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Effect of Cryoprotectants on the Functional Properties of Proteins from Tilapia (*Oreochromis mossambicus)* during Frozen Storage

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Abstract

Tilapia *(Oreochromis mossambicus)* whole fish (dressed), washed meat and washed meat with cryoprotectants were prepared, frozen and stored at -20°C. The stored samples were drawn at 30 day intervals up to 360 days and samples were analyzed for various functional properties of proteins. Gel strength (g cm) decreased gradually for all samples during storage but washed meat showed drastic reduction from initial values of 431.46 to 125.66 g cm. This could be due to aggregation or denaturation of proteins, which lowered the gel forming ability of the meat during the storage period. The effect of freezing on the solubility was to the tune of 25-40% in different samples. The rate of decrease in protein solubility was higher in all the samples immediately after freezing and was minimum in dressed tilapia, where it decreased from 86.46 to 34.94% of total proteins. Free sulphydral group content increased immediately after freezing in all the three samples and subsequently declined, except for the meat with cryoprotectants, where the free sulphydral value remained constant throughout the storage period. Overall, the cryoprotectants were found to be less effective in minimizing deteriorative changes in tilapia meat during frozen storage.

Keywords: Washed fish meat, functional properties, gel strength, solubility, -SH groups

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Introduction

There is an increasing global trend towards sustainable manufacture of surimi by using under-utilized and low commercial value fish species as raw material, alternative to the conventionally used white-fleshed species (Martín Sánchez et al., 2009). Mozambique tilapia (*Oreochromis mossambicus*) which is abundant in inland water bodies of the Indian subcontinent has desirable characteristics to be a potential surimi species (Hall, 2011). To fully harness the potential of this species in surimi manufacture, it is essential to explore functional properties of the muscle proteins as well as their stabilization during frozen storage (Carvajal et al., 2005).

Changes in functional properties of muscle proteins of fish during long term frozen storage have been studied by many workers. Significant reduction in salt extractable protein and apparent viscosity, but increase in water binding and emulsifying capacity has been reported for ray fish (*Raja clavata*) after two years of frozen storage at -18°C (Pastoriza et al., 1994). In earlier works of Iwata et al. (1968), it was observed that the gel-forming ability of surimi made from fresh (1-2 days old) fish such as Alaska pollack, does not change significantly up to one year when held at constant temperature below -20°C. However, when the surimi is stored at -10°C, the gel forming ability gradually decreased and became unsuitable after three months, which is attributed to decrease in extractable actomyosin (Iwata et al., 1974). Similarly, frozen storage induced reduction in gel forming ability of myofibrillar proteins in red hake, amber fish, mackerel and hoki (Jiang et al., 1985; Macdonald et al., 1992). Loss of gel forming ability in frozen

surimi is attributed to denaturation and aggregation of myofibrillar proteins (Suzuki, 1981). An association between loss of solubility and the decrease in –SH reactive groups during frozen storage has been reported for myosin (Chen et al., 1989) and actomyosin (Jiang et al., 1988). Total –SH groups decreased continuously during 15 days frozen storage of fish myosin to reach 33% of the initial value, confirming the importance of disulfide bonds in freeze induced aggregation of fish myosin in solution (Ramirez et al., 2000). In order to minimize the frozen-storage induced denaturation of muscle proteins, blending with cryoprotectants such as sucrose, sorbitol, phosphates or newer forms of carbohydrate polymers has been suggested (Park & Lin, 2005).

The present investigation was taken up to study changes in functional properties of proteins from tilapia meat during freezing and frozen storage that influence suitability of this species in surimi production.

Materials and Methods

Fresh tilapia (*Oreochromis mossambicus*) specimens of length 21-28.5 cm weighting 150-340 g harvested from natural freshwater body near Mysore was used for the study. Immediately after harvest, the fish samples were washed and iced in 1:1 (fish : ice) ratio and transported to the laboratory. Head and entrails were removed manually and washed with chilled water (3°C). After washing, the meat was separated using reciprocatory type of meat separating machine (Toyo Seikan Kaisha Ltd., Tokyo, Japan). Meat was washed with chilled tap water at 1:3 (meat : water) ratio and the slurry was agitated for five min and allowed to settle. Water was decanted and filtered through double layered muslin cloth. Meat was gently squeezed to remove excess water and washing process was repeated three times. At the time of third wash, sodium chloride at a concentration of 0.01% (w/w) of meat was added. After the final wash, excess water was removed by using basket centrifuge (Remi, India). One batch of washed meat and one batch of washed meat with 4% each of sorbitol & sucrose and 0.2% polyphosphate was made and packed in polythene bags. Along with these two categories, samples of dressed-tilapia was also packed individually and all the three categories were frozen at -35°C and stored at -20°C. The frozen stored samples were drawn at 30 days interval and analyzed in triplicates. Solubility of protein in high ionic strength buffer was estimated using the extraction buffer (EB; phosphate buffer, 50 mM; pH 7.5, containing 1M NaCl). The meat:buffer ratio used was 1:10. Meat was homogenized using an Ultra-Turrax homogenizer (Ultra-Turrax, T25, Janke & Kunkel GMBH & Co., Staufen, Germany) at 9000 rpm for 2 min. The homogenate was centrifuged at 9000 x g for 15 min at 4°C using a refrigerated centrifuge (Intl. equipment Co., IEC, B22, Needham Heights, Mass., U.S.A.). The total nitrogen content of the clear supernatant was determined by the Kjeldahl method (Hungerford, 1995). The nitrogen value obtained was multiplied by a factor of 6.25 to obtain the protein content and expressed as a percentage of total protein. Gel was prepared by stuffing into krehalon casings, exposing it to 90°C for 45 min and maintaining it in chilled condition overnight. The strength of the prepared gel was measured using Okada gellometer as per Okada & Yamazaki (1957). A 25 mm thick piece of gel was placed under the plunger of gellometer. Pressure on the gel piece was applied by continuous running water collected into a graduated beaker placed over the plunger. The flow rate of water was adjusted manually to a constant volume (~ 620 – 650 ml) per min by repeated trials. Movement of stylus on the kymograph was recorded and the gel strength was measured by calculating the area under the graph. The strength of the gel was calculated as:

$$G.S. = \frac{1}{2} \times F \times A \times B, \quad \text{where}$$

G.S = gel strength (g.cm)

$$F = \frac{\text{Volume of water rundown in unit time (ml)}}{\text{Distance moved by drum in unit time (cm)}}$$

A = Length of the base of triangle of the kymograph in cm.

B = Length of the height of the triangle of kymograph in cm.

Mean of three or five measurements was reported as gel strength value. Sulphydral group content in the tilapia meat was estimated by the method of Ellman's (1959).

Results and Discussion

Solubility of total proteins as a function of frozen storage period showed significant reduction in all the samples (Fig. 1). The effect of freezing *per se* on the solubility was to the tune of 25-40% in different

samples. Similar reports of reduction in protein solubility is reported in the case of Volador (*Illex coindetii*) during frozen storage at -20°C for 16 months (Ruiz-Capillas et al., 2002). There was no marked difference in total solubility as a function of frozen storage period between washed meat and the meat with cryoprotectants at the end of 300 days of storage. Washed meat had a higher solubility (38.32%) in comparison to meat washed with cryoprotectants, which had the solubility of 26.09% of total proteins. Although the available literature suggests that added cryoprotectants increase the solubility by reducing denaturation, the combination of sucrose and sorbitol at 4% level (w/w) is not effective in achieving the same effects in tilapia. In fact, literature on the effect of cryoprotectants on solubility of proteins indicates higher solubilization during different periods of frozen storage (Sultanbawa & Li-Chan, 1998; Miyura et al., 1992; Park, 1994; Park et al., 1988; Portious & Wood, 1983; Sych et al., 1990, 1991). The reduction in protein solubility is mainly due to aggregation / denaturation caused by freezing out of water from the system. Deterioration of fish protein during frozen storage is reflected mainly by drastic decrease in solubility (Sikorski et al., 1976). Anderson & Ravesai (1970) found that amount of readily extractable myofibrillar proteins in cod stored for 32 weeks at -12°C decreased by about 40% of its original value. Sarma et al. (2000) reported decrease in protein solubility of dressed thread fin bream from 67.77 to 59.09% during frozen storage at -18°C for 12 weeks. In the present study, meat from whole (dressed) tilapia has higher solubility than meat with cryoprotectants throughout storage period. Jeyakumari et al. (2006) reported a similar trend of results in the quality of frozen stored gelatinized product made from pink perch. The surface area of washed meat is higher than that of whole fish meat and it is likely that all deteriorative reactions took place at a much faster rate. It was expected that the addition of cryoprotectants could minimize such deteriorative reaction leading to higher solubility during frozen storage.

Gel forming ability is an important functional property, and the quality of surimi is mainly judged by its ability to form a good gel. In this study, washed meat and meat with cryoprotectants had less gel forming ability than whole fish (Fig. 2). This is mainly due to higher moisture content in the sample. Concomitant reductions in gel strength of all the frozen tilapia samples were recorded and highest reduction was observed in washed meat. Reduction in gel forming ability during frozen storage as a manifestation of the changes in protein structure and extent of denaturation is indicated by the total protein solubility and free -SH content of samples during different periods of storage (Simpson et al., 1994). Gradual loss of gel forming ability of frozen sardine mince (*Sardina pilchardus*) has been reported during 150 days frozen storage (Montero et al., 1996). Studies on the effect of freeze denaturation of protein from herring surimi (Chan et al., 1995) and Alaska pollack surimi (Numakura et al., 1989) on the final gel quality revealed similar

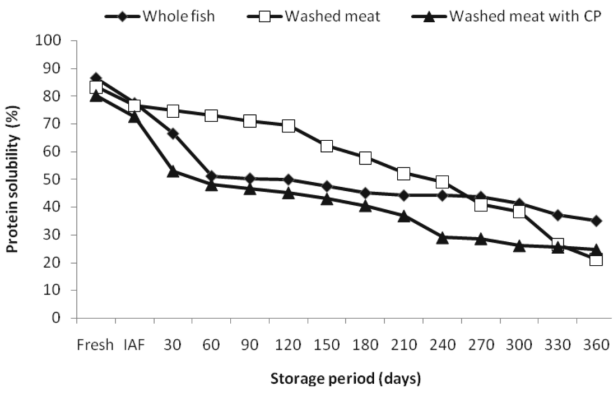

Fig. 1. Changes in total protein solubility* (%) of tilapia during frozen storage
* mean ± sd, n = 3, IAF: immediately after freezing, CP: cryoprotectant

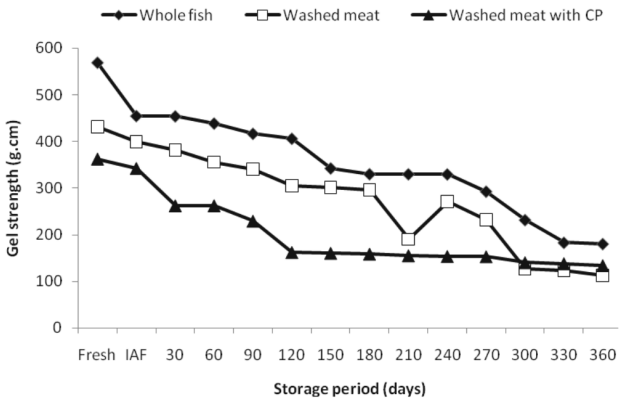

Fig. 2. Changes in gel strength* (g cm) of tilapia meat during frozen storage
* mean ± sd, n = 3, IAF: immediately after freezing, CP: cryoprotectant

observation. Benjakul et al. (2003) observed that the degradation of myosin results in an inferior gel network formation, causing a lower elasticity with poor water holding capacity in the gel matrix. Gel strength data in the present investigation demonstrate that gels of acceptable quality could be prepared from washed meat with cryoprotectants during frozen storage up to 180 days. However, it should be noted that the gel forming ability of tilapia meat is inferior to that of common carp, pink perch and shark meat (Arekere, 1993; Ratnakumar & Shamasundar, 1998; Sijo et al., 2002). The inherent ability to form good gel mainly depends on native protein conformations, amino acid composition and the sequence of amino acids (Kinsella, 1982).

Free sulphydral (mM-SH g^{-1}) content of frozen tilapia samples as a function of frozen storage period is given in Fig. 3. Increase in free sulphydral content immediately after freezing was evident in all the three samples. Washed meat showed an increasing trend up to 90 days of frozen storage after which a gradual decrease was observed. Free sulphydral content of whole fish and meat with cryoprotectants indicated almost similar pattern up to 180 days of storage. After 180 days of frozen storage, a decrease in free sulphydral content of whole fish and washed meat was observed when compared to the meat with cryoprotectants, which clearly indicated formation of disulfide bonds. Changes in sulphydral groups are an indication of either the formation of disulfide bond or fresh exposure of reactive sulphydral groups to the bulk solvent from the interior of the protein molecule. Among various myofibrillar proteins, myosin has 40-45 and actin has 5-8 free -SH residues (Jiang et al., 1988). Chen et al. (1989) reported an initial increase in free -SH content at -20°C in milk fish myosin, which decreased in the later part of the storage period. Similar results were also reported by Jiang et al. (1989) on the formation of disulfide bonds in actomyosin from tilapia. Mohan et al. (2006) reported decrease in -SH groups of Rohu during 11 days of ice storage. In the present investigation, it is evident that washed meat with cryoprotectants could effectively retard the formation of disulfide bonds as evidenced by its constant free sulphydral content throughout the storage period. The washed meat showed a reduction in free sulphydral content after 180 days of storage indicating deteriorative changes. The study demonstrated that tilapia had moderate gel forming ability as revealed by large strain test, solubility and formation of disulfide bonds. It was expected that the addition of cryoprotectants could minimize such deteriorative reaction leading to higher solubility during frozen storage. The present study clearly indicated that sucrose and sorbitol at 4% (w/w) concentration were not effective in minimizing such deteriorative reactions in tilapia. Hence, further studies are needed to standardize the concentration of cryoprotectants suitable for manufacture of tilapia surimi.

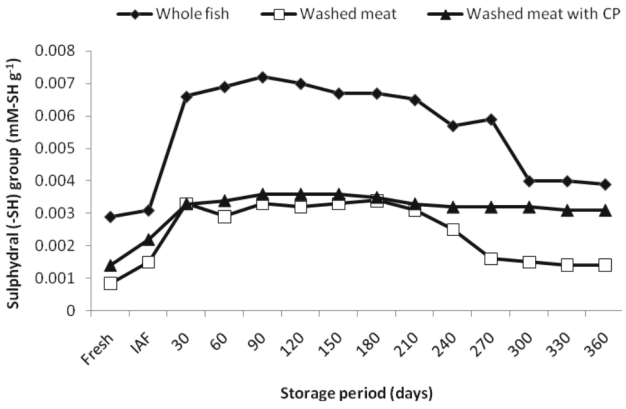

Fig. 3. Changes in sulfhydryl group* (-SH) of Tilapia during frozen storage
* mean ± sd, n = 3, IAF: immediately after freezing, CP: cryoprotectant

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