

**ASSESSMENT OF PHYSICAL AND CHEMICAL
CHANGES IN SOME SMOKED FISH SPECIES DURING STORAGE**

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(FWL/93/4398)

B. Agric Tech. Fisheries (FUTA)

**A THESIS IN THE DEPARTMENT OF FISHERIES AND WILDLIFE
FEDERAL UNIVERSITY OF TECHNOLOGY, AKURE
AS PART OF THE REQUIREMENTS FOR THE AWARD OF THE
MASTER DEGREE IN AGRICULTURAL TECHNOLOGY (FISHERIES)**

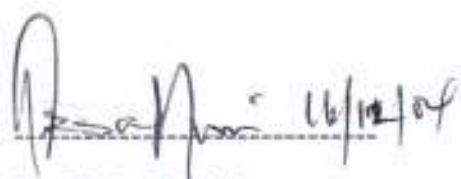


OCTOBER, 2004.

CERTIFICATION

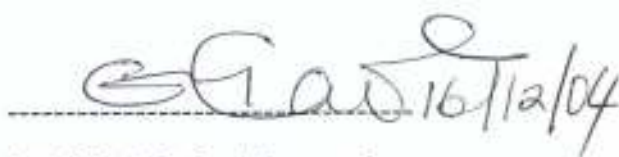
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DEDICATION

This research work is dedicated to the Glory of God and entire members of Mr. and Mrs. J. I. Daramola family.

ACKNOWLEDGEMENT

Foremost, I thank the Almighty God for His grace, provision and mercy on my life. May His name be praised forever.

I also want to thank my indefatigable supervisors Dr. E. A Fasakin and Dr. (Mrs) E. O. Adeparusi for their tutelage and scholastic knowledge impacted on my academic life, you will forever be revered.

My appreciation also goes to the Head of Department, Professor E. A Agbelusi and other member of staff such as Dr. Onibi (APH), Mr. Sumaila (IMC), Mrs Dafiewharae (FWT) and Pastor Mike Ojuola(FWL) for their assistance during the course of this work.

Furthermore, I cannot forget my colleagues such as Ibidun, Bro Tope Olorunfemi, Debola, Sade, Gbolabo, Ken, Bro. Lanre (Pako Ventures) and to Funmi who assisted in typing this project

Special appreciation also goes to Fisheries society of Nigeria (FISON), Ekiti State Chapter and Elder Adewole for their enormous assistance.

In addition, I appreciate immense support of my love, Miss Bolanle Adebayo. Her care and concern was noteworthy.

Finally, Brother Gbenga, Dare and Kunle, Dad and Mum; you played an invaluable role in my life. I love you and God bless us all, Amen.



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ABSTRACT

Physical and chemical changes in five smoked freshwater fish species: *Heterotis niloticus*, *Labeo coubie*, *Parachanna obscura*, *Oreochromis niloticus* and *Clarias gariepinus* during storage were assessed. The fish were smoked-dried to an average moisture content of $10.41 \pm 0.02\%$ and stored without preservative in plastic baskets for 56 days under ambient conditions. Samples of fish were assessed weekly for physical attributes such as colour, fragmentation, odour, taste and texture. Analyses for the proximate composition were based on changes in moisture content, crude protein, lipid and ash content. Chemical assessments were based on Total Volatile Nitrogen (TVN), pH, Peroxide Value (PV) and Free Fatty Acid (FFA). There were significant changes in the physical and chemical characteristics of the smoked fish species as from the 6th week (42 days) of storage, except for the odour which did not differ significantly ($P > 0.05$) during the storage period. There were significant differences ($P < 0.05$) in the initial and final values of proximate and chemical constituents for different species of fish. The study showed that quality of smoked fish stored under ambient conditions decreases with increase in length of storage.

1.0 INTRODUCTION

Fish is an important protein source in the tropics. In Africa, over 17.5% of the animal protein comes from fish while in Nigeria, fish constitutes 40% of the animal protein intake of the people (Olatunde, 1989). The less developed countries capture 50% of the world's harvest and a large proportion of that catch is consumed internally (FAO, 1985). However, fish production in Nigeria has not been able to meet projected demands (Eyo, 2001). Unfortunately, large quantities of available fish catch are wasted yearly in less developed countries due to improper processing and preservation techniques.

Fish is susceptible to spoilage as soon as it is caught. Fish deterioration starts as soon as the fish dies and progresses until the fish is entirely destroyed. According to Eyabi-Eyabi (1996), the limited shelf life of dead fish i.e. 16-20 hours in Southern part of Nigeria and 20-36 hours under conditions in the Northern part are basically due to biochemical changes after death. The speed with which fish spoils depends on hygienic conditions, storage temperature, acidity and the structure of the muscular tissue (Clucas, 1982). Chemical breakdown of protein content, fat content (agent of rancidity and off-flavour) and the water content (water activity) contribute to quick spoilage of fish. However, the extent of fish damage depends on the processing techniques, the type of fish being processed, weather and mode of storage during transportation. (Eyo, 1993).

Post-harvest losses in fish is represented by a net reduction in the amounts of nutrients potentially available to the consumer. This is either by direct physical loss or nutritional loss which is the overall reduction in the value of their chemical components.

These factors have effect on consumer acceptability, commercial value and income of fish farmers/traders i.e economic loss (Bostock *et al*, 1987). In addition, the health implication of consuming spoilt fish cannot be quantified.

Fish storage remains a serious factor affecting fish availability in Nigeria. Tobor (1992) estimated fish loss due to poor preservation to be between 35-40% of the landed weight of fish from the artisanal fishery sector in Nigeria. Also, Jallow (1995) stated that low levels of technical know-how in proper processing techniques, packaging and marketing problems contribute to post-harvest losses to about 20-30% of fish catch. While Oladosun *et al* (1996) reported that 40% of total fish catch in Nigeria are lost annually due to inadequate or poor preservation, processing and handling.

There are different methods or ways of prolonging the shelf-life of fish. These include chilling, freezing, canning, drying, salting and smoking. In Nigeria, the most affordable and widely used method of fish preservation is smoking (Eyo, 1993). The use of smoke from smouldering wood for the preservation of fish dates back to civilization (Clucas, 1982). Smoking enhances flavour and increase utilization of the fish. Meanwhile, deterioration and spoilage still occur in smoked fish during storage. These could be assessed by standardised physical and chemical methods. The essence of this study therefore is to assess these physical and chemical changes with a view of reducing the problem of spoilage during storage.

1.1 JUSTIFICATION

Fish smoking is important in artisanal fisheries in order to prolong the shelf life of the fish. It reduces waste especially when catches are good thereby increasing protein availability to people (Jallow, 1995). Smoked fish is highly favoured in many traditional

dishes in Nigeria. It is used as condiment that greatly enriches the flavour of dishes and as a good alternative to fresh fish, which is scarcely available in many towns and cities that are far away from the coast and riversides.

About 60-65% of fish caught in Nigeria inland waters are preserved by smoke curing (Eyo, 1993). Smoking has preservative effect, impact flavour and drying effect on the fish (Tobor, 1992). The materials (wood, charcoal, wire gauze, e.t.c) needed for the smoking process are cheap and readily available in different parts of the country. Also, the skill involved in smoking fish is relatively low and attainable by local dwellers. Other methods of fish storage such as freezing and canning are still not widely used because of the low technological development and epileptic power supply.

In spite of the positive attributes of fish smoking, losses still occur during and after processing. There is a dearth of information on physical and chemical changes in smoked fish in tropics with increasing storage length. There is, therefore the need for more research work to assess fish quality and proffer possible solutions to post-harvest loss. This would improve nutritional status, enhance good economic returns, thereby eliminating the need to import fish into developing countries. This is imperative in view of the general decline in fish supply and increasing human population and the demand for fish food protein.

1.2 AIMS AND OBJECTIVES:

The broad objective of this study therefore is to evaluate changes in physical and chemical characteristics of smoked fish during storage.

The specific objectives are to:

- (i) assess physical and chemical changes of some smoked fish species during storage.
- (ii) evaluate the loss in quality of the smoked fish during storage.
- (iii) determine the appropriate length of storage for the smoked fish.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Fish Production and Demand in Nigeria

The effective average annual fish demand in Nigeria between 1980 and 1990 was estimated at 1.2 million tonnes and was projected to increase approximately to 2 million tonnes by the year 2000 (Shimang, 1990). However, he reported that in practice, the actual catch covering 1980-1987 averaged 0.6 million tonnes. Nigeria is a large country both in size (923 000km²) and population with over 120 million citizens. It has about 120 million hectares of water bodies, which are made up of natural (Chad) and man-made lakes (Kainji), Rivers (Benue and Niger) and Reservoirs e.g Shiroro, Bakolori, Goronyo and Jebba (Shimang,1990). He posited that fish production in these water bodies has been relatively low and constitutes less than 40% of the total national needs .

In Nigeria, historical fishery statistical data on both fresh and processed fish and fishery products are lacking or unreliable. Ezenwa (1994), reported that accurate estimates of fish production in Nigeria is fairly difficult mainly because there is no clear distinction between capture fishery and aquaculture. However, FAO (1999) estimated the production from aquaculture in Nigeria to have increased between 1991 and 1997. In the year 2000, Fagbenro and Adebayo (2002) reported aquaculture production of 30, 776 tonnes. Also Akinyemi (1998) in estimating fish consumption in Nigeria from 1991 to 2010 (based on the population census of 1991), made a projection that Nigeria would require over 1.3 million metric tonnes of fish and a per caput consumption of 13kg from the year 2000 (Table 1). However, the present level of fish consumption of fish in Nigeria is put at 7kg

(Faturoti, 1999); which is far below the projected requirement. Hence, there is the need to increase fish supplies in Nigeria while post-harvest losses of the little produced are drastically reduced or avoided. This is inevitable in an already malnourished country like Nigeria.

Furthermore, as the world population expands, the demand for fish also grows; with the world population reaching the 6.0 billion mark by the year 2000AD (Campbell-Platt, 1984). Therefore a lot of pressure is being mounted on the world wild fish population. This demand is greater in the tropics with rising human population which accounts for 90% of the world's population growth (Eyo, 2001). Also, in estimating the demand for fish in the world, Pedini (2000) observed that the per caput consumption of fish in Africa is low in spite of the high preference for fish. In view of this, a corresponding increase in fish supply is imperative to sustain the population.

Table 1: Nigeria Projected and Estimated Demand for Fish 1991-2010.

Year	Projected population (million)	Per Caput Consumption (Kg)	Projected fish Demand (metric tones)
1991	88.50	11.00	973,500
1992	90.36	11.00	993,960
1993	92.22	11.00	1,014,420
1994	94.08	11.00	1,034,880
1995	95.94	11.00	1,055,340
1996	97.80	11.00	1,075,800
1997	99.66	11.00	1,096,260
1998	101.52	11.00	1,116,720
1999	103.38	11.00	1,137,180
2000	105.24	13.00	1,358,120
2001	107.10	13.00	1,392,300
2002	108.96	13.00	1,416,480
2003	110.82	13.00	1,440,660
2004	112.68	13.00	1,464,840
2005	114.54	13.00	1,489,020
2006	116.40	13.00	1,513,200
2007	118.26	13.00	1,537,380
2008	120.12	13.00	1,561,560
2009	121.98	13.00	1,585,740
2010	123.84	13.00	1,609,920

Source – Akinyemi, O. (1998).

2.2 Fish Deterioration and Spoilage

The quality of fish begins to deteriorate as soon as it dies. Fish only remain in its good quality for a short period after death. Within this period, the fish is firm and tender and the skin is bright with firm scales. The gills are bright red exuding fresh odour and the eyes are bulging or convex and iridescent. Thereafter, the fish become stiffened and hard due to rigor mortis. After a few hours, depending on the species and the ambient temperature, the flesh begins to soften and later become flabby. The skin lose its sheen and the scale become loose, the gill change colour from bright red to pink and later greenish with ammonia odour and the eyes become sunken into the cranium (Eyo, 2001). At this stage, the fish has deteriorated or spoilt and is unacceptable or unfit for human consumption. Consequently, such fish are used for the production of compound feed or discarded as wastes.

2.2.1 Causes of fish deterioration.

There are three causes of fish deterioration or spoilage:

- (i) Microbiological spoilage, caused by bacteria
- (ii) Autolytic spoilage, caused by enzymes
- (iii) Chemical spoilage

2.2.1a Microbiological Spoilage: This is caused by the bacteria on the surface and intestines of the fish. Their effect is quick on fresh and non-acidic products such as fish. The bacteria reproduce rapidly and act on the fish causing infections, poisoning and spoilage. A number of bacteria are easily destroyed by preservation techniques; however, they can start to grow again after insufficient heat treatment (Colby et al, 1993). The factors affecting bacterial growth on fish include damage to fish flesh, water content (water activity), oxygen, acidity, chemical composition and temperature.

2.2.1b. Autolytic spoilage: This is caused by enzymes in fish. When fish dies, the enzymes inside them are not immediately deactivated. These enzymes then start breaking down compounds into smaller units. This affects the texture, smell and tastes (Eyo, 2001). Some hours later, rigor mortis (stiffening of the muscle) occurs. Thereafter, the flesh gets soft again due to enzymatic reactions (autolysis); at this point, the fish is spoilt. Wastage or spoilage of fish can be salvaged if the guts are removed and fish preserved on or before rigor mortis. However, heat treatment can inactivate the enzymes.

2.2.1c Chemical spoilage: This is caused mainly by the reaction between the fat in the fish and oxygen in the air (oxidation). The spoilage occurs when fish is exposed for a long time, giving rise to unpleasant odours and flavours i.e rancidity (Maas-van Berkel, 1996). Fish oil is more liable to spoilage than other oils due to the greater number of unsaturated fatty acids (Eyo, 2001). The greater the degree of unsaturation, the greater the biochemical changes, which precedes rancidity.

2.3 Quality Assessment of Fish

Quality refers to the aesthetic appearance and freshness or degree of spoilage which the fish has undergone. It is the characteristics or attributes which make fish to be acceptable or rejected by the consumer. Assessment of fish quality is done with reference to freshness, presence or absence of pathogenic organisms (e.g bacteria or moulds), palatability, e.t.c. Fish quality influences consumers demand. A product which does not meet the required standard of quality is rejected at the market level and the producer suffers economically.

The assessment of fish quality is broadly divided into two (Bostock *et al*, 1987):

- Objective (instrumental) methods i.e the chemical, biochemical, microbiological and physical tests.
- Subjective method i.e sensory tests

Since the consumer is the ultimate judge of quality, the accurate objective tests has to be correlated with subjective (sensory) evaluation. However, sensory test must be performed scientifically under carefully controlled conditions so that the effects of test environment, personal bias, e.t.c., may be reduced minimally.

2.3.1 Objective Methods

These are the quality assessment tests which rely on the use of instruments and reagents for their determination. Objective test have the advantage of being reproducible (Eyo, 2001).The tests include chemical, biochemical, microbiological and physical tests.

2.3.1a Proximate Test

This is the determination of the proximate composition of fish products at different stages of processing and storage. It involves the analyses of components such as moisture, protein, lipid, ash and minerals.

2.3.1b Chemical Test

The establishment of tolerance levels of biochemical spoilage indicators eliminate the need to base decisions regarding product quality on personal evaluation alone. Chemical tests are useful in resolving issues regarding products of marginal quality. It is less time consuming unlike the microbiological methods. The chemical compounds to be measured should increase or decrease with the level of spoilage (Maas-van Berkel *et al*, 1996). Also, the compounds to be measured must not be affected by processing.

Chemical evaluation include the test for Total Volatile Bases (TVB) or Total Volatile Nitrogen (TVN), Ammonia, Trimethylamine (TMA), Dimethylamine (DMA), Biogenic Amines (BA), Nucleotide catabolites-Hypoxanthine (Hx), Inosine (Ino) and K-value, Ethanol level, Rancidity:Peroxide value (PV), Thiobarbituric acid (TBA) and Free fatty Acids (Eyo, 2001).

23.1c Microbiological methods

This method is used to assess degradation of the tissue brought about by indigenous fish enzymes and by micro-organisms which are present on the surface of the skin, on the gills and in the intestines. The main microbe are bacteria. Also, the aim of microbiological examinations of fish products is to evaluate the possible presence of bacteria or organisms of public health significance and to give an impression of the hygienic quality of the fish. High microbial load in the skin and gills is associated with fast spoilage since bacteria can penetrate into the tissues of the fish and multiply causing putrefaction. The presence of organisms such as *Clostridium botulinum*, *Salmonellases* and *Vibrio parahaemolyticus* which are pathogenic to man, indicates that such fish may cause illness through infection or poisoning (Hobbs, 1987). Low microbial count (number) is not an indicator of good quality unless the types of microbes colonising the medium is known.

The number of specific spoilage bacteria in a fish determines its remaining shelf life (Eyo, 2001). However, bacteriological tests or examinations are laborious, time consuming, expensive, destructive and require skill in execution and interpretation of the results. It is therefore recommended that such analyses be limited in number and extent.

2.3.1d Physical methods

(i) **Electrical Properties:** The electrical properties of skin and tissue change after death and this has provided a means of measuring post-mortem changes or degree of spoilage (Clucas, 1982). The cell walls of dead fish become increasingly permeable due to enzymatic breakdown and gradually lose their capacitance.

Various electronic instruments such as interelectron, GR Torrymeter and recently RT freshness Grader have been developed for the estimation of freshness of fish. However, Torrymeter is widely used.

These devices measure the dielectric properties of fish flesh (Jason and Richards, 1975).

This is signified by a factor called Q that is calculated by the equation:

$$Q = 2\pi FCR.$$

Where:

F = Frequency of alternating current (AC) which is standardised

C = Capacitance = Ability to retain electrical charge

R = Impedance (AC resistance) or Resistance X Inductance

Q value depends on temperature and the instrument is compensated automatically for fish temperature.

(ii) **Acidity or Alkalinity (pH) and Redox Potential (Eh):** The determination of pH can give some indications of the condition of fish post-mortem. Measurements are carried out with a pH-meter by placing the electrodes (glass-calomel) either directly into the flesh or into a suspension of fish flesh in distilled water (Eyo, 2001).

Redox potential (Eh) of a system is the difference between the reduction potential of the reaction and a hydrogen electrode which by definition has a reduction potential of zero Volt (Huss, 1988). It has also been reported that Eh value of a fresh fish is always positive.

Also, when fish is judged organoleptically unacceptable, Eh decreases rapidly and stays at low negative values as the fish continues to spoil.

Furthermore, it has been observed that there is a relationship between Eh and TMAO (in marine fish) indicating that TMAO is the main factor inducing post-mortem changes in redox potential in the muscle tissues (Huss, 1988).

(iii) Measuring Texture: Texture is an important property of fish muscle, whether fresh or processed. Fish muscle may become tough as a result of freezing or soft and mushy as a result of autolytic degradation (Maas-van Berkel *et al*, 1996). Texture may be monitored organoleptically but there has been need for the development of a reliable objective rheological test which accurately reflect the subjective evaluation of well-trained panel of judges (Bostock *et al*, 1987). In line with this objective, several methods such as the ones evaluating the formaldehyde-induced toughening of frozen fish muscle, measuring hardness/softness of fish flesh, measuring the shear force of fish flesh and a rapid portable non-destructive penetrometer which measure both firmness and resilience have been developed (Colby *et al*, 1993).

2.3.2 Subjective or Sensory methods

This form of evaluation is the scientific discipline used to evoke, measure, analyse and interpret reactions to characteristics of fish as perceived through the senses of sight, smell, taste and touch. It is important because, in many ways, the quality requirement of consumers depend on their subjective analyses i.e by sensory evaluation of the products appearance, colour, odour, taste and texture, plus visual appeal of its packaging and presentation (Eyo, 2001).

Sensory characteristics can only be measured meaningfully by humans, but suffer from the limitation of being subject to bias of the assessor and hence may not be reproducible (Bostock *et al*, 1987). Therefore, advances are being made in the development of instruments that can measure individual quality changes. These include the Instron, Bohlin Rheometer (measures texture and other rheologic properties) and the Microscopic method combined with image analysis, is used to assess structural changes while the "artificial nose" is used to evaluate odour profile (Huss, 1988). Scientifically, the sensory process can be divided into three steps. Detection of a stimulus by the human sense organs, evaluation and interpretation by a mental process and the response of the assessor to the stimuli.

Sensory method can be objective (discriminative or descriptive) or subjective tests. The discriminative test investigates whether there is a difference; it includes the triangle test and ranking. Descriptive test probes what the difference is and include the quality index method, structured scaling and profiling while the subject test involve the market test (Connell, 1995). The commonest subjective (sensory) test is the organoleptic test which involve the constitution of trained panel of assessors; providing a tasting room devoid of distractions; preparing samples in the most uniform and un-adulterated form; presenting samples in a non-bias way and collating result in a manner which can be interpreted, (Bostock *et al*, 1987).

2.4 Fish Preservation Methods

These are the means of keeping fish in highest quality for consumption. Majority of the fish stock is sold either live or fresh while a small fraction is preserved by drying, salting, smoking or freezing.

Table 2: Methods of fish utilisation in Nigeria.

(A) **Artisanal sector**

Fish Production	Percentage (%)
Live fish	7
Fresh but unchilled	18
Smoke-dried	45
Sun-dried	20
Salted & sundried	10

(B) **Industrial sector**

Ice /chilled fish	80
Frozen fish	20

Source: Tobor, 1984.

Essentially, preservation techniques is divided into two major categories, low and high temperature techniques.

2.4.1 Low temperature techniques:

This is a means of preserving fish under a low temperature condition. Though in this part of the world electricity supply is epileptic and unreliable, making this techniques a rarely used means of preservation. However, low temperature preservation techniques includes chilling and freezing.

2.4.1a Chilling: This involves using ice as a cooling medium for fresh fish. Ice is portable and possesses a very large cooling capacity per unit volume. It may be in form of blocks, flakes, cubes or rolls. The amount of ice required for a particular weight of fish can be calculated using:

$$\text{Amount of ice required} = Q/q = M.s.t/q$$

Where Q is the thermal capacity (m.s.t), M the weight of fish, S the specific heat of fish, t the temperature difference and q the amount of heat required to melt 1kg of ice (latent heat of fusion of ice).

Refrigerated sea water (RSW) and chilled sea water (CSW) have also been used in chilling (Amos, 1981). Furthermore, biogas fuelled ice - making machines and compact solar refrigeration system have been developed to compensate for the cost and lack of electricity supply in most rural communities.

2.4.1b Freezing: Fish freezing is a method of fish preservation in which the product is brought into contact with refrigerated air or refrigerated surfaces in a compartment. As heat is removed from the fish, its temperature falls steadily until the fish begins to freeze. There are two major freezing techniques namely blast and plate freezing.

In blast freezing, the product is surrounded by refrigerated gas, usually air, at a temperature between - 30°C and - 40°C forced at an air velocity of 5m sec⁻¹ through the fish. The classic deep-freezer operates at an air temperature of about - 4°C to -12°C (Eyo, 2001).

Plate freezing involves the use of plate freezers in which contact is made between the fish and the two refrigerated metal plates. The air temperature is about - 40°C, which makes the plate freezer faster and more efficient than the air blast freezer.

Cold storage (temperature between -15°C and -28°C) follows freezing. If there is power failure, spoilage can still occur in cold room. This causes evaporation followed by precipitation with an accompanying dehydration. In all, freezing arrests fish deterioration faster than chilling and is commonly used in the industrial sector.

2.4.2 High temperature techniques: This preservation technique aim at reducing free-available water (a_w) from fish muscle. It involves traditional processes of salting, sun drying and smoking which preserve fish by reducing the water activity (a_w) and reduces the range/number of microbes that can attack and cause spoilage (Bostock *et al*, 1987).

Due to high adaptability, low energy costs and suitability for the tropical environment, high temperature preservation techniques constitute the most suitable at the rural level. Modification of these methods to save on cost will be the best for preservation and processing of fish at the subsistence level.

2.4.2a Fish Drying

Drying or dehydration is used to describe any process involving the removal of water from fish or fish product by evaporation. Apart from sun drying, the action of salt and application of pressure will remove water from fish. In Nigeria, fish drying is largely practiced in Northern part where the ambient temperature is high, ranging from 32°C - 33°C coupled with low relative humidity, about 10%-20% (Bostock *et al*, 1987). These environmental conditions enhance quick drying.

The process of drying fish involves the inward transfer of heat and the outward movement of water. The sequence of these process is illustrated below:

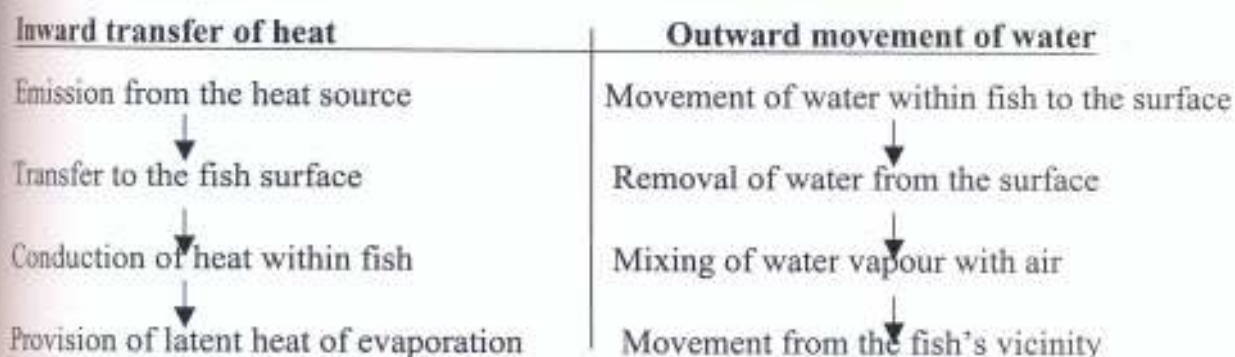


Figure 1: Flow chart of heat transfer and water movement

Bostock *et al*, 1987.

The thermal energy required to remove the water can be obtained from a variety of sources, e.g. the sun, controlled burning of oil, gas or wood; microwave electromagnetic radiation or ultrasonic heat. The rate of drying depends on temperature, the speed of air movement, relative humidity of the air, water content of the fish, size and fattiness (Eyo,2001). While the traditional method involves the action of sun and wind for effective evaporative drying, controlled artificial dehydration devices have been developed. These include the mechanical driers, freeze driers and solar driers.

2.4.2b Salting of fish

Common salt (Sodium chloride) as a preservative has been used globally for centuries in fish curing, fermentation and smoking (Eyo, 2001). Salt is not an antiseptic per se but acts as a preservative by reducing the water activity of fish tissues through osmosis. Salt extracts water from fish cells, retards activities of bacteria, enzymes and chemicals in fish. High salt concentration also decrease the solubility of oxygen thereby retarding fat oxidation (Maas-van Berkel *et al*, 1996). There are three common methods of salting fish. These are dry and kench salting, wet or pickle curing and brining (Poulter, 1980).

(i) **Dry and kench salting:** These methods of salting are used for low fat fish. The fish are beheaded, gutted, splitted and made ready for salting.

In dry salting, granulated salt is applied on the fish by rubbing or sprinkling over the surface of the fish before they are left in the sun to dry. The advantages of dry salting are that, it is a simple operation, with high rate of salt penetration and fast drying. However, the disadvantages are unattractive appearance of the product, uneven penetration of salt and fast fat oxidation due to the exposure of fish.

Kench salting involves the application of salt (40-50% by weight of fish) between layers of fish and the water extracted is allowed to drain away. By the third day, the salt would have penetrated into the flesh to saturate the residual water to give a "green" cure. The fish is then allowed to lie in that position referred to as "wet stack" for months. At frequent intervals, the fish are re-stacked, the top layers going to the bottom and vice versa; this produces an even cure. At the end of wet stacking, the moist content ranges between 53 and 58% while the salt content approximates 20%. The fish are grayish-white in colour and subsequently dried in drying chamber/solar drying on racks. After drying, the fish becomes white in colour with moisture content of 10-30% and salt content ranging from 25-35%.

(ii) **Wet salting or pickle curing:** This method is normally used for fatty fishes. The fish is beheaded, gutted and "roused" with salt. The fish are then layered alternately with dry salt crystals in a container such as a barrel or tank with lid, until it is full. The fish is then allowed to stay for 1 or 2 days when the fish shrink in volume and the space is re-filled with the same day's cure. The lid is put on and the fish is left for 8-10 days. By this time, the brine has become reddish-brown in colour due to the presence of blood in it called

"blood prickle", which eventually cover all the fish. It is said that the blood pickle contains anti-oxidants, which prevent the fish from going rancid. At this stage, the barrel is ready for storage possibly for months.

(iii) **Brining:** The fish to be brined is beheaded, gutted, splitted, and washed. A concentrated salt solution is made by stirring an excess salt into a container of clean water until no more salt would dissolve, making a saturated salt solution. This gives approximately 360g of salt per litre of water (Maas-van Berkel *et al*, 1996). The fish are left in the saturated brine for days and within 6 days, the fish and brine will reach an equilibrium salt content.

(Eyo, 2001). Brining has the advantages of uniform penetration of salt, better taste and appearance of fish and less spoilage from rancidity. The disadvantages are the extra labour needed to stir from time to time, more expensive than the dry method and could be a source of bacterial contamination if the same brine is re-used.

However, the factors affecting salt uptake include the purity and physical properties (fine/coarse grain) of salt temperature, fat content, thickness and fish quality.

Table 3: Quality storage life of dried fish with different water and salt contents

Water content % Weight	Salt Content (% Wet Weight)	Minimum good quality Storage life
40	5	half week
40	10	one week
40	15	three weeks
40	20	one & half months
30	5	half week
30	10	one & half months
30	15	two months
30	20	two months
20	5	three months
20	10	four weeks
20	15	four months
20	20	three & half months
15	5	more than 1 yr
15	10	more than 1 yr
15	15	more than 1 yr
15	20	more than 1 yr

Source: Poulter, 1980.

2.4.2c Fish smoking

Salt curing gives better result when combined with drying or smoking. The use of smoke from smouldering wood for the preservation of perishable foods dates back to civilization (Eyo, 2001). The bacteriostatic, antioxidative and dehydrative effect of smoking process were used inadvertently by the early processors in the preservation of fish. Smoking imparts desirable colour and flavour on fish. The obtained flavours depend both on the raw material used and the length of time the fish are smoked (Ihekoronye and Ngoddy, 1985). It was reported that in the 1970's, before the advent of drought most of the fishes caught in lake Chad were smoked (Shimang, 1990). Gnanadoss and Aderounmu (1982) reported that about 95% of the catches of the coastal fishermen are normally

smoked, the limited quantities which are sold fresh are mostly consumed within a range of 25km from the coast.

Smoking is a method of preserving fish which combines three effects:

- i. **Preservative value of smoke:** the smoke produced from burning wood contains a large number of compounds, some of which kill bacteria e.g phenols.
- ii. **Drying:** The fire which produces the smoke also generates heat and this dries the fish.
- iii. **Cooking:** If the fish are smoked at a high temperature, the flesh is cooked and this destroys enzymes and kill bacteria.

There are two main types of smoking processes:

- (a) **Cold smoking:** The temperature of the smoke does not exceed 30°C and
- (b) **Hot smoking:** the temperature varies between 65 and 100°C

A. Cold Smoking: This is practiced in places where alternative means of preserving the fish such as canning or freezing/refrigeration are available. The method is geared at improving the flavour of the fish, while retaining its nutritive value. During cold smoking, the fish is not cooked, has shorter shelf life and easily infected with micro-organism, if not properly stored. Moisture retention is high in the order of 35-45% (Eyo, 2001) it is mostly practiced in technologically developed countries.

B. Hot Smoking: This is the traditional method of fish smoking in tropical countries. During hot smoking, the fish is cooked in the process. This method extends the shelf life, even as alternative preservation methods are unreliable (Bostock *et al*, 1987). In order to produce a product of low moisture content (< 20%) which is safe from mould and bacteria

infestation during short term storage at ambient tropical temperature, the fish is dried (smoke-dried).

Hardwood is preferred for fish smoking (Eyo, 2001). Hardwood yields more acids as a result of higher hemicelluloses content which produce lower pH. This gives the smoke from it greater preservative power and a more stable, product. Also, over 300 chemical compounds have been detected in wood smoke and liquid smoke preparations. They include more than 45 phenols, 70 carbonyls, 20 acids, 11 furans, 13 alcohols and esters, 13 lactones and 27 polynuclear aromatic hydrocarbons (Hamm, 1977). These compounds leads to production of flavour, colour, antioxidative, bacteriostatic and preservative properties of smoked fish. Furthermore, absorption of smoke by fish tissues is affected by the type of wood, wood combustion temperature, smoke house parameters – type of smoking kiln/method and temperature, smoke density and relative humidity (Eyo, 2001).

Fish smoking is practiced in tropical countries in smoke house and smoking oven or kilns. The smoking equipment has been classified into traditional, improved traditional and mechanical types (Eyo, 1993). Traditional ones include the smoke house, pit oven, mud and drum kiln. The improved traditional kilns include modified attona or watanable kiln, chorkor oven, Magbon-Alade and Kainji Gas Kiln, while an example of the mechanical kilns is the Torry smoking kiln.

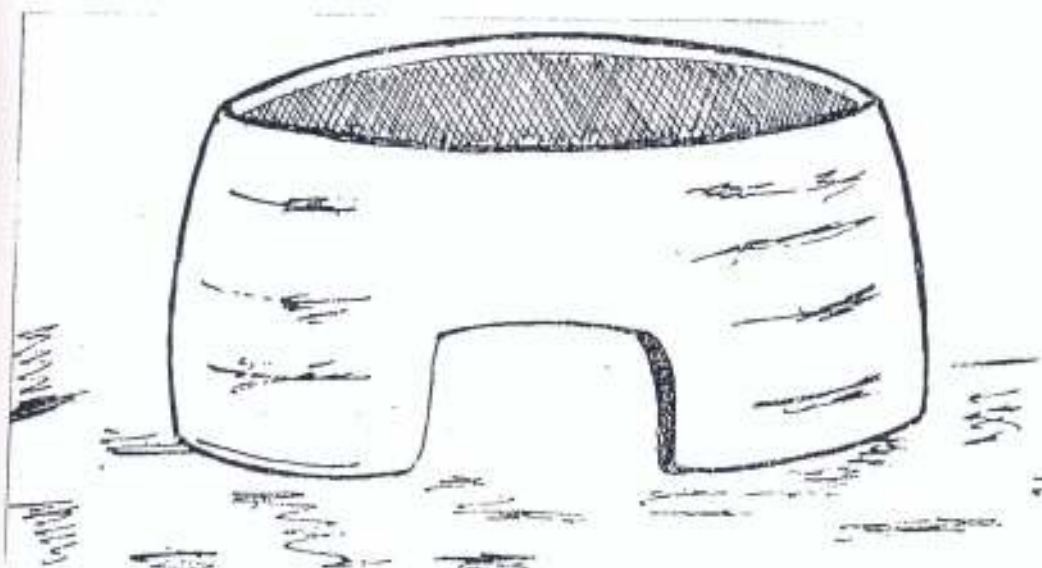


Fig:2 Traditional Conical Mud Kiln



Figure3 : Traditional drum kiln.

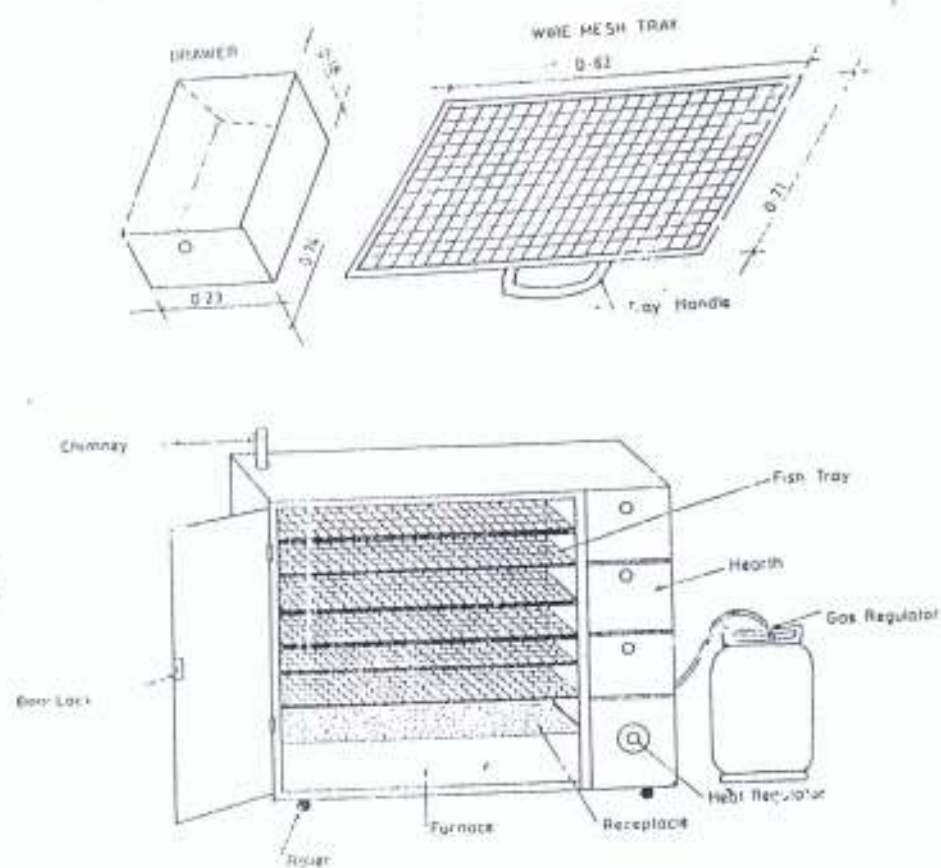


Fig:4 KAINJI GAS KILN

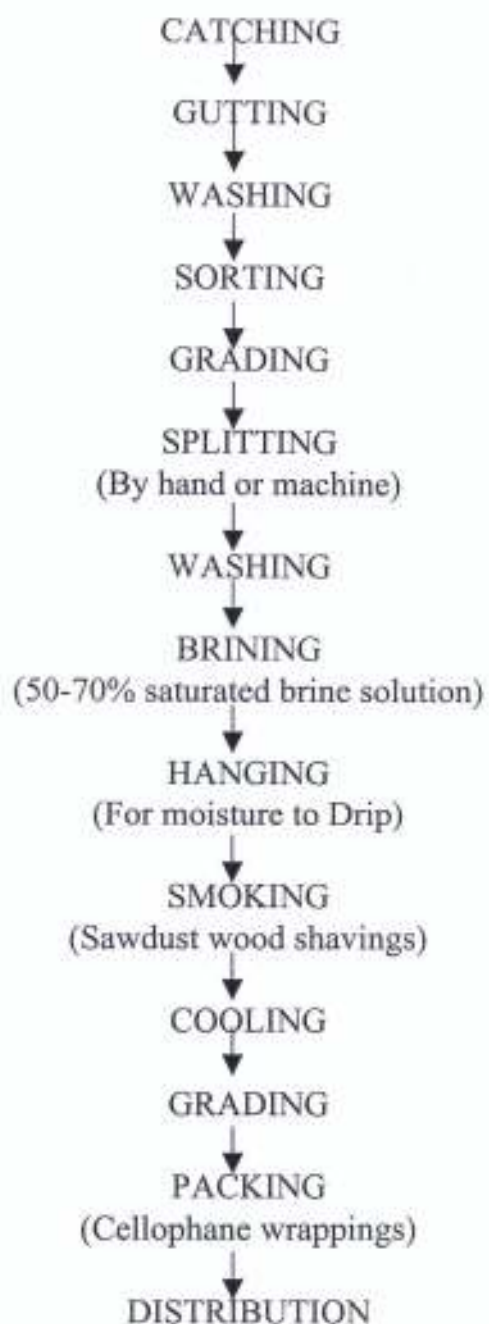


Fig. 5: Flow Chart of the Modern Smoking Process

Source: Eyo, 2001.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Fish Samples and their Preparation

Five fresh fish species were purchased in Akure from a fishmonger.

The different fish species are *Heterotis niloticus* (Cuvier, 1829), *Labeo coubie* (Ruppell, 1832), *Parachanna obscura* (Gunther, 1861), *Oreochromis niloticus* (Linne, 1758) and *Clarias gariepinus* (Burchell, 1822). The choice of these fishes was based on their availability and commercial importance in Akure.

Their corresponding family, scientific and common names are presented in the Table 4.:

Table 4: Fish species used

Family Name	Scientific Name	Common Name
Osteoglossidae	<i>Heterotis niloticus</i>	Bony tongue
Cypinidae	<i>Labeo coubie</i>	African carp
Channidae	<i>Parachanna obscura</i>	Snakehead
Cichlidae	<i>Oreochromis niloticus</i>	Tilapia
Clariidae	<i>Clarias gariepinus</i>	African catfish

The fish were gutted, scales removed, washed, cut into chunks (50 g) and soaked in a 7.5% brine solution (75 grams Sodium chloride in 1 litre of water), drained and placed on a wire guaze. Smoking was done using red-hot charcoal obtained from Gmelina (*Gmelina aborea*) wood. The fish chunks were turned at intervals and smoking was done 6 hours daily for 2 days to an average moisture content of $10.41 \pm 0.02\%$. Smoke-dried fish samples

were packed into polythene bags and kept in covered baskets under ambient conditions in the laboratory.

Physical and chemical evaluations were carried out on the fish species at the beginning of the experiment (week 0) and weekly thereafter. Storage and assessment were carried out over a 56-day period.

3.2 Parameters Assessed

3.2.1 Physical changes:- The following physical attributes of the smoked fish were assessed for changes during the storage period:

- Colour
- Fragments
- Odour
- Texture
- Taste

3.2.2 Proximate composition:- Changes in proximate constituents of the fish were based on the following:

- Moisture content
- Ash content
- Crude protein
- Fat content

3.2.3 Chemical changes:- The following parameters were determined for the chemical changes in the smoked fish:

- pH level
- Total Volatile Nitrogen (TVN)
- Peroxide Value (PV)
- Free Fatty Acid (FFA)

3.2.1 Physical Assessment

Five men panel was constituted and they assessed the smoke-dried fish species based on a structured scoring system (hedonic scale).

To prevent bias on the part of the judges, the fish species were arbitrarily coded as follows:

<i>Heterotis niloticus</i>	-	Sample B
<i>Labeo coubie</i>	-	Sample E
<i>Parachanna obscura</i>	-	Sample N
<i>Oreochromis niloticus</i>	-	Sample K
<i>Clarias gariepinus</i>	-	Sample Z

- (a) Colour was observed as either attractive brown/golden appearance or dark/charred colour
- (b) Taste was adjudged either sweet/salty or sour/bitter
- (c) Fragments: This was based on whether there are cracks or no cracks.
- (d) Odour i.e. pleasant or offensive
- (e) Texture i.e. hard/firm or soft/flabby

Procedure for organoleptic assessment was strictly followed with reference to Bostock *et al* (1987) and Eyo (2001). A copy of the questionnaire used is presented as Appendix 1.

3.2.2 Proximate Analysis

Proximate analyses were carried out at the initial stage after smoking and weekly for 56 days. The moisture, ash, lipid and protein content were determined as follows:

i. Moisture Content

5 grams of smoke-dried fish sample was grinded and oven-dried at 105⁰C. The weights of the samples were checked at intervals for 24 hours until a constant weight was obtained for the individual samples. Samples were cooled in a dessicator and re-weighed.

The difference between the initial and final weight gave the moisture content; expressed in percentage (%).

$$\% \text{ Moisture content} = \frac{\text{weight loss}}{\text{weight of sample}} \times \frac{100}{1}$$

ii. Ash Content

2 grams each of fish samples obtained from moisture determination were put in a muffle furnace at 600°C, until the ash was grey or near white in colour. This took 6 hours. The ash was cooled in a dessicator and weighed (A.O.A.C. 1990).

$$\% \text{ Ash content} = \frac{\text{weight of Ash}}{\text{Weight of sample}} \times \frac{100}{1}$$

iii. Lipid Content

The lipid content was determined by subjecting 5 grams of powdered fish samples to a continuous extraction with petroleum ether using soxhlet apparatus (A.O.A.C. 1990). Petroleum ether was left to evaporate at the end of extraction by placing the flask on the table and the oil residue represented the lipid content.

iv. Crude Protein

The percentage crude protein was determined from the nitrogen content of the smoke-dried fish samples using microkjedahl distillation method (A.O.A.C. 1990). 2 grams each of powdered fish samples was boiled with Tetraoxosulphate (VI) acid, which converted the protein to Ammonium sulphate. Selenium catalyst was used for digestion. Equal amount of Sodium hydroxide was added to neutralize the acid; it digested the nitrogen and separated the ammonium ion. The product was then titrated with standard Hydrochloric acid and the resulting percentage value multiplied with a factor of 6.25.

$$\% \text{ Total Nitrogen} = \frac{\text{Vol. of HCl} \times \text{Molarity} \times 0.0014 \times \text{Dilution factor} \times 100}{\text{Weight of fish sample}}$$

$$\text{Crude Protein} = \% \text{ Nitrogen} \times 6.25$$

3.2.3 Chemical Analyses

i. pH level

A pH meter (Jenway, 3015 model) was used weekly to assess the pH of each fish sample. A solution of 5 grams of each fish sample was made before analyses. The meter was standardized with standard solutions of pH 4 and pH 7; rinsed with distilled water and wiped with tissue paper before each test.

ii. Total Volatile Nitrogen (TVN)

This was determined using the method described by Pearson (1982). A kjedahl distillation apparatus with a 500ml receiving flask was set up. 10 grams of well grinded fish sample was weighed. Then, 100ml distilled water was added and transferred into the distilling flask with 200ml water. 2 grams of magnesium oxide, an antifoaming agent (alcohol), 25ml of 2% boric acid and few drops of methyl red indicator were added to the receiving flask. The mixture was heated to boil in 10minutes and distilled for 25 minutes. The distillate was titrated with 0.1M Tetraoxosulphate (VI) acid. A blank experiment (water) was also carried out.

$$\text{TVN} = \frac{(\text{ml of Acid} - \text{ml of Blank}) \times \text{Molarity} \times 14 \times 100}{\text{Grams of fish sample}}$$



iii. Peroxide Value (PV)

2 grams of fish oil was put in a boiling tube. This was determined using the method described by Pearson (1982). An addition of 1 gram of Potassium iodide and 20ml of solvent mixture (2 glacial acetic acid:1 chloroform) was made.

The boiling tube was placed in boiling water bath for 30 seconds and poured into a flask containing 20ml of 5% Potassium iodide solution; then washed with 25ml water. 0.002M Sodium thiosulphate solution was used to titrate the mixture using 0.5ml starch indicator. A blank experiment (water) was performed at the same time.

Peroxide value was calculated as :

$$\therefore PV = \frac{1000 (V-X)M}{W} \text{ (meq/kg)}$$

Where
V = Vol. of Na_2SO_4 used for titration
X = Vol. of Na_2SO_4 used for the blank
M = Molarity of Sodium thiosulphate
W = weight of fish oil used.

iv. Free Fatty Acid (FFA)

It was determined on the oil extracted from the fish samples using the method described by Pearson (1982). Oil residue from the fat content analysis was used and the weight of the fish oil extracted recorded. 50ml of 95% ethanol was placed in the conical flask and heated to boiling point. Few drops of phenolphthalein indicator were added and then neutralized using Caustic soda (0.1M NaOH).

Then, the neutralized solvent was poured into the flask containing the oil and the flask re-warmed to boiling point. The free fatty acid was then titrated with the 0.1M NaOH until the first permanent pink colouration was obtained.

$$\% \text{FFA (calculated as oleic acid)} = \frac{\text{Vol. of NaOH} \times 282 \times 0.1}{\text{Weight of fish oil}} \times 10$$

Data Analysis

Analysis of Variance (ANOVA) was carried out on all the physical and chemical parameters measured to test for variability at 5% level of significance. Duncan Multiple Range Test (DMRT) was used to separate means. Statistical Package for Social Science (SPSS version 10.00) was used.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Physical Assessment

The result of the physical assessment as it relates to taste, odour, colour, texture and fragmentation is presented in Table 5. There were significant differences ($P < 0.05$) in the values recorded for the species as the weeks progress. However, there was no significant difference ($P > 0.05$) in the odour of the smoked fish.

There was no significant difference ($P > 0.05$) in the taste values for *Heterotis niloticus* and *Oreochromis niloticus*. However, the best taste was recorded for *O. niloticus* (3.60), followed by *H. niloticus* (3.53) *C. gariepinus* (3.49), *P. obscura* (3.29) and lastly *L. coubie* (3.16). There was no significant difference ($P > 0.05$) in the odour of the five species. However, on weekly basis, values recorded for odour gradually decreased.

Assessment of colour revealed significant changes ($P < 0.05$), for all species. Colour changes occurred on the smoked fish with increasing period of storage. Overall, *O. niloticus* had the best colour appearance while the least mean value was recorded for *L. coubie*. The physical assessment of texture showed that *P. obscura* was the most firm. There was no significant difference ($P > 0.05$) in the mean values for *H. niloticus*, *O. niloticus* and *C. gariepinus* while the least mean value was recorded for *L. coubie*. The highest value of fragmentation was recorded for *H. niloticus*, while there was no significant difference ($P > 0.05$) in values obtained for *L. coubie*, *P. obscura* and *C. gariepinus*.

Table 5: Physical (Organoleptic) Assessment of the smoked fish species during storage.

Quality Parameters	Fish Species	Length of Storage (Weeks)									
		0	1	2	3	4	5	6	7	8	Means
Taste	<i>H. niloticus</i>	4.60±0.55	4.40±0.55	4.20±0.45	3.80±0.55	3.60±0.55	3.40±0.55	2.80±0.45	2.60±0.45	2.40±0.71	3.53±0.89 ^a
	<i>L. coubie</i>	4.40±0.55	4.20±0.45	4.00±0.71	3.60±0.45	3.40±0.55	2.80±0.55	2.60±0.55	2.00±0.55	1.40±0.71	3.16±1.09 ^c
	<i>P. obscura</i>	4.60±0.55	4.20±0.45	4.00±0.71	3.80±0.45	3.40±0.55	2.80±0.55	2.60±0.45	2.40±0.71	1.80±0.71	3.29±1.01 ^{bc}
	<i>O. niloticus</i>	4.80±0.45	4.60±0.55	4.00±0.45	3.80±0.45	3.60±0.55	3.20±0.45	3.00±0.55	2.80±0.84	2.60±0.55	3.60±0.84 ^a
	<i>C. gariepinus</i>	5.00±0.45	4.80±0.55	4.20±0.45	3.80±0.71	3.60±0.45	3.40±0.55	2.80±0.55	2.20±0.55	1.60±0.84	3.49±1.16 ^{ab}
Odour	<i>H. niloticus</i>	4.60±0.55	4.40±0.55	4.40±0.55	4.00±0.55	3.60±0.71	3.60±0.55	3.00±0.55	2.60±0.55	2.40±0.55	3.62±0.94 ^a
	<i>L. coubie</i>	4.80±0.45	4.40±0.55	4.00±0.71	3.60±0.55	3.40±0.55	3.20±0.45	2.80±0.55	2.40±0.55	1.80±0.84	3.38±1.05 ^a
	<i>P. obscura</i>	5.00±0.45	4.40±0.55	4.00±0.71	3.80±0.71	3.60±0.45	3.40±0.55	2.80±0.45	2.60±0.55	2.00±0.71	3.51±1.01 ^a
	<i>O. niloticus</i>	4.60±0.55	4.40±0.55	4.00±0.71	3.80±0.45	3.60±0.55	3.40±0.45	2.80±0.55	2.40±0.55	2.20±0.45	3.47±0.94 ^a
	<i>C. gariepinus</i>	4.80±0.55	4.40±0.55	3.80±0.45	3.60±0.55	3.40±0.55	3.20±0.55	2.80±0.55	2.20±0.45	2.00±0.84	3.36±1.00 ^b
Colour	<i>H. niloticus</i>	4.60±0.55	4.40±0.55	4.20±0.55	3.60±0.84	3.40±0.84	3.20±0.71	3.00±0.71	2.60±0.55	2.40±0.55	3.49±0.89 ^{bc}
	<i>L. coubie</i>	4.40±0.55	4.00±0.89	3.80±0.55	3.40±0.71	3.20±0.55	3.00±0.71	2.80±0.84	2.00±1.30	1.80±0.84	3.13±1.01 ^d
	<i>P. obscura</i>	4.20±0.84	4.00±0.55	3.80±0.55	3.60±0.55	3.40±0.71	3.00±0.71	2.80±0.84	2.60±0.71	2.20±1.14	3.29±0.89 ^{cd}
	<i>O. niloticus</i>	4.60±0.55	4.40±0.55	4.00±0.55	4.00±0.71	3.80±0.55	3.60±0.71	3.40±0.84	3.00±0.84	3.00±0.55	3.76±0.74 ^a
	<i>C. gariepinus</i>	4.80±0.45	4.60±0.55	4.40±0.55	3.80±0.55	3.60±0.55	3.40±0.55	3.00±0.84	2.80±0.71	2.00±0.84	3.60±1.01 ^{ab}
Texture	<i>H. niloticus</i>	4.80±0.45	4.60±0.55	3.80±0.45	3.80±0.71	3.60±0.55	3.40±0.55	3.20±0.55	3.00±0.55	2.60±0.45	3.64±0.80 ^b
	<i>L. coubie</i>	4.40±0.45	4.00±0.55	3.80±0.45	3.40±0.55	3.20±0.55	3.00±0.55	2.60±0.55	2.40±0.55	2.00±0.45	3.24±0.91 ^c
	<i>P. obscura</i>	5.00±0.55	4.80±0.55	4.60±0.45	3.80±0.55	3.60±0.55	3.40±0.45	3.40±0.89	3.20±0.45	3.00±0.55	3.87±0.81 ^a
	<i>O. niloticus</i>	4.80±0.45	4.60±0.55	3.80±0.55	3.60±0.45	3.40±0.55	3.20±0.55	3.00±0.55	3.00±0.55	2.80±0.45	3.58±0.78 ^b
	<i>C. gariepinus</i>	4.60±0.45	4.40±0.45	4.20±0.45	4.00±0.55	3.60±0.55	3.20±0.55	2.80±0.89	2.60±0.55	2.20±0.45	3.51±0.92 ^b
Fragment	<i>H. niloticus</i>	4.60±0.55	4.60±0.55	4.40±0.84	4.20±0.71	3.80±0.55	3.40±0.71	2.80±0.55	2.60±0.55	2.40±0.55	3.64±0.96 ^a
	<i>L. coubie</i>	4.80±0.45	4.40±0.45	3.80±0.55	3.60±0.55	3.60±0.55	3.40±0.55	2.80±0.55	2.60±0.71	2.40±0.55	3.49±0.89 ^{ab}
	<i>P. obscura</i>	4.60±0.55	4.40±0.55	4.20±0.55	3.60±0.55	3.40±0.55	3.20±0.55	3.00±0.45	2.60±0.84	2.20±0.84	3.47±0.92 ^{ab}
	<i>O. niloticus</i>	4.80±0.45	4.60±0.55	4.40±0.55	3.60±0.55	3.40±0.55	3.00±0.45	2.80±0.45	2.60±0.55	1.60±0.84	3.40±1.10 ^b
	<i>C. gariepinus</i>	4.60±0.55	4.60±0.55	4.00±0.71	3.80±0.84	3.40±0.55	3.20±0.45	3.00±0.55	2.80±0.55	2.60±0.45	3.56±0.87 ^{ab}

- Means of replicate values with standard deviation
- Means with different superscripts differs significantly ($P < 0.05$).

4.2 Proximate Assessment

The result of the changes in the proximate composition (Initial and final) of the stored smoked fish species is presented in Table 6.

Moisture content of the fish species showed appreciable difference between week 0 (initial) and week 8 (final). In each species, moisture continued to increase weekly. *O. niloticus* gained the highest percentage of moisture (2.02%), followed by *H. niloticus*, (1.83%), *C. gariepinus* (1.64%), *P. obscura* (1.63%), and lastly *L. coubie* (1.54%).

The crude protein decreased with length of storage. In between week 0 and week 8, there were percentage losses in the protein content, for all the species. The loss was highest in *H. niloticus* (5.18%) while the least was in *P. obscura* (3.09%).

There was a gradual decline in lipid content of the smoked fish with increasing length of storage. *C. gariepinus* had the highest loss (6.32%), followed by that of *P. obscura* (5.43%), *L. coubie* (5.03%), *O. niloticus* (4.36%) and *H. niloticus* (3.37%).

The percentage difference between values for week 0 and week 8 revealed increase in the ash content for all the species. The highest percentage difference was recorded for *H. niloticus*, then *C. gariepinus*, *O. niloticus*, *L. coubie* and *P. obscura* in descending order.

Table 6: Mean Proximate composition (%) of stored smoked fish species

Proximate Parameters	Fish Species	Length of Storage (Weeks)								
		0	1	2	3	4	5	6	7	8
Moisture content	<i>H.niloticus</i>	10.40±0.14	10.42±0.01	10.44±0.03	10.46±0.03	10.47±0.01	10.48±0.09	10.49±0.03	10.52±0.03	10.59±0.06
	<i>L. coubie</i>	10.41±0.03	10.45±0.06	10.48±0.01	10.49±0.04	10.52±0.03	10.54±0.03	10.55±0.07	10.57±0.04	10.58±0.01
	<i>P.obscura</i>	10.42±0.04	10.46±0.07	10.47±0.03	10.48±0.06	10.50±0.03	10.53±0.01	10.55±0.06	10.56±0.06	10.58±0.09
	<i>O. niloticus</i>	10.41±0.01	10.47±0.04	10.49±0.03	10.51±0.03	10.54±0.07	10.55±0.07	10.56±0.04	10.59±0.01	10.62±0.02
	<i>C.garipinus</i>	10.39±0.06	10.41±0.09	10.43±0.03	10.46±0.06	10.47±0.07	10.49±0.04	10.51±0.04	10.53±0.03	10.56±0.01
Crude Protein	<i>H.niloticus</i>	72.78±0.03	72.75±0.04	72.72±0.03	72.70±0.01	72.68±0.13	72.63±0.04	70.78±0.03	69.11±0.01	69.01±0.03
	<i>L. coubie</i>	58.95±0.07	58.93±0.04	58.87±0.03	58.84±0.06	58.81±0.03	58.77±0.01	56.95±0.04	56.42±0.03	56.87±0.07
	<i>P.obscura</i>	54.71±0.03	54.68±0.03	54.64±0.01	54.61±0.04	54.57±0.03	54.55±0.01	53.71±0.04	53.26±0.03	53.02±0.01
	<i>O. niloticus</i>	69.24±0.01	69.12±0.18	69.10±0.17	69.18±0.03	69.12±0.01	69.11±0.03	67.24±0.01	66.90±0.06	66.35±0.03
	<i>C.garipinus</i>	56.82±0.03	56.80±0.04	56.79±0.04	56.73±0.04	56.69±0.03	56.66±0.03	55.91±0.06	55.43±0.03	54.74±0.03
Fat content	<i>H.niloticus</i>	11.32±0.03	11.26±0.03	11.20±0.01	11.16±0.04	11.11±0.03	11.09±0.04	11.04±0.03	10.99±0.01	10.94±0.03
	<i>L. coubie</i>	16.12±0.04	15.90±0.03	15.84±0.01	15.83±0.04	15.80±0.03	15.78±0.01	15.71±0.03	15.50±0.03	15.31±0.06
	<i>P.obscura</i>	16.58±0.01	16.51±0.03	16.46±0.04	16.41±0.01	16.35±0.04	16.33±0.03	16.29±0.01	16.14±0.03	15.68±0.03
	<i>O. niloticus</i>	14.22±0.01	14.19±0.04	14.16±0.03	14.10±0.06	14.03±0.04	13.92±0.03	13.78±0.04	13.71±0.03	13.60±0.04
	<i>C.garipinus</i>	20.24±0.06	20.20±0.04	20.17±0.01	20.11±0.03	20.08±0.07	20.01±0.03	19.63±0.03	19.39±0.04	18.96±0.04
Ash content	<i>H.niloticus</i>	8.50±0.07	8.52±0.01	8.54±0.04	8.57±0.10	8.60±0.03	8.62±0.04	8.65±0.03	8.68±0.10	8.72±0.03
	<i>L. coubie</i>	9.53±0.04	9.56±0.06	9.57±0.01	9.59±0.04	9.62±0.07	9.65±0.06	9.69±0.03	9.71±0.11	9.74±0.04
	<i>P.obscura</i>	8.31±0.01	8.35±0.03	8.37±0.06	8.39±0.04	8.41±0.01	8.43±0.03	8.44±0.07	8.47±0.06	8.49±0.01
	<i>O. niloticus</i>	9.14±0.06	9.18±0.01	9.23±0.03	9.26±0.07	9.29±0.04	9.32±0.04	9.33±0.01	9.35±0.06	9.36±0.06
	<i>C.garipinus</i>	8.54±0.03	8.60±0.04	8.62±0.04	8.65±0.06	8.66±0.03	8.68±0.04	8.70±0.06	8.72±0.04	8.75±0.06

• Means of replicate values with standard deviation

4.3 Chemical Assessment

The chemical characteristics of the five fish species stored under ambient conditions are presented in figures 6-9. The parameters i.e pH, TVN, PV and FFA were measured weekly and comparison was made between the initial (WK0) and the final (Wk 8).

(a) pH

Generally, the five species showed a drop in their pH values from initial nearly neutral values to a more acidic values. The pH for *Heterotis niloticus* dropped from 6.45 in week 0 to 5.98 in week 8. *Labeo coubie* dropped from 6.38 to 5.94; *Parachanna obscura*, 6.12 to 5.35; *Oreochromis niloticus*, 6.47 to 6.09 and *clarias gariepinus*, 6.21 to 5.75.

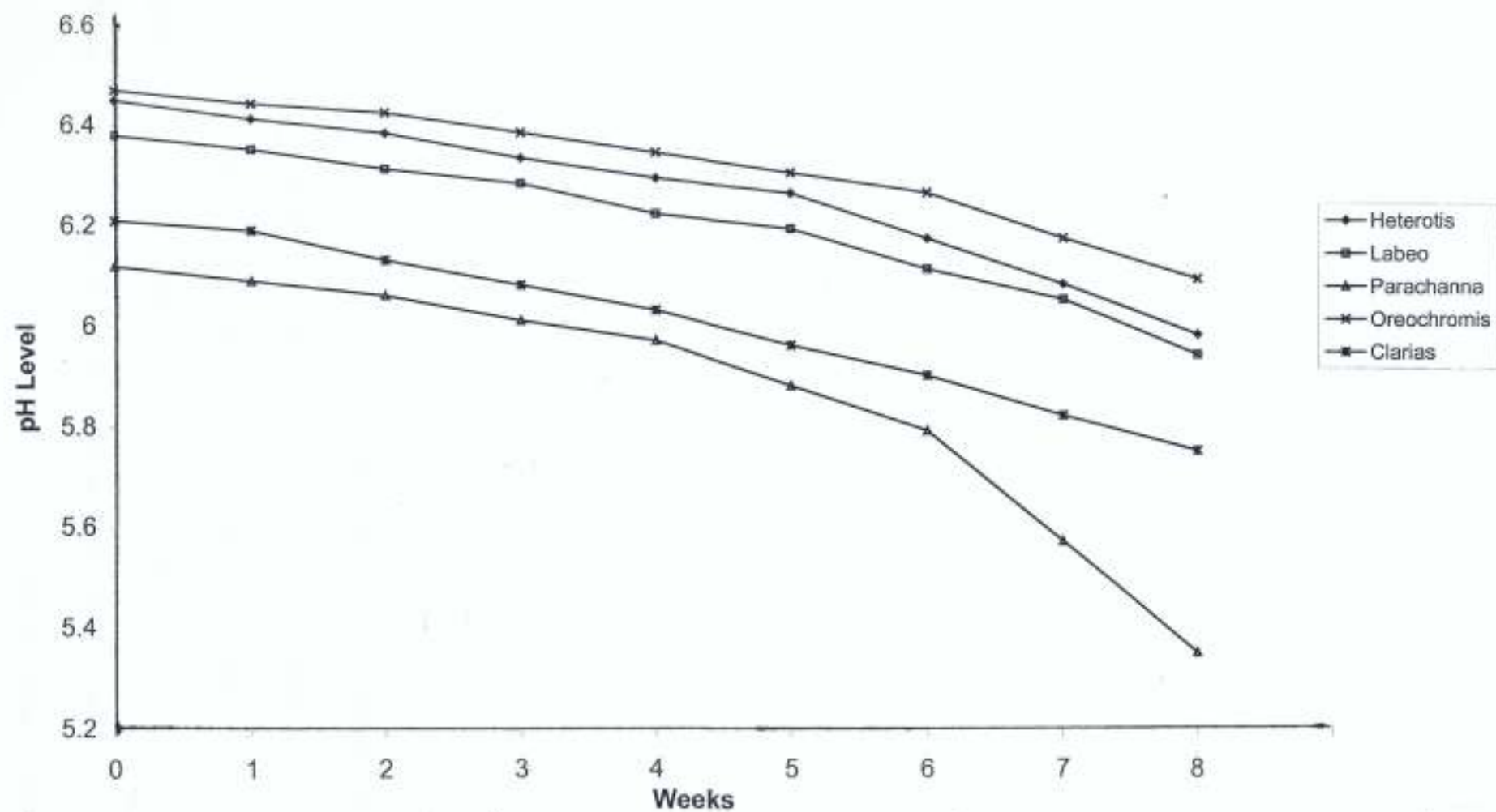


Fig.6: Weekly Changes in pH level of the smoked fish species during storage

(b) **Total Volatile Nitrogen**

There was an increase in TVN values for all the species with increasing length of storage. *Heterotis niloticus*, rose from 7.10mgN/100gm in week 0 to 21.03mgN/100gm in week 8. TVN for *Labeo coubie* rose from 6.40 to 20.08mg N/100gm; *Parachanna obscura*, from 4.91 to 19.12mgN/100gm; *Oreochromis niloticus*, from 6.88 to 20.29mg N/100gm and *Clarias gariepinus*, 5.20 to 20.01mgN/100gm.

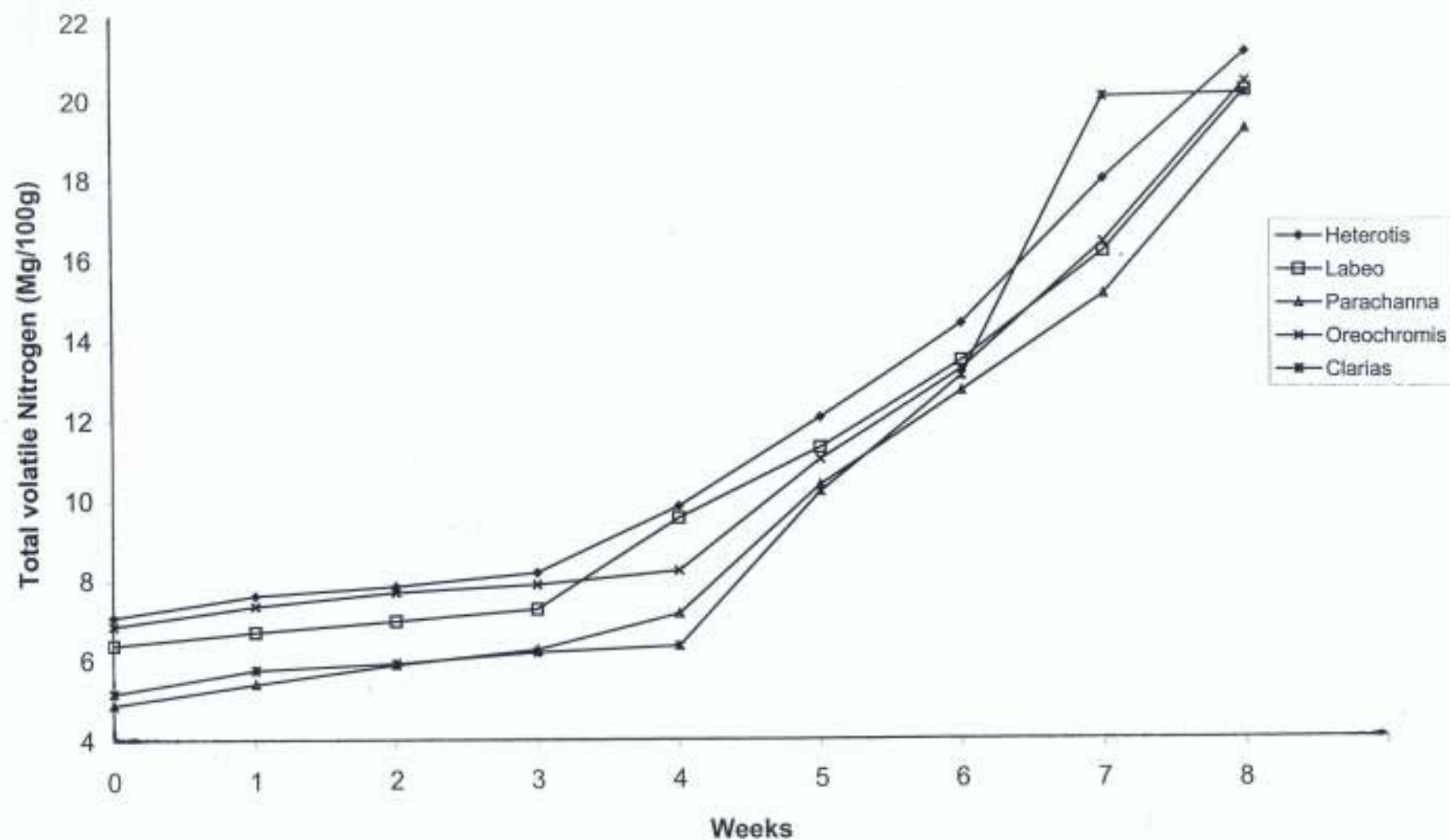


Fig.7: Weekly Changes in TVN of the smoked fish species during storage

(c) **Peroxide Value**

The Peroxide value increased for the five species. For *Heterotis niloticus*, PV increased from 2.41ml/kg of sample in week 0 to 21.27 ml/kg in week 8; *Labeo coubie*, from 3.95 to 24.09ml per kg; *Parachanna obscura*, from 6.20 to 25.98ml per kg; *Oreochromis niloticus*, from 4.98 to 21.75ml per kg and *Clarias gariepinus*, from 6.62 to 27.66 ml per kg of fish sample.

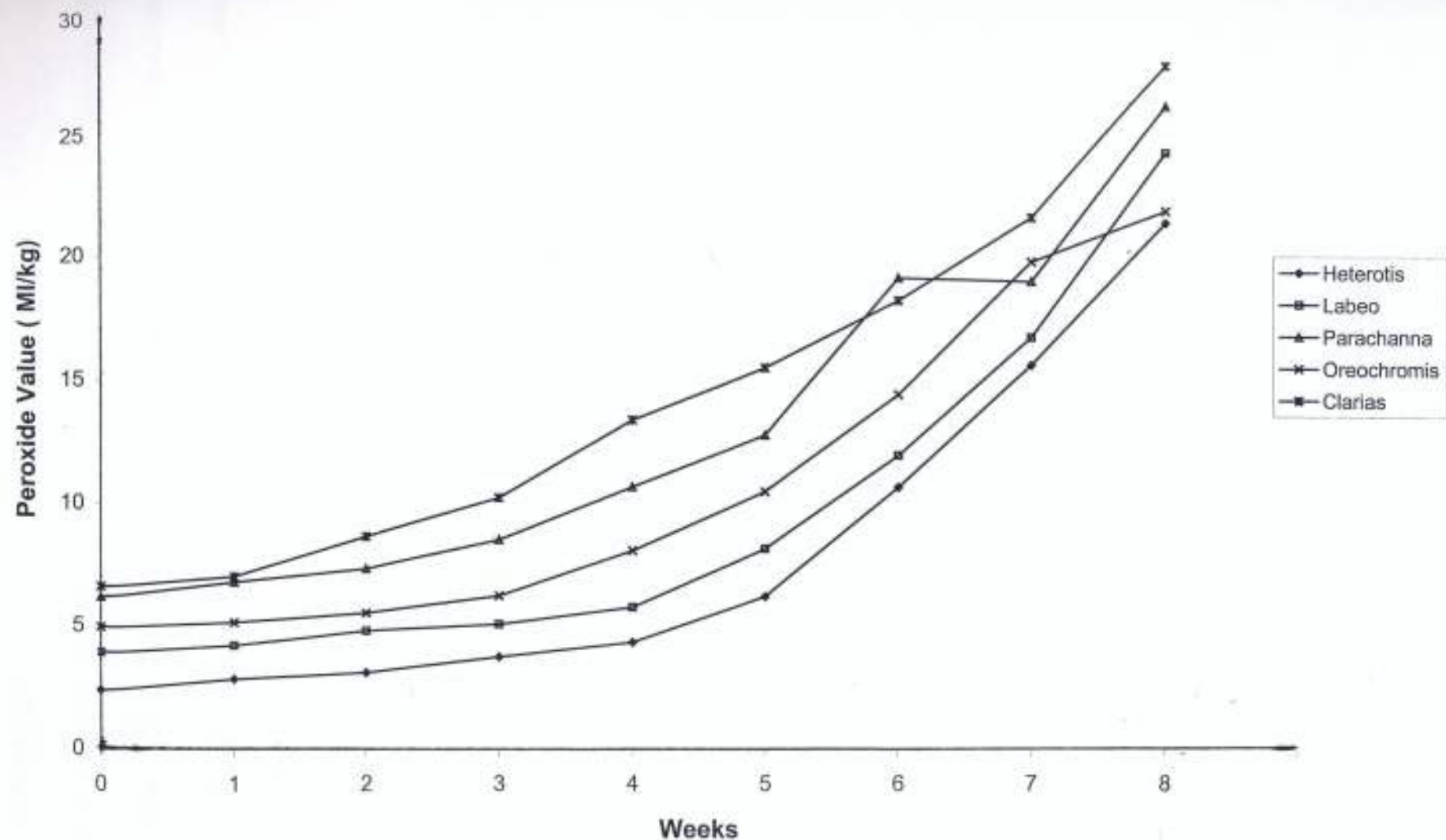


Fig.8: Weekly Changes in Peroxide values of the smoked fish species during storage

(d) Free Fatty Acid

The percentage free fatty acids increased for all the species. *Heterotis niloticus* increased from 0.17 to 0.91%; *Labeo coubie* ,from 0.27 to 1.52%; *Parachanna obscura*, from 0.42 to 1.74%; *Oreochromis niloticus*, from 0.19 to 1.02% and *Clarias gariepinus*, from 0.41 to 1.96%.

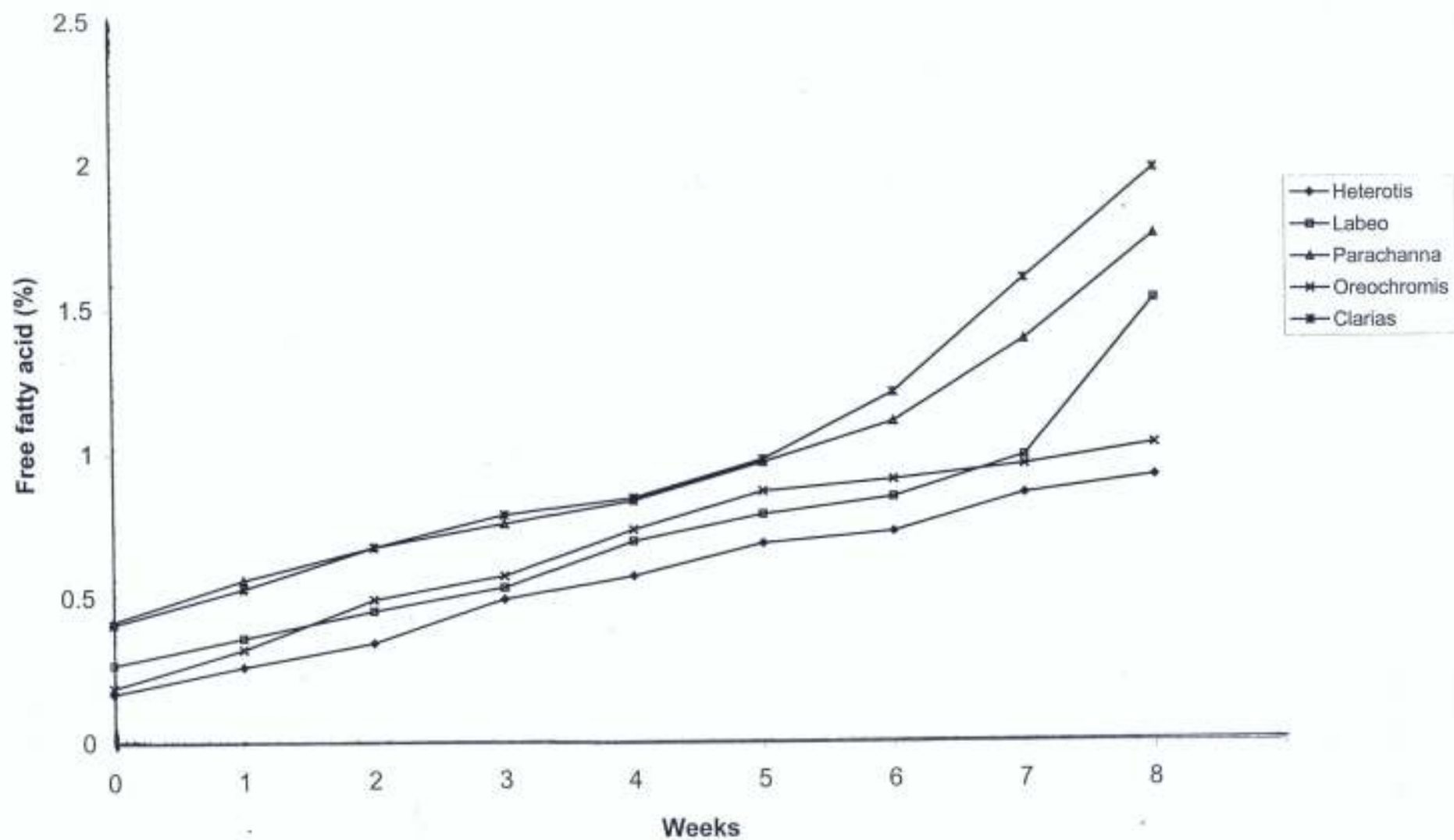


Fig. 9: Weekly Changes in Free fatty acid of the smoked fish species during storage

4.4 DISCUSSION

The shelf life of processed fish e.g smoked is an important problem facing processors. In this study, the five smoked fish species savoured well and looked stable till the 5th week (35 days of storage). Thereafter, degradation in terms of the physical and chemical characteristics set in. This has been attributed to different factors. According to Horner (1992), the shelf life of smoked fish during storage depends on the level of water activity and uptake of bactericidal and antioxidant components of wood smoke. Bernaseek (1991) found out that the shelf life of the smoked fish depends more on the cooking and the state of dryness of the fish than the smoke itself. Also, brining and prolonged smoking has been found useful in increasing the storage life of hot smoked fish by retarding the development of mould growth without increasing the development of lipid oxidation (Stround, 1988). In this study, the fish species were brined in a 7.5% salt solution, as recommended by Maas-van Berkel *et al* (1996) ,while smoking was carried out 6 hours daily for 2 days.

The production hygiene and packaging materials are also important to shelf life (Sveinsdottir, 1998). In this case, proper hygiene was maintained during processing while the smoked fish were packed in polythene bags and stored in covered perforated-plastic baskets. Furthermore, the smoked fish were stored under ambient conditions, without further drying. This could have contributed to the spoilage as the shelf-life is reported to be affected by storage temperature (Njai, 2000).

The effect of the wood type used in smoking cannot be overlooked. According to researchers, different wood types impart different colour and flavour on fish (Eyo, 2001). In this study, Gmelina (*Gmelina arborea*) a hardwood was used. It burns producing heat

which smoke-dried the fish. Hardwoods are found to be more preferable for smoke generation than softwoods because they yield more acid as a result of their hemicellulose content and may thus give products with lower pH and more bacteriological stability (Clifford *et al*, 1980). The smoke compounds reduce the surface pH and thereby make the smoked fish a less favourable environment for most bacteria (Njai, 2000). The typical smoke flavour results from a number of chemicals found in the smoke, but it is mostly attributed to phenols (Horner, 1992). Some phenolic substances also have preserving effects on the smoked product by delaying oxidation and rancidity of the highly unsaturated fish lipids (Sveinsdottir, 1998).

Based on results of the physical assessment of this study, certain inferences can be drawn. A general depreciation in all the physical attributes i.e colour, fragment odour, taste and texture was recorded. Presence of moulds were noticed on the *Clarias gariepinus* from the 5th week. The colour assessment result also revealed a significant colour change in most species as from the 6th week. Also, the taste values for *Oreochromis niloticus* and *Heterotis niloticus* was declared best by the panelist.

Apart from *Parachanna obscura*, the other fatty species *C. gariepinus* and *L.coubie* become less firm as the weeks progress. This corroborated the assertion of Sefa-dedeh *et al* (1995) that differences between fish species may also be reflected in the quality of the smoked fish. Also, it has been reported that as the weeks increase, the spoilage agents gained more potential for their action (Llobreda *et al*, 1986). Reilly *et al* (1986) who worked on shrimp confirmed the quality loss during storage period for samples stored at ambient temperature and chilling.

The procedure of smoking decrease the water activity in the tissue due to salting, pre-drying and amount of smoke (Sveinsdottir, 1998). The percentage moisture content (water activity) determines the storage life of fish. Under this study, the moisture content increased weekly in the five smoked fish species from the initial average of $10.41 \pm 0.02\%$. This was attributed to the difference in the moisture of the smoked fish relative to the surroundings. Hence, this necessitated the absorption by the smoke-dried fish from the vapour-laden wet season. Results from previous studies have shown that the hot smoked process which took about 1-3 hours yields a product with about 35-45% moisture content, but with a limited shelf life of 1-3 days at ambient temperatures. While smoke-dry process which took about 10-18 hours and sometimes 3-4 days yield fish of 10-15% moisture content, sometimes even below 10% with a shelf life of 3-9 months when stored properly (Jallow, 1995). Also, a standard moisture content of 12% was reported by FAO/APHCA (1989) as the level beyond which fish products begin to grow moulds after few days.

In addition, the percentage crude protein reduced over the period of storage. This showed a degradation of the crude protein in the fish species gradually to more volatile products such as Total Volatile Bases (TVB), Hydrogen sulphide and Ammonia (Eyo, 2001). Hence the crude protein content was seen to have been denatured during storage. This agreed with Stround (1988), who reported that smoking process has been found to affect the nutritional value of fish, mainly by reducing the biological availability of proteins. According to Emokpae (1979), changes observed in protein and lipid content during storage may have been due to leaching out of some extractable soluble protein fraction and hydrolysis of some of the lipid fractions.



Furthermore, the percentage lipid content was observed to reduce over time. This was attributed to the oxidation of poly-unsaturated fatty acids (PUFA) composed in the fish tissue to products such as peroxides, aldehydes, ketones and the free fatty acids (Horner, 1992). However, the rate of fat deterioration was found to be very gradual. Also, fish oil has been found to be more liable to spoilage than other oils due to the greater number of unsaturated fatty acids as shown by the lower specification number and higher iodine value (Eyo, 1993). The greater the degree of unsaturation, the greater the tendency for fat oxidation (rancidity), Bernaseek (1991) reported that there may be high risks of rancidity during prolonged storage conditions due to the fatty nature of fish.

The chemical analyses showed that the pH continued to reduce over the weeks. In all the species, it moved from the nearly neutral values to more acidic values. Eyo (1993), stated that pH is an indicator to the extent of microbial spoilage in fish and that some proteolytic microbes produce acid after decomposition of carbohydrate, hence, increasing the acid level of the medium. He concluded that the pH was a reasonably reliable indicator of the degree of freshness or spoilage. The accumulation of acids has been found to retard fermentation and increase putrefaction. (Eyo, 2001). It can however be concluded that the decrease in the pH level is due to the fact that carbohydrate components of the fish species were fermented to acids and thus a decrease in pH towards the acidic region, indicating spoilage.

The Total Volatile Nitrogen also increased during storage. Huss (1988) recommended the ratio of Volatile Basic Nitrogen to the Total Nitrogen as a useful index of quality of fish. Pearson (1982) in his own research recommended that the limit of acceptability of fish is 20-30mg N per 100g. This was further corroborated by Kirk and

Sawyer (1991), who suggested a value of 30-40mg N per 100g as the upper limit. Also, Connell (1995) stated that the limit of acceptability of fish is 30mg N per 100g. He reported that beyond this level, white fish and prawns should be regarded as unacceptable. The work of Trinidad and Estrada (1986) revealed an increase in value of TVN produced in smoked Tilapia fish from 4.2 mg to 11.00mg under fifteen hours. However, in this research work, the highest value of Total Volatile Nitrogen recorded was 21.03 mg N per 100g for *Heterotis niloticus* in the 8th week, which showed incipient of spoilage.

Furthermore, the Peroxide value which is a primary indicator of oxidation of fat (rancidity) was shown to increase weekly. According to the analysis, the Peroxide values of the smoked fish species ranged from 21.27 to 27.66ml per Kg at the end of the 56-day period of storage and assessment. Eyo (2001) reported that since fish oils are highly unsaturated and therefore prone to oxidation, the peroxide values corresponding to incipient spoilage are usually in the order of 20-40 milliequivalents of oxygen per Kilogram of sample (ml per Kg). However, Connell (1995) reported that when peroxide value is above 10-20, then, fish develop rancid taste and smell. Thus, it can be concluded that the values from this research indicated the beginning of spoilage.

In addition, the Free Fatty Acid (FFA) value, which is a tertiary product of rancidity revealed an increase during storage. The FFA is a measure of hydrolytic rancidity-the extent of lipid hydrolysis by lipase action. According to Eyo (2001), with most fish oils, rancidity is noticeable when the FFA calculated as oleic acid is in the region of 0.5-1.5%. In this study, the percentage FFA for the five fish species at the peak of the 56 days of storage ranged between 0.91-1.96%, showing a high rate of FFA production.

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

Physical or subjective methods of analysis may suffer from bias from the assessor and hence may not be reproducible (Eyo, 2001). However, the chemical methods measures freshness or state of deterioration of product directly. This is so as the concentration of chemicals (in fish) are dependent on storage time and temperature (Lima des santos *et al* 1981). It was observed that the five smoked fish species undergone loss in quality i.e physical and chemical/nutritional during storage. There was a high degree of correlation between mean values for the chemical parameters. Therefore, the appropriate storage length of the smoked fish under natural condition at 10% moisture content is 4-5weeks, a prolonged level may be attained at reduced moisture content or higher salt content.

RECOMMENDATIONS

Based on the results of this study, the following recommendations are made:

- Intermittent sundrying or mild smoking can be carried out on smoked fish to extend its shelf life.
- Preservatives such as pirimiphos - methyl (actelic) can be applied to preserve smoked fish.
- The salt concentration could be raised to a level above 30% as this inhibits growth of insect larvae.
- In areas where electricity supply is constant, smoked fish can be oven-dried, refrigerated or freezed, inhibiting some spoilage bacteria.
- Moisture content less than 10% should be maintained in stored smoked fish to reduce the growth of bacteria and mould.

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ASSESSMENT OF THE PHYSICAL CHANGES IN SOME SMOKED FISHES DURING STORAGE (QUESTIONNAIRE).

EXAMINER:.....

Product and Sensory Evaluation Worksheet

Week	Product	Colour	Fragments	Odour	Texture	Taste	Total	Remarks (Acceptable/ Unacceptable)
	Sample B							
	Sample E							
	Sample N							
	Sample K							
	Sample Z							

Evaluation Key:

5 = Excellent, 4 = Good, 3 = Fair, 2 = Poor, 1 = Bad

Description of Grade points for Organoleptic Assessment of the smoked fish species

Scores / Terms					
Attributes	1 (Bad)	2 (Poor)	3 (Fair)	4 (Good)	5 (Excellent)
Taste	Distasteful	Stale	Mild taste (bland)	Tasty	Very tasty
Odour	Unpleasant	Putrid	Satisfactory	Pleasant	Very pleasant
Colour	Unattractive dark colour	Dull colour	Slightly brown	Brown colour	Attractive Brown colour
Texture	Very soft	Flabby	Slightly soft	Firm	Very firm
Fragment	Highly Fragmented	Cracked skin	Fairly Smooth	Smooth skin	Very smooth (no cracks)

APPENDIX 2

Correlations

Correlations

		% Moisture Content	% Ash Content	% Crude Protein	% Fat Content	pH Level	Total Volatile Nitrogen	Peroxide Value	Free fatty Acid
Moisture Content	Pearson Correlation	1.000	.249**	-.060	-.104	-.342**	.497**	.268**	-.048
	Sig. (2-tailed)		.004	.492	.228	.000	.000	.002	.581
	N	135	135	135	135	135	135	135	135
Ash Content	Pearson Correlation	.249**	1.000	.123	-.178*	.466**	.021	-.298**	.618**
	Sig. (2-tailed)	.004		.156	.038	.000	.813	.000	.000
	N	135	135	135	135	135	135	135	135
Crude Protein	Pearson Correlation	-.060	.123	1.000	-.860**	.537**	-.364**	-.726**	-.699**
	Sig. (2-tailed)	.492	.156		.000	.000	.000	.000	.000
	N	135	135	135	135	135	135	135	135
Fat Content	Pearson Correlation	-.104	-.178*	-.860**	1.000	-.301**	.330**	.682**	.769**
	Sig. (2-tailed)	.228	.038	.000		.000	.000	.000	.000
	N	135	135	135	135	135	135	135	135
pH Level	Pearson Correlation	-.342**	.466**	.537**	-.301**	1.000	-.615**	-.769**	-.629**
	Sig. (2-tailed)	.000	.000	.000	.000		.000	.000	.000
	N	135	135	135	135	135	135	135	135
Total Volatile Nitrogen	Pearson Correlation	.497**	.021	-.364**	.330**	-.615**	1.000	.770**	.442**
	Sig. (2-tailed)	.000	.813	.000	.000	.000		.000	.000
	N	135	135	135	135	135	135	135	135
Peroxide Value	Pearson Correlation	.268**	-.298**	-.726**	.682**	-.769**	.770**	1.000	.640**
	Sig. (2-tailed)	.002	.000	.000	.000	.000	.000		.000
	N	135	135	135	135	135	135	135	135
Free fatty Acid	Pearson Correlation	-.048	-.618**	-.699**	.768**	-.629**	.442**	.640**	1.000
	Sig. (2-tailed)	.581	.000	.000	.000	.000	.000	.000	
	N	135	135	135	135	135	135	135	135

*Correlation is significant at the 0.01 level (2-tailed).

**Correlation is significant at the 0.05 level (2-tailed).

		Colour Change	Fragment	Odour	Taste	Texture
Colour Change	Pearson Correlation	1.000	.621**	.677**	.694**	.636**
	Sig. (2-tailed)		.000	.000	.000	.000
	N	225	225	225	225	225
Fragment	Pearson Correlation	.621**	1.000	.698**	.722**	.675**
	Sig. (2-tailed)	.000		.000	.000	.000
	N	225	225	225	225	225
Odour	Pearson Correlation	.677**	.698**	1.000	.771**	.717**
	Sig. (2-tailed)	.000	.000		.000	.000
	N	225	225	225	225	225
Taste	Pearson Correlation	.694**	.722**	.771**	1.000	.742**
	Sig. (2-tailed)	.000	.000	.000		.000
	N	225	225	225	225	225
Texture	Pearson Correlation	.636**	.675**	.717**	.742**	1.000
	Sig. (2-tailed)	.000	.000	.000	.000	
	N	225	225	225	225	225

Regression: Physical Parameters

Variables Entered/Removed^a

Model	Variables Entered	Variables Removed	Method
1	Texture, Colour Change, Fragment, Odour, Taste		Enter

a. All requested variables entered.

b. Dependent Variable: Weeks

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.953 ^a	.907	.905	.80

a. Predictors: (Constant), Texture, Colour Change, Fragment, Odour, Taste

ANOVA^a

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1361.031	5	272.206	428.967	.000 ^a
	Residual	138.969	219	.635		
	Total	1500.000	224			

a. Predictors: (Constant), Texture, Colour Change, Fragment, Odour, Taste

b. Dependent Variable: Weeks

Coefficients^a

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	15.277	.243		62.765	.000
	Colour Change	-.412	.085	-.149	-4.845	.000
	Fragment	-.834	.089	-.305	-9.408	.000
	Odour	-.612	.095	-.233	-6.441	.000
	Taste	-.642	.099	-.251	-6.506	.000
	Texture	-.453	.101	-.151	-4.483	.000

a. Dependent Variable: Weeks

Regression: Proximate

Variables Entered/Removed^a

Model	Variables Entered	Variables Removed	Method
1	% Fat Content, % Moisture Content, % Ash Content, % Crude Protein		Enter

a. All requested variables entered.

b. Dependent Variable: Weeks

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.666 ^a	.444	.427	1.96

a. Predictors: (Constant), % Fat Content, % Moisture Content, % Ash Content, % Crude Protein

ANOVA^a

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	399.282	4	99.821	25.916	.000 ^a
	Residual	500.718	130	3.852		
	Total	900.000	134			

a. Predictors: (Constant), % Fat Content, % Moisture Content, % Ash Content, % Crude Protein

b. Dependent Variable: Weeks

Coefficients^a

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-155.805	23.845		-6.534	.000
	% Moisture Content	14.061	1.781	.558	7.893	.000
	% Ash Content	.127	.341	.025	.373	.710
	% Crude Protein	-.157	.048	-.437	-3.259	.001
	% Fat Content	-.327	.114	-.385	-2.856	.005

a. Dependent Variable: Weeks

APPENDIX 5

Result of Chemical Analyses

Weekly changes in pH (1-14) of the smoked fish species with storage length

Weeks	Heterotis	Labeo	Parachanna	Oreochromis	Clarias
0	6.45	6.38	6.12	6.47	6.21
1	6.41	6.35	6.09	6.44	6.19
2	6.38	6.31	6.06	6.42	6.13
3	6.33	6.28	6.01	6.38	6.08
4	6.29	6.22	5.97	6.34	6.03
5	6.26	6.19	5.88	6.3	5.96
6	6.17	6.11	5.79	6.26	5.9
7	6.08	6.05	5.57	6.17	5.82
8	5.98	5.94	5.35	6.09	5.75

Weekly changes in TVN (MgN/100g) of smoked fish species with storage length

Weeks	Heterotis	Labeo	Parachanna	Oreochromis	Clarias
0	7.1	6.4	4.91	6.88	5.2
1	7.62	6.71	5.42	7.36	5.77
2	7.84	6.97	5.88	7.69	5.91
3	8.17	7.26	6.25	7.87	6.18
4	9.82	9.53	7.13	8.21	6.33
5	12.02	11.27	10.34	10.98	10.17
6	14.34	13.42	12.67	13.21	13.04
7	17.89	16.11	15.05	16.34	19.92
8	21.03	20.08	19.12	20.29	20.01

Weekly changes in Peroxide value (MI/Kg) of the smoked fish species during storage

Weeks	Heterotis	Labeo	Parachanna	Oreochromis	Clarias
0	2.41	3.95	6.2	4.98	6.62
1	2.87	4.23	6.82	5.17	7.04
2	3.12	4.81	7.34	5.54	8.65
3	3.75	5.07	8.51	6.23	10.22
4	4.34	5.76	10.66	8.07	13.37
5	6.2	8.14	12.75	10.46	15.48
6	10.63	11.92	19.09	14.39	18.19
7	15.56	16.68	18.93	19.72	21.5
8	21.27	24.09	25.98	21.75	27.66

Weekly changes in Free Fatty Acid (%) of the smoked fish species during storage

Weeks	Heterotis	Labeo	Parachanna	Oreochromis	Clarias
0	0.17	0.27	0.42	0.19	0.41
1	0.26	0.36	0.56	0.32	0.53
2	0.34	0.45	0.67	0.49	0.67
3	0.49	0.53	0.75	0.57	0.78
4	0.57	0.69	0.83	0.73	0.84
5	0.68	0.78	0.96	0.86	0.97
6	0.72	0.84	1.1	0.9	1.2
7	0.85	0.98	1.38	0.95	1.59
8	0.91	1.52	1.74	1.02	1.966