Effects of dehairing treatment on gelatin yield and quality from bovine hides

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Research Article

Keywords: Gelatin, Bovine hides, Gel strength, Amino acid, Emulsifying properties

Posted Date: April 28th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-392199/v1

Version of Record: A version of this preprint was published at Waste and Biomass Valorization on September 22nd, 2021. See the published version at https://doi.org/10.1007/s12649-021-01582-z.

Abstract

Purpose Hides are the by product of slaughter houses which are mostly used for leather production. In Canada, the hides are either disposed of with other slaughter waste or sold at a very low price. Dehairing of hides is a prerequisite for either leather or gelatin production from it. Therefore, the effect of hide dehairing method on subsequent gelatin extraction and quality was investigated.

Methods Bovine hides (BH) were dehaired using either 5% acetic acid (AA), 10% calcium hydroxide (CH), 0.02% keratinase (KTN), 2.5% papain (PP), or not at all (control; CT), with control BH subsequently treated with 5% AA (CTAA).

Results Mean bovine hide gelatin (BHG) yields (dry basis) were 11.37%, 54.25%, 45.07%, 18.88% and 55.02% for CT, AA, CTAA, CH, and KTN, respectively. Gel strength was highest in AA followed by the CTAA, CT and CH and KTN treatments. The molecular weight (MW) distribution pattern showed that dehairing of BH with enzymes degraded the collagen extensively, increased proportions of low MW peptides that translated into low gel strength.

Conclusions Acetic acid, which is extensively using in food industry, can be used to dehair BH as pre-treatment to extracting high quality gelatin.

Statement Of Novelty

To our knowledge, there is no study has been carried out on dehairing pre-treatment effect on bovine hides for gelatin extraction. The novelty further lies in the application of acetic acid, which is commonly used in the food industry, is an environmentally–friendly chemical for dehairing bovine hides for gelatin extraction and maintaining extracted gelatin quality.

Introduction

Canada is one of the largest producers of beef in the world. In Canada, the hides of cattle are either disposed of with other slaughter waste or sold at a very low price ($ 8–10/per hide). Bovine hide (BH) is rich in collagen and is used as a source material in gelatin production. Generally, gelatin is produced by the controlled hydrolysis of its parent molecule collagen, and extraction of gelatin from BH first requires the hair to be removed. A pre–treatment is always performed on the raw material either with acid or alkali. The pre–treatment is useful for removing non-collagenous substances as well as dehairing so as to obtain a high gelatin yield. The pre–treatment process mainly aims to maximize the yield without compromising the desirable properties of the extracted gelatin.

Dehairing and preparation of BH for gelatin extraction is a chemically-intensive process that involves a lime or lime sulfide bath. For this, hide is generally soaked in 10% hydrated lime with 1.5% sodium sulfide at a minimum pH of 12 for about 40 days. The hides are then acidulated in 0.1M sulfuric acid at pH 1.8 for 2–3 days and washed with water [1]. After removal of the hair from the hide, it is ready for gelatin extraction using heat [2].

Research has focused on eliminating lime sulfide from the process of dehairing ultimately to create a safe working environment for workers and to remove the risk of environmental pollution. Others have used bacterial keratinases [3] which do not degrade collagen, thereby preserving the length and integrity of the collagen α–chains [4]. The enzyme papain, which has both collagenolytic and keratinolytic activities, has been shown to have a depilatory effect [5]. Acid pre–treatment is invariably used for raw materials (pig skin, and fish skin and bone) that have fewer inter or intra–molecular collagen cross–links to extract gelatin [6, 7], but it is not usually used in the preparation of raw materials from bovine animals slaughtered close to maturity which have collagen cross-links that are resistant to acid solubilization. For BH, usually an alkali pre–treatment is used to extract gelatin but due to genetic improvement, cattle are now slaughtered at young ages (< 30 months old) and so acid pre–treatment may be a viable option for gelatin extraction.

Shortening the time frame for dehairing of the BH would also serve to increase product throughput, although it may compromise gelatin yield as lengthy conditioning of BH with lime sulfide is considered important for maximizing gelatin yield [8]. Gelatin quality may also be negatively impacted by alternative dehairing methods, particularly those that are enzymatic and simultaneously collagenolytic. Consequently, a preliminary, proof of concept study focusing on short-term dehairing and its impact on subsequent gelatin yield and quality was conducted to preliminarily test the efficacy of enzymes for dehairing BH. This preliminary study tested the hypotheses 1) that papain is comparable to keratinases, calcium hydroxide and acetic acid for dehairing of BH and increasing gelatin yield; 2) that acetic acid is as efficacious as calcium hydroxide for dehairing; and 3) that subsequent extraction with acetic acid following heat extraction of BH for gelatin increased gelatin yield without reducing gelatin quality.

Materials And Methods

Collection of bovine hides

Five fresh BH from cattle < 30 months old were collected from a local abattoir and stored at –20°C until used.

Experimental design and sample handling

Each frozen BH was thawed at 4°C and divided into a left and right half. The five areas were numbered as R1, R2, R3, R4 and R5 from cranial to caudal (Fig. 1). For randomization, R1, R2, R3, R4 and R5 locations from the five different BH were randomly assigned to different treatments (Table 1) for dehairing and gelatin extraction in a manner balanced for hide area. The selected parts of BH were soaked in and washed with water to remove blood and extraneous materials. The flesh side of the hide was scrubbed to remove fat and meat and cut into squares of approximately 5 × 5 cm^2.

Dehairing treatments

BH pieces were incubated in the randomly assigned dehairing solvents at a ratio of 1:10 (w/v) with vigorous stirring for 24h at room temperature (23°C) to maintain same condition for all treatments. The BH pieces were incubated in deionized (DI) water (negative control) (CT), 10% calcium hydroxide (pH 12–13) (positive control) (CH), 5% acetic acid (AA), 0.02% aqueous keratinase enzyme (from *Bacillus licheniformis*, Wako Chemicals Inc) (pH 8–9) (KTN), and 2.5% (w/v) aqueous papain enzyme (papain from papaya latex, Sigma) (pH 8–9)

(PP). A sixth treatment evolved from the CT as the BH of the CT group after gelatin extraction was subsequently incubated with 5% acetic acid and then used for gelatin extraction (CTAA).

After dehairing treatment, the BH pieces were washed with DI water to remove reactants and both sides of the hide were scrubbed to remove hair and any residual flesh or fat. Fifteen (15) fields of 1.1 × 1.4cm^2 on the hide surface of each BH piece were randomly photographed and total hairs were counted for each field. Means of the 15 fields were used for statistical analysis. The BH pieces were then stored at –20°C until gelatin extraction.

Gelatin extraction

The frozen, post-treatment BH pieces were thawed at 4°C. The BH pieces were immersed in DI water at a ratio of 1:5 (w/v) and heated at 80°C for 4h. The soluble fraction (extracted gelatin) was filtered through 4 folds of cheesecloth, cooled in ice water, dialyzed against DI water until the conductivity was lower than 50 µSiemens, frozen and freeze-dried.

The yield of gelatin was calculated as follows:

$$\text{Gelatin yield (\%) Wet tissue basis} = \frac{\text{Weight of freeze–dried gelatin (g)}}{\text{Weight of wet hides used for gelatin extraction (g)}} \times 100$$

$$\text{Gelatin yield (\%) Dry tissue basis} = \frac{\text{Weight of freeze–dried gelatin (g)}}{\text{Weight of wet hides used for gelatin extraction (g)} \times \text{dry matter in wet hides (\%)}} \times 100$$

Proximate composition

The proximate composition of BH and bovine hide gelatin (BHG) were measured by following official methods of analysis AOAC (moisture; 950.46, fat; 960.39 and ash; 920.153) [9]. Crude protein was determined with a TruSpec carbon/nitrogen determinator (Leco Corp., St. Joseph, MI). Conversion factors of 6.25 and 5.4 [10] were used to calculate protein in BH and BHG, respectively. All measurement was done in duplicate and means were used for statistical analysis.

Gelatin solution characteristics

The pH was measured according to the British Standard Institution method [11] with a glass electrode (Orion 2 Star™ and Star Plus Meter) in a 2% BHG solution. The pH meter was calibrated with standard commercial buffers (pH 4.0, 7.0 and 10.0). The percent transmittance was measured in a 2% BHG solution. For this, the spectrophotometer (Evolution™ 60S UV–Visible Spectrophotometer, Thermo Scientific) was calibrated with DI water as a blank representing 100% transmittance and was measured at 620nm. The isoelectric point (P^I) of

the 2% BHG solution was determined by measuring the percent transmittance at different pH (from 2 to 10) at 660nm by following the method of Roy et al. [12].

Dynamic viscoelastic properties

Dynamic viscoelastic properties of 6.67% BHG gel were measured using a Physica MCR 301 rheometer (Anton Paar GmbH) following the method of Roy et al. [12]. Briefly, the linear viscoelastic range (LVR) of the gel was tested by the amplitude sweep test with an increasing oscillatory strain (from 0.1 to 20%) at a constant frequency (1 hertz) and temperature (10°C). The frequency sweep test was conducted at 10°C, employing a constant strain of 5% and varying the frequency from 0.2 to 100 hertz within the identified LVR region. The viscoelastic properties of BHG gel were measured at a constant strain of 5% and a constant frequency of 1 hertz (at the LVR) under oscillation (25mm parallel plates with a 1 mm gap between the plates) and the temperature was maintained from 45 to 10°C with a heating/cooling rate of 2°C/min. Gelling and melting point of the gel was determined by the sharp increase of storage modulus (G′) during cooling and the sharp decrease during heating, respectively.

Color of gelatin gel

Gel color was measured using the 6.67% BHG gel with a Konica Minolta Chroma Meter CR–410 colorimeter and expressed as lightness (L*), redness (a*), yellowness (b*). The colorimeter was calibrated with the standard white calibration plate provided by the manufacturer prior to measurement and the D65 illuminant was used. Color of gelatin was measured in triplicate and means were used for statistical analysis.

Gel strength

The gel strength of the 6.67% BHG solution was determined by following the British Standard Institution method [11]. Freeze–dried gelatin (7.50g) was allowed to swell in DI water (105mL) for 3h in a standard Bloom jar (150mL, Schott, Mainz, Germany) and then dissolved completely by heating at 45°C for 15min. It was then cooled to room temperature, matured at 10°C for 18h and then gel strength was determined using a texture analyzer (TA–XT2, Stable Micro Systems, Surrey, UK) equipped with 2kN load cell and a 1.27cm diameter flat faced cylindrical Teflon plunger. The gel strength was recorded in gram when the plunger penetrated 4mm into the gel at a speed of 0.5 mm/sec. Gel strength was measured in triplicate and means were used for statistical analysis.

Gelatin thermal properties

The thermal properties of BHG were measured with a multi–cell differential scanning calorimeter (TA Instruments, New Castle, Delaware) following the method of Roy et al. [12]. The matured BHG after gel strength measurement was weighed (about 500mg) into an ampoule, capped and were run at a heating rate of 1 °C/min from 20 to 60°C. An empty ampoule was used as reference. The helix–coil transition temperature was calculated from the endothermic peak and the enthalpy change was measured by integrating the area under the peak. Samples were measured in triplicate and means were used for statistical analysis.

Foaming properties

Gelatin solutions (0.5, 1 and 2%) were prepared and from each concentration 25mL were placed in a tube, homogenized (1 min at 20000 rpm) with an Ultra–Turrax Ika T–18 homogenizer to incorporate air and increase the volume. The total volume was measured at 0, 30 and 60 min of homogenization and the foam expansion (FE) and foam stability (FS) were calculated by the following equations:

$$FE\ (\%) = \frac{(V_T - V_0)}{V_0} \times 100 \qquad FS\ (\%) = \frac{(V_t - V_0)}{V_0} \times 100$$

Where, V_T = total volume after homogenization (mL); V_0 = volume before homogenization; and V_t = total volume after storage at room temperature for 30 and 60 min.

Emulsifying properties

Gelatin solutions (0.5, 1 and 2%) were prepared with DI water and from each concentration, 8mL gelatin solution were added with 2mL of edible sunflower oil and homogenized for 1 min at 24000 rpm at room temperature. Then, 50µL of homogenized emulsion were taken from the bottom of the tube at 0 and 30 min after emulsion formation, added to 5mL of 0.1% sodium dodecyl sulphate (SDS) solution and mixed thoroughly by vortex for 10 sec. The absorbance was measured at 500nm and 0.1% SDS solution was used as the blank at 0 (A_0) and at 30 min (A_{30}) after homogenization. The emulsion activity index (EAI) and emulsion stability index (ESI) of BHG were determined by the following equations:

$$EAI\ (m^2 / g) = \frac{2 \times 2.303 \times A_{500} \times DF}{C \times \varphi \times 10^4} \qquad ESI\ (min) = \frac{A_0 \times \Delta T}{\Delta A}$$

Where, A_{500} = absorbance at 500nm, DF= dilution factor (100), C= protein concentration (g / mL) before emulsification, φ = oil volume fraction (v/v) of the emulsion (i. e., the volume of emulsion droplets divided by the total volume of emulsion, φ = 0.2), and A_{30} and A_0 represent the absorbance after 30min and at time zero, respectively, at 500nm after emulsification, $\Delta A = A_{30} - A_0$ and ΔT = 30min.

Water holding capacity (WHC) **and fat binding capacity** (FBC)

Freeze–dried BHG was dissolved in DI water or sunflower oil (10 mg/mL), and allowed to stand for 1h with stirring every 15min. Then, the supernatant was decanted and the tubes were inverted at 45° for 30 min to drain the water or oil completely. The WHC and FBC were calculated using the following equation:

$$WHC \text{ or } FBC\ (\%) = \frac{\text{Weight of the contents in the tube after draining (g)}}{\text{Weight of the freeze-dried gelatin (g)}} \times 100$$

Molecular weight distribution

Molecular weight (MW) distribution of dehaired BH and BHG was performed using sodium dodecyl sulfate polyacrylamide gel electrophoresis following the Laemmli [13] method. Dehaired BH and BHG were diluted with sample buffer (5 mg/mL), vortex, heated at 95 °C for 5 min and centrifuged at 5000*g* for 5 min. Ten µL of each sample and MW marker standard (Bio–Rad Laboratories Inc.) were loaded on a precast 4 to 20% gradient gel (Bio–Rad Laboratories Inc.). The samples were run at a constant voltage of 170V on a Power Pack Basic^{TM} electrophoresis apparatus (BioRad Laboratories Inc.). After electrophoresis, the bands in the gel were stained with Coomassie Brilliant Blue R–250 and de–stained with a mixture of DI water, methanol and acetic acid at a ratio of 50:40:10.

Amino acid composition

The amino acid composition of BHG was determined using the method of Roy et al. [12]. Gelatin solution (1 mg/mL) was hydrolysed at 160°C for 1h in 6M HCl containing 0.1% phenol. An AccQ–Tag Ultra Derivatization Kit (Waters, Milford, MA) was used to label the hydrolysates. Hydrolysates were analyzed by high performance liquid chromatography (HPLC) (Agilent 1200 Series) using an AccQ–Tag C18 column (3.9×150mm, Waters) at 254nm for detection. Norleucine (Sigma–Aldrich Inc) was used as an internal standard and hydroxyproline from a calorimetric assay standard kit (BioVision Incorporated) as an external standard.

Fourier transform infrared spectroscopy (FTIR)

Freeze-dried BHG were analyzed using FTIR with a Nicolet™ iS50 FTIR Spectrometer (Thermo Fisher Scientific) equipped with an attenuated total reflection accessory. The spectra were recorded at a resolution of 4 cm^{-1} and measured from 400 to 4000 cm^{-1} against a background spectrum performed with a clean empty cell. The automatic signals were collected with accumulation of 32 scans per spectrum. Spectral data were analysed with the data collection software program (OMNIC 7.3, Thermo Electron Corporation). Samples were measured in duplicate and means were used for statistical analysis.

Statistical analysis

One-way analysis of variance was performed to compare the effect of dehairing treatment on the efficacy of dehairing, the recovery of gelatin and the various quality measurements performed on BHG. Data were analyzed with the Statistical Analysis Software (SAS Institute Inc., Cary, NC). Tukey's post–hoc test was used when the differences between means were found significant ($P < 0.05$).

Results And Discussion

Dehairing efficacy

All treatments showed evidence of the ability to dehair the BH in the allotted 24 h time frame (Fig. 2). The number of hairs on BH from CT and CTAA were too numerous to count; therefore, these treatments were not included in the statistical analysis comparing number of hairs remaining after dehairing. Within those treatments where the numbers of hairs were able to be quantified, there was no significant difference due to

treatment (Table 1, Fig. 2), and so the hypothesis was that papain is comparable to keratinases for the dehairing of BH was accepted. We also accepted the hypothesis that acetic acid is as efficacious as calcium hydroxide for dehairing BH because the number of hairs remaining after treatment were not different ($P > 0.05$) (Table 1). Acetic acid provides a more environmentally–friendly alternative to the lime-sulfide baths traditionally used to dehair BH [14].

Gelatin yield and physical properties

Acetic acid at the concentration used in this study (5%) produced gelatin yields on a wet tissue basis that were lower than that of KTN, but greater than that of CH and the control (DI water) (Table 2). On a dry tissue basis, KTN, AA and CTAA showed higher yields than CH and CT, indicating that the use of AA or KTN improved gelatin yield over the conventional CH in the experimental time frame of 24 h, a time frame much shorter than that used by the gelatin industry. Increased yield of gelatin is an indication of increased collagen denaturation [15] and these results agreed with those of others who have shown that the use of enzymes can increase gelatin yield, but this is the first report of acetic acid doing so. Acetic acid swells collagen, making it easier to be denatured by heat [16] or attacked by enzymes such as pepsin [17].

Regardless of differences in protein and crude fat (Table 2), all the gelatins produced in the treatments with the exception of that from the papain treatment were comparable to that of commercial gelatin (90.22% crude protein, 8.52% moisture, 0.21% crude fat and 0.29% ash) [18] which agrees with the present results. As expected, the pH values of the BHG from the AA and CTAA treatments were the lowest, while that from the CH treatment had the highest pH value, followed by the KTN and CT treatments (Table 2). According to GMIA [19], BHG of CT and CTAA are type A (pH 7–9), while those from CH and KTN treatments are type B (pH 4.8–5.4). Both the pH and the P^I (Fig. 3) of gelatin depend on the type, strength and pH of the chemicals used in pre–treatment processes [19, 20, 21]. In bovine heart gelatin, pH values near the P^I resulted in a lower gel strength [12]. At the P^I, protein molecules in gelatin solutions form aggregates, reducing the amount of water that can interact with the protein molecules [22] which reduces gel strength.

Despite the efficacy of papain with regard to dehairing of BH, papain produced an inferior gelatin product even in the limited time that the BH was exposed to it. The gelatin from the PP treatment was unable to form a gel at the standard Bloom strength test concentration of 6.67%, and so it was not further characterized in this study. Dehairing with papain and keratinase appeared to promote cleavage of α-chains (α1 and α2), increasing the amount of low MW fragments following gelatin extraction (Fig. 4). Gelatin from BH treated with papain was extremely degraded and exhibited very low MW peptides (< 25 kD). Gelatins with low MW peptides are usually unable to form a gel [23] because of the restricted electrostatic junctions [24]. Gelatins extracted using enzymes have showed lower gel strength than gelatins extracted using only heat [25] and this corroborates with the results of the present study. Dehairing with AA also produced gel with greater strength than that of CH, with gel strength highest in AA and CTAA gelatin and lowest in KTN gelatin (Table 2). The BHG of CT, AA and CTAA contained high MW components (α, β and γ–chains) as the major protein constituents with fewer low MW peptides than CH and KTN gelatin, and this was associated with increased gel strength (Fig. 4). This result substantiated that gelatin with an increased proportion of high MW components (α, β and γ–chains) has increased gel strength [26] because low MW peptides cannot form the lengthy junction zones needed to develop a strong protein network [27]. Methionine and phenylalanine proportions were reduced only

in the gelatin extracted using acid or enzymatic methods, suggesting that erosion of the collagen α-chains was more extensive than in the CT or CH treatments, but it did not appear to affect gel strength of AA BH (Table 3). Gel strength is considered the utmost quality parameter of gelatin [23] and gelatin that contained increased hydroxyproline also shows increased gel strength [26] but in this study there was no difference in hydroxyproline content between treatment groups. Hydroxyproline in gelatin helps to form three-dimensional hydrogen bonds between water molecules and free hydroxyl groups of amino acids, which affords the strength and rigidity to the gelatin gel [28], and directly hydrogen bonds to the carbonyl groups [15]. The stability of gelatin is proportional to the glycine content just as it is for the parent molecule collagen [29], but in the present study, increased glycine proportion was not related to gel strength.

The spectral differences of the BHG (Fig. 7 and Table 6) may be related to the differences in their MW distribution [29]. The lower wavelength range of amide A peak in the present study might be due to fewer exposed amino groups in extracted gelatin involved in hydrogen bonding [30]. Gelatin extracted after alkali and enzymes treatment (KTN and CH) showed the amide B peak at a lower wavenumber range (Table 6) compared to the CT, AA and CTAA gelatins, which suggested that the $-NH_3$ group interacts between peptide chains [31]. These results support the conclusion that the dehairing treatment affected the secondary structure of BHG.

Acetic acid dehairing produced gelatin with quality and properties comparable or superior to that of the CH treatment. Gelatin from the AA treatment exhibited greater transmittance (clarity) and L* values than that of the KTN and CT treatments, which were comparable to those achieved using CH and indicated that gelatins from AA, CTAA and CH-treated BH were paler than that of other treatments (Table 2). Generally, color of gelatin has no influence on functional properties but a light color is preferred as it allows gelatin to be incorporated into foods without compromising the original color of the product [10]. The transmittance of gelatin is very important in food applications because the gelatin with the highest transmittance has no negative effect on the color or opacity of the final product [32]. Low transmittance of gelatin is mainly caused by inorganic substances, proteins, mucopolysaccharides and other contaminants [33] and these contaminants can be removed by filtration [27]. These results indicated that dehairing of BH with acetic acid, calcium hydroxide, or keratinase assisted with the removal of contaminants before gelatin extraction, increasing the transmittance of the gelatin.

Rheological properties also indicated that AA gelatin was comparable or superior to that of the CH dehairing treatment. Although gelling and melting temperature, storage (G′) and loss modulus (G″, not shown) of BHG (Fig. 5) showed no differences due to dehairing treatments, the elastic response (measured by G′) was highest in gelatin from AA followed by CTAA, CH, CT and lowest in KTN (Fig. 6). Also, the thermal denaturation properties of the BHG from AA and CTAA treatments were comparable to or showed more stability than that of BHG from the CH treatment (Table 4). The onset of denaturation temperature of the BHG was higher in CT than in CH and KTN treatments. The enthalpy of BHG was higher in AA than in CH and KTN treatments. Compared with CT, gelatin from AA and CTAA treatments showed no differences in enthalpy and thermal denaturation temperature. This indicated that dehairing with acetic acid (AA and CTAA) cleaved the intra–molecular cross–links of the parent molecule collagen with limited damage to the integrity of the triple helix portion of the collagen molecule as reflected by the MW distribution (Fig. 4). On the other hand, dehairing with CH and KTN damaged the integrity of the triple helix portion of the collagen α–chains as evidenced by the

increased proportion of low MW peptides (Fig. 4), reduced enthalpy and thermal denaturation temperature. The peak denaturation temperature of BHG in different treatments did not differ and was not expected to as the proline and hydroxyproline content also did not differ which agreed with the report of Kittiphattanabawon et al. [24].

The foam expansion (FE) and foam stability (FS) did not differ due to treatments (Table 5), and were therefore not affected by MW distribution (Fig. 4). Foaming properties of gelatin are important when it is to be incorporated into foods like marshmallows [34]. Ahmed and Benjakul [35] reported that gelatin with low MW peptides showed poor foaming properties because they are unable to form a well–ordered film at the air–water interface. Gelatins derived from BH dehaired using acetic acid (AA and CTAA) had the highest ESI at 2 and 1%, concentrations, respectively, although differences between the treatments were limited (Table 5). The EAI of BHG at 0.5% and 1% concentrations was lowest in CT compared with other treatments.

The AA showed the highest WHC followed by the CTAA and CT, with the lowest being from the CH and KTN (Table 5). The WHC of gelatin is a desirable trait in some food items (sausages, custards and dough) because these items absorb water without dissolving proteins, and by that mechanism attain body thickening and viscosity properties [36]. The BHG regardless of treatment showed a high value of WHC, as one gram of dry gelatin was capable of retaining 4.51–34.39 g of water (Table 5) and agreed with the results of Wasswa et al. [37]. The primary function of a hydrocolloid is to retain water and the high WHC of BHG suggests that it has a large number of pores in its structure [38]. The CT showed the highest FBC and this difference may be due this gelatin having the highest leucine content (Table 5). According to Ninan et al. [39], FBC increased with the increased degree of exposure of the hydrophobic residues and the increased amount of leucine, isoleucine, tyrosine and valine.

Conclusions

BH was dehaired successfully by all treatments, but gelatin quality was profoundly lowered when dehairing involved enzymes, with low gel strength observed when BH was dehaired with keratinase and papain. Dehairing with KTN increased gelatin yield, but the use of 5% acetic acid for dehairing yielded quantities of gelatin comparable to that of CH, which had an acceptable range of gel strength and quality and gel strength that exceeded that of the conventional standard CH. The results of this study should be viewed with caution, as conditions for dehairing were not optimized and did not reflect standard industry practices, but acetic acid, which is already used in the food industry, is a promising chemical to dehair BH and increase gelatin yield from the highly cross–linked collagen of BH.

Declarations

Acknowledgements

The authors would like to thanks Alberta Livestock and Meat Agency (Grant No. 2011R030R) for financial support.

Conflict of interests

There are no conflicts of interest to report

Availability of data and material

Data will be available on request at anytime

Author contributions

Bimol C. Roy and Chamali Das jointly performed assays and laboratory work, and Bimol C. Roy wrote the manuscript. Heather L. Bruce performed experimental design, statistical analysis, and manuscript review and editing. Hui Hong assisted with FTIR data analyses and manuscript editing and review. Mirko Betti assisted with the Rheometer run, editing and review of the manuscript.

Ethics approval

Not applicable

Consent to participate

All authors provided consent for their participation

Consent for publication

All authors provided their consent for publication

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Tables

Table 1. Experimental design for dehairing of bovine hides as a pre–treatment prior to gelatin extraction (locations of R1, R2, R3, R4 and R5 on each bovine hide are indicated in Fig. 1) and mean number of hairs (with standard error) on bovine hides after application of the dehairing treatments.

Hide Number	Dehairing treatment applied to bovine hide pieces prior to gelatin extraction					
	Control (CT)	5 % Acetic Acid (AA)	Control plus 5 % acetic acid (CTAA)	10 % Calcium hydroxide (CH)	0.02 % Keratinase enzyme (KTN)	2.5 % Papain enzyme (PP)
1	R1	R3	R1	R2	R5	R4
2	R5	R2	R5	R1	R4	R3
3	R4	R1	R4	R5	R3	R2
4	R3	R5	R3	R4	R2	R1
5	R2	R4	R2	R3	R1	R5
Number of hairs/ cm^2 after dehairing[1]	ND[2]	22.6 (8.03)	ND	23.2 (8.03)	17.8 (8.03)	3.6 (8.03)

[1] Means (with standard error of the mean) for number of hairs. Analysis of variance indicated the probability of the F value was P = 0.3118.

[2] ND, not determined.

Table 2. Means (with standard errors of the mean) for gelatin yield and proximate composition of bovine hide gelatin extracted after application of different dehairing treatments.

Measurements		Treatments applied to dehair bovine hides prior to gelatin extraction					P value
		Control (CT)	5 % Acetic Acid (AA)	Control plus 5 % acetic acid (CTAA)	10 % Calcium hydroxide (CH)	0.02 % Keratinase enzyme (KTN)	
Gelatin yield (%)	Wet tissue basis	3.40 (1.22) c	10.16 (1.22) b	12.45 (1.22) b	4.86 (1.22) c	17.65 (1.22) a	< 0.0001
	Dry tissue basis	11.37 (5.35) b	54.25 (5.35) a	45.07 (5.35) a	18.88 (5.35) b	55.02 (5.35) a	< 0.0001
Proximate composition of gelatin (%)	Crude protein	98.39 (1.02) ab	98.66 (1.02) ab	101.24 (1.02) a	99.14 (1.02) ab	95.94 (1.02) b	0.0279
	Crude fat	0.19 (0.07) ab	0.014 (0.07) b	0.002 (0.07) b	0.02 (0.07) b	0.32 (0.07) a	0.0324
	Ash	0.35 (0.10) b	0.026 (0.10) c	0.00 (0.10) c	0.66 (0.10) a	0.22 (0.10) bc	0.0005
	Moisture	3.82 (0.95)	4.31 (0.95)	2.99 (0.95)	4.23 (0.95)	7.06 (0.95)	0.0683
pH		6.29 (0.25) b	5.01(0.25) c	4.86(0.25) c	7.72(0.25) a	6.75(0.25) b	<0.0001
Transmittance (%)		23.78 (3.94) c	91.40 (3.94) a	91.74 (3.94) a	66.36 (3.94) ab	55.12 (3.94) b	0.0001
Gel color	L*	40.36 (1.54) bc	45.92 (1.54) a	47.48 (1.54) a	44.33 (1.54) ab	39.55 (1.54) c	0.0057
	a*	1.73 (0.37) ab	0.57 (0.37) c	0.97 (0.37) bc	2.05 (0.37) ab	2.19 (0.37) a	0.0267
	b*	4.07 (0.69) c	12.15 (0.69) a	12.40 (0.69) a	9.83 (0.69) b	4.61 (0.69) c	< 0.0001
Gel strength (g)		201.09 (29.31) b	300.08 (26.21) a	235.28 (26.21) ab	172.96 (26.21) b	74.31 (29.31) c	0.0004
Rheological properties	Gelling temperature (° C)	21.31 (2.40)	20.21 (2.14)	20.48 (2.14)	20.74 (2.14)	16.60 (2.40)	0.656
	Melting temperature (° C)	31.52 (2.19)	29.57 (1.94)	30.51 (1.94)	29.31 (1.96)	25.81 (2.19)	0.4396
	Storage modulus (G, pa)	1353.25 (387.88)	1889.00 (346.93)	1438 .00 (346.93)	1488.24 (346.93)	441.39 (387.88)	0.1331

	Loss modulus (G⍰, pa)	25.53 (5.24)	27.69 (4.69)	18.63 (4.69)	25.69 (4.69)	13.68 (5.24)	0.2799

[a, b, c] Means in the same row with different letters are significantly different (P < 0.05) according to least squares means differences.

Table 3. Mean amino acids (% mol) composition of gelatin extracted from bovine hide after application of different treatments for dehairing

Amino acids (% mole)	Treatments applied to dehair bovine hides prior to gelatin extraction					SE[1]	P value
	Control (CT)	5 % Acetic Acid (AA)	Control plus 5% acetic acid (CTAA)	10 % Calcium hydroxide (CH)	0.02 % Keratinase enzyme (KTN)		
Alanine	10.55	10.62	10.61	10.26	10.30	0.19	0.5200
Arginine	5.37	5.36	5.49	5.56	5.45	0.07	0.2900
Aspartic acid	4.69 a	4.22 ab	3.79 bc	3.35 c	3.53 bc	0.26	0.0109
Glutamic acid	7.54	7.22	6.96	6.96	7.52	0.2	0.1453
Glycine	31.4b	32.5 a	33.5 a	33.14 a	32.70 a	0.31	0.0017
Histidine	0.58	0.60	0.81	0.85	0.77	0.13	0.5200
Hydroxyproline	9.89	10.43	10.55	10.55	10.37	0.19	0.1362
Isoleucine	1.29	1.22	1.26	1.27	1.31	0.02	0.2152
Leucine	2.77 a	2.54 b	2.42 c	2.59 b	2.61 b	0.03	<0.0001
Lysine	2.84	2.67	2.66	2.62	2.75	0.07	0.2819
Methionine	0.60 ab	0.54 b	0.56 ab	0.63 a	0.55 b	0.02	0.0402
Phenylalanine	1.41 a	1.37 ab	1.23 c	1.39 ab	1.26 bc	0.04	0.0182
Proline	12.30	12.49	12.52	12.37	12.30	0.11	0.5600
Serine	3.71 ab	3.63 ab	3.57 b	3.57 b	3.76 a	0.04	0.0380
Threonine	2.13	1.86	1.93	2.00	2.01	0.07	0.1800
Tyrosine	0.50	0.40	0.32	0.57	0.52	0.06	0.1049
Valine	2.36	2.28	1.76	2.24	2.22	0.17	0.1730

[1]SE = Standard Error

[a, b, c] Means in the same row with different letters are significantly different (P < 0.05) according to least squares means differences.

Table 4. Thermal denaturation properties (means with standard errors of the means in parentheses) of BH gelatin extracted after the various dehairing treatments as measured by differential scanning calorimetry

Measurements	Treatments applied to dehair bovine hides prior to gelatin extraction					
	Control (CT)	5 % Acetic Acid (AA)	Control plus 5 % acetic acid (CTAA)	10 % Calcium hydroxide (CH)	0.02 % Keratinase enzyme (KTN)	P value
Denaturation onset temperature (°C)	27.69 (.78) a	25.64 (0.70) ab	25.86 (0.70) ab	24.75 (0.70) b	23.50 (0.78) b	0.0189
Denaturation peak temperature (°C)	33.14 (1.01)	32.19 (0.90)	31.94 (0.90)	31.66 (0.90)	30.20 (1.01)	0.3817
Denaturation end temperature (°C)	40.29 (1.09)	39.64 (0.97)	39.16 (0.97)	38.33 (0.97)	39.20 (1.09)	0.7427
Enthalpy (kJ/mol)	1046 (101) abc	1319 (90) a	1230 (90) ab	949 (90) bc	837 (101) c	0.0110

[a, b, c] Means in the same row with different letters are significantly different (P < 0.05) according to least squares means differences.

Table 5. Foaming, emulsifying, water holding and fat binding capacities (means with standard error of the mean in parentheses) of gelatin extracted from bovine hides dehaired using different treatments

Measurements	Treatments applied to bovine hides to dehair prior gelatin extraction					SE	P value
	Control (CT)	5 % Acetic Acid (AA)	Control plus 5 % acetic acid (CTAA)	10 % Calcium hydroxide (CH)	0.02 % Keratinase enzyme (KTN)		
Foam expansion (FE)							
At 0.5 % concentration	32.80	30.00	32.00	34.00	26.00	3.38	0.5082
At 1 % concentration	40.40	40.80	42.80	45.20	34.80	4.45	0.5692
At 2 % concentration	44.80	39.60	51.60	51.20	40.00	5.78	0.4253
Foam stability (FS)							
At 0.5 % concentration for 30 min	29.20	19.40	25.20	20.80	18.40	4.43	0.4207
At 1 % concentration for 30 min	38.80	22.00	30.00	24.40	25.60	5.33	0.2298
At 2 % concentration for 30 min	42.40	19.60	38.40	27.60	29.60	6.58	0.1524
At 0.5 % concentration for 60 min	27.20	14.40	20.40	18.40	16.80	4.33	0.3180
At 1 % concentration for 60 min	37.20	16.00	23.60	20.00	23.20	5.41	0.1090
At 2 % concentration for 60 min	40.00	12.80	25.60	25.60	25.60	6.81	0.1396
Emulsion stability index (ESI, m^2/g)							
At 0.5 % concentration	56.80	55.60	51.80	41.00	38.40	6.82	0.2276
At 1 % concentration	54.20 ab	47.60 ab	67.80 a	51.20 ab	34.00 b	6.78	0.0341
At 2 % concentration	47.20 c	92.60 a	66.20 b	40.00 c	34.00 c	4.48	<0.0001
Emulsion activity index (EAI, minutes)							
At 0.5 % concentration	9.03 b	18.30 a	19.43 a	18.68 a	18.32 a	1.69	0.0016

At 1 % concentration	6.20 b	9.42 a	10.08 a	8.90 a	9.89 a	0.78	0.0146
At 2 % concentration	3.16	3.66	4.62	4.98	4.31	0.44	0.0602
Water holding capacity (%)	1176.0 c	3439.4 a	2709.8 b	792.4 cd	451.2 d	233.6	<0.0001
Fat holding capacity (%)	5061.00 a	3833.60 b	2871.40 b	3052.20 b	2813.00 b	382.31	0.0021

[a, b, c] Means in the same row with different letters are significantly different ($P < 0.05$) according to least squares means differences.

Table 6. The wavenumbers of ATR-FTIR spectra peaks of gelatin extracted from bovine hides dehaired using different treatment

Measurements	Wavenumbers (cm^{-1}) [Range from to]				
	Control (CT)	5 % Acetic Acid (AA)	Control plus 5 % acetic acid (CTAA)	10 % Calcium hydroxide (CH)	0.02 % Keratinase enzyme (KTN)
Amide I	1632-1633	1631-1632	1631-1633	1631-1632	1632
Amide II	1521-1537	1532-1533	1528-1531	1525-1529	1530-1532
Amide III	1230-1233	1232-1233	1232-1233	1232-1235	1233
Amide A	3289-3295	3297-3298	3299-3300	3290-3295	3294-3297
Amide B	2933-2935	2935-2936	2937-2938	2922-2928	2927-2929

Figures

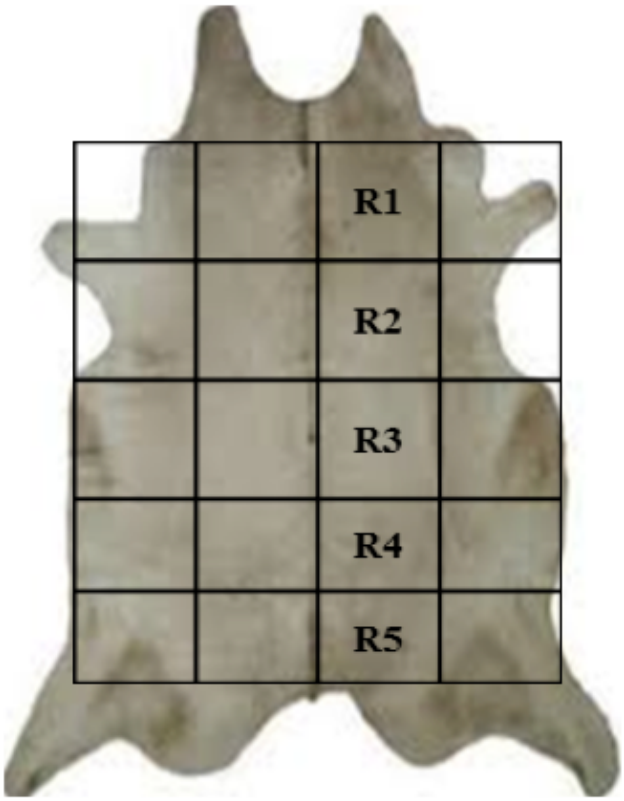

Figure 1

Locations on bovine hides (R1, R2, R3, R4 and R5) in which different chemicals were used as pre-treatment for dehairing and subsequently gelatin extraction

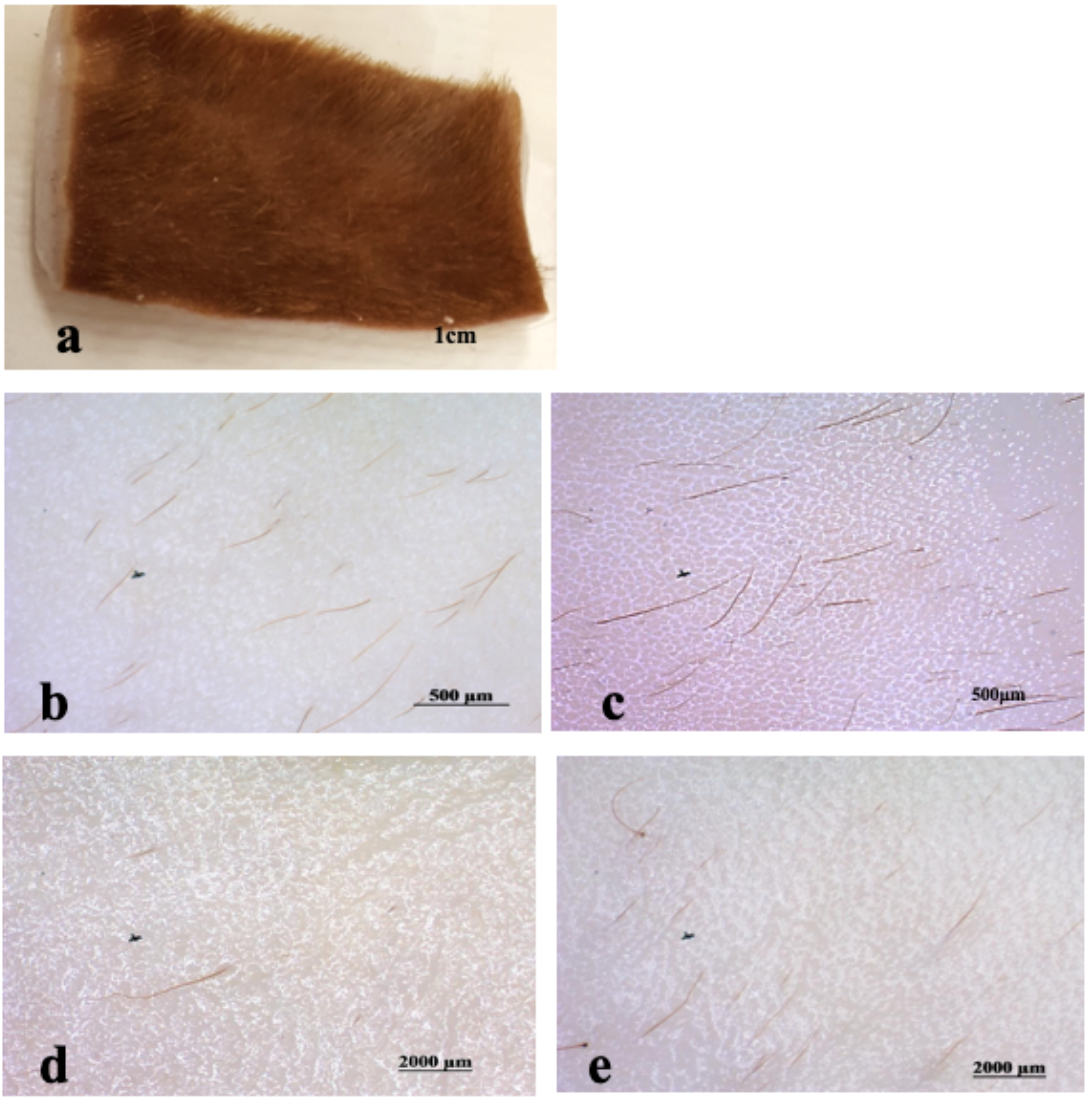

Figure 2

Dehaired bovine hides with different treatments. a; control/ control plus 5% acetic acid (CT/ CTAA), b; 10% calcium hydroxide (CH), c; 5% acetic acid (AA), d; papain enzyme (PP) and e; 0.02% keratinase enzyme (KTN)

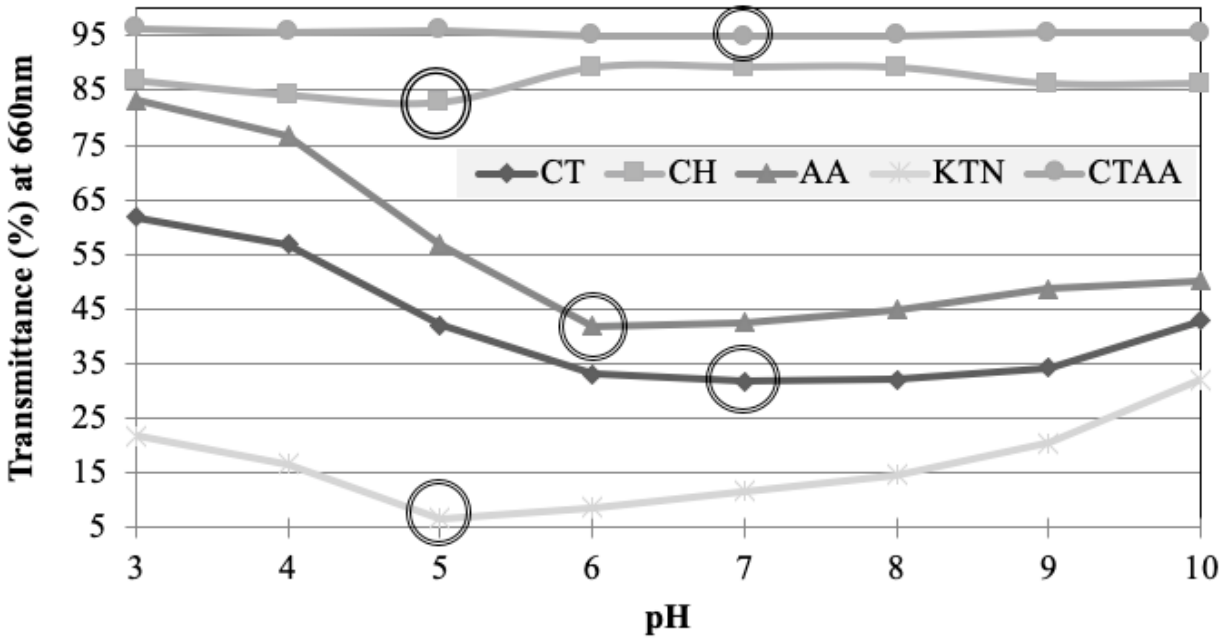

Figure 3

Isoelectric point of gelatin extracted from bovine hides. pH at Lowest transmittance indicate isoelectric point (double lined compound circles). Control (CT); 10% calcium hydroxide (CH); 5% acetic acid (AA); 0.02% keratinase enzyme (KTN) and control plus 5% acetic acid (CTAA).

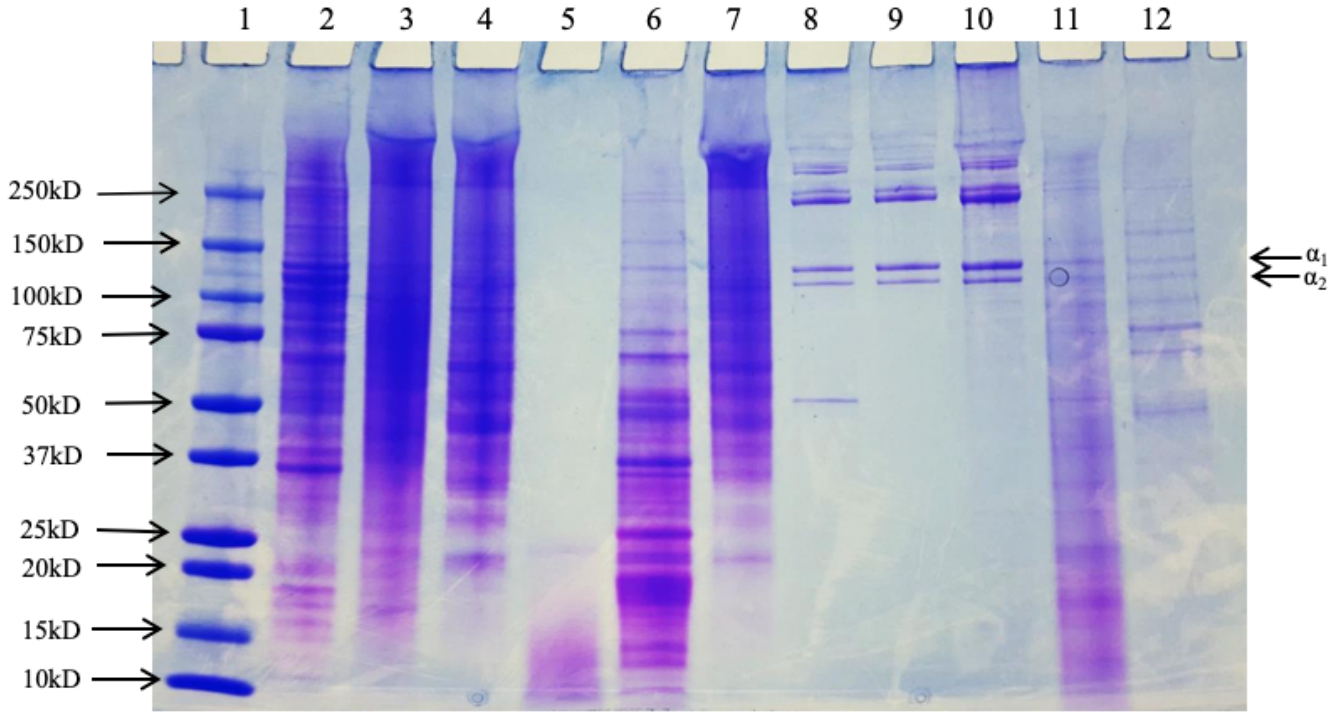

Figure 4

SDS–PAGE pattern of Bovine hides gelatin and Bovine hides. Lane 1: Standard; Protein concentration was 5 mg/ml; Lane 2: (CT) control; Gelatin, Lane 3: (CH) 10% calcium hydroxide; gelatin, Lane 4: (AA) 5% acetic acid; gelatin, Lane 5: (PP) papain; Gelatin, Lane 6: (KTN) 0.02% keratinase; gelatin, Lane 7: (CTAA) control plus 5% acetic acid; gelatin, Lane 8: (CT) control; hides, Lane 9: (CH) 10% calcium hydroxide; hides, Lane 10: (AA) acetic acid; hides, Lane 11: (PP) papain; hides, Lane 12: (KTN) keratinase; hides, and 12 µl of each sample was loaded.

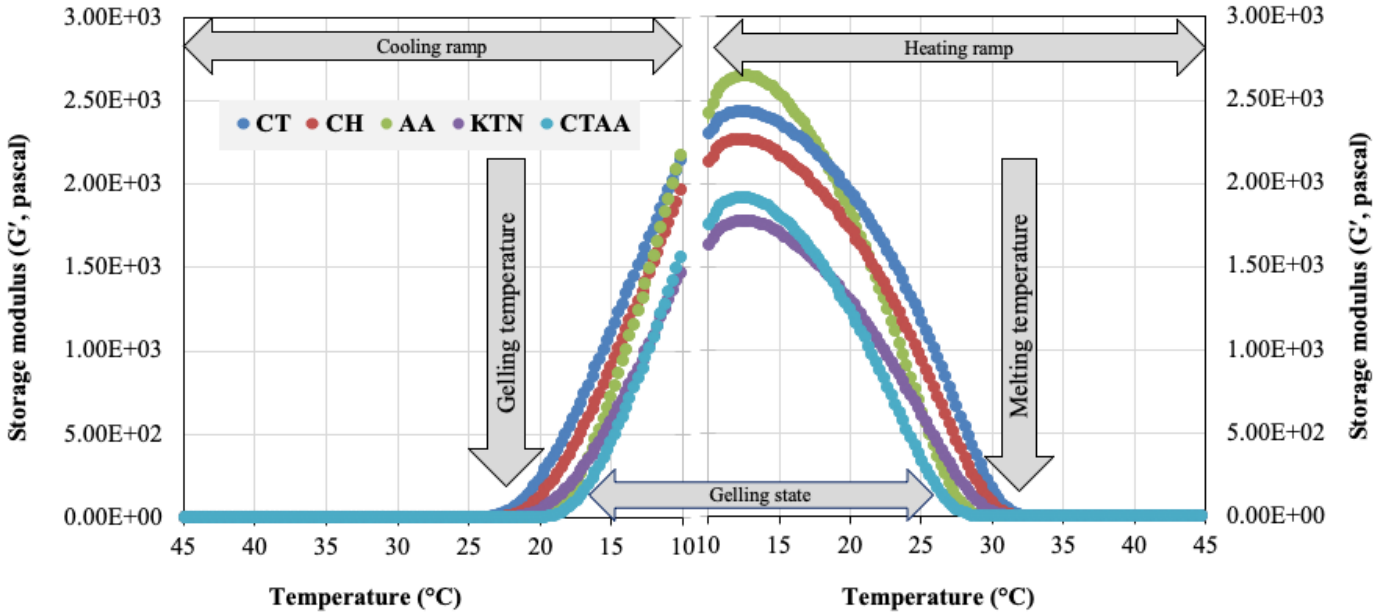

Figure 5

Temperature sweep curve of gelatin gel extracted from Bovine hides in different treatments. Control (CT); 10% calcium hydroxide (CH); 5% acetic acid (AA); 0.02% keratinase enzyme (KTN) and control plus 5% acetic acid (CTAA).

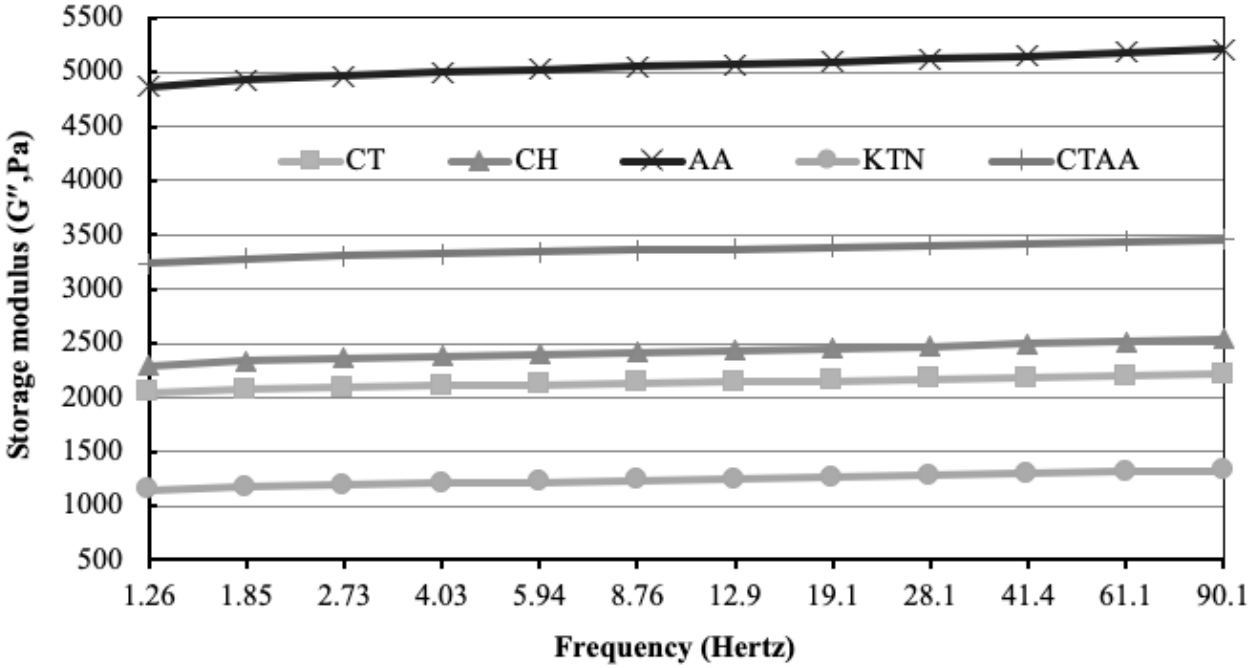

Figure 6

Frequency sweep curves for the frequency dependence of storage modulus (G′) of gelatin gel (6.67%) extracted from Bovine hides in different treatments. Control (CT); 10% calcium hydroxide (CH); 5% acetic acid (AA); 0.02% keratinase enzyme (KTN) and control plus 5% acetic acid (CTAA).

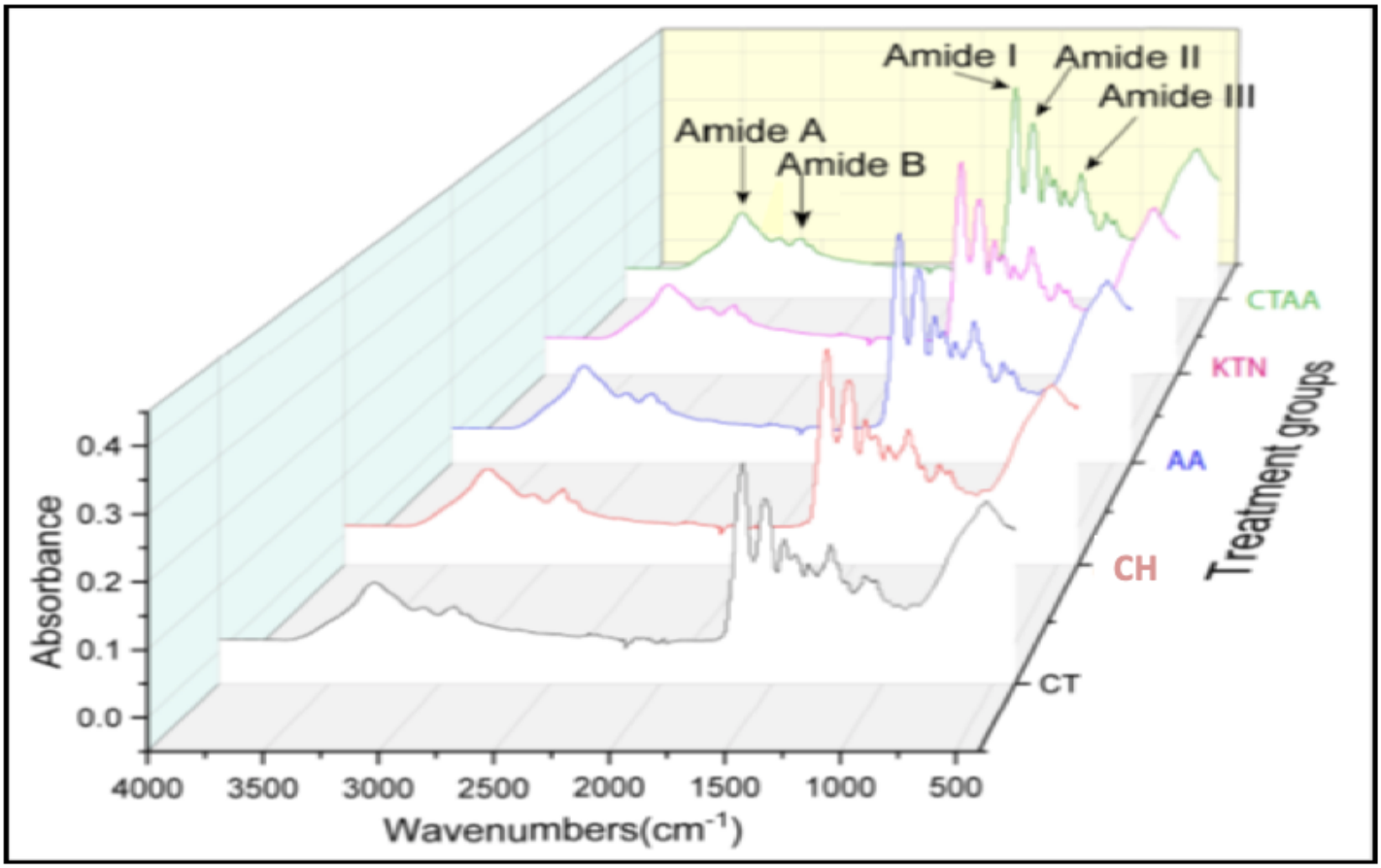

Figure 7

The ATR-FTIR spectra of gelatin extracted from bovine hides in different treatment groups. Control (CT); 10% calcium hydroxide (CH); 5% acetic acid (AA); 0.02% keratinase enzyme (KTN) and control plus 5% acetic acid (CTAA).

Supplementary Files

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