INFLUENCE OF CRYOPROTECTANT LEVELS ON STORAGE STABILITY OF SURIMI FROM A TROPICAL FISH, *NEMIPTERUS JAPONICUS* (BLOCH), AND QUALITY OF SURIMI-BASED PRODUCTS

By

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THESIS

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COLLEGE OF FISHERIES

PANANGAD, KOCHI

To

My Family and Friends

With

Love and Gratitude

DECLARATION

I hereby declare that this thesis entitled "INFLUENCE OF CRYPROTECTANT LEVELS ON STORAGE STABILITY OF SURIMI FROM A TROPICAL FISH, *NEMIPTERUS JAPONICUS* (BLOCH), AND QUALITY OF SURIMI-BASED PRODUCTS" is a bonafide record of research work done by me during the course of research and that the thesis has not formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

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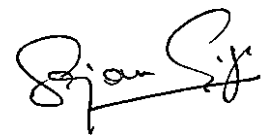

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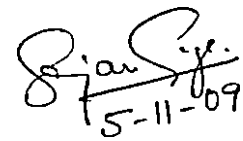

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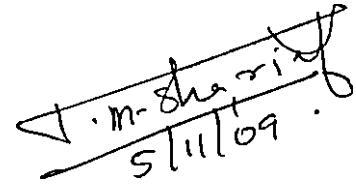

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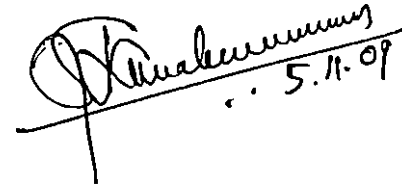

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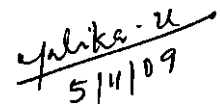

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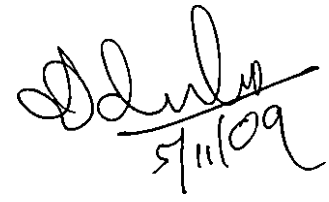

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Parvathy U.

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INTRODUCTION

1. INTRODUCTION

Surimi technology has its historical roots in Japan through centuries of experience in producing fine quality kamaboko-type products. Surimi is a Japanese term denoting the ground fish meat paste. Large scale production of frozen surimi became a commercial reality since a group of scientists at Hokkaido Fisheries Laboratories discovered the role of cryoprotectants in preserving the functionality of frozen fish proteins (Lanier and Lee, 1992). Cryoprotectants are food additives that prevent protein denaturation in surimi during frozen storage. This development together with the introduction of a new generation of surimi-based shellfish analogue products in the West led to world wide recognition of this technology.

Since the beginning of 1980's greater interest in surimi has been generated throughout the seafood and other food industries by the rapid growth through surimi-based products. According to Okada (1992) following characteristics of surimi have stimulated this development: underutilized and less utilized species can be used successfully as raw materials; frozen surimi has a long shelf life and is a highly functional protein ingredient; a variety of surimi-based products can be manufactured; advanced technology permits mass production with consistent quality.

According to recent FAO statistics, global production of surimi in the year 2004 was between 8,60,000 and 11,50,000 metric tonnes. The five main countries producing surimi are Japan, South Korea, Thailand, USA and China. The European Union has now emerged as a major producer. In the year 2002 it was estimated that about 2,00,000 t of surimi was manufactured from tropical fish of which India's contribution was about 20% (40,000 t).

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. Today surimi serves as a convenient raw material for the manufacture of various comminuted fish products like sausages, snacks, paste fishery products (kamaboko-type products) and a variety of fabricated products such as crab sticks and shrimp analogues. Suzuki (1981) reported that the purpose of using frozen surimi, rather than whole fish, is not only to cut down the processing procedure but also to ensure a standard quality supply.

It is necessary to distinguish surimi from minced fish. When fish flesh is separated from bones and skins, it is called minced fish, the starting material for surimi production and an ingredient for some processed fish products, such as fish sticks and cakes. Although whole fish can be preserved long by freezing, minced fish meat has a very low shelf life. The proteins exposed easily get denatured resulting in excessive drip and fibrous structure within a short period, making it unsuitable for preparing various products.

When the minced fish is water washed to remove fat and water soluble components it becomes raw surimi. This material possesses enhanced gel forming, water holding, fat binding and other functional properties relative to minced fish. However, the myofibrillar proteins in the raw surimi will lose their functional properties rapidly once they are frozen as in the case of ordinary minced meat. When the raw surimi is mixed with cryoprotectants such as sugars or sugar alcohols and quick frozen into a block form, frozen surimi is obtained. The myofibrillar proteins in the frozen surimi will retain their functional properties for several months if properly stored.

The relative distribution of fish proteins indicates that myofibrillar proteins are the dominant group which account for over 65% of the total proteins followed by sarcoplasmic proteins (20-30%) and stroma proteins (3-6%). Stroma proteins form the connective tissue and it is not soluble in water, acid, alkali or neutral salt solution. They do not possess the functional properties required for various products. On heating the collagen gets gelatinized. Sarcoplasmic proteins are soluble in water and in salt solution of low ionic strength. Being highly water-soluble, they influence neither the water holding capacity nor the texture of the fish muscle (Suzuki, 1981).

To the fish processing technologist the myofibrillar proteins are the most important group as they are responsible for the textural qualities such as fibrousness, water holding capacity, gel forming ability and plasticity of various products. These properties are often referred to as functional properties which are in a way responsible for the vital qualities of a variety of fabricated fish products and fish paste products. Thus during preparation of surimi, most of the highly soluble sarcoplasmic proteins are to be washed off. The myofibrillar proteins are very sensitive to heat, light, chemical reagents and processing conditions. Hence, surimi should always be prepared from extremely fresh fish and stored at low temperatures. As reported by Offer *et al.* (1984) myofibrils are the largest water holding compartment of the muscle and swelling or water uptake is associated with expansion of the myofibrillar filament lattices.

However, one of the major constraints associated with the utilization of surimi is the high content of sugar required to prevent protein denaturation during frozen storage. Sugars such as sucrose and sorbitol are to be added at 8 – 10% levels for proper stabilization of the material. This can impart considerable sweetish taste to any surimi-based products making them unacceptable to most consumers, particularly Indians. The high

sweetness may be one of the major constraints in introducing surimi or surimi-based products in the internal market.

Surimi-based products are manufactured by grinding surimi with salts and other ingredients followed by extrusion, fiberization or composite moulding depending upon the desired form of final product, and finally heated to get the shape, develop the texture, and pasteurize the product. The type of heat treatment used is altered to vary the flavour, texture and appearance desired in the final product. The different heat treatments include steaming, broiling, boiling, deep fat frying, etc. Although there can be reduction in the sugar concentration in the final products the sweetness will prevail in most cases.

It has been recently reported that muscle proteins of tropical fish have a greater stability to frozen storage conditions than those of cold water species (Park, 2005). Studies have shown that the concentration of sugar in fish meat could be substantially reduced in the tropical species without affecting stability. The main tropical fish species used for surimi manufacture are threadfin bream (*Nemipterus* spp.), big eye snapper (*Priacanthus* spp.), croakers (*Pennahia* and *Johnius* spp.), lizard fish (*Saurida* spp.) and goat fish/red mullet (*Upenius* spp., *Parupenaeus* spp.). Almost the entire surimi manufactured in India is being exported although a few surimi-based products have entered the Indian market recently.

The project was aimed at reducing the concentration of cryoprotectants in surimi without adversely affecting stability of the product during frozen storage. Meat of Japanese threadfin bream (*Nemipterus japonicus*) was used for the study as this species serves as an important raw material for surimi production in India. Surimi was prepared using various concentrations of cryoprotectants, frozen stored and were subjected to various tests to assess the storage stability. Various surimi-based products

were prepared to evaluate the consumer acceptability. As pointed out earlier, surimi is yet to become popular in India. The outcome of the study is expected to make surimi-based products acceptable in the domestic market.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 Denaturation of fish proteins

Proteins found in the fish muscle have been classified into different categories according to their distribution, organization, solubility and function in the living muscle tissue. Based on solubility the fish proteins are classified into three categories viz., sarcoplasmic proteins, myofibrillar proteins and stroma proteins (Goll *et al.*, 1977).

Striated fish muscle is composed of muscle fibres, which in turn contain myofibrils. The myofibrils are constructed of end-on-end contractile units called sarcomeres, which contain three types of filaments – thick, thin and connecting – arranged in such a fashion as to impart the striated appearance of muscle under the microscope. Suzuki (1981) reported that the predominant protein in the sarcomere, found in the thick filament system, is myosin, constituting approximately 55 – 60% of total myofibrillar protein content. The A bands of the myofibrils are composed of thick filaments and the I bands of thin filaments as shown in fig.1. Each thick filament is formed from an ordered arrangement of about 400 myosin molecules.

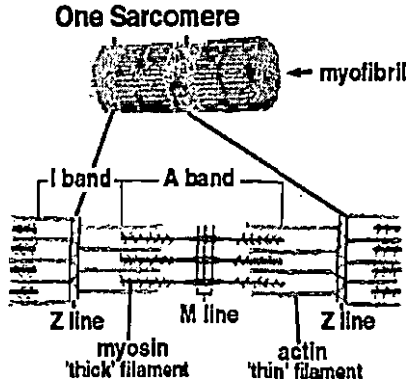

Fig. 1: Structure of myofibril

When myosin is digested by trypsin or chymotrypsin for a short period, myosin is divided into two components, a rapid sediment component called H-meromyosin (HMM) and a slow sediment called L-meromyosin (LMM). When HMM is treated with trypsin or chymotrypsin or papain it is divided into a head and neck part (Squire, 1981). The head part is called S_1 and neck part is called S_2. The HMM has ATPase activity and actin binding ability, but LMM does not have any biological functions. It is considered that the unstable structure of fish myosin exists in S_1 part of HMM.

There are three accepted theories describing alterations in the protein microenvironment that cause protein denaturation during freezing and frozen storage - an increase in solute concentration; dehydration of the cell; auto-oxidative changes that alter the balance of protein-protein and protein-water interactions (Matsumoto, 1980).

Apart from lipid oxidation products ice crystal formation can denature fish proteins during freezing and storage. It is well established that large ice crystals formed extracellularly by slow freezing can cause greater cell damage than small intracellular ice crystals resulting from rapid freezing (Shenouda, 1980). In addition, Deman (1980) observed that freezing results in concentration of solutes and restructuring of water molecules bound to proteins. Martes *et al.* (1982) observed that denaturation of proteins in this way can expose reactive groups which can interact to form aggregates.

According to Park *et al.* (1987) as freezing progresses proteins are exposed to increased ionic strength in the non frozen aqueous phase that leads to extensive modification of protein native structure. Factors influencing protein denaturation during freezing and frozen storage include salt concentration, pH, ionic strength, surface tension and the physical

effects of ice and dehydration. The mechanism of protein denaturation caused by drying can be considered to be the same as the mechanism of freeze denaturation because in both processes water molecules in the cells are removed.

Franks (1995) explained that in a dehydrated state protein-water interactions in tissue are disrupted and protein molecules are exposed to an organic environment that is less polar than water. These changes result in an increased exposure of hydrophobic side chains leading to changes in protein conformation. Recent studies by Srinivasan and Xiong (1996) have demonstrated that lipid autooxidation is involved in denaturation and deterioration of muscle protein functionality by causing cross linking between protein and lipid oxidation products.

The changes in protein structure associated with the preparation and frozen storage of surimi were investigated by Moosavi-Nasab *et al.* (2005). Raw surimi was prepared by repeatedly washing Alaska pollock flesh with chilled water. The product was either slowly frozen or underwent rapid freezing using liquid air; in either case it was then subjected to frozen storage at -20 °C for 24 months. Electrophoresis and differential scanning calorimetry (DSC) results revealed a loss of myofibrillar proteins from surimi after three washing cycles, suggesting that three washing cycles were adequate to prepare surimi. Fourier transform infrared/attenuated total reflectance (FTIR/ATR) spectroscopy indicated a significant decrease in a-helix after two years of storage at -20 °C. The loss of a-helical content was more significant in slowly frozen surimi compared to rapid-frozen surimi samples.

The myofibrillar proteins of most fish, being cold blooded, are known to be liable to denaturation. Park (2005) reported that stability of fish myosin differs considerably among fish species, being closely associated with temperature at which the fish lives; that is, colder the environmental

temperature, more liable (less stable) the myosin. He also reported that 6% sucrose is typically used in surimi manufactured from warm water species perhaps due to higher thermal stability.

2.2 Water leaching

Surimi is prepared by water leaching of mechanically separated fish muscle followed by the addition of cryoprotectants to improve protein stability during frozen storage (Lee, 1984). Lanier (1986) reported that the washing procedure is of great importance on the final quality of surimi, not only for removing fat and undesirable material such as blood, pigments and adourous substances, but more importantly for increasing the concentration of myofibrillar proteins (actomyosin) thereby improving gel forming ability and decreasing protein denaturation during frozen storage.

Gomez and Mendes (1997) conducted studies to determine the effects of wash water parameters such as pH, hardness and sodium tripolyphosphate (STPP) content on water holding capacity and gelation properties of sardine (*Sardina pilchardus*) mince. Two experiments designed by response surface methodology were carried out. Water holding capacity of the mince was maximum when washed in the pH range of 6.5-7.0, with STPP greater than 1.7 g/l and $CaCO_3$ between 30 and 60 mg/l. Variation of water hardness did not induce any significant changes in rheological parameters. Use of relatively high concentrations of STPP in the leaching water contributed to a detrimental effect on breaking force and consequently also on gel strength.

A beneficial decoloration effect resulted for horse mackerel mince washed with ozonized water within 10–20 min, but a longer washing time was required to improve the colour properties when cold water or alkaline solution was used (Chen *et al.*, 1997). Increase in pH as well as

improvements in gel forming ability occurred for mince washed with alkaline solution. Maximal gel strength was obtained for surimi washed for 90 min. A marked decrease in pH and an undesirable gel strength of mince as well as oxidation of the fish oil occurred during ozone treatment. Since the salt-soluble protein concentration increased for all minces washed with the three methods, improvement in gel-forming ability of washed mince was attributed to increase in pH rather than to oil removal. According to Mendes *et al.* (1998) leaching horse mackerel meat using a solution of tetrasodium pyrophosphate (0 - 0.2 %) and $CaCO_3$ (50 mg/l) at a pH of 6.5 resulted in good gelatin characterized product.

Effects of washing and storage on the quality of *Selaroides leptolepis* and *Aristicthys nobilis* surimi kept at -20 °C for 24 weeks were investigated by Siah *et al.* (1998). Results showed that twice washed surimi of both species were generally more stable than once washed and unwashed surimi. The quality in terms of texture, colour, elasticity, moisture content, pH and salt soluble protein value for all samples showed significant decrease during storage. There were increase in expressible moisture, trimethylamine, total volatile base nitrogen and thiobarbituric acid values. It was concluded that twice washed minced flesh from both these species were more stable as a raw material for the production of surimi.

Studies conducted by Sankar and Ramachandran (2002) on mrigal flesh indicated absorption of water by 1-3 %, loss of fat by 49 % and loss of salt soluble proteins by 35 % upon washing. Approximately 75 % of salt soluble proteins were retained after three washing cycles. The rheological properties of the washed flesh were however significantly better than that of unwashed mince. Bindu *et al.* (2004) prepared surimi from black tilapia (*Oreochromis mossambicus*) after storing the fish in ice for different periods. Two washing cycles were given under two different mince-water wash ratios, viz., 1:2 and 1:3. The gel strength and compressibility of tilapia

surimi were higher than those of Indian major carps and were comparable to that of surimi from marine species. Two successive washings at a mince: water ratio of 1:2 (w/v) for a period of five min each was found to be ideal for optimum yield and quality of the surimi.

The effect of the number of water washing cycles on the proximate composition, physicochemical and functional properties of proteins from threadfin bream meat was studied by Karthikeyan *et al.* (2006). There was a significant decrease ($P < 0.05$) in the contents of proteins (3.03% to 2.06%), fat (3.45% to 1.23%), ash (1.13% to 0.15%) and non-protein nitrogen (315.97 mg/100g to 83.33 mg/100g) with the number of washing cycles.

2.3 Cryoprotectants

Okada (1968) reported carboxylic acids such as citric acid to be effective as a cryoprotectant in frozen surimi. The cryoprotective effect of carboxylic acids appear to be closely related to their solubility in water and melting point: higher the solubility, or lower the melting point, higher will be the cryoprotective properties.

Glycitols (sugar alcohols) which are more chemically stable than saccharides were also systematically studied by Noguchi (1974). Glycerol, adonitol, arrabitol, xylitol, and sorbitol exhibited a cryoprotective effect, while erythritol, mannitol, and dulcitol did not. These results suggest the importance of the special structure and physical properties of additives in relation to their cryoprotective effect. Sorbitol is widely used in commercial surimi processing because of its excellent cryoprotective properties, relatively low cost, and low sweetness.

Most of the monosaccharides, disaccharides and several low molecular weight polyols as well as many amino acids and carboxylic acids

were found to be cryoprotective (Noguchi, 1974). In addition high molecular weight compounds such as polydextrose and maltodextrose were found to be cryoprotective. The first amino acid studied by Noguchi and Matsumoto (1975) for its cryoprotective effect on frozen surimi was sodium glutamate. Some of the dicarboxylic amino acids such as glutamic acid and aspartic acid shows good cryoprotective properties.

Noguchi *et al.* (1976) observed that hexoses (glucose and fructose) and disaccharides (sucrose, lactose and trehalose) are amongst the most effective cryoprotectants. Trehalose has only 45% sweetness of sucrose and treatment of surimi with it, but without phosphate, also showed improved frozen stability. Trehalose gave better protein stability at lower concentrations. Pentoses (xylose and ribose) were less effective than hexoses and disaccharides. The content of free aldehyde groups is higher for pentoses than for hexoses and that aldehyde groups bring about the amino-carbonyl reaction. Therefore, the above results suggest that chemically reactive substances are not suitable as cryoprotectants though the reactivity of additives is not so pronounced at low temperature.

Hexoses, disaccharides, and tetrasaccharides were effective, while inulin had a slight effect and starch had none. These observations by Matsumoto *et al.* (1982) suggest that the preventive activity of saccharides does not vary with molecular size when the molecule is not very large. However, several large polymers have been shown to exhibit a cryoprotective effect of fish muscle protein such as polydextrose and maltodextrins. Arakawa and Thimasheff (1982) found out that cryoprotectant increases the surface tension of water as well as the binding energy, preventing withdrawal of water molecules from the protein, thus stabilizing the proteins.

Structural changes occurring during frozen storage of surimi lead to protein denaturation and subsequent loss of gelling capacity. Therefore the inclusion of cryoprotectant is required to ensure long-term frozen stability of surimi (Lee, 1984). This in turn assures good functionality of the material in food manufacture, expressed primarily as gel forming potential with its manifestations of texture formation and water binding properties.

Addition of starch considerably enhances the textural property of surimi gel by increasing the extent of gelatinization as observed by Lee (1984). Starch facilitates greater water absorption. Among the various starches used in surimi manufacture potato starch imparts best firmness and cohesiveness. Strong elastic gels are produced from unmodified starches like potato and wheat starches. This is because of their ability to bind a larger amount of water or to swell to a bigger granular size. Modified starches are seen to improve freeze-thaw stability but give gels of poor elasticity and firmness. Hence a combination of half-modified and half-unmodified starch is recommended along with egg white to produce desirable balance between gel strength and freeze- thaw stability.

According to Offer *et al.* (1984) sodium chloride is added to fish mince to enhance water uptake and swelling in the manufacture of surimi and other products such as sausages, burgers and hams. Salt is essential in extracting myofibrillar proteins. Sodium chloride improves the binding ability of proteins by increasing the amount of salt-extractable proteins as well as altering the ionic strength and pH of the medium facilitating the formation of a coherent three dimensional structure during the process of heating the proteins. Based on the addition of salt surimi can be classified as *mu-en* surimi (free of salt) and *ka-en* surimi (with salt).

Burbot fillets (*Lota lota*) were treated with STPP, sodium glutamate (MSG), a high pH buffer or an antioxidant mixture (BHA, propyl gallate

and citric acid) under high pressure, then frozen and stored at -12 °C by Krivchenia and Fennema (1988a). Similar treatment was given to whitefish fillets (*Coregonus cupleaformis*) by Krivchenia and Fennema (1988b). Control samples included untreated fillets stored at -12 °C and -60 °C, and STPP dipped fillets. Samples treated with STPP or the high-pH buffer had better textural properties than untreated control samples.

Freeze-induced protein denaturation of pollock surimi as affected by the addition of sugar and/or polyol, including a starch hydrolysate product, and/or phosphates during eight months' frozen storage was investigated by Park *et al.* (1988). Polydextrose appeared to be a substitute for sucrose or sorbitol having the same cryoprotective effect. The maltodextrin adversely affected gel-forming properties, although it maintained the salt-soluble protein extractability nearly same as sucrose, sorbitol or polydextrose. The cryoprotective effects of phosphate addition seemed to depend upon the pH and/or specific phosphate ion used.

Yoon and Lee (1990) observed that there were no significant differences in the cryoprotectant effect amongst crystalline sorbitol, liquid sorbitol alone and the latter in combination with sucrose. Optimum sweetness was obtained with either crystalline sorbitol and liquid sorbitol at 8% level or liquid sorbitol with sucrose at 3% level each. Variables evaluated included gel forming properties, cooking loss, drip loss and ice crystal formation. Addition of 8% sorbitol resulted in the highest water holding capacity and gel strength, and least ice crystal formation. A better cryoprotective effect was shown in uncooked products rather than in cooked ones.

Freeze-induced protein denaturation of cod surimi was studied by Sych *et al.* (1990) as affected by carbohydrates. Good cryoprotective effect was achieved from sorbitol, glucose syrup, sucrose and 1:1 mixture of

sucrose and sorbitol at 8% concentration in surimi. Oligosaccharides have been widely used as a food ingredient due to their favourable properties such as high water holding capacity, low sweetness, low viscosity and low calories (Park *et al.*, 1992).

The most commonly used cryoprotectants in the surimi industry have been low molecular weight sugars and polyols such as sucrose and sorbitol, typically added at 8 %, alone or in 1:1 mixture, to leached fish muscle (Mac Donald and Lanier, 1991). Sucrose is usually combined with sorbitol to reduce sweetness (Matsumoto and Noguchi, 1992). These carbohydrates were chosen because of their relatively low cost, availability and less tendancy to cause maillard browning in white gel products. However, 6 % sucrose is typically used in surimi manufactured from warm water species perhaps due to higher thermal stability.

In addition, a mixture of STPP and tetrasodium pyrophosphate (TSPP) in 1:1 ratio at 0.2-0.3% is commonly used as both a chelating agent making the metal ions in surimi inactive, and a pH adjusting agent. Matsumoto and Noguchi (1992) observed that polyphosphates have no cryoprotective effect but works as a synergist to the cryoprotective effect of the carbohydrate additives which gives enhanced protein extractability, which in turn enhances gel forming and water binding properties.

Uijttenboogaart *et al.*, (1993) conducted a study to determine whether stabilization of myofibrillar protein isolates (MPI) during frozen storage could be achieved by addition of certain cryoprotectants. For two to four weeks at - 21°C MPIs were exposed to different freezing and thawing treatments to determine the extent of prevention of denaturation of the protein isolates by cryoprotectants. Overall evaluation of color, weight loss of gels during cooking, and texture proved that 2.8 % sorbitol in combination with 4 % starch was the best cryoprotectant. A positive effect

was also noted for a mixture of 2.8 % sorbitol and 4% sucrose. In contrast, the addition of a dextrose polymer mixture to MPI was not effective in maintaining product integrity.

Jasmine *et al.* (1994) processed fresh threadfin bream (*Nemipterus beekeri*) minced meat and divided into four lots. Cryoprotectants such as sorbitol 4% (w/w), ascorbic acid 0.1% (w/w) and mixture of sorbitol and ascorbic acid were added to each lot. Minced meat mixed with mixture of sorbitol and ascorbic acid represented better quality during frozen storage. The cryoprotective effect of various additives (sucrose, sorbitol and phosphates) on frozen surimi made from *N. tolu* was investigated by Yu *et al.* (1994) who showed that overall acceptability was highest in surimi containing 5% sucrose whereas STPP was more effective than sodium pyrophosphate.

Cryoprotectants (5-6 %) are used to stabilize functional properties of minced meat (Jeremiah, 1996). Salt (4 % NaCl) accelerates the destabilization of muscle proteins with subsequent decrease in functional properties, but this effect was reduced by cryoprotectants. Stabilization of fish myofibrillar proteins by low molecular weight carbohydrates and polyols during frozen storage forms the basis of the surimi process. Studies done by MacDonald *et al.* (1996) have shown that certain divalent cations, notably zinc, may enhance the effectiveness of cryoprotective solutes for stabilizing labile enzymes. Chung and Regenstein (1997) observed that a combination of sucrose and sorbitol (4% each of mince weight) and sodium hexametaphosphate (SHMP) (0.5% of mince weight) improved protein functionality and textural properties of mince during frozen storage.

The control of undesirable changes in the functional properties of meat proteins by cryoprotectants were studied by Dziomdziora and Krala (1998). Composition of various cryoprotectants such as sorbitol, sucrose,

starch and starch hydrolysates was cited. Beef heart surimi was prepared by Wang and Xiong (1998) in the presence or absence of propyl gallate and blended with or without cryoprotectants (sorbitol, sucrose) and frozen stored at temperatures of -15 °C, -29 °C, and -70 °C for upto 52 weeks. Protein solubility, gelling characteristics, water holding capacity, cooking yield, and emulsifying properties decreased during storage at -15°C and -29°C for control surimi (without cryoprotectants). Propyl gallate alone did not influence functionality changes. However, functional properties were largely protected by cryoprotectants as well as at the temperature of -70°C independent of cryoprotectants. Thus, unless extremely low temperatures are used, beef heart surimi subjected to long term cryogenic storage should be mixed with cryoprotectants and antioxidants to preserve functionality.

The effectiveness of various cryoprotectants consisting of polyol blends in maintaining quality of frozen ling cod surimi stored at -18°C was investigated by Sultanbawa and Li-Chan (1998) and compared with the commercial cryoprotectants mix (4% sucrose and 4% sorbitol). Results showed no significant changes in the gel strength, colour, pH or myosin to actin ratio of surimi with any of the cryoprotectant blend used. Tomaniak *et al.* (1998) examined various carbohydrate cryoprotectants which could be added to frozen ground raw meat. Data indicated that polydextroses should be chosen for use in red meat, as it was least sweet in solutions, its taste was suppressed by inclusion in meat, its duration of sweetness was lowest and its total flavour impact was smallest.

Freeze-thaw studies by Auh *et al.* (1999) on different concentrations of actomyosin solution extracted from Alaska pollock revealed that an 8% (w/v) solution of oligosaccharides mixture (HBOS) was most effective in cryoprotection. During frozen storage at a temperature of -18°C, HBOS showed cryoprotective effects similar to those of sucrose and sucrose-sorbitol mixture (1:1). Surimi gel prepared with HBOS showed higher

hardness and denser microstructure than others; although water holding capacity was slightly lower than the gel with sucrose-sorbitol mixture. Gel containing HBOS showed lower whiteness than that containing sucrose but no difference was noticed with sucrose-sorbitol mixture. HBOS appeared to have good potential as a non-sweet cryoprotectant of fish protein.

Surimi and natural actomyosin (NAM) from ling cod (*Ophiodon elongatus*) were subjected to frozen storage studies by Sultanbawa and Li-Chan (2001) in the absence or presence of cryoprotectants (sorbitol, sucrose, lactitol, and litesse), either individually or in combination. Commercial blends of cryoprotectant (4% sucrose and 4% sorbitol), individual cryoprotectants at 8%, and optimal blends at 4, 5.5, 6, and 8%, were effective in maintaining the gel strength of surimi and NAM gels stored at -10 °C for a period of 10 days. Surimi or NAM frozen in the absence of cryoprotectants or with only 4% individual cryoprotectants, showed increased percent a-helical content by Raman analysis.

To investigate the role of antioxidants and cryoprotectants in minimizing protein denaturation in frozen lean fish, Badii and Howell (2002) treated cod fillets with antioxidants or vitamin C and vitamin E, antioxidants with citrate, cryoprotectants (4% sucrose and 4% sorbitol), or a mixture of antioxidants, citrate and cryoprotectants. Results indicated that protein denaturation and texture changes were minimized in the presence of cryoprotectants as well as in the presence of antioxidants with citrate, antioxidants alone or as mixture of antioxidants, citrate, and cryoprotectants. According to them ice crystal formation and lipid oxidation products are the major factors that cause protein denaturation in lean frozen fish, and antioxidants in addition to cryoprotectants can be used to minimize toughness.

Extent of stabilization of trout myofibrillar proteins during 90 days' storage at -20°C and after soaking fillets in water, 8.0% sucrose and sorbitol, or 1.0% sodium lactate, in the presence or absence of 0.5% phosphate and 0.05% $MgCl_2$, was investigated by Jittinandana *et al.* (2003). Treatment with cryoprotectants increased total protein and myofibrillar protein solubility and decreased surface hydrophobicity, total, free, and myosin susceptibility to thermal denaturation. Phosphate minimized frozen storage effects on actin solubility and reduced protein surface hydrophobicity and myosin susceptibility to thermal denaturation, while $MgCl_2$ increased the negative effects of frozen storage.

Cryoprotectants other than sucrose and sorbitol were evaluated by Jittinandana *et al.* (2005) for their effects in reducing the sweetness of restructured trout products during frozen storage. Bacterial growth, lipid oxidation, thaw loss, cook yield, color, and texture were evaluated after 1 day, 3 months, and 6 months of storage at -20 °C. Sucrose-sorbitol mixture, trehalose, and trehalose-sorbitol mixture at 8% levels equally exhibited a cryoprotective action and minimized thaw loss and texture changes during six months of frozen storage.

2.4 Action of cryoprotectants in surimi

Studies on the mechanism of freeze denaturation and its prevention by cryoprotectants have been reviewed by many workers like Meryman (1968) and Nemethy (1968). Some theories focus on the water-ice structure and its change by the presence of cryoprotectants while others emphasize the contribution of the surface functional groups of protein molecules in the process of freezing.

DSC studies reveal that glucose and sodium glutamate behave very differently with respect to their effects on the freezing of water. Love (1968)

reported that glucose addition depresses the freezing point of water in minced fish meat whereas sodium glutamate has no effect on the amount of frozen water at comparable temperatures.

A hypothetical mechanism for the cryoprotective effect of compounds other than sugars and polyols was developed by workers like Matsumoto *et al.* (1977). In such cases the cryoprotectant molecules interact and bond with the protein molecules via functional groups on the surfaces. Water molecules are hydrated onto the other remaining functional groups of the cryoprotectant, and thus each protein molecule is covered by hydrated cryoprotectant molecules. In this manner the frequency of mutual contact between protein molecules is lowered resulting in increased hydration and decreased aggregation of the proteins.

In the case of cryoprotectants that possess ionic functional groups, particularly those having anionic groups like dicarboxylic acids and dicarboxylic amino acids, the protein molecules become covered by anionic charges. These anionic surface charges result in a repulsive force between the protein molecules and an increased hydration of the protein-cryoprotectant conjugate via other anionic groups on the cryoprotectant molecules. The higher effectiveness of anionic compounds as cryoprotectant additive was explained by Matsumoto *et al.* (1977), and Matsumoto (1980) as follows. At natural pH the muscle protein possesses a negative net surface charge due to the prevalence of exposed anionic residues. Thus it would require less anionic additive to block the remaining cationic surface groups than cationic additive to block the more prevalent anionic surface groups of the protein. Additionally, the anionic groups tend to become more hydrated than cationic groups. Thus, an ionic coating is more extensively achieved when anionic additives are used. Arakawa and Timasheff (1982) concluded that the denatured structure of proteins is thermodynamically less

favourable in sugar solution than in water. This theory appears to involve the increase in hydration of protein molecules in sugar-added water.

Evidence for the coating of protein molecules by the nonsugar-polyol cryoprotectant additives were obtained under electron microscope by Matsumoto and Noguchi (1992). Actomyosin filament suspensions to which sodium glutamate is added show stretched filaments separated from one another, while those to which glucose is added are curled or lightly entangled. Stretching and separation of the filament indicates a mutual repulsion due to an ionic coating of the same kind of ionic charge, namely, anionic one, along the protein molecules.

Ice formation concentrates reactants, particularly salts and hydrogen ions (pH depression), which thermodynamically destabilize proteins. The volume of ice formed increases with decreasing temperature below the freezing point. Thus according to Park (2005) by depressing the freezing point with low molecular weight solutes, the deleterious effects of freeze concentration on proteins at any sub-freezing storage temperature should be lessened.

Stabilization by increased surface tension of the medium appears to be the dominant factor leading to the thermal stability of proteins, particularly globular proteins. In addition, the preferential hydration of proteins in the presence of sugars is due to the ability of sugar to increase the surface tension of water (Park, 2005). Increased surface tension of water in the presence of polyols and sugars has been attributed to stronger or more extensive hydrogen bonding between solute hydroxyl groups and water molecules.

2.5 Frozen storage of surimi

Hsu (1990) applied a split plot design to study the effect of frozen storage on the quality attributes of surimi. Gel strength of surimi products as well as whiteness of fish sausages were shown to be significantly ($P<0.01$) affected by storage and its interactions with leaching, grinding, setting and heating processes.

Verma and Srikar (1994) noticed during frozen storage of pink perch mince a non-significant decrease in crude protein, total lipid and water soluble protein during initial storage, whereas salt soluble protein content decreased significantly ($p < 0.05$) throughout the storage. Free fatty acids (FFA), peroxide value (PV), trimethylamine nitrogen (TMAN), total volatile base nitrogen (TVBN) were found to increase significantly throughout the storage period of 180 days. A significant ($p < 0.05$) inverse correlation was observed between salt soluble protein content and PV, FFA, TMAN and TVBN. An increase in peroxide value (PV), freefatty acid (FFA) content and thiobarbituric acid reactive substances (TBARS) was observed to the extent of 3.9-, 3.8- and 4.8-fold respectively, of their initial values during storage indicating oxidation and hydrolysis of tissue lipid. The increase in PV was less during initial stages whereas the increase in FFA and TBARS were more pronounced and amounted to 68 % and 48 % respectively.

Surimi prepared from *Selaroides leptolepis* and *Aristicthys nobilis* kept at -20°C for 24 weeks were investigated by Siah *et al.* (1998). Results showed that twice washed surimi of both species were generally more stable than once washed and unwashed surimi. The quality in terms of texture, colour, elasticity, moisture content, pH and salt soluble protein value for all samples showed significant decrease during storage. There were increases in expressible moisture, TMAN, TVBN contents and TBARS value.

Changes in TVBN, pH, salt soluble protein (SSP), moisture content, and expressible moisture of channel catfish mince during storage at -20, 0, and 5°C were investigated by Suvanich *et al.* (2000). Refined mince was either unwashed or washed twice. Mince designated for frozen storage was mixed with cryoprotectant. TVBN increased during refrigerated storage while SSP decreased during frozen storage. Expressible moisture increased during frozen storage but not during refrigerated storage. Moisture content and pH of mince did not change during storage. Results indicate that mince should be stored for no longer than three days at 0 or 5 °C to maintain optimal quality. Frozen mince with cryoprotectant would remain acceptable for at least three months at -20 °C.

The structural changes occurring during frozen storage, leading to storage induced myosin aggregation and consequent loss of gelling capacity of fish muscle systems, have been investigated by Sultanbawa and Li-Chan (2001) using model systems of myosin or actomyosin. According to them the globular heads of myosin are responsible for its enzymatic (ATPase) activity which is sensitive to changes in the configuration of the molecule around the enzymatic site. Many studies on fish have established that there is a significant loss in the ATPase activity following frozen storage. However, loss of ATPase activity is not necessarily synonymous with frozen storage induced aggregation of myosin. Since sulfhydryl (SH) groups are easily oxidized to disulfide (SS) groups, they are considered to be the most reactive functional group in proteins.

The effects of freezing rate and storage temperature on calcium ATPase activity and salt-solubility of myofibrillar protein in silver carp muscle were studied by Wang and Su (2001). The results showed that freezing rate might have an effect on freeze denaturation of muscle protein in round fish with intact muscle tissue to a certain degree, but almost no effect on protein denaturation of minced fish or surimi as muscle structure

was destroyed no matter whether cryoprotectants were added or not. However, lower the frozen storage temperature the less the extent of freeze denaturation of round fish, minced fish or surimi. It was also observed that freeze denaturation of myofibrillar protein in fish muscle could be efficiently reduced by the addition of cryoprotectants especially in minced fish or surimi.

Surimi is the base for a variety of food formulations. Physico-chemical, organoleptic and microbiological changes taking place in surimi prepared from four tropical fishes, viz., common carp (*Cyprinus carpio*), tilapia (*Oreochromis mossambicus*), threadfin bream (*Nemipterus japonicus*) and shark (*Scoliodon sorrakowah*), during frozen storage were discussed by Hassan *et al.* (2003). The compressibility of shark surimi was more compared to other species. Its organoleptic and physical qualities were good even after a year's storage. Microbiological parameters showed insignificant variation between samples.

Investigations by Singh and Balange (2003) on factors responsible for spoilage of surimi prepared from pink perch (*Nemipterus japonicus*) and stored at various temperatures, in terms of organoleptic quality, bacterial load and biochemical indices, indicated that at ambient temperature ambient (25 to 28 °C) surimi could be preserved for about eight hrs while at refrigerator temperature (6.5 to 7.5 °C) up to five days and at deep freezer temperature (-8 to -10 °C) up to 60 days. Bacterial count, trimethylamine and total volatile nitrogen values increased with storage time whereas organoleptic score decreased. Nutrient contents and gel strength of surimi decreased while moisture content and whiteness increased with storage period.

The frozen storage (-20°C) behaviour of surimi prepared from big eye snapper (*Priacanthus hamrur*) was investigated for 36 weeks by Singh *et al.* (2004). TVBN, TMAN, PV and TPC values of surimi increased

gradually from 1.75 to 4.2 mg %, 0 to 1.4 mg %, 0 to 0.7 meq/kg and 6.26 x 10^4 to 9.8 x 10^4 nos/g, respectively, during frozen storage but were within the acceptable limits. There were increasing trends for non protein nitrogen (NPN), free alpha amino nitrogen as well as whiteness while a decreasing trend was observed for moisture, crude protein, fat, ash, total nitrogen, pH, gel strength during frozen storage. The surimi was in acceptable condition at the end of the 36-week storage period. Rodriguez *et al.* (2006) reported that PV and TBARS index were significantly lower for the cryostabilized samples than for the control throughout the period of storage.

2.6 Gel strength

Iwata (1959) studied the relation between the amount of expressible water and sensory evaluation (elasticity) of Alaska pollock kamaboko and found a high degree of correlation which was inverse in nature.

Suzuki (1981) observed that the gel texture varied with the fish species used, salt concentration needed for protein solubilization, temperature and time for which the surimi was blended with salt, frozen surimi moisture content and heat treatment given. The gel forming ability of surimi varied with the functionality of the myofibrillar proteins and it is well documented that the protein functionality is species dependent. Gel forming ability can also be assessed by sensory testing (Suzuki, 1981). The sensory tests included folding the gel between the thumb and index finger and looking for the extent of cracking and a bite test. Folding test has been widely used to study the heat induced gelation of fish muscle proteins.

The surimi quality is a function of its rheological properties, in particular, the force and strain at failure of gels obtained by thermal processing. The higher the extent of rheological parameters the better the surimi quality (Lanier, 1986). The proportion of amylose and amylopectin

and the rheological behavior of eight types of starch were correlated with the textural properties of starch-containing surimi gels. Findings by Kim and Lee (1987) included the following: increased firmness and cohesiveness with increase in water holding ability and viscosity of the starch; increased expressible moisture and penetration force with an increase in the amylose fraction due to increased retrogradation; increased tensile force with an increase in the amylopectin fraction; and increased cohesiveness and chewiness after refrigerated storage for all starches with a greater increase for high amylose starches. Surimi gels containing potato starch were the firmest and most cohesive.

Surimi batter was prepared by Babbitt and Reppond (1988) using various mixing machines with or without vacuum. Rheological properties of the cooked gels were then tested by punch and torsion methods. Alaska pollock was headed, gutted, and frozen at sea in pre- and post rigor condition by Scott *et al.* (1988). Surimi made from this fish and stored at a temperature of - 29°C showed a gradual loss in gel-forming ability with time of storage. This loss in gel-forming ability was accompanied by a loss in viscosity and Ca-ATPase activity of the surimi over the nine-month storage period. The gel strength of kamaboko gels showed an inverse linear relationship with gel moisture over a limited moisture range. Simply freezing and thawing pollock resulted in surimi with significantly lower gel strength than that from fresh pollock.

The protease activity in mechanically deboned pacific whiting flesh was reduced to 56.3% by two water-minced flesh (3:1) wash exchanges and refining. Surimi prepared using this (91.7% refined flesh, 4.0% sorbitol, 4.0% sucrose, and 0.3% condensed phosphate) produced very poor gels but the strength was markedly improved by the addition of egg white. Hardness increased 1.5-fold and elasticity 4.5-fold over those of control with the addition of 3.0% egg white. Further improvement in gel strength was

observed by Chang-Lee *et al.*, (1989) with the addition of 5.0% potato starch in combination with egg white.

Yoon and Lee (1990) reported that gels prepared using surimi having a low level of cryoprotectants had a sponge-like microstructure with relatively large ice crystal voids whereas gels made using surimi having a high level of cryoprotectants had a more compact and uniform network with smaller and more numerous ice crystal voids. The possibility of using frozen hoki to make surimi was investigated by MacDonald *et al.* (1992). Fish were thawed under controlled conditions and gels were then made from minces of the flesh. Both puncture and torsion tests showed that the quality of gels declined with duration of storage of frozen hoki. This decline was matched by a decrease in pH and an increase in formaldehyde concentration in the frozen flesh.

Studies conducted by Hsu *et al.* (1990) showed that gel strength and whiteness of surimi products were significantly affected by storage, leaching, grinding, setting and heating processes. Pipatsattayanawong *et al.* (1995) prepared frozen surimi with four levels of cryoprotectants (0, 3, 6, 9%) and observed that the gel functionality remained unchanged throughout the storage time.

Surimi produced from male Pacific herring (*Clupea harengus pallusi*), a by-product of the roe fishery, formed gel comparable to that formed by lower-grade pollock surimi but was darker in colour. Reppond *et al.* (1995) found linear relationships between moisture content of surimi and punch force, torsion stress, torsion strain, and compression force at failure. Low temperature setting or heating at 40 °C, prior to cooking at 90 °C resulted in stronger gels as measured by punch test.

Park *et al.* (1996) observed that gel forming ability, measured as cooked gel hardness, and water holding capacity of surimi-like pork were enhanced by addition of NaCl at 1.5 or 3% levels although higher levels did not further increase hardness. Addition of cryoprotectants (3% or 6% sorbitol, 3% glycerol, or 3% sucrose) before freezing had no effect on gel forming ability. Gel hardness was not increased by preheating prior to cooking. Use of relatively high concentrations of STPP in the leaching water contributed to the achievement of high breaking deformation, though beyond 2.5 g/l a detrimental effect on breaking force and consequently also on gel strength was observed (Gomez and Mendes, 1997).

Fish jelly products prepared from six species of freshwater fish were examined by Chang *et al.* (1997) and the characteristics of heat gelation in meat paste and a leaching method for enhancing the gel forming ability were investigated. The gel forming ability of meat paste of these fishes were lower than that of marine fishes such as lizard fish. The temperature for promoting maximum gelation of meat paste from the freshwater fishes was found to be between 50 and 70°C. This is higher than that for marine fishes which was between 30 and 40°C. Further it was found that the gel forming ability was enhanced by freshwater leaching method in the case of carp, rainbow trout, catfish and grass carp and by the alkaline salt water method in case of tilapia and crucian carp. The gel strength of surimi made from Alaska pollock, common carp and silver carp were determined and compared by Luo *et al.* (2001) for different incubation temperature and periods. Gel strength and setting of the freshwater species were found to be inferior to those of Alaska pollock.

The gel strength of unwashed, once washed and thrice washed pink perch mince was compared by Karthikeyan *et al.* (2006) and found that unwashed sample had an elasticity of 304.93 g. cm, once washed with 511.85 g. cm and thrice washed with 604.47 g. cm indicating the

significance of water leaching. Expressible water (%) decreased from 29.47 (unwashed) to 21.12% in once washed and 19.73% in thrice washed sample which indicated an inverse relation between expressible water and gel strength.

2.7 Surimi-based products

Surimi-based products are made possible because of gel formation due to the presence of actin and myosin which have been concentrated in surimi process. Surimi can be used to prepare a variety of products such as traditional paste products (kamaboko-type), fish ham, fish sausage, fish patty, fish sauce, etc. Shellfish analogues have now emerged as one of the most demanding surimi-based products.

Kamaboko, one of the most favourite fish products in Japan, is a sort of jelly prepared by grinding fish meat with some salt into a paste before cooking. The determination factors of quality of kamaboko lie in its elastic property or cohesiveness. Well-bound, elastic products have high jelly strength, good moisture holding capacity, long keeping life as well as excellent palatability. Okada (1963) studied the structure of kamaboko by means of light microscopy and electron microscopy. However his reports were limited to confirming the presence of a network structure in kamaboko and did not clarify the particular type of structure(s) which contributed to an elastic texture in the gels.

Fish sausage is a product in which fish flesh is mixed with additives, stuffed into suitable casings and heat processed. Mince from both freshwater and marine species has been shown to be useful in sausage making (Young, 1982).

Because of its unique gel-forming ability, surimi can be formulated into seafood analogues. These products are made from chopped surimi mixed with salt, starch, flavour and aroma compounds. The surimi mix is coloured, textured and cooked in two stages to set the gel (Yoon *et al.*, 1988). Different seafood analogues include imitation shrimp, imitation lobster tails, breaded scallops, imitation breaded crab claws, sushi products, sushi sticks, imitation crab shreds, minced sticks, filament sticks, etc.

Sato and Tsuchiya (1992) described that kamaboko has a unique, highly elastic texture termed *ashi* (elasticity). The value of a fish paste product depends on the quality of this textural attribute. The elasticity of kamaboko is developed by heating a salted fish meat sol to form a three dimensional gel structure. Thus it is important to understand how the structure of kamaboko relates to this textural quality *ashi*. Fish ball is a popular and nutritious fish jelly product of Malaysia. It is made from fish meat that is ground with salt to a smooth sticky paste. Yu (1995) reported that other ingredients like 2 % whey protein concentrate and 0.5% carrageenan are added to enhance the texture and flavour of the paste that is then shaped and cooked. Mince of threadfin bream (*Nemipterus* spp.) is a common raw material for preparing fish cake. They could be stored for a period of eight months at 4 - 6 °C and could be consumed after frying.

Yu and Siah (1996) observed that sausage containing sugar at the rate of 1.5% and polyphosphate at the rate of 0.2% had moisture content of 68 %, protein 17 % and fat 5.5 %, gel strength of 250 g. cm and expressible water above 6 %. Nisin produced by lactic acid bacteria, *Lactobacillus lactis,* has been examined as an antimicrobial in fish sausage by Raju *et al.* (2003). Fish sausage treated with 50 ppm of nisin enhanced the storage life of sausage from 2 to 22 days at room temperature while at refrigerated temperature the enhancement was from 30 to 150 days.

Lizard fish (*Saurida tumbil*), threadfin bream (*Nemipterus japonicus*) and purple spotted big eye (*Priacanthus tayenus*) surimi were freeze dried to produce surimi powder (Huda *et al.*, 2001). The resulting surimi powder contained 72.8-73.4% protein and 16.8-17.5% carbohydrate. Functional properties such as solubility, gelatin capacity, water holding capacity, emulsification, foaming properties and colour varied from species to species. The surimi powder formed gels and produced about 90% emulsification at a concentration of 1%. Threadfin bream was found to be the best source for surimi powder production, followed by purple spotted big eye and lizard fish. Nurul *et al.* (2001) noted that the resulting powder contained 73% protein and 17% carbohydrate.

Muraleedharan (2003) reported that surimi's water binding and elastic properties and bland taste make it ideal as a protein supplement in pasta, in formed or extruded products such as intermediate moisture pet foods. It is also useful to control freeze-thaw stabilization of icings and filling in bakery items. Dried surimi or frozen surimi can function as a glue-type binder to hold chunks of meat together. It has recently found application as an ingredient in high protein drink powders and high fibre snack bars. Recently surimi has been employed as a carrier for nutritionally important omega-3 fatty acids as reported by Park (2004). Surimi from cod (*Gadus morhua*) containing fatty acids was prepared by the addition of 500 mg of oil as an oil-in-water emulsion to 85 g of surimi.

Sen (2005) explained that there are a number of varieties of kamaboko which differ in shape, in ingredients used and also in the way they are heated. More common shapes are ball, bar, square shaped, leaf shaped, rolled, noodle shaped and chipped.

Surimi can be integrated into poultry and meat products for replacement of fat, and also acts as an emulsifier and binder (Venugopal,

2006). Surimi from fishes like threadfin bream, lizard fish and purple spotted big eye fish are used to prepare functionally active powder. Such powders have been prepared by drying surimi.

Since there are a number of surimi-based products, classification of these seems to be essential. According to Sen (2005), surimi-based products may be grouped into the following three categories: products with smooth gel structure- e.g. different varieties of kamaboko; products with parallel aligned fibres (fiberized products); and products with randomly aligned fibres (composite moulded products).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Preparation of surimi

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 within six hours. Surimi was prepared following the method given in Fig. 2.

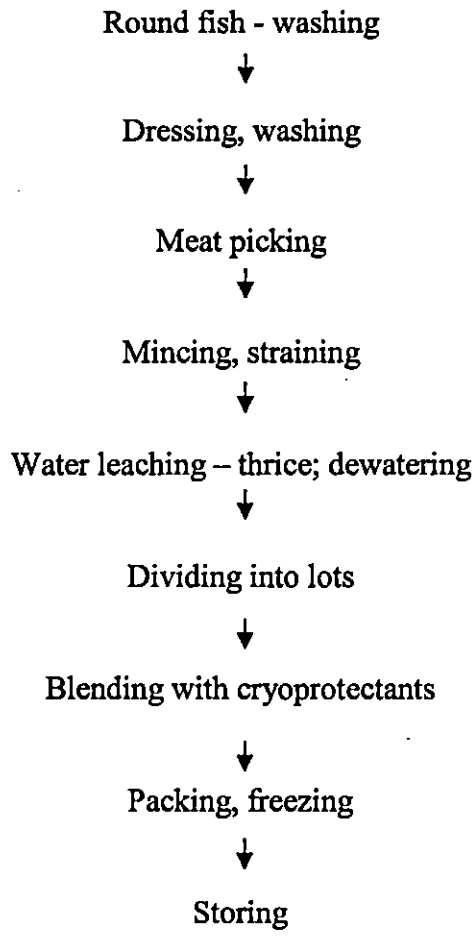

Fig. 2: Flow chart for preparation of fish surimi

The fish was headed and gutted followed by thorough washing in iced potable water. The fish was then filleted and the meat manually picked. The picked meat was minced in a meat mincer using a disc of 0.55 cm diameter perforations. The meat was strained using an extruder with perforations of 0.30 cm diameter. The meat was then water leached using iced water, added at the rate of eight times the weight of meat and at a temperature of about 15 °C, and stirred for a period of 10 min using a mechanical stirrer. The meat particles in the suspension were settled and the supernatant was decanted. Leaching was repeated two more times. For the last wash a solution of 0.05 % NaCl was used along with water. The material was dewatered by wrapping in a cloth bag and pressing using a basket-type press to a final moisture content of about 80 %. The material was then divided into five lots of approximately 1.2 kg each. Each lot was mixed with the required amount of ingredients as given in Table 1.

Table 1: Ingredients used in surimi

Lot No.	% by weight of meat		
	Sucrose	Sorbitol	*STPP
A	0	0	0.25
B	1	1	0.25
C	2	2	0.25
D	3	3	0.25
E	4	4	0.25

* Sodium tripolyphosphate

Sugar and sorbitol were mixed in 1:1 proportion by weight (henceforth called as sugar mixture). The required amount of the mixture was weighed out for each lot. This together with STPP were mixed with meat in a silent cutter for a period of 10 min. Each lot was further divided into six sublots of 200 g each and packed in polypropylene bags of 50 micron thickness as two cm slabs, sealed and quick frozen at -35 °C in an

air blast freezer for a period of two hrs. The slabs were then stored in a cold store maintained at a temperature of -20 °C.

3.2 Tests

Samples were drawn every month for a period of five months for conducting various tests. For this one bag from each lot was withdrawn and the surimi was thawed at room temperature. All tests other than sensory tests and pH were done in triplicate. All chemicals used for the tests were either of AR or GR grade. All food ingredients used were of food grade.

3.2.1 Moisture content

The moisture content was determined by the AOAC (1975) oven drying method. A sample of about 10 g was weighed in a pre-weighed tared petridish. The dish was placed in a hot air oven at a temperature of 100°C for a period of six hrs, cooled in a desiccator and weighed. Drying was continued until a constant weight was obtained and the moisture content was calculated as percentage loss of weight.

3.2.2 Ash content

The method of AOAC (1984) was followed for ash content estimation. About two g of the wet surimi sample was transferred to a pre-weighed silica crucible. The sample was carbonized by burning at low red heat and was placed in a muffle furnace at a temperature of 550°C for about four hrs until a white ash was obtained. Crucibles were weighed after cooling in a desiccator. The weight of ash was expressed as a percentage of the initial sample weight.

3.2.3 Crude fat content

The method of Radin (1981) was followed for determining crude fat content. About one g of surimi sample was taken in a mortar and homogenised with 18 ml of the extraction solvent (hexane: isopropanol :: 3:2, v/v). It was then filtered into a pre-weighed beaker. The residue was washed two or three times with minimum volume of solvent mixture. The solvent was evaporated off on boiling water bath, then cooled to room temperature in a desiccator, and weighed. From the difference in weight the percentage lipid content was calculated.

3.2.4 Total nitrogen and crude protein contents

Total nitrogen and crude protein contents were estimated by the Micro Kjeldahl method (AOAC, 1984). About one g of the well-minced wet sample was transferred to a kjeldahl flask of 100 ml capacity. A few glass beads and a pinch of digestion mixture (K_2SO_4 and $CuSO_4$ = 8:1) and 10 ml concentrated sulphuric acid were also added. It was digested over a digestion unit (Pelican make) until the solution turned colourless. To the digested and cooled solution distilled water was added in small quantities with intermittent shaking and cooling until the addition of water did not generate heat. It was transferred quantitatively to a 100 ml standard flask and made upto the volume. Two ml of the solution was pipetted out into the reaction chamber of the micro kjeldahl distillation apparatus. 10 ml of 40% (w/v) sodium hydroxide solution was added to the sample. Distillation was carried out for a period of two minutes and ammonia liberated was absorbed into 2% boric acid solution taken in a conical flask containing 1-2 drops of Tachiro's indicator. The amount of ammonia liberated was determined by titrating with $N/_{70}$ standard sulphuric acid. Total nitrogen and crude protein contents were calculated as follows.

$$\text{Total nitrogen (\%)} = \frac{V \times 14 \times 100 \times 100}{1000 \times 70 \times 2 \times W},$$

Where V = volume of standard sulphuric acid required for titration in ml, and W = weight of sample taken in g.

Crude protein (%) = % total nitrogen x 6.25

3.2.5 Salt soluble nitrogen content

Salt soluble nitrogen content was determined by the AOAC method (1984). About five g of the sample was weighed accurately in a 250 ml conical flask and 200 ml of chilled NaCl solution (5% NaCl buffered with 0.02M Na_2CO_3) was added. pH of the suspension was adjusted to 7.0 and chilled to 10 °C. It was then homogenised in chilled condition for 2-3 min and made upto a volume of 250 ml. An aliquot was taken and centrifuged at 6000 rpm for 20 min. 10 ml of the supernatant was taken and digested with sulphuric acid and digestion mixture. It was transferred quantitatively to a 100 ml standard flask and made upto the volume. Two ml of the solution was pipetted out into the reaction chamber of the micro kjeldahl distillation apparatus. The procedure given under section 3.2.4 was followed for estimating the nitrogen content. Salt soluble nitrogen content was expressed as percentage of total nitrogen content.

3.2.6 pH

A sample of five g thawed surimi was blended with 45 ml distilled water and the pH of the homogenate was measured using a pH meter as according to the method followed by Suzuki (1981).

3.2.7 Gel strength

The method described by Suzuki (1981) was followed. A test piece of sausage of about 25 mm thickness was placed under a plunger (diameter 5 mm) of an Okada-type gelometer. By applying pressure using the plunger the testing material was gradually deformed and broken. A kymogram showing the stress-strain curve was obtained (Fig. 3).

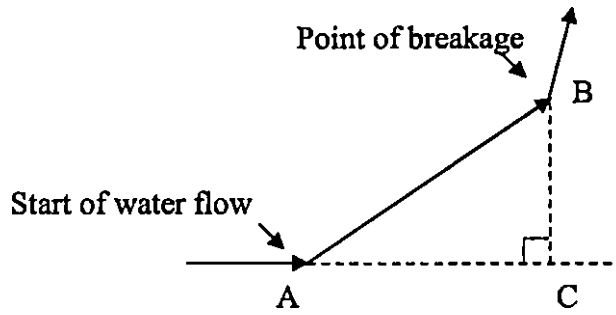

Fig. 3: Okada Gelometer's kymogram

The gel strength was calculated as:

$$\text{Gel strength (g.cm)} = \tfrac{1}{2}\, AC \times BC \times F$$

where, $\tfrac{1}{2}\, AC \times BC =$ area of the triangle, and

$$F = W/D$$

where, W = rate of water flow in g/sec and D = speed of kymogram in cm/sec.

3.2.8 Expressible water content

Expressible water content was measured according to the method of Suzuki (1981). A slice of sausage of about one g was weighed and placed between two filter papers and pressed by a small hydraulic press at a pressure of 10 kg/cm^2 for a period of 20 sec. The weight of material was

again noted. The weight difference was expressed as a percentage of the weight of the sample before pressing.

3.2.9 Folding test

A modified method of Suzuki (1981) was followed for the folding test. Sausage was sliced into five mm thickness pieces using a sharp knife. The circular piece was folded twice, first to half and then to quarter. Elasticity was graded based on any breakage noted along the margin of folding after each fold.

AA: no breakage at all at first and second fold (high elasticity).

A: crack appears at second fold, but not at first.

B: cracking of part of folded edge at first fold.

C: crack runs through entire folded edge on first fold.

D: easily disintegrated on finger pressing (very poor elasticity).

3.2.10 Total plate count (TPC)

All media and diluents were sterilized by autoclaving at a temperature of 121°C for 15 min and all glasswares at 160°C in hot air oven for two hrs. Total plate count was determined according to the method of Surendran *et al.* (2006). A sample of 10 g was aseptically transferred to a sterile blender and homogenized with 90 ml sterile diluent (normal saline). Appropriate serial decimal dilutions of the homogenate were made using the diluent and dilutions of 10^{-1}, 10^{-2} and 10^{-3} were plated by pour plate technique in triplicate. The medium used was Nutrient Agar of SRL make (composition in g/l: beef extract-10, peptone-10, NaCl-5 and agar-12). The plates after solidification were inverted and incubated at a temperature of 37°C for 24 hrs. Plates showing 30 to 300 colonies were used for counting the colonies. TPC was calculated using the formula:

$$TPC = \frac{\text{Average number of colonies x dilution factor}}{\text{Weight of the sample taken}}$$

The count was expressed as number of colony forming units (cfu) / g sample.

3.2.11 Sensory evaluation

A taste panel of about 10 members was selected. Fish sausages prepared from surimi containing different levels of cryoprotectants were fried in refined vegetable (sunflower) oil at 180 ^{o}C for a minute and presented to the judges. They were assigned to evaluate the extent of sweetness, elasticity and preference of each sample. Similarly fish patty and fish cake were evaluated for sweetness and preference. Thawed surimi samples were presented for evaluating whiteness. The score sheets (Appendix I, II and III) provided to the taste panelists for recording their judgements were designed according to Rousseau (2004) with slight modifications.

3.3 Preparation of surimi-based products

3.3.1 Sausage

A modified method of Chandrasekhar and Manisseri (1976) was followed for sausage preparation. Frozen surimi was taken, thawed and weighed. Additives as given in Table 2 were weighed and mixed in a silent cutter. Meat alone was mixed for two min followed by other additives and finally oil was added. Ice was added frequently in small amounts during mixing period of 12-15 min. The paste was then stuffed into cellulose casings of 1.7 cm diameter at the rate of 30 g per piece. The ends of the

Table2: Ingredients used for various surimi-based products (in grammes)

Ingredients(g)	Fish sausage	Fish cake	Fish patty	Batter
Surimi	720	800	1000	-
Maida	90	80	-	30
Table salt	20	20	15	2
Vegetable oil	50	-	70	-
Red chillies	2	-	-	-
Coriander	2	-	-	-
Nutmeg	0.5	-	-	-
Mace	0.5	-	-	-
Mustard	0.5	-	-	-
Cardamom	0.5	-	-	-
Clove pieces	0.5	-	2	-
Cinnamon pieces	0.5	-	2	-
Pepper powder	4	-	3	-
Turmeric powder	-	-	3	-
Ginger paste	2	-	30	-
Garlic paste	2	-	-	-
Green chillies	-	-	30	-
Onion pieces	-	-	300	-
Potato(cooked)	-	-	375	-
Egg white	-	50	-	-
Whole egg	-	-	-	100
Water	105	50	-	100
Bread crumbs	-	-	-	100

casings were clipped using aluminum clips. The sausages were boiled in waterbath at a temperature of 88-90 °C for 40 min, followed by fan drying and labelling. It was further packed in bags and stored in chilled store at a temperature of 1 - 2 °C for a day.

3.3.2 Fish patty

Fish patty was prepared according to a modified method of Gopakumar (2002). Surimi was taken and steamed for 30 min. The additives used for fish patty are given in Table 2. Potatoes were cut, steamed for 30 min, peeled and mashed. Oil was taken in a frying pan and heated to 180°C to which chopped onion, green chillies and ginger were added and fried to a light brown colour. The mixture was then cooled and coarsely ground in a mixie. Meat, potatoes, fried material, salt and spices were mixed manually followed by mixing in a silent cutter for five minutes. The mixture was moulded into 15 g round cakes using a patty moulder. Batter was made by mixing refined wheat flour (*maida*) with water to which beaten eggs and salt were added. Cakes were battered using this followed by breading using bread crumbs. The battered and breaded patties were then fried in refined vegetable (sunflower) oil at 180 °C until the surface attained a medium brown colour.

3.3.3 Fish cake

The method of Suzuki (1981) was slightly modified to prepare fish cake. Surimi was ground for a few minutes. Salt was added, followed by starch and egg white, and the grinding was continued for a period of 15 min. Iced water was added every few minutes. This paste was filled in stainless steel trays without air pockets to 1.5 cm thickness, steamed for 30 min and cooled. Square pieces of dimensions 3 × 3 cms were cut using a sharp knife.

The composition of various ingredients used is given in Table 2. The cakes were fried in oil at 180 °C for a minute.

3.4 Statistical Analysis

The experiments were carried out using Completely Randomized Design (CRD). Data obtained were analyzed using Analysis of Variance (ANOVA) technique (Snedecor and Cochran, 1968). Pair wise comparison of treatment means were done wherever necessary using least significant difference.

RESULTS

4. RESULTS

4.1 Proximate composition

Proximate composition of threadfin bream meat before and after water leaching is presented in Table 3. Moisture content was found to increase from 79.87% to 83.23% upon water leaching whereas crude protein, crude fat and ash contents decreased from 18.38 to 15.5%, 1.0 to 0.75% and 0.77 to 0.57%, respectively upon leaching.

4.2 Whiteness of surimi

As indicated in Table 4 the sensory evaluation scores for whiteness of thawed surimi remained within a narrow range of 2.73 – 3.25, irrespective of the cryoprotectant concentration.

4.3 Product acceptability studies

Sausages prepared from surimi having different levels of cryoprotectants were evaluated. It was found to have a steady increase in the perception of sweetness with increase in sugar concentration as in Fig. 4a. Product was acceptable even at 8 % cryoprotectant level (Fig.4b). However lower concentrations like 0 %, 2 % and 4 % were preferred over 6 % and 8 %. Slight sweetness (2 %) seemed to be preferred over control.

In fish patty also sweetness was found to increase with cryoprotectant level. Upto 2 % level there was a low detection of sweetness similar to 0 % as indicated in Fig. 5a. When preference was evaluated it was found that up to 2 % level there was high preference, but on increasing the sugar levels, the preference was lowered even though the product was moderately accepted (Fig. 5b).

Table 3: Proximate composition of meat of *Nemipterus japonicus* before and after water leaching

Component	Fish meat	Leached meat
Moisture	79.87 %	83.23 %
Crude protein	18.38 "	15.50 "
Crude fat	1.00 "	0.75 "
Ash	0.77 "	0.57 "

Table 4: Sensory evaluation scores for whiteness of surimi treated with different levels of cryoprotectants

Sugar mixture concentration (%)	Score*
0	2.98
2	3.25
4	3.02
6	2.73
8	3.10

* Minimum score = 1 (poor colour)
Maximum score = 5 (extremely white)

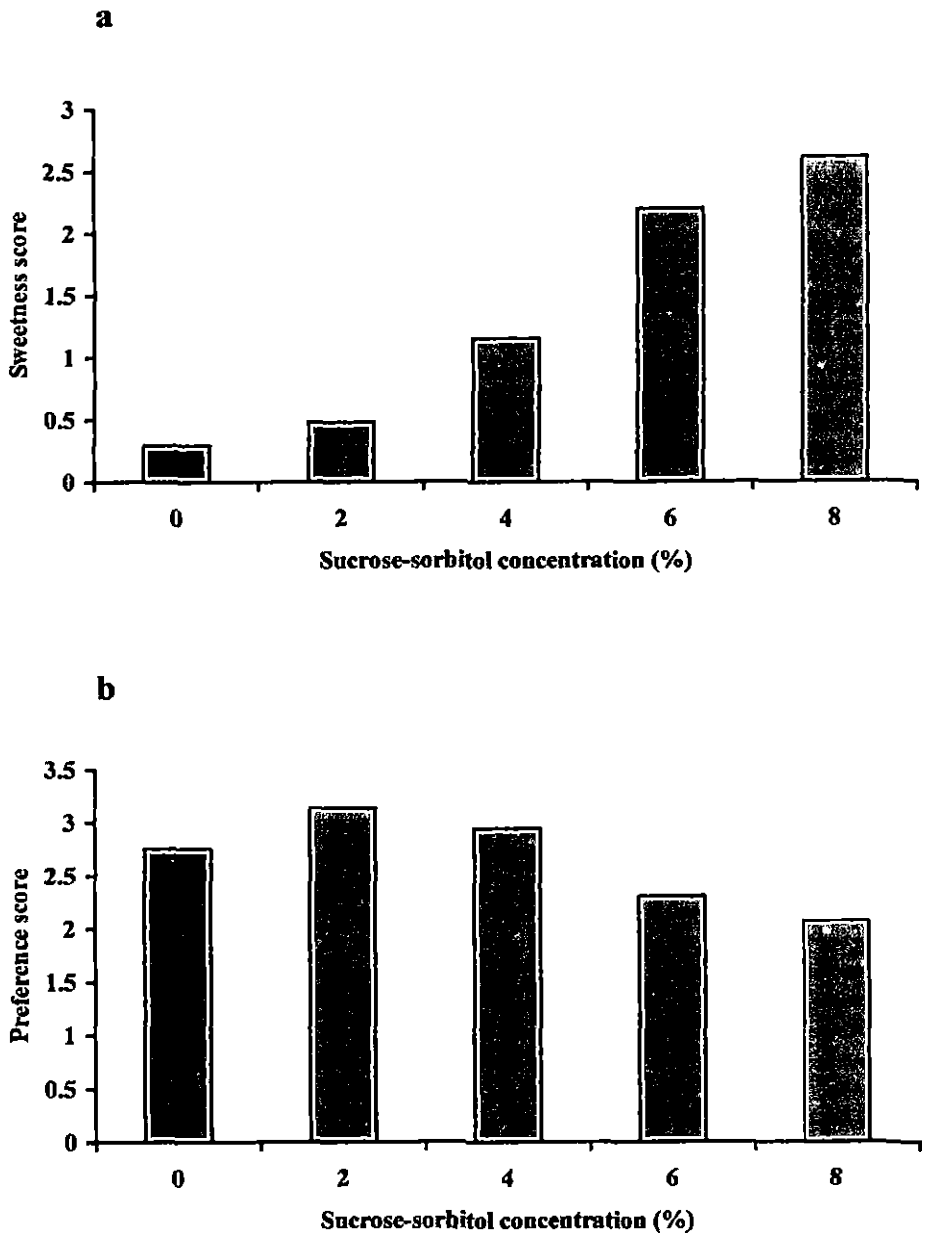

Fig. 4: Taste panel evaluation of sweetness and preference of surimi sausage treated with various concentrations of sugar mixture

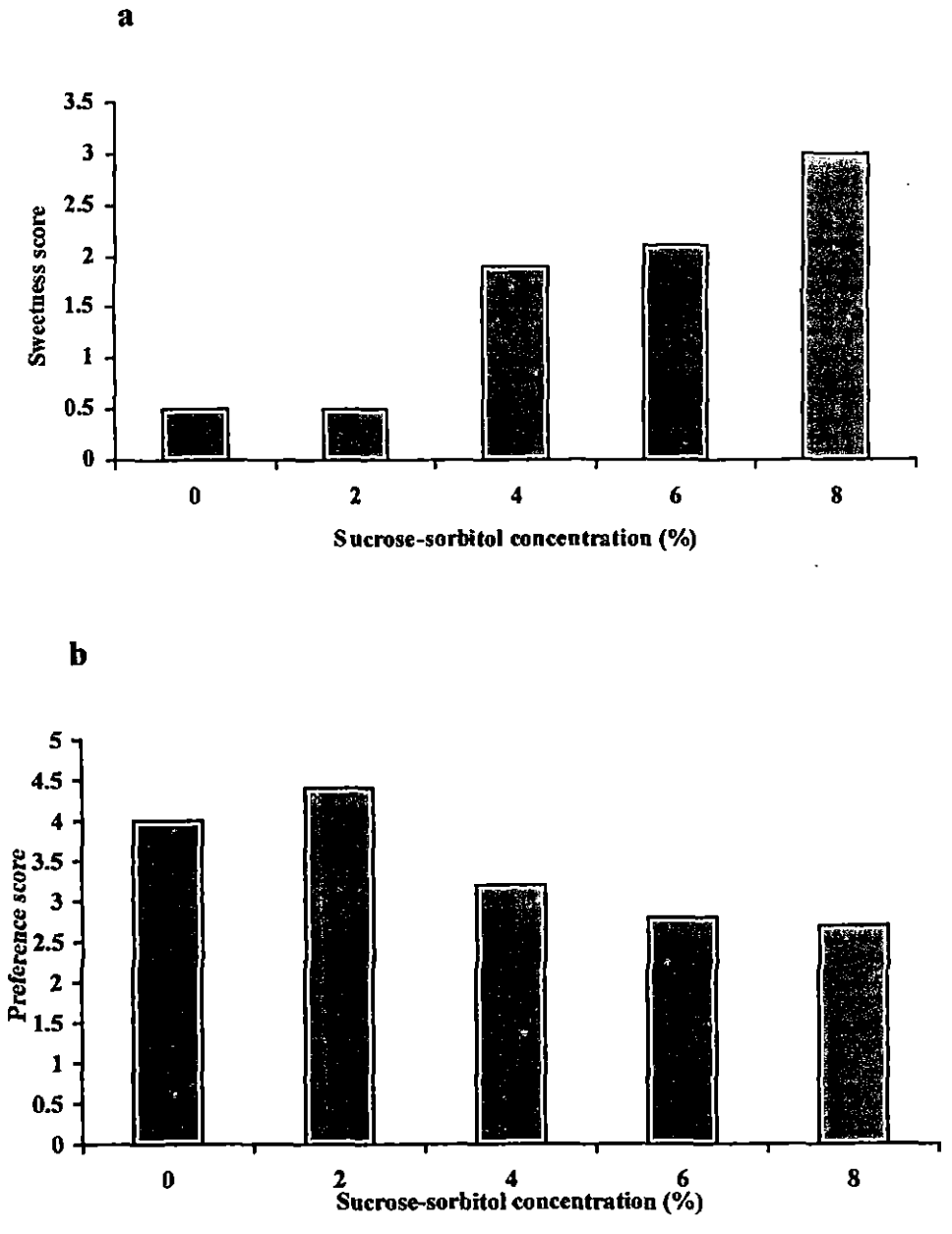

Fig. 5: Taste panel evaluation of sweetness and preference of surimi patty treated with various concentrations of sugar mixture

Similar pattern was followed for sweetness in the case of fish cake also. Preference was more for lower concentration but it was acceptable even at 8 % (Fig. 6a and b).

4.4 Storage studies

4.4.1 pH

pH value of the frozen stored surimi was found to remain within a narrow range of 6.6 to 7.06 throughout the storage period irrespective of the cryoprotectant concentration as shown in Fig. 7.

4.4.2 Total plate count (TPC)

Fig. 8 shows the variations in TPC of surimi samples treated with different levels of cryoprotectants and frozen stored at -20°C for different periods. TPC appeared to fluctuate during the period of study ranging between 3.7 to 4.92 log cycles, but showed a slight decreasing trend towards the end of the storage period.

4.4.3 Moisture content

The variation in the moisture content of surimi during frozen storage is shown in Fig. 9a. The moisture content remained more or less the same throughout the storage period in all the samples containing sugar. However, a steady fall was noticed in the case of control after the second month of storage. A definite decrease in moisture content with increase in sugar concentration was noticeable irrespective of the storage period as shown in Fig. 9b. It varied from about 83.96% in control to about 79.01% in surimi with 8% sugar. Statistical analysis for moisture content showed significant difference between cryoprotectant concentrations (Table 5).

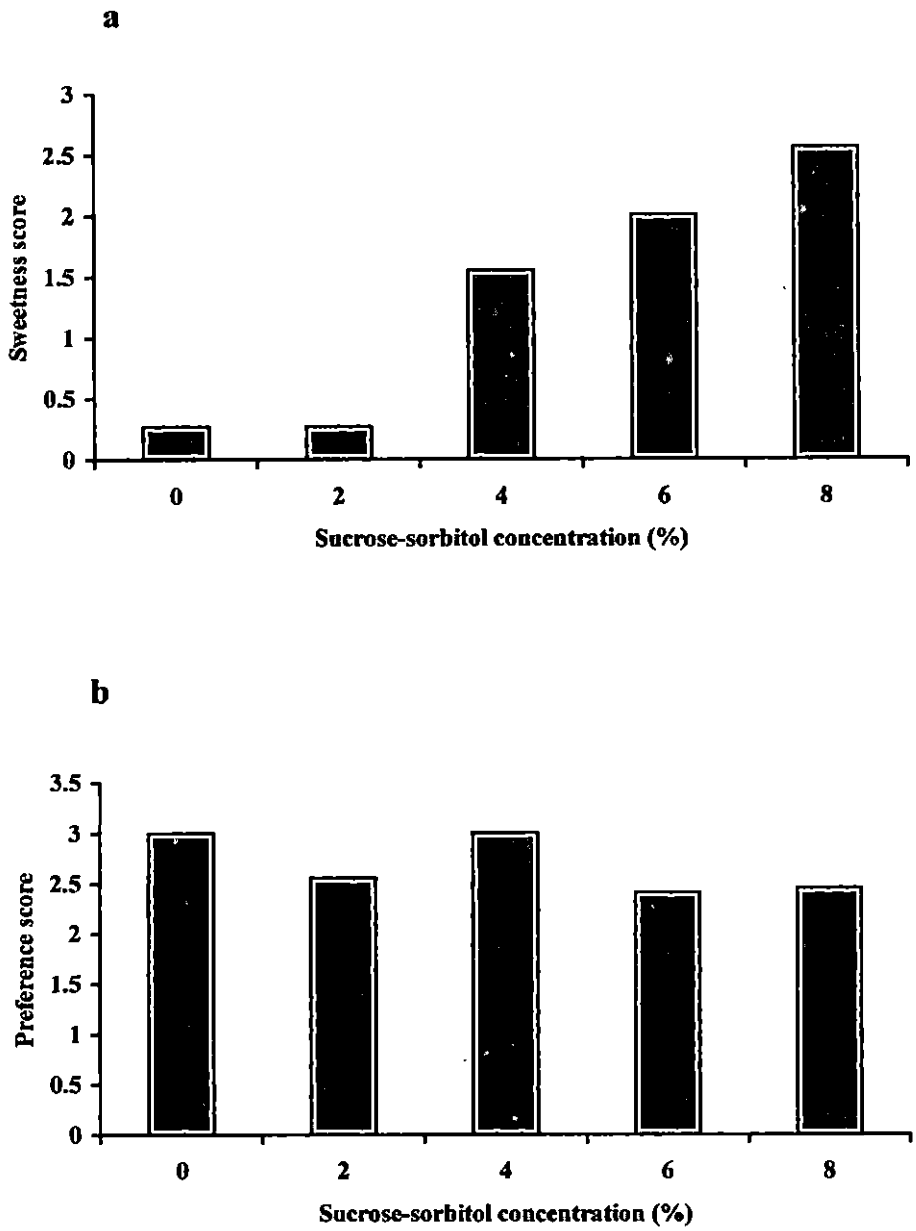

Fig. 6: Taste panel evaluation of sweetness and preference of surimi cake treated with various concentrations of sugar mixture

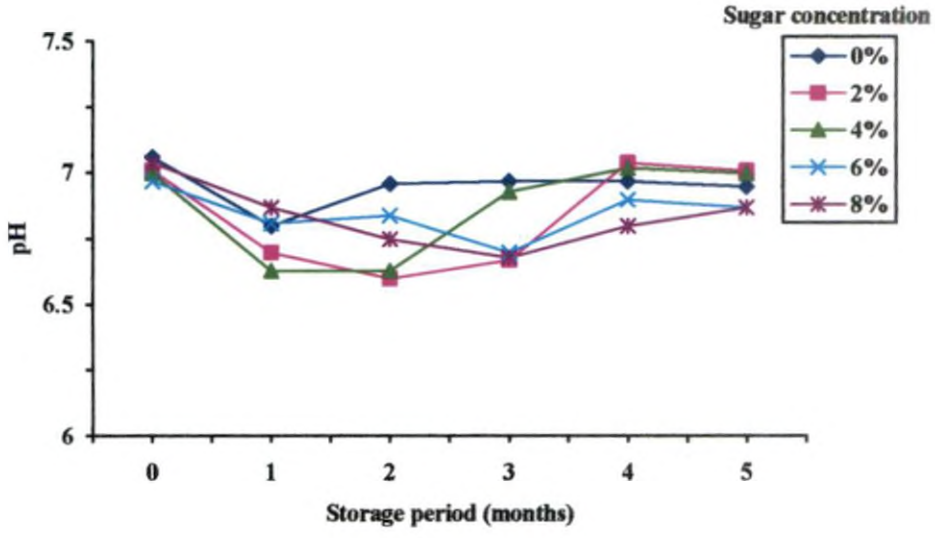

Fig. 7: Variations in pH of surimi containing different concentrations of sugar mixture during storage at -20°C

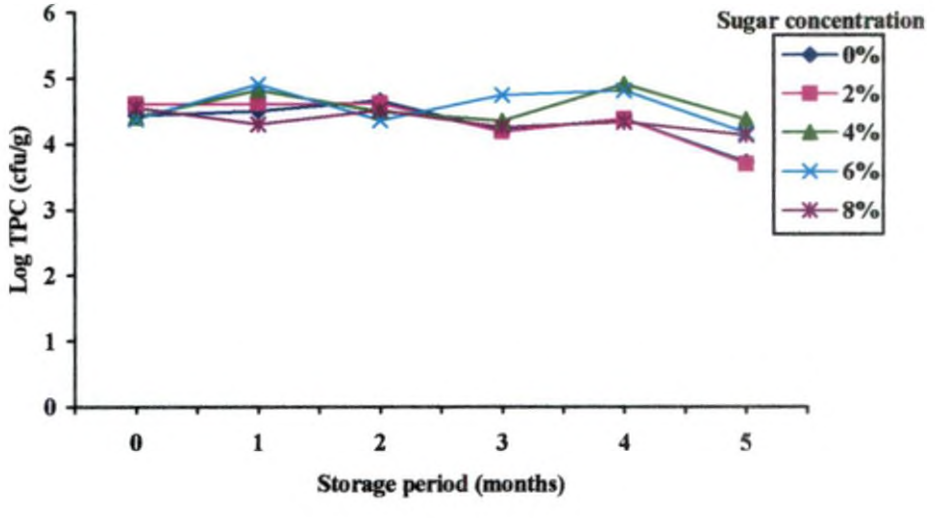

Fig. 8: Variations in TPC of surimi containing different concentrations of sugar mixture during storage at -20°C

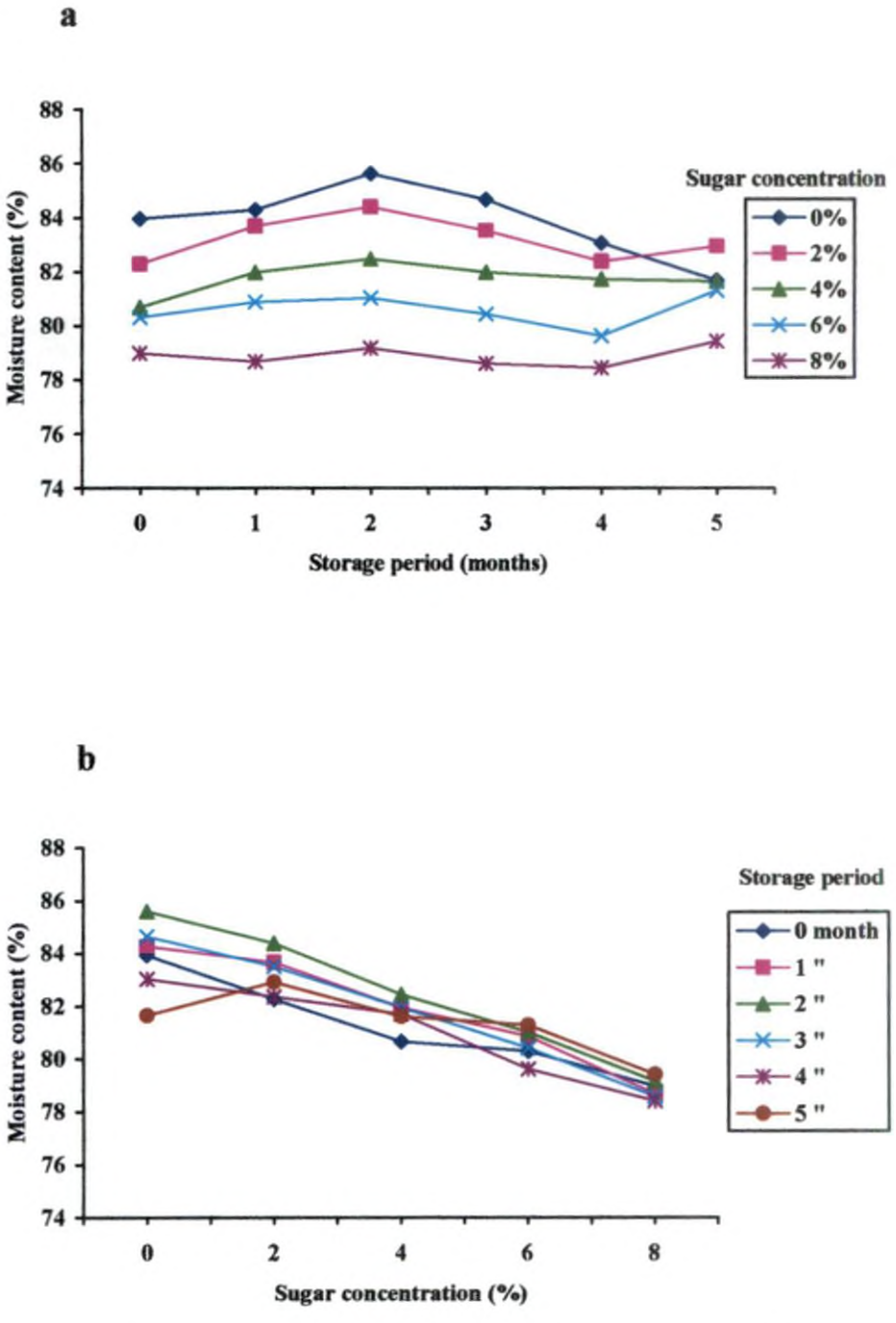

Fig. 9: Variations in moisture content of surimi containing different concentrations of sugar mixture during storage at -20°C

Table 5: ANOVA for variations in moisture content of surimi containing different concentrations of sugar mixture during storage at -20°C

Source of variation	F value	F critical	Significance at 5 % level
Concentration	414.2573	2.525212	Significant

Concentration:	C_4: 8%	C_3: 6%	C_2: 4%	C_1: 2%	C_0: 0%
Means:	78.908	80.609	81.756	83.213	83.882

Critical difference = 113.403

Underscored means are not significantly different

4.4.4 Salt soluble nitrogen (SSN) content

A decreasing trend was observed in the case of SSN content for all the samples of surimi with storage period, whereas for the same period of storage an increase was noticeable with increase in cryoprotectant concentration as shown in Fig. 10a and b, respectively.

The results of statistical analysis are given in Table 6 which indicate a significant difference in SSN between cryoprotectant concentration and storage period. SSN was found to decrease from 54.54 % to 27.92 % in surimi without cryoprotectant in five months, whereas the variations during the same period were 57.69 % to 33.5 %, 52.9 % to 34.15 %, 59.4 % to 34.09 % and 57.7 % to 34.885 % in the case of surimi with 2, 4, 6 and 8% sugar, respectively.

4.4.5 Expressible water content

Expressible water content was found to increase with storage period in all the samples whereas with increase in sugar concentration, it was found to decrease from 30.53 % for control to 19.69 % for surimi having 8 % cryoprotectant in the initial month. Increase in expressible water content ranged from 30.53 % to 33.3 % for surimi without cryoprotectant, 23.3 to 30.38 % for 2 % cryoprotectant concentration, 23.10 % to 27.91 % for 4 % concentration, 20.74 % to 26.39 % for 6 % concentration and 19.69 % to 23.27 % for 8 % concentration during the storage period as shown in Fig. 11a and 11b.

The observations for expressible water content were statistically analysed (Table 7) which showed a significant difference between cryoprotectant concentration as well as storage period, except between first and second month, and between 4 and 6% sugar concentration.

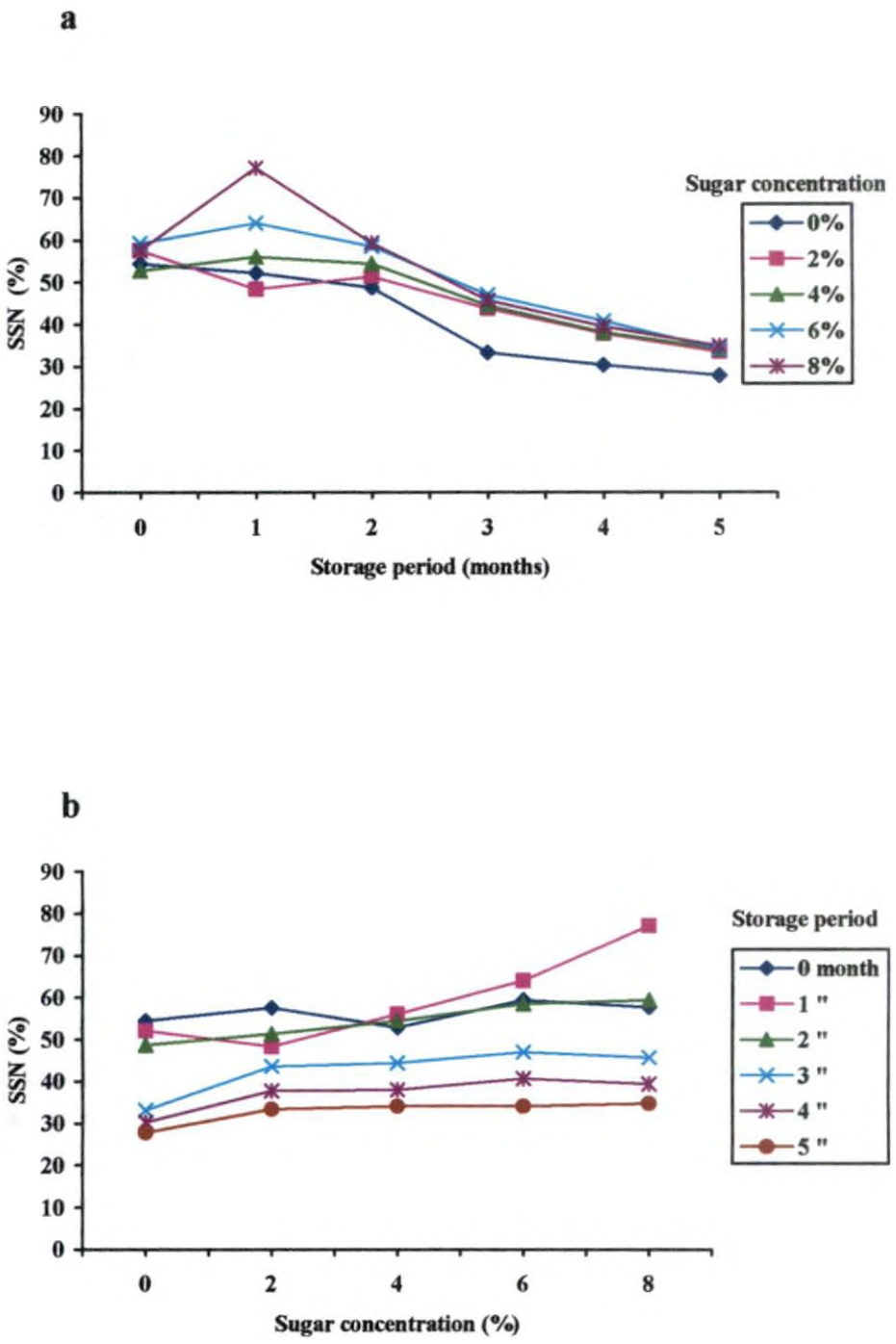

Fig. 10: Variations in salt soluble nitrogen (as % of total nitrogen) content of surimi containing different concentrations of sugar mixture during storage at -20°C

Table 6: ANOVA for variations in salt soluble nitrogen content of surimi containing different concentrations of sugar mixture during storage at -20°C

Source of variation	F value	F critical	Significance at 5 % level
Month	723.6389	2.368267	Significant
Concentration	138.2549	2.525212	Significant

Month:	M_5: 5	M_4: 4	M_3: 3	M_2: 2	M_0: 0	M_1: 1
Means:	32.908	37.32	42.88	54.525	56.446	59.624

Critical difference = 1.165

Concentration:	C_0: 0%	C_1: 2%	C_2: 4%	C_3: 6%	C_4: 8%
Means:	41.208	45.447	46.701	50.668	52.395

Critical difference = 1.064

Underscored means are not significantly different

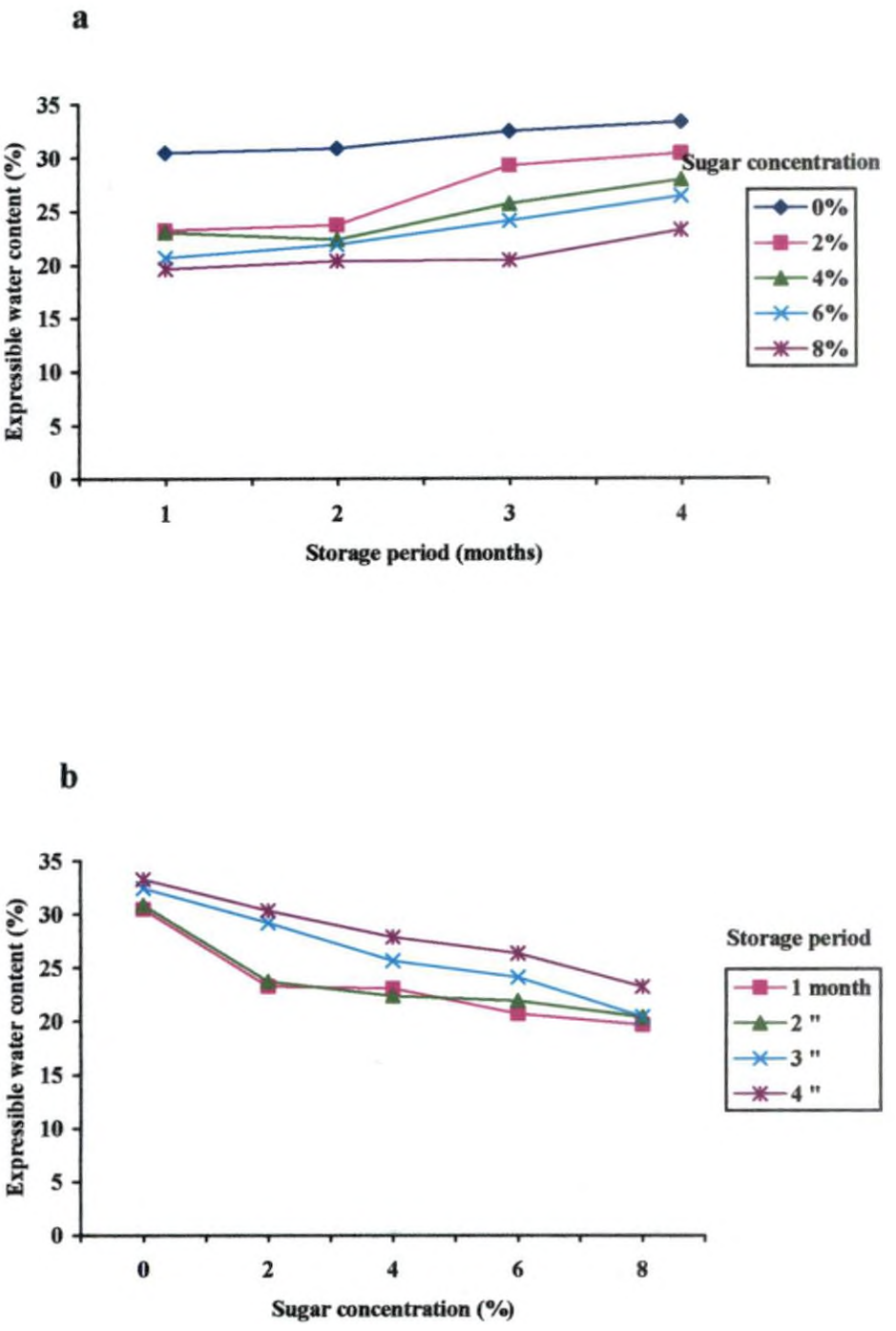

Fig. 11: Variations in expressible water content of surimi sausage containing different concentrations of sugar mixture during storage at -20°C

Table 7: ANOVA for variations in expressible water content of surimi sausage containing different concentrations of sugar mixture during storage at -20°C

Source of variation	F value	F critical	Significance at 5 % level
Month	138.6313	2.557179	Significant
Concentration	70.15648	2.557179	Significant

Month:	M_1: 1	M_2: 2	M_3: 3	M_4: 4
Means:	23.472	23.884	26.405	28.251

Critical difference = 1.22

Concentration:	C_4: 8%	C_3: 6%	C_2: 4%	C_1: 2%	C_0: 0%
Means:	19.303	21.713	22.731	24.416	28.926

Critical difference = 1.22

Underscored means are not significantly different

4.4.6 Gel strength

Gel strength of sausage prepared from the control sample of surimi decreased steadily from 783.71 to 528.29 g.cm, whereas in the cases of 2%, 4%, 6% and 8% sugar levels, the gel strength decreased from 886.9 to 841.8 g.cm, 1027.3 to 988.08 g.cm, 1236.4 to 1106.2 g.cm and 1352.6 g.cm to 1077.45 g.cm, respectively during the storage period as indicated in Fig. 12a. Gel strength was found to increase with increase in sugar concentration, varying from about 783 g.cm to about 1352 g.cm, respectively, for 0 to 8 % sugar concentration during the initial month (Fig. 12b).

Statistical analysis (Table 8) did not show any significant difference in gel strength between first and second month and between second, third and fourth months. Between cryoprotectant concentrations 0 and 2% and between 6 and 8% no significant difference was observed.

4.4.7 Folding test

Folding test conducted on sausage indicated an increase in elasticity with cryoprotectant concentration irrespective of the storage period as given in Table 9. The grade AA indicating high elasticity was obtained for sausage prepared using surimi containing 4 % to 8 % cryoprotectants whereas grades B and C were obtained for lower concentrations of 2 % and 0 %, respectively.

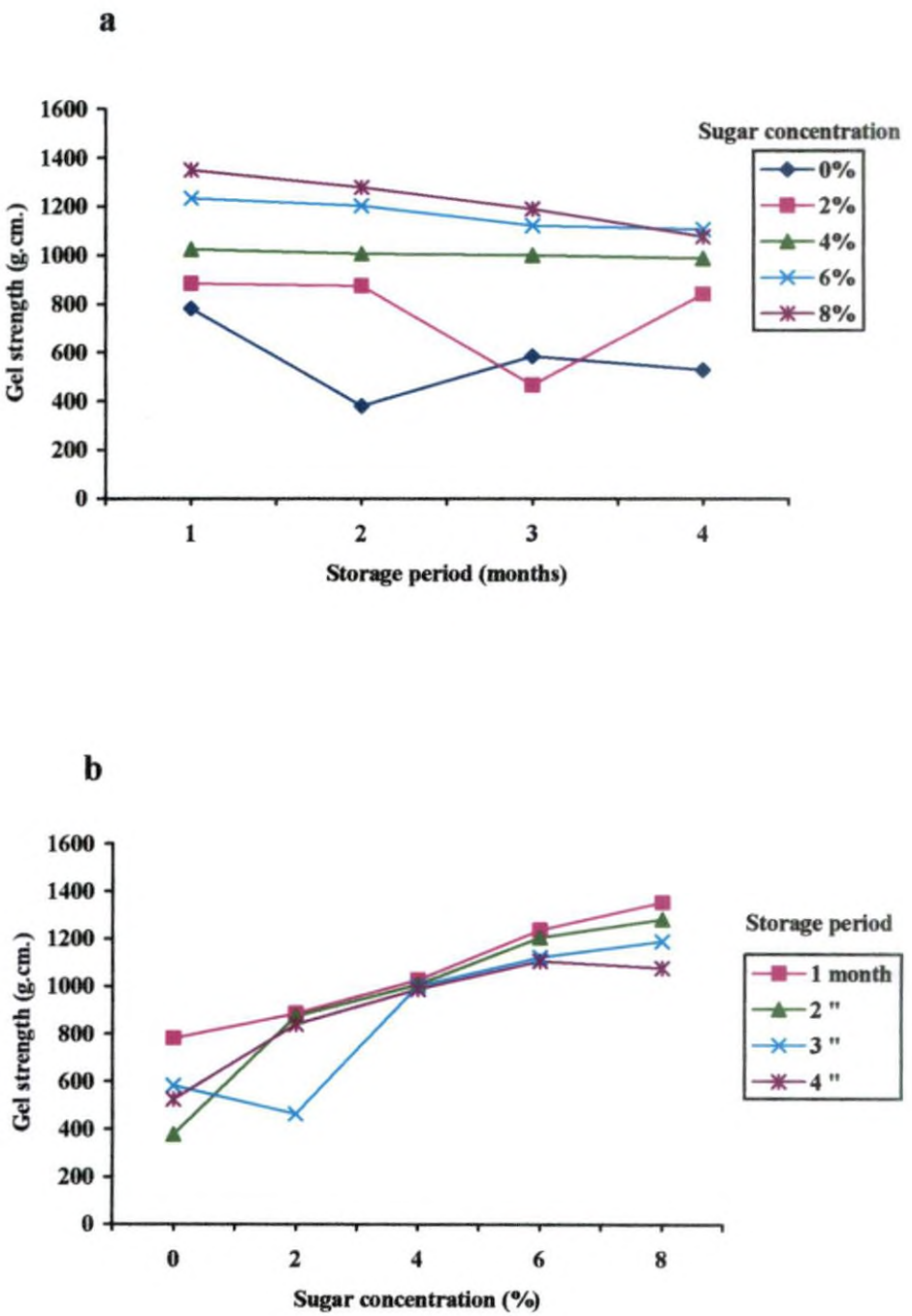

Fig. 12: Variations in gel strength of surimi sausage containing different concentrations of sugar mixture during storage at -20°C

Table 8: ANOVA for variations in gel strength of surimi sausage containing different concentrations of sugar mixture during storage at -20°C

Source of variation	F value	F critical	Significance at 5 % level
Month	38.96699	2.368267	Significant
Concentration	50.2909	2.525212	Significant

Month:	M_3: 3	M_4: 4	M_2: 2	M_1: 1
Means:	872.932	908.364	949.944	1057.371

Critical difference = 113.403

Concentration:	C_0: 0%	C_1: 2%	C_2: 4%	C_3: 6%	C_4: 8%
Means:	717.895	801.628	1127.167	1262.186	1361.659

Critical difference = 113.403

Underscored means are not significantly different

Table 9: Variations in folding test grades of surimi sausage containing different concentrations of sugar mixture during storage at -20°C

Concentration	Month				
	1	2	3	4	5
0%	C	C	C	C	C
2%	B	B	B	B	B
4%	AA	AA	AA	AA	AA
6%	AA	AA	AA	AA	AA
8%	AA	AA	AA	AA	AA

Grade AA: no breakage at all at first and second fold (high elasticity).

A: crack appears at second fold, but not at first.

B: cracking of part of folded edge at first fold.

C: crack runs through entire folded edge on first fold.

D: easily disintegrated on finger pressing (very poor elasticity).

4.4.8 Sensory evaluation

Figs. 13, 14 and 15 show variations in the sensory parameters - sweetness, elasticity and preference, respectively - of sausage prepared from samples of surimi with varying concentrations of sucrose-sorbitol mixture and frozen stored for various periods. The parameters, sweetness and elasticity, were found to increase with sugar concentration whereas preference was found to decrease with sugar concentration. Scores for all the three parameters did not vary much throughout the storage period for each concentration of sugar mixture and no significant difference was observed between storage period intervals (Table 10, 11 and 12, respectively). However, a decreasing trend in the elasticity scores was noticed in the case of surimi sausage with 0 and 2% concentrations of sugar (Fig. 14a). Irrespective of the storage period, significant variations in sweetness and elasticity were noticed with sugar concentration (Fig. 13b, 14b, Table 10 and 11), the scores ranging from 0.1 to 2.86 for sweetness and 1.0 to 3.44 for elasticity. On the other hand, the preference scores increased from 0% to 2% sugar concentration from about 2.1 to 3.43, followed by a significant decrease.

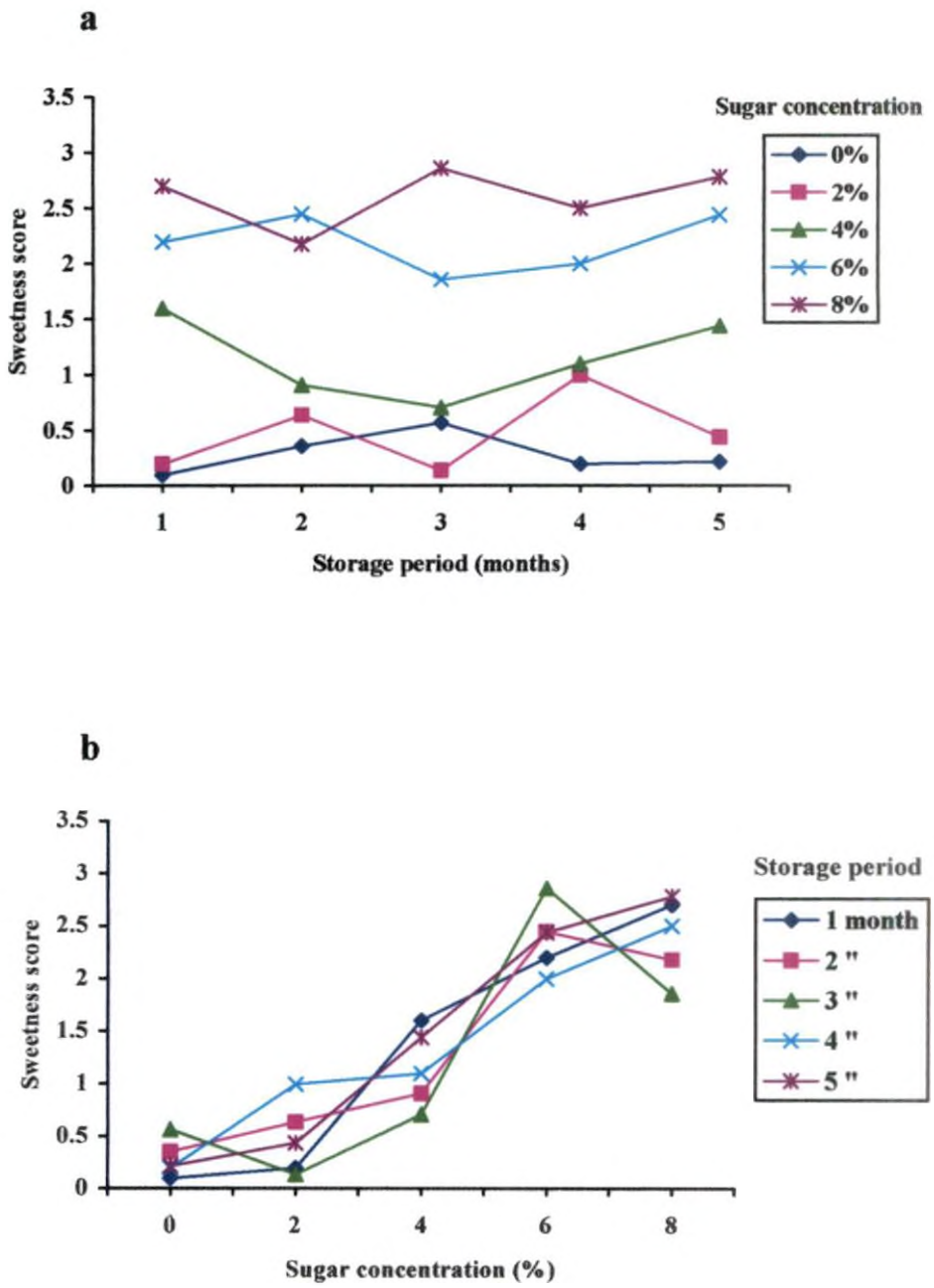

Fig. 13: Variations in sweetness of surimi sausage containing different concentrations of sugar mixture during storage at -20°C

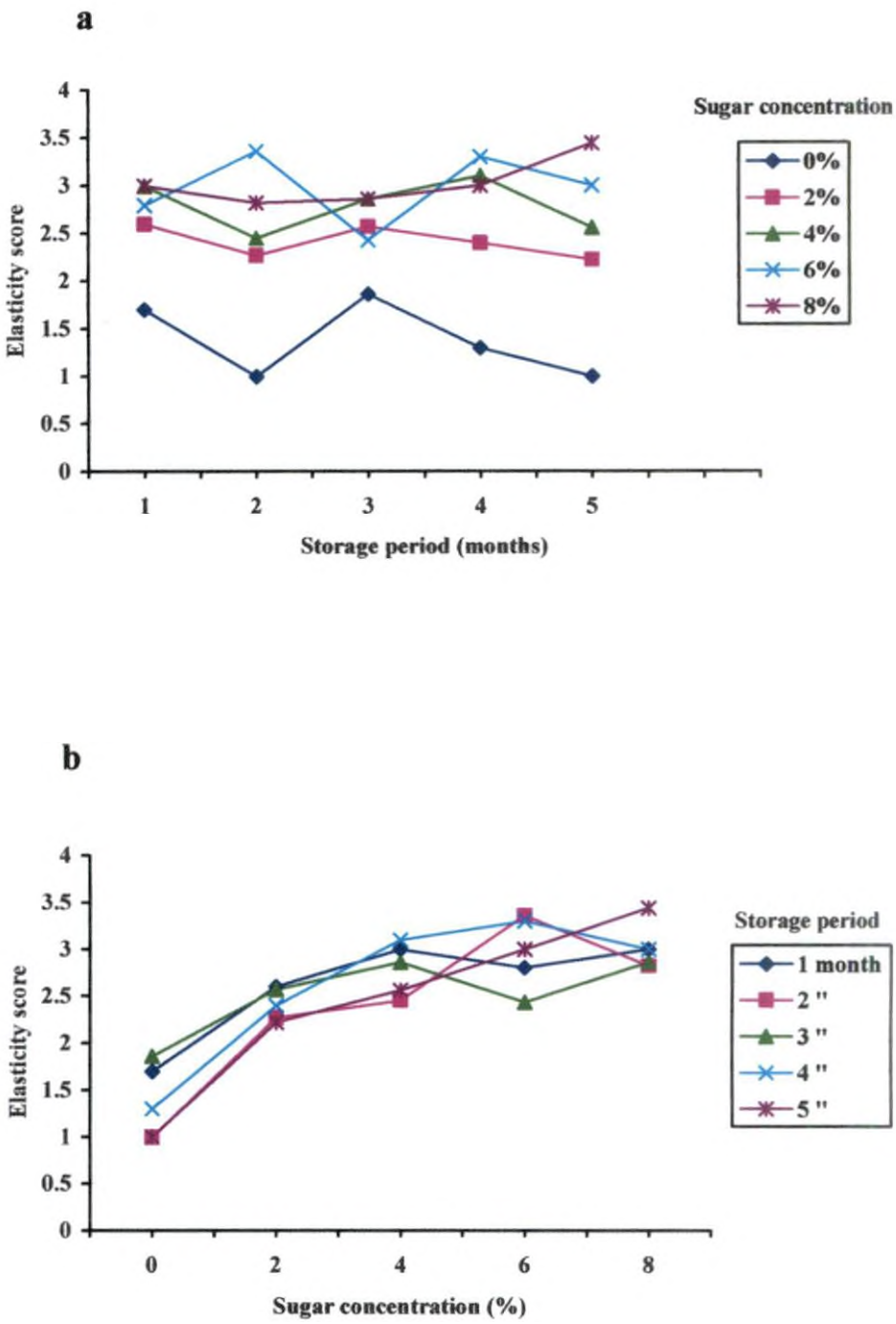

Fig. 14: Variations in elasticity of surimi sausage containing different concentrations of sugar mixture during storage at -20°C

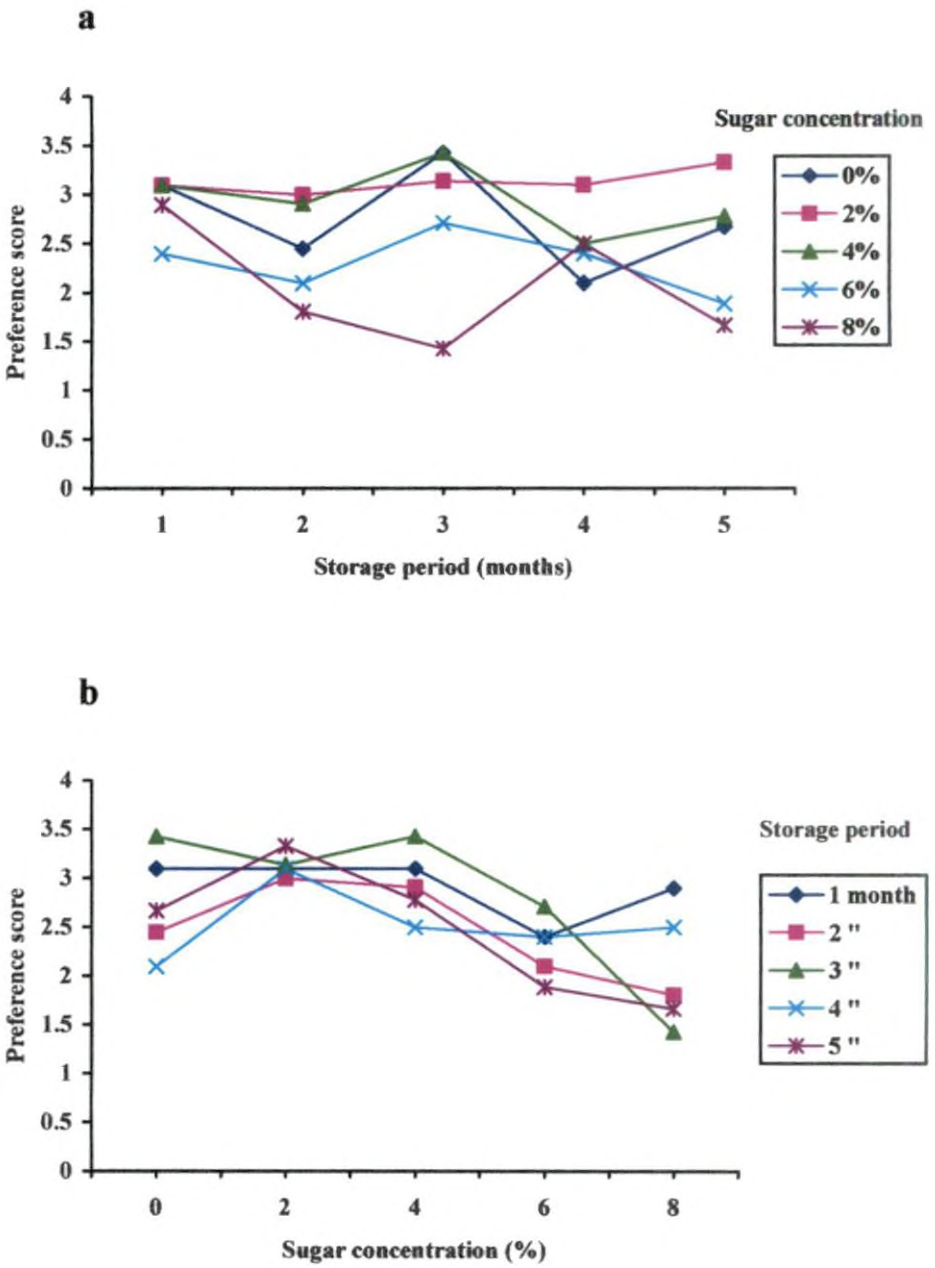

Fig. 15: Variations in preference of surimi sausage containing different concentrations of sugar mixture during storage at -20°C

Table 10: ANOVA for variations in sweetness of surimi sausage containing different concentrations of sugar mixture during storage at -20°C

Source of variation	F value	Fcritical	Significance at 5 % level
Month	0.373743	3.006917	Not significant
Concentration	52.7995	3.006917	Significant

Concentration:	C_0: 0%	C_1: 2%	C_2: 4%	C_3: 6%	C_4: 8%
Means:	0.29	0.484	1.152	2.19	2.604

Critical difference = 0.422

Underscored means are not significantly different

Table 11: ANOVA for variations in elasticity of surimi sausage containing different concentrations of sugar mixture during storage at -20°C

Source of variation	F value	Fcritical	Significance at 5 % level
Month	0.546943	3.006917	Not significant
Concentration	22.55201	3.006917	Significant

Concentration:	C_0: 0%	C_1: 2%	C_2: 4%	C_3: 6%	C_4: 8%
Means:	1.372	2.412	2.794	2.978	3.024

Critical difference = 0.431

Underscored means are not significantly different

Table 12: ANOVA for variations in preference of surimi sausage containing different concentrations of sugar mixture during storage at -20°C

Source of variation	F value	Fcritical	Significance at 5 % level
Month	1.482912	3.006917	Not significant
Concentration	6.155275	3.006917	Significant

Concentration:	C_4: 8%	C_3: 6%	C_0: 0%	C_2: 4%	C_1: 2%
Means:	2.062	2.3	2.75	2.944	3.134

Critical difference = 0.540

Underscored means are not significantly different

DISCUSSION

5. DISCUSSION

Meat of Japanese threadfin bream (*Nemipterus japonicus*) was used for the study as this species serves as an important raw material for surimi production in India. The proximate composition of fish meat (Table 3) indicates that the fish is lean, having only 1.0% fat. This was further reduced by water leaching which is a critical step in the manufacturing of surimi. Protein content decreased on water leaching, which may be chiefly due to the loss of water soluble proteins during the leaching process. For the same reason, mineral content had also reduced. Sijo *et al.* (2002) attributed the reduction in ash content to the removal of water soluble mineral constituents from meat. An increase of 4.21% in moisture content was noticed probably due to the hydration of myofibrillar proteins. Similar results have been reported by Suvanich *et al.* (2000). Many workers (Suzuki, 1981; Hultin, 2002; Park, 2005) have recommended water leaching to the extent of two to three times for lean meat with the last wash using dilute sodium chloride solution in order to prevent excessive water absorption. The same method was adopted in the present study; however, a slightly high hydration of proteins seemed to have taken place.

Another important purpose of water leaching is to develop sufficient whiteness in surimi. Removal of pigments, fat, water soluble minerals, etc., results in white colour as reported by workers like Kim *et al.* (1996), and Roussel and Cheftel (1988) who consider it as an important index of surimi quality. In this study, whiteness of all the lots of surimi remained more or less the same within a narrow range of whiteness score (2.73 – 3.25) as given in Table 4. From the score sheet for whiteness (Appendix III), it could be inferred that all the lots of surimi (with cryoprotectant contents ranging from 0 to 8%) had a moderately white colour. The additives used were only sucrose, sorbitol and sodium tripolyphosphate (STPP). As indicated by Park (1995) these may not have any influence on colour. According to him

whiteness of surimi is dependent mainly on water leaching and the same extent of leaching was given to all the lots of surimi in the present study.

The most commonly used combination of additives was used as cryoprotectants for the study, viz., sucrose-sorbitol mixture in 1:1 proportion (sugar mixture). Surimi was prepared with varying concentrations (from 0 to 8%) of this sugar mixture keeping the concentration of STPP at 0.25%. This combination was supported by Mac Donald and Lanier (1991) who reported that the most commonly used cryoprotectants in the surimi industry are low molecular weight sugars and polyols such as sucrose and sorbitol, typically added at the rate of 8 %, alone or in 1:1 mixture, to leached fish muscle. Sucrose is usually combined with sorbitol to reduce sweetness, as explained by Matsumoto and Noguchi (1992). These carbohydrates were chosen because of their relatively low cost, availability and less tendancy to cause maillard browning in white gel products.

As a preliminary step for the study, a panel of 10 was constituted for testing the preference. The main parameter tested was sweetness of the product. The preference parameter for a fish product by a consumer is influenced by the sweetness of the product and the major objective of this study was to minimize the sugar concentration in surimi without significantly affecting its stability during storage. The test was carried out in order to fix the appropriate sugar concentration for further studies. For this purpose a pilot study on consumer preference was conducted preparing various products, viz., sausage, patty and cake, which had different composition (Table 2) and preparation methods.

Fish sausage (surimi sausage) is a paste product containing approximately 70% surimi the rest being additives such as salt, starch and spices. Although not available currently in the domestic market it is possible to popularize this product on account of its similarity with other meat

sausages. Sausages prepared from surimi having different levels of cryoprotectants were evaluated. A steady increase in the extent of perception of sweetness with increase in sugar concentration was observed (Fig. 4a). The product was acceptable to the judges at all concentrations of sugar, including the maximum level of 8% sugar in surimi (Fig. 4b). This may be on account of other ingredients added to the sausage mix which can not only reduce the sugar concentration in the final product but also can partially mask the sweet flavour, thereby making the product more acceptable. However, lower sugar concentrations of 0 to 4% were definitely preferred over higher concentrations. Slight sweetness (2%) seemed to be more preferred than control (0% sugar) showing a score of 3.13. It seemed that sugar can positively influence the overall taste of a product.

Fish patty or fish cutlet is a popular battered and breaded product. Unlike fish paste products such as sausage and cake, in this product the fish meat or surimi is cooked prior to mixing with additives. When raw surimi is cooked a certain amount of water will be lost on account of protein coagulation. Along with this a certain amount of sugars can be expected to be lost. Sweetness can hardly be detected at 2% level of sugar mixture, but beyond this it could be definitely detected (Fig. 5a). Patty being a spicy snack-type product, a small amount of sugar will be beneficial to the overall flavour of the product. This must be the reason for the highest preference score obtained when surimi with 2% sugar was used (Fig. 5b). Beyond this concentration the acceptance reduced even though the product was still moderately accepted.

A pattern similar to that of sausage or fish patty was obtained for sweetness in the case of fish cake also (Fig.6a). Fish cake is a typical Japanese kamaboko-type product and therefore is a totally new product to the taste panelists. It does not have any typical fish or spicy flavor. The panelists therefore probably did not mind a sweet taste. This is indicated by

the products being moderately accepted by the panelists irrespective of the sugar content (Fig. 6b). However a low level of sweetness was definitely preferred here also.

Although the degree of acceptability varied with sweetness, none of the products prepared from any of the concentrations of surimi were rejected by the taste panelists. Therefore, for subsequent studies on frozen storage of surimi, all concentrations of sugar mixture along with the control were used.

Commercially surimi is quick frozen and frozen stored. Along with sugar, phosphates are also added that aids in the cryoprotective action of the former. Matsumoto and Noguchi (1992), and Jeremiah (1996) observed that polyphosphates have no cryoprotective effect but works as a synergist to the cryoprotective effect of the carbohydrate additives which gives enhanced protein extractability, which in turn, enhances gel forming and water binding properties. For studies on frozen stored surimi therefore, the recommended amount of STPP (0.25%) was mixed with the sugar mixture. The same amount of polyphosphate was added to the control (0% sugar) lot of surimi also.

No marked variations in pH and total plate count (TPC) occurred on account of either storage period or sugar concentration (Figs. 7 and 8, respectively). pH values fluctuated within a range of 6.6 to 7.06 during storage of all samples with no definite trend in variation. Scott *et al.* (1988) also observed that the pH of Alaska pollock surimi varied only slightly between 7.0 and 7.3 but showed no trend of increase or decrease with prolonged storage, whereas Chang and Regenstein (1997) observed a gradual increase in cod mince pH with frozen storage time. The fairly steady pH can be an indication of absence of chemical changes or microbial activity during the period of study. The variations in TPC were insignificant, either with storage time or with sugar concentration; with the counts ranging

between 3.7 and 4.92 $\log_{10}$ cfu/g. Bacterial counts within 3 to 5 $\log_{10}$ cfu/g is common amongst frozen raw fish products; Sikorski (1990) reported that the number of bacteria in high quality fresh fillets varied from 3 to 4 $\log_{10}$ cfu/g. According to Jittinanda *et al.* (2005) frozen storage time does not affect psychrotrophic counts of surimi treated with any cryoprotectant. In the present study, although not significant, a slight reduction in count was seen during storage irrespective of the treatments (Fig. 8) which is possibly due to destruction of microorganisms, probably mesophiles, by the low temperature. A greater extent of destruction of mesophilic organisms compared to psychrophiles has been reported by George and Mohankumar (1979) during frozen storage of prawn.

Moisture content of surimi is generally about 80 % of its weight (Karthikeyan *et al.*, 2006). Moisture content remained more or less the same throughout the period of storage in every cryoprotectant-treated lot of surimi (Fig. 9a). In properly stored surimi, moisture content is not expected to vary during storage. Suvanich *et al.* (2000) also found that moisture content of surimi did not change during storage. The control however showed a decreasing trend after the second month of storage. The moisture content was estimated after thawing the surimi sample. The reduction in moisture content in control is possibly due to drip loss during thawing on account of the protein denaturation that had occurred during storage. Similar observations were made by Scott *et al.* (1988) who observed a decrease in water holding capacity of fish protein during frozen storage. As seen in (Fig. 9b) there was definite reduction in moisture content with increase in sugar concentration. For instance the moisture content values of zero month surimi were 83.96%, 82.3%, 80.7%, 80.33% and 79.01% for 0%, 2%, 4%, 6% and 8% sugar concentrations, respectively. This can be attributed to the addition of sugar mixture itself which reduces the moisture content that is expressed as percentage by weight of surimi. Findings of Chang-Lee (1990)

support this who observed that the moisture content of surimi decreased from 83.93% to 77.28% due to incorporation of cryoprotectants.

Salt soluble nitrogen (SSN) content of frozen raw meat can be used as an indicator of the extent of freeze denaturation of myofibrillar proteins. This has been supported by Moosavi-Nasab *et al.* (2005) who reported the changes in fish proteins during frozen storage as reflected by a significant decrease in solubility of the myofibrillar fraction. The main function of a cryoprotectant in surimi is to prevent this denaturation process. In the present study a definite reduction in the % SSN with period of storage was seen in all the lots of surimi. But as may be observed in Fig. 10a, the rate of reduction was greatest in the control lot indicating a greater rate of protein denaturation. The decreasing trend of moisture content of thawed surimi without sugar (Fig. 9a), probably due to increased drip, can also be on account of protein denaturation. It can also be observed that even a sugar concentration as low as 2% could substantially reduce the protein denaturation process. The tropical fish proteins are more stable to frozen storage conditions and the concentration of sugar required for adequate cryoprotection can be substantially reduced (Park, 2005). With increase in the sugar concentration proteins were increasingly protected as indicated by the higher SSN values throughout the storage period. Statistical analysis also showed significant variation in % SSN with concentration as well as storage period (Table 6). This finding is supported by similar observations made by Sych *et al.* (1990) on cod surimi. Park *et al.* (1988) recorded a 43% decrease in SSN content of Alaska pollock surimi treated with 0.5% STPP stored from one to three months at -20°C. A significant loss of SSN was also observed by Verma and Srikar (1994) throughout the frozen storage of surimi which amounted to 43.9% of the initial value at the end of 180 days.

The quality of surimi can be best evaluated by preparing a food product out of it and assessing its various textural and sensory quality

characteristics. Fish sausage is an important fish paste product that can be prepared using surimi. Therefore for every sampling, after conducting tests on raw surimi, sausages were prepared adopting the recipe given under section 3.3.1.

One test conducted on the sausage was the expressible water content. This parameter has an inverse relationship with the elasticity of the product and %SSN of the surimi. Iwata (1959) studied the relation between the amount of expressible water and elasticity of Alaska pollock kamaboko and found a high degree of correlation which was inverse in nature. Higher expressible water content therefore can be a consequence of increased protein denaturation on frozen storage of surimi leading to corresponding decrease in water holding capacity of the product. Fig. 11a shows an increase in expressible water content of the sausage with storage period of the surimi in all the cases of sugar concentration. However the increase was not statistically significant till the second month, but later showed a definite increase (Table 7). (Data for the fifth month could not be obtained as an error was encountered in the preparation of the product). It can be understood that protein denaturation becomes more evident only in the later stages of storage. These observations were supported by the findings of Siah *et al.* (1998) and Suvanich *et al.* (2000) who also obtained increased expressible water content with storage for their products. In addition, expressible water content was highest for 0% (control) and significantly reduced with increase in cryoprotectant concentration as can be seen in Fig.11b and Table 7. For instance, in the first month sample, the expressible water content was 30.53%, 23.3%, 23.1%, 20.74% and 19.69% for sugar mixture concentrations of 0%, 2%, 4%, 6% and 8%, respectively. It may be noted that compared to control even a small amount of sugar (e.g. at 2% level) could substantially reduce the expressible water content.

Fig. 12a and b respectively shows the variation in gel strength of sausage as influenced by storage period and sugar concentration. The gel strength was measured by an Okada-type gelometer that is widely used particularly for paste products (Suzuki, 1981). It is reported to be a good indicator of the texture of the product and has good correlation with the sensory grading of elasticity. Higher gel strength was obtained for sausage prepared from one month old surimi and it showed a statistically insignificant but decreasing trend with storage period (Table 8 and Fig. 12a). A definite increase in gel strength was observed with sugar concentration (Fig. 12b). Variation in gel strength could be correlated well with the changes in %SSN (Fig. 10) suggesting that gel strength is influenced by the extent of protein denaturation. This is supported by an inverse relationship observed between gel strength and expressible water content (Fig. 11). Workers like MacDonald *et al.* (1992) also have obtained similar results on fishes such as hoki. According to them the gel strength of hoki mince tends to decrease with frozen storage. Sultanbawa and Lichan (2001) reported that gel strength of samples with individual cryoprotectants added at 4% level were generally significantly lower than samples frozen with 8% individual cryoprotectants or with optimal blends containing 8, 6, 5.5 and 4% cryoprotectants. They found an increase in cohesiveness of the gel with an increase in the level of cryoprotectant from 4 to 8%.

The folding test conducted on surimi sausages also indicated the effectiveness of cryoprotectant concentration on elasticity (Table 9). Folding test is a simple but dependable test for the elasticity of fish paste products. The control lot of surimi always resulted in a lower grade (grade 'C') product, but a concentration as low as 4% or above could result in the highest grade of 'AA'. It may be noted that high elasticity is preferred by the Japanese, but in India this may not be the case. A lower grade such as 'A' or 'B' would probably be sufficient for the domestic market, which could be provided by sugar concentrations of 2% or more.

For any manufactured food product the ultimate evaluation is done by the consumer himself. Thus it becomes inevitable to conduct sensory evaluation tests for assessing the quality of products from surimi. Surimi sausage was prepared by boiling. However, generally it is consumed after further preparations such as frying, grilling, etc. Therefore, the products were mildly fried in vegetable oil and presented to a panel of judges. Evaluation was done based on three parameters, viz., sweetness, elasticity and preference or degree of acceptability.

No significant variation in sweetness scores with storage period of surimi was observed in all the cases of sugar mixture concentrations (Fig. 13a, Table 10). This could be expected as there cannot be any loss of sugar during frozen storage. Slight fluctuations in sweetness scores could be observed but these may be only on account of slight sample variations and may not be indicating any particular trend. (Sausages could not be prepared using zero month sample of surimi on account of an error that occurred during processing). From Fig. 13b it could be seen that sweetness was hardly detected by the taste panelists at low sugar levels of 0% and 2%. However, sweetness was judged definitely high and significant for sausage prepared using surimi containing 6 or 8% sugar, throughout the period (Table 10).

Elasticity is an important textural characteristic of paste products such as sausage. The elasticity as judged by the panelists was substantially lower in the control sample compared to sausages prepared using surimi containing any concentration of sugar mixture, throughout the storage period (Fig. 14a). However, for each concentration of sugar mixture no significant variation due to storage was evident. Generally surimi prepared using higher concentration of sugar have frozen storage stability for several months (Park, 2005). The period of five months may not be sufficient to provide a significant difference in the elasticity quality particularly since the

surimi has been prepared from a tropical fish. However, it may be observed (Fig. 14a) that sausage prepared using surimi at 0% and 2% sugar mixture levels showed a decreasing trend after three months of storage. This could be related to a greater rate of protein denaturation occurring in these treatments as indicated by a decrease in % SSN content of surimi with storage period (Fig. 10a). It was evident from Fig. 14b that addition of a small amount of cryoprotectant such as 2% sugar mixture was sufficient to protect the proteins and thereby the elasticity, and that with further increase in sugar concentration there was a corresponding increase in elasticity scores (Table 11). The elasticity scores obtained could be related to other parameters that influence/ indicate texture of sausage, viz., %SSN (Fig. 10), expressible water content (Fig.11) and folding test grades (Table 9). The elasticity scores could be seen inversely related to %SSN and expressible water content, and directly related to gel strength and folding test grades.

The ultimate judgement of the consumer about a product is, to what extent he "likes" the product. This can be evaluated by the preference test which is based on various quality characteristics of the product such as taste, texture, colour and appearance. The only variations that could be expected in the different sausage samples used in the study were sweetness and elasticity on account of the different cryoprotectant levels used. Rest of the additives and treatments were maintained constant for all products. In the case of each concentration of sugar mixture, preference scores did not vary significantly during storage as seen in Fig.15a possibly because sweetness and elasticity did not vary significantly during storage. However, from Fig. 15b and Table 12 it could be inferred that products prepared from surimi containing lower concentrations of sugar mixture (0, 2 and 4% sugar) were definitely preferred over higher concentrations (6 and 8% sugar).

Considering the frozen storage stability of surimi and consumer acceptability of products prepared from surimi, it is desirable to reduce the

sugar level substantially from the commercially used level of 8%, but without sacrificing the storage stability. According to the present investigation, a sugar-sorbitol mixture level of 2 to 4% can be suggested as a suitable concentration for a storage life of at least five months at -20°C. However, 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.

SUMMARY

6. SUMMARY

1. The main objectives were to study the effect of different concentration of cryoprotectants, viz., sucrose and sorbitol, in surimi prepared from a tropical fish on acceptability of surimi-based products and the influence of cryoprotectant levels on surimi stability during frozen storage.

2. Fresh threadfin bream (*Nemipterus japonicus*), a tropical fish commonly used for surimi preparation in India, was used as raw material for the study and it was transported from the fish market/ landing centre to the laboratory in an insulated box.

3. Surimi was prepared from minced meat which was further strained, water leached, dewatered, divided into lots to which different levels of cryoprotectants (mixture of sucrose-sorbitol in 1:1 ratio) were added, packed, quick frozen and stored at a temperature of -20 ^{o}C for various periods.

4. Comparison of proximate composition of threadfin bream meat before and after water leaching was done which showed that moisture content of the meat increased while all the other components viz., protein, fat and ash contents decreased on water leaching.

5. Raw surimi was subjected to evaluation for whiteness which showed that all the samples irrespective of the cryoprotectant concentration gave moderately white colour.

6. For evaluating the acceptability of surimi-based products, different types of products that varied in composition and preparation method viz., surimi sausage, patty and cake were prepared and were subjected to sensory evaluation for sweetness and preference.

7. It was observed that these products with lower sugar concentrations of 0% (control), 2% and 4% were more preferred compared to 6% and 8% levels eventhough higher concentrations where also fairly acceptable. A slight sweetness of that of 2% sugar mixture in the case of surimi sausage and surimi patty, and that of 4% in the case of surimi cake were more preferred over their respective controls (0% sugar mixture).

8. For evaluating the stability of surimi during frozen storage, studies where carried out on surimi containing different concentrations of sugar mixture frozen stored at -20 ^{o}C for a period of five months. These included tests for pH, total plate count (TPC), moisture content and salt soluble nitrogen (SSN) content of surimi. Sausage prepared using surimi was tested for gel strength, expressible moisture content, folding test characteristics and sensory quality for sweetness, elasticity and preference.

9. The values of the quality parameters such as SSN and gel strength showed a decreasing trend while expressible water content showed an increasing trend with storage period. pH, TPC, moisture content, sensory quality for sweetness, elasticity and preference of the samples with 4%, 6% and 8% sugar levels remained almost constant throughout the storage period whereas elasticity for control and sample with 2% sugar level showed a decreasing trend throughout the storage period. The decreasing trend of SSN content could be on account of protein denaturation. This in turn could increase the expressible water content of the product and reduce the gel strength (elasticity).

10. The values of the quality parameters such as SSN, gel strength, sweetness and elasticity significantly increased with cryoprotectant concentration while moisture content and expressible moisture content significantly decreased. The product was well accepted up to a

concentration of 4% sugar mixture in surimi, beyond which the acceptability significantly reduced, probably on account of the increased sweetness. However, pH and TPC remained almost constant irrespective of the sugar concentration indicating that chemical or microbial activity did not have much influence on the quality of surimi during this period.

11. Sausages prepared from surimi stored till the end of the fifth month were still acceptable at all levels of cryoprotectant. However, acceptability of the control (0% sugar) was much lower compared to any other concentration of sugar mixture. From this it may be concluded that even a concentration as low as 2% could substantially stabilize surimi during frozen storage at least for five months at -20^{o}C. Products prepared from such surimi may be well accepted by the consumers, as the taste (sweetness) quality of the product will not be adversely affected at such low concentrations of sugar.

12. This study also supports the finding that unlike surimi prepared from temperate water fish, tropical fish surimi such as that prepared from threadfin bream, has good stability in the frozen condition. Thus only a lower concentration of cryoprotectant is necessary for surimi from such species as their proteins are more tolerant to higher temperatures (or frozen storage conditions).

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ABSTRACT

INFLUENCE OF CRYOPROTECTANT LEVELS ON STORAGE STABILITY OF SURIMI FROM A TROPICAL FISH, *NEMIPTERUS JAPONICUS* (BLOCH), AND QUALITY OF SURIMI-BASED PRODUCTS

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ABSTRACT OF THESIS

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ABSTRACT

A study was undertaken with the aim of reducing the concentration of cryoprotectants in surimi without adversely affecting stability of the material during frozen storage. The concentration of sugar in commercially produced surimi is generally about 8% which may result in high sweetness in the products prepared. A tropical fish, *Nemipterus japonicus* (Bloch) was used as the raw material. Minced fish meat was strained, water leached, mixed with different levels (0%, 2%, 4%, 6% and 8%) of sucrose-sorbitol mixture in 1:1 ratio, quick frozen at -35 ^{o}C and frozen stored at -20 ^{o}C.

Water leaching resulted in a slight absorption of water by meat and reduction in protein, fat and mineral contents. Surimi was found to have a moderately white colour. Sensory evaluation studies were conducted on three products, viz., sausage, patty and cake, prepared using surimi containing different concentrations of sucrose-sorbitol mixture. Sugar content varying from 0% (control) to 4% in surimi resulted in products that were more acceptable to the taste panelists compared to those prepared using surimi with 6% and 8% sugar mixture. A slight sweetness in the product (2% to 4% sugar in surimi) was more preferred by the panelists than their respective controls prepared using surimi containing no sugar.

Frozen storage studies were carried out at -20^{o}C on surimi treated with various concentrations of sugar mixture as mentioned above for a period of five months. pH and total plate count remained nearly steady for all sugar concentrations and throughout the storage period indicating insignificant microbial activity. Moisture content appeared to remain constant during storage, but decreased with increase in sugar concentration probably on account of addition of the sugar mixture. 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 concentration, while expressible water content of the sausage showed an increasing trend with storage period and a decreasing trend with cryoprotectant concentration. These indicate a greater extent of protein denaturation occurring at low concentration of surimi samples. Sensory evaluation parameters - elasticity, sweetness and preference - remained more or less steady during storage. However, elasticity of sausage prepared from surimi with no cryoprotectant (control) and with 2% sugar level showed a decreasing trend with storage. Elasticity and sweetness increased while preference decreased with cryoprotectant concentration. Elasticity and gel strength seemed to be much lower for control (1.7 and 783.71 g.cm, respectively) compared to even the lowest concentration of sugar (2%) used (2.6 and 886.9 g.cm, respectively). From the study it may be concluded that a concentration of 2 to 4% sucrose-sorbitol mixture is well accepted by the consumers in products such as surimi sausage, patty and cake. At this range of concentration, surimi can be well-preserved in frozen condition at -20°C for at least five months.

APPENDIX I

SENSORY EVALUATION SCORE SHEET

You are given samples of fish patty/fish cake, some containing sugar and some without. Kindly taste the samples and indicate (by a tick mark) the extent of sweetness of each.

Score*	Sweetness	Sample				
		A	B	C	D	E
0	Not sweet					
1	Doubtful					
2	Slightly sweet					
3	Moderately sweet					
4	Highly sweet					

Indicate to what extent you like each product by putting a tick mark at the appropriate cell.

Score*	Preference	Sample				
		A	B	C	D	E
0	Do not like					
1	Neither like nor dislike					
2	Like slightly					
3	Like moderately					
4	Like much					
5	Like very much					
6	Like extremely					

Signature :
Name : Date:

* Scores were not given in the original score sheets

APPENDIX II

SENSORY EVALUATION SCORE SHEET

You are given samples of fish sausage. Kindly taste the samples and indicate (by a tick mark) the extent of sweetness and elasticity.

Score*	Sweetness	Sample				
		A	B	C	D	E
0	Not Sweet					
1	Doubtful					
2	Slightly Sweet					
3	Moderately Sweet					
4	Highly Sweet					
Score*	**Elasticity**	**Sample**				
		A	B	C	D	E
1	Poor Elasticity					
2	Slightly Elastic					
3	Moderately Elastic					
4	Highly Elastic					
5	Extremely Elastic					

Indicate to what extent you like each product by putting a tick mark in the appropriate cell.

Score*	Preference	Sample				
		A	B	C	D	E
0	Do not Like					
1	Neither Like nor Dislike					
2	Like Slightly					
3	Like Moderately					
4	Like Much					
5	Like Very Much					
6	Like Extremely					

Signature:
Name : Date:

* Scores were not given in the original score sheets

APPENDIX III

SENSORY EVALUATION SCORE SHEET

You are given samples of surimi. Kindly evaluate their colour and indicate the extent of whiteness of each. Put a tick mark in the appropriate cell for each sample.

Score*	Whiteness	Sample				
		A	B	C	D	E
1	Poor Colour					
2	Slightly White					
3	Moderately White					
4	Very White					
5	Extremely White					

Signature :
Name :

Date:

* Scores were not given in the original score sheets