EVALUATION OF SOME NIGERIA CORN VARIETIES FOR FUEL ETHANOL PRODUCTION.

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DEPARTMENT OF MICROBIOLOGY
UNIVERSITY OF NIGERIA, NSUKKA

SUPERVISOR: PROF .J. C. OGBONNA

APRIL, 2014.

TITLE PAGE

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A DISSERTATIONS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE MASTER OF SCIENCE (M.SC) DEGREE IN MICROBIOLOGY

TO

DEPARTMENT OF MICROBIOLOGY
UNIVERSITY OF NIGERIA, NSUKKA

SUPERVISOR: PROF .J. C. OGBONNA

APRIL, 2014.

CERTIFICATION

ANI, NGOZI PEACE, a postgraduate student in the Department of Microbiology, majoring in Industrial Microbiology and Biotechnology, with a registration number PG / M.Sc / 08 / 49246, has satisfactorily completed the requirement for the degree of Master of Science (M.Sc) in Microbiology. The work embodied in this research project is original and has not been submitted in part or full for any diploma or degree of this or any other University.

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DEDICATION

This project is dedicated to my Lord and personal Saviour Jesus Christ, my beloved father, Mr. S.C. Ani, my sister Charity and my brother, Great.

ACKNOWLEDGEMENT

My sincere regards and special thanks go to my project supervisor, Prof J.C. Ogbonna for his concern and encouragement during this work. He was not just a supervisor, but a father and adviser

I wish to express my joy to Prof A.N. Moneke, Head of Department, lecturers and all members of staff of Microbiology Department, fellow colleagues and the management of the University of Nigeria Nsukka (UNN) for their immense contributions.

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I am greatly indebted to my Father (Mr. S. C. Ani), my sister Charity and my little brother Great for their tireless and sacrifice efforts which made my stay here a success.

ABSTRACT

Fossil fuels are non-renewable, and their excessive use has resulted in a lot of environmental problems. There is an urgent need to develop alternative renewable and environmental friendly energy sources. The objectives of this study were to: (i) compare the carbohydrate contents of some corn varieties grown in Nigeria. (ii) determine their amylose : amylopectin ratios. (iii) compare ethanol productivities and yields from the corn varieties, and (iv) make pragmatic economic analysis of corn ethanol production in Nigeria. Amongst the four corn varieties, *Zea mays var. Indurata* gave the highest ($P<0.05$) starch content of 64 % followed by *Zea mays var. Praecox* with 62 %, *Zea mays.var Indentata* with 60 % and *Zea mays var. Ceratina* with 57 %. Statistically, the starch contents of the corn varieties can be ranked as *Zea mays var. Indurata* > (*Zea mays var. Praecox* = *Zea mays var. Indentata*) > *Zea mays var. Ceratina*. The ethanol concentration obtained from 10 g of corn flour and 10 g of koji were (g/l) 24.70, 23.11, 22.38 and 21.30 for *Zea mays.var Indurata*, *Zea mays var. Identata* and *Zea mays var. Ceratina*, respectively. Fedbatch fermentation gives higher ethanol concentration of 47.08 g/l in 9 days. Economic analysis showed that with *Zea mays var. Indurata* at 6,000/100 kg market price, the cost of production is ₦276.42/L as at 2012. However, if the market price of corn can be reduced to ₦2,000/100kg, the cost of production will decrease to N92.00/L, which is profitable provided that the current fuel subsidy on fossil fuel is removed.

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CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Fuel ethanol is an alcohol produced for use as fuel. It is either used as a main source of energy or as an octane enhancer (Rausch *et al;* 2006). Some vehicles can run on 100% ethanol while others use blends of ethanol and gasoline (gasohol) in various proportions (Farrell *et al;* 2006). It is a very important agricultural product. It burns cleaner for the environment (Lubert *et al;* 2005). Fuel ethanol is a renewable energy source produced mainly by the fermentation process but can be synthesized by chemical processes such as reacting ethylene with steam (Anuj *et al;* 2007). Fuel ethanol produced from different biomass materials is called bio-ethanol. It is renewable and it has lower energy density that gasoline and availability of feedstock for bio- ethanol can vary considerably from season to season. Fossil fuels are non-renewable, and their excessive use has resulted in a lot of environmental problems. Thus, there is an urgent need to develop alternative renewable and environmental friendly energy sources (Adenola *et al;* 2008). Furthermore, the current cost of production is still very high due to high cost of raw materials. Selection of raw materials for bio ethanol production is based on availability and cost which may vary from region to region (Van Ejantten, 2005). There is therefore a need to look for cheap and easily available raw material in Nigeria for bio-ethanol production. Fuel ethanol can be produced from different starch sources such as corn and cassava.

1.2 Raw Materials used for Ethanol production.

Different raw materials are used for ethanol production such as bee, sugar cane, sweet sorghum and fruits. Starchy materials such as corn, milo, wheat, rice, potatoes, cassava, sweet potatoes etc. Cellulose materials like wood, used paper, crop residues (Krishan *et al*; 2011). Corn is commonly used because of the high starch contents it has in order to yield more ethanol. Corn is produced in almost all parts of the country. It is an important cereal being cultivated in the rainforest and the derived savannah zones in Nigeria. There are different uses of corn which include cornmeal, popcorn, corn flakes, starch, cooking oil, corn syrup and ethanol (Van Ejantten, 2005). In order to sustain corn production in Nigeria, it is necessary to expand markets for corn by developing methods of processing the corn into value-added products in the country. The interest in production of bio-ethanol as an alternative fuel is increasing due to the non-renewable nature of fossil fuels and the environmental problems associated with their use. In order to produce bio-ethanol as bio-fuel, the production cost has to be as low as possible. The cost of raw material (carbon source) represents more than 70 % of the total production cost (Krishan *et al;* 2011). Therefore production of bio-ethanol at competitive price requires the use of cheap raw material. Ethanol yield from corn depend on such factors as starch contents as well as on their amylase and amylopectin ratios.

1.3 Methods used for bio-ethanol production

Corn ethanol is ethanol produced from corn. There are two processes by which corn ethanol can be produced. There are Wet Milling and Dry Milling.

Wet Milling Method

In wet milling the grain is soaked or ñsteepò in water and dilute with sulfurous acid (65 %) for 24 to 84 hours. This steeping facilitates the separation of the grain into its many component pats. After steeping the corn slurry is processed through a series of grinders to separate the corn germ. The corn oil from the germ is either extracted on site or sold to crushers who extract the corn oil. The remaining fiber gluten and starch components are further segregated using centrifugation screen and hydrochloric separators.

The steeping liquid is concentrated product, heavy steep water, is co-dried with the fiber component and is then sold as gluten feed to the livestock industries. Heavy steep water is also sold by itself as a feed ingredient and is used as a component of Ice Ban, an environmentally friendly alternative to salt for remaining Ice from road (Singh, 2008). The glutton component (protein) is filtered and dried to produce corn gluten meal co-product. The starch and any remaining water from the mash can processed in one of the three ways; fermented into ethanol dried and sold as dried and modified corn starch, or processed into corn syrup.

Dry Grind Method

In the dry-grind ethanol process, the whole grain is processed; the residual components are separated at the end of the process. There are five major steps in the dry-grind method of ethanol production.

These Includes:

- Milling
- Liquefaction
- Saccharification

- Fermentation
- Distillation and recovery

Milling

Milling involves processing corn through a hammer mill (with screens between 3.2 to 4.0 mm) to produce a corn flour (Raush *et al*; 2006). This whole corn flour is slurried with water.

Liquefaction.

This slurry is cooked, also known as ñLiquefactionò. Liquefaction is accomplished using a beaker that injects steam into the corn flour slurry to cook it at temperatures above 100 ^{0}C (212 ^{0}F). The heat and mechanical shear of the cooking process break apart the starch granules present in the Kernel endosperm, and the allowed to cool to 80 ï 90 ^{0}C (175-195 ^{0}F), additional enzyme (alpha amylase) is added, and the slurry is allowed to continue liquefying for at least 30 minutes.

Saccharification

After liquefaction, the slurry, now called ñcorn mash,ò is cooled to approximately 30 ^{O}C (86 ^{O}F), and the koji enzyme is added to complete the breakdown of the starch into simple sugar (glucose). This step, called ñsaccharificationò often occurs while the mash is filling the fermentor in preparation for the next step (fermentation) and continues throughout the next step.

Fermentation

In the fermentation step, yeast cells grown in the conical flask (*S. cerevisiae*) are added to the corn mash to being the process of converting the simple sugars to ethanol (Wang, 2008). The other components of the corn Kernel (Protein, oil etc) remain largely unchanged during the fermentation process.

In most dry-grind ethanol plants, the fermentation process occurs in batches. A fermentation tank is filled, and the batch ferments completely before the tank is drained and refilled with a new batch.

The up-stream processes (grinding, liquefaction, and saccharification) and downstream processes (distillation and recovery) occur continuously (grain is continuously processed through the equipment). Thus, dry-grind facilities of this design usually have three fermentors (tanks for fermentation) where, at any given time, one is filling, one is fermenting (usually for 48 hours), and one is empting and resetting for the next batch (Farrell *et al;* 2006). Carbon-dioxide is also produced during fermentation. Usually, the carbon-dioxide is not recovered and is released from the fermenters to the atmosphere. If recovered, this carbon-dioxide can be compressed and sold for carbonation of soft drinks or frozen into dry ice sold product storage and transportation.

Distillation and Recovery

After fermentation, the liquid portion of the slurry has 8-12 % ethanol by weight. Because ethanol boils at a lower temperature than water does, the ethanol can be separated by a process called ñdistillationò.

Conventional distillation/rectification systems can produce ethanol at 92 ï 95 % purity. The residual water is then removed using molecular sieves that selectively adsorb the water from an ethanol/water vapor mixture, resulting in nearly pure ethanol (99 %). The residual water corn solids that remain after the distillation process are called ñstillageò. This whole stillage is then centrifuged to separate the liquid (thin stillage) from the solid fragments of the kernel (wet cake or distillersô grains). Some of the thin stillage (backset) is recycled to the beginning of the dry-grind process to conserve the water used by the facility.

The remaining thin stillage passes through evaporators to remove a significant portion of the water to produce thickened syrup (Farrell *et al;* 2006). Usually, the syrup is blended with the distillersô grains and dried to produce an animal feed called ñdistillersô dried grains with solubleò (DDGS). When markets for the feed product are close to the plant, the byproduct may be sold without drying as distillers for the feed product are close to the plant, the byproduct may be sold without drying as distillersô grains or wet distillersô grains.

The advantage of dry-grind process over wet-milling process is that plants can be built at a smaller scale for smaller investment. It is also less expensive (i.e lower costs because less energy is required), lower emissions and greater co-product output because the mash is more highly concentrated.

1.4 Energy Balance in Bio-ethanol Production/Energy Efficiency

Fossil energy inputs and emissions levels from bio-fuel production are sensitive to process and feedstock, to energy embedded in fertilizers, and to local condition. Production of ethanol from sugar cane (Brazil) is energy ï efficient since the crop produces high yields per hectare and the sugar is relatively easy to extract. If bagasse is used to provide the heat and power for the process, and ethanol and bio-diesel are used for crop production and transport the fossil energy input needed for each ethanol energy unit can be very low compared with 60 % - 80 % for ethanol from grains. As a consequence, ethanol well ï to wheels Co$_2$ emission can be as low as 0-2 ï 0.3 kg Co$_2$ / liter ethanol compared with 2.8 kg

Co_2 / liter for conventional gasoline (90 % reduction). Ethanol from sugar beet requires more energy input and provides 50 % - 60 % emission reduction compared with gasoline (Wang, 2008).

Ethanol production from cereals and corn (maize) can be even more energy intensive and debate exists on the net energy gain (Krishan *et al*; 2011). Estimates, which are very sensitive to the process used, suggest that ethanol from maize may displace petroleum use up to 95 % but total fossil energy input currently amounts to some 60 % - 80 % of the energy contained in the final fuel (20 % diesel fuel, the rest being coal and natural gas) and hence in comparison with gasoline, the Co_2 emissions reduction may be as how low as 15 % (Mueller *et al;* 2008).

1.5 Ethanol from lingo-cellulosic feedstock. At present, the total energy input needed may be even higher than that of bio-ethanol from corn. However in some cases most of such energy can be provided by the biomass feedstock itself. Net Co_2 emission reduction from lingo-cellulosics ethanol can therefore be close to 70 % and could approach 100 % if electricity co-generation displaced gas or coal-fired electricity. Current R & D aims to exploit the large potential from improving efficiency in enzymatic hydrolysis. (Singh, 2008). Energy input and overall emissions for bio-diesels production also depend on feedstock and process. Typical values fossil fuels inputs of 30 % and Co_2 emission reductions of 40 % - 60 % in comparison with diesel using recycled oils and animal fats reduces the Co_2 emission.

Cost of production of bio-fuels: Costs of bio-fuels are highly dependent on feedstock, process land and labour costs, credits for byproducts, agricultural subsidies, food (sugar) and oil market. Ethanol energy content by volume is two thirds that of gasoline, so costs should be based on liter of gasoline equivalent.

Ethanol is a fuel with a high octane number and a low tendency to create knocking in spark ignition and engines. Oxygen in its molecule permits low temperature combustion with reduction of CO and Nox emissions. Low percentage ethanol gasoline blends (5% - 10 %) can be used in conventional spark-ignition engine with almost no technical change (David *et al;* 2005). New flex fuel vehicles of which there are over 6 million running mainly in Brazil, United State and Sweden, can run on up to 85 % ethanol blends having had modest changes made during production. Ethanol combustion offers fuel and emissions savings due to the high octane number, the high compression ratio and the combustion benefits from ethanol vapour cooling which partly offsets its lower energy content per liter. Further hydrolysis, microorganisms and to improve conversion of 5-carborn sugar to ethanol. Securing sufficient low cost biomass supply over a long period will need to be resolved. Ethanol could experience rapid expansion in North American and Europe by leap fogging a number of traditional barriers faced by alternative fuels for transport. In the period 2004-2005 global ethanol production increases by 8 % a year from 30.5 to 33 billion liters (Gabby, 2007). The end of 2005, there were 95 operating plants in the United State with total capacity of 16.4 billion liters per year (bnl per year). In mid 2000, 35 additional plants were under constitution with further capacity of 8 bnl per year. Brazil has over has 3000 plants in operation, of which 8 licensed in 2005, and is expected to increase sugar cane production by 40 % by 2009 as a part of a new national plan. The potential market for bio-ethanol is estimated at around 45 EJ by 2050.

Several countries have adopted policies such as tax exemptions, mandates and incentives for bio-fuels in 2005-2006. For example targets 5.75 % bio fuels by 2008 and 10 % by 2015; Germany requires 2 % ethanol and 4.4 % bio-diesel in 2007, increasing to 5.75 % by 2012; Italy mandates 1 % blend for both ethanol and bio-diesel in 2006; and in the beginning of 2007, the European Commission proposed at 10 % target by 2020. In U.S, fuel distribution are required to increase the annual volume of bio-fuels up to nearly 30 bnl by 2012 with the target for ñrenewable and alternative fuelsò raised in 2007 to 140 bnl by 2017. Target and mandates also exist in non OECD countries (e.g; Brazil, China).

1.6 Criticisms on bio –fuel production

Ethanol, o ethyl alcohol, is an alcohol made by fermenting and distilling simple sugars. As a result, ethanol can be produced from any biological feedstock that contains appreciable amount of sugar or materials that can be converted into sugar such as starch or cellulose sugar beets and sugar cane are examples of feedstock that contain sugar. Corn contains starch that can relatively easily be converted into sugar. In the United State, corn is the principle ingredient used in the production of ethanol; in Brazil (traditional the worldôs largest ethanol producer), sugar cane is the principle feedstock. A significant percentage of trees and grasses are made up of cellulose which can also be converted to sugar, although with more difficulty than required to converted starch. In addition, sorghum and potatoes as well as crop residues and animal wastes, are potential feed stocks. Ethanol production has shown rapid growth in the United States in recent years.

1.7 Ethanol Production Cost (Economic Efficiency).

Ethanolês primary fuel competitor is gasoline. Whole sale ethanol prices, before incentives from the federal state governments, are generally significantly higher than those of their fossil fuel counter parts (Gabby, 2007). Apart from government incentives the economics underlying corn-based ethanol market competitiveness hinge on the following factors:-

- The price of feedstock.
- The price of processing fuel, primary natural gas or electricity, used at the ethanol plant.
- The cost of transporting feedstock to the ethanol plant and transporting the finished ethanol to the user.
- The price feedstock co-products (for dry-milled con: distillers dried grains; for wet-milled corn. Corn gluten feed, corn gluten meal, ands corn oil).

Higher prices for corn, processing fuel and transportation hurt ethanolês market for competitiveness, while higher prices for corn by product and gasoline improve ethanolês competitiveness in the marketplace. Feedstock cost is the largest single cost factor in the production of ethanol (Lubert *et at;* 2005). Each kilogramme of corn yield approximately 2.7 liter of ethanol. As a result, the relative relationship of corn to gasoline prices provides a strong indicator of the ethanol industryês well-being.

Government support: - There is a need for Government support in terms of research, income tax credit and incentives for research on renewable fuels.

Energy Efficiency: The net energy balance (NEB) of a fuel can be expressed as a ratio of the energy produced from a production process relative to the energy used in the production process (Haefele *et at;* 2004). An output/input ratio of 1.0 implies that energy output equals energy input. The critical factors underlying ethanol energy efficiency or NEB include:

- Corn yield per acre.
- The energy efficiency of corn production, including the energy embodied in inputs such as fuels, fertilizer, pesticides, seed corn, and cultivation practices.
- The ethanol yield from the raw material for the case of corn, the yield is 2.64 gallons / bushel (for dry mill) and 2.68 gallons / bushel (for wet mill).

1.8 STATEMENT OF PROBLEM

- Fossil fuels are non-renewable. Although ,Nigeria is a major fossil oil producing country in the world. It is estimated that the present Nigeria oil reserve will last for less than 40 years.
- At Global level, on the average, the oil reserve will be depleted in less than 50 years.
- Combustion of fossil fuels leads to increase in atmospheric Co_2 which is a major cause of global warming.
- Bio-ethanol is renewable and environmental friendly. Fuel ethanol is already commercially- produced in many countries but there is no commercial production yet in Nigeria and most other African countries.
- To make production of bio-ethanol economically viable, it is necessary to select cheap and readily available raw material because the cost of raw material represents more than 60 % of the total production cost.
- Nigeria has a lot of potential raw materials for fuel ethanol production but there is yet no detailed evaluation in terms of productivities and yields.

1.9 AIMS AND OBJECTIVES

- To compare the carbohydrate content of some corn varieties growth in Nigeria.
- To determine the amylose: amylopectin ratio in some corn varieties grow in Nigeria.
- To compare ethanol production and yields from the some varieties.
- To make pragmatic economic analysis of corn ethanol production in Nigeria.

1.9.1 LITERATURE REVIEW

Microorganisms for production of fuel ethanol: Many organisms have been found to produce ethanol (i.e. yeast and bacteria). *Saccharomyces cerevisae, Saccharomyces exiguous. Saccuharomyces florentinus, Saccharomyces markii, Sacchramyces diastatics, pichia burtonii, Cadida Sp., including cadida mycoderma; C. tropicalis*, (Swings and Deley, 2006).

Besides yeasts, it is known that several bacteria produce ethanol from palm wine and carbohydrate (Olawale *et al;* 2010), especially species belonging to the genera *Lactobacillus, Streptococcus, Zymomonas, Pedococcus, Sacrina, Corynebacterium, Micrococcus and Klebsiella.* During the ő70sô oil was cheap, and fuel ethanol was produced industrially from this source. Today, this production is not economical.

Although many microorganisms can be employed to produce fuel ethanol, *Saccharomyces cerevisiae* is still the main industrial and predominant ethanol producing yeast. It is largely responsible for the alcohol contents and for the frothing because of carbon (IV) oxide formation. In fact, specific strains that are able to overproduce fuel ethanol in different types of fermentation process have been developed. The theoretical yield is 90 % and 40 g/l ethanol tolerance.

The advantage of *Saccharomyes cerevisiae* over other microorganisms with respect to ethanol yields and productivities includes:

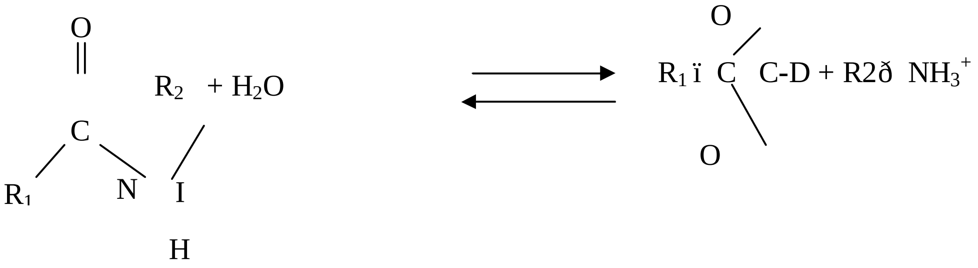
- It is capable of producing ethanol reliably in larger bioreactors.
- It is robust.
- It is well suited to fermentation.
- It ferments mannose and after adaptation, also galactose.
- It has high ethanol tolerance and high rate of fermentation activity (Xiang-yang *et al;* 2005).

1.9.2 Biochemistry of Hydrolysis and fermentation.

Hydrolysis and fermentation are the two basic bio-reactions that take place during the productions of bio-ethanol. This is so because the enzyme (gluco-amylase) produced by some microbes such as *Aspergillus niger* and *Aspergillus* awamori help carryout hydrolysis which makes the sample easily accessible by the yeast strain *(Saccharomyces cerrevisiae)* during the fermentation sate of the entire production process.

HYDROLYSIS

Hydrolysis reaction is the addition of molecule of water to a peptide bond.



The above reaction renders the starch/protein held by this peptide bond to become lose thereby making the starch accessible by other substances (yeast strain).

Microbial enzymes are often used in the hydrolysis of starch in the hydrolytic stage of the entire process. The enzyme gluco-amylase or acid resistant amylase which is produced by *Apergillus niger* and *A. awamori* is used in the hydrolysis of starch.

BIOCHEMISTRY OF FERMENTATION BY YEAST

Fermentation is a general term for anaerobic degradation of glucose and other wort carbohydrates to obtain fermented products (Ethanol) and energy conserved as ATP.

$$\text{Glucose} + N_2 \quad \text{Source} \xrightarrow[\text{Anaerobic Condition}]{\text{Yeast}} \text{Ethanol} + Co_2 + \text{Energy}$$

Anaerobic breakdown of glucose is probably the most ancient biological mechanism obtaining energy from fuel molecules. The biochemistry behind the conversion of glucose and other glucose related nutrients is termed **Glycolysis.** The thermodynamic principle and the types of regulatory mechanism that governs glycolysis are common to all pathways of cells metabolism (Gabby, 2007).

An overview of glycolysis shows that it has three (3) stages. The breakdown of glucose into two molecules of the 3-carbon pyruvate occurs in ten (10) stages. In stage one, glucose is trapped and destabilized; In stage two, two inconvertible 3- carbon molecules are generated by cleave of the 6-carbon fructose; and in stage three ATP is generated and pyruvate is formed which will further break down depending the condition it further finds itself. The fate of the pyruvate thus formed in yeast is broken down to Ethanol as shown in the pathway below.

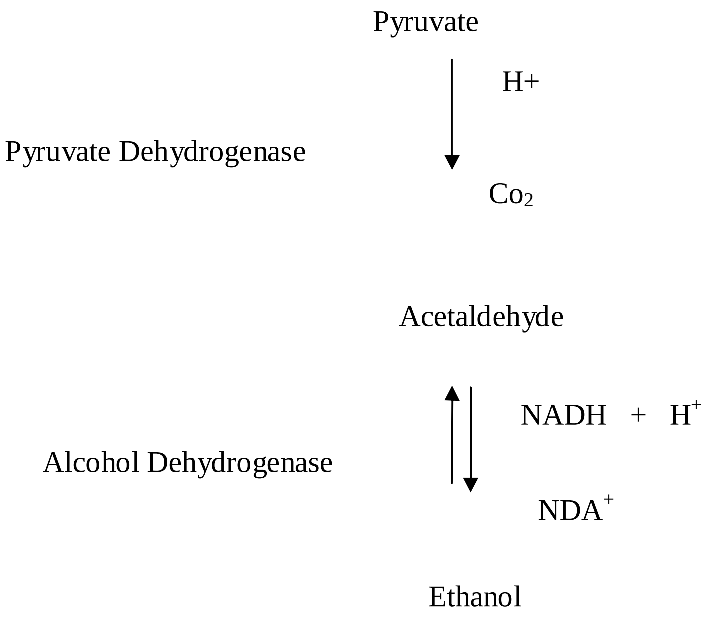


Fig. 1: Fate of pyruvate in yeast (anaerobic condition).

The above breakdown (Glycolysis and fate of pyruvate in yeast) to yield ethanol is the result expected from yeast fermentation of glucose related nutrients simple sugar that are found in the corn meal.

Substrates for Ethanol production

Several raw materials such as starch materials, sugar e.t.c. have been employed as substrate for submerged fuel ethanol production. However, fuel ethanol is mostly produced from starch or sucrose base medium using submerged fermentation. Some other substances such as molasses, hard and soft woods and Jerusalem Artichoke Tubers have also been investigated (Xiang ï Yang Get *et al;* 2005).

Starchy materials are preferably used as the source of sugar for microbial production of ethanol due to high sugar contents. According to Oyeleke and Jibrin, (2009) reported on guinea corn husk and millet husk; ethanol yield from guinea corn husk (26.83 g/1) and millet husk (18.31 g/1) were obtained. S.A. Ado, 2009 reported on corn cobs using *S.cerevisiea* and *A.niger.* The corn cobs used gave 4. 17 % maximum yield. Nwachukwu *et al*; 2008 reported on high ï ethanol yield. *S.cerevisiea* using protoplast fusion and obtained 16 % v/v maximum yield. However, Nathan *et al;* 2005 have investigated work on corn also among others but there has not been works on different corn varieties with A. niger hydrolyzing the starch and *S.cerevisiae* producing the ethanol.

1.9.3 Fermentation Techniques.

Various fermentation techniques, such as submerged, solid state and surface cultures, and modes of fermentation operation such as batch, fed-batch and continuous, have been developed and used for the improvements of various fermentation processes (Mathew *et al*; 2003).

Submerged fermentation:

The submerged fermentation processes is widely used for ethanol production. Mixing in submerged processes can be by mechanical agitation on by air circulation (Rokem *et al;* 2011). It is estimated that 90 % of world production of ethanol is obtained by submerged fermentation. Submerged fermentation can be carried out in batch, fed-batch or continuous system, although the batch mode is more frequently used. Ethanol fermentation is normally concluded in 6 to 10 days, depending on the conditions of process.

However, composition surface culture, submerged cultures are somewhat less sensitive to changes in the composition of media, that is an advantage when using starch having a highly variable composition, simpler, requires lower volumes of waste water and requires low cost for downstream processing (Rokem, 2011). On the other hand, a typical problem of submerged culture is the formation of foam, which can be avoided using antifoam agents and chambers with volume up to one third of the total fermentation volume and more sophisticated technology and the high cost of energy, which in turn requires expertise in the field.

Solid State fermentation

Solid Substrate fermentation (SSF) is defined as the growth of microorganisms on moist solid substrates (termed solid substrate fermentation) or on inert substrate used as solid support. In both cases enough water is present to maintain microbial growth and metabolism (Olawale *et al;* 2010). There is free ï moving water is and air is the continuous phase. SSF technology provides many new opportunities as it allows for the use of agricultural waste products as fermentation substrates without the need for extensive pretreatment. Solid state culture is characterized by the development of microorganism in a low-water activity environment on an insoluble material that acts as physical support and source of nutrient.

As compared to submerged fermentation, solid substrate fermentation offers these advantages:

- High surface exchange for air/substrate.
- Limited consumption of water.
- Low energy consumption.

Although, various fermentation techniques can be applied for ethanol production, it was proven that, the submerged fermentation gives the highest efficiency in terms of yield, maximum ethanol concentration and also overall productivities. The main disadvantage of solid state fermentation is that they are hard to reproduce compared to submerged fermentation (Olawale *et al;* 2010).

1.9.4 Ethanol and the Environment

Increasing industrial activity and population growth has resulted in a rising concentration of ógreenhouse gasesô in the atmosphere that contribute to the óGreenhouse Effectô. These gases include carbon dioxide, methane, and nitrous oxide. The term óGreenhouse Effectô refers to the Earthôs trapping of the sunôs incoming solar radiation, causing warming of the Earthôs atmosphere. This offsets the Earthôs natural climatic equilibrium, and results in a net increase in global temperatures. óGlobal Warming ôis a term used to describe the increasing average global temperature. The term óClimate Changeô refers to a wide range of changes in weather patterns that result from global warming. A substantial increase in the Earthôs average temperature could result in a change in agricultural patterns and melting of polar ice caps, raising sea levels and causing flooding of low-lying coastal areas. The Earthôs climate is ready adjusting to past greenhouse gas emissions, and the average global temperature is expected to rise by 1^{O}C to 3.5^{O}C by the year 2100 (This increase in average temperature is larger than that which has been experienced). By 2100, the Earthôs average sea level is predicted to rise by approximately 50 cm. These phenomena could have serious repercussions on the natural and physical environments as well as on human health with the threat of global warming &energy crises in todayôs environment the need for clean, ñgreenò fuels is quickly becoming a necessity. The U.S. Environmental protection Agency considers ozone to be the most wide spread air pollution problem. To combat this problem, ethanol is widely used in reformulated gasolineôs to help urban cities meet public health standards for ozone. Because itôs produced form renewable resources, ethanol is the only transformation fuel that reduces greenhouse gas emissions from cars. Fossil fuels release carbon dioxide, a greenhouse gas that traps the earthŝ heat, contributing to global warming. Ethanol is made from agricultural crops, which ñbreatheò carbon dioxide and give off oxygen. This maintains the balance of carbon dioxide in the atmosphere.

Increased use of renewable fuels ethanol will help counter the pollution and global warming effects of burning gasoline (Lubert *et al;* 2005) under current conditions, use of ethanol blended fuels as E85 can reduce the net emissions of greenhouse gases by as much 30 ï 36 % and can further contribute by decreasing fossil energy use by 42-48 %. Ethanol blended fuel as E10 reduces greenhouse gases by 2.4-2.9 % and fossil energy use by 3.3-3.9 %. The E10 blend reductions are lower because a smaller fraction of the blend is ethanol. With improved Technologies and use of ethanol made from cellulose, these reductions in emissions will increase (Iken *et al;* 2002).

Environmental Benefits of Fuel Ethanol

Carbon Dioxide

Carbon dioxide from the burning of fossil is the largest single source of greenhouse gases from human activities, representing about half of all greenhouse gas emissions. Use of 10 % ethanol-blended fuels results in a 6-10 % CO_2 reduction and higher levels of ethanol can further reduce the net quantity of CO_2 emitted into the atmosphere (Farrel *et al*; 2006) More CO_2 is absorbed by crops growth than is released by manufacturing and using ethanol. The carbon dioxide produced during ethanol production and gasoline combustion is extracted from the atmosphere by plants for starch and sugar formation during photosynthesis. It is assimilated by the crops in its roots, stalks and leaves, which usually return to the soil to maintain organic matter, or to grain, the portion currently used to produce ethanol. Only about 40 percent or less of the organic matter is actually removed from the farm field for ethanol production. The rest is returned to the soil as organic matter, increasing fertility and reducing soil erosion. With modern conservation farming practices, this soil organic matter will build up, representing a net removal of carbon dioxide from the atmosphere. An increase of only 1% in the soil organic matter level means an atmospheric reduction of over 40 tones of CO_2 per hectare of farmland (/Heafele *et al*; 2004). Canada has vast areas of agriculture cropland. Most of these soils could benefit from increasing soil organic matter by several percentage points. Ethanol use in gasoline has tremendous potential for a net reduction in atmospheric carbon dioxide levels.

Carbon Monoxide

Carbon monoxide, formed by the incomplete combustion of fuels, is produced most readily from petroleum fuels which contain no oxygen in their molecular structure. Because ethanol and other ñOxygenatedò compounds contain oxygen, their combustion in automobile engines is more complete. The result is a substantial reduction in carbon monoxide emissions. Research shows that reductions range up to 30 %, depending on type and age of automobile emission system used, and the atmospheric conditions in which the automobile operates (Watson *et al*; 2000).

Nitrous Oxide (N_2O)

Agricultural grain production for ethanol may generate a slight increase in nitrous (N_2O) emissions resulting from heavy fertilizer use. However, research and advances in agricultural technology in grain production are resulting in a reduction of these emissions, often to levels below other common crops.

Other Octane Additives

Because of its high octane rating, adding ethanol to gasoline can permit the reduction or removal of aromatic hydrocarbons (such as benzene), and other hazardous high-octane additives commonly used to replaced tetra-ethyl lead in Canadian gasoline.

Ozone.

Because of its effects in reducing hydrocarbons and carbon monoxide in exhaust (that causes respiratory problems), adding ethanol to gasoline results in an overall reduction in exhaust ozone-forming potential (Krishan *et al;* 2011). Adding ethanol to gasoline can potentially increase the volatility of gasoline. This potential is controlled if all ethanol-blended gasoline sold in Canada meets the volatility standards required for other types of gasoline. In contrast, the U.S. Clean Act allows gasohol (gasoline plus 10 % ethanol) to have a higher volatility than that of gasoline. This results in greater ñvolatile organic compoundsò emissions. Therefore, the Canadian ethanol blend has less potential to form ozone than the American counterpart.

Adding of ethanol to gasoline does create slightly greater amounts of aldehydes during fuel combustion. Yet the resulting concentrations are extremely small and are effectively reduced by the three-way catalytic converters in the exhaust systems of all recent-model cars. The

Royal society of Canada termed the possibility of negative health effects caused by aldehyde emissions with the use of ethanol blended gasoline as being ñremoteò.

Environmental Behavior

Recent reviews of the environmental behavior of gasoline oxygenates generally note that ethanol is not likely to accumulate or persist for long in the environment. For example, the interagency Assessment of Oxygenated Fuels observes that ethanol is expected to be rapidly degraded in groundwater and is not expected to persist beyond source areas (Watson *et al*; 2000). Ethanol in surface water is also expected to undergo rapid biodegradation, as long as it is not present in concentrations directly toxic to micro-organisms. The half-life of ethanol in surface water is reported to range from 6.5 to 26 hours. Atmospheric degradation is also predicted to be rapid.

Health Effects

Ethanol, the active ingredient of alcohol beverages, has been part of the human diet and the human environment ï for thousands of years. It is produced by fermentation by fungi and other micro-organisms, and is found at low levels in the blood and breath of persons who do not drink alcohol. Ethanol is widely ingested in alcoholic beverages, usually with only mild effects. However, at sufficiently high doses, ethanol can cause toxic effects in humans, both short-term (such as inebriation) and long-term (such as cirrhosis of the liver). If ethanol becomes a common fuel additive, there may be opportunities for exposure by inhalation: ethanol vapors might be inhaled at gasoline stations or in automobiles, for example. Thus, concern has been raised about the possible health consequences of using of ethanol for this purpose.

Energy Values

When evaluating the energy value many areas need to be researched including Carbon Monoxide, Ozone, Octane Enhancement, Carbon Dioxide, and Aldehydes.

An example of the reductions that can be observed when using ethanol blends in your engine is the following (per vehicle mile). Current Corn and Ethanol Production case for Corn-based Ethanol Use of E10 (10 % ethanol and 90 % gasoline by volume) achieves:

- 6 % reduction in petroleum use,
- 1 % reduction in GHG emissions, and
- 3 % reduction in GHG in fossil energy use.

Use of E85 (85 % ethanol and 15 % gasoline by volume) achieves:

- 73 ï 75 % reduction in petroleum use,
- 14 ï 19 % reduction in GHG emissions, and
- 34 ï 35 % reduction in fossil energy use.

Use of E95 (95 % ethanol and 5 % gasoline by volume) achieves:

- 85 ï 88 % reduction in petroleum use,
- 19 ï 25 % reduction in GHG emissions, and
- 42 ï 44 % reduction in fossil energy use.

Energy Balance of Ethanol

Many questions have been raised as to the net energy efficiency of ethanol production from corn. Over the last decade, mush progress has been made in terms of energy-efficient ethanol production methods. Ethanol contains about 32,000 (high heating value) BTUs per litre. It takes about one quarter of that amount to grow the corn and about one third of that amount to process the corn in a modern ethanol production facility. Based on the ethanol ñlife-cycleò, the net energy balance is positive.

Ethanol around the World

Today, many countries around the world are testing both oxygenated and neat (near 100 %) alcohol fuels.

Brazil

Brazil is the world leader in the use of ethanol as an automobile fuel. More than 11 billion liters of ethanol for fuel are produced each year. About 15 % of the vehicles with spark ignition engines (the types normally fueled by gasoline) run on neat ethanol and the rest use a blend of 20 % ethanol in gasoline. Ethanol was introduced to reduce Brazilôs dependence on expensive foreign oil, and provides an additional market for domestic sugar producers. Beneficial effects on air quality have been an added bonus (Kim *et al;* 2003).

United States

The U.S, ethanol blends make up about 12 % of the total gasoline market. In some part of America, projects are underway to test the viability of replacing diesel fuel with ethanol; a project by greater Peoria Transit is documenting ethanolôs usefulness in fighting urban air pollution with its fleet of 14 ethanol-powered buses. Support for fuel ethanol is a key component in the current U.S. ñClean Air actò because of its beneficial effect on air quality. ñOxygenated fuels,ò such as ethanol blends, are mandated in certain regions to reduce carbon monoxide emissions and/or one. Today there are more than 55 domestics fuel ethanol production facilities located in 22 states across the country with annual capacity of approximately 1.8 billion gallons (Singh, 2008). Ethanol production facilities are largely modular, should certain demand for ethanol arises, and expansions could be done quickly by simply adding new equipment to existing production facilities. Expansion to existing facilities could easily add 600 million gallons of production could be available in the near term. Furthermore, the U.S. department of Agriculture has suggested that grain-based ethanol production could grow to as high as 3.3 billion gallons a year by 2004.

In addition, the next generated of ethanol production facilities will include production from cellulose and biomass feedstock. Earlier this year, there was a ground breaking for a new ethanol production plan in Jennings, Lousiana which, when completed, will produce ethanol from rice hulls and bagasse (Kohl, 2004). Three other plants are currently planned in California that will produce ethanol from rice straw. Already, ethanol is being produced from wood waste by Georgia pacific in Washington State and production from forest residues is not far behind.

Canada

In Canada, the ethanol industry is developing momentum, now that ethanolôs environmental, economic; energy rural development and renewable attributes are being more widely recognized. Although the industry still in its infancy, with only a few small scale plants producing ethanol consumer demand has prompted several gasoline retailing chains to feature ethanol blends, with demand for ethanol exceeding our domestic production. Ethanol-blended gasoline now available at over 700 gas bars across Canada from Quebec to the pacific, including the Yukon Territory. In many regions, ethanol blends are available for bulk delivery for farm and fleet use. The federal government and server provinces offer tax incentives based on environmental, economic development and/or energy diversity benefits, for the production and /or marketing of ethanol-blended gasoline to encourage development of an alternative fuel industry. Also, Environment Canada has designated ethanol blended gasoline, which meets their specifications, as an Environmental Choice trade; product on the basis of reduced toxic emissions, reduced use of non-renewable resources, and reduced carbon dioxide emissions. However, a firm and road-base (i.e., multiple departments/ministries) commitment to procurement and incentives for ethanol in the near-term is essential to ensure the successful establishment of this fledging renewable energy industry.

Canada typically produces fewer than 50 million tones of grain (wheat, barley, corn oat, and rye) annually, and exports about half of this. If all Canadian gasoline consumption (presently about 33 ï 35 billion liters annual) contained 10 % ethanol, the maximum grain requirement would be 8 ï 9 million tones. Canada would remain a major grain exporter.

Canada is a major importer of high- protein animal feed ingredients. The value of import is typically about $200 million annual. The by-product resulting from ethanol production from Canadian grain would serve to reduce this importation (Wang, 2008). How much fuel ethanol is being produced? Canadaôs current annual ethanol production, for all markets (1998) is approximately 234 million liters a year. With additional proposed development of ethanol production plants, Canadian potential production in the next few years is at 664 million liters per year. How much fuel ethanol is being used? It is difficult to ascertain current levels of the fuel ethanol use in Canada. In the U.S., it now represents about 90 % of total gasoline sales, or the equivalent of the total Canadian gasoline consumption. Over two trillion kilometers have been traveled using ethanol blends.

BENEFITS FROM ETHANOL TO THE ECONOMY

- There are many benefits to the economy when ethanol is involved. The following is a list of some these benefits.
- Ethanol can substitute for aromatic hydrocarbons, which include the carcinogen benzene. Ethanol can also replace MMT.
- Canadaôs growing dependence on imported light crude oil needed for gasoline and diesel fuel manufacture.
- The cost (financial and environmental) of mega-projects for enhancing Canadian domestic supplies of light oil crude oil.
- The abundant, and renewable, supply of Canadian grain available foe fuel ethanol production.
- The by-product ethanol production from grain, which is a high-protein livestock feed ingredient.
- The effect of adding ethanol to gasoline on environmental quality.
- Many societal costs associated with petroleum energy, such as respiratory and other health problems, crop yield losses and damage to vegetation, environmental disasters (e.g tanker misshapes), etc.

1.9.5 Already Done Works on Economic Analysis

A descriptive engineering spreadsheet model (DM model) was developed to model the dry mill ethanol production process. This model was created to better understand the economics of the ethanol dry mill production process and hoe the profitability of dry mill plants is affected under different conditions. It was also developed to determine the economic and environmental costs and benefits of utilizing new and different technologies in the dry mill process. Specifically, this model was constructed to conduct an economic analysis for novel process of obtaining greater alcohol yields in the dry mill process (Rhys *et al*; 2006)

The Dm model is more technically precise, and more transparent, and then other models of the dry mill process that have been constructed for similar purposes. The Tiffany and Eidman model (TE model) uses broad generalities of the dry mill process, based on the current state production, to approximate the sensitivities of the process to changes in variables. The TE model parameters were well researched, but the model suffers from several drawbacks. The main limitations of this model are that the results are very sensitive to the input values chosen by the user. Unlike the DM model, complex manipulations, such as determining the effect of new technologies would require accurate parameter estimates using the TE model.

The McAloon model uses highly technical engineering software (ASPEN) that acts essentially as a ñblack boxò in the dry mill production process. This very exact model does not aloe for a more general examination of the dry mill process. Changes in the production process would necessitate precise engineering plans.

Similar to the TE and McAloon models, the DM model is a spreadsheet model, but unlike the McAloon model it is completely self-contained. The DM model is a feed backward model input requirements (corn, enzymes, chemicals, utilities etc) are calculated based on the user entered values for annual production and process parameters.

However, according to Krishan *et al*; 2011 the ethanol production from dry mill corn starch using the fluidizing ï bed bioreactor (FBR) in a separate hydrolysis fermentation (SHF) savings are very sensitive to the residence time in the bioreactor or, in other words, to size of the bioreactor. The other sensitive parameters are ethanol concentration and ethanol yield. This analysis also shows that lowering the residence time in the FBR to 20 mins (by improvements in biocatalyst activity) would allow a higher cost savings of 3 ï 12 cents/gal.

Comparison with other studies

Quite a number of assessments of corn based ethanol have been performed during the past decade (Shapouri *et al;* 2003), Patzek (2004) and pimentel (2003) use ñenergy balancesò as basis for comparison and the present study has included this indicator to make comparison of energy results possible. ñDisplacement methodò, ñReplacement value methodò and ñSystem expansion methodò refers to the same principle when handling of co-products is considered, and all studies except (Patzek, 2004) apply the same principle that environmental impacts from the products that co-products of corn ethanol displace are subtracted. (Patzek, 2004) sees co-products from ethanol factory as waste and gives zero credit to the co-products. With respect to conclusions, the present study is in agreement with (Wang, 2008) at points where the results are comparable, namely use of fossil fuels and apparently also global warming. Other indicators cannot be compared directly because (Wang, 2008) provide results as emission of selected substances and the present study apply recalculation of a broader spectrum of emissions into environmental impact potentials. Fossil energy input determined in the present study is about the same level as thermal energy content of ethanol, and the results of the present study in terms of energy balance study ends Emissions considered by (Wang, 2008) all contribute to the environmental impact categories considered in this study. As recommended in the ISO standards for LCA up between (Shapouri *et al;* 2004) who find a positive energy balance and those who find a negative energy balance (Patzek, 2004) and (Pimentel, 2003). Essential inputs and outputs in corn ethanolôs product chain in terms of energy have been addressed in a review by Shapouri, 2003) for a number of studies from the past decade.

Corn ethanol conversion rate has increased gradually over the past decade and it is not surprising that the conversion rate applied in the present study is among the highest because the most recent and efficient technology intentionally has been applied. It is more surprising that energy input per produced unit of ethanol recorded in the present study is quite high compared to most other studies. Explanations can be different calculation principles, different background data and many other things and cannot be revealed without digging deep into each study.

The total energy use calculated for corn ethanol production in the present study is at the same level as in most other studies. Significant variation exists between different studies when it comes to co-product energy credits. This can be explained by application of different assessment principles in different studies, ranging from various allocation principles to differences in system expansion basis. In the present study, application of DDGS as replacement for corn in swine diet has been used as basis for system expansion and energy credit comes out in the lower end of the interval reported in the literature. Sensitivity analyses where DDGS displaces soy meal in cattleô diet indicate, however, that energy credit can vary significantly depending on the specie considered and the suggested energy credit can in fact be overestimated significantly.

ESTIMATED PRODUCTION COST OF ETHANOL FROM DIFFERENT RAW MATERIALS.

The cost of ethanol production and its value depends on plant location, feedstock, production scale, and end use. It also depends on the availability of feedstock, plant location, feedstock transportation cost, method of pretreatment, hydrolysis, fermentation techniques and ethanol market price. Most of the ethanol produced from North America is from cereal grains such as corn and wheat. In a corn-based ethanol industry, the cost of feedstock would be about 50-60 % of the total production cost (Kaylen *et al;* 2001). The price of corn was estimated to increase by US $ 1.20 ï 2.00/ton for every 2.5 million tones of corn used to make ethanol (Elander and putsche 2006). The cost of biomass feedstock will be in the range of US $ 25/ton to US $ 40/dry ton. (Sokhansanj *et al;* 2002) estimated that the cost of corn Stover feedstock delivered to an intermediate storage facility could range from a low of US $ 23/dry t (US $ 21/dry ton) to a high of US $ 45/dry t (US $ 41/dry ton). Therefore, the utilization of cellulosic biomass as feedstock for ethanol production reduces the operating cost of ethanol plants. The biomass feedstock cost will be about 21 % of the total production cost compare to corn which costs 50 ï 60 % of total ethanol production cost (Kaylen *et al;* 2001).

McAloon and co-workers (2000) reported the comparative cost of corn and cellulosic biomass on ethanol production. The cost is much a larger part of the production cost that Stover. A reduction in cost of ethanol can be also achieved by reducing the cost of cellulose enzyme. It is predicted that the use of genetically engineered raw materials with higher carbohydrate content combined with efficient conversion technology can reduce the cost of ethanol production by US $ 0.11 per liter over the next 10 years (Wooley *et al*; 2006). The cost of ethanol production from shelled corn is about US $ 0.88 per gallon. Whereas the cost of ethanol production from cellulosic biomass was about US $ 1.50 per gallon (McAloon *et al;* 2000). Although biomass conversion to ethanol involves several processes, selecting appropriate pretreatment method, efficient hydrolysis process and fermentation technique can reduce the ethanol price competitive to gasoline price. Integration of process operations, such as utilization of lignin for steam production in boilers and the production of furfural from hemicelluloses, could reduce the cost of ethanol production from biomass. Furfural is the valuable chemical solvent often used in the production of furfural alcohol, refining lubricating oils, butadiene extraction and furfural-based production of tetra-hydrofuran. Furfural alcohol is the major feedstock for the production of furan resins, which in turn are used to produce foundry sand binders. Kaylen and co-workers (2000) reported that co-production of ethanol and furfural would make ethanol production profitable. This represents a step toward the development of a biomass refinery, which could be used to produce various product combinations, depending on market demand, price and other factors. They also indicated that crop residues were the choice feedstock for ethanol production because of their low cost and relatively high percentage of hemicelluloses, a source of furfural production.

Improvements in pretreatment processes and breakthrough in enzyme technology will have an impact on the competitive industrial production of fuel ethanol in processes such as continuous process without or with cell recycling. Simultaneous saccharification and co-fermentation, utilization of immobilized cells, etc. enable higher yield of ethanol and reactor productivity (Kosaric *et al*; 2005). Economic conversion of cellulosic biomass to ethanol will also reduce the production cost that can be achieved by making ethanol fermentation process faster, particularly xylose fermentation, reducing the formation of by-products and developing genetically engineering yeasts to produce various high-value by-products (Ho *et al;* 2009).

However, ethanol costs could be reduced dramatically if efforts to produce ethanol from biomass are successful. Biomass feedstock, including forest residue, agricultural residue, and energy crops, are abundant and relatively inexpensive, and they are expected to lower the cost of producing ethanol and provide stability to supply and price. Biomass has to be fractionated into cellulose, hemicelluloses and lignin and the integration of the process byproducts will lead to economically feasible production of ethanol. Enzymatic hydrolysis of cellulose appear to have the most potential for achieving the goals, but substantial reductions in the cost of producing cellulose enzymes and improvements in the fermentation of non-glucose sugars to ethanol still are needed. Significant barriers to the success of cellulose-derived ethanol remain. For example, it may be difficult to create strains of genetically engineered yeast that are hard enough to be used for ethanol production on a commercial scale. In addition, genetically modified organisms may have to be strictly contained. Recent work by (Ho *et al*; 2009) on successful development of genetically reengineered *Saccharomyces* yeast for effective co-fermentation of glucose and xylose to fuel ethanol, and

(Nigam, 2001) work on hydrolysis of wheat straw hemicelluloses strengthen the economic feasibility ethanol production.

Table 1: Comparison of estimated ethanol production costs for various feed stock (s/gal.)

Cost item	U.S corn wet milling	U.S corn dry milling	U.S sugar cane	U.S sugar beets	U.S molasses	U.S raw sugar	U.S refined sugar	Brazil sugar cane	E.U sugar beets.
Feed stock cost	0.04	0.53	1.48	1.58	0.91	3.12	3.61	0.30	0.97
Processing costs	0.63	0.52	0.92	0.77	0.36	0.36	0.36	0.51	1.92
Total cost	1.03	1.05	2.40	2.35	1.27	3.48	3.97	0.81	2.89

CHAPTER TWO

Materials and Methods

2.0 Microorganisms

Aspergillus niger was used for the production of amylase enzyme. It was obtained from pure stock culture of Prof. J. C Ogbonna, Department of Microbiology, University of Nigeria, Nsukka. The *Aspergillus niger* was used for starch hydrolysis while the yeast *Saccharomyces cerevisae* was used for ethanol production. The *S.cerevisae* was isolated from palm wine. It was isolated by inoculation of palm wine into Yeast Peptone Glucose (YPG) agar plates in Petri dish and incubated at 37 ^{0}C for 24 hr. It was when stained with lacto phenol cotton blue stain and viewed under (X 40) microscope for proper identification.

2.1 PREPARATION OF REAGENTS.

Acid Dichromatic solution (0.01 mol/1 in 5.0 mo/1 sulphuric acid):

A 125 ml of water was added to a 500 ml conical flask and then 70 ml of concentrated sulphuric acid was added to a 500 ml conical flask carefully with constant swirling. The flask was allowed to cool in water and 0.75 g of potassium dichromate was added and made up to 250 ml with distilled water.

Starch indicator solution (1.0 % solution):

A 1 g of starch was dissolved in 100 ml of boiled water.

Sodium thiosulphate solution (0.03 mol/1)

A 7.44 g of sodium thiosulphate was added to a 1L volumetric flask, dissolved in distilled water and diluted up to the mark.

Potassium Iodide Solution (91.2mol/L)

A 5 g of KI was dissolved in 25 ml of distilled water.

2.2 MEDIA PREPARATION AND INOCULATION OF MICROBES.

Potato Dextrose Agar (PDA)

The fungi *Aspergillus niger* was grown in potato Dextrose Agar (PDA) medium. PDA powder (3.9 g) was mixed with 100 ml of water and then autoclaved. The medium was aseptically poured into several test tubes (15 ml each) which were then slanted and allowed to cool and solidify. *Aspergillus niger* was then inoculated from the stock culture into the prepared PDA media using a wire loop under aseptic condition. After this, it was incubated for 48 hr at 37 ^{0}C.

Preparation of YPG medium and inoculation of yeast

The yeast extract peptone glucose agar medium was prepared measuring 1 g of glucose, 3 g of yeast extract, 5 g of peptone and 1.5 g of agar agar and dissolving them in 100 ml of distilled water. It was then autoclaved for 1.5 minutes. The medium was aseptically poured into test tubes (15 ml each) which were then slanted and corked with cotton wool or foam and allowed to cool and solidify.

Saccharomyces cerevisiae were then inoculated from the stock culture into the prepared YPG medium using a wire loop and this was done with great caution in front of the Bunsen burner flame to prevent contamination. After inoculation, it was then incubated for 48 hours at 37 ^{0}C.

Yeast Peptone Glucose Broth (for yeast cells).

A broth of the YPG medium (1 g of glucose, 0.3 g of yeast extract, 0.5 g of peptone without the agar agar in 100 ml of distilled water) containing 250 mg of chloramphenicol was prepared in 250 ml conical flask and autoclaved at 121 ^{0}C for 15 mins. It was allowed to cool. Three loops of the yeast were inoculated and incubated for 72. hrs. The medium was decanted and the cells left in the flask.

Nutrient Broth (for yeast cells)

A broth of the nutrient medium (2.5 g of nutrient broth in 100 ml of distilled water) containing 250 mg of chloramphenicol was prepared in a 250 ml conical flask and autoclaved at 121^{0}C for 15 mins. It was allowed to cool. Three loops of the yeast were inoculated and incubated for 72 hrs. The medium was decanted and the cells left in the flask.

Sample Collection

Different corn varieties were soured from the department of crop science, University of Nigeria Nsukka They includes:

Yellow corn-flint maize ï Zea mays var. *Indurata*
Igbo corn ï Dent maize ï Zea mays var. *Indentata*
Popcorn ï Zea mays var. *Praecox*
White corn ï waxy maize ï Zea mays var. Ceratina
The rice was purchased from Ogige Market, Nsukka.

2.3 METHODOLOGY

PREPARATION OF CORN FLOUR

A 1 kg of flint maize ï Zea mays var. *Indurata*, waxy maize ï Zea mays var. *Ceratina,* Popcorn ï Zea mays var. *Praecox* and dent maize ï Zea mays var. *Indentata* were measured and grinded and the chaff was sieved off.

PREPARATION OF ENZYME FOR HYDROLYSIS

A cup of rice was steam to cook and allowed to cool. After cooling, 2000 g was weighed out of the cooked rice and poured into the slant of *Aspergillus sp* and wire loop was used to harvest (suspend or scup) the spores. The spore suspension was poured into 200 g of the cooked rice and mixed very well to ensure that the spores were evenly distributed in the rice. The rice was wrapped with the spores in a clean and sterilized cloth and then incubated for 24 hrs after which it was mixed again with a spoon to ensure that the spores were well distributed. It was wrapped again and left for another 24 hrs.

Heavy productions of black spores were observed at the end of 3 days. The wrapped clean clothes containing the produced enzyme was then stored in the refrigerator. The resulting solid state culture (koji) was used as a source of enzyme for starch hydrolysis.

DETERMINATION OF STARCH CONTENT USING KI-I METHOD by (Bailey & Olis, 1986)

A 1 g of starch sample was weighed and homogenized in 100 ml distilled water. It was boiled for 30 mins, allowed to cool, made up to 100 ml distilled water and filtered. The filtrate (0.1 ml) was added in a test tube mixed with 0.5 ml of potassium iodide and iodine, was up to 10 ml with water. The absorbance was taken at 680 nm. The starch concentration was calculated from the absorbance, using a calibration curve.

Determination of Amylose : Amylopetin Ratio using solubilization method by (Evan Heisman, 1999)

A 10 g of corn was weighed and homogenized in 100 ml of distilled water and gelatinized for 30 min at 70 ^{0}C ï 3 ml of $Cacl_2$ was added and allowed to stand for 10 min after which 5 Iodine was added and diluted- the blue color was read at 620 nm. The amylopectin was obtained by subtracting the amylase content from the starch content for each of the corn variety. They amylase was calculated from the absorbance reading as follows.

$$\frac{\text{Mean x df}}{\text{Slope}} = \text{mg/ml}$$

Then to convert to percent from $\frac{\text{mg/ml}}{100} = \%$

Where df = dilution factor.

Gelatinization of corn varieties

A 10 g of the various corn varieties was weighed and homogenized in 100 ml of distilled water. It was mixed while heating under low flames. After gelatinization; it was allowed to cool before adding the yeast inoculums and the koji enzyme.

Hydrolysis

The gelatinized starch (corn flour) was poured into a conical flask, then the enzyme was added to the gelatinized starch and this was done to hydrolyze the gelatinized starch.

Fermentation

The hydrolysates of the corn varieties were in conical flasks and then inoculated with *S.cerevisiae* cells from the decantation of YPG broth prepared earlier. Samples were taken every 24 hrs for 6days and the starch, glucose and ethanol concentrations were determined.

Determination of starch concentrations by k1 – method

A 0.5 ml of the sample was taken into test tube and then made up to 5 ml with water. 0.5 ml of iodine was added and finally made up to 10 ml with. The absorbance was read at 680 nm. The starch concentration was calculated as follows:

Using $\frac{y}{a} = \text{g/1}$

Where y = mean of the absorbance reading

A = slope of the standard

g/l = unit

Determination of glucose concentration using DNSA method by (mollies, 1959)

A 0.3 g of sample was added to 0.3 ml of DNSA in a test tube. It was allowed to stand with the test tube in boiling water for 10 mins. 3 mls of water was added and the absorbance read at 540 nm. The glucose concentration was calculated as follows:

using y/a = g/l

where y = mean of absorbance reading

a = slope of the standard
g/l = unit.

Determination of ethanol concentration using AO – AC Titration method

A 10 ml of acid dichromate was transferred to a 250 ml conical flask with matching rubber stopper and 1 ml of sample was pipette into the sample holder which was suspended over the dichromate solution and held with rubber stopper at 25^{o}C-30^{o}C. The stopper was loosed, removed after the incubation and the walls of the flask were rinsed with 100 ml distilled water. KI solution (1 ml) was added and mixed. Titration with sodium thiosulphate was done, 1ml starch was added and titration continued till the blue colour disappeared. The ethanol concentration was calculated as follows:

Using y/a x 10/1 = g/l
Where y = mean of titre value
a = slope of standard
g/l = unit

DETERMINATION OF ENZYME ACTIVITIES

Gluco-Amylase Enzyme Activity: The amylase assay was carried out by incubating a reaction mixture containing 0.5 ml of 1 % soluble starch, 0.2 ml of 0.1 mg sodium acetate buffer (ph 5.6) and 0.3 ml crude enzyme soluble from koji at room temperature for 30 minutes. After this, 1 mg of DNSA was added to terminate the reaction, and the tubes were boiled in boiling water for 10 minutes. After cooling, 4 ml of distilled water was added and readings were taking at 540 nm. The amount of reducing sugar was calculated as follows:

$\frac{\text{Mean}}{\text{Slope}} \times \frac{100}{1} = \text{mol/dl}$ (itôs divided by 100 because itôs in mg/ml)
S.I units = 0.0555
= mg/dl x 0.055 = mol/L

Gluco- amylase activity was defined as the amount of enzyme that releases one micromole sugar in 1 min under the assay condition.

Alpha – Amylase Enzyme Activity: The assay was carried out by incubating a reaction mixture containing 0.5 ml of 1 % soluble starch, 0.2 ml of 0.1 mg sodium acetate buffer (ph

5.6) and 0.3 ml crude enzyme from koji at room temperature for 30 mins. After this, 1M acetic acid was added to stop the reaction, followed by the addition of 8 ml distilled water and 1 ml iodine solution. The absorbance readings were taken at 680 nm. The amount of starch was calculated as follows:

$$\frac{\text{Mean x df}}{\text{Crude-enzyme x time}} = \text{mg/ml/min}$$

$$:.\ \frac{\text{Mg/ml/min x 1000}}{162} = \text{micromole}$$

Where df = dilution factor.
162 = molecular weight of starch.

Alpha amylase activity was defined as the amount of enzyme that releases 1micromole of sugar in 1 minute under the condition of assay

STATISTICAL ANALYSIS

The experimental were done in triplicates. The results were subjected to Analysis of Variance (ANOVA). The means where there were significance differences, the means were separated using Least Significance Difference (LSD).

CHAPTER THREE

3.0 RESULTS

3.1 Starch, amylose and amylopectin contents of the corn varieties.

In figures 2, 3, and 4; the starch, amylose and amylopectin contents of the corn varieties were shown. The Zea mays var. *Indurata* gave the highest ($P<0.05$) starch content of 64 % followed by Zea mays var. *Praecox* with 62 %, Zea mays var. *Indentata* with 60 % and Zea mays var. *Ceratina* with 57 %. However, the Zea mays var. *Indurata* also gave the highest amylose and amylopectin while Zea mays var. *Ceratina* has the lowest content. Table 2, shows the amylose : amylopectin ratios of each of the corn varieties; Zea mays var. *Indurata* gave the highest ratio while Zea mays var. *Ceratina* gave the lowest ratio. In fig 9 and 10, the amount of starch liberated and the glucose utilized were analyzed during the solid ï state fermentation of the koji enzym

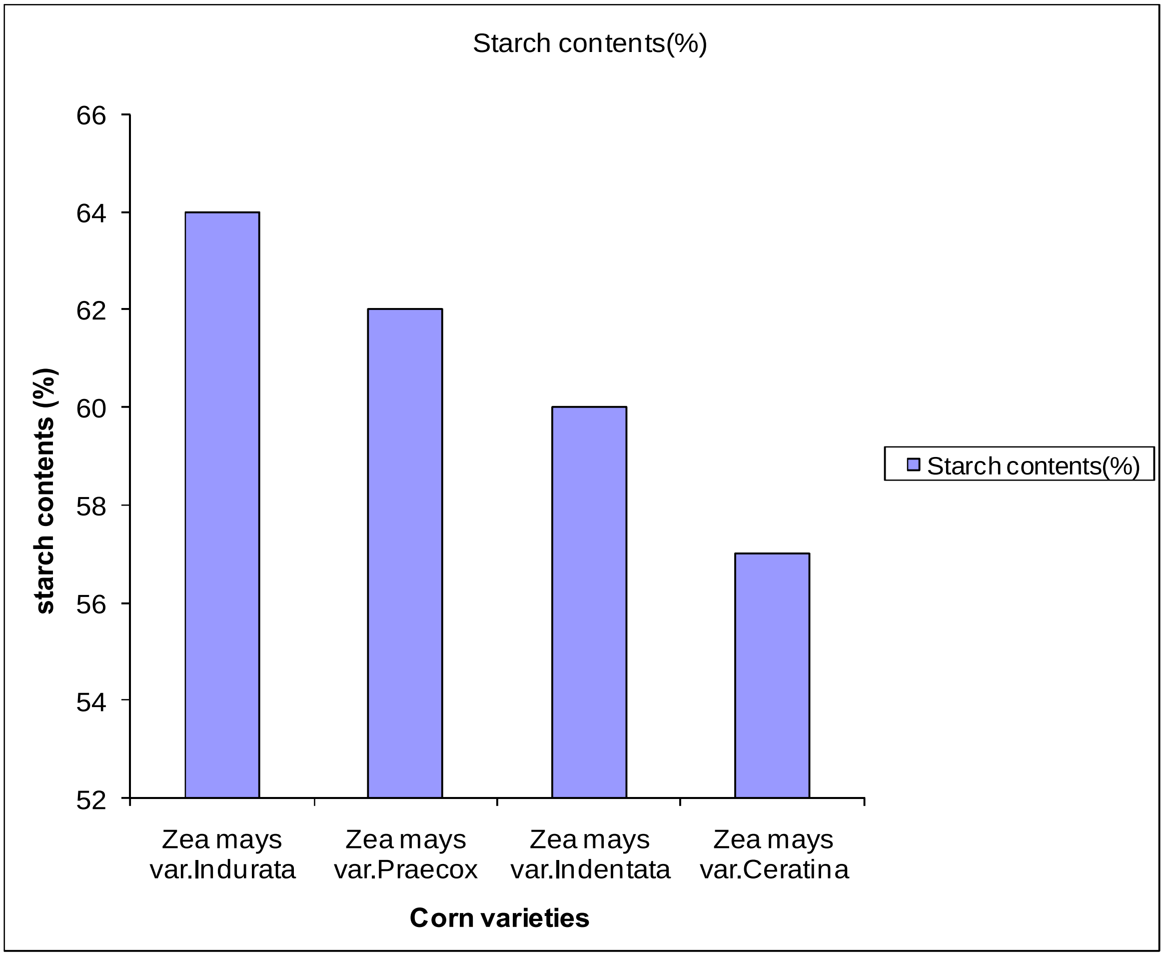


Fig. 2: Starch contents of Raw Zea mays var. *Indurata*, *Praecox*, *Indentata* and *Ceratina* varieties.

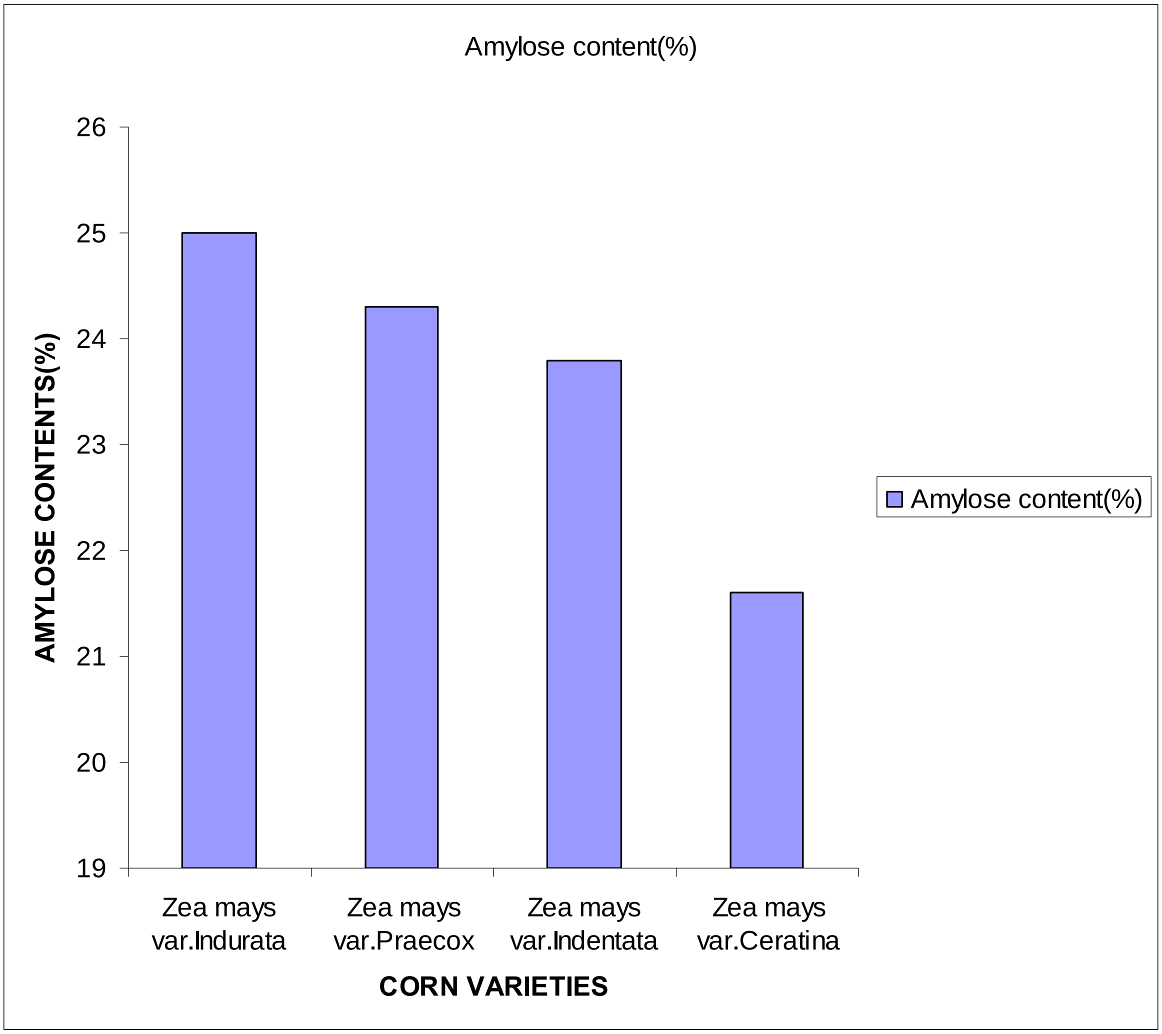


Fig. 3: Amylose contents of Raw *Zea mays var. Indurata, Praecox, Indentata* and *Ceratina* varieties.

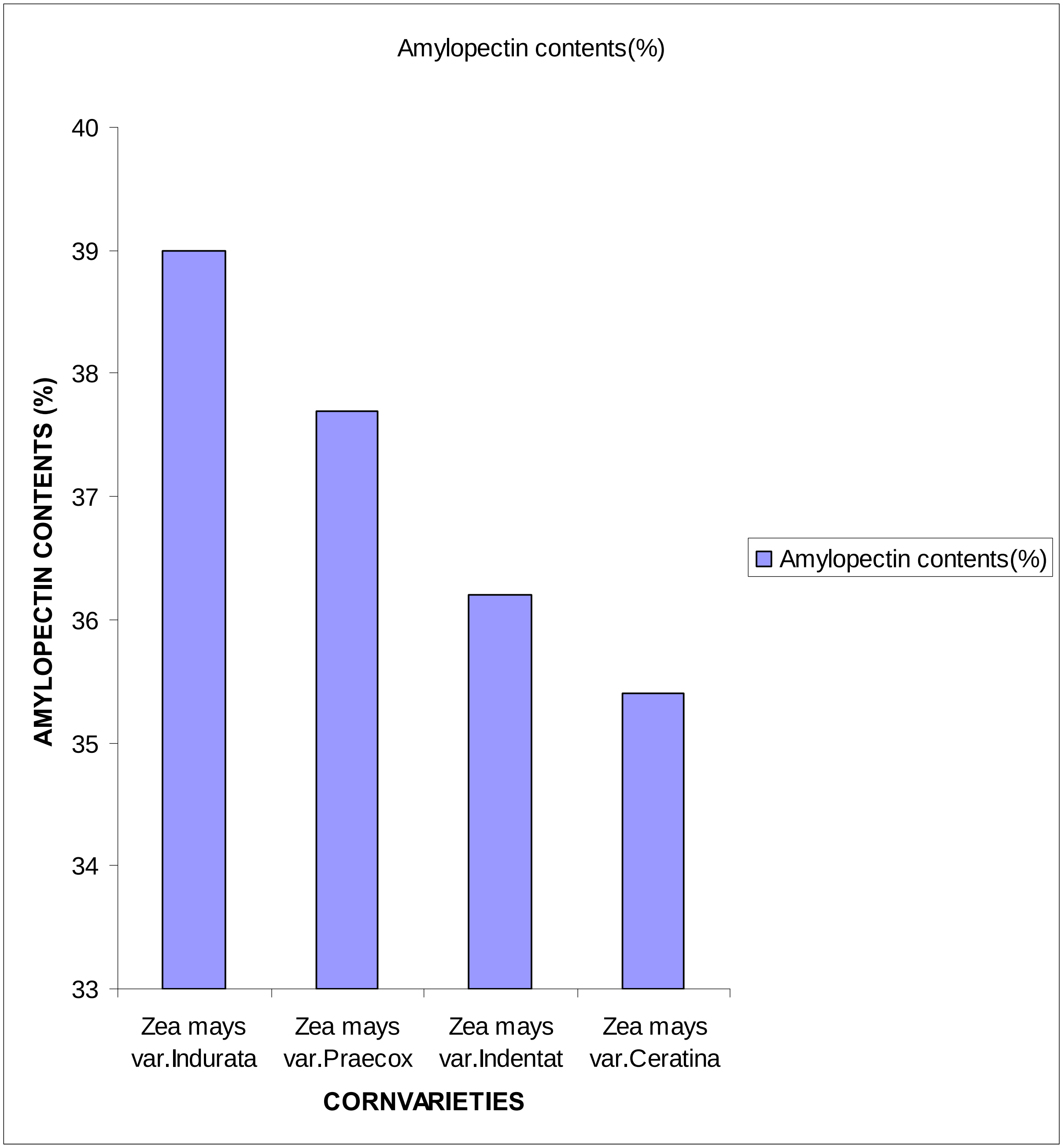


Fig.4: Amylopectin contents in *Zea mays var. Indurata, Praecox, Indentata* and *Ceratina* varieties.

Table 2: Summary of Amylose : Amylopectin Ratios of the corn varieties.

Corn varieties	Amylose (%)	Amylopectin (%)	Amylose: Amylopectin ratios
Zea mays va. Indurata	25.0	39	1.1.56
Zea mays var. Praecox	24.3	37.7	1.1.55
Zea mays var. Indentata	23.8	36.2	1.1.52
Zea mays var. Ceratina	21.6	35.4	1.1.53

3.2 Ethanol production from the corn varieties.

The results of production of ethanol from the different corn varieties under submerged culture condition are shown in figures 5, 6, 7 and 8. The ethanol concentrations increased with the fermentation time up to 72 hours and started to decline. Zea mays var. *Indurata* was the best substrate for ethanol production, giving a concentration of 24.70 g/l in 72 hours. This compares with 23.11 g/l, 21.30 g/l and 22.38 g/l obtained from the other corn varieties within the same cultivation period. Production of ethanol can be ranked statistically as follows, *Zea mays var. Indurata* > (*Zea mays var. Praecox* = *Zea mays var. Indentata*) > *Zea mays var. Ceratina.*

3.3 Alpha and gluco- amylase activities during ethanol production.

The results of enzyme activities from koji are shown in figures 9 and 10. The results show that as the days of the fermentation progressed, the starch was broken down to glucose and the glucose was utilized immediately while ethanol production increased and later started to decline when the starch finished. This shows also the amount of enzyme that degrades one micromole of starch in one minute and the amount of enzyme that releases one micromole sugar in one minute under condition of assay.

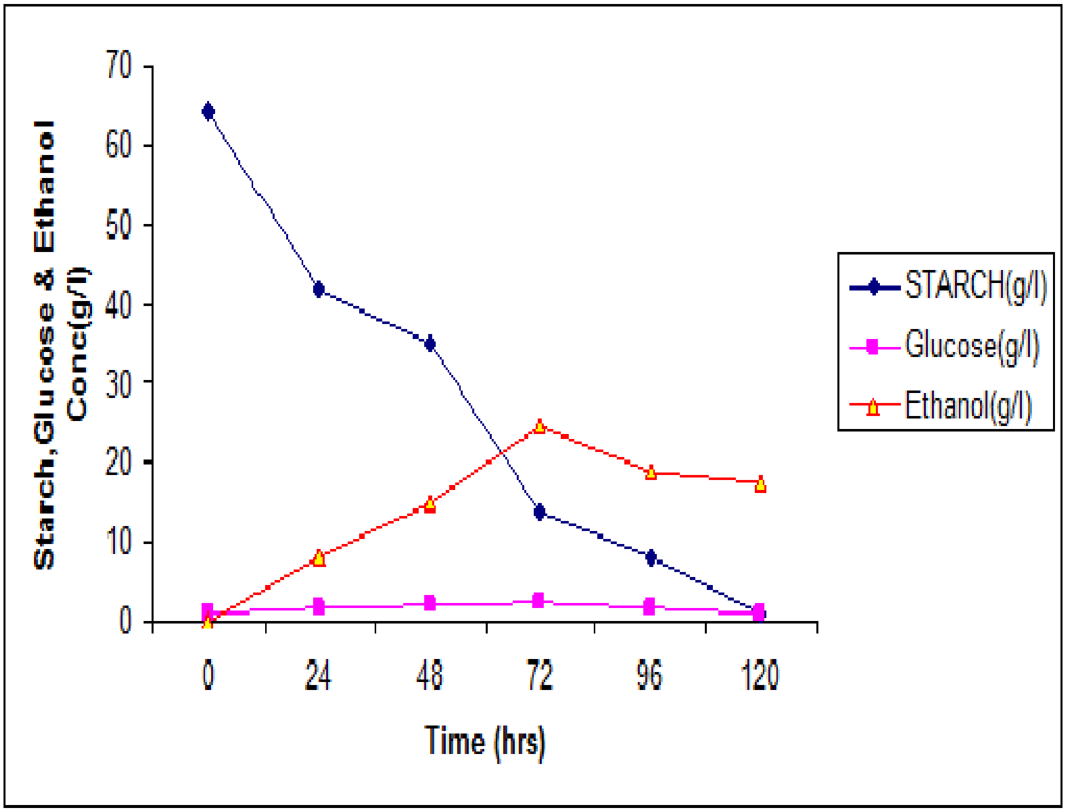


Figure 5: **Simultaneous hydrolysis of starch, glucose and ethanol fermentation of gelatinized *Zea mays var. Indurata* using koji and <u>*S.cerevisiea*</u>. The ratio of koji to flour was 1:1 w/w while the fermentation was done at room temperature. (27°C).**

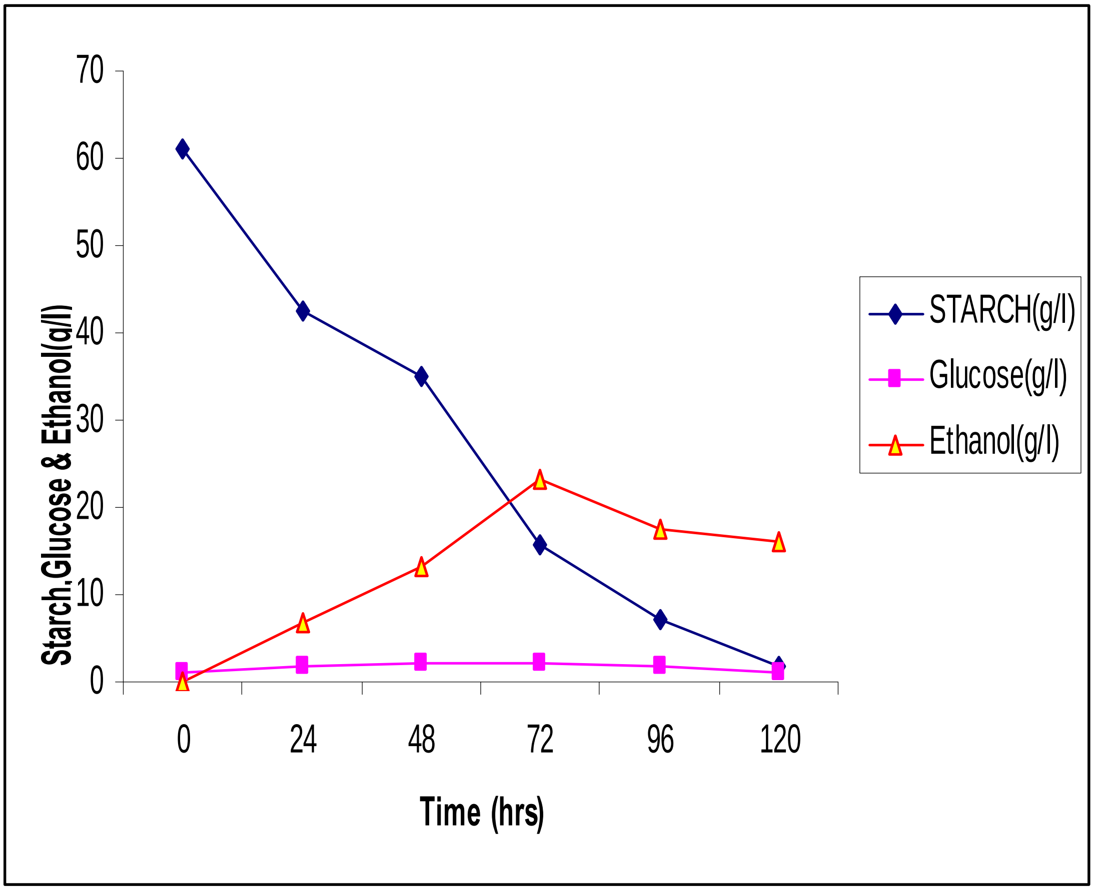


Figure 6: Simultaneous hydrolysis of starch, glucose and ethanol fermentation of gelatinized *Zea mays var. Praecox* using koji and *S.cerevisiea* . The ratio of koji to flour was 1:1 w/w while the fermentation was done at room temperature. (27°C).

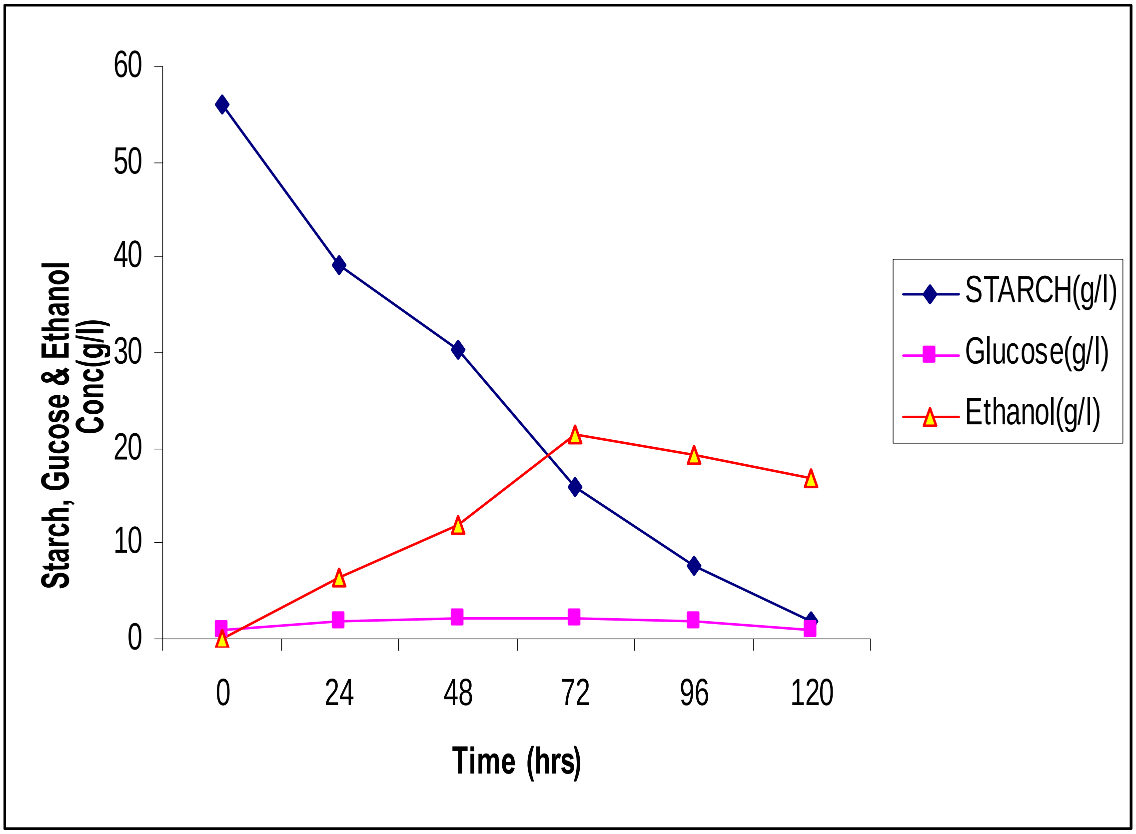


Figure 7: Simultaneous hydrolysis of starch, glucose and ethanol fermentation of gelatinized *Zea mays var. Indentata* using koji and S.cerevisiea . The ratio of koji to flour was 1:1 w/w while the fermentation was done at room temperature (27^{o}C).

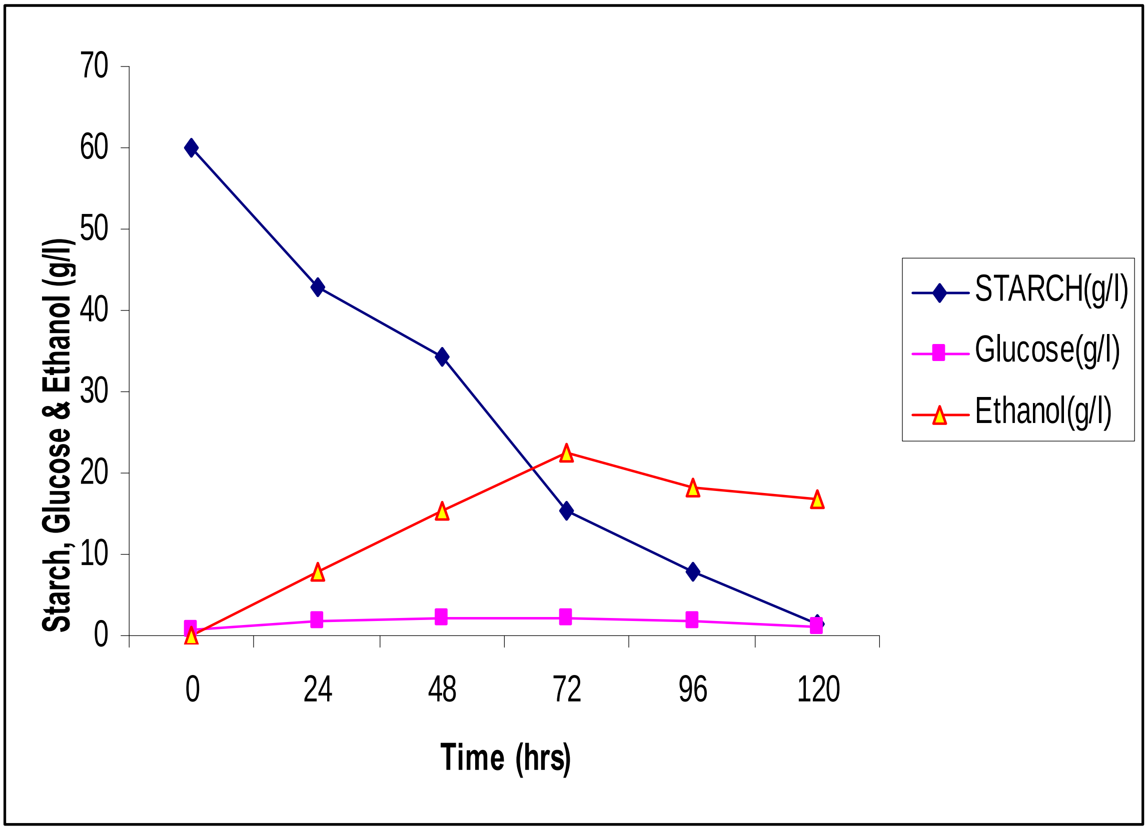


Figure 8: Simultaneous hydrolysis of starch, glucose and ethanol fermentation of gelatinized *Zea mays var. Ceratina* using koji and *S.cerevisiae.* The ratio of koji to flour was1:1 w/w while the fermentation was done at room temperature (27^{o}c).

3.4 Effects of the ratios of koji and corn flour on ethanol production from the corn varieties.

The results of ethanol production using different ratios of koji to corn flour are shown in figures 11 and 12. The results show that ratio 10:30 of koji to corn flour (10 g koji with 30 g flour) gave the highest ethanol concentration of 40.08 g/l with *Indurata*. On the other hand, a ratio of 10:05 gave the best ethanol concentration.

3.5 Fed-batch ethanol production from *Zea mays var. Indurata*

The result of fed batch ethanol production from *Zea mays var. Indurata* is shown in Fig 13. The ethanol concentration increased with fermentation time and a high concentration of 47.08 g/l was obtained after 192 hours of fermentation. Subsequently the ethanol concentration decreased gradually.

Table 3: Ethanol Yield of the Corn Varieties

Corn Varieties	**Ethanol Concentrations(g/l)**	**Flour Concentrations(g/l)**	**Ethanol Yield(g Ethanol/g flour)**
Zea mays var. Praecox	**4.70**	**100**	**0.247**
Zea mays var. Indentata	**23.11**	**100**	**0.231**
Zea mays var. Indentata	**22.38**	**100**	**0.223**
Zea mays var. Ceratina	**21.30**	**100**	**0.213**

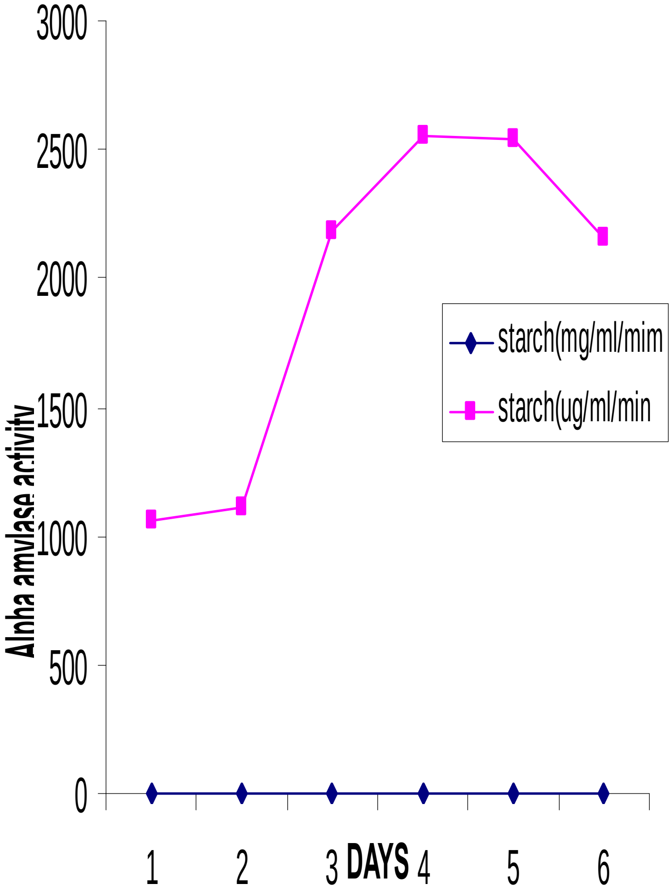


FIG 9: shows the rate of alpha-amylase enzyme activities on starch during fermentation.

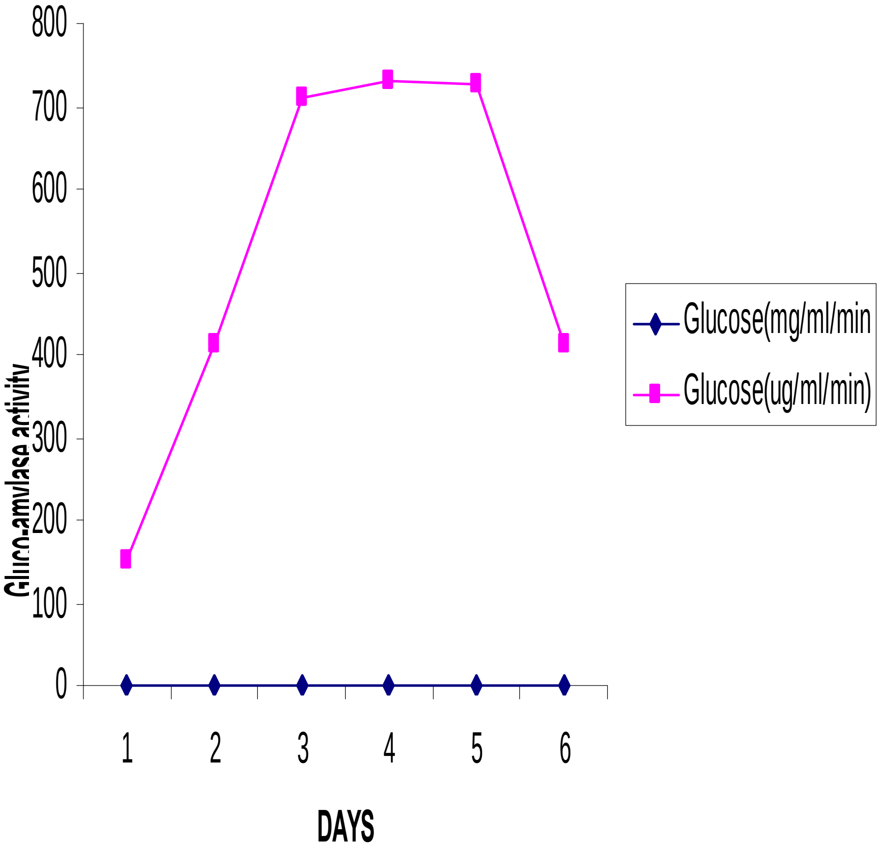


FIG 10: shows the rate of gluco-amylase enzyme activities on glucose during fermentation.

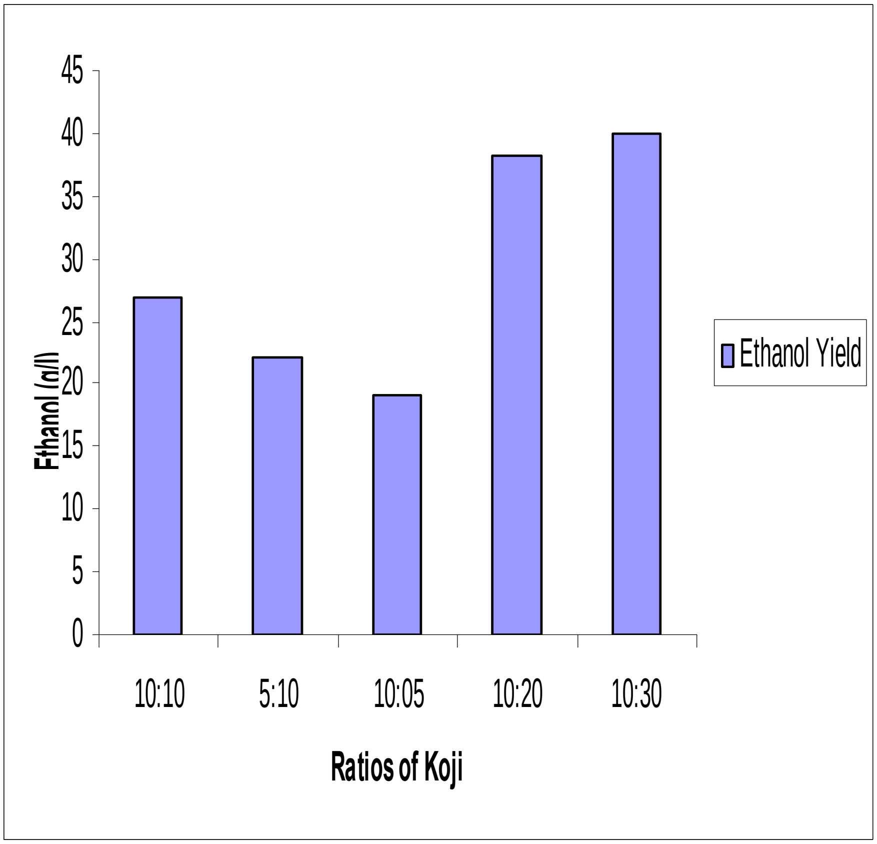


Figure 11: Effect of ratio of koji to flour on ethanol production from *Zea mays var. Indurata*.

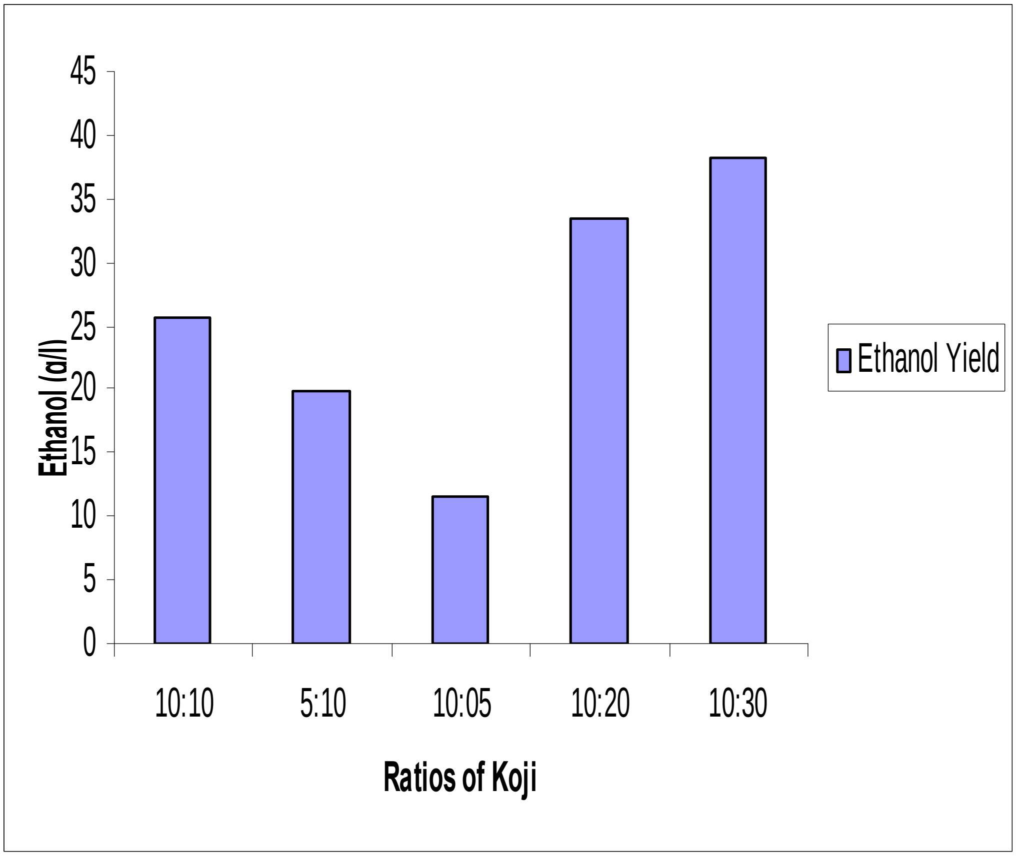


Figure 12: Effect of ratio of koji to flour on ethanol production from *Zea mays var. Praecox*.

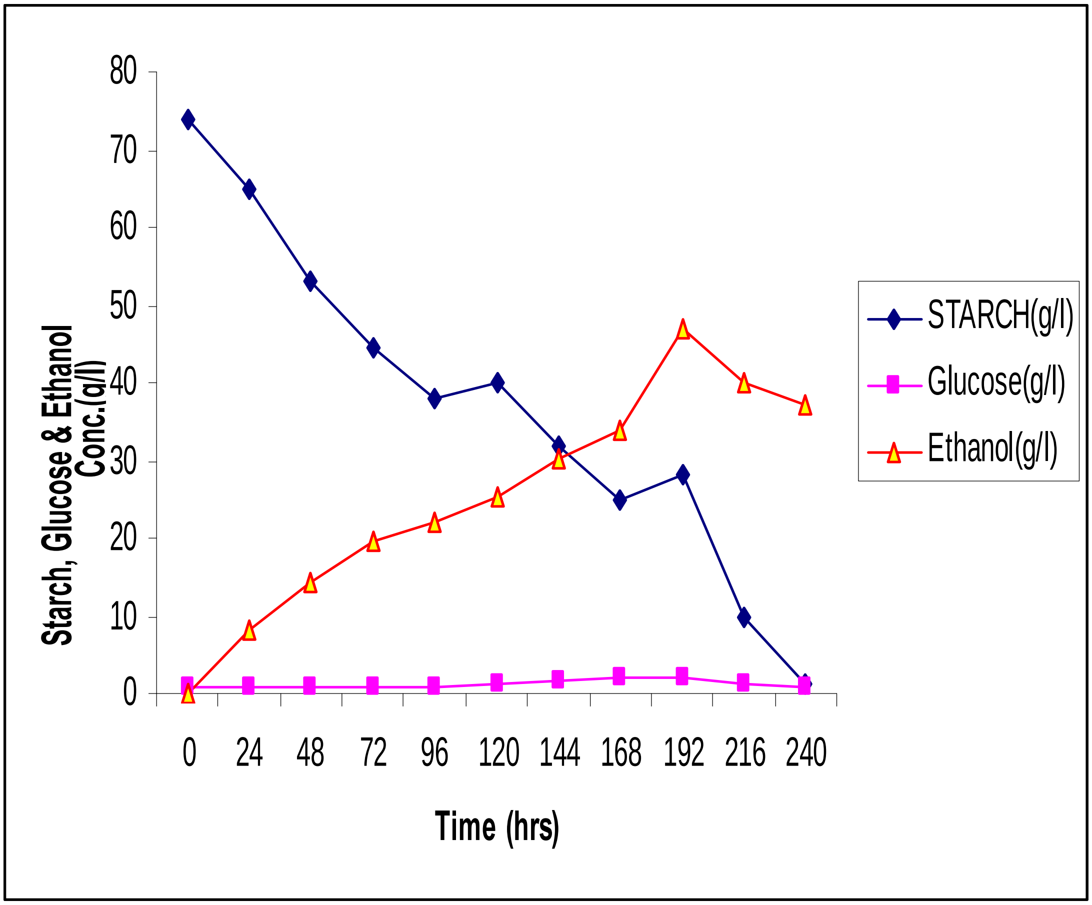


FIG 13: Fed - Batch production of ethanol from *Zea mays var. Indurata*

3.6 Economic analysis of corn ethanol production

Tables 4 to 9 show economic analysis of the corn ethanol production. Table 4 shows the yields of corn flour from the corn varieties while table 5 shows the cost of the various corn varieties in different markets within Nigeria.

As shown in Figure 5, 24. 70 g/l of ethanol was obtained from 100 g/l of *Zea mays var. Indurata* flour. This means that 24.70 g of ethanol was obtained from 100 g of flour. Since the total amount of flour obtained from 1.0 kg of *Zea mays var. Indurata* was 993.57 g, the total amount of ethanol from 1.0 kg of *Zea mays var. Indurata* is 245.41 g. Using a density of 99.5 % ethanol which is 0.789, the volume of ethanol produced from 1.0 kg of *Zea mays var. Indurata* is 311.0 ml (0.31 L).

Based on the cost of *Zea mays var. Indurata* at Nsukka (₦6, 000/100 kg), it can be estimated that the cost of *Zea mays var. Indurata* used to produced 1 L of ethanol is ₦193.5. The cost raw materials usually represent about 70 % of the total production cost. Thus the cost of bio-ethanol from *Zea mays var. Indurata* can be estimate to ₦276.42.00 / L.

As shown in Figure 6, 23.11 g/l of ethanol was obtained from 100 g/l of *Zea mays var. Praecox* flour. This means that 23.11 g of ethanol was obtained from 100 g of flour. Since the total amount of flour obtained from 1.0 kg of *Zea mays var. Praecox* was 992.78 g, the total amount of ethanol from 1.0 kg of *Zea mays var. Praecox* is 229.43 g using a density of 99.5 % ethanol which is 0.789, the volume of ethanol produced from 1.0 kg of *Zea mays var. Praecox* is 290.78 ml (0.29 L).

Based on the cost of *Zea mays var. Praecox* in Nsukka (₦9, 000/100 kg), it can be estimated that the cost of *Zea mays var. Praecox* used to produced 1 L of ethanol is ₦309.6. the cost of raw materials usually represents about 70 % of the total production cost. Thus the cost of bio-ethanol from *Zea mays var. Praecox* can be estimated to be ₦442.28 / L.

As shown in Figure 7, 22.38 g/l of ethanol was obtained from 100 g/l of *Zea mays var. Indentata* flour. This means that 22.38 g/l of ethanol was obtained from 100 g of flour. Since the total amount of flour ethanol from 1.0 kg of *Zea mays var. Indentata* was 993.48 g, the total amount of ethanol from 1.0 kg of *Zea mays var. Indentata* is 222.38 g using a density of

99.5 % ethanol which is 0.789, the volume of ethanol produced from 1.0 kg of *Zea mays var. Indentata* is 281.7 ml (0.28 L).

Based on the cost of *Zea mays var. Indentata* in Nsukka (₦7, 000/100 kg), it can be estimated that the cost of *Zea mays var. Indentata* used to produce 1 L of ethanol is ₦249.9. The cost of raw materials usually represents about 70 % of the total production cost. Thus the cost of bio-ethanol from *Zea mays var. Indentata* can be estimated to be ₦357.00 / L.

As shown in Figure 8, 21. 30 g/l of ethanol was obtained from 100 g of *Zea mays var. Ceratina* flour. This means that 21.30 g/l ethanol was obtained from 100 g of flour. Since the total amount of flour ethanol from 1.0 kg of *Zea mays var. Ceratina* was 992.55 g, the total amount of ethanol from 1.0 kg of *Zea mays var. Ceratina* is 211.41 g using a density of 99.5 % ethanol which is 0.789, the volume of ethanol produced from 1.0 kg of *Zea mays var. Ceratina* is 267.9 ml (0.26 L).

Based on the cost of *Zea mays var. Ceratina* in Nsukka (₦5,800/100 kg), it can be estimated that the cost of *Zea mays var. Ceratina* used to produce 1 L of ethanol is ₦222.7. The cost of raw materials usually represents about 70 % of the total production cost. Thus the cost of bio-ethanol from *Zea mays var. Ceratina* can be estimated to be ₦318.14/L.

In summary, the costs of ethanol production (N/L) from the flour corn varieties were 276.42, 442.28, 357.00 and 318.14 naira per liter for *Indurata, Praecox, Indentata and Ceratina,* respectively (Table 7).

Table 4: Flour and chaff yield from 1 kg of corn obtained in one gram

Corn Varieties	Flour (g)	Chaff (g)
Zea mays var.Indurata	993.57	6.23
Zea mays var.Praecox	992.78	6.12
Zea mays var.Indentata	993.48	6.22
Zea mays var.Ceratina	992.55	6.15

Table.5: prices (Naira) of Corn Grain in Different part of Nigeria.

Weight (kg)	**Zea mays var. *Indurata***	**Zea mays var. *Indentata***	**Zae mays var. *Ceratina***	**Zea mays Var. var. *Praecox***	**Part of Nigeria**
100	**N5,900**	**N6,700**	**N5,600**	**N8,800**	**New Market, Enugu State**
100 kg	**N6,000**	**N6,800**	**N5,700**	**N8,900**	**Ogbete Market, Enugu State**
100 kg	**N6,000**	**N7,000**	**N5,800**	**N9,000**	**Ogige Market Nsukka.**

Table 6: Summary of Commercial Production of Ethanol from Corn.

CORN VARIETIES	COST OF PRODUCTION (NAIRA)
Zea mays var. Indurata	276.42/L
Zea mays var. Praecox	442.28/L
Zea mays var. Indentata	357.00/L
Zea mays var. Ceratina	318.14/L

Table 7: Current price of fuel ethanol in Nigeria

The content of gasoline are 32MJ/L for LHV and 35MJ/L for HHV. In the case of ethanol, the energy contents are 21.1MJ/L for LHV and 32.4MJ/L for HHV (www.ocean.washington.edu 2005). The energy content of fuel ethanol is 66-67% of gasoline (Joss Goldbemberg, 2008). Thus energy content of IL ethanol = energy content in 0.67 of gasoline. IL of gasoline = ₦97.

Price of 1.0L of ethanol = 0.67 x 97 = 64.99 = N65. Thus equivalent current price of ethanol is ₦65.Production cost = ₦276.42. Thus with this current subsidy; there will be a loss of ₦32 per 1 of ethanol. However, with total removal of subsidy, the cost of IL of ethanol will be 0.67 x 141 = ₦94.47. Even without considering sales from byproducts there will be a profit of ₦181.95 per L of corn ethanol. However, if the market price of corn can be reduced to ₦2, 000/100kg, the cost of production will decrease to ₦92.00/L; which is profitable provide that the subsidy is removed. These results are summarized in Table 9.

Table. 8: Effect of Corn Price on Economic Viability of Corn Ethanol

Zea Mays Var *Indurata* at different price of corn/100 kg (₦)	Cost of Production (₦)	Current equivalent price (0.67 x 97)	Loss or gain at Present cost (₦)	With Subsidy removal (0.67 x 141 = 94.47) (₦)
₦6,000	₦276.42/L	₦65.00	211.42/L Loss	-181.95 Loss
₦2000	₦92.00/L	₦65.00	27.00/L Loss	2.47 Gain
₦1,600	₦73.6/L	₦65.00	-8.6/Loss	20.87 Gain
₦1,400	₦64.4/L	₦65.00	0.6/L Gain	30.07 Gain
₦1,200	₦55.2/L	₦65.00	9.8/L Gain	39.27 Gain
₦1,000	₦46.00/L	₦65.00	19.00/L Gain	48.47 Gain

CHAPTER FOUR

4.1 DISCUSSION

From the above observation, it can be deduced that most of the starch content of the corn flour was converted to ethanol by *Saccharomyces cerevisiae* within 7 days while A.niger was capable of hydrolyzing the starch. The maximum ethanol (47.08 g/l) produced from *Zea mays var. Indurata* at 72 hours is in agreement with Curtis *et al*; (2004) who also reported maximum ethanol yield (89.6 %) at 72 hours from raw corn using *Saccharmoyces cerevisiea*.

Furthermore, the production cost of 1 L of ethanol was deducted and confirmed to be 33 e (₦50.33) (McCain, 2007). Though US Government still pays subsides to large corporations that produce ethanol in USA. (McCain, 2007).Brazil the largest producer of ethanol in the world use sugarcane to produce ethanol and sugar cane is a more efficient raw material for ethanol production than corn grain but the Brazilian government still subsidizes the ethanol industry to continue its production in their country. (McCain, 2007) because they know that fossil fuel will deplete soon and also sugarcane is more readily available and the cheap raw material in their country.

The Federal Government of Nigeria should see reasons with those developed countries that still against all odds continue to embark on ethanol production using the available renewable raw materials. The corn varieties used in this study were all developed by the Department of Crop Science University of Nigeria Nsukka and are cultivated in various parts of Nigeria. Since the cost of corn represents more than 70 % of the total ethanol production cost from corn (Krishan *et al,* 2011; Zhang *et al,* 2003), efforts must be made to increase both corn yield and starch contents of corn varieties in Nigeria. Nigerians should be encouraged to go into agricultural production (i.e corn production) the more and in that case the corn will serve both for human consumption and fuel ethanol production.

4.2 CONCLUSION

Amongst the four varieties of corn investigated, *Zea mays var.Indurata* is the best substrate for bio-ethanol production. The total starch as well as the amylose content was higher than those of the other three corn varieties. It also gave the highest ethanol productivity and yield. The cost of production with a market price of corn at ₦6, 000/100 kg is ₦276.42/L. This is not profitable with the current equivalent price of N65.00/L.

However, if the subsidy is completely removed or the same subsidy extended to fuel ethanol, it will be profitable at the price of N2, 000/ 100 kg. Reducing the cost of corn will make corn ethanol profitable.

REFERENCES

Adenola, O.A., Akinwumi, J.A. and Alika, J.E. (2008). Maize Production and Utilization in Nigeria: Biofuels Production. *African Journal of Biotechnology* **65** (2):223 ï 232.

Ado, S.A., Kachalla, G.U., Tijjani, M.B. and Aliyu, M.S. (2009). Ethanol Production from Corn cobs by co-cultures of *Saccharomyces cerevisae* and *Aspergillus niger*. *Bayero Journal of Pure and Applied Sciences* **2** (2): 99 ï 101.

Anuji, K.C., Chan, E.S., Ravinder, R. and Lakshmi, N.M. (2007).Economics of *Environmental Impact of Biotechnology and Molecular Biology Review*.**2** (1): 14-32.

Curtis, W., Steinberg, M.P. and Rodda, E.D. (2004). Fuel Ethanol from Raw corn. Biological Systems Engineering. *International Journal of Food Microbiology* **4** (13): 177-184.

David,K.C., Pimentel, D.A. and Patzek, T.W. (2005). Ethanol production using Corn, Switchgrass and Wood; Biodiesel Production using Soybean and Sunflower. *Journal of Natural Resources Research* **14.** (1): 100-112.

Elander, R.T. and Hsv, T. (2006). Processing and Economic Impacts of Biomass Delignition for Ethanol Biotechnology. *Pakistan Journal of Nutrition*. **4** (7): 463-478.

Elander, R.T. and Putshe, S.A. (2006). Structure and Composition Corp: Chemistry and Technology. Biology Bioenergy. Nigerian Journal of Biotechnology **5** (3): 82-84.

Fakorede, M.B., Kim, S.K., MAreck, J.H. and Iken, J.E. (2003). Breeding Strategies and potentials of available varieties in relation to attaining self sufficiency in maize production in Nigeria . *African Journal of Biotechnology* **3** (6): 15-39.

Farrell, A.E., Plevin, R.J., Turner, B.T., Jones, A.D., OôHare, M. and Kammen, D.M. (2006). Ethanol can Contribute to Energy and Environmental Goals. *African Journal of Biotechnology* **311** (5): 506-508.

Gabby, C.O., Bruz, B.K. and Owens, K.A. (2007). Energy Economics. National Development and Reform Commission: Institute for Energy Economics. *Journal of Applied Microbiology* **6** (4): 73-77.

Haefele, D.F., Owens, K.A., OôBryan, C.T. and Sevenich, D.A. (2004). Selection and Optimization of Corn Hybrids for Fuel Ethanol Production. *Industrial Microbiology* 2nd Edition. P. 72.

Ho, NWY., Chen, Z., Brained, A. and Sedlak, M. (2009). Successful Design and Development of Genetically Engineered *Saccharomyces* yeasts for Effective Fermentation of Glucose and Xylose from Cellulosic Biomass to Fuel Ethanol, *Journal of Adv. Biochemical Engineering and Biotechnology* **65** (3): 164-192.

Iken, J.E., Amusa, N.A., Obatolu, V.O. and Efron, Y.S. (2002). Evaluation of Some Newly Developed Maize Varieties in Nigeria: Food Technology. Journal of Food Technology **65** (7): 25 ï 28.

Joss Goldbemberg, F., Pederson, C.S. and Owen, D.A. (2008). Microbiology of Food Fermentation: *Industrial Microbiology*. 2nd Edition. P. 84.

Kaylen, M., Van Dyne, D.L., Choi, Y.S. and Blaise, M. (2001). Economic Feasibility of Producing Ethanol from Lignocellulosic Feed Stocks. *Journal of Bioresource Technology* **72** (2): 19-32.

Kim, T.H. and Kim, J.S. (2003). Pretreatment of Corn Stover by Aqueous Ammonia. *Journal of Bioresource Technology* **55** (3): 110-111.

Kohl, S. (2004), Techanical Conversions on Ethanol: Food Insights. *World Journal of Microbiology and Biotechnology* **26** (8): 10 ï 17.

Kosaric, N., Duvnjak, Z. and Stewart, G.G. (2005). Fuel Ethanol from Biomass: Production Economics and Energy. *Journal of Advanced Biochemical Engineering* **20** (2): 119-151.

Krishan, M.S., Taylor, F., Davison, B.H. and Nghiem, N.P. (2011). Economic Analysis of

Fuel Ethanol Production From Corn Starch using Fluidized-bed Bioreactor. *Journal of Bioresources Technology* **75** (6): 99-105.

Lubert, W.H., Stryer, S.A., Obi, I.U. and Klim, S.K. (2005). Biochemistry of Ethanol Fermentation. *World Journal of Microbiology and Biotechnology* **21** (3):731-733.

Mathew, C., Rendlemon, U. and Hosein Shapouri, S. (2003). New Technologies in Ethanol Production Phytochemical Analysis. *Journal of Biological Sciences* **34** (2): 79-85.

McCain, S. (2007). The Chemical Analysis of Food: Food Insights. *African Journal of Biotechnology* **26** (3): 10 ï 17.

McALoon, P., Benjack, U. and Klim, A.O. (2000). Fuel Ethanol from Biomass: Production Economics and Energy. *Journal of Advanced Biochemical Engineering* **20** (4): 119-151.

Mueller, N., Claire, Z. and Jackson, G.H. (2008). Ethanol from Biomass Production: Economics and Energy. *Journal of Advanced Biochemical Engineering* **64** (1): 94-99.

Nathan, S., Moser, E. and Klein, I. (2005). How Fuel Ethanol is made from Corn: Department of Agriculture. *Journal of Biological Sciences* **32** (8): 91-98.

Nigam, J.N. (2001). Ethanol Production from Wheat Straw Hemicelluloses Hydrolystate by *Pichia stipitis*. *Journal of Biotechnology* **87** (1):17-27.

Nwachukwu, I.N., Ibekwe, I.V., Nwabueze, R.N., Anyawu, B.N., Kalu, I. and Chinakwe, E. (2008). Production of High Ethanol Yielding *Saccharomyces cerevisiae* of Palm Wine: Origin by Protoplast Fusion. *Journal of Bioresource Technology* **5** (4): 64-68.

Olsson, L., Hahn-Hagerdal, J.E. and Zacchi, G. (2004). Kinetics of Ethanol Production by Recombinant *Escherichis coli* k011. *Journal of Biotechnology and Bioengineering* **45** (2**):** 336-365.

Oyeleke, S.B. and Jibrin, N.M. (2009). Production of Bioethanol from Guinea Corn Husk and

Millet Husk. *African Journal of Microbiology Research* **3**: (4): 147-152.

Patzek, T.W. (2004) Sustainability of the Corn-Ethanol Biofuel Cycle: College of Engineering Submitted. *Critical Reviews of Plant Science* **65** (3): 416-421.

Pimentel, D. (2003). Ethanol Fuels: Energy Balance, Economics and Environmental impacts. *Journal of Natural Resources Research* **12** (2): 720-728.

Rausch, K.D., Belyea, R.L., Ellersieck, M.R., Zingh, V., Johnston, D.B. and Tumbleson, M.E. (2006). Particle Size Distributions Ground Corn and DDGS from Dry Grind Processing. *Journal of Agricultural and Biological Engineering* **48** (1): 273 ï 277.

Rhys, T.D. and Wallace, E.T. (2006). Economic and Technical Analysis of Ethanol Dry Milling: Model Description. *Journal of Applied Microbiology* **95** (4): 470-475.

Rokem, J.S. (2011). Molecular Genetics and Biotechnology: Food Fermentation. *Journal of Biotechnology* **6** (2): 10-14.

Shapouri Hosein, S., Gallagher, E.C. and Michael S. G. (2003). Ethanol Cost of Production Survey United State: Department of Agriculture. *Journal of Food Science and Agriculture* **77:** (1) 127-132.

Singh, C., Ken, A.D and Klein E.U. (2008). National Oil Seeds and Vegetable Oils Development Board India. *Journal of Food Technology* **76** (6): 82-88.

Sokhansanji, S., Turhollow, E.C. and Perlack, R. (2002). Stochastic Modeling of Cost of Corn Stover: *Costs Delivered to and Intermediate Storage Facility Review* **20** (2):40- 44.

Swings, D. and Deley, J. (2006). Ethanol Fuels: Energy Economics and Environmental Impacts: *Journal of Biotechnology* **34** (4): 110-119.

Szczodrak, J. (2008). The Enzymatic Hydrolysis and Fermentation of Pretreated Wheat Straw to Ethanol. *Journal of Biotechnology and Bioengineering* **32** (6): 771-776.

Van Eijnathen, L.M., Lale, N.E. and Kang, B.T. (2005). Improvement of Maize in Nigeria. *Bioscience Research Communication Review* **11** (2): 79 ï 84.

Wang, M.Q. and Greet, M.U. (2008). Transportation Fuel-cycle Model: Methodology, Development, Use and Results, enter for Transportation Research, Argone National Laboratory. *Food Control Review*. **19** (2): 829-832.

Watson, S.A., Nathan, S.M. and Klein, C.Y. (2000). Structure and Composition Corp: Chemistry and Technology. *Biology Bioenergy and American Association of Cereal Chemistry*. **Pp**. 53 ï 82.

Wooley, A.C., Robert, H., Mark,O., Ruth, N., David, D.E., Glassner, G. and John S.P. (2006). Process Design and Costing of Bioethanol Technology: A Tool for Determining the Status and Direction of Research and Development. *African Journal of Biotechnology Progress* **15** (5) : 794-803.

Xiang-Yang Ge, H. and Wei-Gw Zhang, J.U. (2005). A Shortcut to the Production of High Ethanol Concentration from Jerusalem Artichoke Tubers. *Journal of Biotechnology* **43** (3): 241 ï 246.

Zhang, J.G., Wei-Gw Zhang, J.U., Glassner, G. and Claire, Y.S. (2003). Evaluation of some Newly Developed Maize Varieties in Nigeria. *African Journal of Food Technology* **7** (4): 25 ï 28.