

Dept. of Food Hygiene.  
Animal Health Research Institute

## **SHELF LIFE OF TILAPIA NILOTICA STORED IN ICE** (With 3 Tables and 3 Figures)

By  
**HODA A.E. AWAD**

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### **فترة الصلاحية للبلطى النيلى المحفوظ فى الثلج**

**هدى عبد الغنى عوض**

تم حفظ اسماك البلطى النيلى فى الثلج باستخدام معدلات ١ : ١ ، ١ : ٢ ، ١ : ٣ ثلج الى اسماك وتم تعيين فترة الصلاحية للمجموعات الثلاثة بالتقييم الحسى والكيميائى والبكتيرى وصلت الاسماك الى وقت الرفض فى اليوم ١٨ ، ١٨ ، ١٥ معدلات ثلج الى اسماك ١ : ١ ، ١ : ٢ ، ١ : ٣ على التوالي فى حين كان الأس الايدروجينى ٦,٣ ، ٦,٤ ، ٦,٤٥ والنيتروجين الكلى المتصاعد ٢٤,٤ ، ٢٥,٢ ، ٢٦,٤ مليجرام لكل ١٠٠ جرام بالنسبة للمجموعات الثلاثة على التوالي . تعدى العد البكتيرى عند وقت الرفض الحدود المقبولة لكل البكتريا المعدودة وشملت العد البكتيرى للميكروبات الهوائية والبكتيريا المحبة لدرجات الحرارة المنخفضة والبكتيريا المعوية والبكتريا الاهوائية . تمت التوصية باستخدام معدل ثلج : اسماك ١ : ٢ لحفظ اسماك البلطى .

### **SUUMARY**

Tilapia Nilotica fish were stored in ice using 1:1 , 1:2 and 1:3 ice-fish ratios. shelf life was determined to the three fish groups by sensory , chemical and bacterial evaluation . Fish reached the rejection time at day 18 , 18 and 15 for 1:1 , 1:2 and 1:3 ice-fish ratios respectively where pH values were 6.3 , 6.4 and 6.45 , TVBN were 24.4 , 25.2 and 26.4 mg/100g for the three ice-fish ratios groups respectively . The bacterial counts exceeded the acceptability limits for all flora studied (total bacterial count , psychrotrophic , enterobacteriaceae and anaerobic bacteria) at rejection time . The use of ice-fish ratio 1:2 for keeping stored Tilapia was recommended.

**Key words:** *Tilapia Nilotica - Ice store - Shelf life*

## INTRODUCTION

Fresh fish has always been a consumer's primary choice in sea food because it is easier to judge by eye the freshness of whole fish than filleted or processed ones. Icing fish immediately after being caught seems to be the more simple, cheaper and common method used world-wide to keep fish fresh during marketing. Although a detailed organoleptic inspection is usually sufficient to determine the freshness of iced fish, laboratory examinations are indispensable and must rapidly provide data for the assessment of quality.

pH value, trimethylamine (TMA) and total volatile basic nitrogen (TVBN) are widely used to estimate the degree of decomposition of fish and its efficiency assay has been confirmed by several fish scientists and technologists (Sumner and Magno-Orejana, 1985, Bennour *et al.*, 1991 and Reddy *et al.*, 1995).

Fish decomposition is mainly due to bacterial growth which results in the production of various substances, some of which are not normally found in alive muscle tissue (Mendes and Lajolo, 1975).

Although much information is available on the spoilage mechanism and the number and types of microorganisms associated with marine fish and shellfish, very few data have been published on the microbial flora of fish reared in fresh water, (Acuff *et al.*, 1984).

Disney (1971) was the first who alerted fish scientists to the fact that fish from tropical waters might have longer storage life in ice than fish taken from cold waters. Later, Lima do Santos (1981) and Poulter *et al.* (1981) presented storage data for a number of studies carried out by FAO and Tropical Products Institute staff which indicated that while cold / temperate species can be kept an average of 14 days in ice, tropical species can be kept 21 days.

The aim of this study is to determine the shelf life of *Tilapia Nilotica* during ice storage as well as the suitable ice-fish ratio which can be used to get longer shelf life.

## MATERIAL and METHODS

*Tilapia Nilotica* used in this study were obtained from fishermen in Nile River at Monieb, Giza Governorate. Fish were transported in a cooler containing crushed ice to the laboratory where 6 fishes were used to make the first sample (day 0), the others were divided to three groups and stored in

woody boxes with alternating layers of fish and ice . 1:1, 1:2 and 1:3 ice-fish ratios were used (w/w). Water was drained regularly from orifices made in the bottom of the storing boxes and ice was replenished when necessary . The storage of Tilapia fish groups under ice lasted 24 days, samples were periodically taken (every three days) for sensory, chemical and bacteriological examinations . The experiment was repeated for three times.

### **Sensory evaluation**

Fish were evaluated by three members of fish Hygiene Research unit, Department of food hygiene research. The following organoleptic tests attribute appearance, odour, texture and taste were examined to determine the rejection time at which fish no longer appropriate for consumption using a rating scale from 5 to 1. The scale is based on the Torry scale as originally described by Shewan *et al.* (1953). The fish were judged unfit for consumption when the mean value for sensory score was below 3.

### **Chemical analyses**

A 10 grams portion of fish flesh was homogenized in 50 ml of distilled water, and its pH was measured. (Anon 1977). Total volatile bases (TVB) content was determined by the micro diffusion method of Conway (Conway, 1947). Histamine level was determined colorimetrically using the technique recommended by Hardy and Smith (1976).

### **Bacterial analysis**

Fish-flesh (10-grams) were homogenized with 90 ml of sterile peptone water (0.1%) diluent then serial dilutions were made in the same diluent. The aerobic plate count (APC) and the psychrotrophic flora count (PFC) were obtained on plate count agar (PCA) medium incubated for 3 days at 30°C and at 4°C, respectively as recommended by A.O.A.C. (1990) . Anaerobic bacterial count on reinforced clostridial medium (RCM) was applied using the method described by Gudkov and Sharp (1966). Most probable number (MPN) of coliforms using multiple tube fermentation technique in MacConkey broth tubes was carried out according to A.P.H.A. (1992). Total enterobacteriaceae count using pouring technique on violet red bile glucose agar was applied as recommended by ICMSF (1980).

## **RESULTS**

The obtained results are illustrated in Tables (1,2 &3 and Figures 1,2 & 3).

## **DISCUSSION**

The sensory evaluation of the examined samples (Fig. 1 and Table 1) indicated that shelf life of iced Tilapia Nilotica was affected by the ice-fish

ratio used, samples with 1:1, 1:2 and 1:3 ice-fish ratios reached the rejection times at 18, 18 and 15 days of storage respectively. Thus samples stayed accepted for consumption when more ice was used. Nearly the same results were obtained by Poulter *et al.* (1981) and Maia *et al.* (1983) concerning warm fresh water fish, while Gallardo *et al.* (1983), Klausen and Lund (1986) and Bennour *et al.* (1991) recorded shorter shelf-life for cold water fish. The most widely-accepted theory for longer storage life of tropical fish species has been propounded by Shewan (1977); colder water are thought to have higher numbers of psychrotrophs which in turn, coat the fish and shorten the shelf life compared with tropical species.

The present study recorded the same shelf life (18 days) for different treated ice-fish ratio samples, 1:1 and 1:2. The fact that fish out of contact with ice had a longer shelf life and better acceptability than fish in contact with ice recorded by Subrata and Khasim, (1985) could explain the similarity of the two shelf lives instead of using two different ice-fish ratios.

The average values of pH at 0 day were 5.8, 5.85 and 5.8 for ice-fish ratios 1:1, 1:2 and 1:3 respectively (Fig 2 and Table 2), the pH increased at the rejection time to 6.3, 6.4 and 6.5 respectively. The increase in pH between days 0 and day 24 were only 0.85, 0.65 and 0.85, the obtained result was observed by other authors. Reppond and Collins, (1983), EL-Marrakchi *et al.* (1990) and Bennour *et al.* (1991).

Concerning TVBN, average values were 17.3 mg/100g for the three ice-fish ratios groups at day 0, then increased to 24.4, 25.2 and 26.4 mg/100g for 1:1, 1:2 and 1:3 ice-fish ratio respectively at the rejection time as determined by sensory evaluation (day 18 - day 18 - day 15) as shown in (Fig 2). The recorded values did not exceed 30 g/100g proposed by Connell, (1975) as a limit of acceptability for fish of cold and temperate waters. At day 24, the mean values of TVB was 25.8, 26.9 and 28 mg/100g and were lower than the proposed acceptable limit although the fish samples judged as spoiled. TVB content is not a sensitive index of freshness because of its high variability and the test is usually reversed for fish near the limit of acceptance as mentioned by Howgate (1982).

The production of histamine in flesh of iced Tilapia were slow during the storage period and recorded <5 mg/100g of flesh as determined by the colorimetric method. The inhibitory effect of cold on histamine production was reported by several workers, Eitenmiller *et al.*, 1982 and Gallardo *et al.*, 1983) and it could explain the obtained results.

Total aerobic count averaged  $1 \times 10^3$ ,  $1.3 \times 10^3$  and  $1.2 \times 10^3$  CFU/g at 0 day for ice-fish ratios 1:1, 1:2 and 1:3 respectively, (Fig 3 and table 3) after

3 days , there was very slight increase in total bacterial count which may be due to the disappearance of cold environment organisms that could not adapt cold environment as explained by Acuff *et al.* (1984). At the rejection times total bacterial count, psychrotrophic count, anaerobic count and enterobacteriaceae count exceeded the limits of acceptability recommended by ICMSF (1974). Thus, this may indicate the presence of good correlation between the sensory analysis and the bacterial counts and agreed with results obtained by (EL-marrakchi *et al.* (1990) and Bennour *et al.* (1991). Concerning anaerobic bacterial counts, they were  $2.4 \times 10^5$ ,  $6.2 \times 10^5$  and  $3 \times 10^6$  at the rejection times. The importance of anaerobic bacteria is that they are considered as a criterion for fish spoilage, Gram *et al.* (1987).

### CONCLUSION

The preservative effect of ice on *Tilapia nilotica* can depend on ice-fish ratio used. There is 3 days increase in shelf-life when 1:1 and 1:2 ice fish ratios used than 1:3. Economically the ice-fish ratio 1:2 can be recommended because no significant differences were recorded by sensory, chemical and bacterial evaluations between 1:1 and 1:2 ice-fish ratios. We can conclude also that pH of fish during icing storage can't be depended on to evaluate its quality. Also TVB content is a valuable index in assessing the degree of *Tilapia* deterioration rather than evaluating different freshness degrees. Finally, sensory evaluation of *Tilapia* fish can reflect the bacterial load that exists.

### REFERENCES

- Acuff, G.; Azat, A.L. and Finne, G. (1984): Microbial flora of pond-reared *Tilapia* (*Tilapia aurea*) held on ice. *J. Food Port.* 47. 10 : 778-780.
- American Public Health Association APHA (1992): Compendium of methods for the microbiological examination of foods : 3<sup>rd</sup> Ed ; Edwards brothers , Washington .
- Anon (1977): A collection of analytical methods and testing procedures for the assessment of fish and shellfish quality . Paper presented at the CIDA/FAO/CECAF training course on fish handling, plan simulation, quality control and fish inspection. Dakar, Senegal, 10 October - 4 November 1977 .
- Association Official Analytical Chemists AOAC (1990): Official methods of analysis, 15 th Ed . Washington , DC .
- Bennour, M.; EL-Marrakchi, A.; Bouchriti, N.; Hamama , A. and -EL-Ouadaa, M. (1991): Chemical and microbiological assessments of

- mackerel (*Scomber Scombrus*) Stored in ice. *J. Food Prot.* 54. 10: 784, 789-792 .
- Connell, J.J. (1975):* Control of fish quality. Fishing News (Books) Ltd. London.
- Conway, F. (1947):* Micro diffusion analysis and volumetric error. Crosby Lockwood and Sons. London PP. 157-159.
- Disney, J.G. (1971):* Quality assessment in *Tilapia* species. In fish inspection and quality control , edited by R . Kreuzer . London , Fishing News (Books) Ltd .
- Eitenmiller, R.R.; Orr, J.H. and Wallis, W.N. (1982):* Histamine formation in microbiological and biochemical conditions. PP. 39-50. In R.E.Martin ed. , chemistry and biochemistry of Marine food Products. AVI publishing Co., Westport , Co.
- EL-Marrakchi, A.; Bennour, M.; Bouchriti, N., Hamama, A . and Tagafait, H. (1990):* Sensory, chemical and microbiological assessments of Moroccan sardines (*Sardina pichardus*) stored in ice. *J. Food Prot.* 53: 600-605 .
- Gallardo, M.J.; Montemayor, M.I. and Perez-Martin, R. (1983):* Formation de histamine. en caballa (*Scomber scombrus*) y listado (*katsuwonus pelamis*), espccies de alto contenido en hislidine Libre. *Rev. Agroquim . Technol . Alim .* 23: 269-275 .
- Gram, L.; Trolle, G. and Huss, H.H. (1987):* Detection of spoilage bacteria from fish stored at low (0°C) and high (20°C) temperatures . *Int. J. Food Microbiol.* 4: 65: 72.
- Gudkov, A.V. and Sharp, M. (1966):* A preliminary investigation of importance of clostridia in production of rancid flavour in cheeder cheese, *J. Food Microbiol.*4:65:72.
- Hardy, R. and Smith, J.G.M. (1976):* The storage of mackerel (*Scomber seombrus*). Development of histamine and rancidity. *J. Sci. Food Agric.* 27: 295-299.
- Howgate, P.F. (1982):* Quality assessment and quality control. In fish handling and processing (2<sup>nd</sup> ed) (A. Aitken *et al.*, Eds.). PP . 177-186. Her Majest's stationary, Edinburgh.
- International Commission of Microbiological Specification for Food. ICMSF (1980):* Factors affecting life and death of microorganisms. Vol.1:5 Academic Press. INC. (London) LTI.
- International Commission on Microbiological Specifications for Foods (1974):* Microorganisms in foods. University of Tronto press. Tronto.

- Klausen, N.K. and Lund, E. (1986):* Formation of biogenic amines in herring and mackerel. *Z. Lebensm. Unters. Forsch.* 182: 459-463.
- Lima do Santos, C.A. M ( 1981):* The storage of tropical fish in ice. A review. *Trop. Sci.*, 23: 97-127.
- Maia, E.L.; Rodriguez-Amaya, D.B. and Moraes, M.A.C. (1983):* Sensory and Chemical evaluation of keeping quality of the Brazilian fresh water fish *prochilodus scrofa* in ice storage. *J. Food Sci* 48 (4) 1075-1077.
- Mendes, M.H.M. and Lajola, F.M. (1975):* Evlucao das bases volateis totais e da trimethylamina em pescados e o seu uso como indicador de qualidade. *Rev. Farm Bioquim. Univ.* 13: 303-322.
- Poulter, R.G.; Curran, C.A. and Disney, J.C. ( 1981):* Chill storage of tropical and temperate water fish, differences and similarities. In *Advances in the refrigerated treatment of fish. Sci. Tech. Froid / Refei. Sci. Technol., Paris, (1984-1):* 111-24 .
- Reddy, N.R.; Villanueva, M. and Kautter, D.A. (1995):* Shelf life of modified-atmosphere-packaged fresh *Tilapia* fillets stored under refrigeration and temperature-abuse conditions. *J. Food Prot.* 53 . 8: 908 - 914.
- Reppond, K.D. and Collins, J. (1983):* Pacific cod (*Gadus macrocephalus*) change in sensory and chemical properties when held in carbon dioxide modified refrigerated sea water. *J. Food Sci* , 48: 1552-1553 .
- Shewan, J.M.; MacIntosh, R.G. ;Tucker, C.G. and Ehrenberg, A.S.C. (1953):* The development of a numerical scoring system for the sensory assessment of the spoilage of wet white fish stored in ice . *J. Sci. Food Agric.* 4: 283 - 298.
- Shewan, J.M. (1977):* The bacteriology of fresh and spoiling fish and the biochemical changes induced by bacterial action. In proceedings of the conference on handling, processing and marketing of tropical fish. London, Tropical Products Institute, PP. 51-66.
- Subrata Basu and Imam Khasim , D . (1985):* Studies on the effect of leaching on the quality of ice-stored fish. *Fishery Technology*, 22 (2) 105-108.
- Sumner, J. and Magno-Orejana , F. (1985):* Do tropical fish keep longer in ice than temperate fish; the circumstantial and definitive approaches. *Food Sci & Tech. Abs. FAO Fisheries report:* 62 -70.

Table (1) : Organoleptic changes during ice storage of *Tilapia nilotica*

Day	Appearance			Odour			Texture			Taste		
	1 <sup>st</sup> group	2 <sup>nd</sup> group	3 <sup>rd</sup> group	1 <sup>st</sup> group	2 <sup>nd</sup> group	3 <sup>rd</sup> group	1 <sup>st</sup> group	2 <sup>nd</sup> group	3 <sup>rd</sup> group	1 <sup>st</sup> group	2 <sup>nd</sup> group	3 <sup>rd</sup> group
0	4	5	5	5	5	4.9	5	5	5	5	5	5
3	4.7	4.9	4.5	5	4.8	4.7	4.9	4.7	4.8	4.9	4.7	4.6
6	4.4	4.7	4.2	4.8	4.6	4.1	4.8	4.5	4.5	4.7	4.4	4.3
9	4	4.4	4.1	4.5	4.1	3.7	4.6	4.3	4.2	4.3	4	4.1
12	3.6	3.4	2.6	4.2	3.7	3.5	4.2	4.1	3.8	4.1	3.6	3.8
15	3.2	3.6	2.2	4	3.3	3	3.8	3.7	3.5	3.8	3.1	3.6
18	2.9	3.2	1.2	3.8	3	2.8	3.5	3.1	3.1	3.4	2.8	3.2
21	2.5	3	1	3.4	2.8	2.4	3.1	2.6	2.9	2.8	2.4	2.6
24	2.1	2.8	1	3.2	2.4	2	2.7	2.1	2.6	2.5	1.6	2.1

Table (2) : Changes in pH and TVBN during ice storage of *Tilapia nilotica*

Day	pH			TVBN		
	1 <sup>st</sup> group	2 <sup>nd</sup> group	3 <sup>rd</sup> group	1 <sup>st</sup> group	2 <sup>nd</sup> group	3 <sup>rd</sup> group
0	5.8	5.85	5.8	17.3	17.3	17.3
3	5.85	5.95	6	18	18.5	19.2
6	5.9	6.1	6.15	19.7	20.6	21.8
9	5.95	6.1	6.3	20.3	23.2	23.1
12	6.1	6.2	6.4	22.2	24	24
15	6.25	6.35	6.45	23.6	24.6	25.8
18	6.3	6.4	6.5	24.4	25.2	26.4
21	6.45	6.45	6.55	25	26	27
24	6.65	6.5	6.65	25.8	26.9	28



Table (3) : Bacterial counts changes during ice storage of *Tilapia nilotica*

Day	APC			Psychrotrophic count			Enterobacteriaceae count			Anaerobic count		
	1 <sup>st</sup> group	2 <sup>nd</sup> group	3 <sup>rd</sup> group	1 <sup>st</sup> group	2 <sup>nd</sup> group	3 <sup>rd</sup> group	1 <sup>st</sup> group	2 <sup>nd</sup> group	3 <sup>rd</sup> group	1 <sup>st</sup> group	2 <sup>nd</sup> group	3 <sup>rd</sup> group
0	10 <sup>3</sup>	1.3x10 <sup>3</sup>	1.2x10 <sup>3</sup>	3.2x10 <sup>3</sup>	3.1x10 <sup>3</sup>	3.6x10 <sup>3</sup>	5x10 <sup>3</sup>	4.8x10 <sup>3</sup>	4.8x10 <sup>3</sup>	<10 <sup>2</sup>	<10 <sup>2</sup>	<10 <sup>2</sup>
3	2.2x10 <sup>3</sup>	3.1x10 <sup>3</sup>	4.6x10 <sup>3</sup>	4.6x10 <sup>3</sup>	5x10 <sup>3</sup>	4.1x10 <sup>4</sup>	6.2x10 <sup>3</sup>	1x10 <sup>4</sup>	3x10 <sup>4</sup>	6x10 <sup>2</sup>	<10 <sup>2</sup>	2x10 <sup>3</sup>
6	7.1x10 <sup>4</sup>	4x10 <sup>4</sup>	6x10 <sup>5</sup>	6x10 <sup>4</sup>	1.2x10 <sup>4</sup>	4.8x10 <sup>4</sup>	1.1x10 <sup>4</sup>	2x10 <sup>4</sup>	7.1x10 <sup>4</sup>	3.4x10 <sup>3</sup>	2x10 <sup>2</sup>	4.7x10 <sup>3</sup>
9	3.2x10 <sup>5</sup>	6.2x10 <sup>5</sup>	7.2x10 <sup>5</sup>	8x10 <sup>4</sup>	3.7x10 <sup>4</sup>	5.2x10 <sup>5</sup>	2.8x10 <sup>4</sup>	4.6x10 <sup>4</sup>	9.3x10 <sup>4</sup>	7.4x10 <sup>3</sup>	2.2x10 <sup>3</sup>	3x10 <sup>4</sup>
12	4.3x10 <sup>5</sup>	8x10 <sup>5</sup>	2x10 <sup>6</sup>	2.1x10 <sup>5</sup>	2x10 <sup>5</sup>	3x10 <sup>6</sup>	4.2x10 <sup>4</sup>	8x10 <sup>4</sup>	5.1x10 <sup>5</sup>	3x10 <sup>4</sup>	1.7x10 <sup>4</sup>	1.6x10 <sup>5</sup>
15	7.3x10 <sup>5</sup>	2x10 <sup>6</sup>	4.3x10 <sup>7</sup>	4.8x10 <sup>5</sup>	4x10 <sup>5</sup>	3.7x10 <sup>6</sup>	8x10 <sup>4</sup>	2.2x10 <sup>5</sup>	9.6x10 <sup>5</sup>	1.1x10 <sup>5</sup>	3x10 <sup>5</sup>	3x10 <sup>6</sup>
18	2x10 <sup>6</sup>	3.2x10 <sup>7</sup>	3x10 <sup>8</sup>	2x10 <sup>6</sup>	8.2x10 <sup>5</sup>	1.2x10 <sup>7</sup>	9.1x10 <sup>4</sup>	3.9x10 <sup>5</sup>	4.6x10 <sup>6</sup>	2.4x10 <sup>5</sup>	6.2x10 <sup>6</sup>	8.2x10 <sup>6</sup>
21	4x10 <sup>7</sup>	5x10 <sup>8</sup>	7x10 <sup>9</sup>	3x10 <sup>6</sup>	1.3x10 <sup>6</sup>	2.8x10 <sup>7</sup>	3x10 <sup>5</sup>	6x10 <sup>5</sup>	7.9x10 <sup>6</sup>	7x10 <sup>6</sup>	8.1x10 <sup>6</sup>	9.2x10 <sup>6</sup>
24	8x10 <sup>7</sup>	9.6x10 <sup>8</sup>	9x10 <sup>9</sup>	9x10 <sup>6</sup>	2x10 <sup>6</sup>	4.6x10 <sup>7</sup>	6x10 <sup>5</sup>	9x10 <sup>5</sup>	9x10 <sup>6</sup>	10 <sup>6</sup>	9x10 <sup>5</sup>	3x10 <sup>7</sup>

Abbreviations :

APC : aerobic plate count

1<sup>st</sup> group : ice fish ratio 1:1

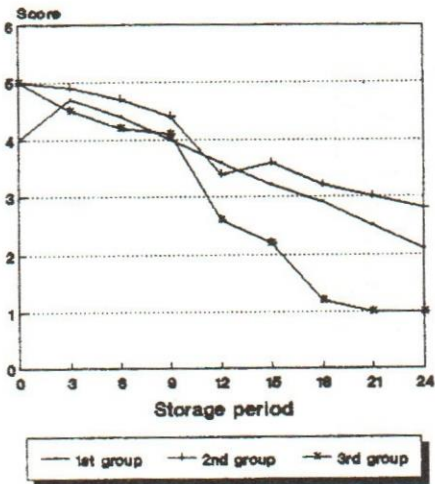
2<sup>nd</sup> group : ice fish ratio 1:2

3<sup>rd</sup> group : ice fish ratio 1:3

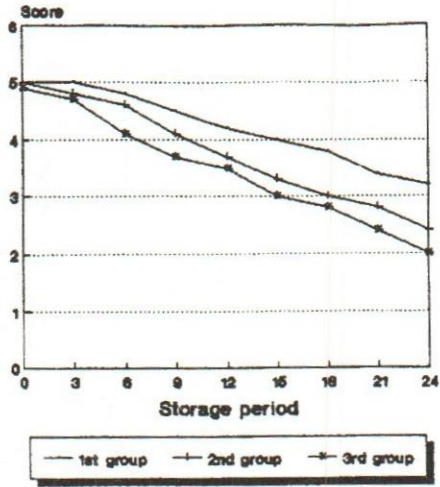
pH : Hydrogen ion concentration

TVBN : Total volatile basic nitrogen

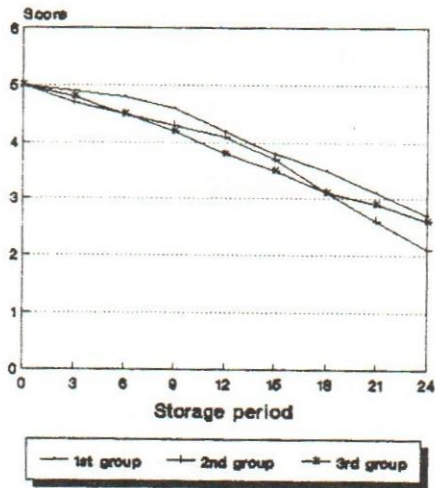
1: Appearance



2: Odour



3: Texture



4: Taste

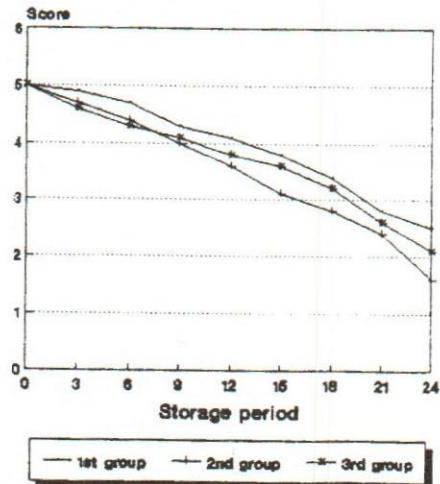


Fig ( 1 ) : Organoleptic changes in appearance , odour , texture and taste .  
Score scale : 5 (very good) , 4 (good) , 3 (moderate) , 2 (bad)  
and 1 (decomposed)

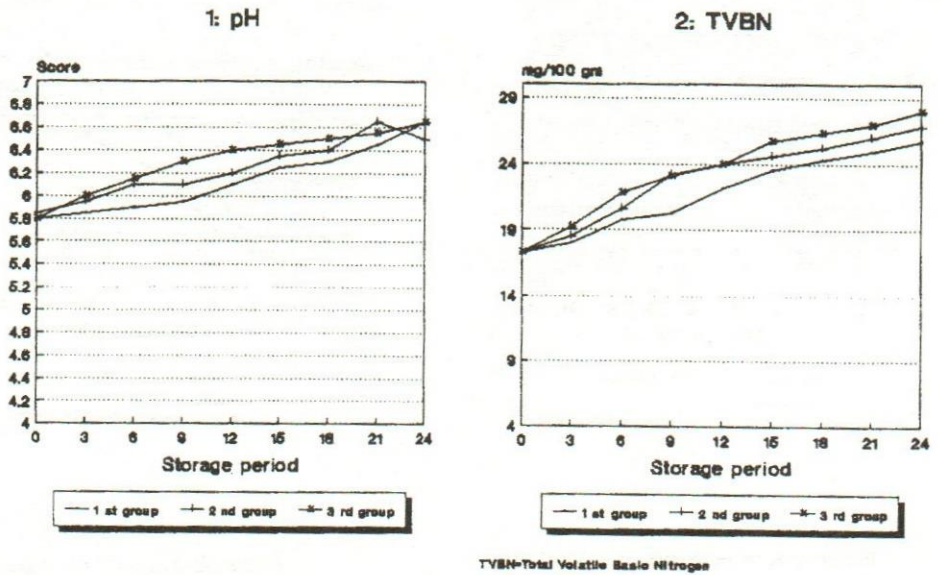
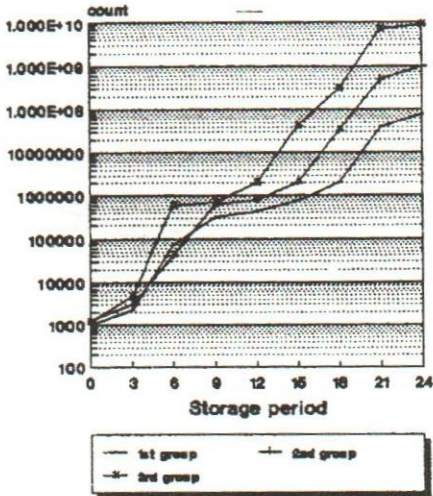
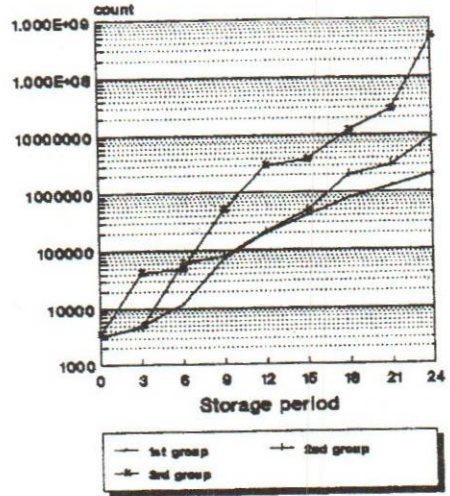


Fig (2) : Changes in pH and TVBN during ice storage of *Tilapia Nilotica*.

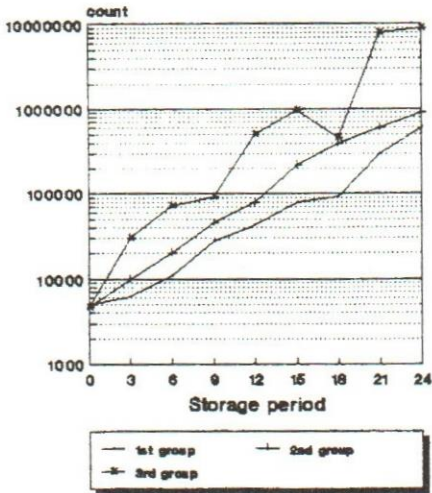
Aerobic plate count changes



Psychrotrophic count changes



Enterobacteraeae count changes



Anaerobic count changes

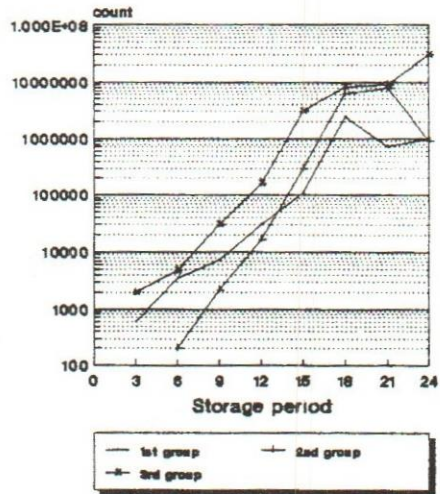


Fig (3) : Changes in APC, Psychrotrophic, Enterobacteriaceae and Anaerobic counts during ice storage of *Tilapia Nilotica*