Research Article

Novel Collagen Isolated from Indonesian Local Sheepskin Using *Aspergillus oryzae* and *Bacillus subtilis* Enzymes

Dita Prameswari Trenggono Putri[1], Ibni Mitha Utami[1], Mohammad Zainal Abidin[1], Nanung Agus Fitriyanto[1], and Yuny Erwanto[1,2*]

[1]Department of Animal Products Technology, Faculty of Animal Science, Gadjah Mada University, Yogyakarta, 55281, Indonesia; [2]Institute for Halal Industry and System, Gadjah Mada University, Yogyakarta, Indonesia.

Abstract | Collagen is widely used in food and pharmaceutical products, but efficient extraction methods are still under exploration. This study was conducted to utilize Indonesian Local sheepskin by extracting the collagen using protease from *Aspergillus oryzae* and *Bacillus subtilis*. Sheepskin collagen was characterized, and the result was compared with commercial collagen. Indonesian Sheepskin collagen was successfully extracted by protease from *Aspergillus oryzae* and *Bacillus subtilis*. The yield of collagen extracted using protease from *Aspergillus oryzae* (C1) was higher than *Bacillus subtilis* (C2), which reached 20.69 ± 0.60% and 16.09 ± 0.39%, respectively. The result of SDS-PAGE analysis on Garut sheepskin collagen (C1 and C2) shows the same motif as the protein band profile of commercial collagen type I, which has α1 and α2 chains with molecular weights of around 140 kDa and 100 kDa, respectively. The result of FTIR analysis displays a similar five-amide band motif in all sample, including Amide A (±3300 cm^{-1}), B (±2900 cm^{-1}), I (±1650 cm^{-1}), II (±1500 cm^{-1}) and III (±1230 cm^{-1}), which indicates a collagen structure. The melting temperature of the samples in this study remained within the range of the literature in the prior research, which reached 145.43°C and 167.05°C for C1 and C2 respectively. The pH and viscosity of the C1 sample and the C2 sample were quite similar. Overall, collagen extracted using *Aspergillus oryzae* protease exhibited the most desirable characteristics, suggesting its potential as a high-quality local collagen source.

Keywords | Sheepskin, Collagen, Extraction, Characterization, Protease, Animal by-product

Received | May 20, 2025; **Accepted** | July 19, 2025; **Published** | September 05, 2025
***Correspondence** | Yuny Erwanto, Department of Animal Products Technology, Faculty of Animal Science, Gadjah Mada University, Yogyakarta, 55281, Indonesia; **Email:** yunyer@ugm.ac.id
Citation | Putri DPT, Utami IM, Abidin MZ, Fitriyanto NA, Erwanto Y (2025). Novel collagen isolated from indonesian local sheepskin using *Aspergillus oryzae* and *Bacillus subtilis* enzymes. Adv. Anim. Vet. Sci., 13(9): 2032-2040.
DOI | https://dx.doi.org/10.17582/journal.aavs/2025/13.9.2032.2040
ISSN (Online) | 2307-8316

INTRODUCTION

Increased animal consumption has led to higher livestock slaughter rates, including sheep. According to Ministry of Agriculture of The Republic of Indonesia (2023), the development of sheep meat production in Indonesia over the period 1983–2023 has shown a significant upward trend, with an average annual increase of 24.40%. The increase of meat production, generating a larger volume of by-products like sheepskin. However, not all of this by-product was utilized, potentially causing environmental issues and necessitating corrective measures.

Indonesia has several local sheep breeds from different areas, including Garut, Wonosobo, Batur, and Kisar. According to Hartatik (2016), Garut sheep is one of the breeds of local Indonesian sheep that is the result of crossbreeding between local sheep and Kaapstad sheep from Africa

and Merino sheep from South Australia. Ministry of Agriculture of The Republic of Indonesia (2011) stated that physical characteristics of Garut sheep include body and head color combination of black and white, large and long curve horns, small ears, and a triangular-shaped tail.

Garut sheep have been traditionally bred in West Java Province. Moreover, Ministry of Agriculture of The Republic of Indonesia (2023) reported that West Java is the leading province in Indonesia in terms of sheep population, contributing an average of 12.06 million heads annually. This represents approximately 34.52% of the national small ruminant population, with sheep accounting for nearly 90% of the total. This suggests that the Garut sheep breed constitutes a significant portion of the sheep population in Indonesia. Alhuur *et al.* (2023) state that the Garut sheep breed has a larger body size than other local sheep in Indonesia, which potentially generates more meat and by-products, including skin. Generally, Garut sheepskin are utilized for leather, however, a significant amount of skin remains unused and becomes waste. Van der Merwe *et al.* (2021) stated that sheepskin typically accounts for about 7–13% of their body weight Furthermore, animal skin consists of approximately 60% collagen on a dry weight basis, making it a promising raw material for collagen protein production (Matinong *et al.*, 2022). Therefore, Garut sheepskin was chosen as a potential source of local collagen production compared to the other local breeds.

Furthermore, collagen is the most abundant structural protein with a triple-helix structure found in animals, with 28 types identified to date (Tang *et al.*, 2022). Advances in technology and changing lifestyles have expanded its use as a key raw material in the food, pharmaceutical, biomedical, and cosmetic industries (Li *et al.*, 2020). Indonesia imports significant quantities of collagen, much of which is untraceable in terms of halal certification. Since most animal slaughtering practices in Indonesia follow halal standards, there is an abundant supply of halal by-product material for collagen production.

Collagen hydrolysis using enzymes was preferable due to the milder reaction conditions than hydrolysis using base or acid. Moreover, the products are relatively predictable and controllable since enzymes typically target specific peptide bonds for cleavage, which also creates specific sequences that can play a role in bioactivity analysis for further research. Based on prior studies, proteases from animals were usually used to extract collagen, such as pepsin (Devita *et al.*, 2021), but the animal-based enzymes were harder to obtain due to the farming and slaughtering processes compared to plant or microorganism-based enzymes. Plant- and bacteria-derived enzymes may also offer an alternative for halal and cheaper enzyme sources. In this study, *Aspergillus oryzae* and *Bacillus subtilis* were chosen as microorganism to produce alternative enzyme to extract collagen. Both of them were classified as Generally Recognized As Safe (GRAS) by the Food and Drug Administration (FDA), and their enzymes are widely used in the food and pharmaceutical industries (Daba *et al.*, 2021; Stülke *et al.*, 2023). This makes them suitable for processes where the final product is intended for human use. Enzymes from *Aspergillus oryzae* and *Bacillus subtilis* are commercially available, affordable, and easy to produce via fermentation, making them practical for scale-up collagen extraction from animal by-products like sheepskin than other microorganism enzymes.

Aspergillus oryzae is a fungus that can produce enzymes that have high proteolytic activity. This enzyme remained stable between pH 4.5 and 6.0, indicating its classification as an acid protease (Daba *et al.*, 2021). Naeem *et al.* (2022) stated that proteases from *Aspergillus oryzae* prefer hydrophobic amino acids as specific cutting sites. *Bacillus subtilis* bacteria can also produce proteolytic enzymes that have been used in so many industries. Peterle *et al.* (2020) reported that this enzyme was categorized as a serine protease and cleaves amino acids at leucine and threonine.

The information on microorganism-based enzymes, such as protease from *Aspergillus oryzae,* for extracting collagen is still limited. Moreover, the use of protease from *Bacillus subtilis* to extract collagen is never been done. Accordingly, the main objectives of this study were the extraction and characterization of collagen from the skin of Garut Sheep using proteases from *Aspergillus oryzae* and *Bacillus subtilis*.

MATERIALS AND METHODS

Ethical approval

Ethical approval was not required as the sheepskin was obtained from a local market and did not involve live animals.

Materials

Skins from eighteen-month-old purebred male Garut sheep were purchased from a local market in Cirebon, West Java, and preserved by ice during transportation. Proteases from *Aspergillus oryzae* (500kHUT/g) and *Bacillus subtilis* (100kNPU/g) were purchased from Sisco Research Laboratories, India. Commercial collagen type I from bovine achilles was purchased from Sigma Aldrich. The chemical reagents used in this study were analytical grade.

Sample preparation

Sheepskin hair was removed using an animal hair remover and a cutter knife, followed by fleshing to remove residual

meat and fat. The skins were then sliced into small segments, with 50 g allocated to each sample pouch. The prepared skin was preserved in the freezer at -18°C for a week, then thawed before the extraction was conducted.

Sheepskin collagen extraction process

The extraction of the samples was carried out following the method of Wahyuningsih *et al.* (2018). Extraction using *Aspergillus oryzae* (C1) and *Bacillus subtilis* (C2). The extraction process graph was presented in Figure 1.

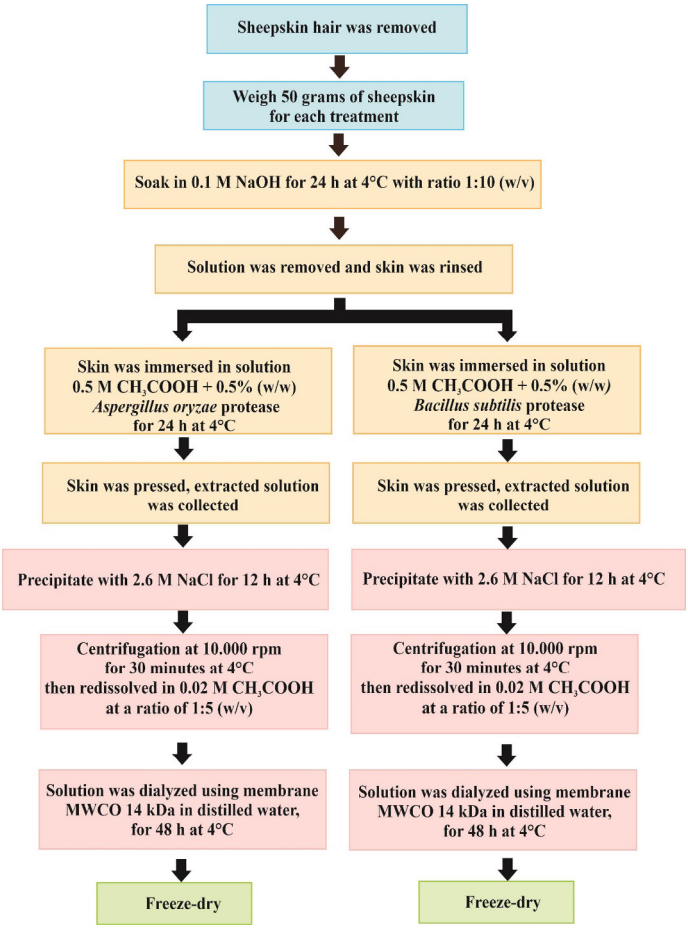

Figure 1: Schematic of sheepskin collagen extraction protocol.

Collagen characterization

SDS-PAGE analysis

The molecular weight determination was done using SDS PAGE analysis by Kuwahara (2021), with some modifications. Initially, 10 mg of extracted collagen was dissolved in 0.1 M acetic acid. Meanwhile, the dilution of commercial collagen was done in distilled water. The sample was then mixed with loading buffer and heated at 55°C for 15 minutes. A 10 µL of denatured solution was loaded onto an SDS-PAGE with a 4-15% gradient gel, which had been set in the analysis chamber. Electrophoresis was conducted at a constant voltage of 170 V for 1.5 hours. Then, the gel was stained with Coomassie blue and destained by rinsing with distilled water overnight.

Fourier transform infrared spectroscopy analysis (FTIR)

The FTIR analysis was conducted following the method described by Li *et al.* (2020). The collagen sample was prepared according to the specified procedure and analyzed using an FTIR device. Measurements were performed within the wavenumber range of 4,000 to 500 cm^{-1}. The functional groups of the collagen sample were determined by correlating the observed absorption peaks with the characteristic absorption ranges of protein functional groups.

Differential scanning calorimetry (DSC) analysis

The thermal stability of the sample was assessed using differential scanning calorimetry (DSC) according to the method described by Faralizadeh *et al.* (2021). Approximately 5-10 mg of the collagen was wrapped in an aluminium pan and subjected to heating from 20°C to 300°C at a rate of 10°C per minute. The resulting DSC thermogram displayed characteristic isothermal peaks associated with collagen.

Evaluation of collagen yield

The methodology of collagen yield determination was described by Akram and Zhang (2020) with slight modification. It was calculated by dividing the wet weight of the extracted collagen by the initial sample weight (50 g). The yield percentage is calculated as follows:

$$\textit{Collagen yields (\%)} = (\textit{Wet weight of collagen} / \textit{Initial weight}) \times 100\%$$

pH analysis

The pH value was measured following the method outlined by Devita *et al.* (2021). The analysis involved using a pH meter to test the wet collagen sample. The pH meter was initially set off and permitted to stabilise. The electrode was afterwards immersed in the sample, and the pH value was collected once it stabilised and remained constant.

Viscosity analysis

The viscosity of the samples was measured according to the method described in reference (Vidal *et al.*, 2020) with modifications. Briefly, 0.03 g of each sample was dissolved in 0.1 M acetic acid. The viscosity of the resulting solutions was then determined using a viscometer at a speed of 100 rpm with spindle number 61.

Experimental design and data analysis

The Yield, pH, and viscosity data were collected in triplicate and analyzed. A mixed sample of three batches was used to collect SDS-PAGE, DSC, and FTIR data. All the data results were analyzed descriptively. Statistical analyses were performed using SPSS and Excel software.

RESULTS AND DISCUSSION

Collagen is a crucial ingredient in the food, medicine, and cosmetics industries. Indonesian local sheepskin offers a potential good quality and halal alternative to collagen sources. To assess the quality of this local collagen, its characteristics must be compared to those of commercially available collagen. In this study, various analyses, including molecular weight, functional groups, thermal stability, pH, viscosity, and total yield, were conducted to evaluate the quality of collagen derived from Garut sheepskin compared with the purchased commercial collagen and data from the previous study.

The molecular weight of collagen (SDS-PAGE)

The SDS-PAGE results showed the molecular weight of collagen from all samples including Garut sheepskin that extracted using protease from *Aspergillus oryzae* (C1) and *Bacillus subtilis* (C2) compared with commercial collagen type I (CC) is shown in Figure 2, C1 and C2 exhibited the same characteristic bands as type I collagen, including α1, α2, and β chains. As shown in Figure 1, the molecular weight of the α1 chain for C1, C2, and CC was approximately 125 kDa, the α2 chain was about 110 kDa, and the β chain was about 200 kDa. Overall, the molecular weight profiles of the extracted collagens (C1 and C2) and the commercial collagen (CC) exhibit similar patterns. According to Reátegui-Pinedo *et al*. (2022), tilapia skin collagen shows a comparable molecular weight distribution to that of commercial type I collagen derived from bovine skin. The molecular weight of the α1 chain was 120 kDa, while the α2 chain was at 116 kDa. Other bands of higher molecular weight were β chains, which have an approximate weight of around 200 kDa. The results of extracted collagen obtained from this study show a similar molecular weight pattern with commercial collagen type I in SDS-PAGE analysis and previous literature.

Analysis of functional group (FTIR)

Figure 3 and Table 1 illustrate the peak regions of the infrared spectrum for collagen samples. Both sample-extracted collagen (C1 and C2) and commercial collagen display a similar functional group pattern, characterized by five distinct amide bands, including Amide A, B, I, II, and III, which are typical of collagen structures. The spectral peaks appeared in the ranges of 3322.65 to 3412.80 for amide A, 2924 to 2926.71 for amide B, 1652.12 to 1655.96 for amide I, 1545 to 1550.76 for amide II, and 1238.73 to 1239.40 for amide III. This result is similar to the FTIR result of collagen from sheep by-product by Vidal *et al*. (2020), which reported that sheep by-product collagen has five amide bands. Amide A at approximately 3325 cm^{-1}, amide B at 2924 cm^{-1}, amide I at 1659 cm^{-1}, amide II at 1553 cm^{-1}, and amide III at 1231 cm^{-1}. The presence of these absorption bands indicates the retention of the triple-helix structure, further supported by an IR absorption ratio of approximately 0.84, close to the value expected for native collagen.

Table 1: Peak positions of FTIR spectra of commercial collagen type I (CC), sheepskin collagen extracted using protease from *Aspergillus oryzae* (C1) and *Bacillus subtilis* (C2).

	Wavenumber (cm^{-1})		
	C1	C2	CC
Amide A	3412.80	3338.96	3322.65
Amide B	2926.71	2926.63	2924.00
Amide I	1655.90	1655.96	1652.12
Amide II	1550.76	1548.46	1545.98
Amide III	1239.38	1238.73	1239.40

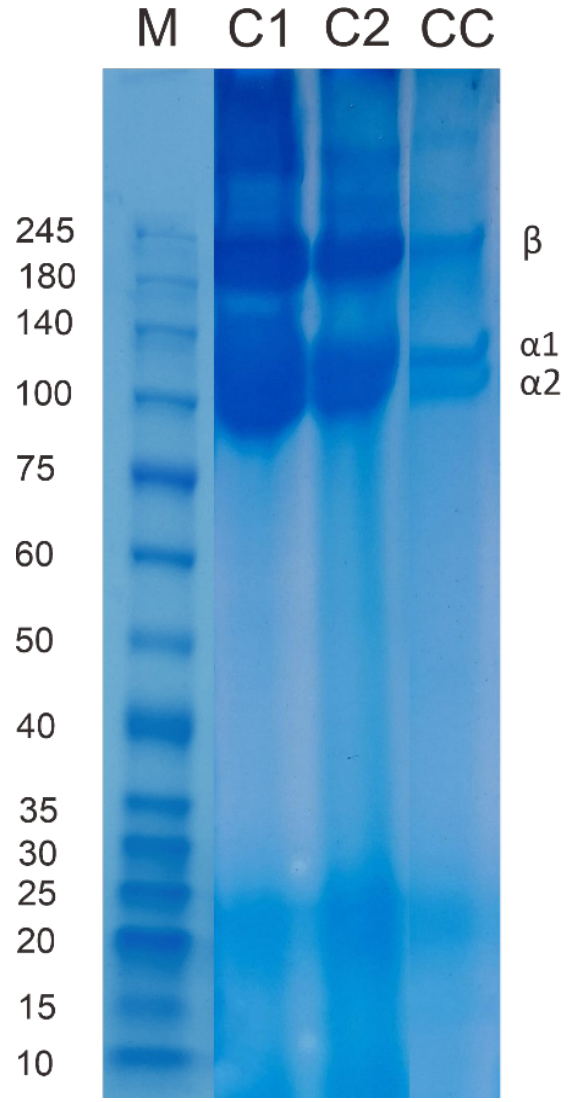

Figure 2: SDS PAGE results of marker (M), commercial collagen type I (CC), sheepskin collagen extracted using protease from *Aspergillus oryzae* (C1) and *Bacillus subtilis* (C2).

Table 2: Thermal stability of sheepskin collagen and commercial collagen type I.

Collagen sample	Collagen sources	Melting temperature (°C)
Collagen extracted using protease from *Aspergillus oryzae* (C1)	Sheepskin	145.43
Collagen extracted using protease from *Bacillus subtilis* (C2)	Sheepskin	167.05
Commercial collagen type 1 (CC)	*Bovine achilles*	153.66

Table 3: Yield, viscosity and pH of sheepskin collagen.

Extracting agent	Total yield (%)	Viscosity (cP) in 0.1 M CH_3COOH	pH of wet collagen
Acetic acid (0.5 M) + Protease from *Aspergillus oryzae* (C1)	20.69 ± 0.60[b]	5.18± 0.03	4.4 ± 0.05
Acetic acid (0.5 M) + Protease from *Bacillus subtilis* (C2)	16.09 ± 0.39[a]	5.18 ± 0.02	4.5 ± 0.05

[a,b] superscript shows significant differences in the same column (P<0.05).

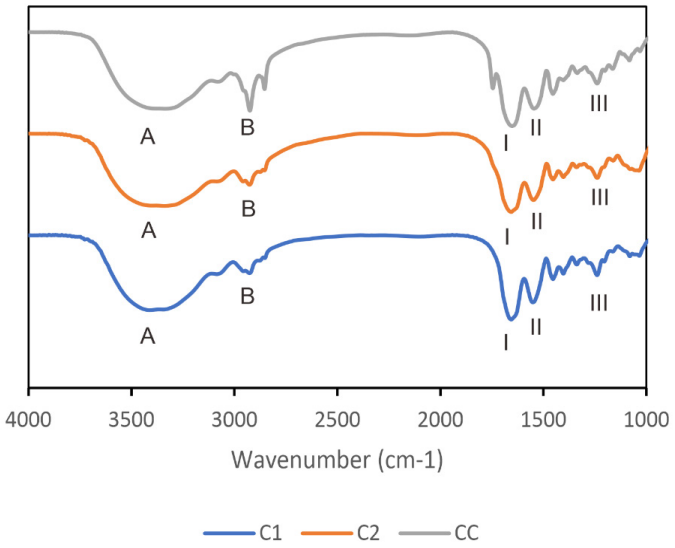

Figure 3: FTIR spectra of commercial collagen type I (CC), sheepskin collagen extracted using protease from *Aspergillus oryzae* (C1) and *Bacillus subtilis* (C2).

These findings of functional group profiles of extracted sheepskin collagen also closely resemble commercial collagen in a previous study. According to Gao *et al.* (2018), Amide A is associated with N–H stretching vibrations, and Amide B arises from asymmetric CH_2 stretching. Generally, Amide A wavenumbers ranged from 3440-3400 cm^{-1}. However, the band shifts to a lower wavenumber at around 3300 cm^{-1}. Furthermore, Devita *et al.* (2021) reported that amide B of collagen generally ranged from 2938-2658 cm^{-1}.

Amide I exhibits stretching vibrations of carbonyl groups (C= O bonds) which correspond with the four secondary structures of proteins, including α-helix, β-sheet, β-turn, and irregular structure. The α-helix structure ranged from 1659 to 1645 cm^{-1}, the β-sheet or non-stranded extended structure was around 1640 to 1620 cm^{-1}, the β-turn structure was from 1700 to 1660 cm^{-1}, and the last was an irregular structure, ranging from 1644-1640 cm^{-1} (Gao *et al.*, 2018). Depending on the literature, the secondary structure of sheep collagen in this study is categorized as α-helix.

Amide II is associated with N–H bending and C–H stretching, and Amide III is linked to intermolecular interactions in collagen, involving C–N stretching and N–H bending. The Amide II band in collagen typically appears in the range of 1600 to 1550 cm^{-1} and Amide III ranges from 1400 to 1200 cm^{-1} (Gao *et al.*, 2018). According to Reátegui-Pinedo *et al.* (2022), commercial collagen shows characteristic absorption bands at specific wavenumbers: Amide A at 3304 cm^{-1}, Amide B at 2925 cm^{-1}, Amide I at 1631 cm^{-1}, Amide II at 1547 cm^{-1}, and Amide III at 1235 cm^{-1}.

The ratio of the Amide III band to the CH_2 bending vibration at 1450 cm^{-1} in both C1 and C2 samples was approximately 1, showing that the triple helix structure of sheep collagen remained and was not harmed by enzymatic extraction. This finding aligns with the report by Gao *et al.* (2018), which stated a ratio close to 1 is indicative of an intact collagen triple helix. The preservation of this structural feature in the extracted samples suggests that the enzymatic extraction process successfully maintained the native integrity of the collagen, making it comparable in quality to commercial collagen. The FTIR analysis results for the extracted samples C1 and C2 have the same pattern as the commercial ones, and all the spectra peaks are still in the range of collagen structure according to the literature.

Thermal stability analysis (DSC)

The DSC analysis was used to analyse the thermal stability of extracted sheepskin and commercial collagen. The results of the DSC analysis of the samples can be seen in Figure 4, and the specific value is in Table 2. All the samples show different thermal peak values. Among the samples analyzed, C1 exhibited the lowest thermal transition, with an endothermic peak at 145.43°C. The CC sample displayed an intermediate peak at 153.74°C, while C2 showed the highest thermal stability, reaching an endothermic peak at 167.05 °C. According to Faralizadeh *et al.* (2021) thermal peak is associated with modifications in collagen's cross-linked structure, leading to the unfolding of its triple-

helical structure, commonly referred to as the melting temperature (Tm). In comparison, Capella-Monsonís *et al.* (2018) reported that commercial Type I collagen has a significantly lower Tm of 116.94°C. Additionally, Ferraro *et al.* (2017) documented that collagen derived from different bovine bone regions exhibited melting peaks between 162.42 °C and 180.01 °C. In this present study, commercial collagen shows higher thermal stability than in the previous study. On the other hand, C2 shows higher thermal stability than C1 and CC. Bhuimbar *et al.* (2024) reported that *Bacillus subtilis* protease selectively cleaves collagen, preserving the core triple helix structure and maintaining thermal stability.

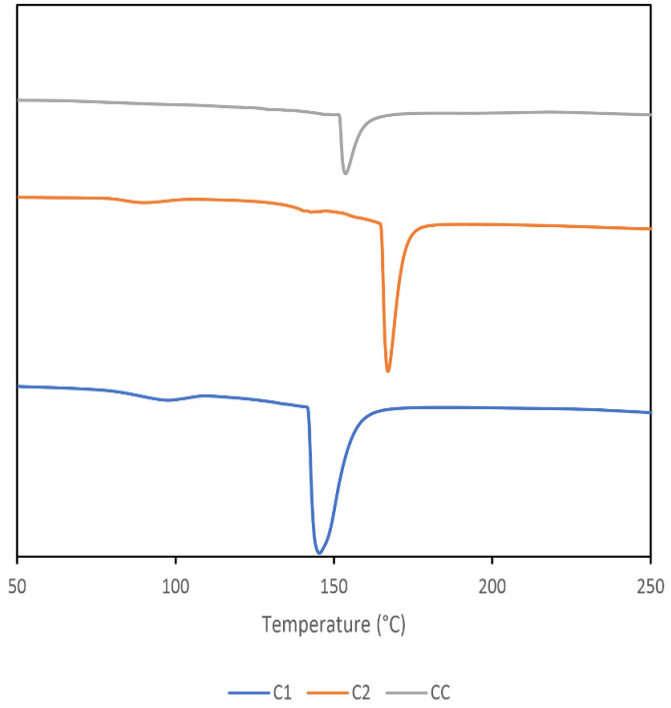

Figure 4: DSC spectra of commercial collagen type I (CC), sheepskin collagen extracted using protease from *Aspergillus oryzae* (C1) and *Bacillus subtilis* (C2).

Higher melting temperatures indicate superior collagen quality, reflecting greater heat resistance. Gauza-Włodarczyk *et al.* (2017) asserted that the variation in denaturation temperatures of the examined materials may be attributed to the hydroxyproline concentration. The concentration of this amino acid is associated with the thermal stability of collagen. Hydroxyproline is a specific amino acid found in collagen that contributes to thermal stability through the formation of hydrogen bonds characterised by the structure –O–H…O=C=. Furthermore, the stabilising influence of hydroxyproline is associated with the distinctive stereochemical characteristics of the pyrrolidone ring.

Yield, viscosity, and pH of extracted collagen

The total yield of extracted collagen from Garut sheepskin is presented in Table 3. The yield of extracted collagen using protease from *Aspergillus oryzae* (C1) was significantly higher than collagen extracted with protease from *Bacillus subtilis* (C2), which reached 20.69 ± 0.60 and 16.09 ± 0.39%, respectively, indicating protease from *Aspergillus oryzae* was more effective in extracting the collagen from sheepskin. C1 shows a higher yield than sheepskin collagen extracted by an acidic method (17.03%), but C2 shows a lower value (Putri *et al.*, 2024). However, both outcomes demonstrated a greater value in comparison to the collagen extracted through the acid method in a previous research conducted by Devita *et al.* (2021), which reported that the yield of fish skin collagen obtained through the acid method was 3.05%. Moreover, C1 is higher than fish skin collagen extracted using papain (15.20%) and trypsin (13.83%), but has a lower yield value than collagen extracted by pepsin (52.02%) and bromelain (42.76%) (Devita *et al.*, 2021).

Differences in collagen extraction yield are largely influenced by the species and type of tissue used as raw material, as well as by factors such as solvent concentration and the extraction method applied. Generally, thinner and less fibrous skins, such as those from fish, are more susceptible to enzymatic or chemical hydrolysis, resulting in higher collagen yields. In contrast, thicker and more cross-linked tissues, such as ovine or bovine skins, present greater structural resistance, making collagen extraction more challenging and often resulting in lower yields (Amirrah *et al.*, 2022). Wu *et al.* (2019) reported that enzymatic methods, can improve collagen yield compared to acid extraction. In acid-enzyme extraction, the acidic solution swells the sample, enabling pepsin to efficiently cleave cross-linked molecules at the telopeptide region without damaging the triple helical structure. This process results in higher yields. The numerous inter-chain and intra-chain crosslinks at the telopeptide region also contribute to the yield difference between the two methods.

The viscosities of C1 and C2 have similar values (Table 3), reaching 5.18 ± 0.03 and 5.18 ± 0.02 cP. The viscosity of the extracted collagen in this work was similar than that reported by Said *et al.* (2018), who reported that the viscosity values of extracted collagen from Bali cattles's hide range from 5.53 to 7.52 cP. Hadfi and Sarbon (2019) state that multiple variables influence viscosity, including the temperature of the solution and hydrogen bonding. The presence of hydrogen bonds at varying concentrations of acetic acid may also influence the viscosity of collagen. The rise in temperature results in an augmentation of thermal energy. The molecules exhibit increased mobility, resulting in decreased viscosity. The method used to measure viscosity, including the combination of the viscometer spindle type and speed, can significantly influence the results (Mariano *et al.*, 2024). The Standardized equipment

and procedures are very important to get consistent and comparable viscosity results.

The pH of the isolated collagen remains consistent with the range seen in prior experiments. The pH value of both extracted collagens in wet conditions was also not significant, which shows 4.4 ± 0.05 and 4.5 ± 0.05. Devita *et al*. (2021) reported that the pH value of bromelain-soluble collagen derived from big-eye tuna (*Thunnus obesus*) skin varied between 4.30 and 4.40. Nonetheless, the value of the pH obtained in this investigation was inferior to that of the commercial collagen reported in the previous work by Gao *et al*. (2018). A separate study conducted by Hadfi and Sarbon (2019) reported that commercial collagen possesses a pH of 6.25 ± 0.04. The reduction in pH may result from the amount of acidic amino acids generated during the enzymatic hydrolysis. The variation in pH value may also result from the characteristics of the amino acids comprising the collagen. León-López *et al*. (2019) explained that the sequence and the arrangement of amino acid residues vary according to the type and duration of hydrolysis. Moreover, the length of time of hydrolysis can influence the pH level of collagen.

The findings and discourse reveal that the isolated Garut sheepskin collagen is categorised as type I collagen. It may serve as an alternate source of high-quality collagen derived from local livestock. Collagen derived from the protease of *Aspergillus oryzae* (C1) exhibited superior outcomes relative to alternative treatments. However, this study was limited by the use of a single extraction condition and a relatively small sample size, which may affect the generalizability of the results. Future research should explore multiple extraction parameters and larger sample sets to validate the findings and assess the scalability and industrial applicability of the process.

CONCLUSION

Two proteases derived from *Aspergillus oryzae* and *Bacillus subtilis* successfully extracted collagen. Based on the characterization data, sheepskin collagen was identified as type I collagen. Sheepskin collagen extracted using protease from *Aspergillus oryzae* produces a higher yield than *Bacillus subtilis*. Functional group, Thermal stability, pH, and viscosity of sheepskin collagen were still in the range of collagen structure, based on the literature. Collagen extracted from Garut sheepskin using *Aspergillus oryzae* protease exhibited optimal characteristics, suggesting its potential as an alternative high-quality halal collagen source.

ACKNOWLEDGMENT

The author would like to thank the Indonesian Ministry of Education, Culture, Research, and Technology for its financial support of this research. Appreciation is also extended to Rifqi for valuable assistance in the laboratory work.

NOVELTY STATEMENT

This work is the first to demonstrate the successful extraction of collagen from Garut sheep skin using *Aspergillus oryzae* and *Bacillus subtilis* enzymes, establishing its potential as a sustainable and high-quality halal collagen source.

AUTHOR'S CONTRIBUTION

DPTP was involved in designing the study, collecting the data, interpreting the data, and drafting the manuscript. IMU was involved in the collection of data, YE designed the study, and contributed to manuscript preparation. MZA and NAF took part in preparing and critically checking this manuscript.

List of abbreviations

SDS-PAGE: Sodium dodecyl-sulfate polyacrylamide gel electrophoresis; DSC: Differential Scanning Calorimetry; FTIR: Fourier Transform Infra-Red; SPSS: Statistical Product and Service Solution; kHUT/g: kilo Hemoglobin Unit Tyrosine per gram; kNUP/g: kilo Novo Unit per gram.

Funding

The research funding was granted by the Ministry of Education, Culture, Research, and Technology, Indonesia, under the Programe Master to Doctoral for Excellent Undergraduate (PMDSU) with contract number 0354/E5/PG.02.00/2024; 008/E5/PG.02.00/PL.PMDSU/2024; 2087/UN1/DITLIT/PT.01.03/2024.

Generative AI or AI-assisted Technology Statement

The author(s) declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The writer states there is no conflict of interest.

REFERENCES

Akram AN, Zhang C (2020). Effect of ultrasonication on the yield, functional and physicochemical characteristics of collagen-II from chicken sternal cartilage. Food Chem., 307: 125544. https://doi.org/10.1016/j.foodchem.2019.125544

Alhuur GKR, Ardhiwirayuda H, Nurmeidiansyah AA, Heriyadi D (2023). The distribution of local ewes' breeds, coat color

patterns, and horns type in Bandung Regency. J. Agric. Sci. Vet., 11(2): 289-298. https://doi.org/10.31949/agrivet.v11i2.8207

Amirrah IN, Lokanathan Y, Zulkiflee I, Wee MFMR, Motta A, Fauzi MB (2022). A comprehensive review on collagen type I development of biomaterials for tissue engineering: From biosynthesis to bioscaffold. Biomedicines, 10(9): 2307. https://doi.org/10.3390/biomedicines10092307

Bhuimbar MV, Jalkute CB, Bhagwat PK, Dandge PB (2024). Purification, characterization and application of collagenolytic protease from *Bacillus subtilis* strain MPK. J. Biosci. Bioengin., 138(1): 21–28. https://doi.org/10.1016/j.jbiosc.2024.03.003

Capella-Monsonís H, Coentro JQ, Graceffa V, Wu Z, Zeugolis DI (2018). An experimental toolbox for characterization of mammalian collagen type I in biological specimens. Nat. Protocols, 13(3): 507–529. https://doi.org/10.1038/nprot.2017.117

Daba GM, Mostafa FA, Elkhateeb WA (2021). The ancient koji mold (*Aspergillus oryzae*) as a modern biotechnological tool. Bioresour. Bioproc., 8: 52. https://doi.org/10.1186/s40643-021-00408-z

Devita L, Nurilmala M, Lioe HN, Suhartono MT (2021). Chemical and antioxidant characteristics of skin-derived collagen obtained by acid-enzymatic hydrolysis of bigeye tuna (*Thunnus obesus*). Mar. Drugs, 19: 222. https://doi.org/10.3390/md19040222

Faralizadeh S, Rahimabadi EZ, Bahrami SH, Hasannia S (2021). Extraction, characterization, and biocompatibility evaluation of silver carp (*Hypophthalmichthys molitrix*) skin collagen. Sustainable Chem. Pharm., 22: 100454. https://doi.org/10.1016/j.scp.2021.100454

Ferraro V, Gaillard-Martinie B, Sayd T, Chambon C, Anton M, Santé-Lhoutellier, V (2017). Collagen type I from bovine bone. Effect of animal age, bone anatomy, and drying methodology on extraction yield, self-assembly, thermal behaviour, and electrokinetic potential. Int. J. Biol. Macromol., 97: 55–66. https://doi.org/10.1016/j.ijbiomac.2016.12.068

Gao L, Wang Z, Li Z, Zhang C, Zhang D (2018). The characterization of acid and pepsin soluble collagen from ovine bones (*Ujumuqin* sheep). J. Integr. Agric., 17: 704–711. https://doi.org/10.1016/S2095-3119(17)61751-9

Gauza-Włodarczyk M, Kubisz L, Mielcarek S, Włodarczyk D (2017). Comparison of thermal properties of fish collagen and bovine collagen in the temperature range 298–670 K. Mater. Sci. Eng. C, 80: 468-471. https://doi.org/10.1016/j.msec.2017.06.012

Hadfi NH, Sarbon NM (2019). Physicochemical properties of silver catfish (*Pangasius* sp.) skin collagen as influenced by acetic acid concentration. Food Res., 3(6): 783–790. https://doi.org/10.26656/fr.2017.3(6).130

Hartatik, T (2016). Analisis genetika ternak lokal. Gadjah Mada University Press, Indonesia.

Kuwahara J (2021). Extraction of type I collagen from tilapia scales using acetic acid and ultrafine bubbles. Processes, 9: 288. https://doi.org/10.3390/pr9020288

León-López A, Fuentes-Jiménez L, Hernández-Fuentes AD, Campos-Montiel RG, Aguirre-Álvarez G (2019). Hydrolysed collagen from sheepskins as a source of functional peptides with antioxidant activity. Int. J. Mol. Sci., 20: 3931. https://doi.org/10.3390/ijms20163931

Li PH, Lu WC, Chan YJ, Ko WC, Jung CC, Le Huynh DT, Ji YX (2020). Extraction and characterization of collagen from sea cucumber (*Holothuria cinerascens*) and its potential application in moisturizing cosmetics. Aquaculture, 515: 734590. https://doi.org/10.1016/j.aquaculture.2019.734590

Mariano A, Scotto d'Abusco A, Ammendola SA (2024). Rheological study of creams and gels containing n-acetyl glucosamine in nanoparticle form: The advantages of a bioengineered strategy for natural anti-inflammatory substance vehiculation. Appl. Sci., 14: 11752. https://doi.org/10.3390/app142411752

Matinong AME, Chisti Y (2022). Pickering KL, Haverkamp RG Collagen extraction from animal skin. Biology, 11: 905. https://doi.org/10.3390/biology11060905

Ministry of Agriculture of The Republic of Indonesia (2011). Penetapan rumpun domba garut: Keputusan menteri pertanian. No.: 2914/Kpts/OT.140/6/2011. Ministry of Agriculture of The Republic of Indonesia, Indonesia.

Ministry of Agriculture of The Republic of Indonesia (2023). Outlook komoditas peternakan daging domba: center for agricultural data and information systems, secretariat general. Ministry of Agriculture of the Republic of Indonesia, Indonesia.

Naeem M, Manzoor S, Abid MUH, Tareen MBK, Asad M, Mushtaq S, Ehsan N, Amna D, Xu B, Hazafa (2022). A Fungal proteases as emerging biocatalysts to meet the current challenges and recent developments in biomedical therapies: An updated review. J. Fungi, 8(2): 109. https://doi.org/10.3390/jof8020109

Peterle D, Pontarollo G, Spada S, Brun P, Palazzi L, Sokolov AV, Spolaore B, Polverino de Laureto P, Vasilyev VB, Castagliuolo I, de Filippis VA (2020). Serine protease secreted from *Bacillus subtilis* cleaves human plasma transthyretin to generate an amyloidogenic fragment. Commun. Biol., 3: 764. https://doi.org/10.1038/s42003-020-01493-0

Putri D, Pangestika V, Ilyas H, Abidin M Z, Fitriyanto N, Erwanto Y (2024). Collagen properties of Indonesian local sheepskin isolated using acid and enzymatic methods. J. Adv. Vet. Anim. Res., 11: 722. https://doi.org/10.5455/javar.2024.k823

Reátegui-Pinedo N, Salirrosas D, Sánchez-Tuesta L, Quiñones C, Jáuregui-Rosas SR, Barraza G, Cabrera A, Ayala-Jara C, Martinez RM, Baby AR, Prieto ZA (2022). Characterization of collagen from three genetic lines (gray, red, and f1) of *Oreochromis niloticus* (tilapia) skin in young and old adults. Molecules, 27: 1123. https://doi.org/10.3390/molecules27031123

Said MI, Burhan B, Tensi T, Haerati H (2018). Synthesis of collagen from Bali cattle's hide using a combination of acid and alkali on the extracting process. J. Indonesian Trop. Anim. Agric., 43: 247. https://doi.org/10.14710/jitaa.43.3.247-256

Stülke J, Grüppen A, Bramkamp M, Pelzer S (2023). *Bacillus subtilis*, a Swiss army knife in science and biotechnology. J. Bacteriol., 205(5): e00102-23. https://doi.org/10.1128/jb.00102-23

Tang C, Zhou K, Zhu Y, Zhang W, Xie Y, Wang Z, Zhou H, Yang T, Zhang Q, Xu B (2022). Collagen and its derivatives: From structure and properties to their applications in food industry. Food Hydrocolloids, 131: 107748. https://doi.org/10.1016/j.foodhyd.2022.107748

Van der Merwe DA, Brand TS, Theron PG, Hoffman LC, Jackson-Moss CA (2021). Sheepskin eather quality characteristics of south African breeds. Small Rumin. Res., 199: 106365.

https://doi.org/10.1016/j.smallrumres.2021.106365

Vidal AR, Duarte LP, Schmidt MM, Cansian RL, Fernandes IA, Mello RDO, Demiate IM, Dornelles RCP (2020). Extraction and characterization of collagen from sheep slaughter by-products. Waste Manage., 102: 838–846. https://doi.org/10.1016/j.wasman.2019.12.004

Wahyuningsih R, Rusman, Nurliyani, Pertiwiningrum A, Rohman A, Fitriyanto NA, Erwanto Y (2018). Optimization of conditions for extraction of pepsin-soluble collagen from indonesian local "Kacang" goatskin by response surface methodology. Am. J. Anim. Vet. Sci., 13: 70–75. https://doi.org/10.3844/ajavsp.2018.70.75

Wu J, Kong L, Zhang J, Chen W (2019). Extraction and properties of acid-soluble collagen and pepsin-soluble collagen from silver carp (*Hypophthalmichthys molitrix*) scales: Prerequisite information for fishery processing waste reuse. Polish J. Environ. Stud., 28: 2923–2930. https://doi.org/10.15244/pjoes/93742