Extraction of Gelatin from Cattle Hides by Using Acetic Acid

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ABSTRACT

Gelatin is a natural substance made by partially hydrolyzing collagen-rich materials that are present in connective tissues, animal skin, and bones. The objective of this investigation was to ascertain the yield and physicochemical characteristics of gelatin that was extracted from cattle hides that had been exposed to varying concentrations of acetic acid. To attain the highest extraction efficiency, pretreatments are typically performed before the gelatin extraction procedure. Fresh hides of cattle were gathered from a nearby slaughterhouse. In this study, cattle hides treated with 1% acetic acid were categorized as AA-1 group, 3% acetic acid treated hides as AA-3 group, and 5% acetic acid treated hides as AA-5 group with three replicates in each group or treatment. A significant difference ($p<0.001$) in acetic acid concentration was observed in yield, gel strength/ Bloom, viscosity but did not differ significantly ($p>0.05$) for moisture, crude protein, crude fat, ash, pH and color (L*, a* and b* values) of the gelatin extracted from cattle hides. Yield of gelatin was highest in AA-5 group (16.98%), followed by AA-3 group (13.99%) and lowest in AA-1 group (11.57%). The highest bloom was observed in AA-3 group (205.52g), followed by AA-5 group (195.52g) and lowest in AA-1 group (154.90g). Bloom increased almost 25.00% in case of AA-3 group compared to AA-1 group. The physiochemical and technological properties of gelatin extracted from cattle hides indicate that high quality gelatin can be extracted from cattle hides after acid pretreatment for application in a variety of fields.

Keywords: Acetic acid, Amino acid, Bloom, Color, Viscosity

INTRODUCTION

Gelatin is a polymer and multipurpose component that is made by heat denaturing or controlled hydrolysis of collagen found in skin, bones, and connective tissue (Alipal et al., 2021). Bangladesh imports gelatin & derivatives, isinglass, glues is about 3716 USD from Brazil, China People'S Republic (P.R), India, Germany, Italy etc during 2022-2023 fiscal year (Dept. of Statistics, BB, 2023) which indicating a domestic deficit that could be addressed by valorizing local bovine hides. Gelatin extraction can be performed using hot water, enzymes, dilute acids, or alkalis. When heated up, collagen in connective tissues is partially hydrolyzed and solubilized to produce gelatin. Mammalian sources, such as pig skin and cowhides, account for 46% of global gelatin production, followed by bones (23%), hooves (29%), and marine sources (1%) (Rakhmanova et al., 2018). According to reports, gelatin contains all amino acids except tryptophan (Duconseille et al., 2015). But its composition and sequence vary from source to source. Gelatin has multiple functional qualities, including rheology (Santana et al., 2020), foaming and stabilizing capacities (Chakka et al., 2017), bioactive properties (Said and Sarbon, 2022), fat replacing properties (Almeida et al., 2016), and film-forming properties. Despite its unique functional and technological properties, it is used as a gelling, emulsifier, and thickener for many goods in the food sector, such as bakery items, sweets, ice cream, and meat products (Zhang et al., 2020) as well as in a wide variety of other industries. Skins and hides from meat animals such as cattle, sheep, and goats

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are valuable byproducts of slaughterhouses that are primarily used to produce leather (Jayathilakan et al., 2012). Most of the hides and skins that dealers in Bangladesh gather originate from rural families and butchers, similar to other developing countries without access to slaughterhouses (Brautigam et al., 2018). In turn, traders sell these hides to leather tanning companies, who process them, starting with pickling and ending with finishing. It might be that there are many alternatives to leather on the international market (Patel et al., 2022) which might decrease the demand and price of hides domestically. In consequence, hides no longer appear to be in high demand for leather production. Nowadays, even farmers or butchers cannot sell cattle hides to traders, so they bury them or send them to landfills. This ultimately increases environmental pollution. A boost in the market price and commercial value of these naturally nutritionally rich hides is imperative to prevent environmental pollution and ensure their commercial viability. Additionally, cow hides are not only rich in nutrients (crude protein = 33%, crude fat = 2 to 6%, ash = 0.5%, and water = 65% (Honig et al., 2022) and also very rich in collagen which is the parent molecule for gelatin (Ashokkumar and Ajayan, 2021). Due to the high collagen content in hides, they can be an excellent source of gelatin extraction (Roy et al., 2021).

It is believed that it requires alkali pre-treatment to produce gelatin from bovine sources to obtain gelatin (Rabiatul et al., 2020). But in a recent study, it was shown that gelatin can be extracted from cattle hides after pretreatment with acetic acid (Roy et al., 2022). Acetic acid is generally used in many food products for processing and preservation. Due to this, it has become increasingly imperative to use naturally occurring cattle hides for gelatin production. In light of recent trends of lower prices for cow hides due to alternative leather and to find a source, which could be a Halal option for Muslims, this study aimed at utilizing cattle hides for gelatin extraction and characterizing the extracted gelatin for possible applications in the future.

MATERIALS AND METHODS

Laboratory activities were performed at Meat Processing Laboratory, Animal Production Research Division, Bangladesh Livestock Research Institute, Savar, Dhaka.

Cattle hides collection

Fresh hides from 2.5-3 years old cattle were purchased from a slaughter house in Savar, Dhaka and transported to the laboratory immediately on ice for processing. Upon receiving in the laboratory, the hides were cut into small pieces and soaked with cold tap water for 1-2 hours and finally wash the hides with tap water to remove manure, mud, blood, and other things stuck to the hair sides. To remove fat and meat from hides, the flesh side was cleaned with a blunt knife. They were then sliced into small 5 x 5 cm^2 pieces. Finally, the small cattle hide pieces were put on a metal sieve to remove loose water and then frozen and stored at -20˚C until used.

Pre-treatments of the cattle hides

Frozen cattle hides pieces were thawed at 4˚C for 16 hours and loose water was drained. The designated hides pieces were subjected to three different treatments (1%, 3% & 5% acetic acid namely AA-1, AA-3 & AA-5, respectively at a 1:10 (w/v) ratio and treated at ambient temperature with vigorous stirring for 24 hours. After being treated with acetic acid, to get rid of reactants, distilled water was used to wash the hide pieces. The hides were cleaned on both sides to get rid of any remaining meat or fat and hair. The hide pieces were subsequently washed with distilled water and kept at -20°C until the gelatin was extracted.

Gelatin extraction

The post-treatment frozen hide pieces were thawed at 4˚C. The designated mass of hides pieces was weighed, recorded the weight and submerged at a 1:5 (w/v) ratio in distilled water in a glass beaker and then heated in a hot water bath (WNB 14, F-Nr.: L412.0806, Memmert, Germany) for five hours at 80˚C. The soluble fraction (extracted gelatin) was collected in another beaker after being filtered through a 4 folds of cheese cloth. In order to concentrate the extracted gelatin, filtrates were heated at 60˚C for five hours in a water bath, cooled, frozen at –20˚C and finally freeze-dried in a freeze dryer (CHRIST, Alpha 2-4 LSC basic, Germany). The freeze-dried gelatin weight was recorded for yield calculation and stored in a sealed plastic jar for further characterization.

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Gelatin quality determination

Gelatin yield: The yield was calculated on wet basis by using the following equation-

$$\text{Gelatin Yield (\%, wet basis)} = \frac{\text{Weight of the freeze dried gelatin}}{\text{Weight of wet hides used for gelatin extraction}} \times 100$$

Determination of pH

pH was determined using a method adapted from (BSI, 1975). Deionized (DI) water was used to prepare a 2% (w/v) gelatin solution, which was then heated to 40˚C for 20 minutes and allowed to cool to room temperature. After calibrating the pH meter using standard buffers of pH 4.0 and 7.0 (Hanna Instrument 98107, v1.04, Romannia), the pH was measured using a glass electrode.

Determination of proximate components

AOAC methods (AOAC, 2005) were used to quantify moisture, ash, and fat content in freeze-dried gelatin. The amount of protein content of extracted gelatin was determined by the Kjeldahl method by estimating total nitrogen.

Color measurement

A Konica Minolta Chroma Meter CR-410 colorimeter was used to measure the color of the 6.67 percent gelatin gel following gel maturation. A standard white calibration plate with an aperture of 8 mm and illumination of D65, supplied by the manufacturer, was used to calibrate the colorimeter. The Commission International de l'eclairage (CIE) supplied the coordinates for describing colors, which were as follows: L* (lightness), a* (redness/greenness), and b* (yellowness/blueness). Three separate measurements of the gelatin's color were made, and the means were computed for statistical analysis.

Gel strength

The gel strength was measured using a slightly modified version of the procedure (BSI, 1975) (Bloom). Using a glass rod, 7.50 g of freeze-dried gelatin and 105 mL of DI water were combined in a standard Bloom jar (Schott, Mainz, Germany) to create a 6.67 percent gelatin gel. For three hours, the gelatin bottle covered with aluminium foil was left at room temperature to absorb the water and swell. To ensure complete dissolution, bottles were submerged in a 45˚C water bath for 20 minutes while shaking periodically. The bottles were allowed to cool at ambient temperature for 15 minutes before being refrigerated at 10˚C for 16–18 hours to allow the gel to mature. A TA-XT2 Texture Analyzer (Stable Micro Systems, Surrey, UK) equipped with a 2 kN load cell was used to evaluate the gel strength. At a maximum force (g) and a penetration distance of 4 mm, an absolute bloom strength measurement is performed at a speed of 0.5 mm/sec. Three separate measurements of gel strength were made, and the means were analyzed statistically.

Viscosity

Viscosity of gelatin samples was measured by following (AOAC, 2000). For this analysis, a gelatin solution (6.67 %; w/v) was made for gel strength measurement was prepared by heating in a temperature-controlled water bath for 30 min at 60˚C. Finally, the viscosity of a 20 ml gelatin solution was measured three times with a Brookfield Viscometer (Model LV-DV-II, Brookfield Engineering, MA, USA) at room temperature and expressed in cP. The mean of the three readings was used for statistical analysis.

Amino acid composition

The amino acid composition of the samples was determined by acid hydrolysis method (SYKAM S433 Amino Acid Analyzer). At first, about 200 mg gelatin samples were taken. Then dissolved in 500 milliliters of hydrolysis solution (300 milliliters of 37% HCL, 200 milliliters of DI water, and 0.5 grams of phenol). After soaking and mixing samples, the resultant was stored at 120 ℃ for 24 hours. Then hydrolysate was cooled and the pH adjusted within the range of 2.9 to 3.1. After adjusting the pH, the samples volume was adjusted to 250 ml. From this 250 ml sample stock, 100 µl of samples was taken from each which was being filtered by 0.45

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µM syringe filter (Syringe filter, labeled, CHROMAFIL Xtra PTFE-45/25, Germany). With this 100 µl of samples, 900 µl of sample dilution buffer (Na-acetate buffer, pH 2.9-3.1) was added and ready for run. Two types of columns were used: (a) pre-column for trapping ammonia (LCA K04/Na 4.6×150 mm, Sykam, GmbH, Eresing, Germany) and (b) Post column for amino acid separation (LCA K04/Na 4.6×100 mm, Sykam, GmbH, Eresing, Germany). Using an auto sampler, 100 µL of sample was injected into the system. After that, the sample moved through the reaction chamber and interacted with the ninhydrin reagent. Two distinct buffers with varied pH values served as the baseline for the amino acid isolation process, one with acidic pH of 1.9-3.1 and the other with basic pH of 10.50-11.85.

Melting point

Gelatin melting point in the study was determined based on (Muyonga et al., 2004). Test tubes with thin walls (12 mm × 75 mm) screw caps were used to create solutions with 6.67 percent (w/v) gelatin. After filling the test tubes to a certain headspace, they were sealed. After 16–18 hours of being kept in a refrigerator (7˚C), the dissolved samples were moved into a water bath (10˚C) and inverted such that the headspace was at the bottom. At intervals of roughly 60 seconds, heated (45˚C) water was added to the water bath to gradually warm it up (approximately 1˚C each minute).

The melting point was determined by measuring the temperature at which the gel melted and the gas in the headspace began to rise.

Setting point

Setting point and setting time of gelatin were determined by the method (Muyonga et al., 2004). 0% (w/v) gelatin solutions dissolved in thin wall (12 mm × 75 mm) test tubes were used to calculate the setting point and time. To give the gelatin time to absorb water and swell, the mixture was stirred and left to stand at room temperature for half an hour. After dissolving in the warm water bath, the samples were moved to a second water bath that was kept at 40˚C. After that, chilled water (-2˚C) was added to the bath at 15-second intervals to gradually cool it down. At intervals of 15 seconds, a thermometer was placed within the sample and removed. The setting temperature was determined by measuring the mixture's temperature at which the gelatin solution stopped dripping from the thermometer's tip.

Statistical analysis

Data were analyzed using SPSS (IBM SPSS Statistics, version XX). Prior to analysis, normality of data within treatments was assessed using the Shapiro–Wilk test, and homogeneity of variances among treatments was evaluated using Levene's test. Variables meeting these assumptions were analyzed using one-way analysis of variance (ANOVA) with treatment as the fixed factor. When minor deviations from normality were detected, ANOVA was applied due to equal sample size and homogeneous variances. Differences among treatment means were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Prior to statistical analysis, data were evaluated for normality and homogeneity of variance. Shapiro Wilk tests indicated that yield, crude protein, crude fiber, ash, pH, bloom strength, viscosity, and color attributes (L*, a*, b*) were normally distributed across treatments ($p > 0.05$), whereas moisture content in Treatment 1 showed a slight deviation from normality. However, Levene's test confirmed homogeneity of variances for all measured parameters ($p > 0.05$), indicating that treatment comparisons were statistically valid. Given the equal sample size among treatments and the robustness of ANOVA to minor departures from normality, one-way ANOVA was considered appropriate for evaluating treatment effects. These results suggest that the observed differences among treatments reflect true treatment responses rather than violations of statistical assumptions.

Gelatin yield

One of the most crucial factors in the manufacturing of gelatin is yield, which is also unquestionably significant for large-scale industrial production. Highest yields indicate that the pretreatment for gelatin

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conversion from collagen was economical and successful. However, pre-treatments and hide quality (species, age, and sections of the hides utilized) may affect the amount of gelatin produced. The production of gelatin increases with the concentration of acetic acid. It was found that AA-5 had the highest gelatin production, followed by AA-3 and AA-1, which had the lowest (Table 1). In 2002, Jamilah and Harvinder stated that incomplete hydrolysis of collagen may contribute to low gelatin yields due to collagen crosslinking via covalent bonds, whereas in 2021, Roy said that incomplete hydrolysis could decrease the amount of extractable gelatin since cattle in Bangladesh are generally slaughtered when they are older than 2 years old, and older animals have higher levels of trivalent collagen crosslinks (Roy et al., 2015). Table 1 shows that pretreatments with varying level of acetic acid significantly affects gelatin production which ranged from 11.57% to 16.98%. Increasing level of acetic acid means a rise in H^+ ions that accelerate the production of gelatin through the hydrolysis of collagen molecules (Zhou and Joe, 2006). The gelatin production for 0.1 M and 0.3 M acetic acid was 5.95 percent and 9.2 percent, respectively (Wulandari et al., 2016).

Table 1: Physiochemical properties of extracted gelatin from bovine hides

Parameter		**Treatments**			**Level of significance**
		(AA-1)	**(AA-3)**	**(AA-5)**	
n		3	3	3	
Gelatin yield (%; wet basis)		11.57 ± 0.54^{c}	13.99 ± 0.37^{b}	16.98 ± 0.12^{a}	***
Moisture content (%)		8.51 ± 2.84^{a}	10.72 ± 0.14^{a}	12.10 ± 0.11^{a}	NS
CP (%)		95.97 ± 0.76^{a}	97.38 ± 0.09^{a}	96.49 ± 0.26^{a}	NS
CF (%)		0.19 ± 0.04^{a}	0.17 ± 0.05^{a}	0.22 ± 0.03^{a}	NS
Ash (%)		0.59 ± 0.05^{a}	0.69 ± 0.03^{a}	0.63 ± 0.07^{a}	NS
pH		4.25 ± 0.13^{a}	4.17 ± 0.25^{a}	4.12 ± 0.15^{a}	NS
Bloom (g)		154.90 ± 0.62^{c}	205.52 ± 0.90^{a}	195.52 ± 0.32^{b}	***
Viscosity (cP)		6.02 ± 0.05^{c}	7.04 ± 0.02^{a}	6.77 ± 0.07^{b}	***
Color	L*	43.64 ± 0.27^{a}	42.00 ± 0.42^{b}	42.36 ± 0.27^{b}	*
	a*	1.44 ± 0.03^{b}	0.96 ± 0.01^{c}	1.61 ± 0.03^{a}	***
	b*	10.48 ± 0.04^{b}	12.08 ± 0.05^{a}	9.68 ± 0.08^{c}	***

Values are mean ± SE, AA-1 = Hides pretreated with 1% acetic acid; AA-3 = Hides pretreated with 3% acetic acid; AA-5 = Hides pretreated with 5% acetic acid, Cp= Centipoise, *** = $p < 0.001$, * = $p < 0.05$, NS = Non-significant

Proximate composition and pH

The proximate composition and pH of the recovered gelatin were not significantly impacted by the variation in acetic acid concentration throughout the hides' processing (Table 1). According to Balti et al. (2011), food-grade bovine gelatin has 90.22% protein, 8.52% moisture, 0.21% fat, and 0.29% ash, while for commercial bovine skin gelatin contains 95.86 % protein, 7.44 % moisture, 1.16 % ash and 0.24 % fat (See et al., 2010). In spite of the acetic acid concentration used for pretreatment, the extracted gelatins had less moisture than the prescribed 15% limit (GME, 2020). Ash contents in extracted gelatin ranged between 0.59 and 0.69 %, significantly lower than the 2% threshold advised for food applications (GME, 2020). Chemical compositions of gelatin obtained in this study are comparable to those of commercial gelatin, which contains crude protein (CP) 90.22%, crude fat (CF) 0.21%, and ash 0.29 % (GMIA, 2019). Results indicate that gelatin derived from bovine hides that has been pretreated with acetic acid can be used in many different foods applications as proximate compositions of commercially acceptable quality. Since the pH of gelatin can influence its qualities and determine how it will be used later, it is extremely important for its chemical properties. Statistical analysis indicated that the value of pH of extracted gelatin did not affect by acetic acid concentration (Table 1). According to the pH of the extracted gelatins (Table 1), they were Type B. How closely gelatin relates to its isoelectric point (pI) determines how it functions. According to Schrieber and Gareis (2007), pH is the point at which an amphoteric molecule's net charge is zero and its solubility is at its lowest.

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Gel strength

Gelatin's gel strength is a crucial aspect of its physical characteristics. The production of gelatin molecules during heating is the cause of gel strength formation (Ulfah, 2011 & Said et al., 2011). Heat causes the gelatin molecules' connections to break, trapping free-flowing liquids inside the structure and creating a thick gel. The gel strength was significantly impacted by variations in acetic acid content. Table 1 displays the gel strength of various gelatin gels produced under various conditions. The gelatin from AA-3 had the highest mean gel strength among the various treatments, followed by AA-5 and AA-1. The gelatin strength of AA-3 was higher than that of AA-5, which indicates that higher concentrations of acetic acid increase yield but decrease gelatin strength. According to Boran and Regenstein (2010), gel strength is essential for recognizing gelatins in the gelatin sector. Gelatin quality is divided into three categories based on its gel strength, or bloom value: low (150 g), medium (151-220 g), and high (225-300 g). Water molecules and the free hydroxyl groups of amino acids in gelatin form a three-dimensional hydrogen bond, which gives the protein its strength and stiffness (Karim and Bhat 2008). Gelling points and the amino acid concentration of gelatin are correlated based on the ratio of proline to hydroxyproline in the original collagen molecule (Ninan et al., 2011 & Rawdkuen et al., 2013). At pH 3-5, the bloom in bovine skin gelatin varied between 193.49g and 251.03g (Raja Mohd Hafidz et al., 2011). The gel strength of hides gelatin ranged from 154.9 to 205.52 g bloom, which is consistent with the ISO 75-300 g bloom requirements (Said et al., 2011). The gel strength of the gelatin obtained in this investigation under various treatments shows that the gel is robust, pliable, and not easily broken.

Viscosity

Viscosity is essentially the amount of friction or drag within a fluid that affects its flow (Andarwulan et al., 2011). Viscosity differ significantly among the treatment groups. The average values of extracted gelatin viscosity ranged from 6.02cP-7.04cP, and were significantly higher in AA-3, followed by AA-1 and AA-5 (Table 1). The increase may have been caused by higher concentrations of H+ ions in collagen molecules breaking peptide bonds. The level of acetic acid used in this study was high enough to break these bonds completely and produce an acceptable viscosity range. This finding is comparable to the Alaska Pollock skin gelatin values (Zhou and Regenstein, 2006). The range of these results is 1.56 to 6.62 cP, contingent on the extraction method used. Gelatin derived from the skin of silver carp also had similar outcomes (Boran and Regenstein, 2009). It is believed that gelatin's viscosity is an indicator of its quality, and gelatin with a high viscosity indicates superior quality gelatin. The viscosity of gelatin rises with its gelling temperature, melting point, and gel strength—all of which are markers of superior quality (Ratnasari et al., 2013).

Color

Instrumented color measurements of gelatins are shown in Table 1. Depending on its intended application, color is important for gelatin's aesthetic qualities, but it has no bearing on its practical qualities (Lassoued et al., 2014). However, gelatin's light color is favoured because it makes it easy to assimilate into food systems without giving them any noticeable colur. The basic ingredients and extraction conditions have an impact on the colour of gelatin. Gelatin extracted from cow skin using varying concentrations of the pepsin enzyme showed L*, a*, and b* values ranging between 47.57-68.19, 1.10-2.19, and 5.65-12.12, respectively (Ahmad et al., 2021). These values are similar to the ones found in this investigation. In Table 1, AA-1's L* value or lightness value is noticeably higher than AA-3's and AA-5's. The AA-5 has the greatest a* value that means higher redness, followed by the AA-1 and the AA-3. AA-5 had the lowest b* value that means less yellow than AA-3 and AA-1 treatment group. These results showed that extraction conditions influenced the color of extracted gelatin. When gelatin was recovered from zebra blenny skin using crude acidic protease, the b* value was 13.97, indicating a light-yellow color (Ktari et al., 2014). Given that the b* values of the several gelatin samples ranged from 9.68 to 12.08, it may be claimed that the samples had a pale-yellow hue. For many foods, this color is appropriate because it won't give the finished product a prominent color.

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Amino acid composition

Table 2: Composition of extracted gelatin's amino acids

Amino acids (%)	AA-1	AA-3	AA-5
Aspartic acid	7.2	7.37	7.44
Threonine	2.17	2.24	2.23
Serine	4.1	4.21	4.18
Glutamic acid	12.25	12.74	12.92
Glycine	24.88	23.03	22.86
Alanine	10.68	10.95	11.02
Cystine	0.79	0.52	0.55
Valine	1.48	1.52	1.48
Methionine	0.99	0.82	0.93
Isoleucine	0.93	1.05	1.02
Leucine	3.03	3.14	3.19
Tyrosine	0.95	0.85	0.26
Phenylalanine	2.52	2.56	2.5
Histidine	1.07	0.95	0.99
Lysine	3.94	4.08	4.13
Arginine	8.57	9.07	9.24
Proline	14.45	14.9	15.06
Total	100	100	100

*Results obtained from duplicate readings

The amino acid makeup of gelatin is essentially the same as that of collagen because gelatin is generated from collagen. In particular, amino acids are essential for estimating the viscosity and gel strength of gelatin. High viscosity and gel strength are correlated with gelatin's high amino acid concentration. Table 2 displays the amino acid contents of gelatins derived from bovine hide. Gelatin from AA-5 had the highest mean total amino acid content among the various treatments, followed by AA-3 and AA-1. Compared to gelatins from cattle hides treated with AA-3 and AA-5, the gelatins from cattle hides treated with AA-1 often have lower amino acid contents. The main amino acids were alanine, glutamic acid, proline, and glycine (Table 2). According to GMIA, commercial gelatin contain glycine: ~160–230 mg/g, Proline: ~80–115 mg/g, Glutamic acid: ~70–110 mg/g, Arginine: ~50–70 mg/g, Alanine: ~50–85 mg/g. Additionally, Pranoto et al. (2016) reported a similar outcome. The gelatin from calf skins treated with AA-3 had 191.872 mg/g of glycine. A significant component of gelatin, glycine made up one-third of the amino acids found in collagen (Amertaningtyas et al., 2019). The second most prevalent amino acid in our investigation, glutamic acid, was found in 108.108 mg/g of gelatin from calf hides treated with AA-5. None of the three samples contained any detectable tryptophan. When synthesizing amino acid monomers from gelatin, tryptophan's indole ring is destroyed by acid hydrolysis (Shyni et al., 2014). By encouraging and stabilising the production of triple-helical structures, higher amounts of proline, hydroxyproline, and alanine in gelatin raise the viscoelasticity of the gelatin solution (Sarbon et al., 2013).

Melting temperature and setting temperature

Table 3: Melting and setting temperature of extracted gelatin

Parameter	Treatments			
	AA-1	AA-3	AA-5	Level of significance
Melting temperature (°C)	25.67 ± 0.22^{b}	27.93 ± 0.09^{a}	27.67 ± 0.28^{a}	***
Setting temperature (°C)	17.70 ± 0.25^{b}	21.53 ± 0.72^{a}	19.07 ± 0.09^{b}	**

Values are mean ± SE

AA-1 = Hides pretreated with 1% acetic acid; AA-3 = Hides pretreated with 3% acetic acid; AA-5 = Hides pretreated with 5% acetic acid The setting and melting temperatures of gelatin vary depending on the species utilized as the raw material, which may have varying habitat temperatures and life conditions. The study found that the AA-1 treatment group's gelatin melting temperatures were lower than those of the AA-2 and AA-3 treatment groups, being 25.67˚C, 27.93˚C, and 27.67˚C, respectively. In relation to viscosity and gelatin bloom strength, the melting temperature of gelatin is the temperature at which the gel softens and permits the carbon tetrachloride to drop. This indicates that AA-1 has lower viscosity and bloom strength than AA-2 and AA-3 (Table 1), which caused the melting temperature to be low. A higher melting temperature may cause the gel to stay in place longer, improving the mouth feel of the finished product. Compared to the AA-2 and AA-3 treatment groups, the AA-1 treatment group's setting temperature was lower (Table 3). The gelling point of gelatin was affected by pretreatment conditions before gelatin extraction (Mad-Ali et al., 2016).

CONCLUSION

Considering physiochemical properties of gelatin extracted from hides, it is concluded that high quality gelatin can be extracted after acid pretreatment and was found better for AA-3 treatment group. This Halal gelatin can be utilized in a variety of food applications and it is crucial for Muslims and other consumers seeking ethically produced products.

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Conflicts Of Interest

The authors declare that they have no competing interests in publishing this article.

Author’s Contribution

B.K.R. designed, supervised the research, data analysis and reviewed the manuscript; S.A.T. designed the study, performed the work, accomplished the data analysis and wrote the manuscript as well; B.C.R. Writing, literature search, review & editing, M.M.B. & A.A.D wrote, review and editing the manuscript. All authors read and approved the final manuscript.

Conflicts Of Interest

The authors declare that they have no competing interests in publishing this article.

Ethical Consideration

Ethical approval not applicable as samples (fresh cattle hides) were collected from a local slaughter house.

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