J Food Sci Technol (July–August 2010) 47(4):432–436
DOI: 10.1007/s13197-010-0071-8

ORIGINAL ARTICLE

Effect of sodium lactate as cryostabilizer on physico-chemical attributes of croaker (*Johnius gangeticus*) muscle protein

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Revised: 15 February 2010 / Accepted: 19 April 2010

Abstract Effect of sodium lactate as cryostabilizer on physico-chemical attributes of croaker (*Johnius gangeticus)* fish muscle protein was studied during freezing and frozen (–20 ± 2°C) storage for 3 months. Minced meat was mixed with 4% sucrose, 4% sorbitol, and 0.3% sodium tri poly phosphate (STPP) (T_1), minced meat was mixed with 6% (w/v) sodium lactate and 0.3% STPP (T_2) and control (C) was without any additive. The decreasing rate of Ca^{2+} ATPase activity, thaw drip, water holding capacity and relative viscosity in T_1 and T_2 samples from that of C was significantly lower, indicating higher protective effect of additives. In case of cryoprotectant treated samples, the degradation of myosin heavy chain was much lower than that of C which prevents the aggregation and subsequent insolubilization of myosin during frozen storage. The sodium lactate prevented Ca^{2+}ATPase activity more than that of sucrose/ sorbitol during isothermal storage at –20 ± 2°C for 3 months. This inferred that sodium lactate can effectively be used as an alternative cryostabilizer to sucrose/sorbitol for stabilization of croaker muscle protein native structure.

Keywords Protein denaturation · Dewatered mince meat · Physico-chemical properties · Frozen storage · Sodium lactate · Ca-ATPase activity

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Introduction

Freeze induced protein denaturation and related functional loss are commonly observed in frozen surimi products (Sikorski et al. 1976). Integrity of myofibrillar protein is of prime importance for surimi products (An et al. 1994). During freezing and/or frozen storage, the unfolding of myofibrillar proteins (mainly myosin) exposes non polar amino acids, which become available for formation of hydrophobic interactions with like groups in the vicinity. This process leads to protein aggregation, textural changes and loss of gelling and water-holding functionality in fish muscle (Niwa 1992). Although cryoprotectants such as sucrose, sorbitol and phosphates have been used in surimi industry (Pigott 1986), the problems of sweetness and high calorific content are of greater concern (Park and Lanier 1987, Park and Morrissey 2000) to which many Western consumers have found objectionable for certain product applications. Thus efforts have been made to select non-sweet additives with a cryoprotective effect equal to that of sucrose or sorbitol. MacDonald and Lanier (1994) reported the effect of sodium lactate on tilapia actomyosin and suggested that sodium lactate was potentially a good cryoprotectant and actomyosin stabilizer. Still not much of work has been taken up till date. Among various species, *Johnius* sp. dominate the catch along the coast of West Bengal and available throughout the year. Hence, for production of acceptable surimi, this underutilized lean fish has been used. With this backdrop, effect of sodium lactate as an alternative cryostabilizer on the physicochemical attributes of croaker muscle protein was studied during freezing and frozen storage which has reports of reduced sweetness and less Milliard browning reaction.

Materials and methods

Croaker (*Johnius gangeticus*, ~402 g) fish caught along Kakdwip coast of West Bengal was stored in refrigerated

seawater and off loaded within 12–16 h of capture. They were then iced on board and brought to the processing hall in an insulated icebox, immediately washed in chilled water and dressed. Meat was picked using roll type fish meat picker and minced. The minced meat was washed using chilled water as described by Gopakumar et al. (1992). Minced meat after washing and dewatering divided to experimental units to which following treatments were randomly assigned and the moisture content was adjusted to 81%: C (control, no treatment), T_1 (4% sucrose, 4% sorbitol, and 0.3% sodium tripolyphosphate, STPP) and T_2 (6% (w/v) sodium lactate and 0.3% STPP). Additives were incorporated into mince by mixing at low speed in a bowl chopper for 2 min. Surimi samples (500 g each) were divided into 7 equal blocks of 7 × 14.5 × 0.7 cm in size and packed in 7 low density polyethylene pouches (thickness: 200 gauge) separately for fortnight interval study, which were then subjected to freezing at (–35°C) in air blast freezer. Then they were stored at –20 ± 2°C in horizontal deep freezer. All experiments were done in triplicate.

Proximate composition analysis: Proximate (AOAC1995), total nitrogen (TN) and salt soluble nitrogen (SSN) (Dyer et al. 1950), water soluble nitrogen (WSN) (AOAC 1995), non-protein nitrogen (NPN) (Srikar and Chandru 1983), trimethyl amine (TMA) (Beatty and Gibbons 1937), peroxide value (PV) (Jacobs 1958) and free fatty acid (FFA) (Takagi et al. 1984) and total plate count (TPC) (APHA 1984) were determined. pH of meat was determined using pH meter (Mettler Toledo Pvt. Ltd., India) after mixing 10 g of minced meat with 50 ml distilled water.

Electrophoretic analyses of salt extractable protein: Myosin heavy chain (MHC) or actin degradation during frozen storage was examined by SDS-PAGE. Three g of muscle was homogenized with 20 ml of 5% NaCl in 40 mM Tris HCl buffer of pH 7. The mixture was homogenized at 1800 rpm for 1 min on ice in Sorvall Omnimixer (Ivan Sorvall Inc., New York). The homogenate was incubated at 85°C for 1 h to dissolve total proteins. The supernatant was decanted after centrifuging at 10,000 x **g** for 10 min at 4±2ºC and 10 ml sample was taken for SDS-PAGE analysis. Electrophoresis was carried out according to the procedure of Laemmli (1970). Samples of 40 µg protein per track, measured by the method of Lowry et al. (1951), were applied on 4% stacking gel and 10% separating polyacrylamide gel. Ten µl of high range molecular weight marker (Bangalore Genei, India) including rabbit muscle myosin (205,000 Da), phosphorylase b (97,400 Da), bovine serum albumin (66,000 Da), ovalbumin (45,000 Da) and bovine erythrocytes carbonic anhydrase (29,000 Da) were used as standard. Protein bands were stained in 0.125% Coomassie brilliant blue (R-250) and destained in 25% methanol and 10% acetic acid (v/v) solution. The gels were scanned in a Phast Image TM gel analyzer (TM II, Biorad, India). Quantitative analysis of protein bands was done for each lane in the electrophoretogram by analyzing the densitometric graph, which was made using a HP Deskscan II print using Phast Image software version 1.0. (Biorad, India). MHC and actin degradation of the 3 samples in the consecutive lane of electrophoretogram were calculated by the equation:

$$\% \text{ MHC or Actin degradation} = (A_c - A_s)/A_c$$

where, A_c = Area of MHC (actin) peak in the control (RMM, 0°C, 0 day) , A_s = Area of MHC (actin) peak in the samples

Preparation of natural actomyosin (NAM)*:* Actomyosin was prepared according to the method of MacDonald and Lanier (1994). Croaker muscle (4 g) was homogenized in 40 ml chilled (4°C) 0.6M KCl, (pH 7.0) for 4 min using a homogenizer (RHC, REMI, India). The beaker containing the sample was placed in ice and each 20 sec of blending was followed by a 20 sec rest interval to avoid overheating during extraction. The extract was then centrifuged at 5,000 x g for 30 min at 4°C. Three volumes of chilled (0–2°C) deionized water were added to precipitate natural actomyosin. Actomyosin was collected by centrifuging at 5,000 × g for 20 min at 0°C and the pellet was dissolved by stirring for 30 min at 0°C in an equal volume of chilled 1.2 M KCI, (pH 7.0). Undissolved debris was removed from the preparation by centrifugation at 5,000 × g for 20 min at 0°C. Natural actomyosin was kept in ice during analysis.

Ca^{2+}-ATPase activity of NAM: NAM from 4 g muscle was prepared and Ca^{2+}ATPase activity of NAM in the extract was measured using modified method of MacDonald and Lanier (1994). The inorganic phosphate liberated in the supernatant after centrifugation was measured by the method of Fiske and Subbarow (1925) and expressed as µmoles inorganic phosphate released/mg protein/min.

Relative viscosity: Relative viscosity was measured every fortnight for 3 months on triplicate samples according to Borderias et al. (1985).

Thaw drip (TD) *and water holding capacity* (WHC)*:* TD of frozen mince and surimi was determined by the procedure of Mishra and Srikar (1989). WHC was measured according to the method of Porteous and Wood (1983) and calculated by the following formulae.

$$\text{WHC (\%)} = \frac{\text{Final wt. of sample (g)} - \text{Original wt. of sample (g)}}{\text{Original weight of sample (g)}} \times 100$$

Statistical analysis: Significant differences among means (3 replicates) of experimental results were evaluated by one way ANOVA using the EXCEL programme (Microsoft, Tulsa, USA). The level of confidence was determined at $p \leq 0.05$.

Results and discussion

The results of proximate analysis, TPC including other chemical characteristics of croaker fish is shown in

Table 1. The results obtained are in concurrence with the earlier studies (Revankar et al. 1981, Reddy et al. 1992).

Electrophoretic analysis of salt extractable protein: The band pattern of salt extractable protein analyzed by SDS-PAGE (Fig. 1) showed that the degradation of myosin heavy chain (205 KD) increased ($p < 0.05$) continuously up to 48% (Fig. 2) by the end of 3 months frozen storage in case of control (C). But in case of surimi prepared by T_1 and T_2, the degradation pattern is evident but to a lesser extent (21.7 % and 19.7 %) than that of control (Fig. 2). The trend of actin degradation is similar to that of MHC, but to a lesser extent ($p > 0.05$) as the actin (45 KD) band was strong up to 3 months of storage. The disappearance of the MHC band and retention of actin band in the SDS-PAGE profiles of the dewatered minced meat sample without cryoprotectant is consistent with the hypothesis of Matsumoto (1980), that dissociation of actomyosin into F-actin and myosin occurs immediately after freezing and that it is primarily the myosin component which undergoes aggregation and insolubilization. These data indicated that the muscle protein, mainly myosin, important for protein functionality was degraded during storage, which might be due to the autolytic degradation of muscle protein by cathepsin and serine proteases, which remained active at low temperature. Benjakul et al. (1997) also found by SDS-PAGE profile that MHC was hydrolysed continuously throughout low temperature storage of Pacific Whiting muscle protein. Though in case of cryoprotectant treated samples the degradation of MHC was much lower than that of control, which indicated that the cryoprotective formulations were effective in preventing the aggregation and subsequent insolubilization of myosin during frozen storage.

Table 1 Characteristics of fresh croaker fish

Protein, %	16.6 ± 0.21
Fat, %	1.9 ± 0.08
Moisture, %	80.0 ± 0.56
Ash, %	1.3 ± 0.24
TN, mg/100g	3.8± 0.88
SSN, as % TN	74.7 ± 0.04
WSN, as % TN	4.1 ± 0.66
NPN, mg/100g	387.5 ± 0.82
TMA, mg /100 g	0.57 ± 0.06
PV, meq of O_2 / kg of fat	1.9 ± 0.01
FFA, % of oleic acid	2.0 ± 0.44
pH	6.8 ± 0.32
TPC, log cfu/g	5.4 ± 0.02

TN=Total nitrogen, SSN = Salt soluble nitrogen,
WSN= Water Soluble nitrogen, NPN=Non-protein nitrogen,
TMA= Trimethylamine, PV=Peroxide value,
FFA=Free fatty acid, TPC= Total plate count

Ca^{2+}-ATPase activity of NAM: Ca^{2+}ATPase activity of NAM extracted from croaker muscle showed a continuous decrease during the storage period. For the DWM, Ca^{2+}ATPase activity decreased by 63.2 % during 3 months storage ($p < 0.05$). In case of surimi prepared by T_1 and T_2, Ca^{2+}ATPase activity decreased by 38.3% and 30.6% ($p < 0.05$) (Table 2). The decrease in Ca^{2+}ATPase activity was possibly due to the conformational changes of myosin globular head as well as the aggregation in this portion. According to Ochaiai and Chow (2000) myosin head possesses the ATPase enzymatic activities. Also Benjakul and Bauer (2000) have reported that rearrangement of protein via protein-protein interactions during frozen storage was also presumed to contribute to loss in ATPase activity. Cryoprotectant has the ability to recover some myosin ATPase activity and sodium lactate prevented loss of Ca^{2+}ATPase activity more than that of sucrose/sorbitol during isothermal storage at (-20±2ºC), which is quite evident from the result of present study. MacDonald and Lanier (1994) have reported that 6% (w/v) sodium lactate concentration recovered almost 80% Ca^{2+}ATPase activity in case of freeze thaw Tilapia muscle and appeared to be four times more effective than sucrose on a percentage basis.

TD and WHC: WHC and resultant TD of the freeze-thawed muscle are the important functional properties in

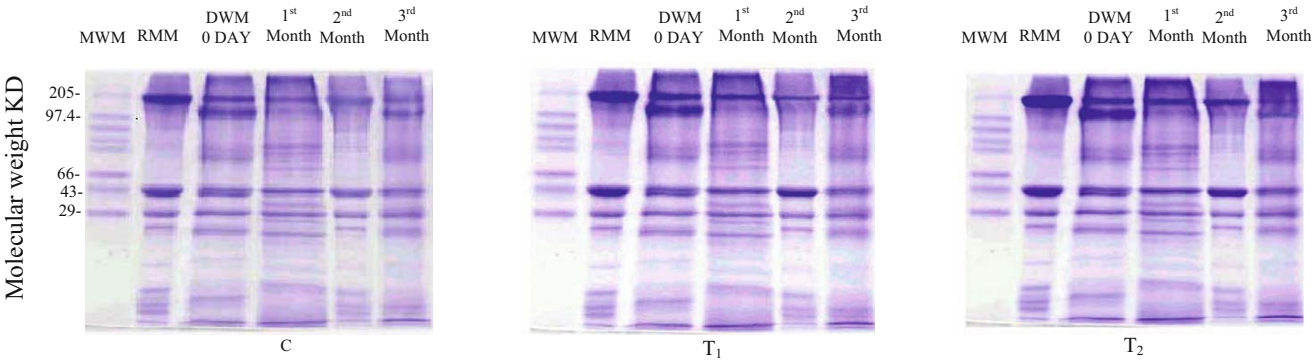

Fig. 1 Polypeptide profiles of salt extractable proteins of Croaker raw minced meat (RMM), dewatered minced meat (DWM) and surimi samples (C, T_1, T_2) during frozen storage. 1.5 µl sample containing 40µg of protein given in each track of 10% gradient polyacrylamide gel. C, T_1, T_2 : See Table 2.

protein gel based products (Chung and Lee 1991). WHC of C, T_1 and T_2 surimi samples decreased by 71.7, 55.5 and 62.1 %, respectively and thus TD increased by 141.7, 85.3 and 108%, respectively (Table 2). This was probably due to the susceptibility of muscle protein to freeze denaturation as observed by marked decrease in Ca^{2+}ATPase activity. From the result, the decrease in WHC and increase in TD was more prevalent in case of DWM than that of surimi treated with two different formulations of cryoprotectants, which might be due to the increase in hydration of the muscle protein in presence of cryoprotectants. According to Benjakul et al. (2003), the ice crystals formed during freez-

Table 2 Physico-chemical characteristics of croaker muscle treated with cryoprotectants

Storage, days	Samples	Ca^{2+}ATPase activity, μ moles Pi /mg protein/min	Relative viscosity, Centipoises	Thaw drip, %	Water holding capacity, g %
0	C	0.38 ± 0.0654	1.255 ± 0.0051	8.3 ± 0.76	1.52 ± 0.32
	T_1	0.36 ± 0.0142	1.391 ± 0.003	8.2 ± 0.63	1.62 ± 0.04
	T_2	0.36 ± 0.0023	1.384 ± 0.008	8.0 ± 0.61	1.74 ± 0.53
15	C	0.35 ± 0.0012	1.216 ± 0.03	10.2± 0.49	1.13 ± 0.46
	T_1	0.34 ± 0.0631	1.360 ± 0.062	9.2 ± 0.08	1.48 ± 0.82
	T_2	0.35 ± 0.0278	1.376 ± 0.013	9.1 ± 0.08	1.58 ± 0.13
30	C	0.30 ± 0.0698	1.198 ± 0.854	12.5 ± 1.44	0.94 ± 0.05
	T_1	0.31 ± 0.0098	1.335 ± 0.02	11.0 ± 0.23	1.23 ± 0.16
	T_2	0.33 ± 0.0432	1.328 ± 0.56	11.7 ± 0.43	1.39 ± 0.08
45	C	0.27 ± 0.0035	1.153 ± 0.006	13.8 ± 0.95	0.78 ± 0.16
	T_1	0.30 ± 0.0464	1.296 ± 0.231	11.9 ± 0.01	1.07 ± 0.07
	T_2	0.30 ± 0.0062	1.306 ± 0.004	12.5 ± 0.24	1.19 ± 0.3
60	C	0.21 ± 0.0391	1.093 ± 0.102	15.7 ± 1.69	0.67 ± 0.07
	T_1	0.28 ± 0.0321	1.279 ± 0.169	13.1 ± 0.04	0.96 ± 0.6
	T_2	0.29 ± 0.0854	1.261 ± 0.02	14.3 ± 0.06	1.03 ± 0.37
75	C	0.18 ± 0.0064	1.074 ± 0.001	18.9 ± 0.65	0.55 ± 0.6
	T_1	0.25 ± 0.0075	1.226 ± 0.007	14.4 ± 0.42	0.83 ± 0.18
	T_2	0.27 ± 0.0392	1.230 ± 0.001	15.9 ± 0.03	0.92 ± 0.05
90	C	0.14 ± 0.0382	1.005 ± 0.045	20.1 ± 0.89	0.43 ± 0.59
	T_1	0.22 ± 0.0169	1.173 ± 0.901	15.1 ± 0.09	0.72 ± 0.07
	T_2	0.25 ± 0.0078	1.203 ± 0.756	17.0 ± 0.82	0.85 ± 0.43

(n=3)

C: Control; T_1 : Surimi sample treated with 4% sucrose, 4% sorbitol, and 0.3% sodium tri poly phosphate (STPP); T_2 : Surimi sample treated with 6% (w/v) sodium lactate and 0.3% STPP

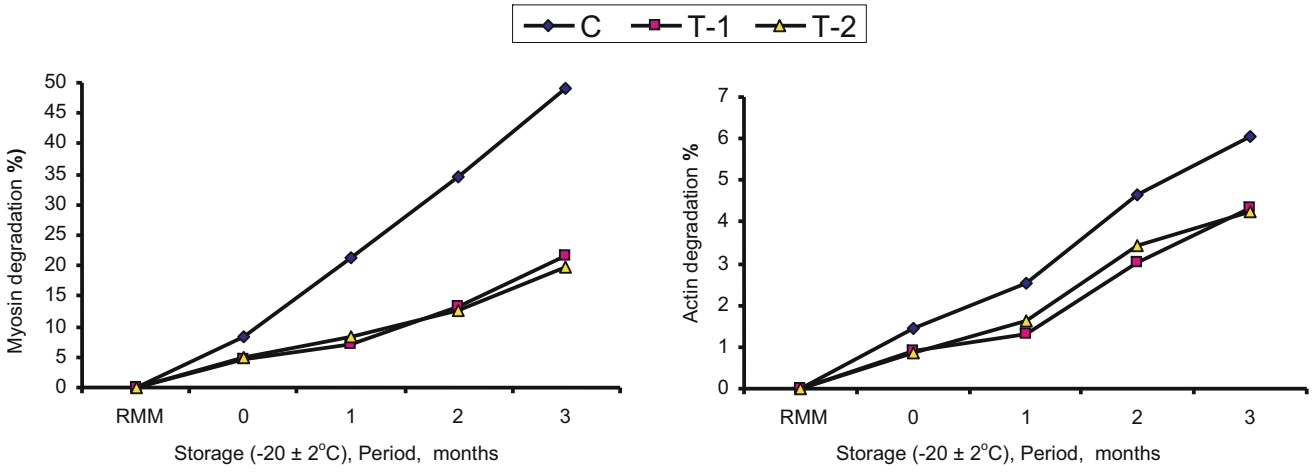

Fig. 2 Changes in myosin heavy chain and dewatered minced meat in C, T_1 and T_2 samples during frozen storage C, T_1, T_2 : See Table 2.

ing or subsequent frozen storage result in the tissue damage and the leakage of various organelles as a result water could be released from muscle more easily particularly when the frozen storage time is increased.

Relative viscosity: Relative viscosity also depends on the shape and specific volume of solvent that is hydrodynamically associated with protein molecules. Relative viscosity for control sample (C) decreased by 20% (Table 2), which is due to molecular interaction and protein aggregation during 3 months of freeze denaturation. This decrease in T_1 and T_2 samples was comparatively less than control (C) which might be due to their respective cryoprotective effect. In contrast, T_2 sample showed greater relative viscosity than T_1 that can be predicted on the basis of comparison of molecular weight of sodium lactate (112.1) to that of sucrose (342.3). Similar result was observed by MacDonald and Lanier (1994) while working with Tilapia actomyosin.

Conclusion

Important physico-chemical parameters assessing protein quality of croaker muscle viz., TN, SSN, TD, MHC degradation, calcium ATPase activity and WHC have been significantly affected by freeze denaturation of protein during 3 months of frozen storage. The effect of cryoprotectants in stabilizing protein from freeze denaturation is also evident from the results. Also sodium lactate and STPP tried out as cryostabilizer showed almost equal or greater efficacy for parameters like Ca^{2+}ATPase activity, WHC, TD, relative viscosity to sucrose / sorbitol / STPP cryoprotectant combination in stabilizing muscle protein during frozen storage. Thus, it may be inferred that sodium lactate can effectively be used as an alternative cryostabilizer to sucrose and sorbitol for stabilization of croaker muscle protein native structure and is less sweet than other cryoprotectants.

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