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Characterization and functional properties of gelatin extracted from goatskin

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Abstract

Gelatin from goatskin pretreated with hydrochloric acid and extracted with distilled water at 60°C for 9 hours was characterized and compared to that of bovine skin gelatin (BSG). A yield of 10.26% (wet weight basis) was obtained. Goatskin gelatin (GSG) had high protein (86.58%), suitable moisture (9.58%), low fat (1.46%) and low ash (0.11%) content. The functional properties of GSG including gel strength (301 g bloom) and emulsion activity index (94.27%) were higher than the functional properties of BSG including gel strength (192 g bloom) and emulsion activity index (49.74%). The foaming property of GSG (102%) was lower than that of BSG (164.67%). This study shows that GSG has a high potential for application as a source of commercial gelatin.

Introduction

Gelatin is one of the most common biopolymers. It is a denatured fibrous protein obtained either by partial acid hydrolysis (type A gelatin) or by partial alkaline hydrolysis (type B gelatin) of animal collagen (Karim and Bhat, 2008; Cheng *et al*., 2012). Because of its unique properties, gelatin is widely used in the pharmaceutical, food and cosmetic industries (Zhang *et al*., 2009; Hashim *et al*., 2010). In the pharmaceutical industry, gelatin is used as a major material in hard or soft gelatin capsules, as a biodegradable matrix material in implantable delivery systems, as a binder in tablets and as a matrix in the microencapsulation of drugs (Rowe *et al*., 2009). In the food industry, it has been used as an ingredient in jellies, desserts, aspics, milk products like yogurt, ice cream, desserts and sweets like marshmallows (Venien and Levieux, 2005).

The most important sources of collagen for gelatin production are cartilages, bones, tendons and skins of bovine and porcine origin (GMIA, 2012). A recent report released that production of gelatin from pigskin is the highest (44%), followed by bovine hides (28%), bovine bones (27%) and other sources (1%) (Ahmad and Benjakul, 2011). However, products containing gelatin from pigskin are prohibited for use by followers of religions such as Islam and Judaism. The use of gelatin from bovine sources is also problematic due to threats from outbreaks of bovine spongiform encephalopathy (BSE). Moreover, bovine products are prohibited to use by Hindus. Since bovine skin gelatin is usually more expensive than porcine gelatin, manufacturers prefer the use of porcine gelatin (Rohman and Che Man, 2012). In the last decade, fish gelatin has received increasing attention as an alternative to bovine and porcine gelatin (Gudmundsson and Hafsteinsson, 1997; Jamilah and Harvinder, 2002; Arnesen and Gildberg, 2007; Ahmad and Benjakul, 2011; Gomez-Guillen *et al.*, 2011; Shyni *et al*., 2014). However, the properties fish gelatin differ from that of mammalian gelatins. The physicochemical and functional properties of fish gelatin are sub-optimal compared to mammalian gelatin (Karim and Bhat, 2008). Commercially, the skin of fish as a source of gelatin is less favorable.

Gelatins from land mammalian sources are preferred due to their superior physicochemical and functional properties (Shyni *et al*., 2014). One mammal that has not been explored as a source of gelatin is goat. Goats are land mammals and are widely available in Indonesia, with goat populations increasing at a rate of approximately 1.29% per year. According to data from Statistics Indonesia,

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the number of goats in Indonesia was 18.879.596 in 2015. Goat meat is used by Indonesians for food and for Islamic religious ceremonies called Qurban and Aqiqah. Goatskin is a resource that should be utilized as much as possible, including as a potential source of gelatin. To our knowledge, reported studies on the production and detailed physicochemical and functional properties studies of gelatin from goatskin are very limited.

The objectives of this study were to extract and characterize goatskin gelatin (GSG) and to compare the physicochemical and functional properties of the extracted gelatin with commercially available bovine skin gelatin (BSG). The hypothesis is that GSG has similar physicochemical properties to BSG due to it being derived from land mammals.

Materials and Methods

Materials

Fresh goatskin was obtained from the Center for Slaughtering House in South Tangerang, Indonesia. The goatskin was put in polyethylene bags and taken to the laboratory, Department of Pharmacy, Faculty of Medicine and Health Sciences, Syarif Hidayatullah State Islamic University, Jakarta, Indonesia, using an ice box which was kept at 4°C. Upon arriving at the laboratory, the goatskin was washed using detergent to clean and remove any smells. The goatskin was washed with tap water for an hour to rinse off the detergent and stored at -20°C until used in further experiments. The chemicals used in this study include sodium sulfide (VWRChemicals, Belgium), calcium hydroxide, hydrochloride acid, HPLC grade acetonitrile and sodium dodecyl sulphate purchased from Merck, Germany and alpha aminobutyric acid, bovine skin gelatin and soybean oil puchased from Sigma-Aldrich, USA.

Unhairing process of goatskin

The unhairing process was conducted according to the manual by Schrieber and Gareis (2007). The goatskin was thawed prior to experiment. Then, the goatskin was soaked in 3%(w/v) sodium sulfide and 2%(w/v) calcium hydroxide for 2 hours to remove hair and non-collagenous protein. Next, the goatskin was washed with tap water until the washing water was clear. The goatskin was cut into 1-2 cm pieces. The goatskin that was cut into pieces was used as raw material for extracting gelatin.

Extraction of gelatin from goatskin using acid hydrolysis

The extraction procedure was conducted according to Gudmundsson and Hafsteinsson (1997) with slight modifications based on preliminary extraction trials. The goatskins were soaked in 2%(v/v) hydrochloric acid at 5°C for 48 hours. The acid-treated goatskins were washed with distilled water until the washing water was clear. The final extraction was carried out in distilled water at 60°C for 9 hours. The clear extract obtained was filtered through a Buchner funnel with Whatman filter paper No.1. The filtrate was evaporated in an oven at 60°C for 2 hours. The filtrate was then cooled in the refrigerator at 5°C until a liquid gelatin was formed. The liquid gelatin was dried using an oven at 60°C for 20 hours. The dried gelatin was ground to produce powdered gelatin. The powdered gelatin was weighed to calculate yield.

Yield of gelatin

The yield of gelatin obtained was determined as follows:

Yield(%) = (dry weight of the gelatin/wet weight of raw materials) x 100%

Proximate analysis

The moisture, ash and fat contents of extracted dried gelatin were determined according to AOAC (2000) methods number 927.05, 942.05 and 920.39 B, respectively. The crude protein content was determined by estimating its total nitrogen content using the Kjeldahl method according to AOAC (2000) method number 984.13. All measurements were performed in triplicate.

Determination of pH

The pH values of gelatin solutions were measured using the British Standard Institution method (1975). The extracted gelatin (1 g) was dissolved in distilled water and adjusted to 100 ml. The mixture was heated at 45°C. The solution was allowed to reach room temperature before pH was measured using a pH meter (Metrohm pH Lab, Swiss).

Determination of gel clarity

Gel clarity was determined according to the method of Avena-Bustillos *et al*. (2006) by measuring transmittance (%T) at 620 nm in a spectrophotometer (Hitachi U-2910 Spectrophotometer) using a 6.67% (w/v) gelatin solution in distilled water heated to 60°C for 1 hour.

Determination of amino acid composition

Prior to measurements, gelatin was hydrolyzed to obtain free amino acids. Gelatin (0.1 g) was added to 5 ml 6N HCl in screw cap tubes and mixed using a vortex for 5 minutes. The tubes were sealed under

nitrogen and then heated in an oven at 110°C for 22 hours. The hydrolyzed gelatin was cooled to room temperature.

Hydrolyzed gelatin was moved to a volumetric flask and adjusted to 50 ml with distilled water. The solution was filtered through a 0.45 µm membrane filter. Filtrate (500 µL) was added to 40 µL AABA (alpha-aminobutyric acid) in 0.1M HCl and 460 µL distilled water. The solution (10 µL) was added to 70 µL AccQ Fluor borate and mixed using a vortex for 5 minutes. The mixture was heated at 55°C for 10 minutes, then cooled to room temperature.

The sample was injected into the amino acid analyzer High-Performance Liquid Chromatography (Waters), detector PDA, flow rate 0.7 mL/min, eluent AccQTag Eluant A- HPLC grade 60% acetonitrile with gradient system, temperature 49°C, column Waters AccQ Tag Ultra C18,4µm (3.9x150 mm). Determinations were performed in triplicate and data correspond to mean values. Standard deviations were in all cases lower than 2%.

Determination of gel strength

Gel strength of gelatin was measured according to the method of Gomez-Guillen *et al.* (2002). The powdered gelatin (6.67 g) was dissolved in distilled water at 60°C for 30 minutes and the solution was cooled in a refrigerator at 7°C for 16 hours until a gel was formed. The gel strength was determined using a Model TA-XT Plus Texture Analyzer with a 5 kN load cell equipped with a 1.27 cm diameter flat-faced cylindrical Teflon plunger. Gel strength was expressed as the maximum force (in grams), required for the plunger to depress the gel by 4 mm at a rate of 0.5 mm/s. The measurement was performed in triplicate.

Determination of emulsifying properties

The emulsion activity index (EAI) of the gelatin was determined according to the method of Pearce and Kinsella (1978) with slight modifications. The gelatin solutions were prepared by dissolving 1% (b/v) powdered gelatin in distilled water at 60°C for 30 minutes. The gelatin solutions 1% (30 mL) were mixed with 10 ml of soybean oil and then homogenized for 1 min at room temperature using a homogenizer (Ika RW 20 digital homogenizer). The emulsion (50 mL) was taken after homogenization and diluted 100-fold with 0.1% SDS solution. The diluted solutions were mixed thoroughly for 10 seconds using a vortex mixer. The absorbance of the diluted solutions were measured at 500 nm using a spectrophotometer (Hitachi U-2910 Hitachi High-Technology Corporation). The absorbance measured immediately (A) after emulsion formation was used to calculate the emulsifying activity index (EAI) as follows (Ahmad and Benjakul, 2011) :

$$EAI\ (m^2/g) = (2 \times 2.303 \times A \times DF)/l\theta C$$

Where : A is A_{500},
DF is dilution factor,
l is path length of cuvette (m),
ϴ is oil volume fraction and
C is protein concentration in aqueous phase (g/m^3).

Determination of foaming properties

Foam expansion (FE) and foam stability (FS) of the gelatin solutions were determined according to the method of Cho *et al.* (2004). The gelatin solution 1% (100 mL) was homogenized for 1 minute at room temperature (25°C) using homogenizer (Ika RW 20 digital homogenizer) to incorporate air. The homogenized solution was then immediately poured into a 250 ml measuring cylinder, and the total volume was measured at 0, 30 and 60 minutes after whipping. Foam capacity was expressed as foam expansion at 0 min, which was calculated according to the following equation :

$$\text{Foam Expansion (\%)} = (V_t - V_o)/V_o \times 100$$

Foam stability was calculated as the volume of foam remaining after 30 and 60 min.

$$\text{Foam Stability (\%)} = (Vt - V_o)/V_o \times 100$$

Where: V_t is the total volume after whipping (ml);
V_o is the volume before whipping;
Vt is the total volume after leaving the foam at room temperature for different times (30 and 60 min).

All determinations are means of three measurements.

Results and Discussion

Yield of gelatin

The yield of extracted goatskin gelatin was 10.26%±1.07 (wet weight basis). Collagen found in connective tissue is difficult to dissolve in water, even at high temperatures. This is due to the strong bond of the triple helix which is a constituent chain of collagen. Therefore, hydrolysis is necessary. The principle of hydrolysis is to break down the triple helix of collagen, whilst keeping the chain of protein intact– so called partial hydrolysis (Karim and Bhat, 2008). In this study, partial hydrolysis of collagen was done using hydrochloric acid, resulting in type

A gelatin. Hydrolysis was carried out by soaking the goatskin in 2% hydrochloric acid for 48 hours. The ionic strength of the solution at acidic pH facilitates the swelling process. With the loss of the bond, warm water is able to penetrate effectively into the matrix (Ahmad and Benjakul, 2011).

This result was higher than yields of cuttlefish gelatin reported by Balti *et al*. (2011), gelatin from grey triggerfish (Jellouli *et al.*, 2011), gelatin from chicken skin (Sarbon *et al*., 2013) and gelatin from red and black tilapia (Jamilah and Harvinder, 2002) – 7.84%, 5.67%, 2.16%, 7.81% and 5.39% respectively. However, our yields were lower than the yields of gelatin extracted from unicorn leatherjacket as reported by Ahmad and Benjakul, (2011) and gelatin from dog shark, skipjack tuna and rohu as reported by Shyni *et al.* (2014) which are 11.54%, 19.7%, 11.3% and 17.2%, respectively. The different types of skin, pH, acid concentration and duration of hydrolysis that affects the swelling process are factors that account for differences in the yields obtained (Ratnasari *et al.*, 2013).

Proximate analysis

The proximate compositions of goatskin gelatin and bovine skin gelatin are shown in Table 1. Dry gelatin is stable in air. When moist, it is easily decomposed by microorganisms. The moisture of gelatin meeting standard requirements is 8-13% (GMIA, 2012). The moisture of goatskin gelatin was 9.58%±1.11, indicating that goatskin gelatin had suitable moisture. The recommended maximum value of ash in gelatin is 2.5% (Jones, 1977). The ash content of goatskin gelatin was 0.11%±0.02. Low ash content in goatskin gelatin indicated the absence of inorganic salt in the gelatin, which might be generated during pretreatment with acid (Ahmad and Benjakul, 2011). The fat content of goatskin gelatin was 1.46%±0.74. The extracted goatskin gelatin was almost free of fat. This indicated that the de-fattening process had eliminated the fat in goatskin gelatin. The protein in goatskin was 19.8%±0.34. In contrast, the protein content of goatskin gelatin extracted was 86.58%±1.34, showing that protein was a major component. The hydrolysis reaction and extraction process increases protein content. The presence of protein at very high levels and ash, lipid and other impurity at very low levels are important measurements for the quality of gelatin.

pH

Gelatin meeting the standard requirements needs to have a pH range of between 3.8 to 5.5 (GMIA, 2012). pH affects the properties of gelatin as an excipient. pH also affects the gel strength (Shyni *et al*., 2014). The pH value of goatskin gelatin obtained was 5.03±0.32 and bovine skin gelatin pH was 5.14±0.10. Reports of gelatin from different sources provide various pH values. The pH of gelatin from shark was 4.34 (Shyni *et al*., 2014), the pH of gelatin from the skin of red tilapia and black tilapia were 3.05 and 3.91, respectively (Jamilah and Harvinder, 2002) and the pH of gelatin from catfish was 5.8 (Ratnasari *et al*., 2013).

Table 1. Proximate composition of goat skin gelatin and bovine skin gelatin

Composition	Goat skin gelatin (%)	Bovine skin gelatin(%)
Moisture	9.58±1.11	8.12±0.58
Ash	0.11±0.02	0.39±0.16
Fat	1.46±0.74	0.47±0.03
Protein	86.6±1.34	90.7±0.77

Values are given as mean ± SD from triplicate determinations

Clarity of gelatin

The clarity of gelatin gels is important for aesthetic purposes. Clarity is compared against the clarity of water at 100%T (transmittance) value. The %T of goatskin gelatin was 62.6%±0.36, and bovine skin gelatin was 71.03%±0.46. The %T of goatskin gelatin is lower than bovine skin gelatin. This means that the bovine skin gelatin is clearer than goatskin gelatin. The factors that influence clarity are the contaminants introduced or not removed (inorganic and proteinaceous substances), the filtration process during gelatin extraction (Shyni *et al*., 2014) and the color of gelatin. The color of gelatin depends on the method of extraction and the raw materials used (Ockerman and Hansen, 1999).

Amino acid analysis

The amino acid composition of goatskin gelatin and bovine skin gelatin are shown in Table 2. The major amino acid composition of goatskin gelatin is glycine (29.05%), followed by proline (13.48%), arginine (10.12%), glutamic acid (9.42%) and alanine (8.47%). The major composition of bovine skin gelatin is glycine (31.15%), glutamic acid (12.31%), arginine (9.64%) alanine (9.62%) and proline (8.57%). However, the major composition of chicken skin gelatin as reported by Sarbon *et al*, 2013 is glycine (33.7%), proline (13.42%), alanine (10.08%), glutamic acid (5.84%) and arginine (5.57%). Based on the glycine and proline content as the major components in gelatin, the goatskin gelatin seemed to be similar to chicken skin gelatin and differed from bovine skin gelatin.

Tabel 2. Amino acid composition of goat skin gelatin (GSG) and bovine skin gelatin (BSG)

Amino Acid	GSG (%)	BSG (%)
	Non Polar	
Alanine	8.47 ± 0.13	9.62 ± 0.06
Valine	2.36 ± 0.04	2.54 ± 0.02
Leusin	3.46 ± 0.06	3.11 ± 0.03
Isoleucine	1.41 ± 0.02	1.68 ± 0.01
Phenylalanine	3.78 ± 0.05	2.22 ± 0.03
Methionine	1.13 ± 0.01	1.46 ± 0.01
Proline	13.5 ± 0.21	8.57 ± 0.01
Total	34.1	29.2
	Polar	
Glysine	29.1 ± 0.44	31.2 ± 0.13
Serine	3.92 ± 0.07	4.14 ± 0.02
Threonine	3.03 ± 0.04	2.39 ± 0.02
Tyrosine	1.11 ± 0.01	0.38 ± 0.01
Cystine	0.01 ± 0.01	0.00 ± 0.00
Total	37.2	38.1
	Acid	
Aspartic	4.48 ± 0.07	5.99 ± 0.02
Glutamic	9.42 ± 0.16	12.3 ± 0.06
Total	13.9	18.3
	Base	
Lysine	3.72 ± 0.06	4.79 ± 0.03
Arginine	10.1 ± 0.16	9.64 ± 0.05
Histidine	1.05 ± 0.01	0 ± 0
Total	14.9	14.4

The minor components (less than 2%) of goatskin gelatin extracted are: isoleucine (1.41%), methionine (1.13%), tyrosine (1.11%), histidine (1.05%) and cystine (0.01%). Amino acids that are not commonly present in gelatin are cystine and tryptophan. However, cystine was detected in this study. Cystine in goatskin gelatin may have been detected from fur left over after the unhairing process. Cystine is not detected in gelatin derived from fish skin. Sarbon *et al.* (2013) report that cystine is detected in gelatin derived from chicken skin at 0.16% and in bovine skin gelatin at 0.47%.

Based on polarity, amino acids are classified into 4 classes, namely: nonpolar, polar, acidic and alkaline amino acids (Hafidz and Yaakob, 2011). They contribute to the functional properties of gelatin such as gel strength, foaming properties and emulsion activity index. The total amount of nonpolar amino acids in goatskin gelatin and bovine skin gelatin were 34.10% and 29.20%, respectively. The total content of polar amino acids in goatskin gelatin was 37.12% and in bovine skin gelatin was 38.07%.

Gel strength

Gel strength is the most important functional property of gelatin. Gel strength is determined by the amino acid composition. Gel strength of goatskin gelatin obtained and bovine skin gelatin were 301±2.64 g bloom and 192±3.05 g bloom, respectively. The gel strength of gelatin is categorized into 3 groups, namely: low (<150), medium (150-220) and high (220-300) bloom values (Sarbon *et al.*, 2013). The gel strength of goatskin gelatin is higher than that of bovine skin gelatin. Based on the values obtained, the goatskin gelatin is classified as high bloom. However, bovine skin gelatin is classified as medium bloom. In contrast, the gel strength of fish gelatin is classified as medium and low bloom. The gel strength of some fish gelatin are as follows: Sepia officinalis -181 (Balti *et al*., 2011), shark skin gelatin-206 (Shyni *et al.*, 2014), red and black tilapia -128.1 and 180.8, respectively (Jamilah and Harvinder, 2002), and Aluterus monoceros - 121.92 g bloom (Ahmad and Benjakul, 2011).

Proline is an amino acid that is responsible for the stability of the collagen structure. The bonds that are formed are the hydrogen bonds between water molecules and hydroxyl groups in hydroxyproline gelatin. This bond influences the properties of gelatin gel strength. Gelatin with low levels of the amino acids proline and hydroxyproline have a lower gel strength (Balti *et al.*, 2011).

Emulsion activity index (EAI)

Emulsion activity index of goatskin gelatin obtained was 94.27% and bovine skin gelatin was 49.74%. EAI of goatskin gelatin was higher than that of bovine skin gelatin. Gelatin is used as a foaming, emulsifying and wetting agent in the food industry in medicine and cosmetics. It is also used in other surface activity related functions. Therefore, it is necessary to measure the value of emulsion activity index. Emulsion activity index is the interface region which is stabilized by protein. EAI is different for different types of gelatin due to variations in the intrinsic properties, protein conformation and composition of amino acids (Balti *et al*., 2011).

Foaming properties

The foaming properties of goatskin gelatin and bovine skin gelatin are shown in Table 3. The foaming property of bovine skin gelatin (164.67%) is higher than that of goatskin gelatin (102%). Foaming ability is another important property that must be possessed by gelatin to be applied in the drug, food and cosmetic industries. There is a positive relationship between hydrophobic proteins and foaming properties. For adsorption to occur at the interface of air and water, a molecule must contain hydrophobic regions (Balti *et al*., 2011). The differences in ability to foam is caused by differences in the amount of hydrophobic amino acids, namely: alanine, valine, isoleucine, leucine, proline, methionine, phenylalanine and tyrosine.

The foaming properties of goatskin gelatin was not too different from that of leatherjacket gelatin -118.15% (Ahmad and Benjakul, 2011) and cuttle

Tabel 3. Foaming ability (FA) and foaming stability (FS) of goat skin gelatin (GSG) and bovine skin gelatin (BSG).

Samples	FA (%)	FS(%)		
		10 min	30 min	60 min
GSG	102.1±0.07	84.12±0.12	78.67±0.12	72.67±0.13
BSG	164.7±0.10	146.6±0.06	128.7±0.12	115.2±0.50

Values are given as mean ± SD from triplicate determinations

fish gelatin -113.77% (Balti *et al*., 2011). However, the foaming properties of shark gelatin is lower -21.5% (Shyni *et al.*, 2014).

Conclusion

Gelatin was extracted with good yield from the skin of goat. Our results followed the hypothesis that goatskin gelatin has good characteristics and fuctional properties due to its mammalian source. There is a difference in the amino acid composition between goatskin gelatin and bovine skin gelatin. The functional properties, including gel strength and emulsifying properties of goatskin gelatin were higher than that of bovine skin gelatin. On the contrary, the foaming property of goatskin gelatin was lower than that of bovine skin gelatin. This report indicates that goatskin gelatin could be a very good potential source of gelatin for use as a additive in the food, pharmaceutical and cosmetic industries.

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