Purification and Characterization of Proteinase from Bigeye Snapper, *Priacanthus macracanthus* and *Priacanthus tayenus* Muscle

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Thesis Title	Purification and Characterization of Proteinase from Bigeye Snapper, *Priacanthus macracanthus* and *Priacanthus tayenus* Muscle.
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Abstract

Proteolysis of muscles from two species of bigeye snapper was studied. Autolysis of mince and washed mince at 50 and 60 °C was compared. A higher degradation of myosin heavy chain (MHC) was observed in both mince and washed mince from *P. macracanthus*, compared to that of *P. tayenus*, especially when incubation time increased. Mince from both species showed a higher degradation than washed mince, indicating a higher proteolytic activity in mince. The result suggested that *P. macracanthus* muscle had a higher activity of either sarcoplasmic or myofibril-associated proteinases. Myofibril-associated proteinases in both species were inhibited by soybean trypsin inhibitor, suggesting that those proteinases were serine proteinases. When sarcoplasmic proteinases in *P. macracanthus* muscle were characterized by using casein-TCA-Lowry assay, two activity peaks with an optimum temperature of 60 °C were observed at pHs of 6.5 and 8.5. Activity of 70-80% was inhibited by soybean trypsin inhibitor, suggesting that the major proteinase belonged to serine protienase. For *P. tayenus* sarcoplasmic proteianse, two activity peaks with an optimum temperature of 60 °C were found at pHs of 5.0 and 8.5. The first peak activity of 75% was inhibited by pepstatin A, while another peak activity was inhibited by various inhibitors including E-64, soybean trypsin inhibitor, lactacystin, EDTA, etc. The result indicated that different sarcoplasmic proteinases were present in *P. tayenus*

muscle. Therefore, *P. macracanthus* muscle generally had the higher proteolytic activity, compared to *P. tayenus* muscle.

Sarcoplasmic proteinase was purified from *P. macracanthus* ordinary muscle by a heat treatment and a series of chromatographies on phenyl-Sepharose 6 fast flow, Source 15Q and Superose 12 HR 10/30. It was purified to 5,180 folds with a yield of 0.8%. The molecular weight of purified proteinase was estimated to be 72 kDa by Superose 12 HR 10/30 gel filtration. On non-reducing SDS-substrate gel, this proteinase appeared as two proteinase activity bands with a molecular weights of 66 and 13.7 kDa. Accordingly, it was found to consist of two different subunits. The optimum pH and temperature for the hydrolysis of casein were 8.0-8.5 and 60^{o}C, respectively. The proteolytic activity was strongly inhibited by soybean trypsin inhibitor (82.7%) and partially inhibited by EDTA, while pepstatin A and E-64 showed no inhibition. Purified proteinase hydrolyzed Boc-Phe-Ser-Arg-MCA, but slowly hydrolyzed Z-Phe-Arg-MCA and Z-Arg-Arg-MCA. In addition, it mainly degraded myosin heavy chain, not actin. These results suggest that purified proteinase was heat activated serine proteinase, which was probably involved in gel weakening of bigeye snapper surimi.