Fishery Technology 50 (2013) : 50 - 59

Cryoprotective Effect of Shrimp Waste Protein Hydrolysate on Croaker Surimi Protein and Gel Characteristics during Frozen Storage

Satya S. Dey[1], Krushna C. Dora[1*], Utpal Raychaudhuri[2] and Subha Ganguly[1]

[1] *Department of Fish Processing Technology, West Bengal University of Animal and Fishery Sciences, Kolkata - 700 094, India*

[2] *Department of Food Technology & Biochemical Engineering, Jadavpur University, Kolkata - 700 032, India*

Abstract

Effect of shrimp waste protein hydrolysate (SWPH, 7.5% dried matter, T_2) on quality of Croaker fish surimi protein was examined in terms of nitrogeneous parameters, myosin and actin degradation, Ca^{2+}-ATPase activity and unfrozen water content in comparison to surimi with sucrose-sorbitol blend (T_1) and control (C, no additive) during frozen storage for 120 days at -25°C. Significant variation ($p<0.05$) of these parameters between storage days and difference of T_1 and T_2 sample from control suggested muscle protein as succeptible to freeze denaturation and application of SWPH as an alternative cryoprotectant to sucrose-sorbitol (SuSo). Textural properties including gel strength, hardness, cohesiveness, springiness, chewiness and gumminess of kamaboko gel prepared from surimi samples decreased with storage days though SWPH was efficient enough to reduce effect of freeze denaturation on gel characteristics during initial three months of storage.

Keywords : Shrimp waste, protein hydrolysate, surimi, cryoprotective

Received 18 July 2011; Revised 21 August 2012; Accepted 20 September 2012

* *E-mail: kc_dora@yahoo.co.in*

Introduction

Cryoprotectants prevent protein denaturation during frozen storage by increasing the surface tension of water as well as the amount of bound water, which prevents ice crystal growth and migration of water molecules from the protein, thus stabilizing the protein in its native form during frozen storage (Carpenter & Crowe 1988). A 1 : 1 blend of sucrose and sorbitol is commonly used as a cryoprotectant for fish products, but several studies have also shown cryoprotective potential of polydextrose, lactitol, glucose syrup (Herrera & Mackie 2004), as well as trehalose and sodium lactate (Zhou et al., 2006). Products containing these carbohydrate-based cryoprotectants would not be acceptable by consumers suffering from diabetes and their tendency to impart a sweet taste to the final product might also not be desirable in some cases (Sych et al., 1990). The application of protein hydrolysates has attracted much attention in the past decade mainly due to their high nutritive value, low cost waste recycling and improved functionalities arising from the production of short peptides and free amino acids during hydrolysis (Clemente, 2000). Protein hydrolysates produced from fish scrap (Khan et al., 2003), squid (Hossain et al., 2004) and shrimp chitin (Somjit et al., 2005) displayed cryoprotective ability through increasing the proportion of unfrozen water and decreasing the loss of Ca^{2+}-ATPase activity in myofibrillar protein extracts. It was estimated that nearly 1 50 000-1 75 000 t shrimp waste per annum would be generated from shrimp processing companies in India (MPEDA, 2006) which is of great environmental concern. Various studies have described the functional and nutritional properties of shrimp waste extract. No studies thus far have investigated the cryoprotective effects of protein hydrolysates produced from total shrimp waste consisting of head, shell and tail. The present investigation was carried out to study the effect of shrimp waste protein hydrolysate (SWPH) as a cryoprotectant on the quality changes of croaker fish

surimi and its gel properties with special emphasis on the muscle protein characteristics during frozen storage at -25ºC.

Materials and Methods

Shrimp waste of *Fenneropenaeus monodon* and *F. indicus* collected from a processing plant in Kolkata was washed under running water, homogenized and freeze dried. It was thawed, suspended (1:1, w/v) in distilled water and homogenized. The pH was adjusted to 8.5 with 1 N NaOH at 60°C for enzymatic hydrolysis following the method of Holanda & Netto (2002) using enzyme 2.4 L Alkalase which is a bacterial serine endopeptidase preparation from *Bacillus licheniformis* strain. Reactions were done in duplicates in 1L polyethylene jacketed glass vessel in a thermostatically controlled water bath with an automatic temperature compensator (ATC) probe, a pH electrode and a mixer shaft for addition of alkali to maintain the desired pH. The hydrolytic reaction was conducted at pH 8.5, 60ºC using pH stat method as described by Adler–Nissen (1986). The samples were hydrolysed for 2 h and terminated by heating at 90°C for 15 min in a water bath with occasional stirring. After filtering of the hydrolysate, the filtrate was heated at 80ºC for 10 min after which, lipid layer was removed from the surface. Samples were cooled and then centrifuged at 16000 g for 15 min at 4ºC using REMI centrifuge. The supernatants were collected, concentrated and freeze dried (Vertis Freeze Mobile, 6ES, USA) and stored in dark bottle under vaccum at room temperature in dessicator until use.

Croaker fish (*Johnius gangeticus*) having average weight and length of 402.5 g, and 31.5 cm respectively, caught along Kakdwip coast of West Bengal were washed in chilled water and iced on board. Meat was picked using roll type fish meat picker and minced using a mincer (Stadler, Mumbai). The minced meat was washed with chilled water, and dewatered so that moisture content was adjusted to 80% (Gopakumar et al., 1992). Surimi was prepared from fish mince by blending with 4% sucrose, 4% sorbitol and 0.3% sodium tri-poly phosphate (STPP) (Treatment 1) and SWPH dried powder (different percentage viz. 2.5, 5.0. 7.5 and 10 were used of which 7.5% sample showed the best result, which is only shown here) at 7.5% dry weight (Treatment 2) basis in a mortar at 5ºC for 30 min at pH 7.0, following the method of Muraleedharan *et al.* (1996). The dewatered mince without additive was used as control. The surimi mixture was made into separate blocks with 7.0 × 14.5 × 0.7 cm in size, placed in a polythene bag, frozen in an airblast freezer and stored at -25ºC for 120 days. Each surimi sample was taken out at regular intervals of 20 days for quality analysis.

Proximate compostion, total nitrogen (TN) and total volatile base nitrogen (TVBN) were estimated by AOAC (1995). Salt soluble nitrogen (SSN) and total protein solubility (TPS) were estimated at 20 days interval during frozen storage (-25ºC) according to the method of Dyer et al. (1950) and Gornall et al. (1949). pH of surimi samples was determined after mixing 10 g of minced meat with 50 ml distilled water using pH meter (Cyberscan, 510). Variation of total plate count and sensory characteristics like appearance, colour, taste, texture, odour and overall acceptability were studied following the method of APHA, (1984) and using 10-point hedonic scale (CIFE, 2001).

Changes in protein pattern was examined by SDS-PAGE analysis where 3 g of thawed surimi sample taken at monthly intervals from frozen storage were used for electrophoresis (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 ml of high range molecular weight marker (Bangalore Genei, India) was 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. 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).

Changes in unfrozen water of each surimi sample during frozen storage (-25ºC) was examined at regular intervals using a MC-DSC model 4207 (Calorimetry Science Corp., Del., U.S.A.) following the method of Wakamatu & Sato (1979). Natural Actomyosin (NAM) was prepared using the modified method of MacDonald & Lanier (1994). Ca^{2+}-ATPase activity was determined using modified method of MacDonald & Lanier (1994) and inorganic phosphate liberated in the supernatant was measured by the method of Fiske & Subbarow (1925). Specific Ca^{2+}- ATPase activity of the NAM was expressed as µ moles inorganic phosphate released/mg protein/min. A blank solution was prepared by adding chilled trichloroacetic acid prior

to addition of ATP. The relative and total Ca^{2+}-ATPase activity of surimi sample was expressed as the ratio of the activity before and after frozen storage.

Heat-induced kamaboko gels were prepared from each surimi sample according to the method of Ian et al. (1995). Various texture profile attributes were determined using a TA-XT2i Stable Micro Systems texturometer (Viena Court, England) with software XTRA^{TM} dimension (XTRAD) following the method of Chung & Merritt (1991b). 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 (-25^{o}C). The samples were allowed for a double compression of 40% with a trigger force of 0.5 kg during which the various textural parameters were recorded from a typical force deformation curve generated. Expressible moisture content of the kamaboko gel was determined in triplicate according to the method by Feng (2000).

The colour of kamaboko gels (L*a*b* values) was determined with a colorimeter (Miniscan-XE+, Hunter lab, Virginia, USA) Six colour readings were taken for each gel. The whiteness of the surimi gel was calculated by using equation of Fujii et al. (1973).

$$\text{Whiteness} = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2}$$

All data were expressed as mean ± standard deviation. One-way ANOVA was used and mean comparison was performed by Duncan's multiple range test (Steel & Torrie, 1980).

Results and Discussion

Proximate composition viz., moisture, protein, fat and ash of SWPH and croaker surimi was 8.05, 63.29, 5.59, 24.53, 80.02, 13.83, 1.73 and 0.62% respectively which was in conformity with the studies done by Holanda & Netto (2002) and Muraleedharan et al. (1996). The decrease in TN in case of control sample was 44.87% ($p<0.05$) compared to T_1 and T_2 samples, where decrease was much lower viz., 23.66 and 14.88% respectively (Table 1). This could be expected due to loss of some water soluble protein and other non-protein nitrogen constituents in the free drip after thawing. Salt soluble nitrogen and total protein solubility are considered as an index of protein denaturation in fish (Shyamsunder & Prakash, 1994). TPS of control sample decreased substantially as also reflected from the data of SSN given in (Table 1) whereas limited depletion of SSN and resulting TPS were observed in case of cryoprotectant treated samples. Decrease in protein solubility during frozen storage was reported in minced cod (Tejada et al., 1996) and hake actomyosin (Delmazo et al., 1999). Decreasing rate of SSN can be attributed to the aggregation leading to insolubilization of myofibrillar fraction. In the present study, SSN decreasing rate of sample treated with SWPH was significantly lower ($p< 0.05$) (17.43 %) than sample treated with sucrose-sorbitol (23.5%), which might be due to higher cryoprotective effect. Similar decreasing trend of SSN of surimi prepared from silver carp was noticed by Chakraborty (1984). TVBN values increased with the storage period but at the 120th day all were within the limit of acceptability as suggested by Lakshmanan (2000) viz., 35 - 40 mg%. The pH of surimi samples decreased significantly specially for the control sample (Table 1) though sample added with cryoprotectants maintained the pH during initial stages of frozen storage but slightly decreased later. A significant decrease in organoleptic scores ($p<0.05$) was noticed for all the sensory qualities adjudged for the samples throughout the period of storage (Table 2). Sensory evaluation data showed 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.

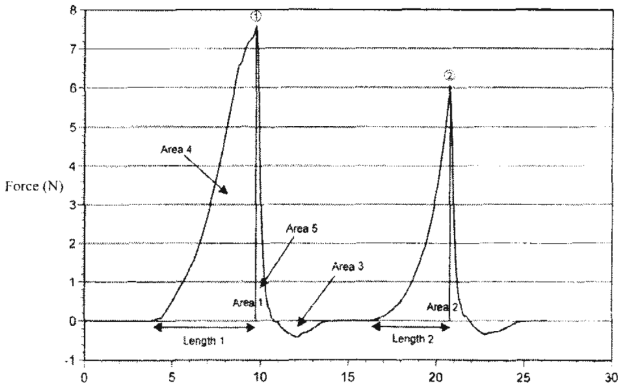

Fig 1. Typical force by time curve plot to determine texture profile analysis parameters.
Peak force 1 and 2 is Hardness 1 and 2; Cohesiveness = (Area 2 / Area 1); Gumminess = (Hardness 1 × cohesiveness) ; Springiness = (Length 2 / Length 1); Chewiness = (Hardness × springiness × cohesiveness)

Table 1. Changes of different nitrogenous parameters and muscle pH of three surimi samples during frozen storage of 120 days at -25°C.

Storage Days	Samples	TN (%)	SSN (% of TN)	TPS (%)	TVBN (mg %)	pH
0	C	3.32±0.68	82.32± 0.23	2.63± 0.63	4.82 ± 0.03	7.23
	T_1	3.38±0.94	82.69± 0.09	2.66± 0.53	4.28 ± 0.06	7.28
	T_2	3.36±0.36	83.43± 0.64	2.61± 0.78	4.36 ± 0.34	7.356
20	C	3.30±0.03	73.11± 0.1	2.57± 0.02	5.08 ± 0.01	7.18
	T_1	3.31±0.09	80.92± 0.61	2.69± 0.06	5.12 ± 0.21	7.255
	T_2	3.40±0.56	81.87± 0.06	2.60± 0.09	5.02 ± 0.05	7.32
40	C	3.26±0.07	67.42± 0.94	2.68± 0.07	5.89 ± 0.56	7.125
	T_1	3.27±0.52	79.04± 0.03	2.65± 0.12	5.63 ± 0.37	7.2
	T_2	3.27±0.07	79.56± 0.32	2.57± 0.4	5.74 ± 0.01	7.29
60	C	2.59±0.61	63.28± 0.56	2.72± 0.35	6.78 ± 0.49	7.12
	T_1	3.09±0.36	76.34± 0.01	2.62± 0.07	6.71± 0.04	7.156
	T_2	3.22±0.01	78.32± 0.07	2.55± 0.39	6.39 ± 0.67	7.25
80	C	2.18±0.08	58.33± 0.7	2.58± 0.21	8.62 ± 0.01	7.1
	T_1	2.92±0.04	70.65± 0.42	2.58± 0.36	8.07 ± 0.23	7.152
	T_2	3.11±0.58	75.62± 0.51	2.53± 0.06	7.84 ± 0.38	7.21
100	C	1.98±0.01	52.07± 0.06	2.47± 0.07	12.06 ± 0.08	7.05
	T_1	2.64±0.08	65.48± 0.67	2.53± 0.13	11.54 ± 0.07	7.08
	T_2	2.98±0.72	71.25± 0.75	2.51± 0.04	10.90 ± 0.43	7.16
120	C	1.83±0.52	48.37± 0.05	2.41± 0.25	18.32 ± 0.32	6.99
	T_1	2.58±0.01	63.26± 0.08	2.51± 0.08	14.66± 0.28	7.01
	T_2	2.86±0.34	68.88± 0.04	2.49± 0.9	13.81± 0.06	7.16

C : Control;T_1 : Surimi sample treated with 4% sucrose, 4% sorbitol, and 0.3% STPP, T_2 : Surimi sample treated with shrimp waste protein hydrolysate. Results are mean of three determination with S.D.

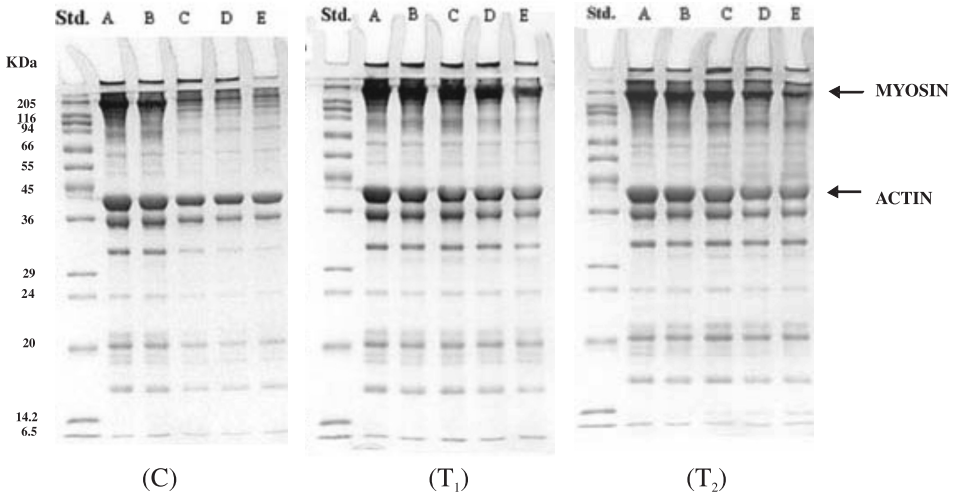

Fig 2. Polypeptide profiles of salt extractable proteins of croaker surimi without additive **(C)**, Surimi added with sucrose sorbitol **(T_1)**, Surimi added with shrimp waste protein hydrolysate **(T_2)** during 120 days of frozen storage at -25°C where A,B, C, D and E denote samples of 0, 30, 60, 90 and 120 days. 1.5 µl sample containing 40 µg of protein given in each track of 10% separating polyacrylamide gel where 1st track of each image represents standard marker.

Table 2. F value representing effects of different treatments and storage period on changes in sensory and microbiological parameters of croaker surimi during frozen storage of 120 days at -25°C.

Characteristics	F-value	
	Between treatments	Between storage days
Appearance	5.08643*	23.62238**
Colour	1.65498	20.54076**
Taste	2.98452	52.54632**
Texture	3.12456*	5.36489*
Odour	0.12458	45.88889**
Overall acceptability	3.45897*	167.66667**
Total plate count	1.21452	2.34518

*Significant at 5% level. **Significant at 1% level.

From Fig. 2, increased myosin degradation was evident for T_2; however, the densitometric analysis indicated higher value for T_1 though difference was minimum. The degradation of myosin heavy chain (205 KD) increased ($p<0.05$) continuously upto 48% (Fig. 3) by the end of 120 days of frozen storage in the case of control (C) sample. But for T_1 and T_2 surimi samples, the degradation pattern was evident but to a lesser extent (21.7and 19.7% respectively) than that of control. The trend of actin degradation was similar to that of MHC, but to a lesser extent as the actin (45 KD) band was prominent up to 120 days of frozen storage (Fig. 3). The disappearance of the MHC band and retention of actin band in the SDS-PAGE profiles of the surimi without additive 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. In the case of cryoprotectant treated samples, the degradation of MHC was much lower than that of control, which indicated that the cryoprotective formulations were effective enough in preventing the aggregation and subsequent insolubilization of myosin during frozen storage.

Frozen storage increases myosin succeptibility to thermal denaturation by decreasing the transition temperature and the decrease was less in cryoprotectant-treated sample (Jittinandana et al., 2003). Fig. 4 shows that the amount of unfrozen water decreased with the increase in storage time, regardless of the presence or absence of additives. On the initial day, the amount of unfrozen water of T_1 and T_2 surimi samples were 1.201 and 1.175 respectively, while that of the control was 0.824 mg H_2O mg^{-1} dried matter, thus indicating that the addition of SWPH or SuSo, irrespective of their deviation markedly increased the amount of unfrozen water as compared to the control. Moreover, throughout the frozen storage period of 120 days, surimi with SWPH and SuSo exhibited the amount of unfrozen water approximately 1.38 – 1.46 folds higher than that of the control. It is worth noting that sample prepared with 7.5% SWPH showed the maximum amount of unfrozen water up to 120 days of frozen storage. SWPH and SuSo probably constructed and stabilized bound water of myofibrillar protein during frozen storage which resulted in decrease in frozen water. The increase in the proportion of unfrozen water was reported, upon addition of other cryoprotective ingredients such as fish-scrap protein hydrolysate (Khan et al., 2003), chitin hydrolysate (Yamashita et al., 2003) and squid protein hydrolysate (Hossain et al., 2004).

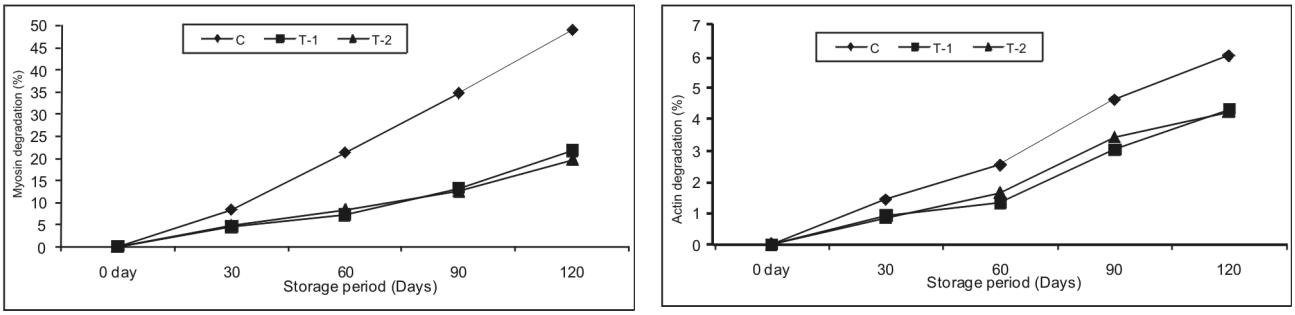

Fig 3. Extent of changes in myosin and actin degradation in croaker surimi protein without additive **(C)**, Surimi added with sucrose sorbitol **(T_1)**, Surimi added with shrimp waste protein hydrolysate **(T_2)** during 120 days of frozen storage at -25°C

Table 3. Changes in textural parameters of kamaboko gel prepared from three croaker surimi during frozen storage of 120 days at -25°C:

Storage Days	Samples	Hardness 1 (kgf.)	Hardness 2 (kgf.)	Cohesiveness	Chewiness (k.gf mm^{-1})	Springiness (mm)	Gumminess (kgf)	Shear force (k gf g^{-1})
0	C	1.87± 0.18	1.12 ± 0.08	0.96 ± 0.13	2.94 ± 0.13	1.64± 0.25	1.79 ± 0.12	1.74 ± 0.08
	T_1	1.84± 0.14	1.09 ± 0.03	0.94 ± 0.09	2.27 ± 0.16	1.32± 0.15	1.72± 0.09	1.70± 0.15
	T_2	1.78± 0.16	1.04 ± 0.01	0.90 ± 0.04	2.36± 0.18	1.48± 0.21	1.60± 0.13	1.72± 0.13
20	C	1.68± 0.03	1.03± 0.09	0.85 ± 0.1	1.60± 0.12	1.12± 0.06	1.43± 0.03	1.61± 0.14
	T_1	1.73± 0.09	1.01± 0.12	0.91± 0.11	1.94± 0.06	1.24± 0.04	1.57± 0.08	1.68± 0.16
	T_2	1.69± 0.13	0.86± 0.04	0.88 ± 0.06	2.07± 0.09	1.39± 0.06	1.49± 0.15	1.65± 0.07
40	C	1.41± 0.07	0.89± 0.03	0.73± 0.04	0.97± 0.07	0.94± 0.08	1.03± 0.04	1.43± 0.06
	T_1	1.49± 0.12	0.92± 0.07	0.82 ± 0.03	1.32± 0.12	1.09± 0.06	1.22 ± 0.06	1.55± 0.11
	T_2	1.46± 0.07	0.85± 0.05	0.80 ± 0.02	1.34± 0.04	1.15± 0.01	1.17± 0.08	1.56± 0.12
60	C	0.79± 0.01	0.51± 0.02	0.61 ± 0.16	0.41± 0.15	0.85 ± 0.03	0.49± 0.07	1.28± 0.14
	T_1	1.05± 0.06	0.65± 0.01	0.70 ± 0.01	0.71± 0.07	0.98 ± 0.03	0.73± 0.06	1.39± 0.07
	T_2	1.09± 0.01	0.66± 0.08	0.73 ± 0.07	0.83± 0.09	1.04 ± 0.06	0.80± 0.01	1.44± 0.05
80	C	0.68± 0.08	0.36± 0.11	0.54 ± 0.04	0.28± 0.01	0.77± 0.07	0.37± 0.08	1.03± 0.07
	T_1	0.90± 0.04	0.52± 0.03	0.62 ± 0.02	0.50± 0.06	0.91± 0.08	0.56± 0.02	1.17± 0.11
	T_2	0.94± 0.08	0.56± 0.04	0.66 ± 0.01	0.58± 0.06	0.95 ± 0.04	0.62± 0.03	1.22± 0.02
100	C	0.45± 0.01	0.31± 0.02	0.46± 0.06	0.13± 0.07	0.64± 0.06	0.20± 0.04	0.89± 0.04
	T_1	0.69± 0.08	0.42± 0.06	0.54 ± 0.07	0.32± 0.03	0.86 ± 0.04	0.37± 0.07	1.02 ± 0.01
	T_2	0.89± 0.02	0.48± 0.03	0.61 ± 0.15	0.48± 0.04	0.88 ± 0.07	0.54± 0.03	1.06 ± 0.04
120	C	0.43± 0.03	0.28± 0.01	0.39 ± 0.05	0.08± 0.05	0.51 ± 0.05	0.17 ± 0.07	0.81± 0.02
	T_1	0.58± 0.01	0.36± 0.08	0.51± 0.08	0.24± 0.08	0.78 ± 0.07	0.30± 0.02	0.98± 0.07
	T_2	0.71± 0.34	0.42± 0.04	0.56± 0.04	0.31± 0.09	0.80 ± 0.01	0.39± 0.04	1.03± 0.03

C : Control; T_1 : Surimi sample treated with 4% sucrose, 4% sorbitol, and 0.3% STPP, T_2 : Surimi sample treated with shrimp waste protein hydrolysate. Results are means of three determinations with S.D.

Specific Ca^{2+}ATPase activity of NAM extracted from croaker surimi samples showed a continuous decrease ($p<0.05$) with the increase of storage period resulting in the decrease of total Ca^{2+}ATPase activity (Fig. 5). The residual Ca^{2+}ATPase activity of NAM from Control, T_1 and T_2 surimi samples were 57.54, 99.15 and 100.84% respectively on the 20^{th} day of storage and remained higher than 50% throughout 120 days of storage except control which was 10.24% at 120^{th} day of storage. The decrease in Ca^{2+}ATPase activity was possibly due to the conformational changes of myosin globular head responsible for ATPase enzymatic activity as well as the aggregation in this portion (Ochaiai & Chow 2000). The results indicated that croaker muscle proteins were succeptible to denaturation and aggregation unless mixed with cryoprotectants such as SuSo or SWPH and samples with 7.5 and 10% SWPH showed optimum cryoprotective effect almost nearer to the sucrose-sorbitol. Cryoprotective effect of SWPH can be attributed to the active short chain peptide groups with molecular weight less than 1600 KD and hydrophilic amino acids which inhibit denaturation of myofibrillar protein and have water constraining effect. Ruttanapornvareesakul et al., (2006) showed that shrimp head protein hydrolysate reduced the effect of freeze denaturation on Ca^{2+}ATPase activity of lizard fish muscle. A low correlation was observed at the initial stage of frozen storage but a high positive correlation was observed between these two variables in later stages ($Y = 0.9954x - 1.3044$, $r = 0.8548$, $n = 16$, p d" 0.001) despite the differences. These results indicated that the denaturation of myofibrillar Ca^{2+}-ATPase was correlated with the amount of unfrozen water, irrespective of the presence or absence of added hydrolysate.

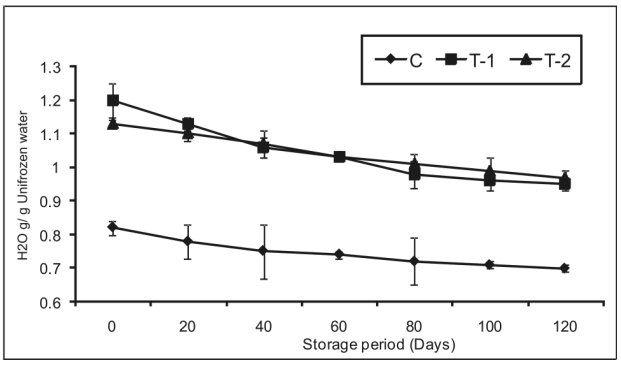

Fig 4. Changes in unfrozen water in muscle protein of croaker surimi without additive **(C)**, Surimi added with sucrose sorbitol **(T_1)**, Surimi added with shrimp waste protein hydrolysate **(T_2)** during 120 days of frozen storage at -25°C

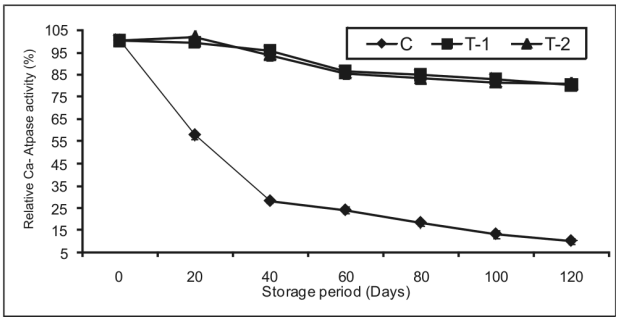

Fig 5. Changes in relative Ca-ATPase activity of natural actomyosin extracted from croaker surimi without additive **(C)**, Surimi added with sucrose sorbitol **(T_1)**, Surimi added with shrimp waste protein hydrolysate **(T_2)** during 120 days of frozen storage at -25°C

It was possible to prepare kamaboko gel from Croaker surimi regardless of the absence or presence of cryoprotectants. According to the puncture test, gel strength values before frozen storage for control, T_1 and T_2 surimi samples were 342, 471 and 473 g.cm respectively. The relative gel strength values, ratio of gel strength before and after frozen storage has been presented in Fig 6, for easy comparison of the effect of each additive on gel strength with the length of frozen storage. Relative gel strength of all samples decreased ($p<0.05$) with the increase in storage period. Specifically, the relative gel strength of the control dropped dramatically to 42.54% of the initial value within first 20 days of frozen storage, whereas kamaboko gels of T_1 and T_2 samples were 83.46 and 85.75% respectively. The result indicated that freezing and frozen storage had a prominant effect on the gel forming ability of croaker fish surimi without additive (control). Kim et al. (1986)

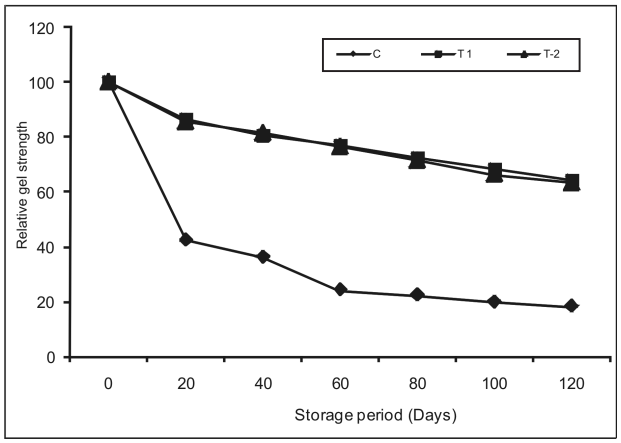

Fig 6. Changes in relative gel strength (%) of kamaboko gel produced from croaker surimi without additive **(C)**, Surimi added with sucrose sorbitol **(T_1)**, Surimi added with shrimp waste protein hydrolysate **(T_2)** during 120 days of frozen storage at -25°C

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 were exposed by freezing, resulting in the substantial decrease in force and deformation. After 120 days of frozen storage, residual gel strength of T_1 and T_2 samples were 52.82 and 56.64% while the control showed the lowest value 23.48% ($p<0.05$). The results also indicated that SWPH (7.5%) successfully stabilized gel forming ability in croaker surimi during frozen storage which is comparable to sucrose/sorbitol. A high positive correlation ($Y= 0.8017x - 1.6495$, $r=0.894$, n- 16, $p<0.001$) was also found between relative Ca^{2+}-ATPase activity and relative gel strength of surimi with or without additives. This result is in agreement with Macdonald & Lanier (1994), who reported a close correlation between the ability of actomyosin to form cohesive gels and its Ca^{2+}-ATPase activity.

Typical force by time curve plot given in Fig. 1 was used to calculate different textural parameters of kamaboko gel given in Table 3. Hardness 1 value was always higher than hardness 2 as the intact sample had a firm texture compared to an already compressed one. Though, hardness of the samples showed reduction during frozen storage addition of SWPH controlled the reduction better than SuSo especially towards the end of frozen storage evident from the result of T_1 and T_2 samples ($p<0.05$). Cohesiveness, also got reduced during frozen storage. Treatment 2 was able to give ideal

cohesiveness value during frozen storage. Gumminess decreased with the length of storage though the reduction was less for T_1 and T_2 samples compared to control (Table 3). SWPH gave higher springiness compared to the commercial cryoprotectant which gave the evidence of its gel enhancing property. Gel enhancing agents such as potato starch and egg white caused increase in the springiness values of surimi gels (Munizaga and Canovas, 2004). Chewiness of the surimi gel samples were reduced during frozen storage. The treatment with commercial cryoprotectant blend and SWPH improved the chewiness of the samples and the latter was found to be more effective especially during the initial months of frozen storage. The shear force values were found to decrease with the frozen storage as can be seen from Table 3, which agrees with the hardness values of TPA, indicating that the product was getting soft with the extent of frozen storage. However, factors related to tissue softening, e.g. release of proteases from lysosomes and cell disruption by ice crystals formation, may also exist in frozen and thawed seafoods and counteract the tissue-toughening factors (Srinivasan et al., 1997).

Expressible moisture (EM) increased 141.7, 85.3 and 108% ($p < 0.05$) for control, T_1 and T_2 samples respectively during 120 days of frozen storage (Fig. 7). This was probably due to the succeptibility of muscle protein to freeze denaturation, loosing its water holding capacity after each freeze thaw cycle. The increase in EM was more prevalent in the case of control than surimi treated with two different

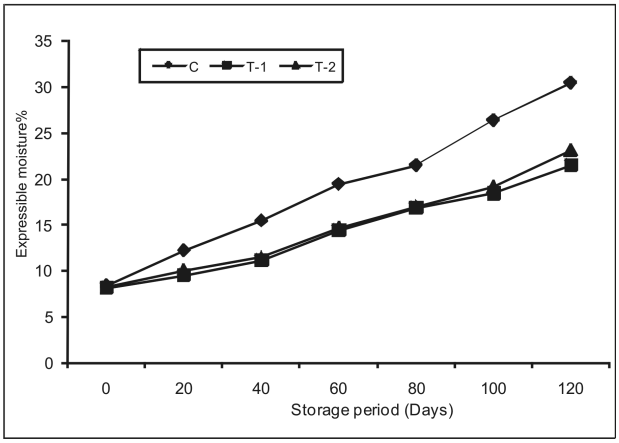

Fig 7. Changes in expressible moisture (%) of kamaboko gel produced from croaker surimi without additive **(C)**, Surimi added with sucrose sorbitol **(T_1)**, Surimi added with shrimp waste protein hydrolysate **(T_2)** during 120 days of frozen storage at -25°C

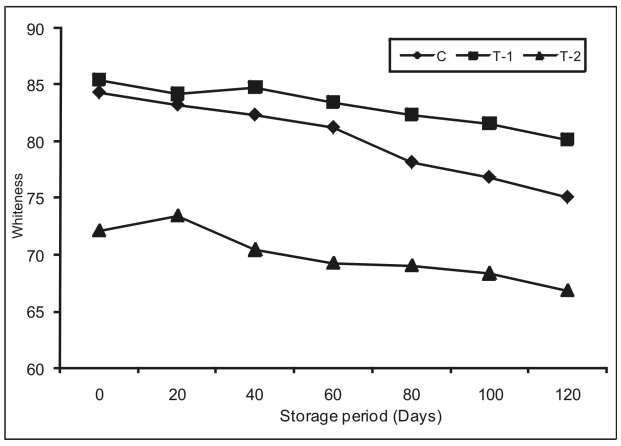

Fig 8: Changes in whiteness (%) of kamaboko gel produced from croaker surimi without additive **(C)**, Surimi added with sucrose sorbitol **(T_1)**, Surimi added with shrimp waste protein hydrolysate **(T_2)** during 120 days of frozen storage at -25°C

additives, which may be due to the increase in hydration of the muscle protein in the presence of cryoprotectants. According to Fennema et al. (1973) decrease in water holding capacity in fish muscle directly relates to the denaturation in myofibrillar protein. No significant change in whiteness of kamaboko gel samples was seen throughout 120 days of storage (Fig 8). The whiteness of kamaboko gel (T_1) during frozen storage was 81-82%, and was not significantly different from control (78-80%). However, the whiteness of all kamaboko gels with SWPH decreased and varied between 67- 71% regardless of concentration. The result indicated that SWPH affected the whiteness of kamaboko gels rather than length of frozen storage. Park (2000) proposed that all protein additives had affected the colour of surimi gels as a result of the slight reduction in L value (lightness) and large increase in b value (yellow hue). The addition of SWPH also decreased L* value and increased b* value. Decreased whiteness may be due to some remaining pigments that exhibited a light yellow colour.

Textural properties of kamaboko gel prepared from surimi samples decreased with storage days, though SWPH was efficient enough to reduce effect of freeze denaturation on gel characteristics during initial three months of storage.

References

APHA (1984) Compendium of Methods for the Microbiological Examination of Foods. (Speck, M.L ed.), 2nd edn., American Public Health Association, Washington, DC

Adler-Nissen, J. (1986) Enzymatic Hydrolysis of Food Proteins, p. 427, Elsevier Applied Science Publishers Ltd., London

AOAC (1995) Official Methods of Analysis. 16th edn., Association of Official Analytical Chemists, Arlington, VA

Azuma, Y. and Konno, K. (1998) Freeze denaturation of carp myofibrils compared with thermal denaturation. Fish Sci. 64: 278-290

Carpenter, J.F. and Crowe, J.H. (1988) The mechanism of cryoprotection of proteins by solutes. Cryobiology. 25(3): 244–55

Chakraborty, R. (1984) Changes in the muscle of thre IMC during frozen storage. Fish. Technol. 21(2): 91-93

CIFE (2001) Training Manual on Fish Processing and Product Development. 20-26 Nov. compiled by Basu, S.and Chouksey, M.K. CIFE, Mumbai

Chung, S.L. and Merritt, J.H. (1991b) Physical measures of sensory texture in thawed sea scallop meat. Int. J. Food Sci. Technol. 26: 207-210

Clemente, A. (2000) Enzymatic protein hydrolysates in human nutrition. Trends Food Sci Technol. 11(7): 254–62

Del-mazo, M.L., Torrejon, P., Careche, M. and Tejada, M. (1999) JFS is available in searchable form at www.ift.org Characteristics of the salt-soluble fraction of hake (*Merluccius merluccius*) fillets stored at –20 and –30 °C. J. Agric Food Chem. 47(4): 1372-7

Dyer, W.J., French, H.V. and Snow, J.M. (1950) Proteins in fish muscle: Extraction of protein fraction in flesh. J. Fish. Res. Bd. Can. 7: 585-593

Feng, Y.M. (2000) Effect of pH on the Functional Properties of Myofibrillar Proteins at Reduced Salt Concentration. PhD dissertation, Mass: University of Massachusetts/ Amherst

Fennema, O.R. (1973) Nature of Freezing Process in Low Temperature Preservation of Foods and Living Matter, New York, pp 151-239

Fiske, C.H. and Subbarow, Y. (1925) The colorimetric determination of phosphorus. J. Biol. Chem. 66: 375-400

Fuijii, Y., Watanabe, K. and Maruyama, Y. (1973) Relation between the ATP break down in ice stored Alaska Pollock meat and the quality of frozen surimi. Bull tokai. Reg. Fish. Res. Lab. 75: 7-11

Gopakumar, K., Muraleedharan, V. and Bhattacharya, S.K. (1992) Preparation and properties of surimi from tropical fish. Food Control. 3(2): 109-112

Gornall, A. G., Bardawill, C. T. and David, M. M. (1949) Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. 177: 751–766

Herrera, J.R. and Mackie, I.M. (2004) Cryoprotection of frozen-stored actomyosin of farmed rainbow trout (*Oncorhynchus mykiss*) by some sugars and polyols. Food Chem. 84(1): 91–7

Holanda, H.D. and Netto, F.M. (2002) Optimization of the conditions for the enzyme hydrolysis of shrimp residue, using response surface methodology (RSM). In: Book of Abstracts, 2002 IFT Annual Meeting, P 194, Anaheim, Calif, USA

Hossain, M.A., Khan, M.A.A., Ishihara, T., Hara. K., Osatomi, K., Osako, K. and Nozaki, Y. (2004) Effect of proteolytic squid protein hydrolysate on the state of water and denaturation of lizardfish (*Saurida wanieso*) myofibrillar protein during freezing. Innovat Food Sci. Emerg. Technol. 5(1): 73–9

Ian, Y.H., Novakofski, J. M., Cusker, R.H., Brewer, M.S., Carr, T.R. and Mc keith, F.K. (1995) Thermal gelation of pork, beef, fish, chicken and turkey muscles affected by heating rate and pH. J. Food Sci. 60 (5): 936-940

Jittinandana, S., Kenney, P.B., Slider, S.D. and Kiser, R.A. (2003) Cryoprotection affects physiochemical attributes of Rain bow trout fillet. J. Food Sci. 68: 4

Khan, M.A.A., Hossain, M.A., Hara, K., Osatomi, K., Ishihara, T. and Nozaki Y. (2003) Effect of enzymaticfish-scrap protein hydrolysate on gel-forming ability and denaturation of lizard fish *Saurida wanieso* surimi during frozen storage. Fish Sci. 69(6): 1271–80

Kim, B.Y., Hamman, D.D., Lanier, T.C. and Wu, M.C. (1986) Effects of freeze thaw abuse on the viscosity and gel forming properties of surimi two species. J. Food Sci. 61: 951-956, 1004

Lakshmanan, P. T. (2000) Fish spoilage and quality assessment. In: Quality Assurance in Seafood Processing (Iyer, T.S.G., Kandoran, M.K., Mary Thomas and Mathew, P.T. (Eds), pp 26-40, Society of Fisheries Technologists (India), Cochin

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 337: 680-685

Lowry, O.H, Rosebrough, N.J., Farr, A.L. and Randal, R.J. (1951) Protein measurement with folin phenol reagent. J. Biol Chem. 193: 256-275

Macdonald, G.A. and Lanier, T.C. (1994) Actomyosin stabilization to freeze-thaw and heat denaturation by lactate salts. J. Food Sci. 59(1): 101-105

Matsumoto, J. (1980) Chemical deterioration of muscle protein during frozen storage. In: Chemical Deterioration of Proteins. (Whitakers, J,R. and. Fujinaki, L.M. Eds), pp 95-124, Am Chem Soc Series., Vol. 123, American Chemical Society, Washington DC

MPEDA (2006) Marine export review. Available from www.mpeda.com (Accessed 20 October 2006)

Munizaga, G.T. and Canovas, G.V.B. (2004) Colour and textural parameters of pressurized and heat treated surimi gels as affected by potato starch and egg white. Food. Res. Int. 37(8): 767-775

Muraleedharan, V., Antony, K.P., Perigreen, P.A. and Gopakumar, K. (1996) Utilization of unconventional fish resources for surimi preparation. In: Proceedings of the Second Workshop on Scientific Results of FORV Sagar Sampada (Pillai, V.K., Abidi, S.A.H., Ravidran, V., Balachandran, K.K. and Agadi, V.V. Eds), pp 539-543, Department of Ocean Development, New Delhi

Ochaiai, Y. and Chow, C. (2000) Myosin ATPase. In: Seafood Enzymes Utilization and Influence on Post-harvest Seafood Quality. (Haard, N.F. and Simpson, B.K. Eds), pp 69 –89, Marcel Dekker Inc., New York

Park, J.W. (2000) Ingredient technology and formulation development. In: Surimi and Surimi Seafood (Park, J.W. ed), pp 343-391, Marcle Dekker, New York

Ruttanapornvareesakul, Y., Somjit, K., Otsuka, A., Hara, K., Osatomi, K., Osako, K., Kongpun, O. and Nozaki, Y. (2006) Cryoprotective effects of shrimp head protein hydrolysate on gel forming ability and protein denaturation of lizardfish surimi during frozen storage. Fish Sci. 72(2): 421-8

Shyamsunder, B.A. and Prakash, V. (1994) Physico-chemical and functional properties of prawns (*Metapenaeus dobsoni*). J. Agric. Food Chem. 42: 169-173

Somjit, K., Ruttanapornwareesakul, Y., Hara, K. and Nozaki, Y. (2005) The cryoprotectants effect of shrimp chitin and shrimp chitin hydrolysate on denaturation and unfrozen water of lizardfish surimi during frozen storage. Food Res. Int. 38(4): 345-55

Srinivasan, S., Suzanne, Y.L., Blanchard, P., Tidwell, J.H. (1997) Physicochemical changes in prawns (*Machrobrachium rosenbergii*) subjected to multiple freeze-thaw cycles. J. Food Sci. 62: 124-127

Sych, J., Lacroix, C., Adambounou, L.T. and Castaigne, F. (1990) Cryoprotective effects of lactitol, palatinit and polydextrose on cod surimi proteins during frozen storage. J. Food Sci. 55(2): 356-60

Steel, R .G. D. and Torrie, J. H. (1980) Principle and Procedure of Statistics 2nd edn., McGraw-Hill, New York

Tejada, M., Careche, M., Torrejon, P., Del-mazo, M.L., Solas, M.T., Garcia, M.L. and Barba, C. (1996) Protein extracts and aggregates forming in minced cod (*Gadus morhua*) during frozen storage. J. Agric. Food Chem. 44(10): 3308-14

Wakamatu, T. and Sato, Y. (1979) Determination of unfreezable water in sucrose, sodium chloride and protein solutions by differential scanning calorimeter. J. Agric. Chem. Soc. Jap. 53(12): 415-420

Yamashita, Y., Zhang, N. and Nozaki, Y. (2003) Effect of chitin hydrolysate on the denaturation of lizard fish myofibrillar protein and the state of water during frozen storage. Food Hydrocolloids. 17(5): 569-76

Zhou, A., Benjakul, S., Pan, K., Gong, J. and Liu, X. (2006) Cryoprotective effects of trehalose and sodium lactate on tilapia (*Sarotherodon nilotica*) surimi during frozen storage. Food Chem. 96(1): 96-103