ORGANOLEPTIC AND MICROBIAL ANALYSIS OF *CHRYSICHTHYS NIGRODIGITATUS* FROM IMO RIVER, NIGERIA SMOKED USING DIFFERENT SOURCES OF HEAT

BY

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SEPTEMBER, 2024

DECLARATION

I hereby declare that this study "*ORGANOLEPTIC AND MICROBIAL ANALYSIS OF CHRYSICHTHYS NIGRODIGITATUS FROM IMO RIVER, NIGERIA SMOKED USING DIFFERENT SOURCES OF HEAT*" is my original work and has not been previously submitted to any institution, either in part or in full thereof for the award of any other certificate, diploma or degree.

_____ _____ _____

Jude Chiemela Jude Jr. Date

CERTIFICATION

This is to certify that the study " ***ORGANOLEPTIC AND MICROBIAL ANALYSIS OF CHRYSICHTHYS NIGRODIGITATUS FROM IMO RIVER, NIGERIA SMOKED USING DIFFERENT SOURCES OF HEAT*** carried out by Jude Chiemela Jude Jr (20204256808/M.Sc) in the Department of Fisheries and Aquaculture Technology, School of Agriculture and Agricultural Technology, Federal University of Technology Owerri is original and has been examined and accepted for the award of the degree of Master of Science (M.Sc) in Fisheries and Aquaculture Technology of the University.

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DEDICATION

This work is dedicated to my Dad, Sir Jude Amaliri Snr and my wonderful Mum, Late Mrs Martha Ukachi Amaliri.

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ABSTRACT

This study is aimed to assess the microbiological and sensory quality of smoke–dried fish processed, using four different sources of heat (Charcoal, Firewood, Sawdust and Oven). A total of 300 fresh fish samples were randomly collected from Imo River for the study. The fish were divided into four batches of 75 fish per batch and processed using four different methods: Smoke-dried with charcoal (T^1), Smoke-dried with firewood (T^2), Smoke-dried with sawdust (T^3) and Smoke-dried with oven (T^4). Organoleptic assessment using a 5-point hedonic scale was also employed using a sensory evaluation while Microbial quality was determined from total coliform count and types of bacterial isolates. Data from Organoleptic assessment were analyzed using simple descriptive statistics such as percentages, pie charts and bar charts while microbiological data were statistically evaluated using the one-way analysis of variance (ANOVA). Results of organoleptic studies which evaluated the perception of four sensory criteria showed that for appearance/colour quality score was 9.0 (Charcoal), 7.2 (Firewood), 7.8 (Sawdust) and 9.5 (Oven). For Aroma, score was 7.8 (Charcoal), 7.6 (Firewood), 7.8 (Sawdust) and 9.4 (Oven). For Texture as sensory quality, score was 7.8 (Charcoal), 7.6 (Firewood), 7.8 (Sawdust) and 9.3 (Oven). Similarly, for Taste, the score was 9.0 (Charcoal), 7.4 (Firewood), 9.0 (Sawdust) and 9.7 (Oven). The result revealed that the organoleptic quality of fish dried using the oven as heat source gave the best sensory quality of excellence in terms of appearance/colour (9.5), aroma (9.4), texture (9.3) and taste (9.7). Results of Microbial analysis based on plate count method (cfu/g) and total viable bacterial count (TVBC) of pathogen food bacteria isolates, *Escherichia coli* and *Klebsiella spp* were as follows: For total coliform (cfu/g), Charcoal was (2.6×10^2), Firewood (2.9×10^2), Sawdust (2.6×10^2), Oven (2.2×10^2). *E. coli* was 1.5×10^2 (Charcoal), 1.8×10^2 (Firewood), 1.6×10^2 (Sawdust) and 1.2×10^2 (Oven). For *Klebsiella spp*, total viable bacterial count (cfu/g) was 1.1×10^2 (Charcoal), 1.1×10^2 (Firewood), 1.0×10^2 (Sawdust) and 1.0×10^2 (Oven). The results of microbial analysis showed that fish processed using the oven gave the lowest microbial load 1.0×10^2. It is thus recommended that where available and economical, oven processing/drying method should be adopted by fish producers. The use of oven in fish processing/drying will also mitigate the effect of deforestation which is the common source of charcoal, firewood and sawdust.

Keywords: Chrysichthys nigrodigitatus, organoleptic and microbial composition, different sources of heat.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Fishes are any collection of faunae (animals) that entail all gill-bearing aquatic craniates that lack limbs with distal appendages such as fingers or toes. They form the majority of cold-blooded aquatic vertebrates. Fish is one of the aquatic animals, which serve as food which contains some chemical elements (compositions) in them. These chemical compositions (elements) can be in form of minerals and vitamins which are supplement to the body when fishes are taken as food (F.A.O, 1981). In general, smoking promotes an environment low in spoilage microflora, with the exception of heterofermentative lactic acid bacteria, yeasts and moulds. Despite massive world-wide development efforts, in terms of many laudable programmes and ways to reduce poverty and improve livelihoods in fisheries and other sectors of the economy, poverty remains a nightmare for millions of Africans. In West Africa alone, seven million people are involved in fishing, aquaculture and related activities, such as processing and trading because it is a major source of livelihoods in many coastal communities, both inland and on the Atlantic coast. In addition to providing employment and income, fisheries play a very important role in local and national economies (Komolafe and Arawomo, 2011). Nigeria is the largest consumer of fish in Africa with an approximately 1.2 million tonnes of fish needed annually to satisfy the demand of the ever increasing population. FAO (2000) estimated fish demand for Nigeria from 1997 – 2025, based on projected population and gave an average of 1.11 million tonnes for a decade (2000 – 2010). The country is highly blessed and endowed with vast expanse of inland freshwater and brackish ecosystems with abundant fish species, which have potentials for culture. These water bodies also

play an important role in the provision of protein to Nigerians, especially now that imported fish is becoming expensive to the common man (Komolafe and Arawomo, 2011). Freshwater is a very important natural resource crucial for the survival of all living beings. UNESCO (2003) reported that water is the most vital resource for all kinds of life on earth and essential for sustainability of the earth's crust ecosystem. The quality of life depends on the quality of water. Adaka, *et al.,* (2015) reported negative allometric of some fish species from Oramiri-Ukwa. Physico – chemical factors are important in estimating the constituents of water and concentration of pollutants or contaminants. These factors are interrelated and interdependent with biological factors (plants and animals). Similarly, these factors immensely influenced the uses as well as the distribution and richness of biota (Unanam and Akpan, 2006). Physical parameters of water bodies include water movement, depth, turbidity, transparency, temperature and suspended solids. Growth is simply defined as change in size (length, weight and bulk) with time and can also be change in numbers with time in the case of population (Abowei and Ezekiel,2013). *Chrysichthys nigrodigitatus*, popularly known as Silver Catfish, is widely consumed in some parts of West Africa especially Nigeria and Ghana (F.A.O, 1981). The silver catfish, *Chrysichthys nigrodigitatus* (Lacépède, 1803) is among the dominant African commercial fishes of high economic value and widely serves as food for human consumption in West Africa. *Chrysichthys*: From the Greek chrysos, meaning golden and ichthys, meaning fish. *Chrysichthys nigrodigitatus* is a prominent member of the *Claroteids* and occurred abundantly in some Benin inland waters (Lake Nokoué, lake Ahémé, Costal lagoons, Porto-Novo lagoon etc.) where this species made about 7-26% of the total annual fish catches. This catfish belongs to the genus *Chrysichthys,* family *Claroteidae, Siluriformes* order and *Ostariophysi* super order.

As the human population inevitably increases, the demand for fish as a source of protein is growing. Fishes such as those in the family *Claroteidae* are highly used and commercialized. The commercially important fish species in this family are the Catfish (*Chrysichthys* species) known as "Inanga" in Ibibio language. *Chrysichthys nigrodigitatus* (Lacepede 1802) is a common silver colored African catfish occurring in Nigeria and several West African countries. It is a highly valuable fish species amongst the indigenous African populations.

Chrysichthys nigrodigitatus species has been found to be a typical example of fish without strict feeding habit. It is regarded as an omnivore, because of its ability to use just any food material present in its environment.

All fish require energy which must be obtained from its food sources for growth, reproduction and migration. Understanding food and feeding habits of fish is useful to all scientists who are concerned with any aspect of fisheries.

Spoilage of fresh fish can be attributed to series of metabolic processes that cause the fish to be undesirable and unacceptable for human consumption due to changes in sensory and biochemical characteristics. The noxious smells from spoilt fish have been suggested to be produced by microbes to repulse large animals, thus reserving the food resource for them (Sherrat *et al*., 2006, Braun and Sutherland 2005). Fresh fish is normally considered a safe food however it can be a significant source of bacterial food poisoning (Shewan *et al*., 1962). Fish is a highly perishable, high protein food that typically contains high levels of free amino acids. Microbes multiply using these amino acids, producing ammonia, biogenic amines such as putrescine; histamine, cadaverine, organic acids, ketones and hydrogen sulphide compounds (Dalgaard *et al* 2006, Emborg *et al*

2005). Some marine fish and fresh water fish contain trimethylamine oxide that is degraded by several spoilage bacteria to trimethylamine (TMA) the compound responsible for fish odors.

Traditional smoking kilns used in Nigeria cart from simple pit to the drum or mud-walled type which may be circular or rectangular in shape. According to Eyo (1997), modern smoking kilns which have been tested in Nigeria's inland fisheries include Modified Altona or Watanabe Smoking Kiln (WSK), Altona Smoking Kiln (ASK), Chorkor oven, and Kainji Gas Kiln (KGK). There is paucity of research on the processing and preservation of *C. nigrodigitatus*. Fish is a rich source of lysine suitable for supplementing high carbohydrate diet. It is also a valuable source of vitamins A, B, and E, iodine and oils containing polyunsaturated fatty acids (Eyo, 2001, da Silva, 2002, Abolagba and Melle, 2008). In Nigeria, fish smoking is the most practiced preservation method. Practically all species of fish available in the country can be smoked and it has been estimated that 70-80 percent of the domestic marine and freshwater catch is consumed in smoked form (Akinyemi *et al.*, 2011). Smoked fish constitutes a major source of animal protein for a vast majority of the population in Nigeria, particularly the rural population (Eyo, 1992). Traditional smoking techniques involve treating of pre-salted, whole, or filleted fish with wood smoke in which smoke form colorations in intensively heated products. This was supported by Ova *et al.* (1998) who reported that the PAH levels were significantly higher in the fish skins than in the edible parts.

In Nigeria, fish smoking is one of the most widely used traditional fish-processing methods. Studies on fish consumption pattern in Nigeria show that fish is consumed more in the smoked form (Asiedu and Okeke, 1994). It is estimated that 85 per cent of processed fish is smoked.

The objective of this study is to determine how four different sources of heat (firewood, sawdust, charcoal and oven), used for fish smoking affect the smoked product by evaluating the organoleptic and microbial quality of the smoked *Chrysichthys nigrodigitatus*. The fish smoking process is conducted traditionally based on the skills passed from generation to generation. Smoke-drying is the most popular method of fish processing and preservation in rural fishing villages where coldrooms and ice plants are not easily available, and helps to reduce post-harvest losses to fishermen. It has become imperative that more efficient methods of fish smoking should be experimented on with a view to recommending them to the fishers to increase their productivity. It is expected that such improved smoking methods will produce smoked fish that are more organoleptically acceptable with less bacterial flora.

The present study therefore investigates the organoleptic and microbial attributes of smoke-dried *Chrysichthys nigrodigitatus*, using different sources of energy, which are; firewood, oven, sawdust and charcoal, from Imo State, Nigeria.

1.2 JUSTIFICATION OF STUDY

The information obtained from this study shall be helpful for fishery managers to implement adequate adoption-centric regulations for sustainable fishery management in the water body of Imo River as well as other parts of the country. The escalating rate of emerging diseases in recent times has become a serious cause of worry.

1.3 AIM AND OBJECTIVES OF STUDY

The aim of this study is to assess the Organoleptic and Microbial quality of *Chrysichthys nigrodigitatus*, from Imo River, Nigeria, with the following Specific objectives:

1) To determine the organoleptic quality of smoke-dried *Chrysichthyes nigrodigitatus* from Imo River.

2) To determine the microbial flora of smoke-dried *Chrysichthyes nigrodigitatus* of Imo River using different sources of heat (firewood, charcoal, sawdust, oven.

3) To compare the effectiveness of the different smoke-drying methods based on organoleptic and microbial quality.

1.4 SCOPE OF STUDY

The specific study was undertaken for a period of six months, in the Imo River. The Organoleptic and Microbial analysis of *Chrysichthys nigrodigitatus* was analysed.

The microbial quality analysis of fish tissues to identify microbial isolates were also carried out.

The different sources of energy, the Oven-dried, firewood, sawdust and charcoal were compared with the international reference values.

CHAPTER TWO

LITERATURE REVIEW

2.1 Fish Marketing and Importation in Nigeria

Fish is an important source of food and income for both the rich and poor people in Nigeria. A lot of people in the riverine area depend wholly or partly on the fisheries sector for their livelihood. Due to the lack of adequate technology processes and locally available methods such as mud bricks stone and firewood the Nigerian fish smoking business is yet to gain enough recognition. Consequently, the quantity, quality control, and hygienic condition are affected while market value diminishes that result in damage and non-attractive appearance of the processed fish. The mechanisms used by traditional fish smokers have a lot of limitations. Smoking contains substances that kill bacteria and destroy enzymes because of high temperature thus helping to preserve the product while the heat dries the fish. The smoking efficiency of a Mechanical smoking kiln or drier can be improved to produce high-quality fishes with uniform heat distribution using forced-draft through an appropriate distribution of the heat generated by electricity, gas or oil from the drying chamber and air passing over it.

The demand for fish in Nigeria is estimated at 1.55million tonnes. Of this estimated demand, domestic production caters for 511,000 tonnes and the remaining is taken care of by importation. Nigeria is one of the largest importers of fish with official records indicating an average of 560,000 tonnes annually. This was estimated at N30billon ($US400million) in 2002 (Presidential Forum, 2005).

Aquaculture is about a century old in Africa. The yield from this subsection in Africa has remained low over the years despite the vast potentials in the continent. (Jamu & Ayinla, 2003, Machena &

Moehl, 2001). Nigeria is the largest fish producer in Africa with production ranging from 17,700 to 25,000 metric tonnes, (Machena & Moehl, 2001; Ridler & Hishamunda 2001). Nigeria is endowed with many large rivers, man-made lakes, creeks and about 200 nautical miles of marine water under the Exclusive Economic Zone (EEZ). A survey by the Federal Department of Fisheries (FDF,1986), showed that 65,000 hectares of freshwater aquaculture potential were yet to be utilized. According to Olomola (1991), Nigeria has a great potential to increase the availability of fish by supporters and expanding aquaculture and as noted by Idachaba (1991), the fishery sub-sector performed below expectations in large part due to over-reliance on imported inputs. Aquaculture projects in Nigeria have a chequered history, dating back to the 1950's. The first project was commissioned by the British colonial government in 1954 in Panyam, near Jos, Plateau State. This was followed by the one in Maska, in Funtua district of Katsina State, Agodi farms in Ibadan, Oyo State, and Umuna farms in Okigwe, Imo State (Nigera Institute of Freshwater Fisheries Research (2000).

The Federal Ministry of Agriculture through its agencies, Federal Department of Fisheries, Nigeria Institute of Oceanography and Marine Research (NIOMR) and Nigeria Institute of Freshwater Fisheries Research, River Basin Development Authorities and proscribed Directorate of Food, Road and Rural Infrastructure (DFFRI), established several aquaculture projects. These include the ones in Olupona, Osun State; Lafiaji, Plateau State; Makurdi, Benue State; Enugu-Aboh, Enugu State; Dwam, Adamawa State; Mando, Kaduna State; Wuhan, Niger State; Oyo, Oyo State; Zuru, Sokoto State; Ilenuwa, Ogun State; Sepateri, Oyo State;; Igede, Ekiti State; New Yidi, Kwara State; EqualLogic, Anambra State and Gubi, Bauchi State (Adikwu, 1999).

It is Important to evaluate marketing systems of fish because they indicate how the various market participants are organized to accomplish the movement of commodity from the producer to the ultimate consumer (Olukosi *et al*., 2007). Marketing of fish is not usually on the basis of the fisherman to consumer (Lawal & Idega, 2004). There are several middlemen in the link between producers (fishers) and consumers (Adegeye & Dittoh, 1985). Therefore, price of fish changes as it passes through middlemen such that by the time it reaches the final consumer, it has increased considerably

The fishery industry has, however, not attained the desired level of self-sufficiency in fish production in Nigeria. The problem is that the total domestic fish production is far less than the total domestic demand. The fear is that the unsatisfied demand will continue to be met through importation unless policy actions are geared towards improving domestic production in a sustainable way through aquaculture (Rahji *et al.,* 2001). In addition to this, the fishery resources of the century are far from being fully utilized. According to Olomola (1991), a review of aquaculture in Nigeria showed that only infinitesimal proportions of the resources available were being utilized. It is believed that the desire of drastically reducing fish importation can be attained through the harnessing and exploration of the existing potentials (Rahji *et al.,* 2001). Today there are two main methods of smoking fish: the traditional method and the mechanical method.

The traditional method involves the fish being suspended in smokehouses over slowly smouldering wood shavings. The fish are left overnight to be naturally infused with smoke.

In the mechanical method smoke is generated through the use of smoke condensates, which are created by the industrial process of turning smoke into a solid or liquid form. The flow of smoke

in the mechanical kiln is computer controlled and the fish generally spend less time being smoked than in a traditional kiln.

2.2 *Chrysichthys nigrodigitatus*

2.2.1 Description

Chrysichthys nigrodigitatus is a species of ray-finned fishes in the family Claroteidae. They are associated with freshwater habitat. They have sexual reproduction.

C. nigrodigitatus is an opportunistic benthic feeder that incorporated in its diet, a broad range of prey items dominated by aquatic insects, detritus, sand particles, seeds, algae and minor food resources such as copepod, cladocera and mollusks. *Chrysichthys nigrodigitatus* is a species of ray-finned fishes in the family *Claroteidae*. They are associated with freshwater habitat. They are omnivorous, feed on seeds, insects, bivalves and detritus. Feeding becomes specialized with age and size, larger fish may feed on decapods. *Chrysichthys nigrodigitatus* is an omnivore with moderate gut length. The colour is quite drab in this species with a basic grey/silver body colouration and a white underside. It has a quite large dorsal fin and a deeply forked caudal fin. It is basically a food fish in its native African waters where it can grow to excess of 50cm and individuals can grow to 65.0 cm. The males when fully grown usually have a broader head which they use to dig out their breeding nests in their native habitat. *Chrysichthys nigrodigitatus* exhibited a pointed snout slightly longer than or equal to the width of the mouth and the pre-maxyllary tooth plate width made 20-30% of the head length.

C. nigrodigitatus has a high potential in aquaculture, it does not reproduce or barely reproduce in captivity in small fish ponds. The numerous abandoned and unmanaged sand-dragged man-made lakes (exemple: Lake Ahozon) throughout the country could serve as alternative medium-

environments and could be valorized for the development of *C. nigrodigitatus* aquaculture and particularly to serve as spawning and nursery grounds. Though widespread in the natural inland waters where the species grows and spawns actively, it is quite difficult to reproduce *C. nigrodigitatus* in fish pond in order to supply aquaculture center with fingerlings (seeds). Therefore, the numerous man-made lakes, established throughout the country are alternative environment mediums that may contribute to aquaculture development and particularly, to serve as spawning grounds for some delicate species such as *C. nigrodigitatus*. In December, 2011, about 265 live individuals (15 genitors and 250 figerlings) of *C. nigrodigitatus* has been introduced by the fishers in the sand-dragged man-made lake of Ahozon (South-Benin) where the species, currently, made about 11.22% of the fish assemblages and hence constituted in this artificial lake, the second dominant species after the cichlid *Sarotherodon galilaeus.*

Fig 1: Picture of ***Chrysichthys nigrodigitatus*** (Lacepède, 1803)

2.2.1.1 Age composition and structure of *Chrysichthys nigrodigitatus*

The exact age determination of fish is one of the most important elements in the study of their population dynamics. It forms the basis for calculations leading to the knowledge of growth, mortality, recruitment and other fundamental parameters of their populations. Age and growth determination of fish species is an essential component in understanding the changes in fishery of any water body (Ogueri *et al.,* 2009). Selection of appropriate method for age and growth determination in fishes often requires balancing precision and accuracy of the method with the limitation in sample size (Zymonas and McMahon, 2009).

There are basically three methods used to determine the age of fishes. The first is recovering the marked fish of a known age that requires the capture, release, and recapture of fish. This is otherwise known as mark-release-capture method, which is the most accurate method under natural conditions. It is expensive and time consuming, thereby limiting its application in fisheries. (Zymonas and McMahon, 2009). The second, referred to as Peterson method, involves comparisons of length-frequency distributions of fish population samples. It requires measuring the length of a large number of fish in a population. This method is most reliable in younger year classes of fish, older fish grow more slowly in length and variation among individuals tends to obscure variation between year classes (Cailliet *et al.,* 1986). Peterson method can best be used in temperate species with seasonal spawning activities that can be adequately sampled. Tropical water fishes spawn throughout the year, therefore distinct year classes are difficult to be recognized in such setting. These methods are based on the assumption that each modal class in a frequency distribution will correspond to a cohort and represent different age classes determined at regular intervals (Cailliet *et al.,* 1986).

The presence of modes in the length distribution depends on the distance between the medians, the extent of the variance, the proportion of each age class in the population and the size of the sample studied (Zymonas and McMahon, 2009).

The third method is anatomical approach, which is commonly used, involve counting marks that develop periodically on some hard parts of fishes. Several kinds of parts in fishes that are calcified can be used to determine age, which are as follows; otoliths, scales, cross sections of ray fins, operculum and vertebrae. Ages of fish are estimated by comparison of the readings from various bony structures by different readers. Metin and Ilkyaz (2011) reported that age determination in fish can be carried out by counting seasonal growth annuli appearing on hard structures such as otolith, scale, fin ray and spine. According to Gomez-Marquez *et al.,* (2008), annual growth rings may not be formed in most tropical fishes as in temperate fishes, which make fish ageing difficult.

Attempt had been made to correlate spawning with annual growth rings on scales amongst others (Adeyemi *et al*., 2009).

Operculum

The operculum of a bony fish is the hard bony flap that covers and protects the gills. In most fish, the rear edge of the operculum marks the division between the head and the body. It is composed of four fused bones-opercle, and sub-opercle. The morphology of operculum varies greatly between species and in some species is vital in obtaining oxygen. Both the left and right operculum should be collected, cleaned and observed under the microscope with a dark background (Winter *et al.,* 2002). Jimenez-Badillo (2006) reported complete growth rings on the opercular bones of *Oreochromis aureus*. Similarly, Gomez-Marquez *et al.* (2008) derived age data from opercular

bone readings of *Oreochromis niloticus*, cleaned and observed under the microscope with a dark background (Winter *et al.*, 2002).

Otoliths

Otolith has been used to determine the age of both tropical and temperate fishes in recent times. It is also known as 'ear bone'- hard structure located in the cranial bones near the brain, which composed of calcium carbonate crystal embedded in an organic matrix. Fresh or frozen otolith are best used for age determination, which must be removed as soon as the fish dies and fixed immediately to avoid the loss of the growth structures. Both left and right otoliths should be collected and kept separate until their morphology can be differentiated and also used if either is damaged during handling, or is crystalline. The growth rings in both otoliths are usually identical. The way in which otoliths are removed depends on the type of fish and is usually examined on black background with reflected light in an aqueous medium (Jones, 1992). Similarly, Jones (1992) reported that otolith had been the most reliable indicator of age for most fish because it shows annual rings, and for younger fish, daily pattern of rings. Many studies have documented the use of otoliths for age determination, which provides the most precise and accurate estimates. However, its removal can be difficult, preparation quite time consuming and fish sacrifice may not be visible in some situation. Fagade (1980) examined the gross morphology of otoliths of *Chrysichthys nigrodigitatus* from Asejire Lake and used it to determine its age. Brown *et al.* (2007) validated the use of otolith as a structure for age determination. Phelps *et al.* (2007) reported that otoliths have gained favour over other body structures because of their lack of absorption and its growth is acellular rather than by calcification.

2.2.1.2 Feeding intensity of *Chrysichthys nigrodigitatus*

The rate at which fish feed can be determined by comparing the frequency of stomachs fullness to that of empty stomachs. These stomachs can be categorized as almost empty, half full, almost full and full for those with food as the case may be, and empty for those without food. If the number or frequency of individuals with half (1/2) full stomach is higher than containing food, then the rate of feeding or feeding intensity is also higher. Individuals are said to have fed poorly when the frequency of less than half (1/2) full stomach is higher than or equal to half (1/2) full stomach (Ogbeibu and Ezeunara, 2005). Feeding intensity is influenced by a number of factors such as season and food availability. Ogbeibu and Ezeunara (2005) found that feeding intensity and diet of *C. nigrodigitatus* varied among sampling sites because of unequal feeding rate. Ogbeibu and Ezeunara (2005) reported high feeding intensity in both rainy and dry seasons because of high percentage of full stomachs. Similarly, Dada and Araoye (2008) and Yem *et al*. (2009) recorded high feeding intensity in both seasons. Spatial feeding intensity and diets showed that *C. nigrodigitatus* had high feeding intensity with chironomid and plant parts as its diet. However, Offem *et al.* (2008) reported spatial heterogeneity, with fish and fish remains been dominant, followed by insects, crustaceans, worms, rotifers and plant materials. Classification of *Chrysichthys nigrodigitatus* based on feeding habits. Fish species can be classified into broad categories according to their predominant feeding habits. However, the validity of any classification can be of doubt because each species feeds on a variety of food items. Several workers have made attempt to classify fish based on feeding habits. The morphology of the species is adopted for bottom feeding although the contents of the stomach may prove otherwise as the different kind of food found in fish stomach often reflect their ability to obtained food from different locations. Ajani (2001) reported the species as a carnivore; *Chrysichthys* species were

omnivorous (Dada and Araoye, 2008), while Ogbeibu and Ezeunara (2005) reported these species as bottom- feeding meso-predators. The ability to feed at the shoreline, open water surface and the bottom is contributory to this non-specialization. In Ikpoba River, *C. nigrodigitatus* were detritivores (Oboh *et al.*, 2003; Ogbeibu and Ezeunara, 2005). Similarly, in Ethiope, a tropical river, *C. nigrodigitatus* was an omnivorous detritivore (Oronsaye and Nakpodia, 2005). The species was considered detritivores because it fed on vegetable debris and associated animal communities. This was observed by Oboh *et al.* (2003) for Jamieson River, Ogbeibu and Ezeunara (2005) for Ikpoba River and Yem *et al.* (2009) for Lake Kainji. Lawal *et al*. (2010) reported *Chrysichthys nigrodigitatus* as omnivore feeder and that its flexibility in trophic level gave the fish ecological advantage to feed effectively on different categories of diet based on the availability of the food items. Atobatele and Ugwumba (2011) reported the species as general mesopredators. *Chrysichthys nigrodigitatus* has also been reported as an omnivore in Nun River (Akinsanya *et al.*, 2007).

2.2.1.3 Classification of *Chrysichthys nigrodigitatus* based on gut length

The structure and length of the intestine are closely related to the diet of fish (Hickman 45 *et al.*, 2001). In fishes, just like in many other vertebrates, the intestine length is an indicator of diet (Akombo *et al.*, 2010). Miller and Harley (2002) reported that the structure, length and conformation of the intestine length are closely related to fish diet. Apart from being an indicator of fish diet, it can be used for interspecific dietary comparisons (Karachle and Stergiou, 2010). For a given body length, intestine in herbivorous species is longer than in omnivorous, and that of omnivorous species is longer than in carnivorous ones (Karachle and Stergiou 2010). Similarly, Malami *et al.* (2004) noted that body structures and fish diet could be important for predicting fish diet, how fishes feed and the mechanics of feeding. This could also help to ascertain the adaptive

food of the fish species and to select the right ingredients to formulate balanced feed. Any fish species with high ratio of gut length to body length will require more proportion of plant items to animal items in its diet, while those with low ratio needs more proportion of animal to plant materials. German and Horn (2006) reported that in all vertebrates, herbivores exhibit longer digestive tracts than carnivores and the pattern is consistent among mammals. Omnivores, which eat both plants and animal materials, have longer intestines than species that eat only other animals. Carnivores have low ratio compared to herbivores and omnivores. Edema and Ojeh (2006) identified herbivores, carnivores and omnivores Edema and Ojeh (2006) reported a ratio range of 0.22-1.00 as carnivores, 2.18-9.27 as herbivores, while 1.41-1.62 fitted between the two but noted that there was no rigid demarcation ratio for carnivores, herbivores and omnivores, where the carnivores could extend to 2; ratio range for *Chrysichthys nigrodigitatus f*rom Kainji Lake as 1.21 - 2.13 with mean ratios of 1.65:1 was reported. This correlated with the feeding habit and was therefore classified as omnivore.

Table 1: Scientific Classification of *Chrysichthys nigrodigitatus*

Scientific Classification

Kingdom	Animalia
Phylum	Chordata
Class	Actinopterygii
Order	Siluriformes
Family	Claroteidae
Genus	Chrysichthys
Specie	Nigrodigitatus
Scientific name	Chrysichthys nigrodigitatus
Common name	Silver Catfish

Source: A. Oletuju Abidemi-Iromini (2019)

2.2.1.4 Abundance and Distribution of *Chrysichthys nigrodigitatus*

Fish distribution and abundance is reported to be influenced by factors, such as, conductivity or habitat availability (Melo, 2000), flow velocity and heterogeneity (Willis *et al.,* 2005), water temperature (Cetra and Petrere, 2006), altitude (Suárez and Petrere Jr, 2007) and other physico-chemical parameters such as dissolved oxygen and pH (Araújo *et al.,* 2009). Riparian vegetation provides food and also increases availability of aquatic refuges for small fish species through the falling of tree trunks and branches into the water course (Casatti *et al.,* 2003). Plant leaves incorporated into water channels also favour the establishment of a diverse fauna that can be used as food by fish (Uieda and Uieda, 2001) as well as influence fish abundance and distribution. Several catfish genera are widespread and common in Afro-tropical waters. The genus *Chrysichthys* with approximately thirty species is the largest of the family Claroteidae and is distributed from the Senegal River in the West, across the Sahelo-sudanian region to the Nile, south to the Zaire Basin and occurs in most major rivers of Africa (Paugy *et al.,* 2003). They are more prominent in low brackish water areas and major rivers of Nigeria, particularly the lagoon, where it is more commonly captured. It is widely distributed in fresh and brackish waters in West Africa. In Jebba Lake, *C. nigrodigitatus* is also among the fishes in commercial catches (Abiodun and Odunze, 2011). The fish, commonly known as silver catfish, and inhabits both freshwater and brackish water of West Africa from Senegal to Angola and fills a different ecological niche, 56 being most common in off shore part of lakes. It has been reported as a *euryhaline species*, inhabiting waters in the salinity range of 1-12 percent and its abundance in the brackish zones of coastal rivers, creeks and estuaries (Odum, 1995). Ekanem (2003) reported that as salinity increases, the fish migrate towards freshwater or low salinity area. Mature adults with ripe gonads are abundant in the headwaters especially during the first half of the rainy season. Therefore,

salinity also plays a major role in their distribution. The abundance and distribution of *C. nigrodigitatus* has been documented. Allison and Okadi (2009) reported the species as being highest by number at the bottom of Lower River Nun, with 33 (0.99%), followed by 30 (0.84%) in mid water and 29 (0.70%) at the surface. Ufodike and Zakari (1992) reported 13 fish families with 28 species in Dadin Kowa Reservoir, where *C. nigrodigitatus* had 0.02% abundance in the total catch and the least within the family with 0.2% abundance. Ogbeibu and Ezeunara (2005) reported 14 families with 28 species in Ikpoba River, where the family Bagridae (*C. nigrodigitatus* formerly belong) dominated (26%) followed by Malapteruridae (16%), Mochokidae (14%), Cichlidae (13%) and Clariidae (10%). Yem *et al.,* (2005) reported the species in a sampling site on Kainji Lake to make up 0.24% and 0.05% in terms of number and weight, respectively. Idowu and Ayoola (2008) reported that the Lagoon systems of Ogun waterside had 70.20% and 52.50% of the total catch in dry and wet season, respectively, with 55.70% of the overall catch to be *C. nigrodigitatus* and concluded that the species contributed the highest number of fish in the two seasons. Agboola *et al*. (2008) reported in Badagry Creek (Nigeria) that *C. nigrodigitatus* had the highest relative abundance with 15.7% followed by *Ethmalosa fimbriata* 15.5% and *Tilapia zillii* 8.5% while *Caranx hippos* with 0.9% was least. Offem *et al.* (2009) found that *C. nigrodigitatus* was the most abundant fish species in Cross River wetlands and 57 asserted for 12.8% and 4.3% of the overall catch in the wet and dry seasons, respectively. Allison and Okadi (2009) reported relative abundance of 0.82% of *C. nigrodigitatus*, which ranked 4th in abundance and scored as common in the lower Nun River in Niger Delta. Odo *et al.,* (2009) reported that out of 576 fish caught in Anambra River Basin, 13 in number were *C. nigrodigitatus*, which contributed only 2.3% to the overall catch from three sampling sites. Adeosun *et al. (*2011) reported that the species in Ikere Gorge contributed 96.7% and 34.51% in number and weight, respectively, and were more

abundant at the peak of the wet season in four sampling stations. *Chrysichthys nigrodigitatus* is known to be the most dominant fish, accounted for considerable number in both artisanal and trawl fishery of Cross River estuary (Victor *et al.,* 2013).

2.2.1.5 Freezing and Quality assurance

Freezing is a process carried out in appropriate equipment in such a way that the range of temperature of maximum crystallization of fish fluid is passed quickly at a temperature of -18 ºC or lower (Njoku, 2006). If the fish is frozen fast, protein denaturation which takes place in fish at a temperature of between -2 ºC to -5 ºC is skipped, thereby keeping the quality of the frozen fish intact.

The phrase "fish quality" refers not only to its freshness and appearance, but also the absence of several bacterial pathogens (*Mycobacterium* spp., *Vibrio vulnificus, Campylobacter jejuni*, *Photobacterium damselae*, *Clostridium botulinum*, *Streptococcus iniae,* and *Edwardsiella tarda*) that could be harmful to consumers. Thus, fish quality involves the elimination of chemical and microbiological hazards as well as the preservation of sensory, nutritional, and physico-chemical aspects that are appropriate to its intended function. Fish quality evaluation requires multiple approaches for assessment, such as sensory, biochemical, microbial, and physical, because of its decomposition complexity.

Sensory analysis is a scientific approach that elicits, analyzes, measurements, and deduces to expert panelist to food items. These responses include taste, appearance, texture, and odor (Bernardo *et al.,* 2020). There are some quality assessment methods that are present, such as the Quality Index Method (QIM) and the Torry method. The QIM is one of the most widely used sensory methods for assessing fish quality (QIM). The method quantifies the alteration in sensory

attributes, which is further utilized to find the shelf-life and equivalent days of storage (Esteves & Aníbal, 2021). The seafood group (crustaceans, bluefish, selachii, whitefish, and caphalopods), storage procedure, temperature, as well as the maximum number of QIM demerit points, all have a substantial influence on the correlation coefficients obtained between QIM and storage time (Esteves & Aníbal, 2021). However, QIM categorization is not justified, and hence the species-specificity of QIM schemes was concluded (Esteves & Aníbal, 2021). Moreover, QIM is an effective scheme to analyze the raw fish, whereas the Torry method is efficient to assess the freshness of processed fish fillets (Wu *et al.*, 2019). The Torry scheme can be used on the following parameters: 10 points (fresh in odor and taste), 5.5 points (limit for ingestion), 3 points (spoiled), and 0 points are considered as inappropriate for human consumption (Wu *et al.*, 2019). Other sensory methods, such as the sensory descriptive method classifies the magnitude and sensory differences, which can be used to assess fish quality (Bernardo *et al.*, 2020). For the sensory descriptive methods, a group of at least 10–12 assessors must be familiar with all of the sensory attributes being assessed on the product, as well as its strengths and weaknesses.

Protein denaturation as the name implies, is a slow irreversible change in the nature of the protein constituents of the flesh which alters the appearance, texture and flavour of frozen fish and increases the amount of thaw drip (Njoku, 2008). Fish is considered as properly frozen if after storage in the cold store, it cannot be differentiated from fresh fish on thawing (Eyo, 2001). Thus, a properly frozen fish must be well protected from microbial deterioration, dehydration, toughening and rancidity.

2.2.1.6 Plant and Animal Communities of Imo River

Imo River is blessed with a lot of both plants and fish species, which also reflects the composition of the Niger River being the major source of the lake. There are many aquatic plants in the lake. Among those identified are Pistia sp. and Salvinia sp. These are obligate or free floating water plants that require all vegetative parts to be submerged or supported by water in order to complete their generative cycle. Others include *Echinochloa crassipes*, *Vossia cuspidate*, Lemna sp., *Ipomoea aqautica*, *Vetivera nigritana*, Polygonum sp., *Typha australis*, *Sesbania dalzel*, *Nymphea lotus, Minosa pigra, Ludwigia decurrens* and *Eichhornis crassipes* etc. Adesina *et al.* (2011) reported 46 plant species in Imo River that belongs to 22 families, which included *Vossia cuspidate, Sesbania dalzielii, Eichhornia crassipens, Mimosa pigra, Marisans longbracteatus, Ipomoeo aquatica, Vetiveria nigritana* and *Polygonum lanigerium*. Aquatic macrophytes found in any water body play a significant role in nutrient cycle because they accumulate and release nutrients slowly, which are the major causes 58 of eutrophication of flood plains, associated lakes, swamps and ponds. Their stands can also serve as suitable spawning sites and feeding grounds for fish in the lake. Similarly, these plants also provide breeding substrate for a large number of insects and other invertebrates, which serve as food for fish.

Some of the fish species commonly found in the lake include *Synodontis membranaceus, Mormyrops deliciosus, Malapterurus electricus, Schilbe intermedius, Mormyrus rume,* Hydrocynus spp., *Hyperopisus bebe, Citharinus citharus,Alestes dentex,Bagrus bayad, Oreochromis niloticus, Clarotes laticeps, Labeo senegalensis, Auchenoglanis occidentalis, Brycinus nurse, Lates niloticus, Bagrus docmac, Mormyrops deliciosus, Clarias gariepinus, Alestes baremose, Tilapia zillii, Chrysichthys nigrodigitatus* (Abiodun and Odunze, 2011).

Plankton algae recorded on the lake include green (Chlorophyceae), blue green (Myxophyceae) and diatoms (Bacillariophyceae), while Cladocera, copepods and rotifer are the zooplankton. Others include fish larvae, ostracods, chironomid larvae and pupae, chaoborid larvae and pupae. Blue greens algae increased in number and produced a layer of bloom on the lake surface (Adesina *et al.,* 2011).

2.3 Sources of Energy

2.3.1 Smoke-dried with Charcoal

One major way by which catfish can be dried is the traditional open fire with either firewood or charcoal.

Charcoal is another source of energy for fish smoking.

On a basic level, charcoal is produced by burning wood or other organic matter in a low oxygen environment. Doing so removes water and other volatile elements, allowing the finished product, the charcoal, to burn at high temperatures with very little smoke. This is because charcoal is not wood cinders but is made by burning wood slowly in an oven with little air, turning it into carbon. Wood is made of fiber (cellulose) and minerals (metals). Charcoal can be made from any type of wood, and other organic matter, such as:

Coconut Shells

Groundnut Shells

Dry Leaves

Lump charcoal should be made from real wood (usually hardwoods).

Charcoal is mostly pure carbon known as char that is been produced by cooking of woods in low oxygen environment which takes some days and burns some volatile compounds like water, methane, hydrogen and tar, but when been produced in commercial quantities this burning takes place in large hole, concrete, bricks and steel silos with little oxygen's and it's been stopped halfway to avoid over burning that will turn it to ash. It contains black lumps and powder with about 25% of the original weight. It gives a historical explanation of wood energy and household perspectives in the rural setting and the history behind the production of wood charcoal dates back to the ancient period, about 4000 BC, in China and West Asia. North and South America, Africans and Europeans also made use of charcoal. (Mba, 2018). In ancient times, wood was piled up in small quantities, set on fire and then covered with dirt to ensure a long, slow burn with very little oxygen. As societies evolved and progressed, charcoal played an increasingly prominent role, being used for writing and drawing, as well as smelting metals, creating glass and as an important component of early gunpowder.

For all of these reasons, charcoal production has been extremely important throughout recent history. As every village needed charcoal, charcoal-burners created charcoal on a local level, slowly improving their production methods. In the last few years, economic hardship, poverty, unemployment, and an increase in the price of oil have necessitated the need for people to find alternative means of making a living in respect of domestic cooking energy in Nigeria. During the colonial periods, many people used firewood as domestic energy fuel, after the colonial era; there was a change in the status quo. People embarked more on using electricity, fossil fuels such as kerosene, and gas as cooking energy. At present, millions of households now use charcoal as domestic and outdoor recreational cooking energy due to epileptic power supply, scarcity and increase in the price of oil and gas (Tobias, 2007). Earth mound kilns were more efficient than

basic pit kilns where the charcoal was burned below ground level. However, as the carbonization process is very long, requiring continuous attention over ten or more days, kilns and charcoal production were later improved with the addition of chimneys for improved air control.

2.3.2 Smoke-dried with Firewood

Firewood is one the sources of energy used for fish smoking. Although many wood types may be used as for fish smoking, among the many factors influencing the choice of wood, what is used depends on local availability. The firewood preferences of most fish smokers are also related to the physical characteristics of the wood and how they affect the smoked product (Kordy las *et al.*, 1982; Nerquaye-Tetteh, 1985; Lartey, Asiedu & Okeke, 1994). Different firewood may affect the quality of the smoked-dried fish differently. It was from the method of Firewood that the term "smoked catfish" was coined. This is because, with this method, the heat required is quite low (as opposed to regular cooking heat) and most times there's a lot of smoke during the process. This is the most common method of drying fish in Nigeria and it involves the drying of the fresh fish on a smoking grate (smoking rack) with a bit of heat underneath from the burning of wood or charcoal. The rack might be placed on an open metal barrel or a mud structure. Ensure that the rack is slightly oiled before putting the fish on it and do not stack the fish on each other as this would make sum dry before others. Start a fire beneath the smoking rack and ensure that the heat is low especially for the first two hours. After the first two hours, you can increase the heat slightly. The drying time depends on the thickness of the fish, nature of the fuel, customer demand and weather. Avoid using wood or saw dust as these generate a lot of smoke while drying. The generated smoke if too much can cause the fish to be bitter and increase the concentration of the cancer causing PAHs. Charcoal is the preferred option. This method requires the regular flipping of the fish from one side to another in order to ensure that all parts of the fish are properly dried.

The organic constituents of wood are reported to include cellulose, hemicellulose, and lignin. When wood is burnt, the chemical compounds formed are broken down into many smaller compounds as a result of incomplete combustion (Cutting, 1965; FAO, 1970; Storey, 1982; Wheaton & Lawson, 1985). The characteristics of traditionally smoked products are to some extent dependent on the source of the smoke. A study on smoking fish with Eucalyptus wood in Zambia showed that the smoked product was golden-brown, and had desirable texture as well as an appealing smoky aroma. There was no bitter taste when eaten, and the product could sell well (Mvambazi *et al*., 1995).

Wood smoke is composed of vapours and particles that are easily taken up by moisture on the fish surface during smoking, and they contribute to the characteristic smoke smell and colour (Foster, Simpson & Campbell, 1961; Gilbert & Knowles, 2007; Hamm, 1977; Daun, 1979). In addition to smoke imparting colour and flavour enhancing ingredients to the smoked product, it also has anti-oxidative and bactericidal properties (Barylko-Pikielna, 1977). However, the effect of wood smoke on smoked fish is poorly documented. Smoking as a method of preservation produces commonly acceptable products as it imparts desirable colour and flavour (Idah and Nwakwo (2013). A combination of drying and smoking at temperatures of about 20-30°C for cold smoking and 70-80°C for hot smoking is used for smoked products (Food Reference Website, 2004, Obodai *et al*., 2009). Tawari and Abowei (2011) reported that when wood and sawdust are burnt, smoke is produced as a result of incomplete combustion. The results indicate that the different type of firewood affects the flavour of produced smoked fish. Not all wood chips are the same when it comes to smoking. Certain types of wood should never come near your smoker. Softwoods such as Cyprus, elm, redwood, fir, or spruce contain resins that leave residues on your smoker and create a thick, unpleasant smoke that can leave toxic residue on your fish. For this reason, you must use

store-bought wood chips or planks specifically for use with smokers so that you can be sure the type of wood you are using is safe for smoking.

In this research, Alder wood was used for fish smoking because after some research about woods, Alder wood is the best type of firewood used for fish smoking rather than the other woods. The taste is better and the flavour is smokier which give a distinct characteristic of a smoked fish. Many woods for smoking give off strong, bold flavours to their food which is great sometimes. And other times, you are looking for something balanced and delicate do not overpower the natural flavours of your food. That's where Alder wood comes in. It is not as widely known as a wood like Hickory, but it should be a top contender in any kitchen. Alder wood has a great flavour profile. The smoke is delicate, subtle and slightly sweet, making it the perfect go-to flavour. Other woods, like Hickory, have very strong smoke flavours, which can be overpowering for some meals. Whereas Alder should be a staple smoke flavour in any household. Its versatility is unmatched in the wood smoke world.

Table 2: Scientific Classification of Alder Tree

Kingdom	Plantae
Subkingdom	Tracheobionta: Vascular plants
Superdivision	Spermatophyta: Seed plants
Division	Magnoliophyta: Flowering plants
Class	Magnoliopsida: Dicotyledons
Subclass	Hamamelididar
Order	Fagales
Family	Betulaceae: Birch family
Genus	Alnus Mill
Specie	Incana

How to identify an alder tree. Alder trees are broadly conical in shape and have dark and fissured bark. Twigs are light brown with orange spots, and young twigs are sticky (as implied by the glutinosa part of alder's botanical name). Leaves grow from purple or grey leaf buds and are fresh green and rounded.

Fig 2: Images of Alder tree and its leaves for identification

2.3.3 Smoke-dried with Sawdust

Sawdust is made from woodchips. Woodchips and other smoke sources can vary significantly in the amount of heat and smoke they give off which makes getting consistently delicious results from fish smoking almost impossible. These woodchips that come from hardwood have a wide variation in both size and density. A few companies do make hardwood chips that are very close to uniform in size, but even then the density can be very different because they are made from natural hardwoods. On the other hand, sawdust, when it is compressed correctly, is one of the ideal materials to use for fish smoking. It burns slow so you can use it for both cold and hot smoking to produce an even and consistent smoke. The material offers less heat and prevents the smoke ring from forming under the fish top layer. However, they need to be replenished fairly often since they turn black quicker than other materials.

You can use hardwood sawdust with all types of food smoking from delicate fish and chicken. You can penetrate the smoke in various ways, but not all of them provide the same tender and juicy results you are looking for. If you ever experienced cutting wood, you will notice a large amount of sawdust, settling on the ground. Sawdust is a byproduct of wood and is great when it comes to penetrating smoke in the fish. It adds to the taste and does not burn the upper surface of the fish. Sawdust of the right type and amount adds a rich flavor to the fish you are smoking. Red Gum sawdust is used throughout the food industry for smoking smallgoods, fish and other products. This sawdust is sold per bag and is packed by volume and not weight which can vary with moisture content.

2.3.4 Oven-dried

Smoke and oven drying of fish concentrate can increase the nutrient value of fish since most of the moisture are lost during processing. Both smoke and oven drying are acceptable methods of processing fish. The overall preference and higher nutrient values of oven dried fish to smoke dried fish indicates that oven drying of fish is better and could complement fish smoking which is presently the popular method. The oven dried fish were also neater in appearance. One major way by which catfish can be dried is Kiln (oven) with either electricity, gas, wood or charcoal as the source of heat. Oven drying is the future of fish smoking because it is faster, healthier, neater, more environmentally friendly and has more capacity than smoking over an open fire. However, it is more expensive to set up. Generally, most kilns use gas with the option of electricity, charcoal or wood. Some extra features such as a thermometer (to monitor the temperature), blower (for proper heat distribution), and insulation (to prevent heat loss) can be added and that would cost you extra. These extras will reduce your drying time, ensure even output as well as reduce the effort required to dry the fish. The kiln has all the above features so once you put everything in motion, you can spend up to three hours without checking on the fish in the kiln.

2.4 Quality Assessment of Fish

Several methods are used to determine the quality of fish. These can be classified into sensory or organoleptic and instrumental methods. The latter comprises of organoleptic and microbiological methods.

2.4.1 Organoleptic assessment

Organoleptic or sensory assessment of food, according to Huss, (1995) and Meilguard *et al.,* (1991), is defined as the means of qualifying and interpreting variations in the quality of food or characteristics of food, (taste, appearance, odour) by using human senses of sight, smell, taste and touch. Studies have shown that assessment of food freshness characteristics using sensory methods are capable of giving objective and reliable results when assessments are done under a controlled condition. Generally, trained and experienced taste panelist is essential to obtain accurate and representative result (Connell, 2001). Sensory methods are divided into two groups, discriminative and descriptive test. However, the most commonly used is the descriptive test which measures the difference or absolute value indicating the different quantitative levels (Meilguard *et al.,* 1991; Huss 1995).

Sensory methods are known to be irrationally expensive due to the high training requirement of the panel, running cost, need for individual scheme for individual fish species given different spoilage patterns and physiological and psychological limitations of the analyst (Connell 2001).

2.4.1.1 Microbial method of fish quality assessment

The major changes in fish freshness for instance, unattractive changes in food characteristics such as flavour, colour and odours are largely due to bacterial growth and activity (Huss, 1995, Connell 1990).

Microbial methods are used to estimate bacterial numbers, in order to determine fish freshness, hygiene and or evaluate the possible presence of bacteria or organisms of public health importance (Huss, 1994, Huss 1995).

Microbial prediction/estimation of bacterial numbers therefore, in order to serve the purpose of food safety and shelf life determination, is expected to relate quantitatively to the characteristics of food during storage (Dalgaard, 2002).

According to Bourgeois & Mafart (1995), the various ways that can be used to determine bacteriological contamination in food/fish include: total plate count (TPC), and other instrumental methods e.g (ATP, microscopy, turbidometry, conductance, others). Total viable count/total plate count/standard plate count/aerobic plate count SPC, APC, all meaning the number of bacteria (colony forming units cfu/g or ml) in a food product under specified standard and uniform condition of culturing. In general, these methods rely on the estimation of the fraction of microflora able to produce colonies in the medium used under specified incubation conditions (Huss *et al.,* 2004). Therefore the temperature during the incubation of the plates has greater influence on the number of colonies developing in the sample thus, in the examination of psychrophilic bacteria, plating a 3-4 day incubation period at 25 degrees centigrade is recommended other than at 30 degrees centigrade or 37 degrees centigrade (Huss, 1995; Huss, 1994).

The enumeration of bacterial counts in food can be done using a variety of media. For instance, plate count agars (PCA) is commonly used for enumeration of bacteria (Huss, 1995; ICMSF, 1998).

2.4.1.2 FAO/WHO Recommendations on Fish/Food Standards and Safety

The 1996 world food summit plan of actions recognized the importance of food safety, as it defined food security as "when all people have access to sufficient, safe and nutritious food."

An overview of the components of food safety management system in Africa including Nigeria and actions required to address deficiencies (FAO/WHO, 2005) include:

i. National food safety policy:

Coherent national food safety policies are the foundation for effective food safety management systems. In general, food safety concerns are not adequately addressed in national governmental policies in most African countries; therefore, coordinated and sustainable approaches to the holistic management of food safety cannot be adopted. As previously indicated, most countries of the region do not appreciate the major public health and economic implications of food safety, so food safety remains a low priority in national policy making. Therefore, governments of the region must work to understand the public health and economic benefits of improving food safety systems, and, accordingly, develop coherent national food safety policies, in consultation with all stakeholders, including the food industry, relevant research institutions and consumers (FAO/WHO, 2005).

ii. Food legislation

The traditional food control systems in most African countries do not provide the concerned agencies with a clear mandate and authority to prevent food safety problems. Furthermore, food legislation that is in line with international requirements (Codex) is lacking in many African countries. Enforcement of food legislation is also problematic, often resulting in insufficient consumer protection against fraudulent practices and contaminated food products, and leading to the importation and domestic production of substandard food items as well as trade rejections of food exports from the region. The informal sector, which is often a significant producer and distributor of fresh and processed food products (including street foods) for direct consumption, is

often outside the scope of official control systems and remains the least controlled, except by municipal environmental hygiene authorities. Governments are encouraged to utilize tools and advice provided by FAO and WHO in the development of food legislation as well as all other aspects of national food control systems. In particular, the 2003 FAO/WHO guidelines for strengthening National food control systems offers interesting options that may be considered in this field (FAO/WHO 2003).

iii. Development of national food standards

Globalization of food markets compels nations to develop food standards that are responsive to the needs of users as well as being accepted and recognized internationally. The WTO SPS Agreement stipulates that national sanitary and phytosanitary standards that are based on internationally agreed Codex Alimentarius, IPPC or OIE standards do not require further scientific justification. Some of the countries of the region have national standards bodies that establish food standards, often based on the relevant Codex standards. However, the food standards authorities in many other countries are not well defined and are not actively engaged in the establishment of national food standards.

As part of the overall food safety management system, national governments should establish food standards based on the Codex Alimentarius. Similar to food safety policies and legislation, all stakeholders, including consumers, must be involved in the development of national food standards.

iv. Science-based risk assessment of food safety issues:

While there is an almost universal agreement that a sound scientific risk assessment is an essential part of the basis for any food safety risk management decision, meeting the need for competent, timely and independent risk assessments presents a considerable challenge to most African countries. Risk assessments are needed for establishing relevant food safety legislations, as well as to assist in the establishment of food inspection priorities and other food safety policies. FAO and WHO have recently developed a Food Safety Risk Analysis Manual that further describes the concept and process of risk assessment, as well as risk management and communication.

However, the number of food safety hazards whose risk must be assessed is large, and expanding. The magnitude of adverse health effects associated with food contaminants continues to expand as scientific research develops additional ways to measure harm. Almost all African countries face similar problems of lack of expertise and difficulty in collecting their own toxicological and exposure assessment data to conduct risk assessments.

Governments of the region should utilize the risk assessments carried out by the FAO/WHO risk assessment bodies in their food safety decision-making. Countries must also actively supply their national data on contaminant levels, food consumption patterns, and all other data requested by the FAO/WHO risk assessment bodies so that these international assessments accurately reflect the situation in countries of the region.

v. Inspection mechanisms/schemes

An effective food safety management system requires clear inspection policy and procedures that are applied by inspectors who are well trained not only to apply these procedures but also to act as quality assurance advisors and extension officers to the food industry. Food inspectors in Africa

suffer generally from (i) a low professional status which is not commensurate with their responsibilities, (ii) a lack of logistical support to carry out the inspections (transport, inspection equipment, etc.) and (iii) the cumulative tasks often requested from them (price control, inspection of non-food consumer items, weights and measures, environmental hygiene, etc.). National food inspection services are often located in the capitals and major cities, with little if any control exercised in small towns and rural areas. Few countries of the region have efficient national import/export inspection and certification systems. Some countries do conduct partial inspections of meat and/or fish imports and exports. In countries where a strong export market exists in a particular sector, the inspection services are often engaged in the control of the concerned products. In order to benefit from potential food export earnings and to protect themselves against sub-standard imported foods, governments of the region must actively upgrade their inspection systems, in both quality and quantity, to meet their national needs in this field.

vi. Laboratory support service:

Effective enforcement of food legislation and the implementation of food-borne disease surveillance systems require sound and efficient food analysis capabilities at national and sub-national levels. Unfortunately, food control laboratories in the African region are generally very weak. The majority of public health laboratories do not have the capacity to test for chemical contaminants and naturally occurring toxins. Some identified causes of this weakness are as follows:

• Inadequate resources in terms of funding, equipment and personnel;

• Lack of recurrent expenditure to enable the repair of equipment and to maintain adequate supplies of chemicals and materials needed for analyses; and

• Inadequate quality assurance procedures.

Only a few of the testing laboratories in Africa are accredited for specific tests in accordance with the quality, administrative and technical requirements of ISO 17025, the international standard that provides general requirements for the competence of testing and calibration laboratories. As a result, competence in terms of equipment and operator skills, as well as reliability of results may not be satisfactory. Furthermore, food exporters may need to send samples of their products to accredited laboratories outside the country for testing in order to be accepted by the importing country. This adds to the cost and inconvenience of the process of exporting foods from the region.

The countries of the region must give greater priority to strengthening food control laboratories. Neighbouring countries could also work together to develop inter-laboratory testing programmes, joint training programmes or even sub-regional laboratories that could serve the needs of multiple countries. Governments could also work to strengthen public-private partnerships between laboratories to better utilize scarce resources within a country.

vii. Information network on food safety issues

An increasingly important role for national food control systems is the delivery of information and advice to stakeholders across the farm-to-table continuum, both within the country and in other countries. These activities include the following:

• the provision of balanced factual information to consumers and the media;

• the supply of information packages and educational programmes for key officials and labourers in the food industry;

• the provision of reference literature to extension workers in the agricultural and health sectors;

• sharing relevant food safety information with other countries, especially within the region.

viii. Training/education in food safety

It is generally recognized that knowledge related to food safety provides the basis for the development of intervention strategies and initiatives aimed at preventing food-borne illness. However, no single country in the region has established on-going educational programmes for government food control officials, food industry officials and/or consumers. Training/education that does exist is sporadic, not focused and not based on actual and/or possible food safety problems.

ix. Customer awareness raising:

The importance of consumer education in the prevention of food-borne illness is universally recognized. When consumers are quality and safety conscious, they are able to complement the efforts of food control agencies in encouraging the food industry to provide good quality and safe food.

In view of the catalytic role played by consumer associations in promoting the quality and safety of food supplies, governments of the region should facilitate the establishment and sustainability of these associations. These associations are active in some parts of Africa, but should be encouraged to increase their efforts to educate consumers and to hold the food industry and governments accountable for safe and high quality food.

x. Coordination of food safety activities:

Assuring food safety in a global economy requires a high degree of communication, coordination, and cooperation within and between countries. Management of food safety is a multi- sectoral affair, often involving the ministries of health, agriculture, trade/industry and at times fisheries, tourism, and local governments. In the absence of a well-defined national food safety policy with implementation plans, these organizations tend to operate in accordance with their own aspirations of food safety. Furthermore, without well-established responsibilities for these organizations, the scarce resources available in the countries of the region often dissipate through the duplication of efforts. When agencies are nominated to coordinate national food safety activities, they often lack the required resources to perform assigned duties effectively.

xi. Epidemiological surveillance of food-borne diseases:

As previously indicated, many food-borne disease incidents are reported every year in Africa. Numerous factors, many of which are discussed in this document, contribute to this high number of incidents. However, it is extremely important to note that most cases of food-borne disease in the region are not reported, so the true extent of the problem is unknown.

In most countries in the region, the surveillance infrastructure for food-borne diseases of both microbiological and chemical etiology is weak or non-existent. With the exception of cholera (which is subject to the WHO International Health Regulations), there is no obligation to report food-borne disease internationally. Only some of the countries of the region require national reporting of food-borne disease incident and even fewer actually have accurate reporting. This absence of reliable data on the burden of food-borne disease impedes understanding about its public health importance and prevents the development of risk based solutions to its management.

2.4.1.3 Public health significance of diseases of fish in fishing industry

A few bacterial diseases and certain parasitic infection of fish are known to be transmissible to man; the known viral and mycotic diseases of fish are not transmitted to a man, however, when considering fish as food, their diseases are important for two main reasons (1) the palatability or wholesomeness of diseased fish maybe impaired; (2) the available quality of saleable fish maybe reduced. Either condition may make fish unacceptable for human consumption (Emikpe e*t al*., 2011).

But man is able to detect and control and sometimes prevent these diseases, such activities cannot be undertaken in the natural environment, thus Capture fisheries is therefore more vulnerable (Njoku, 2006).

2.4.1.4 Bacteria diseases in fish:

Bacteria are responsible for many fatal diseases in fishes like *furunculosis, columnaris,* fin and tail rot, vibriosis, dropsy, cotton mouth disease and tuberculosis.

Some bacterial diseases include;

a. Furunculosis Disease:

Furuculosis disease is caused by Aeromona salmonicida in salmon fishes. It is a non-motile, gram-negative bacterium.

This disease frequently appears to infect fishes living in the dirty waters containing a large amount of decaying matter.

The first symptoms of this disease are appearance of boil like lesions.

Others symptoms are blood-shot fins, blood discharge from the vent, haemorrhages in muscles and other tissues and necrosis of the kidney.

Bursting of boils allow the spread of this disease among other fishes and also offer suitable areas for fungus growth.

Fishes severely infected with the bacteria die in good number.

b. Columnaris Disease:

Columnaris disease is caused by *Chondroccus columnaris* and *Cytophaga columnaris* in many freshwater aquarium fish.

It is a long, thin, flexible, gram-negative slime bacterium (myxobacteriales).

This disease is often associated with low oxygen level.

Initially it is marked by appearance of grayish-white or yellowish-white patches on the body.

The skin lesions change to ulcerations and fins may become frayed.

Gill filaments are destroyed and eventually lead to the death of the fish.

Addition of 1 ppm copper sulphate in the pond to control this disease is effective.

c. Fin and Tail Rot Disease:

Fin and Tail Rot disease is caused by *Aeromonas salmonicid* and *A. liquefaciens.* However, protozoans and fungi may also be involved.

It is characterized by appearance of white lines along the margins of fins, the opacity usually progresses towards the base eroding them and causing haemorrhage.

The fin rays become brittles first and later break leading to the complete destruction of the fins. The infection may also spread on the body surface.

d. Vibriosis Disease:

Vibrio bacteria are the causative agents of vibriosis disease in salmon and many other fishes.

This disease may occur in waters with low oxygen. These bacteria are small gram-negative bacilli, characteristically curved.

Diseased fishes show large, bright coloured, bloody lesions in the skin and muscles, haemorrhages in eyes, gills may bleed with slight pressure, and inflammation of the intestinal tract.

e. Dropsy Disease:

Pseudomonas punctata is the causative agent of this disease. It is characterized by accumulation of yellow coloured fluid inside the body cavity, protruding scales and pronounced exopthalmic conditions. This is known as “Intestinal Dropsy”.

In case of ulcerative dropsy, ulcers appear on the skin, deformation of back bone takes place and show abnormal jumping. This is a fatal disease in culture systems.

f. Cotton Mouth Disease:

The filamentous bacteria, Flexi bacteria are the causative agent of this disease. The main symptom is appearance of fungus like tuft around the mouth.

g. Tuberculosis Disease:

Mycobacterium is a disease causing agent which is difficult to diagnose without pathological examinations.

The symptoms are ulcers on body, nodules in internal organs, fin and tail rot, loss of appetite and loss of weight of fish.

h. Bacterial Gill Disease:

This disease is caused by Myxobacteria in salmon fish.

Many bacteria are found in swollen gill lamellae which show proliferation of the epithelium, and symptoms are lack of appetite.

This disease is transmitted through water from infected fish.

g. Viral Diseases:

Descriptions of viral diseases of fish are rapidly expanding. Viruses are being reported in new species, and interpretation of the significance of findings is also changing. Several viral diseases of ornamental fish are reportable**.**

Although viruses of homeothermic animals are cultured at uniform temperatures, fish viruses have wider, but specific, temperature tolerances in fish cell cultures at lower temperatures. Because of this relatively defined temperature range, variation in temperature may enable control, although often it merely induces latency. Because many viral diseases of fish are geographically limited, regulatory agencies and fish farms in disease-free areas consider them exotic diseases and require certification of introduced stocks. Many result in high mortality in

young fish and little or no losses in adults, which may become carriers. For these reasons, avoidance of carriers and certification of specific pathogen-free replacement stocks are frequently required.

Specific testing procedures are available. Most vaccines used for control of fish diseases are for bacterial agents; however, use of vaccines to control some viral diseases is being introduced. Drugs are not effective; however, antimicrobials and other drugs may be used to control secondary bacterial infections. Management techniques that minimize stress and crowding, biosecurity measures, and temperature manipulation hold the greatest promise for control of piscine viral diseases.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

Imo River (Fig 3) originates from Okigwe in Imo State, Southeastern Nigeria and flows about 240 kilometers into the Atlantic Ocean via the River Niger drainage system (Amangabara 2015). The maximum elevation is found in the upper part of the basin with a height of 417m above mean sea level (MSL), and the minimum height is in the lower part of the basin with a height of 5m above MSL (Amangabara 2015). The river serves as source of water for domestic uses, fishery, recreational activities, and agricultural irrigation programs for more than 5 million people settling close to the water body. Apart from the afore listed uses, the river serves as recipient of industrial effluent discharges and oil spill from oil exploration activities, dumping site for domestic wastes including sewage and industrial solid waste, and runoffs from agricultural lands. Fish sampling was carried out at Owerrinta axis of the river. Owerrinta is a border town between Imo and Abia States which the river traverses on its way to Rivers State.

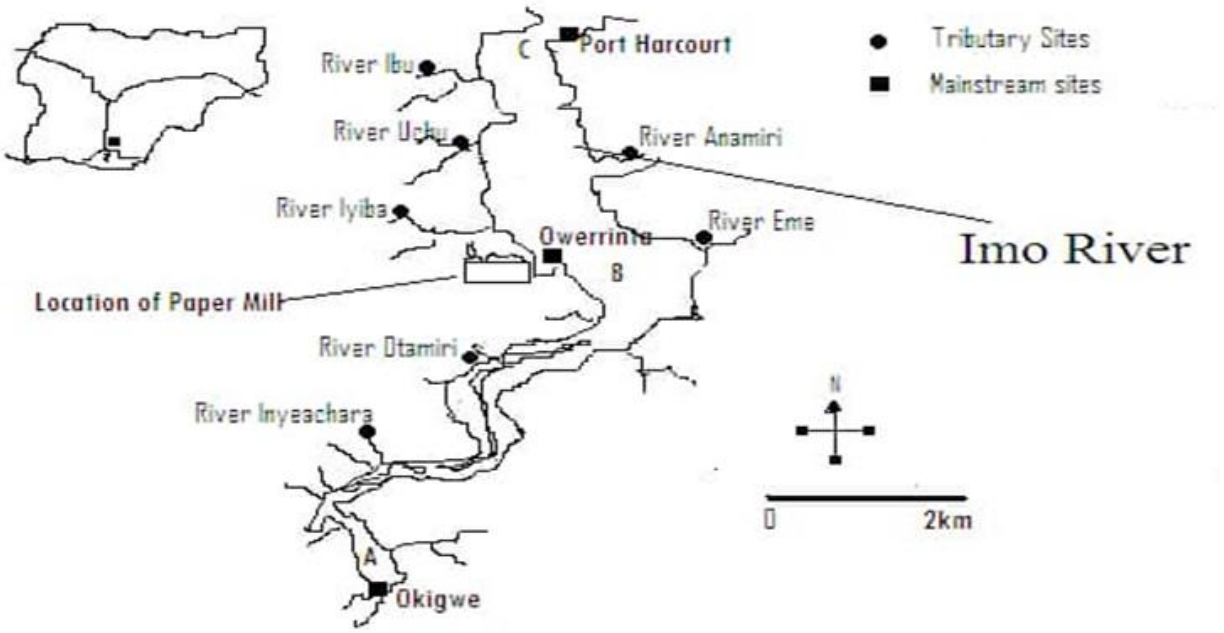

Figure 3.1: Map of Imo River showing the sampling site at Owerrinta, the study area. (Source : Ukagwu *et al.*, 2012)

3.2 Experimental Design

Three hundred adult individuals of *C. nigrodigitatus*; were collected from commercial catches in Imo River around Owerrinta/Obowo axis of the river. The fish were identified in the field using the fish identification key for freshwater species by Olaosebikan and Raji (2004). The fish were preserved in iced boxes and gradually transferred to the Fisheries and Aquaculture wet laboratory of Federal University of Technology Owerri. The fish were then divided into 4 equal batches of 75 fish each and randomly assigned to each of the processing/drying methods as treatments and designated as Treatment – T1 (Charcoal), T2 (Firewood), T3 (Sawdust) and T4 (Oven). Each treatment was subsequently divided into three replicates of 25 fish per replicate in a 4×3 completely randomized experimental design (CRD). The design layout of the experiment is presented in Table 3.1.

Table 3.1 Design layout of the experiment

Replicates	Treatments (Drying method)				Replicates in Total
	T1	T2	T3	T4	
	Charcoal	Firewood	Sawdust	Oven	
1	25A	25B	25C	25D	100
2	25B	25C	25D	25A	100
3	25C	25D	25A	25B	100
Treatments total	75	75	75	75	300

ABC = Treatments randomly assigned to replicates

3.3 Experimental Procedure

3.3.1 Collection of Fish samples and Identification

300 adult fish samples of *C. nigrodigitatus* were used for the experiment. The fish samples were brought from fishers at landing sites around Owerrinta/Obowo. The fish were identified with the aid of standard identification keys by Reed *et al* (1967); Loveque *et al* (1991) and Olaosebikan and Raji (2004). Fish identification was carried out at the landing sites.

Fig 4: Iced *C. nigrodigitatus* in a container

3.3.2 Fish Processing and Smoking

(i) Smoking Kiln and Oven

The fish samples were gutted and washed thoroughly with very clean water, then salted and placed in the smoking trays/chamber.

The Altona-type of smoking kiln at the fish processing unit of the Department of Fisheries and Aquaculture Technology, Federal University of Technology Owerri was employed. It has three compartments and designed to utilize different sources of heat smoke, from firewood, charcoal or sawdust. The kiln has a holding capacity of 250kg of fish in a batch. The oven at the processing unit can be fired either by gas or charcoal and has a capacity of over 300kg of fish per batch.

(ii) Arrangement of fish in the smoking Kiln/Oven

A whole fish was washed under running water before processing. All slime, blood and scales were removed from the cut surfaces of the fish and was placed on the oven floor while the fish were placed on the oven racks above. The smoking kiln or oven were then closed to expose the fish to the drought temperature and wood smoke and smoke components until the fish was fully cooked. While smoking, the fish are inspected every 20 minutes to ensure that the fish are smoked properly and to know when it was due to turn the fish in the racks to avoid charring.

(iii) Smoking/drying temperature and duration

The hot smoking method is adopted for the trial. The smoking temperature (drought) was 80°C in the kiln and oven with the thermal process lasting for 2 hours. Thermal process for charcoal and firewood employed a drought temperature of 70°C with a thermal process period of 3 hours. The moisture content of finished product was about 15 – 20%.

(iv) Smoking Process

Smoking of the fish was carried out for three hours or less, from the smoke generated by the combustion of the charcoal, firewood, sawdust and oven from alder wood. The processed fish after smoke-drying were packed in different labelled cartons to prevent microbial spoilage and stored at room temperature of between 24^0C and 27^0C in the Fisheries Laboratory of Federal University of Technology, Owerri, Imo State for further analysis.

Fig 5: Diagram of Altona-type smoking kiln

Fig 6: Fish Smoke-dried with Charcoal

Fig 7: Fish Smoke-dried with Firewood

Fig 8: Fish Smoke-dried with Sawdust

Fig 9: Oven-dried Fish

3.4 Laboratory analysis

The wholesomeness (quality status) of the smoke-dried fish, was determined using two standard analytical methods namely, organoleptic and microbial techniques.

3.4.1 Organoleptic analysis

The organoleptic or sensory assessment of the fish samples was in accordance with Eyo (2001) for smoked-dried samples.

Sensory evaluation of the smoked-dried *C. nigrodigitatus* using the four heat sources (T1, T2, T3 and T4), was carried out by 20 panelists randomly selected, from amongst the staff and students of the Department of Fisheries and Aquaculture Technology of Federal University of Technology Owerri.

Questionnaires for the panelists (Appendix A) were prepared using a 5-point hedonic scale in accordance with Eyo (2001)

The 20-man panelist compared and graded the quality status of the smoked fish based on their appearance, taste, aroma and texture, using a 5-hedonic scale as described by Eyo (2001) as follows: (Excellent = 9-10, Very Good = 7-8, Good = 5-6, Fair = 3-4, and Bad = 0-2). The grades were allotted depending on the perception of each panelist. The opinion of the panelist was recorded and analysed.

Table 3: The 5-points hedonic scale employed for organoleptic assessment of the smoke-dried fish samples

Quality Score	Remark
9-10	1st quality (Excellent)
7-8	2nd quality (Very good)
5-6	3rd quality (Good)
3-4	Limit of acceptance (Fair)
0-2	Unacceptable/rejected

Source: Eyo, (2001).

3.4.2. Microbial Analysis

Plate count and total viable bacterial count (TVBC) methods were employed in microbial evaluation of the smoked-dried fish. The total coliform count of bacteria was determined according to the method used by Fawole and Oso (1995). Laboratory analysis was carried out at the Federal University Teaching Hospital, Owerri.

1 gram of the smoke-dried fish was blended in a sterile blender differently and the blender was carefully cleaned and disinfected in between samples to prevent any cross contaminations.

Furthermore, the one in ten-fold dilution of each of them were made, using one part of the blended fish and nine parts of sterile peptone water. It was mixed and allowed for 2 miniutes. After 2 minutes, each of them was inoculated in a plate differently. Macconkey agar medium was used to culture the organisms. After inoculation, it was incubated at 37ºC for 24 to 48 hours in an incubator in the laboratory, after which the organism was identified.

3.4.3. Identification of microbial isolates

Microbial isolates of the four differently smoked fish were identified after they were cultured in the same media under similar culture conditions. To identify the organisms, gram staining technique was carried out to isolate *Klebsiella spp* and *Escherichia coli* which may be gram negative rods or gram negative bacilli. This was followed by a mortality test to determine the mortality or otherwise of *Escherichia coli* and *Klebsiella spp*. This was followed by indole test of *Escherichia coli* and *Klebsiella spp*. The two organisms were differentiated by carrying out the citrate test. *Escherichia coli* is citrate negative, while *Klebsiella spp* is citrate positive.

Microbiological characteristics of the various bacteria isolates were noted in agar plate and after staining reactions, individual microbial species were identified as described by Slaby *et al;* (1981). The colony-forming unit (cfu/g) per gram of sample was determined using the formular.

Viable bacterial count (cfu/g) = N/V×D

Where N = Mean number of colonies

V = Volume of dilution pour as plated

D = Dilution factor

Gram staining :

The Gram staining process include four basic steps, including: Application of a primary stain (crystal violet), addition of Gram's iodine, rapid decolorization with ethanol, acetone or mixture of both, and counterstaining with safranin. Gram staining is a common technique used to differentiate two large groups of bacteria based on their different cell wall constituents. The Gram stain procedure distinguishes between Gram positive and Gram negative groups by coloring these cells red or violet. Gram positive bacteria stain violet due to the presence of a thick layer of peptidoglycan in their cell walls, which retains the crystal violet these cells are stained with. Alternatively, Gram negative bacteria stain red, which is attributed to a thinner peptidoglycan wall, which does not retain the crystal violet during the decoloring process.

Materials

- Crystal violet (primary stain)
- Iodine solution/Gram's Iodine (mordant that fixes crystal violet to cell wall)
- Decolorizer (e.g. ethanol)
- Safranin (secondary stain)
- Water (preferably in a squirt bottle)

Procedure

(1) A slide of the cell sample was stained. The slide was heated carefully by passing the slide over a bursen burner three times.

(2) The primary stain crystal violet was added to the sample and incubated for one minute. The slide was rinsed with a gentle stream of water from pipette for a maximum of five seconds to remove unbound crystal violet. Gram'a iodine was added and left for one minute. The sample/slide was rinsed with acetone or alcohol for three seconds, followed by a stream of water. The secondary stain (safranin) was added to the slide and incubated for one minute. It was washed with a gentle stream of water for five seconds. Where the bacteria is gram positive, it retained the primary stain safranin. Where the bacteria is gram negative, it loses the primary stain and takes the secondary stain causing it to appear red when viewed under a microscope.

(3) Cultural characteristics were carried out and they were the same. Both *Escherichia coli* and *Klebsiella species* grow as large colonies on the plate and both of them also ferment lactose on Macconkey agar. Their colonies are moist and raised and some of them are muccored, but *Klebsiella species* is more muccored than *E. coli.* Isolates were gram stained and subjected to appropriate tests which include; Indole test, Mortality and Citrate test (Slaby *et al;* 1981)

(4) It is usually viewed under the microscope using the oil immersion lens (x100). The gram reaction was noted.

The method employed was that of Slaby *et al* (1981) for the identification of specific spoilage organisms and they include:

(i) Indole test:

This was used in the identification of some entero-bacteria like; *E.coli.* The test organism was cultured in peptone water at 37ºC for 24 hours. Indole test was used for characterization of coliforms. Indole test is one of the ideal methods used to distinguish the *E.coli* from *Enterobacter and Keibsiella*. The method distinguishes and

characterizes the bacteria based on the ability of bacteria to degrade the tryptophan (an amino acid) into indole.

Procedure:

1. On the first day, the tryptone broth was prepared as per the mentioned composition, and the pH was adjusted to 7.5. Then, the media test tubes were dispensed and the test tubes containing the prepared media were autoclaved.
2. Under sterile condition, the test organism in the media (dispensed in the test tubes) was inoculated and the test tubes were incubated for 24 hours at 37°C.
3. On the second day, the incubated tryptone broth test tubes were collected from the incubator and the biochemical Kovac's reagent was then added. The test tubes were allowed to stand for 5 to 10 min.
4. It was also observed that the cherry red colour ring is either formed or not formed. If Cherry red colour ring is formed then it is Indole positive and if there is no ring formation it is indole negative.

(ii) **Citrate test:**

The citrate utilization test differentiates organisms on the basis of their ability to use citrate as a sole source of energy.

- The citrate test is performed to differentiate Gram-negative bacilli of the Enterobacteriaceae family.
- It is an important test that allows the species-level identification of the members of the Enterobacteriaceae family.
- The test is also called Simmon's citrate test as it utilizes Simmon's citrate agar that contains citrate as the major source of energy.

Procedure:

- In a beaker, 24.28 grams of the dehydrated powder or lab-prepared media was added to 1000 milliliters of pure distilled or deionized water. The solution was then heated to bring it to a boil in order to dissolve the medium completely and the dissolved medium was then dispensed into tubes and sterilized in an autoclave at 15 lbs pressure (121°C) for 15 minutes.
- When the autoclaving process was completed, the tubes were taken out and cooled at a slanted position to a temperature of about 40-45°C. The position was maintained in order to obtain butts of 1.5 – 2.0 cm depth.

Fig 10: The researcher identifying the micro organisms

3.5 Statistical Analysis

Results of organoleptic assessment was analyzed using simple descriptive statistical methods such as percentages, histograms, bar charts and pie charts in accordance with Njoku, (2004), while microbial evaluation results were analyzed with one-way analysis of variance (ANOVA) in accordance with Njoku *et al* (1998).

The mathematical model is as follows:

CRD, xij = M + Eij

Where;

Xij = Value of independent observations

M = Uknown population parameter

Ti = Treatment effect (Source of heat)

Eij = Experiment error

Mean treatment separation was carried out using the Duncan's multiple range test (DMRT), while mean treatment effects was evaluated at Fά = 0.05. For the purpose of this, the computer statistical package for social science (SPSS) version 16, window 8 was employed.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Organoleptic Assessment

4.1.1.1 Orgonoleptic Assessment of Charcoal-dried fish

Table 4.1 shows the result of the average score opinions of the panelist on organoleptic assessment of fish using charcoal. The color/ appearance and taste of fish under charcoal shows the same mean score of 9.0 ± 0.10 respectively which implies excellent. Also, the aroma and texture of fish under charcoal shows a mean score of 7.8 ± 0.06 and 7.8 ± 0.12 respectively which implies very good aroma respectively.

Table 4.1: Organoleptic Assessment of Charcoal dried-fish

Sensory index	Mean ($\bar{x}$ ± SE)	Std. Deviation
Color/Appearance	9.0 ± 0.10	0.641
Aroma	7.8 ± 0.06	0.405
Texture	7.8 ± 0.12	0.758
Taste	9.0 ± 0.10	0.641

4.1.1.2 Organoleptic Assessment of Firewood-dried fish

Table 4.2 shows the result of the average score opinions of the panelist on organoleptic assessment of fish using firewood. The color/ appearance and taste of fish using firewood gave a mean score of 7.2 ± 0.16 and 7.4 ± 0.13 respectively which implies excellent. Also, the aroma and texture of fish using firewood gave a mean score of 7.6 ± 0.08 and 7.6 ± 0.13 respectively implying that the flavour and texture of fish dried using firewood was very good.

Table 4.2: Organoleptic Assessment of Firewood-dried fish

Sensory index	Mean ($\bar{x}$ ± SE)	Std. Deviation
Color/Appearance	7.2 ± 0.16	0.992
Aroma	7.6 ± 0.08	0.496
Texture	7.6 ± 0.13	0.810
Taste	7.4 ± 0.13	0.810

4.1.1.3 Organoleptic Assessment of fish dried with Sawdust

Table 4.3 shows the result of the average score opinions of the panelist on organoleptic assessment of fish using sawdust. The color/ appearance and taste of fish using sawdust shows a mean score of 7.8 ± 0.06 and 9.0 ± 0.14 respectively which implies excellent. The flavour and texture of fish using sawdust gave a mean score of 7.8 ± 0.06 and 7.8 ± 0.12 respectively which implies that the flavour and texture of fish dried using sawdust was very good.

Table 4.3: Organoleptic Assessment for Sawdust

Sensory index	Mean ($\bar{x}$ ± SE)	Std. Deviation
Color/Appearance	7.8 ± 0.06	0.405
Odour	7.8 ± 0.12	0.758
Texture	7.8 ± 0.12	0.758
Taste	9.0 ± 0.14	0.906

4.1.1.4 Organoleptic Assessment of Oven-dried fish

Table 4.4 shows the result of the average score opinions of the panelist on organoleptic assessment of fish using oven. The color/appearance and taste of fish using oven shows a mean score of 9.5 ± 0.12 and of 9.7 ± 0.11 respectively which implies excellent color/appearance and taste. Similarly, the aroma and texture of fish using oven gave a mean score of 9.4 ± 0.12 and 9.3 ± 0.16 respectively which implies very good aroma and texture of fish.

Table 4.4: Organoleptic Assessment of Oven-dried fish

Sensory index	Mean ($\bar{x}$ ± SE)	Std. Deviation
Color/Appearance	9.5 ± 0.12	0.514
Odour	9.4 ± 0.12	0.502
Texture	9.3 ± 0.16	0.686
Taste	9.7 ± 0.10	0.461

4.1.1.5 Comparative Assessment of quality criteria of fish smoked using different sources of heat

Fig 4.1 is a multiple bar chart showing the relative sensory quality of fish smoke-dried using the different sources of heat. In terms of appearance/colour of the finished product, Charcoal (T^1) and Oven (T^4) gave the best result of 9.0 and 9.5 scores respectively which represents excellent product. This was followed by Sawdust (7.2) and Firewood (7.2). The best aroma came from Oven with a score of 9.4 (excellent), followed by Charcoal and Sawdust (both 7.8) and Firewood (7.6) implying very good product. In terms of texture, the Oven-dried fish was adjudged best with a score of 9.3 (excellent), followed by Charcoal and Sawdust (both 7.8) or very good product, then Firewood (7.6). The best product in terms of taste were obtained from Oven-drying (9.7), Charcoal (9.0) and Sawdust (9.0) all implying excellent products, followed by Firewood (7.4) or very good.

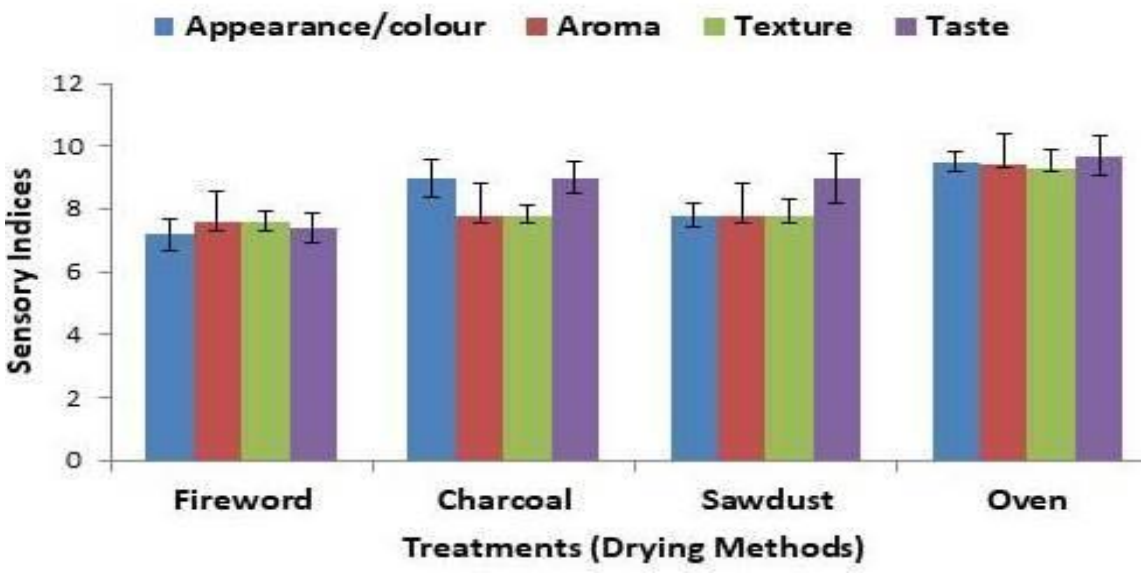

Fig 11 showing multiple bar chart

4.2 Microbial Evaluation

4.2.1 Microbial Isolates of differently smoke-dried fish

Table 4.5 shows the microbial load of processed *C. nigrodigitatus* using the different sources of heat. The result shows that average Coli form count in fish dried using charcoal was 2.6 x 10^2 cfu/g, 2.9 x 10^2 cfu/g (firewood), 2.6 x 10^2 cfu/g (sawdust) and 2.2 x 10^2 cfu/g (oven). The mean coli form count was 2.575 x 10^2. For *E.coli*, 1.5 x 10^2 cfu/g was estimated in fish dried using charcoal, 1.8 x 10^2 cfu/g (firewood), 1.6 x 10^2 cfu/g (sawdust) and 1.2 x 10^2 cfu/g (oven). The mean coliform count for *E.coli* was 1.525 x 10^2. Similarly for *K. spp*, 1.1 x 10^2 cfu/g was estimated in fish dried using charcoal. Others are 1.1 x 10^2 cfu/g (firewood), 1.0 x 10^2 cfu/g (sawdust) and 1.0 x 10^2 cfu/g (oven). Mean coliform count for *K. spp* was 1.05 x 10^2. In addition, the total plate count and total viable bacterial count (TVBC) was also estimated in fish dried using the different sources of heat.

Table 4.5: Microbial Load of a processed *C. nigrodigitatus*

Heat Sources	Coliform (cfu/g)	*E Coli* (cfu/g)	*Kleb* (cfu/g)	TVBC (cfu/g)
Charcoal	2.6 x 10^2	1.5 x 10^2	1.1 x 10^2	7.7 x 10^2
Firewood	2.9 x 10^2	1.8 x 10^2	1.1 x 10^2	8.6 x 10^2
Sawdust	2.6 x 10^2	1.6 x 10^2	1.0 x 10^2	7.9 x 10^2
Oven	2.2 x 10^2	1.2 x 10^2	1.0 x 10^2	5.9 x 10^2
Mean	2.575 x 10^2	1.525 x 10^2	1.05 x 10^2	7.52 x 10^2
St. Dev	0.287 x 10^2	0.25 x 10^2	0.0577 x 10^2	1.15 x 10^2

4.2.2 Comparative Assessment of microbial loads of smoke-dried fish using the different sources of heat

Table 4.6 – 4.8 shows the statistical comparison of microbial loads of fish smoked-dried using different sources of heat.

4.2.2.1 Comparison of Coliform counts in smoke-dried *C. nigodigitatus* using (Charcoal, Firewood, Sawdust and Oven)

Table 4.6 presents the statistical comparison of Coliform counts in smoke-dried fish using different sources of heat. The result shows that microbial load was the same in fish smoked with Charcoal and Sawdust (2.5×10^2 ± cfu/g) and (2.47×10^2 ± cfu/g) approximately (2.5×10^2 ± cfu/g) respectively. This value was significantly higher from bacterial load of 2.2×10^2 ± cfu/g recorded from fish, smoked by oven-drying which gave the least bacterial load (2.2×10^2 ± cfu/g). The highest bacterial flora (2.7×10^2 ± cfu/g) was recorded in fish smoked with firewood.

Table 4.6: **Statistical comparison of coliform counts in smoke-dried *C-nigodigitatus* using (Charcoal, Firewood, Sawdust and Oven)**

Treatments (cfu/g)

Replicates	T1	T2	T3	T4
	Charcoal	**Firewood**	**Sawdust**	**Oven**
Rep 1	2.6×10^2	2.9×10^2	2.6×10^2	2.2×10^2
Rep 2	2.4×10^2	2.7×10^2	2.5×10^2	2.1×10^2
Rep 3	2.5×10^2	2.5×10^2	2.3×10^2	2.3×10^2
($\bar{x}$ ± SE)	$2.5\times10^2 \pm^{a}$	$2.7\times10^2 \pm^{b}$	$2.47\times10^2 \pm^{a}$	$2.2\times10^2 \pm^{c}$

4.2.2.2 Comparison of *E. coli* load of smoke-dried *C. nigodigitatus* using (Charcoal, Firewood, Sawdust and Oven)

Table 4.7 presents the statistical comparison of *E. coli* of smoke-dried fish using different sources of heat. The result shows that microbial load was the same in fish smoked with Charcoal and Sawdust (1.4×10^2 ± cfu/g) and (1.47×10^2 ± cfu/g) respectively. This value was significantly higher from bacterial load of 1.2×10^2 ± cfu/g recorded from fish smoked using oven-drying which gave the least bacterial load (1.2×10^2 ± cfu/g). The highest bacterial flora (1.6×10^2 ± cfu/g) was recorded in fish smoked with firewood.

Table 4.7: Statistical comparison of *E. coli* in smoke-dried *C. nigodigitatus* using (Charcoal, Firewood, Sawdust and Oven)

	Treatments (cfu/g)			
Replicates	T^1	T^2	T^3	T^4
	Charcoal	**Firewood**	**Sawdust**	**Oven**
Rep 1	1.5×10^2	1.8×10^2	1.6×10^2	1.2×10^2
Rep 2	1.3×10^2	1.6×10^2	1.5×10^2	1.1×10^2
Rep 3	1.4×10^2	1.4×10^2	1.3×10^2	1.3×10^2
($\bar{x}$ ± SE)	1.4×10^2 ± [a]	1.6×10^2 ± [b]	1.47×10^2 ± [a]	1.2×10^2 ± [c]

4.2.2.3 Comparison of *K. species* loads of smoke-dried *C-nigodigitatus* using (Charcoal, Firewood, Sawdust and Oven)

Table 4.8 presents the statistical comparison of *K. spp* loads of smoke-dried fish using different sources of heat. The result shows that microbial load was the same in fish smoked with Charcoal and Oven (1.0×10^2 ± cfu/g) and (1.0×10^2 ± cfu/g) respectively. This value was significantly higher from bacterial load of (0.87×10^2 ± cfu/g) and (0.87×10^2 ± cfu/g) recorded from smoke-dried fish using Firewood and Sawdust respectively. The highest bacterial flora (1.0×10^2 ± cfu/g) and (1.0×10^2 ± cfu/g) were also recorded in fish smoked with charcoal and oven.

Table 4.8: Statistical comparison of *Klesiella spp* in smoke-dried *C-nigodigitatus* using (Charcoal, Firewood, Sawdust and Oven)

	Treatments (cfu/g)			
Replicates	T^1	T^2	T^3	T^4
	Charcoal	**Firewood**	**Sawdust**	**Oven**
Rep 1	1.1×10^2	1.1×10^2	1.0×10^2	1.0×10^2
Rep 2	0.9×10^2	0.9×10^2	0.9×10^2	0.9×10^2
Rep 3	1.0×10^2	0.6×10^2	0.7×10^2	1.1×10^2
($\bar{x}$ ± SE)	$1.0\times10^2 \pm$ [a]	$0.87\times10^2 \pm$ [b]	$0.87\times10^2 \pm$ [b]	$1.0\times10^2 \pm$ [a]

4.2.2.4 Comparison of the Mean values of all the parameters, Coliform, *E. coli* and *K. species* in smoke-dried *C-nigodigitatus* using (Charcoal, Firewood, Sawdust and Oven)

Table 4.9 presents the statistical Mean comparison of *Coliform, E. coli* and *K. spp* loads of smoke-dried fish using different sources of heat. The result shows that in Coliform, the mean value for Charcoal is $2.5x10^2 \pm 5.77$, Firewood ($2.7x10^2 \pm 11.55$), Sawdust ($2.5x10^2 \pm 8.82$) and Oven ($2.2x10^2 \pm 5.77$). The mean values recorded in Charcoal ($2.5x10^2 \pm 5.77$) and Sawdust ($2.5x10^2 \pm 8.82$) are the same. The mean value was significantly higher from the value of $2.2x10^2 \pm 5.77$ recorded in fish smoked by oven-drying which gave the least value of ($2.2x10^2 \pm 5.77$), while the highest mean value was recorded in fish smoked with firewood ($2.7x10^2 \pm 11.55$).

Similarly, for *E. coli*, the mean value for Charcoal is $1.4x10^2 \pm 5.77$, Firewood ($1.6x10^2 \pm 11.55$), Sawdust ($1.4x10^2 \pm 8.82$) and Oven ($1.2x10^2 \pm 5.77$). The result shows that the mean value was the same in fish smoked with Charcoal and Sawdust ($1.4\times10^2 \pm$ cfu/g) and ($1.47\times10^2 \pm$ cfu/g) respectively. This value was significantly higher from the mean value of $1.2\times10^2 \pm$ cfu/g recorded from fish smoked using oven-drying which gave the least value ($1.2\times10^2 \pm$ cfu/g). The highest mean value ($1.6\times10^2 \pm$ cfu/g) was recorded in fish smoked with firewood.

For *K. spp*, the mean value for Charcoal is $1.0x10^2 \pm 5.77$, Firewood ($0.87x10^2 \pm 14.53$), Sawdust ($0.87x10^2 \pm 8.82$) and Oven ($1.0x10^2 \pm 5.77$). The result shows that the mean values are the same in fish smoked with Charcoal and Oven ($1.0\times10^2 \pm$ cfu/g) and ($1.0\times10^2 \pm$ cfu/g) respectively. This value was significantly higher from the mean values of ($0.87\times10^2 \pm$ cfu/g) and ($0.87\times10^2 \pm$ cfu/g)

recorded from smoke-dried fish using Firewood and Sawdust respectively. The highest mean value (1.0×10^2 ± cfu/g) and (1.0×10^2 ± cfu/g) were also recorded in fish smoked with charcoal and oven.

Table 4. 9: Mean values of *Coliform* count, *E. coli* and *Klebsiella spp* in smoke-dried *C. nigrodigitatus* using firewood, sawdust, charcoal and oven

	Bacteria		
Treatment (Cfu/g)	**Coliform**	***E. coli***	***Klebsiella spp***
Charcoal	$2.5x10^2±5.77^b$	$1.4x10^2±5.77^{ab}$	$1.0x10^2±5.77^a$
Firewood	$2.7x10^2±11.55^a$	$1.6x10^2±11.55^a$	$0.87x10^2±14.53^a$
Sawdust	$2.47x10^2±8.82^{ab}$	$1.4x10^2±8.82^{ab}$	$0.87x10^2±8.82^a$
Oven	$2.2x10^2±5.77^b$	$1.2x10^2±5.77^c$	$1.0x10^2±5.77^a$

Data are presented as ±SE. Mean values in column with different superscripts are significantly different at Fά =0.05

4.3 DISCUSSION

4.3.1 Organoleptic Assessment

Anywhere in the world, fish serves as a very important food commodity in the international trade but they deteriorate rapidly especially when there are poor storage facilities. It is highly accepted as a very good source of protein and other elements necessary for the maintenance of healthy body. (Adebayo-Tayo *et al.,* 2012). For Organoleptic as was shown in the results, the mean colour/appearance of *C. nigrodigitatus* using Charcoal was 9.0 ± 0.101. The mean colour/appearance using Firewood was 7.2 ± 0.157. The mean colour/appearance using Sawdust was 7.8 ± 0.064 and the mean colour/appearance using Oven was 9.5 ± 0.121. The fish dried using oven had significantly higher score than those dried using charcoal, firewood and sawdust respectively. Similarly, the mean aroma using Charcoal was 7.8 ± 0.064, the mean aroma using Firewood was 7.6 ± 0.078, the mean aroma using Sawdust was 7.8 ± 0.120, and the mean aroma using Oven was 9.4 ± 0.118. Again, *C. nigrodigitatus* smoked dried using oven, had significantly higher score than the other sources of heat. For Texture using Charcoal, the mean was 7.8 ± 0.120, the mean texture using Firewood was 7.6 ± 0.128, the mean texture using Sawdust was 7.8 ± 0.120 and the mean texture using Oven was 9.3 ± 0.162. Here again, mean texture using oven is significantly higher than other mean textures. In Taste, the mean taste using Charcoal was 9.0 ± 0.101, the mean taste using Firewood was 7.4 ± 0.128, the mean taste using Sawdust was 9.0 ± 0.143 and the mean taste using Oven was 9.7 ± 0.109. The mean taste using oven is also significantly higher than the rest. In all the mean values for all the different sources of heat, Oven drying was adjudged best product than the other sources of heat. This means that oven drying was very excellent, followed by charcoal which is also very good product and followed by sawdust and firewood. This result agreed with the observation of Shearer, (1994), that the protein composition

of fish is affected by diversity of factors such as size, sexual maturation, temperature, salinity, exercise, ration, time and frequency of feeding, starvation, type and amount of dietary ingredient. Smoked *Chrysichthys nigrodigitatus* is a good source of pure protein and would be adequate to prevent malnutrition in children, and as well desirable for a growing child and adult who feed solely on fish as a main source of protein. The high acceptable sensory attributes recorded in this study are in line with the findings of (Goulas and Kontominas, 2005; Wu and Mao, 2008 and Kumolu –Johnson *et al.,* 2010), which showed that fish dried in improved fish kilns or dryers generally have good sensory qualities (Govas and Kontominas, 2005; Wu and Mao, 2008 and Kumolu –Johnson *et al.,* 2010). Food attributes are an important factor in predicting consumers' perceptions in food choices decision. The study of food attributes indicated that the satisfaction levels of ethnic foods varies depending on diverse food attributes such as taste, fresh, colorful, uniqueness, and healthiness. The values of the overall acceptability showed the effectiveness of the fish kilns in producing good quality dried fish. From the ANOVA result in Appendix C, since the p-value is less than the significant level ($0.000 < 0.05$) this means that the null hypothesis is rejected which implies that the average microbial load in a processed *Chrysichthys* when various types of heat are applied are not the same. The temperature profiles of the fish kiln indicated that the fish kiln was able to attain the required temperature for fish drying since the temperature attained was well above the ambient temperature which allowed for evaporation of moisture from the fish. The profile showed that while the gas powered kiln was able to maintain uniform temperature over a relatively long period (which is required for effective and oven drying), the temperature trend for the charcoal powered kiln on the other hand was different. This is because the temperature rose steadily as the charcoal, firewood and sawdust are ignited until it fully glowed at a point which is at the maximum temperature of 72°C after 30 minutes.

conformity with previous studies carried out on drying of agricultural commodities. (Mujaffar and Sankat, 2005, and Kilic, 2009).

4.3.2 Microbial Evaluation

Generally, bacteria are abundant in any environment in which fish lives and it is therefore totally impossible to avoid them being a component of their diet. Osungbemiro (2005), observed that quantitatively the microbial flora in fish is a function of the environment. The bacteria entering along with the diet of fish during injection may adapt them in the gastro-intestinal tract and form a symbolic association. The weight loss observed in the fresh fish after smoking was as a result of moisture evaporation. Davies and Davies (2009) reported that weight loss during smoking of fish was due to evaporation resulting from burning charcoal. Omodara and Olaniyan (2012) stated that drying rate increases with increase in drying temperature showing that temperature is a major factor affecting the drying rate of a product. The microorganisms of interest that were studied include Coliforms, *E. coli*, and *Klebsiella*. The fishes were analyzed based on its degree of acceptability based on microbial load in it after smoking.

In this study, the mean values of the total coliform, *E. coli* and *Klesiella spp* counts of the smoked fish samples were that, for the smoked-dried *Chrysichthys nigrodigitatus*, the highest coliform count ($2.9x10^2$ cfu/g) was found in the fish dried using Firewood, while the lowest count ($2.2x10^2$ cfu/g) was found in the fish dried using Oven. A similar trend was also observed in the coliform count of the fish smoke-dried using Charcoal and Sawdust with the same value ($2.6x10^2$ cfu/g) respectively. The highest *E. coli* ($1.8x10^2$ cfu/g) and ($1.6x10^2$ cfu/g) counts were obtained from the Firewood and Sawdust sources of heat, in comparison to the lower counts of $1.2x10^2$ cfu/g (oven) and $1.5x10^2$ cfu/g (charcoal) respectively. For *Klebsiella spp*, the highest

coliform counts were found in fish smoke-dried using Charcoal and Firewood with same value ($1.1x10^2$ cfu/g) respectively, while the lowest coliform counts for *K. spp,* were recorded from Sawdust and Oven with the same value ($1.0x10^2$ cfu/g) respectively. The pairwise comparison using the SPSS LSD Method shows that the average microbes (Coliform, *E. Coli, Kleb and* TVBC) are different. This is because each of the microbes shares a different alphabet. (See Appendix B). In the pairwise comparison of the microbial loads, apart from comparison between *Kleb* and *E. Coli*, *E. Coli* and Coliform, *Kleb* and coliform, TVBC and Coliform, TVBC and *E. Coli*, *Kleb* and *E.coli* are significant. Fish received increased attention as a potential source of animal protein and essential nutrients for human diets (Fawole *et al*., 2007). Protein forms the largest quantity of dry matter in fish (Steffens, 2006). Bacteriological quality is of importance to public health as it directly relates to spoilage of fish and becomes the cause of food poisoning. Microbial hazards causing infections and poor health are closely related to food safety concerned with animal protein derived from marketed food fish, fishery products, meat and meat products (Saima *et al.,* 2012). Fish species serving as passive hosts (*Salmonella* and *Shigella*) are apparently due to injuries on the part of the fish species or environmental stress such as high temperature, poor quality and faecal contamination of water from where fishes are harvested.

Food-borne disease results from the injection of bacteria and the toxins produced by micro-organisms present in the marketed food and the intensity of the signs and symptoms may vary with the amount of contaminated food ingested and susceptibility of the individual to the toxin (Clarence *et al.,* 2009). Smoked fish maybe contaminated with different types of bacteria such as coliform, *E. coli, Klebsiella*, etc and these are responsible for causing cholera and other food-borne diseases (Mobin *et al.,* 2001). It is also well documented that frozen fishes or raw fishes provide important epidemiological pathways for food-borne disease transmission (WHO, 2002). The

hygienic conditions of the fish-smoking environment in the selected study sites were commonly unclean and the contamination of fish species by *E. coli* mostly results from the unhygienic condition of the smoking environment. Concerning this, there is a higher prospect of cross-contamination from the processing environment onto processed fish species. Therefore, the quality of the marketed smoked-dried fish is of a major concern to fish processors and public health authorities. The bacterial density in fish apparently gives an idea of the quality of fish samples. The processed fish or fish products are considered as spoiled when the total bacterial count (TBC) reach 10^6 cfu/g or more in food items (Shewan 1970). Generally, the drying temperature increases as the drying approaches the desired moisture content due to the reduction in the moisture content of the fish samples. This confirms the results of previous studies by Olayemi *et al.* (2013) and who pointed out that as the moisture content of the fish decreases, the drying temperature increases because the drying air no longer carries much moisture. The drying curve obtained show that drying of fish like any other agricultural material occurs in the constant rate and falling rate period. The constant drying rate period was observed in the first three (3) hours due to the initial high moisture content irrespective of the type of kiln. Thereafter the drying phase progresses into the falling rate period as more moisture was removed from the fish samples. This shows that moisture is the driving force for the falling rate period as reported by Omodara and Olaniyan (2012). Although due to the variation in the temperature profile of the kiln the drying rate differs. In fish there is an inverse relationship between moisture and lipid, when the lipid is high, the moisture will be low (Nestel 2000). The processing method, quality and smoking conditions could also contribute to the variation. Aminullahi *et al* (2006) observed that weight variation during fish processing is determined by the type of raw material, manual processing methods, brine type,

quality and smoking conditions (temperature, moisture, air flow and drying). Yuseng and Poulsen (1988) stated that internal water movement controls the drying rate of samples.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The smoked fish samples were generally more nutritively richer. The smoking process which promises to be an age long strategy for preserving fish even in the absence of social amenities like electricity together with the heat generated from the burnt wood drastically dehydrated water content from the fish samples to enhance longer shelf-life. The alder wood contains high complex phenolic compounds released in the fuel smoke; and which act both as antioxidants to slow down rancidity of animal fats and as antimicrobials that lowers bacterial growth; thereby preventing spoilage. The heat generated from the burnt wood dehydrates and kills bacteria in the smoked sample. Smoking and phenolic compounds prolong the keeping qualities of smoked fish, produced high quality nutritive products with brownish skin, good flavour/aroma, taste and attractive appearance. Smoking makes fish easier to pack, transport, improves market value and enhance market income generation. The performance evaluation showed that the model of fish kiln used, was effective in smoke-drying *C. nigrodigitatus* because all the fish samples had high acceptance value. The study has established that the electrically powered kiln is the most effective with respect to drying rate and sensory attributes of the dried fish. The gas powered kiln is more effective than the charcoal, firewood and sawdust powered smoke kiln. Also the gas powered kiln required less monitoring than the charcoal, firewood and sawdust powered kiln. The performance of the charcoal powered fish kiln can be improved by regular charging of the charcoal box. In conclusion, this study showed that smoking methods affects the studied parameters, organoleptic assessment and microbial loads of smoked silver catfish. The bacterial counts on fish species throughout the

study were at satisfactory levels. Smoke-dried fish has higher lipid and protein contents than fresh fish, which is advantageous to fulfil daily nutritional requirements. The richness of dried fish in vital fats and proteins make it suitable for consumption as a meat-substitute, in addition to plant-derived alternatives. Moreover, fish has an array of health benefits; consuming it can be advantageous to prevent cardiovascular disease and osteoporosis, and it is also good for foetal development and as a source of antioxidants. These highlight the importance of smoke-drying fish, as an effort to reduce fish waste and optimize its nutritional benefits through preservation. The lipid oxidation of dried fish increased steadily over time, and can be accelerated during storage, especially in ambient temperature. Thus, it is recommended that packaging methods such as vacuum packing and active packaging via oxygen absorber should be applied to combat lipid oxidation and protein degradation to avoid rancidity. This will lead to improvement of the value chain of fish, reduce impact damage on smoked fish, and provide additional business opportunity for investors as well as enhancing the export value of smoked fish. Other than physico-chemical and biochemical changes, the sensory characteristics of smoke-dried fish are also an important element, as they determine the marketability of the products. More often, the colour, appearance, odour, palatability, flavour, texture, and overall acceptability determine the sensory score of various dried fish. Microbial characteristics of the smoke-dried fish are another vital aspect, especially in determining the safety of the product for consumption. The effectiveness of various drying methods to decrease the microbial load to a safe level have been studied extensively over the years. The most common microorganisms that have been detected were *Escherichia coli* spp and *Klebsiella* spp. Smoke-drying contributed to more microbial activity than oven-drying methods. The safety aspect of smoke-dried fish has been a tremendous challenge, as this industry is made up mostly by traditional drying facilities. The challenges involve microbial safety, heavy

metals, and use of preservatives. Hence, natural preservatives such as neem leaf, paprika, turmeric, and shallots have become more desirable to control the parameters that ensure food safety. Smoke-drying fish involves a number of methods; such as firewood, sawdust, charcoal and oven as mentioned in this review.

The present study concluded that different processing techniques improve some sensory attributes, like flavor, juiciness and tenderness, thereby increasing the overall acceptability of the finished product. It is concluded that the fish species i.e. *Chrysichthys nigrodigitatus* has very good colour, aroma, texture and taste after smoke-drying but has adverse effect after smoking with different sources of heat. When overall analysis was conducted of the processed specimen, the best was observed in oven-drying after every processing method as samples were neither burnt nor fresh and hence possessed good cooking attribute while the lowest value was observed in firewood. As smoking is employed by remote fishing communities due to traditional preference of the local people due to lack of sophisticated preservation techniques so it is less recommended as wood smoke produce microscopic particles, have dull and unattractive colour and due to overall less acceptability which is observed during organoleptic analysis. The health risk may also be faced due to inappropriate smoking. Although Salmonella and Shigella species were not detected in the fish species analyzed in the current study, the high *Escherichia coli* and Staphylococcus species count suggests that the smoked fish studied may be unfit for human consumption due to contamination by bacteria. However, a comparison with the different sources of heat showed that the bacteria load observed were lower than the permissible level for human consumption. Be that as it may, there is a need for effective foodborne infection or disease control measures to be planned and implemented to help improve the microbiological quality.

5.2. Recommendations

i. This study recommends the need for the adoption of good processing practices and storage methods of smoked fish. In other words, the people that are involved in the processing and selling of smoked fish should maintained hygienic environment and practices, so as to ensure that safety standards are maintained in smoked fish industry in order to preserved market worthiness of the products.

ii. Both government and non-governmental bodies should develop the use of automated smoke generators is strongly recommended. In order to provide a consistently uniform volume and quality of smoke throughout the smoking process.

5.3. Contribution to knowledge

Fish is a highly perishable commodity that must be properly preserved to ensure its wholesomeness, not only immediately after capture, but also during storage, transportation and marketing.

The present study has revealed the following scientifically;

i) The prevailing emerging diseases ravaging our society today may be partly attributed to the consumption of fish products. If equipment is left wet after cleaning micro-organisms may grow in the water film. It is important to ensure that equipment is left dry as soon as possible after cleaning and where possible to allow equipment to air-dry naturally. Single use tissue or absorbent materials may be used for drying but they should be used once and discarded.

ii) Adequate drainage points should be provided in equipment that cannot be dismantled and drying racks provided for small pieces of equipment that are dismantled for the purpose of cleaning. Any equipment that unavoidably remains wet for a period during which significant microbial growth might occur should be disinfected immediately before use. They include *Escherichia coli*, *Klebsiella sp* etc.

I am therefore convinced that this study has made useful contribution to public health aspect of fishery technology.

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APPENDICES

APPENDIX A

Organoleptic Assessment Criteria and Quality scores from Panalists

FEDERAL UNIVERSITY OF TECHNOLOGY, OWERRI

PMB 1526

SCHOOL OF AGRICULTURE AND AGRICULTURAL TECHNOLOGY

DEPARTMENT OF FISHERIES AND AQUACULTURAL TECHNOLOGY

QUESTIONNAIRE FOR ORGANOLEPTIC ASSESSMENT OF EXPERIMENTAL FISH CHRYSICHTHYS NIGRODIGITATUS PROCESSED USING DIFFERENT SOURCES OF ENERGY; CHARCOAL, FIREWOOD, OVEN AND SAW DUST.

A. INTRODUCTION

The need to improve the health condition of our people through the consumption of wholesome fish and need to reduce post-harvest storage losses of fish suffered by artisanal fisher folk necessitated this study. It is aimed at ascertaining the wholesomeness of fish processed using different energy sources; charcoal, firewood, oven and saw dust.

It is expected that the resulting information from this research will help profer recommendations that will impact positively on the livelihood of the local fish producer. I therefore solicit your cooperation in objectively attending to the questions as a scorer/Assessor.

B. BIO-DATA/ EXPERIENCE IN FISH QUALITY

1. Name of panelist: Dr. C. N Anyanwu
2. Department: FAT Status: Snr Lecturer
3. Sex: Male Age: +60
4. Do you delight eating fish? Yes ✓ No. Tick ✓ as appropriate
5. Are you able to detect wholesome fish from spoiled fish? Yes ✓ No.
   Tick ✓ as appropriate
6. Have you served as an organoleptic assessment panelist before? Yes ✓ No.
   Tick ✓ as appropriate
7. Were you previously trained as a panelist? Yes ✓ No tick ✓ as appropriate

C. ORGANOLEPTIC ASSESSMENT CRITERIA AND QUALITY SCORES

SCALE RATING

9-10 = 1st quality (excellent)

7-8 = 2nd quality (very good)

5-6 = 3rd quality (good)

3-4 = limit of acceptance (fair)

0-2 = Unacceptable

Quality scores for WHOLE FISH (Tick ✓ as appropriate)

A. Charcoal

Quality criteria: color/ appearance, odour, texture and taste

S/N	Criteria	9 - 10	7 - 8	5 - 6	3 - 4	1 - 2	0
1	Color/appearance		✓				
2	Odour		✓				
3	Texture			✓			
4	Taste		✓				

B. Fire wood

S/N	Criteria	9 - 10	7 - 8	5 - 6	3 - 4	1 - 2	0
1	Color/appearance			✓			
2	Odour		✓				
3	Texture			✓			
4	Taste		✓				

C. Saw dust

S/N	Criteria	9 - 10	7 - 8	5 - 6	3 - 4	1 - 2	0
1	Color/ appearance			✓			
2	Odour			✓			
3	Texture			✓			
4	Taste		✓				

b. Oven

S/N	**Criteria**	9 - 10	7 - 8	5 - 6	3 - 4	1 - 2	0
1	Color/ appearance	✓					
2	Odour		✓				
3	Texture	✓					
4	Taste		✓				

D. CONCLUSION

Thank you very much for your cooperation

Name: Jude, Chiemela Jude

(Researcher)

APPENDIX B

ANOVA Table for Coliform

Descriptive						
Values						
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Firewood	3	270.00	20.000	11.547	220.32	319.68
Sawdust	3	246.67	15.275	8.819	208.72	284.61
Charcoal	3	250.00	10.000	5.774	225.16	274.84
Oven	3	220.00	10.000	5.774	195.16	244.84
Total	12	246.67	22.293	6.435	232.50	260.83

Descriptive		
Values		
	Minimum	Maximum
Firewood	250	290
Sawdust	230	260
Charcoal	240	260
Oven	210	230
Total	210	290

ANOVA					
Values					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3800.000	3	1266.667	6.080	.018
Within Groups	1666.667	8	208.333		
Total	5466.667	11			

Post Hoc Tests

Homogeneous Subsets

Values			
Duncan			
Treatment Coliform	N	Subset for alpha = 0.05	
		1	2
Oven	3	220.00	

Sawdust	3	246.67	246.67
Charcoal	3		250.00
Firewood	3		270.00
Sig.		.053	.094

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.

APPENDIX C

ANOVA Table for *E. coli*

Descriptive						
Values						
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Firewood	3	160.00	20.000	11.547	110.32	209.68
Sawdust	3	146.67	15.275	8.819	108.72	184.61
Charcoal	3	140.00	10.000	5.774	115.16	164.84
Oven	3	120.00	10.000	5.774	95.16	144.84
Total	12	141.67	19.462	5.618	129.30	154.03

Descriptive		
Values		
	Minimum	Maximum
Firewood	140	180
Sawdust	130	160
Charcoal	130	150
Oven	110	130
Total	110	180

ANOVA					
Values					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2500.000	3	833.333	4.000	.052
Within Groups	1666.667	8	208.333		
Total	4166.667	11			

Post Hoc Tests

Homogeneous Subsets

Values			
Duncan			
Treatment Ecoli	N	Subset for alpha = 0.05	
		1	2

Oven	3	120.00	
Charcoal	3	140.00	140.00
Sawdust	3	146.67	146.67
Firewood	3		160.00
Sig.		.062	.142

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

APPENDIX D

ANOVA Table for Klebsiella spp

Descriptive						
Values						
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
Firewood	3	86.67	25.166	14.530	24.15	149.18
Sawdust	3	86.67	15.275	8.819	48.72	124.61
Charcoal	3	100.00	10.000	5.774	75.16	124.84
Oven	3	100.00	10.000	5.774	75.16	124.84
Total	12	93.33	15.570	4.495	83.44	103.23

Descriptive		
Values		
	Minimum	Maximum
Firewood	60	110
Sawdust	70	100
Charcoal	90	110
Oven	90	110
Total	60	110

ANOVA					
Values					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	533.333	3	177.778	.667	.596
Within Groups	2133.333	8	266.667		
Total	2666.667	11			

Post Hoc Tests

Homogeneous Subsets

Values		
Duncan		
Treatment klebsiella spp	N	Subset for alpha = 0.05
		1
Firewood	3	86.67
Sawdust	3	86.67
Charcoal	3	100.00
Oven	3	100.00
Sig.		.373

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 3.000.