FATTY ACIDS, HEAVY METAL CONCENTRATION, AND CHARACTERISTICS OF SELECTED OIL SEEDS GROWN IN WEST NILE, UGANDA

BY

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DECLARATION

I declare that this dissertation is my own original work and that it has never been submitted to any University or Institution of higher learning for any academic award. Where other people's work has been used, this has properly been acknowledged and referenced in accordance to Kyambogo University requirement.

Signature: ……Date: ……

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APPROVAL

This is to certify that this dissertation was under my supervision and is now ready for submission to the Graduate School of Kyambogo University with my approval.

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DEDICATION

I dedicate this work to the Almighty God who made all things possible and to all men and women of God who have given my life a meaning.

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LIST OF ACRONYMS

AAS:	Atomic Absorption Spectrophotometer
GC/MS:	Gas Chromatography/Mass Spectrometry
MUFA:	Monounsaturated Fatty Acids
PUFA:	Polyunsaturated Fatty Acids
SFA:	Saturated Fatty Acids
HDL:	High Density Cholesterol
LDL:	Low Density Cholesterol
FAMEs:	Fatty Acid Methyl Esters
FAO:	Food and Agricultural Organizations
WHO:	World Health Organization
EU:	European Union
MRL:	Maximum Residue Limit
EFSA:	European Food Safety Authority
SR:	Shear Rate
SA:	Surface Area
PV:	Peroxide Value
IV:	Iodine Value
AV:	Acid Value
AOACS:	Association of American Chemist Society
FFA:	Free Fatty Acid
NAADS:	National Agricultural Advisory Services
UK:	United Kingdom
LDPE:	Low Density Polyethylene
RD:	Relative Density
ASTM:	American Society of Testing and Materials
AR:	Analytical Grade
N:	Normality
M:	Molarity
SD:	Standard Deviation
ND:	Not Detected
MPL:	Maximum Permissible Limit

ABSTRACT

In this study vegetable oils extracted from three types of oil seeds namely; sunflower *(helianthus annus)*, sesame *(sesamum indicum)* and peanut (*Arachis Hypogaea)* grown in west nile sub-region were investigated.

Fatty acid composition, heavy metal content, cadmium (Cd), zinc (Zn), iron (Fe), lead (Pb) and characteristics of the oils were determined using GC/MS, AAS and AOAC standard methods respectively. Results for fatty acids showed that oleic, linoleic, palmitic, stearic, behenic, lignoceric, and linolenic acids were present in all the oils.The contents of the major fatty acids ranged from (44.06 to 44.12%) oleic, (30.29 to 31.51%) of linoleic, and (10.93 to 13.07%) palmitic acids. This result demonstrates that sesame, sunflower, and peanut oils are of unsaturated type, but belonging to mid-oleic acid subclass. The polyunsaturated to saturated fatty acid ratios of the oils were >1, the minimum recommended by FAO.

Results of heavy metal determination revealed that iron and lead were detected in all the oil samples and the concentration ranged from 2.330-14.982mg/kg for iron and 1.944-2.126mg/kg for lead (Pb). The highest concentration of lead ((Pb) and iron (Fe) were in sunflower and sesame oils respectively. Cadmium and zinc were not detected in all the oils.The levels of Lead and Iron were above the maximum permissible limit of (0.1mg/kg) for Lead, and (1.5mg/kg) for Iron, respectively. Heavy Metals like iron (Fe) in vegetable oils enhance the oxidation of fatty acids to esters, which affect the nutritional value and properties of oil. Lead (Pb) is toxic to the body even at a low concentration.

Results of the oil quality characteristics showed that the oils exhibited satisfactory results regarding density, viscosity, iodine value, peroxide value, acid value and saponification value. The peroxide value was found in acceptable range of 1.42 to 9.25 meq/kg. While iodine value varied from 79.64 to 126.59gI_2/100g.

Keywords: Fatty Acids, Heavy Metal, Vegetable Oils, Oil characteristics

CHAPTER ONE

1.0 INTRODUCTION

1.1BACKGROUND

Vegetable oils are plant oils extracted from oil bearing seeds/fruits of plants, and the most common ones include soybean, sunflower, peanut, rapeseed (canola oil), sesame, and safflower oils(Nzikou *et al*., 2010). They contain 98% triglyceride (triacylglycerol), which is an ester made up of glycerol and different kinds of fatty acids in varying proportion. Vegetable oils, also contain minor components (2%) such as phosphatides, sterols, fat soluble vitamins, tocopherols, pigments, waxes, and free fatty acids (O'brien, 2018). Vegetable oils are widely used in cooking, food processing, pharmaceuticals, and chemical industries. Nutritionally, they are major sources of essential fatty acids, but also carriers of fat-soluble vitamins like as A, D, E, and K (Koski*et al.*, 2002).

Vegetable oils contain fatty acids, which are important components of human body, having biological, structural, and functional roles. Besides their roles as source of energy, they act as main constitute of cellular membranes in this case as part of membrane phospholipids, they assure the fluidity, flexibility and permeability of the membranes(Tiuca, 2017).

Fatty acids such as Omega-3 and Omega-6 are also known as polyunsaturated fatty acids (PUFA) plays important biological roles in the body, which includes anti-inflammatory properties, reducing oxidative stress, neuroprotection and cardiovascular protection(orosavova, 2015).

The composition of fatty acid in vegetable oils may vary depending on factors such as plant genotype, geographical location, environmental conditions, and agricultural practices (Maxia *et al*., 2009; Gecgel *et al*., 2007; Bansal *et al*., 1993; Alizadeh, 2010).Fatty acid composition of vegetable oils is formed by a mixture of saturated and unsaturated fatty acids, and each

vegetable oil has specific fatty acid distribution depending on their plant source (Orosavova, 2015). Vegetable oils have a high percentage of unsaturated fatty acids such as Oleic and Linoleic acids in the triacylglycerol. Hwang, (2005) reported that fatty acid composition in sesame seed oil has 80% unsaturated fatty acid consisting of oleic (35.9-42.3%) and Linoleic (41.5-47.9%) acids and less than saturated fatty acid 20%. Fatty acid composition of peanut oils from Uganda has been reported as 39.71-55.89% Oleic acid, 20.21-35.59% linoleic acid and 11.91-17.16% Palmitic acid(Musalima, Ogwok, & Mugampoza, 2019).

Akkaya,(2018) reported that sunflower oil contains approximately 15% saturated and 85% unsaturated fatty acids consisting of 14-43% Oleic acid and 44-75% Linoleic acid.

Fatty acid composition also determines chemical stability, and hence quality of the vegetable oils. It has been reported that polyunsaturated fatty acid in vegetable oils though physiologically important in human nutrition, are the most chemically unstable or more susceptible to oxidative degradation (Rajko *et al*., 2010). Oxidation of fatty acids may lead to loss of nutritional value and alteration of sensory properties like aroma, flavor, and colour of vegetable oils during processing and storage.

Besides chemical stability, other chemical parameters that affect quality of oils include peroxide value, acid value, iodine value, and saponification value. Physical parameters like smoke point, moisture content, viscosity, refractive index, density, colour also affect quality and purity of vegetable oils (Mohammed and Alli, 2015).

Ansari et al (2009) reported that vegetable oils contain Heavy metals such as cooper, zinc, iron, manganese and Nickel.

The sources of heavy metals contamination in vegetable oils come from soil as natural metal sources, manufacturing and packaging process and environmental pollution (Mendil et al, 2009, Zeiner et al, (2005).

Muhammad et al, (2018) reported the levels of cadmium (Cd), lead (Pb), Iron (Fe) copper (Cu), Zinc (Zn),Manganese (Mn),and Arsenic (As) in vegetable oilsas 0.11, 0.04, 0.23, 0.02, 0.16, and 0.12mg/kg respectively.

It is well known that Heavy metals have adverse effects on nutritional value, toxicity and oxidative stability of oils, therefore it is critical to determine the concentration of heavy metals in various oils (Zhu et al, 2011).

Heavy metal toxicity can have several health effects in the body.The heavy metals can damage and alter the functioning of organs such as brains, kidney, lungs, liver and blood (Unak *et al*., 2007).

Heavy metal toxicity can either be acute or chronic. Long term exposure of body to heavy metals can progressively lead to muscular, physical, and neurological degenerative processes.

Toxicity due to lead (Pb) exposure is called lead poisoning, and is mostly related to gastrointestinal tract and central nervous system in children and adults (Jaishanka, *et al*, 2014).

Long term exposure to cadmium leads to its deposition in bones and lungs,hence causing bone and lung damage (Bernard, 2008).

Iron (Fe) toxicity is related to gastro-intestinal effects such as vomiting, diarrhea, and increased risk of cancer.

The aim of this study was to analyze fatty acids, heavy metal concentration, and characterization of oils from selected oil seeds grown in west Nilesub region Uganda.

1.2 PROBLEM STATEMENT

Fatty acid composition of vegetable oils may differ with changing environmental growth conditions and plant location. It is likely that vegetable oils from different localities have different fatty acid composition, and hence different qualities. The fatty acid profile of the vegetable oils are known but the quantities of individual fatty acids in the vegetable oils may differ due to environmental conditions.

Therefore, there is need to establish and update the quantities of individual fatty acid of vegetable seed oils obtained from west Nile.

Vegetable oils may also contain substantial amounts of environmental contaminants such as heavy metals (Mendil et al, 2009). Artificial fertilizers and pesticide are known to be potential sources of heavy metal contamination in soils where they are applied (He, 2005). Heavy metals enhance oxidation of fatty acids to esters, hence affecting the nutritional value and properties of vegetable oil.

In West Nile, there is extensive use of fertilizers such as super triple phosphate, diammonium phosphate and pesticides in commercial farming, therefore crops grown are at a risk of being contaminated with heavy metals. Hence there is need to determine the concentration of the heavy metals, fatty acid composition and characteristics of oils from oil seeds grown in West Nile region.

1.3 GENERAL OBJECTIVE

To analyze fatty acids, heavy metal concentration, and characteristics of selected oil seeds grown in west Nile, Uganda.

1.3.1 Specific Objectives

1. To analyze the physicochemical properties in the sunflower (*helianthus annus)*, sesame (*sesamum indicum)* and peanut (*Arachis Hypogaea)*oils from different locations in West Nile region.
2. To analyze fatty acid content and composition of sunflower, sesame, and peanut oils from different locations in West Nile region.
3. To analyze the concentration of cadmium (Cd), zinc (Zn), iron (Fe), and lead (Pb) in the selected vegetable oil sources from different locations in West Nile region.

1.4 SCOPE OF STUDY

The study focused on the determination of fatty acids, concentration of cadmium (Cd), lead (Pb), iron (Fe), zinc (Zn),and physicochemical properties of selected vegetable oils sources namely, peanut, sesame, and sunflower seed oils purchased from Arua, Yumbe, Nebbi,and Zombo districts in West Nile sub region. The study was carried out from August 2017 toAugust2018.

1.5 HYPOTHESIS

Hypothesis (1): there is no significant variation in the physico-chemical characteristics of the vegetable seed oils produced in west Nile region.

Hypothesis (2): There is no significant variation in the fatty acid composition of vegetable oils from seeds produced in different localities in West Nile sub region.

Hypothesis (3): There is no significant heavy metal contamination of vegetable oils from seeds produced in West Nile sub region.

1.6 SIGNIFICANCE OF THE STUDY

Vegetable oils are sources of important fatty acids, embracing all groups of fatty acids.

The findings of this study will help consumers to compare quality of vegetable oil both locally produced and imported.

This study will also aid government and policy makers to develop safety regulation concerning production, refining and consumption of vegetable oils and also data from this study will help researchers get information on fatty acid composition of oils from west Nile region.

Data from this study will help in understanding contamination of vegetable oils by heavy metals and how heavy metals may also accelerate the process of rancidification of oil. Heavy metals are considered as serious inorganic pollutants because of their toxic effects to life when it enters the body through inhalation and ingestion.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 VEGETABLE OILS

Vegetable oils are obtained from plant seeds such as seeds such as sunflower, peanut, sesame, soy beans cotton seeds (Nzikou *et al.*, 2010).They belong to a class of organic compounds called lipids. Vegetable oils contain a major component known as triglyceride which makes up 98% of its content and also the minor components (2%), comprising of free fatty acids, mono- and diglycerides, phosphatides, glycolipids, pigments, sterols, tocopherols, flavonoids, tannins, and trace metals (O'brien, 2018). Vegetable oils are widely used in food preparation, cosmetics and pharmaceutical industries, margarine, soap and detergent, paint and varnishes(Othman and Ngassapa, 2010).

Besides vegetable oils, other plant oils known as essential oils are obtained from plant materials such as flowers, leaves, bark, wood, roots or peels(Bansal, 2016).

2.2 FATTY ACIDS IN VEGETABLE OILS

Fatty acids are a class of aliphatic monocarboxylic acids composed of a carboxyl group and a hydrocarbon chain. Fatty acids are distinguished from one another by the nature of their hydrocarbon chain which can vary in length from 4to 24 carbon atoms which can be saturated or unsaturated (onedouble bond, two or more double bonds). The most common fatty acids in vegetable oils are those containing18 carbon atoms which includes stearic acid, oleic acid, linoleic, and linolenic acids (Ratnayake and Galli, 2009).

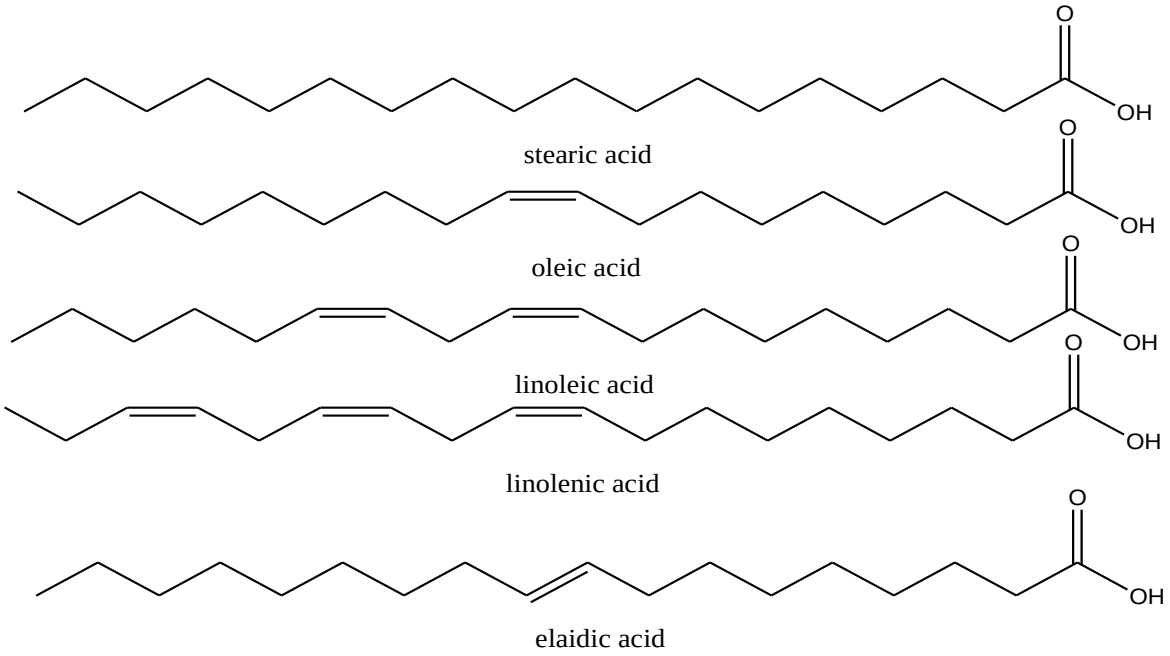

Figure 1: Structures of some C18 common fatty acids in vegetable oils (Ratnayake and Galli, 2009)

2.2.1 Nomenclature of Fatty Acids

The chemical nomenclature requires that carbon atoms be counted from the carboxyl end of the fatty acid (Davidson and Cantrill, 1985). However, for biological activity, carbon atoms are numbered from the terminal methyl group to the first carbon of the ethylenic bond. Such a classification is designated by the symbol ϖ-x, ϖx, or n-x, nx, where x denotes the position of the double bond closest to the terminal methyl group. Fatty acid abbreviations are made according to the number of carbon atoms in the molecule and the number of *cis* ethylenic double bonds. The general assumption is that all multiple double bonds are methylene interrupted (IUPAC-IUB commission on Biochemical nomenclature, 1978).

For example, linoleic acid with two double bonds, where one is located on the sixth carbon atom counted from the methyl group, will be abbreviated as C18:2n-6(Mead and Fulco, 1976).

2.2.2Formation of a Triglyceride in Vegetable Oils

A triglyceride (triacylglycerol) is an ester formed by combination of three fatty acid molecules and a glycerol alcohol. The reaction occurs when three hydroxyl (OH^-) groups of a single

glycerol molecule react with the carboxyl group ($COOH^-$) of three fatty acids to form an ester bond. The fatty acids maybe the same or different in structure.

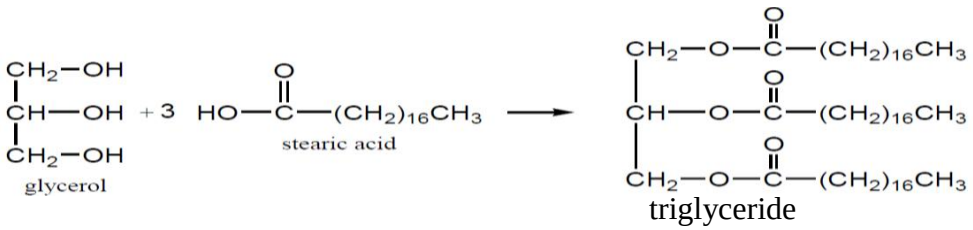

Figure 2: Formation of triglyceride molecule(Boudreaux, 2020)

2.3 CLASSIFICATION OF FATTY ACIDS

Fatty acids may be classified according to the length of the hydrocarbon carbon chain as short, medium or long chained. Besides the hydrocarbon chain length, fatty acids may also be classified as unsaturated (Containing carbon-carbon double bond) and saturated (containing carbon-carbon single bond)(Turicka et al., 2011).

2.3.1 Saturated Fatty Acids

Saturated fatty acids contain only carbon-carbon single bonds in the hydrocarbon chain and naturally occurring saturated fatty acids usually have even number of carbon atoms(table 2.1) shows some major saturated fatty acids in vegetable oils.

Table 2.1:Major saturated fatty acids in vegetable oils(Gunstone, 1996)

Systematic Name	Common Name	Formula	Abbreviation
Butanoic	Butyric	$CH_3(CH_2)_2$-COOH	4:0
Pentanoic	Valeric	$CH_3(CH_2)_3$-COOH	5:0
Hexanoic	Caproic	$CH_3(CH_2)_4$-COOH	6:0
Hepatanoic	Enanthic	$CH_3(CH_2)_5$-COOH	7:0
Octanoic	Caprylic	$CH_3(CH_2)_6$-COOH	8:0
Nonanoic	Pelargonic	$CH_3(CH_2)_7$-COOH	9:0
Decanoic	Capric	$CH_3(CH_2)_8$-COOH	10:0
Undecanoic		$CH_3(CH_2)_9$-COOH	11:0
Dodecanoic	Lauric	$CH_3(CH_2)_{10}$-COOH	12:0
Tridecanoic		$CH_3(CH_2)_{11}$-COOH	13:0
Tetradecanoic	Myristic	$CH_3(CH_2)_{12}$-COOH	14:0
Pentadecanoic		$CH_3(CH_2)_{13}$-COOH	15:0
Hexadecanoic	Palmitic	$CH_3(CH_2)_{14}$-COOH	16:0
Heptadecanoic	Margaric or daturic	$CH_3(CH_2)_{15}$-COOH	17:0
Octadecanoic	Stearic	$CH_3(CH_2)_{16}$-COOH	18:0
Nonadecanoic		$CH_3(CH_2)_{17}$-COOH	19:0
Eicosanoic	Arachidic	$CH_3(CH_2)_{18}$-COOH	20:0
Docosanoic	Behenic	$CH_3(CH_2)_{20}$-COOH	22:0
Tetracosanoic	Lignoceric	$CH_3(CH_2)_{22}$-COOH	24:0
Hexacosanoic	Cerotic	$CH_3(CH_2)_{24}$-COOH	26:0

(Source: Gunstone, 1996)

2.3.2 Unsaturated Fatty Acids

Unsaturated fatty acids contain one or more carbon-carbon double bond in the hydrocarbon chain and the double bonds may be located in the cis or trans configuration. Unsaturated fatty acids with only one double bonds are referred to as monounsaturated fatty acids (MUFA),for example, oleic acid (18:1n9).Whereas those with two or more double bonds are called polyunsaturated fatty acids (PUFA),for example, linoleic acid (18:2n6) and linolenic acid (18:3n3). Table2.2summarizes some major unsaturated fatty acids.

Table 2.2: Major unsaturated fatty acids in vegetable oils(Gunstone, 1996)

Systematic Name	Common Name	Abbreviation
c-9-Dodecenoic	Lauroleic	12:1n3
c-9-Tetradecenoic	Myristoleic	14:1n5
c-9-Hexadecenoic	Palmitoleic	16:1n7
c-6-Octadecenoic	Petroselinic	18:1n12
c-9-Octadecenoic	Oleic	18:1n9
t-9-Octadecenoic	Elaidic	18:1n9t
c-11-Octadecenoic	Ascetic (cis-Vaccenic)	18:1n7
t-11-Octadecenoic	Vaccenic	18:1*trans*-11
c-9, c-12-Octadecadienoic	Linoleic (LA)	18:2n6
c-9, c-12, c-15-Octadecatrienoic	α-Linolenic (ALA)	18:3n3
c-6, c-9, c-12-Octadecatrienoic	γ-Linolenic (GLA)	18:3n6
c-6, c-9, c-12, c-15-Octadecatetraenoic	Stearidonic	18:4n3
c-8, c-11, c-14-Eicosatrienoic	Dihomo-γ-linolenic	20:3n6
c-5, c-8, c-11, c-14-Eicosatetraenoic	Arachidonic	20:4n6
c-5, c-8, c-11, c-14, c-17-Eicosapentaenoic	Eicosapentaenoic (EPA)	20:5n3
c-13-Docosenoic	Erucic	22:1n9
c-7, c-10, c-13, c-16, c-19-Docosapentaenoic	Docosapentanoic (DPA)	22:5n3
c-4, c-7, c-10, c-13, c-16, c-19-Docosahexaenoic	Docosahexaenoic (DHA)	22:6n3

(Source: Gunstone, 1996)

2.3.3 Essential Fatty Acids (EFAs)

These are polyunsaturated fatty acids that are not biosynthesized by the human body, and therefore must be obtained from the diet. They are called essential because they are required for important biological processes like synthesis of prostaglandins and hormones (Glick and Fischer, 2013). Essential fatty acids have been reported to suppress production of pro-inflammatory compounds, and hence very important in control of diseases. Vegetable oils such as sunflower and sesame are some of the major and cheap sources of essential fatty acids, for example, alpha-linolenic acid (an omega-3 fatty acid) and linoleic (an omega-6 fatty acid).

2.3.3.1 Omega-3 Essential Fatty Acids

Vegetable oils are the main sources of omega-3 fatty acids especially alpha-linolenic (ALA). This fatty acid possesses health benefits such as anti-inflammatory, antiarrhythmic and antithrombotic properties (Weylandt *et al*., 2012). Fish and fish oil are rich sources of other omega-3 fatty acids specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

2.3.3.2 Omega-6 Essential Fatty Acids

Vegetable oils, seeds, and nuts are dietary sources of omega-6 fatty acids,for example,linoleic acid. Al-Khudairy *et al*. (2015) reported that omega-6 fatty acids are important in the body for reducing risk of heart disease, lowering total cholesterol levels, lowering "bad" (LDL) cholesterol levels, raising "good" HDL cholesterol levels, and reducing cancer risks.

The essential fatty acids in vegetable oils are nutritionally important, but most susceptible to oxidative degradation during storage, which may cause deterioration of oils leading to loss of nutritional value, alteration of sensory properties such as aroma, flavour, and colour of vegetable oils.

2.3.3.3 Previous studies on fatty acid composition

Various studies have reported fatty acid composition of different vegetable oils.

Musalima et al., (2019) reported fatty acid composition of peanut oil from Uganda contained (39.71-55.89%) oleic acid, (20.21-35.59%) linoleic and (11.91-17.16%) palmitic acids.

Achola et al., (2017) reported a range of (14.61 to 18.61%) palmitic acid, (26.79 to 33.44%) linoleic acid and stearic acid (21.9-34.46) to in peanut oils from Uganda.

Flagella et al., 2002 reported that standard sunflower oil contains approximately 15% saturated, 85% unsaturated fatty acid consisting of (14-43%) oleic acid, and (44-75%) linoleic acid.

In recent years, high quality sunflower oils have been produced with a range of composition via development of mid-oleic type (43.1 to 71.8%) and high oleic type (75 to 90.7%) sunflower varieties that has high oleic acid content than standard sunflower type (flagella et al., 2002).

Hwang, (2005) reported that Fatty acid composition in Sesame seeds consist of abundant unsaturated fatty acid, like oleic acid (35.9-42.3%) and linoleic acid 41.5 - 47.9% from 80% of total fatty acids. Yoshida et al., (2000) reported fatty acid composition of seasame oil as 44% oleic, 34% linoleic and 10% palmitic and 7% stearic acids. High contents of linoleic acids and linolenic acids are another merit of sim sim oils as food source (Park, 2010)

Sesame seeds also contains less than 20% saturated fatty acids mainly palmitic (7.9 to 12% and stearic acid 4.8 to 6.1%. worldwide, fatty acid composition in sesame oil is variable among the different varieties of sesame seeds such as black, brown and white.

Rahman et al., (2007) reported that Fatty acid composition of sesame seeds depends on different factors such as climatic situations, soil conditions, and ripeness of plants.

2.4 IMPACT OF FATTY ACID COMPOSITION ON HUMAN NUTRITION AND HEALTH

The ratio of unsaturated to saturated fatty acids in edible oils and fats is very important for human nutrition. While high levels of saturated fatty acids are desirable to increase oil stability, on the other hand, nutritionally they become undesirable, because high levels of saturated fatty acids are frequently considered to have an influence in increasing the concentration of low density lipoproteins (LDL), affecting the ratio of LDL to HDL (high density lipoproteins), a risk marker for Cardio Vascular Disease (CVD) (Barbour *et al*., 2015).Oleic acid, a monounsaturated fatty acid(MUFA) is however, thought to reverse the above effects. Oleic acid (C18:1n9) has also been associated with several human health benefits, including; decreased risk of cardiovascular disease (CVD) by reducing the levels of serum low-density lipoproteins (LDL)cholesterol; and maintaining the levels of high-density lipoproteins (HDL), without causing significant weight gain (Barbour *et al*., 2015).

Kris-Etherton (1999) reported that MUFAs decrease plasma triglyceride levels in comparison with carbohydrates. In addition, the MUFAs helpin hindering the development of adrenoleukodystrophy (ALD) (Rizzo *et al*.,1986) and reversing inhibitory effects of insulin production (Vassiliou *et al*., 2009). MUFAsmay also decrease platelet aggregation and increase ûbrinolysis, thereby protecting against thrombogenesis (Kris-Etherton, 1999). It also has anti-inflammatory properties that activate different pathways of immune competent cells(Carrillo *et al*., 2012).

Polyunsaturated fatty acids (PUFAs) suchas linoleic (C18:2n6) are recognized for their susceptibility to oxidative rancidity because when heated at high temperatures makes it dangerous for human consumption (Isleib *et al*.,2006), and this instability leads to formation of trans-fatty acid, which has detrimental effect on human health as it causes cardiovasculardisease (CVD) (Wang *et al*.,2015). In addition, linoleic acid is a metabolic

precursor to arachidonic acid and eicosanoids, which has been associated with an increased risk of inflammation, cancers, CVD, and neurological disorders (Whelan, 2008).

2.4.1 Nutritional Index of Oils

The relationship between saturated and polyunsaturated FA content is expressed as nutritional index (P/S index). This value is an important parameter for determination of nutritional value of vegetable oils (Kostik et al, 2008). Vegetable oils with P/S index greater than 1 are considered to have a high nutritional value. Lawton *et al*. (2000) indicated that higher value of P/Sindex means a smaller deposition of lipids in the body. Other studies elsewhere have reported the P/S index value for some vegetable oils such as sunflower, peanut, soybean, and safflower as6.76, 1.04, 4.26, and 10.55,respectively(Zambiazi *et al*., 2007;Daniewski, 2003).

2.5 BIOSYNTHESIS OF FATTY ACIDS

Fatty acid biosynthesis is a complex process due to compartmentalization of the biosynthesis pathway in different organelles in plant cells, and the extensive lipid movement from one organelle to another (Buchanan, 2000). An overview of various steps in the fatty acid biosynthesis pathway in plants is illustrated by Buchanan (2000) in (Figure3).

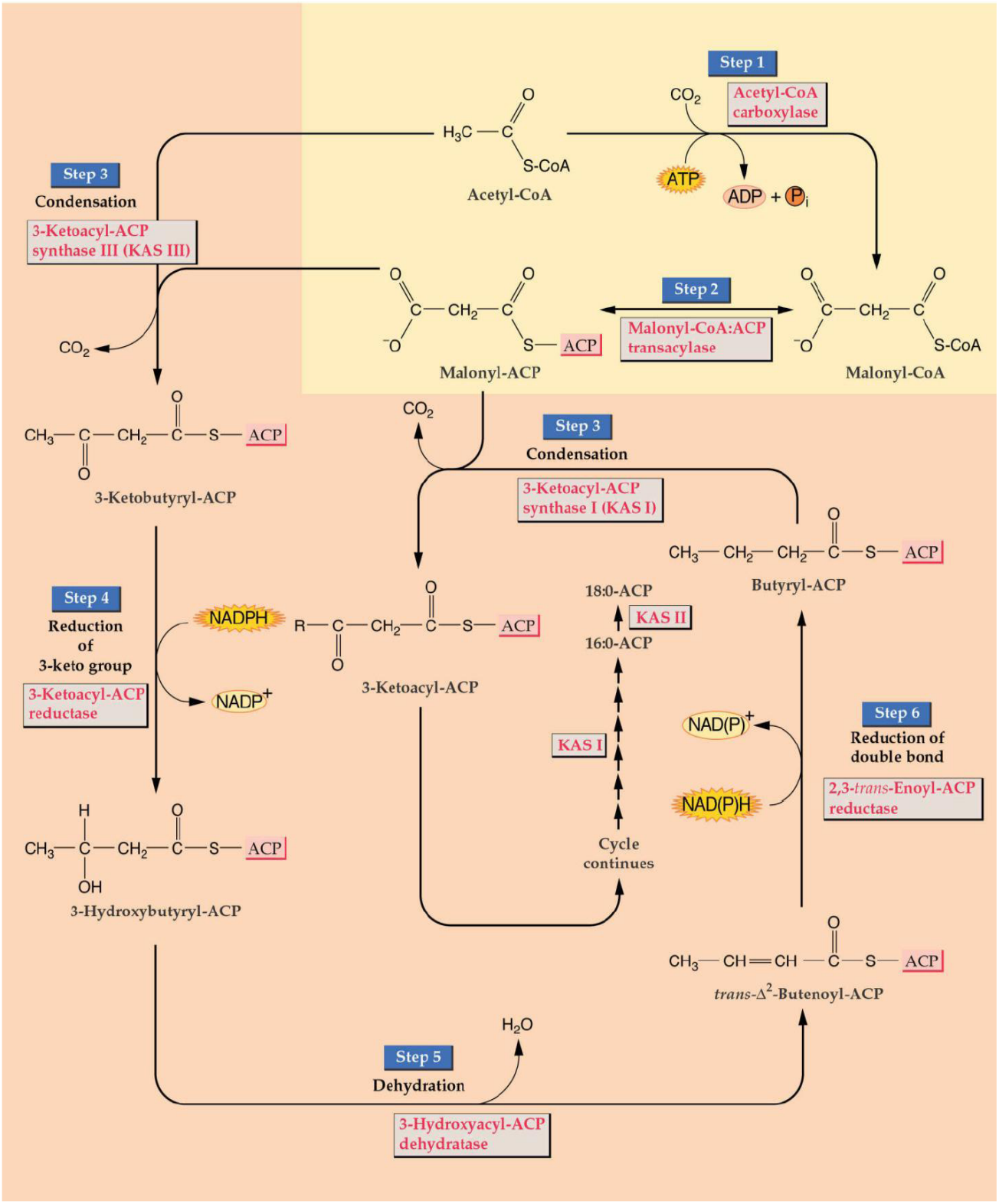

Figure 3: Fatty acid biosynthesis in plants (Buchanan, 2000)

Steps involved in fatty acid biosynthesis in plants; -

Step 1: Fatty acid biosynthesis begins with an ATP-dependent carboxylation process in which Acetyl-CoA carboxylase (also known as ACCase) catalyses the formation of malonyl-CoA (also known as activated Acetyl-CoA). Biotin carboxylase activates CO_2 by attaching it to nitrogen in the biotin ring of the biotin carboxyl carrier protein (BCCP). The flexible biotin arm of BCCP carries the activated CO_2 from the biotin carboxylase active site to the carboxyl transferase site and this enzyme transfers activated CO_2 from biotin to Acetyl-CoA, producing malonyl-CoA. ACCase is highly regulated enzyme which is active in light and inhibited in dark conditions. Also, being a part of the first step of lipid synthesis, this enzyme can be inhibited by a class of herbicides called ACCase inhibitors. The herbicide compounds affect meristem of the grasses and kill them by ceasing production of cell membrane.

Step 2: Malonyl-CoA transacylase (MT) exchanges the CoA for the ACP (Acyl Carrier Protein) which is an essential protein co-factor in fatty acid biosynthesis.

Step 3: The 3-ketoacyl-ACP synthase (KAS) enzyme has three isoforms – (KAS) I, II and III. KAS III is utilized during the first condensation reaction, which includes a carbon-carbon bond formation between C1 of an acetate primer and C2 of the malonyl-ACP. KAS I is active with C4-C14 acyl-ACPs and during production of C6:0 to C16:0. KAS II accepts only longer carbon chains and elongation of 16:0-ACP to C18:0-ACP requires KAS II.

Step 4: The 3-ketoacyl-ACP reductase (KR) enzyme reduces the keto group from 3-ketoacyl-ACP to form 3-hydroxyacyl-ACP.

Step 5: The 3-hydroxyacyl-ACP dehydratase catalyzes the removal of water from 3-hydroxyacyl-ACP to form the 2,3-*trans*-enoyl-ACP and in the next step, enoyl-ACP reductase converts 2,3-*trans*-enoyl-ACP to its corresponding saturated acyl-ACP.

Step 6: Enoyl-ACP reductase (ER) reduces the double bond and the repetitive condensation of 2 carbon units produces 16:0=ACP and 18:0-ACP. Each cycle of fatty acid synthesis adds two carbons to the acyl chain and the reaction stops at 16:0 or 18:0 when thioesterase terminates the biosynthesis cycle. 18:1-ACP is produced using 16.18:0-ACP using a stearoyl-ACP desaturase as a catalyst. 16:1-Δ9 is desaturated by using a soluble acyl-ACP Δ9 desaturase enzyme. Fatty acid elongation happens in the endoplasmic reticulum.

Several desaturase enzymes catalyze the formation of *cis*-double bonds which causes kinks in the fatty acid chain (Figure 4) and generate a diverse variety of unsaturated fatty acids in plant membranes and storage reserves.

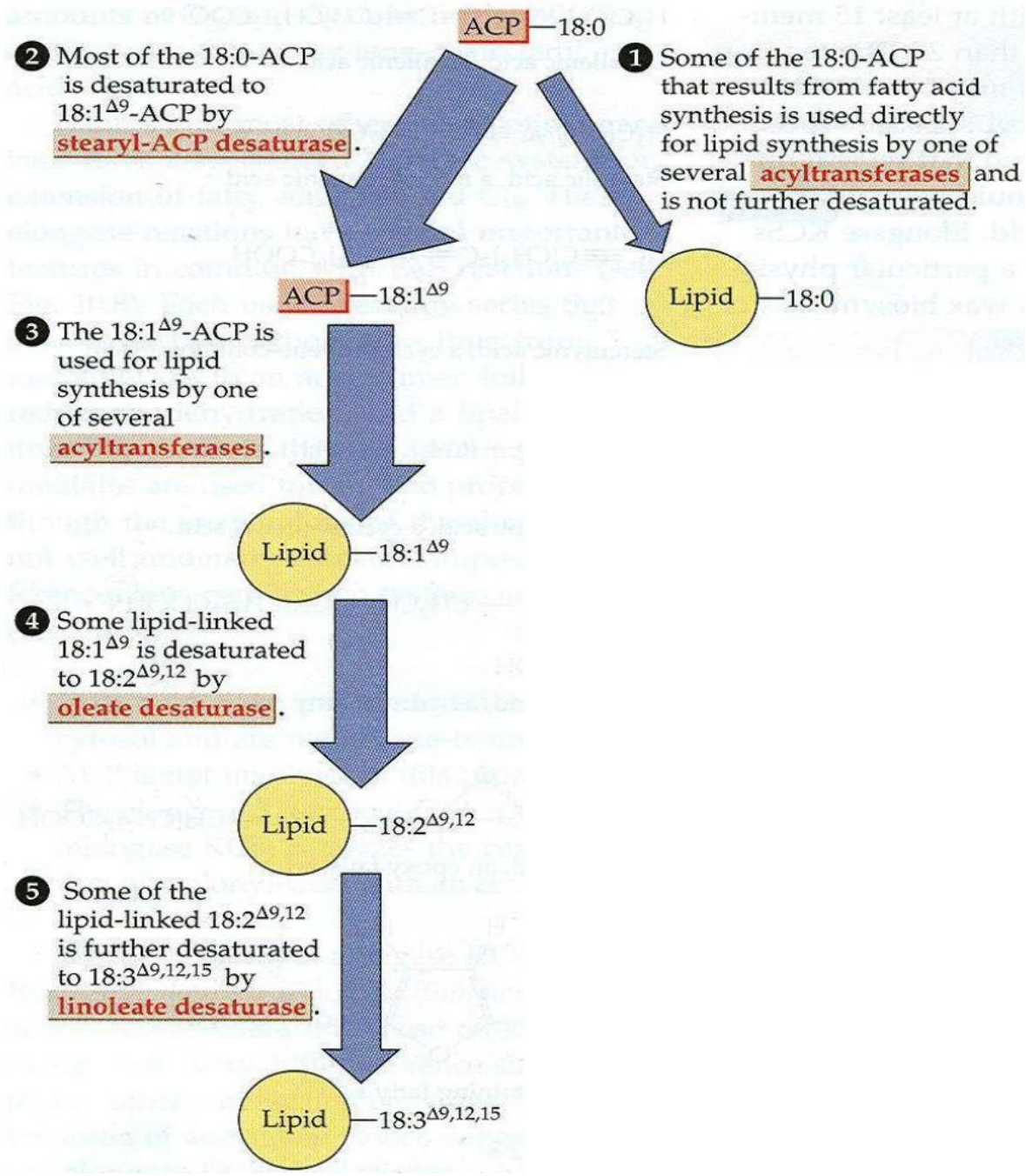

Figure4: Introduction of double bonds in plant lipids (Buchanan, 2000)

2.6 FACTORS AFFECTING THE FATTY ACID COMPOSITION IN VEGETABLE OILS

Fatty acid composition determines the physical and chemical characteristics of vegetable oils, and the fatty acid composition is not always constant but influenced by factors such as genetic, environmental, and oil extraction methods.

2.6.1 Genetic Factors

Fatty acid biosynthesis in plant cells is controlled by a group of genes identified as sad1, sad2, fad2a, fad2b, fad3a, and fad3b, which are collectively known as desaturase (Thambugala *et al*., 2013). The genes act by encoding enzymes that perform fatty acid synthesis in the plant cells. Variation in fatty acid composition in the different vegetable oils could be due to the differential expression of the genes during seed development and maturation (Baud and Graham, 2006).

In peanut, sesame and sunflower seeds, it has been observed that increase in the oleic acid content leads to the reduction in the levels of palmitic, stearic, and linoleic acids, which suggests that there is close linkage of genes controlling these fatty acids (Barkley *et al*., 2013). Variations in fatty acid compositions among different crop varieties of the same species is due to the cumulative effect of several minor genes that modify the expression of fad3a and fad3b (Vrintel *et al*., 2005). Baud and Lepiniec (2009) reported that the genes expression programme related to the fatty acid synthesis are activated during the maturation phase of the seed, and most genes encoding fatty acid synthesis display a bell shaped pattern of expression during seed development.Thefad2 genes are thought to be the rate limiting genes of the fatty acid biosynthesis pathway, and are highly influenced by environment (Fofana *et al*., 2006), and(Esteban *et al*., 2004) also reported that there is a significant gene environment interaction which determines the fatty acid composition differences.

2.6.2 Environmental Factors

Environmental growth conditions of plants such as temperature, soil, moisture, altitude, and light affects the expression of the alleles and relative activity of the genes responsible for fatty acid biosynthesis in plant cells. Therefore, vegetable oils obtained from plants seeds of the same species but from different localities may have different fatty acid compositions(Alizadeh, 2010).

2.6.2.1 Temperature

Varying temperatures during growth and maturation seasons of plants affects the level of gene expression which cause alteration in quantities of fatty acids of vegetable oil(Fofana *et al*., 2008). Byfield, Upchurch (2007) also confirmed that at high temperature during growth and maturation seasons of plant seeds, linoleic acid content reduces while oleic acid content increases because fatty acid desaturase enzymes, encoded by three genes converts linoleic acid to oleic acid, and hence altering the fatty acid composition in some seeds such as sunflower, soybeans, peanut, and sesame.

2.6.2.2 Soil

Soil contains nutrients such as nitrogen, potassium, and phosphorous which are used in the biosynthesis of fatty acids, and the concentration of these nutrients in the soils may influence the quality and composition of fatty acids in plant seeds(Stepien*et al*., 2017).

Kaptan *et al*. (2017) reported that the application of NPK fertilizers to the soils significantly increased the concentration of lignoceric and arachidic acids, whereas myristic and palmitic acids were decreased. On the other hand, fertilization had no significant change on unsaturated fatty acid content observed, except eicosenoic acid.

2.6.2.3 Water

Igbadun *et al.* (2006) showed that the crop yield response is very much dependent on the amount of water applied at different crop development stages than the overall seasonal water applied. Drought stress in the flowering stage of a plant leads to reduced availability of carbohydrates (glucosinolate) for fatty acid biosynthesis, and may cause reduction in the concentration of saturated fatty acids in the plant seeds (Geogel *et al.*, 2007). Drought stress also leads to oxidation of polyunsaturated fatty acids in the plant seeds thus altering the fatty acid composition in plant seeds (Singh and Sinha, 2005).

2.7 FACTORS AFFECTING NUTRITIONAL QUALITY OF VEGETABLE OILS

There is still no standard defining the quality characteristics of vegetable oils for use as food and industrial applications, but there are a number of factors such as fatty acid compositions, oil extraction and processing methods, seed quality and storage systems that are used to evaluate the quality of a good vegetable oil (Okparanta *et al.*, 2018; Abulude *et al.*, 2007).

2.7.1 Fatty Acid Composition and Oil Oxidation

The quality of vegetable oils largely depends on the quantity of oleic and linoleic acids, which constitutes more than 80% of total fatty acids. Good quality vegetable oil contains high proportion of fatty acids like oleic, palmitic, and stearic acids which are resistant to autoxidation. The linoleic and linoleic acids are nutritionally important, but are susceptible to oxidation because their acyl residues are chemically reactive which adversely impacts oil stability and quality. Oxidative degradation of oil leads to loss of nutritional value, alteration of sensory properties like flavour, aroma, and colour. The degree of autoxidation and the potential for deterioration are important parameters of the vegetable oils. It has been reported that factors such as temperature, light, processing procedures, and oxygen concentration are the main causes of oxidation in vegetable oils (Kamal, 2006; Merrill *et al.*, 2008).

2.7.2 Extraction and Processing Methods of Vegetable Oils

The traditional methods are crude, largely unscientific, inefficient, and yielding poor quality oil that contain substantial amount of contaminants.

The solvent extraction method is commonly applied to oilseeds with low oil content (< 20%), like soybean. This method has some disadvantages such as poor product quality caused by high processing temperatures, and a relatively high number of processing steps (Dawidowicz *et al*., 2008; Takadas and Doker, 2017).

Mechanical press methods are often used to extract vegetable oil from oilseeds having oil content higher than 20% (Sinha *et al*., 2015). There are two types of mechanical press method namely, cold-press and hot-press methods.

Cold-press or scarification method is carried out at low temperature below 50^0C. Cold-pressed method are safer than hot-pressed method because high temperature is avoided in the cold press method. In cold-pressed oils, the purity and natural properties of oils are preserved (Azadmard- Damirchi *et al*., 2011; Bhatol, 2013).

The hot-press method is carried out at elevated temperature and pressure. This method results in decreased oxidative stability, degradation of valuable oil components and reduced oil shelf-life of the vegetable oil.

2.7.3Refining and Storage

Inadequate handling and storage facilities for oilseeds and vegetable oils during refining and transportation may lead to contamination of oil products by soil, insects, rodents or microorganisms, and may cause loss or gain of moisture, odor or flavour (Ngassapa and Othman, 2001). The longer the seeds are stored, the higher the contamination and deterioration, and the higher the free fatty acid contents of the oil.

Oil refining processes may lead to contamination of vegetable oils with heavy metals which accelerates oxidation reaction. Oil refining removes antioxidants which leads to acceleration of oxidation reaction that reduces shelf-life. Addition of synthetic antioxidants such as BHT, BHA and BTHQ may also lower oil quality. To retain oil quality, care must be taken while storing vegetable oils for a period of time to prevent their deformation as they easily undergo oxidative deterioration, hence shortening their shelf life.

Bukola *et al*. (2015) reported that oil stored in a refrigerator has a greater nutritional quality than that of a cupboard and shelf upon long storage because the peroxide and acid values obtained from such oils are lower than the other means of storage.

2.7.4 Pro-oxidants

Pro-oxidants in oil have a detrimental effect on oil stability. Metals act as pro-oxidants by electron transfer whereby they liberate radicals from fatty acids or hydroperoxides as in the following reactions (Gordon, 1990):

$M^{(n+l)+} + RH \rightarrow M^{n+} + H^{+} R\cdot$

$ROOH + Mn+ \rightarrow RO\cdot + OH^{-} + M^{(n+l)+}$

$ROOH + M^{(n+l)+} \rightarrow ROO\cdot + H+ + M^{n+}$

Two of the more active metals to induce oxidation are copper and iron of which copper is the most pro-oxidative (Garrido, Frias, Diaz and Hardisson, 1994).

2.8VEGETABLE OIL EXTRACTION METHODS

Most vegetable oils are extracted from plant seeds, and the oil yield will depend on seed variety, soil, and environmental conditions around the oil-bearing plant, as well as on pretreatment procedure, and on the particular extraction method (s) used(Mariana *et al*., 2013). Selection of suitable extraction method(s) is a key factor, and may depend on whether small or

large scale oil extraction is intended. Vegetable oil producers widely use methods like traditional, solvent extraction, and mechanical expression to extract vegetable oils from plant seeds. The percentage yield and percentage oil recovery are evaluated using the expressions;

$$\% \text{ Oil yield} = \frac{\text{Moil}}{\text{Mseed}} \text{x} 100\% \quad (1),$$

$$\% \text{ Oil recovery} = \frac{\text{Moil}}{\text{XMseed}} \text{x} 100\% \quad (2),$$

Where Moil = mass of oil (kg),

Mseed= mass of oilseed (kg),

X = oil content of oil seed.

2.8.1 Traditional Method

Oluwole *et al*. (2012) reported that the traditional method of vegetable oil extraction involves, collection of seed pods, shelling of the pods/winnowing, roasting/drying of seeds to reduce moisture, grinding the roasted seeds by mortar and pestle or in between two stones to form a paste. The paste is mixed with water, and boiled to obtain the oil by floating and skimming. The oil is then dried by further heating.

The traditional method is tedious, time consuming, energy sapping, largely unscientific, inefficient, and yielding poor quality oil (Olaniyan and Yusuf, 2012). This method is mainly practiced among the rural communities to obtain vegetable oils on a small scale from peanut and sesame seeds, and also sheanut.

2.8.2 Solvent Extraction Method

In soxhlet extraction, normally a solid material containing the desired compound is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the

Soxhlet extractor. The soxhlet extractor is placed onto a flask containing the extraction solvent-hexane and is then equipped with a condenser, and the solvent is heated to reflux. The solvent vapour travels up a distillation arm and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material.

The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle may be allowed to repeat many times, over hours or days.

During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles, the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled.

After extraction, the solvent is removed, typically by means of a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble and is usually discarded.

The solvent extraction method is commonly applied to plant seeds with low oil content (<20%). This method is considered as one of the most efficient methods in vegetable oil extraction, with less residual oil left in the cake or meal (Tayde *et al*., 2011). The choice of solvent is based mainly on the maximum leaching characteristics of the desired solute substrate (Dutta *et al*., 2015). Solvents commonly used are hexane, diethyl ether, petroleum ether, and ethanol. Other considerations are high solvent-solute ratio, relative volatility of solvent to oil, oil viscosity and polarity, and cost (Muzenda *et al*., 2012; Takadas and Doker,

2017).Hexane a hydrocarbon, with chemical formula $C_6H_{14,}$ boiling and melting points 68.7°C and -95.3°C respectively, has become the solvent of choice for solvent extraction (liquid-liquid extraction) because of its high stability, low evaporation loss, low corrosiveness, little greasy residue, and better odor and flavour of the extracted products(Muzenda *et al*., 2012). Solvent extraction using hexane has several drawbacks, including high capital equipment cost and operational expenditures, the perpetual hazard of fire and/or explosion, as well as the residual solvent associated with both oil and meal.

The primary prerequisite for solvent extraction of oils is the rupturing of the seed or feed material to render the cell wall more porous, and complete rupturing of the cell wall is necessary for rapid extraction. Soxhlet based solvent extraction process is the primary means of extracting vegetable oil from oleaginous materials. The Soxhlet process is also widely used in laboratory scale oil extraction (Abdelaziz *et al*., 2014), but large scale operation of this process would require a commercial solvent extractor (Ogunniyi, 2006). The major advantage of the Soxhlet process is solvent recycling (over and over) during extraction. However, disadvantages of the Soxhlet method include high solvent requirement, time, and energy consumption (Takadas and Doker, 2017), as well as sample being diluted in large volumes of solvent (Rassem *et al*., 2016).

2.8.3 Mechanical Expression Method

Mechanical expression involves the application of pressure using hydraulic or screw presses to force oil out of an oil-bearing material (Arisanu, 2013). Mechanical press methods are often used to extract vegetable oil from plant seeds having oil content higher than 20% (Sinha *et al*., 2015). There are two types of mechanical press methods namely, cold-press and hot-press methods. Cold-press or scarification method is carried out at low temperature (below 50^oC). In cold-pressed oils, the purity and natural properties of seed oils are preserved (Azadmard-Damirchi *et al*., 2011; Bhatol, 2013), and this includes the retention of valuable nutraceuticals

like phytosterols and tocopherols in the extracted oil (Kittiphoom *et al*., 2015). Because of these attractive qualities, there is growing global demand for cold-pressed oils. Cold-pressed oils are safer than hot-pressed seed oils because adverse effects caused by high temperatures are avoided. Some of the likely adverse effects of hot pressed oils are decreased oxidative stability, degradation of valuable oil components, and reduced oil keeping quality(Matthaus, and Brühl, 2003).

The hot-press method is carried out at elevated temperature and pressure and results into higher oil yield largely due to decreased oil viscosity at high temperatures. This enhances oil flow during extraction, thus high temperature increases the efficiency of the extraction process and oil yields of up to 80% possible (Patel *et al*., 2016).

Mechanical expression methods have the advantage of low operation cost and producing high quality light colored oil with low concentration of free fatty acids. However, it has a relatively low yield compared to solvent extraction, and is therefore comparatively inefficient, often with a large portion of oil left in the cake or meal after extraction (Bhuiya *et al*., 2015).

2.9 ANALYTICAL METHODS OF FATTY ACIDS AND HEAVY METALS

Worldwide, the most commonly employed techniques for analysis of fatty acid profiles of vegetable oils are gas chromatography (GC) and high performance liquid chromatograph (HPLC) (Botinestean *et al*., 2012).

HPLC is the most commonly used tool for the analysis of pharmaceutical products, but it is less successful in the quantification of fatty acids due to the absence of chromophores or fluorescent functional groups. Compared with the standard GC method, this method achieves acceptable separation and precision, but has poor sensitivity (Tsuyama *et al*., 1992).

GC is the most commonly used technology for the analysis of fatty acids which require derivatization to fatty acid methyl esters (FAME) due to the high boiling points of fatty acids, which are difficult to evaporate (Laakso *et al*., 2002).

2.9.1 Principles of Gas Chromatography

Chromatography is a separation technique based on the affinity difference between two phases, the stationary and mobile phases. A sample is injected into a column, either packed or coated with the stationary phase, and separated by the mobile phase based on the difference in interaction (distribution or adsorption) between compounds, and the stationary phase. Compounds with a low affinity for the stationary phase move faster through the column, and elute earlier. The compounds that elute from the end of the column are determined by a suitable detector. The mobile phase is always composed of an inert gas such as Nitrogen, Hydrogen or Helium which does not interact with the components in a mixture, but carries or transports them through the column, and the stationary phase is a "solid" which is either a packed column or a capillary column (Al-Bukhaiti et al., 2017).

This technique enables the separation, identification, and purification of components of a mixture for qualitative and quantitative analysis. However, the use of Gas Chromatography is limited to volatile and thermally stable compounds or molecules that may undergo derivatization reactions to form volatile and thermally stable products (Lehotay and Hajslova, 2002).

During a Gas Chromatography separation process, the sample is vaporized and carried by the mobile gas phase (i.e., the carrier gas) through the column. The quality of separation (resolution) depends on how long the components to be separated stay in the stationary phase, and on how often they interact with this phase. The type of interaction between component and

phase (selectivity) is determined by the functional groups. The polarity of the phase is a function of stationary phase substituents (Grob and Eugene, 2004).

To measure a sample with unknown concentration, a standard sample with a known concentration is injected into the Gas Chromatography. The standard sample peak retention time and area are compared to the test sample to calculate the concentration.

Major components of GC include; -

Gas supply - involves two types of gases, carrier gas to carry the sample through the column, and a detector support gas to support the flame in the flame ionization detector.

Introduction of sample - can be by hand or auto sampler. The most common type of sample introduction involves syringe injection of the sample into a heated injection port.

Column - separation of components of the sample takes place in the heated column, a long tube running from the sample injector to the detector.

Detector - detector function involves detection of sample components as they pass through the end of the column. The most common detector is a Flame Ionization Detector (FID), in which molecules of the sample are burned in the flame with produced ions detected.

A computer system - is used by most GC systems to collect and analyze the data. Signals from the detector are collected and converted into user friendly information. Generally, the system generates a chromatogram which is plotted using information on the amount of the component as peak area with elution time as retention time. Contents and concentration of various components of the sample are determined by comparing various chromatograms.

Chromatography/Flame Ionization Detector (GC/FID) is widely used analytical technique in quantitative analysis of fatty acids in vegetable oils, petrochemical, pharmaceuticals, and natural gas. The FID detector typically uses hydrogen/air flame into which the separated

sample is oxidized and produce electrically charged particles (ions) which are collected and produce an electrical signal which is then measured. The GC/FID technique is robust, and of low cost compared to the Mass Spectrometry. This system has a challenge because the FID is extremely sensitive to hydrocarbon impurities from the hydrogen and air supply for the flame. The impurities can cause increased baseline noise and reduce the detector sensitivity.

Gas Chromatography/Mass Spectrometry (GC/MS) is a hyphenated technique developed from the coupling of GC and MS. The two instruments are highly compatible with each other, however, GC operates at high pressure (760 torrs) while the MS operates at a vacuum (5-l0torr). During the identification of compounds in the MS, the mass spectra acquired by this hyphenated technique offer more structure related information on the interpretation of fragments of the ions. The fragments ions with different relative abundances can be compared with the library spectra. Nowadays, GC/MS is integrated with various online MS databases for several reference compounds with search capabilities that could be useful for spectra match for the identification of separated components. Compounds that are adequately volatile, small, and thermostable in GC conditions can be easily analyzed by GC/MS. Sometimes polar compounds, with a number of hydroxyl groups, need to be derivatized prior to any analysis. The most common derivatization technique is the conversion of the analyte to its trimethylsilyl derivative. It is suitable for analysis of both volatile and nonpolar compounds.

GC/MS technique is limited in that, the compounds analyzed must be sufficiently volatile to allow transfer from liquid phase to mobile carrier gas and thus to elute from analytical column to the detector. Hence many compounds are too polar or too large to be analyzed with this technique. GC/MS has positive attributes in that it offers high efficiency separation with numerous columns, excellent limit of quantification, and also allows use of mass spectral library for identification of samples. During GC/MS analysis, vaporized sample is carried through the GC column with the help of heated carrier gas through the column where the

components are separated. The separated components then enter MS through an interphase, and this is followed by ionization, mass analysis, and detection of mass-to-charge ratios of ions generated from each component by the mass spectrometer. The process of ionization not only ionizes the molecule, but also break the molecule into the positive or negative modes (Ruchira *et al.*, 2012).

2.9.1.1 Selection of the Chromatographic Column

An appropriately selected column can produce a good chromatographic separation which provides an accurate and reliable analysis. An improperly used column can often generate confusion, inadequate, and poor separations which can lead to results that are invalid or complex to interpret. There are over 10,000 compounds that can be analyzed by Gas Chromatography, over 400 Gas Chromatography capillary columns(Jennings, 1990). The selection of proper capillary column for any application should be based on four significant factors which are stationary phase, column internal diameter, film thickness, and column length.

The differences in the chemical and physical properties of injected organic compounds, and their interactions with stationary phase are the basis of separation process. When strength of the analyte-phase interactions differs significantly for two compounds, one is retained longer than the other. How long they are retained in the column (retention time) is a measure of these analyte-phase interactions. Two compounds that co-elute (do not separate) on a particular stationary phase might separate on another phase of different physical and chemical property (Kupiec, 2004).

As a general rule, it is advisable to use similar polarities for phase and target compounds, for example, nonpolar molecules require nonpolar polysiloxane phases in the column.For FAME analysis, for example a capillary column with DB-23, 50% Cyanopropyl 50%

methylpolysiloxane or HP-88, 88% Cyanopropyl 12% arylpolysiloxane can be used as a stationary phase because they are polar(Rahman *et al*., 2015).

2.9.1.2 Fatty Acids Methyl Ester Preparation (Derivatization)

Methyl esters are the favorite derivatives for Gas Chromatography analysis of fatty acids because they are more volatile than fatty acids (Christie, 1998). Derivatization is a process of converting fatty acids into fatty acid methyl esters, and this can be carried out through two possible approaches;

1. By a two-steps reaction involving saponification followed by esterification.
2. By a single step reaction known as transesterification.

The two main chemical reactions that occur during methylation are hydrolysis and esterification.

2.9.1.3 Hydrolysis of Fatty Acids

Hydrolysis results in a mixture of fatty acids and glycerol from triglycerides as indicated by the reaction infigure 5. Where R is a linear carbon chain.

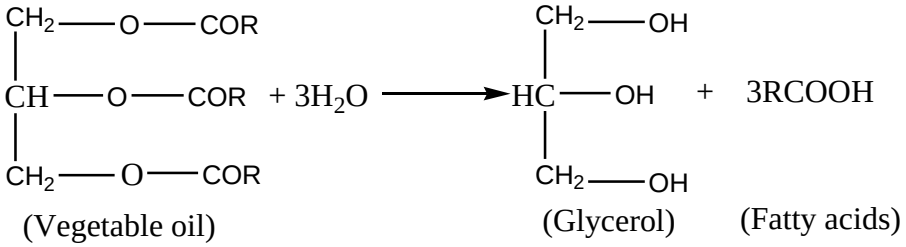

Figure 5: Hydrolysis of fatty acids(Sigma, 2008)

2.9.1.4 Esterification (Methylation) of Fatty Acids

The esterification of fatty acids to fatty acid methyl esters is performed using an alkylation derivatization reagent. The esterification reaction involves the condensation of the carboxyl

group of an acid, and the hydroxyl group of an alcohol in the presence of a catalyst. The catalyst protonates an oxygen atom of the carboxyl group making the acid much more reactive. An alcohol then combines with the protonated acid to yield an ester with the loss of water. The catalyst is removed with the water. Esterification is illustrated in reactions (2) and (3). The alcohol that is used determines the alkyl chain length of the resulting esters, for example, the use of methanol will result in the formation of methyl esters, whereas the use of ethanol will result in ethyl esters.

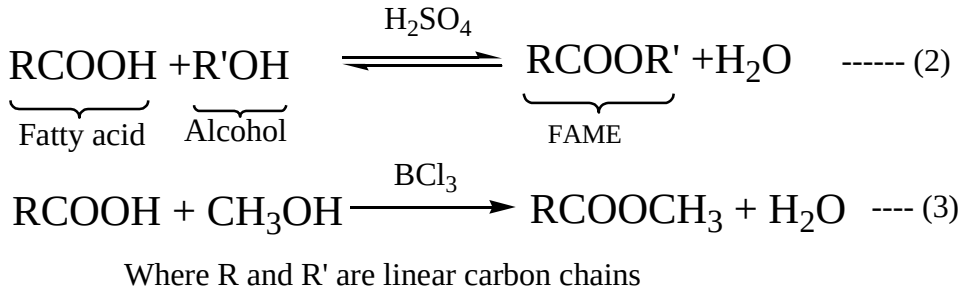

Figure 6: Methylation of fatty acids(Sigma, 2008)

2.9.1.5 Methylation Methods

2.9.1.5.1 Base Catalyzed and Acid Catalyzed (Two-Step) Method

In this procedure, a known amount of oil sample is placed into centrifuge tubes to which potassium hydroxide (10M) solution, and methanol are added. The reaction is performed at 55°C for 1.5h with mixing for 5s every 20min. After cooling to room temperature, 0.5mL sulphuric acid (10M) solution is added, and the reaction is continued at 55°C for 1.5h with mixing for 5s every 20min. After cooling to room temperature, *n*-hexane is added, and the mixture centrifuged for 5min. The hexane phase from the top layer of the solution is extracted, and transferred to screw cap for Gas Chromatography (GC) analysis (Christie, 1998).

2.9.1.5.2 Borontrifluoride/Methanol (BF_3/CH_3OH)

An aliquot of lipid extract is mixed with Borontrifluoride –methanol mixture and heated to a maximum temperature of 100^0C for 2–90 min depending on the type of lipid. After cooling to ambient temperature, water and a non-polar solvent are added, vortexed, and the two phases separated by centrifugation. The upper organic phase containing the methyl esters is carefully removed to a new vial where it is removed under a stream of nitrogen gas (N_2). The remaining residue containing fatty acid methyl esters is dissolved in n-hexane prior to GC analysis.

2.9.1.5.3 Sodium Methoxide/Methanol ($NaOCH_3/CH_3OH$)

Aliquot (20–40mg) is dissolved in dry toluene, 2ml 0.5M sodium methoxide (metallic sodium in anhydrous methanol) added and the samples heated at 60 ^{o}C for 20 min. This esterification step can also be carried out at room temperature (20^0C). A few drops of glacial acetic acid are added followed by 2ml saturated NaCl solution to eliminate excess methoxide. With 50ppm butylated hydroxy toluene (BHT) 2 ml n-hexane are added, and vortexed vigorously. The upper organic phase containing the extracted methyl esters is taken, dried over anhydrous sodium sulphate or 100 µl of a water scavenger such as 2, 2 dimethoxypropane added, and dried under a gentle stream of N_2 at 40 ^{0}C. The fatty acid methyl esters are resuspended in 2 ml of n-hexane with 50ppm BHT and injected for GC analysis. Dilution of the sample in n-hexane may be necessary, depending on the GC response.

2.9.1.5.4 Potassium hydroxide/Methanol (KOH/CH_3OH)

The oil sample is dissolved in n-hexane and KOHmethanolic reagent added and the samples heated at 50^0C for 10–15min. A few drops of glacial acetic acid are added followed by water and n-hexane. The solution is vortexed vigorously and the upper organic phase taken and dried over anhydrous sodium sulphate prior to injection for GC analysis.

2.9.1.5.5 Sulphuric Acid/Methanol (H_2SO_4/CH_3OH)

An aliquot is added to a 0.1MH_2SO_4 in methanol and the tubes heated to 100 $^oC$for 30–60min. Sodiumbicarbonate solution is added to neutralize the reagent and n-hexane to extract the FAMEs. The organic layer is carefully removed, then dried over anhydrous sodium sulphate. Successive n-hexane extracts are added and evaporated under a stream of N_2. The residue is re-dissolved in n-hexane for GC analysis. (Wang et al., 2014).

2.10 ANALYTICAL INSTRUMENTS USED IN FATTY ACID ANALYSIS.

GCis extensively used in food analysis for routine qualitative and/or quantitative determination of components such as fatty acids, sterols, alcohols, oils, and low mass carbohydrates. Further applications in food analysis are devoted to the detection and quantification of food contaminants such as pesticides, environmental pollutants, natural toxins, veterinary drugs, and packaging materials. GC is reliable, efficient, and cost effective method of separationwhich requires small amount of sample, and also provides fast and highly accurate quantitative analysis.

2.11 THE PHYSICOCHEMICAL PROPERTIES OF VEGETABLE OILS

Physical and chemical properties of vegetable oil is determined in order to evaluate the nutritional quality and stability of the vegetable oils. These properties include viscosity, density, acid value, saponification value, iodine value, and peroxide value.

2.11.1 Viscosity

Oil viscosity is defined in two ways, either based on its absolute viscosity or its kinematic viscosity. The absolute viscosity is the resistance of oil to flow and shear due to internal friction, and it is measured in SI unit of Pa*s. Other units include (1poise = 100cP = 1 g*cm-^{1}*s-^{1} = 0.1Pa*s).

The kinematic viscosity is the resistance of oil to flow and shear due to gravity, and it is measured in SI unit of m^2/s. kinematic viscosity of oil can be obtained by dividing the oil absolute viscosity with its corresponding density (Singh and Heldman, 2001).

Viscosity of oil is used to determine the degree of unsaturation and molecular weight of the fatty acid. Viscosity increases with molecular weight of fatty acids and decreases with unsaturation (Kumar *et al*., 2010).

Viscosity is an important factor in selecting a good vegetable oil for food frying. The higher viscosity of frying oils the greater content of oil in the fried foods (Dana and Saguy, 2006), and this is because, high viscosity allows the oils to be accumulated more easily on the surface of fried foods, and enter inside the food during the cooling period (Maskan, 2003).

It has been well established that temperature has a strong influence on viscosity of vegetable oils, with viscosity generally decreasing with increase in temperature. The absolute viscosity of oil is an important parameter in determining quality of oils with regard to its fatty acid composition.

Yalcin *et al*. (2012) reported the viscosities of different vegetable oils from Turkey as olive (61.2mPa*s), hazelnut (59.7mPa*s), cottonseed (57.3mPa*s), canola (53.5 mPa*s), soybean (48.7mPa*s) and sunflower (48.2mPa*s) respectively.

The Principles of Viscosity Measurement is based on Ostwald's viscometer, also known as U-tube viscometer or capillary viscometer, which is a device used to measure viscosity of a liquid with a known density. The method of determining viscosity with this instrument consist of measuring the time for a known volume of the liquid to flow through the capillary under influence of gravity. The instrument must first be calibrated with material of known density such as pure (deionized) water. Knowing the value of viscosity of one liquid, viscosity of other liquids can then be calculated.

$$\frac{\eta_1}{\eta_2} = \frac{t_1 d_1}{t_2 d_2}$$

η_1= is viscosity of liquid 1.η_2 = is viscosity of liquid 2.

t_1= flowtime of liquid 1. t_2= $flowtimeofliquid$ 2.

d_1= density of liquid 1. d_2= $densityofliquid$ 2.

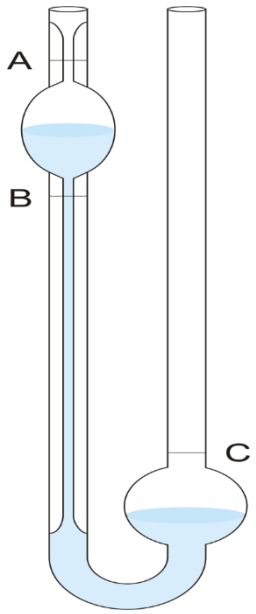

Figure 7: Ostwald's Viscometer (Generali, 2018)

2.11.2 Density

Density of oil is defined as mass per unit volume at specified pressure and temperature. The oil relative density or specific gravity of oil is defined as the ratio of the density of oil to that of water at the same specified pressure and temperature. Density of vegetable oils is temperature dependent, and decreases in value when temperature increases. The range is from 0.91 to 0.93g/cm^3 between the temperature of 15^{o}C and 25^{o}C.

Ackman and Eaton (1977) indicated that a different proportion of fatty acids could be a major factor for the increase in density of vegetable oil. For example, the high density of soybean oil

0.9193g/cm^{3}reported was attributed to the higher content of linoleic acid (Ackman and Eaton, 1977).

Density is an important factor which influences oil absorption as it affects the drainage rate after frying, and also the mass transfer rate during the cooling stage of frying.The codex standard for density of vegetable oils range between 0.919 to 0.925gm/cm^3 (Stahidi, 2005).

2.11.3 Acid Value (AV)

Acid value represents the mass of potassium hydroxide (KOH) in milligrams required to neutralize the free fatty acid in 1g of oil sample. Acid value (AV) is a good indicator of oil degradation caused by hydrolysis or enzymes (Othman and Ngassapa, 2010). Acid value increases with days of storage under ambient conditions. Acid value of oils determines the purity of oils.

The maximum recommended acid value for all vegetable oils is 4mgKOH/g of oil(FAO/WHO, 2019). A low acid value indicates the oil is stable and pure for human consumption (Orthoefer, 2007).

2.11.4 Saponification Value (SV)

Saponification value represents the number of milligrams of potassium hydroxide (KOH) required to saponify (hydrolyze) one (1g) of fat under the conditions specified. Saponification value is used as a measure of the average molecular weight (Chain Length) of all fatty acids present in the vegetable oil sample (Mohammed and Alli, 2015; Fazal *et al*., 2015).

The long chain fatty acids found in fats have a low saponification value, while the short chain fatty acids have a high saponification value (Musa *et al*., 2012). The recommended saponification values for different vegetable oils range between187-196mgKOH/g for peanut,185-193mgKOH/g for sesame, 188-194mgKOH/g for sunflower (FAO/WHO,

1999).Ezeagu *et al*. (1998) reported that a saponification value of 200mgKOH/g indicates a high proportion of short chain fatty acid of low molecular weight in a vegetable oil, and this property makes the vegetable oil potential for use in soap making, cosmetic industry, and sources of essential fatty acids in the body.

2.11.5 Peroxide Value (PV)

The peroxide value is defined as the weight of active oxygen contained in one gram of oil or fat (Horwitz, 1975), and It determines the degree of oxidation of oil which is an indication of the level of deterioration of oils and fats (Okechalu *et al*., 2011). A freshly refined oil should have nil peroxide value.

WHO/FAO (2019) recommended maximum peroxide value of 10meq/kg for all vegetable oils. A high peroxide value indicates high level of oxidative rancidity of the oil which may be caused by increase in storage time, temperature, and air contact with the oil (Kamau and Nanua, 2008). Oils exposed to both atmospheric oxygen and light may show a much larger increase in peroxide value during storage.

Low peroxidative value are indicative of low levels of oxidative rancidity of the oils and also suggest strong presence or high levels of antioxidant (Adelaja, 2006). Low peroxide value may also be used as a measure of shelf-life and freshness of the oil.

2.11.6 Iodine Value (IV)

Iodine value is the mass of iodine in grams that is required to react with 100g of fat or oil sample. The iodine value is used as a measurement of the total unsaturation of vegetable oils, and also as an indicator of their susceptibility to oxidation (Knothe, 2006). Vegetable oils can be divided into four major categories depending on their iodine values namely, saturated oils (iodine value between 5 and 50), mono-unsaturated oils (50 and 100), di-unsaturated oils (100 and 150), and tri-unsaturated oils (over150).

The higher the iodine value, the greater degree of unsaturation, and the more the oil becomes susceptible to oxidation (Onyeike, 2003). Vegetable oils with low iodine values have a high proportion of saturated fatty acids which are stable to oxidative degradation. The recommended iodine value for different vegetable oils range between 7.7–107mg of I_2/100g for peanut oil, 105-120mg of I_2/100g for sesame oil, and 110-143mg of I_2/100g for sunflower oil respectively.

2.12HEAVY METALS IN VEGETABLE OILS

Heavy metals are defined as metallic elements that have a relatively high density compared to water (Ferguson, 1990).Heavy metals can be classified as potentially toxic metals such as Arsenic (As), cadmium (Cd), and lead (Pb)which can be very harmful even at a low concentration when ingested over a long time period (Unak *et al*., 2007).

Micro essential elements such as iron (Fe), copper (Cu), and zinc (Zn)arerequired in small quantities for various biochemical and physiological functions. The essential elements can also produce toxic effects when the metal intake is excessively elevated (Gopalani *et al*., 2007).

2.12.1 Sources of Heavy Metals in Vegetable Oils

All heavy metals are naturally occurring elements that are found throughout the earth's crust. Anthropogenic activities such as mining and smelting operations, industrial production and use, domestic and agricultural use of metals, and metal-containing compounds cause environmental contamination by heavy metals(He, 2005; Shallari*et al*., 1998).Environmental contamination can also occur through metal corrosion, atmospheric deposition, soil erosion of metal ions, and leaching of heavy metal ions, sediment re-suspension and metal evaporation from water resources to soil and ground water (Nriagu, 1989). Natural phenomena such as weathering and volcanic eruptions have also been reported to significantly contribute to heavy metal pollution (Ferguson, 1990; Bradl, 2002; He, 2005).

The presence of heavy metals and their chemical forms in vegetable oils depend on several factors. The metals might originate from the soil and fertilizers, and be absorbed by the plant roots. Heavy metals might be introduced during the production processes such as bleaching, hardening, refining, and deodorization. The metals may also be introduced into the vegetable oils by contamination through metal processing equipments (Leonardis *et al*., 2000).

For many years, it has been discovered that vegetable oil may contain heavy metals such as iron, nickel, lead, cadmium, copper, and Arsenic (Mendil *et al*, 2009). Various studies on vegetable oils consumed in different countries such as India, China, Nigeria, and others reported presence of heavy metals such as lead, cadmium, arsenic, nickel in vegetable oils sold in markets (Juszczak 2008).

Cadmium: This toxic element is easily transferred from sosil to plants, which are increasingly contaminated by cadmium from phosphate-based fertilizers. Cadmium can also be present in vegetable oils, as a result of contamination through refining process, the storage tank or the packing material such as a colorant or stabilizer in plastics(Mendil *et al*., 2009).

Copper: Copper is known to be both vital and toxic for many biological systems, and may enter the food materials from soil through mineralization by crops, food processingand environmental contamination such as application of copper based pesticides which are in common use in some countries (Onianwa *et al*., 2001; Koc *et al*., 2008).

Zinc: The presence of zinc in vegetable oil could be due to absorption by plants from soil or contamination of the vegetable oils during refining process, storage and packaging materials). According to WHO (2003) the Maximum Permissible Limit (MPL) of zinc in vegetable oils is 10000µg/g or mg/g.

Reported zinc levels in different vegetable oils from other parts of the world are in the ranges of 0.04-0.70 µg/g and 0.0484-0.2870 mg/kg (Garrido *et al*., 1994; Pehlivan *et al*., 2008).

Iron: Iron may enter vegetable oils from the soil through uptake of minerals by the roots, and also the reaction between the relatively high-unsaturated portion of the oil with the surface of iron containers used during transportation, storage, and processing of vegetable oils.

Lead:research study shows that the presence of lead in vegetable oil could be attributed to deposition or bioaccumulation from soil containing phosphate base fertilizer that were used in the plantation or may be due to water used for irrigation (Ansari*et al*., 2009). Other reports also reveal that contamination of vegetable oil with lead may also be due to industrial emission, combustion of fuel in refinery process, and from packaging material such as stabilizer and colorant in plastic (La Pera *et al*., 2004).

2.12.2Effects of Heavy Metals on Human Health

Various studies around the world have confirmed the presence of heavy metals such as cadmium, lead, copper, iron, nickel, zinc, chromium, cobalt in vegetable oils, and reports indicate that toxic elements such as cadmium and lead can be very harmful even at low concentration when ingested over a long time period (Unak *et al*., 2007). The essential metals like zinc, copper, iron, and nickel can also produce toxic effects when the metal intake is excessively elevated (Gopalani *et al*., 2007).

Zinc: Zinc is an essential functional and structural element in most metabolic pathways in humans, and is regarded as cofactor for many enzymes. However, excessive levels of zinc in the body harms some physiological processes like breathing. Zinc deficiency can lead to growth retardation and immunological abnormalities (Tahsin and Yankov, 2007). According to WHO (2003) the Maximum Permissible Limit (MPL) of zinc in vegetable oils is 10000µg/g.

Cadmium: Cadmium is a highly toxic heavy metal with very low absorption levels in humans (3-5%) after exposure with contaminated foodstuffs. Cadmium accumulates in the human body and damages mainly the kidneys and liver. Cadmium is retained in the human body

specifically in liver and kidney with a long biological half-life (10-30yrs). Joint expert committee on food additives of FAO and WHO offers the authorized index of provisional tolerable weekly intake for cadmium as 7μg/kg per body weight.

Iron: Iron deficiency is frequently associated with anemia, and thus reduces working capacity and impairs intellectual development (Schumann *et al*., 2007). It is known that adequate iron in diet is very important for decreasing the incidence of anemia. WHO (2003) established a Maximum Permissible Limit (MPL) for iron as 1.5mg/kg of body weight.

Lead: Lead is similar to cadmium because it has no beneficial role in human metabolism, and mainly producing progressive toxicity. The presence of lead in the body can lead to toxic effects, regardless of exposure pathway. Some researchers have suggested that lead continues to contribute significantly to socio-behavioral problems such as juvenile delinquency and violent crime (Needleman *et al*., 2002). WHO (1993) has established a provisional tolerable weekly intake (PTWI) for lead of 0.025mg/kg of body weight.

2.12.4 Determination of Heavy Metals in Vegetable Oils

2.12.4.1 Atomic Absorption Spectrometry (AAS)

Atomic absorption spectrometry (AAS),is an analytical technique that is used to determine the concentration of metals in samples. Itis based on the principle that an atom in the ground state absorbs the light of wavelengths that are characteristic to each element when light is passed through the atoms in the vapour state, and since this absorption of light depends on the concentration of atoms in the vapour, the concentration of the target element in the sample is determined from the measured absorbance. The Beer-Lambert law describes the relationship between concentration and absorbance. Absorbance is directly proportional to concentration of the sample.

$A=\varepsilon bc$,

Where A= absorbance (no unit),

ε=molar absorptivity constant ($Lmol^{-1}cm^{-1}$),

b=Path length of the sample (cm),

c= Concentration of sample in solution ($molL^{-1}$).

Analyzing a sample to see if it contains a particular element means using light from that element. For example, with lead, a lamp containing lead emits light from excited lead atoms that produce the right mix of wavelengths to be absorbed by any lead atoms. Some of the radiation is absorbed by the lead atoms in the sample, and the greater the number of atoms in the vapour, the more radiation is absorbed.

The amount of light absorbed is proportional to the number of lead atoms. A calibration curve is constructed by running several samples of known lead concentration under the same conditions as the unknown.

The amount the standard absorbs is compared with the calibration curve and this enables the calculation of the lead concentration in the unknown sample. Consequently, an atomic absorption spectrometer needs the following three components: a light source; a sample cell to produce gaseous atoms; and a means of measuring the specific light absorbed.

2.12.4.1.1 Flame Atomic Absorption Spectrometry (FAAS)

In flame atomic absorption spectrometry, a sample is aspirated into a flame and atomized. A light beam from a hollow cathode lamp of the same element as the target metal is radiated through the flame, and the amount of absorbed light is measured by the detector. This method is much more sensitive than other methods, and free from spectral or radiation interference by

co-existing elements. Pretreatment is either unnecessary or straightforward. However, it is not suitable for simultaneous analysis of many elements, because the light source is different for each target element.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 DESCRIPTION OF STUDY AREA

Dry clean seeds of peanut, sesame, and sunflower were purchased from farmers from four different locations Nebbi, Arua, Yumbe, and Zombo in West Nile sub region, Northern Uganda between the period of September to December 2017. The seeds (5kg) of peanut, sesame, and sunflower were packed in black polythene bags, and were transported to the Department of Chemistry Kyambogo Universityfor analysis.

3.2 SAMPLE PREPARATION

Clean undamaged seeds were selected, and sundried for 24hrs to get rid of moisture. Sunflower and peanut were de-hulled and de-shelled respectively before grinding of the seeds. Seeds were then ground mechanically into a fine homogeneous powder using electric grinder machine (Brooks Crompton series 2000, UK). The powders were then packed in plastic bags, and stored in a cool dry cupboard at 25^{o}C for two days before oil extraction.

3.3 OIL EXTRACTION USING SOXHLET APPARATUS

Each sample (50g) was placed into a thimble, and extracted using n-hexane (bp 68^{o}C) in a 5L soxhlet extractor for 8hrs using adapted method of Pena *et al*. (1992). The oil was then recovered by evaporating the solvent using a rotary evaporator, and residual solvent was removed by drying in an oven at 60^{o}C for 1hr, and flushing with 99.9% nitrogen gas. The oil obtained after the extraction was transferred into a measuring cylinder which was placed over water bath for 30min at 70^{o}C to ensure complete evaporation of solvent, andv olume of the oil was recorded and expressed as oil content(%).

The oil content was calculated as follows;

$$\text{Oil content} = \frac{\text{weight of the oil}}{\text{weight of the sample}} \text{x} 100\%$$

The extracted oil was stored in a freezer at -2^0C for immediate determination of physicochemical parameters, fatty acid profile, and heavy metal contents.

3.4 DETERMINATION OF PHYSICOCHEMICAL PROPERTIES

3.4.1Density

The densities of different vegetable oils were determined by the pycnometer method using standard method described in AOCS (2009).

The pycnometer was cleaned, dried,and weighed to determine its mass. Dry pycnometer was filled with oil sample at 27^oC and the weight recorded. The pycnometer was then filled with water at 27^oC and weighed. The sample weight was then compared with the weight of water to determine its density (AOAC, 1998);

$$\text{Density } (\rho) = \frac{\text{Weight of sample filled pycnometer} - \text{Weight of empty pycnometer}}{\text{Weight of water filled pycnometer} - \text{Weight of empty pycnometer}}.$$

3.4.2 Viscosity

Absolute viscosity (dynamic viscosity) is the product of kinematic viscosity and density of the oil. The kinematic viscosity f the oil sample was determined with a viscometer at 27^oC.Oil sample (25mL) was placed in a viscometer placed in a temperature controlled vessel equipped with a thermostat which maintained a temperature with an accuracy of $\pm 0.1^o$C. Kinematic viscosities (ν) expressed in centistokes were calculated from the measured flow time (t), and instrument constant (c) by means of the equation below;

$\nu = ct.$

Density was measured using a25mL pycnometer immersed in a temperature-controlled circulating water bath. Dynamic viscosities (η) expressed in centipoise (cP), were calculated from the kinematic viscosities (ν), and the densities (ρ) by using equation below;

$\eta = \rho\nu$.

3.4.3Peroxide Value (PV)

The oil sample (3g) was accurately weighed into a conical flask, and chloroform (10mL) was added to dissolve the oil by swirling. Glacial acetic acid (15mL) and freshly prepared saturated aqueous potassium iodide solution were also added, and the flask was stoppered and shaken for 1 minute and placed in a water bath at 40^{o}C. Water (75mL) was added, and the mixture was titrated with standard sodium thiosulphate solution (0.002M) using soluble starch solution (1%) as an indicator. Titration was also performed for the blank. The peroxide value was calculated using the formula given below;

Peroxide Value (PV) = (S-B) xWxN,

Where B= the volume of sodium thiosulphate used for theblank,

W = the weight of the sample,

S = the volume of sodium thiosulphate consumedby the sample oil,

N = the normality of standard sodium thiosulphate.

3.4.4Saponification Value (SV)

The oil sample(2g) was placed in a conical flask to which 25mLalcoholic KOH (0.5M) was added. The mixture was heated in a reserved condenser and cooled. After cooling the mixture, phenolphthalein (1mL) was added and titrated with HCl (0.2M) until a pink end point was reached. A blank titration was performed under the same time conditions.

Saponification Value $= \frac{(B-T)xNx\ 56.1}{W}$

WhereB= Volume (mL) of HCl required by blank,

T= Volume (mL) of HCl required by oil sample,

N= Normality of HCl,

W=Weight of oil in gm.

3.4.5Iodine Value(IV)

Carbon tetrachloride (200mL) was added to oil sample(0.4g), and 25mL of Wijs solution (8g) of iodine trichloride in 200mL of glacial acetic acid was also added to the mixture in the flask using a safety pipette in a fumed chamber. The flask was stoppered, and the content of the flask was vigorously swirled. The flask was placed in the dark for 2hr30min, at the end of this period, 20mL of potassium iodide solution (10%) and water(120mL)wasadded to the flask. The content of the flask was titrated with sodium thiosulphate solution (0.1M)using starch indicator until end point. A blank titration was performed for other samples. The iodine value (IV) was calculated by the formula;

Iodine Value $= \frac{12.69C(V1-V2)}{M}$

Where C= concentration of sodium thiosulphate,

V_1= volume of sodium thiosulphate used for blank,

V_2= volume of sodium thiosulphate used for oil sample,

M= mass of the oil sample.

3.4.6Acid Value (AV)

An oil sample(5g)was weighed into a conical flask and then neutral ethyl alcohol (25mL)was added to it and then the mixture was boiled on water bath. Phenolphthalein indicator solution

(1-2 drops) was added to the mixture while hot and was titrated against standard potassium hydroxide solution with shaking until end point when the first pink colour persisted for 30 seconds. The acid value was calculated by the formulae below;

$$\text{Acid value} = \frac{\text{VxNx } 56.1}{\text{W}}$$

Where V= volume of standard KOH solution in mL,

N= normality of standard KOH solution,

W= weight of oil sample in grams.

3.5 FATTY ACIDS ANALYSIS

The fatty acid profile was determined using Gas Chromatography/Mass Spectrometry after the oil samples were esterified into Fatty Acid Methyl Esters (FAMEs) suitable for analysis.

3.5.1 Preparation of FAME Standards

The fatty acid methyl ester standards (FAMEs): Palmitic (C16:0), Palmitoleic (C16:1), Stearic (C18:0), Oleic (C18:1), Linoleic (C18:2), Linolenic (C18:3) and Erucic (C22:0)acid were purchased from Sigma (Sigma-Aldrich, Germany), and were used to prepare the stock solution. Individual FAMEs standards were used for preparation of stock standard mixture (50 mg/mL). Identification and contents of the fatty acids were carried out by comparing sample FAME peak retention times with those obtained for FAME mix standard and by mass spectrometry.

3.5.2Preparation of Fatty Acid Methyl Esters (FAMEs)

Derivatization was performed according to standard reference method (AOAC, 2000). Oil sample (50mg)was saponified (esterified) for 5 min at 95^{o}C with 3.4mL ofKOH (0.5M) in dry methanol. The mixture was then neutralized by using HCl (0.7M). 3mL of boron trifluoride (14%) in methanol was added. The mixture was heated for 5min at 90^{o}C to achieve complete

methylation process. The fatty acid methyl esters were thrice solvent extracted from the mixture with redistilled n-hexane and pooled together.

3.5.3Gas Chromatography/Mass spectrometry (GC/MS)Analysis

The fatty acid methyl esters (FAMEs) were analyzed in gas chromatography/mass spectrometry (GC/MS) using an Agilent Technologies gas chromatograph (GC-5975T, Little Fall, NY, USA) equipped with an Agilent auto sampler 7683-B injector and Mass selective (MS-5975) detector. The pooled extract was concentrated to 1mL for GCMS analysis and 1μL was injected on Agilent J&W GC capillary column, HP-88 containing 88% cyanopropyl arylpolysiloxane as stationary phase (30m, 0.25mm i.d., 0.25μm film thickness). The injector and detector temperatures were 240^{o}C and 260^{o}C, respectively. The initial temperature of 140^{o}C was maintained for 2 min, raised to 230^{o}C at the rate of 4^{o}C/min, and kept at 230^{o}C for 5 min. The split ratio was 1:50, and helium was used as a carrier gas with a flow rate of 0.8mL/min.

The mass spectrometer was operated in the electron impact (EI) mode at 70eV; with an ion source temp: (230^{o}C), a quadruple temp: (150^{o}C), and a translating line temperature of 270^{o}C. The mass scan was found in the range between 50 and 550*m/z* with an em voltage, 1035V. Peak identifications for the FAs in oil samples were performed by the comparison with MS spectra and retention times (Rt) of the standards.

3.6 DETERMINATION OF HEAVY METAL CONTENTS IN THE OIL SAMPLES

3.6.1 Sample Preparation

Samples of vegetable oils were weighed, and subsequently digested using a microwave unit. After digestion with a mixture of nitric acid and hydrogen peroxide clear solutions were obtained, and the analytes were determined using FAAS. In the procedure, each sample of oil (1g) was weighed into the digestion vessels. The digestions were performed by adding 3.5mL

of HNO3 (68%) conc. and 1.0mL H2O2 (30%) to the sample. The microwave oven heating programme was performed in five steps using 35 Bar of pressure, as depicted in Table 1. The fifth step was a cooling down procedure of the system through forced ventilation over 20 min. After cooling all the digests were transferred into 10mL volumetric flasks, and diluted to volume with HNO3 (1% v/v). The digestion procedure was done in triplicate for each sample and reagent blanks were prepared similar to the samples.

The different oil samples were digested before analysis using the procedures described by Anwar*et al.* (2004) below;

Oil samples (1g) were weighed into separate digestion flasks. 5mL of concentrated nitric acid (65%) was added, and the contents heated at 70-80^{o}C for 2-3 hours on a hot plate. Heating was continued at about 150^{o}C for 3hours, 3-5mL of concentrated sulphuric acid (98%) and hydrogen peroxide (30%) each was added, and the mixture heated to completely decompose the organic matter. All contents of the flasks were evaporated until a semi-dried mass was obtained, and this mass was dissolved in a small amount of deionized water (approx. 5mL), filtered through Whatman filter paper No42 and made up to a final volume of 25mL in volumetric flasks with 2M nitric acid.

3.6.2Preparation of Standard Calibration Curves

Preparation of standard calibration curve working standards of lead, iron, zinc, and cadmium metals were prepared from the certified standard solutions in freshly prepared 2M nitric acid. A series of standard solutions for lead, iron, zinc, and cadmium of each metal ion in the range of absorbance noted for unknown samples were simultaneously run on FAAS model AA-6300 Shimadzuunder the same set of analytical conditions. Standard calibration curves were obtained for concentrations verses absorbance data that was statistically analyzed using fitting of straight line by least square method.

3.6.3Method Validation and Quality Control

In order to validate the analytical method, the following method validation parameters such as instrumental detection limit, limit of detection, limit of quantification, precision and accuracy studies were carried out.

3.6.4Instrumental Detection Limit

Instrumental detection limit (IDL) is the smallest signal above background noise that an instrument can detect reliably. The IDL is calculated to be the concentration equal to three times the standard deviation of the blank signal. In this study, IDL for each metal was determined from analysis of seven replicates of calibration blank, and the concentration was calculated as;

$IDL = 3xS_{bl}$

3.6.5 Limit of Detection

Limit of detection (LOD) is the minimum concentration of analyte that can be detected, but not necessarily quantified with an acceptable uncertainty. LOD for each metal was determined from analysis of seven replicates of method blanks which were digested in the same digestion procedure as the actual samples. LOD was calculated as;

$LOD = 3xS_{bl}$

Where S_{bl} = Is the standard deviation of the method blank

3.6.6 Limit of Quantification

The limit of quantification (LOQ) is the lowest concentration of an analyte in a sample which can be quantitatively determined with acceptable uncertainty. LOQ was obtained from

triplicate analysis of seven method blanks which were digested in the same digestion procedure as the actual samples. The LOQ was calculated as;

$LOQ = 3xS_{bl}$

where S_{bl} = Is the standard deviation of the method blank

3.6.7 Precision and Accuracy

Precision and accuracy of the results were assessed by determining recovery and repeatability of the analysis of matrix spike, matrix spike duplicate, and laboratory control samples. For doing so, each sample was spiked in replicates of five at near mid-range calibration concentration. The spiked sample were digested and analyzed following the same analytical procedure as the oil samples. Precision was expressed as relative standard deviation (RSD) of replicate results. The relative standard deviations of the sample were obtained as;

$$\%RSD = \frac{\text{Standard Deviation}}{\text{Mean Value}} x100$$

The percentage recoveries of the analyte were calculated to evaluate the accuracy of the analytical procedure. Recovery was then calculated as;

$$\%R = \frac{\text{Concentration spiked sample} - \text{Concentration in unspiked sample}}{\text{Actual spike concentration}} x100.$$

3.6.8 Analysis of Matrix Spike and Matrix Spike Duplicate Sample

Both matrix spikes (MS) and matrix spikes (MSD) were prepared by spiking 0.5g of each oil sample with 2mL of a mixture of spiking standards so that the spike level was 4 mg/L of Feand 2 mg/L of Zn, and 1 mg/L of Cd and Pb. They were all carried through the same digestion and analysis steps as the unspiked sample. The relative percent differences (RPD) between the MS and MSD results were calculated as;

$$\text{RPD} = \frac{\text{MS sample result} - \text{MSD sample result}}{\text{MS sample result} + \text{MSD sample result}} \text{x}100.$$

3.6.9 Analysis of Laboratory Control Sample

Five replicates of 0.5g lithium carbonate spiked with 2mL of a mixture of spiking standards so that the spike level were 4 mg/L of Fe, 2 mg/L of Zn and 1 mg/L of Cd and Pb. Thesewere undergoing the same digestion procedure described for the oil sample but with no addedsample. The % LCS recoveries for each metal were calculated as;

$$\%\text{R} = \frac{\text{LCS}}{\text{S}} \text{x}100$$

where %R = Percent recovery,

LCS = Laboratory Control Sample Results,

S = Amount of spike added.

3.6.10Sample Analysis

The absorbance of the clear supernatant was measured using FAAS model AA-6300 Shimadzu under the following operating conditions; Deuterium arc background correction equipped with a hollow cathode lamp was used for the determination of Cd, Pb, Fe,and Zn, and an air-acetylene burner was used, the wavelengths and (spectral band pass) were for Cd: 228.8nm (0.5nm), Pb: 217.0nm (0.5nm), Fe:238.2 (0.5nm) and Zn: 213.9nm (0.5nm). The nebulizer flowrate was 5.0mLmin^{-1}.

Final concentrations of the metals in the vegetable oil samples were calculated using the following formula;

$$\text{Concentration (mg/kg)} = \frac{\text{C x V}}{\text{W}}$$

WhereC= Concentration of metal ions (mg/L),

V = Final volume (50mL) of solution,

W= Initial weight (0.5g) of sample measured.

3.6.11 Statistical Analyses

In this study, all measurements were carried out in triplicate and reported as means±standard deviation. Statistical analysis were performed using minitab Versin 13.3, statistical package (Minitab Inc. state college, P.A. USA).All variables were subjected to a one way ANOVA to test statistical significant difference among the different categories. A probability value at $P<0.05$, was considered to denote the statistically significant difference.

3.6.12 Limitations of the study

The findings of this study have been seen in light of some limitation , the purchase of oil seeds were done based on random sampling which is subject to biases and sampling error. This may influence overall analysis towards null hypothesis.

The study was limited to only three heavy metals and only oil seeds crops.

CHAPTER FOUR

4.0 RESULTS AND DISCUSIONS

4.1 PHYSICOCHEMICAL PROPERTIES OF THE VEGETABLE OILS

The quality of sunflower, peanut, and sesame oils were analyzed by determining the physicochemical properties such as viscosity, density, peroxide, iodine, acid, and saponification values according to the methods of (AOAC, 1990).Results are presented (Table 4.1).

Table 4.1: Physicochemical parameters of vegetable oil samples.

physicochemical characteristics	**area**	**viscosity at 27^{0}C (CP)**	**Density at 27^{0}C**	**iodine value (g of i_2 /100g)**	**peroxide value (meq0_2/kg)**	Acid value (mgKOH/g)	**saponification value (mgKOH/g)**
Sunflower	Arua	48.80±0.0002	0.87±0.18	134.51±0.01	9.20±0.15	3.20±0.06	202.58±0.01
	Yumbe	56.80±0.0001	0.86±0.58	117.23±0.00	7.64±0.01	2.60±0.40	187.00±0.57
	Nebbi	47.70±0.0003	0.85±0.58	121.38±0.00	12.15±0.29	4.52±0.57	192.14±0.57
	Zombo	46.90±0.0002	0.86±0.58	133.25±0.02	7.67±0.18	2.12±0.20	184.98±0.51
Mean±SD		**50.05±4.57**	**0.86±0.01**	**126.59±8.59**	**9.25±2.12**	**3.11±1.04**	**191.68±7.87**
Sesame	Arua	52.50±0.0007	0.87±0.08	107.87±0.03	4.03±0.36	3.45±0.14	196.35±0.10
	Yumbe	53.40±0.0008	0.87±0.08	102.29±0.00	0.32±0.02	2.38±0.54	187.37±0.42
	Nebbi	52.10±0.0003	0.86±0.67	111.99±0.06	0.86±0.00	2.83±0.40	168.94±0.01
	Zombo	52.10±0.0001	0.85±0.15	104.06±0.33	0.45±0.00	1.48±3.09	168.30±0.06
Mean±SD		**52.53±0.61**	**0.86±0.01**	**106.55±4.31**	**1.42±1.76**	**2.53±0.83**	**180.24±12.05**
Peanut	Arua	57.40±0.0002	0.83±0.04	76.82±0.07	8.74±0.03	5.15±0.46	174.57±0.27
	Yumbe	58.90±0.0002	0.83±0.02	86.26±0.20	0.85±0.01	4.15±0.32	169.15±0.45
	Nebbi	57.60±0.0001	0.91±0.03	71.82±0.56	2.62±0.06	3.47±0.06	168.30±1.28
	Zombo	52.40±0.0004	0.86±0.08	83.66±0.16	1.34±0.15	4.45±0.06	193.75±0.52
Mean±SD		**55.78±3.049**	**0.85±0.04**	**79.64±6.56**	**3.39±3.64**	**4.31±0.69**	**176.44±11.87**

4.1.1 Viscosity and Density

Viscosity and density are used to monitor compositional quality of vegetable oils. Oils with lower values of viscosity and density are highly appreciable to consumers (Ceriani *et al*., 2008;

Mousavi *et al*., 2012). Results tabulated in (table 4.1) revealed that at 27^{o}C, the viscosities ranged from 50.05cP in sunflower oil to 55.78cP in peanut oil. Richard (1986) reported the viscosity of crude sunflower oil at 25^{o}C as 50cP. Murwan (1994) reported viscosity of sesame oil in the ranged of 18.90 - 26.43cP at 40^{o}C. Balla (2001) reported viscosity of peanut oil in the range of 46.0 - 52.43 cP at 30°C.

Fazal *et al*. (2015) reported that vegetable oils are a mixture of triglycerides and their viscosity depends on the nature and arrangement of the fatty acids on the glycerol backbone of the triglyceride molecule, and oil viscosity has a direct relationship with degree of unsaturation and chain length of the fatty acids in lipids, and it increases with increasing degree of saturation. Kim *et al*. (2010) also indicated that viscosity and density decreases with increase in level of unsaturation, and also increases with high levels of saturation and polymerization. Prasad and Dutt (1989) reported that viscosity of oil is influenced by temperature changes, and it generally decreases exponentially with increase in temperature. Wakeham (1954) also reported that hydrogenation increases oil viscosity.

Based on data presented in (table 4.1) at 27^{o}C, the density values covered a small range of 0.85-0.86g/mL, with density value being lowest in peanut oil and highest in sunflower and sesame oils. The small range in the values of density of the vegetable oils analyzed could be attributed to a small difference in their composition and content of fatty acids. Wiess (1983) reported that density of peanut at 15°C was 0.917 - 0.921 and at 25°C was 0.910 - 0.915. While the relative density of peanut oil recommended by joint FAO/WHO (2019) ranges from 0.914 to 0.917 at 20°C.

The density of sesame oil reported by Bernardini (1986) ranged between 0.889and 0.894 at temperature 60°C, which is higher than density of sesame oil (0.86) of the present study at 27^{o}C. Bailey (1996) reported that density of sesame oil between 0.915and 0.924 at 20^{o}C, which is higher than density of sesame oil of the present study. The recommended density

standard for sesame oil at 20°C/ water at 20°C by the joint FAO/WHO (2019) is 0.915 - 0.923.Hui (1996) reported relative density of regular sunflower oil in the range of 0.909 to 0.915 (20^oC /water at 20^oC), which is higher than the density of sunflower oil (0.86) of the present study at 27^oC.The joint FAO/WHO recommends a relative density of regular sunflower oil in the range of 0.918 to 0.923 (20^oC /water at 20^oC). Therefore, the density of all the analyzed oils is lower than the values recommended by the joint FAO/WHO (2019). The differences in the oil densities could be attributed to fatty acid compositions, minor components in the oils, and temperature (Fakhri and Qadir, 2011).

4.1.2 Iodine Value (IV)

Iodinevalue (IV) measures the degree of unsaturation in fats or vegetable oils. It determines the stability of oils to oxidation, and allows the overall unsaturation of oils to be determined qualitatively (AOCS, 1999; Asuquo *et al*., 2012). Onyeike (2003) indicated that the higher the iodine value, the greater degree of unsaturation, and the lower the stability, and the more susceptible the oil to oxidation.The iodine value obtained for the analyzed oils (table 4.1) showed 126.59mg I_2/100g for sunflower oil, 106.55mg I_2/100g for sesame oil, and 79.64mg I_2/100g for peanut oil. Matola *et al*. (2015) found that the iodine value for sunflower oil ranged from 124.8 to 125.7mg I_2/100g, which is in agreement with result of the present study of 126.55mg I_2/100g. Mohammed and Hamza (2008) reported iodine value of sesame oil in the range of 103 - 116mg I_2/100g, and this value is in agreement with the findings of the present study. Sulaiman *et al.* (2012) reported iodine value of peanut oil in the range of 43.72 to 45.12mg I_2/100g, and this finding is not in agreement with result of the present study. Therefore, the difference in iodine values may be attributed to fatty acid composition of the different oils (Atasie and Akinhanmi, 2008).

The recommended ranges of iodine value for different vegetable oils are110-143, 105-120, and 7.7-107mg of I_2/100g of fat for sunflower, sesame, and peanut oils respectively(FAO/WHO,

1999).The iodine values obtained in the present study are within the recommended range of iodine values for edible oils specified by joint FAO/WHO (2019).The low iodine value of peanut oil obtained in the present study demonstrates that it has greater oxidative storage stability. The oxidative and chemical changes of oil during storage are characterized by a decrease in the total unsaturation of oil (Perkin, 1992).

4.1.3 Peroxide Value (PV)

Peroxide value (PV) is used as a measure of the extent to which rancidity reactions have occurred during storage, and it is used as a good criterion for the prediction of the quality and stability of oils (Nangbes *et al*., 2013). The peroxide values tabulated in (table 4.1) for all oils range from 1.42-9.25meq/kg, with sunflower oil at (9.25), peanut oil at (3.39), and sesame oil at (1.42). Jar Elnabi (2001) reported the peroxide value for refined and crude sunflower oil as 4.4 and 6.1meq/kg respectively, and these peroxide values are lower than the peroxide value (9.25) for sunflower oil of the current study. SSMO (2002) reported the peroxide value of unrefined sesame oil as 15meq/kg and refined sesame oil as 10meq/kg, which is higher than the peroxide value of sesame oil (1.42) of the current study. Balla (2001) reported the peroxide value of crude and refined peanut oils in the range of 0.7 to 1.2meq/kg and 2.9 to 4.1meq/kg, respectively, and these values are not in agreement with peroxide value particularly for crude peanut oil (3.39) of the present study. The joint FAO/WHO (2019) recommended maximum peroxide value of 10meq/kg for all vegetable oils. Mohammed and Ali (2015) indicated that high peroxide value could be due to high degree of unsaturation, and found to increase with the storage time, temperature, light, and contact with atmospheric oxygen.

Sunflower oil exhibited higher value than the rest. Therefore, sunflower oil is relatively more susceptible to oxidative rancidity than the other oil samples. However, the values for all the samples were within the recommended range of the joint FAO/WHO(2019). In general, the

analysis showed that sesame oil, peanut oil and sunflower oil samples exhibited excellent, good, and acceptable qualities, respectively.

4.1.4 Acid Value (AV)

Acid value represents the weight of KOH in mg needed to neutralize the free fatty acids present in 1g of fat, while free fatty acid is the percentage by weight of a specified fatty acid such as the percentage of oleic acid in oil (Amos *et al*., 2012). An increment in the amount of free fatty acid in a sample of oil or fat indicates hydrolysis of triglycerides, and such reactions occur by action of lipase enzyme, which is an indicator of inadequate processing and storage conditions like high temperature, moisture, and tissue damage (Othman and Ngaasapa, 2010). Acid value therefore, isa good indicator of oil degradation caused by hydrolysis or enzymes, which is the also an indicator of level of rancidity and edibility of oils.The joint FAO/WHO (2019) recommenced maximum acid value of 4mgKOH/g for all vegetable oils.

The acid values tabulated (table 4.1) for all the analyzed oils of the present study range from 2.42- 4.31mgKOH/g, with 2.42mgKOH/g for sesame oil, 3.11mgKOH/g for sunflower oil, and 4.31mgKOH/g for peanut oil. As it is seen in (table 4.1), except peanut oil, the acid values for oil samples analyzed were below the maximum value of 4mgKOH/g recommended by the joint FAO/WHO (2019). Negash *et al.* (2019)reported the acid value of refined sunflower oil as 1.2mgKOH/g, and this value is lower than the acid value for sunflower oil (3.11) of the current study. Mohammed and Hamza (2008) reported the acid value of white and brown sesame oil as 0.5 and 0.45mgKOH/g respectively, and these values are lower than the acid value for sesame oil (1.42) of the present study. Akhtar *et al.* (2014) reported acid value for peanut oil in the range of 0.6 to 0.99nmgKOH/g of oil, and these values are also lower than the acid value for peanut oil (3.39) in the current study.

The high acid values of the analyzed oils mainly for peanutoil could be attributed to high moisture content, poor extraction techniques, use of damaged seeds, and incorrect or lengthy

storage that can be accelerated by light and temperature. Nevertheless, the most common factor for high acidity is the nature of unrefined oils which easily hydrolyses under storage (Rajko *et al*., 2010; Fazal *et al*., 2015). According to the results, edibility of sesame oil, sunflower oil and peanut oil is excellent, good, and acceptable, respectively.

4.1.5 Saponification Value (SV)

Saponification measures the average chain length of the fatty acid that makes up the oil. In other words, saponification values are useful in providing information as to the quantity, type of glycerides, and mean weight of the acids in a given oil sample (Mohammed and Ali, 2015; Fazal *et al*., 2015). The lower the saponification value, the larger the molecular weight of fatty acids in the glycerides,and the lower the mean molecular weight (Musa *et al*., 2012). The saponification value obtained for the oil samples in (table 4.1) are 191.68mgKOH/g for sunflower oil, 180.24mgKOH/g for sesame oil, and 175.19mgKOH/g for peanut oil. Sulaiman *et al.* (2012) reported saponification value of sunflower oil in the range of 188.9 to 189mgKOH/g, Mohammed and Hamza (2008) reported saponification values of white and brown sesame seed oils as 189 and 191mgKOH/g, respectively. Matola *et al.* (2015) reported saponification value of peanut oil as 189.4. Therefore, the findings of the present study for saponification value of all the analyzed oils are not in agreement with those reported from other studies. The difference in the saponification values could be attributed to the chain length or molecular weight of the fatty acids in the triglycerides.

The recommended ranges of saponification value for different vegetable oils are 187-196mgKOH/g for peanut oil, 185-193mgKOH/g for sesame oil, and 188-194mgKOH/g for sunflower oil (FAO/WHO, 2019). The saponification values of the current study are within the recommended value of the joint FAO/WHO (2019).

All the vegetable oils analyzed showed relatively high saponification values characterized by presence of relatively high concentration of low molecular weight fatty acids which can be used as valuable raw materials for soaps and cosmetics (Nangbes *et al*., 2013).

4.2 FATTY ACID COMPOSITION

The fatty acid composition of analyzed oils is shown (table 4.2 and table 4.3) respectively. The mean of total saturated fatty acid (SFA), monounsaturated fatty acids (MFA), polyunsaturated fatty acids (PUFA), and the nutritional indexes (P/S) are shown(table 4.3).

Table 4.2: Fatty acids (%) in vegetable oils analyzed

Fatty acids	Formula	Sunflower	Sesame	Peanut
Palmitic	C16:00	12.46 ± 0.90^{a}	10.93 ± 0.13^{b}	13.07 ± 0.34^{c}
Stearic	C18:00	6.88 ± 0.29^{a}	6.87 ± 0.04^{a}	7.37 ± 0.19^{b}
Oleic	C18:1n-9	44.07 ± 1.59^{a}	44.12 ± 1.10^{a}	44.06 ± 0.81^{a}
Linoleic	C18:2n-6	31.26 ± 1.45^{a}	$31.5\ 1\pm 0.68^{a}$	30.29 ± 0.86^{b}
Linolenic	C18:3n-3	0.33 ± 0.00^{a}	0.36 ± 0.00^{a}	nd
Arachidic	C20:00	1.69 ± 0.05^{a}	1.74 ± 0.03^{b}	1.61 ± 0.38^{a}
Behenic	C22:00	2.23 ± 1.27^{a}	4.06 ± 1.81^{b}	2.77 ± 0.58^{a}
Lignoceric	C24:00	1.33 ± 0.83^{a}	0.76 ± 0.03^{b}	1.09 ± 0.22^{c}

Data are expressed as percentages of total fatty acid methyl esters; values are means of triplicate determinations, values followed by the same letter with in each row are not significantly different (P>0.05), nd: not detected

Table 4.3: Total fatty acids (%) and liquid health index (P/s) of the vegetable oils

Class of Fatty acids	Sunflower	Sesame	Peanut
∑SFA (%)	24.59 ± 4.77	24.37 ± 4.12	25.91 ± 5.05
∑MUFA (%)	44.07 ± 1.59	44.12 ± 1.10	44.06 ± 0.81
∑PUFA (%)	31.59± 1.45	31.87 ± 0.68	30.29 ± 0.86
P/S Index	1.28	1.31	1.17

SFA: Saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: Polyunsaturated fatty acids; P/S : nutritional index, which is ratio of polyunsaturated to saturated fatty acids

Results of fatty acid composition(Table 4.2) show that 8 fatty acids were present in all the analyzed vegetable oil samples, but only four fatty acids, oleic, linoleic, palmitic and stearic fatty acids make up about 95% of total fatty acid content. Linolenic, arachidic, behenic,and lignoceric fatty acids accounted for only <5%. This finding is close to the data by Ahmed and Young(1982) who reported that palmitic, stearic, linoleic, and oleic acids accounted for 95% of total fatty acid content in peanut and sunflower oils. There were no significant differences ($P>0.05$) in the levels of different fatty acids based on location or type of oil seeds.

In Table 4.2,There is an inverse relation between oleic and linoleic acids in the analyzed oils and this finding is consistent with data by Hassan and Ahmed (2012), Achola *et al*. (2017), Asubo *et al*. (2008), Ashish (2013) and Azharudheen *et al*. (2013) who reported inverse relationship between oleic acid and linoleic acid in sunflower, peanut, and sesame oils. This could be due to action of desaturase enzymes, encoded by three genes which converts linoleic acid to oleic acid, and hence altering the fatty acid composition in plant seeds.

Nutritional quality of any vegetable oil is based on the proportions of SFA, MUFA, and PUFA in the oils. Vegetable oils and fats are perceived as a dietary component associated with risk of diseases, the problem is not the oil but their overconsumption, especially in the unbalanced intake of fatty acids (Babinská and Béderová, 2012).

The results from this study presented (Table 4.3) show that the percentage of total saturated fatty acids(SFA) ranged from 24.37% to 25.91% in all the analyzedoils with predominant presence of palmitic and stearic acids. Peanut oil showed the highest saturated fatty acid content of 25.91%,comprising of palmitic 13.07%, stearic 7.37%, behenic 2.77%, arachidic 1.61, and lignoceric 1.09%. The saturated fatty acid content of sunflower oil (24.59%) and sesame oil (24.37%) showed similar pattern of fatty acid composition, except behenic and lignoceric contents which were significantly different at $P<0.05$. According to FAO/WHO report of an expert consultation on fats and fatty acids in human nutrition, the recommended

human consumption of SFA should cover <10% of the available energy from food (Velíšek and Hajšlová, 2009). Keresteš *et al*. (2011) reported that SFA with longer chain (above 14 carbons) have a tendency to agglomerate with negative effect to the cardiovascular disease occurrence. Studies done in both mice and humans indicate that high levels of long chain saturated fatty acids such as palmitic acid in the diet may adversely affect mood, and reduce physical activities (Kien *et al*., 2003). High levels of palmitic acid also raise the levels of LDL (bad) cholesterol, which is associated with increased risk of heart diseases and cardiovascular complications. Myristic acid is a long chain saturated fatty acid, and its consumption raises the LDL cholesterol more than other fatty acids (Zock *et al*., 1994). Stearic acid lowers the LDL cholesterol slightly as such it may be healthier than other fatty acids (Zock*et al*., 1994). Medium chained saturated fatty acids such as capric, caprylic, and caproic acids are unique because they promote weight loss, increase in insulin sensitivity, and reduce risk of seizers (Dullo *et al*., 1996). Studies show that replacing SFAs with other macronutrients particularly, PUFA has a favorable effect on the blood lipid profile, including lowering of LDL cholesterol levels (WHO, 2016).

Besides the negative impact of SFA on human health, the human body uses SFA up to the length of 12 carbon atoms, mainly for the energy production. Saturated fatty acids (SFA) are also chemically very stable and change either by prolonged heating or at high temperatures. Saturated fatty acids are also recommended to people with digestive problems or liver disorders, because they are not stored in body as fat and also due to their easy digestibility.

All the analyzed oils showed similar patterns of unsaturated fatty acid composition (Table 4.2 and 4.3).The total unsaturated fatty acid content ranged from 74.35to 75.99% in all the analyzed oils, with the predominant presence of oleic acid, a monounsaturated fatty acid (MUFA). Sesame oil showed the highest percentage of total unsaturated fatty acid of 75.99%, comprising of oleic acid (MUFA) 44.14%, linoleic acid (omega-6) 31.51%, and then linolenic

acid (omega-3) 0.36%. This finding is close to data by Yoshida et al., (2000) who reported FA composition of sesame oil as 44% oleic acids, 34% linoleic acid 10% palmitic acid and 7% stearic acid. The total unsaturated fatty acid content of sunflower oil (75.66%) and peanut (74.35%) had almost equal amounts of oleic (MUFA) and linoleic (omega-6) acids, except linolenic acid (omega-3). This finding is similar to data by Musimenta et al., 2019) who reported oil from peanut in Uganda contained 39.71% to 55.89% oleic acid, 20.21 to 35.5% linoleic acid and 11.91-17.16% palmitic acid. Liu *et al.* (2017) reported that unsaturated fatty acids have a wide range of biological roles, and cellular functions which include formation of phospholipid bilayer of cellular membranes, transport of proteins and cellular receptors for hormones and neurotransmitters, and used as substrates for eicosanoid synthesis.

Glick and Fischer (2013) reported that human body is capable of producing all the unsaturated fatty acids,except the essential fatty acids like linoleic acid (omega-6 fatty acid) and alpha-linolenic acid (omega-3 fatty acid). These fatty acids are necessary for growth and development, and are also used as starting materials for the manufacture of other fatty acids (e.g. arachidonic acid (AA) is formed from LA) (Elleuch *et al*., 2007).

FAO/WHO (1998) recommended that human consumption of linoleic (omega-6) should be within 4-8% on average of total energy intake of food. The recommended intake of linolenic acid (omega-3) is about 0.6-1.2% of daily energy intake or 1-2 g per day **(**Frej, 2014; Nitrayová *et al*., 2014).

Various studies have revealed that vegetable oils like sunflower, soybean, peanut, sesame contain abundant dietary unsaturated fatty acids that can supply the nutritional needs of humans (Achola *et al*., 2017; Kowalski *et al*., 2004; Kamal, 2006). Through selective breeding and manufacturing processes, oils of differing proportions of unsaturated fatty acids can be produced (Fernandez-Martínez *et al*., 2004). Based on fatty acid composition, three types of sunflower oils exist with varying levels of oleic and linoleic acids. High oleic type, which

contains nearly (80%) oleic acid, high linoleic typecontains(70%) linoleic acid, and mid oleictype contains (42% to 65%)(Fernandez-Martynez *et al*., 2004).The results presented (table 4.3)show that sunflower oil in the current study contained oleic acid 44.07%, linoleic acid 31.26%, and linolenic acid 0.33%. The findings of this study show that the sunflower oil analyzed is mid oleic type.Thambugala *et al*. (2013) reported that fatty acid biosynthesis in plant cells is controlled by a group of genes identified as sad1, sad2, fad2a, fad2b, fad3a, fad3b,which are collectively known as desaturase. Research studies have also reported that differences in fatty acid composition of oils could be due to differential expression of desaturase genes during seed development and maturation (Baud and Graham, 2006). Morrison *et al*. (1995) reported that fatty acid composition of sunflower is dependent on where the crop is grown. Cooler climates produce higher amounts of linoleic acid compared with warmer climate, where oleic acid is more dominant. Fernandez-Martynez *et al*. (2004) indicates that mid oleic type retains high enough levels of linoleic acid to remain an excellent dietary source, but the relatively high levels of oleic acid make it less prone to rancidity. Various research studies have reported that oleic and linoleic acids are hypocholesterolemic, although linoleic acid is an essential fatty acid, oils rich in oleic acid (MUFA) are preferred as it combines the hypocholesterolemic effect (Mensink and Katan, 1989), and a greater oxidative stability than linoleic acid (PUFA) (Yodice, 1990). However, high linoleic acid oils have alternative nutritional advantages such as the production of conjugated linoleic acid (CLA), associated with a wide range of positive health benefits (Belury, 1995; Ip, 1997).

Sunflower and sesame oils in the current study showed similar presence of linolenic, ranging from 0.33% for sunflower to 0.36% for sesame oil. Linolenic acid is an omega-3 PUFA that plays an important role in regulation of biological functions, prevention and treatment of a great numbers of human diseases such as heart and inflammatory diseases. The low content of

linolenic acid (PUFA) in both sesame and sunflower oil makes them resistant to oxidation and suitable for human consumption.

The total unsaturated fatty acid content of peanut oil is 74.5%, with predominant presence of oleic acid 44.06%, followed by linoleic acid 30.29%, (Table 4.3), this findings were consistent with data by Achola*et al.* (2017) who reported oleic acid and linoleic acid contents of S.7T peanut variety in Uganda as 43.19% oleic acid,and33.44%linoleic acid. The differences in fatty acid composition may be attributed to differences in genotype, growing season (Singkham *et al.*, 2010), location and planting date (Andersen and Gorbet, 2002), soil nutrient, soil temperature, and maturity (Dwivedi *et al*., 1993).

Sesame oil is a superior vegetable oil, and has a pleasant flavour. It ranks second after olive oil with regard to nutritional value. Worldwide, fatty acid composition of sesame oil is variable among the different cultivars of sesame seeds such as black, brown and white, and in this study, sesame oil was extracted from brown sesame seeds. Sesame seeds contain mainly mono, and polyunsaturated fatty acids accounting for almost 85% of total fatty acid (Unal and Yalcin, 2008). Unal and Yalcin (2008) reported that sesame is a rich source of proteins and several health promoting compounds such as phytosterols, tocopherols, and lignans,and this peculiar biochemical composition makes sesame oil the most resistant oil against oxidation though it is highly unsaturated.

Table 4.2 and Table 4.3show that the sum of mono- and polyunsaturated fatty acids accounted for 75.99%of the total fatty acid content of sesame oil, with predominant presence of oleic acid with 41.12%, followed by linoleic acid 31.51%, and linolenic acid 0.36%.The findings of the present study are similar to the results reported by Yoshida *et al.* (2000) who indicated that oleic acid and linoleic acid contents were 44% and 34%, respectively. Contrary to our findings, Thakur *et al*., (2017)reported oleic acid content of 41.36%, linoleic acid content as 41.25%, and linolenic acid content of 0.35%.This implies that Fatty acid composition of

sesame oil depends on different factors such as climatic conditions, soil conditions, and ripeness of plants (Rahman *et al.*, 2007).

The nutritional index (P/S)is the relationship between saturated (SFA) and polyunsaturated fatty acid (PUFA)content, which is an important parameter for determining nutritional value of vegetable oils. The vegetable oilsshown in (table 4.3) covered a small range of nutritional index from1.17 to 1.31, with the P/S value being highest for sesame oil, and lowest for peanut oil.

The polyunsaturated to saturated fatty acid ratio (P/S) measures the tendency of the diet to influence the incidence of coronary heart diseases (Simat et al., 2015) this ratio is also important in determining cholosteromic effect of dietary lipids.

Foods with P/S ratio above 0.45 (FAO, 2010) are considered beneficial due to their potential to lower serum cholesterol (Kostik et al., 2013). The beneficial effect is even more significant when the PH ratio are >1. The P/S ratios of the vegetable oils analyzed ranged from 1.17 to 1.31 (Table 4.3).

Johnson et al., (2009) obtained PS ratios of 1.8 and 2.28 for peanut oil. The high P/S ratios in this study suggest that consumption of diet rich in peanut, sesame and sunflower oil is beneficial for human health.

Oils with high P/S ratios are of high nutritional value than the ones with less (Kostik et al., 2013).

Ramprasath et al., (2012) reported that the serum cholestoral concentration are linked with diets rich in SFA while the opposite effect is provided by diets containing high levels of BUFA.

4.3 HEAVY METAL CONTENTS

In table 4.4, all the vegetable oils were contaminated with substantial amounts of iron (Fe) and lead (Pb) metals, while cadmium (Cd) and zinc (Zn) metals were not detected. The concentration of iron ranged from (0.263mg/kg to 14.982mg/kg). sesame oil from Nebbi had the lowest iron level, whereas sesame oil from Yumbe had the highest.

The concentration of lead (Pb) ranged from 0.585mg/kg to 2.035mg/kg. Peanut oil and sesame oil from Nebbi had the lowest and same levels of lead, whereas sunflower oil from Zombo had the highest level of lead.

Table 4.4: Average amount of heavy metal in vegetable oils (mg/kg)

Oil Type	Location	Cd	Zn	Fe	Pb
Sunflower	Zombo	nd	nd	6.717±0.002	2.035±0.000
	Nebbi	nd	nd	2.314±0.001	nd
	Yumbe	nd	nd	2.300±0.002	2.126±0.000
	Arua	nd	nd	14.017±0.002	nd
Sesame	Zombo	nd	nd	3.728±0.002	1.401±0.000
	Nebbi	nd	nd	0.263±0.001	1.944±0.000
	Yumbe	nd	nd	14.982±0.002	0.585±0.000
	Arua	nd	nd	7.590±0.001	nd
	Zombo	nd	nd	6.624±0.002	nd
Peanut	Nebbi	nd	nd	5.778±0.001	0.585±0.000
	Yumbe	nd	nd	7.087 ± 0.002	nd
	Arua	nd	nd	5.156±0.000	0.766±0.000

("nd" means below detection limit)

Table 4.5: Linear regression equations, coefficient of determination, limit of detection

Metal	IDL (mg/L)	LOD (mg/kg)	LOQ (mg/kg)	Coefficient of determination (r2)	Regression Equation
Fe	0.005	0.203	0.771	0.9999	Y = 0.0116x + 0.0003
Zn	0.005	0.083	0.303	0.9999	Y = 0.0801x + 0.0018
Cd	0.005	0.078	0.35	0.9998	Y = 0.1018x + 0.0004
Pb	0.006	0.095	0.459	0.9997	Y = 0.0054x + 0.0007

Various studies have reported the presence of heavy metals such as iron, nickel, lead, cadmium, copper and Arsenic in vegetable oils (Mendil *et al*., 2009; Juszczak 2008). The presence of heavy metals in vegetable oils may depend on several factors, which includes

uptake by the plant roots from soils contaminated with metals, the metals may also be introduced during the refining processes such as bleaching, hardening, refining, and deodorization (Zeiner *et al*., 2005; Jamali *et al*., 2008). The metals may also be introduced into the vegetable oils by contamination during oil extraction, storage, and transportation (Leonardis*et al*., 2000).

Iron (Fe) is considered an essential mineral because it is needed to make hemoglobin, a part of blood cells which is responsible for carrying oxygen in the body. Therefore, sufficient amount of iron in the diet is necessary for manufacture of hemoglobin in order to reduce incidence of anaemia (Ashraf and Mian, 2008).Anemia is also commonly associated with a decrease in working power, and damaged intellectual development (Ettle *et al*., 2007). High levels of iron in the body has also been associated with some negative effects such as tissue damage, as well as formation of free radicals(Schümann et al., 2007). The Maximum Permissible Limit (MPL) of iron is 5.0mg/kg in all vegetable oils(WHO/FAO, 2003).

The concentration of iron (table 4.4) in most samples exceeded the maximum permissible limit. The high iron content in this study may be attributed to uptake of iron by roots from contaminated soil, reaction between the relatively high-unsaturated portion of the oil with the surface of iron containers used during transportation, storage, and processing of vegetable oils(Duran *et al*., 2013).

Pelivan *et al*. (2009) reported low iron content in different vegetable oils in the range of 0.0039-0.0352mg/kg. Mendil *et al*. (2009) also reported the content of iron in different vegetable oils in the range of 52.0-291.0µg/g. The difference in the iron content reported to that in the present study could be attributed to oil extraction methods, storage and processing, and soil factors (Leonardis*et al*., 2000).

Lead (Pb) is a toxic heavy metal which serves no useful purpose in human body. Its presence in the body may cause chronic and acute poisoning, which may lead to failure of heart and

liver, and other health disorders such as tiredness, sleepiness, hear, and weight loss (Needleman *etal*., 2002). The maximum permissible concentration of lead is 0.1mg/kg for all vegetable oils (FAO/WHO, 2019).

From table4.4, lead level was highest in sunflower oil from Zombo (2.035mg/kg), and lowest in peanut oil from Nebbi and sesame oil from Yumbe (0.585mg/kg). Sunflower oil samples from Nebbi and Arua, sesame oil from Arua, peanut oil from Zombo and Yumbe, did not contain lead.

The reported concentration of lead in different vegetable oils from Saudi Arabia ranged from 0.007-0.015mg/kg (Ashraf, 2014). Asemave *et al*. (2012) reported concentration of lead in palm oil, peanut oil, and soybean oil from Nigeria as 0.1780mg/kg, 0.1631mg/kg and 0.1631 mg/kg, respectively. Findings of the present study reveals higher levels of lead than in the reported result. Research studies have reported that contamination of vegetable oils with lead maybe from soil through mineralization by crops, oil processing or environmental contamination, as in the application of agricultural inputs such as fertilizers and pesticides, which are in common use in farms (Onianwa*et al*., 2001; Mendil *et al*., 2009). Contamination of vegetable oil with lead may also be due to industrial emission, combustion of fuel in refinery process, and from packaging materials such as stabilizer and colorant in plastic (Dugo *et al*., 2004).

Zinc(Zn) is an essential element known to be involved in most metabolic pathways, and is regarded as an important cofactor for many enzymes that participates in metabolism. Zinc deficiency is the most ubiquitous micronutrient deficiency in crops. Zinc is essential for both plants and animals because it is a structural constituent and regulatory cofactor in enzymes, and proteins involved in many biochemical pathways (Alloway, 2009). Millions of hectares of crop land are affected by zinc deficiency, and approximately one third of human population suffers from inadequate intake of zinc. The main soil factors affecting availability of zinc to

plants are low total zinc content, high pH, high calcite and organic matter content, and high concentrations of sodium, calcium, magnesium, bicarbonate, and phosphate in the soil solution.

Zinc was not detected in all the analyzed vegetable oils (table 4.4). This result is in agreement with the findings by Alloway (2009) who revealed that zinc deficiency to crops is a major problem in the world, and affecting millions of hectares of crop land and one third of human population. Other studies elsewhere have reported Zinc levels in the range of 9.1-31.8mg/kg (Ewuzie and Nnorom, 2015), and 0.0484-0.287mg/kg (Pehlivan *et al*., 1998). High levels of zinc in vegetable oil could be attributed to absorption by plants from soil or contamination of the vegetable oils during refining process, storage tank and packaging materials.

Cadmium (Cd) is a highly toxic heavy metal with a natural occurrence in soil, but it spreads in the environment due to human activities. Needleman *et al*. (2002) reported that excessive exposure to cadmium may lead to renal and reproductive effects. Table 4.4 shows cadmium was not detected in all the analyzed vegetable oil samples. Reported concentration of cadmium in different vegetable oils from Turkey were in the range of 0.09-4.57µg/kg (mendil *et al*., 2009). Zhu *et al*. (2011) reported the levels of cadmium in peanut oil, sesame oil, and sunflower oils from China as 3.81, 3.44 and 3.51µg/g,respectively.

The absence of cadmium in the present study may be attributed to low levels of pollution or crop variety. High levels of cadmium in vegetable oils of the previous studies may be attributed to uptake by plants from contaminated soils, contamination through refining process, storage tank or the packing material such as a colorant or stabilizer in plastics (Mendil *et al*., 2009).

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

In this study, fatty acid composition, physicochemical properties, and heavy metal concentration of sunflower, sesame, and peanut oils from West Nile sub region were evaluated using GC/MS, AOCS, and AAS techniques. From the findings of this study, the following conclusions were drawn;

The composition of fatty acids of the oils revealed the presence of high amounts of unsaturated fatty acids and also similar levels of major fatty acids like oleic, linoleic, palmitic and stearic acids. This signifies that the type of oil seed or location may not affect fatty acid composition. The polyunsaturated to saturated fatty acid ratios (P/S) of all the oils analysed were greater than one, which is an indicator that the oils are of good health and beneficial for human consumption.

All the vegetable oils exhibited acceptable and desirable physicochemical qualities of edible vegetable oil. Although sesame and peanut oil contain more unsaturated fatty acids, the oils showed a remarkable stability to oxidation. The low acid values and peroxide values of the oilsare indicators of the ability of oils to resist hydrolytic and oxidative rancidity and remain fresh with long shelf life.

Results showed that lead Pb and Iron (Fe) content had a higher concentration of the mean values than the permissible limits set by FAO/WHO, However, it appears that the heavy metals are not potential health risks through consumption of the vegetable oils from west Nile region. Besides this heavy metal concentrations can vary from place to place.

High concentration of lead (Pb) in sunflower and sesame may cause lead (Pb) poisoning.

5.2 RECOMMENDATIONS

In the light of the findings of the study and discussion the researcher has the following recommendations; -

1) The High P/S ratios of sesame and sunflower oils suggest that consumption of diet rich in this oils would be more beneficial for Human health.
2) Sunflower oil with high saponification value has a potential for use in soap making and cosmetic industry, this potential could be exploited by government to support large scale production of sunflower oil.
3) Further research is important to determine the health risks of heavy metals with dietary intake of other frequently consumed foods such as cassava, beans, cereals and vegetables because intake of Heavy metals, will increase with dietary intake.

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APPENDIX ONE

PHYSICOCHEMICAL PROPERTIES OF VEGETABLE OILS

Table 7.1: Density g/cm^{3}at 27^{o}C

Density				
LAB NO	Wt. of Empty Flask	Wt. of Water + Flask, g	Wt. of Sample in Flask, g	Density, g/cm^3
FAME 1	11.56	17.3	4.9900	0.8693
FAME 2	11.56	17.3	5.0000	0.8711
FAME 3	11.56	17.3	5.2500	0.9146
FAME 4	11.56	17.3	4.7800	0.8328
FAME 5	11.56	17.3	4.9700	0.8659
FAME 6	11.56	17.3	5.0100	0.8728
FAME 7	11.56	17.3	4.9000	0.8537
FAME 8	11.56	17.3	4.9000	0.8537
FAME 9	11.56	17.3	4.7500	0.8275
FAME 10	11.56	17.3	4.9100	0.8554
FAME 11	11.56	17.3	4.8800	0.8502
FAME 12	11.56	17.3	4.9700	0.8659

Table 7.2: Iodine value (mg of I_2/100g)

Iodine Value				
SPL ID	WT, gm	Titre Blank, Ml	SPL AVG Titre, Ml	Iodine Value (Wijs)
FAME 1	0.28	33.6	9.8	107.865
FAME 2	0.2	33.6	12.4	134.514
FAME 3	0.493	33.6	5.7	71.816
FAME 4	0.331	33.6	11.1	86.261
FAME 5	0.223	33.6	13	117.226
FAME 6	0.232	33.6	14.9	102.286
FAME 7	0.2	33.6	12.6	133.245
FAME 8	0.253	33.6	9.4	121.383
FAME 9	0.451	33.6	6.3	76.815
FAME 10	0.399	33.6	7.3	83.646
FAME 11	0.2	33.6	17.2	104.058
FAME 12	0.264	33.6	10.3	111.999

Table 7.3: Peroxide value (meq/kg)

Peroxide Value,					
SPL ID	WT, gm	Titre Blank, mL	Actual Molarity of Thiosulphate	SPL AVG Titre, mL	Peroxide Value, mEq/kg
FAME 1	4.1060	0.10	0.0023	7.30	4.0331
FAME 2	4.0020	0.10	0.0023	15.30	8.7356
FAME 3	3.6900	0.10	0.0023	19.60	12.1545
FAME 4	3.8530	0.10	0.0023	12.90	7.6408
FAME 5	4.0570	0.10	0.0023	1.60	0.8504
FAME 6	5.0150	0.10	0.0023	0.80	0.3210
FAME 7	4.8210	0.10	0.0023	2.90	1.3358
FAME 8	3.9540	0.10	0.0023	4.60	2.6176
FAME 9	4.0010	0.10	0.0023	16.10	9.1977
FAME 10	3.9600	0.10	0.0023	13.30	7.6667
FAME 11	4.0640	0.10	0.0023	0.90	0.4528
FAME 12	3.9920	0.10	0.0023	1.60	0.8642

Table 7.4: Acid value (mgKOH/g)

Acid value				
SPL ID	WT, gm	Titre Blank, mL	SPL AVG Titre, mL	Acid Value, mg KOH/g
FAME 1	10.2330	0.00	6.300	3.4538
FAME 2	9.6400	0.00	5.500	3.2007
FAME 3	9.5460	0.00	5.900	3.4673
FAME 4	10.0020	0.00	7.400	4.1506
FAME 5	9.0410	0.00	4.200	2.6061
FAME 6	9.6450	0.00	4.100	2.3848
FAME 7	10.5880	0.00	4.000	2.1194
FAME 8	8.5600	0.00	6.900	4.5221
FAME 9	10.2400	0.00	9.400	5.1498
FAME 10	10.4510	0.00	8.300	4.4554
FAME 11	13.2300	0.00	3.500	1.4841
FAME 12	12.3000	0.00	6.200	2.8278

Table 7.5: Saponification (mgKOH/g)

Saponification value				
SPL ID	WT, gm	Titre Blank, mL	SPL AVG Titre, mL	Saponification value, mg KOH/g
FAME 1	1.50	36.20	25.70	196.3500
FAME 2	1.98	36.20	21.90	202.5833
FAME 3	1.65	36.20	26.30	168.3000
FAME 4	1.99	36.20	24.20	169.1457
FAME 5	2.01	36.20	22.80	187.0000
FAME 6	2.50	36.20	19.50	187.3740
FAME 7	1.85	36.20	24.00	184.9784
FAME 8	2.00	36.20	22.50	192.1425
FAME 9	1.43	36.20	27.30	174.5769
FAME 10	1.94	36.20	22.80	193.7474
FAME 11	1.75	36.20	25.70	168.3000
FAME 12	1.76	36.20	25.60	168.9375

FATTY ACID COMPOSITIONS OF THE VEGETABLE OILS

Table 7.6: Fatty acids in sunflower %

Results of determination of fatty acid content in sunflower oil						
Fatty Acids	Formula	District				Average ± SD
		Zombo (A)	Nebbi (B)	Yumbe (C)	Arua (D)	
		%	%	%	%	
Palmitic	16:0	13.55	12.83	11.96	11.51	12.46 ±0.90
Stearic	18:0	7.27	7.09	6.61	6.59	6.88 ± 0.29
Oleic	18:1n-9	44.73	42.19	45.88	43.46	44.07 ±1.59
Linoleic	18:2n-6	30.83	31.63	29.57	33.02	31.26± 1.45
Linolenic	18:3n-3	-	-	0.33	-	0.33 ± 0.00
Arachidic	20:0	1.71	1.75	1.66	1.63	1.69 ± 0.05
Behenic	22:0	1.11	3.65	1.22	2.97	2.23 ± 1.27
Lignoceric	24:0	0.85	0.89	2.77	0.82	1.33 ± 0.83
Total %		100	100	100	100	

Table 7.7: Fatty acids in sesame oil %

Results of determination of fatty acid content in sesame oil						
Fatty Acids	Formula	District				Average ± SD
		Zombo (A)	Nebbi (B)	Yumbe (C)	Arua (D)	
		%	%	%	%	
Palmitic	16:0	11.12	10.87	10.90	10.83	10.93 ± 0.13
Stearic	18:0	6.89	6.82	6.91	6.87	6.87 ± 0.04
Oleic	18:1n-9	44.37	43.07	45.56	43.48	44.12 ± 1.10
Linoleic	18:2n-6	32.35	31.03	30.88	31.77	31.51± 0.68
Linolenic	18:3n-3	0.33	0.35	0.40	-	0.36 ± 0.04
Arachidic	20:0	1.75	1.72	1.77	1.71	1.74 ± 0.03
Behenic	22:0	2.41	5.36	2.82	4.62	4.06 ± 1.81
Lignoceric	24:0	0.78	0.78	0.76	0.72	0.76 ± 0.03
Total %		**100**	**100**	**100**	**100**	

Table 7.8: Fatty acids in peanut oil %

Results of determination of fatty acid composition in peanut oil						
Fatty Acids	Formula	District				Average ± SD
		Zombo (A)	Nebbi (B)	Yumbe (C)	Arua (D)	
		%	%	%	%	
Palmitic	16:0	13.57	12.92	12.93	12.85	13.07 ± 0.34
Stearic	18:0	7.61	7.31	7.15	7.42	7.37 ± 0.19
Oleic	18:1n-9	43.18	44.75	44.76	43.56	44.06 ± 0.81
Linoleic	18:2n-6	30.31	29.40	30.02	31.45	30.29 ± 0.86
Arachidic	20:0	1.84	2.02	1.23	1.33	1.61 ± 0.38
Behenic	22:0	2.57	3.62	2.30	2.59	2.77 ± 0.58
Lignoceric	24:0	0.92	0.98	1.42	1.07	1.09 ± 0.22
Total %		**100**	**100**	**100**	**100**	

APPENDIX TWO: CALIBRATION CURVES FOR HEAVY METALS (Cd, Zn, Fe and Pb)

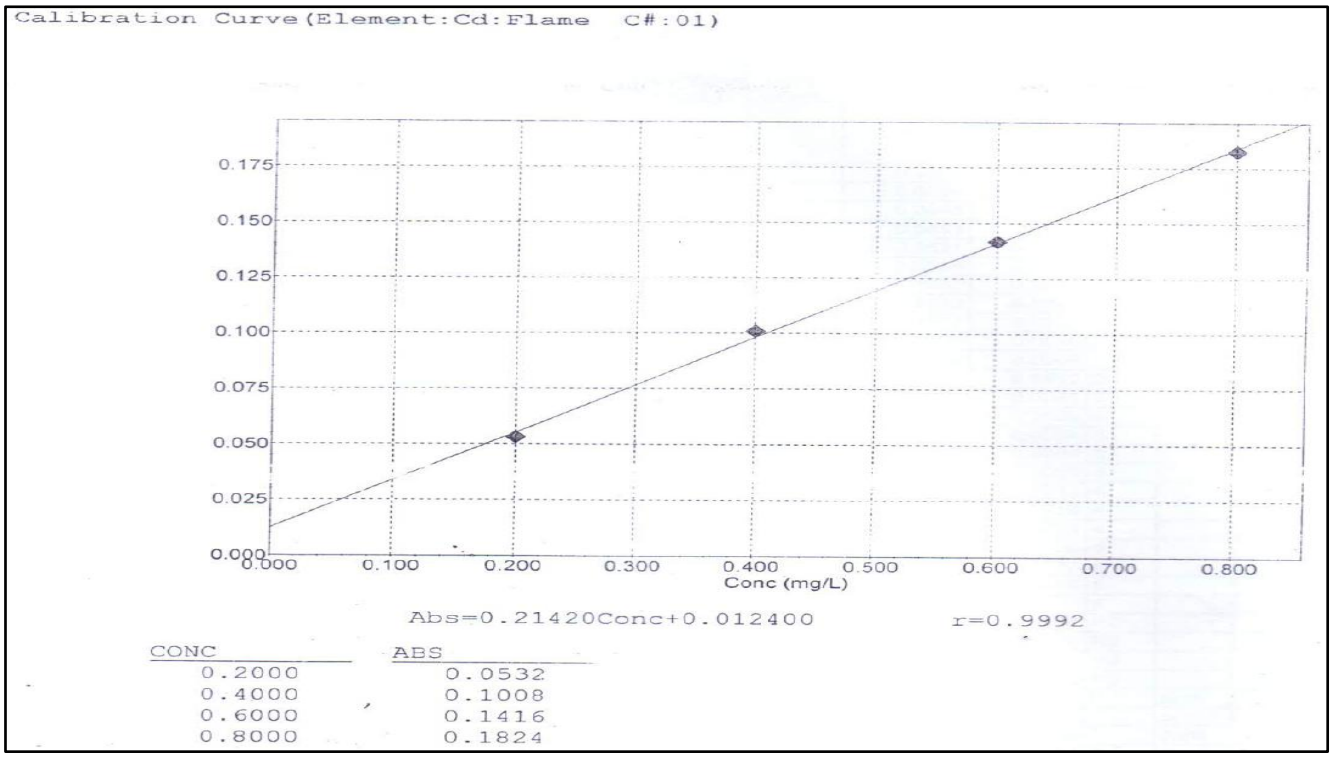


Figure 8: Calibration curve for Cadmium (Cd)

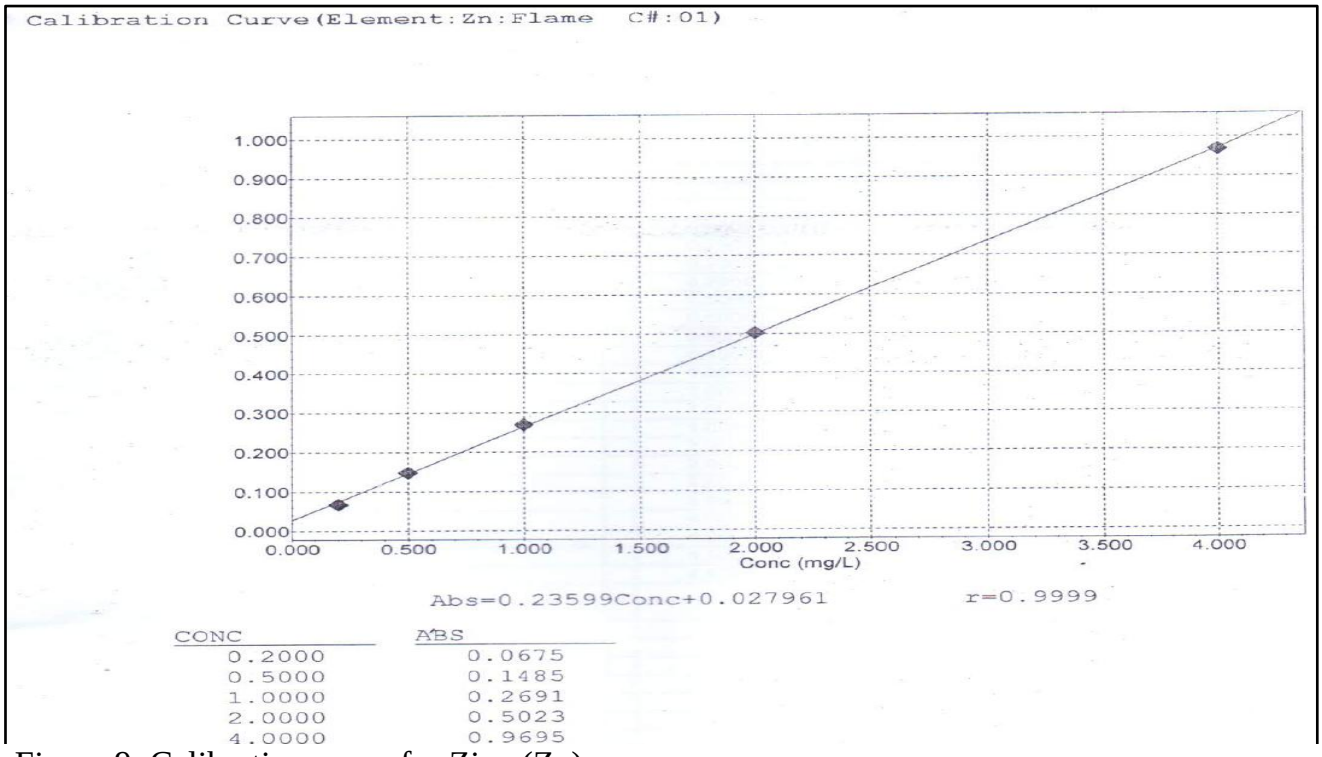


Figure 9: Calibration curve for Zinc (Zn)

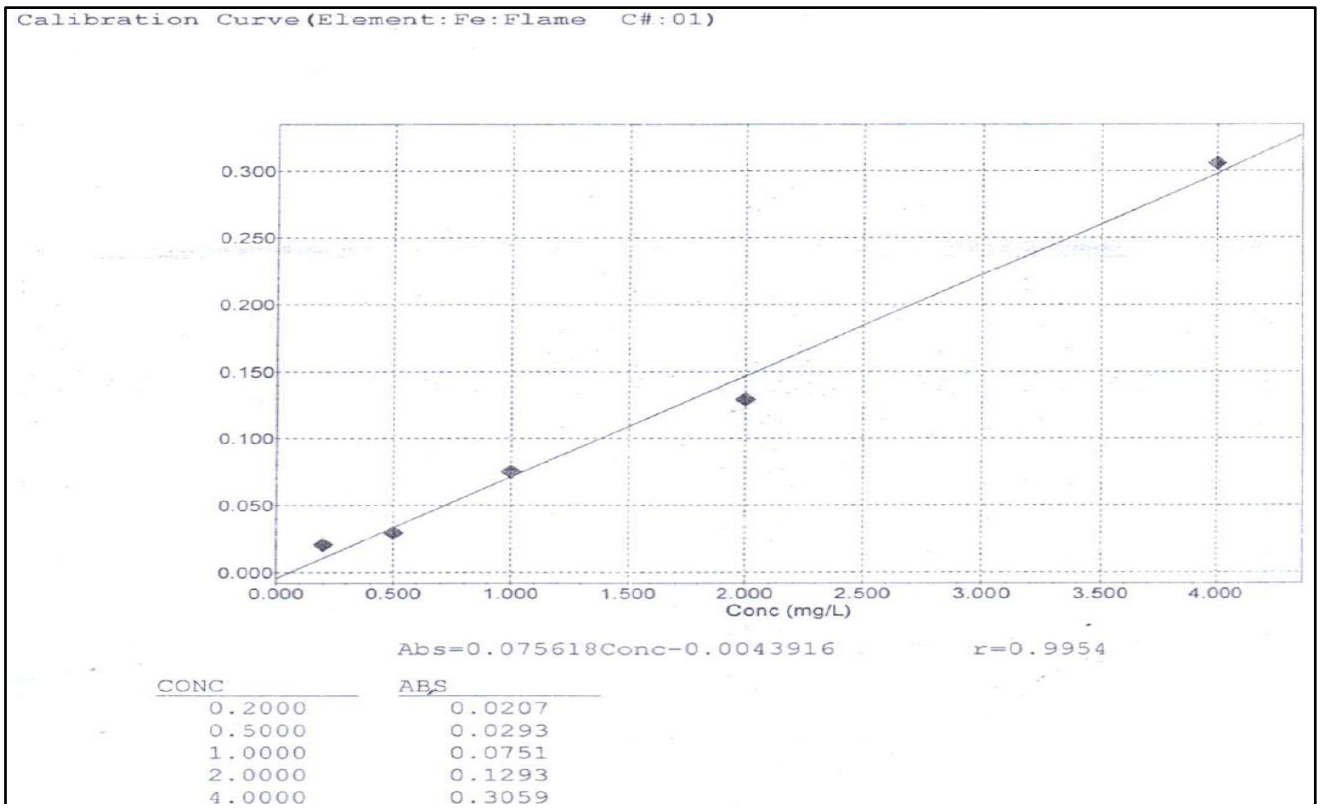


Figure 10: Calibration curve Iron (Fe)

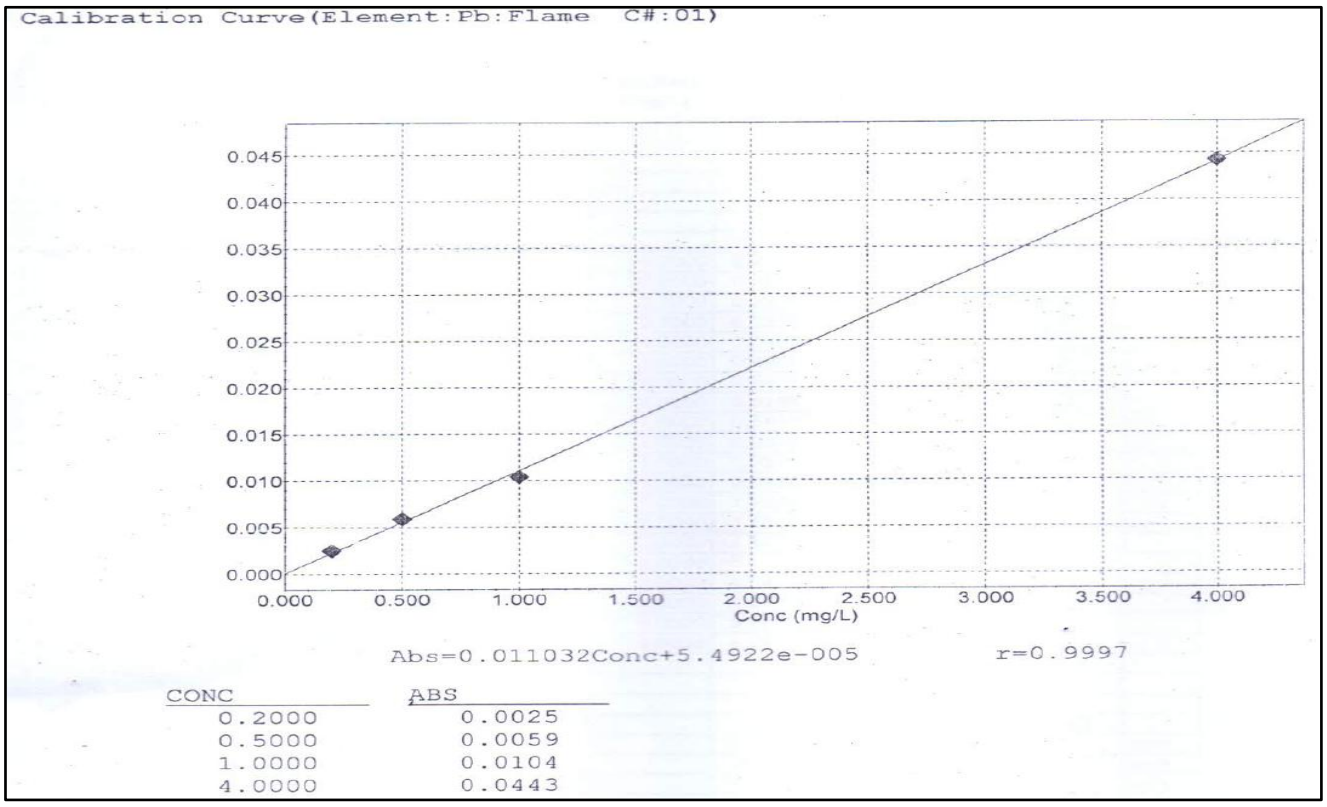


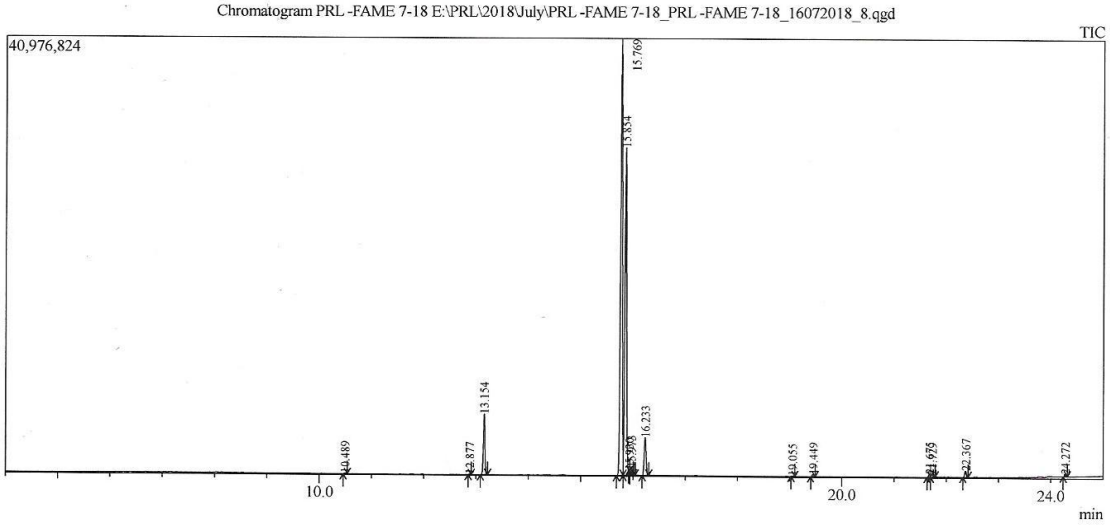
Figure 11: Calibration curve for Lead (Pb)

APPENDIX THREE: STANDARD SAMPLES

Sample Information

Analyzed by	: Ssegawa
Analyzed	: 17/07/2018 00:00:49
Sample Type	: Unknown
Level #	: 1
Sample Name	: PRL -FAME 7-18
Sample ID	: PRL -FAME 7-18
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 13
Injection Volume	: 1.00
Data File	: E:\PRL\2018\July\PRL -FAME 7-18_PRL -FAME 7-18_16072018_8.qgd
Org Data File	: E:\PRL\2018\July\PRL -FAME 7-18_PRL -FAME 7-18_16072018_8.qgd
Method File	: E:\PESTICIDES\Pesticides mix method 1 Scan_SPLIT.qgm
Org Method File	: E:\PESTICIDES\Pesticides mix method 1 Scan_SPLIT.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\03_07_18_CID OFF.qgt
[Comment]	
Fatty acid profile	
Modified by	: Ssegawa
Modified	: 17/07/2018 00:25:49

Chromatogram PRL -FAME 7-18 E:\PRL\2018\July\PRL -FAME 7-18_PRL -FAME 7-18_16072018_8.qgd



Peak Report TIC

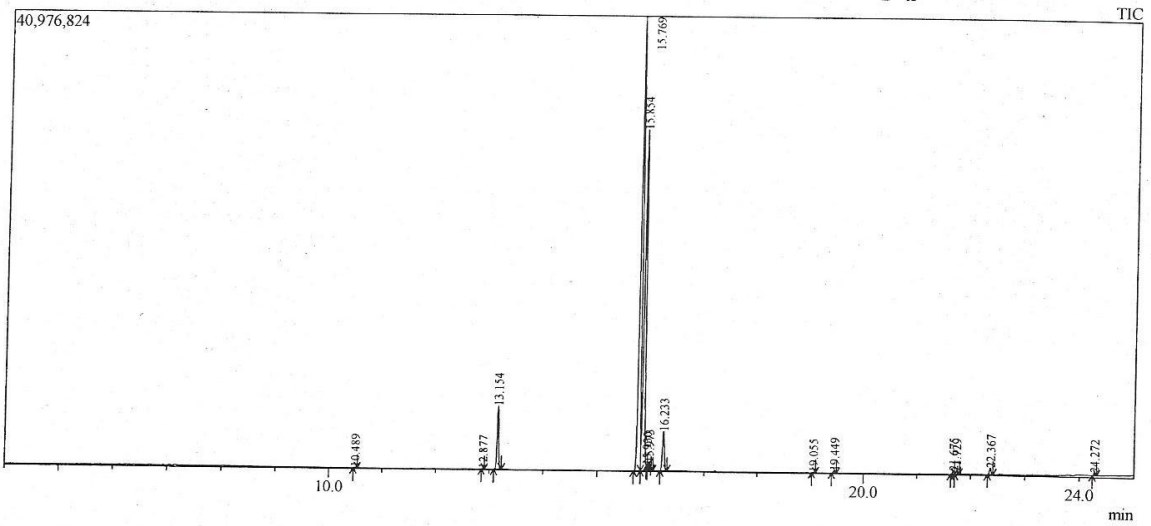
Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	10.489	10.460	10.540	103096	0.05	58304	0.07	1.77		Methyl tetradecanoate
2	12.877	12.850	12.910	77488	0.04	41961	0.05	1.85		9-Hexadecenoic acid, methyl ester, (Z)-
3	13.154	13.085	13.220	12041911	6.09	5661731	6.83	2.13		Hexadecanoic acid, methyl ester
4	15.769	15.685	15.810	98321819	49.69	40902524	49.33	2.40		Methyl 10-trans,12-cis-octadecadienoate
5	15.854	15.810	16.045	74594134	37.70	30659463	36.98	2.43	SV	9-Octadecenoic acid, methyl ester, (E)-
6	15.930	15.920	15.940	100566	0.05	91299	0.11	1.10	T	9-Octadecenoic acid (Z)-, methyl ester
7	15.973	15.940	16.015	1141343	0.58	495653	0.60	2.30	TV	Methyl 9-cis,11-trans-octadecadienoate
8	16.233	16.175	16.305	8341321	4.22	3527802	4.25	2.36		Methyl stearate
9	19.055	19.025	19.100	111871	0.06	51973	0.06	2.15		cis-Methyl 11-eicosenoate
10	19.449	19.400	19.495	395158	0.20	168338	0.20	2.35		Methyl 18-methylnonadecanoate
11	21.675	21.630	21.700	568086	0.29	258780	0.31	2.20		1,8,11-Heptadecatriene, (Z,Z)-
12	21.729	21.700	21.790	480436	0.24	196819	0.24	2.44	V	9-Octadecenoic acid (Z)-, oxiranylmethyl (
13	22.367	22.315	22.415	1175625	0.59	570807	0.69	2.06		Methyl 20-methyl-heneicosanoate
14	24.272	24.235	24.315	406128	0.21	232187	0.28	1.75		Tetracosanoic acid, methyl ester
				197858982	100.00	82917641	100.00			

Sunflower

Sample Information

Analyzed by	: Ssegawa
Analyzed	: 17/07/2018 00:00:49
Sample Type	: Unknown
Level #	: 1
Sample Name	: PRL -FAME 7-18
Sample ID	: PRL -FAME 7-18
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 13
Injection Volume	: 1.00
Data File	: E:\PRL\2018\July\PRL -FAME 7-18_PRL -FAME 7-18_16072018_8.qgd
Org Data File	: E:\PRL\2018\July\PRL -FAME 7-18_PRL -FAME 7-18_16072018_8.qgd
Method File	: E:\PESTICIDES\Pesticides mix method 1 Scan_SPLIT.qgm
Org Method File	: E:\PESTICIDES\Pesticides mix method 1 Scan_SPLIT.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\03_07_18_CID OFF.qgt
[Comment]	
Fatty acid profile	
Modified by	: Ssegawa
Modified	: 17/07/2018 00:25:49

Chromatogram PRL -FAME 7-18 E:\PRL\2018\July\PRL -FAME 7-18_PRL -FAME 7-18_16072018_8.qgd



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	10.489	10.460	10.540	103096	0.05	58304	0.07	1.77		Methyl tetradecanoate
2	12.877	12.850	12.910	77488	0.04	41961	0.05	1.85		9-Hexadecenoic acid, methyl ester, (Z)-
3	13.154	13.085	13.220	12041911	6.09	5661731	6.83	2.13		Hexadecanoic acid, methyl ester
4	15.769	15.685	15.810	98321819	49.69	40902524	49.33	2.40		Methyl 10-trans,12-cis-octadecadienoate
5	15.854	15.810	16.045	74594134	37.70	30659463	36.98	2.43	SV	9-Octadecenoic acid, methyl ester, (E)-
6	15.930	15.920	15.940	100566	0.05	91299	0.11	1.10	T	9-Octadecenoic acid (Z)-, methyl ester
7	15.973	15.940	16.015	1141343	0.58	495653	0.60	2.30	TV	Methyl 9-cis,11-trans-octadecadienoate
8	16.233	16.175	16.305	8341321	4.22	3527802	4.25	2.36		Methyl stearate
9	19.055	19.025	19.100	111871	0.06	51973	0.06	2.15		cis-Methyl 11-eicosenoate
10	19.449	19.400	19.495	395158	0.20	168338	0.20	2.35		Methyl 18-methylnonadecanoate
11	21.675	21.630	21.700	568086	0.29	258780	0.31	2.20		1,8,11-Heptadecatriene, (Z,Z)-
12	21.729	21.700	21.790	480436	0.24	196819	0.24	2.44	V	9-Octadecenoic acid (Z)-, oxiranylmethyl e
13	22.367	22.315	22.415	1175625	0.59	570807	0.69	2.06		Methyl 20-methyl-heneicosanoate
14	24.272	24.235	24.315	406128	0.21	232187	0.28	1.75		Tetracosanoic acid, methyl ester
				197858982	100.00	82917641	100.00			

Peanut

Sample Information

Analyzed by	: Ssegawa
Analyzed	: 16/07/2018 20:18:05
Sample Type	: Unknown
Level #	: 1
Sample Name	: PRL -FAME 1-18
Sample ID	: PRL -FAME 1-18
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 7
Injection Volume	: 1.00
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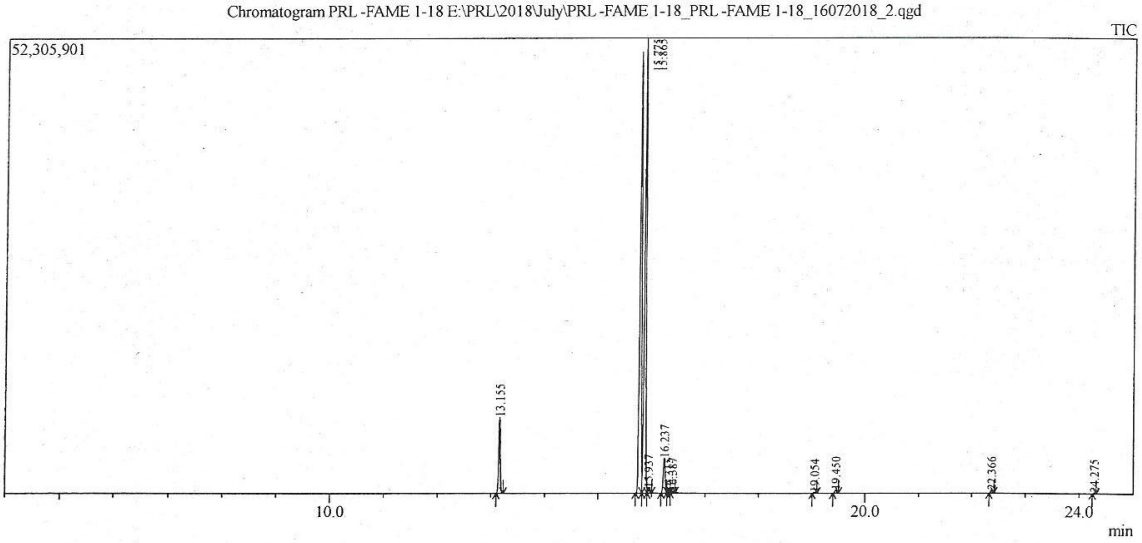
Org Data File	: E:\PRL\2018\July\PRL -FAME 1-18_PRL -FAME 1-18_16072018_2.qgd
Method File	: E:\PESTICIDES\Pesticides mix method 1 Scan_SPLIT.qgm
Org Method File	: E:\PESTICIDES\Pesticides mix method 1 Scan_SPLIT.qgm
Report File	:
Tuning File	: C:\GCMSsolution\System\Tune1\03_07_18_CID OFF.qgt
[Comment]	
Fatty acid profile	
Modified by	: Ssegawa
Modified	: 16/07/2018 20:43:05

Chromatogram PRL -FAME 1-18 E:\PRL\2018\July\PRL -FAME 1-18_PRL -FAME 1-18_16072018_2.qgd



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	13.155	13.100	13.225	17464430	6.69	8727116	7.43	2.00		Hexadecanoic acid, methyl ester
2	15.775	15.695	15.815	114461558	43.83	50696587	43.19	2.26		Methyl 10-trans,12-cis-octadecadienoate
3	15.865	15.815	15.915	115402678	44.19	52249872	44.51	2.21	V	9-Octadecenoic acid, methyl ester, (E)-
4	15.937	15.915	16.005	1342127	0.51	572140	0.49	2.35	V	11-Octadecenoic acid, methyl ester
5	16.237	16.175	16.290	9733686	3.73	3986560	3.40	2.44		Methyl stearate
6	16.315	16.290	16.350	270945	0.10	113056	0.10	2.40	V	9(E),11(E)-Conjugated linoleic acid
7	16.387	16.350	16.445	460821	0.18	171839	0.15	2.68	V	Oleic Acid
8	19.054	19.010	19.100	273615	0.10	103289	0.09	2.65		Methyl 9-eicosenoate
9	19.450	19.395	19.510	891778	0.34	346414	0.30	2.57		Eicosanoic acid, methyl ester
10	22.366	22.320	22.415	663926	0.25	314453	0.27	2.11		Methyl 20-methyl-heneicosanoate
11	24.275	24.240	24.315	167152	0.06	98750	0.08	1.69		Tetracosanoic acid, methyl ester
				261132716	100.00	117380076	100.00			

Sesame