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, 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).

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1. Introduction

Indian anchovy (Stolephorus spp.) is a pelagic small fish containing high proteinase activity and are popularly used as a raw material for fish sauce production in Southeast Asia. Fish sauce is a traditional condiment prepared by mixing anchovies with 25–30% salt and fermenting at ambient temperature (30–40 °C) for about 12–18 months. Protein solubilization slowly occurs during fermentation by the action of proteolytic activity to produce amino acids and small peptides. Proteins involved in muscle degradation have been studied. Martinez and Gildberg (1988) reported that proteolytic enzymes from the digestive tract of anchovies (Engraulis encrasicholus) degraded abdominal tissue. Heu, Pyeun, Kim, and Godber (1991) reported that two alkaline proteinases isolated from viscera of anchovy (E. japonica) were identified to be chymotrypsin-like serine proteinases. Two trypsin-like enzymes isolated from the digestive tract of anchovy were important for muscle degradation (Martinez, Olsen, & Serra, 1988). Two neutral serine proteinases were purified from salted anchovy (E. japonica) (Ishida, Sugiyama, Sato, & Nagayama, 1995). However, proteinases from Indian anchovy have never been purified and characterized.

Trypsin-like proteinase activity was observed in the first 2 months of the Philippino fish sauce (patis) fermentation (Orejana & Liston, 1982). In addition, trypsin-like proteinase activities were predominantly found in Indian anchovy (Stolephorus spp.). These enzymes hydrolyzed muscle proteins at high salt content (4.0 M NaCl) (Siringan, Raksakulthai, & Yongsawatdigul, 2006a). Thus, these proteinases might play an important role in protein hydrolysis during fish sauce fermentation. Furthermore, activities of these enzymes were detected in fish sauce throughout 12 months of fish sauce fermentation (Siringan, Raksakulthai, 0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved. doi:10.1016/j.foodchem.2005.11.049