Effect of Temperature on Thiol Group of Myofibrillar Protein from Common Carp

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The free sulfhydryl groups present in proteins determine the structural and functional properties of seafood. Thermal processing affects the structural and functional properties of protein. This necessitates the requirement of optimum level of thermal treatment in the processing of seafood. In the present work, an attempt has been made to study the effect of temperature (10°-70°C) on functional properties of myofibrillar proteins extracted from common carp (*Cyprinus carpio*) with respect to the exposure of sulfhydryl groups to the surface. Maximum sulfhydryl content was noticed between 30° and 40°C.When the temperature increased or decreased from this range, a decrease in sulfhydryl content was noticed. A marked decrease in sulfhydryl content was observed when temperature increased from 50° to 60°C.These may be attributed to the formation or breakdown of disulfide bonds. Though the formation of disulfide bonds contributes positively to the gel strength and elasticity of protein foods, it also indirectly causes nutritional loss by depleting the free sulfhydryl groups.

Key words : Myofibrillar proteins, sulfhydryl groups, disulfide bond, thermal denaturation

Sulfhydryl groups are usually considered to be the most reactive functional groups in proteins (Wallenfels & Streffer, 1964), but under certain conditions the reactivity of these groups can be more or less inhibited. Sulfhydryl groups and disulfide bonds are important in maintaining structure and functions of native proteins and play important roles in functional properties of proteinaceous foods (Opstvedt *et al.*, 1984).

Fish muscle myosin contains in the range 29-49 moles SH/$5x10^5$g protein (Suzuki, 1981). The sulfhydryl group containing aminoacids, cysteine and cystine in proteins, determine the nutritive value of foods although they do not belong to the group of essential amino acids. A deficiency of "total cystine" (sum of cysteine and cystine) in nutrition increases the requirement of one of the essential amino acids, methionine, which can be metabolized to cysteine. The methionine content of food proteins also limits their nutritive

value. Any destruction of cysteine or cystine during the treatment of foods denotes a detrimental effect on nutritive value (Hofmann & Hamm, 1978). Normally thermal processing leads to loss of nutritional value due to changes in the structural and functional properties of protein. However, Opstved *et al.* (1984) have reported that on heating at 95°C for 20 min or drying at 145°C, the disulfide bonds increased whereas the digestibility of amino acids and protein decreased only marginally. Disulfide cross-linking in proteins during protein denaturation occur due to oxidation of sulfhydryl groups and sulfhydryl - disulfide interchange (Cecil & Mcphee, 1959) and the similar finding has been reported during thermal processing of fish (Opstvedt *et al.*, 1984). In carp actomyosin, the reactive -SH, which appears on the molecular surface by heating, contributes to gel formation through some bonding between the protein molecules (Itoh *et al.*, 1979a). Earlier reports indicate that the sulfhydryl groups are mainly involved in the gel formation of carp actomyosin on heating at 40° and 80°C (Itoh *et al.*, 1979b, 1980).

In the present study, the effect of temperature on sulfhydryl groups of actomyosin has been analysed with respect to the changes in solubility and thiol groups as a result of thermal denaturation.

Materials and Methods

Common carp (*Cyprinus carpio*) was collected from a freshwater farm in absolutely fresh condition, iced and kept chilled overnight for resolution of rigor. The post rigor fish was thoroughly washed to remove blood, slime, dirt etc, and skin-free fillets were made, minced and taken for protein extraction. The temperature of the fish and the mince were maintained below 5°C throughout the experiment.

Actomyosin (AM) was extracted according to the procedure of Jiang *et al.* (1987). 5 g portion of meat was homogenised using a Polytron homogeniser for 1 min with 45 ml of chilled buffer solution (0.6M NaCl; pH 7.2) The extract was centrifuged at 10,000-x g, 4°C for 20 min. The supernatant was diluted with 2 vol. of chilled distilled water. The precipitated actomyosin was separated by centrifugation at 10,000-x g, 4°C for 20 min was then suspended in chilled 0.6 M NaCl, pH 7.2, an ionic strength almost equivalent to that of fish muscle.

The actomyosin was diluted to a concentration of 3 $mg.ml^{-1}$. 10 ml portions of the actomyosin solution was pipetted out into a series of test

tubes and kept in ice. Test tubes were then incubated in a water bath maintained at different temperatures, viz., 10, 20, 30, 40, 45, 50, 60 and 70°C. The test tubes were removed to the ice bath once the protein solution attained the respective temperatures and then subjected to analysis.

Solubility represented by the protein content was estimated by Biuret method (Gornall *et al.*, 1949). The total reactive sulfhydryl groups in the protein samples were estimated based on the method of Sedlak & Lindsay (1968). The treated protein solutions were subjected to SDS-PAGE to understand the effect of temperature on actomyosin (Laemmli, 1970).

Results and Discussion

The soluble protein content was found to be 3.02 $mg.ml^{-1}$ at 10°C, which more or less remained constant up to 30° C and then decreased (Fig. 1). The highest rate of decrease in protein content was found in the temperature range 40-45°C (about 3.5%). Above this temperature, protein content decreased at a lower rate. The least protein content, 2.9 $mg.ml^{-1}$was observed at 70°C. This relatively low degree of insolubility observed could be due to the extremely short duration of exposure of the protein solution to different temperatures. The result showed a difference compared to that in major carps where up to 40% loss in solubility of actomyosin was noticed during almost identical treatment (Sankar, 2000). The ordinary myofibrillar proteins (not dark muscles) of sardine incubated at 35°C for 30 min, showed a 40% decrease in solubility irrespective of pH (Kamal *et al.*, 1990). The myosin rod responsible for the salt solubility of myosin got denatured very fast during thermal denaturation (Azuma & Konno, 1998). According to Zayas (1997),

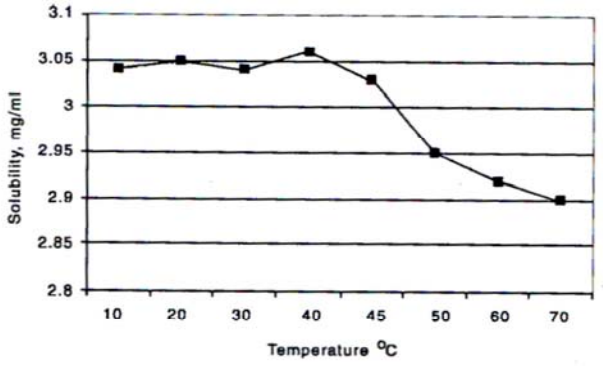

Fig. 1. Changes in the solubility of carp actomyosin during heat treatment

at temperatures of 40-50°C or greater, protein denatured with decrease in solubility, unfolding of polypeptide chains and the formation of new electrostatic hydrogen bonds. Howgate & Ahmed (1972) suggested that insolubility of protein in the muscle during heat treatment might be due to the formation of inter and intra molecular links. They found cod myofibrils quite stable at 0°C and insoluble at a fast rate at 30°C. The changes observed in salt solubility on exposure to temperature were mainly due to the changes in myosin component, since the actin remained unchanged.

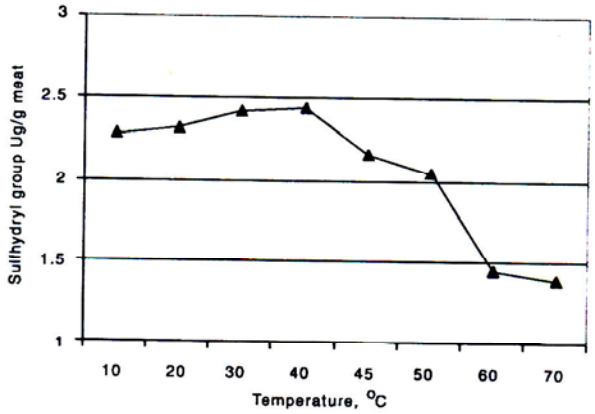

Fig. 2. Changes in SH groups in the carp actomyosin during heat treatment

The sulfhydryl group (Fig. 2) showed an increasing trend from 10 to 40°C (about 6.6% increase from the initial value). This could be due to unfolding of protein structure and exposure of sulfhydryl groups at the molecular surface. Above 40°C, the sulfhydryl content decreased (about 11.48%) followed by a sharp decline (29%) in the range 50-60°C. Almost similar result was reported in rohu (*Labeo rohita*) during heat treatment, while a loss up to 63% in SH groups were noticed in catla and mrigal (Sankar, 2000). In sardine muscle above 60-70°C, the bulk of protein-protein interaction took place through the head portion of myosin molecules by means of either disulfide bonds or hydrophobic bonds (Montero & Guillen, 1996).

The electrophorogram (Fig. 3) of the protein solution (10°C) revealed 2 high molecular weight bands - one faint and other prominent and four low molecular weight bands. Above 30°C appearance of a faint high molecular weight band was noticed. At 60°C, the prominent high molecular band started disappearing and at 70°C the band altogether disappeared. No significant variations were observed in the low molecular weight protein

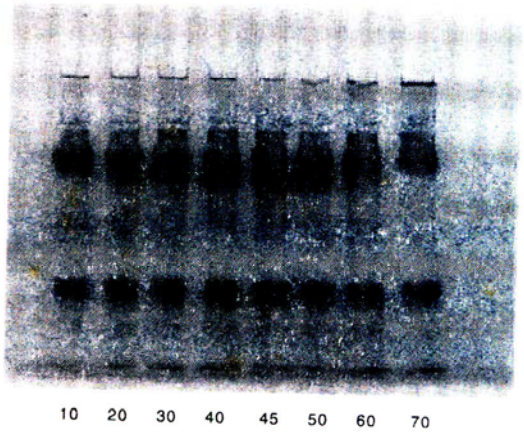

Fig. 3. Electrophoretic pattern of carp actomyosin during heat treatment

fraction, when the temperature was increased from 10°C to 70°C. Almost similar findings were reported by Sano *et al.* (1994) during the thermal denaturation of carp proteins. In the case of freshwater major carps (catla, rohu and mrigal), SDS-PAGE showed 2 intense bands corresponding to myosin heavy chain (MHC) and actin, and no insolubilisation occurred below 40°C. When temperature was increased to 45°C, the insolubilisation occurred as shown by the decrease in the intensity of MHC and actin (Sankar, 2000).

Thus, changes in the sulfhydryl groups were noticed during the thermal denaturation of carp actomyosin. However, the reduction in sulfhydryl groups did not have much effect on the functional property, *viz.*, solubility of actomyosin.

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