



Journal of Biological Sciences

ISSN 1727-3048

science
alert

ANSI*net*
an open access publisher
<http://ansinet.com>

Influence of Ice Storage on the Gel Forming Ability, Myofibrillar Protein Solubility and Ca²⁺-ATPase Activity of Queen Fish (*Chorinemus lysan*)

M.I. Hossain, M. Kamal, M.N. Sakib, F.H. Shikha, M. Neazuddin and M.N. Islam
Department of Fisheries Technology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

Abstract: An investigation was carried out on the quality changes in surimi prepared from ice stored Queen fish (*Chorinemus lysan*) in time interval during 20 days of storage in ice storage condition and the ratio of the fish to ice was maintained 1:1. Mince was prepared in both washed and unwashed condition. To evaluate the gel-forming characteristics, a portion of the mince was washed with water containing 0.1% NaCl. Both washed and unwashed mince were ground with 3% NaCl for 20 min at 4°C. The ground paste was stuffed into polyethylene tube and incubated at various temperatures (40, 50 and 60°C) for 2 h. The quality changes during ice storage of mince prepared from the ice stored fish muscle were evaluated with time interval by determining the gel-forming ability, myofibrillar Ca²⁺-ATPase activity, protein solubility and pH value of fish muscle. The resulting gels were subjected to the puncture test, teeth cutting test and folding test. Maximum breaking force was obtained from both washed and unwashed mince at the incubation temperature of 50°C. The gel strength of both unwashed and washed meat paste gradually declined with lapse of storage period and washed meat paste showed higher gel forming ability than unwashed meat paste throughout the study period. Myofibrillar Ca²⁺-ATPase activity, protein solubility and pH of the ice stored muscles decreased from 0.847 to 0.309 $\mu\text{mol pi min}^{-1} \text{mg}^{-1}$, 87 to 12% and 6.69 to 5.89, respectively, during 20 days of ice storage.

Key words: Surimi, gel forming ability, temperature, ATPase activity, protein solubility

INTRODUCTION

Chilling by icing is an important method of short-term preservation and it is widely used for keeping the raw materials in good condition before use in any value added products. Storage of fish at low temperature sometimes causes insolubilization of myofibrillar proteins and loss of functional properties such as the kamaboko forming ability of fish meat. Myofibrillar proteins play an important role on the gel forming ability. Any change particularly denaturation of myofibrillar protein will affect the gel forming ability. Many studies have been reported on sardine and other fishes on fish muscle protein denaturation caused by various factors during ice storage which influences markedly the quality of kamaboko gel^[1,2].

Queen fish (*Chorinemus lysan*) is abundantly available in Bangladesh marine water but it has limited value in the fresh fish market due to less attractive in appearance and taste. This species might be provided an attractive source of raw materials for production of surimi. It is of interest to see the changes in solubility, myofibrillar ATPase activity and gel forming ability of queen fish during ice storage.

Myofibrillar ATPase activities have been widely used as measure of actomyosin integrity^[3]. Factors that cause denaturation or degradation of the protein can affect ATPase activities. Such activities have been widely used to monitor post-mortem changes during iced or frozen storage^[4,5]. Myofibrillar proteins are susceptible to degradation of lysosomal enzymes and calcium activated neutral proteinases^[6]. Therefore degradation of myofibrillar proteins can be indirectly measured by changes in ATPase activity, solubility.

The objectives of present study were to investigate the changes of gel properties of Queen fish surimi paste during ice storage and to study the changes of pH, protein solubility and ATPase activity of Queen fish muscle during ice storage.

MATERIALS AND METHODS

Raw materials: Queen fish (*Chorinemus lysan*) was collected from Cox's Bazar BFDC landing center. The fish samples were stored in an insulated box containing ice with the ratio of 1:1. The box had a hole at the bottom to drain out the melt water. Storage in the box consisted of a

bottom layer of ice about 5 cm thick layer of fish with ice and a final top layer again about 5 cm thick. The melted ice replaced every morning with fresh ice. At selected time intervals (0, 5, 10, 15 and 20 days) the samples were obtained for preparation of gel and myofibrils. Gel-forming ability, pH, Ca²⁺-protein solubility and ATPase activity of myofibrillar protein were assayed immediately after preparation of myofibrils.

Preparation of meat paste: The fishes were decapitated and gutted before washing by chilled water. Sufficient time was lapsed to drain out excessive blood. The washed fish was filleted very carefully eliminating scales, skin, red muscles, belly flaps and kidney tissue. The fillet was deboned, minced by a manually operated meat mincer. Remaining bones and connective tissue fibers were removed from the meat by fine mesh sieve. The mince was washed two times using chilled fresh water containing 0.1% NaCl. For washing the mince was stirred in 4 volumes of the washing solution for 2 min and then allowed to settle for 10 min before dewatering. The water was removed through bag made of cotton cloth at the pressure of 5 kg cm⁻² for 10 min and finally at 10 kg cm⁻² for 15 min. The unwashed and washed minces were ground with 3% NaCl and 20% iced water by a mortar for 20 min at 4°C. All the operation was done in cold condition.

Preparation of gel: The salt ground meat paste was then carefully stuffed into the heat stable polyethylene tube and the both ends of the tubes were tightened. The paste in the polyethylene tube was heated at 40, 50 and 60°C for 120 min in water bath. The resulting gels were taken out of the water bath and immediately kept in iced water for 1 h.

Preparation of myofibrils: Myofibrils were prepared from ordinary muscle immediately after excision according to Perry and Grey^[7] with slight modification. The muscle was chopped by meat grinder and chilled minced muscle (50 g) was homogenized for 1 min in 5 volumes of 39 mM borate buffer (pH 7.1) containing 25 mM KCl and 0.1 mM DTT. The homogenate was centrifuged for 15 min at 600×g. The residue obtained was again homogenized and centrifuged for 15 min. The light colored upper layer of residue consisting mainly of myofibril was recovered with small volume of 39 mM DTT. The suspension was centrifuged for 15 min to remove the supernatant. Myofibrils were diluted with 4 volumes of 39 M borate buffer (pH 7.1) containing 0.1M KCl and 0.1 mM DTT and coarse materials were removed by centrifugation again for 15 min at 600×g to sediment myofibrils. After the pellet was washed three times in the same way, myofibrils were suspended with a desired volume of 39 mM borate

buffer (pH 7.1) containing 0.1 M KCl to make a concentration of 10-15 mg mL⁻¹.

Measurement of gel strength: The gel-strength of the products was assessed by organoleptic methods. A 10 person panel as described by Poon *et al.*^[8] was constituted for the organoleptic assessment. Panelists were teachers and graduate students about the assessments and had participated in such organoleptic tests. The gel was removed from the tube and subjected to puncture test, folding test and teeth cutting test for physical measurement of the gel.

Puncture test: The test was done by removing the gels from the tube and cut into equal pieces of 2 cm. The breaking force of the gel was measured against the penetration of a ball type spherical plunger (6 mm diameter) on the pan of an electronic balance. The force in g required to break the gel by the plunger was recorded from the balance.

Folding test: For folding test a spherical disc of 1 mm thick gel was cut off and placed on the index and middle finger of the right hand, the disc was folded first into halves and then quarter with the help of thumb and index finger. The gel was graded using scores presented in Table 1, as suggested by Poon *et al.*^[8].

Teeth cutting test: The disc gel of same size used in folding test was supplied to the panelist to recognize the test by cutting it through incisor for teeth cutting test. Gel-strength was evaluated by the following numerical scores as suggested by Shimizu *et al.*^[9], which are presented in Table 2.

Ca²⁺-ATPase activity: The reaction mixture for Ca²⁺-ATPase assay contained 25 mM Tris, 5 mM CaCl₂,

Table 1: Grade used in the folding test of the gel

Grade	Results on folding
AA	No crack visible when disc is folded into quarter
A	No crack when disc is folded into half but one or more cracks or breaks are visible when folded into quarter
B	One or more cracks are visible when disc is folded into half
C	Breaks, but does not split into halves
D	Splits into halves when folded into half
O	Sample too soft to evaluate

Table 2: Score used in the teeth-cutting test of the gel

Scores	Characteristics of the gel
0-1	Paste or mud like gel
2-3	Very frail gel
4-5	Frail
6	Medium gel strength
7-8	Strong gel strength
9-10	Very strong gel

0.1 MKCl or 0.5 MKCl and 0.25 mg myofibril mL. ATPase activity was measured at 25°C for 6 min. After preparation of the reaction mixture, an appropriate quantity of myofibril suspension was pipette to the reaction mixture followed by two minutes pre-incubation. The reaction mixture was started by the addition of 1 mM ATP and 2 mL portion of the reaction mixture was withdrawn at different time intervals. The reaction was terminated by adding 1 mL of 10% trichloroacetic acid. The supernatant obtained by 5 min centrifugation at 3000×g was analyzed for the liberation of inorganic phosphate (Pi) according to the method described by Fiske and Subbarow^[10].

Myofibrillar protein solubility: Two milliliter of myofibrillar suspensions (5 mg mL⁻¹) were homogenized with 2 mL of 1 M KCl plus 100 mM phosphate buffer (pH 7.0) and tris buffer (0.6 M KCl-0.3 M Tris-HCl) using a homogenizer. The homogenate was allowed to settle at refrigerated temperature (4°C) overnight. The suspension was centrifuged for 30 min at 400×g in cold condition. The protein in supernatant was determined by the Biuret method^[11].

pH measurement: Two grams of Queen fish muscle was homogenized with 10 mL distilled water in a blender and the pH was measured using a pH meter (Corning model 250).

RESULTS AND DISCUSSION

Changes in gel forming ability during ice storage: To investigate the optimum heating temperature for gel-forming ability of Queen fish (*Chorinemus lysan*) both washed and unwashed fish paste in the polyethylene tube was heated in water bath at various temperatures of 40, 50 and 60°C for 120 min (Fig. 1). At 40°C the breaking force was found 425 and 534 g for unwashed and washed paste, respectively. The highest gel-forming ability in both washed and unwashed paste was found at 50°C for 120 min showing the maximum value 685 g for unwashed paste and 736 g for washed mince paste. The gel strength decreased with the rising of heating temperature at 60°C. This result is in agreement with Nowsad *et al.*^[12] who reported that heated for 120 min at 50°C in single-step heating treatment had the highest gel-strength of queen fish.

To evaluate the changes in gel-forming ability of Queen fish (*Chorinemus lysan*) during ice storage the fish paste in the polyethylene tube was heated in water bath at 50° for 120 min. Both washed and unwashed mince were used for this purpose. Initially the breaking force was found 730 g for washed mince and 628 g for unwashed

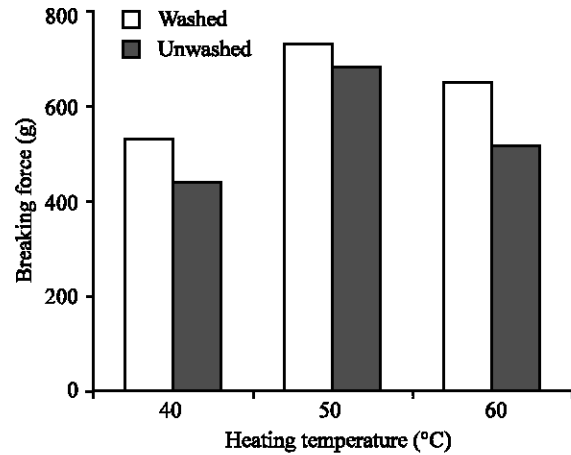


Fig. 1: Changes in gel forming ability of washed and unwashed meat of Queen fish (*C. lysan*) at different heating temperature

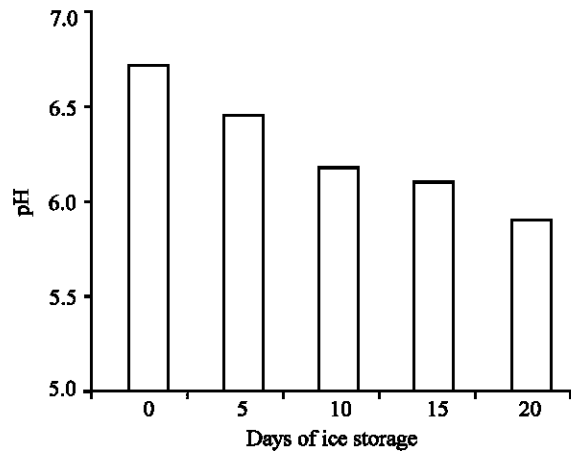


Fig. 2: Changes in pH of Queen fish (*C. lysan*) during ice storage

Table 3: Changes in gel-forming ability of washed and unwashed mince prepared from Queen fish (*C. lysan*) during ice storage

Temperature	Storage time in ice (days)	Breaking force (g)	Teeth cutting test	Folding test
120 min at 50°C				
Washed	1	730	8	AA
	5	683	8	AA
	10	545	7	A
	15	509	5	A
	20	345	4	B
Unwashed	1	628	8	AA
	5	482	7	A
	10	432	6	A
	15	324	5	B
	20	210	3	C

mince (Table 3). Breaking force of gel prepared from washed mince declined gradually from 730 to 683 g up to first 5 days of storage. The decrease in gel-forming ability was more rapid from 683 to 345 g during last 15 days of ice storage. On the other the breaking force of

unwashed mince (Table 3) decreased more rapidly 628 to 482 g up to first 5 day of ice storage period and gradually 482 to 210 g during last 15 days of ice storage.

Initially the folding tests have been judged as grade “AA” and at the end of experiment it was judged as grade “B” for washed mince and “C” for unwashed mince (Table 3). Teeth cutting score was “8” for both washed and unwashed mince initially which declined about “4” for washed mince and “3” for unwashed mince.

It was markedly evident that the breaking force i.e. gel-forming ability of washed mince was higher than unwashed mince. Washed mince surimi is always superior to unwashed mince in gel quality^[13]. Ismail *et al.*^[14] found higher gel-forming ability in the washed mince of silver carp and pangas than those of unwashed mince. Washing is necessary to remove water-soluble substances, mainly sarcoplasmic proteins, fat and other undesirable materials like pigments. The removal of sarcoplasmic proteins concentrate myofibrillar proteins, which is the primary component in the formation of three-dimensional gel structure, responsible for the gel forming ability of surimi.

From the study we found that gel-forming ability of Queen fish (*Chorinemus lysan*) decreased day by day during ice storage. The result obtained from the present study is in agreement with those reported for other fishes under similar storage condition^[15-17].

Changes in pH value during ice storage: The initial value of pH was 6.69, which decreased to about 5.89 at the end of the storage period (Fig. 2). The pH falls more rapidly during first 10 days of storage and then gradually declined with the lapse of storage period.

Many researchers agree that pH is one of the most important factors associated with changes in meat texture^[18]. According to Ishikawa *et al.*^[1] the rapid pH decline in sardine muscle during post-mortem ice storage markedly influenced the Kamaboko gel-forming ability. Fukuda *et al.*^[2] reported that pH value of chub mackerel decrease during ice storage. Shimizu *et al.*^[19] reported that the gel-forming ability of fresh fish muscle is optimal at neutral pH, decreasing with decrease in pH. In the present study the, lower pH value during ice storage is probably one of the contributing factors for poor gel-forming ability of the fish. It is apparent that at pH less than 6.0 the myofibrillar proteins during ice storage are unstable and rapidly lose the gel forming ability and also lose their ATPase activity, which is an indicator of gel forming ability.

Changes in protein solubility during ice storage: The solubility of Queen fish muscle protein was studied both in Tris-buffer (0.6 M KCl-0.3 M Tris-HCl, pH 7.6)

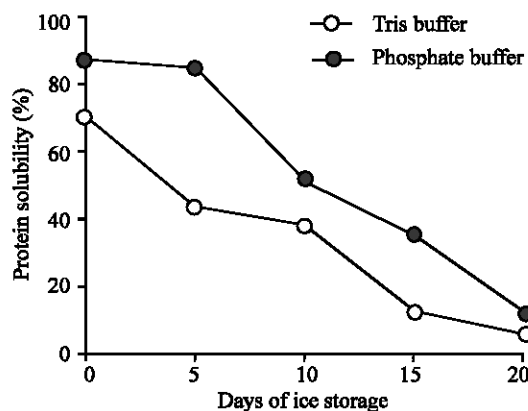


Fig. 3: Changes in protein solubility of Queen fish (*C. lysan*) muscle during ice storage

and phosphate buffer (1 M KCl plus 100 mM phosphate buffer, pH 7.0) as to measure the rate of denaturation of protein during 20 days of ice storage period at every five days of interval (Fig. 3). The initial solubility in phosphate buffer was 87%, remained unchanged during the first 5 days after that solubility declined sharply with the lapse of storage period and at the end of the storage time it declined to allow 12%.

Incase of Tris-buffer the initial protein solubility was 70.58%, which decreased rapidly with the lapse of storage time and fallen to about 5.92% at end of 20 days of storage period. A similar decrease in protein solubility has been reported by Joseph and Perigreen^[20]. Decrease in protein solubility was also observed by Gill *et al.*^[21] in red hake due to formation of products of trimethylamine and di-methylamine oxide by enzymatic reaction. Formation of disulphide bonds has been known to the loss of protein extractability during frozen storage of halibut mince^[22]. Jiang *et al.*^[23] found that solubility of milkfish actomyosin in 0.6 M KCl decreased during frozen storage.

From the present study, the decreased solubility in Queen fish indicated the aggregation as well as denaturation of proteins caused by ice storage. Tejada *et al.*^[24] reported that aggregates formed during frozen storage of minced cod are mostly linked by secondary interaction and disulfide bridges.

The present study clearly showed a relationship between the gel forming ability and the protein extractability of queen fish i.e. the gel strength decrease with the decreases in protein solubility during ice storage. Some studies indicated that the myofibrillar proteins of fish muscle aggregated into a high molecular weight polymer during low temperature ice storage or very low temperature storage^[23,25].

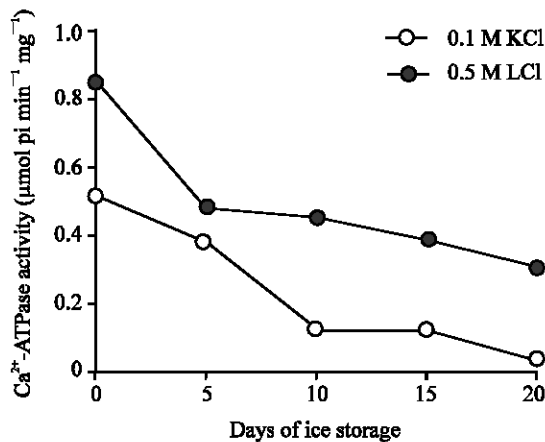


Fig. 4: Changes in Ca²⁺-ATPase activity of Queen fish (*C. lysan*) muscle during ice storage

From the result, the decreased solubility in Queen fish muscle during ice storage indicated the aggregation as well as denaturation of protein caused by low temperature storage.

Change in Ca²⁺-ATPase activities: The initial Ca²⁺-ATPase activity in presence of 0.1 M KCl was 0.847 µmol pi min⁻¹ mg⁻¹, which declined rapidly with the lapse of storage period (Fig. 4). Similarly, the initial activity in presence of 0.5 M KCl was 0.516 µmol pi min⁻¹ mg⁻¹, which also decreased sharply during storage period. The results obtained from the present study indicate the denaturation of myofibrillar proteins particularly myosin underwent denaturation during ice storage. The loss in ATPase activity was due to the tertiary structural changes caused by ice storage and increase in ionic strength of the system. Rearrangement of protein via protein-protein interactions was also presumed to contribute to the loss in ATPase activity^[26].

In the present study the measurement of Ca²⁺-ATPase activity was found good indicator in relation to breaking force to understand denaturation profile of Queen fish (*Chorinemus lysan*) muscle during storage. According to Bendall^[27] and Portzehl *et al.*^[28] the ATPase activity of myofibrillar protein reduces at saturation concentration of Ca²⁺ due to increase of acidity during post-mortem changes. Some lysosomal protease might be responsible for degradation of certain muscle proteins at low pH. Therefore, we hypothesized that the decrease in Queen fish myofibrillar Ca²⁺-ATPase activities due to decrease of pH value during 20 days ice storage. The results are in agreement with the finding of Kamal *et al.*^[4], the

decreased in fish myofibrillar-ATPase activities was reported to be directed function of pH.

In conclusion, maximum breaking force of Queen fish gel was obtained at the incubation temperature of 50°C. The gel strength of both unwashed and washed meat paste gradually decreased with the increase of ice storage time and washed meat paste showed higher gel forming ability than unwashed meat paste. Myofibrillar Ca²⁺-ATPase activity, protein solubility and pH declined with the lapse of storage period.

REFERENCES

1. Ishikawa, S., K. Nakamura and Y. Fuji, 1977. Fish jelly product (Kamaboko) and frozen minced meat (frozen surimi) made of sardine. I. Freshness and handling of the fish material affecting its kamaboko forming ability. Bull. Tokai Reg. Fish Res. Lab., 90: 59-66.
2. Fukuda, Y., Z. Tarakita and K. Arai, 1984. Effect of chub mackerel on the freeze-denaturation of myofibrillar proteins. Nippon Suisan Gakkaishi, 50: 845-852.
3. Roura, S.I., C. Montecchia, A.L. Goldemberg, R.E. Truco and M. Crupkin, 1990. Biochemical and physicochemical properties from pre- and post-spawned hake (*Merluccius hubbsi*) stored in ice. J. Food Sci., 55: 688-692.
4. Kamal, M., S. Watabe and K. Hashimoto, 1991. Post-mortem changes of myofibrillar ATPase of sardine ordinary and dark muscle. Nippon Suisan Gakkaishi, 57: 1177-1184.
5. MacDonald, G.A and T.C. Lanier, 1994. Actomyosin stabilization to freeze-thaw and denaturation by lactate salts. J. Food Sci., 59: 101-105.
6. Ouali, A. and C. Valin, 1981. Effect of muscle lysosomal enzymes calcium activated neutral proteinases on myofibrillar ATPase activity: Relationship with aging changes. Meat Sci., 5: 233-245.
7. 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.
8. Poon, K.H., P.Y. Lim, M.C. Ng and P.C. Ng, 1981. The suitability of leached meat of small demersal fish for making fish jelly products. Singapore J. Pri. Ind., 9: 28-37.
9. Shimizu, Y., R. Machida, and S. Takenami, 1981. Species variation in the gel forming ability characteristics of fish meat paste. Nippon Suisan Gakkaishi, 47: 95-98.

10. Fisk, C.H. and Y. Subbarow, 1925. The colometric determination of phosphorus. J. Biol. Chem., 66: 375-400.
11. Gomall, A.G., C.J. Bardawill and M.M. David, 1949. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem., pp: 751-766.
12. Nowsad, A.K.M., S.C. Chanda, S. Kanoh and E. Niwa, 2000. Gel forming ability and other properties of eleven underutilized tropical marine fish species. J. Aquatic Product Technology, 9: 71-89.
13. Suwansakornkul, P., Y. Itoh, S. Hara and A. Obatake, 1993. The gel forming characteristics of fish meat paste. Nippon Suisan Gakkaishi, 59: 1029-1037.
14. Ismail, M.I., M.M. Kamal, F.H. Shikha and M.S. Hoque, 2004. Effect of washing and salt concentration on the gel forming ability of two tropical fish species. Intl. J. Agril. Biol., 6: 762-766.
15. Haard, N.F. and J.E. Warren, 1985. Influence of Halding Fillets from Undersize Atlantic Cod (*Gadus morhua*) at 0 or 3°C on the Yield and Quality of Surimi. In Proceedings of the International Symposium on Engineered Seafood Including Surimi. R.E. Martin and E.V., Collette (Ed.). National Fisheries Institute, Washington, DC., pp: 92.
16. Noguchi, S., 1982. Science of Frozen Surimi I, II, III. In: Practical Technical Handbook for Kneaded Seafoods, Nippon. Shokuhin Kelzai-sha, Tokyo, pp: 40.
17. Kurokawa, T., 1979. Kamaboko-forming ability of frozen and ice stored lizard fish. Bull. Japan. Soc. Sci. Fish, 45: 1551.
18. Kramer, D.E. and M.D. Peters, 1981. Effects of pH and pre-freezing treatment on the texture of yellowtail rockfish (*Sebastes flavidus*) as measured by the Ottawa texture measuring system. J. Food Technol., 17: 493-504.
19. Shimizu, Y., W. Simidu and T. Ikeuchi, 1954. Studies on jelly strength of kamaboko-III. Influence of pH on jelly strength. Bull. Jap. Soc. Sci. Fish, 20: 209-212.
20. Joseph, J. and P.A. Perigreen, 1983. Studies on frozen storage of minced fish from thread fin bream. Fish Technol., pp: 20:13.
21. Gill, T.A., R.A. Keith and B. Smith, 1979. Textural deterioration of red hake and haddock muscle in frozen storage as related to chemical parameters and changes in the myofibrillar proteins. J. Food Sci., 44: 661.
22. Lim, H.K. and N.F. Haard, 1984. Protein insolubilization in frozen Greenland halibut (*Reinhardtius hippoglossoides*). J. Food. Biochem., 8: 163-187.
23. Jiang, S.T., D.T.Y. Chu, C.C. Lan and T.C. Lee, 1984. New approach to improve the quality of minced fish products using freeze-thawed cuttle fish. Proceeding of Chinese-American Academic and Professional Convention. S3.3.1-S3.3.5, Los Angeles, CA June 29-July 2, 1984.
24. Tejada, M., C.P. Torrejon, M.L. DelMazo, M.T. Solas, M.L. Garcia and C. Barba, 1996. Protein extracts and aggregates forming in minced cod (*Gadus morhua*) during frozen storage. J. Agric. Food Chem., 44: 3308-3314.
25. Anderson, M.L. and Ravesi, 1970. On the nature of the association of protein in frozen stored cod muscle. J. Food Sci., 35: 551.
26. Benjakul, S. and F. Bauer, 2000. Physicochemical and enzymatic changes of cod muscle proteins subjected to different freeze-thaw cycles. J. Food Sci. Agric., 80: 1143-1150.
27. Bendall, J.R., 1960. Post-Mortem Changes in Muscle. Structure and Function of Muscle Vol. III (Bourne G.H.M. Ed.), Academic Press, New York, pp: 227.
28. Portzehl, H., P. Zaoralek and J. Gaudian, 1969. The activation by Ca²⁺ of the ATPase of extracted muscle fiber with variation of ionic strength pH and concentration of Mg²⁺ ATP. Biochim. Biophys. Acta., 189: 440-448.