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The Potential of Gelatin Hydrolysate as Antioxidant from Wood Grasshopper

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Abstract
Gelatin hydrolysate has the potential as an antioxidant for functional food production. This study aimed to analyse the effect of enzyme concentration on the antioxidant activity of wood grasshopper gelatin hydrolysate. In this study, the hydrolysis of gelatin extracted from wood grasshoppers by enzymatic treatment with various bromelain concentrations has been carried out. Several parameters including the degree of hydrolysis, soluble protein content, and antioxidant activity using the DPPH ((1,1-diphenyl-2-picrylhydrazyl) method were then investigated. The statistical analysis indicated that the bromelain concentration (0.1 and 0.2% (b/V) used to break down the gelatin had a significant effect on the three parameters we analyszed. The greater bromelain concentration results in increased hydrolysis (2.04% and 2.60%) and soluble protein content (1,81% and 4,40%), leading to slightly increased antioxidant activity although categorized as very weak (more than 8000 ppm). Further purification process to recover the short peptide fragment is required to increase the antioxidant capacity.

Keywords:Gelatin; Insect; Hydrolysate; Bromelain; Antioxidant.

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INTRODUCTION

Gelatin is a high-molecular-weight biopolymer produced by the partial hydrolysis of collagen (Ahmad et al., 2017). Apart from its functional properties, gelatin is also used in functional food manufacturing. Functional foods usually contain bioactive compounds such as antioxidants, vitamins, natural extracts, and prebiotics that can provide essential nutrients for the body and exert beneficial effects on human health (He et al., 2021). Gelatin can be hydrolysed by enzymatic treatment to produce bioactive peptides that have antioxidant activity (He et al., 2021; Mirzapour-Kouhdasht et al., 2021; Shiao et al., 2021) as ingredients for functional food products. Previous study showed that the peptide fraction of commercial bovine, porcine, and tilapia gelatin hydrolysate potentially inhibited ACE, acetylcholinesterase, and prolyl endopeptidase activities to mitigate hypertension (Panjaitan et al., 2024).

Nowadays, alternative food sources are urgently needed due to the rapid expansion of the global population, civil conflicts, environmental issues, and pandemics. Insects can serve as food sources to address these issues (Egonyu et al., 2026). Previous research indicated that gelatin can be extracted from melon bugs (*Coridius viduatus*), sorghum bugs (*Agonoscelis versicoloratus*) (Mahamud et al., 2023; Mariod & Fadul, 2015), and black soldier fly (*Hermetia illucens*) (Chua et al., 2023).

Wood grasshopper is one of the insects that is commonly consumed in Indonesia as a fried dish, especially in Gunungkidul, Yogyakarta Special Region (Agustina & Pinasti, 2020). Study on Mexican grasshoppers showed that the body contains a high protein portion, between 52.60 and 75.87% (Torruco-Uco et al., 2019) so that they are potential to be developed as gelatin. A hadith which was narrated by Ahmad, Ibnu Majah, Ad-Daru Quthni, and At-Tirmidhi also supported the idea of using wood grasshoppers as they are one of the carcasses that are halal to be consumed:

ُأحِلَّتْ لَنَا مَيْتَتَانِ وَدَمَانِ، فَأَمَّا الْمَيْتَتَانِ فَالْحُوتُ وَالْجَرَادُ، وَأَمَّا الدَّمَانِ فَالْكَبِدُ وَالطِّحَال

Two types of carcasses and two types of blood are considered permissible. The permitted carcasses are fish and grasshoppers, while the lawful blood-derived organs are the liver and spleen (H.R. Ahmad, Ibnu Majah, Ad-Daru Quthni and At-Tirmidhi).

In our prior study, we were able to extract gelatin from wood grasshopper, which exhibits a functional group profile comparable to that of commercial gelatin derived from bovine (Dewi, 2023). In this study, the extracted gelatin was hydrolyzed using bromelain. The plant-derived enzymes bromelain and papain were used to prepare the buffalo skin collagen hydrolysate. The radical scavenging ability test against 2,2-diphenyl-1-picrylhydrazyl (DPPH) indicated that the hydrolysate produced by the bromelain 50 U/g skin had the highest % activity (Ahmad et al., 2024). Further, we analysed the soluble protein, degree of hydrolysis, and the antioxidant activity of gelatin hydrolysate.

RESEARCH METHODS

Materials

The materials used in this study included wood grasshoppers, technical-grade n-hexane, NaOH (Merck), distilled water, Lowry A, Lowry B, Lowry C, Lowry E, bromelain enzyme (HIMEDIA), sodium dihydrogen phosphate (Merck), disodium hydrogen phosphate (Merck), trichloroacetic acid (TCA), hydrochloric acid (HCl), ethanol, and 2,2-diphenyl-1-picrylhydrazyl (DPPH).

Gelatin Extraction and Hydrolsis

Wood grasshopper gelatin extraction was performed according to the previous research (Dewi, 2023). Dry gelatin was then hydrolyzed based on He et al. (2021) with slight modification using bromelain enzyme (0; 1; 2 g/L) in phosphate buffer solution at $55°C$ for 4 hours and heated at $100°C$ for 10 minutes to inactivate the bromelain. Gelatin hydrolysate then was dried using a freeze dryer.

Degree of Hydrolysis Estimated by SN (Soluble Nitrogen)-TCA

A gelatin hydrolysate sample was added with 10% trichloroacetic acid (TCA), homogenized, incubated for 30 minutes, and centrifuged to recover the filtrate. The filtrate was analysed using the Kjeldahl method, which consists of destruction, distillation, and titration processes with HCl solution (Hoyle & Merritt, 1994; Khirzin et al., 2020). The degree of hydrolysis was calculated as the ratio of TCA-soluble N to the total N in the sample and expressed as a percentage.

Soluble Protein Content Analysis

Freeze dried gelatin hydrolysate sample was dissolved and then centrifuged. A 1 mL of the supernatant was transferred to the test tube, then 1 mL of Lowry B reagent (Lowry A: Lowry B: Lowry C at a ratio of 10: 0.5: 0.5) was added and incubated for 10 min. A 3 mL of Lowry E reagent was added to the reaction mixture, followed by homogenization and incubation for 20 min. The absorbance was measured at 590 nm (Purwanto, 2014). The soluble protein concentration was then determined using a bovine serum albumin (BSA) standard curve prepared and analysed using the same procedure.

Antioxidant Activity Assay Using DPPH

The hydrolysate solution was mixed with an equal volume of 0.2 mmol/L ethanolic DPPH solution. After incubation in darkness at room temperature for 30 min, the absorbance was recorded at 517 nm (He et al., 2021).

RESULT AND DISCUSSION

Degree of Hydrolysis Corresponds to the Soluble Protein Content

The degree of hydrolysis represents the proportion of peptide bonds broken during hydrolysis. In this research, we estimated the degree of hydrolysis by measuring the TCA (trichloroacetic acid)-soluble nitrogen, which corresponds to the amino acids and small peptides produced from the hydrolysis reaction. This method has been used also in calculating the degree of hydrolysis of protein hydrolysate from Nile Tilapia (Mendes et al., 2026) and mung bean (Promjeen et al., 2025). Trichloroacetic acid will precipitate unhydrolyzed protein, while soluble nitrogen will remain in the supernatant, which can be recovered through centrifugation. The soluble N and the total N in the sample then can be determined by the Kjeldahl method (Rutherfurd, 2010).

In this study, the degree of hydrolysis was higher in the wood grasshopper gelatin hydrolysate sample generated from enzymatic treatment with more concentration as shown in Figure 1. Analysis of variance showed that enzyme concentration had a significant effect on the degree of hydrolysis $(p - value < 0.05)$. The degree of hydrolysis from the control sample of gelatin, which was treated under similar conditions but without adding an enzyme in this study, is notably high at 90.72%. This could be due to the degraded gelatin sample, resulting in many fragments that are not precipitable by TCA. However, we cannot verify this as we did not test for TCA-soluble nitrogen beforehand or analyze the protein using SDS-PAGE. Since the SN-TCA doesn't directly quantify the cleaved peptide bonds which can result in misleading, the combination with other

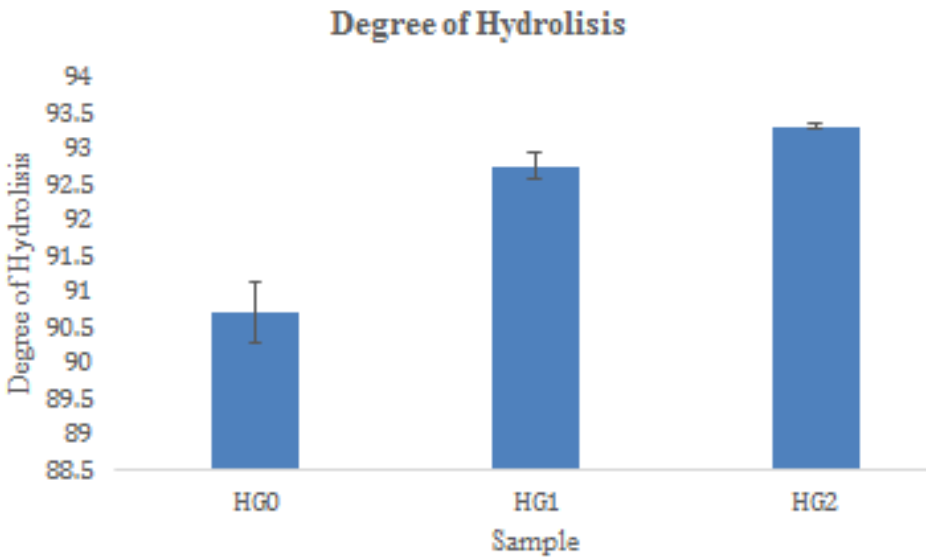


Figure 1: Degree of hydrolysis of wood grasshopper gelatin hydrolysate (HG0: without hydrolysis; HG1: hydrolysed using bromelain 1 g/L; and HG2 2 g/L)

method such as TNBS (trinitro-benzena-sulfonic acid) or OPA (ortho-phthaldialdehyde that will react with primary amino group produced during the hydrolysis (Rutherfurd, 2010)) is encouraged to generate the more reliable result.

Higher degree of hydrolysis results in the higher soluble protein content (Shaik et al., 2021) as shown in Figure 2. The analysis shows that higher bromelain concentration led to an increase in the soluble protein of wood grasshopper gelatin hydrolysate. The hydrolysis of high-molecular-weight proteins into smaller peptides can improve protein solubility (He et al., 2021). The presence of more enzymes resulted in the increasing of the hydrolysis reaction (high degree of hydrolysis) than can lead to the shorter fraction formation. Enzymatic hydrolysis could produce lower-sized fragments (1–20 kDa) that are more hydrophilic, thereby increasing the amount of protein remaining soluble in the aqueous phase. The soluble protein content of protein hydrolysates may be improved by polar amino acids, including asparagine, serine, threonine, and glutamine (Shaik et al., 2021). The statistical analysis showed that enzyme concentration significantly affects the soluble protein content ($p - value < 0.05$).

Antioxidant Activity of Wood Grasshopper Gelatin Hydrolysate

Figure 3 shows that the higher concentration of bromelain used during wood grasshopper gelatin hydrolysis leads to higher antioxidant activity (lower $IC50$ value), although the number is very low (higher than 8000 ppm). The statistical analysis indicated that enzyme concentration significantly affects the antioxidant activity (p-value < 0.05). Besides the extremely low activity, this study also has a limitation due to the absence of positive control such as ascorbic acid, BHT, or Trolox. Therefore, the antioxidant activity should be interpreted as relative activity under these test conditions. The hydrolysate that contains larger short fragments has the higher antioxidant activity against radical DPPH (2,2-diphenyl-1-picrylhydrazyl) (Shiao et al., 2021). The antioxidant activity of gelatin hydrolysates may be associated with the presence of basic and aromatic amino acids. The radical-scavenging activity of basic amino acids such as lysine, arginine, and histidine may be attributed to their ability to donate hydrogen atoms. Aromatic amino acids, particularly tyrosine and tryptophan, can also stabilize free radicals (He et al., 2021). Since we did not perform the purification process to recover the shorter peptide, there are still some larger fragments that hinder the antioxidant activity. Further purification processes to separate the peptide fraction from protein hydrolysate mixture need to be taken into account. Several separation and purification methods can be employed, including ultrafiltration, gel filtration,

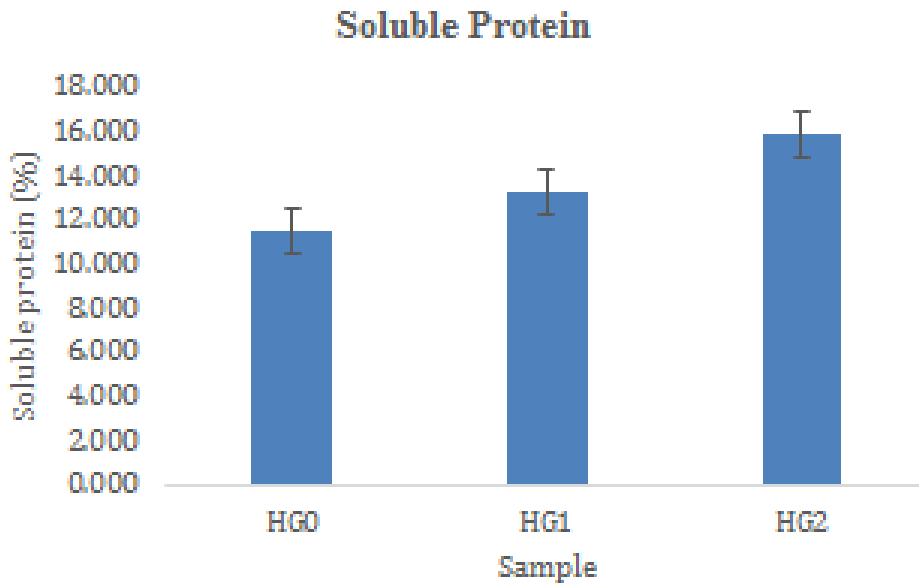


Figure 2: Soluble protein of wood grasshopper gelatin hydrolysate (HG0: without hydrolysis; HG1: hydrolysed using bromelain 1 g/L; and HG2 2 g/L)

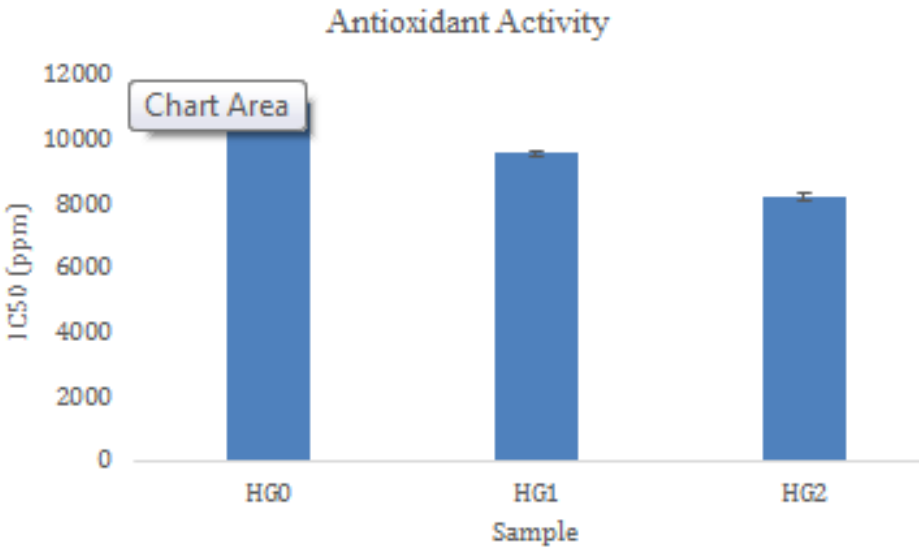


Figure 3: Antioxidant activity of wood grasshopper gelatin hydrolysate (HG0: without hydrolysis; HG1: hydrolysed using bromelain 1 g/L; and HG2 2 g/L)

ion-exchange chromatography, and reversed-phase high-performance liquid chromatography (RP-HPLC) (Panjaitan et al., 2024).

CONCLUSION

The concentration of bromelain used to hydrolyse wood grasshopper gelatin significantly influenced the degree of hydrolysis, soluble protein content, and antioxidant activity of the resulting hydrolysate although the numbers are low. Further enzymatic concentration optimization during the hydrolysis treatment needs to be conducted to determine the optimum condition. The purification of short peptide fragments will further boost the antioxidant activity.

References

Agustina, S. H. & Pinasti, V. I. S. (2020). Walang goreng sebagai ikon wisata kuliner gunungkidul. *Dimensia: Jurnal Kajian Sosiologi*, 8(2):149–164, DOI: 10.21831/dimensia.v8i2.36369.

Ahmad, T., Ismail, A., Ahmad, S. A., Khalil, K. A., Kumar, Y., Adeyemi, K. D., & Sazili, A. Q. (2017). Recent advances on the role of process variables affecting gelatin yield and characteristics with special reference to enzymatic extraction: A review. *Food Hydrocolloids*, DOI: 10.1016/j.foodhyd.2016.08.007.

Ahmad, T., Kumar, Y., Muzaddadi, A. U., Kumar, V., Singhal, S., Kumar, D., et al. (2024). Extraction and characterization of collagen hydrolysate from buffalo (bubalus bubalis) skin including its antioxidant properties and antiarthritic effect. *Waste and Biomass Valorization*, 15(7):4159–4173.

Chua, L. K., Lim, P. K., Thoo, Y. Y., Neo, Y. P., & Tan, T. C. (2023). Extraction and characterization of gelatin derived from acetic acid-treated black soldier fly larvae. *Food Chemistry Advances*, 2, DOI: 10.1016/j.focha.2023.100282.

Dewi, D. K. (2023). Isolasi dan karakterisasi gelatin dari belalang kayu (valanga nigricornis). Master's thesis, UIN Sunan Kalijaga Yogyakarta, Yogyakarta.

Egonyu, J. P., Darr, D., Niassy, S., Pohle-Fröhlich, R., & Kleinke, M. (2026). Conserving, farming, and commercializing edible insects for a circular food and bioeconomy: Multidisciplinary perspectives. *International Journal of Tropical Insect Science*, DOI: 10.1007/s42690-025-01706-1.

He, L., Gao, Y., Wang, X., Han, L., Yu, Q., Shi, H., & Song, R. (2021). Ultrasonication promotes extraction of antioxidant peptides from oxhide gelatin by modifying collagen molecule structure. *Ultrasonics Sonochemistry*, DOI: 10.1016/j.ultsonch.2021.105738.

Hoyle, N. T. & Merritt, J. H. (1994). Quality of fish protein hydrolysates from herring (clupea harengus). *Journal of Food Science*, 59(1):76–79.

Khirzin, M. H., Hilmi, M., Prastujati, A. U., Mawardi, N., & Rahayu, R. (2020). Karakteristik hidrolisat gelatin tulang itik dengan enzim tripsin sebagai penghambat alfa amilase ($\alpha - amylaseinhibitor$). $JurnalIlmiahInovasi, 20(3)$.

Mahamud, N., Santiworakun, N. Y., Chaovasuteeranon, S., & Boonmalert, F. (2023). Halal alternative sources of gelatin: A review. *Journal of Halal Science, Industry, and Business*, 1(2):43–56, DOI: 10.31098/jhasib.v1i2.1987.

Mariod, A. A. & Fadul, H. (2015). Extraction and characterization of gelatin from two edible sudanese insects and its applications in ice cream making. *Food Science and Technology International*, 21(5):380–391, DOI: 10.1177/1082013214541137.

Mendes, P. D. S., Reitz Cardoso, F. A., Giugiolli, M. F., Wolhmuth, G., Oliveira, M. I. C., Fante, V. D. F., et al. (2026). Enzymatic hydrolysis and characterization of protein concentrate obtained from mechanically separated meat of nile tilapia (oreochromis niloticus). *Journal of Food Science*, 91(1):e70799.

Mirzapour-Kouhdasht, A., Moosavi-Nasab, M., Kim, Y. M., & Eun, J. B. (2021). Antioxidant mechanism, antibacterial activity, and functional characterization of peptide fractions obtained from barred mackerel gelatin with a focus on application in carbonated beverages. *Food Chemistry*, DOI: 10.1016/j.foodchem.2020.128339.

Panjaitan, F. C. A., Shie, S. T., Park, S. H., Sevi, T., Ko, W. L., Aluko, R. E., & Chang, Y. W. (2024). Bioactive properties of enzymatic gelatin hydrolysates based on in silico, in vitro, and in vivo studies. *Molecules*, 29(18), DOI: 10.3390/molecules29184402.

Promjeen, K., Phongthai, S., Singh, K., Chaisan, W., Pakakaew, P., Srichairatanakool, S., et al. (2025). Optimization of enzymatic protein hydrolysate from mung bean (vigna radiata l.), and its functional properties. *Foods*, 14(14):2459.

Purwanto, M. G. M. (2014). Perbandingan analisa kadar protein terlarut dengan berbagai metode spektroskopi uv-visible. *Jurnal Ilmiah Sains & Teknologi*, 7(2):64–71.

Rutherfurd, S. M. (2010). Methodology for determining degree of hydrolysis of proteins in hydrolysates: A review. *Journal of AOAC International*, 93(5):1515–1522.

Shaik, M. I., Mohd Noor, S. N. A. A., & Mohd Sarbon, N. (2021). In vitro angiotensin converting enzyme (ace), antioxidant activity and some functional properties of silver catfish (pangasius sp.) protein hydrolysate by ultrafiltration. *Biocatalysis and Agricultural Biotechnology*, DOI: 10.1016/j.bcab.2021.102100.

Shiao, W. C., Wu, T. C., Kuo, C. H., Tsai, Y. H., Tsai, M. L., Hong, Y. H., & Huang, C. Y. (2021). Physicochemical and antioxidant properties of gelatin and gelatin hydrolysates obtained from extrusion-pretreated fish (oreochromis sp.) scales. *Marine Drugs*, DOI: 10.3390/md19050275.

Torruco-Uco, J. G., Hernández-Santos, B., Herman-Lara, E., Martínez-Sánchez, C. E., Juárez-Barrientos, J. M., & Rodríguez-Miranda, J. (2019). Chemical, functional and thermal characterization, and fatty acid profile of the edible grasshopper (sphenarium purpurascens ch.). *European Food Research and Technology*, DOI: 10.1007/s00217-018-3160-y.

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