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SEEDS BY GC-MS

BY

ODOEMELAM CHIDIEBERE VICTOR EDEMIEOBI
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DEPARTMENT OF PURE AND INDUSTRIAL CHEMISTRY
UNIVERSITY OF NIGERIA, NSUKKA

MAY, 2010
DETERMINATION OF FATTY ACID PROFILES OF OILS OF *MONODORA myristica* AND *MONODORA tenuifolia* SEEDS BY GC-MS

BY

ODOEMELAM CHIDIEBERE VICTOR EDEMIEOBI
PG/M.Sc/03/34016

A RESEARCH PROJECT SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTERS IN ANALYTICAL CHEMISTRY IN THE DEPARTMENT OF PURE AND INDUSTRIAL CHEMISTRY, FACULTY OF PHYSICAL SCIENCES, UNIVERSITY OF NIGERIA, NSUKKA.

MAY, 2010
This research project has been approved for the department of Pure and Industrial Chemistry, Faculty of Physical Sciences, University of Nigeria Nsukka.

BY

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DATE:..........................

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EXTERNAL EXAMINER
DEDICATION

To my Son; Chimdiegwu and my father; Tasie Ikeadachie Odoemelam.
ACKNOWLEDGEMENTS

“Then I returned and considered all the oppression that is done under the sun: And look! The tears of the oppressed but they have no comforter. On the side of their oppressors there is power, but they have no comforter. Therefore I praised the dead who were already dead more than the living who are still alive. Yet better than both is he who has never existed, who has not seen the evil work that is done under the sun” says the book of Ecclesiastes 4:1-3. Implicitly, the world and life therein is full of battles. Those who neither believe and accept the Holy Bible as the only True WORD of God nor accepted CHRIST JESUS as God’s only SON and Saviour of this wicked/sinful world and those who make CHRIST JESUS “a Passenger” instead of “Master” in their lives, will continue to be power-cheated and remain in perpetual spiritual slavery thus have locked the Doors of Holy heaven against themselves. Consequently, I thank and worship God for His SON: CHRIST JESUS whom without His POWER and WILL, the completion of my M.Sc programme would have been a mirage.

I am effusive in my gratitude to my supervisor, Dr. C. O. B. Okoye who understands the battles of this life and have stood by my side. God by him gave me this project assignment hence creating a spiritual forum to disavow my trust and hope in science as god!

I immensely appreciate him for his advice, guidance and constructive criticism that have goaded me into working harder. May God protect him and his family. I equally thank Dr. Ukoha and Dr. Akpanisi of Chemistry Department, UNN for their technical questions, as regards the interpretation of the mass spectra which forced me to go extra mile in finding solutions and answers.

I am greatly indebted to my true friend, Agbo Hillary of Texas A&M University, Texas USA, who supplied all the journals and through whom it was possible to carry all the GC – MS analysis in their Biological Mass Spectrometry
Laboratory. I also thank my good friends indeed, Agbo Mathias and Nnadi Joseph of STC, UNN.

I thank Emma Mbaorji of Chemistry Dept. UNN for his assistance in oil extraction especially for taking time to design the steam distillation unit used in this work.

I wish to express my praise of my father, Odoemelam Tasie Ikeadachie; a gentle lion indeed, through whom God provided almost all the finance spent in this project work.

My mother, my God – given wife and my Children; Chimdiegwu and Chinekwu even my lovely sister and brother inlaws; Ogonnaya Ijere and Colonel Arinze, I thank you all for your moral and financial encouragement. My best friend; Ifeanyi Umunnakwe, my uncle; Osita Ofo: CEO Osikandi Resources LTD cannot be forgotten for their financial and moral support. I love you all. It is forceful enthronement!

Odoemelam Chidiebere Victor Edemieobi
Dept. of Pure & Industrial Chemistry,
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May, 2010
ABSTRACT

Volatile and non-volatile seed oils of local varieties of *Monodora myristica* and *Monodora tenuifolia* were extracted from achenes of both seeds by steam distillation and soxhlet solvent extraction. Some physical parameters of the oils were determined. The percentage yield of *Monodora myristica* volatile and non-volatile oils were 0.66% and 27.68% (v/w) respectively while 7.56% was obtained for non-volatile oil of *Monodora tenuifolia* whose volatile oil could not be methodically obtained. Fatty acid composition of both seed oils were determined after formation of fatty acid methyl and butyl esters. GC-MS analysis was performed on a ThermoScientific® GC-MS instrument, with a Trace GC Ultra gas chromatograph with a vaporization method in splitless mode, connected to a ThermoScientific DSQ II mass spectrometer. The oven temperature was programmed to hold at 50°C for 2 mins, then increased at 15°C/min to 320°C, and held isothermally for 5 mins. High purity helium was used as carrier gas at 1.2 mL/min, and a Restek ® DB-5 ms (30 m x 0.25 mm, with 0.25 μM) column coated with 5% diphenyl 95% dimethyl polysiloxane was used. The ion source was held at 200°C and the transfer line at 325°C. Electron ionization in the positive mode in the range of 40-400 dalton was recorded at 70 eV with emission current of 250 uA. Six scans per second were collected. The multiplier potential was 3 kV and the source pressure was 10⁻⁵ torr. All data were recorded by computer and compounds were identified by comparison with the NISTdemo mass spectral database. Area of each peak was used for quantitative analysis. Their methyl and butyl esters confirmed the presence of large amount of linoleic acid (EFA) as well as other fatty acids (NEFAs), with *Monodora myristica* possessing higher percentage (EFA) than *Monodora tenuifolia* but the GC-MS spectra of the volatile oil revealed no trace of fatty acids but what identified were terpenes like IR-α -Pinene, β -mycrene, α -phellandrene, p-cymene, D-Limonene, β -linalool etc. There were few artifacts peaks revealed by MS, which could have mistakenly termed low molecular weight or unusual fatty acids if only GC was used, thus justifies application of the GC/EI-MS-Full Scan Mode for confirmation of results obtained by using GC alone.
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CHAPTER ONE

1.0 INTRODUCTION

Lipids are used on a daily basis in our diet, consumed either as food constituents, condiment or spices and they have a fundamental importance in keeping us in good health. From a physiological standpoint, they play a role in several biochemical membranes, vitamins, hormones, bile acids and as energy suppliers.¹

In the last several years, the correlation between lipidic intake and health has been highlighted by several works,² ³ regarding the links between cardiovascular diseases and a diet rich in saturated fatty acids [SFAs]. This has led some nutritionists to emphasize the potential benefits of the Mediterranean diet rich in vegetables, sea products and extra virgin olive oil. All these food products, contain a consistent amount of monounsaturated fatty acids [MUFAs] and “essential fatty acids” (EFAs) the “good fat or lipids” due to among other functions, their capability of hindering the formation of atheromatose plaques. The consumption of an n-3 polyunsaturated fatty acid (PUFA) rich diet has been positively linked with optimal “infant development”, cardiovascular protection, prevention of neurodegenerative diseases and behavioural disorders as well as improvement of immune defenses.

For infant formula to act as a substitute for human milk, it is important that the formula and human milk contains fairly equivalent amounts of fat. About 98% of lipids in human milk fat are triglycerides, with less than 1% each of diglycerides, free fatty acids, and sterols. Infant formula, if used to replace human breast milk, should have the necessary lipids to help form cellular membrane layers. Some of the long chain polyunsaturated fatty acids that contribute to membrane synthesis of the brain and nervous system are Linoleic and Linolenic acids.⁴ It has also been observed that skin lesions have been found to develop in infants who are fed milk-based formula of low linoleic acid content. Although data vary among different researchers, human milk fat appears to be made up of about 3.1% palmitoleic acid (C16:1), 35-36% oleic acid
(C18:1), 8-10% linoleic acid (C18:2), and 1.2% linolenic acids (C18:3). Only small
to trace amounts of C_{20}-C_{22} unsaturated fatty acids are present. Chief among non-
essential fatty acids in human milk is palmitic acid (C16:0) which is present at levels of
20-25%. The C_{4}-C_{8} fatty acids make up scarcely 1.5% of human milk fat.\(^5\)

However, these essential fatty acids cannot be synthesized in the human body
but a good number of them are found in most vegetable oils.\(^6\) A breast-feeding mother
obtains them from her “diet” and then passes them on to the newborn. A mother who
chooses not to breast feed must therefore use some type of infant formula that has the
aforementioned essential fatty acids in them.\(^7\)

For the aforementioned reasons there is a need to increase the knowledge about
fatty acids in our seed oils and Analytical chemistry can be a precious support.

During determination, fatty acids (FAs) are converted into fatty acids methyl
esters (FAMEs) and analyzed by gas chromatography/flame ionization detection (GC-
FID).\(^8\) Presently, a large number of lipid analysts use the FID to measure FAME
separated by GC. Though FID has proven to be a robust tool for FAME determination,
the lack of any selectivity can limit the usefulness of this detector when applied to
complicated samples, since only instrument response and “retention time” information
may be gathered.\(^9\)

Owing in large part to this limitation, misidentification of FAME in the
presence of contaminants, artifacts or co-eluting compounds is still of concern when
using FID\(^10\) thus much work has been done to maximize the usefulness of retention
time for FAME identification through methods such as retention time locking, retention
time prediction and the dependence of retention time on FAME equivalent chain
length.\(^11\)

However, FAME identification assigned by such methods are generally
considered tentative.\(^12\) Hence, FID analysis of complex biological extracts may prove
inadequate in some situations particularly for FAME of relatively low abundance.\(^13\)
With the coupling of MS; mass spectrometry to GC, much has been accomplished in the area of qualitative characterization of FAME mixtures. It provides a means of analyte selectively; thus, detection with MS also represents a potentially powerful fool for quantitative analysis of FAME especially in the presence of a convoluted biochemical background. Consequently, GC/electron ionization-mass spectrometry analysis in the full scan could be used for verification of results obtained by GC/FID.\(^{14}\)

1.1 **Monodora myristica and Monodora tenuifolia**

The monodoras’ are an important food items in most parts of West Africa and is mostly used as spices or condiments. *Monodora myristica* gaertn commonly called “Ehuru” in Igbo, “Abo lakoshe” in Yoruba and “Ebenoyoba” in Bini, is a large tree which grows to a height of about 24-27 meters. It is widely distributed mainly in sub-terrenean sub-tropics and tropics. They are also eaten in Uganda.\(^{15}\) The fruit is suspended on a long green stalk with numerous seeds embedded in whitish sweet smelling pulp, each oblong and pale brown when fresh, with a thin seed coat, hard kernel and contain up to 36% fixed oil.\(^{16}\) The flowering and seed production is seasonal. It bears flowers between November and May and bears fruits between August and November.

*Monodora myristica* because of its long history in African traditional medicine had spurred so many researches into its many parts and their compositions; the leaves, seed, bark, roots, volatile oils and less commonly it’s fixed (non-volatile) oils.

They are used in treatment of sores including those from guinea worm. They are equally used in treatment of constipation and as a stimulant with palm oil.\(^ {17}\) The volatile oils of *Monodora myristica* have been shown to contain predominatly monoterpen (75%).

The analysis of the seed have shown to contain protein (19.70%), fats (23.49%) and carbohydrates (34.30%).\(^ {18}\)
Saponification followed by preferential crystallization and gas chromatography of the fixed oil revealed large amounts of C18:1 and C18:2 fatty acid and traces of C14:0 and C16:1.\textsuperscript{19}

Equally \textit{Monodora tenuifolia} Benth commonly called Ehuru-ofia in Igbo, “Lakesin” in Yoruba, “Uyenghen” in Bini is an important food item in most parts of West Africa and is mostly used as spices or used as condiments in soup\textsuperscript{20} it flowers between October and May and bears fruits between April and June. It is found in tropical Africa in the northern, western and eastern part of Nigeria. It is found in the forest regions of east Indies, Malaysia and Srilanka. \textit{Monodora tenuifolia} seeds after grinding and roasting are rubbed on the skin for unspecified skin diseases.\textsuperscript{17} Oily extract has insecticidal activity and is thus applied on bruises in yam tubers.\textsuperscript{21} It is also widely used to relieve tooth ache as well as in the treatment of dysentery.\textsuperscript{22} The volatile oils of the root had been analysed to contain predominantly sesquiterpenes (33.4\%).\textsuperscript{23}

1.2 \textbf{Aim of the Study}

Traditionally these two plant seeds having been used as spices were predominantly investigated for their antimicrobial, antifungal and flavour potentials for drugs, cosmetics and foods etc. There nutritional potentials have been overlooked or better still disdainfully investigated especially as sources of essential fatty acids.

The present investigation had the following aims:

(1) To determine the fatty acid profile of the oils of these locally grown seeds.

(2) To determine their essential fatty acids.
CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Lipids, Fats and Oils

There is no satisfactory universally accepted definition of a “lipid” although most chemists and biochemists who work with these fascinating natural products have a firm intuitive understanding of the term. Most general text books describe lipids rather loosely as a chemically heterogeneous group of substances having in common the property of insolubility in water but solubility in non-polar solvents such as chloroform, hydrocarbons or alcohols. This definition encompasses steroids, terpenes, carotenoids and bile acids (a generic term in their own right!) in addition to fatty acids and glycerolipids. This concept of a lipid is unnecessarily broad, therefore a definition was proposed recently which hearkens back to the origins of the term “lipos” meaning fats. It simply puts it, “lipids are fatty acids and their derivatives and substances related biosynthetically or functionally to these compounds”. 24

Fats and oils are constructed of building blocks called “triglycerides or triacylglycerols” resulting from the combination of one unit of glycerol and three units of fatty acids. They are insoluble in water but soluble in many organic solvents. They have lower densities than water (ie float in water) and may have consistencies at ambient temperatures of solid, semi-solid or clear liquid. Depending on the percentage composition of the fatty acids component, they are “solid” appearing at a normal room temperature referred to as “fats” and “liquid” at that temperature are referred to as “oils”.

Importantly, all fats become liquid at higher temperatures. Therefore, temperature-based distinction between oils and fats is “imprecise” since the temperatures of room vary and typically, any one substance has a melting range instead of a single melting point. A fake advantage for dubious manufacturers in tropics where purely saturated fats are packaged in the name of polyunsaturated oils. Weather! Accordingly, the word fats and oils are used interchangeably.

2.2 Composition and Uses of Vegetable Oil

The main constituents of vegetable oils and animal fats are triglycerides. Triglycerides also called triacylglycerol (TAG) is a chemical compound formed from one molecule of glycerol and three fatty acids. If all three fatty acids are
identical. It is a simple triglyceride. The more common forms however are the mixed triglycerides in which two or three kinds of fatty acids are present in the molecule.

One hundred grams of the fat or oil will yield approximately 95 grams of fatty acids. Both the physical and chemical characteristics of fats and oils are influenced greatly by the kinds and proportions of the component fatty acids and the way in which these are positioned on the glycerol molecule.

Unequally, crude vegetable oil or fat contains approximately 2% of minor components. These minor components include;

(i) **Mono and Diglycerides**

These are mono and diesters of fatty acids and glycerol. They are formed in the intestinal tract as a result of the normal digestion of triglycerides. They occur naturally in minor amounts in both animal fats and vegetable oils. Oil composed mainly of diglyceride has also been used as a replacement for oil composed of triglycerides.

(ii) **Free Fatty Acids (FFAs)**

As the name suggests, they are the unattached fatty acids present in a fat or oil. Most unrefined oils contain relatively high levels of FFAs. A typical level for crude soybean oil is from 0.5 to 1.5%, crude palm oil may contain 3.0-5.0%. Refined oils and fats that are ready for use in foods usually have FFAs level of less than 0.05%.

(iii) **Phosphatides**

They are also known as phospholipids. It consists of an alcohol (usually glycerol) combined with fatty acids and a phosphate ester. Majority of the phosphatides are removed from oil during refining. Phosphatides are important source of natural emulsifiers marketed as lecithin.

(iv) **Sterols**

They are found in both animal fats and vegetable oils but there are substantial biological differences. The much dreaded cholesterol is the primary animal fat sterol and is found in vegetable oils in only trace amounts. Infact, it is widely believed scientifically that plants doesn’t contain cholesterol. Vegetable oil sterols are collectively called “phytosterols”. Stigmasterol and sitosterol are
the best known vegetable oil sterols. They have been shown to reduce both serum and LDL cholesterol. The type and amount of vegetable oil sterols vary with the source of the oil.

(v) Tocopherols and Tocorienols

They are important minor constituents of most vegetable oils and fats. They serve as antioxidant to retard rancidity and as sources of the essential nutrient: Vitamin E.

The common types are alpha, beta, gamma and delta.

They vary in anti oxidation and vitamin E activity.

(vi) Pigments

The characteristic yellow-red color of most vegetable fats and oils is principally derived from carotenoid pigment. Examples Lycopene and xanthophylls. Palm oil which is unusually high in color contain, abut 0.2% β-carotene. These pigments are readily reduced to low levels by alkali refining of the oil and adsorption bleaching.

(vii) Fatty Alcohols

Long chain fatty alcohols are of little importance in most edible fats and oils. A small amount esterified with fatty acids is present in waxes found in some vegetable oils.

Uses of Fats and Oils

Apart from food uses of fats and oils, there are many other use and applications of this commodity. In recent times technological progress have played a decisive role in increasing the value and uses of fats and oils in the edible and industrial market. Fatty acid methyl esters are the predominant esters consumed, with a year 2000 global consumption of 1 million metric tones. In addition, FAMEs, are the favoured starting material for the production of fatty amides, more complex esters, ester sulfonates and fatty alcohols. Production and consumption of the methyl esters of FA are rapidly increasing due to their growing use as biodiesel; a renewable replacement for petroleum based diesel engine fuel.

2.3 Fatty Acids

Fatty acids are long-chain molecules with a carboxyl and a methyl end. The fatty acids of plants, animals and microbial origin generally contain even
numbers of carbon atoms in straight chains, with a carboxyl group at one extremity and with double bonds of the cis configuration in specific positions in relation to this. In animal tissues, the common fatty acids vary in chain length from 14 to 22, but on occasion can span the range from 2 to 36 or even more. Individual groups of micro-organisms can contain fatty acids with 80 or more carbon atoms but higher plants usually exhibit a more limited chain length distribution. Fatty acids from animal tissues may have one to six double bonds, those from algae may have up to five, while those of the higher plants rarely have more than three. Microbial fatty acids are synthesized in some animal tissues but fatty acids with other functional groups when present have usually been taken up from the food chain. Plant and microbial fatty acids, on the other hand can contain a wide variety of functional groups including trans-double bonds, acetylenic bonds, epoxyl, hydroxyl, keto and ether groups and cyclopropene, cyclopropane and cyclopentene rings.

Saturated fatty acids are highly flexible due to relativity free rotation about the carbon to carbon single bond thus easy packing while fatty acid double bonds commonly have the cis configuration. This puts a rigid 30° bend in the hydrocarbon chain of unsaturated fatty acids that interferes with their efficient packing to fill space.

2.4 Chemistry of Fats and Oils

Fats and oils also called triglycerides are esters of the trihydric alcohol and various fatty acids.

To describe precisely the structure of a fatty acid molecule, one must give the length of the carbon chain [i.e carbon number], number of double bonds and also the exact position of these double bonds. This will define the “biological activity” of the fatty acid molecule and even of the lipid containing the fatty acid studied. Two ways are offered namely the chemist way and biochemist way.

Chemist Way

i. The carbon atoms are counted from the carboxyl group which put the emphasis on the double bond closest to this group denoted Δ [delta].

Example 18:2 Δ 9, 12 or Cis-9 Cis-12-Octadecadienoic acid
ii. The number to the left of the colon indicates “the number of carbons in the fatty acid”.

iii. The number immediately to the right of the colon indicates the number of the double bonds in the fatty acid”.

iv. The number following the delta sign indicates the location of the double bonds counting from the carbon at the carboxyl end of the fatty acid which the designated as to number one carbon. If the number after the colon is 0, the fatty acid has no double bond and is saturated.

Thus 18:2 Δ9, 12 is a fatty acid of 18 carbons with two double bond that are found between the 9th and 10th carbons and between the 12th and 13th carbons in the fatty acid molecule. Its familiar or trivial name is linoleic acid or linoleate. The double bonds have usually a Z (Cis) configuration but can have also E(trans) configuration as illustration below:

\[
\text{CH}_3(\text{CH}_2)_4 \text{CH}^\text{Z} \text{CHCH}_2\text{CH}^\text{Z} \text{CH(CH}_2)_7 \text{COOH} \\
\text{Linoleic acid, 18:2 (9z, 12z)}
\]

**Biochemist Way**

Because of increasing awareness of the importance of unsaturated fats especially EFAs, this system of numbering is frequently used in nutrition and in public. It was agreed upon by the international commission on Biochemical Nomenclature because of its interest in describing the fatty acid metabolism.

The double bonds are counted from the methyl group determining the metabolic family noted by Greek last alphabet; Omega (ω) or n-x.n group of a particular fatty acid is determined by the number of carbons between the methyl end of the molecule and the nearest double bond.
Illustration:

\[
\text{CH}_3(\text{CH}_2)_4 \text{CH} = \text{CHCH}_2 \text{CH} = \text{CH} (\text{CH}_2)_7 \text{ COOH}
\]

\[
18:2 \ (n - 6)
\]

Linoleic acid thus has 18 carbon atoms, 2 double bond and 6 carbon atoms from the last double to the terminal methyl group.

\[
n = \text{total number of carbon}
\]

\[
x = \text{position of the distal double bond}
\]

To deduce the other double bonds: Just add 3 carbons to the right.

Most fatty acids naturally are non – conjugated in nature.

so 18:2(n-6) = 18-6 = 12, 12-3 = 9 hence Δ9,12.

2.5 Classification of Fatty Acids

Fat has a bad reputation. It is a word associated with Obesity, heart diseases and many other diseases state. This have made the whole issue of fat in the diet very confusing mainly because there are so many different types of fat. However, not all fats are bad especially those from vegetable origin. There are two broad categories of fatty acids: namely saturated and unsaturated fatty acids.

Recall that fatty acids are the building block of fats and oil (i.e triglycerides), thus fats and oil are fatty acids. Importantly, for the purpose of Nutrition and fat metabolism, fatty acids are classified into Essential and Non – essential fatty acids.

2.5.1 Essential Fatty Acids [EFAs]

Nutrition is an integral component of our daily life routine and has the potential to determine our life and death. The human body can manufacture most of the fats it needs including cholesterol, saturated fatty acids and unsaturated fatty acids but there are two class of fatty acids which cannot be manufactured in the body and are very essential for the life and growth and which must be obtained in diet. They are called
Essential fatty acids (EFAs). Linolenic acid or the n-3 family does not only maintain normal skin conditions in babies. It is also essential for sexual reproduction. Good nutrition sustains the physical life to a limit.

FAO slogan today, “Food for All”, if food the hope of the world sustains life to a limit. Then the hope of the world is a hopeless hope. No wonder Alexander the great, it was told “went down to Ethiopia in search of food that gives Eternal life on perceiving that Ethiopian lives longer life span than any other people as of that time. But after seeing the princess of Sudan, who took him down to a cave to see what could make him not to die. He came out disappointed and later died”.

Where is the food that can keep us forever? It has entered into man’s conscience that there is ETERNAL LIFE but the way, how and where has eluded the falling man and science. Science thought that oxygen gives eternal life yet people died even in a pool of oxygen. Science has blamed it on environmental pollution among other factors. Reincarnation as taught by some can not even give Eternal Life. Convincingly, yet true, He said “if anyone keeps my WORD, he shall never taste death”.

Arguably, EFAs support the cardiovascular, reproductive, immune and nervous systems. The human body needs EFAs to manufacture and repair cell membranes, enabling the cells to obtain “optimum nutrition and expel harmful waste products. A primary function of EFAs is the production of prostaglandins: which regulate body functions such as heart rate, blood pressure, blood clothing, fertility, conception, and play a role in immune function by regulating inflammation and encouraging the body to fight infections. EFAs are also needed for proper growth in children particularly for neural development and maturation of sensory systems with male children having higher needs than females. Foetuses and breast-fed infants also require an adequate supply of EFAs through their mother’s dietary intakes. Thus we are what we eat!
Importantly all EFAs are polyunsaturated fatty acids (PUFAs) but all PUFAs are not EFAs. We have two main families of EFAs n-3 family (Linolenic) and n-6 (Linoleic) family.

**n-3 (Linolenic acid) Family**

The enzymes in animals are only able to insert new double bonds between an existing double bond and the carboxyl group but the enzymes in plant tissues are capable of inserting a double bond in the terminal region of an existing unsaturated fatty acid. Linolenic acid 18:3(n-3) or Cis-9, Cis-12, Cis-15-Octadecatrienoic acid is the end point of biosynthesis in most higher plants.

Linolenic acid occurs in linseed oil (about 60%) and is the major fatty acid of green leaves. It is also found in several other highly unsaturated oils like flaxseed oil (has the highest linolenic content of any food), flaxseeds, flaxseed meal, hempseed oil, hempseeds, walnuts pumpkin seeds, brazil nuts, sesame seeds, sesame oil [queen of oils] arocas, canola oil (cold pressed and unrefined) soyabean oil, wheat germ oil,
salmon, mackerel, sardines, anchovies, albacore tuna etc. unripe flaxseeds contain a natural form of cyanide so must be harvested when ripe.

**n-6 (Linoleic acid) Family**

Linoleic acid is the parent of other members of n-6 family. They include gamma linolenic acid (GLA), Dihomo-Gamma-linolenic acid (DGLA), Arachidonic acid (AA), etc. A healthy human with good nutrition will convert linoleic acid into GLA.\(^3\)

Though most people can obtain an excess of linoleic acid often it is not converted to GLA because of metabolic problems caused by diets rich in alcohol, or trans fats from processed foods, as well as smoking, pollution, stress, aging, viral infection and other illnesses such as diabetes.\(^4\)

Linoleic acid when consumed the enzyme convert it either by chain elongation and/or desaturation into other members of the family. It is normally found in foods like flaxseed oil, flaxseeds, flaxseed meal, hempseed oil, hempseeds, grape seed oil, pumpkin seeds, pine nuts, pistachio nuts, sunflower seeds, olive oil, olives, borage oil, evening primrose, black current seed oil, chestnut oil, corn oil, safflower oil, soyabean oil, cotton seed oil, chicken etc.

Animals must be fed with feeds that contain EFA before one can get appreciable amount of EFAs from their meat. It has been found that cattle and poultry feed with increased EFA will produce meat and eggs higher in EFA.
PRODUCTION OF ESSENTIAL FATTY ACID DERIVATIVES

20-carbon (eicosanoid) fatty acids shown in bold

OMEGA-6 FAMILY

Diet

Linoleic Acid

Δ-6-desaturase

Gamma-Linolenic Acid (GLA)

Δ-6-desaturase blocked by alcohol and in diabetes

Dihomo-Gamma-Linolenic Acid (DGLA)

Δ-5-desaturase

Prostaglandins & Thromboxanes

(series 1)

Arachidonic Acid

Lipoxygenase

Cyclo-oxygenase

Leucotrienes & Lipoxins (series 4)

Prostaglandins & Thromboxanes (series 2)

EicosaPentanoic Acid (EPA)

Lipoxygenase

Cyclo-oxygenase

leucotrienes (series 5)

Prostaglandins & Thromboxanes (series 3)

DocasaHexaenoic Acid (DHA)

Δ-4-desaturase

OMEGA-3 FAMILY

Alpha-Linolenic Acid

Δ-6-desaturase

Prostaglandins & Thromboxanes

Cyclo-oxygenase

Δ-5-desaturase

Figure 1: Illustration for Production of EFA and Products Derivatives
2.5.2 Non-Essential Fatty Acids [NEFAs]

Primordially all that God created was good. The non-essential fatty acids are essential but technically are not EFAs because the body can manufacture a good amount provided EFAs are present. When a particular diet or food is avoided or modified to deal with one nutritional problem, new nutritional problems arises, so NEFAs has its own role but for the fact that deficient of them normally doesn’t cause any disorder when not included in our diet means that it is not essential in the diet. Hence it was advised prudently to reduce fat intake of non essential fats to as low a quantity as possible, so long as absorption of oil-soluble vitamins is not impaired.\(^{35}\) Rats reared on diets deficient in EFA ceased to gain weight at approximately 3 months, became infertile, developed dermal lesions, and have an increased water uptake compared to roots reared on normal diets. So fatty acids that relieve the symptoms produced in animals reared on a fat-free diet are said to have “EFA activity”. So NEFAs do not have EFA activity and consuming it will amount to “excess fat”. In fact they are major source of excess body fat.

They include:

(i) n:0 (saturated) family. It includes all Saturated Fatty Acids (SFAs).

(ii) n-9 (oleic) family.

2.6 Metabolism of Fats and Oils

Fats and oils are too big to pass from the digestive tract to the blood. Therefore they must be broken down to their constituents fatty acids and glycerol. Some monoglycerides can be absorbed intact.

In the intestine, triglycerides are split into glycerol and fatty acids in a process called “Lipolysis” with the help of Lipases; (an enzyme) and bile secretions which can then move into blood vessels. The triglyceride are rebuilt in the blood from their fragments and become constituents of lipoprotein which deliver the fatty acids to and from fat cells among other functions. Various tissues can release the free fatty acids and
take them up as a source of energy. When the body requires fatty acids as an energy
source, the hormone, glucagon signals the breakdown of the triglycerides by hormone-
sensitive lipase to release free fatty acids. All lipoproteins have triglycerides,
phospholipids, cholesterol and protein. Interference with the metabolism of EFAs by
saturated fats, cholesterol and transfats, glucose, insulin deficiency, viruses, alcohol,
and ageing reduces the formation of GLA, DGLA, AA, EPA, and DHA and their
beneficial metabolites.\textsuperscript{36}

2.6.1 Cholesterol

\begin{center}
\includegraphics[width=0.5\textwidth]{cholesterol.png}
\end{center}

This entity or rather compound is the most dreaded among all fats and oils
family. Because of its connection with oil, it is widely thought though ignorantly that
all fats and oils are bad yet people eat and dine with it every day in their diet but in a
disquized form.

Our body needs fats to function properly even cholesterol. Nearly half of the dry
weight of the brain is fat and a quarter of this is cholesterol. Cholesterol is an essential
part of sex hormones, bile acids, vitamin D and steroid hormones. Just like NEFAs,
cholesterol need not be included in our diet.

Most importantly, the liver and other tissues can manufacture cholesterol from
saturated fatty acids. Ignorantly, most people avoid cholesterol in their diet yet
consume more saturated fats (fatty acids) in the form of the so called vegetable oil
which in turn are converted to cholesterol by the liver. Yet our so called manufacturers
and media will only half truth the issue by labeling the vegetable oil or fat “No
cholesterol” when the oil itself is completely saturated fat. Consequently, high saturated fats/or oil (fatty acid) results in excessively “high blood of levels of cholesterol that can end up being deposited in atherosclerotic plagues on blood vessels leading to cardiovascular disease.

High blood cholesterol depresses the immune system and thereby increases the incidence of cancer. 37

2.7 Reactions of Fats and Oils

The most important chemical reactions of fats and oils occur at the points of unsaturation on the fatty acids chain and the point where the fatty acid are attached to the glycerol molecule (ester linkage).

2.7.1 Hydrolysis

This is the most important reaction of the fats and oil to the producers of fats and oils, since this lowers the quality of the oil and hence market price. Like other esters, glycerides on partial hydrolysis will yield mono and diglycerides and free fatty acids but when the reaction is carried to completion with water in the presence of acid catalyst, the mono-, di- and triglycerides will undergo hydrolysis to yield glycerol and free fatty acids (FFAs).

In the digestive tracts of humans and animals and in bacteria, fats and oils are hydrolyzed by enzymes. Any residues of these enzymes present in some edible oils sources are deactivated by the elevated temperature normally used in oil processing.

\[ \text{Oil} + 3 \text{H}_2\text{O} \rightarrow \text{Glycerol} + 3 \text{Fatty acid} \]

With aqueous NaOH or KOH (strong base), glycerol and salt of the component fatty acids (Soap) are obtained.
2.7.2 Hydrogenation

This is the process of adding or inserting hydrogen with the aid of a metal catalyst to an unsaturated bond of a triglyceride.

\[
R\text{C}═\text{C}—\text{C}═\text{O} \rightarrow \text{R}\text{C}═\text{C}—\text{C}═\text{OH} + \text{H}_2
\]

Commercially, hydrogenation is used to add more hydrogen to natural unsaturated fats and oils to decrease the number of double bonds and retard or eliminate the potential for rancidity. Hydrogenation is the traditional way of hardening oils for the production of shortenings, margarines, confectionaries, soaps, fats and others. The high temperatures and catalysts used for this chemical reaction weaken the double bonds and as a side effect, cause a large percentage of the natural cis double bonds to change to “Trans double bonds”. So hydrogenation destroys EFAs. Researchers have reported that people who ate partially hydrogenated oils or fats, which are high in trans fats or oils, worsened their blood lipid profiles and had nearly twice the risk of heart attacks compared with those who did not consume hydrogenated oils. Trans fatty acids that are incorporated into cell membranes create denser membranes that alter the normal functions of the cell.

2.7.3 Oxidation

When oils and fats are exposed to air, little change takes place for a period of time that varies from oil to oil depending upon the amount and type of unsaturation and the content of natural antioxidants. It is generally accepted that the major part of oxidation proceed through “free-radical” chain formation. Once the free radical is produced the high reactivity of the radicals with oxygen causes a rapid conversion to a peroxide or hydroperoxide.
It is known that the three main unsaturated fatty acids are oxidized at different rates viz linoleic acid oxidized 64 times faster than oleic acid and linolenic acid is oxidized 100 times faster than oleic acid. At room temperature, this reaction is induced by air (autoxidation) and slowly though to a limited degree form hydroperoxides which may break into secondary oxidation products like hydrocarbons, ketones, aldehydes and smaller amounts of epoxides and alcohols. Some of these products possess the purgent, disagreeable odours characteristics of Rancid Fats.

At high temperatures like during frying, this reaction proceeds faster. The extent of it depends on the frying conditions principally the temperature, aeration and duration. Frying condition do not however saturate the unsaturated fatty acids, although the ratio of saturated to unsaturated fatty acids will change due to degradation and polymerization of the unsaturated fatty acids. Oxidation and consequent rancidity does not however proceed so fast in vegetable oils as in animal fats, owing to the presence of naturally occurring protective materials called “antioxidants”. High rates of lung cancer
among women in China have been associated with lipid peroxidized oils in fumes from cooking polyunsaturated vegetable oils in a wok. Hot oil in open air is subject to much lipid peroxidation, thus our fast-food restaurants that fry foods in the same oil all day serve lots of lipid peroxides to their customers.

### 2.7.4 Polymerization

All commonly fats and oils particularly those higher in polyunsaturated fatty acids tends to form large molecules known broadly as polymers when heated under extreme conditions of temperature and time (thermal-induced condensation). If polymerization is allowed to proceed to extremes, it can result in foaming of the fats and oils when an appreciable amount of polymer is present, there is a marked increase in viscosity.

### 2.7.5 Esterification

In its simplest form, esterification may be considered to be the reverse of hydrolysis. It is the combining or recombination of FFAs with glycerol to form glycerides. Monoglycerides and diglycerides may also be produced by esterification. Fats and oils also react with ethylene or propylene oxide, acetylene or vinylacetate, monohydric alcohols. The principal monohydric alcohols used are methyl, ethyl, propyl, butyl and isobutyl alcohols.

### 2.7.6 Halogenation

The halogen include chlorine, bromine, iodine. They can readily add to the double bonds of unsaturated fatty acid.
This reaction is used as a measure of the proportion of unsaturated constituents present in a fat or oil and the quantity of halogen absorbed is expressed in terms of iodine.

2.8 Physical Properties

2.8.1 Solubility and Miscibility

Fats and oils are freely miscible with most organic solvents including ether, benzene, carbon tetrachloride, chloroform except alcohols at temperatures above their meeting points. However as a result of predominance of non-polar groups, they are insoluble in water. At temperatures far below their melting points, fats are very slightly soluble in solvents. Generally there is a linear relationship between solubility and temperature. Normally fatty acids exhibit appreciable solubility in water compared to the corresponding hydrocarbons due to the presence of the polar carboxyl group up to 6 or 4 carbon atoms, organic acid are considered “short-chain” is substantially soluble in water but those with more than 10 carbon atoms are practically insoluble in water.

However fats and oil can be emulsified in water especially on addition of colloids or emulsifiers e.g lecithin e.g milk is an oil-in-water emulsion.

2.8.2 Heat of Combustion

The heats of combustion of the saturated fatty acids increase with increase in the chain length of the acids and vary from about 5900 cal/g for butyric acid to about 8900 for lauric, 9600 for stearic. Triglycerides are substantially the same heats of combustion as the fatty acids of which they are composed.

Treybal has given the following formular for calculating the approximate heat of combustion of fatty acids, in gram-cal/gram in terms of constant volume at 15ºc.

\[
\Delta H_c = 11,380 - (\text{IV}) - 9.15(\text{S.V})
\]

I.V = iodine value

S.V = saponification value
A value of 9500 gram-cal/gram is ordinarily taken for common edible fats. Such as lard and cotton seed oil.

2.8.3 Melting and Solidification Points

The melting point of fats is the temperature at which it completely liquidifies or solidifies. Glycerides have several polymorphic forms with different melting or transition points. In general, triglycerides reflect the melting points of their constituent fatty acids. The melting point of fatty acids vary with the type of fatty acids, increasing with chain length and decreasing with degree of unsaturation. Thus, a low degree of unsaturation, a high molecular weight, and the presence of trans rather than cis isomers of unsaturated fatty acids, all contribute to a relatively high melting point.

2.8.4 Density/Specific Gravity

The specific gravity of an oil is defined as the ratio of the apparent mass determined by weight in air of a given volume of oil at t°c to that of the same volume of water at 20°C while apparent density at t°c is the apparent mass in grams, determined by weighing in air of 1ml of the oil at t°c. There are no great differences in the densities or specific gravities of different fats and oils in the liquid state, although both the degree of unsaturation (as measured by the iodine value) and the average molecular weight (as measured by the saponification value) affect this properties slightly.

The relationship between iodine value, saponification value and density as correlated by Lund is show:

\[ d = 0.8467 + 0.0003 (S.V) + 0.00014(I.V). \]

Because of the unusual structure of their fatty acids, conjugated fatty acid oils and hydroxy containing oils do not conform to this relationship. Specific gravity is usually measured at 15°c in the tropical regions, this temperature is not easily obtain and where not possible a correction factor is used as shown below.

Specific gravity at 15°c = (t°c-15°c) x 0.000069. specific gravities of oils vary with a narrow range of 0.91 to 1.00.
2.8.5 Specific Heat and Heat of Fusion

The specific heat of liquid fats increases slightly with decrease in the iodine value and varies almost linearly with the temperature. The heat of fusion is a function of the compactness of the fat crystals being higher for fats of higher molecular weight than those of low molecular weight.

Higher for simple than for mixed saturated triglycerides and higher for saturated than for unsaturated glycerides.

2.8.6 Viscosity

Viscosity is a measure of internal friction in molecule. Oils owe their relatively high viscosities to the intermolecular attractions of the long chain of their glycerides molecules. In general, the viscosity of oils decreases slightly with increase in unsaturation, thus viscosity is increased slightly by hydrogenation. Oils containing fatty acids of low molecular weight are slightly less viscous than oils of an equivalent degree of unsaturation containing only high molecular weight acids. The viscosity of oil accesses its property as a fuel or lubricant than for analytical purposes polymerization increases markedly the viscosity of oil and an increase in temperature reduces viscosity. A hot liquid thus has less internal friction, owing to greater mobility and distance between the molecules and will flow more readily than when cold.\(^\text{42}\)

2.8.7 Refractive Index

The refractive index of an ordinary fat is dependent upon its average molecular weight (and to a minor degree upon its glycerides structure), as well as its degree of unsaturation. It is useful for estimating the iodine value because of the ease and rapidity with which it can be determined and because there is little variation in the average molecular weight among many common fats. Determinations of the refractive index are particularly useful the control of hydrogenation. Refractive index is easily determined for small samples usually at 20°C for oil and 40°C for solids (fats). A correction factor can be included where it is not possible to work at the stipulated temperature.
Refractive index = R + 0.00380Ø

Where R = refractive reading

Ø = number of degree in centigrade by which the measurement temperature is above the specific temperature. 

2.8.8 Crystal Formation

Most natural fats contain a great diversity of high and low melting glycerides which melt or solidify over a wide range of temperatures. Limited cooling of a liquid fat results in the deposition of the higher melting glycerides in the form of needle like crustals which interlace and cohere to form a plastic material with the lower melting liquid glycerides. The plasticity of fat which thus changes progressively with temperature is a highly important property of many industrial fat products.

To a large degree, the size of the crystals and the plasticity are dependent upon the rate of cooling, with the smallest crystals being produced by rapid cooling. Pure triglycerides are polymorphic that is, they are capable of existing in more than one crystal form, each of which has a distinctive melting point, density, heat of fusion. Polymorphism is not ordinarily observed in the complex glyceride mixtures comprising most commercial fats.

2.8.9 Smoke Fire and Flash Points

The smoke, fire and flash points of fat are determined primarily by its content of free fatty acids. These are measure of its thermal stability when heated in contact with the air.

The smoke point is the temperature at which smoking is detected in the laboratory apparatus protected by drafts and provided with special illumination.

The flash point is the temperature at which an oil sample, when heated under prescribed conditions, will flash when flame is passed over the surface of the oil but not maintain ignition.
The fire point is the temperature at which an oil sample, when heated under prescribed conditions, will ignite for a period of at least five seconds (spontaneous combustion).

2.9 Characterization of Fats and Oils

CHEMICAL PROPERTIES

2.9.1 Acid/FFA Value

This is a measure of the amount of free fatty acid present in a fat. Some of the deterioration that take place during storage of either the raw material from which the fat is obtained, or in the fat itself after isolation, results in hydrolysis of the triglycerides to yield free fatty acids (FFAs). So it is expressed in terms of percentage FFA provided mineral acid and other non-fatty acids are absent. It is often calculated as lauric acid for coconut and palm kernel oils and as oleic for most other oils. It is the number of milligrams of potassium (or sodium) hydroxide required to neutralize the FFA in 1 gram of fat. The relationship between acid value and % FFA calculated as oleic acid is

1 unit of acid value = 0.503% FFA

Free fatty acid content of oils varies with the source of oil and level of refining. It can occur in refined oils at about 0.1% (w/w) and typically between 5-15% in crude oils.44

2.9.2 Iodine Value (I.V)

It is a measure of the proportion of unsaturated fatty acids present. There is no iodine present in oils and fats but the test measure the amount of iodine which can be absorbed by the unsaturated fatty acids. As the concentration and types of unsaturated fatty acids present in the fat are fairly constant, the iodine value will give a figure for the total degree of unsaturation, expressed as the percentage of iodine absorbed by the fat. However, not all unsaturated bonds are the same in reactivity. Conjugated double bonds react more slowly than non-conjugated double bonds. Saturated fatty acids with no double bond at the fatty acid chain have iodine value of zero.41 Iodine value only
tells the total unsaturation not specific unsaturated fatty acid. The higher the iodine value, the greater is the unsaturation in the substance. Thus it is used to classify oil. Oils with I.V around 180-190 like linseed oil and tung oil are known as drying oils. Oils with I.V around 100-120 are semi-drying while oils with less than 100 is known as non-drying oil.. In practice, iodine value is determined by using iodine chloride or iodine bromide as a reagent rather than free iodine.

2.9.3 Saponification Value (S.V)

Saponification value is the number of milligrams of potassium hydroxide required to neutralize the fatty acids liberated on the complete hydrolysis or saponification of 1g of the fat or oil. It is a measure of the mean molecular weight of the fatty acids present in the fat. The process of saponification is the hydrolysis of triglycerides into glycerol and the potassium salt of the fatty acids, using a solution of potassium hydroxide in alcohol. This process measures the amount of alkali which is required to combine with the fatty acids liberated by the hydrolysis of the fat, and from this, the equivalent weight of the fatty acids can be determined thus its value or information on the fatty acid composition may be neglected when Gas-Chromatography-MS is applied.

2.9.4 Peroxide Value (P.V)

Peroxide value is a determination of the ability of compounds produced by oxidation to liberate iodine from potassium iodide in glacial acetic acid and is expressed in terms of millimoles of peroxide or milliequivalents of oxygen per 1000 grams of fat.

As oxidation takes place, the double bonds in the unsaturated fatty acids are attacked forming peroxides. These break down to produce secondary oxidation products which indicate rancidity. The P.V can therefore be used to estimate oxidation or deterioration (Rancidity) of fats but as the compound formed is unstable and oxidation proceeds further, it is not a complete measure of oxidation.
2.9.5 **Anisidine Value (An.V)**

The peroxides in an oxidized oil are of course transitory intermediates, which decompose into various carbonyl and other compounds. This composition is accelerated as the temperature is raised thus reducing the peroxide and increasing the secondary products. Hence a badly oxidized oil may be processed to give a product with a low P.V and acceptable but perhaps inferior flavour. Therefore An.V estimates the levels of the secondary products especially aldehydes (2-alkenals) present in the oil. An.V is defined as 100 times the absorbance of a solution resulting from the reaction of 1g of fat or oil in 100ml of a mixture of solvent and p-anisidine, measured at 350nm in a 10-mm cell under the conditions of the test.

2.9.6 **Totox Value**

Totox value simply means total oxidation value. It combines the Anisidine value and peroxide value.

\[ \text{Totox value} = 2\text{PV} + \text{An.V}. \]

It had been experimented that 1PV unit decomposed to give an increase of 2An.V units. Equally peroxides have two oxygens per molecule while aldehydes have only one. Thus Totox value is often considered useful in estimating rancidity, since it combines evidences from An.V and PV.

However, totox value drops when an oil is refined, owing to simultaneous reductions in both PV and AV. Infact the totox value should fall by about half during both bleaching and deodorization.

2.9.7 **Reichert-Meissl Value**

It is expressed as the number of milliliters of 0.1N alkali required to neutralize the water soluble volatile fatty acids (largely caproic, butyric and caprylic) obtained from 5g of the fat or oil by a specified method of saponification, distillation and
acidification. It is used to determine the quantity of low molecule weight acids present as esters in a substance. The acids concerned are lauric (C12:0), Capric (C10.0), caprylic (C8:0), caproic (C6:0), and butyric (C4:0).

The larger the Reichart-meissl value, the greater the proportion of low-molecular weight acids present as esters in the sample.

2.9.8 Unsaponifiable Matter

It is a measurement of the water insoluble components produced after heating the fat with potassium hydroxide (Saponification) to yield glycerol and potassium salts of the fatty acids. Both of these products are water soluble and the insoluble hydrocarbons, sterols, and fatty alcohols can be extracted from the aqueous solution and their proportion measured. The amount of unsaponifiable matter found in edible fats is usually small and high figures may indicate contamination or adulteration. For most oils the value is as low as 0.2% but can be as high as 17% for some.

2.9.9 Ester Value

This is the number of milligrams of alkali (NaOH or KOH) required to saponify the ester contained in 1 g of oil or fat.

Ester value (E.V) = SV-AV

Where SV = Saponification value

AV = Acid value

2.10 Processing of Fats and Oils

Foods fats and oils are derived from oilseed and animal sources. Animal fats are generally heat rendered from animal tissues to separate them from protein and other naturally occurring materials. Rendering may be accomplished with either dry heat or steam.

Vegetable oils are obtained by the extraction or the expression of the oil from the oilseed.
The first step in oilseed processing is to de-shell or de-hull and crush or mill the oilseed and then separate the flakes by extraction into its crude oil and meal components. It is also very important to dry the de-shell seed either in oven or sun.

Extraction methods of oil is largely chosen depending on the oil content and nature of the oil and seed. We have:

- Mechanical Expression/pressing (cold or hot)
- Solvent extraction
- Steam distillation
- Super critical carbon dioxide.

**Steam distillation** is a method for extracting or distilling oils which are volatile and heat sensitive. This process involves using bubbling steam through a heated mixture of the de-hulled and crush seeds. The vapour mixture is cooled and condensed usually yielding a layer of oil and water. This kind of oils is called “essential or volatile oils”. They are not to be confused with essential fatty acids (EFAs). In fact essential oils are responsible for odour rather than taste of the plant material because of its volatile constituents. Terpenes are by far the most dominant constituents of essential oils. They do not consist of glyceryl esters of fatty acids. Hence do not leave a permanent grease spot on paper and cannot be saponified with alkalies. Volatile oils do not become rancid as the fixed oils (i.e non-volatile oils) but instead, on exposure to light and air, they will oxidized and resinify.

However, most volatile oils contain trace amounts of various free acids. This free fatty acids increases with the age of the oil due to oxidation of other constituents or hydolysis of certain esters.

**Supercritical carbon dioxide** is the most recent method of extraction especially volatile oils. Instead of steam, supercritical CO₂ is used as solvent. The CO₂ is generated into the plant material or seed (crushed). It is the best method for fine powders (i.e finely crushed seeds) that refuses steam distillation. Infact where steam
failed, supercritical CO$_2$ thrives. It also has among other things the advantages of low
temperature operating conditions which prevents the decomposition and denaturing of
oils and provides for a superior volatile oils. When the extraction is complete, the
pressure is reduced to ambient and the carbondioxide reverts back to a gas, leaving no residue.

**Solvent extraction** is the commonest and most economical for extraction of
oils. Solvent extraction is employed for materials with low oil contents. This method
has largely replaced cold or hot expression due to its high percentage oil yield but cold
pressed is the best method that gives a nutritious oils. In this process, the oil is extracted
from the oil seed by hexane and the hexane is then separated from the oil, recovered
and reused. Because of its high volatility, hexane does not remain in the finished oil
after processing.

Hence, in selecting a solvent for extraction the solubility of the fats and oil in
the solvent, solvent, solvent toxicity cost and intended use of the oil are of utmost
important.

The fats and oils obtained directly from solvent extraction of the oilseed are
termied “crude fats or oils”. This contains varying but relatively small amounts of
naturally occurring non-glyeride components or material that are removed through a
series of processing steps.

This non-glycerides components includes protein, free fatty acids phosphatides,
vitamins, water etc. most importantly, not all of the non-glycerides are undesirable
elements. Tocopherols for example, perform the important function of protecting the
oils from oxidation and provide vitamin E. Ideally, processing is carried out in such a
way as to control retention of some of these important non-glycerides. These processes
includes viz; degumming, Refining, bleaching deodourization, fractionation and
winterization, partial hydrogenation/hydrogenation etc.
2.10.1 Degumming

Crude oils having relatively high levels of phosphatides may be degummed prior to refining to remove the majority of those phospholipid compounds. These gums are good emulsifying agents and if left in the oil could give rise to serious losses of oil. The process generally involves treating the crude oil with a limited amount of water to hydrate the phospholipids and make them separable by centrifugation. This phospholipids in developed countries are often recovered and further processed to yield a variety of lecithin products.

In a batch process, crude oil is pumped into a neutralizing tank or vessel comprising a large vessel cylinder with a cone shaped bottom fitted with an agitator and heating coils. Steam heating is used in the coils to cause the oil temperature to increase to about 95°C. Hot water is then sprayed into the oil while is being agitated. Because of the affinity of phosphatides for water, the water hydrates the phosphatides and gums to form a material of higher specific gravity than that of the oil. When water (about 4-5%) has been added, the agitator is stopped and the mass allowed to come to rest and settle. If the operation has been performed correctly, the hydrated gums will settle at the bottom of the vessel and can then be run-off. The settling process is sometimes enhanced by scattering salt on the surface of the oil in order to speed split emulsions and increase the specific gravity of the aqueous phase. 48

In some processes, instead of adding water, concentrated phosphoric acid is added which effectively coagulated protein matter and precipitate the dispersed phosphatides. The resulting fats or oils is either centrifuged or filtered immediately or passed direly to neutralizing or refining stage. Animal fats do not contain appreciable quantities of gums and phosphatides especially when compared with the amount present in vegetable oils. Thus it is often assumed that animal fat need not degumming.

A relative new process in the United States of America is enzymatic degumming. An enzyme phospholipase converts phospholipids present in crude oil into
lysophospholipids that can be removed by centrifugation. In this process, crude oil, pre-heated with a combination of sodium hydroxide and citric acid is mixed with water and enzymes by a high shear mixer, creating a very soluble emulsion.

The emulsion allows the enzyme to react with the phospholipids transforming them into water soluble lysophospholipids. This emulsion is broken by centrifugation, separating the gum and phospholipids from the oil. This process generates a better oil yield than traditional degumming/refining.

### 2.10.2 Refining/Neutralization

Neutralization is the second stage in oil refining and is known as alkali refining. The main objective is to remove the free fatty acids (FFAs) which have developed in the oil. The selection of type and strength of alkali will influence the efficiency of neutralization and the ability of the process to accomplish other desirable targets such as removal of pigment and residual amounts of phosphatides and gums not removed by degumming.

Caustic soda and soda ash are the two main agents used in the neutralization process. Hence the name “alkali refining”. Caustic soda is widely used as it gives not only the maximum reduction in FFA but also is effective in making some of the pigments soluble in the aqueous phase. The concentration, temperature of operating condition and duration of the treatment varies from oil to oil and is designed to achieve a balance between maximum refining and minimum oil loss.

Unfortunately, the treatment with caustic soda in order to remove free fatty acids is not selective, not only does that caustic soda react with the FFAs but it also attack the neutral glycerides, splitting them to produce soap and glycerol.

Because of these limitations in soda methods which certainly brings a huge financial loss and reduction in EFAs, other methods which are mainly physical have been employed viz steam refining, solvent refining etc.
Steam refining in theory will only remove FFA though there will be destruction of carotenoid pigments which are heat labile. Other non-desirable like the phosphatides and gum are coagulated by the heat and steam to form particles of solid matter in the oil. However, there is equally some hydrolysis of neutral oil so that the FFA cannot be reduced to zero.

2.10.3 Bleaching

The term bleaching refers to the process for removing colour producing substances and for further purifying the fat or oil. Normally, bleaching is accomplished after the oil has been refined. The colour producing substances are naturally occurring components of the fats and oil mainly pigments. Like carotenoids which gives yellow and red colours, chlorophylls gives green colours. However, colour change or deterioration can equally be cause during extraction of the fat or oils from the oil-bearing seeds, or by solvent effect of the oil itself. Removal of these colours is very imperative, not only because a pale-coloured fat or oil has an appeal of purity, but also because the colour of the fat may affect the appearance (colour) of he finished food products in which the oil is used. There are many notable methods of bleaching viz Heat-treatment, chemical treatmen, Adsorption.

Among these methods, Adsorption is often preferred. It involves adsorption of the colour producing substances on an adsorbent material. Acid-activated bleaching earth or clay sometimes called Bentonite is the adsorbent material that has been used most extensively. This substance consists primarily of hydrated aluminum silicate. Anhydrous silica gel and activated carbon also are used as bleaching adsorbents to a limited extent. After the bleaching the oil or fat gives a lighter coloured oil.

2.10.4 Deodorization

This process is normally accomplished after refining and bleaching. It is a vacuum steam distillation process for the purpose of removing trace constituents that give rise to undesirable flavours, colours, and odours in fats and oils. In addition the
process destroys peroxides in the oil, removes aldehydes and ketones, or other volatile products resulting from oxidation and reduces the colour by the destruction of Carotenoid pigment.\textsuperscript{45}

This process is feasible because of the great differences in volatility between the substances that gives flavours, colours and odours to fats and oils and the triglycerides. It is carried out under vacuum to facilitate the removal of the volatile substance, to avoid undue hydrolysis of the fat or oil and to make the most efficient use of the steam. Deodourisation have any significant upon the fatty acid composition of most – fats and oils. Depending upon the degree of unsaturation of the oil being deodourized small amounts of trans fatty acids may be formed.

2.10.5 Fractional Crystallization

This is the removal of solids by controlled crystallization and separation techniques involving the use of solvents or dry processing. The resulting oil is usually referred to as the “Olein” fraction and the solid is called “Stearin” fraction. This process relies upon the differences in melting points to separate the oil fractions.

2.10.6 Winterization

This is a special form of fraction crystallization of fats in which the melting saturated acylglycerols are removed.\textsuperscript{41} It is a process whereby material is crystallized and removed from the oil by filtration to avoid clouding of the liquid fraction at cooler temperatures. A similar process called “dewaxing” is utilized to clarify oils containing trace amounts of clouding constituents. Winterization is done by holding the oil at 4-5°C for 2-4 days to crystallize the solid fats which are removed by filtration thus producing a clear and brighter oil.

2.10.7 Hydrogenation

Hydrogenation is the process by which hydrogen is added to points of unsaturation in the fatty acids. It was developed as a result of the needs to; convert
liquid oils to the semi-solid form for greater utility in certain food uses and increases
the oxidative and thermal stability of the fat or oil.

In the process of hydrogenation, hydrogen gas reacts with oil at elevated
temperature and pressure in the presence of a catalyst like nickel-which is removed
from the fat after the hydrogenation process is completed. The hydrogenation process is
easily controlled and can be stopped at any desired point. Thus it could be partially or
completely hydrogenated. Partially hydrogenated oils are typically used to produce
institutional cooking oils, liquid shortenings and liquid margarines. They may contain
“trans fatty acids” but complete hydrogenated oil is a hard brittle solid thus fat and
contains no “trans fatty acids”.

The hydrogenation conditions can be varied by the manufacturer to meet certain
physical and chemical characteristics desired in the finished product. This is achieved
through selection of the proper temperature, pressure, time, catalyst and starting oils.
Both positional and geometric (trans) isomers are formed to some extent during
hydrogenation, the amounts depending on the conditions employed.

Biological hydrogenation of polyunsaturated fatty acids occurs in some animal
organisms, particularly in ruminants. This account for the presence of some trans
isomers that occur in the tissues and milk of ruminants.

2.11 Health Impact of EFAs and NEFAs

2.11.1 Cell

It is the smallest structural, functional and biological unit of all organism. It is
an autonomous self replicating unit that may exists as functional independent unit of
life (as in the case of unicellular organism) or as subunit in a multicellular organisms
such as in plants and animals that is specialized into carrying out particular functions
toward the cause of the organism as a whole.

So life begins at the cellular levels! Anything that happens to the cells will
automatically affects or reflects to the entire body. The gate way into the cells is the
“cell membrane”. These membranes not only separate cells from the external environment but also compartmentalize cells and provide a special milieu for many important biochemical processes. With an unhealthy cell, critical nutrient have a hard time getting in and waste has a hard time getting out. Hence the cell operates inefficiently.

Compositionally, nearly all cell membranes are chiefly of lipids. The lipids include glycerolipids, sphingolipids and steroids. Among these, glycerolipids are quantitatively by far the most important group. These glycerolipids contains fatty acids esterified at the sn-1, sn-2 or sn-3 positions of the glycerol. Thus the type of fatty acids contains in these compounds will determine the function and fluidity of the cell membrane. Dietary lipids especially EFAs may modulate immune reactions either by modifying the composition of cell membrane and membrane surface components or by influencing the nature of the precursor pool for eicosnoids. 49

2.11.2 Cancer, Heart Attack and Stroke (CVD)

Recent researches have indicated that the quality or type of fat may be more important than the quantity of fat in reducing CVD risk.50 Polyunsaturated fats have often been recommended to reduce coronary heart disease.51

Occasionally when certain cells in the body undergo a change that is abnormal and thus begin a process of uncontrolled growth and spread thus growing into masses of called Tumors. These tumours can be benign (non-cancerous) or malignant (cancerous). Implicitly sickness, disorder and healthy life begins in the cell. The danger of cancer is that it invades and destroys normal tissue. In the beginning, cancer cells usually remain at the original site and the cancer is said to be localized later cancer cells may metastasize (that is, they invade distant or neighbouring organs or tissues. This occurs either by direct extension of growth or by cells becoming detached and carried through the lymph or blood systems to other parts of the body.
Dietary fat may be a modulator of carcinogenesis rather than a promoter (that is it may enhance or inhibit depending on the experimental design and only linoleic acid has been shown clearly and unequivocally to enhance tumor growth when fed to rodents on an excessive amount of calories. It has been theorized that linoleic acid causes cancer by chronic over production of the inflammatory arachidonic acid eicosanoids which stimulate the proliferation of mutated cells while Omega-3 fatty acids like DHA reduce cancer risk by markedly inhibiting activator protein1, a transcription factor which promotes cancerous proliferation and metastasis. Also omega-6 fatty acid promote cancer by blocking Omega-3 activity.

Some evidence even suggest that risk of breast cancer and to a positive greater extent colon, prostate, and ovarian cancers is associated with saturated fatty acids (SFAs). Although too much, linoleic acid have shown to increase the risk of cancer, a form of linoleic acid in which the double bonds are closer together known as “conjugated linoleic acid” (CLA) actually reduces cancer risk. CLA has been shown to significantly inhibit prostate cancer proliferation and breast cancer formation.

However, while breast cancer and colon cancer have been linked on high fat diets, more recent prospective studies have found little, if any relationship between the two. Some animal studies have suggested that polyunsaturated fatty acids (PUFAs) may increase tumor growth, no relationship has been found between PUFAs and cancer in humans. A study at Yale University of 1119 women who were breast cancer patient revealed that there were no significant trends associating any fatty acid or macro nutrient to the risk of breast cancer.

Unfortunately, dietary fats and fats is been parochially linked and blamed for incidence of cancer perhaps due to the fact that cell membranes are chiefly lipids. So any alterations or change in the cell is fastly attributed to the fats without looking at other factors. Apart from fat or dietary fat, there are other factors like aging (old age), smoking, excessive exposure to sunlight, sex life, free radical damage that can cause
tumour growth. All these risk factors affects the activities of Δ6 desaturase and Δ5 desaturase enzymes which converts the EFAs to their metabolites or derivatives. Since diet and exercise may be controlled by man, they are the basis for recommendations to reduce risk factors for CVDs. 56

2.11.3 Brain

AA is essential for growth. 57 DHA and AA are the predominant EFAs in the human brain. In the last third of pregnancy and in the first four months after birth, rapid brain growth in the human infant requires large amounts of omega-3 and omega-6 EFAs. It have been reported that by (or just before) age 8, children who had been breast-fed as infants had an 8.3-point IQ advantage over children who had received formula. 58 Human milk contains (total fatty acids by weight) 12% linoleic acid, 0.5% alpha-linolenic acid, 0.6% arachidonic acid (AA) and 0.3% DHA. 59

Support for the idea that DHA is critical for brain development came from an experiment which studies the effects of adding DHA (in the form of fish oil) to infant formula. At both 16 and 30 weeks of age the breast-fed and supplement-formula fed infants showed significantly better visual acuity than the placebo-formula-fed infants. 60 Evidently an infants desaturase and elongase enzymes are not fully developed because no amount of alpha-linolenic acid supplement can provide enough DHA for neural development. 61 Thus it has firmly been recommended to include AA and DHA in the formula of premature babies. 51 Although fat serves an infant’s need for energy and makes a significant contribution to the activity of the central nervous system, at excessive levels in diet, fat can interfere with calcium absorption and absorption of certain fat-soluble vitamins. 7

The ability of enzymes to produce the omega-6 and omega-3 families declines with age. 62 It seems reasonable to wonder if dietary fat or fat supplements affects the mental function of adults A diet high in saturated fat was shown to impair a wide range
of learning and memory functions.\textsuperscript{63} Too much saturated fat (SFAs) or trans fat in the diet leads to stiff, rigid membranes and a loss of membrane fluidity.\textsuperscript{64}

The influence of EFAs supplement on brain membrane content declines with maturity as adult cell membrane content of DHA and AA is only slowly altered by diet or supplement. As brain cell membranes ages, the ratio of cholesterol to phospholipids increases and membrane fluidity decreases.\textsuperscript{35}

As one gets older, DHA levels naturally decline, if dietary intake of EFAs is low, the body will sacrifice EFA content of cell membranes outside the brain before neurons are affected thus function sub-optimally. Perhaps this may explain why old men and women behave like infants and societies who diets are high in DHA have a lower incidence of neurodegenerative disorders. DHA is also reduced when the brain of rats were experimentally exposed to high oxygen levels, suggesting that free radical oxidation causes depletion of DHA.

Consequently, diet, aging and free radical damage are major factors that causes a depletion in DHA in the brain. It become imperative that Vitamin E and vitamin C be taken to protect DHA from free radical damage and also avoid heavy metals.

2.11.4 Obesity, Diabetes and Alcoholics

Losing weight not only makes a person look good, it can reduce the danger of getting heart disease, diabetes and cancer\textsuperscript{65} thus obesity or overweight is objectionable. It is nearly always due to overeating without over working. It makes arthritis of the hips and knees worse because of the abnormally great pressure it puts on joints and articular cartilages.

Carbohydrates ingested into the body is converted to glucose and unused glucose then converted and stored as fats, so additional consumption of NEFAs which the body can sill provide on itself when needed will amount to double excess fat-thus obesity arises. So eating too much carbohydrates and saturated fats causes obesity
though some are genetically triggered. Infact NEFAs are mostly responsible for obesity since the body provides a good amount of them.

Over weight individuals tends to have higher total cholesterol, higher low density lipoprotein (LDL), triglycerides and lower High Density Lipoprotein (HDL). This is largely due to dietary NEFAs especially SFAs which are converted to cholesterol by the liver which may end up being deposited in atherosclerotic plagues on blood vessels leading to cardiovascular disease. High blood cholesterol also depresses the immune system and thereby increases the incidence of cancer. 34 Hence blood cholesterol could reduced by eating good oils.

So NEFAs especially SFAs need not to be taken excessively, since it will finally amounted to excess cholesterol.

Today, our so called good vegetable oil slogan “No cholesterol !” possesses excess disquized cholesterol because of its high amount of saturated fatty acids. So cholesterol is more often acquired by eating too many SFAs than by eating cholesterol itself.

However, not all SFAs have the same effect on cholesterol synthesis in the liver. Only the saturated fats of chain length 12, 14 and 16 (lauric acid, myristic acid and palmitic acid) have shown to elevate blood cholesterol, of these myristic acid (high in coconut and palm oil) elevates cholesterol the most. 39 Stearic acid has been shown to lower cholesterol by 21% even more than oleic acid which lowers LDL by 15%. 66

Insulin stimulates the delta-6-desaturase enzyme thus this enzyme activity is much reduced in diabetes, thus affecting the omega-6 products like AA. it is essential for leucocyte (white blood cell) function. So reduced AA, inturn reduce leucocyte function making diabetes more vulnerable, to infection. 67 This is perhaps why diabetic wounds hardly heals. Dietary intake of EFAs especially n-3 from infancy reduced the risk for type I diabetes68 confirming previous animal studies that EFAs prevent chemical-induced diabetes. 69 The fatty acid content of muscle membranes is critical in
determining insulin sensitivity. A high fat diet can increase insulin resistance, but both dietary n-3 and n-6 fatty acids can increase membrane fluidity and thereby improve insulin sensitivity.  

Alcoholics suffer from disturbances of fat metabolism notably in the liver. The liver is the most active site of delta 6-desaturase enzyme activity in the body. Infact, many (if not most) cells in the body have no delta-6-desaturase enzymes and are dependent upon the liver for omega-3 and omega-6 desaturase and elongase products thus alcoholics are most deficient in AA. Interference with the metabolism of EFAs by saturated fats, chochsterol trans fats, in sulin deficiency, viruses alcohol and ageing reduces the formation of GLA, DGLA, AA, EPA and DHA and their beneficial metabolites, that could account for the initiation and progression of atherosclerosis, and failure of the healing process, thus alcoholic are easily prone to suffer coronary heart disease, diabetes, cancer, stroke etc.

2.11.5 HIV and AIDS

There is evidence to suggest that PUFAs can inactivate Human immunodeficiency virus (HIV) an enveloped virus and thus is of benefit in Acquired immunodeficiency syndrome; AIDS. Patients with AIDS and intravenous drug abusers have been reported to have low plasma phospholipids DGLA, AA and DHA concentrations that could favour the onset and development of AIDS. Increasing concentrations of n-6 DGLA and AA in breast milk reduced the risk of mother-to-child transmission of HIV suggesting that EFAs have anti HIV actions.

2.12 Analysis of Fatty Acids

We own a considerable debt to ancient investigators who prior to about 1935, made enormous contributions to our knowledge of the fatty acid composition of natural lipids despite primitive equipments and analytical techniques.
Then chemists isolated lipids using only solubilities properties of solvents, the formation of salts of fatty acids which were further characterized by their raw formulae and ebullition or fusion temperatures.

Progressively, period following, 1935, there has been markedly new and more efficient procedures for separating and studying fatty acid mixtures. These procedures include ester distillation, crystallization of urea complexes or of various metallic salts, various forms of chromatography and countercurrent distribution. Conventionally, methods for the profiling of fatty acids consists of several steps: viz, lipid extraction with organic solvents, hydrolysis (saponification), methylation/derivatization and finally, analysis and quantification with GC. Other conventional techniques generally used in lipid including thin-layer chromatography impregnated with silver-nitrate (AGNO₃-TLC), silver-ion liquid chromatography (Ag-LC), fourier transform infrared spectroscopy (FTIR) and mass spectrometry (MS). These techniques when used in combination are more than adequate for full characterization of fatty acids including complete identification and quantitation. Nevertheless, choice of one technique or combination of techniques over another depends on several factors, for example the complexity of the sample, sample amounts, and the purpose of the analysis.76

However, the application of these conventional procedure to biological samples is, however bedeviled with disadvantages such as high risk of contamination and recovery losses in multi-step procedures. Further more, these methods become cumbersome and impractical for analysis of large series of samples especially for limited sample amounts.77 To overcome these disadvantages methods that combine extraction and derivatization in a single step have been developed.78,79

2.12.1 Extraction of Fatty Acids

The analysis of fatty acid containing compounds require previously their hydrolysis, separation of the non-acidic constituents and the liberation of the acids from the mixture of soap.
For more natural fats; saponifying 100 parts by weight of fat with a solution of 30 parts of KOH in 500 parts of alcohol (95-100%) boiling under reflux for 3 hours followed by removal of most of the alcohol by distillation. A small amount of water must be present to affect rapid and complete saponification of glyceride oils.

Fats can equally be saponified readily at room temperature. The process may be carried out by dissolving KOH (260g) in water (250ml). After some minutes oil (1 litre) to be saponified is added to this solution. Ethanol (10ml) is then added. This process was applied to fats containing easily altered fatty acids (conjugated highly unsaturated). After saponification approximately half of the alcohol is removed under vacuum, the residue being diluted with water and the unsaponifiable matter extracted by shaking with an organic solvent and the fatty acids are liberated by the addition of mineral acid, usually of H₂SO₄ or HCL (10%). When very short-chain acids are present, the alcohol is removed prior acidification under vacuum with the addition of sufficient water to keep soaps in solution. If the amount of acid is important, long chain unsaturated acids are separated from the aqueous medium by allowing the mixture to stand in the cold (i.e Low temperature crystallization) and the fatty acid crystals recovered by filtration.

Unfortunately, hydrolysis is reported to be the main cause of why well-established methodologies fail when sample size and reagent volumes are scale down.

2.12.2 Chemical Derivatization in Gas Chromatography

When considering chemical derivatization in GC, it is expedient to distinguish between two causes that can affect the volatility of a substance. Low volatility can be due either to the fact that the substance has large molecules or that the molecules are mutually associated through polar groups. In the first instance, the intermolecular cohesion is the result of interactions by dispersion forces and the volatility of such compounds obviously cannot be increased by derivatization. In the second instance,
however, compounds with relatively small molecules can have very low volatility if the molecule has functional groups that provide for polar interactions, especially interactions through hydrogen or ionic bonds.

Hence derivatization is usually carried out in order to increase the volatility of substances with boiling-points that are too high, to reduce the adsorption of solutes on the support and column surface and to improve the separation, thereby reducing long retention times or non-elution of the compounds. So fatty acids are derivatized to obtain new compound(s) with different properties especially its volatility in a process commonly known as Methylation. There are other number of ways in which the volatility of a given compound or solute component can be controlled. These aspects have been studied since the advent of GC and in many respect it was these problems that led to new concepts in GC techniques and instrumentation and to the development of new chromatographic materials. Examples are high-temperature GC, temperature programming, the use of sorbents of high selectivity, operation in systems with a low content of the sorbent, high-pressure and supercritical-fluid chromatography unlike chemical derivatization, the compound or analyte properties is not altered.  

Special derivatives often provide for the selective detection of certain species of compounds or the separation of chemically very similar compounds, such as optical isomers.

**FATTY ACID ESTERS**

The three most commonly used methodologies in the preparation of fatty acid esters are:

- Acid-catalyzed esterification (methylation)
- Acid catalysed transesterification
- Base catalyzed transeserification
Initially, two organizations that mark rules in the analytical methods: Association of official analytical chemists and American oil chemists society, recommended the use of sulphuric acid – methanol reagent\textsuperscript{81, 82} for preparing fatty acids methyl esters (FAMEs). However, both organizations accepted the later use of the boron trifluoride (BF\textsubscript{3})-methanol reagent. Evidently there is no need to hydrolyze lipid to obtain the fatty acids before preparing the esters as most lipid can be transesterified directly even \textit{In situ}.\textsuperscript{83}

Normally fatty acids are acid-catalysed transesterified to their esters using hydrogen chloride (5\%), or sulfuric acid (2\%) or boron trifluoride (7\%) in methanol.\textsuperscript{84} This gives fatty acid methyl esters (FAMEs). FAMEs are the most commonly used derivatives of the carboxylic group. The volatility of methyl esters is sufficiently high to permit the G.C determination of even higher fatty acids. However, with short chain fatty acids, the volatility of the methyl esters is unsatisfactory as it can cause losses of the derivatives before analysis, hence higher esters are for the GC of short chain acids.\textsuperscript{85} Butyl and benzyl esters are very frequently used. The method of the preparation of higher esters are similar to those for methyl esters.

Methanolic hydrogen chloride or sulfuric acid are the best general purpose esterifying agents because they methylate even free FFAs very rapidly. However, if FFAs alone are to be methylated no solvent other than methanol is necessary (i.e need no solubilizing agent like toluene).

Caution should be exercised in using the BF\textsubscript{3} reagent, which has a limited shelf-life, even when refrigerated because the use of old or overly concentrated (>7\%) solutions might result in the production of artifacts and the loss of appreciable-amount of highly unsaturated fatty acids. For fat samples such as refined vegetable oils, which contain primarily triglycerides with no or very small amounts of FFAs, phospholipids or any other polar lipid classes, basic catalysed transesterification is recommended for speed and simplicity. Sodium or potassium hydroxides or methoxides are the common
reagents for the base-catalyzed transesterification but do not normally esterified FFAs unlike acid-catalysed.

Acid-catalyzed methylation is commonly used because it is effective under many conditions and methylates all fatty acids, including esterified, unesterified and those in salt form, where as alkaline methylation only transesterifies fatty acids that are in glyceride form.\(^{86}\)

Though acid-catalysed methylation is commonly used because of its effectiveness under many conditions and methylates all fatty acid, it has been criticized for its potential to cause migration of conjugated double bonds in unsaturated fatty acids.\(^{87}\)

However, fatty acids methyl esters (FAMEs) with unsaturation suffers rearrangement of the double bonds under electron ionization (EI) and gives mass spectra with extensive fragmentation which may not be interpreted to locate the original double bond positions. Hence other derivatives are formed as an aid to methyl esters or butyl esters but not as an alternative namely picolinic esters (3-hydroxy methyl pyridine, 4, 4-dimethoxyoxazoline (DMOX) derivatives.

Both picolinyl ester and DMOX derivatives are best considered as complementary. DMOX derivatives are only slightly less volatile than methyl esters and can be subjected to GC analysis on polar stationary phases under comparable conditions and give equivalent resolution.\(^{88}\)

Picolinyl esters on the other hand require column temperatures about 50\(^\circ\)c higher than FAMEs.\(^{89}\). Picolinyl esters must be prepared from FFAs or FAME samples must first be hydrolysed. It is prepared by reacting oxalyl chloride overnight with 3-hydroxy methyl pyridine in dichloromethane. It could equally be prepared by reacting FFAs with 2-amino-2-methyl-1-propanol in a nitrogen atmosphere at 180\(^\circ\)c for 2hours or 18hours for FAMES.\(^{90}\)
The prolonged high temperatures required for DMOX preparation gives cause for concern, and there must be some risk to polyunsaturated fatty acids or any other compound with a liable functional group. Hence it is complementary.

2.12.3 Gas Chromatography

The first of many important advances in the early development of GLC for analytical purpose was the separation and determination of FAs reported by James and Martins in 1952. Polar GC columns phases are employed in both packed and capillary column analysis as these afford separation according to:

i. Carbon chain number

ii. Degree of unsaturation in the alkyl chain

These reflects their retention behaviour or properties on the column. Direct identification of an unknown substance (FA) on the basis of retention behaviour (or data on a single stationary phase is very unreliable and this approach is more often used for a negative purpose (i.e demonstrating that a certain substance is not present in a sample). Because of huge structural variety there are some substances that have the same retention behaviours on the same columns under the same operating conditions. Example on a cyanopropyl-polysiloxane stationary phase; there is co-elutions of FAMES of 18:3n-3 and 20:1n-9. On a carbowax stationary phase, 22:6n-3 and 24:1n-9 co-elutes.

So GC identification of complete unknown substances fails and additional methods must be used. Thus it is said “GC is one of the quickest ways of getting the wrong answer in qualitative analysis.”

The reliability of the qualitative analysis is improved when the retention data are obtained in two or better three stationary phases differing in their selectivity on the same operating conditions. However, there are retention concepts and modification which has helped to make GC qualitative results more reliable namely Equivalent chain length, factional chain length, temperature programming, retention time locking.
Quantitatively, most GC FAMEs analysis is done with flame ionization detector (FID). This is a mass flow sensitive detector in that the area of a given peak is proportional to the mass of single carbon fragments.

An often held assumption among lipid analysts is that, in a FID chromatogram, the peak area of an analyte FAME (A_{FAME}) divided by the total peak area of all FAME (A_{FAME}) is equal to the ratio of the corresponding analyte FA mass ((M_{FA}) to the total mass of FA present in the sample (\Sigma M_{FA}):

\textbf{Mathematically:} \quad \frac{A_{FAME}}{\Sigma A_{FAME}} = \frac{M_{FA}}{\Sigma M_{FA}}

This statement is clearly based on a number of assumptions, not the least of which is that for the FID, the relative responses toward all FAME are equal. On the contrary, FID responses toward all FAME cannot be assumed equivalent strictly argued in terms of the mechanism of detection. Since carbonyl carbons are not FID susceptible, it would follow that for an equal mass of two different FAME, the FAME of lower carbon number would have the smaller response factor owing to a higher proportion of FID-inactive carbon.\textsuperscript{6}

Hence, the FID response factor for FAME are expected to depend on both the carbon number and the number of unsaturation of the analyte FAME in question. In practice, a number additional factors prevent the FAME area percentage from equating to mass percentage. Example, injector bias results in a lesser percentage of more volatile component being successfully loaded on-colum.\textsuperscript{92}

\textbf{Equivalent Chain Length (ECL)}

ECL is defined as:

\text{ECL (x) } = \log_{1R}(x) - \log_{1R}(z) - \log_{1R} - \log_{1R}(z)
\[ Z = \text{Carbon number in the saturated FAME eluting immediately before the analyte of interest (x)} \]

\[ Z+1 = \text{Number of carbons in the saturated FAME eluting immediately after analyte x} \]

\[ t'_r = \text{adjusted retention time.} \]

The ECL concept uses the saturated straight chain FAME as reference compounds. By definition 18:0 has ECL value of 18. 20:0 has an ECL value of 20. only the carbon in the fatty acid chain of the FAME molecule are counted.

Even though the ECL value may be characteristics for a fatty acid molecule, it is not unique. It has been reported that the polarity of cyanopropyl phases is high temperature dependent. Hence significant increase in ECL values of unsaturated fatty acids with increasing column temperatures has been reported. A method to monitor this shift in ECL values has been devised.\textsuperscript{11}

### 2.12.4 Mass Spectrometry

MS is an analytical tool mainly use for structural analysis by degrading submicrogram quantities of a compound and recording the fragmentation pattern according to mass. Sample vapour diffuses into the low pressure system of the MS where it is ionized with sufficient energy to also cause fragmentation of the chemical bonds in the original molecule. The resulting positively charged ions are accelerated into a magnetic field which disperses and permits relative abundance measurement of ions of a given mass-to-charge ratio. The resulting record of ion abundance versus mass constitutes a fragmentation pattern. This pattern is usually sufficiently distinct to identify the molecule. MS just like GC needs volatile (volatilize) samples.

As a GC detector, MS is unique because it is both a universal (i.e full scan mode) and tunable (selective ion monitoring) selective detector. When operated in the universal detection mode, its response verses retention time resembles that of a FID.\textsuperscript{93}

The number, relative abundance and structures of the ions formed depends upon the
ionizing energy, the molecular structure and the ion source temperature and pressure. The distribution of internal energies and consequently the appearance of the mass spectrum is strongly dependent on the nominal electron beam energy ($E_{ei}$). The standard $E_{ei}$ is set at 70eV and $10^{-6}$-Torr source pressure for a number of reasons viz.

a. For most compounds, this is the electron energy at which maximum ion formation occurs.

b. The appearance of the mass spectra changes little with changes in $E_{ei}$

c. A relatively intense molecular ion as well as a large number of fragment ions are formed, giving both molecular weight and structural information.

d. The internal energy distribution of the ions formed should be the same from instrument to instrument giving rise to instrument-independent spectra.

Thus a compound’s mass spectrum will be identical regardless of the MS used, providing conditions in the ion source are the same. Most of the mass spectrometric structure work on fatty acids has been performed on the corresponding (usually methyl) esters. The methyl esters spectrum is not complicated by additional fragmentation as it is for long ester alkyl groups.

### 2.13 Hyphynated technique (GC-EI-MS)

Purity is probably the second most important physical characteristics of the sample to be considered in analysis of MS. One or two abundant fragment ions from a low level purify could conceivably misdirect the structure elucidation of the analyte. Sample purification and isolation can be difficult and time consuming. Some samples are so small that the isolation of the minor components are impractical. Further more, owing to the tedious manipulation involved, the investigator (analyst) is tempted to ignore most of the minor components in a complex mixture.

Equally, gas chromatography has long been appreciated for its ability to separate small amounts of compound having only subtle differences in structure.
However, the ambiguity or uncertainty of retention data for qualitative purposes has always been a short coming but MS molecules are analysed in the vapour phase prompted the development of combined instrumentation commonly known as “Hyphynated techniques”.

Thus GC-MS combines the advantages and eliminates most disadvantages of the individual techniques. Complex mixtures are separated by the GC and component are individually presented to the MS for identification with the development of highly polar stationary phases, it has becomes possible to separate even the unseparated isomers which can be convincingly identified with MS.

The unseparated 8 isomers of linolenic acid have been separated with highly polar polysiloxane stationary phase.  

It implies that, though MS are capable of producing vast quantities of data useful for trace chemical analysis and compound identification, the importance of the chromatographic separation cannot be ignored.

The main difficulties for the analysis of fatty acids by GC-MS are encountered in the determination of the position and geometry of the double bonds of monounsaturated (MUFA) and polyunsaturated fatty acid (PUFA) methyl esters.

Long-chain saturated FAMEs are easily identified. The Mass spectrum of methyl n-octadecanoate at 70 ev showed

Molecular ion (M+) m/z 298 are fairly abundant (about 20% of the base peak). Detailed studies have revealed that M+ is always present in spectra of unbranched saturated FAME, although its intensity relative to the base peak at m/z 74 varies considerably with chain length. It passes through a minimum at five carbon atoms in the chain and then increases as the chain becomes longer. Ions of type, +OCH3 (M-31) is usually present in low abundance but possess diagnostic value since they are characteristics of the methoxyl group in the FAME. In the corresponding ethyl ester spectrum; a peak occurs at m/z= M-45 (loss of the ethoxyl group).
Fragments of general formular \([(\text{CH}_2)^n(\text{COOCH}_3)]^+\) where \(n = 2, 3, 4\) etc are found for all \(n\) in spectra of normal chain FAME. Ions of \(m/z\) 87 \((n = 2)\), 101 \((n = 3)\), 115 \((n = 4)\) are present. \(m/z\) 143 \((n = 6)\) and \(m/z\) 199 \((n = 10)\) separated by four methylene units are considerably higher than others. Electron impact ionization (EI) spectra of saturated and monoenoic FAMEs are dominated by the representative fragment ion \((\text{CH}_3\text{C(OH)OCH}_3)^+\) at \(m/z\) 74 caused by McLafferty rearrangement.

**Illustration of McLafferty Rearrangement:**

![McLafferty Rearrangement](image)

Losses of neutral aliphatic radicals give rise to a series of ion \([(\text{CH}_2)^n\text{CO}_2\text{CH}_3]^+\) where \(m/z\) 87 usually the most abundant. The most abundant ions in monoenes are a series with molecular formular \((\text{C}_n\text{H}_2n-1)^+\), \(m/z\) 55 \((\text{C}_4\text{H}_7)\) being usually the base peak. In methylene interrupted dienes also exists a series with the molecular formulary \((\text{C}_n\text{H}_{2n-3})^+\), where \(m/z\) 67 is usually the base peak. In spectra of fatty acids with three or more methylene interrupted double bonds the series with molecular formular \((\text{C}_n\text{H}_{2n-5})^+\) is dominating and \(m/z\) 79 \((\text{C}_6\text{H}_7)^+\) is usually the base peak.

The fragmentation patterns of unsaturated FAMEs are not indicative for the position of double bonds. Double bond positions are determined by converting unsaturated viz pyrrolidine, picolinyl and 4, 4-dimethyloxazoline (DMOX). These derivatizations steps are time consuming. The moisture level has to be minimized in the case of picolinyl and DMOX derivatives, so an additional step is necessary. Therefore temperatures equal or higher than 100\(^\circ\)C are necessary for the formation of pyrrolidine derivatives. The use of chemical degradation methods such as ozonation which requires...
an ozonization equipment, could be a second option for the identification of positional and geometrical isomers. Additional derivatization procedures can be of value including hydrogenation and deuteration and preparation of dimethyl disulfide and 4-methyl-1, 2, 4-triazoline-3, 5-dione adducts.

Nevertheless, because of high sensitivity and selectivity of GC-MS and shortcomings in preparing some of the derivatives, samples are analysed by conversion into FAME and using a specific capillary column for isolation and identification.
3.0 MATERIALS AND METHODS

3.1 Seed Samples

Seeds of *Monodora myristica* were purchased from Nsukka spice market in Enugu State, while *Monodora tenuifolia* seeds were purchased from a local spice market in Ngodo Isuochi, Umunneochi L.G.A of Abia State. The identity of the seeds were authenticated by the Taxonomical Section of the Department of Botany, University of Nigeria, Nsukka. The seed coats were removed and their achenes (seeds without coat) were carefully sun-dried and then ground until analysed.

3.2 Oil Extraction

Steam distillation: The samples of both seeds were separately submitted to steam distillation for 6 hours. Steam was generated in a round bottom flask (5 litres) with distilled water and connected to another round bottom flask (2 litres) containing the sample via a glass pipe. The sample flask was then connected to a condenser. The resultant vapour mixture was continuously condensed and collected forming a layer of water and volatile oils. The extraction was done in triplicate for each sample.

*Monodora tenuifolia* did not yield volatile oil. The volatile oil of *Monodora myristica* was collected, dried over anhydrous calcium chloride, and kept in an air-tight sample bottle in a refrigerator.

Solvent Extraction:

Both samples after steam distillation were collected and dried again in an oven at 65°C for 72 hours. The dried samples were again weighed and submitted to soxhlet extraction. The extracted non-volatile oils of both seed samples were collected. The solvent was removed using a rotary evaporator leaving light brown coloured oil of *Monodora myristica* and a brownish yellow coloured oil of *Monodora tenuifolia*. The
solvent free oils were dried, put in an air-tight container, then kept in a refrigerator until needed. The percentage oil yields, densities and refractive indices of both oils were determined.

3.3 Percentage Oil Yield

The volume of oils extracted were measured and the weight recorded for each seed. Then percentage calculated by multiplying with the densities of each oil.

3.4 Determination of Physical Properties

3.4.1 Refractive Index

The instrument used was Abbe’s refractometer. The instrument was reset using a light compensator. A small amount of oil was smeared on the lower prism of the instrument, light was passed through by means of an angled mirror. The reflected light appeared inform of a dark background. With fine adjustments, the telescope tubes were moved until the black shadow appeared central in the cross wire indicator, the instrument reading was then noted.

3.4.2 Density

Apparatus:

i. Analytical balance weighs up to four decimal places of accuracy.

ii. Density bottles

iii. Thermometer

iv. Ice block and water bath

The density sample bottles weight were obtained at room temperature (26°C) for five each. After that their weights were obtained again at 20°C for the same number of times to obtain their actual weight using ice block in water bath. The density sample bottles were filled with the oils and re-weighed to obtain their weights. The differences between the weight of oil samples in density bottles and density bottles at 20°C after which the formular.
Density = \frac{\text{Weight}}{\text{volume}}

was used for the calculations.

3.5 Derivitization

3.5.1 Fatty Acid Methyl Esters [FAMEs]:

Apparatus

- Measuring cylinder
- Reflux condenser
- Thermostated heating mantle
- Retort stand and clamp
- 25ml conical flask
- Pipettes
- Stop watch

Reagents and Method

- Sulphuric acid in Methanol
- Toluene
- n – Hexane
- Potassium bicarbonate
- Nacl
- Anhydrous calcium chloride
- Separating funnel
- Bucunor funnel
- Filter paper

Fatty acid methyl ester (FAME) derivatives were prepared by transesterification using 2% sulphuric acid in methanol.\(^8\) Non-volatile oil of *Monodora myristica* (NOMM) 1ml was dissolved in toluene (5ml) and 2% sulphuric acid in methanol (10ml) added. This was refluxed for 2hours at 80\(^\circ\)C. Then sodium chloride solution (5ml) and hexane
(20ml) were added and the organic phase including the FAMEs was separated, washed with potassium bicarbonate solution, dried; and stored in a refrigerator until ready for GC-MS analysis. The same procedure was used for the non-volatile oil of *Monodora tenuifolia* (NOMT).

### 3.5.2 Fatty Acid Butyl Esters [FABEs]

Fatty acid butyl ester (FABE) derivatives were also prepared by transesterification using the same method above since methods of the preparation of higher esters are similar to those for methyl esters.\(^8\) However, the concentrations and volumes of reagents were scaled up. Both samples were converted, dried and stored in refrigerator. The volatile oil of *Monodora myristica* (VOMM) needed no conversion for GC-MS analysis. In all 5 samples were obtained.

### 3.6 GC-MS ANALYSIS

5 µL of each sample was dissolved in 1.5 mL of dichloromethane (EMD, HPLC grade) and injected into a ThermoScientific ® GC-MS instrument consisting of a Trace GC Ultra gas chromatograph with a vaporization method in splitless mode, connected to a ThermoScientific DSQ II mass spectrometer. The oven temperature was programmed to hold at 50°C for 2 min, then increased at 15°C/min to 320°C, and held isothermally for 5 min. High purity helium was used as carrier gas at a flow rate of 1.2 mL/min, and a Restek ® DB-5 ms (30 m x 0.25 mm, with 0.25 uM) column was used. The ion source was held at 200°C and the transfer line at 325°C. Electron ionization in the positive mode in the range of 40-400 dalton was recorded at 70 eV with emission current of 250 µ A. Six scans s\(^{-1}\) were collected. The multiplier potential was 3 kV and the source pressure was 10\(^{-5}\) torr. All data were recorded by computer and compounds were identified by comparison with the NISTdemo mass spectral database.
3.6.1 Quantification

The peak area of an analyte FAME ($A_{FAME}$) divided by the total peak area of all FAMEs ($\sum A_{FAME}$) is equal to the ratio of the corresponding mass of analyte FA ($m_{FA}$) to the total mass of FA present in the sample ($\sum m_{FA}$).\textsuperscript{12, 98, 99} Mass abundance could equally be used for quantification.\textsuperscript{100} In this work, Measured Area (MA) of the peak was used (ie area under the peak) as determined using an X-caliber integrator software. The MA of all the peaks in each GC chromatogram was added and the percentage of each fatty acid obtained relative to the total MA. Thus:

$$\% \text{ of component A} = \frac{MA_{FAME}}{\sum MA_{FAME}} \times \frac{100}{1}$$
CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

The results of the oil physical properties and % yield is shown in table 1 and table 2 shows the % fatty acids composition of Monodora myristica and table 3 shows the result of % fatty acid composition of Monodora tenuifolia.

Table 1: % oil and some physical properties

<table>
<thead>
<tr>
<th>Parameters</th>
<th>VOMM</th>
<th>NOMM</th>
<th>NOMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil content (%v/v)</td>
<td>0.66%</td>
<td>27.68%</td>
<td>7.5%</td>
</tr>
<tr>
<td>Density</td>
<td>0.785</td>
<td>0.890</td>
<td>0.843</td>
</tr>
<tr>
<td>Refractive index</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Percentage fatty acid composition of Monodora myristica

<table>
<thead>
<tr>
<th></th>
<th>FAME</th>
<th>FABE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid</td>
<td>1.7</td>
<td>0.92</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>nd</td>
<td>0.75</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>92.7</td>
<td>98.33</td>
</tr>
</tbody>
</table>

Table 3: Percentage fatty acid composition of Monodora tenuifolia

<table>
<thead>
<tr>
<th></th>
<th>FAME</th>
<th>FABE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid</td>
<td>15.62</td>
<td>18.73</td>
</tr>
<tr>
<td>Sterculic acid</td>
<td>nd</td>
<td>8.32</td>
</tr>
<tr>
<td>Nonadecenoic acid</td>
<td>70.32</td>
<td>45.64</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>14.06</td>
<td>20.42</td>
</tr>
</tbody>
</table>

nd = not detected

VOMM = Volatile oil of Monodora myristica
NOMM = Non-Volatile oil of Monodora myristica
NOMT = Non-Volatile oil of Monodora tenuifolia
FAME = Fatty Acid Methyl Ester
FABE = Fatty Acid Butyl Ester
Figure 2: Gas Chromatogram of the FAMEs of *Monodora myristica*
Figure 3: Gas Chromatogram of the FABEs of *Monodora myristica*
Figure 4: Gas Chromatogram of the FAMEs of *Monodora tenuifolia*
Figure 5: Gas Chromatogram of the FABEs of *Monodora tenuifolia*
Figure 6: Mass spectrum of methyl n-hexadecanoate of *Monodora myristica*
Figure 7: Mass spectrum of methyl linoleate of *Monodora myristica*
Figure 8: Mass spectrum of butyl n-hexadecanoate of *Monodora myristica*
Figure 9: Mass spectrum of butyl linoleate of *Monodora myristica*
Figure 10: Mass spectrum of butyl octadecanoate of *Monodora myristica*
Figure 11: Mass spectrum of methyl n-hexadecanoate of Monodora tenuifolia
Figure 12: Mass spectrum of methyl linoleate of *Monodora tenuifolias*
Figure 13: Mass spectrum of methyl nonadecenoate of *Monodora tenuifolia*
Figure 14: Mass spectrum of butyl hexadecanoate Monodora tenuifolia
Figure 15: Mass spectrum of butyl linoleate *Monodora tenuifolia*
Figure 16: Mass spectrum of butyl sterculate *Monodora tenuifolia*
Figure 17: Mass spectrum of butyl nonadecenoate *Monodora tenuifolia*
4.2 Discussion

*Monodora myristica* volatile oil has very light yellow colour and its non-volatile oil has light golden brown colour while *Monodora tenuifolia* non-volatile oil has dark brownish yellow colour. The oils of both seeds have strong appetizing odour with *Monodora tenuifolia* smelling like house hold maggi cube. *Monodora myristica* appears to be a good source of seed oil better than *Monodora tenuifolia* because it has 27.68% oil content.

The oil content of NOMM is higher than NOMT but lower than what is obtained in the literature probably because of steam distillation and loss during handling. Their refraction index and density were also relatively high suggesting high iodine value which may be the reason, the oils remained liquid during density determination even at about 5°C the oils were still liquid.

The gas chromatogram of the FAME of *Monodora myristica* (Figure 2), showed four compounds. Two were identified as Hexadecanoic acid methyl ester (i.e. methyl n-hexadecanoate) and linoleic acid methyl ester i.e methyl, 9,-12 – octadecaenoate at retention time of 16.77 and 17.90 respectively. The other two compounds were artefacts.

The mass spectrum of methyl n-hexadecanoate (Figure 6) showed a representative fragment at m/z 74 caused by Mc lafferty rearrangement. The molecular ion, M+ 270.2 is about 20% of the base peak confirming the molecular weight of the compound. There is also ion type +OCH3 (M-31) at m/z 239.2 which is usually present in low abundance but possess diagnostic value. There were long homologous series of related ions (14 amu apart) at m/z 87, m/z 101.0, m/z 129.1, m/z 199.1, m/z 143.1 with general formular [(CH2)n COOCH3]+ showing evidence that there were not likely to be other functional groups in the chain. There was an ion at m/z 227.2 (M-43)+ which represents loss of a C3 unit (carbons 2 to 4).
The mass spectrum of methyl linoleate (Figure 7) showed the molecular ion M⁺ at m/z 294.2 which corresponds to the molecular weight of methyl linoleate. There were also diagnostic peaks of general formular (CₙH₂ₙ-₃)⁺ accounting for m/z 67, m/z 81.1, m/z 95.1, m/z 123.1. There was also a peak at (M-31) m/z 263.2 representing loss of methoxyl group and confirming that it is indeed methyl linoleate.

The gas chromatogram of FABEs of Monodora myristica (Figure 3) three compounds were separated and identified as butyl n-hexadecanoate, butyl linoleate and butyl octadecanoate at retention times of 18.40, 19.42 and 19.53 respectively.

The mass spectrum of butyl n-hexadecanoate (Figure 8) showed a molecular ion, M⁺ at m/z 312.3 just 42 amu higher above methyl n-hexadecanoate confirming that it is a butyl ester of hexadecanoic acid. There was also Mc lafferty rearrangement at m/z 116.1, instead of m/z 74 due to the 42 amu difference between butyl ester and methyl ester of the same fatty acids. There was a diagnostic fragment ion type +OC₄H₉ (M-73) at m/z 239.3 and a homologous series of related ions (14 amu apart) with general formular [(CH₂)ₙCOOC₄H₉]+ accounting for m/z 129.1, m/z 171.1, m/z 185.1.

The mass spectrum of butyl linoleate (Figure 9) showed a molecular ion, M⁺ at m/z 336.2 just 42 amu higher above methyl linoleate confirming that it is a butyl ester of linoleic acid thus giving its molecular weight as 336.2. There was a diagnostic peak of a diene at m/z 67.0 and representative fragments of hydrocarbon ions of general formular (CₙH₂ₙ-₃)⁺ accounting for m/z 81.1, m/z 95.1, m/z 137.1. There was also (M-73) at m/z 263.2 due to loss of (+OC₄H₉)

The mass spectrum of butyl octadecanoate (Figure 10) showed the molecular ion, M⁺ at m/z 340.3 thus giving the molecular weight. There was a characteristics ion type +OC₄H₉ at (M-73) m/z 267.2 and representative fragments of general formular [(CH₂)ₙCOOC₄H₉]+ accounting for m/z 129.1, m/z 185.1, and m/z 241.2.

The gas chromatogram of FAMEs of Monodora tenuifolia (Figure 4), three compounds were separated and identified as methyl n-hexadecanoate (hexadecanoic
acid methyl ester), methyl linoleate (9, 12 – octadecadienoic acid methyl ester) and methyl nonadecenoate (Nonadecenoic acid methyl ester) at retention times of 16.77, 17.90 and 18.61 respectively.

The mass spectrum of methyl n-hexadecanoate (Figure 11) showed the molecular ion M$^+$ 270.2 about 20% of the base peak confirming the molecular weight of the compound. There were representative fragments of Mc lafferty rearrangement at m/z 74 and loss of methoxy $^+$OCH$_3$ (M-31) at m/z 239.2. There was a loss of carbons 2 to 4 at m/z 227.2. There were representatives of long homologous series of related ions (14 amu apart) with general formlar [(CH$_2$)$_n$ COOCH$_3$]$^+$ at m/z 87, m/z 129.1, m/z 143.1, m/z 171.1 and m/z 185.1 showing evidence that there was unlikely to be other functional groups in the chain.

The mass spectrum of methyl linoleate (Figure 12) showed the molecular ion, M$^+$ at m/z 294.2 confirming the molecular weight of the compound. There were diagnostic peaks of diene FAMEs at m/z 67, m/z 55, m/z 150.1 and also representative fragment hydrocarbon ions of general formlar (C$_n$H$_{2n}$-3)$^+$ accounting for m/z 81.1, m/z 95.1, m/z 109.1 and m/z 123.1. There was also a diagnostic peak at (M-31) m/z 263.2 confirming that the compound was a methyl ester.

The mass spectrum of methyl nonadecenoate (Figure 13) showed the molecular ion, M$^+$ at m/z 310 and a diagnostic peak at (M-31) m/z 279.2. There was a loss of Mc lafferty at (M-74) m/z 236.2. There were representative ions with general formlar (C$_n$H$_{2n}$-1)$^{+101}$ accounting for m/z 55, m/z 69, m/z 97.1 and m/z 111.1.

The gas chromatogram of FABEs of Monodora tenuifolia (Figure 5), four compounds were separated and identified namely; butyl hexadecanoate, butyl linoleate, butyl sterculate, and butyl, nonadecenoate with retention times of 18.40, 19.41, 19.56, 20.04. The peak with retention time 21.40 could not be identified.

The mass spectrum of butyl hexadecanoate (Figure 14) showed the molecular ion, M$^+$ at m/z 312.3 confirming the molecular weight of the compound. There was the
representative loss of ion type $^+\text{OC}_4\text{H}_9$ (M-73) at m/z 239.2 showing that it was a butyl ester. There were representative fragments of long homologous series of related ions (14 amu apart) with general formula $[(\text{CH}_2)_n\text{COOC}_4\text{H}_9]^+$ at m/z 129.1, m/z 171, m/z 185, m/z 199 and m/z 213 showing evidence that other functional groups are unlikely. There was a Mc lafferty rearrangement at m/z 116.1.

The mass spectrum of butyl linoleate (Figure 15) showed the molecular ion, $M^+$ at m/z 336.3 confirming the molecular weight. There were diagnostic peaks of diene at m/z 55, m/z 67, m/z 150.2 and a loss of Mc lafferty at m/z 220.2. There were representative fragment ions of general formula $(\text{C}_n\text{H}_{2n-3})^+$ accounting for m/z 81.1, m/z 95.1, m/z 109.1 and m/z 123.1. There was also a diagnostic peak (M-73) at m/z 263.2 confirming that it was a butyl ester.

The mass spectrum of butyl sterculate (Figure 16) showed the molecular ion, $M^+$ at m/z 352 confirming the molecular weight of the compound and also a loss of butoxy group $(\text{OC}_4\text{H}_9)^+$ (M-73) at m/z 279.2 confirming that the compound was a butyl ester of the fatty acid.

Having similar spectra with monoenes, there are hydrocarbon ions with general formular $(\text{C}_n\text{H}_{2n-1})^+$ accounting for ions at m/z 55, m/z 97.1, m/z 111.1 and m/z 125.1.

The mass spectrum of butyl nonadecenoate (Figure 17) showed the molecular ion, $M^+$ at m/z 352 confirming the molecular weight. There are diagnostic peak at m/z 279.3 as a result of loss of butoxy group, $(\text{OC}_4\text{H}_9)^+$ and a loss of MC lafferty at (M-116) m/z 236.2. There are representative fragment ions with general formular $(\text{C}_n\text{H}_{2n-1})^+$ accounting for m/z 55, m/z 69.1, m/z 83.1, m/z 97.1, m/z 111.1, m/z 125.1.

The gas chromatogram of the volatile oil of Monodora myristica (Appendix 1) showed no trace of fatty acids but what identified were terpenes like IR-α -Pinene, β -mycrene, α-phellandrene, p-cymene, D-Limonene, β -linalool etc.
Tables 2 & 3 showed that the use of two different types of esters of the same oil for GC-MS analysis gives more accurate and precise results. Some fatty acids which could not be detected in the FAME showed up in the FABEs suggesting that the particular fatty acid(s) may have been lost during transesterification or its percentage in the original sample was too small for esterification as FAME.

4.3 CONCLUSION

Evidently, both *Monodora myristica* and *Monodra tenuifolia* contains linoleic acid with myristica having higher amount thus a powerful potential commercial source of linoleic acid. Nursing and expectant mothers may eat it raw since their cynogenic glucoside content had been reported low.\(^{103}\)

Nevertheless, because of high percentage of nonadecenoic acid in the *Monodora tenuifolia* could serve as a replacement for oleic acid since both are members of n-9 family. It could also be good for the manufacturing of margarine and soap because of relatively high level of palmitic acid.
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