Sudan University of Science and Technology

College of Graduate Studies

Microbial Assessment ofFresh and Frozen (for Four Days)Marine Najil fish (*Plectropomus Pessuliferus*)

تقييم ميكروبي لسمكة الناجل البحرية (Plectropomus Pessuliferus) الطازجة و المجمدة (لمدة أربعه أيام)

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science (M.Sc) in Fish Science and Technology

By:

KHANSAA BDR ALDIEN OSMAN AHMED

SUPERVISOR:

DR. SAMIA HAMID AHMED HAMID

September 2017

بسم الله الرحمن الرحيم

الإستهلال

قَالَ تَعَالَىٰ:

﴿ وَهُوَ ٱلَّذِى سَخَّرَ ٱلْبَحْرَ لِتَأْكُلُوا۟ مِنْهُ لَحْمًا طَرِيًّا وَتَسْتَخْرِجُوا۟ مِنْهُ حِلْيَةً تَلْبَسُونَهَا وَتَرَى ٱلْفُلْكَ مَوَاخِرَ فِيهِ وَلِتَبْتَغُوا۟ مِن فَضْلِهِۦ وَلَعَلَّكُمْ تَشْكُرُونَ ﴿١٤﴾ ﴾

النحل: ١٤

Dedication

I sole dedicate output of this work to:

My beloved father (Bdr aldein Osman),

(Who helped me and still guided me)

My beloved Mother (Nabeha AlSiddig),

(Who give me the ability to guide community, love and kindness)

My Brother (Mohamed, Shymaa, Fatima,

(Whom I love them too much)

MY beloved Supervisor:

Dr.Samia Hamid Ahmed Hamid

Teachers,

(Whom I respect them and I hope to become like them in the future)

Colleagues,

(Whom I spend with them beautiful time in my study)

KHANSAA (2017)

Acknowledgment

Firstly I say "praise be to Allah, the cherisher and sustainer of the worlds", the most gracious and the most merciful, who give me knowledge and power for successful completion of this research work.

Foremost,myheartfelt appreciation to my reverent teacher and supervisorDr. Samia Hamid Ahhmed.For here infinite patience and enthusiasm, extensive knowledge, the valuable and constructive suggestions and advices during the development of this project. Words cannot express my gratitude for their encouragement and willingness to help me all over the time of my research and writing of this dissertation, especially during last weeks. I cannot imagine having a better supervisor and mentor than you and it was a great opportunity to work with you. I am grateful for all your guidance. I learnt a lot from you how to work with maximum efficiency.

My sincere gratitude goes toDr.Ahmed El-aabid Hamad, Dr. Abdalla Nasir Elawad for their strong support and assistant. Also it extend to the staff of the Directorate general of preventing medicine and PHC, Epidemiology Dept, Port Sudan for providing a good working environment and giving me warm welcome.

Most special thanks to my parent (bdr aldein and nabiha), my second father Amar Osman, my second mother (khadiga), grandfather and mother, my dearest aunt and uncle.And my deepest thanks extent to

Prof. Hamid Agab (Sudan University of Science and Technology), Dr. Mona (head of fish research centre, Port Sudan) and Dr. Sheikhaldein (college of marine science and fisheries) for their assistant, my friends for their support, love and understanding.

Table of contents

Table Index

Figure Index

Abstract

The present study was conducted at Directorate General of Preventing Medicine and PHC, Epidemiology Dept, Ports and quarantine health unit, Port Sudan, endeavors for Microbial evaluation (bacteriological load) of marine fish Najil (***Plectropomus Pessuliferus)***. And to determine the occurrence of some contaminated bacteria.

This study was carried out from 23/1/2017 to 2/2/2017. 20 samples of Najil fish (***P. Pessuliferus***) were collected from Port Sudan fish market (in sterile plastic bags) and pre chilled with ice in thermostatic container. All the samples were tested immediately, and instantly froze for four days. Then the studied samples were tested microbiologically and results showed:

For fresh fish total bacterial count (TBC) was (**5.68×10^5**) CFU/gm, and for frozen samples was (**4.38×10^5**) CFU/gm respectively.

The study concluded that,Najil fish (***P. Pessuliferus)***showed the highest level of contamination between fresh fish. While in frozen fish freeze seem have the higher effect (less number). And the results showed highly significant differences between fresh and frozen fish.

The samples were also tested for contaminant bacteria and the result indicated the presence of *Staphylococcus* and *Ecoli*, while the *salmonella* and *vibrio* were absent at each of it.

Keywords: Microbial evaluation, Frozen fishes, Najil (*plectropomus pessuliferus*) fish.

ملخص البحث

أجريت هذه الدراسة بالإدارة العامه للطب الوقائي والرعاية الصحية الأولية, إدارة الوبائيات, وحدة الموانئ والمحاجر الصحية , بورتسودان , وهدفت إلي التقييم الميكروبي (الحمل الميكروبي) لسمكة الناجل (بلكتروبوماس بسيليفيرس) البحرية الطازجة والمجمدة. وكذلك تحديد وجود بعض أنواع البكتريا الملوثة.

أجريت هذه الدراسه علي 20 سمكة . في الفتره من1/23 الي2/2/ 2017 . تم جمع هذه العينات منسوق بورتسودان للأسماك (في أكياس بلاستيك معقمة) ثم بردت العينات تبريد مبدئي بالثلج في حافظه ثابته درجه الحرارة. تم فحص كل العينات ثم جمدت لمدة أربعه أيام. فحصت العينات ميكروبيولوجيآ وقد أظهرت النتائج الآتي:

الأسماك الطازجة العدد الكلي للميكروبات ($\mathbf{5.68 \times 10^5}$) خلية مكونة للمستعمرات في الجرام . وفي حالة الأسماك المجمدة لمدة أربعة أيام كان العدد ($\mathbf{4.38 \times 10^5}$) خلية/الجرام علي التوالي .

خلصت الدراسة إلي أن أسماك الناجل (بلكتروبوماس بسيليفيرس) أظهرت مستوي عالي من الميكروبات بين الأسماك الطازجة . بينما في الأسماك المجمدة كان للتجميد الأثر الأكبر(العدد أقل) . وقد أظهرت النتائج فروق معنوية عالية جدآ بين الأسماك الطازجة والمجمدة.

كما فحصت العينات فحص بيوكيميائي للبكتريا الملوثة وخلصت النتائج الي وجود كل من الاستافيلوكوكسوالإيكولاي, ولم توجد السالمونيلا والفبريو في كل منها.

كلمات مفتاحية : تقييم ميكروبي , أسماك مجمدة , ناجل (بلكتروبوماس بسيليفيرس) .

CHAPTER ONE

INTRODUCTION

Fish accounts for approximately 17% of the global animal protein intake. Globally, the production of fish compete the growth of world population (FAO, 2014).

Sea foods have traditionally being a popular part of the diet in many parts of the world and in some countries constituted the main supply of animal protein. Today, even more people are turning to fish as a healthy alternative to real meat. Seafood differs from other types of foods in a number of ways, most seafood is still collected from wild population, and the fishermen are hunters with no influence on handling of their prey before it is caught .Thus, it is not possible to irritate the situation for slaughter animals, selecting only the most suitable specimen for slaughter and to rest and feed them well before killing (Adebayo-Tayo *et al*., 2012a). Also fish and Seafood supply a number of essential vitamins and minerals, including: vitamins A, B3, B6, and B12, and D, and the minerals calcium, iron, selenium, and zinc.

Seafood consumption is linked with improvements in health conditions including cardiovascular disease (stroke), arthritis, Cognitive function, Hearing loss, Depression/mood, Osteoporosis, and cancer. However, consumption of fish and shell fish may also cause diseases due to infection or intoxication, some of these diseases have been specifically associated with pathogens which are resistant to antibiotics (Adebayo-Tayo *et al*., 2012a).

Some seafood commodities are more risky than others owing to many factors, including the nature of the environment from which they come, their mode of feeding, the season during which they are harvested, and how they are prepared and served. Fish are conditioned by their environment if the growing and harvesting environment of fish is polluted chemically or microbiologically, the fish will be also polluted (Begum *et al*., 2010). When frozen seafood products are consumed raw, there is the likelihood of endangering the health of the consumer especially when the micro-organism present includes pathogenic ones (Adebayo-Tayo *et al*., 2012a).

Fisheries sector plays an important role in food security, poverty alleviation and economic development of Sudan. Inland and marine fisheries are the major fisheries sectors of Sudan. These sectors support not only the domestic needs but also contribute to world export markets (Begum, 2014).

In Sudan because of lacking facilities, bad handling and processing most of the fish landing sites where subjected to very poor conditions. Thus the competent authority that is supposed to intervene to improve this quality unfortunately has very limited role and effort as a result of lacking in technical and financial capabilities.

Sea basses (Serranidae) are among the most important families of commercially harvested tropical marine fishes worldwide (Elamin *et al.,* 2011). Because coral trouts are favored for consumption and sale in commercial and subsistence fisheries in Sudanese water, they have been main target species for artisanal fisheries in Sudanese marine fisheries for the past two decades (Elamin *et al.*,2014)

One of the species that are most commonly caught in Sudanese water is coral trout of species *plectropomus pessuliferus,* the species which caught by handlines, heavy

exploiteted, sold and bring high market prices (Beets, and Hixon,1994; Morris *et al.,* 2000).

However, as demands for and production of seafood increased, it becomes important to be more attention to seafood safety and seafood related diseases.

Aims of the study:

General objectives:

The general objectives of this study are firstly to increase microbiological information on marine fish species by determine microbial qualities, carry out trials for microbiological shelf life of freshly and frozen hygienically handled Najil (*p. pessuliferus,*in laboratory study) as well as the prevalence of certain human pathogens. Also to understanding, be aware about fish quality, HACCP system concerns. And finally to reduce quality loss of fish.

Specific objectives:

The present study was therefore aimed firstly for comparative analysis of microbiological evaluation (estimate the microbial load) in the available consumable marine fish fresh and frozen Najil (*p. pessuliferus*), Secondly to findout the occurrence of some pathogenic bacteria such as *vibrio* and *salmonella* spp.

CHAPTER TOW

LITERATURE REVIEW

2.1Nutritional benefits 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.

Consumption of fish is increasing globally due to increasing evidence of positive benefits for medical conditions like constipation, cardiovascular diseases, obesity, hypertension and certain variety of cancer. World per capita apparent fish consumption increased from an average of 9.9 kg in the 1960s to 19.2 kg in 2012 (Mohan *et al.,* 2016).

Fish contain high-quality protein and other nutrients our bodies require. It is low in saturated fat and cholesterol (EFSA, 2014). 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). Fish and seafood constitute an important food component for a large section of world population (Bark, *et al*., 2011; Sakthivel and Fernando, 2012); Trivedi, *et al*., 2012; Ozcan, *et al*., 2013; Varadharajan, *et al*., 2013).
Seafood products have attracted considerable attention as a source of high amounts of important nutritional components like high-quality protein, essential vitamins and minerals and healthful polyunsaturated fatty acids to the human diet (Ackman, 1989;INA- Smith and Simpson, 1996).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.

Raw fish and seafood is a highly perishable commodity compared to other fresh meat commodities and have short lifetimes even at refrigeration temperature (Lauzon *et al.*, 2010; Popovic *et al.*, 2010; Can, 2010). To maximize its value, freshness quality must be maintained and shorter lifetime is a big hurdle.

Societies with high fish intake have considerably lower rates of acute myocardial infarctions, other heart diseases and atherosclerosis, arthritis, asthma and other inflammatory or self- immune illnesses or certain types of cancer (Bang and Dyerberg, 1980; Shahidi and Botta, 1994).

The above mentioned benefits are thought to be due to rich omega-3 poly unsaturated fatty acids content (Ω-3 PUFA). Furthermore, dietary intake of Ω-3 PUFA was inversely related to the risk of impaired cognitive function. Ω-3 PUFAs are also critical for normal neural and visual development in the human foetus. The two important -3 PUFAs, EPA (Eicosapentaenoic acid) and DHA (Docosahexaenoic acid) are available to consumers mainly through a diet rich in fish. Studies also indicated that dietary factors such as DHA have neuroprotective effects which help in preventing Alzheimer's disease (Clemens and Pressman, 2007).

Fish, which have highly contribution of net protein utilization in Sudanese homes, is still below the recommended requirement by the World Health Organization of 40.0g/day. (WHO, 2015).

Najil (*Plectropomus pessuliferus*):

Sea basses (Serranidae) are among the most important families of commercially harvested tropical marine fishes worldwide, Coral trout (*P. pessuliferus*"Najil"isthe main target species for artisanal fishers in Sudanese marine fisheries (Elamin *et al*., 2011). Coral trouts of the genus Plectropomus is members of the serranid subfamily Epinephelinae, which are commonly known as groupers (Frisch and Hobbs, 2007).

*P. pessuliferus*colours isthat the head, body, and fins brown to orange red, with numerous small dark-edged blue spots; some spots on head and sides of body of adults are elongated (those on body usually vertically elongate); spots few or absent on ventral part of body; edge of orbit often blue (may be broken into segments).And his habitat and biologyoccurs on or near coral reefs at depths of 25 to 147 m. *P. pessuliferus* differs from *P. maculatus* in having the longest gill rakers shorter than the longest gill filaments, some spots on body vertically elongate, and pelvic fins with blue spots (FAO species catalogue).

2.2 Microbial flora of fish:

The natural habitat of fish is extremely susceptible to pollution from domestic, industrial and agricultural discharges. Therefore, fish and other aquatic life forms are vulnerable to all environmental hazards (Raufu *et al*., 2014).

Fish is soft and easily to damaged, therefore rough handling and bruising results in contamination of fish flesh. Within one day of capture fish will become unfit for human consumption unless it is subjected to some form of processing or preservation. Fish are much more perishable than any other high protein muscle foods and this high degree of perishability is primarily due to the large amounts of non-protein nitrogen NPN) e.g. free amino acids, volatile nitrogen bases i.e.

ammonia, TMA, creatine, taurine, uric acid, carnosine and histamine (Mayer and Ward, 1991).

Fish are extremely susceptible to microbial contamination, although the flesh itself is normally sterile, Millions of bacteria many of them potential spoilers, are present in the surface slime, on the gills and in the intestines of life fish (Abolagba and Uwagbai, 2011).

All seafood can be susceptible to surface or tissue contamination originating from the marine environment. Bivalve mollusks feed by filtering large volumes of seawater. During this process, they can accumulate and concentrate pathogenic microorganisms that are naturally present in harvest waters, such as vibrios (Popovic *et al.,* 2010). Human pathogens of exogenous origin in seafood include *Escherichia coli*, *Salmonella*, *Shigella*, *Yersinia enterocolitica*, *Campylobacter* spp., *Staphylococcus aureus* and *Bacilluscereus* introduced through poor personal hygiene or cross contamination with other contaminated foods (Popovic *et al*., 2010).The microfloras 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).

2.3 Fish and sea food spoilage

Seafood's in their natural environments are associated with a variety of microorganisms. The microbiology of fish skin and gastro intestinal tract has been subjected to many researches. Fish can spoil from both outer surface and inner surfaces as fish stomach contain digested and partially digested food which can pass into the intestine (Emikpe *et al*., 2011). Although the flesh itself is normally sterile, Bacterial growth and invasion on the fish are prevented by the body's natural defense system during life but after death the defense system breaks down and the bacteria multiply and invade the flesh (Abolagba and Uwagbai, 2011).

Seafood contamination occurs naturally from the environment where fish are harvested, during harvesting, processing or during food preparation. Contamination of fish from enteric bacteria of human and animal origin may also be responsible for various food spoilages (Emikpe *et al*., 2011).

Various compounds such as Trimethylamine (TMA), total volatile base nitrogen (TVB-N), sulphuric compounds, aldehydes, ketones, esters, etc., are being produced by various microorganisms during the fish spoilage (Gram and Huss, 1996).Apart from spoilage the safety of seafood also has to be controlled in terms of the presence of possible food–borne pathogens such as the human pathogenic *Vibrio* species, *C. botulinum*, *Aeromonas hydrophylla*, *B.cereus*, *Salmonella* spp., *Y. enterocolitica*, *L.monocytogenes* (Francoise, 2010).

In the technology of marine animal processing by cooking, the following critical aspects of marine animals are significant, the duration of cooking, temperature of steam, water and other media racy of thermometer and other monitoring and timing devices. For unpreserved fish, spoilage is a result of gram-negative, fermentative bacteria (such as Vibrionaceae), whereas psychrotolerant gram-negative bacteria (such as *Pseudomonas* spp. and *Shewanella* spp.) tend to spoil chilled fish(Gram

and Huss, 2000). It is therefore important to distinguish non spoilage microflora from spoilage bacteria as many of the bacteria present do not actually contribute to spoilage (Huss, 1996).

2.3.1Fish 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).

Fish is extremely perishable. It spoils easily. “Spoilage” can be defined as a change in fish or fish products that renders them less acceptable, unacceptable or unsafe for human consumption.

Aquatic food products deteriorate rapidly due to post-mortem as a consequence of biochemical and microbial breakdown mechanisms (FAO, 2014;Botta 1995) defines fish spoilage as a change in a fish or fish product that makes it unsafe, less acceptable, or unacceptable to the consumer for its original intended purpose.

In tropical conditions, fish spoils quite rapidly within a few hours of landing if not properly cooled (Akinneye *et al*., 2010).Chemical breakdown of protein, fat and water contents contribute to quick spoilage of fish. 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.

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 colonize fish skin surfaces and gills are predominantly aerobes however, facultative bacteria may occur in high counts on pelagic fish.

Bacteria from skin and gills are predominantly aerobic although facultative bacteria particularly *Vibrio* may occur in high numbers on pelagic fish.
Gomez and Balcazar (2008) stated that obligate anaerobic bacteria are not common on the surfaces of fish but their occurrence is significant in the intestines.
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 characterized 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).

2.3.2Microbiological spoilage

Fish flesh provides an excellent substrate for the growth of most heterotrophic bacteria with compositional attributes that affect bacterial growth and the related biochemical activities.Live fish is normally considered to be sterile, but microorganisms are found on all the outer surfaces (skin and gills) and in the alimentary tract of live and newly caught fish in varying numbers.Bacteria are characterized according to their optimal growth requirements, which include among others water, oxygen, pH, temperature and redox potential (Eh). Their distribution in the environment is therefore dependent on their requirements.

Bacteria living on the surface of marine animals are phenotypically capable of utilizing amino acids, peptides and other non-carbohydrate sources. Utilization of these substrates normally leads to the production of slightly alkaline conditions especially in the stored fish products (Liston, 1980).

Spoilage bacteria are characterized by their ability to produce Hydrogen Sulphide (HS), reduce trimethlyamine oxide (TMAO) to trimethlyamine (TMA) and convert urea to ammonia. 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 (Aboh, 2015). Trimethlyamine (TMA), ammonia and hypoxanthine are chemical compounds produced by bacterial action (Carlos, 2008; FAO, 2013),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 al*., 2010).

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

2.3.3 Chemical spoilage

Chemical spoilage processes are changes taking place in the lipid fraction of the fish. Lipids are oxidised to peroxides, aldehydes, ketones and lower aliphatic acids. The main reactants in these processes involves atmospheric oxygen and fish lipid but the reactions are initiated and accelerated by heat, light (especially UV-light) and several organic and inorganic substances like copper and iron ions. The end products are aldehydes and ketones, which impart the strong rancid flavour normally, associated with spoilt fatty fish (Huss, 1994).

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.

2.3.4 Autolytic spoilage

Rigor mortis disappears in a short time even under the most favourable storage conditions. Immediately thereafter, the decomposition of the highly complex protein of the fish muscle into simpler protein, polypeptides and amino acids starts

to take place. These changes are known as autolysis. While autolysis is proceeding, bacterial decomposition begins, which is the most complex and important of all the changing processes (Mohan *et al.*, 2016). According to (Huss. 1994), autolytic changes are responsible for the early quality loss in fresh fish but contribute very little to spoilage of chilled fish and fish products. However, under frozen conditions, autolytic enzymes break down TMAO to dimethylamine (DMA) and formaldehyde (FA). The more these enzymes get in contact with the Fish's flesh the greater the spoilage. Adenosine triphosphate (ATP) is broken down through a series of products such as adenosine diphosphate (ADP), inosine monophosphate (IMP), inosine and hypoxanthine (HX).

2.4Factors that influence the rate of fish spoilage

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 effect of time/temperature, post-mortem handling procedures processing facilities, initial microbial load in association with fishing ground, method of capture, mode of storage. Literally, every stage of handling from harvest to consumption affects spoilage in one way or another.

The rate at which spoilage occur varies with species of fish, sanitary conditions, methods of handling and storage (Flowra *et al.*, 2012).

2.4.1 Effects of time/temperature conditions on microbial growth

Consumers demand for fresh or fresh like fish products without altering its natural quality attributes will be met by using low temperature preservation particularly

chilling. Low temperature preservation is widely practiced in the industry to overcome the spoilage of fish (Mohan *et al.,* 2016).

Temperature is the primary factor controlling spoilage in fish because it has direct effect on the growth of microorganisms.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.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.

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).

2.4.2 Post-mortem handling practices and storage procedures

Different species have different storage life, which varies depend upon the oil content, catch, fishing area, season and duration of the rigor mortis (Gopalakrishnan *et al.,* 2016). 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).

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. The bacteria may also be transferred from the processing environment namely, contaminated surfaces, knives and machines. It has been estimated that fillets and other products from fresh fish processors usually carry counts of 10^3-10^5/g or more (Abowei, 2011).

Insufficient cleaning may lead to bacterial build up which in turn will act as a source of subsequent contamination.

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.

Offloading operations provide opportunities for bacterial contamination via offloading equipment, pumps, conveyors, baskets and boxes that redistribute surface contamination (FAO, 2011).

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 ambient temperatures are more than $20C^0$ permits multiplication of spoilage bacteria. Delays in chilling when ambient temperatures are high can significantly shorten shelf life during subsequent storage(Abowei, 2011).

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 al.,* 2010).The rate of fish spoilage depends on handling during processing, acidity level, species of fish (Round fish deteriorate faster than flat fish (Flowra *et al.,* 2012), weather, mode of storage and temperature during transportation (FAO, 2013).

2.4.3 Initial bacterial load

The initial microflora 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).

The flesh of the freshly caught fish is regarded as sterile and their surfaces will carry contaminants, which are easily transferred to the flesh. The fresh and minimally processed fish products provide a good substrate for microbial growth. Such substrate may allow proliferation of human pathogenic organisms. Ensuring the safety of food products depends on minimizing the initial contamination with pathogenic microorganisms and inhibiting their development during handling and storage.

The microflora on tropical fish often carries a slightly higher load of Gram-positives and enteric bacteria but otherwise is similar to the flora on temperate-water fish. Basically, bacteria populations on temperate fish are predominantly psychrotrophic reflecting water temperatures of about $10c^0$ while fish from the tropics have largely mesophilic bacteria.

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 *Shewanella putrefaciens* (Lauzon *et al., 2010*). Nilla *et al.,* (2012) studied that the total bacterial count of marketed Mola (*Amblypharyngodon mola)* ranged from $1.8 \pm 0.25 \times 10^4$ to $6.5 \pm 0.75 \times 10^6$ cfu/g for fresh sample and $5.5 \pm 0.55 \times 10^3$ to $7.0 \pm 0.80 \times 10^5$ cfu/g for frozen . The highest total coliform count of mola was $8.0 \pm 0.55 \times 10^4$ and $6.1 \pm 0.40 \times 10^3$ cfu/g for local market and departmental chain shop, respectively.Also Oramadike *et al.,* (2010) studied microbiological qualities of some frozen fishes available in some reputable supermarkets in Lagos State and reported that total bacterial count ranged between 2.0×10^3 to 7.4×10^3 cfu/g, total coliforms per gram ranged between 0 and 53 MPN/g and did not exceed acceptable total coliforms limit per gram for frozen fish. The sanitary, storage and hygienic conditions of the supermarkets were relatively the same.

Noor *et al.,* (2013) studied the prevalence of pathogenic microflora along the two major sea fish samples, Rupchanda (*Pampus chinensis*) and Surmai (*Scomberomorus guttatus*) and reported that the total bacterial count was 2.5×10^6cfu. /g in fish blend samples.

2.4.4 Methods of capture

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).

Fish caught by hook and line die or are killed relatively rapidly when brought into the air (ICMSF, 1998) and the method minimizes stressed 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 utilize 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. Bacteria may gain access through puncture wounds and bruises during the death struggle and may multiply rapidly in these localized 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). A method that inflicts stress or struggle hastens the onset of spoilage.

2.4.5 Rigor mortis

Rigor mortis is the process through which fish loses its flexibility due to stiffening of fish mussels after few hour of its death (Adebowale *et al.*, 2008).

Temperature and rigor mortis are the main underlying factors in fish spoilage (Flowra *et al.*, 2012). 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. The change in pH affects the enzymatic and other chemical reactions in fish muscle cell, which may result in flavour and odour changes.

2.5Quality, Hazard Analysis Critical Control Point (HACCP) system and principles of fish preservation

2.5.1Quality and HACCP system

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).

Quality can be assessed by the determination of storage life, which is the amount of time which seafood remains palatable.There are two main methods of assessing fish quality to determine its freshness and shelf life and these are i. Sensory and Non-sensory methods. Sensory methods rely mostly on appearance, odour, texture and taste of the Fish while non-sensory methods use physical, biochemical, chemical and microbiological means (Huss, 1995). 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. Shelf life of most marine fishes have been observed to range between 2-24 days in ice, 5 days at $5C^0$ and 3 days at $10C^0$ (Lauzon *et al*., 2010).

Fish of lower quality, which is rejected either from the marked or at the fish processing plant, accounts for 10 - 25% of the total global wild-captured fisheries (FAO, 2014a, b).

The Food and Drug Administration (FDA) regulations mandated (starting December 1997) the application of the Hazard Analysis and Critical Control Point (HACCP) principles to seafood processing. The first line of control of the presence of bacterial pathogens in seafood is the use of Good Manufacturing Processes (GMP) (Vasconcellos, 2004a). Secondly most seafood processors have effective safety and quality assurance systems in place in most cases based on the Hazard Analysis Critical Control Points (HACCP) principles (Vasconcellos, 2004b), (Ward, 2002).

The four most prominent driving forces for use of HACCP are: (1) HACCP is focused on food safety, (2) is science based, (3) relies on preventive controls rather than retrospective end-product testing, and (4) focuses control on those food safety hazards that are reasonably likely to occur. Although HACCP holds great promise for minimizing the risk of foodborne disease, application of HACCP principles to a few foods and food processes is challenging because no useful strategies are available to control some identified food safety hazards. Smoked fish and the process generally used for this product are examples of foods and processes that pose such challenges (Ward 2001).

HACCP can be applied throughout the food chain from primary production to final consumption and its implementation should be guided by scientific evidence of risk to human health. Any HACCP system is capable of accommodating change, such as advances in equipment design, processing procedures or technological developments.

Several methods to remove, reduce, kill or inhibit the growth of pathogens in seafood and water are employed. One of the main functions of the HACCP system is to ensure the effectiveness and robustness of these methods. Proper sanitation of the food processing environment using the right sanitizers in correct concentrations is paramount to keeping the initial microbial load to the minimum. In addition upholding the personal hygiene of the personnel and training in sanitation methods, as well as the use of potable clean water in all cleaning and sanitation procedures are indispensable in attaining this objective. Once this goal has been achieved other methods to be used in successive steps will be successful in reducing or eliminating spoilage organisms and pathogens to acceptable limits hence attaining the desired shelf life and safety of the final product (Ray and Bhunia, 2008).

2.5.2Fish Preservation

The most important aspect in post harvest technology of fish is to prevent and retard the deteriorative changes in fish muscle.

The two major problems with respect to marketing and distribution of seafood's are their high perishability and poor hygienic quality (Olafsdottir *et al.*, 1997). To overcome these problems various preservation methods are being practiced. The principle aim of fish preservation is to delay, reduce or inhibit the enzymatic, chemical and microbial spoilage. This is achieved by controlling the storage temperature, maintaining proper water activity, pH or by using chemical preservatives (Gould, 1989). Therefore, the efficiency of methods depends on its effect in reducing the spoilage rate and thus increasing the shelf life and improving final quality.

The selection of a method of preservation is based on the nature and type of fish, economic viability of the method and technical knowledge.

2.5.2.1 Methods of fish preservation

Preservation technique by temperature control is of two ways, either by lowering or increasing the temperature. Methods involving the lowering of temperature are chilling and freezing. In chilling, the temperature of the fish is lowered immediately to reduce the autolytic and bacterial changes. To slow down the mechanisms involved in quality loss, the fish should be refrigerated immediately after capture until consumed (Gram and Huss, 1996; Sumner *et al*., 1984)

Apart from these various other methods such as drying and salting, smoking, irradiation by gamma radiation, addition of chemical preservative agents, high pressure processing, sous-vide, pulsed electric field are employed as a means of preserving fish and fish products and have shown some degrees of effectiveness (Ahmed*et al*., 1997; Kumta *et al.*, 1973). Canning is another method, in which the temperature is increased to more than 1000C to inactivate all the enzymes and bacterial including spores (Ball and Olson, 1957; Biji *et al.*, 2015).

Each preservation method possesses some significant disadvantages like deterioration of fish quality and modification of freshness, lack of stability, and excessive cost (INA- Smith and Simpson, 1996). However global demand for fresh mildly preserved, convenient with better keeping quality fishery products is increasing. This can be achieved by frozen storage of fish.

2.5.2.2Freezing of fish

Shelf life extension of fish is of importance to allow the transport of products to distant markets at lower cost.

At room temperature, the seafood deteriorates in a faster rate, therefore freezing and frozen storage are important methods for the preservation of fish species, it is a

common practice in the meat fish because it preserved the quality for an extended time, minimum deterioration in products color, flavor and texture.

The freezing and frozen storage of fish have been largely used to retain their sensory and nutritional properties before consumption (Erickson, 1997; Lakshmisha*et al*., 2008).

Freezing is a transition phase in which a liquid turns into a solid when its temperature is lowered below its freezing point, During frozen storage of fish, deterioration in quality due to microorganisms and some biochemical processes is decreased because in freezing, the products are cooled below a temperature of -35C^0 to arrest the enzymatic and bacterial activities completely to prolong the shelf life (Clucas and Ward, 1996). Properly frozen and packed good quality lean fish can normally be stored at -200C^0 to -300C^0 for more than 1 year without much loss in consumer acceptability. But the quality of fish deteriorates during storage as shown by organoleptic, chemical and physical changes.

Frozen fish stored for extended period can have reduced palatability by loss of flavour or texture. The denaturation and aggregation of fish muscle proteins particularly myofibrillar fraction are associated with the texture deterioration of frozen fish. According to (Huss, 1994) autolytic changes are responsible for the early quality loss in fresh fish but contribute very little to spoilage of chilled fish and fish products. However, under frozen conditions, autolytic enzymes break down TMAO to dimethylamine (DMA) and formaldehyde (FA), Therefore maintenance of proper storage temperature and freezing properties of different species must be given for great importance on the quality of fish. These mean that fish if necessary should be stored, for a short period of time to retain the taste, and provide both the protein and fat at optimal level (Mazrouh, 2015).

Many factors influence the deterioration during frozen storage, like rate of freezing, temperature and time of frozen storage, post harvest history of fish prior to freezing etc. (Sravani, 2011).

2.5.2.2.1Advantages 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).

2.5.2.2.2Disadvantages 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).

2.6 Fish contamination

Fish take a large number of bacteria into their gut from water sediment and food (Emikpe *et al.,* 2011). Fish contamination can also be linked to raw material, personnel, processing tools such as forklifts through leakage, opening in building

and pests. Some pathogens may even become established in the processing plants from niches where they can survive for a long period of time (Adebayo-Tayo *et al*., 2012b). The tissue of a healthy fish is normally considered sterile until bacterial inversion that leads to spoilage.

Adebayo-Tayo *et al.,* (2012) determined microbial quality of frozen fishes from three different markets using standard methods. The Bacteria isolated from frozen fish samples were *Staphylococcus aureus,Esherichia coli*, *Vibrio* spp., *Salmonella* spp., *and Pseudomonas* spp., *Micrococcus* spp.

Saima *et al.,* (2012) examined the bacteriological quality of mola fish (*Amblypharyngodon mola*) from three local fish markets as fresh and as frozen and revealed that *Salmonella-Shigella* was identified in 67% samples (75% of fresh and 58% of frozen samples) varied from $0.9 \pm 0.00 \times 10^2$ to $5.3 \pm 0.30 \times 10^3$ CFU/g.

Joseph *et al.,* (2013) found the presence of *Aeromonas spp.,* which has been recognized as a potential health risk and surveillance of this pathogen is crucial for successful disease management and control.

Noor *et al.,* (2013) studied the prevalence of pathogenic microflora along the two major sea fish samples, Rupchanda (*Pampus chinensis*) and Surmai (*Scomberomorus guttatus*) and reported that the total bacterial count was 2.5×10^6cfu./g in fish blend samples and the samples were highly contaminated with *Shigella* spp., *Listeria* spp., *Staphylococcusaureus.*

Noorlis *et al.,* (2011)explained that the high frequency of *Vibrio* spp. in the samples may be due to the use of contaminated ice to cover the fish on the display bench or from long holding time on the display rack at the retail level without proper temperature control and mishandling by fish sellers.

Rahman *et al.*, (2012) assessed the health hazard microbes in raw and finished product of coral (*Lates calcarifer*) and reported that total coliform was between 15 MPN/g and 20 MPN/g in the finished product of coral, faecal coliform in raw and finished product of coral was found <3 MPN/g and *Salmonella* spp. and *Vibrio cholerae*, both were absent.

Vignesh *et al*. (2012) isolated *Vibrio, Staphylococcus, Lactobacillus, Salmonella* and *Klebsiella* species. Another study done by Adebayo-Tayo *et al.*, (2012) aimed to microbiological and physicochemical analyses of fresh catfish from different markets in Uyo, Akwa Ibom State, showed the microorganisms isolated from sea foods were identified as *Staphylococcus aureus*, *Aeromonas hydrophila*, *Escherichia coli*, *Shigella* spp, *Listeria monocytogenes*, *Yersinia enterocolitica* and *Salmonella* spp. It's also showed that *Staphylococcus aureus* was the most predominant organisms isolated from seafood samples.

2.7 Microbial contamination of fishes

The structure of marine bacterial communities is a result of both habitat (spatial) filtering (Pontarp *et al*., 2012) and temporal patterns influenced by both biotic and abiotic factors (Fuhrman *et al*., 2006).Infection as a result of microbial contamination does not usually result in a disease but environmental stresses may upset the balance between the potential pathogens and their hosts (Iqbal *et al*., 2012).

2.7.1 Contamination of fish with *Enterobacteriaceae*

2.7.1.1*Salmonella*

Salmonella are motile rod and gram negative bacteria,occurs commonly in domestic animals and birds andbelongs to *Enterobacteriaceae* family. They are

common waterborne bacterium which may be apparently present in the tissues of normal fishes (Newaj-Fyzul *et al.*, 2008).

Members of the genus *Salmonella* are ubiquitous, found in all organisms including humans (Lotfy *et al.*, 2011). *Salmonella* survival in water depends on biological (macro and micro-invertebrate) and physical factors (e.g. temperature). There are two species, *S. bongori*and *S.enteric* (De Freitas Neto *et al*., 2010) and around seven subspecies and various serovars of *Salmonella* (Porwollik *et al.*, 2004). According to the Center for Food Safety and Applied Nutrition in Washington (2001), most fish related food borne illness are traced to *Salmonella, Staphylococcus spp., Escherichia spp*., *Vibrioparahemolyticus, Clostridium perfringens,Clostridium botulinum E,* and *Enteroviruses* (Yagoub, 2009).

Salmonella is a second leading cause of foodborne illness worldwide (Wong and Chen, 2013). The majority of 1.3billion annual cases of *Salmonella* cause human gastroenteritis, through the ingestion of undercooked eggs, shell fish and fish (Awuor *et al.*, 2011).The major reservoirs of the *Salmonella* spp. are aquatic environment; however, fish and fishery products have been renowned as a carrier of food-borne pathogens (Upadhyay *et al.*, 2010).

Fishes serve as a host to a variety of parasites including *Salmonella*. *Salmonella*, usually, is not a fish pathogen, rather the consumption of *Salmonella* contaminated feed and water causes this infection. Deep-sea fish are generally *salmonella* spp. free but susceptible to contamination post-catch. Water temperature has been proposed as playing an important role in the long-term survival of salmonella in the environment (FAO, 2010).

Salmonella enteric can cause asymptomatic carrier state, gastroenteritis, bacteremia and enteric fever (Ryan and Ray, 2010; AbMutalib *et al*., 2014). It is more common in children under the age of 5, and patients of 70 or above.

Gastroenteritis is commonly identified by unexpected nausea, abdominal cramps, diarrhea, continuous pain in head, cold and fever up to 39ºC (Newton and Surawicz, 2011). The symptoms of salmonellosis can be insignificant to severe and may last between 5 to 7 days (Ryan and Ray, 2010). Enteric fever or typhoid fever is caused by serotypes of *Salmonella*, which includes *Typhi* and *Paratyphi* (Lotfy *et al.*, 2011).

Contamination of seafood with *Salmonella* is a major public health concern. In Croatia, *Salmonella* spp. was recorded as the primary microbial pathogens responsible for the majority of food-borne illnesses (Wafaa *et al*., 2011). Also according to (FAO, 2010), aquaculture products can becomes contaminated with salmonella through the use of unsanitary ice, water, containers, and poor hygienic handling practices(FAO, 2010).

Adebayo-Tayo *et al.,* (2012) isolated *Salmonella* spp., from frozen fish samples in study of determined microbial quality of frozen fishes from three different markets using standard methods.

Ahmadoon (2013)carried study about marine fishes, *Plectropomus areolatus* (Najel, Sulimani) first class fishes, and the study revealed that before and after chilling of ice all samples are free from *salmonella spp.* Also Rahman *et al.,* (2012) assessed the health hazard microbes in raw and finished product of coral (*Lates calcarifer*) and reported that *Salmonella* spp. was absent.

2.7.1.2*Escherichia coli*

Coliforms are gram negative bacteria which ferment lactose and produce gas and acid. Faecal coliforms are generally found in gastrointestinal tract of human and animals. So, if faecal coliforms are found in fish or fish products, then it can be said that these are contaminated by man or animal excreta. There are three genera of faecal coliforms, e.g. *Escherichia, Klebsiella* and *Enterobacter*.

Escherichia coli cause dysentery. Normal fish and human skin is a complex organ and the bacterial populations associated with it are complex in kind and number.

Saima *et al.,* (2012) examined the bacteriological quality of mola fish (Amblypharyngodon mola) from three local fish markets as fresh and as frozen and revealed that all fresh and frozen samples were observed having high quantity of E. coli above 10^2 cfu/g. Also Adebayo-Tayo *et al.,* (2012) isolated *Esherichia coli,* from frozen fish samples in study of determined microbial quality of frozen fishes from three different markets using standard methods.

Ahmadoon (2013)carried study about marine fishes, *Plectropomus areolatus* (Najel, Sulimani) first class fishes, and revealed that before and after chilling of ice *Ecoli* has been isolated.

Nilla *et al.,* (2012) found that all fresh and frozen samples were observed having high quantity of *E. coli*. Also study done by Sujatha *et al., (*2011) concluded that different species of bacteria were isolated and identified and among them, the human pathogens that were found in the culture include *E. coli*.

2.7.2 Contamination of fish with *Staphylococcus aureus*

Study done by Adebayo-Tayo *et al.,* (2012) showed the microorganisms isolated from sea foods were identified as *Staphylococcus aureus*, It's also showed that *Staphylococcus aureus* was the most predominant organisms isolated from seafood samples. In another study by Adebayo-Tayo *et al.*, (2012) to determined microbial quality of frozen fishes from three different markets using standard methods and one of bacteria isolated from frozen fish samples was *Staphylococcus aureus*. Also Saima *et al.,* (2012) examined the bacteriological quality of mola fish (*Amblypharyngodon mola*) from three local fish markets as fresh and as frozen and revealed that *Staphylococcus spp.* was confirmed in 79% samples with 83% of fresh and 58% of frozen samples (63% of total samples) beyond 10^3 cfu/g.

Noor *et al.,* (2013) studied the prevalence of pathogenic microflora along the two major sea fish samples, Rupchanda (*Pampus chinensis*) and Surmai (*Scomberomorus guttatus*) and reported that *Staphylococcusaureus.*has been isolated. While, Ahmadoon (2013) carried study about marine fishes, *Plectropomus areolatus* (Najel, Sulimani) first class fishes, and revealed that before and after chilling of ice all samples are free from *staphylococcus spp*.

2.7.3 Contamination of fish with Pathogenic *Vibrio* Spp.

Pathogenic *Vibrios* have been a public health concern for seafood consumers and have been cause of import bans, detentions and rejections in international fish trade (Wafaa *et al.*, 2011).

Species such as *V. cholerae, V. parahaemolyticus, V. vulnificus, V. alginolyticus, V. mimicus, V. fluvialis, V. furnissii, V. metschnikovii, V. hollisae* and *V. damsel* are human pathogens, Theyaccount for a significant proportion of humaninfections

such as gastroenteritis, usually associatedwith consumption of raw or undercooked seafood,wound infections, septicemia and ear infections(Adeleye *et al.,* 2010).

Vibrio frequently occurs in polluted water. After transmission these organisms multiply rapidly in the intestines of the victims.

Vibrio cholerae is gram negative, comma shaped bacteria, and itis the most commonly occurring pathogenic *Vibrio* species, followed by *V. parahaemolyticus*. Cholera is characterised by profuse watery diarrhoea with flakes and mucus, dehydration and sometimes death when adequate medical intervention is not instituted (Jay *et al.*, 2005; Kaper*et al.*, 1995; Talkington *et al.*, 2011).

Huq *etal.* (1990) have suggested that *V. cholerae* is wide spread in estuarine and marine waters around the world, although the numbers may be low in sea water throughout the year. They are found in areas where salinity is between 4 to 17% and their presence does not correlate with either *E. coli* or *Salmonella*. They establish symbioses with planktons as a means of overcoming low temperatures that prevail during winter in temperate regions (Huq *et al.,* 1990; Montanari *et al.,* 1999). *V.cholerae* is sensitive to temperatures, higher than 45°C, and to many disinfectants used in the food industry.

Vibrio parahaemolyticus and *Vibrio vulnificus* are part of the natural flora of estuarine and coastalmarine environments worldwide and have beenisolated from sea and brackish water of both tropicaland temperate regions, sediments, and a variety ofseafood especially shellfish and bivalve mollusks(Kirs *et al.,* 2011;Wafaa *et al.,* 2011).

V. parahaemolyticus is rarely isolated in water where temperatures are below 15°C (Matches *et al*., 1971). The bacteria are moderately sensitive to freezing and can persist in frozen food for long periods (Vasudevan *et al*., 2002).

V.parahaemolyticus is very sensitive to heat (killed at 47 to 60C^0) to ionizing radiation, and to halogens (Adams and Moss 2008). Food poisoning associated with this organism arises from gross mishandling during preparation and from temperature abuse (Nickelson and Finne, 1992).

Adebayo-Tayo, *et al.,* (2012) determined microbial quality of frozen fishes from three different markets using standard methods, The Bacteria isolated from frozen fish samples was *Vibrio* spp.

In study done by Saima *et al.,* (2012) to examine the bacteriological quality of mola fish (*Amblypharyngodon mola*) from three local fish markets as fresh and as frozen. The study revealed that *Vibrio* spp. was confirmed in 79% samples (83% of fresh and 75% of frozen samples) of which 90% samples exceeded 10^2 CFU/g. Also study done by Sujatha *et al., (*2011) concluded that different species of bacteria were isolated and identified and among them, the human pathogens that were found in the culture include *Vibrio cholerae*.

Nilla *et al.,* (2012) found that *Vibrio* spp. was confirmed in 79% samples.

Noorlis *et al.,* (2011)explained that the high frequency of *Vibrio* spp. in the samples may be due to the use of contaminated ice to cover the fish on the display bench or from long holding time on the display rack at the retail level without proper temperature control and mishandling by fish sellers.

Rahman *et al.,* (2012) assessed the health hazard microbes in raw and finished product of coral (*Lates calcarifer*) and reported that *Vibrio cholerae*, was absent. Also Ahmadoon (2013)carried study about marine fishes, *Plectropomus areolatus* (Najel, Sulimani) first class fishes, and concluded that before and after chilling of ice all samples are free from *vibriospp.*

CHAPTER THREE

MATERIALS AND METHODS

3.1 Laboratory of investigation:

This study was carried out at Directorate general of preventing Medicine and PHC, Epidemiology Dept, Ports and quarantine health unit, Port Sudan Laboratory, is an accredited laboratory which was accredited by SSMO. The laboratory follows ISO 17025 standards.

3.2 collections of samples:

A total of 20 samples of Najil *(Plectropomuspesulliferus)* fish were collected randomly from different sellers at Port Sudan fish market.

The fish samples were identified, measured and processed following the methods used by the association (personal communication).Then transported to the laboratory by collecting in sterilized plastic bag put into ice box (thermostatic containers) and directly analyzed as fresh, froze (put into freezer for 4days) and then analyzed.

Fig (1) Najil *(Plectropomuspessulliferus)*

3.3 Equipments and materials:

The following items were used for bacteriological count and these include: fish sample, plastic container, freezer to froze fish sample, sensitive balance, sterile bottles, flasks, beakers ,measuring cylinders, flame for sterilizing, sterile tubes (10 ml),sterile pipettes, normal saline, Petri dishes, glass (20×100 mm), Test-tube racks to hold tubes in incubator and during storage, Wire loops for inoculating media, Container for used pipettes, plate count agar, stomacher ,autoclave, water bath, plate count agar, nutrient agar, incubator $37c^0 \pm 1c^0$. All the used glass wares such as conical flasks, beakers, measuring cylinders, test tubes were washed, dried and sterilized in autoclave at a temp of 121c for 15 min at 15 Ib/inch pressure (Ahmed *et al* 2014).

3.4 processing of fish samples:

Aseptic measures and conditions were undertaken during the sampling procedure to prevent contamination of the samples; 25g of each sample was weighted and homogenized by a stomacher blender for 2min at 160 revolution/min with 225ml. of peptone water (ph 7.0).

Each of three tubes was filled with nine milliliters of buffer saline solution (BS) of first tube to prepare 10^{-1} dilutions. The 1ml was taken from the first tube and mixed to the second test tube to prepare 10^{-2} dilutions. The 10^{-3} dilution was prepared by these subsequent dilution techniques. Then sealed in polyethylene bags to store in frozen condition at -20oc for further analysis, After 4 days fish samples were taken out of the refrigerator and thawed at room temperature. Then 20 g of each sample was blended with 180 ml of sterile bacteriological peptone water in a stomacher blender. Then 1 ml of this 10^{-1} dilution was transferred to a screw cap vial containing 10 ml of sterile dilute of bacteriological peptone to make a dilution of

10^{-2}. Then the vial was shaken gently. This process was repeated progressively to prepare of 10^{-3}.

3.5 Bacteriological examination:

3.5.1 Culture media used for bacteriological evaluation

BPW (Bacteriological peptone water): Used for serial dilution of fish samples.

PCA (Plate Count Agar): Used for total bacterial count.

APW (Alkaline Peptone Water): Used for 1st enrichment of *Vibrio* spp.

TCBS (Thiosulphate Citrate Bile Salt Sucrose agar): Used for 2nd enrichment of *Vibrio* spp.

VRB (Violet Red Bile agar): Used for enrichment of *E.coli.*

LB (Lactose Broth): Used as pre-enrichment broth during testing of *Salmonella* spp.

BPW (Buffer Peptone Water): Used for 1st enrichment of *Salmonella* spp.

SCB (Selenite Systine Broth): Used for enrich the cultivation of while inhibiting other organisms.

XLDA (Xylose Lysine Deoxycholate): Used for 3rd enrichment of *Salmonella* spp.

3.5.2Occurrence of total bacterial count

25grams of fish muscle was blended with 0.1% of 225ml peptone water in a sterile blender jar for 5 minutes. To make decimal dilution, 1ml mixture was transferred with the help of sterilized pipette to a test tube containing 9 ml of sterilized peptone water. In this way decimal dilutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} were

prepared. One ml of sample from 10^{-3}, 10^{-4} and 10^{-5} dilution were pipetted into previously prepared agar plates. After mixing and solidification the media, the Petri-dishes were incubated at inverted position for 72 hours at 30ºC**.** After 72 hour of incubation, forming colonies were counted which had a range between 30 and 300. As there is sufficient evidence that 10^{-1} and 10^{-2} dilution contain more than 300 colonies, these two dilutions were ignored for determining total bacterial load.

3.5.2.1 Plate count agar media preparation:

For isolation of TBC (Total Bacterial Count), Bacteriological peptone plate count agar (BPCA) media was used. To dilute the fish sample Bacteriological peptone water was prepared by mixing 1g of media with 1000 ml distilled water (according to the manufacturer's instructions). Then PCA was prepared by mixing 11.75 g of medium with 500 ml of distilled water and heated properly (according to the manufacturer's instructions). Both of the media were, then autoclaved at $121C^0$ for 15 minutes.

3.5.2.2Test Procedures

(i) Each of 1 ml of solution from 10-^3, 10-^4 and 10-^5 dilutions was plated by pipette into sterile plates.

(ii) About 15 ml of sterile PCA was poured into the plates.

(iii) After solidification of the media, the plates were inverted and incubated in incubator at 30˚C for 72 hours.

(iv) Bacterial colonies were counted by using digital colony counter; andthe total number of bacteria per gram of sample was obtained by multiplying the average number of colonies on Petri dishes by the respective dilution factor.

3.5.3 Occurrence of *Vibrio* spp.

To know the presence or absence of *Vibrio spp.* in the sample, 25 grams of fish sample was homogenized with 225 ml. of Alkaline Peptone Water (APW) and kept for enrichment over night in an incubator at 370C ± 10C. Later a loop full of enriched sample was streaked on to the Thiosulphate Citrate Bile Salt Sucrose agar (TCBS) plates and incubated at 370C ± 10C for 24 hrs for the appearance of positive colonies.

3.5.3.1Media preparation

For *vibrio spp.* isolation, Alkaline Peptone Water (ABW) was prepared by mixing 20mg of media with 1000ml of distilled water. Then 225ml of the first enrichment was transferred to culture bottles and sterilized at $121c^0$ for 15minutes.

3.5.3.2Test Procedures

(i) For preparation of first enrichment, 25g of sample was taken and diluted with 225ml of sterile APW(Alkaline Peptone Water).This suspension was incubated at $37c^0$for 6hours.

(ii) For selective enrichment, TCBS (Thiosulphate Citrate Bile Salt Sucrose) agar was prepared and sterilized. Then the culture of the second enrichment was streaked by means of a loop on the surface of TCBS agar plates.

(iii) After streaking, the plates were inverted and incubated in an incubator at $37c^0$ for 24hours.

(iv) After incubation, the presence of typical colonies of *vibrio spp.* were examined and marked on the bottom of the dish (typical colonies of *vibrio cholera* growth on TCBS agar are smooth, yellow and 2mm to 3mm in diameter.

3.5.4 Occurrence of *E.coli.*

3.5.4.1Media preparation

For *E.coli* spp. isolation, VRB (Violet Red Bile) agar was prepared by mixing 41,53gms of media with 1000ml of DW (Distilled Water), and then 225ml of the first enrichment was transferred to culture bottle and sterilized at $121c^0$ for 15minutes.

3.5.4.2Test procedures

(i) For preparation of initial suspensions, 25g of sample was taken and diluted with 225ml of sterile peptone water. Then it was incubated at $37c^0$ for 24hours.

(ii) 1ml of each three suspensions (containing sample) was transferred to duplicate Petri dishes, VRB broth was added to this Petri dishes.

(iii) Then it was incubated at $35c^0$ for 24hours.

(iv) After incubation *E. coli* colonies was grown under the media and can easily be isolated.

3.5.5 Occurrence of *staphylococcus spp.*

3.5.5.1Media preparation

For *staphylococcus spp.* isolation, BBAB (Baired Barker Agar Base) was prepared by mixing 36gms of media with 950ml DW (Distilled Water).heat to boiling, sterilized in autoclave at 151Ibs,pressure ($121c^o$) for 15minutes,cool to $55c^o$ then supplement called egg tellurite emulsion yolk (50ml) was added.

3.5.5.2Test procedures

(i) For preparation of initial suspensions, 25g of sample was taken and diluted with 225ml of sterile peptone water (first dilution),10ml of first dilution was transferred to bottle containing 90ml peptone water (second dilution), finally 2ml of second dilution was transferred to bottle containing 18ml peptone water (third dilution).

(ii) 0.1ml of this three diluents was transferred to ready duplicate dishes contain Baired parker agar media and incubated at $35c^0$ for 24hours.

(iii) After incubation, *staphylococcus spp. (staphylococcus aureus)* colonies were growth and can easily be isolated.

3.5.6 Occurrence of *salmonella* spp.

To know the presence or absence of *Salmonella* in the sample, primary enrichment was done in lactose broth (LB). Secondary enrichment was done using Fluid selenite cysteine broth (SCB). Later a loop full of enriched sample was streaked on Brilliant Green Agar (BGA) plates and incubated at 370C ± 10C for 24 hrs for the appearance of positive colonies.

3.5.6.1Media preparation

For *salmonella spp.* isolation, pre-enrichment media BPW (Buffered Peptone Water) was prepared by mixing 20g of media with 1000ml of DW (Distilled Water). Then 225ml of the pre-enrichment broth was transferred to culture bottle and sterilized at $121c^0$ for 15minutes.

For LB (Lactose Broth) preparation, 13gm of media mixed with 1000ml DW (Distilled Water), heat, sterilized by autoclave at $121c^0$ for 15 minutes.

3.5.6.2Test procedures

(i) For preparation of initial suspension, 25g of sample was taken and diluted with 225ml of sterile LB (Lactose Broth) and incubated at $37c^0$ for 24hours.

(ii) 1ml this suspension (containing sample) was transferred to tube contain 90ml of SCB (Selenite Cystine Broth) and also incubated for another 24hours.

(iii) By loop, sample from this tube was taken and cultured in betri dish with XLD (Xylose Lysine Deoxycholate), then incubated for 24hours at $37c^0$.

(iv) For identification of salmonella spp. solid media (XLD) was prepared, and then the culture obtained after incubation of SCB was streaked by means of a loop, on the surface of XLD plate.

(ivi) After streaking, the plates were inverted and incubated at $37c^0$ for 24hours.

(ivii) After incubation, the presence of typical colonies of *salmonella* spp. was examined on the bottom of the dish (typical colonies of *salmonella* spp. growth on XLD agar plate have a black centre and a lightly transparent zone of reddish colour.

3.6 Bacterial identification

3.6.1 Culture method:

3.6.2 First isolation:

Fish was isolated in nutrient agar. Supplemented plates were incubated at $37c^0$ for 24 hours depending on the appearance of bacterial growth on the surface of the media.

3.6.2.1Preparation of nutrient agar:

32g of nutrient agar dissolved in 400ml distill water then, it was sterilized by autoclaving at 15 pound per square inch pressure (121c^{o}) for 15 minutes.

3.6.3 Biochemical test:

3.6.3.1 Catalase test:

The test described by the (Barrowand Felltham, 1993). Drop of hydrogen peroxide was placed on microscopic slide. Using sterile glass. Rod small part of isolates colony was taken and emulsified in the hydrogen peroxide drop. The production gas bubbles were considered appositive reaction.

3.6.3.2 Indol test:

The test was performed according to the method described by (Barrowand Felltham, 1993) using sterile tube isolate and incubated at 37c^{0} and examined to addition covacs solution was occur red ring in the surface of tube.

3.7 Statistical analysis:

The Obtained results were analyzed statistically using **IBM6 SPSS** statistic **version (23).**

CHAPTER FOUR

RESULTS

The result obtained that the bacterial count for fresh Najil fish (*P.Pessuliferus*) was $5.68\pm1.57\times10^5$, and for frozen Najil fish (*P. Pessuliferus*) was $4.38\pm1.96\times10^5$cfu/g respectively.

The result revealed that there was highly significant difference in total bacterial count ($p<0.05$) between fresh and frozen Najil (*P. Pessuliferus*) fish, and indicates that *Staphylococcus aureus* and *E.coli* were isolated as contaminant bacteria; while *salmonella*, and *vibrio*were not isolated from both fresh and frozen Najil (*P. Pessuliferus*) fish. As the result shown in table (1) and (2)

Table (1): Microbial load for fresh and frozen Najil(*P. Pessuliferus*) fish:

Items	**Microbial load for fresh Mean±Std. Deviation**	**Microbial load for frozen Mean± Std. Deviation**
N=20	**5.68±1.57 cfu/g**	**4.38±1.69 cfu/g**
Significant	**highly significant**	**

****= highly Significant at (P<0.05).**

Table (2) pathogens that have been isolated from Najil fish (*P. Pessuliferus*) samples:

Organism / Treatment	staphylococcus	salmonella	E.coli	vibrio
Fresh fish	+ ve	- ve	+ ve	- ve
Frozen fish	+ ve	- ve	+ ve	- ve

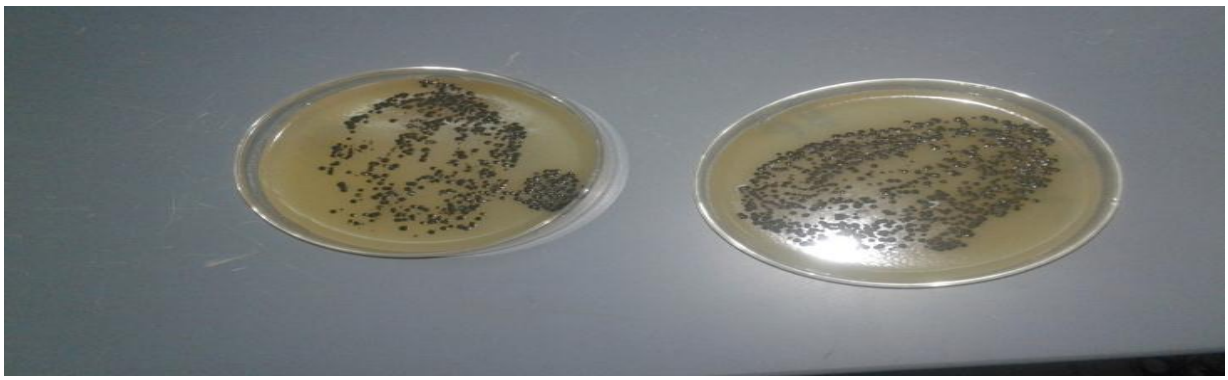


Fig (2)*Staph*colonies at Baird Parker (BP) agar plates for fresh Najil (*P. Pessuliferus*) fish.

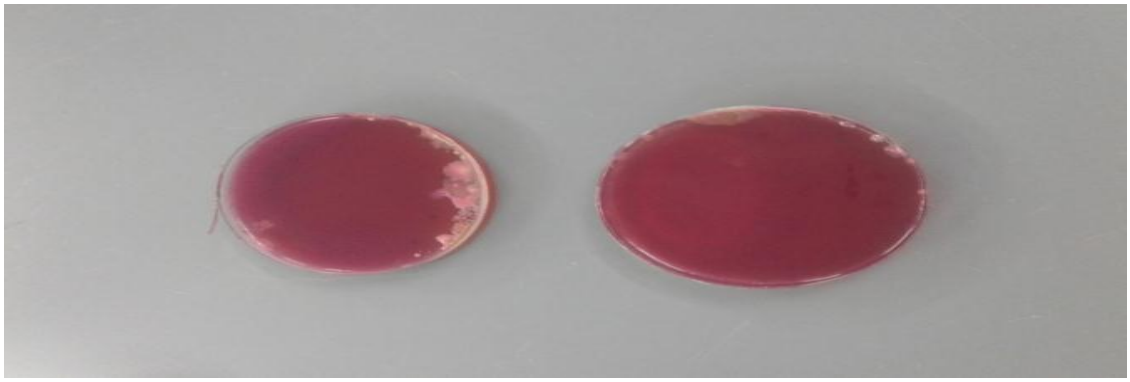


Fig (3)*E.coli* colony at Violet Red Bile (VRB) agar plates for fresh Najil (*P. Pessuliferus*)fish.

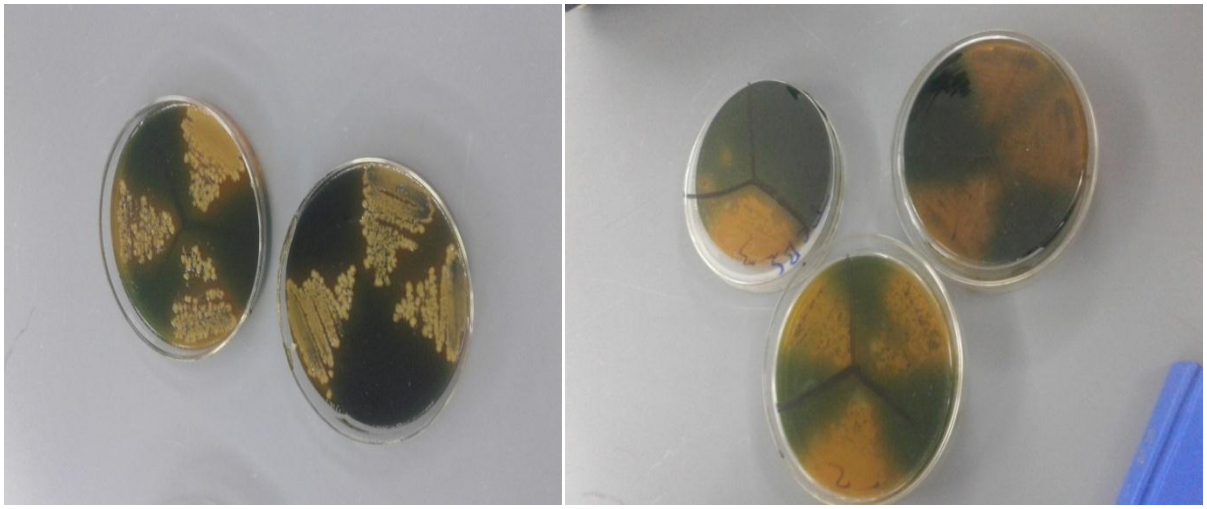


Fig (4)Colonies of *V. SPP.* on Thiosulphate Citrate Bile Salt Sucrose (TCBS) after Incubation for fresh Najil (*P. Pessuliferus*)fish.

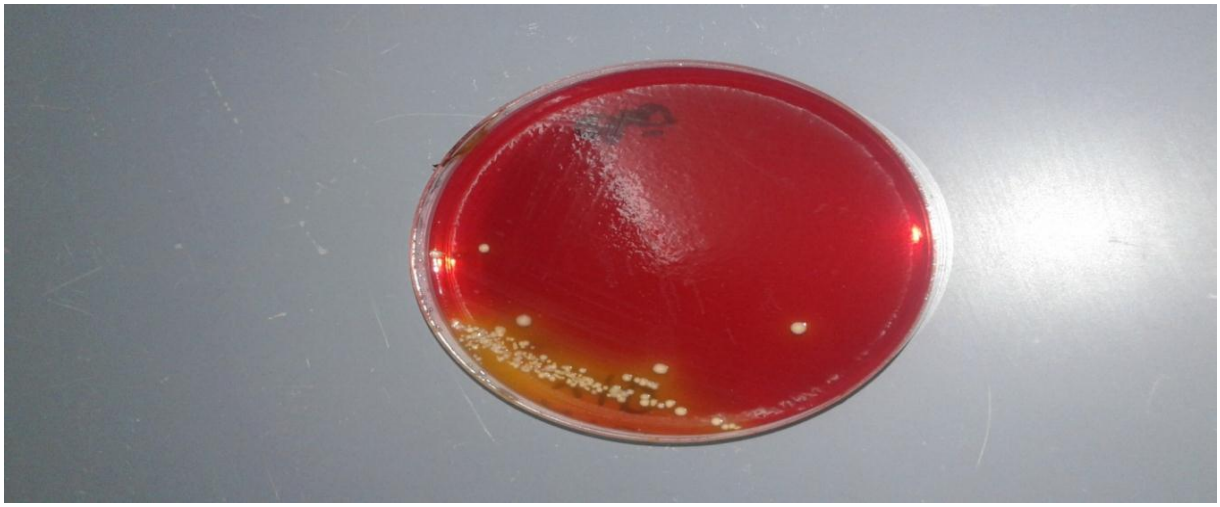


Fig (5) *Salmonella* colonies at Xylose Lysine Deoxycholate (XLD) agar plates for fresh Najil (*P. Pessuliferus*)fish.

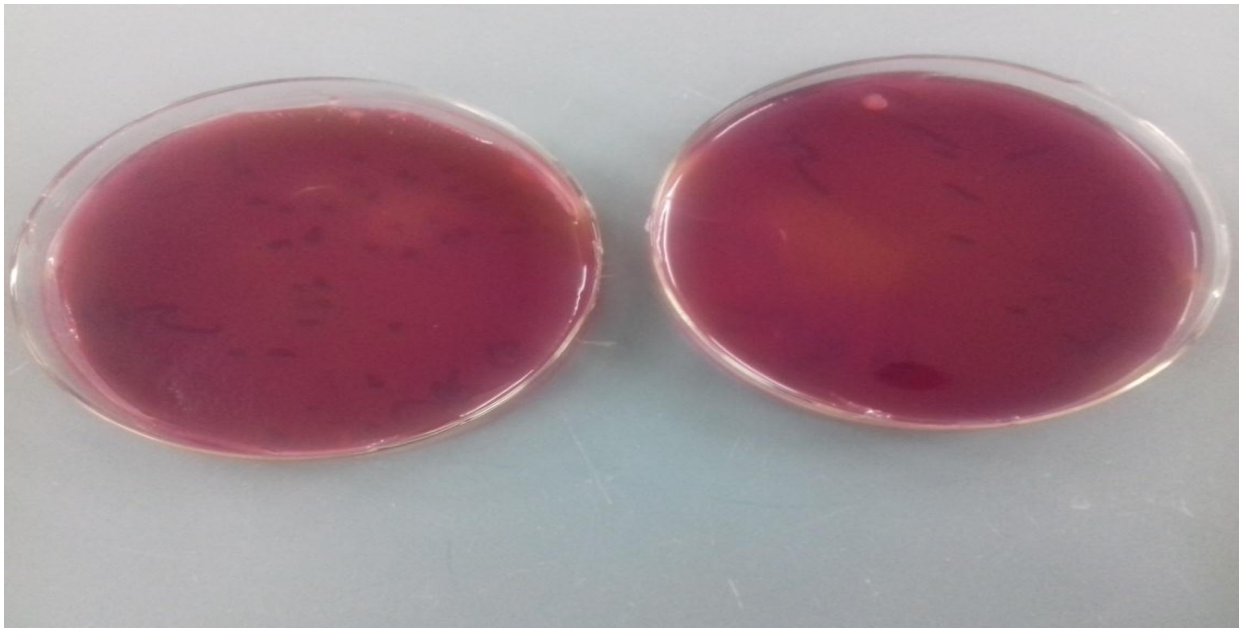


Fig (6) *E.coli* colonies at Violet Red Bile (VRB) agar plates for frozen Najil (*P. Pessuliferus*)fish.

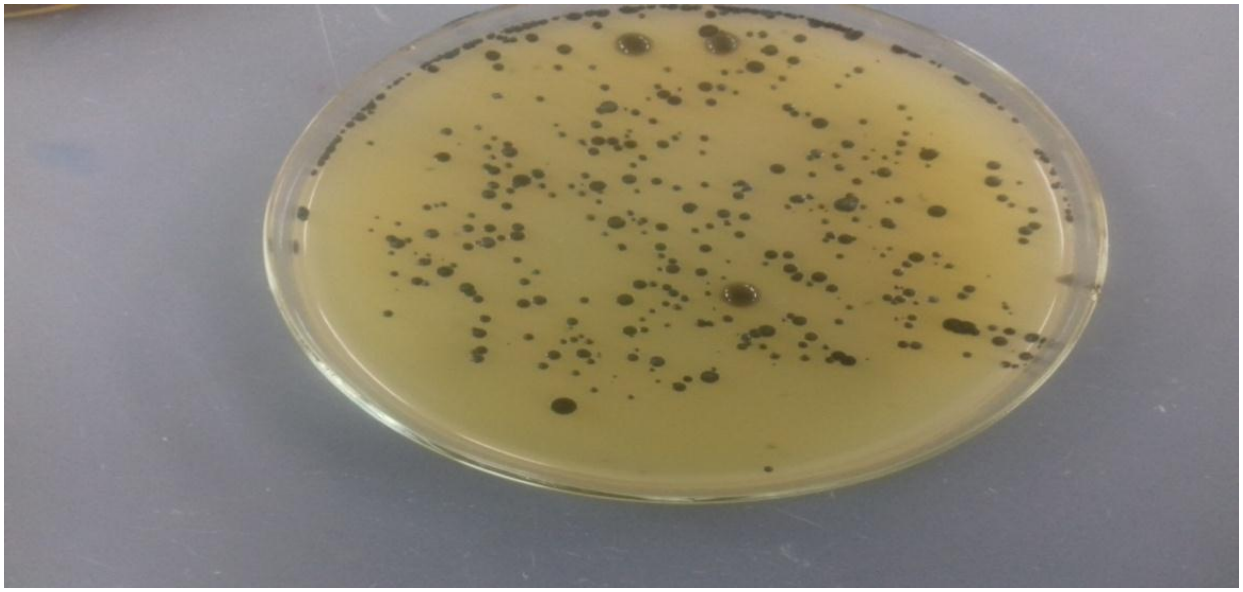


Fig (7)*Staph* colonies at Baird Parker (BP) agar plates for frozen Najil (*P. Pessuliferus*)fish.

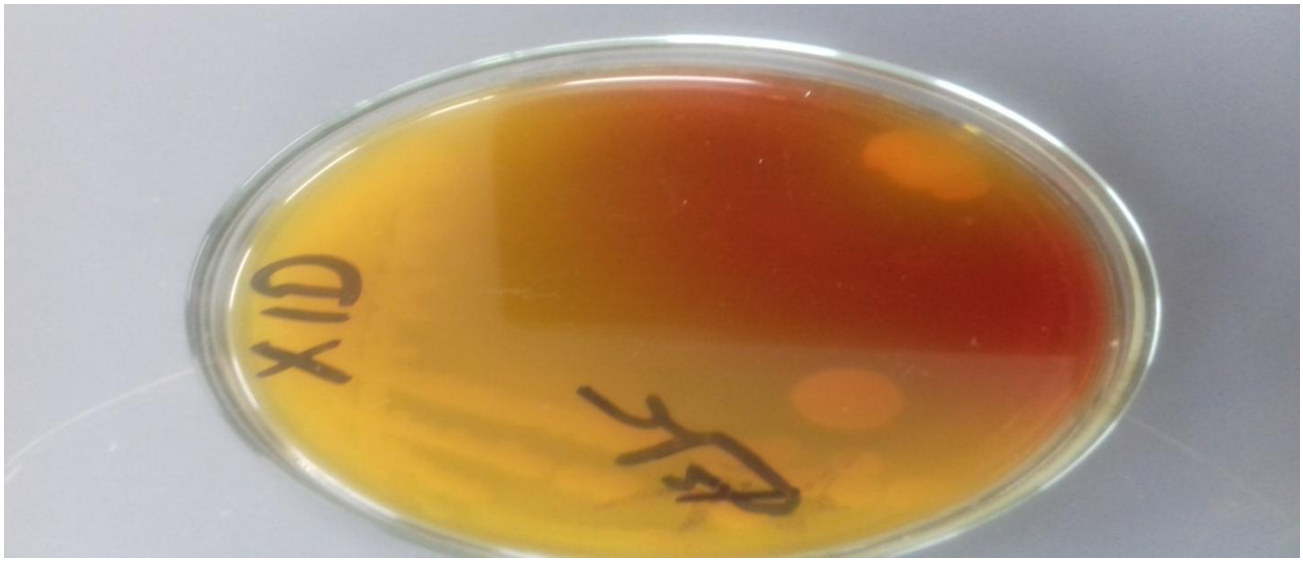


Fig (8) *Salmonella*colonies at Xylose Lysine Deoxycholate (XLD) agar plates for frozen Najil (*P. Pessuliferus*)fish.

Biochemical tests:

Table (3) revealed a biochemical test of the *Eshcerichia coli and Staphylococcus aureus* respectively.

Table (3): Biochemical test for fresh and frozen Najil (*p.pessuliferus)* fish:

Type of bacteria	Shape	Indole test	Catalase
Staphylococcus aureus	Sphere	- ve	+ ve
E.coli	Rod	+ ve	- ve

CHAPTER FIVE

DISCUSSION

Registered bacterial species in fresh and processed fish samples, warn high risk situation for human health due to ingest of fish, especially when it is consumed raw or with short baking time, and also because the presence of *enterobacteria* was predominant (Jorge, *et al.,* 2016).

A t-test comparing the fresh and frozen conditions of Najil (*P. pessuliferus*)showed that there was a highly significant different. On the other hand, the total bacterial count of Najil (*P. pessuliferus*) fish stored under frozen condition for four days showed a significant decrease through the time of preservation. The bacterial load showed decreases from5.68×10^5 to 4.38×10^5 cfu/g. This numbers was in the acceptable limit mentioned by SSMO (Sudanese Standards Metrology Organization, SDS357) which was 5×10^5-10^6 for fish products.

The International Commission on Microbiological Specifications for Food (ICMSF) recommends that during fish storage at lower temperature, the total plate count should never exceed log mean 7 log CFU/g of wet weight (ICMSF, 1978). According to the result, Najil fish did not loss its microbiological quality acceptance level (microbiological basedshelf-life). The initial load (5.86×10^5 cfu/g) may have contributed to the accepted number(acceptable level of ICMSF) of total bacterial count in short storage time.

This result is coincide with finding of Adebayo-Tayo*et al*., (2012) who studied the microbial quality of frozen fishes: Shinna (*Auxis thazard*), Bonga (*Ethmalosa fimbriata*) and Mackerel (*Scomber scombrus*) obtained from three different markets were carried out using standard methods revealed that, The total

heterotrophic bacterial count ranged from 3.0 x10^5 to 5.0 x 10^5 cfu/g, 3.0x 10^5 to 4.8 x10^5 cfu/g and 3.0 x10^5 to 6.3 x10^5 cfu/g for Shinna, Bonga and Mackerel respectively.

This in differ from the finding of Noor *et al.,* (2013) who studied the prevalence of pathogenic microflora along the two major sea fish samples, Rupchanda (*Pampus chinensis*) and Surmai (*Scomberomorus guttatus*) and reported that the total bacterial count was 2.5×10^6 cfu./g in fish blend samples. Also it is in partial agreement with result obtained by Nilla *et al.,* (2012) who studied that the total bacterial count of marketed Mola (*Amblypharyngodon mola)* ranged from 1.8 ± 0.25 ×10^4 to 6.5 ± 0.75 × 10^6 cfu/g for fresh sample and 5.5 ± 0.55 × 10^3 to 7.0 ± 0.80 × 10^5 cfu/g for frozen . Also it is differed from the result of Oramadike *et al.,* (2010) who studied microbiological qualities of some frozen fishes available in some reputable supermarkets in Lagos State and reported that total bacterial count ranged between 2.0 ×10^3 to 7.4×10^3 cfu/g.

According to FAO (1979) good quality fish should have count of total bacteria less than 10^5 per gram. This study within the acceptable limit recommended by food and agricultural organization.However, in this study the fish samples of Najil (*P. pessuliferus*) were contaminated with bacteria load, this might be due to the contamination of water that fish found or might be due the contamination during the time of handling as well as transporting of fishes in boats, preservation boxes materials or contaminated ice.

Study done by Okoro*et al*., (2010) aimed to quality assessment of a typical Nigerian marine fish species, *Liza falcipinnis* (Mullet) was carried out at various storage temperatures such as ambient (28C^o), Refrigeration (4 C^o) and Frozen state (-5 C^o) using sensory, microbiological and biochemical method of evaluation. At

ambient temperature, the bacterial load of the tissue was 6.2 $x10^4$ cfu/g, at refrigeration 2.4 $x10^4$ and at freezing point 3.2 x 10^4 .This in partial agreement with results of this study.

Also study done by Ahmed*et al.,* (2014) tend to determine the microbial load on fresh and chilled fish and to identify some contaminant bacteria on *Oreochromis niloticus* and *lates niloticus* at Elmourda fish market, revealed that the bacterial count in fresh fish in *Oreochromis niloticus* and *lates niloticus* is $4.69\times10^5\pm1.35\times10^5$ and3.69×10^5 $\pm0.89\times10^5$ and in chilled fish of *Oreochromis niloticus* and *lates niloticus* is 5.65×10^5 $\pm1.88\times10^5$and3.81×10^5 $\pm1.22\times10^5$cfu/g respectively. In addition, the result indicates that *Staphylococcus* and *E.coli* were isolated as contaminant bacteria, while *Salmonella* was not isolated from both fresh and chilled *Oreochromis niloticus* and *lates niloticus*.

Shamsuzzaman *et al.,* (2011) concluded that in study to determine microbial quality of hilsa shad (*Tenualosa ilisha*) at different stages of processing, *Escherichia coli*, fecal coliform, *Vibrio cholerae,* Salmonella and total load of bacteria were identified from 5 stages of processing and they observed that 80.69 % of total bacterial load, 77.29% of total coliform and 58.33 % of fecal coliform were destroyed during different processing stages. Also there was no evidence of presence of Salmonella and Vibrio cholera at any stages of processing.

Jianadasa*et al.,* (2014) examined the chemical, microbiological quality and safety of fresh fish obtained from the retail and supermarkets of Sri Lanka. Fresh fish samples of yellow fin tuna (*Thunnus albacares*), sailfish (*Istiophorus platypterus*), salaya/ sardine (*Sardinella gibbosa*), shrimp (*Fenneropenaeus indicus*) and *squid ssp.* (*Loligo duvaucelii*),furthermore, the presence of selected pathogens such as *Escherichia coli* and *Salmonella spp*. were detected. In microbiological analysis of

samples total plate counts were obtained in the range of 7 x 10^3-1.2 x 10^8 cfu/g and 28% of the samples had < 5 x 10^5 cfu/g. Eight percent of the samples contained > 10^3 MPN/g of *E. coli* and 1 sample contained 500 MPN/g and rest of the samples had < 200 MPN/g with 33% of the samples without *E. coli.*

Following human pathogenic bacteria were isolated (*Salmonella typhi, Pseudomonas aeruginosa, Escherichiacoli, Staphylococcus aureus* and *Enterococcus faecalis*). Austin and Austin (1999), have demonstrated the presence of pathogenic enterobacteria in fish that lives in contaminated marine water by domestic wastewater, such as: *Escherichia coli, Enterobacter cloacae, Citrobacter* sp., *S paratyphi A and B, S. enteritidis, S. amsterdam,S. give, P. vulgaris, P. rettgeri, Proteus mirabilis,Proteus morganii, Clostridium botulinum*, C group *Enterobacter*, *Streptococcus faecalis* and *Strptococcus faecium.* In study done by Jorge *et al.*,(2016) aimed to determine the presence of human pathogenic bacteria in fish muscle, recollected at moment of their capture and during their process for sale in marketing sites, species of different bacterial families were identified, such as: Enterobacteriaceae; *Salmonella hirschfeldii, Salmonella schottmulleri, Salmonella parathipi, Salmonella typhi, Salmonella enteritidis, Proteus miriabilis, Proteus rettgeri, Proteus vulgaris, Citrobacter freundii, Citrobacter amanolaticus* and three varieties of *Escherichia Coli*; Pseudomonadaceae: *Pseudomonas fluorescens;* Aeromonadaceae: *Aeromonas hydrophila* and Vibrionaceae: *Vibrio fluviales, V. cholerae El Tor* and *V. parahaemolyticus.* The 70.6% of the samples presented a bacterial growth; 46% in recently captured fish, and 54% in market fish.

Table (2) and Figure (2,3,4,5,6, 7 and 8) shows the occurrence of isolated pathogenic bacteria in Najil fish from Port Sudan fish market. And the result also

showed that the isolated contaminant bacteria include*Staphylococcus* and *Ecoli*, while the salmonella and *vibrio* were not isolated (absent).

Many pathogens isolated in this study not necessarily come straight away from the aquatic environment for the fishes tested. Fishes may be spoiled after the entry in to the market area were spoilage causing organism, become permanent inhabitant, because of the poor environmental hygiene and human fecal pollution. Contaminants and other spoilage-causing agent would have entered from the fishes transported earlier or in the past to the marketed area.

At freezing temperature (-7C^0), the storage life of the fish sample was predicted to be 1week and this was based mainly on the microbiological evaluation which were within the recommended maximum limits of acceptability. This shows that the frozen condition has successfully kept the total bacterial count under the recommended level throughout the storage days. It confirms the inhibitory effect of frozen condition on the growth of microorganisms on fish, but it does not eliminate the microbial population during 4days of storage.

Ahmadoon (2013) carried study about marine fishes, *Plectropomus areolatus* (Najel, Sulimani) first class fishes, and they revealed that before and after chilling of ice all samples are free from *salmonella spp.* and this result in agreement with findings of this result. Also Rahman *et al.*, (2012) assessed the health hazard microbes in raw and finished product of coral (*Lates calcarifer*) and reported that *Salmonella* spp. was absent.

This result is differ from the finding of Adebayo-Tayo *et al.*, (2012) who isolated *Salmonella spp*., from frozen fish samples in study of determined microbial quality of frozen fishes from three different markets using standard methods.

Also *E.scherichia coli* have been isolated from this study, and this in agreement with findings of Ahmadoon (2013) who carried study about marine fishes, *Plectropomus areolatus* (Najel, Sulimani) first class fishes, and they revealed that before and after chilling of ice*Ecoli* has been isolated and with Saima *et al.,* (2012) who examined the bacteriological quality of mola fish (*Amblypharyngodon mola*) from three local fish markets as fresh and as frozen and revealed that all fresh and frozen samples were observed having high quantity of *E. coli* above 10^2 CFU/g. and with Nilla *et al.,* (2012) who found that all fresh and frozen samples were observed having high quantity of *E. coli*. instudy of marketed Mola (*Amblypharyngodon mola).*

Adebayo-Tayo *et al.,* (2012) also isolated *Esherichia coli,* from frozen fish samples in study of determined microbial quality of frozen fishes from three different markets using standard methods. Another study done by Sujatha *et al., (*2011) concluded that different species of bacteria were isolated and identified and among them, the human pathogens that were found in the culture include *E. coli.*

Staphylococcus aureus have been isolated from both fish fresh and frozen. This result with agreement with the findings done by (Ahmadoon 2013). Also study done by Adebayo-Tayo *et al.,* (2012) showed that the microorganisms isolated from sea foods were identified as *Staphylococcus aureus*, It's also showed that *Staphylococcus aureus* was the most predominant organisms isolated from seafood samples.

Saima *et al.,* (2012) examained the bacteriological quality of mola fish (*Amblypharyngodon mola*) from three local fish markets as fresh and as frozen and revealed that *Staphylococcus spp.* was confirmed in 79% samples with 83% of fresh and 58% of frozen samples (63% of total samples) beyond 10^3 cfu/g. and

Noor *et al.,* (2013) determined microbial quality of frozen fishes using standard methods.

The obtained result was in agreement with Ahmadoon (2013) who carried study about marine fishes, *Plectropomus areolatus* (Najel, Sulimani) first class fishes, and they revealed that before and after chilling of ice all samples are free from *vibriospp.,* and with Rahman*et al.,* (2012) who assessed the health hazard microbes in raw and finished product of coral (*Lates calcarifer*) and reported that *Vibrio cholera*was absent. while it was disagreeing with results that reported by Adebayo-Tayo *et al.,* (2011) who reported *Vibrio spp*. were identified from (52.6%) of freshly harvested seafood. Also it disagree with Noorlis *et al.,* (2011) whom explained that the high frequency of *Vibrio spp*. in the samples may be due to the use of contaminated ice to cover the fish on the display bench or from long holding time on the display rack at the retail level without proper temperature control and mishandling by fish sellers.

Saima *et al.,* (2012) examained the bacteriological quality of mola fish (*Amblypharyngodon mola*) from three local fish markets as fresh and as frozen and revealed that *Vibrio spp*. was confirmed in 79% samples (83% of fresh and 75% of frozen samples) of which 90% samples exceeded 10^2 cfu/g. Also Nilla *et al.,* (2012) found that *Vibrio spp*. was confirmed in 79% samples.Adebayo-Tayo *et al.,* (2012)determined microbial quality of frozen fishes from three different markets using standard methods, The Bacteria isolated from frozen fish samples was *Vibrio* spp.

Also study done by Sujatha *et al., (*2011) concluded that different species of bacteria were isolated and identified and among them, the human pathogens that were found in the culture include *Vibrio cholerae*.

Noorlis *et al.,* (2011) explained that the high frequency of *Vibrio spp*. in the samples may be due to the use of contaminated ice to cover the fish on the display bench or from long holding time on the display rack at the retail level without proper temperature control and mishandling by fish sellers.

Noorlis *et al.,* (2011) and Adebayo-Tayo *et al.,* (2011) reported much higher results (98.7%, 90.0%) respectively of *Vibrio* spp. isolated from fish commonly sold in markets. However, Noorlis *et al.,* (2011) used high precision methods (Most Probable Number-PCR) for detection.

Although cultural methods have long been used in detecting human pathogens including *Vibrio* species in fish, these methods are time consuming and sometimes inaccurate. Also some pathogens have the propensity to change into the Viable but non culturable (VBNC) state in unfavorable environments.

CONCLUSION

Food borne pathogens are a growing concern for human illness and death (Losito *et al.,* 2012).

The total viable counts for fresh samples of Najil (*p. pessuliferus*)(**5.68×10^5**) CFU/gm, and for frozen samples was (**4.38×10^5**) CFU/gm is strongly suggest the urgent need to improve the quality control and assurance systems.It has also shown that samples of fresh fish and frozen fishes used in this study were grossly contaminated by pathogenic organisms such as *Staphylococcus aureus, Escherichia coli,* which indicated post-harvest contamination probably due to mishandling, improper storage and using of contaminated containers during transportation, and thus, constitute potential public health hazard due to the unhygienic nature of fish handlers which predisposes frozen fishes to contamination by pathogenic microorganisms. This call for public health concerns and improvements in handling and processing are needed to minimize the prevalence of the pathogens.

Higher rate of Staphylococcus *aureus and Escherichia coli* was isolated from the fresh and frozen fish, which pose high risk of food borne illness, as this fish commonly eaten by high numbers of consumers as first class fish.

Also it was concluded from this study that fish markets harbored bacteria of zoonotic importance that may constitute potential hazards to fish handlers.

The results of this study also constitute an indicator of bacteriological contamination of one of important marine fishes. However, fishes should be properly cooked before consumption and good quality control measures should be adopted in culturing, processing, harvesting and consumption of sea foods.

Frozen conditionstorage could maintain the fish fillet qualities for longer time. In general, the results obtainedshowed that storing at frozen condition can significantly improve the shelf life of fish qualitycompared to fresh storage. Besides this, fish catching, handling andprocessing need to be evaluated and improved to assure quality and safety, and to minimize loss.

To limit the microbial loads of frozen fish and fish product, I suggest the provision of the adequate storage facilities i.e. refrigerator by retailer so as to avoid the multiplication of microbes under atmospheric temperature in the market.

Finally, messages warning consumers of the potential risks of infection associated with consumption of good and hygienic fish and sea food products should be considered as educational strategies. Also fish handler's education of the importance of personal hygiene should be implemented as control measures to prevent contamination during postharvest handling, processing and distribution of fish.

This finding may be considered as additional knowledge to enhance proper controlling of the storage life of fish, and fish products quality in Constantine. Safety of this kind of seafood can be guaranteed mainly by preventive measures and application of appropriates procedures of hygiene, because the surveillance of potential contaminant bacteria in harvested seafood is crucial for sustenance of public health.

RECOMMENDATIONS

♦It's better to preserve fish in ice during handling and short storage period and to maximize freshness quality, fish should be held at the temperature of melting ice i.e. 0°C.

♦Frozen condition storage could maintain the fish fillet qualities for longer time (frozen condition can significantly improve the shelf life of fish quality compared to refrigeration temperature storage).

♦Identification of bacterial type should be made in order to make well fish preservation standards.

♦In addition, raw material quality, cooling methods, processing, packaging, transporting and storage conditions should maintain freshness and shelf life extension.

♦Also the private water sources used for ice production should be based on the microbiological standards for potable water should be fit to be directly used in food items intended for human consumption and the ice should be cleaned and changed daily to achieve good safety and avoid contamination of fish.

♦Marine fishshould be subjected to better preservation method due to its fast deterioration rate.

♦ Appling of HASSP system and other programs and practices (GMPs, GHPs, GAPs,SSOPs).

♦ Inspection must be done regularly and safe the record keeping and all the equipments, machine and utensils should be calibrated regularly.

♦Extension, awareness of all the stakeholders must be done to become very hygiene and careful during all the stages of fish processing.

♦Further study is required to address important questions surrounding fish and fish products of marine environment. The studies need to include; identification and characterization of specific spoilage organisms, potential pathogenic microorganisms and parasites, and further studies to establish fish quality acceptance levels, detailed quality analysis and addressing the other species.

♦ Further study also required to use more sophisticated genetic engineering (transgenic) tools (recombinant DNA, DNA cloning, BCR, Sequencing technique) to trace, enhance fish growth and identify the pathogenic bacteria.

CHAPTER SIX

REFERENCES

Abbas, K. A., Mohamed, M., Jamilah, B and brahimian, M. 2008.A Review on correlations between fish freshness and pH during cold storage.*American Journal of Biochemistry and Biotechnology*; 4:416-421.

AbMutalib, N., Chan, K and Learn Han, L. 2014.Rapid methods for the detection of foodbornebacterial pathogens: Principles, applications,advantages and limitations. *Frontiers inMicrobiology [E]*, *5*, Article number770; 1-19.

Aboh, E.A.2015. Biochemical qualities of some imported frozen marine fish sold in Zaria, Nigeria. Msc.Ahmadu Bello University, Zaria; 1-74.

Abolagba, O. J and Uwagbai, E. C. 2011. A Comparative analysis of the microbial load of smoke-dried fishes (*Ethmalosa Fimbriata* and *Pseudotolithus Elongatus*) sold in Oba and Koko markets in Edo and Delta States, Nigeria at different seasons. *Australian Journal of Basic and Applied Sciences;* 5 (5): 544-550.

Abowei, J. F. 2011. Some principles and requirements in fish nutrition.*British Journal of Pharmacology and Toxicology*;2 (4): 165-167.

Ackman, R. G. 1989. Marine biogenic lipids, fats and oils. CRC Press, USA; pp. 472.

Adebayo-Tayo, B. C., Odu, N. N., Anyamele, L. M., Igwiloh, N. J and Okonko, I. O. 2012. Microbial quality of frozen fish sold in Uyo Metropolis. *Nature and Science;* 10 (3):71-77.

Adebayo-Tayo, B. C, Odu, N. N, Igiwiloh, N. J and Okonko, I. O. 2012.Microbiological and physicochemical level of freshCatfish (*Arius hendelotic)* from different markets in Akwa Ibom State, Nigeria.*New York Science Journal*;5(4):46-52]. (ISSN: 1554-0200). http://www.sciencepub.net/newyork. 7.

Adebayo-Tayo, A.C., Odu, N. N., Michael, M. U and Okonko, I. O. 2012a. Multi-Drug Resistant (MDR) organisms isolated from Sea-foods in Uyo, South-Southern Nigeria. *Nature and Science;* 10 (3): 61-70.

Adebayo-Tayo, B. C., Odu, N. N., Anyamele, L. M, Igwiloh, N. J and Okonko, I. O. 2012b. Microbial quality of frozen fish sold in Uyo Metropolis. *Nature and Science*; 10 (3): 71-77.

Adebowale, B. A., Dongo, L. N., Jayeola, C. O and Orisajo, S. B.2008. Comparative quality assessment of fish (Clariasgariepricuns) smoked with cocoa pod husk and three other different smoking materials. *J Food Technol*; 6:5-8.

Adeleye, I. A., Daniels, F. V and Enyinnia, V. A. 2010. Characterization and pathogenicity of *Vibrio spp*. contaminating seafoods in Lagos, Nigeria. *Internet Journal of Food Safety;* 12: 1-9.

Ahmadoon, H. G. A. 2013. Quality changes in marine fish (*Plectropomus areolatus*) during preservation in different rates of ice.*Red Sea University Journal refereed scientific biannual Journal;* (3): 7-20.

Ahmed, S., Bdr eldien, K and Abdella, R. 2014.Survey of contaminant bacteria on *Oreochromis niloticus* and *lates niloticus* at Elmourda fish market.*Direct Research Journal of Agriculture and Food Science (DRJAFS);* 2 (7):98-101.

Ahmed, I. O., Alur, M. D., Kamat, A. S., Bandekar, J. R and Thomas, P.1997. Influence of processing on the extension of shelf-life of Naglifish (*Sillagosihama*) by gamma radiation. *Journal of Food Science and Technology;* 32(4): 325-331.

Ajiboye, A. E., Kolawole, O. M., Oladosu, T. O., Adedayo, M. R and Akintunde, J. K. 2011. Studies on the microorganisms associated with dried meat (Tinko) sold in Ilorin, Nigeria. *African Journal of Microbiology Research;* 5 (24): 4150-4154.

Akinneye, J. O., Amoo, I.A and Bakare, O. O. 2010.Effect of drying methods on the chemical composition of three species of fish.*African Journal of Biotechnology*; 9 (28); 4369-4373.

Alemu, L. A. 2013. Microbiological and chemical changes of nile tilapia (*Oreochromisniloticus*L.) fillet during ice storage. *Advance Journal of Food Science and Technology*;5(10): 1260- 2013.

Austin, B and Austin D. A. 1999.Bacterial pathogens of fish.*Journal of Applied Bacteriology;* 58 (6): 483-506.

Awuor, W. S., Miruka, O. D and Eliud, W.N. 2011. Charaterisation of *salmonella* isolated from nile tilapia (*Oreochromis niloticus*) along Lake Victoria Beaches in Western Kenya. *World Acad. Sci. Engin. Technol*; 5: 08-28.

Bakr, W.M., Hazzah, W. A and Abaza, A. F.2011.Detection of *Salmonella* and *Vibrio* species in some seafood inAlexandria. *Journal of American Science*; 7(9): 663-668.

Ball, C. O and Olson, F. C. 1957. Sterilization in food technology. McGraw-Hill Book Co, New York, USA; pp. 654.

Bang, H. O and Dyerberg, J. 1980. Lipid metabolism in Greenland Eskimos.*Advances in Nutritional Research;* 31: 1-32.

Barrow, G. L and Feltham, R.K. 1993.Cowan steel manual of Identification of medical microbiology bacteria 3d edition Cambridge University press 315.

Beets, J. and Hixon,A. 1994. Distribution,persistence, and growth of groupers (Pisces:Serranidae) on artificial and natural patch reefs inthe Virgin Islands. Bull. Mar. Sci., 55: 470-483.

Begum,K. 2014.Comparative microbiological assessment of export oriented fishes and locally marketed fishes.MSC. Dhaka University Institutional Repository; 1-85.

Begum, M., Abu Ahmed, A., Das, M. and Parveen, S. 2010. Acomparative microbiological assessment of five types of selected fishes collected from two different markets. *Advances in Biological Research;* 4 (5): 259-265.

Botta, J. R. 1995. Evaluation of seafood freshness quality. VCH Publishers Inc.

Biji, K. B., Shamseer, R. M., Mohan, C.O., Ravishankar, C.N and Mathew, S. 2015. Effect of thermal processing on the biochemical constituents of green mussel (*Pernaviridis*) in Tin-free-steel cans.*Journal of Food Science and Technology;* 52(10): 6804-6809.

Can, O. P. 2010. Evaluation of the microbiological, chemical and sensory quality of carp processed by the sous vide method. *International journal of agricultural and biological sciences;* 1 (2): 99-104.

Carlos, R. 2008. Evaluation of suitable chemical methods for seafood products in mozambique.Maputo- Mozambique; pp.16.

Centerfor Food Safety andApplied Nutrition. 2001.Food and Drug Administration. 3 rd edition, Washington pp.145-166.

Chuck, C. 2012. Variation in fish Composition: cause and effect changes in the live animal.*Advance Journal of Food Science and Technology;* 5 (10): 1260-1268.

Clucas, I. J and Ward, A. R. 1996. Post-harvest fisheries development: A Guide to Handling, Preservation, Processing and Quality. Kent, ME44TB: Chatham Maritime. pp. 84-128.

Clemens, R and Pressman, P. 2007. Food and Alzheimer's disease.*Food Technology;* 61(10): 16-22.

De Freitas Neto, O., Penha Filho, R., Barrow,P and Berchieri Junior, A. 2010. Sources of human nontyphoidsalmonellosis: a review. Revis.Brasi. De Ciência Avíc; 12(1): 01-11.

Dominique, P. B. 2014. Fish nutrition, aquafeeds and companion animal nutrition in ontario. University of Guelph.

European Food Safety Authority (EFSA), 2014.Panel on dietetic products, nutrition and allergies.*European Food Safety Authority Journal*; 12 (11): 38 - 94.

Elamin, S. M., Ambak, M. A., Ibrahim, S and Hamza, M. E. 2011.Some morphometric relationships of *Plectropomus areolatus* in Sudanese Red Sea. *Empowering Science, Technology and Innovation Towards a Better Tomorrow;* 349-363.

Elamin, S. M., Ambak, M. A., Samoilys, M. A and Hamza, M. E. 2011.Some morphometric relationships of coral trouts *Plectropomus Pessuliferus* and *Plectropomus Areolatus* inhabiting Sudanese Red Sea. *Advances in Environmental Biology;* 5 (9): 2860-2865.

Elamin, M. S., Ambak, M.A., Zakaria, M. Z., Ibrahim, S and Hamza, M.E. 2014.Intraspecific morphometric comparisons between the two populations of coral trout, Plectropomus pessuliferus and Plectropomus areolatus, in two different locations in the Sudanese Red Sea. *Red Sea University Journal*; (6):7-22.

Emikpe, B. O., Adebisi, T and Adedeji, O. B. 2011. Bacteria load on the skin and stomach of *ClariasGariepinus* and *Oreochromis Niloticus* from Ibadan, South West Nigeria; Public health implications. *J. Microbiol. Biotech. Res;* 1 (1): 52-59.

Erickson, M. 1997. Lipid oxidation: Flavor and nutritional quality deterioration in frozen foods. In: M Erickson, et al. (Eds.), Quality in Frozen Food. Chapman and Hall, New York, USA; pp. 141-173.

FAO.Food and Agriculture Organization. 1979. Manual of good quality control for microbiological analysis. Food and Agriculture Organization of the United Nation, Roma, Italy, ISBN-13:9789251008492, page: 144.

FAO.Food and Agriculture Organization. 2010. Export workshop on the application of biosecurity measures to control salmonella contamination in sustainable aquaculture. FAO fisheries and aquaculture Report No.937.ISSN 2070, Mangalore, India, 19-21.

FAO.Food and Agriculture Organization.2011. Post-harvest fish loss assessment in small-scale fisheries. Rome, Italy.

FAO. Food and Agriculture Organization. 2013. The State of World Fisheries and Aquaculture. Rome, Italy.

FAO.Food and Agriculture Organization. 2014. The state of World Fisheries and Aquaculture. Rome. 223.

FAO. Food and Agriculture Organization. 2014. The State of World Fisheries and Aquaculture; P. 109 ISSN 1020-5469 E-ISBN 978-92-5-108276-8.

FAO.Food and Agriculture Organization of the United Nations. 2014. The State of World Fisheries and Aquaculture; Opportunities and Challenges, Canada.

FAO. Food and Agriculture Organization 2014a.Post-harvest losses in artisanal fisheries.http://www.fao.org/focus/e/fisheries/proc.htm [2014-01-29].

FAO. Food and Agriculture Organization.2014b. Reducing post-harvest losses.

http://www.fao.org/fishery/topic/12369/en [2014-01-29].

FAO species catalogue**.** Vol. 16. Groupers of the world (Family Serranidae, Subfamily Epi-nephelinae). An annotated and illustrated catalogue of the grouper, rockcod, hind, coralgrouper and lyretail species known to date.FAO fisheries Synopsis.No. 125, Vol. 16. Rome, FAO. 1993.382 p., 522 figs, 31 colour plates.
Food and Drug Administration (FDA).2015. Evaluation and definition of potentially hazardous foods.

FAO. 2015.Nitrogen containing extractives. FAO, Rome.

Faisal,K., Mosammad, S. S and Heri, K. 2015. Antimicrobial activities of grape (*Vitisvinifera*L.) pomace polyphenols as a source of naturally occurring bioactive components. *African Journal of Biotechnology*; 14 (26): 2157-2161.

Flowra, F. A., Dil, G. N., Anannya, S. T and Tariqul, M. I. 2012. Biochemical analysis of five dried fish species of Bangladesh. *University Journal Zoological;* 31, 9-11.

Francoise, l.2010.Occurence and role of lactic acid bacteria in seafood product.*Food microbial;* 27: 698-701.

Fuhrman, J. A., Hewson, I., Schwalbach, M. S., Steele, J. A., Brown, M. V and Naeem, S. 2006. Annually reoccurring bacterial communities are predictable from ocean conditions. *P Natl Acad Sci USA;* 103: 13104-13109.

Frisch, A. J and Hobbs, J. A. 2007. In vitro hybridization of coral trouts, *Plectropomusleopardus* (Lacepe`de, 1802) and *Plectropomusmaculatus* (Bloch, 1790): a preliminary investigation. *Aquaculture Research;* (38): 215-218.

Ghaly, A. E., Dave, D., Budge, S and Brooks, M. S. 2010. Fish spoilage sechanisms and preservation techniques. *American Journal of Applied Sciences*;7 (7): 859-877.

Gomez, G. D and Balcazar, J. L. 2008. Interactions between gut microbiota and innate immunity of fish. FEMS.*Immunology and Medical Microbiology*;52: 145-154.

Gopalakrishnan, S., Sasidharan, V., Sunder, J., Mudavath, M and Kumar, R. R. 2016. Effect of storage temperature on sensory and microbial quality of *Rastrelliger kanagurta* (Cuvier, 1816) from Andaman Coast. *Adv. Anim. Vet. Sci;*4 (9): 468-475.

Gould, G. W. 1989. Mechanisms of action of food preservation procedures. Elsevier Applied Science, London, UK.

Gram, L and Huss,H. 2000. Fresh and processed fish and shellfish, in: the microbiological safety and quality of foods. Lund, B.M. A.C. Baird Parker and G.W. Grould (Eds.) Champinan and Hall, London. pp. 472-506.

Gram, L and Huss, H. 1996.Microbiological spoilage of fish and fish products.*International Journal of Food Microbiology*;33: 121-137.

Huq, A., Colwell, R., Rahman, R., Ali, A., Chowdhurry, M., Parveen, S., Sack D and Russek-Chen, E. 1990. Detection of *Vibrio cholerae* O1 inthe aquatic environment by fluorescent monoclonal antibody andculture methods. Appl. Environ. Microbiol; 56: 2370-2373.

Huss, J. 1996. Microbial and biochemical spoilage of foods: An overview. Int J Food Microbiol; 33:1-18.

Huss, H. H. 1995. Quality and Quality changes in fresh Fish. FAO fisheries technical paper 348;FAO, United Nations,Rome.48pp.

Huss, H. H. 1994. Assurance of seafood quality.FAO Fisheries Technical paper 334.Rome.

Huss, H. H. 1994. Quality and quality changes in fresh fish. FAO Fisheries Technical paper 348. Rome.

ICMSF (International Commission on Microbiological Specifications for foods). 1998. Micro-organisms in foods: Microbial ecology of food commodities. London: Blackie Academic and Professional.

INA- Smith, J. P, Simpson, B. K. 1996. Spoilage and shelf life extension of fresh fish and shellfish.*Crit Rev Food Sci Nutr;* 36(1-2): 87-121.

Iqbal, Z. Sheikh, U and Mughal, R. 2012. Fungal infections in some economically important freshwater fishes. *Pakistan Vet. J;*32 (3), 422-426.

Janvier, K., Victor, B. A., Vieira-Dalode, G., Akissoe, H. N and Jacobs, A. 2014. Production, consumption, and quality attributes of lanhouin, a fish-based condiment from West Africa. *Food Chain*;2 (1):10.3362/2046-1887.

Jay, J. M., Loessner, M.J and Golden, D. A. 2005.Foodborne gastroenteritiscaused by *Vibrio*, *Yersinia* and *Campylobacter* species. In: ModernFood Microbiology. (7th edn). Springer Science, New York, pp. 657-664.

Jinadasa, B. K. 2014. Determination of quality of marine fishes based on total volatile nitrogen test (TVN).*Nature and Science*; 12 (5): 106-107.

Jianadasa, B. K. Ginigaddarage, P. H and Ariyawansa, S. 2014. A comparative quality assessment of five types of selected fishes collected from Retail Market in Sri Lanka. *American Journal of Food Science and Technology;* (2) 21-27.

Jorge, R. J., Redondo, P. N and Dosta, M. d. 2016. Bacterial load comparison of marine fish collected and commercially obtained for human consumption in western region of Yucatan Peninsula, Mexico.*International Journal of Aquatic Science;* 7 (1) 6-12.

Joseph, A. V., Sasidharan, R. S., Nair, H. P and Bhat, S. G. 2013.Occurrence of potential pathogenic *Aeromonas* species in tropical seafood, aquafarms and mangroves off Cochin coast in South India, *Vet. World;* 6 (6):300-306.

Kaper, J. B., Morris, J. G and Levine, M. M.1995.Cholera.Clin.Microbiol. Rev; 8: 48-86.

Kindossi, J. M. Anihouvi, V. B. Vieira-Dalode, G. Akissoe, N. H. and Hounhouigan, D. J. (2012). Production, consumption, and quality attributes of lanhouin, a fish-based condiment from West. *Food Chain*; 2 (1) 10.3362/2046-1887.

Kirs, M. A., Depaola, R., Fyfe, J. L., Jones, J., Krantz, A. V., An Laanen, D. Cotton, A. and Castle, M. 2011. A survey of oysters (Crassostrea gigas) in New Zealand for *Vibrio parahaemolyticus* and *Vibrio vulnificus*.*International Journal of Food Microbiology;* 147 (2): 149-153.

Kumta, U. S., Savagaon, K. A, Ghadi, S. V., Doke, N. S and Gore, M. S. 1973. Radiation preservation of sea foods.Review of Research in India.*International Atomic Energy Agency;* pp. 402-425.

Lakshmisha, I. P., Ravishankar, C. N., Ninan, G., Mohan, C. O and Srinivasa Gopal, T. K. 2008.Effect of freezing time on the quality of Indian mackerel (*Rastrelliger kanagurta*) during frozen storage.*J Food Sci;* 73 (7): S345- 353.

Lauzon, H. L., Margeirsson, B., Sveinsdottir, K., Guojonsdottir, M., Karlsdottir, M. G and Martinsdottir, E. 2010.Overview on fish quality research.Impact of fish handling, processing, storage and logistics on fish quality deterioration.Iceland: Matis;21-22.

Liston, J. 1980.Microbiology in fishery science. In Connell, J. J. ed. *Advances in Fish Science and Technology;* 23-27.

Losito, F., Bottini, G., de Ascentis, A., Priolisi, F., Mari, A., Tarsitani, G and Antonini, G. 2012.Qualitative and quantitative validation of the microbiological survey method for *Listeria spp.*, *Salmonella spp.*, *Enterobacteraceae and Staphylococcus aureus* in food samples. *A M.J. Food Technol*; 7:340-351.

Lotfy, N., Hassanein, M., Abdel-Gawad, F., El-Taweel, G and Bassem, S. 2011. Detection of*salmonella* spp. in aquatic insects, fish and waterby MPN-PCR. World J. Fish. Marine Sci. 3: 58-66.

Makri, M. 2009. The biochemical textural and sensory properties of frozen stored (-22°C) king scallop (Pectenmaxinus) meats. *African Journal of Biotechnology*; 8 (16): 3893-3903.

Matches,J., Liston, J and Daneault, L. 1971.Survival of *Vibrioparahaemolyticus* in fish homogenates during storage at lowtemperatures. Appl. Environ. Microbiol; 21: 951-952.

Mayer, B. K and Ward, D. R .1991.Microbiology of finfish and finfish processing.In Ward, D. R and Hackney, C. eds. *Microbiology of marine food products*. New York: Van Nostrand Reinhold.

Mazrouh, M. M. 2015. Effects of freezing storage on the biochemical composition in muscles of *Saurida undosquamis* (Richardson, 1848) comparing with imported frozen. *International Journal of Fisheries and Aquatic Studies 2015;* 3 (2): 295-299.

Mohan, C. O., Ravishankar, C. N and Srinivasa Gopal, T. K. 2016.Packaging interventions in low temperature preservation of fish.A Review. MOJ Food Process Technolp; 2 (1): 00026. DOI: 10.15406/mojfpt.2016.02.00026.

Montanari, M. P., Pruzzo, C., Pane, L and Colwell, R. 1999. *Vibrios*associated with plankton in a coastal zone of the Adriatic Sea (Italy).FEMS Microbiol. Ecol; 29: 241-147.

Morris, A.V., Roberts, C.M and Hawkins, J.P. 2000.The threatened status of groupers (Epinephelinae).Biodiversity Conserv., 9: 919-942.

Newaj-Fyzul, A., Mutani, A., Ramsubhag, A and Adesiyun,A. 2008. Prevalence of bacterial pathogens and their anti-microbial resistance in Tilapia and their pond water in Trinidad.Zoono.Publ. Health; 55(4): 206-213.

Newton, J. M and Surawicz, C.M. 2011.Infectiousgastroenteritis and colitis Diarrhea.Springer.33-59.

Nickelson, R and Finne, G. 1992. Fish, crustaceans, and precooked seafoods In: Vanderzant C, Splittstoesser D. (eds) Compendium of methods for the microbiological examination of foods. APHA, Washington D.C; pp. 875-895.

Nilla, S. S., Khan, N. A., Khan, M. M., Ahsan, D. A and Mustafa, M. G. 2012.Bacteriological quality of marketed Mola Fish, *Amblypharyngodon Mola* from Dhaka Metropolis.*Bangladesh J. Zoo;* 40 (1): 77-88.

Noor, R., Acharjee, M., Ahmed, T., Das, K. K., Paul, L., Munshi, S. K., Urmi, N. J., Rahman, F and Alam, M. Z. 2013. Microbiological study of major sea fish available in local markets of Dhaka City, Bangladesh. *Journalof Microbiology, Biotechnology and Food Sciences;* 2 (4) 2420-2430.

Noorlis, A., Ghazali, F. M., Cheah, Y. K.,Tuan Zainazor, T. C., Ponniah, J., Tunung, R., Tang, J. Y. H., Nishibuchi, M., Nakaguchi, Y and Son, R. 2011. Prevalence and quantification of *Vibrio* species and *Vibrio parahaemolyticus* in fresh water fish at hypermarket level. *International Food Research Journal*; 18: 689-695.

Okoro, C. C., Aboaba, O. O and Babajide, O. J. 2010. Quality assessment of a nigerian marine Fish, Mullet (*Liza falcipinnis*) under different Storage Conditions. *New York Science Journal;* 2010; 3 (8):21-28.

Olafsdottir, G., Martinsdottir, E., Oehlenschlager, J., Dalgaard, P and Jensen, B. 1997. Methods to evaluate fish freshness in research and industry. *Trends in Food Science and Technology;* 8 (8): 258-265.

Oramadike, C. E., Ibrahim, A. O and Kolade, O. Y. 2010.Biochemical and microbiological quality of frozen fishes available in some supermarkets in Lagos State, Nigeria.*Journal of Life and Physical Sciences;* 3 (2): 48 – 51.

Ozcan, T., Erdogan, H and Ozcan, G. 2013. A contribution to a knowledge of the freshwater decapods of Hatayregion, Turkey. *Arthropods*; 2(1): 42-44.

Popovic, N. T., Skukan, A. B., Dzidara, P., Coz-Rakovac, R., Strunjak-Perovic, I., Kozacinski, L., Jadan, M and Brlek-Gorski, D. 2010. Microbiological quality of marketed fresh and frozen seafood caught off the Adriatic coast of Croatia. *Veterinarni Medicina-Czech;*55 (5): 233-241.

Pontarp, M., Canback, B., Tunlid, A and Lundberg, P. 2012. Phylogenetic analysis suggests that habitat filtering is structuring marine bacterial communities across the globe. *Microb Ecol;*64: 8-17. doi: 10.1007/s 00248-011-0005-7.

Porwollik, S., Boyd, E., Choy, C., Cheng, P., Florea, L., Proctor, E and McClelland, M. 2004.Characterization of *Salmonella*enteric subspecies I genovars by use of microarrays.*J.Bacteriol*; 186(17): 5883-5898.

Rahman, M. M., Majumder, M. Z., Sarker, B. S and Shamsuddin, M. 2012. Microbiological quality assessment of raw and frozen product of Coral (*Latescalcarifer*) in a fish processing plant, *Bangladesh J. Prog. Sci. and Tech;* 10 (1): 045-048.

Raufu, I. A., Lawan, F., Bello, H., Musa, A., Ameh, J and Ambali, A. 2014. Occurrence and antimicrobial susceptibility profiles of *Salmonella* serovars from fish in Maiduguri, sub-Saharah, Nigeria. *The Egyptian J. Aqua. Res;* 40 (1): 59-63.

Ray, B and Bhunia, A. 2008.Microbial foodborne diseases opportunistic pathogens, parasite, and algal toxins.In *Fundamental Food Microbiology (4th edn.)London: CRC.* pp. 315-347.

Ryan, K. J and Ray, C. G. 2010.Sherris medicalmicrobiology. McGraw Hill Medical New York.

Saima, S. N., Khan, R. A., Mahmudur, R., Ahsan, A. D and Mustafa, G. M. 2012.Bacteriological quality of marketed mola fish, *Amblypharyngodon mola* from Dhaka metropolis.*Bangladesh Journal of Zoology;* 40:77-88.

Sakthivel, K and Fernando, A. 2012. Brachyuran crabs diversity in Mudasal Odai and Nagapattinam coast of southeast India. *Arthropods*; 1(4): 136-143

Shahidi, F and Botta, J. 1994. Seafoods: Chemistry, processing technology and quality. Chapman and Hall, UK, pp. 3-9.

Sallam, K. I. 2007. Chemical, sensory and shelf life evaluation of sliced salmon treated with salts of organic acids. *Food Chem;* 101 (2): 592–600.

Shamsuzzaman, 1. M. M., Mazumder,S. K., Siddique, M. A and Miah, M. N. 2011.Microbial quality of hilsa shad (*Tenualosa ilisha)* at different stages of processing.*J. Bangladesh Agril. Univ;* 9 (2): 339-344.

Sravani, K. 2011. Quality assessment of rohu (*labeo rohita*) stored at different temperatures - 524 344.

Sujatha, K., Senthilkumaar, P., Sangeetha, S and Gopalakrishnan, M. D. 2011. Isolation of human pathogenic bacteria in two edible fishes, Priacanthus hamrur and Megalaspis cordyla at Royapuram waters of Chennai, India, *Indian Journal of Science and Technology;* 4:539-541.

Sumner, J. L., Gorczyca, E., Cohen, D and Brady, P.1984. Do fish from tropical waters spoil less rapidly in ice than fish from temperate waters? *Food Technology Australia;* 36: 328-329.

Talkington, D., Bopp, C., Tarr, C., Parsons, M., Dahourou, G., Freeman, M.,Joyce, K., Turnsek, M., Garret, N., Humphrys, M., Gomez, G., Stroika, S.,Boncy, J., Ochieng, B., Oundo, J., Klena, J., Smith, A.,Keddy, K and Gerner-Smit, P. 2011.

Characterisation of toxigenic *Vibrio cholera* from Haiti.Emerg. Infect. Dis., 17: 2122-2129.

Trivedi, J.N., Gadhavi,M. K and Vachhrajani, K. D. 2012.Diversity and habitat preference of brachyuran crabs in Gulfof Kutch, Gujarat, India.*Arthropods*; 1(1): 13-23.

Tawari, C. C and Abowei, J. F. 2011.Traditional fish handling and preservation in Nigeria. *Asian Journal of Agricultural Sciences*;3 (6): 428-429.

Upadhyay, B., Utrarachkij, F., Thongshoob, J., Mahakunkijcharoen, Y., Wongchinda, N., Suthienkul, O and Khusmith, S. 2010. Detection of *Salmonella* invA gene in shrimp enrichment culture by polymerase chain reaction. *Southeast Asian J. Trop. Med. Public Health;* 41(2): 426-435.

Varadharajan, D., Soundarapandian, P and Pushparajan, N. 2013.The global science of crab biodiversity fromPuducherry coast, south east coast of India.*Arthropods*; 2(1): 26-35

Vasconcellos, J. A. 2004a. Food Plant Sanitation: Good Manufacturing Practice Audits. In *Quality Assurance for the Food Industry A Practical Approach*. Washington D.C., Florida: CRC Press, LLC. pp 201-263.

Vasconcellos, J. A.2004b. Hazard Analysis and Critical Control Points.In Quality Assurance for the Food Industry A Practical Approach. Washington D.C.,Florida: CRC Press, LLC. pp 289-298.

Vasudevan, P., Marek, P., Daigle, S., Hoagland, T and Venkitanarayan, A. 2002.Effects of chilling and Freezing on survival of *Vibrioparahaemolyticus* on fish fillets.*J. Food Safety*; 22: 209-217.

Vignesh, S. M., Badhul, H. M and Selva, P. A. 2012. Pilot study and first report on the Jelly fish (*Crambionella Stuhlmanni*) associated bacteria and its amylase producing properties. *International Journal of Pharm Tech Research;* 4:427-431.

Wafaa M.K Bakr, Walaa A Hazzah, Amani F Abaza. 2011. Detection of *Salmonella* and *Vibrio* species in some seafood in Alexandria. Journal of American Science; 7 (9):663-668.

Wang, T., Zhang, J and Zhang, X. 2010. Fish product quality evaluation based on temperature monitoring in cold chain. *African Journal of Biotechnology*; 9 (37): 6146-6151.

Ward, D. R. 2001. Description of the situation.In Processing Parameters Needed to Control Pathogens in Cold-smoked Fish. Especial Supplement to *the Journal of Food Science*; 66 (7) S-1067-1071.

WHO. 2015. Diet nutrition and the prevention of chronic diseases. World Health Organization Technical Report Series; 916.

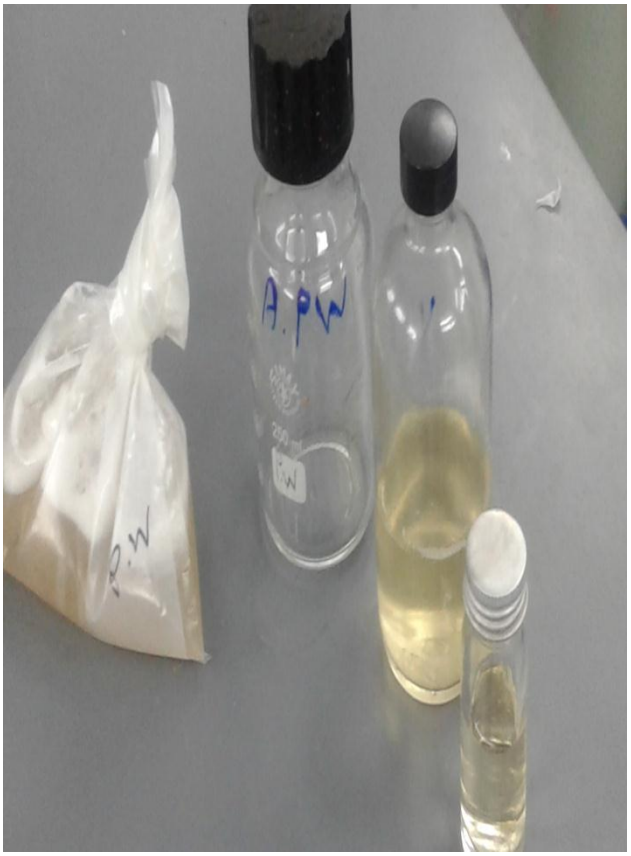
Wong, M and Chen, S. 2013. First detection of oqxAB in Salmonella spp. isolated from food. Antimicro.Agen. Chemothera; 57(1): 658-660.

Yagoub, S. O 2009. Isolation of *Enterobacteriaceae* and *Pseudomonas spp*. from raw fish sold in fishmarket in Khartoum state. *Journal of BacteriologyResearch;* 1(7): 085-088.

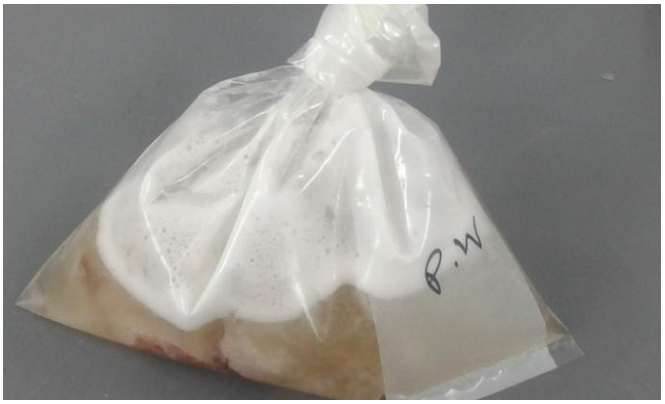
Zhimin, Z and Lianhua, L. 2014. Lipid contents, fatty acid profiles and nutritional quality of nine wild caught freshwater fish species of the yangtze Basin, China. *Journal of Food and Nutrition Research*; 2 (7): 388-394.

Appendix

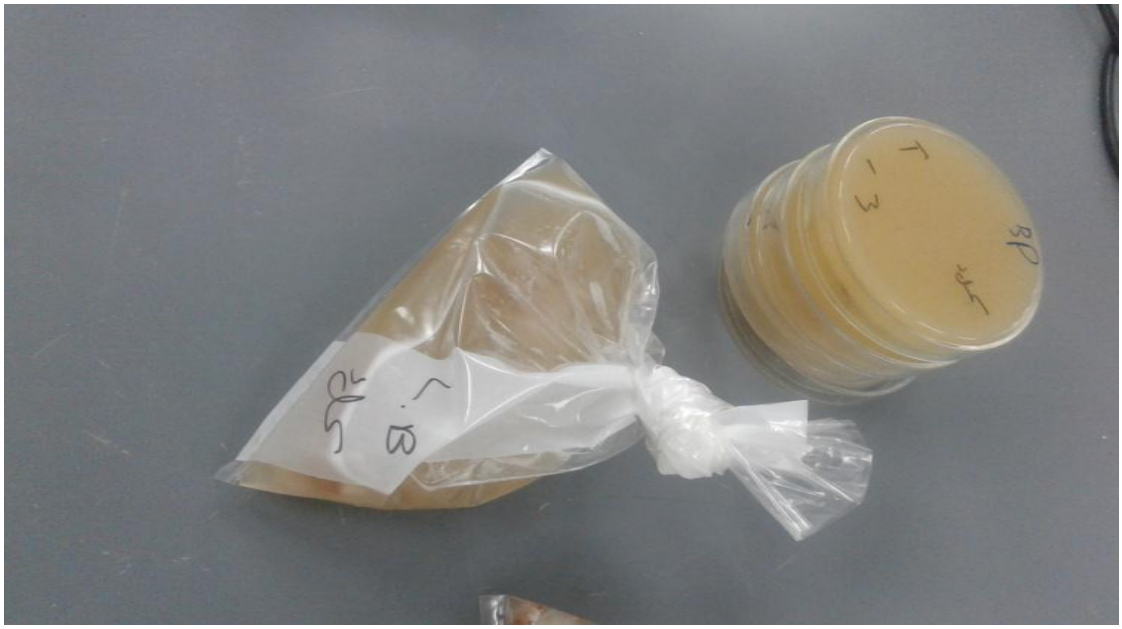
SAMPLE PREPERATIONS



VRB and (PCA) and three diluents of alkaline peptone water broth

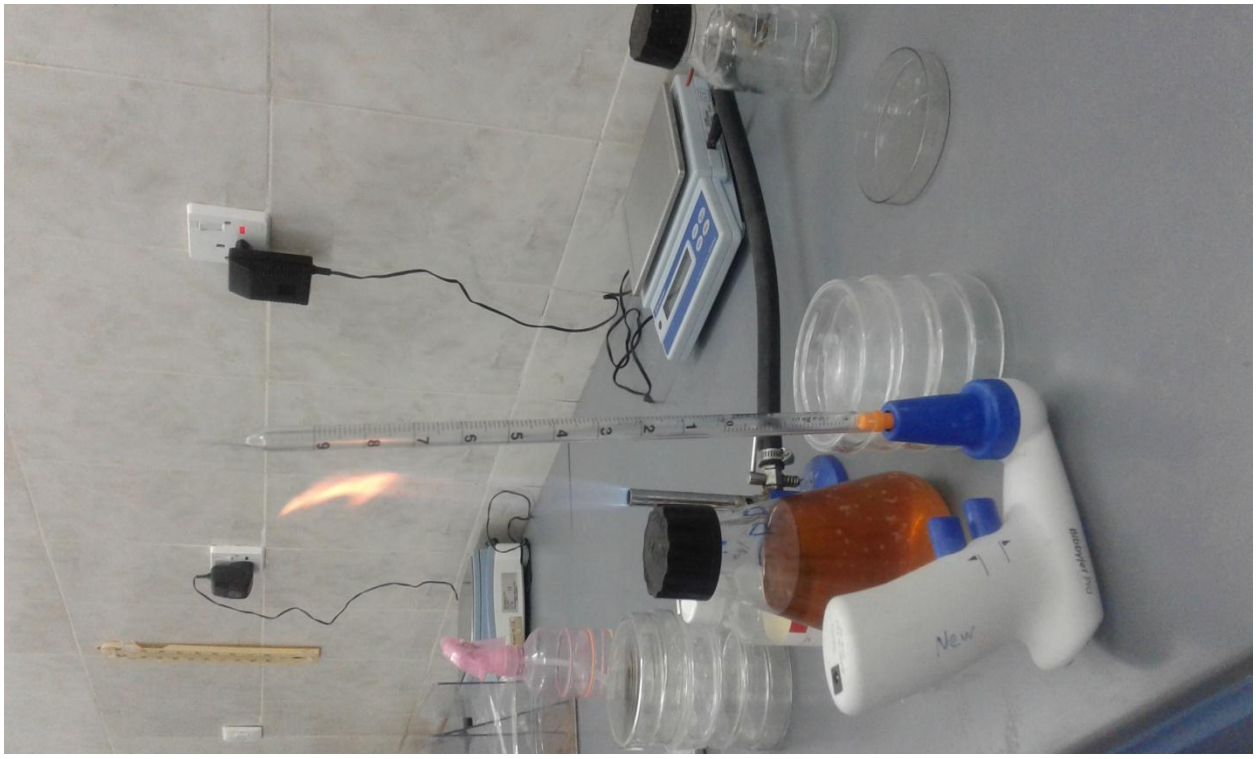


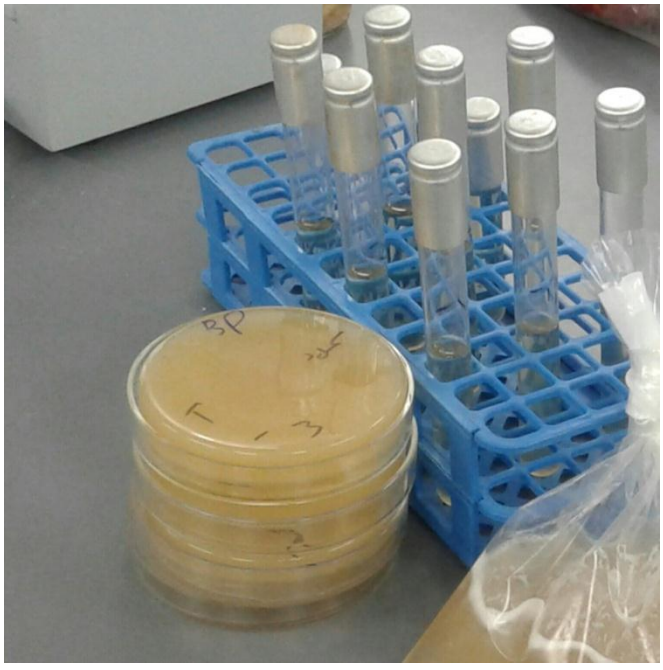
Fish samples containing peptone water after homogenized in stomacher



Fish samples containing Lactose Broth (LB) after homogenized in stomacher for salmonella initial suspension and three petri dishes containing Baird Parker agar for staph.

Selenite cysteine broth SCB (use prior streaking of sample culture on XLD) without sample.





Sample preparations and nine tubes (three diluents)

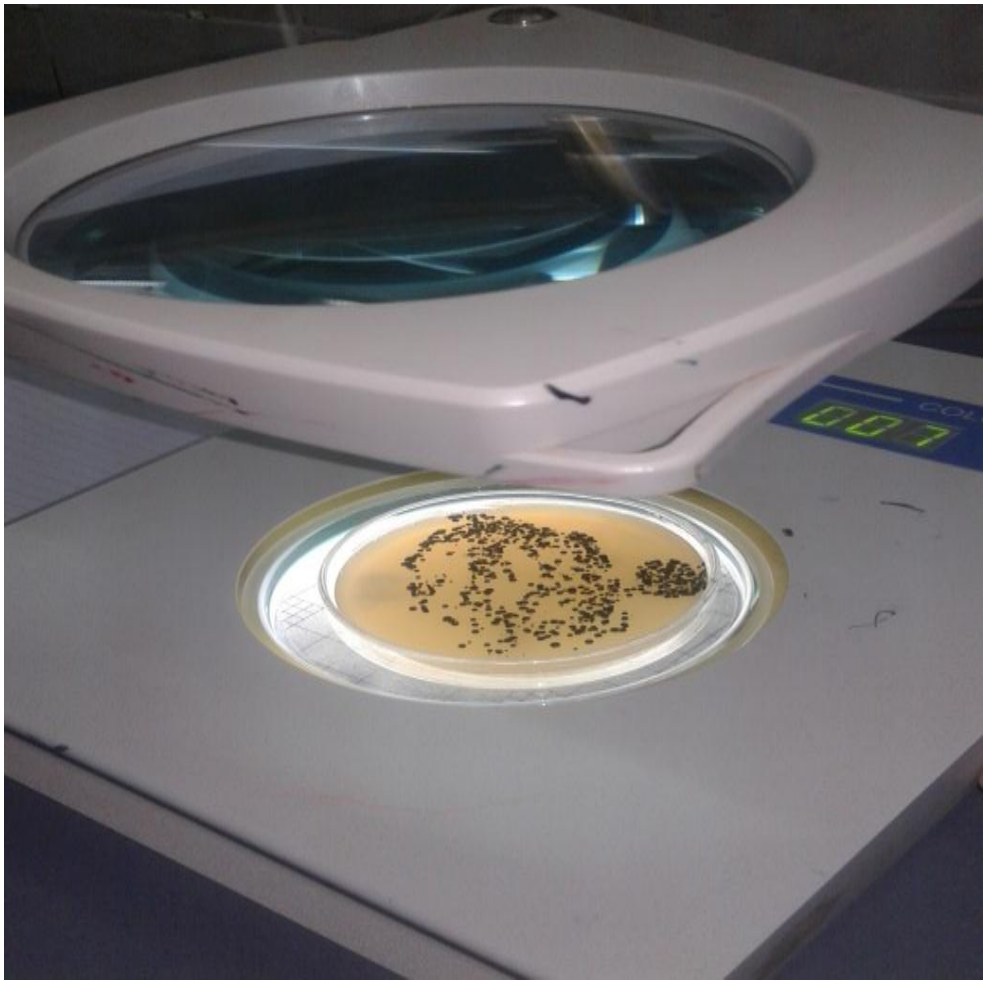
EQIPMENTS

Flame

Incubator

Sensitive balance

Stomacher



Digital colony counter

Bad Hygienic Practices

Display of fish at the market

Washing of fish prior their display

Preservation of Najil species at iced water prior the display to consumers

Fish preservation and one of the fish buyers

UN hygienic Shrimp preparation prior to distribution to the restaurants and consumers

UN hygiene fish cleaning practices