Sudan University on Science and Technology

College of Animal Production Science and Technology

Department of Fisheries Science and Wildlife

Microbial load and Proximate Composition of Fresh Nile Tilapia (*O .niloticus*) Collected from Different Market at Khartoum State.

A Thesis Submitted in Partial Fulfillment of the Requirement of the B.Sc. Degree in Fisheries and Wildlife Science (Honor).

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Quran

ح ْر َ ل ِت َأ ْك ُل ُوا م ِن ْه ُ ل َح ْم ً ا ط َر ِي ًّا و َ ت َس ْ ت َخ ْر ِج ُ وا م َ ِة ً ن ْت َه َل ْب َح ِس ل ُي ْو َن َه ا َل ْف ُو ل ْك َ ت َر م َ ْى و َ ا ْه ِ و َ ل ْف ِت َي ْب َ ت َغ ُوا
م ِن ْ ف َض ْ ل ِه ِ و َ ل َع َ ل َّك ُم ْ ت َش ْ ك ُر ُ ون َ

)الاية 14(سورة النحل

DEDICATION

To our Fathers for their encouragements.

To our Mothers for their continuous encouragement and blessing and to whom we are always indebted

To ours brothers, sisters, relatives and friends who support us.

To all whom we love…

ACKNOWLEDGMENT

Our greatest thanks to Allaha Almighty, the most merciful who gave us the health, strength and patience to conduct this study.

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Finally our thanks go to our colleagues, relatives and

friends for their encouragement

Abstract

This study was designed to investigate the proximate composition and the bacteria load on the gills, skin and intestines associated with fresh Nile tilapia (*Oreochromis niloticus*) wear collected from different markets of Khartoum State during summer season,. Fish samples were collected from EL-Mawrada and EI Markzy market. A total of10 *Oreochromis niloticus* samples (20 to 235 cm) were collecting between March to May 2016.

The result shows that the bacterial load varial from the two markets fish analyzed the skin, gills and intestine. . The bacterial load in all sample was high (Gills, 7.5×10^4, skin 6.2×10^4 and intestines 5.0×10^4) in fish sample collected from El Mawrada fish market respectively, and the bacterial load in all sample was high (Gills $5.6\times10^{4,}$ skin 6.4×10^4 and intestine 4.7×10^4) in samples collected from El-Markazy market. The obtained results were analyzed statistically using ANOVA one way variance. The test was used to evaluate the mean differences among different treatments at the 0.05 significance level using SPSS (17).

الخلاصة

هدفت هذه الدراسة للتعرف على التركيب الكيميائي والتعداد الكلي للبكتيريا على الخياشيم والجلد والأمعاء في أسماك البلطي النيلي التي تم جمعها من مختلف الأسواق في ولاية الخرطوم في من سوق الموردة بام موسم الصيف خلال مارس - مايو عام 2016. .تم جمع عينات من الأسماك الخرطوم تم جمع عدد 20 عينة من أسماك البلطي النيلي متوسط طولها .درمان والسوق المركزي اوضحت النتائج المتحصل عليها أن التعداد الكلي للبكتريا يختلف بين الأسماك .بين 20-235 سم كان التعداد الكلي للبكتيريا في اعينات التي تم جمعها من سوق الموردة التي تم فحصها من السوقين. في (الخياشيم، 7.5 × 104، والجلد 6.2 × 104 والأمعاء 5.0 × 104) على التوالي، وكان العدد والجلد 6.4 × 104 و الأمعاء 4.7 × 104) في العينات 104 × الكلي للبكتيريا في (الخياشيم 5.6 وقد تم تحليل النتائج إحصائيا باستخدام تحليل التباين التي تم جمعها من السوق المركزي. تم استخدام اختبار اقل فرق معنوي لمعرفة الفروقات بين المتوسطات عند مستوى .(ANOVA) 0.05) باستخدام . SPSS (17).

Table of Content

List of Table

List of Figure

CHAPTER ONE

INTRODUCTION

Fish are one of the main foods for humans for many countries and still constitute an important part of the diet in many countries (**Leisner *et al*., 1995**). In Africa, the short supplies of animal protein together with the increasing human population have raised the cost of animal protein to a level almost beyond the reach of the low income group (**Ezeri *et al*., 2001**). As a result, there is a considerable increase in the demand for fish being the cheapest source of animal protein. (**Ladipo *et al*., 1981**).

The advantages of fish as a food are its digestibility and high nutritional value (**Leisner *et al*., 1995**). These important attributes makes the commodity readily susceptible to microbial attack particularly bacteria (**Adams *et al*., 1999**).

Fish flesh naturally contains very low levels of carbohydrates and these are further depleted during the death struggle of the fish (**Adams et al., 1999**). This has two important consequences for spoilage. Firstly, it limits degree of post mortem acidification of the tissue so that the ultimate pH of the muscles is 6.2-6.5 (**Adams *et al.,* 1999**.

Secondly, the absence of carbohydrate means that bacteria present on the fish will immediately resort to use the soluble pool of readily assimilated nitrogenous material, producing off-odour. (**Adasms *et al.,* 1999**) fin fish such as Tilapia have a particular large pool of nitrogenous extractives and are even more prone to raid spoilage, a factor which accounts for the common practice of keeping them alive until immediately prior to consumption (**Adams *et al*., 1999**). The speed with which a product spoils is also related to the initial microbial load on the product: the higher the count, the sooner spoilage occurs (**Adams *et al*., 1999**).

The fresh water or rivers and lakes have a complex flora of microorganisms which include genuinely aquatic species as well as component introduced from terrestrial, animal and plant sources. (**Adams *et al*., 1999**). The scale of a human activity has had a detrimental effect on coastal waters. Many fin fishes used for food out particles from large volume of waters. If these waters have been contaminated with sewage, there is always the risk that enteric organisms from infected individuals may be present and will be concentrated by the filter feeding activities of shell fish (**Adams *et al.*, 1999**). Also during handling of the commodity, the natural flora of the environment may be contaminated with organisms associated with man such as members of the *enterobacteriaceae* and *Staphylococcus aureus* which can grow well at 30-37oc (**Miceal *et al.*, 2007**).

By monitoring the bacteria contents of fish organs, the quality of fish can be measured since these will affect the storage life and quality of the fishery products.

Objectives:

1. To investigating the bacteria load on the gills, skin and intestines associated with fresh fish (Nile tilapia)
2. To determine proximate composition of Nile tilapia (*Oreochromis niloticus*) fish collected from different market in Khartoum State within the summer season.

CHPTER TWO
LITERATURE SURVEY

2.1. Bacterial load on fish

Fish are known for their high nutritional quality, relatively low in fat, saturated fat, cholesterol and high in poly-saturated fatty acids, protein and minerals such as calcium, phosphorus, sodium, potassium and magnesium (**Salihu et al ., 2012**). Millions of people in the world today depend on fish for protein. Fish contributes about 60% of the world supply of protein and 60% of the developing world derives more than 30% of their animal protein from fish (**FAO, 1994**).

In sub-Saharan Africa, fish accounts for 10% of the animal protein consumed and 98% of this is fin-fish (**Imam et al., 2010**). In Africa, fish is widely consumed as a remarkable source of animal protein. Thus, the average per capita world of fish in Africa in 1992 was about 8 kg having increased from an average of 7 kg per annum, from 1969 – 1974 (**Ahmed, 199**7). In Nigeria, the short supplies of animal protein together with the increasing human population have raised the cost of animal protein to a level almost beyond the reach of the low income group (**Ezeri, 2001).** The resultant effect is a considerable increase in the demand for fish as an alternative, source of animal protein in the face of the ever increasing population. Fish is more fancied and widely consumed than meat in the Niger Delta area of Nigeria. In Mali, **Breuil and Quensiere (1995)** stated that the fish consumption in the area was about 10.5kg/person/year compared to 10kg/person/year elsewhere in Africa.

Aquatic environments are easily polluted by both wastes from homes, farmlands and industries. This endangers the life of aquatic biota such as fish since fish take in a large quantity of bacteria into their alimentary tract from water and food. However, Infection due to microbial contamination does not according to **Salihu et al . (2012)** result in disease; environmental stresses may upset the balance between the potential pathogens and their hosts.

Fish are usually infected with a wide range of microbes in aquatic ecosystems. The types of micro-organisms found associated with fish depend on the aquatic habitats of fish and are known to be affected by certain factors like the saltiness level and bacterial load of the habitat (**Salihu et al., 2012**).

Bacteria often occur in parts of fish such as scales, gills, gut and alimentary tract. The bacteria present on the body or internal organs of fish indicate the extent of pollution of aquatic ecosystems.

In faecal polluted water, Aeromonas sp., Coliform, Shigella flexineri, Salmonella sp. etc are common bacteria which can penetrate the body of fish through different routes including wounds, natural openings, ingestion and engulfment with food water. **Rodricks (1991)** classified bacterial pathogens into non-indigenous and indigenous pathogens.

The indigenous pathogens include Closteridium botulinum, Listeria monocytogenes, Staphylococcus aureus, Salmonella species, Escherichia coli and others (**Salihu et al ., 2012**).

However, non-indigenous pathogens which are not harmful to fish may on consumption be harmful to man. Oreochromis niloticus, Clarias gariepinus and Synodontis budgetti are highly cherished for their tasty and social significance, particularly among the Igbo people of Nigeria. They are also used in educational institutions because of their readily availability.

Bacteriological quality is of importance to public health since it directly relate to fish spoilage and may cause food poisoning. It is therefore important to monitor the quality of harvested freshwater fish from Oguta Lake and Agulu Lake to ensure that the fishery products does not pose health risks to end users and that the level of potential organisms is within the limits of acceptable product shelf-life. By monitoring the bacteria content of fish organs, the quality of fish can be measured since these will affect the spoilage life and quality of fishery products.

Fish had long been regarded as a desirable and nutritional source of high quality protein and generous supply of minerals and vitamins constituting the major part of human diet. (**Hastein et al., 2006**).

Bacterial diseases in fish are a serious threat to aquaculture systems that cause severe damage and mortality in Egypt (**Noor El-Deen et al., 2010**). Enterobacteriaceae in fish are considered as an indicator to sewage pollution and has been reported as opportunistic pathogen in fish (**Rajasekaran, 2008**). The pathogenic strains of Enterobacteriaceae may cause diarrhea in fish (**Shender et al., 2009**).

Enterobacteriaceae are widely distributed in nature and found in feces of human, poultry and animals (**Wogu and Maduakol, 2010**). Enterobacteriaceae are a common water-borne bacterium, which may be present in the tissues of apparently normal fish (**Newaj et al., 2008**) .Whenever fish are exposed to environmental stress, or injury, it causes serious outbreaks of disease with mortalities. Environmental stresses such as high temperature, poor water quality and high organic content primarily contribute to the onset and severity of Enterobacteriaceae infections in fish (**Sekar et al., 2008).**

Some human pathogens such as, *Escherichia, Klebsiella and Salmonella spp.* have been found to survive and multiply in the gut, mucus and tissues of fish and that render fish acting as potential vector of human disease over long periods (**Onyango et al., 2009**).

The particular isolation of some most pathogenic organisms such as *Salmonella spp., E. coli* and potential pathogenic organisms *as Klebsiella spp., Citrobacter spp. and Proteus spp.*, which when isolated from fish and fish products gives an indication about environmental fecal pollution of fish (**Wogu. and Maduakol, 2010**)..

Thampuran et al. (2005) reported that the microbial quality of the tilapia indicated that all tissue samples except muscle tissues were contaminated with fecal coliform were *Escherichia coli* is the most common contaminant and is often encountered in high numbers. The presence of *E. coli* as well as verotoxigenic *E. coli* O157:H7 in fish meal was investigated by (**Ristori et al., 2007**).

Ristori et al. (2007) isolated *Aeromonas spp., Plesiomonas shigelloides, Vibrio cholerae* 01, *Vibrio parahaemolyticus, and Vibrio vulnificus* from different organs of fishes. It was found that the hygienic quality and freshness of fish and shellfish decreased in summer, especially for clam and mussel (**Hwang et al., 2004**).

Bacterial microbiota associated with fresh raw shrimp was *Aeromonas, Pseudomonas, Vibrio, Flavobacterium* and *Serratia* (**Jeyasekaran et al., 2006**).

Hood et al. (1983) found that fecal coliform levels were above the recommended wholesale level suggested by the National Shellfish Sanitation Program (less than or equal to 230/100 g). **El Hadi et al. (2004)** detected the presence of eight potentially pathogenic *Vibrio* species, with overall

incidence in the samples as 4.6% for *V.cholerae*, 4.7% for *V. parahaemolyticus*, 6.0% for *V.vulnificus*, 11% for *Vibrio alginolyticus*, 9.9% for *Vibrio metschnikovii*, 1.3% for *Vibrio mimicus*, 13% for *Vibrio damsela*, 7.6% for *Vibrio fluvialis*, and 52% for a combined population of all of the above.

Heinitz et al. (2000) found that 10% of imported and 2.8% of domestic raw seafood was positive for *Salmonella. Enterococcus sp* and *Aeromonas sp*, fecal and total coliform, the presence of *Listeria sp* and *Salmonella spp* from the external surface of tilapias were shown by **Morales et al. (2004).**

Håstein et al. (2006) outlined and discussed the hazards and challenges associated with handling fish during farming and capture and the environmental contaminants in seafood that may pose a risk to human health.

Fish has become an increasingly important source of protein and other element necessary for the maintenance of and healthy body. Many species of fish (Nile tilapia) normally live in fresh water lakes and rivers. The term or word "Fish" are generally defined as aquatic vertebrate that are typically cold blooded covered with scales and equipped with two sets of paired fins and several unpaired fins that use gills to obtain oxygen from humbler of skeletal called Fin-Rays, put together and thus various kinds of fish greatly in shape sizes and colour (**Ayres, 1995**).

Fish is one of the most highly perishable food products, during handling and storage, quality deterioration of fresh fish rapidly occurs and limits the shelf life of the product, Marketing of Fish in Africa is mostly carried out by local fish sellers at ambient temperature, therefore knowledge of spoilage patterns of tropical fishes and their shelf life under ambient conditions is very important (**Okoro et al., 2010**).

The fresh water or rivers and lakes have a complex flora of microorganisms which include genuinely aquatic species as well as component introduced from terrestrial, animal and plant sources. (**Adams et al., 1999).** The scale of a human activity has had a detrimental effect on coastal waters. Many shell fishes used for food out particles from large volume of waters. If these waters have been contaminated with sewage, there is always the risk that enteric organisms from infected individuals may be present and will be concentrated by the filter feeding activities of shell fish **(Adams et al 1999**) .Also during handling of the commodity the natural flora of the environment may be contaminated with organisms associated with man such as members of the enterobacteriaceae and Staphylococcus aureus which can grow well at 30-37o c (**Miceal et al., 2007**.) By monitoring the bacteria contents of fish organs, the quality of fish can be measured since these will affect the storage life and quality of the fishery products (**Kaneko, 1971).**

The indigenous pathogens include *Closteridium botulinum, Listeria monocytogenes, Staphylococcus aureus*, Salmonella species, Escherichia coli and others (**Salihu et al., 2012).** However, non-indigenous pathogens which are not harmful to fish may on consumption be harmful to man.

Spoilage bacteria differ somewhat for freshwater and marine fish and for temperate and tropical water fish. Storage and processing conditions also affect microbial growth **(Doyle, 2007).**

Pseudomonas and *Shewanella* are the predominant species on chilled fresh fish under aerobic conditions (**Fitzgerald et al., 2003; Hozbor et al., 2006; Doyle, 2007).**

Contamination of fish from enteric bacteria of human or Bangladesh in 1974 from Thailand Because of their animal origin may also be

responsible for various food faster growth rates, tolerance to harsh environment and spoilages. Faecal coli form in fish demonstrates the Bangladesh as an alternative and additional species level of pollution of their environment because coli forms among farmed fishes are not the normal floras of bacteria in fish.

The enteric Most of the tilapia production in Bangladesh bacilli include *E. coli*, *Klebsiella* spp. *Citrobacte*r spp. comes from aquaculture which largely depends to a *Enterobacter* spp., *Serratia* spp. and *Edwarsiella* spp. greater extent on aquatic environment, Water However, bacterial density in Nile tilapia sampled quality is the main factor that determines the from pond, *gher* and market has never been reported. Degree of production. Contaminated water is not suitable Thus the present study was designed to investigate the for aquaculture. Contamination may result from rupturing occurrence of viable coli forms quantitatively in different fish intestine during poor processing or inadequate organs of Nile tilapia reared in ponds, *ghers* and waters of washing the same is the causative agent for food spoilage .

Refrigeration temperatures are also relevant because they are used by most households in Africa for temporary storage of fish (**Okoro et al., 2010**). Frozen state condition is also important since most fishes consumed in Africa are imported and they usually come in frozen state (**Okoro et al., 2010**).

Spoilage bacteria differ somewhat for freshwater and marine fish and for temperate and tropical water fish. Storage and processing conditions also affect microbial growth (**Doyle, 2007**). *Pseudomonas* and *Shewanella* are the predominant species on chilled fresh fish under aerobic conditions (**Hozbor et al., 2006**).

Packing under carbon dioxide and addition of low concentrations of sodium chloride favour growth of lactic acid bacteria and *Photobacterium phosphoreum* (**Doyle, 2007**). Heavily wet-salted fish support growth of yeasts while dried and salted fish are spoiled by moulds (**Doyle, 2007**).
Addition of organic acids selects for lactic acid bacteria and yeasts (**Lyhs et al., 2004**).

Pasteurization kills vegetative bacteria but spores of *Clostridium* and *Bacillus* survive and may grow, particularly in unsalted fish (**Gram and Dalgaard, 2002**).

Varieties of quality attributes have been used to assess fish freshness in many cold water fish species as sea bream, sea bass, sardine and European eel (**Liu et al., 2010**).

Many indices have been used for the assessment of fish quality during storage (**Sallam, 2007**). Such indices comprise changes in the microbial population, (**Sallam, 2007**), chemical changes (**Sallam, 2007**), as well as changes in sensory attributes (**Sallam, 2007**). However, few researches were reported on quality assessment for tropical freshwater fish species (**Liu et al., 2010**).

The effect of physical condition and chemical agents on the growth of microorganism in fish has been investigated and well documented and not much of information on the spoilage of fish. Some reports on the storage quality of frozen/chilled tilapia were still not comprehensive on spoilage mechanism and quality assessment (**Sil *et al*., 2008**). In the recent time, modern biotechnology have introduced new techniques that can detect early fish contamination, improve the taste, modify the quality of fish and prolong the shelf life and also impact disease resistance to the fish (**Okoro *et al*.,2010**).

The chief sources of fish contamination were water, soil and fish handlers ***(Youssef etal., 1981).***

Microbiological guidelines for cooked ready-to-eat fish products may be include APC, *E.coli* and *Staph aureus* evaluation, these parameters were useful to evaluate faulty processing and/or handling practices such as inadequate heating, raw materials, and workers which may create hazards ***(National Academy of Sciences, 1985)***. The most important bacteria causing food poisoning are Escherichia, Salmonella and Staphylococcus ***(Jay, 1992)***. Cooking fish at very high temperature for short time kills all the vegetative bacteria except those that from heat-resistant spores, however, when the conditions become suitable, the growth rate of germinating spores would be high ***(*Abd-El Rahman *et al., 2003).*** Center for disease control (DCC) and U.S. Department of agriculture stated that sea food is twenty five times likely than poultry meat to cause serious outbreak, and they added that 5000 cases were reported as fish food poisoning **(FAO, 2001)**. **Sarker *et al.,(1985)*** reported the seasonal distribution of *Vibrio parahaemolyticus* in fresh water environments in association with fresh water fish and the incidence of its presence in fresh water fish is related to their association with a biological host particularly fish, most of isolates of this organism occurred during summer months. Many species of Vibrio were incriminated in human illnesses ranging from gastroenteritis in healthy persons to septecimea in debilitated patients in the last 30 years.

2.2. Chemical composition of Nile fishes

The study of chemical composition of fish is an important aspect of fish flesh quality since it influences both keeping quality and the technological characteristics of the fish (Huss, 1988).

Data on chemical composition of many of the freshwater fishes of our country is not available and hence an attempt has been made to analyze as many as thirty-six species. The chemical composition of fish varies widely from species to species and season to season. There is also individual variation in the same species. Knowledge of chemical composition is essential in order to compare its value as food with other protein foods (Stansby, 1954**)** has elaborated on the importance of chemical analysis.

Various studies have examined the effects of temperature, light, salinity, pH and oxygen concentration on the proximate composition of fish but these factors would seem to have very limited effects. On the other hand, endogenous factors are genetic and linked to the life stage, age, size, sex and anatomical position in the fish (Huss, 1995).

The variations in the chemical composition of fish are closely related to the environment of rearing in ponds or nature and completely depend on feed intake. During periods of heavy feeding, at first the protein content of the muscle tissue will decrease very slightly and then the lipid content will show a marked and rapid increase. Fish will have starvation periods for natural and physiological reasons (Bendall, 1962).

The fish´s chemical composition can be affected by many factors, including species, environmental conditions, fish size, level of protein in the diet, and feeding rate (Ogata& Shearer, 2000).

Numerous studies on tilapia show that body composition approximates the diet composition, but little information has been produced by comparing the entire and the fillet composition of different genetic groups (Lugo et al., 2003).

Proximate composition of body muscles of *Puntius stigma* (male and female) analyzed shows that the moisture content was found to be higher in

female, while protein, fat, ash, carbohydrate and minerals contents were higher in male. Moreover, different sexes were observed to have varying chemical composition (Biro *et al*., 2009).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study area

This study was conducted at microbiology laboratory, collage of animal production science and Technology, Sudan University of Science and Technology.

3.2. Materials

Flask,test tub,swab,distle water,petry dish,cotton,loops,tips,outoclave Oven,incubation,injection,sensitive balance,pepatte

3.3. Collection of fish

Fish samples were collected from EL-Mawrada and central fish market. A total of10 samples of *Oreochromis niloticus* range between 20to 235 cm in length s. Conducted through March to May 2016.

Table (1) Samples size and sites

Sites	**Coordinates**	**Type & No. of fish**	**Organs & No. of samples tested**		
			Gill	**Skin**	**Intestine**
El-Mawrada Market	N: **15 14 .229** E: **032**	5 *O. niloticus*	5	5	5
EL. Markazy Market	N: **15 30907** E: **032 28752**	5 *O. niloticus*	5	5	5
Total No. of fish		**30**	10	10	10
			Total No. of samples tested 30		

3.4. Examination of fish

The samples were examined externally from skin and gills and internally from intestine for bacteriological examination.

3.5. Bacteriological examination:

Solid media were used for bacteriological investigation.

Broth agar: 1.3 grams of broth agar extract to dissolve in 100 ml distilled water. Then it was sterilized by autoclaving at 1 hour still cool.

3.6Bacteriological method:

3.5.1 **Preparation of the sample:** 5 ml broth agar was added for each swap from the 15 swaps and inoculated for at 37C° for 18 hour.

3.5.2Preparation 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).

3.5.3Total viable count (TVC):

The test was done according to Guinn *et al.,* (1999). 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±2 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.

3.6Proximate Composition Analysis:

Moisture content, crude protein, fat and ash were determined for wet sample according to standard methods of Association of Official Analytical Chemists **(AOAC) (1990**) as follows:

3.6.1 Moisture Content Determination:

The samples were first weight (Initial weight) then dried in an electric oven at 1050C for 24-30 hours to obtain a constant weight. The moisture content was calculated as follows:-

$$\textbf{Moisture content (\%)} = \frac{\text{Initial weight} - \text{Dry weight}}{\text{Initial weight}} \times 100$$

3.6.2 Crude Protein Determination:

The Kjeldal method for estimation of nitrogen was applied. Nitrogen content was converted to protein percentage by multiplying by 6.25 as follows:

$$\textbf{Protein \%} = \frac{(Va - Vb) \times N \times 14 \times 6.25}{1000 \times Wt} \times 100$$

Whereas:

Va = volume of HCL used in titration

Vb = volume of sodium hydroxide of known normality used in back titration

14 = conversion factor of ammonium sulfate to nitrogen

6.25= conversion factor of nitrogen to protein

Wt= weight of sample

N= normality of NaoH

3.6.3 Crude Fat Determination:

Fat content of each sample was determined according to Soxhlet method by ether extract using 2 gm of fish samples. Extraction continued for

5 hours at 100 0C before finding the weight of the extract fat. Fat percentage was then calculated as follows:

$$\textbf{Fat \%} = \frac{\text{Extracted fat weight}}{\text{Sample weight}} \times 100$$

3.6.4 Ash Content Determination:

Ash was determined by heating 1 gm at 5500C in muffle furnace until a constant weight was obtained. Ash content percentage was given by the following formula:

$$\textbf{Ash \%} = \frac{\text{Ash weight}}{\text{Sample weight}} \times 100$$

$$\text{N.F.E} = 100-(\text{A}+\text{C.F}+\text{M}+\text{C.P}+\text{E.E}+\text{D.M})$$

3.7. Statistical analysis:

The obtained results were analyzed statistically using ANOVA one way variance. The test was used to evaluate the mean differences among different treatments at the 0.05 significance level using SPSS (17).

CHAPTER FOUR

RESULTS

Table 1 showed the means of the chemical composition of *O.niloticus* collected from different fish market at Khartoum state.

From the results obtained table 1, 2 and 3 shows the result of the chemical composition of the studied fish and total viable count of bacterial isolates from the gills, skin and intestine of ten sampled tilapia fish.

The mean total viable count revealed 7.5 x10^4 cu/g from the gills 6.2 x 10^4 (cfu/g) from the skin and 5.0 x 10^4(cfu/g) from the intestine in O.niloticus collected from El-Mawrada fish market.

A range of total viable count from the three sites analyzed revealed 5.6 x 10^4 cfu/g from the gills, 6.4 x 10^4 (cfu/g) from the skin and 4.7 x 10^4 (cfu/g) from the intestine in *O.niloticus* collected from El-Markazy fish market. The mean count computed for each fish part sampled show that gill had the least count of 7.5 x 10^4 cfu/g in the fish sample collected from El-Mawrada fish market and skin had the highest count of 6.4x 10^4 cfu/g in fish sample collected from El-Markazy fish market-Table 3.

Table (1): Means illustrate approximate chemical omposition of *Oreochromis niloticus* collected from different fish market.

Parameters	EI-Markazy	EI-Mawrada
M	78·18±0.03	79.04±0.04
D.M	21.82±0.03	20.98±0.01
C.P	18.49±0.02	19.49±0.04
C.F	0.01±0.00	0.01±0.00
E.E	6.11±0.01	6.27±0.04
Ash	1.13±0.02	2.42±0.02
N.F.E	26.094±0.01	23.12±0.07

Whereas**:**

D.M = Dry matter,A=Ash, C.P = Crude protein, C.F=Crude fat, E.E = Esther extract, N.F.E = Nitrogen free extract. M=moistiore

Table (2): illustrate mean of total bacteria count different organs of *Oreochromis niloticus* collected from different samples.

Markets	**El-Mawrada market**			**Central fish market**		
Samples	Gill	Skin	Intestine	Gill	Skin	Intestine
1	6.4×10^4	8.7×10^4	5.7×10^4	6.5×10^4	8.4×10^4	7.4×10^4
2	1.12×10^5	6.3×10^4	3.6×10^4	4.6×10^4	8.4×10^4	4.0×10^4
3	5.1×10^4	5.9×10^4	6.4×10^4	3.7×10^4	3.5×10^4	7.2×10^4
4	4.4×10^4	3.7×10^4	3.0×10^4	8.1×10^4	4.9×10^4	1.3×10^4
5	1.06×10^5	6.5×10^4	6.6×10^4	5.4×10^4	7.0×10^4	3.7×10^4
P-value	*	*	*	*	*	*

There is significance at the level ($p<0.05$).

Table (3): illustrate mean of total bacteria count different organs of *Oreochromis niloticus* collected from different areas.

Parameters / **Markets**	**Gill**	**Skin**	**Intestine**
Mawrada market	7.5×10^4	6.2×10^4	5.0×10^4
El Markazy market	5.6×10^4	6.4×10^4	4.7×10^4
P – value	*	NS	*

There is significance at the level ($p<0.05$).

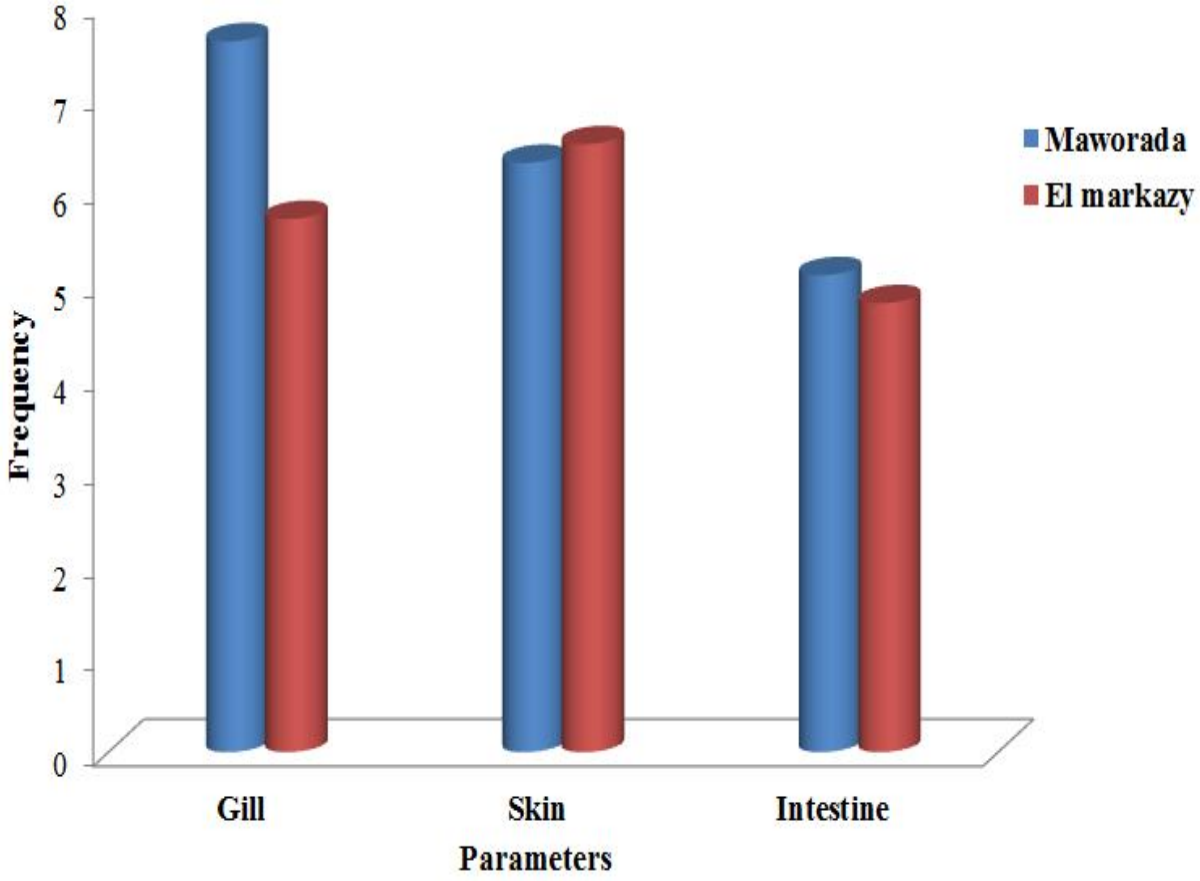

Figure (1): Illustrate mean of total bacterial count different organs of *Oreochromis niloticus* collected from different areas.

CHAPTER FIVE
DISCUSSION

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 count for fresh Tilapia (*Oreochromis niloticus*) was 8.4×10^5 cfu\g of fish meat, and this number was in the accepted limit mentioned by SSMO (Sudanese Standards and Metrology Organization, SDS 357) which was $5\times10^5 - 10^6$ cfu\gm for fresh fish products. Also this number was in the normal range stated by **Liston (1980)** which was $10^2 - 10^7$ cfu\g of fish meat.

Also **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.

The result from this research shows that the Bacterial load varies in the two markets of the fishes tasted in the skin, gills and intestine. The bacterial load in all sample was high (Gills, 7.5×10^4, skin 6.2×10^4 and intestines 5.0×10^4) in fish samples collected from El Mawrada fish market respectively, and the bacterial load in all sample was high (Gills 5.6×10^4, skin 6.4×10^4 and intestine 4.7×10^4) in samples collected from El-Markazy fish market and this may be attributed to the high ambient temperature in the river during summer season and this study was agreement with (**Hossain *et al*, 1999**) who reported that bacterial load in fish might increase with the increase of water temperature .This is accepted limit compared to **Anon (1991)** who said that the total mesophilic aerobic bacterial counts over 10^6 cfu\g are regarded as the acceptability limit for seafoods. The same limit is also accepted for freshwater fish (**Turantas, 2000).**

Also the result is in agreement with **Jeyasekaran *et al*., (2006**) who reported that the initial total bacterial count was found to be 10^5 cfu\g, when the fish were chilled with ice. The total viable bacterial count of refrigerated Tilapia (*O. nilticus)* for 4 and 7 days were 2.1×10^6 and 1.1×10^7 cfu\g of fish meat, respectively.

The intestine had the lowest bacterial load compared to the gill and skin this result was disagree with (**Ezeri *et al.*, 2001**) who stated that the number of bacteria associated with the gills are actively maintained at low level, there by implying that fish probably has mechanism which enables it to keep the bacteria number low, and therefore afford it some degree of protection against bacteria invasion by the gill microflora.

The result obtained from this study showed that there was no significant difference in proximate composition of Nile tilapia (O.niloticus**)** collected from the two markets at Khartoum state this results was agreement with (**Stansby, 1954)** who reported that the chemical composition of fish varies widely from species to species and season to season. There is also individual variation in the same species. Knowledge of chemical composition is essential in order to compare its value as food with other protein foods has elaborated on the importance of chemical analysis.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1. Conclusion

Recently, the demand of Tilapia fish (*Oreochromis niloticus)* consumption have increased continuously because, these fish are of low price but, high nutrition food. The mean total viable count revealed 7.5 x10^4 cu/g from the gills 6.2 x 10^4 (cfu/g) from the skin and 5.0 x 10^4(cfu/g) from the intestine in O.niloticus collected from El-Mawrada fish market and 5.6 x 10^4 cfu/g from the gills, 6.4 x 10^4 (cfu/g) from the skin and 4.7 x 10^4 (cfu/g) from the intestine in *O.niloticus* collected from El-Markazy fish market. Therefore, total bacterial counts results are below the standard value in TACFS 7001-2004.

6.2. Recommendation

Based on the findings during this study, the following recommendations are suggested:

1. More study was needed to determine microbial load on other fish species
2. To determine pathological bacteria in *O.niloticus*
3. To determine fungi in fresh water fish

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