ASSESSMENT OF QUALITY AND HANDLING PROCEDURES OF IMPORTED FROZEN FISH IN OYO STATE, NIGERIA

BY

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ABSTRACT

The deficit of domestic fish supply necessitates importation of frozen fish to supplement protein intake of Nigerians. Adequate quality standards and control measures are in place for frozen fish at the point of entry to the country. However, there is dearth of information on quality control along the distribution chain to the final consumer. Therefore, quality and handling procedures of imported frozen fish within Oyo State were investigated.

Three-stage sampling procedures were used for the study. Oyo State was stratified into four Agricultural Development Programme zones (Ibadan/Ibarapa, Ogbomoso, Oyo and Saki). All cold store operators (n=67) and 5.0% of registered retailers per zone (n=150) were randomly selected and assessed using 217 structured questionnaires to obtain information on compliance level on temperature management, personal hygiene, facilities' sanitation and handling. *Sardinella species* and *Micromesistius poutassou* were selected for sensory and non-sensory assessment based on their availability in all zones. Overall acceptability was based on 7-point hedonic scale (very much liked (7) - very much disliked (1). Chemical tests such as Hypoxanthine (Hx), Peroxide value (PV), Trimethylamine (TMA) and Free fatty acid (FFA) were carried out using standard methods. Bacteria and Fungal counts were determined using standard procedures. Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$.

Compliance level to quality measures by cold store operators in all the zones were 65.7%, 83.6% and 92.5% for temperature management, personal hygiene and facilities' sanitation, respectively. Ibadan/Ibarapa zone had the highest cold storage capacity of 7,433 tonnes and the least was Saki zone with 61 tonnes. Transportation of fish to retail points were by motorcycle (43.3%), taxi cab (31.1%) and by head load (24.0%). Fish was displayed by retailers using bowls (50.0%), wooden tables (36.7%) and wooden boards (10.0%). Only 57.3% of retailers washed their tables daily and none used chlorinated water. Overall acceptability was 5.47±0.20 (*Sardinella spp*.) and 5.57±0.13 (*M. poutassou*). The Hx (25.54±0.41 mg/100g; 26.28±1.06 mg/100g), PV (19.47±0.90 meq/kg; 20.03±0.53 meq/kg,) TMA (23.79±0.52 mg/100g; 23.45±0.89 mg/100g) and FFA (1.85±0.31%; 1.82±0.19%) were recorded for *Sardinella spp*. and *M. poutassou*, respectively. Oyo zone had the least PV (17.48±0.81; 17.90±0.60 meq/kg), TMA (20.58±0.91; 17.90±0.60 mg/100g), bacteria count (1.2 $x10^5$±0.49; $1.5x10^5$±0.43cfu/g) and fungal load ($7.9x10^4$±0.35; $1.4x10^5$±0.38 cfu/g) in both *Sardinella spp*. and *M. poutassou*, respectively. Ogbomoso had highest PV (18.83±0.60 meq/kg), FFA (1.84±0.13%), TMA (23.62±0.60 mg/100g), bacteria and fungal load ($3.1x10^5$±1.34 cfu/g; $1.8x10^5$±0.81cfu/g) for *Sardinella spp*. and highest FFA (1.81±0.19%) and bacteria load ($3.2x10^5$±0.78cfu/g) in *M. poutassou*. Hypoxanthine and TMA were significantly different for *Sardinella spp.* and *M. poutassou* across the zones, respectively. Predominant bacteria isolates were *Shewanella putrefaciens* and *Streptococcus faecium* while fungi included *Penicillium notatum* and *Aspergillus niger*. Total viable count

for bacteria and fungal load were $3.1x10^5$±1.34 cfu/g and $1.8x10^5$±0.81 cfu/g, respectively for *Sardinella spp*. and $3.2x10^5$±0.78 cfu/g and $2.4x10^5$±0.41 cfu/g for *M. poutassou*.

Quality of frozen fish was best in Oyo zone. Most cold stores met the recommended temperature for cold storage. All sensory and non-sensory indicators of fish quality were within acceptable limits.

Keywords: Frozen fish, Fish storage, Cold store operators, Fish retailers

Word Count: 492

DEDICATION

This research is gratefully dedicated to:

My creator, for His grace and mercy that sustained me throughout this research work.

My parents, the vessel that gave me the opportunity to grace this beautiful world.

My family for their unflinching support throughout the period of this research work.

My mentors, whose examples and inspirations at various stages of my life had been invaluable.

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Fawole Olaolu Olalekan.

CERTIFICATION

I certify that that this work was carried out by Mr. Olaolu Olalekan FAWOLE in the Department of Aquaculture and Fisheries Management, University of Ibadan, Ibadan, Nigeria.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

LIST OF ACRONYMS

FDF	Federal Department of Fisheries
HIV	Human Immunodeficiency Virus
FDA	US Food and Drug Administration
FAO	Food and Agriculture Organization
ICES	International Commisssion for the Exploration of the Sea
IGFA	International Game Fish Association
HACCP	Hazard Analysis and Critical Control Point
NASA	US National Aeronautical and Space Administration
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid
TMAO	Trimethylamine oxide
TMA	Trimethylamine
CFU	Coliform forming unit
SPC	Standard plate count
TVC	Total viable count
AOAC	Association of Official Analytical Chemists
TVN	Total Volatile Nitrogen
TVB	Total volatile base
Hx	Hypoxanthine
FFA	Free fatty acid

PV	Peroxide value
ATP	Adenosine triphosphate
DCPIP	Dichlorophenol indophenol
IMP	Inosine monophosphate
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
GLC	Gas-liquid chromatography
DMAB	Dimethylamino benzoic acid
KI	Potassium Iodide
PCA	Plate Count Agar
MCA	Mac Conkey Agar
MSA	Mannitol Salt Agar
SSA	Salmonella Shigella Agar
PDA	Potato Dextrose Agar
BA	Blood Agar
MRS	de Man Rogosa Sharpe
ONPG	Ortho-Nitrophenyl-BD-galactopyranosidase
ADH	Arginine Dihydrolase
LDC	Lysine Decarboxylase
ODC	Omithine Decaboxylase
URE	Urease

CIT	Citrate
GLU	Glucose
MAN	Mannitol
INO	Inositol
SOR	Sorbitol
RHA	Rhamnose
SAC	Saccharose
MEL	Melibiose
AMY	Amygdaline
TDA	Tryptophane Deaminase
IND	Indole
VP	Voges Proskauer
API	Analytical Profile Index
ANOVA	Analysis of variance
NAFDAC	National Agency for Food and Drug Administration and Control
SON	Standards Organization of Nigeria
NIS	Nigerian Industrial Standard
THSC	Total haemolytic streptococcus count
TLAB	Total Lactic acid Count
TEBC	Total Enterobacteriaceae Count
TSSC	Total Salmonella Shigella Count

LAB	Lactic Acid bacteria
ICMSF	International Commission on Microbiological Specification for Foods
DMRT	Duncan Multiple Range Test
SIFAR	Support Unit for International Fisheries and Aquatic Research
NACMCF	National Advisory Committee on Microbiology Criteria for Food
NPC	National Population Commission

CHAPTER ONE

1.0 INTRODUCTION

Historically, fish has been an important component of the diet of human beings for the past 30,000 years. The Greeks were the first to fillet fish and to discover oysters. Populations settled near rivers or lakes not because of transportation, but because of the steady food supply. The Neolithic hunter, who moved to the Nordic countries after the Ice Age settled down in areas with good fishing opportunities (Simopoulos, 1997).

Fish is a major source of protein and its harvesting, handling, processing and distribution provide livelihood for millions of people as well as providing foreign exchange earning to many countries (Al-Jufaili and Opara, 2006). Although meat and poultry products are probably on the whole more sought after by consumers, they are also far more expensive. The comparative low price of fish is undoubtedly the main reason that it is eaten in much greater quantities than the other forms of animal protein. This is the reason fish is often referred to as the cheapest and most important source of animal protein (Reynolds and Kirema-Mukasa, 1991).

The sources of domestic fish production in Nigeria are capture fisheries (from rivers, lakes, coastal water and seas) and fish farming (FDF, 2012a). During the period 2007-2011, average annual domestic fish production was put at 0.77 million metric tonnes (Table 1). Nigeria, according to Akande (2002) is one of the biggest importers of fish and fishery products. This is due to the need to meet the shortfall in domestic demand for fish.

The need for balanced diet and appropriate protein intake in any economy cannot be over-emphasized. The average Nigerian staple food is deficient in protein such that a large proportion of the people feed on foods richer in carbohydrate than protein to the detriment of their health.

Table 1: Fish Production in Nigeria by Sectors (2007-2011)

S/No	SECTOR/YEAR	2007	2008	2009	2010	2011
	ARTISANAL: SUB-TOTAL	**504,226**	**511,382**	**598,211**	**616,981**	**638,486**
1	Coastal & brackish water	260,098	164,988	309,981	328,332	346,381
	Inland: Rivers & lakes	244,128	246,394	288,230	288,649	292,105
2	**AQUACULTURE (fish farm)**	**85,087**	**143,207**	**152,796**	**200,535**	**221,128**
	INDUSTRIAL (Commercial Trawlers)	**26,193**	**29,986**	**229,698**	**31,510**	**33,485**
3	Fish (inshore)	18,040	18,585	18,820	19,961	19,736
	Shrimp (inshore)	5,995	9,881	10,878	12,249	13,749
	EEZ	2,158	1,520	-	-	-
	GRAND- TOTAL	**615,506**	**684,575**	**780,705**	**849,026**	**893,099**

Source: Federal Department of Fisheries, Abuja (2012a)

Fish, to many people in Nigeria represents a significant proportion of the animal protein in their diet, whether as fresh, frozen, canned, or cured in a variety of ways such as smoking, salting and drying. Fish constitutes 40% of the animal protein intake of most human beings (Olatunde, 1998). Fish is rich in most of the vitamins, a good selection of minerals and the proteins; contains all essential amino acids in the right proportions (Murray and Burt, 1977). Fish is less tough and more digestible compared to beef, mutton, chicken and bush meat. This is possible because of a greater ratio of muscle protein of fish to connective tissue. Fish is a food with a high proportion of polyunsaturated fatty acids of the (n-3) family and low cholesterol in its fat content (Ohlenschlager, 1997a).

Generally, fish are good sources of vitamins B12 and B6. It is also a good source of fluorine and iodine which are needed for development of strong teeth and the prevention of goiter (Eyo, 2001). Fish is usually recommended to patients with digestive disorder like ulcer and it is available in most markets either as fresh, smoked, dried, canned, chilled or frozen and as such the problem of scarcity is removed (Eyo, 2001).

1.1 Statement of Research Problem

The average Nigerian consumer is dependent on fish as a source of protein supply because livestock production has been hindered by cattle rustling; farmers-fulanis clashes and recurring terrorists' attacks in the northern part of the country with resultant scarcity and high cost of meat. Also, the persistent bird flu scare in the poultry industry has put more pressure on fish demand. Fish is the preferred source of much desired animal protein compared to poultry, beef, mutton, pork and veal. It is comparatively cheaper and highly acceptable, with little or no religious bias, which gives it an advantage over pork or beef (Johnston *et al*., 1994 and Feldhusen, 2000).

Increase in human population and the resulting increase in demand for animal protein necessitated that fish production be widened in scope with the active participation of all stakeholders in the fisheries sub-sector. The multi-disciplinary approach to fishery production which involves the government, research organizations and the organized private sectors should take advantage of scientific research findings to improve production if it will succeed in its bid to feed the ever-increasing human population in Nigeria.

According to Food and Agricultural Organisation, FAO (2004), fish quality, including safety is a major concern facing the food industry today. Great number of socio economic changes such as increased urbanization (overcrowding), migrations and population demographics are further contributing to the interest in the safety of foods. The population of highly susceptible persons is expanding worldwide because of ageing, malnutrition, HIV infections and other underlying medical conditions with a weakened immune system. During seafood handling and processing, there is the possibility of contamination with pathogenic microorganisms and their subsequent proliferation. Generally, human illness caused by food borne bacteria often result from feacal contamination of food by the food handlers, however, seafood can naturally harbour a variety of human pathogens (Twiddy and Reilly, 1995).

Ensuring safety of seafood presents special challenges to both the industry and the regulator. It is therefore of utmost importance that those who handle and process seafood commercially understand the hazards associated with this type of food (FDA, 2002). Fish unfortunately is the most perishable of marine resources, especially in less developed countries like Nigeria, with its attendant problem of processing and preservation before marketing.

1.2 Justification for the study

Fish are marketed in two forms in Nigeria, namely: fresh fish and preserved fish (Emokpae, 1979). The preserved fish could be smoked, sun-dried or frozen. Fresh fish is mostly available to people in the production areas while frozen fish is imported by the industrial sector. A clearly defined network of frozen fish distribution exists through a chain of intermediaries who handle products at different levels (Talabi and Makanjuola, 1977).

Frozen fish has been a reliable major source of animal protein to the public because it provides a viable alternative, and a measure of profitability. In a recent survey by the Federal Department of Fisheries and Federal Ministry of Agriculture and Rural Development on fish price according to states in Nigeria (appendix 10), frozen fish rated as the cheapest of all fisheries products which makes it most widely accepted among the predominantly low income Nigerians**.** An important social impact of this on the economy is the job opportunity offered to the teeming number of people involved in this sub-sector (Agbon *et al*., 2000). The importance and quality of frozen fish

consumed in Nigeria is likely to increase and cannot be underestimated because Nigeria is a leading importer of frozen fish in the world (Akande, 2002). The bulk of the fish consumed in the country is frozen and are imported. Fish demand in Nigeria is about 2 million metric tonnes, while the current domestic fish production is about 0.9 million metric tonnes. The shortfall between demand and supply is made up by importation of more than 600,000 metric tonnes of fish **(**FDF, 2007), thus making frozen imported fish the bulk of fish consumed in Nigeria.

Fish, being a highly perishable commodity, requires continuous reassurance of its quality. There is therefore a need for the study of preservation techniques and determination of spoilage parameters to minimize losses, thereby increasing the quality of fish available for human consumption in many of the developing countries. This will help to mitigate dietary problems and health hazards resulting from the intake of low quality contaminated fish (Oyelese, 1992).

Consumers are becoming more aware of possible hazards, malpractices and mistakes arising from the food they consume and are individually and collectively becoming more demanding in respect of freshness, naturalness, microbial safety, freedom from pollutants, protection from damage and convenience. The freezing process cannot improve the quality of fish, therefore the best products are those made from first class raw materials (Horde, 1973). However, quality deterioration of stored fish is inevitable with length of storage period (Jeon *et al*., 2002). Large quantities of seafood are harvested from cold waters; therefore, their microflora is not inhibited as effectively by refrigeration as in the normal microflora of warm-blooded animals (Nickelson *et al*., 1980). Frozen fish displays third order biotic activity. It belongs to the class of foods in which the respiration process is suspended, but in which biochemical, microbial and other decomposition processes which must be taken into account still proceed (Huss *et al.*, 1992). Even after freezing, checks need to be frequently made on the temperature of the frozen product during handling, transportation and cold storage as a means of quality control (FAO, 1994).

The distance between the point of production and the areas of consumption calls for efficient distribution channels, which can provide the consumer with a flow of fish and fish products of the type, quality and quantity they desire at minimum cost. Due examination of fish and its products for evidence of spoilage, damage,

adulteration or disease is a long established practice. The objectives being partly to guard the health and pocket of consumers; and also ensure the goodwill of vendors.

In order to ensure that standardization and proper handling procedures are followed in the processing of frozen fish for consumption and to protect the health of the fish consumers in Oyo state, this study intends to address the following research questions:

(a) How will the frozen fish sellers improve the transportation of products?
(b) How will they improve handling of frozen fish?
(c) What are the recommendations on handling and transportation for frozen fish sellers?

1.3 Objectives

This study aimed principally to evaluate the facilities and operations of the frozen fish depots at both wholesale and retail levels in various locations and identify factors that could increase efficiency of production and provision of good quality and wholesome frozen fish in Oyo state.

The specific objectives of the study are to:

1. Examine the constraints to frozen fish distribution and management in Oyo state.
2. Evaluate operations of the frozen fish business across zones in Oyo state and their effects on wholesomeness and microbiological quality of products.
3. Determine the length of storage of frozen fish across the zones in Oyo state.
4. Determine the wholesaler-retailer relationship and its effects on storage of frozen fish in Oyo state.

1.4 Hypotheses

The hypotheses tested and stated in the null form are:

i. Ho: There are no constraint to distribution and management of frozen fish in Oyo state.
   H_A: There are constraints to distribution and management of frozen fish in Oyo state.
ii. Ho: There is no significant difference in the wholesomeness of frozen fish across the zones in Oyo state.

H_A: There is significant difference in the wholesomeness of frozen fish across the zones in Oyo state.

iii. Ho: There is no marked difference in the quality of frozen fish due to handling and storage.

H_A: There is marked difference in the quality of frozen fish due to handling and storage.

iv. Ho: The storage of frozen fish is not affected by wholesaler-retailer relationship.

H_A: The storage of frozen fish is affected by wholesaler-retailer relationship.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Habitat and Biology of Sardine (*Sardinella* species)

Sardinella is a genus of fishes in the family Clupeidae, the herrings and sardines. This genus currently contains 21 recognized species (Froese and Pauly, 2011). It is distributed in both sides of the Atlantic Ocean, throughout the Mediterranean Sea and in the Indian and the Western Pacific Oceans (Whitehead, 1985). *Sardinella* evolved from the small herring genus *Harengula*. *Sardinella* are distinguished from other Clupeidae genera by several characteristics. They have terminal mouth, lack a median notch in the upper jaw, lack radiating grooves in their smooth opercula, lack scales on the predorsal ridge and have no more than 24 rays on the anal fin. They have a unique dermal fold on the anterior edge of the cleithrum, a bone attached to the skull (Chan, 1965).

Species are distinguished by their ranges and by specific body features. They include *Sardinella aurita* (round sardinella) (Valenciennes, 1847), *Sardinella maderensis* (African sardine) (Lowe, 1838), *Sardinella longiceps* (Indian oil sardine) (Valenciennes, 1847) and *Sardinella rouxi* (yellowtail sardinella) (Poll, 1953).

2.2 Habitat and Biology of Blue Whiting (*Micromesistius poutassou*)

The blue whiting (*Micromesistius poutassou*, Risso, 1827) is a member of the cod family Gadidae found throughout the NorthEast Atlantic. The core of the distributional range is from the Bay of Biscay along the continental shelf edge to the Norwegian Sea. The edges of distribution range from the Iberian Peninsula and the Mediterranean in the South to the Barents Sea in the North, from the North Sea to the Mid-Atlantic ridge East-Greenland and East coast of North America (Bainbridge and Cooper, 1973; Bailey, 1982; Monstad, 2004; Heino *et al*.; 2008; Pointin and Payne, 2014).

Blue whiting are bathypelagic oceanodromous fish that occupy depth ranges from 150-3000m, but are mostly found at 300-400m (Svetoridov, 1986; Riede, 2004). It has a long, narrow body and a slivery underbody and reaches total lengths up to 50cm (common from 15-30cm) and can weigh up to 830g (Cohen *et al*., 1990; International Game Fish Association (IGFA), 2001. The three dorsal fins are widely spaced and the interspace between the second and third fin is larger than the base length of the first dorsal fin. The mouth and gill cavities are black and the lower jaw is somewhat protruding with very big eyes (Russell, 1976; Muus and Nielsen, 1999). Blue whiting females are usually larger than males. They prey on small crustaceans but larger individuals also forage on small fish and cephalopods. The meat is sold both fresh and frozen and is also processed as oil and fishmeal (Cohen *et al*., 1990). The blue whiting stocks are the target of the largest fishery in the Atlantic (International Commisssion for the Exploration of the Sea (ICES), 2004.

2.3 Fish Quality

Quality according to the Longman Dictionary of Contemporary English (2000), is the degree to which something is good or bad. Fish quality is a complex concept involving a whole range of factors which for the consumer include for example: safety, nutritional quality, availability, convenience and integrity, freshness, eating quality and the obvious physical attributes of the species, size and product type (Abass *et al*., 2008). Fish quality is undoubtedly the most important factor, which influences consumer demand. In assessing the quality of a fish, a consumer may consider its freshness, safety of the fish in terms of the microbial load and the presence or absence of pathogenic organisms as well as the palatability of the fish (Eyo, 2001). According to Connell (1995), fish quality is that attribute which consciously and unconsciously the fish eaters or buyers consider should be present in a fish. Quality he said, embraces intrinsic composition, degree of spoilage, damage, deterioration during processing, storage, distribution, sale and presentation to the consumers, hazard to health, satisfaction on buying and eating, aesthetic considerations, yield and profitability to the producer and middlemen.

The most well known modern method of managing quality and safety of food as recommended by Huss *et al*., (2004) include:

- Good Hygiene Practice (GHP)/Good manufacturing practice(GMP)

- Quality Control (QC)
- Quality Assurance (QA)/ Quality Management (QM)
- Quality System
- Total Quality Management (TQM)
- Hazard Analysis and Critical Control Point (HACCP)

Quality control as defined by Jouve *et al*., (1998) is the operational techniques and activities that are used to fulfill quality requirements. It is an important subset of any quality assurance system and is an active process that monitors and modifies the production system so as to consistently achieve the required quality. Quality control is the maintenance of quality at a level that satisfies the consumer and that is economical to the producer or seller (Connell, 1995).

Quality management is often used interchangeably with quality assurance. This can be defined as all activities and functions concerned with the attainment of quality in a company (Huss *et al*., 2004). Total quality management is an organization's management approach, centered on quality; based on the participation of all its members and aimed at long term success through customer satisfaction and benefit to the members of the organization and to society (Jouve *et al*., 1998).

2.3.1 Hazard Analysis and Critical Control Point (HACCP)

Hazard Analysis and Critical Control Point was conceived in 1960 when the US National Aeronautical and Space Administration (NASA) asked Pillsbury to design and manufacture the first foods for the space flights. Since then, HACCP has been recognized internationally as a logical tool for adapting traditional inspection methods to a modern science-based food safety system. Based on risk assessment, HACCP plans allowed both industry and government to allocate their resources efficiently in establishing and auditing safe food production practices (FDA, 2002). According to Notermans *et al*., (1995), HACCP is a systematic approach to the identification, assessment, and control of hazard in a particular food operation. It principally aims to identify problems before they occur, and establish monitors for their control at stages of production that are critical to ensuring the safety of the food. HACCP is an effective means by which food manufacturers can identify the key steps for preventing, controlling or eliminating hazards associated with their products, thereby minimizing food safety problems (Buchanan, 1995).

The objective of HACCP is to ensure that safe, wholesome and unadulterated foods reach the consumer. This depends on the process control throughout the product life, through the identification of potential hazards and establishment of critical control point (CCP) in the food system to minimize the presence of unacceptable health risks (Alonge, 2001).

The HACCP system that seafood processors will have to follow according to FAO (1995) will help weed out seafood hazards with the following seven steps:

a. Identify potential hazards and assess their risk (likelihood) of occurrence.
b. Determine the Critical Control Points (CCPs). Determine steps that can be controlled to eliminate or minimize the hazards. A CCP that can completely control a hazard is designated CCP-1, while CCP that minimizes but not completely control a hazard is designated CCP-2.
c. Establish the criteria (tolerances, target level) that must be met to ensure that a CCP is under control.
d. Establish a monitoring system.
e. Establish the corrective action when CCP is not under control.
f. Establish procedure for verification.
g. Establish documentation and record keeping.

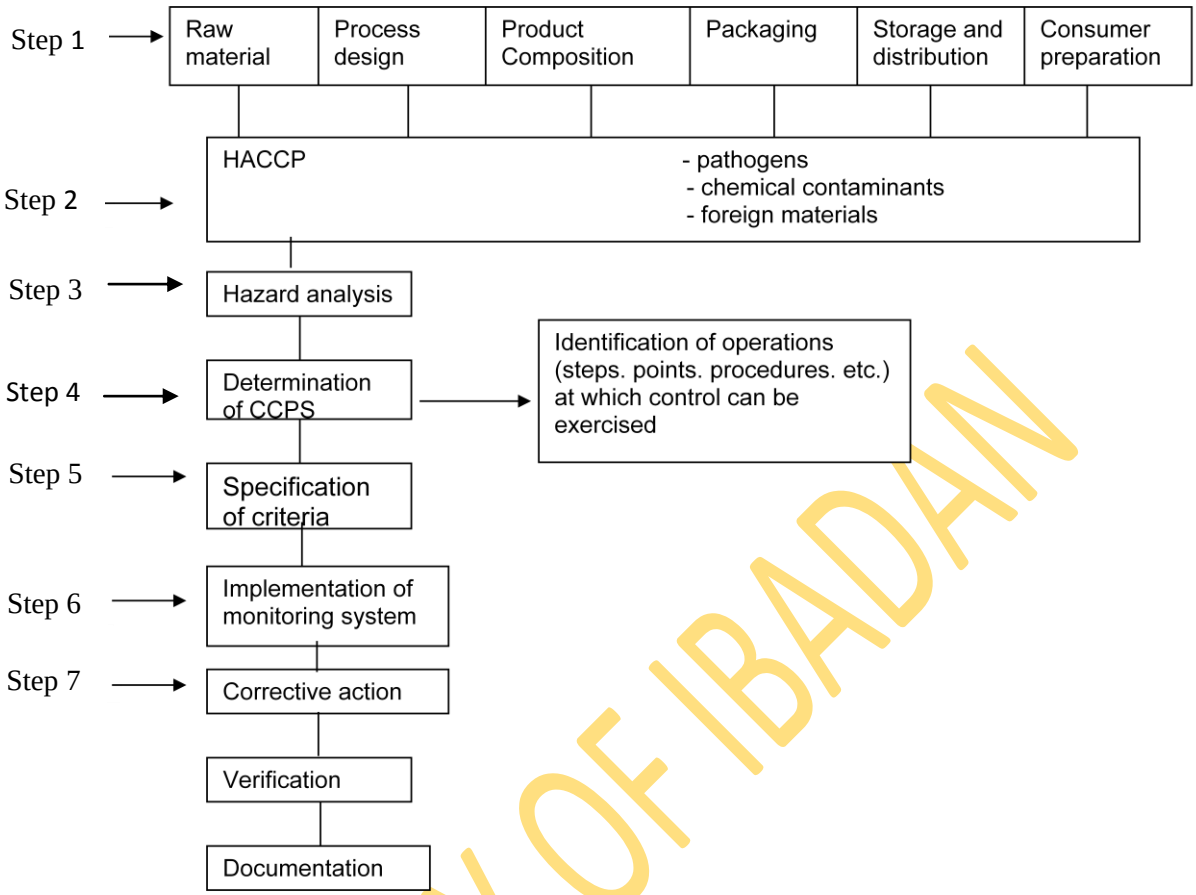

Figure 1: The HACCP Concept and its Seven Steps

Source: Codex Alimentarius Commission (1991)

2.4 Fish Freezing

Frozen storage has been widely employed to retain fish properties before it is consumed or employed in other technological processes (Pigott and Tucker, 1987; Erickson, 1997).

Fish freezing is a method of fish preservation in which the product is brought in contact with refrigerated surfaces in a compartment. As heat is removed from the fish, its temperature falls steadily until the fish begins to freeze. Three types of freezers commonly used for industrial freezing of fish and fish products are: air blast freezer, contact or plate freezer and immersion or spray freezer (Johnston *et al*., 1994). Heat is removed from fish in the freezing process by surrounding the fish with a stream of cold air; by placing the fish in contact with a cold surface or by spraying with certain liquid refrigerants (Horde, 1973). The operating temperatures of these freezers are shown in Table 2. The full potential of freezing fish can only be realized through a comprehensive 'cold chain' distribution. Frozen fish is primarily distributed in the developing countries in unsophisticated bulk packs and its distribution depends in many instances on the traditional fish trade in the public markets or sometimes in fishmongers' shops (Menon, 1977).

According to FAO/Support Unit for International Fisheries and Aquatic Research, SIFAR (2001), temperature of storage at the wholesale depot should be -30^{o}C for frozen fish and temperature on delivery should be a little above this as possible, certainly not above -18^{o}C, the recommended maximum temperature in the retail market.

The storage life of frozen fish depends on the temperature of storage and any increase in temperature even for a very short period has a bad effect on the quality of the product (Nicol, 1975).

2.4.1 Fish freezing Techniques

Water forms about 60% -80% of the weight of fresh fish. Pure water freezes at 0^{o}C but a fish begins to freeze at -1^{o}C due to the presence of salt which depresses the freezing point of fish. At -5^{o}C most of the water become frozen and only about 20% of the water in the fish remain unfrozen. Even at -30^{o}C, up to 10% of the water in the fish muscle is still unfrozen (Eyo, 2001). The interval of -1^{o}C to -5^{o}C when freezing begins

is known as the critical zone since the speed at which the fish is frozen within this range determines the quality of the fish on thawing. The fish that spends longer than 2hours at this temperature interval is regarded as being slowly frozen. The average temperature at the end of the freezing process should be -30^{o}C, while the warmest part of the fish should be -20^{o}C (Johnston *et al*., 1994). The variation of the proportion of water (which is converted to ice) in the muscle of fish against temperature is shown in Fig. 2. In general, the faster the freezing rates, the higher the end-product quality. The more rapidly the freezing interface advances, the smaller and more numerous the ice crystals, hence, cause little damage to the tissue structure, minimizing drip losses (Yen-Con Hung, 1997).

Extremely slow freezing causes the formation of large ice crystals which might result in a porous texture with tiny holes in the fish after thawing; hence slow freezing should be avoided because of its damaging effect on fish quality. The temperature of maximum denaturation activities is approximately -1^{o}C to -2^{o}C; hence it is necessary to pass through this temperature quickly since the time spent in this temperature zone determines the difference between slow freezing and quick freezing (FAO, 1994). Fish is considered properly frozen if after storage in a cold store, it still retains its freshness even after thawing (Eyo, 2001).

There are three stages in fish freezing; Stage 1: the temperature falls fairly rapidly to just below 0^{o}C; Stage 2: the temperature remains fairly constant at about -1^{o}C, the period when the temperature of the fish will not drop (i.e. the 'thermal arrest' period). This period lasts until approximately 75% of water is frozen. It is a critical period that the fish should pass through quickly to produce a good frozen product. During Stage 3, the temperature again drops and most of the remaining water becomes frozen. This 3-stage reduction in temperature during freezing is as illustrated in Fig. 3 (Graham, 1977).

As water in fish freezes into pure crystals of ice, the remaining unfrozen water contains an ever increasing concentration of salts and other compounds which are naturally present in fish flesh. The effect of this ever increasing concentration is to depress the freezing point of the unfrozen water. The result is that, unlike pure water, the complete change to ice is not accomplished at a fixed temperature of 0^{o}C, but proceeds over a range of temperature. The variation of the proportion of water (which

is converted to ice is shown in Fig. 2. (Graham, 1977). Some freezing codes and recommendations define freezing rate in terms of the thickness of frozeness in unit time. Average freezing rates vary between 2 and 1000 mm/h as shown in Table 3.

2.5 Composition of Fish.

Fish is not a single homogenous product. There are considerable differences between fish species as regards their content of nutrients, especially fat (Simopoulos, 1997). Fish is a very nutritious part of a man's diet, it is rich in most of the vitamins, has a good selection of minerals and proteins contain all essential amino acids in the right proportions (Murray and Burt, 1977). Fish is highly digestible and its protein efficiency ratio ranges from 2.7-3.2. The *in vitro* digestibility of fish is not influenced by the protein denaturation and aggregation that occurs with storage (Pigott and Tucker, 1990). According to Olayide and Abidogun (1979), the protein gain in humans per gramme from fish per day is far higher than gain from any animal products (Table 4).

Fish has a skeleton or cartilaginous structure, which provides support for the body. The muscles, which form the edible part, account for most of the weight of the fish. The skin forms a cover, often with an outer layer of scales, and secretes slimy mucus, which lubricates the fish and seals the surface (FAO, 1994). Flesh from healthy fish contains about 60-84 % water, 15-24% protein and 0.1-22.0% fat. Minerals usually constitute 1-2%, while the carbohydrate content in the muscle of fresh fish is insignificant. The proportions of the constituents are species specific and the main variations between species are in the fat content. Seafood however, is a low fat food and most fish have less than 5% fat (Clucas, 1990).

In addition to being low in fat and cholesterol, seafood is the richest source of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the omega-3 fatty acids that are beneficial to health. Omega-3 content is higher in deep cold water fish and varies with season and age of the fish (Van Vliet and Katan, 1990). The proximate composition of fish may vary depending on certain factors such as the geographical location, season of the year, the feed intake, and metabolic efficiency of the fish, the energy expended by the fish, sex, species, age and size (Eyo, 2001).

Table 2: Operating Temperature of Fish Freezers

Type of freezer	Operating temperature
Batch Air blast	-35^0C to -37^0C
Continuous Air Blast	-35^0C to -40^0C
Batch Plate	-40^0C refrigerant
Continuous Plate	-40^0C refrigerant
Liquid Nitrogen	-50^0C to -196^0C refrigerant
Liquid Carbondioxide	-50^0C to -70^0C refrigerant
Sodium Chloride brine	-21^0C refrigerant

Source: Johnston *et al*., (1994)

Table 3: Freezing Rate of Fish

Time taken	Type of freezing
2 mm/h	Slow bulk freezing in a blast room
5 to 100 mm/h	Quick freezing in a tunnel air blast or plate freezer
50 to 100 mm/h	Rapid freezing of small products
100 to 1000 mm/h	Ultra rapid freezing in liquefied gases such as nitrogen and carbon dioxide

Source: FAO (1994).

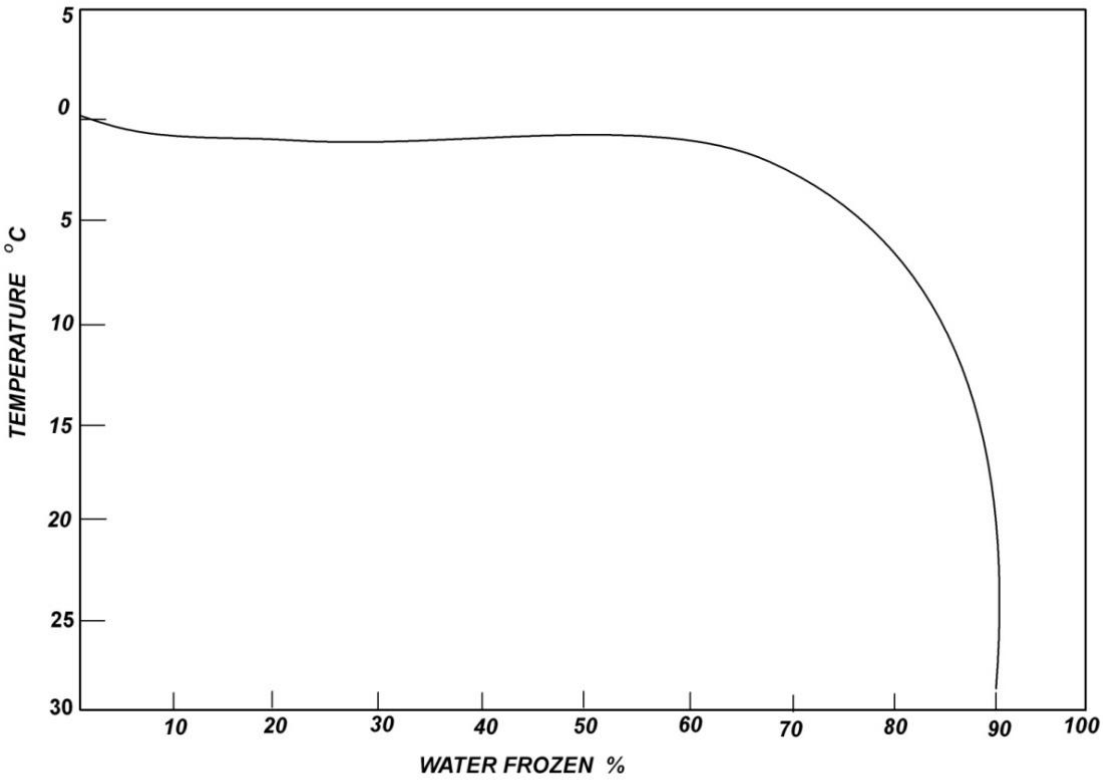

Figure 2: Percentage of Water frozen at different Temperatures in Fish Muscle

Source: Graham (1977).

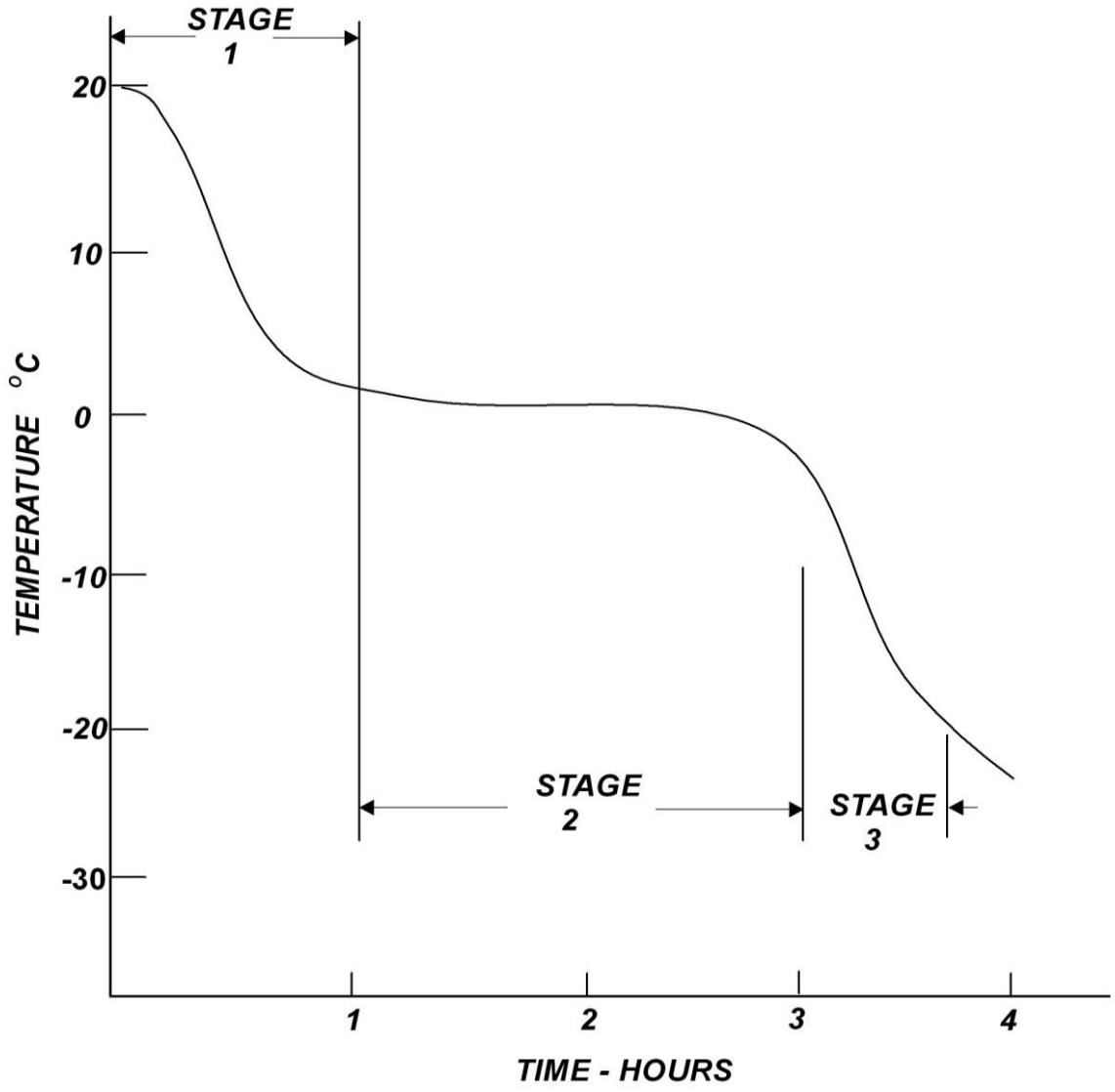

Figure 3: Typical Fish Freezing Curve

Source: Graham (1977)

Table 4: Protein Gain per Day from Animal Source

Animal product	Protein(g)per output/day	Percentage
Beef	1.77	9.13
Goat meat	0.92	4.75
Mutton	0.18	0.93
Cheese	0.02	0.01
Poultry	0.40	2.06
Pork	0.15	0.77
Offal	3.24	16.75
Bush met	2.18	11.26
Fish	9.29	47.98
Egg	0.38	1.96
Milk	0.85	4.40
Total	**19.38**	**100.0**

Source: Olayide and Abidogun (1979).

Table 5: Chemical Composition of various Fish Species

Specific name	Common name	Water%	Lipid%	Protein%
Marine				
Anguilla Anguilla	Eel	60-71	8.0-31.0	14.4
Carcharhinus brachyurus	Shark	75-80	0.1	18.9
Clupea harengus	Herring	60-80	0.4-22.0	16.0-19.0
Gadus morhua	Cod	78-83	0.1-0.9	15.0-19.0
Merluccius capensis	South African hake	80	0.4-1.0	17.8-18.6
Micromesistius poutassou	Blue whiting	79-80	1.9-3.0	13.8-15.9
Nephrops nopregicus	Norway lobster	77	0.6-2.0	19.5
Pleuronectes platersa	Plaice	81	1.1-3.6	15.7-17.8
Sardine pilchardus	Pilchard	60-80	17.0-20.0	1.0-23.6
Scomber scombrus	Mackerel	56-74	1.0-23.5	16-20
Solea solea	Sole	78	1.8	18.8
Thunnus sp.	Tuna	71	4.1	25.2
Fresh water				
Citharinus citharinus	Moonfish	76.80	1.18	20.04
Clarias gariepinus	Catfish	78.13	4.22	18.63
Cyprinio carpio	Carp	78.80	2.0-2.2	17.5-18.9
Hemichromis fasciatus	Cichlid	74.37	0.25	18.41
Lates niloticus	Nile perch	74-79	1.2-9.9	15.1-22.0
Mormyrus rume	Trunkfish	74.77	4.97	19.36
Oreochromis niloticus	Tilapia	78.11	4.22	18.63
Salmo solar	Salmon	67.11	0.3-14.0	21.5
Salmo trutta	Trout	70-79	1.2-10.8	18.8-19.1
Sarotherodon galilaus	Tilapia	78.11	3.66	18.62
Tilapia mosambicus	Tilapia	78.20	2.2	18.60
Tilapis zilli	Tilapia	77.50	2.75	18.88

Source: (Murray and Burt, 1977; Afolabi *et al*., 1984; Eyo, 1998).

2.6 Spoilage of Fish

As soon as fish dies, spoilage begins. Spoilage of fish is a rather complex process; and is caused by a number of inter-related systems (FAO, 1994). Fish spoilage as defined by Botta (1995) is a change in fish and fish product that makes it unsafe, less acceptable or unacceptable to the consumer for its original intended purpose. The prime cause of spoilage in fish according to Eyo (2001) are bacteria, enzymatic action which result in the production of various volatile compounds; and chemical action involving the oxygen of the air and fat in the flesh of the fish.

It was observed by Frazier and Westhoff (1988) that of all flesh foods, fish is the most susceptible to autolysis and hydrolysis of fats and extractible lipids especially in the tropics where extremely high temperature catalyses spoilage activities. Seafoods are highly susceptible to both microbiological and chemical deterioration due to their high water content, neutral pH, relatively large quantities of free amino acids and natural presence of autolytic enzymes (Jeyasekaran *et al*., 2006).

Fish spoilage occurs when the enzyme of bacteria diffuses into the flesh muscle and the nutrients from the flesh muscle diffuse to the outside. This happens more rapidly for fish species with a thin skin layer (Aberoumand, 2010). Commonly, fish spoilage due to autolysis occurs first and is followed by spoilage due to bacteria and rancidity, but sometimes they overlap (Gram, 1992; Gram and Huss, 1996; Frederiksen *et al*., 1997).

Obvious signs of spoilage are slime formation, detection of off-flavour, gas production, discolouration and changes in texture and these developments are due to a combination of microbiological, chemical and autolytic phenomena (Daczkowska-Kozon, 1993; Huss, 1994 and Connell, 1995). Most fish flesh are more perishable than meat because of the less acid reaction of the flesh that favours microbial growth. Factors influencing the kind and rate of spoilage as highlighted by Twiddy and Reilly (1995) include the kind of fish, the condition of fish when caught, the kind and extent of contamination of fish flesh with bacteria, use of an antibiotic or dip and temperature. The pH of the fish flesh also has an important influence on the growth of bacteria. The lower the pH of the fish flesh; the slower is general bacteria decomposition will be. The causes of various types of spoilage are summarized in Table 6.

Table 6: Causes of Fish Spoilage

Signs of spoilage	Types of spoilage			
	Microbiological	**Chemical**	**Autolytic**	**Physical**
Off odours/off flavours	+	+	+	-
Slime formation	+	-	-	-
Gas formation	+	-	-	-
Discolouration	(+)	+	+	+
Change of texture	(+)	-	+	+

Source: Huss (1994).

2.6.1 Autolytic Spoilage

Enzymatic spoilage also known as autolysis (self digestion) is a process whereby enzymes against which the fish is normally protected alive, under optimal conditions for enzymatic activity, *post mortem*, digest the fish tissue (Eyo, 2001). Enzymes act as catalysts to chemical reactions both in the gut and in the flesh and they remain active after death, resulting in self-digestion affecting the flavour, texture and appearance of the fish (FAO, 1994).

According to Connell (1995), the viscera (gut) contain enzymes, which while the fish is alive are responsible for digesting food. On death; these powerful digestive proteolytic enzymes attack the organs themselves and surrounding tissues. The rate of attack is particularly great in fish that fed heavily such that the organs quite quickly become degraded to a structureless mass and the belly walls either rot away or become weakened so that the slightest abrasion or pressure causes them to tear. The well known phenomenum 'belly burst' which can occur in only a few hours after catch in sardines, herrings and some other fish is caused simply by weakening of the belly wall due to self digestion (FAO, 1994).

In frozen fish, bacteria action is inhibited and autolytic enzymes breakdown trimethylamine oxide (TMAO) to dimethylamine (DMA) and formaldehyde (FA). The effect of FA in frozen fish is increased denaturation of fish tissue, changes in texture and loss of water binding capacity. It has been reported that enzymes from fish adapted to low temperatures are less sensitive to control by refrigerated storage and thus are more active at 0^{o}C (Haard, 2000). Autolytic enzymes are active even at -20^{o}C and below, but proceed at faster rate at high sub-zero temperatures (Huss, 1994). Autolytic enzymes include Cathepsin, Chymotrypsin, Trypsin, Caboxypeptidase, Calpain, Collagenase and TMAO-demethylase (Howgate, 1982; Huss, 1995).

2.6.2 Microbiological Spoilage

Fish and bacteria exist in a state of equilibrium and it is only after death that bacteria can invade the tissue and spoil the fish (Clucas, 1990). Bacteria according to Eyo (2001) secrete digestive juices and enzymes which breakdown the tissue and cause spoilage of fish. This results in loss of flavour and odour and is replaced by a sour and stale odour. During spoilage, a characteristic flora develops, but only a part of

this flora contributes to spoilage. The specific spoilage organisms are producers of metabolites responsible for the off-flavour associated with spoilage (Huss, 1994). Bacteria also reduce TMAO to give TMA, which impacts an off-odour on the fish. Other bacterial by-products are ammonia and hydrogen sulphide, both of which have objectionable smells (Clucas, 1990).

Though individual bacteria are microscopically small, they are present in such large numbers on the skin at the time of spoilage that they can be seen in aggregate as the yellow slime (Aitken *et al*., 1982). Fish carry a flora of psychrotrophic bacteria, most of which survive freezing; and are ready to grow on thawing (Twiddy and Reilly, 1995). Fish may harbor a number of biohazards as well as chemical contaminations such as biogenic amines, biotoxins, pathogenic bacteria and viruses if not properly handled (Ashie *et al*., 1996 and Gram *et al*., 2000).

2.6.3 Chemical Spoilage

Fish oil is more liable to spoilage than other oils due to the greater number of unsaturated fatty acids as shown by the lower saponification number and higher iodine value (Eyo, 2001). An oxidative process, autooxidation as shown in Fig. 4 is a reaction involving only oxygen and unsaturated lipid. The first step leads to formation of hydroperoxides, which are tasteless but very reactive; and cause brown and yellow discolouration of the fish tissue. The degradation of hydroperoxides gives rise to formation of aldhydes and ketones – compounds with strong rancid flavor (Ackman and Ratnayake, 1992). Oxidation may be initiated and accelerated by heat, light (especially UV-light) and several organic and inorganic substances (e.g. Cu and Fe). Also a number of antioxidants with the opposite effect are known (alpha-tocopherol, ascorbic acid, citric acid, carotenoids) (Huss, 1994).

Chemical examination of spoiling fish muscle has shown that most important constituents are the volatile sulphur compounds such as hydrogen sulphide (H_2S), dimethylsulphide $(CH_3)_2S$ and methylmercaptan (CH_3SH). Ester of lower fatty acids such as acetic, propionic, butyric and hexaenoic acids are also produced (Lakshmanan, 2000). The overall qualitative chemical picture of spoiling fish is summarized in Table 7.

Table 7: Chemical production by Bacteria during Fish spoilage

Substrate	Compounds produced by bacterial action
Inosine	Hypoxanthine
Carbohydrate and Lactate	Acetic acid, CO_2 and H_2O
Methionine and Cysteine	H_2S, CH_3SH and $(CH_3)_2S$
Tryptophan	Indole
Glycine, Leucine and Serine	Esters of acetic, propionic, butyric and hexanoic acids
Trimethylamine oxide	Trimethylamine
Urea	Ammonia
Lipids	Carbonyls
Protein	Tyrosine, indole, skatole, putrescine, cadaverine
Histidine	Histamine

Source: Fish spoilage and quality assessment (Lakshmanan, 2000).

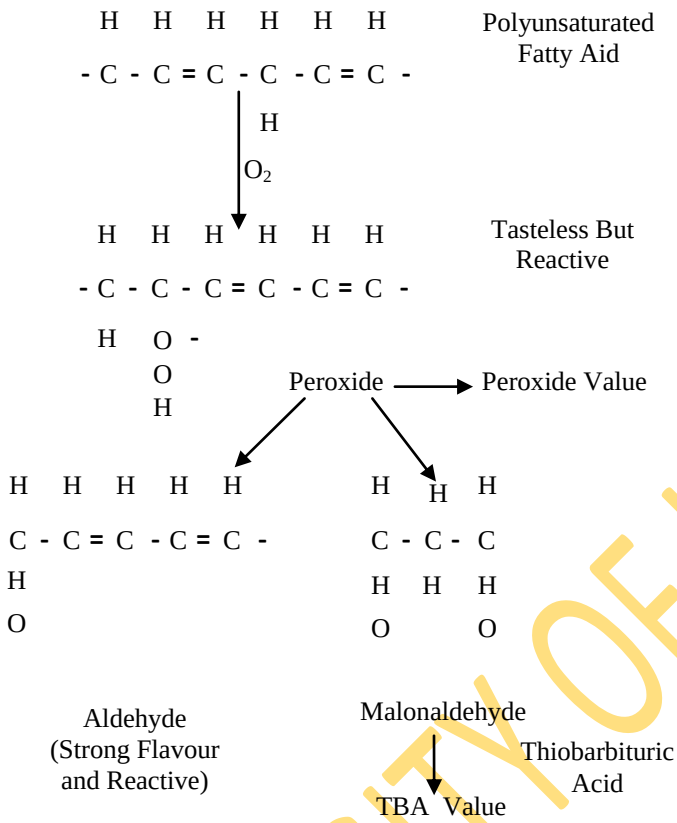

Figure 4: Basic Process for Oxidation of Polyunsaturated Fatty Acids found in Fish Tissue.

Source: Ackman and Ratnayake (1992)

2.6.4 Indicators of Deterioration in Frozen Fish

During freezing, the temperature falls below that of ice (0^{o}C), microbial activity is slowed down as the temperature goes further down to -30^{o}C, and most bacteria may die out. Freezing therefore is responsible for drastic reduction in surviving bacteria in fish and enhances microbial stability and thereby extends its shelf life (Baranowski *et al*., 1990; Eyo, 2001). The main factors affecting the quality of frozen fish are the freshness of the fish at the time of freezing, the rate of freezing, the storage temperature, the amount by which the storage temperature is allowed to fluctuate and desiccation during cold storage. Even temporary rise in temperature of a few degrees can have a marked effect on quality or storage life (FAO, 1983).

Indications of deterioration in frozen fish include marked changes in texture and flavour when thawed out. The thawed flesh may feel rubbery and appear dense white instead of translucent. After cooking, they are found to be tough and fibrous or stringy (Horde, 1973).

Storage of frozen fish brings about a decrease of extractability of mycofibrillar proteins. There is also deterioration of the texture and functional properties of the flesh. In model systems, aggregation of myosin, actin, tropomyosin, and whole myofibrils have been described. These changes are caused by concurrent action of partial dehydration due to the freezing out of water, exposure of the proteins to inorganic salts which are concentrated in the remaining nonfrozen fluid, interactions with free fatty acids liberated from phospholipids and lipid oxidation products, and cross-linking by formaldehyde produced in some species of fish as a result of enzymic decomposition of trimethylamine oxide. The extent of protein alterations increases with time and temperature of storage as well as advanced disintegration of the tissues and intermixing of their component (Sikorski *et al.*, 2009).

The protein changes in fish frozen under poor conditions can be recognized in the thawed fish. The normally bright, firm and elastic product becomes dull and spongy. The flesh will tend to sag and break and there will be substantial losses of fluid which can be squeezed out easily. When cooked, the fish will be dry and fibrous (FAO, 1994).

In frozen fish stored at a low temperature, the bacteria are kept in a state of suspended animation. Immediately it begins to thaw out however, they become active again and continue the process of bacteria spoilage (Burgess *et al*., 1967).

According to Twiddy and Reilly (1995), the predominant type of bacteria causing spoilage varies with the temperature at which the fish are held. At freezing temperature, species of Pseudomonas are most likely to predominate with *Acinetobacter*, *Moraxella* and *Flavobacterium* species next in order of importance. *Micrococcus* and *Enterobacteriaceae* and *Bacillus* largely replaced dominant microflora of *Pseudomonas* type as temperature increase (Huss *et al*., 1992). There are marked changes in texture and flavour on thawing, when fish is frozen very slowly at temperature only a little below 0^{o}C (Horde, 1973).

Shewanella putrefaciens according to Huss (1994) is typical for the aerobic chill spoilage of many fish from temperate waters and produces Trimethylamine (TMA), hydrogen sulphide (H_2S) and other volatile sulphides which give rise to the fishy, sulphidy cabbage like off-odours and flavours. *Vibrionaceae* and *Enterobacteriaceae* form similar metabolites during spoilage at higher temperatures. *Aeromonas hydrophilia* has also been reported in spoilage microflora of mackerel held at 5^{o}C, 10^{o}C and 15^{o}C, while traditional spoilage species occurred in the same fish species held at 0^{o}C (Huss *et al*.,1992).

Frozen fish may dry slowly in cold storage; fish get badly dehydrated and surface becomes dry, opaque, spongy and loses weight. This is known as freezer burn. Drying also accelerates denaturation of the protein oxidation of the fat in the fish (FAO, 1994). Some of the reactions that occur in unfrozen fish continue in the frozen state, albeit much more slowly, but in general, the deteriorative changes in frozen fish are quite different. A badly stored fillet or a fillet from a badly stored whole fish will feel hard stiff and will release copious drip. There is a loss of the sweet meaty flavour of fresh fish that is variously described as musty turnip, leathery or singed (Aitken *et al*., 1982).

2.6.5 Spoilage Bacteria

Bacteria are unicellular microorganisms, which occur almost everywhere in nature; and require a source of food, moisture and suitable temperature to grow (Cheesbrough, 1984; Olutiola *et al*., 1991). The flesh of newly caught healthy fish is sterile, however, the skin, gills and intestines in fish which have recently been feeding, carry considerable bacterial loads (Shewan, 1977).

According to Adebayo-Tayo *et al.,* (2012), the microbial composition of fish depends upon the microbial count of water in which they live. Both quantitatively and qualitatively, the microflora is a function of the environment in which fish are caught (Kriss, 1971). The environmental microflora introduced by cooling medium and by handling is also responsible for spoilage after the initial phase of self-digestion (FAO, 1994).

The number of microorganisms on the skin of fish can be influenced by the method of catching. For example, trawling fishnets along the bottom of the sea for long periods result in exposure of the fish to high bacteria counts in the disturbed bottom sediment (Twiddy and Reilly, 1995).

Contamination concern has been on high loads of unsuspected spoilage microorganisms like *Salmonella* spp., *Staphylococcus aureus, Pseudomonas aureginosa* and *Escherichia coli* (Bramsnacs, 1999 and Gram *et al*., 2000).

2.6.6 Bacterial Food Poisoning in Seafood

It has now been proven that bacterial contamination is the most important and frequent type of food poisoning (Thornton and Gracey, 1974). The term bacterial food poisoning is usually applied to gastro-intestinal disturbance caused by bacteria that have multiplied in food during storage at too low a temperature. The common symptoms are acute abdominal pain and diarrhoea often accompanied by vomiting and headache. The organisms may cause their effect either by direct infection of the alimentary tract or by a toxin produced in the food during storage (Aitken *et al*., 1982).

There is evidence of fish poisoning in different cases of food poisoning and illness depending on the types and species of the bacteria; such case of shewanellosis,

traveler's diarrhea, self-limiting gastroenteritis and bacteraemia. The patients with liver disease and the immuno-compromised individuals appear to be at higher risk. (Connell, 1995; Palumbo *et al.*, 1992; Morais *et al.*, 1997; FDA, 2002; Krsnik *et al.*, 2002; Otsuka *et al.*, 2007).

Causative organism of food poisoning in fish include *Staphylococcus aureus; Salmonella spp; Clostridium perfringes (welchii); Clostridium botulinum; Vibrio parahaemolyticus; Vibrio cholerae* and *Bacillus cereus* (Aitken *et al*., 1982; Clucas, 1990). Other bacteria include scomboid-food poisoning, which is usually associated with mackerel in the UK (Aitken *et al*., 1982). If *Clostridium botulinum* is excluded, only *Vibrio parahaemolyticus* among the remaining pathogens can be said to occur naturally in fish. Botulism is a neuro-paralytic disease resulting from the ingestion of toxins produced by *Clostridium botulinum*; and for the disease to occur, the organism must be present in the fish and must have an opportunity to grow and produce toxin. Even then, the fish must be consumed without or with only inadequate cooking since the toxin is heat-labile (Hobbs, 1987). There are seven types of *Clostridium* referred to as types A, B, C, D, E, F and G (Clucas, 1990).

Fish botulism is most often caused by strains producing type E toxin, though the other psychrophilic strains producing type B and F toxins are associated with fishery products (Hobbs and Hodgkiss, 1982).

In raw fish, the level of contamination with *C. botulinum* is low and if stored at temperature where growth and toxin production can occur, the fish is normally spoilt before significant amounts of toxin accumulate (Hobbs, 1987).

Vibrio parahaemolyticus is an infection agent, which causes gastroenteritis in man, usually severe but of short duration and usually with complete recovery (Hobbs and Hodgkiss, 1982). *V. parahaemolyticus* is probably the fastest growing bacterium known to man, it can double its number every 10 to 15 minutes under the best condition for growth (Aitken *et al*., 1982). *Clostridium perfringes* is unable to grow in the presence of air and at certain times produces within its body a specialized resisting structure called spore. Spores are extremely resistant to heat, freezing, dehydration and chemicals (Clucas, 1990).

Escherichia coli according to Hobbs (1987) has been used as index of feacal contamination. *E. coli* could be a motile or non-motile rod, which causes gastroenteritis, bacteriaemia, wound infection and urinary infection in human (Cheesbrough, 1984). *Pseudomonas* is naturally present in fish. According to Huss (1994), some fresh water fish and others from tropical waters during iced/aerobic storage are characterized by a pseudomonas type of spoilage, which is described as fruity, sulphydryl and sickening. *Pseudomonas* is a gram-negative motile rod, which causes urinary infections and septicaemia (Cheesbrough, 1984).

Micrococcus acidophilus replaces *Pseudomonas* when there is increase in temperature of fish caused by poor storage. *Proteus* is also added during handling (Huss *et al*., 1992). There are spore forming species of bacteria like *Bacillus* cereus and *Bacillus licheniformis*. According to Cheesebrough (1984), the spores enable them to survive when conditions for vegetative growth are not favourable, especially when carbon and nitrogen become unavailable. *Bacillus* causes anthrax in human (Cheesebrough, 1984).

Lactobacillus acidiophilus serve a preservative function. According to Clucas (1990), *Lactobacillus spp.* have the ability to reduce the pH to a level where the normal spoilage flora is inhibited, the usual mechanism being the production of lactic acid bacteria from the carbohydrates in the food thereby elongating the shelf life.

Streptococcus bacteria produce extra cellular enzymes called streptokinase, which assist the organisms to spread in the body by breaking down fibrin, which is formed by the host as a protective barrier. *Streptococcus faecium* are cocci in chains and they cause wound and ulcer infection, septicaemia and urinary tract infections (Cheesbrough, 1984).

Staphylococcus aureus produces a poisonous toxin, which differs from most other microbial toxins. The toxins can withstand boiling for up to thirty minutes before being destroyed. The number of *S. aureus* in fish is also currently used in commercial specification and standard (Aitken *et al*., 1982).

Table 8: Characteristics of Bacterial Food Poisoning

Causative organisms	Period of incubation in hours	Duration
Salmonellae (infection)	12-36	1-8days
Staphylococcus (toxin)	2-6	6-24hours
Clostridium welchii (toxin)	8-22	12-24hours
Clostridium botulinum (toxin)	9-72	Death in 1-8 days or convalescence over 6-8months
Vibrio parahaemolyticus	24-72,	1-14days
Bacillus cereus	1-16	12-24hours

Source: Fish handling and processing (Aitken *et al*., 1982).

2.6.7 Microbial Assay

Estimation of bacteria number in food is frequently used in the retrospective assessment of microbiological quality or to access the presumptive safety of foods. This procedure requires that samples be taken from the food, microbiological tests or analysis performed and the result evaluated (Huss, 1994). Three types of bacteria indices are used in standard specifications and limits. First is the general purpose index which expresses the total number of living bacteria present in a foodstuff, which is reported as total viable count (TVC), standard plate count (SPC) or colony forming unit (CFU). The second group known as organism of public health significance comprises: *Escherichia coli*, *Enterobacteriaceae* and faecal *Streptococci* or *Enterococci*. The third group consists of the specific food poisoning bacteria of which only Salmonella and *Staphylococcus aureus* are commonly incorporated in standards for fishery products (Aitken *et al*., 1982).

Table 9: Specification applied to Fish Products.

Standard plate count at 35^{o}C	**10^{5}/g**
Escherichia coli	NIL/g
Staphylococcus aureus	NIL/g
Salmonella	NIL/25-50g

Source: Fish handling and processing (Aitken *et al*., 1982)

2.7 Fish Quality Assessment

Due examination of fish and its products before sale for evidence of spoilage, damage, adulteration or disease is a long established practice. The objective being partly to guard the health and "pockets" of consumers and also to ensure goodwill of vendors.

Quality assessment of the fish includes sensory evaluation, chemical tests and microbiological tests (Official Methods of Analysis of the Association of Official Analytical Chemists, AOAC (2002). The main categories of quality assessment commonly encountered in fish industry as identified by Eyo (2001) are objective and subjective methods. Objective methods include chemical, biochemical, bacteriological and physical tests, while the subjective test is the organoleptic or sensory test. The methods of assessing freshness can be classified into sensory, biological, chemical and physical methods (Aitken *et al*., 1982).

2.7.1 Sensory or Organoleptic Tests

The oldest and still the most widespread means of evaluating the acceptability and edibility of fish are the senses (Farber, 1965). Sensory assessment is the use of one or more of the five senses to judge or form an opinion on some aspects of quality. The senses in question are sight, smell, taste, touch and hearing (FAO/Codex, 1999). Tests are either used singly or in combination with each other frequently to assess the appearance of the fish including the gill colour and presence or absence of indentations.

As fish spoils, it goes through a sequence of changes that are readily detectable by the human sense of sight, touch, smell and taste (Aitken *et al*., 1982). The reason for preferential use of sensory tests are obvious: no special laboratory equipment is needed, the fish can be examined wherever they happen to be, assessment can be carried out quickly and many samples can be evaluated in relatively short time (Borgstrom, 1965). Sensory as opposed to non-sensory methods offer the best opportunity of getting a valid idea of what the consumer wants. The human sense of smell can differentiate between good and bad quality fish. (Connell, 1995).

According to Lakshmanan (2000), taste panel study is most satisfactory for quality assessment of fish. A hedonic scale or scoring method could be adopted. A special taste panel room which should be free of odour, with proper lightning and well screened with individual booths is recommended, to prevent the testing environment from influencing the result.

In testing the fish, particular attention would be paid to general appearance including the condition of the skin, presence of scales, appearance of eyes, gills and belly, the texture appearance and odour of the fish, the appearance of the belly cavity including the integrity of viscera and odour from the gills (Nyagambi, 1982). The sense of smell is very unique in that so far no instrument is capable of performing this task. Sensory evaluation for organoleptic changes in the raw chilled and frozen fish is conducted on the exposed organs such as the eyes, gills, scales and the entire body of the fish (Eyo, 2001).

The quality scores and organoleptic changes for trunkfish *Mormyrus rume* raw samples are shown in Table 10.

Table 10: Quality Scores and Organoleptic Changes for Trunkfish, *Mormyrus rume* Raw Samples.

Score	Eyes	Gills	Skin	Flesh	Grade
10	Convex, dark pupil, white cornea, iridescent	Dark red, fresh odour	Dark grey at dorsal region, silvery at ventral region	Firm, elastic, slime	1-First quality
8	Convex, dark pupil, white cornea, loss of brightness	Pale red, fresh odour	Dark grey at dorsal region, silvery at ventral region	Less firm, elastic	2-Second quality
6	Flat white pupil and cornea	Pinkish sour, slightly rancid	Dark grey at dorsal region, silvery at ventral region	Less firm, slightly elastic	3-Third quality
4	Slightly concave, white pupil, grey cornea	Bleached, sour/rancid	Dark grey at dorsal region, silvery at ventral region	Soft no elasticity	Limit of acceptability
2	Concave, white pupil, grey cornea	Pale greenish, very sour/strong rancid	Dark grey at dorsal region, creamy at ventral region	Soft, no elasticity	Rejected
0	Concave white pupil, grey cornea	Greenish, strong rancid	Dark grey at dorsal region, creamy at ventral region	Very soft and flabby	Rejected

Source: Eyo (1998)

According to Howgate (1989), a finer degree of measurement of freshness can be obtained with a scoring system such as that used in Torry Research Station. Separate descriptive scales are used for the senses of sight, smell, touch and taste. For the more discriminating senses of smell and taste, scales ranging from 10 to 0 are used, with 10 denoting absolutely fresh fish and 0 completely putrid fish. Most people would consider fish below a freshness of 4 to 5 on the scales unacceptable.

Sensory test of eating quality according to Connell and Shewan (1980) are undoubtedly those, which ultimately count because they come close to assessing those consumer responses, which matter. The sensory attributes of frozen fish such as off-flavour and firmness changes in a progressive fashion with time of storage and these changes can be arranged into fully descriptive scoring system intensive scales (Baines *et al*., 1969). Sensory methods are difficult to standardize and the result can be subject to the personal whims and biases of the assessors (Howgate, 1989).

However, since sensory tests express to a large extent the opinion of the consumer, they are the tests frequently carried out in the fishing industry for on the spot assessment of fish quality (Eyo, 2001). In fact, taste may influence food market habits and according to Lickow and Delahunty (2004), consumers judge the acceptability of a product based on taste rather than on other attributes such as health benefits.

2.7.2 Objective Method of Fish Quality Assessment

Objective methods of quality assessment are those tests which rely on the use of instrument and reagents for their determination. Objective methods have the advantage of being usually reproducible or comparable within different laboratories. According to Connell (1967), the ideal method would be one that avoids the subjectivity of sensory methods and would be cheap, non destructive, easy to use, not subject to variation or fatigue, have rapid response and wide application. Only an instrument can satisfy these requirements.

As long as 1891, Eber proposed a simple chemical test for putrefaction based on volatile amines and since then numerous chemical, biochemical and microbiological tests have been investigated (Connell and Shewan, 1980).

2.7.3 Chemical and Biochemical Tests

Chemical methods according to Eyo (2001) are often used to determine the composition of fish products at different stages of processing. The chemical elements most frequently analyzed are moisture, protein, lipid, ash and mineral elements.

According to FAO (1982), chemical assays were made of ash content, moisture content, iodine value, total nitrogen, non-protein nitrogen and total volatile bases with pH. Measurements of the products of biochemical reaction that take place in fish after death have been used to determine the level of spoilage (Eyo, 2001). Such products of biochemical reaction include hypoxanthine (Hx), trimethylamine (TMA), total volatile nitrogen (TVN) and total volatile bases (TVB). Protein content is assessed by determining the nitrogen content of the sample and multiplying it by a factor (6.25) representing the inverse of the known nitrogen content of protein (Connell, 1995).

It was observed by Adebona (1978) that indices of spoilage including iodine value, peroxide value, volatile bases and non-protein were found to be dependent on the duration of the storage, and composition of these indices vary from gutted to ungutted stored fish. Mineral content which is a good measure of bone, shell or salt content of a product can be determined by burning off at a high temperature, the organic part of a known quantity of the product and the residue of ash (Connell, 1995).

2.7.4 Hypoxanthine (Hx)

Hypoxanthine (Hx) is a normal constituent of fish flesh. It is the end product of a series of enzymatic reactions going on in the flesh. On the death of the fish, the balance of the enzymatic reactions is disturbed and hypoxanthine increases (Aitken *et al*, 1982). Hypoxanthine is a degradation product of adenosine triphosphate and the level present reflects evidence of autolytic deterioration (Kirk and Sawyer, 1999).

According to Burt (1977), the degradative changes in fish muscle tissues associated with the hydrolysis of adenosine triphosphate (ATP) have shown that the hypoxanthine content rises throughout the commercial important periods of storage at chill temperatures. The nucleotide adenosine triphosphate has a widespread and a near uniform degradative pathway in most tissues and hypoxanthine is a result of this ATP degradation (Eyo, 2001). The way in which hypoxanthine concentration increases with

storage time is more variable and is a better way of spoilage over a wide range of quantities; and is applicable to a wide range of species products than TMA and TVB (Howgate, 1982).

Hypoxanthine begins to accumulate shortly after fish dies; however, changes in TVB and TMA are related to the microbiological activities and they do not increase at the beginning of storage. Thus nucleotide and hypoxanthine measurements have some advantages over TMA and TVB analysis (Metin *et al*., 2001). The total volatile bases and trimethylamine tests measure the various stages of spoilage caused by bacteria, but the assay of nucleotide and Hx reflects enzymatic spoilage (Spinelli, 1967).

According to Kassemsam *et al*., (1963) Adenosine triphosphate is degraded at the post mortem stage by endogenous enzymes in the fish flesh.

ATP → ADP→ AMP → IMP → HxR→ Hx

Hypoxanthine is regarded as the major catabolite of adenosine triphosphate (ATP) and is a useful freshness indicator because of its gradual accumulation in flat fish (Greene and Bernalt-Byrne, 1990). The usual trend according to Eyo (2001) is that the formation of hypoxanthine begins slowly at first following the activities of autolytic enzymes, and increases rapidly as spoilage progresses through the activities of bacteria enzymes. The presence of higher levels of IMP in the muscle indicates relatively high quality, while accumulation of inosine and Hx is an indication of poor quality (Lakshmanan, 2000). These autolytic degradative products have been found to possess different organoleptic properties. Inosine monophosphate (IMP) produces a sweet flavor at very low concentrations and is regarded as a strong flavour enhancer. Inosine is flavourless while hypoxanthine imparts a bitter flavour to spoiling fish (Spinelli, 1965). Burt (1977) also points out that hypoxanthine itself has a bitter flavour and that the degradative pathway involves inosine 5-monophosphate at an intermediate stage and it is a flavour enhancer, hence the hypoxanthine index has a special significance in relation to the organoleptic assessment of fish staleness.

Methods for the analysis of hypoxanthine include Colorimetric, Gravimetric, K-value and the test paper methods (Lakshmanan, 2000; Eyo, 2001). According to Burt (1977), Colorimetric method involves the use of the enzymes xanthine oxidase and 2, 6- dichlorophenol indophenols (DCPIP), Gibb's reagents, as colour indicator.

The colour change is measured at 618nm wavelength using a spectrometer. The k-value is the ratio (expressed in percentage) of Inosine plus hypoxanthine to total amount of ATP-related compounds (Saito, *et al*., 1959; Ehira, 1976).

2.7.5 Trimethylamine (TMA)

Spoilage changes result in the gradual accumulation in the flesh of compounds, the quantity of which provides a measure of the progress of spoilage that is independent of sensory assessment. The most well known of these compounds is the trimethylamine (TMA) derived possibly partly by intrinsic enzymes but certainly by bacteria action from trimethylamine oxide (TMAO) (Connell, 1995).

Trimethylamine (TMA) is formed in spoilt fish by the action of certain species of bacteria on TMAO. The oxygen is used up by aerobic bacteria (Aitken *et al*., 1982). A number of well defined spoilage bacteria (*Aeromonas* spp., psychrotolerant Entrobacteriaceae, *Photobacterium phosphoreum*, *Shewanella putrefaciens* and *Vibrionaceae*) are able to utilize TMAO as the terminal electron acceptor in an anaerobic respiration resulting in ammonia-like and 'fishy' off-odours and off-flavours due to the formation of TMA (Lerke *et al*., 1963; Ringo *et al*., 1984; Gram *et al*., 1990; Dalgaard, 1995). Beatty and Gibbons (1936) as stated by Eyo (2001) were the first to propose the use of TMA as index of fish freshness.

Determination of TMA could provide information on both pre-freezing spoilage and deterioration on frozen storage (Castell *et al*., 2011). In most marine fish and pike among the freshwater fish, which contain substantial amount of TMAO, the determination of the degradation product, TMA has been used extensively as a more specific index of bacteria spoilage (Connell and Shewan, 1980). According to FAO (1994), bacteria action in frozen fish is inhibited and TMAO is broken down by autolytic enzymes to DMA and formaldehyde (FA).

TMA is the product of the breakdown of TMAO by the enzyme triaminase oxidase which is associated with *Shewanella putrfaciens*. Method of measuring TMA includes micro-diffuse, distillation and colorimetric methods; Gas-liquid chromatography (GLC) and the use of TMA-sensitive electrode (Eyo, 2001).

According to Connell (1980), the spoilage of whole white fish beyond fitness for human consumption was chemically indicated by the surpassing of flesh total trimethylamine (TMA) of 150mg/kg-1. However, there are situations where the TMA limit is passed well before the limit of edibility or even the limit of sensory desirability Horner *et al.*, (1997). This stated by Oehlenschlager (1997a), the amines are present in freshly caught marine fish on a very low level and develop in later stages of storage depending on species, temperature, hygiene regime and other factors. The deeper the fish lives, the higher the TMAO content. Pelagic species like herring, mackerel normally do not exceed 30mg/100g of fish. Gadois, hakes and red fish species can contain up to 120mg/100g wet weight and elasmobranchs show even higher value (Amano *et al*., 1963; Tokunaga, 1980 Agustsson and Strom, 1981; Regenstein *et al.*, 1982; Sikorski and Kostuch, 1982; Hebard *et al*., 1982).

2.7.6 Total Volatile Base (TVB)

According to FAO (1982), Total volatile base (TVB) has been found to be useful for estimating the freshness of fish; and it has been suggested that the upper limit for TVB in fresh water fish should be about 40mgN/100g. The measurement of TVB is often used as an alternative to measuring TMA content because fish, during spoilage, contains several bases that are volatile when its extract is made alkaline (Howgate, 1989).

TVB could be measured by Conway micro-distillation technique or by the micro-distillation method proposed by Lucke and Geidel (1935). The use of an apparatus designed by Antonacopoulos (1968) for the routine determination of TVB in fish is another method (Eyo 2001). The methods differ mainly in the way the bases are released from the fish and distilled, but the absorption and neutralization of the bases are similar (Aitken *et al*., 1982).

2.7.7 Peroxide Value (PV)

During the frozen storage of fish, lipid hydrolysis and oxidation have been shown to occur and become an important factor of fish acceptance; and influencing rancidity development, protein denaturation and texture changes (Mackie, 1993; Verma *et al*., 1995).

Under chemical measurement of oxidative rancidity, there are two tests of roughly equal but limited value: Peroxide value (PV) and Thiobarbituric Acid value (TBA) (Connell, 1995). PV monitors the early stage of oxidation of the lipids, while TBA measures the end products of oxidation. Both are indicators of rancidity (Howgate, 1989; Lakshmanan, 2000).

The measurement of PV depends on the release of iodine from potassium iodide by hydroperoxides and trimetric determination of the iodine. The units are the number of 0.002N sodium thiosulphate required to titrate the iodine librated by 1g fat extract from the fish. Peroxide value above 10.20mg and TBA above 1-2(1x 7.8 or 2x 7.8 Matonaldehyde) in fish result in objectionable rancid flavour (Connell, 1995).

Yellowing of fish flesh associated with lipid oxidation and carbonyl-amine reaction is observed during frozen storage. Brown or yellow discoloration is caused by the reaction of protein or amino acids with product of lipid oxidation. Discoloration due to protein-lipid browning is greater in fatty fish than lean fish (Lakshmanan, 2000).

2.7.8 Acid Value and Free Fatty Acid Content

It has been proven that not all fat changes are mainly oxidative in nature. Some involve the hydrolytic production or liberation of fatty acids as well as other organic acids. Free Fatty Acid (FFA) formation as a result of lipid (triglyceride and phospholipid classes) hydrolysis has provided a suitable means for assessment of fish damage during frozen storage (Aubourg, 1999).

Formation of FFA itself does not lead to nutritional losses. However, its accumulation in frozen fish is related to some extent with lack of acceptability of frozen fish, because FFA are known to cause texture deterioration by interacting with protein (Mackie, 1993; Sotelo *et al.*, 1995) and have been shown to be strongly interrelated with lipid oxidation (Miyashita and Takagi, 1986; Han and Liston, 1988).

2.7.9 Microbiological test

A number of microbiology tests for fish and fish products are used to check that the microbiological status is satisfactory. The purpose of these examinations is to detect for pathogenic bacteria (*Salmonella*, *V. parahaemolyticus*, *Staphylococcus*

aureus, Listeria monocytogenes, E. coli) or for organisms that are possible indications of feacal contamination (*E. coli*) or other types of general contamination or poor manufacturing practices (coliform bacteria, feacal *Streptococci*, aerobic plate count) (Huss, 1994).

According to Connell (1995), all recommended methods of this kind depend upon the microbial community. The sample should be grinded very finely in suitable aqueous medium to release the organism, diluting the suspension so formed and then mixing it with a layer of agar jelly containing nutrients. When the layer is incubated at a suitable constant temperature between 20^{o}C and 40^{o}C**,** single individuals multiply into visible colonies that can be counted by eyes. The count can then be related to a given unit weight of sample. Alternatively, the sample is inoculated into a special medium that on incubation indicates only whether the organism is growing.

A method of assessing bacteria load of the fish flesh was devised by Lima dos Santos (1992). A representative area of the fish flesh of Kamongo (*Protopterus ethiopicus*) was aseptically removed from each fish sample at each sampling occasion, using a sterile template. For Furu (*Haplochromis* spp.), a transverse section from the part immediately anterior to the tail section of the fish was aseptically cut with a scissors; the total sample at each sampling occasion consisted of approximately 10g of skin, flesh and bones. Finally, for Saranga (*Lates* spp.), a piece of flesh weighing approximately 5g was aseptically removed from each fillet. Each fish sample was then homogenized with 9ml of a peptone water solution (0.01%) for one minute in an electric blender. After a suitable serial dilution of the sample employing the same diluents (0.1% peptone water), the resulting suspension was later paired into Standard Plate Count Agar, (SPA). Duplicate plates were then incubated at room temperature (23^{0}C-27^{0}C) for three days and the colour recorded.

As explained by Adebona (1978), bacteria count of the muscle tissue of ungutted samples was found to be in the same order, with a tendency for higher counts in gutted samples. Two types of methods according to Connell (1995) are used for routine examination, one that measures the total number of organisms present in the sample and capable of growing under the incubation conditions adopted and those that measure the numbers of special groups of organisms like pathogens. The first type is Standard Plate Count (SPC). It gives a comparative measure of the overall degree of

microbiological contamination. The second type utilizes special media that favour nearly exclusively the growth of the peculiar group of organisms being measured. Thus, details of sampling, diluting, plating out, composition of media, temperature and time of incubation technique of counting should all be kept standard.

Total Viable Count (TVC) or Aerobic Plate Count (APC) according to Huss (1994) is the number of bacteria (cfu/g) in a food product obtained under optimum conditions. It is a measure of the fraction of the microflora able to produce colonies in the medium used under the conditions of incubation. *Escherichia coli* are particularly useful as indicator of contamination (small number) or mishandling such as temperature abuse in product handling (large number). Contamination of food with *E. coli* implies a risk that one or more enteric pathogens may have gained access to the food; however, failure to detect *E. coli* does not ensure the absence of enteric pathogen (Mossel, 1967; Silliker and Gabis, 1976).

Recent investigation have shown that *E. coli* and feacal coliform bacteria can be found in unpolluted warm tropical waters and that *E. coli* can survive indefinitely in this environment (Hazen, 1988; Fujioka *et al*., 1988; Toranzos *et al*., 1988). Feacal *Streptococci* and *Enterococci* are contained in foods and fish products as normal part of their flora and unlike *E. coli;* they are relatively resistant to freezing, which makes them potentially useful as indicator organisms for evaluating plant hygiene during processing of frozen food (Huss, 1994).

Staphylococcus aureus is included in a number of microbiological criteria and its enumeration presents no problem. Baird-parkers egg yolk medium and incubation for 30 hours at 37^{o}C is a mostly reliable method. Positive culture needs to be confirmed by testing for coagulase activity (Huss, 1994).

According to FAO (1994), a standard is a microbiological criterion that is part of a law or ordinance and is a mandatory criterion. A microbiological guidance is a criterion used to assess microbiological conditions during distribution and marketing of foods, hence, it is mostly an advisory criterion. A microbiological specification is used in purchase agreements between buyer and vendor. Nevertheless, it is generally true that if fish have on them more than 10^6 bacteria in 1gram, there is a good chance that spoilage is well advanced, and if the count exceeds 10^8/g the fish will be inedible (Aitken *et al*., 1982).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Area of Study

The study area was Oyo state, which was stratified into four Agricultural Development Programme (ADP) zones, including Ibadan/Ibarapa, Ogbomoso, Oyo and Saki zones. Oyo state of Nigeria, otherwise known as 'Pacesetter State' was formed on 3rd February, 1976 from the former Western State, and originally included Ọsun State, which was split off in 1991. The state lies within the longitude 3°E and 4.25°E and latitude 7°N and 9°N with total land area of 28,454km^2. It is bounded in the South by Ogun state and in the North by Kwara state, in the west by the Republic of Benin while in the east it is bounded by Osun state (Fig. 5). It has 33 Local Government Area and population of 5,591,589 (National Population Commission, NPC, 2006).

The landscape consists of old rock and dome shaped hills which rise gently from about 500m in the southern part and reaching a height of about 1,219m above sea level in the northern part. Some principal rivers such as Ogun, Oyan, Erinle and Osun take their source from these highlands. The climate is equatorial, notably with dry and wet seasons, and relatively high humidity. The dry season lasts from November to March, while the wet season starts from April and ends in October. Average daily temperature ranges between 25°C and 35°C almost throughout the year.

Oyo State is homogenous, mainly inhabited by the Yoruba ethnic group who are primarily agrarian but have a predilection for living in high density urban centers. The indigenes mainly comprise the Oyos, the Oke-Oguns, the Ibadans and the Ibarapas, all belonging to the Yoruba family and indigenous cities in Africa, South of the Sahara. Ibadan had been the centre of administration of the old Western Region, Nigeria, since the days of the British colonial rule. Other notable cities and towns in Oyo State include: Oyo, Ogbomoso, Iseyin, Kishi, Okeho, Saki, Eruwa, Lanlate, Sepeteri, Ilora, Awe, Ilero, Igbeti, Igboho and Igbo-Ora. The climate in the state favours the cultivation of crops like maize, yam, cassava, millet, rice, plantain, cacao

tree, palm tree and cashew. There are a number of government farm settlements in Ipapo, Ilora, Sepeteri, Eruwa, Ogbomoso, Iresaadu, Ijaiye, Akufo and Lalupon.

3.2 Questionnaire Administration

The areas of study covered the four zones of Oyo state viz: Ibadan/Ibarapa, Ogbomoso, Oyo and Saki zones (Fig. 5). The zones are taken as the sampling frames for the study. A sample survey was thereafter carried out to draw the sample needed for subsequent empirical analysis. A total number of 217 respondents for the study included wholesalers and retailers. The wholesalers are the frozen fish dealers, who operate cold stores, while retailers buy from the cold stores and sell to the final consumers.

The administration of questionnaire lasted six months and was in two stages. The first stage was the selection of all cold store operators (wholesalers) in the four zones of the study area totaling 67. Sequel to this was the random selection of 150 retailers at five percent representation per zone of all registered fish retailers in Oyo state (Table 11).

Questionnaire schedule developed for the study (appendices 4 and 5) consists of items on personal characteristics of respondents such as age, gender and educational qualifications. Socio-economic parameters, group activities of the fish sellers, transportation, handling and physical facilities among others were evaluated. The questionnaires were completed by the respondents but where necessary, further in-depth face to face interaction was employed in obtaining accurate data.

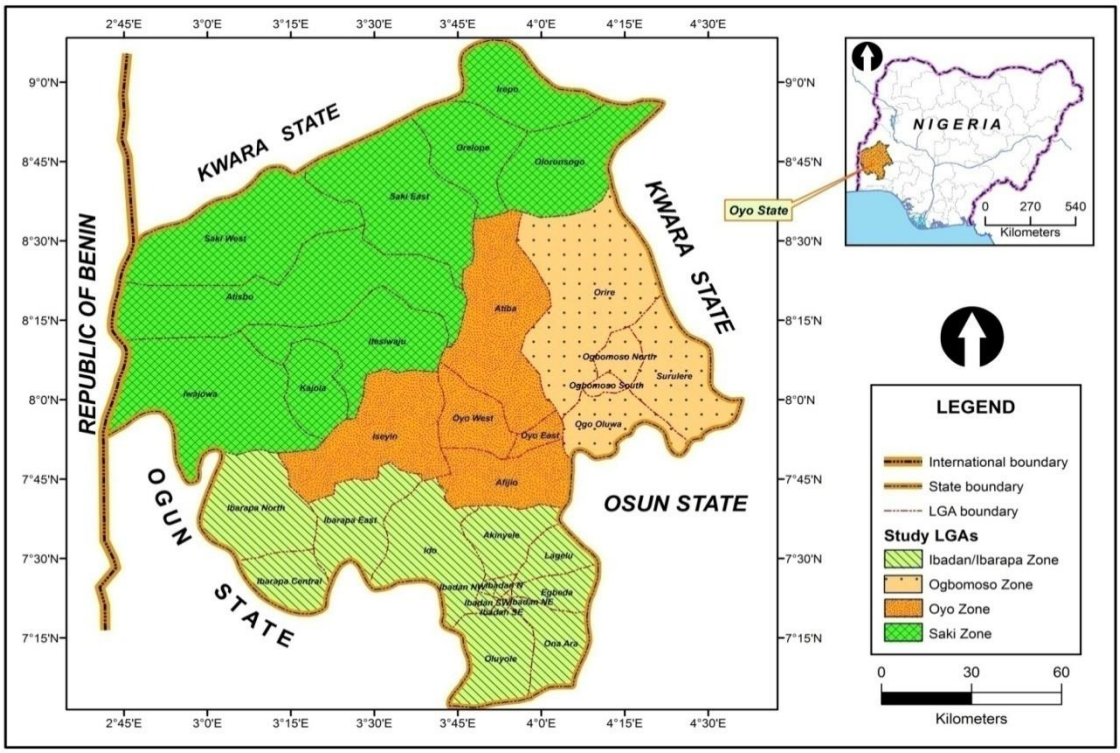

Figure 5: Map of Oyo State showing Study Area

Table 11: Area of Study and Distribution of Respondents by Zone

Zone	No of wholesalers interviewed	No of registered retailers	No of retailers interviewed (5% representation)
Ibadan/Ibarapa	39	2047	102
Ogbomoso	16	584	29
Oyo	7	303	15
Saki	5	83	4
Total	**67**	**3017**	**150**

Source: Field data (2011)

3.3 Descriptive Statistics

The socio-demographic and socio-economic variables such as gender, age, level of education, group activities, pricing policy and income were presented in frequency tables and analyzed using simple percentages.

3.4 Sample Collection

Sardinella species (sardine) and *Micromesistius poutasssou* (blue whiting) were selected from among the commercially important species of imported frozen fish supply to Oyo state (appendix 2). This was due to their availability, spread and acceptability to the consumers in all the four zones of the study area.

Several samples of *Sardinella* species and *Micromesistius poutasssou* averaging 190g weight and between 25-30cm long were collected from retail depots from four different zones of the study area. These fish depots were located in Ibadan/Ibarapa, Ogbomoso, Oyo and Saki zones.

The samples were collected in suitable aluminum foil, placed in sterile plastic containers with ice and immediately taken to the laboratory for tests to be carried out on them. The biochemical analysis was carried out in the Department of Chemistry, University of Ibadan, while the microbial tests took place in the Department of Microbiology, University of Ibadan. This was done for the four zones at every two weeks per zone for a period of twelve months.

Plate 1: Freshly Collected Frozen Sample of *Sardinella* species

Plate 2: Freshly Collected Frozen Sample of *Micromesistius poutassou*

3.5 Organoleptic Assessment

This assessment was carried out on both the uncooked and the cooked fish samples. The assessment was based on the scoring system, which involved measurement of certain parameters on a 7 point hedonic scale on the determination of characteristics (Minim, 2006). Seven categories were ranked: very much liked (7), liked a lot (6), liked (5), liked and did not like (4), disliked (3), much disliked (2) and very much disliked (1).

Fish samples from different zones of the study areas were presented bi-monthly to a semi-trained five-man panel for organoleptic assessment in a well lit environment free of odour. The fish were presented to the taste panel immediately they are brought from retailers.

For the cooked fish, the samples were gutted and steamed whole for about 20 minutes; and allowed to cool to a comfortable tasting temperature. They were then presented to the taste panel in a clean plate. For the uncooked, each fish sample was placed on a clean plate for assessment. Each assessor was given a score sheet to record their observation (appendix 9). The parameters assessed by the panelists were as follows:

Uncooked fish:

- Appearance or external characteristics, which include mucus, shape of the eye and rigidity of the abdominal wall.
- Colour of the gills and skin.
- Texture
- Odour

Cooked fish:

- Texture
- Odour
- Taste

3.6 Proximate Analysis

This was determined on wet matter basis. Proximate analysis for crude protein, crude fibre, crude fat, ash and moisture was according to AOAC (2002). Proximate composition was determined bi-monthly on each sample of fish from all the zones.

3.7 Biochemical Analysis

Biochemical analysis carried out on the samples includes:

- Hypoxanthine (Hx)
- Peroxide value (PV)
- Trimethylamine (TMA)
- Free fatty acid (FFA)

3.7.1 Determination of Hypoxanthine

2g of crushed fish samples was weighed into a 250ml beaker, 1g of active carbon, 100ml distilled water and 5ml of Carez solutions I and II were added and mixed for 30 minutes. The mixture was filtered through a Whatman No. 2 filter paper.

5ml of clear colourless filtrate was pipetted into 15ml test tube, 5ml of 4 dimethylamino benzoic acid (DMAB) solution added, mixed and placed in the water bath at 20^{o}C. The absorbance of mixture was taken after colour development on a spectronic 21D spectrophotometer at a wavelength of 460nm. Standard hypoxanthine of range 2ppm-10ppm was also treated as above and absorbance taken at the same wavelength.

$$Hx\ (mg/100g) = \frac{\text{Absorbance of sample x Gradient factor of standard x Dilution factor}}{\text{Weight of sample}}$$

3.7.2 Determination of Peroxide Value

2g of crushed fish sample was weighed into a 250ml of chloroform and 10ml of glacial acetic acid was added to the fish sample in the beaker and mixed.

The mixture was filtered into 250ml conical flask, 1ml of 5% (aq) saturated potassium iodide (KI) solution was added and shaken thoroughly. The homogenous mixture was placed on the hot plate to boil for 30 seconds. 25 ml distilled water was

added and shaken; 1 ml of 1% starch was added and the hot mixture titrated against the 0.002M Na_2SO_3. A blank determination was also carried out at the same time. Peroxide value is the number of mls of $Na_2S_2O_3$ (0.002M) used for the titration in milli equivalent per kilogram (Meq/kg).

$$Pv\ (Meq/kg)\ = \frac{\text{Titre value of sample} - \text{Titre value of blank x } MNa_2S_2O_3x10^3}{\text{Weight of sample}}$$

3.7.3 Free Fatty Acid Determination

1g of well- macerated flesh sample was weighed into a 100ml beaker. 50ml of chloroform was added and stirred for 5 minutes to ensure a complete extract of fat from the fish flesh sample. The mixture was filtered through a Whatman No.1 filter paper into a 250ml conical flask. 25ml of the filterate was dissolved in 25ml of mixed neutral solvent (mixture of diethylether and alcohol neutralized with 0.1N NaOH). 1 ml of 1% phenolphthalein solution was added and titrate against 0.1N NaOH until a pink colour, which persisted for 15 seconds was obtained.

$$FFA\ (\%) = \frac{1}{2}X\ \frac{\text{Titre value x 5.61}}{\text{Weight of sample}}$$

3.7.4 Determination of Trimethylamine

2g of well ground fish samples was homogenized with 6ml of 5% TCA (Trichloroacetic acid). The mixture was properly homogenized to obtain uniform slurry. The slurry was filtered into a 50ml volumetric flask to obtain a clear filtrate. 5ml of the clear filtrate was pipetted into a semi distillation apparatus to which 5ml of 2M NaOH was added. The mixture was then steam-distilled in the distillation apparatus into 15ml of 0.01M HCl solution in 50ml conical flasks. 15 drops of rosolic acid indicator solution were added to give a bluish colour. The mixture was then titrated to give a pale pink end point with 0.01M NaOH solution to obtain V_2. To every 10ml liquid in the titration flask, 1ml of 16% neutralized formaldehyde solution was added.

$$TMA = \frac{14\ (300\ +\ W)\ x\ V_2}{500}\ mg/100g$$

V_2 = volume standard acid released in 2nd titration

W = water content of the sample in g/ml.

3.8 Microbiological Analysis

Frozen samples of *Sardinella* species and *Micromesistius poutasssou* were purchased from the retail depots at different zones on bi-monthly basis for a period of twelve months between January and December 2011. The two different samples were wrapped with aluminum foil and placed in sterile plastic containers with ice and conveyed to the laboratory for microbiological analysis. The samples of *Sardinella* species and *Micromesistius poutasssou* were aseptically removed from the plastic container and were placed on a sterile tray and with the aid of a sterile knife; cuts were made from the edible parts of the samples. 90ml peptone water was added to 10g of fish flesh and homogenized in a blender for a minute at high speed. 10ml of the original homogenate fluid was then taken for microbiological analysis.

3.8.1 Culture Media

Plate Count Agar (PCA), Mac Conkey Agar (MCA), Mannitol Salt Agar (MSA), *Salmonella Shigella* Agar (SSA), Potato Dextrose Agar (PDA), Blood Agar (BA) and De Man, Rogosa Sharpe (MRS) Agar were weighed and distilled water was added according to Manufacturer's instruction. The solution was homogenised in a water bath for 10 minutes. The medium was then sterilized in an autoclave at 121^{o}C for 15 minutes and allowed to cool to 45^{o}C before use.

3.8.2 Enumeration and Isolation of Microorganisms

Serial dilutions were made from the samples using sterile pipette. This was done by mixing 1g of the sample thoroughly with 9ml of sterile distilled water to give 1:10 dilution. The dilution was made up to 10^{-6}. Using sterile pipette of 1ml, appropriate dilutions were plated out using different culture media (Harrigan and McCance, 1976). The plates were inoculated in duplicates and allowed to set. After solidifying, MRS plates were incubated in a carbon-dioxide enriched jar at 37^{o}C for 48 hours. Other bacterial plates were incubated aerobically at 30^{o}C for 24 hours, while Potato Dextrose Agar plates were incubated for 3-5 days. At the end of incubation, representative colonies were selected at random and sub- cultured repeatedly to obtain pure cultures.

The culture media were used in the isolation and enumeration of the microbial loads of the fish samples. PCA for Total Viable Count, MCA was used for

Enterobacteriaceae count, SSA for *Salmonella* and *Shigella* count, MSA for *Staphylococcus* count, MRS for Lactic acid bacteria, BA for haemolytic *Streptococci* count and PDA for fungi load using the pour plate technique. All isolated colonies were calculated and expressed as colony forming units (CFU/g) per gram of fish.

3.8.3 Characterization of the Isolates

Characterization of the isolates was carried out by employing macroscopic, microscopic, biochemical and physiological tests as follows:

3.8.4 Macroscopic Examination

The cultural characteristics of each of the isolates were observed. The appearance, colour, shape and size of each of the colonies was noted.

3.8.5 Microscopic Examination

3.8.6 Gram's staining:

A thin smear of each bacteria isolate was prepared and heat fixed. The slide was then flooded with crystal violet solution and left to react for 60 seconds, after which it was drained quickly and rinsed with tap water. Two drops of Gram's iodine was then added to act as a mordant. The solution was left for 60 seconds on the slide and washed off under tap water. 95% ethanol was used to wash the slide until it appeared free of the violet stain after which it was rinsed again with tap water to prevent excessive discolouration. The slide was then flooded with Safranin and left for 30 seconds. The slide was rinsed again with tap water and blot dry. The slide was examined with oil immersion under x100 objective lens objective to determine the Gram reaction and cellular characteristics (Willey *et al.*, 2008).

3.8.7 Biochemical Tests

3.8.8 Catalase test:

Catalase is an enzyme found in most bacteria. After the incubation of the isolates in their respective media for 18 hours, a sterile wire loop was used to pick young bacteria isolate from a colony and placed on a grease-free sterile slide. A drop of freshly prepared 3% hydrogen peroxide was added to each plate (Seeley and Van

Dermark, 1972). Evolution of frothy white gas indicated a catalase positive reaction, while absence of froth indicated negative reaction.

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

3.8.9 Characterization of *Enterobacteriaceae* using API 20E

After Gram staining of the isolates, the use of conventional procedures for further identification of members of the family *Enterobacteriaceae* and other Gram negative bacilli was employed.

The API 20E system is a standardized miniaturized version with plastic strips which hold twenty mini-test chambers containing dehydrated substrate having chemically defined composition for each test. The ONPG contained an ingredient that functions as an internal indicator. The ADH, LDC, ODC, and URE tubes contained phenol red as the indicator. The CIT, GLU, MAN, INO, SOR, RHA, SAC, MEL, AMY, and ARA tubes contained bromothymol blue as indicator. The GEL tube contained charcoal and the H_2S tube contained iron salts as indicators. The TDA, IND and VP contained no indicator. All the tubes contained buffers and all tubes, with the exception of the CIT; while URE tubes contained peptone.

The incubation box (tray and lid) was set up and distilled water dispensed into the honey-combed wells of the tray with squeeze bottle to provide a humid atmosphere. The strip was then placed in the tray and labeled at the elongated flap with specimen information. A sterile swab was then used to remove a single well-isolated colony from a young culture. The inoculum was thoroughly mixed with 5ml tube saline to obtain a homogeneous bacterium suspension. The suspension was immediately distributed into the micro tubes of the strip with a sterile Pasteur pipette. The tip of the pipette was placed against the side of the capsule after the strips had been slightly tilted forward. This was done to prevent bubbles at the base of the tubes. Both tube and capsule were filled for CIT, VP and GEL tests. For tests ADH, LDC, ODC, H_2S and URE, the micro tubes were over-laid with mineral oil to create anaerobiosis. The incubation tray was then covered with the lid and incubated at 37^0C for 24hours.

After incubation, the strips were read using the Reading Table and the reaction recorded. Reagents were later added to TDA, VP and IND tubes. TDA and VP reactions were immediate and recorded; while IND reaction was recorded after 10

minutes (appendix 7).

Identification of the isolates through interpretation of the result obtained was done by using numerical profile. On the result sheet, the tests were separated into 3 groups and a value of 1, 2 and 4 was indicated for each. A 7 digit profile number was obtained for the 20 tests of the API 20E strip. The Analytical Profile Index (5^{th} Edition) was used to interprete the 7- digit profile number and the strains identified.

3.8.10 Characterization of Lactic Acid Bacteria using API 50 CH Strips and Medium.

Lactic acid bacteria were initially differentiated based on colonial morphology, Gram staining, catalase reaction and spores staining. The ability of the isolates to ferment carbohydrates was studied using the API 50 CHL (Bio Merieux) system and it was used to differentiate the isolates into strains level.

The rapid identification of different strains of lactic acid bacteria isolated was done using the API 50 CH (Bio Merieux) which is a standardized system, associating 50 biochemical tests for the study of the carbohydrate metabolism by microorganisms. The kit contained 10 incubation boxes (tray and lid), 10 API 50 CH strips, 10 API 50 CHL medium, identification table and result sheets. API 50 CH was used in conjunction with API 50 CHL medium. One API 50 CH strip consisted of 50 micro tubes used to study fermentation of substrates belonging to the carbohydrate family; and its derivatives (heterosides, polyalcohols, uronic acids). The holes in the incubation boxes were filled with sterile distilled water to create a humid atmosphere, the incubation tray was put on it and the strips were placed on the trays by arranging them according to the numbers on them, starting from 0-9, 10-19, 20-39, 30-39, 40-49. Pure culture of the test organisms were cultured on MRS agar plates for 18 hours and harvested into 5ml sterile peptone water. The suspension prepared had a turbidity equivalent to 2 McFarland. It was then dispensed into the mediums for immediate use, and the suspension was dropped into the strip's microtubules; the strip was labeled accordingly and incubated. The fermentation tests were inoculated with API 50 CHL medium which rehydrated the substrates present in the strip's micro tubes. Anaerobiosis in the inoculated strips was obtained by overlaying with sterile paraffin oil and the strips were incubated at 30^0C and results read after 24 hrs to 48 hrs. A

change in colour of the indicator was recorded as positive, while tubes with the colour of the indicator were recorded negative. Identification was done using the identification chart for the kit (Zwadyw *et al.,* 1977).

3.8.11 Characterization of *Staphylococcus* Isolates

Staphylococcus species was isolated using Mannitol Salt Agar. The stock culture was preserved on nutrient agar slant and kept in the fridge at 4^0C. The stock culture was later withdrawn and sub cultured and fresh isolates obtained.

The tests employed in characterizing the young isolates included Gram reaction, motility test, production of catalase, coagulase, starch hydrolysis, utilization of glucose, sucrose, lactose, mannitol, maltose, xylose, fructose and galactose. Identification to the generic level was done using Bergey's manual of determinative bacteriology (Holt *et al.,* 2000) as a reference of identification based on the result of the various biochemical tests obtained.

3.8.12 Cultural and Morphological Characterization of Fungal Isolates

After 72 hours of incubation, potato dextrose agar plates were observed for growth. Microbial colony counts were taken for the identified growth. Colonies of each suspected fungal species were sub cultured in fresh potato dextrose agar plates. This was used to characterize the isolates based on the pigmentation of the spores, nature of the mycelia and spores formed.

Microscopic details were studied by performing a wet mount using lactophenol cotton blue mounting fluid. The preparation was examined under objective lens. The fungi were identified as detailed by Barnet and Hunter (1972), Rhode and Hartmann (1980), Kulwant *et al*. (1991) and Larone (2002).

3.9 Statistical Analysis

The statistical programme, SPSS, 2003 VERSION 16.0 was used to analyze the result of the treatments. T-test was used to determine whether there was a difference in quality parameters of the two frozen fish species examined. Analysis of variance (ANOVA)/Duncan multiple range test (DMRT), correlation and regression analysis were also carried out on the result of the frozen fish samples across zones.

CHAPTER FOUR

4.0 RESULTS

4.1 Frozen Fish Distribution and Management

The structure of imported frozen fish handling and management in Oyo state as observed from the study is presented tracing the pattern of distribution from the firm to the consumer.

From the study, three types of intermediaries were identified in the frozen fish distribution chain. They included wholesalers, agents and retailers. However, this research work zeroed on the wholesalers and retailers because they were the main players in the handling and management of imported frozen fish in the state.

Ibadan/Ibarapa zone had the highest number of cold stores (39) representing 58.2% out of a total number of 67 and the largest capacity of 7,433 tonnes, while the least was Saki zone (5) with capacity of 61 tonnes as shown in Table 12.

4.1.1 Gender and Age Distribution of Wholesalers and Retailers

Out of the 67 wholesalers interviewed, there were more men (83.6%) in the frozen fish distribution business than women (16.4%). However, out of the 150 retailers interviewed across the zones, none was male (Table 13). On age distribution, 19.4% of wholesalers were above 60 years, 51-60years (25.4%), 41-50years (34.3%), 31-40years (11.9%) and 9.0% fell within 21-30years. Only 3.3% of retailers were older than 60years, 51-60years (12.0%), 41-50years (24.0%), 31-40years (38.7%) and 22.0% were within 21-30 years (Table 14).

Table 12: Distribution and Capacity of Cold Stores in Oyo State

Zone	Number	%	Capacity (tonnes)	%
Ibadan/Ibarapa	39	58.2	7,433	87.03
Ogbomoso	16	23.9	973	11.39
Oyo	7	10.4	74	0.87
Saki	5	7.5	61	0.71
Total	**67**	**100.0**	**8,541**	**100.0**

Source: Field data (2011)

Table 13: Gender Distribution of Wholesalers and Retailers across Zones

Zone/Gender	Male		Female	
	Wholesaler	**Retailer**	**Wholesaler**	**Retailer**
Ibadan/Ibarapa	31	0	8	97
Ogbomoso	15	0	1	29
Oyo	5	0	2	15
Saki	5	0	0	4
Total (%)	**56 (83.6)**	**(0.0)**	**11 (16.4)**	**150 (100.0)**

Field data (2011)

Table 14: Age Distribution of Wholesalers and Retailers

Age (Years)	Wholesaler (%)	Retailer (%)
Above 60	13 (19.4)	5 (3.3)
51-60	17 (25.4)	18 (12.0)
41-50	23 (34.3)	36 (24.0)
31-40	8 (11.9)	58 (38.7)
21-30	6 (9.0)	33 (22.0)
Total (%)	**67 (100.0)**	**150 (100.0)**

Field data (2011)

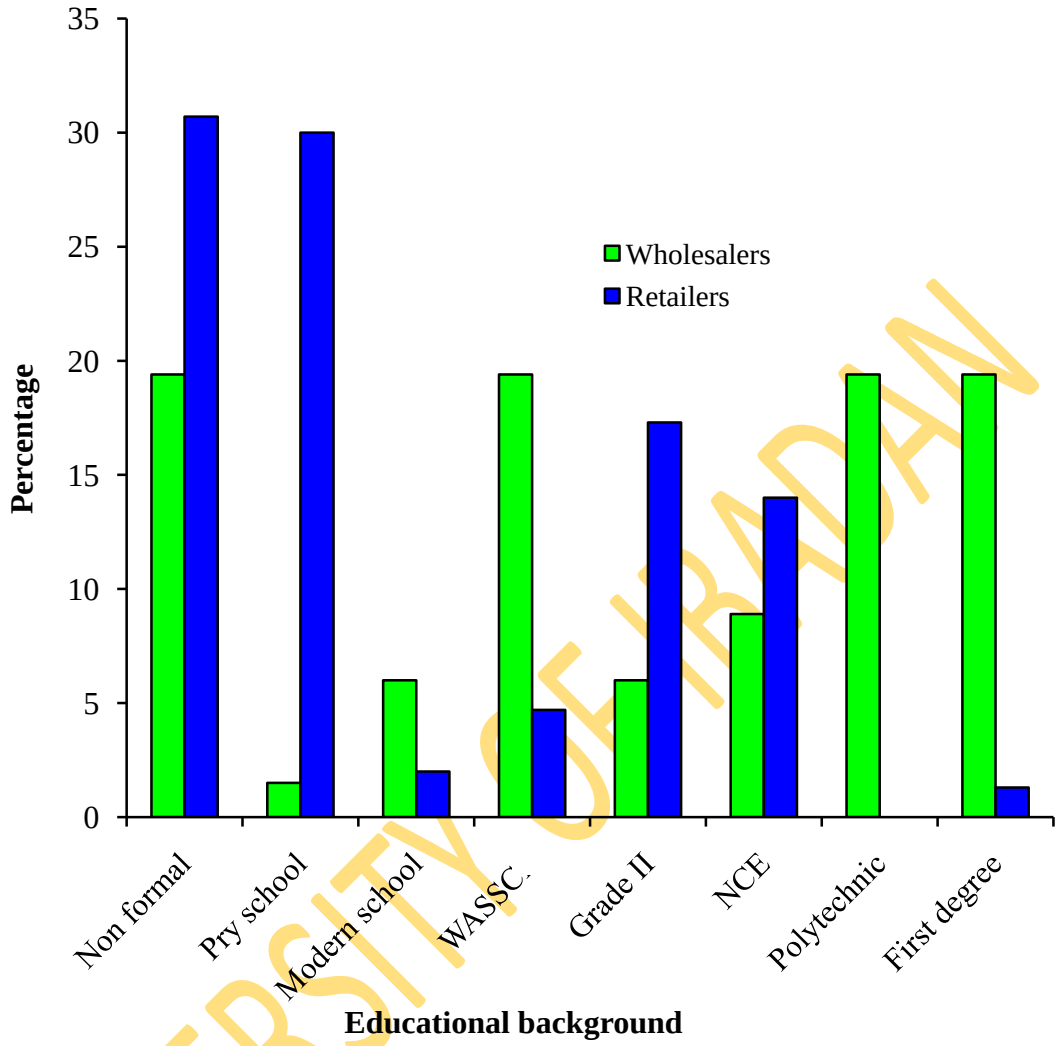

Figure 6: Educational level of Wholesalers and Retailers

Table 15: Sources of Fish to Wholesalers across Zones

	Zone				Total	Percentage
	Ibadan/Ibarapa	**Ogbomoso**	**Oyo**	**Saki**		
Lagos	35	15	7	4	61	91.0
Port Harcourt	4	1	0	1	6	9.0
Total	**39**	**16**	**7**	**5**	**67**	**100.0**

Source: Field data (2011)

Table 16: Means of Fish Transportation by Wholesalers across Zones

	Zone				Total	Percentage
	Ibadan/Ibarapa	**Ogbomoso**	**Oyo**	**Saki**		
Refrigerated truck	36	14	7	3	60	**89.6**
Taxi cab	3	2	0	0	5	**7.5**
Bus	0	0	0	2	2	**3.0**
Total	**39**	**16**	**7**	**5**	**67**	**100.0**

Source: Field data (2011)

1.2 Level of Education of Respondents

Educational level attained by respondents as shown in Figure 6 indicates that 30.7% of the retailers had no formal education, 30.0% had primary education, 2.0% with modern school education and 14.0% with National Certificate of Education (NCE). A total of 69.3% of the retailers had received western education. However, 80.6% of the wholesalers had western education including 19.4% with first degree.

4.1.3 Transportation and Physical facilities

Majority of the fish stock distributed in Oyo state came from Lagos (91.0%), while 9.0% got their stock from Port Harcourt (Table 15). Refrigerated truck (89.6%) was most commonly used by wholesalers to transport frozen fish round the state (Table 16); while other means were taxi cab (7.5%) and bus (3.0%). Motorcycle (43.3%) remained the major means of transporting frozen fish from cold stores by retailers. Others were taxi cab (31.3%) and head load (24.0%) as shown on Table 17.

On Table 18, majority of the retailers sold fish at fish market stalls (51.3%), roadside (20.7%), hawking (23.3%), while 4.7% supplied customers and agencies like hotels and institutions. More than sixty two percent of the retailers attracted customers by calling the attention of prospective buyers, 23.3% by conspicuous display and 4.7% through their recognition as fish sellers, while 9.3% sold by granting credit to their customers (Table 19). Handling facilities for the display of fish by the retailers included bowls (50.0%), wooden tables (36.7%), wooden boards (10.0%) and others 3.3% (Table 20).

Most of the fish traders kept the fish stall clean individually (71.3%), 21.3% cleaned collectively, 6.0% used hired labour, while local government officials (1.3%) were sometimes involved in cleaning (Table 21). More than fifty seven percent of the retailers cleaned their table/slab daily, 25.3% twice a week, 15.3% thrice weekly, while 2.0% cleaned their tables once per week (Table 22). It was observed that none of the retailers used chlorinated water in washing their tables/slabs.

Compliance to facilities' sanitation by wholesalers was 92.5% satisfactory (Table 23), while compliance to personal hygiene by cold store workers was 83.6% (Table 24).

Table 17: Means of Transportation of fish to market by Retailers

	Zone				Total	Percentage
	Ibadan/Ibarapa	**Ogbomoso**	**Oyo**	**Saki**		
Taxi cab	24	15	8	0	47	**31.3**
Motorcycle	59	1	3	2	65	**43.3**
Head load	17	13	4	2	36	**24.0**
No response	2	0	0	0	2	**1.3**
Total	**102**	**29**	**15**	**4**	**150**	**100.0**

Source: Field data (2011)

Table 18: Methods of Selling fish by Retailers across Zones

	Zone				Total	Percentage
	Ibadan/Ibarapa	**Ogbomoso**	**Oyo**	**Saki**		
Supplies to people and agencies	3	2	2	0	7	**4.7**
Public hawking	16	10	7	2	35	**23.3**
Roadside sales	30	0	1	0	31	**20.7**
Fish market stalls	53	17	5	2	77	**51.3**
Total	**102**	**29**	**15**	**4**	**150**	**100.0**

Source: Field data (2011)

Table 19: Methods of Attracting Customers by Retailers

	Zone				Total	Percentage
	Ibadan/Ibarapa	**Ogbomoso**	**Oyo**	**Saki**		
Conspicuous display	25	11	4	0	35	**23.3**
Granting of credit/discount	8	3	3	0	14	**9.3**
Calling attention of prospective customers	69	15	8	2	94	**62.7**
Recognition as fish seller	5	0	0	2	7	**4.7**
Total	**102**	**29**	**15**	**4**	**150**	**100.0**

Source: Field data (2011)

Table 20: Methods of Displaying Fish by Retailers

	Zone				Total	Percentage
	Ibadan/Ibarapa	**Ogbomoso**	**Oyo**	**Saki**		
Wooden table	39	9	5	2	55	**36.7**
Bowls	56	15	2	2	75	**50.0**
Wooden board	8	0	8	0	15	**10.0**
Others	0	5	0	0	5	**3.3**
Total	**102**	**29**	**15**	**4**	**150**	**100.0**

Source: Field data (2011)

Table 21: Methods of Keeping the Fish Stalls clean by Retailers

	Zone				Total	Percentage
	Ibadan/Ibarapa	**Ogbomoso**	**Oyo**	**Saki**		
Retailers Collectively	27	3	2	0	32	**21.3**
Hired Labour	0	5	4	0	9	**6.0**
Individual Retailer	75	20	8	4	107	**71.3**
Local Government	0	1	1	0	2	**1.3**
Total	**102**	**29**	**15**	**4**	**150**	**100.0**

Source: Field data (2011)

Table 22: Frequency of Washing of Display facilities by Retailers

	Zone				Total	Percentage
	Ibadan/Ibarapa	**Ogbomoso**	**Oyo**	**Saki**		
Daily	69	10	5	2	86	57.3
Once a week	0	1	2	0	3	2.0
Twice a week	20	18	0	0	38	25.3
Thrice a week	13	0	8	2	23	15.3
Total	**102**	**29**	**15**	**4**	**150**	**100.0**

Source: Field data (2011)

Table 23: Compliance to Facilities' Sanitation by Wholesalers across Zones

	Zone				Total	Percentage
	Ibadan/Ibarapa	**Ogbomoso**	**Oyo**	**Saki**		
Satisfactory	35	16	7	4	62	**92.5**
Not Satisfactory	4	0	0	1	5	**7.5**
Total	**39**	**16**	**7**	**5**	**67**	**100.0**

Source: Field data (2011)

Table 24: Compliance to Personal Hygiene by Cold store Workers

	Zone				Total	Percentage
	Ibadan/Ibarapa	**Ogbomoso**	**Oyo**	**Saki**		
Satisfactory	36	12	5	3	56	**83.6**
Not Satisfactory	3	4	2	2	11	**11.0**
Total	**39**	**16**	**7**	**5**	**67**	**100.0**

Source: Field data (2011)

4.1.4 Pricing Policy

Qualities that influenced price by wholesalers as shown in Table 25 were species (46.3%), size (43.3%) and taste (10.4%). Daily price of fish was determined mainly by cost of purchase (91.0%) while demand (3.0%), supply (1.5%) and processing costs (1.5%) followed in that order (Table 26). In retailing, price of fish was determined mainly by cost of purchase (88.7%), demand (4.7%), supply (4.0%) and 2.7% by the cost of transportation (Table 27).

Source of information to fellow trader on change in price is mainly mobile phone (53.7%) by wholesalers while fellow traders (78.0%) are the most common source for retailers (Fig. 7). As shown in Table 28, 52.2% wholesalers saved money in the bank against 23.3% retailers.

4.1.5 Group Activities

Figure 8 shows that percentage of wholesalers in trade association was highest in Ogbomoso zone, while Saki zone had no Trade association.Altogether, 86.0% of retailers belonged to Trade associations. The reason for joining the association by retailers as shown in Table 29 included general welfare of members (88.0%), members' access to help in time of difficulty (9.3%) and other reasons (2.7%).

4.1.6 Constraints to Frozen Fish Distribution and Management

Problems encountered by wholesalers in fish distribution, handling and management as shown in Figure 9 included power outage (electricity), scarcity of fish, debt by customers, transportation cost, fuel cost and shortage of technical personnel. Ninety seven percent across the zones identified electricity as a problem in fish business.

Eighty two percent of the wholesalers did not experience fish scarcity. Most of the wholesalers sold on credit to retailers; therefore the problem of debt recovery from retailers was 65.0%, while fuel cost was 87.0%. More than eighty percent identified fuel cost as a problem because the fuel used to power generating sets increased the bill of maintaining cold store facilities. The problem of technical personnel handling the repairs of facilities was 17.4%, while 47.8% of the wholesalers identified transportation as a problem.

Table 25: Qualities that Influenced Fish Pricing by Wholesalers

	Zone				Total	Percentage
	Ibadan/Ibarapa	Ogbomoso	Oyo	Saki		
Size	16	7	3	3	29	**43.3**
Taste	4	3	0	0	7	**10.4**
Species	19	6	4	2	31	**46.3**
Total	**39**	**16**	**7**	**5**	**67**	**100.0**

Source: Field data (2011)

Table 26: Determinants of Fish Pricing by Wholesalers in a Day

	Zone				Total	Percentage
	Ibadan/Ibarapa	**Ogbomoso**	**Oyo**	**Saki**		
Supply	1	0	0	0	1	**1.5**
Demand	1	1	0	0	2	**3.0**
Cost of purchase	35	14	7	5	61	**91.0**
Processing cost	0	1	0	0	1	**1.5**
No response	2	0	0	0	2	**3.0**
Total	**39**	**16**	**7**	**5**	**67**	**100.0**

Source: Field data (2011)

Table 27: Factors that Determined Price of fish by Retailers

	Zone				Total	Percentage
	Ibadan/Ibarapa	**Ogbomoso**	**Oyo**	**Saki**		
Supply	4	2	0	0	6	**4.0**
Demand	1	5	1	0	7	**4.7**
Cost of purchase	95	22	12	4	133	**88.7**
Transport cost	2	0	2	0	4	**2.7**
Total	**102**	**29**	**15**	**4**	**150**	**100.0**

Source: Field data (2011)

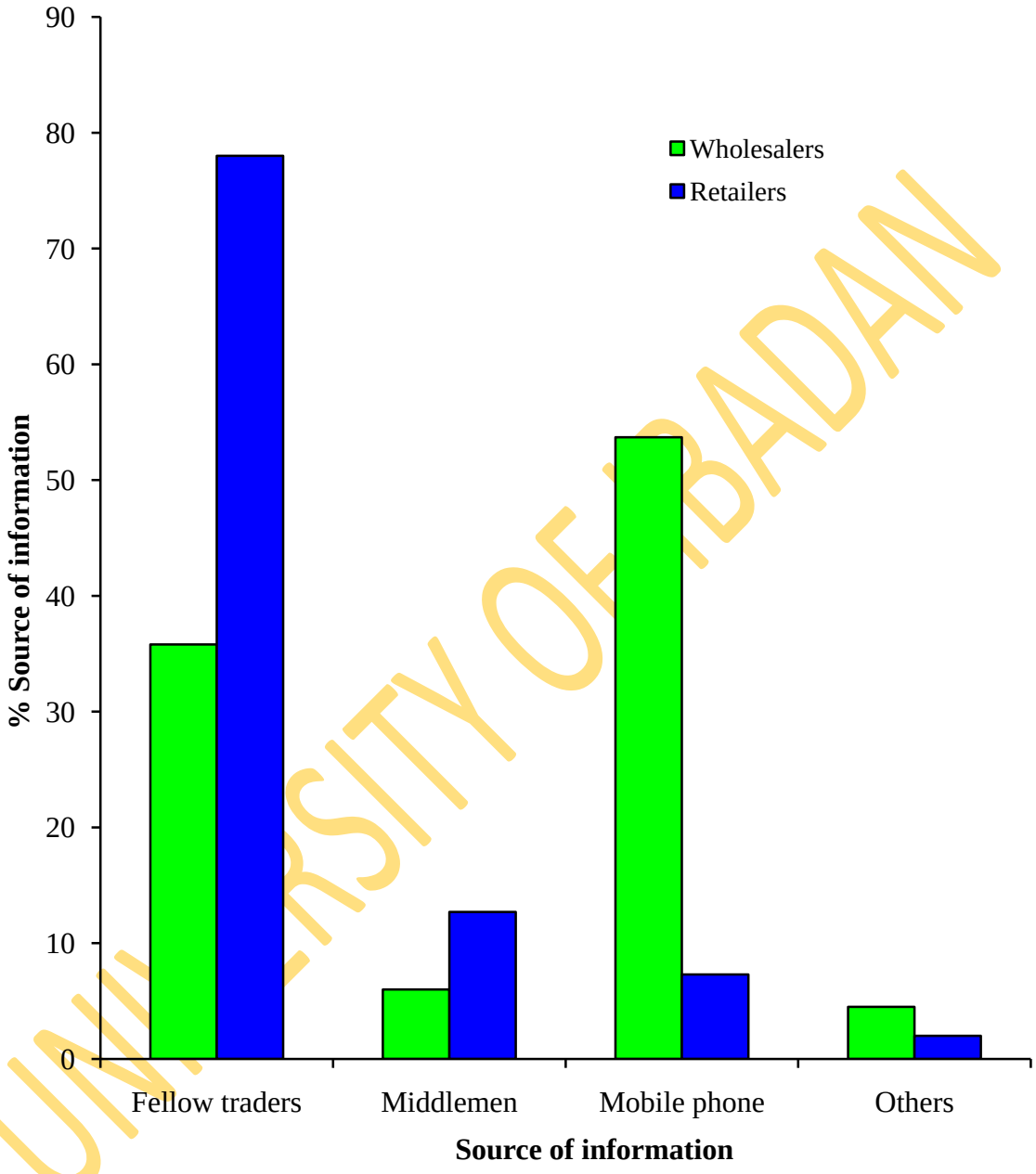

Figure 7: Source of Information to Wholesalers and Retailers on Price change

Table 28: Distribution of Wholesalers and Retailers that saved money with bank

Zone	Wholesaler	Retailer
Ibadan/Ibarapa	22	13
Ogbomoso	11	17
Oyo	2	5
Saki	0	0
Total (%)	**35 (52.2)**	**35 (23.3)**

Field data (2011)

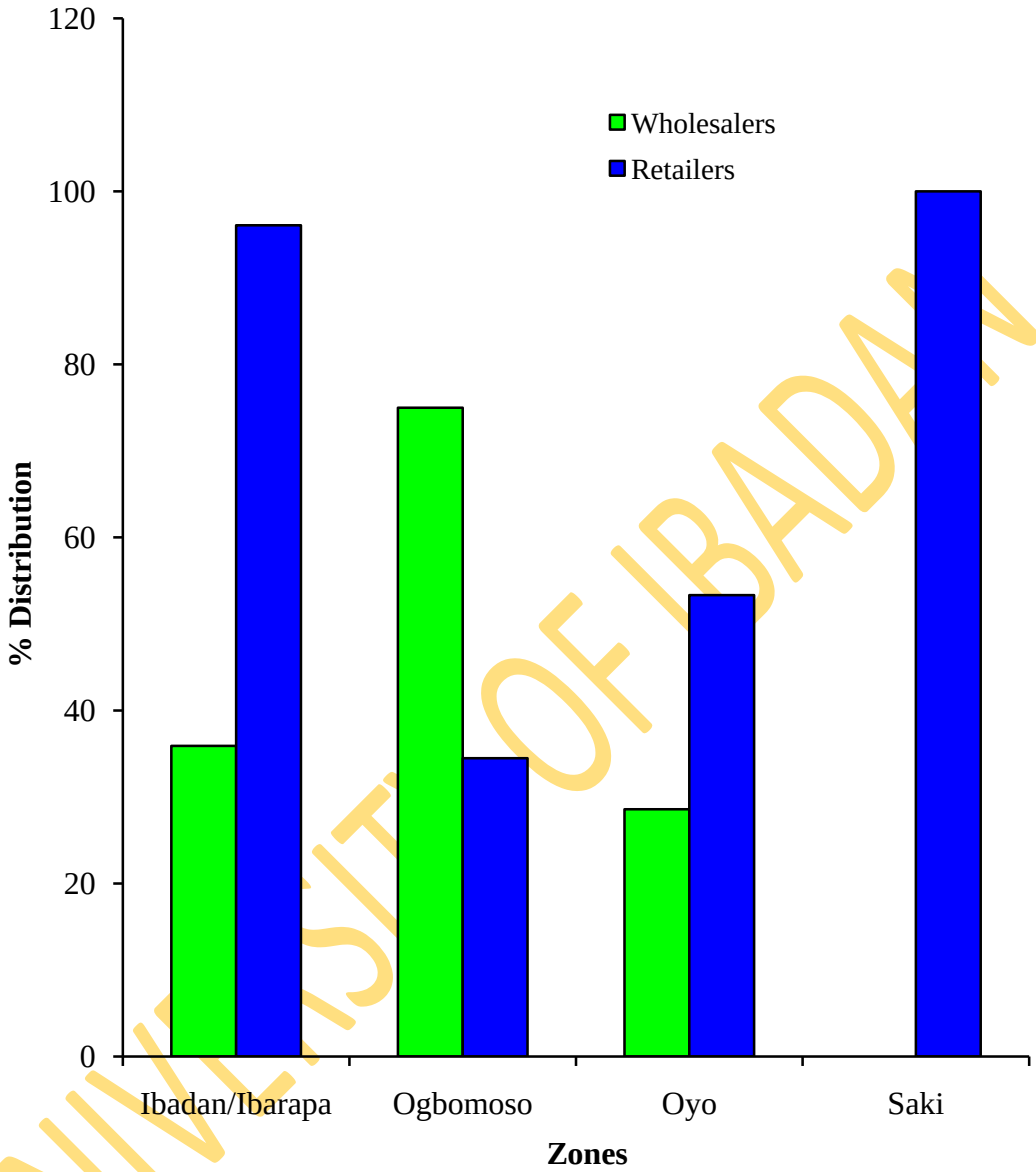

Figure 8: Distribution of Wholesalers and Retailers in Trade Association

Table 29: Reason for joining Trade Association by Retailers

	Zone				Total	Percentage
	Ibadan/Ibarapa	**Ogbomoso**	**Oyo**	**Saki**		
Access to Help in difficulty	5	8	1	0	14	**9.3**
General welfare	95	21	12	4	132	**88.0**
Others	2	0	2	0	4	**2.7**
Total	**102**	**29**	**15**	**4**	**150**	**100.0**

Source: Field data (2011)

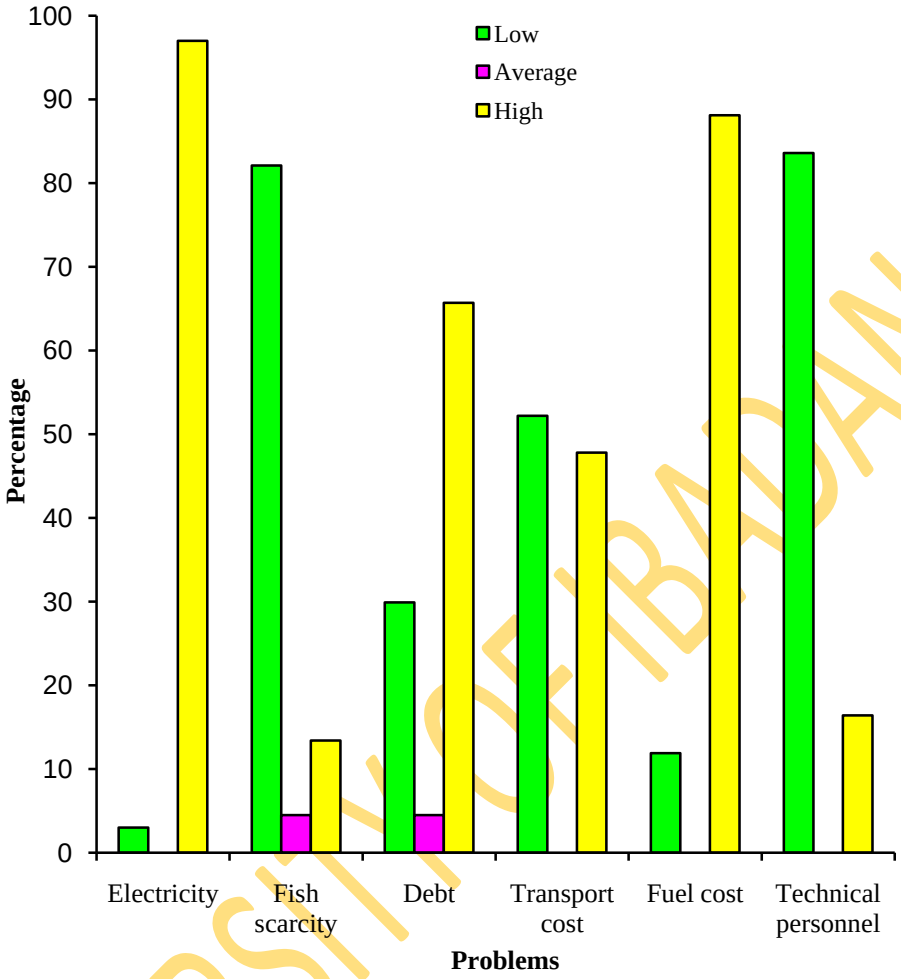

Figure 9: Problems encountered by Wholesalers of Imported Frozen Fish in Oyo state

Plate 3: A refrigerated truck loaded with frozen fish ready to be off loaded into a cold store.

Plate 4: Activities at a local cold store.

Plate 5: Frozen fish being mounted on a motorcycle by a Retailer

Plate 6: Frozen fish being loaded into a taxi cab by a Retailer

4.2 Analysis of the Quality Parameters

In order to achieve the aim of this study, Oyo state was divided into four zones namely Ibadan/Ibarapa, Ogbomoso, Oyo and Saki according to Agricultural Development Programme (ADP) geographical distribution. For easy access to the research zones and major towns alike, random samples were selected for the two species of fish over a period of twelve months with the relevant chemical and microbial assessment carried out, including organoleptic assessment.

4.2.1 Reason for Choice of Imported Frozen Fish for this Research Work

The most common imported frozen fish sold in Oyo state markets included *Sardinella* species, *Micromesistius poutasssou*, *Merluccius capensis*, *Scomber japonicus*, *Scomber scombrus*, and others. The consumer preference for these wide range of frozen fish varied; and as shown in Fig. 10, *Sardinella* species was most preferred (52%) followed by *Scomber japonicus* (17%), *Micromesistius poutasssou* (16%) and *Scomber scombrus* (15%) in that order.

Research showed that *Scomber japonicus* is highly priced and out of reach of the common man, while *Scomber scombrus* (horse mackerel) is seasonal. The area of concentration of this research therefore was for the low priced fish: *Sardinella* species and *Micromesistius poutasssou*. The fish species were available all year round, widely spread across the zones, affordable and consumed by a large percentage of the populace in the state.

4.3 Sensory Evaluation of the Samples

The mean hedonic scores obtained for the taste, odour, texture, appearance, colour and overall acceptability of the two fish samples are presented in Fig. 11. There were no significant differences ($p>0.05$) between the mean scores of the two samples across zones. The values were within tolerable limits of 4.0 with a range of 5.47±0.20 - 5.90±0.08 recorded for *Sardinella* spp. and 5.57±0.13 - 5.80±0.10 for *M. poutassou* respectively.

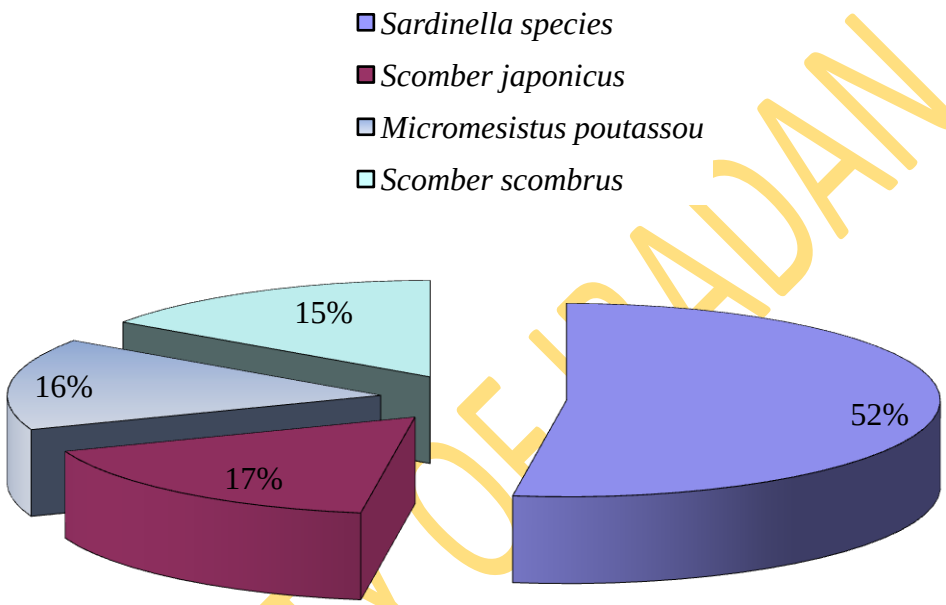

Figure 10: Pie chart showing consumer preference for common frozen fish in Oyo State

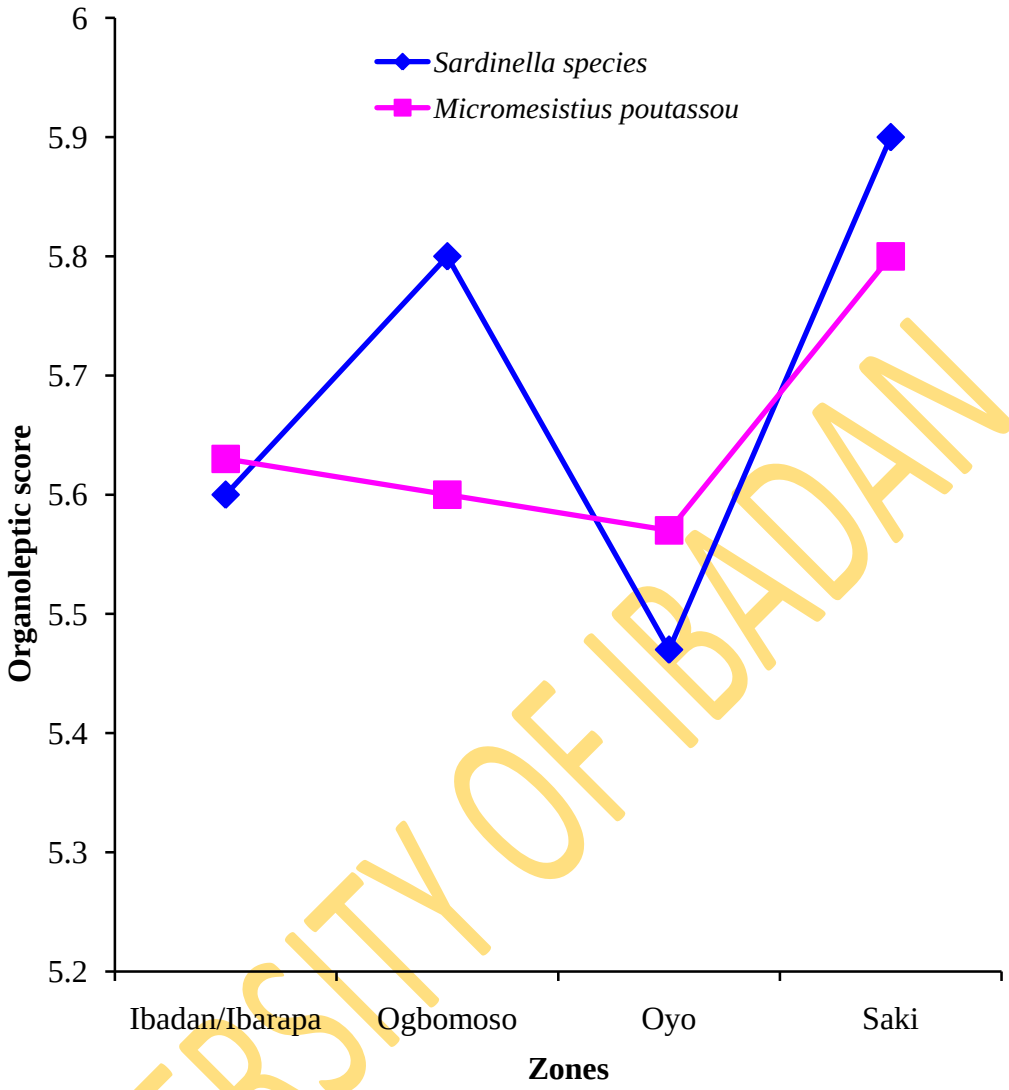

Figure 11: Mean Organoleptic score of imported frozen fish samples across zones

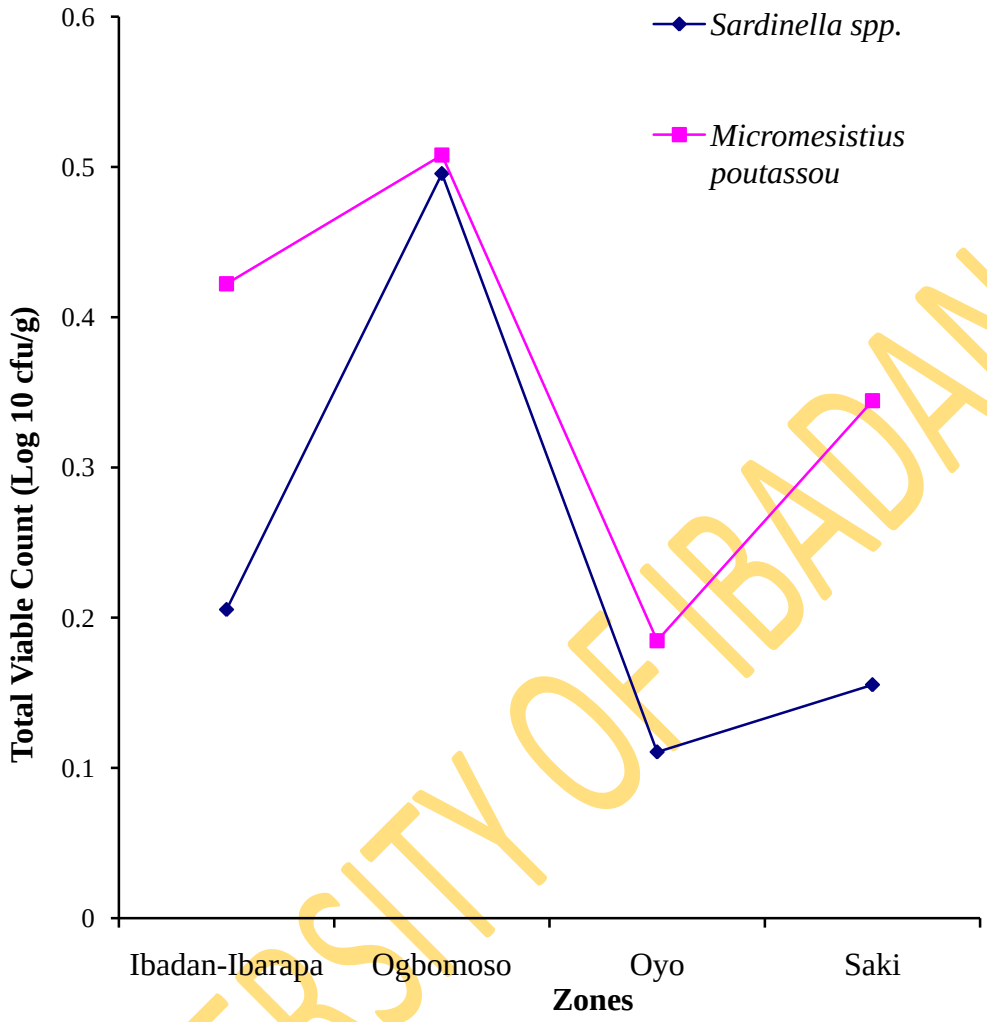

Figure 12: Total Viable Count of imported frozen fish collected from different zones

4.4 Microbiological Evaluation of Samples

The Total Bacteria Count of imported frozen fish across the four zones of the study area as shown in Fig. 12 indicates that Total Viable Count (TVC) in *Sardinella* species was highest in Ogbomoso zone (3.1 $x10^5$ cfu/g), while the lowest was Oyo zone (1.2 $x10^5$cfu/g). In *M. poutasssou*, the highest TVC was also in Ogbomoso zone (3.2 x 10^5cfu/g) and the lowest in Oyo zone (1.5 $x10^5$cfu/g) (Table 34).

As indicated in Fig. 13, the highest Total *Salmonella-Shigella* Count (TSSC) in *Sardinella* species was recorded in Ogbomoso zone (6.9 $x10^3$cfu/g) and the least was Oyo zone (4.1 $x10^3$cfu/g). The highest TSSC in *M. poutasssou* was recorded in Saki zone (1.0 $x10^4$cfu/g) and the least was Oyo zone with (4.9 $x10^3$cfu/g).

Total Haemolytic Streptococci Count (THSC) in *Sardinella* species was highest in Oyo zone (3.5 $x10^4$cfu/g) and the least was in Saki zone (2.8 $x10^4$cfu/g). Ogbomoso zone (3.5 $x10^4$cfu/g) recorded the highest in *M. poutasssou*, while Ibadan/Ibarapa recorded the least value of 3.1$x10^4$cfu/g (Fig. 14).

Total Lactic Acid Bacteria Count (TLAB) in *Sardinella* species as shown in Fig. 15 was lower in Ibadan/Ibarapa, Ogbomoso and Saki zones than in *M. poutasssou*. Oyo zone was the only zone that recorded a higher TLAB count of 6.8 x 10^3cfu/g in *Sardinella* spp. than *M. poutasssou* with 3.1 $x10^3$cfu/g.

In the same vein, Fig. 16 showed that *M. poutasssou* had higher total *Enterobacteriaceae* Count **(**TEBC**)** in all zones compared to *Sardinella* species. The highest TEBC was recorded in Ogbomoso zone for both *M. poutasssou* (4.1 $x10^4$cfu/g) and *Sardinella* species (3.1 $x10^4$ cfu/g) while the least was 2.3 x 10^4cfu/g in Ibadan/Ibarapa zone and 1.2x $x10^4$cfu/g in Oyo zone respectively.

Highest Total *Staphylococcus* Count (TSC) for *Sardinella* species was recorded in Oyo zone (3.0 $x10^3$cfu/g), while the least was in Saki zone (1.0x $x10^4$cfu/g). *M. poutasssou* recorded highest count of 6.6 $x10^3$cfu/g in Ibadan/Ibarapa zone and the least count of 1.0 x 10^3cfu/g in Oyo zone (Fig. 17).

However, *M. poutasssou* had higher *Staphylococcus* count than *Sardinella* species in Ibadan/Ibarapa, Ogbomoso and Saki zones, while the case was different in Oyo zone where *Sardinella* species had higher counts than *M. poutasssou* (Fig. 17).

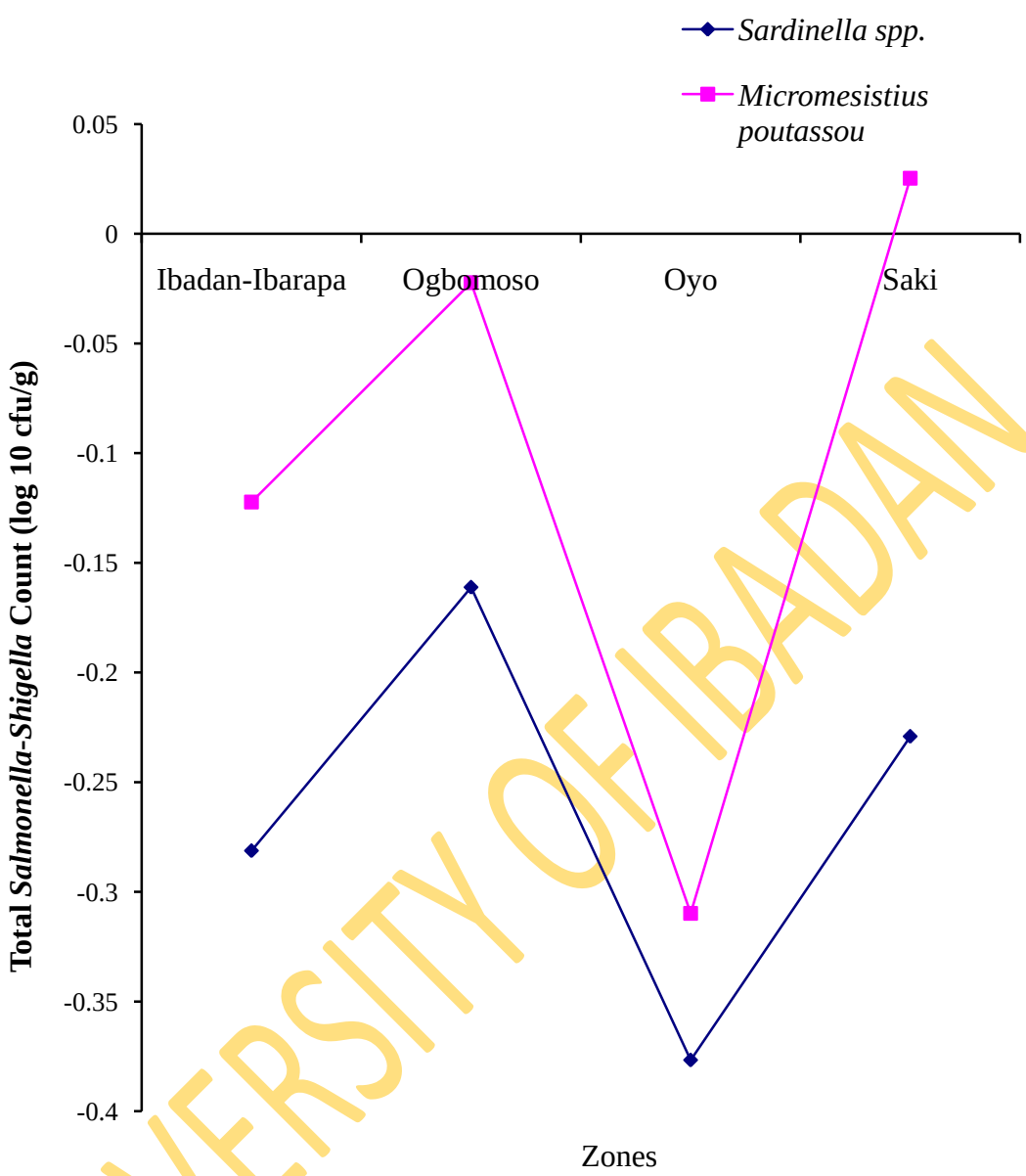

Figure 13: Total *Salmonella-Shigella* Count of imported frozen fish collected from different zones

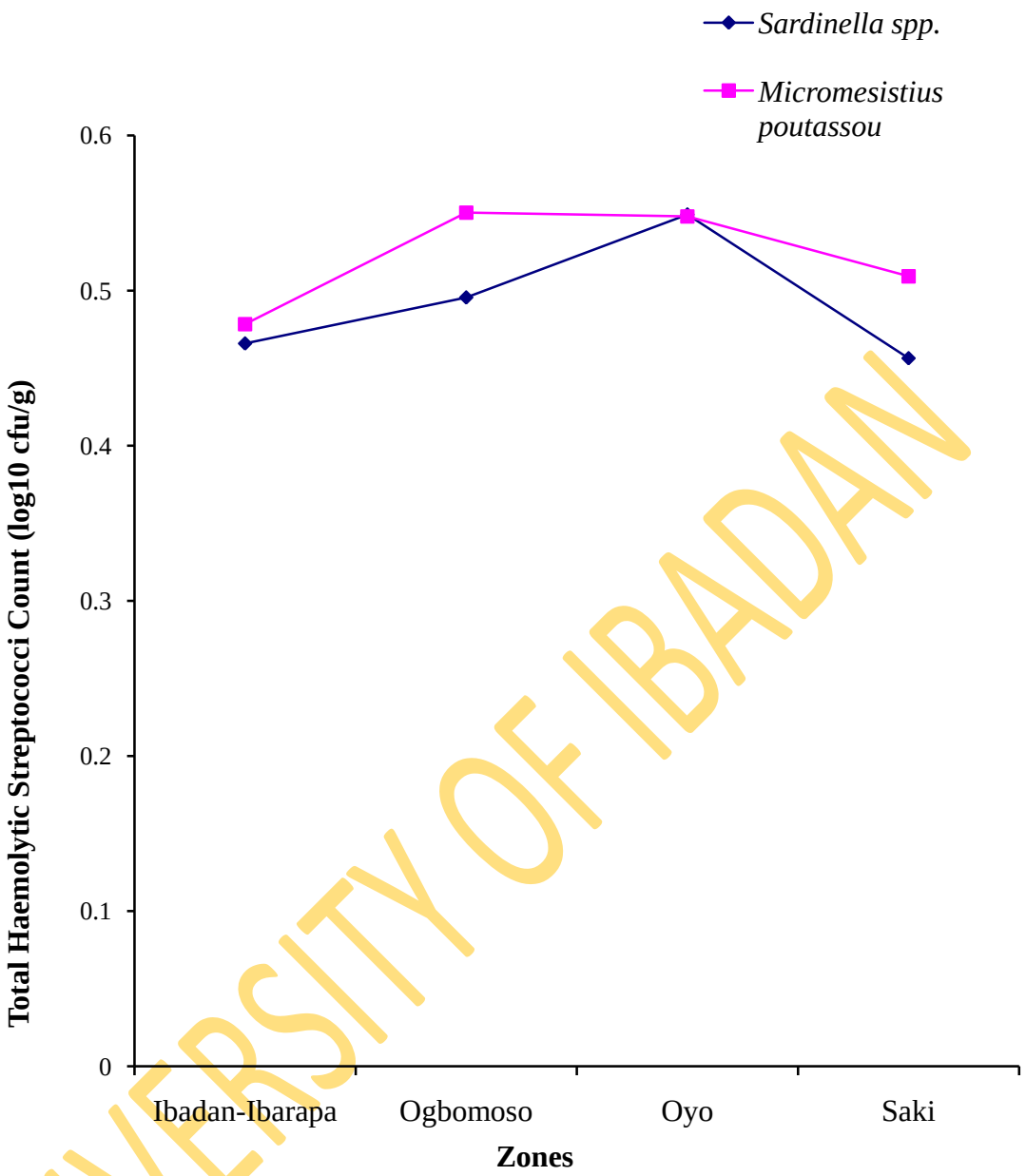

Figure 14: Total Haemolytic Streptococci Count of imported frozen fish collected from different zones

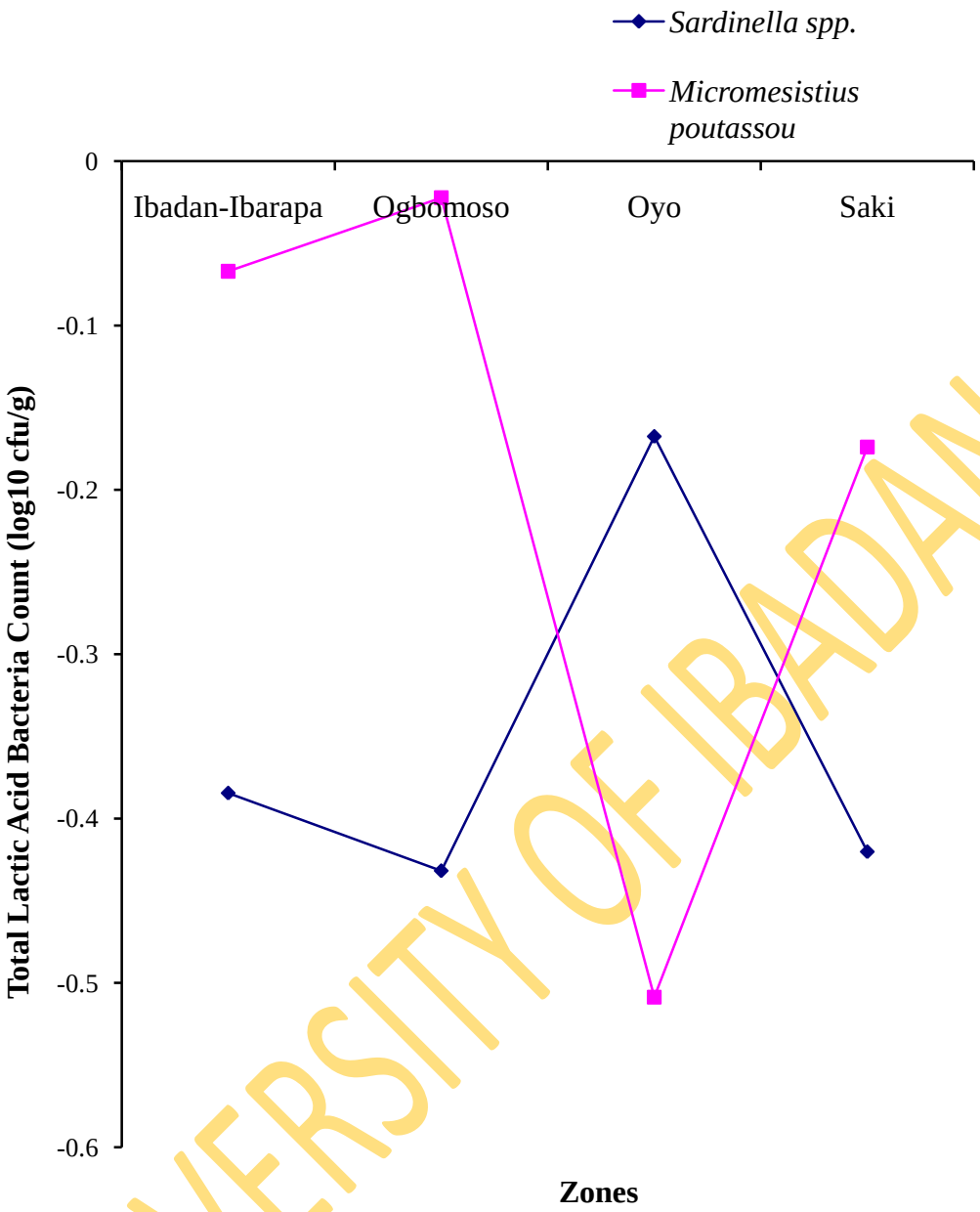

Figure 15: Total Lactic Acid Bacteria Count of imported frozen fish collected from different zones

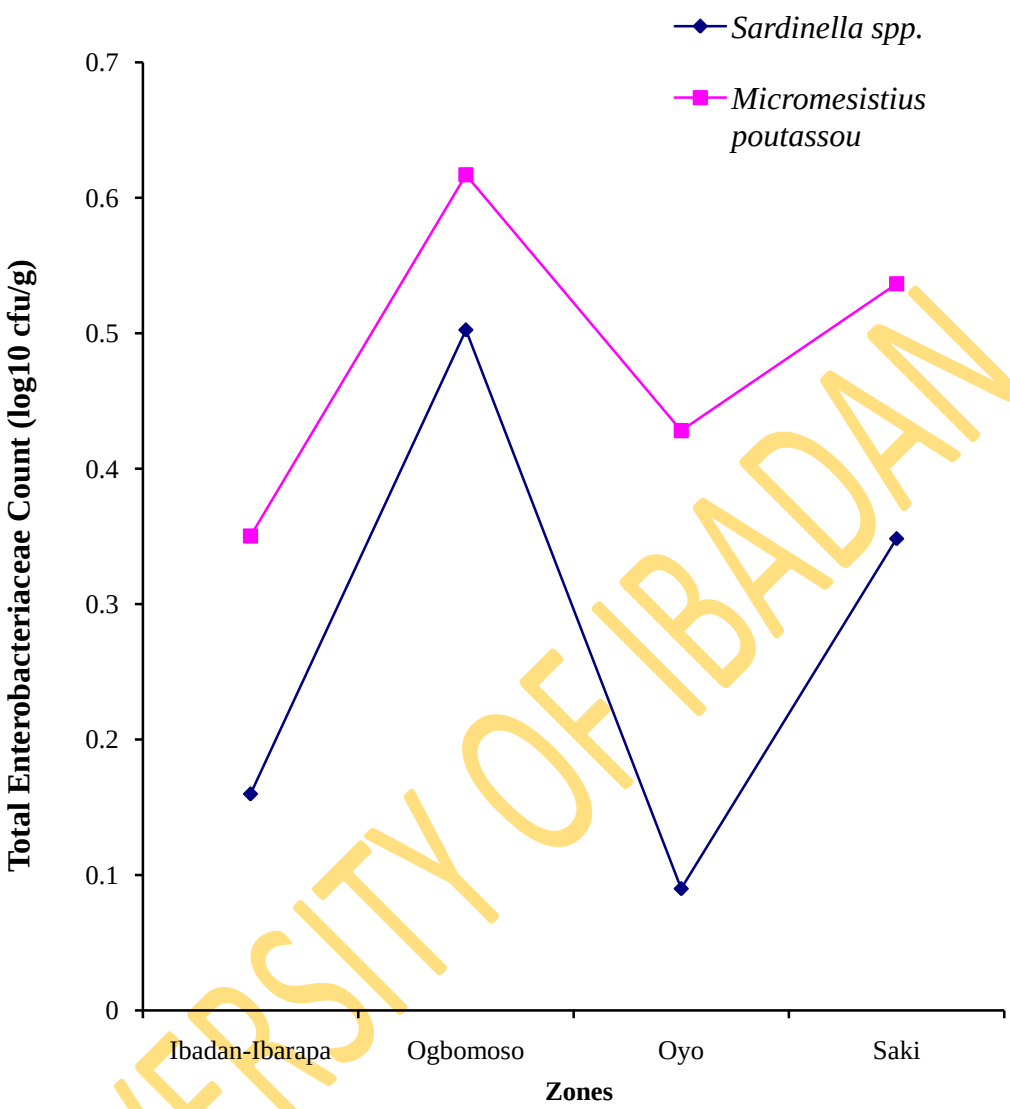

Figure 16: Total Enterobacteriaceae Count of imported frozen fish collected from different zones

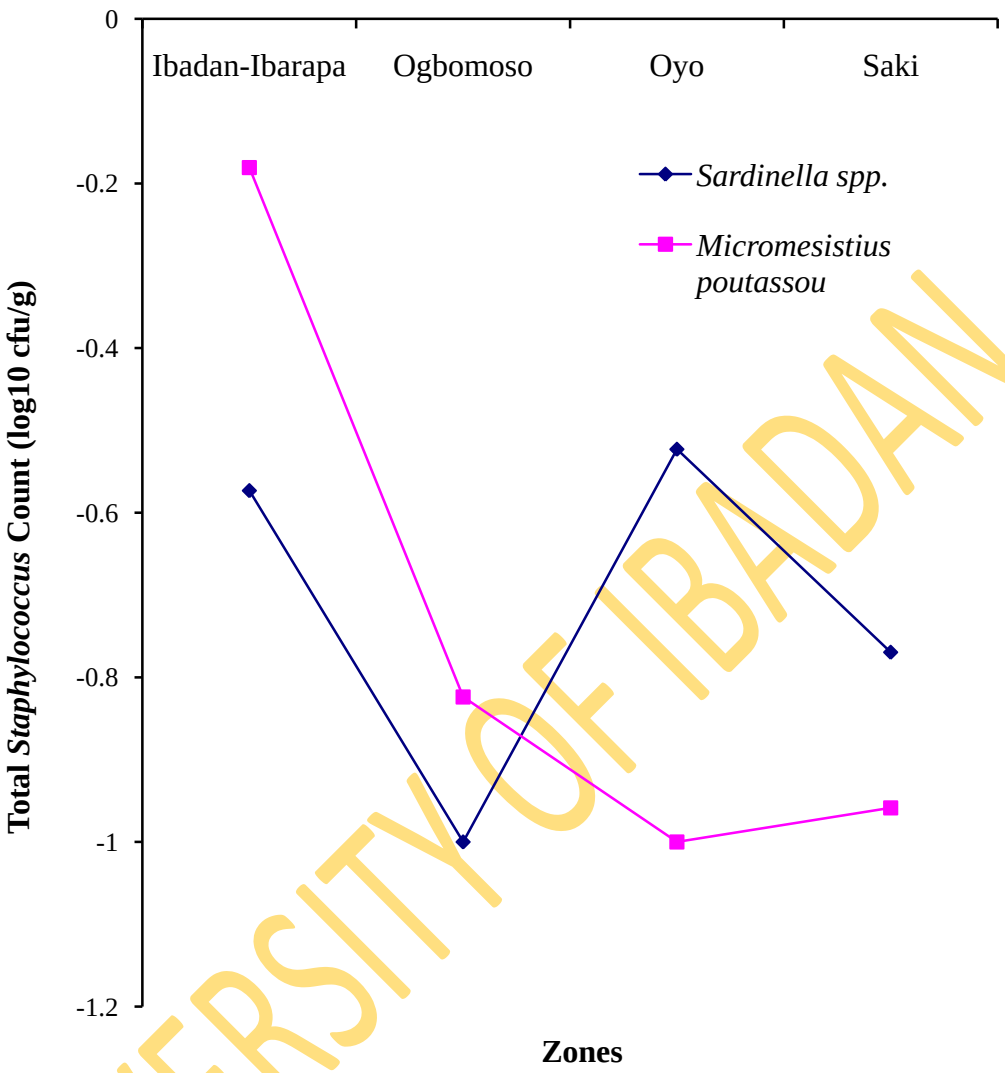

Figure 17: Total *Staphylococcus* Count of imported frozen fish collected from different zones

The frequency of occurrence of bacteria isolated in *Sardinella* species showed that *Streptococcus faecium* was highest with 16.8% followed by *Shewanella putrefaciens* 16.1% and *Salmonella typhi* (14.8%), while the least bacteria count of 1.9% was recorded in *Leuconostoc mesenteroides* (Fig. 18).

For *M. poutasssou*, *Shewanella putrefaciens* also recorded the highest value of 16.5% followed by *Salmonella typhi*, 15.9% and *Streptococcus faecium* 14.1% while the least value of 2.9% was recorded in *Pediococcus damnosus* (Fig. 19).

All the 10 bacteria species recorded in this study showed their occurrences in both *Sardinella* species and *M. poutasssou* with *Shewanella putrefaciens*, *Streptococcus faecium* and *Salmonella typhi* showing higher frequencies of occurrence in both fish species.

Colonially, the size of the test isolates ranged from small to medium; and their forms ranged from circular to irregular shapes. They had creamy, brownish to pinkish colouration with entire edges. Opacity ranged from translucent to opaque. They had raised elevation and consistency varied from friable, butryous to viscoid. The bacterial population of the frozen fish samples consisted of both Gram negative and Gram positive rods and cocci. The Gram negative bacterial isolates were members of the family *Enterobacteriaceae*: catalase and oxidase negative rods, producing acid aerobically and anaerobically form glucose in Hugh-Leifson's medium (Hugh and Leifson, 1953). The representative organisms were identified as *Salmonella typhi*, *Shewanella putrefaciens*, *Enterobacter asburiae*, *Pseudomonas aeruginosa* and *Proteus vulgaris* (Table 30).

Gram positive isolates were catalase and oxidase negative, non-endospore forming fermentative organisms recognized as members of the lactic acid bacteria group. They included *Lactobacillus acidophilus*, *Leuconostoc mesenteroides*, *Pediococcus damnosus* and *Streptococcus faecium* (Table 31).

The *Staphylococcus aureus*, also isolated from the samples was found to be coagulase and catalase positive and did not ferment lactose, glucose and fructose, but were found to ferment only sucrose. The strains were also mannitol-positive, but found to be indole and motility negative gram-positive cocci bacteria (Table 32).

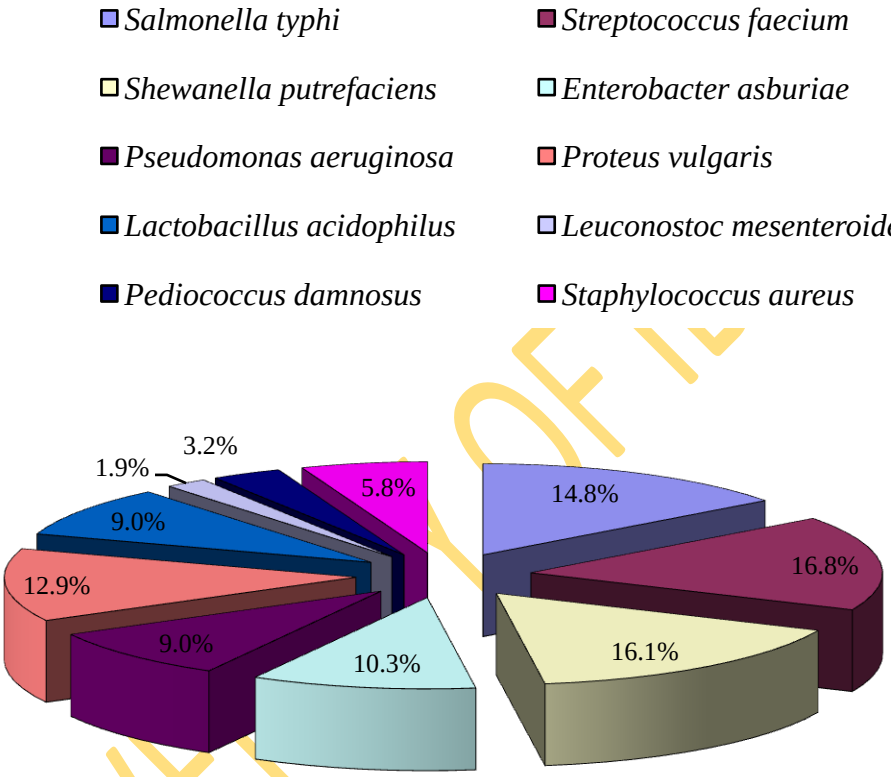

Figure 18: Frequency of occurrence of Bacteria isolated from *Sardinella* species

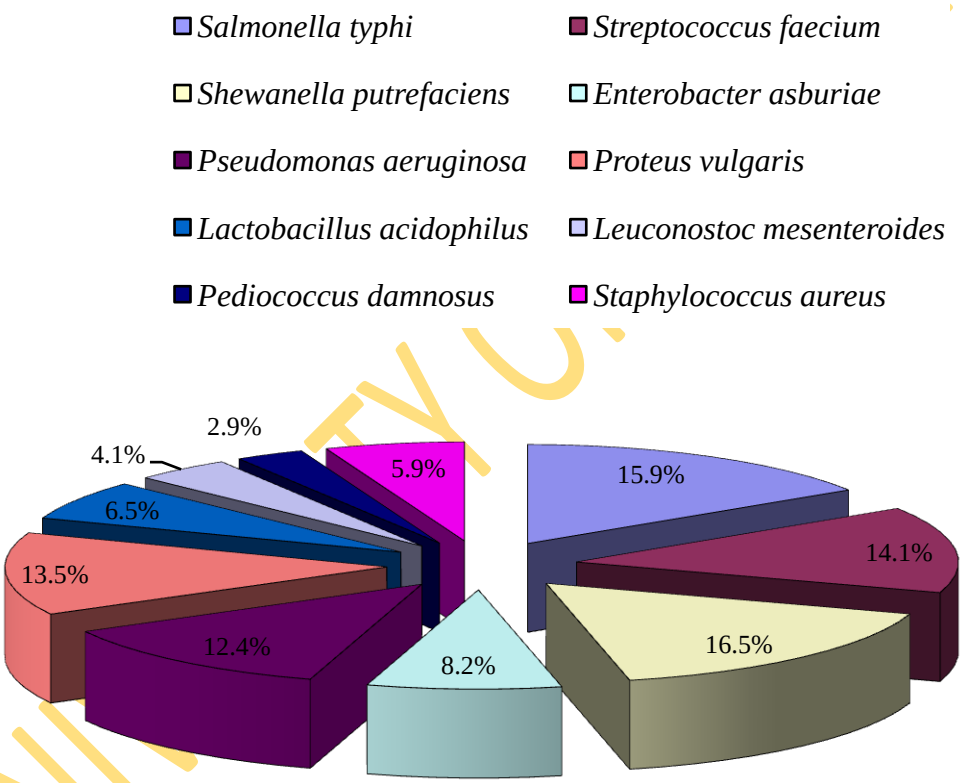

Figure 19: Frequency of occurrence of Bacteria isolated from *Micromesistius poutassou*

Table 30: Sugar Fermentation Test on *Enterobacteriaeceae* Isolated from *Sardinella* species and *Micromesistius poutassou* using API 20E kit

TESTS	SSA1	MCA1	MCA2	MCA3	MCA4
2-nitrophenyl-BD-galactopyranose	-	-	+	-	-
Arginine dihydrolase	-	-	-	+	-
Lysine decarboxylase	+	-	-	-	-
Ornithine decarboxylase	-	+	+	-	-
Citrase	-	+	+	+	-
Hydrogen sulfide	-	+	-	-	+
Urease	-	-	-	-	+
Tryptophan deaminase	-	-	-	-	+
Indole	-	-	-	-	+
Voges-Proskauer	-	-	-	-	-
Gelatin Liquefaction	-	+	-	+	+
Glucose	+	-	+	+	+
Mannose	+	-	+	-	-
Inositol	-	-	-	-	-
Sorbitol	+	-	+	-	-
Rhamnose	-	-	-	-	-
Sucrose	-	-	+	-	+
Melibiose	+	-	-	-	-
Amygdalin	-	-	+	-	+
Arabinose	-	-	+	-	-
Probable organism	***Salmonella typhi***	***Shewanella putrefaciens***	***Enterobacter asburiae***	***Pseudomonas aeruginosa***	***Proteus vulgaris***

Source: Field data (2011)

Table 31: Sugar Fermentation Test on LAB Isolated from *Sardinella s*pecies and *Micromesistius poutassou* fish samples using API 50 CH kit.

Sugars/isolate code	**MRS1**	**MRS2**	**MRS3**	**BA1**
Control	-	-	-	-
Glycerol	-	-	-	-
Erythritol	-	-	-	-
D-Arabinose	-	-	-	-
L- Arabinose	+	+	-	-
D-Ribose	+	W	-	W
D-Xylose	+	+	-	-
L-Xylose	-	-	-	-
D-Adonitol	-	-	-	-
Methyl-BD-Xylopyranose	-	-	-	-
Galactose	+	+	+	-
D-Glucose	+	+	+	+
D-Fructose	+	+	+	+
D-Mannose	+	+	+	-
L-Sorbose	-	-	-	-
L-Rhannose	-	-	-	-
Dulcitol	-	-	-	-
Inositol	-	-	-	-
D-Mannitol	W	-	-	-
D-Sorbitol	-	-	-	-
Methyl-aD-mannopyranoside	-	-	-	-
Methyl-aD-glucopyranose	-	+	-	-
N-acetylglucosamine	+	+	+	-
Amygdalin	+	+	+	-
Arbutin	+	+	W	-
Esculin ferric citrate	+	+	+	-
Salicin	+	+	+	-
D-Celibiose	+	+	+	-
D-Maltose	+	+	W	-
D-Lactose	+	W	-	+
D-Melibiose	+	+	-	-
D-Saccharose	+	+	-	+
D-Trehalose	+	+	+	-
Inulin	W	-	-	-
D-Meleziotose	-	-	-	-
D-Raffinose	W	W	-	-
Amidon	-	-	-	-
Glycogen	-	-	-	-
Xylitol	-	-	-	-
Gentiobiose	+	+	+	-
D-Turanose	-	+	-	-
D-Lyxose	-	-	-	-
D-Tagatose	-	-	-	-
D-Fucose	-	-	-	-
L-Fucose	-	-	-	-
D-Arabitol	W	-	-	-
L-Arabitol	-	-	-	-
Potassium gluconate	+	W	-	-
Potassium 2-KetoGluconate	W	-	-	-
Potassium 5-ketogluconate	-	-	-	W
Probable organism	***Lactobacillus acidophilus***	***Leuconostoc mesenteroides***	***Pediococcus damnosus***	***Streptococcus faecium***

(+) positive;(-) negative; (w) weak

Source: Field data (2011)

Table 32: Morphological and Biochemical properties of Staphylococci Isolated from the samples

Tests/Isolate code	**MSA1**
Colony morphology	Yellow, flat, smooth and entire colony
Gram staining	Gram positive cocci in cluster
Motility	-
Spore stain	-
Catalase	+
Coagulase	+
Citrate	-
Starch hydrolysis	-
O/F	F
Glucose	A
Sucrose	-
Lactose	A
Manitol	A
Maltose	A
Xylose	-
Fructose	-
Galactose	A
Probable organism	***Staphylococcus aureus***

A: Acid production, **F**: Fermentation, **-**: Negative, **+**: Positive

Source: Field data (2011)

4.4.1 Mould Isolates in the Imported Frozen Fish Samples.

The fungal load as shown in Fig. 20 picked Oyo zone with the lowest count in both *Sardinella* species ($7.9x10^4$) and *M. poutassou* ($1.4x10^5$) respectively. Ogbomoso zone had the highest fungal load ($1.8x10^5$) in *Sardinella* species and Ibadan/Ibarapa and Saki ($2.4x10^5$) in *M. poutassou*.

In Figure 21, frequency of occurrence of fungal isolates for *Sardinella* species showed that *Penicillium notatum* had the highest occurrence of 63.9% and *Geotrichum species* the least (11.1%) and *Aspergillus niger* (25.0%). In *M. poutasssou*, *Aspergillus niger* had the highest occurrence (39.1%), while both *Penicillium notatum* and *Geotrichum species* had equal percentage of occurrence of 30.4% each (Fig. 22).

Based on the pigmentation of the spores, nature of the mycelia and spore formation, the following fungal species were observed in the frozen fish samples: *Aspergillus niger*, *Penicillium notatum* and *Geotrichum* species (Table 33).

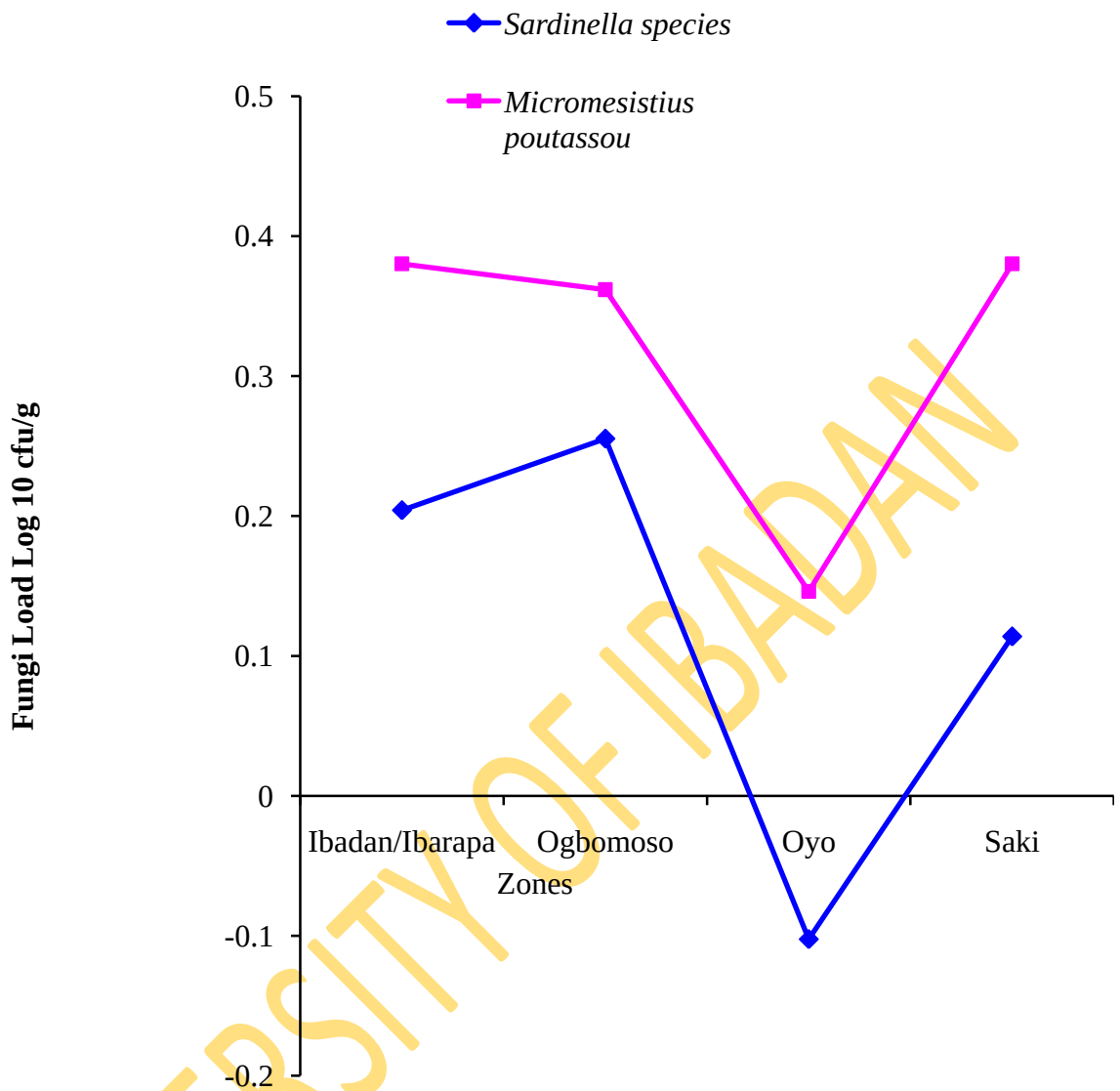

Figure 20: Total Fungal load of Imported frozen fish samples across the zones

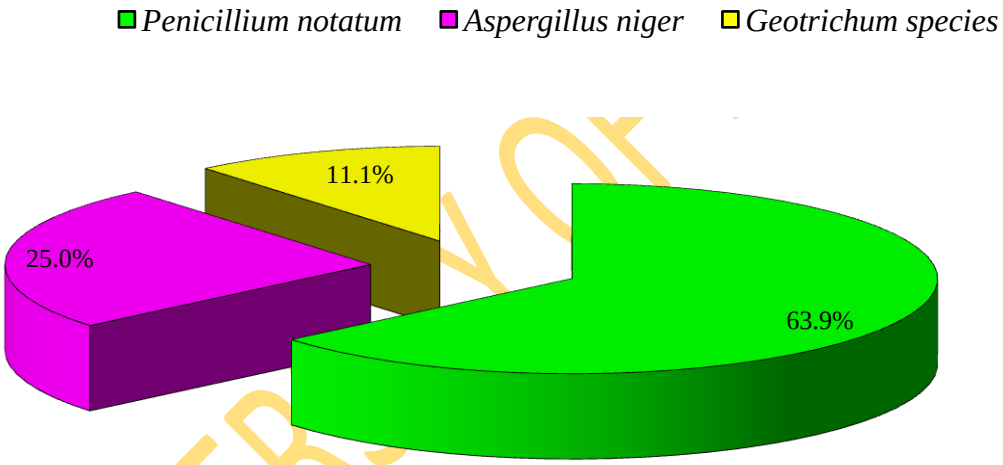

Figure 21: Frequency of occurrence of Fungal Isolates from *Sardinella* species

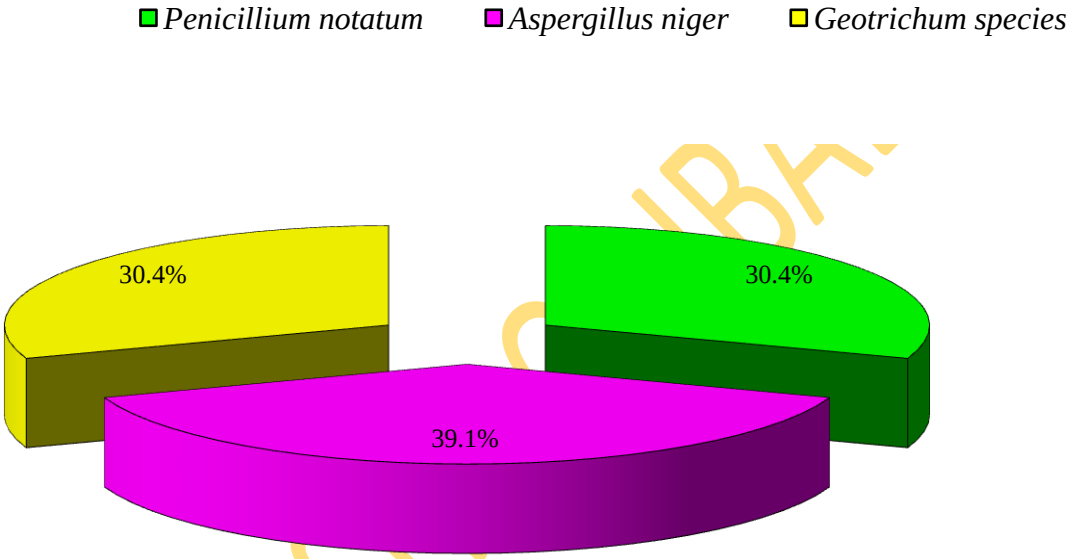

Figure 22: Frequency of occurrence of Fungal Isolates from *Micromesistius poutassou*

Table 33: Cultural and Morphological Characteristics of Fungal Isolates

Isolates codes	Growth rate	Spore colour	Colony morphology	Microscopy	Probable organism
PDA 1	4days	Green	Powdery/Velvety surface with fin peripheral extensions	Septate hyphae, conidia on conidiosphore in multi-chains like a paint brush	***Penicillium notatum***
PDA 2	Rapid mature in 4days	Whitish but later turned black	Velvety surface due to marked sporulation	Septate hyphae, conidia borne in chain on sterigmata	***Aspergillus niger***
PDA 3	4 days	White moist	Initially yeast-like, later formation of air mycelium	Septate hyphae, pronounced arthrospores, no blastospores	***Geotrichum* species**

Source: Field data (2011)

4.5 Chemical Evaluation of Imported Frozen Fish Samples

4.5.1 Peroxide Value and Free Fatty Acid Concentration

Results indicated that Oyo zone recorded the lowest peroxide value in both *Sardinella* species (17.48±0.81) and *M. poutassou* (17.90±0.59). Ogbomoso zone had the highest PV (18.83±0.60) in *Sardinella* species and Saki zone (19.79±1.03) in *M. poutassou*. Peroxide value concentration is generally lower in the four zones for *Sardinella* species than *M. poutassou* (Fig. 23).

Ogbomoso zone as shown by Fig. 24 had the highest mean concentration of FFA (1.85±0.36; 1.82±0.19) in both *Sardinella* species and *Micromesistius poutasssou* respectively. The lowest mean value was also found in Ibadan/Ibarapa zone (1.52±0.14; 1.38±0.07) respectively. *Sardinella* spp. had higher values in three zones of Ibadan/Ibarapa, Ogbomoso and Oyo than *M. poutasssou* except in Saki zone.

4.5.2 Trimethylamine level in Imported Frozen Fish Samples

As shown in Fig. 25, the mean concentration by zones showed Ibadan/Ibarapa zone as having the highest concentration of TMA (22.96±0.49) in *Micromesistius poutassou*. This is followed by Saki zone (21.79±3.32) and the least was in Oyo zone (19.03± 0.65). The highest concentration of TMA in *Sardinella* species was in Ogbomoso zone (23.63±0.89) and the lowest was 20.58±0.91 in Oyo zone. However, Table 35 showed that there is significant difference ($p<0.05$) in TMA value in *Micromesistius poutasssou* across the four zones [$F(3,26) = 3.674$; $p<0.05$].

From Table 36, Ibarapa/Ibarapa and Saki zones were not significantly different in their mean Trimethylamine (TMA) ($p>0.05$). Also, Ogbomoso, Oyo, and Saki zones were not significantly different in their mean TMA content ($p>0.05$). However, Ibadan/Ibarapa significantly had higher mean Trimethylamine acid (TMA) ($p<0.05$) than Ogbomoso and Oyo zones.

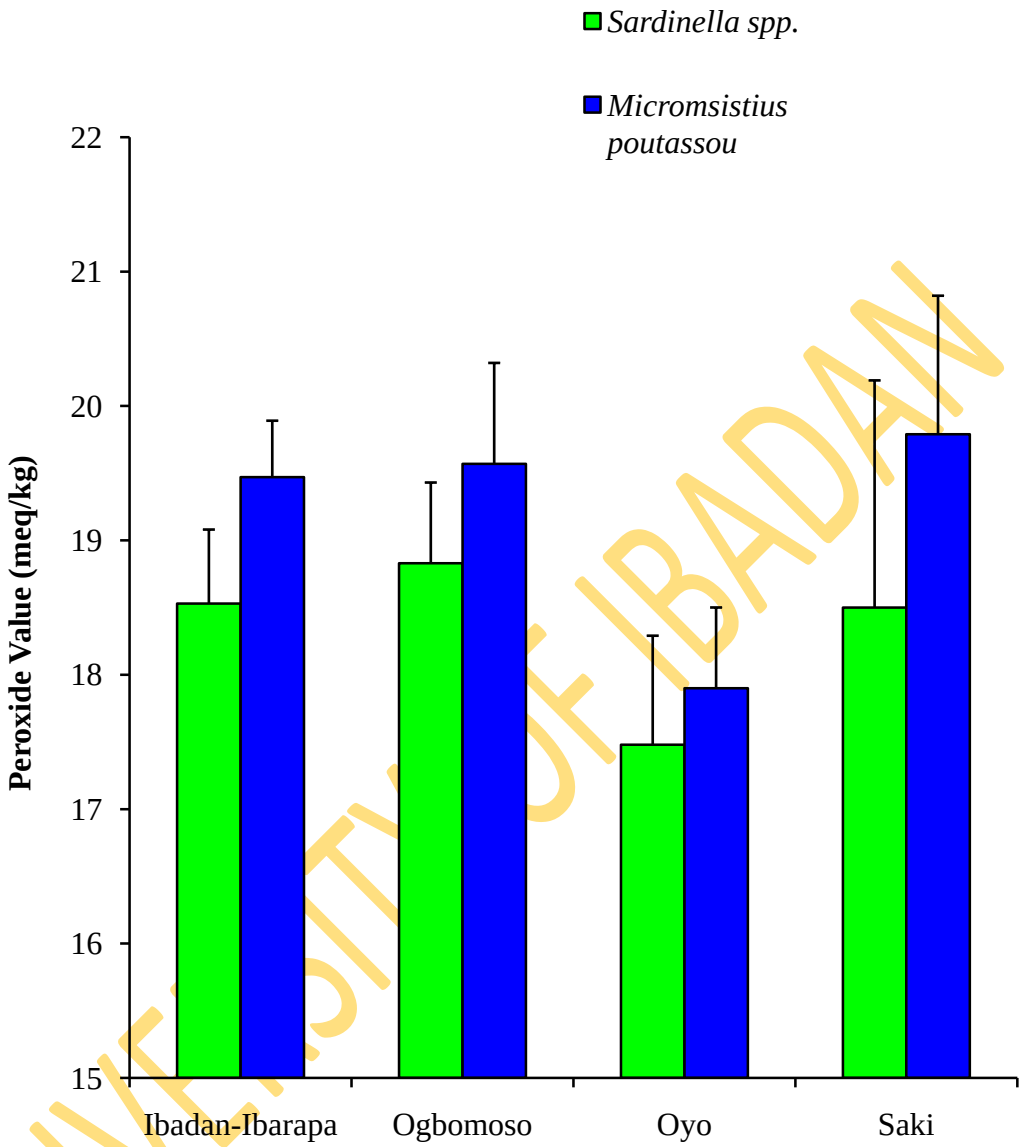

Figure 23: Mean concentration by zone of Peroxide value of imported frozen fish samples in Oyo state

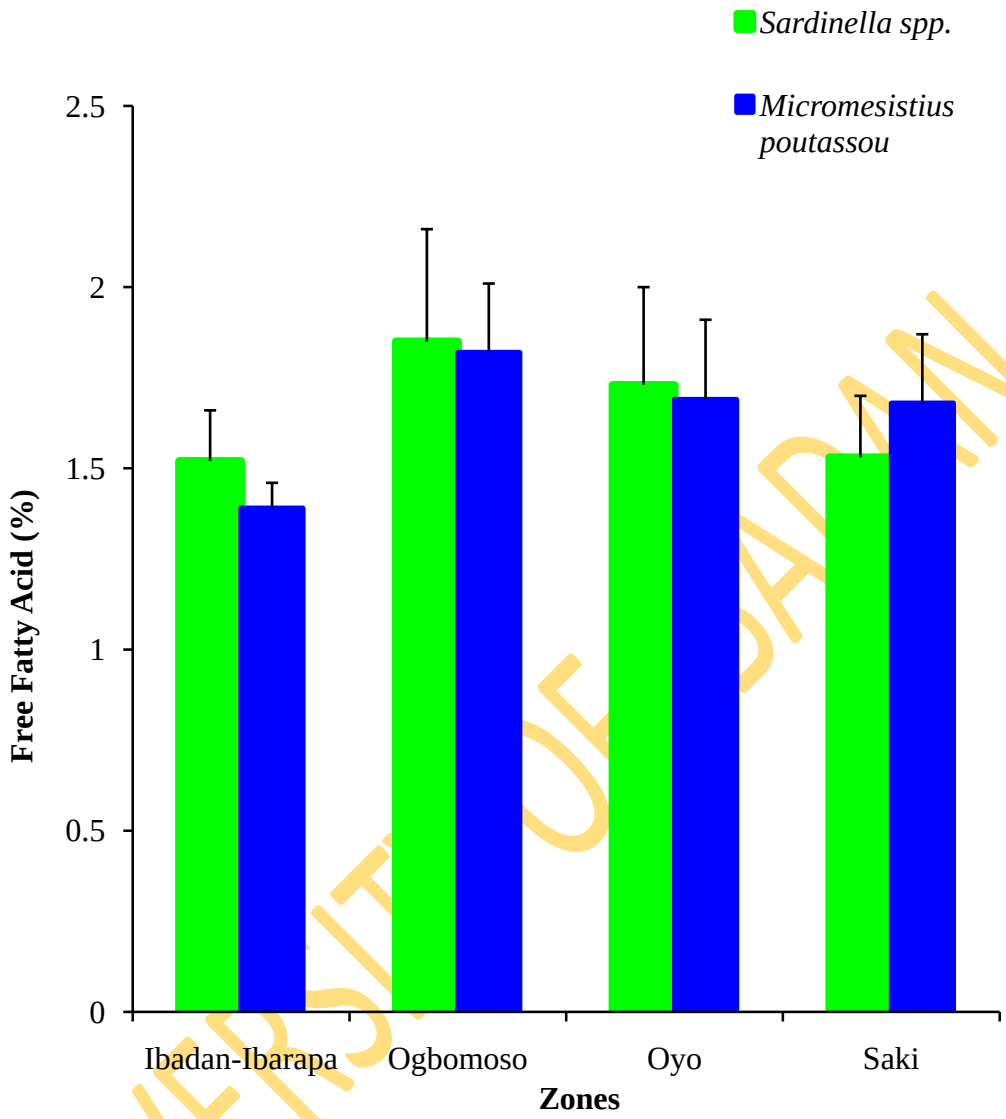

Figure 24: Mean concentration by zone of Free Fatty Acid content of imported frozen fish samples collected in Oyo State

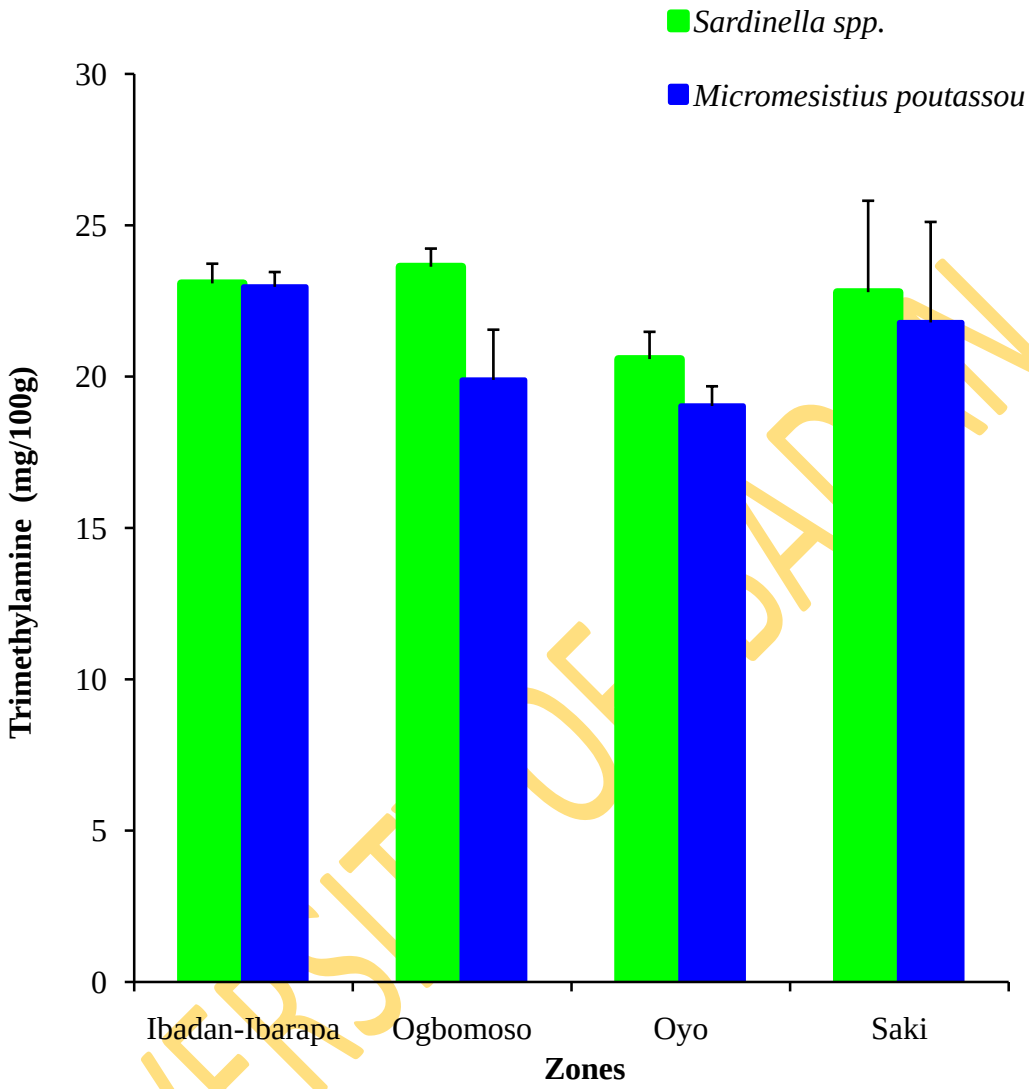

Figure 25: Mean concentration by zone of Trimethylamine Acid content of imported frozen fish samples collected in Oyo State

Table 34: Mean Concentration of Quality Parameters in Imported Frozen Fish in Oyo state compared with International Standards

Parameters	International Standard	Zone	Oyo state		Remark
			Sardinella species	*Micromesistius poutassou*	
TVC	5.0 x 10^5cfu/g ICMSF, 2005	Ibadan/Ibarapa	1.7 x 10^5	2.6 x 10^5	A/A
		Ogbomoso	3.1 x 10^5	3.2 x 10^5	A/A
		Oyo	1.2 x 10^5	1.5 x 10^5	A/A
		Saki	1.4 x 10^5	2.2 x 10^5	A/A
Hx	29.58mg/100g (Oyelese, 2012)	Ibadan/Ibarapa	24.48± 0.56	25.49 ±0.65	A/A
		Ogbomoso	24.48± 0.77	25.69 ±1.01	A/A
		Oyo	25.54 ±0.41	25.96± 0.53	A/A
		Saki	22.15± 1.04	25.77 ±0.49	A/A
TMA	30mg/100g (Regenstein *et al*., 1982)	Ibadan/Ibarapa	23.07± 0.65	22.96 ±0.49	A/A
		Ogbomoso	23.63± 0.60	19.89 ±1.66	A/A
		Oyo	20.58 ±0.91	19.03± 0.65	A/A
		Saki	22.79± 3.02	21.79 ±3.32	A/A
PV *	10-20 meq/kg (Lakshmanan,2000); 18.21meq/kg (Aubourg *et al*.,2005)	Ibadan/Ibarapa	18.52±0.55	19.47 ±0.42	A/A
		Ogbomoso	18.83 ±0.60	19.56± 0.75	A/A
		Oyo	17.48 ±0.81	17.90 ±0.60	A/A
		Saki	18.50 ±1.69	19.79± 1.03	A/A
FFA	1.85% (Oyelese,2012)	Ibadan/Ibarapa	1.52± 0.14	1.38 ±0.07	A/A
		Ogbomoso	1.85 ±0.31	1.82± 0.19	A/A
		Oyo	1.73± 0.27	1.69± 0.22	A/A
		Saki	1.53± 0.17	1.67 ±0.19	A/A
Organoleptic Assessment	4.0 (Minim, 2006)	Ibadan/Ibarapa	5.60 ±0.15	5.63± 0.11	A/A
		Ogbomoso	5.80 ± 0.12	5.60 ± 0.13	A/A
		Oyo	5.47 ± 0.20	5.57 ± 0.13	A/A
		Saki	5.90 ± 0.08	5.80 ± 0.10	A/A

A: Acceptable; **NA**: Not acceptable.

* The PV in all the zones were within the acceptable range as posited by Lakshmanan (2000)

Table 35: Analysis of Variance (ANOVA) for Quality Parameters across zones in *Micromesistus poutassou*

		S.S	Df	M. S	F	P-level	(<0.05)
Log TVC	Zone	.469	3	.156	1.431	.256	(>0.05)
	Error	2.840	26	.109			
	Total	3.309	29				
Organoleptic	Zone	.195	3	.065	.524	.670	(>0.05)
	Error	3.220	26	.124			
	Total	3.415	29				
Peroxide value	Zone	13.879	3	4.626	1.433	.256	(>0.05)
	Error	83.938	26	3.228			
	Total	97.817	29				
Trimethylamine	Zone	77.714	3	25.905	3.674	.025	(<0.05)
	Error	183.310	26	7.050			
	Total	261.023	29				
Free fatty acid	Zone	.895	3	.298	1.692	.193	(>0.05)
	Error	4.583	26	.176			
	Total	5.478	29				
Hypoxanthine	Zone	.973	3	.324	.547	.082	(>0.05)
	Error	102.648	26	3.948			
	Total	103.622	29				

Table 36: Mean Trimethylamine, TMA (mg/100g) across zones in *Micromesistius poutassou*

Zones	N	$\overline{X}$ ± SEM*
Ibadan/Ibarapa	12	22.96 ± 0.49[a]
Ogbomoso	6	19.89 ± 1.66[ab]
Oyo	6	19.03 ± 0.65[b]
Saki	6	21.79 ± 1.36[ab]

* Means with same superscript are not significantly different according to Duncan Multiple range test (DMRT) at p=0.05

4.5.3 Hypoxanthine Level in Imported Frozen Fish Samples

Figure 26 indicated that *M. poutasssou* had higher hypoxanthine values in all the zones (Ibadan/Ibarapa, Ogbomoso, Oyo and Saki) than *Sardinella* spp. The highest hypoxanthine values were recorded in Oyo zone (25.54±0.41; 25.96±0.53) for both *Sardinella* spp. and *M. poutassou* while Saki zone had the lowest (22.15±1.04) in *Sardinella* spp. and Ibadan/Ibarapa zone (25.49±0.65) in *M. poutassou* respectively.

Table 37 indicated that there was significant difference in hypoxanthine values in *Sardinella species* across the four zones [F (3,26) = 3.294, p< 0.05].

From Table 38, Ibadan/Ibarapa, Ogbomoso, and Oyo zones were not significantly different (p>0.05) in their mean hypoxanthine values. However, Ibadan/Ibarapa, Ogbomoso and Oyo zones significantly had higher mean hypoxanthine (p<0.05) than Saki zone.

As shown in Table 39, there was positive correlation between TMA and PV in *Sardinella* species (r = 0.69, p < 0.01). This implies that increase in TMA will lead to increase in PV value. Also, there is positive correlation between FFA and organoleptic quality, (r = 0.501, p < 0.01). This implied that increase in FFA led to increase in organoleptic value.

Table 40 shows a positive correlation between TMA and PV in *Micromesistius poutasssou* (r = 0.448, p < 0.05). Also there was positive correlation between FFA and Hx (r = 0.441, p < 0.05). However, there was negative correlation between FFA and TMA (r = -0.39, p < 0.05).

In Table 41, the result of the t-test conducted on the mean values of the quality indices of the fish species showed that there was no significant difference in organoleptic parameters, PV, TMA and FFA (p > 0.05). There was however significance in TVC and hypoxanthine values between the two fish species (p < 0.05).

This implied that there was marked difference in the mean microbial load and hypoxanthine contents of both *Sardinella* species and *Micromesistius poutassou*.

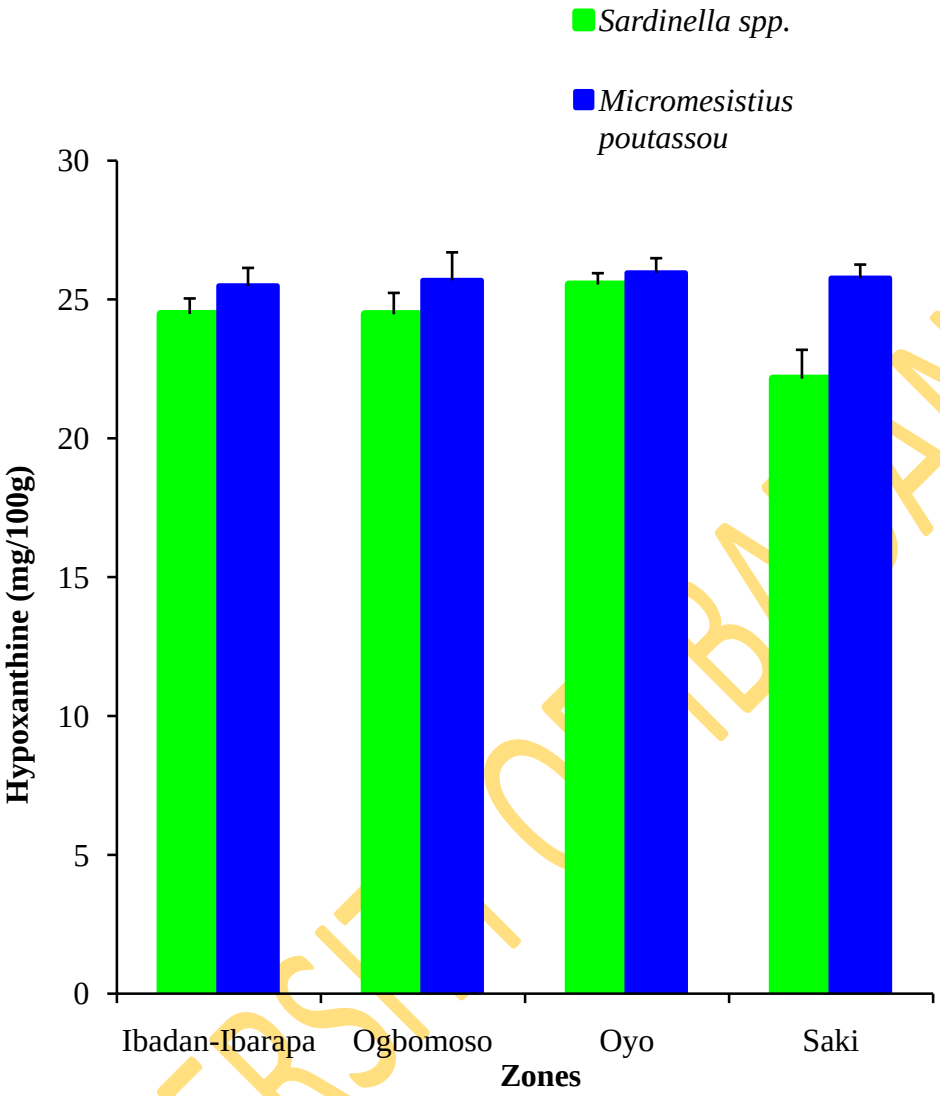

Figure 26: Mean concentration by zone of Hypoxanthine content of imported frozen fish samples collected in Oyo State

Table 37: Analysis of Variance (ANOVA) for Quality Parameters across zones in *Sardinella* species

		S.S	Df	M. S	F	P-level	(<0.05)
Log TVC	Zone	.125	3	.042	.126	.944	(>0.05)
	Error	8.592	26	.330			
	Total	8.717	29				
Organoleptic	Zone	.725	3	.242	1.279	.302	(>0.05)
	Error	4.913	26	.189			
	Total	5.639	29				
Peroxide value	Zone	6.410	3	2.137	.353	.788	(>0.05)
	Error	157.554	26	6.060			
	Total	163.964	29				
Trimethylamine	Zone	33.856	3	11.285	.772	.520	(>0.05)
	Error	379.882	26	14.611			
	Total	413.738	29				
Free fatty acid	Zone	.536	3	.179	.510	.679	(>0.05)
	Error	9.108	26	.350			
	Total	9.645	29				
Hypoxanthine	Zone	37.437	3	12.479	3.294	.036	(<0.05)
	Error	98.506	26	3.789			
	Total	135.943	29				

Table 38: Mean Hypoxanthine, Hx (mg/100g) across the zones in *Sardinella* species

Zones	**N**	$\overline{X}$ **± SEM***
Ibadan/Ibarapa	12	24.48 ± 0.56^{a}
Ogbomoso	6	24.47 ± 0.77^{a}
Oyo	6	25.54 ± 0.41^{a}
Saki	6	22.15 ± 1.04^{b}

* Means with same superscript are not significantly different according to Duncan Multiple range test (DMRT) at p=0.05

Table 39: Correlation among Quality indices across Zones in *Sardinella* species.

		1.	2.	3.	4.	5.	6.
1.	**Peroxide value**						
2.	**Trimethylamine**	.690**					
3.	**Free fatty acid**	-.140	-.035				
4.	**Hypoxanthine**	.027	.255	.351			
5.	**Log TVC**	-.091	-.145	-.295	.114		
6.	**Organoleptic**	-.176	-.084	.501**	-.167	-.084	

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

Table 40: Correlation among Quality indices across Zones in *Micromesistius poutassou*

		1.	2.	3.	4.	5.	6.
1.	**Peroxide value**	.					
2.	**Trimethylamine**	.448*					
3.	**Free fatty acid**	-.239	-.390*				
4.	**Hypoxanthine**	-.009	-.071	.441*			
5.	**Log TVC**	-.014	-.056	-.063	-.035		
6.	**Organoleptic**	.104	.309	.327	.179	-.058	

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

Table 41: T- Test for Mean values of Quality indices in *Sardinella* species and *Micromesistus poutassou* across Zones

Parameter	Sample	N	$\bar{x} \pm SEM$	Df	t-value	P Level	(<0.05)
Log Total viable count	*Sardinella* spp.	30	0.2±0.10	58	-2.32	0.024	(<0.05)
	M. poutasssou		0.29±0.06				
Organoleptic	*Sardinella* spp.	30	5.67±0.08	58	0.26	0.795	(>0.05)
	M. poutasssou		5.65±0.06				
Peroxide value	*Sardinella* spp.	30	18.38±0.43	58	-1.58	0.120	(>0.05)
	M. poutasssou		19.24±0.34				
Trimethylamine	*Sardinella* spp.	30	22.63±0.69	58	1.48	0.145	(>0.05)
	M. poutasssou		21.33±0.55				
Free fatty acid	*Sardinella* spp.	30	1.63±0.11	58	0.30	0.768	(>0.05)
	M. poutasssou		1.59±0.08				
Hypoxanthine	*Sardinella* spp.	30	24.23±0.40	58	-2.78	0.007	(<0.05)
	M. poutasssou		25.68±0.35				

4.6 Storage of Frozen Fish

Duration of storing fish in the cold store between 1-2 weeks by wholesalers was 92.5% (Table 42), after which new fish was brought in. On temperature management, most cold stores (71.6%) were maintained at -11 to -18^{0}C (Table 43). The total percentage of cold stores that maintained the recommended storage temperature for frozen fish (-18^{0}C) across the four zones as shown in Table 44 was 65.67%. Eighty six percent of the cold stores were maintained below -18^{0}C in Oyo zone, followed by Ibadan/Ibarapa (74.3%), while the least was Saki zone with 40.0%.

4.7 Daily Handling of Unsold fish by Retailers

Findings showed that not all the fish were sold daily by retailers. More than sixty three percent of retailers refrigerated the unsold fish, 24.7% smoked the fish and 1.3% gave fish out after daily sales (Table 45). Virtually all retailers (98.7%) sold the left-over fish the following day (Table 46).

The consequences of not immediately cold storing the left over fish after daily sales by retailers as discovered in the course of this research work included:

- Obvious drip loss from the frozen fish when not stored in time. As the fish thawed, a large amount of fluid is lost; resulting in dry tough flesh and the fish usually became difficult to cut.
- There was incidence of dehydration of fish which resulted in the unusual dryness of the fins, most prominently the caudal fin.
- The fish lost its economic value as it did not appeal to customers due to changes in physical appearance.

Table 42: Duration of Fish Storage by Wholesalers across Zones

	Zone				Total	Percentage
	Ibadan/Ibarapa	**Ogbomoso**	**Oyo**	**Saki**		
1- 2 wks	37	14	6	5	62	**92.5**
3-4 wks	2	2	0	0	4	**6.0**
Others	0	0	1	0	1	**1.5**
Total	39	**16**	**7**	**5**	**67**	**100.0**

Source: Field data (2011)

Table 43: Temperature of Cold stores across Zones

Temperature	Zone				Total	Percentage
	Ibadan/Ibarapa	**Ogbomoso**	**Oyo**	**Saki**		
-1 to -5^{0}C	0	2	0	1	3	**4.5**
-6 to -10^{0}C	3	2	0	1	6	**9.0**
-11 to -18^{0}C	29	10	7	2	48	**71.6**
Unknown	7	2	0	1	10	**14.9**
Total	**39**	**16**	**7**	**5**	**67**	**100.0**

Source: Field data (2011)

Table 44: Percentage Temperature range of Cold Stores above and below -18^{0}C across Zones

	Zone				Total/ (%)
	Ibadan/Ibarapa/(%)	**Ogbomoso/(%)**	**Oyo/(%)**	**Saki/(%)**	
-18^{0}C and below	29(74.35)	7(43.75)	6(86.0)	2(40.0)	**44(65.67)**
Above -18^{0}C	3(7.69)	7(43.75)	1(14.29)	2(40.0)	**13(19.40)**
Unknown	7(17.95)	2(12.5)	-	1(20.0)	**10(14.93)**
Total	**39(58.21)**	**16(23.88)**	**7(10.45)**	**5(7.5)**	**67(100.0)**

Source: Field data (2011)

Table 45: Methods of preserving the unsold fish by Retailers

	Zone				Total	Percentage
	Ibadan/Ibarapa	**Ogbomoso**	**Oyo**	**Saki**		
Smoking	22	11	2	2	37	**24.7**
Refrigeration	70	13	10	2	95	**63.3**
Give out	0	2	0	0	2	**1.3**
Others	10	3	3	0	16	**10.7**
Total	**102**	**29**	**15**	**4**	**150**	**100.0**

Source: Field data (2011)

Table 46: Response on whether Retailers disposed of Unsold fish the following day or not

	Zone				Total	Percentage
	Ibadan/Ibarapa	**Ogbomoso**	**Oyo**	**Saki**		
Yes	102	27	15	4	148	**98.7**
No	0	2	0	0	2	**1.3**
Total	**102**	**29**	**15**	**4**	**150**	**100.0**

Source: Field data (2011)

CHAPTER FIVE

5.0 DISCUSSION

5.1. Frozen Fish Distribution and Management

The fish firm is the sole source and major distributor of frozen fish in Nigeria. Most of these firms engaged in charter arrangements with foreign firms which travel to distant waters to fish. The Nigerian firms pay the foreign firms in foreign exchange for specific quantities of fish.

As discovered from the field survey, amongst the major importers in Nigeria, the Stallion Group is the biggest in terms of infrastructure (cold rooms), transport and staff. Other active firms are Seafood Products Limited, POF Nigeria Limited, African Fish Limited, Onward Fisheries, CIC Limited, Fiogret Nigeria, Agro Allied, Joma Foods, Globe Fishing, Agromar, Fola Foods, Bharat ventures and Primlax Nigeria.

All these firms have their headquarters in Lagos, with distributors or vendors all over the country (appendix 3).

5.1.1 Gender and Age Distribution of Wholesalers and Retailers

Dominance of men in the wholesale sector could be attributed to the heavy capital investment required for procuring and maintaining the equipment needed for the smooth running of the business which included cold store and refrigerated trucks. The strong gender linkage in frozen fish retailing was probably due to the existence of gender bias for women in the South West Nigeria, occasioned by cultural belief that retailing is not strenuous and is a woman's job.

Age distribution also showed that there were more youths in fish retailing than wholesaling, suggesting that older people are better placed financially than the young.

5.1.2 Level of Education of Respondents

Since education is vital to taking appropriate investment decisions, educational level was higher among wholesalers than retailers. However, literacy level was generally high in both wholesalers (80.6%) and retailers (69.3%).

5.1.3 Transportation and Physical Facilities

Transportation was a critical activity in frozen fish distribution, linking the production area and the consumption centers. A little hitch in transportation could affect frozen fish distribution. The most common means of transportation among wholesalers was refrigerated truck. It was discovered that they employed chartered truck to convey fish from Lagos and Port Harcourt. The preference for refrigerated trucks for transportation by wholesalers was due to the large quantity of consignment delivered at a time, the distance from the firms to the cold stores and the perishable nature of the product which required precautionary measures and maintenance of cold chain by wholesalers to conserve fish quality. The trucks were however maintained at -18^{0}C and below. Motorcycle was the major means used by the retailers.

Fifty seven percent of the retailers washed their slab daily as shown in Table 22. There was considerable high level of hygiene but none of them used chlorinated water. Majority of the retailers sold fish at fish market stalls, while others sold by the roadside or hawked in public using handling facilities such as bowls, wooden tables and wooden boards. This corroborated earlier work by Krone (1977) who stated that frozen fish was primarily retailed in developing countries in unsophisticated bulk packs and its distribution depended in many instances on the traditional fish trade in public markets or sometimes in fishmongers' shops. This possibly informed the submission of Talabi and Makanjuola (1977) that any technological measures aimed at improving frozen fish distribution and marketing in Nigeria must concentrate on the retailers' practices and tools. However, most of the cold store workers across the zones (83.6%) wore insulated clothing into the refrigerated trucks and cold stores during loading and unloading.

5.1.4 Pricing Policy

Cost of purchase was the main determinant of the price of fish by both wholesalers (91.0%) and the retailers (88.7%). A change in price was mainly communicated amongst the wholesalers through the use of mobile phone (53.7%) while the major means of communication amongst retailers was word of mouth (78.0%). The wholesalers made better use of the new information technology than the retailers possibly because of their stronger economic power and their higher level of education. While the majority of the wholesalers saved money in the bank (92.5%) due to larger volume of sales in the wholesaling, only 23.3% of the retailers did. However, the wholesalers were reluctant in giving the financial status of their business (appendix 8).

5.1.5 Group Activities

It was observed that membership of Trade associations was not mandatory in the wholesale trade. Only 45.2% of wholesalers were in trade association, but 86.0% of the retailers were members of fish retailers association embracing members across the four zones in the state. The retailers communicated well with each other through their associations. Once accepted into the Trade Association, retailer, paid an entry fee and made monthly or weekly contributions to the association. Entry into retail trade is dependent on the applicant's acceptability by the President of the Association. The socio-economic benefits enjoyed by members among others included general welfare and access to help in time of difficulty.

5.1.6 Constraints to Frozen Fish Distribution and Management

Electricity was neither regular nor consistent. Seventy five percent of the wholesalers experienced power outage above 5 hours daily. It was observed that all wholesalers made use of alternative power source (generator). More than eighty percent identified fuel cost as a problem because the fuel used to power generating sets increased the bill of maintaining cold store facilities. Technical personnel handling the repairs of facilities were however readily available as confirmed by 83.6% of the wholesalers.

Frozen fish was transported from the sea port at Lagos and Port Harcourt to Oyo state in refrigerated trucks. The degree of efficiency of transport system was therefore a major determinant of market access, having a critical influence on wholesalers and retailers' prices. This was made worse by the general inflationary trend, incessant fuel scarcity and high cost of motor spare parts. Forty-seven percent of the wholesalers identified transportation as a problem because transportation costs were eventually added to the total running costs. Though most of the wholesalers did not buy on credit, majority sold to retailers on credit. There was therefore problem of debt recovery from defaulters which was as high as 65.0%. The requirement to pay an entry fee before joining the retailers' association also constituted a barrier because majority of the prospective members were relatively poor and could not afford the fee.

In the same vein, inadequate finance limited the number of entrants into wholesale business due to the high cost of construction of cold stores, maintenance and other functional services in transporting fish to the cold stores.

5.2 Analysis of the Quality Parameters

5.2.1 Reason for Choice of Imported Frozen Fish for this Research Work

During field survey, it was discovered that the price of *Scomber japonicus* was relatively high, thus the fish was virtually unavailable in Saki zone due to low demand. It however enjoyed patronage in other zones of Ibadan/Ibarapa, Ogbomoso and Oyo. Also, *Scomber Scombrus* was seasonal and not available all year round. *Sardinella* species and *Micromesistius poutassou* used in this research were available in all the four zones throughout the year. They are relatively cheap and affordable and therefore consumed by larger percentage of the population.

5.3 Sensory Evaluation of the Samples

In all cases, organoleptic assessment confirmed that all the fish samples were fit for consumption throughout the study. Although this was subject to individual judgment of the panelists, the organoleptic properties of the examined samples indicated that the products were acceptable according to the panel's evaluation using a 7-point hedonic scale (Minim, 2006).

The T- test conducted on the mean proximate composition of the two fish samples (appendix 11) showed significant differences ($p<0.05$) in crude fibre and ether extract (crude fat). The implication is that *Sardinella* species had higher fat content than *Micromesistius poutasssou*. This is in agreement with Stroud (1972) who identified *Sardinella* species as a pelagic fish with high fat content. The organoleptic properties of the samples as reported by the taste panel was in line with previous works (Ryan, 1979; Love, 1980 and Borresen, 1992).

5.4 Microbiological Evaluation of Samples

The tests carried out in this research were in line with international standards. The presence of contaminating bacteria in seafoods could be attributed to cross-contamination from the environment, source, and handling along the distribution chain (Bryant *et al*., 1988). The microorganisms reported in this study were similar to what had been reportedly isolated in other studies in Nigeria (Okonko *et al*., 2008; 2009; Chukwuka *et al*., 2010; Akinmusire, 2011; Akinjobi *et al*., 2011, Adebayo-Tayo *et al*., 2011, 2012).

The mean viable count of the organisms from the study was found to be within international standards (SON, 2004 and ICMSF, 2005) for frozen fish products which are between 5.0 $x10^5$ and 1.0 $x10^6$ CFU g^{-1}. The mean viable count although cannot be taken as an absolute figure. This is because the number and type of bacteria found on frozen fish is dependent on many factors, of which, source of the fish contributes the major factor and this supports Thatcher and Clark (1973) earlier report which stated that the kind and number of microorganisms found on frozen fish is dependent on the source of the fish, additional contamination introduced in the fishing boat, freezing temperature during storage, severity of freezing process with respect to lethality to microorganisms and contamination by handlers and market sellers.

In this study, *Pseudomonas species* was isolated from the fish samples collected from the four zones. The isolation of *Pseudomonas spp*. from experimental samples is important because *Pseudomonas* is a potential pathogen and spoilage agent. (Koutsoumanis and Nychas, 2000; Jeyasekaran *et al*., 2006 and Yaqoub, 2009). *Pseudomonas* is the most common spoilage organism in fish (Vanderzant *et al*., 1970). The species are capable of causing spoilage because of two important characteristics. First, they are psychrotrophic and thus multiply at refrigeration temperatures.

Secondly, they attack various substances in the fish tissue to produce compounds associated with off flavor and off odour (Miller *et al*., 1973). *Pseudomonas* can survive freezing temperature and will resume growth when thawed (Frazier and Westhoff, 1988). This also corroborated Ryser *et al*., (1984), who isolated *Pseudomonas fluorescens* from raw Sashimi tuna.

Shewanella putrefaciens isolated from the fish samples is also a well defined spoilage bacteria. It utilizes TMAO as the terminal electron acceptor in an anaerobic respiration resulting in off-odours and off- flavours due to formation of TMA (Lerke *et al*., 1963, Ringo *et al*., 1984, Gram *et al*., 1987 and 1990; Dalgaard, 1995). Jorgensen *et al*., 1988 and 1989 also isolated *S. putrefaciens* in packed fish products and was observed to be capable of producing TMA and is indeed a specific spoilage organism. *S. putrefaciens* and *Pseudomonas* were identified as specific spoilage organism of different types of fresh chilled fish when stored aerobically (Chai, 1968; Gram *et al*., 1990).

Lactic acid bacteria (LAB) were also isolated from the fish samples. As observed by Schroder *et al*., (1980), LAB occurs naturally in fish and they are easily outgrown by the Gram-negative bacteria during iced, aerobic storage. This could have accounted for why there were more *Enterobacteriaceae* isolates in the fish samples examined.

Staphylococcus aureus encountered in this study is in agreement with previous studies on fish within and outside Nigeria. According to Adams and Moss (2000), *Staphylococcus aureus* is not a part of the normal flora of fish and fish products and the enumeration of *S. aureus* in food products is employed generally as a sanitation index. Presence of large number indicates the possible presence of enterotoxin or faulty sanitary or production practices (FAO, 1994). Enterotoxin production is typically associated with coagulase positive *Staphylococcus aureus* when cell population is 10^{-5} g^{-1} (Jablonski and Bohach 1997). *Staphylococcus aureus* as an indicator of contamination of processed foods could come from the skin, mouth or nose of handlers (Clucas and Ward, 1996; Acco *et al*., 2003,). *Staphylococcus aureus* causes many outbreaks of food poisoning resulting from hand contact (Bryant *et al*., 1988). *Staphylococcus* according to Cheesbrough, (1984) causes meningistis, pneumonia, abscesses and secondary infections.

However, neither tactile, visual nor organoleptic changes occur in foods containing high levels of *Staphylococcus aureus* or their enterotoxin (Collins and Lyne, 1986; Bergdoll, 1989). Since large numbers, typically $>10^6$ CFU g^{-1} are required for the production of enough toxin to cause illness, contamination is necessary but is not sufficient for an outbreak to occur (Adams and Moss, 2000).

Streptococcus faecium was also isolated from the fish samples in agreement with FAO (1994) which observed that the organism is relatively resistant to freezing, which makes it potentially useful as an indicator organism for evaluating plant hygiene during processing of frozen food. However, many foods including fish products contain these organisms as normal part of their flora and they are also able to establish themselves and persist in a food processing plant.

Escherichia coli, a useful indicator of contamination was not isolated throughout the period of the research, which shows some level of hygiene in the handling of the products. However, *E. coli* is a less useful indicator of frozen fish and is less resistant than *Salmonella* in frozen products (Mossel *et al*., 1980). The presence of other indicator organisms like *Enterobacter* and *Salmonella* might be as a result of possible contamination during sale or unhygienic handling of seafood right from the processing plants (Okonko *et al*., 2008).

However, *Salmonella* discovered in the samples is not tolerable at all according to the Nigerian Industrial Standard (NIS) approved by the Standard Organization of Nigeria (SON). *Salmonella* is not to be detected at all in frozen fish products. *Salmonella* is known all over the world as an agent of food-borne disease; and animal foods are still the major source of human salmonellosis characterized by fever, abdominal pain, diarrhoea, prostration and frequent vomiting (Rao, 1983; Bachhil and Jaiswal, 1988). *Salmonella* is normally not present on fish, but fish products may become contaminated during processing, storage, distribution or preparation for consumption (Huss *et al*., 1987). The heat resistance of Salmonella is however very low (Clucas, 1990).

5.4.1 Mould Isolates in the Imported Frozen Fish Samples.

The presence of fungal isolates in the fish samples is in agreement with previous findings by Wogu and Maduakor (2010), who reported the presence of

similar fungal species in fresh fish samples. Three fungi species were isolated in each of the two frozen fish samples. This was fewer compared to bacteria isolates which were ten. This corroborated earlier submission by Clucas (1990) that where water is abundant, bacteria grow much more rapidly and moulds are only of secondary importance. He concluded that yeast are not important as far as sea foods are concerned but moulds, due to their ability to grow where water is limited, could be a problem on smoked or dried fish.

5.5 Chemical Evaluation of Imported Frozen Fish Samples

5.5.1 Peroxide Value and Free Fatty Acid Concentration

The Peroxide value recorded for Ibadan/Ibarapa, Ogbomoso and Saki zones were higher than the acceptable limit of 18.21 meq/kg (Aubourg, 2005), while Oyo zone had the lowest PV of 17.48 meq/kg and 17.90 meq/kg in both *Sardinella* species and *Micromesistius poutassou* respectively.

According to Lakshmanan (2000), if the Peroxide value of fish is above 10-20 meq/kg, fish would probably smell and taste rancid. Peroxide values for frozen fish across the four zones fell within acceptable limit; and frozen fish from Oyo zone had the lowest tendency to go rancid.

In the same vein, Free Fatty Acid concentration in both fish samples across the zones, were within acceptable limit of 1.8% as established by Oyelese (2012).

5.5.2 TMA level in Imported Frozen Fish Samples

The fish samples used in this study were marine fish. Trimethylamine is a well accepted indicator of freshness or spoilage in marine fishes (Connell, 1980). TMA level in the two frozen fish samples fell within the acceptable limit of 30mg/100g fish across zones (Regenstein *et al*., 1982).

5.5.3 Hypoxanthine Level in Imported Frozen Fish Samples

Hypoxanthine is a good spoilage index in fish. It begins to accumulate shortly after the death of fish. This corroborated Howgate (1982), who opined that the way in which hypoxanthine concentration increases with storage time is more variable and is a better predictor of spoilage over a wide range of quantities and it is applicable to a

wide range of species products than both TMA and TVB. Hypoxanthine content rises throughout the commercially important period of storage (Burt, 1977).

There was significant difference in the T-test conducted on Total Mean Concentration of hypoxanthine in the two fish species as shown in Table 40. A similar work by Greene and Bernatt-Byrne (1990) reported that accumulation of hypoxanthine in Pacific cod (*Gadus macrocephalus*) fillets was slower than that in Atlantic cod (*Gadus morhua*), but similar to that in North sea cod (*Gadus callarias,L*). The hypoxanthine levels were negatively correlated with flavor and desirability of the fish. Metin *et al.,* (2001) stated that organoleptic spoilage became obvious at the highest level of hypoxanthine. In the present study, hypoxanthine level in the two fish samples were within acceptable limits as reported by Oyelese (2012).

5.6 Storage of Frozen fish

This is aimed at maintaining the keeping quality of the fish. Storage performs a critical function since it allows for the period of availability of a certain product to be extended and at acceptable quality condition. This is necessary in view of the time lag between production and consumption of the commodity, particularly for perishable foods like fish.

This study found that facilities for storing fish were cold stores and deep freezers. All cold stores had standby power generators due to incessant power outage common to all the zones. Majority of cold stores however met the recommended temperature (-18^0C) for cold storage. The wholesalers were able to offer a product of fairly uniform quality for sale because of availability of good storage and transport facilities in all zones across the state. This supports the report of Nicol (1975) that the storage life of frozen fish depended upon the temperature of storage and any increase in temperature even for a very short time had a bad effect on the quality of the product.

The boxed or cartoned fish came in packs weighing between 10kg to 30kg depending on the species. Preservation functions were however undertaken by the wholesalers to reduce waste. The wholesaler sold to the retailers who in turn sold to the final consumers, hence the need for special attention on the quality of fish presented for sale. The retailer was the last link to the consumers. Retailers had permanent stalls in the market places while some hawked around for prospective

buyers. Majority of the retailers however operated a small volume of business; purchasing as much as they could sell per day.

Although the study indicated that there was less number of wholesalers dealing with large number of retailers, the wholesaler-retailer relationship operated on a system of minimum wastage and the storage facilities helped to maintain the frozen fish quality.

5.7 Daily Handling of Unsold fish by Retailers

Good handling and storage practices were needed by the retailers to ensure that the consumer received safe seafood that would keep for a reasonable time, was fresh in appearance and colour and palatable when consumed. The unsold fish was smoked, given out to attract customers or returned to the cold store at little or no cost. Charges however depended on the relationship with the owner or manager of cold store and the quantity of fish. The wholesalers gave preservative assistance to the retailers to enable them to sell on subsequent days. This is in agreement with the findings of Ladipo and Fabiyi (1986) that in frozen fish marketing, arrangement were usually made by wholesalers to enable the retailers return the left-over fish to the cold store for preservation.

The drip loss experienced in left-over fish was corroborated by Aitken *et al*., (1982) who posited that a badly stored fillet or fillets from a badly stored whole fish would feel hard, stiff and would release copious drip. Temporary rise in temperature of a few degrees could have a marked effect on quality or storage life of frozen fish (FAO, 1983).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

This research work which lasted for eighteen months investigated the handling, distribution and management, as it affects the keeping quality and shelf life of imported frozen fish products marketed in Oyo state, Nigeria. It was carried out in order to proffer solution to challenges in frozen fish marketing in Oyo state; and improve the quality of frozen fish consumed in Nigeria in general.

The result of analysis indicated that the wholesale business of fish was dominated by male, while the female dominated the retailing sector. The age distribution also showed that the population of youth in the fish retailing business is higher than in the fish wholesaling business. The wholesalers were older and better placed financially. The wholesalers were also educationally better positioned than retailers.

The medium of transportation by wholesalers was the refrigerated truck, while most retailers preferred motorcycle. The price of fish was determined by cost of purchase in both wholesale and retail sectors. The frozen fish was usually packaged in plastic film (bags) and transported in cartons or boxes of between 10kg to 30kg depending on species. The facilities for storing the products were cold stores and deep freezers and majority (65.67%) met the recommended temperature for cold storage. Imported frozen fish moved from the firm to wholesalers and then to the retailers and finally to the consumers.

The organoleptic properties of the examined samples indicated that the products were acceptable. However, closer chemical studies and microbial assessment proved that Oyo zone had the best keeping quality which was a function of the storage quality, which was also a function of the handling procedures of frozen fish distribution by stake holders in the zone as 86.0% of distributors stored their fish below -18^{0}C.

All chemical quality indices including TMA, FFA, Hx and PV had their values within acceptable international standards in the two frozen fish species examined.

Also, Total viable count (TVC), Total Staphylococcus count (TSC), Total haemolytic streptococcus count (THSC), Total lactic acid bacteria count (TLAB) and Total enterobacteriaceae count (TEBC) were all within the standard microbiological limit of 5.0 $x10^5$ and 1.0 $x10^6$ CFU g^{-1} across zones except for Total *Salmonella Shigella* count (TSSC) which was higher than the acceptable limit. However, for all the quality indices evaluate across zones, *Micromesistius poutassou* had higher values than *Sardinella* species, which implied that *M. poutasssou* had higher spoilage potential than *Sardinella* spp. in the study area.

Quality of frozen fish was best in Oyo zone, while Ogbomoso zone had the least quality amongst the four zones in the state.

Although all the fish samples were within acceptable limit for consumption and their threshold values in terms of microbial load, chemical and organoleptic assessment were not exceeded, the number of bacteria isolated showed that the quality of frozen fish did not reach expected standard in Oyo state, however these products did not constitute any health risk or hazard since they would still be properly cooked before consumption.

6.1 Contribution to Knowledge

At the end of this study, the following were achieved:

- It was established that handling and storage procedures affect the shelflife of frozen fish.
- It was also established that Oyo zone had the best quality frozen fish in Oyo state.
- Wholesalers of frozen fish in Oyo state were mainly men while retailers were all women.
- It was established that 65.67% of cold stores in Oyo state complied with -18^0C recommended temperature in frozen fish handling and distribution above which frozen fish quality is compromised.

- Consequences of not immediately cold storing left-over fish after daily sales were discovered; including accelerated quality deterioration of the fish and economic loss to retailers.

6.2 Recommendations

Generally, the consumer will pay more for the fish that he or she considers to be of good quality; and will continue to buy it as long as the quality remains constant. It is important that business men/women involved in getting fish from the site of primary production to the final consumer should have an awareness of the part they can play in maintaining or improving quality. For successful handling, distribution and marketing of frozen fish, the following points should be noted:

Advice to Producers

- It is necessary for the producers to be aware of the economic factors that affect quality, such as cost, supply and demand. Some improvements in quality can be effected at no extra cost.
- A high standard of hygiene must be maintained during the handling of raw materials at the pre-freezing stage.
- Freezing should be rapid so that the critical zone (-1 to -5^0C) is passed within a short time.
- Frozen fish should remain in storage at a temperature lower than -4^0C but preferably -18^0C to -30^0C, which should bring the quality parameters to a tolerable limit.
- Temperature fluctuation during storage, transportation, loading and unloading should be avoided.

Advice to the Government

- Since the objective of fish quality control is the protection of consumers' health, then government should recognize that they must assume ultimate responsibility for frozen fish quality.
- Government should organize fish sellers into cooperative bodies to enable them derive all possible assistance from the government.

- Government should ensure that public health problems arising from the consumption of fish products are controlled by national or local food laws, the enforcement of which should fall on official government inspectors.
- Government should assume responsibility for ensuring the operation of fair trading practices, which affect the fish industry in such matters as correct descriptions, labeling, weights and measures.
- Government should assume interventionist role by defining the minimum standards for frozen fish meant for human consumption; and ensure through the activities of Inspectors, that end products comply with these standards.

Advice to Wholesalers

- Cold store operators should record the product temperature of each lot of frozen fish received and accept custody only in accordance with good commercial practices.
- Each cold store should be of adequate capacity and be equipped with suitable mechanical refrigeration facilities to maintain a reasonably steady air temperature of -18^{0}C or lower.
- Each cold store should be equipped with two or more accurate and calibrated temperature-measuring devices. The temperature of each store should be recorded and dated each day.
- It is recommended that refrigeration equipment/facilities should include an audible or visual alarm system that will activate when refrigeration failure occurs.
- Frozen fish should be promptly moved through non-refrigerated load and unloading areas to minimize exposure to humidity, elevated temperatures or other adverse conditions.
- Store manager should not tender to a retailer any container which has been damaged or defaced to the extent that the frozen product is in unsellable condition.
- Store managers should dispose of stock on a first-in-first-out (FIFO) basis.

Advice on Transportation

- The cargo area of all vehicles used to transport frozen fish should be clean and free from debris, odour or any substance that could contaminate food.
- The refrigerated truck should be constructed, insulated and equipped with adequate refrigeration capacity to continuously maintain product temperature of -18^{0}C or lower.
- Refrigerated trucks should be equipped with tight fitting doors and suitable closures for drain holes to prevent air leakage.
- Refrigerated trucks should be pre-cooled to -18^{0}C prior to loading and the temperature monitoring device of the truck should be mounted in a readily visible location that can be conveniently read from outside the cargo area.
- The thermostat on the vehicle's refrigeration unit should be set to maintain a return air temperature of -18^{0}C or lower.

Advice to Retailers

- Retailers should maintain a steady temperature of -18^{0}C or lower.
- The display facilities should be regularly emptied, cleaned/sanitized with chlorinated water.
- Retailers should minimize the introduction of warm humid air into their freezers/display facilities by limiting the number of times they are opened.
- Non-frozen food should not be placed in display facilities containing frozen fish.
- Unsold fish should be well wrapped or sealed before placing them in storage facility.

Advice to Consumers

The decision about what constitute quality rests ultimately with the consumer. In order to maintain good health and prevent ill-health arising from fish consumption, the consumer must:

- Assume the role of the quality controller by ensuring that only fish of high quality is bought for consumption.
- Ensure that only properly cooked fish is consumed.

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APPENDIX 1

Compositon of media

Mac Conkey Agar (Biotec)

Composition	
Lactose	10.0g
Peptone mix	20.0g
Bile salt (sodium taurocholate)	5.0g
Neutral red	0.05g
Distilled water	1000ml.

Mannitol salt agar (Lab M)

Composition	
Beef extract	1.0g
Balanced Peptone No.1	10.0g
Sodium chloride	75.0g
D-Mannitol	10.0g
Agar No.2	12.0g
Phenol red (0.4%)	0.025g
Distilled water	1000ml

Potato Dextrose Agar (Lab M)

Composition	
Potato Extract	4.0g
Dextrose	20.0g
Agar No. 1	15.0g
Distilled water	1000ml

MRS Agar (de Man, Rogosa and Sharpe) (Lab M)

Composition	
Peptone	10.0g
Yeast extract	5.0g

Meat extract	10.0g
Glucose	20.0g
Sodium acetate	2.0g
Sodium acetate	5.0g
Ammonium citrate	2.0g
Magnesium sulphate	0.2g
Manganese sulphate	0.05mg
Tween 80	1.08ml
Agar no.1	15.0g
Distilled water	1000ml.

Nutrient Agar (Lab M)

Composition

Peptone	5.0g
Beef extract	3.0g
Sodium chloride	8.0g
Agar No.2	12.0g
Animal blood	500ml

Salmonella Shigella Agar (Lab M)

Composition

Beef extract	5.0g
Balanced peptone No. 1	5.0g
Lactose	10.0g
Bile salt No.3	8.5g
Sodium citrate	8.5g
Sodium thiosulphate	8.5g
Ferric citrate	1.0g
Brilliant green	0.00033g
Neutral red	0.025g

Agar No.2	13.5g

Plate Count Agar (Lab M)

Tryptone	5.0g
Yeast Extract	2.5g
Glucose	1.0g
Agar No.2	12.0g
Distilled water	1000ml.

Malt Extract Agar (Lab M)

Malt extract	30.0g
Mycological peptone	5.0g
Agar No. 2	15.0g

Peptone Water (Lab M)

Composition	
Sodium chloride	0.5g
Peptone (bacteriological)	1.0g
Distilled water	100ml

Gram's Crystal Violet

Solution A: Crystal violet	10.0g
Ethyl alcohol (95%)	100ml
Solution B: Ammonium oxalate	1.0g
Distilled water	100ml

Solution A and Solution B were mixed in equal ratio to give crystal violet

Gram's Iodine

Potassium iodine	2.0g
Iodine	1.0g
Distilled water	1000ml.

API 20E Reagents

NaCl (0.85%)	5ml
Mineral oil	25ml

Individual Reagents

TDA	5ml
JAMES	5ml
VP 1	5ml
VP 2	5ml
Oxidase	5ml

APPENDIX 2

Common frozen fish species found in Oyo state markets

Local name	Common name	Scientific name
Titus/ Alaran	Mackerel	*Scomber japonicus*
Agbodo	Bonga	*Etmalosa fimbriata*
Panla	Blue whiting	*Micromesistus poutassou*
Panla osan	Alaska/ hake	*Merluccius capensis*
Apo	Croaker	*Pseudotholitus species*
Kote	Horse mackerel	*Scomber scrombrus*
Sawa	Herring	*Sardinella species*
Epiya	Tilapia	*Tilapia species*
Pilchard	Pilchard	*Sardine pilchardus*
Express	Argentine hake	*Merluccius hubbsi*
Lady fish	Lady fish	*Albula species*

Source: Field survey (2011)

APPENDIX 3

Major Sources of Frozen Fish Supply to Oyo state (Lagos and Port Harcourt)

Names of frozen fish importing Firms
Stallion Fisheries
Seafood Products Limited
African Fish Company Limited
Onward Fisheries
Fiogret Fisheries Nigeria
Agro Allied Fisheries
Magulf Fisheries
Joma Foods
Globe Fishing
Agromar Fisheries
Fola Foods
Primlax Nigeria
CIC Fisheries
Lafigal Fisheries
Paramount Fisheries
Bharat Ventures
Premium Seafood Ltd
Unifish Ltd
Admiralty Overseas Nig. Ltd
Karflex fisheries Limited

Source: Field data (2011)

APPENDIX 4

UNIVERSITY OF IBADAN

DEPARTMENT OF WILDLIFE AND FISHERIES

ASSESSMENT OF QUALITY AND HANDLING PROCEDURES OF IMPORTED FROZEN FISH IN OYO STATE

QUESTIONNAIRE: WHOLESALER

A. GENERAL INFORMATION

1. Name of fish market ……..

2. Town ……..

3. L.G.A. ……..

4. State Of Origin ……

5. Gender: Male ( ) Female ( )

6. Age: Below 20yrs ( ) 21-30yrs ( ) 31-40 ( ) 41-50 ( ) 51-60 ( ) above 61 ( )

7. Marital Status: Single ( ) Married ( ) Widowed ( ) Divorced ( )

8. Level of Education: Non-formal Education ( ) First school Leaving Certificate ( ) WASC ( ) Grade II ( ) NCE ( ) First Degree ( ) Modern school ( ) Others ( ) Specify……

9. Vocational training apart from fish selling: Tailoring ( ) Carpentry ( ) Hair dressing ( ) Others ( ) specify…….

10. Size of household:(2) (3) (4) (5) (6) (7) (8) (9) (10) above 10 ( )

11. Number of children of involved in fish selling business? 1-2 ( ) 3-4 ( ) >4 ( ) None ( )

B. SOCIO-ECONOMIC CHARACTERISTICS

Experience of Fish Sellers in the Business

12. Which of the following are you engaged in? a. Fish retail ( ) b. Fish wholesale ( ) c. Middlemen ( ) d. Retail and wholesale ( ) e. Others ( ) specify ……

13. Length of time in fish wholesale business a. 1-5 yrs ( ) b. 6-10 yrs ( ) c. 11-15yrs ( ) d. 16yrs and above ( ).

14. Is fish selling a family business? Yes ( ) No ( )

15. If No, were you apprenticed before qualifying as a fish seller? Yes ( ) No ( )

16. If yes, how long was the training period? a. 1yr ( ) b.2yrs ( ) c. 3yrs and above ( )

17. To which of the following categories of buyers do you normally sell? A. fish retailers ( ) b. final consumers ( ) c. middlemen/agents ( ) d. hotels, hospitals, institutions, etc ( ) e. Others ( ) specify……..

18. Do you deposit/safe your money with Banks/Financial institutions? Yes ( ) No ( )

19. If Yes, which category of bank? a. commercial bank ( ) b. Microfinance ( ) c. agricultural bank ( ) d. Local credit e. Others ( ) specify …….

20. What is the source of your operating capital? a. personal savings ( ) b. commercial bank ( ) c. Microfinance bank ( ) d. Relatives ( ) e. Agric bank ( ) f. Money lender ( ) g. Non-governmental organization ( ) h. Government ( ) i. Others specify ……..

21. Have you ever obtained credit facilities? Yes ( ) N0 ( )

22. If Yes, how much? ……

23. How much did you spend/invest in your business/trade last year?…….

24. What is your annual income(s) from sales? ……..

25. How much do you realize per day/week/month? a ……/day b……../week c……../month.

26. At what interest rate do you procure credit? A. 5-10% ( ) b. 10-20% c. 20-30% ( ) d. 30-40% ( ) e. 50-100% ( )

27. What is the pay-back period for such credit? A. 6 months ( ) b. 1year ( ) c. 2year ( ) d. 3years and above ( )

C. GROUP ACTIVITIES

28. Do you belong to any organized interest group .g. Fish Sellers Association or Co-operative Society? Yes ( ) No ( )

29. If Yes, mention the name ……

30. How many years have you been a member of the association? ……..

31. If No, Why? .....

32. Do you give discount to your customers? Yes ( ) No ( )

33. If yes, to what categories of buyers? ……

34. Which of the following qualities influence the price(s) of fish? a. size ( ) b. taste ( ) c. absence/presence of bones ( ) d. colour ( ) e. freshness ( ) f. Others ( ) specify…….

35. Which of these influences the price(s) you fix for fish each day? a. supply ( ) b. demand ( ) c. cost of purchase ( ) d. transportation cost ( ) e. processing cost ( ) f. Others ( ) specify……

36. Do you buy or sell on credit? Yes ( ) No ( )

37. If Yes, to what categories of buyers? a. Retailers ( ) b. middlemen/agents ( ) c. hotels/institutions d. Others ( ) specify …….

38. Which of the following is the source of information about price, supply and demand? a. fellow trader ( ) b. middlemen ( ) c. mobile phone ( ) d. No response ( ) e. Others ( ) specify……..

E. FISH TRANSPORTATION, HANDLING AND PHYSICAL FACILITIES

39. Where is the source of your fish supply? a. Lagos ( ) b. Warri ( ) c. Port Harcourt ( ) d. Ibadan ( ) e. Others ( ) specify ……..

40. What means do you favour most in transporting your fish to the cold store? a. Refrigerated truck ( ) b. Taxi cab ( ) c. Motor cycle ( ) d. Head load ( ) e. Wheel barrow ( ) f. No response ( )

41. What is the temperature of the RefrigeratedTruck? …….

42. What is the capacity of your cold store? ……..

43. At what temperature do you store your fish in the cold store?
……

44. Do you wear insulated clothing into the cold stores/ refrigerated truck Yes ( ) or No ( ).

45. What is the duration of storage of your fish supply? a. 1-2weeks ( ) b. 3-4weeks ( ) c. 1-2months ( ) d. 3-4months ( ) e. Other, specify ……

46. How long is the period of power outage in the cold store? a. Less than 1 hour ( ) b.2-3 hours ( ) c. 3-4hours ( ) d. 5hours and above ( )

47. Itemize the species of fish available in your cold store

……
……
……
……
……....

48. Rank by ticking (highest (1) and lowest (5) the following problems in order of their importance as they affect your cold store.

Problems	1	2	3	4	5
Inconsistent power(electricity)					
Scarcity of frozen fish					
Problem of debt by customers					
Cost of transportation					
High cost of fuel					
Lack of technical personnel					

49. Give the number of agents/middlemen in your cold store? ……

50. Rate the condition of your cold store?

a. Very good ( ) b. good ( ) c. fair ( ) d. bad ( ) e. very bad ( )

APPENDIX 5

UNIVERSITY OF IBADAN

DEPARTMENT OF WILDLIFE AND FISHERIES

ASSESSMENT OF QUALITY AND HANDLING PROCEDURES OF IMPORTED FROZEN FISH IN OYO STATE

QUESTIONNAIRE: RETAILER

A. GENERAL INFORMATION

1. Name of fish market ……..

2. Town ……..

3. L.G.A. ……..

4. State Of Origin ……

5. Gender: Male ( ) Female ( )

6. Age: Below 20yrs ( ) 21-30yrs ( ) 31-40 ( ) 41-50 ( ) 51-60 ( ) above 61 ( )

7. Marital Status: Single ( ) Married ( ) Widowed ( ) Divorced ( )

8. Level of Education: Non-formal Education ( ) First School Leaving Certificate ( ) WASC ( ) Grade II ( ) NCE ( ) First Degree ( ) Modern school ( ) Others ( ) Specify……

9. Vocational training apart from fish selling: Tailoring ( ) Carpentry ( ) Hair dressing ( ) Others ( ) specify…….

10. Size of household:(2) (3) (4) (5) (6) (7) (8) (9) (10) above 10 ( )

11. Number of children of fish seller involved in fish selling business? 1-2 ( ) 3-4 ( ) >4 ( ) None ( )

B. SOCIO-ECONOMIC CHARACTERISTICS

Experience of Fish Sellers in the Business

12. Which of the following are you engaged in? a. fish retail ( ) b. fish wholesale ( ) c. middlemen ( ) d. retail and wholesale ( ) e. Others ( ) specify
…….

13. Length of time in fish retail business A. 1-5yrs ( ) B. 6-10yrs ( ) C. 11-15yrs ( ) D. 16yrs and above ( )

14. Is fish selling a family business? Yes ( ) No ( )

15. If no, were you apprenticed before qualifying as a fish seller? Yes ( ) No ( )

16. If yes, how long was the training period? A.1yrs ( ) B. 2yrs ( ) C. 3yrs and above ( )

17. Do your children attend school? Yes ( ) No ( )

18. If yes, what level of education have they attended A. Primary school ( ) B. Secondary( ) C. Tertiary institution ( ) D. Combination ( )

19. If No, are they apprenticed for a trade? Yes ( ) No ( )

20. What quantity of fish do you purchase daily for retail?.....

21. Are you involved in any local financial contribution? Yes ( ) No ( )

22. If Yes, how much do you safe daily A. Nil ( ) B. < N100.00 ( ) C. N100-N249.00 ( ) D. N250- N499.00 ( ) E. >N500.00 ( )

23. Do you dposit/save your money with banks Yes ( ) No ( )

24. If Yes , which bank? A.Commercial bank ( ) B. Micro finance bank ( ) C.Relatives ( ) D. local credits ( ) E. Others ( ) specify…….

25. What is the source of your operating capital? A. Personal savings ( ) B. Commercial bank( ) C.Microfinance bank ( ) D. Relatives( ). Local credits ( ) F. Money lender ( ) G. Non-governmental organization ( ) H. Government ( ) I. Others ( ) specify……

C. GROUP ACTIVITIES

26. Do you belong to any organized interest group .g. Fish Sellers Association or Co-operative Society? Yes ( ) No ( )

27. If Yes, mention the name of the association……
…

28. Is membership of the association compulsory in your market? Yes ( ) No ( )

29. How does a new entrant into the trade secure the membership of the association?
.....
..

30. How many years have you been a member of association?
.....

31. Reason(s) for joining the association

A. Activities during burial ceremony ( )B. help members in difficulty ( )

C. general welfare of members ( ) D. Others Specify……

D. PRICING POLICY

32. Which of these qualities influence the price(s) of fish? A. Size ( ) B. Taste ( ) C. Absence /Presence of bones ( ) d. Socio- culture e. Colour ( ) f. freshness ( ) g. Others ( ) Specify …….

33. How do you settle the selling of price to a particular fish or quantity of fish? A. By weight ( ) B. By feel of hand ( ) C. By haggling ( ) D. Intuition ( ) E. Other methods ( ) specify……..

34. Which of these influences the daily price of fish? A. Supply ( ) B. demand ( ) C. Cost of purchase ( ) D. Transportation cost ( ) E. Processing cost ( ) F. Others ( ) specify ……..

35. Does your association fix a price for its members? Yes ( ) No ( )

36. Which of the following is the source of information about price, supply and demand? A. fellow trader ( ) B. Middlemen ( ) C. Mobile phone ( ) D. No response

E. SALES OUTLET

37. Which of the following method employ in selling the fish? A. direct supply to the people/agencies ( ) B. Public hawking ( ) C. Road side sales ( ) D. Fish market stalls ( ) E. Others ( ) specify ……..

38. On which of the following do you display your fish for sale? A. Wooden table ( ) B. Raised cement slab ( ) C. Bowls ( ) D. Wooden board ( ) E. On the ground spread on large leaves/papers ( ) F. Others ( ) specify ……

39. How do you attract your customers? A. Conspicuous display ( ) B. Granting of credit and discount ( ) C. Calling attention of prospective customers ( )

F. PHYSICAL FACILITIES /FISH HANDLING PARAMETERS

40. What is the means of transporting your fish to the market? A. truck ( ) b. Taxi cab ( ) C. motorcycle ( ) D. head load ( ) E. Wheel barrow ( )

41. how do you handle unsold fish products at the end of each day? A. Smoke ( ) B. Refrigerate ( ) C. Give out ( ) D. Cook and eat at home ( ) E. Others ( ) specify……..

42. Is there any storage facility in the fish market? Yes ( ) No ( )

43. If yes, how much do you pay for such storage facilities? …….

44. Do you always sell left over fish from the previous day, on the following day? Yes ( ) No ( )

45. Who keeps the fish stall clean? A. fish retailers ( ) B. hired labour ( ) C. individual retailer clean his/her own part of the stall ( ) D. Local government officials ( )

46. How often in a week do you wash your display facility? A. Daily ( ) b. Once ( ) C. Twice ( ) D. Thrice ( ) E. None ( ) F. Others ( ) specify
……

47. Do you use chlorinated water for washing? Yes ( ) No ( )

48. Are you satisfied with the service at the cold store? Yes ( ) No ( )

49. What are the things you think should be put in place to improve service rendered to you at the cold store?

50. How do you want to rank the condition of the cold store? A. Very good ( ) B. Good ( ) C. Fair ( ) D. Bad ( ) E. Very bad ( ).

APPENDIX 6

LIST OF FUNCTIONAL COLD STORE OPERATORS IN OYO STATE

IBADAN/IBARAPA ZONE

S/N	Name & address	LGA	Owner	Capacity (tonnes)
1	Ade Usat, Oje	Ibadan North	Alhaja Afusat Adenekan	20
2	Ade Usat, Bodija market	Ibadan North	Alh. Afusat Adenekan	4
3	Bola fisheries, Sango	"	Alh. Mukaila Bolarinwa	12
4	Layi frozen foods, Sango	"	Mr. Olayiwola	20
5	Balanced diet foods, Mokola	"		35
6	Balanced diet foods, Eleyele	"		75
7	Ekimis, Yemetu	"	Mr.Meriki	200
8	3TS, Yemetu	"	Alh. Abiodun atolani	30
9	Golden Orchard	"	Alh. Olayiwola Ademola	30
10	Ade Usat, Oja Oba	Ibadan S/W	Alh.Afusat Adenegan	44
11	Sharko fisheries, Oke Ado	"	Mr. Michael Apanpa	20
12	Kollington fisheries, Oke Ado	"	Chief kolawole Adeleye	135
13	Blemmy fisheries, Idi Arere	" Ibadan S/E	Mr. Folorunso Okeowo	8
14	Ekimiks, Idi Arere	"	Mr. Meriki	20
15	Blemmy fisheries Popo Yemoja	Ibadan N/W	Mr. Folorunso Okeowo	8
16	Kollington fisheries, Idi Ikan	"	Chief Kolawole Adeleye	10
17	Lazmos. Basorun	Ibadan N/E	Mrs. C. Lasisi	12
18	Favour frozen fish, Ogbere	Ona ara	Pastor Dele Ogunyemi	3
19	Progress Seafoods, Ogbere	"	Alaja Simiat Jimoh	130
20	Ade Usat, Olorunsogo	"	Alaja Afusat Adenegan	40
21	Yaishol frozen fish, Olodo	Lagelu	Alh Y.A. Ishola	750
22	Ade Usat, Monatan	"	Alaja Afusat Adenegan	12
23	Akinwale & Sons, Monatan	"	Alh. A.O. Akinwale	750
24	Morounfolu Nig. Enterprise Akingbile	Akinyele	Mr. B. O. Okunoye	16
25	Ekimiks, Ojoo	Akinyele	Mr. meriki	12
26	Alhaja Risikat frozen foods, Ojoo	Akinyele	Alh. Risikat Shittu	8
27	Supreme frozen foods, Apata	Ido	Mrs. B.O.Falade	20
28	Ade Usat, Apata	Ido	Alaja Afusat Adenegan	7

29	Bharat Seafoods New Ife road	Egbeda	Bharat ventures	500
30	Kollington Fisheries, Gbagi	Egbeda	Chief Kolawole Adeleye	1,900
31	CIC, Podo	Oluyole	CIC Limited	2,400
32	Ekimiks, Orita Challenge	Oluyole	Mr. Meriki	100
33	Owotutu Investment, Molete, Ayete	Ibarapa North	Alh. Fasasi Adesope	14
34	Owotutu Investment, Igangan	"	"	12
35	Owotutu Investment, Sagoun, Igboora	Ibarapa central	"	20
36	Owotutu Investment, Paako,Igboora	"	"	12
37	Owotutu Investment, Ita Bale, Igbora	"	"	10
38	Owotutu . Towobowo, Eruwa	Ibarapa East	"	12
39	Owotutu investment, Sango, Eruwa	"	"	20

OGBOMOSO ZONE

S/N	Name & Address	LGA	Owner	Capacity (tonnes)
1	Bol-Raib Investment, Aroje	Ogbomoso North	Alh. Y.A.Ishola	500
2	Oluade resources. Owode	"	Mr. Segun Ogundijo	15
3	Adefak Ventures,Tara,	"	"	12
4	Sola-Funmi frozen fish. Taki	"	Pastor & Mrs Solafunmi Ogunniyi	15
5	Sibesibe fisheries, Bolanta	"	Alh. Muraina Ajagbe	14
6	Sibesibe fisheries, Oke Owode	"	"	84
7	MOAA ventures, Sabo	"	"	14
8	Obanijesu ventures, Aje	"	Mr. Sola Adekola	12
9	Adunbu Nig. Ltd.General hosp	"		10
10	Success frozen food, Sabo	"	Mr. Rufus Adebayo	8
11	Ajikeola Nig. Ent. Ilorin road	"	Alh. S. Oladimeji	150
12	Mayowa frozen foods, Agboin	"	Mr. Isaac Ilesanmi	15
13	Akeju fisheries, Sunsun	Ogbomoso south	Mr. Temitope Alalade	10
14	Sibesibe fisheries, Ayegun	"	Alh. Muraina Ajagbe	7
15	Ekundayo fisheries, Maternity area	"	Elder David Ogundijo	100
16	Anuoluwapo fisheries, Caretaker	"	Mr. Rashidi	12

OYO ZONE

S/N	Name & Address	LGA	Owner	Capacity (tonnes)
1	Ibru seafoods,Owode	Oyo west	Ibru Seafoods	4
2	Al barka frozen fish,Idiope	"	Mr Kabir Akano	6
3	Eagle coldstore, Isokun	"	Mr. Dele Bolaji	10
4	Mercy frozen fish, Durbar	Oyo east	Mr. Adebisi Josiah	2
5	Phalom seafoods,Oke esa	Iseyin	Mrs Obiorah	15
6	Mastass ventures	"	Alhaja. R.O. Bello	24
7	Aduratimisola	"	Mr. Johnson Abiodun	13

SAKI ZONE

S/N	Name & Address	LGA	Owner	Capacity (tonnes)
1	Ogo Oluwa frozen foods, Ajegunle	Saki west	Abraham Akins & Sons	14
2	Albarka frozen foods, Igboro	"	Mr. Kabir Akano	18
3	Farayola coldstore, Isiya	Kajola	Mr. Farayola	7
4	Gbanigbani frozen foods	"	Alh. Hamzat Gbanigbani	20
5	Mumini frozen depot, Oja oba	"	Mr. Mumini Tijani	2

APPENDIX 7

Identification Reading Table for API 20E

Tests	**Active ingredients**	**Quantity**	**Reactions-enzymes**	**Result**	
				Negative	**Positive**
ONPG	2-nitrophnyl-BD-galactopyranoside	0.223	Ortho-Nitrophenyl-BD-galactopyranosidase	Colourless	Yellow
ADH	L-arginine	1.9	Arginine DiHydrolase	Yellow	Red-orange
LDC	L-lysine	1.9	Lysine Decarboxylase	Yellow	Red-orange
ODC	L-omithine	1.9	Omithine Decaboxylase	Yellow	Red-orange
CIT	Trisodium citrate	0.756	CITrate utilization	Pale green-yellow	Blue-green
H_2S	Sodium thiosulphate	0.075	H_2S production	Colourless-greyish	Blck deposit-thin line
URE	Urea	0.76	UREase	Yellow	Red-orange
TDA	L-Tryptophan	0.38	Tryptophane DeAminase	Yellow	Reddish-brown
IND	L-Tryptophan	0.19	INDole production	Colourless-pale-green-yellow	Pink
VP	Sodium pyruvate	1.9	Acetone production (Voges Proskauer)	Colourless-pale pink	Pink red
GEL	Gelatin(bovine origin)	0.6	GELatinase (GELatine)	No diffusion	Diffusion ofblack pigment
GLU	D-glucose	1.9	GLUcose	Blue-blue green	Yellow-greyish yellow
MAN	D-mannitol	1.9	MANnitol	Blue-blue green	Yellow
INO	Inositol	1.9	INOsitol	Blue-blue green	Yellow
SOR	D-sorbitol	1.9	SORbitol	Blue-blue gren	Yellow
RHA	L-Rhamnose	1.9	RHAmnose	Blue-blue green	Yellow
SAC	D-sucrose	1.9	SACharose	Blue-blue green	Yellow
MEL	D-Melibiose	1.9	MELibiose	Blue-blue green	Yellow
AMY	Amygdaline	0.57	AMYgdaline	Blue-blue green	Yellow
ARA	L-arabinose	1.9	ARAbinose	Blue-blue green	Yellow

APPENDIX 8

WHOLESALERS RESPONSE TO THEIR FINANCIAL STATUS

Table II: The amount of loan obtained from the bank

Amount		Zone			Total	Percentage
	Ibadan/Ibarapa	Ogbomoso	Oyo	Saki		
LessN1million	9	5	0	0	14	20.9
N1-N2million	0	0	1	0	1	1.5
N2-N5million	0	0	0	0	0	0
N5-N10million	6	0	0	0	6	8.9
No response	**24**	**11**	**6**	**5**	**46**	**68.7**
Total	39	16	7	5	67	100

Table III: Amount spent on business in previous year

Amount		Zone			Total	Percentage
	Ibadan/Ibarapa	Ogbomoso	Oyo	Saki		
LessN1million	0	2	0	0	2	3.0
N1-N2million	0	2	2	0	4	6.0
N2-N5million	2	1	0	0	3	4.5
N5-N10million	8	3	2	0	13	19.4
No response	**29**	**8**	**3**	**5**	**45**	**67.1**
Total	39	16	7	5	67	100.0

Table IV: Amount realized daily/weekly

Amount		Zone			Total	Percentage
	Ibadan/Ibarapa	Ogbomoso	Oyo	Saki		
<N300,000.00	0	5	1	0	6	9.0
<N500,000.00	0	1	0	0	1	1.5
N1-N2m	4	0	0	0	4	6.0
N2-N5m	13	0	0	0	13	19.4
No response	**22**	**10**	**6**	**5**	**43**	**64.1**
Total	39	16	7	5	67	100.0

Table V: The interest rate on the loan

		Zone			Total	Percentage
	Ibadan/Ibarapa	Ogbomoso	Oyo	Saki		
5-10%	6	3	1	0	10	14.9
10-20%	9	1	0	0	10	14.9
20-30%	0	3	0	0	3	4.5
50-100%	0	1	0	0	1	1.5
No response	**24**	**8**	**6**	**5**	**43**	**64.2**
Total	39	16	7	5	67	100.0

Table VI: Terms of interest on the loan obtained

		Zone			Total	Percentage
	Ibadan/Ibarapa	Ogbomoso	Oyo	Saki		
1 year	12	4	1	1	18	26.9
2 years	1	1	0	0	2	2.9
No response	**26**	**11**	**6**	**4**	**47**	**70.2**
Total	39	16	7	5	67	100.0

APPENDIX 9

Template of Score sheet for Organoleptic Assessment by Taste Panel

Months & Parameters	**Sample**	**1st**	**2nd**	**3rd**	**4th**	**5th**	**6th**
Taste (cooked)	*Sardinella* species						
	Micromesistus poutassou						
Odour	*Sardinella* species						
	Micromesistus poutassou						
Texture	*Sardinella* species						
	Micromesistus poutassou						
Appearance	*Sardinella* species						
	Micromesistus poutassou						
Colour	*Sardinella* species						
	Micromesistus poutassou						

KEY

7- Very much liked , 6- Liked alot, 5- Liked, 4- Liked and did not like, 3- Disliked, 2- Much disliked, 1- Very much disliked

APPENDIX 10
FISH PRICE BY STATES (2007 - 2011)

States	2007			2008			2009			2010			2011		
	FRESH	SMOKED	FROZEN	FRESH	SMOKED	FROZEN	FRESH	SMOKED	FROZEN	FRESH	SMOKED	FROZEN	FRESH	SMOKED	FROZEN
ABIA	450.00	1,080.00	350.00	400.00	1,015.00	300.00	385.00	1,000.00	290.00	516.00	831.00	340.00	800.00	1,400.00	600.00
ADAMAWA	650.00	1,050.00	280.00	512.50	1,015.00	277.50	500.00	965.00	230.00	659.00	802.00	285.00	700.00	750.00	630.00
AKWA IBOM	550.00	1,000.00	295.00	450.00	850.00	247.50	450.00	755.00	235.00	450.00	800.00	400.00	800.00	1,200.00	670.00
ANAMBRA	600.00	1,050.00	350.00	500.00	925.00	318.50	465.00	895.00	250.00	700.00	993.00	427.00	750.00	1,400.00	700.00
BAUCHI	545.00	950.00	300.00	497.50	900.00	300.00	885.00	1,150.00	330.00	700.00	993.00	427.00	750.00	1,400.00	750.00
BAYELSA	520.00	980.00	330.00	520.00	855.00	272.50	620.00	1,100.00	310.00	450.00	830.00	448.00	850.00	1,350.00	680.00
BENUE	500.00	1,100.00	370.00	450,00	1,000.00	340.00	555.00	1,145.00	385.00	710.00	930.00	452.00	800.00	1,000.00	600.00
BORNO	457.00	900.00	400.00	416.00	832.50	350.00	575.00	1,010.00	375.00	743.00	909.00	426.00	750.00	1,300.00	620.00
CROSSRIVER	600.00	950.00	350.00	500.00	875.00	304.00	620.00	1,075.00	345.00	550.00	971.00	423.00	800.00	1,200.00	660.00
DELTA	500.00	1,100.00	350.00	475.00	951.00	325.00	560.00	1,150.00	355.00	500.00	798.00	445.00	820.00	1,300.00	620.00
EDO	550.00	1,050.00	320.00	525.00	932.50	320.00	465.00	935.00	340.00	450.00	634.00	448.00	750.00	1,200.00	635.00
EBONYI	470.00	1,00.00	375.00	445.00	862.00	375.00	385.00	755.00	335.00	600.00	714.00	387.00	820.00	1,100.00	670.00
EKITI	500.00	980.00	300.00	425.00	900.00	299.00	410.00	860.00	275.00	450.00	687.00	323.00	750.00	1,000.00	600.00
ENUGU	502.00	950.00	302.00	458.50	882.50	303.00	430.00	825.00	250.00	600.00	883.00	361.00	700.00	1,400.00	650.00
GOMBE	550.00	1,000.00	300.00	445.00	945.00	325.00	535.00	1,075.00	320.00	650.00	964.00	350.00	740.00	1,200.00	690.00
MO	389.00	950.00	300.00	434.50	965.00	257.00	440.00	1,120.00	300.00	650.00	825.00	392.00	900.00	1,300.00	710.00
JIGAWA	612.00	1,100.00	320.00	563.50	1,000.00	300.00	530.00	965.00	285.00	660.00	907.00	446.00	750.00	800.00	680.00
KADUNA	650.00	1,050.00	300.00	585.50	985.00	275.00	675.00	1,075.00	310.00	430.00	975.00	388.00	700.00	800.00	650.00
KANO	479.00	1,000.00	300.00	439.50	990.00	267.50	500.00	1,150.00	300.00	625.00	643.00	405.00	750.00	900.00	700.00
KATSINA	485.00	1,000.00	300.00	467.50	875.00	293.50	465.00	780.00	310.00	620.00	900.00	345.00	800.00	1,000.00	700.00
KEBBI	490.00	1,000.00	315.00	451.50	850.00	296.50	510.00	1,050.00	275.00	630.00	695.00	360.00	750.00	800.00	680.00
KOGI	600.00	1,200.00	320.00	525.00	1,005.00	317.50	480.00	830.00	285.00	620.00	710.00	360.00	750.00	1,000.00	660.00
KWARA	520.00	980.00	290.00	511.00	897.50	270.50	440.00	860.00	255.00	650.00	618.00	364.00	800.00	1,100.00	650.00
LAGOS	500.00	1,200.00	265.00	530.00	952.00	270.50	450.00	750.00	260.00	400.00	703.00	392.00	700.00	1,200.00	550.00
NASSARAWA	489.00	1,220.00	300.00	502.00	995.00	294.00	500.00	825.00	270.00	600.00	671.00	375.00	800.00	1,000.00	800.00
NIGER	520.00	980.00	315.00	510.00	880.00	307.50	445.00	785.00	296.00	550.00	812.00	396.00	800.00	1,200.00	680.00
OGUN	600.00	1,150.00	280.00	535.00	915.00	298.50	595.00	1,185.00	285.00	400.00	716.00	377.00	755.00	1,000.00	655.00
ONDO	612.00	1,200.00	300.00	566.00	947.00	295.00	550.00	900.00	265.00	650.00	689.00	650.00	780.00	1,300.00	690.00
OSUN	700.00	980.00	285.00	550.00	846.00	295.00	520.00	785.00	240.00	400.00	720.00	298.00	850.00	1,250.00	650.00
OYO	720.00	900.00	280.00	627.00	793.00	285.00	620.00	860.00	320.00	400.00	997.00	459.00	700.00	1,200.00	610.00
PLATEAU	505.00	950.00	300.00	477.50	850.00	300.00	670.00	1,145.00	330.00	600.00	895.00	427.00	820.00	1,000.00	680.00
RIVERS	698.00	1,200.00	250.00	602.50	958.50	265.00	730.00	1,075.00	255.00	650.00	810.00	369.00	700.00	800.00	620.00
SOKOTO	702.00	850.00	300.00	596.00	750.00	257.50	570.00	935.00	310.00	650.00	863.00	448.00	800.00	950.00	600.00
TARABA	450.00	1,000.00	245.00	400.00	890.00	247.50	480.00	1,050.00	290.00	550.00	926.00	366.00	680.00	980.00	640.00
YOBE	500.00	890.50	250.00	437.50	905.25	222.50	550.00	1,100.00	295.00	600.00	926.00	402.00	750.00	1,380,00	635.00
ZAMFARA	520.00	900.00	330.00	432.50	925.00	265.00	480.00	865.00	285.00	600.00	744.00	348.00	820.00	1,250.00	650.00
FCT	600.00	1,050.00	281.50	550.00	950.00	268.75	535.00	1,320.00	375.00	700.00	1,108.00	536.00	800.00	1,000.00	600.00

Source: Federal Ministry of Agriculture and Rural Development, Federal Department of Fisheries (2012)

APPENDIX 11

Mean Concentration of Proximate Analysis in Imported Frozen Fish samples collected across zones

Species	Month	Moisture Content	Ash content	Crude Protein	Crude Fibre	Ether Extract
Sardinella	1	64.63±1.28	2.86±0.03	15.60±1.14	1.79±0.19	16.19±0.89
species	2	64.15±1.15	2.25±0.07	15.75±1.42	1.41±0.10	9.58±0.80
	3	66.16±0.75	1.83±0.08	16.28±0.46	1.16±0.09	7.90±0.77
	4	58.48±1.98	1.95±0.15	13.75±0.63	1.94±0.11	6.91±0.35
	5	40.76±3.35	2.89±0.20	14.29±0.38	1.55±0.08	4.38±**050**
	6	62.39±0.62	1.63±0.05	12.08±0.24	1.15±0.04	3.02±0.16
Micromesistius	1	62.60±1.33	2.43±0.02	13.11±0.63	2.52±0.13	3.96±0.17
Poutassou	2	62.67±1.07	2.14±0.07	13.59±0.37	2.31±0.12	3.47±0.48
	3	68.78±0.40	1.78±0.10	17.26±0.61	1.65±0.12	2.84±0.19
	4	64.31±2.01	2.27±0.10	15.67±0.37	2.51±0.23	3.96±0.74
	5	54.53±1.48	2.39±0.13	16.02±0.26	1.74±0.14	3.52±0.61
	6	61.96±0.41	1.56±0.07	12.91±0.08	1.17±0.05	2.99±0.04

T-test for Total Mean Value of Proximate Analysis in *Sardinella species* and *Micromesistus poutassou* across zones

Parameter	Sample	N	$\bar{x} \pm SEM$	df	t-value	P level	(<0.05)
Moisture content	*Sardinella spp.* *M. poutasssou*	24	59.43±1.92 62.48±0.99	46	1.410	0.165	(>0.05)
Ash content	*Sardinella spp.* *M. poutasssou*	24	2.23±0.11 2.9±0.08	46	-1.046	0.301	(>0.05)
Crude protein	*Sardinella spp.* *M. poutasssou*	24	14.62±0.42 14.76±0.38		0.237	0.814	(>0.05)
Crude fibre	*Sardinella spp.* *M. poutasssou*	24	1.50±0.07 1.98±0.12		3.523	0.001	(<0.05)
Ether extract	*Sardinella spp.* *M. poutasssou*	24	7.99±0.92 3.46±0.19		-4.854	0.000	(<0.05)

APPENDIX 12

Table 1.1: Projected Human Population, Fish Demand and Supply in Nigeria, 2000-2015.

Year	Projected population (millions)	Projected fish demand (tonnes)	Projected domestic fish supply (tonnes)	Deficit (tonnes)
2000	114.4	1,430,000	467,0980.00	962,902.00
2001	117.6	1,470,000	480,163.60	989,836.40
2002	121	1,412,500	507,928.20	1,004,572.00
2003	124.4	1,555,000	522,627.10	1,032,373.00
2004	128	1,600,000	536,917.60	1,063,082.40
2005	131.5	1,643,750	552,533.10	1,091,317.00
2006	135.3	1,691,250	567,948.60	1,123,301.00
2007	139.1	1,738,750	583,872.40	1,154,878.00
2008	143	1,787,500	600,612.80	1,186,887.00
2009	147.1	1,838,750	617,353.20	1,221,397.00
2010	151.2	1,890,000	634,560.20	1,225,440.00
2011	155.5	1,943,750	652,606.60	1,291,143.30
2012	160	2,000,000	671,492.30	1,328,508.00
2013	164.4	2,055,000	689,958.00	1,365,042.00
2014	169.1	2,113,750	707,683.10	1,404,067.00
2015	174	2,175,000	730,248.00	1,444,752.00

Source: Federal Department of Fisheries (FDF), Abuja 2007.

APPENDIX 13

Total Bacteria Count of Imported Frozen Fish across the Four Zones in Oyo State

Fish sample	Zones	TVC (cfu/g)	TSSC (cfu/g)	THSC (cfu/g)	TLAB (cfu/g)	TEBC (cfu/g)	TSC (cfu/g)
Sardinella	Ibadan/Ibarapa	$1.7x10^5$	$5.5 x10^3$	$3.0 x10^4$	$4.8 x10^3$	$1.4 x10^4$	$2.9 x10^2$
Species	Ogbomoso	$3.1 x10^5$	$6.9 x10^3$	$3.1 x10^4$	$3.7 x10^3$	$3.1 x10^4$	$1.0 x10^3$
	Oyo	$1.2 x10^5$	$4.1x10^3$	$3.5 x10^4$	$6.8 x10^3$	$1.2 x10^4$	$3.0 x10^3$
	Saki	$1.4 x10^5$	$5.9 x10^3$	$2.8 x10^4$	$3.8 x10^3$	$2.2 x10^4$	$1.7 x10^3$
Micromesistius	Ibadan/Ibarapa	$2.6 x10^5$	$7.5 x10^3$	$3.1 x10^4$	$8.6 x10^3$	$2.3 x10^4$	$6.6 x10^3$
Poutassou	Ogbomoso	$3.2 x10^5$	$9.5 x10^3$	$3.5 x10^4$	$9.5 x10^3$	$4.1 x10^4$	$1.5 x10^3$
	Oyo	$1.5 x10^5$	$4.9 x10^3$	$3.5 x10^4$	$3.1 x10^3$	$2.6 x10^4$	$1.0 x10^3$
	Saki	$2.2 x10^5$	$1.0 x10^4$	$3.2 x10^4$	$6.7 x10^3$	$3.4 x10^4$	$1.1 x10^3$

TVC: Total Viable Count

TSSC: Total Salmonella Shigella Count

THSC: Total Haemolytic Streptococci Count

TLAB: Total Lactic acid bacteria Count

TEBC: Total Enterobacteriaceae Count

TSC: Total Staphylococcus Count

Source: Field data (2011)

APPENDIX 14

Total Fungal load of imported Frozen Fish across the Four Zones in Oyo state

Fish sample	Zones	Fungal load (cfu/g)
Sardinella species	Ibadan/Ibarapa	1.6×10^5
	Ogbomoso	1.8×10^5
	Oyo	7.9×10^4
	Saki	1.3×10^5
Micromesistius poutassou	Ibadan/Ibarapa	2.4×10^5
	Ogbomoso	2.3×10^5
	Oyo	1.4×10^5
	Saki	2.4×10^5

Source: Field data (2011)

APPENDIX 15

Mean Concentration of Chemical Parameters in Imported Frozen Fish Samples Collected across Zones.

Species	Zones	PV (meq/kg)	TMA (mg/100g)	FFA (%)	Hx (mg/100g)
Sardinella species	Ibadan/Ibarapa	18.52±0.55	23.07± 0.65	1.52± 0.14	24.48± 0.56
	Ogbomoso	18.83 ±0.60	23.63± 0.60	1.85 ±0.31	24.47± 0.77
	Oyo	17.48 ±0.81	20.58 ±0.90	1.73± 0.27	25.54 ±0.41
	Saki	18.50 ±1.69	22.79± 3.02	1.53± 0.17	22.15± 1.04
Micromesistius poutasssou	Ibadan/Ibarapa	19.47 ±0.42	22.96 ±0.49	1.38 ±0.07	25.49 ±0.65
	Ogbomoso	19.56± 0.75	19.89 ±1.66	1.82± 0.19	25.69 ±1.01
	Oyo	17.90 ±0.60	19.03± 0.65	1.69± 0.22	25.96± 0.53
	Saki	19.79± 1.03	21.79 ±3.32	1.67 ±0.19	25.77 ±0.49

Source: Field data (2011)

APPENDIX 16

Mean hedonic scores for *Sardinella* species and *Micromesistius poutassou* across the zones

Species	Zone	Taste (cooked)	Odour	Texture	Appearance	Colour	Overall acceptability (Organoleptic)
Sardinella	Ibadan/Ibarapa	5.83±0.16	5.75±0.17	5.67±0.14	5.50±0.23	5.25±0.25	5.60±0.15
species	Ogbomoso	6.17±0.16	5.83±0.16	6.00±0.25	5.67±0.21	5.33±0.42	5.80±0.12
	Oyo	5.67±0.21	5.67±0.21	5.50±0.22	5.33±0.33	5.17±0.40	5.47±0.20
	Saki	6.17±0.30	5.83±0.16	6.00±0.00	5.83±0.16	5.67±0.21	5.90±0.08
Micromesistius	Ibadan/Ibarapa	5.75±0.25	5.67±0.18	5.67±0.18	5.67±0.18	5.42±0.22	5.63±0.11
poutassou	Ogbomoso	6.17±0.16	5.67±0.21	5.50±0.22	5.33±0.21	5.33±0.21	5.60±0.13
	Oyo	6.00±0.25	5.83±0.16	5.50±0.22	5.50±0.22	5.00±0.00	5.57±0.13
	Saki	5.83±0.30	6.00±0.25	5.67±0.21	5.67±0.21	5.83±0.16	5.80±0.10

Source: Field data (2011)