MICROBIAL LOAD OF FROZEN AND SMOKE DRIED *Merluccius merluccius* AND *Scomber scombrus* SOLD IN NEW BENIN MARKET. OREDO LGA. BENIN CITY. NIGERIA

BY

Gift AYEMWENRE (Miss)

AGR2004368

DEPARTMENT OF AQUACULTURE AND FISHERIES MANAGEMENT.
FACULTY OF AGRICULTURE
UNIVERSITY OF BENIN
BENIN CITY

NOVEMBER 2025

MICROBIAL LOAD OF FROZEN AND SMOKE DRIED *Merluccius merluccius* AND *Scomber scombrus* SOLD IN NEW BENIN MARKET. OREDO LGA. BENIN CITY. NIGERIA

BY

Gift AYEMWENRE (Miss)

AGR2004368

SUBMITTED TO

THE DEPARTMENT OF AQUACULTURE AND FISHERIES MANAGEMENT, FACULTY OF AGRICULTURE, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE.

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF AGRICULTURE DEGREE B. AGRIC (AQUACULTURE AND FISHERIES MANAGEMENT)

NOVEMBER 2025

CERTIFICATION

This is to certify that this research work was carried out by Gift AYEMWENRE (AGR2004368) and has been certified by the undersigned in the Department of Aquaculture and Fisheries Management, Faculty of Agriculture, University of Benin, Benin City, Edo State, Nigeria.

DR. K. OMORUYI (Project Supervisor)	**DR. M. EGWENOMHE** (Head of Department)
Date	**Date**

DEDICATION

I dedicate this work first and foremost to God Almighty, my all sufficient Father, who in his infinite mercy kept me this far and also to my wonderful family, for their love and support both morally and financially all through my stay in the University of Benin, Benin City, Edo State, Nigeria.

ACKNOWLEDGEMENT

My heartfelt gratitude goes to God Almighty for his grace, mercy, knowledge and wisdom throughout my years in the University of Benin.

My sincere appreciation goes to my project supervisor, Dr. K. Omoruyi for his assistance, recommendations and suggestions in the process of preparing this report, as well as for the time spent reading and correcting my errors.

I would also like to appreciate all lecturers in the Department of Aquaculture and Fisheries Management, Dr. M. Egwenomhe (H.O.D), ,Prof. O. J. Abolagba, Prof. V. A. Okonji, Dr. B. S. Aliu, Prof. (Mrs.) A. E. Odiko, Dr. O. M. Wangboje Dr. A. Orowe, Dr. J. N. Nuntah and Dr. S. E. Osayi, Dr. M. O. Sado, Dr. E. Izevbigie, for their guidance, corrections and tireless efforts in imparting knowledge. I also want to appreciate the farm personnel, Mr. Nosa Iriomwen , God bless you all.

I would not fail to acknowledge the efforts of my lovely parents, Mr. and Mrs. Ayemwenre for their love, prayers, care, financial support, encouragement, taking my Education seriously and ensuring that I complete my degree program. I am forever grateful to God for the privilege of having parents who cares for me both materially, spiritually, financially and morally. Thanks to my siblings and entire family for their unwavering support in one way or the other .

My sincerest gratitude goes to my special person, Ifeakachukwu David Ebinum who actively contributed towards the success of this project by helping, supporting and encouraging me in every possible ways. I am as well thankful to my roommate; Audrey Unabor who contributed to my success directly and indirectly. I pray God Almighty blesses and reward you guys.

Finally, I want to thank my wonderful course mates and friends who made my stay in the University of Benin a memorable one. I'm really thankful for the love and support they contributed to the success of this work. I really appreciate you all for everything.

TABLE OF CONTENT

LIST OF TABLES

LIST OF PLATES

ABSTRACT

This study was carried out to assess the bacterial and fungal load of selected frozen and smoked-dried fish species sold in the New Benin Market, Oredo Local Government Area, Benin City, and to isolate and characterize the associated microorganisms. A total of fourteen (14) fish samples were randomly purchased from the market in Benin, Edo State, Nigeria.

The samples were collected in sterile polythene bags to prevent contamination and transported for microbial analysis. Smoked-dried fish samples were obtained from various fish sellers, while frozen fish samples were sourced from different cold rooms. Frozen fresh smoked-dried samples were used as control. All samples were handled carefully to avoid contamination with dirt or other microorganisms. Microbiological analysis was conducted using Nutrient Agar to determine bacterial load and Potato Dextrose Agar for fungal load estimation.

The results revealed that smoked-dried fish samples for *Scomber scombrus* had a high bacterial counts ($9.6x10^3$ cfu/g) compared to the frozen samples ($8.0x10^3$ cfu/g) and the control samples recorded the lowest bacterial load ($3.4x10^3$ cfu/g). Similarly, the smoked-dried fish samples showed higher fungal counts ($7.0x10^2$ cfu/g) than the frozen ones ($6.33x10^2$ cfu/g). The elevated microbial levels observed in smoked fish from the market are likely due to poor handling practices, inadequate smoking techniques employed by processors and sellers, exposure to contaminated air and septic environments.

For *Merluccius merluccius*, the frozen fish samples had a high bacterial counts ($1.5x10^4$ cfu/g) compared to the smoke dried fish samples ($3.4x10^3$ cfu/g) and the control samples recorded the lowest bacterial load ($2.0x10^3$ cfu/g). Similarly, the frozen fish samples showed higher fungal counts ($5.67x10^2$cfu/g) than the smoke dried ones ($2.0x10^2$ cfu/g). The level of microbes observed in the frozen fish depends heavily on the initial contamination level, the

speed of freezing, temperature fluctuations during storage, and handling practices during thawing. Frozen fish contains a residual microbial population from its initial state (harvesting, handling, processing), once the fish thaws, these surviving microbes can become active again and multiply rapidly if the temperature is favorable, potentially reaching levels that cause spoilage or illness.

A total of ten (10) microorganisms, five (5) bacterial species and five (5) fungal species were isolated from both frozen and smoked-dried fish samples. The bacterial isolates included Bacillus sp., Staphylococcus sp., Proteus sp., Pseudomonas sp., and Enterobacter sp., while the fungal isolates included Aspergillus sp., Saccharomyces sp., Penicillium sp., Mucor sp., and Rhizopus stolonifer. Most of the fungal isolates identified are known producers of mycotoxins. There were significant differences ($P > 0.05$) between the frozen and smoked-dried fish samples collected from the same market, varying from one fish seller to another. This variation indicates that different handling, preservation, and marketing practices are used by fish vendors. Consequently, the microbial load reflects the hygiene standards and processing methods adopted. To ensure consumer safety, it is recommended that mechanized smoking systems be employed to completely dehydrate fish and reduce contamination risks associated with residual moisture and the need for proper hygienic conditions during processing, handling, distribution, packaging and storage of the fish.

CHAPTER ONE

1.0 INTRODUCTION

Fishes are fully aquatic and ectothermic vertebrates, distinguished by their streamlined bodies and the presence of lateral line sensory structures (Verma and Prakash, 2020). They are found in both freshwater and marine ecosystems. Fishes are a valuable source of essential macronutrients such as proteins, lipids, and ash as well as micronutrients like vitamins and minerals. These nutrients are vital for maintaining health and well-being, as they provide energy and play important roles in bodily repair and regulatory processes (Srivastava and Srivastava, 2008).

Fish is particularly rich in high-quality proteins and beneficial fats, which is why it is often described as a "rich food for poor people" (Sujatha *et al.,* 2013). Proteins and fats are the primary components that define the nutritional composition of fish. It offers excellent nutritional benefits by providing superior proteins, healthy fats, and essential vitamins and minerals such as magnesium and phosphorus (Ali *et al.,* 2020). The combination of both macro- and micronutrients makes fish nutritionally preferable to many other sources of animal protein.

Fish is considered a healthier meat alternative due to its high content of Long Chain Polyunsaturated Fatty Acids (LCPUFAs), which are linked to better health and the prevention of age-related diseases. In Nigeria, fish contributes to 40% of animal protein intake and is especially important in areas where livestock is scarce. Fish also offers significant nutritional advantages, with superior protein quality that helps combat malnutrition, particularly in comparison to other animal-based foods like meat, milk, and eggs (Adeniji and Okedeyi, 2017). Moreover, the presence of Omega-3 unsaturated fatty acids in fish imparts medicinal

benefits, including the management of conditions like arthritis, asthma, coronary heart disease, and cancer (Abolagba and Igbinevbo, 2010).

However, due to their high-water content and soft tissues, fish are highly vulnerable to microbial contamination and spoilage (Olayemi *et al.,* 2012). Once fish dies, it remains in prime condition for only a brief period before spoilage sets in, aggravated by rising ambient temperatures that promote the growth of microorganisms. Additional factors contributing to spoilage include the poikilothermic nature of fish, which allows bacteria to thrive across a range of temperatures, a high postmortem pH in the flesh, the presence of non-protein nitrogen (NPN) in large quantities, and trimethylamine oxide (TMAO). These elements, upon decomposition, produce the off-flavors commonly associated with spoiled fish. Autolytic enzymes like protease break down the fish muscle, softening it and leading to the development of undesirable flavors. Microorganisms, such as bacteria, further degrade the fish by producing volatile compounds like trimethylamine (TMA) and hydrogen sulfide (H2S). Additionally, lipid oxidation causes the breakdown of triacylglycerols and phospholipids, producing fatty acids that result in rancidity, which can make the fish less appealing to consumers. To extend shelf life and preserve quality, traditional and modern preservation methods such as drying and freezing are commonly employed.

Freezing fish helps preserve it for extended periods by halting the growth of microorganisms and stopping the enzymatic processes that cause spoilage. Most pathogens do not multiply at freezer temperatures, and freezing also converts available water into solid ice crystals, reducing microbial growth. However, despite the benefits of freezing, fish sold in open markets, including both imported frozen fish and locally caught fish frozen on board (Oramadike *et al.,* 2010), can still be subject to quality degradation. Research by Arannilewa *et al.,* (2005) indicated that protein content decreases with prolonged frozen storage, with fresh, unfrozen fish having higher protein levels. The quality of frozen fish can also be

compromised by issues such as dehydration, rancidity, drip loss, and bleaching, which negatively affect its overall quality (Kropf and Bowers, 1992).

Smoking fish for preservation dates back to ancient times and provides desirable flavor, odor, and extended shelf life by its antibacterial and oxidative effects, as well as by lowering the pH, imparting color, and accelerating the drying process. However, in many local settings, hygienic standards are not properly followed, leading to the spoilage of smoked fish as easily as non-smoked fish (Jeyasanta *et al.,* 2015). Fish are rich in lipids, minerals, and vitamins, including riboflavin, vitamin A, and vitamin D, all of which promote the growth of microbes (Olaleye and Abegunde, 2015). In several communities in Benin, smoked fish is often sold or hawked openly in markets without proper regard for potential microbial contamination from the environment (Akinwumi and Adegbehingbe, 2015).

1.1 Justification of The Study

Fish is a highly perishable, protein-rich food that typically contains high levels of free amino acids. Microorganisms metabolize these amino acids, resulting in the production of ammonia, biogenic amines like putrescine, histamine, and cadaverine, as well as organic acids, ketones, and sulfur compounds (Emborg *et al.,* 2005; Dalgaard *et al.,* 2006). Microbial hazards, which can lead to infections and health issues, are closely tied to food safety concerns related to animal proteins found in fish and other seafood products. Freezing is a widely used method in the meat, fish, and other animal protein industries, as it helps preserve the quality of products for extended periods. It offers several benefits, such as minimal changes in product size and negligible deterioration in color, flavor, and texture (Obuz and Dikeman, 2003; USDA Food Safety Information, 2013). Some bacteria present in frozen fish can survive heat. Although freezing slows down bacterial growth, it doesn't kill all bacteria, and some can survive and even multiply once thawed (melted) and heated. Smoking is another traditional method used

to process fish, aiming to prevent or reduce post-harvest losses. The application of heat during smoking removes water and inhibits bacterial and enzymatic actions that can affect the fish (Jeyasanta *et al.,* 2015).

In Benin, the majority of frozen and dried fish are sold in open markets, exposing them to dust and contaminated air. This exposure creates favorable conditions for microbial growth, which may increase the transmission and prevalence of pathogens. Additionally, poor personal hygiene, such as failing to wash hands after handling contaminated materials, further contributes to the contamination of frozen and dried fish with harmful microorganisms.

Several studies have been carried out on the microbial quality of frozen and smoke-dried fish. Different authors have documented the microbial characteristics of various fish species in Nigeria. Tama *et al.,* (2023) reported a higher bacterial load which was observed in frozen Taki (5.75÷0.098 Log CFU/g) than those of dried (4.28+0.510Log CFU/g) sample although both were complied with ICMSF standard. Significantly higher Total coliform count (TCC) was calculated in raw (155.67# 44.261 MPN/g) fish whereas lesser number was shown in frozen (22.92 ÷ 2.884 MPN/g) and dried (14.89# 1.655 MPN/g) Taki, respectively. Adebayo-Tayo et al., (2012) reported higher microbial levels in frozen mackerel, ranging from 3×10^5 to 6.3×10^5 cfu/g. Similarly, Popovic *et al.,* (2010) examined 30 frozen fish samples, including sea bass (*Dicentrarchus labrax*), sea bream (*Sparus aurata*), sprat (*Sprattus sprattus*), pilchard (*Sardina pilchardus*), red scorpionfish (*Scorpaena scrofa*), bluefin tuna (*Thunnus thynnus*), hake (*Merluccius merluccius*), and striped red mullet (*Mullus surmuletus*). Their findings showed that 20 of the samples had total viable counts (TVCs) ranging between 3 and 5 log cfu/g. Taiwo *et al.,* (2017) conducted a microbial analysis on five frozen fish species, *Trachurus trachurus, Scombers combrus, Larimichthys crocea, Gadus chalcogrammus and Oreochromis niloticus*, where *S. scombrus* and *G. chalcogrammus* had the highest (5.30 x 102 cfu/g) and lowest microbial loads (1.85 x 102 cfu/g) respectively. The microbiological

quality of *T. trachurus* was reported by Adebayo-Tayo *et al.,* (2008) along with several other fish species. Adegunwa *et al.,* (2013) examined the microbial quality of smoked herring (*Sardinella eba*) in Odeda Local Government Area, Ogun State, Nigeria, and reported total plate counts ranging from 1.26×10^6 to 3.00×10^7 cfu/g, total fungi between 0 and 3.50×10^4 cfu/g, and coliform counts from 0 to 2.00×10^3 cfu/g. Ibrahim *et al.,* (2014) investigated the microbial diversity of smoked fish (*C. gariepinus*) in Minna Metropolis, Niger State, Nigeria, and found microbial counts ranging between 1.85×10^6 and 2.41×10^6 cfu/g. The microbiological quality of smoked T. trachurus sold in Benin City, Nigeria, was documented by Daniel *et al.,* (2013). Abolagba and Uwagbai (2011) assessed the microbial load of smoke-dried fishes (*E. fimbriata and P. elongatus*) sold in Oba and Koko markets in Edo and Delta States and reported mean bacterial counts of 9.2×10^6 cfu/g during the dry season and 1.2×10^7 cfu/g during the rainy season, while fungal counts were 2.5×10^6 cfu/g in both seasons. Eze, E. I., Echezona, B. C., and Uzodinma, E. C. (2011) carried out a study on the isolation and identification of pathogenic bacteria associated with frozen mackerel (*Scomber scombrus*). They reported a mean bacterial load of 1.135×10^8 CFU/g, which was considerably higher than the public health and safety limit of 5.0×10^5 CFU/g set by the Nigerian National Agency for Food and Drug Administration and Control (NAFDAC).

Nevertheless, limited studies have focused on comparing the microbial analysis of the smoke dried and frozen form of merluza (*Merluccius spp*.) and *Scomber scombrus* fsh therefore, the present research was undertaken to evaluate the microbial quality of smoked dried and frozen form of Merluccius sp. and *Scomber scombrus* and to see if there is much difference in the level of microbes between the frozen and smoked dried form., understand the safety or potential risks associated with smoked Merluccius and *S. scombrus*, and also help to ascertain the level of microbes in these fish consumed by the local populace as well as to check for the level of exposure to these micro-organisms. The findings of this study will be used to

enlighten fish vendors and processors on the risks associated with poor hygiene and improper handling, as well as the potential hazards of inadequately frozen or insufficiently smoked Merluccius merluccius and Scomber scombrus. Additionally, the results will help in developing strategies aimed at extending the shelf life of smoked fish products.

1.2 Aim and Objectives of The Study

The aim of this study is to identify the microbial load of frozen and smoked dried fish species sold in New Benin market, Oredo LGA. Benin City.

The specific objectives of the study are to determine the:

1. fungal load of various commercially important frozen and smoked dried fish species sold in new Benin market in Oredo LGA, Benin City.
2. bacteria load of the commercially important frozen and smoked dried fish species sold in new Benin market in Oredo LGA, Benin City.
3. Percentage occurrence and diversity of fungi and bacteria present in the frozen and smoked dried forms of the commercially important fish species in the study area

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Perishability and Shelf Life of Fish

Fish are highly perishable commodities with a notably short shelf life, which refers to the period from the time of capture to the point when the fish remains acceptable for sale and consumption (Regenstein and Regenstein, 1991). Consequently, proper handling and preservation are essential to prolong shelf life while maintaining quality and nutritional value. According to the Food and Agriculture Organization (FAO), quality is primarily judged by visual appeal and freshness or the extent of spoilage. Once fish are harvested, they quickly lose their natural resistance to microbial invasion and undergo physical and chemical transformations, altering their appearance, flavor, odor, and texture.

2.2 Freshness Indicators

Freshness in fish is typically assessed through sensory evaluation based on appearance, odor, and texture (Karube *et al.,* 2001). These organoleptic factors include:

1. Overall appearance, particularly the eyes, gills, surface slime, scales, and firmness of the flesh.
2. Odor of the gills and the abdominal cavity.
3. Discoloration, especially along the backbone.
4. Presence or absence of rigor mortis.
5. Condition of the belly walls (Bate and Bendall, 2010).

2.3 Fish Spoilage

Fish spoilage is a complex process involving physical, chemical, and microbiological factors (Adebayo-Tayo *et al.,* 2012b; Pal, 2012). A variety of spoilage-causing bacteria including Aeromonas, Alcaligenes, Bacillus, Enterobacter, Enterococcus, Escherichia coli, Listeria, Pseudomonas, and Shewanella as well as fungi such as Aspergillus, Candida, Cryptococcus, and Rhodotorula, have been isolated from both fresh and spoiled fish and other seafood (Pal, 2012). In fatty fish, lipid degradation leads to rancid odors. Furthermore, marine and some freshwater fish contain trimethylamine oxide, which is broken down by spoilage bacteria into trimethylamine (TMA) a compound responsible for the characteristic fishy smell. Iron, being a limited nutrient in fish tissue, encourages the growth of bacteria like Pseudomonas, which produce siderophores to scavenge iron. Spoilage results from a combination of enzymatic and bacterial activity occurring post-mortem (Pal, 2012). This deterioration begins immediately after the fish dies. In warm climates, spoilage can occur within 15–20 hours, depending on the species and capture method (Adedeji and Adetunji, 2004). Due to its high-water content, fish is highly perishable (Pal and Mahendra, 2015). Spoilage refers to any alteration in fish or fish products that makes them less acceptable or unfit for consumption (Pal, 2012). Typical signs of spoilage include discoloration, slime formation, textural changes, off-odors, off-flavors, and gas production (Adedeji and Adetunji, 2004; Pal, 2010). Compared to fresh fish, spoiled fish may exhibit a strong odor, dark-red gills covered in slime instead of bright red ones, soft flesh with brownish blood rather than firm flesh with red blood, and cloudy, red-tinged pupils instead of clear ones (Pal, 2010). The spoilage process begins the moment the fish dies. In warmer regions, the rate of deterioration is faster. Enzymes from the fish's digestive system, originally meant to process food, begin breaking down the stomach after death and later migrate into the flesh, leading to softening and stronger odors (Lima Dos Santos *et al.,* 2011). Although the fish's skin, gills, and intestines naturally harbor bacteria,

these microorganisms begin to multiply soon after death. Within two to four days, even a well-chilled fish can start decomposing due to enzymatic and bacterial action (Karube *et al.,* 2001). The initial bacterial load is influenced by the fish's health, the cleanliness of its environment, and its method of capture. Fish from clean waters tend to last longer than those dragged through muddy, contaminated conditions.

Spoilage involves both enzymatic and microbial degradation, which trigger chemical reactions responsible for the distinct off-odors and flavors (Putro, 2005). Additionally, oxidation of oils in the flesh contributes to rancidity. The primary goal of fish preservation is to slow down or halt these enzymatic, bacterial, and oxidative processes, thus maintaining the freshness and quality of the fish for as long as possible (Bate and Bendall, 2010).

2.3.1 Causes of Fish Spoilage

Fish spoilage occurs due to the activity of enzymes, bacteria, and naturally occurring chemicals within the fish. Several additional factors also contribute to this process (Abbas and Saleh, 2009):

1. Elevated moisture levels
2. High fat content
3. Fragile muscle structure
4. High protein content
5. Exposure to ambient temperatures
6. Poor hygienic practices during handling

2.3.2 Fish Spoilage Process

Fish is a highly nutritious food, appreciated for its flavor, which is due to its key components: water, protein, and fat (Adebowale *et al.,* 2008). Spoilage in fish is a complex process influenced by enzymatic activity, bacterial growth, and chemical changes. This process begins immediately after the fish dies and occurs in three distinct stages (Amos, 2007):

1. Rigor mortis
2. Autolysis
3. Bacterial invasion and putrefaction

2.3.3 Types of Fish Spoilage

2.3.3.1 Microbiological Spoilage:

Although live fish are generally considered sterile internally, microorganisms are commonly present on their external surfaces such as the skin and gills as well as in their digestive tracts. Newly caught fish can carry microbial loads ranging from 10^2 to 10^7 colony-forming units (cfu) per cm^2 on the skin, and from 10^3 to 10^9 cfu per gram in the gills and intestines (Adedeji and Adetunji, 2004). The composition of this microbial flora largely reflects the microbial environment of the water in which the fish lived. Common bacterial genera found include Pseudomonas, Alcaligenes, Vibrio, Serratia, and Micrococcus (Gram and Huss, 2000). Microbial activity is one of the primary causes of fish spoilage. As bacteria grow and metabolize, they generate various spoilage compounds such as biogenic amines (e.g., putrescine, histamine, and cadaverine), organic acids, sulphides, alcohols, aldehydes, and ketones many of which result in unpleasant odors and flavors (Dalgaard *et al.,* 2006; Emborg *et al.,* 2005; Gram and Dalgaard, 2002). In unpreserved fish, spoilage is mainly driven by Gram-negative, fermentative bacteria like those in the Vibrionaceae family. However, in

chilled conditions, spoilage is typically caused by psychrotolerant Gram-negative bacteria such as Pseudomonas spp. and Shewanella spp. (Gram and Huss, 2000). It's essential to differentiate spoilage bacteria from the general microflora, as many bacteria present on fish do not actually contribute to spoilage (Huss, 2005). One key indicator of spoilage is the concentration of trimethylamine (TMA), which forms when specific bacteria reduce trimethylamine oxide (TMAO) a compound fish use for osmoregulation. This microbial reduction process, carried out by species like Shewanella putrefaciens, Aeromonas spp., psychrotolerant Enterobacteriaceae, Photobacterium phosphoreum, and Vibrio spp., leads to the development of ammonia-like off-odors (Gram and Dalgaard, 2002). During the early stages of spoilage, bacteria such as Pseudomonas putrefaciens and fluorescent pseudomonads multiply rapidly, producing proteolytic and hydrolytic enzymes that further break down fish tissue (Shewan, 2001).

2.3.3.2 Enzymatic Spoilage

After harvest, fish undergo chemical and biological changes due to enzymatic breakdown of key molecules (FAO, 2005). Autolytic enzymes, while not causing off-odors, reduce texture quality early in spoilage (Hansen *et al.,* 2003). This limits shelf life even with minimal microbial activity. Digestive enzymes contribute to autolysis, causing softening, belly wall rupture, and leakage of protein- and oil-rich fluids (FAO, 2005). Proteolytic enzymes in muscle and viscera accelerate post-mortem degradation and alter product quality (Engvang and Nielsen, 2001). Poor storage enhances proteolysis, leading to protein breakdown, solubilization, and spoilage through microbial growth and biogenic amine formation (Lin and Park, 2006; Fraser and Sumar, 2008). Belly bursting results from enzyme leakage from the intestines. These enzymes are most active at neutral to alkaline pH but degrade more slowly at 0°C and pH 5 (Martinez and Gildberg, 2011).

2.3.3.3 Chemical Spoilage

Lipid oxidation is a major cause of spoilage in oily pelagic fish like mackerel and herring (Fraser and Sumar, 2008). It follows a three-phase free radical process: initiation (formation of lipid radicals via heat, metal ions, or irradiation), propagation (reaction with oxygen to form hydroperoxides and new radicals), and termination (radicals combine to form stable products) (Frankel, 2005; Khayat and Schwall, 2003; Hultin, 2004). Due to their high polyunsaturated fat content, fish lipids are especially prone to oxidation. Molecular oxygen, activated by transition metals, drives this process (Hultin, 2004). Oxidation can be enzymatic or non-enzymatic. Enzymatic fat breakdown, or lipolysis, involves lipases (from fish tissue or microbes) that release free fatty acids, causing rancid odors and lower oil quality (Huis in't Veld, 2006; FAO, 2005). Key enzymes include triacyl lipase, phospholipase A2, and B (Audley *et al.,* 2008; Yorkowski and Brockerhoft, 2005). Non-enzymatic oxidation is catalyzed by hematin compounds like hemoglobin and myoglobin, forming hydroperoxides (Fraser and Sumar, 2008). The resulting fatty acids can denature proteins in the muscle (Anderson and Ravesi, 2009). Hemoglobin, especially in its deoxygenated or oxidized state, is a strong promoter of oxidation (Undeland *et al.,* 2005).

2.3.4 Factors Influencing Fish Spoilage

2.3.4.1 Time and Temperature Effects on Microbial Growth:

Time and temperature are key in determining fish quality. Higher temperatures accelerate microbial growth and spoilage, while temperatures near 0ºC slow it down, extending shelf life. Rapid cooling after harvest is essential to preserve freshness, texture, and nutrition. The spoilage rate increases with temperature and is measured by the Relative Rate of Spoilage (RRS) (Spencer and Baines, 1964).

2.3.4.2 Impact of Hygiene on Fish Quality During Handling:

Fish quality can be significantly affected by hygiene, with contamination increasing due to poor handling and unclean storage equipment. Studies comparing different handling methods (aseptic, clean, and normal) found that unwashed fish in dirty containers had higher bacterial contamination and shorter shelf life (Huss *et al.,* 1974). Additionally, proper fish hold design is essential for hygiene, as it should allow for easy purge collection. Higher purge levels at 5–7ºC promote bacterial growth, accelerating spoilage (Hermansen, 1983).

2.3.4.3 Rough handling:

Rough handling speeds up spoilage by causing physical damage to the fish, allowing easier access for enzymes and bacteria. Mishandling, such as large catches, stepping on fish, or placing heavy items on them, can lead to bruising and ruptured blood vessels. In rigor mortis, rough handling can cause gaping (Huss 1995).

2.3.4.4 Initial Bacterial Load:

Tropical fish typically carry a slightly higher proportion of Gram-positive and enteric bacteria compared to temperate-water species, though their overall microflora is quite similar (Liston 1980). The key difference lies in bacterial type: temperate fish harbor mainly psychrotrophic bacteria due to cooler water temperatures around 10°C, while tropical fish are dominated by mesophilic bacteria (Gram and Huss 1996).

2.3.4.5 Mode of storage

In bulk storage, the weight of stacked fish can crush those at the bottom, causing physical damage and reduced yield. For example, haddock stored in a 3-foot-deep pile may lose up to 15% of their weight, compared to the typical 3–8% loss from normal biochemical changes

that reduce water-holding capacity (Regenstein and Regenstein 1991). Pressure from ice or other fish can also release gut enzymes into the muscle, speeding up autolytic spoilage.

2.4 Methods of Fish Preservation

Fish can be preserved for both short- and long-term durations (Eyo, 2002).

2.4.1 Short-Term Preservation

2.4.1.1 Chilling

Chilling is the simplest and most common short-term preservation method, achieved by covering fish with ice. Cooling slows spoilage, although fish will still deteriorate within hours if not properly chilled (Tawari and Abowei, 2011). Ice lowers the temperature, slowing enzymatic activity (FAO, 2007), and is especially useful for transporting fish to markets or processing facilities. However, melting ice can leach flavor compounds from the flesh, making it unsuitable for long-term storage.

Most fish are iced during processing. Well-iced fish stored for up to six or seven days can still taste fresh to trained panels, and shelf life can be slightly extended by adding antibiotics to the ice. Ice preserves fish in two main ways (Idachaba, 2001):

1. It reduces bacterial growth by lowering temperature.
2. It washes away bacteria and slime as it melts making drainage of meltwater essential.

2.4.2 Preservation for Long Duration

2.4.2.1 Salting

Various types of salt can be used for curing fish, with some being more effective than others. However, in remote or island areas, people often rely on whatever salt is available whether

purchased, locally produced, or extracted from naturally salty soil. Salting techniques generally fall into two main categories: wet salting and dry salting (FAO, 2005).

2.4.2.2 Wet Salting

Wet salting involves preserving fish by soaking it in brine for an extended period. This requires a watertight container such as a tin, drum, canoe, or barrel. Brine is prepared by mixing one part salt with four parts clean water (either fresh or seawater). If coarse salt is used, it should be ground before dissolving. Proper brine is strong enough to make a fish float (Tys and Peters, 2009). Fish preparation depends on the type and size. Typically, the head is removed, and the fish is gutted and cleaned. Small fish can be salted whole, while large ones should be cut open preferably with the backbone removed. Thick-scaled fish must be descaled, and deep cuts should be made into thick flesh to allow the brine to penetrate. Very large fish are best cut into thin fillets. Once the fish has been prepared based on its size, it should be thoroughly cleaned and then placed into the brine solution (FAO, 2008). And then covered with a plank or matting, and weighted down with rocks to keep it fully immersed. The fish should be stored in a dark or shaded area (Leistner and Gould, 2002). The brine can be reused up to three times, but salt and water must be replenished each time to maintain its strength. However, fresh brine always yields the best results.

2.4.2.3 Dry Salting

In dry salting, fish is coated with salt, and the resulting juices, slime, and brine are allowed to drain away. This method can be done in an old canoe, on mats, leaves, or boxes, as long as the brine formed is allowed to escape. For every two parts of fish, one part of salt is used (Kauffeld *et al.,* 2005). Fish layers should be separated by salt. This method is useful when containers are unavailable, such as for salting flying fish on open boats at sea, where the fish

remain whole. The salted fish can be soaked in fresh water to remove excess salt before consumption, depending on taste preferences (FAO, 2005).

2.4.2.4 Drying

Small, thin fish can be dried directly in the sun if caught early in the morning and the weather is clear. If not, they should be soaked in brine or dry salted overnight before drying the next day (Deepchill, 2010). In case of rain, the fish should be washed in fresh or sea water and dried later once the weather clears. Fish are typically sun-dried on mats or suspended; they should be turned every two hours to ensure quick drying and prevent maggot infestation. For larger fish, hanging is preferred if they are split (Ananou *et al.,* 2007). Dry salted fish should be cleaned before drying, usually after three days. If a large quantity is being preserved, it should be stored in a dark, dry place on wooden boards, covered with a sack or mat. After two weeks, the fish can be briefly sun-dried before being stored again. These are general guidelines, and variations in the drying process are possible (Leistner and Gould, 2002).

2.5 Smoking

Fish can be smoked using various methods, with three primary techniques:

(a) Smoking and roasting;

(b) Hot smoking;

(c) Long smoking.

2.5.1 Smoking and Roasting

This simple preservation method allows for fish to be consumed immediately after curing or within twelve hours. Re-smoking and roasting can extend the product's freshness for an additional twelve hours (Kauffeld *et al.,* 2005). Fresh, unsalted fish is placed over a small fire made from wood or coconut husks, with the fish being turned every five minutes. After

approximately 30 minutes, the fish is ready for consumption or, if longer storage is desired, it should be placed in an aerated container (Tys and Pieters, 2009).

2.5.2 Hot Smoking

Hot smoking preserves fish for up to 48 hours and is ideal for immediate consumption. Small fish are salted for 30 minutes, dried for another 30 minutes, and then smoked using an oil drum. A fire of hardwood or coconut husks is used, with the smoking process taking about one hour until the fish skin turns golden yellow (Tys and Pieters, 2009). For larger fish, they are split and hung on racks above a controlled fire: a slow fire for 30 minutes, a brisk fire for 1 hour, and a small fire for 6 hours (Alasalvar *et al.,* 2011). The smoked fish remains good for 2–3 days, commonly used in the Celebes for skipjack and other tunas (Ananou *et al.,* 2007).

2.5.3 Long Smoking

For long-term preservation (up to several months), smoking can be effective, provided the fish is not oily. A small closed shed made from palm leaves or other local materials can be used, with a height of at least six feet. Inside, racks are built to hang or lay the fish. The fish can be hung on spits, or placed on loosely woven matting, with hanging starting three feet from the bottom (Deepchill, 2010). Coconut husks should be used for slow-burning smoke, and the fish should be dry smoked for 48 hours to ensure thorough drying. If the fish needs to be transported, they should be wrapped in dry leaves and reinforced with bamboo or sticks (Idachaba, 2001).

2.6 Fish Canning

Fish canning involves heat treatment in sealed containers, such as tin, aluminum, or glass, to sterilize the product. The heat treatment kills bacteria and spores, inactivates enzymes, and ensures the fish remains safe and consumable during long-term storage (FAO, 2005). Proper heat treatment and airtight sealing allow canned fish to stay preserved for years without

refrigeration (Leistner and Gould, 2002). Typically, small pelagic species like herrings, sardines, mackerels, and tunas are used for canning (Gopakumar, 2010). The fish must be of high quality, as poor-quality fish can result in undesirable odors, flavors, and textures (Burt, 2003).

2.7 Disadvantages of Fish Preservation

While fish preservation and processing are vital to the fishing industry, they come with several drawbacks (Bate and Bendall, 2010):

1. Chilling: Ice crystal formation during chilling damages muscle tissue, leading to cell rupture, deformation, dehydration, and loss of flavor and texture (FAO, 2008).
2. Poor Hygiene: Inadequate hygiene during steps like washing, gutting, and evisceration can introduce more bacteria, reducing the safety and quality of preserved fish.
3. Histamine Formation: Incomplete preservation can cause histidine in fish flesh to convert to histamine and related compounds (saurine), which may lead to food poisoning (Karube *et al.,* 2001).
4. Drying: This method reduces the weight, nutritional value, and digestibility of fish.
5. Salting: Excessive salting promotes the growth of salt-tolerant bacteria, potentially causing spoilage known as “pink eye.”
6. Salting and Smoking: This combination results in protein loss 1–5% from salting and 8–30% from smoking.
7. Smoking Alone: Smoking can accelerate fat rancidity, reducing the digestibility of fatty fish.

8. Canning: This process causes significant losses of essential nutrients like vitamin B1, pantothenic acid, vitamin C, and folate (FAO, 2005).

Plate 1: Frozen form of *Merluccius merluccius*

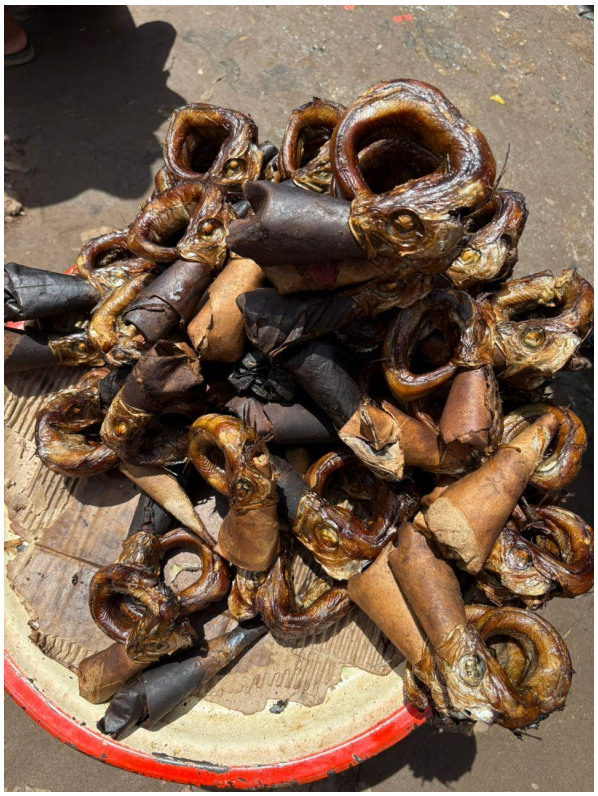

Plate 2: Smoke-dried form of *Merluccius merluccius*

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of study area

The study was carried out in New Benin Market, Oredo Local Government Area (LGA) within Benin Metropolis, Edo State, Nigeria. Located in the South-South region of Nigeria. Edo State is the 20th most populous State as of 2024, with an estimated population of approximately 5.25 million, up from 4.78 million in 2021. It ranks 21st in land area across the country. The state capital, Benin City, is Nigeria's fourth largest city and a key center for the rubber industry. It was established in 1991 from the former Bendel State, Edo is known as the "Heartbeat of the Nation." It shares borders with Kogi State to the north (133 km), Anambra State to the east (about 4 km across the Niger River), Delta State to the south and southeast (350 km), and Ondo State to the west. Edo State consists of 18 local government areas, with Benin City as its main urban center, encompassing primarily four LGAs: Ovia North-East, Oredo, Egor, and Ikpoba-Okha.

3.2 Experimental Design

The experimental design was conducted as a factorial experiment which was laid down as Completely Randomized Design (CRD), and each treatment was conducted in triplicates.

The experiment was made up of two main factors; Species of fish which include; Scumbia *(Scomber scombrus)* and Merluza (*Merluccius merluccius),*and two preservative methods (Frozen and dried fish samples) which were collected randomly at three different points (beginning, middle and end of the market), market and 2 control which include one of the frozen form of each fish species which was dried personally.

The experiment was carried out with 2 (species) x 2(preservation methods) x 3(different points) x 1(market) and 2 control factorial in a CRD. Replicated 3 times.

3.3 Collection of samples

Two fish species in frozen and smoked dried forms were collected from new Benin market. Three of the smoked dried form of each species were collected randomly from three sampling locations (at the beginning of the market, at the middle of the market and the end of the market) from different fish sellers. Three of the frozen form were also collected randomly from three sampling location from different cold rooms. And one of the frozen form of each species were collected randomly too to be used as my control. At the end of the day, a total of 14 fish samples in the frozen and dried form were collected. The dried fish samples were labeled and kept in sterile polythene bags and transported to the laboratory for microbial analysis while the frozen fish samples were also labeled and kept in a bag containing ice and transported to the laboratory for microbial analysis.

Table 1: Collection of samples from different sampling stations in New Benin market, Benin city.

MARKET	FISH SPECIES	Sampling Stations Sampling Station 1	Sampling Station 2	Sampling Station 3
	Scomber scombrus			
New Benin Market	Frozen	XXX	XXX	XXX
	Dried	OOO	OOO	OOO
	Merluccius merluccius			
	Frozen	XXX	XXX	XXX
	Dried	OOO	OOO	OOO

Where;

x = Frozen Fish

o = Dried Fish

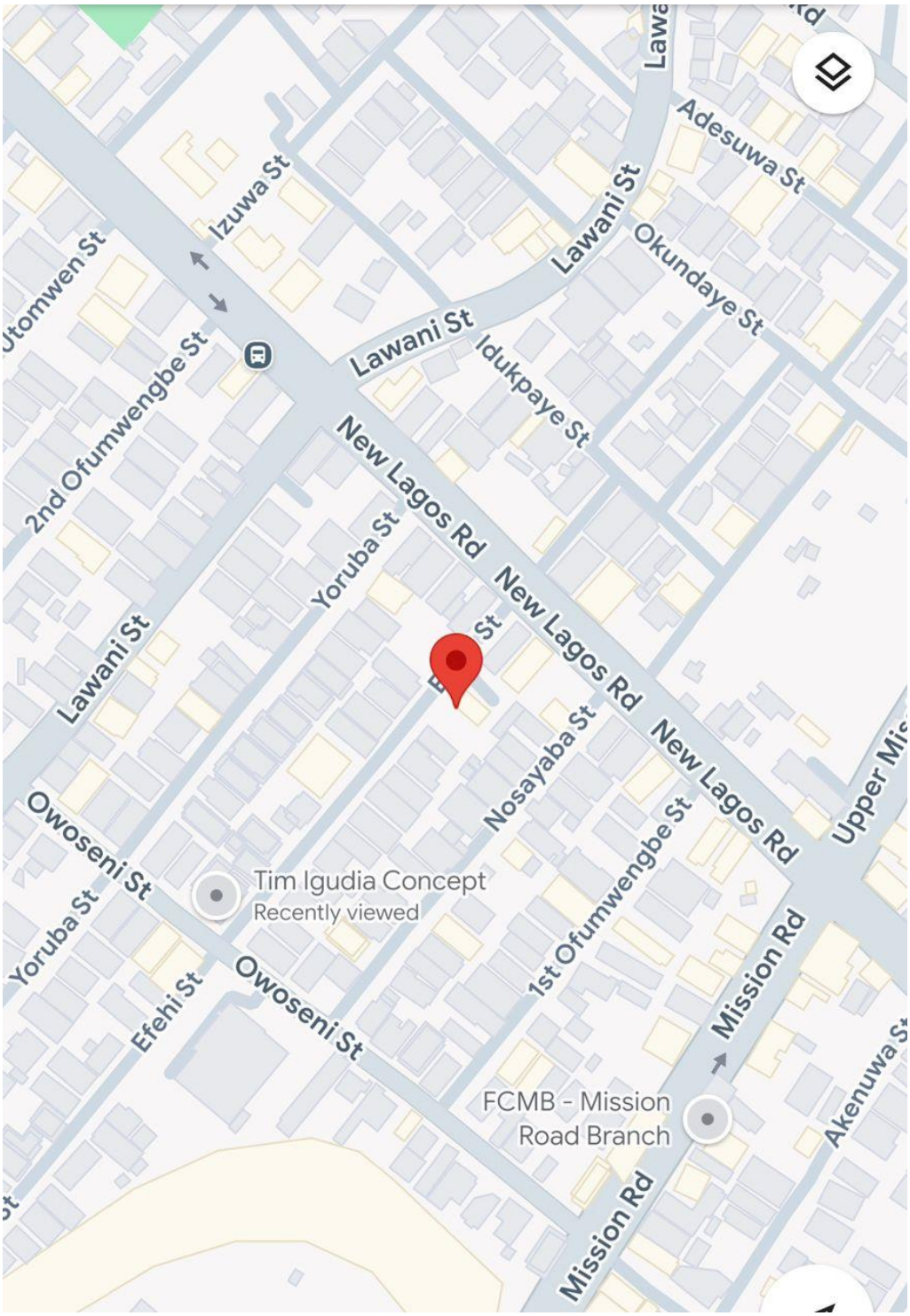

Plate 3: GPS Showing Sampling Location

3.4 Microbial Analysis

3.4.1 Materials

Hand gloves, sample collection jars, Petri dishes, Bunsen burner, antibiotic mixture, cotton wool, disinfectants (Dettol), aluminum foil, autoclave, masking tape, McCartney bottles, marker pens, test tubes, conical flasks, beakers, measuring cylinders, Gram stain reagents, glass slides, standard wire loops and needles, light microscope, weighing balance, and spatula were the materials used for the microbial analysis

3.4.2 Sterilization of materials

To ensure that all glassware, including pipettes, that was used in this study is free from grease, the items were thoroughly washed with detergent, rinsed with clean water, and allowed to drip dry before being placed in canisters. Sterilization was carried out using an autoclave set at 121°C for 15 minutes under 15 psi pressure. Screw caps were sterilized as well, with the caps loosely fitted on the glass containers to allow proper steam penetration. Inoculating loops will be disinfected before and after use by heating them to red-hot using a Bunsen burner flame. Commercially available pre-sterilized Petri dishes were utilized.

3.4.3 Method

The method used for the microbial analysis of the materials was that outlined by Roberts (1978). To facilitate the isolation and confirmation of bacteria and fungi, Nutrient Agar and Potato Dextrose Agar were prepared following the manufacturer's instructions.

3.4.4 Nutrient agar

Fourteen grams (14 g) of Nutrient Agar were dissolved in 500 ml of distilled water and thoroughly stirred to ensure complete mixing. The prepared medium was sterilized in an autoclave at 121°C for 15 minutes and allowed to cool to 45°C. Once cooled, the medium

was poured into sterile disposable Petri dishes. The plates was inoculated using a pasture pipette and incubated at 37°C for 24 hours. After solidification, the plates was inverted to prevent condensation from dripping onto the agar surface, which could cause the merging of colony-forming units. Following incubation, a portion of the microbial growth was sub-cultured and Gram stained, as described by Roberts (1978), and the slides was examined under a microscope at 100x magnification. Nutrient Agar will specifically be used for the isolation of bacteria.

3.4.5 Potato dextrose agar

Potato Dextrose Agar (PDA) was used for the isolation of fungi, following the method described by Roberts (1978). The medium was prepared by dissolving 20g of PDA in distilled water within a 500 ml conical flask and stirring thoroughly to ensure complete mixing. The mixture was sterilized in an autoclave at 121°C for 15 minutes and allowed to cool to 45°C. Once cooled, the medium was poured into sterile, pre-inoculated disposable Petri dishes and left to solidify. Fungal isolates were identified through Gram staining and examined under a microscope at 100x magnification.

3.4.6 Preparation of sample and serial dilution

The microbial analysis for both fungi and bacteria, as well as sample preparation, was conducted using the procedure outlined by Roberts (1978). One gram (1 g) of fish sample was cut with a sterilized blade, pounded, and transferred into a test tube containing 10 ml of distilled water to serve as the stock solution. Additionally, five other test tubes, each containing 9 ml of distilled water, were arranged in a test tube rack for serial dilution. Using a pipette, 1 ml of the stock solution was transferred to the second test tube, and the process was repeated sequentially up to the sixth tube, resulting in dilution levels of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}. Dilution factors 10^{-3} and 10^{-5} were selected for plating. From each of these dilutions,

1 ml was transferred into sterilized Petri dishes in duplicates. All plates were incubated at 37°C for 24 hours prior to colony counting and isolation.

3.4.7 Isolation of microorganisms

The Microorganisms were isolated using the serial dilution technique on Nutrient Agar (NA) for bacteria and Potato Dextrose Agar (PDA) for fungi. In this method, 1.0 g of the sample was added to 10 ml of distilled water, thoroughly mixed for 15 minutes, and vortexed. The resulting suspension was serially diluted to 10^{-3} and 10^{-5}. From each dilution, 0.1 ml was pipetted onto NA and PDA plates, evenly spread using a sterile glass spreader, and incubated at 37°C for bacterial growth and 28°C for fungal growth. Each visible colony on the plate were counted as one colony-forming unit (CFU).

3.4.8 Inoculation of plates

Duplicate Nutrient Agar plates were inoculated with 0.1 ml of the 10^{-5} diluted solution using the pour plate technique. The plates were incubated at 37°C for 24 hours, after which colony enumeration and isolation were carried out, following the method described by Baker *et al.,* (1998).

3.4.9 Isolation of bacteria

The isolated bacterial colonies were purified by re-streaking them onto sterile Nutrient Agar plates, followed by incubation at room temperature (28 ± 2°C) for 24 hours.

3.4.10 Isolation of fungi

Discrete sub-cultured colonies were purified by re-planting onto Potato Dextrose Agar and incubated at 28 ± 2 °C for 3–5 days.

3.4.11 Characteristics and identification of microorganism

Isolates were identified through Gram staining, physiological and biochemical tests, as well as sugar fermentation, following standard taxonomic procedures outlined by Fawole and Oso (2001).

3.4.12 Morphological test

Morphological characteristics were observed using a microscope.

3.4.13 Gram staining

A smear of each bacterial isolate were prepared on a clean slide. Using a sterilized inoculating needle, a small amount of bacterial colony was picked and mixed with a drop of sterile distilled water on the slide. The bacterial cells were spread evenly to form a thin layer, air-dried, and then heat-fixed. The heat-fixed smear was stained with crystal violet for 1–2 minutes, after which the excess stain was gently rinsed off. The slide was decolorized with 95% alcohol until no more violet color washes off. After rinsing the slide with moderate running tap water, it was counterstained with safranin for 1–2 minutes, rinsed again with water, blotted dry, and examined under an oil immersion microscope (Fawole and Oso, 2001).

3.4.14 Biochemical test

The biochemical characteristics of the isolates were determined using the following tests on fresh cultures: Catalase test, Coagulase test, Citrate utilization test, Urease test, Indole formation test, and Triple Sugar Iron test.

3.4.15 Catalase test

A thick emulsion of each suspected bacterial isolate was prepared on a clean slide. Several drops of 3% hydrogen peroxide were added to each slide. The presence of effervescence, caused by the release of catalase enzyme from the bacteria, indicates a positive result. In

contrast, bacteria that do not produce catalase will show no gas bubble formation (Fawole and Oso, 2001).

3.4.16 Oxidase test

A piece of filter paper was soaked in a 1% sodium oxalate solution. Each bacterial colony was picked up and rubbed onto the filter paper. A color change to blue within 10 seconds indicate a positive reaction for the enzyme oxidase.

3.4.17 Urease test

The medium used was urea agar base, which was autoclaved and cooled to 30°C before adding 5 ml of a 40% urea solution to each tube. The test bacterial isolates were inoculated into the tubes containing the urease reagent and incubated at 37°C for 24–48 hours. This test is designed to determine whether the organisms can produce the enzyme urease, which catalyzes the breakdown of urea into ammonia.

3.5 Statistical Analysis

Data were analyzed using two-way analysis of variance (ANOVA) with the aid of computer software (Genstat Version 8.1, 2005). Significant differences between means will be identified using Duncan's multiple range test at a significance level of $P < 0.05$.

CHAPTER FOUR

RESULTS

4.1 Total Bacteria Count (TBC)

The mean Total Bacteria Count (TBC) expressed in Colony Forming Units per gram (CFU/g) of the fresh and smoked dried fish samples is presented in Table 2. The TBC across the analyzed samples ranged from 1.5 $\times 10^4$ CFU/g (Merluza Frozen) to 9.6 $\times 10^3$ CFU/g (Scumbia Dried), indicating a high degree of microbial presence. There was a significant difference ($P < 0.05$) in the TBC between the fresh and smoked dried fish samples. For *Scomber scombrus*, the Dried sample (9.6 $\times$ 10^3 CFU/g) was significantly higher than the Frozen sample (8.0 $\times 10^3$ CFU/g).

Table 2: Bacteria Counts (cfu/g) of Fish Sample.

Market	Species	Preservation Method	Sampling Locations			Mean (ẍ)	SED
			A	B	C		
New Benin Market	*Merluccius merluccius*	Frozen	1.8×10^4	1.4×10^4	1.3×10^4	1.5×10^{4b}	3477.1
		Control				2.0×10^{3a}	
		Dried	6.1×10^3	2.6×10^3	1.5×10^3	3.4×10^{3a}	
	Scomber scombrus	Frozen	1.0×10^4	1.7×10^4	6.0×10^3	8.0×10^{3ab}	
		Control				3.4×10^{3a}	
		Dried	6.4×10^3	8.4×10^3	1.4×10^4	9.6×10^{3ab}	

Mean Values (ẍ) with the same superscripts across the table are not significantly different ($P < 0.05$)

S.E.D: Standard error of the differences of mean

Cfu/g: Colony-Forming Units (CFU) per gram

4.2 Total Fungi Count (TFC)

The mean Total Fungi Count (TFC) is presented in Table 3. The TFC ranged from 5.67 $\times 10^2$ CFU/g (Merluza Frozen) to 7.0 $\times 10^2$ CFU/g (Scumbia Dried). Statistical analysis using DMRT indicated no significant difference ($P > 0.05$) in the mean TFC among the fresh and smoked dried fish samples.

There was no significant difference in the TFC of the samples with dried *Scomber scombrus* (7.0 $\times$ 10^2 CFU/g) recorded the highest TFC, and frozen *Merluccius merluccius* (5.67 x 10^2 CFU/g) recorded the lowest TFC.

Table 3: Fungi Counts (cfu/g) of Fish Sample.

Market	Species	Preservation Method	Sampling Locations			Mean (ẍ)	SED
			A	B	C		
New Benin Market	*Merluccius merluccius*	Frozen	1.2×10^3	2.0×10^2	3.0×10^2	5.67×10^{2a}	471.2
		Control				2.0×10^{2a}	
		Dried	1.0×10^2	2.0×10^2	3.0×10^2	2.0×10^{2a}	
	Scomber scombrus	Frozen	3.0×10^2	1.3×10^3	3.0×10^2	6.33×10^{2a}	
		Control				3.0×10^{2a}	
		Dried	1.0×10^2	1.0×10^2	1.9×10^3	7.0×10^{2a}	

Mean Values (ẍ) with the same superscripts across the table are not significantly different ($P < 0.05$)

S.E.D: Standard error of the differences of mean

Cfu/g: Colony-Forming Units (CFU) per gram

4.3 Diversity and Percentage Occurrence of Microbial Isolates

4.3.1 Diversity and percentage occurrence of microbial isolates

As seen in table 4, a total of five bacterial genera were isolated from the fish samples, contributing to a total bacterial frequency of 61.84% of all isolated microorganisms. The most frequently isolated bacterium was Enterobacter sp., with a frequency of 14.47% (11 isolates). Enterobacter was observed in most dried fish samples (DM A, B, C, and DS A, B, C) but was largely absent from the fresh/frozen samples. *Staphylococcus sp*. with a frequency of 13.16% (10 isolates) was the second most frequent isolates. *Proteus sp.*, and Bacillus sp. both at 11.84 (9 isolates each) and *Pseudomonas sp*. (10.53%) were also frequently isolated.

4.3.2 Fungal Isolates

A total of five fungal genera were isolated, contributing to a total fungal frequency of 38.16% of all isolated microorganisms. The most frequently isolated fungi were *Penicillium sp.* and *Aspergillus sp.*, with a frequency of 13.16 (10 isolates) and 9.21 (7 isolates) respectively. *Aspergillus sp.* was consistently found in many dried and some frozen samples. *Saccharomyces sp., Mucor sp*., and *Rhizopus stolonifer* were also isolated, each at a frequency of 5.26% (4 isolates each).

Table 4: Frequency Of Occurrence Of Microbial Isolates In Fish Samples

Bacterial isolate	F(%)	FM A	FM B	FM C	DM A	DM B	DM C	Ctrl M	FS A	FS B	FS C	DS A	DS B	DS C	Ctrl S
Bacillus sp.	9(11.84)	+	+	-	-	-	-	+	-	+	+	+	+	+	+
Staphylococcus sp.	10(13.16)	+	+	+	+	+	+	+	+	-	-	-	+	+	-
Proteus sp.	9(11.84)	+	+	+	-	-	-	-	+	+	+	-	+	+	+
Pseudomonas sp	8(10.53)	-	+	-	+	+	+	+	-	+	-	+	-	+	-
Enterobacter sp	11(14.47)	+	+	+	+	+	-	-	+	+	+	+	+	-	+
Bacterial frequency	47	4	5	3	3	3	2	3	3	4	3	3	4	4	3
Bacterial % frequency	61.84	8.51	10.64	6.38	6.38	6.38	4.26	6.38	6.38	8.51	6.38	6.38	8.51	8.51	6.38
Fungal isolate															
Aspergillus sp.	7(9.21)	+	-	-	-	+	+	-	+	+	-	-	-	+	+
Saccharomyces sp	4(5.26)	-	-	-	+	-	-	+	-	+	-	+	-	-	-
Penicillium sp	10(13.16)	+	+	+	-	-	+	+	+	+	+	-	-	+	+
Mucor SP.	4(5.26)	-	-	+	-	-	+	-	-	-	-	-	+	+	-
Rhizopus stolonifer.	4(5.26)	+	-	-	-	+	-	-	-	-	+	-	-	+	-
Fungal frequency	29	3	1	2	1	2	3	2	2	3	2	1	1	4	2
Fungal % frequency	38.16	10.34	3.45	6.90	3.45	6.90	10.34	6.90	6.90	10.34	6.90	3.45	3.45	13.79	6.90
Microbial frequency	76	7	6	5	4	5	5	5	5	7	5	4	5	8	5
Microbial % frequency	100	9.21	7.89	6.58	5.26	6.58	6.58	6.58	6.58	9.21	6.58	5.26	6.58	10.53	6.58

+ =Present, - = Not present, F = frequency, CTR = ControlFM A = Fresh Merluza A, FM B = Fresh Merluza B, FM C = Fresh Merluza C, DM A= Dry Merluza A, DMB = dry Merluza B, DM C = Dry Merluza C, Ctrl M = Control Merluza. FS A = Fresh Scumbia A, FS B = Fresh Scumbia B, FS C = Fresh Scumbia C, DS A = Dry Scumbia A, DS B = dry Scumbia B, Dry Scumbia C, Ctrl S = Control Scumbia

CHAPTER FIVE

DISCUSSION

5.1 Fungal Load of Frozen Fish Species

The results revealed that the fungal counts in frozen fish samples ranged between 2.0×10^2 cfu/g and 1.3×10^3 cfu/g, with *Merluccius merluccius* showing slightly higher counts than *Scomber scombrus*. Although the counts were generally low, their presence indicated possible post-processing contamination. This agrees with the findings of Egbere *et al.,* (2010), who reported that improper handling and repeated thawing of frozen fish could facilitate fungal growth despite low temperatures. The relatively low fungal counts could be attributed to the inhibitory effects of freezing on microbial proliferation, as low temperatures reduce enzymatic activity and metabolic processes (Olayemi and Adebayo, 2018). However, fungi such as Aspergillus and Penicillium were still isolated, showing their ability to survive under cold and nutrient-limited conditions (Okonta and Ekelemu, 2005). Though the fungal load of the frozen form of *Merluccius merluccius* (5.67 x 102 cfu/g) was higher than that of the smoked dried form (2.0 x 102 cfu/g), the occurrence of these fungi suggests contamination from the air, storage containers, or handlers during packaging and sales. Similar studies by Abdullahi *et al.,* (2020) on frozen fish from Kano markets also reported the persistence of fungal spores even in properly frozen fish, emphasizing the role of poor sanitary conditions and cross-contamination in fish processing and marketing environments.

5.2 Bacterial Load of Frozen Fish Species

For *Merluccius merluccius*, the bacterial count of the frozen form (1.5 x 10 4 cfu/g) was higher than that of the smoked dried form (3.4 x 10 3 cfu/g). The bacterial counts obtained from frozen

samples ranged between 6.1× 10^3 cfu/g and 1.8 × 10^4 cfu/g. These values are within the acceptable limits for fresh fish as recommended by the International Commission on Microbiological Specifications for Foods (ICMSF, 2011). Nevertheless, their presence suggests that contamination occurred during handling, transportation, or retailing.

The bacteria isolated included Staphylococcus sp., Proteus sp., Pseudomonas sp., Bacillus sp., and Enterobacter sp. Among these, Staphylococcus sp. and Enterobacter sp. had the highest frequency of occurrence. This aligns with findings by Adebayo-Tayo and Ogunjobi (2008), who noted that Staphylococcus aureus is commonly associated with fish surfaces due to frequent human handling, while Enterobacter species are indicators of poor hygiene and water contamination.

The presence of Pseudomonas sp. is particularly significant as this genus is a well-known spoilage bacterium responsible for off-odors and slime formation in stored fish (Gram and Dalgaard, 2002). Pseudomonas species are known pathogens capable of causing nosocomial infections in humans (Todar, 2012). The Staphylococcus species identified in this study belong to a bacterial group commonly associated with human diseases, although they naturally inhabit the skin and mucous membranes without causing harm. This agrees with the report of Ayuba *et al.,* (2013), who noted that Staphylococcus aureus is among the most frequent causes of human infections. Besides superficial and systemic infections such as boils, impetigo, and folliculitis, S. aureus can also lead to more severe conditions, including pneumonia, bacteremia, and various bone and wound infections. The toxins and enzymes it produces may worsen certain conditions, resulting in food poisoning, septic shock, or toxic shock syndrome. Moreover, the severity of disease symptoms often depends on both the amount of contaminated food ingested and an individual's sensitivity to the toxin. Since S. aureus is strongly linked to food poisoning, its

presence indicates poor handling or cross-contamination of smoked fish products. Although freezing can inhibit bacterial activity, it does not completely destroy microorganisms; once thawed, bacterial growth can resume (Bremner, 2002). Therefore, maintaining an unbroken cold chain is essential to prevent microbial proliferation.

5.3 Fungal Load of Dried Fish Species

The fungal counts of dried fish samples (*Scomber scombrus*) were slightly higher than those of frozen samples, ranging between 1.0×10^2 cfu/g and 1.9×10^3 cfu/g. The presence of species such as Aspergillus, Penicillium, Mucor, Rhizopus, and Saccharomyces indicates contamination likely occurring during drying, storage, or marketing. in the fish samples were Fusarium spp., Mucor piriformis, Aspergillus flavus, Aspergillus niger, Rhizopus stolonifer, Saccharomyces spp., Candida spp. and Penicillium spp. Adebayo-Tayo *et al.,* (2008) identified Aspergillus flavus, Aspergillus terreus, Aspergillus fumigatus, Absidia spp., Rhizopus spp., A. niger, Mucor spp., Cladosporium spp., Penicillium italicum, Penicillium viridatus, Candida tropicalis, and Fusarium moniliforme as fungal isolates present in smoked-dried fish sold across different markets in Uyo, Nigeria. Similarly, Akise *et al.,* (2013) reported P. italicum, Penicillium oxalicum, Mucor, Saccharomyces, Rhodotorula spp., and Aspergillus spp. as fungi isolated from three anatomical parts of smoked-dried fish during storage. The occurrence of Aspergillus sp., Rhizopus sp., and Penicillium sp. in smoked fish species may result from the reabsorption of environmental moisture during storage, which promotes microbial growth, along with contamination during processing, handling, and market display.

According to Ayeloja *et al.,* (2011), in artisanal fisheries, freshly caught fish are often covered with moist bags or mixed with wet grass and aquatic weeds to lower their temperature. However, this practice increases the risk of contamination by microorganisms such as Salmonella and

Aspergillus, suggesting that fish spoilage can begin in the aquatic environment. Processed fish are further exposed to microbial invasion due to unhygienic processing and preservation practices, particularly common in artisanal fisheries.

Most of the fungal isolates reported in these studies are known producers of mycotoxins, which are secondary metabolites synthesized by microfungi capable of causing disease. Aspergillus species, for instance, produce various mycotoxins such as aflatoxin, ochratoxin, and sterigmatocystin, as noted by Hashem (2011). Cases of acute aflatoxicosis in humans have been recorded in several parts of the world, showing symptoms such as vomiting, abdominal pain, pulmonary edema, convulsions, coma, and even death due to cerebral edema and fatty degeneration of the liver, kidney, and heart (Akinyemi *et al.,* 2011).

Adebayo-Tayo *et al.,* (2008) further reported that aflatoxins occur in numerous protein sources of both plant and animal origin, where they contribute to the reduction of nutritional value. This observation supports the findings of Pratiwi *et al.,* (2015), who revealed that aflatoxins in food materials negatively affect both nutritional quality and food safety. The significant presence of fungi, especially aflatoxigenic molds, in smoked fish species is therefore a serious concern, as these molds produce mycotoxins known to be harmful to humans particularly affecting the liver and kidneys and potentially leading to death.

According to Olayemi *et al.,* (2012), the traditional sun-drying method commonly practiced in Nigerian markets exposes fish to dust, air, and insects, which serve as carriers of fungal spores. Aspergillus niger and Penicillium sp. are particularly known for their ability to thrive in low-moisture environments and produce mycotoxins such as aflatoxins and ochratoxins (Adeyeye, 2016).

Furthermore, the variation in fungal load across sampling points (A, B, and C) may reflect differences in storage duration, environmental humidity, and vendor hygiene. Similar patterns were observed by Nwankwo *et al.,* (2019) in their study on dried fish sold in Onitsha Market, where fungal contamination was linked to extended storage and poor packaging. According to these reports from multiple authors, these microbial contaminants are not limited to the fish species under research, as contamination by bacteria and fungi occurs in a variety of smoked fish species.

5.4 Bacterial Load of Dried Fish Species

The bacterial counts in dried fish samples ranged between 1.5×10^3 cfu/g and 1.4×10^4 cfu/g, with *Scomber scombrus* generally showing higher values than *Merluccius merluccius*. It was observed that the bacteria count for smoke dried *Scomber scombrus* was higher than that of the frozen form. The predominant bacteria identified were Staphylococcus sp., Proteus sp., and Enterobacter sp. The survival of these organisms in dried fish suggests that the drying temperature and duration were insufficient to destroy bacterial spores (Olafadehan and Adewumi, 2015).

Drying reduces water activity, making the environment unfavorable for most microorganisms; however, spore-forming bacteria such as Bacillus sp. can withstand desiccation and later become active under favorable conditions (Okoro *et al.,* 2011). According to Odu and Imaku (2013), S. aureus is resistant to heat, drying, and radiation and generates toxin that cannot be eliminated by heat. The presence of Proteus sp. and Enterobacter sp. also implies post-drying contamination from handling and display surfaces.

This finding corroborates the work of Eyo (2001), who observed that bacterial contamination in dried fish often results from exposure to unhygienic surfaces and the absence of adequate packaging. Vendors in open markets such as New Benin often keep fish exposed to air and dust, encouraging microbial colonization.

According to the findings of this study, the smoke dried *Scomber scombrus* had a higher microbial contamination than the frozen form, with the control having the lowest microbial contamination. While for *Merluccius merluccius*, the frozen form had a higher microbial contamination than the smoke dried form, with the control having the lowest microbial contamination too. The high microbial load observed may be linked to the market's polluted environment, which is affected by various human activities such as the absence of a proper drainage system, proximity to refuse dumps, inadequate smoking practices by fish processors, and poor hygiene and handling methods employed by smoked fish sellers. In Uyo, Nigeria, Adebayo-Tayo *et al.,* (2008) reported a similar situation where retailers displayed smoked-dried fish in open trays close to gutters and waste heaps. Likewise, Adegunwa *et al.,* (2013) found that smoked fish obtained from a camp in Odeda, Ogun State, Nigeria, exhibited higher microbial loads compared to other locations, largely due to improper handling, frequent exposure, and unsanitary environmental conditions. The elevated microbial growth observed in smoked fish samples may therefore be attributed to poor handling practices by sellers both before and after smoking. The microbial load found in the frozen fish is largely influenced by the initial level of contamination, the rate of freezing, temperature variations during storage, and the handling methods used during thawing. Although freezing slows microbial activity, a residual population from earlier stages such as harvesting, handling, and processing remains present. Once the fish is

thawed, these surviving microorganisms can reactivate and multiply rapidly under favorable temperature conditions, potentially leading to spoilage or foodborne illness.

5.5 Percentage Occurrence and Diversity of Microbial Isolates

The microbial diversity data showed that bacteria had a higher percentage occurrence (61.84%) compared to fungi (38.16%) across all fish samples. This indicates that bacteria were more dominant contaminants than fungi. Among the bacterial isolates, Enterobacter sp. recorded the highest occurrence (14.47%), followed by Staphylococcus sp. (13.16%) and Bacillus sp. (11.84%). Similarly, Penicillium sp. (13.16%) and Aspergillus sp. (9.21%) were the most frequent fungi identified.

The dominance of bacteria may be due to their rapid multiplication rates under favorable conditions and their ability to survive short-term environmental stress. The relatively lower fungal occurrence could be associated with the reduced moisture content of the fish samples and the antimicrobial effect of salt used during preservation (Agbabiaka *et al.,* 2020).. This microbial diversity reflects the combined effects of environmental exposure, handling, and preservation methods on fish quality in local markets. The findings are consistent with those of Nwabueze and Okocha (2008), who also reported a higher bacterial prevalence in both fresh and dried fish sold in Benin City markets.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study revealed that both frozen and dried fish sold at New Benin Market harbored varying levels of bacterial and fungal contamination. The predominant bacterial isolates identified were *Staphylococcus sp., Enterobacter sp., Proteus sp., Pseudomonas sp.,* and *Bacillus sp.,* while fungal isolates included *Aspergillus sp., Penicillium sp., Mucor sp., Rhizopus sp.,* and *Saccharomyces sp*.

The high frequency of *Enterobacter sp*. and *Staphylococcus sp.* in both frozen and dried fish suggests that contamination likely occurred through poor handling, processing, and exposure to unsanitary environments.

Smoke dried fish obtained from the market had a high occurrence of microorganisms, whereas the smoked control fish sample had a low occurrence of microorganisms due to good sanitary conditions observed prior to and during the smoking process, which is generally lacking in the smoked fish obtained from the markets. This indicates that fish that has been properly smoked and preserved is safe to eat and free of microbial contamination. Although the control samples had significantly lower microbial counts, their detection of some microorganisms indicates that even under controlled storage conditions, contamination may occur if hygiene practices are not strictly followed.

The smoke dried form of *Scomber scombrus* recorded a higher microbial counts compared to the frozen form. This may be due to exposure to airborne spores, dust, and moisture absorption during open-air drying and market display. Also, maybe the fish was not properly smoke dried

and still has some moisture in it or the dried fish were able to absorb moisture from the environment, which would then support the growth and multiplication of the microorganisms.

The microbial count for the frozen form of *Merluccius merluccius* was higher than the dried form. This may be because the dried *Merluccius merluccius* was properly smoked to remove moisture as properly dried fish, especially with salt-curing, has a significantly lower microbial count because the conditions do not support microbial growth.

The overall microbial loads obtained were within or slightly above acceptable limits for ready-to-eat foods as recommended by the International Commission on Microbiological Specifications for Foods (ICMSF, 2011) which ranges from 5.0×10^5 and 1.0×10^6 CFU/g. However, the results of the study emphasize the importance of maintaining good hygiene during fish processing, preservation, and marketing to ensure consumer safety.

6.2 Recommendations

1. Fish handlers and vendors should adopt strict hygiene measures during processing, drying, and display. Clean utensils, sanitized surfaces, and protective coverings should always be used to minimize contamination.

2. Frozen fish should be kept under constant cold chain conditions to prevent microbial growth. Dried fish should be stored in dry, clean, and well-ventilated environments to discourage fungal proliferation.

3. Routine microbial assessments should be conducted by relevant health authorities such as NAFDAC and environmental health officers to ensure that fish products meet safety standards before sale to consumers.

4. Awareness programs should be organized for fish vendors and processors on the dangers of poor handling and the importance of food safety practices.

5. Market authorities should provide facilities such as clean water supply, waste disposal systems, and designated hygienic areas for fish display to reduce environmental contamination.

6. The government, through the Ministry of Health and Environmental Sanitation, should enforce strict regulations on fish handling, preservation, and marketing, with penalties for defaulters.

REFERENCES

Abbas, K. A., Saleh, A. M., Mohamed, A., and Lasekan, O. (2009). The relationship between water activity and fish spoilage during cold storage: A review. *Journal of Food and Agricultural Environment, 7,* 86–90.

Abdullahi, I. O., Maiangwa, M. W., and Aliyu, A. M. (2020). Microbiological quality assessment of frozen fish sold in Kano metropolis, Nigeria. Bayero *Journal of Pure and Applied Sciences,* **13**(1): 132–136.

Abolagba, O. J., and Igbinevbo, E. E. (2010). Microbial load of fresh and smoked fish marketed in Benin metropolis, Nigeria. *Research Journal of Fisheries and Hydrobiology, 5(2),* 99–104.

Abolagba, O. J., and Uwagbai, E. C. (2011). A comparative analysis of the microbial load of smoked dried fishes (Ethmalosa fimbriata and Pseudotoliyhus elongates) sold in Oba and Koko markets in Edo and Delta states Nigeria at different seasons. *Australian Journal Basic and Applied Sciences,* 5 (5), 544, 550.

Adebayo-Tayo, B. C., and Ogunjobi, A. A. (2008). Comparative studies of microbial load of smoked-dried fishes (*Scomber scombrus* and *Clarias* gariepinus) sold in Uyo, Nigeria. *Journal of Food and Agricultural Environment*, **6**(2): 65–69.

Adebayo-Tayo, B. C., Odu, N. N., and Okonko, I. O. (2012). Microbiological and physiochemical changes and its correlation with quality indices of tilapia fish (*Oreochromis niloticus*) sold in Itu and Uyo markets in Akwa Ibom State, Nigeria. *New York Science Journal, 5,* 38–45.

Adebayo-Tayo, B. C., Onilude, A. A., and Patrick, U. G. (2008). Mycofloral of smoke-dried fishes sold in Uyo, Eastern Nigeria. *World Journal of Agricultural Sciences*, 4(3), 346-350.

Adebowale, B. A., Dongo, L. N., Jayeola, C. O., and Orisajo, S. B. (2008). Comparative quality assessment of fish *(Clarias gariepinus)* smoked with cocoa pod husk and three other different smoking materials. *Journal of Food Technology, 6*, 5–8.

Adedeji, O. B., and Adetunji, V. O. (2004). Pests in farm animals and stored animal products. In *Agriculture, renewable natural resources, animal husbandry and health* (pp. 141–151). General Studies Programme (GSP), University of Ibadan.

Adegunwa, M. O., Adebowale, A. A., Olisa, Z. G., and Bakare, H. A. (2013). Chemical and microbiological qualities of smoked herring (sardinella eba, valenciennes

Adeniji, C. A., and Okedeyi, O. O. (2017). Preliminary assessment of heavy metal concentrations in selected fish feed ingredients in Nigeria. *Journal of Fisheries and Livestock Production, 5*(1), 1–4.

Adeyeye, S. A. O. (2016). Fungal mycoflora and aflatoxin contamination of some dried fish species sold in major markets of Ogun State, Nigeria. *Croatian Journal of Fisheries*, **74**(4):149–156.

Agbabiaka, T. O., Onwumere, B. G., and Daminabo, I. C. (2020). Assessment of microbial flora associated with smoked-dried fish sold in selected markets in Port Harcourt, Nigeria. *Nigerian Journal of Fisheries and Aquaculture*, **8**(1): 45–53.

Akinwumi, F. O., and Adegbehingbe, K. T. (2015). Microbiological analysis of three smoked fish obtained from Ondo State, Nigeria. *Food and Public Health, 5*(4), 122–126.

Alasalvar, C., Miyashita, K., Shahidi, F., and Wanasundara, U. (2011). *Handbook of seafood quality, safety, and health applications* (p. 349). John Wiley and Sons.

Ali, S. S. R., Abdhakir, E. S., Muthukkaruppan, R., Sheriff, M. A., and Ambasankar, K. (2020). Nutrient composition of some marine edible fish species from Kasimedu Fish Landing Centre, Chennai (TN), India. *International Journal of Biological Innovations, 2*(2), 165–173.

Amos, B. (2007). *Analysis of quality deterioration at critical steps/points in fish handling in Uganda and Iceland and suggestions for improvement* (p. 45). United Nations University.

Ananoui, S., Maqueda, M., Martínez-Bueno, M., and Valdivia, E. (2007). Bio preservation, an ecological approach to improve the safety and shelf-life of foods. In A. Méndez-Vilas (Ed.), *Communicating current research and educational topics and trends in applied microbiology* (p. 456). Formatex.

Anderson, M. L., and Ravesi, E. M. (2009). Reactions of free fatty acids with protein in cod muscle frozen and stored at -26°C after aging in ice. *Journal of Fish Research, 26,* 2727–2736.

Anthonio, G. B. O. (1970). *Fish marketing survey in the Kainji Lake Basin*: Yelwa area study. Nigerian Institute of Social and Economic Research.

Audley, M. A., Shetty, K. J., and Kinsella, J. E. (2008). Isolation and properties of phosphilase A from Pollock muscle. *Journal of Food Science, 43*, 1771–1775.

Ayuba, V. O., Alhassan, M. I., and Jimmy, U. U. (2013). Assessment of the microbial load of smoked sardine (Sardina pilchardus, Walbaum, 1792) sold in Makurdi Markets. *International Journal of Current Microbiology and Applied Sciences*, 2(9), 277-287.

Ayuba, V. O., and Omeji, N. O. (2006). Effect of insect infestation on the shelf life of smoked dried fish. In *Proceedings of the 21st Annual Conference of the Fisheries Society of Nigeria (FISON), Calabar, 13–17 November* (pp. 357–359).

Azeza, N. I. (1976). Fish handling, processing and marketing in the Lake Chad Basin (North South shores). In *Proceedings of the Conference on the Handling, Processing and Marketing of Tropical Fish* (pp. 348–352). Tropical Product Institute.

Bate, E. C., and Bendall, J. R. (2010). Changes in fish muscle after death. *British Medical Bulletin, 12,* 2305.

Bello, R. A., Adeyeye, S. A. O., and Adebayo, E. F. (2014). Mycoflora and potential mycotoxin contamination of smoked-dried fish sold in major markets of Ogun State, *Nigeria. Journal of Food Safety*, **34**(2): 145–152.

Bremner, H. A. (2002). Safety and quality issues in fish processing. Woodhead Publishing Series in Food Science, Technology and Nutrition. Woodhead Publishing.

Burt, J. R. (2003). Hypoxanthine: A biochemical index of fish quality. *Process Biochemistry, 11*(10), 23–25.

Clucas, I. J. (1981). In R. Kreuzer (Ed.), *Fish inspection and quality control* (pp. 1–40). Fishing News (Books).

Dalgaard, P., Madsen, H. L., Samieian, N., and Emborg, J. (2006). Biogenic amine formation and microbial spoilage in chilled garfish (Belone belone): Effect of modified atmosphere packaging and previous frozen storage. *Journal of Applied Microbiology, 101,* 80–95.

Daniel E. O., Ugwueze A. U. and Igbegu H. E. (2013). Microbiological quality and some heavy metals analysis of smoked fish sold in Benin City, Edo State, Nigeria.

Davies, R. M. (2006, August 19). *Mechanization of fish industry in Nigeria: Status and potential for self-sufficiency in fish production.* Paper presented at the one-day workshop on intensive fish farming.

Davies, R. M., Davies, O. A., Inko-Tariah, M. B., and Bekibele, D. O. (2008). Mechanization of fish farms in Rivers State, Nigeria. *World Applied Sciences Journal, 3*(6), 926–929.

Egbere, O. J., Akubor, P. I., and Adeyemi, I. A. (2010). Microbiological quality of traditionally smoked fish sold in Jos and its environs. *Journal of Food Safety,* **30**(4): 714–728.

Emborg, J., Laursen, B. G., and Dalgaard, P. (2005). Significant histamine formation in tuna (Thunnus albacares) at 2°C: Effect of vacuum- and modified-atmosphere-packaging on psychrotolerant bacteria. *International Journal of Food Microbiology, 101,* 263–279.

Engvang, K., and Nielsen, H. H. (2001). Proteolysis in fresh and cold-smoked salmon during cold storage: Effects of storage time and smoking process. *Journal of Food Biochemistry, 25*(4), 379–395.

Eyo, E. E. (2002). *Fish processing and utilisation*. Paper presented at the National Workshop on Fish Processing, Preservation, Marketing and Utilisation, New Bussa, 4–5.

Eze, E. I., Echezona, B. C., & Uzodinma, E. C. (2011). Isolation and identification of pathogenic bacteria associated with frozen mackerel fish (Scomber scombrus) in a humid tropical environment. *African Journal of Agricultural Research*, 6(21), 4891–4897.

FAO Fisheries and Aquaculture. (2008). *Globalisation and fisheries: Proceedings of an OECD-FAO workshop development.* OECD Publishing.

FAO. (2005). *Post-harvest changes in fish.* FAO Fisheries and Aquaculture Department, Food and Agriculture Organization.

FAO. (2007). *Survey methods of appraising evaluation of traditional solar dry system in Nigeria fisheries resource* (Fish Technical Paper, p. 171).

Food and Agriculture Organization. (n.d.). *Quality and changes in fresh fish.* FAO.

Frankel, E. N. (2005). Chemistry of free radical and singlet oxidation of lipids. *Progress in Lipid Research, 23*(2), 197–221.

Fraser, O., and Sumar, S. (2008). Compositional changes and spoilage in fish. *Nutrition and Food Science, 5*(4), 275–279.

Gram, L., and Dalgaard, P. (2002). Fish spoilage bacteria-problems and solutions. Current Opinion in Biotechnology, **13**(3): 262–266.

Gram, L., and Dalgaard, P. (2002). Fish spoilage bacteria—Problems and solutions. *Current Opinion in Biotechnology, 13*(3), 262–266.

Gram, L., and Huss, H. H. (2000). Fresh and processed fish and shellfish. In B. M. Lund, A. C. Baird-Parker, and G. W. Gould (Eds.), *The microbiological safety and quality of foods* (pp. 472–506). Springer.

Hansen, T. L., Gill, T., Rontved, S. D., and Huss, H. H. (2003). Importance of autolysis and microbiological activity on quality of cold smoked salmon. *Food Research International, 29*(2), 181–186.

Hermansen. (1983). In C. E. Regenstein and M. J. Regenstein (1991), *Introduction to fish technology* (p. 64). Van Nostrand Reinhold.

Huis, T., and Veld, J. H. J. (2006). Microbial and biochemical spoilage of foods: An overview. *International Journal of Food Microbiology, 33*, 1–18.

Hultin, H. O. (2004). Oxidation of lipids in seafoods. In F. Shahidi and J. R. Botta (Eds.), *Seafoods: Chemistry, processing technology and quality* (1st ed., pp. 49–74). Blackie Academic and Professional.

Huss, H. H. (2005). *Quality and quality changes in fresh fish* (FAO Fisheries Technical Paper No. 348). Food and Agriculture Organization.

Huss, H. H. (Ed.). (1995). *Quality and quality changes in fresh fish* (FAO Fisheries Technical Paper No. 348). FAO.

Huss, H. H., Dalgaard, D., Hansen, L., Ladefoged, H., Pedersen, A., and Zittan, L. (1974). The influence in catch handling on the storage life of iced cod and plaice. *Journal of Food Technology, 9,* 213–221.

Ibrahim, B. U., Baba, J., and Sheshi, M. S. (2014). Isolation and identification of bacteria associated with fresh and smoked fish (Clarias gariepinus) in Minna Metropolis, Niger State. Nigeria. *Journal of applied and environmental microbiology*, 2(3), 81-85.

ICMSF. (2011). Microorganisms in foods: Sampling for microbiological analysis-Principles and specific applications (2nd ed.). International Commission on Microbiological Specifications for Foods.

Idachaba, F. S. (2001). The Nigerian food problem: Of processed fish and varied sources of proteins. *Journal of Agriculture, Science and Technology, 1*(1), 5–16.

Karube, I., Marouka, H., Suzuki, S., Watanabe, E., and Toyana, K. (2001). *Journal of Agricultural and Food Chemistry, 32,* 314–319.

Khayat, A., and Schwall, D. (2003). Lipid oxidation in seafood. *Food Technology,* 37, 130–140.

Leistner, L., and Gould, G. W. (2002). *Hurdle technologies: Combination treatments for food stability, safety, and quality* (p. 334). Springer.

Lin, T. M., and Park, J. W. (2006). Protein solubility in Pacific whiting affected by proteolysis during storage. *Journal of Food Science, 61*(3), 536–539.

Liston, J. (1980). Microbiology in fishery science. In J. J. Connell (Ed.), *Advances in fishery science and technology* (pp. 138–157). Fishing News Books.

Martinez, A., and Gildberg, A. (2011). Autolytic degradation of belly tissue in anchovy (*Engraulis encrasicolus*). *International Journal of Food Science and Technology, 23*(2), 185–194.

Nwabueze, A. A., and Okocha, R. C. (2008). Microbial flora of fresh and smoked fish sold in some markets in Benin City, Nigeria. *Journal of Applied and Natural Science*, **2**(1): 7–11.

Nwankwo, C. U., Okeke, P. O., and Ezenwaka, C. S. (2019). Fungal contamination of dried fish sold in Onitsha main market, Nigeria. *International Journal of Fisheries and Aquatic Studies*, **7**(2): 52–57.

Odu N. N. and Imaku L. N. (2013). Assessment of the microbiological quality of street-vended ready-to-eat bole (roasted plantain) fish (*Trachurus Trachurus*) in Port Harcourt metropolis, Nigeria. Researcher 5(3):9-18.

Okonko, I. O., Ogunjobi, A. A., Fajobi, E. A., Ogun, A. A., and Onajobi, F. (2010). Comparative studies of microbial load of fresh and frozen fish (*Tilapia nilotica* and *Clarias gariepinus*) sold in Ibadan, Nigeria. *Nature and Science,* **8**(3): 55–62.

Okonta, A. A., and Ekelemu, J. K. (2005). A preliminary study of micro-organisms associated with fish spoilage in Asaba, Southern Nigeria. Proceedings of the 20th Annual Conference of the Fisheries Society of Nigeria (FISON), Port Harcourt, 557–560.

Okoro, C. C., Eke, S. O., and Uche, A. (2011). Comparative analysis of microbial load of traditionally and oven-dried fish (*Clarias gariepinus*). *Nigerian Food Journal*, **29**(2): 1–5.

Ola, I. A., and Olasupo, N. A. (2011). Assessment of microbiological quality of smoked fish sold in Ibadan markets, *Nigeria. African Journal of Food Science*, **5**(13): 790–796.

Olafadehan, O. A., and Adewumi, O. O. (2015). Evaluation of microbial quality of smoked fish sold in Ilorin, Kwara State, Nigeria. *Nigerian Journal of Fisheries and Aquaculture*, **3**(1): 76–82.

Olajire, A. A., Lawal, A. T., and Adewuyi, G. O. (2020). Microbial assessment and safety evaluation of frozen fish sold in selected Nigerian markets. *Journal of Food Microbiology*, **7**(2): 23–31.

Olaleye, O. N., and Abegunde, T. A. (2015). Microbiological safety assessment of selected smoked fish in Lagos metropolis. *British Microbiology Research Journal, 9*(3), 1–5.

Olayemi, F. F., Adebayo, E. F., and Adegoke, G. O. (2012). Microbiological and chemical quality of sun-dried fish from markets in Kwara State, *Nigeria. African Journal of Food, Agriculture, Nutrition and Development*, **12**(6): 6613–6625.

Olayemi, F. F., and Adebayo, E. F. (2018). Effects of frozen storage on microbial and sensory quality of fish species in Ilorin metropolis, *Nigeria. African Journal of Food Science,* **12**(3): 45–52.

Pal, M. (2010). *Fish hygiene* (MSc lecture notes). Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit, Ethiopia.

Pal, M. (2012). *Food spoilage* (Ph.D. lecture notes). Addis Ababa University, College of Veterinary Medicine, Debre Zeit, Ethiopia.

Regenstein, M. J., and Regenstein, C. E. (1991). *Introduction to fish technology*. Van Nostrand Reinhold.

Shewan, J. M. (2001). The microbiology of sea water fish. In G. Borgstrom (Ed.), *Fish as food* (pp. 487–560). Academic Press.

Spencer, R., and Baines, C. R. (1964). The effect of temperatures on the spoilage of wet fish: 1. Storage at constant temperature between -1°C and 25°C. *Food Technology Campaign, 18,* 769–772.

Srivastava, R., and Srivastava, N. (2008). Changes in nutritional value of fish Channa punctatus after chronic exposure to zinc. *Journal of Environmental Biology, 29*(3), 299–302.

Sujatha, K., Joice, A. A., and Senthilkumaar, P. (2013). Total protein and lipid content in edible tissues of fishes from Kasimodu fish landing centre, Chennai, Tamil Nadu. *European Journal of Experimental Biology, 3*(5), 252–257.

Tatcher, R.S., and Clark, D.S. 1973. Microorganisms in food. Toronto press. pp. 57-115.

Tawari, C. C., and Abowei, J. F. N. (2011). Traditional economics of fish production in Kaduna State, fish handling and preservation in Nigeria. *ARPN Journal of Agricultural and Biological Science, 3*(6), 427–436.

Tys, D., and Pieters, M. (2009). Understanding a medieval fishing settlement along the southern Northern Sea: Walraversijde, c. 1200–1630. In L. Sicking and D. Abreu-Ferreira (Eds.), *Beyond the catch: Fisheries of the North Atlantic, the North Sea and the Baltic, 900–1850* (pp. 91–122). Brill.

Undeland, I., Hall, G., Wendin, K., Gangby, I., and Rutgersson, A. (2005). Preventing lipid oxidation during recovery of functional proteins from herring (*Clupea harengus*) fillets by an acid solubilization process. *Journal of Agricultural and Food Chemistry, 53*, 5624–5634.

Verma, A. K., and Prakash, S. (2020). Status of animal phyla in different kingdom systems of biological classification. *International Journal of Biological Innovations, 2*(2), 149–154.

World J. Fish Marine Sci. 5(3):239-243.

Yorkowski, M., and Brockerhoft, H. (2005). Lyso lecthinase of cod muscle. *Journal of Fish Research, 22,* 643–652.