

The effect of citric and acetic acid treatment on gelatin production from catfish skin

Nami Lestari^{1,2*}, Lamhot P. Manalu¹, Taufik Hidayat¹, Lukman Junaidi¹, Eddy Sapto Hartanto¹, Rienoviar¹, Suroto Hadi Saputra³, Armen Zulham⁴, and Dheni Mita Mala⁵

¹Research Centre for Agroindustry, Research Organization for Agriculture and Food, National Research and Innovation Agency (BRIN), 16911 Cibinong, Indonesia

²School of Postgraduate Studies, Universitas Djuanda, 16720 Bogor, Indonesia

³Research Center for Biomass and Bioproduct, Research Organization for Life Sciences and Environment, National Research and Innovation Agency (BRIN), 16911 Cibinong, Indonesia

⁴Research Center for Cooperative, Corporation, and Peoples Economy, Research Organization for Governance, Economy, and Community Welfare, National Research & Innovation Agency, 12710 Jakarta, Indonesia

⁵Centre for Agro-based Industry (BBSPIA), Ministry of Industry, 16122 Bogor, Indonesia

Abstract. The demand for halal gelatin is increasing, but the supply is decreasing. One solution to this problem is to produce halal gelatin from fishing waste, particularly catfish skin. A study was conducted to determine the impact of adding acid to the gelatin extraction process. Citric and acetic acid were used at concentrations of 0.05%, 0.075%, and 0.1%. The yield of gelatin varied from 14.97% to 24.1% depending on the concentration of acid used. The highest yield was obtained using a concentrated solution of acetic acid. Gelatin extracted using a citric acid concentration of 0.075% had the lowest water content. The pH of the solution ranged from 4 to 5, and the viscosity ranged from 10 to 14 MPa. The resulting gelatin exhibited a strength of between 200 and 259 blooms. The production process that used acetic acid on immersion was the most efficient because it resulted in high quantities of gelatin, low water content and viscosity, and good gel strength.

1 Introduction

Gelatin is a protein that is derived from the hydrolysis of collagen found in animal bones or skins [1, 2, 3]. It is a peptide mixture with protein. Gelatin is widely used in the food and non-food industries [3-4] due to the characteristics of the hydrocolloid, which play a vital role in the properties of the products. According to Said [3], the characteristics of gelatin are influenced by the properties of the collagen that make it up. Collagen is a derivative of high molecular weight fibrous proteins [2], with long polypeptide chains that form about 50 to 1000 amino acid chains. These chains are mainly composed of the amino acids glycine, proline, and hydroxyproline [3, 5].

The global market demand for standard food gelatin products was \$1638 million in 2021, according to Straits Research [6]. This demand is predicted to rise to \$2389 million in 2030,

* Corresponding author: namilestari65@gmail.com

with a cumulative annual growth rate (CAGR) of 4.3% during the period 2022-2030. Although there is an increase in global gelatin market demand, the demand for halal gelatin products required by halal industries, such as the food, cosmetics, and pharmaceutical industries [4], is also growing. The State of the Global Islamic Economy (SGIE) report of 2022 suggests that Muslims around the world spent \$2 trillion on food, medicine, and cosmetics in 2021. This spending reflects a year-to-year growth of 8.9% and is expected to reach \$2.8 trillion by 2025 with a CAGR of 7.5% [7]. The Global Islamic Economy Indicator report, which covers 81 countries, shows that Indonesia is the fourth-largest country in demand for halal products, after Malaysia, Saudi Arabia, and the United Arab Emirates [7]. Indonesia, as the world's largest Islamic country [8], needs many halal products to be consumed, including gelatin that is much needed in the Indonesian halal industry. However, Indonesia still imports gelatin from countries such as the United States, Spain, Japan, and China. The US is the world's largest gelatin producer, with a 31.26% market share, followed by Spain with a market share of 12.72%. The demand for gelatin-containing foods and beverages in Indonesia, such as ice cream, yogurt, and chocolate, as well as cosmetics and pharmaceuticals [4, 9], is increasing rapidly, which is why Indonesia needs to produce more halal gelatin.

Indonesia is blessed with abundant biological resources that can be utilized as raw materials for the production of halal gelatin. These include cowskin, goatskin, camelskin, and fishskin. Biopolymers of partial hydrolysis nature that produce biodegradable packaging films with excellent mechanical properties, high quality, good biocompatibility, non-toxicity, and excellent film-forming properties can be derived from fish gelatin [10]. Given the varied and widely distributed fishery products throughout Indonesia, catfish is a potential raw material for gelatin production, according to Indonesian local wisdom. The Director-General of Marine Competitiveness and Fisheries of the Ministry of Marine Affairs and Fisheries (KKP) has dismissed the idea that catfish (*Pangasius sp*) is not a fish that is in great demand in the world market. On the contrary, catfish is one of the most sought-after fish in the global market, with a projected Indonesian production of 380,000 tons by 2022, most of which is absorbed by the domestic market. This strengthens the potential for using catfish as a raw material for gelatin production [4, 10]. The skin part of the catfish can be used to produce gelatin [11].

Gelatin from the skin of the *Pangasianodon gigas* fish contains 89.1 g of protein/100 g and 0.75 g of fat/100 g, with high amounts of proline and hydroxyproline amino acids (211 residues per 1000 residues) [12]. The amino acid composition of catfish gelatin is slightly different from bovine gelatin, but the gelatin from catfish skin has greater gelatine flowering strength, viscosity, foam capacity, and foam stability compared to cow skin gelatin. SDS-PAGE gelatin of catfish leather also shows a high band intensity on the main protein components, particularly the α -, β -, and γ -components, which are similar to standard type I or type A collagen. Furthermore, it was found that catfish leather gelatin has a high gel strength (276 ± 5 g) and a different amino acid composition compared to pig leather gelatin [11].

The success of gelatin extraction from catfish skin depends on the type of solution used during the extraction process [11-12]. The other research has also expressed a similar view, stating that the method of gelatin isolation affects the success of the process [13]. It also has identified acetic acid and citric acid as the most commonly used solvents in the pre-treatment of raw materials [13]. The type of solvent used in pre-treatment determines the type of gelatin produced, with acid pre-treatment producing A-type gelatin, while base pre-treatment produces B-type gelatin [3, 13, 14, 15]. Moreover, the previous researchers have noted that the type and concentration of acid solvent used in pre-treatment affect the characteristics of the resulting gelatin products [16-17].

Based on the description provided above, the problem that this study aims to solve is about the effects of citric acid and acetic acid treatment during the extraction process of gelatine from catfish skin, which can result in high yield and good quality gelatin type A. The objective of this study is to identify the effects of using different types of acids and concentrations during the extraction process, which can help researchers and gelatin business industry professionals to determine the appropriate pre-treatment method to obtain high-quality and high-yield gelatin products from catfish skin.

2 Materials and methods

2.1 Materials

The study primarily uses the skins of catfish (*Pangasianodon sp*) obtained from small and medium-sized industries (SMEs) located in Pasuruan, East Java (as shown in Figure 1) as the main raw material. The chemicals used in the study include sodium hydroxide (NaOH), citric acid ($C_6H_8O_7$), acetic acid (CH_3COOH), aquades (H_2O), filing fabrics, and other laboratory analysis materials obtained from the Process Laboratory of the Grand Chamber of Standardization and Agro-Industrial Services (BBSPJIA) located in Cikaret, Bogor District, West Java, Indonesia.



Fig. 1. The skins of catfish.

2.2 Preparation of gelatin from catfish skin

The process of extracting gelatin from the catfish skin involves modified techniques described in the other research [16-18]. The catfish skin is cleaned by continuously flowing water to remove any residual meat, blood, adipose tissue, abrasions, and extraneous substances. The cleaned catfish skins are stored in a freezer at a temperature of $-18^{\circ}C$ before use.

The next step is to remove the non-collagen components from the skin by immersing it in a solution of 0.01 N sodium hydroxide for an hour, followed by rinsing with aquades until the pH level reaches 7. The skin is then immersed in solutions containing acetic acid and citric acid at different concentrations for 12 hours. The substance is filtered, and then cleansed using aquades until the pH level reaches 7. The extraction process is conducted using aquades in an $80^{\circ}C$ water bath for four hours with a ratio of 1:3 between the catfish skin and aquades. The liquid gelatin produced is filtered using a specialized fabric designed for filtration purposes. The resulting liquid gelatin is then dried in an Excalibur dehydrator at a

temperature of 55°C for 20 hours to produce a dry gelatin sheet. The parameters of the extracted gelatin product are determined by analyzing dried gelatin sheets, taking into account the treatment involving acetate and citric acid concentrations. Table 1 presents the treatment code utilized in this investigation.

Table 1. Experimental code of research.

Treatment code	Acid type	Acid concentration (%)
A1	Acetic Acid	0,100
A2	Acetic Acid	0,075
A3	Acetic Acid	0,050
B1	Citric Acid	0,100
B2	Citric Acid	0,075
B3	Citric Acid	0,050

2.3 Gelatin characterization

Gelatin products are characterized based on their physical and chemical properties. Physical properties are determined through visual analysis (color), yield calculation, viscosity and gel strength measurement. On the other hand, chemical properties are determined through water content, ash content, protein content, pH, and metal resin (Cd, Hg, Pb, and As) analysis. Gelatin product characterization is conducted in the Process Laboratory of the Grand Chamber of Standardization and Agro-Industrial Services (BBSPJIA) in Cikaret, Bogor District, West Java, Indonesia, Agroindustrial and Biomedical Laboratory (LAPTIAB) in Serpong District, South Tangerang City, Banten, Indonesia, and Saraswanti Laboratory Indo Genetech (SIG) in West Bogor district, City of Bogor, West Java, Indonesia. The results of the characterization will be compared with the Indonesian National Standard (SNI) on Fish Gelatin (SNI 8622:2018) [19] and the British Standards Institution on methods for sampling and testing gelatine (physical and chemical methods) (BS 757:1975) [20].

The yield of gelatin produced from the skin of catfish is calculated by comparing the dry weight of gelatin to the wet weight of the skin. This calculation is based on the characterization of the physical properties of the gelatin product and can be expressed using equation 1.

$$\% \text{ yield} = \frac{\text{Dry weight of gelatin (g)}}{\text{Wet weight of catfish skins (g)}} \quad (1)$$

The gel strength and viscosity of gelatin products were determined using the Rheoner RE 3305 device and Brookfield Syncro-Lectric Viscometer, respectively, following the Official Procedure of the Gelatin Manufacturers Institute of America as described by the British Standard Institution [21]. For gel strength determination, a 5 mm cylinder probe of size 400 x 0.01 mm was used with a speed of 0.5 mm/s, a sensitivity of 0.2 V. For viscosity measurement, a gelatin sample with a concentration of 6.67% (w/w) was prepared in fine water (7 g of gelatin in 105 ml of fine water) and its viscosity was measured at a temperature of 60 °C and a sliding speed of 60 rpm using a spindle.

The chemical properties of gelatin products are characterized by measuring their acidity (pH) at room temperature using a pH meter [21] according to the standards set by the British Standard Institution. Water levels, ash levels, and protein levels are determined using the standard method of the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC) [22]. The presence of heavy metal resins in gelatin products is identified

by following the guidelines of SNI 7387:2009, which specifies the maximum limit of heavy metal resin permissible in foodstuffs [23].

3 Results and discussions

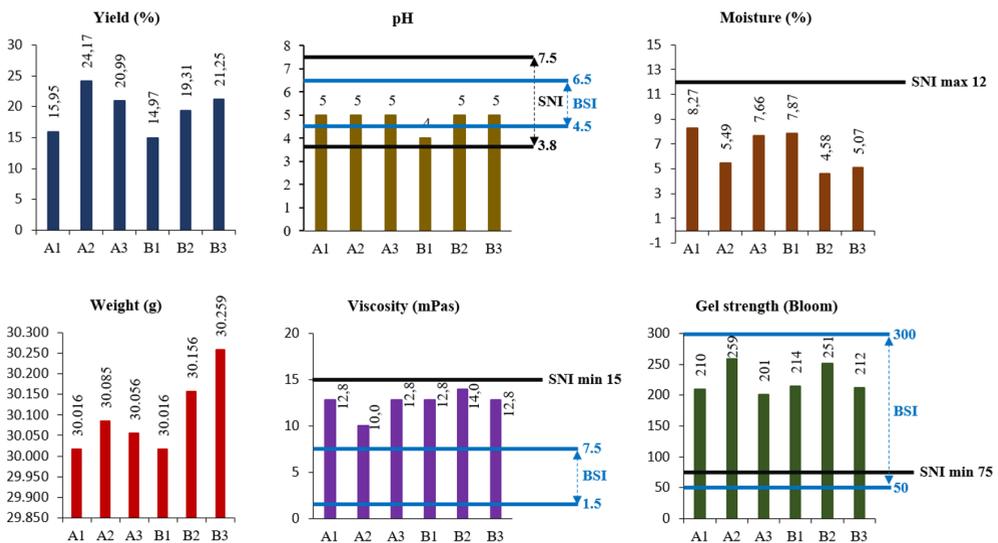
3.1 Physical and chemical properties of gelatin

The results of the study indicated that varying the concentration of acetic acid and citric acid during the immersion process did not have an impact on the color of the gelatin produced. All treatments produced a transparent and yellowish gelatin color, as shown in Figure 2. This meets the standard set by SNI 8622:2018 [19], which requires the gelatin to be colored up to pale yellow. Similar results were observed when compared to the standard set in BSi 757-1975 [20], which also specifies a pale yellow color.



Fig. 2. The gelatin of catfish skin.

Characterization of the physical and chemical properties of gelatin consists of yield, pH value, water content, viscosity, and gel strength, where the analysis results are shown in Figure 3.



Note: A1 is the symbol for treatment with the addition of 0.100% acetic acid; A2 is the symbol for treatment with the addition of 0.075% acetic acid; A3 is the symbol for treatment with the addition of 0.050% acetic acid; B1 is the symbol for treatment with the addition of 0.100% citric acid; B2 is the symbol for treatment with the addition of 0.075% citric acid; and B3 is the symbol for treatment with the addition of 0.050% citric acid

Fig. 3. Physical and chemical properties of gelatin.

According to Figure 3 the concentration levels of acetate and citric acid used during the immersion process have an impact on the yield of gelatin. The results indicate that adding 0.075% acetic acid during the immersion process produces higher gelatin yields compared to adding 0.050% acetic acid. This suggests that a higher concentration of acetic acid modifies more collagen and helps to transform it into gelatin. However, increasing the concentration of acetic acid to 0.1% does not increase the yield of gelatin. In a previous study, adding 0.05 M of acetate acid to the skin of catfish produced a gelatin yield of 69% [15]. This is greater than the yield produced in this study using 0.075% acetic acid. When citric acid is used instead of acetic acid, the yield of gelatin decreases as the concentration of acid increases. This result contradicts the findings of the other research, which found that higher acid concentrations lead to higher yields during the immersion process [24]. Increasing the concentration of acid during the immersion process increases the number of hydrogen ions (H^+) [25]. Increasing the acid concentration speeds up the hydrolysis process, which converts collagen molecules into gelatin more quickly. The faster the hydrolysis, the more collagen molecules are converted, increasing the yield.

According to the study, an increase in H^+ ions speeds up the rate of collagen hydrolysis, resulting in more triple helix being fragmented into α , β , and γ chains. As a result, the collagen is turned into gelatin more efficiently. Figure 3 shows that the pH of gelatin remains unaffected by the difference in acetate and citric acid concentrations used during the immersion process. The resulting gelatin had a pH of 5 for all treatments except for the one with 0.1% citric acid immersion, which dropped to 4. This shows that there is a possibility that washing material (catfish skin) after immersion with 0.1% imperfect citric acid causes the gelatin pH to be still low. This could be due to the acetic and citric acid remaining in the gelatin after rinsing. It was found that the type and concentration of acid used can affect the acidity of the produced gelatin [24]. This supports the findings of our study. However, the pH values for all treatments fall within the range of gelatin-sensitive values specified in BSI 757-1975 [20].

Based on the water level identification study, the concentration of acetic acid and citric acid employed during the immersion process has a direct impact on the water level in the resulting gelatin, as shown in Figure 3. The treatments that included 0.075% and 0.05% citric acid and 0.075% acetate acid resulted in the lowest water content in the gelatin, with values of 4.58%, 5.07%, and 5.49%, respectively. The other three alternative therapies, on the other hand, resulted in much higher water content, ranging from 7.66% to 8.27%. It is worth noting that the water content of all treatments stayed below the maximum amount of gelatin water defined by the SNI of Fish Gelatin (SNI 8622:2018) [19].

Adjusting the concentration of acetate and citric acid during the immersion process does not significantly affect gelatin viscosity, as observed in Figure 3. When 0.075% acetic acid and 0.075% citric acid were added during immersion, viscosity measurements differed. The observed viscosity values for acetic acid were 10 mPa and 14 mPa for citric acid. It was previously discovered that increasing the concentration of acid used in the immersion process increases the viscosity of the resulting gelatin products [24]. This increase in viscosity value can be attributed to the interconnection of polypeptide chains, which in turn increases the molecular weight [26]. The viscosity identification analysis showed that the viscosity values of gelatin for all treatments were within the range of 10 to 12.8 mPa, which exceeded the vulnerability limits specified in BSi 757-1075. The specified range in the BSi 757-1075 is 1.5 to 7.5 mPa. This indicates that the acid concentration provided during the immersion process is excessively high, leading to an increased value of gelatin viscosity.

The results of the gel strength identification reveal that the concentration of acetate and citric acid used in the immersion process affects the strength of the gel produced (as shown in Figure 3). Treatments that included 0.075% acetic acid and 0.075% citric acid during immersion showed the highest gel strength values, at 259 and 251 blooms respectively. It is

suspected that the increased acid concentration used in the immersion process improves the gel formation process by breaking down the polymer chain of amino acids. This is supported by Said [3]. The gel strength of all treatments was found to be within the vulnerability value of gel strength, which is a minimum of 75 bloom according to SNI Standard [19], and above 50 – 300 bloom according to BSI Standard [20].

Based on the treatments depicted in the study, it can be concluded that the two most effective treatments were the ones that incorporated 0.075% acetic acid (A2) and 0.075% citric acid (B2). Additional testing will be conducted on both treatments to determine the chemical properties of the most optimal gelatin products.

3.2 Chemical properties of gelatin A2 and B2

According to the results presented in Table 2, both Gelatin A2 and Gelatin B2 meet the standards outlined in SNI 8622:2018 [19]. Neither of the gelatin products contained heavy metal resin, indicating that the catfish skins used in manufacturing were free from heavy metallic resin prior to processing. Additionally, there was no evidence of migration or contamination from the production equipment during manufacturing. Typically, metallic debris in food items arises from contaminated raw materials with heavy metals during the cultivation process [27].

After analyzing the ash and protein content of two gelatin products, A2 and B2, it was observed that B2 had a lower ash value but a higher protein level compared to A2. Research conducted related to application of acid, indicates that an acidic solution can effectively break down the polymer chain of amino acids under optimal conditions, resulting in optimal protein extraction [3]. The other research also suggest that the concentration of the acidic solution used during the immersion process plays a significant role in breaking down the amino acid polymer chain at the right threshold [24]. Specifically, under the same process conditions, a 0.075% concentration of citric acid was found to be more effective than a 0.075% concentration of acetate acid.

Table 2. Chemical properties of gelatin A2 and B2.

Parameter	Gelatin A2	Gelatin B2	SNI 8622:2018
Ash Content (%)	0.68	0.37	Max 3
Protein Content (%)	88.92	93.22	-
Heavy Metal Contamination:			
- Mercury (Hg) (mg/kg)	Not detected	Not detected	Max 0.1
- Timbal (Pb) (mg/kg)	Not detected	Not detected	Max 1.5
- Cadmium (Cd) (mg/kg)	Not detected	Not detected	Max 0.5
- Arsenic (As) (mg/kg)	Not detected	Not detected	Max 0.5

4 Conclusion

The study found that soaking gelatin in different concentrations of acetic acid and citric acid did not affect the color of the gelatin produced. All treatments resulted in transparent, light yellow gelatin. The extraction process yielded gelatin ranging from 14.97 to 24.1%, with the highest yield obtained when using a higher concentration of acetic acid. The use of 0.075% citric acid resulted in the least amount of water in the extracted gelatin. The pH of the gelatin ranged from 4 to 5, with viscosities between 10 and 14 MPa. The gelatin produced had a strength range of 200-259 blooms. The best treatment for gelatin production was the use of

a higher concentration of acetic acid, which resulted in high yield, low water content and viscosity, and high gel strength.

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