BIOCHEMICAL QUALITIES OF SOME IMPORTED FROZEN MARINE FISH SOLD IN ZARIA, NIGERIA

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BY

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DECEMBER, 2015

DECLARATION

I declare that this dissertationentitled "**BIOCHEMICAL QUALITIES OF SOME IMPORTED FROZEN MARINE FISH SOLD IN ZARIA, NIGERIA**" has been written by me in the Department of Biological Sciences, Ahmadu Bello University, Zaria.The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other Institution.

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Signature Date

CERTIFICATION

This thesis entitled **"BIOCHEMICAL QUALITIES OF SOME IMPORTED FROZEN MARINE FISH SOLD IN ZARIA, NIGERIA**", by EnenwaAchem ABOH meets the regulations governing the award of the degree of Master of Science in Biology of the Ahmadu Bello University, Zaria and is approved for its' contribution to knowledge and literary presentation.

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DEDICATION

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ABSTRACT

This study assessed the biochemical qualities of some imported frozen marine fish sold in Zaria, Nigeria. A total of 60 samples per species of frozen marine fish were collected from Sabo-Gari fish depot, Zaria,. The samples were subjected to biochemical tests to determine their quality for human consumption. The fish species were *Clupeaharengus*, *Sardinapilchardus*and *Trachurustrachurus*. The biochemical qualities assessment of the fishes was conducted according to the methods of Riquixo (1998), Undeland*et al.* (2005) and Vyncke (2006). Chemical compound, Total volatile nitrogen (TVN), Trimethylamine (TMA), Thiobarbituriic acid (TBA) and Peroxide value (PV) of the samples were analysed. Result showed that*Trachurustrachurus* had a total of 57 biochemical compounds, TVN of 18.64 mg-N/100g, TMA value of 9.87 mg-N/100g, TBA value of 1.51mg/kg and PV of 4.68 meq/kg.*Sardinapilchardus*had atotal of 73 biochemical compounds, TVN value of 22.87 mg-N/100g, TMA value of 12.64 mg-N/100g, TBA value of 1.88 mg/kg and PV of 4.18 meq/kg; while *Clupeaharengus* had atotal of 73 biochemical compounds, TVN value of27.10 mg-N/100g, TMA value of 16.12 mg-N/100g, TBA value of 2.44 mg/kg and PV of 2.72 meq/kg.This study revealed that TVN, TBA, and TMA concentrations in the three fish species were within the acceptable limits, therefore the fish sold in Sabo-gari Zaria, are safe for human consumption although *Trachurustrachurus* have significantly higher quality indices than *Clupeaharengus* and *Sardinapilchardus*.

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LIST OF ABBREVIATIONS

ANOVA ----------Analysis of variance

CANDU ----------Canada Deuterium Uranium

CAS----------Chemical Abstracts Service

CNS----------Central Nervous System

DHA ---------- Docosahexaenoic Acid

DMA----------Dimethylamine

EPSA ---------- European Food Safety Authority

EU ---------- European Union

FAO---------- Food and Agriculture Organisation

FDA---------- Food and Drug Administration

GC/MS ----------Gas Chromatography/Mass Spectrum

HPF ---------- Horizontal Plate Freezers

ICMSF ---------- International Commission on Microbiological Specifications for foods

LNF ---------- Liquid Nitrogen Freezer

NARICT----------National Research Institute for Chemical Technology

NPN----------Non-Protein Nitrogen

OSHA----------Occupational Safety and Health Administration

PV ----------Peroxide value

SIFAR ----------Support unit for International Fisheries and Aquatic Research

TBA ----------Thiobarbituric acid

TCA ----------Trichloroacetic

TMA ----------Trimethlyamine

TMAO---------- Trimethlyamine oxide

TVN----------Total Volatile Nitrogen

UNECA----------United Nations Economic Commission for Africa

USAID----------United States Agency for International Development

USEPA----------United State Environmental Protection Agency

USFDA----------United States Food and DrugAdministration

VPF ----------Vertical Plate Freezers

WHO ----------World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Fish is the cheapest animal protein source and it is being used increasingly because of its availability, palatability and health provisions (Azam*et al.,* 2004; Akinwumi, 2011). Fish is a highly perishable commodity. The quality of fish and fishery products has become a major concern in fish industry all over the world (Huss *et al*., 2003); partly due to the role it plays in international trades and food sector (Jinadasa, 2014). Fish quality is a complex concept involving a whole range of factors, which for the consumer include: safety, nutritional quality, availability, convenience, integrity, freshness and eating quality (Abbas *et al*., 2008; Jinadasa, 2014). Freshness is one of the most important attributes of fish quality and at the same time, it is not a single attribute. However, it can be measured by different analytical methods. These methods can be divided into two categories; namely, sensory methods using the quality index method and instrumental methods such as chemical, physical and bacteriological analyses.

In tropical conditions, fish spoils quite rapidly within a few hours of landing if not properly cooled (Akinneye*et al*., 2010). The rate at which spoilage occur varies with species of fish, sanitary conditions, methods of handling and storage (Flowra*et al.,* 2012). The rate of spoilage is temperature dependent and lowering the temperature will reduce the rate of spoilage. Different species of fishes have different shelf life depending on the oil levels, duration of rigor mortis, intrinsic conditions of the fish and how it was captured and handled; Shelf life of most marine fishes have been observed to range between 2-24 days in ice, 5 days at 5^{o}C and 3 days at 10^{o}C (Lauzon*et al*., 2010).

Spoilage bacteria are characterized by their ability to produce Hydrogen Sulphide (H_2S), reduce trimethlyamine oxide (TMAO) to trimethlyamine (TMA) and convert urea to ammonia (http://www.manmadeprroject, 2014). Marine fish is characterized by the presence of an odourless compound called trimethlyamine oxide (TMAO). TMA is produced in fish muscle slowly at first then at greater speed in fish stored at ambient temperature, in ice or in refrigerated seawater (http://www.*manmadeproject, 2014*).Trimethlyamine (TMA), ammonia and hypoxanthine are chemical compounds produced by bacterial action (Carlos, 2008; FAO, 2013).

Time and temperature are the most critical factors to control, to ensure that seafood retains high freshness quality as long as possible (Adams and Moses, 2008). With the growing world population and the need to store and transport fish from one place to another where it is needed, fish preservation is carried out in order to increase its shelf life and maintain its nutritional value, texture and flavour. Adequate preservation techniques do not prevent spoilage of fish without affecting its quality. A spoilage of fish and fish products can be due to chemical, enzymatic or microbial activities. Chemical deterioration and microbial spoilage are responsible for loss of 25% of gross primary agricultural and fishery products every year (Baird-Parker, 2000). The spoilage rate of fish may be reduced by good handling practices and effective temperature control immediately they are harvested.

1.2 Statement of Research Problem

Fresh fish spoilage can be very rapid after it is caught. The spoilage process will start within 12 hours of their catch in the high ambient temperatures of the tropics (Berkel*et al*., 2004). Around 4-5 million tons of trawled and shrimp fish are lost every year due to enzymatic and microbial spoilage because of improper onsite storage (Unklesbay,

1992). Fish are highly perishable, and prone to vast variation in quality due to difference in species, environmental and feeding habitats and action of autolysis on the fish muscle (Venugopal, 2002). Johnson *et al*. (1994) stated that freezing and cold storage are inefficient methods of fish preservation since they degrade product quality.

1.3 Justification

During fish spoilage, there is breakdown of various components and the formation of new compounds. Some of these new compounds such as trimethylamine, hypoxanthine are responsible for the changes in odour, flavour and texture of the fish meat. This spoilage can be as a result of transportation especially when there is fluctuation in temperature during transportation, thus the need to investigate TMA, biogenic amine levels.

Demand for high quality fish products is increasing. Most of the fish consumed by Nigeria is imported. Estimated fish demand is over one million metric tonnes whereas maximum production ever is about 756, 400 kg(FAO, 2013). The ever increasing deficit is met from importations which at of times are of poor quality marine fish(FAO, 2013). The quality of fish handling in the country is generally poor and wastage is high. Knowledge regarding improved fish handling and post-harvest practices could positively contribute to the quality of fish and fishery products (FAO, 2013).

Marketing of frozen fish in Zaria is mostly carried out by local fish sellers who have little or no knowledge of temperature fluctuations. Therefore, knowledge of the fish and associated biochemical activity likely to cause spoilage of the fish is paramount, thus the need to investigate the biochemical qualities of imported frozen fish.

1.4 Aim of the Study

The aim of this study was to assess the biochemical qualities of imported frozen marine fish species sold in Zaria.

1.5 Objectives of Study

The specific objectives of the study were to:

1. Determine the morphometrics of imported frozen *Clupeaharengus, Sardina pilchardus* and*Trachurustrachurus* sold in Zaria.
2. Determine the influence of the morphometrics of *Clupeaharengus, Sardina pilchardus*and *Trachurustrachurus*on their biochemical qualities.
3. Determine the biochemical qualities of imported frozen *Clupeaharengus, Sardinapilchardus*and *Trachurustrachurus*sold in Zaria.
4. Determine the quality indices of these imported frozen species.

1.6 Hypotheses

1. There is no significant difference on the morphometrics of imported frozen *Clupeaharengus, Sardinapilchardus*and *Trachurustrachurus* sold in Zaria.
2. There is no significant difference on the influence of the morphometrics of *Clupeaharengus, Sardinapilchardus*and *Trachurustrachurus*on their biochemical qualities
3. Imported frozen *Clupeaharengus*, *Sardinapilchardus*and *Trachurustrachurus* fishes sold in Zaria are not of significantly high biochemical qualities.
4. There is no significant difference in the quality indices of imported frozen*Clupeaharengus, Sardinapilchardus*and *Trachurustrachurus* sold in Zaria.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Nutritional Composition of Fish

Fish and fish products are known worldwide as very important dietary components because of their high nutritive quality and significance in improving human health. Fish, which contributes 36.6g/day of net protein utilization in Nigerian homes, is still below the recommended requirement of 40.0g/day by the World Health Organisation (WHO, 2015).

Fish plays a vital role in feeding the world's population and contribute significantly to the dietary protein intake of hundreds of millions of the populace. On a global scale, almost 16% of total average intake of animal protein was attributable to fish in 1988 (FAO, 1990). In developing countries, fish is a highly acceptable food that supplies as much as 40% of all animal protein available in the countries where fish is the main source of animal protein as 39 out of the top 40 countries are found in the developing world. Moreover, the poor spend proportionally more on fish than on meat and other sources of animal protein (Amao*et al.*, 2006).

Fish freshness is the most important and fundamental single criterion for judging the quality of fish and fishery products (Rodríguez *et al*., 2004). Aquatic food products deteriorate rapidly due to post-mortem as a consequence of biochemical and microbial breakdown mechanisms (FAO, 2014). The characteristic changes occurring post-mortem in fish, especially in its sensory properties and composition, vary considerably depending on fish species and storage method (FAO, 2002; Martinsdóttir, 2002), leading to divers deterioration rate. Fish should be refrigerated immediately after catch

and should be generally stored and transported in ice in order to slow down the mechanisms involved in quality loss (Rodríguez *et al.,* 2004).

Most fish and other marine species give rise to products of great economic importance in many countries. Along the latest decades, fatty fish has captivated a big attention from consumer because of the positive role of marine lipids on human nutrition and health (Zhimin*et al.,* 2014). Great efforts are being carried out by fish traders and food technologists in being able to store and commercialize fatty fish products in a safe and high quality state.

2.2 Fish Demand and Supply

Total fish supply is used for export and re-exports to generate foreign exchange income, for human consumption and as a major component of feeds for animals. Except for imports and products from aquaculture, a high percentage share of the supply of fish from commercial and municipal fisheries is used for human consumption (FAO, 2013).

There is an increasing awareness for aquaculture in Nigeria, leading to an increase in the turnover of the aquaculture industry.Importation provides 80% of fish consumed in Nigeria as a result of the deficit in fish demand and supply (Ozigbo*et al.*, 2014). Fish prices are influenced by demand and supply factors, including the costs ofproduction and transportation (FAO, 2014). Yet the importation of frozen fish plays a key role in bridging the gap between the demand for fish and the supply of fish from domestic sources. The major species imported into Nigeria are *Sardinellaeba*(sardines)and *Mackerel* spp.(Mackerel), among others. (Jo, 2014).

Fish products play a significant role in the diets of the populations of West African countries. It is estimated that between 15 to 20% of all animal proteins come from aquatic sources (FAO, 2000). Post-harvest losses of fresh fish are estimated to be about 20% in West African countries (Olayemi, 2012).

2.3 Benefits of Fish Consumption

Fish contain high-quality protein and other nutrients our bodies require. It is low in saturated fat and cholesterol(EFSA, 2014). Fatty fish, such as salmon, mackerel, herring and, to a lesser extent, tuna and trout, have high levels of Omega-3 fatty acids (Kris-Etherton, 2002). Fish have lower cholesterol when substituted for saturated fatty acids such as that in meat. According to the European Food Safety Authority, among the essential nutrients contained in seafood in substantial amounts, docosahexaenoic acid (DHA) and iodine have a well-established role in the development of the central nervous system (CNS) of the foetus during pregnancy (EFSA, 2014).Fish improve learning ability in children; decrease triglycerides; lower blood pressure; reduce blood clotting and enhance immune function(EFSA, 2014).

2.4 Importation of Marine Fish into Nigeria

Importation refers to the bringing of goods into a customs territory. Between 2011 and 2012, all European (EU) countries registered an increase of their extra-European exports. In terms of volume, Nigeria and Norway were the main countries of destination, accounting for around 250,000 tonnes in 2012 (FAO, 2013). Some 90% of exports to Norway were covered by non-food use products, destined for aquaculture plants. Total value amounted to EUR 395 million for Norway and almost EUR 250 million for Nigeria (pelagic), with both reaching their highest amount since 2006(FAO, 2014). Horse mackerel is the most exported species to extra-European countries, after

non-food use products(FAO, 2014). Volumes of horse mackerel exported in 2012 amounted to 206,000 tonnes, increasing by almost 20,000 tonnes with respect to the previous year(FAO, 2014). Herring is one of the top five commercial fish species exported in volume terms. In 2012, over 170,000 tonnes were exported to extra-EU countries, an increase of 54,000 tonnes with respect to 2011, for a value of EUR 175 million. It was mostly exported to Nigeria, at an average price of 0.98 EUR/kg, an increase of 15% (FAO, 2014). Nigeria spends N100 billion on fish importation annually and the current fish demand consumption in Nigeria stands at over 2.66 million tonnes per annum, while the present importation rate is over 750,000 metric tonnes (Oota, 2012). With importation of more than 750,000million tonnes of fish, more than USD 600 million is spent (USAID, 2010).

2.5 International Trade in Seafood

About 33% of live weight fish production for 1998 was exported, with developing countries accounting for almost 20% of exports (FAO, 2000). Total fish and fishery products exports for the year were US $51 billion, a 3.8% decrease compared with 1997 (FAO, 2000). Net export trade from developing countries increased from US$10 billion in 1990 to US $18 billion in 2000, corresponding to growth of 45% (FAO, 2002). In 1995, Africa produced 5% of the world's production of fishery products but supplied 0.2% of the fishery items in international trade (Lokuraka, 2000). More than 90% of global trade in fish and fishery products consists of processed products (excluding live and fresh whole fish) (FAO, 2000).

Frozen and chilled fish/fish products make up the majority of exports. Although live, fresh or chilled fish represents only a small share of world fish trade and owing to its perishability, trade is growing, reflecting increased demand (FAO, 2000). More than

77% of total world seafood export value is concentrated in the three areas of shrimp, tuna and groundfish, seafood categories that are often implicated in incidences of scombroid food poisoning (Becker *et al.,* 2001). Emerging African economies that are capable of exporting considerable quantities of fishery products are assured of earning considerable revenue as long as they can meet food safety requirements and can compete effectively with similar products from other parts of the world. Although a choice has to be made between domestic and international export markets, it seems wise to develop both because the markets in developed countries are sometimes volatile, and world politics often unpredictably affects the functioning of markets. Fishery product which non-African markets will not need or are currently being met by European imports can be consumed by intra-African trade.

A sizeable market for shrimp and other molluscs, tuna and small pelagic exists in North America, Japan and Europe, inclusion of components dealing with fishery products in international trade agreements would benefit Africa's fishery products exporters. The stringent standards of quality and safety required by the Centre for Food Safety and Nutrition of the Food and Drug Administration (FDA) of the U.S.A. before fishery products are allowed into the U.S.A. will be an impediment Africa's fishery products exporters would have to overcome. Many African countries are currently working hard to attract foreign investment but the pace of investment in the fisheries sector is slow, despite the incentives offered to would-be investors. The incentives include the unimpeded repatriation of profits, low domestic labour costs and low tariffs on fishery related imported technology (FAO, 2003). An extra impediment to be overcome by African countries wanting to enter the seafood export markets includes improvement of catch handling through the application of post-harvest procedures acceptable to

importing countries. Often, the Codex Alimentarius Commission assists adapt the necessary regulations to the prevailing situation in member countries (FAO, 1997).

Zango-Daura (2000) stated that Nigeria's importation of fish is worth ₦12 billion annually. The country is known to require 750,000 tonnes of fish per year. Domestic production amounts to 350,000 tonnes while importation makes up the balance of 400,000 tonnes. There is the research need, therefore to confirm whether or not consumers in Nigeria consider the farmed species as close substitutes to the imported species. If affirmatively confirmed, then trade restriction on the importation of fish and the encouragement of local production of fish will be beneficial to the economy. The current import dependent situation is deemed to be unfavourable and non-optimal to the Nigerian economy and remains unacceptable in view of the natural fish habitat endowment of the country (Teslim, 2010).

2.6 Fish Spoilage

The rate of fish spoilage depends on handling during processing, acidity level, species of fish, weather, mode of storage and temperature during transportation (FAO, 2013). Chemical breakdown of protein, fat and water contents contribute to quick spoilage of fish.

Quality losses might occur very rapidly after catch, especially in hot climates and tropical areas where cold preservation techniques are often missing. Saliu (2008) reported that fish spoilage in Nigeria is influenced to a large extent by high ambient temperatures, considerable distances of landing ports to points of utilization and poor as well as inadequate infrastructure for post-harvest processing and handling.

Biochemical tests have been used to establish quantities of different spoilage compounds in fish. Howgate (1982) stated that these have stood the test of time as

reliable i.e. determination of trimethylamine (TMA) and total volatile nitrogen (TVN). They are related to bacterial activity in the fish flesh. There are other methods such as peroxide value, thiobarbituric acid, iodine value and Anisine, which measure rancidity in fish and fish products (Haimd, 2011).

2.6.1 Autolytic spoilage

Enzymes are natural chemicals in the body muscles. They are protein catalysts, which change substances without being changed. They continue to function even when the fish is dead; the enzymes continue to function in the body of the dead fish in a process called autolysis (self-destruction). Since they do not die, their function after the death of the fish is to break down other compounds into smaller units. This breaking down of body tissues into smaller units also affects the fish flavour.

2.6.2 Bacterial spoilage

When the fish dies, the bacteria present on surface, gills and gut rapidly multiply and invade the flesh which provides an ideal medium for growth and further multiplication. Bacteria can also breakdown skin and muscles, feeding on smaller units produced by autolysis. The increase in number and feeding activity of bacteria can result in heavy slime on the skin and gills, an unpleasant ammonia sour odour and taste. Eventually, the fish flesh softens.

2.6.3 Oxidation

The oils and fats of fish generally have as much high level of unsaturated fatty acids than other food stuffs. For this reason, there is the likelihood that a reaction between oxygen in the air, the fats and oils will occur. In fatty fish such as mackerels, chemical changes involving oxygen and fats may produce rancid odour, off flavour and colour

change. As the spoilage process continues, changes occur. Such changes include off-flavour, change in body texture and body appearance.

2.7 Factors Influencing Spoilage of Fish Products

Apart from intrinsic factors such as moisture content, pH, onset and resolution of rigor mortis, chemical composition, redox potential, and other factors may influence spoilage in fish, if not eliminated or reduced considerably. These include temperature, fishing method, initial microbial load in association with fishing ground, post-mortem handling procedures and processing facilities. Literally, every stage of handling from harvest to consumption affects spoilage in one way or another.

2.7.1 Temperature

The most important factor influencing the composition of micro flora is temperature. After capture and death, fish is normally chilled or iced, giving rise to changes in the micro flora. Bacterial populations on fish from temperate waters are predominantly psychrotrophic reflecting water temperatures of $\leq 10^{\circ}$C in the main water mass, while fish from the tropics, especially pelagic have mesophilic bacteria (Baffone*et al*., 2001). This can lead to rapid spoilage if chilling is delayed. On the other hand, because the natural incidence of psychrotrophic bacteria on tropical fish is low, rapid chilling and sustained low temperature storage can result in long shelf life for tropical species (Davies *et al*., 2008).

The micro floras of marine fish are predominantly halotolerant, able to grow over a wide range of salt concentrations but display optimal growth at 2-3% salt concentration. This is enhanced by the common use of ice to chill the fish, thus exposing the bacterial populations to decreasingly saline conditions during storage. This seems to favour the survival and growth of halotolerant species e.g. *Vibro spp*.

(ICMSF, 1998). Temperature is the primary factor controlling spoilage in fish because it has direct effect on the growth of microorganisms. Connell (1990) reported that increasing the temperature from 0°C to 5°C at least doubled the rate at which cod and similar species spoil. Wang *et al.* (2010) stated that temperature contributes immensely to the perishability of fish as evidenced by the effective inhibition of bacterial growth on fish harvested from warm waters by refrigeration. Chilling exerts selective pressure on the bacterial populations on the surface of the fish resulting into mesophiles failing to grow and psychrotrophs increasing in abundance. Mc (2002) observed that psychrotrophs were able to grow and utilise the non- protein nitrogen (NPN) compounds relatively rapidly at 0°C. Fish from temperate regions were held at 0°C there was a bacterial lag phase from 1-5 days and an exponential growth from 6-14 days. Sensory evaluation of shelf life showed a rapid deterioration from about 12 days onwards.

The bacterial counts on tropical fish handled properly, chilled and held at 0°C show some extension of the lag phase and marked decrease in growth rate (Hassan*et al.*, 2011).This results in a shelf life of ≥ 30 days for a number of species (Mc, 2002). The effect appears to be due to both the low concentration of bacteria on tropical fish that are able to grow at low temperatures and an intrinsically lower growth rate for the naturally occurring psychrotrophic species (Connelly, 2006). Storage at adequately low temperature will not prevent spoilage, but will limit spoilage in psychrotrophic microorganisms. Tawari (2011) attributed the importance of temperature in fish spoilage, to their wide distribution in the environment, their ability to utilise a wide range of materials as substrates for growth and the ability to contaminate a product from many sources.

2.7.2 Rigor mortis

In live fish, there are different enzymes naturally present in fish flesh and engaged in normal processes like tissue building and muscular contraction and relaxation. After fish die, they become involved in predominantly degradation reactions. One of these reactions is the gradual hydrolysis of glycogen to lactic acid, resulting in the fall of pH from the normal neutral to acidic i.e. 7 to 5.8-6.8 depending on the species and condition of the fish. The decline in pH and the imbalance in the biochemical reactions within the fish musculature brought about by the post mortem continuation of enzymatic activity, initiates a phenomenon referred to as rigor mortis (Makri, 2009). It involves the stiffening of the fish muscles, which subsequently may pose processing problems. It affects the quality of the fish, as the texture of the flesh is rendered firmer because of its tendency to lose moisture. From the microbiological viewpoint, it is an advantage to have a low pH as it retards bacterial activity but in practice, the degradation of non-protein nitrogen (NPN) compounds raises the pH during the post-rigor considerably. The increase in pH from 6-8.0 in some species provides a favourable environment for bacterial proliferation (Violeta, 2007). The change in pH affects the enzymatic and other chemical reactions in fish muscle cell, which may result in flavour and odour changes.

2.7.3 Methods of capture

Fish caught by hook and line, die relatively rapidly when brought into the air (ICMSF, 1998) and the method minimises stress and its associated deterioration attribute. Fish caught by a seine net, has a better quality index than fish caught in a trawl net, which tends to compact the fish and in so doing presses out guts with their high bacterial contents. The spilled bacteria utilise the available substrate and their metabolic products constitute deterioration(Chuck, 2012). Gill netting entails fish struggling,

which in turn quickens the onset of rigor and subsequent deterioration (Chuck, 2012). Bacteria may gain access through puncture wounds and bruises during the death struggle and may multiply rapidly in these localised areas. The degree of struggling before death reduces the levels of glycogen in the fish muscles, which has a negative impact on quality as far as the texture, is concerned. A method that inflicts stress or struggle hastens the onset of spoilage(Chuck, 2012).

2.7.4 Initial microbial load

The initial micro flora on the surface of the fish is directly related to the water environment while the flora in the gastro-intestinal tract corresponds to the type of food and the condition of the fish (Abowei, 2011). Jay (2000) stated that intrinsic and extrinsic factors determine the initial bacterial contamination. This is further enhanced by the poikilothermic nature of the fish, the presence of TMAO which increases the redox potential, the presence of large amounts of NPN and low carbohydrate content which increases the pH in the flesh post-mortem. Bacterial numbers range from 102 to 106 CFU/cm^2 on the skin, 103-105/g on the gills and in the intestines from very few in non-feeding fish to 107 or greater in feeding fish (Karwowska*et al.,* 2003). During storage however, the counts on a given morphological entity, may increase by a minimum of one logarithm (Alemu, 2013). Regardless of the differences in the initial micro flora, the spoilage patterns of fish during ice storage are usually quite similar and are caused by *Pseudomonas sp*. and *Shewanellaputrefaciens*(Lauzon*et al.,2010*).

2.8.5 Post-mortem handling practices and storage procedures

Various post-harvest practices and storage procedures enhance microbiological contamination, and subsequently influence spoilage (Sierra *et al.,* 2009). During capture operations, fish come in contact with nets, ropes, deck boards, human hands

and clothing. This contact continues during packing and storing operations below deck. Excessively handled fish may carry significant numbers of Gram positive bacteria, some of which may be spoilage bacteria or pathogenic. Most of these Gram positive bacteria are naturally derived from human and avian sources (Faisal, 2015).

Gutting of fish is one of the practices, where fish is exposed to agents of spoilage. In some species, gutting is avoided especially on board fishing vessels either because the fish is too difficult or too small and numerous to handle within the available time (Violeta, 2007). Although Nile perch and other fish species in Uganda are fewer per boat they are not gutted because the fishing vessels are not adequately equipped to handle the gutting and subsequent operations (Mtti, 2006).

Offloading operations provide opportunities for bacterial contamination via offloading equipment, pumps, conveyors, baskets and boxes that redistribute surface contamination (FAO, 2011). Insufficient cleaning may lead to bacterial build up which in turn will act as a source of subsequent contamination. In public auction markets where fish may be displayed on/in wooden, metal or plastic containers in the open, potential bacteriological dangers abound. For example, exposure of catch to direct sunrays especially in the tropics where temperatures are more than 20□C permits multiplication of spoilage bacteria. Delay in chilling when temperatures are high can significantly shorten shelf life during subsequent storage (Abowei, 2011).

The type of production material and design of the containers may not allow them to be cleaned and disinfected effectively. The presence of vermin e.g. rodents, birds, domestic animals and people (handlers and auctioneers) are additional sources of contamination. Jacqueline *et al.* (2013) reported similar sources of contamination around most landing sites in Uganda. At the fish processing plant, further handling

occurs during wet processing operations like sorting, filleting and trimming. These operations transfer Gram positive bacteria usually associated with humans, directly from fish skin and gut to filleted flesh surfaces. It has been estimated that fillets and other products from fresh fish processors usually carry counts of 103-105/g or more (Abowei, 2011).

If the product is refrigerated later on, the dominant bacteria on the product after a day or two of storage will be Gram negative because of the inability of Gram positive bacteria to grow competitively under refrigeration (ICMSF, 1998). The bacteria may also be transferred from the processing environment namely, contaminated surfaces, knives and machines.

2.8 Quantification of Spoilage

With the current market demands, it is imperative to have reliable methods of detecting contamination or remaining shelf life of individual consignments under storage or on display to avoid unnecessary financial losses. UNECA(2008) recognise the importance of rapid and effective means of identifying the causes of spoilage as a basis for instigating quick remedial action. Loss of freshness followed by spoilage is a complex combination of microbiological, chemical and physical processes influenced by species type and different storage conditions (Ozogul*et al*., 2005). A single spoilage index cannot therefore be used as a measure of freshness but rather a combination of selected indices representative of different changes that occur during spoilage. Some particular feature of the spoilage process is measured, used as an index of spoilage and it gives an optimal information on a given food can be obtained (Koutsoumanis, 2009). Each of these methods measure different spoiling indicators or freshness in fish and/or fish

products. Sensory evaluation gives direct measurements while the other three give indirect measurement, though the measurements are closely related.

The biochemical composition of foods and their interrelationships with extrinsic factors during storage, contribute significantly to their freshness and quality partly for the reason that they determine and enhance the initial microbial growth (Sivertsvik*et al.,* 2002). With regard to fish, the inherent characteristic of the presence of non-protein nitrogen components, such as, trimethylamine-oxide (TMAO), creatine, methionine, free amino acids, cystine, histamine, carnosine, volatile nitrogen bases such as urea especially in cartilaginous fishes support microbial growth and the production of their related metabolites; responsible for fish spoilage during storage (Ghaly et.,, 2010).

Trimethylamine-oxide (TMAO) is one of the major non-protein nitrogen (NPN) fractions found in almost all marine fish species, but in varying quantities (1 to 5 %) of the dry muscle weight. The variation in the amount of TMAO in fish muscle depends on; fish species, the fishing ground and season (Huss, 2004). With regard to the amount of TMAO in various fish species, elasmobranchs and squids, are known to contain the highest quantities of TMAO (75-250 mgN/100g), whereas cod has a relatively low amount (60-120 mg N/100g) while flatfish and pelagic fish have the least. However, the concentration has been found to be more in the dark muscles whereas in demersal fish, higher concentrations are in the white muscle (Baixas-Nogueras*et al.,* 2003). However, early studies found that Nile perch and tilapia from Lake Victoria comprises of 150-200 mg TMAO/100g of fresh fish (Gram*et al*., 1989).

FAO (2015) stated that the source of Trimethylamine-oxide (TMAO) in fish is known to be from the biosynthesis of certain species of zooplanktons. These organisms possess an enzyme, monooxygenase, which oxidizes trimethylamine (TMA) commonly found

in marine plants as well as many other methylated amines (mono-methylamine and dimethyl amine) to TMAO.Thus, the plankton-eating fish obtain their TMAO from these zooplanktons. The reduction of TMAO to TMA is not independently the function of TMAO-reducing bacteria, but other systems too have been found to participate in its reduction. The TMAO-reducing system is known to exist in dark muscles of some pelagic fish species and certain fish species are able to convert TMAO to TMA but this is regarded as of minor importance.

Abowei (2011) stated that the highly nutritious properties of fish flesh provides an excellent substrate for the growth of most heterotrophic bacteria and the composition affects the bacterial growth and related biochemical activities. The poikilothermic nature and its aquatic surroundings of fish favour the proliferation of a wide range of bacteria on fish. The ultimate pH of fish after death is normally neutral or slightly acidic pH and therefore favours the growth of a wide range of microorganisms.

The presence of highly unsaturated fatty acids in triglycerides (long–chain fatty acids and the phospholipids) in fish in general and especially in the pelagic fish species have important part in the spoilage processes of fish under aerobic storage (Huss, 1994).
The physical, chemical, biochemical and bacteriological characteristics of fish tend to differ with species, feeding habits, seasonality, spawning cycles, methods of catching, fishing grounds, size, age, microbiological load and geographical location (Huss, 1995). Temperature and rigor mortis are the main underlying factors in fish spoilage (Flowra*et al.,* 2012). Round fish deteriorate faster than flat fish (Flowra*et al.,* 2012).

Dominique (2014) stated that storage of fish caught during their heavy feeding and or spawning seasons show wide variations in spoilage rate. Heavily feeding fish tend to be more susceptible to autolytic tissue degradation than the little feeders. The type of feed

/food on which fish feed on may similarly have an effect on their spoilage rate during storage. Low feeding fish have been found to have low levels of bacteria in the intestines as compared to the heavily feeding fish.

Spawning fish tend to use most of their glycogen and the effect of depletion is reflected in their susceptibility to rapid deterioration during storage due to faster onset and resolution of rigor mortis (Huss, 1994).The geographical location, and or the type of waters, has a remarkable influence on the type of micro fauna that will grow on the fish. Ajiboye (2011) states that microorganisms associated with food spoilage are found and can thrive under a wide spectrum. The fishing grounds and geographical locations exclusively tend to determine fish spoilage in so far as temperature, food types, the level of pollution of micro environments are concerned (FAO, 2003).

Bacteria associated with fish vary according to their optimal growth requirements, which among others include; oxygen or redox potential, pH, temperature and water activity thus, their distribution in the environment is dependent on their requirements. Dominique (2014) stated that bacteria living on the surface of marine fish are phenotypically capable of utilizing amino acids, peptides and non-carbohydrate sources for growth. Their activity on these substrates normally leads to the production of slightly alkaline conditions in stored fish products. Bacteria which colonise fish skin surfaces and gills are predominantly aerobes however, facultative bacteria may occur in high counts on pelagic fish. Gómez and Balcázar (2008) stated that obligate anaerobic bacteria are not common on the surfaces of fish but their occurrence is significant in the intestines.

The method of fishing as well has implication on the level of glycogen level at the time of fish death.More exhausted fish, for instance those caught by trawl method exhibit

lower glycogen reserves in the muscle cells thus, higher ultimate pH at the resolution of rigor mortis and are therefore, more prone to higher rates of spoilage as high pH favours microbial proliferation (FDA, 2015).

The growth of specific spoilage bacteria and the accumulation of their metabolic by-products contribute to the major spoilage changes in fish during storage. Essentially, freshly caught fish are usually characterised sensorally by "fresh fish flavours" during storage, a period is reached where the odours and flavours are described as neutral or non-specific, (the first indications of off-odours and flavours) which progressively become more pronounced and ultimately renders the fish unacceptable for consumption (Gram and Huss, 1996).

2.9 Fish freezing and Frozen Storage

Freezing and frozen storage have largely been employed to retain fish sensory and nutritional properties (Aubourg*et al.,* 2004). Marine fish species have shown a highly unsaturated lipid composition and an important presence of prooxidant molecules that facilitate the rancidity development (Richards and Hultin, 2002). In frozen conditions, lipid oxidation compounds have shown to interact with proteins leading to protein denaturation and nutritional losses (Aubourg*et al.,* 2004). Freezing is a transition phase in which a liquid turns into a solid when its temperature is lowered below its freezing point while refrigeration is a process in which work is done to move heat from one location to another. The work of heat transport is traditionally driven by mechanical work.

2.10 Type of Freezing

There are different types of freezers available for freezing fish, and freezer operators are often uncertain about which type is best suited to their needs. Threefactors may be initially considered when selecting a freezer; financial, functional and feasibility.

The three basic methods of freezing fish are: air blast freezers, contact or plate freezers, and immersion or spray freezers.

2.10.1 Air blast freezers

The advantage of the blast freezer is its versatility. It can cope with a variety of irregularly shaped products and whenever there is a wide range of shapes and sizes to be frozen, the blast freezer is the best choice(FAO, 2001). However, because of this versatility it is often difficult for the buyer to specify precisely what he expects it to achieve and, once it is installed, it is all too easy to use it incorrectly and inefficiently (FAO, 2001).

2.10.2 Plate freezers

Plate freezers and air blast freezers are the types of freezers most commonly used for freezing fish in industrial countries. Plate freezers do not have the versatility of air blast freezers and can only be used to freeze regularly shaped blocks and packages. Plate freezers can be arranged with the plates horizontal to form a series of shelves and, as the arrangement suggests, they are called horizontal plate freezers (HPF). When the plates are arranged in a vertical plane they form a series of bins and in this form they are called vertical plate freezers (VPF) (Makri, 2009).

2.10.3 Liquid nitrogen freezer

In this freezer, the product is brought into direct contact with the refrigerant. The fish on the stainless steel conveyor belt initially come into contact with the counter current

flow of nitrogen gas at a temperature of about -50°C. As the fish progress through the precooling stage of the freezer, the gaseous nitrogen partially freezes the fish and up to 50% of the product's heat is extracted. The product then passes below the liquid spray where freezing is completed by the boiling liquid. The last stage in the freezer provides a few minutes for the fish temperature to reach equilibrium before the fish are discharged (Makri, 2009).

The advantage of the liquid nitrogen freezer is that freezing is very quick and the physical size of the freezer is correspondingly small. The freezer is operated without the need for compressors, condensers or coolers; therefore maintenance requirements are minimal and the power required to operate the freezer is very low. Liquid nitrogen must be retained in a vacuum insulated pressure vessel with continuous venting to keep the contents cool and the internal pressure down. One estimate given is that 0.5% of the stored contents are lost each day by this method (Iverson, 2004). About 10% has been estimated to be lost during the transfer of liquid from the tanker to the storage vessel although the customer is not charged directly for this loss. This method of freezing is more expensive than most others, being up to four times more costly than conventional air blast freezing. The main disadvantage of this type of freezer in most developing countries is that delivery of nitrogen could be expensive and there may be no guarantee of regular supplies (Gameiro, 2002).

2.10.4 Carbon dioxide freezer

This type of freezer has been known for a long time and uses liquefied carbon dioxide which is usually a by-product of another industrial process. The liquefied carbon dioxide is injected into the freezer and comes into direct contact with the product. In this respect, it is similar in operation to a liquid nitrogen freezer (LNF) (AP, 2011).

With large units, it is economically feasible to recover the carbon dioxide and about 80% of the refrigerant used can be liquefied. Carbon dioxide can be contained in insulated vessels at a moderate pressure and losses during storage are therefore negligible. High levels of carbon dioxide in the factory air are dangerous, therefore a freezer using this refrigerant must be vented and the gas discharged outside the building. Freezers which rely on regular supplies of refrigerant such as carbon dioxide freezers would not be suitable for use in remote areas (Iverson, 2004).

2.10.5 Immersion freezers

This involves using a liquid for the removal of heat from a product; favourable freezing rates can be achieved. Liquid can remove more heat per unit volume than gas, but, like gas, a stagnant boundary layer is formed, which slows the transfer of the heat. Liquids used for heat transfer must therefore be circulated over the product. Difficulties due to high viscosity often arise when a low temperature liquid is used. Many liquids that have suitable refrigeration and heat transfer properties are not allowed to be used in direct contact with food. Those that are available are limited in their use because they may cause changes in texture and taste in the food with which they are in direct contact (Kolbe*et al.,* 2004).

Immersion in sodium chloride brine was one of the very first methods used to freeze fish since it was a logical progression from the method used to freeze ice. Brine immersion freezing may still be used for such fish as tuna which are intended to be marketed as a canned product. The fish are large and have a thick skin; therefore the uptake of salt is not great. The little salt that is absorbed is not detrimental to the canned product since salt is usually added to the product before canning in any case. For many other fish freezing applications, adverse effects on texture and taste of the

fish due to the absorption of brine have proven to be unacceptable. Even without excessive brine uptake, the surface of the fish will be coated and handling the product after freezing is difficult and messy. Some fish products such as shrimp have been frozen in syrup and salt solutions and sugar and salt solutions but again there is some degree of absorption with changes in flavour (Martinsdóttir*et al.*, 2001).

2.11 Advantages of Freezing

Food can be cooked and baked in bulk or in separate portions. Such portions can be used as you need them thus saving time and energy. Food or fish can be bought while in season and at its best and cheapest either raw or cooked. In tropical regions, particularly in West Africa the practice of cold storage limits the problem posed by the extreme perishability of fish in developed countries (Janvier*et al.*, 2014).

2.12 Disadvantages of Freezing

Disadvantages such as product dehydration, rancidity, drip loss and product bleaching have an overall effect on the quality of frozen food (Kindossi*et al.*, 2012). Knowledge of the nutrient composition of freshwater fishes and the relationship between their chemical composition, food value and stability while being processed into acceptable products is of significant practical interest. Freezing is relatively costly and if poorly done can adversely affect fish quality(Kindossi*et al.*, 2012).

2.13 Biochemical Quality Assessment of Fish and Fish Products

Biochemical studies of fish tissue are of considerable interest for their specificity in relation to the food values of the fish and for the evaluation of their physiological needs at different periods of life(Huss, 2004). Changes in chemical composition of body have been known to reflect storage or depletion of energy reserves. The values of body composition in fishes vary considerably within and between species, with fish size,

sexual condition, feeding, time of the year and activity (Saliu, 2008). Food composition, environment and genetic trait are also known to influence chemical composition of fish (Huss, 2004).

Chemical compounds such as total volatile nitrogen (TVN) and biogenic amines (e.g. histamine, cadaverine and putrescine) which normally did not exist in the living tissues of fish are formed by autolysis and microbial actions (Brad, 2002). Chemical tests measure the amounts of breakdown product derived from enzymatic, bacterial or oxidative activity, and trimethylamine, histamine, ammonia and several other breakdown products can be chemically recorded to evaluate the fish quality (Anup, *et al.,* 2013).

2.14Biogenic Amine/Compound

Biogenic amines are important nitrogenous compounds in plant, microbial and animal cells and therefore can be detected in raw as well as in processed foods (Beutling, 1996). They may be formed by bacterial activity during fermentation or spoilage. Food poisoning has been associated with histamine in certain fish species (Anderson, 2010). Symptoms that can occur after excessive oral intake of biogenic amines include nausea, respiratory distress, hot flush, sweating, heart palpitations, headache, bright red rash, burning sensations in the mouth and alterations in blood pressure (Krizek*et al.*, 2004). Putrescine and cadaverine are often mentioned as potentiators of the effects of histamine, for example, in the case of scombroid fish poisoning (Jorgensen *et al.*, 2000). Putrescine is also related to tumour growth (Karovicová*et al.,* 2005). Putrescine and cadaverine can form carcinogenic nitrosamines with nitrite in meat products (Ruiz-Cappillas*et al.*, 2004). The United States Food and Drug Administration (USFDA) specified limits in fish (European Commission, 2005; Food and Drug Administration,

2011) stated that no tolerable levels for putrescine and cadaverine in foods have been elaborated. However, national limits can be established, based on the principles of risk assessment. This may be achieved by relating the amounts of orally administered amines that do not cause health effects to amounts of food consumed for histamine, tyramine and phenylethylamine as reported in fermented sausages, fish and cheese (Rauscher-Gabernig*et al.,* 2010).

Fish muscle has the ability to support the bacterial formation of a wide variety of amine compounds which result from the direct decarboxylation of amino-acids. Most spoilage bacteria possessing decarboxylase activity do so in response to acidic pH, presumably so that the organisms may raise the pH of the growth medium through the production of amines (Ellis *et al.,* 2006).

Histamine, putrescine and cadaverine are produced from the decarboxylation of histidine, ornithine, lysine and tyrosine, respectively. Histamine has received most of the attention since it has been associated with incidents of scombroid poisoning in conjunction with the ingestion of tuna and mackerelAlberto *et al.* (2002). The absence of histamine in scombroid fish (tuna, mackerel, etc.) does not ensure the wholesomeness of the product since spoilage at chill storage temperatures does not always result in the production of histamine. Alberto *et al.* (2002) proposed a chemical quality index based on biogenic amines, which reflects quality loss in canned fish with an increase in its quality index ratio.

Magwamba (2010) followed the production of histamine, cadaverine and putrescine in skipjack and yellowfin tuna at 20°C and found that cadaverine and histamine increased exponentially after an initial lag period of about 36 hours. However, putrescine increased slowly after an initial lag period of 48hours. Levels of cadaverine and

histamine increased to maximum levels of 5-6µg/g tuna but the authors reported that the absence of such amines in raw product did not necessarily mean that the products were not spoiled. Tuna, bonito, mackerel etc. are most frequently incriminated in scombroid poisoning. They have a high concentration of free histidine in their tissues. Bacteria producing histidinedecarboxilase convert histidine to histamine and possibly other biogenic amines. *Proteus morganii* appears to be the most active species in producing the scombrotoxin but members of the genera *Salmonella, Shigella, Clostridium,* and *Escherichia* are also able to do so (Richard, 2013).

Biogenic amines are stable to thermal processing, so their presence in finished canned products is a good indication that the raw material was spoiled prior to heating. Histamine and other heat-stable biogenic amines appear to be the responsible agents. The histamine content of toxic fish is usually insufficient, by itself, to cause scombroid poisoning. Usually the fish does not become toxic unless it is held for many hours to a few days at room temperature (WHO, 2015). Toxic canned tuna undoubtedly has become toxic prior to canning, probably as a result of inadequate refrigeration on the fishing vessels. Since histamine is neither volatile nor destroyed by cooking, a convenient method of detecting it in seafood samples is needed, particularly where decomposition is suspected. Spoiled scombroid fish containing about 100 mg or more of histamine per 100g fish muscle is generally toxic to human. Five to ten mg could be a sign of histamine poisoning; 50-100 mg will always give histamine poisoning (Riquixo, 1998).

2.15Lipid Oxidation

2.15.1 Oxidative rancidity

Fats and oils of many kinds of fish are composed of unsaturated fatty acids and hence are subjected to oxidative changes producing oxidative rancidity and sometimes undesirable changes (Frazier, 1996). The highly unsaturated fatty acids found in fish lipids are very susceptible to oxidation. The primary oxidation products are the lipid hydroperoxides. These compounds can be detected by chemical methods, generally by making use of their oxidation potential to oxidize iodide to iodine. The methods for peroxide value (PV) determination are empirically based, and comparisons between PVs are only possible for results obtained using identical methods (Frazier, 1996). The thiocyanate-method gives values 1.5 - 2 times higher than the iodine titration method (Barthel and Grosch, 1974). The peroxide value may indicate a potential for a later formation of sensorial-objectionable compounds (Barthel and Grosch, 1974). Lipid break down with time, and a low PV at a certain point during the storage of a product can indicate both an early phase of autoxidation and a late stage of a severely oxidized product, where most hydroperoxides have been broken down.Kanner and Rosenthal (1992), stated that the PV has not been lowered through extended storage or high temperature exposure. Peroxide value (PV) should not be above 10-20meq/kg fish fat (Connell, 1975).

In later stages of oxidation, secondary oxidation products will usually be present and thus be indicative of a history of autoxidation. Secondary oxidation products such as aldehydes, ketones, short chain fatty acid and others, many of which have very unpleasant odours and flavours, and which in combination yield the fishy and rancid character associated with oxidized fish lipid. Some of the aldehydic secondary oxidation products react with thiobarbituric acid, forming a pinkish coloured product

that can be determined spectrophotometrically. Foods with Thiobarbituric acid (TBA) values above 1-2 µmol MDA-equiv/g fat will probably have rancid flavours (Ke*et al.,* 1976).

2.15.1.1 Peroxide value

During storage of fish, hydroperoxide production is present. Increase of peroxide value (PV) in frozen fish in contrast with fresh fish showed development of rancidity during frozen storage (Ben-Gigirey*et al.,* 1999). Development of off-flavour is one of the major effects of lipid oxidation (Fagan *et al.,* 2003) and at the further stage of lipid peroxidation, changes in colour and nutritional value (Dragoev*et al.,* 1998). Oxidized products of lipids formed during storage of fishery products are known to influence the soluble proteins i.esarcoplasmic and myofibrillar proteins (Sarma*et al.,* 2000). In comparison with sharks, mackerel contributed to the more accelerated oxidative degradation of lipids. Lipid content ranged in mackerel between 6.35 - 3.89 meq/kg (Sahari, 2009).

2.15.1.2 Thiobarbituric acid

Fatty fish under conditions of frozen storage lose quality through oxidative deterioration of their lipid components. Susceptibility and rate of oxidation depends to a large extent on fatty acid profile; the greater the unsaturation, the more easily a fat will oxidize (FDA, 2011). The skin and muscle of mackerel (*Scomberscombrus*) are particularly susceptible to oxidation (Vyncke, 2006) while herring (*Clupeaharengus*) of similar unsaturation appear more resistant. Accumulation of oxidation by-products is responsible for objectionable flavours perceived in rancid foods. The TBA number is a measure of malonaldehyde, a by-product of lipid oxidation. TBA records revealed an increased rate of lipid oxidation during the frozen storage of the fish species examined

(Siddaiah*et al.*,2001). Significant increase in TBA of fish flesh in mackerel, during frozen storage, indicated oxidative deterioration; and this vary with species of fish (Sarma*et al.,* 2000). Tarladgis*et al.* (1960), reported that rancidity occurred when TBA value exceeded 4mg malonaldehyde/kg. Thiobarbituric acid (TBA), Peroxide (PV), and total volatile nitrogen (TVN) values increase with storage time.

2.16Total Volatile Nitrogen

Total volatile nitrogen (TVN) is used to estimate the degree of decomposition of fish. TVN analysis reflects on the later stages of advanced spoilage and is generally considered unreliable for the measurement of spoilage during the first ten days of chilled storage of several species (Anup*et al.,* 2013**).** It is particularly useful for the measurement of quality in cephalopods such as squid, industrial fish for meal and silage and crustaceans. The level of TVN for white fish is generally considered to be fresh if the TVN is less than 20 mg N/100 g sample(Zaid*et al.,* 2013). If the TVN reaches 35 mg N/100 g most authorities consider the fish to be stale, whilst at level of 40 mg N/100 g the fish is regarded as unfit for consumption (Zaid*et al.,* 2013).

The Codex Alimentarius Committee (1968), proposed the TVN assay by steam distillation (Egan *et al.,* 1997). TVN concentrations of mackerel during frozen storage were 4.83mg/100g. At the first month of storage, the TVN values showed an increasing trend up to 35.58mg/100g for mackerel. TVN level is a poor indicator of fish freshness (Castro *et al.,* 2006). It has been commonly used to evaluate fish muscle spoilage (Mazorra*et al.,* 2000). For several fish species, TVN values were reported to increase linearly with time (Mazorra*et al.,* 2000). The rate of enzymatic cleavage of TMAO to Formaldehyde and Dimethylamine (DMA) depends on the substrate (Sikorski and Kostuch, 2003). In cod (*Gadusmorhua*), TMA constitutes most of the so-called total

volatile bases until spoilage(Castro *et al.,* 2006). Besides, TVN levels still rise due to formation of ammonia and other volatile amines. In some fish that do not contain TMAO, or where spoilage is due to a non-TMAO reducing flora, a slow rise in TVN is seen during storage, probably resulting from the deamination of amino-acids (Castro *et al.,* 2006).

2.17Trimethylamine (TMA)

Trimethylamine is a pungent volatile amine often associated with the typical "fishy" odour of spoiling seafood. Its presence in spoiling fish is due to the bacterial reduction of trimethylamine oxide (TMAO) which is naturally present in the living tissue of many marine fish species. Although TMA is believed to be generated by the action of spoilage bacteria, the correlation with bacterial numbers is often not very good. This phenomenon is now thought to be due to the presence of small numbers of "specific spoilage" bacteria which do not always represent a large proportion of the total bacterial flora, but which are capable of producing large amounts of spoilage-related compounds such as TMA. One of these specific spoilage organisms, *Photobacteriumphosphoreum,* generates approximately 10 - 100 folds the amount of TMA than that produced from the more commonly-known specific spoiler, *Shewanellaputrefaciens*(Dalgaard, 1995).

Trimethylamine(TMA) is not a particularly good indicator of edibility with respect to herring quality but is useful as a rapid means of objectively measuring the eating quality of many marine demersal fish. Trimethylamine(TMA) index and eating quality have been excellent in some cases (Wong and Gill, 1987). The advantages of TMA analysis over the enumeration of bacterial numbers are that TMA determinations can be performed far more quickly and often reflect more accurately the degree of spoilage

than do bacterial counts **(**Anup, *et al.*, 2013). For example, even high quality fillets cut with a contaminated filleting knife may have high bacterial counts. However, in such a case the bacteria have not had the opportunity to cause spoilage, thus TMA levels are bound to be low. The chief disadvantages of TMA analyses are that they do not reflect the earlier stages of spoilage and are only reliable for certain fish species. Mamman*et al.*, (2000) stated that in a fresh fish, the concentration was usually less than 3mg per 100g fresh weight. For a spoilt fish, the concentration was usually greater than 8mg per 100g fresh weight, while a value of 5mg per 100g could be taken as an indicator of doubtful quality. It was observed that fish flesh was easily perishable and the concentration of trimethylamine increases with storage time.

The consumer of such fish containing toxic substances such as trimethylamine may suffer from food toxicity such as diarrhoea, abdominal cramps, fever, vomiting, nausea, fibrosis and botulism (Gaman, 1981). Various kinds of fish differ considerably in their perishability. The consumer of spoilt fish containing toxic substances such as trimethylamine suffer from food poisoning such as Botulism, Vibriosis, e.t.c. (Mossel, *et al.*, 1975).

Trimethylamine(TMA) determination has become one of the established procedures for determining fish quality. It has been proposed that TMA levels between 5 and 10 mg/100 g tissue should be considered the maximum allowable levels in international trading. It should be observed that TMA, as would be expected of the bacterial product, is not useful in determining quality deterioration, which occurs during frozen storage. It should also be observed that the TMA values are dependent upon the storage temperature of the fish and will vary accordingly (FAO, 2012).

The total volatile bases developed during the storage of unfrozen fish consist primarily of ammonia and trimethylamine (TMA). Depending on the species (benthic fish, pelagic species, and shellfish), TMA has been observed to be a useful measure of freshness quality (particularly flavour and odour) of a variety of seafood, but this usefulness depends upon the location of catch, stage of spoilage, type of processing, and/or storage, and method of analysis (Leo *et al.*, 2010).

Trimethylamine provides an accurate indication of bacterial spoilage in some species (Anup, *et al.,* 2013). It is a pungent volatile amine often associated with the typical (fish odour of spoiling seafood. Its presence in spoiling fish is due to the bacterial reduction of trimethylamine oxide (TMAO) which is naturally present in the living tissue of many marine fish species. TMA is believed to be generated by the action of spoilage bacteria. TMA is useful as rapid means of objectively measuring the eating quality of many marine demersal fish.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Source of Fish Samples

Marine fish species that were used in this study include *Clupeaharengus*(also known as herring), *Sardinapilchardus*(Sardine) and*Trachurustrachurus*(also known as horse mackerel). The table-size fishes were purchased from the fish depot located at Sabongari, Zaria, Kaduna state, Nigeria. These fishes were chosen because they are readily available and their affordability cuts across the various economic classes of Nigerian.

3.2 Identification and Preparation of Fish Samples

After purchase, the fish were immediately brought in ice boxes to the Fisheries Laboratory, Department of Biological Sciences, Ahmadu Bello University,Zaria. The collection was carried out in such a way that from 20 fish samples, 2 pooled samples of fish was drawn. Collection was carried out thrice. A total of 60 fish per species was collected for analysis. The fish species were identified as *Clupeaharengus, Sardinapilchardus*and *Trachurustrachurus* using the keys of Burchard*et al.*, (1967) and Froese and Pauly, (2012). Weight was measured in grams, using Sartorius CP820 model, while the standard length and total length in centimetres were measured with the aid of a measuring board. The fish samples were gutted, beheaded, filleted, thoroughly rinsed with distilled water and kept in air-tight labelled plastic container and stored at freezing temperature of 0□C to -4□C

3.3 Biochemical Methods of Fish Quality Assessment

3.3.1 Oxidative Rancidity

3.3.1.1 Determination of peroxide value (PV)

The analysis was carried out in the Biochemical Laboratory, National Research Institute for Chemical Technology (NARICT), Zaria.Two grams of each fish sample was dissolved in 10ml chloroform and 30ml glacial acetone saturated potassium iodide was added. The sample was kept for 30minutes to enable reaction with the chemical. Then 20ml of 5% KI was added followed by 3 drops of starch solution. The solution containing the sample was then titrated with 0.1N sodium thiosulphate. The colour changed from dark brown to white after titration. Blank samples were prepared using the above procedure but without the sample.

PV was determined using the published ferric thiocyanate method (Undeland*et al*., 2005).

PV was calculated by the following equation:

$$\text{PV (mEq peroxide kg} - 1) = \frac{(\text{A} - \text{B}) \times 0.1\text{N} \times 1000}{\text{W}}$$

Where,

A =Amount of sample used

B = Blank concentration

W = weight of sample in grams

0.1N= Concentration of sodium thiosulphate

3.3.1.2 Determination of thiobarbituric acid

Thiobarbituric acid (TBA) was determined according to the method of Vyncke (2006) and the analysis was carried out in the Animal Science Laboratory, Faculty of Agriculture, Ahmadu Bello University, Zaria. Ten grams of fish flesh was

homogenised with 50 ml of 7.5% trichloroacetic acid for 1minute using an electric blender. The mixture was filtered using Whatman No.1 filter paper. The filtrate was distilled until 50ml of distillate was obtained. Then, 5ml of thiobarbituric acid reagent (0.02M of 2-thiobarbituric acid in distilled water) was added to 5ml distillate and 5ml distilled water in a test tube. Five millilitres of TBA reagent and 5ml distilled water were used for the blank. The test-tubes were covered using screw caps and further placed at 90^0C in a water bath for 40minutes, after which the test tubes were cooled under tap water. The absorbance was measured at 538 nm using Jenway 6405 uv/vis. The optical density of the diluted solution was measured with a spectrophotometer.

TBA (mg malonaldehyde kg-1) = Absorbance value of sample x 7.8.

3.3.2 Determination of Total Volatile Nitrogen

Total volatile nitrogen was determined by using trichloroacetic (TCA) extract steam distillation method (Riquixo, 1998). The analysis was carried out in the Animal Science Laboratory, Faculty of Agriculture, Ahmadu Bello University, Zaria.Fifty millilitres of 7.5% aqueous TCA solution was added to 25g of fish muscle and was then homogenised for 1minute using an electric blender, the mixture was filtered using Whatman no 1 filter paper. 25 ml of filtrate was then loaded into the distillation tube followed by 6 ml of 10% NaOH. A beaker containing 15 ml of 4% boric acid and 2 drops of methyl red indicator was placed under the condenser until 100ml of the distillate was obtained after distillation. The boric acid solution was titrated with 0.03N sulphuric acid solution using a graduated burette. The colour changed from grey to red. The quantity of TVN in mg was determined from the volume of sulphuric acid (ml) obtained after titration.

The TVN mg-N/100g will be determined using following calculation

$$\text{TVN mg- N/100g} = \frac{(A)\ (0.055\ \text{mol/l})\ (14\ \text{N /mol})\ 100}{8.333}$$

A = amount of sulphuric acid used

14 = Molar mass of nitrogen

3.3.3 Determination of trimethylamine by picric acid method

Trimetylamine was determined by using by picric acid method (Riquixo, 1998). In the Animal Science Laboratory, Faculty of Agriculture, Ahmadu Bello University, Zaria the analysis was carried out. Trimethylamine (TMA) standard stock solution was prepared by adding 0.7ml of TMA to which 1ml of HClwas added and diluted to 100 ml with distilled water using a volumetric flask. Working solution was prepared by adding 1ml stock solution to 1ml HCl and it was then diluted to 100ml with distilled water in a volumetric flask. Out of the working solution, 4ml of distilled water was used as blank for TMA standard. For the standard 1ml, 2ml and 3ml of working solutions were used and diluted to 4ml with distilled water. Absorbance was read at 410nm.

Forty millilitresof 7.5% trichloracetic acid aqueous solution was added to 20g of each fish sample and blended for 1min using an electric blender. The mixture was filtered using Whatman No. 1 filter paper to receive clear solution. One millilitres of the sample, 3 ml distilled water, 1 ml 20% formaldehyde, 3 ml 45% KOH and 10 ml clean toluene were placed into a reagent glass. The reaction glass was shaken 10 times. Seven to eight millilitres of solution was decanted using pump pipette and introduced to second glass containing 0.3g of dry Na_2SO_4. The mixture was shaken for 15mins at 3rps using Gallenkamp flask shaker machine. Four millilitres of the solution was put into new reagent glass containing 4 ml of 0.2% picric acid. The TMA in mg-N/100g was determined using following calculation:

$$\text{TMA mg-N /ml standard solution} = \frac{\text{Concentration of standard}}{\text{Absorbance of standard}} \times \text{Volume of standard}$$

TMA mg-N/100 g sample = (A–B) x 3 x TMA mg-N/100 standard x C

A = Absorbance of the sample

B = Absorbance of blank

C = Dilution of sample

3.4 Data Analyses

Data was subjected to one-way analysis of variance (ANOVA) by using the general linear model procedure. Differences between sample means where present, were ranked using Duncan's multiplerange test (DMRT).The values at $P \leq 0.05$ were considered statistically significant. Correlation analysis (r) was carried out to determine the relationship between the biochemical parameters and the morphometrics of fish.

CHAPTER FOUR

4.0 RESULTS

4.1 Analysis of the Biochemical Qualities of Fish Samples

In this study, 60 samples of *Clupeaherangus* wereanalysed with an average weight of 274g (range 176.9-262.1g), and 60 samples of *Sardinapilchardus* with an average weight of 353.93g (range 224.9–507.4g) and 60 samples of *Trachurustrachurus* with an average weight of 523.23g (range 423.8–602.5g).

4.1.1 Total volatile nitrogen (TVN)

InTable 4.1: for*S.pilchardus*, the TVN value within the samples collected showed significant difference ($P \leq 0.05$). The third collection showed the highest TVN value of 40.20mg-N/100g which indicates that it is spoilt according to the quality index. The first and second collections were very good according to the quality index, the TVN value of the first and second collections are 9.24mg-N/100g and 19.17mg-N/100g respectively which indicate that they were very good according to the quality index.

In Table 4.1:for *C.harengus,* the mean of the TVN value within the samples collected were significantly different ($P \leq 0.05$). The third collection had the highest value of TVN 55.44mg-N/100g which indicates that the third collection was spoilt according to the quality index. The first and second collection with TVN values of 8.78mg-N/100g and 17.10mg-N/100g respectively indicates that they were very good. The first collection has the lowest TVN value.

In Table 4.1:for *T.trachurus*, the TVN values within the collection samples were highly significantly different ($P \leq 0.01$). The second collection had the highest TVN value of 24.49mg-N/100g, while the first and third collections had 9.47mg-N/100g and

Table 4.1: Total Volatile Nitrogen (TVN) within three collections of theFish Samples

Collection	*Sardinapilchardus*	Quality index	*Clupeaharengus*	Quality index	*Trachurustrachurus*	Quality index
1st	9.24 ± 0.53^{c}	Very good	8.78 ± 1.10^{b}	Very good	9.47 ± 1,27^{b}	Very good
2nd	19.17 ± 1.02^{b}	Very good	17.10 ± 1.22^{ab}	Very good	24.49 ±1.22^{a}	Very good
3rd	40.20 ± 1.39^{a}	Spoilt	55.44 ± 21.10^{a}	Spoilt	21.95 ± 2.60^{a}	Very good
P value	0.000*		0.050*		0.001*	

Means along columns with the same superscript were not significantly different at $P \leq 0.05$
* = significant
ns = not significant.

21.95mg-N/100g respectively. According to the quality index, all the *Trachurustrachurus*samples collected were very good.

4.1.2 Trimethylamine (TMA)

InTable 4.2it showed that for *Sardinapilchardus*, the TMA value within the samples collected was significantly high. The highest value of TMA was observed in the third collection 20.77mg-N/100g, while the lowest is the second collection 6.55mg-N/100g. The first collection had TMA value of 10.62mg-N/100g.

In Table 4.2 it showed that for *Clupeaharengus*, the samples collected had highly significant TMA values ($P \leq 0.05$). The third collection had the highest TMA value of 40.67mg-N/100g, while the first collection had the lowest TMA value of 2.13mg-N/100g. The second collection had TMA value of 3.37mg-N/100g.

In Table 4.2 the TMA value within the *Trachurustrachurus* samples collected was significant. The third collection had the highest TMA value of 2.64mg-N/100g while the second collection had the lowest value of 1.39mg-N/100g.

In Table 4.2, the TMA values for each fish species indicate that the quality is spoilt for *Sardinapilchadus*and*Clupeaherangus*but*Trachurustrachurus* is very good.

4.1.3 Peroxide value (PV)

In Table 4.3 it showed that for*Sardinapilchardus,* the PV for the samples collected was significantly high. The third collection had the highest PV value of 8.80meq/kg, indicating that it is above limits according to the quality index. The first collection had the lowest PV of 2.40meq/kg.

Table 4.2: Trimethylamine(TMA) within three collections of each of the fish samples

Collection	*Sardinapilchardus*	Quality index	*Clupeaherangus*	Quality index	*Trachurustrachurus*	Quality index
1st	10.62 ± 0.07[ab]	Marketable	4.37 ± 0.67[b]	Marketable	1.80 ± 0.15[b]	Very Good
2nd	6.55 ± 1.33[b]	Marketable	3.37 ± 1.60[b]	Good	1.39 ± 0.29[b]	Very Good
3rd	20.77 ± 2.62[a]	Spoilt	40.67 ± 3.53[a]	Spoilt	2.64 ± 0.03[a]	Very Good
P valve	0.001*		0.002*		0.000*	

Means along columns with the same superscript were not significantly different at $P \leq 0.05$
* = significant

Table 4.3**:** PerioxideValue (PV) within three collections of the Fish Samples

Collection	*Sardinapilchardus*	Quality index	*Clupeaherangus*	Quality index	*Trachurus trachurus*	Quality index
1st	2.40 ± 0.46^{b}	Good	4.00 ± 0.92^{a}	Good	7.20 ± 0.81^{a}	Good
2nd	2.55 ± 0.32^{b}	Good	1.75 ± 0.18^{b}	Good	2.85 ± 0.75^{b}	Good
3rd	8.80 ± 0.00^{a}	Spoilt	2.40 ± 0.46^{ab}	Good	4.00 ± 0.69^{b}	Good
P value	0.000*		0.068		0.007*	

Means along columns with the same superscript were not significantly different at $P \leq 0.05$
ns = not significant.

In Table 4.3 it showed that for *Trachurustrachurus*, the PV for the sample collected showed significant difference at P≤ 0.05. The first collection had the highest PV of 7.20meq/kg while the lowest PV value was 2.85meq/kg observe in the second collection.

In Table 4.3 it showed that for *Clupeaharengus*, the PV for the sample collected is not significantly different. Although, the first collection had the highest PV of 4.00meq/kg indicating that it was very good according to the quality index and the lowest value was observed in the 1.75meq/kg which was the second collection.

In Table 4.3 it showed that the PV values for each of the three fish species indicate that the quality was good, although*Clupeaherangus*has the least value. The PV value among the three fish species was not significantly high at P > 0.05.

4.1.4 Thiobarbituric acid (TBA)

In Table 4.4 it showed that for*Sardinapilchardus*, the TBA values for the collected samples were significantly high. The 3rd collection had the highest TBA value of 3.71mg/kg indicating that it was very good according to the quality index. The 1st collection had the lowest TBA value of 0.50mg/kg indicating that it was very good according to the quality index. The 2nd collection had TBA value of 1.44mg/kg indicating that it was also very good according to the quality index.

In Table 4.4 it showed that for*Clupeaharengus,* the TBA values for the collected samples showed significant difference at p ≤ 0.05. The 3rd collection had the highest TBA value of 4.00mg/kg indicating that it was very good according to the quality index. The 1st collection had the lowest TBA value of 0.90mg/kg indicating that it was very good according to the quality index. The 2nd collection had TBA value of 2.42mg/kg indicating that it was also very good according to the quality index.

Table4.4:Thiobarbituric Acid (TBA) within ThreeCollections of the Fish Samples

Collection	*Sardinapilchardus*	Quality Index	*Clupeaherangus*	Quality index	*Trachurustrachurus*	Quality index
1st	0.50 ± 0.02^{b}	Very Good	0.90 ± 0.06^{b}	Very Good	0.39 ± 0.03^{a}	Very Good
2nd	1.44 ± 0.43^{b}	Very Good	2.42 ± 0.12^{ab}	Very Good	1.33 ± 0.08^{a}	Very Good
3rd	3.71 ± 0.84^{a}	Very Good	4.00 ± 1.38^{a}	Very Good	2.82 ± 1.37^{a}	Very Good
P value	0.007*		0.046*		0.15	

Means along columns with the same superscript are not significantly different at $P \leq 0.05$
* = significant
ns = not significant.

In Table 4.4 it showed that for*Trachurustrachurus*, the TBA values for the collected samples were not significantly different at P> 0.05. The 3rd collection had the highest TBA value of 2.82mg/kg indicating that it was very good according to the quality index. The 1st collection had the lowest TBA value of 0.39mg/kg indicating that it was very good according to the quality index. The 2nd collection had TBA value of 1.33mg/kg indicating that it was also very good according to the quality index.

In Table 4.4 it showed that the TBA values for each of three fish species indicate that the quality is very good although*Trachurustrachurus* has the least value of 1.51mg/kg.

4.2 Comparative Analysis of the Biochemical Qualities among *Clupeaharengus, Sardinapilchardus*and *Trachurustrachurus*

In Table 4.5there are non-significant differences between *C. harengus, S.pilchardus*and *T.trachurus*in terms of their biochemical qualities. In the present study the quantity of the biochemical qualities present among the three frozen marine fish species namely *C.harengus, S.pilchardus*and *T.trachurus*were investigated. The total volatile nitrogen (TVN) component of the fish content has maximum amount been seen in *C.harengus*27.10mg-N/100g, *S.pilchardus*22.87mg-N/100g and *T.trachurus*has the lowest 18.64mg-N/100g. The Trimethylamine (TMA) component of the fish content has maximum amount been seen in *C.harengus*16.12mg-N/100g, *S.pilchardus*12.64mg-N/100g and *T.trachurus*has the lowest 9.87mg-N/100g. The peroxide value (PV) component of the fish content has maximum amount been seen in *T.trachurus*4.68meq/kg, *S.pilchardus*4.18meq/kg and *C.harengus*has the lowest 2.72meq/kg. The Thiobarbituric acid (TBA) component of the fish content has maximum amount been seen in *C.harengus*2.44mg/kg, *S.pilchardus*1.88mg/kg and *T.trachurus*has the lowest 1.51mg/kg.

4.3 Correlation of Morphometric Parameters Against Biochemical Qualities

InTable 4.6,the correlation of morphometric parameters against biochemical qualities, showed that decrease in the morphometric analysis of *C. harengus, S. pilchardus*and *T.trachurus*brought about increase in TVN, TMA AND TBA value while increase in the morphometric analysis of *C. harengus, S.pilchardus*and *T.trachurus*brought about an increase in the peroxide value.

Table 4.5: Comparative analysis of the biochemical qualities among *Clupeaharengus, Sardinapilchardus*and *Trachurustrachurus*

Species	TVN	Quality Index	TMA	Quality Index	PV	Quality Index	TBA	Quality Index
S.pilchardus	22.87± 3.93[a]	Very Good	12.64 ± 2.01[a]	spoilt	4.18 ± 0.78[ab]	Good	1.88 ± 0.50[a]	Very Good
C.harengus	27.10± 8.85[a]	Good	16.12 ± 5.29[a]	Spoilt	2.72 ± 0.43[b]	Good	2.44 ± 0.54[a]	Very Good
T.trachurus	18.64± 2.19[a]	Very Good	9.87 ± 3.54[a]	marketable	4.68 ± 0.68[a]	Good	1.51 ± 0.51[a]	Very Good
Mean value	22.87	Very Good	12.88	spoilt	4.18	Good	1.94	Very Good
P value	0.58ns		0.52ns		0.10ns		0.45ns	

Means along columns with the same superscript were not significantly different at $P \leq 0.05$
ns = not significant.

Table 4.6: Correlation of morphometric parameters against biochemical qualities

	TVN(mg-N/100g)	TMA(mg-N/100g)	PV(meq/kg)	TBA(mg/kg)	Weight (g)	Standard Length (cm)	Total Length (cm)
TVN(mg-N/100g)	1						
TMA(mg-N/100g)	0.983*	1					
PV (meq/kg)	-0.888*	-0.789*	1				
TBA(mg/kg)	0.979*	0.924*	-0.964*	1			
Weight (g)	-0.979*	-1.000*	0.774*	-0.915*	1		
Standard Length (cm)	-0.968*	-0.905*	0.975*	-0.999*	0.895*	1	
Total Length (cm)	-0.958*	-0.889*	0.983*	-0.996*	0.878*	0.999*	1

* - strong correlation (negative or positive)

CHAPTER FIVE

5.0 DISCUSSION

5.1 Levels of Biochemical Qualities in *Clupeaherangus, Sardinapilchardus*and *Trachurustrachurus*

The biochemical quality differed from one fish to the other and improper handling of fish may lead to fish spoilage. The stressful environment in which the frozen fish undergoes during the long distant journey from India, Germany or China to Zaria could inhibit the freshness of the fish because of breakdown of the freezer or improper trans-loading.

5.1.1 Total Volatile Nitrogen (TVN)

The results of this study have shown in the 1st and 2nd collections of *Sardinapilchardus* that the TVN were within acceptable limit except the 3rd collection which exceeded the acceptable limit. The acceptable limit of TVN in *Sardinapilchardus* is ≤ 35 mg-N/100g.The results of this study have shown in the 1st and 2nd collections of *Clupeaharengus* that the TVN were within acceptable limit except the 3rd collection which exceeded the acceptable limit. The acceptance limit of TVN in *Clupeaharengus* is ≤ 35 mg-N/100g.In *Trachurustrachurus* all the three collections are within the acceptable limit. The acceptable limit of TVN in *Trachurustrachurus* is ≤35 mg-N/100.

Although high levels of TVN have been implicated in fish which may lead to N-nitroso-compound (NOC) induced cancer, diarrhea and it can also trigger respiratory conditions ranging from asthma to even death (FAO, 2004).

5.1.2 Trimethylamine (TMA)

The results indicate that the concentrations of TMA in the 1st and 2nd collections of *Sardinapilchardus* were within acceptable limit except the 3rd collection which exceeded the acceptable limit. The acceptable limit of TMA in *Sardinapilchardus* is ≤ 10 mg/100 g.The results have shown in the 1st and 2nd collections of *Clupeaharengus* that the TMA are within acceptable limit except the 3rd collection which exceeded the acceptable limit. The acceptable limit of TMA in *Clupeaharengus* is ≤10 mg/100 g.In *Trachurustrachurus* all the three collections are within the acceptable limit. The acceptable limit of TMA in *Trachurustrachurus* is ≤ 10 mg/100 g.

There was highly significant difference (P≤ 0.05) in TMA level among the collected samples of *Sardinapilchardus, Trachurustrachurus* and *Clupeaharengus*. The amount of TMA produced is a measure of the activity of spoilage bacteria in the fish flesh and so it is an indicator of spoilage. The increase in the amount of TVN parallels the increase in TMA. The TMA value states the level of spoilage before freezing (FAO, 2001).

5.1.3 Peroxide Value (PV)

The results indicate that the PV in the 1st and 2nd collections of *Sardinapilchardus*were within acceptable limit except the 3rd collection which exceeded the acceptable limit. The acceptable limit of PV in *Sardinapilchardus* is ≤ 8meq/kg. In this study, the PV in all the three collections of *Clupeaharengus*were within acceptable limit. The acceptance limit of PV in *Clupeaharengus* is ≤ 8meq/kg.

The results indicate that in *Trachurustrachurus* all the three collections were within the acceptable limit. The acceptable limit of PV in *Trachurustrachurus* is ≤8meq/kg. Oxidation of the oil in oily fish (*Clupeaherangus*) gives rise to rancid odour and flavor

which limit the storage life of these fish species more quickly. Increase in the peroxide value is most useful as an index of earlier stage of oxidation; as oxidation proceeds the peroxide valve can start to fall (FAO, 1995).

5.1.4 Thiobarbituric Acid (TBA)

The results indicate that the concentrations of TBA in all the three collections of *Sardinapilchardus* were within acceptable limit. The acceptance limit of TBA in *Sardinapilchardus* is ≤ 8 mg/kg. The results indicate that the concentrations of TBA in all the three collections of *Clupeaharengus* were within acceptable limit. The acceptance limit of TBA in *Clupeaharengus* is ≤ 8 mg/kg.The results indicate that the concentrations of TBA in all the three collections of *Trachurustrachurus* were within acceptable limit. The acceptable limit of TBA in *Trachurustrachurus* is ≤ 8 mg/kg.

The P values within *Sardinapilchardus*, *Clupeaharengus*and *Trachurustrachurus* indicate that they are not significant. Increase in TBA value is the measure of the extent of oxidative deterioration in oily fish(herring) as in the case of peroxide value can also fall at the later stage of spoilage (FAO, 2001).

5.2 Comparative Analysis among the Three Species

Among the three species evaluated in this study, there was no significant difference ($p>0.05$) in TVN, TMA, PV and TBA. Although,*Clupeaharengus*apparently had the highest values, followed by *Sardinapilchardus*and *Trachurustrachurus* for (TVN, TMA, and TBA. The reasons could be that the lower the weight the higher the value of TVN, TMA, and TBA (Clancy, 1995).With respect to the reverse were the case whereby *Trachurustrachurus*had the highest value, followed by *Sardinapilchardus* and thirdly *Clupeaharengus*. The reason could be that the higher the fish weight the higher the PV (meg/kg).

High TVN and TMA concentrations could be as a result of fish handling after catch and the seawater showers defrosting method, with the addition of storage time for these species. Ababouch*et al.* (1996) found that TVN and TMA quantities accumulate rapidly at ambient temperature (21□C – 27□C) in the flesh of frozen sardines and reach can values of 11.2 mg TMA⁄ 100 g and 57.5 mg TVN⁄ 100g. Ruiz-Capillas (2004) reported that the growth of the same parameters in two experiments done with frozen sardines was rapid; reaching 2.72 mg TMA⁄100g and 5.20mg TVN⁄ 100g of sardine flesh per day. TMA quantities depend on the condition of individual fish before catch, season, handling after catch, freezing method and storage time; these could be possible causes for the variations and differences among individual samples.

Low TVN and TMA values in *Trachurustrachurus* samples were detected. In fish stored for a longer period at -18□C, growth of the volatile amines was present. If kept at room temperature after defrosting, TMA and TVB quantities in fish would increase over the limit of tolerance within a few hours. These values continue as a linear growth (Fitzgerald and Bremner, 1994). Studies have shown that TVN quantities above the limit of acceptance do not always follow at the beginning of the spoilage process (Shakila*et al.*, 2003). The increase of TVN quantities in fish controls the exponential growth of the TMA concentration; the spoilage process is considered present when TMA in the fish crosses the limit of acceptance. Fraser and Sumar (1998) and Ozogul*et al.* (2002) concluded that storage of any kind increases TVN values and when those values reach the highest level of tolerance(25–35mg ⁄ 100g) the fish is considered spoiled due to increased bacterial activity.

The speed of deterioration can be linked to the history of the fish prior to freezing, where metabolism has initiated the process that progresses more rapidly when defrosted

than it should when freezing was done immediately and the storage was correct.Protein requirements generally are higher for smaller fish. As fish grow larger, their protein requirements usually decrease, protein requirements also vary with rearing environmental water temperature and water quality(FAO, 1996).

Increase of TVN can be attributed to a decreasing trend of volatile nitrogen analyser enzymes or a decreasing amount of a substrate such as trimethylamine or dimethylamine or non-protein nitrogen. Connell (1980) and Pearson (1997) reported that samples could be considered consumable if the TVN level is less than 20 mg/100 g fish and that a level of more than 30 mg determines the product as not consumable. The increasing of TVN value during storage is related to bacterial spoilage and activity of endogenous enzymes (Chomnawang*et al*., 2007).

Decrease in TBA values indicated decrease in level of lipid oxidation in fish (Abramson, 1949).Decrease of TBA values in fish could be attributed to the interaction of decomposition products of protein with malonaldehyde to give tertiary products (Hernandez-Herrero*et al*., 1999). Increase in the peroxide value of the fish during frozen storage might be due to mechanical mincing of fish meat which accelerates oxidation due to the incorporation of oxygen in the tissue or the disruption and intermixing of tissue components. A similar increase in the PV content was observed by Ninan*et al*. (2010).

The three different fish species have different shelf life which is dependent on their oil level, catch area condition of the seafood, fishing ground and how they have been handled since capture *Tracharustracharus* has a storage life of 21-24 days at

0^{o}C*Clupeaherangus* 2-6 days at 0^{o}C and *Sardine pilchardus* 7-12 days at 0^{o}C(Baixas-Nogueras et al., 2002).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The biochemical indices:TVN, TMA, PV and TBA, of *Sardinapilchardus, Trachurustrachurus* and *Clupeaharengus*are strongly correlated to their morphometrics especially the weight (-0.979, -1.000, 0.774 and -0.915 respectively).

This study revealed that TVN, TBA, and PVmean concentrations of 22.87, 1.94 and 4.18, respectively in the three fish species are within the acceptable limit of FAO, except TMA (12.88) which is not within the acceptable limit. Therefore, the biochemical qualities of imported frozen *Clupeaharengus*, *Sardinapilchardus* and *Trachurustrachurus* sold in Zaria is suitable for human consumption.

Trachurustrachurus has significantly higher quality indices of TVN, TMA, PV and TBA (18.64, 9.87, 4.68 and 1.51 respectively) than *Sardinapilchardus* (22.87, 12.64, 4.18 and 1.88 respectively) and *Clupeaharengus*(27.10, 16.12, 2.72 and 2.44 respectively).

6.2 Recommendations

1. Relevant agencies should ensure strict compliance to recommended biochemical limits of frozen fish imported into Nigeria
2. *Clupeaharengus* should be subjected to better preservation method due to its fast deterioration rate.

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Appendix I: Identification of Fish Species Used.



Plate I:*Sardinapilchardus*



Plate II: *Clupeaherangus*



Plate III:*Trachurustrachurus*

Appendix II: *Raw data of biochemical qualities for Sardinapilchardus*

	TVN	**TMA**	**PV**	**TBA**
SAMPLE 1	9.24	5.12	1.6meq/kg	0.499
SAMPLE 2	9.24	5.2	3.2meq/kg	0.495
SAMPLE 3	20.79	4.83	3.1meq/kg	0.702
SAMPLE 4	17.56	8.26	2.0meg/kg	2.184
SAMPLE 5	40.67	17.46	8.8meq/kg	2.785
SAMPLE 6	39.73	24.09	8.8meq/kg	4.637

Appendix III: Raw data of biochemical qualities*Clupeaherangus*

	TVN	**TMA**	**PV**	**TBA**
SAMPLE 1	6.933	2.69	2.4meq/kg	0.796
SAMPLE 2	10.63	1.56	5.6meq/kg	0.998
SAMPLE 3	17.56	4.8	2.05	2.613
SAMPLE 4	16.63	1.94	1.45	2.223
SAMPLE 5	18.94	37.14	3.2meq/kg	1.856
SAMPLE 6	91.94	44.19	1.6meq/kg	6.131

Appendix IV: Raw data of biochemical qualities*Trachurustrachurus*

	TVN	**TMA**	**PV**	**TBA**
SAMPLE 1	11.55	2.02	5.8	0.351
SAMPLE 2	7.39	1.58	8.6	0.44
SAMPLE 3	22.64	1.27	3.65	1.25
SAMPLE 4	26.34	1.5	2.05	1.4
SAMPLE 5	18.02	2.61	5.2	1.28
SAMPLE 6	25.87	2.67	2.8	4.99

Appendix V: Quality Index of the Biochemical Qualities of Fish

Quality index	TVN[a]	TMA[b]	TBA[c]	PV[d]
Very good	□ 25 mg-N/100g	□ 2mg-N/100g	□ 3 mg/kg	
Good	25-30 mg-N/100g	4 mg-N/100g	5-8 mg/kg	
Marketable	30-35 mg-N/100g	10 mg-N/100g	5-8 mg/kg	
Spoilt	> 35 mg-N/100g	≥ 12 mg-N/100g	≥ 8 mg/kg	> 8meq/kg
Acceptable limit	□ 35 mg-N/100g	□ 12 mg-N/100g	□ 8 mg/kg	□ 8meq/kg

Sources

a = Zaid*et al.*, 2013

b = Mammane*t al.*, 2000

c = FAO, 2012

d = FAO, 2012