

ABSTRACT

Aurora Fara Nabila Shifa Khoirunnisa. 24020122140169. **Protease Enzyme Activity during Fermentation of Black Soybean Tempeh (*Glycine max* (L.) Merrill).** Under the guidance of Arina Tri Lunggani and Sri Pujiyanto.

Tempeh is a fermented soybean-based food product produced through the activity of *Rhizopus* sp. mold, which generates hydrolytic enzymes including proteases that break down proteins into peptides and amino acids. Protease activity during fermentation is dynamic and influenced by the duration of fermentation. This study aims to analyze the dynamics of protease enzyme activity during fermentation, determine the effect of varying fermentation times on protease enzyme activity, and identify the optimal fermentation time for black soybean tempeh (*Glycine max* (L.) Merrill). This study employed a laboratory experimental design using a one factor completely randomized design (CRD), with fermentation times of 0, 12, 24, 36, 48, 60, and 72 hours, each with three replicates. Protease enzyme activity was analyzed using the Anson method with casein as the substrate, while soluble protein content was determined using the Lowry method. Absorbance measurements were performed using a UV-Vis spectrophotometer based on standard curves of L-tyrosine and Bovine Serum Albumin (BSA). Data were analyzed using the Shapiro–Wilk normality test, Levene’s homogeneity test, One-Way Analysis of Variance (ANOVA), and Tukey HSD post-hoc test at a 5% significance level. The results showed that variations in fermentation time had a significant effect on protease enzyme activity ($p < 0.05$). Protease enzyme activity increased gradually until reaching a maximum value of 0.23167 U/mL at 48 hours of fermentation, then decreased at 60 and 72 hours of fermentation. Soluble protein levels showed a similar pattern of change, with the highest value of 587.75 $\mu\text{g/mL}$ observed at 48 hours of fermentation. Thus, 48 hours of fermentation is the optimal duration for producing the highest protease enzyme activity in black soybean tempeh. This information can be used as a reference for improving the nutritional quality and overall quality of tempeh products.

Keywords: black soybean tempeh, fermentation, protease enzyme, fermentation time, *Rhizopus* sp.