MJoCE/Vol 15 No 1/January 2025/Hal. 27-33

p-ISSN 2722-8258
e-ISSN 2722-8266

EFFECT OF SOAKING AND HYDROLYSIS TIME VARIATIONS ON GELATIN CHARACTERISTICS FROM COW BONES

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Received: 16 September 2024 / Accepted: 18 October 2024 / Published: 10 January 2025

ABSTRACT

Gelatin extraction from cow bones has been conducted with variations in both acid and alkaline soaking. The gelatin extraction process from cow bones consists of two steps: soaking the cow bones and collagen hydrolysis. During the soaking step, continuous soaking was performed, beginning with soaking in a 4% NaOH alkaline solution, followed by acid soaking using a 1.2% citric acid solution, and further acid soaking with 4% HCl, with variations in soaking times of 1 hour, 12 hours, and 24 hours. The soaked cow bones were then hydrolyzed at temperatures of 60°C and 80°C for 5 hours. The resulting gelatin was characterized using FTIR and SDS-PAGE techniques. The best-characterized gelatin, with a band thickness in the molecular weight range of about 120 kDa, confirmed as the α chain, was obtained from soaking for 24 hours and hydrolyzing at 80°C.

Keywords: *Gelatin, Cow bones, Hydrolysis, Acid, Alkaline.*

INTRODUCTION

Cow bones are a common waste material in society, particularly in Indonesia, where beef consumption is widespread. This is because almost the entire population consumes beef. These cow bones are usually discarded without further utilization. Cow bones contain a significant amount of collagen protein, which can be used as a raw material for producing gelatin. Gelatin is defined as a denatured fibrous protein obtained from collagen connective tissue through partial hydrolysis (Das et al., 2017). Gelatin is commonly used in the food industry as an additive to enhance properties such as elasticity, consistency, and product stability. It also functions as a thickening agent in foods such as jelly, cakes, and marshmallows, as well as in dairy products like yogurt, ice cream, and candy (Zilhadia et al., 2018).

Gelatin is produced from animal sources and obtained through acid or alkaline hydrolysis of collagen with thermal denaturation (Cao et al., 2020). The structure of gelatin is illustrated in Figure 1. Based on the processing method, gelatin extraction is divided into two types: type A, derived from soft materials such as pig skin, and type B, derived from harder materials such as aged skin and bones. In the production of type A gelatin, the raw materials undergo immersion in an acid solution, making this process known as the acid process, with a relatively short soaking time of several hours or a few days. On the other hand, in the production of type B gelatin, an alkaline treatment is applied. This soaking process takes longer, lasting several weeks or even months (Rehman et al., 2016).

Soaking in an acid or alkaline solution is necessary because insoluble collagen must first be converted into a soluble form through acid or alkaline pretreatment, which causes swelling and disrupts collagen's native orderly structure. The conversion into gelatin occurs during the extraction process, which involves breaking hydrogen and covalent bonds through heat. The hydrolysis process itself involves the breakdown of substances through the addition of water

p-ISSN 2722-8258
e-ISSN 2722-8266

(H_2O), leading to a hydrolysis reaction in which ions separated from H_2O bind to collagen to form gelatin.

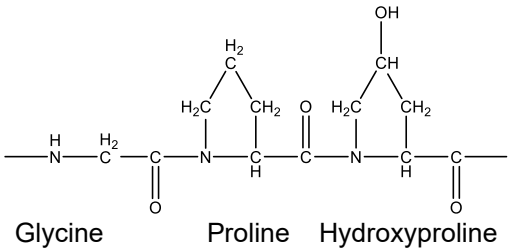

Figure 1. Gelatin Structure (Imeson, 1992)

Several studies have been conducted on extracting gelatin from cow skin and bones using variations of acid and alkaline soaking. The extraction of gelatin from cow skin, as carried out by Ahmad et al. (2018), involved soaking the cow skin in a 0.1 M NaOH solution for 6 hours, followed by acid soaking using a 1% HCl solution for 20 hours. Then, bromelain enzyme was added, and the mixture was incubated at 35.5°C for 48 hours. After that, the extraction process was carried out at 60°C for 2, 4, and 6 hours (Ahmad et al., 2018).

Meanwhile, the extraction of gelatin from cow bones, conducted by Arioui et al. (2018), involved soaking the cow bones in a 2% HCl solution, followed by alkaline soaking in a 1.0 N NaOH solution for 48 hours at 4°C, with the solution being replaced every 12 hours. The extraction process was then carried out at 100°C for 90 minutes (Arioui et al., 2018).

The gelatin extraction process begins with a soaking stage using an acid or alkaline solution, followed by the hydrolysis stage. The soaking stage aims to expand collagen fibers, making them more accessible for extraction and conversion into gelatin during hydrolysis. The duration and type of soaking, as well as the hydrolysis temperature, significantly influence the properties of the resulting gelatin (Sitepu & Fatimah, 2022). Generally, the soaking process takes a long time when using materials derived from animal bones, such as cow bones.

In addition to its applications in the food and cosmetic industries, gelatin can also be used as a template in the synthesis of mesoporous materials (Trisunaryanti et al., 2016). The type of gelatin suitable for this purpose contains α-chain collagen with a molecular weight ranging from approximately 120 to 160 kDa (Atma & Ramdhani, 2017). Based on this, the author conducted gelatin extraction from cow bones using a combination of acid and alkaline pretreatment, with variations in soaking and extraction times, to obtain gelatin with the desired characteristics.

RESEARCH METHOD

Material

The materials used in this research include distilled water (aquades), sodium oxide (NaOH), hydrochloric acid (HCl), and citric acid. Additionally, cow bones were sourced from Jangkang Widodomartani Market, Ngemplak, Sleman, Yogyakarta.

Tools

The equipment used in this research includes a set of glassware, a reflux set, a desiccator, a Buchner funnel, pH indicator (Merck), an analytical balance (AT 200), and an oven (Memmert). The instruments used for material characterization are Fourier Transform Infrared Spectroscopy (FTIR, Shimadzu Prestige FTIR 21), and SDS-PAGE.

Procedure

The preparation of gelatin material begins with the cleaning and drying of cow bones. The cow bones are cleaned of any remaining meat and and washed repeatedly with water until clean. Then, the bones are crushed into smaller bone pieces. Next, the bone pieces are soaked in water at a temperature below 80°C for several hours to dissolve any remaining fat. After that, the cow bones are dried at a temperature below 80°C to remove any residual water.

p-ISSN 2722-8258
e-ISSN 2722-8266

The prepared cow bones are then consecutively soaked in 4% NaOH solution, 1.2% citric acid solution, and 4% HCl solution (the ratio of bones to each of these solutions is 1:6) with variation soaking times of 1; 12; 24 hours (for base dan acid). Before soaking in these three solutions, the pH of the bones is adjusted to neutral. The bones, after being soaked under neutral pH conditions, are then hydrolyzed with water at 60 and 80°C for 5 hours. The obtained gelatin solution is subsequently dried at a temperature of 50°C to obtain solid gelatin. The solid gelatin obtained is then identified using IR spectroscopy and electrophoresis (SDS-PAGE).

RESULTS AND DISCUSSION

Gelatin extraction through hydrolysis method

The gelatin extraction process from cow bones has been carried out, starting with an alkaline soaking treatment using 4% NaOH, which aims to demineralize the bones, thereby increasing OH and accelerating the hydrolysis process (Sitepu & Fatimah, 2022). This is followed by acid soaking, intended to expand the collagen fibers so that during the hydrolysis process, gelatin can be extracted effectively. The acid solution used consists of a weak acid, namely 1.2% citric acid, followed by a strong acid, 4% HCl. The soaking stages can be seen in Figure 3. This sequential soaking treatment was conducted at each stage for 1, 12, and 24 hours. After that, the osein hydrolysis process was carried out for 5 hours at temperatures of 60 and 80°C.

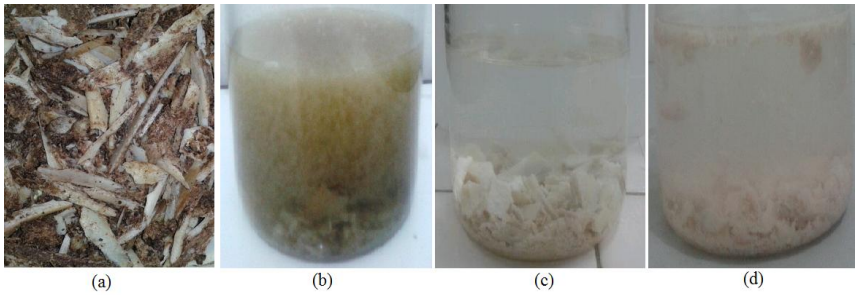

Figure 2. (a) Cow bones, Cow bones soaking with (b) NaOH, (c) Citric Acid, (d) HCl

Characterization Results of Gelatin Using FTIR

There are five main absorption regions identified as characteristic absorptions of gelatin. These include absorption at a wavenumber of 3600–3400 cm^{-1}, known as amide A; absorption at 3000–2800 cm^{-1}, known as amide B; absorption at 1656–1644 cm^{-1}, known as amide I; absorption at 1560–1335 cm^{-1}, known as amide II; and absorption at 1240–670 cm^{-1}, known as amide III (Muyonga et al., 2004). The amide A absorption band indicates the presence of stretching vibrations from NH groups and hydrogen bonds, while amide B shows stretching vibrations from CH_2. Amide I corresponds to C=O stretching vibrations, amide II indicates NH bending vibrations and C-N stretching vibrations, and amide III is associated with a complex system related to CH_2 group residues from glycine and proline (Abedinia et al., 2020). In Figure 3 and Table 1, it can be seen that the hydrolysis results of cow bones, which began with sequential soaking using NaOH, citric acid, and HCl for 1, 12, and 24 hours, exhibit absorption in the wavenumber range corresponding to the characteristic absorption of gelatin as reported by Muyonga et al. (2003).

p-ISSN 2722-8258
e-ISSN 2722-8266

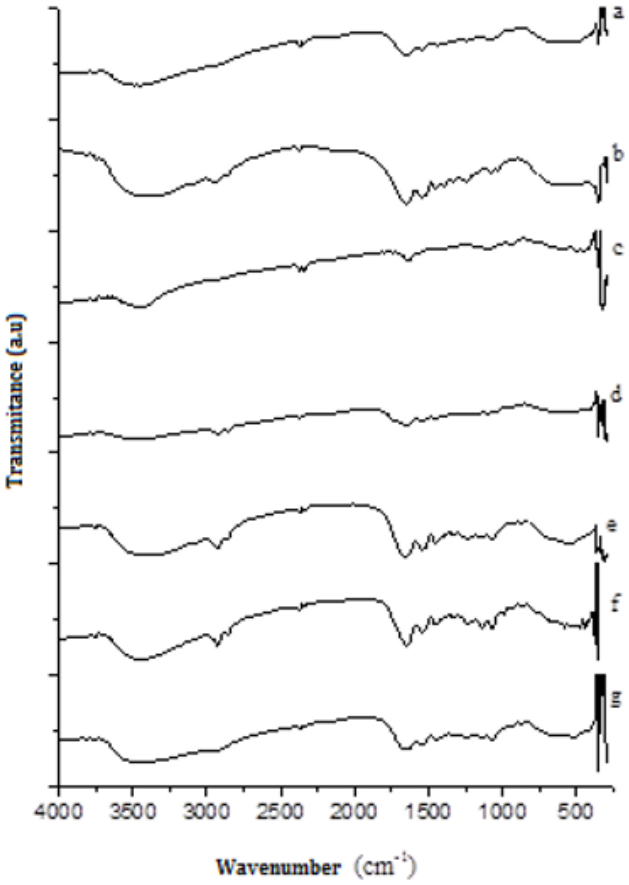

Figure 3. FTIR Spectra: (a) Synthetic Gelatin, Gelatin from Hydrolysis at 80°C with Soaking Time: (b) 24 hours, (c) 12 hours, (d) 1 hour, and Gelatin from Hydrolysis at 60°C with Soaking Time: (e) 24 hours, (f) 12 hours, (g) 1 hour.

For all six variations, there is an amide A absorption at wavenumbers 3348, 3410, 3441, 3425, 3402, 3410, and 3464 cm^{-1}. Amide B, which corresponds to asymmetric stretching vibrations of the CH_2 group, is also present in all six variations at wavenumbers 2939, 2931, and 2924 cm^{-1}. However, the symmetric stretching vibration of the CH_2 group is absent in synthetic gelatin, gelatin with a 24-hour soaking variation at a hydrolysis temperature of 80˚C, and gelatin with a 1-hour soaking variation at a hydrolysis temperature of 60˚C. The amide I, II, and III absorptions are present in all six variations. Amide I is found at wavenumbers 1635 and 1651 cm^{-1}, amide II at 1543, 1527, and 1550 cm^{-1}, and amide III at 1080, 1095, 1242, and 1234 cm^{-1}. This confirms that the collagen hydrolysis results from cow bones in all variations conducted in this study can be identified as gelatin.

p-ISSN 2722-8258
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Table 1. Interpretation of FTIR Spectra from Gelatin Extraction

Type of Vibration	Wavenumber (cm^{-1})						
	Synthetic gelatin	Gelatin resulting from hydrolysis at a temperatur of 80 ˚C			Gelatin resulting from hydrolysis at a temperatur of 60 ˚C		
		P.24 h	P.12 h	P.1 h	P.24 h	P.12 h	P.1 h
Amide A: Stretching vibration of NH groups and hydrogen bonding	3448	3410	3441	3425	3402	3410	3464
Amide B: Asymmetric stretching vibration of CH2 groups	2939	2931	2931	2924	2924	2924	2924
Symmetric stretching vibration of CH2 groups	-	-	2862	2854	2854	2854	-
Amide I: Stretching vibration of C=O	1635	1651	1635	1651	1651	1651	1635
Amide II: Bending vibration of NH and stretching vibration of C-N	1543	1543	1527	1527	1543	1543	1550
Amide III: Residues of CH2 groups from glycine and proline	1080	1080	1095	1242	1234	1234	1234

Results of Gelatin Electrophoresis Test

Electrophoresis testing was conducted on gelatin extracted from cow bones, with the aim of determining the range of molecular weights present in the gelatin. The key characteristics of gelatin are greatly influenced by amino acid composition, molecular weight distribution, and structure (Jellouli et al., 2011). Gelatin extracted from cow bones through the continuous soaking phase using NaOH, citric acid, and HCl with varying soaking times of 1, 12, and 24 hours, followed by hydrolysis at temperatures of 60 and 80°C, was analyzed using SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis). The results of the SDS-PAGE analysis are presented in Figure 4.

p-ISSN 2722-8258
e-ISSN 2722-8266

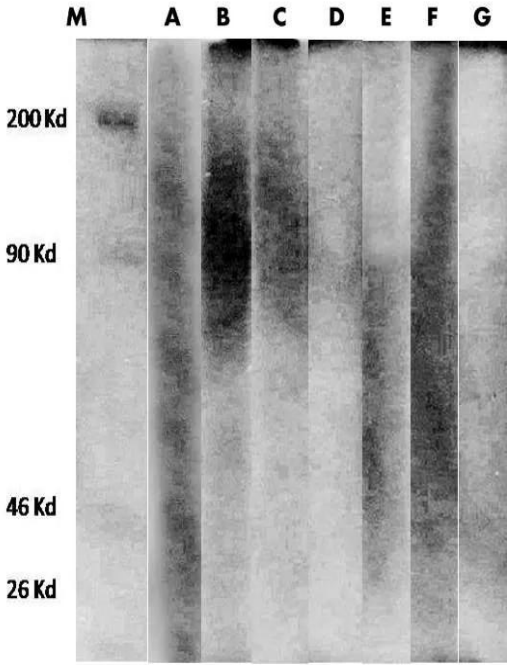

Figure 4. Electrophoresis Results (SDS-PAGE): (A) Synthetic Gelatin, Gelatin from Cow Bone Hydrolyzed at 80°C with Initial Treatment (Soaking) Time Variations of (B) 24 hours; (C) 12 hours; (D) 1 hour, and Hydrolyzed at 60°C with Initial Treatment (Soaking) Time Variations of (E) 24 hours; (F) 12 hours; (G) 1 hour, and (M) marker.

From the electrophoresis data (Figure 4), it is known that the highest protein content is sequentially found in gelatin variations B, F, C, and E. Meanwhile, variations D and G are predicted to have the lowest protein content. This is because the stepwise soaking process with NaOH, citric acid, and HCl for variations D and G was only carried out for 1 hour. As a result, the process of dissolving non-collagen proteins and the expansion of collagen fibers did not occur effectively. This led to suboptimal extraction and conversion of collagen protein into gelatin during the hydrolysis process. Among the six variations tested, variation B had the highest protein content, as evidenced by the thickness of the band in the electrophoresis results. The band thickness in variation B appears more prominent in the molecular weight range of 120 kDa. This molecular weight range has been confirmed as the molecular weight of the α-chain (Atma & Ramdhani, 2017). Thus, it can be stated that the gelatin composition in this variation is dominated by α-chains. This indicates that the cow bones subjected to the soaking process and hydrolysis temperature in variation B where bones were soaked stepwise for 24 hours and hydrolyzed at 80°C successfully removed non-collagen proteins and effectively converted collagen proteins from a triple-helix structure into a single-helix structure. This aligns with the findings of Díaz-Calderón et al. (2017), who stated that gelatin molecular weight is highly influenced by the soaking treatment process (Díaz-Calderón et al., 2017).

CONCLUSION

In the process of extracting gelatin from cow bones with sequential soaking in NaOH, citric acid, and HCl for 24 hours, followed by hydrolysis at a temperature of 80°C, the resulting gelatin exhibited the best characteristics. This was evidenced by the FTIR spectra, which matched that of synthetic gelatin, and a molecular weight distribution in the range of 120 kDa.

p-ISSN 2722-8258
e-ISSN 2722-8266

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