CELLULOSIC ETHANOL SYNTHESIS FROM WHEAT STRAW AND GALLANTS SOLDIER USING INDIGENOUS BASIC MINERAL SALT HYDROLYTIC REGIMES

ALOYS MOSIMA OSANO

DOCTOR OF PHILOSOPHY

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Aloys Mosima Osano

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DECLARATION

This Thesis is my original work and has not been presented for a degree in any other University

Signature……..Date……

Aloys Mosima Osano

This Thesis has been submitted for examination with our approval as University Supervisors.

Signature……..Date……

Prof. Eric R. Okong'o

JKUAT, Kenya

Signature……..Date……

Prof. Oyaro Nathan

Maasai Mara University, Kenya

Signature……..Date……

Dr. Jackson Kiptoo

JKUAT, Kenya

DEDICATION

I sincerely recognize the support I received from my beloved Children; Gloria Gesare, Sharon Bosibori, and John Francis Osano. They gave me a peace of mind and encouragement throughout the research period. My beloved Mum, Teresiah Kerubo for her continuous prayer which really supported me.

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LIST OF ABBREVIATIONS

Abbreviation	Meaning
HPLC	High performance liquid chromatography
TLC	Thin layer chromatography
HMF	Hydroxymethyl furan
UFLC	Ultra-Fast liquid chromatography
XRF	X-ray Fluorescence spectrophotometer
FTIR	Fourier transform infrared spectrometer
GC	Gas Chromatography
DP	Degree of polymerization

ABSTRACT

Production and use of ethanol for fuel can reduce air pollution, and reduce global warming. This research emphasizes on the plant biomass salt pretreatment and breakdown for microbial fermentation for cellulosic ethanol production. Studies of indigenous basic salt hydrolysis of two non-woody agricultural lignocelluloses; wheat straw and gallants soldier has been determined. The hydrolysis study was done using basic salts; 'magadi', 'Lebek' and 'Para,' salts. Trace metallic ionic composition, pH, percent concentration of CO_3^{2-}, and HCO_3^- and the degree of hydrolysis data are presented for the three salts found in Kenya and the complete randomized block design was used in sampling. Salts were analyzed for metallic ions; K, Na, Ca, Mg, Co, Fe, Mn, Cu, Pb, Cd, Zn, anions CO_3^{2-}, HCO_3^-, and pH values and structures analysis was done. The following analytical instruments were used; AAS, Flame Photometer, XRF, FTIR, UFLC, and a pH meter. Titrimetric method was employed for CO_3^{2-} and HCO_3^- determinations. All were of recorded pH greater than 9.98 hence were alkaline. The FTIR charts of all the salts indicated presence of bicarbonate group. The total carbonate levels recorded were; magadi (47.2%wt/wt), para (113.9%wt/wt) and Lebek (11.8% wt/wt). The main metallic ion concentrations recorded for the salts were; Magadi had Na^+ (71.52 mg/g), para had Ca^{2+} (52.56 mg/g), while Lebek had Ca^{2+} (166.09 mg/g). Degree of salt hydrolysis of the lignocellulose samples was greater than 20.94±0.01% with the highest being 44.99±0.03% with neat salts. There was a clear indication that bicarbonate group play an important role in lignocellulose hydrolysis. Higher bicarbonate levels showed higher percentage of hydrolysis. Para salt was found to be a better ligno-cellulosic hydrolyser in oxidative media than the other salts tested. Wheat straw para salt hydrolysis recorded total sugars of 30.12% wt/wt and ethanol of 64.07% vol./wt. Gallants soldier para salt recorded slightly lower amounts, with sugars of 18.09%wt/wt and ethanol of 49.74%vol./wt.

CHAPTER ONE

1.0: INTRODUCTION AND LITERATURE REVIEW

1.1. INTRODUCTION

One of the attractions of biofuels is that they can be used in most internal combustion engines with little or no modification. Ethanol and biodiesel are the two most immediate candidates for adoption in the existing petroleum fuel infrastructure. Domestic production and use of ethanol for fuel can decrease dependence on foreign oil, reduce trade deficits, create jobs in rural areas, reduce air pollution, and reduce global warming due to carbon dioxide buildup (Birur, (2010). For decades, ethanol has been used alone and with petroleum-based gasoline in internal combustion engines. The renewable nature of its feedstocks has attracted research and government assistance on ethanol as a renewable fuel. Ethanol can be made synthetically from petroleum or by microbial conversion of plant biomass materials through fermentation. In Kenya, ethanol production is from the bioconversion of the grain from corn and wheat, and in some instances, sugarcane molasses. However, grain-based production is limited by available grain feedstocks and their prices as valuable multi-use commodities while molasses exhibits low production yields.

For this reason, researchers and industrialists have sought to make conventional, grain-based ethanol systems more efficient by not just fermenting the grain, but by bioprocessing as much of the plant (cellulose and hemicellulose) as possible. Owing to their abundance, polysaccharides, and especially cellulose and hemicellulose, have the potential to be major sources of small molecules for a variety of applications. Two polysaccharides, cellulose and hemicellulose, make up approximately 70–85% of lignocellulose, (Verendel, Tamara, & pher, 2011; Kennedy, Knill, & Taylor, 1998) which in turn makes up the vast majority of woody biomass and agricultural residues. Additionally, cellulose cannot be digested by humans, so its use as chemical feedstock need not compete with food resources.

This technology aims at digesting (chemical digestion) much of the plant into usable subunits that can then be efficiently converted (chemical-catalysis) to sugars for fermentation processing. The less usable co-products (lignin, ash and hard-to-process proteins) can be combusted to provide power and heat for the ethanol production

facility and the residual non-combustible ash and gypsum can become a marketed co-product for field fertilizer. This is the basic system envisioned for cellulosic ethanol production. Cellulosic (plant fiber) "conversion," along with hydrogen, is viewed by many environmental and social policy organizations as being the transportation fuel of the future.

With respect to conventional ethanol, technological research primarily is concentrated in two areas: ethanol yield improvement and overall production efficiency. Both seek to reduce production cost and create a viable technology to replace imported oil, as well as make crop, crop residue and alternative feedstock-based ethanol a sustainable, domestic biofuel. Much of this research is applicable to converting or transitioning existing conventional grain-based production facilities to cellulosic systems.

Conversion of ligno-cellulosic residues to fermentable sugars is a difficult process because of the close association among lignin, hemicellulose and cellulose (Ruigang *et al.*, 2005). Enzymatic hydrolysis of this material is possible but requires treatment of the ligno-cellulosic material before the enzymes can access the sugar polymers. Various treatment processes increase the accessibility of the cellulose portion to the hydrolytic action of cellulose enzymes. These methods can be grouped into four categories: Physical, chemical, biological or a combination of physical and chemical. Two treatments that use a combination of physical and chemical effects are steaming process and the ammonia freeze explosion process (Mes-Hartree, Dale & Craig, 1998).

Kenya has abundant agricultural residues that can be converted to biofuels. These resources can be used to produce enough ethanol to displace the current petroleum consumption. This study will therefore use cellulosic materials (wheat straw and Gallants Soldier agricultural weed) to produce fermentable sugars, which will then be subjected to fermentation to produce ethanol. This will provide a great opportunity for technology to ensure sustainable and environmental quality products.

1.1.1. Statement of the Problem

The use of ethanol as an alternative motor fuel has been steadily increasing around the world for a number of reasons. Domestic production and use of ethanol for fuel can decrease dependence on foreign oil, reduce trade deficits, create jobs in rural areas, reduce air pollution, and reduce global climate change carbon dioxide buildup. Ethanol,

unlike gasoline, is an oxygenated fuel that contains 35% oxygen, which reduces particulate and NOx emissions from combustion. Ligno-cellulosic ethanol synthesis worldwide has remained in the pilot and demonstrative stages because of the cost of production and the low yields associated. In Kenya, research into cellulosic ethanol has not taken momentum and the cost of farming is on the upward trend because of low yields, non-use of the abundant agricultural residues, which goes to waste or have low economic use and high cost of agricultural inputs.

In Kenya, there exist catalysts, which our citizens have used limitedly in their daily chores for decades. This has remained untapped and their use has been limited to that of a domestic and crude spectrum. Fairly true is the fact that alkaline hydrolysis has not featured much in cellulosic ethanol synthesis. And yet it could be the key to efficient hydrolysis and hence high yields with low operational costs.

Currently available hydrolytic systems in ligno-cellulosic ethanol synthesis are very costly due to the high temperatures and pressure required for the process, produces inhibitors for enzymatic fermentation and hence low yields. This calls for urgent need for the search of efficient less costly catalytic systems and procedures, which will help increase cellulosic ethanol yields from non-woody vegetal sources and less polluting.

1.1.2. Justification of research

Cellulosic ethanol production systems emit far lower net levels of greenhouse gases (GHG). Conventional, grain-based ethanol uses fossil fuel to produce heat for fermentation and other aspects of processing and produces GHG emissions. Cellulosic ethanol production uses part of the input-biomass feedstock (lignin, hard-to-process proteins) instead of fossil fuel.

Cellulosic ethanol also may provide additional positive environmental benefits in the form of reductions in GHG emissions and air pollution. Some researchers calculate that since lignin is a renewable fuel with no net GHG emissions, the GHG produced by the combustion of biomass are essentially offset by the CO_2 absorbed by the plant material (biomass crop) because it sequesters carbon during its growth.

Cellulosic ethanol is a potential replacement for gasoline and grain-based ethanol in cars and trucks. Additionally, cellulosic ethanol is promoted, as are other biofuels, to reduce the nation's dependence on imported oil, increase energy security and reduce the

trade deficit. Rural economies will benefit from new job creation and in the form of increased farming incomes.

1.1.3. Research hypothesis

i. Locally available agricultural residues; wheat straw and sugarcane bagasse do not have the potential to produce cellulosic ethanol
ii. Locally available hydrolytic basic and acidic catalysts are not applicable and capable of producing cellulosic ethanol
iii. Cellulosic ethanol produced from locally available agricultural residues is of low quantity.

1.1.4. Objectives

1.1.4.1. Main objective:

To assess the potential synthesis of cellulosic ethanol from locally available agricultural residue and weed (wheat straw and gallants soldier) using solution mixtures of basic inorganic salts (Trona-sodium carbonate-sodium bicarbonate and other salt mixtures) hydrolytic catalysts to provide sustainable and environmental quality fuels and other products that will reduce greenhouse gases emission and improve livelihoods.

1.1.4.2. Specific Objectives

i) To determine the chemical composition of the hydrolytic salt catalysts and characterize the salts
ii) To evaluate the potential of locally available agricultural weeds and residues; Gallants soldier and wheat straw to produce cellulosic ethanol
ii) To determine the applications and hydrolytic potential of locally available indigenous salt catalysts in cellulosic ethanol synthesis.
iii) To determine the quantity of fermentable sugars and cellulosic ethanol produced from locally available agricultural residues and weeds.

1.2. LITERATURE REVIEW

1.2.1. Lignocelluloses

Lignocellulosic materials from agriculture, forest management and urban waste are the largest sources of hexose (C-6) and pentose (C-5), sugars with a potential for the production of biofuels, chemicals and other economic by-products (Wu *et al.*, 2006). Lignocellulose composed of cellulose (40–50%), hemicelluloses (25–35%) and lignin (15–20%) is extremely resistant to enzymatic digestion (Iuliana, 2009). Pretreatments are usually necessary to disrupt the plant cell wall (lignin) in order to improve enzymatic digestibility (Mosier *et al.*, 2005 ; Tortosa *et al.*, 2007).

Unlike starch, the specific structure of cellulose favors the ordering of the polymer chains into tightly packed, highly crystalline structures that is water insoluble and resistant to depolymerization (Johansson & Samuelsson, 1975). The other carbohydrate component in lignocellulosics is hemicellulose which is dependent on the species, is a branched polymer of glucose or xylose, substituted with arabinose, galactose, fluctose, mannose, or glucuronic acid (Kim &Holtzapple, 2006). Some of the sidechains may also contain acetyl groups of ferulate (Holtzapple, 1993). Hemicelluloses hydrogen-bonds to cellulose microfibrils, thus forming a network that provides the structural backbone to plant cell wall (Kim & Holtzapple, 2006). Cellulose and hemicellulose are potential sources of fermentable sugars (Johansson, and Samuelsson, 1975; Kim & Holtzapple, 2006; Michael *et al*., 2009; and Jeffries, 2004). The presence of lignin in some cell walls imparts further strength, and provides resistance against pests and diseases (Kim & Holtzapple, 2006). This lignin, however, impedes enzymatic hydrolysis of the carbohydrates (Michael *et al*., 2009).

The crystallinity of cellulose, accessible surface area, protection of cellulose by lignin, the heterogeneous character of biomass particles, and cellulose sheathing by hemicellulose all contribute to the resistance of lignocellulosic biomass to hydrolysis (Kumar *et al.*, 2009; Lee *et al*, 2008). However, crystallinity alone is insufficient to prevent significant hydrolysis if sufficient enzyme is used. For example, hydrolysis of Avicel, microcrystalline cellulose, proceeded to 80% hydrolysis in 6 days when incubated with 72 units of Genencor Cytolase (CL) cellulase per gram (Katzen *et al*., 2006).

Various processes are employed for lignocellulosic conversion. A general process includes size reduction and pretreatment, hydrolysis, fermentation and separation. The first step in bioconversion of lignocellosics to bioethanol is size reduction and pretreatment. Size reduction and pretreatment are required to alter the biomass structure and increase the accessible surface area of cellulose, so that hydrolysis of the carbohydrate fraction to monomeric sugars can be achieved more rapidly and with greater yield (Wiesenthal *et al.*, 2006). The goal of any pretreatment technology is to alter or remove structural and compositional impediments hydrolysis in order to improve the rate of chemical or enzyme hydrolysis and increase yields of fermentable sugars from cellulose or hemicelluloses (Balat *et al.*, 2008). Pretreatment is required to alter the structure of cellulosic biomass to make more accessible to the chemicals or enzymes that convert the carbohydrate polymers into fermentable sugars and to cellulose producing microorganisms (Deker *et al.*, 2003). A successful pretreatment must meet the following requirements: improve formation of sugars or the ability to subsequently form sugars in hydrolysis, avoid degradation or loss of carbohydrate, and avoid formation of byproducts inhibitory to subsequent hydrolysis and fermentation processes (Andersen *et al.*, 2007). The purpose of the pretreatment is to remove lignin and hemicellulose, reduce cellulose crystallinity, and increase the porosity of the materials (Wiesenthal *et al.*, 2006). Rigorous process economic analysis is necessary to determine the best pretreatment process options for a particular feedstock and product opportunity.

Pretreatment methods are either physical or chemical. Physical pretreatment methods include; mechanical reduction in biomass particulate size, steam explosion, and hydrothermolysis. Acids or bases promote hydrolysis and improve the yield of glucose recovery from cellulose by removing hemicelluloses or lignin during pretreatment (Michael *et al*., 2009). Hydrolysis includes the processing steps that convert the carbohydrate polymers into monomeric sugars. During the fermentation process, the monomeric sugars are converted to ethanol and then ethanol is recovered from the fermentation broth, usually by distillation (Iuliana, 2009).

1.2.2. Chemical Structure and Composition of Lignocelluloses

1.2.2.1 Cellulose

The cellulose fraction of native wood is about 40 % (on dry weight), depending on the species. Cellulose is a homopolysaccharide on the molecular level, composed solely of β-D-glucopyranose units, joined together by (1→4) - glycosidic bonds (Kim & Holtzapple, 2006) to form cellobiose units, the smallest repeating unit in cellulose (Figures 1.1a and 1.1b). The degree of polymerization (DP) of cellulose present in the living tree is unknown, but the size of the native molecule is often stated to be 5000-10000 glucopyranose units (Kim & Holtzapple, 2006).

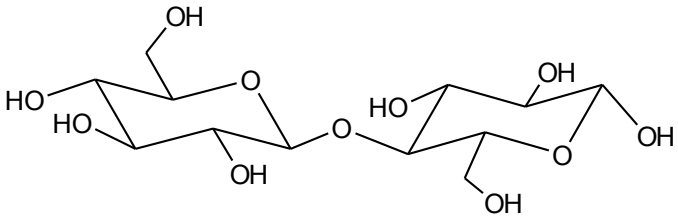

Figure 1.1a. Chemical structure of cellobiose

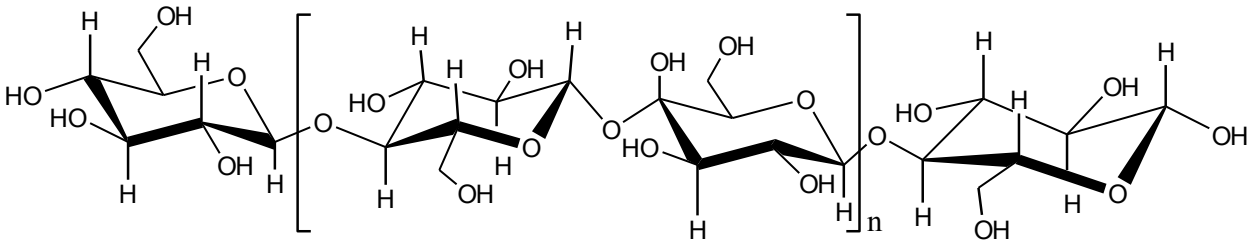

Figure 1.1b: Structural unit of cellulose

1.2.2.2: Hemicellulose

A number of different molecules present in wood are called hemicelluloses and the compositions of the hemicelluloses in hardwoods and softwoods differ slightly (Kim and Holtzapple, 2006). In hardwood, such as birch, the main hemicellulose is *O*-acetyl-4-*O*-methylglucuronoxylan, called glucuronoxylan or simply xylan. The content of xylan in hardwood fibers is about 15-30%, depending on the species (Kim and Holtzapple, 2006).

In softwood, the main hemicelluloses are *O*-acetyl-galactoglucomannan and arabino-(4-*O*methylglucurono) xylan (Figure 1.2), simply called glucomannan and xylan, respectively.

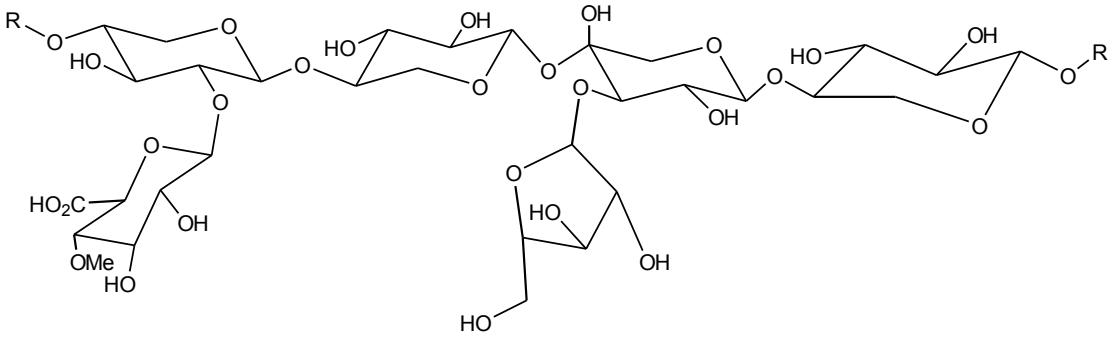

Figure 1.2: Principal composition of arabinoglucuronoxylan.

The content of glucomannan and xylan in softwood is 20% and 10%, respectively. The hemicellulose polymers in wood are much smaller in size than the cellulose, as the hemicelluloses have degree of polymerizations (DPs) of 100-200 (Kim and Holtzapple, 2006).

1.2.2.3: Lignin

Lignin is a complex three-dimensional molecule that gives the wood fiber rigidity. It enhances the tree's resistance towards microorganisms while it acts as a chemical adhesive joining the fibers together in the stem (Sarkanen *et al*. 1971). Softwood lignin is composed of the precursor trans-coniferyl alcohol, hardwood lignin is composed of both trans-coniferyl and trans-sinapyl alcohol, and grass lignin is composed of trans-*p*-coumaryl alcohol. These precursors are joined together to form a polymeric macromolecule (Sarkanen *et al*. 1971), which is assumed to have an infinite molar mass in the living tree. An example of a proposed softwood lignin structure is shown in Figure 1.3.

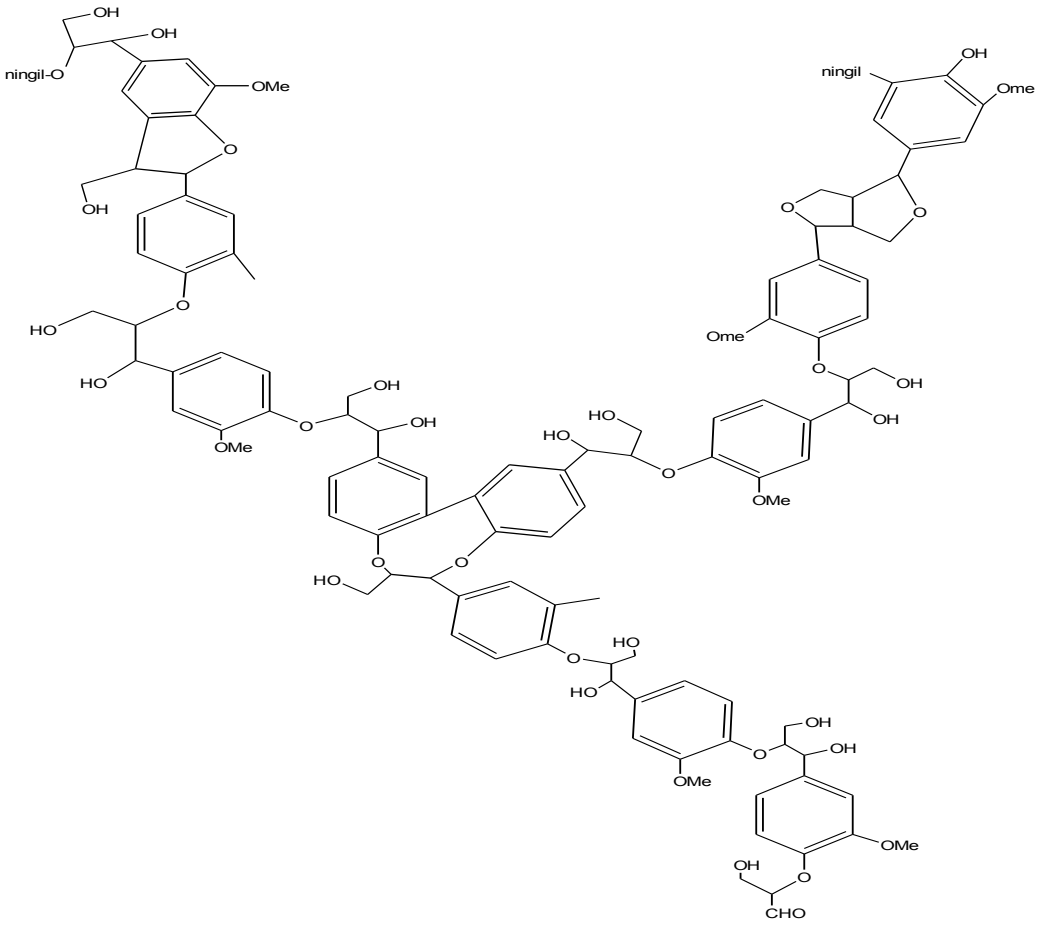

Figure 1.3. Model of softwood lignin structure.

1.2.3. Description of Indigenous Salt Catalyst

Magadi(kiswahili, Para(Luo) and Lebek(Kalenjin) are commonly used salts in several countries in East, West, and Central Africa and they are most commonly used salts in Kenyan homes (Mavura *et al*, 2010; Ankrah *et al*, 1978). The main use of these indigenous salts is cooking tough food materials such as beans and maize utilising its ability to fasten the softening and the digestive property of the food during cooking (Nielsen *et al*, 2010). In addition the salts are used as prophylactic agents and a feed supplement to cattle and goats, as reported by Mavura *et al*, 2010. Furthermore, in some places, magadi is ground with tobacco in the preparation of snuff (Ankrah *et al*, 1978).

In Eastern Rift Valley, magadi is formed, as crystals ($Na_2CO_3.NaHCO_3.2H_2O$), in the alkaline lakes due to chemical weathering of rock minerals and high evaporation of the lake waters (Nielsen *et al*, 2010). Magadi is also formed, as the so-called scooped magadi, on the surface soil formed due to capillary evaporation of soil water (Nielsen *et al*, 2010).

Analyses of both crystalline and scooped magadi from Nigeria and Ghana have shown that crystalline magadi consists essentially of trona mixed with minor contents of halite (NaCl). The scooped magadi is rich in trona but it also contains admixtures of quartz, clays, chlorides, and sulphates (Sodipo, 1993; Nielsen *et al*, 2010).

IR spectroscopy analysis of naturally occurring carbonates and hydrogen carbonates has shown that neutral H_2CO_3 presence is indicated by a band near 1730 cm^{-1} in the spectra for C= O stretching (Bruno et al. 1992). The vibrational spectrum of the HCO_3^- anion can be interpreted on the basis of a planar tetratomic group CO_2 (OH), which has a C_2 symmetry (Teleb, *et al*, 2004)). By referring to the IR transmission spectra of alkali metal bicarbonate solids, bands for free HCO_3- ion in aqueous solution (unavailable in the literature) were assigned as follows: stretching of C-OH at 1010 cm^{-1}, symmetric stretching of CO_2 at 1360 and 1310 cm^{-1}, asymmetric stretching of CO_2 at 1668 and 1605 cm^{-1}, and out-of-plane bending of CO_3 at 843 cm^{-1}. The rest of the fundamental vibration modes, symmetric in-plane bending of CO_3 and asymmetric in-plane bending of CO_3 should appear below 800 cm^{-1}, a spectral region which could not be observed (Teleb, *et al*, 2004), Bruno et al. 1992).

The FTIR difference spectra of uncomplexed Na_2CO_3 solutions at a pH ~11.2 in 1.0 M Na_2CO_3, of only about 10% of total C is in the form of HCO_3^-, exhibit asymmetric stretching at 1680 and very weakly at 1560 cm^{-1} (Teleb, *et al*, 2004). Since the dominant carbonate species in Na_2CO_3 solution is the free CO_3^{2-} anion, which has a trigonai planar symmetry ((Teleb, *et al*, 2004), the IR absorption bands at 1065, 887 and 1383 cm^{-1} assigned to the vibrational modes of symmetric stretching, out-of-plane bending and asymmetric stretching, respectively (Nakamoto, 1978, Teleb, *et al*, 2004, Bruno et al. 1992).

1.2.4. Chemical Treatment of Lignocelluloses

The catalytic degradation of natural polysaccharides to obtain glucose and other small sugars has long been studied. Most frequently, dilute or concentrated acid has been used to hydrolyze wooden material to glucose and pentoses (Ye *et al*., 2002). Sugars such as glucose (the monomer unit of cellulose) and xylose (a common monomer unit of hemicellulose) can be used as obtained, or converted into a wide variety of compounds (Jeffries, 2004). Historically, ethanol has been the most common target of

sugar degradation, though the obtainable products are quite diverse and have been extensively reviewed (Lange, 2007).

Unfortunately, polysaccharides can require harsh conditions to degrade. This is especially true for cellulose, which is largely crystalline and held together by a network of hydrogen bonds (Figures 1.4a and 1.4a b) (Mosier *et al*. 2005; Verendel *et al.,* 2011). Thus the conditions required to hydrolyze polysaccharides to sugars often degrade these same sugars (Bobleter, *et al.,* 2005, Abatzoglou *et al.,* 1998). This incompatibility between high feedstock stability and lower product stability has been a long-standing challenge for chemists and engineers, who have sought technical and chemical processes that operate under milder conditions, and thus produce higher yields of glucose and other sugars. Some progress toward this goal has been achieved.

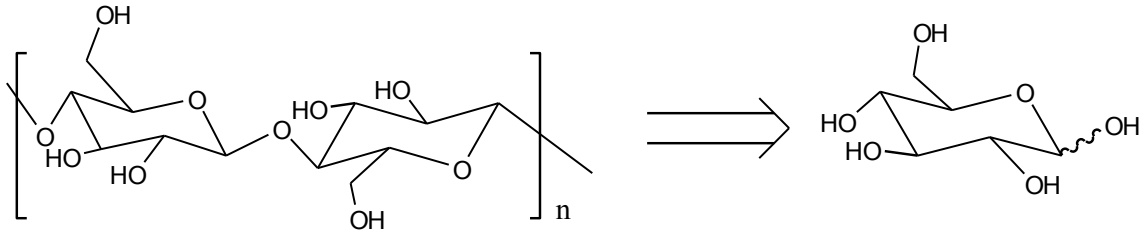

Figure 1.4. (a) Cellulose polymer of hydroglucose units bound via **β**-(1-4)-glycoside linkages.

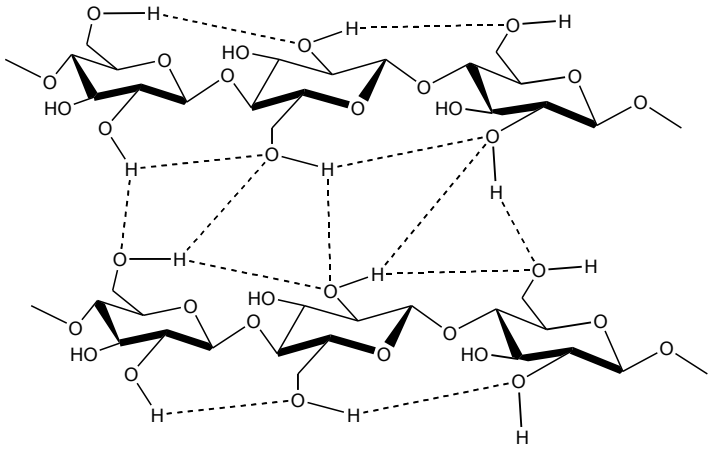

Figure 1.4. (b) A network of intra and inter-chain hydrogen bonds in cellulose molecules.

Today, biomass samples that contain both hemicellulose and cellulose are first subjected to a milder hydrolysis, which degrades hemicellulose, before the more resistant cellulose is hydrolyzed under harsher conditions. This procedure improves the yields of pentoses (mainly xylose and arabinose) and hexoses (mainly mannose and

galactose) from hemicellulose, which is a branched, homogeneous polymer Figure 1.5, (Wyman *et al.,* 2005; Nevell, 1985).

Additionally, pretreatments that decrease cellulose crystallinity and increase its surface area and porosity, such as ball-milling (Krässig, 1993) or steam explosion, (Nevell, 1985) are often used to permit lower operation temperatures, thereby minimizing glucose decomposition. Nevertheless, the hydrolysis of cellulose to glucose in high selectivity and conversion remains difficult.

During the course of efforts to degrade polysaccharides to monosaccharides for use as renewable sources of organic chemicals and fuels, a myriad of 'byproducts' have been identified; the character and amount of these byproducts depend on the conditions used for polysaccharide degradation (Verendel *et al*, 2011).

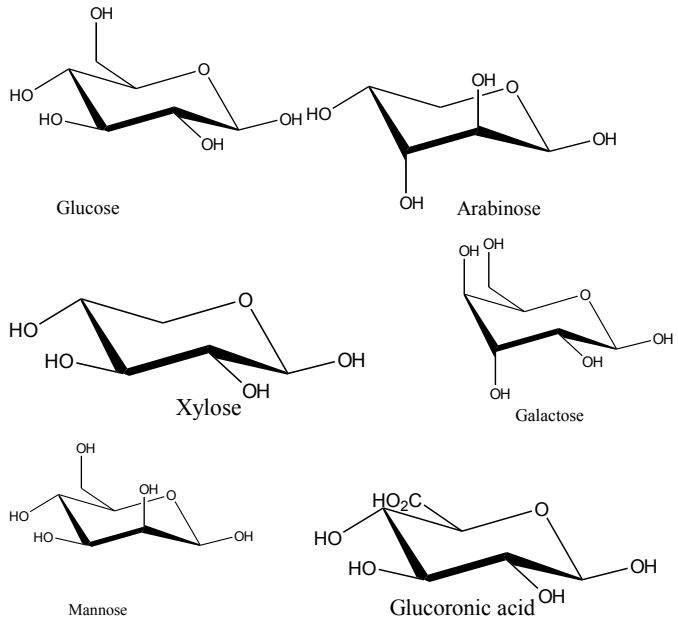

Figure 1.5: Components of a hemicelluloses

1.2.4.1. Acid-Catalyzed Processes (T < 250 °C)

1.2.4.1.1: Hydrolysis to Monosaccharides

Garves, 1979, demonstrated the solubilization of cellulose with concentrated Brønsted acids and this process works because the highly ionic solvent is able to penetrate and disrupt the hydrogen bonds between and within the cellulose polymer strands (Figure 1.5(b)). Additionally, strong acids can derivatize the hydroxyl groups along the cellulose chain, disrupting intermolecular hydrogen bonding and producing cellulose derivatives that are more reactive than cellulose itself (Abatzoglou *et al*, 1998).

The fate of cellulose (or its derivatives) dissolved in strong acid depends upon the reaction conditions and if the solution is diluted immediately after the cellulose is dissolved and the system is not heated, very little chain-breaking occurs and cellulose is regenerated (Bienkowski *et al*, 1984). On the other hand, diluting the strongly acidic solution with water and heating to ~100 °C for a few hours yields glucose, typically in >80% yield (Durawalla *et al*, 1962). This process is commonly denoted 'concentrated acid hydrolysis' or 'homogeneous acid hydrolysis', and is shown in Figure1.6. The acid-catalyzed hydrolysis of polysaccharides is a specific acid- catalyzed process, so the particular acid used is irrelevant; the pH alone determines the rate of hydrolysis (Bienkowski *et al*, 1984).

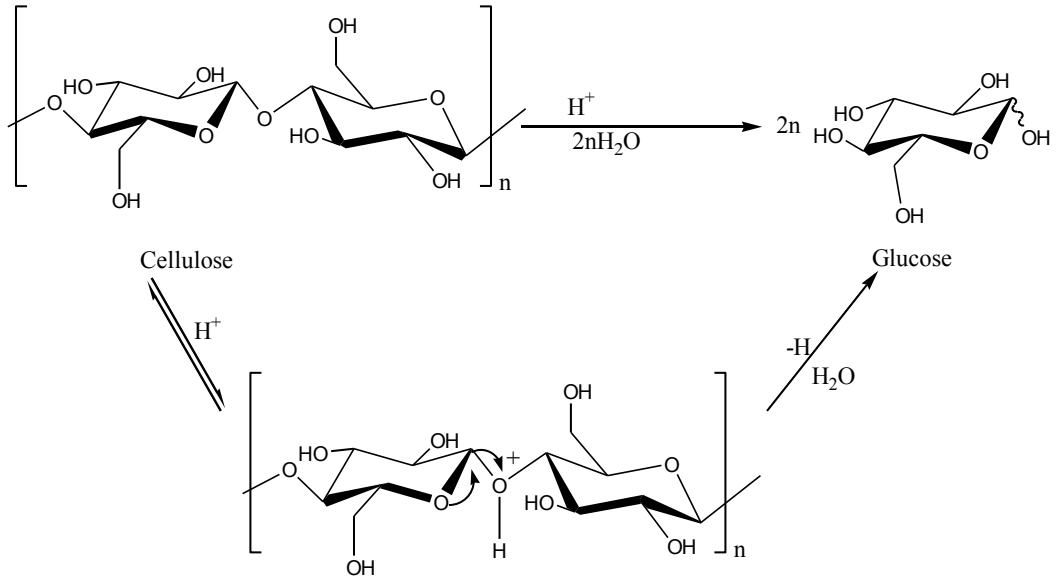

Figure 1.6. A scheme of acid-catalyzed hydrolysis of cellulose to glucose

Sulfuric acid was traditionally chosen to mediate cellulose hydrolysis because of its low cost and strong acidity (Garves, 1979). The dissolution of cellulose in aqueous sulfuric acid is fast even at room temperature when the acid concentration is ≥ 65% w/v and the acid-to-cellulose ratio is >2 mL/g (Lee, 2008; Lange, 2007). The hydrolysis of solubilized cellulose is rapid at temperatures between 50 and 100 °C and results in very little degradation of the formed monosaccharides because of the low temperature (Lee, 2008).

Despite their selective nature, concentrated acid processes suffer from several serious disadvantages: the acids are corrosive, and the waste products contain large amounts of acids that must be neutralized (Lee, 2008; Iuliana, 2009). These issues prevented the

concentrated acid hydrolysis of polysaccharides from being extensively industrialized, even though it offers access to very high sugar yields (Lee, 2008).

Dilute acid solutions can also degrade polysaccharides, though high temperatures must be used to compensate for the weaker acid solutions (Lee, 2008). This comes at the cost of selectivity, as monosaccharides are not stable at high temperatures, especially not under acidic conditions. The dilute acid hydrolysis of cellulose is usually performed between 150 and 250 °C and using ~1 wt% of a strong, inorganic Brønsted acid (Lee, 2008).

Weak acids such as acetic and carbonic acids have been used, but yielded inferior results because they, as weaker acids, are less suitable for specific-acid-catalyzed processes (such as cellulose hydrolysis) where the reaction rate is directly correlated to the pH (Lee, 2008). Cellulose is not significantly dissolved in dilute acid at temperatures below 250 °C, so the degradation seen in these reactions is attributed to a combination of the rather fast diffusion of water and protons into amorphous parts of the cellulose, and slower reactions on the crystal surface that liberate soluble glucose and oligosaccharides (Lin *et al.*, 1993). Consequently, the degree of polymerization of cellulose declines in the early stages of dilute acid hydrolysis, but gradually levels off as the fast hydrolysis of the sensitive amorphous regions is completed and is followed by slow erosion at the surface of the inert crystallites, (Marchessault *et al.*, 1959) There are also indications that both chemical modifications of the solid cellulose and the formation of unreactive oligosaccharides occur under these conditions, further lowering the monomer yield and prohibiting full conversion of poly- and oligosaccharides to monomers (Figure 1.7) (Lai, 2001; Lai *et al*., 1967). Hence, in most reports, the decrease in degree of polymerization diminishes quickly at first, but eventually becomes relatively constant when the system consists of glucose, soluble oligosaccharides and insoluble crystalline cellulose (Katzen *et al.*, 1995; MacDonald et al., 1983).

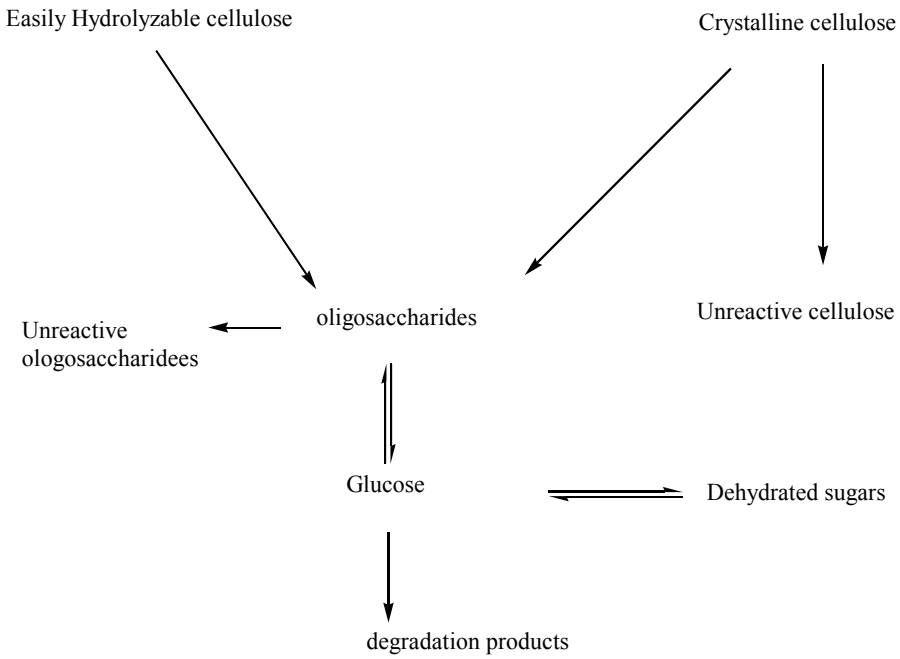

Figure 1.7: A scheme of a Model for the dilute acid hydrolysis of cellulose.

Despite problems in achieving complete cellulose degradation from dilute-acid hydrolysis, the most serious drawback of the process is that the desired products are unstable to the reaction conditions. At 200 °C, for example, the hydrolysis of oligosaccharides is only acid-catalyzed for pH < 4 (Verendel *et al.,* 2011). Under these conditions, the pentoses and hexoses formed from the degradation of lignocellulosic material decompose quickly (Verendel *et al.,* 2011). Thus the maximum yield of glucose from dilute acid cellulose hydrolysis was long considered to be ca. 60–70%. The quick decomposition of monosaccharides in hot acid and the desire to obtain high yields of monosaccharides, which are essentially reaction intermediates under these conditions, prompted the development of several modified processes over the past century. For example, cellulose pretreatment can be used to shorten the hydrolysis time and therefore improve glucose stability (Fan, *et al.*, 1982; Fan, *et al*., 1987 ; Gustavsson, & Al-Dajani 2000).

Additionally, multi-step hydrolysis processes have been used to improve the total sugar yields from lignocellulose. In the first step, hemicellulose and easily hydrolyzable cellulose regions are hydrolyzed and the products collected; the more robust crystalline cellulose is then hydrolyzed under more extreme conditions (Kim & Holtzapple, 2006; Verendel *et al.,* 2011). Hemicellulosic sugars can be produced selectively from lignocellulosic material by dilute acid hydrolysis. Under carefully controlled

conditions, high yields of xylose and other hemicellulosic monosaccharides can be obtained without significantly altering the cellulosic fraction (Wyman *et al.***,** 2005).

1.2.4.1.2. Decomposition of Sugars in Hot, Dilute Acid

The degradation of glucose itself in hot, dilute acid has seen significant research, and occurs primarily by dehydration (Wyman *et al.,* 2005). The main degradation product under these conditions is HMF, which can be formed both from glucose and from its isomer, fructose (Figure 1.8). The formation of hydroxymethylfuran (HMF) from fructose and glucose involves three consecutive dehydration steps and is acid catalyzed (Wood *et al.,* 1989). Just as HMF is formed from glucose, furfural is a main degradation product of pentoses such as xylose and arabinose in acidic solution (Wickholm *et al*., 2001; Kim & Holtzapple, 2006). Consequently, the acidic degradation of hemicellulose, a more chemically diverse compound than cellulose, generates xylose, arabinose, and furfural, as well as acetic acid from the acetyl ester groups that are also present (Popa *et al.* 1998). Furfural formation from xylose also occurs via dehydration (Ruigang *et al*. 2005), and its rate increases with lower pH and higher temperature (Wickholm *et al*., 2001).

Thus, HMF and furfural are often byproducts of polysaccharide hydrolysis in acid (Wickholm *et al*., 2001; Verendel *et al.,* 2011). High yields of hemicellulosic monosaccharides can be obtained from lignocellulosic material by dilute acid hydrolysis because hemicellulose can be hydrolyzed under milder conditions, thus avoiding furfural formation (Harris, et al., (1984).

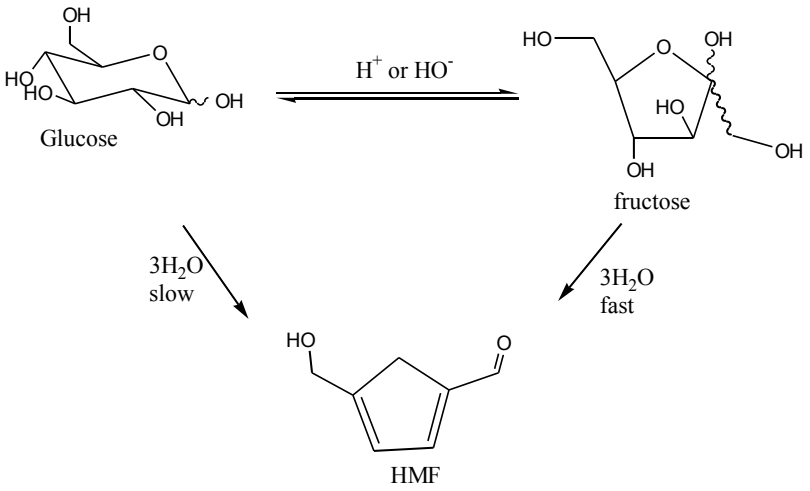

Figure 1.8. A scheme for the formation of HMF from glucose and fructose.

The degradation of glucose during the dilute acid hydrolysis of cellulose is summarized in Figure 1.9.

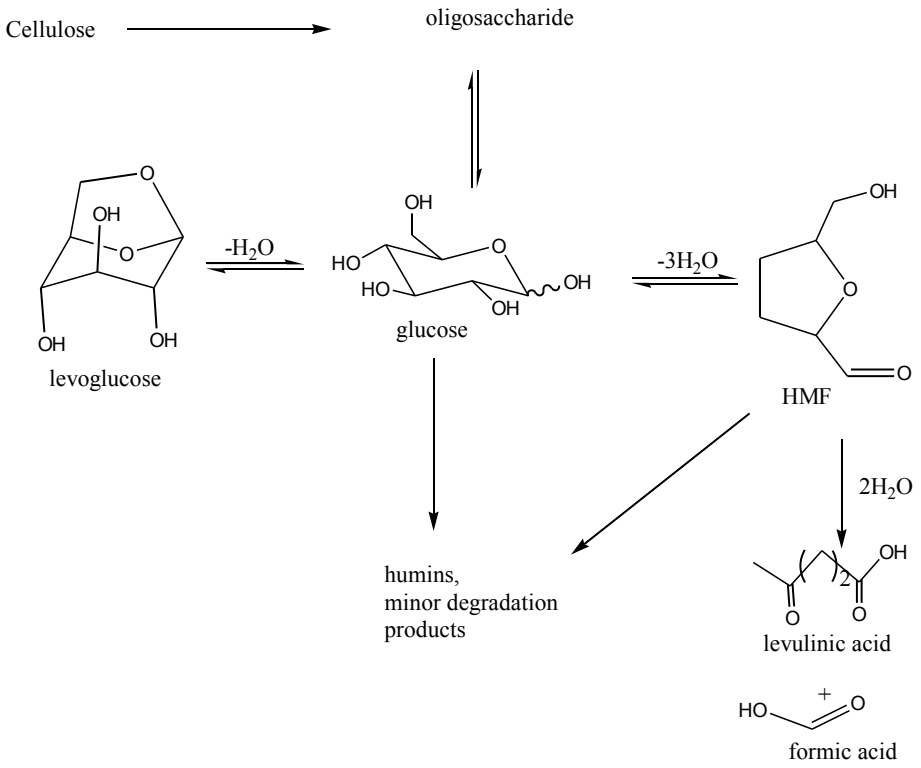

Figure 1.9: A scheme of Major pathways for glucose degradation in dilute acid hydrolysis of cellulose.

1.2.4.2. Alkaline Hydrolysis (T < 250 °C)

1.2.4.2.1. Background

Cellulose swells in concentrated aqueous base ($[HO^-]$ > 2.0M), making it more accessible for hydrolysis, and alkaline cellulose hydrolysis has half the activation energy of acidic cellulose degradation (Verendel *et al.,* 2011). The challenge, however, lies in producing a single (or strongly predominant) compound from the reaction (Werpy, & Petersen, 2004). Although some work has aimed to manipulate the reaction to improve its selectivity, most studies have sought to identify the products of alkaline polysaccharide decomposition and to understand their formation (Verendel *et al.,* 2011; Werpy & Petersen, (2004).

Below 170 °C and under an inert atmosphere, the aqueous alkaline hydrolysis of cellulose is dominated by 'peeling', the successive removal of monomer units from the end of the carbohydrate polymer (Ruigang *et al*. 2005). This reaction produces one

equivalent of a saccharinic acid, most often D-glucoisosaccharinic acid (4H, Figure 1.10) for each glucose monomer that is depolymerized, and occurs even at ambient temperature, albeit slowly (Verendel *et al.,* 2011).

Between 170 and 250 °C, the situation is similar, except that β-(1→4)-glycosidic bonds, which link the anhydroglucose monomers in cellulose, are randomly cleaved as well. This reaction, caused by hydroxide ion attack at β-(1→4)-glycosidic bonds (Xiang, *et al*,.2004) Verendel *et al.,* 2011), Results in a rapid decrease in the degree of cellulose polymerization, (Ruigang *et al*. 2005) and increases the number of end groups present, thus allowing new chains to begin peeling. Conversions are therefore higher for alkaline cellulose hydrolyses carried out above 170 °C (Xiang, *et al*., 2004).

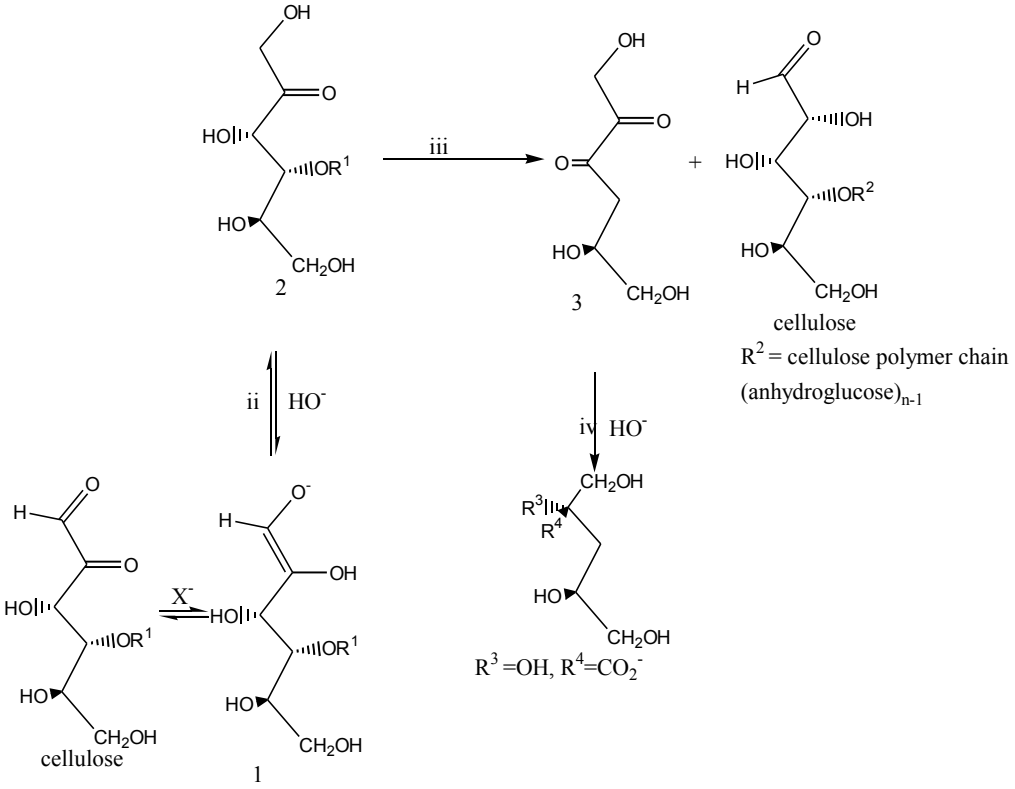

Figure 1.10. A scheme of primary mechanisms of cellulose peeling in alkaline solution.

1.2.4.2.2. Mechanisms

Some bases can also be used for pretreatment of lignocellulosic materials and the effect of alkaline pretreatment depends on the lignin content of the materials (Katzen *et al*. 1995; Anne *et al*., 2000). The mechanism of alkaline hydrolysis is believed to be saponification of intermolecular ester bonds cross linking xylan hemicelluloses and other components, for example, lignin and other hemicellulose (Mosier *et al*., 2005).

The porosity of the lignocellulosic materials increases with the removal of the cross links. Dilute NaOH treatment of lignocellulosic materials caused swelling, leading to an increase in internal surface area, a decrease in the degree of polymerization, a decrease in crystallinity, separation of structural linkages between lignin and carbohydrates, and disruption of the lignin structure (Yi, *et al*., 2009; Verendel *et al.,* 2011).

Alkaline treatment causes lignocellulosic materials to swell; increased swelling leads to higher susceptibility of cellulose to saccharification (Yi, *et al*., 2009). In the presence of alkaline chemicals (NaOH or NH_3OH), cellulose, hemicellulose, and lignin bonds can be disrupted, which permits cellulose to swell beyond normal water-swollen stages (Anne *et al*., 2000). Consequently, the pore size, intraparticle porosity, and capillary size are increased. There is also a phase change in the cellulose crystalline structure. Sodium hydroxide is the most commonly used chemical in the treatment of lignocellulose. It is a strong swelling agent for cellulose. The amount of NaOH used for treatment ranges from 2 to 20%, and the temperature for the treatment ranges from ambient to 120^0C. Under mild conditions (low concentration and low temperature) substrate components remain unchanged. Under harsher conditions, most of the lignin and hemicellulose are solubilized (Gentile *et al*., 1987; Verendel *et al*., 2011).

Alkali pretreatment reduces the lignin and hemicelluloses content in biomass, increases the surface area, allowing penetration of water molecules to the inner layers, and breaks the bonds between hemicellulose and lignincarbohydrate (Yi *et al*., 2009; Verendel *et al*., 2011). Dilute NaOH is usually used for alkali pretreatment. Considering economic and environmental aspects, dilute NaOH treatment would be much more suitable than the concentrated NaOH pretreatment (Lai, 2001). Combination of dilute NaOH treatment and other treatments seems more efficient (Yi, *et al*., 2009).

Alkali pretreatment may be carried out at ambient conditions, but pretreatment time is measured in terms of hours or days rather than minutes or seconds (Iuliana *et al*., 2009). Unlike acid-catalyzed pretreatments, a limitation occurs because some of the alkali is converted to irrecoverable salts or incorporated as salts into the biomass by the pretreatment reactions (Verendel *et al*., 2011). The process of lime pretreatment involves slurring the lime with water, spraying it onto the biomass material, and storing

the material in a pile for a period of hours to weeks. The particle size of the biomass is typically 10 mm or less. Elevated temperatures reduce contact time (Katzen *et al*., 1995).

1.2.5. Producing Cellulosic Ethanol

In general, the bioconversion of cellulose to ethanol requires three major processing steps: pretreatment, saccharification and fermentation. In order to use woody wastes, it is first necessary to break down the "woody compounds" into fermentable sugars. This has taken the form of a number of different pretreatment (pre-fermentation) strategies. Pretreatment requirements vary with the feedstock and are often substantially less in the case of various paper and hydrolysis waste streams.

1.2.5.1. Pretreatment of Raw Materials.

Pretreatment is an essential step for bioconversion of most lignocellulosic materials. Roughly two-thirds of the lignocellulosic materials is present as cellulose and hemicellulose (the two main components of plants that give them structure), and lignin makes up the bulk of the remaining dry mass (Ruigang *et al*., 2005). To efficiently and economically produce cellulosic ethanol, the complex polymeric structures must be separated into fermentable sugars. The sugars in cellulose and hemicellulose are locked in complex carbohydrates called polysaccharides (long chains of monosaccharides, or simple sugars). Pretreatment breaks apart the structure of biomass to allow for the efficient, effective hydrolysis of cellulosic sugars, but this involves extremely complex chemical engineering. Usually the systems use processes to disrupt the hemicellulose/lignin sheath that surrounds the cellulose in plant material (Mes-Hertree *et al*., 1998).

Pretreatments maximize subsequent bioconversion yields and minimize the formation of inhibitory compounds (Klinke *et al*., 2004). They include acid hydrolysis (a controlled breakdown using dilute acid), alkali treatment, ammonia fiber explosion (liquid ammonia under moderate heat and pressure to separate the biomass components), autohydrolysis, chemical pulping, heat, mechanical size reduction, solvent extraction, steam, steam explosion, weak acid hydrolysis and various combinations of these separate processes. These technologies have different strategies for accessing the cellulose and hemicellulose and then dealing with the lignin, smaller

amounts of other proteins, lipids (fats, waxes and oils) and ash (Yang, & Wyman, 2008).

Katzen, *et al*., 1995 reported that, acid hydrolysis is often used as a pretreatment because it can be adapted to a wide variety of feedstocks. Except for strong hydrochloric acid hydrolysis, at higher acid concentrations, it can be carried out at temperatures as low as 30 degrees centigrade. Generally an inexpensive process, acid hydrolysis may also produce large quantities of degradation byproducts and undesirable compounds that inhibit other areas of cellulosic processing (Klinke, *et al*., 2004).

Autohydrolysis is the process of converting lignocellulose into fermentable sugars by exposure to high-temperature steam. Many lignocellulosic materials contain significant quantities of acetylated hemicellulose. Steam releases these in the form of acetic acid, which subsequently carries out a partial hydrolysis of the hemicellulosic and cellulosic sugars (Mes-Hartree, *et al.*, 1988). The principal disadvantage of this approach is that sugar yields are generally very low.

In a laboratory study, enzymatic hydrolysis of maize stover pretreated with Bayer Process Sand (BPS) which is a waste with high alkalinity generated in aluminum production was compared with that of maize stover pretreated with sodium hydroxide. After pretreatment at a BPS loading of 0.093 g NaOH equiv/g maize stover, a temperature of 35 °C for 24 h, and at a cellulase enzyme loading of 15 FPU/g glucan, 95.6% of the total amount of glucan and xylan contained in the pretreated maize stover was enzymatically hydrolyzed. In combination of the pretreatment and enzymatic hydrolysis stages, 93.2% of the glucan and 94.5% of the xylan were converted into glucose and xylose, respectively, similar to those obtained by means of sodium hydroxide pretreatment. These results suggest that BPS should replace sodium hydroxide for the pretreatment of maize Stover (Michael *et al*., 2009). Corn cobs and stover were found to be particularly well suited to pretreatment by hemicellulose hydrolysis (Torget *et al*., 1991).

1.2.5.2: Hydrolysis Methods to Produce Cellulosic Ethanol

Cellulose hydrolysis is the process of turning polymeric lignocellulosic materials into fermentable sugars and can be accomplished by a number of processes including acid, alkaline, alkaline-oxidation, solvent and enzymatic hydrolysis.

$$\ldots[\text{-}C_6H_9O_5\text{-}]_n \quad + \quad H^+ / OH^- \quad \rightarrow \quad nC_6H_{12}O_6$$

Cellulose hydrolytic catalyst Glucose

Acid hydrolysis and enzymatic hydrolysis are currently the main two processes used to create fermentable sugars from cellulosic biomass. Acid hydrolysis processing breaks down the complex carbohydrates into simple sugars. Enzymatic hydrolysis processing uses a complex pretreatment processing stage to reduce the size of the material, making it more efficient than acid hydrolysis (Ohgren *et al*. 2006). In both processes, enzymes are used to convert the cellulosic biomass into fermentable sugars and then microbial fermentation is used to produce ethanol. As with current corn-based systems, carbon dioxide is produced as a co-product in this final stage of production.

Ohgren, *et al*. 2006 documented that, dilute acid hydrolysis with 1 percent to 5 percent sulfuric acid is generally considered the most cost-effective means of hydrolysing wood and agricultural residues. Yields of hemicellulosic sugars can be 80 percent to 95 percent of theoretical. Yields of glucose from cellulose are generally less than 50 percent but can approach 55 percent at elevated temperatures (Schell, *et al*., 2003).

Strong acid hydrolysis, often using a concentrated form of sulfuric acid, usually separates and recycles the acid catalyst, limiting the total acid losses to approximately 3 percent, or the same as the dilute process. Use of the concentrated acid, however, allows lower temperature and pressure hydrolysis with fewer byproducts produced (Schell, *et al*., 2003). Concentrated hydrochloric acid at a concentration of about 47 percent is sometimes used for strong acid hydrolysis because it is relatively easy to recover. Hydrolysis with concentrated hydrochloric acid gives one of the highest sugar yields of any acid hydrolysis process. It is carried out at room temperature. The chief drawback is that it is highly corrosive, volatile, expensive and almost complete recovery is essential to make the process economical (Thring R W and Chorent E, 1990).

Alkali pretreatment processes utilize lower temperatures and pressures compared to other pretreatment technologies. Alkali pretreatment may be carried out at ambient conditions, but pretreatment time is measured in terms of hours or days rather than minutes or seconds. Unlike acid-catalyzed pretreatments, a limitation occurs because some of the alkali is converted to irrecoverable salts or incorporated as salts into the

biomass by the pretreatment reactions. Lime has been used to pretreat wheat straw (85 ^{0}C for 3 h), poplar wood (150 ^{0}C for 6 h with 14-atm oxygen), switchgrass (100 ^{0}C for 2 h), and corn stover at 100 ^{0}C for 13 h, (Karr, 1998 and Holtzapple, 2000).

Azzam, (2009) treated sugarcane bagasse with lime at ambient conditions for up to 192 h to improve the enzyme digestibility of the cellulose from 20% before pretreatment to 72% after pretreatment. Higher temperatures and shorter reactions times were also shown to effectively pretreat lignocellulose with lime. Chang *et al*. (1998), obtained similar digestibility results by pretreating bagasse with lime at 120 ^{0}C for 1 h. Other alkali pretreatments use sodium, potassium, calcium, and ammonium hydroxide as reactants. Sodium hydroxide has received the most attention (Sharples, 1957; MacDonald *et al*., 1983). Lime (calcium hydroxide) has the additional benefits of low reagent cost and safety and being recoverable from water as insoluble calcium carbonate by reaction with carbon dioxide. The carbonate can then be converted to lime using established lime kiln technology (Michael *et al*., 2009).

The addition of air/oxygen to the reaction mixture greatly improves the delignification of the biomass, especially highly lignified materials such as poplar (Holtzapple, 2000). Oxidative lime pretreatment of poplar wood at 150 ^{0}C for 6 h removed 77.5% of the lignin from the wood chips and improved the yield of glucose from enzymatic hydrolysis from 7% (untreated) to 77% (treated) compared to the untreated and pretreated poplar wood (Karr *et al*., 2000). The process of lime pretreatment involves slurrying the lime with water, spraying it onto the biomass material, and storing the material in a pile for a period of hours to weeks. The particle size of the biomass is typically 10 mm or less. Elevated temperatures reduce contact time (i.e., 3 h at 85 ^{0}C for wheat straw and 13 h at 100 ^{0}C for corn stover) (Karr *et al*., 2000).

Alkali pretreatment technologies, including lime pretreatment, are rather similar to the Kraft paper pulping technology. The major effect of the alkaline pretreatment is the removal of lignin from the biomass, thus improving the reactivity of the remaining polysaccharides (Holtzapple, 2000; Richards, 1971). Alkali pretreatments remove acetyl and the various uronic acid substitutions on hemicellulose that lower the accessibility of the enzyme to the hemicellulose and cellulose surface ((Schell, *et al*., 2003); Holtzapple, 2000). For lesser ligninified materials such as corn stover, the

addition of oxygen appears to only marginally improve the digestibility of pretreated corn stover with lime at a 1:0.075 ratio (Stover: lime) at 120 ^{0}C for times up to 6 h, (Karr, 1998 and Holtzapple, 2000). Corn stover pretreated for the optimal time (4 h) at 120 ^{0}C loses 32% of the lignin. Hydrolysis yielded 88% of the cellulose as glucose after 7 days at an enzyme loading of 25 FPU per gram of biomass (Karr *et al*., 2000).

Azzam, 2009, showed that cane bagasse with alkaline hydrogen peroxide greatly enhances its susceptibility to enzymatic cellulolysis and thus the ethanol production from it and results obtained showed, that about 50% of lignin and most of hemicellulose content was solubilized. A 2% Alkaline hydrogen peroxide at 30^{0}C within 8 h while the cellulose content was consequently increased from 42% in the original cane bagasse to 75% in the oxidized pulp and saccharification of this pulp residue with cellulase from Trichorderma viride at 45^{0}C for 24 h, yielded glucose with 95% efficiency (Richards, 1971). The efficiency of ethanol production from the insoluble fraction with S. cervisiae was 90% compared to about 50% for untreated cane bagasse (Azzam, 2009).

Anne *et al*., 2000 in their research found that, under wet alkaline oxidation conditions; the fermentation inhibitors (furfural and hydroxymethyl-furfural) were not produced or even to be stable in such a system. Furthermore, they described a new consideration of the wet alkaline oxidation process resulting in a hemicellulose fraction directly substantial for microbial growth. The fungus, ***aspergilus*** *niger,* a known producer of cellulases and hemicellulases, should be able to use the process water with dissolved hemicellulose as a carbon source when no inhibitors are generated (Sues, *et al*., 2002; Anne *et al*., 2000). Thus, the usefulness of the filtrates as fermentation substrates could be evaluated. This was found to improve the ethanol yields drastically since the process provides enzymes cellulase and hemicellulase (Anne *et al*., 2000).

1.2.5.3: Potential Feed Stocks for Cellulosic Ethanol Synthesis

Cellulosic ethanol can be produced from a wide variety of cellulosic biomass feedstocks (Mohammad, et al., 2007). These include agricultural plant wastes (corn stover, cereal straws, and sugarcane bagasse), plant wastes from industrial processes (sawdust, paper pulp, distiller's grains) and energy crops grown specifically for fuel production, such as switchgrass (Katzen *et al*., 1995 and Perlack, *et al*. 2005). Growing

energy crops and harvesting agricultural residuals are projected to increase the value of farm crops, potentially eliminating the need for some agricultural subsidies (Taherzadeh et al., 1997). Perennial grasses, such as switchgrass and miscanthus, have been discussed as promising feedstocks for cellulosic ethanol production and they use water efficiently and do not need a lot of fertilizers or pesticides (Lynd, 1996 and Salmén and Olsson, 1998). In the case of agricultural residues, particle size reduction can often be done simply with grinding (Salmén and Olsson, 1998). For dry corn or soybean residues, density is often lower than what is desirable, and particle size reduction is not an issue (Taherzadeh et al., 1997). Municipal solid wastes present a particular problem because of their extremely heterogeneous nature. The dominant sugar in hemicellulose derived from crop residues is usually xylose (Karimi et al., 2006; Taherzadeh et al., 1997).

Kosaric et al., (1983) in their research found that the properties of the substrate can affect the hydrolysis. These properties were found to be: neutralizing capacity, proportion of easily hydrolysable hemicellulose and cellulose. Similarly the amount and rate of hydrolysis of the difficult-to-hydrolyze materials, the length of the macromolecules, degree of polymerization of cellulose, configuration of the cellulose chain, and association of cellulose with other protective polymeric structures within the plant cell wall such as lignin, pectin, hemicellulose, proteins, mineral elements (Richards, 1971). Another parameter affecting the hydrolysis is the acidity of the system. The acidity is dependent on the amount of acid (e.g. acetic acid) released from the biomass during hydrolysis, liquid to solid ratio, the neutralizing capacity of the lignocellulose, and movement of the solution during heating (Kosaric et al., 1983). It has been shown that the diffusivity of sulfuric acid is significantly higher in agricultural residues than in hardwood (Kim and Lee 2002).

The rate of decomposition of the products during the hydrolysis process depends on temperature, acidity, reaction time, and the concentration of sugars (Mohammad, et al., 2007). Under hydrolysis conditions that produce a solution containing in excess of 10 percent glucose, reversion phenomena are suggested to be very important. The reversion phenomena result in much of the glucose being present not as free glucose but as dimers, oligomers, and anhydrosugars which are unavailable to the microorganisms used in fermentation (Harris et al., 1984).

It was recently reported that metals and/or metal ions can also catalyze glucose decomposition during the acid hydrolysis of lignocellulosic materials. (Xiang et al., 2004). Over the course of the reaction, the formed organic acids lower the reaction pH to 3–4. (Harris et al., 1984) This was due the decomposition of glucose under reflux yielding both acid- and base-catalysis products even in the presence of 0.02 M sulfuric acid or sodium hydroxide (Mohammad, et al., 2007).

1.2.5.4. Ethanol Fermentation

In ethanol fermentation, one glucose molecule breaks down into two pyruvates (Polakowski, *et al*., 2008). The energy from this exothermic reaction is used to bind inorganic phosphates to ADP and convert NAD+ to NADH. The two pyruvates are then broken down into two acetaldehydes and give off two CO_2 as a waste product. The two acetaldehydes are then converted to two ethanol by using the H- ions from NADH; converting NADH back into NAD+ (Tony *et at*., 1989).

Alcoholic fermentation, also referred to as ethanol fermentation, is a biological process in which elements such as glucose, fructose, and sucrose are converted into cellular energy and thereby produce ethanol and carbon dioxide as metabolic waste products. Because yeasts perform this conversion in the absence of oxygen, alcoholic fermentation is considered an anaerobic process (Tony *et at*., 1989).

1.2.5.5. Biochemical Process of Fermentation of Glucose and Sucrose

The chemical equations below summarize the fermentation of sucrose ($C_{12}H_{22}O_{11}$) into ethanol (C_2H_5OH). Alcoholic fermentation converts one mole of sucrose into four moles of ethanol and four moles of carbon dioxide, producing two moles of ATP in the process (Tony *et at*., 1989)

The overall chemical formula for alcoholic fermentation is:

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

Sucrose is a dimer of glucose and fructose molecules. In the first step of alcoholic fermentation, the enzyme invertase cleaves the glycosidic linkage between the glucose and fructose molecules.

$$C_{12}H_{22}O_{11} + H_2O + \text{invertase} \rightarrow 2\ C_6H_{12}O_6$$

Next, each glucose molecule is broken down into two pyruvate molecules in a process known as glycolysis. Glycolysis is summarized by the equation:

$C_6H_{12}O_6 + 2\ ADP + 2\ P_i + 2\ NAD^+ \rightarrow 2\ CH_3COCOO^- + 2\ ATP + 2\ NADH + 2\ H_2O + 2\ H^+$

The chemical formula of pyruvate is CH_3COCOO^-. P_i stands for the inorganic phosphate. As shown by the reaction equation, glycolysis causes the reduction of two molecules of NAD^+ to NADH. Two ADP molecules are also converted to two ATP and two water molecules via substrate-level phosphorylation (Polakowski, *et al*., 2008).

1.2.5.6. Economic Importance of Cellulosic Ethanol

Agricultural wastes, agricultural residue material that is ploughed into the soil for fertilizer, composted, burned or disposed of in landfills, are more abundant and contain greater potential energy than simple starches and sugars (Lynd, 1996; Farrell, *et al*., 2006). Additionally, the collection, transportation and perhaps processing of these residues would present farmers and agricultural services providers with another lucrative crop-based profit center (Lynd, 1996).

The problem is that the complex chemical and biological engineering needed to optimally convert (in a manner suitable for profitable, large-scale fuel production) a single homogeneous feedstock, for example, wheat straw, may be very different from another homogeneous feedstock, for example, corn stalks (Kosaric et al., 1983). Lynd, 1996 said that, cellulosic conversion to a specific feedstock could be a great solution for taking some waste materials from a landfill and converting them to fuel and usable co-products. This “bio-remediation” of an industrial waste stream has the potential to eliminate a costly expense for a company and turns it into a new profit center (REN21, 2009).

CHAPTER TWO

2.0. MATERIALS AND METHODS

2.1. Sample Collection and Pretreatment

2.1.1. Plant Biomass

Wheat straw and Gallants soldier samples were collected from several places in Kenya where they are locally available. Six regions were selected randomly according to the availability of raw materials whereby three regions represent each raw material. Wheat straw was collected from Narok, Nakuru, and Eldoret while Gallants soldier was collected from Narok, Kisii and Eldoret. Approximately three 100g of dry raw material was collected from each region and was packaged in plastic paper bags and some cases gunny bags, labeled well and taken to the laboratory for analysis. All the samples whether wet or dry were cut into small pieces (2.0 mm size) then shredded and ground into powder respectively. The shredded samples were shade dried then ground into powder. The ground samples were then stored in well corked and dried polystyrene plastic sample bottles.

2.1.2. Mineral Salts

Three (3) Salt samples of 500 g each from the regions where they occur were collected. Magadi samples from Lake Magadi, (n=3), Para samples from Kendu-bay at the shores of Lake Victoria (n=3), Lebek samples from Kerio-valley (n=3) and market places (n=3) were collected, packaged dry in paper bags and taken to the laboratory.

In the laboratory each sample were crushed and homogenized in a mortar. About 10.00-20.00 g of each salt sample was oven dried in a 100.00 mL beaker at a temperature of 110 ^{o}C for 2.0 hours. Another portion of about10.00-20.00 g of each salt sample fresh (not oven dried) was taken and stored in plastic bottles. Oven dried samples were to be used for metallic ion and anionic ion analyses and pH measurements, while the non-oven dried were to be used for anionic ion analysis and pH measurements.

2.2. Chemicals and Reagents

2.2.1. Chemicals

The chemicals used in this research were of analytical grade. Acids: sulphuric acid, hydrochloric acid, acetic acid, formic acid, nitric acid and peroxy-acetic acid. Bases: Sodium hydroxide, and ammonia solution. Inorganic salts: Lead nitrate, Copper nitrate,

Zinc nitrate, Ammonium ferrous nitrate, Cobalt nitrate, Nickel nitrate, Potassium permanganate, Hydrogen peroxide, Sodium hypochlorite, and Chromium sulphate hydrate.

Sugar Standards; Pure saccharide samples of D(-)-arabinose, D(-)-ribose, D(+)-xylose, D(+)-galactose, D(+)-glucose, D(+)-mannose, D(-)-fructose, D(+)-sucrose, D(+)-maltose, and D(+)-lactose were used as standards (Aldrich Chemical Co.).

Other chemicals: Absolute ethanol, acetonitrile, methanol, acetone, distilled water, local yeast, boiling chips

2.2.2. Standard Reagents

Bennedicts solution, pH buffers of 4.0 and 7.0, methyl orange and phenolphthalein indicators

2.3. Apparatus and Instrumentation

2.3.1. Apparatus

Apparatus used; crucible with lid, test tubes, test-tube holder, pair of tongs, stirring rods, hot plates, beakers of ranging volumes 50 mL-500 mL, volumetric flasks of ranging volumes 10 mL-100 ml, Refluxing apparatus, fractional distillation apparatus, titration apparatus, fermentation tanks, TLC plates, filter papers; numbers 42 and 1 whatman type.

2.3.2. Instrumentation

Buck Scientific Model 210VGP Atomic Absorption Spectroscopy (AAS). Fourier Transform Infrared Spectroscopy (FTIR), High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC)-Flame ionization detector (FID), X-ray fluorescence (XRF) spectroscopy, Corning Flame 840 photometry.

2.4. Chemical Analysis of Salts and plant samples

2.4.1. Metallic Ions in Salts

A triplicate mass of 2.000 g of each of the salt sample was weighed and dissolved in 100.00 mL beaker with 50.0 mL of de-ionized water. The solution was then filtered using Whatman filter paper No. 42 as per the method developed by Makanjuola *et al*, (1975) and Sharp, (1970). The pH of the clear filtrate was measured after which the filtrate solution was acidified with 1.0 mL 0.1M HNO_3, placed into a 100.0 mL volumetric flask and filled to the mark with more de-ionized water. The solutions were corked and mixed

thoroughly and were ready for metallic ions analysis (Sharp, 1970). Buck Scientific Model 210VGP Atomic Absorption Spectroscopy and Corning Flame 840 photometry were used to analyze the metallic ion composition of the salts. The ions investigated were; metallic ions; $Na^{+,}$ K^{+}, Ca^{2+}, Mg^{2+}, Cd^{2+}, Pb^{2+}, Fe^{2+} Cu^{2+} and $Zn^{2+.}$

2.4.2. Non-Metallic Ion, and Structural Analysis of Salts

The pH of each filtrate of oven dried and non-oven dried salts were measured after which the filtrate solution of each treatment type was used analyze CO_3^{2-} and HCO_3^{-}. A triplicate mass of 0.700 g of each of the dried salt sample was weighed and dissolved in 100.00 mL beaker with 50.00 mL of de-ionized water. The solution was then filtered using Whatman filter paper No. 42. The CO_3^{2-} and HCO_3^{-} were analyzed by titrimetric method using 0.1M HCl solution (Sharp, 1970).

The carbonate (CO_3^{2-} and HCO_3^{-}) concentrations were measured using the end point titration method according to the standard methods where the end point of pH = 4.5 was used. An aliquot of 5.00 mL of the magadi solutions (2.00 g/100.0 mL) was diluted to 50.0 mL and titrated automatically with 0.1 N HCl (Sodipo, 1993; Nielsen *et al*, 2010).

2.4.3: Determination of Metal Ions in Plant Samples

Residues were weighed (2.000 g), put in 250 mL conical flasks. 30 mL concentrated nitric acid was added then soaked overnight (avoid foaming When digesting, it also shorten the digestion period to only 1 hour from 2 hours. After which they were digested to dryness in a hood chamber using a hot plate. It was done until all the solids dissolve. It was cooled and distilled water added then filtered [weight of the filter paper should be known] into 100 mL volumetric flask, and filled to the mark. Analysis of the filtrate was done using AAS and flame photometer for metals; K, Na, Li, Ca, Mg, Co, Cu, Ba, Fe, Zn, Cd, Pb, Mn, Al.

2.4.4. Preparation of 1000 ppm of Standard Stock Solutions

Standard stock solutions for each of the element to be determined was prepared. The procedure done below for Cadmium was repeated for all the other elements.

Procedure for Cd^{2+} standard solution from $Cd(NO_3)_2$ salt is shown below;

It needed 1g Cd to dissolve in 1.0 litrre of distilled water. But Cd is in the form of $Cd(NO_3)_2$ which means.

112.4g Cd (RAM Cd) →308.47g $Cd(NO_3)_2$

1g Cd→?

= 308.47/112.4g $Cd(NO_3)_2$

= 2.7444g $Cd(NO_3)_2$ which is 99% pure.

To get 100% purity we need to weigh more as follows 2.7444x100/99 (purity) =2.7721 g $Cd(NO_3)_2$ all the other salts followed same method.

2.4.5. FTIR Profiling

Functional group characterization of the powdered oven dried indigenous salt samples was done using FTIR Spectrophotometer model FTIR 8400.

Solid sample preparation

Solid sample preparation method followed the Potassium bromide (KBr) method; Three (3) mg of powdered sample or extract were mixed with 200 mg KBr powder and pressed into a KBr disk which was then mounted on the sample holder in the FTIR spectrometer scan.

For the paste sample, Sample was smeared on the KBr disk and mounted on the FTIR sample holder and then to the sample cell holder in the FTIR spectrometer and the spectra was run. The spectra were recorded in the 4000 to 400 cm^{-1} range. Functions of smoothing, normalization and baseline correction were carried out to obtain the first derivative IR spectra. The characteristic peaks of the spectra were noted.

2.4.6. XRF Samples Preparation and Analyses Procedures for Salt.

Step1. Sample grinding and digestion

1. Ground and oven dried soil samples passed through a sieve of <100µM
2. A mass of between 0.3 and 0.5 g was weighed into a digestion vessel and 2.5 mL and 7.5 mL of concentrated HNO_3& HCL were added respectively.
3. The instrument temperature was set to 200 ^oC and samples were digested for 30 minutes.
4. The digested samples were transferred into a clean volumetric flask and topped up to 50 mL with double distilled water.

Step 2. Adding internal standard and spiking on the carrier

i. Ten (10 mL) of each sample was Pipetted into a clean vial.

ii. Twenty (20) µL of 1000 ppm Gallium (or any std) stock solution (as internal standard) was added resulting into a concentration of 2 ppm Ga in sample.
iii. Shaking for a minute to homogenize was done.
iv. Ten (10) µL of sample was pipetted using a micro-pipette onto a clean quartz carrier.
v. Dry carrier in an oven or hot plate to evaporate the liquid.

Step 3: Sample spectrum acquisition and quantitative analysis

a. S2 PICOFOX TXRF Spectrometer was used to acquire sample spectrum (sample irradiated for between 300 - 1000 seconds using a 50 kV and a current of 1000 µA.
b. The measured spectrum using S2 PICOFOX software on the basis of the chosen elements was evaluated.
c. The same software (S2 PICOFOX) was used to determine concentration of elements. The calculation of element concentrations based on the net intensities of the element peaks as per the following formula;

$$Cis = \frac{Nx / Sx}{Nis / Sis} xCis$$

Where,

Cx --Concentration of the analyte; *Cis* -Concentration of the internal standard

Nx----- Net intensity of the analyte. *Nis*----- Net intensity of the internal standard

Sx---Relative sensitivity of analyte. *Sis*---Relative sensitivity of internal standard

2.5. Salt Hydrolysis of Biomass Samples

2.5.1. Hydrolytic Measurements

Portions of 20 to 30 g earlier dried plant samples were dried in the oven at 110 ^{o}C for 2hrs in 100 mL beaker, then cooled for 30 minutes before weighing the exact mass needed for any analysis. A mass of 3.00 g dried powder of each sample in 100 mL beaker was weighed and carefully placed into a 500.00 mL quick-fit round bottomed flask and a relevant salt solution was added. The refluxing set up erected and the refluxing was done for varied; times, conditions of temperature, salt: plant sample ratios and hydrolytic medium for each type of salt and plant biomass tested. Refluxing was done for a given time after which filtration was done in order to measure the potential degree of hydrolysis of each salt versus the biomass. Weight of oven dried

filter paper was always noted. First washing was done using a dilute solution of the same hydrolyzing salt, followed by distilled water. The pulp obtained was oven dried at 110 ^{o}C for 3hrs, cooled then weighed. The loss-in-weight method of Meller for the determination of the hydrolysis rate was used in these experiments (Mahamadi *et al.,* 2007).

2.5.1.1. Hydrolysis at Various Time Intervals

The degree of hydrolysis of a given salt with the lignocelluloses was done at different times. Time was varied from 1 hour to 5 hours while the salt: plant ratios, temperature of hydrolysis were kept constant. This experimental procedure was repeated for all the salt samples with lignocellulose samples. These experiments were to investigate on the effects of different hydrolytic time periods on the percentage degree of hydrolysis.

2.5.1.2. Hydrolysis at Various Salt: Plant Ratios

The degree of hydrolysis of a given salt with the lignocelluloses was done at salt: plant sample ratios. Ratios were varied from 1:20, 1:15, 1:10, to 1:5 in an increasing order of salt concentration levels while the time, temperature of hydrolysis were kept constant at 2 hours and 100 ^{0}C. This experimental procedure was repeated for all the salt samples with lignocellulose samples. These experiments were to investigate on the effects of different hydrolytic concentration levels of the salts on the percentage degree of hydrolysis.

2.5.1.3. Hydrolysis at Various Temperatures

The degree of hydrolysis of a given salt with the lignocelluloses was done at different temperature conditions. Temperature was varied at intervals of at least 10 ^{0}C gap from 49 ^{0}C, 57 ^{0}C, 67 ^{0}C, 80 ^{0}C, to 100 ^{0}C. The salt: plant ratios, time period of hydrolysis were kept constant at 1:5 and 3 hours respectively. This experimental procedure was repeated for all the salt samples with lignocellulose samples. These experiments were to investigate on the effects of different hydrolytic temperature regimes on the percentage degree of hydrolysis.

2.5.1.4. Hydrolysis at Various Hydrolytic Media

The degree of hydrolysis of a given salt with the lignocelluloses was done at different hydrolytic media; acidic, oxidative and alkaline at constant time period, salt: plant ratio of 1:5 and temperature of 100^{0}C. The amounts of each medium solutions were

systematically varied while measuring the percentage degree of hydrolysis for each case. The medium solution volumes added were varied depending on the nature of the experiment. For the oxidative experiments, the solution volumes varied from 2.00 mL, 4.00 mL, 8.00 mL, and 12.00 mL to 16.00 mL for each experiment. The alkaline experiments the solution volumes were; 2.00 mL, 4.00 mL, 8,00 mL, 12.00 mL, 16.00, to 20.00 mL. The media solution volumes for the acidic medium varied as per nature of the salt; magadi had volumes varied from 10.00 mL, 16.00 mL to 21.00mL, para had media solution volumes from2.00 mL, 4.00 mL to 6.00 mL, while Lebek had media solution volumes varied from 2.00 mL, 4.00 mL to 5.80 mL. For the acidic media, the end point neutralization reactions of the salts with acids determined what solution volumes were to be used. The maximum volume was the equivalence volume for each salt. These experiments were to investigate on the effects of different hydrolytic media environments of the percentage degree of hydrolysis.

2.5.2. Lignocellulose Hydrolysis for Sugar Production

2.5.2.1. Sugar Extraction for Fermentation

The treated lignocelluloses were subjected to salt solution hydrolytic processes to produce fermentable sugars. Ground and oven dried plant samples of 50.0 g were first soaked in 300.0 mL distilled water for 1.0 hours. It was then boiled at water boil off temperatures for another 1.0-2.0 hours to ensure water molecules penetrates the lignocellulose matrix. This could help delivering the salt ions into the lignocellulose matrix. Then a clear 10.0 g para-salt solution was slowly decanted and added to the boiling mixture of the water-lignocellulose. Steady boiling of the digesting adduct was continued for at least 48.0 hours. At this point the colloidal solution was clarified by adding 15.0 mL of a decolorizing agent. Boiling of the digest solution was continued for a further 3.0 hours in order to complete the decolorizing process and remove the decolorizing so that it does not affect the fermentation process. This solution was allowed to cool for 1.0-2.0 hours then strained with a clean cloth and then filtered to produce a clear solution ready for the fermentation process.

2.5.2.1. Sugars Extraction for Chromatographic Analysis

Ground and oven dried plant samples of 15.0 g were first soaked in 300.0 mL distilled water for 1.0 hour. It was then boiled in distilled water at water boil off temperatures

and at atmospheric pressure for another 1.0-2.0 hours to ensure water molecules penetrates the lignocellulose matrix. This could help delivering the salt ions in the solution into the lignocellulose matrix. Then the clear para-salt solution was slowly decanted and added to the boiling mixture of the water-lignocellulose. Boiling was continued for at least 48.0 hours. At this point the colloidal solution was clarified by adding 15.0 mL of a decolorizing agent. This solution was allowed to cool for 1.0-2.0 hours then strained with a clean cloth and then filtered to produce a clear solution extract. The extract was rotary-evaporated to dryness. Then the residue was placed in an air-tight glass container covered with 200 mL of boiling 80% Ethanol, which dissolved only simple carbohydrates and not the complex polysaccharides. After simmering for several hours in a steam bath, the container was sealed and stored at room temperature. For the analysis, the sample was homogenized in a blender for 3-5 min and then suction-filtered. Then it was extracted with 80% Ethanol (2 x 50 mL), the syrup was concentrated and further extracted in a separatory funnel using a mixture of Methanol- dichloromethane-water (MDW) (0.3:4:1, v/v/v). The Methanol-water phase containing sugars evaporated. Then residue was oven-dried at 50 °C overnight to remove the residual solvent and stored at –2 ℃ for chromatographic analysis.

2. 5.3: Fermentation of Sugars and Ethanol Production

The digested sugar solution mixture from section 2.5.2 was strained with a clean cloth then filtered. The 300.0 mL clear filtrate was put in a fermentation tank, equal volume of warm distilled water was added and then 20.0 g local yeast (*Saccharomyces cerevisiae* from finger millet) was added to ferment it. It was incubated for 72 hours. The fermented adduct was then cloth strained before the alcohol was distilled off at 80 ^{o}C using fractional distillation apparatus. The mother liquor after distilling off the alcohol was re-incubated for 72 hours by adding half its volume of distilled water, plus 10.0 g local yeast (*Saccharomyces cerevisiae* from finger millet). Distillation of the re-incubated adduct was done collecting more ethanol. This was done to ensure all the fermentable sugars were converted to ethanol.

2.6. Analysis Sugar and Ethanol

2.6.1. Chemical Standards for Sugar Analysis

Pure A.R grade sugar samples of D(-)-arabinose, D(-)-ribose, D(+)-xylose, D(+)-galactose, D(+)-glucose, D(+)-mannose, L(-)-sorbose, D(-)-fructose, L(+)-rhamnose, D(+)-sucrose, D(+)-maltose, and D(+)-lactose were used as standards (Aldrich Chemical Co.).

2.6.2. TLC Analysis of Sugars

2.6.2.1. Preparation of Chromatoplates

The extracted sugar mixture was concentrated and subjected to thin-layer chromatography. The sugar fraction was analyzed with one-dimensional TLC on a cellulose MN 300 G layer plate. Each plate was activated at 110℃ prior to use for 10 min.

2.6.2.2. One-Dimensional Chromatography

One mL aqueous solution of 10-mg sample of each of standard sugars or the analyte sugars was prepared. Then, 1 μL of each sugar solution was applied or spotted on the TLC plate. The chromatoplate was placed in a TLC chamber containing the developing solvent and the lid placed. The solvent system used was n-butanol-acetone-pyridine-water (10:10:5:5, v/v/v/v). The plates were developed in an almost vertical position at room temperature. After the elution, the plate was dried under warm air and sprayed with a mixture of 5% diphenylamine in EtOH, 4% aniline in EtOH and 85% phosphoric acid (5:5:1v/v/v). or p-anisaldehyde/H_2SO_4 (acentic acid, 5ml; conc. H_2SO_4, 25 mL; ethanol, 425 mL; water, 25 mL). The plate was heated for 10 min at 105 ℃ in an oven to develop the colors. The colored spots eluted were visualized after which R_f values were calculated.

2.6.3. HPLC Analysis of Sugars

2.6.3.1. Preparation of Sugar Sample

The extracted sugar solution mixture (100.0 mL) was concentrated by evaporating to dryness in a water bath to remove ethanol. The sugar crystals were then re-dissolved by adding 100.0 mL 25% acetonitrile water mixture and the sugar sample solution was kept in a plastic container ready for HPLC sugar analysis.

2.6.3.2. HPLC Sugar Analysis

Ten (10.0) mL aqueous solution of 100-mg sample of each of standard sugars or the analyte sugars was prepared. Then, 1 μL of each sugar solution was injected into the HPLC Colum. The sugar solution from section 2.6.3.1 was done in duplicate then filtered with micro filters. The monosaccharaides were then quantified by HPLC (HPLC-10Avp Shamadzu column (column-c8) with a matching pre-column at 63^{o}C. The sugars was eluted with Acetonitrile (80%) at a flow rate of 1.0 mL per minute, pressure- 64-69Kgf and detected by their differential refractometer index (RID (Refractive Index Detector), RID 6A))

2.6.4. GC Analysis of Ethanol in Fermented Hydrolyzates.

The products of fermentation mixture was distilled normally first before fractionally distilling it again. Determination of concentration of bioethanol produced was carried out using GC-FID head-space analysis. Perkin Elmer gas chromatograph equipped with FID detector using chromos orb 101 column at 151 ^{o}C was used. Nitrogen gas was used as a carrier gas while injector and detector temperatures were 200 and 220 ^{o}C respectively. Fifty (50.0) mL of standard absolute ethanol A.R grade and 50.0 mL of standard methanol A.R grade were measured and mixed together to form 100.0mL of standard mixture. Portions of 10.0mL of the stock solution mixture were serially diluted with 1.0 mL, 2.0 mL, 3.0 mL, 4.0 mL, 5.0 mL, 6.0 mL and 7.0 mL of distilled water to give concentrations of standard solutions of; 90.0, 80.0, 70.0, 60.0, 50.0, 40.0 and 30.0 % of the ethanol-methanol mixture respectively. These standard solutions were put in glass containers and corked tightly ready for GC-FID analysis. The analysis of each concentration was measured at GC conditions and the readings used to developed standard ethanol and methanol curves. Then 10.0 mL of each bioethanol samples were put in glass sample containers and GC analyzed. The chromatograms of the mixture was used to measure the percentage ethanol and methanol content of the sample from the standard curve.

2.7. Data Analysis

Mstat Statistical data analysis package was used to analyze data in which Factor analysis tool was used to carry out the analysis of the various parameters. Here the coefficient of variance (CV) and t-test between various test parameters was done. T-test

of the hydrolytic potential between the salts and the quality and quantity of alcohol produced was done. Coefficient of variance was used to test the significance level of differences between the agricultural residue (wheat straw and gallants soldier) (Miller, J. C. and J. N. Miller 1993).

CHAPTER THREE

3.0. RESULTS AND DISCUSSIONS

3.1. pH Analysis of the Salts

The values for pH of the salts are presented in Table 3.1. From the data all the oven dried salts significantly ($p \leq 0.01$) recorded higher pH compared to the non-oven-dried salts. The pH values recorded for Magadi salt showed the highest variance, increasing from pH 9.98 for non-oven-dried salt to pH 11.26 for oven-dried salt, a clear 1.28 margin difference. The other two salts, para and Lebek had a marginal difference of about 0.8 on average.

The salts recorded the lower pH in their raw form probably because of losing much of the acidic hydrogen in the CO-H of the bicarbonate group due to the bicarbonate decomposing to CO_2 and H_2O during oven drying period (Chunming *et al*., 1997). The large difference between oven dried and non-oven dried clearly shows that, there was decomposition and loss of most of acidic hydrogen during the drying stage, increasing the pH proportionately with HCO_3^- lost. Magadi salt had the highest pH after oven drying probably substantial amounts of hydrogen carbonates in it as compared to both para and lebek salts. Tatiana et al, (2010) in their studies found that carbonate salts tend to lose some degree of the bicarbonate group.

Table 3.1. The pH values of oven-dried and non-oven-dried salts.

Salt sample	**Sample treatment**		**(p≤0.05)**
	Oven dried (OD)	**Non-oven dried**	$t_{cal.}$
Magadi	11.26 ± 0.03	9.98 ± 0.01	10.651
Para	10.88 ± 0.06	10.08 ± 0.02	4.707
Lebek	10.85 ± 0.06	10.09 ± 0.02	6.58
(cv) (P≤0.01)	0.47%	0.17%	

3.2. FTIR Analysis Data for the Salt Samples.

FTIR spectra of HCO_3^- had the absorption bands in the spectral range of 3400-800 cm^{-1} (Figures 3.1, 3.2 and 3.3). Differences in position and number of absorption bands between the spectra of the three salts indicated that the salts had the same basic anionic

structure.

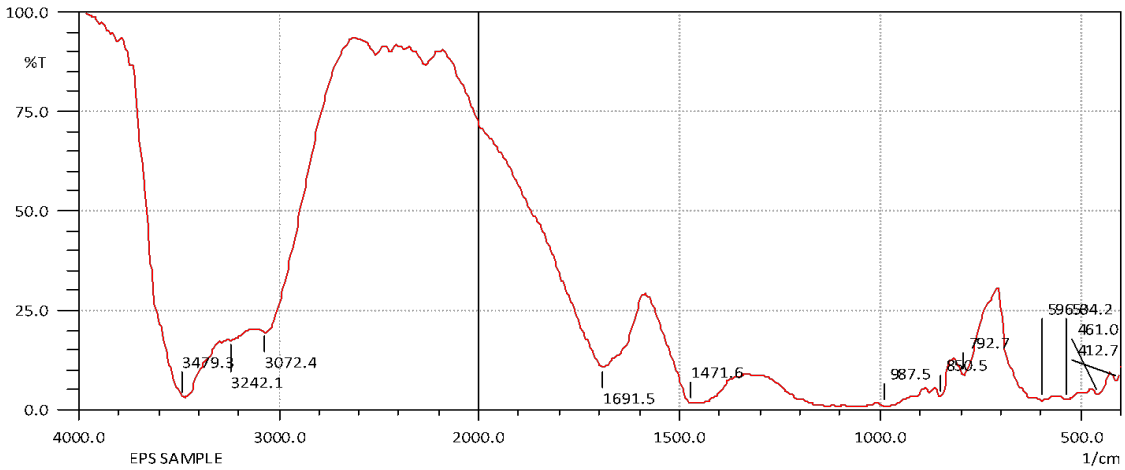

Figure 3.1: FTIR spectrum of Para-salt

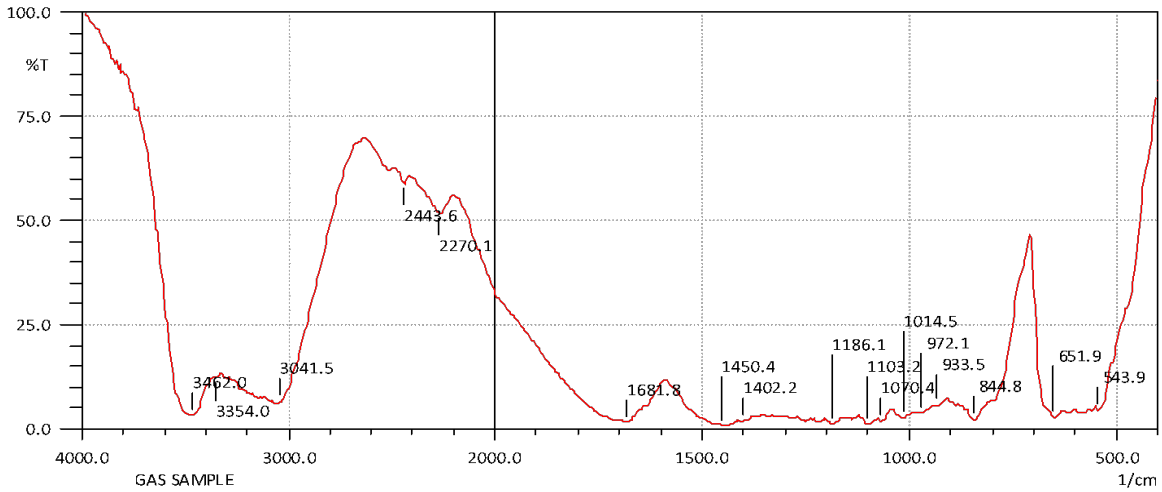

Figure 3.2: FTIR spectrum of Magadi-salt

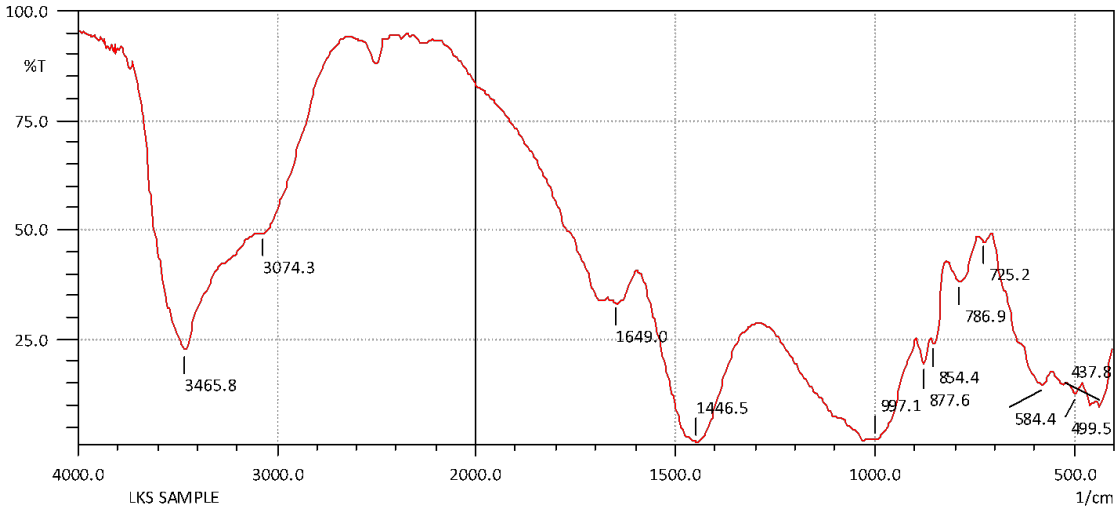

Figure 3.3: FTIR spectrum of Lebek-salt

From Figures 3.1, 3.2 and 3.3, it was observed that all the salts showed peaks at 3479-3482cm^{-1} , 1650-1690cm^{-1} and 950-1000 cm^{-1} ranges due to the –O-H , C=O and C-O stretches respectively. This indicate the presence of carbonate and bicarbonate groups (Huanc *et al*., 1960). In their studies on metal carbonates and bi-carbonates Chunming, *et al.,* 1997 and Teleb, et *al*., (2004) found that the C=O was commonly appearing in the range of 1640-1780cm^{-1}. In the FTIR transmission spectra of metal bi-carbonate salts, bands for free HCO_3^- ion in aqueous solution were assigned as follows: stretching of C-OH at 1010 cm^{-1}, symmetric stretching of CO_2 at 1360 and 1310 cm^{-1}, asymmetric stretching of CO_2 at 1668 and 1605 cm^{-1}, and out-of-plane bending of CO_3^{2-} at 843 cm^{-1} (Chunming, *et al.,* 1997). The rest of the fundamental vibration modes, (symmetric in-plane bending of CO_3^{2-}) and (asymmetric in-plane bending of CO_3^{2-}) appeared below 800 cm^{-1}. This is correlates well with earlier studies (Tatiana *et al*., 2010; Chunming *et al*., 1997; Teleb, et *al*., 2004)

3.3. Carbonate and Bicarbonate Content in Salt Samples

Table 3.2 below shows the percentage concentration levels of the carbonate and bicarbonate anions in dry salt samples.

Table 3.2. % wt/wt CO_3^{2-} and HCO_3^- Content in dry salt samples

Samples	Anionic radicals Content % wt/wt		
	Carbonate CO_3^{2-}	Hydrogen carbonate HCO_3^-	Total Carbonate CO_3^{2-} & HCO_3^-
Magadi	21.9 ± 0.5	25.3 ± 0.3	47.2 ± 0.2
Para	6.5 ± 0.4	7.3 ± 0.2	13.9 ± 0.3
Lebek	5.5 ± 0.1	6.2 ± 0.1	11.8 ± 0.4
Cv	3.7	4.0	3.8
Lsd	2.4	2.6	2.5

From Table 3.2 all the salts recorded higher $\%HCO_3^{2-}$ levels than the $\%CO_3^{2-}$ levels. Magadi salt recorded the highest values of both the $\%HCO_3^{2-}$ levels and the $\%CO_3^{2-}$ with values of 25.3% and 21.9% respectively. The other two salts; para and lebek recorded values below 7.5% for both HCO_3^{2-} and CO_3^{2-}. This results correlates very well with values recorded in Table 3.1 where the pH values of non-oven-dried salts were lower than oven-dried. This indicate that all the salts had a substantial amounts of the acidic HCO_3^{2-}. And the pH level of margin on the differences between the oven-dried salts and non-oven-dried showed higher HCO_3^{2-} than CO_3^{2-} (Table 3.1). Also the results of the Table 3.2 above agrees with those of FTIR Figures 3.1, 3.2, and 3.3, which showed a strong presence of the two anions; HCO_3^{2-} and CO_3^{2-} on the FTIR spectra peaks. The HCO_3^- contents of mineral salts has been found to be higher than the CO_3^{2-} content. This could be due to the fact that, the final major reaction that could influence the HCO_3^- contents in the deep aquifer is the addition in the system of CO_2 of deep origin (Makanjuola et al, 1975, Teleb *et al*., 2004).

3.4. Major Elemental Composition in Salt Samples

Table 3.3 below shows the composition of alkali and alkaline earth metals in dry salt samples. The concentration levels of the metals was determined in mg/g as some of the elements in the salts was of substantial amounts.

Table 3.3. Composition in mg/g of alkali and alkaline earth elements in dry salt

Samples	Elements mg/g				
	Na	K	Li	Ca	Mg
Magadi	71.52 ± 1.96	0.31 ± 0.04	0.21 ± 0.02	0.25 ± 0.12	0.02 ± 0.00
Para	27.23 ± 0.87	2.69 ± 0.41	0.29 ± 0.02	52.56 ± 8.03	2.89 ± 0.13
Lebek	25.83 ± 0.38	1.37 ± 0.05	0.15 ± 0.02	166.09 ± 6.55	0.16 ± 0.00
Coefficient of variance (P≤0.01)	2.91%	18.04%	0.00%	10.01%	7.33%

From the data, it shows that each salt had at least one element that was dominant with substantial concentration amounts than all other elements in the salt. Magadi salt significantly (P≤0.01) had a higher concentration of Na (71.52 mg/g) compared with the other elements in the salt. Magadi with high values of Na compared to very small concentration levels of the other elements clearly shows it is a sodium salt. In para the dominant element was Ca (52.56 mg/g), though this salt had a good amounts of Na (27.23 mg/g). This shows para as a mixed salt of Ca and Na, since the concentration levels of the other species of elements were found to be below 3.0 mg/g. Lebek salt had high levels of Ca (166.09 mg/g), with the other elements having values less than 26.0 mg/g. Thus, Lebek is a calcium salt. The composition of a given mineral salt could be attributed to the calcite rock source from which the hot spring brines originate (Eugster, 1970, Hill, 1964). Studies done on Lake Brines in Africa and other regions of the world showed a clear difference in the concentration levels and composition of the salt brines obtained (Sodipo, 1993, Talling *et al*., 1965, Tata chemicals, 2003).

3.5. Trace Elemental Content in the Salt Samples

Table 3.4 below shows the composition of trace metallic elements in dry salt samples. The elemental concentrations were recorded in µg/g because most of the elements in all the salts were of trace nature, except Fe and Mn which had higher values.

Table 3.4. Composition in µg/g of trace metallic elements in dry salt samples

Samples	Trace Metallic elements in µg/g						
	Zn	Co	Cu	Pb	Cd	Fe	Mn
Magadi	9.06 ±2.73	20.69 ± 1.02	2.91 ± 0.38	16.16 ±0.43	11.15 ± 1.20	214.8 ± 16.6	10.2 ± 3.0
Para	15.33 ±2.43	15.74± 0.60	8.44 ±0.32	12.83 ±1.30	2.87 ±0.27	1650.6 ±133.9	396.9 ±35.8
Lebek	23.01 ±3.09	20.49 ±1.49	10.07 ±1.04	9.50 ±1.37	2.24 ±0.15	5068.6 ±461.0	346.8 ±0.5
Cv	4.7	2.2	5.6	8.5	6.5	8.7	7.3

-Cv - Coefficient of Variations ($P \leq 0.01$).

Table 3.4 above recorded a significantly ($p \leq 0.01$) high values of Fe concentration levels in all the salts, ranging from a minimum concentration value of 214.8 µg/g in magadi to the highest value of 5068.6 µg/g in lebek. The concentration values for Mn also recorded substantial amounts compared to the trace a mounts of the other salts (Table 3.4). The range of Mn in salts was a minimum of 10.2 µg/g in magadi salt to to a maximum of 396.9 µg/g in para salt, the concentration levels in lebek quite comparable to that of para salt recording a value of 346.8 µg/g. All the other trace elements; Zn, Co, Cu, Pb, and Cd recorded a relatively low values, with the ranges in the salts of 9.06 µg/g to 23.01 µg/g Zn, 15.74 µg/g to 20.69 µg/g Co, 2.91 µg/g to 10.07 µg/g Cu, 9.50 µg/g to 16.16 µg/g Pb, and 2.24 µg/g to 11.15 µg/g Cd. The values of Co the salts were quite comparable with a narrow margin of range while Cd recorded a wide margin of the range betwee the minimum concentration and the highest concentration.

This kind of trends of the concentration in mineral salts varying has been attributed to salts rock of origin, clay rock which is rich in iron and manganese (Makanjuola *et al*., 1975, and Sharp, 1970). Other research findings had attributed it to the underlying rock source, and the conditions of the underground brine fluids (Tata Chemicals, 2003). Also the trace nature of these elements in solution could be attributed to the insoluble nature of most carbonates. Tables 3.1, 3.2 and 3.3 registered high values of pH, CO_3^{2-} and HCO_3^- values and high Na and Ca contents in the salts respectively, a clear show that it's the alkali and most of the alkaline earth metallic carbonates which are mostly solution in water (Sharp, 1970).

3.6. Trace Elements in Plant Samples

Dried plant samples were acid digested and analyzed for heavy metal ion compositions. The results were statistically analyzed and recorded in the tables below.

Table 3.5. Composition in µg/g of trace metal elements in dry Plant samples

Samples	Trace Metallic elements in µg/g dry plant sample						
	Zn	Co	Cu	Pb	Cd	Fe	Mn
Wheat straw	42.97 ± 2.08	7.77 ± 0.23	5.87 ±0.45	4.30 ±0.00	0.79 ±0.00	336.94 ±17.56	66.85 ± 0.38
Gallants soldier stem	92.79 ±12.36	11.78 ±1.89	10.16 ± 1.17	12.39 ±1.35	2.12 ±0.41	4027.37 ±68.74	167.68 ±1.93
Gallants soldier roots	137.33 ±20.22	8.54 ±1.44	7.50 ±1.92	19.64 ±0.67	1.03 ±0.13	3287.10 ±210.33	119.97 ±10.78
Cv(p≤0.01)	17.93%	9.17%	15.44%	8.50%	21.44%	6.69%	5.53%

Gallant soldier stem was found to have a significantly (p≤0.01) the highest concentration of iron with a mean concentration of 4027.4 ± 68.7µg/g, while Gallants soldier roots residue had the significant (p≤0.01) second highest of 3287.1 ± 210.3µg/g residue sample, while Wheat straw residue had the least significant (p≤0.01) concentration of 336.9 ± 17.6µg/g sample. Iron is an essential element for growth of animals and plants (Balaji *et. al.,* 2000). Its deficiency can hinder metabolism. Plant samples of gallants soldier and gallant soldier roots contained comparatively higher amounts of Fe >3287 µg/g while the highest concentration found in the other analyzed samples of wheat straw was 336.94 µg/g. In their studies; Ghosh *et al*., 2009, Hashmi *et.al.,* 2007, Başgel and Erdemoğlu, 2006, Balaji *et.al.,* 2000 and Mahwas *et al.*, 2011found that concentrations of iron in various plant samples ranged between 8.8 ± 0. 01 µg/g in *Brassica rapa* to 194.9 ± 3.960 µg/g *Trachyspermum ammi.* The concentration of iron in *gallants soldier* was found in order of stem > roots (Table 3.5).

Gallants soldier stem were found to have a highest concentration of manganese with a mean concentration of 167.68 ± 1.93µg/g while gallants soldier roots had a second highest of 119.97 ± 10.8µg/g, while Wheat straw had a significant (p≤0.01) the least

concentration, 66.85 ± 0.38µg/g sample. In their studies; Mahwas *et al.*, 2011found that concentrations of manganese in various plant samples ranged between 6.860 ± 1.401 µg/g in *Brassica rapa* to 40.89 ± 2.170 µg/g *Trachyspermum ammi.* These high amounts could be attributed to the fertilizer applications in the farms (Mahwas *et al.*, 2011, Hashmi *et. al.,* 2007).

Gallants soldier roots were found to have a significantly ($p \leq 0.01$) higher concentrations of zinc with a mean concentration of 137.33 ± 20.22 µg/g as in table 3.5. It was followed by Gallants soldier stem that had a significant ($p \leq 0.01$) mean concentration of 92.79 ± 12.36 µg/g. Wheat straw had a significantly ($p \leq 0.01$) the least concentration, with a mean concentration of 42.97 ± 2.08 µg/g. It was noted that gallant stem, gallant root and wheat straw had a significantly ($p \leq 0.01$) higher amounts of zinc compared to the salts. Zinc levels in the current study were higher as compared with those obtained by Hashmi *et al.,* 2007, Balaji *et al.,* 2000 who reported values of less than 4.1 ± 0.02 µg/g in all the plants they analyzed. Such concentrations level of zinc could be due to the addition of fertilizer additives designed to control plant growth as reported by Hashmi *et al.,* 2007, Başgel and Erdemoğlu, 2006, and Mahwas *et al.*, 2011. Concentration of zinc in this study ranged from 42.97 to 137.33 µg/g these were comparable with the studies of Mahwas *et al*, 2011who found that concentrations of zinc in various plant samples ranged between 4.940 ±1.228 µg/g in *Brassica rapa* to 53.74 ± 1.706 µg/g *Trachyspermum ammi*

Gallants soldier roots were found to have significantly ($p \leq 0.01$) the highest concentrations of lead with a mean concentration of 19.64 ± 0.6 µg/g as in Table 3.5. It was followed by Gallants soldier stem that had a significantly ($p \leq 0.01$) mean concentration of 12.39 ± 1.35 µg/g. Wheat straw had significantly ($p \leq 0.01$) the least concentration, with a mean concentration of 4.3±0.0µg/g. Lead levels in the current study were quite comparable with those obtained by Ghosh et. *al*., 2009 who reported similar values (16.53 µg/g) in all the plants they analyzed. Such concentrations level of lead could be due to the addition of fungicides and additives designed to control plant growth and smelting as reported by Hashmi *et al.,* 2007, Başgel and Erdemoğlu, 2006, and Mahwas *et al.*, 2011. Concentration of lead in this study ranged from 4.3±0.0µg/g to 19.64± 0.6µg/g these were comparable with the studies of Mahwas *et al.*, 2011who found that concentrations of lead in various plant samples ranged between 9.515 ±

0.276 µg/g in *Brassica rapa* to 24.02 ± 2.027 µg/g *Murraya koenigii*. Concentration of lead in *gallants soldier* roots is greater than that of *gallants soldier stem* which may be due to high concentration of lead in soil than in air (Mahwash *et al*., 2011).

Gallant soldier stem was found to have a significantly ($p \leq 0.01$) the highest concentrations of copper with a mean concentration of 10.16±1.17 µg/g as in table 3.5. It was followed by Gallants soldier roots which had a significant ($p \leq 0.01$) mean concentration of 7.50 ± 1.92 µg/g. Wheat straw had significantly ($p \leq 0.01$) the least concentration, with a mean concentration of 5.87±0.45µg/g. Copper significant ($p \leq 0.01$) concentration levels were found in gallant soldier stem and gallants soldier root and *wheat straw* were higher as compared to that obtained by Hashmi *et. al.,* 2007, Balaji *et al.,* 2000. This high concentration of copper could be to contamination from copper pipes, as well as additives in fertilizers designed to control algal growth as reported by Hashmi *et.al.* 2007, Başgel and Erdemoğlu, 2006, and Mahwas *et al.*, 2011.

Gallant soldier stem was found to have a significantly ($p \leq 0.01$) highest concentrations of cadmium with a mean concentration of 2.12 ± 0.41µg/g as in table 1.0. It was followed by Gallants soldier roots which had a significant ($p \leq 0.01$) mean concentration of 1.03 ± 0.13 µg/g. Wheat straw had a significantly ($p \leq 0.01$) the least concentration with a mean concentration of 0.79±0.00µg/g (Ghosh *et al*., 2009, Hashmi *et. al.,* 2007, Balaji *et.al.* 2000 and Mahwas *et al.*, 2011).

Gallant soldier stem was found to have a significantly ($p \leq 0.01$) highest concentrations of Cobalt with a mean concentration of 11.78 ± 1.89 µg/g. It was followed by Gallants soldier roots which had a significantly ($p \leq 0.01$) mean concentration of 8.54 ± 1.44 µg/g. Wheat straw had a significantly ($p \leq 0.01$) the least concentration, with a mean concentration of 7.77 ± 0.23 µg/g. It was found concentration of cobalt in the plant samples significantly ($p \leq 0.01$) ranged between 8.54 ± 1.44-11.78 ± 1.89 µg/g these were quite high as compared with the studies of Mahwas *et al*, 2011who found that concentrations of lead in various plant samples ranged between 0.654 ±0.040 µg/g in *Brassica rapa* to 1.837 ±0.187 µg/g *Murraya koenigii*.

Gallant Soldier Concentration of metals was usually high in the roots as compared with other stem parts of the plant for some metals this correlates well the study done by (Hashmi *et.al.* 2007, Başgel and Erdemoğlu, 2006, and Mahwas *et al.*, 2011). Lead and

contents found in gallant soldier (root) were lower than that of its stem which may be due to the fact that different parts even different leaves of the same plant may contain different proportion of same metals, depending upon age of plant and because of other factors. Each element has its individual impact in the structural and functional integrity of the living cells and organisms. Results shown in Table 3.5 verify the presence of variable amounts of these metals in the agricultural plant samples.

3.7. Alkali and Alkaline Earth Metal Content in Plant Samples

Dried plant samples were acid digested and analyzed for alkali and alkaline earth metallic ion compositions. The results were statistically analyzed and recorded in Table 3.6 below.

Table 3.6. Composition in mg/g Alkali and alkaline earth metallic elements in dry Plant samples

Samples	Elements mg/g				
	Na	K	Li	Ca	Mg
Wheat straw	17.45 ± 0.67	31.82 ±0.62	0.80 ±0.00	2.30 ±0.29	0.39 ±0.01
Gallants soldier	39.12 ±1.08	67.09 ±1.33	1.38 ±0.04	44.53 ±2.16	0.40 ±0.01
Gallants soldier roots	22.78 ±0.95	36.27 ±2.02	0.94 ±0.04	12.69 ±2.62	0.40 ±0.00
Cv($p \leq 0.01$)	3.58%	1.55%	2.22%	5.19%	2.52%

Gallant soldier stem was found to have the highest sodium concentrations with a mean concentration of 39.12±1.08mg/g as shown in Table 3.6. Gallants soldier roots had a significant ($p \leq 0.01$) mean concentration of 22.78±0.95mg/g while Wheat straw had a significant ($p \leq 0.01$) mean concentration of 17.45±0.67mg/g. The concentration of sodium was found to be relatively higher in all the plant samples, this correlated well with studies done by Mahwash *et al*., 2011. In these studies, the concentration levels of sodium were relatively higher than other metallic elements in the other plant samples analyzed.

Gallant soldier stem was also found to have significantly ($p \leq 0.01$) the highest calcium concentrations with a mean concentration of 44.53±2.16g/g as shown in Table 3.6. Gallants soldier roots had a significantly ($p \leq 0.01$) mean concentration of 12.69±2.62g/g

while Wheat straw had a significantly ($p \leq 0.01$) mean concentration of 2.30±0.29mg/g. Calcium usually enters in plants from soil as fertilizers contain a lot of calcium (Mahwash *et al*., 2011). The concentration of calcium was found to be significantly ($p \leq 0.01$) very higher in all the species as compared with other metals. Highest concentration of Potassium was recorded in *gallants soldier (*44.53 mg /g) while in other plant samples it ranges from 2.3 to 12.69 mg/g these compared well with studies by Mahwash *et al*., 2011 who reported great variatons of metal concentration levels in plant species.

Gallant soldier stem was found to have a significantly ($p \leq 0.01$) higher potassium concentrations with a mean concentration of 67.09 ± 1.33 mg/g as shown in Table 3.6. Gallants soldier roots residue had a significantly ($p \leq 0.01$) a mean concentration of 36.27 ± 2.02 mg/g and this result was not in line with studies done by Mahwash *et al.,* 2011 who found high metallic levels in roots than other parts of the plant. Wheat straw residue had a significantly ($p \leq 0.01$) mean concentration of 31.82±0.62mg/g. The concentration of Potassium was found to be very high in all the species (Mahwash *et al*., 2011). Gallant soldier had the highest significantly ($p \leq 0.01$) concentration of potassium was recorded in *gallants soldier was* 90.38 µg /g while in other plant samples it ranges from 1494-25.18 µg/g except in *gallants soldier* (root) in which concentration of Potassium is found to be 45.55 µg/g which may be due to the contact of roots of *gallants soldier* with the potassium rich fertilizer (Mahwash *et al*., 2011). The concentration levels in the residues were dramatically high than those of the salts. Potassium concentrations in these residues were higher than those of sodium and were the highest metal concentrations in all the residues as in shown in table 3.6.

Gallant soldier stem was found to have highest magnesium concentrations with a mean concentration of 0.40 ± 0.01 mg/g as shown in table 3.6. Gallants soldier roots had significantly ($p \leq 0.01$) mean concentration of 0.40 ± 0.00 mg/g while wheat straw residue had a mean concentration of 0.39 ± 0.01 mg/g. Gallant soldier stem was found to have lithium concentrations with a mean concentration of 1.38 ± 0.04 mg/g. Gallants soldier roots had a mean concentration of 0.94±0.04mg/g while Wheat straw residue had a mean concentration of 0.80 ± 0.00 mg/g. Some of the metals contents found in *Gallants soldier (*root) were lower than that of its stem which may be due to the fact

that different parts same plant may contain different proportion of same different metals, depending upon age of plant.

3.8. XRF Elemental Composition Analysis of the Salt Samples.

Dried salts were subjected to XRF elemental analysis. The results of selected elements analyzed are presented in the Table 3.7.

Table 3.7. XRF Metallic Analysis of the salts samples in mg/g of dry sample

Samples	Elemental composition in samples in mg/g dry sample						
	Si	Al	Fe	Ca	Mg	S	K
Magadi	19.0 ±0.8	15.5 ±2.8	0.0 ±0.0	33.3 ±4.8	2.0 ±0.2	3.7 ±0.8	0.0 ±0.8
Para	91.9 ±3.5	32.4 ±4.4	42.5 ±0.6	91.5 ±1.5	56.6 ±3.3	1.3 ±0.1	3.0 ±0.2
Lebek	89.8 ±8.2	33.3 ±1.8	43.6 ±2.8	161.8 ±1.3	25.5 ±2.8	1.5 ±0.2	1.2 ±0.1

Lebek salt significantly ($p \leq 0.01$) recorded very high calcium concentration levels as compared with the other salts. It had a mean concentration of 161.8±1.3 mg/g, the other salts followed in the significantly ($p \leq 0.01$) decreasing order of Para then magadi. This could be attributed to the calcite bedrock source from which the hot spring bines originate. This is in line with other studies done on Lake Brines in Africa (Sodipo, 1993, Talling et al, 1965, Tata chemicals, 2003). Potassium concentration in Para-salt had the highest amounts with a mean concentration of 1.2±0.1 mg/g. The concentration of potassium ions in all the three salts was significantly ($p \leq 0.01$) less than 1.5.00 mg/g. This is in line with studies done in Lake Magadi (Eugster, 1970) and Lake Albert (Tatiana et al, 2010) which showed that potassium is in low concentration because of leaching during salt precipitations. Magnesium concentration was significantly ($p \leq 0.01$) highest in Para-salt with a mean concentration of 56.6±3.3 mg/g .The concentration of magnesium ions in all the three salts was significantly ($p \leq 0.01$) low, as compared to studies done in Lake Magadi (Eugster, 1970). Silcon and aluminum concentrations in Para-salt had significantly ($p \leq 0.01$) the highest amounts with a mean concentration of 91.9 ± 3.5 mg/g and 32.4±4.4 mg/g. From the data, elemental concentration levels in Lebek and para were significantly ($p \leq 0.01$) comparable. These

results were comparable to those obtained (Makanjuola et al, 1975) and this could be due to the trace nature of lithium in mineral rocks.

3.9.0: Neat Salt Hydrolysis of the Plant Samples

3.9.1: Hydrolysis at Constant Conditions of Ratio, Time and Temperature

The values for percent hydrolysis are presented in the Table 3.8. The coefficient of variation (cv) from the data Magadi salt significantly ($p \leq 0.01$) had higher percent hydrolysis compared to the other two salts. The percent hydrolysis followed the pattern Magadi salt >para salt >Lebek salt. The Gallants soldier stem had significantly ($p \leq 0.01$) higher degree of hydrolysis compared to wheat straw. There was significant ($p \leq 0.01$) interaction between the salts and the residues in terms of percentage hydrolysis. The t-test analysis showed that the percent degree of hydrolysis levels of the three salts were significantly ($p \leq 0.01$) different and the percent degree of hydrolysable levels of the two plant sample residues were significantly ($p \leq 0.01$) different.

Table 3.8. Salt hydrolysis of dry plant samples in % wt/wt at constant conditions; ratio 1:5, time 2.0 hours and temperature 100^0C

Type of sample and ratio		%wt/wt Hydrolysis			
		Magadi	Para	Lebek	Cv
Gallants Soldier	1:5	44.99 ±0.01	32.28 ±0.01	29.98 ±0.02	0.49%
Wheat straw	1:5	30.75 ±0.05	21.08 ±0.02	20.94 ±0.01	0.09%
Cv		0.07%	0.74%	0.08%	($p \leq 0.01$)
Lsd		0.16	1.13	0.12	($p \leq 0.01$)

3.9.2: Gallant's Soldier-Residue

Magadi salt with a delignification of 44.99%, exhibits significantly ($p \leq 0.01$) the highest degree of hydrolyzing the lignin and hemicelluloses from a Gallant's soldier. Para salt and Lebek salt had percentage degree of delignification of less than 33%. The high degree of hydrolysis of magadi in its neat for could be attributed to the high amounts of $NaHCO_3^-$ levels as recorded in Tables 3.2 and 3.3. The HCO_3^- has been associated with

hydrolytic characteristics (Mahamadi *et al. (*2007*),* and Yan, *et al*, 1956). Mahamadi *et al. (*2007*)* reported in their studies that, higher degrees of hydrolysis were recorded when an inert salt was added in the hydrolyzing regime than when it was absent. Osano, *et a*l (2013) recorded high amounts of sodium in Magadi salt, followed by Para salt then Lebek salt, a trend witnessed in the degree of hydrolysis.

3.9.3: Wheat Straw-Residue

Magadi salt with a significant ($p \leq 0.01$) percent delignification of 30.75±0.05%) exhibited the highest degree of hydrolyzing the lignin and hemicelluloses from a Wheat straw-residue. This value was slightly lower than that recorded with gallants soldier, Table 3.8. This explains the fact that different plant samples hydrolyze differently due to differences in structure and form (Ananda *et al.*, 2012). Para salt and Lebek salt had significant ($p \leq 0.01$) degrees of delignification of 21.08±0.02%) and 20.94±0.01% respectively and were close to each other. Also as for Gallant's soldier-residue, Magadi salt recorded the highest percent hydrolysis with Wheat straw as compared to both Para salt and Lebek salt. Osano, *et a*l 2013 have shown that Magadi had higher HCO_3^- concentration of neutral salts than Para and Lebek salts.).

Gallants soldier recorded the highest degree of delignification with Magadi salt. This produced more hydrolysates than Wheat straw-residues. This could be attributed to lingo-cellulosic plant sample structure. In their studies Fuess, *et al*, 1931 and Ananda *et al*,2012 reported that, high percentage hydrolysis in Wheat rye (over 52%) were recorded than in Spruce wood at 20% to 30% using the same acid hydrolysis.

3.10. Salt Hydrolysis of Plant Samples at Various Time Intervals.

3.10.1. Salt to Plant Sample Ratio of 1:10

The values for percent hydrolysis are presented in the Table 3.9. The coefficient of variation (CV%) from the Magadi and Para salts significantly ($p \leq 0.01$) had higher percent hydrolysis compared to the Lebek salt at ratio 1:10. The percent hydrolysis was in the order magadi salt >para salt >Lebek salt. The Gallants soldier plant residue had higher degree of hydrolysis compared to wheat straw. There was an interaction between the salts and the residues in terms of percent hydrolysis within a given time period. This meant that varying either the type of salt or time period had a significant ($p \leq 0.01$) effect on the percent hydrolysis trends. The t-test analysis showed that the percent

degree of hydrolysis levels of the three salts were significantly different and the percent degree of hydrolysable levels of the two plant sample residues were different.

Table 3.9. Salt hydrolysis of plant samples in % wt/wt at various time intervals at constant ratio and temperature.

Samples		***Time Durations (Hrs)*** Ratio 1: 10; Temperature at 100°C					(p≤0.01)	
salt	Residue	1.0	2.0	3.0	4.0	5.0	Cv	Sld
Magadi	Gallant soldier	36.33 ±0.12	37.44 ±0.12	40.25 ±0.14	36.46 ±0.15	36.77 ±0.12	0.38	0.34
	Wheat straw	18.56 ±0.10	25.59 ±0.08	23.22 ±0.15	28.00 ±0.23	27.01 ±0.08	1.19	0.69
Para	Gallant soldier	35.05 ±0.13	36.12 ±0.07	36.13 ±0.09	35.39 ±0.08	36.11 ±0.06	0.23	0.20
	Wheat straw	19.31 ±0.14	18.91 ±0.08	17.94 ±0.07	19.52 ±0.09	19.5 ±0.15	0.47	0.21
Lebek	Gallant soldier	33.69 ±0.13	34.62 ±0.12	33.21 ±0.12	36.81 ±0.07	34.63 ±0.14	0.08	0.06
	Wheat straw	16.3 ±0.21	18.7 ±0.07	18.88 ±0.14	18.84 ±0.07	19.32 ±0.09	0.79	0.33

The Table 3.9 showed that gallants soldier with magadi-salt recorded significantly (p≤0.01) high percent delignification and hemicellulose removal of all the salts tested. The highest recorded value of magadi-salt was 40.25% in 3.0 hours. This value was slightly lower the value of 44.9% recorded in Table 3.8 at a 2 hours hydrolysis period and a higher salt to plant sample ratio of 1:5. This could be attributed to concentration levels of the hydrolyzing regime. The higher the concentration of the hydrolyzing component the higher the %wt/wt degree of hydrolysis (Fuess, *et al*, 1931).

There was almost a constant percentage degree of delignification of magadi-salt irrespective of the delignification period as shown in Table 3.9 above. Para-salt recorded significantly (p≤0.01) the second highest percent delignification of all the salts tested. The highest recorded value in Para salt with gallants soldier was 36.13% in 3.0 hours. There was a constant percent degree of delignification of Para-salt irrespective of the delignification period as shown in table 3.9. The highest recorded value of Lebek-salt was 36.81% in 4.00 hours. There was also constant percent degree of delignification of lebek salt as can be seen from Table 3.9.

Generally, there was significant ($p \leq 0.01$) no trend in percent delignification of the various salts increasing time period of hydrolysis. This could be explained from the low concentration of the hydrolyzing solution mixture. Dilute solutions of the hydrolyzing medium influencesd the amounts of hydrolysates obtained during lignocelluloses digestion (Gustavsson, and Al-Dajani, 2000). In their studies Anne et al., (1997) observed that the treatment time with <2% NaOH had no clear maximal efficiency with pretreatment time producing reducing sugars of percentage hydrolysis from office paper.

3.10.2. Salt to Plant Sample Ratio of 1:5

The values for percent hydrolysis are presented in the Table 3.10 below. The coefficient of variation from the Magadi salt significantly ($p \leq 0.01$) had higher percent hydrolysis compared to the para and Lebek salts at ratio 1:5. The percent hydrolysis was in the order magadi salt >para salt and Lebek salt. The Gallants soldier plant residue had higher degree of hydrolysis compared to wheat straw. There was an interaction between the salts and the residues in terms of percent hydrolysis within a given time period. This meant that varying either the type of salt or time period had a significant ($p \leq 0.01$) effect on the percent hydrolysis trends. The t-test analysis showed that the percent degree of hydrolysis levels of the three salts were significantly different and the percent degree of hydrolysable levels of the two plant sample residues were different.

Table 3.10. Salt Hydrolysis of plant Samples at various times at constant temperature and ratio.

Samples		**Time variation** (Ratio 1:5, Temperature, 100^0C)						($p \leq 0.01$)	
Salt	Plant sample	0.5Hr	1HR	2HRS	3HRS	4HRS	5HRS	Cv %	lsd
Magadi	Gallants soldier	28.19 ±0.32	34.03 ±0.64	45.63 ±0.23	47.90 ±0.43	50.00 ±0.11	49.18 ±0.15	1.04	1.01
	Wheat straw	23.58 ±0.25	27.30 ±0.56	28.89 ±0.73	33.54 ±0.11	35.42 ±0.34	35.34 ±0.14	0.83	0.59
Para	Gallants soldier	36.14 ±1.21	36.79 ±0.84	36.93 ±0.65	37.74 ±0.13	38.64 ±0.12	38.24 ±0.11	2.08	1.80
	Wheat straw	19.73 ±0.12	21.51 ±0.33	22.17 ±0.23	24.45 ±0.32	25.12 ±0.45	24.34 ±0.55	0.99	0.51
Lebek	Gallants soldier	37.54 ±0.26	37.51 ±0.25	37.98 ±0.19	38.71 ±0.16	38.86 ±0.18	38.79 ±0.28	0.47	0.43
	Wheat straw	18.43 ±0.18	20.47 ±0.23	21.17 ±0.53	23.13 ±0.32	23.93 ±0.16	23.67 ±0.18	0.65	0.36

The Table 3.10 showed that gallants soldier with magadi-salt recorded significantly ($p \leq 0.01$) the highest percent delignification and hemicellulose removal of all the salts tested. The recorded values showed a steady increase in the degree of delignification from 30 minutes to 4.00 hours. The highest recorded value of Magadi-salt being 50.00% in 4.00 hours, with the least recorded being 28.19% in 30 minutes. Wheat straw with magadi-salt recorded values lower than those with gallants soldier with magadi salt. This clearly showed that hydrolysis does not depend only on the type of hydrolyzing species but also on the lignocellulse structure and composition (Gustavsson, and Al-Dajani, 2000).

Para and lebek salts with the two plant samples significantly ($p \leq 0.01$) recorded lower values than the values recorded with magadi salt, Table 3.10 above. Para and lebek salts at 4 hours both recorded a % degree of hydrolysis of about 39.0%, while for the same time magadi salt gallants soldier recorded a value of 50.o%. The same trend replicated for para and lebek salts with wheat straw recording % degree of hydrolysis of less than 25.5% at 4 hours, while magadi recorded a value of 35.42% at the same time period. These could correlates well with their low % concentration values of HCO_3^- in Table 3.2. This clearly showed that, %wt/wt concentration values of HCO_3^- in the salts

positively correlated with the %wt/wt degree of hydrolysis of lignocelluses. The higher the %wt/wt concentration values of HCO_3^- higher %wt/wt degree of hydrolysis of lignocelluses (Gustavsson, and Al-Dajani, 2000).

The recorded values showed a steady increase in the degree of delignification from 30 minutes to 4.00hours for magadi gallants soldier but for magadi salt with wheat straw there was no % degree of hydrolysis increase with increasing time. There was no clear pattern of percent delignification with increasing time and the recorded values were almost a constant for para and lebek salts with the two plant samples.

The data from Table 3.10 above showed that maximum hydrolysis was achieved at time of 4.00 hours. Studies by Anne et al., (1997) observed that the treatment time with 2% NaOH had maximal efficiency after a one hour pretreatment producing highest reducing sugars of 0.48mg/mL (48% hydrolysis) from office paper. Other studies have also shown that the longer the reaction time the more hemicellulose was solubilized, but also the more hemicellulose was degraded (Schaleger & Brink, 1978). Schmid and Bjerre, 1997 observed that, hydrolysis levels increased from 33% to 46% by varying time from 5.0 minutes to 15.0minutes at a constant temperature of 150^{o}C.

3.11. Salt Hydrolysis of Plant Samples at Various Concentration Ratios

The Table 3.11 below shows data of the salts-plant samples % degree of hydrolysis at varied salt to plant samples ratios. Parameters kept constant were; temperature was kept at 100^{o}C and time at 2.0 hours.

Table 3.11. Hydrolysis of plant Samples at constant temperature (100^{o}C) and time (2.00 hours) while varying salt: plant sample ratios.

Samples		Various Ratios				($p \leq 0.01$)	
Salt	Plant Sample	1:5	1:10	1:15	1:20	Cv %	lsd
Magadi	Gallants Soldier	45.63 ±0.13	35.50 ±0.49	32.00 ±0.31	36.68 ±0.14	0.49	0.59
	Wheat straw	28.89 ±0.12	26.88 ±0.68	24.56 ±0.24	21.14 ±0.43	1.64	1.34
Para	Gallants soldier	36.43 ±0.15	36.06 ±0.17	34.25 ±0.32	32.08 ±0.22	0.22	0.24
	Wheat straw	22.17 ±0.35	20.81 ±0.01	15.43 ±0.46	16.53 ±0.03	1.21	0.73
Lebek	Gallants soldier	36.98 ±0.22	34.20 ±1.31	35.21 ±0.53	34.93 ±0.11	1.53	1.74
	Wheat straw	29.17 ±1.65	19.28 ±0.55	16.67 ±0.05	22.80 ±0.52	3.13	2.21

Table 3.11 had gallants soldier with magadi salt ratio of 1:5 recording significantly ($p \leq 0.01$) high % delignification and hemicellulose removal of all the salts ratios tested. The recorded values showed a steady decrease in the % degree of delignification and hemicellulose from ratio 1:5 to lowest ratio of 1:20. The highest recorded value of magadi-salt being 45.63 % in 1:5 ratio, followed by 35.50 % in 1:10 ratio, then 32.00 % in 1:15 ratio, the least was 28.68 % in a 1:20 ratio. Para-salt ratio (1:5 and 1:10) recorded the second highest percent delignification of all the salts ratios tested. The recorded values showed a decrease in the degree of delignification from ratio 1:5 to the lowest ratio of 1:20.The highest recorded value of para-salt with gallants soldier was 36.43 % in 1:5 ratio and the least was 32.08 % in 1:20 ratio. Lebek-salt in all its ratios recorded % degree of hydrolysis values similar to those of para salt in its ratios.

Wheat Straw with magadi-salt ratio (1:5) recorded significantly ($p \leq 0.01$) the highest percent delignification of all the salts ratios tested but was far lower than that of gallants soldier with magadi. The recorded values showed a steady decrease in the degree of delignification from ratio 1:5 to the lowest ratio of 1:20.The highest recorded value of magadi-salt being 28.89% in 1:5 ratio and the least was 21.14 % in a 1:20 ratio. Lebek-salt ratio (1:5) recorded the second highest percent delignification of all the salts ratios tested. The recorded values showed a steady decrease in the degree of

delignification from ratio 1:5 to the lowest ratio of 1:20.The highest recorded value of Lebek-salt being 29.17 % in 1:5 ratio followed and the least was 16.67±0.05% in 1:15 ratio. Para-salt ratio (1:5) recorded the third highest percent delignification of all the salts ratios tested. The recorded values significantly ($p \leq 0.01$) showed a steady decrease in the degree of delignification from ratio 1:5 to the lowest ratio of 1:20.The highest recorded value of Para-salt being 22.17±0.35% in 1:5 ratio followed by in 1:10 ratio 20.81 % and the least was 16.53±0.03% in 1:20 ratio Table 3.11. The degree of hydrolysis increased with the concentration level increase. This compared very well with the studies of Millet *et al*., (1976) who observed that the digestibility of NaOH-treated hardwood increased from 14% to 55% with the with increased NaOH concentration levels.

Generally there was a significant ($p \leq 0.01$) decrease in percent delignification with a decrease in the salt: plant sample ratio. Magadi-salt significantly ($p \leq 0.01$) recorded a clearer pattern, while Para-salt and Lebek-salt significantly ($p \leq 0.01$) recorded a few non-conforming values. This could be attributed to higher bicarbonate levels in magadi than in para and lebek which recorded lower bicarbonate levels. From studies done earlier, the lignocellulose pretreatment involved mineral acids H_2SO_4 or alkali NaOH in concentration of 2% (w/w) gave an optimal delignification and hemicellulose removal (Iuliana, 2009). The data clearly correlated positively with studies by Hui *et al*., 2010, who found that the degree of hydrolysis varied with the type of lignocellulose sample, and the nature of the hydrolyzing chemical and the concentration levels of the hydrolyzing chemical to the lignocellulose dry mass.

At high salt concentration hydrolysis took a shorter time to reach higher percent hydrolysis levels. This shows that alkalinity also affects the relative rates of hydrolysis (Katzen et al., 2006). The salts showed variable degrees of hydrolysis as different paramenters were implemented. This could be attributed their various compositions depended on their source and nature. Yi et al., 2009 in their research observed that, when Ba^{2+} or Sr^{2+} is used as the counter ion, the conversion decreases with increasing alkalinity in the range 0.1 M [HO–] 0.4 M.

3.13. Salt Hydrolysis of Plant Samples at Various Temperatures

The data recorded on Table 3.12 shows the trend of the % degree hydrolysis of the plant samples using the mineral salts for various temperatures at constant concentration ratio of 1:5 and time of 3.0 hours.

Table3.12. Percent degree of delignification and hemicelluloses removal from dry samples, at various temperatures (^{o}C) at time (3.hrs) and ratio of 1:5.

Samlpes		variation of temperatures, ^{0}C					($p \leq 0.01$)	
Salt	Residue	49	57	67	80	100	Cv %	lsd
Magadi	Gallants soldier	29.73 ±0.10	33.90 ±0.12	35.57 ±0.11	36.84 ±0.14	40.25 ±0.14	0.05	0.04
	Wheat straw	14.17 ±0.10	15.50 ±0.02	16.95 ±0.15	18.83 ±0.03	23.22 ±0.15	0.38	0.15
Para	Gallants soldier	30.15 ±0.12	33.28 ±0.07	33.97 ±0.09	34.80 ±0.01	36.13 ±0.09	0.16	0.12
	Wheat straw	12.52 ±0.14	13.62 ±0.06	13.95 ±0.07	16.04 ±0.03	17.94 ±0.07	0.27	0.10
Lebek	Gallants soldier	29.75 ±0.15	31.48 ±0.12	32.82 ±0.17	34.91 ±0.11	35.15 ±0.18	0.17	0.15
	Wheat straw	11.34 ±0.12	13.22 ±0.05	13.64 ±0.06	15.15 ±0.13	17.68 ±0.09	0.26	0.14

The values of % hydrolysis (Table 3.12) showed a steady increase as temperature was increased for all the salts with all the plant samples. The results show that, gallants soldier with magadi-salt recorded the highest percent delignification of all the salts tested. The highest recorded value of Magadi-salt being 40.25 % at a temperature of 100 ^{o}C and the least recorded was 29.73 % in 49 ^{o}C. The highest recorded value of Para-salt being 36.13 % at 100 ^{o}C, the least being 30.15% at 49^{o}C. The highest recorded value of lebek-salt was 35.15 % in100^{o}C and the least being 29.75±0.15% in 49^{o}C. All the salts with gallants soldier recorded higher values than their corresponding values of all the salts with wheat straw. This could be attributed to the fact that gallants soldier lignocellulose composition was easily hydrolyzed than wheat straw. Bobleter, O. (2005) while researching on hydrothermal degradation of polysaccharides found that, the susceptibility of the polysaccharide depolymerization depended on structural diversity of the and structural environment.

Wheat Straw with magadi-salt recorded the highest percent delignification of all the salts tested. The recorded values showed some pattern in the degree of delignification for temperatures of 49^{o}C all through to 80^{o}C.The significant ($p \leq 0.01$) highest recorded value of magadi-salt being 23.22% in100^{o}C and the least was 14.17±0.10% in 49^{o}C. Para and lebek salts recorded similar trends like those of magadi with wheat straw but of lower % hydrolysis values ranging with highest of 17.94 % to a lowest of 11.34%

The maximum % degree hydrolysis was recorded at temperatures of 100^{o}C for all the three salts. This could be attributed to increase in rate of hydrolysis reactions of the salts with the lignocelluloses. Schmid & Bjerre, (1997) in their research on wheat straw hydrolysis found out that, changing temperature from 150^{o}C to 185^{o}C increased the degree of hydrolysis of wheat straw by sodium carbonate from 33% to 77% at constant salt to sample ratio of 1: 10. Also, Kim *et al*., (2005) used lime to pretreat corn stover and obtained maximum lignin removal of 87.5% at 55℃ up from lower values at lower temperatures. Iuliana, 2009, Ghose, 1987 in their studies found that at 120°C temperature, hydrolysis sugar yield increased as compared sugar concentration at low temperature pretreatments. Alkali hydrolysis at Elevated temperatures reduces contact time of hydrolysis (Ghose *et al*, 1987).

3.14. Salt Hydrolysis of Plant Samples at Different Media

Treatment of salt samples with hydrogen peroxide, ammonia and dilute hydrochloric acid reagent solutions done recorded dramatic results. The hydrolytic effects on the plant samples, at room temperature and atmospheric pressure determined had a decrease of pH values with each addition of peroxide volumes. Refluxing time was 4.0 hours and the ratios of salt: residue used was 1:5. Higher values of hydrolysates were recorded in the salt-oxidative regimes then followed by the acidic-salt regimes and finally alkaline salt hydrolysis.

3.14.1: Salt Hydrolysis in Oxidative Media.

Table 3.13. Magadi with wheat straw

Sample Code	pH		H_2O_2/ mL	% Hydrolysates
	Without H_2O_2	With H_2O_2		
12	11.23 ± 0.02	10.32 ± 0.02	2.0 ± 0.2	29.7 ± 0.1
14	11.27 ± 0.02	9.61 ± 0.01	4.0 ± 0.2	28.6 ± 0.1
16	11.30 ± 0.02	9.25 ± 0.02	8.0 ± 0.2	28.9 ± 0.3
18	11.22 ± 0.02	9.17 ± 0.02	12.0 ± 0.2	30.1 ± 0.1
20	11.22 ± 0.02	9.17 ± 0.02	16.0 ± 0.2	32.4 ± 0.3

Table 3.14. Magadi with Gallants soldier

Sample Code	pH		H_2O_2/ mL	% Hydrolysates
	Without H_2O_2	With H_2O_2		
12	11.27±0.02	10.45±0.02	2.0±0.2	30.7±0.3
14	11.26±0.02	10.10±0.02	4.0±0.2	32.8±0.2
16	11.29±0.02	9.63±0.02	8.0±0.2	45.7±0.3
18	11.28±0.02	9.40±0.02	12.0±0.2	48.9±0.4
20	11.24±0.02	9.13±0.02	16.0±0.2	41.1±0.2

Table 3.15. Para with wheat straw

Sample Code	pH		H_2O_2/ mL	% Hydrolysates
	Without H_2O_2	With H_2O_2		
12	10.99±0.02	10.67±0.02	2.0±0.2	18.2±0.2
14	10.87±0.02	9.36±0.02	4.0±0.2	22.3±0.3
16	10.86±0.02	9.02±0.02	8.0±0.2	25.1±0.2
18	10.84±0.02	8.72±0.02	12.0±0.2	23.7±0.2
20	10.77±0.02	8.38±0.02	16.0±0.2	26.1±0.1

Table 3.16. Para with gallants soldier

Sample Code	pH		H_2O_2/ mL	% Hydrolysates
	Without H_2O_2	With H_2O_2		
12	10.84±0.02	9.61±0.02	2.0±0.2	49.3±0.1
14	10.78±0.02	9.31±0.02	4.0±0.2	53.0±0.1
16	10.88±0.02	9.06±0.02	8.0±0.2	64.2±0.3
18	10.79±0.02	8.64±0.02	12.0±0.2	65.9±0.5
20	11.02±0.02	8.33±0.02	16.0±0.2	68.4±0.3

Table 3.17. Lebek with wheat straw

Sample Code	pH		H_2O_2/ mL	% Hydrolysates
	Without H_2O_2	With H_2O_2		
12	10.74±0.02	9.70±0.02	2.0±0.2	16.0±0.3
14	10.83±0.02	9.41±0.02	4.0±0.2	15.1±0.2
16	10.9±0.02	9.06±0.02	8.0±0.2	20.3±0.5
18	10.87±0.02	8.74±0.02	12.0±0.2	27.9±0.6
20	10.79±0.02	8.53±0.02	16.0±0.2	31.0±0.3

Table 3.18: Lebek with gallants soldier

Sample Code	pH		H_2O_2/ mL	% Hydrolysates
	Without H_2O_2	With H_2O_2		
12	10.84±0.02	9.61±0.02	2.0±0.2	36.6±0.5
14	10.88±0.02	9.31±0.02	4.0±0.2	38.1±0.2
16	10.88±0.02	9.06±0.02	8.0±0.2	40.±0.3
18	10.79±0.02	8.64±0.02	12.0±0.2	45.7±0.4
20	11.02±0.02	8.33±0.02	16.0±0.2	52.6±0.2

From the data in Tables 3.13; 3.14; 3.15; 3.16; 3.17 and 3.18, all salt solutions had a significant ($p \leq 0.01$) gradual decrease in pH with subsequent addition of peroxide. Magadi-salt recorded an initial salt pH value of 11.39 that decreased gradually. The pH value of 10.32 was obtained after an addition of 2.0 mL peroxide and lowest pH value

of 9.13 was recorded after adding 16.0 mL peroxide, Table 3.13. Gallant soldier percent hydrolysis increased gradually with increased peroxide volumes and subsequently decreased in pH values with each peroxide addition. Percentage hydrolysis ranged between 41.1±0.2 to 48.9±0.4% with peroxide volume of ranging from 4.0 mL to 16.0 mL, Table 3.14. For wheat straw percentage hydrolysis ranged between 28.6 ± 0.1% to 30.1±0.1% with a peroxide volume ranging from 2.0 mL to 16.0 mL. It showed no clear pattern of percent hydrolysis with increased peroxide volumes and decreased pH values.

Para-salt initial salt pH value of 10.99 decreased gradually, Table 3.15. The pH value of 10.67 was obtained after an addition of 2.0 mL peroxide. The lowest pH value of 8.33 was recorded after adding 16.0 mL peroxide. Wheat straw percent hydrolysis significantly ($p \leq 0.01$) ranged between 18.2±0.2 and 25.1±0.3% with a peroxide volume ranging from 2.0 mL to 16.0 mL. There was no clear pattern of percent hydrolysis with increased peroxide volume and decreased pH values. Gallant soldier percent hydrolysis significantly ($p \leq 0.01$) ranged between 49.3±0.1 and 68.4±0.3% with peroxide volume of between 4.0 mL to 16.0 mL, Table 3.16. Percentage hydrolysis increased gradually with increased peroxide and subsequent decrease in pH values with each peroxide addition.

The initial salt pH value of lebek-salt was 11.74 and the pH decreased gradually on addition of H_2O_2. The pH value of 9.70 was obtained after an addition of 2.0 mL peroxide. The least pH value of 8.53 was recorded after adding a maximum volume 16.0 mL of peroxide to hydrolyzing mixture Table 3.17. Wheat straw percent hydrolysis significantly ($p \leq 0.01$) ranged between 16.0±0.3 and 31.0±0.3% with a peroxide volume ranging from 2.0 mL to 16.0 mL, Table 3.17 and Table 3.18. There was no clear pattern of percent hydrolysis with increased peroxide volume and decreased pH values.

Generally there was increased degree of hydrolysis with increased peroxide additions, which was comparable with studies done by Taylor & Weygandt, (1974) where they found that, the wet alkaline oxidation process convert largely lignin to CO_2, H_2O and carboxylic acids. Ahring et al., (1996) in their studies observed a tendency of high concentration of oxygen solubilizing hemicellulose in alkaline media. In their studies

Schmid & Bjerre, 1997 observed that the base and an oxidizing agent achieved a high solubilisation of the hemicellulose from the wheat straw straw.

3.14.1: Ammonia-Salt Treatment of the Residue Plant Samples

Table 3.19. Magadi-Wheat straw

Sample Code	pH		NH_3/ mL	% Hydrolysates
	Without NH_3	With NH_3		
$magadi_1$-Wheat straw	11.29±0.02	11.45±0.03	2.0±0.2	21.8±0.2
$magadi_2$-Wheat straw	11.28±0.02	11.65±0.02	4.0±0.2	22.1±0.2
$Magadi_3$-Wheat straw	11.22±0.02	11.74±0.02	8.0±0.2	25.8±0.3
$Magadi_4$-Wheat straw	11.29±0.02	11.93±0.02	12.0±0.2	28.2±0.2
$Magadi_5$-Wheat straw	11.25±0.02	11.96±0.02	16.0±0.2	26.5±0.5
$Magadi_6$-Wheat straw	11.25±0.02	12.04±0.02	20.0±0.2	23.3±0.3

Table 3.20. $Magadi_1$-Gallants soldier

Sample Code	pH		NH_3/ mL	% Hydrolysates
	Without NH_3	With NH_3		
$Magadi_1$-Gallants soldier	11.27±0.02	11.53±0.02	2.0	38.7±0.3
$Magadi_2$-Gallants soldier	11.28±0.02	11.78±0.02	4.0	38.9±0.4
$Magadi_3$-Gallants soldier	11.28±0.02	11.87±0.02	8.0	40.1±0.2
$Magadi_4$-Gallants soldier	11.28±0.02	11.89±0.02	12.0	40.7±0.4
$Magadi_5$-Gallants soldier	11.32±0.02	12.08±0.02	16.0	40.7±0.1
$Magadi_6$-Gallants soldier	11.30±0.02	12.08±0.02	20.0	44.0±0.3

Table 3.21. Para$_1$-Wheat straw

Sample Code	pH		NH_3,/ mL	% Hydrolysates
	Without NH_3	With NH_3		
Para$_1$-Wheat straw	10.90±0.02	11.45±0.02	2.0±0.2	18.0±0.3
Para$_2$-Wheat straw	10.89±0.02	11.51±0.02	4.0±0.2	18.8±0.2
Para$_3$-Wheat straw	10.86±0.02	11.72±0.02	8.0±0.2	19.2±0.1
Para$_4$Wheat straw	10.90±0.02	11.87±0.02	12.0±0.2	18.8±0.3
Para$_5$-Whweat straw	10.86±0.02	11.91±0.02	16.0±0.2	19.0±0.2
Para$_6$-Wheat straw	10.84±0.02	11.95±0.02	20.0±0.2	19.6±0.4
Para$_7$-Wheat straw	11.92±0.02	12.00±0.02	24.0±0.2	16.8±0.4

Table 3.22. Para1-Gallants soldier

Sample Code	pH		NH_3,/ mL	% Hydrolysates
	Without NH_3	With NH_3		
Para$_1$-Gallants soldier	10.92±0.02	11.42±0.02	2.0±0.2	30.6±0.3
Para2-Gallants soldier	10.88±0.02	11.54±0.02	4.0±0.2	33.0±0.2
Para$_3$-Gallants soldier	10.78±0.02	11.77±0.02	8.0±0.2	34.7±0.1
Para$_4$-Gallants soldier	10.84±0.02	11.82±0.02	12.0±0.2	35.9±0.1
Para$_5$-Gallants soldier	10.90±0.02	11.93±0.02	16.0±0.2	35.4±0.4
Para$_6$-Gallants soldier	10.92±0.02	11.96±0.02	20.0±0.2	36.6±0.3

Table 3.23. LebeK-Wheat straw

Sample Code	pH		NH_3,/ mL	% Hydrolysates
	Without NH_3	With NH_3		
LebeK$_1$Wheat straw	10.92±0.02	11.42±0.02	2.0±0.2	13.7±0.3
LebeK$_2$Wheat straw	10.85±0.02	11.59±0.02	4.0±0.2	13.8±0.5
LebeK$_3$Wheat straw	10.79±0.02	11.72±0.02	8.0±0.2	16.2±0.2
LebeK$_4$Wheat straw	10.94±0.02	11.93±0.02	12.0±0.2	23.4±0.2
LebeK$_5$Wheat straw	10.90±0.02	12.48±0.02	20.0±0.2	29.5±0.4

Table 3.24. LebeK- Gallants soldier

Sample Code	pH		NH_3,/ mL	% Hydrolysates
	Without NH_3	With NH_3		
$Para_1$.Gallants soldier	10.98±0.02	11.22±0.02	2.0±0.2	29.6±0.2
Para2.Gallants soldier	10.98±0.02	11.44±0.02	4.0±0.2	32.5±0.3
$Para_3$.Gallants soldier	10.88±0.02	11.67±0.02	8.0±0.2	34.8±0.1
$Para_4$.Gallants soldier	10.78±0.02	11.72±0.02	12.0±0.2	35.6±0.1
$Para_5$.Gallants soldier	10.94±0.02	11.93±0.02	16.0±0.2	35.9±0.4
$Para_6$.Gallants soldier	10.97±0.02	11.98±0.02	20.0±0.2	37.7±0.5

The initial pH for magadi-salt was 11.28 and it significantly ($p \leq 0.01$) increased gradually as the ammonia was added, Table 3.19. A pH value of 11.65 was obtained after an addition of 4.0 mL ammonia and the highest pH value of 12.00 was recorded after adding 20.0 mL of ammonia. Wheat straw percent hydrolysis ranged between 22.1±0.2 and 23.2±0.2% with ammonia volume ranging from 4.0 mL to 20.0 mL, Table 3.20. There was significantly ($p \leq 0.01$) no clear pattern of % hydrolysis with increased ammonia volume and increased pH values it was almost constant for all samples irrespective of the amount of ammonia added. Gallants soldier percent hydrolysis ranged between 40.1±0.2 and 44.0±0.3% with ammonia volume ranging from 4.0 mL to 20.0 mL. Percent hydrolysis increased gradually with increased ammonia and subsequent increase in pH values with each ammonia addition, Table 3.20.

The initial para-salt pH value of 10.90 increased gradually as the ammonia was added. The pH value of 11.45 was obtained after an addition of 2.0 mL ammonia. The highest pH value of 12.00 was recorded after adding 24.0 mL ammonia to para-wheat straw. Wheat straw percent hydrolysis varied between 16.0±0.4 and 19.6±0.4% with ammonia volume ranging from 2.0 mL to 24.0 mL, Table 3.21. There was clear pattern of percentage hydrolysis with increased ammonia volume and increased pH values. Gallants soldier percent hydrolysis significantly ($p \leq 0.01$) ranged between 29.3±0.3 and 36.6±0.3% with ammonia volume ranging from 2.0 mL to 20.0 mL. Percent hydrolysis

and pH values increased proportionately with increased ammonia addition volumes, Table 3.22.

The initial Lebek-salt pH value of 10.92 increased gradually as the ammonia was added, and a pH value of 11.42 was obtained after an addition of 2.0 mL ammonia Table 3.22. The highest pH value of 11.98 was recorded after adding 20.0 mL of ammonia. Wheat straw percent hydrolysis ranged between 13.0±0.2 and 29.6±0.4% with ammonia volume ranging from 2.0 mL to 20.0 mL. There was increasing pattern of percentage hydrolysis with increased ammonia volume and increased pH values Table 3.23.

Generally, there was an increased hydrolysis with increased pH but only to a limited range. This was also seen in studies carried out by Tortosa et al (2007) that, the amount of NaOH used for treatment ranges from 2 to 20%, and the temperature for the treatment ranges from ambient to 120 ^{0}C. Also, under mild conditions (low concentration and low temperature) substrate components remain unchanged. (Tortosa et al., 2007) Under harsher conditions, most of the lignin and hemicellulose are solubilized. (Ye sun et al, 2002, Iuliana, 2009).

3.14.3: Acid Treatment of Sample Salt Solutions;

Salt sample solutions were acid treated with a volume before the equivalence point/ end point. All the salts were significantly ($p \leq 0.01$) found to be basic. The salts were acidified with 0.1M HCl to determine the degree of hydrolysis of the lignocellulosic plant samples for a period of 1.00 hour.

Table 3.25a: Magadi salt against gallants soldier plant sample (ratio 1:5) refluxed for 1.0 hr

Salt Code	pH		Acid added /mL	% Hydrolyzates
	initial	After acid addition		
Magadi-I	9.98	9.53	10.0	39.8±0.2
Magadi-2	9.96	9.23	16.0	33.6±0.3
Magadi-3	9.98	8.47	21.0	37.2±0.2

The initial salt pH value of 9.98 decreased gradually as acidification with 0.1M of HCl. The significant ($p \leq 0.01$) pH value of 9.53 was obtained after an addition of 10.0 mL

0.1M HCl. The lowest significant ($p \leq 0.01$) pH value of 8.47 was recorded after adding 21.0 mL of 0.1M HCl. Gallants soldier percent hydrolysis ranged between 33.6±0.3 and 39.8±0.2% with 0.1M HCl volume ranging from 10.0 mL to 21.0 mL. The Percentage hydrolysis had no clear pattern with increased ammonia and subsequent decrease in pH values with each 0.1M HCl addition.

Table 3.25b: Para salt against Gallants plant sample (Ratio 1:5) refluxed for 1.0 hr

Salt Code	pH		Acid added/mL	% Hydrolyzates
	initial	After acid addition		
Para-I	10.06	9.56	2.0	27.4±0.1
Para-2	10.06	9.29	4.0	22.2±0.2
Para-3	10.05	9.06	6.0	28.5±0.3

The initial salt pH value of 10.06 decreased gradually as acification with 0.1M of HCl. The pH value of 9.56 was obtained after an addition of 2.0 mL 0.1M of the HCl and lowest pH value of 9.06 was recorded after adding 6.0 mL 0.1M HCl. Gallants Soldier percent hydrolysis ranged between 22.2±0.2and 28.5± 0.5% with 0.1M HCl volume ranging from 2.0 mL to 6.0 mL. Percent hydrolysis had no clear pattern with increased ammonia and subsequent decrease in pH values with the HCl addition. Hydrolysis was nearly constant.

Table 3.25c: LebeK salt against Gallants soldier plant sample (Ratio1:5) refluxed for 1.0 hr

Salt Code	pH		Acid added/mL	% Hydrolyzates
	initial	After acid addition		
LebeK-I	10.08	9.51	2.0	32.0±0.2
LebeK-2	10.07	9.25	4.0	33.6±0.3
Lebek-3	10.08	9.02	5.8	29.6±0.4

The initial salt pH value of 10.08 decreased gradually as acidification with 0.1M of HCl. The pH value of 9.51 was obtained after an addition of 2.0 mL 0.1M HCl and the lowest pH value of 9.02 was recorded after adding 5.8 mL of HCl. Gallants soldier percent hydrolysis ranged between 29.6±0.4 and 33.6±0.3% with 0.1M HCl volume

ranging from 2.0 mL to 5.8 mL. The percent hydrolysis had no clear pattern with increased ammonia and subsequent decrease in pH values with the HCl addition. Hydrolysis was nearly constant.

3.14.4: Salt Hydrolysis of Plant Samples in Neutral and Acidic Media

Three Solutions of each salt were prepared; to which two were treated with 0.1M HCl and the third no acid was added. To each solution 3.0g of dried plant sample was added and mixed and all were left overnight to soak. In the morning one acid treated sample and one non- acid treated were refluxed for 1.0 hour, filtered and the residue oven dried and weighed to constant mass. The remainder was filtered the residue oven dried to constant weight without refluxing.

Table 3.26. Soaking of Gallants Soldier plant samples in acid treated and non-acid treated salt solutions of each type

Salt Code	pH			Acid/ mL	Time/Hrs		% hydrolysates
	initial	Acid added	After reflux		Soaking	Reflux	
Magadi-1	9.93	9.58	8.75	10.0	16.00	1.0	35.5±0.1
Magadi-2	9.88	9.88	9.01	0.00	16.00	1.0	38.6±0.1
Magadi-3	9.91	9.55	8.93	10.0	16.00	0.0	23.1±0.2
LebekK-I	9.97	9.48	7.64	3.0	16.00	1.0	31.6±0.1
Lebek-2	10.02	9.52	8.05	3.0	16.00	0.0	25.0±0.3
Lebek-3	9.98	9.98	7.70	0.0	16.00	1.0	22.5±0.2
Para-1	9.96	9.55	7.84	3.0	16.00	1.0	24.8±0.2
Para-2	9.97	9.56	8.07	3.0	16.00	0.0	24.6±0.1
Para-3	9.96	9.96	8.06	0.0	16.00	1.0	24.7±0.3

Magadi- salt recorded a percentage hydrolysis of 38.6±0.1% for non-acid treated (neat magadi solution) and refluxed sample as compared to 35.5±0.1% for acid-treated and refluxed. An acid-treated but not refluxed recorded a percentage hydrolysis of 23.1±0.2%. Lebek- salt recorded 22.5±0.2% hydrolysis level for non-acid treated and refluxed sample as compared to 31.6±0.1% for acid-treated and refluxed. The acid-treated but not refluxed was significantly ($p \leq 0.01$) 25.0±0.3% hydrolysis. Para- salt recorded 24.7±0.3% hydrolysis for non-acid treated and refluxed sample as compared

to a 24.8±0.2% hydrolysis for acid-treated and refluxed. The acid-treated but not refluxed was 24.6±0.1% hydrolysis. This meant that the acid added significantly ($p \leq 0.01$) reduced the level of alkalinity thereby reducing the degree of delignification and hemicellulose removal.

Generally there was a decreased pH of the refluxing mixtures after reflux. This could be attributed the generation of acidic compounds and also consumed during the hydrolysis reactions of the hemicellulose and the lignin. These findings correlates well with those of other researchers who reported that, hydroxide anions catalyze cellulose hydrolysis, and also they get consumed during the course of reaction, causing the reaction pH to drop over the course of the reaction (Mosier et al., 2005, McDonald et al., 1983, Gentile et al., 1987).

Soaking of the plant samples improved the degree of hydrolysis levels. The hydrolytic levels were generally higher than when the samples were not soaked first before hydrolysis. This could be attributed to the infiltration of the sample structure by the ionic salt solutions. These findings compared well with those of Kim *et al.*, (2005) where they used lime to pretreat corn stover and obtained maximum lignin removal of 87.5% at 55^{o}C for four weeks with aeration.

There was no significant difference in the hydrolysis between the acid treated and non-acid treated samples. This could be attributed to the fact that both acids and bases possess hydrolytic properties towards hemicelluloses and lignin. In their studies Perez *et al*., (2007), and Fein *et al.*, (1991) showed that, continued to optimize process variables (temperature and residence time) in liquid hot water pretreatment of wheat straw and could achieve 80% hemicellulose up from 53%.

3.15. Chromatographic Analysis of Hydrolysates

3.15.1: TLC Profiles for Plant Samples.

In the TLC analysis using the solvent system of n-butanol-acetone-acetonitrile-water (10:10:5:5, v/v/v/v), a standard mixture of sugars were spotted along with analyte fractions which indicated the presence of sugars, D-fructose, D-Ribose, and D-Xylose in plant residues. This was the first assay of the sugar profiles of plant residues, which can be further evaluated for the sugar composition (Chidan et al., 2012)

Table 3.27. R_f values of hydolysates of plant residues

Samples	***Rf values (***n-butanol-acetone-pyridine-water (10:10:5:5, v/v/v/v)
1. (D-Fructose)	0.636
2. (D-sucrose)	0.717
3. (D-Xylose)	0.620
4. (D-Lactose)	0.930
5. (D-Ribose)	0.687
6.(D-Glucose)	0.709
7. Sample1(PGS)	0.618 and 0.729
8. Sample2 PWS	0.634 and 0.688

Thin layer chromatographic compositional analysis of hydrolysates from the samples gave a range of compounds from one to two. The mixture of n-butanol-acetone-pyridine-water (10:10:5:5, v/v/v/v) gave better results as compared with methanol-ethyl acetate where it gave a better resolution (Pak et al 2004). TLC analyses accurately confirm the presence of sugars, D-ribose and D- xylose in para-gallants soldier, and D-Fructose and D-Ribose in Para-wheat straw, Table 3.27, and Appendix C. Other sugars could not be detected may be their presence was below the detection limits.

3.16.2. HPLC Analysis of Sugars

Table 3.28. Percentage Total simple sugars and hydrolysates extracted from 3.00gm of plant sample from different treatments.

Treatment type		% Hydrolysates	% Total sugars
Peroxide	Magadi-Gallants soldier	48.9±0.4	0.15±0.01
	Magadi-Wheat straw	30.1±0.1	0.20±0.03
	Para-gallants soldier	68.4±0.3	0.12±0.02
	Para-Wheat straw	26.1±0.1	0.14±0.02
	Lebek-gallants soldier	54.7±0.7	0.15±0.03
	Lebek-Wheat straw	27.9±0.6	0.10±0.02
Neat salts	Magadi-Gallants soldier	44.0±0.3	0.53±0.01
	Magadi-Wheat straw	28.5±0.2	0.42±0.02
	Para-gallants soldier	36.5±0.3	0.42±0.02
	Para-Wheat straw	19.6±0.4	0.37±0.01
	Lebek-gallants soldier	37.6±0.4	0.45±0.03
	Lebek-Wheat straw	29.5±0.4	0.44±0.02
Acid	Magadi-Gallants soldier	40.8±0.2	2.32±0.02
	Magadi-wheat straw	32.7±0.3	1.75±0.05
	Para-gallants soldier	28.5±0.3	1.40±0.02
	Para-wheat sraw	22.6±0.2	0.43±0.01
	Lebek-gallants soldier	33.6±0.3	1.79±0.01
	Lebek-wheat straw	23.2±0.4	0.39±0.02

3.16.2.1. Alkaline peroxide

Table 3.28 had magadi with gallants soldier recording a percentage total sugars of 0.15±0.01% for peroxide treated as compared to 0.12±0.02% for para with gallants soldier and 0.15±0.03% of lebek with gallants soldier. Magadi with wheat straw recorded percentage total sugars of 0.20±0.03% for peroxide treated as compared to 0.14±0.02% for para with wheat straw and 0.10±0.02% of lebek with wheat straw.

Alkaline oxidative hydrolysis produced the least amounts of sugars, though it had the highest percentage degree of hydrolysis. This could be attributed to most of the

hydrolyzates being small molecular organic acids and inorganic acids. This compared well with studies done by Taylor & Weygandt, (1974); and McGinnis et al., (1983), who observed that basically, the wet oxidation process converts a large number of organic molecules (lignin and hemicelluloses) to CO_2, H_2O and low molecular weight carboxylic acids

Schmid & Bjerre, (1997) in their research of oxidative alkaline hydrolysis using sodium carbonate, found out that, the total amount of carboxylic acids produced increased with temperature. In their research the acids measured were acetic acid, formic acid, glycolic acid, oxalic, malic, and isobutyric acid. Schaleger & Brink, (1978) in their study concluded that, the wet oxidation process was a balance between solubilisation of the hemicellulose and degradation of the solubilized hemicellulose to other products. Schaleger & Brink, (1978) also found that, the longer the reaction time the more hemicellulose was solubilized, but also the more hemicellulose was degraded.

3.16.2.2. Neat Salts

Magadi with gallant's soldier recorded percentage total sugars of 0.53±0.01% for neat salts as compared to 0.42±0.02% for para with gallants soldier and 0.45±0.03% of lebek with gallants soldier. Magadi with wheat straw recorded percentage total sugars of 0.42±0.02% for neat salts treated as compared to 0.37±0.01% for para with wheat straw and 0.44±0.02% of lebek with wheat straw, Table 3.28.

The neat alkaline salts well compared with oxidative alkaline hydrolysis, producing high amouts of hydrolysates but low amounts of sugars. This could also be attributed to high amounts of organic acids produced during alkaline hydrolysis. This compared well with studies by Schmid & Bjerre, (1997) in their research of alkaline hydrolysis using sodium carbonate alone, found out that, the total amount of carboxylic acids produced increased with temperature. Schmid & Bjerre, (1997 in their research the acids measured were acetic acid, formic acid, glycolic acid, oxalic, malic, and isobutyric acid.

3.16.2.3 Acidic Salts

Magadi with gallant's soldier recorded percentage total sugars of 2.32±0.02% for acidic salts as compared to 1.40±0.02% for para with gallants soldier and 1.79±0.01% of lebek with gallants soldier. Magadi with wheat straw recorded percentage total sugars

of 1.75±0.05 for acidic salts as compared to 0.43±0.01% for para with wheat straw and 0.39±0.02% of lebek with wheat straw, Table 3.28.

Generally the amounts of sugars produced were low compared to the total percentage hydrolysates. This could be attributed to most of the lignin and the hemicellulose being converted to other hydrolytic products. This compared well with research done by Cantarella *et a.l*, (2004) and Weil *et al.*, (1997) who found that, during the pretreatment of lignocelluloses, the hemicellulose is often hydrolyzed to organic acids such as acetic acids and other acids formed from acetyl or other functional groups, in the biomass. Other researchers also found that, the degradations of sugars to acids might happen during uncatalyzed steam-explosion due to acidic conditions (Garcia-Aparicio *et al*., 2006)

3.17. UFLC Sugar Analysis

The lingo-cellulose samples were degraded by indigeneous salt solutions which was able to hydrolyze the lingo-cellulose polymer to its monomeric sugars. The UFLC chromatograms for hydrolysed plant samples; wheat straw and gallants soldier hydrolysates are shown in Appendix A1 and Appendix A2. The amounts of D-Ribose and D-Fructose were more than any other sugars for wheat straw Table 3.29. Fructose registered the highest amounts with a percentage of 13.78%, D-ribose had 4.77%, L-glucose and D-glucose registered amounts of 4.31% and 5.14% respectively, and sucrose registered 1.13%. Para-wheat straw registered a total reducing sugar levels of 30.12%wt/wt.

The amounts of D-Ribose and D-xylose were more than any other sugars for gallants soldier Table 3.30. D-xylose registered the highest amouts with a percentage of 12.85%, D-ribose had 4.22%, L-glucose registered amount of 0.42% and D-glucose was absent, sucrose had 0.60 % concentration. Para-gallants soldier registered a total reducing sugar levels of 18.09% wt/wt.

Generally, the reducing sugar amounts recorded were below the expected amounts since the percent amounts of cellulose and hemicellulose which are responsible for releasing simple sugars is 50 and 30% respectively. This could be associated with; breakdown of most sugars to carbon dioxide and water under high temperature. Also, during sugar extraction for analysis could lost some sugars. Taylor & Weygandt,

(1974); and McGinnis et al., (1983), observed that, during wet oxidation process breaking lingo-celluloses, a large number of organic molecules (lignin and hemicelluloses) converts to CO_2, H_2O and low molecular weight carboxylic acids.

Schmid & Bjerre, (1997) in their research of oxidative alkaline hydrolysis using sodium carbonate, found out that, the total amount of carboxylic acids (acetic acid, isobutyric acid glycolic acid and formic acid) produced increased with temperature. Schaleger & Brink, (1978) in their study concluded that, the wet oxidation process was a balance between solubilisation of the hemicellulose and degradation of the solubilized hemicellulose to other products. Schaleger & Brink, (1978) also found that, the long reaction time breaks down hemicellulose. Carbon dioxide and water.

Table 3.29. Potential reducing sugar types and amounts in para-wheat straw hydrolysates substrate.

Sample	Type of Simple sugar	%concentration wt/wt
Para-Wheat straw	D-Ribose	5.77
	D-Fructose	13.78
	L-Glucose	4.31
	D-Glucose	5.14
	Sucrose	1.13
	Totals	30.12

Table 3.30. Potential reducing sugar types and amounts in para-gallants soldier hydrolysates substrate.

Sample	Type of Simple sugar	%concentration wt/wt
Para-Gallants soldier	D-Ribose	4.22
	D-Xylose	12.85
	L-Glucose	0.42
	Sucrose	0.60
	Totals	18.09

3.18. GC Analysis of Cellulosic Ethanol

Fermentation was carried out for the hydrolyzed samples using Sacharomyces. sp from local yeast at room temperature for 7 days of incubation. Primary distillation of the fermented samples was carried out in fractional distillation apparatus at 80^{o}C and amount of ethanol produced was tabulated (Table 3.31.). A repeat fermentation and fractional distillation of the remains of after distilling ethanol was done in order to maximize sugar ethanol conversion rates to increase ethanol yields. Produced ethanol was determined by GC-FID method and chromatograms recorded (Figures 3.4, 3.5 and 4.6). Standard absolute ethanol were run and chromatograms of ; 30%, 40%, 50%, 60%, 70%, 80% and 98% recorded (Appendix 9.0). Figure 4.7, shows standard chromatogram for Ethanol 80%, while Figure 4.8, shows the standard curve for the absolute ethanol from which the samples concentration levels were determined. The higher value of R^2 (0.9890) suggested a correlation between all the two parameters ((Polakowski, *et al*. (2008))

The amounts of ethanol produced was highest in wheat straw with a 71.23% vol./wt ethanol, while gallants soldier recorded a 47.79% vol./wt ethanol (Table 4.31). These ethanol corresponded well with sugar composition and amounts recorded in Tables 3.29 and 3.30. This showed that wheat straw had higher reducing sugar amounts and more fermentable sugars (fructose, glucose and sucrose), hence high amounts of ethanol percentages. D-Ribose and D-xylose were found to be dominant in gallant's soldier. D-Ribose and D-xylose have been found to have very low sugar-ethanol conversion rates, which only increased with engineered Sacharomyces. Sp, (Polakowski, *et al*. (2008). Tony and co-workers, (1989) in their research reported that, glucose causes a 56% and 74% inhibition of fructose fermentation to ethanol in *Saccharomyces cerevisiae* and *Saccharomyces uvarum (carIsbergensis),* respectively, whereas fructose resulted in a 38% inhibition of glucose uptake in both strains under the conditions employed (Polakowski, *et al*. (2008).

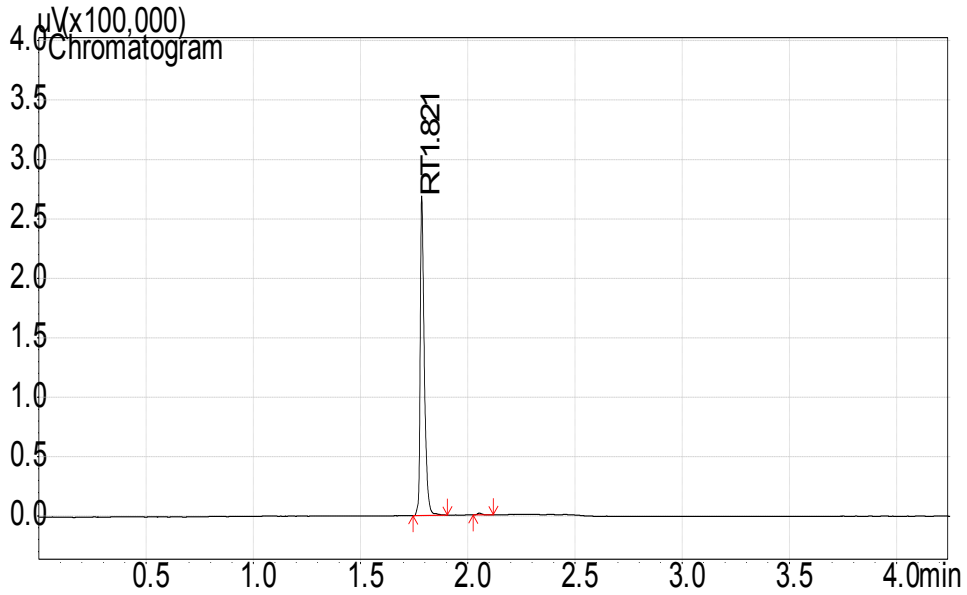

Figure 3.4. GC chromatogram for wheat straw fermented hydrolysates analysis

Peak#	Ret.Time	Area	Height	Conc.	Units	Mark	Compound ID#
1	1.786	358857.5	264659.8	0.00000	ppm		1
2	2.054	2465.2	1526.1	0.00000			

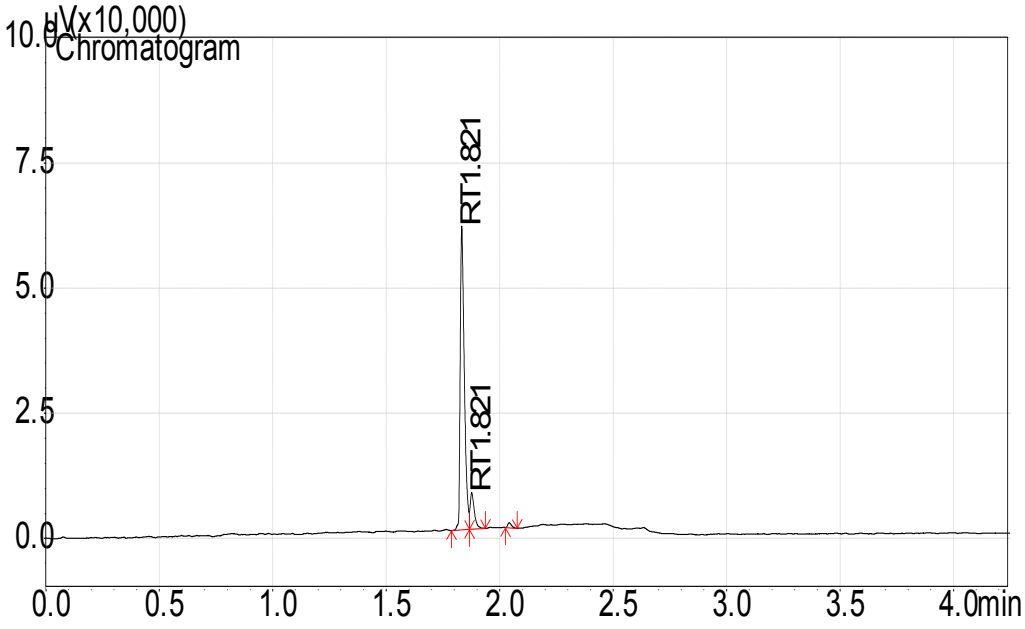

Figure 3.5. GC chromatogram for gallant's soldier second fermented hydrolysates analysis

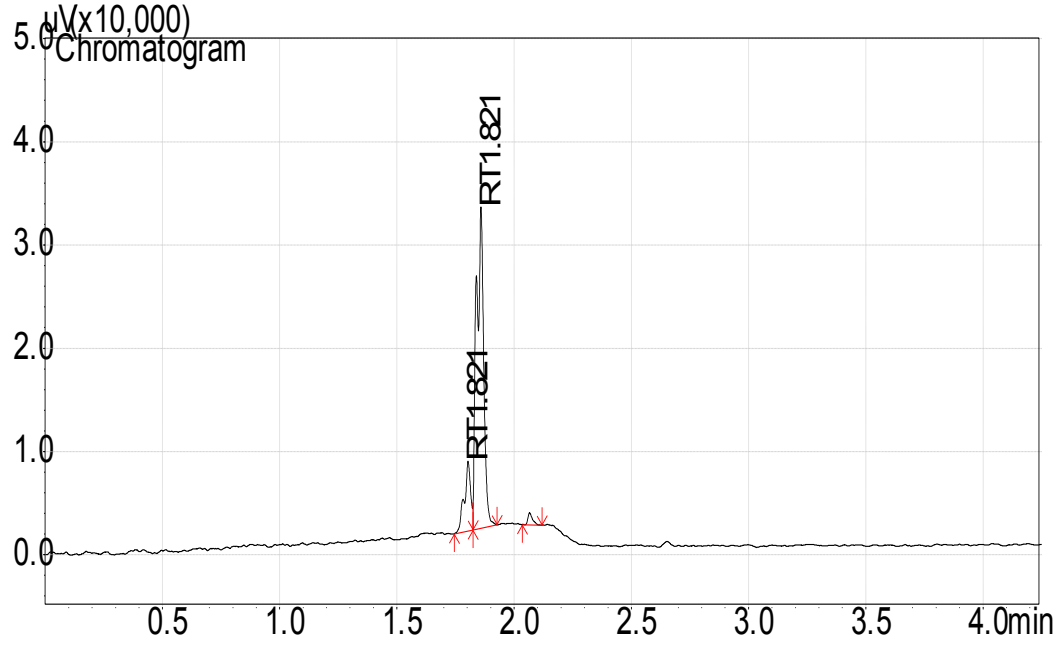

Figure 3.6. GC chromatogram for gallants soldier fermented hydrolysates analysis

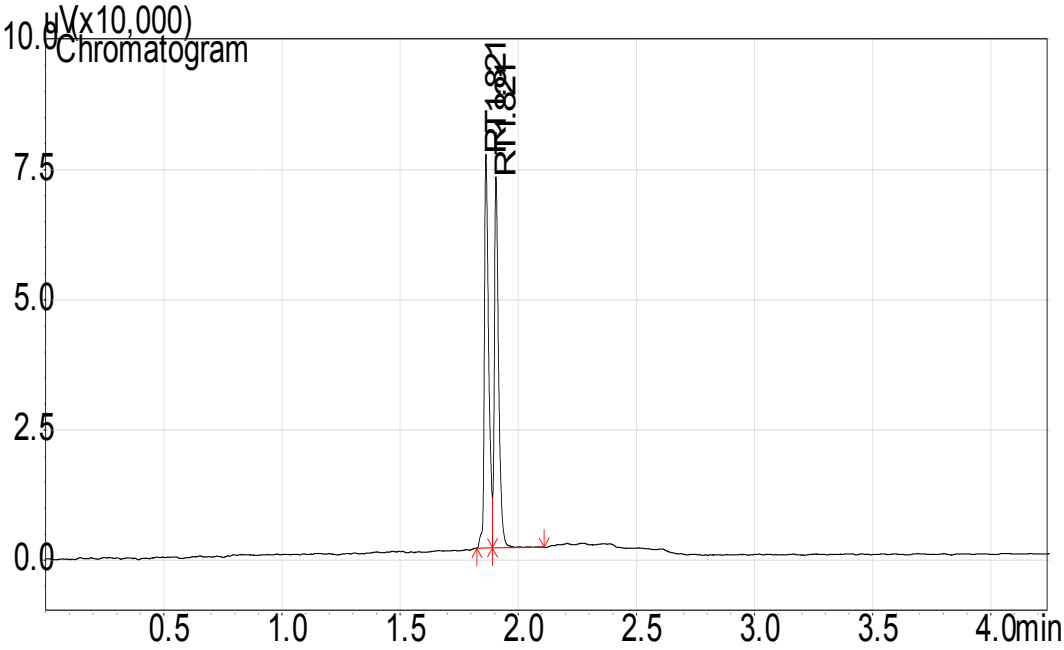

Figure 3.7. GC chromatogram for ethanol standard of 80%.

Table 3.31. Percentage ethanol per gram vol./wt in the two samples studied

Sample and hydrolysis media	Sample weight/ g	Ethanol Volume/ mL	%Ethanol/ vol/wt
Para-Wheat straw	25.54	16.36	64.07
Para-Gallants soldier	54.86	27.29	49.74

3.2.0: Conclusions and Recommendations

3.2.1. Conclusions

The three salts differ in their percent elemental compositional levels greatly (Magadi had 71.53 mg/g Na, and 0.25 mg/g Ca; Para had 27.23 mg/g Na and 52.56 mg/g Ca and Lebek had 25.83 mg/g Na and 166.09 mg/g Ca). The salts have the same carbonate and bi-carbonate structure which only differs in percent concentration levels (Magadi had 47.2%; Para had 13.9% and Lebek had 11.8% total carbonate). Magadi salt is basically a sodium carbonate-sodium bi-carbonate. The bicarbonate content is slightly higher than the carbonate. Para and Lebek salts have lower amounts of carbonates and bi-carbonate and they are more of calcium and iron salts. All the three salts were found to be alkaline salts with pH values greater than 9.8. FTIR spectra showed that all the three salts had bicarbonate in their structure.

The degree of hydrolysis of these salts depended on concentrations of hydrogen carbonate group. Salts with high concentration of bicarbonates and lower pH recorded higher percent degree of lingo-cellulosic hydrolysis. Percent degree of lingo-cellulosic salt hydrolysis depended on the type of ligno-cellulosic plant sample structure. From the above results it shows magadi-salt was best hydrolyser for both residues used. para-salt was slightly better than lebek-salt. Gallants soldier recorded the highest degree of delignification with neat magadi-salt. Thus produced more hydrolysates than Wheat straw-residues Concentration in μg/g sample. The combination of the wet alkaline oxidation process (water, H_2O_2 temperature) hydrolysis was efficient in pretreating wheat straw and gallants soldier. The degree of delignification and hemicellulose solubilisation increased with the reaction temperature and time.

Para hydrolysed plant samples produced better reducing sugars and ethanol amounts. Where wheat straw recorded the highest amounts of sugars and ethanol which was, 30.12% and 64.07% respectively. Gallants soldier produced sugars and ethanol amounts of 18.09% and 49.74% respectively. This was a clear indication that para hydrolyzes non-woody plant biomass to fermentable sugars at ambient temperatures.

3.2.2. Recommendations

1. Investigate on the catalytic species responsible for ligno-cellulosic hydrolysis in the indigenous salts.
2. Study the reaction rate of lingo-cellulosic hydrolysis using indigenous salts
3. Investigate on the kind of volatile materials during ligno-cellulosic breakdown with salt solutions.
4. Determine the role of evaporative solvent removal from extracted sugars in reducing sugar breakdown or conversions to other products.
5. Investigate on a combination laboratory salts to mimic the indigenous salts
6. Investigate on the Quality and performance of cellulosic ethanol with a petrol engine and the blends of the same.
7. The research findings should be piloted to determine its economic and industrial production viabilities.
8. Investigation of the best processing unit for hydrolysis process using the indigenous salts.
9. This method to be applied in researching more on the hydrolysis of other mineral salts on Agricultural weeds and residues or same salts on more other Agricultural weeds and residues.

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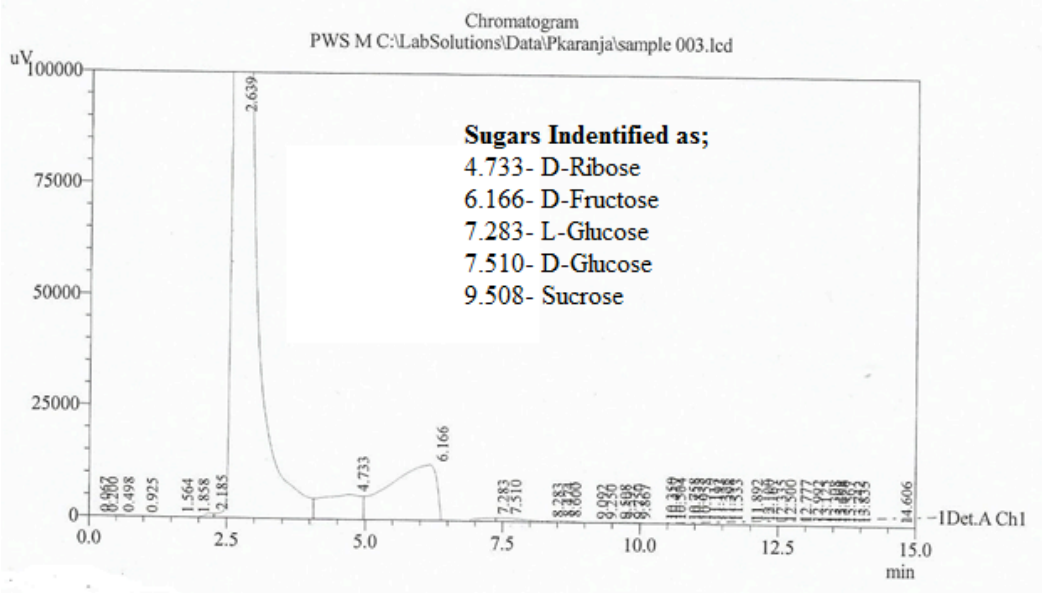
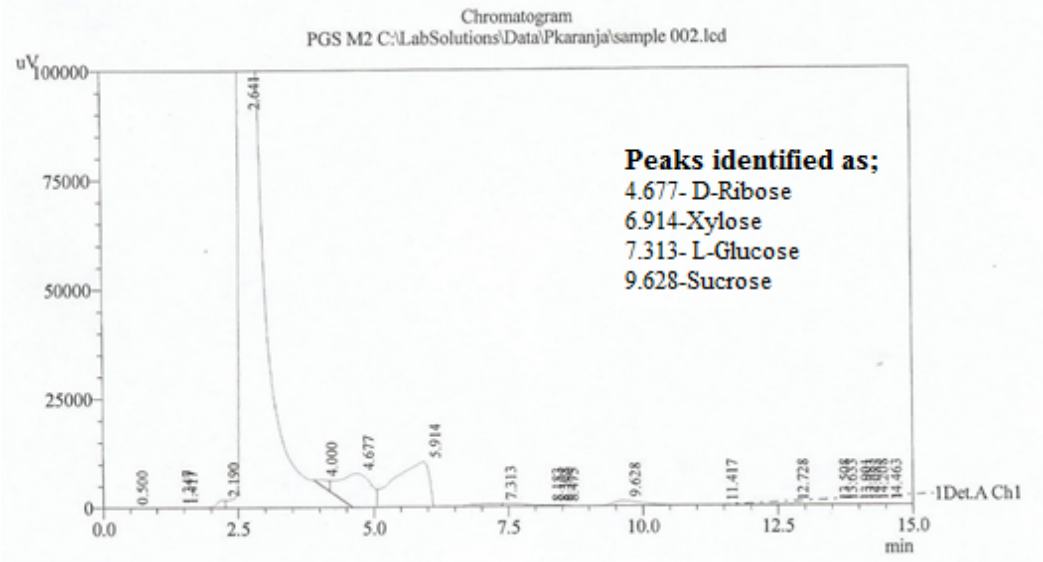
APPENDICES

Appendix A: UFCL Chromatograms for Sugars

Appendix A1. UFLC Chromatogram for para-wheat straw sugar analysis



Appendix A2. UFLC Chromatogram for para-gallants soldier sugar analysis



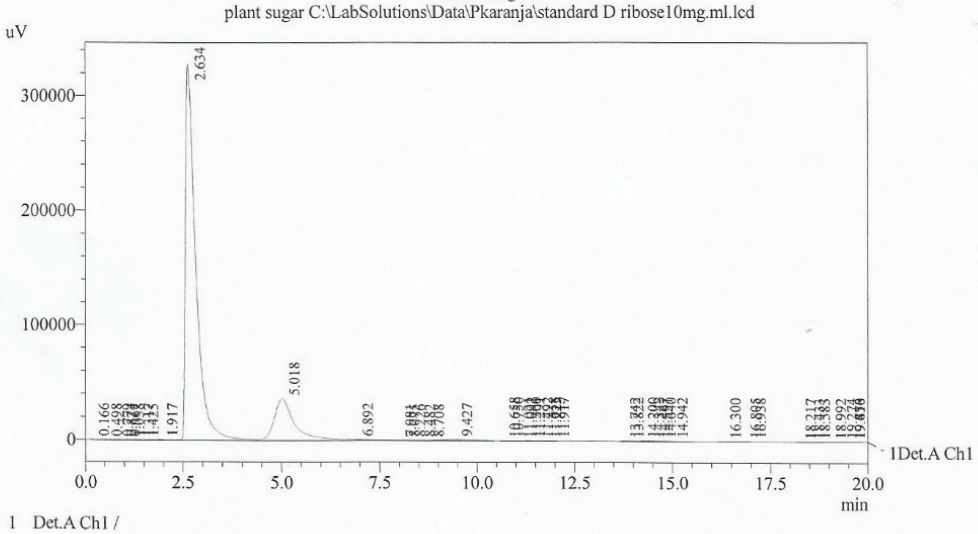
Appendix A3: D-Ribose

Sample Information

Acquired by	: Admin
Sample Name	: plant sugar
Sample ID	: masai mara
Tray#	: 1
Vail#	: 4
Injection Volume	: 20 uL
Data Filename	: standard D ribose10mg.ml.lcd
Method Filename	: mathege sugar.lcm
Batch Filename	: mathenge.lcb
Report Filename	: davidphy.lcr
Date Acquired	: 1/21/2015 4:13:30 PM
Data Processed	: 1/21/2015 4:33:33 PM

Chromatogram

plant sugar C:\LabSolutions\Data\Pkaranja\standard D ribose10mg.ml.lcd



1 Det.A Ch1 /

PeakTable

Detector A Ch1

Peak#	Ret. Time	Area	Height	Area %	Height %
1	0.166	568	45	0.008	0.012
2	0.498	2319	210	0.031	0.057
3	0.779	2158	195	0.029	0.053
4	0.874	1335	208	0.018	0.056
5	0.967	880	187	0.012	0.050
6	1.058	1732	168	0.024	0.045
7	1.317	243	50	0.003	0.013
8	1.425	186	48	0.003	0.013
9	1.917	8049	318	0.109	0.086
10	2.634	5923897	328108	80.404	88.497
11	5.018	1335709	36013	18.129	9.713
12	6.892	152	32	0.002	0.009
13	7.981	523	62	0.007	0.017
14	8.075	450	74	0.006	0.020
15	8.226	1298	119	0.018	0.032
16	8.482	2147	153	0.029	0.041
17	8.708	1163	166	0.016	0.045
18	9.427	46563	941	0.632	0.254
19	10.658	139	28	0.002	0.008

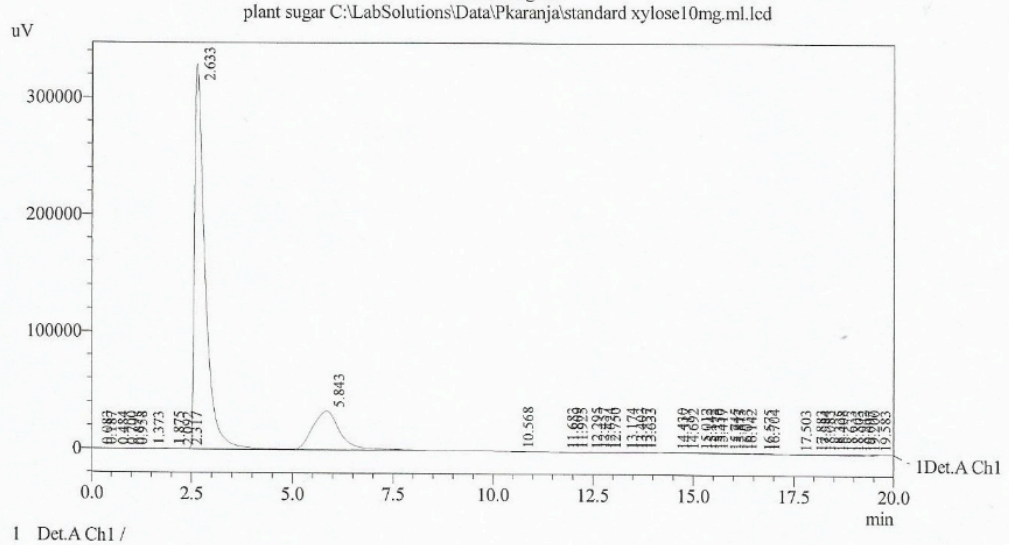
Appendix A4: D-Xylose

Sample Information

Acquired by	: Admin
Sample Name	: plant sugar
Sample ID	: masai mara
Tray#	: 1
Vail#	: 6
Injection Volume	: 20 uL
Data Filename	: standard xylose10mg.ml.lcd
Method Filename	: mathege sugar.lcm
Batch Filename	: mathenge.lcb
Report Filename	: davidphy.lcr
Date Acquired	: 1/21/2015 3:27:17 PM
Data Processed	: 1/21/2015 3:47:18 PM

Chromatogram

plant sugar C:\LabSolutions\Data\Pkaranja\standard xylose10mg.ml.lcd



1 Det.A Ch1 /

PeakTable

Detector A Ch1

Peak#	Ret. Time	Area	Height	Area %	Height %
1	0.083	208	47	0.003	0.013
2	0.187	988	112	0.013	0.030
3	0.484	1901	181	0.025	0.049
4	0.700	1379	130	0.018	0.035
5	0.875	846	110	0.011	0.030
6	0.958	994	99	0.013	0.027
7	1.373	728	75	0.010	0.020
8	1.875	6058	263	0.080	0.071
9	2.092	1885	168	0.025	0.045
10	2.317	631	127	0.008	0.034
11	2.633	5901953	328786	78.361	89.024
12	5.843	1551884	32983	20.605	8.931
13	10.568	335	36	0.004	0.010
14	11.683	165	32	0.002	0.009
15	11.809	497	95	0.007	0.026
16	11.925	569	105	0.008	0.029
17	12.295	4490	267	0.060	0.072
18	12.457	3888	287	0.052	0.078
19	12.624	1970	262	0.026	0.071

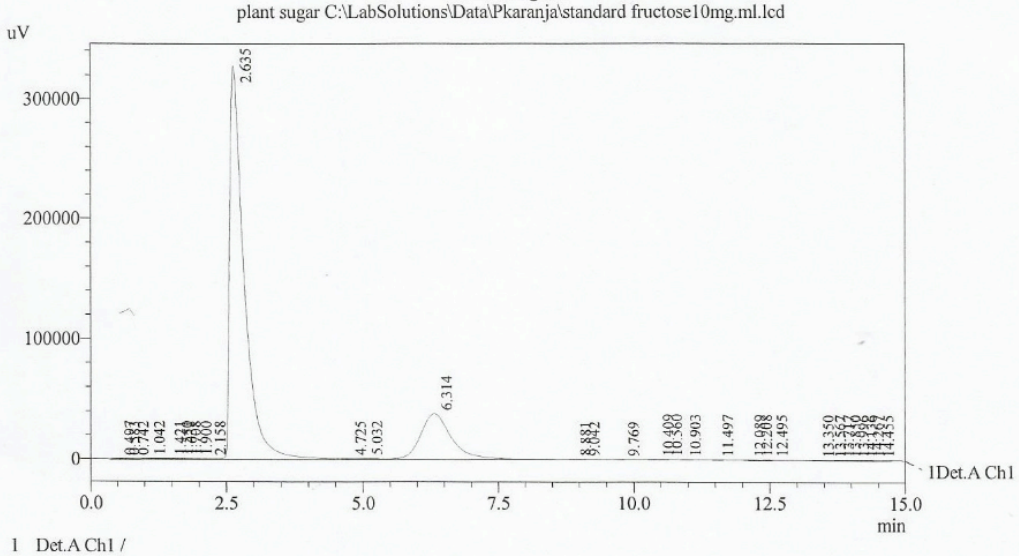
Appendix A5: D-Fructose

Sample Information

Acquired by	: Admin
Sample Name	: plant sugar
Sample ID	: masai mara
Tray#	: 1
Vail#	: 5
Injection Volume	: 20 uL
Data Filename	: standard fructose10mg.ml.lcd
Method Filename	: mathege sugar.lcm
Batch Filename	: mathenge.lcb
Report Filename	: davidphy.lcr
Date Acquired	: 1/21/2015 5:17:14 PM
Data Processed	: 1/21/2015 5:32:18 PM

Chromatogram

plant sugar C:\LabSolutions\Data\Pkaranja\standard fructose10mg.ml.lcd



1 Det.A Ch1 /

PeakTable

Detector A Ch1

Peak#	Ret. Time	Area	Height	Area %	Height %
1	0.497	2055	293	0.027	0.079
2	0.583	1870	290	0.025	0.078
3	0.742	2491	328	0.033	0.088
4	1.042	6858	432	0.091	0.116
5	1.421	12830	530	0.170	0.142
6	1.550	2741	502	0.036	0.135
7	1.631	2761	509	0.037	0.137
8	1.708	1239	501	0.016	0.135
9	1.900	11791	553	0.156	0.149
10	2.158	2020	455	0.027	0.122
11	2.635	6005177	327634	79.403	88.140
12	4.725	320	41	0.004	0.011
13	5.032	3717	169	0.049	0.046
14	6.314	1494050	37920	19.755	10.201
15	8.881	221	34	0.003	0.009
16	9.042	249	38	0.003	0.010
17	9.769	196	42	0.003	0.011
18	10.409	258	39	0.003	0.011
19	10.560	107	27	0.001	0.007

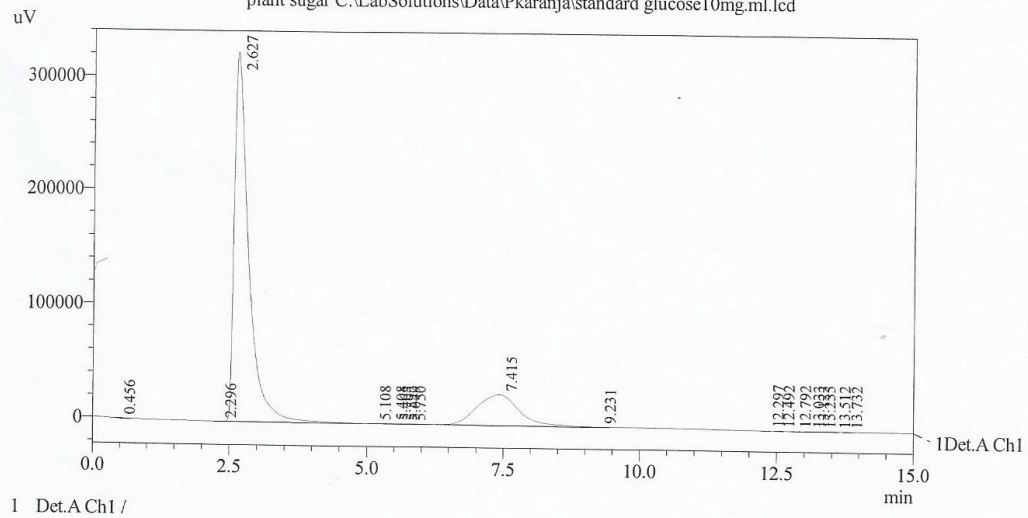
Appendix A6: D- Glucose

Sample Information

Acquired by	: Admin
Sample Name	: plant sugar
Sample ID	: masai mara
Tray#	: 1
Vail#	: 2
Injection Volume	: 20 uL
Data Filename	: standard glucose10mg.ml.lcd
Method Filename	: mathege sugar.lcm
Batch Filename	: mathenge.lcb
Report Filename	: davidphy.lcr
Date Acquired	: 1/21/2015 2:22:20 PM
Data Processed	: 1/21/2015 2:52:29 PM

Chromatogram

plant sugar C:\LabSolutions\Data\Pkaranja\standard glucose10mg.ml.lcd



1 Det.A Ch1 /

PeakTable

Detector A Ch1

Peak#	Ret. Time	Area	Height	Area %	Height %
1	0.456	1154	81	0.016	0.023
2	2.296	159	33	0.002	0.009
3	2.627	5852132	324500	79.871	91.922
4	5.108	365	56	0.005	0.016
5	5.408	2235	178	0.031	0.050
6	5.465	935	193	0.013	0.055
7	5.592	1136	191	0.015	0.054
8	5.649	1018	195	0.014	0.055
9	5.750	3001	188	0.041	0.053
10	7.415	1461825	27027	19.951	7.656
11	9.231	1215	130	0.017	0.037
12	12.297	136	23	0.002	0.007
13	12.492	160	19	0.002	0.005
14	12.792	240	22	0.003	0.006
15	13.033	172	29	0.002	0.008
16	13.123	150	35	0.002	0.010
17	13.235	313	44	0.004	0.012
18	13.512	416	40	0.006	0.011
19	13.732	216	33	0.003	0.009
Total		7326979	353017	100.000	100.000

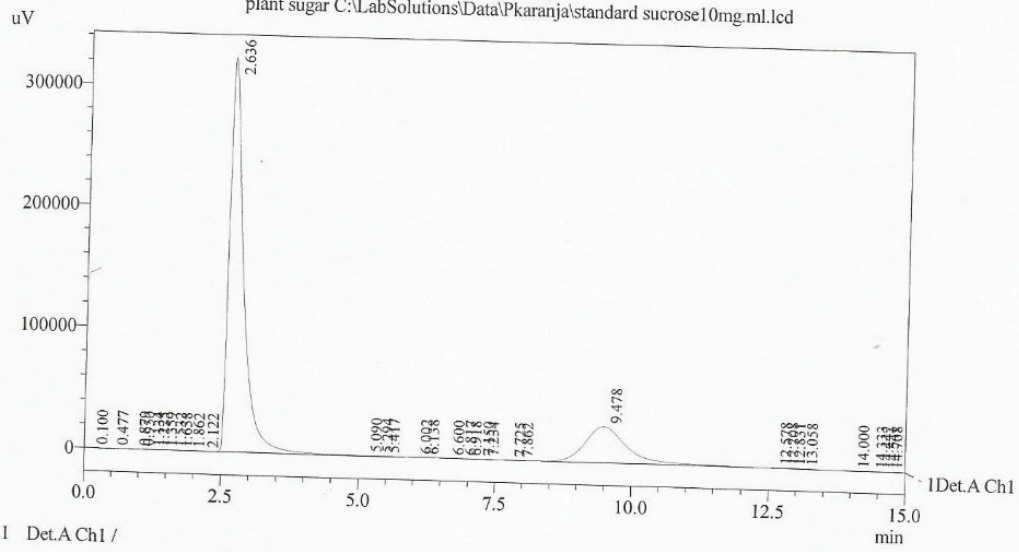
Appendix A7: Sucrose

Sample Information

Acquired by	: Admin
Sample Name	: plant sugar
Sample ID	: masai mara
Tray#	: 1
Vail#	: 3
Injection Volume	: 20 uL
Data Filename	: standard sucrose10mg.ml.lcd
Method Filename	: mathege sugar.lcm
Batch Filename	: mathenge.lcb
Report Filename	: davidphy.lcr
Date Acquired	: 1/21/2015 5:01:45 PM
Data Processed	: 1/21/2015 5:16:49 PM

Chromatogram

plant sugar C:\LabSolutions\Data\Pkaranja\standard sucrose10mg.ml.lcd



1 Det.A Ch1 /

PeakTable

Detector A Ch1

Peak#	Ret. Time	Area	Height	Area %	Height %
1	0.100	1068	70	0.014	0.020
2	0.477	827	118	0.011	0.033
3	0.879	206	43	0.003	0.012
4	0.950	132	37	0.002	0.010
5	1.134	192	37	0.003	0.010
6	1.225	113	27	0.002	0.007
7	1.359	364	41	0.005	0.011
8	1.532	171	40	0.002	0.011
9	1.638	161	33	0.002	0.009
10	1.862	896	75	0.012	0.021
11	2.122	169	23	0.002	0.007
12	2.636	5872375	324404	78.423	90.955
13	5.090	398	61	0.005	0.017
14	5.294	1527	145	0.020	0.041
15	5.417	2209	156	0.030	0.044
16	6.002	138	36	0.002	0.010
17	6.138	531	38	0.007	0.011
18	6.600	625	49	0.008	0.014
19	6.817	395	72	0.005	0.020

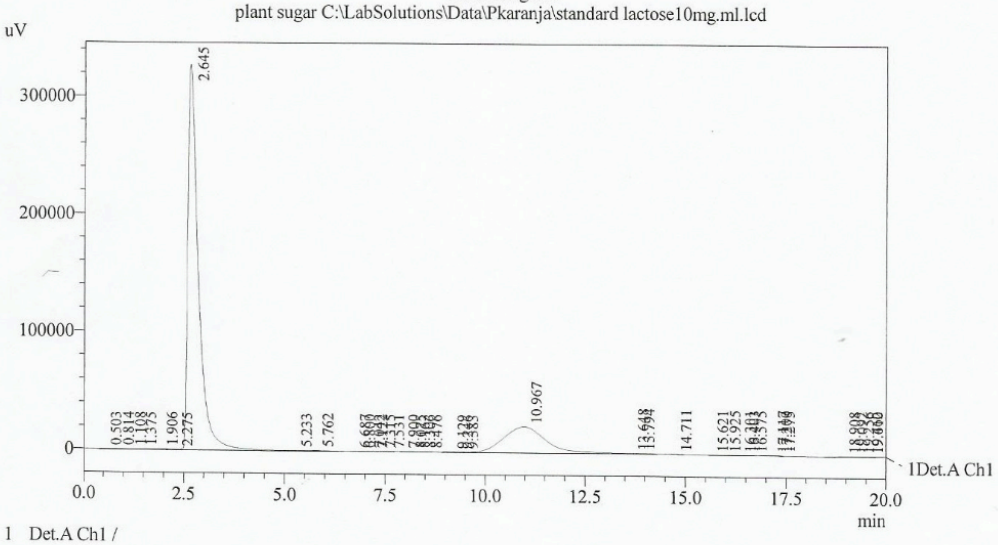
Appendix A8:Lactose

Sample Information

Acquired by	: Admin
Sample Name	: plant sugar
Sample ID	: masai mara
Tray#	: 1
Vail#	: 7
Injection Volume	: 20 uL
Data Filename	: standard lactose10mg.ml.lcd
Method Filename	: mathege sugar.lcm
Batch Filename	: mathenge.lcb
Report Filename	: davidphy.lcr
Date Acquired	: 1/21/2015 4:35:37 PM
Data Processed	: 1/21/2015 4:55:39 PM

Chromatogram

plant sugar C:\LabSolutions\Data\Pkaranja\standard lactose10mg.ml.lcd



1 Det.A Ch1 /

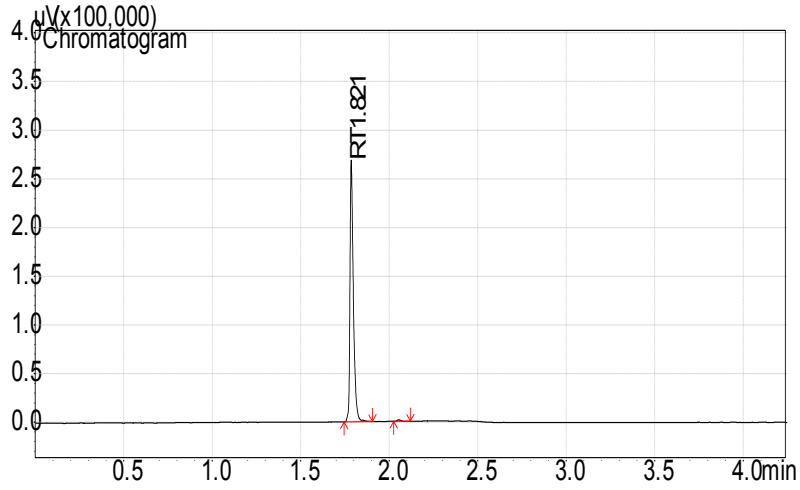
PeakTable

Detector A Ch1

Peak#	Ret. Time	Area	Height	Area %	Height %
1	0.503	3228	277	0.042	0.078
2	0.814	7600	303	0.099	0.085
3	1.108	2066	235	0.027	0.066
4	1.375	2296	259	0.030	0.073
5	1.906	19602	523	0.255	0.147
6	2.275	1888	311	0.025	0.087
7	2.645	5952630	327579	77.582	92.312
8	5.233	1589	142	0.021	0.040
9	5.762	17572	409	0.229	0.115
10	6.687	197	37	0.003	0.010
11	6.800	349	50	0.005	0.014
12	7.042	1057	123	0.014	0.035
13	7.122	1568	132	0.020	0.037
14	7.315	1122	122	0.015	0.034
15	7.531	758	87	0.010	0.025
16	7.900	181	14	0.002	0.004
17	8.075	168	40	0.002	0.011
18	8.163	345	61	0.004	0.017
19	8.306	485	72	0.006	0.020

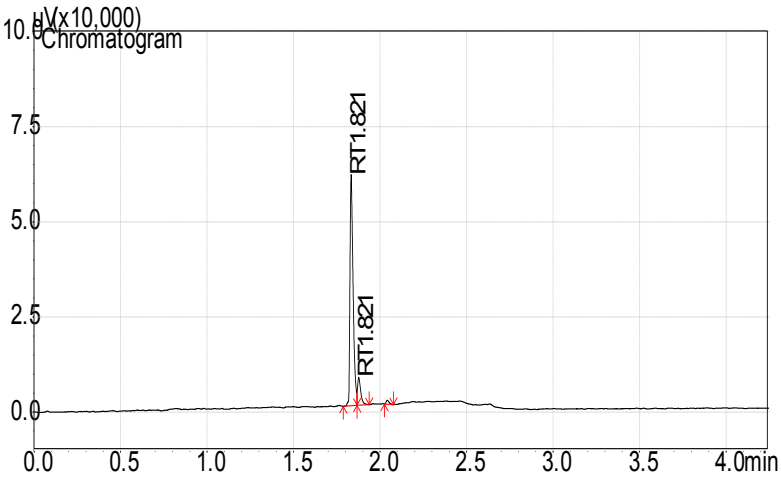
Appendix B: GC-FID Chromatograms of Cellulosic Ethanol

Appendix B1: Exp-pWS-1 para-Wheat straw hydrolysates fermentation analysis.



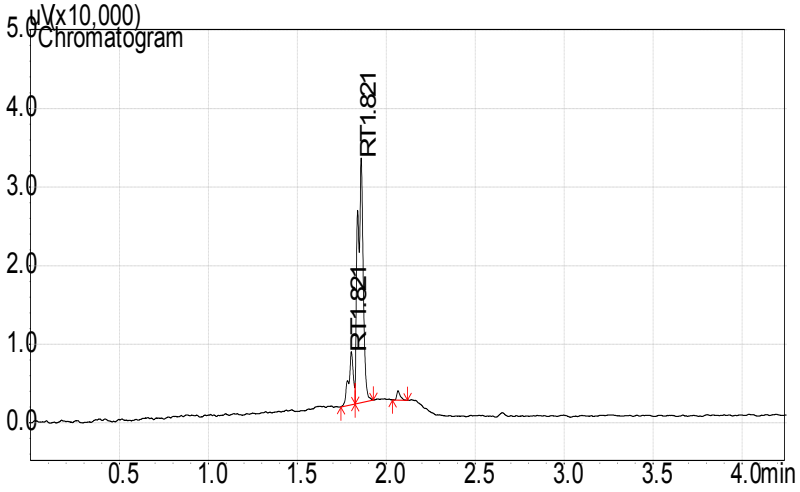
Peak#	Ret.Time	Area	Height	Conc.	Units	Mark	Compound ID#	Compound Name
1	1.786	358857.5	264659.8	0.00000		ppm	1	RT1.821
2	2.054	2465.2	1526.1	0.00000				

Appendix B2: exp-p-gs-1 f2 para-Gallants soldier hydrolysates fermentation analysis .



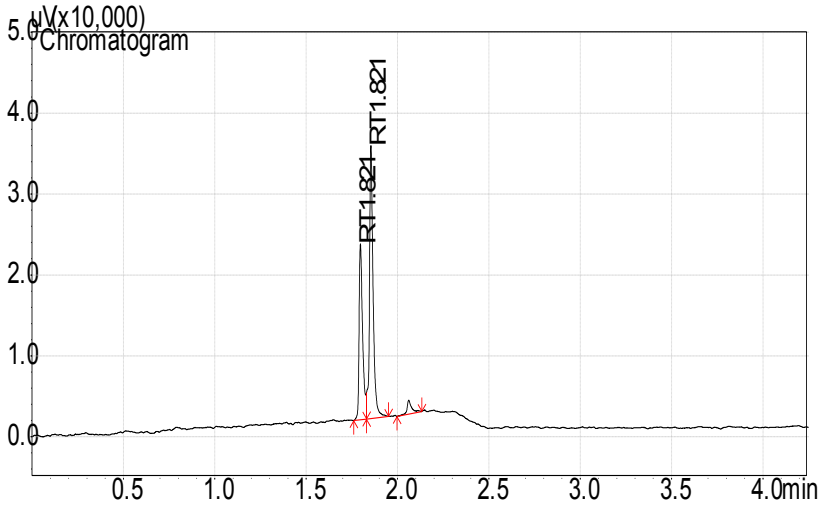
Peak#	Ret.Time	Area	Height	Conc.	Units	Mark	Compound ID#	Compound
1	1.833	76075.2		59375.2	0.70024	ppm	1	RT1.821
2	1.877	9313.8	7063.1	0.08573		ppm	V	1 RT1.821
2	2.042	1190.2	1012.6	0.00000				

Appendix B3: exp-p-gs-1 distillate 1 para-Gallants soldier hydrolysates fermentation analysis .



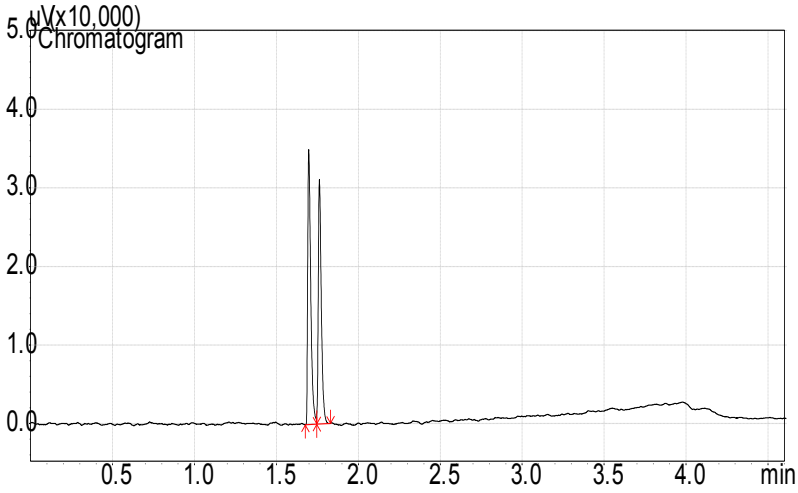
Peak#	Ret.Time	Area	Height	Conc.	Units	Mark	Compound ID#	Compound
1	1.804	12847.3		6644.4	0.00000	ppm	1	RT1.821
2	1.859	66837.7		30283.3	0.00000	ppm V	1	RT1.821
3	2.066	1600.9	1156.9	0.00000				

Appendix B4: Exp-p-gs-1 para-Gallants soldier hydrolysates fermentation analysis.



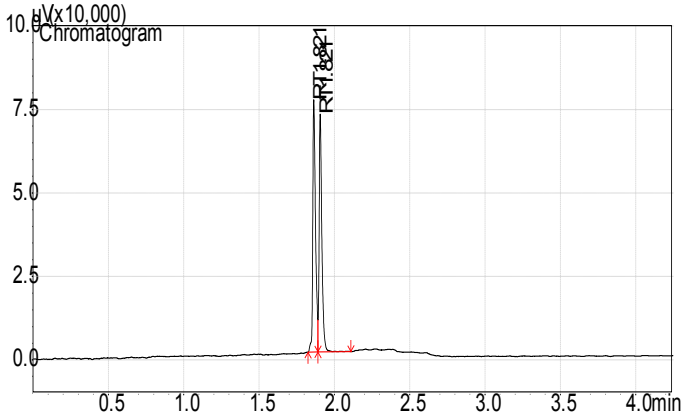
Peak#	Ret.Time	Area	Height	Conc.	Units	Mark	Compound ID#	Compound Name
1	1.797	29344.7		20870.7		0.00000 ppm	1	
2	1.855	46680.3		32712.8		0.00000 ppm V	1	
3	2.062	3206.7	1696.3	0.00000				

Appendix B5: Ethanol absolute 98% analysis**.**



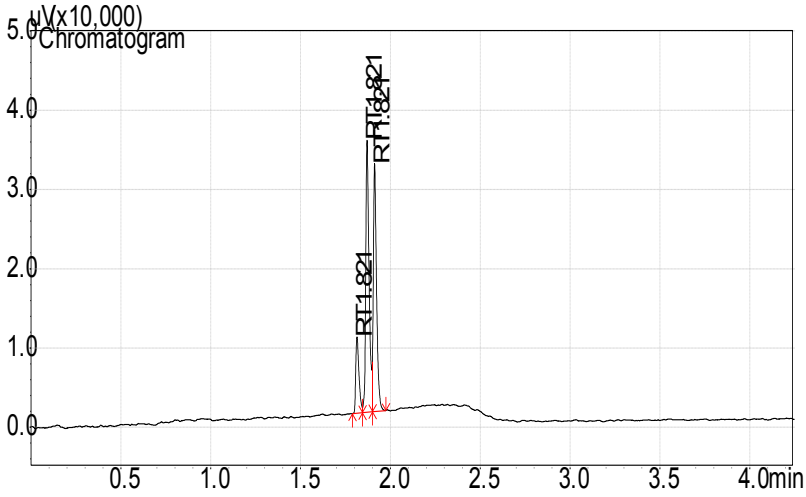
Peak#	Ret.Time	Area	Height	Conc.	Units	Mark	Compound ID#
1	1.698	45323.4	34448.4	53.26426			
2	1.763	39768.2	30704.4	46.73574			V

Appendix B6: Ethanol absolute 80% analysis.



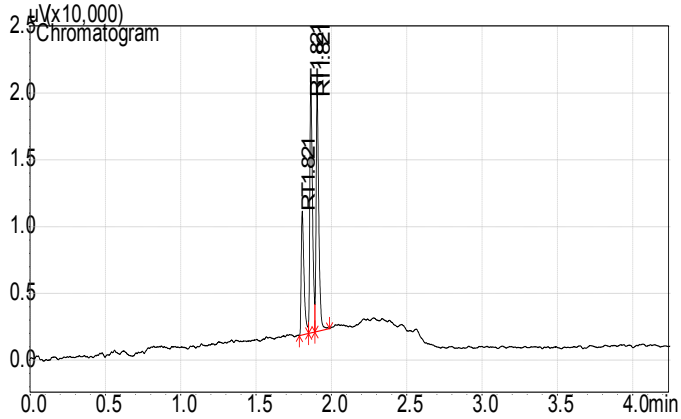
Peak#	Ret.Time	Area	Height	Conc.	Units	Mark	Compound ID#	
1	1.864	96256.6	72463.1	0.36287	ppm		1	RT1.821
2	1.906	89602.7	68783.4	0.33779	ppm	SV	1	RT1.821

Appendix B7: Ethanol absolute 70% analysis.



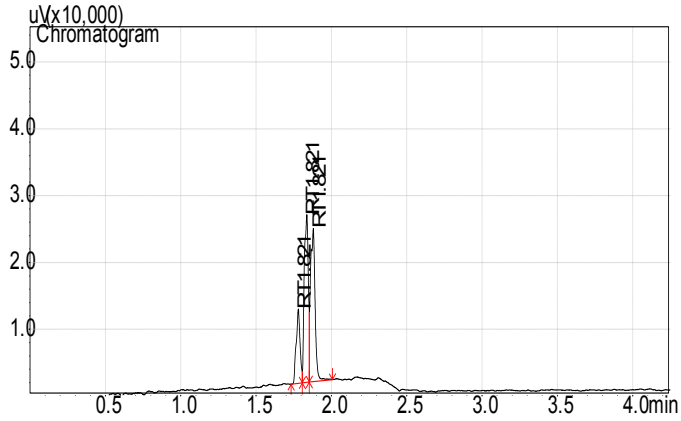
Peak#	Ret.Time	Area	Height	Conc.	Units	Mark	Compound	ID#
1	1.814	12032.5	9489.1	0.09655	ppm		1	RT1.821
2	1.870	44930.6	33796.2	0.36053	ppm	V	1	RT1.821
3	1.912	38237.0	30770.2	0.30682	ppm	V	1	RT1.821

Appendix B8: Ethanol absolute 60% analysis.



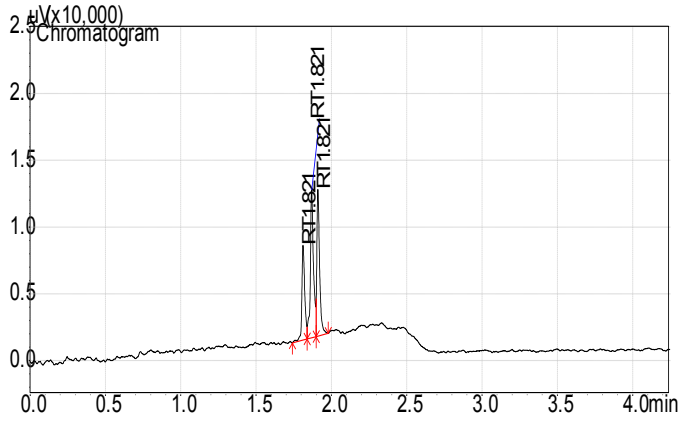
Peak#	Ret.Time	Area	Height	Conc.	Units	Mark	Compound	ID#
1	1.807	12016.7	8829.0	0.12990	ppm		1	RT1.821
2	1.864	23184.9	18651.2	0.25062	ppm	V	1	
3	1.906	22324.0	17465.6	0.24132	ppm	V	1	

Appendix B9: Ethanol absolute 50% analysis.



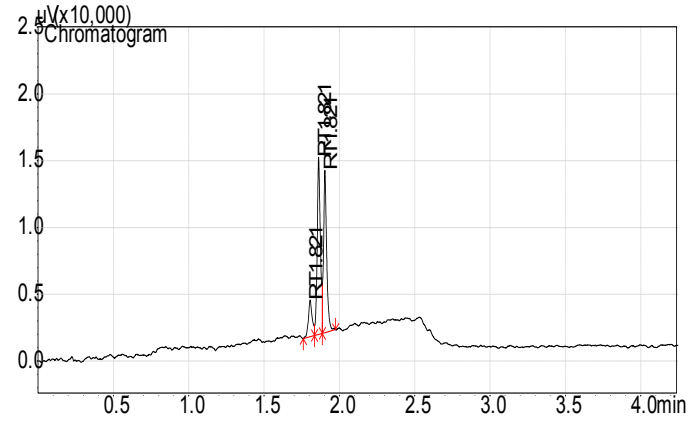
Peak#	Ret.Time	Area	Height	Conc.	Units	Mark	Compound	ID#
1	1.780	19715.1	10889.2	0.13409	ppm			1
2	1.836	46686.6	24837.8	0.31753	ppm	V		1
3	1.879	48587.3	22716.3	0.33046	ppm	V		1

Appendix B10: Ethanol absolute 40% analysis.



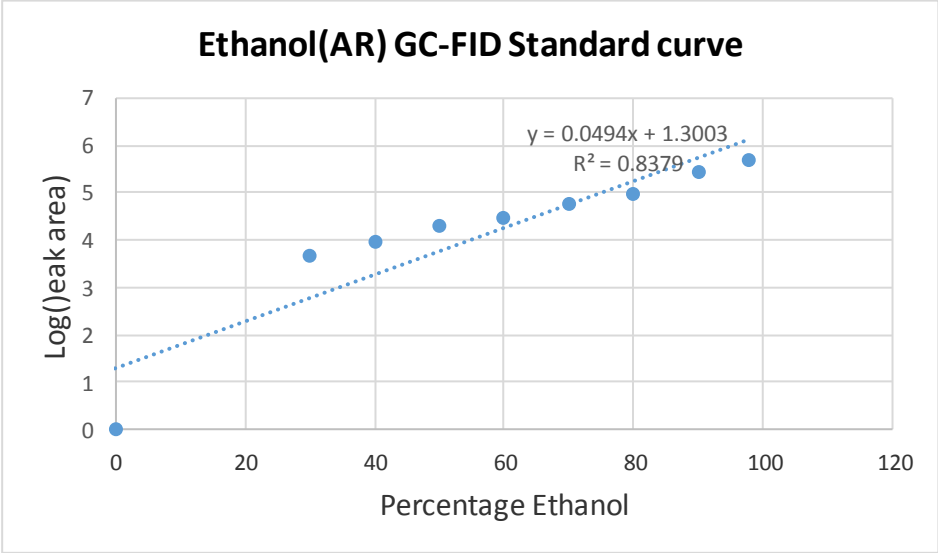
Peak#	Ret.Time	Area	Height	Conc.	Units	Mark	Compound	ID#	
1	1.812	9617.3	6859.2	0.14683	ppm		1		RT1.821
2	1.869	16647.0	10873.6	0.25415	ppm	V	1		RT1.821
3	1.911	13819.1	10747.4	0.21097	ppm	V	1		RT1.821

Appendix B11: Eethanol absolute 30% analysis.

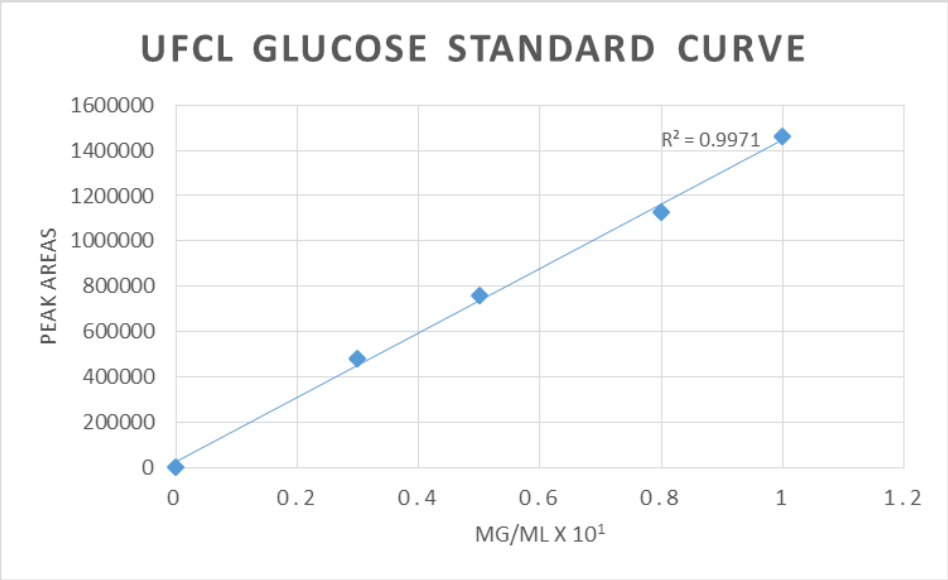


Peak#	Ret.Time	Area	Height	Conc.	Units	Mark	Compound ID#	
1	1.806	4844.1	2715.4	0.07030	ppm		1	RT1.821
2	1.862	19509.6	12896.8	0.28314	ppm	V	1	RT1.821
3	1.904	17739.2	11858.6	0.25745	ppm	V	1	RT1.821

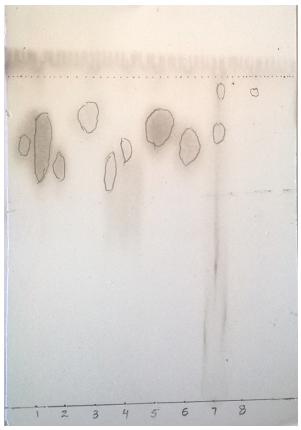
Appendix Bs. Absolute ethanol GC standard curves

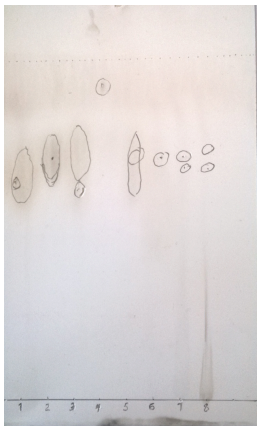


AppendixAs. Glucose standard curve of UFLC sugar analysis



Appendix C: Thin layer Chromatoplates





Appendix D: Publications from the Research Work

Appendix D1: Compositional and Structural Characterization of Three Basic Indigenous Salts Used in Kenya: A Case Study of 'Ebara', 'Magadi' and 'Lebek' Crystalline Salts.

Appendix D2: A Study of the Heterogeneous Dilute Indigenous Carbonate Salt Hydrolysis of the Non-Woody Ligno-Cellulosic Plant Samples

Appendix D3: Studying Indigenous Salt Hydrolysis of two Ligno-Cellulosic Agricultural Residues. Posta presentation 6th Hope Meeting. Tokyo Japan.