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The Effect of Papain Enzyme Concentration on Protein Content and Antioxidant Activity of Skipjack Tuna (*Katsuwonus pelamis*) Protein Hydrolysate

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ABSTRACT

Protein deficiency is one of the most common nutritional problems found in Indonesia. Skipjack tuna is a source of high-quality protein; however, it is highly perishable and therefore requires processing to extend its shelf life. One alternative processing method is the production of protein hydrolysates, which also possess antioxidant activity; nevertheless, optimization of enzyme concentration is required to obtain protein hydrolysates with optimal characteristics. This study aims to analyze the effect of variations in papain enzyme concentration on protein content and antioxidant activity of skipjack tuna protein hydrolysates, where the hydrolysis process was carried out using papain enzyme concentrations of 4%, 5%, and 6% at a temperature of 55°C for 2 hours, and the research data were statistically analyzed using analysis of variance (ANOVA) to determine differences among treatments. Protein content was determined using the Kjeldahl method, while antioxidant activity was analyzed using the DPPH free radical scavenging assay. The results of protein content analysis showed the best value at an enzyme concentration of 6% at 1.22%, and the results of the antioxidant activity test showed the highest value of 66.04% at an enzyme concentration of 6%; in conclusion, an increase in enzyme concentration affects the increase in protein content and antioxidant activity of the hydrolysate, and these findings indicate that optimizing papain enzyme concentration is important in producing skipjack tuna protein hydrolysates with better functional characteristics, thus having the potential to be developed as a functional food ingredient.

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INTRODUCTION

The availability of protein-rich food remains a significant issue in Indonesia. In 2022, data from the World Health Organization (WHO) indicated that approximately 45 million children under five years of age were affected by malnutrition, and the results of the Indonesian Nutritional Status Survey (SSGI) reported that the prevalence of stunting reached 21.6% [1]. Protein deficiency is one of the most commonly encountered nutritional problems in Indonesia. This nutrient plays a crucial role as it functions as a building and regulatory substance, and also acts as a receptor, enzyme, antibody, and tissue-forming component, among others [2].

Fish is a readily available source of animal protein in Indonesia, with a protein content ranging from 20–30% [3]. One type of fish with high nutritional value is skipjack tuna (*Katsuwonus pelamis*), which contains approximately 20.15% protein and is rich in omega-3 fatty acids [4]. With these characteristics, skipjack tuna has strong potential as an alternative source of animal protein.

Skipjack tuna is a leading fishery commodity in North Sulawesi Province with high economic value. However, it is highly perishable after being caught, which can lead to economic losses, especially during periods of abundant harvest [5]. Therefore, processing efforts through product diversification are necessary to increase added value and extend shelf life. Its high protein content makes skipjack tuna a promising raw material for the production of protein hydrolysates.

Fish protein hydrolysate is a product derived from fish through the cleavage of peptide bonds into free amino acids and low-molecular-weight peptide compounds via hydrolysis processes using acids, bases, or enzymes [6–8]. Enzymatic hydrolysis is more commonly preferred because the resulting protein hydrolysates exhibit better nutritional properties. The enzymes used in protein hydrolysis are proteases [9]. Protease enzymes can be obtained from plants, such as papaya, from which the enzyme papain is derived [10]. Protein hydrolysates are known to provide various benefits, including enhanced flavor, improved digestibility, and antioxidant activity, making them potential sources of natural antioxidants [11–13].

Previous research conducted by Nofiandi et al. (2020) [14] showed that enzyme concentration influences the protein content of the resulting hydrolysate. The highest protein content was obtained at an enzyme concentration of 4%, reaching 2.48%, but decreased at a concentration of 6% to 2.33%. This finding indicates that increasing enzyme concentration does not always correspond to an increase in protein content, highlighting the need to optimize enzyme concentration to obtain maximum protein hydrolysate yield.

Research on protein hydrolysates derived from skipjack tuna remains relatively limited, particularly in analyzing the effect of enzyme concentration variations on protein content and antioxidant activity. Therefore, this study aims to analyze the relationship between enzyme concentration, protein content, and antioxidant activity of protein hydrolysates derived from skipjack tuna (*Katsuwonus pelamis*).

RESEARCH METHODS

Materials and Equipment

The equipment used in this study included an analytical balance, heating bath, incubator, supporting glassware, filter paper, micropipettes, vortex mixer, titration apparatus, Kjeldahl digester, Kjel-line, and a UV–Vis spectrophotometer. The materials used in this study were skipjack tuna (*Katsuwonus pelamis*) meat obtained from the traditional market in Tondano, distilled water, papain enzyme, 2,2-diphenyl-1-picrylhydrazyl (Sigma-Aldrich), 23% NaOH, 0.2 N HCl, 95% ethanol, concentrated H_2SO_4, 4% H_3BO_3, Bromocresol Green–Methyl Red indicator, and Kjeldahl catalyst tablets.

Procedure for Preparing Fish Protein Hydrolysate [15]

The fish meat was minced and mixed with water at a ratio of 1:4 and papain enzyme at various concentrations (4%, 5%, and 6%) at pH 7. The mixture was then subjected to hydrolysis at 55 °C using a temperature-controlled water bath. Subsequently, the enzyme was inactivated at 90 °C for 20 minutes, and the mixture was filtered using filter paper to obtain a solution in the form of fish protein hydrolysate.

Procedure for Protein Content Analysis [16]

A total of 0.5 g of protein hydrolysate sample was placed into a Kjeldahl tube. Then, two Kjeldahl catalyst tablets and 20 mL of concentrated H_2SO_4 were added. The mixture was heated for 90 minutes at 500 °C until the solution became clear, after which it was allowed to cool.

The digested solution was then diluted with 80 mL of distilled water and 90 mL of NaOH, followed by distillation for 5 minutes. The distillate was collected in an Erlenmeyer flask containing 25 mL of 4% H_3BO_3 and 2 drops of BCG-MR indicator.

The distillate was titrated with 0.2 N HCl until a light blue color was obtained, and the volume of HCl used was recorded. The same procedure was performed without the sample as a blank.

The protein content was calculated using Equation (1):

$$Protein\ (\%) = \frac{(V1 - V0) \times Ct \times 14 \times 100 \times 6.25}{m \times 1000} \quad (1)$$

Where:
V_1 = Volume of 0.2 N HCl used for sample titration
V_0 = Volume of 0.2 N HCl used for blank titration
C_t = Normality of HCl (0.2 N)
m = Sample mass

Procedure for Antioxidant Activity Test [17]

A total of 100 µL of protein hydrolysate sample solution was mixed with 3,900 µL of 0.075 mM DPPH solution in 95% ethanol. The mixture was incubated in the dark for 30 minutes, after which the absorbance of the sample and blank was measured using a UV–Vis spectrophotometer at a wavelength of 517 nm.

The antioxidant activity of the protein hydrolysate was calculated using Equation (2):

$$\%\ \text{Inhibition} = \frac{Control\ Absorbance - Sample\ Absorbance}{Control\ Absorbance} \times 100\% \quad (2)$$

Data Analysis Technique

All measurements were performed in triplicate for each treatment. The data obtained were analyzed to determine whether there were significant effects of the treatments on the measured parameters. Protein content and antioxidant activity data were statistically analyzed using analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Protein Content

The determination of protein content in this study was carried out using the Kjeldahl method. This method determines protein content based on the nitrogen present in the sample and consists of three main stages: digestion, distillation, and titration.

The digestion stage aims to release nitrogen from the sample through the addition of a strong acid (H_2SO_4) and heating. The reaction occurring during digestion is as follows:

$$NH_2–CH(R)–COOH + H_2SO_4 \rightarrow (NH_4)_2SO_4$$

The distillation stage aims to convert ammonium sulfate into ammonia gas by adding NaOH and heating, producing NH_3 gas that evaporates and reacts with H_3BO_3 to form $NH_4H_2BO_3$. The reactions occurring during distillation are:

$$(NH_4)_2SO_4 + 2NaOH \rightarrow Na_2SO_4 + 2H_2O + 2NH_3$$

$$2NH_3 + 2H_3BO_3 \rightarrow 2NH_4H_2BO_3$$

The final stage is titration, which aims to quantify NH_3 by titrating $NH_4H_2BO_3$ with HCl. The principle of this stage is that $NH_4H_2BO_3$ is titrated with 0.02 N HCl, resulting in the separation of boric acid and the formation of NH_4Cl. The reaction during titration [18] is:

$$NH_4H_2BO_3 + 2HCl \rightarrow 2NH_4Cl + 2H_3BO_3$$

The average results of protein content analysis of skipjack tuna protein hydrolysate are presented in Figure 1.

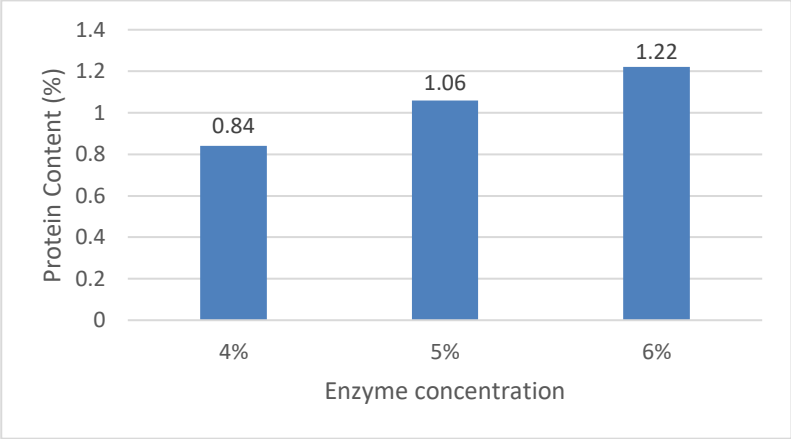

Figure 1. Protein content of skipjack tuna protein hydrolysate at various enzyme concentrations

The results show that different enzyme concentrations affected the percentage of protein in the resulting hydrolysate. Figure 1 indicates that protein content increased with increasing enzyme concentration. The protein content ranged from 0.84% to 1.22%. At 4% enzyme concentration, the protein content was 0.84%, which increased to 1.06% at 5% and reached 1.22% at 6%. This demonstrates a clear upward trend between enzyme concentration and protein content. The highest average protein content was obtained at 6% enzyme concentration (1.22%).

The highest protein content obtained in this study (1.22%) was lower than that reported in previous studies [14] and [19]. These differences may be attributed to several factors, including variations in enzyme concentration, hydrolysis time, solvent ratio, and post-hydrolysis treatment. In this study, 6% papain enzyme was used with a hydrolysis time of 2 hours and a sample-to-solvent ratio of 1:4, resulting in a more diluted hydrolysate and relatively lower measured protein content. In contrast, study [14] on goat lung protein hydrolysate used a longer hydrolysis time (6 hours) and a smaller solvent ratio (1:2), resulting in a higher protein content (2.48%). Meanwhile, study [19] on snakehead fish (*Channa striata*) protein hydrolysate reported a much higher protein content (20.81%) using a higher enzyme concentration (20%). Such a high enzyme concentration accelerates protein breakdown into peptides. In addition, differences in post-hydrolysis treatment, where the hydrolysate was dried prior to analysis, reduced water content and increased the measured protein level. Therefore, differences in sample form represent an important factor contributing to variations in protein content across studies.

In this study, the enzyme concentration range of 4%–6% showed a significant increase in protein content, indicating that increasing enzyme concentration up to 6% remains within an effective range for producing soluble protein without reaching substrate saturation.

Antioxidant Activity

The antioxidant activity in this study was determined using the DPPH method, with results expressed as percentage inhibition. The DPPH method is a quantitative assay used to evaluate antioxidant activity by measuring changes in absorbance. The absorbance of DPPH decreases when its free radical electrons are reduced through the acceptance of hydrogen atoms donated by antioxidants [8].

Based on the results, a color change in the DPPH solution was observed after mixing with the protein hydrolysate sample, from purple to yellow. This is consistent with previous findings [20], which state that antioxidant activity can change the color of DPPH solution from purple to

pale yellow. Antioxidant compounds donate hydrogen atoms to DPPH, causing the purple color to fade and turn yellow. The average antioxidant activity results of skipjack tuna protein hydrolysate are presented in Figure 2.

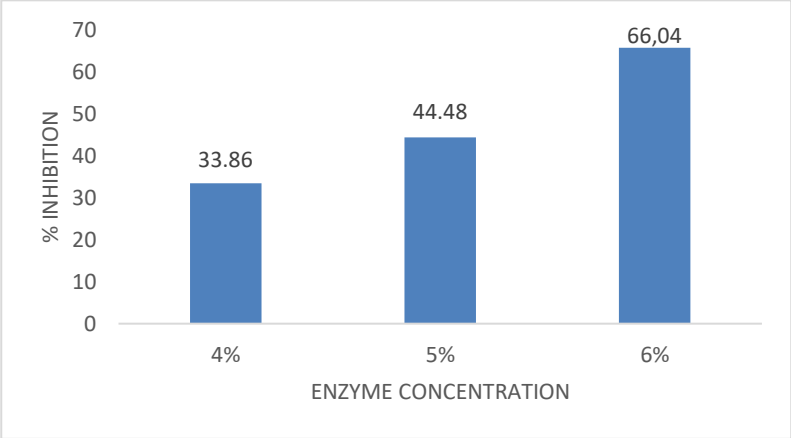

Figure 2. Antioxidant activity of skipjack tuna protein hydrolysate at various enzyme concentrations

Figure 2 shows that antioxidant activity increased with increasing enzyme concentration. The antioxidant activity ranged from 33.86% to 66.04%. At 4% enzyme concentration, the antioxidant activity was 33.86%, increasing to 44.48% at 5% and reaching 66.04% at 6%. This indicates a clear upward trend between enzyme concentration and antioxidant activity. The highest average antioxidant activity was obtained at 6% enzyme concentration (66.04%).

In this study, the antioxidant activity of skipjack tuna hydrolysate increased with increasing enzyme concentration. The highest antioxidant activity (66.04%) was achieved at 6% enzyme concentration. Compared to previous research, this value is higher than that reported in study [21], which showed antioxidant activity of 54.86% in protein hydrolysate from white snapper (*Lates calcarifer*) viscera. This difference may be due to the lower enzyme concentration used in that study (0.1%), which may result in lower antioxidant activity. Additionally, differences in raw materials also influence antioxidant activity, as skipjack tuna and white snapper have different amino acid compositions and protein structures, affecting the type and quantity of bioactive peptides formed during hydrolysis.

Increasing enzyme concentration leads to higher production of peptides and free amino acids, thereby enhancing antioxidant activity. This is because the antioxidant bioactivity of protein hydrolysates is closely related to the presence of aromatic amino acids such as tyrosine, phenylalanine, and tryptophan. These amino acids can donate hydrogen atoms to neutralize free radicals. Tyrosine is considered the most effective due to the hydroxyl (–OH) group in its phenolic ring, which donates hydrogen atoms to form a stable tyrosyl radical. Tryptophan also plays an important role due to its electron-rich indole ring, which stabilizes radicals through resonance [22].

Table 1. ANOVA test results

Parameter	Mean	Significance	F-value	F-table
Protein content	0.84 1.06 1.22	Significance($p < 0.05$)	94.337	5.143
Antioxidant activity	33.46 44.39 65.69	Significance($p < 0.05$)	200.343	5.143

Analysis of variance (ANOVA) was conducted on two parameters: protein content and antioxidant activity. Based on the results presented in Table 1, variations in enzyme concentration produced statistically significant differences ($p < 0.05$) in both protein content and antioxidant activity. This indicates that enzyme concentration significantly affects the protein content and antioxidant activity of the produced protein hydrolysate.

CONCLUSION

Based on the research results, it can be concluded that increasing the concentration of papain enzyme in skipjack tuna protein hydrolysis significantly affected the protein content and antioxidant activity. The best enzyme concentration, 6%, resulted in a protein content of 1.22% and antioxidant activity of 66.04%.

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