OPEN ACCESS Pakistan Journal of Nutrition

ISSN 1680-5194
DOI: 10.3923/pjn.2019.

Research Article
Characteristics of Gelatin Extracted from Indonesian Local Cattle Hides Using Acid and Base Curing

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Abstract

Background and Objective: Gelatin quality is affected by factors including the animal source materials, the age of the animal and curing and extraction processes. However, the effects of the cattle breed on gelatin quality have not been reported. This study aimed to determine the characteristics of gelatin from Indonesian local cattle [Bali, Madura and Ongole Crossbred (OC)] hides with acid and base curing. **Materials and Methods:** The hides were treated with 0.25 M hydrochloric acid and 0.25 M sodium hydroxide. The data were analyzed using a completely randomized design with a nested pattern and three replicates. The mean differences in the data were analyzed using Duncan's multiple range test. **Results:** The highest gelatin yield was 11.04%, the pH was 9.91, the protein content was 83.45%, the soluble protein content was 12.82 mg mL^{-1}, the viscosity was 8 cP and the gel strength was 166 Bloom. The A_w, moisture content, fat content and color values of the gelatins did not differ significantly ($p>0.05$) among the cattle breeds or between the curing treatments. The molecular weight determinations of the HCl-derived gelatin showed clearer bands than those from NaOH, including showing a band for low-molecular weight proteins (size range between 10 and 25 kDa). Fourier transform infrared (FTIR) spectroscopy showed that while the absorbance intensities of the O-H, C-H, C ≡ C, C ≡ N, C = C, C-C and C-O functional groups from the gelatin samples varied, the peaks were still indicative of the functional groups typically present in gelatin. The most abundant amino acid was glycine at 48.73 g/100 g, followed by glutamic acid at 18.69 g/100 g and arginine at 14.77 g/100 g and histidine was not detected. **Conclusion:** The use of 0.25 M HCl was more effective and efficient than using 0.25 M NaOH. The amino acid content from the OC hide treated with NaOH was higher than those obtained with the other treatments.

Key words: Amino acid, Bali cattle, gelatin quality, Madura cattle, Ongole crossbred cattle

Received: Accepted: Published:

Citation: Dedes Amertaningtyas, Zaenal Bachrudin, Jamhari, Koo Bok Chin and Yuny Erwanto, 2019. Characteristics of gelatin extracted from Indonesian local cattle hides using acid and base curing. Pak. J. Nutr., CC: CC-CC.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Gelatin is a hydrocolloid produced from the partial hydrolysis of collagen, a protein in skin and bone. Gelatin has a unique amino acid composition and structure giving it a range of functional properties and when mixed with water, it takes on unique properties due to sol or gel formation[1]. Currently, the demand for gelatin is increasing because it is an important material with various applications in the food, health, cosmetics and pharmaceutical industries. For example, gelatin can be used as a gelling agent, emulsifier, binder, coating, filler, edible film and preservative. However, 90% of the gelatin produced worldwide is derived from pig skin or bones and is therefore not recommended for Muslims. As a result, many researchers are interested in finding alternative sources of gelatin. According to De Wolf[2], there are three main stages in the process of making gelatin. The first stage is preparing the raw material, which includes the removal of non-collagen components. The second stage is the conversion of collagen into gelatin. The final stage is the purification and recovery of dry gelatin. The quality and characteristics of the gelatin are affected by the quality of the raw materials and the production method.

There are two types of gelatin: Type A, which is made from young animals, especially the skin of young pigs and type B, which is made from cattle hides or bone[3]. Type A and B gelatins have different isoelectric points (IEPs); the IEPs of type A and B gelatin are in the pH ranges of 8-9 and 4.8-5.5, respectively[1]. Type A is obtained using an acidic curing solution, such as solutions of hydrochloric acid, sulfuric acid, sulfurous acid or phosphoric acid. The soaking or curing process is rapid, taking 3-4 weeks. However, type B gelatin is obtained using a basic curing solution, such as solutions of lime ($Ca(OH)_2$) or NaOH, so the soaking or curing process requires approximately 3 months, especially when using bone as the raw material[1]. Previous methods have involved soaking bovine skin in 1% HCl at a ratio of 1:10 (w/v) for 20 h with occasional stirring at room temperature to swell the material[4]. Indonesia has locally produced raw materials, including Bali, Madura and Ongole Crossbred (OC) cattle hides, suitable for making gelatin. The quality of the gelatin is affected by two factors: first, the quality of the input raw materials, such as the cattle species, race, age and type of feed and second, the methods used for extraction, including the temperature and the concentration of the curing solution. Gilsenan and Ross-Murphy[5] noted that the gel strength, viscosity and melting point of the resulting gelatin are dependent on the source of the raw materials and the molecular weight distribution and amino acid composition of the gelatin. Previous studies have investigated fish gelatin and observed that the physiochemical characteristics, rheological properties and amino acid composition are dependent on the gelatin isolation procedure[6,7]. However, to date, little research has been performed on the effect of cattle breeds and methods of extraction on the quality and characteristics of gelatin.

As the effects of the breed on the quality of gelatin have not been reported, this study aimed to explore the effect of different curing processes and hides of different breeds of local cattle in the production of gelatin and the resulting quality.

MATERIALS AND METHODS

Gelatin extraction: Gelatin powder is prepared prior to the determination of the quality and characteristics of the gelatin. The gelatin was extracted and produced used a modified version of the method reported by Schrieber and Gareis[1]. Hides were obtained from six male cattle from 2.5-3 years of age, including Bali, Madura and OC cattle. Fresh croupon and shoulder hides were bought from slaughterhouses in East Java, Madura Island and Bali Island. The hides were transported on ice to the laboratory where they were cleaned, the fur was scraped off and they were cut into medium-sized pieces (30×30 cm^2). Then, the hypodermic layer was cleaned and the hides were salted at a concentration of 10% (w/w). The hides were packed in polyethylene bags and kept frozen (-40°C) until further use. The curing solutions were sodium hydroxide (NaOH, 0.25 M) with a pH of approximately 12 and hydrochloric acid (HCl, 0.25 M) with a pH of approximately 2. All the chemicals and reagents used in this study were of analytical grade. Prior to gelatin extraction, the frozen hides were thawed in a room for 6 h and cut into small pieces (2×2 cm^2) weighing an average of 300 g and then soaked in the curing solution. The hides were grouped and soaked in the acidic curing solution or the basic curing solution at a 1:2 ratio and sealed with aluminum foil for 3 days. Next, the hides were washed until they reached a pH of 6-8 and soaked in distilled water (1:2) at 55°C for 6 h. The liquid gelatin was filtered through a gray cloth into a 15×20 cm^2 plastic tray and then dried in a hot air oven at 60°C for 72 h. Finally, the obtained gelatin was weighed, mixed using a blender and filtered through a 24-mesh sieve.

Determination of yield (%): The gelatin yield was calculated using the procedure outlined in Gimenes *et al.*[8]. A digital scale (Sartorius Scientific Instruments, Beijing) was used to compare the mass of the dried gelatin to the total weight of the cattle hide on a wet basis. The formula used to calculate the yield of the extracted gelatin is shown below:

$$\text{Yield (\%)} = \frac{\text{Weight of gelatin powder (g)}}{\text{Weight of fresh cattle hide (g)}} \times 100\%$$

Determination of pH: The pH of the gelatin samples was measured using a pH meter (Mettler Toledo) with a glass electrode. The pH meter was calibrated using certified buffer solutions (pH 4.00±0.01 at 25°C and pH 7.00±0.01 at 25°C). Gelatin samples (0.5 g) were weighed and then dissolved completely in 20 mL of distilled water and the calibrated electrodes were then used to measure the pH of the solution.

Determination of water activity (A_w): The A_w of the gelatin was measured using an A_w meter (Testo 650). Gelatin samples (3 g) were weighed and placed on top of the cylinder, which was then placed at the bottom of the meter. The A_w value was read from the device.

Chemical composition analysis: The chemical composition of the gelatin samples, including the contents of moisture, fat and protein, were analyzed based on the AOAC[9] methods. The protein content was calculated by multiplying the nitrogen content by 5.55.

Determination of the soluble protein content: The soluble protein contents in the gelatin samples were measured using a spectrophotometer and the biuret method described by Owusu-Apenten[10]. The gelatin sample was weighed into 0.1 g portions and transferred to 2 mL Eppendorf vials and 800 µL of the titrant was added [alkaline copper, i.e., 1.5 g copper sulfate ($CuSO_4 \cdot 5H_2O$)]. Then, 6 g of potassium sodium tartrate ($KNaC_4H_4O_6 \cdot {}_4H_2O$) was added to 500 mL of MilliQ water (H_2O) and the solution was added to 300 mL of a NaOH solution (10% w/w, 800 µL). After 30 min, the solution was measured at 540 nm using a spectrophotometer. The soluble protein content was determined by comparison to the absorbance of a standard sample prepared with bovine serum albumin (BSA).

Determination of the viscosity (cP): The viscosity was determined according to the method of Arnesen and Gildberg[11] with an Elcometer 2300 viscometer (viscosity range specification 3-2,000,000 mP with 19 speeds). The viscosity of each gelatin sample was measured using a Stormer Couette Viscometer. Solutions of the gelatin samples (6.67% w/v, 6.67 g sample in 100 mL of distilled water) were prepared by dissolving the gelatin powder in distilled water and heating to 60°C. The gelatin sample solution was poured into the bowl of the viscometer and an external water bath was used to control the sample temperature. The viscosity was determined at room temperature (28°C) and readings were taken in triplicate.

Determination of the gel strength (Bloom): The gel strength (GS) (fmax/cm^2) of the gelatin was determined according to the methods described by Muyonga *et al.*[12] and Liu *et al.*[13] using a universal testing machine (Imada/ZP-200N, digital force gauge with a voltage of 220-240 V and a capacity of 0-200 N equipped with a 13 mm diameter cylindrical plunger). The gelatin solution (6.67% w/v, prepared from 6.67 g sample in 100 mL of distilled water) was heated at approximately 60°C until the gelatin particles were completely dissolved. The gelatin sample solution was then transferred to a container with a diameter of 5 cm and a height of 6 cm with three replicates. The gelatin samples were stored in a refrigerator at 5°C for 16-18 h. The gelatin samples in their containers were placed at the bottom of the plunger for further testing at approximately 20°C. The GS readings of the maximum force of the plunger against the gel are represented as f max values (N/cm^2). The free plunger penetrated the gel at 10 mm min^{-1} to a depth of 4 mm. The GS values of the samples were calculated in units of g Bloom. The formula used to determine GS used f max with units of N/cm^2 (dyne/cm^2), which could be converted to units of g Bloom by using the following equation[14,15]:

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

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

Where

F = The maximum of the graph before breaking the gel (N/cm^2)

G = Constant (0.07)

Determination of the molecular weight distribution (Laemmli[16]): The molecular weight (MW) distribution of the gelatin sample was determined by using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (ATTO Model AE-6530 Serial No. 5125811, Japan) following the method described by Laemmli[16]. The gelatin samples (1 g) were dissolved in 10 mL of 5% SDS solution (w/v) and the mixture was heated at 85°C for 5 min in a water bath to completely dissolve the proteins. Then, after centrifuging the samples at 6,000 rpm for 3 min, the supernatants were collected and mixed with sample buffer (containing 0.5 M tris-HCl, pH 6.8 and 4% (w/v) SDS and 20% (v/v) glycerol at a ratio of 1:1 (v/v)). The protein samples (20 µg) were loaded

into a polyacrylamide gel consisting of 10% running gel and 4% stacking gel and subjected to electrophoresis at a constant current of 15 mA per gel using a Mini Protean II system (Bio-Rad Laboratories, Inc., Richmond, CA, USA). After the electrophoresis, the gel was stained with 0.05% (w/v) Coomassie Brilliant Blue R250 (B7920, Sigma Chemical Co., USA) in 15% (v/v) methanol and 5% (v/v) acetic acid and then destained with 30% (v/v) methanol and 10% (v/v) acetic acid. The individual protein sample bands were identified by comparison with standard protein markers (Precision Plus Protein™ Standards, Dual Color, Bio-Rad Laboratories, USA).

Determination of the color: The gelatin color was measured using a Color Reader CR-10 (Minolta Co. Ltd., Japan) with only an AC-A12 adapter (NUR MIT AC Adapter AC-A12). The color is given by three color coordinates, namely, L* (whiteness or brightness/darkness), a* (redness/greenness) and b* (yellowness/blueness). Two grams of sample was placed on the dish of the instrument and mounted in a precalibrated cup. The color values (L*, a* and b*) were recorded as the means of three determinations[17].

Determination of the functional groups: The functional groups were measured with a Miracle ABB MB 3000 Fourier transform infrared (FTIR) spectrophotometer over a spectral range of 4000-650 cm^{-1} to determine the structural conformation of the gelatin samples. The FTIR spectra were obtained from disks containing 1 mg of gelatin sample and approximately 10 mg of potassium bromide (KBr). All the equipment used to prepare the disks was cleaned with acetone/alcohol (70%). A mixture of gelatin and KBr was then ground and blended well and transferred to a pelletizer. A small, thin disk was formed and inserted into the FTIR instrument. The results were obtained directly from the software used to control the instrument.

Determination of the amino acid content using HPLC: High-performance liquid chromatography (HPLC) was used to determine the amino acid content in the gelatin following hydrolysis with 6 N hydrochloric acid and basification (phosphate) using an ACC Tag column and a fluorescence detector. The final results were obtained directly from the software used to control the instrument. The following pieces of equipment were used: a balance, Teflon reagent tubes, an oven, 5 mL pipettes, 20 µL pipettes, a centrifuge, 0.45 µM PTFE filters, an ACC Tag column, a fluorescence detector and an HPLC (Waters Alliance system 2695). The following reagents were used: nitrogen gas, 6 N HCl, 0.02 N HCl, AccQ-Fluor borate buffer as the eluent, AccQ-Fluor 2A and amino acid standards (Waters WAT088122). To prepare the sample (0.05 g) for HPLC analysis, 5 mL of 6 N HCl was added under flowing nitrogen gas followed by hydrolysis in an oven (116° C) overnight; then, the mixture was passed through filter paper (Whatman no. 4). A 0.5 mL aliquot of the filtrate was removed with a pipette. The residue was dried under nitrogen, redissolved in 3 mL of HCl (0.02 N), centrifuged at 3522 rpm for 15 min and filtered again through filter paper (Millipore PTFE 0.45 µm). Then, 20 µL of the filtrate was removed with a pipette and mixed with 140 µL of AccQ-Fluor borate buffer and 40 µL of AccQ-Fluor 2A. The sample was shaken and heated at 60°C for 10 min to complete the derivatization. The solution of the derivative was inserted into the HPLC system using a tapered vial insert. Before injecting the sample, a standard amino acid solution was injected under the same conditions. Then, the sample solution (5 µL) was injected into the HPLC instrument under the following conditions: Column temperature, 115°C; injector temperature, 270°C; detector temperature, 270°C; N_2 flow rate, 30 mL min^{-1}; and H_2 flow rate, 40 mL min^{-1}. The amino acid content was calculated using the following formula[18]:

$$\text{Sample area} \times \text{Concentration of amino acid standard} \times \text{WM} \times \text{FP}$$

$$\text{Amino acids (\%)} = \text{Standard area of amino acid} \times \text{grams of sample} \times 10{,}000$$

Where

WM = Partial amino acid molecular weight

$$\text{Amino acid concentration standard: } 1 \text{ mg mL}^{-1} = 1{,}000 \text{ µg mL}^{-1}$$

Statistical analysis: This research used 3 types of cattle hides, i.e., Bali cattle (B), Madura cattle (M) and OC cattle (P) and the curing solutions were 0.25 M NaOH and 0.25 M HCl. The experimental design was completely randomized with a nested pattern (curing types nested to cattle breeds) and 3 replications. The mean differences were calculated using Duncan's test.

RESULTS AND DISCUSSION

Yield: The gelatin yields from the hides of the three types of cattle showed no significant differences (p>0.05) and the yields ranged from 7.22±2.19% to 8.52±2.02% (Table 1). However, among the hides from individual breeds of cattle, the HCl and NaOH extractions showed highly significant

Table 1: Data for yield, pH, A_w and moisture content of gelatins from Bali, Madura and OC cattle hides cured with HCl and NaOH.

	Cattle hides							
	B			B		M		OC
		M	OC	HCl	NaOH	HCl	NaOH	HCl	NaOH
Yield (%)	8.23±3.23	8.52±2.02	7.22±2.19	11.04±1.08^{a}	5.42±1.11^{b}	9.97±1.86^{a}	7.06±0.63^{b}	9.08±0.13^{a}	5.36±1.29^{a}
pH	6.80±3.36	6.64±3.18	6.71±3.51	3.73±0.06^{a}	9.86±0.06^{b}	3.79±0.31^{a}	9.50±0.86^{b}	3.51±0.25^{a}	9.91±0.04^{b}
A_w	0.49±0.03	0.49±0.02	0.48±0.03	0.52±0.03	0.47±0.02	0.51±0.02	0.48±0.02	0.48±0.03	0.48±0.03
Moisture content (%)	7.03±0.59	7.16±0.62	6.73±0.98	7.20±0.55	6.86±0.68	6.83±0.45	7.48±0.65	6.24±0.79	7.22±1.03
Fat content (%)	0.27±0.11	0.28±0.12	0.37±0.14	0.20±0.02	0.35±0.12	0.26±0.07	0.29±0.17	0.34±0.19	0.40±0.11
Protein content (%)	78.05±6.31^{b}	73.14±2.81^{a}	74.37±2.30^{ab}	83.45±2.78^{a}	72.64±2.07^{b}	73.88±0.99^{a}	72.39±4.13^{b}	75.28±3.01^{a}	73.45±1.25^{b}
Soluble protein (mg mL^{-1})	11.79±0.82^{a}	11.19±0.60^{b}	12.14±0.81^{a}	11.09±0.04^{a}	12.49±0.44^{b}	10.96±0.83^{a}	11.43±0.20^{b}	11.46±0.28^{a}	12.82±0.39^{b}
Viscosity (cP)	4.67±0.82^{b}	7.17±1.60^{a}	4.16±0.75^{b}	4.67±0.58	4.66±1.16	8.00±1.73	6.33±1.16	3.67±0.577	4.67±0.58
Gel strength (Bloom)	85.99±49.01^{a}	104.59±68.65^{b}	48.39±12.48^{b}	127.63±21.08^{a}	44.37±19.02^{b}	166.93±8.44^{a}	42.26±7.32^{b}	50.29±1.94	46.49±19.37

Values with different letters (^{a}, ^{b} and ^{c}) in the same row are significantly different ($p<0.05$). All values are the mean±standard deviation from three replications

differences ($p<0.01$). The highest yield (11.04±1.08%) was obtained from the Bali hides with HCl extraction and the lowest yield (5.36±1.29%) was obtained from the OC hides with NaOH extraction. The range of yields obtained using HCl was higher than the range of yields observed using NaOH. HCl treatment resulted in a more open collagen structure and more of the collagen was hydrolyzed in the gelatin, resulting in more gelatin being obtained relative to what is obtained from NaOH extraction. Chamidah and Erlita[19] explained that an acidic solution hydrolyzes the soluble collagen, simplifying the gelatin extraction process and the open collagen structure is due to the multiple protein bond cleavages. Handoko *et al.*[20] added that acid curing (e.g., CH_3COOH) causes the skin to swell, which is a result of the facile cleavage of the covalent bonds in the hydrolyzed collagen and this cleavage converts the collagen into gelatin and causes it to dissolve during the extraction. Mulyani *et al.*[21] stated that HCl is a strong inorganic acid (i.e., it produces more hydrogen ions) and thus facilitates the dissolution of the intra and intermolecularly cross-linked collagen. Upon cleavage, the triple-helical structure of collagen converts to random coil to afford gelatin, resulting in a higher gelatin yield. Wulandari *et al.*[22] added that when producing gelatin from a split hide with acid, the diffusion of acid into the skin breaks the covalent crosslinks between collagen molecules, allowing increased hydrolysis of the collagen protein into gelatin and better dissolution of the product during the extraction. On the other hand, Mad-Ali *et al.*[23] explained that gelatin from goat skin pretreated with 0.5 M NaOH for 4 days provided a lower yield than hides pretreated for 2 or 3 days. This result was attributed to the fact that the goat skin is not swollen by pretreated with 0.5 M NaOH for only 1 day or 0.75 M for 2 days. The repulsion between protein chains in the goat skin matrix became more pronounced under alkaline condition, so the solubilization was enhanced and a lower gelatin yield was obtained.

pH: The pH values of the three types of cattle hides were not significantly different ($p>0.05$) but among hides from the different breeds of cattle, the HCl and NaOH treatments showed highly significant differences ($p<0.01$). The lowest pH value (3.51±0.25) was obtained from the OC hide treated with HCl and the highest pH value (9.91±0.04) was obtained from the OC hide treated with NaOH (Table 1). This difference can be attributed to the soaking of the hides in the curing solutions for 3 days, which caused the skins to swell due to the acid. Many basic molecules are present in the skin tissue and although the next step in the process was washing the skin until the pH reached a more neutral value (6-8), some basic molecules remain in the skin tissue, affecting the pH of the

final product[24]. This type of curing solution, which is used to extract gelatin from the skin, significantly affects the final pH of the resulting gelatin. In this study, the pH values ranged from 4.5-6.5, which are still less than those typical of gelatins derived from either HCl or NaOH[25,26].

A_w and moisture content: No significant differences ($p>0.05$) in the A_w value or the moisture content were observed for the three different types of cattle hides using the HCl and NaOH treatments. The A_w values of the gelatins ranged between 0.47 ± 0.22 and 0.51 ± 0.02 and the moisture contents ranged between 6.24 ± 0.79 and 7.48 ± 0.65% (Table 1). The factors that affected the A_w and moisture content of gelatin are thought to be the gelatin temperature during the production process and the storage methods. During this study, the temperature of the extraction and the storage methods had no impact on the gelatins. Said[15] studied the preparation of goat skin gelatin and found that the A_w and moisture content do not change when using acid (CH_3COOH, 0.5 M) or base [$Ca(OH)_2$, 100 g L^{-1}] at concentrations of 3, 6 or 9% for 2 or 4 days. In this research, the value of A_w using HCl tended to be higher than that obtained using NaOH. These results were similar to a study performed by Said[15], which investigated curing with CH_3COOH and $Ca(OH)_2$; these similarities may be due to the strong bonds between the water molecules and the hydroxyl (OH) groups or the carbonyl (C=O) groups in the raw materials under basic conditions or acidic conditions (CH_3COOH), respectively, ensuring that the free water content in the raw materials was larger, leading to higher A_w values. However, if base curing [$Ca(OH)_2$] is used, the interactions only occur between the water molecules and the hydroxyl groups (OH). These bonds are weak, so the free water content in the material is smaller, resulting in a lower A_w value. Rosli and Sarbon[27] explain that the moisture content in eel skin gelatin subjected to pretreatment with NaOH is 18.8%. The moisture contents of the gelatins from the three types of cattle hides with either HCl or NaOH curing were lower (6.62-7.48%) than those of GMIA[26], which had a maximum content of 16%, or ISO, which was 16%[25].

Fat content: The fat contents of the gelatins obtained from the hides of three different types of cattle when using HCl and NaOH treatments showed no significant differences ($p>0.05$) and the contents ranged from 0.20 ± 0.02 to 0.40 ± 0.11% (Table 1). The highest fat content (0.40 ± 0.11) was obtained from the OC hides with NaOH and the lowest fat content (0.20 ± 0.02%) was obtained from the Bali hides with HCl. According to the Indonesian National Standard (SNI), the fat content in gelatin cannot be more than 5%. The overall results of the study were consistent with the SNI. The low fat contents may be due to the longer treatment times, as the extraction temperature was constant. The extraction temperature affects the fat content in the resulting gelatin because the unsaturated fatty acids will oxidize and breakdown into shorter carbon chains. The fat molecules contain unsaturated fatty acid radicals, which are oxidized during heating, forming shorter carbon chains. In this research, the fat contents in the gelatin from the three types of cattle hides treated with NaOH tended to be higher than the contents obtained using HCl because acid curing results in greater cleavage of protein bonds. Thus, HCl will dissolve more of the protein that binds the fat molecules during the curing and neutralization processes and as a result, the fat particles will be removed along with the protein. Therefore, the fat content of the HCl-derived gelatin will be lower than that obtained via base curing, which cannot dissolve the protein as well. The study performed by Wulandari[28] used the shank skin from broilers aged 40 days and observed fat content levels that were higher than those from broilers aged 30 days. According to Muyongga *et al.*[12], the levels of fat found in Nile fish gelatin derived from adult fish and from young fish were not substantially different. The fat contents in the pig gelatins prepared in previous studies increased with increasing age of the pigs because the fat under the skin was more developed[29]. Rosli and Sarbon[27] observed that the fat content in eel skin gelatin is 0.34% if the skin was pretreated with 0.15% (w/v) sodium hydroxide. This gelatin is therefore considered fat-free because the fat content is <0.5%.

Protein content: The protein contents of the gelatins obtained from the hides of the three types of cattle using HCl and NaOH were highly significantly different ($p<0.01$). The gelatin with the highest protein content (83.45 ± 2.78%) was obtained from the Bali hide treated with HCl and the lowest protein content (72.39 ± 4.13%) was obtained from Madura hide treated with NaOH (Table 1). According to the International Organization for Standardization (ISO), the standard protein contents are 85-90 and 84-90%[25,26]. These results were obtained because the curing solution breaks down proteins during the 3-day soaking process. Choi and Regenstain[30] stated that the quality of the gelatin depends on the source of the raw materials used, the species or connective tissues used for extraction and the gelatin production methods used. Wang *et al.*[6] mentioned that the dissolution rate of collagen is affected by the curing material because the curing material will affect the amount of collagen dissolved and an increase in temperature will facilitate the extraction of collagen during dissolution and solubilization. Sompie[29]

showed that the protein content in gelatin from pig skin decreases with increasing concentration of the curing material (CH_3COOH at 2, 4 and 6%) because higher concentrations of acetic acid will hydrolyze stronger peptide bonds, resulting in less skin protein loss during the washing/neutralization process. Ulfah[24] added that curing in a high concentration of acid causes cleavage of hydrogen bonds and the excessive opening of the collagen structure, causing some amino acids to be extracted and separated from the collagen with the wash water, decreasing the protein content in the resulting gelatin. Rosli and Sarbon[27] explained that the protein content of gelatin derived from eel skin subjected to pretreatment with NaOH is 67.64%. On the other hand, Amiza *et al.*[31] said that gelatin derived from cobia (*Rachycentron canadum*) skin treated with acetic acid has a protein content of 89.7%, which is slightly higher than that of the analogous bovine gelatin (84.72%). These results indicate that cobia skin has a high percentage of crude protein, which is probably due to the higher protein content in the collagenous material in the fish skin itself.

Soluble protein: The contents of soluble protein in gelatins from the hides of three types of cattle using HCl and NaOH were highly significantly different ($p<0.01$). The highest soluble protein content (12.82 ± 0.39 mg mL^{-1}) was obtained from the OC hide treated with NaOH and the lowest soluble protein content (10.96 ± 0.83 mg mL^{-1}) was obtained from the Madura hide treated with HCl (Table 1). The use of NaOH produced a higher soluble protein content than that generated by HCl treatment. This difference was related to the nature and capabilities of each curing solution, which indicates that acid curing is better able than base curing to break the bonds of the amino acids that compose the cattle hides. Proteins are long chains of amino acids connected by peptide bonds and they can be broken down into amino acids or small peptides via hydrolysis of the CO-NH bonds. Jamhari[32] reported the soluble protein contents in gelatins from local meat protein sources, including Indonesian Bali beef at 14.00 mg mL^{-1}, "Kacang" goats at 17.13 mg mL^{-1}, native chickens at 18.20 mg mL^{-1} and native ducks at 12.07 mg mL^{-1}.

Viscosity: The viscosities of the gelatins derived from the hides of three types of cattle were highly significantly different ($p<0.01$) but among the individual cattle hides, the HCl and NaOH treatments caused no significant differences ($p>0.05$). The gelatin with the highest viscosity (7.17 ± 1.60 cP) was obtained from the Madura hide and the gelatin with the lowest viscosity (4.16 ± 0.81 cP) was obtained from the OC hide. The range of viscosities in the gelatins from the HCl and NaOH treatments was from 8.00 ± 1.73 to 3.67 ± 0.58 cP (Table 1). The viscosities of the gelatins from HCl treatment tended to be higher than that those obtained from NaOH treatment. HCl is better able to break down the peptide bonds of the longer peptide chains. According to Wulandari[28], the viscosity of the gelatin was influenced by its constituent MW and a higher MW led to a gelatin with a higher viscosity. In this study, the gelatin from the Madura cattle hide was more viscous than that from the OC cattle hide. The SNI set a gelatin viscosity of 2.0-7.5 cP, so the viscosity results obtained in this study were within the standard range. Leiner Davis Gelatin Co[33] explained that the increase in viscosity was driven by the molecular structure of the amino acids that make up the proteins in the gelatin; the composition of the amino acids could enhance the viscosity of the gelatin. Viscosity is a physical property that influences the properties of the gelatin gel, especially the gelation point and the melting point, producing a higher viscosity and a gelation melting rate higher than that of the low-viscosity gelatin. Wulandari[28] reported that a higher gelatin viscosity was observed from a broiler shank that was aged for 40 days than in that obtained from a shank aged for 30 days. Muyonga *et al.*[34] explained that the gelatin from an adult Nile perch fish has a higher viscosity than the gelatin from a young fish. The higher viscosities of gelatins from old animals are due to higher MWs. Schrieber and Gareis[1] added that the viscosity of the gelatin solution is related to the number of components that contribute to the MW of the gelatin. Higher viscosities are associated with gelatins with higher MWs and longer amino acid chains.

Gel strength (GS): The GS values of the gelatins derived from the hides of the three types of cattle and the HCl and NaOH treatments were highly significantly different ($p<0.01$). The highest GS (166.93 ± 8.44 Bloom) was obtained from the Madura hide treated with HCl and the lowest GS (42.26 ± 7.32 Bloom) was obtained from the Madura hide treated with NaOH (Table 1). The GS is a functional property of the gelatin and it is defined as the force required to produce a certain deformation. Gel formation occurs because of the development of gelatin molecules during heating[1]. The use of HCl tends to produce gelatins with higher GS values than those of gelatins derived from NaOH treatment. Arnesen and Gildberg[11] and Bhat and Karim[7] explained that the GS properties were associated with hydrogen bonds between water molecules and the free hydroxyl groups of the amino acids, with the protein chain size and with the concentration and MW distribution of the collagen. Kolodziejska *et al.*[35] added that the GS values are also influenced by the concentration and the curing time and that the GS is one of

the parameters that determines the physical quality of the gelatin product. According to the SNI, the standard GS values for gelatins range from 75-300 Bloom[25]. Binsi *et al.*[36] reported that the GS or Bloom value can be categorized as low (<150), medium (150-220), or high Bloom (220-300). In this study, the gelatins from the Madura and Bali cattle hides cured with base had very low GS values, i.e., 42.26-44.37 Bloom. The GS is related to the length of the amino acid chains and longer amino acid chains will give higher GS values[37]. Sompie[29] explained that the decline in GS was caused by the termination process, which involved the breaking of the amino acid polymer chains with increasing concentrations of curing acid; this process resulted in the breaking of bonds between the polymer molecules that make up the collagen, yielding very short and damaged monomers and reduced collagen formation (low GS value).

Color analysis: The lightness (L*), redness (a*) and yellowness (b*) are the parameters used to characterize color. This study showed that the type of cattle hide has no significant impact ($p>0.05$) on the L* and a* color parameters but the type of cattle hide highly significantly influenced ($p<0.01$) the b* color parameter (Table 2). The L* values ranged between 61.63±0.55 and 63.10±1.87, the a* values ranged between 13.97±0.23 and 14.77±0.32 and the b* values ranged between 18.70±0.82 and 22.60±1.11. The NaOH treatment had a greater influence than the HCl treatment on the L* parameter. The color change is caused by the browning reaction, or the Maillard reaction, that occurs during the production of gelatin, which involves extraction and oven-drying processes. The Maillard reaction causes darkening as a result of acids coming into contact with the C = O moieties in the gelatin and releasing amino acids[38,39]. The Maillard reaction, which occurs during the process of making gelatin, plays a role in the color, flavor, aroma and texture of the food and it impacts the levels of nutrients and harmful components[40,41]. Tyler and Gregory[42] added that the Maillard reaction can be caused by both the acidic and basic treatments. The availabilities of the various protein and carbohydrate components in the skin and the temperature during extraction can alter the rate of the Maillard reaction. Baynes *et al.*[40] and Lee[43] explained that the Maillard reaction is a nonenzymatic reaction that occurs because the aldehyde groups and carbohydrates react with the amino groups of proteins at high temperatures and produce very complex biological effects.

Molecular weight (MW): The MW distribution was determined by using SDS-PAGE. The gelatins produced from the three types of cattle hides with HCl had similar MW distributions (Fig. 1). The gelatins hydrolyzed by acid shows bands that indicate that the proteins in the gelatin have be hydrolyzed into simple peptides. The study showed that the gelatins from the Bali cattle hides treated with HCl (BA) and OC cattle hides treated with HCl (PA) each have 6 bands, with the smallest band being between 10 and 25 kDa. The gelatin from the Madura cattle hides treated with HCl (MA) produced approximately 4 bands, with the smallest having a MW of approximately 25 kDa. The degrees of hydrolysis of BA and PA were higher than the degree of hydrolysis of MA. However,

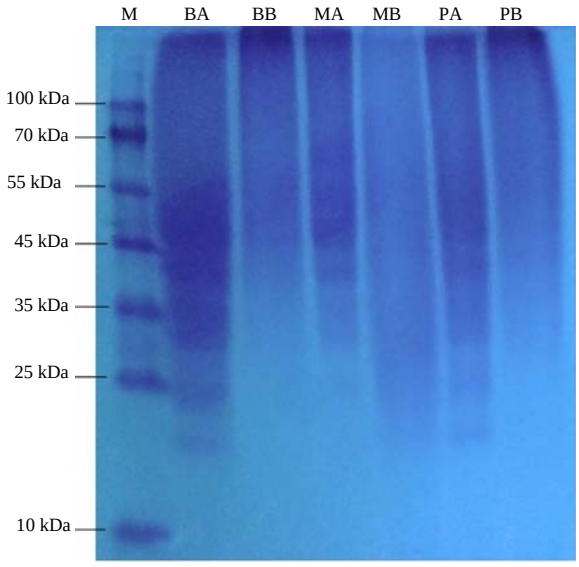

Fig. 1: Results of SDS-PAGE for determining the molecular weights

M: Marker, BA: Bali HCl, BB: Bali NaOH, MA: Madura HCl, MB: Madura NaOH, PA: OC HCl and PB: OC NaOH

Table 2: Color parameters (L*, a* and b*) of gelatins from Bali, Madura and OC cattle hides treated with HCl and NaOH

	Cattle hide			B		M		OC	
	B	M	OC	HCl	NaOH	HCl	NaOH	HCl	NaOH
L color	62.72±1.61	61.93±1.34	62.30±0.52	62.33±1.59	63.10±1.87	61.63±0.55	62.23±1.97	62.40±0.52	62.20±0.61
a* color	14.33±0.48	14.60±0.37	14.27±0.31	13.97±0.23	14.70±0.36	14.43±0.40	14.77±0.32	14.23±0.21	14.30±0.44
b* color	22.07±1.01[a]	20.87±1.19[b]	19.75±1.39[b]	21.53±0.68	22.60±1.11	21.30±1.30	20.43±1.15	18.70±0.82	20.80±0.95

Values with different letters ([a], [b] and [c]) in the same row are significantly different ($p<0.05$). All values are the mean±standard deviation from three replications

gelatin treated with NaOH shows a band that is not clear, nor are the bands able to provide information like what is obtained from the real bands that are characteristic of the Bali, Madura and OC cattle hides. Badii and Nazlin[44] explained that the MW distribution of the gelatin was closely associated with the long amino acid chains and the GS. The GS values of the Madura and OC hides treated with NaOH are very low (42.26±7.32 and 46.69±19.37 Bloom, respectively). When tested using SDS-PAGE, the gelatins produce a band that is not as clear as that of the gelatins obtained from the HCl treatment, showing a very high GS. A gelatin with a greater MW will have longer-chain amino acids and its GS level will be higher. Ali *et al.*[23] added that the components of goat skin gelatin pretreated with NaOH had MWs of 131, 125 and 216 kDa. These results indicated that the α- and β-chains were not degraded by the pretreatment conditions. On the other hand, curing with dilute acid or base affects the polypeptide chain structure, resulting in longer chains and a higher distribution of molecular weights[37]. However, if the curing treatment uses acids or bases at high concentrations, the polypeptide chains will breakdown, resulting in shorter chains and a lower distribution of MWs. The molecular weight distributions of various gelatins include the following: a MW of 100 kDa from Dover sole fish skin (*Solea vulgaris*), a MW in the range of 14-80 kDa from minke whale skin and a MW of 116 kDa from carp fish skin (*Cyprinuscarpio*)[6,45,46]. The MW of gelatin from acid-cured pig skin was in the range of 91-200 kDa and the MWs of goat skin-derived gelatin ranged from 45-102 kDa[15,29]. Azira *et al.*[47] explained that porcine skin gelatin exhibits a wider MW distribution than bovine skin gelatin and contains 10 prominent bands (approximately 125, 120, 114, 106, 96, 87, 76, 70, 64 and 58 kDa) rather than the 2 prominent bands (approximately 135 and 110 kDa) in bovine skin gelatin.

Profiling of the functional groups: The profiles of the functional groups in the gelatin were analyzed using FTIR spectroscopy. A functional group is a special group of atoms within a molecule that influences the characteristic chemical properties of that molecule. The characteristic bands of the common functional groups in the gelatin as determined by FTIR spectroscopy proved that the gelatin samples were true gelatins. According to Muyonga *et al.*[34], the absorption features typical of gelatin can be classified into 4 groups, i.e., the vibrational bands of amide A at 3600-2300 cm^{-1}, amide 1 at 1636-1661 cm^{-1}, amide II at 1560-1335 cm^{-1} and amide III at 1300-1200 cm^{-1}. The gelatin samples from the cattle hides has almost the same FTIR spectra. The amide A region shows peaks at 3564.19, 2935.44, 2360.70 and 2341.41 cm^{-1} (Fig. 2); these absorption peaks are caused by the presence of NH groups, CH groups, OH groups and C ≡ C groups, respectively. The amide I peak at 1631.66 cm^{-1} is attributed to C = C groups, the amide II peaks at 1531.37 and 1400.22 cm^{-1} are attributed to C = C groups and the amide III peaks at 1323.07 and 1234.35 cm^{-1} are attributed to C-C and C-O groups. Gelatin from the Bali hide treated with HCl has stronger absorbances (higher absorption intensities) than those of the other gelatins, while the gelatin from the OC cattle hides treated with HCl has

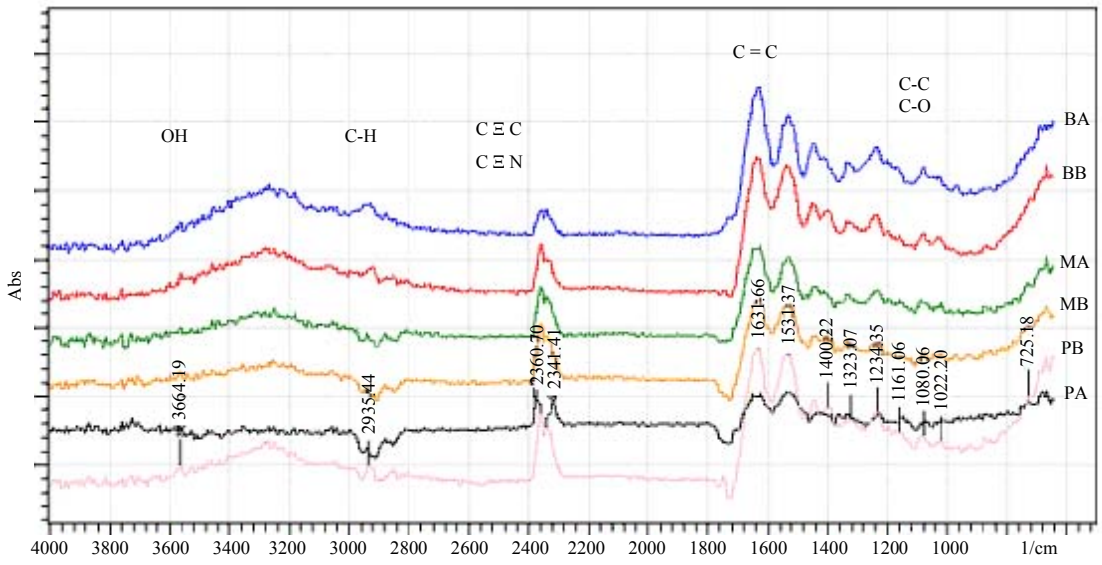

Fig. 2: FTIR spectra of gelatin samples from various breeds
BA: Bali HCl, BB: Bali NaOH, MA: Madura HCl, MB: Madura NaOH, PA: OC HCl and PB: OC NaOH

Table 3: Amino acid compositions (g/100 g) of gelatin samples

	Bali		Madura		OC	
Amino acid	HCl	NaOH	HCl	NaOH	HCl	NaOH
Asp	1.01	4.56	4.56	4.99	3.56	3.43
Ser	2.20	1.15	0.66	1.70	1.68	1.79
Glu	3.89	7.53	5.87	4.95	16.78	18.69
Gly	35.56	32.51	35.80	38.35	47.73	48.73
His	*	*	*	*	*	*
Arg	6.21	12.50	13.72	14.77	4.42	4.72
Thr	3.19	0.78	1.13	1.23	5.63	5.48
Ala	3.72	5.92	6.12	4.48	4.03	3.67
Pro	5.06	7.03	6.67	6.84	5.92	5.62
Cys	*	*	*	0.65	4.43	7.87
Tyr	0.29	0.45	0.37	0.53	0.47	0.58
Val	1.53	1.63	1.56	1.77	1.56	2.09
Met	0.35	0.54	0.50	0.59	0.43	0.48
Lys	1.38	1.24	1.58	1.70	2.65	2.14
Ile	0.70	0.95	0.81	0.98	1.23	1.33
Leu	1.46	2.99	2.77	2.86	3.10	3.24
Phe	1.39	2.81	2.85	3.16	1.97	1.49

*Not detected. Results obtained from duplicate readings

weaker absorption bands. Nurul and Sarbon[48] reported that eel (*Monopterus* sp.) skin gelatin treated with NaOH 0.15% (w/v) and bovine gelatin showed similar spectra, with some peaks in the amide I and II regions being slightly shifted; the amide I and II peaks in the spectrum of eel skin gelatin were observed at 1634.72 and 1538.65 cm^{-1}, respectively, while those for bovine gelatin were found at 1633.94 and 1538.95 cm^{-1}. Rohman[49] explains that compounds with the same functional groups tend to undergo the same chemical reactions. The presence of functional groups associated with the molecular structures of amino acids is also characteristic of prepared gelatin, which is composed of a number of amino acids. The amino acid units contain functional groups, the nature of which are likely influenced by the curing materials that are used. The band indicative of OH group is only seldom visible (at a wavelength of 3650-3200 cm^{-1}) in the obtained spectra compared to the intensities of the bands from other groups such as the band in the region of 3500-3000 cm^{-1} characteristic of N-H groups.

Amino acid profile: This study showed that the amino acid histidine was undetectable in the gelatins from the cattle hides treated with NaOH or HCl (Table 3). The amino acid cysteine was not detected in the Bali cattle hide gelatins prepared using HCl or NaOH, nor was it found in gelatin from Madura cattle hides treated with HCl. Only gelatin from the Madura cattle hides treated with NaOH showed a cysteine content at a level (0.65 g/100 g) lower than those of the gelatins from the OC cattle hides treated with HCl (4.43 g/100 g) or NaOH (7.87 g/100 g). The gelatins from the cattle hides treated using NaOH tends to show amino acid compositions greater than those of gelatins from the cattle hides treated with HCl. Said[15] reported goat skin gelatins prepared with acid curing (0.5 M CH_3COOH) or base curing ($Ca(OH)_2$ at 100 g L^{-1}). The highest concentrations of the amino acid glycine were observed in our gelatins; in particular, the glycine content in the gelatin from the OC cattle hides treated with NaOH was 48.73 g/100 g and the next most abundant amino acid was glutamic acid, which was present at 18.19 g/100 g. Arginine was observed in the Madura cattle hide gelatin prepared with NaOH. Charley[50] explained that the amino acid composition of the gelatin was nearly equal to that of the collagen. The amino acid glycine was a major amino acid and comprised 1/3 of all the amino acids in the collagen and the remainder of the amino acid content consisted of proline and hydroxyproline among others. Mulyani *et al.*[21] added that the GS can be attributed to the high contents of proline and hydroxyproline. On the other hand, the most abundant amino acid in cobia gelatin is glycine (Gly), which accounts for approximately 20.98% of the material, followed by proline (Pro) at 10.08% and hydroxyproline (Hyp) at 7.14%; however, the levels of all the amino acids except for Gly, Pro, Hyp, Tyr and Lys are higher in cobia gelatin than in bovine gelatin[31].

CONCLUSION

This study shows that the use of 0.25 M HCl was more effective and efficient than using 0.25 M NaOH in the production of gelatin from regional Indonesian cattle hides

(Bali, Madura and OC cattle) based on the physicochemical properties of the resulting gelatin. However, the amino acid content tended to be higher when using base for curing than when using HCl (0.25 M) for curing.

SIGNIFICANCE STATEMENT

This study discovered new sources of collagen material for gelatin production and the results in this study are also helpful for the production of acid- and base-derived collagen from cattle hides, which will help meet the high demand for gelatin production for both food and medical applications. This study will help researchers to uncover critical areas of optimization for collagen extraction using combinations of base and acid that very few researchers have applied to certain hides. Thus, the new methodology for gelatin extraction is quite meaningful.

ACKNOWLEDGMENTS

This study was supported by a grant from "Penelitian Hibah Disertasi Doktor (PDD)" 2017, Directorate General of Higher Education, Ministry of Research, Technology and Higher Education of the Republic of Indonesia. The manuscript was edited by Dr. Allan McGee and Brett Alcock from Massey University of New Zealand as part of the "Professional Development Short Course on Academic Writing Skill in English" Brawijaya University Programme on November 4-25, 2017 at the Massey University of New Zealand.

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