

Production and characterization of halal-based gelatin derived from Red Nile Tilapia (*Oreochromis niloticus*) fishbone



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ABSTRACT

Gelatin is one of the chemical products derived from protein hydrolysis. The demand for gelatin in Indonesia increases yearly, but domestic production still needs to meet the demand, leading to imports. International gelatin production statistics indicate that 58% is derived from pig skin, which is prohibited for Muslims. Therefore, this research aims to produce halal-based gelatin that meets quality standards. Red Nile Tilapia (*Oreochromis niloticus*) fishbones are used as the raw material for this study, as their halal status is clear in the Quran and Hadith. The fishbones are soaked in different concentrations of phosphoric acid, namely F1 (4%), F2 (6%), and F3 (8%), for 48 hours, then extracted using distilled water solvent in a water bath for 6 hours. The extracted solution is filtered, and the filtrate is dried in an oven at 50°C for 48 hours. The gelatin yield is statistically analyzed using correlation methods, and its functional groups are identified using FTIR spectroscopy. Compared to existing standards, gelatin characteristics are evaluated through organoleptic tests, such as pH, gel strength, viscosity, moisture content, and ash content. The gelatin yield for F1 is $8.15 \pm 0.18\%$; F2 is $12.08 \pm 0.12\%$, and F3 is $15.66 \pm 0.26\%$. The research demonstrates that phosphoric acid concentration significantly influences gelatin yield, with higher concentrations resulting in higher yields. The FTIR spectra also indicate that the synthesized gelatin resembles commercial gelatin in spectra and absorption peaks. All gelatin variations meet the requirements for organoleptic properties, gel strength, moisture content, and ash content, while only F1 and F2 meet the pH requirements.

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INTRODUCTION

Gelatin is a chemical product derived from the hydrolysis of proteins. Typically, gelatin is hydrolyzed from collagen, the main protein constituent of skin, bones, and cartilage tissues (Wong, 2018). In brief, the conversion of collagen into gelatin occurs through the denaturation process of collagen. The denaturation process involved in gelatin synthesis consists of two stages: the breakdown of the triple helix bonds in collagen and the breakdown of smaller gelatin molecule chains. As a result of the breaking and disruption of covalent and hydrogen bonds in collagen, the triple-helix structure becomes unstable and transforms into a more water-soluble coiled form, which is ultimately referred to as gelatin (Martianingsih & Atmaja, 2010; Nada et al., 2017).

Indonesia's demand for gelatin has been increasing annually, yet domestic production has not kept pace, resulting in reliance on imported products. In 2016, Indonesia imported 11,088.9 tons of gelatin, 12,787.7 tons in 2017, 13,131.3 tons in 2018, and 30,938.4 tons in 2019. In 2020, international gelatin production reached 516.8 thousand tons, with 58% derived from pig skin and the remainder from cow skin and bones, fishbones and skin, and other sources (Arnamalia et al., 2022). Data from the Central Bureau of Statistics shows that Indonesia's gelatin imports in 2023 reached 2.45 million kg, with a value of 20.77 million USD, and it is expected to increase to 4.96 trillion USD by 2030 (Putri et al., 2023; Setyabudi et al., 2024).

Based on the data above, most commercial gelatin is derived from mammalian animals, namely pig skin, which is prohibited according to QS—Al-Maidah verse 3, and cow bones. However, the use of gelatin from cow bones often sparks controversy due to concerns about the slaughtering process not aligning with Islamic law (Junianto et al., 2013). Therefore, there is a need for development and research into alternative gelatin production from non-mammalian animals, such as poultry or fish, which already have clear halal status (Hasan & Dwijayanti, 2022).

According to the hadith narrated by Ibn Majah, the halal status of fish is clear; even in the state of being carrion, fish are permissible for consumption. All types of fish have the potential to be used as raw materials in the production of halal gelatin. However, each type of fish has its characteristics, advantages, and disadvantages. One type of fish known for its high protein content is the Nile tilapia (Arnamalia et al., 2022). Red Nile Tilapia, or *Oreochromis niloticus* in Latin, is a freshwater fish highly favored by various communities. Moreover, its cultivation is relatively easier and more cost-effective (Wardhana & Sugiharto, 2022).

Fishbones are chosen as the main material in halal gelatin synthesis because they are one of the organs with relatively high protein content (Wardhana & Sugiharto, 2022). According to research by Junianto et al. (2013), gelatin from fishbones suits both the food and pharmaceutical industries. Fishbones are also an underutilized waste product in the fishing industry, hence the need for utilization (Panjaitan, 2016). Fish gelatin is obtained from collagen-rich fish tissues, including scales, skin, and bones. The extraction process is adapted to suit the specific characteristics of fish collagen. Fish-based gelatin has distinct properties, such as strong gel formation, low viscosity, and improved solubility, making it suitable for various applications (Reza & Annissa, 2023). According to research conducted by Ridhay et al. (2016) regarding the synthesis of gelatin from skipjack tuna bones using various acids, phosphoric acid resulted in the highest yield. This study used various acids with the same concentration, producing different percentage yields. For instance, hydrochloric acid (HCl) 5% yielded 10.687%; sulfuric acid (H₂SO₄) 5% yielded 8.680%; phosphoric acid (H₃PO₄) 5% yielded 14.658%; acetic acid (CH₃COOH) 5% yielded 4.765%; oxalic acid (H₂C₄O₄) 5% yielded 12.864%; and citric acid (C₆H₈O₇) 5% yielded 7.312%. The differences are attributed to the varying amounts of H⁺ ions in each acid, where a higher concentration of H⁺ ions leads to a higher yield by facilitating the conversion of collagen into gelatin. Therefore, the research investigated the effect of phosphoric acid concentration as a soaking medium on the yield and physicochemical characteristics of gelatin made from Red Nile Tilapia fishbones. Consequently, based on the explanation above, research will be conducted on gelatin synthesis using phosphoric acid as the soaking medium.

RESEARCH METHOD

Materials

The equipment used in this research includes an FTIR Spectrophotometer instrument (Agilent Technologies, Cary 630), Brookfield Viscometer (Ametek), Texture Analyzer (Ametek, CT3), pH meter (Mettler Toledo), Disintegration Tester (Erweka), muffle furnace, oven (Binder), refrigerator, desiccator, water bath (Mettler), hotplate (Heidolph), and grinder/blender (Philips). The materials used include Red Nile Tilapia fishbones obtained from Malang (the whole bone, excluding the head bone), phosphoric acid (H₃PO₄) (Merck, 85%), distilled water (PT. Brataco), and commercial gelatin (Merck).

Methods

1. Sample Preparation

Red Nile Tilapia fishbones are soaked in water at 60-70°C for 30 minutes and then cleaned from any remaining flesh and dirt attached to the bones until thoroughly cleaned. Subsequently, the bones are cut into smaller pieces approximately 2-3 cm in size (Hasan & Dwijayanti, 2022).

2. Demineralization

The fishbones are soaked in phosphoric acid with varying concentrations of 4%, 6%, and 8% in beakers for 48 hours with a ratio of 1:8 (w/v). After the soaking process, the Nile tilapia fishbones are washed with running water (Hasan & Dwijayanti, 2022).

3. Extraction

The fishbones are extracted using distilled water solvent in a water bath at 70°C for 6 hours with a ratio of 1:3 (w/v). The extraction results are filtered using Whatman filter paper (Hasan & Dwijayanti, 2022).

4. Drying

The gelatin filtrate is dried in an oven at 50°C for 24 hours or until dry and forms sheets. Once dried, the gelatin is ground into powder using a grinder (Hasan & Dwijayanti, 2022).

5. Gelatin yield calculation (Ridhay et al., 2016)

Using the ratio of the final weight of the produced gelatin to the initial weight of the cleaned Red Nile Tilapia fishbones used, you can then use the following formula:

$$\text{Rendemen (\%)} = \frac{\text{Final weight of gelatin produced}}{\text{Initial weight of fish bone}} \times 100\%$$

6. Organoleptic test (Ridhay et al., 2016)

The organoleptic test directly involves the human senses to evaluate the appearance, color, smell, and texture of the produced gelatin. A comparison is made with commercial gelatin available in the market and against existing standards or requirements.

7. pH test (Ridhay et al., 2016)

Dissolve 0.2 grams of gelatin in 20 mL of distilled water at 80°C. After a homogeneous solution is formed, test it using a pH meter.

8. Gel strength test (Yusuf, 2021)

Dissolve 6.67 grams of gelatin in 100 mL of distilled water. The gelatin solution is then transferred into a test container, covered, and allowed to stand for 2 minutes. Afterward, the solution is incubated at 10°C for approximately 2 hours. Subsequently, the sample's gel strength is tested using a texture analyzer and calculated using the following formula:

$$D \text{ (dyne/cm}^2\text{)} = F/G \times 980 \text{ N}$$

$$\text{Gel strength (bloom)} = 20 + (2.86 \times 10^{-3}D)$$

Explanation:

F: Curve height; G: Constanta (0.07)

9. Viscosity test (Sumiati et al., 2020)

Dissolve 6.67 grams of gelatin in 100 mL of distilled water, then stir until homogeneous. The homogeneous solution is tested using a Brookfield Viscometer instrument.

10. Water content test (Ridhay et al., 2016)

The moisture content test is conducted using the oven method by drying an empty porcelain dish at 100-105°C for 1 hour, cooling it in a desiccator for 15 minutes, weighing and recording its weight, and labeling the porcelain dish. Next, weigh 5 grams of gelatin using the labeled porcelain dish, then dry it in the oven at a temperature of 100-105°C for 1 hour and cool it in a desiccator for 30 minutes. After cooling, weigh it to determine its weight and apply it to the formula. The formula for moisture content is as follows:

$$\text{Water content (\%)} = \frac{m_1 - m_2}{m_1 - m_0} \times 100\%$$

Explanation:

m₀ : weight of empty cup; m₁ : weight of cup + sample weight before drying; m₂ : weight of cup + sample weight after drying.

11. Ash content test (Ridhay et al., 2016)

The ash content test is conducted by heating a porcelain dish in a muffle furnace at a temperature of 550°C for 30 minutes, then cooling it in a desiccator for 30 minutes. After cooling, the dish is weighed, and the result is recorded. Weigh 2 grams of gelatin sample in the preheated and weighed porcelain dish. The dish containing the sample is then reheated in the furnace at a temperature of 550°C for 3.5 hours until it turns white ash. The dish is cooled in the desiccator for 30 minutes. Afterward, the ash from heating is weighed and applied to the ash content formula. The formula for ash content is as follows:

$$\text{Ash content (\%)} = \frac{\text{ash weight}}{\text{sample weight}} \times 100\%$$

Explanation:

Ash weight: (cup weight + ash) – weight of empty cup

Data analysis

The data is analyzed descriptively and statistically. Descriptive analysis includes pH value, gel strength, viscosity, moisture, and ash content. These data are compared with the requirements for good gelatin as stated in BSN (2018) and GMIA (2019) standards. Statistical analysis involves the yield values of gelatin, using correlation methods to determine the influence of phosphoric acid concentration on the yield of gelatin.

RESULT AND DISCUSSION

The result of gelatin yield

Table 1. Results of Red Nile Tilapia fishbone gelatin yield.

Variation	Raw Material (gr) ($\bar{x} \pm \text{SD}$)	Extract (gr) ($\bar{x} \pm \text{SD}$)	Yield (%) ($\bar{x} \pm \text{SD}$)
F1 (4% Phosphoric acid)	107.63 ± 0.90	8.77 ± 0.12	8.15 ± 0.18
F2 (6% Phosphoric acid)	106.13 ± 1.61	12.82 ± 0.08	12.08 ± 0.12
F3 (8% Phosphoric acid)	106.93 ± 0.21	16.75 ± 0.30	15.66 ± 0.26

Data presented with three replications as mean ± standard deviation.

The calculation of yield in an extract determines the ratio of the extract obtained from a material to the weight of the initial raw material or crude drug used. Additionally, this yield calculation aims to ascertain and determine the amount of bioactive compounds contained within the extracted material (Utami et al., 2020). From Table 1, it can be observed that F3 generates the highest yield with a phosphoric acid concentration of 8%.

The data is consistent with the research conducted by Ridhay et al. (2016), where the higher the concentration of H⁺ ions, or in this case, the higher the acid concentration, the higher the gelatin yield produced. It is thought to be due to the binding of calcium minerals in Nile tilapia bones, allowing the collagen in the bones to be freed. The bonds that may be broken in this process include hydrogen, ionic, hydrophobic, and Van der Waals bonds formed between polypeptide chains, leading to the opening of molecular bonds. The increase in H⁺ ions also affects the rate of collagen hydrolysis, which increases. With a higher rate of hydrolysis, the breakdown of the triple helix into single helix also increases, which in turn increases the amount of collagen converted into gelatin and enhances the yield value (Hasan & Dwijayanti, 2022; Ridhay et al., 2016).

Based on statistical analysis using the correlation method, a correlation value of 0.949 was obtained. It can be concluded that there is a robust and positive correlation (Dahlan, 2013). This shows that the phosphoric acid concentration in the gelatin synthesis process significantly affects gelatin yield. The higher the concentration of phosphoric acid used, the higher the yield of gelatin produced.

Gelatin function groups identification results

Table 2. The absorption peak of commercial gelatin and Red Nile Tilapia fishbone gelatin.

Absorption Area	Range of Absorption (cm ⁻¹)	Absorption Peak (cm ⁻¹)			
		Commercial Gelatin	F1	F2	F3
Amida A	3600 – 2300	3274	3278	3278	3276
Amida I	1700 – 1600	1627	1627	1625	1627
Amida II	1560 – 1335	1522	1528	1541	1526
Amida III	1240 – 670	1235	1235	1235	1235

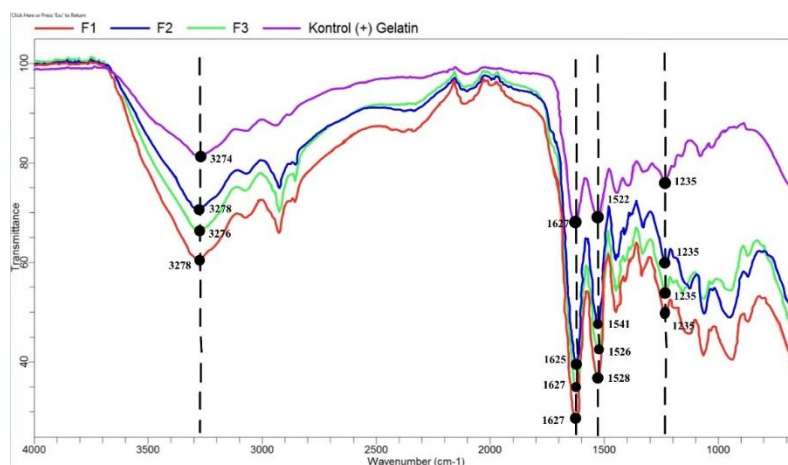


Figure 1. FTIR Spectra of commercial gelatin and Red Nile Tilapia fishbone gelatin.

Table 2 and Figure 1 show that all four gelatins exhibit absorption bands in regions suspected to be the gelatin profile, where absorption regions for amide A, amide I, amide II, and amide III are observed. In the amide A region, absorption is caused by the stretching of N-H bonds of the amide groups associated with hydrogen bonding and the presence of OH groups. The broad peak shape is evidence of the presence of OH groups from hydroxyproline. Furthermore, the amide I absorption region is caused by the stretching of double bonds of carbonyl groups C=O, bending of NH bonds, stretching of CN bonds, and OH groups paired with carboxyl groups. This absorption region is related to the deformation of tropocollagen into α -helix chains. The amide III absorption region indicates the relationship with the triple helix structure of collagen. Therefore, all four gelatins are still suspected of containing collagen and have not been completely converted into gelatin (Fatmawati et al., 2024; Puspawati et al., 2012).

Physical evaluation results of gelatin

Table 3. Physical evaluation results of Red Nile Tilapia fishbone gelatin.

Parameter	Variation of Gelatin			SNI (2018)	GMIA (2019)
	F1 ($\bar{x} \pm SD$)	F2 ($\bar{x} \pm SD$)	F3 ($\bar{x} \pm SD$)		
pH	5.51 ± 0.05	4.75 ± 0.05	3.55 ± 0.13	3.8 – 7.5	3.8 – 7.5
Gel strength (<i>bloom</i>)	154.93 ± 4.49	148.13 ± 3.44	115.83 ± 1.64	> 75 bloom	50-300 bloom
Viscosity (cps)	5.6 ± 0.20	4.6 ± 0.57	4.0 ± 0.35	> 1.5 cps	1.5 – 7.5 cps
Water content (%)	10.45 ± 2.11	8.63 ± 2.93	7.87 ± 2.21	< 12 %	< 15 %
Ash content (%)	0.75 ± 0.12	0.61 ± 0.12	0.58 ± 0.08	< 3 %	0.3-2 %

Organoleptic gelatin

Organoleptic testing aims to determine the characteristics of a material visually using the human senses, focusing on aspects such as color, odor, appearance, and texture (Collins et al., 2023). In this study, the gelatin synthesized using phosphoric acid as the soaking medium is compared with

commercial gelatin available in the market. According to [BSN \(2018\)](#), commercial gelatin is colorless or pale yellow, with a standard or odorless smell, and is acceptable to consumers. [Kemenkes RI \(2022\)](#) states that gelatin comes in sheets, flakes, or pieces or in acceptable to coarse powder form. It has a weak yellow or light brown color, depending on the particle size of the gelatin, and a faint odor.

The gelatin produced in this study exhibits characteristics such as a slightly fishy odor, off-white to yellowish color, and a crystalline powder texture, as shown in Figure 2. The fishy odor detected in the gelatin is presumed to be carried over from the raw fishbone material. This is likely due to the presence of volatile substances, such as ammonia, contained within it, which are carried over into the gelatin. The odor can be masked with additional aroma or flavor ([Simbolon et al., 2022](#)).

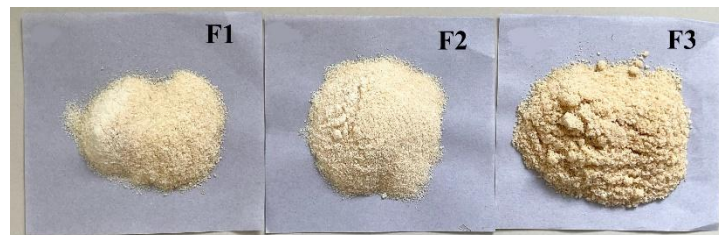


Figure 2. Red Nile Tilapia fishbone gelatin (F1: add 4% Phosphoric acid; F2: add 6% Phosphoric acid; and F3: add 8% Phosphoric acid).

Gelatin pH

The pH value is a crucial parameter to analyze in every material, especially food materials. The pH value of gelatin is an essential parameter to determine its quality. The pH value of gelatin can affect other physical properties, such as viscosity and gel strength ([Hermanto et al., 2014](#)).

As seen in Table 3, the pH measurement results of gelatin from Red Nile Tilapia bones F1 and F2 meet gelatin's pH value requirements according to SNI and GMIA standards. However, gelatin F3 does not meet the requirements because it has a pH value that is too low. The higher the concentration used, the lower or more acidic the pH value produced because residual phosphoric acid from the demineralization stage and inadequate washing of the bones, which is eventually carried over into the extraction stage, thus affecting the pH value of the resulting gelatin ([Hermanto et al., 2014](#)).

Gelatin gel strength

Gel strength is the most crucial parameter in gelatin quality. Gel strength analysis is conducted to determine how well gelatin forms a gel, as one of the properties of gelatin is its ability to transform a sol into a reversible gel ([Yusuf, 2021](#)). Factors influencing the gel strength of gelatin include extraction temperature, pH, and amino acids ([Febriana et al., 2021](#)).

As seen in Table 3, all three gelatin formulations, F1, F2, and F3, meet the requirements for good gel strength according to SNI and GMIA standards. Gelatin F1 has the highest gel strength value because of the influence of the pH of the gelatin used, where the lower the pH of the gelatin, the lower the gel strength produced; in this case, the higher the concentration of phosphoric acid used, the lower the gel strength produced. Further hydrolysis occurs in the protein chains, thereby shortening the amino acid chains in the gelatin ([Febriana et al., 2021](#)). According to [Djunaidi et al. \(2022\)](#), factors influencing gel strength in gelatin include the content of hydroxyproline and proline amino acids. The higher the content of hydroxyproline amino acid in the gelatin, the greater the gel strength.

Gelatin viscosity

Viscosity is a parameter related to gel strength. The purpose of viscosity testing is to assess the thickness of gelatin in forming a solution. Some factors influencing viscosity values in gelatin include extraction temperature, soaking duration, and the concentration of acid used ([Febriana et al., 2021](#)).

As observed in Table 3, the viscosity values of gelatin F1, F2, and F3 meet the requirements for good gelatin viscosity according to SNI and GMIA standards. The higher the acid concentration used, the lower the viscosity value obtained. According to [Febriana et al. \(2021\)](#), acid concentration is one of the factors affecting gelatin viscosity. The acidity can lower the viscosity value because the acid breaks

covalent bonds between amino acids, ultimately causing the gelatin chains to shorten and the molecular weight to decrease, resulting in low viscosity.

Gelatin water content

Water content testing aims to determine a substance's amount or moisture content. The moisture content in gelatin, a food material, should not exceed the specified requirements, as it can affect the quality and integrity of the gelatin. This moisture content test is crucial as it can also serve as a reference in determining the shelf life, where water can act as a medium for microbial growth that can lead to spoilage or degradation of a substance (Febriana et al., 2021).

As seen in Table 3, gelatin variations F1, F2, and F3 meet the requirements for good gelatin moisture content according to SNI and GMIA standards. The moisture content of gelatin is influenced by the soaking duration and the extraction temperature used, where the longer the duration and the higher the extraction temperature, the lower the moisture content produced from the gelatin. Duration and extraction temperature can increasingly open collagen structure, resulting in low binding capacity for free water and high adsorbed water binding capacity. The decreased ability to bind free water leads to easy evaporation from the gelatin during evaporation or drying processes. The decrease in gelatin moisture content due to high extraction temperature is caused by the denaturation process, resulting in changes in molecular structure and a weakening and reduction in the amount of bound water (Yusuf, 2021).

Gelatin ash content

The ash content value is also an essential parameter in the quality and purity of gelatin, particularly its purity. The ash content value of a food substance indicates the amount of minerals contained within that substance, including sodium, calcium, chlorine, phosphorus, and magnesium. The higher the ash content in gelatin, the lower its purity (Hermanto et al., 2014).

As seen in Table 3, all variations of gelatin, whether F1, F2, or F3, meet the requirements for good ash content in gelatin according to both SNI and GMIA standards. A high ash content value may be attributed to mineral elements in the bones that have not been fully decomposed during demineralization, leading to their solubility during extraction. Additionally, mineral components in the bones may pass through during the gelatin solution filtration process, which can increase the ash content (Yusuf, 2021).

CONCLUSION

From the results obtained from the study, it can be concluded that there is an influence of phosphoric acid concentration on the yield of gelatin synthesized from the bones of Red Nile Tilapia (*Oreochromis niloticus*), wherein the higher the concentration of phosphoric acid used, the greater the yield of gelatin produced. Furthermore, the physicochemical characteristics of gelatin synthesized from the bones of Red Nile Tilapia (*Oreochromis niloticus*) using the phosphoric acid immersion method meet the criteria for good gelatin in terms of organoleptic indicators, gel strength, viscosity, moisture content, and ash content. Only gelatin F1 and F2 meet the requirements regarding the pH indicator, while F3 does not.

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