CHEMICAL ANALYSIS AND STORAGE STUDIES OF THREE NIGERIAN FRESH WATER FISHES

BY

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DECLARATION

This is to certify that the work reported in this thesis is original and was carried out by myself. It has not been accepted in any previous application for a higher degree, and all information cited from published and unpublished sources are specifically acknowledged in the text by means of references.

S.A. OTITOLOGBON

CERTIFICATION

This thesis entitled "Chemical Analysis and Storage Studies of three Nigerian Fresh Water Fishes" by Stephen A. Otitologbon meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

TO ORPHANS – Who believe they can do all things through Christ that strengthens them.

QUOTATION

"For the vision is yet for an appointed time. but at the eno it shall speak. and not lie: thouon it tarry, wait for it: because it will surely come. it will not tarry. For he that seeketh findeth".

(Habakuk 2:3: Mat. 7:8)

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ABSTRACT

The proximate compositions of the edible portion of fresh, prefrozen and oven dried Nigerian fresh water fish Mormyrus rume, Clarias lazera and Oreochromis niloticus were determined. The fish have high moisture and protein contents. They are also fairly rich in lipid and ash. Mormyrus rume has the highest lipids and proteins while Oreochromis niloticus has the least.

The edible portions were extracted using four different solvent systems. Of the solvent systems, the chloroform/methanol (2:1 v/v) mixture gave the highest amount of extract irrespective of fish specie and pre-extraction treatment and was subsequently used for all extractions. TLC of the extracts showed that all fish extracts contained same components of six different neutral lipids and eight phospholipid fractions although in different proportions. The determination of the mineral elemental compositions of the fish support their age long usage as an important nutritional source. The studies on drying, freezing and refrigeration of the fish samples indicated different on-set of autoxidation manifested in fish spoilage. The PH, amount of peroxides and free fatty acids of the fish samples were observed to increase with increase in storage time (days) whereas the amount of lipids and proteins decreased with increase in storage time.

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DEFINITION OF TERMS

C.FH Fresh Clarias lazera

C.FO Freshly caught Clarias lazera. frozen at -10°C for 5 days and then oven dried (60°C) for 22-24 hours.

C.FD Oven dried (60°C) for 22 - 24 hours Clarias lazera

M.FH Fresh Mormyrus rume

M.FO Freshly caught Mormyrus rume. frozen at -10°C for 5 days and then oven dried (60°C) for 24 hours

M.FD Oven dried (60°C) for 24 hours Mormyrus rume

O.FH Fresh Oreochromis niloticus

O.FO Freshly caught Oreochromis niloticus. frozen at -10°C for 5 days and then oven dried (60°C) for 24 hours.

O.FD Oven dried (60°C) for 24 hours Oreochromis niloticus.

CHAPTER ONE

1.0 INTRODUCTION

Most past reports on fish in Nigeria were concentrated on the evaluation of their physical parameters The composition of fish fats have been less studied. However in the developed countries, the relation between diet and health has been in recent focus (Oyvind Lie et al, 1994). In this connection fats from fish have received much attention. Of all the fats contained in fish the rapid emergence of omega - 3 fatty acids, as a possible prevention for cardiovascular (Nelson, 1972) and coronary health disease (Glomset, 1985; Chanmugan et al., 1986) has made the study of fish lipids a relevant research topic in the quest for the understanding of its relations to diet and health. Even in more recent times, Omega -3 fatty acids have been linked to foetal development (Oyvind Lie, et al., 1994). Further studies have also indicated that they are vital fatty acids for the development of brain and nervous systems in infants/children. They are similarly implicated in the protection against chronic infections, diabetes and certain types of cancer (Dyerbery, 1986; Kinsella, 1986; Oyvind Lie et al, 1994).

The past three decades have seen a rapid growth in the understanding of the chemical nature of animal lipids and fatty acids. To a large extent, credit for this advance in knowledge can be linked to the development of a variety of

analytical techniques. Some of these techniques include chromatographic analysis which has made the fractionation and analysis of animal lipids and their component acids comparatively simple. (Hilditch & Williams 1964, Flick et al. 1980, Kates, 1986).

The initial problem in the determination of lipid content of animal tissue occur in selecting the best method of extraction. Insufficient attention to this crucial aspect of analytical operation may result in a variety of errors arising either from incomplete extraction of the cellular lipid or from non-physiological alterations in the lipid structure occurring during extraction. The importance of an effective extraction method was highlighted by Entenmann (1961) who indicated that if the lipids are incompletely extracted or altered during extraction, the results will be inaccurate regardless of the efforts spent later or the precision of the apparatus and technique used in the analysis. Therefore, to accurately report lipid composition, it is imperative that the lipid be extracted quantitatively without decomposition from the food products.

The main problems associated with extraction procedures are that some of the solvents used for the extraction are found to be inadequate in solubilising the entire lipid content of the fish. Also, some solvents are incapable of selective extraction of only the lipid amongst the other components such as proteins and carbohydrates. In an attempt

to overcome some of these difficulties, different types of solvent systems have been suggested (Blanck, 1955). These include acetone, diethylether, dichloromethane; methanol mixture; chloroform: methanol mixture and petroleum ether. Of these, the most commonly used solvent system is the chloroform: methanol (2:1 v/v) even though this mixture does not completely extract the acidic phospholipid and free fatty acids (Higgin, 1987).

In addition to extraction procedural problems, other factors that affect fish analysis are sampling procedure, time of analysis, the efficiency of equipment and procedural skills. All of these contribute to the loss of quality or to the rancidity of the extracted lipids.

1.1 ASSESSMENT OF FISH SPOILAGE

The "on the spot" routine assessment of the degree of spoilage in fish by most inspectors rely largely on appearance and odour as indicators of fish spoilage. For instance, the odour deteriorates, the surface becomes dull, the slime on it becomes opaque, the flesh softens, the eye pupils become turbid and a reddish colouration develops along the backbones. Baines and Shewai (1965) have presented organoleptic scales for assessing the raw odour and cooked flavours of white fish. Chemically, the development of rancidity can be traced to the occurrence of autoxidation caused by some environmental effects - heat, air, light, transition metals and some

bacterial effects (Connell, 1979 and Pearson, 1981).

1.2 OBJECTIVE OF THE RESEARCH

With an increase in the daily demand of fish due to its nutritional and medical importance, the chemical analysis of fish is therefore important.

Most works reported have been done on the temperate marine and fresh water fish. Little information is available on the proximate composition of Nigerian fish. Very few research information have been reported on the highly priced tropical counterparts such as Mormyrids, Clarias and Tilapia species. Therefore considerable information on many aspects of chemical and nutritional values of fish found in Nigerian water is desirable.

The objectives of this research therefore include:

(a) The determination of proximate composition of Mormyrus rume; Clarias lazera, and Oreochromis niloticus (Tilapia nilotica).

(b) The determination of the physicochemical properties and fatty acid composition of the fish lipids.

(c) The determination of elemental compositions (micro and macro-elements) of the fish and

(d) The effects of drying, freezing and refrigeration on the proximate compositions.

CHAPTER TWO

2.0 LITERATURE REVIEW

Approximately 19,000 species of fish exist but only about 200 species are commercially important (Gurr and James, 1975; Finar, 1982). The typical consumer is familiar with only a few of these varieties. In Nigeria almost half of the fresh water fish catch is made up of Tilapias, Aletes, Clarids, Mochokids, Bagrids and Mormyrids (Orji and Akobuche, 1989, Oni, 1979).

Fish could well occupy a more prominent place in the diet in view of the fact that nearly three-quarter of the earth's surface is covered with water. However, Fish consumption is closely linked to traditional food and feeding habits. The well informed discriminating consumer can infact find a wide choice of fish and fishery products to provide pleasure for the palate as well as the necessary proteins, vitamins, and minerals in desirable proportion (Piggott, 1976).

2.1 CLASSIFICATION OF FISH

Fish are sometimes classed on the basis of anatomical differences as fish with vertebrae and fins or as shell fish. Fin fish can be grouped further into lean (fat content under 5%), and fat fish (fat content 5-20%). Bass, flounder, halibut, Perch and sole are examples of lean fish. Fatty fish include albacore, herring, mackerel, salmon (Chinook and red), shad, sardine, smelt and tuna. Fin fish are also classed on

the basis of the kind of water in which they spend their adult lives as either fresh or salt water types (Peterson, 1978).

Shell fish have, instead of a skeleton, a hard shell on the outside of the soft tissues and they are mainly crustaceans (segmented). Examples are crabs, cray-fish, lobsters, prawns and shrimps. Molluscs constitute the other group of shell fish (unsegmented body in a calcified shell) and these include abalone, clams, mussels, oysters and scallops, and most of these are found in Nigerian waters (Gurr & James 1982).

2.2 COMPOSITION AND NUTRITIVE VALUE OF FISH

Nutritionists agree that fish is an excellent source of protein. Flesh of fish contains all the ten essential amino acids, these are lysine, threonine, methionine, phenylamine, valine, arginine, histidine, leucine, isoleucine and tryptophan (Peterson, 1978,) in desirable concentrations for human beings. Fish contain minerals such as iodine, potassium, phosphorus, iron, copper, sodium, and calcium (Paul and Southgate, 1978, Ruiter et. al. 1995; Oyvind et al. 1994).

Shell fish is a good source of selenium, iodine and fluorine, most of the other mineral elements required in nutrition are present in appreciable amounts (Maage & Julshamn, 1987; and Oyvind et al. 1994).

2.2.1 PROTEIN:

Fish is an excellent source of protein with a well-balanced amino acid composition. The nutritive value of protein in fish flesh is equal to or better than milk protein, casein (Blanck, 1955, Peterson 1978). Fish muscle is highly digestible (Thorpe Dictionary 1949 and Blanck, 1955). It is high in lysine, therefore, it is a good supplement to a high cereal diet. The outstanding food value of fish protein has stimulated interest in the preparation of canned fish as baby food (Thorpe, 1949).

2.2.2 LIPID/FAT:

Fish lipid differs from that of most other naturally occurring oils and fats in:

(a) having a greater variety of lipid compounds

(b) possessing larger quantity of fatty acids with chain lengths exceeding 18 carbons (James and Gurr, 1978).

(c) containing a much greater proportion of highly unsaturated fatty acids (Ackman, 1967).

(d) having polyunsaturates, primarily at the omega-3 rather than omega-6 position (Ackman, 1988, a,b) Ackman and Eaton, 1988).

The polyunsaturated fatty acids have important nutritional roles. Fish lipids contain 2-9% linoleic acids (Ackman, 1966, Connell, 1979). The linolenic acids of the omega-3 fatty acids series not only supports growth but also

functions in the transport action of lipid materials such as cholesterol (Rodolfo Paoletti, 1964, Susan E, 1987). As lipids serve as a convenient means of rapid heat transfer, fish lipids is now used in commercial frying operation (Blanck, 1955, Peterson, 1978).

Dietary lipids represent the most compact chemical energy available to man (Thorpe, 1949). They contain twice the caloric value of an equivalent weight of sugar. They are vital to the structure and biological functions of cells. The linoleic acids of fish have both a structural and functional roles in animal tissues and are carriers of the nutritionally essential fatty soluble vitamins (Sussan E. 1987).

In Europe, marine fish oils are used in the manufacturing of edible fats such as margarine, though in North America the existing large vegetable oil resources practically prelude their use for this purpose (Hilditch Williams, 1964; Kummerov, 1960).

2.2.3 MINERALS:

In general sea fish have higher iodine and lower iron contents than lean beef (Hilditch Williams, 1964). Canned fish which contain the bones usually have over 0.2% of both calcium and phosphorus.

Fish muscle in addition contains most of the elements present in sea water which includes the nutritionally important ones like Zn, Cr, and Ni, Fe and Cu are needed for

the synthesis of haemoglobin. Iodine is nutritionally important for the maintenance of and function of the thyroid gland in the production of thyroxine and in the prevention of goitre. Cr functions in glucose and fat metabolism. Unfortunately other elements that are injurious to the body when present in excess, examples include Pb, Hg, As, Se and Cd may also be present.

2.2.4 VITAMINS:

Fish, especially their viscera, are collectively a source of most of the vitamins required by man. Fish muscle, liver and visceral organs are the richest natural supplier of the vitamins A and D (Petersons, 1978, Oyvind, 1994). Comparative vitamin data for fish based on both raw and processed samples of similar origin are very rare. (Bhuiyan A et al., 1992). The distribution of vitamins A and D is extremely variable with species, seasons, and sexual maturity. The fillets of some fish such as char, herring, mackerel, plaice, salmon and sprat contain high value of vitamin B12 (Oyvind lie et al., 1994 and Ackman, 1991).

2.3 NATURE OF FISH LIPID

The basic lipid of fish muscle is cellular in nature. Fat generally carries natural flavour and preserves others generated during cooking, pickling or other processing. As few as ten or eight fatty acids, fairly accurately, describe the

composition of most fat or fish oil (Hilditch and Williams, 1964).

After death, the lipids in fish are subjected to two major changes namely - lipolysis and autoxidation. The effects produced by these changes are considered undesirable and are often the major cause of spoilage (Pearson, 1981). Of the two processes, autoxidation is the more important, particularly in the deterioration of frozen fish products resulting in flavour, colour, and possible textural changes (Connell, 1979; Pearson, 1981).

The most unsaturated lipids in all fish are the phospholipid which do not oxidise rapidly because of their physical disposition which makes it difficult for them to participate in the oxidation chain reactions.

Lipid hydrolysis, as a common post-mortem feature in fish and fish products, is influenced by increase in temperature of the fish. It is also known that lipolytic activity of fish tissues may be species dependent and it leads to undesirability in the fish oils (Ackman, 1966). The major products are free fatty acids (ffas) and glycerol. Thus all species containing phospholipid are subject to lipolysis with those in some elasmobranch remarkably stable.

The component acids of fats in various tissues of a British, Indian and South American fresh water fish have been determined (Hilditch and Williams, 1964). distinctive in fresh water fish flesh fats are the small proportions (as compared

with many marine fish fats) of unsaturated C_{20} acids. With the tropical fresh water fish fats, an increase in proportions of palmitic and stearic acid and predominace of unsaturated C18 acid (about 30% upward) are observed (Hilditch and Williams, 1964).

In the C_{20} and C_{22} groups, the average unsaturated vary considerably, being greater in the fat of fresh water fish from temperate water than from tropical. In some of the latter, unsaturated C_{22} acids are absent or only present to the extent of 1-2% of the total acids.

Similarly, in these fats from warm climates, Oleic acids frequently form the greater part of the unsaturated C_{18} acids and the proportions of palmitic and stearic acids may concurrently rise respectively to as much as 25 - 28% and 10 - 12%. Marine animal oils differ from those of most other known fats especially in their very unsaturated character and avidity with which they absorb oxygen from the air and become partially converted in to viscous and gum-like materials (Connell, 1979).

One of the most prominent specific component acids of marine animal oils, hexadecanoic acid C_{14} H_{32} O_2 was first recorded in a fish by Bull (1906). Monoethenoic acids (gadoleic acid, (C_{20} H_{38} O_2) of fish was identified by Bull and later by Toyama and Tsujimoto (1927). The components to which the ready autoxidation of fish oils is due to are mainly polyethenoid acids of (C_{20} C_{22} C_{22}) groups and they are by

far the most abundant. Clupanodinic acid, belonging to this series was isolated by Tsujimoto (1927) from Japanese sardine oil and subsequently a number of other individual polyethenoid were reported by Japanese workers (Hilditch and Williams, 1964) including stearidonic ($C_{18}H_{28}O_4$) (octadecatetraenoic), arachidonic; ($C_{20}H_{32}O_2$) (eicosatetraenoic), hiragonic acid, ($C_{16}H_{24}O_2$) (hexaderatrienoic), nisinic, ($C_{24}H_{34}O_2$) (tetracosahexaenoic), shibic, ($C_{26}H_{42}O_2$) (hexaco-hexaenoic) acids. Therefore the constitution of these polyethenoid acids have continued to be the subject of much investigation.

It should be noted that the livers of many fish, in contrast to those of land animals, are often rich in fats and frequently serves as the main depot of the fish (Peterson 1978, Blanck, 1955). Studies have been carried out on the fats of fresh water fish which had been kept on low fat diet or on the latter supplemented with cotton - seed oil or manheden oil. Neither the low nor the cotton seed oil diet had any effect on the 'natural' fat of the fish. The authors inferred that marine and fresh water fishes can synthesize polyethenoid acids from known non-fatty sources and that differences between marine and fresh water might be from differences in their dietary fats (Peterson, 1978).

2.4 AUTOXIDATION AND OXIDATIVE RANCIDITY OF FISH

Oxidative deterioration of fats results in the destruction of essential fatty acids, vitamins (A,C,D and E)

and development of a pungent and offensive off-flavour (Aurand and Wood, 1973; Pearson, 1981). Fat oxidation of all non-conjugated olefinic compounds proceeds through the formation of hydroperoxides. These are however not the exclusive primary products of autoxidation of fats (Swern, 1979; Reinert, Hunter and Sabatino (1983); Hiatt (1983).

There are quite a number of other factors that affect autoxidation. Some of these are: The total number of unsaturated linkages in the fish, the degree of unsaturation within a given molecule, irradiation from all forms of light, temperature, moisture and some trace metals such as Cu, Pb, Zn acting as peroxidants to fats. In some cases some unsaturated and highly reactive fatty acids can each reflects on autoxidation rate of the other as well as the disposition of an acid within the glyceride molecules. The understanding of autoxidation in fish is made more complicated by the observation that some proteins especially haem protein do encourage autoxidation (Banks, 1939; Rouchand & Lutete, 1968, Catalano et al., 1970, Hammand and Johnson, 1972, Raghveer and Hammond, 1967).

There is also evidence to suggest that in some fatty fish species, oxidation in cold storage is probable in long term ice storage (Shenoy & Pillai, 1971; Hardy and Smith, 1976; O. Keefe and Noble, 1987), whether this is due to the introduction of free radical generating such as hydroperoxides or the development of more reactive lipids such as free fatty

acids, or the activation of catalysts through the lysis of cells or removal of an antioxidant is not known. Salt water used for fish treatment prior to freezing causes a somewhat analogous effects (Banks, 1939, Nair et al., 1978).

Spoilage assessment of fish, though often monitored by physical and chemical methods, must of necessity therefore rest finally on sensory evaluation. In wet fish, storage components introduced primarily by bacterial spoilage but also by enzymatic reactions contribute more to the flavour than those derived from lipid oxidation.

2.5 NUTRITIONAL AND HEALTH IMPORTANCE OF FISH OIL

There has been a special interest in the use of fish and fish oil for health (Avault, 1986; Budowski, 1981). Some of these effects concern the use of fish oil in the diet to reduce the risk of coronary health diseases, arthritis, atherosclerosis, asthma, auto immune diseases, cancer and multiple sclerosis (Kinsella, 1986, Zamula, 1986, Weiner et al., 1986; Foxall et al., 1990,; Bernsohn and Stephanides, 1967; Allen et al., 1987). It has also been found that there is reduction in cardiovascular and coronary mortalities through long term regular fish (Omega-3 fatty acids) consumption (Kromhout et al., 1985; Kinsella, 1987; Rosemont, 1989). High consumption of polyunsaturated fatty acids, a necessary precursor to arachidonic acid have been linked to reduction in (a variety of) human diseases, such as thrombosis

and asthma (Williams, 1967). Polyunsaturated fatty acids result in an increased production of hormone-like arachidonate, which may be responsible for antagonising certain disease states such as atherosclerosis and thrombosis. The effects of the arachidonate on serum lipids have been discussed from the perspective of ischemic heart disease (IHD) (Kinsella, 1986).

Fish oil has been found to increase erythrocyte membrane fluidity in diabetic patients (Kamada, 1988). It also contributes to the optimal development of the brain in new born infants and decrease blood pressure (Susan, 1992, Artemis, 1995), a controlled study of polyunsaturated fatty acid supplements in essential hypertension have been found to lead to a decrease in blood pressure (Knapp et al., 1989). Fish oils, when included in the diet, have been shown to be effective in depressing the concentration of cholesterol in the blood (Peterson, 1978, Hanfman & Facinoli, 1991).

Unsaturated fatty acids derived from dietary source are required for eicosanoid formation and are integral part of cell wall and mitochondrial membrane (Singh, 1988). Fish oil has been implicated in the altering of the brain polyunsaturated fatty acid composition (Bourre, et al., 1988). Studies also shown that ingestion of Omega-3 fatty acid has beneficial effects on coronary arteries, lowering blood pressure, reducing arthritis symptoms and cancer risks (Zaidins, 1989). α - linolenic acid (Omega-3) caused a

reduction of the size and number of turmours (Artemis, 1995) and this is an area where a lot of research is needed in order to improve the understanding of the cellular mechanism involved.

Omega-3 (PUFA) are now regarded as so important that among the food recommendations, there is a specific recommendations that people should be eating 'at least' two portions of fish daily, and of which one should be oily fish.

2.6 EXTRACTION OF LIPID OF FISH

The solvent extraction of lipids from tissue depend on the degree of association of the lipid with the tissue protein. Successful extraction requires that bonds between lipids and other compounds be broken so that the lipids are freed and solubilised. Generally, such solubility is attained when polarities of the lipids and the solvent are similar. (Marinetti, 1962). During extraction it is important to keep the chemical, physical and enzymatic degradation of the fish lipids to a minimum through the control of temperature, chemical environment and time of exposure of the material to each solvent. Several solvent systems have been used but the presence of water in the fish tissue adds a complicating factors which led to the early workers prefering dehydrated materials for lipid extraction (Robin et al. 1956). There is a growing tendency to use petroleum ether because it is more selective towards true lipids. But its use is affected by the

moisture content, as the only part of lipids that can be extracted is from the moist materials. The solvent cannot penetrate the tissue as it becomes quickly saturated with water. To overcome this, diethylether is judged to be a fairly better solvent for fat extraction as it will dissolve oxidised lipids though also with some disadvantages.

A more rapid method for fish lipid extraction was proposed originally by Folch (1957) for the isolation and purification of total lipid from animal tissues with chloroform: Methanol (2:1 v/v) mixture to remove most of the phospholipid in the tissue. This mixture is a ternary one which lies close to the "tieline" in a three phase diagram of $CHCl_3$: CH_3OH:H_2O. Thus it is inconvenient to extract very wet or aqueous suspension (Bonner, 1910) and this was later modified by Bligh and Dyer (1959) and then Tsen et al. (1962).

Other solvent systems in use include;

(1) the official AOAC method that involve a 16-hour extraction with acetone in a soxhlet extraction

(2) extraction with Bligh - Dyer Method of chloroform - methanol (1:1 & 2:1 v/v).

(3) extraction with chloroform: methanol: Water (Higgins, 1987, Kates, 1986).

(4) alkaline saponification method and

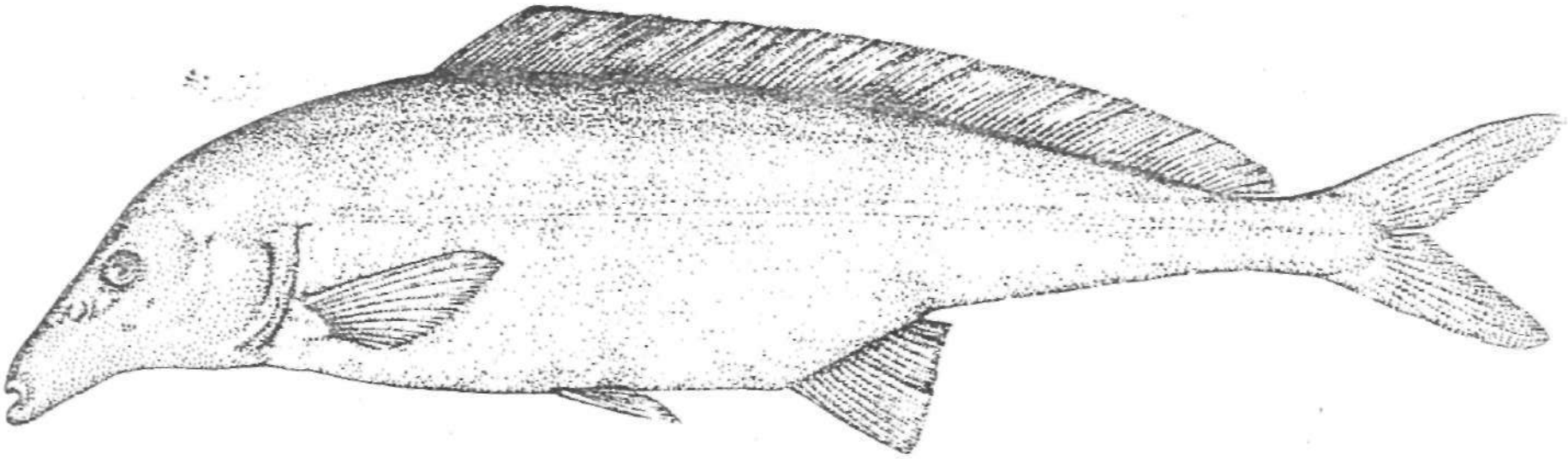
(5) the mojonnier method (Hara & Rabin, 1978; Maxwell, 1980; Rabin, 1981; Chen et al., 1981; Anderson, 1982; Burton et al., 1985; Kinsella, 1987, Kalorovic et al. 1986

Erickson, 1993.)

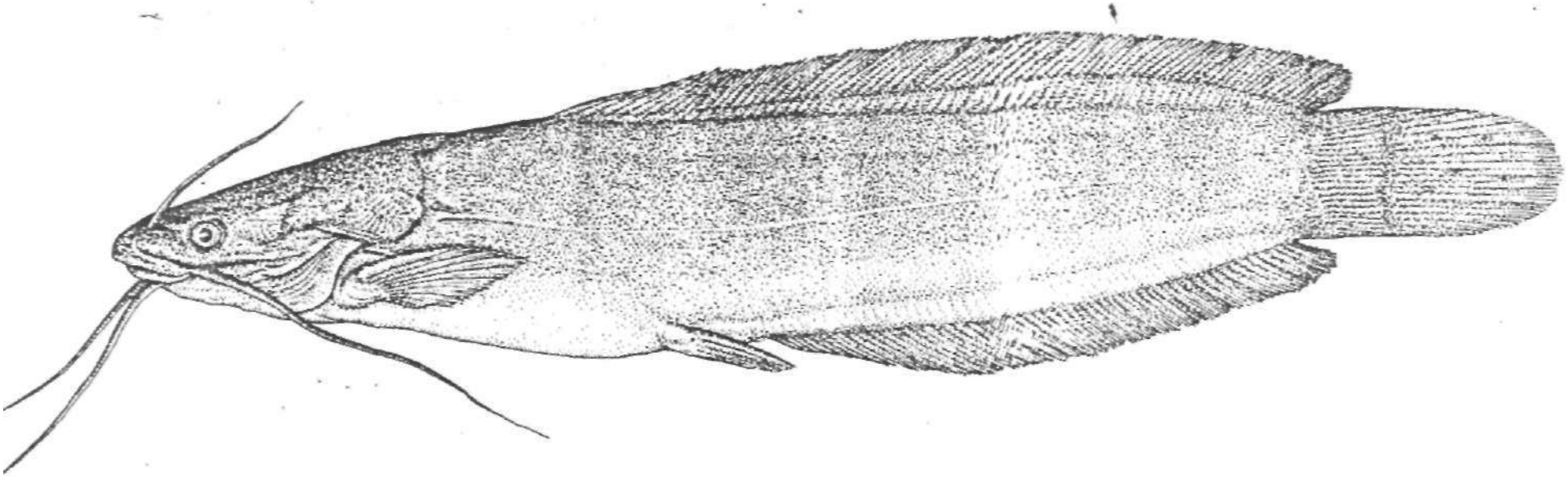
2.7 TYPES OF FISH ANALYSED

2.7.1 Mormyrus rume:

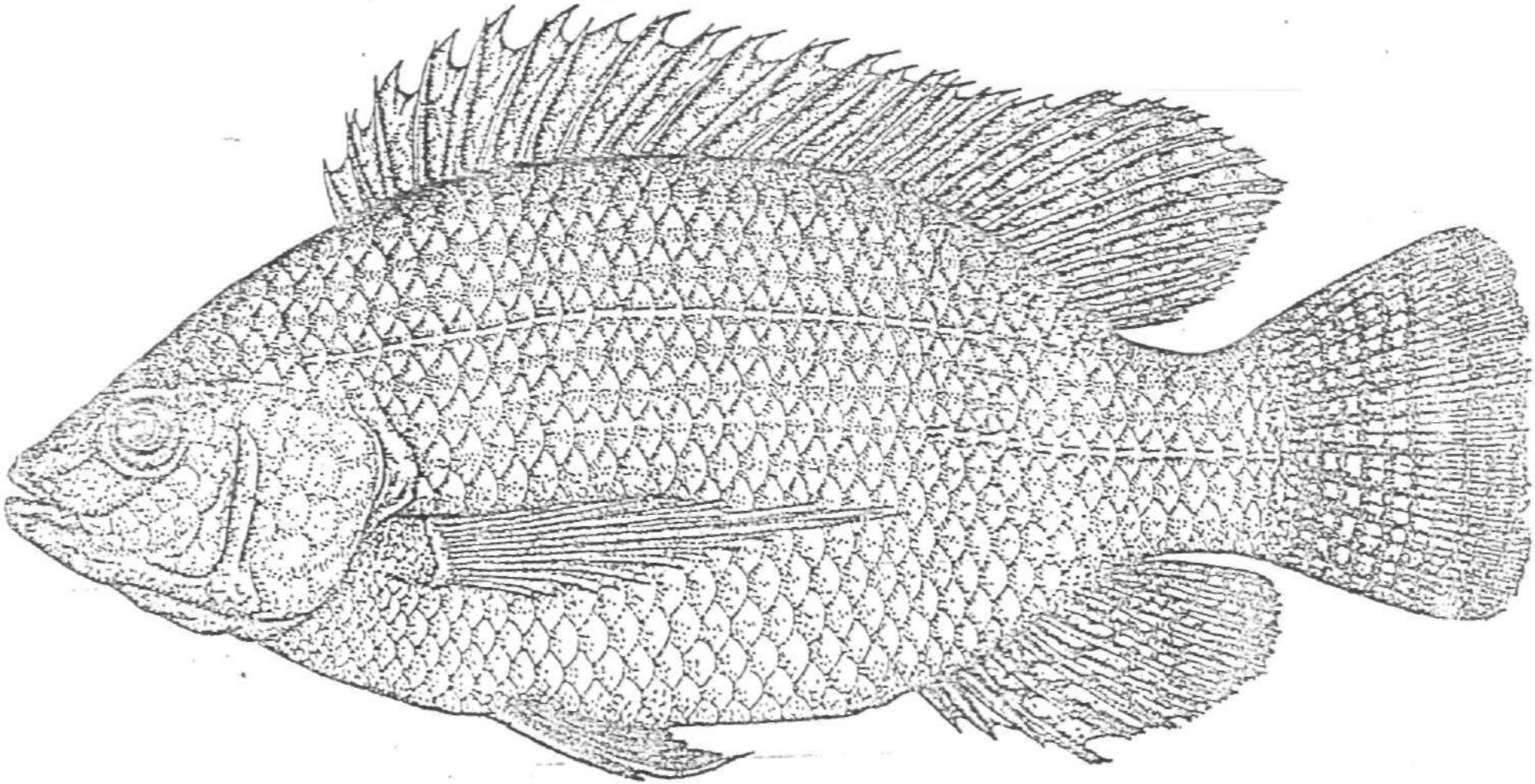
This is from the aberrant family of mormyridae which has about twenty-six species belonging to six genera. The species have the anal fins less than half of the dorsal. They have a trunk-like snout and a small terminal mouth. There are usually a uniformity greyish-yellow colour on the back and sides and light ventrally. The species grows to about a metre in length and a weight of more than six kilogram when fully matured. It is fairly common in Zaria water dam. Large species are capable of giving quite noticeable electric discharge (William et al. 1967).



Mormyrus rume



Clarias lazera



Oreochromis Niloticus

2.7.2 Clarias lazera

Belongs to the family of clariidae (teloests), widely distributed throughout Asia minor and Africa. The normal habitat of C lazera is tropical swamps and rivers. C. lazera is capable of existing in mud throughout dry season and it is able to survive on dry land for several hours, thus enabling it to migrate on occasions from dried-up pools to reach fresh water. The head is long, but the body is 3-3.5 times as long as the head.

Many exceed 100 cm but an average adult measure 50-60 cm and weighs approximately 3-6kg. The skin is smooth, tough and lack scales. It is darkly pigmented on the dorsal and lateral parts of the body. The species becomes lighter in colour, or mottled in appearance when exposed to bright light due to the concentration of pigment carrying cell called chromatophore. It is fairly more abundant than catches would suggest.

2.7.3 Oreochromis niloticus (formerly Tilapia niloticus):

belongs to the family of cichlidae. It is more elongated and less compressed than others. The colouration is brown olive with faint dark vertical bars on the flanks. The posterior part of the dorsal fin, the caudal fin and anal fin bear numerous dark vertical bars on a pale back ground, a characteristic which serves to distinguish it from all other species. Oreochromis niloticus is mainly microphagus and are abundant in Zaria dam and rivers.

CHAPTER THREE

3.0 MATERIALS AND METHODS

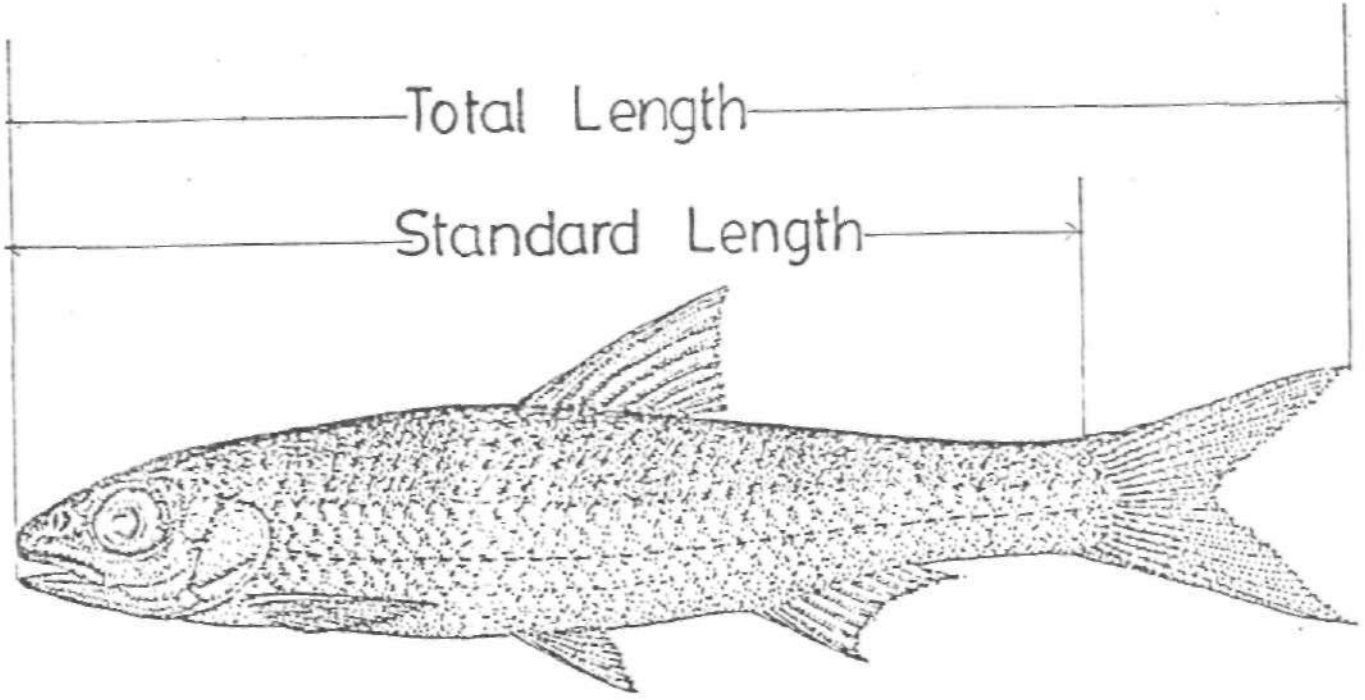
All chemicals were of analytical grade unless other wise stated. Distilled water was used throughout.

3.1 SAMPLE COLLECTION:

The fish samples used in the work described are fresh water species caught from River Galma, Sabon Gari - Zaria at various times throughout the year. The species were caught in the lower part of the river. The three species used are (i) Mormyrus rume, (ii) Clarias lazera and (iii) Oreochromis niloticus.

The fish were caught using small mesh nets. Thereafter, they were quickly transferred to the laboratory alive in some quantities of the river water. They are killed in the laboratory. The standard lengths, total lengths as well as the weights of each fish sample were quickly determined as described by Owodehinde as shown in figure (iv). The fish were cleaned, the heads, tails fins, scales were removed and then eviscerated before being minced and then weighed.

Some of the fish were refrigerated at 4°C, some were frozen at -10°C, while others were oven dried at 60°C. For the analyses involving fresh fish, the fish were immediately processed and analysed. The dried samples were grinded with mortar and stored in air tight labelled containers (Polythene bags) in the desiccators.



Method Used For Measuring Fish.

Fig.(IV).

3.2 EXTRACTION AND DETERMINATION OF LIPID CONTENT

The extraction of the fresh and the dried samples were carried out in the following solvent systems using soxhlet extractor.

i. Petroleum ether (40° - 60°C)

ii. Petroleum ether (60° - 80°C)

iii. Chloroform: Methanol (1:1 v/v)

iii. Chloroform: Methanol (2:1 v/v)

Thirty grammes (30g) of each wet sample and twenty grammes (20g) of each oven dried sample were weighed into pre-weighed thimbles and sealed with cotton wool to prevent escape of the sample from the thimbles during the course of extraction. These were transferred into soxhlet apparatus and extracted for about four hours using 250cm³ of the solvent systems. The solvents were then removed using rotatory evaporator. The remaining solvents were completely evaporated on the water bath and the flasks containing the extracts were then weighed. The extracts were stored in chloroform (antioxidant) and then quickly transferred into freezer (-10°C) to avoid autoxidation of the extracts. Three determinations were made for each solvent system. The percentage extracts were calculated based on the following formula.

$$\% \text{ extract} = \frac{\text{wt of lipid extract}}{\text{wt. of the sample taken}} \times 100\%$$

OR

$$= \frac{\text{wt. gain in flask}}{\text{wt of sample}} \quad 100\%$$

3.2.1 MOISTURE (WATER) CONTENT DETERMINATION (PEARSON'S 1981)

Twenty grammes (20g) of the prepared samples of each fish was weighed into a dry and pre-weighed metal dish containing 15.0g acid - washed (acetic acid) sand. The sample mass was mixed with $5cm^3$ ethanol using a glass rod. heated to 70°C, with occasionally stirring and then evaporated to apparent dryness. The dish with the content was placed in the oven at 105°C for about two hours after which it was cooled in a desiccator, weighed and then heated and cooled repeated until a constant weight was obtained. The percentage moisture was calculated using the following formula.

$$\% \text{ Moisture} = \frac{\text{loss in wt. on drying (g)}}{\text{initial Sample wt (g)}} \times 100\%$$

$$\% \text{ Dry matter} = \frac{\text{Oven dry wt. (g)}}{\text{initial Sample wt (g)}} \times 100\%$$

Three determinations were made for each sample.

3.2.2 DETERMINATION OF CRUDE PROTEIN CONTENT (GILCHRIST 1967)

Crude protein content is the figure obtained by multiplying the nitrogen content of the food by 6.25 (for fish). The factor 6.25 owes its origin to the assumptions that all the feed protein contain 16% nitrogen and that all the

nitrogen in a feed is present as protein. Other values are milk = 6.38, oil seed = 5.4, 5.7 - 5.9 and 6.6 are used for cereals and plant leaf protein respectively.

MATERIALS:

1. Conc. H_2SO_4 (about 98%), sp.gr. 1.84
2. HCl, 0.01M standardised.
3. Boric acid solution. Prepared by dissolving 4g of boric acid in water and diluting to 100cm^3
4. Sodium hydroxide solution: Prepared by dissolving 40g in 100cm^3 of distilled water.
5. Mixed catalyst - prepared by mixing together 16g of anhydrous K_2SO_4, 1.0g of $CuSO_4:5H_2O$ and 0.3g of selenium powder.
6. Indicator: Screened methyl red.
7. Antibumping granules.

METHODS:

DIGESTION: - (0.25g) of each sample was weighed into a dried Kjeldahl flask. One gramme of the mixed catalyst, 6cm^3 of conc. H_2SO_4 and antibumping granules were added to the flask. The mixture was heated until a clear greenish and later colourless solution was obtained. The solution was transferred quantitatively into a 50cm^3 volumetric flask and made up to the mark with distilled water.

DISTILLATION: Ten (10)cm^3 of each of the above solutions was transferred to Kjeldahl distillation apparatus. Sodium hydroxide solution (10cm^3) was added and the ammonia liberated in each was steam distilled into 5cm^3 boric acid solution containing 4 drops of the indicator.

TITRATION: The ammonia collected in boric acid solution was titrated with 0.01M HCl.

CALCULATION: Total Nitrogen (%) = $\frac{5 \times V \times M \times 0.014 \times 100}{W}$

Where W = wt (g) of sample.

V = Vol. (cm^3) of HCl required to neutralise the ammonia liberated by 10cm^3 aliquot of the digest (after correcting for blank)

M = Molarity of HCl.

6.25 = the conversion factor for fish protein (Pearson's 1981 & Gilchrist, 1967).

Three determinations were made for each sample analysed.

3.2.3 ASH CONTENT DETERMINATION (PEARSON'S 1981).

One gramme of each sample (fresh, dried and frozen and dried) was heated in a preweighed evaporating dish with 0.5cm^3 of 25% (w/v) magnesium acetate solution on a water bath. When dried, the mass was incinerated at 550°C for about four hours (i.e. until a white ash was obtained), then removed and cooled in a desiccator. The ash was then weighed. The % ash was then determined using the following formula.

$$\text{Ash \%} = \frac{\text{wt. of ash (g)}}{\text{wt. of (oven dried) sample (g)}} \times \frac{100\%}{1}$$

Three determinations were made for each sample.

3.2.4 SPECIFIC GRAVITY DETERMINATION (PETERSON, 1978, AOAC, 1970).

The specific gravity bottle of known weight was filled with the oil sample and the stopper was inserted. The filled bottle was immersed in a water bath kept at room temperature. Excess of oil was removed from the stopper and the bottle was removed from the bath and cleaned and dried. This was then weighed with care. The procedure was repeated using distilled water. The specific gravity was then determined using the following formular.

$$\text{Specific gravity} = \frac{\text{wt of oil (g)}}{\text{wt of distilled water}}$$

Three determinations were made for each sample.

3.2.5 REFRACTIVE INDEX DETERMINATION (AOAC, 1970)

The refractive index was determined using Abbe refractometer and sodium vapour lamp at 21.°C. The instrument was charged by opening the double prism, and placing few drops of samples on it, closing it firmly and then allowing it to stand for about 10 mins before reading so that the temperature of the sample and instrument could be the same.

Method of measurement was based upon observation of position of border line of total reflection in relation to faces of flint glass prism. This border line was brought into

the field of vision of telescope. The border line was adjusted until it fell on point of intersection of cross hairs. The refractive index was then read directly on the scale of sector.

Three determinations were made for each sample.

3.2.6 DETERMINATION OF pH (AOAC, 1980)

The hydrogen ion concentration of each sample was determined by homogenising 1g of sample in 10cm³ distilled water for 5 minutes. AGB 2000 pH meter was then used to read the pH.

3.2.7 PEROXIDE VALUE DETERMINATION:

The peroxide values of the lipids were determined by the use of the micro method of St. Angelo et al. (1972). This method is an adaptation of the official method of the American oil chemists' Society (1960) and Pearson's Chemical analysis of food (1981), but greatly scaled down to permit determination of smaller quantity of oil (150mg of oil).

REAGENTS: 5% KI, 2:1 glacial acetic acid: chloroform mixture 0.002M $Na_2S_2O_3$, deionised water, starch indicator (1%), oil sample, micro stirring bar and magnetic stirrer.

Preparation of Solutions:

i. 5% KI : 5g of KI was weighed into 100cm³ volumetric flask and then made up to the mark with distilled water.

ii. 0.002M $Na_2S_2O_3$: 0.079g $Na_2S_2O_3$ dissolved in 250cm³ of distilled water.

iii. Starch Indicator (1%): 1g of soluble starch was dissolved in 100cm³ of boiled distilled water, stirred and then cooled.

PROCEDURE:

An oil sample (0.15g) was dissolved in 1cm³ of solvent in a 10cm³ erlenmeyer flask. One drop of freshly prepared saturated aqueous Potassium iodide was added and the mixture stirred for exactly one minute with a micro stirring bar and a magnetic stirrer. The reaction was then quenched with 1cm³ of boiled deionised water. The liberated iodine was titrated with 0.002M $Na_2S_2O_3$ to a colourless endpoint with starch indicator.

Blank titration was carried out. The results were calculated as milliequivalent of peroxide for kg of oil as in the official method of the American oil chemists society (1960).

CALCULATION:

$$\text{Peroxide Value} = \frac{(V - Vo)\,T}{\text{wt. of oil.}} \times 1000\text{mEq/kg}$$

Where V = Volume of thiosulphate used to titrate with iodine

Vo = Volume of thiosulphate used for the blank titration.

T = Molarity of $Na_2S_2O_3$

Three determinations were made for each sample.

3.2.8 IODINE VALUE DETERMINATION (HANUS METHOD: IUPAC, 1979).

REAGENTS:

i Hanus solution: This was prepared by 4.33g of iodine solid (Analar) into a 100 cm_3 volumetric flask with a small amount of glacial acetic acid. 0.9 cm^3 of bromine added from a measuring cylinder. The flask was then made up to the mark with glacial acetic acid and then stored in an amber - coloured bottle (brown).

ii. 10% KI solution: This was prepared by dissolving 10g of KI in a 100cm^3 volumetric flask and then made up to the mark. Also stored in amber - coloured bottle.

iii. 0.1M $Na_2S_2O_3$ solution: 8.28g of $Na_2S_2O_3$ was dissolved in distilled water and made up to 100cm^3 in a 100cm^3 volumetric flask and then stored.

iv. 2% starch indicator solution: starch (1g) was dissolved in hot water and made up to the mark in a 50cm^3 flask.

PROCEDURE:

The lipid sample (0.3g) was weighed into a glass stoppered flask. 25cm^3 of the Hanus the solution was added from the burette, then allowed to stand for about 1¼ hours in the dark. 15cm^3 of 10% aqueous KI and 50cm^3 of water were then added. The iodine solution was titrated with 0.1M $Na_2S_2O_3$ to a pale yellow colour. The blank without the oil was done simultaneously. This was done in triplicate for each sample.

CALCULATION:

$$\text{Iodine Value} = \frac{1.27 \times (a-b)}{\text{wt. of Sample.}}$$

Where a = Volume of 0.1M $Na_2S_2O_3$ used on blank

b = Volume of 0.1M $Na_2S_2O_3$ used on oil Sample.

3.2.9 ACID AND FREE FATTY ACID VALUES DETERMINATION (Pearson's 1981).

REAGENTS: Diethylether, ethanol, Potassium hydroxide, 1% Phenolphthalein etc.

PROCEDURE

The lipid sample (0.5g) was accurately weighed into the mixed solvent made up of equal volumes of diethylether and ethanol and then neutralised with 0.5cm³ of phenolphthalein, and then titrated with 0.1M KOH, shaken constantly until a pink colour which persisted for about 15 sec was obtained. Triplicate results were obtained for each sample.

CALCULATION:

(i) $$\text{Acid value} = \frac{\text{Titre value (cm}^3\text{)}}{\text{wt. of Sample (g)}} \times 5.61$$

(ii) $$\text{FFA} = \frac{\text{Titre} \times 5.61}{1000} = X$$

Then $$\frac{X}{\text{wt. of Oil}} \times 100 = \text{FFA \%}$$

Where X = wt. of base that is present in the titre used to neutralise the free fatty acid.

3.2.10 SAPONIFICATION VALUE DETERMINATION (Pearson's 1981; IUPAC, 1979).

REAGENTS: HCl, KOH, Ethanol, Phenolphthalein.

PREPARATION OF SOLUTION:

0.5M HCl Solution: 4.345cm³ Hcl was dissolved in 100cm³ distilled water.

1% Phenolphthalein: 0.1g of phenolphthalein was dissolved in absolute ethanol and some drops of distilled water, contained in a 10cm³ volumetric flask, then made up to the mark.

PROCEDURE:

18g of KOH was dissolved in 10cm³ of water and was diluted to a 1dm³ mark with ethanol (96%). The mixture was allowed to stand overnight and decanted using separating funnel. The lipid sample (0.80g) was weighed into condensing bottles (quickfit flasks) and 10cm³ of the ethanolic potassium hydroxide solution added. The refluxing condenser was attached and the flask heated to boiling on water bath for one hour with frequent, shaking. 1% phenolphthalein solution (0.4cm³) was added and this was titrated hot with 0.5 M HCl (titre = a cm³). The blank was carried out (titre = b cm³).

CALCULATION:

$$\text{Saponification Value} = \frac{b - a \times 28.05}{\text{wt. of sample (g)}}$$

3.2.11 ESTER VALUE DETERMINATION (Pearson's 1981).

The ester values were calculated from the expression:

Ester value = Saponification value - Acid Value.

3.2.12 UNSAPONIFIABLE VALUE DETERMINATION (Pearson's 1981).

REAGENTS

Alcoholic KOH, diethylether, Phenolphthalein etc.

PROCEDURE: The lipid extract (1g) was weighed into a 250 cm^3 flask. Alcoholic KOH solution (12.5cm^3) 96% ethanol) was added and boiled gently under reflux for one hour. The saponified sample solution was transferred into a 250 cm^3 Separating funnel and the flask was rinsed with three (20cm^3) of water. The extracted solution in the ether layer was washed with 10cm^3 of aqueous 0.5M KOH solution and also washed with three (10cm^3) water until the wash water was neutral to phenolphthalein. The ether layer was poured into a weighed flask and weigh to constant weight.

CALCULATION:

$$\% \text{ Unsaponifiable Value} = \frac{\text{wt. gain by flask (g)}}{\text{wt of oil (g)}} \times 100\%$$

The determinations were made in triplicate for each sample.

3.3. CHROMATOGRAPHY

3.3.1 THIN LAYER CHROMATOGRAPHY (TLC)

TLC is a method of resolving a mixture of components using plate coated with thin layers of certain substances called absorbent e.g. silica gel, alumina, keiselghor and so on. In this analysis Keiselghor (60%) was used impregnated with 1M silver nitrate solution.

PREPARATION OF PLATES

The glass plates used were of sizes 10x20cm^2. They were cleaned with acetone to make them grease free. They were dried and arranged in place on the spreading bed.

PROCEDURE

Forty grammes of Keiselghor was weighed into a conical flask with screw cap, 80cm^3 of water added with 5cm^3 of 1M $AgNO_3$ solution and shaken vigorously for about 90 seconds to obtain a smooth slurry. This was poured into a spreading device (0.25mm thick), which was drawn across the glass plates, depositing a uniform coating on them. The plates were air dried, then activated in the oven at 110°C for 30 minutes, after which they were kept in a desiccator until required for use.

SPOTTING:

The chromatographic plates were arranged in the TLC spotting plane (templates). From the mark (10cm distance), a

line was drawn through the chromatographic plates that were made as solvent fronts. From the holes on the template (plane) using the micropipette (syringe) or capillary tubes (0.5mm), the samples were then spotted.

SOLVENT SYSTEMS USED:

The solvent systems used are:

i. Benzene: Chloroform (80:20; 70:30; 60:40)

ii. Chloroform: Methanol: Water (70:30:5; 70:30;10).

iii. Chloroform: Methanol: Acetic Acid: Water (70:30:1:4; 70:30:2:3).

iv. Benzene: ethanol (80:20)

Benzene chloroform (70:30) and Chloroform: Methanol: Acetic acid: Water (70:30:2:3) gave the best resolutions for neutral and phospholipid components respectively.

DEVELOPMENT OF PLATES AND THEIR VISUALIZATION

The development tanks were left or allowed to saturate with each solvent system. The spotted chromatoplates were then arranged (dipped) into the developing tank (each), then covered and untouched until the solvent system reached the desired solvent fronts (15cm distance) after about 50 minutes of the developments.

The plates were then removed and air dried using air drier. The dried plates were subjected to eye visualization, UV lamp (light) at 254 and 320nm and later with the use of

iodine vapour tanks. The colour produced were noted, the number of component were observed. The Rf, values of the components were then calculated using the following formular which is basic measurement in TLC.

$$Rf = \frac{\text{Distance Moved by Lipid from origin}}{\text{Distance Moved by the Solvent front}}$$

3.4.1 MACRO-AND MICRO-ELEMENTS DETERMINATION

The analytical methods for the determination of these elements are based on Flame photometry, Atomic Absorption spectrometry and colorimetry, Potassium and sodium were determined by flame photometry, calcium, magnesium, copper, iron, zinc, chromium, manganese and nickel were determined by Atomic absorption spectrometry while phosphorus and Iodine were by colorimetry (spectronic 20).

3.4.2 SAMPLE PREPARATION

The mineral elements of the fish samples were taken into solution by wet digestion described below.

3.4.2.1 WET DIGESTION OF FISH-SAMPLES FOR MINERAL ANALYSIS (Harris, 1979).

REAGENTS:

1. Digestion acid (1 Vol. $HCLO_3$ (63%): 4Vol. HNO_3).
2. Nitric acid concentrated
3. Hydrogen Peroxide and Sulphuric acid concentrated.

PROCEDURE:

One gramme of the grounded sample was weighed into a 30cm³ capacity micro kjeldahl flask and 20cm³ digestion acid was added. The flask was fixed in a clamp and kept over night. The flask was placed on a heating mantle and heated slowly to a temperature of about 180°C with occasionally swirling. This was continued until there was no visible particles and the digestion acid was clear. The temperature was increased to about 240°C and the digestion acid was evaporated until dense white fumes were formed within the digestion flask. The flask was then removed from the heating source, the content filtered through an acid-washed filter paper in a 25cm³ capacity volumetric flask using deionised water. This was kept in a dust proof glass chamber ready for further analysis.

3.4.3 PREPARATION OF THE STANDARD SOLUTIONS (White Side, 1975).

i. Sodium Stock Solution (100 ppm):

Prepared by dissolving 0.2542g of dry sodium chloride in 100 cm³ distilled water. Working standards contained 0 to 5 ppm of sodium.

ii. Potassium Stock Solution (1000 ppm).

This was prepared by dissolving 1.9068g of potassium chloride in water in a litre volumetric flask. Working standard range between 0 to 10 ppm.

iii. Calcium Stock Solution (1000 ppm).

2.4973g of anhydrous calcium carbonate was disolved in

about 200cm³ of water containing 5cm³ concentrated hydrochloric acid. The mixture was warm to drive off carbondioxide, cooled and made up to 1000cm³ with water. A working standard of concentrations between 0 to 10 ppm calcium were prepared.

iv. Magnesium Stock Solution (1000 ppm).

This was prepared by dissolving 3.9106g magnesium chloride in 200 cm³ water and diluting the solution to 1000 cm³. Working standards contained 0 to 3 ppm magnesium. Sufficient stock of strontium chloride (5000 ppm) were added to the standard samples and blank solutions to obtain 400 ppm in each. Appropriate quantity of 10% sulphuric acid were also added to the sample and blank solutions to obtain an acid concentration of 1% (v/v).

v. Copper Stock Solution (1000 ppm).

0.3939g copper sulphate was dissolved in water a litre volumentric flask. Working concentrations were between 0 to 10 ppm.

vi Zinc Stock Solution (1000 ppm).

1.0g of zinc metal was dissolvedin 30 cm³ 5M hydrochloric acid. The resulting solution was diluted to the mark with water in a litre volumetric flask. Working standards ranged between 0 to 1 ppm.

Phosphorus Determination (Allen, 1974).

REAGENTS:

i. Phosphorus Standards:

A 100 ppm Stock solution was prepared by dissolving 0.4393g dry potassium dihydrogen orthophosphate in water and in a litre flask. An intermediate working standard was freshly prepared when required from the stock solution.

ii. Vanadate - Molybdate Composite reagents:

50g Ammonium molybdate in 500cm³ warm water. In a separate beaker, 2.5g Ammonium vandadate was dissolved in 500cm³ boiling water. On cooling, 350cm³ of conc. HNO_3 was added with stirring. Then the molybdate solution was added to the acid - vanadate solution with stirring and the resulting solution was diluted to 2.5 litre with water.

1:2 Nitric acid solution:

100cm³ of conc. nitric acid (63% w/w, sp. gr. 1.42) was diluted with trice its volume of water.

Collins & Polkinhorne, 1952 photometer was used for the determination of sodium and potassium.

PROCEDURE

Working standards containing 0 - 03mg phosphorus were prepared in 50cm³ volumetric flask. Suitable aliquot of the sample solution were pipetted into 50cm³ volumetric flasks. These flasks were filled to two-third capacity with water, 2 cm³ of ammonium molybdate reagent was added to each followed

by 2 cm^3 1:2 nitric acid solution. The contents of each were mixed and made up to volume. The absorbance reading were taken after 30 minutes at 450nm using Bausch-Lomb, spectronic 20. A calibration curve was plotted using the standard solutions. The amount of phosphorus in the samples were determined from the graph and the mg/100g calculated as below.

$$\text{Phosphorus (mg/100g)} = \frac{\text{C } (cm^3) \times \text{Solution volume} \times 100}{\text{Aliquot } (cm^3) \times \text{sample weight(g)}}$$

Where C (mg) = amount of phosphorus in milligram obtained from the calibration curve. Factors of dilution were applied where necessary.

3.5 EFFECTS OF PRESERVATION (STORAGE) ON FISH

REAGENTS: As stated under the analysis of protein, lipids, FFA and pH determinations. See section 3.2.6 on page 28.

PROCEDURE:

All the samples used were degutted. Some were put into a labelled cellophane bag, and then refrigerated at 4°C. Others were bagged in a labelled cellophane and stored in the deep freezer at -10°C while some portions were oven dried at 60°C for 24 hours, bagged in a cellophane bags, kept in desiccators and stored in the cupboard at ambient temperature (23°C). All the three sets of the samples were stored for a period of thirty days. Lipid, protein, % FFA and pH were determined at intervals of five and ten days.

3.6 EFFECTS OF ATMOSPHERIC AIR ON FISH LIPIDS

REGENTS: Similar to the reagents listed under section 3.2.7 and 3.2.9 on pages 28 and 31 respectively.

PROCEDURE

The fish samples were oven dried at 60°C for between 22-24 hours. The lipids were extracted with chloroform: methanol (2:1 v/v) mixture and the extracts were exposed to the atmosphere twenty four hours at ambient temperature (23°C). The peroxide and free fatty acid values were determined at interval of one hour and ten hours.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 PHYSICAL CHARACTERISTICS OF THE FISH SAMPLES

The lengths and weights of the fish analysed are given in Table 1.

M. rume and C lazera are of similar lengths 13.37cm and 13.30cm respectively. M rume however has the largest total weight (195.93g). O. niloticus is the shortest (10.46 cm) but has a larger total weight than C. lazera (139.95). From the results C. lazera and M rume have similar % total weight as edible part 76.62 and 74.77% respectively. The values show that the species analysed are not fully matured compared with the standard (average) size of the species given in literature: M. rume as one metre and 6 kg; C. lazera, 50-60cm and 3-6 kg lengths and weights respectively.

TABLE 1: PHYSICAL CHARACTERISTICS OF THE FISH SAMPLES ANALYSED

SAMPLE	TOTAL LENGTH (cm)	STANDARD LENGTH AS % TOTAL LENGTH	TOTAL WEIGHT (g)	EDIBLE PART AS % TOTAL WEIGHT
C. lazera	13.30 ± 0.66	76.74 ± 0.47	117.98 ± 6.09	76.62 ± 5.77
M. rume	13.37 ± 0.47	81.20 ± 0.44	195.93 ± 7.28	74.77 ± 10.15
O. niloticus	10.46 ± 0.21	70.17 ± 0.06	135.95 ± 12.17	59.49 ± 2.28

Nine Samples determinations were made for each fish.

4.2. PROXIMATE COMPOSITION OF THE FIST SAMPLES

4.2.1 MOISTURE CONTENT

The moisture content for the fish samples are given in Table 2. The fish samples contained high amount of water, over 75%. The values are higher than those of beef (64%), lamb (53%) and pork (54%). (Brian and Alan, 1977) but are comparable with those of other fish (both) in the marine and fresh water species. For example, haddock, aleste, synodontis, Tilapia Zilli have 82%, 74.62%, 77.56% 72.56 - 80.28% respectively (Oni 1979, Brian & Allan 1977). the spring water species of fish also have about 79.70% (Bhuiyan et al. 1986).

The appearance, keeping quality and dry matter content of foods are closely related to their moisture content (Whyte and Selvery, 1969), with the amount of dry matter having an inverse relationship with moisture content; thereby preventing the growth of spoilage micro-organisms and also reducing the chemical and biochemical reactions to the minimum during storage. The moisture content of food can be used as an index of stability and susceptibility to fungal infections (Ishola, 1988). Thus this high moisture content in fish is responsible for its deterioration and storage problems (Blanck, 1955, Sidwell 1962).

TABLE 2: PHYSICAL CHARACTERISTICS OF THE FISH SAMPLES ANALYSED

SAMPLE	MOISTURE CONTENT (%)	CRUDE LIPID (%)	CRUDE PROTEIN (N X 6.25%)	ASH CONTENT AS (%)
MC. FH	74.41 ± 0.98	3.70 ± 0.14	18.81 ± 0.18	1.28 ± 0.12
C. FD	-	13.58 ± 0.60	65.80 ± 0.32	6.67 ± 0.06
C. FD	-	14.00 ± 0.18	68.57 ± 0.16	6.46 ± 0.03
MM. FH	75.25 ± 0.75	4.16 ± 0.20	19.22 ± 0.70	1.17 ± 0.16
M. FD	-	14.54 ± 1.11	69.70 ± 0.17	6.40 ± 0.01
M. FD	-	15.17 ± 1.37	72.22 ± 0.22	6.35 ± 0.02
MC. FH	77.21 ± 1.55	2.64 ± 1.00	16.15 ± 0.10	2.15 ± 0.06
D. FD	-	9.74 ± 0.21	62.81 ± 0.06	9.89 ± 0.06
D. FD	-	9.81 ± 0.32	65.83 ± 0.25	10.44 ± 0.11

Three determinations were made for each sample.

* Determined on wet basis. Others were determined on dry basis of the samples.

4.2.2 CRUDE LIPID CONTENT

The percent crude lipid contents are as shown in Table 2. In each of the samples the lipid is semi solid at room temperature. M. rume contains the highest amount of lipid both on wet and dry weights basis and O. niloticus the least. The results also show that O. niloticus with a lipid content of 2.64% on wet weight basis and C lazera with 3.70% belong to the low-fat class (2-4%), while M rume belongs to medium class of fat. The fish analysed have lower lipid values than beef (17%), Mutton (30%), Pork (30%), herring (18%) and sardine (14%) but are comparable to those obtained by Oni (1979) for similar fresh water fish (aleste, 17.40% synodontis, 9.55%, and tilapia, 12.33%) as determined on dry weight basis. The result also falls within the range (0.3 - 15%) lipid content given by Pearson, 1981 for fresh water fish.

The lipid contents of the pre-frozen samples were however lower than the lipid content of the dried samples for each fish. This might be due to some bacterial or enzymatic actions during storage.

Comparing the moisture contents and crude lipid contents, the data confirmed the known inverse relationship of the body fats to water content already established in fish by Gerking (1955), Brown (1957) and Lagler, 1962).

4.2.3 CRUDE PROTEIN CONTENT:

The protein contents of the non-defatted fish samples are given in Table 2. These values show that all the samples are rich in protein (>60% on dry weight basis). The values are comparable with beef (18%), Lamb (16%), Pork (16%), and some marine fish (Haddock, 17%, Sardine, 20%, Mackerel 17-23% and Oyster, 11%), all determined on wet basis (Brain and Allan, 1977; Pearson, 1981, Blanck, 1955). The M. rume contains the highest percent crude protein both on wet and dry bases while O. niloticus has the least values. The crude protein contents of the samples analysed are about the same as in lean meat. Fish protein however differs from that of meat in having less connective tissue and no elastin. The absence of tough elastin and conversion of collagen into gelatin which occurs during cooking, make the protein of cooked fish easily digestible (FAO/WHO, 1973. Brain & Allan, 1977).

4.2.4 ASH CONTENT

Oreochromis niloticus contains the highest ash contents both on dry and wet bases, while Mormyrus rume has the lowest values. (Table 2) The results obtained are in agreement with those obtained by Oni (1979), for Aleste (9.00 - 10.80%),Synodontis (10.00 - 17.55%) and Tilapia Zilli (9.81 - 16.31%) determined on dry weight basis. Also the result compare well with those obtained for spring water and fall water fishes 1.9% and 1.6% respectively, determined on wet

weight basis. The ash content for any particular fish has been reported to be relatively constant during the growth of the fish, thus indicating that the relative proportion of skin, scales and bones in the fish does not change appreciably with age (Denton et. al., 1976).

4.3 THE pH VALUES OF THE FISH SAMPLES

The pH values are given in Table 3. The pH of each sample is least for the fresh samples and highest for the pre-frozen samples. The increase in pH is due to changes that occur during storage or drying, amounting to degradation of the fish components (e.g. protein which produce ammonia and make the fish more alkaline in solution).

The values are comparable with the value reported by Orji (1989) for Tilapia specie as 6.8).

TABLE 3: THE PH VALUES OF CLARIAS, MORMYRUS & OREOCHROMIS

SAMPLE	pH AT 21°C
C.FH	6.87 ± 0.02
C.FD	6.91 ± 0.02
C.FO	7.04 ± 0.02
M.FH	6.78 ± 0.01
M.FD	7.04 ± 0.04
M.FO	7.10 ± 0.01
O.FH	6.54 ± 0.01
O.FD	6.96 ± 0.04
O.FO	7.04 ± 0.02

Three determinations were made for each fish sample analysed.

4.4 COMPARISON OF DIFFERENT SOLVENT SYSTEMS FOR LIPID EXTRACTION

The criteria for the suitability of method for lipid extraction are the quality and quantity of lipid extracted.

During extraction, it is important that all linkages of lipids with other components be broken or disrupted while at the same time ensuring that solvent used do not cause degradation of the extracted lipid. The results for four different solvent systems used for the extraction are shown in Table 4. From the results, it is evident that petroleum ether (60-80°C) extracted the least quantities compared with others solvent systems. The chloroform; methanol (2:1 v/v) solvent systems extracted the largest quantities of lipids from the fish samples.

Both Petroleum ethers 40-60°C and 60-80°C have low extracts especially for fresh samples due to the possibility of loss of some volatile components during extraction. The inability of the solvent to penetrate the tissues as well as its becoming saturated with water. Thus both petroleum ether are rendered inefficient for lipid extraction from wet materials. The high values obtained for the chloroform; methanol (2:1 v/v) also is in agreement with the report of Chen (1981). It was reported that chloroform is effective and can be used interchangeably with dichloromethame in the extraction of lipids from food products (Higgins, 1987).

TABLE 4: EXTRACTION OF FISH LIPIDS

SAMPLE	PET ETHER (40-60°C)(%)	PET ETHER (60-80°c)(%)	$CHCl_3$:CH_3OH(1:1) (%)	$CHCl_3$ CH_3OH (2:1) (%)
*C. FH	1.31 ± 0.09	0.42 ± 0.06	3.12 ± 0.40	4.00 ± 0.53
C. FD	11.81 ± 0.28	11.53 ± 0.23	12.05 ± 0.15	12.19 ± 0.29
*M. FH	1.60 ± 0.37	0.56 ± 0.14	3.06 ± 0.17	3.95 ± 0.22
M. FD	14.40 ± 0.82	13.47 ± 0.84	14.46 ± 1.11	14.65 ± 0.35
*O. FH	1.00 ± 0.06	0.27 ± 0.10	2.15 ± 0.25	2.62 ± 0.19
O. FD	10.02 ± 0.26	7.81 ± 0.27	10.06 ± 0.33	10.21 ± 0.33

* Determinations were made on wet basis

Three determinations were made for each fish analysed.

For this study, chloroform: methanol (2:1 v/v) was used to extract the lipids of fish analysed.

4.5 PHYSICAL PROPERTIES OF FISH LIPIDS

4.5.1 SPECIFIC GRAVITY

The specific gravities of the fish lipids are given in Table 5. M. rume has the highest values and is therefore expected to contain components with the highest molecular weights. This followed by O. niloticus with C. lazera having the least. The values obtained are in agreement with the range of values given for fish oils generally as 0.9020 - 0.9223 (Pearson, 1978, Thorpe, 1949), cod liver oil (0.922 - 0.929), herring (0.917 - 0.930), sardine (0.932 - 0.935), whale (0.021 - 0.926) menhaden (0.929 - 0.931) (Brain & Allan, 1977). The values are also comparable to those for some vegetable oils like maize oil (0.917 - 0.925), Groundnut oil (0.914 - 0.917), Cottonseed oil (0.918 - 0.926) and soyabean oil (0.919 - 0.925) (Thorpe 1949 and Blanck 1955). The values are however a little higher than the recommended values for lard (0.896-0.904) and rendered pork fat (0.894 - 0.906) (Thorpe, 1949 and Pearson, 1981).

4.5.2 REFRACTIVE INDEX:

The values obtained for the refractive indices for the fish lipids analysed are given in Table 5. The refractive index can be used as a test of the purity of the oil samples.

TABLE 5: SPECIFIC GRAVITY & REFRACTIVE INDEX OF FISH LIPIDS AT 21°C

SAMPLE	SPECIFIC GRAVITY	REFRACTIVE INDEX
C.FH	0.9139 ± 0.0002	1.456 ± 0.003
C.FD	0.9142 ± 0.0001	1.460 ± 0.002
C.FO	0.9161 ± 0.0002	1.459 ± 0.002
M.FH	0.9179 ± 0.0003	1.456 ± 0.002
M.FD	0.9186 ± 0.0002	1.467 ± 0.002
M.FO	0.9197 ± 0.0004	1.467 ± 0.002
O.FH	0.9144 ± 0.0002	1.450 ± 0.002
O.FD	0.9154 ± 0.0002	1.466 ± 0.002
O.FO	0.9175 ± 0.0002	1.466 ± 0.002

the values range between 1.450 and 1.467 and can be compared with vegetable oils like groundnut (1.460 - 1.465), soyabean, (1.465 - 1.469), cotton seed, (1.458 - 1.465) and maize, (1.465 - 1.468) at 40°C (Pearson, 1981). The values are also related to those of lard (1.448 - 1.460) at 40°C and pork (1.448 - 1.461) at 40°C (Pearson, 1981).

4.6 THIN LAYER CHROMATOGRAPHY OF THE FISH LIPIDS

The Rf values obtained from the thin layer chromatographic separation of extracted lipids are given in Table 6.

Benzene: chloroform (70:30) and chloroform: methanol: Acetic acid: water (70:30:3.2) were used for the separation of the neutral lipids and the phospholipid respectively. The neutral lipids are separated into six (6) components, while the phospholipid are eight (8) components. The identification is made difficult due to lack of standard lipids for comparison. The suspected neutral lipids are the glyceride (mono-,di-and tri-), cholesterol, cholesterylester, free fatty acids and sterol. The phospholipid are likely to be phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, sphingomychin, sphinolipids and phosphoglycerides as all the above are commonly found in fish lipids and most of the above phospholipid components have been reportedly found in mackerel (Body et al., 1987).

TABLE 6: Rf OF THE NEUTRAL AND PHOSPHOLIPID USING TLC.

Rf	NEUTRAL LIPID (C_6H_6:$CHCl_3$;70:30)	PHOSPHOLIPID ($CHCl_3$:CH_3OH:CH_3CH_2COOH:H_2O;70:30:2:3)
Component 1	0.039	0.058
Component 2	0.092	0.142
Component 3	0.250	0.242
Component 4	0.329	0.333
Component 5	0.362	0.400
Component 6	0.980	0.533
Component 7		0.725
Component 8		0.983

*** Three readings were obtained (determined) and all the samples contained the same components but with different relative intensities.

4.7 CHEMICAL CHARACTERISTICS OF FISH LIPIDS

Data on oil compositions tend to be variable and they are affected by such factors as, mode of breeding, the fish samples and the part analysed, method of storage, body compositions and species of the fish analysed. The fish lipid is yellowish in colour and they are semi solids at room temperature (21°C).

4.7.1 PEROXIDE VALUE

The peroxide values are as shown in Table 7. These values are least in the fresh fish lipids (2.21, 2.50 and 2.89 mEq/kg) for M. rume, C. lazera and O. niloticus respectively) and highest in the pre-frozen fish lipids (M. rume; 5.02; C. lazera 3.10; and O. niloticus, 5.31 mEq/kg). These values are below the 10 mEq/kg given by Pearson (1981) as maximum value for consumable fats and oils in fish. Rancidity can only be noticed when the peroxide value is between 20-40 mEq/kg for food generally, though this range depend on the type of oil or lipid. The results also indicate that there was little effect of autoxidation and lipolysis on the samples analysed, even after frozen for five days.

Peroxide is formed when a food material is exposed to oxygen, peroxide and free radicals are produced. The formation of these free radicals is responsible for the spoilage of food (Gurr and James 1971).

TABLE 7: CHEMICAL CHARACTERISTICS OF FISH LIPIDS

SAMPLE	PEROXIDE VALUE (mEq/kg)	IODINE VALUE	ACID VALUE (mgKOH/g)	SAPONIFICATION VALUE)(mgKOH/g)	ESTER VALUE (mgKOH/g)	UNSAPONIFIABLE MATTER (%)
C.FH	2.50±0.09	112.60±0.05	18.61±0.12	203.83±0.46	185.22±0.5	1.36±0.03
C.FD	3.10±0.03	107.63±0.11	19.29±0.08	198.67±0.25	179.39±0.6	1.41±0.02
C.FD	2.81±0.03	109.18±0.13	19.07±0.15	205.63±0.12	186.56±0.5	1.38±0.01
M.FH	2.21±0.02	126.20±0.72	16.19±0.18	197.06±0.15	180.87±0.12	1.27±0.02
M.FD	5.02±0.03	112.53±1.14	18.11±0.07	195.18±0.11	177.07±0.09	1.37±0.02
M.FD	2.58±0.02	116.82±0.06	16.81±0.11	202.91±0.13	186.10±0.42	1.23±0.03
B.FH	2.89±0.03	116.69±0.02	21.11±0.13	206.66±0.09	185.74±0.20	1.05±0.05
B.FD	5.31±0.02	95.86±0.13	25.22±0.07	204.33±0.14	179.11±0.16	1.02±0.02
B.FD	3.37±0.08	98.31±0.04	23.07±0.10	209.80±0.13	186.63±0.13	1.96±0.02

4.7.2 IODINE VALUE

Iodine values measure the degree of unsaturation and therefore the reactivity of the oil. The iodine values of the fish lipids analysed range from 95.86 - 126.20. It is lowest in the lipid of pre-frozen Oreochromis niloticus and highest in the lipid of the fresh Mormyrus rume (Table 7). The iodine values of the fish lipids analysed compare with those of herring (105 - 125) and whale (108 - 130). Sardine (173 - 190), menhaden (160 - 180) and cod liver (155 - 173) are of higher iodine values than the analysed fish samples while lard (45-70) and pork (45-70) are of lower iodine values (Thorpe, 1978; Pearson, 1981).

The lipids of the samples analysed are of low iodine value hence low degree of unsaturation. Thus it can be classed as semi drying oils The drying oils have iodine values between 130 - 200 and non drying oils have iodine values of below 100. (Pomeranz and Meloan, 1978). In general, the greater the degree of unsaturation, the greater the iodine values and the greater the tendency of the lipid to oxidative rancidity. This implies that among the samples analysed the M rume (fresh, 126.20) is most prone to oxidative rancidity and contains the highest amount of poly unsaturated fatty acids while O. niloticus is least prone.

4.7.3 ACID VALUE AND FREE FATTY ACID:

The acid value of a lipid is a measures of the extent to which the constituent glyceride have been decomposed by lipase action. This decomposition is usually accelerated by heat. light, air, moisture and bacteria.

The results of the acid values range from 16.19 mg KOH for fresh mormyrus to 25.25 mg KOH/g for pre-frozen O. niloticus. (Table 7). The values show that little quantities of the lipid glyceride have been converted even after frozen for five days. The analysed samples are of low acid values when compared with herring (2-8%), sardine (1-4%), whale (up to 2%), menhaden (2-7%) and cod liver (up to 0.6%) (Thorpe, 1949). Also they are lower than the one reported by Orji (1989) for tilapia species (5.6%).

Though the acid value and free fatty acid are not necessarily related to the extent to which the rancidity has progressed (Pearson, 1981), the formation of free fatty acids is usually accompanied by rancidity. The determination is often used as a general indication of the condition and edibility of the oils. This is because an increase in acid value is accompanied by the development of objectionable flavours and odours which reduce the consumer acceptance of the lipid/ oil.

4.7.4 SAPONIFICATION VALUE:

Saponification value is an indication of the average molecular weight of the lipid. A high saponification value

indicates a greater proportion of fatty acids of low molecular weight (Thorpe et al., 1970).

The results given in Table 7 show high saponification values for all the samples and it is highest in the lipid of dried O. niloticus (208.80 mgKOH.g). Though the results show little variation from fresh to oven dried samples of each of the fish analysed, the M. rume has the least saponification values in each of the category of the fish analysed. This is consistent with the result obtained from the specific gravity study (Section 4.5.1) in which the M. rume lipid is said to have the most components of high molecular weight.

The saponification values of fish analysed are comparable with the values for some animal and other fish lipids. For example, lard, rendered pork fat, cod liver, herring, sardine, whale, menhaden, butter fat, beef tallow have 192 - 203, 192-203, 180 - 190, 184 - 194, 188 - 192, 193 - 197, 188 - 194, 210 - 230, 196 - 200 mg KOH/g Maize (187 - 195 mg KOH/g, cotton seed (185 - 195 mg KOH/g) have also some comparable values with the fish lipids analysed (Pearson, 1981), while coconut (252 - 258 mg KOH/g), palm kernel (244-249 mg KOH/g) have somewhat higher values (Brian and Allen, 1977; Ishola, 1988, Thorpe, 1949).

4.7.5 ESTER VALUE:

The results obtained for ester values are given in Table 7, and it is the difference between the saponification value

and acid value. The result shows that all the samples contain high ester values. This implies that more glyceride are still retained in the samples even after frozen for five days, thus the oils are of good quality. The results shows that the oven dried samples contained the highest amount of glyceride followed by fresh samples and then prefrozen samples, the least.

4.7.6 UNSAPONIFIABLE MATTER:

The results of unsaponifiable matter are as shown in Table 7. The results ranged from 0.96 (dried O. niloticus) to 1.41 (pre-frozen C. lazera). The values obtained are within the range of values (0:2%) reported for fish oil by Williams, (1964) and Peterson, (1978). The values obtained in this study can be compared with cod liver (upto 1.5%), herring (1-2.5%) sardine (0.5 - 0.9%), whale (1.5%), menhaden (0.5 - 1.5%) (Brian and Allan, 1977, Thorpe, 1949). They are however lower than the values for some vegetable oils like groundnut oil (10%), cotton seed oil (15%), Maize (28%) (Pearson, 1981, Blanck, 1955 and Thorpe, 1949).

These unsaponifiable matters may include squalene, some waxes, phospholipid, sterol, higher alcohol, vitamins and some hydrocarbons - but consist principally of steroids and terpenes (Christie, 1973).

4.8 MACRO - AND MICRO - ELEMENT/ MINERAL COMPOSITIONS

The results of twelve different elements investigated for

the three fish samples are shown in Table 8. From the results these elements can be classified into two main groups.

i) The macro elements which include Ca, P,K, Na and Mg. and these decrease in that order in the sample analysed.

ii) Micro elements which include Zn, Fe,I,Ni,Mn,Cr, and Cu which also decrease in that order.

The M. rume species contained the smallest amounts of each element analysed except in phosphorus.

4.8.1 CALCIUM AND PHOSPHORUS:

All the fish samples indicate high level of calcium followed by phosphorus except in M. rume where this is reversed O. niloticus contained the highest amount of Ca (3875 mg/100g) followed by C. lazera (3250 mg/100g) while M. rume has the smallest amount (1450 mg/100g). C. lazera has the lowest amount of phosphorus (2018 mg/100g) while M. rume has the highest value (2662.90 mg/100g). The calcium values were much higher than values obtained for most fruits and some leafy vegetables such as spinach (400mg/100g), baobab leaves (562 mg/100g), cocoyam (409.10mg/100g), soyabean (220mg/100g) and locust bean seed (310 mg/100g) (Ishola, 1988). Cereals reported to be the major source of phosphorus in African diet contain 140-330 mg/100g phosphorus (Ishola, 1988).

The results imply that fish can adequately supply the calcium and phosphorus required by the body. The average amount of calcium and phosphorus required by man are 1000 - 1500mg and 600 - 900mg respectively. It has been

TABLE 8: MACRO - AND MICRO - ELEMENTS FROM DRIED FISH SAMPLES (mg/100g).

SAMPLE	Ca	P	K	Na	Mg	Zn	Fe	I	Ni	Mn	Cr	Cu
C. lazera	3250.00	2018.00	1450.00	317.00	137.00	31.75	13.25	0.46	12.50	4.05	1.563	1.088
M. rome	1450.00	2662.90	1299.00	138.00	72.50	23.75	6.00	0.45	2.00	0.86	0.188	0.063
O. niloticus	3875.00	2228.19	1180.00	316.00	157.50	31.25	10.63	0.46	6.25	4.15	0.188	0.135

estimated that pregnant and lactating women usually required large amounts of calcium and this ranges from 800mg - 2000mg daily (FAO/WHO, 1962). It would therefore mean that fish can contribute a significant amount of calcium and phosphorus to the diet.

Calcium is needed in the blood for clotting and for successful functioning of nerves and muscles. Phosphorus is also required for complex formation (process) whereby energy is obtained from oxidation of nutrient (adenosine triphosphate). Phosphorus is essential to the structure of every cell in the body and also for body fluid. Both are required for calcification of bones in the body (Brian and Allan 1977; Neuhaus, 1975).

4.8.2 POTASSIUM, SODIUM AND MAGNESIUM:

The values of the three minerals are given in Table 8. C. lazera has the highest amount of potassium (1450mg/100g) and sodium (317.00mg/100g) while O. niloticus contained the most magnesium (157.50 mg/100g). M. rume contained the least of the three minerals (K. 1299.00; Na, 138.00; Mg, 72.50mg/100g). The average amounts required by man (adult) of these minerals per day are potassium (125mg), sodium (80mg) and magnesium (25mg). Thus fish can provide these minerals. Potassium is used in softening tissues, the potassium ions help to control the pH of intracellular fluids and maintain osmotic pressure within the cell. Sodium is responsible for fluid outside the cells.

While magnesium is used to form the bone structure of the body with calcium and phosphorus.

4.8.3 ZINC, IRON, AND IODINE:

The levels of these mineral elements are relatively low, though the body requires them in minute quantities. An average amount required by an adult are Zinc (10 - 20 mg/day; Iron (4mg/day); and iodine (0.06 - 0.15mg/day) (Brian and Allan, 1977, Fritz et al. 1975, Blanck, 1955). Thus the fish analysed can contribute significantly to these values.

Zinc is required by the body for enzyme formation, e.g. carbonic anhydrase found in the red blood cells where it assists in quick liberation of carbondioxide from the lungs. Iron is required in the haemoglobin of the red blood cells which transport oxygen from the lungs to the tissues and also needed in the formation of new red corpuscles. The cell enzyme cytochrome contain iron. The iron obtained in the fish analysed is higher than the values reported for sardine (2.9 mg/100g), spinach (3.2 mg/100g), beef (1.9 mg/100g) and egg (2.7 mg/100g) (Brian & Allan, 1977). Iodine is essential to the body for hormone formation (thyroid gland). It combines with thyrosine and the compound formed converts into hormone thyroxine and thus prevents goitre (Blanck, 1955).

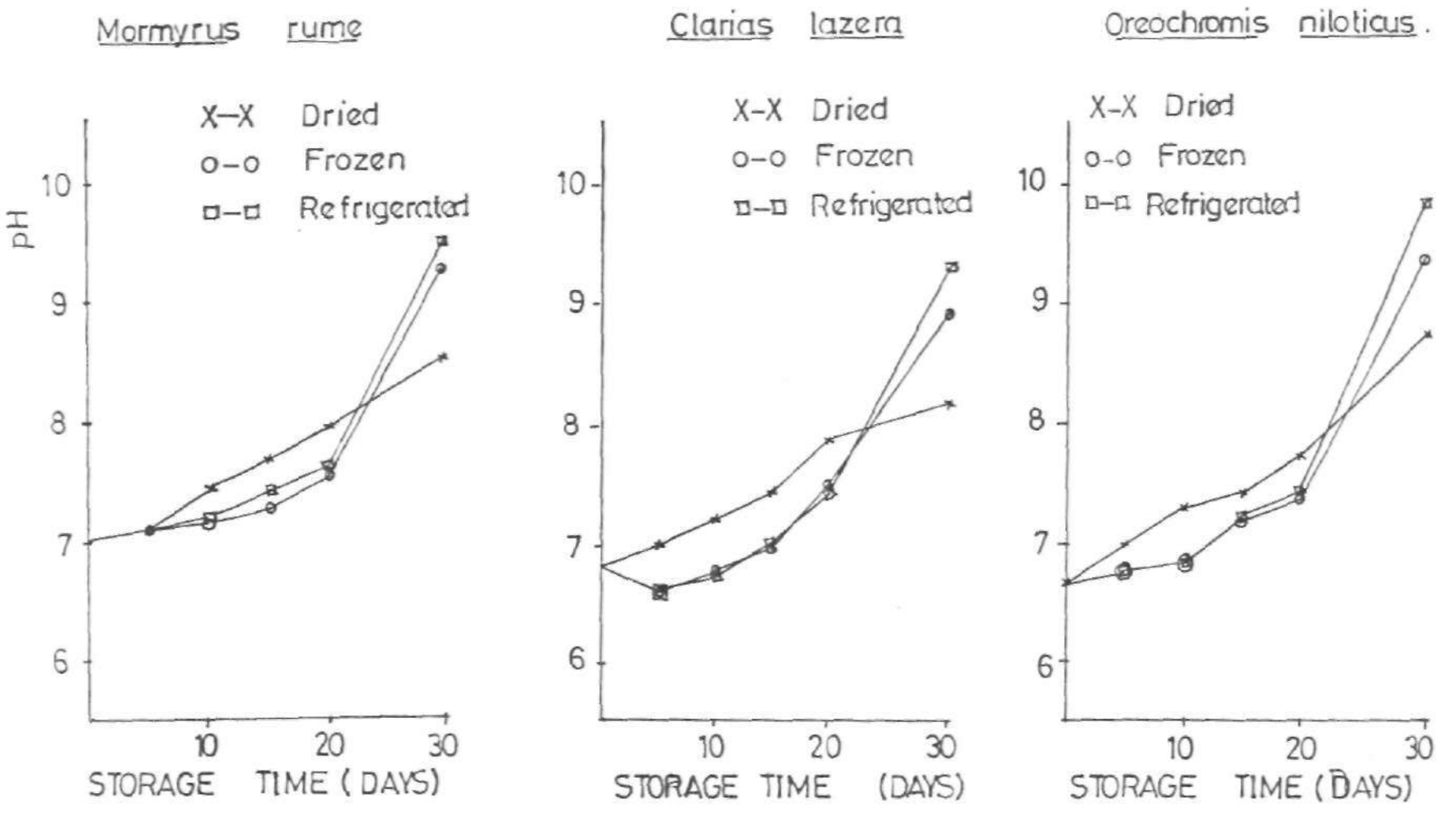
4.8.4 NICKEL, MANGANESE, CHROMIUM AND COPPER:

These are in low concentrations. C. lazera contained the largest amounts of the four elements. These minerals are equally important to both human and poultry animals though in small quantities. Manganese is required in poultry feeding where a deficiency produces the bones deformity called 'perosis'. The lesion is in the developmental cartilage of the bones. Eggs laid by hens fed diets low in manganese have a poor hatchability and the embryo have shortened legs, wings bones and beaks. Thus fish can be used as supplement in their diets. Manganese activates phosphatase and arginase which are concerned with bone and urea formation respectively. The daily requirement of copper is 2mg (WHO report, 1973). Nickel is not known for any function in plants and animals.

4.9 DRYING, FREEZING AND REFRIGERATION OF FISH SAMPLES

4.9.1 EFFECTS ON pH VALUES:

The pH of the fish samples gradually increased with time and this is more pronounced in the refrigerated fish samples (Fig.1). The pH at the end of the storage time (30 days) is highest for O. niloticus (9.81) even though it has the least pH value (6.67) at the beginning of the storage. The increase in pH as the storage progressed may be due to ammonia production as proteins and amino acids of the fish samples are broken down through the process of deamination. Similar observations have been made by Mjelde and Urdehl (1974).



FIG·1: EFFECTS OF DRYING, FREEZING AND REFRIGERATION ON pH OF THE FISH SAMPLE

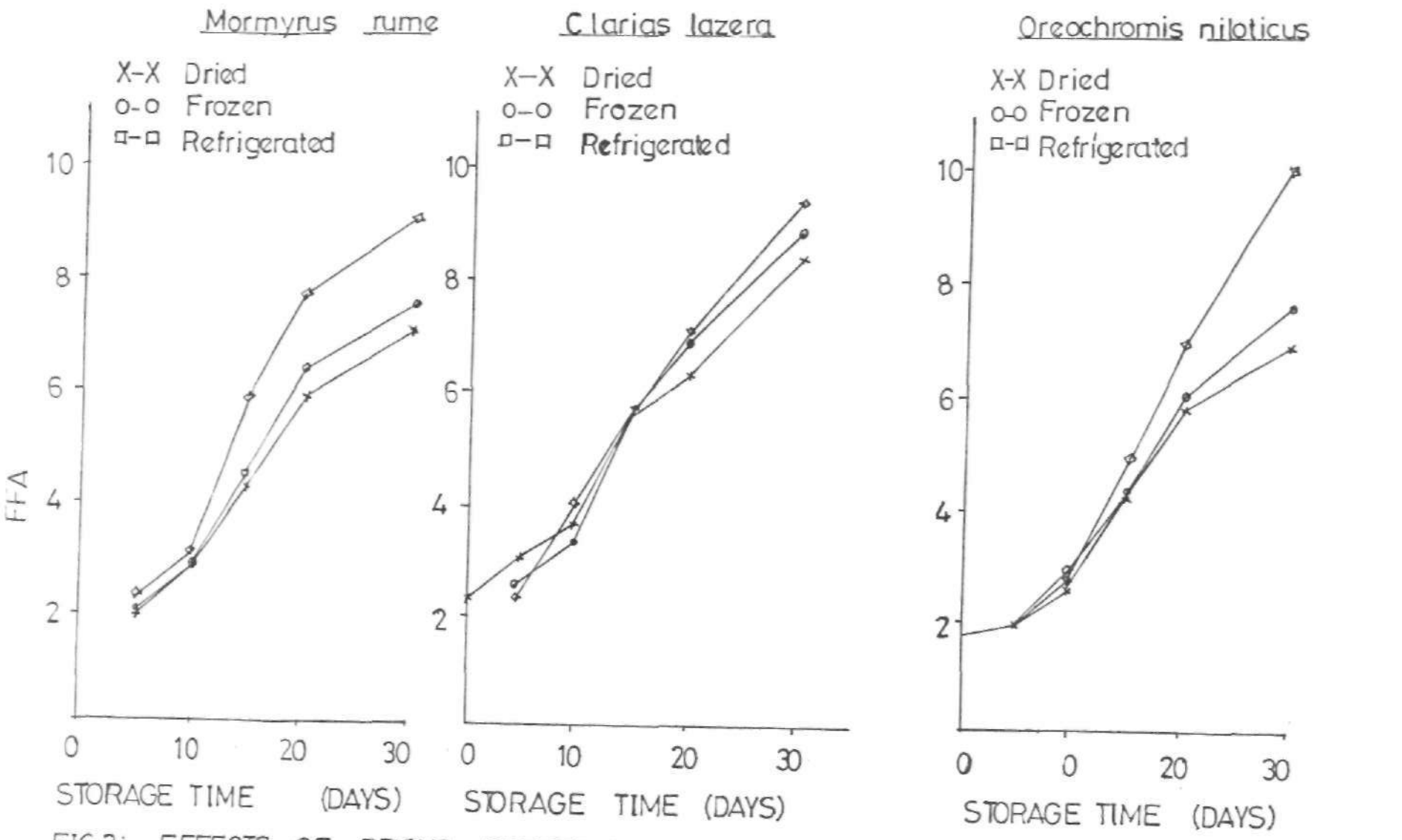


FIG.2: EFFECTS OF DRYING, FREEZING AND REFRIGERATION ON FFA OF THE FISH SAMPLES

4.9.2 EFFECTS ON FREE FATTY ACID:

As storage time increased, there was also an increase in the percent free fatty acids of all the fish samples analysed (fig. 2). The result shows that the refrigerated samples had more free fatty acid towards the end of the storage time. Earlier workers (Mjelde and Urdehl, 1974, Orji, 1989) made similar observations. The frozen fish samples had higher values (C. lazera, 7.61; M. rume, 7.53 and O. niloticus, 9.35) than the oven dried samples (C. lazera, 6.98, M. rume, 8.53 and O. niloticus, 7.02) at the end of the storage time.

This increase in the tissue free fatty acids can be attributed to the hydrolysis of the phospholipid by lipase and phospholipase into free fatty acids during storage (Olley et al., 1962). Olcott (1962) reported an increase in free fatty acid and a decrease in lipid content of Salmon gairdneii during storage. Also Mjelde and Urdehl (1974), Orji (1989) attributed an increase in free fatty acid in stored fish to extracellular bacterial lipase.

4.9.3 EFFECTS ON PERCENT CRUDE PROTEIN:

A general reduction in the percent crude protein of the fish was observed (Fig. 3). The largest amount of protein was lost by the refrigerated samples at the end of the thirty days storage process.

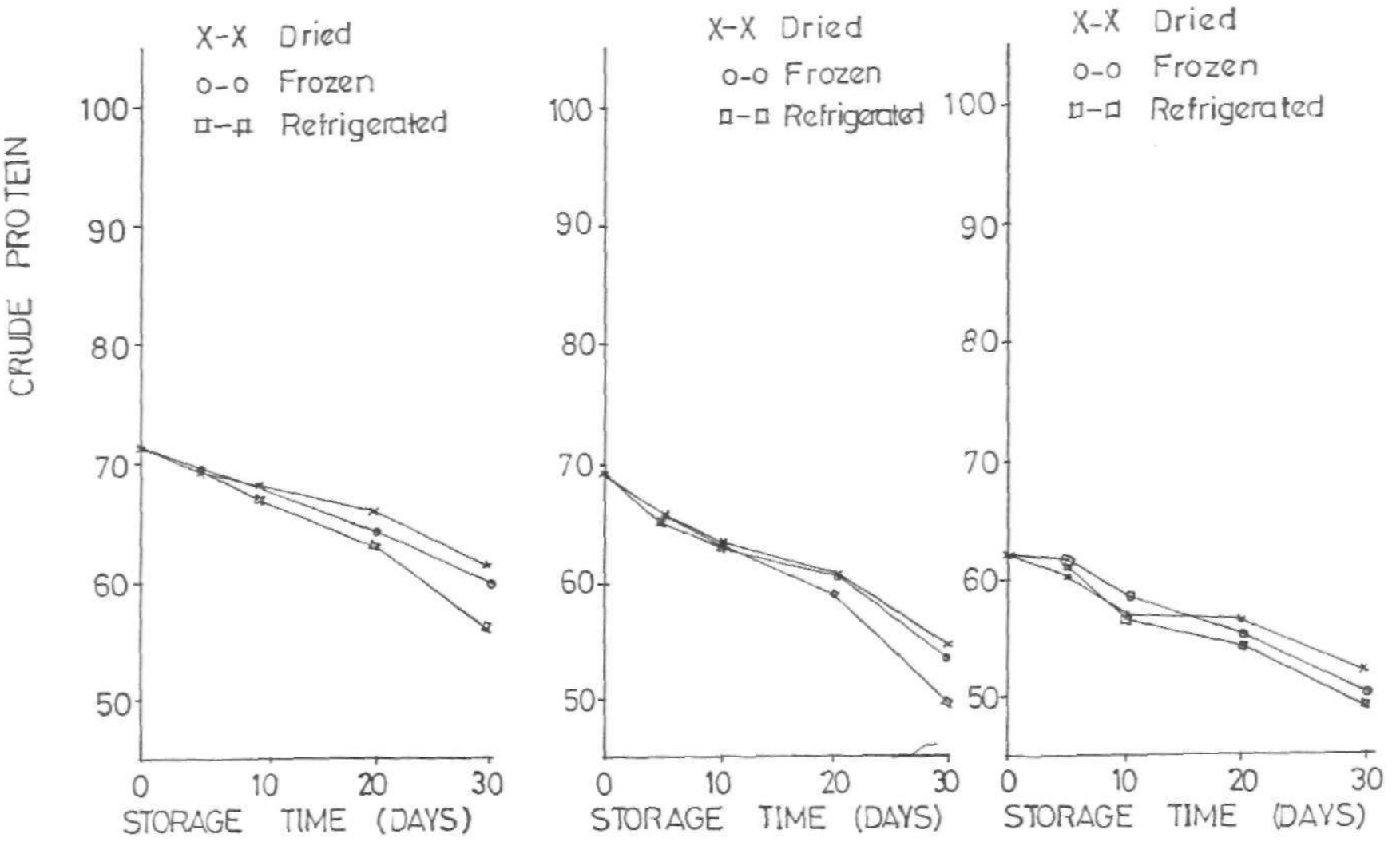


FIG 3: EFFECTS OF DRYING, FREEZING AND REFRIGERATION ON PROTEIN OF THE FISH SAMPLES

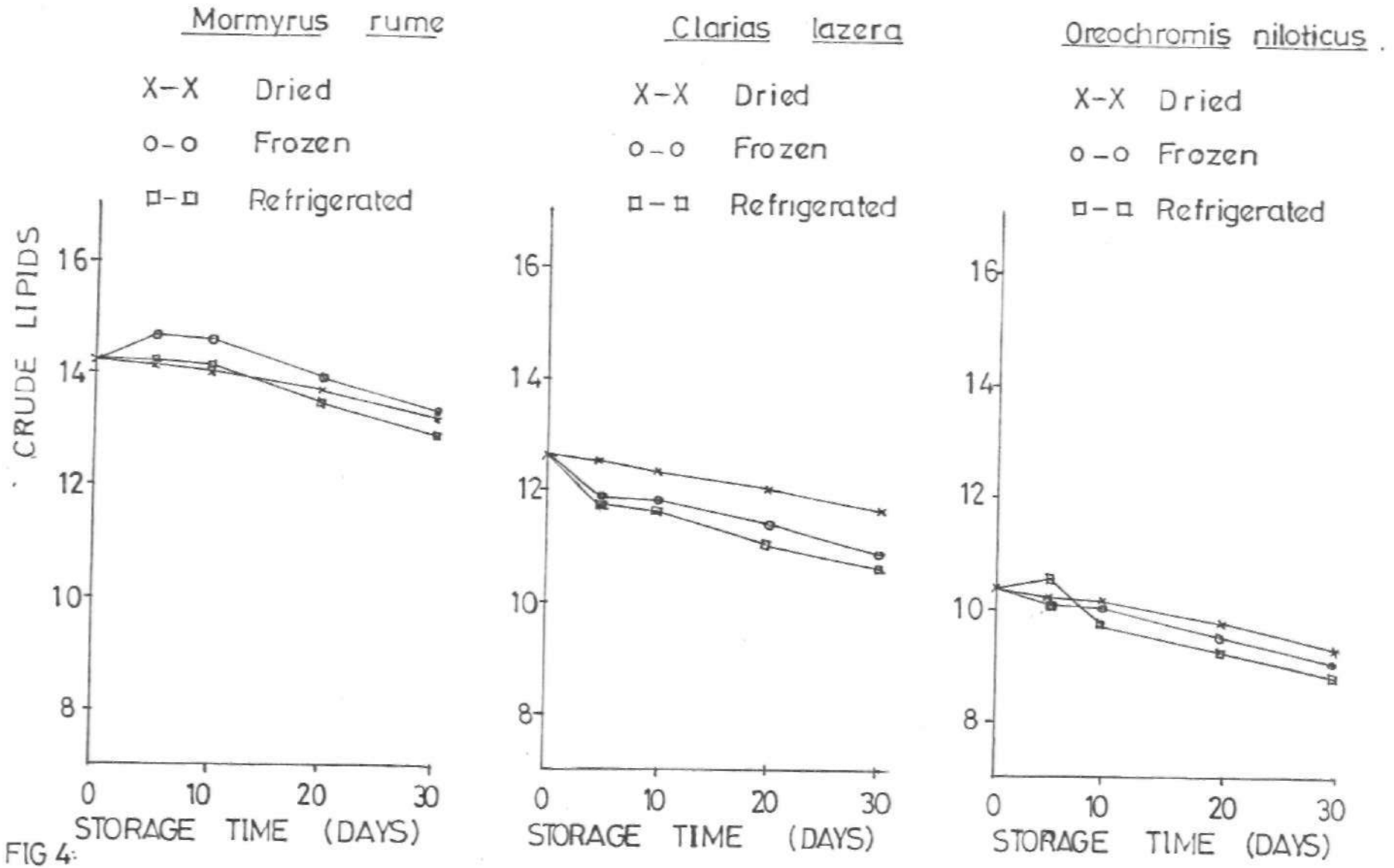


FIG 4: EFFECTS OF DRYING FREEZING AND REFRIGERATION ON THE LIPIDS OF THE FISH SAMPLES

4.10 EXPOSURE OF THE FISH LIPID TO AIR

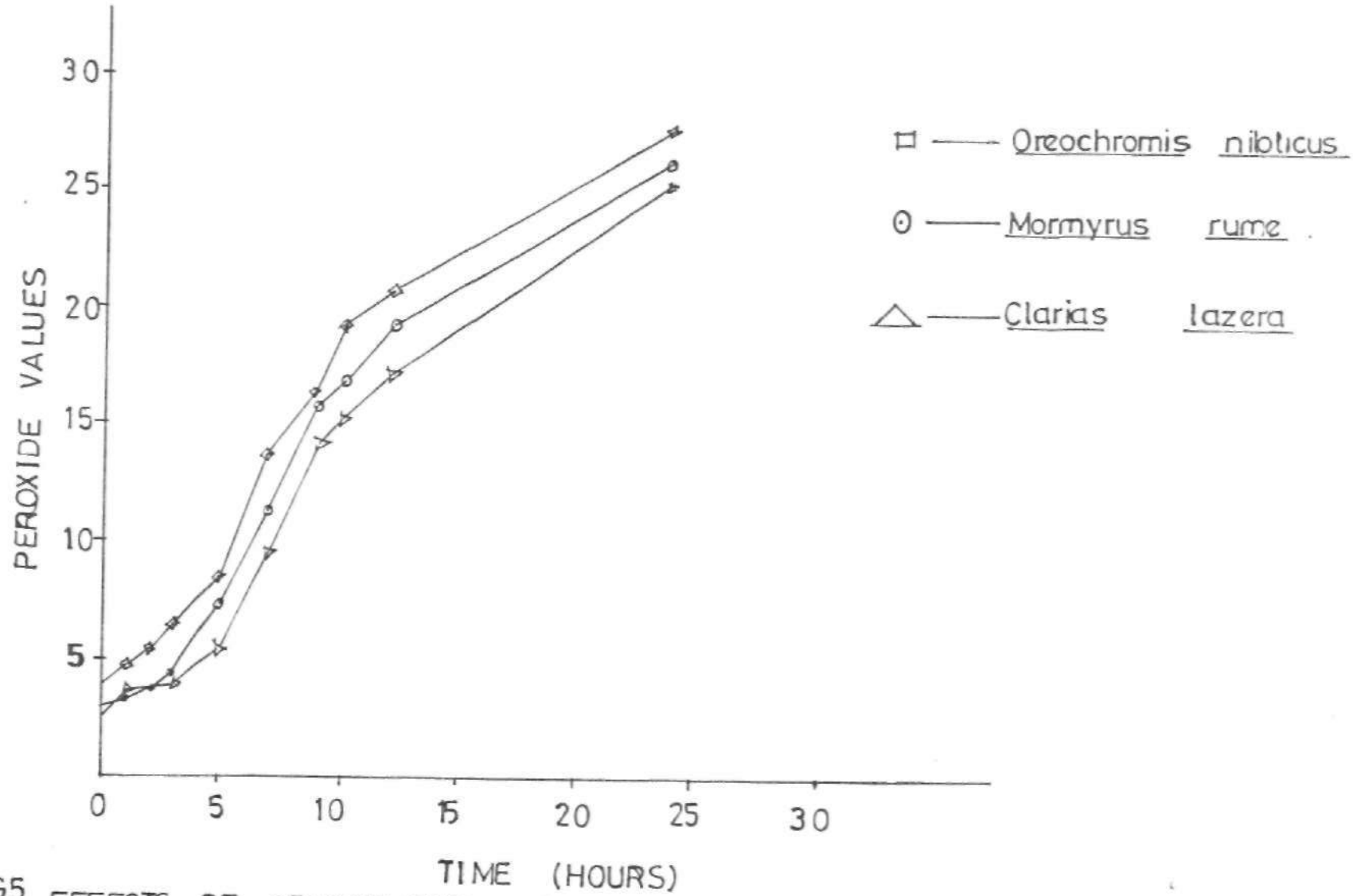
4.10.1 EFFECT ON THE PEROXIDE VALUE:

The peroxide value of the fish samples analysed increased with time (Fig. 5). With increasing time the components of the fish lipids are broken down by the process of oxidation producing more hydrogen peroxide. Thus the rate of rancidity or deterioration of the fish lipids increased and this decreases the quality and acceptability of the fish.

After twelve hours, the level of rancidity (odour and off flavour) becomes noticeable because the values have exceeded the acceptable range of 0-10 mEq/kg given by Pearson (1981). Actually, it took O. niloticus, M. rume and C. lazera 5.5 hours, 6.25 hours and 7.25 hours respectively to exceed the acceptable range.

4.10.2 EFFECT ON PERCENT FREE FATTY ACID:

The free fatty acid is directly related to the peroxide values. This observation is as a result of the break down of higher lipid components to free fatty acids. This increase in fatty acids enhances the rate of rancidity of the samples. The trend of the effects on the fish lipids analysed are similar to each other. The values show that the acid value/free fatty acids occur slowly at the initial stage in all the samples but as storage progressed, the rate of formation increased rapidly (Fig.6).



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FIG5 EFFECTS OF ATMOSPHERIC AIR ON PEROXIDE VALUES OF THE FISH LIPIDS

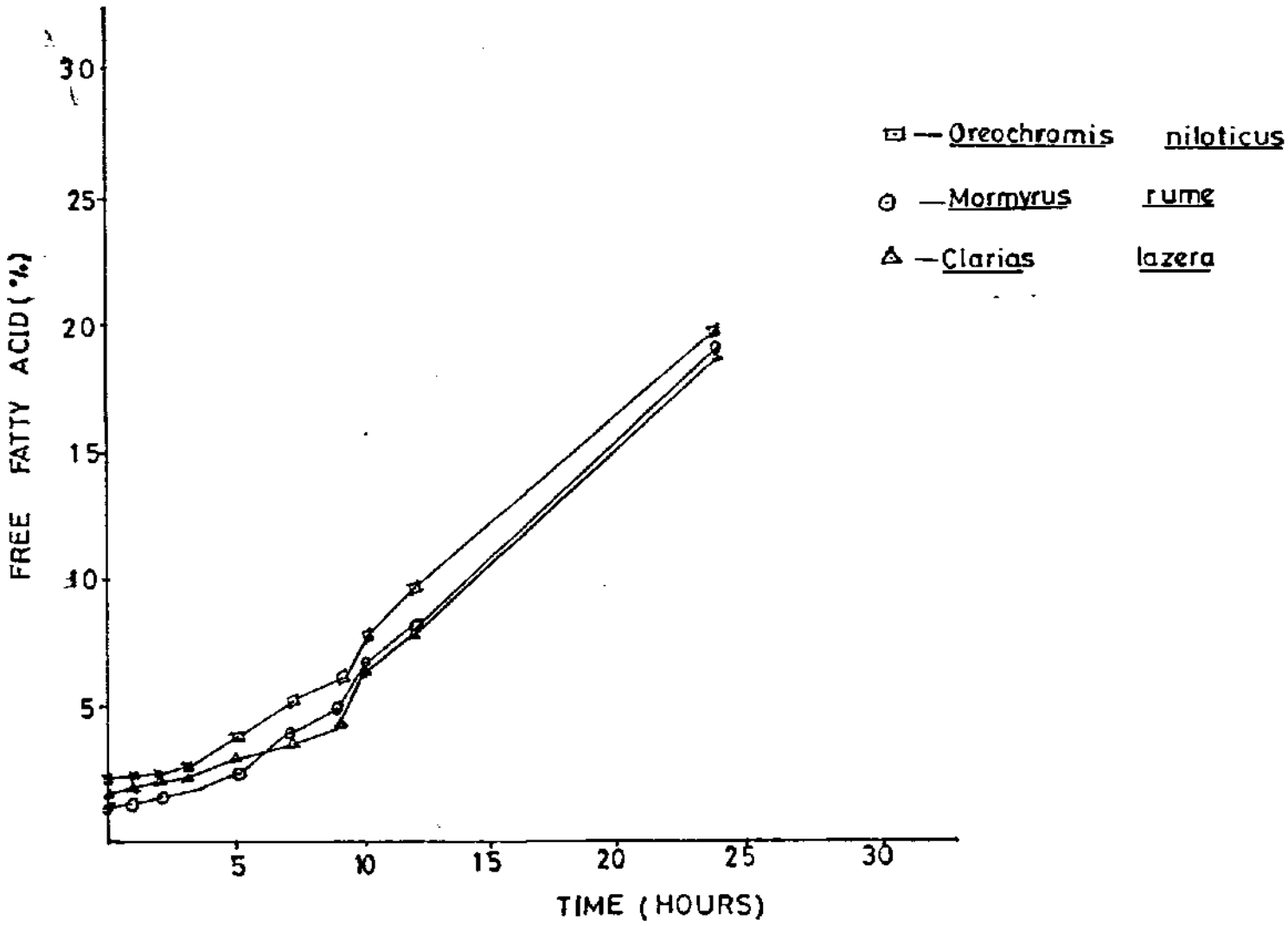


FIG.6: EFFECT OF ATMOSPHERIC AIR ON FREE FATTY ACID OF LIPIDS OF THE FISH SAMPLES

CONCLUSION AND RECOMMENDATIONS

With fish fast becoming a mainstream of the world and Nigerians diet and with the great potential for fish oil as a health food and therapeutic substance, the need for analysing fish and especially fish oil quality is becoming increasingly important. Chloroform: methanol (2:1 v/v) gave the best result of all the solvent systems used for extraction, however, better methods of extractions (especially for wet samples) would be useful. Cabrini et al. (1992) have recently found that minor fish tissue components may not be recovered as effectively as major components even when widely accepted solvent systems are employed.

The results show that fish samples analysed will be good fish meal for purpose of human and poultry consumptions as all of them contain similar components. The results also show that Mormyrus rume has the highest merit or nutritional values. It has the highest amount of protein, lipid, phosphorus, and iodine values. It has least values of peroxide and acid values and hence is least affected by autoxidation and this means its rate of rancidity/deterioration is the least. Clarias lazera contained the highest amount of potassium, iron, nickel, chromium and copper and therefore can provide more mineral supports to the body. While Oreochromis niloticus has the highest amount of total mineral contents (e.g Ca and Mg). O. niloticus has the highest saponification and ester values and thus may be more useful for soap production than others. It

contained more of moisture contents, peroxide and acid values. and this made it to be more liable to autoxidation and rancidity than any other ones.

From the results, it is evident that oven drying at 60°C. is the best way of increasing the shelf-life of fish than freezing or refrigerating. However, preservation of flesh of the fish by any of the three processes investigated still led to deterioration in quality. Consumption of fresh fish would therefore be more appropriate for obtaining the most nutrient.

It is suggested for further work, that the amino acid composition of the fish proteins, which could not be investigated in this study due to the unavailability of equipment, be investigated. The vitamins and the fatty acids should also be further investigated to determine their components.

With the high cost of some certain fish notably sardine and Mackerel and the fact that prices have not been determined by nutrient value, it has become necessary to establish nutrient compositions of all the other fish consumed in Nigeria to enable consumers make their choice on the basis of nutrient values. And considering the nutritional and health importance of fish, an all-out determination and effort to improve production must be mounted through efficient pond management based on biological information and knowledge. Thus this should engage the urgent attention of fishery and infact food scientists in general. This also would mean that efforts

to develop "improved technologies" for fish including storage facilities have to be stepped up. The technologies have to be acceptable to fish farmers and should include the development and breeding of species of fish that are;

(a) high yielding and of high nutritional quality

(b) responsive to cultural improvement

(c) resistance to diseases

(d) cultivatable in mixture with other aquatic animals.

The fish analysed have been shown to be high in quality, it is therefore suggested that they be included in efforts at developing improved species (varieties).

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