported increase in bioethanol yield at temperature 30^{o}C from 72 to 96 hours but decrease from 96 to 144 hours when solid state fermentation method was carried out at pH 4.5 using potato wastes.

There was no significant difference in terms of bioethanol concentration and yield but highly significant difference with regards to bioethanol productivity in fruit substrates. For fermentation durations of 48 and 96 hours, temperature

19°C gave the highest bioethanol but at 144 hours, temperature 21°C recorded the highest in all parameters used to determine the amount of bioethanol.

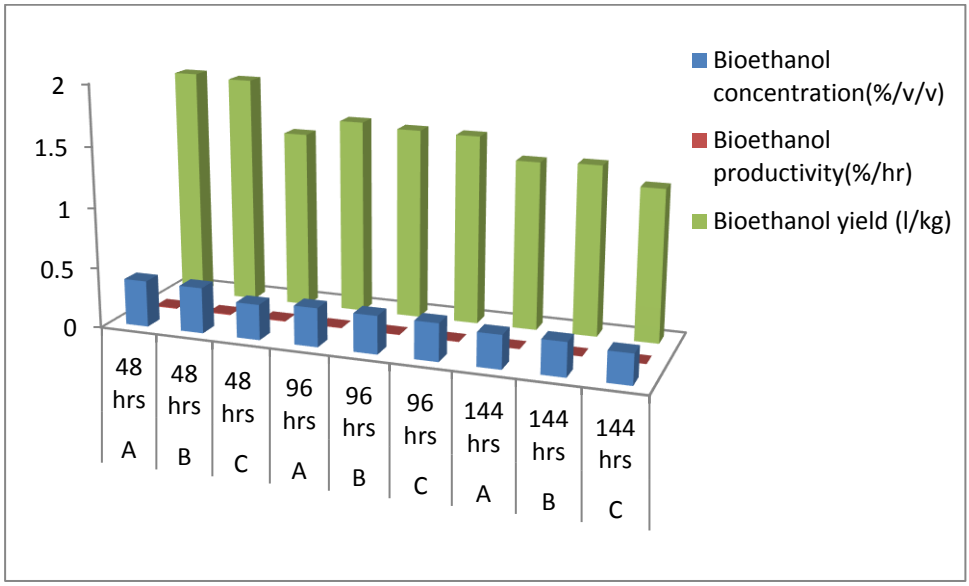

Fig.4.7: Effects of different incubation temperatures and fermentation times on bioethanol productivity, concentration and yield of fruit wastes
*A= Temperature 19°C; B= Temperature 21°C and C = Temperature 30°C

The decrease in bioethanol concentration, productivity and yield at 144 hours with temperature 19°C as compared to temperature 21°C might be due to the fact that substrate concentration of fruit hydrolysate under soil at that duration was lower than that of dark room leading to lower bioethanol concentration and yield at 19°C as compared to 21°C. There was decrease in bioethanol productivity with increasing incubation time in all fermentation temperatures. For ethanol concentration and yield, there were decreases at temperatures 19°C and 21°C as incubation duration increased but at temperature 30°C, there was an increase from 48 to 96 hours but a decrease from 96 to 144 hours as seen from figure 4.7. The increase is because yeast cells at that temperature were still in their logarithm growth phase from 48 to 96 hours but approached the stationary phase from 96 to 144 hours causing the decrease.

A similar work by (Sharma *et al*., 2007) reported increase in bioethanol yield in kinnow and banana wastes at the beginning of the incubation hours but a decrease as the incubation time was approaching the end at temperature 30ºC. Temperature 30ºC gave the least amount of bioethanol at all incubation durations making the incubator not suitable for bioethanol production using fruits wastes. The best incubation techniques for fruit substrates in this study were under soil and dark room.

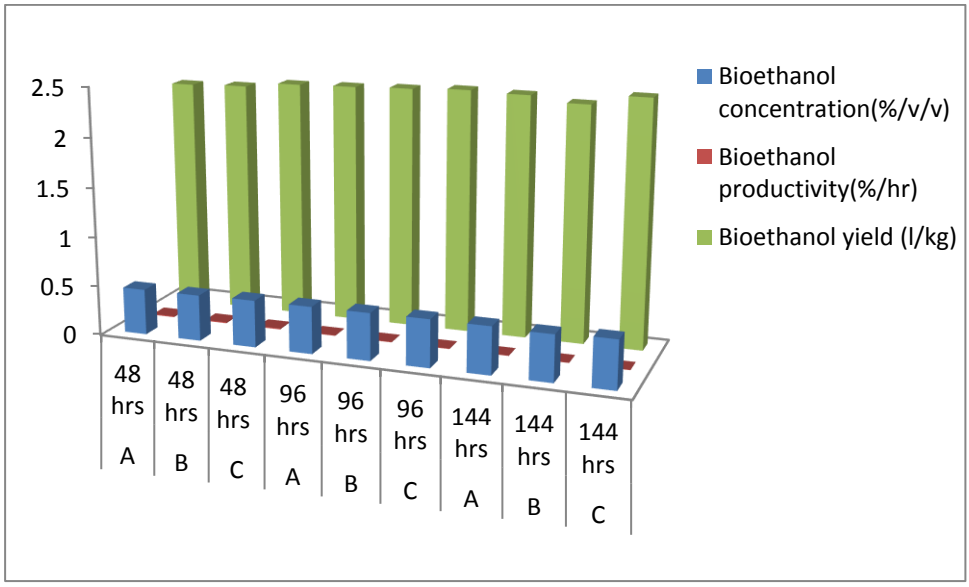

Fig 4.8: Effects of different incubation temperatures and fermentation durations on bioethanol content of cereal wastes
*A= Temperature 19ºC; B= Temperature 21ºC and C = Temperature 30ºC

In cereals substrates, there was no significant difference in bioethanol concentration, productivity and yield. Temperature 30ºC recorded the highest bioethanol concentration, productivity and yield at all incubation durations followed by incubation temperature 21ºC at fermentation durations of 48 and 96 hours but at 144 hours, bioethanol concentration, productivity and yield in temperature 19ºC was higher than that of temperature 21ºC.

Bioethanol productivity of cereals decreased with increasing incubation durations at all fermentation temperatures. There were increases in ethanol yield and concentration with increasing fermentation durations for

temperatures 30^{o}C and 19^{o}C but for temperature 21^{o}C there were decreases from 96 to 144 hours. This is because substrate composition at 21^{o}C temperature has reduced and the yeast cells have approached their stationary phase. The use of incubator gave the best results in ethanol production from cereal wastes as compared to under soil and dark room. Ahamad (2016); Benjamin *et al.* (2014) both reported increases in bioethanol production as incubation time increased from 1-7 days at temperature 30^{o}C using different agricultural waste products.

CHAPTER FIVE: SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 Summary

1) Findings from the research revealed cereals wastes as the best substrate for bioethanol production followed by fruits and vegetables wastes respectively. The high amount of bioethanol in cereal wastes is due to their rich starch and cellulose content, the substantial amount of cellulose and available sugars in fruits wastes also accounted for their bioethanol amount whilst the presence of low starch, cellulose and sugar contents in vegetables wastes resulted in their low amount of ethanol.

2) Results showed the optimum incubation temperature conditions for cereals, fruits and vegetables to be 30^{o}C (incubator), 19^{o}C (under soil) and 21^{o}C (dark room) respectively. This shows the conduciveness of using incubator, under soil and dark room for bioethanol production in cereals, fruits and vegetables wastes respectively.

3) The optimum fermentation durations were 144 hours for cereals wastes and 48 hours for fruits and vegetables wastes. Availability of nutrients in cereal hydrolysate leading to increase yeast cell numbers resulted in increased bioethanol concentration and yield with time. The accumulation of inhibitors and toxic metabolic by-products affected yeast growth causing decrease in bioethanol concentration, yield and productivity with time in fruit and vegetable hydrolysates.

5.2 Conclusions

1) The highest amount of bioethanol was achieved in cereal wastes followed by fruit and vegetable wastes respectively.
2) The optimum incubation temperature condition for maximum bioethanol in cereal wastes was incubator (30^{o}C) whilst under soil

(19^{o}C) and dark room (21^{o}C) were optimum for high bioethanol production in fruit and vegetable wastes respectively.

3) The optimum fermentation duration for maximum bioethanol production in fruit and vegetable wastes was 48 hours whilst 144 hours was the optimum duration for high bioethanol content in cereal wastes.

5.3 Recommendations

1) High amounts of bioethanol were achieved from agricultural food wastes especially fruit and cereal wastes. It is recommended that these wastes should be used in biofuel production because of their abundance and renewability other than energy crops that threaten food security.
2) The optimum incubation temperature conditions for high bioethanol production in fruit and vegetable wastes were under soil and dark room. It is therefore recommended that under soil and dark room which are more economical should be incorporated as part of the incubation techniques for fermentation especially in developing countries.
3) The optimum fermentation duration for high bioethanol production in fruit and vegetable wastes was 48 hours whilst cereal waste was 144 hours. It is recommended that fermentation should be carried out at short durations in fruit and vegetable substrates but relatively long durations in cereal substrates.

5.4 Areas for Further Research

Although this study has proven the feasibility of generating bioethanol from agricultural wastes using rare conditions like dark room and under soil as incubators for fermentation, there are still loopholes and other research works should be done by keeping in view the following aspects:

1) The conditions in the soil and dark room should be checked before and after the fermentation process. This will enhance our knowledge about the conditions that influence the production of bioethanol aside temperature.
2) The media composition to undergo fermentation should also be analysed to determine their constituents aside the sugars present. This will give a clear

idea about certain substances that can act as inhibitors to reduce the amount of bioethanol that is being produced.

3) For economic feasibility, further research should focus on the production of enzymes on-site for the enzymatic hydrolysis stage.
4) Other rare conditions aside soil and dark room should be explored to determine their feasibility in bioethanol generation.

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APPENDICES

Appendix 1: Raw data of bioethanol concentration, productivity and yield from vegetable wastes

Fermentation duration in hours under different conditions	Average Absorbance	Bioethanol conc (%v/v)	Bioethanol productivity (%/hr)	Bioethanol yield (ml/kg)
1(a) Fermentation duration of 48 hours under different conditions				
Soil (19^{o}C)	0.262	0.1406	0.00293	70.3091
	0.378	0.2029	0.00423	101.4384
	0.522	0.2802	0.00584	140.0815
	0.23	0.1234	0.00257	61.7218
	0.312	0.1675	0.00349	83.7269
	0.3	0.1610	0.00335	80.5067
	0.335	0.1798	0.00375	89.8991
	0.339	0.1819	0.00379	90.9725
Averages	0.3348	0.1796	0.00374	89.8320
Dark room (21^{o}C)	0.327	0.1755	0.003656344	87.7523
	0.34	0.1825	0.00380	91.2409
	0.622	0.3338	0.00695	166.9171
	0.267	0.1433	0.00299	71.6509
	0.35	0.1878	0.00391	93.9244
	0.299	0.1605	0.00334	80.2383
	0.338	0.1814	0.00378	90.7042
	0.331	0.1777	0.00370	88.8257
Averages	0.3593	0.1928	0.004017	96.4067
Incubator (30^{o}C)	0.167	0.089630743	0.00187	44.8154
	0.284	0.1524	0.00318	76.2130
	0.304	0.1632	0.00340	81.5801
	0.196	0.1052	0.00219	52.5977
	0.176	0.0945	0.00197	47.2306
	0.183	0.0982	0.00205	49.1091
	0.178	0.0955	0.00199	47.7673
	0.204	0.1095	0.00228	54.7445
Averages	0.2115	0.1135	0.00236	56.7572
1(b) Fermentation duration of 96 hours under different conditions				
Soil (19^{o}C)	0.231	0.1240	0.00129	61.9901
	0.203	0.1090	0.00113	54.4762
	0.313	0.1680	0.00175	83.9953
	0.222	0.1191	0.00124	59.5749
	0.167	0.0896	0.000934	44.8154

	0.185	0.0993	0.00103	49.6458
	0.2	0.1073	0.00112	53.6711
	0.224	0.1202	0.00125	60.1116
Averages	0.2181	0.1171	0.00122	58.5350
Dark room (21^oC)	0.228	0.1224	0.00127	61.1851
	0.266	0.1428	0.00149	71.3826
	0.334	0.1793	0.00187	89.6307
	0.24	0.1289	0.00134	64.4053
	0.187	0.1004	0.00105	50.1825
	0.184	0.0988	0.00103	49.3774
	0.203	0.1090	0.00113	54.4762
	0.213	0.1143	0.00119	57.1597
Averages	0.2319	0.1245	0.00130	62.2249
Incubator (30^oC)	0.177	0.0950	0.00099	47.4989
	0.332	0.1782	0.00186	89.0940
	0.315	0.1691	0.00176	84.5320
	0.199	0.1068	0.00111	53.4027
	0.177	0.0950	0.00099	47.4989
	0.215	0.1154	0.00120	57.6964
	0.179	0.0961	0.00100	48.0356
	0.208	0.1116	0.00116	55.8179
Averages	0.2253	0.1209	0.00126	60.4471
1(c) Fermentation duration of 144 hours under different conditions				
Soil (19^oC)	0.161	0.0864	0.00060	43.2052
	0.176	0.0945	0.00066	47.2306
	0.298	0.1599	0.00111	79.9699
	0.22	0.1181	0.00082	59.0382
	0.14	0.0751	0.00052	37.5698
	0.155	0.0832	0.00058	41.5951
	0.172	0.0923	0.00064	46.1571
	0.18	0.0966	0.00067	48.3040
Averages	0.1878	0.1008	0.00070	50.3837
Dark room (21^oC)	0.174	0.0933	0.00064	46.6939
	0.184	0.0988	0.00069	49.3774
	0.27	0.1449	0.00101	72.4560
	0.236	0.1267	0.00088	63.3319
	0.118	0.0633	0.00044	31.6660
	0.184	0.0988	0.00069	49.3774
	0.183	0.0982	0.00068	49.1091
	0.18	0.0966	0.00067	48.3040
Averages	0.1911	0.1026	0.000712	51.2894

Incubator (30^{o}C)	0.122	0.0655	0.00045	32.7394
	0.192	0.1030	0.00072	51.5243
	0.214	0.1149	0.00080	57.4281
	0.191	0.1025	0.00071	51.2559
	0.172	0.0923	0.00064	46.1571
	0.172	0.0923	0.00064	46.1571
	0.137	0.0735	0.00051	36.7647
	0.162	0.0869	0.00060	43.4736
Averages	0.1703	0.0913	0.00063	45.6875

Appendix 2: Raw data showing bioethanol concentration, yield and productivity of cereal wastes

Fermentation duration in hours under different conditions	Average Absorbance	Bioethanol conc (%v/v)	Bioethanol productivity (%/hr)	Bioethanol yield (ml/kg)
2(a) Fermentation duration of 48 hours under different conditions				
Soil (19^{o}C)	0.829	0.4449	0.00927	222.4667
	0.844	0.4530	0.00944	226.4921
	0.914	0.4906	0.01022	245.2769
	0.796	0.4272	0.00890	213.6110
	0.894	0.4798	0.01000	239.9098
	0.894	0.4798	0.01000	239.9098
	0.896	0.4809	0.01002	240.4465
	0.871	0.4675	0.00974	233.7377
Averages	0.8673	0.4655	0.00970	232.7313
Dark room (21^{o}C)	0.88	0.4723	0.00984	236.1529
	0.895	0.4804	0.01001	240.1782
	0.894	0.4798	0.01000	239.9098
	0.791	0.4245	0.00884	212.2692
	0.898	0.4820	0.01004	240.9832
	0.879	0.4718	0.00983	235.8845
	0.883	0.4739	0.00987	236.9579
	0.868	0.4657	0.00971	232.9325
Averages	0.8735	0.4688	0.00977	234.4085
Incubator (30^{o}C)	0.93	0.4991	0.0104	249.5706
	0.901	0.4836	0.01017	241.7883
	0.916	0.4916	0.01024	245.8137
	0.824	0.4422	0.00921	221.1249
	0.894	0.4798	0.01000	239.9098
	0.899	0.4825	0.01005	241.2516
	0.903	0.4847	0.01010	242.3250
	0.876	0.4701	0.00979	235.0794
Averages	0.8929	0.4792	0.00998	239.6079
2(b) Fermentation duration of 96 hours under different conditions				
Soil (19^{o}C)	0.895	0.48035	0.0050	240.1782
	0.859	0.4610	0.0048	230.5173
	0.921	0.4943	0.00515	247.1554
	0.894	0.4798	0.0050	239.9098
	0.914	0.4906	0.00511	245.2769
	0.915	0.4911	0.00512	245.5453
	0.903	0.4847	0.00505	242.3250
	0.879	0.4718	0.00491	235.8845

Averages	0.8975	0.4817	0.00502	240.8491
Dark room (21^oC)	0.894	0.4798	0.0050	239.9098
	0.923	0.4954	0.0052	247.6921
	0.942	0.5056	0.0053	252.7909
	0.888	0.4766	0.00496	238.2997
	0.894	0.4798	0.0050	239.9098
	0.918	0.4927	0.00513	246.3504
	0.897	0.4814	0.00501	240.7149
	0.872	0.4680	0.00488	234.0060
Averages	0.9035	0.4849	0.00505	242.4592
Incubator (30^oC)	0.931	0.4997	0.00520	249.8390
	0.916	0.4916	0.00512	245.8137
	0.925	0.4965	0.00517	248.2289
	0.891	0.4782	0.00498	239.1048
	0.897	0.4814	0.00501	240.7149
	0.919	0.4932	0.00514	246.6187
	0.921	0.4943	0.00515	247.1554
	0.897	0.4814	0.00501	240.7149
Averages	0.9121	0.4895	0.00510	244.7738
2(c) Fermentation duration of 144 hours under different conditions				
Soil (19^oC)	0.923	0.4954	0.00344	247.6921
	0.914	0.4906	0.00341	245.2769
	0.924	0.4959	0.00344	247.9605
	0.888	0.4766	0.00331	238.2997
	0.879	0.4718	0.00328	235.8845
	0.93	0.4991	0.00347	249.5706
	0.918	0.4927	0.00342	246.3504
	0.885	0.4750	0.00330	237.4946
Averages	0.9076	0.4871	0.00338	243.5662
Dark room (21^oC)	0.884	0.4745	0.00329	237.226
	0.902	0.4841	0.00336	242.0567
	0.914	0.4906	0.00341	245.2770
	0.839	0.4503	0.00313	225.1503
	0.897	0.4814	0.00334	240.7149
	0.911	0.4889	0.00340	244.4719
	0.889	0.4771	0.00331	238.5681
	0.869	0.4664	0.00324	233.2009
Averages	0.8881	0.4767	0.00331	238.333

Incubator (30^{o}C)	0.948	0.5088	0.00353	254.4010
	0.937	0.5029	0.00349	251.4491
	0.946	0.5078	0.00353	253.8643
	0.896	0.4809	0.00334	240.4465
	0.898	0.4820	0.00335	240.9832
	0.938	0.5034	0.00350	251.7175
	0.942	0.5056	0.00351	252.7909
	0.902	0.4841	0.00336	242.0567
Averages	0.9259	0.4969	0.00345	248.4637

Appendix 2: Raw data showing bioethanol concentration, productivity and yield produced from fruit wastes.

Fermentation duration in hours under different conditions	Average Absorbance	Bioethanol conc (%v/v)	Bioethanol productivity (%/hr)	Bioethanol yield (ml/kg)
3(a) Fermentation durations of 48 hours under different conditions				
Soil (19^{o}C)	0.754	0.4047	0.00843	202.3401
	0.608	0.3263	0.00680	163.1602
	0.895	0.4804	0.01001	240.1782
	0.625	0.3354	0.00699	167.7222
	0.689	0.3698	0.00770	184.8970
	0.731	0.3923	0.00817	196.1679
	0.702	0.3768	0.00785	188.3856
	0.706	0.3789	0.00789	189.4590
Averages	0.7138	0.3831	0.00798	191.5388
Dark room (21^{o}C)	0.762	0.4090	0.00852	204.4869
	0.622	0.3338	0.00695	166.9171
	0.886	0.4755	0.00991	237.7630
	0.568	0.3049	0.00635	152.4259
	0.709	0.3805	0.00793	190.2641
	0.679	0.3644	0.00759	182.2134
	0.707	0.3795	0.00791	189.7274
	0.698	0.3746	0.00780	187.3122
Averages	0.7039	0.3778	0.00787	188.8887
Incubator (30^{o}C)	0.64	0.3435	0.00716	171.7475
	0.546	0.2930	0.00611	146.5221
	0.651	0.3494	0.00728	174.6994
	0.542	0.2909	0.00606	145.4487
	0.444	0.2383	0.00496	119.1498
	0.551	0.2957	0.00616	147.8639
	0.497	0.2667	0.00556	133.3727
	0.5	0.2684	0.00559	134.1778
Averages	0.5464	0.2932	0.00611	146.6227
3(b) Fermentation duration of 96 hours under different conditions				
Soil (19^{o}C)	0.716	0.3843	0.004003	192.1426
	0.529	0.2839	0.00296	141.9601
	0.712	0.3821	0.00398	191.0691
	0.606	0.3252	0.00339	162.6234
	0.508	0.2726	0.00284	136.3246
	0.543	0.2914	0.00304	145.7170
	0.558	0.2995	0.00312	149.7424
	0.606	0.3252	0.00339	162.6234
Averages	0.5973	0.3206	0.00334	160.2753

Dark room (21^{o}C)	0.664	0.3564	0.00371	178.1881
	0.444	0.2383	0.00248	119.1498
	0.756	0.4058	0.00423	202.8768
	0.56	0.3006	0.00313	150.2791
	0.505	0.2710	0.00282	135.5195
	0.552	0.2963	0.00309	148.1322
	0.597	0.3204	0.00334	160.2082
	0.601	0.3226	0.00336	161.2817
Averages	0.5849	0.3139	0.00327	156.9544
Incubator (30^{o}C)	0.642	0.3446	0.00359	172.2842
	0.59	0.3167	0.00330	158.3298
	0.671	0.3601	0.00375	180.0666
	0.553	0.2968	0.00309	148.4006
	0.46	0.2469	0.00257	123.4435
	0.708	0.3800	0.00396	189.9957
	0.501	0.2689	0.00280	134.4461
	0.519	0.2786	0.00290	139.2765
Averages	0.5805	0.3116	0.00325	155.7803
3(c) Fermentation duration of 144 hours under different conditions				
Soil (19^{o}C)	0.611	0.3279	0.00228	163.9652
	0.321	0.1723	0.00120	86.1421
	0.704	0.3778	0.00262	188.9223
	0.586	0.3145	0.00218	157.2563
	0.425	0.2281	0.00158	114.0511
	0.526	0.2823	0.00196	141.1550
	0.494	0.2651	0.00184	132.5676
	0.466	0.2501	0.00174	125.0537
Averages	0.5166	0.2773	0.00193	138.6392
Dark room (21^{o}C)	0.64	0.3435	0.00239	171.7475
	0.384	0.2061	0.00143	103.0485
	0.696	0.3736	0.00259	186.7754
	0.516	0.2769	0.00192	138.4714
	0.453	0.2431	0.00169	121.5650
	0.534	0.2866	0.00199	143.3018
	0.501	0.2689	0.00187	134.4461
	0.45	0.2415	0.00168	120.7600
Averages	0.5218	0.2800	0.00194	140.0145

Incubator (30°C)	0.447	0.2399	0.00167	119.9549
	0.394	0.2115	0.00147	105.7321
	0.521	0.2796	0.00194	139.8132
	0.536	0.2877	0.00200	143.8386
	0.417	0.2238	0.00155	111.9043
	0.547	0.2936	0.00204	146.7905
	0.478	0.2565	0.00178	128.2739
	0.398	0.2136	0.00148	106.8055
Averages	0.4672	0.2508	0.00174	125.3891

Appendix 4: Quantity of vegetable wastes collected from Githurai market, Kiambu County, Kenya

Month	Amount collected (kg)
June	1.867
	1.712
Total	3.579
July	2.921
	3.955
Total	6.876
August	3.620
	2.931
Total	6.551
September	3.421
	3.651
Total	7.072
Overall Total	**24.078**

Appendix 5: Quantity of fruit wastes collected from Githurai market, Kiambu County, Kenya

Month	Amount collected (kg)
June	3.322
	5.987
Total	9.309
July	4.461
	4.193
Total	8.654
August	4.420
	4.512
Total	8.932
September	5.092
	4.850
Total	9.942
Overall Total	**36.837**

Appendix 6: Quantity of cereal wastes collected from Githurai market, Kiambu County, Kenya.

Month	Amount collected (kg)
June	3.254
	1.388
Total	4.642
July	1.249
	2.367
Total	3.616
August	1.257
	1.255
Total	2.512
September	1.524
	2.125
Total	3.649
Overall Total	**14.419**

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Appendix 8: Published paper from the study

Osei, J.A., Manohar, S. & Kitur, E. (2020). Effects of different incubation methods on ethanol production from selected food wastes products. *Indonesian Journal of Environmental Management and Sustainability*, 4(3), 64-69 doi: https://doi.org/10.26554/ijems.2020.4.3.64-69