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Tissue Proteinase Activity in Indian Mackerel (*Rastrelliger kanagurta*) during Iced Storage

Leema Jose and M.R. Raghunath*
Biochemistry and Nutrition Division
Central Institute of Fisheries Technology
P.O. Matsyapuri, Cochin - 682 029, India

Tissue proteinase activity (TPA) during iced storage of mackerel (*Rastrelliger kanagurta*) and its effect on tissue proteins were followed by assay of proteinase activity and SDS-PAGE of tissue homogenates, respectively. TPA in mackerel is predominantly expressed at pH 3, 4, 9 and 10. The activities at pH 3 and 4 were initially higher than that at alkaline pH. TPA at the acid pH range were observed to decrease during iced storage and were always at lower than original levels, although there was a transient increase at 4-6 days of iced storage. TPA in the alkaline pH range (9 and 10) remained mostly unaffected till 6-8 days of iced storage after which, a slight decrease was observed. However, at both pH ranges, the activity increased sharply after 11 days, owing to diffusion of visceral/microbial proteinases from the belly cavity due to spoilage. Electropherogram of the muscle homogenates revealed early breakdown of high molecular weight proteins followed by lower molecular weight proteins.

Key words: Tissue proteinase, iced storage, Indian mackerel, *Rastrelliger kanagurta*

Autolysis in the living animal muscle, expressed as the tissue proteinase activity (TPA), is a highly controlled process which aids in protein turnover and metabolic regulation. However, following the death of the animal, TPA becomes unregulated and causes post-mortem tenderisation of mammalian meat. Autolysis or TPA in fish and shellfish is a major problem as it leads to textural degradation of muscle in fish (Yamashita, 1993; An *et al.*, 1992; Matsumiya *et al.*, 1990). TPA is also responsible for the 'modori' phenomenon which results in kamaboko of poor strength or ashi (Suzuki, 1981). The presence of numerous proteinases in fish tissues, active at acid, neutral and alkaline pH ranges have been well documented (Jayan *et al.*, 1998; An *et al.*, 1992 Matsumiya *et al.*, 1991; Makinodan *et al.*, 1984).

Indian mackerel *(Restrelliger kanagurta)* has been observed to undergo rapid textural degradation even when irradiated to control microbial spoilage (Lewis, 1979). The presence of TPA in mackerel has also been demonstrated previously (Jose & Raghunath, 1998; Jose *et al.*, 1998). The activity of acid proteinases in mackerel muscle and other organs has been shown to decrease during frozen storage (Pratapachandran *et al.*, 1988), while in carp muscle, acid, neutral and alkaline tissue proteinases were not observed to vary significantly during iced storage (Makinodan *et al.*, 1984). However, the fate of major tissue proteinases in Indian mackerel during iced storage and its effect on major muscle proteins have not been so far elucidated.

Materials and Methods

Indian mackerel was purchased from local markets in fresh and post-rigor condition and brought to the laboratory within an hour. After a brief wash, the fish were iced (1:1) with crushed block ice and stored in an insulated box. On alternate days, five mackerel were removed from the ice and re-icing was done if necessary. General condition of the fish was observed, the fish washed, and muscle from the

* Present address: Peninsular Aquaculture Division, Central Institute of Freshwater Aquaculture, Hessarghata lake, P.O. Bangalore-560 089, India

posterior portion of fish (without skin) was carefully excised and pooled. Pooled muscle was minced in a chilled blender and taken for analysis. Minced muscle was homogenized with 4 volumes of distilled water at 10000 rpm for 3 min in a Polytron homogenizer at 0-5°C. Two ml of homogenate was incubated with 4 ml of buffer (0.3M citrate phosphate buffer at pH 3.0 and 4.0 and 0.3M Tris-HCL buffer at pH 9.0 and 10.0) for 1h at 50°C. Reaction was terminated with 5ml TCA (final concentration, 5%) and the Folin positive material in supernates were estimated as per Heriott (1955). For blanks, the order of addition of homogenate and TCA was reversed. Activity was expressed as μmol Tyr. g muscle^{-1}.min^{-1}. Aliquots of homogenates were derivatized and subjected to SDS-PAGE as per Laemmli (1970) on 0.75 mm thick, 6.5% acrylamide gels with 5% cross-linking. A slab system (Biorad, Miniprotean) was used at a constant voltage of 120 V and the gels were stained with Comassie brilliant blue (0.1% in 50% methanol and 10% acetic acid) for 2 h and destained with 10% acetic acid.

Results and Discussion

The principal TPA in mackerel muscle was at pH 3.0 and 4.0 in the acid range and at pH 9.0 and 10.0 in the alkaline range as previously reported (Jose & Raghunath, 1998), although traces of TPA were observed at other pH (2, 5, 6, 7 and 8). The fate of TPA in mackerel tissue during iced storage is shown in Fig. 1. TPA in the acid range (pH 3.0 & 4.0) was initially at higher levels than that in alkaline range (pH 9.0 & 10.0).

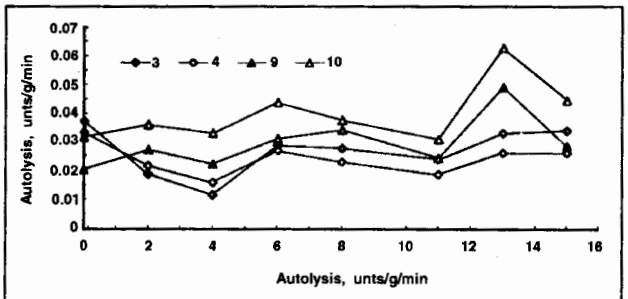

Fig. 1. Autolytic activity in Indian mackerel (*Rastrelliger kanagurta*) muscle during iced storage

However, TPA in acid range declined in the muscle as iced storage progressed, reaching 30-47% of the original levels by the 4th day. Subsequently, it increased transiently at the sixth day of iced storage, but continued to decline till the 11th day. Acid proteinase activity in the muscle and other organs of Indian mackerel during frozen storage has been generally found to decrease (Pratapchandran *et al.*, 1988). The lack of any significant variations in carp (*Cyprinus carpio*) TPA at acid, neutral and alkaline pH during iced storage observed earlier (Makinodan *et al.*, 1984) may be due to the limited iced storage period (3 days) and the different species studied.

TPA in the alkaline range of pH 9.0 and 10.0 increased in 6-8 days of iced storage (138-171%). Subsequently, the activity declined, but still remained almost equal to or slightly higher than original activity levels. Thus, TPA in the alkaline range continued to be as strong as, or even slightly higher, than the original levels during iced storage. On the other hand, TPA in the acid range were always lower than original levels during iced storage. This difference in behaviour indicates that these two TPA might arise from different parts of the muscle cell. Yamashita & Konagaya (1992) have reported that TPA can arise from the cytosolic as well as lysosomal portions of the cell.

In both the pH ranges, however, there was a sharp increase in TPA after 11 days of iced storage. This sharp increase roughly coincided with the near total disintegration of the peritoneal membrane in the ice stored fish as observed visually. The texture of the muscle had also deteriorated considerably by 6-8 days, as has been seen in earlier studies of ice stored mackerels where deterioration was observed even on the 4th day itself (Madhavan *et al.*; 1970). Hence, the sharp increase in TPA observed after 11 days of iced storage is attributable to the diffusion of digestive and microbial proteinases from visceral cavity into the fish muscle.

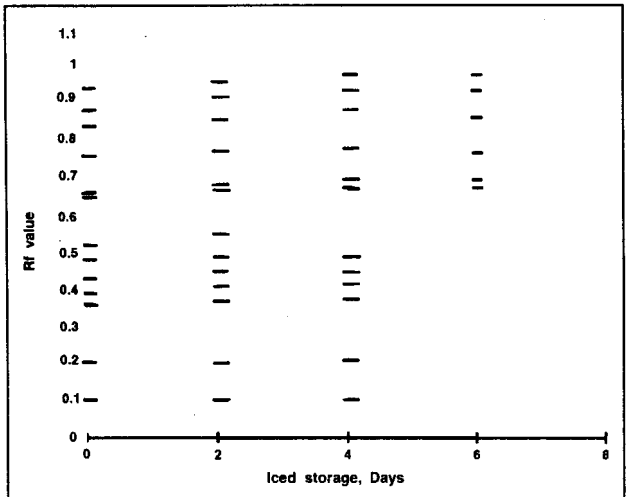

Fig. 2. Electrophoretic pattern of tissue proteins during iced storage of Indian mackerel (*Rastrelliger kanagurta*).

Changes in the electrophoretic pattern of the major protein fractions during iced storage of mackerel are shown in Fig. 2. In the fresh mackerel muscle, 13 protein bands could be observed. Although all these bands were also observed on the 2nd day of iced storage, the molecular weights of all the proteins had decreased as evidenced by the increase of Rf values. These increases were even greater on the 4th day. As the tissue proteinases hydrolyze the muscle proteins, their size is reduced and the molecular weight decreases. However, as the hydrolysis became extensive with progress in iced storage, nearly all the higher molecular weight protein bands could not be detected by 6th day. Finally on the 8th day, no protein bands, including low molecular weight ones, were visible. The hydrolysis of muscle proteins during iced storage also leads to increase in non-protein and *a*-amino nitrogen as has been observed earlier in cook-drips of mackerels canned after iced storage (Madhavan *et al.*, 1970). Such rapid modification of muscle proteins even in early stages of iced storage would indicate that ice stored mackerel is unlikely to be suitable for critical applications like gel and paste type products, where functionality of muscle proteins is essential.

Thus, TPA in ice stored mackerel decreases only in the acidic range, while the activity was higher than the original levels in the alkaline range. Extensive breakdown of major muscle proteins, which have high molecular weights, occurred by the 6th day of iced storage.

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