

## QUALITY ASSURANCE OF SOME FATTY FISHES (With 9 Figures)

By

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(Received at 11/6/2003)

### توكيد جودة بعض الأسماك الدهنية

أنور درويش ، محمد خالد المسلمي ، رأفت البسيوني

تم جمع ١٠٢ عينة من الأسماك النيلية مكونة من (٦٦ عينة من أسماك البياض، ٣٦ عينة من ثعبان السمك) وتم فحصها بإجراء الاختبارات الحسية والكيميائية والبكتريولوجية وكذلك لتحديد أقصى مدة حفظ عند درجة -١٨°م. وأظهرت النتائج أن المجموع الكلي لنقاط عيوب الجودة (total demerit points) كانت صفر في الأسماك المصمادة حديثاً في كلتا المجموعتين من سمك البياض و ثعبان السمك الكاملة والمجوفة وأن هناك إزدياد في المجموع الكلي لنقاط عيوب الجودة أثناء فترة الحفظ و التجميد حتى وصلت إلى ١٨,٥، ١٤,٥، ١٦,٥، ١٤ على الترتيب في نهاية فترة التخزين حيث ظهرت علامات الفساد على الأسماك. كما أوضحت النتائج أن أقصى مدة للحفظ عند درجة -١٨°م لأسماك البياض الكاملة والمجوفة و ثعبان السمك الكامل والمجوف كانت ٢٦، ٣٢، ١٢، ١٦ أسبوع على التوالي مما يدل على أن هناك إختلاف في فترة الحفظ لكل من سمك البياض و ثعبان السمك نظراً لإختلاف المحتوى الدهني في كلا النوعين ، و أن إزالة الأحشاء الداخلية تزيد من فترة الصلاحية ٦ ، ٤ أسابيع في سمك البياض و ثعبان السمك على التوالي. كما لوحظ إزدياد تدريجي في متوسط قيم التركيز الأيدروجيني ، نسبة النيتروجين الكلي المتصاعد، حمض الثيوباربيتورك وكذلك قيمة (K) "اختبار الطزاجة" أثناء حفظ الأسماك بالتجميد بينما قل تدريجياً متوسط المحتوى الدهني أثناء فترة التخزين. وخلصت النتائج إلى التوصية بأن قيمة (K) هي ٥٥% كحد أقصى للقبول. وأوضحت الفحوص البكتريولوجية أن الميكروبات بدأت في الظهور عند الأسبوع الثاني من فترة التخزين في كلا المجموعتين. حيث كانت نسبة الميكروبات المظلة للدهون في أسماك ثعبان السمك الكاملة والمجوفة أعلى بكثير من أسماك البياض. أما ميكروب المكور العنقودي الذهبي ، الكورليفورم والفيريوباراهيموليتكس فلم يتم عزلهم في كلا النوعين من الأسماك المفحوصة.

### SUMMARY

One hundred and two of fresh fish, composed of 66 Bagrus bajad 'Bajad-Bagar' and 36 Anguilla anguilla 'Thobban Elsamak – Hanash' were investigated for its sensory, chemical and bacteriological

parameters beside the maximum frozen storage life at  $-18^{\circ}\text{C}$ . Organoleptic scores (total demerit points) of newly caught whole and gutted *Bagrus bajad* and *Anguilla anguilla* fish were 0.0 (excellent degree). There was a significant increase in the total demerit points has been occurred during the successive weeks of frozen storage, where it reached a values of 18.5, 14.5, 16.5 and 14.0 respectively at the end of frozen storage period when the fish became distinctly spoiled. The shelf-life of whole and gutted *Bagrus bajad* and *Anguilla anguilla* fish, after which the fish became distinctly spoiled, were 26, 32, 12 and 16 weeks (about 6.0, 7.5, 3.0 and 3.5 months) respectively. Gutting, will extend shelf-life for approximately 6 and 4 weeks in *Bagrus bajad* and *Anguilla anguilla* fish respectively (about 21.5% and 28.5% more than whole fish respectively). There was a significant gradual increase in the pH, TVB-N, TBA and K-value mean values which had occurred during the successive weeks of frozen storage. There was a significant decrease in fat content during the frozen storage period. According to the obtained results K-value of 55% is recommended as the maximum limit for acceptability. The microorganisms begin to appear in the second week of frozen storage in the both groups of the two fish species and gradually increased during the frozen storage period. The percentage of lipolytic bacteria in whole and gutted *Anguilla anguilla* fish were much higher than in *Bagrus bajad* fish. *Staph. aureus*, Coliforms and *Vibrio parahaemolyticus* failed detection in the muscles of both fishes.

*Key words: Quality assurance, fatty fishes.*

## INTRODUCTION

Fish occupies one of the foremost places among the food products of animal origin in nutritive value because of the presence of high biological valuable protein, lipids rich in vitamins and essential fatty acids as well as several kinds of minerals especially phosphorus, magnesium, calcium, copper and iron. However, fish is a highly perishable food, changes in freshness are a result of many complex changes owing to autolytic and microbial activity.

Spoilage starts as soon as a fish dies as a result of a complex series of chemical, physical, bacteriological and histological changes that occur in the muscle tissue. These interrelated processes are usually accompanied by the gradual loss or development of different compounds that affect fish quality.

These quality changes are influenced by many factors, the most important of which is temperature. If not properly controlled, exposure of fresh fish to temperature abuse can cause serious deterioration in fish quality.

Freezing is considered an excellent process for preserving the quality of fish for longer periods of time. However, it is now recognized that deteriorative changes take place in fish and seafood during freezing, frozen storage and thawing, which influence the quality and shelf stability of the finished product (Santos-Yap, 1996).

During frozen storage, microbiological changes in fish and seafoods are very minimal. On the other hand, series of changes such as protein denaturation, lipid oxidation, texture deterioration, loss of fresh odour and flavour, various enzymatically induced reactions, loss of volatile constituents, nutritional losses and changes in moisture take place in fish and seafood when subjected to excessively prolonged frozen storage.

Umemura *et al.* (2000) stated that 2 weeks of 80-100g daily fatty fish consumption had no effect on serum saturated fatty acids consumption, whereas monounsaturated fatty acids level was reduced from 24.2 to 22.1% and polyunsaturated fatty acids (PUFA) increased from 50.0 to 51.2% of total fatty acids, n-3 PUFA levels were particularly increased from 6.8 to 9.7% and the n-3 : n-6 ratio increased significantly from 0.16 to 0.24. Iso *et al.* (2001) stated that the woman with higher intake of fish had a lower risk of total stroke compared with woman who ate fish less than once a month.

Magnusson and Martinsdottir (1990) assessed the effect of frozen storage on the survival of mesophilic, psychrotrophic and coliform bacteria in cod fillets stored at  $-25^{\circ}\text{C}$ . They found that one day storage didn't result in reduction of the counts of examined bacteria. However, with increasing frozen storage time, the reductions of bacterial numbers become greater. Thus 23 and 50 weeks of storage resulted in 53, 43, 80% and 70, 90, 91% reduction in mesophilic, psychrotrophic and coliform counts respectively. Meanwhile, the psychrotrophic flora composition showed increase in the Gram-positive bacteria after 23 weeks of storage.

Fish which is before or during the rigor mortis could be acceptable for freezing and to be such fish, it is important to extend holding time as long as possible before reaching the stage of rigor mortis. For the extension of holding time, cooling down the material fish with crushed ice is essential. The recent trend is to freeze fish on board as soon as they



are caught and sometimes while they are alive being successful in improving the quality of the frozen fish. (Motohiro, 1992)

The spoilage changes in fresh fish occur most commonly as a result of bacterial activity. The species of bacteria vary according to storage temperature. In fish stored in ice, *Alteromonas*, *Achromobacter* and *Flavobacter* spp. predominate. At temperature between 35 and 55°C, *Micrococcus* and *Bacillus* spp. constitute the main microflora. Some of these microorganisms produce very active proteolytic enzymes, which produce odor, flavor and textural problems. (Santos-Yap, 1996).

Dines (1999) assessed the microbial quality of seafoods considering the health of the consumer. Estimation of *Escherichia coli* counts gives the status of the sanitary hygienic conditions in processing plants and indicates the occurrence of other pathogenic bacteria.

The general objectives of the study is to investigate the quality changes as measured by sensory, chemical and bacteriological parameters and the maximum suitable frozen storage life at -18°C (home freezer) of whole and gutted bayad (*Bagrus bajad*) and *Anguilla anguilla* (Eel).

## **MATERIALS and METHODS**

### **I- Materials:**

One hundred and two of fresh fish, composed of {66 *Bagrus bajad* (Baiaid - Bagar) and 36 *Anguilla anguilla* (Thobban Elsamak - Hanash), each fish weight about 250 - 400 g.} were purchased alive by special agreement with fisher man in Giza fish market and transferred immediately to the laboratory in special box within half hour.

All fish samples were left to die by struggling. Fish samples were divided into two groups. The first group was washed by clean running water then packed individually in polyethylene bags while, the second group was gutted (eviscerated) and washed also by clean running water then packed individually in polyethylene bags. Both groups were chilled for two hours at 7°C before to be frozen and stored at -18°C.

Organoleptic, bacteriological and chemical tests to estimate the keeping quality and to record the changes which occurred during the storage period of such tested fishes were carried out by taking the mean values of two examined fish samples just after fish dying and at two weeks intervals till the detection of decomposition and spoilage by sensory assessment.

## **II- Methods:**

### **1- Preparation of the sample:**

Frozen fish were thawed for overnight at refrigerator shelf (7°C). Judge the completion of thawing by gentle squeezing of the fish sample until no hard core or ice crystal can be felt.

### **2. Sensory Examination:**

The Quality Index Method (QIM) originally developed by the Tasmanian Food Research unit for fresh and frozen fish and developed by Branch and Vail (1985) for Freshness Quality Assessment System for round fish has been used, while for gutted fish the system recommended by Larsen *et al.* (1992) has been applied.

### **3- Chemical examination:**

#### **3.1- Preparation of the fish sample:**

The thawed fish sample was rendered into a uniform mass after removal of head, fins, tail, gut and bones, then the following tests were done.

- 1- Determination of total fat content (Soxhlet extraction); (Koniecko, 1985);
- 2- Measurement of pH (ISO, 1974);
- 3- Determination of Total Volatile Bases Nitrogen (TVB-N) FAO, 1980.
- 4- Determination of Thiobarbituric acid number (TBA) (Tarladgis *et al.*, 1960 with modification of Pikul *et al.*, 1983)
- 5- Determination of the K-value (Ehira *et al.*, 1970).

#### **4- Bacteriological examination:**

Fish homogenate was prepared and the following counts were determined.

- Aerobic plate count (APC) according to ICMSF (1978) using nutrient agar. The inoculated Nutrient agar plates were incubated at 30±1°C.
- Pseudomonas species count (ICMSF, 1978) using Pseudomonas selective agar with glycerol and incubated at 25°C for 3 days.
- Lipolytic count (APHA, 1992) using Tributyrin agar plates which were incubated at 25°C for 3 days.
- Staphylococcus aureus count (FAO, 1992) using Baird-Parker agar plates incubated at 37°C for 48 hours, suspected *S. aureus* colonies were tested for coagulase production.
- Coliforms counts (MPN); ICMSF (1978) using Lauryl sulfate tryptose broth with inverted Durham's tubes.
- *Vibrio parahaemolyticus* count (ICMSF, 1978) Thiosulphate citrate bile salt sucrose (TCBS) agar was used, inoculated plates were incubated at 35°C for 24 hours.

## **RESULTS and DISCUSSION**

### **I – Organoleptic parameter and shelf life:**

The presented data revealed that the mean Organoleptic scores (Total demerit points) of newly caught whole and gutted *Bagrus bajad* and *Anguilla anguilla* fish were 0.0 (Excellent degree).

There was a significant increase in the Total demerit points which had been occurred in whole and gutted *Bagrus bajad* and *Anguilla anguilla* fish during the successive weeks of frozen storage, where it reached values of 18.5, 14.5, 16.5 and 14.0 respectively at the end of frozen storage period when the fish became distinctly spoiled.

Results achieved in figure (1) pointed out that the shelf-life of whole and gutted *Bagrus bajad* and *Anguilla anguilla* fish, after which the fish became distinctly spoiled and unfit for human consumption, were 26, 32, 12 and 16 weeks (about 6.0, 7.5, 3.0 and 3.5 months) respectively. It is noted that the whole and gutted *Bagrus bajad* fish had approximately double shelf-life than that of whole and gutted *Anguilla anguilla* fish. This difference may be attributed to the big variation in fat content between the two species. In this respect, Bramnaes (1969) cleared that fish composition has an appreciable effect on shelf-life stability of frozen fish. Fatty fish had a shelf-life of 2-3 months at  $-18^{\circ}\text{C}$ , whereas lean fish exhibited storage stability up to 4 months at the same storage temperature. Shirley and Margy (1994) recommended storage times at  $-18^{\circ}\text{C}$  of 6 and 3 months for lean and fatty fish respectively. Johnston *et al.* (1994) reported that storage life for frozen fish varies considerably according to fish species. Fatty fish had storage life of 4 months at  $-18^{\circ}\text{C}$ , while lean fish had storage life of 8 months at the same storage temperature.

It could be concluded from the above mentioned data that there was significant difference in shelf-life between whole and gutted fish in both species. Gutting, will extend shelf-life for approximately 6 and 4 weeks in *Bagrus bajad* and *Anguilla anguilla* fish respectively. On contrary, Karacam and Boran (1996) and Kassem (2001) reported that evisceration had no significant effect on fish quality.

### **II – Chemical parameters:**

#### **I- fat content:**

The achieved data (figure 2) cleared that the mean values of fat content of the whole and gutted newly caught (zero time) *Bagrus bajad* were 7.34 and 7.41 % respectively.



There was a significant decrease in these mean values which had occurred during the successive weeks of frozen storage, where it reached values of 5.68 and 5.50 % respectively at the end of frozen storage time.

The decrease was about 22.61 and 25.78% from the original values in whole and gutted *Bagrus bajad* fish respectively.

Regarding *Anguilla anguilla*, the results given in the same figure showed that the mean fat content values of the two groups were 17.16 and 16.97 % respectively at zero time. These values gradually decreased in both groups during the frozen storage period to become 14.08 and 13.83 % respectively at the end of the storage period. The decrease was approximately 17.95 and 18.50 % from the original values in whole and gutted *Anguilla anguilla* fish respectively.

#### **2- pH value:**

The mean pH value of the two groups of newly caught (zero time) *Bagrus bajad* were 5.89 and 5.78 respectively (Figure 3). These values gradually increased in both groups during the frozen storage period to become 6.57 and 6.79 respectively at the end of the storage period.

Concerning *Anguilla anguilla*, the results presented in the same figure showed that the mean pH values of the whole and gutted fish were 5.68 and 5.72 respectively at zero time. There was a significant increase in these mean values that had been occurred during the successive weeks of frozen storage, where it reached a values of 6.66 and 6.70 respectively at the end of frozen storage time.

These results agreed with those recorded by Galli *et al.* (1993), Hassan (1995), Cakli (1996), Hassan (1998) and Kassem (2001).

#### **3- Total volatile bases-nitrogen (TVB-N):**

The data given in figure (4) cleared that the mean values of TVB-N content of the two groups of newly caught (zero time) *Bagrus bajad* were 4.33 and 3.84 mg/100g.fish muscle respectively. There was a significant increase in these mean values which had occurred during the successive weeks of frozen storage, where it reached values of 25.15 and 28.30 mg/100g.fish muscle respectively at the end of frozen storage time.

Regarding *Anguilla anguilla*, the results given in the same figure pointed out that the mean TVB-N content values of the two groups (whole and gutted) were 5.96 and 5.39 mg/100g.fish muscle respectively at zero time. These values gradually increased in both groups during the frozen storage period to become 30.02 and 27.62 mg/100g.fish muscle respectively at the end of the storage period when the fish become distinctly spoiled.

The aforementioned results were in agreement with those reported by Sengupta *et al.* (1971), Connell (1995), Hassan (1995), Simeonidou *et al.* (1997), Hassan (1998), Sarma *et al.* (1998) and Kassem (2001).

On contrary, Ryder *et al.* (1984), Gelman *et al.* (1990), and Aubourg *et al.* (1998) reported that there is no correlation between sensory characteristics and TVB-N content.

#### **4- Thiobarbituric acid value:**

The achieved results in figure (5) illustrated that the mean TBA values of the whole and gutted newly caught (zero time) *Bagrus bajad* were 0.004 and 0.006 mg MD/1000g fish flesh respectively. These values gradually increased in both groups during the frozen storage period to become 4.073 and 3.900 mg MD/1000g fish flesh respectively at the end of the storage period.

With respect to *Anguilla anguilla*, the results presented in the same figure showed that the mean TBA values of the two groups were 0.011 and 0.014 mg MD/1000g fish flesh respectively at zero time. There was significant increase in these mean values occurring during the successive weeks of frozen storage, where it reached values of 3.272 and 3.592 mg MD/1000g fish flesh respectively at the end of frozen storage time.

These results agree with those reported by Lovell (1972), Moral *et al.* (1978), Galli *et al.* (1993), Cakli (1996), Jayasinghe *et al.* (1997), Simeonidou *et al.* (1997), Hassan (1998), Sarma *et al.* (1998) and Kassem (2001).

It could be concluded from the above mentioned results that there is a good correlation between sensory assessment and TBA values. Same finding was reported by Lovell (1972), Hassan (1995), Aubourg (1999) and Kassem (2001).

#### **5 - Nucleotide degradation:**

It is evident from the data presented in figure (6) that the mean K-values of the whole and gutted newly caught (zero time) *Bagrus bajad* fish were 12.49 and 11.68 % respectively. These values gradually increased in both groups during the frozen storage period to become 57.80 and 52.76 % respectively at the end of the storage period when the fish became distinctly spoiled.

Concerning *Anguilla anguilla* the results presented in the same figure illustrated that the mean K-values of the whole and gutted fresh fish (zero time) were 16.17 and 16.21 % respectively. There was a significant increase in these mean values during the successive weeks of frozen storage of these two groups where it reached values of 56.72 and 60.05 % respectively at the end of frozen storage time.



The results of the two groups of *Bagrus bajad* and *Anguilla anguilla* agree with those of Cho (1981) who found that the K-value depends on the death type, instant death had value of 5.8%, whereas death after struggle had 14.3%.

Okada (1992) reported that the K-value is a very convenient and useful index of freshness regardless of fish species, whereas Chang *et al.* (1998) found a maximum K value of 50% was appropriate for sea bass shelf-life determination.

It could be concluded from this investigation that the K -value can be used as a mean of evaluating the freshness of fish. Same finding was reported by Barile *et al.* (1985), Ehira and Uchiyama (1987), Surette *et al.* (1988), Nunes *et al.* (1992) and Yamagata and Low (1995).

Gopakumar (2000) confirmed after analysis of several species of cold water and tropical fish that there is an excellent correlation between freshness of fish and K-values and the mean K-value, if prime quality, should be around 20%.

Finally, from the achieved results it is recommended to recognize K-value of 55% as the maximum value for acceptability of fish freshness in the Egyptian standard.

### **III- Bacteriological parameters :**

#### **1- Aerobic plate count (APC):**

The presented data in fig. (7) revealed that the muscles of the whole and gutted of both newly caught (zero time) *Bagrus bajad* and *Anguilla anguilla* fish were free from any microorganisms. The microorganisms begin to appear in the second week of frozen storage in the both groups of the two fish species and gradually increased during the frozen storage period to become  $4.85 \times 10^4$ ,  $1.05 \times 10^4$ ,  $6.35 \times 10^3$  and  $1.65 \times 10^3$  /g. fish muscles respectively at the end of the storage period when the fish became distinctly spoiled.

From the aforementioned results it is cleared that the muscles of the newly caught fish were free from any microorganisms. These results were in agreement with those reported by Shewan (1962), Gram *et al.* (1989), Hayes (1992) and Graham *et al.* (1993).

From the previous results it is also illustrated that there was a significant increase in the APC occurring during the successive weeks of frozen storage. These results were in agreement with those reported by Hultin (1992) and Hassan (1998). On contrary, Bogges *et al.* (1971), Ilieva (1977), Magnusson and Martinsdottir (1990) and Kassem (2001) observed a significant decrease in APC with successive frozen storage

time.

**2- Pseudomonas species count:**

It is evident from the results achieved in figure (8) that the *Pseudomonas* species begin to appear in the second week of frozen storage in the whole and gutted *Bagrus bajad* fish muscles and slowly increased during the frozen storage period to become  $2.25 \times 10^4$  /g. (46.4% from APC) and  $5.65 \times 10^3$ /g (53.8% from APC). respectively at the end of the storage period when the fish became distinctly spoiled.

With respect to *Anguilla anguilla*, the presented data in the same figure revealed that the *Pseudomonas* species begin to appear in the six week of frozen storage in fish muscles of both groups and slowly increased through the frozen storage period to become  $2.30 \times 10^3$  (36.2% from APC) and  $8.75 \times 10^2$ /g (53.0% from APC) respectively at the end of the storage period when the fish became obviously spoiled.

**3- Lipolytic count:**

Data achieved in figure (9) pointed out that the lipolytic microorganisms begin to appear in the second week of frozen storage in the whole and gutted *Bagrus bajad* fish muscles and slowly increased during the frozen storage period to become  $4.50 \times 10^3$ /g. (9.3% from APC) and  $2.45 \times 10^3$ /g. (23.3% from APC) respectively at the end of the storage period when the fish became distinctly spoiled.

Regarding *Anguilla anguilla* fish the presented data in fig. (9) revealed that the Lipolytic microorganisms begin to appear in the six week of frozen storage in fish muscles of both groups and slowly increased through the frozen storage period to become  $2.35 \times 10^3$ /g. (37.0% from APC) and  $5.50 \times 10^2$ /g (33.3% from APC) respectively at the end of the storage period when the fish became obviously spoiled.

It could be concluded from the previous results that the percentage of lipolytic bacteria in whole and gutted *Anguilla anguilla* fish were much higher than that of both groups of *Bagrus bajad* fish. This difference in Lipolytic count may be due to the difference in fat content between both species, (17.16 and 16.97) and (7.34 and 7.41) in whole and gutted *Anguilla anguilla* and *Bagrus bajad* fish respectively. Concerning *Anguilla anguilla* fish, the same results were reported by Jayasinghe *et al.* (1997).

**4- Staphylococcus aureus, Coliforms and Vibrio parahaemolyticus:**

The achieved results revealed that the muscles of the whole and gutted *Bagrus bajad* and *Anguilla anguilla* fish were free from any *Staphylococcus aureus*, Coliforms and *Vibrio parahaemolyticus* microorganisms during all the frozen storage period. Same findings were

reported by many investigators (Shewan, 1977; Jayasinghe *et al.*, 1997; Hassan, 1998 and Kassem, 2001).

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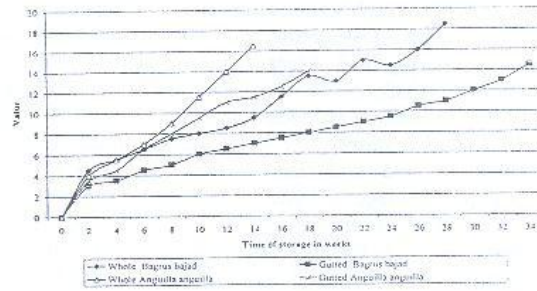


Fig ( 1 ) : Mean Organoleptic scores of whole and gutted Bagrus bajad and Anguilla anguilla fish during frozen storage at  $-18^{\circ}\text{C}$ .

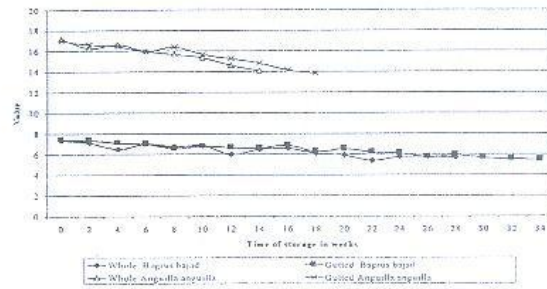


Fig ( 2 ) : Mean values of Fat content ( % ) of whole and gutted Bagrus bajad and Anguilla anguilla during frozen storage at  $-18^{\circ}\text{C}$ .

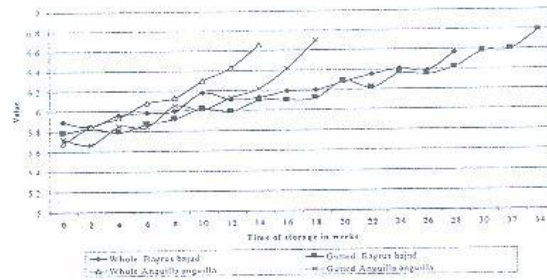


Fig ( 3 ) : pH Mean values of whole and gutted Bagrus bajad and Anguilla anguilla fish during frozen storage at  $-18^{\circ}\text{C}$ .

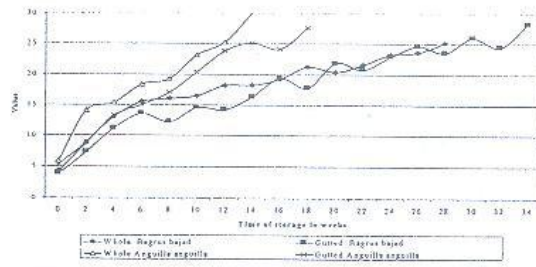


Fig. ( 4 ) : Mean values of TVB-N content ( mg/100g ) of whole and gutted Bagrus bajad and Anguilla anguilla fish during frozen storage at  $-18^{\circ}\text{C}$ .

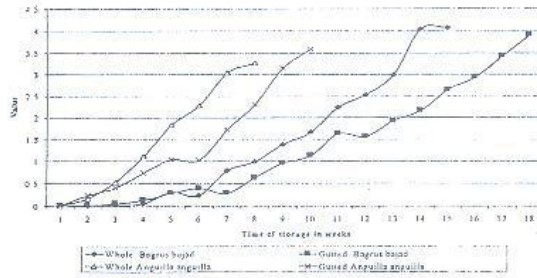


Fig. ( 5 ) : Mean TBA values ( mg MD/1000g ) of whole and gutted Bagrus bajad and Anguilla anguilla during frozen storage at  $-18^{\circ}\text{C}$ .

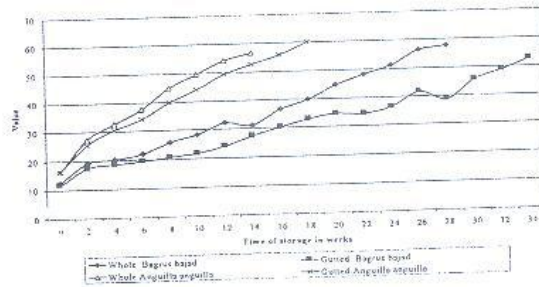


Fig. ( 6 ) : Mean K-values ( % ) of whole and gutted Bagrus bajad and Anguilla anguilla fish during frozen storage at  $-18^{\circ}\text{C}$ .



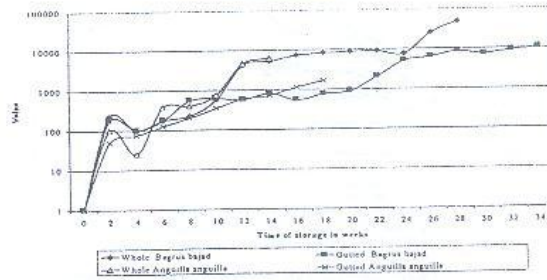


Fig. ( 7 ) : Mean APC ( count / g ) of whole and gutted Bagrus bajad and Anguilla anguilla fish during frozen storage at  $-18^{\circ}\text{C}$ .

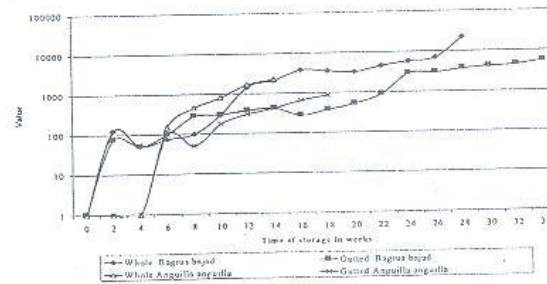


Fig. ( 8 ) : Mean counts of Pseudomonas species ( count / g ) of whole and gutted Bagrus bajad and Anguilla anguilla fish during frozen storage at  $-18^{\circ}\text{C}$ .

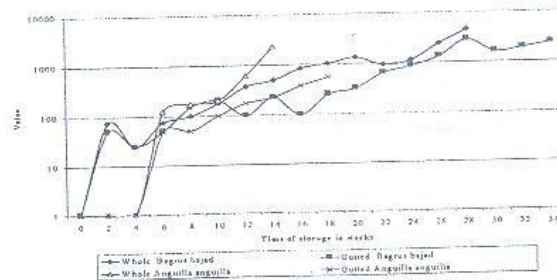


Fig. ( 9 ) : Mean counts of Lipolytic bacteria ( count / g ) of whole and gutted Bagrus bajad and Anguilla anguilla fish during frozen storage at  $-18^{\circ}\text{C}$ .