http://www.pjbs.org

PJBS

ISSN 1028-8880

# Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 4 (9): 1144-1148, 2001


# Studies on the Post-mortem Changes in Genetically Improved Farmed Tilapia (*Oreochromis niloticus*) During Ice Storage

L. Yasmin, [1]Kamal M., [1]S.A.K. Ahmed, K.M. Azimuddin , M.N.A. Khan and [1]M.N. Islam
Bangladesh Fisheries Research Institute (BFRI), Mymensingh 2201, Bangladesh
[1] Department of Fisheries Technology, Bangladesh Agricultural University (Bau), Mymensingh 2202, Bangladesh

**Abstract:** Studies were conducted on the post-mortem changes in genetically improved farmed tilapia (*Oreochromis niloticus*) during ice storage. Fish sample kept at room temperature, rigor started 1 hr. after spiking, reached full rigor (100%) within 3 hr which continued for 3 hr. . In ice stored fish, rigor started within one hour and attained full rigor (100%) within 2 hr that continued for 16 hrs. The pH of the muscle was about 7 immediately after catch and started to decrease gradually with the lapse of storage period. But the decrement of pH in samples stored at room temperature was much rapid than those stored in ice. Organoleptically the fish were in acceptable conditions for 16 days. The initial TVB-N and peroxide values were 5.3 mg/100g of fish and 5 meq/kg of fish respectively. These values gradually increased with the lapse of storage time but remained within the recommended limit up to 16 days. $Ca^{2+}$ ATPase activities in presence of 0.1M KCl and 0.5 M KCl were 0.349 and 0.139 μmol.pi/min. mg, respectively, and decreased gradually with storage period. $Mg^{2+}$ ATPase activities in presence and absence of $Ca^{2+}$ were 0.418 and 0.183 μmol.pi/min. mg respectively. The myofibrillar solubility decreased gradually from around 85.33% to 38.6% at the end of the 16 days of ice storage. The bacterial loads in muscle of ice stored GIFT varied from $7.6 \times 10^3$ to $7.1 \times 10^3$ cfu/g at 2nd day of storage and then gradually increased with storage period. At the end of the 16 days of ice storage, bacterial load increased to $4.6 \times 10^6$ cfu/g and at this stage the fish were organoleptically in acceptable condition. After 18 days of storage the bacterial load was $3.8 \times 10^8$ cfu/g that exceeded the acceptable recommended limit.

**Key words:** GIFT, rigor-mortis, storage, quality

## Introduction

Genetically Improved Farmed Tilapia (GIFT) (*Oreochromis niloticus*) is a high yielding variety and very delicious fish, resulted from genetic development of tilapia. The production of GIFT is about 50% more than tilapia and there is a great potential of developing various valuable products from GIFT. The quality of fish is influenced by a number of factors of which the most important one is the post-mortem changes and development of rigor-mortis soon after death. There are three stages of rigor mortis: pre-rigor, in-rigor, and post-rigor. The time involved in each stage of development, duration and subsequent resolution of rigor mortis depends on many factors such as species, physical condition, size of the fish, the degree of exhaustion before death, catching method, the amount of post-harvest handling and the temperature at which it is kept. It is generally accepted that the longer the duration of rigor, the longer is the shelf life of fish. Thus a prolongation of rigor mortis period, consequently, is of great economic importance. The state of rigor in association with other biochemical changes influence the meat quality of fish and higher animals (Penny, 1967). The progress of rigor-mortis in association with ATP depletion and lactate accumulation is dependent on temperature and varies from species to species. It is generally accepted that low temperature delays the onset of rigor-mortis but several tropical fish species, such as tilapia, red sea bream and plaice are reported to have a shortened pre-rigor period when stored at 0 $^0$C (Iwamoto *et al.*, 1988, Iwamoto and Yamanaka, 1986).

Considerable information is available on the keeping qualities of most of the species from temperate region but the scientific and practical knowledge is very limited on commercially important tropical fish species. An essential pre-requisite for designing in infrastructure for fish handling, storage, transport and marketing is to know how long each particular species in the catch will keep edible condition under various storage conditions.

Very few studies have been conducted on the post-mortem changes of freshwater fish in storage condition in contrast to the lot of information that is available for marine fish species.

## Materials and Methods

**Fish sample:** Live specimens were obtained from pond of BFRI, Freshwater Station, Mymensingh and were transported to the Department of Fisheries Technology, Faculty of Fisheries, Mymensingh. For rigor mortis study, three live specimens (total length 10-20 cm) were selected randomly and after cranial spiking, the fish were stored in ice in an insulated box (1:1). The box had a number of holes at the bottom to drain out the melted water. The samples were obtained with time interval for organoleptic and biochemical assessment.

**Determination of rigor index:** "Rigor index" of the fish was measured according to the method described by Bito *et al.* (1983) and used as a parameter of rigor tension. At selected time intervals, rigor index was calculated by the following formula:

$$\text{Rigor index (\%)} = \frac{D_0 - D}{D_0} \times 100$$

Where $D_0$ and D represent the distances of the base of caudal fin from horizontal line of the table at the start of the experiment and at subsequent storage period respectively.

**Organoleptic quality assessment:** Sensory methods were used to assess the degree of freshness based on organoleptic characteristics such as odour, colour, general appearance, eyes, slime and consistency of flesh. These characteristics were judged by panel members and the changes in quality of chilled fish during storage were assessed at every alternate

day. The grading of fish was done using score on the characteristics, following EC freshness grade for fishery products with slight modification (Howgate *et al.*, 1992) to judge the quality of fish (Table 1A and B).

**pH measurement:** Two grams of fish muscles were homogenized with 10 ml distilled water in a warring blender and the pH was measured using a pH meter (Corning Model 250).

**Total volatile base nitrogen (TVB-N) Determination:** Total volatile base nitrogen (TVB-N) was determined according to the standard methods described by EC (1995).

**Method for peroxide value estimation:** The peroxide value was determined according to the method of Lima dos Santos *et al.* (1981).

**Myofibril preparation and ATPase assay:** Myofibrils were prepared from ordinary muscles immediately after excision according to Perry and Grey (1956), with slight modification. $Ca^{2+}$-ATPase activity in presence of 0.1M and 0.5 M KCl and $Mg^{2+}$-ATPase activity in presence of $Ca^{2+}$and absence of $Ca^{2+}$(EGTA) were assayed according to the standard method.

**Myofibrillar protein solubility:** Two ml of myofibrillar suspension (5mg/ml) was homogenized with 2 ml of 1M KCl plus 100 mM phosphate buffer (pH 7.0) using a homogenizer. The homogenate was allowed to stand at refrigerated temperature (4 °C) overnight. The suspension was centrifuged for 30 min at 400 x g in a refrigerated centrifuge. The protein in supernatant was determined by the Biuret method (Gonnall *et al.*, 1949).

**Aerobic plate count (APC) of the ice stored SIS samples:** About 10-15 g of whole fish samples were blended with appropriate volume of 0.2% peptone water in warning blender for few minutes until a homogenous slurry is prepared. Total aerobic plate count (APC) expressed as colony forming units per gram of shrimp muscle (cfu/g) of the representative samples were determined by standard plate count methods on plate count agar (Hi-media, India).

## Results and Discussion

Rigor-index of GIFT stored in ice (0 °C) and at room temperature are shown in Fig. 1 and 2, respectively. Fish sample kept at room temperature, started rigor, 1 hr after spiking and it reached full rigor (100%) within 3 hr. The state of full rigor continued for 3 hr and then started to relax from rigor. Almost complete relaxation was occurred within 16 hr. after death. In ice-stored fish, rigor started in tilapia within one hour. Rigor increased gradually with the lapse of storage time. The fish sample attained full rigor (100%) within 2 hours and the state of full rigor continued for 16 hours and then started to relax from rigor. Fifty-percent relaxation was found at 66 hours after death and almost complete relaxation, at 102 hr after death without showing any sign of spoilage. The values of standard errors for this study were in the range of 0.82 to 6.65 during rigor mortis progress. The results obtained in present study are in agreement with some previous reports which stated that several fish species such as tilapia, red sea bream and plaice had short pre rigor periods, when stored in ice (0 °C) (Poulter *et al.*, 1981; Iwamoto and Yammanaka, 1986), although it is quite known that the progress of rigor

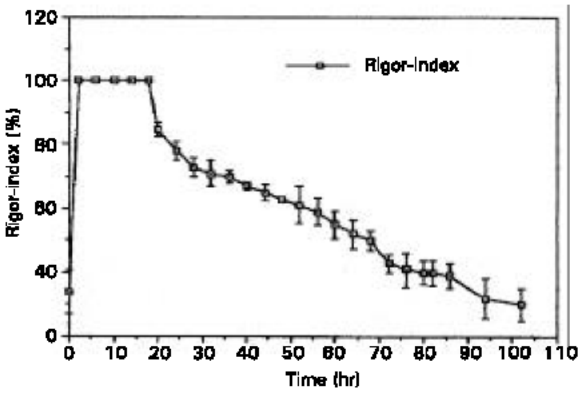


Fig. 1: Rigor-mortis progress in Tilapia during ice storage

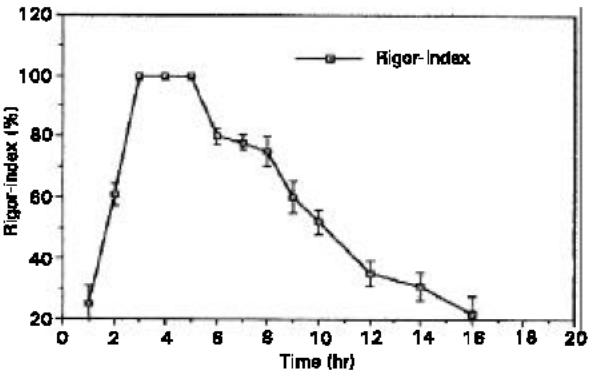


Fig. 2: Rigor-mortis progress in Tilapia stored at room temperature (25°C)

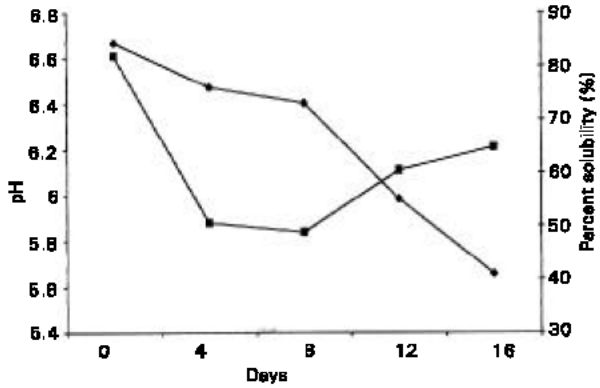


Fig. 3: Changes in pH and solubility during ice storage in Telapia

mortis is influenced by various factors such as species, size, catching method, handling of the fish and storage temperature. A small range of variation in rigor mortis progress, duration and relaxation from rigor was observed within the same species of GIFT.

The results of the organoleptic quality assessment of GIFT during ice storage in an insulated box are presented in Table 2. The quality of fish was graded using the score from 1-5. On

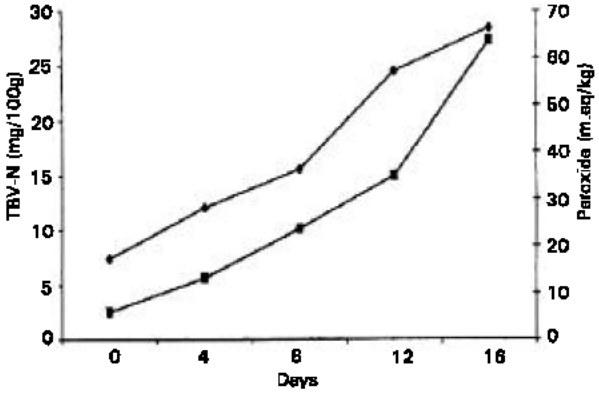


Fig. 4: Changes of TBV-N and peroxide value during ice storage of Telapia

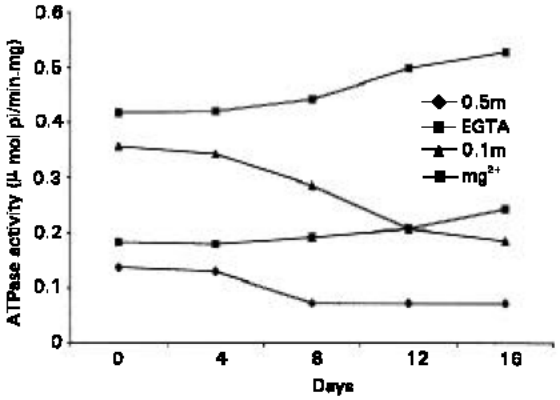


Fig. 5: Changes in myofibrillar ATPase activities of Tilapia during ice storage

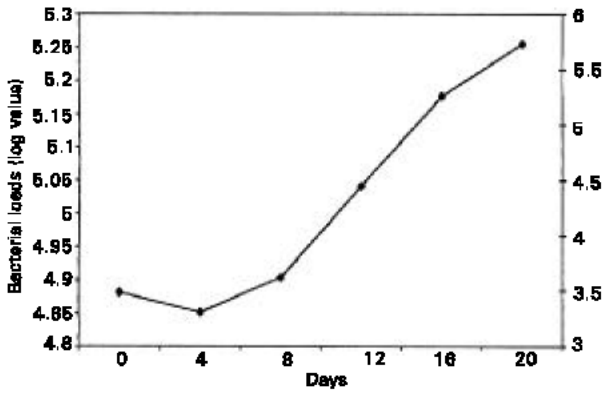


Fig. 6: Changes in bacterial and coliform loads during ice storage in Telapia.

the basis of the scores the fish were found in acceptable conditions for 16 days in ice storage. During this period, changes in quality can roughly be divided into 4 phases corresponding to 0 to 4, 5 to 8, 9 to 12 and 13 to 16 days in ice. Little or no changes occurred in phase 1 without loss of natural flavour and odour but in phase 2 there was little

Table 1A: Grading of fresh fish.

| Grade | Points | Degree of freshness |
|---|---|---|
| A | <2 | Excellent/Acceptable |
| B | <2 to 5 | Good/Acceptable |
| C | 5 | Bad/Rejected |

deterioration without showing definite signs of spoilage and off-flavour. There were signs of early spoilage with slight off-flavour in phase 3 and in phase 4 the fish begins to taste stale, its appearance and texture begins to show signs of spoilage and moderate sour odour in gills and body cavity. The results obtained in present study are in agreement with those reported for Indian major carps and other commercial fish species where the fish samples were found in acceptable conditions for 2-3 weeks (Bandyopadhyay *et al.*, 1986; Kamal *et al.*, 1994; Faruk, 1995).

The pH of the muscle was about 7, immediately after catch. It started to decrease gradually with the lapse of storage period regardless the storage temperature (0°C or 25°C) but the decrement in samples stored at room temperature was much rapid than those stored in ice (Fig. 3). The ultimate pHs were 5.98 and 5.86 for the samples stored at room temperature and ice, respectively after 16 hr of storage. It can be concluded that when rigor sets quickly and its duration is shorter, pH values decline much faster than that of rigor develops slowly and last longer. Studies were also conducted on the changes in solubility during ice storage. As shown in Fig. 3, the solubility decreased gradually from around 85.33% to 38.6% at the end of 16 days of ice storage. The result is in agreement with that reported by Seki *et al.* (1979) for carp where myofibrillar solubility decreased from 95 to 20% during 2 to 3 days of ice storage.

The results of TVB-N (mg/100g) and peroxide value (m.eq/kg of oil) are presented in Fig. 4. The initial TVB-N values were 5.3 mg/100g, which gradually increased with the lapse of storage period. At the end of 16 days of ice storage TVB-N values increased to 28.4 mg/100g, which is within the range of recommended values of 25 to 30 mg TVB-N/100g for fresh fish. However, at the end of 18 days, the TVB-N values were 35.2 mg/100g, that exceeded the recommended values (Connell, 1980). The increase in TVB-N with the lapse of storage may be attributed to bacterial spoilage.

The initial peroxide values were below 5 m.eq/kg of oil, which increased gradually with the lapse of storage period. At the end of 16 days of storage, the peroxide values were 19.2 m.eq/kg of oil which was within recommended values of 10-20 m.eq/kg of oil as suggested by Connell (1980). At the end of 18 days of storage, the peroxide values were 41 m.eq/kg of oil that exceeded the recommended values.

Changes in ATPase activity of myofibrillar protein of GIFT during 16 days of ice storage at 0 $^{o}$C were examined (Fig. 5). Different curves were obtained for different ATPase assays which all changed continuously with storage period. $Ca^{2+}$ ATPase activities in presence of 0.1M KCl were 0.349 µmol.pi/min. mg which were more or less stable up to first 4 days of storage and then gradually decreased with storage period. After 16 days of storage, the ATPase activity in presence of 0.1M KCl declined to 0.183 µmol.pi/min. mg. The initial $Ca^{2+}$ ATPase activities in presence of 0.5 M KCl was 0.139 µmol.pi/min mg, which gradually decreased to 0.076 µmol.pi/min. mg, at the end of 16 days of storage. However, the large fall of myofibrillar $Ca^{2+}$ ATPase activities during ice storage indicated denaturation of muscle protein. $Mg^{2+}$ ATPase activities in presence and absence of $Ca^{2+}$ (EGTA) were 0.418 and 0.183 µmol.pi/min. mg respectively. The

Table 1B: Determination of defect points.

| Characteristics of whole fish | Defect characteristics | Defect points | Grade |
|---|---|---|---|
| **Odour of neck when broken** | a) Natural odour | 2 | Acceptable |
| | b) Faint or Sour odour | 5 | Rejected |
| **Odour of gills** | a)Natural odour | 1 | Excellent |
| | b)Faint sour odour | 2 | Acceptable |
| | c)Slight moderate sour odour | 3 | Acceptable |
| | d)Moderate to strong sour odour | 5 | Rejected |
| **Colour of gills** | a)Slight pinkish red | 1 | Excellent |
| | b)Pinkish red or brownish red, some c)mucus may be present | 2 | Acceptable |
| | d)Brown or gray colour covered with mucus | 3 | Acceptable |
| | e)Bleached; thick yellow slime | 5 | Rejected |
| **General appearance** | a)Full bloom; bright; shining; iridescent | 1 | Excellent |
| | b)Slight dullness and loss of bloom | 2 | Acceptable |
| | c)Definite dullness and loss of bloom | 3 | Acceptable |
| | d)Reddish lateral line; dull; no bloom | 5 | Rejected |
| **Eye** | a)Bulging with protruding less; transparent eye cap | 1 | Excellent |
| | b)Slight clouding of lens and sunken | 2 | Acceptable |
| | c)Dull, sunken, cloudy | 3 | Acceptable |
| | d)Sunken eye covered with yellow slime | 5 | Rejected |
| **Consistency of flesh** | a)Firm and elastic | 1 | Excellent |
| | b)Moderately soft and some loss of elasticity | 2 | Acceptable |
| | c)Some softening | 3 | Acceptable |
| | d)Limp and floppy | 5 | Rejected |

Table 2: Changes in organoleptic qualities of GIFT during ice storage in an insulated box.

| Days of storage | Organoleptic qualities | Defect points | Grade | Overall qualities |
|---|---|---|---|---|
| 0 | Fresh, bright appearance, soft and firm texture with characteristics of natural fishy odour | 1.25 | A | Excellent |
| 4 | A decrease in the brightness; slightly softer texture, natural fishy odour | 2.1 | B | Acceptable |
| 8 | Some loss in brightness; slight loss of the natural flavour some slime in surface | 2.3 | B | Acceptable |
| 12 | Slimy surface and soft texture; considerable loss of flavour odour | 2.8 | B | Acceptable |
| 14 | Soft texture and slime on surface; moderate loss of flavour and odour | 3.5 | B | Acceptable |
| 16 | Fish has dull appearance with blood In the limit and slime on surface; texture begin to show obvious signs of spoilage | 4.4 | B | Acceptable |
| 18 | The fish is putrid by all of the characteristics | 5.0 | C | Rejected |

activities almost remain unchanged during first 4 days of ice storage and then increased gradually with the increase in storage period. Seki *et al.* (1979) reported that carp myofibrillar $Ca^{2+}$ and $Mg^{2+}$ ATPase activities decreased gradually with the exception that EGTA modified $Mg^{2+}$ ATPase activity increased during 16 days of ice storage. Seki *et al.* (1980) reported that sardine myofibrillar EDTA-ATPase activity had lost about 70% activity in 1 day storage in ice.

The results of the study on total bacterial load in muscle (with skin) of tilapia during ice storage are shown in Fig. 6. The bacterial loads in muscle of ice stored GIFT was initially 7.6 x $10^3$ cfu/g which slightly decreased to 7.1 x $10^3$ cfu/g at 2nd day of storage and then gradually increased with storage period. At the end of 16 days of ice storage, bacterial load increased to 4.6 x $10^6$ cfu/g and at this stage the fish were organoleptically in acceptable condition. After 18 days of storage the bacterial load was 3.8 x $10^8$ cfu/g which exceeded the acceptable recommended limit. During the whole period of study very few coliforms were detected in fish samples. The initial decrease in bacterial population in fish muscle after the first day of storage might be due to some sorts of cold shock or leaching of surface flora by washing with melted ice. This is in agreement with those reported for some ice stored fresh water fish (Bandyopadhyan *et at.*, 1985).

In conclusion the results of the above studies revealed that the duration of rigor-mortis was faster at room temperature than that in ice. The pH of muscle of GIFT was about 7 immediately after catch which decreased gradually with the lapse of storage period regardless the storage temperature (0°C or 25°C) but the decrement in samples stored at room temperature was much rapid than those stored in ice. Freshly caught GIFT was found in acceptable condition up to 16 days in ice storage in an insulated box by deteriming organoleptic, TVB-N, peroxide values and bacterial loads. There was a large fall of ATPase activities and solubility of myofibrillar proteins during 16 days of ice storage, which indicated denaturation of muscle protein.

## References

Bandyopadhyan, J.K., A.K. Chattopadhyan and S.K. Bhattacharyya, 1985. Studies on the ice storage characteristics of commercially important freshwater fishes. In: Harvest ad Post-harvest Technology of Fish. Society of Fisheries Technologists (India). Cochin, India, pp: 381-385.

Bandyopadhyan, J.K., A.K. Chattopadhyan and S.K. Bhattacharyya, 1986. Studies on the ice storage characteristics of *Catla catla* and *Labeo fimbriatus*. Fish. Tehnol. Soc., 23: 140-142.

Bito, M., K. Yamada, Y. Mikumo and K. Amono, 1983. Studies on rigor-mortis of fish-1. Differences in the mode of rigor-mortis among some varieties of fish by modified cuttings method. Bull. Tokai Reg. Fish. Res. Lab., 109: 89-96.

Connel, J.J., 1980. Quality deterioration and extrinsic quality defects in raw material In: control of fish quality, 2nd. ed. Fishing News books Ltd. Surrey, England, pp: 31-35.

European Communities (EC), 1995. Determination of the concentration of volatile nitrogenous bases (TVBN) in fish and fish products: A reference procedure. Official Journal of the European Communities. Commission Decision, P:15.

Faruk, M.A.R., 1995. Studies on the post-mortem changes in Rohu fish (*Labeo rohita*) M. Sc. Thesis, Dep. Fish. Technol. Bangla. Agric. Univ., Mymensingh, Bangladesh, p: 47.

Gornall, A.G., C.J. Bardawill and M. M. David, 1949. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem., 751-766.

Howgate, P., A. Johnston and K.J. Whittle, 1992. Multilingual guide to EC freshness grades for fishery products. Torry Research Station, Food Safety Directorate, Ministry of Agriculture, Fisheries and Food, Aberden, Scotland.

Iwamoto, M. and H. Yamanaka, 1986. Remarkable difference in rigor-mortis between wild and cultured specimens of the red sea bream. Nippon Suisan Gakkaishi, 52: 125-279.

Iwamoto, M., H. Yamanaka, H. Abe, H. Ushio, S. Watanabe and K. Hashimoto, 1988. ATP and creatine phosphate breakdown in spiked plaice muscle during storage. J. Food Sci., 53: 1662-1665.

Kamal, M., S. Gheyasuddin, S.C. Chakraborty, M.A. Hossain, M.A.R. Faruk and M.I. Hossain, 1994. Development for handling, transportation and processing of high quality hilsa fish. Studies on organoleptic characteristics on the quality changes in hilsa during ice-storage. BAU. Res. Progr. No. 8.

Lima, dos Santos, C.A.M., 1981. The storage of tropical fish in ice – a review. Trop. Sci., 23: 97-127.

Penny, I.F., 1967. The influence of pH and temperature on the properties of myosin. Biochem. J., 104: 609-615.

Perry, S. V. and T.C. Grey, 1956. A study on the effect of substrate concentration and certain relaxing factors on the magnesium activated myofibrillar adenosine triphosphate. Biochem. J. 64: 184-192.

Poulter, F.G., C.A. Curran and J.G. Disney 1981. Chill storage of Tropical and Temperate Water Fish-Differences and Similarities. In " Advances in Technology in the Chilling, Freezing, Processing, Storage and Transport of Fish, Especially Underutilized Species". Int. Inst. Refrigeration, Paris, pp: 111-123.

Seki, N., Y. Oogane and T. Watanabe, 1980. Changes in ATPase activities and other properties of sardine myofibrillar proteins during. Nippon Suisan Gakkaishi, 46: 697-615.

Seki, N., M. Ikeda and N. Narita, 1979. Changes in ATPase activities of myofibrillar proteins during ice storage. Nippon Suisan Gahhaishi, 45: 791-799.