Sudan University of Science and Technology

College of Graduate Studies

Sensory and Microbial Evaluation of Quality of Fish (*Orechromis sp Bagrus sp Claris sp*)

From ALmawrada Fish Market.

التقييم الحسى والميكروبى لجوده اسماك البلطى والقرموط والبياض فى سوق المورده

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الآيـــــــــة

قَالَ تَعَالَىٰ:

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

صدق الله العظيم

النحل: ١٤

Dedication

THIS THESIS IS DEDICATED TO SOUL OF MY LATE FATHER ALTAHIR MOHIELDIN ALTAHIR AND BELOVED MOTHER BAYA MOHAMMED ALGONI AHMED AND MY LOVELY HUSBAND KHALED OSMAN . FOR THEIR LOVE AND ENCOURAGEMENT AND PATIENCE DURING THE PERIOD OF THE STUDEY. IT IS ALSO DEDICATED TO MY BROTHERS AND SISTER.

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OUR GREATEST THANKS TO ALLAHA ALMIGHTY, THE MOST MERCIFUL,,,,

WHO GAVE ME THE HEALTH, STRENGTH AND PATIENCE TO CONDUCT THIS STUDY,,,

GRATEFUL THANKS TO MY SUPERVISOR DR HARAM HASSAN

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OF SCIENCE AND TECHNOLOGY OF ANIMAL PRODUCTION IN

DEPARTMENT OF FISHERIES SCIENCE AND WILDLIFE

FINALLY MY THANKS GO TO MY COLLEAGUES, RELATIVES AND FRIENDS ,,,

Abstract

This study was conducted through (Nov.2016) to evaluate the quality of fresh fish *Orechromis sp*, *Bagrus sp*, *Claris sp* **at** almawrada fish market based on sensory and microbial testing **,** Sensory examination using the European scheme in this scheme the fish were classified in to four categories depending the firshness of fish excellent quality (E) , very good grade (A) , good grad (B) grad, undesirable grad (C).And Microbial taste for the total bacterial count and isolated pathological bacteria (Escherichia coli ,Salmonella) .

165sample were collected from almawrada fish market ,55 sample from each species , drawn from 10 ton and total of 30 swabs samples were obtained 10 samples from each species for microbial and analysis.

the result found that there was highly significant difference in sensory evaluation between fresh fish *Orechromis sp*, *Bagrus sp*, *Clarias sp*. As the average skin of fishes at respectively (1.5±7 , 1.8±.9 , 2.9±.9) and outer slime (1.5±.8 , 2.2±1, 2.9±1) and eyes (1.8±.8 , 2.3±.9 , 2.9±.9) and gill color (1±.7 , 2±.7 , 3±.7) peritoneum (1.7±.1 , 2.1±.9 , 2.9±.9) and gill odour (1.1±.7 , 2±1 , 2.9±.7) the *Clarias sp* is beast quality**,** and the total number of bacterial load for fresh fish *Orechromis sp*, *Bagrus sp*, *Clarias sp* respectively at ($5.9 \times 10^5 \pm .18 \times 10^5$c.f.u./g , $4.05 \times 10^5 \pm .31 \times 10^5$c.f.u./g, $4 \times 10^5 \pm .47 \times 10^5$c.f.u./g**)**. Result indicates no significant difference in toatal bacterial count from the studied ssp.

The result indicates tha salmonella and E.coli were isolated as Pathogenic bacteria.

Generally the handling, preservation and procsing of fish is un hygienic and un safe. The poor condition in almawrada fish matket should draw attention of the public authority consider all this deviation which no doubt well affect the environment and human health.

الخلاصه

اجريت هذه الدراسه خلال نوفمبر (2016) لتقييم جوده اسماك البلطى والبياض والقرموط بسوق المورده عن طريق الفحص الحسى والميكروبى .

الفحص الحسى باستخدام النظام الاوربى الذى يقوم بتقيم جوده الاسماك الى اربعه درجات حسب الطزاجه وهى الاولى الممتازه والثانيه جيده جدا والثالثه مقبوله والثالثه غيرصالحه لاستهلاك الادامى والفحص الميكروبى لمعرفه العد الكلى لبكترياوعزل البكتريا الممرضه.

اجريت هذه الدرسه على 165 عينه تم اخذها من 10 طن من الاسماك وايضا تم اخذ 30 اسواب عشره من كل نوع من الاسماك لاجراء الفحص الميكروبى . اظهرت النتائج وجود فرق معنوى بين اسماك البلطى والبياض والقرموط حيث كانت النتائج على التوالى متوسط الجلد على التوالى (2.9±.9، 1.8±.9،1.5±.7) ثم المخاط(1.5±.8 , 2.2±1 , 2.9±1) والعيون(1.8±.8،2.3±.9 , 2.9±.9) لون الخياشيم (1±.7 ,2±.7, 3±.7) والتجويف البطنى (1.7±.1، 2.1±.9، 2.9±.9) ورائحه الخياشيم (1.1±.7، 2±1 , 2.9±.7) .

حيث توصلت الدراسه ان سمكه القرموط هى افضل سمكه من حيث التقيم الحسى . واظهرت ايضا نتائج التحليل الميكروبى لسمكه البلطي والبياض والقرطوط ان العد الكلى لحمل الميكروب لاسماك على التوالى ($4\times10^5\pm.47\times10^5$، $4.05\times10^5\pm.31\times10^5$، $5.9\times10^5\pm.18\times10^5$)

لايوجد فرق معنوى بين الانواع المدروسه من حيث العد الكلى لبكتريا كما تم عزل الايكولاى والسالمونيلا من جميع الانواع .

وبشكل علم يمكن القول ان طر يقه تسويق وعرض وتحضير الاسماك بسوق المورده تتم تحت ظروف غير صحيه تفتقر الى ابسط قواعد السلامه مما يدعو الامر الى اتخاذ التدابير اللازمه تجاه تصحيح هذا الوضع الذى يوثر بلا شك على البئه وصحه الانسان.

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CHAPTER ONE

INTRODUCTION

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INTRODUCTION

Back ground of the study:-

Fish is one of the most important foods and is valued for its nutritional qualities. Fish protein is a good source of high quality protein containing essential amino acids in the amount and proportion required for good nutrition. It also provides a good source of vitamins and minerals *(***Onyia, *et al,* 2013).** It will also enhance the proper mental and immunity development against disease among growing children **(NAFDAC, 2003).**

In low-income countries, staple foods such as rice, wheat, maize, and cassava make up the bulk of the food consumed by people and they supply majority of energy. However, some essential nutrients (essential amino acids and micronutrients) are not found in these staples. These important nutrients can be supplied by fish because they contain very light connective tissue (**Eyo, 2001).**

Fish is an indispensable source of micronutrients, such as iron, iodine, zinc, vitamin A and B (**Haruna, 2003)**.

The quality of fish and fishery products has become a major concern in fish industry all over the world (**Huss *et al*., 2003)**.

Fish, being one of the exceptionally perishable foods and as a result of globalization of food trade fish products tend to be more susceptible to rejection due to poor quality especially if the initial raw materials are of poor quality despite the technological developments in fish production (**Huss *et al*, 2004).**

Sensory evaluation is defined as the scientific discipline used to evoke, measure, analyze and interpret reactions to characteristics of food as perceived through the senses of sight, smell, taste, touch and hearing.

Most sensory characteristics can only be measured meaningfully by humans. However, advances are being made in the development of instruments that can measure individual quality changes (**Connell, 2001; Alejandra *et al,* 1992).**

In sensory analysis appearance, odour, flavour and texture are evaluated using the human senses Scientifically, the process can be divided into three steps. Detection of a stimulus by the human sense organs; evaluation and interpretation by a mental process (**Connell, 2001; Alejandra *et al,* 1992).**.

Microbiological quality assessment (we use the term quality in its most wide sense including safety as one more criteria) is an essential point of seafood processing and distribution chains. Bacterial populations found in skin and digestive tract of seafood are considered as natural populations. This indigenous flora is composed of several micro-organisms, some of them can be human pathogens, and others are spoilage micro-organisms. As seafood is often processed in fish industries, contamination with non-indigenous micro-organisms is a very real possibility that increases with handling and storage steps. Microbial contamination originated from human activity in specific coastal areas also considered as non-indigenous **(Huss, 1995; Connell, 2001)**.

Seafood microbiological quality assessment depends greatly on the processing conditions within the seafood chain. Processing parameters modify the original physic-chemical properties of seafood, favoring and/or avoiding the growth of several micro-organisms (**Huss, 1995; Connell, 2001)**.

Aim of the study:-

The main objective of this research to draw attention to the status of fish quality at almawarada fish market .

Specific objectives:-

1-To determine the quality of the fish spices Tilapia sp, Cat fish, sp Bagrus sp Using sensory evaluation skin, outer slime, eye, gill, peritoneum , gill and internal ordure by EU (European Scheme).

2-To determine total bacterial load and isolate the pathological bacteria of fish (*Ecoli-Salmonila*).

3-To assist competent authority to have the necessary knowledge and abilities to identify the potential hazards and bad practices involved and consequently establish appropriate control measures.

4-To familiarize the trainees and concern party with the appropriate procedure and knowledge required for the evaluation of fish quality using sensory and microbical method.

5-To help and assist inspectors to have necessary knowledge and abilities to inspect fish and fishery products.

CHAPTER TWO

LITERATURE REVIEW

CHAPTER TWO
LITERATURE REVIEW

2.1 General:-

The natural fisheries of Sudan are divided into two main sectors the inland fisheries (fresh water fisheries) and the marine fisheries of the Red Sea. The inland fisheries are composed of the main Nile and its tributaries which are 6500 km long. And especially the reservoirs formed by the dams on the rivers Jebel Aulia reservoir on the White Nile, Rosaries and Sennar reservoirs on Blue Nile, Khashm Algerba reservoir on Atbara River and Nuba Lake, which is the Sudan portion on Nasir reservoir. It lies in the northern part of Sudan. It is the richest source of fish in the Main Nile inside the Sudan in addition to the Sub region at Upper White Nile **(Awad Elkarim, 1999).**

On the other hand, the marine fisheries are at the Sudanese coast line on the Red Sea, which extends to 720 km, and a continental shelf of about 98,000 km2, which is unsuitable for trawling due to its irregular coral beds **(Awad Elkarim, 1999.**

This area is endowed with fine fishes, shelf fishes, 'crap' and crustacean 'shrimp' and lobster. The total sustainable fish stock of Sudan is about 110,000 ton **(Awad Elkarim, 1999)**.

Khartoum State covers an area of 21000 km2 and the fish storage in it is estimated around 15,000 tons. But the amount exploited is not more than one thousand tons. The fish production is found in the fisheries inside Khartoum State in Jabal Awlia, Kalakla, Fetiah Al-Agaleen, El-Mawrada, the island of Al-Fitihab, Al-Sagai, AlSabalwaga and Al-Jeriaf area on the Blue Nile The process of handling and distribution of fish is carried by fishermen and traders. **(Degebassa, 2010**).

The fish section in Khartoum State is characterized by being traditional in general and the ways and equipment of fishing did not find their chance to be modernized effectively. Add to that there are no enough means of storing, refrigeration and suitable transportation. On the other hand, the fish marketing activity is concentrated on only two markets out of the three fruit and vegetable central markets that exist in the state. Even in those two markets, there are very simple ways of preserving, showing and circulating the fish. In the state there are two stations for fish services which are regarded as a centre for the teams of the statistics. What is observed in these two centers is that there infrequency in the studies and researches that are concerned with the development of the fish section in the state (**Awad Elkarim, 1999**).

The *Orechromis sp* is highly valued as a seafood source due to its many beneficial qualities, which are attributed to its wealth of nutrients, vitamins, and minerals, including significant amounts of protein, omega -3 fatty acids, selenium, phosphorous, potassium, vitamin B12, niacin,vitamin B6 , and pantothenic acid (**FAO, 2007**).

The *Clarias sp* of the family Candida is generally considered to be one of the most important tropical catfish species for aquaculture. It has an almost Pan-African distribution, ranging from the Nile to West Africa and from Algeria to Southern Africa, up to the Mediterranean coast including Asia Minor. It is a slow moving omnivorous predatory fish, which feeds on a variety of food items from microscopic zooplankton to fish half its length or 10% of its own body weight (**Welcome, 1979).**

The *Bagrus sp* is more or less elongated The dorsal fin has a smooth spine, and the pectoral fins have spines with serrations on the inside. There are four pairs of barbell anatomy barbells. The maxillary barbells may reach to the ventral fin or pelvic fins. This fish is yellow-greenish or blackish with a white belly. The fins are darker, sometimes reddish purple Juveniles have

little black spots on the sides.The bayed has a maximum size of about 112 centimeters (44.1 in) FL. It has a maximum published weight of 12.5 kilograms (27.5 lb), but is reputed to reach 100 kg (220 lb). Mean sizes and weight of males are less than those of females from the same age. Males grow up to 7 years old, females 8 years (**Srivastava, 1959*)*.

2.2 Fish Quality :-

One general definition is 'degree of excellence'. In commerce, quality limits are set by what the customer is prepared to pay for generally the customer will pay more for fish that he considers to be of higher quality, and will continue to buy as long as quality remains constant. **(Parisi *et al*,2002).**

Fish quality is a very difficult concept to explain due to the variety of factors that must be considered. Population, fish species, spawning period, season, nutrition, post-harvest handling, and storage are some of the key factors that will impact the quality of a fish product ***(*Parisi *et al*,2002).**

Quality of fish involves nutritional, microbiological, biochemical and physicochemical properties, however, consumers will decide to buy a fresh seafood product based solely on its “freshness”. To determine freshness of a fish product, consumers use their senses for evaluation and will make a decision based on appearance (color, surface appearance) aroma, flavor and texture. Sensory analysis is considered to be the most important tool to determine freshness of a fish product by inspection services and fish industry in the European Union ***(*Parisi *et al*,2002).**

2.2.1Quality control (QC):-

Quality control can be defined simply as 'maintenance of quality at a level that satisfies the customer and that is economical to the producer or seller'.

This definition could apply to almost any procedure involving the quality control of fish or fish products (**Huss *et al,* 2003).**

However, QC usually means something more formal, based on written agreed procedures or specifications which are designed to reduce mistakes, and the term QC is used in this sense in 'what follows. Quality is normally controlled by designated trained staff who have a clear knowledge of what the customer wants (**Huss *et al,* 2003).**

Inspection is part of QC and means examination of raw material or finished product to make sure it meets the specification. Inspection is normally a commercial activity, but some official inspection of fish and fish products is required by law; for example in the UK fish is examined at port and inland markets by environmental health officers (**Huss *et al,* 2003)**.

This mandatory inspection is not part of industrial QC, but industry should know what the law requires in this respect .Process control is part of QC; it means checking the process, as distinct from the raw material or finished product, to ensure that all operations on the fish are done correctly and consistently to a set standard that is usually described in the process specification quality is difficult to define, since it means different things to different people (**Nielsen *et al.,* 2002)**

2.3 Fish spoilage:-

Fish spoilage is brought about mainly by, the enzymes present in the live fish. The enzymes begin to break down fish tissues. Prior to death, the enzymes were involved in the digestion of ingested food and all enzymatic reactions are controlled. In the dead fish, the control system fails and the enzymes begin to act on the alimentary system and fish flesh, thereby resulting in soft destructive changes. This process is referred to as autolytic spoilage **(FAO, 1985).**

Bacteria are present in the gut, gills and skin surfaces of live fish. The live fish defense mechanism is able to combat the action of these bacteria. However, some after death, this defense mechanism also fails. Consequently, the bacteria invade the gut, gills and skin, and cause the decomposition from within and the exposed surfaces of the fish (**Davies *et al,* 2008).**

2.4 Prevention ,Reduction of Spoilage:-

Enzyme and bacteria spoilage of fish can be reduced or temporarily halted by various techniques. The traditional and popular methods employed include:-

2.4.1 Chilling:-

Chilling may be defined as cooling of fish to low temperatures without necessarily hardening fish. Chilling does not prevent spoilage.

However, the colder the fish the better and the lower are the incidences of microbial or enzymatic spoilage. Bacteria or enzyme action are not completely stopped but they may be temporarily halted by chilling. To chill fish, the fish has to be surrounded by colder medium, which could be solid such as ice or liquids such as refrigerated water **(Ita, 1972).**

2.4.2 Freezing:-

Freezing is distinct from chilling of fish. Freezing can keep products in near perfect condition for very prolonged periods. Freezing is essential for export purposes. Freezing becomes extremely effective, if it is combined with cold storage **(FAO/UN, 1970)**.

2.4.3Drying:-

Drying is defined as the removal of water by evaporation. When applied to fish, drying is the removal of water by any method as a means of fish

preservation to prolong the shelf life. In areas where sun drying is used traditionally, the effects of wind and weather conditions are important. Basically, the drying effect of the sun depends on the emission of heat from the sun. This is transferred to the fish and; it is accompanied by, heat transfer within the fish. During drying, the fish shrinks and undergoes irreversible changes. Water is removed from the surface in the following sequence. Firstly, water on the surface of fish evaporates. Water migrates to the surface of the fish from within fish tissues and evaporates. The air surrounding the fish then experiences a drop in temperature. This is accompanied by cooling of the surface of the fish. The energy required to drive the moisture from the surface of the fish can be obtained from a variety of sources including wood smoke, sun drying, solar drier electricity and mechanical driers (**Davies, *et al,* 2008).**

2.4.4 Smoking:-

Smoking is a popular traditional method of fish preservation in most developing countries. Smoking combines the effect of the destruction of bacteria by compounds in the smoke, such as phenols and the cooking of the fish, since, high temperatures will be generated. Smoked fish products have long shelf life, which has been attributed to the drying and cooking effects. When wood and sawdust are burnt, smoke is produced as a result of incomplete combustion. The smoke produced depends on the amount of air availabl and the quality of wood or sawdust. Soft woods produce a lot of smoke, which may lead to blacking of the finished products. Wood smoke is a mixture of complex chemical product gases, vapor and volatile substances. The volatile substances are absorbed on the wet surfaces of fish during the smoking and produce the characteristic aroma **(FAO/UN, 1970)**.

2.4.5 Salting:-

There are four standard methods for salting fish. These are brine, dry, kench and pickle salting methods. In brine salting, the fish are immersed in a solution of salt in water. Where granular salt is rubbed into the surface of fish, the process is referred to as dry salting. Granular salt is also used in kench salting. In this process, the salt is rubbed into the surface of split fish and the fish are stored with salt placed between each layer of fish **(FAO, 1971).** .

The liquid formed is not allowed to drain off the fish, which will eventually become covered with the liquid. The liquid is referred to as pickle. In pickle salting, the fish are packed in watertight containers with salt between each layer of fish. If the pickle formed does not cover the fish within 4 h, saturated brine is added to the fish so that, it becomes immersed by the pickle. Otherwise, the fish may spoil **(FAO, 1971).**

2.4.6 Fermentation:-

Majority of the methods used in fish preservation involve the removal of water. These processes involve drying by the use of either heat or heat and smoke. The method that may be employed determines the end product flavor and texture. Fermentation methods have been widely employed to conserve or utilize surplus products. **(Ita, 1972).**

2.5 Sensory evaluation:-

Sensory evaluation of food, according tois defined as the scientific means of quantifying and interpreting the variations in food characteristics (odour, taste, tactile, appearance) by using human senses of sight, smell, taste, touch and hearing (**Huss, 1995**).

Studies have shown that assessment of food freshness/ characteristics using sensory methods are capable of giving objective and / reliable results when assessments are done under controlled conditions. Generally, trained

and experienced taste panel is essential to obtain accurate and reproducible result **(Connell, 2001; Alejandra *et al*, 1992**). Sensory methods are divided into two groups; discriminative and descriptive tests however, the most commonly used is the descriptive test which measures the difference or absolute value indicating the different quantitative levels **(; Huss, 1995).**
However, sensory methods in general are known to be irrationally expensive due to the high training requirement of the panel; cost of running, need for individual scheme for individual fish species given the different spoilage patterns and physiological and psychological limitations of the analyst (**Connell, 2001**).

2.5.1 Importance of sensory evaluation of fish:-

Sensory evaluation is one of the most important methods for assessing freshness and quality in the fishing sector and in fish-inspection services. Sensory methods performed in a proper way are a rapid and accurate tool providing unique information about the food (**Huss, 1995**).

They can be very fast, reliable, non-destructive on raw fish and no expensive instruments are needed. They give direct measurement of the perceived attributes and provide information assisting in better understanding of consumer responses. However the panelists need training and retraining under the supervision of experienced panel leaders using fish samples of known freshness stage **(Martinsdottir, 2002).**

Sensory evaluation can be practiced at different levels in fish processing such as after landing, arriving at the fish plant (whole), at the reception, or processing halls of fish factories; evaluation of raw/cold and cooked fillets at the reception, or processing halls of fish factories, or at auction sites, very common in Europe (**Martinsdottir, 2002).**

Traditionally, sensory methods have been seen as a subjective assessment of the quality. However, they can be turned into an objective tool Progress has been made in sensory evaluation during the last years mainly because of the use of computers and data analysis. The work of collecting and analyzing data is not very time-consuming and the information on the results can be used and correlated with other information on the products as well. No single instrumental method has so far been (**Huss, 1995**).

2.5.2 Quality Index Method (QIM):-

The method is based on characteristic changes that occur in raw fish. These relate to the outer appearance attributes of the eyes, skin, gills and odour and a score system from 0 to 3 demerit (index) points. The scores for all of the characteristics are summarised to give an overall sensory score, the so-called Quality Index. (**Frederiksen, 2002).**

QIM has to be adapted to each fish species. The scientific development of QIM for various species aims at having the Quality Index increase linearly with the storage time in ice. QIM has several advantages, including estimation of past and remaining storage time in ice. (**Frederiksen, 2002).**

The descriptions of each score for each parameter are listed in the QIM scheme. The assessor must evaluate all the parameters involved in the scheme. As the Quality Index increases linearly with storage time in ice, the information is well suited to use in production management. QIM is well suited to teach inexperienced people to evaluate fish, train panellists and monitor performance of panelist **(Frederiksen, 2002).**

2.5.3 European scheme:-

In Europe today, the method most used and recommended for quality assessment of raw fish in the industry and the inspection service is the European scheme In this scheme, three grades of freshness are established: E, A and B, corresponding to various stages of spoilag E (Extra) is the highest possible quality, while below B is the level where fish is considered unfit for human consumption **(Frederiksen, 2002).**

This method gives rather limited information about the condition of the fish, as it is not species-related and does not therefore take into account the differences between species. The EU-scheme is commonly accepted at auction levels however its use has been disputed. **(Frederiksen, 2002).**

2.5.4 Fish freshness rating:-

Fish freshness is evaluated according to the rating desing each type of fish The detrmined approach has been adopted in another scheme for assessing freshness or degree of deterioration. In this grades show in the table are replaced by demerit scores thus Extra,A,B, and C are replaced by scores 0,1,2,and3 all components of fish (skin , outer slime ect) are score speartely and the scored summed to give an overall score are (perfectly fresh) fish would score 0,completely deteriorated **(Frederiksen, 2002).**

2.6 Microbiological methods:-

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

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

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

According to (**Huss, 1995**) the various ways that can be used to determine bacteriological contamination in food/fish include.

Total Viable Count / Total Plate Count/ Standard Plate Count/ Aerobic Plate Count SPC, APC, all mean the number of bacteria (colony forming units, cfu/g or ml) in a food product under specified standard and uniform conditions of culturing. In general, these methods rely on the estimation of the fraction of the microflora able to produce colonies in the medium used under specified incubation conditions **(Huss *et al.*, 2004).**

Therefore, the temperature during incubation of the plates has greater influence on the number of colonies developing in the sample thus, in the examination psychrophilic bacteria, pour plating and a 3-4 day incubation period at 25oC is recommended other than at 30 or 37oC **(Huss, 1995**).

The enumeration of bacterial counts in food can be done using a variety of medium, thus the classification of these methods for instance; plate count agars (PCA) are commonly used for enumeration of bacteria **(Huss, 1995)**

However, it is recommended that a more nutrient rich agar, such as (Iron Agar, Lyngby) be used when analysing seafood to obtain reproducible results (**Huss *et al.*, 1994**).

2.6.1 Food borne pathogens associated with fish and fish products:-

From the standpoint of microbiology, fish and related products are a risk foodstuff group. Particularly Clostridium botulinum type E and Vibrio parahaemolyticus rank among pathogenic bacteria associated with fish. (**Whipple and Rohovec, 1994**).

Other potentially pathogenic bacteria associated with fish and shellfish include C. perfringens, Staph. spp., Salm. spp., Shigella spp., V. cholerae and other vibrios. Outbreaks usually occur due to the ingestion of insufficiently heat-treated fish or products contaminated after/during their processing Freezing fish and related products in the seawater, intensive handling, long-time transport or cooking in fishing containers straight on the deck contributes to their contamination with microorganisms. (**Whipple and Rohovec, 1994**).

2.6.2 Escherichia coli:-

E. coli is a classic example of enteric bacteria causing gastroenteritis. E. coli including other coliforms and bacteria as Staphylococcus spp. and sometimes enterococci are commonly used as indices of hazardous conditions during processing of fish. Such organisms should not be present on fresh-caught fish (**Chattopadhyay, 2000).** The contamination of food of fish origin with pathogenic E. coli probably occurs during handling of fish and during the production process **(Ayulo *et al.*, 1994;**). An outbreak of diarrhoeal illness caused by ingestion of food contaminated with enterotoxigenic E. coli was described in Japan (**Mitsuda *et al.*, 1998**). The illness was strongly associated with eating tuna paste.

Brazilian authors isolated 18 enterotoxigenic strains of E. coli (ETEC) from 3 of 24 samples of fresh fish originating from Brazilian markets; 13 of them produced a thermolabile enterotoxin. The authors explained the presence

of toxic strains of E. coli in samples collected from fish (not from water) from one fish market by a longer survival of bacteria on an adequate substrate, i.e. inside the living organism. The isolation of 317 E. coli isolates tested for thermostabile (ST) and thermolabile (LT) toxins has been described in another Brazilian study (**Ayulo *et al.*, 1994).**

Only one produced ST and none produced LT toxin. Infection with verocytotoxin-producing strains of E. coli (VTEC) after ingestion of fish was recorded in Belgium **(Pierard *et al.*, 1999).** An outbreak caused by salted salmon roe contaminated, probably during the production process, with enterohaemorrhagic E. coli (EHEC) O157 occurred in Japan in 1998 **(Pierard *et al.*, 1999).**

The roe was stored frozen for 9 months but it appears that O157 could survive freezing and a high concentration of NaCl and retained its pathogenicity for humans (**Semanchek and Golden, 1998)**

2.6.3 Salmonellosis:-

Fish and shellfish appear to be passive carriers of salmonella, demonstrate no clinical disease and can excrete Salmonella spp. without apparent trouble. The contamination of this organism derives from terrestrial sources and fish may serve as a vector for *Salmonella spp*. **(Metz 1980, Minette 1986, Chattopadhyay 2000).**

An outbreak of Salmonella. blockley infections following smoked eel consumption was described in Germany. The consumed eel came from four different local smokehouses, but could be traced back to fish farms in Italy **(Fell, *et al* 2000).**

This outbreak indicates that eel may be a vector of Salmonella spp. infection and that the smoking process may not eliminate bacterial contamination from raw fish. Salm. enterica serotype Paratyphi B var. Java phage type Dundee was isolated from the stool of a 14-month old boy who suffered from diarrhoea, vomiting, and fever for two days **(Senanayake, *et al* 2004)**.

The same isolate was identified from the water of home fish tank fish was the vector of Salmonella spp. in this case. Unusual Salmonella spp. serotypes were found in eight of 100 tropical aquariums sampled in Wales. In a Canadian outbreak of Salmonella enterica serotype ParatyphiB linked to aquariums, five of seven cases were in children aged less than 10 years **(Gaulin *et al* 2002).**

(**Samia, *et al* 2014)** studied contaminant bacteria on *Orechromis sp Clarias sp* at Elmourda fish market . They found that the results of the bacterial count in fresh fish in Tilapia sp Cat fish sp**.** is $4.69\times10^{5}\pm1.35\times^{5}_{10}$ and $3.69\times^{5}_{10}\pm0.89\times^{5}_{10}$. In addition, the result indicates that Salmonella and E.coli were isolated as contaminant bacteria, while Staphylococcus was not isolated from both fresh Tilapia sp Cat fish sp.

(Arafat,2013) studied of Microbiological assessment of three types of fresh fish (Tilapia sp Cat fish sp and Bagrus sp) they found results . Total Viable counts of bacteria and Salmonella in fishes Skin were ranged from 2.8 $x\ ^{3}_{10}$ to 9.8 $x\ ^{4}_{10}$ cfu/g and 0.0 to 7.2 x^{2}_{10} cfu/g, respectively.

(Awatif Mohamed,2012) studied in Assessment of fish quality based on sensory evaluation methods of *Orechromis sp*, *Bagrus sp*, *Clarias sp* the result found that the mean skin of fish at respectively (2.5±8 , 3±.9 , 4±.91) and outer slime (2.6±.7 , 3.5±8, 4.6±9) and eyes (2.8±.53 , 3.3±.66 ,

4.5±.79) and gill color (2.68±.76 , 3±.77 , 4±.8) peritoneum (2.78±.16 , 3.18±.91 , 4.14±.93) and gill odour (2.18±.78 , 3.83±1 , 4.85±.78).

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER THREE
MATERIALS AND METHODS

f3.1 The study area:-

This study was conducted at Sudan University of Sciences and Technology college of Animal production science and technology department of fishers and wild life science through November 2016.

3.2 Fish sampling:-

165sample were collected from almawrada fish market to evaluate the fish quality and level of freshness of the fish species *Orechromis sp*, *Bagrus sp*, *Clarias sp*.

These sample were drawn from 10 ton inspected and rated according to European scheme. and total of 30 swabs samples were obtained from skin and gill 10 sample from each species

Preserved in ice and transferred to microbiology laboratory.

Table (1) Show the European scheme used in sensory evaluation for studied fish sp in almawrada fish market.

Grade	EXTRA	A	B	C(unfit)
Skin	Bright, shining iridescent, or opalescent , no bleaching	Waxy ,slight loss of bloom, very slight bleaching	Dull, some bleaching	Dull, gritty marked bleaching and shrinkage
Outer Slime	Transparent or water white	Milky	Yellowish gray some clotting	Yellow-brown, Very clotted and thick

Eyes	Convex black pupil, transient cornea	Plane, slightly, opaque, pupils, slightly opalescent cornea	Slightly concave, drey putil, opaqye cornea	Completel y sunken, grey pupil, opaque discolored cornea
Gill	Bright red mucus, translucent	Pink, mucus slightly opaque E	Grey bleached, mucus opaque and thick	Brown bleaching mucus yellow grey and clotted
Peritoneum	Glossy, brilliant, difficult to tear from flesh	Slightly dull, difficulty to tear from flesh	Gritty, fairly easy to rear from flesh	Gritty, easily torn from flesh
Gill and internal odors	Fresh	No oudour neutral oudour trac, of mausy etc	Definite musty etc brady, maly	Acetic, fruitg amines, sulphide faecal

3.3 microbial analysis:-

3.3.1 Materials:-

Flask,test tub ,swab ,distle water ,petry dish, cotton, loops, tips, outoclave Oven ,incubation ,injection, sensitive balance ,pepatte , broth agar ,nutrient agar, DCA agar ,EMB agar.

3.3.1.1 Preparation of Broth agar:-

2.6 grams of broth agar extract to dissolve in 250 ml distilled water. Then it was sterilized by autoclaving at 1 hour still cool .

3.3.1.2 Preparation of Nutrient Agar:-

7grams of nutrient agar extract to dissolve in 250 ml distilled water. Then it was sterilized by autoclaving at 1 hour still cool .

3.3.1.3 Preparation of DCLS Agar: Deoxycholate Cholate Citrate Lactose Sucrose:-

7grams of DCLS Agar extract to dissolve in 200ml distilled water. Then it was sterilized by autoclaving at 1 hour still cool .

3.3.1.4 Preparation of EMB Agar (Eosin Methylene Blue Agar):-

3.5grams of EMB Agar extract to dissolve in 100ml distilled water. Then it was sterilized by autoclaving at 1 hour still cool .

3.3.2 Preparation of the sample:-

5 ml broth agar was added for each swap from the 30 swaps and inoculated for at 37C° for18 hour (all night).

3.3.3 Preparation of serial dilutions:-

Separate sterile pipettes were used, decimal dilution of 10-2, 10-3, 10-4, 10-5 and others were prepared, and sample was homogenized by transferring 1ml of previous dilutions to 9ml of diluents. Samples foam avoided, all dilution were shacked 25 times within 7seconds. 1ml of each dilution was pipeted into separate duplicate, appropriately marked Petri dishes. Two plates were inoculated per dilution 15-20 ml plate count agars were added (after cooled to 45ºc ±1) to each plate within 15 min. of original dilution (**AOAC, 1980**)

3.3.4 Total viable count (TVC):-

The test was done according to (**Gaulin *et al.*,2002**). Immediately sample dilutions and agar medium were mixed thoroughly and uniformly by alternate rotation and back and forth motion of plates on flat level surface. The poured agar let to solidify; the solidified Petri dishes were inverted and incubated promptly for 48 hrs at 37 ºc. Thirty to three hundreds colonies were counted. The total colony count per milliliter was calculated by multiplication of the number of colonies counted by dilution level.

3.3.5 Salmonella isolated

had taken 1ml from sample by micro pipette , then added to surface of Petri dish contain D.C.A agar incubated overnight at 37 °C for 24 hours *Salmonella* Show colonies pale yellow color Then the colonies counted .

3.3.6 Escherichia coli isolated

had taken 1ml from sample by micro pipette , then added to surface of Petri dish contain EMB Agar incubated overnight at 37 °C for 24 hours *Escherichia coli* Show colonies green color Then the colonies counted**.**

CHAPTER FOUR

RESULTS

CHAPTER FOUR
RESULTS

4.1 sensory results:-

The results obtained in table(2) showed that there is a highly significant difference between the three types of fish *Orechromis sp*, *Bagrus sp Clarias sp*,.

the *Clarias sp* is best quality from where **(**Skin **,** Outer Slime **,** Eyes **,** , Gill Color, Peritoneum, Gill odour) and then followed by **a** *Bagrus sp* and then *Orechromis sp*.

Table (2) shows sensory evaluation of **(***Orechromis sp*, *Bagrus sp*, *Clarias sp*) From Almawrada Fish market to using European scheme.

Fish type	No.s	Skin M±SD	Outer Slime M±SD	Eyes M±SD	Gill Color M±SD	Peritoneum M±SD	Gill Odour M±SD
Orechromis sp	*55*	$1.5\pm.7^{c}$	$1.5\pm.8^{c}$	$1.8\pm.8^{c}$	$1\pm.7^{c}$	$1.7\pm.1^{c}$	$1.1\pm.7^{c}$
Bagrus sp	55	$1.8\pm.9^{b}$	2.2 ± 1^{b}	$2.3\pm.9^{b}$	$2\pm.7^{b}$	$2.1\pm.9^{b}$	2 ± 1^{b}
Clarias sp	55	$2.9\pm.9^{a}$	2.9 ± 1^{a}	$2.9\pm.9^{a}$	$3\pm.7^{a}$	$2.9\pm.9^{a}$	$2.9\pm.7^{a}$
Siginficant		**	**	**	**	**	**

M=mean

SD=stander deviation

**= highly significant difference

a,b,c, =in the same column bearing the differen superscripts are significantly different (p>0.01**)** **.**

4.2 microbical result:-

The result obtained revealed the bacterial load in fresh fish *Orechromis sp*, *Bagrus sp*, *Clarias sp* respectively is ($5.9 \times 10^5 \pm .18 \times 10^5$ and $4.05 \times 10^5 \pm .31 \times 10^5$ and $4 \times 10^5 \pm .47 \times 10^5$) c.f. u/g. the result analyzed show the that there was no significant difference in total bacterial load ($p<0.01$).

Table (3)shows microbical load of *Orechromis sp*, *Bagrus sp*, *Clarias sp* from almawrada fish market.

Fish Type sp	**NO.S**	**M±SD**
Orechromis sp	10	$5.9 \times 10^5 \pm .18 \times 10^5$c.f.u./g
Bagrus sp	10	$4.05 \times 10^5 \pm .31 \times 10^5$c.f.u./g
Clarias sp	10	$4 \times 10^5 \pm .47 \times 10^5$c.f.u./g
Siginficant		NS

NS= no significant difference($p<0.01$)

Table (3) shows the pathological bacteria *Ecoli* isolated from almawrada fish market studied fish spp *Orechromis sp*, *Bagrus sp*, *Clarias sp* .

the result analyzed show the that there was no significant difference in specis.

Fish Types sp	NO.S	Ve+	Ve-
Orechromissp	10	3	7
Bagrus sp	10	5	5
Clarias sp	10	5	5
Siginficant		NS	NS

NS= no significant difference($p<0.01$)

Table (4) shows the pathological bacteria *Salmonilla* isolated from studied fish spp *Orechromis sp*, *Bagrus sp*, *Clarias sp* at almawrada fish market.

the result analyzed show the that there was no significant difference amoung the species.

Fish Type	NO.S	Ve+	Ve-
Orechromis sp,	10	4	6
Bagrus sp	10	4	6
Clarias sp	10	3	7
Siginficant		Ns	Ns

NS= no significant difference($p<0.01$)

CHAPTER FIVE

DICUSSION

CHAPTER FIVE
DICUSSION

The sensory evaluation is the most important method today for freshness evaluation in the fish sector (**Huss, 1995)**.

EU scheme has been used by many research laboratories and in now being implemented in the fish industry . the main advantage is that it is specific for each species and the fluctuation between assessors is diminisished. (**Huss, 1995)**.

This result is agree with(**Awatif Mohamed,2012)** the result found that there was highly significant difference in sensory evaluation between fresh fish *Orechromis sp, Bagrus sp, Clarias sp* the mean skin of fish at respectively (2.5±8 , 3±.9 , 4±.91) and outer slime (2.6±.7 , 3.5±8, 4.6±9) and eyes (2.8±.53 , 3.3±.66 , 4.5±.79) and gill color (2.68±.76 , 3±.77 , 4±.8) peritoneum (2.78±.16 , 3.18±.91 , 4.14±.93) and gill odour (2.18±.78 , 3.83±1 , 4.85±.78).

Microbiological tests is to [illegible] and characterize the micro-organisms most important in fish and fishery products by looking at the factors that affect their growth and survival and where they are mostly likely found in the processing plant(**Mitsuda *et al.*, 1998**).

Bacterial growth is the main cause of fish spoilage; therefore it is logical to use bacterial number as an index of fish quality.in this study, the total number of bacterial load for fresh fish *Orechromis sp, Bagrus sp, Clarias sp* respectively $5.9\times10^5\pm.18\times10^5$c.f.u./g , $4.05\times10^5\pm.31\times10^5$c.f.u./g, $4\times10^5\pm.47\times10^5$c.f.u./g**).** and this number was in the accepted limit mentioned by SSMO (Sudanese Standards and Metrology Organization, SDS357) which was 5×10^5 to 5×10^6 cfu\g for fresh fish products. In addition, this number

was in the normal range stated by **(Liston ,1980)** which was 10^2 to 10^7 cfu\g of fish meat. This is accepted limit compared to (**Anon 1991**) who said that the total mesospheric aerobic bacterial counts over 10^6 .cfu/g was regarded as accepted limit for sea foods.

(Shewan 1977) reported that the bacterial flora on freshly caught fish depends on environment rather than fish species, and this reflects the wide range of bacterial count. Also fish spoil at very different rates, and differences in surface properties of fish have been proposed to explain this. Skins of fish have very different textures. Thus, Tilapia sp may have a very fragile integument spoil rapidly compared to Bagrus sp and Cat fish that has a very robust dermis and epidermis.Furthermore, the latter group has a very thick slime layer, which includes several antibacterial components, such as antibodies, complement and bacteriolytic enzymes. This finding may coincides to some extent with result of **(Hielmland and Christie, 1983**) who claimed that Although, very wide variations occur, tropical fish species often have prolonged shelf lives when stored in ice when comparisons are made, data on fatty fish like herring and mackerel.

The result indicates tha salmonella and E.coli were isolated as contaminant Pathogenic bacteria. Bacteria associated with fish and fishery product can be categorized into two general groups: (1) bacteria (indigenous bacteria) that belong to the natural microflora of fish (Clostridium botulinum, pathogenic Vibrio spp., Aeromonas hydrophila); (2) enteric bacteria (no indigenous bacteria) that are present due to fecal contamination (Salmonella spp., Shigella spp., pathogenic Escherichia coli, Staphylococcus aureus) (**FDA, 2010**). In polluted waters, high numbers of Enterobacteriaceae may be found. In clean temperate waters, these organisms disappear rapidly, but it has been shown that Escherichia coli and Salmonella can survive for very long periods in tropical waters and once introduced may almost become indigenous

to the environment (**Fujioka *et al.*, 1988**). Each area including amount and type of different available nutrients, pH and nature of adhesion factors for each bacterial groups in the epithelial cells. Control of enteropathogenic E.coli and other food borne pathogens such as Salmonella and Staphylococcus aureus could be achieved. Precaution should include adequate cooking and avoidance of recontamination of cooked meat by contaminated equipment, water or infected food handlers.

This result is agree with (**Samia, *et al* 2014)** studied contaminant bacteria on *Orechromis sp Clarias sp* at Elmourda fish market . They found that the results of the bacterial count in fresh fish in Tilapia sp Cat fish sp**.** is $4.69\times10^{5}\pm1.35\times^{5}_{10}$ and $3.69\times^{5}_{10}\pm0.89\times^{5}_{10}$. In addition, the result indicates that Salmonella and E.coli were isolated as contaminant bacteria, while Staphylococcus was not isolated from both fresh Tilapia sp Cat fish sp.

CONCLUSION AND RECOMMENDATIONS

CONCLUSION AND RECOMMENDATIONS

- **Conclusion:**

Sensory evaluation and microbial is very important for the evaluation of the fish freshness as consumer became more concerned about quality of fish and food .this study reached to the following conclusion and recommendation:-

The quality of the fish At Almawrada fish market is different responding of the way of preservation ,transportion ,and handling. The results show that the *Calris sp* the best quality among the species that have been inspected.and total bacteria load of *Orechromis sp, Bagrus sp, Clarias sp*.

the result analyzed shows the that there was no significant difference .

and indicates tha *salmonella* and *E.coli* were isolated as contaminant bacteria.

- **Recommendation:**

I recommendation the following point -

- Fisheries authority should conduct quality inspection to ensure the fish quality of landing is safe and good human consumption.
- Landing site almawrade market should be clear, and complying with the principle of food hygiene and safety.
- Fishermen, handling processing should be trained on the practice ,of fish handling processing and distribuation .
- Adequate facility including water ,ice , and utensils should be proceeded at the landing site.
- Fish waste and offal should be properly managed and disposed on aregulae basis in order to avoid contamination.
- processing area should be separated for the receiving area.

- Will More study will needed to determine microbial load on other fish species and determine pathological bacteria in fish market.

CHAPTER SIX

REFERENCES

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REFERENCES

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Appendixes

Appendix (1):

Sensory evaluation of *Orechromis sp* gill in almawrada market

Appendixes (2) :

Swap sample from studied spp in almwrada fish market

Appendixes (3) :

swap sample from skin spp studied im almwrada fish market