Journal of Natural Sciences and Mathematics Research
J. Nat. Scien. & Math. Res. Vol. 12 No. 1 (2026) 56-62
ISSN: 2460-4453 (Online); 2614-6487 (Printed)
Available online at http://journal.walisongo.ac.id/index.php/jnsmr

Microwave-assisted extraction of gelatin from Barramundi (*Lates calcarifer*) scales: effect of extraction time and quality evaluation

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ABSTRACT
Barramundi (*Lates calcarifer*) has economic value and is highly sought after in Indonesia. One of the by-products of barramundi is its scales, which are used to make gelatin products. Gelatin production can be increased by exploring alternative extraction processes that use environmentally friendly green technologies, such as microwave processing. This study aims to determine the effect of microwave extraction time on gelatin production from barramundi and to compare the evaluation results with those of standard gelatin per SNI and GMIA. The method in this study was an experiment with three extraction-time treatments (10, 20, and 30 minutes) to determine gelatin product yields and characterize the products. Results showed that curing time significantly affected yield, water content, ash content, and Zn content ($P < 0.05$), but did not significantly affect viscosity, pH, or Cu content ($P > 0.05$). Variations in extraction time indicate that it affects gelatin quality, including yield, organoleptic water content, ash content, pH, viscosity, and Cu and Zn levels. Gelatin with a microwave extraction time of 30 minutes produced the highest amount, namely 11.68 ±0.02%, with a water content of 12.77±0.01%, ash content of 2.16±0.15%, pH of 5.44±0.05, viscosity of 3.51±0.04 cps, and Cu and Zn metal content of 8.61 ±0.04 mg/Kg and 4.61±0.13 mg/Kg, respectively. The characterization results were based on SNI and GMIA standards. The FTIR results for gelatin also showed compliance. This study concluded that extracting gelatin from barramundi fish scales with lime juice in a microwave for 30 minutes is a promising method for obtaining alternative halal gelatin raw materials.
Keywords:
Barramundi Scales; Extraction Time; Gelatin; Lime Juice; Microwave.

Introduction

Barramundi (*Lates calcarifer*) is a high-value, rapidly growing aquaculture species. Still, its industrial processing generates substantial waste, with byproducts such as scales, skin, and bones accounting for roughly 75% of the fish's total weight. Transforming this low-value waste into premium resources is crucial for sustainable aquaculture, particularly through the production of fish-scale gelatin, which offers superior characteristics compared to those of other fish species (Alfio et al., 2021; Hariyanti, Nafi'ah, et al., 2024a). This alternative source effectively addresses the religious restrictions associated with dominant, mammalian-derived commercial gelatins from pigs or cattle, providing a globally acceptable and culturally inclusive option for the pharmaceutical and food industries (Fajar Deliaz et al., 2025). Furthermore, incorporating natural acids, such as lime juice, into the collagen hydrolysis process is an eco-friendly green technology that extracts gelatin without altering its core structural bonds, matching the quality of synthetic acid extractions (Wonganu, 2020). One green extraction technology increasingly used in gelatin extraction is microwave-assisted extraction (Fauziyah et al., 2023; Kholis et al., 2025).

Microwave-assisted extraction is a gelatin extraction process that combines microwave energy with solvent to break down compounds in the sample to be analyzed. The principle of this method is that the microwave effect increases temperature, breaking down the cell wall and allowing the compound to flow toward the solvent (Nam et al., 2017; Pinto et al., 2021). Microwave extraction is not only a modern technology with short extraction times and reduced energy consumption, but also offers significant environmental benefits. Using water-solvents and ethanol minimizes environmental impact and improves extraction efficiency. This sustainable approach is a key aspect of our research and will likely interest the audience. Gelatin extraction using microwave technology has attracted attention for its potential to improve efficiency and reduce environmental impact. This method is being explored across various sources, including duck skin (Kang et al., 2023; Nam et al., 2017), fish (Kang et al., 2023), and rabbit skin (Liu et al., 2019), with a focus on optimizing yield and functional properties.

While microwave-assisted extraction offers several benefits, it also presents challenges. The method requires careful optimization of parameters, such as microwave power and treatment time, to avoid overhydration and degradation of gelatin properties. Furthermore, the development of sophisticated systems for large-scale production remains a challenge (Noor et al., 2021). Therefore, researchers are interested in conducting research on gelatin extraction from barramundi scales (*Lates calcarifer*) with variations in extraction duration and lime juice solution concentration (1: 3), which can affect the hydrolysis process in barramundi scales (*Lates calcarifer*) and is the primary source of making safe and halal gelatin. A characterization test of the gelatin was conducted to determine the quality of barramundi scale gelatin (Lates calcarifer) obtained by microwave extraction.

Methods

Materials

The material used is barramundi (*Lates calcarifer*) scale by-products obtained from the Cilincing Traditional Fish Market. Other ingredients used are lime juice (concentrate solution from *Citrus aurantifolia*), sodium lauryl sulfate (Sigma-Aldrich), and Aquadest. All the chemicals or solvents used in this study were of analytical grade and used directly without further purification.

The equipment used is a Microwave (R-21D0(S)-IN; SHARP, frequency 2450 MHz, power 450 W), a Fourier Transform-Infrared Spectrophotometer (Shimadzu Excellence in Science), an atomic absorption spectrophotometer (Shimadzu), an oven (Mammert), a pH meter (Hanna), and an Ostwald viscometer.

Degreasing and Demineralization of Fish Scales

The degreasing procedure follows the method of Erizal et al. (2018) and was modified. Waste barramundi scales (200 grams) are cleaned of surface impurities by soaking in 21% sodium lauryl sulfate at 1:2 (w/v) for 24 hours and refrigerating. Then, they are washed with aqua dest and dried. After the fish scales are dried, they are weighed (Erizal et al., 2018). Fish scales are dried and then soaked with acid. The acid is squeezed lime at a 1:3 (w/v) ratio for 24 hours and stored in the refrigerator. Then, rinse under running water until the pH of the barramundi scales is neutral (Erizal et al., 2018; Hariyanti, Nafi'ah, et al., 2024b).

Extraction of Gelatin from Fish Scales

The acquired demineralized fish scales (ossein) are added to Aquadest in a 1:2 ratio. The extraction is then carried out in a microwave (Sharp, 450 W) at 30% power for 10, 20, and 30 minutes. The extraction result is filtered and laid out on a flat tray. The extraction results were placed on a tray and dried in an oven at 50-60 °C for 24 hours, until a gelatin sheet formed. The formed gelatin sheets were cut into small pieces and blended until a gelatin powder formed (Hariyanti, Nafi'ah, et al., 2024b).

Analysis of gelatin product characteristics

The gelatin was tested for characteristics to determine the best results in accordance with the 1995 SNI and 2019 GMIA standards. The characteristics observed in this research were gelatin's organoleptic, physical, and chemical properties. The physical properties of yield percentage and viscosity were analyzed (Zhang et al., 2016). The chemical properties were pH, water content, ash content, Cu content, and Zn content (SNI, 1992).

Identification of gelatin using an FTIR Spectrometer

The determination of gelatin's wavelength is based on several modifications (Das et al., 2017). The sample was scanned 20 times at a resolution of 2 cm-1 over the wavenumber range of 4000 cm^{-1} to 400 cm^{-1}. Gelatin samples formed a powder that was characterized directly by FT-IR spectrophotometers.

Results and Discussions

The Organoleptic Observation of Gelatin aims to find out its characteristics. Organoleptic observations, based on SNI standards, included color, odor, and shape statements. The results of the organoleptic observations are shown in Table 1.

Table 1. Organoleptic Gelatin Fish Scales

Parameter	SNI 1995	Extraction Time (minutes)		
		10	20	30
Color	Colorless - yellowish	Pale yellow	Pale yellow	Pale yellow
Smell, taste	Acceptable	Acceptable	Acceptable	Acceptable

The gelatin extraction yield from barramundi scales is expressed as a percentage. The yield was calculated as the weight of the gelatin extract divided by the weight of the fish scales (Hariyanti, Nafi'ah, et al., 2024b). The output from the microwave extraction of each acid soak with time variations (10, 20, and 30 minutes) is shown in Figure 1. Based on the data in Figure 1, extraction with lime juice (1:3) can yield a reasonably high amount. The highest yield of gelatin products was obtained after 30 minutes of extraction, at 11,68±0.02%. This shows that the longer the extraction time, the more collagen is hydrolyzed into gelatin, and is in line with previous research on the pressure-cooking extraction of chicken breasts (Miskiyah et al., 2022). These results show that soaking in lime juice (1:3) using microwave technology has a high extraction efficiency, as previous studies using citric acid and water bath extraction reported a maximum yield of 7.14% (Penniston et al., 2008). The development is also supported by research on lime juice (1:3) that can be used as a natural hydrolytic acid for gelatin extraction (Hariyanti, Gantini, et al., 2024; Wonganu, 2020). Lime contains a weak tricarboxylic acid called citric acid with a concentration of 1.38 g/oz, equivalent to 0.0475 g/mL (Penniston et al., 2008). The yield of gelatin can be significantly influenced by the extraction method. For example, the microwave-freezing-thawing coupling method showed that lower freezing temperatures could enhance yield by facilitating the dissolution of subunit components.

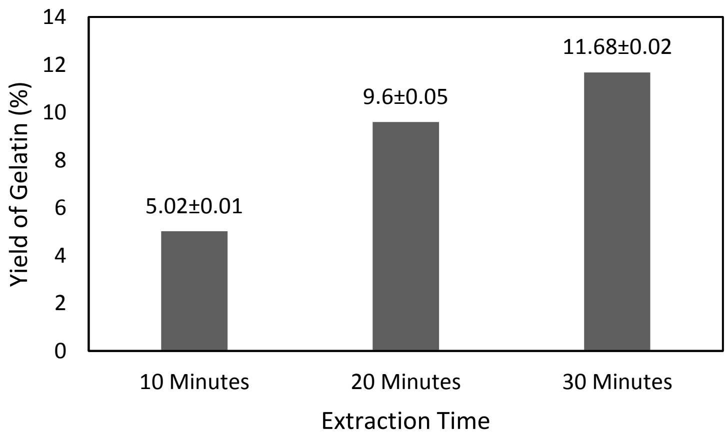

Figure 1. Value of the Yield of Barramundi Scales Gelatin

Measurement of water and ash content aims to meet SNI standards (Hariyanti, Gantini, et al., 2024). Table 2 shows the results for ash content. Determining the water content provides a minimum limit or range for the material. Choosing the water content is essential in testing the quality of food products. The result is that water is a habitat for microbes, bacteria, enzymes, and chemicals that alter texture, color, taste, and aroma. The water yield from gelatin extracted with lime juice (1:3) is relatively high. The water content at extraction decreases as the time variation increases. The high water content in the gelatin is due to an insufficiently thorough drying process. The water content of gelatin is regulated by SNI 1995 at 16%, indicating that the gelatin extract used meets this standard.

The determination of ash content is known from organic compounds; their derivatives are deformed and evaporated by heating, so only mineral and inorganic elements remain (Kementerian Kesehatan Republik Indonesia, 2020). Based on data from gelatin extraction using lime juice (1:3), the ash content of the entire sample is, on average, below 6%, and the ash purity decreases (Table 2). In a previous study, the ash content of barramundi bone gelatin (*Lates calcarifer*) in 7% HCl was 3%. According to the Indonesian national standard SNI No. 06-3735-1995, the ash content of the gelatin produced from barramundi (*Lates calcarifer*) bones remained within the specified limits. The ash content of the catfish gelatin extracted with citric acid solvents was 0.38%. Results indicate that lower ash content is associated with higher material purity (Hariyanti, Nafi'ah, et al., 2024b).

Table 2. Value of Parameters of Barramundi Scales Gelatin Characteristics

Extraction Time (minutes)	**Value of Gelatin Characteristics**					
	Water Content (%)	**Ash Content (%)**	**pH**	**Viscosity (cps)**	**Cu content (mg/Kg)**	**Zn content (mg/Kg)**
10	16.31±0.22^{c}	5.27±0.03^{c}	5.06±0.03^{a}	3.32±0.01^{a}	5.74±0.03^{a}	20.38±0.01^{b}
20	15.01±0.04^{b}	1.90±0.02^{a}	5.19±0.01^{a}	3.56±0.23^{a}	7.96±0.02^{b}	24.56±0.06^{c}
30	12.77±0.01^{a}	2.16±0.15^{b}	5.44±0.05^{a}	3.51±0.04^{a}	8.61±0.04^{b}	4.61±0.13^{a}
SNI 1995	Max 16	Max 3.25	-	-	Max 30	Max 100
GMIA	-	0.3 – 2.0	3.8 – 5.5	1.5 – 7.5	-	-

All values were mean ± standard deviation (n=3). The same letter (a, b, and c) in the same row indicates no significant differences between means.

Gelatin pH value measurement aims to meet the GMIA-determined pH standard of 3.8-5.5 (GMIA, 2025). Based on the measurements, the gelatin pH ranges from 5.06 to 5.44. This value still meets the standards for type A gelatin (acid-soaking). These results follow previous studies (Hariyanti, Gantini, et al., 2024). The lower the acid's pH, the lower the gelatin solution's pH. The pH results are shown in Table 2.

Viscosity measurement aims to determine the viscosity of gelatin using an Ostwald viscometer. The results showed that the average viscosity value ranged from 2.7 to 3.9 (cps). This value still meets the GMIA standard for type A gelatin (soaking process with acid), with a range of 1.5-7.5 (cps) (GELITA North America, 2019). The previous study showed that red snapper scales extracted with 5% acetic acid soaking yielded the highest viscosity of 15.54 (cps). The results of the viscosity measurements are shown in Table 2. Microwave extraction methods have been shown to increase gelatin viscosity, which is beneficial for certain applications. This is consistent with the use of microwave acid on chicken feet, which also produces high viscosity (Esmaeili-Kaliji et al., 2025). The viscosity of gelatin extracted by microwave methods was higher than that of gelatin extracted by other methods, such as water bath extraction. Additionally, MAE can lead to numerous holes on the gelatin surface, potentially affecting its structural properties (Liu et al., 2019).

Based on ash content analysis, good results were obtained, with ash content below the SNI standard (Liu et al., 2019). The Atomic Absorption Spectrometry (AAS) analysis showed that the highest Cu metal content at an extraction time of 30 minutes was 8.61±0.04 mg/kg. Based on the SNI standard of Cu metal in gelatin, the maximum was 30 mg/kg. The results of the AAS analysis showed that the highest Zn metal content at an extraction time of 20 minutes was 24.26 mg/Kg. According to the SNI standard for Cu metal in gelatin, the maximum is 100 mg/kg. The results of measuring Cu and Zn levels are shown in Table 2.

Identification of gelatin products is performed by FT-IR analysis to detect functional groups indicative of the gelatin structure (Table 3). Proteins such as gelatin generally have a structure consisting of an amine group (N-H), a hydroxyl group (-OH), a nitrile group (C-N), an alkane group (C-H), and a carbonyl group (C=O). Based on the reading of the gelatin spectrum of barramundi scales in Table 3, the gelatin results in the FTIR test show the existence of a functional group that is the same as the specific active group of gelatin (Hariyanti, Gantini, et al., 2024).

Table 3. FT-IR Gelatin Spectrum Results Data Barramundi Scales

Amida	Functional Groups	Wavenumber Range (cm^{-1})	Wavenumber Peak (cm^{-1})		
			10 minutes	20 minutes	30 minutes
Amide A	N-H Stretching	3600-3000	3274.5	3278.2	3278.2
Amide B	C-H Stretching	3300-2700	2933.4	2933.6	2935.3
Amide I	C=O Stretching	1900-1600	1628.8	1628.8	1628.8
Amide II	N-H Bending	1650-1500	1533.8	1522.6	1522.6
Amide III	O-H Bending	1450-1200	1436.9	1437.0	1436.9

Conclusion

Based on the study's results, it can be concluded that variations in microwave-assisted extraction time affect gelatin yield. Variations in extraction time showed that drying time significantly ($P < 0.05$) affected the yield, moisture content, ash content, and Zn content, but did not significantly ($P > 0.05$) affect the viscosity, pH, or Cu content. The optimal extraction time was 30 minutes to achieve the highest yield, and all parameters met the requirements of SNI (1995) and GMIA. Analysis (FTIR) showed that all amide bands were present in acidic gelatin obtained by microwave-assisted extraction. This indicates that extracting gelatin from barramundi fish scales using lime juice and microwave assistance is a promising approach to producing environmentally friendly gelatin.

Acknowledgments

This work was supported by the Research and Development Department of UHAMKA in 2022 (No. 711/F.03.07/2021), for which we are grateful.

Conflicts of interest

The authors declare no conflicts of interest.

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