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Enzymatic hydrolysis of chicken feet for bioactive peptides: method validation and protein quantification

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Background: Stunting affects 21.5% of Indonesian children under five, primarily due to chronic nutritional deficiencies. Chicken feet, underutilized poultry byproducts rich in protein, represent potential sources of bioactive peptides for nutritional interventions.

Objective: This study evaluated two enzymatic hydrolysis methods for producing bioactive peptides from chicken feet and validated analytical methods for protein quantification.

Methods: Chicken feet were hydrolyzed using bromelain and sequential papain-alcalase treatments. Soluble proteins were quantified using the Biuret assay. Analytical methods were validated for accuracy, precision, and linearity according to ICH guidelines.

Results: The analytical method demonstrated satisfactory performance with accuracy values of 98-102%, RSD values below 2%, and linearity (R^2 = 0.9977) across the 3-11 mg/mL range. Bromelain treatment significantly increased soluble protein content to 24.39 ± 0.65 mg/mL, representing a 1.71-fold increase compared to the non-hydrolyzed control (14.24 ± 0.20 mg/mL, $p < 0.001$). Conversely, papain-alcalase treatment decreased soluble protein content to 10.92 ± 0.05 mg/mL, likely due to excessive peptide degradation into free amino acids undetectable by the Biuret assay.

Conclusion: Bromelain hydrolysis demonstrated superior protein solubilization efficiency compared to papain-alcalase treatment. These preliminary findings suggest bromelain's potential for converting chicken feet into functional ingredients, though further peptide characterization and bioactivity assessment are required for nutritional applications.

Keywords: bioactive peptide, bromelain, papain, alcalase, validation

Introduction

Stunting remains a significant public health challenge in Indonesia, affecting 21.5% of children under five years of age, or approximately 6.5 million cases in 2023, with a substantial gap remaining to achieve the national target of 14% by 2024 [1][2]. Stunting primarily results from chronic nutritional deficiencies during the first 1,000 days of life, a critical window for child development [2]. Research on nutritional intake in stunted toddlers and children indicates that inadequate consumption of macronutrients, particularly carbohydrate and protein, alongside deficiencies in key micronutrients–in particular iron, zinc, calcium, vitamin A, vitamin D, and omega-3 fatty acids–are associated with stunting and wasting [3-5]. Consequently, increasing the intake of these essential nutrients during this crucial development period represents a critical intervention action against stunting. Despite extensive research on nutritional interventions for stunting, there remains a significant gap in developing locally-sourced, affordable, and sustainable protein solutions that can be readily implemented within the Indonesian context, particularly those utilizing undervalued food industry by-products.

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Given the crucial role of protein intake in preventing stunting, identifying an affordable and sustainable protein source is imperative. Chicken ranks as the second most consumed protein in Indonesia after eggs, with the poultry industry generating substantial by-products, including chicken feet [6]. In the Indonesian context, chicken feet are particularly relevant as they are accepted in various traditional dishes such as *soto* and *semur*, widely available throughout the archipelago's diverse regions, and represent an underutilized protein source in nutritional interventions. Despite their rich in collagen and protein content, chicken feet are generally considered to have low economic value, yet they represent a promising source of bioactive peptides [7-10].

Bioactive peptides are protein fragments that positively affect the body's functions, condition, and health [11]. Bioactive peptides typically consist of 2 to 20 amino acid residues, with molecular weights ranging from 0.4 to 2 kDa [12]. More than 4,800 bioactive peptides have been catalogued in the Biopep database [13]. These peptides play a crucial role in the metabolic functions of living organisms and contribute to overall health. As a result, bioactive peptides hold great potential for development as functional food ingredients (nutraceuticals) or as therapeutic agents. Studies investigating bioactive peptide effects on malnutrition and stunting have demonstrated that increased amino acid and peptide intake could improve serum protein, hemoglobin, and insulin-like growth factor (IGF-1) levels in animal models [14].

The immunomodulatory and antioxidant properties of bioactive peptides increase growth factors such as IGF-1 and promote cellular metabolism via protein synthesis, glycolysis, mitochondrial functions, and lipid synthesis [15]. Furthermore, when used as animal feed supplements, bioactive peptides can enhance their nutrition, gut function, and capacity to combat infectious diseases [16], which are crucial in addressing stunting.

Numerous approaches have been developed to produce bioactive peptides from diverse protein sources. These sources include marine organisms (mollusks, crustaceans, and fish, including fishery waste), meat products (bovine and porcine), plant-based sources (plum, flaxseed, and soybean), eggs, dairy products, microorganisms, and edible insects [11,17]. Enzymatic hydrolysis, particularly using digestive enzymes, represents the most common production method. Alternative approaches include hydrolysis using enzymes extracted from microorganisms or plants, microbial fermentation, chemical synthesis, and recombinant production. Chemical synthesis involves reacting amino acid units under specific conditions but is limited by lengthy and complex processes required to obtain pure products. Recombinant production, conversely, generates peptides within specific in vivo or in vitro expression systems [16].

Given the potential of bioactive peptides in addressing stunting, chicken feet as low-value by-products of the poultry industry could be developed into a sustainable source of peptides. This study aimed to produce bioactive peptides from chicken feet using enzymatic hydrolysis and compare the effectiveness of two hydrolysis methods. It seeks to contribute to developing affordable and sustainable solutions for mitigating stunting.

Methods

Materials and sample preparation

All enzymatic reagents (papain 4000 U/g, alcalase 5000 U/g, and bromelain 5000 U/g) were of analytical grade. Bovine serum albumin (BSA) was used as a protein standard. Fresh broiler chicken feet were obtained from a local poultry processing facility, cleaned thoroughly, and stored at 4°C until use.

Enzymatic hydrolysis of chicken feet using sequential papain-alcalase

The hydrolysis of chicken feet was adapted from Nadzri et al. [18] and Selamassakul et al. [19] with modification. Broiler chicken feet (100 g) were finely minced using a blender and homogenized with distilled water to achieve a 30% (w/v) suspension. The homogenate underwent a two-stage enzymatic hydrolysis process. In the first stage, papain (4000 U/g) was added to the suspension, and the pH 7.5 using 1 M NaOH. The mixture was incubated at 65°C for 2.25 hours. For the second stage, after the papain treatment, hydrolysis was conducted using alcalase (5000 U/g) at 65°C and pH 10.0 for 2 hours. The hydrolysis reaction was terminated by heating the mixture in a water bath at 95°C for 15 minutes. The hydrolysate was filtered through a nylon sieve, and the filtrate was centrifuged at 5600×g for 20 minutes. The resulting supernatant was collected and stored at -18°C until further analysis.

Enzymatic hydrolysis of chicken feet using single-enzyme bromelain process

For comparison, a single-enzyme hydrolysis method was performed. Minced chicken feet (100 g) were homogenized with distilled water to achieve a 30% (w/v) suspension. The pH was adjusted to 8.0, and bromelain (5000 U/g) was added. The mixture was incubated at 50°C for 4 hours. Enzyme inactivation, filtration, centrifugation, and storage were performed as described in the sequential hydrolysis method.

Preparation of non-hydrolyzed control solution

To establish a baseline for comparison, a non-hydrolyzed control solution was prepared. Minced chicken feet (100 g) were homogenized with distilled water to achieve a 30% (w/v) suspension. The suspension was heated in a water bath at 95°C for 15 minutes to simulate the enzyme inactivation step without prior enzymatic treatment. The heated mixture was filtered through a nylon sieve, centrifuged at 5600 × g for 20 minutes at 4°C, and the supernatant was stored at -18°C.

Protein quantification using Biuret method

BSA was used as the protein standard. A stock solution (10 mg/mL) was prepared by dissolving 100 mg of BSA in 10 mL of purified water. This stock solution was serially diluted with purified water to prepare standards with concentrations of 3, 5, 7, and 11 mg/mL.

Protein content was determined using a modified Biuret method as described by Trang [20]. Aliquots (10 µL) of appropriately diluted sample supernatants or BSA standards were mixed with 1 mL of Biuret reagent. The mixtures were vortexed for 30 seconds and incubated at room temperature (25 ± 2°C) for 5 minutes. Absorbance measurements were performed at 545 nm using a Shimadzu UV 1780 spectrophotometer. Protein concentrations were calculated using the standard calibration curve.

Analytical method validation

The spectrophotometric method was validated according to ICH guidelines for analytical procedures [21]. The following parameters were assessed:

Linearity was evaluated by analyzing the BSA standard solutions across the concentration range of 3-15 mg/mL. The calibration curve was constructed by plotting absorbance values against corresponding concentrations, and the correlation coefficient (r^2) was calculated.

Intra-day precision (repeatability) was determined by analyzing six replicate measurements of samples at three concentration levels (low, medium, and high) within the same day. Inter-day precision (intermediate precision) was evaluated by analyzing the same samples on three consecutive days. Results were expressed as relative standard deviation (RSD, %).

Accuracy was assessed using the standard addition method at three concentration levels. Known amounts of BSA were added to pre-analyzed samples, and the percentage recovery was calculated.

Statistical analysis

All experiments were performed in triplicate, and results are presented as mean ± standard deviation (SD). Statistical analyses were conducted using GraphPad Prism version 10.0 (San Diego, CA, USA). Differences between groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. A p-value < 0.05 was considered statistically significant.

Results

Analytical method validation

Accuracy. Accuracy was assessed by the standard addition method at three concentration levels (80%, 100%, and 120% of the target concentration). As shown in Table 1, the mean percent recovery values ranged from 97.44% to 101.88%, falling within the acceptance criteria of 98-102%. The relative standard deviation (RSD) values for all concentration levels were below 2% (ranging from 1.09% to 1.75%), confirming that the accuracy of the methods was satisfactory.

Precision. Method precision was evaluated through six replicate measurements of a 10 mg/mL BSA standard solution. The results presented in Table 2 demonstrate consistent concentration determinations with values ranging from 9.644 to 9.903 mg/ml. The calculated RSD of 1.177% was well below the acceptance limit of 2%, confirming high measurement precision and repeatability of the analytical method.

Linearity. The linearity of the Biuret spectrophotometric method was evaluated across the protein concentration range of 3-11 mg/mL. The calibration curve demonstrated excellent linearity with a coefficient of determination (R^2) of 0.9977, meeting the validation requirements.

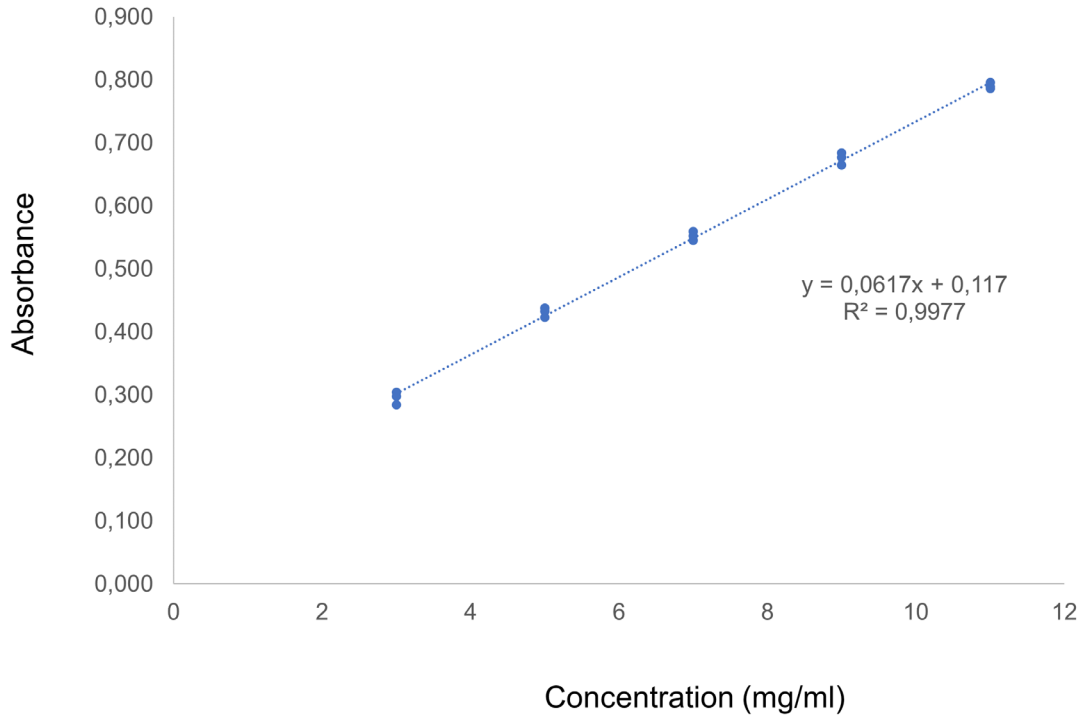

Figure 1. Linear regression analysis of BSA standard solutions measured by the Biuret spectrophotometric method. The calibration curve demonstrates excellent linearity (R^2 = 0.9977) across the concentration range of 3-111 mg/mL

Table 1. Accuracy validation of the Biuret spectrophotometric method for protein quantification

Concentration (mg/mL)	Measured concentration (mg/mL)	Recovery (%)	Mean recovery (%)	SD	RSD (%)
80% (5.6 mg/mL)	5.608	100.15	99.96	0.0174	1.75
	5.495	98.12			
	5.689	101.60			
100% (7.0 mg/mL)	7.018	100.26	100.65	0.0109	1.09
	7.132	101.88			
	6.986	99.80			
120% (8.4 mg/mL)	8.396	99.95	99.37	0.0171	1.73
	8.461	100.72			
	8.185	97.44			

Table 2. Precision validation of the Biuret spectrophotometric method for protein quantification

Replicate	Concentration (mg/mL)	Mean ± SD (mg/mL)	RSD
1	9.693	9.763 ± 0.115	1.177%
2	9.903		
3	9.676		
4	9.757		
5	9.644		
6	9.903		

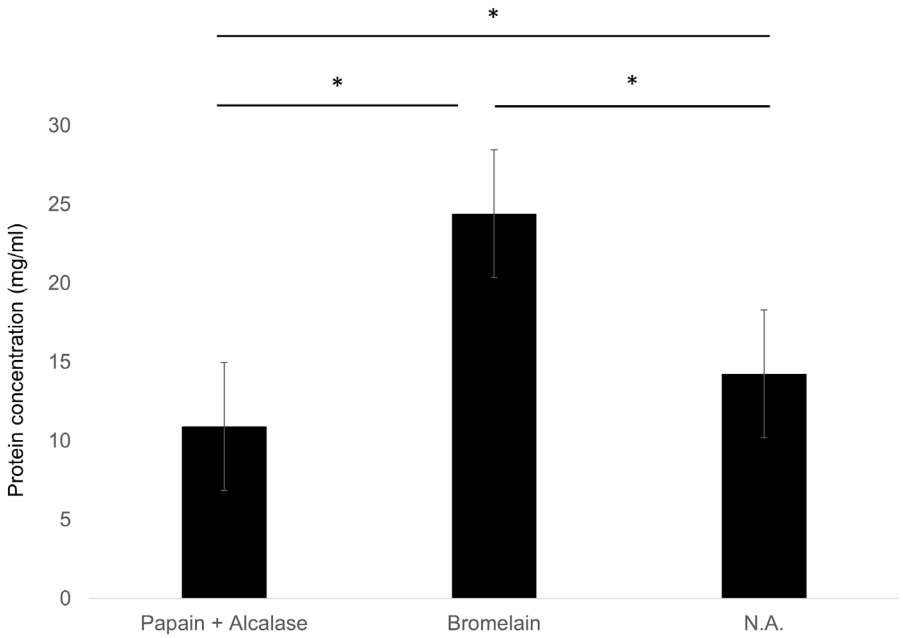

Figure 2. Soluble protein content in chicken feet preparations following different treatment methods. Values represent mean ± SD (n=3). Control (N.A): non-hydrolyzed preparation (14.24 ± 0.20 mg/mL); bromelain: single-enzyme hydrolysis (24.39 ± 0.65 mg/mL); Papain+Alcalase: sequential enzymatic hydrolysis (10.92 ± 0.05 mg/mL). Statistical analysis using one-way ANOVA followed by Tukey's HSD post-hoc test showed significant differences between all treatment groups ($p < 0.05$)

Protein content analysis following enzymatic hydrolysis

The soluble protein content in chicken feet preparations was quantified using the validated Biuret method, with markedly different outcomes observed between the two enzymatic hydrolysis approaches. The non-hydrolyzed control preparation yielded a baseline soluble protein content of 14.24 ± 0.20 mg/mL.

Enzymatic hydrolysis with bromelain significantly increased soluble protein content to 24.39 ± 0.65 mg/mL, representing a 1.71-fold increase compared to the non-hydrolyzed control. This significant increasing demonstrates the effectiveness of bromelain in hydrolyzing chicken feet proteins and improving protein solubilization.

Conversely, the sequential papain-alcalase treatment resulted in decreased soluble protein content to 10.92 ± 0.05 mg/mL, representing 77% of the control value. This 23% reduction compared to the control indicates that the combination treatment may have proceeded beyond optimal peptide formation, potentially degrading peptides into free amino acids that are not detected by the Biuret assay.

One-way ANOVA revealed statistically significant differences between all treatment groups. Subsequent Tukey's Honestly Significant Difference post-hoc analysis demonstrated that all pairwise comparisons between the three preparations were statistically significant ($p < 0.05$), confirming distinct protein profiles for each treatment approach (Figure 2).

Discussions

The validation of the analytical method was conducted to ensure the analytical method used is suitable and reliable for protein analysis. The validation results demonstrate good performance across the key parameters of accuracy, precision, and linearity, confirming their suitability for protein analysis in this study. The accuracy results confirmed the reliability of the method in quantifying protein concentrations. The low RSD values (<2%) show the consistency of the measurements, indicating minimal variability during replicate analyses. This level of accuracy ensures that the method can reliably reflect the actual concentrations of the analytes without significant bias, a critical factor for generating reproducible data in protein studies [21].

The precision validation resulted in RSD <2% across all replicates, indicating excellent repeatability of the methods. This level of precision minimizes the risk of analytical error due to instrumental or procedural inconsistencies. It also underscores the robustness of the method in handling routine sample measurements.

The linearity results (R^2 = 0.9977), highlight the methods' ability to establish a strong correlation between absorbance and protein concentration across the tested range. This ensures the methods can accurately predict protein concentrations. The R^2 value exceeding the commonly accepted threshold (0.995) further emphasizes the reliability of the regression model and the methods' ability to handle diverse sample concentrations effectively [21].

The current study explored the production of bioactive peptides from chicken feet using two enzymatic hydrolysis methods: bromelain and a combination of papain and alcalase. This investigation aimed to identify the method that yields higher levels of soluble proteins, providing insights into the effectiveness of enzymatic treatment in protein hydrolysis.

The fold-change analysis relative to the control revealed that bromelain treatment achieved the highest protein extraction efficiency, while the papain-alcalase combination unexpectedly reduced measurable protein content below baseline levels. These contrasting outcomes highlight the critical importance of optimizing enzymatic conditions to achieve desired hydrolysis endpoints while preventing excessive protein degradation. The superior performance of bromelain hydrolysis in terms of soluble protein yield suggests its potential advantage for bioactive peptide production from chicken feet, warranting further investigation of peptide profiles and bioactive properties.

The analysis data showed a significant increase in soluble protein content for bromelain treatment, as indicated by ANOVA results ($p < 0.001$). This confirms that bromelain enzymatic hydrolysis effectively converted insoluble proteins into shorter, water-soluble protein fragments. However, the unexpected decrease in measurable protein content observed with the papain-alcalase treatment requires comprehensive mechanistic analysis.

The counter-intuitive finding that bromelain, typically characterized by moderate proteolytic activity, outperformed the more aggressive papain-alcalase combination challenges conventional expectations based on enzyme activity profiles. This phenomenon likely reflects the complex relationship between enzyme specificity, hydrolysis kinetics, and optimal peptide formation. Bromelain's relatively controlled hydrolytic action may have achieved an optimal balance between protein solubilization and peptide integrity, maintaining peptide bonds detectable by the Biuret assay while generating bioactive fragments.

Conversely, the sequential papain-alcalase treatment, despite employing enzymes known for high proteolytic efficiency, appears to have proceeded beyond optimal hydrolysis endpoints. The observed reduction in measurable protein content to 77% of control levels suggests extensive peptide degradation into free amino acids, which are not detected by the Biuret assay due to the absence of sufficient peptide bonds [20]. This over-hydrolysis phenomenon has been documented in other protein hydrolysis studies, where excessive enzymatic treatment leads to diminished functional peptide recovery [22].

The Biuret assay played a crucial role in quantifying soluble proteins. The Biuret reagent specifically reacts with peptide bonds, allowing for the selective measurement of proteins and peptides while remaining unaffected by interfering substances such as free amino acids, DNA, or RNA. This specificity enhances the reliability of peptide quantification, particularly in complex matrices like enzymatically hydrolyzed chicken feet [20]. The assay's selectivity for peptide bonds makes it particularly valuable for monitoring hydrolysis progression and identifying optimal endpoints for bioactive peptide production.

Given the study's ultimate objective of developing nutritional interventions for stunting prevention, the superior performance of bromelain hydrolysis presents significant practical advantages. Stunting primarily results from chronic nutritional deficiencies during the first 1,000 days of life, particularly inadequate protein intake [2,3]. The enhanced protein solubilization achieved through bromelain treatment could improve the bioavailability and digestibility of chicken feet-derived proteins, making them more suitable for incorporation into nutritional interventions targeting vulnerable populations.

Several limitations of the current study must be acknowledged. The reliance on a single protein quantification method, while appropriate for initial screening, provides limited insight into peptide size distribution and bioactive potential. The absence of peptide profiling through techniques such as liquid chromatography-mass spectrometry prevents comprehensive characterization of the hydrolysis products and identification of specific bioactive sequences.

The study's focus on total soluble protein content, while informative for process optimization, does not

address the functional properties or bioactivities of the generated peptides. Future research should incorporate assessment of antioxidant activity, antimicrobial properties, and other bioactivities relevant to nutritional applications. Additionally, the study did not evaluate the digestibility or bioavailability of the hydrolyzed products, factors critical for determining their effectiveness in addressing malnutrition.

The unexpected superior performance of bromelain necessitates detailed investigation of the underlying mechanisms. Future studies should examine the specific peptide profiles generated by different enzymatic treatments, compare the molecular weight distributions of hydrolysis products, and assess the relationship between peptide characteristics and bioactive properties. Optimization of hydrolysis conditions, including enzyme concentration, temperature, and duration, represents another critical research priority.

Conclusion

This study evaluates two enzymatic hydrolysis methods, bromelain and papain-alcalase combination for producing bioactive peptides from chicken feet. Enzymatic hydrolysis using bromelain shows superior results, while the papain-alcalase combination yielded lower protein concentrations, likely due to the excessive peptide degradation. Further studies are necessary to compare the peptide profiles, bioactivity, and functional properties of the hydrolysates from both methods to determine the most effective approach. These findings highlight the potential of enzymatic hydrolysis for converting poultry by-products into functional food ingredients, promoting sustainability in food and nutraceutical applications.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

AM: conceptualization, methodology, data curation, validation, writing – original draft; AR: formal analysis, data curation ; SD: formal analysis, data curation ; RS: conceptualization, writing – review & editing.

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References

1. Kementerian Koordinator Bidang Pembangunan Manusia dan Kebudayaan. Pemerintah Optimis Target Penurunan Stunting 14 Persen Tercapai di 2024. 2024 Nov 23 [cited 2024 Nov 23]. Available from: https://www.kemenkopmk.go.id/pemerintah-optimis-target-penurunan-stunting-14-persen-tercapai-di-2024
2. Tim Percepatan Penurunan Stunting. TP2S - Tim Percepatan Penurunan Stunting. 2024 Nov 21 [cited 2024 Nov 21]. Available from: https://stunting.go.id/
3. Fikawati S, Syafiq A, Ririyanti RK, Gemily SC. Energy and protein intakes are associated with stunting among preschool children in Central Jakarta, Indonesia: a case-control study. Mal J Nutr. 2021 Mar;27(1):081–91. https://doi.org/10.31246/mjn-2020-0074
4. Widyawardani N, Bustamam N, Aprilia CA, Hardini N, Prasetio PO, Yusuf KN, et al. Analysis of macronutrient and micronutrient intake with the incidence of stunting and wasting in toddlers 0-59 months of age at public health center Bojong, Bogor regency. World Nutr J. 2024 Feb;7(i2):i2. https://doi.org/10.25220/WNJ.V07.i2.0010
5. Elisanti AD, Jayanti RD, Amareta DI, Ardianto ET, Wikurendra EA. Macronutrient intake in stunted and non-stunted toddlers in Jember, Indonesia. J Public Health Res. 2023 Jul;12(3):22799036231197178. https://doi.org/10.1177/22799036231197178
6. Badan Pusat Statistik Indonesia. Rata-rata Konsumsi Perkapita Seminggu Menurut Kelompok Bahan Makanan Lainnya Per Kabupaten/kota - Tabel Statistik. 2024 Mar 13 [cited 2024 Mar 13]. Available from: https://www.bps.go.id/id/statistics-table/2/MjEwNiMy/rata-rata-konsumsi-perkapita-seminggu-menurut-kelompok-bahan-makanan-lainnya-per-kabupaten-kota.html
7. Ozturk-Kerimoglu B, Heres A, Mora L, Toldrá F. Antioxidant peptides generated from chicken feet protein hydrolysates. J Sci Food Agric. 2023;103(14):7207–17. https://doi.org/10.1002/jsfa.12802
8. Bravo FI, Mas-Capdevila A, Margalef M, Arola-Arnal A, Muguerza B. Novel Antihypertensive Peptides Derived from Chicken Foot Proteins. Mol Nutr Food Res. 2019 Jun;63(12):e1801176. https://doi.org/10.1002/mnfr.201801176
9. Casanova-Martí À, Bravo FI, Serrano J, Ardévol A, Pinent M, Muguerza B. Antihyperglycemic effect of a chicken feet hydrolysate via the incretin system: DPP-IV-inhibitory activity and GLP-1 release stimulation. Food Funct. 2019 Jul;10(7):4062–70. https://doi.org/10.1039/C9FO00695H
10. Mas-Capdevila A, Iglesias-Carres L, Arola-Arnal A, Suarez M, Muguerza B, Bravo FI. Long-term administration of protein

hydrolysate from chicken feet induces antihypertensive effect and confers vasoprotective pattern in diet-induced hypertensive rats. J Funct Foods. 2019 Apr;55:28–35. https://doi.org/10.1016/j.jff.2019.02.006

11. Akbarian M, Khani A, Eghbalpour S, Uversky VN. Bioactive Peptides: Synthesis, Sources, Applications, and Proposed Mechanisms of Action. Int J Mol Sci. 2022 Jan;23(3):3. https://doi.org/10.3390/ijms23031445
12. Zaky AA, Simal-Gandara J, Eun JB, Shim JH, Abd El-Aty AM. Bioactivities, Applications, Safety, and Health Benefits of Bioactive Peptides From Food and By-Products: A Review. Front Nutr. 2022 Jan;8:815640. https://doi.org/10.3389/fnut.2021.815640
13. BIOPEP-UWM - Katedra Biochemii Żywności. 2024 Mar 13 [cited 2024 Mar 13]. Available from: https://biochemia.uwm.edu.pl/en/biopep-uwm-2/
14. Sabrina N, Rizal M, Nurkolis F, Hardiansyah H, Tanner MJ, Gunawan WB, et al. Bioactive peptides identification and nutritional status ameliorating properties on malnourished rats of combined eel and soy-based tempe flour. Front Nutr. 2022 Sep;9:963065. https://doi.org/10.3389/fnut.2022.963065
15. Salindeho N, Mokolensang JF, Manu L, Taslim NA, Nurkholis F, Gunawan WN, et al. Fish scale rich in functional compounds and peptides: A potential nutraceutical to overcome undernutrition. Front Nutr. 2022 Dec;9:1072370. https://doi.org/10.3389/fnut.2022.1072370
16. Zaky AA, Simal-Gandara J, Eun JB, Shim JH, Abd El-Aty AM. Bioactivities, Applications, Safety, and Health Benefits of Bioactive Peptides From Food and By-Products: A Review. Front Nutr. 2022 Jan;8:815640. https://doi.org/10.3389/fnut.2021.815640
17. Jakubczyk A, Karaś M, Rybczyńska-Tkaczyk K, Zielińska E, Zieliński D. Current Trends of Bioactive Peptides-New Sources and Therapeutic Effect. Foods. 2020 Jul;9(7):7. https://doi.org/10.3390/foods9070846
18. Selamassakul O, Laohakunjit N, Kerdchoechuen O, Yang L, Maier CS. Bioactive peptides from brown rice protein hydrolyzed by bromelain: Relationship between biofunctional activities and flavor characteristics. J Food Sci. 2020 Mar;85(3):707–17. https://doi.org/10.1111/1750-3841.15052
19. Ahmad Nadzri FN, Tawalbeh D, Sarbon NM. Physicochemical properties and antioxidant activity of enzymatic hydrolysed chickpea (Cicer arietinum L.) protein as influenced by alcalase and papain enzyme. Biocatal Agric Biotechnol. 2021 Sep;36:102131. https://doi.org/10.1016/j.bcab.2021.102131
20. Trang CH, Le DTC, Lieu DT, Minh PQ. Determination of total protein content in biologicals using biuret method. jcvb. 2021;1(1+2):118–30. https://doi.org/10.56086/jcvb.vi1.13
21. Suhendi A, Rohman A, Cahyaningrum S. Validasi Metode Analisis Penetapan Kadar Protein Ekstrak Ikan Gabus dengan Metode Lowry dan Bromocresol Green. J Kefarm Indones. 2023 Feb;13(1):50–8. https://doi.org/10.22435/jki.v13i1.6219
22. Bradauskiene V, Vaiciulyte-Funk L, Cernauskas D, Dzingelevivinene R, Lima JPM, Braudauskaite A, et al. The Efficacy of Plant Enzymes Bromelain and Papain as a Tool for Reducing Gluten Immunogenicity from Wheat Bran. Processes. 2022 Oct;10(10):10. https://doi.org/10.3390/pr10101948