



Changes in Electrophoretic patterns of Sarcoplasmic Proteins and Myofibrillar Proteins in *Caranx melampygus* (Cuvier, 1833) during chilled storage

Femeena Hassan and K. V. Nija

ICAR-Central Institute of Fisheries Technology, P. O. Matsyapuri, Cochin - 682 029, India

Abstract

SDS-PAGE studies were conducted to apprehend the changes in the electrophoretic pattern of myofibrillar and sarcoplasmic proteins of *Caranx melampygus* (Cuvier, 1833) (Blue fin Trevally) during chilled storage. The study revealed that the intensity of Myosin bands were getting reduced during storage. The relative front of myosin band was getting decreased during storage. The relative front on initial day for myosin is 0.331 and it decreased to 0.303 on 12th day of storage. In protein profile, molecular weights of protein bands were found to be 191.8 kDa, 99.1 kDa, 52.0 kDa for myofibrillar proteins on initial day, and 200.0 kDa, 101.4 kDa, 51.5 kDa on 12th day. Molecular weights of protein bands were found to be 97.4 kDa, 60.3 kDa, 50.9 kDa, 30.4 kDa, 29.2 kDa, 26.8 kDa, 25.6 kDa, 21.5 kDa for sarcoplasmic proteins on initial day and 97.4 kDa, 61.1 kDa, 52.1 kDa, 45.8 kDa, 34.6 kDa, 30.0 kDa, 26.1 kDa, 21.5 kDa respectively on 12th day. The number of bands for sarcoplasmic proteins and myofibrillar proteins of *Caranx melampygus* were same on initial day and final day of storage (9 and 3 respectively).

Keywords: SDS- PAGE, salt soluble protein, water soluble protein

Introduction

Fish, which is the primary source of animal protein is more susceptible to spoilage and hence should be preserved immediately after capture. It is estimated that 60% of people in developing countries obtain

40-100% of the animal protein in their diets from fish (Clucas & Ward, 1996). Protein content varies from one species to another. Denaturation is the most characteristic phenomena associated with proteins giving rise to definite changes in chemical, physical or biological properties. The degradation of fish meat is mainly caused by the enzymatic proteolysis of the myofibrillar proteins (Jasra et al., 2001). The quality deterioration in frozen products was found to be due to the deteriorative biochemical reactions in lipids and proteins (Suzuki, 1981). Long-term storage in fish leads to alteration in textural properties termed denaturation-aggregation (Shenouda, 1980; Jiang & Lee, 1985).

In recent times, chilled fish has become a popular product in supermarkets worldwide. Urbanisation and improvements in the quality of life has made the market for chilled seafoods promising. However, the shelf life of fish during chilled storage is short due to its high water activity, neutral pH, high amino acid content, bacteria and autolytic enzymes. The postmortem changes in fish muscle results in deterioration of quality and brings down its shelf life. On frozen storage of fish, as a results of protein denaturation, particularly of myofibrillar proteins changes in texture like extra firmness, springiness, toughness, dryness, loss of water holding capacity etc. happens. About 65% of the protein fraction of fish comprises myosin and actin. Most of the changes in frozen storage occur in the myosin-actomyosin system (Sirkorski et al., 1976). Jiang & Lee (1985) reported that during frozen storage of fish, the myofibrillar proteins of muscle gets aggregated in to high molecular weight polymers. The functional and textural properties of fish mainly depends on myofibrillar proteins (Goll et al. 1971) and hence change in myofibrillar proteins affects the quality of fishery products. Fish proteins contain many bioactive peptides that can be used for

Received 05 July 2018; Revised 30 October 2020; Accepted 30 October 2020

*E-mail: femeenahassan@rediffmail.com

metabolic activities. They are having influence on water holding capacity, oil absorption, gelling activity, foaming capacity and emulsification property. Chilled storage of fish changes the texture and functional properties.

All the way through chilled storage, one of the most imperative quality attributes of fish muscle is the degradation of muscle proteins caused by proteolysis. There is a link between protein oxidation and degradation of myofibrillar proteins, and their proteolysis in fish, which is stimulated by oxygen radicals during postmortem chilled storage. The present study was an attempt to seek changes in protein structure on chilled storage of *C. melampyus*. Fish sarcoplasmic proteins contain myogens and enzymes which are soluble in water or low ionic strength solution. During ice storage of fishes, the oxidation of sulphhydryl groups was found to form mainly MHC dimers between myosin rod portions. The changes taking place in actomyosin, the major portion of myofibrillar proteins is responsible for its functional role. Sompag et al. (1996) stated that during ice storage, the sulphhydryl content of the actomyosin increased while the solubility decreased. Because of these changes in protein, the functional properties also changes during storage in ice and hence the studies of changes in sarcoplasmic and myofibrillar proteins on chilled storage have relevance. In the present study this aspect was looked into in *C. melampyus*, which is a commercially important tropical fish.

Materials and Methods

Blue fin Trevally, *C. melampyus* weighing 150 -180 g were procured in fresh condition from Cochin Fisheries Harbour and were iced immediately in 1:1 ratio and brought to the laboratory in insulated boxes. Then it was de-iced, washed in potable water, re-iced in 1:1 ratio and kept in chilled store (0-2°C). After initial sampling in real time (fresh), periodic sampling were done in predetermined intervals viz., on third day, sixth day, ninth day and twelfth day for analysis. Sarcoplasmic and myofibrillar proteins were estimated as per Sankar & Ramachandran (2000).

Sodium dodecyl sulfate polyacrylamide gels (SDS-PAGE) were run using a BIO-RAD protein 16cm model system (Richmond, CA). The gel concentration was 7.5%. The solutions were made as per Yates & Greaser (1983). All the chemicals were procured

from BIO-RAD grade. The protein samples were mixed with sample buffer containing 10% SDS, 0.4 ml β -mercaptoethanol, 0.4 ml 0.5% bromophenol blue, 1.0 ml TrisHCl pH 6.8, 1.6 ml glycerol, 3.0 ml distilled water in the ratio 1:4. Heat at 95°C for four minutes. 10 μ l of protein was loaded on to the gel. After running of electrophoresis, the gel was stained with 0.1% Coomassie Brilliant Blue R-250 in 10% CH_3COOH and 40% methanol). Molecular weights of protein bands were determined using a high molecular or low molecular weight standard. The molecular weight of the protease was determined by comparison of the migration distances of standard marker proteins consisting of myosin, 200 kDa; galactosidase, 116 kDa; phosphorylase b, 97.4 kDa; serum albumin, 66.2 kDa; ovalbumin, 45.0 kDa; carbonic anhydrase, 31 kDa; trypsin inhibitor, 21.5kDa and lysozyme, 14.4 kDa.

Results and Discussion

The study revealed that the number of bands for sarcoplasmic proteins were same on the initial day and 12th day of storage (number of bands-9). Also the number of bands for myofibrillar proteins are same on the initial day and 12th day of storage (number of bands -3). In the electrophoretic pattern, the molecular weight of myofibrillar proteins were 191.8, 99.1, 52.0 kDa on initial day and 200.0, 101.4, 51.5 kDa on 12th day of storage. Similarly for sarcoplasmic proteins, the molecular weight were 97.4, 60.3, 50.9, 30.4, 29.2, 26.8, 25.6, 21.5, 21.5 kDa and 97.4, 61.1, 52.1, 45.8, 34.6, 30.0, 26.1, 21.5, 21.5.kDa respectively on 12th day of storage.

Change in sarcoplasmic and myofibrillar proteins of *C. melampyus* on chilled storage is shown in Table 1.

Table 1. Change in sarcoplasmic and myofibrillar proteins of *Caranx melampyus* on chilled storage

Days of storage	Myofibrillar protein content (%)	Sarcoplasmic protein content (%)
0	78.40	38.21
3	49.94	31.40
6	46.03	30.69
9	42.03	28.71
12	38.42	23.68

The myofibrillar protein content is 78.40% on initial day and it is decreased to 38.42% of total protein on 12th day of storage. The sarcoplasmic protein content is 38.21% and it is decreased to 23.68% on 12th day of storage. In *C. melampygu*s the percentage decrease in sarcoplasmic proteins is less compared to myofibrillar proteins during postmortem chilled storage. High molecular weight polymers via disulfide cross linking and/or degradation/digestion by proteolysis could be formed in the myofibrillar proteins during postmortem chilled storage. This suggest that there is a probable link between protein oxidation and protelytic degradation of myofibrillar proteins during postmortem storage, and the effects of chilled storage on oxidation and possible changes in myofibrillar and sarcoplasmic proteins could be different. Myofibrillar protein (MFP) solubility showed an increasing trend up to 9 days of storage followed by a decrease. This could be connected to the probable unfolding of MFP revealing the hydrophobic groups to the periphery. Another potential contributing aspect could be the development of the cross link between myosin and actin in

the muscle. Existence of structural proteins like M-line and Z-line proteins, which are accountable for the integrity of muscle structure, decrease the solubilization of myofibrillar proteins in fish. Solubility of sarcoplasmic protein (SPP) showed a decreasing trend throughout the chilled storage period. Drop in the solubility of SPP could be related to its instability due to aggregation behavior under the conditions of chilled storage.

The relative front on initial day for myosin is 0.331 and it is decreased to 0.303 on 12th day of storage. Fig. 1 and Fig. 2 represent the electrophoretic pattern of sarcoplasmic and myofibrillar proteins of *C. melampygu*s respectively. In Fig. 1 the lanes numbering from 1 to 6 denotes the low molecular weight protein marker (M), 0th day, 3^d day, 6th day, 9th day and 12th day of storage for sarcoplasmic proteins of *C. melampygu*s and in Fig. 2 the lanes numbering from 1 to 6 denotes the 12th day, 9th day, 6th day, 3rd day, 0th day of storage and high molecular weight protein Marker (M) respectively for myofibrillar proteins of *C. melampygu*s.

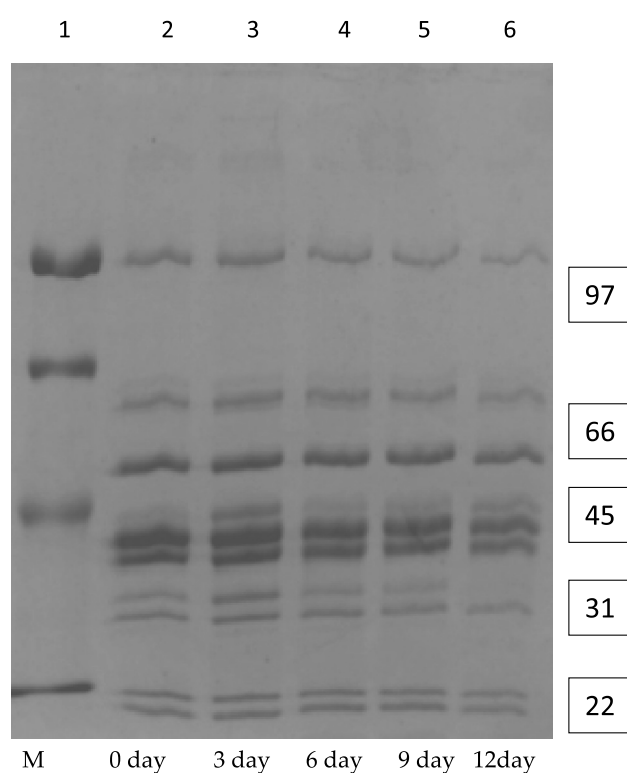


Fig. 1. SDS- PAGE profiles of Sarcoplasmic proteins from *Caranx melampygu*s

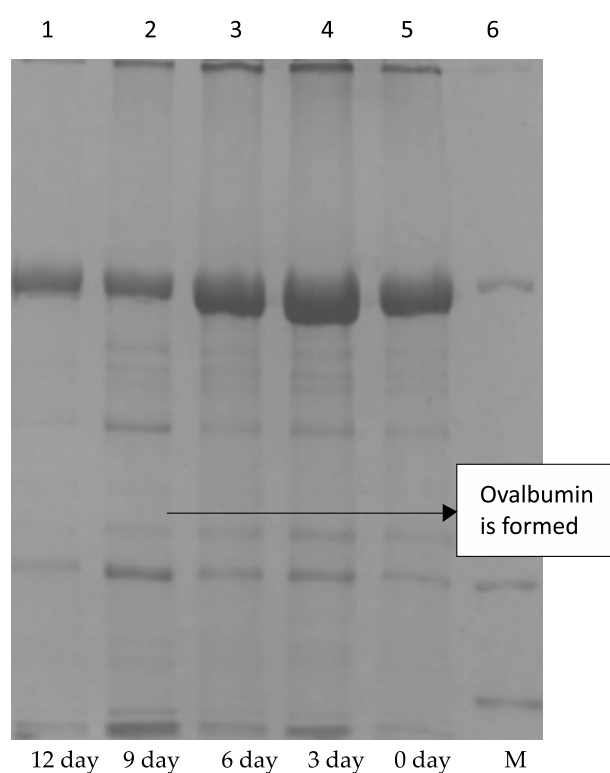


Fig 2: SDS-PAGE profiles of Myofibrillar proteins from *Caranx melampygu*s

In Fig. 1 the lane 1 represents the low molecular weight protein marker consists of Phosphorylase b (MW-97.4 kDa), BSA (MW-66.2 kDa), Ovalbumin (MW-45.0 kDa), Carbonic anhydrase (MW-31.0 kDa), Trypsin inhibitor (21.5 kDa) and the lane 2 represents the electrophoretic pattern obtained on initial day and it consists of 9 bands of molecular weights 97.4, 60.3, 50.9, 30.4, 29.2, 26.8, 25.6, 21.5, 21.5 and relative front in the order 0.272, 0.486, 0.582, 0.691, 0.718, 0.771, 0.800, 0.916, 0.941 respectively. The lane 3 represents the 3rd day of storage consisting of 10 bands of molecular weights 97.4, 60.5, 51.0, 44.0, 30.5, 29.2, 26.6, 25.4, 21.5, 21.5 with relative front 0.276, 0.484, 0.580, 0.652, 0.689, 0.718, 0.775, 0.805, 0.923, 0.948 respectively. The lane 4 denotes the 6th day of storage consisting of 10 bands with molecular weights 97.4, 61.4, 51.8, 45.4, 31.0, 29.6, 27.1, 25.7, 21.5, 21.5 with relative front 0.269, 0.476, 0.571, 0.645, 0.679, 0.708, 0.765, 0.797, 0.914, 0.938 respectively. The lane 5 represents the 9th day of storage consisting of 10 bands with molecular weights 97.4, 61.2, 52.0, 45.3, 33.1, 29.9, 27.3, 25.9, 21.5, 21.5 and its relative front obtained are 0.276, 0.477, 0.570, 0.647, 0.674, 0.701, 0.760, 0.792, 0.914, 0.936 respectively. The lane 6 represents the 12th day of storage consisting of 9 bands with molecular weights 97.4, 61.1, 52.1, 45.8, 34.6, 30.0, 26.1, 21.5, 21.5 and its relative front obtained are 0.274, 0.479, 0.568, 0.640, 0.671, 0.699, 0.788, 0.916, 0.939 respectively. From the electrophoretic pattern obtained for sarcoplasmic proteins of *C. melampyngus*, a new band corresponding to 45.0 kDa is obtained during storage. On the initial day, a band corresponding to molecular weight (MW) 45.0 kDa (i.e. corresponding to molecular weight protein marker Ovalbumin) is not seen. But during storage it is produced. Also, the intensity of bands corresponding to 26 kDa was decreasing during storage. The degradation of low-abundance proteins can alter protein configuration in fish during chilled storage. The increase in hydrophobic interactions and disulfide bonds during chilled storage can decrease both the immobilized water content and mobility, which results in the deterioration of fish quality. The Ca²⁺, Mg²⁺ and EDTA ATPase activities decreased during ice storage. The Ca²⁺ ATPase activity decreased considerably indicating the loss of integrity of myosin structure. The Mg²⁺ ATPase activity decreased only marginally indicating that no structural alteration has taken place in actin myosin complex

In Fig. 2 the lane 6 represents the high molecular weight marker consists of Myosin (MW-200.0kDa),

b-galactosidase (MW-116.2 kDa), Phosphorylase b (97.4 kDa), BSA (MW-66.2) and Ovalbumin (MW-45.0 kDa). The lane 5 represents the initial day of storage consisting of 3 bands with molecular weights 191.8, 99.1, 52.0 with relative front 0.331, 0.740, 0.969 respectively. The lane 4 represents the 3rd day of storage consisting of 5 bands with molecular weights 188.1, 136.7, 107.7, 99.3, 51.5 kDa and its relative front are 0.342, 0.526, 0.677, 0.739, 0.971 respectively. The lane 3 represents the 6th day of storage consisting of 4 bands with molecular weight 191.8, 108.1, 99.7, 53.0 and its relative front are in the order 0.331, 0.674, 0.735, 0.966 respectively. The lane 2 represents the 9th day of storage consisting of 6 bands with molecular weights 198.3, 138.3, 108.6, 99.7, 61.3, 52.5 kDa and its relative front are 0.311, 0.519, 0.671, 0.735, 0.942, 0.968 respectively. The lane 1 denotes the 12th day of storage consisting of 3 bands with molecular weights 200.0, 101.4, 51.5 kDa and its relative front are 0.303, 0.723, 0.971 respectively. During storage of *C. melampyngus*, the intensity of myosin band in the myofibrillar proteins is getting decreased during storage. During storage of myofibrillar protein, the bands corresponding to molecular weight 107.7 kDa (relative front 0.677), 108.1 kDa (relative front 0.674) and 108.6 kDa (relative front 0.671) are produced on 3rd, 6th and 9th day of storage respectively which are not seen on initial day and final day (12th) of storage. On 9th day of storage, a band corresponding to 61.3 kDa is obtained and it represents the BSA (MW-66.2kDa) of high MW protein marker. From the study, it is revealed that some new bands are developing and some are diminishing during storage of *C. melampyngus* under chilled condition. This indicates that the chilled storage might cause degradation or digestion of the myofibrillar proteins by proteolysis. Also the oxidation may results in reduced band intensity of some myofibrillar and sarcoplasmic proteins, suggesting its cleavage into smaller peptides, or by cross-linking through non-disulphide covalent bonds to form higher molecular weight polymers. Yi Sun et al. (2018) also supports this view on studying the texture characteristics of Mandarin fish (*Siniperca chuatsi*) during chilled storage.

From the study, it is concluded that during chilled storage of the fish *C. melampyngus*, some new bands are produced (bands of molecular weight 108 kDa in myofibrillar proteins and 45 kDa in sarcoplasmic proteins) and some bands are diminished (band of molecular weight 26.8 kDa) and the protein percent

is decreased during storage. But the number of bands on initial day and final day of storage for sarcoplasmic proteins and myofibrillar proteins are same (9 bands for sarcoplasmic proteins on initial and final day and 3 bands for myofibrillar proteins on initial and final day of storage). There is no much variation in the intensity of bands corresponding to molecular weight 97.0 kDa (Phosphorylase b), and bands corresponding to trypsin inhibitor of molecular weight 21.5. There are two bands corresponding to trypsin inhibitor throughout the storage of *C. melampygu*, but its relative front were different.

References

- Clucas, I. J. and Ward, A. R. (1996) Post-Harvest Fisheries Development: A Guide to Handling, preservation, processing and Quality. Natural Resources Institute (NRI), U. K
- Goll, D. E., M. H. Stromer, R. M. Robson, J. Temple, B. A. Eason, and W. A. Busch (1971) Tryptic digestion of muscle components simulates many of the changes caused by postmortem storage. J. Animal Sci. 33: 963-982
- Jasra, S.K., Jarsa, P.K. and Talesara, C.L. (2001) Myofibrillar protein degradation of carp (*Labeo rohita*) muscle after post-mortem unfrozen and frozen storage. J. Sci. Fd. Agric. 81: 519-524
- Jiang, S. T., Ho, M.L. and Lee, T. C. (1985) Optimization of the freezing conditions on mackerel and amberfish for manufacturing minced fish. J. Food Sci. 50: 727-732
- Sankar, T.V. and Ramachandran, A. (2000) Biochemical and storage characteristics of myofibrillar protein (Surimi) from freshwater major carps. Doctoral thesis, Cochin University of science and Technology, Cochin
- Shenouda, S. Y. K. (1980) Theories of protein denaturation during frozen storage of fish flesh. Adv. Food Res. 26: 275-311
- Sompongse, W., Itoh, Y. and Obatake, A. (1996) Effect of cryoprotectants and reducing reagent on the stability of actomyosin during ice storage. Fish. Sci. 62: 110-113
- Suzuki, T. (1981) "Fish and Krill Protein Processing Technology." Applied Science Pub. Ltd., London. 30p
- Yates, L. D., and M. L. Greaser (1983) Quantitative determination of myosin and actin in rabbit skeletal muscle. J. Mol. Biol. 168: 123-141
- Yi Sun, Liang Ma, Mingsi Ma, Hong Zheng, Xiaojie Zhang, Luyun Cai, Jianrong Li & Yuhao Zhang (2018) Texture characteristics of chilled prepared Mandarin fish (*Siniperca chuatsi*) during storage. Int. J. Food Prop. 21: 242-254
- Yi Sun, Liang Ma, Mingsi Ma, Hong Zheng, Xiaojie Zhang, Luyun Cai, Jianrong Li and Yuhao Zhang (2018) Texture characteristics of chilled prepared Mandarin fish (*Siniperca chuatsi*) during storage. Int. J. Food Prop. 21: 242-254