Partial purification and characterization of trypsin-like proteinases in Indian anchovy (*Stolephorus* spp.)

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Abstract

Four fractions (P111, P21, P31, and P4) of proteinases were obtained from various purification steps including heat treatment (60 °C, 10 min), 30–60% ammonium sulfate precipitation, anion exchange, hydrophobic interaction, and gel filtration chromatography. Optimal temperature and pH of all fractions were 50–60 °C and 8.5, respectively. All partially purified proteinases preferably hydrolyzed substrates containing Arg at the P_1 position. All proteinases were inhibited by soybean trypsin inhibitor, leupeptin, and *N*-tosyl-L-lysine chloromethyl ketone. Partially purified proteinases were stable at 35 °C up to 12 h. However, their activity decreased about 40% when incubated at the optimal temperature (50–55 °C) for 2 h. Only P111 was stable at its optimal temperature (60 °C) up to 12 h. Molecular weight (MW) of P111, P21, and P31 was estimated to be 27, 33, 37, 43, 48, 55, 60, and 65 kDa, while MW of P4 was 39 kDa based on activity staining. All partially purified proteinases hydrolyzed washed anchovy mince at 4.0 M NaCl, pH 8.5, at 35 °C and at their optimal temperatures (50–60 °C).

Keywords: Indian anchovy (*Stolephorus* spp.); Trypsin-like proteinase; Partial purification; Characterization