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Tamarind Fruit Shell Inhibits Autolysis in Mackerel and Squid Muscle

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Abstract : Tamarind *(Tamarindus indica)* is a traditional culinary additive in India whose preservative effect is generally attributed to its tartaric acid acidulant. The shell of the tamarind fruit is usually discarded. Our report shows that the shell has a powerful protease inhibitor which can retard autolysis in muscle of the Indian mackerel *(Rastrelliger kanagurta)* and Squid (*Loligo duvauceli*) which pose special problems to the fish processing technologist on account of their high rate of proteolysis and consequent early spoilage during postmortem storage. The powder of the dried fruit shells (Tamarind Fruit shell powder, TFSP) was incubated with tissue homogenates in buffered media. Tissue proteinases of mackerel and squid at acid as well as alkaline pH were inhibited to extents ranging from 41 to 100% in the presence of TFSP. The inhibitory principle in the shell powder could be extracted by buffers without losing its inhibitory activity. The inhibitory activity of extracts was only partially lost upon autoclaving the extracts indicating it was stable to some extent upon heat treatment. The broad range of inhibitory activity of tamarind fruit shell powder shows it has a preservative potential as a cheap and effective preservative for fish.

Introduction

Autolysis in tisues of animals is a controlled process which aids in protein turnover and metabolic regulation. However, following the death of the animal, the process is unregulated and is sometimes beneficial as in tenderization of mammalian meat. But it can also be a major problem as in fish and shellfish where autolytic activity in muscle can make it unsuitable for critical applications as in paste products or reduce the shelf life because of autolysis induced belly bursting. As the progress of autolysis paves the way for rapid microbial spoilage of seafood, ways to arrest such proteolytic breakdown are the basic need for extension of shelf life. Inhibitors of proteases occur widely in plants and animals as metabolic regulators and defense mechanisms. Such natural inhibitors have been used to improve the shelf life of iced fish by retarding the growth of spoilage microflora (Gowda and Karunasagar, 1985; Abraham *et al.*, 1995) or reducing proteolytic breakdown in ground fish (Aksnes, 1989). The pulp of tamarind fruit is traditionally used as a culinary additive in India and its preservative action is generally attributed to the lowering of pH due to its tartaric acid constitutent. But the shell of the fruit pods are usually discarded. In this paper we report the inhibitory properties of tamarind fruit shell on proteolytic activity in tissues of Mackerel and Squids both of which are noted for their high tissues proteolytic activity and rapid spoilage.

Materials and Methods

Mackerel (*Rastrelliger kanagurta*) and Squid (*Loligo duvauceli*) were procured in iced post-rigor condition

from retail market and landing centers respectively of Cochin. They were brought within an hour to the laboratory, eviscerated, washed thoroughly to remove any visceral contamination, skin removed and the meat extracted manually. Meat from 7-10 fish were pooled, minced in a chilled mincer, frozen and kept at - 20°C pending analysis. Tamarind fruits (*Tamarindus indica*) harvested from trees were dried in the sun (2 days) and the fruit shells carefully seperated free of any adhering pulp. The separated shells were ground finely in a mortar and stored in a desiccator pending analysis. All chemicals used were of AR grade or equivalent.

Autolytic activity determinations were done by incubating 4 ml of 0.3M buffer with 2 ml of muscle homogenate. Homogenates were prepared by homogenizing muscle with cold distilled water (muscle: water ratio, 1:4 for mackerel, 1:6 for squids) at 9000 rpm at 0-5°C for 3 min. in a Polytron homogenizer. Incubations were done at 50°C with pH 2, 3, 9 & 10 buffers for mackerel and at 40°C with pH 3 & 8 buffers for squid. Reactions were terminated after 1h by the addition of 5 ml tricholoroacetic acid (TCA, final concentration 5%). The Folin +ve material in the supernates were estimated as per Herriott (1955) and expressed as μM Tyr released $min^{-1}g^{-1}$ muscle. For blanks the order of addition of homogenate and TCA were reversed.

Inhibition studies with shell powders were carried out by adding 60mg of powder (1% of reaction volume) to buffers 10 min prior to the addition of homogenates. Aqueous extracts of shell powders were prepared by keeping 1% dispersions of powder with respective buffers at 5°C overnight and centrifuging at 5000 rpm for 10min. to get the supernates. Heat treatment of extracts were carried out by autoclaving the prepared extracts at 121°C for 30 min. Extracts were included in the reaction mixtures by replacing 1 ml of buffer with the extract. Seperate blanks were run in presence of inhibitors to compensate for coloring matter released by the powder.

Results and Discussion

The pH of assay for mackerel and squid were selected on the basis of previous trials. Figure 1 shows that the inhibitory activity of Tamarind Fruit Shell Powder (TFSP) can effectively inhibit tissue proteinases from diverse sources such as fish and shellfish. Acidic as well as alkaline proteinases of both animals were found to be inhibited when the powder was directly added to the reaction mixture. This would indicate that the inhibitory action is either of a general nature, active against a wide range of proteinases or that more than one type of inhibitor is present in the TFSP. The inhibitory action

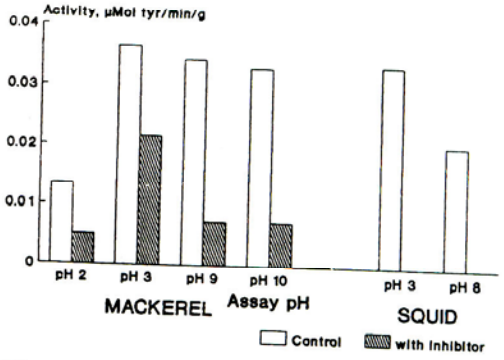

Fig. 1. The inhibition of autolysis in muscle of ***Rastrelliger kanagurta*** *and* ***Loligo duvauceli*** *by tamarind fruit shell powder assayed at different pHs.*

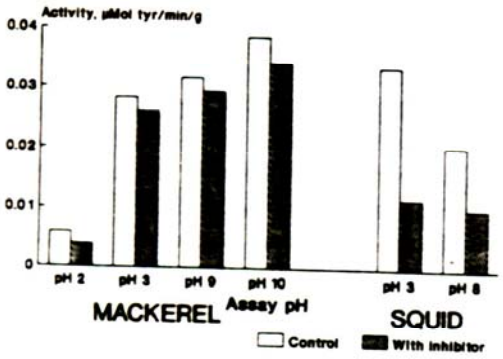

Fig. 2. The inhibition of autolysis in muscle of ***Rastrelliger kangaurta and Loligo duvauceli*** *by aqueous extracts of tamarind fruit shell powder assayed at different pHs.*

was very strong against the tissue proteinases of Squid mantle, completely arresting the proteolysis. In comparison, proteinases of mackerel muscle were only partially inhibited. Thus tissue proteinases from different sources appear to differ in their sensitivity towards inhibition by TFSP.

The soluble nature of the inhibitory compounds in TFSP is clearly

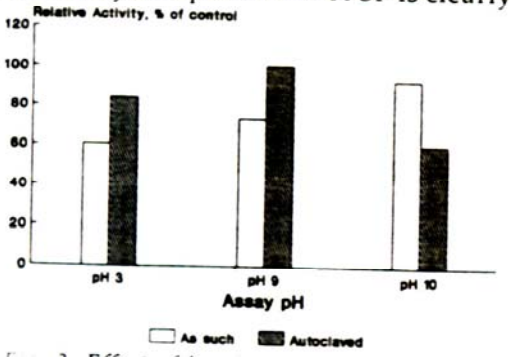

Fig. 3. Effect of heating aqueous extracts of tamarind fruit shell powder on inhibition of autolytic acitvity in muscle of ***Rastrelliger kanagurta*** *assayed at different pHs.*

seen from the results in Figure 2. The inhibitory principle was soluble in acidic as well as alkaline buffers. The difference in the intrinsic autolytic activities between the mackerel tissues in Fig. 1,2&3 is probably due to varying degrees of freshness of mackerel.

Experiments aimed at finding out whether the inhibitory activity was liable to heat did not give conclusive results (Fig. 3). When the TFSP extracts were autolaved (121°C for 30 min) and then tested for inhibition, the inhibitory activity was found to decrease at pH 3 and 9. In fact, at pH 9, inhibition appeared to be completely overcome and a little amount of activation could be observed. But at the higher pH of 10, the inhibition was found to increase as a result of heat treatment. This differential behaviour could either be due to the presence of more than one type of inhibitory principle in TFSP or the release of bound metallic ions by the pH/heat treatment. This however, needs confirmation by further testing. Potato tuber extract for example, has been shown to contain inhibitors to more than one type of proteinases (Aksnes, 1989). In any case, it is quite apparent that the inhibitory activity at pH 3 and 10 were not completely lost upon heat treatment. The inhibitory principle was also found to be resistant to oxidation as extracts of TFSP heated overnight at 150°C in an oven still retained their inhibitory activity (data not shown).

The nature of proteinease inhibitors are diverse; peptides diazoketones, heavy metals, flouorophosphates and chelators have all been shown to inhibit different types of proteineases (Walsh, 1975; Barrett, 1977). Previously, extracts from natural sources like potatoes (Aksens, 1989; Abraham *et al.*, 1995), and *Adenanthera pavonia* (Gowda and Karunasagar, 1985) with proteinase inhibitory activity have been used to enhance keeping quality of ice stored fish.

The protease inhibitors from potato in particular are known to be effective against cathepsins of trout muscle (Busse and Belitz, 1976). Our results indicate that TFSP has a preservative potential in fish with its broad range of inhibitory activity to tissue proteinases. In view of its easy availability and low cost, this observation can be commercially significant.

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