Sudan University of Science and Technology

College of Graduate Studies and Scientific Research

Effects of steaming and chilling on the Quality and Chemical Composition of (*Oreochromis .niloticus*)Fish.

تاثير المعالجة بالبخار والتبريد علي الجودة والتركيب الكيميائي لأسماك البلطي النيلي

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الآية

قال تعالي :

(يا ايها الذين امنو اذا قيل لكم تفسحوا في المجالس فأفسحوا يفسح الله لكم وإذا قيل أنشزوا فانشزوا يرفع الله الذين أمنوا منكم والذين اوتوا العلم درجات والله بما تعملون خبير)

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Dedication

Dedication this study to the beloved of my Mother, to my soul Father, brother, sister, Husband, friends, To everyone believed in the value of science with respect and love

Sara

Acknowledgment

I blessed first to Allah for giving assistance, health patience and power to complete this work.

This work could not be accomplished without the guidance and direction of my supervisor Dr.Haram Hassan Abbas so all my appreciation and gratitude expressed to her.

I acknowledge with gratitude the workers of the microorganism laboratory and chemical laboratory Khartoum university for their assistance support .
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Abstract

This study was conducted in Department of Fisheries and Wildlife Science, (DFW)at Sudan University of science and technology. To identify the bacterial load and chemical composition and sensory evaluation of processed steamed and chilled (*O. niloticus*) fish.50 fish samples were collected from Hatchery of (DFW), processed by steaming and chilled for ten days; random samples were analyzed for microbial, chemical and sensory evaluation.

The results showed the bacterial loads of steamed chilled and row chilled fish werein the range of (3.3×10^{2} - $1.4\times10^{4)}$cfu/g, (5×10^{4} - 8.4×10^{3}) cfu/g respectively. There wassignificance different ($p>0.05$) between steamed chilled and row chilled fish at all storage time.

On the other hand *Total coliform, salmonella and Ecoli* were absent in all analyzed samples in different storage time.

The chemical composition (%) of steamed chilled fish were,moisture content (70.1-78), protein (26.3-28), fat (1.1-1.3) and ash(.2-1.4) while the row chilled fish were moisture (78-79),protein (21.2 -26.3),fat (1.3-1.6%) and ash (.2- 2.1%) .There was significance different ($p>0.05$). In chemical composition between steamed chilled and row chilled fish in different storage time.

Sensory evaluation of steamed chilled fish was (6.6 -8.4) for texture, (8.6-10) for odor and (7.4-9.6)for color while row chilled fish was (9.3-9.8) for texture, (9.2 -10) for odor and(8.6 -9.8)for color. There was significance different ($p>0.05$) in sensory evaluation in steamed chilled and rowchilled fish in different storage time.

Therefore, steaming and chilling is a new introduced method for fish preservation, and they are preventing the quality deterioration.

ملخص البحث

اجريت هذه الدراسة في قسم علوم الاسماك والحياة البرية بجامعة السودان للعلوم والتكنولوجيا بهدف التعرف علي الحمل البكتيري والتركيب الكيميائي والتقييم الحسي لاسماك البلطي المعالجة بالبخار والمبردة ، اجريت هذه الدراسة علي 50 سمكة بلطي تم جمعها من مفرخ قسم الاسماك والحياة البرية في جامعة السودان للعلوم والتكنولوجيا .
اظهرت النتائج ان الحمل بكتيري للاسماك الاسماك المعالجة بالبخارالمبردة و الاسماك الخام المبردهكان ($10^7\times$ 1.4 - $10^2\times$3.3) و($10^3\times$ 8.4 - $10^4\times$5) علي التوالي.كما اوضحت الدراسه وجود فروق معنويه في جميع فترات التخزين.

ومن ناحية اخري وجد ان بكتريا كوليفورم والسالمونيلا و الاشريشيا القولونيه غائبة في جميع العينات التي تم تحليلها. حلال فتره التخزين

كما اظهرت الدراسة نسب التركيب الكيميائي(%) للاسماك المعالجة بالبخارالمبردة كان كالاتي (70.1-78)رطوبة ،(26.3-28)بروتين ،(1.1-1.3%)دهون و(1.4%-2.) رماد.اما الاسماك الخام المبردة كانت كالاتي: (78-79)رطوبة ،(21.2 -26.3)بروتين ،(1.3- 1.6)دهون و(2.1 -2.) رماد.وكانت هنالك فروق معنوية ($p>0.05$) بين الاسماك المعالجة بالبخارالمبردة و الخام المبردة خلال جميع فترات التخزين.
التقييم الحسي للاسماك المعالجة بالبخاركان كالاتي -6.6) (8.4للملمس،(8.6-10) للقوام و (7.4-9.6)للون اما الاسماك الخام المبردة فقد كانت قيم التقييم الحسي كما يلي-9.3)(9.8 للملمس، (9.2 -10)للقوام و (8.6 -9.8)للون وقد اظهرت النتائج اختلافات معنوية ($p>0.05$) في التقييم الحسي للاسماك المعالجة بالبخار والمبردة المبردة خلال جميع فترات التخزين.
ولذلك وجد ان استخدام طريقه المعالجه بالبخار قبل التبريد هي وسيله جديده لحفظ الاسماك والوقايه من تدهور جودتها.

CHAPTER ONE

INTRODUCTION

Fish are one of the main sources for the provision of animal protein for a growing demand in a world of ever growing population and increasing consumption. In this respect, the Sudan is not exception and the various aquatic resources (marine,fresh water, brackish, groundwater and others) are tapped in order to fulfill the needs in this direction(**Ghada*et al*;2012).**

Fish is widely consumed in many parts of the world by humans because it has high protein content, low saturated fats and also content essential fatty acids known to support good health. Marine foods are very rich sources of minerals component **(Sikorski *et al.*, 1990).** The global contribution of fish as a source of protein is high, ranging from 10% to 15% of the human food basket across the world **(Wilson *et al.*, 2007).** Despite the fact that the nutritional value of fish is well known, it nevertheless plays only a limited role in the diet of many countries .There fore, it would seem appropriate to find new processing methods for this compared valuable raw material so as to increase consumer interest. Compared to mammalian meat, fish meat has more water and less connective tissue, which contains very little elastin **(Kolakowska, 2001).**

Fish is most important source of meat that may play an appreciable role in solving food problem in the world especially in the developing countries **(FAO, 1991).**

Tilapia **(***Oreochromiusniloticus***)**recently, the demand of *Oreochromius niloticus* consumption has increased continuously because it is low in price with high nutrition food. The whole fish and fillet are admirable for consumers. As a result, it affects the trend of both domestic and export consumption. Moreover, the *Oreochromius niloticus* has many outstanding advantages such as easy to culture, high growth rate, easy breeding, high protein, good taste, white cotton meat like

sea bass fish, high nutrition and having more Omega-3than other wild freshwater fishes and wild estuarine fishes (**Aquatic Animal Research centreCharoenpokph and, 1999).**

The rate of deterioration in fish is highly temperature dependent and can be by inhibited by use of low temperature **(Sivertsvik *etal* ,2002).** Chilling generally slow down the deterioration of sea food .the prevalent method of retarding spoilage in the tropical countries is storage in ice **(Surendran *etal* ,1989).**When the atmospheric surrounding of the product is modified to reduce oxygen concentration ,the shelf life is increase due to the reduction in the rate of the chemical oxidation by oxygen as well as in the growth of aerobic microorganisms **(stile ,1991).**

Among the various methods of processing fish, application of heat is one of the most important methods available for fish preservation, quality improvement and consumer convenience and/or to increase their market value. Cooking has the additional benefits of inactivating endogenous enzymes and stopping microbial growth. Although fish in India are commonly consumed as pan-fried, the consumer has minimum or no knowledge about the nutritive values of raw and cooked fish. In the canning industry, cooking is used mainly to reduce excess moisture, so that the total exudates released in thermal canned / retort products are minimum, thereby improving the sensory, physical and chemical qualities of the product and increasing the shelf life.(**SP Aubourg. Food Sci. & Technol. Int.2001) .**

The main objectives of this study were:

- To determine the effect of steaming and chilling on microbial load of (*Oreochromis.niloticus*).
- To investigate pathological bacteria on the steamed and chilled fish.

- To determine the chemical composition of (*Oreochromis. niloticus*) fish as row chilled and steamed chilled fish.
- To asses sensory evaluation of Nile tilapia (*Oreochromis. niloticus*) as row chilled and steamed chilled fish.

CHAPTER TWO

Literature review

2.1 Background

The establishment of a balance micro flora is important in animal health and digestive function and it can possibly occur in fish where asresident microflora has been suggested **(munro,1994) .** The microbiology of fish gastrointestinal tract has been subjected to many investigators .the characteristics of the different microorganisms differ at various location throughout the alimentary tract influence both the taxonomic composition and the numerical abundance of bacterial presence **(Horsley ,1977) .** The gastrointestinal tract of fish can influence nutrition , growth and disease susceptibility , the micro flora may be essential in fish metabolism that feed on material lacking vitamin , which the micro flora can synthesize **(trust and sparrow , 1974).**

2.2 Microbial flora of fish

The flora living fish depends upon the microbial content of the water in which they live. The slime that covers the outer surface of the fish has been found to contain bacteria of genera *pseudomonas ,alcaligens , micrococcus ,flavor bactrium ,corynebactrium ,yersinia ,serrata , vibrio , bacillus , clostridium , and Escherichia***(frazier and Wsthoff, 1978)** also **Brogstorm (1961)** mentioned that the aerobic flora of salmonid fish in various parts of the world is of type normally regarded as autochthonous to soil, air, water and indicates too wide geographical distribution . **Jay (1978)** reported that bacteria that exist on fresh water fish are generally found in three places, the water slime, gill and intestine.

The total number of microorganism found on all outer surfaces (skin and gills) and in the intestine of live and nearly caught fish vary enormously. **Liston (1980)** stated that a normal range of $10^2 – 10^7$c.f.u/cm^2,of the skin surface. The gills and intestine both contain between 10^3 and 10^9c.f.u/g (colony forming unit)**(Shewan, 1962). Hoffman (1971)** reported a very wide range of numbers of microorganisms that are found on all outer surface, skin ($10^2 – 10^7$c.f.u/cm^2), gill (10^3 -10^9c.f.u/g) and intestines ($10^3 – 10^9$ c.f.u/g) for live and freshly caught fish and this wide range reflects the effect of environment. Also **Shewan (1977)** stated that the bacterial flora in freshly caught fish depend on the environment rather than the species. Fish caught in very cold clean water carry the lower numbers whereas fish caught in warn water have slightly higher count.

Many different bacterial species can be found on the fish surfaces .the bacterial on temperate water fish are all classified according to their growth temperature ranges as either *psychographs* or *psychrophiles* .psychographs (cold –tolerant)are bacteria capable for growth at 0C° *psychrophiles* (cold – loving) are bacteria with maximum growth at temperature around 20C° and optimum temperature at 15C°**(monita,1975).**

In warm water higher number of *mesophiles* can be isolated. The micro flora on temperate water fish is dominated by *psychotropic* gram – nagtive rod bacteria belonging to the genera*Pseudomonas, Moraxella, Acinetobacter showanella* and flavor bacterium.Member of the viborionaceae (vibrio and photo bacterium) and the *Aeromonadaceae (Aeromonasspp)*are also common aquatic bacteria and typical of fish flora .gram – positive organisms are *bacillus, micrococcus , clostridium, lactobacillus and coryneforms*can also be found in varying proportions but general gram negative dominate the micro flora. **Shewan (1977)** concluded that gram positive bacillus and micrococcus dominate fish in

tropical water .however, this conclusion has later been changed by several studies which have found that the micro flora on tropical fish species is very similar to the flora on temperature species **(lima dos Santos ,1981, surendran et al ., 1989 gram et ., 1990)** several authors like **liston (1980)** concluded that the micro flora on tropical fish often carry slightly higher lode of gram positive bacteria and enteric bacteria, but otherwise is similar to the flora and temperate water fish .

Aeromonasspp is typical bacteria of fresh water fish;whereas a number of bacteria require sodium for growth and are thus typical marinwater . These include Vibrio, photo bacterium and Shewanella strain she wanellaputrefaceinscan also be isolated from fresh water environments **(Gram *et al.*, 1990;Dichristina and Delong 1993 ;spangaard*etal*., 1993).**AlthoughS .puterfaceins has been isolated from tropical fish, it is not important in the spoilage of fresh water fish**(Lima dos santos, 1981 ; Gram,1990).**

In polluted water, high numbers of *enterobacteriae* may be found. In clean temperate water these organisms disappear rapidly, but it has been shown that *Escherichia coli* and *salmonellina spp*. can survive for a long period in the tropical water and once introduced may almost became indigenous to the environment **(Fujioka *et al* ., 1988)** . The Japanese studies has shown very high number of microorganisms ingastrointestinal tract of fish and such number higher in the surrounding water, this indicates the presence of favorable ecological niche for the microorganisms. reported that up to 10^7c.f.u/g of Vibrio like organisms from intestine of Turbo . Also photo bacteria which can be isolated in high number form intestinal tract of the some fish species **(Dolgaarad ,1993).**on the contrary, some authors believed that , the micro flora of gastrointestinal tract is merely reflection of environment and food intake . Also **Gilmour and McCallum (1976) and allen *et al.*, (1993)**reported

that such bacteria as Pseudomonas Flourescens ,aromonashy drophila,Edwardsiellatrada, vibrio,*spp* and Myxobacteriaspp is ubiquitous in the environment .**Hossian et *al.*, (1999)**suggested that bacterial load in fish might be increased with increase of water temperature .

2.3 Microbial load on Chilled Fish

Chemical, Enzymatic, and Microbial spoilages occur in refrigerated and iced fish, but microbial spoilage in considered the main factor affecting quality **(Davidson,1975).**

Microorganisms are the important reason for spoilage because they breakdown the food into a form that they can utilize .Therefore, food quality decrease and spoilage starts at this stage and estimation of the quality of food products relies on the quantification of total number of microorganisms **(Brownsell*etal*,. 1989)** .The total Viable count of fish products is $10^7 – 10^8$c.f.u/g at the point of sensory rejection. However ,standards often use alower total viable count for safety **(Olafsdottir*et al.*, 1997)** . The acceptability limit is 10^6c.f.u/g for mesophilic aerobic bacteria is **(Anon, 1991)** Determination of the number of the mesophilic aerobic bacteria is a valid method to estimate fish freshness **(Dogan and Tukel ,2000).**

Quantitative analysis of the loads total aerobic bacteria, Escherichia coli in *Oreochromis.niloticus* Production was sex steps of processing which were tested **(wilaiphan and lawhavinit , 2007)** .the result of the total aerobic bacteria and E.coli indicated that the highest contamination step was the process that packed Tilapia *Oreochromis. niloticus* , in polyethylene bags after chilled fish at 4 °C for 7days and that Tilapia (*Oreochromis. niloticus*)showed the level of contamination $4.4/10^4$,< 10 c.f.u / cm^2 respectively .therefore ,total aerobic bacteria and E.coli

result were not cover the standard value in that agriculture commodity and food standards**(TACFS,2004)** Anyway ,the coliform and staphylococcus aurous indicated that the highest contamination step was the process in which fish was wiped with sponges before being packed showed the level of contamination 6.6×10^3 and 3.4×10^2c.f.u/cm, respectively .No reported was known before about the *E. coli form* and *staphlococcus* in (*Oreochromis*. *niloticus*).the result would be used to assess the critical point of chilled and fresh (*Oreochromis*.*niloticus*)which was contaminated with this pathogenic bacteria that agricultural commodity and food standards **(TACFS,2004).**

2.4 Microbiology of Nile Tilapia(*Oreochromis*. *niloticus*)

Tilapia (*Oreochromis niloticus*) has evidence epidemic problem .Also this type of fishes can be cooked for many types of food with a good price. However one important problem that the importers concerned for Tilapia was the microbiological quality .it should be met with standard each country sets. This standard was very important and each country will use it to be making sure about the aquatic animal quality **(Sanjindawong , 1995).** After the fish died, the bacteria from viscera could even get into the fish meet directly through the skin and alimentary canal. The great amount of bacteria at the skin of the fish at the beginning would cause the fish to be rotten more rabidly. Therefore the management could control and reduce the initial number of microorganism in the process of chilling fresh fish maintain the freshness of fish it also reduced the problem about the spread of cross contamination **(Watanasinthu , 2000).**

2.5Chemical Composition of Fish Meat

The chemical composition of fish meat varies greatly from one species to another and one individual to another depending on age, sex, environment and season. The variation in the chemical composition of fish is closely related to feed intake, migratory swimming and sexual changes in connection with spawning **(FAO, 1995).** Although the protein fraction is rather constant in most species, variation had been observed such as protein reduction occurring in salmon during long spawning migration **(Ando *et al*., 1985;Ando and Halton, 1986)** and pelagic code during spawning season, **(Borresen, 1992)** .

Some tropical fish also showed a marked seasonal variation in chemical composition. West African shad **(***Ethmalosadorsalis* **)** showed range in fat content of 2.7% (wet weight) over the year with maximum in July **(Watts,1957).**

Corvina *(Micropogonfurieri)* and pescada *(Mardonancylodon)* captured off Brazillian coast had a fat content range of 2-8.7 % and 0.1-5.4%respectively **(Ito and Watanabe ,1968)** . It has also been observed that oil content of these species varies with size,, larger fish containing about 10%more oil than smaller ones . **Watanabeel at., (1997)**examined fresh water fish from Zambia and found variation from 0.1%to 5% in oil content of four species including both pelagic and demersal .

Fish raised in aquaculture may also show variation in chemical composition . it had been reported that factors such as feed composition ,environment, fish size and genetic trait all have impact on the composition and quality of the aquaculture fish meat **(Reinitz*et al* ;1979).**

the presence of the basic protein has long been known ,and it is also noticed that they are not present in all fish species ,the best source are

salmonids and herring ,whereas ground fish like code are not found to contain protamine's **(Kossel ,1928)** .The amount of minerals is species-specific and can furthermore vary with seasons .Fish meat is regarded as valuable source of calcium and phosphorus in particular but also of iron, copper and selenium and salt water fish had high content of iodine.

Murry and Burt (1969) reported some minerals constituents of fish muscle in mg /100g as sodium 30-134 potassium 19-50, calcium 19-88, magnesium54-452 and phosphorus 68-550 In aquaculture fish, the component in the fish feed **(Maage*et al.*, 1991**).**Mohamoud (1977)** studied the meat quality of common Nile fishes.He reported that the proximate composition of fish species were in the range of 14 _ 17 %, 2-10% and 70_79% for protein , fat ash and water content respectively . He also found that the female shows more moisture, fat ash and less protein content than male. Also **Awouda (1984)** worked on *Oreochromis nloticus* , and found no significant differences between fish speciesin term of sex. **Agab and Babiker (1987)** studied traditional salted fermented fish (fassiekh of the Sudan). They mentioned that the approximate chemical composition results were in the range of

18.12-28.5%, 10.6-22.5% and 3.2-5.5% for protein, moisture, fat and ash respectively. **Eltom (1989)** studied the microbiology and biochemistry of fasseik fermentation . He reported that, the proximate chemical composition of fresh fish were about 74% moisture, 19% protein, 4.02 % fat and 2.5 ash.

FAO (1989) reported that the common carp contains 17.5 %, 4.79% and 36% for protein, fat and fillet respectively. **Eltay (1994)** studied the chemical composition and quality grading of the Nile fishes

(byad, tilapia and Gnathonnemus)as related to environmental condition in the Blue Nile and White Nile. She reported that there were no

significant differences (P>0.05) in protein content between all species studied .

The composition of a particular species often appears to vary from one fishing ground to another , and from season to season , but the basic causes of change in composition are usually variation in the amount and quality of food that the fish eats and the amount of movement it make . For example, fish usually stop feeding before they spawn, and draw on their reserves of fat and protein. Again when fish are overcrowded, there may not be enough food to go round; intake will be low and composition will change according. Reduction in basic food resource, plankton for example, can affect the whole food chain **(FAO, 2001)**. Plankton –eating species such as herring will then naturally experience another seasonal variation than that caused by spawning, since plankton production depends on the season and various physical parameters in the oceans**(FAO,1995).**

There is usually considerable seasonal variation in the fat content of fatty fish, for example a starved herring may have as little ½ percent fat, where as one that has been feeding heavily to replenish tissue may have a fat content of over 20 %. Sardines, sprats and mackerel also exhibit this seasonal variation in fat content. As the fat content rises ,so the water content falls ,and vice versa ;the sum of water and in fatty fish is fairly constant at about 80% Although protein content falls very slightly when the fat content falls it nevertheless remains fairly constant , some where between 15 and 18%**(FAO, 2001).**

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental site

This study was conducted at department of fisheries and Wildlife Science (DFW) College of science and technology of animal production Sudan University of Science and Technology.

3.2 Materials

The following materials have been used for detection of microbial loads on steamed and chilled fish. fish sample, plastic container ,ice ,refrigerator to cool fish sample at 5^0Cice for chilling sensitive balance ,knifes ,heater , thermometer ,bottle, ethanol ,flame for sterilizing ,sterile tube , Petri dishes, glass plate count agar, autoclave water bath ,oven media .

3.3 Fish Samples collection and preparation

A total of fifty fresh samples of *Oreochromisniloticus* weighted *(*115 – 200g) were collected from hatchery of DFW. Fish samples were gutted,washed with clean tap water and stored at 5^OC in refrigerates for further chemical and microbial testing.

The ice preserved fish were divided into two groups . (25 fish in any groups), Random samples from one group were stored in refrigerator form,while random samples from the second group were processed by steaming for20minutes.and then put into refrigerator at 5^oC. The samples from each group were analyzed at zero, 2^{th}, 4^{th}, 6^{th}, $8^{th}$and 10^{th} days and analyzed for chemical and microbiological parameters .

3.4 Sensory evaluation

Sensory evaluation tests were conducted with volunteer panel consisting of five participants students, they assess the two groups of samples (chilled and processed forms)in different periods at zero, 2^{nd}, 4rth, 6th, 8th and 10th day. Samplewere randomized and identified by a numerical code that was unknown to panel member .The attributes evaluated were color, texture, and odor. A numerical scoring system was used and the scores consisted of numerical value ranging from one (1, lowest) to ten (10, highest). Each panel member recorded on an individual score sheets, the numerical value that best described the attributes of the sample were summarized among panel participants.

3.5 Bacteriological Examination

Solid media were used for bacteriological investigation.

3.5.1 Plate count Agar:

this was non - selective medium for general viable counts of bacteria in food **(Harrigan ,1998).**it was obtained in dehydrated from the medium was composed of yeast extract , tryptone ,D- glucose and granulated agar . It was prepared according to the manufacturer's instruction by using 17.5g in one liter distilled water. The medium was allowed to boil in water bath until it was completely dissolved. The pH was adjusted to pH 7.0 and then the medium was sterilized in an autoclave at $121C^0$.

3.5.2 Nutrient agar

This was a general - purpose culture medium for sub culturing of bacteria it was obtained in dehydrated form. The constituents of the medium were lab– lemcopowder, yeast extract, peptone sodium chloride and agar it was prepared according to the manufacturers instruction by suspending 40 g in on liter distilled water. The medium was allowed to boil until it was completely dissolved .the pH of medium was adjust

to7.0 and then the medium was sterilized in an autoclave at 121C °. For 20 minutes **(Harrigan ,1998)** .

3.5.3 Bismuth sulpite agar

This was used for salmonella detection. it was obtained in a dehydrated from .the medium was composed of Bacto- beef extract ,peptone , Bacto – dextrose ,disodium phosphate ferrous sulphate ,bismuth sulphate indicator ,Bacto –agar and brilliant green . It was prepared according to the manufactures instruction by suspending 52g in one liter distilled water. The medium was allowed to boil in a water bath until it was completely dissolved. The PH of the medium was adjusted to pH7.0 and then the medium was sterilized in an autoclaved at 121 C°.

3.5.4 Eosin methylene blue agar (E M B)

This medium was used for the detection of coliform bacteria and /or *Escherichacoli* .It was obtained in a dehydrated from the constituents of the medium were peptone, lactose, dipotassium hydrogen phosphate, eosin ,methylene blue and agar . It was prepared according to the direction of the manufacturers by suspending 37.5g in one liter distilled water and bringing to the boil to dissolve completely. The pH of medium was adjust to pH6.8 and then the medium was sterilized at 150 C° for 20 minutes **(Cowan and steel , 1974).**

3.5.5 Triple sugar agar

This medium was used for differentiation of the Entero bacteriaceae (Salmonella). it consisted of peptone , lactose ,sucrose, D- glucose ,sodium chloride sodium thiosulphate pentahydrate , di ammonium ion (II)sulphate hexahydrate ,phenol red and agar .it was prepared according to manufactures instruction by suspending 65g in one liter dispensed in 7ml amounts in test tube .it was sterilized in an autoclave at 121C° for

15 minutes . it was then allowed to set as slope with as 3cm butt (**Harrigan ,1998).**

3.6 Liquid media

3.6.1 Nutrient broth (N B)

This medium was used for detection of salmonella .the consisted peptone, sodium chloride and lab– lemco extract (Oxoid) .The ingredients were dissolved in distilled water by heating at 100C°. Then the PH was adjust to PH7.6 and medium was sterilized in an autoclave at 121C° for 15 minutes **(Harrigan, 1998).**

3.6.2 MacConkey broth

This medium was used for the detection and enumeration of lactose – frmentingentero bacteria (coliform bacteria) by the multiple tube technique **(F A O ,1992)** it was obtained in a dehydrated from . The medium consisted of peptone, lactose, bilesalt , sodium chloride and bromocresolpurple. it was prepared according to the manufactures instruction by suspending 40 g in one liter distilled water .the medium was distributed in test tube with inverted Durham tubes .the pH was adjust to pH 7.0 and the medium was sterilized in an autoclave at 121C ° for 20 minutes .

3.6.3 Selenite cystine broth

This was used as an enrichment medium for salmonella detection the medium was composed of peptone lactose sodium hydrogen selenite , disodium hydrogen phosphate ,and cystine . the ingredients were dissolved in one liter distilled water , distributed in suitable amounts and sterilized in boiling water bath for 10 minutes **(Harrigan ,1998).**

3.6.4 Brilliant green bile lactose broth

This was selective medium for isolation and counting of coliform and Escherichia coli by the multiple tube technique .it was obtained in a dehydrated form. The medium was composed of peptone ,lactose purified bile and brilliant green .it was prepared according to the manufactures instruction by suspending 40g in one liter distilled water. the PH was adjusted to PH 7.4 ,distributed in test tube with inverted Durham tubes and the medium was sterilized in autoclave at 121C°for 15 minutes **(Harrign ,1998)** .

3.6.5 E.C medium broth

This was used for the detection of coliform bacteria and/or Escherichia coli by the multiple tube technique. It was obtained in a dehydrated from the medium was composed of tryptose ,lactose bile salts ,dipotassium hydrogen phosphate ,potassium dihydrogen phosphate and sodium chloride . it was prepared according to the manufactures instruction by suspending 37g in one liter distilled water .the pH of the medium was adjusted to pH7 distributed in test tubes with inverted .

3.6.6 Determination of coli form bacteria

3.6.6.1 Presumptive coli form test.

One ml each of the three first dilutions (10^{1-},10^{2-} and $1O^{3-}$) was inoculated aseptically in triplicates of ml sterilized MacConkey broth using the three. Tube technique with Durham tube .the tube was incubated at 37°C for 48 hours. Positive tube gave in the Durham tubes.

3.6.7 Confirmed coli form test

All tube the dilution showing gas fermentation in 24 hours,were submitted to the confirmed test using brilliant green bile lactose broth

fermentation tube with Durham tube and then incubated at C° for 48 hours. the most probable number (MPN)was recorded the most probable number (MPN)table were according to FAO (1992)to record the coliform s number **(FAO, 1992) .**

3.6.8 Faecal coliform test

At least 3 loopfuls each confirmed positive tube were sub –cultured in to EC broth medium and then incubated at 44.5 C° for 24 hours.Tubesshowingany amount of gas production were considered positive . The most probable number (MPN) was recorded

3.6.9 Differentiations of fecal coliform

For further confirmation of fecalcoliforms , tubes giving reaction at 44.5 C° for were streaked on EMB agar . Colonies with green metallic sheen indicated appositive test .

3.7 Proximateanalysis

Analysis for the determination of the proximate composition (crude protein, total lipid, ash and moisture)for fillet fish, for the two groups was performed by the methods described below include methods recommended by the Association Of Official Analytical Chemists 1995 **(AOAC,1995).**

3.7.1 Moisture content

Two grams of well –mixed sample were weighed accurately using sensitive balance in clean dry and pre –weighed crucible and then placed in an oven at 105 C° .the crucible was transferred to desiccator and allowed to cool and then weighed additional placement in the oven were carried out until a constant weight was obtained moisture was calculated using the following formula :

$$MC(\%) == \frac{(W2-W1)-(W1-W3)}{(W2-W1)} \times 100$$

Where:

MC = moisture content

W1 = Weight of empty curable
W2 = Weight of empty curable with sample
W3 = Weight of empty curable with dry sample

3.7.2 Ash content determination

Two grams of sample were placed in clean dry pre –weighed crucible, and then the crucible with it is content ignited in a muffle furnace at 505 °C for 3 hours or more until light grey ash was obtained. The crucible a moved from the furnace to desicator to cool and then weighed Ash content as calculated using the following equation:

$$AC = \frac{W2\text{-}W1}{W3} \; X\ 100$$

Where:
AC = Ash content
W1= Weight of empty curable
W2= Weight of empty curable with ash
W3= Weight of sample

3.7.3Crude Protein determination

The Curde protein was determined by using the micro – Kjeldahl method according to AOAC (1995) as follows :

1. Digestion :
   about 0.2 gram of the sample was weighed and placed in small digestion flask (250 ml).two catalysts tablet (anhydrous sodium sulphate + copper sulphate) were added to the sample , 3.5 ml of approximately 98% H2SO4 was added .the content of flask was then heated on electrical heater for two hour till the colour changed to

blue – green . the tube were then removed from digester and allowed to cool .

2. Distillation :

the digested sample was transferred to the distillation unit and 15 ml of 40% NAOH were added . the ammonia was received in 100 ml conical flask containing 10 ml of boric acid plus 3 - 4 drops of methyle red indicator . the distillation until the volume reached 50 ml .

3. Titration :

   the content of the flask were titraed against 0.02 N HCL . The titration reading was recorded. The crude protein was calculated using following equation:

$$CP\% = \frac{(T - B) \times N \times 14\ 100 \times 6.25}{WS}$$

Where:

CP = Crude Protein
T= Titration reading
B = Blank titration reading
N = Normality of HCL
WS = Weight of sample
100 = To convert mg
6.25 = Protein factor

3.7.4 Fat content determination

An empty clean and dry extraction round bottomed flask was weighed About two gram of sample was weighed and placed in a clean extraction thimble and covered with cotton wool .The thimble was placed in extractor. Extraction was carried out for 8 hours with petroleum either. The heat was regulated to obtain at least15siphoning per hour. The residual ether was dried by evaporation. The flask was placed in an oven at 105 °C . Til it dried completely and then cooled in adesicator and weighed. The fat content was calculated using the following equation:

$$CF\% = \frac{W2 - W1}{W3} X100$$

Where :
FC = fat content
W1 = weight of extraction flask
W2 = weight of extraction with fat
W3 = weight of sample

3.8Statistical Analysis

The obtained results were analyzed statistically using one ways analysis of Varian's (ANOVA) followed by LSD.The test was used to evaluate the mean differences among different treatment at the 0.05 significance level **(Dowdy and Wearden , 1991).**

CHAPTER FOUR

RESULTS

4.1 proximate composition results

Table (1) present the mean values of proximate composition analysis, of processed fish there is significant difference ($p<0.05$) in different periods.

Table (1) shows proximate analysis of steamed chilled fish (*Oreochromis niloticus)*in different storage time(mean± SD).

time / parameters	Day 0 M±SD	Day 2 M±SD	Day 4 M±SD	Day 6 M±SD	Day 8 M±SD	Day 10 M±SD	Sig
Moisture	70.1 ± .6[a]	70.5 ± .05[a]	77.5 ±.1[d]	78.5± 1.5[e]	71.8 ± 1.4[b]	75.3 ±1.05[c]	**
Protein	26.4 ± .25[a]	26.3 ± .3[a]	26.3± .3[a]	27.2 ± .6[b]	28.06± 1[c]	28.2 ±.2[c]	**
Fat	1.3 ± .15[a]	1.4 ± .2[a]	1.1 ± .2[a]	1.3± 05[a]	1.3 ± .2[a]	1.1 ± .05[a]	*
Ash	2.12± .17[c]	.2 ±.19[a]	1.2 ±.01[b]	1.3± .1[b]	1.3± .3[b]	1 .4 ±.05[b]	**

M= mean, SD = stander deviation

a.b.c means in the same row in different letters is significantly different.

Table (2) present the mean values of proximate composition analysis, there is significant difference ($p<0.05$) between chilled and processed samples.

Table (2) shows proximate analysis on raw chilled fish (*Oreochromis niloticus)* in different storage time(mean± SD).

time / parameters	Day 0 M±SD	Day 2 M±SD	Day 4 M±SD	Day 6 M±SD	Day 8 M±SD	Day 10 M±SD	Sig
Moisture	**78.9 ± .3^{a}**	**79 ± 1.05^{b}**	**78.7 ±.5^{a}**	**78.5± .4^{a}**	**79.1± 1.3^{b}**	**78.5 ± .3^{a}**	*
Protein	**21.2 ± .15^{a}**	**23.9 ± .14^{c}**	**22.5 ± .15^{b}**	**26.3 ±. .15^{f}**	**25.4 ± .15^{e}**	**24.2 ± .19^{d}**	**
Fat	**1.5± .05^{a}**	**1.6 ± .14^{a}**	**1.3 ± .17^{a}**	**1.5 ±.2^{a}**	**1.3± 05^{a}**	**1.4 ±.1^{a}**	*
Ash	**2.12± .17^{b}**	**1.2 ±.19^{a}**	**1.2 ±.01^{a}**	**1.4 ±.05^{a}**	**1.3± .1^{a}**	**1.3± .3^{a}**	**

= mean, SD = stander deviation

a.b.c means in the same row in different letters is significantly different

4.2 bacteriological results

Table (3) shows bacterial load(CFU/G) of steamed chilled fish (*Oreochromis niloticus)*in different storage time(mean± SD).

time / bacteria	Day 0	Day2	Day4	Day6	Day8	Day10	sig
Total count cfu\g	3.3×10^{2} ±.56 a	4.8×10±5.2 b	6.1×10^{2} ± 6.1 c	8.3×10^{2} ± 3.5 d	2.6×10^{3} ±4.2 e	1.4×10^{4} ±1 .9 f	*
Total coliform MPN\g	.000±.000	.000 ±.000	.000 ± .000	.000 ± .000	.000±.000	.000± .000	
EcoliMPN\g	.000±.000	.000 ±.000	.000 ± .000	.000 ± .000	.000±.000	.000± .000	
Salmonella	.000±.000	.000 ±.000	.000 ± .000	.000 ± .000	.000±.000	.000± .000	

cfu/g =colony forming unit / gram , = mean, SD = stander deviation

a.b.c means in the same row in different letters is significantly different
MPN=most probable number

Table (4) shows bacterial load (CFU/G) on raw chilled fish *(Orecohromis nilotics)* in different storage time(mean± SD)

time / Bacteria	Day 0	Day 2	Day 4	Day 6	Day 8	Day10	Sig
Total count cfu\g	5.0×10^{4} ± .6 a	7.5×104 ± .5 b	3.7×104± .7 c	5.7×10^{5} ±. 86 d	3.4×10^{5} ± 45 e	8.4×10^{3} ± .4 f	*
Total coliform MPN\g	.000±.000	.000 ±.000	.000 ± .000	.000 ± .000	.4± 1	4.3±1.5	**
EcoliMPN\g	.000±.000	.000 ±.000	.000 ± .000	.000 ± .000	.000±.000	.000± .000	
Salmonella	.000±.000	.000 ±.000	.000 ± .000	.000 ± .000	.000±.000	.000± .000	

cfu/g =colony forming unit / gram, , = mean, SD = stander deviation,

a.b.c means in the same row in different letters is significantly different

MPN =most probable number

4.3 sensory examination results

Table (5) shows sensory evaluation of steamed chilled fish *(Oreochromis nilotics)*in different storage time (mean± SD).

time / parameters	Day o	Day2	*Day4*	*Day6*	*Day8*	*Day10*	Sig
texture	7.2 ±1.9 [a]	9.4 ±.5	6.6 ±.8 [a]	8.4 ± 1.9	6.6 ± .54 [a]	7.4 ± 1.5[b]	**
Oder	8.4±2.07[a]	9.4 ±1.3[b]	8.6 ± .4[a]	9.8 ±.4[b]	10±.00[c]	9.2±1.2[b]	*
Color	9 ±1.2[c]	9.6 ± .8[c]	7.6± 1.5[a]	7.6 ± 1.5[a]	7.4±.5[a]	8.4 ±1.5[b]	*

mean, SD = stander deviation

a.b.c means in the same row in different letters is significantly different.

Table (6) shows sensory evaluation of raw chilled fish (*Oreochromis nilotics)*in different storage time(mean± SD).

time / parameters	Day o	Day2	*Day4*	*Day6*	*Day8*	*Day10*	sig
Texture	9.8 ± .4[a]	9.4± .5[a]	9.2 ±.8[a]	9.8±.4[a]	9.4 ±.4[a]	9.3±.8[a]	**
Oder	10 ±.00[b]	9.4 ± .5[a]	9.2 ±.8[a]	9.6 ±8[a]	9.6 ±9[a]	9.2 ± 1.3a	*
Color	8.6 ± 1.9[a]	9.2±.8[b]	8.6 ± .8[a]	9.8 ±.4[b]	9.4 ±.4[b]	9 ± .5[b]	*

mean, SD = stander deviation. a.b.c means in the same row in different letters is significantly different

Chapter Five
Discussion

Fish has been widely accepted as good source of protein and other element necessary for the maintenance of healthy body **(FAO,1985),**The chemical composition of fish is an important aspect in fish processed as influences both the keeping quality and the technological characteristics of the fish .it is directly related to the moisture ,protein ,fat and ash content of the muscle **(Huss,1988).**These parameters were taken in consideration during the comparative study on microbial growth and sensory evaluation and chemical composition of fresh chilled and processed chilled fish(*Oreochromis niloticus*).

The results obtained from chilled and processed (*Oreochromis niloticus)*

at different storage time (0-10 days) the moisture content of chilled fish(78-79%),protein (21.2 -26.3%),fat (1.3-1.6%),ash (2.0- 2.1)and theprocessed fish moisture content (70.1-78%) , protein (26.3-28%),fat (1.1-1.3%),ash (.2-1.4%)this values is agree with the finding of **Mahmoud(1977)** , who found the value of moisture and fat in range of 70-79% , 2-10%respectively.And agree with **Eltay (1994)**who found the protein ,moisture ,ash was 33.1% ,76%, 5.1% respectively . and also agree with **Agab and Babiker (1987)**they mentioned that the proximate chemical composition results were in range 18.1-28.5%, 20.7-54.5%protein ,moisture respectively while this study results disagree with **Eltom (1989)** who found the value about 19% protein ,74% moisture,4.02% fat and 2.5% ash, this result agree with **(Melaku,2007)** who stated that freezing changes less than processing other methods .

the fluctuation of the chemical composition during different study periods may be due to the difference in the sample portion from the fish.

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 raw chilled fish *(Oreochromis niloticus*)meat was $5\times10^4 – 5.7\times10^5$ Cfu/g , and 3.3×10^2 .1.4×10^7Cfu/g

for processed chilled fish this result agrees with the finding of **(Ahmed , 2007)** who stated that the bacterial count of fresh tilapia was 3.7×10^5 Cfu/g .This number was Also in the accepted limited mentioned by **SSMO (Sudanese Standards and Meterlology Organization ,SDS357**). Which was $5\times10^5 - 10^6$ Cfu/g for fresh fish products .

Also this result was in the same range of the finding of**(Musa and Ahmed ,2011)** who found that the bacterial load of fresh *Oreochromis niloticus* ranges between 1.8×10^5- 2×10^6 Cfu/g .Also the result agree with the finding of **(Jeyasekaran*et al* .,2006)** who reportedthat the initial total bacterial count was found to be 10^5 when the fish were chilled with ice .it was considered normal range of fresh as mentioned by**(Hoffman, 1971)** who stated that bacterial count 10^2 -10^7 Cfu/g of skin surface is normal range Also it is in accepted limit compared to **(Anon 1991)** who said that the total mesospheric aerobic bacterial count over 10^6 Cfu/g is regard as accepted limit for sea foods. The same limit also accepted for fresh water fish **(Turantas ,2000)**. in this case it was considered in the normal range of fresh as mentioned by **(Hoffman, 1971)** who stated that the bacterial count is $10^2 - 10^7$ Cfu/g of skin surface is in the normal range the number of bacteria was in critical point in case of bacterial contamination as mentioned by Thai Agriculture Commodity and Food Standard **(TACFS,2004)** which indicated that the critical point of tilapia in case of bacterial contamination of fish meat , the total bacterial count is not greater than1×10^7 Cfu/g of fish meat , and it considered as critical point .

Conclusion and Recommendations

Conclustion

- Steaming and chilling was affected the chemical composition in different storage time.

- The study results indicated that highest contamination level of the total bacterial count were in processed tilapia .

- Coliform, Ecoli and salmonella were absent in all levels ,except the coliform 8^{th} and 10^{th} days on raw chilled fish.

- Steaming is a proper technique to reduce the bacterial contamination .

Recommendation

- More research are needed in chilling aspect for different fish species and different storage time.

- It's better to preserve fish in ice during handling and short storage periods.
- Fish must be preserved clearly after gutting and change daily to achieve good safety and avoid contamination of fish.

- Identification of bacterial type should be made in odor to meet well fish preservation standards.

- Fish Inspection must be done regularly .

- Preservation methods and storage should be based on scientific knowledge .

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Appendix (1)

Processing and preparing of fish chilling

Appendix (2)

Steaming process of fish

Appendix (3)

Measurement of Temperature ice