INVESTIGATION OF THE COMPONENT FATTY ACIDS OF SOME FRESHWATER FISHES COMMON IN NIGERIA AND A COMPARATIVE STUDY OF THEIR NUTRITIONAL QUALITY WITH THOSE OF MARINE FISHES

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A thesis report submitted to the Postgraduate School, Ahmadu Bello University, Zaria in partial fulfillment for the award of Master Degree in Organic Chemistry

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SEPTEMBER, 2008

DECLARATION

I declare that the work in the thesis report entitled 'Investigation Of The Component Fatty Acids Of Some Freshwater Fishes Common In Nigeria And A Comparative Study Of Their Nutritional Quality With Those Of Marine Fishes' has been performed by me in the Department of Chemistry, Ahmadu Bello University, Zaria under the supervision of DR. G. I. NDUKWE and DR. O. T. AUDU. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis report was previously presented for another degree or diploma at any university.

UGOALA Emeka

| --- | --- | --- |
| Name of student | Signature | Date |

CERTIFICATION

This thesis entitled INVESTIGATION OF THE COMPONENT FATTY ACIDS OF SOME FRESHWATER FISHES COMMON IN NIGERIA AND A COMPARATIVE STUDY OF THEIR NUTRITIONAL QUALITY WITH THOSE OF MARINE FISHES by UGOALA, EMEKA meets the regulations governing the award of the degree of Master of Science in Organic Chemistry of AHMADU BELLO UNIVERSITY, ZARIA and is approved for its contribution to knowledge and literary presentation.

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In conclusion, I will say that the world of tomorrow will result from the interplay of powerful forces when the cases for change, for alternatives that involve uncomfortable personal efforts, are argued as never before. But in all, the Creator of the universe has the final decision and unto Him be the glory, honour and majesty for the eventful transformation of mankind into a body of wisdom and knowledge.

UGOALA Nnaemeka

DEDICATION

Dedicated to CHIDIEBUBE, who was diagnosed with a heart defect, and CHINUA, who was born during the time of this work.

Also to the memory of my beloved grandfather, Late Pa BENSON OGBONNA NJOKU UGOALA, for encouraging and inspiring me to highest educational pursuits.

ABSTRACT

In the process of exploiting the economic potentials of fish as a source of quality protein among others, adequate identification and characterisation of the diverse fatty acids components of the fish species is very necessary. Therefore, the fatty acids compositions of freshwater fishes (*Hyperopisus bebe*, *Mormyrus rume*, *Mormyrops anguilloides*, *Oreochromis niloticus, Sarotherodon galilaeus, Clarias gariepinus, Cla*rias *anguillaris, Heterobranchus bidorsalis, Lates niloticus, Clariheterobranchus* and *Hydrocynus forskali*) was studied and compared with that of the twelve marine species. The fatty acids were determined by GC-MS and were identified using retention time locked methods and retention time databases. The fatty acids profiles include minor amounts of odd-number, branched-chain, and even-number fatty acids as well as saturated components, the MUFA and PUFA. The major SFA were C14:0 and C16:0. The C18:1 was the prominent MUFA. The dominant PUFA are of the Omega-6 series and are found chiefly in C18:2 fatty acids. The essential fatty acids compositions showed prominence in C18:3n-3 and C18:2n-6. The overall significance of this study was that the marine fish are better sources of Omega-3 EFA while freshwater fish are good source of Omega-6 EFA. The regular pattern of marine fish FA compositions makes them of advantage to the Hydrogenator. The high percent of branched and saturated FA in freshwater fish gives them an advantage in processing. A systematic review of the scientific medical literature was included, in order to appraise, and synthesize the evidences for the economic potentials accruable from these natural resources as regard their contents of EPA and DHA.

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ABBREVIATIONS AND SYMBOLS

CLA Conjugated Linoleic acid

DHA Docosahexaenoic acid

DNA Deoxyribonucleic acid

EFA Essential fatty acid

EPA Eicosapentaenoic acid

FAME Fatty Acid Methyl Ester

GC-MS Gas Chromatography- Mass Spectrometry
GM_1 Monosialoganglioside

IFFO International Fishmeal and Fish oil Organization
IgG Immunoglobulin G

IgM Immunoglobulin M

IQ Intelligent Quotient

MCFA Medium Chain Fatty Acid

MUFA Monounsaturated Fatty Acid

MVA Multi-Variate Analysis

OxPAPC 1-palmitoyl-2-arachidonoyl- sn-glycero-3-phosphorylcholine

PA Phosphatidic Acid

PAF Platelet-Activation Factor

PC Phosphatidylcholine

PCS Principal Component similarity analysis
PE phosphatidylethanolamine

PEG polyethylene glycol

PG Phosphatidylglycerol

PGPC 1- palmitoyl-2- glutaroyl- sn-glycero-3-phosphorylcholine

PI Phosphatidylinositol

POVPC 1-Palmitoyl-2- (5-oxovaleroyl)- sn-glycero-3-phosphorylcholine

PPARalpha Peroxisome Proliferator-Activated Receptor Alpha

PPARgamma Peroxisome Proliferator-Activated Receptor gamma

PS Phosphatidylserine

PUFA Polyunsaturated Fatty Acid

SCFA Short Chain Fatty Acid

SFA Saturated Fatty Acid

TNF Tumor Necrosis Factor

CHAPTER ONE
GENERAL INTRODUCTION

1.1. INTRODUCTION

Commodities have different elasticity with respect to the development of any economy. Therefore the progressive development of a nation depends on its recognition of the importance of each commodity to its developmental needs. This implies that information processing mechanisms capable of detecting trends, competitors, markets and technological developments relevant to economic development must be accorded priority. In this regard, the strength of research is therefore an essential weapon to strategic developmental plans.

Lipid research has in the recent past assumed a different dimension, thanks to a series of application successes. These successes have raised the stakes of lipids and as well brought high expectations for this emerging area of research. Although this current interest in lipids chemistry had been influenced to some degree by years of classical work in statistics, pattern recognition techniques and gas chromatography-mass spectrometry (GC-MS), a combination of factors have caused the recent resurgence of interest. These factors include, among others, the continuing impressive advances in technology, lipid biochemical and analytical methods, chemical synthesis of radioactive and fluorescent lipid analogs, quantitative fluorescence microscopy of living cells, laser scanning confocal microscopy, immunofluorescence-staining of cells, microinjection of antibodies, DNA, fluorescent probes into cells, tissue culture of various cell types, including human fibroblasts and rat lung epithelial cells, protein isolation-biochemistry, antibody production/purification, two-dimensional protein gel electrophoresis, gene cloning, sequencing, Northern-, Western-,

Southern blot analysis, protein over expression systems, cell transfection/expression of tagged proteins in living cells and computer graphics.

Adequate identification and characterisation of the diverse lipids is vital in the selection of the different lipids for specific purposes. The diglyceride and tocopherol contents of fats and oils could be used as indicators of quality, the levels of 5,8,11,14,17-eicosapentaenoic acid (EPA) and 4,7,10,13,16,19-docosahexaenoic acid (DHA) as well as the ratio of Omega-3 to Omega-6 in the oils could be used to compare their use in nutrition and health. The use of pattern recognition techniques based on the content of the respective fatty acids of the oils could be used to achieve correct classification.

The capacity of the lipid industry to extend itself beyond classical investigation could rapidly accelerate investments. Lipids could generate economic growth because of their varied subsidiary or ancillary activities that may impinge on them. Lipids have potential applications in the health, agriculture and industrial sectors. However, the problems of the industry in terms of its contribution to growth may stem not so much from the demand as from the supply side.

1.1.1 LIPIDS DISCUSSED

Lipids are fatty acids and their derivatives, and substances related biosynthetically or functionally to these compounds. Lipids consist of fatty acids esterified with alkanols to which other compounds may also be bound. Depending on the nature of the alkanols and the attached compounds, lipids are divided into different classes-phospholipids and neutral lipids. The neutral lipids (triglycerides) constitute the main part. The phospholipids serve in the structural and metabolic purposes in the body while the triglycerides are used mainly

for storage of energy as fat. Consequently, the content of a triglyceride may fluctuate depending on the energy status, while the content of phospholipids remains fairly constant.

Lipids are important dietary constituents because of their high energy value, fat soluble vitamins and the essential fatty acids which are found with the fat of natural foods.

1.2. CLASSIFICATION OF LIPIDS

1.2.1. PHOSPHOLIPIDS

The backbone of phospholipids is the same glycerol molecule that forms the backbone of triglycerides. Phospholipids consist of two fatty acids (a phosphate group and an alkanol). Phospholipids and cholesterol are the principal components of nearly all cell membranes, although different cells have varied quantities of phospholipids in their membranes. The alkanols portion of phospholipids protrudes away from the membrane, whereas the two fatty acids jut into the membranes. The middle fatty acid (in the second position) is usually unsaturated whereas the end fatty acid (in the first position) is usually saturated. Phospholipids include lecithin, cephalin, sphingomyelin, phosphatidyl inosital, cardiolipins and lysolecithins.

Sphingolipids include Sphingosine and Derivatives (Egg, Brain & Milk), D-erythro-Sphingosine, Sphingomyelin, Ceramides, Cerebrosides, Brain Sulfatides, Ganglioside - Porcine Brain. The Sphingosine Derivative (Soy Bean) is Glucosylceramide while the Phytosphingosine and Derivatives (Yeast) are Phytosphingosine D-ribo-Phytosphingosine-1-Phosphate, N-Acyl Phytosphingosine C2, N-Acyl Phytosphingosine C8 . N-Acyl Phytosphingosine C18 also belongs to this group.

The phospholipids occurring in fish are mainly lecithin and cephalin, which account for about 60% and 20% of the total phospholipids respectively (Young, 1986). In most marine fish, phospholipids have been found to be higher in their content of the polyunsaturated fatty acids (PUFA) C20:5 and C22:6 than the triglycerides from the same fish.

The phospholipids or phosphatides content in commercial fish oils are needed as emulsifying agents and as catalyst poison in hydrogenation of fish oils. The quantity of phospholipids present in oil is usually stated in terms of parts per million (ppm) or microgrammes per gramme (μg/g) of phosphorus found in the oil. The phosphorus content of fish oils is largely dependent on the efficiency of cleaning of the extracted oils.

Phosphatidyl cholines find applications as phosphalipase inhibitors, platelet-activating factors, central nervous system agents, cardiac agents and antitumour agents. This is due to the absence of reactive functional groups that would require protection in synthesis.

1.2.2. NEUTRAL LIPIDS

The neutral lipids constitute the main part of fish oils and may fluctuate depending on the energy status and contain less polyethene fatty acids but more of the monoethylenic fatty acids. They are used mainly for storage of energy as fat. Because they are uncharged glycerides, cholesterol, and cholesterol esters are termed neutral lipids.

1.2.3. DERIVED LIPIDS

These include substances derived from the above groups by hydrolysis of fatty acids (both saturated and unsaturated), glycerol, steroids, alkanols in addition to glycerol and sterols, fatty alkanals and alkanones.

Biologically relevant synthetic glycerol-based lipids include Phosphatidylcholine (PC), Phosphatidylethanolamine (PE), Phosphatidylserine (PS), Phosphatidylinositol (PI & PIP's), Phosphatidic Acid (PA), Phosphatidylglycerol (PG),Cardiolipin (CA), Diacylglycerides (DG), Cholesterol (Plant-Derived). *Head Group Modified Derivatives* PEG Lipids, Functionalized Lipids for Conjugation, Phospholipids with Multifarious Headgroups, Polyphenolic Lipids, Lipids for pH Sensitive Liposomes, Metal Chelating Lipids, Antigenic Phospholipids, Doxyl Lipids

Fatty Acid Modified Derivatives include Lyso Phospholipids (Monoacyl), Alkyl Phosphocholine, Oxidized Lipids, Biotinylated, Ether Lipids, Plasmologen Lipids, Diphytanoyl Phospholipids(Black Lipid Membranes), Polymerizable Lipids, Brominated Phospholipids, Fluorinated Phospholipids, Deuterated Lipids, Doxyl Lipids, Fluorescent Lipids

Glycerol Based are Choline (Phosphatidylcholine and Platelet-Activation Factor (PAF), Ethanolamine (Phosphatidylethanolamine), Glycerol (Phosphatidyl-DL-glycerol), Inositol (Phosphatidylinositol and Phosphatidylinositol (pi(4)p, pi(4,5)p2), Serine (Phosphatidylserine (Sodium Salt)), Cardiolipin (Cardiolipin (Sodium Salt)), Phosphatidic Acid, Egg Derived, Lyso (Mono Acyl) Derivatives (Lysophosphatides), Hydrogenated Phospholipids, Lipid Tissue Extracts (Brain & Egg, Escherichia Coli & Heart, Liver & Soy).

1.3.0. PROPERTIES OF LIPIDS

Lipids are found in all animal cell membranes and they play important roles in a wide variety of cellular functions, including cell-cell interactions, cell growth and differentiation, transformation, and signal transduction. Synthetic lipids are used for bilayer stabilization,

temperature/pH sensitive liposomal drug delivery, tumor imaging, and two-dimensional crystallization on lipid bilayers. To better understand the roles of

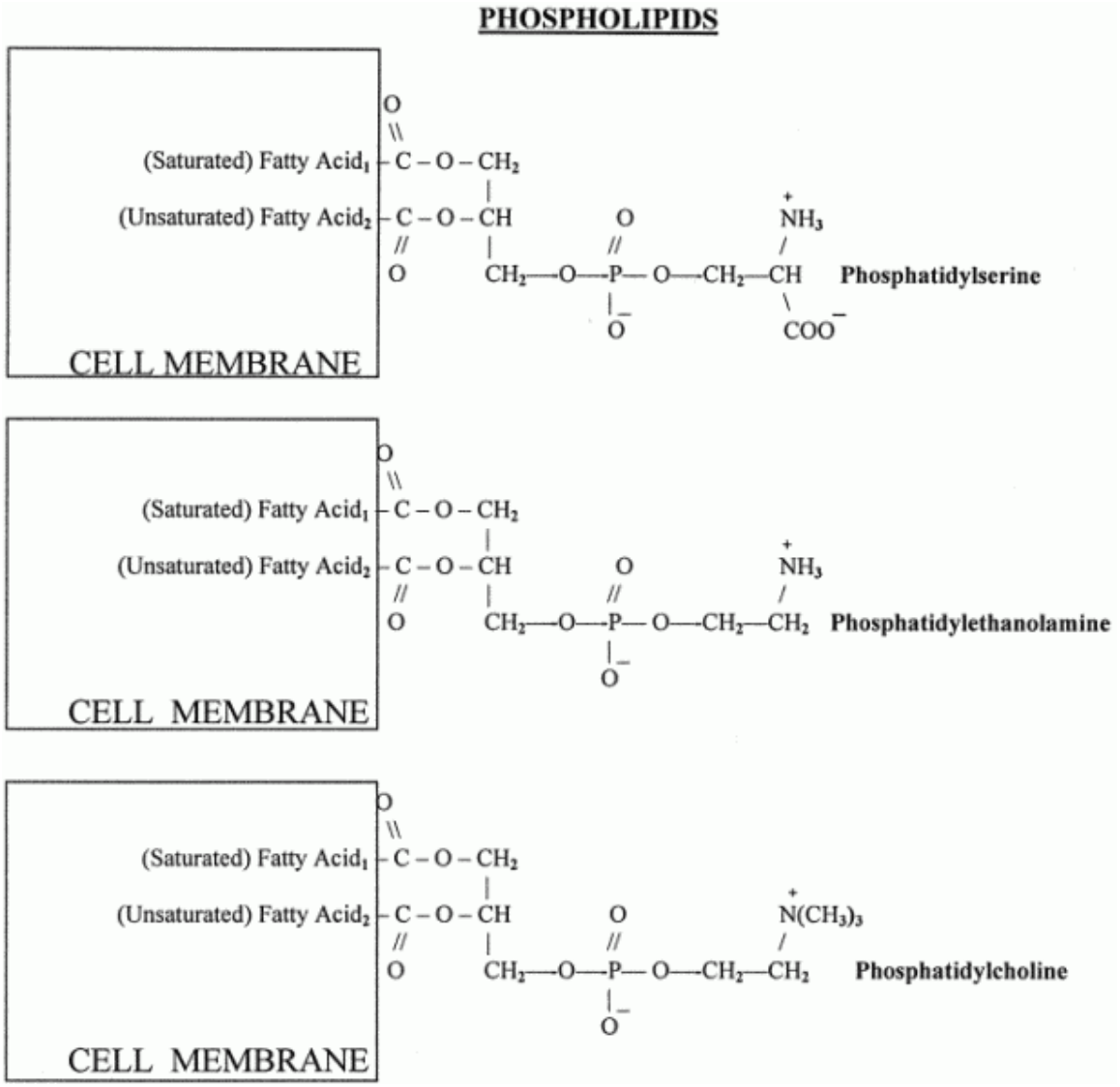


Fig 1.1: Phospholipids

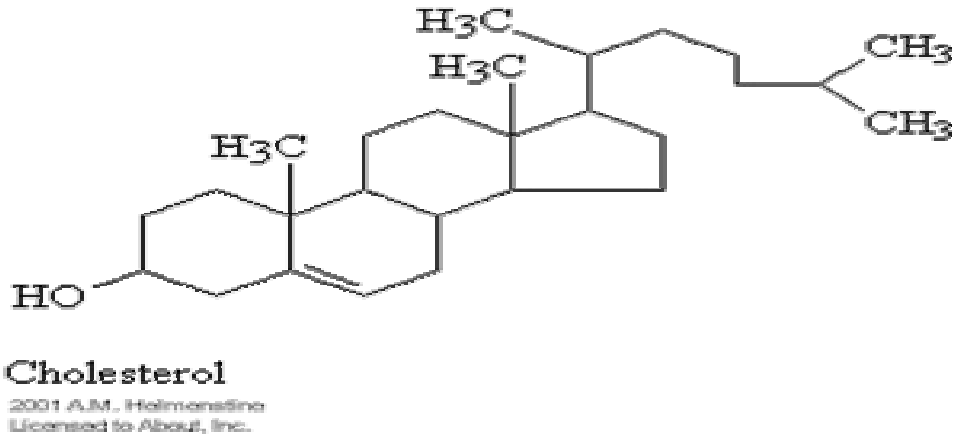


Fig. 1.2: Structure of Cholesterol

lipids in these complex processes, it is necessary to elucidate the mechanisms responsible for generating and maintaining the intracellular distribution of lipids in cell membranes.

In lipids, the presence of particular groupings is associated with activities of varying kinds but not a guarantee of biological activity. However individual chemical groupings can be of significance in two ways. They may be essential for the manifestation of a particular type of biological activity, due perhaps to a special type of chemical reactivity or stereochemical arrangement. They may not be replaced by alternate groupings without loss of their characteristic function unless the alternative groupings produce a molecule in which the chemical and physical properties are very close to those of the prototype. Secondly, individual groups may exert characteristic effects in modifying the intensity of a type of biological activity which is exhibited in compounds having a common molecular basis.

When a particular grouping is responsible through its chemical reactivity for producing biological activity, it is important to bear in mind the quantitative aspect of chemical reactivity. If the grouping is very reactive, the substance may react easily with a variety of cell constituents, perhaps even with water and in consequence it may not be able to reach a

site within the organism where a specific cell constituent can be affected. At the other extreme, if the chemical reactivity is too low, biological activity will be diminished, perhaps to the point of disappearance (Ross, 1958).

Alkyl groups may have a determining effect on the physical properties of an organic substance. The longer the chain or in the case of branched chains the higher the molecular weight, the greater the effect. In cases where biological activity is largely influenced by the physical properties of the substance, such as its solubility, diffusibility or surface activity, the nature of the alkyl group will largely influence the biological activity. The methyl group is unique among the alkyl groups because it plays a special part in metabolism. It can also affect biological activity by the replacement of active hydrogen atoms, thus distorting the general chemical reactivity of the molecule, either by blocking chemically reactive positions or by activating other positions (Preussmann, 1958).

The function of hydroxyl groups in biologically active compounds may depend whether the OH group confers or modulates activity by virtue of its effect on physical properties or whether it undergoes a significant chemical reaction. The substitution of OH for H affects biological activity profoundly. Polyhydroxyl compounds are characterized by their capacity to enter into union with other molecules, and within themselves, by H-bonding. Such unions can by the stereochemical orientation of the OH groups determine such properties as specific enzyme affinities and, at the intramolecular level. Also OH groups' reactions are based on their capacity to accept activated groups through the action of group-transferring enzymes (Belleau, 1959).

The physicochemical properties of molecules are fundamentally changed by the introduction of COOH group. These changes may result in the creation of conditions necessary for biological activity or for altering the nature of the biological effect, or they may have the reverse effect, destruction of any activity which was originally present (Belleau, 1959).

Unsaturation in compounds may influence its stereochemical considerations especially where the possibility exists in the unsaturated compound of *cis-trans* isomerism. One of the isomers of the unsaturated form may have some characteristic activity which is either not shown by the saturated substance or is only shown to a diminished degree. Unsaturation in compounds may also affect their physical properties. Chemically, an isolated double bond does not appear to possess a sufficient degree of reactivity to enter into ready unions with cell constituents, for if it were otherwise, the unsaturation hydrocarbons might be expected to exhibit a greater variety of actions than they actually do. However, when the double bond is chemically activated by a neighbouring group, a different state of affairs prevails (Brookes, 1960).

1.4.0. PRODUCTION OF LIPIDS

Fish oil is usually produced by the wet-rendering process, which involves cooking the fish with steam to rupture the fat cells and pressing the cooked fish to separate the lipids fraction from the solid fraction. The solid fraction is dried to produce fishmeal while the liquid fraction is processed into crude fish oil.

1.4.1. HEALTH FOOD-GRADE FISH OILS

Health food-grade fish oils are usually produced from small short-lived fish (sardines and anchovies). This is because these short-lived fishes are less prone to accumulated environmental pollutants. Most health-food grade fish oils are fish body oils extracted from the fish.

A slightly more purified type of health-food grade fish oil includes those that have been subjected to a very limited amount of molecular distillation to remove some of the cholesterol in order to be labelled 'cholesterol-free'. Another type of higher-grade health-food fish oil is known as "fish oil concentrate". This type of fish oil consists of ethyl esters of the fish oil that has been subjected to fractional cooling. The more saturated fats will solidify, leaving behind a more concentrated solution of long-chain omega-3 fatty acids. However, this fractional cooling does not remove the organic pollutants or the long-chain monoene fatty acids that give rise to significant gastric distress. A one-gram capsule of this thermally fractionated health-food grade fish oil will contain up to 500 mg of long-chain omega-3 fatty acids. Health-food grade fish oils are therefore acceptable in small amounts while higher doses require a much more purified type of oil; ultra-refined fish oil.

1.4.2. MANUFACTURE OF PHARMACEUTICAL GRADE FISH OIL CONCENTRATES (FISH OIL DERIVATIVES)

Producing pharmaceutical-grade fish oil concentrates requires state-of-the-art manufacturing capabilities to assure maximum 5,8,11,14,17-eicosapentaenoic acid (EPA) and 4,7,10,13,16,19-docosahexaenoic acid (DHA) purity and concentration of long chain omega-3 fatty acids essential for eicosanoid modulation. A variety of fish oil derivatives include interesterified glycerides, resynthesized triglycerides, mixed methyl or ethyl esters,

mixed fatty acids, concentrates of Omega-3 fatty acids, and pure compounds of ester or fatty acid form. The production of these derivatives requires further processing steps as shown in Figure 1.3

1.5.0. AIMS OF PROJECT

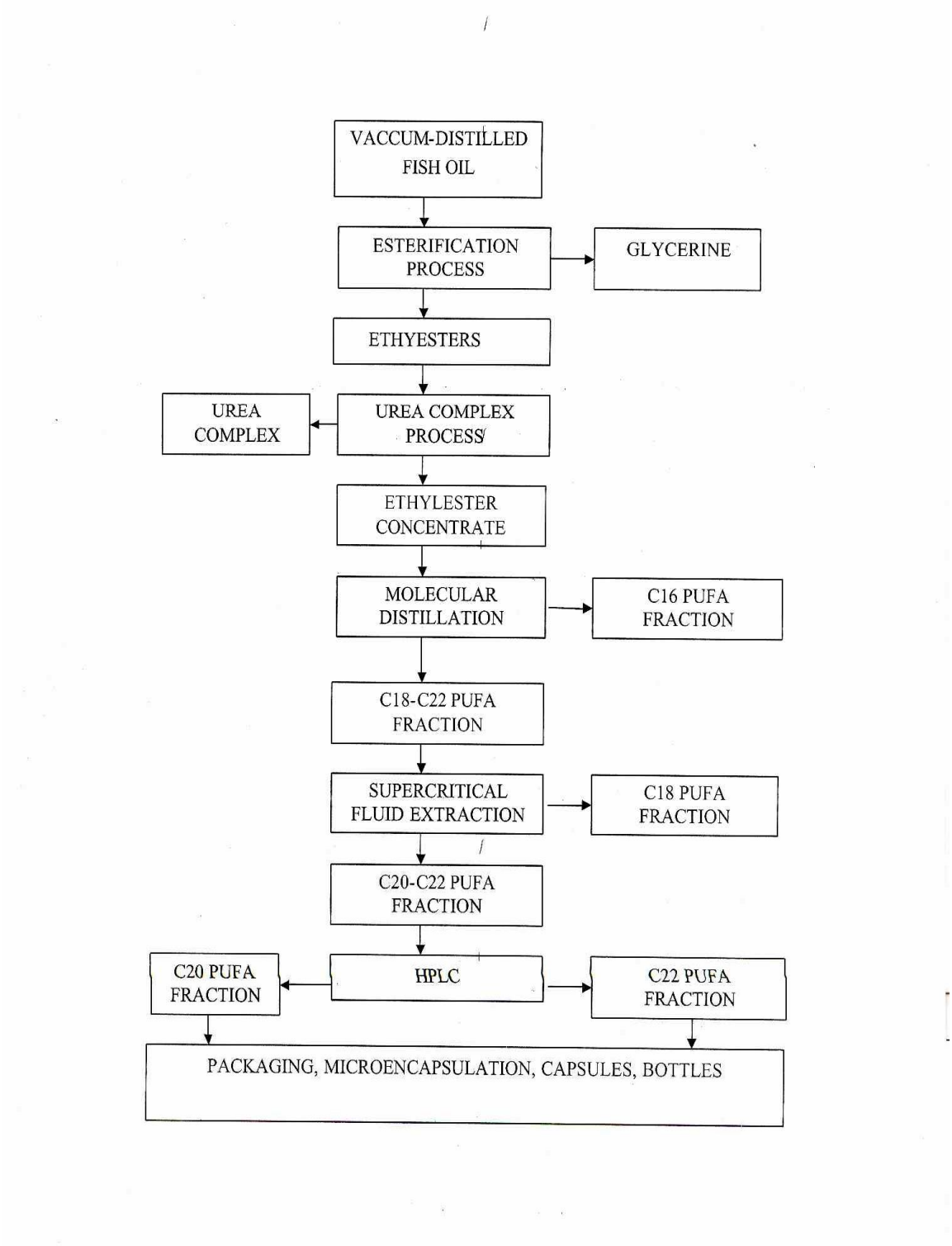
1. To compare the nutritional quality of freshwater fish oil with that of marine fish oil by comparing the levels of essential fatty acids present.
2. To characterise some Nigerian freshwater fish oils through the identification of the component fatty acids of the triglyceride molecule as well as the branched chain fatty acids
3. To identify oils that contained a mixed fatty acids which might have a common value as well as evaluate differences in fatty acids composition among species
4. To encourage the massive cultivation of fish as an employment generation strategy for the populace
5. To systematically review the evidences to aid in the development of a research agenda for the possible health benefits of Omega-3 fatty acids.

1.6.0. JUSTIFICATION FOR THE STUDY

Since fish is recognised as a health promoting food and the cheapest source of quality proteins to man and animals, there is need to develop technologies to help harness this resource. But to foster any appreciable technological growth from this resource, the characteristics, chemical and physical composition must be studied and known. The determination of the basic components of this resource is therefore, fundamentally important for the application of different technological processes in harnessing the diversity of usage of Nigerian freshwater fish oils. Fish oil development will generate general

economic growth because of the varied subsidiary or ancillary activities in the economy like labour force and the manufacturing sector that will impinge on it.

Fig 1.3. Production of pharmaceutical-grade fish oil (derivatives and concentrates).



When the fundamental fatty acids distribution patterns found in natural triglycerides are deciphered, it may well be possible to estimate the composition of many fish triglyceride mixtures directly from their fatty acids composition.

This study may also help in the detection of adulterated fish oil, identification of specific triglycerides causing graininess in shortenings and margarines, and the identification of specific triglycerides giving some fish oils unique suitability for confectionary purposes.

1.7.0. SCOPE OF THE STUDY

In order to achieve the set objectives, this study will determine or analyse some freshwater fish oils for the following parameters:

1. Content and type of triglycerides like the saturated, monounsaturated, polyunsaturated and branched fatty acids in the fish oils.
2. In order to identify the economic importance as well as the current research direction of lipids research, the scientific medical database will be thoroughly searched for evidence to that effect.

CHAPTER TWO

LITERATURE REVIEW

2.1. INTRODUCTION

Information is a basic building block necessary for the rational management, growth and optimum use of any resource. Relevant and up-to-date information propel the process of decision-making. This is sequel to the fact that the influx of ideas on a subject in some areas does provide new perspectives and stimulates new thinking about potential alternatives on same subject in other ways. Therefore, exploring a technical subject usually means exploring the literature; whether historical background to a problem or results of most recent research in a field is needed.

2.2. FRESHWATER FISH AND QUALITY

Fish, as a perishable commodity, is rich in proteins, lipids, minerals and vitamins. However these nutrients vary with species and varieties and can deteriorate very rapidly during processing. Oladapo *et al* (1984) observed that the amino acid compositions of traditionally smoked (TS), traditionally solar dried (TD), oven dried (OD), Ife solar dried (ISD) of *Clarias lazera, Sarotherodon niloticus, Sarotherodon galilaeus, Tillipia zilli* and *Hemichromis fasciatus* decreased in percent of available lysine in the order OD≈ISD>TS>TD. They however noted no systematic variation in the fatty acid compositions. Analysis of nucleotides and nucleotide related products revealed no systematic variation in the individual nucleotide content. However a pooled percent of the total nucleotides shows that TD has the highest value of hypoxanthine.

2.3.0. LIPID COMPOSITION OF FRESWATER FISH

Achionye-Nzeh and Omoridion (2002) studied the lipid composition of the fishes *Heterotis niloticus, Brycenus nurse, Gnathonemus cyprinoids* and *Sarotherodon galilaeus* and noted that *Heterotis niloticus* had the lowest lipid content (13%) while *Gnathonemus cyprinoids* had a lipid content of about 26%. The common neutral lipids were cholesterol, free fatty acids and cholesterol esters, while diphosphatidyl glycerol, phosphatidyl glycerol and phosphatidyl ethanolamine were the most predominant phospholipids.

2.4.0. IMPORTANT FATTY ACIDS

POLYUNSATURATED FATTY ACIDS (PUFA). These are long chain fatty acids (C14 to C24). Long chain fatty acids (LCFA) are absorbed and metabolized more slowly than either medium chain fatty acids (MCFA) (C6 to C12) or short chain fatty acids (SCFA) (C2 to C6); much of the LCFA may be lost as calcium fatty acid soap in the feces (Boon *et al*, 2002).

Fatty acid that cannot be synthesized by the body from other fatty acids and therefore must be obtained from food is called Essential Fatty Acids (EFA). The human body lacks the desaturase enzymes required for their production. EFA is classified into two families: Omega-3 and Omega-6.

These two families are the result of increasing chain length and of forming double bonds. They compete for same enzymes for forming double bonds (desaturase enzymes) and enzymes for lengthening the carbon chain (elongase enzymes). Elongase enzymes always add carbon atoms (in pairs to the delta end of the fatty acids). Tilapia possess enzymes that desaturate and elongate fatty acids of the Omega-3 and Omega-6 series to provide

sufficient levels of the long chain polyunsaturated fatty acids necessary for membrane function and fluidity (Kanazawa, 1980, Takeuchi, 1983)

CONJUGATED LINOLEIC ACID (CLA). This is a naturally occurring fatty acid with a conjugated double bond and has a number of documented beneficial properties for humans and animals. The hydrogenation of vegetable oils with high quantity of CLA might provide the possibility for their utilization as health-promoting food ingredients. However, Watkins *et al* (1996) are of the opinion that commercial sources of food grade quantities of this unusual non-methylene interrupted fatty acids will be required before the therapeutic benefits are realized in humans.

TRANS FATTY ACIDS (TFA). TFA are unsaturated fatty acids with at least one double bond in the Trans configuration. The smaller double bond angle and a linear acyl chain results in a more rigid and straight molecule with a high melting point that approaches a corresponding saturated fatty acids. The structural similarity with saturated fatty acids, lack of specific metabolic functions of their parent polyunsaturated fatty acids and its competition with essential fatty acids in many complex metabolic pathways increases low density lipoprotein (LDL) to a degree similar to that of saturated fats but reduces high density lipoprotein (HDL) cholesterol (Mann, 1994). High density lipoprotein picks up excess fats and carries them to the liver while low density lipoprotein drops fats on blood vessel walls rather than carry them to the cells where they are needed. Trans fatty acids show competitive interactions with essential fatty acids metabolism by inhibiting its incorporation into membrane phospholipids and by reducing the conversion of essential fatty acids to eicosanoids in different cells. Therefore, trans fatty acids ingestion results in essential fatty acids deficiency. However, the incorporation of trans fatty acids into

membrane phospholipids may influence the physical properties of the membrane as well as the activities of the membrane-associated enzymes (Holman *et al*; 1991)

Breast-fed infants have a greater intake of trans fatty acids through their mothers' milk (2-5% of total milk fatty acids) which reflects the maternal consumption of these fatty acids (Koletzko *et al*; 1988).

2.5.0. CURRENT RESEARCH DIRECTIONS IN LIPIDS

2.5.1. CRITERIA FOR LITERATURE REVIEW

The following online databases were searched to identify literature: Cyberlipids, International Fishmeal and Fish Oil, Agency for Healthcare Research and Quality, MEDLINE®, PreMEDLINE®, EMBASE, Cochrane Centre Register of Controlled Trials, CAB Health.

All articles identified through the literature search were screened using eligibility criteria as follows:

1. Studies that had to describe a difference between Omega-3 fatty acid content in study arms for all study designs except case series and describe the effect of Omega-3 fatty acid on any of the diseases
2. Studies that evaluated potential interactions between Omega-3 fatty acid supplements and commonly used drugs, including but not limited to hormone replacement therapy, medications, aspirin, and anticoagulants.
3. Studies that had to investigate at least one pertinent clinical outcome (e.g., symptom improvement, incidence of a disorder).

4. Specific sources of Omega-3 fatty acid considered acceptable included fish oils, dietary fish, canola (rapeseed) oil, soybean oil, flaxseed or linseed oil, walnuts or walnut oil, and mustard seed oil. Other sources were considered if Omega-3 fatty acid levels were reported to be greater than the control.
5. Human controlled clinical trials (randomised and non-randomised), prospective cohort studies, case control studies, and case series were included.
6. Studies that described human populations of any age, involved any type of study design, and investigated the use of any food or extracts known to contain Omega-3 fatty acid as a treatment, primary or secondary prevention.
7. Studies that examined the association between dietary Omega-3 fatty acid intake and tissue levels of Omega-3 fatty acid to the larger randomised trials that met eligibility criteria for either intermediate or clinical outcomes.

The results of the review are presented below.

2.5.2. THE IMPACT OF DIETARY SOURCES OF POLYUNSATURATED FATTY ACIDS ON THE FA COMPOSITION OF BRAIN, LIVER AND REDBLOOD CELLS

Royneberg (2005) examined the effects of Omega-3 polyunsaturated fatty acids of different marine origin and the incorporation of 5,8,11,14,17-eicosapentaenoic acid (EPA) and 4,7,10,13,16,19-docosahexaenoic acid (DHA) in rat brain, liver and red blood cells (RBC). She observed that the fatty acids profile changed profoundly in the liver and RBC of animals receiving marine Omega-3 polyunsaturated fatty acids compared to Omega-6 diets, whereas the changes in the brain were only moderate. Animals fed the marine diets showed increased levels of EPA and DHA with a concomitant lower level of Omega-6 fatty acids compared to animals fed vegetable diets. The brain and liver responded to the structural

forms of the fatty acids whereas the RBC responded in a dose dependent matter. The brain, the liver and the RBC differed in their response to the different diets. The results showed that the incorporation of EPA and DHA from capsules containing the fatty acids as ethyl esters was poor while the incorporation from natural sources was better.

2.5.3. ANTIINFLAMMATORY PROPERTIES OF FATTY ACIDS

Oxidized 1-palmitoyl-2-arachidonoyl- sn-glycero-3-phosphorylcholine (OxPAPC), a component of minimally modified low density lipoprotein (MM-LDL), activates endothelial cells to bind monocytes. 1-Palmitoyl-2-5-oxovaleroyl)-sn-glycero-3-phosphorylcholine (POVPC) and 1-palmitoyl-2-glutaroyl-sn-glycero-3-phosphorylcholine (PGPC) is a potent regulator of monocyte-specific endothelial interactions and play a dominant role in a number of chronic inflammatory processes where oxidized phospholipids are known to be present. Structurally similar oxidized phospholipids differentially regulate endothelial binding of monocytes and neutrophils.

Vaccines: Vaccine development is dependent on immunological adjuvants that mediate and promote a wide variety of immune responses. The immunological response to detoxified lipid A is dependent on the vehicle formulation. Either an aqueous vehicle or an oil-in water emulsion can be used to modulate the vaccine strategies being investigated today.

Laboratory animals fed with diets rich in Omega-3 fatty acid (linseed or fish oil) were found to have a reduction in natural killer cell and cytotoxic T-lymphocyte activity (Peterson., 1998), but stimulates the more antigen-specific immunoglobulins lgM and lgG (Watanabe, 1994). An experiment showed that both fish oil and safflower oil reduced the

secretion of interleukin-6 (a cytoxine that activates lymphocyte immune-cells and increases antibody production), but that only fish oil inhibited the secretion of Tumor Necrosis Factor alpha (a cytokine that increases fever, shock and blood vessel permeability) (Helmut, 1994).

2.5.4. EFFECTS OF FATTY ACIDS ON LIPIDS AND GLYCEMIC CONTROL IN TYPE II DIABETES AND THE METABOLIC SYNDROME AND ON INFLAMMATORY BOWEL DISEASE, RHEUMATOID ARTHRITIS, RENAL DISEASE, SYSTEMIC LUPUS ERYTHEMATOSUS, AND OSTEOPOROSIS

The quantity and strength of evidence for effects of Omega-3 fatty acids on outcomes in the conditions assessed varies greatly. The findings of many studies among type II diabetes provide strong evidence that Omega-3 fatty acids reduce serum triglycerides but have no effect on total cholesterol, high density lipoprotein cholesterol, and low density lipoprotein cholesterol. For rheumatoid arthritis, the available evidence suggests that Omega-3 fatty acids reduce tender joint counts and may reduce requirements for corticosteroids, but does not support an effect of Omega-3 fatty acids on other clinical outcomes. There are insufficient data available to draw conclusions on the effects of Omega-3 fatty acids on inflammatory bowel disease, renal disease, bone density, or fractures or the effects of omega-3 fatty acids on insulin resistance among type II diabetes (MacLean *et al*, 2004).

2.5.5. EFFECTS OF FATTY ACIDS ON CARDIOVASCULAR DISEASE

The intake of Omega-3 fatty acids in any population varies. A number of studies offer evidence to support the hypothesis that fish, fish oil, or 9,12,15-octadecatrienoic acid (18:3n-3) supplement consumption reduces all-cause mortality and various cardiovascular disease outcomes, although the evidence is strongest for fish or fish oil. The adverse events

due to fish oil or 9,12,15-octadecatrienoic acid supplement consumption appear to be minor. The specific effects on different cardiovascular disease outcomes, especially stroke and myocardial infarcation are uncertain (Wang *et al*, 2004).

However studies were heterogeneous with regard to the methods of estimating fish or Omega-3 fatty acid intake, background diets, and background risk for heart disease. Furthermore, the optimal quantity and type of Omega-3 fatty acid, and the optimal ratio of Omega-3 to Omega-6 fatty acid, if any, still remain undefined. Different types of fish and the method of food preparation may cause different effects. The potential effect of 9,12,15-octadecatrienoic acid is unknown. The relative effect of 9,12,15-octadecatrienoic acid versus fish oil is also unknown.

2.5.6. EFFECTS OF FATTY ACIDS ON CARDIOVASCULAR RISK FACTORS AND INTERMEDIATE MARKERS OF CARDIOVASCULAR DISEASE

Numerous studies have examined the relationship between dietary fat and cardiovascular disease. Most early epidemiology studies noted very low cardiovascular mortality in populations with high fish consumption. The apparent benefit of dietary fish is explained by the intake of very long chain, highly polyunsaturated Omega-3 fatty acids. There is strong evidence that fish oils have a strong beneficial effect on triglyceride that is dose-dependent. There is also evidence of a very small beneficial effect of fish oils on blood pressure and possible beneficial effects on coronary artery restenosis after angioplasty, exercise capacity in patients with coronary atherosclerosis, and possibly heart rate variability, particularly in patients with recent myocardial infarctions. No consistent beneficial effect is apparent for other cardiovascular disease risk factors or intermediate

markers. However there is also no consistent evidence of a detrimental effect of Omega-3 fatty acids on glucose tolerance (Balk *et al*, 2004).

2.5.7. HEALTH EFFECTS OF FATTY ACIDS ON ASTHMA

Omega-3 fatty acid supplementation intended to influence asthma has little probability of harm beyond occasional mild discomfort. Troublesome events are produced by the delivery of the oils in large numbers and sizes of capsules Lack of sufficiently consistent evidence suggests that no definitive conclusion can yet be drawn regarding the efficacy of Omega-3 fatty acid supplementation as a treatment for asthma in children and adults. Likewise, nothing specific can be concluded regarding the role of specific sources, types, or doses of Omega-3 fatty acid content in producing significant clinical effects (Schachter *et al*, 2004).

2.5.8. EFFECTS OF FATTY ACIDS ON CANCER

An increasing number of physiological functions have been attributed to Omega-3 fatty acids, including movement of calcium and other substances into and out of cells, relaxation and contraction of muscles, and regulation of clotting and secretion of substances that include digestive enzymes and hormones. Omega-3 fatty acids also play a role in the control of fertility, cell division, and growth, suggesting they may protect against certain types of cancer or may alter the response to cancer treatment (Jones and Papamandjaris, 2003; James *et al*, 2000). However, studies carried out by MacLean *et al* (2005) showed that Omega-3 fatty acids do not appear to decrease overall cancer risk.

Conjugated 9,12-octadecadienoic acid prevents or cure cancer (Ha *et al*. 1987; Liew *et a*l, 1995; Ip *et al*, 1996), atherosclerosis (Lee *et al*, 1994), and type II diabetes (Houseknecht *et al* 1998), because of their unique chemo protective and antioxidant actions. Conjugated 9,12-octadecadienoic acid may regulate cytokines production to strengthen muscle and

bone activity in humans (Cook *et al,* 1993). Fat partitioning activity of Conjugated 9,12-octadecadienoic acid reduces fat synthesis in mice and abdominal circumference in humans (Park *et al*, 1999; Riserus *et al*, 2001).

Phosphatidyl cholines find applications as phosphalipase inhibitors, platelet-activating factors, central nervous system agents, cardiac agents and antitumour agents. This is due to the absence of reactive functional groups that would require protection in synthesis.

2.5.9. EFFECTS OF FATTY ACIDS ON COGNITIVE FUNCTION WITH AGING, DEMENTIA, AND NEUROLOGICAL DISEASES

Omega-3 fatty acids may play an important role in brain development and function (American Dietetic Association, 2003). 4,7,10,13,16,19-docosahexaenoic acid is the precursor to a newly described metabolite called 10,17S-docosatriene, which is part of a family of compounds called resolvins (Marcheselli *et al*, 2003; Serhan *et a*l, 2002). They are released in the brain in response to an ischemic insult and counteract the pro-inflammatory actions of infiltrating leukocytes by blocking interleukin 1-beta-induced NF-kappaB activation and cyclooxygenase-2 expression. 4,7,10,13,16,19-docosahexaenoic acid also plays a role in retinal rod outer segments by influencing membrane fluidity so as to optimise G protein coupled signalling (Hong *et al,* 2003; Niu *et al*, 2004).

Total Omega-3 fatty acid consumption and consumption of 4,7,10,13,16,19-docosahexaenoic acid (DHA) (but not 9,12,15-octadecatrienoic acid or 5,8,11,14,17-eicosapentaenoic acid (EPA) were associated with a significant reduction in the incidence of Alzheimer's and child's cerebral palsy.

2.5.10. EFFECTS OF FATTY ACIDS ON EYE HEALTH

The brain and eye are highly enriched with Omega-3 fatty acids which accumulate in these tissues during late foetal and early neonatal life (Innis, 2003). Very high levels of 4,7,10,13,16,19-docosahexaenoic acid (DHA) are present in the retina, especially in the disk membranes of the outer segments of photoreceptor cells. DHA accounts for over half the total fatty acyl groups present in the phospholipids of rod outer segment membranes, a proportion higher than that is found in any other tissues (Stone *et al*, 1979). Its specific role, however, is not well understood. The role of DHA may be related to its biophysical effects on the cell membrane. DHA influences the biophysical properties of membranes via its high polyunsaturation, and may help to create a membrane that accommodates the dynamic behaviour of rhodopsin during the photoreceptive process (Gibson and Brown, 1993; Brown, 1994; Litman *et al*, 2001). In addition, DHA may modulate the activity of membrane bound enzymes and receptors, and the kinetics of membrane transport systems, as well as being a precursor for the syntheses of other biologically active molecules.

2.5.11. EFFECTS OF FATTY ACIDS ON MENTAL HEALTH

The mechanism by which diet may affect health, including depression or cardiovascular disease, has been thought to involve low levels of Omega-3 fatty acid content in biomarkers like the red blood cells (Edwards *et al*, 1998; Peet and Horrobin, 2002). An Omega-3 fatty acid deficiency hypothesis of depression has been put forward, which has helped justify treatment with Omega-3 fatty acid supplementation (Horrobin and Bennet, 1999). The membrane phospholipids hypothesis of schizophrenia has been proposed in an attempt to develop a model explaining its etiology (Horrobin, 1998). It described the presumed biochemical dynamics underpinning a neurodevelopment theory. Some of the evidence

used to substantiate this perspective suggests the existence of phospholipids and polyunsaturated fatty acid metabolic abnormalities in schizophrenia (Horrobin, 1998; Mahadik *et al*, 1994; Fukuzako *et al*, 1999). It has been posited that modifications to diet could mitigate or even aggravate an underlying abnormality of phospholipids metabolism (Horrobin, 1998).

The 4,7,10,13,16,19-docosahexaenoic acid plays a very important role in brain development and the maintenance of normal brain function in adults (Horrocks and Yeo, 1999). Impaired development of brain and visual acuity, reduced intellectual capacity in infants can also be prevented by this fatty acid. 4,7,10,13,16,19-docosahexaenoic acid (DHA) increases the IQ of children at age four by an average of 4.1 points (Helland *et al;* 2003). 4,7,10,13,16,19-docosahexaenoic acid is preferentially taken up by the brain compared to other fatty acids, where it then enhances membrane fluidity, which in turn changes the signalling properties of neurons and affects the function of the blood brain barrier (Farkas *et al*; 2002).

2.5.12. EFFECTS OF FATTY ACIDS ON BODY COMPOSITION

Fish oil is also beneficial in humans by its improvement in body composition by activation of peroxisome proliferator-activated receptor alpha (PPARalpha) and peroxisome proliferator-activated receptor gamma (PPARgamma), which in turn increases the production of various enzymes that break down fat (Tsuboyama-Kasarka *et al*; 1999; Jang *et al* 2003; Nakatani *et al* 2002). It also deactivates a number of lipogenic (fat forming) enzymes (Jang *et al* 2003; Nakatani *et al* 2002; Kim *et al* 2003).

Biotin phosphatidylethanolamine (PE) has been used for bilayer stabilization, temperature/pH sensitive liposomal drug delivery (Ferraretto *et al*; 1996), tumor imaging (Ogihara-Umeda *et al*; 1994), two-dimensional crystallization on lipid bilayers (Darst *et al*; 1991, Hemming *et al*; 1995, Qin *et al*; 1995) and immobilization of liposomes on gel beads for chromatographic analysis of drug-membrane partitioning (Yang *et al*; 1998). Its use for *in vivo* targeting applications utilizing liposomes containing monosialoganglioside (GM_1) or polyethylene glycol (PEG) lipid derivatives has also been studied.

2.5.13. EFFECTS OF FATTY ACIDS ON AGRICULTURE

There is increasing evidence that inclusion of fish lipids in the diet of animals also improves their health. Both fish oil and fish meal supply lipids rich in Omega-3 fatty acids. In poultry, balancing Omega-6: Omega-3 fatty acids composition were reputed to have had the following improvement results viz

1. The Specific immunity was increased and immune reaction was moderated towards better growth and feed conversion (Billiar *et al.,* 1988; Fritsche *et al.,* 1991).
2. Improves bone development (Watkins *et al.*, 1996)
3. Improves broiler performance and also condemnation of carcasses from sepsis, inflammatory processes and cellulites were significantly lower with fish meal (Pike, 1999).
4. Improves fertility rates and hatchability of eggs (Blesbois *et al.,* 1997;Pike, 1975).

Hen eggs for example contain excellent, low cost high quality protein and a balanced distribution of minerals and vitamins (Moreng and Avens, 1985; Shrimpton, 1987). These eggs have relatively high cholesterol content and attempts at reducing the cholesterol content have not been successful (Naber, 1983; Hargis, 1988). However, the fatty acids

composition and contents of the fat-soluble vitamins and certain trace minerals can be altered by diet (Jiang *et al.,* 1994). Relatively high 4,7,10,13,16,19-docosahexaenoic acid (DHA) levels in the egg can be achieved by feeding poultry with fish oil, fishmeal or algal products without adversely affecting flavour (Van Elswyk, 1995; Maurice, 1994; Farrell, 1993).

In livestock, fatty acids are beneficial in the following regard

1. Better litters with fish oil feeding of sows Improves resistance to diseases in growing pigs and in ruminant (Miller and Newby, 1984; Miggdal and Kaczmarczyk, 1991; Silflow *et al.,* 1992; Van Houtert *et al.,* 1995)
2. Improves fertility (Bruckental, 1989; Armstrong *et al.,* 1990).

Fish oil and/or fishmeal can be used to introduce and/or boost 4,7,10,13,16,19-docosahexaenoic acid (DHA) and 5,8,11,14,17-eicosapentaenoic acid (EPA) levels in meat and eggs. The level of these fatty acids in the diet will determine levels in the meat. Care has to be taken to stabilise the diet with antioxidant, and to supplement it with vitamin E so as to prevent oxidation.

Fish lipids are more effective generally than vegetable lipids as source of EPA and DHA. Vegetable lipids tend to deposit C18 Omega-3 (9, 12, 15 – octadecatrienoic acid), which has limited use in today's human diet. Algal lipids are effective concentrated sources of EPA and DHA but are expensive to produce. Fish oil with its low price and high-energy value comes into most diets on a least cost basis.

2.5.14. INDUSTRIAL APPLICATIONS OF LIPIDS

A wide variety of fatty acids and fatty acids derivatives have found numerous applications in both biochemical and industrial fields. These include:-

1. Detection of adulterated fats and oils;
2. Identification of specific triglyceride causing graininess in shortenings and margarines;
3. Identification of specific triglycerides giving some fats and oils unique suitability for confectionary;
4. Increased utilization of dietary fats in infant feeding;
5. Defining mechanisms of intestinal fat absorption;
6. Defining pathways for triglyceride biosynthesis;
7. Identification of specific triglycerides which are active substances producing aggregation;
8. Diagnosis of diseases by characterization of distinctive phytanate triglycerides found in plasma which might possibly reflect metabolic disorders.
9. Manufacture of Esters. The esters produced are subdivided into the M series, H series, C series and E series, based on the alkanols used, and the properties of the product ester. The M series comprise FA esters of monohydric alkanols, which display low odour levels and light colours. The H series comprise FA esters of neopentyl polyol, and include both full esters and partial esters. The full esters are thermally stable, display excellent viscosity-temperature characteristics and lubricating properties and also offer low pour point, high flash point, and excellent biodegradability. They are widely used products, mainly as synthetic lubricants. The partial esters with high polarity and display good compatibility with resins are used as internal lubricants or self-emulsifying oils. The C series offer unique combination of high viscosity and low pour point, have been unattainable with

conventional ester oils. The E series comprise diesters of ethylene glycol, which are used mainly as pearling agents in cosmetic products.

10. Manufacture of Amides from fatty acids and amines. Octadecanoic acid is the main fatty acid used, although the range also includes amides of duodecanoic acid, octadecenoic acid and docosanoic acid, thereby satisfying demands across a wide range of different properties and functionalities. Examples of potential uses of these amides include lubricants, plasticizers, mould release agents, and antifoaming agents.
11. Production of Metallic soap. This is formed using a salt of a fatty acid and an alkali earth metal (Al, Ca, Zn, Mg or Ba) by double decomposition or direct method. Applications include lubricants, water repellents, resin lubricants, PVC stabilisers, thickeners, matting agents, mould release agents, blocking prevention agents, vulcanization accelerators, base materials for greases, compounding agents for cosmetics, food additives, and as base for the manufacture of floating feeds.
12. Manufacture of fatty acid chlorides (the hydroxyl group at the terminal of the fatty acid molecule substituted with a chlorine atom), are important acylating agents. The dimerisation of fatty acid chlorides produces products that are used as sizing agents in the paper manufacturing industry, and also to prevent ink bleeding.
13. Manufacture of Aliphatic amines (produced by replacing the carboxyl group at the terminal of a fatty acid molecule with an amine group) include aliphatic primary amines, dimethylamine-based aliphatic tertiary amines, polyoxyethylene alkyl amine-based aliphatic tertiary amines produced by the addition of ethylene oxide, and aliphatic alkyl propylenediamines. These are used as intermediates in the manufacture of industrial chemicals, antistatic agents, additives for lubricating oils,

and water treatment agents. The tertiary amines are used as raw materials in the manufacture of surfactants.

14. Development of functional phospholipids. This technology seeks to replace fatty acids found in plant phospholipids with essential Omega-3 fatty acids. Important effects of functional phospholipids on humans may include better memory, learning, concentration and reducing or delaying the ageing processes in the brain. By improving on traditional Omega-3 triglycerides, the incorporation of long-chain Omega-3 fatty acids in phospholipids helps the product to increase the elasticity of cell membranes and thus their penetrability when substances are transported into and out of cells. In addition to increased absorption and improved bio efficiency, the innovation also facilitates stable and predictable product quality. The technology paves the way for unique benefits by enabling the composition of phospholipids to be tailored to produce optimal health-promoting effects suitable for the pharmaceutical, neutraceutical, food ingredient, and animal feed markets.
15. Traditional packaging films usually provide protective functions such as oxygen barrier, water vapour barrier and mechanical properties. Hydrophilic edible films have oxygen and carbon dioxide barrier properties at low relative humidity while lipid edible films have water vapour barrier properties but less effective mechanical properties. Foods containing fats are susceptible to oxidation; therefore oxygen barrier characteristics for protective films are important. Waxes and fatty acids had been found to be effective raw materials for the packaging industry, although they lack film-forming characteristics due to ineffective mechanical properties. Laminated edible films made with methylcellulose and corn zein-fatty acids show good vapour barriers and mechanical properties. The film being insoluble in water

was a useful wrap for high moisture foods. Laminated edible films from methylcellulose and corn zein-fatty acids have water vapour permeability and tensile strength affected by fatty acids type and concentration. A decreased chain length of edible films and increased concentration of fatty acids, oxygen permeability of laminated films increases.

2.5.15. SOME POSSIBLE DISADVANTAGES OF USE OF LIPIDS

Polyunsaturated fatty acids can be a health hazard because C = C can lead to free radical generation and reactions with oxygen to form unstable lipid peroxide compounds containing the same unstable oxygen-oxygen bond found in H_2O_2. Lipid perioxidation and free radicals can cause cancer and may accelerate ageing. High rates of lung cancer among women in China have been associated with lipid peroxidized oils in fumes from cooking polyunsaturated vegetable oils (Shields *et al.*, 1995). Hot oil in open air is subject to much lipid peroxidation. Fast-food restaurants that fry foods in the same oil all day serve lots of lipid peroxides to their customers.

The use of saturated oil in deep fat frying enhances the formation of heterocyclic amines in the field products. The saturated oil may undergo hydrolysis to form a large amount of free fatty acid (FFA) during heating, which in turn facilitates the degradation rate of lipid and thus promote formation of heterocyclic amines (Tai *et al.*, 2001). Frying may as well increase the ratio of polyunsaturated fatty acid to saturated fatty acid and that of Omega-6 to Omega-3, giving rise to a negative effect on the benefits related to intake of 4,7,10,13,16,19-docosahexaenoic acid (DHA) and 5,8,11,14,17-eicosapentaenoic acid (EPA). Therefore, vegetable oils rich in Omega-6 polyunsaturated fatty acid should be avoided in pan and deep fat frying if an increase of Omega-3 polyunsaturaed fatty acid is

desired (Agren and Hanninen, 1993). Sebedio *et al*. (1993) asserted that longer chain Omega-3 polyunsaturated fatty acid may not be affected by deep-fat frying due to the formation of geometrical fatty acids isomers of long-chain highly polyunsaturated fatty acids.

High doses of fish oil may decrease immune function, impair bacterial resistance, increases low density lipoprotein cholesterol and decrease high density lipoprotein cholesterol. It also could cause the thinning of blood and therefore may increase the likelihood of bleeding.

Increased amounts of polyunsaturated fatty acids in diets and eggs increase the degree of unsaturation and may increase susceptibility to lipid oxidation. There could also be some potential human health risks associated with increased consumption of Omega-3 polyunsaturated fatty acids due to increased consumption of lipid oxidation products and depletion of tissue levels of vitamin E (Ajuyah *et al.*, 1993).

To minimise lipid peroxidation, essential fatty acids supplements should be taken with no less than 500mg of vitamin E per day. But for smokers, vitamin E may not be adequate to reduce oxidation of fish oil in low density lipoprotein cholesterol, and some medical authorities have cautioned against too much fish oil consumption by smokers (Harats, 1991).

2.5.16. THE USE OF STATISTICS IN FISH COMPOSITION CHARACTERISATION

The chemical composition of fish varies greatly from one species and one individual to another depending on sex, age, environment and season. Therefore, a substantial normal variation is observed for the constituents of fish muscle and oil as well.

During the starvation periods, for instance, fish uses the energy depots in the form of lipids and also may utilize protein, thus depletion of these reserves results in a general diminution of biological condition. This condition may influence the quality of the different fish products and may also condition the yield and process efficiency (Yeannes and Almandos, 2003). A concise description of the raw data therefore, is important to identify fundamental characteristics of their distribution and thus describe the generated numerical material (Ugoala, 2006). This has fundamental importance in the application of different technological processes, quality of raw material, sensory attributes and storage stability.

Yeannes and Almandos (2003) used a series of mathematical equations with water content as the independent variable, based on a statistical study of proximate composition of fish, to estimate the percentage (%) protein having the analytical determination of percent moisture for lean species. For fat species, a second-order regression equation obtained was used to estimate percent lipid content based on the analytical determination of percent moisture content.

Statistical tests – correlation matrix, t-tests, principal component analysis and cluster analysis were used by Coll *et al* (1994) to study the lipid fractions of laboratory and commercial fried squid.

Nakai *et al.* (1994) compared the most commonly used multi-variate analysis (MVA) techniques in food sciences – principal component, discriminant and cluster analyses, using non-food related and food protein structure – function data. According to the study, although discriminant analysis is the most efficient and useful method of classifying samples into groups, there could be a risk of discarding useful information in the analysis if

discriminant technique only is used. Discriminant technique uses only that portion of data variation that is useful for the classification purpose. Therefore, they recommended discriminant technique of multivariate analysis only when the groups are clearly defined a priori. To group continuous data without a clear distinction between groups, principal component regression was recommended. Principal component similarity analysis (PCS) comprises a group of sequentially applied MVA techniques proposed for classification of samples when the grouping cannot be clearly defined in advance or classifies samples with minimal loss of data variation.

CHAPTER THREE

METHODOLOGY OF STUDY

3.1. SAMPLE COLLECTION AND PREPARATION

Fresh captured fishes were sorted and identified. They include *Mormyridae* (*Hyperopisus bebe*, *Mormyrops anguilloides* and *Mormyrus rume*), *Cichlidae (Orechromis niloticus* and *Sarotherodon galilaeus), Claridae (Clarias gariepinus* and *Cla*rias *anguillaris*), *and Heterobranchus bidorsalis, Centropomidae* (*Lates niloticus*), *Clariheterobranchus* and *Characidae* (*Hydrocynus forskalii*). They were obtained from Fishermen at the Kainji Lake Dam site. The fishes were weighed, beheaded, eviscerated and cleaned prior to freezing. In an attempt to obtain a homogeneous sample from each species, their fleshes were removed from their backbones, minced, blended and immediately extracted using chloroform-methanol mixture in the ratio of 2:1.

3.2. LIPID EXTRACTION

Lipid extractions were performed on minced fish samples (10g each) using the extraction methods of Folch *et al* (1957). In this method, chloroform-methanol was used in the ratio of 2:1. The solvent mixtures were concentrated in vacuum using a rotary evaporator maintained at $35^{O}C$ and the extracts were acidified and stored in sample bottles respectively.

3.3. FATTY ACIDS ANALYSES

The method of derivatisation of the fatty acids was used and essentially that of methylation (Park and Goins 1994). In this method, fatty acids methyl esters were prepared by heating lipids with large excess of either acid- or base- catalyzed reagents. Acid-catalyzed reagents form fatty acid methyl esters from free fatty acids and O-acyl lipids while the base reagents

cause only transesterification. Methylene chloride (100µL) and 1 mL 0.5M NaOH in methanol were added to oil extracts in a test-tube and heated in a water bath at 90^{O}C for 10 min to prepare fatty acids methyl esters. The test tubes were removed from the water bath and allowed to cool before the addition of 1 mL 14% BF_3 in methanol. The test tubes were heated again in a water bath for 90^{O}C for 10 min, and cooled to room temperature. One mL of distilled water and 200µL hexane were added to the test tubes respectively and then fatty acid methyl esters were extracted by vigorous shaking for one minute. After centrifugation, the top layer which is the fatty acids methyl esters was collected and transferred into a sample bottle for analysis. The fatty acids profiles were determined using an Agilent Gas Chromatograph, Model 6890N fitted with an Agilent Mass Selective Detector, 5973 series. Separation was carried out in a capillary column (30 x 0.25mm internal diametre x 0.25µm DB wax). The starting temperature was 150^{o}C maintained for 2minutes at a heating rate of 10 ^{O}C/minute. The total running time was 22 minutes. Helium was the carrier gas while the injection volume was 1µL.

3.4. REAGENTS/SOLVENTS USED

All the solvents used were of analytical grade. All solvents were redistilled to achieve purity. They include Methanol, Chloroform, NaOH, $BF_{3,}$ Hexane, Methylene chloride supplied by the British Drug House (BDH).

CHAPTER FOUR

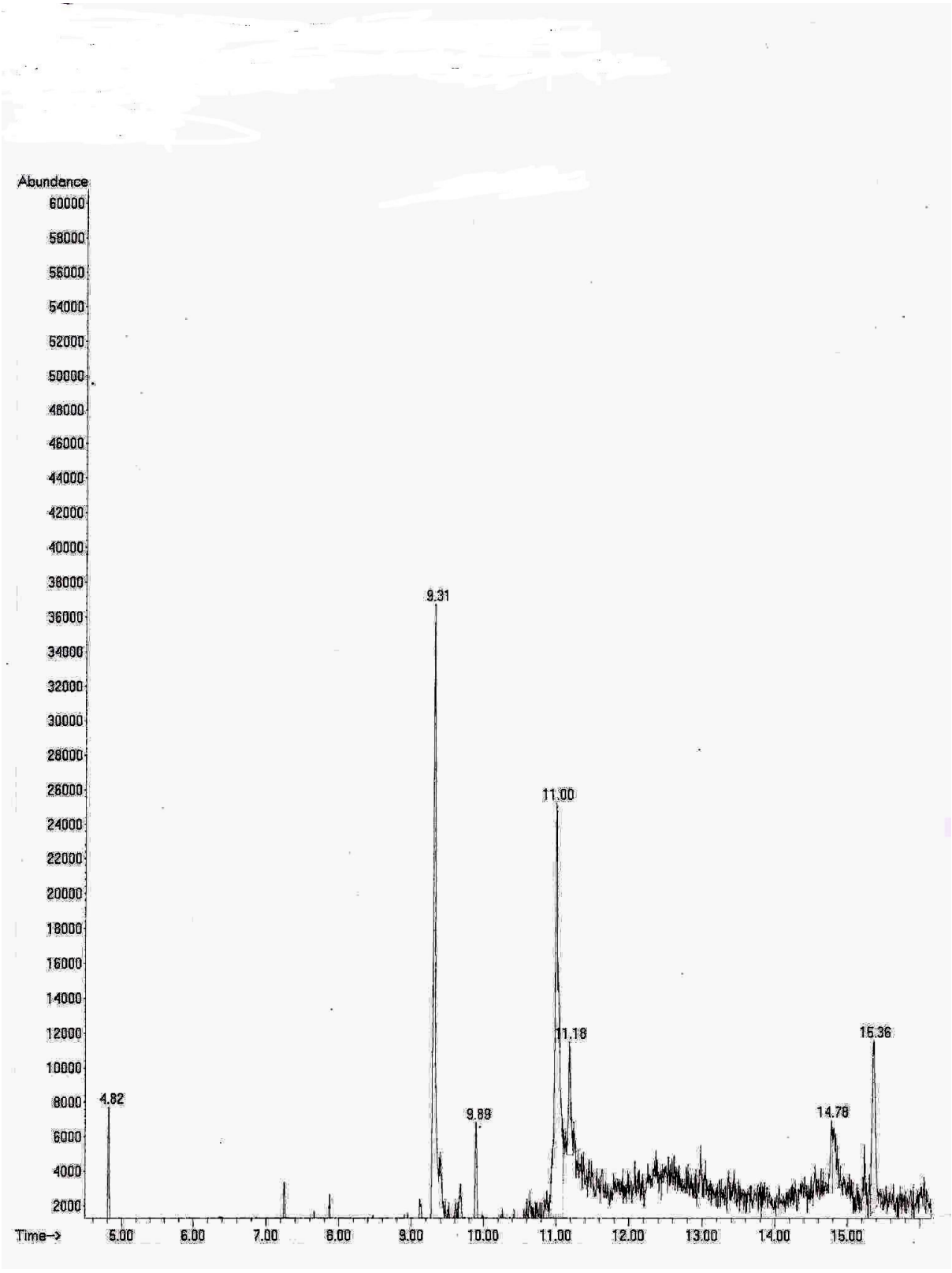
RESULTS

4.1. GAS CHROMATOGRAMS OF STUDIED FRESHWATER FISH OILS

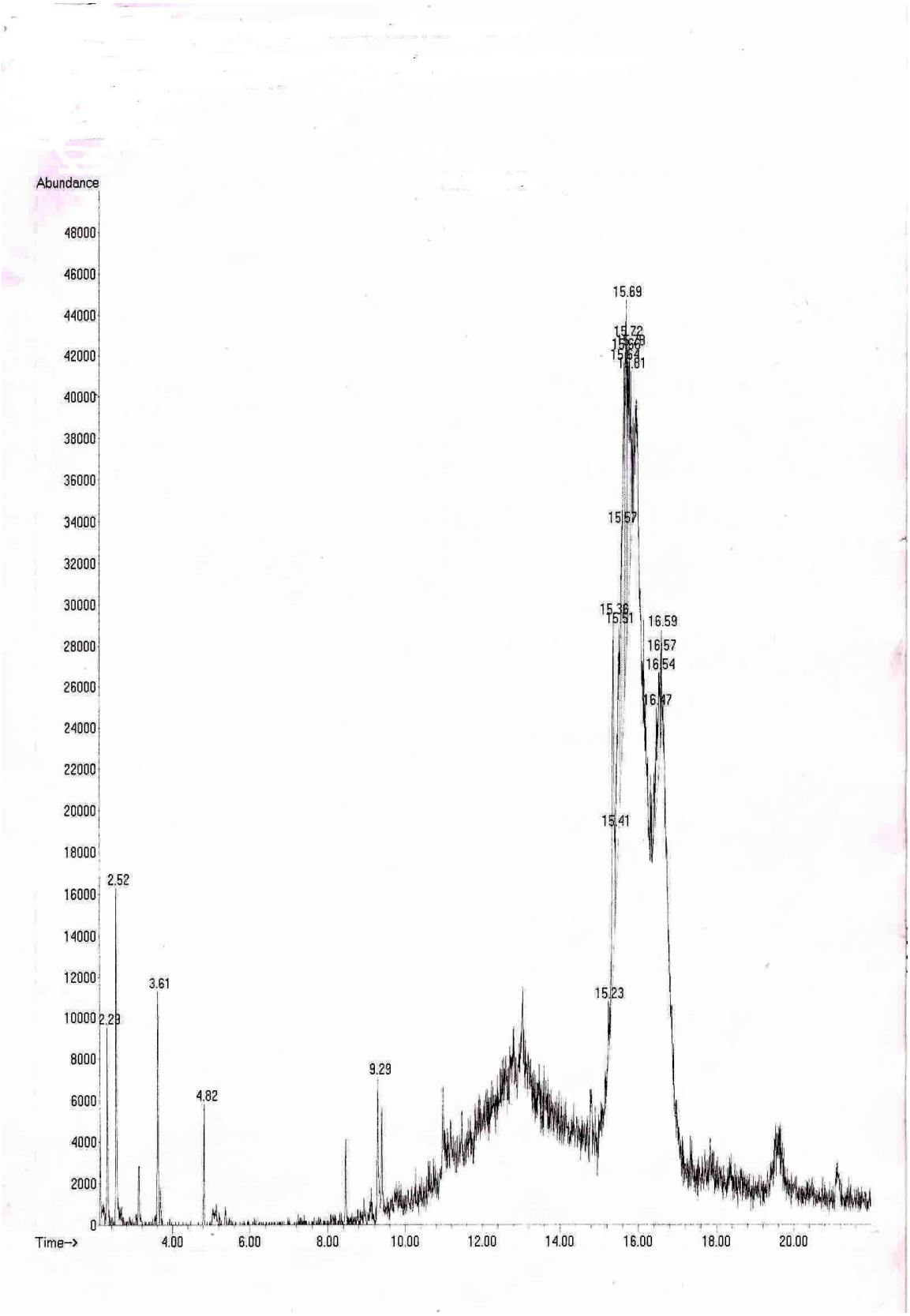
Chromatograms obtained from the analyses of the freshwater fishes used in this work are shown below. Good separations are obtained from each sample. The DB-wax column used in the analyses could not resolve clearly some complex mixtures. It however, separated fatty acids from C4 according to carbon number and unsaturation (Frank *et al*, 2007). The retention times of the fatty acids are listed below for the samples; *Hyperopisus bebe*, *Mormyrops anguilloides*, *Mormyrus rume*, *Cla*rias *anguillaris*, and *Heterobranchus bidorsalis* were same.

Table 4.1: Common retention times obtained from chromatograms of freshwater fishes

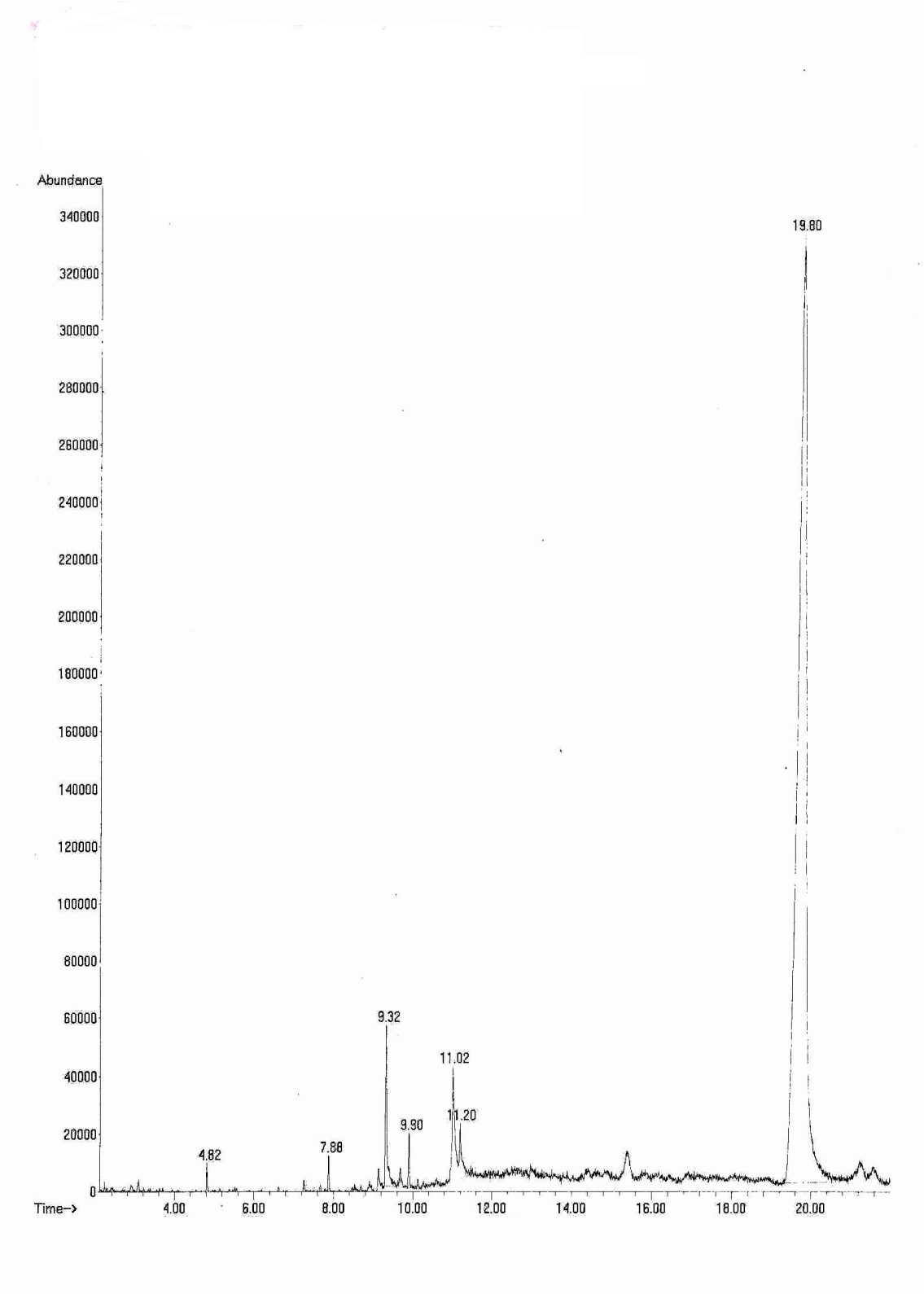
FATTY ACID	RETENTION TIME (Minutes)
Butanoic acid	4.82
Duodecanoic acid	9.32
Tridecanoic acid	9.90
Pentadecanoic acid	11.01
9,12-octadecanoic acid	11.20
9,12,15-octadecatrienoic acid	15.36



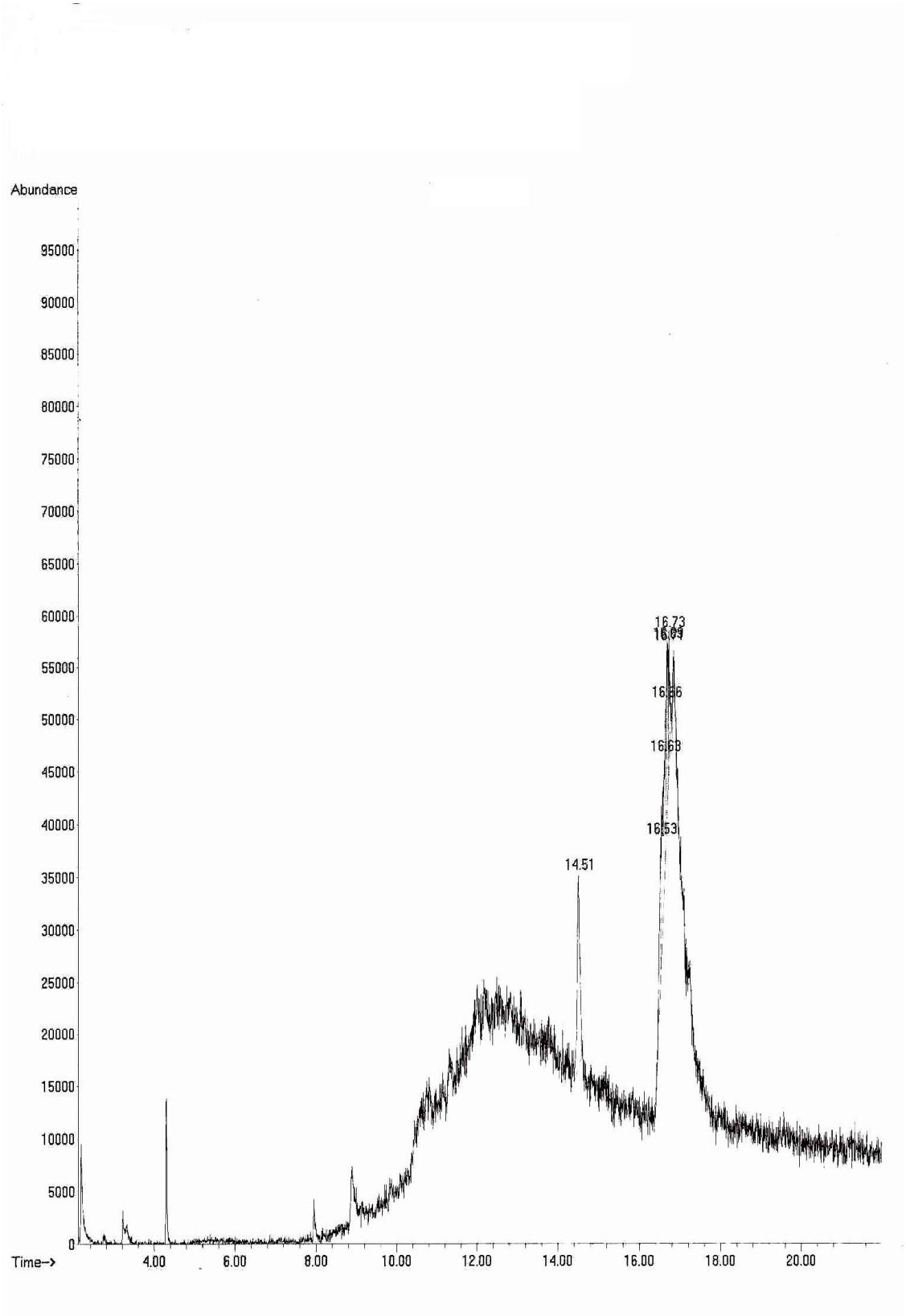
4.1. Chromatogram for *Hyperopisus bebe*



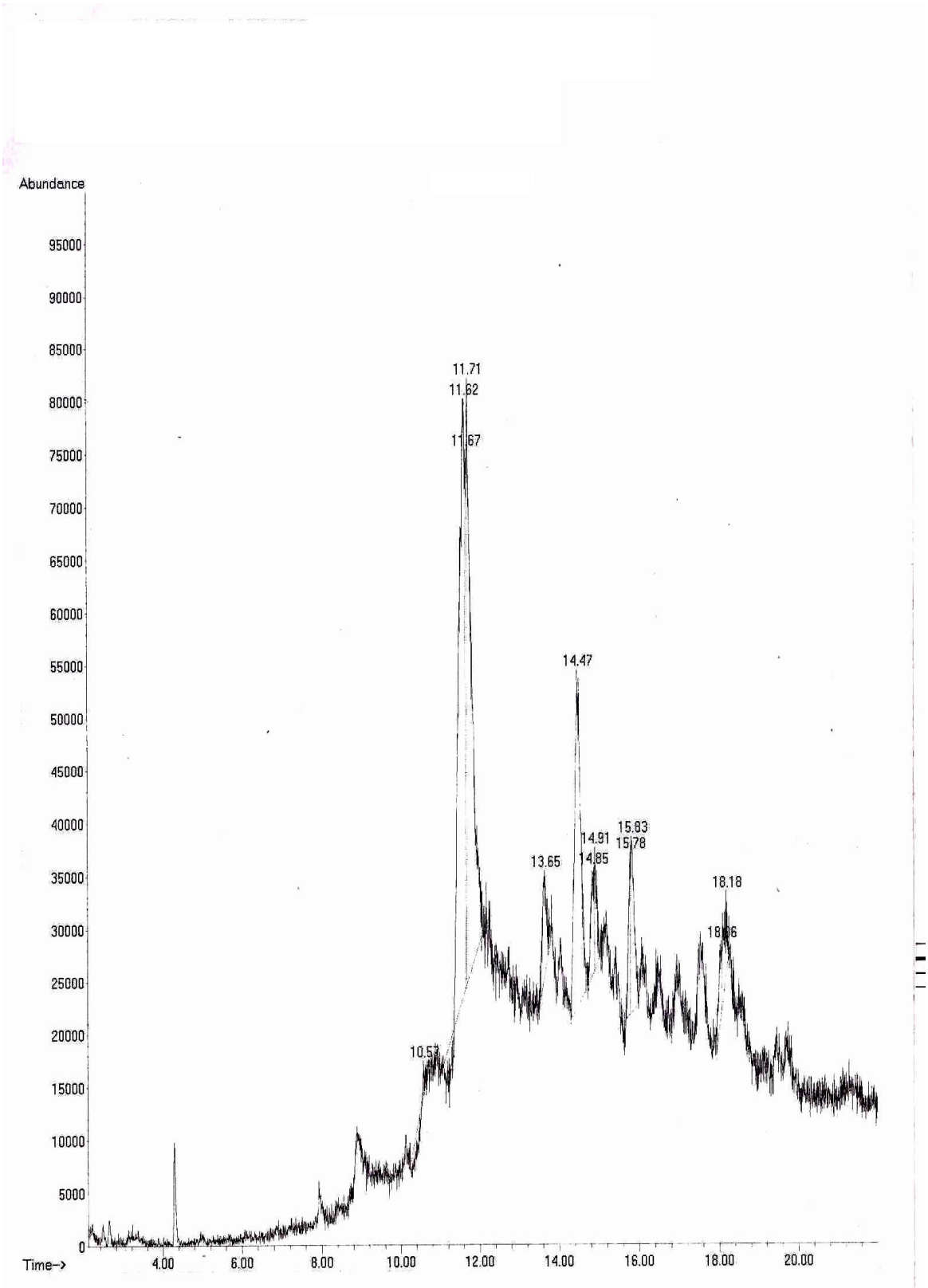
4.2. Chromatogram for *Mormyrops anguilloides*



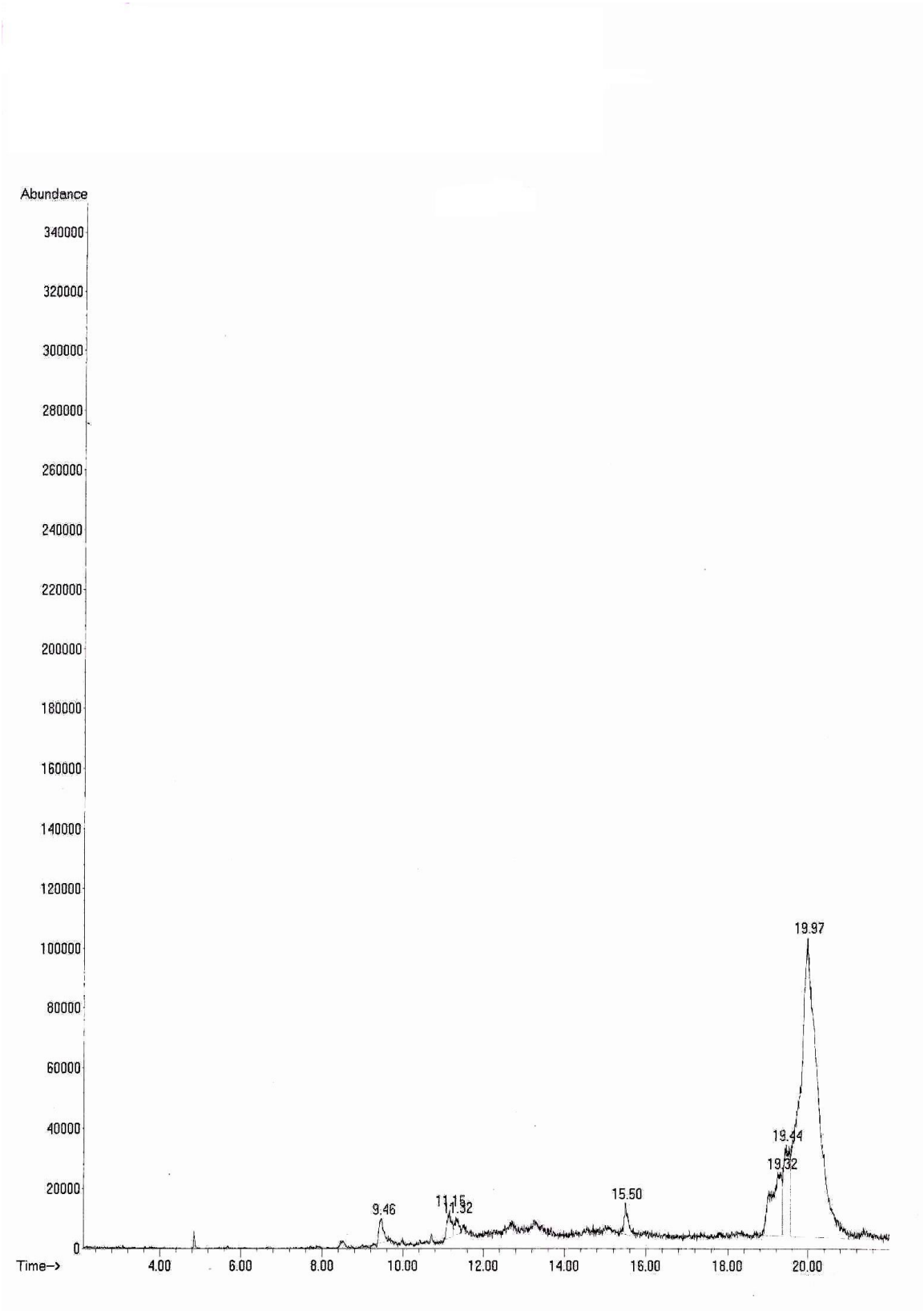
4.3. Chromatogram for *Mormyrus rume*



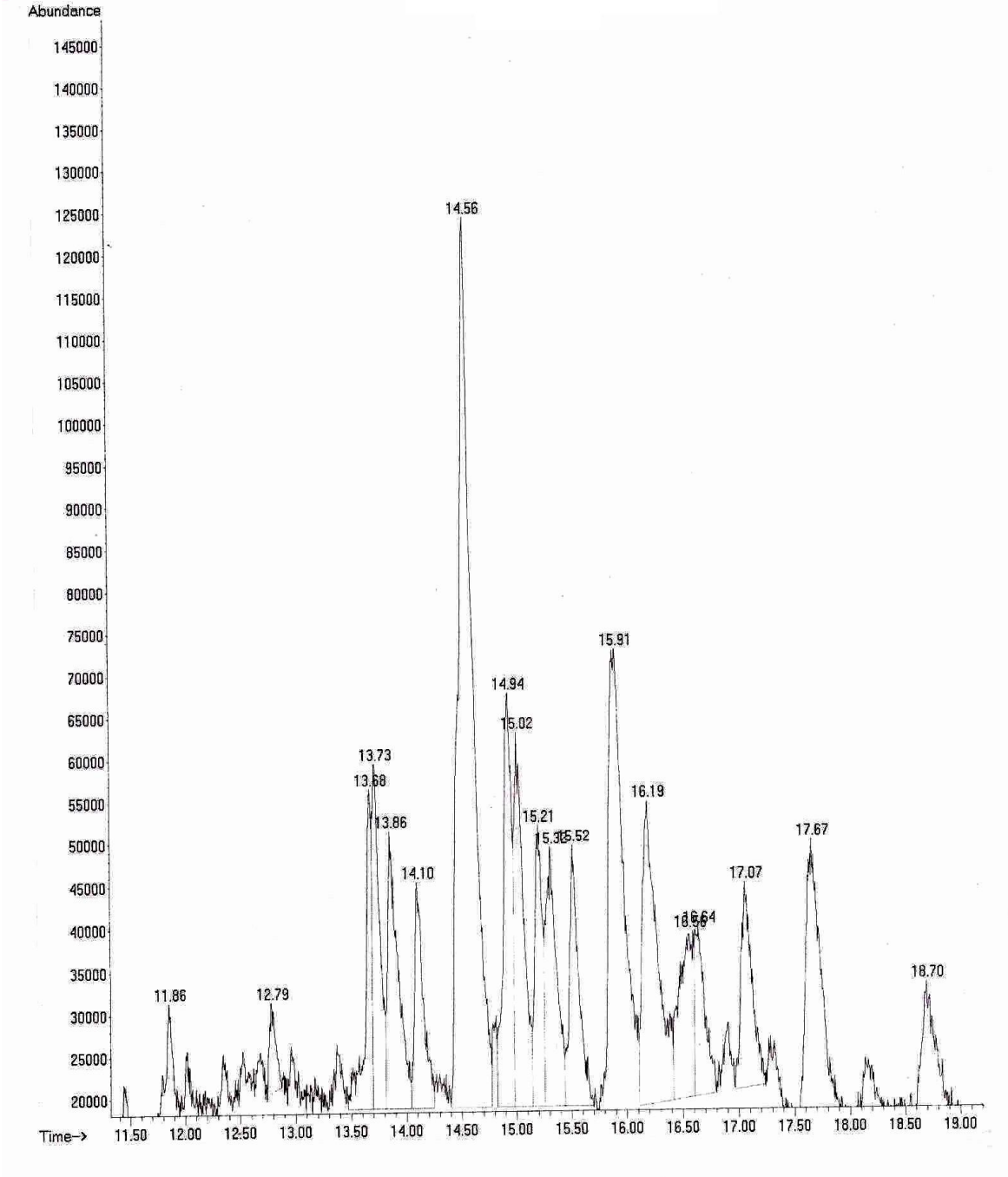
4.4. Chromatogram for *Clariheterobranchus*



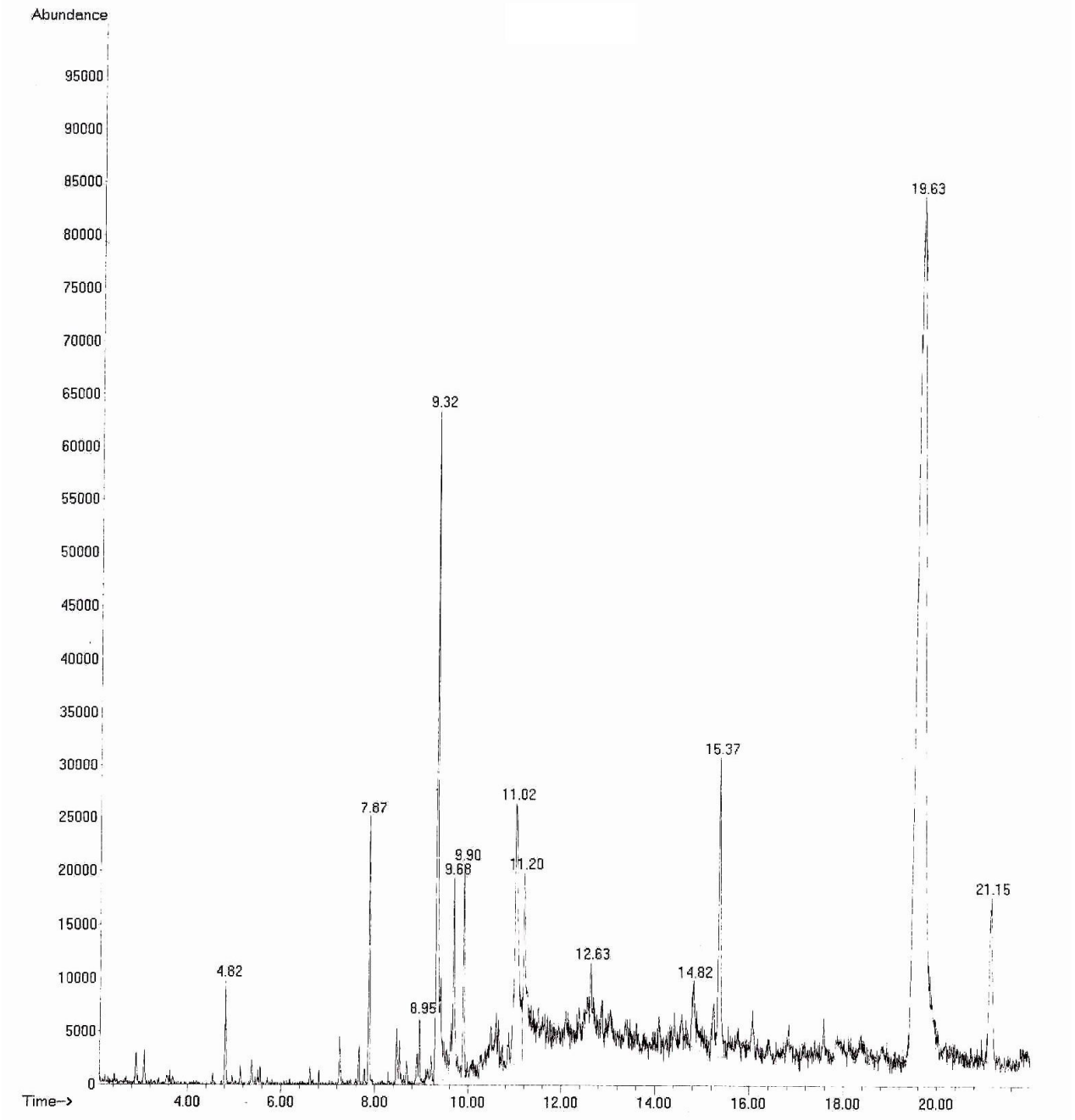
4.5. Chromatogram for *Clarias gariepinus*



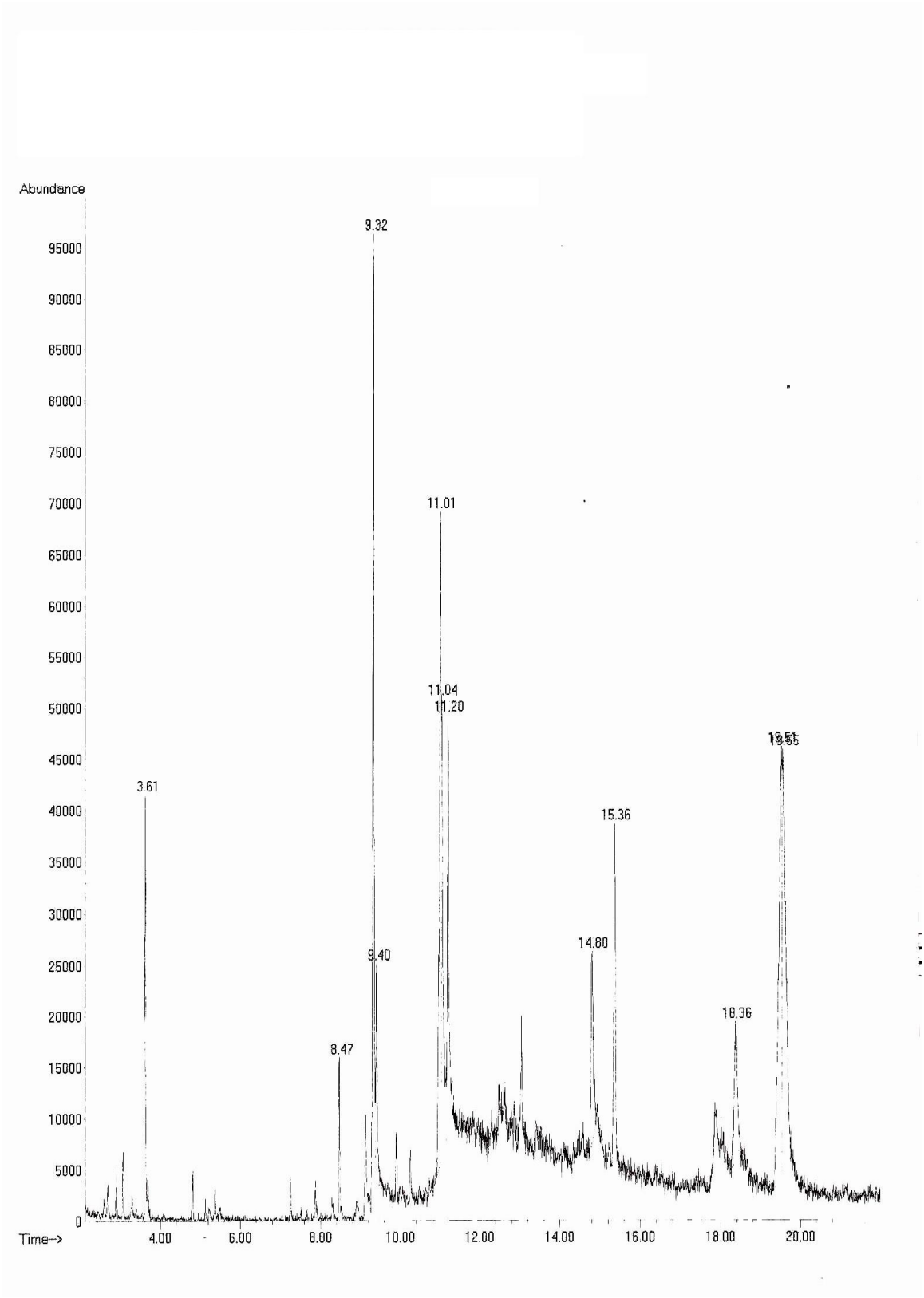
4.6. Chromatogram for *Oreochromis niloticus*



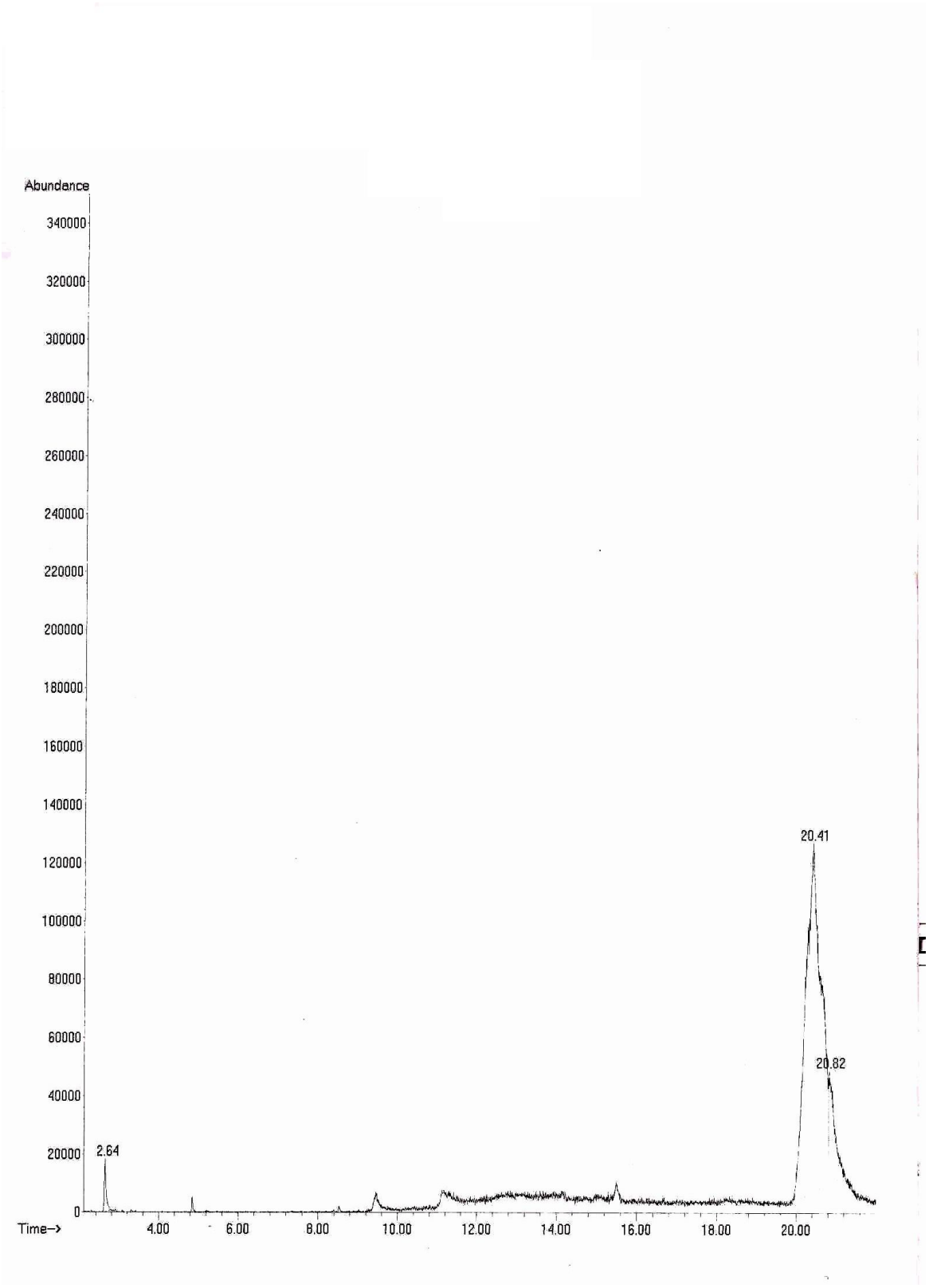
4.7. Chromatogram for *Lates niloticus*



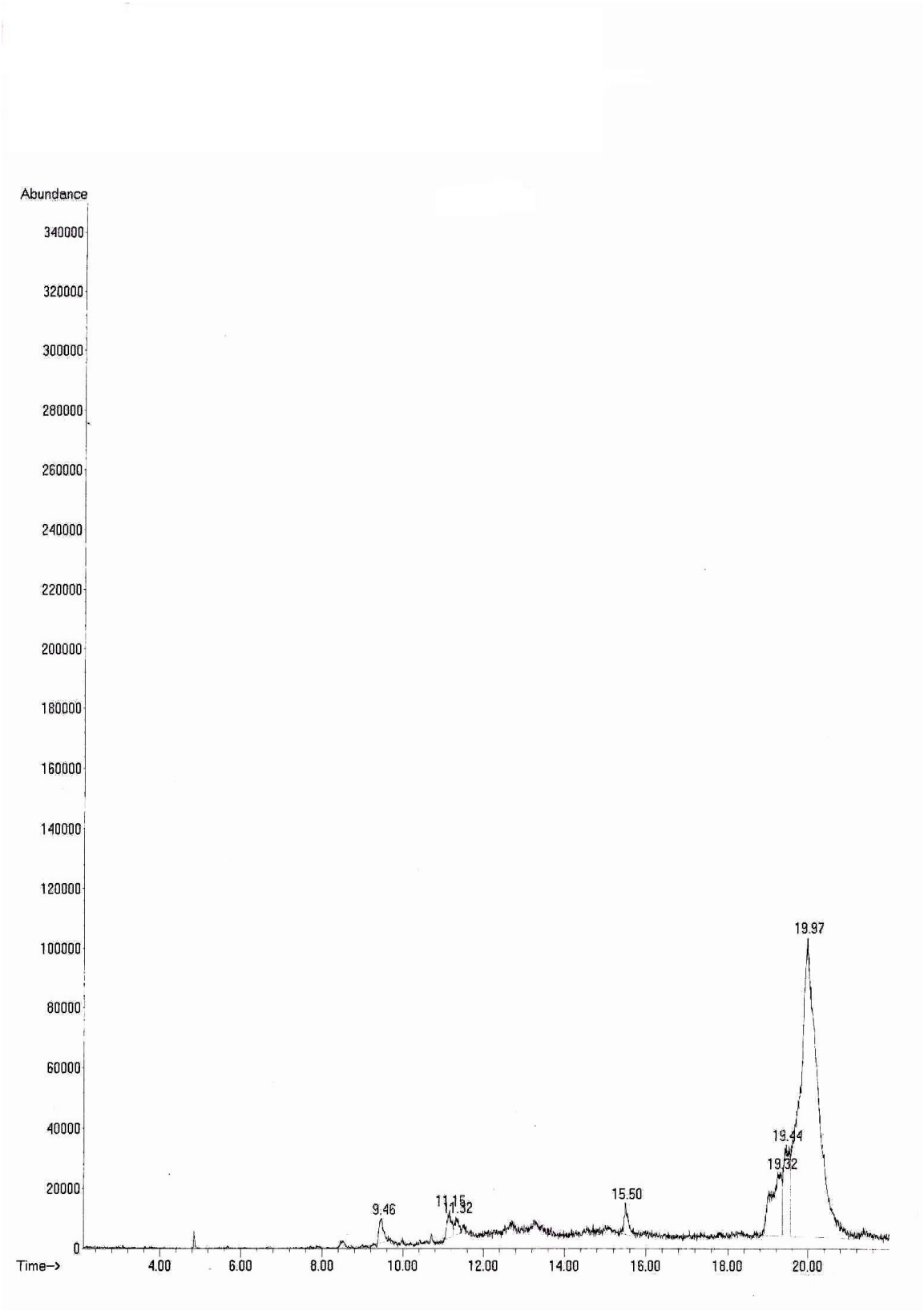
4.8. Chromatogram for *Clarias anguillaris*



4.9. Chromatogram for *Heterobranchus bidorsalis*



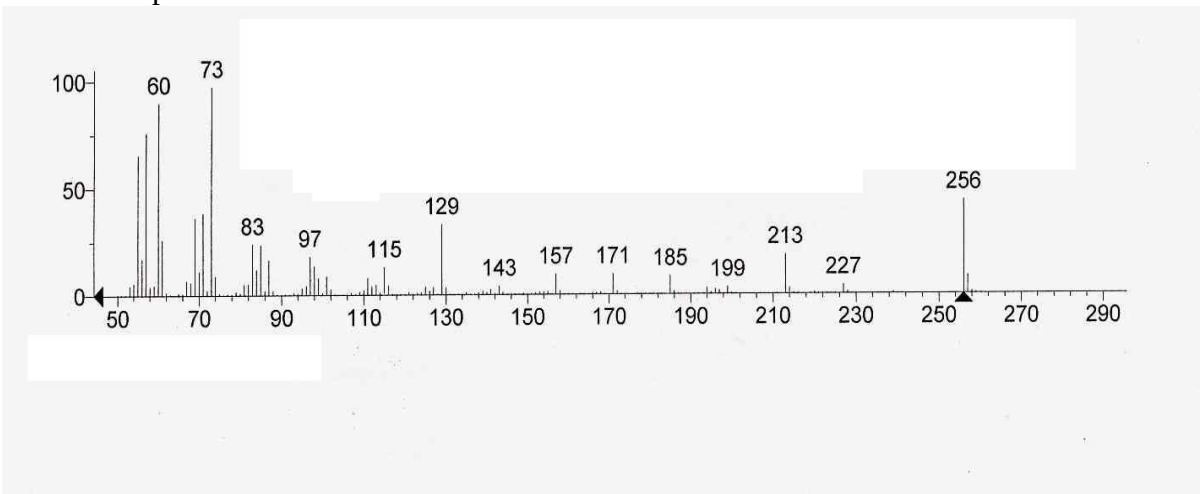
4.10. Chromatogram for *Hydrocynus forskalii*



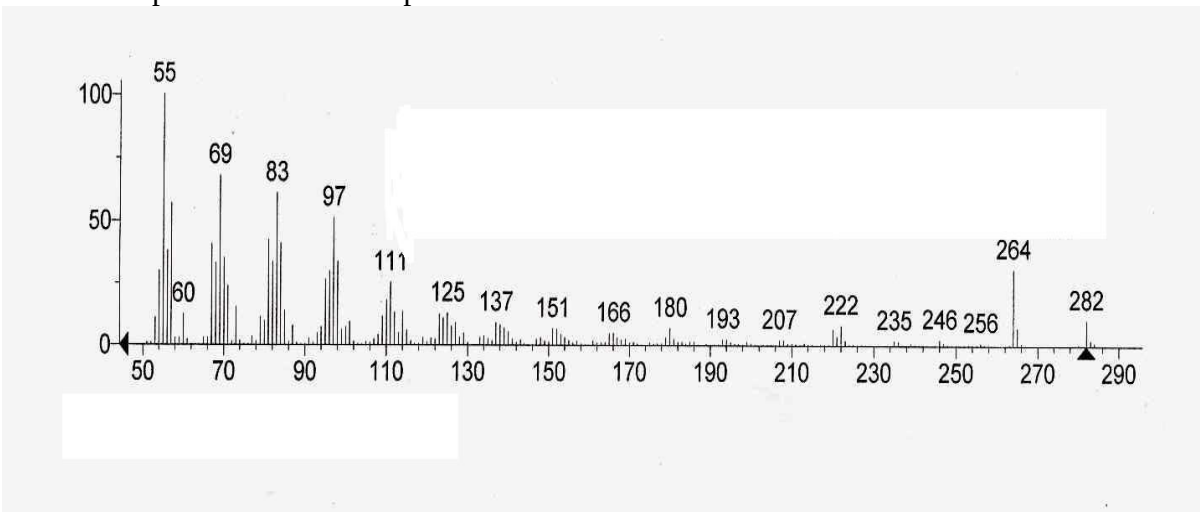
4.11. Chromatogram for Sarotherodon galilaeus

4.2. MASS SPECTRA OF STUDIED FRESHWATER FISH OILS

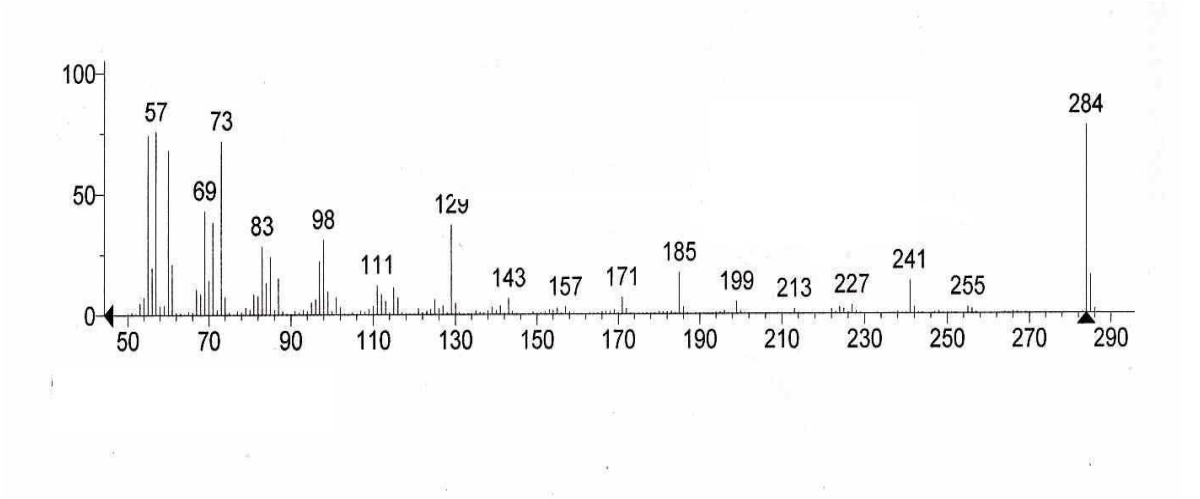
4.1. Mass Spectrum for Hexadecanoic acid



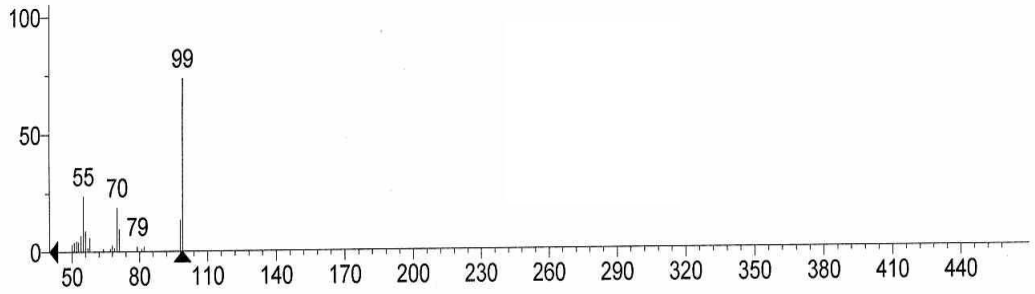
4.2. Mass Spectrum for Mass Spectrum for Oleic acid



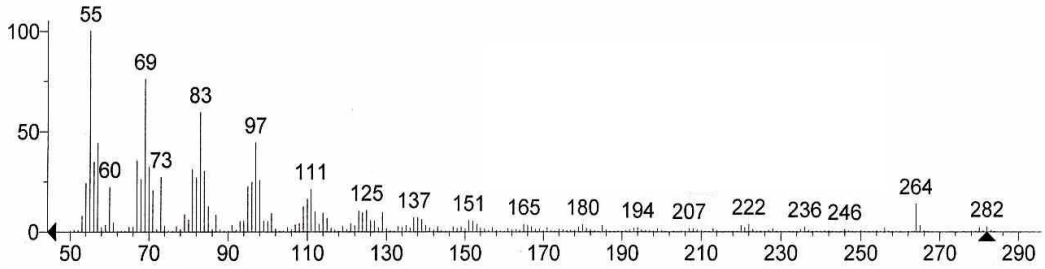
4.3. Mass Spectrum for Octadecanoic acid



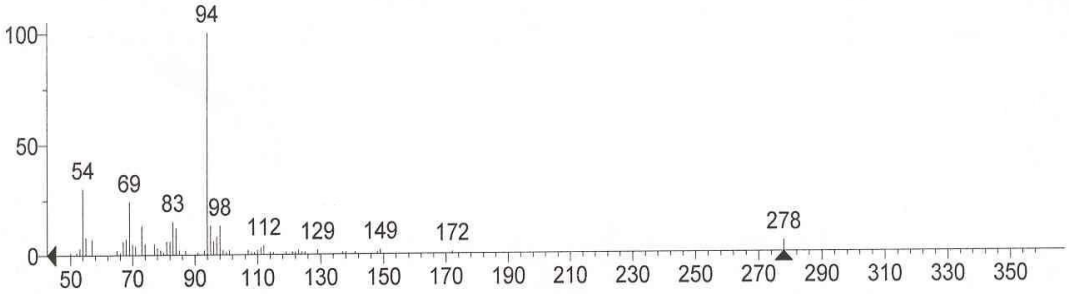
4.4. Mass Spectrum for 2-Piperidnone



4.5. Mass Spectrum for 6-Octadecanoic acid



4.6 Mass Spectrum for Undecanoic acid



CHAPTER FIVE

DISCUSSION

5.1. FRESHWATER FISH SPECIES STUDIED

MORMYRIDAE: This class is well represented in local waters with 26 different species belonging to 6 genera. They are bottom-dwellers around deep, rocky pool or deep water around fallen trees. The fleshes of most species contain excess of fatty oil. The high oil content makes them difficult to cure. These species feed on molluscs, larvae of *chironomid* and *chaoborid* flies.

CHARACIDAE: This class is represented by three genera and fifteen species, all of which are predators. The flesh is white and tasty, dry and not oily, but has excellent keeping qualities after smoking. The young feed on insects and water beetles, while the adults prey on other fishes, especially *Alestes.*

CLARIIDAE: These are divided into two genera-Clarias and *Heterobranchus*, each having three species. Clarias live mostly in swamps, where they feed on weeds, insects' larvae, snails, crustacean, worms and small fishes. They have oily flesh which can be rendered into oil. *Heterobranchus* flesh is less oily than that of *Clarias.*

CICHLIDAE: These are important figure in many fisheries because of their great adaptability, high fecundity, and rapid growth rate. They feed on insects' larvae and plant materials.

CENTROPMIDAE: *Lates niloticus* is the sole local species of this family. It grows to about 2 metres long and could weigh of up to 80kg. *Lates* are carnivores and feed mostly on young fishes.

5.2. FATTY ACIDS PROFILE OF STUDIED FRESHWATER FISH

The fish oils *Hyperopisus bebe*, *Mormyrops anguilloides,Mormyrus rume*, *Oreochromis niloticus*, *Sarotherodon galilaeus, Clarias gariepinus, Cla*rias *anguillaris*, *Heterobranchus bidorsalis, Lates niloticus, Clariheterobranchus* and *Hydrocynus forskalii* are unique in their variety of fatty acids (Table 5.1) of which they are composed and their degree of unsaturation (Figure 5.1). There are high levels of Omega-6 polyethylenic than Omega-3 polyethylenic fatty acids. The most common fatty acids presented below have even number of carbon atoms per molecule and seldom contain functional groups other than cis and trans olefinic unsaturation, which usually occur in a methylene-interrupted pattern in polyenes. Long chain fatty acids (Table 5.1) are ubiquitous constituents noticed. In a given species, saturated and unsaturated fatty acids occur generally side by side, their structures varying widely in chain length and in degree of unsaturation. The fatty acids profiles include minor amounts of odd-number, branched-chain, and even-number fatty acids. These varieties as well as the quantity and quality of fatty acids noticed are due to differences in sub-species, diet, spawning cycle, season and environment.

The saturated components ranged from nine to seventy-six percent. Within these components the major fatty acids were C14:0 and C16:0. The mole percent of each fatty acid seems to vary. The monoene contents ranged from ten to ninety percent with C18:1 as the prominent monounsaturated fatty acids. Polyunsaturated fatty acids attained the highest value of ninety percent. The branched chain fatty acids identified are C15:0, C16:0, C17:0, C18:2 and C20:0. These fatty acids were fifty-one, forty-four and thirty-one percent respectively in *Sarotherodon galilaeus*, *Oreochromis niloticus* and *Lates niloticus*

respectively and were the highest noticed. This high level of branched chain fatty acids in these species has an important advantage.

Table 5.1: Fatty acids compositions of some freshwater fish species

FATTY ACIDS	*Clarias anguillariass*	*Lates niloticus*	*Mormyrops anguilloides*	*Mormyrus rume*	*Hydrocynus forskali*	*/Heterobranchus bidorsalis*	*Hyperopisus. bebe*	*Clarias gariepinus*	*Clariheterobranchus*	*Sarotherodon galilaeus*	*Oreochromis niloticus*
12:0	11.20		5.23	4.58		19.89	3.58		9.79		
13:0	3.43			0.98		4.30	0.65				
14:0	7.99					14.52		31.61		3.81	17.82
14:1	19.63							1.23			
15:0	4.79			4.58		9.14	3.58	5.43		5.08	4.53
15:1	2.74			1.96		8.60	0.65	27.90	2.25		
16:0		2.15								36.38	34.14
16:1										10.15	12.39
17:0										4.65	1.81
17:1		0.92									
18:0	2.05	3.38						3.46		9.31	5.74
18:1										12.27	9.37
18:1cis		4.92						2.22			
18:1trans	4.11	4.31						15.56			
18:2n-6										4.65	3.02
18:3n-3		6.15	1.31			6.18	0.98	2.22		2.12	3.63
18:2cis		22.15	14.05			4.57					
18:2trans		3.38	1.31				0.33	1.98	19.26		
18:3n-6		5.85	12.09					4.94			
18:4										2.12	1.51
20:0		3.08	6.54						13.16		
20:1n-9		4.31	13.73						7.38		
20:2		3.69	5.23						12.68		
20:3n-6		7.07	4.25					1.48	4.17		
20:3n-3		2.78	4.25			4.30					
20:4n-6		3.69	9.48					1.98	3.85	2.96	1.51
20:5n-3		3.69	3.27							0.17	0.60
21:0		10.46	7.52						3.85		
21:1n-11			2.61			13.98					
22:0	36.53										
22:1n-11				86.27			89.25				
22:2		6.46	0.65		89.57	12.09					
22:5n-3										2.54	1.51
22:6n-3										3.81	2.42
23:0	2.51		1.31		10.43						
24:0		1.54									

2-piperidinone also found in *Mormyrops anguilloides*

Branched chain fatty acids influence lower melting point, lower cholesterol levels, provide energy, and form an integral part of biomembranes. Branched fatty acids, because of their stability at high temperatures play important role in the finished product of hydrogenated fish oils. Esterification of branched chain fatty acids to cholesterol causes the fatty acids to stimulate protein synthesis. The branched chain esters influence some ribosomal functions which are necessary for peptide elongation (Hradec *et al*, 1974).

In all the fish species analysed, the dominant polyunsaturated fatty acids are of the Omega-6 series and are found chiefly in C18:2 fatty acids. The essential fatty acids compositions showed prominence in C18:3n-3 and C18:2n-6. C22:6n-3 was noted in the tilapia species. *Clarias anguillaris* has more of saturated fatty acids than *Clarias gariepinus* and their hybrid *Clariheterobranchus*. *Clarias gariepinus* has more monounsaturated fatty acids while *Clariheterobranchus* is more of polyunsaturated fatty acids (Figure 5.2). *Mormyrus rume* and *Hyperopisus bebe* contain more of monounsaturated fatty acids while *Mormyrops anguilloides* is highest in polyunsaturated fatty acids contents. The tilapia species (*Oreochromis niloticus* and *Sarotherodon galilaeus*) contain more of saturated lipids but comparable amounts of monounsaturated fatty acids, polyunsaturated fatty acids as well as Omega-3 fatty acids. *Lates niloticus* and *Hydrocynus forskali* have their lipid content being more of polyunsaturated fatty acids. *Heterobranchus bidorsalis* is about fifty percent of saturated fatty acids.

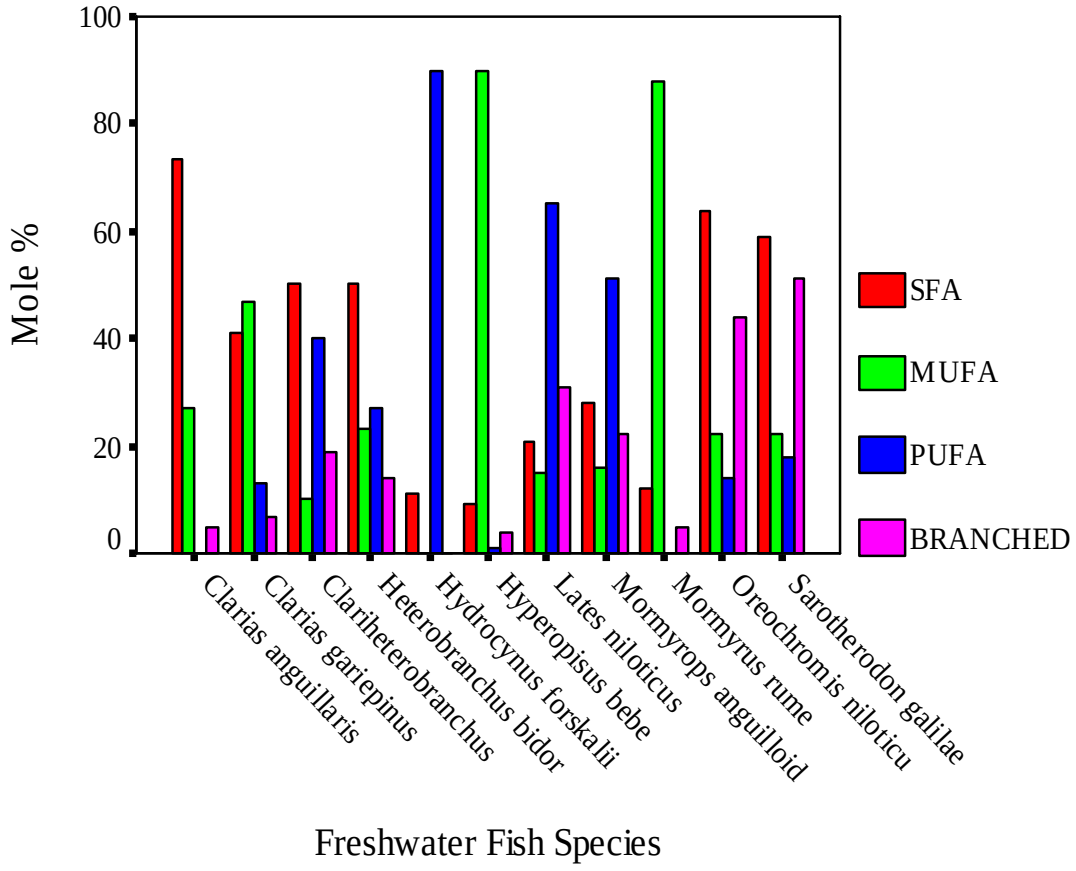


Figure 5.1: Major Fatty Acids groups of studied fishes

Clarias anguillaris, *Mormyrus rume*, *Hydrocynus forskali* and *Clariheterobranchus* showed low values for Omega-3 fatty acids while Omega-6 fatty acids were not detected in *Clarias anguillaris, Mormyrus rume, Hydrocynus forskali*, *Heterobranchus bidorsalis*, and *Hyperopisus bebe*. Wild fish has low Omega-3: Omega-6 ratios. This is of very dietary importance because it will enable the reduction of high levels of Omega-6: Omega-3 in most human diets. The essential fatty acids (figure 5.2) were lacking in *Clarias anguillaris*, *Mormyrus rume*, and *Hydrocynus forskali*. 4,7,10,13,16,19-docosahexaenoic acid (Omega-3) were absent in all the species except the tilapias. The tilapia species contain all the essential fatty acids but in varying composition. 9,12-octadecdienoic acid contents in *Lates*

niloticus and *Clariheterobranchus* as well as *Mormyrops anguilloides* are high in that order. The oils were characterized by low levels of Omega-3 polyunsaturated fatty acids.

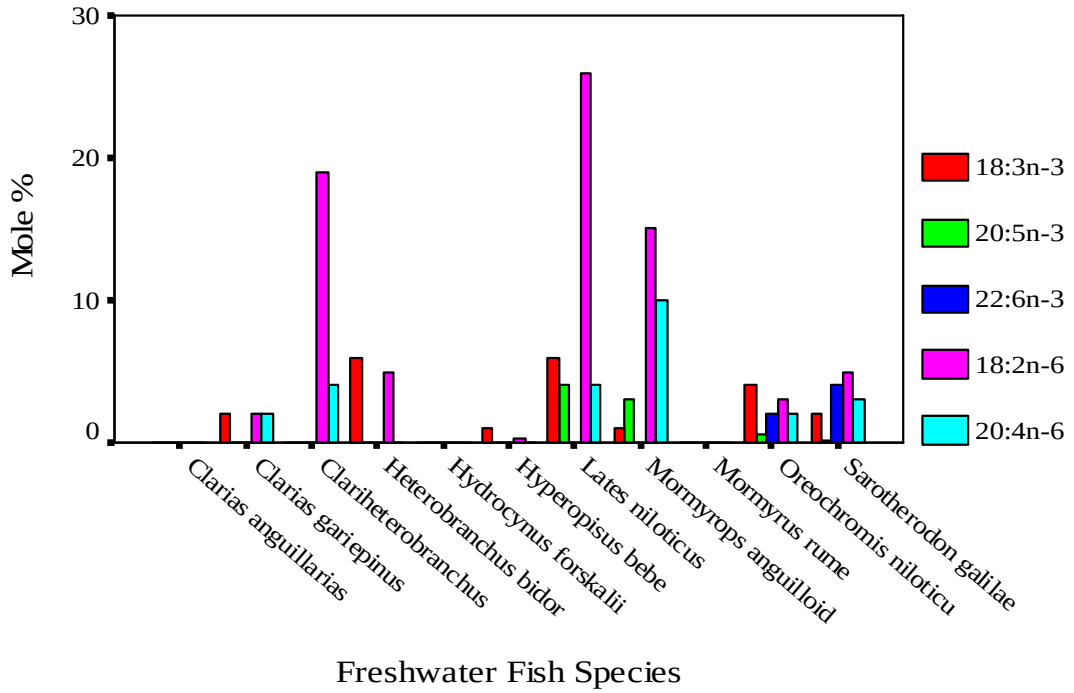


Figure 5.2: Essential Fatty Acids groups of studied fishes

The values of component fatty acids in the figures below are expressed in moles percent because molar composition is frequently more informative than composition by weight since it express the relative number of molecules of each type of acid present in oil.

5.3. COMPARISON OF FRESHWATER FISH AND MARINE FISH

The fatty acids compositions of marine fish species (Table 5.2) are complex and variable. While the marine species have similar fatty acids compositions pattern, the freshwater species differs in their fatty acids compositions pattern. A study of table above places the marine fish species into two groups. Group one include Anchovy, Horse mackerel, Menhaden and Sardine while Capelin, Herring, Mackerel, Norway pout, Sand eel and Sprat constitute group two. Group one are higher in saturated fatty acids, particularly C16:0,

higher in polyunsaturated fatty acids (C20:5), lower in monounsaturated fatty acids (C20:1 and C22:1), similar in fatty acids composition, and may be more susceptible to oxidation compared with group two oils because of the higher level of unsaturation of the former.

Table 5.2: Fatty acids compositions of some marine fish species

FATTY ACIDS	Anchovy	Horse mackerel	Menhaden	Sardine	Capelin	Herring	Mackerel	Norway pout	Sand eel	Sprat
14:0	8.48	7.95	8.95	5.68	6.53	8.50	7.80	5.57		
14:1		0.55	0.30							
15:0	0.73	16.70	0.65							
16:0	19.43	7.35	19.10	17.80	10.23	14.27	16.10	12.30	14.63	16.50
16:1	8.85		11.50	6.80	9.30	6.13	9.00	4.70	7.03	6.80
16:2			1.65							
16:3		1.40	1.90							
16:4		1.65	1.10							
17:0	0.93	0.60	0.90							
18:0	4.45	3.05	3.45	3.09	1.30	1.03	1.80	2.70	1.80	2.17
18:1	14.20	9.30	10.00	7.85	14.03	9.67	12.90	13.90	9.05	15.67
18:2	1.78	1.15	1.25	2.58	1.23	1.37	1.30	1.23	7.23	1.97
18:3	0.68	0.60	1.35	1.87	0.60	1.50	1.10	1.17	5.68	1.73
18:4		1.90	2.70		5.33	3.00	2.50	4.30		
20:0		0.35		0.63						
20:1	3.70	5.55	0.90		12.67	12.60	12.10	10.23	15.08	10.27
20:2					0.40	0.57	0.20	0.60		
20:3		0.30				0.40				
20:4	0.70	0.85	2.25		1.67	5.10		2.57		
20:5	16.23	13.65	14.10	8.43	9.97		7.60	8.23	9.05	6.33
21:5		0.35	0.65							
22:0		0.20								
22:1	1.85	9.55	0.30	5.07	10.63	21.83	13.90	11.70	15.53	14.10
22:2					0.60	0.37		0.60		
22:4										0.67
22:5	0.98	2.00	2.45		0.63	0.80	0.60	10.67	0.73	0.65
22:6	9.55	9.35	8.55	15.53	9.57	6.47	7.70	14.33	8.90	8.73

Values courtesy of IFFO Fish oil bulletin no. 18

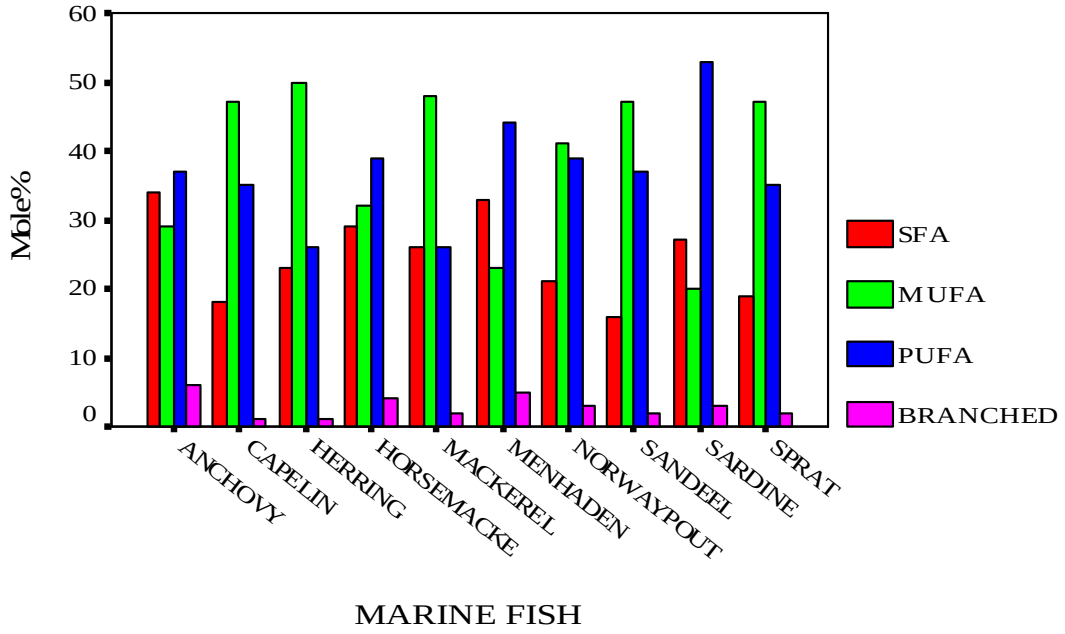


Figure 5.3: Major fatty acids components of marine fish

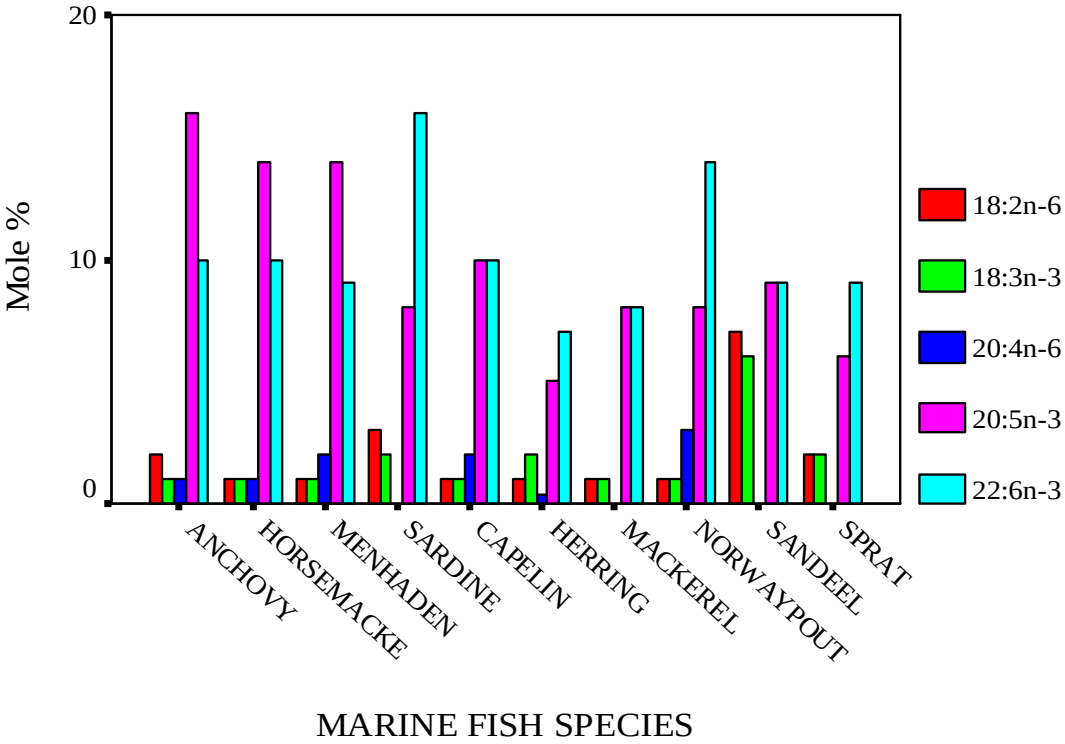


Figure 5.4: Essential Fatty Acids in some Marine Fish

Branched chain fatty acids identified in marine fish (Figure 5.3) are C16:0, C18:2 and C20:0 as against C15:0, C16:0, C17:0, C18:2 and C20:0 in the freshwater species (Figure 5.1). The content of these fatty acids in freshwater species ranged between five to fifty-one moles percent while in the marine species, it is between fourteen to twenty-one percent. This suggests that the freshwater fish oils may be better fats after hydrogenation. Our results revealed that freshwater fish oil is more of Omega-6 series of the polyunsaturated fatty acids while the marine is more of Omega-3 series (Figures 5.2 and 5.4). The prominent Omega-3 being C22:6 while the C18:2 are for the Omega-6 series. This suggests that the dietary essential fatty acids requirements for marine fish for Omega-3 polyunsaturated fatty acids may be higher than that of freshwater fish. It also suggests that marine species are suppliers of C18:3n-3; the initial building blocks for all Omega-3 fatty acids in the human body although *Cla*rias *anguillaris, Lates niloticus*, *Heterobranchus bidorsalis*, *Clarias gariepinus*, *Clariheterobranchus*, *Oreochromis niloticus* and *Sarotherodon galilaeus* compare favourably well with marine fish oil in C18:3n-3. However, only *Lates niloticus* seems to compare well with marine species on C20:5n-3 content. The marine species are better in 4,7,10,13,16,19-docosahexaenoic acid. The freshwater species are rich in C18:2n-6 as well as in C20:4n-6 contents. The C20:4n-6 fatty acid is a major constituent of membrane lipids (phospholipids) and by enzymatic action is the principal precursor of hormone-like compounds known as eicosanoids including the prostaglandins (prostanoids, isoprostanes, and isofurans). The eicosanoids produced from C20:4n-6 cause the strongest inflammatory response in humans. Inflammation is one of the body defense mechanisms that reduce the spread of infection.

5.4. FATTY ACIDS PROFILE AND LIPID DETERIORATION DURING PROCESSING AND STORAGE

High contents of polyunsaturated fatty acids in *Lates niloticus, Mormyrops anguilloides*, *Hydrocynus forskalii* and *Clariheterobranchus* (Figure 5.2) and in the marine species (Figure 5.4) increase the degree of unsaturation of these fish oils and may increase their susceptibility to lipid oxidation. Such fish therefore may not be suitable for preservation through smoking, frying and sun drying except if antioxidants are used. Large or large oily fish require longer periods of processing, therefore cold smoking technique (45-60^{O}C) could be recommended for such fish species.

High proportion of saturated fatty acids accounts for low iodine value. However, substantial amount of 9,12-Octadecadienoic acid and 9-Octadecenoic acid (Figure 5.2) necessitates high iodine value. The presence of 9-Octadecenoic acid and 9,12-Octadecadienoic acid encourage lower melting point. 9,12-Octadecadienoic acid and 9,12,15-Octadecatrienoic acid content among other factors (viscosity, colour, iodine value and peroxide value) determines oil quality for industrial purposes.

Oils rich in Omega-3 polyunsaturated fatty acids (Figures 5.2 and 5.4) containing 9,12-Octadecadienoic acid and 5,8,11,14-eicosatetraenoic acid are essential fats because their constituent nutrients are not synthesized by the body but are required for tissue development.

The very long polyunsaturated fatty acids found predominantly in fish and fish oils; Omega-3 fatty acids have been associated with reduced risk for coronary heart disease. However a negative health aspect of markedly increased consumption of polyunsaturated

fatty acids would be associated with increased lipid oxidation products, increased *in vivo* production of lipid oxidation productions and depletion of tissue levels of vitamin E.

Oxidation of lipids is of economic and nutritional significance to the food industry as well as consumers. It may result in sensory changes (flavour and aroma) and loss of nutritional value (Essential fatty acids, fat-soluble vitamins: A, D, E, K), as well as the production of primary and secondary oxidation products (hydro peroxides, free radicals, epoxides, etc). These products, like most common oxidation products of cholesterol could cause atherosclerotic injury, potent inhibitors of sterol biosynthesis or be carcinogenic (Fontana *et al*, 1993).

5.5. FATTY ACIDS PROFILE AND THE PRODUCTION OF STRUCTURED LIPIDS

Structured lipids are tailored-made, that is, they are fats and oils with improved nutritional or physical properties because of modifications which incorporate new fatty acids or change the position of existing fatty acids on the glycerol framework

The component fatty acids and their positions in the triacylglyerides molecules determine the functional and physical properties, the metabolic fate and the health benefits of a structured lipid. Short chain fatty acids (2-6 carbons) due to their water soluble nature, molecular size and short chain length, are more rapidly absorbed in the stomach than other fatty acids. Short chain fatty acids have lower heat of combustion and lower in calories. The medium chain fatty acids (MCFA) ranges between 6 -12 carbons, are metabolized as rapidly as glucose in the body with little tendency to deposit as stored fats because they are not re-esterified into triacylglycerols. They are useful in the control of obesity and can potentially raise serum cholesterol levels. Therefore, MCFA are most useful in a structured

lipid that combines their inherent mobility, solubility, and ease of metabolism with more healthful polyunsaturated fatty acids (Akoh, 1998). Long chain fatty acids (14-24 carbons) are common to animal fats and vegetable and marine fish oils. Because they are slowly absorbed and metabolized, they are lost as calcium-fatty acid soaps in the feaces (Broun *et al*, 1999).

Fish oils (Tables 5.1 and 5.2) contain highly unsaturated fatty acids which are susceptible to oxidation. High percentage of unsaturated fatty acids as well as the lowered energy of activation in the initiation of free radical formation make the oils most reactive. The oils must therefore be hydrogenated or interesterified to modify such fatty acids before the oil can be used for normal commercial food purposes such as salad oils, frying fats, industrial margarines and shortenings, low calorie spread to make bread, pastries, cakes, cookies, biscuits and synthetic creams. They could also be used in the production of emulsifiers for food applications.

The broad spectrum of component fatty acids as well as the consequent triglycerides make these fish oils when hydrogenated to solidify in the β' category. This makes the oils influence any blends of which they form part and also to crystallize in the β' form, thus giving the desired characteristics to the product (IFFO BULLETIN No. 18).

Fatty acids composition is the surest method of determining the selectivity of a hydrogenation reaction. Therefore fatty acids profiles aids in determining oils suitable for the production of solid fats for industrial purposes.

Knowledge of the degree of unsaturation (Figures 5.1 and 5.4) aid in the careful control of the hydrogenation conditions to ensure that oxidation products responsible for the typical

fishy taste and smell are eliminated and a fat of satisfactory oxidative stability produced for margarines and shortenings through the elimination of 6, 5, and 4 double bond acids as well as the trienes. Polyunsaturated fatty acids serve as precursors for fish flavours (Josephson, 1991). Low levels of polyunsaturated fatty acids ensure low fishy flavour development. However, such fish lack the substantial concentration of nutritionally desirable 5,8,11,14,17-eicosapentaenoic and 4,7,10,13,16,19-docosahexaenoic acids that could be in the edible muscle tissue (Figures 5.2 and 5.4).

5.6. SUMMARY

Lipids are fatty acids and their derivatives, and substances related biosynthetically or functionally to these compounds. Lipids consist of fatty acids esterified with alkanols to which also other elements/functional groups may be bound. The neutral lipid constitute the main part of fish oils and may fluctuate depending on the energy status and contains less polyethene fatty acids but more of the monoethylenic fatty acids.

Lipids are important dietary constituents because of their high energy value, fat soluble vitamins and the essential fatty acids which are found with the fat of natural foods.

Fish oil is usually produced by the wet-rendering process, which involves cooking the fish with steam to rupture the fat cells and pressing the cooked fish to separate the lipids' fraction from the solid fraction. Health food-grade fish oils are usually produced from small short-lived fish. Most health-food grade fish oils are fish body oils extracted from the fish and are acceptable in small amounts while higher doses require much more purified type of oil; ultra-refined fish oil.

The flesh of most freshwater fish species contains excess of fatty oils. This high oil content makes them difficult to cure. Detailed information about lipid components and their fatty acids constituents is needed to understand how to diminish oxidative or hydrolytic factors which affect quality of fish. However, the fatty acids composition of the muscle cell membranes is an important factor in determining the stability because oxidative changes are initiated from the membrane components of muscles.

Fatty acids composition is the surest method of determining the selectivity of a hydrogenation reaction. Therefore fatty acids profiles aid in determining oils suitable for the production of solid fats for industrial uses. It also provides information about the essential fatty acids requirements of fish which would aid in the compounding of adequate protein-to-fat ratios in feeds that would balance energy requirements with caloric intake.

Freshwater and marine fishes have complex compositions of fatty acids but they differ in their fatty acids compositions. They are unique in the variety of fatty acids of which they are composed and their degree of unsaturation. The high level of branched chain fatty acids in freshwater species has an important advantage. Fish oils contain highly unsaturated fatty acids which are susceptible to oxidation. High percentage of unsaturated fatty acids as well as the lowered energy of activation in the initiation of free radical formation make the oils most reactive.

The marine fish contains high levels of C20:5n-3 and C22:6n-3 fatty acids while the freshwater fish have high content of C18:2n-6 and C20:4n-6 fatty acids. The prominent Omega-3 being C22:6 in marine species while the C18:2 are for the Omega-6 series in the freshwater species. This therefore means that the dietary essential fatty acids requirements

for marine fish for Omega-3 polyunsaturated fatty acids are higher than that of freshwater fish. Marine species are therefore suppliers of C18:3n-3 fatty acids; the initial building blocks for all fatty acids in the body (Hilditch and Williams, 1964; Yamada and Hayashi, 1975).

In the systematic review of the scientific-medical literature to identify, appraise, and synthesize the evidence for the economic potentials of omega-3 and omega-6 fatty acids, it was noted that the structural similarity with saturated fatty acids, lack of specific metabolic functions of their parent polyunsaturated fatty acids (PUFA), and its competition with essential fatty acids (EFA) in many complex metabolic pathways increase low density lipoprotein (LDL) to a degree similar to that of saturated fats but reduce high density lipoprotein (HDL) cholesterol

Studies were however heterogeneous with regard to the methods of estimating fish or omega-3 fatty acid intake, background diets, and background risk for disease. Also optimal quantity and type of omega-3 fatty acid, and the optimal ratio of omega-3 to omega-6 fatty acid, if any, still remain undefined. Likewise, nothing specific can be concluded regarding the role of specific sources, types, or doses of omega-3 fatty acid content in producing significant clinical effects. High doses of fish oil may decrease immune function, impair bacterial resistance, increases low density lipoprotein cholesterol and decrease high density lipoprotein cholesterol.

Fish lipids are, however, more effective generally than vegetable lipids as source of 5,8,11,14,17-eicosapentaenoic and 4,7,10,13,16,19-docosahexaenoic acids. Diets rich in Omega-3 fatty acids reduce natural killer cell and cytotoxic T-lymphocyte activity but

stimulate the more antigen-specific immunoglobulin M and immunoglobulin G (Peterson, 1998; Watanabe, 1994).

5.7. CONCLUSION

The aim of this work is to investigate the component fatty acids of some freshwater fish species common in Nigeria's freshwaters as well as compare the nutritional quality of these fishes with those of marine fishes by comparing the levels of essential fatty acids present. A systematic review of the scientific medical literature was included, in order to appraise, and synthesize the evidence for the economic potentials accruable from these natural resources as regards their contents of 5,8,11,14,17-eicosapentaenoic acid and 4,7,10,13,16,19-docosahexaenoic acid as well as to aid in the development of a research agenda for the possible health benefits of fatty acids.

The overall significance of this study is that marine fishes are better sources of Omega-3 essential fatty acids while freshwater fishes are good sources of Omega-6 essential fatty acids. The regular pattern of marine fish fatty acids compositions makes them of advantage to the Hydrogenator. However, the high percentage of branched and saturated fatty acids in freshwater fishes gives them an advantage in curing processing. Fatty acids profile analysis also provide information about the essential fatty acids requirements of fish which would aid the compounding of adequate protein-to-fat ratios feed that would balance energy requirements with caloric intake.

From the literature review, we observed that most of the claims by scientists on the curative/preventive effects of fish oils are not always true. However, Omega-3 fatty acids reduce only serum triglycerides in humans. It could be certain that non-validity of the

claims could be the paucity of solid observational research with which to construct an experimental framework affording the meaningful conduct of randomized clinical trials (RCT). There is strong evidence that fish oil has a strong beneficial effect on triglycerides, small on blood pressure, coronary artery restenosis and possibly heart rate variability, particularly in patients with recent myocardial infarctions. Omega-3 fatty acids had a favourable effect on triglyceride levels relative to placebo (pooled random effects estimate). Studies are also suggestive in the supplemental treatment of schizophrenia as a short term intervention. If a reasonable view is that polyunsaturated fatty acids may play a role in human health generally, then given the observed or proposed interrelationships among Omega-3 and Omega-6 fatty acids contents both in the human diet and metabolism, researchers should consider taking into account the possible therapeutic or preventive influence of the dietary Omega-3 fatty acid ratio.

5.8. RECOMMENDATIONS

1. Much research should be carried on the therapeutic and/or preventive potentials of each source, type, dose, or combination of Omega-3 fatty acids as well as the Omega-6/ Omega-3 fatty acids intake ratio. Also the influence of smoking, alcohol use, and psychotropic medication on the relationship between Omega-3 content and clinical outcomes needs to be investigated and established.

2. All studies that assess the effects of Omega-3 fatty acids should use standard validated instruments to assess clinical outcomes. The measurement techniques for a number of the outcomes evaluated complicate interpretation of individual study findings and limit the ability to compare studies.

3. It is necessary for future studies to reveal the economic and ecological impact of increased fish intake and the potential to initiate changes in the dietary patterns as well as the possible different effects of Omega-3 fatty acids in different sub-population and different effects related to different covariates including dose and duration of intake.

4. There should be the development and dissemination of a consensus statement about goals and standards of research while Observational studies should report data about type of fish consumed and method of preparation.

5. Individuals who have disorders involving bleeding, who bruise very easily, or who are taking blood thinners should consult with medical practitioners before taking supplemental Omega-3 fatty acids. There could be bleeding and hemorrhagic stroke following confirmed Omega-3 supplementation.

6. To minimize lipid peroxidation, essential fatty acids supplements should be taken with no less than 500mg of vitamin E per day. Vitamin E can protect essential fatty acids from lipid peroxidation in capsules and in the bloodstream, as well as in cell membranes. But for smokers, vitamin E may not be adequate to reduce oxidation of fish oil in low density lipoprotein-cholesterol, and some medical authorities have cautioned against too much fish oil consumption by Smokers (Harats, 1991).

7. Undertake collaborative work with the food, nutraceutical and pharmaceutical industries to study the rules that govern the assembly of natural and fabricated food structures and their subsequent disassembly in the gastro-intestinal tract. This will advance understanding of the way that food structure and processing affect macro- and micro-nutrient release and allergic potential and increase the proportion of income that emanates from knowledge transfer and commercial exploitation.

8. The role of the individual human genotype and its impact on phenotype with respect to the interplay between diet and health. This will provide guidance to policy-makers on life-style, life-stage nutritional guidelines as well as for food formulation for target groups.

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APPENDICES

Appendix 1.1: Various Fatty Acids Found in Fish Oils.

Fatty Acid	IUPA Name
SFA	**$C_nH_{2n+1}COOH$**
C4:0	Butanoic acid
C6:0	Hexanoic acid
C10:0	Decanoic acid
C12:0	Duodecanoic acid
C13:0	Tridecanoic acid
14:0	Tetradecanoic acid
15:0	Pentadecanoic acid
16:0	Hexadecanoic acid
17:0	Heptadecanoic acid
18:0	Octadecanoic acid
20:0	Eicosanoic acid
21:0	Uncosanoic acid
22:0	Docosanoic acid
23:0	Tricosanoic acid
24:0	Tetracosanoic acid
MUFA	**$C_nH_{2n-1}COOH$**
14:1	7-Tetradecanoic acid
15:1	Pentadecenoic acid
16:1	8-hexadecanoic acid
17:1	8-heptadecanoic acid
18:1	9-octadecanoic acid
20:1n-9	9-eicosanoic acid
21:1	Uneicosenoic acid
22:1	Docosadienoic acid

PUFA	
Two double bonds	**$C_nH_{2n-3}COOH$**
16:2n-7	7,10-hexadecadienoic acid
18:2 n-6	9, 12-octadecadienoic acid
20:2 n-6	11,14-eicosadienoic acid
22:2 n-6	13,16-docosadienoic acid
Three double bonds	**$C_nH_{2n-5}COOH$**
16:3n-3	7,10,13-hexadecatrienoic acid
18:3 n-6	6, 9, 12-octadecatrienoic acid
18:3 n-3	9,12,15-octadecatrienoic acid
20:3 n-6	8,11,14-eicosatrienoic acid
20:3 n-3	11,14,17-eicosatrienoic acid
Four double bonds	**$C_nH_{2n-7}COOH$**
16:4n-4	7,10,13,16-hexadecatetraenoic acid
18:4 n-3	6,9,12,15-octadecatetraenoic acid
20:4 n-6	5,8,11,14-eicosatetraenoic acid
22:4n-6	7,10,13,16-docosatetraenoic
Five double bonds	**$C_nH_{2n-9}COOH$**
20:5 n-3	5,8,11,14,17-eicosapentaenoic acid
21:5n-2	7,10,13,16,19-uncossapentaenoic acid
22:5 n-3	7,10,13,16,19-docosapentraenoic acid
Six double bonds	**$C_nH_{2n-11}COOH$**
22:6 n-3	4,7,10,13,16,19-docosahexaenoic acid
BRANCHED CHAIN FA	
15:0	5, 7-dimethyltridecanoic acid
16:0	5,7-dimethyltetradecanoic acid
17:0	7-methylhexadecanoic acid
18:0	7, 9-dimethylhexadecadienoic acid
20:0	7, 9-dimethyloctadecanoic acid

Appendix 1.2: Scientific names of marine fish species

Anchovy	*Engraulis capensis*
Horse mackerel	*Trachurus murphyi*
Menhaden	*Brevoortia tyrannus*
Sardine	*Sadirons saga*
Capelin	*Mallotus villosus*
Herring	*Clupea harengus harengus*
Mackerel	*Scomber scombrus*
Norway pout	*Trisopterus esmarkii*
Sand eel	*Ammodytes tobianus*
Sprat	*Clupea sprattus*