ABSTRACT

Mutia Intan Trista Putri, 24020222140085, ***Characterization of Protease Enzymes Produced by Yeast Isolates AH. 6.5 from Mackerel (Rastrelliger sp.)*** (under the guidance of Endang Kusdiyantini and Sri Pujiyanto).

Salted fermented Indian mackerel (*Rastrelliger* sp.) is a traditional fermented fish product involving the activity of various microorganisms, including yeasts that have the potential to produce protease enzymes. Protease enzymes play an important role in hydrolyzing proteins into peptides and amino acids, which contribute to the development of flavor and quality in fermented products. This study aimed to analyze the optimum activity conditions of the protease enzyme produced by yeast isolate AH.6.5 obtained from salted fermented Indian mackerel (*Rastrelliger* sp.) based on variations in pH, temperature, and enzyme reaction incubation time, as well as to evaluate the significance of the effects of these factors on protease activity. The yeast isolate was rejuvenated on PDA + NaCl medium and cultured in Malt Yeast Glucose Peptone (MYGP) medium to obtain crude enzyme extracts. Protease activity was determined using casein as a substrate through the Folin–Ciocalteu method and a tyrosine standard curve. The pH levels tested were 5, 7, and 9; temperature variations were 30°C, 40°C, and 50°C; and enzyme reaction incubation times were 15, 30, and 60 minutes. Data were analyzed using One-Way ANOVA followed by Duncan's multiple range test at a 95% confidence level. The results showed that the highest protease activity among the pH treatments was observed at pH 5, with an average activity of 5.5556 U/mL. For temperature variation, the highest activity was obtained at 30°C, with an average activity of 5.5556 U/mL. The highest activity for incubation time variation was recorded at 30 minutes, reaching 7.2223 U/mL. Statistical analysis indicated that variations in pH, temperature, and enzyme reaction incubation time had a significant effect on protease activity ($p < 0.001$). Based on these findings, the protease enzyme produced by yeast isolate AH.6.5 can be classified as an acidic protease with optimum activity at pH 5, mesophilic characteristics with an optimum temperature of 30°C, and an optimum reaction incubation time of 30 minutes. These results demonstrate the potential of yeast isolate AH.6.5 as a source of protease enzymes for the development of fermented food biotechnology.

Keywords: *Yeast, Peda saltfish, Rastrelliger sp., Protease*