

Collagen Extraction from Snakehead Fish Skin with NaOH and Bilimbi Extract

Marhaini^{1*}, Dewi Fernianti², Delfi Yulandari³

^{1,2,3}Chemical Engineering Program, University of Muhammadiyah Palembang

Corresponding author:

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Abstract

Snakehead fish skin is a rich source of collagen, an important protein that can be processed into gelatin. In this study, collagen was extracted from snakehead fish skin using sodium hydroxide (NaOH) and bilimbi extract. The study aimed to determine the effects of varying the mass of fish skin and the NaOH soaking duration on gelatin properties. Parameters analyzed included moisture, ash, protein, and fat contents, gel strength, viscosity, FTIR spectral characteristics, and overall yield, utilizing a maceration extraction method at 40°C.

The experiment was performed using various fish skin mass (50, 100, 150, 200, and 150 g) and different NaOH soaking durations (1, 2, and 3 hours). All skins were also soaked in bilimbi extract for 3 days. The results showed that 250 g and 3 hours of NaOH soaking obtained gelatin with the yield of 9.4%, moisture of 2.91%, ash of 1.18%, pH of 6.3, and viscosity of 6.09 cps. The optimal condition of protein (73.01%) was achieved from 250 g and NaOH soaking for an hour. The lowest fat content (0.87%) was observed with a 50 g sample soaked with NaOH for 3 hours, whereas the highest gel strength (302.42 g/cm²) was achieved using a 250 g sample with a 1-hour soaking period with NaOH. The FTIR spectrum presents a triple helix structure on amide-III band, indicating collagen as the main component in gelatin formation.

Keywords: Bilimbi, Collagen, Gelatin, NaOH, Skin

1. Introduction

Aquaculture has emerged as a key sector in global food supply and economic development, driven by the growing demand for sustainable aquatic food sources. With the world population projected to reach 9.7 billion by 2050, enhancing the quality and efficiency of food production systems has become increasingly critical [1,2]. Over the past two decades, the aquaculture industry has expanded rapidly, with the intensive culture method in various species, enabling highly controlled production and strong market adaptability to meet rising global demand. In 2024, global aquaculture production was estimated at 122.6 million tons, valued at \$264.8 billion, with 71% of which derived from aquatic animals [3]. Snakehead fish (*Channa striata*) is a

widely-distributed fish across China, India, Sri Lanka, Eastern India, Philippines, Nepal, Myanmar, Pakistan, Singapore, Malaysia, and Indonesia [4]. Palembang, a capital city of South Sumatra Province, is known to possess traditional cuisine processed from snakehead fish, namely *pemek*, *model*, *tekwan*, *laksan*, and *celimpungan*. Approximately, 70% of fishery products are processed for food consumption, resulting in substantial quantities of solid waste, including heads, skins, internal organs, fins, scales, and fillet trimmings [5]. Over 50% of the total fresh fish weight is typically discarded during processing, often disposed of by incineration or landfilling, which contributes to both environmental degradation and economic inefficiency. Only a limited portion of this waste is currently repurposed, primarily as animal feed. Addressing fishery waste requires rapid and innovative solutions. Globally, various initiatives have been implemented to minimize food waste, aiming not only to reduce management costs but also to enable the recovery of valuable biomolecules, such as oils, proteins, pigments, bioactive peptides, amino acids, collagen, chitin, and gelatin [6].

Collagen is an endogenous protein composed of three polypeptide chains arranged in a triple helix structure. These chains are characterized by a repeating (Gly-X-Y)_n sequence, where X and Y are often occupied by proline and 4-hydroxyproline, respectively [7]. Degradation of collagen due to intrinsic aging, ultraviolet (UV) radiation, and tobacco exposure can lead to a range of physiological concerns, including wrinkles, sagging skin, joint stiffness, and dryness of the skin. As a result, the identification of novel collagen sources is crucial for supporting tissue repair and regeneration [8,9,10]. Byproducts of fish processing, particularly skins and bones, have emerged as promising sources of collagen, yielding up to 61.26% of dry weight when extracted using the acid-solubilized collagen (ASC) method [11,12,13].

Fish skin and bones contain type-I collagen, which possesses significant potential as an antioxidant, anti-aging, and skin-brightening agents [14, 15, 16]. Cosmetic products that contain fish collagen have been shown to enhance facial skin hydration, reduce pores and wrinkles, and noticeably brighten the skin when used consistently for at least two weeks. Additionally, fish collagen exhibits antioxidant, anti-aging, and moisturizing properties, and supports damaged skin reparation [17, 18, 19].

In this study, collagen was extracted from snakehead fish skin through a soaking process using a sodium hydroxide (NaOH) solution to remove non-collagenous proteins from the epidermal layer. As an acidifying agent, natural citric acid derived from bilimbi extract was applied as a substitute for synthetic acids, making the process more environmentally friendly and safe for consumption.

2. Methods

2.1. Materials and Equipment

Furnace, soaking container, analytical scales, funnel, hot plate, oven, filtered paper, tray, beaker glass, aluminium foil, Erlenmeyer glass, spatula, dessiccator, knife, pH Meter, cutting tray, snakehead fish skin, NaOH, bilimbi, and aquadest

2.2. Procedures

1) Material preparations

- Snakehead fish skin preparation

The 6 kg of snakehead fish skin were washed with clean water to remove meat and fats attached to the skin. The skins were cut evenly and dried under the sunlight.

- Acid preparation

The 6.2 kg of bilimbi fruits were chopped into smaller pieces and squeezed without water. The juice or extract of the bilimbi fruits was then separated from the pulp using a strainer. Subsequently, the volume, concentration, and pH of the bilimbi extract were analyzed. A total of 4 L extract was obtained, with a concentration of 8.6 M and a pH of 1.2.

- NaOH 0.05 M preparation

The 6.2 kg of bilimbi fruits were chopped into smaller pieces and squeezed without water. The juice or extract of the bilimbi fruits was then separated from the pulp using a strainer. Subsequently, the volume, concentration, and pH of the bilimbi extract were analyzed. A total of 4 L extract was obtained, with a concentration of 8.6 M and a pH of 1.2.

2) Snakehead fish skin soaking with NaOH

The 50 g of dried snakehead fish skin was weighed and placed in a 1000 ml of Beaker glass, before pouring NaOH 0.05 M into the glass at 250 ml. After an hour of soaking, the skin was cleaned from NaOH solution with 200 ml of aquadest. This procedure was performed three times until the pH was remained neutral. Also, this procedure was repeated for 100 g, 150 g, 200 g, and 250 g, with each for soaking durations of 2 and 3 hours.

3) Acid Soaking

The snakehead fish skins, previously treated with NaOH soaking, was subsequently soaked in 250 ml of bilimbi extract for 3 days, with varying skin mass samples.

4) Extraction

The soaked snakehead fish skin in bilimbi extract was cleaned with aquadest until the pH was remained neutral. Extraction from the fish skin was performed in 250 ml of aquadest at 40°C for 2 hours to produce water-soluble collagen.

5) Drying

The gel extract was dried in an oven at 70°C for 12 hours. After drying, the gelatin sheets were grounded and prepared for further analysis, including moisture, ash, fat, and protein contents, pH test, yield production, viscosity, and gel strength.

3. Results

3.1 Effect of Snakehead Fish Skin Mass and NaOH Soaking Duration on Gelatin Yield

The gelatin yield was produced at 6.3–9.4% (Figure 1). The lowest yield was obtained from the 50 g sample with an hour of soaking, while the highest yield was found in 250 g sample in 3-hour soaking. A greater sample mass and a longer soaking duration affect on the increased yield production. Yield is an important parameter in gelatin production. Jakhar et al. (2014) used central composite design (CCD) and RSM methods to optimize four variable processes, namely NaOH concentrations, soaking time, temperature, and extraction time [20]. The three analyzed responses—yield, gel strength, and melting point—demonstrated a good model fit, with R^2 values of 0.867, 0.837, and 0.765, respectively. Meanwhile, Gokula et al. (2018) reported a maximum collagen yield of 19.27 ± 0.05 mg/g of fish skin under optimal extraction conditions using 0.54 M acetic acid, 1.90 M NaCl, a solvent-to-solid ratio of 8.97 mL/g, and an extraction time of 32.32 hours [21].

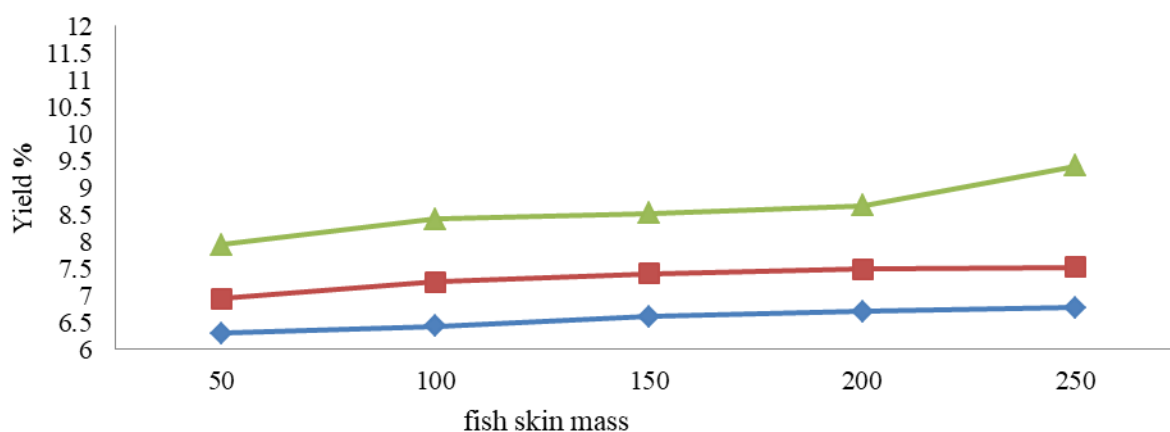


Figure 1. Correlation of Snakehead fish skin and NaOH soaking duration on Gelatin Yield

3.2. Effect of Snakehead Fish Skin Mass and NaOH Soaking Duration on Gelatin Moisture Content

The moisture content of the gelatin was at 2.91-10.6% (Figure 2). The highest value was observed in the 50g sample soaked for an hour, and the lowest value was found in the 250g sample soaked for 3 hours. Increasing both the sample mass and soaking duration led to a reduction in moisture content, that still remained within the acceptable limit set by the Indonesian National Standard (SNI No. 06-3735:1995), with a maximum threshold of 16%. Nurimala et al. (2022) reported that the moisture content of gelatin derived from red Nile tilapia, red snapper, and parrotfish was within the normal range (approximately 8%), thus in line with the typical average moisture content of gelatin produced in this study [22].

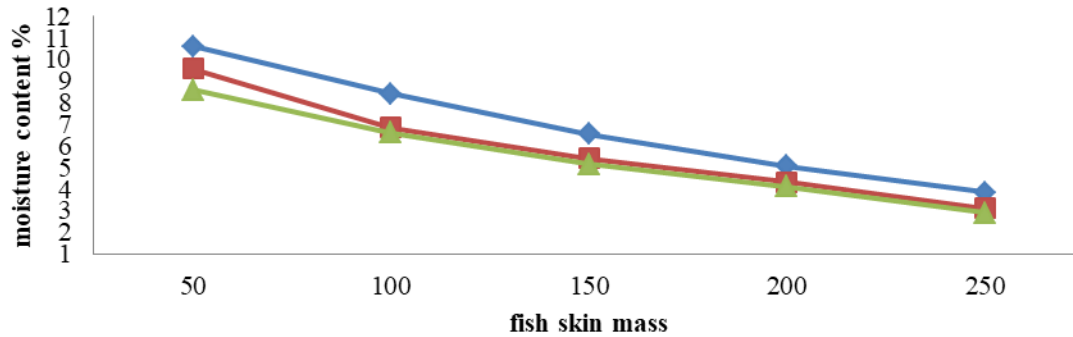


Figure 2. Correlation of Snakehead fish skin and NaOH soaking duration on Gelatin Moisture Content

3.3. Effect of Snakehead fish skin and NaOH soaking duration on Gelatin Ash Content

The ash content of the gelatin was at 1.18-2.72% (Figure 3). The highest value (2.72%) was obtained from the 50g sample soaked for an hour in bilimbi extract and NaOH. In contrast, the lowest value (1.18%) was recorded in the 250-g sample soaked for 3 hours. In general, increasing both the skin mass and soaking duration led to a reduction in ash content. Nevertheless, all values remained within the maximum limit set by the Indonesian National Standard (SNI No. 06-3735:1995) at 3.25%. According to Syahrial et al. (2022), gelatin derived from striped catfish skin pre-treated with acid yielded the lowest ash content of 0.19%, compared to 1.54% using an alkaline method [23]. Muyonga et al. (2004) also reported that a combination of acid and alkaline methods to the fish skin produced gelatin with low ash content, compliant with international standards [24].

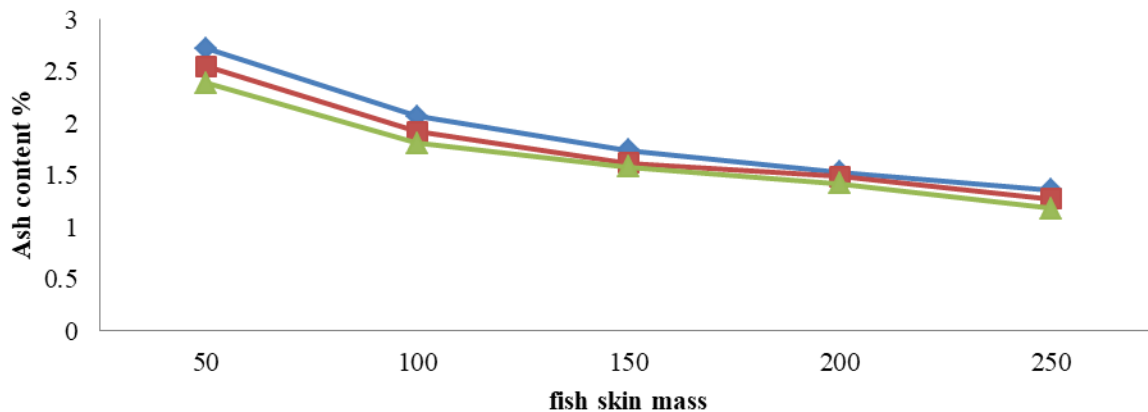


Figure 3. Correlation of Snakehead fish skin and NaOH soaking duration on Gelatin Ash Content

3.4. Effect of Snakehead fish skin and NaOH soaking duration on Protein Content

According to Figure 4, the gelatin contained 63.29-73.01% protein. The lowest protein content was observed in the 50g sample soaked for 3 hours, while the highest protein content was recorded in the 250g sample soaked for an hour with NaOH. All measured protein contents were below the Indonesian National Standard (SNI No. 06-3735:1995), which specifies minimum values of 85.99% for standardized gelatin and 87.25% for commercial-grade gelatin. Thus, the gelatin samples did not meet the standard criteria for gelatin quality. In Hammed et al. (2014), optimization of gelatin extraction from silver carp resulted in a protein recovery of up to 78%, with a purity range of 71.8% to 97% [25]. An acid pretreatment using 0.1 N HCl for 45 minutes was reported to yield optimal conditions for gelatin protein content [26].

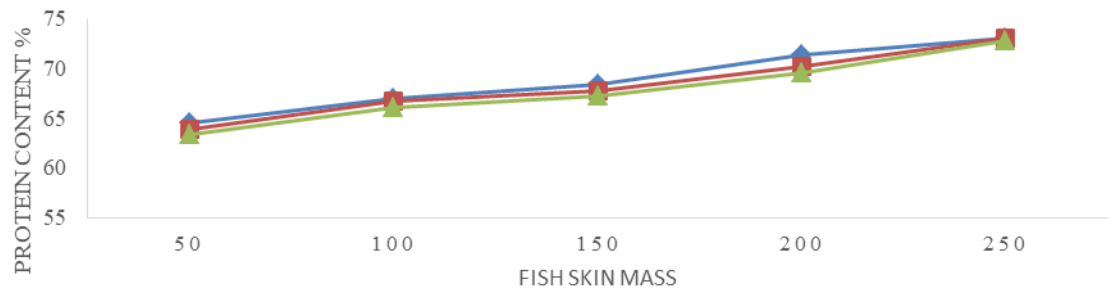


Figure 4. Correlation of Snakehead fish skin and NaOH soaking duration on Gelatin Protein Content

3.5. Effect of Snakehead fish skin and NaOH soaking duration on Gelatin Fat Content

In Figure 5, the fat content of gelatin was achieved at 0.87–3.05%. The lowest fat content at 0.87% was shown in the 50 g sample and 3 hours of soaking using NaOH and bilimbi extract, while the highest fat content at 3.05% was found in the 250 g sample and an hour of soaking.

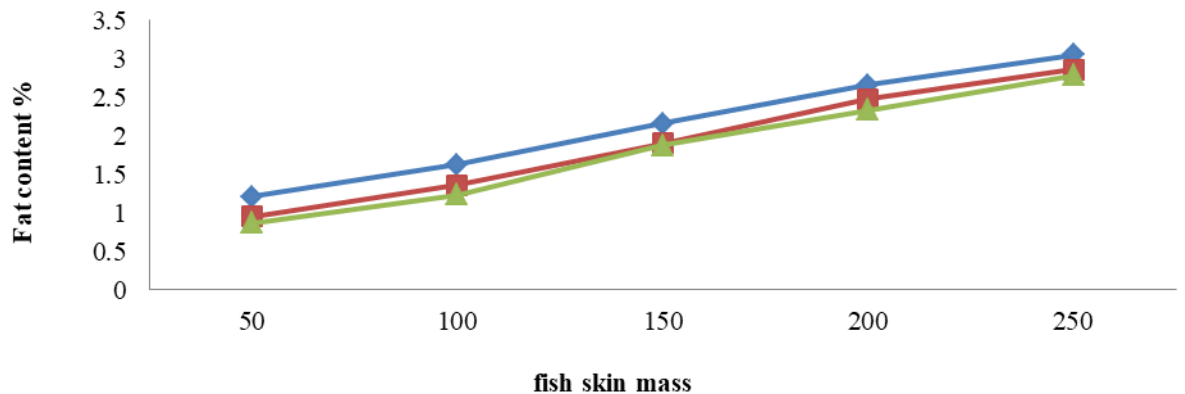


Figure 5. Correlation of Snakehead fish skin and NaOH soaking duration on Gelatin Fat Content

According to Nguyen et al. (2020), different NaOH concentrations and soaking durations significantly influenced fat removal efficiency on gelatin produced from yellowfin tuna skin. Increasing the NaOH concentration from 0.8 N to 0.93 N could reduce the residual fat content. However, excessively high concentrations or prolonged soaking duration led to a decreased dry matter, thereby increasing the percentage of residual fat due to the protein or collagen loss [27].

3.6. Effect of Snakehead fish skin and NaOH soaking duration on Gelatin pH Level

The pH level of gelatin produced from snakehead fish skin was at 6.3 (Table 1). This value is still within the standard range set by the the Indonesian National Standard (SNI No. 3735:1995) at 4.5–6.5, thus all mass and soaking duration variables have met the standard criteria for gelatin product.

Table 1. Effect of Snakehead fish skin and NaOH soaking duration on Gelatin pH Level

| Soaking duration (hours) | pH value | | | | | Quality Standard (SNI No. 3735:1995) |
|--------------------------|--------------------|-----|-----|-----|-----|--------------------------------------|
| | Fish Skin Mass (g) | | | | | |
| | 50 | 100 | 150 | 200 | 250 | |
| 1 | 6.3 | 6.3 | 6.3 | 6.3 | 6.3 | |
| 2 | 6.3 | 6.3 | 6.3 | 6.3 | 6.3 | 4.6 -6.5 |
| 3 | 6.3 | 6.3 | 6.3 | 6.3 | 6.3 | |

According to Roy et al. (2017), alkaline pre-treatment using NaOH could typically yield a type-B gelatin, characterized by a final pH of 4-5, while acidic pre-treatment produced a type-A gelatin with a pH closer to 7-9. For example, in gelatin extracted from bovine lung tissue, the recorded pH ranged from 4.73 to 5.57 (type B), following a mild acid or an alkali treatment. The pH of gelatin significantly influences its helical (conformational) structure, whereas the pH range of approximately 5 to 10 exhibits gelatin with maximum helix content and optimal gel strength. In contrast, apart from highly acidic or alkaline conditions can cause destabilized helix structure, reducing its ability to form a strong gel[28].

3.7. The FTIR Spectrum of Gelatin from Snakehead Fish Skin

The FTIR spectral analysis was conducted to identify the presence of gelatin through its characteristic functional groups. This spectroscopy technique detects specific absorption bands within the infrared region, including Amide-A (3800-3200 cm⁻¹), Amide-B (2450-2390 cm⁻¹), Amide-I (1710-1590 cm⁻¹), Amide-II (1590-1490 cm⁻¹), and Amide-III (1300-1200 cm⁻¹). These absorption bands confirm the presence of gelatin in the sample. The FTIR spectrum results are presented in Figure 6.

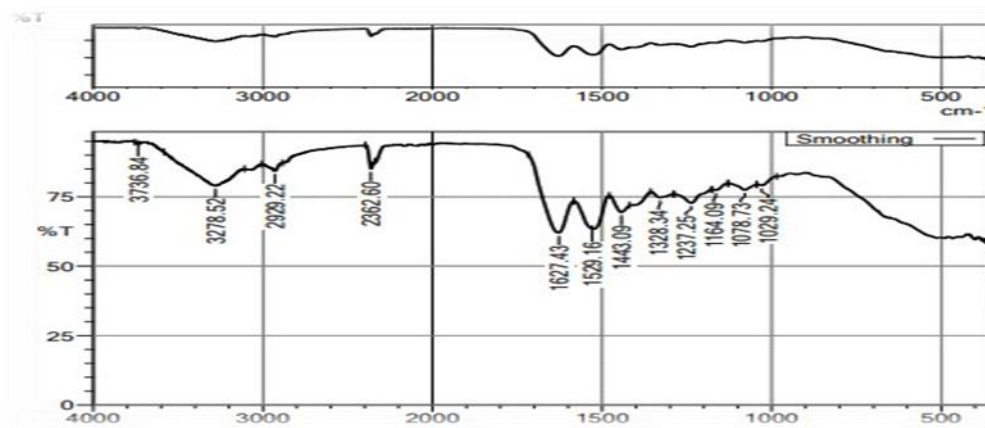


Figure 6. The FTIR Spectrum of Gelatin from Snakehead Fish Skin

Figure 6 illustrates the FTIR spectrum of gelatin from snakehead fish skin, highlighting the characteristics of functional groups in collagen. Absorption peaks were identified in the mid-infrared region, specifically in the Amide-A, Amide-B, Amide-I, Amide-II, and Amide-III regions. The Amide-A peak was observed at 3278.52 cm^{-1} , indicating N–H stretching vibrations associated with hydrogen bonding and the presence of proline amino acid [28]. Additionally, the spectrum that exhibited a peak at 2362.60 cm^{-1} was corresponded to Amide-B, characterized by asymmetric CH_2 stretching as a typical condition of the gelatin structure [29].

4. Conclusion

1. The leaching process by applying 250 g snakehead fish skin soaked in NaOH 0.05 M for 3 hours obtained the best gelatin production, with the yield of 9.4%, moisture of 2.91%, ash of 1.18%, pH of 6.3, and viscosity of 6.09 cps.
2. Under optimal conditions, the highest protein content (73.01%) and gel strength (302.42 g/cm^2) were achieved using 250 g of fish skin soaked in NaOH for an hour, while the lowest fat content (0.87%) was observed in the 50 g of fish skin soaked for 3 hours.
3. The physical characteristics of gelatin, confirmed through FTIR analysis, identified the presence of Amide-A, B, I, II, and III functional groups. The detection of the Amide-III peaks, associated with triple-helix structure, indicates that a portion of the collagen remained partially undegraded during the gelatin extraction process.

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