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Development of Bioactive Peptides from Broiler Chicken Feet Through Enzymatic Hydrolysis

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ABSTRACT

Background: Stunting remains a significant public health issue in Indonesia, with a prevalence of 21.6%, severely affecting child growth and development. Improving amino acid intake is essential for addressing stunting. Bioactive peptides derived from protein-rich food sources show promise due to their potential role in stunting prevention. Chicken feet, a by-product of poultry with high protein content, offer an opportunity for developing bioactive peptides to support child nutrition.

Objective: This study aims to develop bioactive peptides from broiler chicken feet through enzymatic hydrolysis using the proteolytic enzymes papain-alcalase and bromelain.

Research Methods This study utilizes an experimental design involving enzymatic hydrolysis of chicken feet with proteolytic enzymes, bromelain, and a combination of papain and alcalase. The peptide concentration was analyzed by direct spectrophotometer at 280 nm.

Results: The absorbance results revealed that both methods able to increase soluble protein concentrations. However, based on ANOVA (P value < 0.05) with Tukey pairwise comparison, only the papain-alcalase method was able to increase the soluble protein in a significant way. This show that the papain-alcalase combination proved more effective than bromelain. This increased effectiveness could be attributed to the differences in thes enzymatic mechanisms, as papain and alcalase target distinct functional groups during protein hydrolysis.

Conclusion: This study demonstrates the potential of enzymatic hydrolysis, particularly the combined use of papain and alcalase, to produce bioactive peptides from chicken feet.

Keywords: Hydrolysis; peptide analysis; Papain; Alcalase; Bromelain

INTRODUCTION

Approximately 6,5 million or 21,6% of children in Indonesia were classified as stunted in 2023 (Tim Percepatan Penurunan Stunting, 2024). Stunting could cause impaired cognitive and motoric growth, metabolic disorder, impaired nerve and brain function, and increased risk of non-communicable diseases (World Health Organization, n.d.). While stunting is a complex problem with various factors, the root cause is relatively simple: chronic nutrition deficiency in the first 1000 days of life(Rini Puji Lestari, 2023; Tim Percepatan Penurunan Stunting, 2024). Food quality plays a significant role in addressing stunting, as inadequate nutritional intake during critical growth periods directly contributes to impaired development. Enhanced food quality ensures the availability of essential nutrients necessary for cognitive, physical, and metabolic functions, ultimately reducing the prevalence of stunting (World Health Organization, 2014). Thus, improvement in food quality is a crucial corrective and preventive action to erase stunting.

Bioactive peptides are defined as protein fragments that have a positive effect on the function, condition, and health of the body (Sánchez & Vázquez, 2017; Zaky et al., 2022). Bioactive peptides have an important role in the metabolic functions of living things and, indirectly, play a role in health (Ozturk-Kerimoglu et al., 2023). Therefore, bioactive peptides have good potential to be developed into functional food preparations (nutraceuticals) or even as medicines for treating diseases such as hypertension, cancer, obesity, diabetes, ischemic heart disease, and other diseases (Kitts & Weiler, 2003; Sánchez & Vázquez, 2017). Recent research has highlighted the potential of bioactive peptides in improving nutritional quality, especially in populations vulnerable to malnutrition (Sabrina et al., 2022). However, their specific application in addressing stunting remains underexplored. Despite advancements in bioactive peptide research, a significant gap exists in understanding their direct role in mitigating stunting. This study focuses on the possibility of developing chicken feet as material for bioactive peptides for stunting prevention supplements.

Chicken is the most consumed meat in Indonesia, showing positive growth in production and consumption with 3 billion broiler chickens raised in 2022 (Badan Pusat Statistik Indonesia, n.d.-a, n.d.-b). With such a huge number and growth, the number of low-economic value by-products will also rise. One such by-products are chicken feet. Thus, this study attempts to produce bioactive peptides from chicken feet using enzymatic hydrolysis and asses the effectivity by comparing the soluble protein concentration.

RESEARCH METHODS

Materials

Chicken feet were purchased from traditional market in Yogyakarta. Papain, alcalase, and bromelain were purchased from CV. General Labora. Bovine serum albumin (BSA) were purchased from Sigma-Aldrich.

Enzymatic Hydrolysis of Chicken Feet

Chicken feet were enzymatically hydrolyzed using two different enzyme treatments. For the first treatment, 100 g of finely minced broiler chicken feet was mixed with distilled water to achieve a 30% (w/v) concentration. The mixture underwent hydrolysis with papain enzyme (4000 U/g) at 65°C and pH 7.5 for 2.25 hours in waterbath, followed by a second hydrolysis using alcalase enzyme (5000 U/g) at 65°C and pH 10.0 for 2 hours. In the second treatment, 100 g of finely minced broiler chicken feet were similarly mixed with distilled water to achieve a 30% (w/v) concentration and hydrolyzed with bromelain enzyme (5000 U/g) at 50°C and pH 8 for 4 hours. Minced chicken feet were used as control. For all treatments, the hydrolysis reaction was terminated by heating the mixture in a water bath at 95°C for 15 minutes. The hydrolysates were then filtered through a nylon sieve, and the filtrates were centrifuged at 5600 × g for 20 minutes. The resulting supernatants were collected and stored at −16°C.

Direct UV spectrophotometric analysis

A volume of 1 mL of appropriately diluted supernatant. The mixture was vortexed and absorbance was measured at a wavelength of 280 nm using a Shimadzu UV 1780 spectrophotometer. BSA aqueous solution was used as standard.

Statistical analysis

The resulting data was subjected to statistical analysis using minitab statistical software version 22.1. Normality test was conducted using Ryan-Joiner test, followed by ANOVA.

RESULTS AND DISCUSSION

This study attempts to compare the two production methods for bioactive peptides from chicken feet: enzymatic hydrolysis by utilizing bromelain and a combination of papain and alcalase enzymes. The enzymatic hydrolysis is measured by the increase in soluble protein concentration, as the hydrolysis will broke down various proteins into smaller fragments and increase their solubility. Thus, the increase in soluble protein concentration means an increase ini the effectiveness of the hydrolysis process. There are various detection method for peptide, such as mass spectrometry (Andy Lin et al., 2022), hplc (de Souza & Queiroz, 2023), and SDS-PAGE (Zhang et al., 2020). However, direct spectrophotometric analysis was chosen due to its simplicity, although more vulnerable to interruption. This is due to the method is reliant on the aromatic group residue of the amino acids that make up protein, namely histidine, phenylalanine, tryptophan, and tyrosine (Krewing et al., 2020). This made the method easily disrupted by other aromatic compounds present in the matrix, such as DNA and RNA.

Chicken feet were chosen as the source of protein due to its low economic value compared to other source (Santana et al., 2020). As chicken meat is the most consumed meat in Indonesia, the supply of by-products such as chicken feet would be stable and readily accessible (Badan Pusat Statistik Indonesia, n.d.-b). Minced chicken feet were hydrolyzed with the respective enzyme.

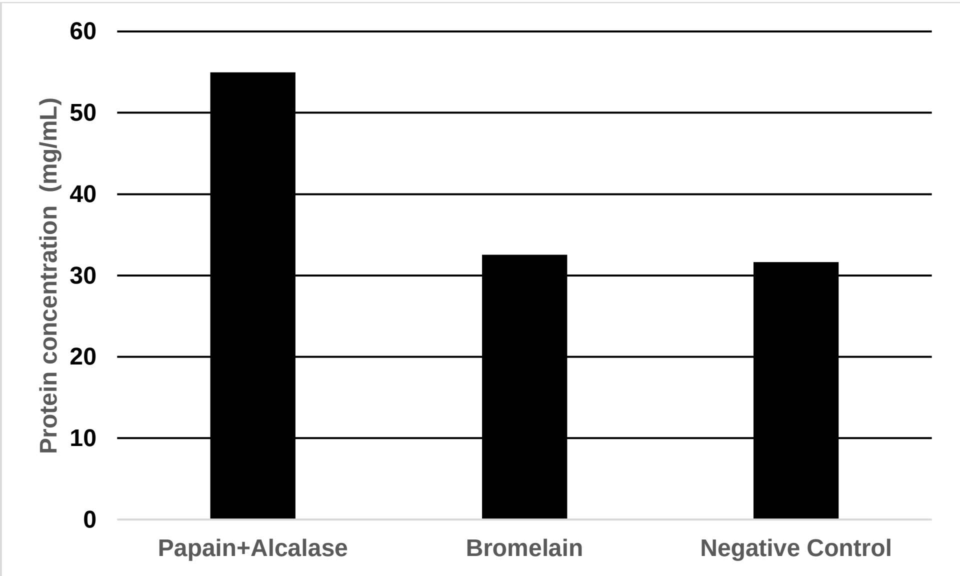

Figure 1. Protein concentration results

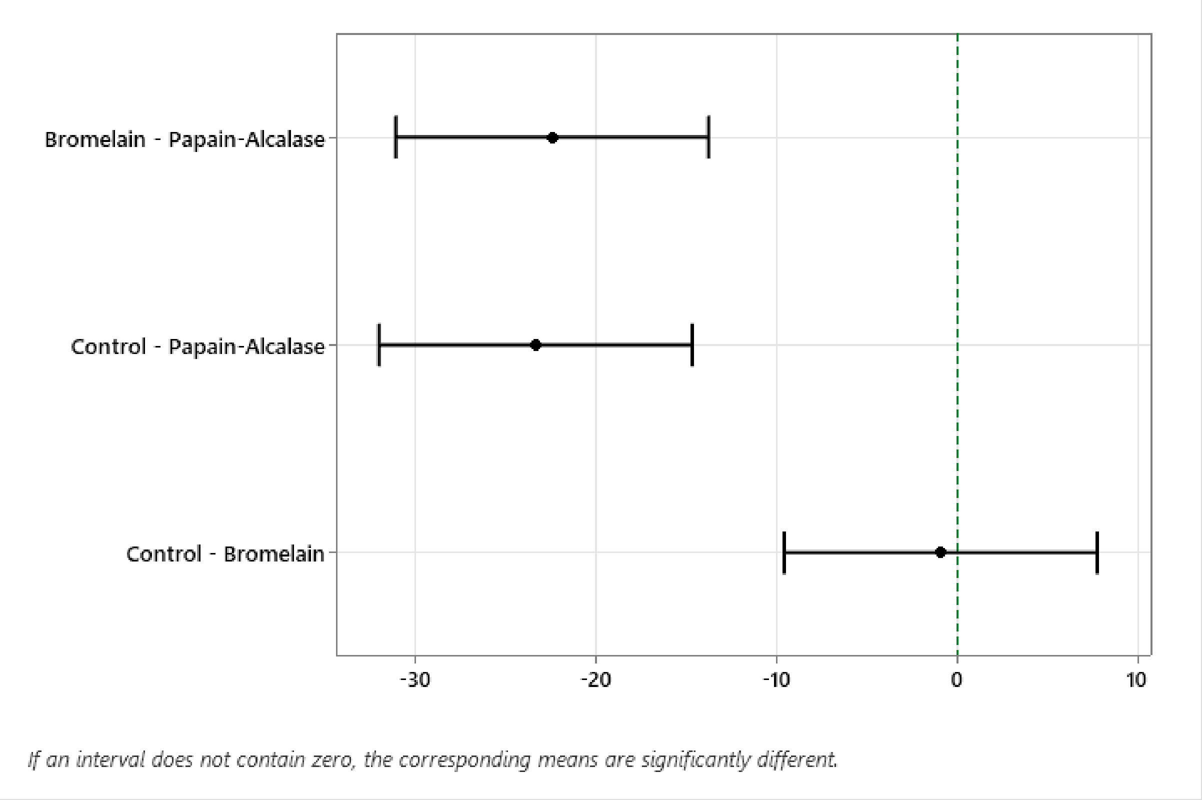

Figure 2. Grouping of data based on the Tukey method on a 95% confidence interval

The resulting data were subjected to the Ryan-Joiner normality test, and all three groups with all three groups show p-value > 0,05, meaning all three groups is normally distributed. The ANOVA result coupled with Tukey pairwise comparison data showed a significant increase in soluble protein content (P-value = 0.000). However, the group contributing to this significance is the papain-alcalase group, whereas the bromelain and control group is not statistically significant.

Both groups show an increase in soluble protein concentration, which confirms that both enzymatic hydrolysis methods could convert insoluble proteins into shorter, water-soluble protein fragments. However, only the combination of papain and alcalase showing a significant increase. This could be due to difference in the respective enzyme mechanism, where papain and bromelain are both cysteine proteases. However, they differ in substrate preferences: papain favors hydrophobic residues, while bromelain prefers more polar arginine residues (Azarkan et al., 2020; Petushkova et al., 2022). Whereas alcalase is a serine protease and prefers more hydrophobic residue (Xiaoqing Xu et al., 2024). The combination of papain and alcalase enzymes has been shown to enhance protein hydrolysis, resulting in increased solubility of the hydrolyzed proteins (Schlegel et al., 2020). In contrast, hydrolysis with bromelain alone has been associated with lower protein solubility (Saad & Dia, 2023).

These findings suggest that the combined use of papain and alcalase can more effectively break down insoluble proteins into smaller, more soluble peptides, thereby increasing the concentration of soluble proteins compared to hydrolysis with bromelain alone. This should be further verified by using other methods such as SDS-PAGE to visualize the difference in protein fragment size between the two methods, or HPLC to help better understand the protein fragmentation process, as this method could easily measure the concentration of free amino acid or short peptide fragments that liberated from the hydrolysis process.

This study highlights the potential of chicken feet as a source of bioactive peptides through enzymatic hydrolysis, particularly with the combination of papain and alcalase enzymes. The observed increase in soluble protein content underscores the potential of enzymatic treatments to enhance the value of poultry by-products. These findings support the use of chicken feet-derived bioactive peptides as a potential nutritional supplement to address stunting in Indonesia.

Despite its promising outcomes, this study has some limitations that warrant consideration. The reliance on spectrophotometric analysis, while simple, is prone to interference from non-protein aromatic compounds present in the sample matrix. Incorporating more robust analytical techniques, such as HPLC or mass spectrometry, in future studies could ensure specificity and accuracy in peptide quantification. Additionally, focusing solely on soluble protein concentration as a measure of hydrolysis efficiency overlooks the functional bioactivity of the peptides. Moreover, exploring alternative by-products as protein sources could enhance the sustainability and expand the impact of this approach.

CONCLUSION

This study demonstrates the potential of enzymatic hydrolysis, particularly the combined use of papain and alcalase, to produce bioactive peptides from chicken feet. The papain-alcalase combination proved to be more effective than bromelain alone in enhancing soluble protein concentration, underscoring its superior hydrolytic capacity. These findings highlight the viability of utilizing poultry by-products as a cost-effective and sustainable source of bioactive peptides, offering a dual benefit of waste valorization and nutritional enhancement. However, further research is essential to confirm the functional bioactivity, bioavailability, and industrial scalability of the peptides. Addressing these aspects will be critical to unlocking the full potential of chicken feet-derived peptides as functional food ingredients or dietary supplements, particularly in addressing malnutrition challenges such as stunting in Indonesia.

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