Comparison of the Physico-Chemical Properties of Type-B Halal Gelatin from Bovine and Goat Skin Material

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Abstract. In this work aims to compare the physico-chemical properties of gelatin derived from bovine skin and goat skin. Bovine skin and goat skin were cured in 10% CaO solution for 30 days then neutralized using 0.1 N HCl. Extraction was carried out in three stages for 4 hours with different temperature ranges namely 60-80 °C for bovine skin and 60-66 °C for goat skin. Extraction results showed a higher yield of bovine skin gelatin compared to goat skin gelatin which results are in line with ash content. The water content and pH produced tend to be constant. The strength of bovine skin gelatin is lower than that of goat skin gelatin. Whereas the viscosity of bovine skin gelatin is higher. In the goat skin gelatin color parameters have a higher L * value which means it has a lighter color.

1. Introduction
Gelatin is a type of protein that results from the partial hydrolysis of collagen that is extracted from the skin tissue, bone or ligaments (connective tissue) of animals. Gelatin has many functional properties that are widely applied in several fields such as industry, cosmetics, food and pharmacy. These functional properties such as stabilizers, textureification, foaming, emulsification, sedimentation, adhesion and encapsulation. [1]. Although gelatin has many benefits, gelatin application is still limited due to concerns about non-halal raw materials.

The potential development of the gelatin industry in Indonesia is quite promising. However, until now Indonesia does not have an industry that produces commercial gelatin both on a small and medium scale. So that the requirement for Indonesian gelatin is entirely dependent on the import results. In an effort to reduce dependence on imported gelatin, the development of the gelatin industry in Indonesia is believed to be a very potential market [2]. The main material for global gelatin production generally uses material derived from pigs. Gelatin production in the world is still dominated by pig skin gelatin with a percentage of about 46% then followed by bovine skin and bones with a percentage of about 29.4% and 23.1% and other sources around 1.5%. However, the use of pig-based material and its derivatives is not acceptable among Muslim communities such as in Indonesia. Halal law in Muslim societies requires food and non-food products to be free of pigs and their derivatives. Halal food products have contributed 20% to world trade and are predicted to continue to increase to 30% by 2025 [1]. Therefore, alternative raw materials besides pigs are needed for gelatin production which can be accepted by the public, especially the Muslim community. Alternative material for gelatin production is...
bovine and goat based material [3]. Bovine and goat based gelatin can be an alternative to pigs gelatin because of its abundant raw material. In addition, mammalian gelatin is preferred over maritime based gelatin because mammalian gelatin has better gel strength, viscosity and melting points [4]. Bovine and goat skin can be used as an alternative raw material for gelatin production. The collagen content in mammalian skin reaches 89% of the total body weight. The abundance of gelatin-based raw material from bovines and goats is supported by slaughterhouse animal waste, for example in each slaughtering goat will produce skin waste of 6.4–11.6% while in bovine will produce skin waste 6.84–8.11% of the total body weight. [5,6].

Functional and physiological characteristics gelatin are strongly influenced by the initial treatment (acidic or alkaline), the extraction conditions (time and temperature) and raw material [4], [7]. Gelatin is divided into two types namely type A and type B. Gelatin Type A is produced from hydrolysis of collagen under acidic conditions while type B gelatin is produced from hydrolysis of collagen under alkaline conditions. Differences in the production process of type A gelatin and type B gelatin cause differences in the content of amino acid composition. In type B gelatin asparagine and glutamine from collagen are converted to aspartic acid and glutamic acid, whereas in gelatin type A asparagine and glutamine do not undergo conversion [8].

In addition to the above conditions the characteristics of gelatin are also influenced by the gelatin raw material because the magnitude of the heat-stable collagen cross-link in the raw material is different from each other [9]. The study conducted in this article discusses the comparison of the physico-chemical properties of gelatin from the skins of bovine and goat.

2. Material and Methods

2.1. Collection and Preparation Skin Material
Bovine and goat skins are obtained from slaughterhouses in Pegirian, Surabaya, Indonesia. Skin samples are packaged using polyethylene bags in an insulated ice box, to avoid physiological damage and transport to the laboratory within 2 hours. After arriving at the laboratory, bovine and goat skins are washed using running water at a temperature of 28–30 ºC then the hair is cleaned. After the skin is free from the hair then put it back in a polyethylene bag and stored in the freezer with a temperature of -20 ºC (max. 2 months). Before being processed, the skin must first be thawed using a water bath at 28-30°C for 15 minutes.

2.2. Pre-Treatment of Skin Material
The prepared skin material is cut with a size of 3 x 1 cm and then washed with water until clean. Pieces of skin that has been cleaned and then soaked in 10% CaO solution, where per kg of skin requires 1 liter of CaO solution. Skin immersion process is carried out for 30 days. During the immersion process, stirring is carried out every two days. This process aims to make the ossein contained in the skin swell. Then the skin is washed with running water for 20 minutes and neutralized using 0.1 N HCl solution for 5 minutes where per kg of skin requires 1 liter of 0.1 N HCl. Neutralized skin is rinsed again using running water until a neutral pH is obtained.

2.3. Gelatin Extraction
The gelatin extraction process is carried out using water where each kg of skin needs 1 liter of soaking water with an extraction time of 4 hours. Extraction was carried out in three stages with an increase in temperature namely 60 º C (first stage); 70 º C (second stage); and 80 º C (third stage) for bovine skin then temperature 60 º C (first stage); 64 º C (second stage); and 66 º C (third stage) for goat skin. A quick look at the gelatin extraction procedure is summarized as a flow diagram in figure 1.

2.4. Yield
The gelatin produced was calculated based on the ratio of gelatin dry weight to the initial raw material (bovine skin material), according to Eq. (1):

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\text{Yield} = \frac{\text{weight of dried gelatin (g)}}{\text{weight of initial bone material (g)}} \times 100\%
\]  

(1)
2.5. Water Content, Ash Content and pH
Gelatin water content is determined by weighing 5 grams of gelatin and then heated for 16-18 hours at a temperature of 105 ± 2 ºC. As soon as the heating is finished, the gelatin sample is weighed again. The water content in this analysis is the percentage of sample weight loss [10]. Gelatin ash levels were measured by heating 5 grams of sample in a furnace at 550 º C for 15 to 20 hours [10]. pH of 1.5% (b/v) gelatin solution was measured by potentiometry at 35 ± 1 ºC using a pH meter.

2.6. Gel Strength and Viscosity
Gelatin water content is determined by weighing 5 grams of gelatin and then heated for 16-18 hours at a temperature of 105 ± 2 ºC. As soon as the heating is finished, the gelatin sample is weighed again. The water content in this analysis is the percentage of sample weight loss [10]. Gelatin ash levels were measured by heating 5 grams of sample in a furnace at 550 º C for 15 to 20 hours [10]. The viscosity of the 6.67% (b/v) gelatin solution at temperature of 60 ºC measured using viscometer bath.

2.7. Color
Colour properties of bovine skin and goat skin gelatin measured using CieLab coordinates (L*, a*, b*) with spectro-photocolorimeter
3. Result and Discussion

The physicochemical properties of gelatin are strongly influenced by the raw material, pretreatment and extraction conditions (time and temperature) [11]. Chemical structure of gelatin shown in Figure 3. In this work, will discuss the quality of gelatin from two different raw materials namely bovine and goat skins on the physico-chemical properties of gelatin. Physico-chemical properties compared include yield, proximate (water content and ash content), pH, viscosity, gel strength and color. Bovine skin gelatin and goat skin gelatin resulting from this work are shown in Figure 2.

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**Figure 1.** Flow chart of type b gelatin production

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**Figure 3.** Chemical structure of gelatin.
3.1. Yield
The yield of gelatin produced from this work shows that gelatin from bovine skin material gives a greater percentage of yield compared to gelatin from goat skin material as shown in table I. The difference in yield of gelatin is caused by differences in collagen in bovine skin and goat skin where according to Ref [9]. Gelatin extraction with heat will produce an optimum yield but the extraction yield is limited by the concentration of collagen available in the raw material used. Besides being influenced by the concentration of collagen, the difference in yield of the resulting gelatin is also influenced by the extraction temperature wherein the bovine gelatin is used to increase the extraction temperature from 60-80 °C while in goat skin gelatin the temperature increase from 60-66 °C. These results are in line with [7], where an increase in yield increases with an increase in extraction temperature. The increase in yield along with the increase in extraction temperature can occur because high extraction temperatures provide more energy to break hydrogen bonds and covalent cross-linking bonds that maintain the collagen matrix. Thus, the triple helix structure is transformed into a simpler structure that is easily extracted by solvents which results in high yield [7][3].

3.2. Proximate
Proximate analysis conducted in this work includes water content and ash content. Based on the standards of GMIA. The water content and ash content in gelatin do not exceed 12% and 2%, respectively. From the results of the study in table I show that the water content in both bovine skin
Gelatin and goat skin gelatin have met the standards [12]. However, both ash levels still exceed the standards [12].

Water content is the percentage of water that is bound by an ingredient to dry weight. Determination of water content is carried out to determine the amount of water bound by the solid component of the material. The water content in a material can determine its appearance, texture and ability to withstand the attack of microorganisms expressed in aw, that is the amount of free water that can be utilized by microorganisms for its growth [12].

The ash content of gelatin varies with the type of raw material and its processing method. Gelatin made from the skin contains a small amount of chloride or sulfate. Bone gelatin contains calcium salts from acids used in neutralization after calcification. Ash content shows the amount of inorganic material contained in organic matter. Ash indicates the amount of inorganic material left during the high combustion process (temperature around 600 oC) [12].

The pH measurement is carried out to determine the condition and type of charge found in gelatin. Gelatin is a polypeptide chain consisting of various amino acids. Amino acids have zwitterion or dipolar properties because in their chemical structure they have a negative functional group (COO-) and a positive function group (NH3+). Amino acids are also amphoteric, which can be acidic, neutral or basic in accordance with environmental conditions [12].

Water content produced from bovine and goat skin gelatin does not have a significant difference tends to be constant. In line with the moisture content the pH value produced tends to be constant. Ash content produced from bovine skin gelatin is higher than goat skin gelatin ash level. This can occur due to the use of higher extraction temperatures in bovine skin gelatin compared to goat skin gelatin.

Table 1. Physico-chemical properties I of bovine skin and goat skin gelatin.

| Properties | Bovine Skin Gelatin | Goat Skin Gelatin |
|------------|---------------------|-------------------|
| Yield (%)  | 13.51               | 11.050            |
| Water content (%) | 10.83 | 10.79 |
| Ash content (%) | 3.28 | 2.4 |
| pH         | 6.87                | 6.91              |

3.3. Gel Strength

The rheological properties of gelatin are the most important properties for the development and characterization of a product in the pharmaceutical, biomedical and food industries. At concentrations between 1-50% gelatin can form a homogeneous gel if cooled to a temperature below the sol-gel transition [13]. One of the rheological properties of gelatin is gel strength. The strength of a gelatin gel is defined as the amount of strength needed by the probe to press the gel until the gel breaks. The unit to show the strength of the gel that results from a certain concentration is called bloom. One of the important physical properties of gelatin is the strength to form a gel called the gel strength. Gel strength is influenced by pH. the presence of electrolyte and non-electrolyte components and other additives [12].

Gel formation (gelation) is a phenomenon of combining or cross-linking polymer chains to form a continuous three-dimensional series. so that it can capture water in it into a compact and rigid structure that is resistant to flow under pressure. When the soles of the gelatin cool down, the consistency becomes thicker. and then the gel will form. The exact mechanism of gel formation from gelatin soles is still unknown. Individual molecules combine in more than one crystalline form to form a three-dimensional intertwine that traps the liquid and is strongly cross-linked. causing sequential gel formation [12]. The strength of the gel or blossom values including low (<150), moderate (150-220) and high bloom (220-300) determine the quality of gelatin and viscoelastic properties such as gel and fusion. Rheological data are needed for the analysis of flow conditions in various food processing operations and texture measurements [14].

Gel strength is an important parameter for evaluating gelatin quality. Gel strength is not only influenced by the type of gelatin but also influenced by the type of pre-treatment and extraction.
conditions [11], [15]. Table II shows the gel strength of bovine and goat skin gelatin. These results indicate that gelatin from bovine skin has a lower gel strength compared to goat skin gelatin. This can occur due to the use of higher extraction temperatures in bovine skin gelatin. Low gel strength is associated with shorter peptide bonds. Higher extraction temperatures cause more protein degradation, resulting in protein fragments and reduced gel-forming ability. This is in line with what was found by Ref. [9], who compared the strength of bovine bone gelatin extracted at a temperature of 45 °C against the gelatin gray trigger fish extracted at a temperature of 65 °C which results showed the gel strength of material bovine bone is higher than that of gelatin from gray trigger fish.

3.4. Viscosity

Viscosity is the second important parameter after gel strength. Viscosity is the flowing power of a molecule in a solution both in water, simple organic solvents, aqueous suspension or emulsion. Viscosity is the physical property of gelatin which is very important after gel strength, because viscosity affects the physical properties of other gelatins such as melting points, general points and emulsion stability [12].

Gelatin viscosity affects gel properties, especially the point of gel formation and melting point, where high gelatin viscosity results in a higher melting rate and gel formation than gelatin with low viscosity. For the stability of the gelatin emulsion a high viscosity is required. Viscosity is influenced in part by hydrodynamic interactions between gelatin molecules, temperature, pH, and concentration [12].

The viscosity and gel strength are parameters related to each other where the high gel strength is due to the longer gelatin peptide bonds. The longer peptide bonds will make gelatin molecular weight higher which causes high viscosity [16]. Data from Table 1 shows that the viscosity of gelatin from bovine skin is higher compared to gelatin from goat skin. This value is contrary to the value of the gel strength obtained from this work. This is due to the fact that bovine gelatin is thought to have shorter peptide bonds but with high hydrolyzed collagen concentrations. Conversely, in goat skin gelatin it is suspected that even though they have longer peptide bonds, the amount of hydrolyzed collagen is lower. The events that occurred were thought to be related to the gelatin material used, pre-treatment conditions and extraction conditions [15].

| Properties          | Bovine Skin Gelatin | Goat Skin Gelatin |
|---------------------|---------------------|-------------------|
| Viscosity (mPs)     | 53.4                | 49.6              |
| Gel strength (Bloom)| 203                 | 211               |
| Color (L*)          | 110.2               | 120.3             |

3.5. Color

Color is an important parameter in gelatin products. The color of the gelatin will affect the color of the food being applied which is generally the lighter gelatin color more needed. Increasing the extraction temperature higher will increase the potential for breaking of some bonds with the obtained gelatin compounds. The occurrence of molecular structural changes that occur in gelatin, especially if it is then accompanied by the presence of unpaired electrons in the gelatin compound. will increase the potential for electron excitation from nonbonding (n) orbitals to δ orbital and orbital π orbitals when adsorbing photons. This condition will automatically cause a shift in the wavelength of absorption of the gelatin product from the ultraviolet (UV) spectrum to the visible UV spectrum (UV Vis) and encourage the appearance of a darker color to the gelatin product obtained with increasing extraction temperature [17]. As shown in table II, it appears that bovine skin gelatin extracted at higher temperatures has a lower lightness value when compared to goat skin gelatin extracted at lower temperatures.
4. Conclusion

Bovine skin gelatin and goat skin gelatin have been compared physicochemical properties. Extraction results showed a higher yield of bovine skin gelatin compared to goat skin gelatin which results are in line with ash content. The water content and pH produced tend to be constant. The strength of bovine skin gelatin is lower than that of goat skin gelatin. Whereas the viscosity of bovine skin gelatin is higher.

In the goat skin gelatin color parameters have a higher L * value which means it has a lighter color. Of the several parameters of the analysis only ash content whose value is still high and has not yet reached the standards set by GMIA.

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