USMANU DANFODIYO UNIVERSITY, SOKOTO

(POST GRADUATE SCHOOL)

COMPARATIVE STUDY OF BIOFUEL POTENTIAL OF COUNTRY MALLOW AND WATER HYACINTH

A DISSERTATION

SUBMITTED TO POSTGRADUATE SCHOOL USMANU DANFODIYO UNIVERSITY SOKOTO, NIGERIA

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By

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DEDICATION

The dissertation work is dedicated to my family.

CERTIFICATION

This dissertation by Muhammad Auwal Ahmad (11211601004) has met the requirements for the award of Master Degree (M..Sc) in Renewable energy of the Usmanu Danfodiyo University, Sokoto, And is approved for its contribution to knowledge.

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ABSTRACT

Comparative study of biofuel pontentials of country mallow and water hyacinth was conducted. Yeast species was isolated from palm wine. The isolate was identified as *Sacchromyces cerevisae.* Proximate analysis was carried out on country mallow and water hyacinth .The following proximate composition for water hyacinth: moisture content (0.02%), ash (1.78%), nitrogen determination (1.18), organic matter (98.2%), carbon content (56.97%) and carbon- Nitrogen ratio (48.27%). Proximate composition for country mallow: moisture content (0.02%), ash (1.50%), nitrogen determination (0.94), organic matter (98.48%), carbon content (51.11% and) carbon-Nitrogen ratio (60.75%). Although the result revealed that the two plants have the potential to generate biogas, Such potential varied among the two plant wastes, with water hyacinth having produced the higher total volume (5787cm^3) than country mallow with total volume (5021cm^3) . The samples were hydrolysed with different concentration of dilute sulphuric acid (3%,4%,5%). The result revealed 4% sulphuric acid concentration to give the highest yield of reducing sugar. Effect of temperature, pH and fermentation days was carried out for bioethanol production .The best temperature for bioethanol production for the two plants was 35^o C, the best pH for water hyacinth was 5 but for country mallow it was found to be 6.The best fermentation period was 3days for both plants.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Nutrition is the relationship of foods to the health of human body i.e all the essential nutrients are supplied and utilized in adequate balance to maintain good health (Klein, 1997). Food is defined as any substance taken into body for the purpose of providing nourishment. Although a large amount of food is consumed, only portions of it (nutrients) nourish our body (Penfield, 1994).

Nutrients are conveniently grouped into these that collectives account for the bulk of the food known as macro nutrients (carbohydrates, fats, proteins and water), and the micronutrients (minerals and vitamins) that are present in only small amount. These nutrients serve mainly for growth, energy, maintenance and protection (Penfield, 1994). Many foods also contain antinutrients toxic materials that can be present naturally or according to the changes taking place during harvesting processing or preservation. (Shekhar *et al*., 2013).

In general, a substance taken as a food must possess a significant amount of these nutrients and minimum amount to toxic materials for it to be able to sustain a healthy body. Any nation that requires development must search to identify areas where it has comparative advantage over other nations and seek to develop the identified areas. Apart from hydrocarbons Nigeria has advantage in agricultural sector where varieties of products are produced due to the favourable climatic condition, good soil and the fact that over 70% of the entire land mass of the country is arable oil seeds is one of these major agricultural produce and serve as main source for production of oil that are normally called vegetable oil (Akubugwo *et al*., 2008) cotton is a natural vegetable fiber obtained from the cotton plant of the genus *gossypium hirsutum* and

belongs to malvacae family. It is a shrub which grows to about 40cm high (Shekhar *et al*, 2013) and grows in tropical and sub-tropical areas which its flowers either red or yellow. The capsules (seeds) of this plant are formed soon as the petals fall off and burst open into four parts upon maturity thereby revealing the cotton seeds after which they are harvested mechanically. The most destructive pest that attacks cotton plant is the boll weevil (Bedigia, 2007). Cotton seed oil is use for cooking or frying at home, use for soap production, body cream and margarine (Zhuan, 2010).

The cakes as by-products after extraction of oil are very useful in the production of animal feeds. Cotton seed meal can be used as a dry organic fertilizer, cotton salad also used in lubricants, paints, bath soaps, etc. the outer cover of cotton seed is a rich source of protein and cellulose. The oil has also been explored as a feedstock for biodiesel production (Saxena *et al*, 2011).

At global level, largest producers of cotton are China, followed by India. White gold cotton is Indians largest cash crop, large number of families in rural areas depends on cotton for their living. Production of cotton seeds in 2012-2013 is 10.2 million tones (Smith ,2006).

In proximate analysis, results obtained vary between or within seed type due to the location factors which appear to influence the chemical components of the seed (Waheed *et al*., 2010)

According to Lyimo *et al*, (2003), the use of seeds as food was also influenced by cultural acceptability and easy methods of processing. However in order to successfully introduce cotton seeds as food or as supplement into food items, there is a need to evaluate its nutritional status, this proximate analysis and other analytical methods are important tools. A lot of researchers reported the proximate composition and nutritional factors of different seeds (Smith, 2006;

Saxena,.*et al*,2011; Olaofe, *et al* 1994; Bediga, 2007; Ikurior and Babatunde,1987; Adeola and Ndudi, 2016).

From the reports, it is clear that cotton seeds food are of great importance to human nutrition and animals. Hence its knowledge of their nutritional potential is desirable.

1.2 LITERATURE REVIEW

The cotton plant is of the genus *gossypium* and belongs to malvacae family also called Auduga and the seeds is gurya in Hausa. It is a shrub which grows to about 40cm high (Shekhar *et al*., 2013) and grows in tropical and subtropical areas with its flowers either red or yellow. The capsules (seeds) of this plant are formed as soon as the petals fall off and burst open into four parts upon maturity thereby revealing the cotton seeds after which they are harvested (Shekhar *et al*, 2013).

Plate 1.1 photograph of Cottonseeds (**Brien**, ***et al*.,2013**).

Plate 1.2 Photograph of Cottonseeds (**Brien**, ***et al*.,2013**).

The seeds is covered with white hairs called the lint which is the main product used to make cotton textile. Depending on the species and variety, cotton lint has different colours (black, brown or red) and may be longer or shorter and thicker (Shekhar,*et al*.,2013).

The cotton seed has outstanding food values as well as pharmacological properties. The seed is rich in carbohydrate, proteins, minerals, fats and vitamins. The cotton seeds is also rich in oil which has high industrial value (Zhuan, 2010), and it's also used to make cotton seed cake which is used as substitute for soybean meal in broiler, because of its fibre content is used as a feed for ruminant animals, like cattle, goats e.t.c, powdered cotton seeds mixed with milk after sieving it are given to those people with headaches problems , or mixed with water and ginger use to treat burns, and is used in the treatment of snake and scorpion bites. In India cotton seeds

used for making bread, shortening(butter), biscuit, cakes, cuisines foods e.t.c. And oil in human body is used to prevents distinct heart vascular diseases and intestinal parasitosis (Boelhoure, 1983).

1.2.1 CARBOHYDRATES

Carbohydrates are polyhydroxy aldehydes or ketones or compounds (Tewari *et al*, 1998). And are the most abundant and widely distributed food components and constitutes a large proportion of the human diet. It's reported that in most part of the developing world, 50-75% of the total dietary energy comes from carbohydrates (Suma and Boelhouwer (1999).

Carbohydrates are widely distributed in plants and animals. And is reported that legumes contain carbohydrates within the range of 23- 66%. Alongside with protein, carbohydrates are involved in both structural and biological functions of the cell. Plants synthesize their carbohydrates via the process of photosynthesis using inorganic compounds, carbondioxide and water (Strov, 1989).

$$6CO_2+6H_2O \xrightarrow[\text{Chlorophyll}]{\text{Sunlight}} C_6H_{12}O_6+6O_2 \quad \text{-----1.1}$$

The glucose formed is stored in a form of starch or converted to the cellulose of the plant supportive tissue while animals derived carbohydrates ultimately from plants through food chain (McDonald *et al*.,1995).

1.2.1.1 Classification of carbohydrates

Naturally, carbohydrates may be present as free molecules, physically or chemically bound to other molecules. Individual molecules can be classified according to the number of

monomers that they contain as mono-, oligo- or poly- saccharides. Molecules in which the carbohydrates are covalently attached to proteins and lipids are known as glycol-proteins and glycol-lipids respectively and collectively known as complex carbohydrates. Some carbohydrates are digestible by humans and therefore provide an important source of energy, whereas others are ingestible (collectively known as dietary fibre), but important to human health (McDonald *et al*.,1995).

Carbohydrates are classified according to their complexity into monosaccharides, oligosaccharides, polysaccharides and complex carbohydrates; the first two known to be sugars while the last two are non-sugars (McDonald *et al*.,1995).

i. Monosaccharide

Monosaccharides are water-soluble, crystalline compounds containing three to seven carbon atoms per molecule. Monosaccharide may be subdivided into trioses, tetroses, pentoses, hexoses or heptoses. According to Ihekoranye and Ngoddy (1985), naturally occurring monosaccharides are mostly hexoses (glucose, fructose, mannose, and galactose). Glucose and fructose are found abundantly in nature, occurring free in some foodstuff and combined with each other as oligo- or polysaccharides (Sanches *et al*, 2000).Monosaccharides are either aldoses or ketoses depending upon whether the aldehyde or ketone group is present.(Avems, 2012).

Hexose

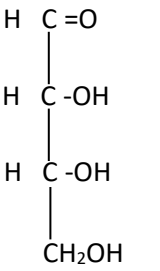
Hexoses have general molecular formula $C_6H_{12}O_6$.andare all aldoses except fructose, which is a ketose. Their formulae may be written in a straight chain or cyclic (ring) from under physiological conditions. Sugars exist mainly in a ring form. In both cases, sugars have two forms of isomers D and L depending upon the orientation of the hydroxyl group on the

penultimate carbon atom (Cs). For straight form, the D-form, has the -OH producing to the right while for L-form the -OH produce towards the left (in ring form, D-glucose, for example can occur as a pyranose ring while fructose may be in pyranose form but is more commonly a furanose with -OH group below or above the plain hence designated α- and β- D-glucose respectively. .(Avems,2012)

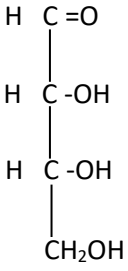
Pentose

Pentoses have general formula $C_5H_{12}O_6$. The most important members are the aldoses L-Arabinose, D-Xylose and D-Ribose, and the ketoses D-Xylulose , D- Rabilose. L-Arabinose and D-Xylose occurs as pentosans in arabinans and xylans respectively and are components of hemicelluloses in herbage. D-Ribose is represent in all living cells as a constituent of ribonucleic acid (RNA) and also as components of several vitamins and coenzymes (Mckinley,2014).

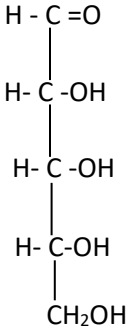
Tetrose **Pentose**



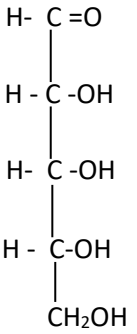
D-Erythrose



D-Therose

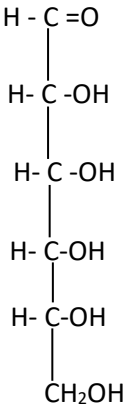


D-Ribose

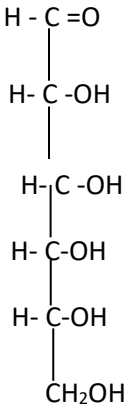


D-Xylose

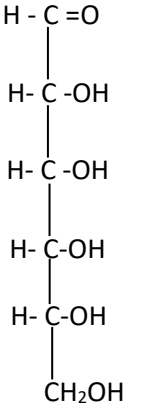
Hexoses



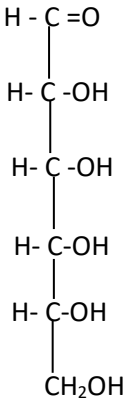
D-Mannose



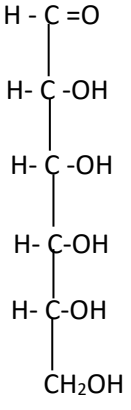
D-Glucose



D-AIdose



D-Allose



D-Altrose

Figure 1.1 Structures of some Monosaccharide

ii. Oligosaccharides

Oligosaccharides are carbohydrates with two to ten monosaccharide units linked by glycoside bonds .The disaccharides, trisaccharides and tetrasaccharides (starchyose) are the most commonly encountered oligosaccharides in food (Ihekoranye and Ngoddy, 1985).

Disaccharides are sugars composed of two monosaccharide residues jointed together by a glycosidic linkage. The most nutritionally important members are sucrose, maltose and lactose. **Sucrose:** Sucrose is the most widely abundant disaccharide in plants and added sugar in food products (Sachez-castillo *et al*., 2000).this sugar has many sources, dominated by sugarcane (200g/kg) sucrose composes of α-D-glucose and β-D-fructose jointed together through an oxygen bridge between their respective anomeric carbon atoms as shown in figure 1.2 as a result of this linkage, sucrose has no active reducing group, that is why it is refered to as a non-reducing sugar (Sachez-castillo *et al*., 2000).

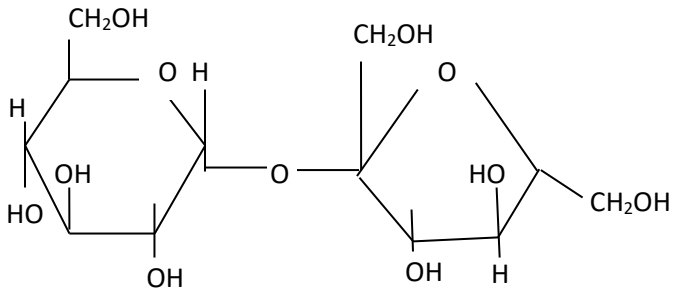


Figure1.2 Structure of Sucrose

Maltose: Maltose, or malt sugar is produced during the hydrolysis of starch by enzyme, amylase are dilute acid in plants. It is water soluble, less sweat than sucrose. It is composed of two α-D-glucose residues linked in the α-(1- 4) positions, hence has one active reducing agent (Sachez-Castillo *et al*, 2000).

Lactose: Lactose or milk sugar is a non-plant disaccharide consisting of one molecule of β-D-glucose jointed to one of β-D-galactose in a β-(1- 4) linkage (figure 1.4).

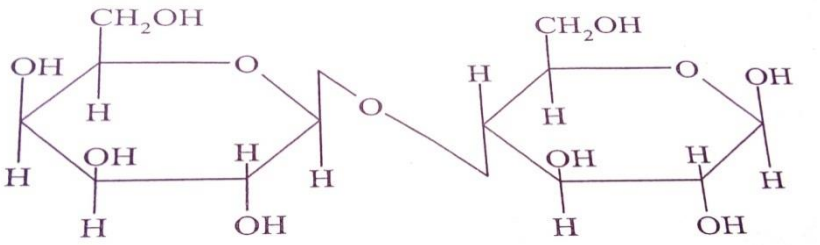


Figure1.3 Structure of lactose

iii. Trisaccharides or triose: The two most important naturally occurring trioses are raffinose and ketone. They are both non-reducing and on hydrolysis produce three molecules of monosaccharide sugars.

Raffinose: Raffinose is the commonest member of the group occurring widely in plant especially legumes (McDonald *et al.*,1995) this sugar is poorly absorbed when digested and results in their fermentation in the large intestine. This leads to gas production. Raffinose contains one molecule each of glucose, fructose and galactose as shown in figure 1.5

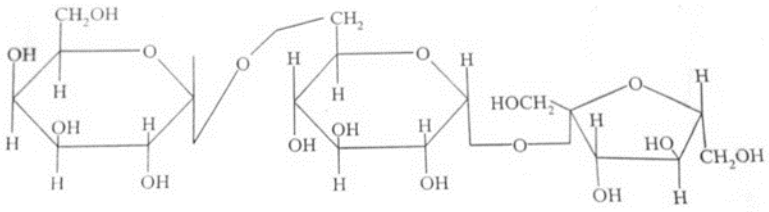


Figure1.4 Structure of Raffinose.

Stachyose: Stachyose is a tetrasaccharide having four monosaccharide residues (2-glucose, 1-glucose and 1-fructose), and is widely distributed in higher plants especially legumes.

iv. Polysaccharides: Polysaccharides are high-molecular weight carbohydrates containing more than ten monosaccharide units linked by glycosides bonds. they differ from one another in the nature of monosaccharides involved, in a molecular mass and in the type of chain-linking glycoside bonds. They may be composed of one type of repeating sugar unit or made up of more than one type. The former are called homopolysaccharides or homoglycans while the latter are heteropolysaccharides or heteroglycans (Avems,2012).

Homoglycans: Homoglycans are high molecular weight carbohydrates composed of large numbers of pentose or hexose residues. Many of these carbohydrates occur in plants either as reserve food materials such as starch or as structural material such as cellulose.

Starch: In most part of the developing countries, 50-75% of total dietary energy comes from starch and sugar, quantitatively, starch is the most abundant (80-90%) of all plant polysaccharides eaten and it is the main storage polysaccharides of staples such as cereal grains, tubers, seed and roots .(Avems,2012)

Starch differs in their chemical composition and is mixture of two structurally different polysaccharides, amylopectin. The ratios of these glucose vary according to botanical origin, although in most starches, amylopectin is the main component amounting to about 80% amylase is water-soluble and is composed of straight chain α-(1- 4) linked glucose unit, while amylopectin is water-soluble starch, which is a branched type molecules at α-(1 - 6) linkage of straight α-(1- 4) linked unit as in amylase form of starch (Dwek and Raymond, 2013).

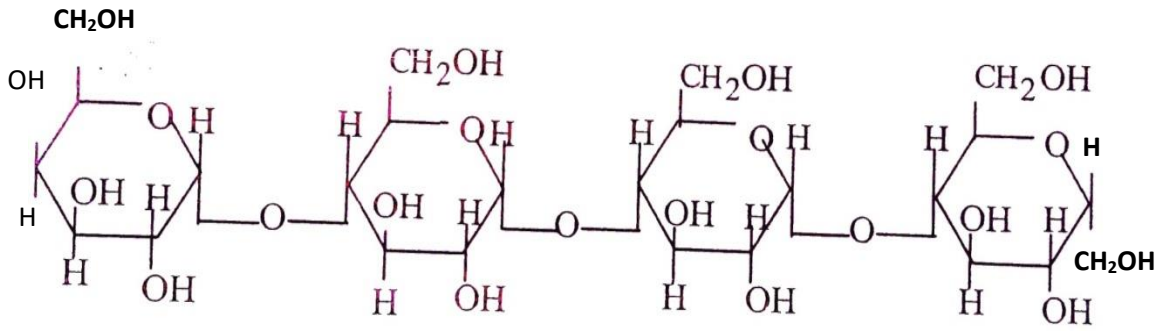


Figure 1.5 Structure of starch

Cellulose: Cellulose is the most abundant single polymer in the plant kingdom, forming the fundamental structure of plant cell wall pure cellulose is a homoglycan of high molecular weight in which the repeating unit is cellobiose. Here the β-glucose residues are 1,4 linked. While in the plant cell wall cellulose is closely associated, physically and chemically, with other components especially hemicelluloses and lignin (Laurentin *et al*,2003).

Others: Other members of homoglycans of plant origin are fructans (a reserve material in roots, stems, leaves and seeds of variety of temperature plants). galactans and mannans (present in cell walls of plants) and xylans (McDonald *et al*.,1995).

Heteroglycans: Heteroglycans are Heteroglycans that contain more than one type of monomer. Members of this class are pectin, hemicelluloses, exudates gums and acidic mucilage. Together with cellulose and lignin, heteroglycans, form an unavailable carbohydrate, because they cannot be digested by human and are termed dietary fibre (McDonald *et al*.,1995). Recent study shows that dietary fibre also contains oligosaccharides and is defined as edible part of plants or analogous carbohydrates that are resistant to digestion and absorption in the human intestine with complete or partial fermentation in the large intestine. There is increasing interest in fibre in

diets, because high-fibre diets are now considered important for specific physiological effect that could be regarded as protective in relation to degenerative diseases such as diabetes, obesity, coronary disease, colon disease and colorectal cancer (Laurentin *et al,2003).*

1.2.1.2 PROTEINS

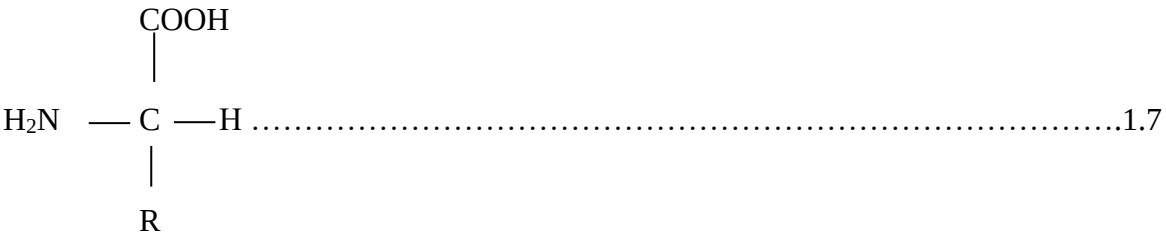
Proteins are high molecular nitrogen-containing organic compounds with complex structural organization, composed of amino acids linked into chain by peptide bonds (Strov, 1989). Proteins occur naturally in all living matter where they play the most important role in all biological functions as chief structural unit of protoplasm, source of amino acids, enzymes, hormones, antibodies and involved in the transport of water, inorganic ions, organic compounds and oxygen (Strove,1989).

Apart from their main function as constituent of all cells, excess protein also yields energy- If carbohydrates and fats do not provide adequate energy, protein is used to provide energy; as a result, less protein is available for growth, cell replacement and other metabolic needs. This effect is more pronounced in infants and children, who need extra protein for growth. Thus, malnutrition results. In developing countries where plant sources of protein are the major means of protein 60% of the populace suffers from protein energy malnutrition (PEM) resulting in high mortality rate (Abdullahi, 2000).

1.2.1.3 Amino Acids

Proteins form amino acids during digestion by enzymes, acids or alkalis. Amino acids are characterized by having a basic nitrogenous group generally an amino group ($-NH_2$) and an acidic carboxyl unit (-COOH). All amino acids involved in protein structures are the ∞-type,

having the amino group attached to the carbon atom adjacent to the carboxyl group with L-configuration (McDonald *et al*, 1995).



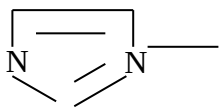
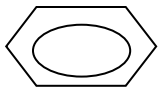
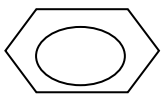
…….1.7

Figure 1.9 Structure of protein

Where R is the side-chain and varies in different amino acids.

Over 200 amino acids have been isolated form biological materials, and only 20 of these are commonly found as components of proteins (McDonald *et al*,1995).

Table 1.1: **Some Naturally Occurring Amino Acids.**

Amino acid (Abbreviation)	**Structure**
Glycine(GIy)	$H_2C(NH_2)COOH$
Alanine (Ala)	$CH_3CH(NH_2)$
Valine (Val) *	$(CH_3)_2CH\ CH(NH_2)\ CQOH$
Leucine (Leu) *	$(CH_3)_2CHCH_2CH(NH_2)\ COOH$
Isoleucine (lie) *	$CH_3CH_2CH(CH3)CH(NH2)COOH$
Serine (Ser)	$HOCH(NH_2)COOH$
Threonine (Thr) *	$CH_3CH(OH)CH(NH_2)COOH$
Cysteine (Cys) *	$HSCH_2CH(NH_2)COOH$
Methionine (Met) *	$CH_3S(CH_2)\ _2CH(NH_2)COOH$
Aspartic acid (Asp)	$HOOCCH_2CH(NH_2)COOH$
Asparagine (Asn)	$H_2NCO(CH_2)CH(NH_2)COOH$
Glutamic acid (Glu)	$HOOC(CH_2)_2CH(NH_2)COOH$
Glutamine (Glu-Q)	$H_2NCQ(CH_2J2CH(NH_2)COOH$
Arginine (Arg)	$H_2NC(=NH)NHCH2CH_2CH_2CH(NH2)COOH$
Lysine (Lys) *	$H_2N(CH_2)_4CHCNH_2)COOH$
Histidine (His)	 $CH_2CH(NH_2)COOH$
	 $CH_2CH(NH_2)COOH$
	

Phenylalanine (Phe) ——

Tyrosine (Tyr)

HO —— ——$CH_2CH(NH_2)COOH$

Essential Amino Acids

***Essential Amino Acids**

McDonald *et al.* 1995

Plants and many microorganisms are able to synthesize protein from simple nitrogenous compounds such as nitrates, however, animals do not have ability rather they derive all the amino acids necessary for building up body protein from consumption of plants and animals. Animals have differing abilities to convert one form of amino acids to another. In human, this ability is limited; hence, of the 20 amino acids known as component of protein, eight categories i.e. isoleucine, leucine, lysine, tryptophan, valine, thronine, aromatic amino acids (phenylalanine and tyrosine) and sulphur containing amino acids (cysteine and methionine) have been found to be essential for human and have been termed essential or indispensable amino acids (Hertweck, 2011).

1.2.1.4 Classification of Proteins

Proteins have been classified from the point of view of their biochemical, chemical or physical properties. However, the most universally applicable system of classification is in term of their physical properties. In this case, proteins are divided into simple and conjugated proteins.

a. Simple protein: these proteins produced only amino acids on hydrolysis. They are sub-divided into two groups, fibrous and globular proteins based on their solubility in aqueous solvents.

i. Fibrous: fibrous proteins have structural roles in animals' cell and tissues. They are insoluble and very resistant to animal digestive enzymes. They are composed of elongated, filamentous

chains jointed together by cross-linkages. This group includes collagens, elastin, and keratins. (Zhang and Skolnick,2010)

●**Collagens** are the main proteins of connective tissues and constitute about 30% of the total protein in the mammalian body. Hydroxypoline is the amino acid present in collagen.

●**Elastin** is the protein found in elastin tissues such as tendons and arteries, which is rich in alanine and glycine.

●**Keratins** are classified into two, α-keratins found in wool and hair and β-keratins which occur in feather, skins, beaks and scales of most birds and reptile and are very rich in cysteine, a sulphur containing amino acids. (Strove,1989).

ii. Globular: Globular proteins are so-called because, their polypeptide chain is folded into compact structures; the group includes all the enzymes, antigens and those hormones which are proteins. Globular proteins are subdivided into albumins, histones and globulins.

●**Albumins** are soluble in water or dilute salt solutions and coagulate when heated. They are found in milk, blood, egg and in many plants.

●**Histones** are basic proteins that occur in cell nuclei where they are associated with DNA.
●**Globulins** occur in milk, egg and blood and are also found in seeds like wheat and rice.

b).Conjugated proteins: These proteins have a non-protein part in there molecule, some important members are glycoprotein, lipoprotein, phosphoprotein,

Chemo and nucleo-proteins containing carbohydrates, lipids, phosphoric acid residue, coloured group (usually metals such as Fe, Cu, Mg e.t.c) and nucleic acid respectively.

1.3 LIPIDS

Lipids are hydrophobic organic biomolecules that are soluble in common organic solvents such as benzene, ether and chloroform (McDonald *et al*, 1995). And are one of the most important constituents of ail food materials where they serve as rich sources of energy, fat-soluble vitamins (A, D, E and K) and essential fatty acids (Ihekoronye and Ngoddv, 2000).

In plants, lipids are of two types, the structural and the storage lipids. The former are present as constituents of various membranes and protective surface layers and make up about 7% of leaves of higher plants. The surface lipids are mainly waxes, with relatively minor contributions from long chain hydrocarbons, fatty acids and cutin. The membrane lipids, present in mitochondria, the endoplasmic reticulum and the plasma membrane, are mainly glycolipids (40-50%) and phospholipids. Plant storage lipid occur in fruits and seeds and are predominantly oil (McDonald *et al*., 1995).

1.3.1 Classification of Lipids

Lipids have been classified into several different ways. However, the most satisfactory classification is based on their backbone structures (Fahy *et al.*,2010).

●Glycerol based lipids

● Simple lipids (fats and oils);

● Compound or complex lipids (glycolipids and phosphoglycerides)

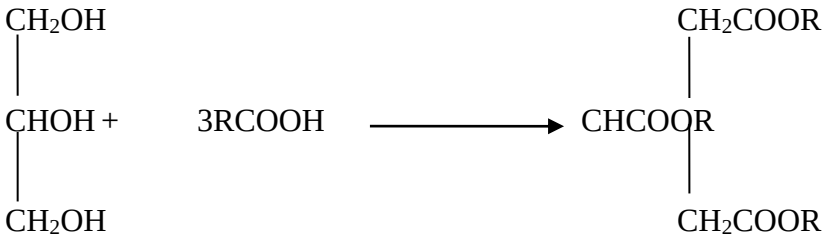
● Non-glycerol based or derived lipids (Cerebrosides, Waxes, Steroids, Terpenes. e.t.c).

Although wide ranges of different compounds are classified as lipids, the nutritionists restrict their interests to the simple lipids (fats and oils) and their component fatty acids. This is

because fats and oils are the predominant form of all lipids associated with food. Over 98% of the fatty acids in meats, fish and vegetable oils are in triglycerides (Fahy *et al*,2010).

a). Triglycerides (Fats and Oils)

Fats and oils are constituents of both plants and animals and are important sources of stored energy. They have the same general structural and chemical properties but have different physical characteristics. At room temperature, fats are solids while oils are liquids. oils are esters of fatty acids with the trihydric alcohol glycerol, and are also referred to as triacylglycerols or triglycerides. (Fahy *et al*,.2010).



Glycerol **Triglyceride**

Triglycerides differ in type according to the nature and position of the fatty acid residues. Triglycerides with three alkyl (R) residues of the same fatty acid are termed simple triglycerides, otherwise called mixed triglycerides. However, naturally occurring fats and oils.

b).Fatty Acids

The physical nature of fats and oils depends on composition of fatty acids. Fatty acids are aliphatic carboxylic acids mostly obtained from the hydrolysis of natural fats and oils. Most

naturally occurring fatty acids are unbranched and have an even number of carbon atoms ranging from 4 to 24 carbon atoms per molecule. However, in meat, fish and vegetable oils, the carbon content range from 14 to 22 (Beintz and Knight, 1994).

Table 1.2: Common Acids of Natural Lipids

Common name (Systematic Name)	Designation	Structural formula
Butyric acid (Butanoic acid)	4.0	$CH_3(CH_2)_2COOH$
Caproic acid (Hexanoic acid)	6.0	$CH_3(CH_2)_4COOH$
Caprylic acid (Decanoic acid	8.0	$CH_3(CH_2)_6COOH$
Cpric acid (Hexanoic acid)	10.0	$CH_3(CH_2)_8COOH$
Lauric acid (Dodecanoicacid)	12.0	$CH_3(CH_2)_{10}COOH$
Myristic acid (Tetradecanoic acid)	14.0	$CH_3(CH_2)_{12}COOH$
Palmitic acid (Hexadecnoic acid)	16.0	$CH_3(CH_2)_{14}COOH$
Stearic acid (Octadecanoic acid)	18.0	$CH_3(CH_2)_{16}COOH$
Arachidic acid (Eiocosanoic acid)	20.0	$CH_3(CH_2)_{18}COOH$
Behenic acid (Docosanoic acid)	22.0	$CH_3(CH_2)_{20}COOH$
Lignoceric acid (Tetracosanoic acid)	24.0	$CH_3(CH_2)_{22}COOH$
Palmitoleic acid (9-hexadecenoic acid)	$16;1)^9$(T-9-16 :1)	$CH_3(CH_2)_5CH=CH(CH_2)_7COOH$
Oleic acid acid (9-octadecenoic acid)	$18;1)^9$(T-9-18:1)	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$
Linoleic acid (9,12-octadecadienoic acid)	$18;2)^{9,12}$(T-6,9-18:2)	$CH_3(CH_2)_4(CH=CH)_2(CH_2)_6COOH$

McDonald *et al*.,1995

Fatty acids could be saturated or unsaturated. The later ones are subdivided into monoenoic and polyenoic (i.e. those with two or more double bond per molecule). While the former are frequently (Fahy *et al*,.2010).

1.3.2 Essential Fatty Acids

Essential fatty acids (EFAs) are long chain polyunsaturated alkanoic acids, which are indispensable in the diet of the mammalian organisms .the three wisely known members are linoleic acid ,α-linolenic acid Plants synthesize linolenic acids from oleic acid because they possess the enzymes that are capable of introducing double bonds beyond carbon-9 and thus desaturate oleic acid towards the methyl end (McDonald *et al*.1995).

Dietary essential fatty acids have long been considered as part of the lipid supply necessary for energy, growth, cellular metabolism and muscle activity. Some EFAs also serve as indispensable dirtary precursors for eicosanoid (prostaglandins, thromboxanes and prostacyclins) formation hence they provided greater significance to the study of their role in health and disease The primary sources of EFAs are terrestrial and marine plants (especially oil seeds) and phytoplantkton. Some marine animals (especially fish) and liver are also sources of elongated and EFAs (Alberto,2016).

1.3.3 Rancidity of lipids

Many fats and oils are prone to lipid deterioration that leads to the formation of off-flavours, colour defects and potentially harmful products. These changes are generally referred to as rancidity. During rancidification, the chemical changes believed to take place are hydrolytic and oxidative rancidities. Plant possess antioxidants, which have certain degree of resistance to

oxidation furthermore, they are also used for the prevention of some human diseases such as cardiovascular disease, cancer and degenerative eye diseases. (Mozaffarian *et al*.,2006).

1.3.4 Waxes

The waxes are monoesters of fatty acids and alcohols other than glycerol and of higher molecular weight (Ihekoronye and Ngoddy, 1985). The two parts of the carboxylic group of one amino acid react with the a-amino group of another to form a peptide bond. The peptide bonds allow many amino acid linkages until a protein is formed (Ihekoronye and Ngoddy, 1985).

1.3.5 Steroids

When plant or animal materials are extracted with non-polar solvents, not only are triglyceroids and complex lipid removed, a group of non saponifiable compounds called steroids are extracted. Steroids are important "biological regulators" that nearly always show dramatic physiological effects when they are

Administered to living organisms (Graham, 1990). They are generally characterized by a polycyclic carbon skeleton. Examples of steroids are cholesterol, male and female sex hormones, adrenocortical hormones, vitamin D and certain cardiac poisons.

Cholesterol is a steroid alcohol or sterol. It is found in nearly all tissues of vertebrates, particularly in the spinal cord, and in the main constituent of gull stones. The structure of cholesterol is shown Figure 1.7. Human beings and higher animals can synthesize cholesterol from acetate units in about 36 steps and in a matter of seconds. Cholesterol is known to serve as an intermediate in the biosynthesis of all the steroids of the body; it is therefore essential to life (Graham, 1990).

1.4 MINERALS

Mineral element gets into our body mainly through the food we eat. Even though large numbers of elements are found in tissues of organism, few are known to..have a metabolic role in the body that is necessary for health and growth consequent!), termed essential mineral elements (Ayodele *et al*, 2003). Essential mineral elements are classified into Major (calcium, phosphorus, potassium, sodium. Microelements contribute to the global burden of disease through increased rates of illness and death from infectious diseases, and of disability such as mental impairment. Thus, their evaluation in food is of great importance in showing the relationship between food, health and environment (Okaka, 2009).

1.4.1 CALCIUM

Calcium is the most abundant mineral elements in the body and it is an important constituent of the skeleton and teeth, in which about 99% of the total body calcium is found (Aganga *et al.*, 2003). Small amount of calcium is also found in the body fluids either as calcium ions or in combination with protein where it activates enzyme systems including those necessary for the transmission of nerve impulses, moreover, it is involved in blood clotting, acid-base regulation and muscular irritability (Aganga *et al.*, 2003); Harvard women's Health watch. 2003). According to US national research council, NRC, (1989), the daily allowance for calcium for adults is 1200mg.

Because calcium play a role in supportive structures of the body (Adeyeye, 2002), diseases or malformation caused by dietary deficiency of calcium (together with phosphorus and vitamin D) are rickets in children, in children, and osteomalacia (adults rickets) (Adeyeye, 2002).

1.4.2 PHOSPHORUS

Phosphorus is always associated with calcium in the body,both contributing to the supportive structures (Adeyeye, 2002). About 80 – 85% of total body phosphorus is found in the skeleton where it exist as hydroxyapatite, $3Ca_3(PO_4)_2.Ca(OH)_2$. When cremated, bone contain 170g phosphorus/kg (McDonald *et al.*,1995). In addition, phosphorus is present in cells and in the blood as soluble phosphate ion as well as in lipids, proteins, carbohydrates, nucleic acids and nucleoproteins (responsible for cell division, reproduction and the transmission of hereditary traits (Adeyeye, 2002). The element is also known as to play a vital role in energy metabolism in the formation of sugar-phosphates, adinosine di- and triphosphate (McDonald *et al.*, 1995), The daily allowance of phosphorus for adults is 1200mg (NRC, 1989).

Phosphorus deficiency is associated with poor fertility, apparent function of the ovaries causing inhibition, depression or irregularity of oestrus (Umar, 2010).

1.4.3 MAGNESIUM

Magnesium is closely associated with calcium and phosphorus. About 70% of the total magnesium is found in the skeleton and remainder distributed in the soft tissue and fluids (McDonald *et al.,* 1995), thus, essential as intracellular fluid where it maintains the electrical potentials in nerves (Adeyeye, 2002) as activator of many enzymes in carbohydrates, protein and lipids metabolism (McDonald *et al.,* 1995); stabilizing some structures and energizing others I n all types of biopolymers e.g DNA and RNA, proteins, polysaccharides and lipids. Magnesium deficiency results in uncontrollable twitching of muscles leading to convolution, which may cause death (Ertan *et al.,* 2013).

1.4.4 POTASSIUM

Potassium is primarily an intracellular cation, in large part this cation is bound to protein and with sodium, chlorine and bicarbonate ions, influences osmotic pressure and contributes to normal acid-base (pH) equilibrium (McDonald *et al.,* 1995; Adeyeye, 2002). Potassium plays an important in nerve and muscle plexibility, and is also concerned with carbohydrate metabolism.

The potassium content of plants is generally very high, consequently, its deficiency is rare in the body (Mc Donald et al., 1995). When deficiency occurs, it results in growth retardation, weakness, paralysis, and is result in death (McDonald et al. 1995).

A dietary excess potassium is normally, rapidly excreted from the body chiefly in the urine. About 8% of potassium intake in retained by the body (Heiman. 1980), however, high intakes may interfere with the absorption and metabolism of magnesium (FND, 2002).

1.4.5 SODIUM

Most of the sodium of the animal body is present in the soft tissues and body fluids (McDonald *et al*., 1995). Like potassium, sodium is the major extra cellular cat ion of the body fluid, where it influences osmotic pressure and pH balance (Adeyeye, 2002). Sodium also plays a role in the absorption of sugars and amino acids from the digestive tract, in the transmission of nerve impulse and in the glycogen and protein synthesis (Garba, 1999; McDonald et al., 1995).

Sodium deficiency is rare I healthy person due to its ingestion in the form of common salt. Nevertheless, its deficiency result in dehydration of the body (McDonald *et al*., 1995). The daily allowance of the element is 500mg (NRC, 1989).

1.4.6 IRON

Iron is an essential trace element in human nutrition (Beyeh *et al.,* 1998) found mostly in the red blood cells as a component of haemoglobin (Cabrera et al., 1996). Iron is also present in myoglobin, a compound occurring mainly in muscles, storage iron ferritin and haemosiderin found mainly in the ,liver and bone marrow (McDonald *et al.*, 1995).

In most living organism, iron is a participating in numerous vital biochemical reaction, and plays an important role in the structure of enzymes responsible for redox processes in living cells such as liberation of energy from carbohydrates, fats and proteins (Ertan *et al*., 2013).

The most common manifestation of iron deficiency is anaemia. According to Black (2003), about 2 billion people were affected by iron deficiency, and anaemia is responsible for a tenth of maternal mortality. Inadequate iron intakes are also associated with poor learning and decreases cognitive development.

1.4.7 COPPER

Copper is an essential trace element in human body (Ysart *et al*., 2000) where it exists as an integral part of copper protein, which occur in erythrocytes where it play a role in oxygen metabolism and cytochrome, an enzyme involved in energy metabolism (McDonald *et al.,* 1995; Adeyeye, 2002). Thus this shows that, copper is necessary for haemoglobin formation and contributes to iron and energy metabolism (Adeyeye, 2002)

In human, copper deficiency is rare since it is widely distributed in food (Adeyeye 2002).

The copper content of normal human adult is 50 – 120mg but above 15mg causes nausea,

vomiting, diarrhea and intestinal pain (Sabir *et al*., 2012). However toxic effects of this element are allergic contact dermatitis from repeated skin contact multi-organs failure and death and the recommended dietary allowance of copper was 15.3 mg/100g for adult (FND, 2002).

1.4.8 ZINC

Zinc has been found in every tissue in the body particularly in the skeleton (Aganga *et al.,* 2003). Skin and hair are also known to contain high amount of zinc (McDonald *et al.,* 1995). Zinc has been found to be essential to human nutrition Adeyeye, 1994; 2002 Amaros, 2003) necessary for the metabolism of nucleic acids, carbohydrates and protein. Therefore, it is necessary for DNA and protein synthesis. It is also involved in production, storage and secretion of hormone (Agnga *et al*., 2003), and in the reproductive function because it participates in the mechanism of action of testosterone (Camera and Amaro, 2003).

Zinc deficiency has an adverse effect on cellular metabolism, that poor zinc status during pregnancy affect the cerebral function of the fetus (Erten, 2012).

1.4.9 MANGANESE

Manganese is an essential trace element for human nutrition. It plays a role as an activator of many enzymes such as hydrolyses and kinases (McDonald et al., 1995 and as a constituent of metalloenzymes pyruvate carbozylase and superoxide dismutase present in mitochondria .Thus, it helps in bone formation (bone have the highest manganese concentrates) and in the metabolism of amino acids cholesterol and carbohydrates (Harvard Women's Health Watch, 2003).

Manganese deficiency is rarely observed owing to the large amount of it in human diet such as legumes, whole grams, nut etc. However inadequate intake can cause abnormalities in the metabolism of carbohydrates and cholesterol. The recommended daily allowance is 2-5mg in adults (FND2002).

1.5 RESEARCH PROBLEM

Due to the economic crisis in Nigeria, which resulted in the increase of food prices like meat, fish and wheat e.t.c ,which are much needed and are rich in proteins ,carbohydrates, fats, minerals, e.t.c (N.R.C,1993).

This problem has become a major source of concern to many Nigerians and urgent solution must therefore be taken to overcome the problem. We must therefore look resourcefully to identify, explore and utilized other resources which are cheaper and obtain affordable. Thus this research is going to be carried out so as to assess the nutritional value of cotton seeds (Okaka*etal*,2009).

The cotton seed has nutritional values and pharmaceutical health benefits. The seeds is rich in carbohydrates, proteins, minerals, fats and vitamins. The cotton seeds is also rich in oil which has high industrial value (Yan-zhuan, 2010), and also used to make cotton seed cake which is used as substitute for soyabeans meal, in animal feeds, cotton seeds oil has health benefits to man in it's reported to prevents distinct heart vascular diseases and intestinal parasitosis (Boelhoure,1983).

1.6 JUSTIFICATION

Due to prevailing difficult economic situations faced by people in the developing countries and the lack of awareness, majority of the populace are disposed to nutritional deficiencies. Although several reports have been published on the nutritional value of many

seeds. (Okonkwo,2014), Liu *et al*., (2002). cotton seed used to produced feeds for broilers, ruminants and fish (Ojewola *et al*.,,2006). Since environment conditions fertilizer application and genetic variation exert significances in chemical composition of plants (Donald, 1981), then it is important to determine the nutritional composition within each local environment. Thus this work will be carried out so as to assess the nutritional value of Whole cotton seeds in Dutsin-ma and Malumfashi local government areas of Katsina State.

1.7 SCOPE AND DELIMITATION

The research will cover the proximate composition of *Gossypium hirsutum* (cotton) seeds which account for the macronutrient (carbohydrate, fats, protein the moisture content, ash content, crude protein content, crude lipid and crude fibre contents, the major elements (such as sodium, potassium, sulphur, calcium), the trace elements include (iron, Iodine, copper, manganese, and zinc). The physicochemical analysis of the oil will also be carried out. But the determination of smoke point, cetane rating and transesterification of the oil will not be carried out.

1.8 AIM AND OBJECTIVES

1.8.1 AIM

The aim of this research is to investigate the proximate composition and physicochemical properties of *Gossypium hirstum* seeds oil.

1.8.2 The aim will be achieved through the following objectives :

1. Collection of cotton seeds samples from Dutsinma and Malumfashi local government areas.
2. Determination of the proximate composition of the two cotton seeds samples.
3. Determination of the physicochemical properties of the cotton seeds oils samples.
4. Determination of the Amino acid and the mineral composition of the two whole seeds.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 INSTRUMENTS/APPARATUS

In addition to the routine laboratory apparatus, the following instruments were used in the analysis.

Table2.1: List of Apparatus Used

Instruments	Model	Manufacturer
Flame Photometer	Corning 490	Korl-Kolb, Germany
kjeldhal Apparatus.	Glass	Korl-Kolb, Germany
Hot plate	Gerhard	Korl-Kolb, Germany
Amino Acid Analyser	Technon sequencial multi sample.	Dublin, UK.
Atomic Absorption Spectrophotometre	PYE-unicam 969	Korl-Kolb, Germany
Hot plate	Gerhard	Korl-Kolb, Germany
Kinematic Viscometer	K23400	Lenton. UK
Spectrophotometre	6100	Jenmay. UK
Muffle Furnace	Lenton Furnace	Jose industry, Germany
Soxhlet Extractor	Glass	Jenmay, UK
Weighing Balance	Mettler 400	Jenmay. UK
Spectrophotometer	6100	Mettler, Switzerland
Pycometer	Glass	Unicam LTD,UK
Crucible	Ceramics	Werthem

Water bath	M.F Refrigerator	U.S.A
Hot Air Oven	Oven PS	Gallen kamp, U.S.A
GC-MS machine	QP-2010 plus	Shimadzu, Japan

2.1.2 REAGENTS/CHEMICALS

Table 2.2 The reagents and chemicals used in this research are:

Name	Formula	% Purity	Grade	Manufacturer
Sodium hydroxide	NaOH	95		BDH
Potassium hydroxide	KOH	99.5	AR	BDH
Potassium iodide	KI	99.5	AR	BDH
Phenolpthalein	C_{20} $H_{14}O_4$	98	LR	BDH
Sodium chloride	NaCI	99.5	AR	BDH
Ethanol	CH_3CH_2OH	99.6	AR	BDH
n-hexane	C_6H_{12}	95	AR	BDH
Sodium thiosulphate	$Na_2S_2O_3.5H_2O$	99	GPR	BDH
Hydrochloric acid	HCI	36	LR	M&B
Bromocresol green	$C_{12}H_{14}Br_4S$	95	LR	M&B
Methanol	CH_3OH	95	AR	BDH
Methyl red	C_{15} $H_{15}N_3O_2$	95	LR	M&B
Boric acid	H_3B0_3	99.5	AR	M&B
Iodine crystals	I_2	99.5	AR	BDH
Ammonium molybdate	$NH_4O_6MO_7O_{24}.4H_2O$	98	AR.	M&B
Magnesium turning	Mg	99.5	LR	M&B
Calcium trioxocarbonate	$CaCO_3$	99.5	LR	M&B
Iron turnings	Fe	99.5	LR	M&B
Cobalt chloride hexahydrate	$CoCI_2.6H_2O$	98	LR	M&B
Sodium acetate	$CH_3COONa.3H_2O$	99.5	AR	M&B
Acetic acid	CH_3COOH	95	LR	M&B

Key: LR=Laboratory Reagent, AR=Analytical Reagent, GPR-General Purpose Reagent, M&B= May&Baker, BDH= British Drug House

2.1.3 Preparation of reagents

a) Sodium Hydroxide (40 %) Solution:

Sodium hydroxide (40 g) pellet was dissolved in volumetric flask with distilled water and diluted to volume in a 100 cm^3 volumetric flask.

b) Mixed Indicator

A mixture of bromocresol green and methyl red indicators (0.1 g) in the ratio (1:2) were dissolved in 95 % methanol and diluted to volume in a 100 cm^3 volumetric flask.

c) 0.01M Hydrochloric Acid Solution:

Concentrated HCl (0.8 cm^3, 36% HCl, S.G 1.18) was diluted to volume with distilled water in a 1000 cm^3 volumetric flask. The solution was standardized with 0.01 M NaOH solution.

d). Boric Acid-Indicator Solution:

Two grams (2 g) of pure boric acid was accurately measured into a 100 cm^3 volumetric flask, 20 cm^3 of methanol and 50 cm^3 of distilled water were added. The mixture was shaken, and 10 cm^3 of the mixed indicator was added and shaken again. Few drops of 0.01 M HCl was added (to adjust the pH to faint red). The mixture was then diluted to volume with distilled water (Krishna and Ranjhan, 1980).

e) Tetraoxosulphate (VI) Acid (1.25 %) (w/v):

Concentrated tetraoxosulphate (VI) acid (7.20 cm^3, 96 % H_2SO_4 S.G 1.835) was accurately measured using measuring cylinder and diluted to volume in a 1000 cm^3volumetric flask with distilled water.

f) Sodium Hydroxide Solution (1.25 %) (w/v):

Sodium hydroxide (12.50 g) pellets were accurately weighed and dissolved with distilled water and diluted to volume in a 1000 cm^3 volumetric flask.

g) Hydrochloric Acid (0.5 M) Solution:

This was prepared by diluting 10.63 cm^3 of concentrated Hydrochloric acid (36 % HCl, S.G 1.16) with distilled water in a 250 cm^3 volumetric flask and the solution was diluted to volume.

h) Alcoholic KOH (0.5 M) Solution:

Twenty eight grams (28 g) of KOH was dissolved with ethanol in a 500 cm^3 volumetric flask and the solution is diluted to volume with ethanol.

i) Phenolphthalein (1 %):

One gram (1.0 g) of phenolphthalein was dissolved in ethanol in a 100 cm^3 volumetric flask and the solution diluted to volume with ethanol.

j) Sodium Hydroxide (0.25 M) Solution:

NaOH (2.5 g) was dissolved with distilled water in a 250 cm^3 volumetric flask and the solution was made to volume with distilled water.

k) **Potassium Iodide (5 %) Solution:**

Five grams (5.0 g) of KI was dissolved with distilled water in a 100 cm^3 volumetric flask and the solution was made to volume.

l) **Sodium Thiosulphate (0.1 M) Solution:**

$Na_2S_2O_3.5H_2O$ (6.2 g) was dissolved in distilled water in a 250 cm^3 volumetric flask and the solution made to volume.

m) **Starch (1 %) Solution:**

Soluble starch (1 g) was dissolved in 100 cm^3 of distilled water and boiled for 1 minute, allowed to cool and 2 g of potassium iodide was added and the solution kept in a stopper bottle.

n) Potassium Iodide (10 %) Solution:

Ten grams (10 g) KI was dissolved with distilled water in 100 cm^3 volumetric flask and the solution made to volume.

2.2 Methods

2.2.1 Sample Collection and Treatment

The matured and dried whole (cotton) *Gossypium hirsutum* seeds from different location in Dutsin-ma and Malumfashi local government areas in Katsina state and was collected in December 2017. For each sample, 2kg seeds were both pulverized ground using mortar and pestle, sieved and stored, in air tight polythene bags until needed for the different analysis.

2.2.2 Proximate Analysis

The AOAC (1990) methods was employed in the determination of moisture, ash, crude lipid, crude fibre, carbohydrate, protein, lipid, amino acid and fatty acid content of the seed samples.

2.2.2.1 Determination of Moisture Content

The moisture content of the processed samples was determined by vacuum oven method. The sample (3.0g) were weighed into crucibles of known weight. The crucibles with the samples were placed into an oven operated at 80^0C for 3 hours. It was then carefully removed from the

oven and allowed to cool in a desiccator before re-weighing. The crucible with the sample was returned to the oven and dried further. The process of drying, cooling and re-weighing was continued until a constant weight was obtained. The moisture content was calculated as percent using equation 2.1.(Akpan *et al.*,2006).

$$\text{Moisture content}(\%) = \frac{w_i - w_f}{W_f} \times 100 \quad \text{----------} \quad 2.1$$

w_i = initial weight of the sample before drying

w_f = final weight of the sample after drying

2.2.2.2 Determination of Ash Content

The sample (2.0g) was weighed into a crucible of known weight. The crucible with its content was placed in a furnace set at 550^0C for 6 hours after which it was carefully removed from the furnace and allowed to cool before being re-weighed.

The process was continued until a constant weight was obtained. The ash content was calculated as percent using equation 2.2 (Akpan *et al.*,2006).

$$\text{Ash}(\%) = \frac{\text{Weight of ash}}{\text{Weight of dry sample}} \times 100 \quad \text{----------} \quad 2.2$$

2.2.2.3 Determination of Crude Protein

The processed sample (2.0g) was transferred and placed into a 100 cm^3 Kjeldahl digestion flasks. Kjeldahl catalyst (0.5g) mixture (Na_2SO_4 +$CuSO_4$) and 15 cm^3 of conc.H_2SO_4 was added. The mixtures were thoroughly mixed and heated under fume cupboard for 2 hours until complete digestion was achieved and a clear solution obtained. The cooled digest was diluted to 100 cm^3 and only 10 cm^3 of the digest was mixed with equal volume of 10mol/dm^3NaOH. The mixture was placed in a micro- Kjeldahl distillation apparatus which was

distilled by steam and the distillate was collected into a conical flask containing 10 $cm^3$of 4% Boric acid. Three (3) drops of mixed indicator (5g bromocrysol green and 1g methyl red in 100 $cm^3$of ethanol) was added into 50cm^3 of the distillate and titrated against 0.1M H_2SO_4 solution. A blank was conducted simultaneously under similar experimental condition.The crude protein (CP) was calculated in percent using the following equation;

$$CP(\%) = \frac{(a-b)x0.01MHCIx14xcx\ 6.25x100}{dxe} \text{- -2.3}$$

a = titre value for the digested sample, b = titre value for the blank, c = volume to which the digest was made up

d = volume of aliquot used in distillation, e = weight of dried sample.

2.2.2.4 Determination of Crude fibre

. The defatted residue from crude lipid extraction (2.0g) was transferred into100 cm^3 conical flask. Sulphuric acid 20cm^3 of 0.25M was then added and the mixture boiled for 30 minutes. The content was filtered and insoluble matter was washed with boiling water till it is freed from acid. The residue was again washed into a conical flask with 20 $cm^3$of 0.313M sodium hydroxide. The contents were boiled for 30 minutes. The insoluble matter was then transferred to filter paper by means of boiling water, followed by 1% hydrochloric acid and again with boiling water until it became free from acid. Then it was washed twice with alcohol .It was then transfered to ash-less filter paper and dried at 110°C to constant weight. The filter paper and its contents were incinerated and ignited to ash in a previously weighed crucible. Increase in weight of crucible was reported as crude fibre (AOAC, 1990).

$$\% \text{ Crude fibre} = \frac{\text{loss in weight on ignition}}{\text{Weight of sample}} x100 \text{- -2.4}$$

2.2.2.5 Determination of Crude Lipid

Dried sample (2.0g) were weighted into porous thimble, to which its opening was plugged with cotton. The thimble was placed in an extraction chamber, which was suspended above receiving flask containing n-hexane and below a condenser. The flask was heated on a mantle and the oil extracted. The extraction lasted for eight hours after which the thimble was removed from the soxhlet and heated over water bath to recover the solvent. The flask containing the crude lipid was disconnected, cleaned with dry cloth and placed in an oven at 100^{o}C for 30 minutes. After the heating, the flask was cooled in desiccators and weighed. And the crude lipid calculated by equation 2.5(Okonko *et al*.,2016).

$$\% \text{ Crude Lipid} = \frac{\text{weight of lipid}}{\text{Weight of sample}} \times 100 \quad \text{-----------------------------------} 2.5$$

2.2.2.6 Determination of Carbohydrate

Available carbohydrate also referred to as soluble carbohydrate is not determined directly but obtained as a difference as show in the equation below:

Available carbohydrate = 100- (% moisture + Ash +% crude protein +% crude fiber +% crude lipid).

2.2.2.7 Determination of energy value

The energy value (KJ/100g) of the samples were estimated by multiplying percentage crude protein, crude lipid and carbohydrate by the recommended factor (3.44, 8.37 and 3.57 respectively) used in vegetable and seed analysis (Akubugwo *et al.,* 2008).

2.3 OIL ANALYSIS

2.3.1 Extraction of oils

Extraction of cotton seeds oil was carried out by soxhlet extraction method. n-hexane (100cm^3) was placed in round bottom glass flask. The sample 50g was placed in thimble and settled in the center of the extractor and soxhlet apparatus was heated at 108^{0}C.

The solvent boiled and vaporized the top. The condensate liquid started dropping into the thimble containing the sample and the extracted oil seeped through thimble into the flask via the siphon. The extraction cycle continue and the oil – solvent mixture was deposited in the flask which later heated at 108^0 C for solvent recovery. The % oil yield was calculated using equation2.7.Akpan *et al*; (2006).

$$\% \text{ Extraction oil yield} = \frac{weight\ of\ oil\ extracted(g)x\ 100\%}{weight\ of the\ sample} \quad \text{.....2.7}$$

2.3.4 Determination of Physicochemical Properties

2.3.4.1 Determination of Saponification Value

To determine the saponification value of the extracted oil, The oil (2.0g) was placed in a 250 cm^3 conical flask and 25cm^3 of 0.5M ethanoic potassium hydroxide solution was added. A reflux condenser is attached and the flask is refluxed for 30 min on a water bath with continues swirling until it simmered. The excess potassium hydroxide was titrated against 0.5M hydrochloric acid using phenolphthalein indicator. A blank titration was carried out under the same condition. The saponification value of the oil was calculated by the equation; (Adeola and Ndidi, 2016).

$$\text{Saponification value} = \frac{(B1-R1)\ xN\ x\ 56.1}{weight\ of\ sample} \quad \text{- - - - - - - - - - - - - - - - - -2.8}$$

Where B_1 and R_1 are volumes of hydrochloric acid used for blank and sample titration respectively And N= normality of the acid,56= molecular weight of KOH.

2.3.4.2 Determination of Iodine Value

This was carry out by placing 2.0g of oil in a 250cm^3 conical flask, adding 10cm^3 of anhydrous chloroform follow by addition of 30cm^3 of the solution. The content is place in a drawer for 30min. potassium iodide solution (10cm^3 of 15% weight by volume) was added to the solution. The entire mixture was titrated against 0.1M $Na_2S_2O_3$ until the solution turns light yellow. After which 2cm^3 of 1% starch indicator was added and titration will continued until the blue color form is finally disappeared. A blank determination was carried out under same conditions. http:// ijcccsenent.org international journal of chemistry vol.8, No. 3 ; 2006.

Iodine value = $\frac{(B2-R2)\,x\,0.1M\;x\;12.6g}{weight\;of\;sample}$……………………………………………………….2.9

Where B_2 and R_2 are volumes of $Na_2S_2O_3$ used for blank and sample titration respectively.

2.3.4.3 Determination of Acid value

Two gram of oil was place in a 250cm^3 conical flask and warm. 25cm^3 of methanol was added with thorough stiring, followed by addition of 2 drops of phenolphthalein indicator and a drop of 0.1N sodium hydroxide solution. The mixture was then titrated until a permanent light pink colour was observed. Av. determined using. Akpan *et al*; (2006)

Acid value = $\frac{titre\;value\;x\;0.1M\;x\;56.1}{weight\;of\;sampple}$- -2.10

Free Fatty Acid = 0.5x Acid value- -- - - - - - - - - - - - - - - - 2.11

2.3.4.4 Determination of Peroxide Value

The peroxide value of the oil is was determined by placing 2.0g of oil in a 50cm^3 conical flask and addition of 30cm^3 glacial acid/chloroform (2.2 v/v). the mixture was then shake until it becomes homogenous. After which 1cm^3 of saturated potassium iodide solution and 0.5cm^3 starch indicator was added. The mixture was then titrated against 0.1N sodium thiosulphate until the dark blue colour was observed. Blank titration was carried out under the same conditions. The peroxide value was determined; washeed *et al*; (2010).

Peroxide value = $\frac{(B2-R2)\ x\ Normality}{weight\ of\ sample}$ - - - - - - - - - - - - - - - - - -- -- -- -- -- - - - - - - - - -- -2.12

Where B2 and R2 are volumes of $Na_2S_2O_3$ used for blank and sample titration respectively.

2.3.4.5 Test for cloud point and pour points

The oil samples (5cm^3) was measured in 5cm^3 test tube and placed in a freezer. The sample was taken out of the freezer every 1 minute to check any visible changes and the temperature was measured using digital mextech thermometer (ASTM, 2008).

Observation for cloud point: at a particular temperature, crystals began to form in the sample, reading was taken (Faduka, 2001). Observation for poor point: At a certain temperature and crystal was dissolved from sample (it's frozen) and then the temperature reading was taken (ASTM, 2008).

2.3.4.6 Test for flash point:

The flash point was determined according to ASMD 93 method, the sample was placed in the test tube to the prescribed mark in the interior of the cup. The cup was mounted on to its positions on the testers. Bunsen burner was used to supply heat to the apparatus at rate of 1^{0}C per

minute with constant stirring. A small test flame plane was directed into the cup intermittently. The flash point was taken as the temperature when the test flame caused the vapour above the sample to ignite (Faduka, 2001).

2.3.4.7 Kinematic Viscosity

The dynamic viscosity was determined using cannon viscometer model 2020. The spindle (spindle number 04 were used) through caliberated spring of viscometer was Rotated in $50cm^3$ of the sample in $50cm^3$ beaker .the viscous drag of the fluid against the spindle was measured by the spring deflection . The deflection was measured with a rotator transduser which provides a torque signal. The viscosity in centipoles was displayed on the screen of the viscometer. To obtain kinematic viscosity in centistokes (1 centistokes $=1mm^2/s$), the dynamic viscosity was divided with density. (Cannon manual, 2017).

2.3.4.8 Determination of Specific Gravity

The empty beaker of $50cm^3$ capacity was weighed (Wo) filled with oil, and then reweighed (Wi). The oil was substituted with water after washing and drying the beaker and weighed (W_2) (Akpan *et al*., 2006).

The express for specific gravity is:

$$\text{Specific gravity} = \frac{\text{Mass of substance}}{\text{Mass of equal volume of water}}$$

$$\textbf{S.G} = \frac{W_1 - W_o}{W_1 - W_o} \quad \text{-- - - - -- - - - - - - - - - - - - - - -- -- -- -- - - - - - - -- -- -- -- - - - -- -2.13}$$

2.4 Mineral analysis

2.4.1 Sample Digestion

The triple acid digestion method described by Sahrawat *et al*., (2002) was adopted. One gram of dried sample was weighed into Kjeldahl digestion flask to which 24cm^3 of a mixture of conc. HNO_3conc. H_2SO_4 and 60% $HCIO_4$ (9:2:1v/v) was added. The flask was placed on a heating block and was digested to a clear solution cooled and the content filtered into a 50cm^{3}volumetric flask through Whatman No. 1 filter paper. The flask was then made-up to the mark with water. Blank was prepared in similar manner without samples being added.

2.4.2 Analysis of metals using AAS

Mineral elements (except Na and K) were determined using atomic absorption spectrometry, The sodium and potassium was determined using Atomic emission spectrometry (AOAC, 1990). In metal analysis (except for sodium and potassium) atomic absorption spectrometry (AAS) was used due to it precision and accuracy (Watchasunder and Nafde, 2001). The principle of the method is based on nebulizing a sample solution into an air-acetylene flame

where it was vaporized. Elemental ions were then atomized and the atoms formed absorb radiation of a characteristic wavelength from a hollow-cathode lamp. The absorbance measured, is proportional to the amount of analyte in the sample solution.

2.4.3 Analysis of Sodium and Potassium using AES

Atomic emission spectrometry (AES) has similar principle with AAS, but they differ from each other in terms of measurement of transmitted radiation. While the AES measured the intensity of emitted radiation, AAS measure the intensity of absorbed radiation.

Procedure

The galvanometer reading of the flame photometer (Model 400, Corning U.K.) was adjusted to zero with water and then to full scale with standard solution of $25mgl^{-1}$. The other standard solutions were subsequently aspirated along with the samples and blank solution. Calibration curves were plotted from which the concentrations of sodium and potassium was determined using equation 2.11.

$$X\ (mg/100g) = \frac{X\ (ppm) \times \text{vol. of sample made}}{\text{Weight of the sample} \times 10} \quad \text{- 2.14}$$

2.4.4 Analysis of phosphorus

The AOAC (1990) method was adopted. 2g of ash was dissolved in $5cm^3$20% hydrochloric acid and diluted to $50cm^3$ with distilled water.

$2cm^3$ of this solution was transferred to $50cm^3$ volumetric flask,$2cm^3$ of ammonium molybdate reagent was added and diluted to the mark with distilled water. The solution was

allowed to stand for 10 minutes for colour development, after which the absorbance of each was recorded at 660nm.

2.4.5 Amino Acid Analysis

The analysis of amino acids was carried out according to Spackman *et al*, (1958). Two gram of each sample were defatted and 30mg of defatted sample weight into a glass ampole to which $5cm^3$ of 6M HCI and 5moles norleocine (as internal standard to correct slight fluctuations of amino acid peaks were added (Eyo *et al*.,1999). The ampoule was evacuated by passing nitrogen gas to remove oxygen so as to avoid possible oxidation of some amino acids during hydrolysis. The ampoule was then sealed with Burnsen burner flame and put in an oven at 110^oC for 24 hours. After the hydrolysis, the ampoule was cooled, and broken at the tip and content was filtered. The filtrate was than evaporated to dryness at 40^oC under vacuum in a rotary evaporator.

The residue was dissolved to SNL for acid and neutral amino acid or $10\mu l$ for basic amino acids with acetate buffer, pH 2.2. The aliquot was then loaded into the cartridge of amino acid analyzer (Technon sequential multi sample). The chromatograms obtained along with automatic pen records. Indicated amino acid peaks corresponding to the magnitudes of their respective concentrations.

Quantification was performed by comparing the peaks area of each amino acid in the sample to the area of the corresponding amino acid standard of the protein hydrolysate (0.025 mole).

Calculation

The net height (NH) of each peak produced by the chart record of the analyzer was measured. The half-hieght (NH/2) of each peak on the chart was found and the width (W) of the peak at the half height was accurately measured and recorded.

Approximate area of each peak was then obtained as NH xW.

The norleocine (Neu) equivalent (NE) for each amino acid in the standard and mixture (AAstd) was calculated using equation 2.14.

$$NE = \frac{\text{area of neu peak}}{\text{Area of each AAStd}} \quad \text{-----2.15}$$

A constant Sstd was calculated for each A-Astd using equation 2.15

Std= NEstd x molecule weight of each AAstd Xμ mole AA std----------2.16

The amount of each amino acid in the sample (AAstd) in g/100g protein is:

$$= \frac{\text{Area of AAsmp}}{\text{Area of new in the sample}} \quad \frac{\text{Sstd X } x \text{ dilution } x \text{ 16}}{\text{sample weight } x \text{ N(\%)} x \text{ 10 } x \text{ volume Buffer solution loaded}} \quad \text{----------2.17}$$

2.4.6 GC-MS Analysis

The analysis was carried out using Schimadzu QP 2010 SE (single squad) gas chromatography. The gas chromatography was programmed as follows: The column was programmed at 50 ^{0}C and held for 8 minutes and then ramped at 8 ^{0}C per minute to 130 ^{0}C and up to 200 ^{0}C with final ramp of 15 ^{0}C per minute to 280 ^{0}C and held for 4 minutes. The injector port was set at 250 ^{0}C and samples injected in split mode with a split ratio of 1:10. Helium gas was employed as the carrier gas at 1 ml/min. flow rate. The mass spectrometer was programmed

as follows: Ion source temperature was set at 200 ^{0}C with an interface temperature of 300 ^{0}C. Scan speed of 2000 at 0.20 secs; scanning through 30 to 400 m/z. The oil sample was diluted with normal hexane and 0.1 microliter was injected into the oven. The components of the oil were identified by comparison of their mass spectra with those of standard mass spectra from Wiley and NIST libraries (Wiley 9 and NIST 11)

2.4.7 Statistical Analysis

Data generated was reported as mean± standard deviation of triplicate results. Two (2) sample T-tests was used to compare means of the whole cotton seeds A and B samples.

CHAPTER THREE

RESULTS AND DISCUSSION

3.1 Results

The result obtained from the analyses were presented in tables 3.1-3.7. Table 3.1 shows the result of proximate analysis of both whole cotton seed A and whole cotton seed B .In both cases, the samples were rich in protein and carbohydrate. But low in ash and moisture content. The ash content was significantly ($p<0.05$) higher in whole seeds B than whole seeds A whereas whole seeds A has significantly ($p<0.05$) higher amount of lipid than whole seeds B.

Table 3.1: Major proximate composition of whole cotton seeds obtained from Dutsin-ma and Malumfashi Local Government Areas (%)*

Components (%)	Whole seeds A	Whole seeds B
Moisture	4.33±0.57	5.87±0.10
Ash content	6.83±0.08	7.55±0.06
Crude protein	24.68±0.35	26.20±0.84
Crude lipids	25.33±4.80	22.09±1.20
Crude fibre	8.33±0.29	10.94±0.28
Carbohydrate *	20.50±5.91	27.35±0.29
Energy value (KJ/100g)	392.65	378.76

All values are the mean of triplicate determination expressed on dry weight basis ± standard deviation

*Calculated by difference

Whole seeds A (Dutsin-ma cotton seeds)

Whole seeds B (Malumfashi cotton seeds)

Table 3.2 shows the physicochemical properties of the oil extracted from the two samples .The results indicates that whole seeds A and B have high iodine value, saponification value, but low peroxide value for both the samples

Table 3.2: Physicochemical Properties of Oil Extracted from Cotton Seeds (*Gossypium hirsutum*)

Characteristics	**Whole seeds A**	**Whole seeds B**
% Oil yield (wt/wt)	25.33±4.00	21.04±1.20
Colour	Dark brown	Dark brown
Odour	Pleasant	Pleasant
Specific gravity at 30^{o}C	1.07±0.01	1.09±1.00
State at 25^{o}C	Semi-liquid	Semi-liquid
Pour point ^{o}C	-16.0±2.00	-17.41±1.54
Flash point ^{o}C	235.0±1.00	230.0±1.00
Kinematic viscosity at 30^{o}C	32.6±1.3	31.50±0.10
ApI gravity	0.74±0.10	1.68±1.19
Cloud point ^{o}C	2.10±0.2	1.80±1.80
Iodine value (g/100g)	86.13±1.72	91.65±1.74
Saponification value (mgKOH/g)	136.26±2.14	128.39±0.23
Acid value (mgKOHg-1)	4.65±0.10	3.88±0.30
Peroxide value(meq/kg)	1.90±0.20	2.20±0.10
Free fatty acid (mgKOH/g)	2.33±0.20	1.94±3.50

Table 3.3 shows the mineral content of *Gossypium* samples .The results indicates that the whole seeds A and B are rich in potassium, calcium and sodium while iron, zinc and copper were detected at low concentrations. In the table whole seed B had significantly ($p<0.05$) higher amount of potassium, calcium and magnesium than whole seed A. While sodium and manganese significantly ($p<0.05$) higher in whole seeds A.

Table 3.3: Mineral Composition of whole Cotton Seeds A and whole Cotton Seeds B in (g/100g D W)

Elements	**Whole seeds A**	**Whole seeds B**
Sodium	128.33±5.77	123.33±5.77
Potassium	5308.33±28.87	5490±56.58
Magnesium	1.98±0.01	2.0±0.05
Calcium	164.28±7.34	172.34±3.18
Phosphorous	3.89± 0.02	3.78±0.50
Iron	3.76±0.01	1.51±0.15
Zinc	0.10±0.01	3.53±0.31
Copper	0.04±0.02	3.69±0.74
Manganese	0.15±0.02	0.08±0.04

All values are the mean of triplicate determination expressed on dry weight basis ± standard

DW= Dry Weight

Table 3.4 shows The level of amino acids content of whole seeds A and B. The results indicates glutamic acid, arginine, aspartic acid and leucine are the predominant amino acids in both A and B samples . The samples also contain appreciable amount of essential amino acids.

Table 3.4: **Amino acid contents of whole cotton seeds A and B (g/100g protein)**

Amino Acid	Whole Cotton Seed A	Whole Cotton Seed B
Essential Amino Acid		
Lysine	4.14	4.51
Threonine (Thr)	3.22	3.00
Tyrosine (Thr)	2.75	3.10
Phyenylalanine (Phe)	5.23	4.97
Cysteine (Cys)	1.51	1.33
Valine (Val)	4.50	4.09
Methionine (Met)	1.33	1.39
Leucine (Leu)	5.95	5.49
Isoleucine (Ile)	3.21	3.14
Non-Essential Amino Acid		
Histidine (His)*	2.62	2.43
Arginine (Ar)*	11.01	10.41
Aspartic acid (Asp)	8.87	7.88
Serine (Ser)	3.94	3.73
Glutamic acid (Glu)	15.59	14.61
Glycine (Gly)	4.32	3.94
Proline (Pro)	3.96	3.25
Alanine (Ala)	4.55	4.17

* Essential for Children

Comparison of the essential amino acids with the reference standard given by FAO/WHO UNU (1991) standard protein is shown in table 3.5. The result shows that whole cotton seeds A is deficient in lysine with score of 71%. The whole cotton seeds B has lysine with limited amino acids score of 78%. And whole cotton seeds A has chemical score above the reference value >100%.

Table 3.5: Essential amino acids composition of whole cotton seeds A and whole cotton seeds B (g/100g protein) compared to FAO/WHO/UNU reference pattern

Amino Acid	Whole Seeds A	Whole Seeds B	Chemical Score		FAO/WHO/UNO Reference Standard
			Whole Seeds A	Whole Seeds B	
Ile	3.21	3.14	115	112	2.8
Leu	5.95	5.49	90	83	6.6
Lys	4.14	4.51	71	78	5.8
Thr	3.22	3.00	95	88	3.4
Val	4.50	4.09	129	117	3.5
Cys+Met	2.84	2.72	101	97	2.8
Tyr+Phe	7.98	8.07	127	128	6.3

Adapted from FAO/WHO/UNU (1991)

* % amino acids content expressed as: $\frac{Amino\ acid\ in\ the\ sample}{standard\ reference\ pattern} \times 100\backslash$

Table 3.6: Fatty Acid Composition of Seed Oil B

The mass spectra of the fatty acids composition of the oil are presented in (appendix), And the percentage composition of each fatty acid present in the oil was assessed and the result presented in Table 3.5.

Table 3.6: Fatty Acid Composition of Seed Oil B

Peak	RT(min)	Compounds	MWt	% in oil
1	10.892	2,5-octadecadienoic acid	285	11.19
2	18.857	9-Hexadecenoic acid	268	14.92
3	21.277	10-Heptadecenoic acid	282	0.40
4	26.05	2-Tetradecanoic acid	258	15.90
5	29.031	10 Nonadecenoic acid	310	3.34

Table 3.7: Fatty Acid Composition of Seed Oil A

The mass spectra of the fatty acids composition of the oil are presented in (appendix), and the percentage composition of each fatty acid present in the oil was assessed and the result presented in Table 3.7.

Table 3.7: Fatty Acid Composition of Seed Oil A

Peak	RT(min)	Compound	MWt	% in oil
1	33.116	Hexadecanoic acid	256	36.4
2	36.135	Docasanoicacid	680	6.80
3	25.642	Octadec-9-enoic acid	611	24.60
4	19.483	14,17-Octadecadienoic acid	294	**14.70**
5	30.491	Tetradecanoic acid	228	9.10

3.2 Discussion

3.2.1 Moisture Content

Moisture is the presence of a liquid, especially water in trace amounts in a substance. The moisture content of the whole cotton seeds A and whole cotton seeds B were determine to be 4.33±0.5 % and 5.87± 0.10 % respectively. This shows that whole cotton seed B have higher moisture content value 5.87± 0.10 % compared with the whole cotton seed A 4.33±0.5 %. These results are higher than the results reported by. (Mohammed.*et al*.,2015) for Ipomoeagenus.Indica (1.57%),and I. *pestigridis* (1.99%).The percent found in the two seeds is lower in comparison with standards (9.18%) (FAO/WHO, 2004).

The high moisture content of whole seeds B could be due to the environmental condition where the seeds are obtained. The relatively low moisture content of the samples are advantage since high moisture content is associated with rise in bacterial action during storage (Badifu, 2001).

3.2.1.2 Ash content

The ash refers to all non-aqueous, non-gaseous residues that remain after sample is burned .which is an index of inorganic minerals. The result showing in table 3.1 indicates that the whole seeds of cotton A and B have the ash content of 6.83±0.08 % and 7.55±0.06 % respectively. This shows that whole cotton seed B have high value 7.55±0.06 % compared with the whole cotton seed A 6.83±0.08 %.(Halilu *et al*., 2017).

The values are higher compared with 4.6% for ,*Annonasquamos a*re reported by Baldwin,(1999).Generally the ash contents were founds to be higher than those value reported for *Ipomoea carnea* 3.33±0.29% (Halilu *et al*., 2017).The percent found in the two seeds is

higher in comparison with standards (4.06%) reported by (Baldwin, 1999).Since ash content represents the index of mineral elements present in the sample. The value obtained for the ash showed that the seeds contained reasonable amounts of mineral elements. For example calcium is needed for healthy bones and teeth, magnesium for protein synthesis, and zinc for wound healing.

3.2.1.3 Crude Protein Content

Crude protein is the measure of how much protein is present in a food substance. The crude protein content of the whole cotton seeds A and B are 24.68±0.35% and 26.20±0.84% respectively. This shows that whole cotton seed B have high protein content 26.20±0.84% value compared with the whole cotton seed A 24.68±0.35%. These values were observed to be higher than that of cowpeas, pigeon peas which are between 23.1-33% (Akanbi *et al*., 2005).The values found for the two seeds ware higher in comparison with standards (19.8%) for the (FAO/WHO,2004). The high protein content will serves as a source of amino acids and protein for both man and animals (Pickel *et al*.,2013).

3.2.1.4 Crude Lipid Content

Crude lipid is a free lipid that can be extracted into less polar solvents such as petroleum ether. The value obtained for the percentage crude lipid in whole cotton seeds A and B in Table 3.1 are 24.6±0.35% and 26.20±0.84% respectively. These shows that whole cotton seed B have higher lipid content 26.20±0.84% as compared with the whole cotton seed A 24.6±0.35%. And are lower than 32.69 % reported by Satishgale and Shruti,(2016) for the same sample. The values found in the two seeds are higher in comparison with cotton seeds standards (18.7%) (FAO/WHO,2004) .The higher value obtained in this research futher justified the use of cotton

seed oil as food, in cosmetics and pharmaceuticals (Ulrich,2002). It also serves as a source of raw materials for oil mills and source of livestock feed. (Ezeagu *et al*., 2000).

3.2.1.5 Crude Fibre Content

Crude fibre is the measure of the quality of indigestible cellulose, lignin and other components present in food substance. The results of the crude fibre analysis of the samples (Table 3.1) shows that the whole cotton seeds B has the higher value of crude fibre content of 10.94±0.28% when compared with 8.33±0.29% of whole cotton seeds A. These values ware however observedbelow the recommended range 12-38 g/day (FND,2002).

The fibre is required in our diet for the maintaining of intestinal problems and reduces the risk of cancers. Therefore, the high level of crude fibre in the seed could render the seed important as livestock feed.(Njoku *et al*., 2007).

3.2.1.6 Carbohydrates

The carbohydrate contents of whole cotton seeds A and whole cotton seeds B are 30.50±5.91% and 27.35±0.29% respectively. These shows that whole cotton seed A have higher carbohydrate content 30.50±5.91% compared with the whole cotton seed B 27.35±0.29%. These values ware observed to be higher than those water Mellon seed (12.96%) reported by Oyolola, (2010) but are in agreement with the 27.27±0.41% reported in the same species by (Kose *et al.,* 2015). Total carbohydrate is considered a good source of energy for the body, The carbohydrate content of the seed makes it a good source of calories (Dwek and Raymond, 2013).

3.2.1.7 Energy Value

The energy value for the whole seeds A and whole seeds B (shown in Table 3.1 in kcal/10g) are 392.65 and 378.76 respectively. The whole seed A have the highest value compared with the value for whole cotton seed B. This variation is due to the high amount of crude lipid in the whole cotton seeds A than in whole cotton seeds B.(http:\\ijc . cesenet.org international journal of chemistry vol.8 No.3 :2016.)

3.2.2 Amino acids content

The amino acids composition of the seed samles A and B as shown in Table 3.4. Considering lysine (4.14) for whole seeds A as compared to (4.51) for whole seeds B, valine (4.50) for whole seeds A as compared B (4.09), leucine for whole seeds A (5.95) as compared to (5.49) for B, phyenalamine for whole seeds A (5.23)) as compared to (4.97) for B. The amino acids are the building blocks of protein (Carrey, 2003) it therefore implies that the whole seeds A has more protein content than whole seeds B. Among the essential amino acids leucine have the highest value in whole seed A 5.95 when compared with 5.49 for whole cotton seed B. The values for leucine where found to be higher than that of calabash fruit 2.75. Donu *et al*;(1986*)* .Also Methionine have the least value in whole cotton seed A 1.33 when compared with 1.39 for whole cotton seed B. Donu *et al*;(1986*).*High amount of leucine helps the body to regulate blood sugar ,aid growth ,repair muscle and bone and for wound healing and the low amount of methionine will affect the growth of nails and hair. (Fust *et al., 2004).*

Considering the individual non-essential amino acids of each sample, glutamic acid was found to be the most abundant amino acid in all the results. Whole seeds A having significantly the highest ($P < 0.05$) value (15.59) as compared to whole seed B (14.61). But these values were found to be lower than that of cashew nut (18.74) (Fetuga *et al*., 2009). And higher than that of

calabash fruit (2.4) (Donu *et al*., 1986). The nutritional quality evaluation of *Gossypium hirsutum* are expressed as the percentage of essential amino acids in the sample which are compared to reference standard set by World Health Organization (FAO/WHO/UNO, 1991).

The results from the samples shows that they have good amino acids profile. This is an indication that *gossypium* protein is of good quality.

3.2.3 Fatty Acid Content of *Gossypium hirsutum*

The results of fatty acid contents of whole seeds A and whole seeds B are shown in table 3.5 and 3.6 .The palmitic acid content of whole seeds A (36.40%) was higher than that of whole seeds B (14.92%).Which is lower compared with (38.68%) reported by Thompson,(2008).

The oleic acid content of whole seeds B (11.19 %) was lower than that of whole seeds A (24.60%) which is in agreement with that was reported in castor seeds oil Gregory *et al.,2017. The* low content of oleic acid in the sample which is unsaturated, indicate that it does not raise the level of cholesterol in the body which is one of the index of heart diseases Saribiyik *et al*.,(2010).

The behemic acid content of whole seeds A(6.80%) was found to be higher than that of whole seeds B (3.34%) in the samples . while the mystric acid content of whole seeds A(9.10%) was found lower than that of whole seeds B (15.90%). The high content of unsaturated fatty acid was responsible for the reduction of blood pressure induced by oil seeds. (Teres *et al*.,2008).

3.2.3 Physicochemical Properties of the Oils

3.2.3.1 Percentage oil Yield

The percentage oil yield of whole cotton seeds A and whole cotton seeds B are (25.33±4.00 and 21.04±1.20%) respectively. Which are almost similar with the 28.5% reported by Sribiyik *et al*. (2010) and lower than Adeola *et al*., (2012). The differences may be due to plant growing environment, fertilizer application and method of extraction employed. The values of this seeds oil is quite promising and suggests that by obtaining commercial quantities it can be used for industries, pharmaceutical, cooking and other purposes.

3.2.3.2 Saponification Value

Saponification Value is a measure of the equivalent weight of fatty acids present in the oil sample. The saponification values of the cotton seed oil for A and B are 136.26±2.14mg/KOH/g and 128.39±0.23mg/ KOH /g respectively. These indicates that, the oils have median weight fatty acids. Oils with saponification value of 200mg/g and above regarded as high molecular weight fatty acids oils which are used in soap making and it fall within the range of values obtained for some vegetable oil 188-35mg/ KOH /g Aremu *et al*.,(2006).

3.2.3.3 Iodine Value

Iodine Value, also called iodine number is define as the measure of the degree of unsaturation of oil and fat. Saturated fats and oil take up no iodine but unsaturated oils and fats take up iodine Adebayo *et al*.,(2011).

The results of iodine value of whole cotton seeds oil and whole cotton seeds B oil are (86.13±1.72 and 91.65±1.74) respectively. iodine value it’s an index of unsaturation which

shows that fatty acids in the two oil samples to be non-drying since their values falls within the range of 86-106 (Abayeti *et al*., 2008) But the values obtained are lower than that of cucurbit foedisssima, 144(Gunstone *et al*., 1986). The iodine values of (96.6-103.63) found in the oil samples indicates that the oils will not have high interest in the industry as drying oils bit could be used in the preparation of soap, cosmetic, lubricants, and soon (Anhawange, *et al*., 2004). Iodine value it's an index of unsaturation which shows that fatty acids in the two oil samples to be non-drying since their values falls within the range of 86-106 (Abayeti *et al*., 2008)

3.2.3.4 Acid Value

Acid value is defined as the weight of KOH in mg needed to neutralize the organic acids present in 1g of fat and oil and is a measure of free fatty acids (FFA) presents in the fat or oil. The acid value of the two oils extracted using soxhlet methods are 4.65±0.10 mg KOH /g for cotton seeds A and 3.88±0.30mg KOH/g for cotton seed B. These shows that whole cotton seed A have high value compared with the whole cotton seed B. The values are higher than the value obtained by Rakesh *et al*.,(2014) and higher than the maximum value of 2mg KOH/g. The high acid value implies that the oil has high sensitivity to decomposition (Omohy and Omale, 2017).

3.2.3.5 Kinematic Viscosity

The kinematic viscosity of the oil affects the fluidity of the oil (Sattanathan, 2013). The kinematic viscosity of whole cotton seeds A and whole cotton seeds B are 32.6±1.30mm^2 at 40^o and 31.50±0.10mm^2 at 40^oc respectively. These shows that whole cotton seed A have high value compared with the whole cotton seed B. Viscosity is one of the most important properties describing the possibility or otherwise of using vegetables oil as an alternative fuel (Aigbodion *et al.,* 2004). The values are lower than 40.25mm^2 at 40^oc reported by Encinar *et al.,* (2011). They

are higher than (7.91cst) for soybeans reported by (Kanosmanoglu and Kunt, 1998). From the results obtained the oils have high viscosity and therefore can be used as biodiesel. (Aigbodion *et al.*, 2004).

3.2.3.6 Free Fatty Acids

The free fatty acids of the cotton seeds oil A and cotton seeds oil B are 2.33±0.20mgKOH/g and 1.94±3.50 mg/KOH/g respectively. These shows that cotton seeds oil A have higher free fatty acids of 2.33±0.20mgKOH/g as compared with B 1.94±3.50 mg/KOH/g .This Indicate that the oils have low deteriorating and high edibility (Aigbodion, *et al.*, 2012).

3.2.3.7 Peroxide Value

The peroxide values of the oils obtained from the whole cotton seeds A and whole cotton seeds B are 1.90±0.20meq/kg and 2.20±0.10meq/kg respectively. When compared with some oil, the value is lower than 5.14 meq/kg of oil in baobab seed (Adetokun, 1996), 3.5meq/kg of oil in *Canarium album* seed (Zhiyong and Wenshui, 2006). The high peroxide values are associated with high rate of rancidity, the low peroxide values obtained in the oils is an indication of the oil to have less rancidity at room temperature (Rakech *et al.*, 2014).

3.2.3.8 Specific Gravity

Specific gravity is the ratio of the density of a substance to the density of a reference substances for the same given volume, and is a property which affect the engine performance Rengasamy *et al.*,(2014)The results of specific gravity at 30^{o}C for the two cotton seeds oils are 1.07±0.01 and 1.09±1.00 respectively. This values, shows that there is no positive impact on the specific gravity of the oil since the values are higher than that of fuel (0.92±0.0)reported by Senatore *et al.*,(2015).

3.2.3.9 Pour Point and Cloud Point

The cloud points is the temperature at which dissolved solids are no longer completely soluble precipitating as a second phase giving the fluid a cloudy appearance. Rengasamy *et al* (2014).The values of pour and cloud point for the whole cotton seeds A and B are -16.0±2.00^{o}C, 2.10±0.20^{o}C and for B are 1.80±0.80^{o}C, - 17.4±1.54^{o}C respectively. These shows that the values of whole cotton seed A and whole cotton seed B are higher compared with -14^{o}C and 12.5^{o}C reported by (Forero, 2010) and are far higher than the values reported for fuels 0-3^{o}C, ASTM (2008).

3.2.3.9 Flash Point

The flash point is the minimum temperature at which oil gives momentary flash on ignition under specified test conditions. The flash point of whole cotton seeds A and whole cotton seeds B are 230.0±1.00^{o}C and 230.0±0.20^{o}C respectively. Which is lower than 286^{o}C that observed by Rengasamy *et al.* (2014). And almost similar with 229^{0}C ICSC (International chemical safety cards) 1452 standard. This indicates that the cotton seeds are safe in handling and storage.

3.2.4 Mineral Compositions of the two Whole Cotton Seeds

The results of the mineral composition of the two sample in Table 3.3

3.2.4.1 Sodium

The result indicates that, whole seeds A with sodium content of 128.33±5.77mg/100g has the higher value while whole seeds B sodium content registered the least value of

123.33±5.17mg/100g. These values are these values are higher than that of Almond seed (3.80mg/100g) reported by Yusuf, (2010). The high level of sodium observed in the samples could be due to high intake of sodium from the soil. The values are slightly low when compared with the recommended daily allowance for adult (1500mg/100g) (FND, 2002). Sodium is associated with potassium in the body in maintaining proper acid base balance and transmission of nerve impulses (Carrey, 2003).

3.2.4.2 Potassium

The concentration of potassium in whole seeds A is 5308±28.87mg/100g. while that of whole seeds B is 5490±56.58mg/100g. The values are very high when compared with the 1826.72mg/100g obtained in the seed of *Hasta la Pasta* (Hassan *et al*.,, 2009, Zhenchangel, *et al*., 2011).

And also are higher than the recommended daily allowance for adult (4700mg/100g) (FND, 2002). The values obtained revealed that the seeds are rich in potassium and could play a vital role in biochemical processes involving potassium such as fluid balance mere and muscle contraction.(Turan *et al*., 2003).

3.2.4.3 Phosphorus

Phosphorous content of whole seeds A is 3.89±0.02mg/100g and whole seeds B is 3.78±0.02mg/100g. The values for cotton seeds was found to be higher than the values(12.2-79mg/100g) reported for some conventional seeds and nuts. (Almustafa *et al.,* 1995).

This is an indication that the samples are not good sources of phosphorus when compared to its RDA value 800mg/day (NRC, 1989). Phosphorus is an import mineral for proper functioning of bones, teeth and muscles (Turan *et al*., 2003).

3.2.4.4 Magnesium

The concentration of magnesium in the whole seeds A and whole seeds B are 1.98±0.01mg/100g and 2.0±0.05mg/1001g respectively. The values are very low when compared with shea nut (222±3.1mg/100g) reported by Almustapha *et al*., 1995 and RDA of 320-420mg/100 (FND, 2002).Magnesium is an essential mineral that is involved in energy production, nucleic acid and protein synthesis (Ladan *et al.*, 1996).

3.2.4.5 Calcium

The concentration of calcium in the whole seeds A and whole seeds B are 164.28±7.34mg/100g and 172.34±3.18mg/100g respectively. The values are very low compared with the recommended dietary allowance of 1000mg/100g for adult (FND, 2002). Calcium is a dietary element needed for the formation of bones and teeth (Guthrie, 1989). The amount of calcium circulating in the blood and in the soft tissue plays a vital role in metabolic process and control of heartbeat (Bello, *et al*., 2008).

3.2.4.6 Iron

The iron content of whole seeds A and whole seeds B are 3.76±0.01mg100/g and 1.51±0.15mg/100g respectively. The values are higher than that of oil bean seeds5.63mg/100g as reported by Almustafa *et al*., (1995).Iron is an essential trace element for haemoglobin formation ,in functioning of central nervous system and in oxidation of carbohydrate and proteins.

Considering the high values of iron in the two samples analyzed, the seed and its oil could serves as a source of iron for man and animals (Almustafa *et al.*, 1995).

3.2.4.7 Zinc

The concentration of zinc whole seeds A and whole seed B are 0.10±0.016mg/100g and 3.53±0.3mg/100g respectively. These shows that whole cotton seed A have high value compared with the whole cotton seed B. The values are low compared with 3.32mg\100g for Agama lizzard Abulude *et al* .,(2005). The two values are low when compared with the recommended daily allowance for an adult (11mg/100g) (FND, 2002).

The dietary requirement for zinc necessary for growth, tissue repair, and obligatory excretion. However, its deficiency lead to growth failure and impaired wound healing (WHO, 2003).

3.2.4.8 Copper

The results indicate that whole seed A with copper content 0.04±0.02mg/100g has the lowest value than whole seeds B with copper content of 3.69±0.74mg/g. The values are low compared with the research by 2.02mg/100g for Barbados (Olaofe, 2004), yam tuber (Ojeka, *et al*., 2004) and RDA 15.3mg/100g (NRC, 1989).Copper is an essential trace element in human body in the energy metabolism (McDonald *et al*., 1995, Adeyeye, 2002).

3.2.4.9 Manganese

The concentration of manganese in the whole seeds A and whole seeds B are 0.15±0.02mg100/g and 0.08±0.04mg/100g respectively. The values are very low compared with 12mg/100g reported for calabash seed (Badifu, 2001). The values are higher than the recommended daily allowance for adult 1.8-2.3mg/100g (FND, 2002).

Manganese plays a structural role in the chloroplast, membrane system and may be responsible for colour, test and smell for fatty acid, DNA and RNA synthesis (Ayeze *et al*., 2006).

CHAPTER FOUR

CONCLUSION AND RECOMMENDATIONS

4.1 Conclusion

In this study nutritional profile of two whole cotton seeds from katsina state were evaluated and there physicochemical properties analyzed. The following conclusions were reached.

(i). The nutrient distribution between the whole seeds A varies with the nutrient distribution of whole seeds B. The whole cotton seeds A (Dutsin-ma) was found to contain high percentage of oil 25.33±4.00 than whole cotton seeds B (Malumfashi) 21.04±1.20 .

(ii). The whole cotton seeds B was found to contain high amount of fibre 10.94±0.28 , protein (26.20±0.84), and ash content 7.55±0.06 . The cotton seeds can therefore considered as important source of oil, fibre, protein and mineral elements.

(iii). The whole cotton seeds was found to contain most of the essential amino acids with leucine which the highest for whole cotton seeds A (5.95) and (5.49) for whole cotton seeds B. With these results the seeds can be sources of protein which can reduce the problem of protein energy malnourishment.

(iv). The oils were found to have low peroxide (2.20±0.10) for whole cotton seeds B and 1.90± 0.20 for whole seeds A and acid value (4.65±0.10) for whole seeds A, (3.88±0.30) for whole seeds B respectively. Indicating that, the oils have good storage quality and also good for edible (vegetable oil) purpose and industrial application.

4.2 Recommendations

From the analysis carried out, The following recommendations as regards to further studies were proposed.

(i). Medicinal Analysis of the seeds should be carried out to determine the medicinal content of *gossypium hirsutum*.

(ii). Performance and emission test for cotton seeds oil that satisfied ASTM standards should be carried out.

(iii). The Toxicological studies of the whole cotton seeds should be investigated.

(iv). The Transesterification of cotton seeds oil into biodiesel and production of lubricants should be carried out.

(v) Biological activities such as antimicrobial should be investigated.

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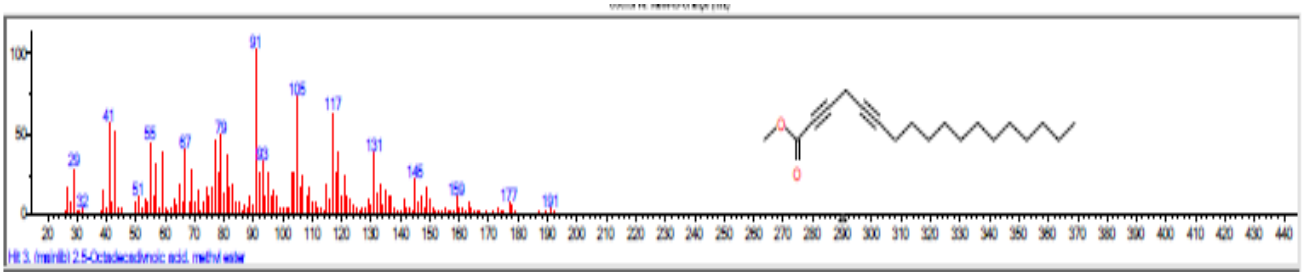
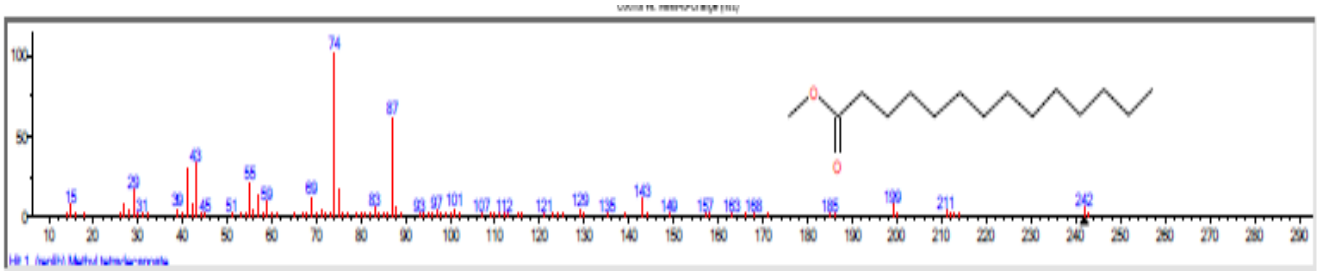
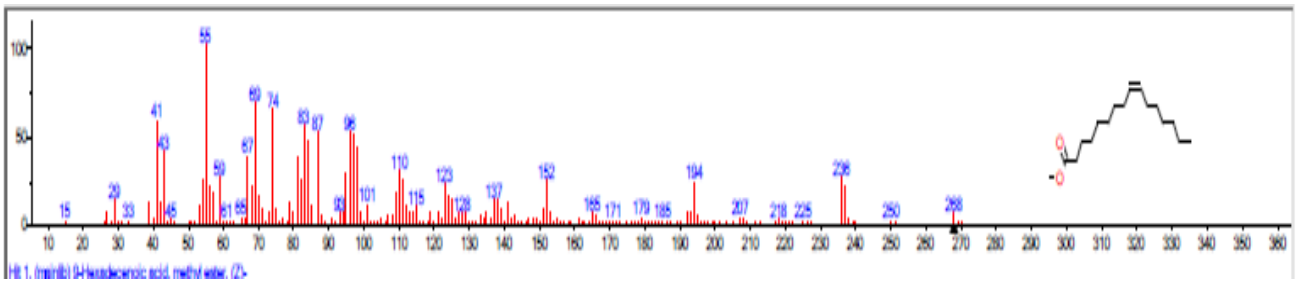
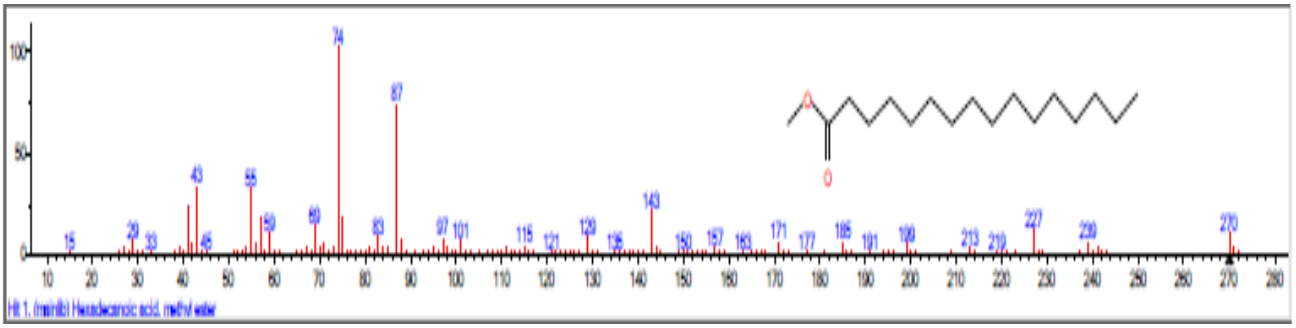
APPENDICES

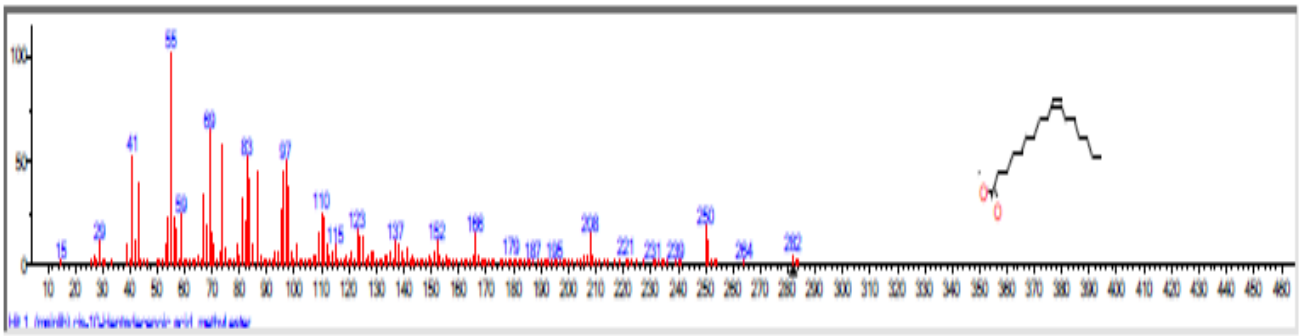
The GC-MS Spectrum of *Gossypium Hirsutum*-Seed Oil

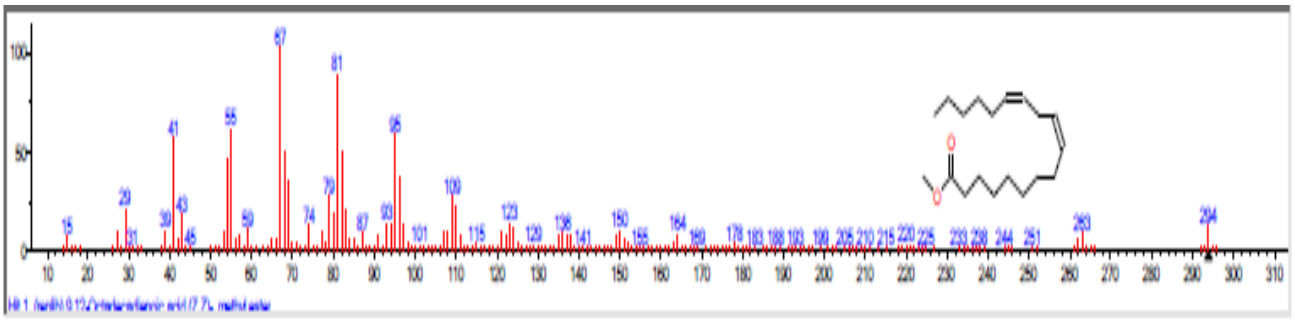


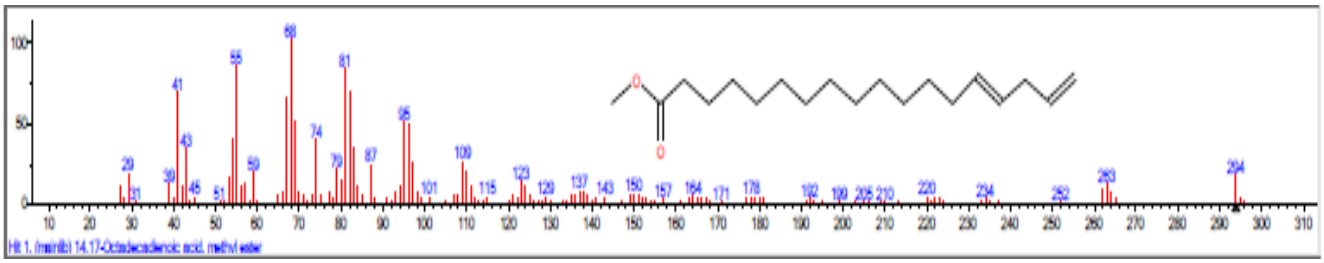


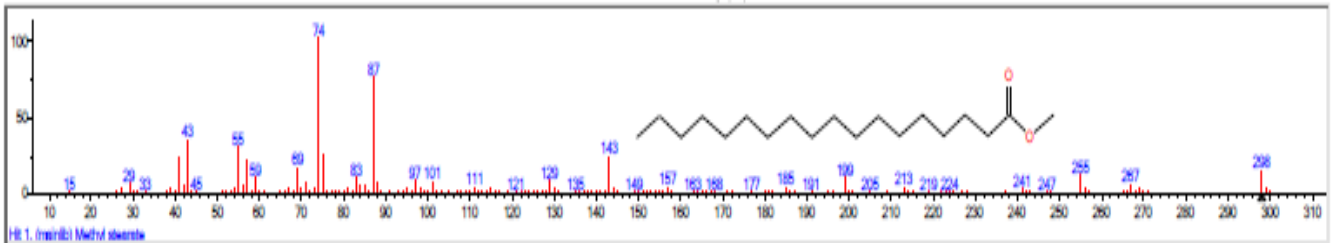


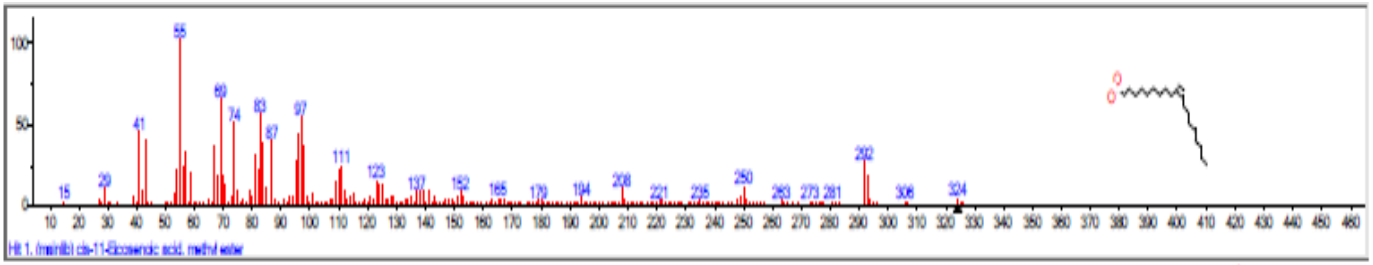


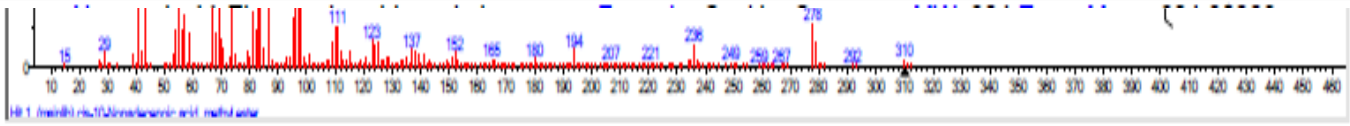




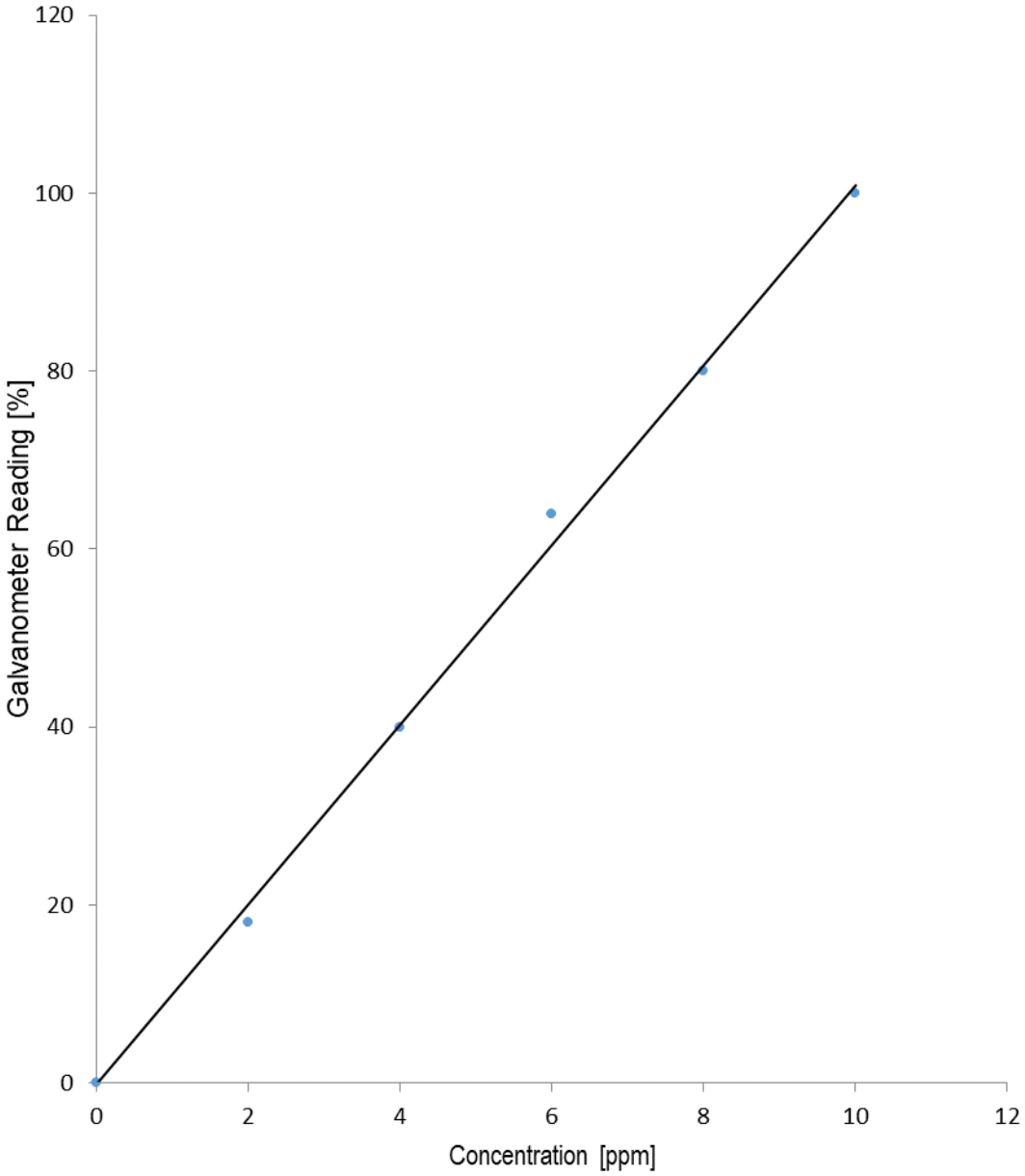




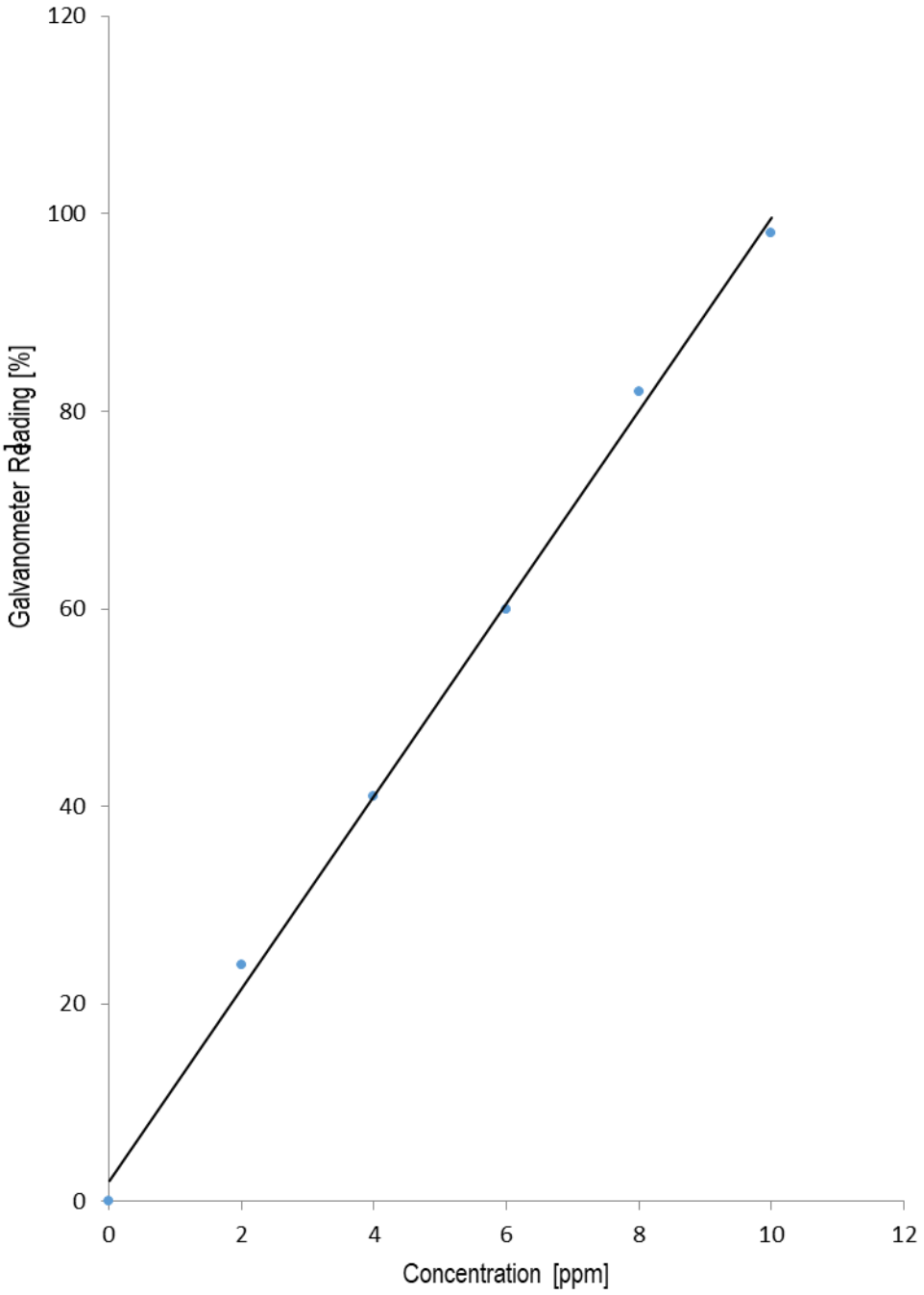




Potassium Calibration Curve



Sodium Calibration



Curve

Phosphorus Calibration Curve