Trop J Nat Prod Res, February 2025; 9(2): 711 - 715 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

The Effect of Solvents and Extraction Techniques on the Quality of Mangrove Crab Shells Gelatin (*Scylla serrata*)

La Ode A. Rasydy[1]*, Zenith P. Dewianti[1], Syifa S. Hayatunnisa[1], Adefia Femalda[1], La Ode M. Anwar[2], Fikria M. Nur[3], Lulu L. Octaviani[1], Nadianti P. Della[1]

[1]*Department of Pharmacochemistry and Science, Faculty of Pharmacy, Muhammadiyah A.R. Fachruddin University, St. KH Syekh Nawawi Km 4 No.13 Matagara, Tigaraksa, Tangerang, Banten 15720, Indonesia*
[2]*Departement of Pharmacy, Faculty of Health Sciences, Suherman Medika University, St. Raya Industri Pasir Gombong, Jababeka Cikarang, Bekasi 17530, Indonesia*
[3]*Departement of Pharmacy, Faculty of Health Sciences, PGRI Adi Buana University, St. Ngagel Dadi III-B/37, Surabaya 60245, Indonesia.*

ARTICLE INFO

Article history:
Received 18 March 2024
Revised 06 April 2024
Accepted 10 January 2025
Published online 01 March 2025

ABSTRACT

Gelatin is frequently used in various aspects such as in pharmacies, cosmetics and food. The need for gelatin increases every year, but it has limitations related to the halal element, especially in Indonesia. Mangrove crab shells are an alternative raw material for making gelatin, which is clearly halal and safe. This research aims to measure the effect of solvent types and extraction techniques on the quality and characteristics of the gelatin produced. This research was designed to optimize the quality of crab gelatin using a completely randomized design. The treatments used in this study were the use of CH_3COOH 1% (A1) and NaOH 1% (A2) solvents and the extraction techniques of an autoclave, 1 atm pressure at 100°C for 1 hour (B1), and a water bath at 70°C for 2 hours (B2). The results show that the mud crab shells had characteristics that were almost similar to the gelatin Standards, so they had the potential to be a natural resource for gelatin production. The best quality of gelatin was shown in the treatment using CH_3COOH 1% and autoclave 1 atm pressure at 100°C for 1 hour, producing a yield of 25.53 ± 3.16%, ash content of 2.81 ± 0.07%, fat content 8.06 ± 1.15%, water content 9.96 ± 0.35%, protein content 61.28 ± 1.23%, pH 5.43 ± 0.46, viscosity 5.98 ± 0.59 cPs, and gel strength 68.16 ± 0.57 bloom. Therefore, the selection of solvents and extraction methods plays a critical role in determining the efficiency and quality of gelatin production, particularly from crab shells.

Keywords: Characteristics, Gelatin, Halal, Mangrove Crab Shells, Extraction, Solvent.

Introduction

Total gelatin production in the world reaches 326,000 tons per year, but its halalness is an issue,[1] 98.5% of gelatin in the world is made from meat, bones and the skin of porks.[2,3,4] The method for making gelatin is divided into type A and type B. Type A gelatin uses an acidic solvent, and type B gelatin uses an alkaline solvent. Pig skin and beef bones are considered the main sources of type A gelatin, while type B gelatin is often derived from beef-related ingredients, sometimes in combination with pork bones.[5] Unfortunately, some are non-halal products. Therefore, it is necessary to develop gelatin production from halal raw materials. The need for gelatin increases every year. Until now, the need for gelatin for food and non-food industrial products worldwide has continued to increase each year.[6] Therefore, other alternative raw materials is needed for making gelatin that are safe and clearly halal, such as mangrove crab shells. Crabs are aquatic animals that live both in seawater and freshwater but do not live or have habitats in both worlds,[7] so it is halal for consumption.[8]

*Corresponding author. Email: rasydyakbar@gmail.com
Tel: +6285341979241

Citation: Rasydy LOA, Dewianti ZP, Hayatunnisa SS, Femalda A, Anwar LOM, Nur FM, Octaviani LL, Della NP. The Effect of Solvents and Extraction Techniques on the Quality of Mangrove Crab Shells Gelatin (*Scylla serrata*). Trop J Nat Prod Res. 2025; 9(2): 711 – 715 https://doi.org/10.26538/tjnpr/v9i2.38

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Crab shells contain 18.70%-32.20% chitin, 53.70%-78.40% calcium carbonate, and 15.60%-23.90% protein.[9] The high protein content in mangrove crab shells contains collagen, used as a raw material to make gelatin.[10] Moreover, the crab shell parts are also underused by people and only become waste.[11] Therefore, gelatin made from crab shells has a low economic value. There were several research of halal raw materials for gelatin, such as milkfish bone,[12] chicken feet,[13] and dried cow skin,[14] and this was the first study of mangrove crab shells to be exploited as raw materials for making gelatin. This latest research used crab shell waste which is rarely used as a potential source of gelatin. Quality gelatin certainly produces a large yield, and the resulting quality can meet the characteristics of gelatin. To produce the best quality, gelatin definitely needs to be optimized both in terms of solvent and extraction technique. Several previous studies showed the best chicken feet gelatin extraction process uses acid solvents, CH_3COOH and NaOH, showing significant differences ($P < 0.05$).[15] This study aims to evaluate the influence of solvent type and extraction technique on the quality and properties of gelatin produced using acidic (1% CH_3COOH) and basic (1% NaOH) solvents. By employing autoclave and water bath extraction methods, this research seeks to identify an alternative source of halal gelatin suitable for applications in food additives and pharmaceuticals.

Materials and Methods

Plant collection and identifications

The materials mangrove crab (*Scylla serrata*) shells obtained from seafood distributors originating from Timika (Papua, Indonesia) in June 2021, GPS location: https://maps.app.goo.gl/v3rTdQsJwSxh9iYDA. The collected animal material was preserved in a Herbarium Bogoriense, Botanical Division, Research and Development Center for

Biology-LIPI Cibinong, Indonesia, and identified by a taxonomy specialist (Voucher number: B-232/V/DI.05.07/10/2021).

Research design
This research design utilized the Completely Randomized Design (CRD) method, which was divided into two factors. The first factor examined the effects of two types of solvents: 1% CH_3COOH (A1) and 1% NaOH (A2). The second factor focused on the extraction technique, which included two methods: extraction using an autoclave (1 atm) at 100ºC for 1 hour (B1) and extraction in a water bath at 70ºC for 2 hours (B2). Consequently, four treatment combinations were obtained.

Production of mangrove crab shell gelatin
Gelatin extraction was performed using a modified method.[16] Mangrove crabs were washed and boiled at 700ºC for 25-30 minutes. The shells were separated from the meat, washed, and then dried by heating them in an oven at 450ºC for 30 minutes. The dried shells were crushed using a blender and sifted through a mesh of 40. Crab shell powder was soaked in an acid or base solution according to the treatment (1% CH_3COOH and 1% NaOH) at a ratio of 1:3 (w/v) for 2 days at 25ºC. The mixture was then filtered, and the residue was washed until the pH was neutral. The residue was subsequently dried for 24 hours at 50ºC. Next, extraction was carried out according to the treatment conditions (using an autoclave at 100ºC for 1 hour and a water bath at 70ºC for 2 hours) with demineralized water at a ratio of 1:4 (w/v). The extract was stored in a refrigerator at 4ºC for 30 minutes, filtered using Whatman 42, placed in a non-stick frying pan, dried in an oven at 70ºC for 1 hour, ground with a blender, packaged in glass bottles, and then tested for gelatin quality parameters.

Organoleptic test
Organoleptic tests were conducted using the five senses to describe the characteristics of mangrove crab (*Scylla serrata*) shell gelatin, including its shape (solid, dry powder, thick, liquid), color, and odor (aromatic, odorless, etc.).[17]

Yield calculation
The percentage yield of gelatin produced (%) was calculated by comparing the weight of the obtained gelatin powder to the weight of the raw material.[17] The test was carried out three times.

$$\textit{Yield (\%)} = \frac{\text{weight of gelatin powder}}{\text{weight of raw materials}}\ x\ 100\%$$

Ash content test
The cup was pretreated in an oven at 100–105°C for 30 minutes, then placed in a desiccator to remove moisture and weighed (A). Two grams of crab shell gelatin were then added to the dried cup (B) and incinerated over a burner flame until smokeless. The process continued until complete ashing was achieved in a furnace at 550–600°C. The resulting ash was cooled in a desiccator (non-vacuum, PDA251114-DESNONN30, Normax, Japan) before being weighed (C). The test was carried out three times.[17]

$$\textit{Ash content (\%)} = \frac{C-A}{B-A} \text{x } 100\%$$

Fat content test
The fat pumpkin was dried in an oven at 105°C for 15 minutes (A). Five grams of crab shell gelatin were weighed (C) and then placed into a fat sleeve. The filter paper containing the sample was inserted into a Soxhlet extraction apparatus connected to a condenser. One hundred fifty milliliters of hexane solvent were added to a fat flask and refluxed for 5 hours. The remaining solvent in the fat flask was removed by heating in the oven (SNB 400, Memmert, Germany), and then weighed (B). The test was carried out three times.[17]

$$\text{Fat content (\%)} = \frac{B-A}{C} \text{x } 100\%$$

Water content test
The test employed the gravimetric method, starting with the weighing of an empty porcelain cup (A). Two grams of crab shell gelatin were placed in the porcelain cup (B) and heated in the oven at 105°C for 30 minutes. Afterward, the gelatin was cooled for 10 minutes in a desiccator and then weighed (C). The heating and weighing process was repeated until a constant weight was achieved.[17] The test was carried out three times.

$$\text{Water content (\%)} = \frac{(A+B)-C}{B} \text{x } 100\%$$

Protein content test
The test utilized the Kjeldahl method. In the digestion stage, 1.00 gram of the ground sample was weighed and placed in a Kjeldahl flask along with a boiling stone, 0.35 grams of copper sulfate ($CuSO_4$), 7.5 grams of potassium sulfate (K_2SO_4), and 15 mL of 0.1 M sulfuric acid (H_2SO_4). The mixture was shaken until homogeneous. All materials were heated in the Kjeldahl flask within a fume cupboard. When the smoking ceased, heating continued until the liquid boiled and became clear, which took approximately 30 minutes.In the distillation stage, the digestion mixture was transferred to a distillation flask, and the Kjeldahl flask was rinsed with distilled water. Fifty milliliters of 50% NaOH, 200 mg of zinc, and 100 mL of distilled water were added. The liquid was gradually heated in the Kjeldahl flask until mixed, and then rapidly heated until it boiled. The distillate was collected in an Erlenmeyer flask, and 50 mL of a standard hydrochloric acid solution (HCl 0.1 N) was added along with 3-5 drops of a 1% phenolphthalein indicator to ensure that the tip of the distillation pipe was submerged in the hydrochloric acid solution (HCl 0.1 N). The process was completed when the solution ceased to be alkaline, which was indicated by a drop in pH. During the titration phase, the distillation product was titrated using sodium hydroxide (NaOH 0.1 N). The endpoint of the titration was achieved when the solution changed to a stable pink color. Subsequently, a blank titration was performed, with the conversion factor set at 6.25.[18] The test was carried out three times.

$$\text{Nitrogen content (\%)} = \frac{\text{(ml NaOH blank} - \text{ml NaOH sample) x N NaOH x 14,008}}{\text{g sampel x 1000}}\ x\ 100\%$$

$$\text{Protein content (\%)} = \text{Nitrogen Content x Conversion Factor}$$

pH test
The measurement was conducted using a calibrated pH meter (SevenExcellence S400-Basic, Mettler Toledo, Switzerland) after the gelatin had undergone a drying and grinding process similar to flour. The pH meter was immersed in the gelatin solution to assess the suitability of the pH range of the produced gelatin. The electrode was rinsed 6–8 times with distilled water, and each different sample was analyzed.[17] The test was carried out three times.

Viscosity test
Gelatin with a concentration of 6.67% (w/w) in distilled water was measured using a Lammy viscometer (B-One Plus, Lamy Rheology, France). This measurement was conducted at 60°C with a shear rate of 60 rpm using spindle no. 1. The viscosity value was recorded in centipoise units (cPs).[19] The test was carried out three times.

Gelatin strength test
The 6.67% (w/v) gelatin solution was heated for 15 minutes at 45°C and then incubated for 2 hours at 10°C. The resulting gel was measured using a Texture Analyzer (CT3, AMETEK Brookfield, USA).[19] The test was carried out three times.

Statistical analysis
The data on gelatin characteristics obtained were analyzed using Analysis of Variance (ANOVA), followed by Duncan's Advanced Test at a significance level of 5% ($\alpha = 0.05$) using the SPSS 21.0 Statistics Software package. The results of the data analysis were compared with the standard gelatin quality and testing methods for gelatin.

Results and Discussion

The effect of treatment on the yield of mangrove crab shell gelatin is illustrated in Figure 1. The results indicate that the use of 1% CH_3COOH (A1) produced a higher yield than 1% NaOH (A2), and the extraction technique using an autoclave (B1) yielded more than the water bath method (B2).

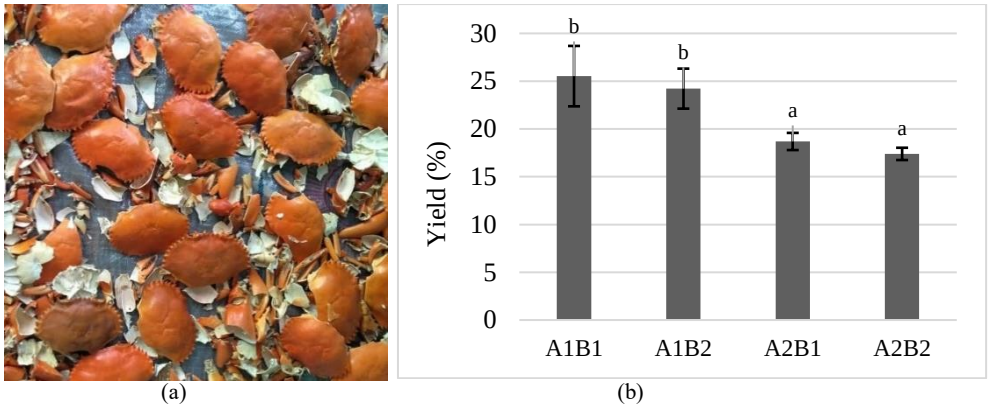

Figure 1: (a) Mangrove crab shell (b) Effect of treatment on gelatin yield.

The use of solvents and extraction techniques resulted in significantly different outcomes ($P < 0.05$), indicating that the type of solvent and extraction technique influenced the yield of mud crab shell gelatin. Previous research on gelatin extraction from dry cowhide using 1% CH_3COOH and 1% NaOH with an autoclave showed that the yield from CH_3COOH was greater than that from NaOH.[15] The use of acidic solvents altered the collagen triple helix structure into a single helix, while immersion in alkaline solvents transformed it into a double helix.[20] This was what caused acidic solvents to produce higher yield values than basic solvents. The use of autoclave as an extraction technique could increase the yield of gelatin produced. That was because gelatin could shorten the extraction time required. It was in a closed and stable condition from external influences so that the yield could increase. The long heating process caused collagen destruction.[21] Based on previous research on jellyfish (*Lobonema smithii*) gelatin, increasing the concentration of acid and using a stronger type of acid could decrease the yield,[22] This occurs because the amino acid peptide bonds, which ware the main structure of collagen, undergo degradation, resulting in the dissolution and loss of collagen during the washing process. Consequently, the overall yield decreases.[22]

The physical characteristics of the gelatin product were similar to those of commercial gelatin. The chemical characteristics of the mangrove crab shell gelatin produced are presented in Figure 2. The water content obtained when using solvents showed significant differences ($P < 0.05$), while the extraction technique did not show any significant differences ($P > 0.05$). Using 1% CH_3COOH (A1) was more effective than using 1% NaOH (A2) because the collagen structure in acidic conditions is opened and weakened, resulting in gelatin with a fragile structure. Consequently, the gelatin's water-holding capacity was reduced.

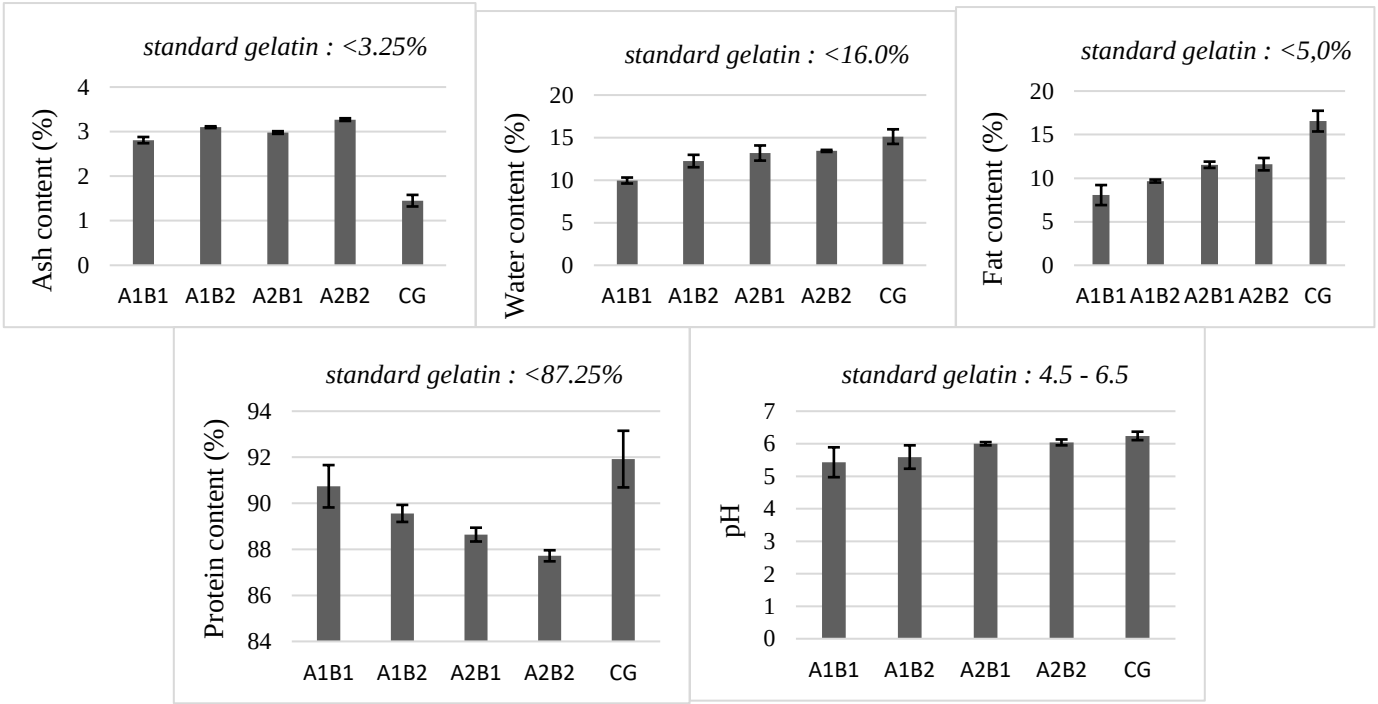

Figure 2: Effect of treatment on ash, water, fat, protein content and pH
(A1: CH_3COOH 1%; A2: NaOH 1%; B1: extraction using autoclave; B2: extraction using water bath; CG: Commercial gelatin

This weak water-holding capacity allowed water to evaporate easily during extraction, leading to lower water content in the dry gelatin. The water content of crab shell gelatin impacts its shelf life, as it is closely related to the metabolic activities that occur while the gelatin is stored.[20] The results of the statistical analysis indicated that the treatments using different solvents and extraction techniques significantly affected ash content ($P < 0.05$). The treatment with 1% CH_3COOH (A1) was more effective than the treatment with 1% NaOH (A2). Using 1% CH_3COOH (A1) as a solvent was optimal for the demineralization process, effectively dissolving the mineral salts found in crab shells.[23] Similarly, the extraction technique using an autoclave (B1) outperformed the technique using a water bath (B2). The autoclave's internal pressure enabled it to dissolve the minerals present in mangrove crab shells more efficiently.[23]

The results of the statistical analysis indicated that the treatments using different solvents and extraction techniques produced significantly different results ($P < 0.05$) regarding fat content. The treatment with 1% CH_3COOH (A1) yielded better fat content compared to the treatment with 1% NaOH (A2). This was because alkaline solvents could only hydrolyze the triple helix into a double helix, resulting in suboptimal fat extraction from the cells and consequently higher fat levels in the gelatin.[15] Meanwhile, the extraction technique using an autoclave (B1) produced better fat content than the water bath method (B2). This was due to the high temperature (100°C) and pressure in the autoclave, which allowed for optimal fat reduction.[15] All treatments in this study resulted in fat levels that did not meet gelatin specifications, remaining below 5%.[24] The high fat content may have been caused by insufficient degreasing or cleaning of the crab shells, which allowed residual fat from the crab meat to be carried over during the extraction process.[25]

The protein content in mangrove crab shell gelatin showed significantly different results ($P < 0.05$) depending on the solvent and extraction technique used. The use of 1% CH_3COOH (A1) resulted in higher protein levels compared to the 1% NaOH solvent (A2). Using an acidic solvent during extraction effectively broke the hydrogen bonds, optimally opening the collagen coil structure, which allowed for greater protein extraction than with an alkaline solvent.[22] Meanwhile, the extraction technique using an autoclave (B1) produced higher protein levels than the water bath method (B2). The autoclave facilitated a shorter extraction time, preventing damage to the protein in the crab shell.[23] All treatments in this study produced protein content by the gelatin specification above 87.25%.[24] All treatments in this study yielded protein content that met gelatin specifications, exceeding 87.25%. Therefore, mangrove crab shells have great potential as a raw material for producing gelatin with high protein content.

The results of the statistical analysis indicated that the treatments using different solvents and extraction techniques showed no significant differences ($P > 0.05$) in pH values. The highest pH value was obtained with the 1% NaOH (A2) treatment using the water bath extraction technique (B2), measuring 6.04 ± 0.09, while the lowest pH value was recorded with the 1% CH_3COOH (A1) treatment using the autoclave extraction technique (B1), measuring 5.43 ± 0.46. The pH values of the gelatin were related to the processes used in its production. All treatments resulted in pH values for mangrove crab shell gelatin that met the specifications, ranging from 4.5 to 6.5.[24]

Physical quality characteristics of mangrove crab shell gelatin

The gelatin product derived from mangrove crab shells was soluble in water at 80°C (Table 1), indicating that the quality of the gelatin was good. This high-quality gelatin was achieved through the degradation of the triple helix structure of collagen protein into a polypeptide mixture that easily dissolves in water at 80°C during the cooling process.[26]

Table 1: Solubility analysis of mangrove crab shell gelatin

Treatment	Solubility
A1B1	Soluble
A1B2	Soluble
A2B1	Soluble
A2B2	Soluble
CG	Soluble
Standard gelatin	Soluble

(A1: CH_3COOH 1%; A2: NaOH 1%; B1: extraction using autoclave; B2: extraction using water bath; CG: Commercial gelatin)

Statistical measurements of gelatin viscosity showed significantly different results ($P < 0.05$) based on the solvents and extraction techniques used (Table 2). The treatment with 1% CH_3COOH (A1) produced a higher viscosity than the treatment with 1% NaOH (A2). This was due to the hydrolysis of the triple-helix amino acid chain, which resulted in longer chains. The longer the amino acid chains, the greater the molecular weight of the gelatin, leading to higher viscosity.[20] When compared to standard gelatin, the treatment using 1% CH_3COOH (A1) with the autoclave extraction technique (B1) produced a viscosity greater than that of commercial gelatin. This demonstrates the great potential of mangrove crab shells as raw materials for producing high-quality gelatin.

Table 2: Viscosity Analysis of mangrove crab shell gelatin

Treatment	Mean ± SD (cPs)
A1B1	5.98 ± 0.59^{c}
A1B2	3.51 ± 0.49^{a}
A2B1	5.18 ± 0.41^{bc}
A2B2	3.93 ± 0.59^{ab}
GK	5.89 ± 0.16
Standard gelatin	1.5 – 7.5

(A1: CH_3COOH 1%; A2: NaOH 1%; B1: extraction using autoclave; B2: extraction using water bath; CG: Commercial gelatin.

Table 3: Strength gell of mangrove crab shell gelatin

Treatment	Mean ± SD (bloom)
A1B1	68.16 ± 0.57^{a}
A1B2	67.83 ± 0.28^{a}
A2B1	67.5 ± 0.50^{a}
A2B2	67.33 ± 0.28^{a}
CG	95.50 ± 9.01
Standard gelatin	50 - 300 bloom

(A1: CH_3COOH 1%; A2: NaOH 1%; B1: extraction using autoclave; B2: extraction using water bath; CG: Commercial gelatin.

The results of the statistical analysis indicated that the solvent treatment and extraction technique showed no significant differences ($P > 0.05$) in the strength of the gelatin gel (Table 3). The use of 1% CH_3COOH produced better gel strength values than the 1% NaOH (A2) solvent. This improvement was due to the optimal hydrolysis of collagen facilitated by the acidic solution.[27] All treatments of mangrove crab shell gelatin, as well as commercial gelatin, produced gel strength values that met the specifications, ranging from approximately 50 to 300 bloom.[24]

Conclusion

The mangrove crab shells have the potential to serve as a halal and safe raw material for gelatin production, exhibiting characteristics that closely align with gelatin standards. It emphasizes the critical role that the choice of solvent and extraction techniques plays in determining the quality and efficiency of gelatin production. By focusing on these factors, the research paves the way for further advancements in the production of high-quality gelatin. Future investigations could explore variations in solvents and extraction methods, as well as test the applications of gelatin in the pharmaceutical and cosmetic industries. Additionally, it is essential to conduct studies on waste processing from the extraction process to enhance the sustainability and efficiency of gelatin production. Thus, this research not only contributes to meeting the demand for halal gelatin but also supports the development of a more environmentally friendly industry.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them

Acknowledgements

Thank you to Muhammadiyah A.R. Fachruddin University (Tangerang, Indonesia) for facilitating the laboratory during this research.

References

1. Karim AA, Bhat R. Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins. Food Hydrocoll. 2009; 23(3): 563–576.
2. Kuan YH, Nafchi AM, Huda N, Ariffin F, Karim AA. Effects of sugars on the gelation kinetics and texture of duck feet gelatin. Food Hydrocoll. 2016; 58: 267–275.
3. Lu Y, Luo Q, Chu Y, Tao N, Deng S, Wang L, Li L. Application of gelatin in food packaging: a review. Polym. 2022; 14(3): 1-19
4. Purwandi S, Yuwono M, Hendradi E. Isolation and characterization of cellulose from siwalan (*Borassus flabellifer*) Fruit Peel Fiber. Trop J Nat Prod Res. 2022; 6(4): 480–483.
5. Alipal J, Mohd Pu'ad NAS, Lee TC, Nayan NHM, Sahari N, Basri H, Idris MI, Abdullah HZ. A review of gelatin: Properties, sources, process, applications, and commercialisation. Mater Today Proc. 2019; 42(02): 240–250.
6. Said MI. Role and function of gelatin in the development of the food and non-food industry: A review. IOP Conf Ser Earth Environ Sci. 2020; 492(1): 1-7
7. Katran HM, Brestovac B, Dye DE. Crab ash extract has anti-proliferative effects on sk-mel-28 melanoma cells and induces a cellular stress response and metabolic changes. Trop J Nat Prod Res. 2019; 3(4): 113–123.
8. Dorairajoo S. Halal crab, haram crab: Understanding islam in southern thailand through the lens of seafood. Glob Eat Cult Mediat Mediat. 2018; 7(13); 267–287.
9. Gani A, Yulianty R, Supiaty S, Rusdy M, Asri GD, Satya DE, Feblina AR, Achmad H. Effectiveness of combination of chitosan gel and hydroxyapatite from crabs shells (*Portunus pelagicus*) waste as bonegraft on periodontal network regeneration through IL-1 and BMP-2 Analysis. Int J Biomater. 2022; 2022(1): 1-8.
10. Cliche S, Amiot J, Avezard C, Gariépy C. Extraction and characterization of collagen with or without telopeptides from chicken skin. Poult Sci. 2003; 82(3): 503–509.
11. Topić Popović N, Lorencin V, Strunjak-Perović I, Čož-Rakovac R. Shell waste management and utilization: mitigating organic pollution and enhancing sustainability. Appl Sci. 2023; 13(1): 1–20.
12. Ismail I, Hamzah N, Qurrataayyun S, Rahayu S, Tahir K, Djide M. Extraction and characteristic of gelatin from milkfish (*Chanos chanos*) scales and bones with variation in acid and base concentrations, extracting and drying method In: 1st International Conference on Science and Technology. Makassar: Universitas Islam Negeri Alauddin Makassar; 2019. 5–10 p.
13. Rahman MN, Jamalulail SA. Chicken-Feet Gelatin : An alternative to halal species gelatin. Born Sci J. 2012; 30(1): 1-13.
14. Mulyani S, Pranoto Y, Setyabudi FMCS, Legowo AM, Santoso U. The Functional Properties of Buffalo skin Gelatin Extracted Using Crude Acid Protease from Cow's Abomasum. J Appl Food Technol. 2019; 6(2): 40–43.
15. Sántiz-Gómez MA, Mazorra-Manzano MA, Ramírez-Guerra HE, Scheuren-Acevedo SM, Navarro-Garcı´a G, Pacheco-Aguilar R, Ramı´rez-Sua´rez R. Effect of acid treatment on extraction yield and gel strength of gelatin from whiptail stingray (*Dasyatis brevis*) skin. Food Sci Biotechnol. 2019; 28(3): 751–757.
16. Shyni K, Hema GS, Ninan G, Mathew S, Joshy CG, Lakshmanan PT. Isolation and characterization of gelatin from the skins of skipjack tuna (*Katsuwonus pelamis*), dog shark (*Scoliodon sorrakowah*), and rohu (*Labeo rohita*). Food Hydrocoll 2014; 39(1): 68–76.
17. Principle A, Apparatus B. Official Methods of Analysis of AOAC International. (18th ed). USA: AOAC International; 2005. 27 p.
18. Kubmarawa D, Magomya AM, Yebpella GG, Adedayo SA. Nutrient content and amino acid composition of the leaves of *Cassia tora* and *Celtis integrifolia*. Int Res J Biochem Bioinform. 2011; 1(9): 222–225.
19. Institution BS. Methods for sampling and testing gelatine (physical and chemical methods). London: B.S.I; 1975. 27 p.
20. Bhattacharjee A, Bansal M. Collagen structure: The Madras triple helix and the current scenario. IUBMB Life. 2005; 57(3): 161-172.
21. Sutanti S, Santo M. Making gelatin from broiler chicken leg bones and milkfish bones using autoclave extraction. CHEMTAG J Chem Eng. 2021; 2(1): 23-29.
22. Chancharern P, Laohakunjit N, Kerdchoechuen O, Thumthanaruk B. Extraction of type A and type B gelatin from jellyfish *(Lobonema smithii*). Int Food Res J. 2016; 23(1): 419–424.
23. Miskiyah N, Juniawati N, Kamsiati E. The effect of solvent use and extraction techniques on the quality of chicken foot gelatin. J Penelit Pascapanen Pertan. 2020; 16(1): 10-18.
24. GMIA. GMIA Handbook. New York : Official procedures of the Gelatin Manufacturers; 2019. 25 p.
25. Ahmed MA, Al-Kahtani HA, Jaswir I, AbuTarboush H, Ismail EA. Extraction and characterization of gelatin from camel skin (potential halal gelatin) and production of gelatin nanoparticles. Saudi J Biol Sci. 2020; 27(6): 1596–1601.
26. Abend A, Curran D, Kuiper J, Lu X, Li H, Hermans A, Kotwal P, Diaz D, Cohen MJ, Zhang L, Stippler E, Drazer G, Lin Y, Raines K, Yu L, Coutant CA, Grady H, Kramer J, Pope-Miksinski S, Saurez-Sharp S. Dissolution Testing in Drug Product Development: Workshop Summary Report. Am Assoc Pharm Sci J. 2019; 21(2): 1-12.
27. León-López A, Morales-Peñaloza A, Martínez-Juárez VM, Vargas-Torres A, Zeugolis DI, Aguirre-Álvarez G. Hydrolyzed collagen-sources and applications. Molecules. 2019; 24(22): 1-16.