ASSESSMENT OF PHYSICAL AND CHEMICAL CHANGES IN SOME FROZEN FRESH WATER FISHES DURING STORAGE

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ABSTRACT

Chemical and physical changes in some fresh water fish species; *Clarias gariepinus (Burchell 1822), Oreochromis niloticus (Trewavas 1983) and Heterotis niloticus (Cuvier 1829)* were investigated under low temperature (-4^{0}C to –10^{0}C) storage conditions in a deep freezer (Mondial frigor) for 7 weeks (49 days). The fish samples were analyzed for proximate composition at the beginning and at the end of the storage period to determine the nutritive changes in fish muscle.The fish were assessed for quality changes using some organoleptic and chemical methods. The organoleptic assessment was based on changes in texture, odour, skin pigmentation, gill tint, eye shape and skin mucous of the fish species using hedonic scale. Weekly chemical assessment was based on changes in total volatile nitrogen (TVN), crude protein, lipid content, peroxide value and pH. The physical assessment showed that the three species of fish kept well for 35days. However, there was a significant difference ($P<0.05$) in changes in physical attributes among the different species of fish. The result of chemical evaluation of the stored fish species showed that there were significant difference ($P< 0.05$) in chemical indices among the different species during storage. However, peroxide value in the fish muscle remained undetectable over the period of storage. The study showed that all the indices measured (physical and chemical parameters), except peroxide value, changed significantly with storage.

CERTIFICATION

This is to certify that Miss Comfort O. Aiyemo carried out this study under supervision in the Department of Fisheries and Wildlife, Federal University of Technology, Akure, Nigeria.

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DEDICATION

This work is dedicated to the Glory of God.

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CHAPTER ONE

1.0 INTRODUCTION

Fish is one of the most valuable sources of high-grade protein available to man (Murray and Burt, 1991) and has been used for food since the beginning of man's recorded history. The main edible portion of a fish is composed of the muscles. The principal components of the fish muscle are water, fat and protein. Water is the main constituent, making up about 80% in lean fish and 70% in fatty fish. Carbohydrates, minerals and vitamins, and some water extractable components constitute other minor substances present (F.A.O., 1981a). All these components must be preserved with little or no changes in quality after the fish have been caught. Fish, however being extremely perishable begins to spoil as soon as it dies, this being reflected in gradual development of undesirable flavours, softening of the flesh and eventually substantial losses of fluid containing protein and fat (F.A.O. 1981a, Huss 1988). In the high ambient temperature of the tropics, spoilage could occur within 12 to 20 hours depending on species, method of catch and chemical composition, while it takes less than 2 days to about 6 days in colder water depending on the temperature (Sutcliffe and Clucas, 1978 as cited by Adejumo, 1997). Fish perishability is due to its high susceptibility to degradative organisms, which are present in the slime, gills, and intestine and on the surface of the fish.

Presently, the only means of preserving fish in its original fresh state is by refrigeration or freezing. Provided the temperature is low enough, i.e. below – 10^0C bacterial action will be hindered by the freezing process. However, chemical, biological and physical processes leading to irreversible changes still occur but at a very slow rate (F.A.O. 1981a). In other words, deterioration during frozen storage in

inevitable. There is for every frozen product a relationship between storage temperature and the time it takes at this temperature to undergo a certain amount of quality changes (F.A.O. 1981a). These changes are more pronounced when fish are stored in a domestic freezer with temperature varying between – 4^0C and-10^0C as compared to fish stored in major cold stores with temperature as low as – 18^0C and 36^0C.

The number of methods currently used for the assessment of frozen fish quality are quite large. This is based on the fact that enzyme activity results in the production of volatile bases particularly trimethylamine (TMA) and ammonia, and also lipid hydrolysis and oxidation occur in frozen fish. Quality parameters that relate to the production of these volatile bases and peroxide have been used for the assessment of the quality of the fish (Kelleher *et al.*, 1982, Kirk and Sawyer, 1991, Bechmann, 1998).

Clarias gariepinus, Oreochromics niloticus and Heterotis niloticus which have been chosen for this study highly relished fresh-water species and are very important in commercial fisheries (Capture fisheries and aquaculture) due to their wide distribution, resilience and fast rate of reproduction and growth. (Hecht *et al.*, 1997, Popma and Masser, 1999, Paugy 1990).

1.1 JUSTIFICATION

Most fish species undergo deteriorative changes in texture, flavour and colour in the frozen state (Shenouda 1980). However, these changes differ depending on the species degree, of muscular integrity, pre-refrigeration treatment conditions and period of storage (Pastoriza and Sampedro, 1994). Most studies on low temperature storage in Nigeria have been on marine species (Bossey and Talabi, 1981). There is

little research information on quality changes in fresh water species during storage. There is therefore the need to carry out a study of this nature to ascertain the chemical and physical changes which do occur in some fresh water fish species during frozen storage. This knowledge will aid the consumer, nutritionist and food processor in ascertaining how long fish could be kept frozen and would still be nutritionally beneficial when consumed, bearing in mind the gradual loss in quality. The processor in particular needs to ensure the use of quality raw material in order to forestall a rejection of the final product.

1.2 OBJECTIVES

The objectives of the study are to:

(a) determine the physical and chemical changes that occur in some frozen freshwater fish during storage

(b) determine the quality loss in some frozen freshwater fish during storage

(c) determine the appropriate storage length for some frozen freshwater fish during storage

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. *Clarias gariepinus* (Burchell 1822)

Order: Siluriformes

Family: Clariidae.

Catfishes constitute a large group of fresh water fishes which are widely distributed throughout the world. The African catfish, *Clarias gariepinus* is the freshwater fish species with the widest laditudinal range in the world (Bruton 1988). *Clarias gariepinus* is eurytrophic and inhabits a very wide range of inland waters including streams, rivers, swamps, underground sinkholes, shallow and deep lakes as well as impoundments. The distinguishing characteristics of *Clarias gariepinus* are; a large and bony head with small eyes; long dorsal and anal fins; no adipose fin; pectoral fin with stout serrated spine, used for defence or "walking" overland; a large terminal mouth; four pairs of barbels and a well developed accessory respiratory organ. The colour varies from sand-yellow through gray, to olive with dark greenish-brown markings, and a white belly. *Clarias gariepinus* is extremely hardy and adaptable, efficiently able to utilize a wide variety of both animal and plant protein under diverse environmental conditions. It is equipped to feed on a variety of food organisms, from plankton to fish. Juveniles feed on chironomid larvae, shrimps and planktonic or benthic crustaceans while adults feed mainly on fishes, crabs and snails. The species is an opportunistic omnivore capable of switching feeding modes, depending on prey availability (Spataru *et al.*, 1987)

Clarias gariepinus is able to withstand adverse environmental conditions and habitat instability. It is hardy and does not easily succumb to disease. In terms of

aquaculture, it has a potential for highly intensive culture without pre-requisite pond aeration or high water exchange rates facilitated by its air-breathing ability and tolerance of poor water quality. It is highly fecund. Spawning takes place after rain with rising water levels. Hatching is temperature dependent, and occurs approximately 18-24 hours after fertilization at 24 – 28 °C. The maximum size reached in most lakes and small rivers is rarely greater than 20kg, but very large specimens sometimes in excess of 40kg may be found in large turbid rivers. Catfish are either sold whole, (dead or alive), as fillets or cutlets, or are processed and sold in several sophisticated ways. They are sold fresh or frozen and can be eaten boiled, fried, baked or smoked. The fish could also be preserved by smoking (Hecht, *et al.*, 1997)

2.2 *Oreochromis niloticus* (Trewavas 1983)

Order: Perciformes

Family: Cichlidae

Oreochromis niloticus are widely distributed in Africa and Asia, and is one of the most widely farmed freshwater fish in the tropics. *Oreochromis niloticus* is laterally compressed and deep-bodied with long dorsal fins. The forward portion of the dorsal fin is heavily spinned. Spines are also found in the pelvic and anal fins. There are usually wide vertical bars down the sides of fry, fingerlings and sometimes in the adults. They have strong vertical bands on the caudal fins. Mature males have gray or pinkish pigmentation in the throat region, however coloration is often an unreliable method of distinguishing the species because environment, state of sexual maturity and food source greatly influence the color intensity. All *Oreochromis* species, are mouth brooders. The male excavates a nest in the pond bottom and mates

with several females. After a short mating ritual the female spawns in the nest, the male fertilizes the eggs and then the female holds and incubates the eggs in her mouth until they hatch. Fry remain in the female's mouth through yolk sac absorption stage and often seek refuge in her mouth for several days after they begin to feed. *Oreochromis niloticus* ingest a wide variety of natural food organisms, including plankton, some aquatic macrophytes, larval fish, detritus and decomposing organic matter. They may reach a size of 60.0 cm and 450 grams. *Oreochromis niloticus* are more tolerant than most commonly farmed freshwater fish to high water temperature, low dissolved oxygen, high salinity (up to 15ppt) and high ammonia concentrations. They are highly resistant to viral, bacterial and parasitic diseases, especially at optimum temperature for growth. They are a good fish for warm water aquaculture because they are easily spawned, use a wide variety of natural foods as well as artificial feeds, tolerate poor water quality and grow rapidly at warm temperatures. Consumers like the firm flesh and mild flavour and so markets have expanded rapidly in the U.S. They are sold fresh or frozen and can be eaten boiled, fried or smoked. In the tropics they are preserved by smoke drying, salt drying and sun-drying. (Balarin and Hatton, 1979, Landau, 1992, Popma and Masser,1999)

2.3 ***Heterotis niloticus* (Cuvier 1829).**

Order: Osteoglossiformes

Family: Osteoglossidae

Heterotis niloticus are widely distributed in Africa. *Heterotis niloticus* are laterally compressed with long dorsal and anal fins positioned at the posterior end of the body, terminating very close to the small caudal fin. *Heterotis niloticus* occur in shallow waters and feed on invertebrates, copepods and chironomid larvae. *Heterotis*

niloticus are the only member of the family Osteoglossidae with an epibranchial spiral organ, apparently used to taste water before microfiltration (filter feeding) is activated. The fish do well in a temperature range of 25 – 30 °C and have a good growth under aquacultural condition. *Heterotis niloticus* creates an elaborate nest of grass during breeding and they grow to a maximum size of 98.0cm and 11kg. They are sold fresh or frozen and can be eaten boiled, fried or smoked. They are preserved by smoking and salt-drying in the tropics (Paugy,1990, Olaosebikan and Raji, 1998)

2.4 CHEMICAL COMPOSITION OF FISH

The major composition of fish tissues are water, lipids, protein and micronutrients. The proximate composition of fish may vary depending on certain factors such as geographical location, season of the year, feed intake, the metabolic efficiency of the fish, the energy expended by the fish, sex, species, age and size (Eyo 2001). The compositions not only vary from fish to fish of the same specie, but also within an individual fish (Murray and Burt 1991).

2.4.1 WATER

The main constituent of fish flesh is water, which accounts for about 50 to 85% (Balogun, 1998). Individual specimens of certain species may at times be found with a water content between the extremes of 30 and 90% (Murray and Burt 1991) Water in fish exists either as free or bound water. While the free water can readily be expelled by slight pressure, the bound water is tightly bound to the proteins in the structure in such a way that it cannot readily be expelled even under high pressure. The water content usually increases and the protein content decreases as spawning time approaches in the living fish. Fish stored in melting ice tends to gain water,

whereas fish frozen and thawed tend to lose water. Generally, the water content varies with the method of handing and storage (Murray Burt 1991, Eyo 2001)

2.4.2 PROTEINS

The protein content of most raw finfish flesh is 20 ± 2% (Sidewells, 1981), but values lower than 15% or as high as 28% are occasionally met with in some species (Murray and Burt 1991). Gordon et al, (1979), as cited by Balogun(1998), reported that sole contained as low as 15% protein. Leu et al (1981), as cited by Balogun (1998), reported that Atlantic mackerel contained 16-18% protein. Balogun and Talabi (1985) reported a range of 22.4 – 25.5% protein for *Katsuwomus pelamis* (Skip jack tuna).

All proteins are chains of chemical units linked together to make one long molecule. These units are called amino acids and certain of them are essential to the human diet for the maintenance of good health (Murray and Burt 1991).

2.4.3 LIPID

Lipids are fat soluble components found in plant and animal tissues. They are made up of fats, phospholipids, sphingomyelins, waxes and sterols. Lipids are made up of the chemical element carbon, hydrogen and oxygen. Fat varies more widely in fish than the water, protein or mineral content. The pelagic species have a higher fat content that the dermersal species. In both species, fat content varies with the behaviour of the fish. When the fish is feeding, fat content increases, when the fish migrates and spawns, it no longer feeds but lives on the reserve of fat and so the fat content falls. A high fat content is usually indicative of a high eating and processing quality. For instance, a starved herring may have as little as ½% fat, whereas one that

has been feeding hearing to replenish tissue may have a fat content of over 20% (Murray and Burt 1991, Eyo 2001).

Balogun (1998), reported that lean fish (low-fat) contain less than 2% (by weight), medium or moderate fat fish, 2-5%, and high fat fish more that 5%. Lean fish is usually white (Tilapia, Croaker, sole , Cod and Tuna) and a major portion of the fat is found in the liver (e.g. Cod and shark). In high – fat fish, the lipids are concentrated mostly in the subcutaneous fatty layer resulting in the flesh being pigmented (e.g. herring, mackerel, sardine). The lipids in fish have significantly higher levels of unsaturated fatty acids because these fatty acids are associated with reduced risk of heart disease

2.4.4. CARBOHYDRATES

Fish contains negligible amount of carbohydrates. In white fish the amount is usually less than 1% but in the dark muscle of some fatty species it may occasionally be up to 2% some molluscs, however contain up to 5% of carbohydrate glycogen (Murrary and Burt 1991).

2.4.5 VITAMINS AND MINERALS

Fish, provide a well balanced supply of minerals in a readily usable form in diet. These minerals include sodium, magnesium, phosphorus, calcium, potassium, iron, fluorine and iodine. These all contribute greatly to good health in man (Eyo 2001). Fish also contains appreciable quantities of vitamins which are required in minute quantities in the diet for the maintenance of good health. Fish contains all the fat-soluble vitamins, A, D, E and K and water-soluble vitamins B and C (Murray and Burt 1991, Eyo 2001). The amounts of these vitamins in individual fish of the same

species and even of different parts of the same fish also vary considerably. The livers of cod *(Gadus morhua)* and halibut (*Hippoglossus hippoglossus)* usually contain almost all of the vitamins A and D present in those species while the same vitamins are found mainly in the flesh of eels *(Anguilla anguilla)*. The water-soluble vitamins are more uniformly distributed, being found in the skin, liver and the gut. The flesh usually contains more than half the total amount present in the fish (Murray and Burt 1991).

2.5 DETERIORATION IN FISH QUALITY

When fish dies a number of physical and chemical changes leading to spoilage take place in its body. These changes are brought about by enzymes within the fish and bacteria. Spoilage of the muscles proceed in three stages, namely, release of mucus/hyperemia, autolysis and bacterial decomposition. Emokpae (1979) stated that the release of mucus set in as a result of a peculiar reaction of the dying fish to an unfavourable surrounding. The slime secreted is made up of glycol proteins which is an effective substrate for bacterial growth as a result the fish soon putrefies and gives off an offensive odour.

2.5.1. RIGOR MOTIS

Rigor motis is the stiffening of fish muscle brought about by biochemical changes post-mortem. It is a very important phenomenon, which determines the shelf life and quality of fresh fish, depending on its duration and intensity (Eyo, 2001). Rigor motis develops as a result of depletion of adenosine triphosphate (ATP) and adenosine diphosphate (ADP) concentrations post mortem resulting in the tension of antagonistic muscles (Hultin, 1985). ATP and ADP act as plasticizers for actin and myosin, thus preventing their interaction and keeping the muscle in a state of

relaxation. When the animal dies both the ATP and ADP concentrations in the muscle drop gradually making the interaction stronger. The result is a progressive loss in muscle extensibility, which translates into tougher texture, thus decreasing the marketability of the product (Colby et al, 1993).

The occurrence of rigor motis is usually divided into three phases; initiation or delay phase, propagation or fast phase and termination or post rigor phase (Eyo 2001). In the initiation phase, the breakdown of glycogen (glycolysis) occurs resulting in a lowering of PH value due to the production of lactic acid, while phosphocreatine formed from reserved phosphagen maintains constant adenosine triphosphate (ATP) level (Eyo 2001). At this stage the fish muscles are totally relaxed. The fish is soft and pliable and the texture is firm and elastic to touch. In the propagation phase, the phosphocreatine becomes exhausted and the level of ATP falls rapidly. The fish enters into rigor and there is a release of ammonia from nucleotide where adenosine monophosphate (AMP) is deaminated to inosine monophosphate (IMP) and ammonia (NH_3) (Eyo 2001). This phase is evidenced externally by the stiffening of the entire body and may last for a few minutes or several hours depending on the species, fish size, degree of exhaustion before the fish dies, environmental temperature and some biochemical factors inherent in the fish. The muscles of the fish become soft and limp again in the terminal or post rigor phase.

Rigor motis, can have a bearing on handling and processing. In some species the reaction can be strong, especially if the fish has not been chilled. The muscles under strain tend to contract, therefore, some of the tissues may break, especially if the fish is roughly handled, leaving the flesh broken and falling apart. If the muscles are cut before or during rigor, they will contract and in this way fillets from fish can shrink and acquire a somewhat rubbery texture (FAO1981a)

Eyo (2001), noted that low temperature prolong rigor considerably. For temperate water fish (eg Cod), temperature of about 17^0C cause a very rapid entry into rigor and a severe contraction which produces a condition in the fillets known as gaping in which the myocommata (connective tissues between the myotomes) are torn. Temperature of about 25^0C may produce the same effect in tropical fish. However, if fish are cooled immediately after capture to a temperature of about -1^0C to -2^0C and held at that temperature, the pre-rigor and rigor motis period will be remarkably increased thus reducing the chances of contraction of the muscles.

When certain species of tropical fish are cooled, the fish may experience a shorter pre-rigor and go into rigor-like stage referred to as cold shock (Curran *et al* 1986). These fish have a higher drip loss (1-2%) and a lower yield (-2%) than untreated fish. Storing the fish at 20^0C for six hours after capture and chilling thereafter can prevent cold shock. Jones (1969) reported that if fish are frozen before the resolution of rigor and stored at low temperatures, rigor may occur in the fish when it is thawed (thaw rigor) and may result in excessive drip loss, shrinkage and gaping.Once the fish is dead and rigor motis has passed, spoilage begins to set in.

2.5.2 BACTERIAL SPOILAGE

When fish is freshly caught, the flesh is sterile, however, bacteria are present in the guts, on the skin and gills of the living fish. While the fish is alive, most of them do no harm but when it dies they begin to increase in number and invade the flesh (FAO 1977). The bacteria lining the gills penetrate the flesh and vascular system. Those lining the gut penetrate the nearby tissues through the peritoneum. Bacteria in the slime penetrate the skin into the surrounding tissues.

The gut enzymes breakdown the lining of the gut and thereby pave way for bacteria to enter into the tissues. The bacteria secrete digestive juices and enzymes which beakdown the tissues and cause spoilage of the fish. The fresh odour and flavour of the fish is then replaced by a sour and stale odour which proceed to ammoniacal, putrid and faecal odours at the later stages of spoilage. The initial texture which was elastic to the touch gives way to a softer flesh with grittiness. Later the fish becomes exceedingly soft and flabby, retains finger indentations and the flesh tears from the backbone (Eyo 20021).

Liston (1982) stated that the flora of cold-water fish is predominantly gram-negative, while those of tropical fish are predominantly mesophilic gram- positive micro-organisms. The dominant microflora of cold-water fish species are *Pseudomonas, Alteromans, Moraxella, Acinetobacter, Vibrio, Flavobacterium and cytophaga.* Colby *et al* (1993) reported that gram-positive organisms found on warm water fish species are predominantly *Microcosms* and *Bacillus.*

The bacteria, breakdown the tissues and produce substances such as ammonia. The bacterial reduction of trimethylamine oxide to trimethylamine and oxygen, and the endogenous enzyme breakdown of trimethylamine (DMA) and formaldehyde (FA) contribute to a gradual increase in total volatile bases in the fish muscles and further predisposes the fish to high bacterial attack (Balogun 1998).

Fish spoil rapidly under tropical conditions with a rate of 25% of fish caught being discarded due to post harvest loss (FAO, 1981b). Gram (1991) as cited by Colby *et al* (1993) stated that bacteria of the family Vibrionaceae are important spoilers of fish at ambient temperature (24 to 30^0C). Shewan (1977) reported that pseudomonad's made up 90% and 100% of the total number of isolates in he flesh and skin of 2-week old refrigerated fish fillets. Pseudomonads are the most

conspicuous bacteria isolated from spoiling fish, that are capable of generating offensive odors at refrigerated temperatures (Colby 1993). By cooling fish to around 0^0C, some of the bacteria groups responsible for spoilage will cease to grow and the rate of spoilage will be reduced (FAO 1981a).

2.5.3 AUTOLYSIS

In live fish, food in the gut is reduced to simple substances such as sugar and amino acids which are absorbed into the blood stream. The blood conveys these essential substances to sites where they are required, notably in the muscles. The reduction is induced by enzymes which act as catalysts to chemical reactions both in the gut and in the flesh. The enzymes remain active after the fish dies and thus bring about self-digestion or autolysis. Emokpae (1979), defined autolysis as the breakdown of carbohydrates, proteins and fats by the action of the enzymes amylase, protease and lipase respectively, in the tissues of fish at low temperature. Autolysis affects the flavour, texture and appearance of the fish (FAO 1981a).

Eyo (2001) noted that proteolytic enzymes are present in the gut, in the skin and in the tissues while digestive enzymes are derived from the gut in the fish. The digestive enzymes attack and destroy the muscles surrounding the gut and the belly cavity post-mortem. If allowed to continue the attack exposes the backbone and cause the belly cavity to burst as a result of which off – odour and off-flavour will be produced.

The proteolytic enzymes in the skin eat up the skin tissues gradually, thus facilitating bacterial invasion. The most active and significant of proteolytic enzymes in autolysis are the cathepsins, which are said to be particularly more active in fish than in other animals. Proteolysis is the breakdown of fish proteins into amino acids

by hydrolysis. Further breakdown of amino acids (lysine, histidine and ornitline) result in the production of toxic compounds such as cadaverine , histamine, putrescine and carbondioxide in the fish. There is also a possibility of the amino group reacting with carbonyl compounds. This results in non-enzyme browning, which affects the colour and flavour of fish. The greater the amount of amine acids produced the greater the browning. (Eyo 2001).

Colby *et al.*, (1993), reported that trimethylamine oxide (TMAO) is readily degraded postmortem to trimethylamine (TMA) by the action of (TMA) – reductase enzymes. TMAO can also be reduced directly to equimolar quantities of dimethylamine (DMA) and formaldehyde (FA) in frozen sea foods (e.g cod, haddock). Eyo (2001) stated that the DMA produced modifies the flavour while the formaldehyde, being a protein precipitant affects the texture of the frozen fish. Proteolysis is undesirable since amino acids degradation can lead to decarboxylation and potential toxicity in fish.

Lipid enzymes involved in autolysis include lipases, lipo-oxidases, phospholipases and B-oxidative enzymes. Balogun (1998) reported that fish lipids are subject to two major changes post mortem, namely lipolysis and autoxidation. Lipid hydrolysis is effected through the action of lipases on triglycerides and phospholipases on phospholipids, the major products being free fatty acids (FFA) and glycerol. .The presence of FFA has been implicated as one of the features causing protein denaturation in fish.

Colby *et al.*, (1993) defined autoxidation as the self – generating chemical degradation of unsaturated lipids in the presence of oxygen. Balogun (1998) observed that autoxidation proceeds in two stages, namely the initiation and the progagation stages. In the initiation phase, free radicals are produced while in the propagation

phase peroxides, hydroperoxides, aldehydes and ketones are produced. The hydroperoxides produced may later be further degraded to produce secondary products such as malonadehyde and other ketone compounds which produce rancid flavours in fish.

Free radicals and peroxides from lipid oxidation, destroy vitamins A, C and E. The reaction of protein with peroxides breaks down pigments, bleaches foods, produce off-flavours and odors and create toxins and/or carcinogens. Hydro-peroxides are responsible for making oxidized lipids toxic, since hydoperoxides may cause death when the pure or concentrated forms are ingested (Colby *et al.*, 1993).

Flick *et al.*, (1992), observed that lipid oxidation in fresh and frozen fish occur in the order: skin, dark muscles and ordinary muscle. Yamaguchi and Toyomizu (1984),as cited by Colby *et al* (1993), noted that lipid oxidation may occur initially in the skin during cold storage of fish in the round. Lipids buried in the skin connective tissue may migrate to the surface from the inner layer when the connective tissue disintegrates, resulting in higher oxidation rates. Siu and Draper (1978), as cited by Colby *et al., (1993),* reported that the susceptibility of seafood to autoxidation during subfreezing storage is related to the high degree of unsaturated long chain fatty acids found in them. Khayat and Schwall (1983), as cited by Colby *et al.*, (1993), noted that the oxidized unsaturated lipids may bind to proteins to form insoluble lipid protein complexes. The result of these complexes is toughened texture, poor flavour and unappealing odor in the frozen seafood.

2.6 METHODS USED FOR ASSESSING THE DETERIORATIVE CHANGES IN FISH

Borgstorm(1965), stated that the primary aim of investigating fish spoilage is to find some methods by which deteriorative changes could be accurately and quantitatively measured throughout their courses. Assessing the quality of fish entails judging the suitability of the fish for eating or processing. This includes evaluating its freshness, intrinsic value (organoleptic quality), nutritive value, degree of contamination with undesirable materials (parasites, chemicals or pathogenic organisms) degree of spoilage, method of storage, presentation to the consumer and sales.

Fish spoilage is a complex combination of microbiological, chemical and physical processes, as such, freshness or spoilage is not easy to define or measure. Different tests may be necessary under different circumstances or conditions in the determination of fish quality. Presently, the methods of assessing fish quality can be classified into (a) Sensory/subjective methods and (b) Non-sensory/objective methods.

The non-sensory methods can be further divided into:

(i) Mechanical and physical methods

(ii) Chemical methods

(iii) Biochemical methods

(iv) Microbiological methods

2.6.1 SENSORY/SUBJECTIVE METHOD

This method involves the use of human senses in evaluating fish. It is the oldest and most wide spread means of evaluating the acceptability and edibility of fish. The parameters used in the assessment include the external appearance, texture,

odour and flavour of the fish samples. Sensory tests can be applied to all species of fish and need no laboratory facility. The tests are quick, non destructive, unless the sample in being cooked, and are closely allied to the criteria the consumer uses in evaluating acceptability (How gate, (1982) as citied by Adejumo 1997).

Subjective methods suffer from the limitation of being subject to bias of the assessor and hence may not be reproducible. Biases are removed to a large extent by the use of specially trained judges who limit their judgment to special well defined attributes of the fish (Eyo 2001).

Emokpae (1979). Highlighted three major sensory methods in assessing the quality of fish;

(i) Those in which certain diagnostic features are rated on intensity scale.

(ii) Those in which tasters are asked to assess the fish on a scale of acceptability.

(iii) Those in which comparisons between two samples are made in order to distinguish the sample under test from a control on the basis of a noticeable difference between them.

Balogun (1986) and Eyo (1998) employed the use of taste panels for the organoleptic assessment of fresh fish. Tables 1 and 2 show the work of Eyo (1988) on various quality changes of two tropical fresh water species, moonfish *(Citharinus citharus)* and trunkfish (*Momyrus rume*) at ambient temperature taken as a typical example of white fish and fatty fish respectively.

Table 1 Quality scores and organoleptical changes for moonfish *Citharinus citharus* raw samples

SCORE	EYES	GILLS	SKIN	FLESH	GRADE
10	Convex, iridescent	Bright red,fresh odour	Bright grey at dorsal region silvery at ventral region scale firm.	Firm, tender	1-frist quality
8	Convex, loss of brightness	Pale red, fresh odour	Pale grey at dorsal region, silvery at ventral region; scale firm.	Less firm	2-Second quality
6	Convex, cloudy	Pinkish, neutral odour	Pale grey at dorsal region, cream colour at ventral region; scales firm.	Neither firm nor soft	3-Third quality
4	Flat, cloudy	Bleached, neutral	Pale grey at dorsal region, dark cream at ventral region; some Loose scales	soft	limit of accept-ability
2	Slighty sunken cloudy.	Bleached, very sour	Pale grey at dorsal region, green patches At the ventral region; Some loose scales	very soft, leaves finger indentation	rejected
0	Very sunken or concave	Greenish, ammoniacal Odour	Pale grey at dorsal region, greenish at ventral region; scales completely loose	very soft and flabby	rejected

Source: Eyo,1998

Table 2. Quality scores and organoleptical changes for trunkfish *Mormyrus rume* raw samples

SCORE	EYES	GILLS	SKIN	FLESH	GRADE
10	Convex, dark pupil, white cornea, iridescent	Dark red, fresh odour	Dark grey at dorsal region, silvery at ventral region	Firm, elastic slime	1-First quality
8	Convex, dark pupil, white cornea, loss of brightness	Pale red, fresh odour	Dark grey at dorsal region, silvery at ventral region	Less firm, elastic	2-second quality
6	Flat white pupil and cornea.	Pinkish sour, slightly rancid	Dark grey at dorsal region, silvery at ventral region.	Less firm, slightly elastic	3-Third quality
4	Slightly concave, white pupil, grey cornea.	Bleached sour/rancid	Dark grey at dorsal region, dark silvery at ventral region	soft, slightly elastic	Limit of acceptability
2	Concave, white pupil grey cornea.	Pale greenish, sour/strong rancid	Dark grey at dorsal region, creamy at the ventral region	soft, no elasticity	rejected
0	Concave, white pupil, grey cornea	Greenish, strong rancid.	Dark grey at dorsal region, creamy at ventral region	very soft and flabby	rejected

Source: Eyo,1998

2.6.2 NON-SENSORY/OBJECTIVE METHODS

These methods employ the use of calibrated scientific instruments and reagents to measure a specific quality parameter usually on a numerical scale (Clucas 1982). Objective tests have the advantage of being reproducible or comparable within and between different laboratories (Eyo 2001).

(A) MECHANICAL AND PHYSICAL METHODS

(i) MEASUREMENT OF pH

The measurement of pH value can be used as an indicator of fish freshness. pH is measured by placing the electrodes of a pH meter either directly into fish flesh or into a suspension of the tissues in distilled water (Eyo 2001). According to FAO (1981b), the early post mortem changes in fish are associated with a drop in pH from 7-07-7.2 to 6.2-6.5 due to the predominant formation of acid compounds. The pH in the muscle increase again during the later phases of spoilage when volatile amines and other basic compounds predominate.

Cowie and Little (1966) reported that at low temperatures (-29^{0}C, -20^{0}C) the rate of changes of extractable nitrogen, texture and liquor loss is dependent on muscle pH. The lower the pH of a fillet the more rapid its deterioration Flores and Crawford (1973) as cited by Colby *et al.*, observed a progressive increase in pH from 7.6 to 8.8 during storage of intact shrimp for 8 days in ice. Hultin (1985) noted that the ultimate pH in fish is muscle and species dependent. In red meat species (e.g mackerel), the value tends to be low, approximately 5.5, while in lean white fish (e.g. flounder) it ranges between 6.2 to 6.6. Generally the ultimate pH is somewhat higher in seafood than in warm-blooded animals or birds; this is the result of lower glycogen reserves in the fish primarily due to changing feeding patterns with the season.

(ii) MEASUREMENT OF REDOX POTENTIAL (Eh)

The redox potential (Eh) can also be used to assess the freshness of a fish sample. The measurement is carried out using platinum and calomel electrodes as a reference. Huss and Larsen, as cited by Eyo (2001) have demonstrated that Eh always seem to be positive in the muscle of a newly caught fish. Eh decreases rapidly and stays at low negative values as the fish continues to spoil. Eyo (2001) reported that a close relationship has been observed between Eh and the presence of TMAO, indicating that TMAO is the main factor influencing the post – mortem changes in redox potential in the muscle tissues of marine fish.

(iii) TORRY FISH METER

The measurement of pH, buffering capacity, refractive index and opacity of eye lens or eye fluid, firmness or viscosity of the flesh, e.t.c in a bid to assess the freshness of fish have not proved particularly effective. A new approach was provided by finding that electrical properties of the skin and underlying musculature of the fish change in a regular manner during spoilage. This principle was eventually embodied in an ingenious instrument which measured the electrical impedance across the body of fish at two different frequencies and displayed the ratio on a dial (Hennings,1965). This design was not entirely suitable for many industrial situations as such further developments led to a more practical instrument (Cheyne 1975)

The most recent and most satisfactory instrument produced so far, is known as the Torry Fish freshness meter (TFM). It measures the flesh impedance (resisstance to alternating current) and capacitance (ability to retain electric charge) of the fish samples (Clucas 1982). The TFM has four electrodes which are placed upon the fish. Two electrodes measure the impedance and capacitance while the other pair ensure good electrical contact and automatically correct the reading to the value,

which would be observed at 0^0C. The reading is displayed digitally in the range 1 to 19. Rarely does the value exceed 16 for U.K fish, such values corresponding to the highest quality fish (Clucas 1982).

Eyo (2001) reported that the biochemical basis for measurement with this equipment is founded on the observation that the cell walls of dead fish become increasingly permeable post mortem due to enzymatic breakdown, and gradually lose their capacitance. The loss in capacitance, recorded in the meter reflects the degree of spoilage of the fish. The dielectric property of fish flesh measured is signified by a factor called Q which is calculated by the equation $Q = 2\pi FCR$

Where F = frequency of alternating current (AC) which is standardized.

C = Capacitance - the ability to hold electrical charge.

R = Impedance (AC resistance) or resistance inductance.

Clucas (1982) reported that the Torry meter works very well with fish that have not been damaged or packed too tightly as to leave abrasions or blemishes on the surface of the skin. Physical damage caused by rough handling and bruising markedly reduces the TFM reading, probably to a greater extent than the physical damage reduces the eating quality, this is considered a disadvantage.

The instrument is finding widespread use in industry and official inspection and has the advantage of giving instantaneous reading obtained without destroying or affecting the sample. It is useful in testing the quality of fish under commercial conditions since it is economical and fast. Sampling can be done with the fish still in boxes. The instrument is easy to use, robust, portable and ideally suited to field operations (Clucas 1982)

(B) CHEMICAL METHODS

Eber (1891), as cited by Adejumo (1997), proposed a simple chemical test for putrefaction based on volatile amines, and since then numerous chemical, biochemical and microbiological methods have been used to investigate fish freshness.

Chemical tests are usually used in determining the chemical composition of fish and fish products at different stages of processing. It is applied in assessing proximate composition, determination of heavy metals and other harmful constituents and identification of fish species in food samples. Chemical tests do not measure freshness directly, however, the concentration of chemical measured is usually dependent on storage time and temperature thus a correlation is made possible.

The chemical elements most frequently analyzed are protein, lipid, moisture, ash and mineral elements.

(i) CRUDE PROTEIN CONTENT

The protein content is of major interest in quality grading of products. The total nitrogen content in a sample is determined by the Kjeldahl method and protein is calculated using a factor of 6.25 (A.O.A.C. 1990)

(ii) LIPID CONTENT

The fat content of a sample is important to the flavour of the product and it is estimated by the Soxhlet extraction method (A.O.A.C. 1990). It can also be measured by the Bligh and Dyer (1959) method which involves the use of methanol, chloroform and water.

(iii) MOISTURE CONTENT

This test is used to determine the degree of loss of moisture in fish. It is determined by drying a known weight of the sample to constant weight in an oven at

105^0C (A.O.A.C 1990). The moisture content is calculated from the weight loss of the sample

(iv) **ASH CONTENT**

The ash of a biological material is an analytical term for the inorganic residue that remains after the organic matter has burnt away. It is determined by burning a known weight of the fish sample in a muffle furnace at 550^0C to 570^0C for one hour after which the sample is cooled and weighed. Ashing is completed when the weight of the ash is constant and no black particles remain in the evaporating dish. The ash content is calculated from the weight loss of the sample.

(v) **MINERAL ELEMENTS**

The ash sample is usually preserved for mineral analysis. The mineral elements are the cations and anions. Cations such as sodium and potassium are determined by the use of a Flame Photometer. Calcium, magnesium and iron are determined by an Atomic Absorption Spectrometer. Anions such as nitrates are determined by the use of a UV/visible spectrophotometer. Amino acids and fatty acids are determined with an Amino Acid Analyzer (AAA), Gas Liquid Chromatograph (GLC) or High-Pressure Liquid Chromatograph (HPLC). The salt content of fermented or salted fish is measured using the Volhard method.

(C) **BIOCHEMICAL METHODS**

Many biochemical reactions occur in the tissues of living fish and these reactions continue post-mortem leading to spoilage.Measurement of the products of these reactions have been used to determine the level of deterioration in fish. These chemical changes are caused by enzymatic reactions and the result is that some constituents of the fish tissues are chemically altered and some even disappear, thus leading to changes in the sensory properties (odour and flavour). Some of these

substances, commonly known as extractives are present in varying amounts from species to species. Herring and mackerel contain large amounts of amino-acid histidine, skates, dogfish and shark on the other hand contain large quantities of urea while trimethylamine oxide is available in all salt water fishes. The products of biochemical reactions include hypoxanthine (from inosine), trimethylamine (from trimethylamine oxide) and total volatile nitrogen or total volatile bases (from amino acids) (FAO 1981a).

(I) TRIMETHYLAMINE (TMA)

Trimethylamine oxide (TMAO) is a quaternary ammonium base that forms part of the osmoregulating system of marine fishes and shellfishes. It is widely distributed in marine fauna and found in relatively large concentrations in elasmobranchs (e.g. sharks) and gadoids (e.g. cod). Its occurrence within the sample species varies with the season, size, age, sex and location. TMAO is readily degraded post mortem to trimethylamine by either the action of TMAO-reductase gram-positive microorganisms or endogenous enzymes. In fresh fish and shellfish reduction of TMAO has been associated with the growth of various gram-negative spoilage bacteria (e.g. Pseudomonads) (Colby *et al.*, 1993). The use of TMA as an index of fish freshness was first proposed by Beatty and Gibbons (1936). Ikeda (1979) reported that the accumulation of TMA in the muscle of various spoiling seafoods had correlated well with sensory scores. Colby *et al.*, (1993) observed that the production of TMA correlated well with bacterial counts in fresh fish particularly with psychotropic plate counts. The close relationship between these two indices has led to the adoption of TMA as a standard of quality in fresh shrimp in Australia and Japan. Colby *et al.*, (1993) reported that concentrations above 5mgN per 100g of tissue have been found to produce offensive off-odours in inoculated samples. It has been

proposed that TMA levels between 5 and 10mg/100g tissue should be considered maximum allowable levels in fish (Kirk and Sawyer 1991). Colby *et al.*, (1993) reported that TMA is not always a reliable estimator of shelf life because blood, viscera and dark muscles of certain species possess relatively high concentrations of the enzymes. This is particularly true during improper handling, cleaning, packing or high ratio of dark to white muscle.

Connel (1978), as cited by Adejumo (1997), observed that only marine species contain TMAO, so that the test for TMA does not apply to fresh water fish species. Huss (1988) also observed that trimethylamine was a product of the early stages of spoilage and may be lost indiscriminately during spoilage. Clucas (1982) reported that if fish are stored or processed in contact with water or melt-water, some TMA will be washed out, masking the degree of spoilage. This occurrence is most pronounced in small prawns which have a large surface area in relation to volume. TMA, can be measured by:

(i) Conway micro diffusion or steam distillation

(ii) Trimethylamine sensitive electrode and pH meter

(iii) Measurement of the colour produced by treating TMA with picric acid.

(iv) Gas Liquid Chromatography.

(II) TOTAL VOLATILE NITROGEN/BASES (TVN/TVB)

This refers to any volatile nitrogen-containing compounds that are produced post-mortem. The main components are TMA and ammonia. In a few species dimethylamine is also produced e.g. in Japanese hake (*Lutella* sp.) the relative amounts of these compounds depend upon the type of fish being examined and its quality.In freshwater fish and crustacean shell fish, the compounds formed are almost entirely ammonia while in bony marine fish and cartilaginous marine fish, the

compounds formed ate ammonia and TMA (Clucas 1982). The ammonia is mainly produced by bacterial attack on proteins and also by attack on amino acids (particularly arginine in Crustacea) and on urea in cartilaginous species.

TVN determinations are carried out by the same methods used in determining TMA, however there is no need to prevent interference by ammonia.

TVB content has been reported as a very useful index of the freshness of freshwater and lean fish (Faber 1965). Kirk and Sawyer (1991) suggested a value of 30-40mgN/100g flesh as maximum limits. White fish is considered to be fresh if the TVN is less than 20mgN/100g. At 30mgN/100g the fish is considered stale while at 40mgN/100g the fish is regarded as unfit for consumption.

(III) BIOGENIC AMINES

Eyo (2001), stated that biogenic amines consist of the diamines, mainly putrescine, cadaverine and histamine formed by the decarboxylation of amino acids and that these are used as indicators of fish spoilage because their content increases with bacterial spoilage. The most important biogenic amine in fish spoilage is histamine. Kirk and Sawyers (1991), reported that the presence of histamine is an indication of decomposition and has been linked to scombroid poisoning. The natural level in fresh fish is less than 5mg/100g and the higher values in decomposing fish are due to the decarboxylation of histidine. The amount that is considered to constitute a hazard to health is of the order 50mg/100g.

(IV) FAT DEGRADATION PRODUCTS

Clucas (1982), reported that the degradation of fat during storage can cause undesirable changes in flavour, odour and texture. Fat degradation (rancidity) is only conveniently measured on extracted oils, it is therefore necessary to extract the oil

from the flesh first. The peroxide value or thiobarbituric acid value of the extracted oil is used to determine the degree of spoilage.

(a) **PEROXIDE VALUE**

This is a measure of peroxides present in the oil. Peroxides are formed in the early phases of lipid oxidation and being odourless and flavourless can be determined chemically before any rancidity becomes organoleptically apparent. Pearson (1976), reported that during storage, peroxide formation is slow at first during an induction period which may vary from a few weeks to several months depending on the oil or fat and the temperature at which it is stored. Fresh oils usually have peroxide values well below 10 Σq/Kg. A rancid taste begins to be noticeable when the peroxide value is between 20 and 40 Σq/Kg.

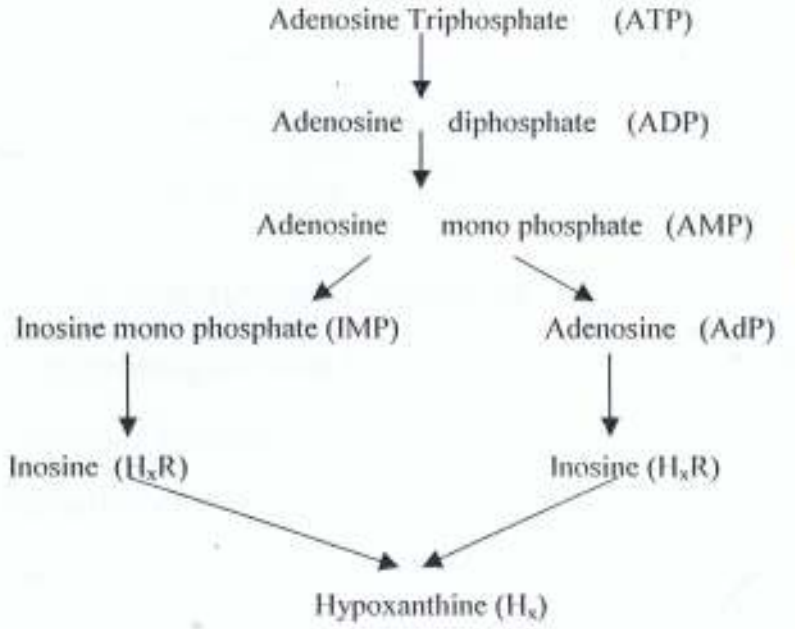
(b). **THIOBARBTURIC ACID VALUE**

The peroxides initially formed from lipid oxidation can be further oxidized to aldehydes and ketones, which have a very disagreeable "fishy" or "rancid" odour and taste. The reaction of the compound formed with thiobarbituric acid is an effective means of measuring the extent of auto-oxidation. Thiobarbituric number above 1-2 units (micromoles of malonaldehyde present in 1g fat) in fish results in objectionable rancid flavour and odour (Pearson 1976, FAO 1981a). Peroxide value and thiobarbituric acid value are measured with volumetric methods.

(V) **NUCLEOTIDE DEGRADATION PRODUCTS**

Adenosine triphosphate (ATP) is the major nucleotide of living muscle. Post – mortem, it is degraded by enzymes and micro-organisms which have invaded the flesh (clucas 1982).

The pathway of ATP breakdown is summarized below;



Source: Clucas, 1982

Spinelli (1965), reported that the autolytic degradative products have been found to possess different organoleptic properties. Inosine monophosphate (IMP), produces a sweet flavour at very low concentrations and is regarded as a strong flavour enhancer. Inosine is flavour less. Hypoxanthine imparts a bitter flavour to spoiling fish. Clucas (1982), reported that a measure of nucleotide breakdown is more closely related to eating quality than a measure of TMA or TVN, especially in the early stages of spoilage. Hypoxanthine is not very soluble in water and so is not easily leached as are TVN and TMA. It is essentially stable during processing and its presence in canned fish is indicative of pre-processing quality. Eyo (2001) noted that in most species studied hypoxanthine concentrations were found to increase steadily during iced storage. At higher temperature the formation of hypoxanthine is more rapid and so will be the loss of freshness.

Hypoxanthine can be measured by;

(i) The use of test papers

(ii) A colorimeter or UV spectrophotometer

(iii) Gravimetric method.

(VI) MICROBIOLOGICAL METHODS

Microbiological action is the main and the fastest cause of spoilage as such great care must be taken to avoid conditions which accelerate the growth of micro-organisms (FAO 1981a). The type and number of bacteria present in a newly caught fish are influenced by the environment from which the fish were caught and subsequent handling. Fish and shellfish caught in polluted water usually carry pathogenic bacteria (Eyo 2001).

Methods used for estimating bacteria population and detecting bacterial growth are total count and viable count. Other methods include membrane filter, roll tube and surface count.

The limits set by the ICMSF (1986) for some microorganisms are; less than 100 *Staphylococcus aureus/g* of fish, less than 100 faecal *Streptococcus/g* fish; less than 100 *Ecsherishia coli/g* fish and less than 100 *Vibrio parahaemohyticus/g* fish. Concentration greater than these limits are considered dangerous to human health.

3.0. MATERIAL AND METHODS

3.1 PREPARATION OF FISH SAMPLES

The study was carried out with freshly caught fish species; *Clarias gariepinus, Oreochromis niloticus and Heterotis niloticus* with average body weight ranging between 200g to 208g; 110g to 114g; 218g to 224g and average body length ranging between 30cm to 34cm; 16cm to 20cm and 26cm to 31cm respectively. *Clarias gariepinus and Oreochromis niloticus* were procured from the Agriculture. Development Programme (ADP) fish pond at Alagbaka, Akure, Ondo State, while *Heterotis niloticus* was purchased from a retailer who procured them freshly caught from the Benue River system, Nigeria.

The fish were observed for freshness, individually weighed, washed with fresh clean water and then gutted. They were again washed with clean water to remove all traces of blood and spilled enzymes. They were then placed immediately in the freezer at $-4^{o}C$ to $-10^{o}C$. The study lasted for 7weeks.

3.2 PROXIMATE ANALYSIS

Proximate analysis of the fresh fish samples was determined at the commencement of the experiment and at the end of the experiment. The protein, lipid, moisture and ash contents were determined before putting the fish in the freezer.

3.2.1 CRUDE PROTEIN

Crude protein content was determined by the micro-kjeldahl distillation method (A.O.A.C 1990).

(i) **Digestion**

About 2g of the fish sample was weighed into a 500ml Kjeldahl flask and 20ml conc. H_2SO_4 was added together with one Kjeldahl catalyst tablet.

The mixture was heated on low heat for about 15minutes, increased to medium heat for about 30minutes and finally at high heat until digested. The digest was allowed to cool and then filtered and made up to 50ml (V_1).

(ii) **Distillation**

5ml of 2% boric acid (HBO_3) was placed into a 100ml conical flask (receiving flask). Three drops of mix indicator was added. The receiving flask was placed such that the tip of the condenser tube was below the surface of the boric acid.

5ml(V_2) of the sample (digest) was pipetted into a markham distiller and 10ml of 40% NaOH was added. About 50ml of the mixture in the distiller was steam distilled into the receiving flasks.

(iii) Titration and Calculation

The distillate was titrated with standard mineral acid (0 1M HCl). A blank was titrated at the same time.

$$\%N = \frac{M \times T \times 0.014}{W} \times \frac{V_1}{V_2} \times 100$$

Where

M	=	Molarity of acid
T	=	control titre (Sample titre-blank titre)
W	=	Weight of sample
V_1	=	volume of digest
V_2.	=	Volume of digest used

Crude Protein content = % N x 6.25

3.2.2 LIPID CONTENT

The lipid content was determined with a soxhlet extraction apparatus. (A.0.AC 1990). About 5g of the dried sample was placed on a thimble and weighed. A 500ml round bottom flask was filled 2/3 up with petroleum ether. The soxhlet

extractor was fitted up with the reflux condenser and the samples The solvent (petroleum ether) was brought to boil gently and left to siphon over 5 hours after which the condenser was detached and the thimble removed. The petroleum ether in the flask was then distilled. The flask containing the fat residue was dried in an air oven at 100^0C for 5 minutes, cooled in a desiccator and weighed. The thimble was placed in a beaker in the oven at 50^0C and dried to constant weight with the sample. It was then cooled in a desicator and weighed.

The percentage of extracted lipid was calculated by the amount of lipid in the flask after extraction.

$$\% \text{ fat} = \frac{W_4 - W_3}{W_2 - W_1} \times \frac{100}{1}$$

Where:
- W_1 = Weight of dry thimble
- W_2 = Weight of thimble plus sample
- W_3 = Wt of round bottom flask
- W_4 = Wt of flask containing the fat residue after the Extraction.

3.2.3 MOISTURE CONTENT

A known weight of the fish sample was placed in a clean dish (weighed) and oven dried at 105^0C for 24hours. The dish was then transferred to the desiccator and cooled for about an hour and weighed. This was repeated to constant weight. The percentage of moisture was calculated as the difference between the initial weight and final weight.

$$\% \text{ moisture} = \frac{\text{Loss in weight}}{\text{weight of sample before drying}} \times \frac{100}{1}$$

$$= \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where W_1 = Weight of clean dried dish

W_2 = Weight of dish plus sample before oven drying

W_3 = Weight of dish plus sample after oven drying and cooling.

3.2.4 ASH CONTENT

About 1gm of the sample obtained from moisture determination were put in a crucible and weighed. The crucible was placed inside a muffle furnace and the temperature was slowly increased from 200^0C to 570^0C. until the sample became whitish in colour (about one hour). The crucible was then removed from the furnace to the desiccator and allowed to cool to room temperature. The crucible and its content were then reweighed.

The percentage ash content was calculated thus;

$$\% \text{ Ash} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

Where W_1 = Weight of crucible

W_2 = Weight of crucible plus sample before ashing

W_3 = Weight of crucible plus sample after ashing and cooling

3.3. ORGANOLEPTIC ASSESSMENT

The assessment was based on a scoring system, which involved the measurement of certain quality parameters on graded scores (hedonic scale). A six-man panel was briefly trained on the organoleptic assessment of fresh fish. The fish were placed on a clean table before grading. The parameters employed by the panelist were as follows:

(a) **Appearance:** Different parts of the fish were assessed;

(i) The skin was assessed for brightness and slime

(ii) The eyes were assessed for brightness, prominence and colour

(iii) The gills were checked for colour and presence or absence of mucus.

(b) **Texture** : The flesh was assessed for firmness or tenderness

(c) **Odour:** The odour of the fish was assessed for freshness (sea weedy) or putrefaction (ammoniacal).

The scores of the panelists were put together and the mean calculated. The freshness or degree of spoilage of the fish samples were assessed with a numerical and descriptive hedonic scale. Scores for each characteristic was on a scale of 0(very bad or extremely unacceptable) to 6 (excellent or extremely acceptable).

TABLE 3: Desciption of Grade Point Attributes of some frozen Fish during preservation

Score/Observation	0 (Very bad)	1 (Bad)	2 (Fair)	3 (Satisfactory)	4 (Good)	5 (very Good)
Texture	Very soft and flabby, retains finger indentations marked grittiness, flesh easily torn from back bone	Soft and flabby, scales easily rubbed off the skin	Soft flesh, definite grittiness, scales easily rubbed off the skin	Softening of the flesh and some grittiness	Slight softening	Firm, elastic flesh, does not retain finger indentation
Skin pigmentation	Lack luster, dull colour, grayish, marked bleaching	Discoloured, lack luster	Faded	Dull colours, loss of bright opalescence, some bleaching	Livid colours	Irridescent lustrous colours, bright opalescent sheet, no bleaching
Skin mucus	Dense, thick yellowish brown	Gritty	Thick knotted	Milky	Opague	Transparent
Eye shape	Completely sunken eyes, concave at the center, white pupils.	Flat eyes, bulky white pupils, opalescent cornea	Sunken eyes, bulky white pupils, opalescent cornea	Slightly sunken, grey pupils, opalescent cornea	Bulging but duller pupils, transparent cornea	Bulging black brilliant pupils, transparent cornea
Gills Tint	Bleached/dark brown discolouration covered with thick mucus	Grayish and covered with mucus	Yellowish with mucus	Discoloured with slight mucus	Slight discolouration	Bright red gills
Odour	Putrid, nauseating	Ammoniacal	Slightly sweet, fruity or chloroform - like	Slightly musty	No odours, neutral	Fresh seaweedy

Sourece: F.A.O. 1981a, Clucas, J.J. 1982, Adejumo, C.O. 1997

TABLE 4: Grade Pattern for Physical Assessment of some frozen Fish during Preservation

Grade	Scores	Assessment
A	>3.5	Excellent
B	3.0 – 3.5	Good quality
C	2.5 – 3.0	Satisfactory quality
D	2.0 – 2.5	Fairly good quality
E	1.5 – 2.0	Fair quality
F	<1.5	Poor quality

3.4 CHEMICAL ASSESSMENT

The fish samples were subjected to chemical analysis on the first day(prior to preservation) and subsequently every week for seven weeks. The filleted sample were macerated and used for the following analysis: Total volatile nitrogen value was determined using methods described by Pearson (1976). pH was determined using a pH meter (Jenway model 3015) and Peroxide value was determined using methods described by Pearson (1976). Analysis were carried out on triplicate samples

3.4.1 TOTAL VOLATILE NITROGEN (TVN)

TVN value was determined using methods described by Pearson (1976)

A macro – Kjeldahl distillation apparatus was set up. About 10g of the fish sample was mechanically macerated with 100ml tap water and washed into a distilling flask with 200ml tap water. 2g magnesium oxide and an anti foam agent were added. 25ml of 2% boric acid solution and a few drops of screened methyl red indicator were put in the 500ml receiving flask. The apparatus was connected up with the receiver tube dipping below the boric acid solution. The distilling flask was heated so that the liquid boiled in exactly 10minutes. The same rate of heating was used to distil for 25minutes. The condenser was then washed down with distilled water. The distillate was titrated with 0.05m sulphuric acid. The TVN value was obtained by multiplying the titration (less blank) by 14, and expressed as mg N per 100g flesh.

TVN = (T x 14) mg N/100g

Where

T = sample titre – blank titre

3.4.2 PEROXIDE VALUE

This was determined using methods described by Pearson (1976) About 1g of the oil was weighed into a clean dry boiling tube and 1g powdered potassium iodide was added. 20ml of solvent mixture (2vol glacial acetic acid + 1 vol chloroform) was also added. The tube was placed in boiling water so that the liquid boiled within 30 seconds. The content of the tube was poured into the titration flask containing 20ml of 5% potassium iodide, and titrated with 0.002m sodium thiosulphate using starch as indicator. A blank was performed at the same time.

$$\text{Peroxide value} = \frac{(V_1 - V_2) \times M \times 1000}{\text{Wt of sample}}$$

Where V_1 = Volume of $Na_2S_2O_3$ required for the sample

V_2 = Volume of $Na_2S_2O_3$ required for the blank

M = Normality of $Na_2S_2O_3$ used

The peroxide value is expressed as the number of ml of 0.002N sodium thiosulphate per g of sample (Meq/kg)

3.4.3 pH VALUE:

This was determined using a pH meter (Jenway 3015 model) About 5 grams of the sample was mechanically macerated and washed into a 100ml conical flask with 25ml of distilled water. The electrode of the meter was placed in the suspension and the pH value was noted and recorded.

3.5 DATA ANALYSIS

The data collected were subjected to a simple Analysis of Variance to test for variability. Differences, were accepted as significant where $P<0.05$. Duncan's

multiple range tests was used to compare means when a significant variation was highlighted. by the Analysis of Variance (SPSS 1999).

CHAPTER FOUR

4.0 RESULTS

4.1 PROXIMATE ANALYSIS

The results of the proximate analysis are presented in Table 5

There was a percentage loss of 1.42%, 1.15% and 0.31% in moisture content for *Clarias gariepinus, Oreochromis niloticus and Heterotis niloticus* respectively.

A percentage loss of 23.83%, 27.60% and 24.99% were recorded for protein content of *Clarias gariepinus, Oreochromis. niloticus* and *Heterotis niloticus* respectively.

A percentage loss in fat content of 12.88%, 30.62% and 26.36% were recorded for *Clarias gariepinus, Oreochromis niloticus* and *Heterotis niloticus* respectively.

Table 5: Initial and final Proximate composition of some fresh fish species subjected to deep freezer preservation for seven weeks.

Species	Moisture Content		Crude Protein		Fat		Ash		N.F.E	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final		
Clarias gariepinus	73.03	71.99	18.38	14.00	2.95	2.57	5.61	5.46	0.03	
Oreochromis niloticus	73.87	73.02	16.92	12.25	2.58	1.79	5.08	4.52	1.55	
Heterotis niloticus	74.13	73.90	17.50	13.25	2.20	1.62	5.31	4.85	0.86	

4.2 ORGANOLEPTIC ASSESSMENT

The scores of the panelists on the effect of frozen storage on physical attributes of the fish species are presented in Table 6. The texture (in terms of softness and elasticity) was accepted for *Clarias gariepins*(score of 1.92) and *Oreochromis niloticus* (score of 1.75) for 4 weeks, after which the texture became unacceptable. For *Heterotis niloticus*, the texture was accepted for the 7 weeks of storageThe analysis of variance showed no significant difference (P>0.05) between textural changes that occurred in *Clarias gariepinus* and *Oreochromis niloticus*, but there was a significance difference (P<0.05) between these two species and *Heterotis niloticus*

The skin pigmentation (in terms of brightness) was accepted by the panelists for 6 weeks for *Clarias gariepinus* (score of 2.00), 5 weeks for *Oreochromis niloticus* (score of 1.75) and 7 weeks for *Heterotis niloticus* (Score of 3.67). The analysis of variance showed a significant difference (P<0.05) among the three species.

The skin mucus (in terms of colour and thickness) became unacceptable for *Clarias gariepinus* and *Oreochromis niloticus* after 6weeks (scores of 1.25) while it was still acceptable for *Heterotis niloticus* for 7 weeks (score of 2. 17). The analysis of variance showed no significant difference in the mucus changes (P>0.05) between *Clarias gariepinus* and *Oreochromis niloticus* but showed a significant difference between these two species and *Heterotis niloticus*

The eye shape (in terms of bulging) remained acceptable for *Clarias garieprinus* (score of 1.83), for seven weeks, for *Oreochromis niloricus* 5 weeks (score of 1.75) and for *Heterotics niloticus* 7 weeks (score of 1.83).The analysis of variance showed a significant difference in changes in eye shape among the three species.

The gills tint (in terms of colour/freshness) for the three species were no longer acceptable after 5weeks (scores of 1.25, 1.25 and 1.58 for *Clarias gariepinus Oreochromis. niloticus* and *Heterotis niloticus* respectively).The analysis of variance showed no significant difference ($P>0.05$),among the three species.

The panelists accepted the odour of *Clarias gariepinus* for 6 weeks (score 1.75), of *Oreochromis niloticus* for 4 weeks (score of 1.8) and of *Heterotis niloticus* for 7 weeks (score of 3.17).The analysis of variance showed a significant difference ($P<0.05$) in odour changes among the three species.

Table 6: Physical assessment of some frozen fish species during deep freezer preservation

QUALITY PARAMETERS	SPECIES	LENGTH OF STORAGE (WEEKS)								
		0	1	2	3	4	5	6	7	Mean
Texture	*Clarias gariepinus*	5.00 ± 0.00	3.92 ± 0.38	3.33 ± 0.26	2.17 ± 0.26	1.92 ± 0.20	1.33 ± 0.26	1.25 ± 0.27	1.00 ± 0.00	2.49 ± 0.71a
	Oreochromis niloticus	4.58 ± 0.38	3.83 ± 0.26	3.17 ± 0.26	2.50 ± 0.32	1.75 ± 0.27	1.67 ± 0.26	1.17 ± 0.27	0.67 ± 0.26	2.42 ± 0.71a
	Heterotis niloticus	4.83 ± 0.26	4.58 ± 0.20	4.50 ± 0.00	4.33 ± 0.26	4.17 ± 0.26	3.83 ± 0.26	3.67 ± 0.27	3.17 ± 0.26	4.14 ± 0.71b
Skin pigmentation	*Clarias gariepinus*	5.00 ± 0.00	4.17 ± 0.26	4.17 ± 0.41	3.50 ± 0.32	2.69 ± 0.23	2.25 ± 0.27	2.00 ± 0.00	2.00 ± 0.00	3.22 ± 0.07b
	Oreochromis niloticus	4.57 ± 0.27	4.17 ± 0.26	3.67 ± 0.26	3.17 ± 0.26	2.33 ± 0.26	1.75 ± 0.27	1.25 ± 0.27	0.92 ± 0.20	2.75 ± 0.07a
	Heterotis niloticus	5.00 ± 0.00	4.75 ± 0.27	4.58 ± 0.20	4.17 ± 0.26	4.17 ± 0.26	4.00 ± 0.00	3.83 ± 0.26	3.67 ± 0.26	4.27 ± 0.07c
Skin Mucus	*Clarias gariepinus*	5.00 ± 0.00	4.25 ± 0.27	3.50 ± 0.45	2.50 ± 0.45	1.75 ± 0.27	1.67 ± 0.26	1.25 ± 0.26	1.00 ± 0.00	2.62 ± 0.50a
	Oreochromis niloticus	4.75 ± 0.27	4.25 ± 0.27	3.50 ± 0.45	2.92 ± 0.38	1.67 ± 0.26	1.58 ± 0.20	1.25 ± 0.26	0.75 ± 0.27	2.58 ± 0.50a
	Heterotis niloticus	4.92 ± 0.20	4.75 ± 0.26	4.08 ± 0.38	3.17 ± 0.26	2.75 ± 0.27	2.76 ± 0.26	2.33 ± 0.26	2.17 ± 0.26	3.34 ± 0.50b
Eye Shape	*Clarias gariepinus*	5.00 ± 0.00	4.50 ± 0.32	4.00 ± 0.32	3.25 ± 0.27	2.83 ± 0.26	2.67 ± 0.26	2.17 ± 0.26	1.83 ± 0.26	3.28 ± 0.05a
	Oreochromis niloticus	4.92 ± 0.20	4.33 ± 0.26	3.50 ± 0.45	2.67 ± 0.26	2.25 ± 0.27	1.75 ± 0.27	1.33 ± 0.27	0.83 ± 0.26	2.70 ± 0.05a
	Heterotis niloticus	4.92 ± 0.20	4.75 ± 0.27	4.17 ± 0.26	3.75 ± 0.27	3.33 ± 0.26	2.75 ± 0.27	2.33 ± 0.26	1.83 ± 0.26	3.48 ± 0.05c
Gills Tint	*Clarias gariepinus*	4.67 ± 0.26	3.92 ± 0.38	2.75 ± 0.52	2.25 ± 0.27	1.75 ± 0.27	1.25 ± 0.27	1.17 ± 0.26	0.67 ± 0.26	2.30 ± 0.05a
	Oreochromis niloticus	4.42 ± 0.49	4.17 ± 0.41	3.08 ± 0.38	2.25 ± 0.27	1.75 ± 0.27	1.25 ± 0.27	0.67 ± 0.26	0.33 ± 0.52	2.24 ± 0.05a
	Heterotis niloticus	4.50 ± 0.32	3.67 ± 0.26	3.17 ± 0.41	2.17 ± 0.26	1.83 ± 0.26	1.58 ± 0.20	1.17 ± 0.26	0.83 ± 0.26	2.36 ± 0.05a
Odour	*Clarias gariepinus*	4.92 ± 0.09	4.08 ± 0.20	3.42 ± 0.20	2.83 ± 0.26	2.17 ± 0.26	2.00 ± 0.00	1.75 ± 0.27	1.50 ± 0.00	2.83 ± 0.06b
	Oreochromis niloticus	4.50 ± 0.32	3.92 ± 0.38	3.08 ± 0.38	2.17 ± 0.26	1.83 ± 0.26	1.17 ± 0.26	0.75 ± 0.27	0.50 ± 0.00	2.24 ± 0.06a
	Heterotis niloticus	4.83 ± 0.26	4.58 ± 0.38	4.25 ± 0.27	3.83 ± 0.26	3.75 ± 0.27	3.33 ± 0.26	3.17 ± 0.26	2.83 ± 0.26	3.82 ± 0.06c

Note: Means with the same alphabet are not significantly different (P>0.05); Means separated by Duncan's test

4.3 CHEMICAL ASSESSMENT

The chemical characteristics of the three fish species at - 4^0C are presented in Figs 1 – 4. The parameters were measured at the initial (zero) state and subsequently every week for seven weeks.

(a) **Total volatile Nitrogen**.

Total volatile Nitrogen (TVN) values rose from 6.07 mgN/100gm to 27.53 mgN/100g for *Clarias gariepinus*. For *Oreochrommis niloticus* it rose from 6.53mgN/100gm to 54.13mgN/100g while for *Heterotis niloticus* it rose from 8.87mgN/100g to 28.00mg/100g. The analysis of variance showed a significant difference ($P< 0.05$) among the three species.

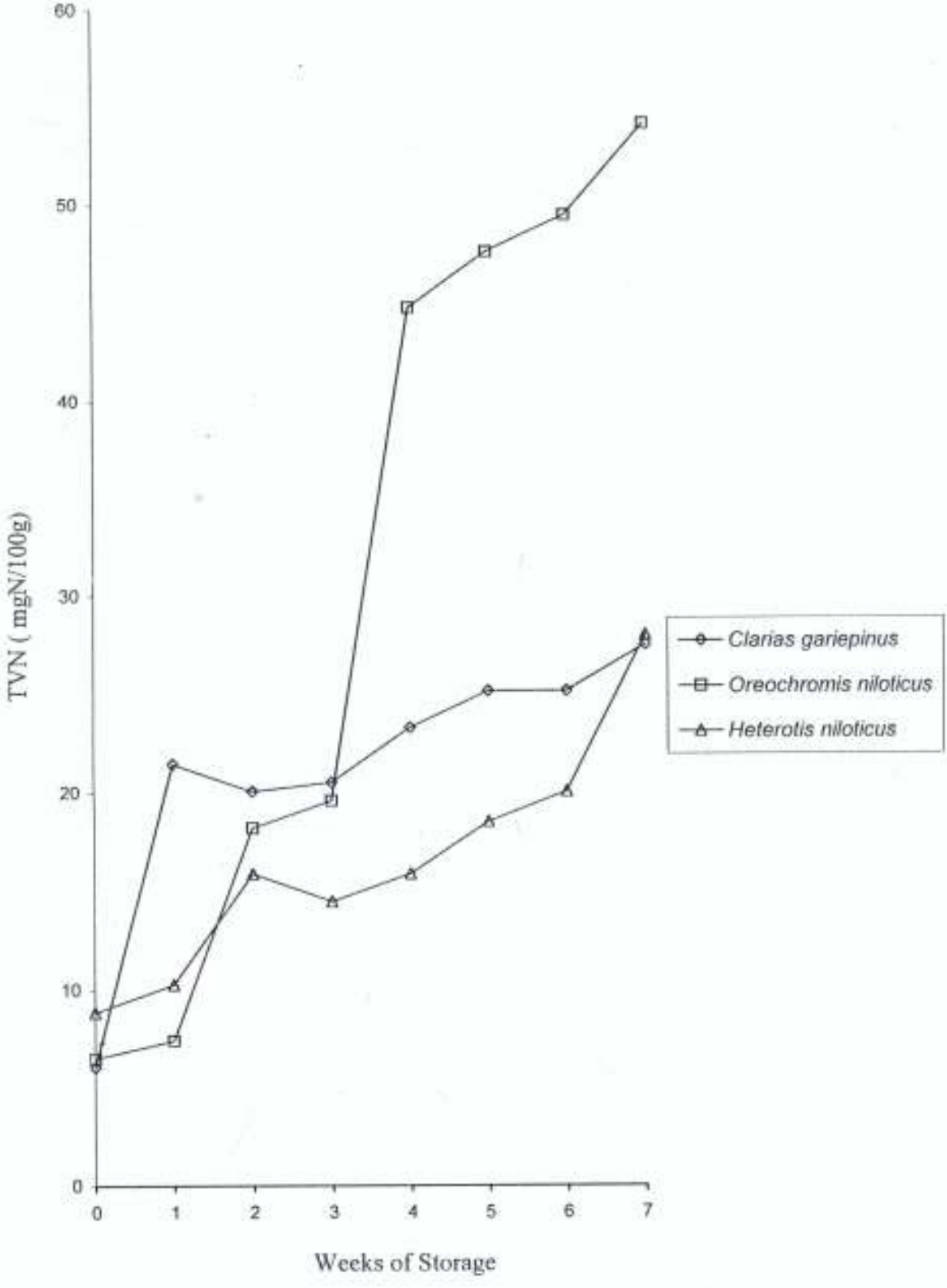


Figure 1. Weekly changes in Total Volatile Nitrogen (mgN/100g) of freshwater fishes during frozen storage.

(b) **pH value**

The pH value of *Clarias gariepinus* ranged between 7.03 to 7.33 during the study period. For *Oreochromis niloticus* pH value ranged between 7.19 to 7.79 while values for *Heterotis niloticus* ranged between 7.83 to 8.15 during the storage period. The analysis of variance showed a significant difference ($P<0.05$) among the three species.

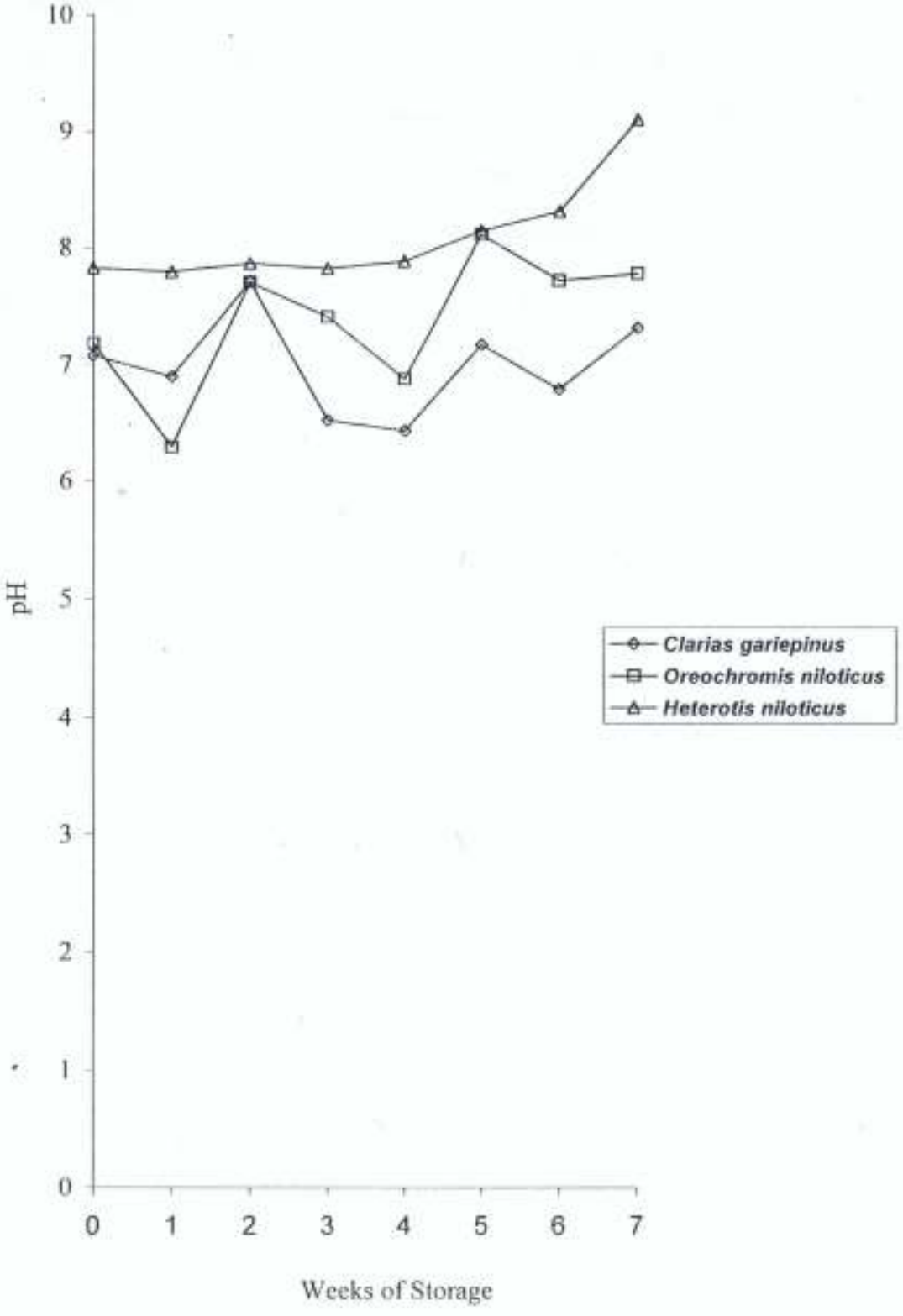


Figure 2. Weekly changes in pH of freshwater fishes during frozen storage

(c) **Crude Protein**

The crude protein value of *Clarias gariepinus* decreased from 18.38 to 14.00 within the 7 weeks of storage. For *Oreochromis niloticus* it decreased from 16.92 to 12.25. For *Heterotis niloticus* there was also a decrease in crude protein value from 17.50 to 13.25. The analysis of variance showed no significant difference ($P< 0.05$) among the three species.

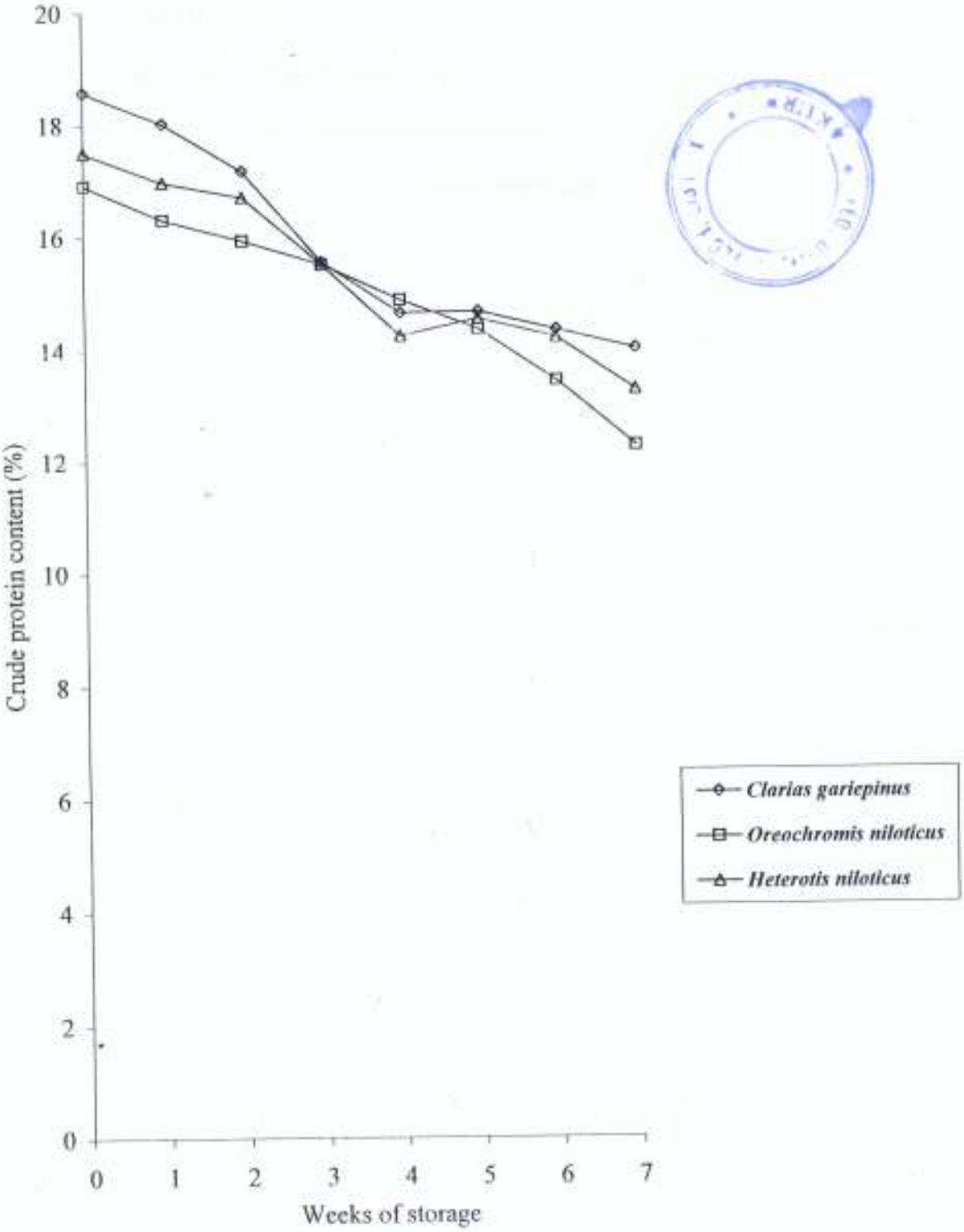


Figure 3. Weekly changes in crude protein (%) (wet weight) of freshwater fishes during frozen storage

(d) **Lipid Content**

The values of lipid content for *Clarias gariepinus* decreased from 2.95% to 2.57%, for *Oreochromis niloticus* from 2.58% to 1.79% and from 2.20% to 1.62% for *Heterotis niloticus*. The analysis of variance showed a significant difference ($P < 0.05$) among the three species.

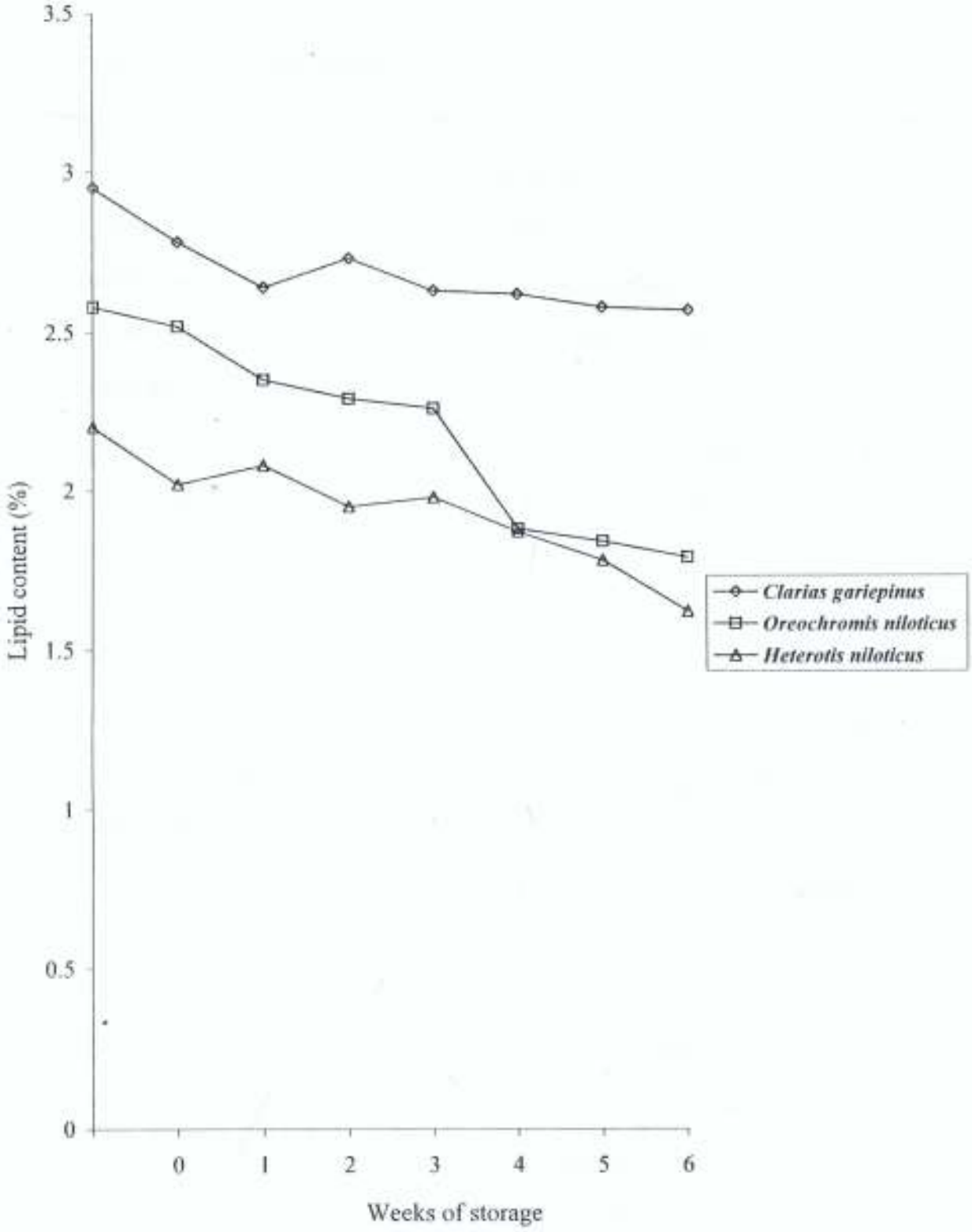


Figure 4 Weekly changes in lipid content (%) (wet weight) of freshwater fishes during frozen storage

4.4. DISCUSSION

The result of the proximate analysis which showed a decrease in protein content, fat content and moisture content for the three species of fish with length of storage infers a loss of some of the principal components of the fish muscle and thus a reduction of the nutritional quality of the fish as reported by Mills (1975) and F.A .O (1981a) Gradual denaturation of protein leads to a decrease in water – holding capacity, thus when frozen fish is thawed drip is produced and nutritive substances are drained away with the drip (Ciarlo *et al* (1985). Balogun (1986) observed that the deterioration of the quality of *Sarotherodon niloticus* stored at – 4^0C was accompanied with a decrease in the levels of total protein nitrogen and total lipid which he attributed to microbial, enzymatic and other decompositions leading to autolysis and lypolysis.

From the results of the panelists, it appears that the three fish species remained in good condition for about 5 weeks (35 days) in the freezer at -4^0C to –10^0C after which deterioration set in. Balogun (1986) observed in his studies with *sarotherodon niloticus* that the limit of acceptability was between fourteen and eighteen days (2-3weeks) when the fish were stored in the freezer at – 4^0C. Uchiyama *et al.*, (1979) and Aleman *et al.*, (1982) as cited by Huss (1988), carried out a number of experiments, storing various fish species at – 3^0C and generally agreed that partial freezing (0^0C to –4^0C) is able to delay microbial growth for an extended period and that the fish remain acceptable for up to 4 to 5 weeks depending on the species.

Several authors (Beaty and Gibbons 1936, Bossey and Talabi 1981) have reported the use of chemical methods to assess freshness or state of deterioration of fish. However, the usefulness of the chemical parameters depend on the species. Most marine fish contain substantial amounts of trimethlyamine oxide (TMAO) and

as such its degradation product, trimethlyamine (TMA) serves as a useful index of spoilage. For fresh water fish and lean fish the total volatile bases (TVB) content is used as a quality index. Kirk and Sawyer (1991) have reported the content of total volatile nitrogen as a useful index for fresh water fish. They suggested a value of 30 – 40 mgN/100g as the upper limit. Balogun (1986) has also provided statistical evidence to show that total volatile bases content is a very useful index of storage quality of fresh water fish. From the results of the chemical assessment, it could be suggested that the value of 23.33mgN/100g for *Clarias gariepinus* and 28.00mgN/100g for *Heterotis niloticus* after seven weeks of storage at -4^{0}C to -10^{0}C are still within acceptable limits while that of *Oreorhromis niloticus* which is 54.13mgN/100g makes it quite unacceptable. As stated earlier the decline observed in protein content and lipid content may have been due to leaching out of some extractable soluble protein fraction and hydrolysis of the lipid fractions. Srikar and Reddy (1991) in pink perch (*Perca sp.)*, Owusu – Ansah and Hultin (1986) in red hake (*Urophyscis chuss)*, Pastoriza and Sampedro (1994) in ray *(Raja clavata)*, Kelleher *et al.*, (1982) in red hake (*Urophyscis chuss)* and Dias *et al.*, (1994) in black scabbard *(Aphanopus carbo)* and silver scabbard *(Lepidopus caudatus)* have shown that the quantity of protein extracted from fish muscle decreases during the storage of the different species at various temperatures.

The fluctuating trend of pH in the three fish species is associated with post mortem changes which occur in fish. Due to the predominant formation of acidic compounds, the pH in the fish muscle has been found to initially drop (from about 7.07-7.20 to 6.2-6.5) and then increase later when volatile amines and other basic compounds predominate (F.A.O. 1981b, Huss 1988). Cowie and Little (1966) observed that the lower the pH of a fillet, the more rapid its deterioration. Pastoriza

and Sampedro (1994) reported an increase in the pH of the muscle of ray fish (*Raja clavata*) stored in ice from 6.24 on the first day to 9.00 on the 15th day.

Changes taking place in the lipids of frozen fish generally slow down as a result of freezing (FAO 1981a) as such, lipid oxidation may not be a problem. Pearson (1976) reported that during storage, peroxide formation is slow at first during an induction period, which may vary from a few weeks to several months, this may account for the undetection of peroxide in the fish species studied.

It would appear from the chemical assessment that the three species of fish remained in fairly good condition for a greater part of the period of storage. *Heterotis niloticus* and *Clarias gariepinus* kept very well, but *Oreochromis niloticus* showed significant signs of deterioration at the end of the study period.

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

The degree of freshness or deterioration that has occurred in frozen fish could be estimated by the use of some physical and chemical parameters. This was the attempt that was made in this study.

The changes that were observed in the physical and chemical parameters assessed in this study indicate that fresh water fish could be stored at -4^{0}C to -10^{0}C for up to 5 weeks and still remain acceptable. However, this may be only possible using freshly caught fish.

Though often marketed as fresh fish, the results obtained indicate that these species show a great potential in the frozen products market, however complementary studies are needed in order to fully characterize their behaviours in the frozen state.

5.2 RECOMMENDATIONS

As a result of the findings during this study the following are recommended:

(1) Only freshly caught and gutted fish should be used in frozen storage.

(2) Where fresh fish is to be transported from one part of the country to another it could be transported in the frozen form instead of in water.

(3) Further research into the behaviour of fresh water fish in the frozen state is needed to serve as a basis for establishing limits of acceptability.

(4) This study could be extended by carrying out further research to control the changes taking place in muscle tissue with the use of external stabilizers that prevent the loss of quality and extend the self-life of fish during storage.

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APPENDIX 1:

ANOVA table for weekly changes in Total Volatile nitrogen (TVN) for some frozen fish species during preservation.

Dependent Variable: T.V.N

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	12663.039[a]	23	550.567	45.749	.000
Intercept	37693.427	1	37693.427	3132.129	.000
FISH	2621.924	2	1310.962	108.934	.000
WEEK	6614.568	7	944.938	78.519	.000
FISH * WEEK	3426.547	14	244.753	20.338	.000
Error	577.653	48	12.034		
Total	50934.120	72			
Corrected Total	13240.693	71			

a. R Squared = .956 (Adjusted R Squared = .935)

1. FISH

Dependent Variable: T.V.N

FISH	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Clarias	21.175	.708	19.751	22.599
Tilapia	30.975	.708	29.551	32.399
Heterotis	16.492	.708	15.068	17.915

FISH

T.V.N

Duncan[a,b]

FISH	N	Subset		
		1	2	3
Heterotis	24	16.4917		
Clarias	24		21.1750	
Tilapia	24			30.9750
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 12.034.

a. Uses Harmonic Mean Sample Size = 24.000.

b. Alpha = .05.

APPENDIX 2

ANOVA table for weekly changes in pH for some frozen fish species during preservation

Dependent Variable: PH

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	30.436[a]	23	1.323	1617.628	.000
Intercept	4044.302	1	4044.302	4943799	.000
FISH	15.063	2	7.531	9206.559	.000
WEEK	9.704	7	1.386	1694.660	.000
FISH * WEEK	5.669	14	.405	494.980	.000
Error	3.927E-02	48	8.181E-04		
Total	4074.777	72			
Corrected Total	30.475	71			

a. R Squared = .999 (Adjusted R Squared = .998)

1. FISH

Dependent Variable: PH

FISH	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Clarias	6.994	.006	6.982	7.005
Tilapia	7.391	.006	7.379	7.403
Heterotis	8.100	.006	8.088	8.111

FISH

PH

Duncan[a,b]

FISH	N	Subset		
		1	2	3
Clarias	24	6.9938		
Tilapia	24		7.3908	
Heterotis	24			8.0996
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 8.181E-04.

a. Uses Harmonic Mean Sample Size = 24.000.

b. Alpha = .05.

APPENDIX 3

ANOVA table for weekly changes in crude protein for some frozen fish species during preservation

Dependent Variable: PROTEIN

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	178.840[a]	23	7.776	2.285	.008
Intercept	17059.816	1	17059.816	5013.679	.000
FISH	10.371	2	5.185	1.524	.228
WEEK	160.811	7	22.973	6.751	.000
FISH * WEEK	7.659	14	.547	.161	1.000
Error	163.327	48	3.403		
Total	17401.983	72			
Corrected Total	342.167	71			

a. R Squared = .523 (Adjusted R Squared = .294)

1. FISH

Dependent Variable: PROTEIN

FISH	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Clarias	15.871	.377	15.114	16.628
Tilapia	14.943	.377	14.186	15.700
Heterotis	15.365	.377	14.608	16.122

FISH

PROTEIN

Duncan[a,b]

FISH	N	Subset 1
Tilapia	24	14.9429
Heterotis	24	15.3646
Clarias	24	15.8713
Sig.		.105

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 3.403.

a. Uses Harmonic Mean Sample Size = 24.000.

b. Alpha = .05.

APPENDIX 4:

ANOVA table for weekly changes in lipid content for some frozen fish species during preservation

Dependent Variable: LIPID

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	9.981[a]	23	.434	5.554	.000
Intercept	372.054	1	372.054	4762.034	.000
FISH	6.863	2	3.431	43.921	.000
WEEK	2.528	7	.361	4.622	.001
FISH * WEEK	.590	14	4.214E-02	.539	.897
Error	3.750	48	7.813E-02		
Total	385.785	72			
Corrected Total	13.731	71			

a. R Squared = .727 (Adjusted R Squared = .596)

1. FISH

Dependent Variable: LIPID

FISH	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Clarias	2.687	.057	2.572	2.802
Tilapia	2.187	.057	2.072	2.301
Heterotis	1.946	.057	1.831	2.061

FISH

LIPID

Duncan[a,b]

FISH	N	Subset		
		1	2	3
Heterotis	24	1.9458		
Tilapia	24		2.1867	
Clarias	24			2.6871
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 7.813E-02.

a. Uses Harmonic Mean Sample Size = 24.000.

b. Alpha = .05.

APPENDIX 5:

ANOVA table for weekly changes in texture of some frozen fish species during preservation

Dependent Variable: TEXTURE

Source	Type I Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	268.222[a]	23	11.662	180.570	.000
Intercept	1308.028	1	1308.028	20253.333	.000
FISH	90.691	2	45.345	702.124	.000
WEEK	152.722	7	21.817	337.819	.000
FISH * WEEK	24.809	14	1.772	27.439	.000
Error	7.750	120	6.458E-02		
Total	1584.000	144			
Corrected Total	275.972	143			

a. R Squared = .972 (Adjusted R Squared = .967)

FISH

TEXTURE

Duncan[a,b]

FISH	N	Subset 1	Subset 2
Tilapia	48	2.4167	
Clarias	48	2.4896	
Heterotis	48		4.1354
Sig.		.162	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type I Sum of Squares
The error term is Mean Square(Error) = 6.458E-02.

a. Uses Harmonic Mean Sample Size = 48.000.

b. Alpha = .05.

APPENDIX 6:

ANOVA table for weekly changes in skin pigmentation of some frozen fish species during preservation

Dependent Variable: PIGMENT

Source	Type I Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	205.457[a]	23	8.933	154.778	.000
Intercept	1678.746	1	1678.746	29087.029	.000
FISH	58.167	2	29.083	503.919	.000
WEEK	125.920	7	17.989	311.681	.000
FISH * WEEK	21.371	14	1.526	26.449	.000
Error	6.926	120	5.771E-02		
Total	1891.129	144			
Corrected Total	212.383	143			

a. R Squared = .967 (Adjusted R Squared = .961)

FISH

PIGMENT

Duncan[a,b]

FISH	N	Subset 1	Subset 2	Subset 3
Tilapia	48	2.7500		
Clarias	48		3.2223	
Heterotis	48			4.2708
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type I Sum of Squares
The error term is Mean Square(Error) = 5.771E-02.

a. Uses Harmonic Mean Sample Size = 48.000.

b. Alpha = .05.

APPENDIX 7:

ANOVA table for weekly changes in mucus of some frozen fish species during preservation

Dependent Variable: MUCUS

Source	Type I Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	249.972[a]	23	10.868	128.282	.000
Intercept	1167.361	1	1167.361	13778.689	.000
FISH	17.774	2	8.887	104.898	.000
WEEK	226.083	7	32.298	381.218	.000
FISH * WEEK	6.115	14	.437	5.155	.000
Error	10.167	120	8.472E-02		
Total	1427.500	144			
Corrected Total	260.139	143			

a. R Squared = .961 (Adjusted R Squared = .953)

FISH

MUCUS

Duncan[a,b]

FISH	N	Subset 1	Subset 2
Tilapia	48	2.5833	
Clarias	48	2.6146	
Heterotis	48		3.3438
Sig.		.600	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type I Sum of Squares
The error term is Mean Square(Error) = 8.472E-02.

a. Uses Harmonic Mean Sample Size = 48.000.

b. Alpha = .05.

APPENDIX 8:

ANOVA table for weekly changes in eye shape of some frozen fish species during preservation

Dependent Variable: EYESHAPE

Source	Type I Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	209.972[a]	23	9.129	126.405	.000
Intercept	1431.361	1	1431.361	19818.846	.000
FISH	15.837	2	7.918	109.639	.000
WEEK	189.778	7	27.111	375.385	.000
FISH * WEEK	4.358	14	.311	4.310	.000
Error	8.667	120	7.222E-02		
Total	1650.000	144			
Corrected Total	218.639	143			

a. R Squared = .960 (Adjusted R Squared = .953)

FISH

EYESHAPE

Duncan[a,b]

FISH	N	Subset 1	Subset 2	Subset 3
Tilapia	48	2.6979		
Clarias	48		3.2813	
Heterotis	48			3.4792
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type I Sum of Squares
The error term is Mean Square(Error) = 7.222E-02.

a. Uses Harmonic Mean Sample Size = 48.000.

b. Alpha = .05.

• APPENDIX 9:

ANOVA table for weekly changes in gills tint of some frozen fish species during preservation

Dependent Variable: GILLSTINT

Source	Type I Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	251.651[a]	23	10.941	101.322	.000
Intercept	763.141	1	763.141	7067.026	.000
FISH	.375	2	.188	1.736	.181
WEEK	247.845	7	35.406	327.880	.000
FISH * WEEK	3.431	14	.245	2.269	.009
Error	12.958	120	.108		
Total	1027.750	144			
Corrected Total	264.609	143			

a. R Squared = .951 (Adjusted R Squared = .942)

FISH

GILLSTINT

Duncan[a,b]

FISH	N	Subset 1
Tilapia	48	2.2396
Clarias	48	2.3021
Heterotis	48	2.3646
Sig.		.080

Means for groups in homogeneous subsets are displayed.
Based on Type I Sum of Squares
The error term is Mean Square(Error) = .108.

a. Uses Harmonic Mean Sample Size = 48.000.

b. Alpha = .05.

APPENDIX 10:

ANOVA table for weekly changes in odour of some frozen fish species during preservation

Dependent Variable: ODOUR

Source	Type I Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	235.243[a]	23	10.228	154.904	.000
Intercept	1266.174	1	1266.174	19176.378	.000
FISH	61.420	2	30.710	465.108	.000
WEEK	159.382	7	22.769	344.837	.000
FISH * WEEK	14.441	14	1.031	15.622	.000
Error	7.923	120	6.603E-02		
Total	1509.340	144			
Corrected Total	243.166	143			

a. R Squared = .967 (Adjusted R Squared = .961)

FISH

ODOUR

Duncan[a,b]

FISH	N	Subset 1	Subset 2	Subset 3
Tilapia	48	2.2396		
Clarias	48		2.8333	
Heterotis	48			3.8229
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type I Sum of Squares
The error term is Mean Square(Error) = 6.603E-02.

a. Uses Harmonic Mean Sample Size = 48.000.

b. Alpha = .05.