Fishery Technology
2005, Vol. 42(2) pp : 155 - 162

Changes in the Functional Properties of Tilapia (*Oreochromis mossambicus*) Protein During Storage in Ice

F. Partiban, T.V. Sankar and R. Anandan
Central Institute of Fisheries Technology
Cochin - 682 029, India

The effect of iced storage on the functional properties namely solubility, emulsion activity index (EAI), foaming, water holding capacity (WHC) and gel forming ability of tilapia protein was evaluated. The K-value of tilapia meat increased from 0.47% to 89% during the storage in ice for 14 days. The solubility of total salt soluble protein (TSP) decreased by 8% in 2 days, remained almost constant up to 7 days and then decreased further during the storage period. EAI (124 m^2/g) increased by 2.3 times during the storage of fish in ice up to 7 days. Only marginal increase in WHC and foaming efficiency were noted. There was a 70% decrease in the gel strength of tilapia meat during the 14 days storage in ice. Even though tilapia is acceptable for 10 days as far as sensory attributes are concerned, the functional properties of tilapia protein started decreasing after 7 days of ice storage.

Key words: Tilapia, functional properties, ice storage, sensory score

Proteins are functional components in processed food, where they contribute to texture and sensory characteristics besides the nutritional properties. Generally fish are kept in ice prior to processing to minimize spoilage. Degradation of muscle protein is a major problem associated with fish (Shenouda, 1980; Jiang et al., 1987; Reddy & Srikar, 1991). The myofibrillar protein contributing to 55-65% of total protein is the important protein fraction responsible for the physicochemical properties of the protein in a food system (Lanier, 1986). The properties of protein undergo changes during different condition to which the protein is exposed.

Post harvest changes in fish muscle affect the quality of protein and hence its functional properties. K value is commonly used as a biochemical index to monitor freshness of fish during processing and storage (Ryder, 1985; Bremner, 1981; Lakshmanan et al., 1996; Yongswawatdigul & park, 2002). K value is related to the degradation of nucleotides and the changes are related to storage temperature and are species dependent (Ehira & Uchiyama, 1986).

Tilapia (*Oreochromis mossambicus*) is a fast growing weed fish in the aquaculture system throughout the world. The total world production from both wild capture and aquaculture increased from 373000tons in 1980 to 1.85 million tons in 2000 (Roderick & Gillespie, 2003). The fish is mainly consumed fresh and has been reported to have good potential for value addition (Gopakumar, 1997). Understanding the functional properties of tilapia proteins is important to increase its utilization in a food system. Limited literature is available on change in functional properties in fish proteins during ice storage (Wang *et al.*, 2003; Roura *et al*, 1995; Reddy & Srikar, 1991) and frozen storage (Carech & Tejada, 1990; Huidobro & Tejada, 1992). The objective of the study is to investigate the functional properties associated with tilapia meat and correlate it with storage in ice.

Materials and Methods

Farm fresh tilapia (*Oreochromis mossambicus*) weighing 110 ± 15g and length 15 ± 2.5 cm were collected from a local brackish water culture pond and brought to the laboratory in iced condition. The fish were washed thoroughly with chilled water and kept in insulated box with ice (1:1). Ice supplementation was done as per the requirement. Samples were drawn every alternative day for a period of 14 d for analysis of changes in physicochemical properties of fish protein. Meat was separated from fish and kept at cold condition (<5°C) pending analysis.

Determination of K value

Muscle (5 g) taken from the anterior dorsal portion of the fish was homogenized in a polytron homogeniser at low temperature (<5°C) for 1 min with 25 ml of chilled 0.6 M perchloric acid (Ryder, 1985). The homogenate was centrifuged at 6000 rpm for 10 min at 4°C in a REMI cooling centrifuge (model CPR 24). 10 ml of the supernatant was neutralized to pH 6.8 with chilled 1M KOH. After keeping at 5°C for 30 min, the solution was filtered through a syringe filter (0.45µ m) and the filtrate was stored at –30°C for subsequent HPLC analysis.

A Shimadzu (LC – 10 ATVP) HPLC attached with Diode Array Detector (SPD – M 10 AVP) and a reverse-phase column (C18 RP – Phenomenax, USA) was used. The nucleotide separation was achieved by isocratic elution with phosphate buffer solution prepared by mixing of 0.04 M KH_2PO_4 and 0.06 M K_2HPO_4 in de-ionised water at a flow rate of 1.5 ml/ min. A 20ml of the sample was injected into the HPLC. Quantification was made by external standard method and K value was calculated as the ratio of the sum of hypoxanthine and inosine to total amount of ATP related compounds.

Biochemical Analyses

Total soluble protein (TSP) and Sarcoplasmic protein (SPP) were extracted as per the methods of King & Poulter (1985) and Sankar & Ramachandran (2001) respectively. Proteins were quantified by biuret method (Gornell *et al.*, 1949) using Bovine Serum Albumin as standard. The difference between total soluble protein and sarcoplasmic protein was taken as myofibrillar protein (Sankar & Ramachandran 2001). To determine the emulsion activity index (Pearse & Kinsella, 1978), viscosity and foaming (Wild & Clark, 1996), a 5mg/ml TSP solution was used. Refined sunflower oil was used as oil source for emulsion studies.

Water holding capacity of mince was carried out by the method of Kocher and Foedging (1993). Changes in viscosity were estimated using Schott Gerate CT 050 (Germany) as per the standard procedure. Heat - induced gels were prepared using washed mince according to a standard procedure (Lee, 1984) and the gel strength of the heat-induced gel was analysed with the help of a Food Texture Analyzer using a 10mm spherical probe using 5 kg load cell. The specification (test speed is 12m/min trigger 50gf and dispersion 10mm) has been given to computer software (Lee, 1984). Formability as foam expansion (%FE) was expressed as percentage volume increase after mixing (Wild & Clark, 1996).

Sensory analysis

The panel of five member evaluated freshness of fish based on the demerit score for every sensory factor (Branch & Vail, 1985). The different organoleptic factors taken in to consideration for sensory assessment were appearance, scale looseness, skin firmness, presence of slime, muscle stiffness, eye condition, gill condition, belly etc. The best quality of each factor was graded to zero and most unacceptable quality scores were 2 to 3.

Statistical analysis

Duncan's multiple comparison test using Windows based SPSS statistical software (SPSS Inc., Chicago, Illinois, USA) was employed to determine the significant difference in the functional properties with refers to storage period.

Results and Discussion

K value

The K-value shot up from 0.47% to 89% during the storage of fish in ice (Fig 1). The value was relatively low for the first four days, which started increasing from 7th day onwards up to 14 d ($p < 0.05$). Among the individual nucleotides IMP decreased gradually up to 7 days followed by sharp fall ($p < 0.05$). The decrease in IMP compared with Hx production and between different days showed significant difference ($p < 0.05$). In fresh tilapia the concentration of Adenosine Triphosphate (ATP), Adenosine diphosphate (ADP), and Adenosine monophosphate (AMP) were relatively low with 0.026, 0.026 and 0.04 m mol/ g tissue respectively. High concentration of Inosine monophosphate (IMP) was recorded indicating that the fish had stressed before death leading to rapid degradation ATP and ADP. IMP gradually decreased throughout the storage period. The accumulation of HxR (Inosine) was very low in tilapia while that of Hx (Hypoxanthine) started increasing significantly after 7th day (Fig 1).

Changes in HxR and Hx have been reported in threadfin bream during ice storage (Yongsawatdigul & Park, 2002). High accumulations of HxR over Hx have been reported in *Nemipterus furcous* during ice storage (Bremner *et al.*, 1988). This leads to infer that in tilapia the nucleotide degradation takes place through the formation of Hx and can be grouped under Hx forming category. Degradation of ATP, ADP, AMP was faster in mullet (*Liza corsula*) than pearl spot (*Eteroplus suratensis*) but the breakdown of HxR to Hx proceeds quickly in pearl spot (Laksmanan *et al.*, 1996). Gill (2000) showed the rate of IMP and Hx formation is the highest at temperature 20°C compared to 0 -15°C.

Based on K-value (35%) the freshness of threadfin bream stored in ice up to 12 d could be regarded as good quality (Yongsawatdigul & Park, 2002). K-value of prawn, squid, pomfret and rainbow sardine exceed 50% after 8 d of storage while in jew fish and ribbon fish, the increase was quite rapid during ice storage (Laksmanan *et al.*, 1996). The K-value from different fish varied from 45 to 89% (Lakshmanan & Gopakumar, 1999). In tilapia the K-value crossed 35% by 9th day indicating the quality of acceptance of the fish.

Solubility

In tilapia, the TSP contributed to 62g per 100g fish meat on dry weight basis. The myofibrillar proteins contributed to about 66% of TSP and the remaining sarcoplasmic proteins. The changes in solubility of protein during iced storage are given in Fig 2. The solubility of TSP decreased by 8% in 2 d and remained almost constant up to 7 d of ice storage. By 10th day TSP decreased further (10%) and by the end of the storage about

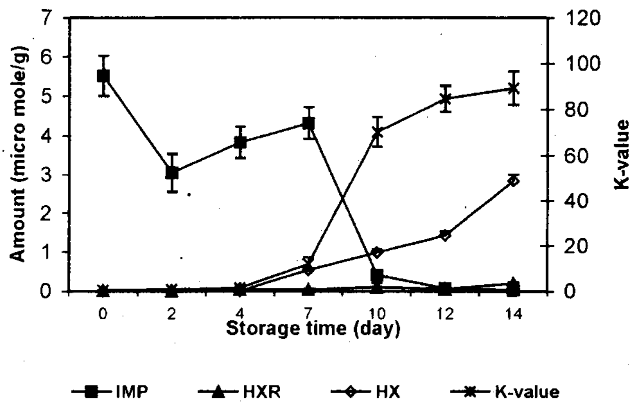

Fig 1. Changes in concentrations of IMP, HxR, Hx and K-value during ice storage

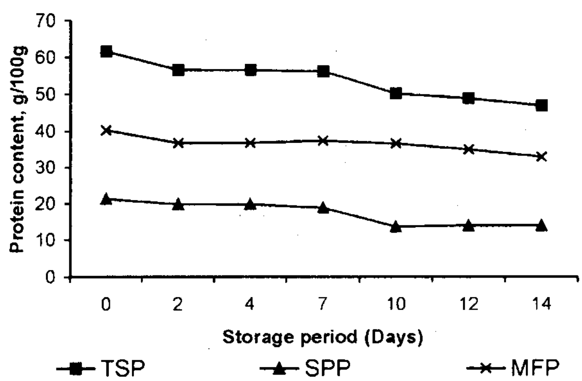

Fig. 2. Changes in protein solubility (DWB) during ice storage

24% of the total salt soluble protein becomes insoluble. Sarcoplasmic protein accounted for about 11.8% and myofibrillar protein 11.95% of this. A 4 % decrease on as is basis in total solubility was reported in pink perch while an 8% loss was reported in oil sardine during 16 and 20 days of storage in ice, respectively (Sarma *et al.*, 1999). The decrease in solubility could be attributed mainly to two factors namely the leaching out of the water soluble SPP in the melt ice and the aggregation and insolubilisation of myofibrillar protein fractions.

The insolubilisation of myofibrillar protein is related to its stability. The exposed hydrophobic residues as a result of protein unfolding could have affected the protein solubility (Wang *et al.*, 2003; Jiang *et al.*, 1988; Jiang *et al.*, 1989). Protein solubility in aqueous medium is essential for protein to impart in functional properties such as emulsion or foaming (Patel & Fry, 1987). Hydrophobic association with oxidised and hydrolysed products of lipids (Reddy *et al.*, 1991), oxidation and reduction of thiol groups, interaction of formaldehyde and secondary products of lipid oxidation with protein lead to denaturation (Shenouda, 1980; Suzuki, 1981).

Emulsion active index

The Emulsion Active Index (EAI) reflects the ability of the protein to rapidly absorb at the water oil interface during the formation of emulsion preventing flocculation and coagulation (Zayas, 1997). The emulsion capacity as EAI (m^2/g) gives an indication of oil utilized by the protein in order to stabilize the emulsion. EAI value was 124 m^2/g for extracted total soluble protein from fresh fish. The EAI increased by 2-3 times during the storage of fish in ice up to 7 d and then decreased.

The unfolding of the 3-D structure of protein at the storage temperature contributes to the increase in surface active SH groups, which facilitates the interaction of protein with non-polar portion leading to higher emulsion capacity (Matsudomi *et al.*, 1982). By the 10th day EAI decreased and continued till the end of storage period. The decrease in EAI could be related to the formation of disulfide bond between the protein molecules by the oxidation of SH groups exposed during protein unfolding during the initial periods of storage. A decrease in the emulsion capacity from the first day has been reported in pink perch and sardine during ice storage (Reddy & Srikar, 1991; Sarma *et al.*, 1999). The emulsion stability (ES) as represented by the time taken for the half O.D at 500nm, was 570 for the fresh sample, which increased marginally by 28% by 2nd day. The stability decreased by the 4th day and remained more less constant during the storage period.

Table 1. Changes in solubility, Emulsion active index, Water holding capacity and viscosity and sensory score

Storage days	TSP	SPP	EAI (m^2/g)	ES (Sec)	WHC (unit/g protein)	Viscosity mm^2/sec	Gel strength g.cm	Forming efficiency (%)	Organoleptic Score
0	61.58 ± 2.58^{a}	21.26 ± 1.53^{a}	124 ± 10.5^{c}	570 ± 5.0^{c}	2.8 ± 0.00^{b}	3.25 ± 0.00^{d}	710 ± 5^{a}	$176.6\pm2.037^{b,c}$	0
2	56.69 ± 2.29^{b}	$19.88\pm1.3^{a,b}$	183 ± 5.9^{b}	730 ± 5.0^{a}	3.0 ± 0.05^{ab}	3.44 ± 0.15^{b}	702 ± 12^{a}	$180\pm1.0^{b},^{c}$	6
4	56.62 ± 1.54^{b}	$19.77\pm1.18^{a,b}$	191 ± 21.0^{b}	450 ± 10.0^{d}	3.1 ± 0.2^{a}	3.55 ± 0.3^{a}	682 ± 7^{a}	186.67 ± 6.9^{b}	13
7	56.28 ± 2.03^{b}	18.85 ± 0.97^{b}	288 ± 18.0^{a}	$620\pm15.0^{b,c}$	2.8 ± 0.1^{b}	3.37 ± 0.01^{c}	682 ± 3^{a}	$200\pm10.0^{a,b}$	18
10	50.29 ± 0.85^{c}	13.66 ± 0.78^{c}	190 ± 10.0^{b}	$600\pm25.0^{b,c}$	2.7 ± 0.05^{b}	3.23 ± 00^{d}	641 ± 2^{b}	213.33 ± 18.0^{a}	19
12	$48.95\pm0.65c^{d}$	13.97 ± 0.62^{c}	189 ± 9.0^{b}	$610\pm10.0^{b,c}$	2.7 ± 0.19^{b}	3.12 ± 0.03^{e}	457.19 ± 26^{c}	160 ± 5.00^{c}	25
14	$46.95\pm0.76c^{d}$	13.99 ± 0.43^{c}	186 ± 10.0^{b}	$600\pm5.0^{a,b}$	2.7 ± 0.28^{b}	3.01 ± 0.03^{f}	220.94 ± 30^{d}	133.33 ± 25^{d}	28

Values with different letters within the columns are significantly different ($p<0.05$).

Foaming

The percentage volume increase after blending the protein solution in grinder is taken as foaming capacity for fresh fish. The foaming efficiency was 176% for fresh fish protein, which increased by 21% during storage up to 4 d and then decreased. The foam remains more or less constant up to 30 min in protein extracts from fresh un-iced fish, which gradually decreased with increase in storage period. The foaming capacity of tilapia compares well with that of horse mackerel and mackerel but lower than that of blue whiting (Huidobro & Tejada, 1992). The alteration of hydrophobicity and low molecular weight of peptides in protein hydrolysates were reported to improve foaming by forming stable interfacial layer (Wild & Clark, 1996). The protein that retains their tertiary structure at the interface will maintain extensive protein-protein interaction forming strong foam. The myofibrillar protein from tilapia is less effective as foaming agent comparing to whey protein (Webb *et al.*, 2002).

Water holding capacity

Water Holding Capacity (WHC) of food is the ability to hold its own or added water during the application of force (Zayas, 1997). In fresh tilapia the water holding capacity was about 2.8 g/g on dry mater basis. Binding of water is related to polar hydrophilic group in the primary structure. The WHC marginally increased up to 4 d of the storage and then decreased. However after 10th d there was no further decrease.

The decrease in water holding capacity after 2 h post mortem was observed by Kijowski *et al.*, (1982) and was reported to be due to denaturation of proteins. Decrease in water holding capacity during ice storage has been reported for pink perch and oil sardine (Sarma, 1999). The water, which is imobilised in the protein structure, is released as a result of protein denaturation consequent of protein unfolding. Post-mortem drop in pH has also influenced the water holding capacity (Zayas, 1997).

Gel strength

Gel strength is the setting of protein sol into a gel as a result of grinding with salt (Suzuki, 1981). The gel strength, a product of distortion and force, was 710g.cm for fresh fish, which decreased gradually with increase in length of storage in ice and a 70% loss in gel strength was noticed at the end of storage period (Table 1). Surimi with appreciable gel strength (641g.cm) could be made from tilapia stored for 10 d in ice. There was good correlation (0.823 at $P<0.01$) between the gel strength and total soluble protein. The low temperature setting of gel is related to the activity of a Ca^{2+} dependant transglutaminase enzyme while a stronger gel is formed as a result of the unfolding of protein and possible interaction of higher temperature (An *et al.*, 1996). Surimi with good gel strength could be obtained from white hake (*Urophycis tenuis*) after 2 d of ice storage (Albert *et al.*, 1991). A decline in gel strength of surimi was observed in ice-stored lizardfish after 3 d (Kurokawa, 1979) and in threadfin bream after 2 d of ice storage (Yean, 1993). Gel strength of Kamaboko made from lizard fish kept in ice for 3 d was 50% of that made from fresh fish due to the formation of formaldehyde and DMA (Kurokawa, 1979; Yasui & Lim 1987). Acceptable quality surimi was made from northern squaw fish stored after 9 d, (Lin & Morrissey, 1995), while good quality surimi could be made from hoki stored in ice up to 10d (Mac Donald *et al.*, 1990).

Sensory evaluation

The sensory evaluation of ice-stored fish by demerit scale system (Table 1) showed acceptability of the fish during the entire length of storage. Accordingly, the resolution of rigor took place by 4th day and the appearance slightly dull by 7th day. The fish has retained freshness up to 10 d as indicated by surface slime, vent fishy smell and firm skin. As storage days advanced the thickness of slime, discoloration and firmness of belly were noticed. The fish became unacceptable after 10^{th} day of storage in ice. Slight spoiled smell however, started by 14^{th} day. Studies indicated freshness of finfish started changing from 3^{rd} day onwards and there is a direct relation between freshness and species of fish (Lakshmanan *et al.,* 1997).

There are changes in the physicochemical properties of tilapia protein during ice storage due to changes in the characteristics of soluble proteins. The acceptability of the fish as per K value was 9 d and it compares well with the organoleptic score. However, the functional properties of tilapia protein started decreasing after 7 d of ice storage. Hence, this important observation needs to be taken in to account while utilizing tilapia for value addition.

References

Albert, R.E., Bligh, E.G. and Spencer, K., (1991). Influence of freshness on quality of whitehake (*Urophycis tenuis*) surimi. *Can. Inst. Food Sci. Technol.,* **24,** pp 36 – 41.

An, H., Peters, M.Y. and Seymour, T.A., (1996). Role of endogenous enzymes in surimi gelation. *Trends in food science and technology.* 7 pp 321-327.

Branch, A.C. and Vail, A.M.A., (1985). Freshness quality assessment for round fish. Bring fish inspection into computer age. *Food Technol. Aust.,* **37** pp 352 –355.

Bremner, H.A., Olley, J., Statham, J.A. and Vail, A.M.A., (1988). Nucleotide catabolism. Influence on the storage life of tropical species of fish from northwest shelf of Australia. *J. Food Sci.,* **53** pp 6-11.

Carech, M. and Tejada, M., (1990). The effect of neutral and oxidized lipids on the functionality in hake (*Merluccius merluccius*), a dimethyl and formaldehyde forming species during frozen storage, *Food chem.,* **36,** pp 113–128.

Ehira, S. and Uchiyama, H., (1986). Determination of fish freshness using the K value and comments on some other biochemical changes in relation to freshness. *In: seafood quality determination,* Proceedings of an International symposium coordinated by University of Alaska. (ed. Kramer, D.E. and Liston, J.). Elsevier Science publishers, Amsterdam, pp: 185-207.

Gill, T., (2000). Nucleotide-degrading enzymes. *In: Seafood Enzymes,* (ed. Haard, N.F. and Simpson, B.K.). Marcell, Dekker Inc., New York, pp. 37-68.

Gopakumar, K., (1997). *Biochemical composition of Indian food fish,* Central Institute of Fisheries Technology, Cochin, 44 pp.

Gornall, A.C., Bardawill, C.J. and David, M.M., (1949). Determination of serum proteins by means of biuret reaction, *J. Biol. Chem.,* **177** pp 751-766.

Huidobro, A. and Tejada, M. (1992). Foaming capacity fish muscle during frozen storage. *In:* Quality assurance in the fish industry (ed. Huss, H. H., et al.). Elsevier science publication, pp. 139 – 147.

Jiang, S.T., Hwang, B.S. and Tsao, C.Y., (1987). Protein denaturation and changes in nucleotides of fish muscle during frozen storage, *J. Agri. Food Chem.,* 35: 22-27.

Jiang, S.T., San, P.C., and Japit, L.S., (1988). Denaturation and changes in SH groups of actomyosin from milk fish during frozen storage at 20°C, *J. Agric. Food Chem.,* **36,** pp 433–437.

Jiang, S.T., San, P.C., and Japit, L.S. (1989). Effect of storage temperature on the formation of disulfide and denaturation of tilapia hybrid actomyosin (Tilapia nilotica x Tilapia aurea). *J. Agric. Food Chem.,* **37,** pp 633–436.

Kijowski, J., Niewiarowica A. and. Kujawska-Biernat. B., (1982). Biochemical and technological characteristics of hot chicken meat. *Food Technol.,* **17** pp 533.

King, D.R. and Poulter, R.G., (1985). Frozen storage of Indian mackerel (*Rastrelliger kanagurta*) and Big eye (*Priacanthus hamrur*). *Trop. Sci.*, **25** pp79-90.

Kocher, P.N. and Foegeding, E.A., (1993). Micro centrifuge based method for measuring water holding of protein gels. *J. Food Sci.*, **58** pp 1040-1046.

Kurokawa, T.,(1979). Kamaboko-forming ability of frozen and ice stored lizard fish. *Bull. Jap. Soc. Sci. Fish.*, **45** pp 1551-1555.

Lakshmanan, P.T. Antony, P.D. and Gopakumar, K. (1996) Nucleotide degradation and quality changes in mullet (Liza corsula) and pearl spot (Etroplus suratensis) in ice and at ambient temperature. Food Control, 7: (6), 227-283.

Lakshmanan, P.T. and Gopakumar, K. (1999) K-value, an index for estimating fish freshness and quality. *Cur. Sci.*, 76 (3): 400-405.

Lanier, T.C. (1986). Functional properties of surimi. *Food Tech.*, 40: 107-114.

Lee, C.M., 1984. Surimi process technology, *Food Tech.*, 38(11): 69-82.

Lin, D. and Morrissey, M.T., (1995). Northern squaw fish (*Ptychocheilus oregonensis*) for surimi production. *J. Food Sci.*, 60: 1245–1247.

Mac Donald, G.A., Lelievre, J. and Wilson, N.D.C. (1990). The strength of gels prepared from washed and unwashed minces of Hoki (*Macrerius novaeelandiae*) stored in ice. *J. Food Sci.*, **55** pp 976 – 978.

Matsudomi, N., Kato, A. and Kobayashi, K., (1982). Conformation and surface properties of deaminated gluten. *Agri. Biol. Chem.*, **46** pp 1583-1589

Patel, P.D. and Fry, J.C., (1987). Standardized methods for protein functionality. *In:* Development in food proteins. (ed. Hudson, B.J.F.)., Elsever Applied science, New York, pp. 302–324.

Pearce, K.N. and Kinsella, J.E. (1978). Emulsifying properties of protein evaluation by a turbidimetric technique. *J. Agri. Food Chem.*, **26** pp 716-723

Reddy, G.V.S. and Srikar, L.N. (1991). Preprocessing ice storage effects on functional properties of fish mince protein. *J. Food Sci.*, **56** pp 965–968.

Roderick, E. and Gillespie, D. (2003). Tilapia goes global, Seafood International, Oct 26-32.

Roura, S.I. and Crupkin, M. (1995). Biochemical and functional properties of myofibrils from pre and post spawned hake (Merluccius hubbsi Marni) stored on ice, *J. Food Sci.*, **60**, pp 269–272.

Ryder, J.M., (1985). Determination of adenosine tri-phosphate and its breakdown products in fish muscle by high performance liquid chromatography. *J. Agric. Food Chem.*, **33** pp 678-680

Sankar, T.V. and Ramachandran, A. (2001). Changes in biochemical composition in Indian major carps in relation to size. *Fish. Technol.*, **38** pp 22-27.

Sarma, J., Srikar, L.N. and Redddy, G.V. (1999). Effect of ice storage on the functional properties of pink perch and oil sardine meat. *J. Sci. Food Agri.*, **79** pp 169-172.

Shenouda, S.Y.K., (1980). Theories of protein denaturation during frozen storage of fish flesh. *Adv. Food Res.*, **26** pp 275-311.

Suzuki, T. (1981). *Frozen minced meat (Surimi). In:* Fish and krill proteins: Processing technology. Applied Sci. Publishers Ltd, London, UK, pp. 10-13.

Wang, H., Liceaga-Gesualdo, A.M. and Li-Chan, E.C.Y. (2003). Biochemical and physiochemical characteristics of muscle nad natural actomyosin isolated from young Atlantic salmon (Salmo salar) fillets stored ayt 0 and 4°C, *J. Food Sci.*, **68,** pp 784 –789.

Webb, M.F., Naeem, H.A. and Schmidt, K.A., 2002. Food protein functionality in a liquid system: a comparison of deaminated Wheat protein with dairy and soy proteins. *J. Food Sci.*, **67** pp 2896–2902.

Wild, P.J. and Clark, D.C., (1996). Foam formation and stability. *In:* Methods for testing protein functionality.(ed. Hall, G.M.). Blackie Acadamic and professional, London, England, pp 110-148.

Yasui, A. and Lim, P.T., (1987). Changes in chemical and physical properties of lizard fish meat during ice and frozen storage. *Nippon shokuhin kyogo gakkaishi*, **34** pp 54–60.

Yean, Y.S., (1993). The quality of surimi made from threadfin bream stored on ice for different periods. *Int. J. Food Sci. Technol.*, **28** pp 343-346.

Yongsawatdigul, J. and Park, J.W. (2002) Biochemical and conformation changes of actomyosin from threadfin bream stored in ice. *J. Food Sci.*, **67** pp 985-990.

Zayas, J.F., (1997). Functionality of proteins in food. Springer-Veerlag Berlin Heidelberg, **6**, 310-331, 81-100.