

Colour and textural parameters of threadfin bream surimi during frozen storage as affected by cryoprotectants and chitosan

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Effect of chitosan on the colour and textural parameters of threadfin bream surimi during frozen storage were studied in comparison to commercial cryoprotectant blend and also the suitability of chitosan to replace polyphosphate in surimi during frozen storage was analysed. The treatment with cryoprotectants and /or chitosan did not have a significant influence on the moisture levels and pH during frozen storage. The addition of chitosan or cryoprotectants did not affect the colour of the samples. The textural parameters including gel strength showed higher values for the chitosan treated samples. The samples treated with sucrose, sorbitol and polyphosphate showed higher gel strength than the control upto about 4 months of frozen storage, but the textural quality was deteriorated towards the end of frozen storage where it was inferior even to the untreated samples. The sucrose-sorbitol-chitosan blend gave better textural properties to surimi than either chitosan alone or sucrose-sorbitol-polyphosphate blend.

Key words : Surimi, threadfin bream, colour, texture, cryoprotectant, chitosan

Surimi is the Japanese term for minced fish where most of the water soluble components including sarcoplasmic proteins have been removed by leaching with potable water. This concentrated myofibrillar protein fraction is the starting material for the traditional fish gel or "kamaboko" and other value added products (Suzuki, 1981). The quality of surimi is a function of its rheological properties, in particular the force and strain at failure of the heat induced gels (Lanier, 1986). During frozen storage, the gel forming ability is reduced as a result of freeze denaturation and aggregation of myofibrillar proteins (Suzuki, 1981). Cryoprotectants such as sucrose, sorbitol and polyphosphate protect fish myofibrillar proteins during long periods of frozen storage (Lee, 1984). To prepare a strong

elastic gel from surimi, gel forming biopolymers and food-grade protein additives have often been incorporated (Niwa *et. al.*, 1988; Park, 1994). Starch is the ingredient most commonly used as filler in the production of surimi. It increases firmness and gel strength (Takagi & Simidu, 1972). Other hydrocolloids like carrageenans (Gomez Guillen *et. al.*, 1997) locust bean and xanthan gum (Ramirez *et.al.*, 2002) are also used to improve mechanical properties.

Chitosan is the name used for low acetyl substituted forms of chitin and is composed primarily of glucosamine, 2-amino-2-deoxy-glucose (Shahidi *et. al.*, 1999). Chitosan has been reported to have a number of functional properties that make it technically and physiologically useful in nutrition (Shahidi *et.*

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al., 1999 and Gallaher *et al.*, 2002). Technically, these include its antimicrobial activity and its ability to form protective films (Cuero, 1999), its texturizing (Benjakul *et al.*, 2001), and binding action (No, Lee, & Meyers, 2000), emulsifying capacity (Cho *et al.*, 1998) and its antioxidant activity (Kamil *et al.*, 2002). Chitosan is used in food as a clarifying agent in apple juice (Boguslawski *et al.*, 1990), enzymatic browning inhibitor in apple and pear juices (Sapers, 1992) and as a food quality enhancer (Shahidi *et al.*, 1999). Japan produces dietary cookies, potato chips and noodles enriched with chitosan because of its hypocholesterolemic effect (Hirano, 1989).

Chitosan has been found to improve the gel strength of surimi (Kataoka *et al.*, 1998), particularly in combination with setting and/or the addition of calcium ions (Benjakul *et al.*, 2001). In these reports, frozen surimi with added cryoprotectants was used for gel formation, after tempering and mixing with chitosan. There are no reports where chitosan is added to surimi prior to freezing and there is little information on the effect on textural parameters during long period of frozen storage. Therefore the objective of this work was to analyse the changes in colour and textural parameters of threadfin bream surimi during frozen storage as affected by the addition of cryoprotectants and chitosan.

Materials and methods

Japanese threadfin bream (*Nemipterus japonicus*) was used for the study. Fresh fish of average size 12 cm were purchased from nearby fishing harbour, iced immediately and on reaching the lab, thoroughly washed and weighed. Surimi was prepared according to the method of Muraleedharan *et al.*, (1996). It was divided into 4 lots and given the following treatments, each lot receiving one among the

four treatments.

1. Control (C) ie. without any additives
2. Treatment with standard cryoprotectants- Sucrose (4%), Sorbitol (4%), and Sodium tripolyphosphate (0.3%), (percentage by weight of surimi) (S)
3. Treatment with Chitosan (1%) (CH)
4. Treatment with Sucrose (4%). Sorbitol (4%) and Chitosan (1%) (CHS)

Surimi samples were thoroughly mixed with the additives in a silent cutter

Keeping the temperature below 10°C, packed in PE pouches as thin blocks and frozen in a blast freezer (-40°C). Samples were stored at -20°C and were drawn at random for analysis at regular intervals of one month for a period of 8 months

Determination of moisture, protein, fat and ash contents were done by the method of AOAC (2000).

The Total soluble protein (King and Poulter, 1985) was obtained by homogenizing 3g meat (mince or surimi sample) with 60ml of 5% NaCl containing 0.02M NaHCO₃ (pH 7.5), for 2 min. The homogenate was centrifuged at 10000 rpm for 20 min in a refrigerated centrifuge at 3-5°C, to collect the salt soluble fraction. The protein separated was quantified by biuret method (Gornall *et al.*, 1949).

The Sarcoplasmic proteins were extracted (Sankar, 2000) by homogenizing 3 g meat in 0.02 M NaHCO₃ (pH 7 to 7.5), instead of plain water. The homogenate was centrifuged at 10000 rpm for 20 min in refrigerated centrifuge at 3-4°C, for 20 minutes to collect sarcoplasmic protein fraction. The protein separated was quantified by biuret method (Gornall *et al.*, 1949)

The difference between total salt soluble protein and sarcoplasmic (water-soluble) protein was taken as total Myofibrillar protein (Sankar, 2000).

Heat induced gels were prepared from myofibrillar protein concentrate according to the method of Ian *et. al.*, (1995) by grinding with 3% (w/w) sodium chloride for 2 minutes using a cooled kitchen mixer. During grinding, the temperature of the gel was kept below 5°C to preserve the functionality of the actomyosin. The paste obtained was stuffed using a laboratory model hand stuffer into polypropylene tubing of 5.0cm diameter, taking care to eliminate the trapped air as much as possible. The ends of the tubes were tied and the specimen cooked by immersing in a water bath maintained at 90°C for 30 minutes. The gels were cooled in chilled water and then kept at 5°C over night.

The gel strength of the heat-induced gel was analysed with the help of a Food Texture Analyzer (Lloyd instruments Ltd, UK) 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 the computer software. Specimens of 2.5 cm length x 5 cm diameter were used for measurement. The gel strength was calculated from load and deformation trigger. The highest point of peak force was load at rupture, in grams, multiplied by deformation trigger, i.e., the distance traveled by the probe, measured in centimeters. The resulting value has the unit of gram-centimeters (g.cm).

The texture of the heat-induced gel was analysed with the help of Food texture analyzer using a 50 mm cylindrical probe with 5 Kg load cell. The specification (test speed was 12mm/min, trigger 50gf and compression 40%) has been

given to the load cell. The texture parameters measured included Cohesiveness, Gumminess, Springiness, Chewiness and Stiffness.

Colour of the frozen surimi and heat-induced gels were analysed using Lovibond tintometer.

The pH was determined by weighing 5 g of surimi and homogenizing in 45 mL distilled water. Measurement was done using a Cyberscan 510 pH meter.

Results are presented as means $\pm$ standard deviation and significance of the differences between mean value was determined by analysis of variance (ANOVA) coupled with the Duncan's multiple range test (Steel and Torrie, 1980) using windows based statistical software SPSS 10. P-value of less than 0.05 were considered to be significant.

Food grade chitosan of 80% degree of deacetylation supplied by Fish processing division, CIFT, Cochin was used for the experiments.

Analytical grade reagents supplied by E. Merck (Damstardt, Germany) and Sigma (St.Louis, U.S.A). were used for the experiments.

Results and Discussion

Table 1 and 2 shows the initial biochemical characteristics of raw mince and surimi. Surimi prepared had a moisture content of 82.13% and a protein content of 15.94%. Surimi from four Indian fish species *Nemipterus japonicus*, *Trichurus savala*, *Epinephelus diacanthus* and *Saurida tumbil* having average protein content of 17% and moisture content of 79% was reported by Muraleedharan *et. al.*, (1996). Slightly higher moisture content observed here may be due to the manual dehydration process employed, which is not as efficient as a screw

Table 1. Proximate composition and characteristics of raw mince and surimi from *Nemipterus japonicus*

Composition / characteristics	Raw Mince	Surimi
Moisture (%)	79.95	82.13
Fat (%)	1.2	0.586
Ash (%)	1.31	0.662
Protein (%)	17.82	15.94
Yield (%)	30.49	29.38
pH	6.98	7.1

Table 2: Total soluble, Sarcoplasmic and Myofibrillar protein contents of raw mince and surimi from *Nemipterus japonicus*

Protein (mg/g)	Mince	Surimi
TSP (total soluble)	173.68 ± 0.125	158.63 ± .015
SPP (sarcoplasmic)	31.25 ± 0.03	16.17 ± .068
MFP (myofibrillar)	142.66 ± .095	142.43 ± .047

press. Fat content has reduced by 45% during the washing procedure in the preparation of surimi from mince. Removal of fat by 38-48% has been reported during washing of Indian major carp mince (Sankar, 2000). Minerals were also removed in the process of washing by 49.4%. Yield of mince from *Nemipterus japonicus* was 30.49% and that of surimi was observed to be 29.38%. Muraleedharan *et. al.*, (1996) reported the yield of mince from different species in Indian water to vary from 27% in *Tachysurus spp* to 56% in *Saurida tumbil*. The surimi produced was white in colour and low in fat content. pH of mince and surimi were close to neutral.

Table 3. Variation in pH of surimi from *Nemipterus japonicus* during frozen storage

Months	C	S	CH	CHS
0	7.13 ± 0.00	7.255 ± .007	7.335 ± .021	7.215 ± .021
1	7.125 ± .035	7.225 ± .035	7.225 ± .035	7.17 ± .042
2	7.1 ± .000	7.215 ± .021	7.235 ± .007	7.115 ± .021
3	7.05 ± .070	7.15 ± .070	7.2 ± .000	7.05 ± .070
4	6.995 ± .007	7.15 ± .000	7.145 ± .007	7.115 ± .021
5	7.05 ± .070	7.14 ± .014	7.15 ± .000	7.145 ± .007
6	7.05 ± .070	7.155 ± .007	7.17 ± .014	7.145 ± .007
7	7.05 ± .070	7.24 ± .014	7.195 ± .007	7.145 ± .007
8	7.115 ± .021	7.2 ± .014	7.31 ± .014	7.125 ± .035

Total soluble protein content was less in surimi compared to mince, which corresponded to the removal of sarcoplasmic proteins during washing. Myofibrillar proteins constituted around 89% of the total salt soluble proteins and the rest by sarcoplasmic proteins. Sarcoplasmic proteins were not completely removed by the washing process and showed a decrease of only 50%. Saeki & Hirata (1994) reported the loss of sarcoplasmic proteins to the tune of 50-60% during surimi production from croakers and walleye Pollock. Not much variation was observed in the myofibrillar protein content of mince and surimi.

pH of all the samples showed a slight decrease during storage upto 4 months and then increased slightly (Table3). Similar observation was made by Berg (1961) where a gradual decrease in pH of cod and haddock muscle upto 40 days of frozen storage followed by an increase and was suggested to be due to the slow precipitation of salts. Precipitation of alkaline calcium, magnesium and sodium phosphates cause a decrease in pH initially followed by an

Table 4: Variation in colour of raw surimi from *Nemipterus japonicus* during frozen storage

Months of storage		Colour			
		C	S	CH	CHS
0	R	1.3	1.3	1.2	1.3
	Y	2	1.7	1.5	2
	B	0.1	0.2	0.2	0.1
2	R	1.4	1.3	1.8	1.5
	Y	2.3	2	2	2.1
	B	0.2	0.2	0.3	0.3
4	R	1.4	1.1	1.5	1.3
	Y	2.1	2.1	2.1	2.1
	B	0.2	0.1	0	0.1
6	R	1.3	1.4	1.4	1.5
	Y	2	1.9	2.1	2
	B	0.1	0.2	0.2	0.1
8	R	1.9	1.5	1.6	1.6
	Y	2.5	2.3	2.2	1.8
	B	0.1	0	0.1	0.1

increase caused by the precipitation of acid potassium phosphate and sodium and potassium citrate. The values remained near to neutral throughout the storage period. The difference in pH between the treatments was of little practical significance. Scott *et al.*, (1988) observed that the pH of Alaska Pollock surimi with commercial cryoprotectants to vary from 7 to 7.3, which showed no trend to increase or decrease with prolonged storage of the fish up to 9 months. The optimum pH for gelation is found to vary between 6.5-7.5. The pH of the samples lied within this range.

Colour of raw surimi and gel samples did not vary significantly during frozen storage

Table 5. Variation in colour of the heat induced gel during frozen storage

Months of storage	Colour	C	S	CH	CHS
0	R	1.1	1.2	1.1	1
	Y	2	1.7	2	1.5
	B	0.1	0.4	0.2	0.3
1	R	1.1	1.2	1.2	1.1
	Y	2.1	1.9	2.1	1.8
	B	0.1	0.3	0.2	0.2
2	R	1.1	1.1	1.5	1.2
	Y	2	2	2.1	2
	B	0.3	0.1	0.3	0.1
3	R	1.2	1.2	1.2	1.2
	Y	2	2	2	1.9
	B	0.2	0.2	0.2	0.1
4	R	1.2	1.1	1	1.1
	Y	2.1	2	2.1	2.1
	B	0.2	0.1	0.1	0.1
5	R	1.1	1.1	1.1	1.1
	Y	2	2	2	2
	B	0.2	0.1	0.1	0.1
6	R	1.3	1.2	1.3	1.4
	Y	2.1	2	2	2
	B	0.1	0.1	0.1	0.2
7	R	1.2	1.1	1.2	1.1
	Y	2.1	2	2	2
	B	0.1	0.2	0.1	0.1
8	R	0.1	0.1	0.1	0.2
	Y	1.2	1.4	1.4	1.1
	B	2.1	2	2	2

(Table 4 and Table 5). The addition of chitosan did not affect the colour of the samples. Similar observation was made by Darmadji & Izumimoto (1994) where 0.2-1% chitosan did not make significant modification on lightness of beef minced meat during incubation at 30 °C for 24 h. No large differences in colour were reported in studies on pork sausages, except slight increase in lightness and yellowness when adding 0.2% chitosan oligomer (Jo *et al.*, 2001) and also no great differences in colour when using 0.1% chitosan dissolved in acetic acid (Lin & Chao, 2001).

Significant reduction in gel strength was observed during freezing and frozen storage (Fig.1). Treatment with the standard cryoprotectants was found to improve the gel strength compared to the control upto around 4 months of frozen storage, but was not effective towards the end of frozen storage period. The gel strength after freezing was about 21% higher in the standard cryoprotectant treated samples compared to the control. Chitosan treatment gave higher gel strength than the commercial cryoprotectant blend ($P < 0.05$). It was almost 58% higher in the chitosan treated samples compared to the control and about 65% higher in the sucrose-sorbitol-chitosan treated samples than



Fig. 1. Variation in Gel strength of surimi from *Nemipterus japonicus* during frozen storage

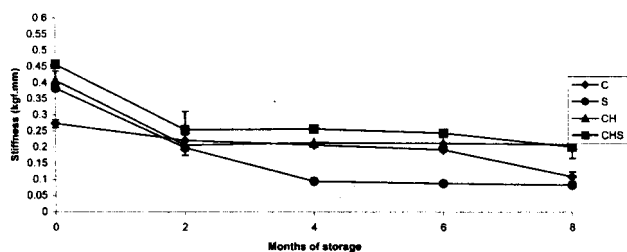


Fig. 2. Changes in stiffness of surimi gel from *Nemipterus japonicus* during frozen storage

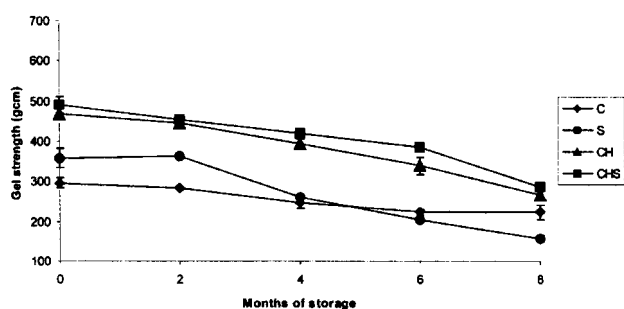


Fig. 3. Changes in Cohesiveness of surimi gel from *Nemipterus japonicus* during Frozen storage

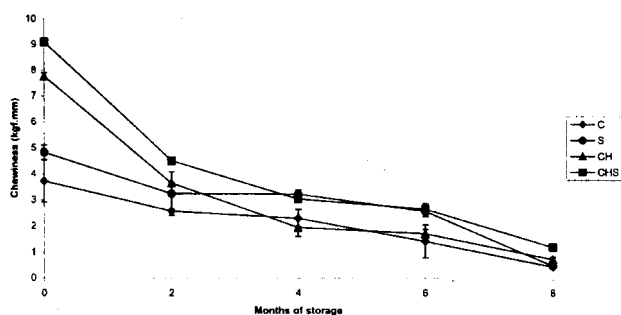


Fig. 4. Changes in chewiness of surimi gel from *Nemipterus japonicus* during frozen storage

the control after freezing. The combination of chitosan with sucrose and sorbitol gave the

highest gel strength throughout storage ($P < 0.05$). Decrease in gel forming ability has been observed during frozen storage of surimi (Yoon & Lee, 1990; Macdonald *et al.*, 1990) and the cryoprotectant treatment which reduces protein denaturation improves the gel strength (Sultanbawa & Li-Chan, 2001). Medina & Garrote (2002) reported a decrease of more than 30% in gel strength after freezing even with the addition of cryoprotectants. The improvement in gel strength by chitosan during frozen storage indicates its ability to stabilize myofibrillar proteins.

Stiffness of the gel is measured as the gradient arising from the change of force in the first 5% compression (Bourne, 1982). Stiffness of the samples showed significant reduction during frozen storage. The addition of chitosan has improved the stiffness ($p < 0.05$). Addition of standard cryoprotectants caused reduced stiffness compared to control especially towards the end of frozen storage. Addition of sugar is observed to cause a decrease in stiffness (Norsker *et al.*, 2000), but this effect was eliminated in presence of chitosan as observed by the improved stiffness of samples containing chitosan, sucrose and sorbitol, where a better stiffness was noticed even after 6 months of frozen storage compared to control and the standard cryoprotectant treatment.

Cohesiveness of the samples reduced significantly during frozen storage (Fig.3). Cohesiveness is the capacity of the sample to maintain the interactions after the first compression cycle of the TPA. Cohesiveness values close to 1 indicate sample recovery after the first compression (Munizaga & Canovas, 2004). Chitosan alone or in combination with sucrose and sorbitol gave higher cohesiveness than other treatments. Improvement of

cohesiveness by the addition of powdered chitosan has been reported in fish patties (Caballero *et. al.*, 2005).

Chewiness of the surimi gel samples showed significant reduction during frozen storage (Fig. 4). The treatment with commercial cryoprotectant blend and chitosan improved the chewiness of the samples and the latter was found to be more effective especially during the initial months of frozen storage. The result compares well with the observation in fish patties where the addition of powdered chitosan

potato starch and egg white have been reported to cause an increase in the springiness values of surimi gels (Munizaga & Canovas, 2004).

The addition of chitosan has improved the gel strength and textural properties of surimi without any adverse effect on colour. The beneficial effect of standard cryoprotectant treatment was not observed during prolonged storage period. The textural quality was deteriorated especially towards the end of frozen storage where it was inferior even to the untreated samples. Since gel strength is the single most important parameter, which determines surimi quality and price, it is concluded that chitosan can replace the commercial cryoprotectant blend in surimi preservation. The sucrose-sorbitol-chitosan blend gave better textural properties to surimi than either chitosan alone or sucrose-sorbitol-polyphosphate blend.

Table 6: Variation in springiness of the surimi gel from *Nemipterus japonicus* during frozen storage

Months of storage	Springiness (mm)			
	C	S	CH	CHS
0	7.549 ± .914	7.329 ± .019	7.479 ± .149	9.08 ± .2
2	6.81 ± .016	7.07 ± .06	7.323 ± .349	7.807 ± .022
4	6.9 ± .009	6.7 ± .49	6.503 ± .196	7.04 ± .44
6	6.73 ± .025	4.56 ± .13	4.96 ± .24	6.04 ± .048
8	4.18 ± .03	3.625 ± .225	4.44 ± .08	4.97 ± .19

(1.5%) has improved the chewiness values (Caballero *et. al.*, 2005). However, Jo *et. al.*, (2001) reported no significant changes in chewiness of the pork sausages when 0.2% chitosan oligomer was added. Chitosan- sucrose-sorbitol combination gave highest chewiness values ($p < 0.05$) compared to all other treatments.

Springiness reduced significantly during storage (Table 6). Chitosan in combination with sucrose and sorbitol gave higher springiness for the product compared to other treatments.

Springiness gives an indication of how fast the heat induced gel sample gets back after compression. In case of viscoelastic materials they recover their original height after the first compression. Gel enhancing agents such as

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