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Suitability of sodium lactate as cryoprotectant and its effect on gel forming ability and protein denaturation of croaker fish surimi during frozen storage

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Abstract
The effect of sodium lactate is compared with sucrose + sorbitol + sodium tri-poly phosphate as cryoprotectant on gel forming ability & protein denaturation of croaker surimi during frozen storage at -20±2°C for 90 days was evaluated. The quality of Croaker surimi with 6% (w/v) sodium lactate was examined in terms of biochemical parameters of muscle protein, thaw drip, gel strength and calcium ATPase activity comparing with those of surimi added with sucrose/sorbitol & without additive as control. Both the cryoprotectants minimized the negative effects of frozen storage on physico-chemical traits of myofibrillar proteins which was evident from the biochemical and sensory parameters. The residual Ca^{2+} ATPase activity and gel strength of surimi with sodium lactate were higher than those of control throughout 90 days of storage. Ca^{2+} ATPase activity and gel strength found a high positive correlation. From the results, it was found that sodium lactate was equally effective in preservation of croaker muscle protein native structure during frozen storage as the sucrose/ sorbitol and also less sweet without any risk of maillard browning.

Key words: Gel forming ability, Sodium lactate, Cryoprotectant, Frozen storage

Introduction

Surimi is an important intermediate product containing stabilized myofibrillar proteins obtained from mechanically deboned fish flesh that is water- washed, blended with cryoprotectants and then used in the production of fabricated seafood products. Cryoprotectants can be used to improve quality and extend shelf life of frozen foods by preventing deleterious changes in myofibrillar proteins caused by freezing, frozen storage, and thawing (MacDonald and Lanier 1997). Although a wide variety of cryoprotectants such as sucrose, sorbitol, phosphates etc. have been used in surimi industry (Pigott 1986), the problem of sweetness and of high calorific content are of greater concern (Park and Lanier 1987 and Park and Morrissey 2000).

Macdonald *et al.* (1996) studied the mechanism for stabilization of fish actomyosin by Sodium Lactate where they hypothesized Sodium lactate as effective stabilizer at low concentration i.e. 6% (w/v) stabilizes fish muscle protein by the mechanism of preferential hydration where the protein solvent contact surface increases due to strong increase of surface tension of the water when they are added. Hence, the surface free energy of the unfolding of surface protein shifts the equilibrium towards the more compact native state of protein. Jittidana *et al.* (2003) also showed the cryoprotective effect of Sodium Lactate at 1% (w/v) on Rainbow trout fillet protein stabilization and its physico-chemical properties.

With this backdrop, in the present study, suitability of sodium lactate [6% (w/v) at pH 7.32] as an alternate cryoprotectant was investigated for controlling quality and physico-chemical changes of croaker muscle protein during frozen storage at -20 ±2°C which has reports of reduced sweetness and less milliard browning reaction.

Materials and methods

Prepartion of surimi samples

Croaker (*Johnius gangeticus*, Avg weight of 402.5 g, Avg length of 31.5 cm.) caught along Kakdwip coast of West Bengal was iced on board and brought to the processing hall in an insulated icebox. They were washed in chilled water and dressed. Meat was picked using roll type fish meat picker and minced with mincing machine . The minced meat was washed using chilled water as described by Gopakumar *et al.* (1992). Minced meat after washing dewatered and moisture level adjusted at 81%. Dewatered mince meat subjected to different treatments. In T_1 (treatment 1) sample, 4% sucrose, 4% sorbitol, and 0.3% sodium tri-poly phosphate (STPP) whereas in T_2 (treatment 2) sample 6% (w/v) sodium lactate was added. The control sample 'C' (no treatment) was taken to study compare the effect of different treatments. To investigate the effect of different treatments on the minced meat during 90 days of frozen storage period, each sample was divided into seven equal portions and packed in seven LDPE bags separately for fortnight interval study, which were then subjected to freezing at -35°C in horizontal plate freezer. Then they were stored at -20 ±2°C in horizontal deep freeze.

Biochemical and organoleptic parameters

Proximate composition analyses were carried out according to AOAC methods (1975). Salt soluble nitrogen (SSN), water soluble nitrogen (WSN) and non-protein nitrogen (NPN) were estimated following the method of Dyer *et al.* (1950), AOAC (1975) and Srikar and Chandru (1983) respectively. Peroxide value (PV) and free fatty acid (FFA) were determined according to Jacobs (1958) and Takagi *et al.* (1984) respectively while alpha amino nitrogen (AAN) and total volatile base nitrogen (TVB-N) were estimated by the method of Beatty and Gibbons (1937) (Table 1).

Table 1. Bio-chemical characteristics of croaker fish surimi samples treated with and without additives

Storage days	Samples	TN (%)	SSN (% of TN)	WSP (%)	NPN (mg%)	TVBN (mg%)	AAN (mg /100gm)	PV milimoles /kg Fat)	FFA (% TL as Oleic acid)	TPC (ln) (/gm of fish)	Overall acceptability (OAA)
0	C	3.32±0.68	82.32± 0.23	2.63± 0.63	364.52± 0.25	4.82 ± 0.03	11.11	2.13	4.21	12.709	7.5
	T_1	3.38±0.94	82.69± 0.09	2.66± 0.53	368.61± 0.25	4.28 ± 0.06	11.12	2.15	4.25	10.747	8.0
	T_2	3.36±0.36	83.43± 0.64	2.61± 0.78	366.28± 0.21	4.36 ± 0.34	11.00	2.11	4.20	10.596	7.8
15	C	3.36±0.03	80.11± 0.1	2.57± 0.02	358.45± 0.46	5.08 ± 0.01	13.24	2.67	8.39	10.598	7.3
	T_1	3.42±0.09	80.92± 0.61	2.69± 0.06	361.94± 0.34	5.12 ± 0.21	12.06	2.65	8.12	10.545	7.7
	T_2	3.40±0.56	81.87± 0.06	2.60± 0.09	362.08± 0.06	5.02 ± 0.05	12.13	2.62	8.56	10.232	7.6
30	C	3.30±0.07	77.42± 0.94	2.68± 0.07	352.66± 0.08	5.89 ± 0.56	14.89	3.25	10.25	10.571	6.8
	T_1	3.31±0.52	79.04± 0.03	2.65± 0.12	358.86± 0.06	5.63 ± 0.37	12.95	3.01	9.13	7.87	7.4
	T_2	3.27±0.07	79.56± 0.32	2.57± 0.4	360.12± 0.01	5.74 ± 0.01	12.74	2.95	9.17	7.83	7.3
45	C	3.26±0.61	73.28± 0.56	2.72± 0.35	343.12± 0.43	6.78 ± 0.49	15.64	4.43	16.38	10.586	6.5
	T_1	3.27±0.36	76.34± 0.01	2.62± 0.07	356.24± 0.13	6.71± 0.04	13.27	3.56	12.42	7.889	7.1
	T_2	3.22±0.01	78.32± 0.07	2.55± 0.39	357.76± 0.53	6.39 ± 0.67	13.32	3.12	10.28	7.803	7.0
60	C	2.84±0.08	65.33± 0.7	2.58± 0.21	335.89 ± 0.7	8.62 ± 0.01	16.21	5.26	24.52	10.214	6.2
	T_1	3.18±0.04	71.65± 0.42	2.58± 0.36	351.03± 0.08	8.07 ± 0.23	14.33	4.04	16.35	7.904	6.9
	T_2	3.17±0.58	75.62± 0.51	2.53± 0.06	355.48 ± 0.4	7.84 ± 0.38	14.17	3.45	13.54	7.897	6.9
75	C	2.36±0.01	62.07± 0.06	2.47± 0.07	328.27± 0.06	12.06 ± 0.08	17.49	5.93	32.98	10.246	5.8
	T_1	3.13±0.08	70.48± 0.67	2.53± 0.13	346.54± 0.64	11.54 ± 0.07	15.87	4.35	21.33	7.915	6.6
	T_2	3.14±0.72	73.25± 0.75	2.51± 0.04	352.17± 0.07	10.90 ± 0.43	15.26	3.53	19.56	7.922	6.5
90	C	2.14±0.52	58.37± 0.05	2.41± 0.25	316.1 ± 0.45	18.32 ± 0.32	18.79	6.32	41.43	10.258	5.3
	T_1	3.08±0.01	68.26± 0.08	2.51± 0.08	342.34± 0.07	14.66± 0.28	16.36	4.97	28.52	7.919	6.1
	T_2	3.09±0.34	71.88± 0.04	2.49± 0.9	349.82 ± 0.1	13.81± 0.06	15.84	3.68	24.17	7.930	6.0

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

Minced meat was steam cooked with 2% salt, cooled and assessed for organoleptic quality by 8 panelists on the basis of appearance, odour, texture and overall acceptability using 10 point hedonic scale range (Reddy and Srikar 1991). Standard method recommended by APHA (1984) was followed to estimate the total plate count (TPC) of the sample.

Thaw drip and preparation of natural actomyosin

Thaw drip (TD) in the frozen mince and surimi was determined by the procedure of Mishra and Srikar (1989). Actomyosin was prepared according to the method of MacDonald and Lanier (1994). Croaker muscle (4g) was homogenized in 40 ml chilled (4°C) 0.6M KCL, pH 7.0 for 4 min using a homogenizer. The beaker containing the sample was placed in ice and each 20 seconds 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 X *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 X *g* for 20 min at 0°C.Natural Actomyosin was kept in ice during all analysis.

Cc^{2+}- ATPase activity of natural actomyosin

Ca^{2+}-ATPase activity was determined using modified methods of MacDonald and Lanier (1994). Natural Actomyosin (NAM) was diluted to 2.5-8 mg/ml,with 0.6 M KCI, pH 7.0. Diluted NAM solution (1 ml) was added to 0.6 ml of 0.5 M Tris-maleate, pH 7.0. The mixture was added with 10 mM $CaCl_2$ to make the final volume 9.5 ml to the assay solution, 0.5 ml of 20 mM ATP was added to initiate the reaction. The final pH was adjusted at 8. The reaction was conducted for 5 min at 27°C and terminated by adding 5 ml of chilled 15% (w/v) trichloroacetic acid. The reaction mixture was centrifuged at 3500 X g for 5 min and the inorganic phosphate liberated in the supernatant was measured by the method of Fiske and Subbarow (1925). Specific Ca^{2+}- ATPase activity of the NAM was expressed as □moles inorganic phosphate released/mg protein/min. A blank solution was prepared by adding chilled trichloroacetic acid prior to addition of ATP. The relative total Ca-ATPase activity of surimi sample was expressed as the ratio of the activity before and after frozen storage.

Preparation of kamaboko gel

The frozen surimi sample was thawed at 4°C for 2 h and placed in a mortar. NaCl, 3% w/w, was sprinkled onto the surimi, and the fish-salt mixture was ground at 5°C for 30 min. The surimi paste was then stuffed into a stainless steel cylindrical mold with a diameter of 3 cm and length of 3 cm. Both ends of mold were sealed tightly using

polyvinylchloride film and rubber bands. Two-step heated gels were carried out by setting at 40°C for 1 h, followed by heating at 90°C for 30 min in a water bath. These gels were referred to as kamaboko gels. The kamaboko gels were cooled in iced water and stored at 4°C overnight. Kamaboko gels were equilibrated at room temperature for 30 min before measuring properties.

Determination of gel forming ability

Gel forming ability of croakerfish surimi was evaluated by measuring rheological properties of kamaboko gel using the rheometer (Universal Texture Analyzer, Stable MicroSystem, England) with spherical plunger (diameter 5 mm; depression speed 60 mm/min). Kamaboko gels were removed from the stainless steel mold and cut into 2.0 cm in thickness. Each gel was subjected to the puncture test by the plunger to measure breaking force (g) and deformation distance. The gel strength (g·cm) was calculated by multiplication of breaking strength (g) by deformation distance (cm). The relative gel strength was defined as the ratio of gel strength before and after frozen storage.

Statistical analysis

All experiments were done in triplicate and represented as mean ± standard deviation. Significant differences among means of experimental results were evaluated by one-way analysis of variance (ANOVA) using the EXCEL programme (Microsoft, USA). The least significant difference test was used to determine differences between samples. The level of confidence interval was determined at $p \leq 0.05$. To find out the relationships between the various parameters multivariate Pearson's correlation coefficient was calculated and linear regression equation was found out.

Results and discussion

Effect of sodium lactate on biochemical & organoleptic parameters

Moisture content of frozen surimi decreased from 80.02% to 77.45% and the decrease was not significant during 90 days of frozen storage ($p>0.05$). The decrease in TN in case of control sample from 3.32% to 2.14 % was significant ($p<0.05$) but in case of T_1 and T_2 samples it decreased from 3.38% to 3.08% and 3.36% to 3.09% respectively during 90 days of frozen storage, which were not significant ($p>0.05$). This could be expected due to loss of some WSP and other NPN constituents in the free drip after thawing. From the result it is evident that the decrease is much less in T_1 and T_2 samples than that of control, which may be due to their respective cryoprotective effect. A similar result has been observed by Garg *et al.* (1982) and Shyamsunder and Prakash (1994).

SSN is considered as an index of protein denaturation in fish (Shyamsunder and Prakash 1994). SSN content of all samples tabulated decrease with the increase in

storage period. Decreasing rate of SSN can attributed to the aggregation leading to insolubilization of myofibrillar fraction. In the present case, decreasing rate of sample treated with sodium lactate was significantly lower than sample treated with sucrose, sorbitol and sodium tripolyphosphate ($p<0.05$), which might be due to higher cryoprotective effect. Similar decreasing trend of SSN of surimi prepared from silver carp was noticed by Chaudhury (2002).

There is a decrease of 8.36%, 5.63% and 4.60% in WSP content in the croaker mince and surimi treated by cryoprotectants (T_1 and T_2 samples) respectively during the period of frozen storage. This decrease could be due to loss of water extractable protein in the free drip. Boderias *et al.* (1985) attributed the decrease in WSP content to the denaturation of sarcoplasmic protein during frozen storage.

The NPN value of control, T_1 and T_2 samples showed decreasing trend during the frozen storage which might be due to the driploss through which considerable amount of NPN is lost. Similar observation was observed by Mishra and Srikar (1989) in clam meat and Mandal (2003) in ribbonfish. Moreover the depletion of NPN might be due to its utilization by the microorganisms (Jhaveri and Constantinides 1982). The AAN content also increased in all the samples during frozen storage. The increase was significant in case of control sample ($p<0.05$). There was no significant difference in AAN content of T_1 and T_2 samples during storage period of 90 days. Changes in freshness parameters *viz.* PV, FFA and TVB-N of frozen stored samples are given in the table. All parameters showed a significant increase during frozen storage ($p<0.05$). Further the rate of increase was maximum in control sample while it was not significant for T_1 and T_2 samples, which might be due to the effect of cryoprotective treatment in surimi necessarily reduced protein denaturation as a result the breakdown products are subsequently reduced. The increase in PV and FFA indicated the oxidation of lipid content of surimi during frozen storage. The results are in conformity with the results obtained by Dora and Chandrasekhar (1998).

A significant decrease in organoleptic scores ($p<0.05$) was noticed for all the sensory qualities judged for the samples throughout the period of storage. Sensory evaluation data showed highly negative correlation between mean panel scores for overall acceptability and storage period. A linear regression equation $Y= - 0.0163 X + 4.1308$ with a correlation coefficient $r = - 0.9907$ was obtained. The decrease in AAN paralleled the loss in flavour (Chakraborty 1984).

Effect of sodium lactate on thaw drip

Changes in thaw drip (%) and proximate composition during frozen storage of surimi are given in Fig 1. Thaw drip loss increased from 4.8% to 14.30%, 4.05% to 10.7% and 4.1% to 10.1% in control, T_1 and T_2 samples respectively. It has been observed that the drip loss in surimi during frozen storage is largely affected by the duration and temperature of frozen storage. The capacity of fish mince to retain water, a property reflected in drip, decreased in storage time. Prolonged storage results in decreased water retention capacity due to denaturation of proteins resulting from

surface dehydration, ice crystal formation and resulting cell rupture.

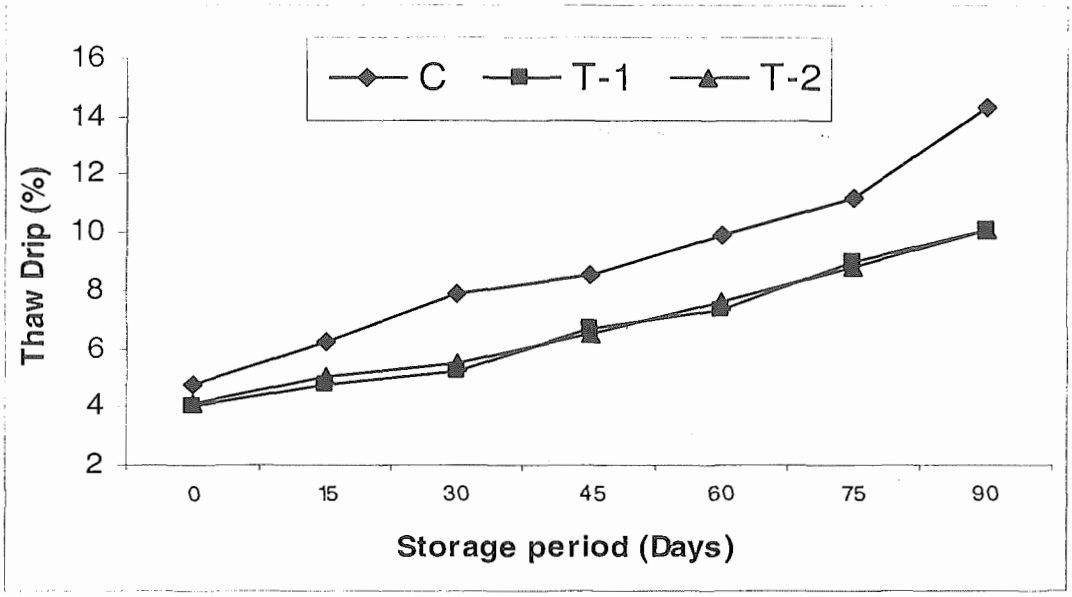

Fig 1. Changes in thaw drip in DWM with no additive (C), surimi prepared by 4% sucrose, 4% sorbitol, 0.3% STPP (T_1) and 6% (w/v) sodium lactate (T_2) during frozen storage at -20 ±2°C for 90 days.

In the present investigation negative correlation exists between thaw drip and overall acceptability score ($p<0.05$). The reduction of thaw drip percentage in T_1 and T_2 samples from that of control samples during the frozen storage period indicated the effect of cryoprotectant in preventing the protein denaturation. Further thaw drip percentage in T_1 and T_2 samples did not vary significantly ($p<0.05$) indicating that the cryoprotectant sodium lactate is quite effective as that of sucrose, sorbitol and sodium tripolyphosphate.

Effect of sodium lactate on relative total ca^{2+}- ATPase activity

Ca^{2+}ATPase activity of NAM extracted from croaker muscle showed a continuous decrease during the storage period. For the control sample, relative Ca^{2+}ATPase activity decreased to 35.64% during 90 days of storage ($p<0.05$). In case of surimi prepared by treatment I and II, relative Ca^{2+}ATPase activity decreased to 61.11% and 59.32% respectively ($p<0.05$) (Fig. 2). The decrease in Ca^{2+}ATPase activity with the length of frozen storage was possibly due to the conformational changes of myosin globular head as well as the aggregation in this portion.

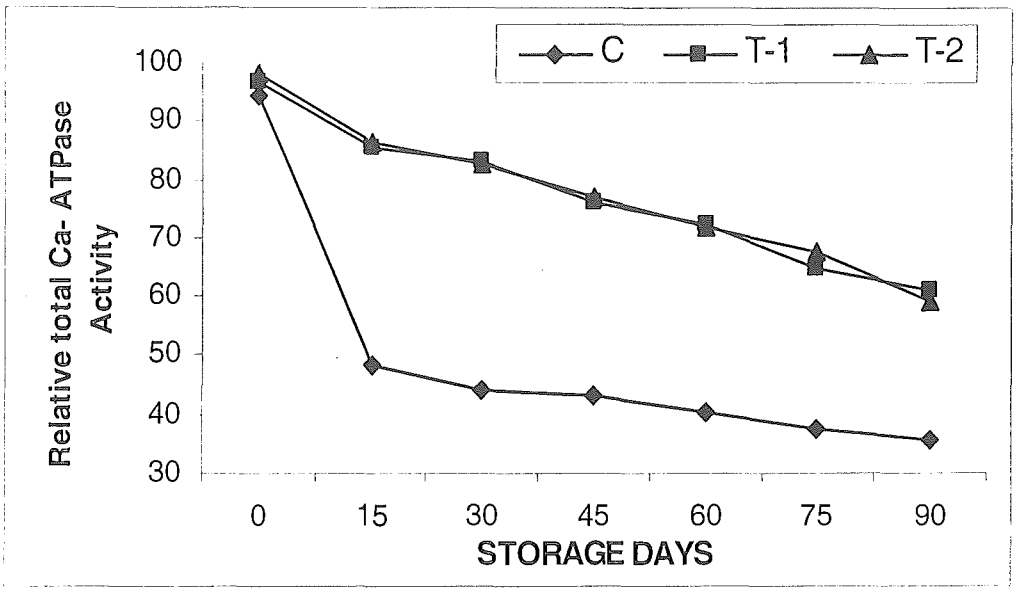

Fig 2. Changes in Relative total calcium ATPase activity in DWM with no additive (C), surimi prepared by 4% sucrose, 4% sorbitol, 0.3% STPP (T_1) and 6% (w/v) sodium lactate (T_2) during frozen storage at -20 ±2°C for 90 days.

According to Benjakul and Bauer, 2000 myosin head possess the ATPase activitiesand the rearrangement of protein via protein-protein interactions in myosin 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 almost same to that of sucrose/sorbitol during isothermal storage at -20 ± 2°C, which is quite evident from the result of present study. MacDonald and Lanier (1994) reported 6% (w/v) sodium lactate concentration recovered almost 80% Ca^{2+}ATPase activity in case of freeze thaw Tilapia muscle and also showed sodium lactate appeared to be four times more effective than sucrose on a percentage basis.

Effect of sodium lactate on gel forming ability

The gel strength values before freezing of kamaboko gels in T_1, T_2 and control samples were 965, 864 and 2088 g.cm respectively. As the initial gel strength values of Croakerfish Kamaboko gels with and without additives were different, therefore the relative gel strength defined as the ratio of the gel strength before and after frozen storage were presented in the study for easy comparison of the effect of each additive which has been shown in (Fig 3). Relative gel strength of all samples decreased with the increase in storage time ($p<0.05$). Specifically, the relative gel strength of the control dropped dramatically to 42.54% of the initial value within 15 days of frozen storage, whereas kamaboko gels of $T_1$and T_2 samples were 83.46% and 85.75% respectively. The results indicate that freezing had a strong effect on the gel forming ability of croakerfish

surimi without additive (control). Kim *et al.* 1986, elucidated that repeated freezing and thawing of surimi, made from Alaska Pollock and sandtrout, denatured myosin, and that the hydrophobic amino acid residues of actomyosin exposed by freezing , resulting in the substantial decrease in gel strength. After 90 days of frozen storage, residual gel strength of $T_1$and T_2 samples were 52.82% and 56.64% ($p>0.05$) while the control gel showed the lowest value 23.48% ($p\leq0.05$). The results indicated that sodium lactate stabilized gel forming ability in croaker surimi during frozen storage which is comparable to sucrose/sorbitol. A high positive correlation ($r=0.894$, $p<0.001$) (data not shown) was also found between relative Ca-ATPase activity and relative gelstrength of surimi with or without additives. This result corresponds to Macdonald *et al.* 1996, who reported a close correlation between the ability of actomyosin to form cohesive gels and its Ca-ATPase activity.

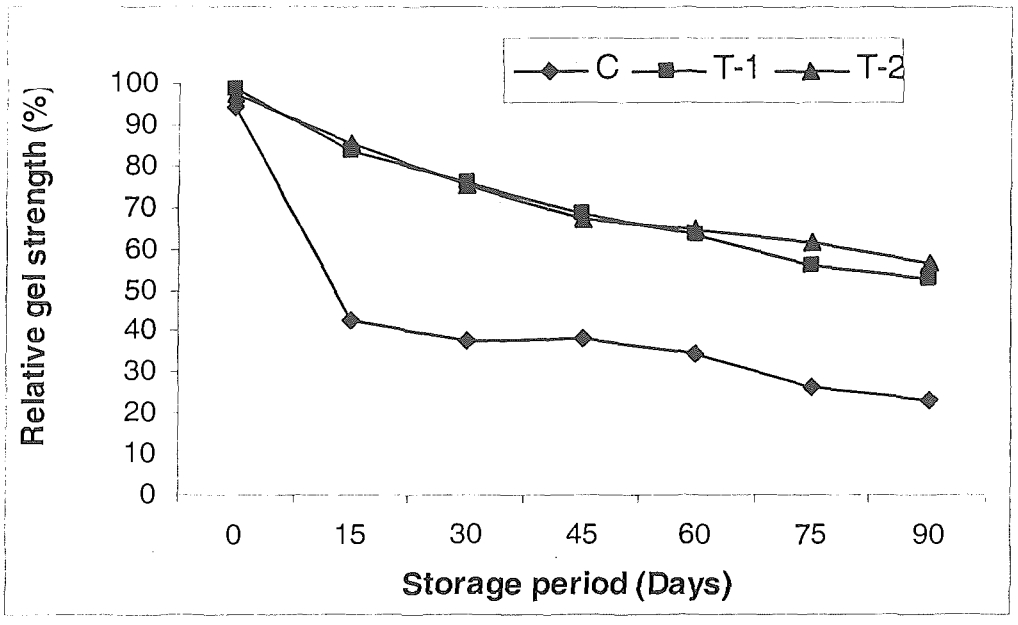

Fig 3. Changes in Relative gel strength (%) in DWM with no additive (C), surimi prepared by 4% sucrose, 4% sorbitol, 0.3% STPP (T_1) and 6% (w/v) sodium lactate (T_2) during frozen storage at -20 ±2°C for 90 days.

Conclusion

In the present study significant changes in protein quality mainly myofibrillar protein during 90 days of frozen storage has been observed. Cryoprotectants used in the study minimized the negative effects of frozen storage on physico-chemical traits i.e. thaw drip, Ca-ATPase activity and gel forming ability of myofibrilar proteins, which is also evident from the bio-chemical and sensory parameters studied. Sodium lactate provided equal effective cryoprotection, rather in some cases greater cryoprotection, in preservation of native protein structure of Croaker fish surimi during 90 days of frozen

storage. Thus it may be inferred that sodium lactate can effectively be used as an alternative cryoprotectant to sucrose sorbitol for stabilization of Croaker muscle protein native structure as it is less sweet than them and also having less risk of maillard browning.

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