Influence of cryoprotectant levels on storage stability of surimi from *Nemipterus japonicus* and quality of surimi-based products

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Abstract A study was undertaken with the aim of reducing the concentration of cryoprotectants in surimi without adversely affecting frozen storage stability. Minced meat from a tropical fish, *Nemipterus japonicus*, was strained, water leached and mixed with different levels of sucrose-sorbitol (1:1) mixture (henceforth called sugar mixture), quick frozen at -35°C and frozen stored at -20°C . The surimi samples were subjected to storage stability studies for a period of 5 months. Water leaching resulted in slight absorption of water by meat and reduction in protein, fat and mineral contents. Surimi was found to have moderately white colour. Sensory evaluation studies were conducted on three products, viz., sausage, patty and cake, prepared using surimi containing different concentrations of sugar mixture. Sugar mixture content varying from 0% (control) to 4% in surimi resulted in products that were more acceptable to the taste panelists compared those with 6% and 8% sugar mixture. During frozen storage of surimi pH and total plate count remained nearly steady for all sugar mixture concentrations and throughout the storage period. Moisture content appeared to remain constant during storage, but decreased with increase in sugar concentration. The salt soluble nitrogen content of surimi and gel strength of sausage prepared from it decreased with storage period in all surimi samples, and increased with sugar mixture concentration. Expressible water content of surimi sausage showed an increasing trend with storage period of surimi and a decreasing trend with sugar mixture concentration. Sensory evaluation parameters—elasticity, sweetness

and preference—remained more or less steady during storage. However elasticity and sweetness increased and preference decreased with sugar mixture concentration beyond 4%. Elasticity and gel strength of surimi sausage seemed to be much lower for control compared to even the lowest concentration of sugar (2%) used. A concentration of 2 to 4% sucrose-sorbitol mixture is well-accepted by the consumers in products—surimi sausage, patty and cake and at this range of concentration surimi could be well-preserved at -20°C for at least 5 months.

Keywords Surimi · Cryoprotectants · *Nemipterus japonicus* · Sausage · Patty · Cake

Introduction

Surimi constitutes a wet frozen concentrate of myofibrillar proteins of fish muscle that is prepared by deboning, washing the fish mince and stabilizing by mixing with cryoprotectants. Muscle proteins, mainly myosin, important for protein functionality gets degraded during frozen storage (Dey and Dora 2010). Cryoprotectants are food additives that prevent protein denaturation in surimi during frozen storage. The mechanism of stabilization of fish muscle protein during frozen storage by cryoprotectant is by interaction and bonding with the protein molecules via functional groups on the surfaces. Thus each protein molecule gets covered by hydrated cryoprotectant molecules resulting in increased hydration and decreased aggregation of the proteins (Matsumoto and Noguchi 1992; Dey and Dora 2010). Today surimi serves as a convenient raw material for the manufacture of various comminuted fish products and a variety of fabricated products. Suzuki (1981) reported that the purpose of using

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frozen surimi, rather than whole fish, is not only to cut down the processing procedure but also to ensure a standard quality supply.

About 2–3 million tons of fish from around the world, amounting to 2–3% of the world fisheries supply, are used for the production of surimi and surimi-based products (Vidal-Giraud and Chateau 2007). In the year 2002 it was estimated that about 200,000 t of surimi was manufactured from tropical fish of which India's contribution was about 20% (40,000 t).

However, one of the major constraints associated with the utilization of surimi is the high content of sugar (8–10%) required to prevent protein denaturation during frozen storage. It has been recently reported that muscle proteins of tropical fish have greater stability to frozen storage conditions than those of cold water species (Park and John Lin 2005) and hence the concentration of sugar in fish meat could be substantially reduced in warm water species without affecting stability.

The study was aimed at reducing the concentration of cryoprotectants in surimi prepared from a tropical fish without adversely affecting the stability during frozen storage. Surimi is yet to become popular in India and the outcome of the study is expected to make surimi-based products acceptable in the domestic market.

Materials and methods

Fresh threadfin bream (*Nemipterus japonicus*, Bloch) that was iced immediately after catching was purchased from the fishing harbour at Kochi and transported in an insulated box in iced condition to the laboratory. Fish was washed, dressed, meat picked, minced, strained followed by water leaching thrice, dewatered and divided into five lots. Cryoprotectants were blended in each lot, packed, frozen and stored.

Mixture of sucrose and sorbitol, in 1:1 proportion by weight (sugar mixture) was mixed with various lots of surimi at the rate of 0 (control), 2, 4, 6 and 8%. Sodium tripolyphosphate (STPP) at the rate of 0.25% was also mixed with each lot in a silent cutter for a period of 10 min. The samples were packed in 50 µm polypropylene bags as 2.0 cm slabs and blast frozen at −35 °C for two hours. The slabs were frozen stored at −20 °C. Various tests were conducted for a period of 5 months. For this, every month one bag from each lot was withdrawn and the surimi was thawed at room temperature.

Proximate composition of the meat before and after water leaching was estimated—moisture content as per AOAC (1975), protein as per AOAC (1984), fat by the method of Radin (1981), and ash as per AOAC (1984). For evaluating the acceptability of surimi-based products, three

products viz., surimi sausage, patty and cake were prepared and were subjected to sensory evaluation for sweetness and preference by a taste panel of 10 judges. For evaluating frozen storage stability of surimi the following tests were carried out: pH according to Suzuki (1981), total plate count (TPC) as per Surendran et al. (2006), moisture content as previously mentioned and salt soluble nitrogen (SSN) content as per AOAC (1984). Sausage prepared using surimi was tested for gel strength, expressible moisture content and folding test characteristics according to the methods given by Suzuki (1981) and sensory quality for sweetness, elasticity and preference as per Rousseau (2004).

A modified method of Chandrasekhar and Manisseri (1976) was followed for sausage preparation. The ingredients used for fish sausage (in g/kg paste): surimi-720, maida-90, table salt-20, vegetable oil-50, red chillies and coriander-2 each, nutmeg, mace, mustard, cardamom, clove pieces, cinnamon pieces- 0.5 each, pepper powder-4, ginger paste and garlic paste-2 each and water-105. Frozen surimi was thawed and to this ingredients were added and mixed for a period of 12 min in a silent cutter. The paste was then stuffed into cellulose casings of 1.7 cm diameter at the rate of 30 g per piece. The sausages were boiled in water bath at a temperature of 88–90 °C for 40 min, cooled using chilled water (15 °C) for about 15 min followed by fan drying till surface drying occurred and labeled.

Fish patty was prepared according to a modified method of Gopakumar (2002). The ingredients used for fish patty (in g/kg paste): surimi-547, table salt-8, vegetable oil-38, clove pieces, cinnamon pieces- 1.0 each, pepper powder, turmeric powder-2 each, ginger paste-16, green chillies-16, onion pieces-164 and cooked potatoes-205. Thawed surimi was steamed for a period of 30 min. Onion, green chillies and ginger were fried in oil at 180 °C to a light brown colour and then coarsely ground in a mixie. Surimi, mashed potato, fried material and other ingredients were then mixed manually followed by mixing in a silent cutter for 5 min. The mixture was moulded into 15 g round cakes using a patty moulder. Cakes were battered in a batter of maida, table salt, whole egg and water (3:0.2:10:10 by weight) and breaded using bread crumbs. They were then fried in refined sunflower oil at 180 °C until the surface attained a medium brown colour.

The method of Suzuki (1981) was slightly modified for preparing fish cake. The ingredients used for fish cake (in g/kg paste): surimi-800, maida-80, table salt-20 and egg white-50. Thawed surimi was ground for a few minutes to which the ingredients were added and further ground for a period of 15 min with addition of ice water (50 g/kg paste). This paste was filled in stainless steel trays to 1.5 cm thickness, steamed for 30 min, cooled, cut into square pieces (3×3 cm) using a knife and were fried in refined sunflower oil at a temperature of 180 °C for a minute.

All tests were done in triplicate and sensory evaluation was done by a team of 10 semi-trained personnels. The experiments were carried out using completely randomized design. Data obtained were analyzed using analysis of variance technique (Snedecor and Cochren 1968). Pair wise comparison of treatment means were done wherever necessary using least significant difference.

Results and discussion

Meat of threadfin bream was used for the study as this species serves as an important raw material for surimi production in India. The proximate composition of meat indicates that the fish used was already lean. Comparison between the proximate composition of fish meat before and after water leaching indicated a significant increase in moisture content from $79.87 \pm 0.77\%$ to $83.23 \pm 0.27\%$, significant decrease in protein, fat and ash from $18.38 \pm 0.39\%$ to $15.5 \pm 0.23\%$, $1.0 \pm 0.03\%$ to $0.75 \pm 0.03\%$ and

$0.77 \pm 0.02\%$ to $0.57 \pm 0.03\%$ respectively. Protein content and mineral content decreased on water leaching, which may be mainly due to the loss of water soluble proteins during the leaching process. A reduction in ash content may be due to the removal of water soluble mineral constituents from meat (Sijo et al. 2002) and an increase in moisture content probably due to the hydration of myofibrillar proteins. A low fat content is a requirement for good quality surimi. Water leaching also influenced gel strength and expressible water content of surimi which indicates its significance in surimi manufacture (Karthikeyan et al. 2006).

As a preliminary step for the study, the preference of three surimi-based products that varied in composition and preparation methods, viz., sausage, patty and cake, as influenced by the sugar mixture concentration, was evaluated. An increase in the perception of sweetness with sugar mixture could be generally observed in Fig. 1a, while the products were found more acceptable at lower concentrations of sugar mixture (upto 4% level in surimi) (Fig. 1b). In patty and cake, the sweetness scores have not

Fig. 1 Taste panel scores for a. Sweetness and b. Preference of surimi-based products prepared with various sugar mixture concentrations; Bars with same superscripts are not significantly different ($p < 0.05$); $n = 10$

shown any difference between 0 and 2% which may be on account of some guess work since in 0% there can't be sweetness detection whereas most of them have not detected sweetness at 2% cryoprotectant level which is an advantage. The higher score given for patty for preference parameter (upto a score of 4.5) could be because it is already a popular battered and breaded product locally whereas fish sausage and cake are currently not available in the domestic market. However, the latter two have also been well-accepted by the panelists, again at lower levels of sugar mixture.

The variations in surimi quality were monitored using various tests. pH and TPC of surimi varied within a narrow range of 6.6–7.06 and 3.7–4.92 log₁₀ cfu/g, respectively,

with no significant difference either with sugar mixture concentration or with storage period, indicating absence of any significant chemical or microbial activity during the period of study. Scott et al. (1988) also observed that the pH of Alaska pollock surimi varied only slightly between 7.0 and 7.3 and showed no trend of increase or decrease with prolonged storage. According to Jittinanda et al. (2005) frozen storage time does not affect psychrotrophic counts of surimi treated with any cryoprotectant.

Significant reduction in moisture content occurred with increasing sugar mixture concentration (Fig. 2a). This can be attributed to the addition of sugar mixture as supported by the findings of Chang-Lee et al. (1990). Rupsankar (2010) reported that reduction of moisture in meat

Fig. 2 Changes in quality parameters of surimi containing different concentrations of sugar mixture stored at -20°C and sausage prepared from surimi; $n=3$ and standard error $\pm 5\%$; *SSN: Salt Soluble Nitrogen

improved the gel formation capacity of Bombay duck. A definite decrease in moisture content was observed in the control (0% sugar) on the second month of storage possibly due to protein denaturation that had resulted in drip loss during thawing. However a significant variation was not observed in any of the sugar mixture treated surimi throughout the storage period indicating cryoprotective effect of the sugar mixture even at low concentrations. Findings of Suvanich et al. (2000) support this.

Variation in SSN content of surimi, and expressible water content, gel strength and elasticity scores of sausage prepared from the surimi appear to be related. Reduction in SSN of frozen raw fish meat can be used as indicator of denaturation of myofibrillar proteins (Moosavi-Nasab et al. 2005). Denaturation affects extractability of proteins and cause drip formation. Such a material can adversely affect the texture and flavour of surimi-based products. In the present study a definite reduction in SSN content was seen in all lots of surimi during storage (Fig. 2b) as supported by similar observations made by Sych et al. (1990) on cod surimi. But the rate of reduction was greatest in the control lot indicating a greater rate of protein denaturation. With increase in the sugar concentration proteins were increasingly protected as indicated by the higher SSN values throughout the storage period (Table 1). Statistical analysis also showed significant variation in % SSN with cryoprotectant concentration as well as storage period. It could be observed that a sugar mixture concentration as low as 2% could substantially reduce the denaturation process. This is supported by the findings of Park and Lin (2005) that tropical fish proteins are more stable to frozen storage conditions and hence require substantially lower amounts of sugar than temperate water fish proteins.

Expressible water content of surimi sausage increased with storage period (Fig. 2c), thus showing an inverse relationship with the elasticity of the product and SSN content of the surimi. Suzuki (1981) studied the relation between the amount of expressible water and elasticity of

Alaska pollock kamaboko and found a high degree of correlation that was inverse in nature. In addition, expressible water content remained highest for control surimi (without sugar mixture) throughout the storage period but significantly reduced even with the addition of as low as 2% of sugar mixture (Fig. 2c, Table 1). This should be on account of a better water holding capacity developed in the product as a result of cryoprotection of the proteins.

Gel strength was determined by using Okado gelometer and the gel strength was calculated from the area under kymogram. Variation in gel strength (Fig. 2d) appears to be correlated with change in SSN content (Fig. 2b) suggesting that gel strength is influenced by the extent of protein denaturation. Workers like Mac Donald et al. (1992) also have obtained similar results on fishes such as hoki. The control lot of surimi sausage always resulted in a lower grade (Grade C: Crack runs through entire folded edge on first fold), B (Cracking of part of folded edge at first fold) for 2% during folding test, whereas sugar mixture concentration as low as 4% or above resulted in the highest grade of AA (No breakage at first and second fold). On account of some experimental error, during third month a fall in gel strength was observed in 2% cryoprotectant concentration. However the general trend indicated significantly higher gel strength for 2% than control. It may be noted that high elasticity is preferred by the Japanese (Suzuki 1981) but a lower grade of A or B is probably sufficient for the domestic market, which could be provided by sugar mixture concentration of 2% or more.

The taste panel evaluation of elasticity seems to agree with the objective parameter. Elasticity was judged highest for 8% sugar mixture and lowest for 0% and the variation in elasticity was found to be significant between different sugar mixture concentrations (Fig. 2e and Table 1). Although the scores did not vary significantly with the storage period a decreasing trend in the elasticity was noticeable after 3 months of frozen storage in the case of 2% and 0% levels. The sweetness and overall preference scores also did

Table 1 Variations in quality parameters of surimi and surimi sausage containing different concentrations of sugar mixture stored for 5 months at -20°C ($n=3$). Means followed by same superscripts in a row are not significantly different ($p<0.05$); *Salt Soluble Nitrogen

Parameters	Cryoprotectant concentration (%)				
	0	2	4	6	8
Moisture(%)	83.9 $\pm$ 0.15 ^a	83.2 $\pm$ 0.12 ^b	81.8 $\pm$ 0.26 ^c	80.6 $\pm$ 0.11 ^d	78.9 $\pm$ 0.32 ^e
SSN*(%)	41.2 $\pm$ 10.66 ^a	45.5 $\pm$ 7.08 ^b	46.7 $\pm$ 7.79 ^c	50.7 $\pm$ 10.02 ^d	52.4 $\pm$ 12.36 ^e
Expressible water(%)	28.9 $\pm$ 2.88 ^a	24.4 $\pm$ 3.14 ^b	22.7 $\pm$ 2.21 ^c	21.7 $\pm$ 2.08 ^c	19.3 $\pm$ 1.65 ^d
Gel strength(g.cm)	717.9 $\pm$ 263.45 ^a	801.6 $\pm$ 133.92 ^a	1127.2 $\pm$ 193.89 ^b	1262.2 $\pm$ 151.93 ^c	1361.7 $\pm$ 218.63 ^c
Elasticity	1.4 $\pm$ 0.33 ^a	2.4 $\pm$ 0.14 ^b	2.8 $\pm$ 0.23 ^c	3.0 $\pm$ 0.29 ^d	3.0 $\pm$ 0.16 ^e
Preference	2.8 $\pm$ 0.41 ^a	3.1 $\pm$ 0.08 ^a	2.9 $\pm$ 0.26 ^a	2.3 $\pm$ 0.24 ^b	2.1 $\pm$ 0.51 ^b
Sweetness	0.3 $\pm$ 0.14 ^a	0.5 $\pm$ 0.27 ^a	1.2 $\pm$ 0.29 ^b	2.2 $\pm$ 0.21 ^c	2.6 $\pm$ 0.21 ^c

not vary significantly with the storage period. However, lower concentrations of sugar mixture (upto 4% in surimi) were preferred by the taste panelists probably because of more acceptable flavour, elasticity and sweetness (Table 1).

Conclusion

Considering the frozen storage stability of surimi and the taste panel acceptability of products prepared, it is desirable to reduce the sugar mixture concentration substantially from the commercially used level of 8%, but without sacrificing the storage stability. According to the present investigation, a sugar mixture concentration of 2 to 4% can be suggested as a suitable concentration for a storage life of at least 5 months at -20°C . However, it is suggested that the exact concentration may have to be decided based on the storage period actually required as well as the type of product to be manufactured from the surimi.

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