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Research Article

Isolation and Characterization of Collagen from Indonesia Local Duck Feet Using Bromelain Enzyme

[1]Rafika Mila Amanah, [1]Dita Prameswari Trenggono Putri, [1]Mohammad Zainal Abidin and [1,2]Yuny Erwanto

[1]Department of Animal Products Technology, Faculty of Animal Science, Gadjah Mada University, Jl. Fauna No. 3, Bulaksumur, Yogyakarta, Indonesia
[2]Halal Materials Development, Institute for Halal Industry & System, Gadjah Mada University, LPPT Building, Jl. Kaliurang Km 4.5, Yogyakarta, Indonesia

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Corresponding Author:
Yuny Erwanto
Department of Animal Products Technology, Faculty of Animal Science, Gadjah Mada University, Jl. Fauna No. 3, Bulaksumur, Yogyakarta, Indonesia
Email: yunyer@ugm.ac.id

Abstract: The utilization of local duck feet as a collagen source to minimize waste and add value to the duck by-product. This research aimed to isolate and characterize collagen from Local duck feet using enzymatic method. The bromelain enzyme in various concentrations: 0% (A), 0.2% (B), 0.4% (C), 0.6% (D), and 0.8% (E). Variables observed were collagen yield, viscosity, pH, Fourier Transform Infrared Spectroscopy (FTIR), and Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE). The results showed the highest collagen yield was 7.77% from D treatment; the viscosity ranges were 2.18-1.99 cP, and the pH ranges were 4.89-4.68. FTIR spectra showed amide A, B, I, II, and III. SDS-PAGE results showed three distinct main bands, consisting of α1, α2, and β chains. In conclusion, collagen could be extracted from Local duck feet and categorized as collagen type I.

Keywords: Duck Feet, Collagen Isolation, Bromelain Enzyme, Characterization

Introduction

Local ducks result from crossbreeding between Peking ducks and Khaki Campbell ducks. This crossbreeding produces high-quality Day-Old Ducks (DOD) as the final stock for broiler ducks (Sjofjan *et al.*, 2021). Both hybrid and Peking ducks are varieties developed explicitly for meat production, making them a viable alternative to meet the demand for animal-based protein (Ridwan *et al.*, 2020). People only consume duck meat, while duck by-products such as feet and head are wasted due to low palatability and utilization. According to Kim *et al.* (2016), duck by-products such as feet can serve as valuable collagen extraction and characterization sources to enhance their potential added value. Collagen is a very important biomolecule and has approximately 30% of the overall protein content in the body of the animal (Pati *et al.*, 2010). Collagen contains distinct characteristics that offer numerous product advantages across different sectors, including food, biomedical, pharmaceutical and cosmetic industries (Tangboriboon *et al.*, 2012). Generally, most commercial collagen is extracted from cattle and pigs using their skin and bones. Collagen derived from bovine sources may carry the risk of biological contamination, including prion-related diseases like Bovine Spongiform Encephalopathy (BSE) and viral infections like foot-and-mouth disease (FMD. Meanwhile, collagen sourced from pigs faces religious and cultural limitations. (Gallo *et al.*, 2020). Islamic and Jewish dietary laws prohibit pork consumption (Baziwane and He, 2003). Islam completely prohibits the consumption of pork and products derived from it. Al-Qur'an provides guidelines regarding halal (acceptable) and haram (forbidden) food, specifically in the verses Al-Baqarah: 172-173 and Al-Maidah: 3, 88. Based on the explanation above, there is an opportunity to find alternative collagen sources, such as local duck feet.

Collagen extraction using enzymes can produce a higher extraction yield. Iltchenco *et al.* (2017) stated that the enzyme used for collagen extraction is a protease enzyme. Various organisms, such as plants, animals and bacteria, can serve as sources of protease enzymes. The ability of protease enzymes depends on the specific properties of the enzyme. Bromelain is one of the protease enzymes that is applicable for collagen extraction. Nanda *et al.* (2023) stated that bromelain enzyme can be found in pineapple and is known to have various applications in food, pharmaceutical and cosmetics. According to Ketnawa *et al.* (2011), bromelain enzyme showed a wide pH range of activity,

maintaining relatively high activity (>60) between pH 3 until 12. It also has the highest activity at a temperature of 50-60°C. Additionally, bromelain sourced from plants is considered halal.

A previous study (Putri *et al.*, 2024) showed that 0.5% of bromelain enzymes can be used for collagen isolation from the local sheep skin, resulting in the highest total yield compared to other protease enzymes. Devita *et al.* (2021) found that a 0.5M acetic acid solution with the bromelain enzyme resulted in a high amount of collagen yield, comparable to pepsin-soluble collagen. However, there was no study has been conducted on the application of the Local duck feet and their optimization for collagen isolation. Therefore, it is important to perform a study on the isolation and characterization of collagen derived from Local duck feet in Indonesia using bromelain enzyme.

Materials and Methods

Materials

The fresh duck feet of local origin were obtained from a Yogyakarta-based slaughterhouse. All the reagents and chemicals utilized in the experimental procedures were of analytical grade standards.

Sample Preparation

The preparation of duck feet follows Cheng *et al.* (2009) with modifications. The duck's feet were thoroughly rinsed with tap water. Followed by the complete removal of claws and the external yellow skin. They were minced into small pieces using a meat grinder. First, the duck feet were submerged in 20% ethanol (1:10 v/w) for 24 h at 4^{o}C, then centrifuged for 15 min at 10.000 rpm. The remaining sediments were immersed in 0.2 M NaOH (1:10 v/w) at 4^{o}C for 24 h. Centrifuged again for 15 min at 10.000 rpm to separate the mixture. Then, the sediment was rinsed with water.

Isolation of Local Duck Feet Collagen

Duck feet were immersed in 0.5 M CH_3COOH and also added with bromelain enzyme (w/w) in various concentrations: 0% (A), 0.2% (B), 0.4% (C), 0.6% (D) and 0.8% (E) for 12 h at 4^{o}C, then centrifuged for 30 min at 10.000 rpm. The supernatant was added with 0.9 M NaCl. Then, the solution was centrifuged for 50 min at 10.000 rpm and redissolved in 10 vol (v/w) of 0.02 M CH_3COOH. Dialysis of the solutions using distilled water for 72 h (exchange distilled water every 24 h). Solvents that contained collagen were freeze-dried until they had a sponge-like texture.

Yield

The total yield was measured by calculating the ratio of the collagen produced (g) to the weight of fresh material that had been cleaned (g) (Shyni *et al.*, 2014):

$$Yield\,(\%) = \left(\frac{Dry\ collagen\ weight}{Dry\ scale\ weight}\right) \times 100$$

Viscosity

The viscosity analysis was performed following the Kittiphattanabawon *et al.* (2005) method. Dissolve 0,03% of collagen samples into 0.1M CH3COOH. The viscosity was determined by a digital viscometer equipped with spindle No.1, operating at 60 rpm.

pH

pH values were evaluated by measuring the wet collagen with a pH meter, following Devita *et al.* (2021) method. The pH meter was turned on to calibrate. The electrode was submerged in the sample solution until the display showed stable readings.

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR analysis was determined according to Woo *et al.* (2008). The spectra of FTIR were derived from a 1 mg collagen sample combined with potassium bromide using an FTIR spectrophotometer (Nicolet Is10, USA) with a wavenumber range of 4000-500 cm^{-1}.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS PAGE)

SDS-PAGE analysis was determined following Putri *et al.* (2024) method with some modifications. First, 1 ml of 0.1M CH_3COOH was mixed with 10 mg collagen. Then, 10µ of the mixture was combined with 3µ of loading SDS buffer and incubated in a water bath at 55-65^{o}C for 15 min. SDS gel was loaded with the protein marker and samples. The samples were subsequently electrophoresed for 150 min at 170 V. SDS gel was stained using 0.25% coomassie blue for 15 min and then rinsed with distilled water overnight.

Statistical Analysis

The statistical results data were analyzed using one-way ANOVA. Subsequently, Duncan Multiple Range Test (DMRT) was used to analyze the significance of differences in the mean values yield, viscosity and pH. The results from the FTIR and SDS PAGE analyses were analyzed using descriptive analysis. The analysis was conducted quantitatively using Statistical Product and Services Solutions (SPSS) software.

Results

Yield

Total yield of Local duck feet collagen using bromelain enzymes was reported in Table (1). Local duck feet collagen samples D (777±038) and E (735±034) have the highest total yield among the other samples A (667±043), B (654±006) and C (625±007) Samples A, B and C do not show any significant

differences, also samples D and E show no significant difference, as indicated by the same superscript However, the comparison between (A, B, C) samples with samples (D and E) demonstrates a statistically significant difference.

Table 1: Yield, viscosity and pH of Local duck feet collagen; [a, b, c] *different superscripts indicated significant differences*

Bromelain enzyme level (%)	yield (%)	Viscosity (cP)	pH
0 (A)	$6.67^{a,b}$±0.43	2.18^{b}±0.05	4.89±0.31
0.2 (B)	6.54^{a}±0.06	$2.10^{a,b}$±0.08	4.85±0.03
0.4 (C)	6.25^{a}±0.07	2.05^{a}±0.04	4.80±0.02
0.6 (D)	7.77^{c}±0.38	1.99^{a}±0.06	4.78±0.02
0.8 (E)	$7.35^{b,c}$±0.34	2.00^{a}±0.08	4.68±0.06

Viscosity

The viscosity measurement result of Local duck feet collagen using bromelain enzymes was reported in Table 1. The viscosities between the samples were 2.18^{b}±0.05, 2.10^{ab}±0.08, 2.05^{a}±0.04, 1.99^{a}±0.06, 2.00^{a}±0.08 cP. The sample with bromelain enzyme (B, C, D and E) has a lower viscosity value than the control treatment (A). The result showed a significant difference in each sample.

pH

pH value of Local duck feet collagen using bromelain enzymes was reported in Table (1). The pH values of A, B, C, D and E were 4.89±0.31, 4.85±0.03, 4.80±0.02, 4.78±0.02, 4.68±0.06. There are no significant differences in each sample.

Fourier Transform Infrared Spectroscopy (FTIR)

The infrared spectra peak positions of duck feet collagen in Figure (1). look similar and Table (2) showed in the wavenumbers range 3331.38-3415.84 (cm^{-1}) for amide A, 2925.62-2957.17 (cm^{-1}) for amide B, 1656.32 to 1659.20 (cm^{-1}) for amide I, 1553.38-1554.61 for Amide II, 1239.50-1240.20 (cm^{-1}) for Amide III.

Table 2: Peak positions of Local duck feet collagen isolated with various bromelain enzyme levels; 0% (A), 0.2% (B), 0.4% (C), 0.6% (D), 0.8% (E), collagen commercial type I (CC)

Amida	Wavenumbers (cm^{-1})					
	A	B	C	D	E	CC
Amide A	3415.84	3334.69	3414.94	3331.38	3334.21	3322.65
Amide B	2926.8	2956.95	2926.5	2925.62	2957.17	2924.88
Amide I	1657.99	1658.69	1656.32	1659.02	1659.2	1652.12
Amide II	1553.76	1554.46	1553.38	1553.81	1554.61	1545.98
Amide III	1240.15	1239.65	1240.2	1239.5	1239.82	1239.4

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS PAGE)

Figure (2) showed the result of the SDS-PAGE analysis. It can be seen that samples extracted with different levels of bromelain enzyme had protein band patterns similar to those of commercial collagen type I. The bromelain-treated samples displayed three distinct main bands, such as α1, α2 and β chains. Local duck feet collagen was categorized as type I collagen due to the existence of α1 and α2 chains. The molecular weights of the α1 and α2 chains ranged from approximately 100–140 kDa, while the β chains had a molecular weight of around 245 kDa.

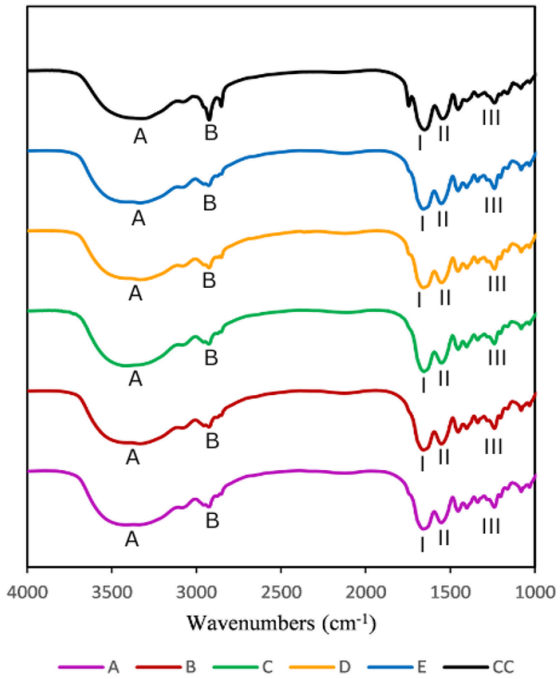

Fig. 1: Local duck feet collagen spectra; 0% (A), 0.2% (B), 0.4% (C), 0.6% (D), 0.8% (E), collagen commercial type I (CC)

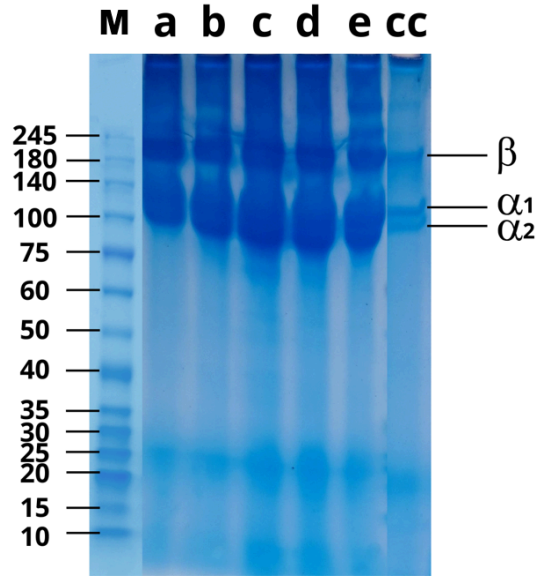

Fig. 2: SDS-PAGE of Local duck feet collagen. Protein marker (M), 0% (A), 0.2% (B), 0.4% (C), 0.6% (D), 0.8% (E), collagen commercial type I (CC)

Discussion

Total yield of isolated collagen from local duck feet using bromelain enzyme at various concentrations was shown in Table 1. It can be seen that the addition of 0.6% bromelain enzyme achieves optimal yields. Increasing the enzyme concentration to 0.8% or higher does not lead to a significant yield value. According to Putri *et al.* (2024), collagen isolated from sheepskin using bromelain enzyme has a higher total yield than collagen obtained using other protease enzymes (such as nutrease and alcalase) or using acid only. According to Devita *et al.* (2021), collagen had non-helical segments in the telopeptide regions at the end of the Amino-terminal and Carboxyl-terminal, created a cross-linked structure. This telopeptide region contained cross-linked structures that bind collagen fibers, making them less soluble in acidic solutions such as 0.5M acetic acid. The presence of the protease enzyme in the extraction process can cleave these cross-link structures, making collagen more soluble and increasing the yield. In this study, the use of bromelain potentially affects the telopeptide region and increases collagen yield. The different yield values are due to differences in the use of extraction methods, soaking time, raw material sources, temperature and solution during the preliminary process to eliminate the non-collagenous proteins (Potaros *et al.*, 2009).

The viscosity value is shown in Table (1). Samples B, C, D and E had a lower viscosity value than the control treatment (A). This result was aligned with collagen sourced from skatefish skin that exhibited viscosity values between 1.8 and 3.4 cP (Shon *et al.*, 2011). Viscosity is a key physicochemical property of collagen, due to the significant presence of protein chains that contribute to a higher molecular weight (Ogawa *et al.*, 2004). Protease enzymes have different activities in cleaving a certain peptide bond. In this study, bromelain might cleave a certain peptide bond, resulting in smaller peptides. Consequently, the molecular weight distribution of collagen decreases, leading to a reduction in collagen viscosity. The increase in viscosity was attributed to variations in the distribution of molecular weight and protein molecule size (Mohtar *et al.*, 2010).

The pH value is shown in Table 1. The pH results in this research are aligned with Putri *et al.* (2024), which indicated that sheepskin collagen had pH values ranging from 4.01 to 4.87; it was also reported that the pH of commercial collagen type I was 6.52. In this research, increasing the level of bromelain enzyme caused a decrease in pH values. The decrease in pH can be caused by the existence of acidic amino acids formed during the enzymatic hydrolysis process (Devita *et al.*, 2021). The non-significant decrease occurs due to the presence of both acidic amino acids (including aspartic acid and glutamic acid) and basic amino acids (including arginine, lysine and histidine). The release of acidic amino acids during hydrolysis does not drastically shift the pH due to compensatory interactions with basic residues (Nalinanon *et al.*, 2010). The appearance of acidic and basic amino acids in collagen contributes to pH stability, even as enzyme concentrations increase. Several factors, such as the isolation method, concentration of CH_3COOH and the duration of isolation, can influence the pH value. The final pH value of collagen may be adjusted through a post-extraction neutralization step. An appropriate neutralization process can reduce the residual acidity, resulting in collagen with a pH level approaching neutrality (Suwarjoyowirayatno *et al.*, 2024).

FTIR spectra of Local duck feet collagen with different levels of bromelain enzyme exhibited the peaks of Amide A, B, I, II and III. These peaks highlight the characteristic structure of collagen presence in the samples. The range of wavenumbers from 3.400-3.440 (cm^{-1}) was typically linked to N-H stretching vibration associated with Amida A. Amida B was associated with asymmetrical stretching of CH_2 was found at 2.925-2.935 (cm^{-1}). Amide I was represented by the stretching vibration of carbonyl groups (C=O) found in peptides and was found at 1.600-1.700 (cm^{-1}). Amide II was indicated by NH bending and CH stretching and was found at 1.550-1.600 (cm^{-1}) (Kim *et al.*, 2016). Amide III was cognate with the triple helix structure of collagen and was found at 1.200–1.300 (cm^{-1}) (Dhakal *et al.*, 2018). In this research, the wavenumbers of Amide A were observed to shift slightly toward lower values (Table 2). According to Hashim *et al.* (2014), the positions shift to lower regions due to interactions between the NH group and the hydrogen bonds within the peptide chain.

Based on Figure (2). The pattern of local duck feet collagen using bromelain enzyme in various concentrations was not significantly different. According to Reátegui-Pinedo *et al.* (2022), the type I commercial collagen had a molecular weight of 120 kDa for the α1 chain and 116 kDa for the α2 chain. Jafari *et al.* (2020) also explained that the α1 and α2 chains of type I collagen had molecular weights of approximately 110–150 kDa, while β chains had a weight of around 200–250 kDa. This research showed that the α1 and α2 chains exhibit a similar pattern of protein bands, so the difference is not too noticeable. According to Fawzya *et al.* (2016), the SDS-PAGE was unable to separate the α2 chains, possibly due to each α chain having similar chemical and electrophoretic properties, caused them to migrate to the same position on the gel. SDS-PAGE is an effective method for separating individual components within complex protein mixtures, making it the most widely used method in laboratories for protein identification (Blanco *et al.*, 2019).

Based on this research, local duck feet have been identified as a type I collagen and a feasible alternative

for producing halal collagen. Naomi *et al.* (2021) explained that although collagen type I is isolated from various sources, it retains a high level of biocompatibility and demonstrates low immunogenicity due to its structural similarity to collagen in the human body. As a result, collagen type I is extensively utilized across multiple fields, such as tissue engineering, medical devices, pharmaceuticals and biomedical applications. Utilizing duck feet for collagen extraction not only provides an alternative source but also contributes to waste valorization.

Conclusion

The conclusion of this research was that collagen could be extracted from local duck feet using bromelain enzyme. The characteristic of the result showed that the extracted collagen sample can be confirmed as collagen type I. These results provide some basic information for further research on collagen from Local duck feet and may be utilized as alternative sources of halal collagen.

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Author's Contributions

Rafika Mila Amanah: Responsible for the original draft writing, conducting experiments, formal analysis and data curation

Dita Prameswari Trenggono Putri and Mohammad Zainal Abidin: Assist the SDS PAGE analysis. Contributes to the processes of writing, reviewing and editing. Provided supervision, project administration, funding acquisition and conceptual development.

Yuny Erwanto: Propose a research idea, supervise an experiment and provide final approval of the manuscript to be submitted and any revised versions thereafter.

Ethics

All of the authors affirm that this article is original and has no ethical issues.

References

Baziwane, D., & He, Q. (2003). Gelatin: The Paramount Food Additive. *Food Reviews International*, *19*(4), 423-435. https://doi.org/10.1081/fri-120025483

Blanco, M., Vázquez, J. A., Pérez-Martín, R. I., & Sotelo, C. G. (2019). Collagen Extraction Optimization from the Skin of the Small-Spotted Catshark (S. canicula) by Response Surface Methodology. *Marine Drugs*, *17*(1), 40. https://doi.org/10.3390/md17010040

Cheng, F.-Y., Hsu, F.-W., Chang, H.-S., Lin, L.-C., & Sakata, R. (2009). Effect of different acids on the extraction of pepsin-solubilised collagen containing melanin from silky fowl feet. *Food Chemistry*, *113*(2), 563-567. https://doi.org/10.1016/j.foodchem.2008.08.043

Devita, L., Nurilmala, M., Lioe, H. N., & Suhartono, M. T. (2021). Chemical and Antioxidant Characteristics of Skin-Derived Collagen Obtained by Acid-Enzymatic Hydrolysis of Bigeye Tuna (Thunnus obesus). *Marine Drugs*, *19*(4), 222. https://doi.org/10.3390/md19040222

Dhakal, D., Koomsap, P., Lamichhane, A., Sadiq, M. B., & Anal, A. K. (2018). Optimization of collagen extraction from chicken feet by papain hydrolysis and synthesis of chicken feet collagen based biopolymeric fibres. *Food Bioscience*, *23*, 23-30. https://doi.org/10.1016/j.fbio.2018.03.003

Fawzya, Y. N., Chasanah, E., Poernomo, A., & Khirzin, M. H. (2016). Isolasi dan Karakterisasi Parsial Kolagen dari Teripang Gamma (Stichopus variegatus). *Jurnal Pascapanen Dan Bioteknologi Kelautan Dan Perikanan*, *11*(1), 91-100. https://doi.org/10.15578/jpbkp.v11i1.284

Gallo, N., Natali, M. L., Sannino, A., & Salvatore, L. (2020). An Overview of the Use of Equine Collagen as Emerging Material for Biomedical Applications. *Journal of Functional Biomaterials*, *11*(4), 79. https://doi.org/10.3390/jfb11040079

Hashim, P., Ridzwan, M. S. M., & Bakar, J. (2014). Isolation And Characterization Of Collagen From Chicken Feet. *World Academy of Science, Engineering and Technology, Nternational Journal of Bioengineering and Life Sciences*, *8*(3), 250-254. https://doi.org/10.5281/ZENODO.1091484

Iltchenco, S., Kempka, A. P., & Prestes, R. C. (2017). Profiles Of Enzymatic Hydrolysis Of Different Collagens And Derivatives Over Time. *Revista Brasileira de Tecnologia Agroindustrial*, *11*(1). https://doi.org/10.3895/rbta.v11n1.2755

Jafari, H., Lista, A., Siekapen, M. M., Ghaffari-Bohlouli, P., Nie, L., Alimoradi, H., & Shavandi, A. (2020). Fish Collagen: Extraction, Characterization, and Applications for Biomaterials Engineering. *Polymers*, *12*(10), 2230. https://doi.org/10.3390/polym12102230

Ketnawa, S., Chaiwut, P., & Rawdkuen, S. (2011). Aqueous two-phase extraction of bromelain from pineapple peels ('Phu Lae' cultv.) and its biochemical properties. *Food Science and Biotechnology*, *20*(5), 1219-1226. https://doi.org/10.1007/s10068-011-0168-5

Kim, H.-W., Yeo, I.-J., Hwang, K.-E., Song, D.-H., Kim, Y.-J., Ham, Y.-K., Jeong, T.-J., Choi, Y.-S., & Kim, C.-J. (2016). Isolation and Characterization of Pepsin-soluble Collagens from Bones, Skins, and Tendons in Duck Feet. *Korean Journal for Food Science of Animal Resources*, *36*(5), 665-670. https://doi.org/10.5851/kosfa.2016.36.5.665

Kittiphattanabawon, P., Benjakul, S., Visessanguan, W., Nagai, T., & Tanaka, M. (2005). Characterisation of acid-soluble collagen from skin and bone of bigeye snapper (Priacanthus tayenus). *Food Chemistry*, *89*(3), 363-372. https://doi.org/10.1016/j.foodchem.2004.02.042

Mohtar, N. F., Perera, C., & Quek, S.-Y. (2010). Optimisation of gelatine extraction from hoki (Macruronus novaezelandiae) skins and measurement of gel strength and SDS-PAGE. *Food Chemistry*, *122*(1), 307-313. https://doi.org/10.1016/j.foodchem.2010.02.027

Nalinanon, S., Benjakul, S., & Kishimura, H. (2010). Collagens from the skin of arabesque greenling (Pleurogrammus azonus) solubilized with the aid of acetic acid and pepsin from albacore tuna (Thunnus alalunga) stomach. *Journal of the Science of Food and Agriculture*, *90*(9), 1492-1500. https://doi.org/10.1002/jsfa.3973

Nanda, R. F., Bahar, R., Syukri, D., Thu, N. N., & Kasim, A. (2023). A Review: Application of Bromelain Enzymes in Animal Food Products. *Andalasian International Journal of Agriculture and Natural Sciences (AIJANS)*, *1*(1), 33-44. https://doi.org/10.25077/aijans.v1.i01.33-44.2020

Naomi, R., Ridzuan, P. M., & Bahari, H. (2021). Current Insights into Collagen Type I. *Polymers*, *13*(16), 2642. https://doi.org/10.3390/polym13162642

Ogawa, M., Portier, R. J., Moody, M. W., Bell, J., Schexnayder, M. A., & Losso, J. N. (2004). Biochemical properties of bone and scale collagens isolated from the subtropical fish black drum (Pogonia cromis) and sheepshead seabream (Archosargus probatocephalus). *Food Chemistry*, *88*(4), 495-501. https://doi.org/10.1016/j.foodchem.2004.02.006

Pati, F., Adhikari, B., & Dhara, S. (2010). Isolation and characterization of fish scale collagen of higher thermal stability. *Bioresource Technology*, *101*(10), 3737-3742. https://doi.org/10.1016/j.biortech.2009.12.133

Potaros, T., Raksakulthai, N., Runglerdkreangkrai, J., & Worawattanamateekul, W. (2009). Characteristics of Collagen from Nile Tilapia (Oreochromis Niloticus) Skin Isolated by Two Different Methods. *Agriculture and Natural Resources*, *43*(3), 584-593.

Putri, D., Pangestika, V., Ilyas, H., Abidin, M., Fitriyanto, N., & Erwanto, Y. (2024). Collagen properties of Indonesian local sheepskin isolated using acid and enzymatic methods. *Journal of Advanced Veterinary and Animal Research*, *11*(3), 722. https://doi.org/10.5455/javar.2024.k823

Reátegui-Pinedo, N., Salirrosas, D., Sánchez-Tuesta, L., Quiñones, C., Jáuregui-Rosas, S. R., Barraza, G., Cabrera, A., Ayala-Jara, C., Martinez, R. M., Baby, A. R., & Prieto, Z. A. (2022). Characterization of Collagen from Three Genetic Lines (Gray, Red and F1) of Oreochromis niloticus (Tilapia) Skin in Young and Old Adults. *Molecules*, *27*(3), 1123. https://doi.org/10.3390/molecules27031123

Ridwan, M., Sari, R., Andika, R. D., Candra, A. A., & Maradon, G. G. (2020). Usaha Budidaya Itik Pedaging Jenis Hibrida dan Peking. *PETERPAN (Jurnal Peternakan Terapan)*, *1*, 8-10. https://doi.org/10.25181/peterpan.v1i1.1473

Shon, J., Eo, J.-H., Hwang, S. J., & Eun, J.-B. (2011). Effect of processing conditions on functional properties of collagen powder from skate (Raja kenojei) skins. *Food Science and Biotechnology*, *20*(1), 99-106. https://doi.org/10.1007/s10068-011-0014-9

Shyni, K., Hema, G. S., Ninan, G., Mathew, S., Joshy, C. G., & Lakshmanan, P. T. (2014). Isolation and characterization of gelatin from the skins of skipjack tuna (Katsuwonus pelamis), dog shark (Scoliodon sorrakowah), and rohu (Labeo rohita). *Food Hydrocolloids*, *39*, 68-76. https://doi.org/10.1016/j.foodhyd.2013.12.008

Sjofjan, O., Adli, D. N., Natsir, M. H., Nuningtyas, Y. F., Bastomi, I., & Amalia, F. R. (2021). The effect of increasing levels of palm kernel meal containing α-β-mannanase replacing maize to growing-finishing hybrid duck on growth performance, nutrient digestibility, carcass trait, and VFA. *Journal of the Indonesian Tropical Animal Agriculture*, *46*(1), 29-39. https://doi.org/10.14710/jitaa.46.1.29-39

Suwarjoyowirayatno, S., Hidayat, C., Wahyuningsih, T. D., & Indrati, R. (2024). Characterization of acid-soluble collagen from peanut worm (Siphonosoma australe) and its inhibitory activity of cyclooxygenase-2. *Journal of Applied Biology & Biotechnology*, *12*(6), 123-129. https://doi.org/10.7324/jabb.2024.192617

Tangboriboon, N., Kunanuruksapong, R., & Sirivat, A. (2012). Preparation and properties of calcium oxide from eggshells via calcination. *Materials Science-Poland*, *30*(4), 313-322. https://doi.org/10.2478/s13536-012-0055-7

Woo, J.-W., Yu, S.-J., Cho, S.-M., Lee, Y.-B., & Kim, S.-B. (2008). Extraction optimization and properties of collagen from yellowfin tuna (Thunnus albacares) dorsal skin. *Food Hydrocolloids*, *22*(5), 879-887. https://doi.org/10.1016/j.foodhyd.2007.04.015