*Fishery Technology* 51 (2014) : 47 - 53

# Effect of Cryoprotectants on the Frozen Storage Stability of Mince and Quality of Mince-based Products from *Nemipterus japonicus* (Bloch, 1791)

**U. Parvathy[1]*, M. M. Jibina[2] and Sajan George[2]**
[1] *Central Institute of Fisheries Technology, P. O. Matsyapuri, Cochin - 682 029*
[2] *Kerala University of Fisheries and Ocean Studies, P. O. Panangad, Cochin - 682 506, India*

## Abstract

A study was undertaken to assess the effect of cryoprotectants on frozen storage stability of mince for a period of 80 days and acceptability of mince-based products from *Nemipterus japonicus* (Bloch, 1791). Mince was mixed with various cryoprotectants viz., 2% sucrose, 2% (1:1) sucrose and sorbitol mixture (sugar mixture) and 3% sugar mixture, quick frozen at -35°C and frozen stored. A steady increase in perception of sweetness and elasticity with increase in cryoprotectant concentration was observed in the products. For sausage, 2% sucrose level was preferred while for patty, control (without cryoprotectant) was the most accepted one. During frozen storage of mince, pH showed a slight increase and total plate count remained nearly steady for all the samples throughout the storage period. Moisture content appeared to remain constant during storage, but decreased with increase in sugar concentration. Salt soluble nitrogen content of mince decreased with storage period, and increased with addition of cryoprotectant. Expressible water content of sausage showed an increasing trend with storage period while a decreasing trend with cryoprotectant concentration was observed. It was vice-versa in case of the gel strength of fish sausage. The study concluded that a 2 to 3% sugar mixture concentration can be considered as optimum for consumer acceptance of mince-based products as well as for enhancing the stability of mince stored at -20ºC for a period of 80 days.

**Keywords:** Mince, cryoprotectants, *Nemipterus japonicus*, sausage, patty

Received 22 July 2013; Revised 11 November 2013; Accepted 13 November 2013

* *E-mail: p.pillai2012@gmail.com*

## Introduction

Fish which is a good source of high quality proteins, poly unsaturated fatty acids, many vitamins and minerals, are becoming increasingly popular in human diet. There is a need for new approaches to meet the growing demand for fish products. The development of value-added products from fish mince is a good option for achieving this requirement. Fish mince is the starting material for the preparation of various comminuted fish products such as fish cutlets, burgers, sticks and cakes. Although whole fish can be preserved by freezing, the frozen storage stability of fish mince is very poor on account of excessive drip resulting in fibrous texture. Surimi is an alternative having good shelf life but is expensive and generates huge volume of organic material rich waste water. The advantage of using mince compared to surimi is its higher protein content and higher yield during processing since there is no leaching of sarcoplasmic proteins. However, proteins on prolonged frozen storage undergo denaturation. This can be reduced to a greater extent by the incorporation of cryoprotectants. The mechanism of stabilization of fish muscle protein during frozen storage by cryoprotectant is through its interaction and bonding with the functional groups on the surfaces of protein molecules wherein each protein molecule gets covered by a hydrated cryoprotectant molecule resulting in increased hydration and decreased aggregation of the proteins. These can be incorporated in mince also, to improve its frozen storage stability.

The cryoprotectant concentration used in commercially produced surimi is about 8% (Mac Donald & Lanier, 1991) which results in high sweetness in the final products that may not be acceptable to the Indian consumer. The incorporation of

cryoprotectants in mince can be at a lower level if it is for domestic market since the frozen storage period considered is less. Hence, a study was conducted with the aim of understanding the consumer acceptance of mince-based products containing cryoprotectants at different concentrations and the frozen storage stability of these cryoprotectant-treated fish mince stored at -20ºC for 80 days.

## Materials and Methods

Fresh *Nemipterus japonicus* (threadfin bream) that was iced in 1:1 ratio immediately after catch was purchased from the fishing harbour at Cochin and transported in an insulated box to the laboratory. Fish was washed, dressed, meat picked manually and mechanically minced in a mincer (Sirman-TC 22E BX) for one min. It was then strained and divided into four lots. Cryoprotectants were blended in each lot, packed, frozen and stored.

Sucrose alone @ 2% (w/w), sucrose and sorbitol mixture in 1:1 proportion @ 2% and 3% (w/w) levels were mixed with various lots of mince. One lot was taken as control (without cryoprotectant). Sodium tripolyphosphate (STPP) @ 0.25% (w/w) was also mixed with each lot in a silent cutter (MADO) for 10 min. The samples were packed in 50 micron polypropylene bags as 2.0 cm slabs and blast frozen in blast freezer (Beeta) at -35ºC for two hours. The slabs were frozen stored in deep freezer (Voltas) at -20ºC. Physical, biochemical and sensory quality tests were conducted for a period of 80 days. For this, every 20 days one bag from each lot was withdrawn and mince was thawed at room temperature.

As a preliminary step for the study, the preference of two mince-based products that varied in composition and preparation viz., sausage and patty, as influenced by the cryoprotectant concentration, was evaluated by a panel of 10 semi-trained judges using 9 point hedonic scale as per Mailgaad et al. (1999). None of the samples were rejected by the panel and hence, all the cryoprotectant treated mince were considered for further storage study. Sensory quality for sweetness, elasticity and preference were done as per Rousseau (2004). For evaluating frozen storage stability of mince, the following tests were carried out: pH according to Suzuki (1981), total plate count (TPC) as per BAM (1998), moisture content as per AOAC (1975) and salt soluble nitrogen (SSN) content as per AOAC (1984). Sausage was prepared from the mince during every sampling and was tested for gel strength and expressible moisture content according to the methods given by Suzuki (1981). Gel strength was determined by using Okado gelometer and was calculated from the area under kymogram obtained.

Sausage was prepared using the modified method of Chandrasekhar & Manisseri (1976). Ingredients used for sausage preparation are given in Table 1. Frozen mince was thawed and to this ingredients were added and mixed for a period of 12 min in the silent cutter. The paste was then stuffed using a fabricated stuffer at the rate of 30 g per piece into cellulose casings of 1.7 cm diameter and heat set in water bath at a temperature of 88-90 °C for 40 min, cooled for about 15 min using chilled water (15ºC), surface dried and labeled.

Table 1. Ingredients used for mince-based sausage and patty (in g)

| Ingredients(g) | Fish sausage | Fish patty |
|---|---|---|
| Mince | 720 | 1000 |
| Maida | 90 | - |
| Table salt | 20 | 15 |
| 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 |
| | - | - |
| | - | - |
| Water (ml) | 105 | - |
| | - | - |

Fish patty was prepared as per modified method of Gopakumar (2002). Ingredients used for fish patty are given in Table 1. Thawed mince was steamed for about 30 min. Onion, green chillies and ginger were fried in oil at 180ºC till light brown and then coarsely ground in a mixie. Mince, mashed potato, fried material and other ingredients were then mixed manually followed by mixing in silent cutter for 5 min. The mixture was moulded into 15 g round cakes using a patty moulder. Cakes were dipped in a batter of maida, table salt, whole egg and water (3:0.2:10:10 by weight), coated using bread crumbs and fried in refined sunflower oil at 180°C until the surface attained medium brown colour.

All quality evaluation tests were done in triplicate. Experiments were carried out using completely randomized design. Data obtained were analyzed using analysis of variance technique (Snedecor & Cochran, 1968) and results expressed as mean ± SD.

## Results and Discussion

Proximate composition of threadfin bream mince (moisture 79.87±0.77%, crude protein 18.38±0.39%, crude fat 1.0±0.03% and ash 0.77±0.02%) indicated that the fish used was lean. Mince was mixed with varying concentrations (from 0 to 3%) of sucrose and sorbitol. Zhou et al. (2006) reported that the most commonly used cryoprotectants in the surimi industry were low molecular weight sugars and polyols such as sucrose and sorbitol. According to Lanier et al. (2004) sucrose was usually combined with sorbitol to reduce sweetness.

Sausages were found to have a steady increase in perception of sweetness and elasticity with increase in cryoprotectant concentration (Fig 1a and c). Slight sweetness (2 %) seemed to be preferred over control and other samples (Fig. 1b). Product was acceptable even at 3% cryoprotectant level. This may be on

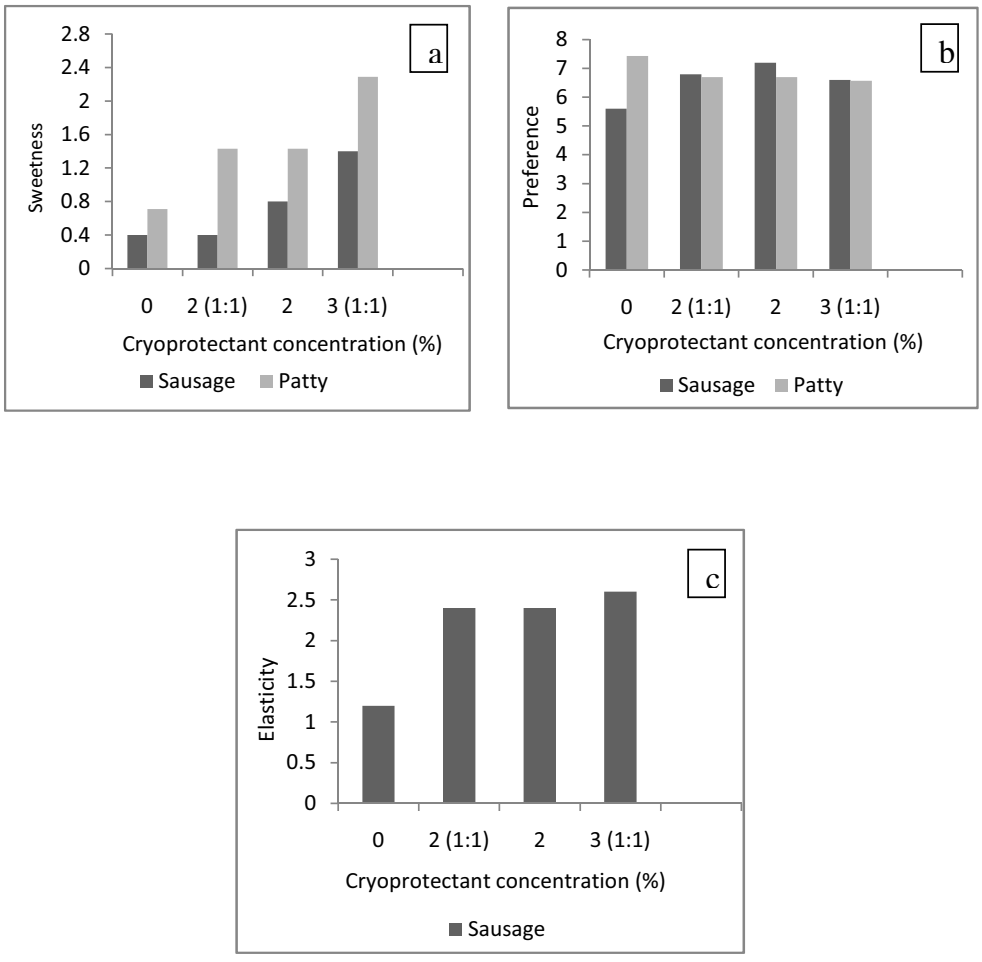


Fig. 1. Sensory score of sausage and patty treated with various concentration of cryoprotectants

account of other ingredients added to the sausage mix which not only reduce the cryoprotectant concentration in the final product but also partially mask the sweet flavour, thereby making the product more acceptable. Although fish sausage is available currently in hypermarkets of metropolitan cities of India, it is not popular in small cities. It is possible to popularize this product on account of its similarity with meat sausages.

In fish patty, sweetness was found to increase with cryoprotectant level (Fig. 1a) whereas in case of preference (Fig. 1b) contrary to sausage, control patty was the most accepted followed by patty with 2% sucrose alone as well as 2% sugar mixture. Fish patty is a popular battered and breaded product. Unlike fish paste products, this product has fish meat or mince cooked prior to mixing with additives. When raw mince is cooked, a certain amount of water will be lost on account of protein coagulation and a certain amount of sugar can also be expected to be lost. Sweetness could hardly be detected at 2% levels of sugar mixture, but beyond this it could be detected. Patty being a spicy snack-type product, a small amount of sugar will be beneficial to the overall flavour of the product.

During frozen storage pH and TPC of mince varied within a narrow range of 6.13-6.78 and 4.83-5.43 $\log_{10}$ cfu $g^{-1}$, respectively, with no significant difference ($p>0.05$) either with cryoprotectant concentration or with storage period (Fig. 2a and 2b respectively). pH of mince showed a slight increase during the period of study which was supported by Undeland et al. (2002), who also observed a gradual increase in cod mince pH with frozen storage time. The fairly steady pH can be an indication of lesser chemical changes or microbial activity during the period of study. According to Jittinandana et al. (2005), frozen storage time does not affect psychrotrophic counts of surimi treated with any cryoprotectant. Bacterial counts within 3 to 5 $\log_{10}$ cfu $g^{-1}$ are common amongst frozen fish products; Ismail et al. (2013) reported that the number of bacteria in high quality fresh fillets varied from 3 to 4 $\log_{10}$ cfu $g^{-1}$.

Moisture content was found to reduce significantly with increasing cryoprotectant concentration (Fig. 2c) which can be attributed to addition of sugar mixture which reduces moisture content that is expressed as percentage by weight of mince. Chang-Lee et al. (1990) observed that the moisture content of surimi decreased from 83.93 to 77.28% due to incorporation of cryoprotectants. A decrease in moisture content was observed in the control during storage period which may be possibly due to protein denaturation that had resulted in drip loss during thawing. However, the variation was not significant in any of the cryoprotectant-treated mince throughout the storage period indicating cryoprotective effect even at low concentrations. Moisture content of channel cat fish mince did not change during frozen storage at -20°C (Suvanich et al., 2000).

During initial 20 days of storage a sharp decline of SSN (about 40%) was noted in all the lots (Fig. 2d). This finding is in agreement with observations made by Sych et al. (1990) in cod surimi and in mechanically deboned silver carp mince during frozen storage (Siddaiah et al., 2001). The rate of reduction was maximum in control lot indicating a higher rate of protein denaturation. During a study conducted by Tejada et al. (1996) in cod mince, a decrease in protein solubility during frozen storage was also observed. Statistical analysis also showed significant variation ($p<0.05$) in SSN % with cryoprotectant concentration as well as storage period. It was observed that a cryoprotectant concentration as low as 2% could substantially reduce the denaturation process. Proteins from fish that inhabit the tropical waters are more stable to frozen storage conditions and hence require substantially lower amounts of sugar than temperate water fish proteins (Park, 2005).

Expressible water content of sausage increased with storage period which was more prominent after 60 days (Fig. 2e). Siddaiah et al. (2001) stated that the amount of expressible moisture generally reflects the extent of protein denaturation resulting from surface dehydration, ice crystal formation and cell rupture. The increase in expressible moisture of mince during frozen storage may have been due to a change in microstructure of myofibrillar proteins from a continuous filamentous matrix to a globular matrix (Smith, 1987). Hernandez-Briones et al. (2009) observed high degree of negative correlation between the amount of expressible water and elasticity of Alaska pollock kamaboko. In addition, expressible water content remained the highest in control throughout the storage period but significantly reduced even with the addition of as low as 2% cryoprotectant (Fig. 2e). According to Hultmann & Rustad (2002) decrease in water holding capacity (WHC) in fish muscle directly relates to the

denaturation in myofibrillar protein and a better WHC in the product as a result of cryoprotection of the proteins. Decrease in WHC leads to increased expressible moisture and protein denaturation (Dey et al., 2013).

Variation in gel strength (Fig. 2f) appears to be positively correlated with change in SSN content (Fig. 2d) suggesting that gel strength is influenced by the extent of protein denaturation. MacDonald et al. (1992) also have obtained similar results on fishes such as hoki where the gel strength of hoki

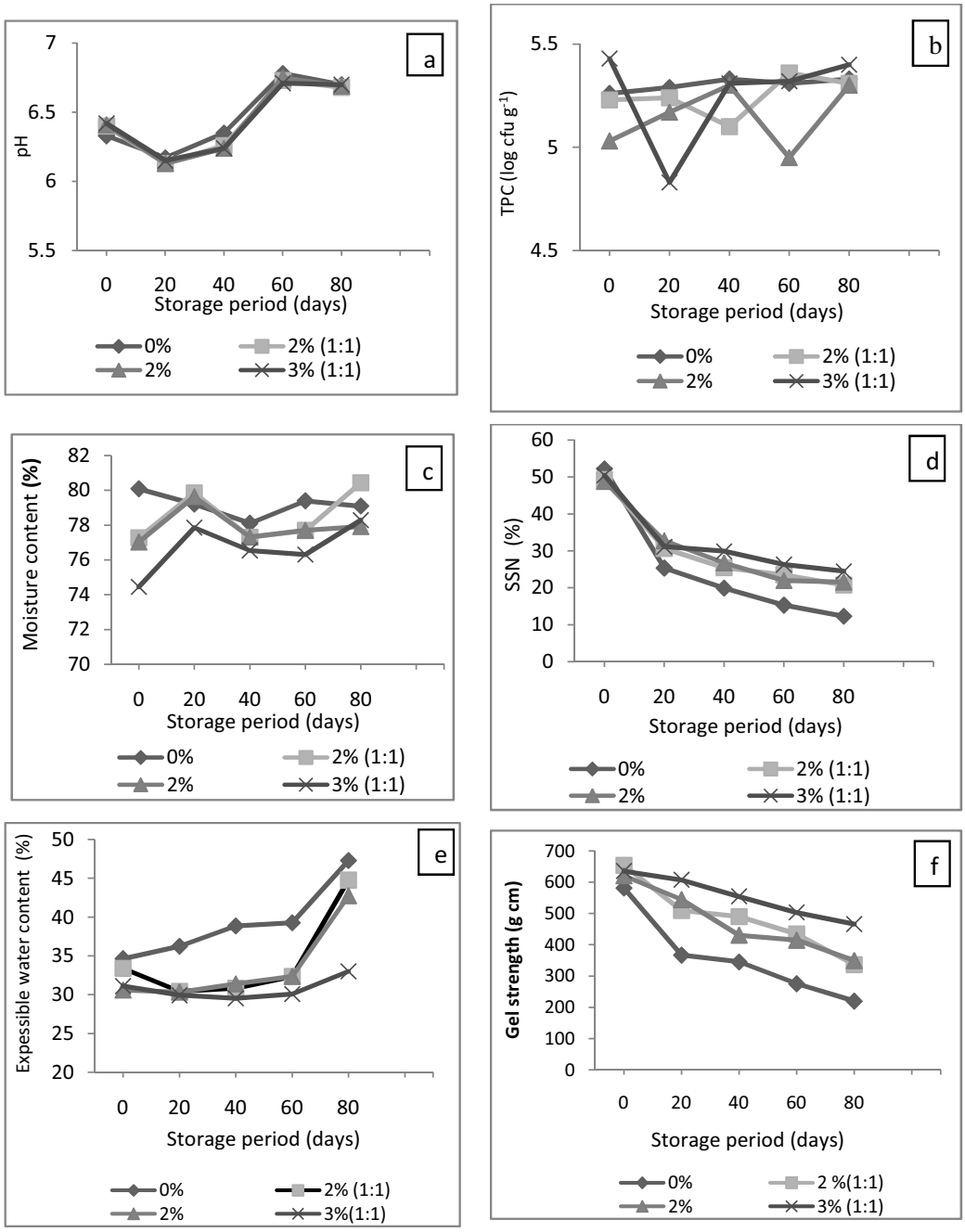


Fig. 2. Variations in quality parameters of mince containing different cryoprotectant concentrations during storage at -20°C

mince decreased with frozen storage. However, the general trend indicated significantly higher gel strength for 2% cryprotectant-incorporated fish mince than for control. According to Dey et al. (2013), freezing and frozen storage have a prominent effect on the gel forming ability of croaker fish surimi without cryoprotectant.

The present study revealed that a sugar mixture concentration of 2-3% can be considered as effective for improving the frozen storage stability of fish mince at -20°C. However, it is suggested that the exact concentration may have to be decided based on the storage period actually required as well as the type of product to be manufactured from the mince.

## Acknowledgements

The authors wish to thank College of Fisheries, Kerala University of Fisheries and Ocean Studies, Cochin for providing the facilities to carry out this work. The first author thanks the Director, CIFT, Cochin-29 for granting permission to publish this paper.

## References

AOAC (1975) Official Methods of Analysis, 12th edn., 1094 p, Association of Official Analytical Chemists, Washington DC

AOAC (1984) Official Methods of Analysis, 14th edn., 1141p, Association of Official Analytical Chemists, Washington DC

BAM (1998) Bacteriological Analytical Manual 8th edn., Revision A. United States Food and Drug Administration

Chandrasekhar, T.C. and Manisseri, M.K. (1976) A Practical Manual of Fishery Products,119 p, Department of Fish Processing Technology, College of Fisheries, University of Agricultural Sciences, Mangalore

Chang-Lee, M.V., Lampila, L.E. and Crawford, O.L. (1990) Yield and composition of surimi from Pacific whiting (*Merluccius productus*) and the effect of various protein additives on gel strength. J. Food Sci. 55 (1): 83-86

Dey, S.S., Dora, K.C., Raychaudhuri, U. and Ganguly, S. (2013) Cryoprotective effect of shrimp waste protein hydrolysate on croaker surimi protein and gel characteristics during frozen storage. Fish Technol. 50: 50-59

Gopakumar, K. (2002) Text Book of Fish Processing Technology, 491 p, Directorate of Information and Publications of Agriculture, ICAR, New Delhi

Hultmann, L. and Rustad, T. (2002) Textural changes during iced storage of salmon (*Salmo salar*) and cod (*Gadus morhua*). J. Aquatic Food Prod. Technol. 11: 105-123

Hernandez-Briones, A, Velazque, G, Vazquez, M., and Ramýrez, j. A. (2009) Effects of adding fish gelatin on Alaska pollock surimi gels. Food Hydrocolloids 23: 2446-2449

Ismail,Y.G., Eduardo, E., Jaime, A. and Abdullah, D. (2013) Effects of chilled storage on quality of vacuum packed meagre fillets. J. Food Eng. 115: 486-494

Jittinandana, S., Kenny, P.B. and Slider, S.D. (2005) Cryoprotection affects on physical properties of restructured trout during frozen storage. J. Food Sci. 70 (1): C35-C42

Lanier, T., Carvajal, P. and Yongsawatdigul, J. (2004) Surimi gelation chemistry. In: Surimi and Surimi Seafood (Park, J. W., Ed), Taylor and Francis, New York, USA

Mac Donald, G.A. and Lanier, T.C. (1991) Carbohydrates as cryoprotectant for meat and surimi. J. Food Technol. 45: 150-159

Mac Donald, G.A., Lelivre, J., and Wilson, N.D.C. (1992) Effect of frozen storage on the gel forming properties of Hoki (*Macruronus novaezelandiae*). J. Food Sci. 57 (1): 69-71

Mailgaad, M., Civille, G.V. and Carr, B.T. (1999) Sensory Evaluation Techniques. Boca Raton, Florida CRS Press

Park, J.W. (2005) Surimi and Surimi Seafood, 923p, Taylor and Francis Publishers, Boca Raton

Rousseau, B. (2004) Sensory evaluation. In: Handbook of Food Analysis, I (Nollet, L.M. L., Ed), pp 21-38, Marcel Dekker, Inc., New York

Siddaiah, D., Vidya S.R.G., Raju, C.V. and Chandrasekhar, T.C. (2001) Changes in lipids, proteins and Kamaboko forming ability of silver carp (*Hypophthalmichthys molitrix*) mince during frozen storage. Food Research Int. 34 (1): 47-53

Smith, D.M. (1987) Functional and biochemical changes in deboned turkey due to frozen storage and lipid oxidation. J. Food Sci. 52: 22-27

Snedecor, G.W. and Cochran, W.J. (1968) Statistical Methods, 6th edn., 258 p, Oxford and IBH Co., New Delhi

Suvanich, V., Jahncke, M.L. and Marshall, D.L. (2000) Changes in selected chemical quality characteristics of channel cat fish frame mince during chill and frozen storage. J. Food Sci. 65 (1): 24-29

Suzuki, T. (1981) Fish and Krill Protein Processing Technology, 260 p, Applied Science Publishers Ltd., London

Sych, J., Lacroix, C., Adambounou, L.T. and Castaigne, T. (1990) Cryoprotective effects of some materials on cod-surimi proteins during frozen storage. J. Food Sci. 55 (5): 1222-1227

Tejada, M., Careche, M., Torrejon, P., Del-mazo, M.L., Solas, M.T., Garcia, M.L. and Barba, C. (1996) Protein extracts and aggregates forming in minced cod (*Gadus morhua*) during frozen storage. J. Agric. Food Chem. 44 (10): 3308-3314

Undeland, I., Richards, M. P. and Hultin, H. O. (2002) Added triacylglycerols do not hasten hemoglobin-mediated lipid oxidation in washed minced cod muscle. J. Agric. Food Chem. 50 (23): 6847-6853

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