

بسم الله الرحمن الرحيم

Sudan University Of science And Technology



College – Graduate studies

Determination of Quality and Shelf Life of Some Commercial Freshwater Fish Based on Total Volatile Basic Nitrogen TVB-N

in Khartoum state

تشديد الجودة ونحرة صلاهية بعض أسماله المياه العذبة التجارية إعتمادًا على

النييتروجين القاعدي الطيار الكلي في ولاية الخرطوم

A dissertation submitted in partial Fulfillment of the Requirement for the Degree of Master of Science (M.S.C) in Fish Science Technology

BY:

Hanyia Elhadi Elzbeer Abdalla

Supervisor

Dr / Siham Elias Saliman Mohammed

<mark>¢</mark>2017

J Library XI

قال تعالى :

{فَوَجَدَا عَبْدًا مِنْ عِبَادِنَا أَثَيْنَاهُ رَحْمَةً مِنْ عِنْدِنَا وَعَلَّمْنَاهُ مِنْ لَدُنَّا عِلْمًا }

صدق الله العظيم

سورة الكهف الآية {65}

Dedication

To mymother To my Brother usbandTo my H Andmysisters

Acknowledgement

Firstly I

say praise is to All a the Expander and Sustainer of the word stog ivem eknowledge and power to complete this research

I would like to express gratitude myto supervisor Dr .Siham EliasMohammed College of veterinary medicine, Sudan University of science and technology, for fruitful suggestion, guidance and encouragement during all stage of this research

Thanks and gratefulness is extended to the staff of Industrial Research and Consultancy Center Laboratory, Ministry of Industry.

Thanks to all members of Fisheries and Wildlife Department and all my friends.

Finally, I would like to thanks my mother, brother, husband and sisters for their continues support.

Table of content

Title	No
Initiation	i
Dedication	ii
Acknowledgement	iii
Table of content	iv
List of table	vi
List of Abbreviation	vii
English abstract	viii
Arabic abstract	ix
Introduction	1
Chapter one Literature Review	3 - 9
1-1 Shelf life	3
1-2 Fish Spoilage	3
1-3 Fish Quality	5
1-3-1 Sensory method	5
1-3-2 Microbiological method	5
1-3-3 Chemical method	6
1-4 Total Volatile Nitrogen	7
1-4-1 Estimation of Total Volatile Base Nitrogen	7

Chapter two Materials and methods	10 - 12
2-1 Experimental area	10
2-2 Sample treatment	10
2-3 Equipment and Material	10
2-3-1Equipment	10
2-3-2 Material	11
2-4 Preparation of sample	11
2-5 Determination of TVB-N	11
2-6 Sensory evaluation	12
2-7 Statistical analyses	12
Chapter three Results	13 - 16
3-1Bayad	13
3-2 Nile tilapia	14
3-3 Nile perch	15
3-4 Statistical analysis of total volatile basic nitrogen	16
Chapter four Discussion	17 - 18
Discussion	17
Cancelation	18
Recommendations	18
Reference	19 - 22

List of Table

Title	No
Table (1)Progressive development of TVB-N over time regarding Bayad	13
Table (2)Progressive development of TVB-N over time regarding Nile tilapia	14
Table (3)Progressive development of TVB-N over time regarding Nile perch	15
Table (4)Statistical analysis of Total volatile nitrogen	16

Abbreviation

TVC	Total Viable Count
TPC	Total Plate Count
APC	Aerobic Plate Count
SPC	Standard Plate Count
SSOs	Specific Spoilage Organisms
SSO	Specific Spoilage Organism
RFID	Ratio Frequency Identification
MRLs	Maximum Recommended Limits
ATP	Adenosine tri phosphate
HX	Hypoxanthine
TVN	Total Volatile Nitrogen
TMAO	Tri Methylamine Oxide
DMA	Di Methyl Amine
SOQI	Single Compound Quality index

Abstract

This study was carried out to evaluated the quality and shelf life of 3 freshwater fish (*Bagrusbagrus ,Orechromisniloticus and Latesnlioticus*) based on total volatile base nitrogen test (TVB-N) .The studied fish was collected a life from South Omdurman kept under ice and immediately transferred to laboratoryand store in deepfreeze(0°c) for 3days . The samples was kept in refrigerator at (4°c) for further analysis. The total volatile basic nitrogen (TVB-N) test become use for assesses the quality and shelf life of fresh fish using Magnesium Oxide Method. This method was repeated every 5 days for 35 days . The result revealed that the TVB-N ratio in the (*Bagrusbagrus*) was reached the recommended limitation which was 30 mg/100 at interval time of 30 days .While the two other species (*Oreochromisniloticus and Latesniloticus*) were reached the recommended limitation in interval time of 25 days . The results showed that there was no significant different between the species (P >0.05).

Generally, the study showed that the expiratory period of the freshwater fish when kept freezer at 4c°was range between 25-30 days.

الخلاصة

اجريت هذه الدراسة لتحديد الجودة وفترة الصلاحية لثلاث انواع لا سماك المياه العذبة (البياض البلطي والعجل) اعتمادا علي اختبار القواعد النيتروجينية الطيارة وقد تم جمع العينات وهي حية من جنوب ام درمان وحفظت سريعا في ثلج ومن ثم تقلها الى المعمل وحفظها في فريزر درجة حرارته صفر درجة مئوية لمدة ثلاث ايام وبعد ذلك تم نقل العينات الى ثلاجة درجة حرارته اربعة درجة مئوية حتي يتم التحليل .

اختبار القواعد النيتروجينية الطيارة اصبح يستخدم لتقييم الجودة وفترة صلاحية الاسماك بعمل طريقة اوكسيد الماغنسيوم. اجري الاختبار في اليوم الاول وكرر كل خمس ايام لمدة خمس وثلاثون يوم واظهرت النتائج ان سمكة البياض وصلت الحد الموصي به من القواعد النيتروجينية الطيارة 30مليجرام لكل100جرام من السمكة في ثلاثون يوما بينما سمكتي البلطي والعجل وصلت الحد الموصي به من القواعد النيتروجينية الطيارة في خمس وعشرون يوما. وقداوضحت النتائج بانه لا يوجد فرق معنوي بين الانواع بمستوى معنوية اقل من (5%). وبشكل عام اوضحت الدراسة ان مدة صلاحية اسماك المياه العذبة وبالأخص اسماك البياض والبلطي والعجل وهي مجمدة في درجة حرارة اربعة درجة مئوية تتراوحما بين (25-30) يوما.

Introduction

Fish are classified as cold-blooded aquatic vertebrate with at least 20,000 known species .Fish are source of protein rich in essential amino acids ,micro and macro elements (calcium, phosphorus, fluorine, iodine), fat that are valuable sources of energy , fat soluble vitamins and unsaturated fatty acids that among other benefits have a hypocholesterolic effect (El.**Dengawy** ,*et al*, **2010**).

Fish is an important food, which contributed about one hundred million tons as total world production for 1989, (55%) was championed by the developing world (**FAO**,1995).

World fish production (catches of wild fish plus production in aquaculture) has increased steadily to approximately 120 million tons (FAO, 2000)

The freshwater fishery resources in Sudan distributed in an area about $100/000 \text{km}^2$, while the Red sea which represents the marine fisheries has coast line of more than seven hundred kilometers. These two water can land up to 110,000 tons\years which is consumed fresh or processed for later marketing and distribution. It was noted that most of the fish landings from this sector were subjected to very poor condition because of lacking facilities, bad handling and processing which negatively affected quality and the value of the products (**Hassan**, *et al*,**2012**).

Fish is one of the most highly perishable food products during handling and storage, quality deterioration of fresh rapidly occurs and limits the shelf life of the product (**Khalid**, 2007).

Shelf life is defined as the period of times under condition of storage for which a food product remains safe and fit for human (**Chuma**, *et al*, **2010**).

Spoilage of foods can be caused by both chemical reaction and physical damage, however the major cause of food spoilage is microbial growth and metabolism resulting in the formation of amines, sulfides, alcohols, aldehydes, ketones and organic acids with unpleasant and unacceptable off flavor (Louis, 2008)

Aim of the Study

1-Todetermaine freshness quality and shelf life of some frozen fish (three species of freshwater fish Bayad, Nile tilapia and Nile perch) when kept in refrigerator at $4c^{\circ}$.

2-To assess fish quality and shelf life based on many quality indexes which include ammonia, mono methylamine, dim ethylamine.

3- To provide knowledge and information on the progressive decline of the fish quality and spoilage.

Chapter (1)

Literature Review

1-1 Shelf life

Shelf life of food is defined as the maximum length of time a given products of fish for human consumption. Fish shelf life is the time from when it's a taken from the Water until it is no longer fit to eat. In marketing the shelf life of fresh and frozen fish is a very important consideration .Knowing the remaining shelf life allows the processor and retailer to plan the length of time a product can be held allowing control of their market. Adding one or two days to the shelf life allows the market to get top dollar and assure repeat sales (**Doyl**, **1995**).

Different species of fish have different shelf life, which also varies depending on the oil levels, catch area, season duration of rigor mortis, intrinsic condition of fish and how it was captured (**Chuma**, *et al*, **2010**).

Temperature and handling practices are the most important factor in determine the shelf life of all species of fish, if the fish product is handled carefully, the temperature at which it is held controls its useful life. Temperature will control the rate of bacteria spoilage and enzyme breakdown an indisputable fact is that the higher the temperature the faster fish spoilage (**Doyle**, 1995).

1-2 Fish Spoilage

Spoilage of food product can be due to chemical, enzyme or microbial activities, chemical deterioration and microbial spoilage are responsible for loss of 25% of gross primary agriculture and fishery products every year (**Barid**, **2000**). Fish contains little carbohydrate but typically has high content of free amino acids; many fish species contain trim ethylamine oxide (TMAO), the seafood (SSOs) produce ammonia, biogenic amine, organic acids and sulfur compounds from amino acids, hypoxanthine from ATP degradation products and acetate from lactate. TMA is produce by some bacteria capable of using TMAO is anaerobic respiration, many microbial metabolites produce in seafood are similar to those observed in meat and poultry products, however in seafood spoilage, TMA in particular contributes to the characteristic ammonia like and fishy off flavours *,P.phosphoreum ,Shewanella*

putrefaciens like organisms and *Vibrio spp* can all reduce TMAO to TMA (Shewan 1977). Some spoilage metabolites can be used as quality index compared with microbiological methods which are slow chemical analysis may be significantly faster, however, for some compounds measurable concentrations are not present until close to spoilage. Classical single compound quality indexes (SCQI) for seafood include measurement of total volatile nitrogen TVN, TMA and hypoxanthine. Ratio between ATP degradation product (k value) and biogenic amine has also been used for some time as quality index (Gram,2002).

Freshness spoilage can be very rapid after it is caught, the spoilage process rigor mortis will start within 12 h of their catch in the high ambient temperature of the tropics, rigor mortis the process through which fish loses its flexibility due to stiffening of fish mussels after few hour of death (Adebowale, *et al*, 2008) During fish spoilage there is breakdown of various compounds and the formation of new compounds these are responsible for the change in odour ,flavor and texture of the fish meat ,this represent a major concern of the freshness of saleable products and the breakdown of protein and lipids (Ghaly, 2010).

The chemical spoilage associated with fish during storage is mainly due to fish lipid degradation (auto-oxidation), in general fish have high degree of unsaturated lipids than other food commodities, during fish storage fish lipids known to susceptible to oxidative rancidity (Huss. 1995)... The main reactants in these processes involves atmospheric oxygen and fish unsaturated lipids leading to the formation of hydropreoxides associated with tasteless, flavor and accompanied by brown yellow discoloration of the fish tissue (Huss, 1994).

1-3 Fish Quality

The determination of fish quality is assessed by three method included under the fallowing

- 1-Sensory methods
- 2- Microbial methods
- 3-chemical methods

1-3-1 Sensory methods

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

Sensory methods are divided into two groups discrimination and descriptive tests however the most commonly used is the descriptive test which measures the difference or absolute value indicating the different quantitative level (Lillian ,2010).

Sensory methods 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 limitation of the analyst (**Connell, 2001**)

1-3-2- Microbiological Methods

The major change in fish freshness for instance unattractive change in food characteristic such as flavors, odour, coloure are largely due to bacterial growth and activity (**Connell**, **1995**).

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

The various way used to determine bacteriological contamination in food fish include Total Plate Count , Total Viable Count , Aerobic Plate Count and Standard Plate Count (TPC ,TVC ,APC ,SPC) and other instrumental methods . All mean number of bacteria (Colony forming unit cfu/g or ml) in food product under

specified and uniform condition of culturing. In general these methods rely on the estimation of the fraction of the micro flora able to produce colonies in the medium used under specified incubation condition (**Huss**, *et al*, **2004**).

1-3-3 chemical methods

Fish death are subjected to a series of changes a result of bacterial and enzymatic activities ,which will result in the breakdown of the storage material and degradation of energy rich compounds these analytic and bacterial changes during fish spoilage are accompanied by the accumulation of a certain constituent and degradation of others. Several methods have been designed to measure one or a group of these constituents and used the result as an indication of fish spoilage. Many chemical tests have been used in the assessment of fish quality. The evaluation of food using chemical methods are considered to be more objective than sensory methods specially when it is done accurately using appropriate method , these methods involve determination of the concentration of specific chemical in the food under study , chemical method of food evaluation are normally used to indirectly predict. The level of a sensory attribute which allows for immediate determination of freshness to use chemical methods to serve this purpose , well set quantified and standardized tolerance levels of chemical spoilage indicators need to be established (**Huss,1995**).

With regard to evaluation of fish quality using chemical methods, the total volatile basic nitrogen (TVBN) constitute to the commonly measured chemical indicators. TVB-N is general phrase used to include volatile amines such as ammonia and trim ethylamine produce by spoilage bacteria, dimethylamine produce by autolytic enzyme (**Huss**, **1988**).

Conversely the measurement of the amount of hypoxanthine (HX) in fish is one of the chemical methods of determining fish freshness, HX is one of the product of nucleotides degradation mediated by bacteria activity (*Proteus bacterium*) is known to be responsible for bitter off flavours of spoilt fish. studies have shown that the degradation of nucleotides progresses vary greatly from one fish to the other but often coincidently progresses with the preserved level of spoilage as

many be determined by trained analysts thus the development of the formula for fish freshness based on these autolytic change that entailed (**Huss, 1995**).

K-Value freshness can be determined by calculating the ratio of inosine and hypoxanthine to the of ATP and all the other products of ATP degradation multiplied by 100, the interoperation is that the smaller the K-Value the more fresh the product is and high K-Value indicate unacceptable fish product(**Harrd,2002**).

1-4 Total Volatile Nitrogen

Volatile amines are the characteristic molecules responsible for the fishy odour and flavor present in fish several days after the catch (**Monique and Ifremer, 2005**).

Total volatile nitrogen is a group of biogenic amine formed in non formed food products during storage, the combined total amount of ammonia (NH₃), dim ethylamine DMA and trim ethylamine TMA in fish is called the total volatile base (TVB) nitrogen content of the fish and is commonly used as an estimate of spoilage and has been widely used as an index for freshness of fish (**David**, **1970**). The increase in the amount of TVN parallel with the increase in the TMA during spoilage .At the activity of spoilage bacteria increases after the death of fish , and subsequent in the reduction of TMAO to TMA (**Sabrina**,**2014**).

During frozen storage ammonia produced by the domination of amino acids and nucleotide catabolites (**Huss**,1995).

1-4-1 Estimation of Total volatile base nitrogen (TVB-N)

TVB-N was determined by a slight modification of Micro diffusion method Conway (1968) .As 25g of the fish sample was chopped and mixed thoroughly with 75ml distilled water in a 250 ml and the pH was adjusted to 5.2 by addition of few drops of Nacl , this was followed by heating at 70c° and cooling to room temperature . After cooling the samples were filtered into conical flask with the aid of whatman No .1 filter,2mls of .025N HCL was transferred to the central compartment of the micro diffusion dish with the aid of a pipette , this was followed by the addition of 2mls of the extract and 1ml of saturated K2CO₃ solution into the outer ring .The dish was covered immediately with the glass plate and the set up was left at room temperature for (24hr). After this ,the HCL in the inner compartment was titrated with (0.025)N NAOH using 2-3drops of methyl/ red methylene blue indicator .The result was expressed in mg TVB-N /100g of fish as described in (Conway ,1968).

The content of TVN in freshly caught fish was typically 5-20 mg/100g of fish and 25-30 mg /100gof fish of muscle which were generally regarded as the limit of acceptability for ice stored cold water fish. TVB-N value increased according to time of storage proposed that the quality classification of fish and fishery products. Regarding TVB-N value would be high quality up to 25mg/100g good quality up to 30 mg/100g limit of acceptable up to 35mg/100g spoilt fish(**Mall,1989**).

The recommended level of TVB-N is 10mg/100g of fish or less for fresh fish 20-30 mg/100g for beginning of spoilage and over 30mg/100g for spoiled fish (**Jinadasa**, **2014**).

In many studies, TVB-N level was very close to the rejection level when bacteria counts reached 7 log cfu/g (**Huss ,1995**).

Previous studies indicated that TVN value fluctuated in both whole ungutted and filleted of rainbow trout chilled storage and it was proposed as a poor indicator of fish freshness (**Chytiris**, *et al*, **2004**).

Frozen tilapia filleted was aimed at evaluating the fish product quality and predicts shelf life through monitoring temperature change inside the refrigerated vehicle with the ratio frequency identification (RFID) technology and analyzes the effect of temperature experience and profile on quality. The contrast experiment under different temperature condition was designed namely: $-18c^{\circ}$ stable, $-18c^{\circ}$ fluctued with $\pm 2c^{\circ}$ and room temperature. Temperature data were collected by RFID record at different points in the vehicle and then product quality of three corresponding groups was evaluated according to sensory analysis and total volatile base nitrogen (TVB-N) value . The result showed that product temperature in different point has no significant difference (P >,05) and product shelf life of the some group also has little different between sensory analysis and TVB-N value , shelf life of product along with fluctuated within $-5c^{\circ}$ was two months longer than within $2c^{\circ}$ meanwhile , the rate of quality deterioration is an accelerate process with passage of storage time (**Tingman, 2010**).

Quality assessment of a typical Nigerian marine fish species was carried out at various storage temperatures such as ambient (28°c) refrigeration (4°c) and frozen state (-5°c) using sensory, microbiological and biochemical method of evaluation. At ambient temperature the shelf life was estimated (12hr) TMA and TVB-N value were 4.8mg/100g and 26.7mg/100g respectively. At refrigeration temperature the shelf life was estimated to be (6) days TMA and TVB-N value were 6.8mg/100g and 28.8mg/100g respectively. At freezing temperature the shelf life estimated of 3 weeks TMA and TVB-N value 9.5 mg/100g and 39,8mg/100g respectively (**Chuma**,*et al*, **2010**).

Determination of quality and shelf life of three marine fish (*Coral trout*, Greasy *grouper and Red mouthed bream*) based in total volatile nitrogen test in Khartoum stated kept under $(4^{\circ}c)$. the shelf life of *Coral trout*, Greasy *grouper* was estimated (25) days and *Red mouthed bream* was estimated (20)days TVB-N value (29.4)mg/100g of fish (Hassan ,*et al*, 2012).

Chapter(2)

Material and methods

2-1 Experimental area

The study was carried out at Industrial Research and Consultancy Center Laboratory at Ministry of Industry in Shambat Area Khartoum Bahri

2-2 Sample treatment

Three samples was collected alive from fisherman from south Omdurman, the live fish killed by placing in ice box for preventing spoilage (Each sample separately) and there average weight of three samples (Bayad , Nile tilapia and Nile perch) were (15-13-16kg) respectively. The fishes were gutted, filleted and washed and then stored in ice box (the ratio of fish to ice 2:1) each sample was identified Name of sample Date of collection were recorded. The samples transferred to laboratory and store in Deepfreeze (0°c) for 3 Days. After 3days the samples were kept in a refrigerator at (4c°) waiting for testing.

2-3Equipment and materials

2-3-1Equipment

- 1-Distillation apparatus
- 2-Water path
- 3-Drying oven 103c°
- 4-Warring blender
- 5-500ml Erlenmeyer flask
- 6-1000ml round bottom flask
- 7-50ml burette
- 8-Three 125ml Erlenmeyer flask
- 9-Watch glass

10-Weighing bottle

2-3-2 Materials

1-Boric acid solution 2% prepared by dissolving 20g boric acid in 1 litter distilled water.

2-Methyl red indicator to 100ml ethanol and 1g of methyl red indicator was added and dissolved.

3-Sulphric acid (5%) to litter distilled water and 1.39 ml concentrated H_2SO_4 was added.

4- Magnesium oxide .

2-4 Preparation of sample

The representative sample was minced and mixed thoroughly and l0g portion of sample was weighted in order to be tested for Total volatile nitrogen

2-5 Determination of TVB-N

Total volatile nitrogen was determined by magnesium oxide method. The sample contained ammonia – mono methyl amine – die methylamine and other volatile amine (**Daivd,1970**).

- The procedure started by dissolved 10 grams of minced fish with 2 g of magnesium oxide in 300ml distilled water.
- The add 25ml of 2%boric acid solution and few drops of methyl red indicator in other flask

- The two flask (heating-receiving) were connected to the evaporator heat the distilling flask so that the liquid boils in exactly 10min and using the same rate of heating distil for exactly 25min
- After25min was stopped the content of receiving flask than was transferred to another flask and titrated to the end point by very week acid(,05H₂SO₄) and from the equation below total volatile nitrogen was determined , was expressed in mg N/100g of fish (**David ,1970**).

$$TVB - N = \frac{V \times N \times 14 \times 100}{W}$$

Where:

V=volume (ml) used for sample

N=normality of H₂SO₄

W=weight of sample in grams

2-6 Sensory evaluation

Sensory evaluation for the three species was determined and the characteristic features of the fish such as coloure ,odor and texture of the frozen product as the acceptability (**Huss,1995**)

2-7 Statistical analyses

Complete randomize design was used to analyze the obtained data from this study and subjected to general linear model (GLM) followed by least significant different (LSD) as 3×8 Factorial arrangement using SPSSV.16 program

Chapter(3)

Result

3-1 Bayad (Bagrus bagrus)

Total volatile base nitrogen for Bayad fish in first day 4.6mg/100 of fish, after 30 days was reached 29.4 mg/100g of fish. After 35days TV B-N reached 34 mg/100g of fish .the maximum recommended limits (MRLs) which set at 30 mg /100g of fish (Table1)

Table (1) Progressive development of TV B-N over time (days) regarding Bayad fish in Khartoum state.

Days	TVB-N mg /100g of fish
1	4.6
5	7
10	11.2
15	14.9
20	19.1
25	24.7
30	29.4
35	34

TVB-N =Total volatile base nitrogen

3-2 Nile tilapia (Oreochromis niloticus)

The consecutive test of total volatile base nitrogen for the fish above also showed progressive declined in the quality over time, the first test estimated TVN at 2.8 g of fish, after 25 days TVB-N increased to reach its marginal acceptable limit which was 29.4 mg/100g of fish. After 30 days TVB-N reached 34.5mg/100 of fish (Table2)

 Table (2) Progressive development of TV B-N over time (days) regarding Nile

 tilapia in Khartoum state

Days	TVB-N mg/100g of fish
1	2.8
5	8.4
10	12.1
15	17.2
20	22.8
25	29.4
30	34.5
35	38.7

TVB-N =total volatile base nitrogen

3-3 Nile perch (Lates Niloticus)

Total volatile base nitrogen for the species above was carried out in the first day was determined at 4.2 mg/100g of fish and after 25days total volatile base nitrogen was reached 29.4mg/100g of fish. After 30 days TVB-N reached 35mg/100g of fish(Table 3)

Table (3) Progressive development of TVB-N over time (days) regarding Nile perch in Khartoum state

Days	TVN mg/100 g of fish
1	4.2
5	9.8
10	13.5
15	19.1
20	25.2
25	29.4
30	35
35	39.6

TVB-N= Total volatilebase nitrogen

Shelf life of three fish

The results of shelf life of *Bagrus bagrus* was 30 days, *Oreochromeis niloticus* 25 days and *Lates niloticus* was 25 days

3-4 Statistical analysis of total volatile nitrogen

Parameters of the results indicated that there was no significant different in total volatile basic nitrogen in three species (Table 4)

Days	Bayad	Nile tilapia	Nile perch
	Mean± SD	Mean \pm SD	Mean ±SD
1	4.6 ±80	2.8±0	4.2±1.4
5	7±0	8.4±0	9.8±1.4
10	11.2±0	12.1±.80	13.5±1.6
15	14.9±.80	17.2±.80	19.1±2.5
20	19.1±.80	22.8±.69	25.2±0
25	24.7±.80	29.4±1.4	29.4±1.4
30	29.4±0	34.5±.80	35±0
35	34±.80	38.7±.80	39.6±.80
Mean		20.2	
SD	15.1		
SE		.34	
Sig		**	

Table (4) Total volatile basic nitrogen in freshwater fish in Khartoum stated.

SD=Standard Deviation

SE=Standard Error

SIG =High Significant (P<0.05)

Chapter (4)

Discussion

The quality assessment of commercial freshwater fish (Bayad, Nile tilapia and Nile perch) was carried out in order to determine the shelf life of the frozen fish samples under kept in refrigerator (4°c) using TVB-N test.

The TVB-N value in *Bagrus bagrus* (Bayad) fish at the first day of the test was recorded (4.6mg/100g) this results in agreement with result of Connell(1995) who recorded that 2to20 mg/100gof fish , the TVB-N value was 29.4 mg/100g in 30days . This finding disagreed with Hussien (2014) who said that shelf life in Bayad that stored in crushed ice is18days. Also the shelf life of fresh water fish stored in refrigerator (13-33days) was recorded Huss (1995).

The TVB-N value in (*Oreochromis niloticus*, Nile tilapia and *Lates niloticus*, Nile perch) fish at the first day of the test was recorded 2.8-4.2 mg/ 100g of fish respectively, this result agreement with result of **Connell**(**1995**) who recorded that 2-20mg/100g of fish ,the TVB-N was 29.4mg/100g of fish in 25 days. This finding agreement with **Hassan**, *et al*, (**2012**) who said that shelf life in *Coral trout and Greasy grouper*)stored in refrigerator to be 25days, and disagree with **Abraham** (**2015**) and **Lillian** (**2010**) the shelf life in Nile tilapia and Nile perch stored in ice to be 19-18 days respectively.

The results degrees with (**Gram**, *et al*, 1989) the shelf life in Nile perch stored in ice to be 4 weeks.

The results above explained that the Bayad fish deteriorated after the Nile tilapia and Nile perch and this might be due to many factor such as handling, flatting and storage or biological activity.

Conclusion

The safe use for refrigerator fish product within 25-30 days when kept at constant temperature below 4c°, the study also revealed that the acceptability of Bayad as refrigerator, product can last 25 days as good quality and 30days marginal acceptable product. The Nile tilapia and Nile perch as refrigerator, product can last for 20 days as good quality and 25days marginal acceptable product. The TVN not exceed than 30mg/100g of fish by international organization namely Codex Aliment Aries EU stander and regulators countries

Recommendations

1-The study recommended and encourages the fish industry to follow the program in order to prevent food problems.

2- Competent authority (fishery department) should play a significant role to increase awareness between the fisheries communities in order to comply with the best practices of fish handling and processing special fish quality control laboratory to test fishery product quality and safety is highly needed.

Reference

- 1- Abraham, A.O (2015) Shelf life of Wild tilapia stored in ice and at ambient temperature American journal of agricultural science 2(1) 8.12.
- 2- Adebowale, B.A (2008) Comparative quality assessment of fish (*Clarias garepnius*) smoked with cocoa pod husk and three other different smoking Journal food technology 6:5-8 DOI 3923.

3- **Baird**, **T.C** (2000) The production of microbiologically safe and stable food in the microbiological safety and quality of food publisher Inc Gaithersburg pp3.18.

4- Chuma, C. Okora, Olusimbo ,O.Aboaba and Ola ,J.Babajide (2010) Quality assessment of Nigerian marine fish mullet (Liza Falcipinnis) under different storage condition –Journal New York science 3(8) 21-28 ISSN 1554-0200.

5- Chytris, Chouliara , I , Savvaidis , N. Kontominas , M.G (2004) Microbiological chemical and sensory assessment of iced whole and filleted aquaculture rainbow trout –food microbial.

6- **Connell** ,**J.J** (2001) -Quality control in fish industry –FAO Torry researches station in partnership with support unit for international fisheries in aquatic .

7- **Connell ,J.J(1995)** Control of fish quality Published by Oxford fishing New book ISBN 0852380682.

8- Conway ,E.L(1968) Micro diffusion analysis and volumetric error Crosby lock wood and sons Ltd London 68pp.

9-David Pearson (1970) The Chemical analysis of foods London.

10 - **Dolye, Joun .P(1995)** Seafood shelf life as function of temperature – Alaska seagrant Marine Advisory program NO 30.

11-EL-Dengawy ,R.A ,El-Shehawy ,S.M ,Kassem ,A.E.M ,El-Kadi ,S.M, Zeninab ,S,Farag (2012) Chemical and microbiological evaluation if some fish product. journal Agriculture , Chem and Biotech ,Monsoura Uni ,V3(8) 247-259 .

12-FAO (1995) Quality change in fresh fish -FAO fisheries technical paper 429 1

13- FAO (2000) The state of world fisheries and aquaculture – FAO fisheries department.

14-Ghaly ,A.E(2010) Fish spoilage mechanisms and preservation techniques – American Journal 7.859 -877

15- Gram Lone(2002) Fish spoilage bacteria problems and solution current Opinion in technology 13.262-266.

16- Gram Lone, Oundo, J and Bon ,J (1989) Storage life of Nile perch dependent on storage temperature initial bacteria lood Tropical science 29.221-236.

17- Harrad, N (2000) The role of enzyme in determining sea food color and texture in A.H Brenner safety and quality issues in food processing(pp221-253).

18-Hassan Mohammed Adam , Lubna Osman and Mohammed Abdelhafiz (2012) Determination of quality and shelf life of three marine fish (Coral trout , Greasy grouper and Red mouthed) based in total volatile nitrogen –Journal Life Sci , Biomed 2(5) 178-191. 18- 19-Huss,H.H (1995) Quality and Quality change in fresh fish FAO fisheries technical paper .

20-Huss, H.H, Ababouez and Gram Lone (2004) Assessment and management of seafood safety and quality FAO fisheries technical paper 444.

21- **Huss H.H** (1988)Fresh fish quality and quality changes –At raining manual prepared for the FAO/ DANIDA training program on fish technology and quality control Rome Italy –FAO of the united Danish international development agency.

22- Huss H.H(1994) Assurance of sea food quality ,Rome FAO fisheries technical paper No334 .

23-Hussien ,A.H.I (2014) Assessment of shelf life of *Bagrus bayad* (Bayad) fish stored in crushed ice in Khartoum state Journal Vo(2) N(4).

24- **Jinadasa B.K.K (2014)** Determination of quality of marine fish based on total volatile nitrogen –Research and Development Agency Colombo 12 (5) Srilanka .

25-Khalid Ibrahim (2007) Chemical, Sensory and shelf life evaluation of sliced Salmon treated with salt of organic acid -University of Monsoura –Egypt.

26- Lillian Chebet (2010) Rapid alternative methods for evaluation of fish freshness and quality – University of Akureyri –Barzel.

27-Louis Lowtence (2008) Bacterial load of fish in the central market Khartoum university.

28- **Mall,P** (1989) Anew chemical criterion for the quality control of fish Trimethylamine / Total volatile amine , Journal of food production 52,419-423 .

29-Monique Etienne and Ifremer Nates (2005) Volatile as criteria for chemical quality assessment – France .

30- **Sabrina Dacosta Silva(2014)** Chemical quality index for freshness evaluation of fish –Journal food studies university of brazil .

31-Shewan ,J.M (1977) The bacteriology of spoilage of fish and SPS spoiling fish and the biochemical change induce by bacterial action ,proceedings of the conference on handling processing and marketing of tropical fish , tropical product In pp51-66 London.

32- **Tingman ,W.jian (2010)**–Fish product quality evaluation based on temperature monitoring in cold chain –African Journal of Biotechnology V9 (37) pp 6146-6151.