COMPARATIVE EVALUATION OF DIRECT FERMENTATION AND SIMULTANEOUS SACCHARIFICATION AND FERMENTATION OF POST HARVEST PINEAPPLE AND WATERMELON WASTE FRUIT FOR THE PRODUCTION OF BIOETHANOL

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BY

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NIGERIA

MAY, 2015

DECLARATION

I declare that the work in this thesis entitled 'Comparative evaluation of direct fermentation and simultaneous saccharification and fermentation of post harvest pineapple and watermelon waste fruit for the production of bioethanol' has been carried out by me in the Department of Chemistry. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or Diploma at this or any other institution.

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(M. Sc/SCIE/14563/11 - 12)	Signature	Date

CERTIFICATION

This thesis entitled 'COMPARATIVE EVALUATION OF DIRECT FERMENTATION AND SIMULTANEOUS SACCHARIFICATION AND FERMENTATION OF POST HARVEST PINEAPPLE AND WATERMELON WASTE FRUIT FOR THE PRODUCTION OF BIOETHANOL' by Uzezi Gerald IGELIGE meets the regulation governing the award of the degree of Masters of Science (M.Sc.) in Analytical Chemistry of Ahmadu Bello University, Zaria and is approved for its contribution to its knowledge and literary presentation.

Dr. P.A Ekwumemgbo	…….	…..……
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DEDICATION

This work is dedicated to Almighty God.

ACKNOWLEDGEMENT

I am most grateful to Almighty God for the opportunity and grace I enjoyed throughout this work.

I am also indebted to my major supervisor Dr P.A. Ekwumemgbo for her patience and guidance in the course of this work. I am particularly grateful for the challenges and suggestions which helped improve the quality and usefulness of this work. Also I am grateful to my supervisor, Prof. G.I. Ndukwe whose support and encouragement has made this work a success.

To my lovely wife Igelige Florence Opoola, I must specifically express my gratitude for your encouragement and support; this work wouldn't have seen the light of day if not for you. I love you so much. Also I wish to thank my parents Mr and Mrs John Igelige, Mr and Mrs. S.A. Opoola for their love and support in making this dream come true.

ABSTRACT

The production of bioethanol from waste pineapple and watermelon, using dried active baker's yeast strain (*Sacchromyces cerevisiae*) was investigated using two methods of production (Direct Fermentation and Simultaneous Saccharification and Fermentation). The proximate composition, pH and the sugar content were determined. The mean value obtained for moisture, ash, crude protein, crude fiber, fat and carbohydrate was 91.52 ± 0.01 %, 0.22 ± 0.01 %, 0.45 ± 0.05 %, 0.19 ± 0.01 %, 0.18 ± 0.01 %, 7.18 ± 0.05 % respectively for watermelon and also the mean obtained for moisture, ash, crude protein, crude fiber, fat and carbohydrate was 82.20 ± 0.07 %, 1.23 ± 0.15 %, 0.45 ± 0.07 %, 0.70 ± 0.01 %, 0.47 ± 0.03 %, 4.06 ± 0.20 % respectively for pineapple waste. The reducing sugar assay of the pineapple waste gave 7.8 g/100 g and watermelon gave 4.5 g/100 g. Effect of yeast concentration, duration of fermentation, pH and temperature as they relate to the optimisation of the ethanol production were also investigated. The result showed that the fermented fruits waste produced ethanol: 5.88 ± 0.03 % and 1.91 ± 0.01 % (v/v) for pineapple and watermelon respectively using direct fermentation and 14.18 ± 0.03 %, 3.07 ± 0.01 % (v/v) for pineapple and watermelon respectively using simultaneous saccharification and fermentation, the result shows that the rate of ethanol production by fermentation of both waste fruits by baker's yeast (*Sacchromyces cerevisiae*) increases with fermentation period and maximum ethanol yield was at 72 h.

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CHAPTER ONE

1.0 INTRODUCTION

One of the oldest biological processes known to mankind is the fermentation of biomass into bioethanol. The process has been known since prehistoric time for the production of alcoholic drinks but has lately gained growing importance due to the interest in producing fuel ethanol.

Industrial production of fuel ethanol started in Brazil during the oil crisis in the 1970s, and has ever since been widely important there. The know-how and achievements from Brazil have been used and further developed around the world. Today ethanol is produced from all sorts of biomass rich in sucrose, starch and cellulose. These are easily hydrolyzed by acids or enzymes to C6 sugars (mainly glucose), which are fermented into ethanol and CO_2.

The largest producers of bioethanol are Brazil and the USA using sugarcane and corn respectively as feedstock. Mainly wheat crops and sugar beet have so far been used in Europe. The world production of fuel ethanol in 2007 was around 50 billion liters (Renewable Fuel Association, 2008). The energy in this ethanol corresponds to less than 1.2 % of the total fuel consumption of 88.2 quadrillion Btu in 2005. In the USA (the largest producer of bioethanol) the production of bioethanol has more than doubled from 2002 to 2007 (Renewable Fuel Association, 2008).

The types of feedstock used today for production of bioethanol are primarily based on edible bio-resources, which can also find use as food for humans and animals (Klass, 1998). This has together with increasing food prices resulted in an almost religious debate about the ethics in using food for fuels (Renewable Fuel Association, 2008). Furthermore it has also increased the interest in the next generation of bioethanol. Second generation bioethanol or cellulosic ethanol as it is also called is made from biomass like agricultural waste, straw and wood. These consist of large amounts of cellulose, hemicelluloses and lignin (termed lignocellulose) and much less starch and sugar

compared to the feeds for first generation bioethanol. Due to the complex structures in which glucose is bound in lignocellulosic biomass more energy is required to release the glucose for fermentation (Klass, 1998).

In particular rice straw in Asia has a huge potential as lignocellulosic feed for second generation bioethanol production (Klinke *et al.,* 2004). These new improved technologies for biomass conversion use less non-renewable energy and give both a higher CO_2 reduction and a higher product yield than first-generation plants due to a higher utilization of the feedstock. Therefore, there is globally an intense research on developing this so-called second generation bioethanol.

Another significant aspect is that abundant coal resources are the primary source of the non-renewable energy in conversion of biomass into ethanol. In that way, a premium liquid transportation fuel is produced from a less valuable energy resource, which further reduces the need for import of petroleum (Shapouri, 2002).The use of ethanol as an automobile fuel is not a new invention. Already in 1908, Ford's model (T) could be adjusted to run on either gasoline or alcohol (Saha *et al.,* 2005). However, after World War II the interest in using ethanol as a fuel declined because cheap gasoline made from petroleum was available. In the 1970's, the interest in fuel ethanol was renewed due to the oil crisis (Aro *et al.,* 2005). More recently, ethanol has become used as an additive in gasoline. MTBE (methyl tertiary butyl ether) is used as a gasoline additive to increase the oxygen content and the octane number. During the last few years, the use of MTBE has been banned in several states in the USA due to the risk of contamination of water. Many companies have replaced MTBE with ethanol to give the gasoline similar clean burning and octane boosting properties as MTBE-blended gasoline (Dien *et al.,* 2006).

Today there are several flexi-fuel automobile models (vehicles that can run on mixtures of ethanol and gasoline containing up to 85 % ethanol) available from various manufacturers (Larsson *et al.,*

1999). About 99 % of the fuel ethanol is produced from cultivated crops. Brazil has for a long time been the leading ethanol producer of the world. However, during the last years USA has increased its production and today both countries have an annual production of about 16000 000m^3 (Nigam, 2002).

1.1 Bio-fuels

These are alcohols, ethers, esters and other chemicals made from cellulose-based biomass. This includes herbaceous and woody plant, agricultural and forestry residues and a large portion of municipal and industrial waste materials. Bio-fuels are renewable since they are produced from biomass—organic matter, such as plants. They generate about the same amount of carbon dioxide (a greenhouse gas) from the tailpipe as fossil fuels, but the plants that are grown to produce the bio-fuels actually remove carbon dioxide from the atmosphere.

Therefore, the net emission of carbon dioxide will be close to zero (Rainer and Dominik, 2007). The bio-fuels industry has evolved from using first generation feedstock (typically food crops) to using second and third generation feedstocks, for both ethanol and biodiesel. While the term bio-fuels denote any fuel made from biological sources, for most practical uses, the term refers to either ethanol or biodiesel. The last few years have seen tremendous growth in biofuel (Rainer and Dominik, 2007)

1.1.1 Types of Biofuel

1.1.2. Biothanol

Biothanol, a distilled colorless liquid fuel obtained from numerous potential feedstock varieties such as sugar beet, wheat, corn, cassava, fruits, baggasse, barley, molasses, skim milk (whey), potatoes, sorghum, switch grass and cellulose biomass such as wood, paper, straw and other

cellulose wastes such as grasses, others includes municipal solid wastes. These various waste streams for bioethanol production have their peculiar properties and generally differ. Feedstocks prices and price of natural gas are predominant influential factors that determine the cost of biothanol production. Biothanol as an alternative fuel, offers a Sustainable economy by reducing the use of imported petroleum, emitting neutral CO_2 (g), boost economy providing value added market opportunities for the Agricultural sector (Shell and Codex, 2006). Ethanol with its high octane count has is currently positively used as an automobile fuels with policies to promote its production most especially in Brazil, United States, majority of the European Union Countries and South Africa.

1.1.3 Biodiesel

This is an ester that can be made from several types of oils, such as vegetable oils and animal fats. Biodiesel is typically used as a blend 20 % biodiesel and 80 % petroleum diesel called B_{20}. B_{20} can be used in a conventional diesel engine with essentially no engine modifications. There is also a growing interest in using biodiesel where workers are exposed to diesel exhaust, in aircraft to control local pollution near airports, and in locomotives that face restricted use unless emissions can be reduced (Rainer and Dominik, 2007).

1.2 Alcohol

The word alcohol derives from Arabic al-kuhul, which denotes a fine powder of antimony produced by distilling antimony and used as an eye makeup. Alcohol originally referred to any fine powder, but medieval alchemists later applied the term to the refined products of distillation, and this led to the current usage. Ethanol, the most widely used bio-fuel, is made in a process similar to brewing beer. The ethanol in the end is blended with gasoline to improve vehicle performance and reduce air pollution.

Blending ethanol with gasoline produces a fuel with a higher octane rating which increases the engine's compression ratio and results in increased thermal efficiency. In high altitude sports, ethanol-gasoline blends are used as oxidizer and with the exhaust gases contributing little to atmospheric pollution. Bioethanol used as fuel is produced from fermentation of sugars derived mainly from corn (primary bioethanol source in the US), sugar beet, straw, sugar cane (primary bioethanol source in Brazil), wheat, molasses and any other rich source of carbohydrate (Demirbas, 2007).

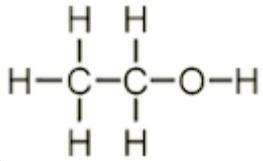
1.2.1 Properties of ethanol

Ethanol, which is also called ethyl alcohol, is a colourless, biodegradable, a high-octane, water-free alcohol. It is low in toxicity and causes little environmental pollution if spilt. Ethanol, being a straight-chain alcohol is often abbreviated as EtOH. It has a widespread usability in alcohol industry as alcoholic beverages, in chemical industry as a base chemical for other organic compounds, in medical as an antiseptic or as a treatment for poisoning by other alcohols (Demirbas, 2007).

The physical properties of ethanol are dictated primarily by the presence of the hydroxyl group and its short carbon chain. The hydroxyl group forms hydrogen bonds which makes ethanol less volatile than other organic compound of comparable molecular weight. The properties of ethanol stem primarily from the presence of its hydroxyl group and the shortness of its carbon chain. Ethanol's hydroxyl group is able to participate in hydrogen bonding, rendering it more viscous and less volatile than less polar organic compounds of similar molecular weight. Ethanol, like most short-chain alcohols, is flammable, colorless, has a strong odor, and is volatile. Ethanol-water mixtures that contain ethanol in excess of 50 % are flammable and ignite easily.

Table 1.0: Physical properties of ethanol

Property	**Value**
Molecular Formula	C_2H_6O, CH_3CH_2OH
Molecular Mass	46.07 g/mol
Density	0.789 g/mol^3
Melting Point	-114.3 ^{0}C
Boiling Point	78.4 ^{0}C
Viscosity	1.20 Cp at 20 ^{0}C

(i) 

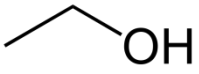
(ii) 

Figure 1.0 Chemical structure of Ethanol and its Functional group

1.3 Crops with Biofuel Potential in Nigeria

1.3.1 Sorghum

Sorghum is one of the high drought resistance crops cultivated in about 50 % of the Nigeria agricultural land, mostly in the Northern region (8 ^{0}N to 14 ^{0}N latitude), accounting for 6.86 million hectares of land. Annual production has been estimated to rise by 45 % from the total production of 4.8 million tonnes in 1978 (Ogbonna, 2002). This Figure gives Nigeria the opportunity to be the highest producer of sorghum in Sub Saharan Africa, accounting for about 70 % of the total production in the region. The commonly grown varieties are the Farfara, Guinea and Kaura, which are all resistance to different killer weeds, and scientifically classified.

Sorghum is currently use in Nigeria for two main categories of purpose classified here as local and industrial. Traditionally, the crop is mostly cultivated by poor farmers to meet their local demands; they mainly use their harvest for food, beverages, and variety of drinks. Non-food uses include roofing and fencing of compounds in local communities. The local application accounts for about 73 % of annual sorghum usage in the country. Industrially, the crop is used in malting. In 1984 and 1985 the demand for industrial sorghum malt in Nigeria was computed as 134170 and 161043 kg, accounting for 64 and 74 million Naira market value respectively (Ilori, 1991). This Figure had since risen by about 45 %, considering the large scale demand of sorghum both locally and industrially, diversion of the crop for fuel ethanol production could have severe consequences.

First, the peasant farmers would definitely shift from cultivating other subsistence crops to sorghum, creating an imbalance in the food circle. Secondly, the objective of the biofuel policy would be defeated by sudden rise in food price and inappropriate use of agricultural land. Thirdly, most of the agricultural land would be exposed to degradation due to continuous mono-cropping, and this can severely add to the already existing problems of soil erosion and desertification in the Northern parts (Galadima, *et al.*, 2007).

1.3.2 Cassava

Cassava is another crop grown on both local and commercial scales in some major parts of Nigeria, especially the rainforest, and the savannah areas of North West and North Central, due to availability of well-drained deep loamy soils. The spread of cassava production in the country could be traced to the period between 1930 and 1946, when yam production was considered unprofitable due to considerable damage by pests. Over sixty different varieties are currently cultivated. Initially, sweet varieties that could be eaten by the local people without further processing were the dominants. However, these were subsequently matched with other improved varieties such as TMS

30572, 4(2)1425, 92/0326 and NR 8082. The annual production was estimated to have increased by about 66 % from 382,000 hectare per year from 1946 (Nweke, 2004). Like the sorghum, cassava is used at both local and industrial scales. Peasant farmers employ the tubers for production of food in form of garri, fufu and fermented flour (Ugwu and Nweke, 1996). Industrially, the crop is used as raw material for starch, chips, pellets, unfermented flour and more importantly in beer manufacture.

Cassava has been given a great emphasis for fuel ethanol production under the current biofuel implementation plan than sorghum. In areas where its production remain the only source of food and household incomes for the local farmers, an imbalance could be created, although may not be very severe if the existing pre-exploited land is used in preference. Careful planning is therefore necessary to ensure that, large scale cassava production is carried out screening out food-to-fuel diversion issues.

1.3.3 Sugarcane

Since its introduction into the country through the eastern and western coasts by the European Sailors in fifteen century, sugarcane has become an important crop grown in many parts of Nigeria. Traditionally, sugarcane is grown on small holdings (usually 0.2 to 1.0 ha) for chewing as juice and preparing livestock feed. However, with the increased demand for sugar in the country, the crop is grown on large scale as raw material for sugar industry.

Around 1997, the major sugar companies operating; Bacita, Lafiagi, Numan and Sunti utilized about 12,000 ha out of the total 30, 000 ha for sugar-based sugarcane production (Agboire, 2002). In the year 2007/2008 an estimate of 100, 000 tonnes were produced compared to 80, 000 tonnes in 2006/2007, however, due to the persistent increased in sugar demand to 1.50 billion tonnes, making Nigeria the second largest in Africa, the local sugarcane production is insufficient to meet the

demand. With the current shift to biofuel ethanol production by the government, more companies were invited to participate in sugarcane production across the country.

In the last few years, a US-based company (Lemna International) proposed to establish the first ethanol production plant in Taraba State. The project analysed to cost 50 million US$ would require a land covering 30,000 to 50,000 ha for local raw material cultivation.

The NNPC have clearly identifies sugarcane and cassava as the major raw materials for the bioethanol production program. Currently, investors have already invested over $3.86 billion for the construction of 19 ethanol bio- refineries, 10,000 units of mini-refineries and feedstock plantations for the production of over 2.66 billion litres of fuel grade ethanol per annum from sugarcane and cassava, leading to land requirement of 859,561 ha (Ohimain, 2010).

Sugarcane-based fuel ethanol production would have very little threat to the local people, as the crop is not used for daily food like sorghum or cassava. However, sudden rise in prices of sugar and sugar products would be a great challenge. To address this, importation and selling of sugar to peasants at a subsidized rate is necessary. Similarly, an unbiased food price *versus* food-fuel feasibility research should be executed simultaneously, such that proper policy modification is carried out in line with real situation.

1.3.4 Jatropha

The policy identifies Jatropha oil as the main pilot raw material for the biodiesel industry. Jatropha is non-edible plant and therefore has not been on the large scale production by either the Nigerian food or commercial farmers. Some few research plantations were established in the recent years, as pilot studies for checking soil desertification. However, with the current biofuels plan some northern states namely Kebbi, Sokoto, Zamfara, Katsina, Kano, Jigawa, Bauchi, Yobe, Borno,

Adamawa and Gombe are selected for large scale production. A number of literature studies have indicated Jatropha to be a very good source of oil for biodiesel production; yielding nearly 100 % of the fuel in short transesterification time under both homogeneous and heterogeneous conditions (Lu, 2009; Sahoo and Das, 2009; Vyas, 2009).

From the economic perspective studies indicated successes in large scale Jatropha plantations in different tropical countries. Studies by Prueksakorn (2010) in Thailand showed that, both 20 years perennial system and annual cultivation method, involving harvesting the trees for wood and the seed for biodiesel could produce up to 4720 and 9860 GJ of net energy per ha.

In India, production and use of Jatropha biodiesel have reported to triggers 82 % decrease in fossil diesel demand and 52 % decrease in global warming potential (Achten, 2010). Therefore, selection of Jatropha in Nigeria would be a multipurpose opportunity. In addition to the sources of energy, soil degradation, desertification, and deforestation problems could be addressed. If only 10 % of the available agricultural land (60,000,000 ha) in the selected states could be utilized, additional revenue of $3 billion, which is more than the annual allocation to these states, could be generated. However, the poor farmers may shift from food crops to Jatropha cultivation due to foreseeable market value, deforming the food circle. Similarly, continuous plantation is associated with soil acidification and eutrophication (Achten, 2010).

1.4 Fuel Ethanol

The production of ethanol from food such as corn, cassava etc. is the most predominate way of producing ethanol. This will definitely lead to a shortage in food, in-balance in food chain, increased food price and indirect land use. In 1925, Henry Ford had quoted ethyl alcohol, ethanol, as "the fuel of the future." He furthermore stated, "The fuel of the future is going to come from apples, sugar

cane, pawpaw, weeds and sawdust – almost anything. There is fuel in every bit of vegetable matter that can be fermented." Today Henry Ford's futuristic vision significance can be easily understood. In the current time, the importance of alternative energy source has become even more necessary not only due to the continuous depletion of limited fossil fuel stock but also for the safe and better environment, with an inevitable depletion of the world's energy supply, there has been an increasing worldwide interest in alternative sources of energy (Wyman, 1999; Lynd, 2004; Herrera, 2004; Lin and Tanaka, 2006).

Keeping in view all the above said advantages, biomass based fuel development technologies should rapidly gain momentum and the barriers imposed earlier should be removed for successfully attempting the production of bioethanol at the commercial level. It is welcome to understand that the use of bioethanol as a source of energy would be more than just complementing for solar, wind and other intermittent renewable energy sources in the long run (Lin and Tanaka, 2006).

During the last two decades, advances in technology for ethanol production from biomass have been developed to the point that large-scale production will be a reality in next few years (Moiser, 2005). The ethanol yields processes and economics along with the technical maturity and environmental benefits of using ethanol blend fuel are the key parameters that determine the feasibility of bioethanol production (Nguyen and Saddler, 1991). The burning fossil fuel at the current rate is likely to create an environmental crisis globally.

Ethanol production process only uses energy from renewable energy sources, and as such no net carbon dioxide is added to the atmosphere, making ethanol an environmentally beneficial energy source (Bull *et al.,* 1992; Kheshgi *et al.*, 2000). Furthermore, fuel ethanol from waste fruits may also open new employment opportunities in rural areas, and thus make a positive socio-economic impact (Wyman, 2003). Producing ethanol as fuel, beyond its current role as fuel oxygenates will require

developing waste fruit biomass as a raw material because of its abundantly available and low cost (Bevan and Franssen, 2006). The world ethanol production in 2004 was estimated to be 40 giga litres (GL) (Berg, 2004; Kim and Dale, 2004).

Brazil and the US are the world leaders, which together accounted for about 60 % of the world ethanol production exploiting sugarcane and corn respectively. In India, lignocellulosic biomass (crop residues, forestry, fruit, vegetable waste and weeds) is available in quantity. The important issue that we wish to address affirmatively here is that the bioethanol production, without doubt, needs an economical approach to address the global fuel needs. Research efforts are needed to design and improve the process, which would produce sustainable and economically feasible transportation fuel. The new designed cellulase enzyme cocktail are important factors to consider in establishing a cost effective technology, besides the low cost of feedstock (Mojovic, 2006; Gray *et al.*, 2006).

1.4.1 Ethanol Production Technologies

Bioconversion of fruit waste to ethanol consists of four major unit operations: pretreatment, hydrolysis, fermentation and product separation/ distillation.

1.4.1.1 Pretreatment

Plant biomass is made up of lignocellulosic materials which make up the majority of plant cell walls (Pandey *et al.,* 2000). This lignocellulose is difficult for microorganisms to break down through direct fermentation (Kamm *et al.,* 2006). Pretreatment is required to alter the biomass macroscopic and microscopic size and structure as well as its submicroscopic chemical composition and structure so that hydrolysis of carbohydrate fraction to monomeric sugars can be achieved more rapidly and with greater yields (Sun and Cheng, 2002; Moiser *et al.,* 2005).

Pretreatment affects the structure of waste by solubilizing hemicellulose, reducing crystallinity and increase the available surface area and pore volume of the substrate. Pretreatment has been considered as one of the most expensive processing step in biomass to fermentable sugar conversion with cost as high as 30 cents/gallon ethanol produced (Moiser *et al.*, 2005).

To assess the cost and performance of pretreatment methods, techno-economic analysis have been made recently (Eggerman and Elander, 2005). There is huge scope in lowering the cost of pretreatment process through extensive R and D approaches. Pretreatment of cellulosic biomass in cost effective manner is a major challenge of cellulose to ethanol technology research and development.

Aqueous or steam pretreatment method is often acid or base catalyzed, the main objective of this method is to process the feedstock biomass with hot water or steam applications at high temperatures and pressures for short periods. In this process the use of acids and other chemicals are minimized with high sugar recovery tendencies and minimal fermentation inhibition.

1.4.1.2 Hydrolysis

After pretreatment there are two types of processes to hydrolyze the feed stocks into monomeric sugar constituents required for fermentation into ethanol. The hydrolysis methods most commonly used are acid (dilute and concentrated) and enzymatic. To improve the enzymatic hydrolytic efficiency, the lignin-hemicellulose network has to be loosened for the better amenability of cellulase to residual carbohydrate fraction for sugar recovery. Dilute acid treatment is employed for the degradation of hemicellulose leaving lignin and cellulose network in the substrate. Other treatments are alkaline hydrolysis or microbial pretreatment with white-rot fungi (*Phaenerochate chrysosporium*, *Cyathus stercoreus*, *Cythus bulleri* and *Pycnoporous cinnabarinus* etc.) preferably

act upon lignin leaving cellulose and hemicellulose network in the residual portion. However during both treatment processes a considerable amount of carbohydrates are also degraded, hence the carbohydrate recovery is not satisfactory for ethanol production (Chandel *et al.*, 2007).

1.4.1.3 Acid hydrolysis

There are two types of acid hydrolysis process commonly used - dilute and concentrated acid hydrolysis. The dilute acid process is conducted under high temperature and pressure and has reaction time in the range of seconds or min. The concentrated acid process uses relatively mild temperatures, but at high concentration of sulfuric acid and a minimum pressure involved, which only creates by pumping the materials from vessel to vessel. Reaction times are typically much longer than for dilute acid

1.4.1.4 Dilute Acid Hydrolysis

In dilute acid hydrolysis, the hemicellulose fraction is depolymerized at lower temperature than the cellulosic fraction. Dilute sulfuric acid is mixed with biomass to hydrolyse hemicellulose to xylose and other sugars. Dilute acid is interacted with the biomass and the slurry is held at temperature ranging from 120 - 220°C for a short period of time. Thus hemicellulosic fraction of plant cell wall is depolymerised and will lead to the enhancement of cellulose digestibility in the residual solids (Sun and Cheng, 2002).

Dilute acid hydrolysis has some limitations, if higher temperatures (or longer residence time) are applied, the hemicelluosic derived monosaccharides will degrade and give rise to fermentation inhibitors like furan compounds, weak carboxylic acids and phenolic compounds (Olsson and Hahn- Hagerdal, 1996). These fermentation inhibitors are known to affect the ethanol production performance of fermenting microorganisms (Chandel *et al.*, 2006).

In order to remove the inhibitors and increase the hydrolysate fermentability, several chemicals and biological methods have been used. These methods include over liming (Martinez *et al.,* 2000), charcoal adsorption (Chandel *et al.,* 2006), ion exchange (Nilvebrant, 2001), detoxification with lactase (Chandel *et al.,* 2006), and biological detoxification (Lopez *et al.,* 2004). The detoxification of acid hydrolysates has been shown to improve their fermentability; however, the cost is often higher than the benefits achieved (Palmqvist and Hahn- Hagerdal, 2000). Dilute acid hydrolysis is carried out in two stages- First-stage and two-stage.

1.4.1.5 First-stage Dilute Acid Hydrolysis

The lignocellulosic material is first contacted with dilute sulfuric acid (0.75 %) and heated to approximately 50°C followed by transferring to the first stage acid impregnator where the temperature is raised to 190°C. Approximately, 80 % of the hemicellulose and 29 % of cellulose are hydrolyzed in the first reactor. The hydrolysate is further incubated at a lower temperature for a residence time of 2 h to hydrolyse most of the oligosaccharides into monosaccharides followed by the separation of solid and liquid fractions. The solid material again washed with plentiful of water to maximize sugar recovery. The separated solid material is sent to second stage acid hydrolysis reactor (Chandel, *et al.,* 2007).

1.4.1.6 Two-stage Dilute Acid Hydrolysis

In two-stage dilute acid hydrolysis process, first, biomass is treated with dilute acid at relatively mild conditions during which the hemicelluose fraction is hydrolyzed and the second stage is normally carried out at higher temperature for depolymerization of cellulose into glucose. The liquid phase, containing the monomeric sugars is removed between the treatments, thereby avoiding degradation of monosaccharides formed. It is very important to avoid monosaccharide degradation

products for improving the ethanol yield. Sanchez *et al.,* (2004) carried out the two-stage dilute acid hydrolysis using Bolivian straw material, *Paja brava*. In first stage, *P. brava* material was pretreated with steam followed by dilute sulfuric acid (0.50 or 1.0 % by wt) hydrolysis at temperatures between 170 and 230°C for a residence time between 3 and 10 min. The highest yield of hemicellulose derived sugars were found at a temperature of 190°C, and a reaction time of 5 – 10 min, whereas in second stage hydrolysis considerably higher temperature (230°C) was found for hydrolysis of remaining fraction of cellulose.

1.4.1.7 Enzymatic Hydrolysis

The acid, alkaline or fungal pretreated lignocellulosic can be saccharified enzymatically to get fermentable sugars (Ghose and Bisaria, 1997; Kedah *et al.,* 1997; Itoh *et al.,* 2003). Bacteria and fungi are the good sources of cellulases, hemicellulases that could be used for the hydrolysis of pretreated lignocellulosic. The enzymatic cocktails are usually mixtures of several hydrolytic enzymes comprising of cellulases, xylanases, hemicellulases and mannanases. In the last decade, new cellulases and hemicellulases from bacterial and fungal sources have continued been isolated and regular efforts have been made for the improved production of enzymatic titers (Foreman *et al.,* 2003). However, the cellulases were produced at a concentration too low to be useful. There is a group of microorganisms (*Clostridium, Cellulomonas, Tricho-derma, Penicillium, Neurospora, Fusarium, Aspergillus* etc.) showing a high cellulolytic and hemicellulolytic activity, which are also highly capable of fermenting monosaccharides. Genetic engineering is used to produce super strains, which are capable of hydrolysing cellulose and xylan along with fermentation of glucose and xylose to ethanol (Lin and Tanaka, 2006). The utilization of cellulose by microorganisms involves a substantial set of fundamental phenomena beyond those associated with enzymatic hydrolysis of cellulose (Lynd *et al.,* 2002).

1.5 Separate Hydrolysis and Fermentation (SHF)

Enzymatic hydrolysis performed separately from fermentation step is known as separate hydrolysis and fermentation (SHF) (Sreenath *et al.,* 2001).The separation of hydrolysis and fermentation offers various processing advantage and opportunities. It enables enzymes to operate at higher temperature for increased performance and fermentation organisms to operate at moderate temperatures, optimizing the utilization of sugars.

1.6 Simultaneous Saccharification and Fermentation (SSF)

The most important process improvement made for the enzymatic hydrolysis of biomass is the introduction of simultaneous saccharification and fermentation (SSF), which has been improved to include the co-fermentation of multiple sugar substrates (Sreenath *et al.,* 2001; Wingren *et al.,* 2003). This approach combined the cellulase enzymes and fermenting microbes in one vessel. This enabled a one-step process of sugar production and fermentation into ethanol. Simultaneous saccharification of both carbon polymer, cellulose to glucose; and hemicellulose to xylose and arabinose; and, fermentation will be carried out by recombinant yeast or the organism which has the ability to utilize both C5 and C6 sugars. According to Alkasrawi *et al.,* (2006) the mode of preparation of yeast must be carefully considered in SSF designing.

A more robust strain will give substantial process advantages in terms of higher solid loading and possibility to recirculate the process stream, which results in increased energy demand and reduced fresh water utilization demand in process. Adaptation of yeast to the inhibitors present in the medium is an important factor for consideration in the design of SSF process. More recently, Kroumov *et al.,* (2006) demonstrated an unstructured model of SSF of starch to ethanol by genetically modified strain *Saccharomyces cerevisiae,* using two hierarchic levels of concept. In

first concept, a mechanism of enzymatic hydrolysis of starch to glucose by combined action of two enzymes (alpha amylase and glucoamylase) secreted by recombinant yeast and the second concept was the enzymatic degradation of starch to glucose and simultaneous utilization of glucose to ethanol by microorganisms. SSF combines enzymatic hydrolysis with ethanol fermentation to keep the concentration of glucose low. The accumulation of ethanol in the fermenter does not inhibit cellulase action as much as high concentration of glucose; so, SSF is good strategy for increasing the overall rate of cellulose to ethanol conversion (Lin and Tanaka, 2006). SSF gives higher ethanol yield while requiring lower amounts of enzyme because end-product inhibition from cellobiose and glucose formed during enzymatic hydrolysis is relieved by the yeast fermentation (Banat *et al.*, 1998).

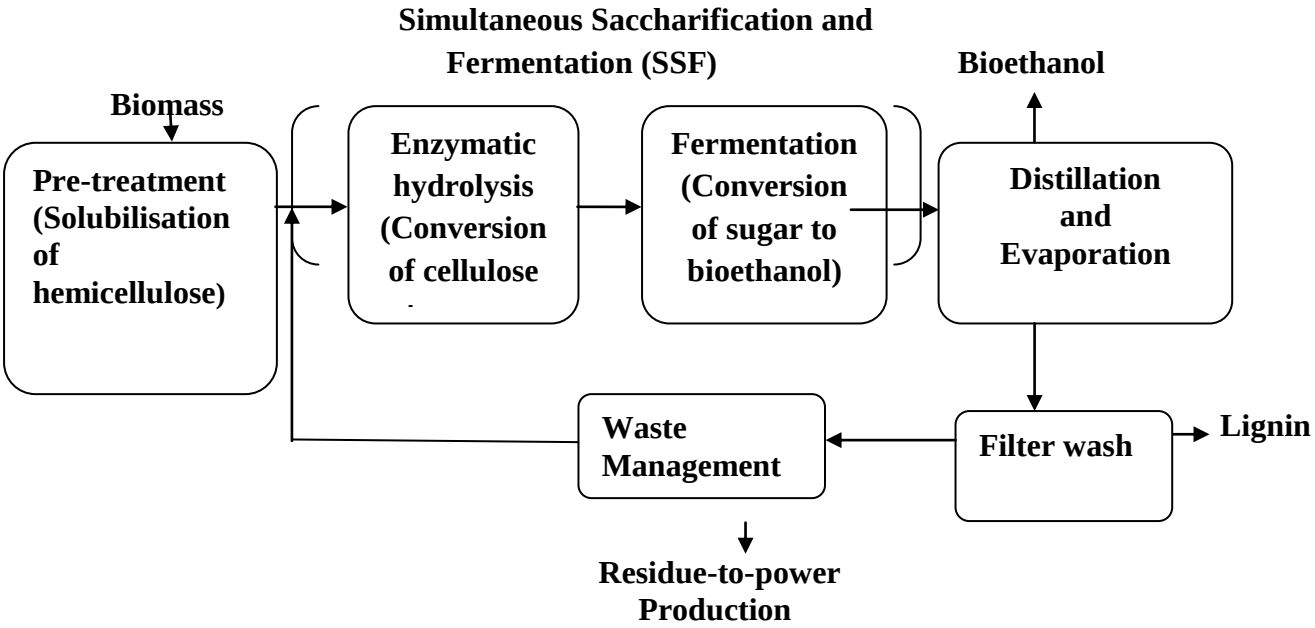


Figure 1.1 A Schematic Design of Bioethanol Production

1.7 Distillation

Distillation is one of the steps of the purifications. Distillation is the method used to separate two liquid based on their different boiling points. However, to achieve high purification, several

distillations are required. This is because all materials have intermolecular interactions with each other, and two materials will co-distill during distillation. This means that proportion between two materials, in this case ethanol and water can be changed, and still, there are two materials in layers, the liquid and the vapor layers (Onuki, 2005).

Whatever method of preparation is used, the ethanol is initially obtained in a mixture with water. The ethanol is then extracted from this solution by fractional distillation. Although the boiling point of ethanol, 78.3 ^{0}C, is significantly lower than the boiling point of water, 100 ^{0}C, these materials cannot be separated completely by distillation. Instead, an azeotrope mixture (i.e. a mixture of 95 % ethanol and 5 % water) is obtained, and the boiling point of the azeotrope is 78.15 ^{0}C. In a distillation, the most volatile material (i.e. the material that has the lowest boiling point) is the first material to distill from the distillation flask, and this material is the azeotrope of 95 % ethanol which has the lowest boiling point. If an efficient fractionating column is used, 95 % alcohol could be obtained first and then a small intermediate fraction of lower concentration, and then water. But no matter how efficient the fractionating column used, 95 % alcohol cannot be further concentrated by distillation because the vapor has exactly the same composition as the liquid; towards distillation, then, 95 % alcohol behaves exactly like a pure compound.

1.8 Feedstock for Ethanol Production

In designing production processes for ethanol fuel, it is important to assess the various sources of feedstock and their utilization. Bioethanol can be produced from a variety of feedstock containing simple fermentable sugars. Bioethanol may also be produced from some complex polysaccharides that can be hydrolyzed to release the fermentable sugars. In the following sections, various feedstock for bioethanol production are discussed.

1.9 Feedstock Containing Simple Sugars

These are feedstock that contains sugar in its simple form. In this category is sugarcane either in the form of cane juice or cane molasses. This form is the most important feedstock utilized in tropical and sub-tropical countries for producing ethanol. In European countries, beet molasses are the most utilized sucrose-containing feedstock (Cardona and Sánchez, 2007). Because of the simple form in which these sugars exist, it is easy to convert them to ethanol during microbial fermentation. The major challenge faced by this source of feedstock for ethanol production is the competition created by the use of this same feedstock as food by the human population. There are indications that continued use of this source can affect food prices in the long run. The availability and cost of procuring raw materials play a vital role in terms of design of ethanol production processes from these kinds of feedstock. Maiorella *et al.,* (1984) performed a detailed economic evaluation of alternative processes for ethanol production from molasses. They observed that the cost of raw materials (molasses) accounted for up to 70 % of the final ethanol sale price.

1.10 Feedstock Containing Starch

Unlike simple sugars, bioconversion of starch to ethanol cannot be accomplished in a single step. This is because starch is a complex polysaccharide which requires hydrolysis (typically enzymatic) to release fermentable sugars which can then be converted to ethanol by a suitable microorganism. This type of feedstock is most utilized in North America and Europe (Cardona and Sánchez 2007). The economic evaluation of different starch containing feedstock is of prime importance with respect to the design of the ethanol production process.

1.11 Lignocellulosic Biomass Feedstock

For countries where the cultivation of crops for biofuels production is not sustainable, lignocellulosic materials are a viable alternative to conventional field crops. The main challenge

facing this prospect comes from the nature of the feedstock. Due to the complex nature of the lignocellulose, there is a need to introduce a pretreatment step for its degradation, removal of lignin, hydrolysis of the hemicellulose and a reduction in the fraction of crystalline cellulose. During this pretreatment step, the cellulose undergoes hydrolysis to release the sugar which is then converted to ethanol during fermentation. Lignocellulosic materials are cheap and readily available because many of them are by-products of agricultural activities, domestic and industrial wastes and this offers considerable possibilities for the production of ethanol fuel on a large scale as well as its global consumption as a renewable fuel (Cardona and Sánchez, 2007). Currently, a considerable amount of research is being carried out on the utilization of lignocellulosic biomass as feedstock for ethanol fuel production. Hamelinck *et al.,* (2005) evaluated the production of ethanol from cellulosic biomass for three stages of technological development (short-term, medium-term and long-term). It was observed that the production process contributes approximately 50 % of the capital investment for the short- and long-term. In another work, McBride *et al.,* (2005) carried out a study to determine the cost of producing ethanol from corn starch and lignocellulosic feedstock.

1.12 Microbial Fermentation

The fermentation of sugars by microorganisms is the most common method of converting sugars present in biomass to liquid fuels such as ethanol. This process occurs by bioconversion or biocatalysis which is the use of microorganisms or enzymes to convert one material to another. Ethanol fermentation, also referred to as alcoholic fermentation, is a biological process by which sugars such as glucose, fructose and sucrose are converted to ethanol and carbon dioxide. The process is accompanied by the release of metabolic energy. Ethanol fermentation is facilitated by microorganisms the most common one being yeast. The microorganisms use glucose as carbon

substrate to produce ethanol, carbon dioxide and more cells. In actual fact, the fermentation process proceeds through a network of intracellular reactions.

1.13 Significance of the Study

All energy sources have an impact on the environment. Concerns about the greenhouse effect and global warming, air pollution, and energy security have led to increasing interest and more development in renewable energy sources such as bio-fuel, solar, wind, geothermal, and hydrogen. The fluctuating oil price has increased the interest of finding other possible ways to produce fuel. And the production of bio-ethanol has grown steadily during the last 25 years.

1.14 The Statement of the Problem

Although bioethanol fermentation from edible, cellulosic feedstock's using yeast cells has been carried out with success, very little research has been done on producing bioethanol from waste fruit. Inadequate municipal and industrial solid waste collection and disposal creates a range of environmental problems in Nigeria. A considerable amount of waste ends up in open dumps or drainage system, threatening both surface water and ground water quality and causing flooding, which provides a breeding ground for diseases-carrying pests. Open air burning of waste, spontaneous combustion in landfills and incinerating plants that lack effective treatment for gas emissions is a major cause of air pollution. The situation is exacerbated in slums where households cannot make use of garbage collection containers. Lack of the most basic solid waste services in crowded, low-income neighbors are a major contributor to the high morbidity and mortality among the urban poor. The adverse effects of inadequate solid waste service on productivity and economic development of the city are significant. Solid waste such as fruit peels and damaged whole fruits largely obtained as a by-product from fruits markets and also from its transportation to hotels,

restaurants and juice processing houses in our country. These wastes can entail serious environmental problems unless they are changed or converted into some useful products or disposed properly.

1.15 Aims

The aim of this work is to transform waste fruits by direct fermentation and simultaneous saccharification and fermentation to bioethanol using the fungus S*accharomyces cerevisiae* as ethanol producing organism.

1.16 Objective

The overall objectives of this work are to:

- Produce bioethanol from fruit wastes.
- Determine the proximate composition of both fruits
- Compare the quality (pH, concentration and specific gravity) of the bioethanol produced by two unique methods (i) direct fermentation and (ii) simultaneous saccharification and fermentation.

  Verify the chemical composition and determine the purity of the bioethanol.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1 Ethanol Production

2.1.1 Continuous Fermentation

The production of ethanol by fermentation in a simple batch reactor is often limited by the performance of the fermenting microorganisms. It is an inhibitory process because the ethanol produced is toxic to the microorganisms. Typically, yeast cannot tolerate ethanol concentrations in excess of 10 % w/v (Taylor *et al.,* 1995), because of this limitation, it is necessary to use large and often expensive fermenters. The large volume of water associated with this process leads to high cost of downstream separation and purification of ethanol. This limitation can be overcome in a number of ways. One approach is to start the process with a relatively dilute glucose solution, usually not more than 16 % by weight in order to have complete conversion of the glucose substrate in a reasonable time. Another approach is to combine fermentation with product separation (Taylor *et al.,* 1995). This means that the ethanol is removed from the fermenter as it is being produced. When this is done, it is possible to achieve a higher conversion of a more concentrated glucose feed. Since less water is associated with this process, the separation costs are greatly reduced. Park and Geng (1992) have recently reviewed methods for combining fermentation with product separation. These include fermentation under vacuum, fermentation coupled with pervaporation (Shabtai *et al.*, 1991), fermentation coupled with liquid-liquid extraction (Matsumura and Märkl 1984), fermentation coupled with perstraction (Christen *et al.,* 1990) and fermentation coupled with adsorption (Lencki *et al.,* 1983).

2.1.2 Fermenting Organisms

Historically, the most commonly used microorganism for fermentation has been yeast. For most ethanol fermentations, *Saccharomyces cerevisiae* is preferred because it can produce relatively high concentrations (up to 10 %) of ethanol in fermentation broth. This species of yeast can utilize simple sugars such as glucose, fructose and sucrose (Lin and Tanaka 2006). The main limitation associated with the use of yeast is that it has a poor yield of ethanol (per fermented glucose). This is probably due to its low resistance to higher concentrations of ethanol. Another disadvantage is the production of various by-products, which include acetic and lactic acid (Lin and Tanaka, 2006). Another fermenting microorganism that appears to hold much promise is *Zymomonas mobilis.* It is very well suited for ethanol production, can tolerate up to 120 g/l ethanol and has a higher yield (5-10 % more ethanol per fermented glucose) than yeast. Despite its attractive features as an ethanol producing microorganism, *Z. mobilis* can only be utilized for a limited range of feedstock because it can only ferment glucose, fructose and sucrose (Lin and Tanaka, 2006).

Production of ethanol fuel from lignocellulosic feedstock remains a challenge with many opportunities for improvement. More robust microorganisms are needed with higher rates of conversion and yield to allow process simplification through consolidated bio-processing steps. This development will reduce capital and operating costs, which remain high when compared with those of simple feedstock like corn (DOE, 2008). Research efforts are being directed towards the development of strains of microorganisms capable of efficiently fermenting both cellulosic and hemicellulosic substrates that will make possible the development of the direct conversion of lignocellulosic biomass to ethanol. Ingram *et al.*, (1999) from the University of Florida have carried out significant research on the development of enteric bacteria catalyst with the view of using them in the biomass to ethanol conversion process.

2.1.3 Thermophilic Ethanol Producing Bacteria

Thermophilic bacteria are microorganisms that thrive well at relatively high temperatures between 55 ^{o}C and 70 ^{o}C. They are usually found in various geothermal habitats of the earth such as the Yellowstone national park mud pots. Over the years, a large number of thermophilic ethanol producing bacteria have been isolated but not many of them have high ethanol yields. The use of thermophiles for ethanol fermentation has been reported extensively in the literature (Wiegel, 1980; Nigam *et al.,* 1981; Payton, 1984). Examples of thermophilic bacteria include: *Thermoanaerobacter brockii, Thermoanaerobacter, Thermohydrosufuricus, Thermoanaerobacter ethanolicus* etc. The characteristics of thermophiles that make them important in ethanol fermentation are outlined in the following sections. The advantages of thermophilic bacteria are presented thus.

2.1.4 Biomass Yield

The yield of biomass (microbial cells) at the temperatures at which they grow is lower for thermophiles compared with yeast and other microorganisms. This is as a result of the higher maintenance energy requirements at such temperatures (Wiegel, 1980).

2.1.5 Substrate Range

Thermophiles are able to utilize a wider range of substrates. Most are able to ferment pentoses, hexoses and starch. A few show promise in the utilization of cellulosic materials.

2.1.6 Growth and Reaction Rates

At their optimal growth temperatures, thermophiles exhibit increased rates of metabolism. Consequently, fermentation times are reduced and production rates increased with an overall increase in process efficiency (Demirbas, 2007).

2.1.7 Continuous Product Removal

The use of thermophiles favours continuous fermentation in which the product can be removed as it is been produced. Operating the fermenter close to the boiling point of ethanol makes it easy to

remove it into the gas phase by applying a mild vacuum or using an inert stripping gas. This is possible because at such high temperatures, ethanol is very volatile and most of it will exist in the gas phase inside the fermenter (Demirbas, 2007)

2.1.8 Condition of the Fermentation Broth

The high temperature necessary to sustain thermophilic fermentation has some benefits on the physical properties of the broth. For example, an inverse relationship exists between the viscosity of the medium and temperature and as such, as the temperature increases, the viscosity decreases. This leads to less energy required for agitation to maintain a uniform medium. Also, oxygen is less soluble under thermophilic conditions. This in effect, supports the maintenance of anaerobic conditions (Payton, 1984).

2.1.9 Risk of Contamination

With thermophilic bacteria, there is less risk of contamination hence sterile conditions are less essential compared to other microorganisms. This is possible because most pathogens do not grow under thermophilic conditions. In spite of the advantages and promise shown by the thermophilic bacteria, there are still some limitations to their use on an industrial scale. Most important amongst these are:

2.2.0 By-product Formation

This is perhaps the most important limitation to the use of thermophiles for ethanol fermentation. In addition to ethanol being produced, other by-products notable organic acids are produced. This in principle reduces the yield of ethanol and increases the complexity and consequently the cost of further downstream separation (Demirbas, 2007).

2.2.1 Growth Inhibition

Thermophilic bacteria are not very tolerant to ethanol hence their growth is severely inhibited by ethanol. Current research is applying genetic engineering in order to develop thermophilic

microorganisms that can exhibit the advantages listed above and possess little or none of the disadvantages listed (Demirbas, 2007).

2.2.2 The Benefits of Fuel Ethanol

A major benefit of a well-functioned bio-fuel system is that it eliminates and converts organic waste into useful and valuable products. One of the main benefits with bio-ethanol is the replacement of expensive fossil fuel. Some advantages are connected to waste removal, like improving hygienic conditions. Protection of soil, water, air and woody vegetation can be mentioned as environmental advantages. If the actual conditions are satisfactorily, the bio-ethanol technology can contribute to conservation and development (Galbe and Zacchi, 2002).

The fact that a bio-ethanol production can be building and operated locally creates opportunities to decrease the waste solid collection volume and land disposal costs. Furthermore, the technology has also a potential to create job opportunities locally for several thousands of people (Galbe and Zacchi, 2002). Over the last 150 years, human activities have caused a dramatic increase in the emission of a number of greenhouse gases such as CO, which has led to changes in the equilibrium of the earth's atmosphere. Fuel ethanol is suggested as a sustainable fuel which can be produced from renewable resources and led to maintain or even reduce the level of greenhouse gases (Galbe and Zacchi, 2002). Finally, the standard of living can be enhanced which directly contributes to social and economical development of the country (Galbe and Zacchi, 2002).

2.2.3 Ethanol Production and Environmental Issues

Ethanol represents closed carbon dioxide cycle because after burning of ethanol, the released carbon dioxide is recycled back into plant material because plants use CO_2 to synthesize cellulose during photosynthesis cycle (Wyman, 1999; Chandel *et al.*, 2006). Ethanol production process only uses energy from renewable energy sources; no net carbon dioxide is added to the atmosphere, making ethanol an environmentally beneficial energy source. In addition, the toxicity of the exhaust

emissions from ethanol is lower than that of petroleum sources (Wyman and Hinman, 1990). Ethanol derived from biomass is the only liquid transportation fuel that does not contribute to the greenhouse gas effect (Foody, 1988). As energy demand increases the global supply of fossil fuels cause harm to human health and contributes to the greenhouse gas (GHG) emission. Hahn-Hagerdal, *et al.*, (2006) alarmed to the society by seeing the security of oil supply and the negative impact of the fossil fuel on the environment, particularly on GHG emissions. The reduction of GHG pollution is the main advantage of utilizing biomass conversion into ethanol (Demirbas, 2007). Ethanol contains 35 % oxygen that helps complete combustion of fuel and thus reduces particulate emission that pose health hazard to living beings. These factors lead to the complete burning of ethanol and less emissions.

With its ability to reduce ozone precursors by 20 - 30 %, bioethanol can play a significant role in reducing the harmful gasses in metro cities worldwide. Ethanol blended diesel (E-15) causes the 41 % reduction in particulate matter and 5 % NOx emission (Subramanian *et al.*, 2005; Chandel *et al.*, 2006). One of the disadvantage in using ethanol as fuel is that aldehyde predominantly acetaldehydes emissions are higher than those of gasoline. However acetaldehydes emissions generate less adverse health effects in comparison to formaldehydes emitted from gasoline engines (Gonsalves, 2006).

The production of ethanol from starch by a co-immobilized mixed culture system of aerobic and anaerobic microorganisms such as *Aspregillus awamorii* and *Zymomonas mobilis* was investigated by Tanaka *et al.*, (1986) and he obtained 25.00 g ethanol from 100.00 g L^{-1} of starch. Herman *et al.*, (1986) obtained maximum ethanol of 41.00 – 42.00 g L^{-1} from concentrated deproteinized whey having a lactose content of 23 % by using *Zymomonas mobilis*, immobilized with sodium alginate. Anthony and Heady (1986) reported that ethanol may be produced from corn or grain sorghum in any region either by dry (or) wet milling process. The current conversion technology allows 387

litres of bioethanol to be obtained from a metric tonne of corn or grain sorghum. Co-fermentation of sweet sorghum juice and grain was studied by Gibbons and Westby (1989) for production of fuel ethanol and obtained 3.50 % (V/V) ethanol from 6.50 - 7.60 % (W/W) reducing sugar after fermentation. Nimbkar *et al.,* (1989) successfully fermented unsterilized juice of sweet sorghum by using *Saccharomyces cerevisiae* strain 3319 and obtained maximum alcohol of 12.45 % (V/V).

Pineapple wastes (containing 11.70 % soluble sugars) was fermented for ethanol production by Bankoffi and Han (1990) and obtained 0.80 % in 48 h. Co-fermentation of glucose and xylose with immobilized *Pichia stipitis* and *Saccharomyces cerevisiae* was studied by Grootjen *et al.,* (1990) and glucose conversion was found to be 0.13 g^{-1} h^{-1}. Czarnecki and Grajek (1991) studied the influence of temperature and incubation time of starch gelatinization in Wheat, Rye and Maize grain and found that rye starch was the most susceptible to enzymatic hydrolysis and produced highest alcohol yield of 65 %. Today most fuel ethanol is produced by either the dry grind or wet mill process. Lynd *et al.,* (2002) obtained 400 billion litres of ethanol by microbial conversion of the sugar residues present in waste paper and yard trash and from US landfills. The waste paper was treated with steam in pressure vessel at 170 - 220 ^{0}C and then enzymatically saccharified and fermented for ethanol production by Capek *et al.,* (1992) and obtained 460 liters per ton of ethanol which was an increase of 29 % over control. The technical microbial production of ethanol from potato waste using *Clostridium acetobutylicum* DSM 1731 was studied by Grobben *et al.,* (1993). The maximum ethanol produced was 4.20 g L^{-1}. Smith and Buxton (1993) showed that, sweet sorghum had potential to produce 3,100-5,235 L per ha and grain maize has the capacity to produce about 2,340 L per ha. A solid state fermentation process was developed by Pandey (1994) for efficient utilization of agro industrial residues such as cassava, bagasse, apple, pomace, coffee pulp and husk for the ethanol production. It was studied by Takahashi *et al.,* (1994) about the potentiality of sugar such as glucose (5.00 g L^{-1}) xylose (80.00 g L^{-1}) and arabinose (5.00 g L^{-1}) for biofuel

production and they reported 42.50 g $L^{-1}$of ethanol was obtained in 96 h, yielding 0.49 of alcohol per gram of sugar and also reported that the first sugar to be consumed was glucose, followed by arabinose and xylose. *Fusarium oxysporum* F3 alone or in mixed culture with *S. cerevisiae* 2541 were used for fermenting soluble and insoluble carbohydrate of sweet sorghum stalk for direct product of ethanol by Lezinou *et al.,* (1995) and reported ethanol yield as high as 24.40 and 33.50 g per 100 g dry stalk. Premidevi and Singh (1995) used acid hydrolysate of water hyacinth with 13 % total reducing sugar for conversion of ethanol by using *Candida shehatae*. Mamma *et al.,* (1996) studied on simultaneous saccharification and fermentation of sweet sorghum carbohydrates to ethanol by a mixed culture of *Fusarium oxysporum* and *Saccharomyces cerevisiae*. The optimum yield of ethanol was 5.20 - 8.40 %. Sheorain *et al.,* (2002) stated that sorghum can potentially give good yield of alcohol of about 380 to 390 litre absolute alcohol from 1 tonne of grains. Hammond *et al.,* (1996) conducted the laboratory experiment to assess the ethanol production from waste banana as a whole fruit, pulp and peel, and obtained ethanol yield of 0.91, 0.082 and 0.006l kg^{-1} respectively.

Marakis and Marakis (1996) used ripened deseeded carrot fruit (*Ceratonea siliqua*) which is rich in soluble sugar (54.7 %) for production of ethanol and fructose syrup of 4.75 % (V/V) and 10.49 % respectively. Srivastava *et al.,* (1997) obtained ethanol from guava pulp by using three different strains of *S. cerevisiae*. Current technologies allows for 2.5 gallons (wet milling process) to 2.8 gallons (dry milling process) of ethanol per bushels of corn (Bothast and Sclicher, 2005).

Spent cherry brine, which is an acidic by-product of maraschino cherry processing and consisting of variable amounts of glucose and fructose of 0.50 - 1.50 % $CaCl_2$, up to 11 % fermentable solids, up to 0.40 % sulphur dioxide, sorbitol and lesser amount of other cherry constituents, was used for ethanol production by Park and Bakalinsky, (1997). All strains of *Saccharomyces cerevisiae* used

for fermentation, were able to ferment all lots of Ca $(OH)_2$ treated and phosphorous-enriched brines efficiently. Highest yield of ethanol was 4.70 % (w/v) in 4 days.

Guava, which is one of the important commercial fruit crops of India, was investigated for ethanol production by Srivastava *et al.,* (1997). The study achieved maximum ethanol production (5.8 % w/v) during 36 h fermentation of *Saccharomyces cerevisiae*. Papi *et al.,* (1998) studied xanthan gum and ethanol production by *Xanthomonas campestris* and *Zymomonas mobilis* from peach pulp. The study suggested that both bacteria grew well and produced 0.10 g/L xanthan gum and 110.00 g/L ethanol.

Steam explosion of potato for the efficient production of alcohol was experimentally studied by Kobayashi *et al.,* (1998). The amount of water soluble starch increased with the increase in steam pressure. The exploded potato was later hydrolysed by amylolytic microorganism (*Aspergillus awamorii*) and fermenting microbe (*S. cerevisiae*).

Biochemical studies on bioconversion of rice straw to ethanol was conducted by Sandhu *et al.,* (1998) and revealed maximum ethanol yield of 0.34 g^{-1} based on sugar utilized after fermentation and fermentation efficiency of 66.87 % was obtained. Shirai *et al.,* (1998) cultivated micro algae in the solution from the desalting process of soy sauce waste treatment and utilized the algae biomass for ethanol production of 11 m g^{-1} of *Dunaliella* cells. Ethanol yield of 2.90 % (V/V) from damaged sorghum and 2.09 % (V/V) was obtained by Suresh *et al.,* (1999a) from damaged rice grain by simultaneous saccharification and fermentation. Amutha and Gunasekeran (2000) attempted production of biofuel from liquefied cassava starch and obtained ethanol yield of 21.10 g/L by batch fermentation process.

Economics of fuel ethanol production from dry milled corn starch using *Zymomonas mobilis* was studied by Krishnan *et al.,* (2000) and obtained ethanol yield of 24 g l^{-1} from a 15 million gallon $year^{-1}$ capacity ethanol plant. Bioconversion of starch co-culture inoculated with *S. cerevisiae* and *S. diastasticus* for ethanol production was done by Verma *et al.,* (2000) and they reported 24.80 g l^{-1} of ethanol which was 48 % higher than the yield obtained by monoculture.

Bvochora *et al.,* (2000) investigated on ethanol production from mixture of sweet sorghum juice and sorghum grains, fermentation was carried out 96 h, using malted and unmalted milled sorghum grains from sorghum cultivars DC-75 and SV-2. Maximum ethanol level were about 16.8 % (V/V) and 11 % (V/V) for media containing malted and unmalted sorghum grains.

Ramanathan (2000) obtained an ethanol yield of 421 kg^{-1} feedstock by fermentation of root crops namely cassava, potato, yam and sweet potato. Shiva *et al.,* (2001) conducted an experiment for fermentation of agricultural waste such as jowar stalk and left over corn stalk to ethanol. Sharma *et al.,* (2002) used amylolytic yeast strain *S. cerevisiae* SJ 31 to hydrolyse potato starch and they reported 48 % hydrolysis and 3.40 % alcohol production with fermentation efficiency of 91 %, (Sharma *et al.,* 2002) isolated yeast from different sources and screened for growth, ethanol production and gluco-amylose activity. The selected yeast strain SM-10 showed maximum gluco-amylose activity of 80 unit/ml and ethanol production from starch (House *et al.,* 2000). From this, it was calculated that 1.20 - 2.30 million metric tons sorghum was used for ethanol production, 3.70 – 7.50 % of the grain for ethanol production was sorghum, and 0.13 – 0.25 billion gallon (0.49–0.95 billion litres) of ethanol originated from sorghum.. Kim and Dale (2003) showed that wasted sorghum grains alone had potential to produce 1.40 GL of bioethanol and also produce 4.90GL of bioethanol globally.

Vidyavathi (2003) reported that pods of *Samanea saman* when treated with different fungal cultures and the maximum reducing sugars were released from the pods. Among them *Aspergillus foetidus* NCIM-515 was efficient in release of maximum reducing sugar (24.86 g L^{-1}).

Patle and Lal (2007) studied ethanol production from hydrolysed agricultural wastes using mixed cultures of *Zymomonas mobilis* and *Candida tropicalis*. They used different fruit and vegetable wastes collected from the market and fruit processing industries. They concluded that among the acid, alkaline and enzymatic hydrolysis processes, enzymatic hydrolysis yielded maximum reducing sugars (97.70 %). They suggested that these wastes were proved to be promising substrates for ethanol production.

Nzelibe and Okafoagu (2007) investigated the optimisation of ethanol production from *Garcinia kola* (bitter kola) pulp agro waste. With acid hydrolysis and saccharification pretreatments, the ethanol yield was maximum at 120 h (70.70 g/L).

In a study, which Chatanta *et al.,* (2008) produced bioethanol from apple pomace left after juice extraction, *S. cerevisiae*, *A. foetidus* and *F. oxysporum* were used as fermentative organisms and 16.09 % (v/w of apple pomace) ethanol was produced from fermented apple pomace with a residual sugar of 0.15 % (w/w of apple pomace). They indicated that the alcoholic fermentation of apple pomace might be an efficient method for alleviating waste disposal.

According to Vendruscelo *et al.,* (2008), apple pomace and its aqueous extract present a great potential for use as substrates in biotechnological processes. Furthermore, in order to use the apple pomace in bioprocesses effectively, several operational variables must be considered and optimised.

Moreover, the yield of pectin substance extraction was studied by Faravash and Ashtiani (2008), using dried mixed varieties of peach pomace. The investigated parameters in this particular study

were acid volume, ethanol-to-extract ratio and acid washing time. All of the factors had significant effect on pectin extraction. The maximum extraction yield obtained was 9.94 ± 0.20 % using 65 cm^3 of HCl, at the ethanol-to-extraction ratio of 1:5 and the acid-washing time of 120 min.

Ponte Rocha *et al.,* (2009) studied the enzymatic hydrolysis and fermentation of pre-treated cashew apple bagasse with alkali and diluted sulphuric acid for bioethanol production. They achieved 52 g/L glucose concentration using hydrolysis (45 ^{0}C, enzyme load of 30 FPU/g bagasse, and solid percentage of 16 (w/v), and using cashew apple juice dilute acid pretreatment followed by lignin removal by NaOH). This hydrolysate was easily fermented by *S. cerevisiae* yeast for the production of ethanol, resulting in a concentration of 20.00 g/L in 6 h of fermentation. Therefore, they concluded that the fermentation of cashew apple pomace hydrolysate stands as an alternative process for fuel ethanol production from lignocellulosic residues.

Except for its use by rural inhabitants in the production of homemade alcoholic beverages, cashew apple has no commercial value (Karuppaiya *et al.,* 2009). Therefore Karuppaiya *et al.,* (2009) studied optimisation of process conditions using response surface methodology for ethanol production from waste cashew apple juice by *Zymomonas mobilis* and determined the optimum process conditions as: substrate concentration 62 % (v/v), pH 5.5, temperature 32 ^{0}C, and fermentation time of 37 h. On these conditions ethanol concentration of 12.64 g/L was obtained.

Pineapple waste was used for vinegar production by semi-solid state fermentation by Gu *et al.,* (2010). Wine yeast for alcoholic and Acetobacter powder for acetic acid fermentation was inoculated together. Under the selected optimum condition, which were 22 ^{0}C for fermentation temperature, sugar content of 16 ^{0}Brix, 3.5 for pH, 6 days of fermentation time and 0.30 % of inoculation ratio, the acid production (calculated as acetic acid) was around 6.78 g/100 g for fermented pineapple waste, and the conversion ratio of acetic acid was 82.50 %. The study

suggested that semi-solid state fermentation gave a higher total acid production in a shorter time in comparison with liquid-state fermentation.

Orange and pineapple wastes were used as potential substrates for citric acid production (Kuforiji *et al.,* 2010). In this study using orange waste, two strains, *Aspergillus niger* strains NRRL 567 and 328 produced 57.60 % and 55.40 % of citric acid respectively at a moisture level of 38.90 %. Highest citric acid yields of 46.40 % and 45.40 % were obtained in pineapple waste at moisture contents of 54.40 % and 63.40 %, respectively.

Oberoi *et al.,* (2010) used orange peels to produce ethanol by two-stage hydrolysis. First hydrolysis was carried out at acid concentrations ranging from 0.00 to 1.00 % (w/v) at 121 ^{0}C and 15 psi for 15 min. Second hydrolysis was carried out at 0.50 % (w/v) acid. They achieved a high volumetric productivity of 3.37 g/L/h. This indicated a significant potential for such a process to commercially produce ethanol from orange peels. Pectinase production in solid state fermentation by *Aspergillus niger* using orange peel as substrate was studied by Mrudula and Anitharaj (2011). The optimum temperature, pH, incubation time, moisture ratio, inoculum size, carbon source and surfactants, were found to be 50 ^{0}C, 5, 96 h, 1:2 (v/w), 2.5 mL, sucrose and Triton-X-100, respectively. The strain produced 232 U/mL in submerged fermentation (Smf) and 1224 U/g solid in solid-state fermentation (SSF). The final optimised production was 5283 U/g solid. Valencia orange (*Citrus sinensis*) peels were used as substrate for the production of citric acid (CA) by *Aspergillus niger* CECT-2090 in Solid-State Fermentation (SSF) (Torrado *et al.,* 2011). 193.20 mg/g dry orange peel resulted into the highest CA concentration, obtained at 85 h of incubation. The inoculum concentration was 0.50 spores/g of dry orange peel and the initial water content of 2.52 mL/g of orange peel, corresponded to 70 % saturation. The study suggested that the results could be of interest for possible future industrial applications.

Fermentation of pre-treated hydrolysates of banana and mango fruit waste was studied by Arumugan and Manikandan, (2011) for ethanol production. According to their results banana fruit pulp had 23.37 % total solids, 1.37 % lipid, 19.75 % ash and 0.63 % starch and mango peels had 18.74 % total solids, 7.96 % protein, 1.48 % lipid, 13.08 % ash and 0.51 % starch. Total dietary fiber content ranged from 3.54 % to 73.04 % in the fruit samples. Pretreatment was performed using dilute H_2SO_4 followed by enzymatic hydrolysis. Maximum reducing sugar yield of 64.27 % was obtained when mixed fruit pulps were used. This fermentation media showed maximum ethanol production of 35.86 % corresponding to a fermentation efficiency of 70.31 % at 48 h of incubation.

Hadeel *et al.,* (2011) studied rambutan fruit waste biomass with different parameters using yeast (*Saccharomyces cerevisiae*) fermentation. The optimum condition of bioethanol yield was having 3 g of yeast at 30°C by following pH 6 for two days of incubation. Glucose content, total soluble solid (TSS) and pH values reduced after fermentation due to the conversion of glucose to ethanol and carbon dioxide in case of all parameters. The chemical content, viscosity and acid values of the bioethanol produced were within ASTM (American Society for Testing and Materials) standard specifications with less hazardous chemical content in produced bioethanol. Furthermore, the engine test result showed that greenhouse gas emission like hydrocarbon (HC), NOx and SO_2 content in E5 and E10 were significantly lower in bioethanol than in 100 % gasoline tested in (proton Gen 2 multi cylinder) car. Thus, it can potentially be used as good biofuel for petrol engine purposes.

Simultaneous Saccharification and Fermentation (SSF) of banana peels to ethanol by co-cultures of *Aspergillus niger* and *Saccharomyces cerevisiae* was investigated by Ajay *et al.,* (2014) at different temperatures (20°C to 50°C) and at different pH (4 to 7). Fermentation was done for 7 days for banana peels and the ethanol content was measured every 24 hours. The optimum pH and

temperature for the fermentation of banana peels was found to be 6 and 30°C. With the optimized pH and temperature, fermentation was then carried out at different yeast concentration 3% to 12%. With the change in the concentration of yeast, the time required for the completion of fermentation decreased dramatically. Using a 12%, 9%, 6%, 3% yeast inoculum, maximum ethanol production was completely achieved in 2, 3, 5, 7 days respectively.

Alessia *et al.,* (2014) investigated the potential to transform such pineapple waste into ethanol after enzymatic saccharification of plant cell walls, and fermentation of the resulting simple sugars using the *Saccharomyces cerevisiae* NCYC 2826 strain. Three different fermentation modes, direct fermentation, separate hydrolysis and fermentation, and simultaneous saccharification and fermentation of the biomass were tested and compared. The results show that the main sugars obtained from pineapple waste were: glucose, uronic acid, xylose, galactose, arabinose and mannose. The highest ethanol yield was achieved after 30 hours of simultaneous saccharification and fermentation, and reached up to 3.9% (v/v), corresponding to the 96% of the theoretical yield.

These numerous studies indicate that waste from fruit can be used as a potential fermentation media for industrial applications.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. Apparatus

Plastic bags, Knife, Crushers, Sieves, Balances, pH meter, Electric Blender, Thermostats, Vessels, Centrifuge, Graduated cylinders of different volumes, Oven, Hydrometer, Shaker, Thermometer, fermentation set up and distillation set up.

3.2. Reagents

All the chemicals used for this research were of analytical grade:

Sigma Aldrich 98 % Tetraoxosulphate (VI) Acid (H_2SO_4), Sodium Hydroxide (NaOH), Sodium Bicarbonate, Baker's yeast (*Saccharomyces cerevisiae*).

3.3. Sample Collection

Waste fruits 2.00 kg each of pineapple and watermelon were collected from Sabo Gari fruit market in Zaria, Kaduna State. The experiment of the production of ethanol from waste fruit was carried out in the laboratory of Chemistry Department, Ahmadu Bello University, Zaria.

3.4 Direct Fermentation of the Fruits (Whole)

3.4.1 Sample preparation

Whole ripped watermelon and pineapple fruits were obtained from the market in Sabo Gari, Zaria, Kaduna State, Nigeria. They were washed, cut in small pieces with their outer coats in place using a knife and then blended with distilled water in ratio of 1:10 respectively using electric blender and stored in a refrigerator prior to use.

3.4.2 Determination of Moisture

Exactly 2.00 g (triplicate) of the waste fruits (whole) were weighed into previously weighed crucibles and dried at 95 – 100 ^{0}C for 2 h in an oven to a constant weight. The samples were removed, cooled in a desiccator, weighed and returned into the oven again for 1 h. The samples were then brought out and cooled in a desiccator before weighing. This was repeatedly done until a constant weight was obtained with three consecutive weighing. The percentage of moisture was determined from the results of the weighing (AOAC, 2000).

$$\% \text{ Moisture} = \frac{W2 - W3}{W2 - W1} \times 100 \quad \text{.....Equation 1}$$

3.4.3 Determination of Ash

Exactly 2.00 g of finely ground samples obtained after moisture determination was weighed into porcelain crucibles. The samples were charred on a heating mantle inside a fume cupboard to get rid of the smoke. The samples were then transferred into a muffle furnace and gradually heated to a temperature of 550 ^{0}C for 6 h until a clear grey ash was obtained and then after which the samples were cooled in a desiccator and weighed. The percent ash was determined from the results of the weighing (Forster *et al.*, 2003).

$$\% \text{ Ash} = \frac{W2 - W3}{W2 - W1} \times 100 \quad \text{.....Equation 2}$$

3.4.4 Determination of Crude Fibre

Exactly 2.00 g of sample was weighed into a round bottomed flask and 100.00 cm^3 of 0.25 moldm^{-3} was added and the mixture boiled for 30 min**.** The solution was quickly filtered under suction. The insoluble matter residue was washed several times with hot water until it was acid free. It was quantitatively transferred into the flask and 100.00 cm^3 of 0.3 moldm^{-3} NaOH solution was added

and the mixture boiled again under reflux for 30 min before it was quickly filtered. The residue was washed with boiling water until it was base free; it was dried to constant weight in the oven at 100 ^{0}C, cooled in a desiccator and weighed (C1). The weighed residue (C1) was then incinerated in a muffle furnace at 550 ^{0}C for 2 h, cooled in the desiccator and weighed (C2). The loss in weight on incineration divided by the original weight of sample multiplied by 100 gave the percent crude fibre (Nelson, 1994).

The loss in weight on ashing (incineration) = C_2 - C_3

Weight of original sample = W

% crude fiber = $\frac{C2 - C1}{W} \times 100$ .....Equation 3

3.4.5 Determination of Lipids

A previously cleaned and dried 500.00 cm^3 round bottomed flask containing a few anti-bumping granules was weighed (W_1) and 300.00 cm^3 of petroleum ether was poured into the flask fitted with soxhlet extraction unit. The extraction thimble containing 20.00 g of the fruit sample was fixed into the round bottomed flask and a condenser connected to the soxhlet extractor. Cold water circulation was put on, the heating mantle switched on and heating rate adjusted until the solvent was refluxing at a steady rate. Extraction was carried out for 6 h, the solvent was recovered and the lipid extract obtained was dried in an oven at 70 ^{o}C for 1 h. The round bottomed flask with lipid extract was cooled and then weighed. Lipid content was calculated and expressed in percentage (AOAC, 2000).

The extractible lipid was calculated as

$\frac{\text{Weight of Lipid extracted}}{\text{Weight of direct sample}} \times 100$ .....Equation 4

Where the weight of lipid extracted (crude fat) is given by the loss in weight (w_2-w_3) of the thimble content after extraction.

3.4.6 Determination of Crude Protein

Exactly 5.00 g of the dried sample was weighed into a 500 cm^3 Kjeldahl flask. 10.00 g of K_2SO_4 and 0.70 g of Ca_2SO_4 (both as catalyst) was added followed by 40.00 cm^3 of 98 % H_2SO_4. The mixture was gently boiled for over 2 h to obtain a clear digest solution. 50.00 cm^3 of the digest was poured into a distillation flask, 20.00 cm^3 of water was added with a few anti-bumping granules. 100.00 cm^3 of 10 % NaOH solution was poured into the funnel of the distillation flask. 100.00 cm^3 of 0.10 M HCl was poured into a receiving conical flask with the funnel tap opened. The flask was heated to gentle boiling and distillation continued for about 40 - 45 min (or until about 150.00 cm^3 of liquid had distilled out). A few drops of mixed indicator (methyl red and bromocresol green) was added and titrated against 0.10 M NaOH solution. A blank titration was carried out with an equal measured volume of 0.10 M HCl acid. Results were expressed as percent nitrogen (Mendham *et al.*, 2006).

% total N per sample was calculated from the formula

$$\% N = \frac{V_1 - V_0 \times M \times 14 \times 100}{2 \times 1000 \times 10 \times 1} \times 100 \quad \text{.....Equation 5}$$

Where:

V_0 = Vol. of HCl required for the blank

V_1 = Vol. of HCl required for 10.00 cm^3 sample solution,

M = Concentration of HCl (0.1 M)

14 = Atomic weight of nitrogen,

100 = Total Vol. of digest,

100 = % conversion,

10 = Vol. of distillate,

2 = weight of sample taken in gram,

1000 = Converting to dm^3

The crude protein (cp) was calculated as

The crude protein = 6.25 x % N

3.4.7 Determination of Carbohydrate

The total carbohydrate contents in the samples were determined using the method of difference (Onwuka, 2005). Thus percentage available carbohydrate was obtained as:

% carbohydrate = 100 – (% moisture + % ash + % crude protein + % lipid extract + % crude fibre).

3.4.8 Fermentation

Fermentation was carried out on the samples (fruit paste) by using baker's yeast (*Saccharomyces Cerevisiae*). 100.00 g of the sample was weighed into 250.00 cm^3 conical flask and 100.00 cm^3 of distilled water was added. The pH was adjusted with 0.3$moldm^{-3}$ NaOH to 4.5 and then pasteurized. Fermentation was monitored for 7 days at 30 ^oC. At the end of the fermentation, the alcohol was recovered by simple batch distillation using laboratory distillation unit. The alcohol content was determined by the determination of the specific gravity using a standard alcohol density table (AOAC, 1970).

3.5 Simultaneous Saccharification and Fermentation

3.5.1 Sample preparation

The fruits were washed, and then peeled, the pulp was cut in small pieces using a knife and then blended with distilled water in ratio of 1:10 respectively using electric blender and stored in refrigerator prior to use. The fresh ripened watermelon and pineapple fruits peels were chopped into small pieces (2.00 – 3.00 cm) and dried in a hot air oven at 65 ºC for 48 h. The dried materials (peel) were ground in a dry compartment blender to pass through a 1.00 mm screen and the powdered materials were stored at room temperature for further analysis (Amenaghawon *et al.*, 2012)

3.5.2 Acid hydrolysis of fruits

10.00 cm^3 of 43 %, 50 %, 60 %, 70 % and 77 % (v/v) diluted sulfuric acid was added to the non-soluble peel component, the fruit peels was then hydrolyzed in a thermostatic water bath varying the temperature between 33 ^{0}C to 70 ^{0}C and also varying the time from 1hr 30 min to 7 h. After hydrolysis, it was neutralized with 10.00 M NaOH until the pH show 7, the solid particles from the liquid in the hydrolyzate were separated using centrifugation (to remove the non-fermentable lignin portion). After separating the solid part, it was washed with distilled water twice. The washing was performed in order to extract all soluble sugars from the waste fruit peel, the soluble component with the previously blended pulp solution was mixed together for the next procedure (fermentation) for both fruits (Amenaghawon *et al.*, 2012).

3.5.3 pH Adjustment

Before addition of yeast to the above prepared samples, the pH of the samples was 3.50 ± 0.40 and 5.53 ± 0.20 for watermelon and pineapple respectively which was adjusted using 0.3 $moldm^{-3}$ of

NaOH. Otherwise the yeast will be denatured in hyper acidic or basic state. A pH of around 5.0 - 5.5 was maintained. The mixed pretreated, hydrolyzed solution was filtered and shaken. The substrate was primarily checked for pH using a digital pH meter. The pH then was adjusted to 5.0 - 5.5, the mixed samples (pretreated and hydrolyzed) were acid hydrolyzed. Then sodium hydroxide solution was added drop wise to the flask containing the samples with constant stirring until the pH reached a range of 5.0 - 5.5 (Amenaghawon *et al.*, 2012)

3.5.4 Fermentation

100.00 g portion of the slurry (pulverized) was weighed into 250.00 cm^3 conical flask and 100.00 cm^3 distilled water was added. This was pasteurized by boiling in a water bath for 15 min. It was allowed to cool and the yeast added. The fermentation was then monitored for 7 days. Different concentrations of the yeast were used and the pH of sample was also adjusted with 0.5 $moldm^{-3}$ NaOH to5.0, at the end of the fermentation, the fermented sample was poured into a cheese cloth to drain out the fermented broth. This procedure was carried out in triplicate (Akin-Osanaiye *et al.*, 2008).

3.5.5 Distillation

In this experiment separation were used by rotary evaporator at a temperature of 78.5 ^oC for 3 h. Some of the components of the distillation set up include; distillation vessel, special top-fit of distillation vessel, condenser, diverting glass that fits at the end of condenser and the top of harvesting vessel, condenser tubing, harvesting vessel, stands and fixing screws, beaker, thermostat, the thermostat supporting flat metal bar. All distillation experiments were carried out at a temperature of 78.5^oC and a distillation time of 3 h.

3.5.6 Alcoholic Content

With the aid of the specific gravity values of the distillate the percentage alcohol was determined using a standard alcohol density table (AOAC, 1974). The overall distillate obtained was then

pooled together and rectified. The percentage purity of the rectified (distillate collected at 78-80 ^{0}C) was determined (AOAC 1974).

3.6 Density Measurements

The glass container and hydrometer were cleaned, and then the glass container was placed on a level surface and filled with the ethanol. It was ensured that the depth of ethanol was equal to the length of the hydrometer. The hydrometer was carefully inserted into the ethanol sample with its weight facing down. When the hydrometer has reached a stable free float in the ethanol sample, the specific gravity from the scale within the hydrometer was read. This was done by visually sighting along the upper surface of the ethanol sample and reading where the scale crosses the ethanol's surface. The temperature was measured simultaneously as the readings were taken. It was noted that specific gravity characteristics of the ethanol change somewhat with temperature. As such, the maximum permissible specific gravity reading changed as the ethanol temperature change (Amenaghawon *et al.*, 2012).

3.7. Data Analysis

Design expert 7 software experimental method was used to determine the effect of three operating variables of the acid hydrolysis in ethanol production from fruit peel. These were time, temperature and acid concentration. The response variable was ethanol yield. Significance of the result was set from analysis of variance (ANOVA).

CHAPTER FOUR

4.0 RESULTS

The tables represented in this chapter shows the results obtained from the comparative evaluation of direct fermentation and simultaneous saccharification and fermentation of post-harvest pineapple and watermelon waste fruit for the production of bioethanol.

Table 4.1 shows the proximate composition of post-harvest watermelon and pineapple waste, Table 4.2 shows the optimisation of ethanol production with direct fermentation this captures the ethanol yield (%) at different duration of fermentation and different yeast concentration of pineapple waste fruit.

Table 4.3 shows the ethanol yield (%) at different duration of fermentation and different yeast concentration of watermelon waste fruit. Table 4.4 shows the chemical and physical properties of watermelon. Table 4.5 shows the chemical and physical properties of pineapple. Table 4.6 shows the percentage of ethanol formed with varying concentration of yeast on the pineapple and watermelon for 72 h. Table 4.7 shows the experimental design for hydrolysis and percent yields of ethanol (Pineapple).Table 4.8 shows the optimum condition and the predicted value of bioethanol production from pineapple slurry, determined from the Best fit model.

Table 4.9 shows the experimental design for hydrolysis and percent yields of ethanol (watermelon). Table 4.10 shows the optimum condition and the predicted value of bioethanol production from watermelon, determined from the best fit model.

Table 4.11 shows the comparison of the methods for the production of ethanol.100g of Waste Fruits (Pineapple and Watermelon) produced optimum yields.

4.1 Proximate Composition

Table 4.1: Proximate composition of Watermelon and Pineapple

Fruits	Moisture (g/100 g)	Ash (g/100 g)	Crude Protein (g/100 g)	Crude fibre (g/100 g)	Fat (g/100 g)	CHO (g/100 g)
Watermelon	91.52	0.22	0.45	0.19	0.18	7.18
	± 0.01	± 0.01	± 0.05	± 0.01	± 0.01	± 0.05
Pineapple	82.2	1.23	0.44	0.70	0.47	4.06
	±0.07	± 0.15	± 0.07	± 0.01	± 0.03	± 0.20

4.2 Optimisation of Ethanol Production with Direct Fermentation

Table 4.2: Ethanol yield (%) at different duration of fermentation and different yeast concentration of pineapple waste fruit

Yeast Concentration (%)	1 day	2 days	3 days	7 days
1	2.58 ± 0.04	3.30 ± 0.01	4.75 ± 0.08	5.23 ± 0.04
2	2.59 ± 0.05	3.33 ± 0.01	4.90 ± 0.03	5.50 ± 0.10
5	2.52 ± 0.04	4.39 ± 0.03	5.73 ± 0.01	5.45 ± 0.04
10	2.70 ± 0.02	4.55 ± 0.02	5.88 ± 0.03	5.30 ± 0.05

Results are means of duplicate experiments. Temp = Ambient temperature (27 ± 3 ^{0}C). Initial pH:5.53

Table 4.3: Ethanol yield (%) at different duration of fermentation and different yeast concentration of watermelon waste fruit

Yeast Concentration (%)	1 day	2 days	3 days	7days
1	0.38 ± 0.04	0.60 ± 0.01	1.58 ± 0.08	1.77 ± 0.04
2	0.25 ± 0.05	0.70 ± 0.01	1.88 ± 0.03	1.29 ± 0.10
5	0.42 ± 0.04	0.77 ± 0.03	1.91 ± 0.01	1.10 ± 0.04
10	0.80 ± 0.02	0.96 ± 0.02	1.76 ± 0.03	1.27 ± 0.05

Results are means of duplicate experiments. Temp = Ambient temperature (27 ± 3 ^{0}C). Initial pH: 3.5

Table 4.4: Chemical and Physical Properties of Watermelon

Parameters	g/100g
pH	3.50 ± 0.40
Alcohol content (% v/v)	1.91 ± 0.08
Percentage purity of ethanol	92 %

Table 4.5: Chemical and Physical Properties of Pineapple

Parameters	g/100g
pH	5.53 ± 0.20
Alcohol content (% v/v)	5.88 ± 0.08
Percentage purity of ethanol	88 %

These parameters were obtained from fermentation of the waste using 10 % yeast for 7 days at its initial pH 3.5 and 5.53 respectively and room temperature (27.3 ^{0}C).

Table 4.6: Percent Ethanol formed with varying Concentration of Yeast on the Pineapple and Watermelon for 72 h.

Concentration of yeast (g)	% ethanol from pineapple	% ethanol from water melon
1.0	4.69 ± 0.03	0.68 ± 0.04
2.0	4.76 ± 0.02	0.77 ± 0.04
3.0	4.80 ± 0.04	1.84 ± 0.02
4.0	5.77 ± 0.01	1.88 ± 0.03
5.0	5.85 ± 0.02	1.65 ± 0.31
6.0	5.70 ± 0.01	1.91 ± 0.03
7.0	5.68 ± 0.04	1.84 ± 0.03
8.0	5.88 ± 0.02	1.90 ± 0.03

Table 4.7: The Experimental design for Hydrolysis and Percent Yields of Ethanol (Pineapple)

Run	Factor 1 A: Temperature	Factor 2 B: Time	Factor 3 C:Concentration	% Yield
1	60	5	70	9.766 ± 0.003
2	50	3.5	60	12.168 ± 0.011
3	60	5	50	12.835 ± 0.003
4	50	3.5	77	13.028 ± 0.002
5	50	3.5	60	12.171 ± 0.009
6	50	3.5	70	12.533 ± 0.001
7	50	5	60	12.313 ± 0.003
8	50	1.38	60	12.985 ± 0.004
9	33.2	3.5	60	14.184 ± 0.005
10	40	3.5	60	9.635 ± 0.008
11	40	5	70	11.501 ± 0.004
12	60	2	50	12.658 ± 0.009
13	50	3.5	60	12.173 ± 0.001
14	40	2	70	8.095 ± 0.002
15	40	5	50	11.643 ± 0.010
16	66.8	3.5	60	10.251 ± 0.003
17	60	2	70	12.259 ± 0.008
18	50	6.02	60	0.111 ± 0.012
19	40	2	50	10.870 ± 0.001
20	50	3.5	43	11.581 ± 0.002

Table 4.8: The Optimum Condition and the Predicted value of Bioethanol Production from Pineapple Slurry, determined from the Best fit model.

Factor	Name		Low	High	Std		
		Level	Level	Level	Deviation	Coding	
A	Temperature	57.984	40	60	0	Actual	
B	Time	2.5025	2	5	0	Actual	
C	Concentration	60.356	50	70	0	Actual	
		SE	95 % CI	95 % CI		95 %PI	95 %PI
Response	Prediction	Mean	Low	High	SE Pred	Low	Low
YIELD	12.85 ± 0.0072	1.52218	9.46553	16.2488	3.43249	5.2091	20.5052

Table 4.9: The Experimental design for Hydrolysis and percent Yields of Ethanol (Watermelon)

Run	Factor 1 A: Temperature	Factor 2 B: Time	Factor 3 C:Concentration	% Yield
1	60	5	70	1.166 ± 0.003
2	50	3.5	60	0.413 ± 0.002
3	60	5	50	1.393 ± 0.004
4	50	3.5	76.82	1.171 ± 0.001
5	50	3.5	60	0.418 ± 0.008
6	50	3.5	70	0.885 ± 0.004
7	50	5	60	0.507 ± 0.004
8	50	1.38	60	1.909 ± 0.001
9	33.18	3.5	60	2.658 ± 0.003
10	40	3.5	60	1.940 ± 0.013
11	40	5	70	2.978 ± 0.011
12	60	2	50	1.430 ± 0.017
13	50	3.5	60	0.416 ± 0.006
14	40	2	70	2.030 ± 0.008
15	40	5	50	2.524 ± 0.003
16	66.82	3.5	60	1.554 ± 0.003
17	60	2	70	3.074 ± 0.004
18	50	6.02	60	0.596 ± 0.007
19	40	2	50	1.430 ± 0.004
20	50	3.5	43.18	0.241 ± 0.002

Table 4.10: The Optimum Condition and the Predicted value of Bioethanol Production from Watermelon, determined from the best fit model.

Factor	Name		Low	High	Std		
		Level	Level	Level	Deviation	Coding	
A	Temperature	50	40	60	0	Actual	
B	Time	3.5	2	5	0	Actual	
C	Concentration	60	50	70	0	Actual	
		SE	95 % CI	95 % CI		95 %PI	95 %PI
Response	Prediction	Mean	low	High	SE Pred	Low	Low
Yield	1.43 ± 0.0038	0.23591	-0.0919	0.95942	0.53778	-0.7645	1.63204

Table 4.11: A Comparison of the Methods for the Production of Ethanol.100g of Waste Fruits (Pineapple and Watermelon) produced optimum yields.

Methods	Samples	% Yield	Specific gravity (g/cm^3)
Direct fermentation	Pineapple	5.88	0.79
	Watermelon	1.90	0.79
SSF	Pineapple	14.18	0.80
	Watermelon	3.07	0.80

CHAPTER FIVE

5.0. DISCUSSION

5.1. Proximate Composition

This study shows that watermelon contains more of moisture as compared to pineapple. This is due to an increase in water content of the pulp, derived from carbohydrates utilized during breathing and osmotic transfer from the peel to pulp due to increase in the sugar content in the pulp (Emaga *et al.,* 2007).The pineapple fruit showed highest ash content of 1.23 % and the watermelon had the lowest of 0.15 %. The observed results is in agreement with the literature findings of Hammond *et al.,* (1996) in the dessert banana with ash content between 1.10 – 1.52 %.

The carbohydrate content of 7.13 to 7.23 % in watermelon and 4.04 to 4.08 % in pineapple observed in this present studyc is similar to the findings of (Emaga *et al.,* 2007; Reginna and Gloria, 2005). This is as a result of the degradation of starch to free sugars during the ripening process by the action of several enzymes. The high protein content and carbohydrate content observed in pineapple and water melon is in agreement with literature findings of Essein *et al.,* (2005), could serve as the main source for fermentative ethanogenic microbial growth. The highest lipid content of 0.47 % was observed in pineapple fruit and the lowest was obtained in watermelon (0.18 %).The results obtained in the present study on the proximate composition are in agreement with the literature findings of Emaga *et al.,* (2007) on pineapple varieties with fat content between of 0.38 – 0.50 %.

5.2 Optimisation

The initial pH of the substrates was 3.5 for watermelon, this is lower than the recommended pH range of 4.5 – 5.0 for optimum ethanol yield and 5.53 for pineapple (Morris and Sarad, 1990). The

reducing sugar assay of the pineapple waste gave 7.8 g/100 g and water melon gave 4.5 g/100 g of the sample which shows that not all carbohydrate in the samples are available for fermentable sugar. The ethanol yield obtained from the substrate when fermented at its initial pH and room temperature (27 ± 3 ^{0}C) at different duration of fermentation and different yeast concentrations are shown in Table 4.2 and 4.3 for watermelon and pineapple respectively. From these results, it is observed that ethanol can be produced from waste pineapple even at initial pH of 4.3 and also that of watermelon which was buffered to 4.3 to allow for optimum fermentation. Also, the yield was increased when the concentration of the yeast was increased, it was observed from Table (4.2 and 4.3) that fermentation was optimum on day 3 (72 h) for direct fermentation.

Table 4.6, shows that there is a significant difference from the increasing yield of ethanol in pineapple with added yeast than when compared to the result obtained from water melon. Appendix I, Figure 4.1 depicts the variation of the yeast with percentage of ethanol formed from the substrate. It was found that when 8.0 g of yeast was added to the substrate of both fruits a significant yield was optimised, giving 5.88 % and 1.90 % for both pineapple and water melon respectively (Akin-Osanaiye *et al.*, 2008).

5.3. Statistical Analysis of the Experimental Results

The process consists of five parts: pretreatment to remove lignin, reduce cellulose crystallinity, sterilize the fruit peel and increase the porosity of the materials, dilute acid hydrolysis and fermentation to produce ethanol, distillation to remove the ethanol. After following the above series of procedures, the experimental outcomes of those particular results are measured for their density using hydrometer to know the yield of ethanol concentration. The effects of acid concentration, temperature and time on dilute acid hydrolysis were investigated. The Design expert® 7 software were used and significance of the result was set from analysis of variance (ANOVA). Box-Behnken

was applied to study the interaction effects, to see the contour and surface plot. The optimum results were determined in both fruits as indicated in Tables 4.7 and 4.9 (using Hydrometer).

5.4. Effects of Experimental Variables on Hydrolysis

Ethanol production can be affected by many parameters starting from sample preparation to distillation, the hydrolysis steps has a complex connection with independent variables. The best way of showing the effects of this parameter for the yield of ethanol are to generate response surface plots of the equation. The three dimensional i.e. interactions, contours and response surfaces effect were plotted in Figures (4.2), (4.3), (4.4), (4.7) and (4.8) below as a function of the interactions of any two of the variables by holding the other value of the variable at middle. For the interaction Figures, black and red line indicates low and high level of parameters respectively.

The effect of acid concentration and temperature on the yield of ethanol, acid concentration was selected at the center point. When the levels of temperature increased, hydrolysis resulted in higher yield of ethanol. However, as observed from the graph after some increments of temperature, the yield of ethanol became decreased since the possible formation of other molecules instead of glucose formation due to high temperature. Similarly, at low and high time, the yield of ethanol decrease. Contour plot graph showing predicted response of ethanol yield as a function of hydrolysis temperature and acid concentration was shown in Figures 4.2 and 4.7. As hydrolysis temperature increases at lower level of acid concentration and as acid concentration increases at low level of temperature gives a positive effect on the yield of ethanol. The response surface Figures 4.2 and 4.7, obtained from hydrolysis temperature and acid concentration was spiral shaped. Hence from the result, there were well defined optimums operating conditions. As hydrolysis temperature increases at lower level of acid concentration and as increase level of acid concentration and lower

level of temperature gives a positive effect on the yield of ethanol. The response surface suggests that there were dominance interactions of these two factors.

From the contour plot graph showing predicted response of ethanol yield as a function of hydrolysis time and hydrolysis temperature was shown in figure 4.4 and 4.6. As hydrolysis time increases at lower level temperature gives positive effect on the yield of ethanol and it decrease when the hydrolysis time and temperature became higher and higher. The response surface figure both Figures, obtained from hydrolysis time and hydrolysis temperature was conical shape. It suggests that there were well-defined optimum operating conditions. The response optimised value for the production of ethanol was based on both in hydrolysis time and temperature. At the lower and higher levels of temperature and acid concentration, the production of ethanol yield decrease since it has effect of the hydrolysis treatment. At lower temperature and acid concentration the cellulose might not hydrolysis to simple glucose and at higher acid concentration and time the cellulose might convert to other molecules which might not be fermentable. Hence both temperature and acid concentration have strong relationship for the yield of ethanol production.

The effect of acid concentration and time on the yield of ethanol, temperature was selected at the center point. At the lower and higher levels of acid concentration and time, the production of ethanol yield level decrease since it has effect of the hydrolysis treatment. At lower acid concentration and time the cellulose might not have hydrolysed to simple glucose and at higher acid concentration and time the cellulose might convert to other molecules which might not be fermentable. Hence both acid concentration and time have strong relationship for the yield of ethanol production. Contour plot graph showing predicted response of ethanol yield as a function of hydrolysis time and acid concentration was shown in Figures 4.3 and 4.8. As hydrolysis time

increases at lower level of acid concentration and as acid concentration increases at low level of time gives a positive effect on the yield of ethanol.

The response surface from both Figures, obtained from hydrolysis time and acid concentration was conical shape. Hence from the result, there were well defined optimums operating conditions. As hydrolysis time increases at lower level of acid concentration and as increase level of acid concentration and lower level of time gives a positive effect on the yield of ethanol. The response surface suggests that there were dominance interactions of these two factors.

5.5. Stepwise Simultaneous Saccharification and Fermentation of Fruits

In this section, the stored and blended pulp solution in addition with the hydrozylate (which becomes slurry) was fermented at room temperature and a hydrometer was used to monitor the difference in their specific gravity.

Saccharification of the dried, pulverized peels and hemicellulose was done at varying temperature (approximate optimum temperature for yeasts), the simultaneous saccharification and fermentation (SSF) process yielded higher results for pineapple and watermelon than when direct fermentation (DF) process was used.

The test for the percentage of ethanol formed from the slurry of pineapple and water melon was done by subjecting the slurry to acid hydrolysis, 10.00 cm^3 of the prepared sulphuric acid was used in each case. An experimental design was setup using the design expert software 7.08 in order to make its analyses easier in finding the most efficient condition or profound method for the successful production of bioethanol from the these fruits.

The sequential model sum of squares[Type I]": with the source model having the highest order polynomial where the additional terms are significant and the model is not aliased.

The source Mean as plotted against Total having a mean square of 2481.10 at df = 1 was sugested, while for the process order the cubic model was selected since the model summary statistics had the higest adjusted R-squared value 0.889.

Figure 4.2 depicts the effect of the varying factors, concentration and time with the yield of bioethanol obtained, it was found that when high concentration of acid is used the longer the broth stays undisturbed the more the sacharrification of the hemicellulose of the fruits, this trend reduces with concentration as well as for the time given. While in Figure 4.3 the three dimensional plot shows that increasing the temperature of the broth positively affects the production of bio ethanol. The longer the time the more of bioethanol obtained, the combined effect of concentration and temperature were checked against the percentage yield of bioethanol obtained and it was found that the use of concentrated acid (H_2SO_4) sulphuric acid negatively affects or impedes bioethanol production as temperature of the broth is increased.

Accordingly in Figure 4.5 the percentage of ethanol formed from the slurry of water melon was done by subjecting the slurry to acid hydrolysis; an experimental design was used in order to make its analyses easier in finding the most efficient condition or profound method for the successful production of bioethanol from the slurry.

From Figure 4.8 it was found that the highest value of bioethanol produced was found to be attributed to the synergy of two opposing factors time and temperature. At low temperature the yeast was able to propagate the production of bioethanol but as the temprature increases the yeast's effect reduced, this show that high temperature destroys or impedes the effort of the yeast in fermenting the broth, similar trend was also found in Figure 4.9 where concentration of sulphuric acid helps to improve the effect of yeast on fermenting the broth, but this property decreases as the temperature of the broth increased. Figure 4.10 in appendix I shows that only about 20 % of the

hemicellulose in the water melon slurry were properly hydrolysed indicating the need for temperature increase since at room temperature the sacharrification stage takes a very long time before its completely achieved.

From Table 4.11, it is inferred that in the production of bioethanol using both methods: direct fermentation yielded less percentage of bioethanol and has very good specific gravity as compared to simultaneous saccharification and fermentation, The high yield of bioethanol from SSF method is due to the hydrolysis of the fruits which is obtained from the peels of the fruits inclusive (Akin-Osanaiye *et al.,* 2008).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 SUMMARY

Hydrolysis of fruit peel waste was carried out with dilute acid and the optimum condition as well as interaction, contour and response surface plot were examined by using Design expert 7.0. These three factors were significant variables for the yield of ethanol. Very high and low acid concentration, temperature and retention time have negative effect on the yield of ethanol. Production of ethanol from fruit peels is feasible from the economic point of view in that its internal rate of return provides a return greater than the current rate of return. Thus it brings no wastes that are dangerous to the environment and health. The by-product is also biodegradable and we can use it for the production of fertilizer. The waste also generates financial revenue in addition to protect the environment from disposed peel wastes in open yard landfill areas.

The research carried out on the production of bioethanol from fruit waste proves lucid and establishes the fact that the quantity of bioethanol production increases when the fruits pineapple and watermelon are subjected to hydrolysis as employed by the SSF method. The percent yield obtain from direct fermentation is less, this is as a result of the inability to break the hemicelluloses nature of the fruits. The quantified amount of bioethanol obtained from pineapple was found to be significantly higher than that found in water melon when SSF method for bioethanol production was used, this was done with reference to the research reported by Zhou *et al.,* (2007) who stated that further hydrolysis of fruit will lead to the degradation of the glucose contained in them and hence negatively affecting the amount of bioethanol produced from the fruit.

The variation of yeast concentration in the fermentation indicates a significant increase between 3.0 and 8.0 g (wt), showing that a steady increase of ethanol production with increasing addition of yeast, the best or most significant production of bioethanol from the pineapple and watermelon was found to be at 0.4 g (wt) where the % of bioethanol produced from pineapple and water melon were (1.899 and 1.120) % respectively. Bioethanol production from pineapple (direct fermentation) was to significantly higher than that in watermelon given that the % of ethanol produced from pineapple is 5.88 % and that of watermelon is 1.917 %, this shows that the percentage of ethanol produced from pineapple is approximately three times (3X) that of watermelon.

The use of design expert software 7.0 has helped in establishing a proper experimental design so as to have a better picture in optimizing the best condition needed for the production of bioethanol from the fruits (Pineapple and watermelon) slurry using the simultaneous saccharification and fermentation method. In the optimisation of conditions by varying important factors such as temperature of the media, fermentation time and concentration of acid used for hydrolysis, the best conditions were found for pineapple slurry when the temperature was set between (33-34) ^{0}C, for 3 h and 5 min and concentration of sulphuric acid in % (V/V) to be 60 %. The yield obtained was 14.184, when modeled as a polynomial function the predicted yield was found to be 12.85 which was significantly higher than the adjusted R-Squared value determined was the highest with a significant value of 0.889 and because the predicted yield of ethanol for the model was the closest to that experimentally determined.

This research gives ideas on the favorable conditions necessary for the optimisation of bioethanol production from pineapple and watermelon fruits (pomace and rind), so it is therefore recommended that pineapple which yields more bioethanol is to be used in cases where the waste from fruits are to be destroyed hence could be converted to bioethanol. It is also recommended that

the best method and condition necessary for the production of bioethanol from this research is to be adopted.

6.2 C0NCLUSION

Conclusively, the present work has clearly shown that stepwise simultaneous saccharification and fermentation of waste materials from pineapple and watermelon to ethanol by a digesting sugar fermenter (*S. cerevisiae*) is feasible. Simultaneous saccharification and fermentation has been found to effectively remove glucose, which is an inhibitor to cellulase activity, thus increasing the yield and rate of cellulose hydrolysis. It is interesting to note that even though ethanol is produce from renewable resource, economic factors such as land availability, labour, taxation, utilities, crop processing costs and transportation are to be put into consideration otherwise there will be no profit for its production.

6.3 RECOMMENDATION

Further researches have to be done to improve the production of high quality and quantity of fruit peel ethanol. Alternative extraction methods of ethanol such as enzymatic extraction have to be done in order to investigate the variation that could be arise on the quality and quantity of the ethanol yield as a result of using different extraction methods. Most of the solid wastes including fruit peel waste in our country have no or very low conversion to different usable products and as such among the major problems of health especially for cities such as Zaria. Hence, it recommended that government or other investor's to recover this very valuable product as well as to contribute to the country in reducing the highly rising quantity of wastes. To conclude the recommendation, there is an urgent need for proper collection, documentation and assessment of fruit peel yields of orange, mango and banana as well as their seasonal variation in our country.

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APPENDICES

Appendix I: A variation of yeast concentration with % volume of ethanol formed from pineapple and water melon (direct fermentation)

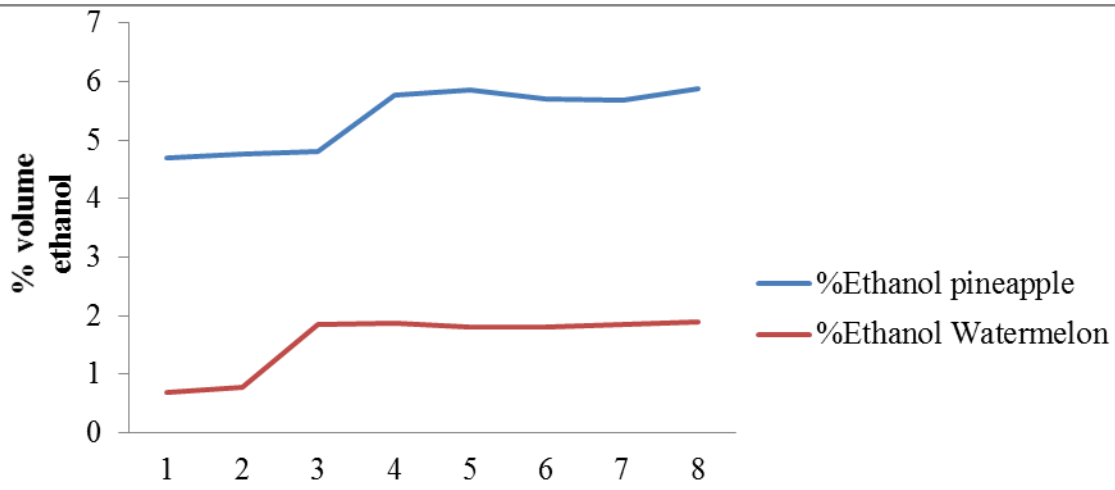


Figure 4.1: A Variation of Yeast Concentration with % Volume of Ethanol formed from Pineapple and Watermelon (direct Fermentation)

Appendix II: Three Dimensional and Cube Plot of Varying Parameters for the Production of Ethanol from Pineapple

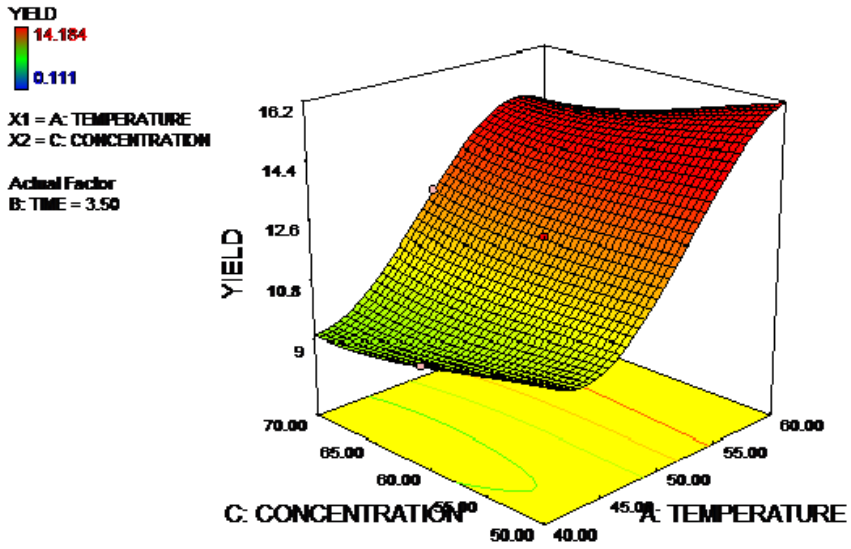
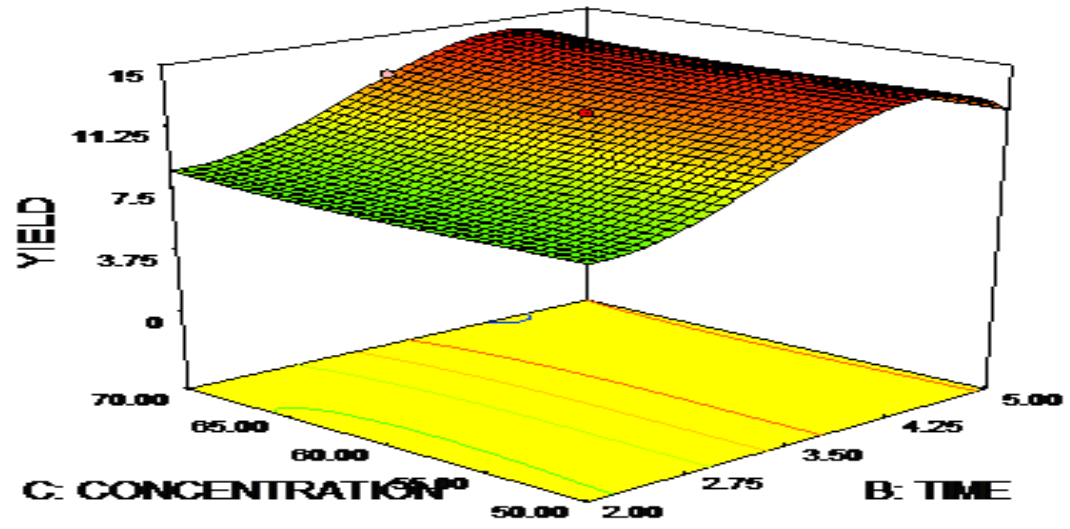


Figure 4.2: A Three Dimensional Plot of % Ethanol Yield Produced in Pineapple Slurry with varying Concentration and Temperature



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Figure 4.3: A Three Dimensional Plot of % Ethanol Yield Produced in Pineapple Slurry with varying Concentration and Time

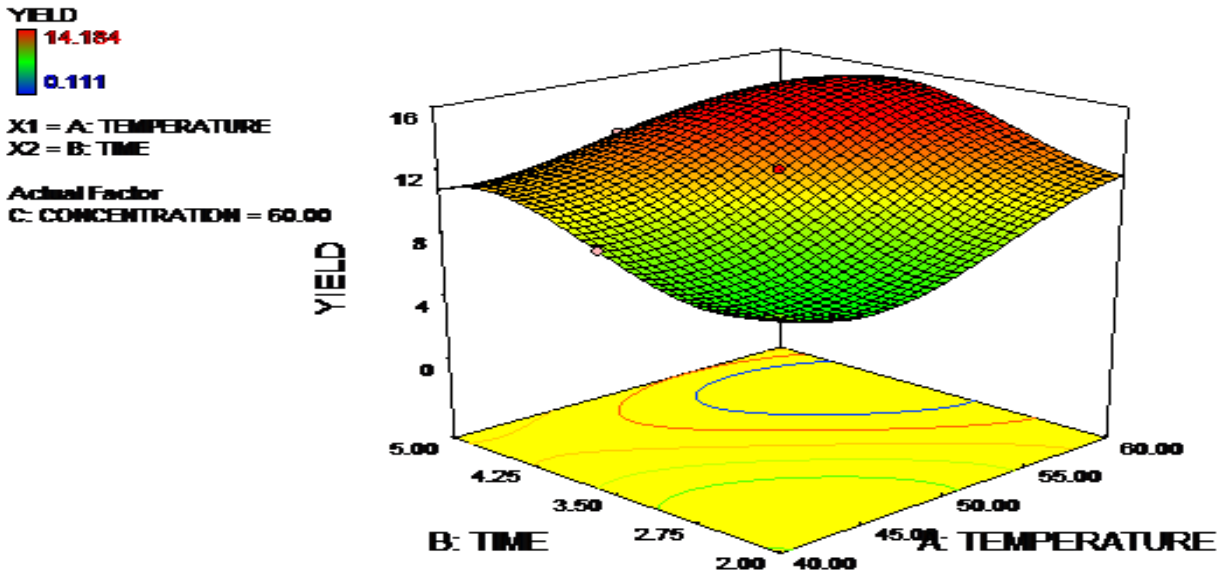


Figure 4.4: A Three Dimensional Plot of % Ethanol Yield Produced in Pineapple Slurry with varying Temperature and Time

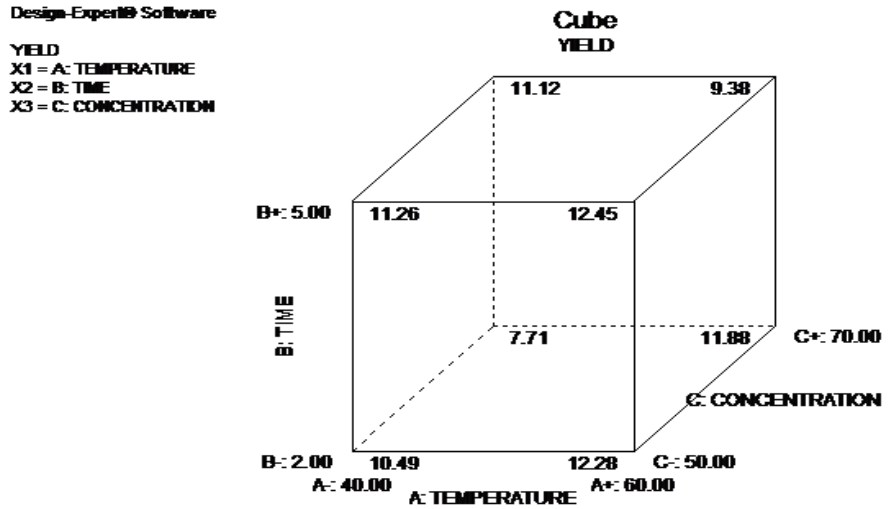


Figure 4.5: A Cube Plot of % Ethanol Yield Produced in Pineapple Slurry while varying all other Factors Considered.

Appendix III: A Three Dimensional Plot and Cube Plot of varying Parameters for the Production of Ethanol from Watermelon

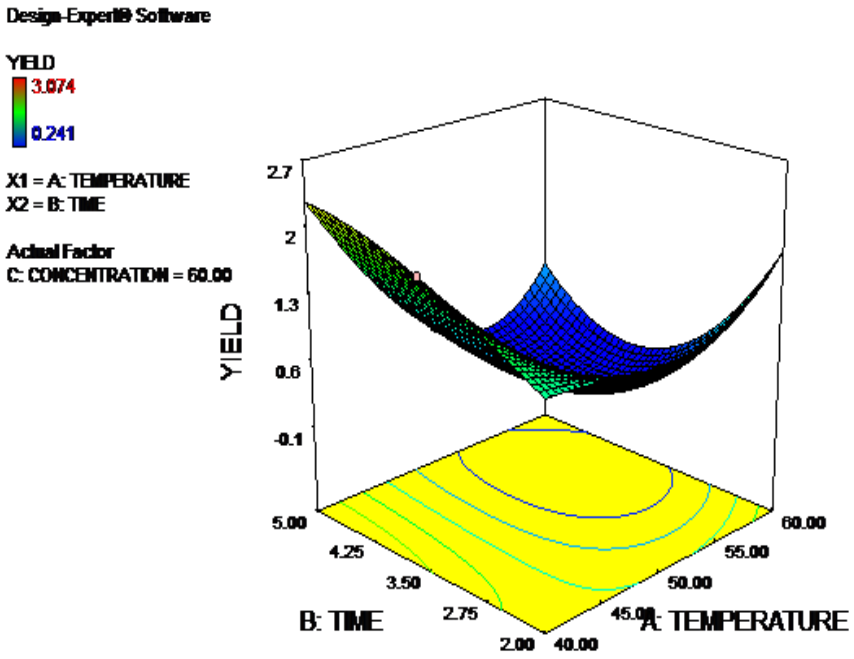


Figure 4.6: A Three Dimensional Plot of % Ethanol Yield Produced in Watermelon Slurry with varying Temperature and Time.

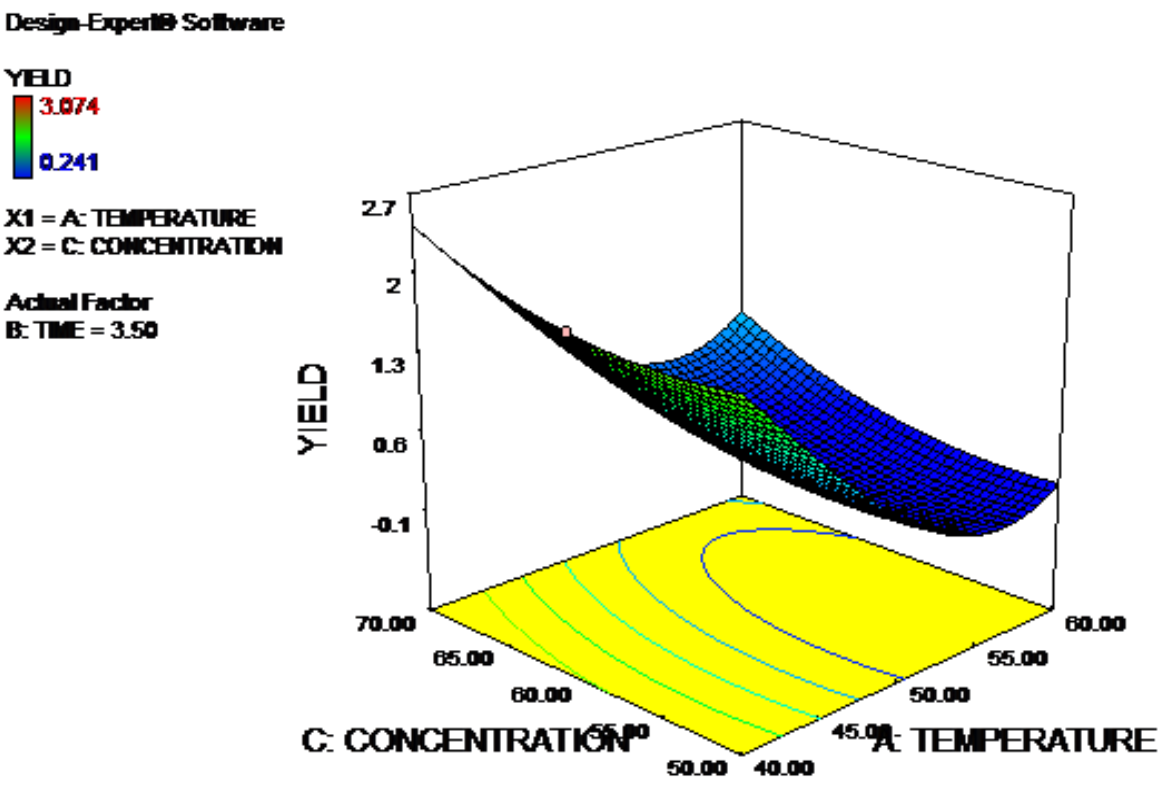


Figure 4.7: A Three Dimensional Plot of % Ethanol Yield Produced in Watermelon Slurry with varying Concentration and Temperature.

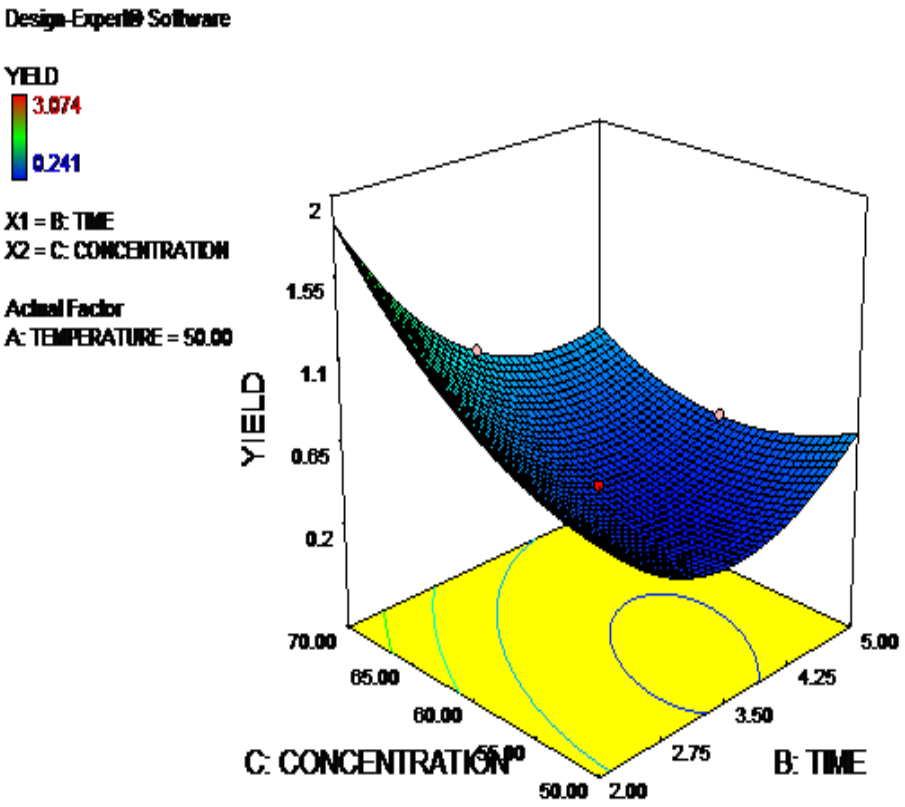


Figure 4.8: A Three Dimensional Plot of % Ethanol Yield Produced in Watermelon Slurry with varying Concentration and Time.

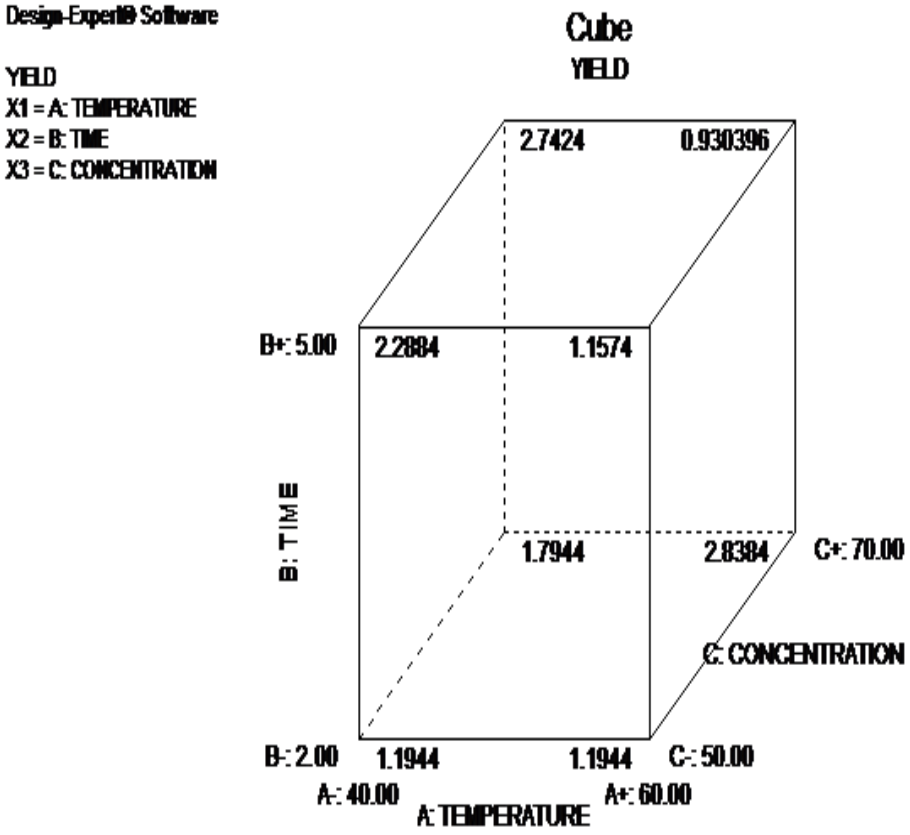


Figure 4.9 A Cube Plot of % Ethanol Yield Produced in Watermelon Slurry while varying all other factors considered.