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Extraction and characterization of gelatin from goat skin

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ABSTRACT

The present study was conducted to optimize the pretreatment and extraction protocols for gelatin from goat skin. The physico-chemical properties of gelatin extracted from goat skin under different pretreatment conditions *viz.,* scalding (65°C for 5 min), 0.1 M NaOH for 2 h, 0.25 M NaOH for 2h and extraction temperatures (60°C and 65°C) were studied. Pretreatment with 0.1 M NaOH and extraction at 65°C (6 h) recorded the highest yield (8.42±0.02%) as well as better gel clarity (53.03±0.18%) and instrumental colour values. The scanning electron microstructure of goat skin gelatin displayed a compact network of protein strands without any void spaces. The gel strength and viscosity of goat skin gelatin were 437.56 g bloom and 15.73 cP respectively. The SDS-PAGE fractionation of gelatin proteins showed the presence of distinct α, β and γ-chains. These findings suggest that goat skin has an immense potential to be employed for gelatin extraction and can be used as an alternative to gelatin from other sources.

Key words: Goat skin gelatin, NaOH pretreatment, Extraction, Yield

INTRODUCTION

Gelatin is a diverse mixture of polypeptides produced through partial hydrolysis of collagen from animal connective tissues using either an acidic or alkaline treatment. Gelatin is used in numerous applications including its use as a stabilizer, thickener, adhesive, water-retaining agent, clarifying agent and setting agent in a wide range of pharmaceuticals, foods and household products. Gelatin has been applied in food, cosmetic and pharmaceutical products because of its gelling, binding, stabilizing and emulsifying properties.

The total annual production of gelatin is estimated to be 3,26,000 tonnes, with pig skin accounting for the largest single contribution (approx. 41%) followed by bovine bone, bovine hide and fish accounting for 29.5%, 28.5% and 1%, respectively (Pradini et al. 2018). When compared to mammalian gelatin, the physico-chemical and functional characteristics of fish gelatin are sub-optimal (Shyni et al. 2014). The outbreak of Bovine Spongiform Encephalopathy, also known as mad cow disease, in 1980 reduced the acceptability of bovine gelatin (Balti et al. 2011). Porcine gelatin is forbidden for use in muslim community because of religious beliefs. So, gelatin from other sources and as a lawful status is necessary (Harahap et al. 2018). Goat skin may be an important source of gelatin to overcome these disadvantages. However, currently there is

no demand for sheep and goat skin and are sold at very low price without any value addition. Therefore, there is a potential to produce gelatin from goat skin that are abundantly available in India.

Among different edible and inedible by-products produced during the slaughter of goats, skin constitutes 6.4 to 11.6 g/100 g of live body weight (Warmington and Kirton, 1990). Extraction of 74.77% of goats during the year 2021-22 has resulted in the availability of 111 million goat skins which is ~ 0.142-0.257 million tonnes. Hence, there is a potential to produce gelatin from goat skin that are abundantly available. However, the extraction and characterization of goat skin gelatin is minimally investigated. Hence, the current study was aimed to optimize the pretreatment steps and the process of gelatin extraction from goat skin and to characterize the goat skin gelatin.

MATERIALS AND METHODS

Source of raw materials

Non-descript (ND), market age goat skins were collected from the Experimental slaughter house, ICAR-National Meat Research Institute, Hyderabad. After collection, the skins were washed thoroughly to remove the dirt, blood clots and fecal debris followed by removal of visible fat and fascia. The cleaned skins were frozen at -20°C until further use. The frozen skins were thawed overnight at 4°C before use.

Pretreatment of goat skin

Scalding: The goat skin was scalded at 65°C for 5 min for loosening of hair follicles and the skin was manually scrapped to remove the hair.

Pretreatment with NaOH: Goat skins were soaked in 0.1M and 0.25M NaOH 1:5 (w/v) for 2 h to ensure dehairing. The solution was replaced with the same volume of freshly prepared NaOH after 1 h. The skins were dehaired manually through scrapping and washed several times under running tap water until the pH of the wash water became neutral. Later the skins were cut into approximately 1×1 cm^2 pieces and subjected to gelatin extraction.

Gelatin extraction

Frozen-thawed skin pieces were soaked in 1.5% HCl solution for 6 h (skin:solution ratio of 1:4 w/v) with intermittent mixing. Acid solution was drained and the skins were washed under running tap water until the wash water pH reached neutral. The washed skins were then subjected to thermo-hydrolysis (skin:distilled water @ ratio of 1:3 w/v) in a water-bath (JEIO TECH BS-11, Korea) at 60°C and 65°C for 6 h. The extract was filtered through double layered muslin cloth. Filtered extract was poured onto glass trays as a thin layer and dried in a hot air oven at 60°C for 24-26 h. The dried gelatin was scrapped from the trays and pulverised to obtain gelatin powder, packed in laminate pouches and stored at room temperature.

Physicochemical properties and microstructure of gelatin

pH: The pH of dried gelatin was determined by mixing 1 g of gelatin powder in 10 ml of distilled water and the mixture was heated in water bath (JEIO TECH BS-11, Korea) at 60°C for 10 mins. It was cooled to room temperature and the pH of gelatin was measured using a standardized electrode attached to a digital pH meter (HANNA Instruments, HI 2216, Europe) (AOAC,1975).

Gelatin yield (%): The yield of gelatin was calculated as per Mulyani et al. (2017)

$$\text{Yield (\%)} = \frac{\textit{Weight of dried gelatin (g)}}{\textit{Weight of wet Skin (g)}} \times 100$$

Gel clarity: Gel clarity was determined by the method of Avena-Bustillos et al. (2006). The 6.67% of gelatin solution was prepared from the dried gelatin powder and placed in water bath (JEIO TECH BS-11, Korea) at 60°C for 1 h. Then it was cooled to room temperature and transmittance (% T) was measured at 620 nm using UV-VIS spectrophotometer (Model: UV-1700 Pharma-Spec, SHIMADZU, Japan).

Instrumental color value: Instrumental colour values of dried gelatin powder was measured using colorimeter (CR-20, KONICA MINOLTA, INC., Japan) according to the method prescribed by Al-Hassan (2020) using illuminant D65 and 10-degree standard observer angle (Hunter and Harold, 1987). The CIE (Commission International d'Eclairage - International Commission on illumination's) L^*(lightness), a^*(redness) and b^*(yellowness) values were measured and recorded.

Microstructure of gelatin: The microstructure of 6.67% gelatin gel was visualized using HITACHI (S-3400-II) scanning electron microscope with an acceleration voltage of 10 kV and emission current of 14000nA. The micrographs were taken at a working distance of 6.5 mm at 200x magnification. Back Scattered Electron (BSE) Detector was used.

Rheological properties and protein characterization

Gel strength: Gel strength of gelatin was determined according to the method described by Fernandez-Diaz et al. (2001). The 30 ml of 6.67 % (w/v) gelatin solution was

prepared with distilled water at 60 °C in a 50 ml beaker. The solution was kept at refrigeration temperature for 16-18 h. The dimensions of the sample were 3.8 cm diameter and 2.7 cm height. Gel strength was measured with a texture analyzer (TA-XT Plus, Stable Micro-Systems, Surrey, UK) using a flat-cylindrical Teflon® plunger [(P/0.5R); (1.27 cm in diameter)], a load cell of 5 KN and a cross-head speed of 1 mm/s. The maximum force (g) at 4 mm of probe penetration was calculated as gel strength.

Viscosity: The method described by Shakila et al. (2012) was used for the measurement of viscosity. The 6.67% gelatin solution was prepared and the viscosity was measured at 25 ± 0.5°C using a digital viscometer (ViscoQC 100-R, Anton Paar, Austria) equipped with a No.2 spindle at 60 rpm.

Molecular weight distribution of gelatin

The SDS-PAGE was performed by using the method of Laemmli (1970) in a mini-gel electrophoresis apparatus (Mini-PROTEAN® 3, BioRad Laboratories, Hercules, CA, USA). The 10 mg of gelatin powder was dissolved in 1 ml of warm distilled water (60°C) in an eppendorf tube. The sample was vortexed and kept overnight at room temperature. The 1x sample buffer was prepared by mixing 5x Laemmli buffer with Tris buffer (pH 6.8) in 1:4 ratio. The 10 µl of gelatin sample was mixed with 10 µl of sample buffer, vortexed and the mixture was heated at 100°C for 5 min. The heated samples were loaded onto 12% acrylamide gel and the electrophoresis was carried out at a constant voltage mode of 80 V/slab at 60 mA for 2 h. The gel was stained with Coomassie brilliant blue for 1 h and thereafter destaining for 2 h.

Statistical analysis

Statistical analysis was performed using SPSS (SPSS version 26.0 for windows; SPSS, Chicago, IL, USA). The data consisting of five replicates (n=5) was subjected to one-way ANOVA (Snedecor and Cochran, 1995) using Duncan's post-hoc test with a significance level of P<0.05.

RESULTS AND DISCUSSION

Gelatin yield and quality

pH: The pH of gelatin is an important parameter which influences the functional properties of gelatin (Bahar et al. 2020). In the present study it was found that the type of pretreatment used had a significant effect (P<0.05) on the pH of the resulting gelatin (Table 1). As the concentration of NaOH increased, the pH of gelatin was increased whereas gelatin from only scalded skin showed significantly lower (P<0.05) pH. This might be due to the acid treatment of skin before the extraction process. It was also found that pH increased as the extraction temperature of thermohydrolysis was increased from 60°C to 65°C. This might be because of the decomposition of the collagen triple helical structure which triggered the release of residual NaOH that has not been neutralised properly (Bahar et al. 2020).

Gelatin yield: Heat treatment is required for gelatin extraction because it breaks the hydrogen bonds in the collagen molecule, causing irreversible structural interference by solubilizing collagen to produce gelatin. The degree of collagen to gelatin conversion is determined by the type of the pre-treatment and boiling techniques, which are generally based on temperature, pH, and extraction time (Alipal et al. 2021). The yield of gelatin extracted from goat skin under different pretreatment conditions and different extraction temperatures are shown in Table 1. A comparatively higher yield was obtained by pretreatment with 0.1 M NaOH than with 0.25 M NaOH or scalding. The lower yield with 0.25 M NaOH pretreatment might be due to the strong alkaline conditions enhancing the repulsion between the protein chains in the skin matrix which resulted in increased solubilization and observable gelatin loss (Mad Ali et al. 2016). A shorter period of mild alkaline conditions was effective to disrupt the cross-links in the skin matrix, improving the yield.

An increase in the extraction temperature from 60°C to 65°C resulted in an increased gelatin yield (Table 2). This is in accordance with the results of Bahar et al. (2020) for goat skin gelatin and Kim et al. (2012) for chicken skin gelatin who reported an increase in the yield with the increase in the extraction temperature. However, further increase of temperature above 65°C has resulted in greater thermo-hydrolysis leading to very smaller peptides/hydrolysates thereby affecting gelatin yield and quality. Hence, in the current study maximum of 65°C was tried.

Table 1: Yield and physico-chemical properties of gelatin subjected to different pretreatment steps

Parameters	Scalded goat skin	0.1M NaOH treated goat skin	0.25 M NaOH treated goat skin
pH	3.83±0.01^{a}	3.96±0.01^{b}	4.14±0.03^{c}
Yield (%)	4.15±0.01^{a}	8.06±0.02^{c}	7.82± 0.03^{b}
Gel Clarity(%T)	26.76±0.03^{a}	49.94±0.28^{b}	50.13±0.03^{b}
L*	54.40±0.49^{a}	75.86±0.95^{c}	72.12±0.14^{b}
a*	1.57±0.12^{c}	0.57±0.03^{a}	0.66±0.03^{b}
b*	9.13±0.14^{c}	6.87±0.09^{a}	7.06±0.06^{b}

Means with same superscripts in a row do not differ significantly (P<0.05), n=5.

Table 2: Yield and physico-chemical properties of gelatin subjected to different thermo-hydrolysis temperature

Parameters	60°C for 6 h	65°C for 6 h
pH	4.46±0.02[a]	4.60±0.01[b]
Yield(%)	8.08±0.02[a]	8.42±0.02[b]
Gel Clarity(%T)	51.21±0.04[a]	53.03±0.18[b]
L*	74.76±0.29[a]	76.23±0.30[b]
a*	0.60±0.05[b]	0.53±0.03[a]
b*	6.73±0.12[a]	6.73±0.09[a]

Means with same superscripts in a row do not differ significantly (P<0.05), n=5.

Gel clarity: In commercial applications, gel clarity is a crucial property for the evaluation of the gel quality. When gelatin is employed as a thickening agent in culinary applications, gel clarity plays a crucial role to ensure that unwanted colour or opacity does not negatively impact the quality of the finished product. The lesser clarity of gel extracted from scalded skin might be due to the inorganic and non-collagenous substances which are not removed during the pretreatment. Previous studies have reported a gel clarity of 40.47 % in chicken skin (Bichukale et al. 2018), 71.3% in goat skin gelatin (Zilhadia et al. 2018), 44.28 % in broiler skin (Aykin Dicer et al. 2017) and 24.71 % in pig skin gelatin (Mishra et al. 2023). Gel clarity was increased by increasing the extraction temperature from 60°C to 65°C. Thermo-hydrolysis of connective tissue at 65 °C might help to remove the impurities and enhance the quality of the filtered gelatin which is in accordance with the results of Roy et al. (2017).

Colour of gelatin: Depending on the desired use of the gelatin, both colour and clarity of gelatin are significant aesthetic characteristics. The basic raw materials and extraction techniques employed will determine the colour of the gelatin (Shyni et al. 2014). The instrumental colour of gelatin from goat skin with various pretreatment conditions and different extraction temperatures are presented in Table 1 and 2. It was found that gelatin from scalded skin showed lower *L** and higher *a** and *b** values compared to alkali pretreated skin. This might be due to the remnants of non-collagenous material as the alkali pretreatment is crucial for the removal of non-collagenous substances. As the extraction temperature increased lightness (*L**) value increased and redness (*a**) value decreased. There was no significant difference (p>0.05) in the yellowness (*b**) value of gelatin. This is in agreement with the results of Kim et al. (2012) who reported an increase in the *L** value and decrease in the *a** and *b** values of chicken skin gelatin as the extraction temperature was increased. The difference in the colour of gelatin can be caused by difference in the raw materials and pretreatment steps and drying methods (Hasdar and Randi, 2020).

Microstructure and rheological properties

Based on the aforesaid findings, pretreatment with 0.1 N NaOH and thermo-hydrolysis at 65 °C was found optimal. Hence, these conditions were chosen and the resulting gelatin was further characterized as explained below.

Microstructure of gelatin: The microstucture of gelatin gel from goat skin is presented in Fig. 1. The goat skin gelatin displayed a compact network of protein strands without any void space. This indicates that they cannot be readily disrupted and are unaffected by the external force. One of the factors influencing the microstructure of gelatin is the distribution of α, β and γ-components. The way that protein molecules are arranged and bonded together within the gel matrix directly affects the gel strength of gelatin (Benjakul *et al.,* 2009). The results are in agreement with Mad-Ali et al. (2017) who found denser microstructure of goat skin gelatin with no voids when compared with commercial bovine gelatin.

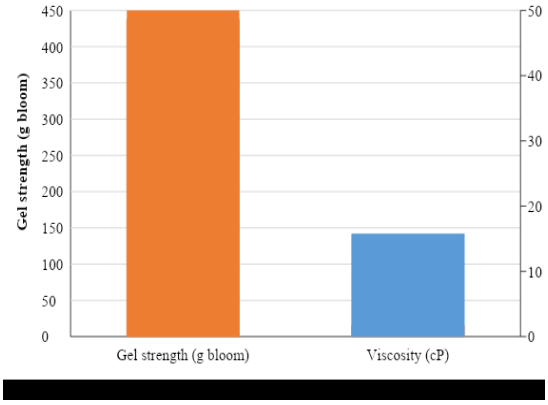

Fig. 1. Scanning electron microstructure of goat skin gelatin

Gel strength: The gel strength of goat skin gelatin was 437.56 g bloom (Fig. 2). It is the force in grams required to press the surface of the gelatin gel by 4 mm. Gel strength, often known as the bloom value, is a common indicator of gelatin quality. There are three levels of bloom: low (<150), medium (150-220), and high (220-300). The mean gel strength value obtained in the present study was higher than that reported by previous researchers. Mad-Ali et al. (2016) reported gel strength of 222.42 g and Zilhadia et al. (2018) reported a value of 219.65 g in goat skin gelatin. This variation might be due to the difference in the source of raw materials which differ in proline and hydroxyproline contents. The hydroxyproline's ability to form hydrogen bonds with the -OH functional group affects the gel strength (Shyni *et al.,* 2014). The shorter chain lengths in gelatin cannot form a strong gel since its inter-junction zones are smaller. Gelation of gelatin was shown to be primarily influenced by its amino acid content and the proportion of its α, β, and γ-components (Mad-Ali et al. 2017).

Viscosity: One of the most important characteristics of gelatin, in addition to gel strength is viscosity, which is a measure of the fluid's resistance to flow. High viscosity gelatin generates harder and extensible gels, whereas gelatin with low viscosity produced short and weak gel. The viscosity of goat skin gelatin in the present study was 15.73 cP (Fig. 2). Viscosity is influenced by molecular size and molecular weight distribution of gelatin. Sompie et al. (2019) reported a viscosity of 8.6-9.24 cP in pig skin gelatin and Tumerkan et al. (2019) reported a viscosity 7.5 cP in chicken skin gelatin. The variation in raw material properties, gelatin extraction techniques and measurement protocols have a significant impact on viscosity (Tumerkan et al. 2019). The higher viscosity of goat skin gelatin can be correlated with the higher molecular weight proteins in gelatin as evident from the SDS-PAGE profile (Fig. 3).

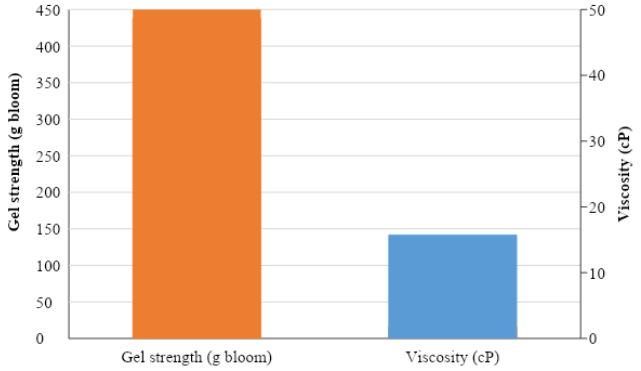

Fig. 2. Gel strength and viscosity of goat skin gelatin

Molecular weight distribution of gelatin

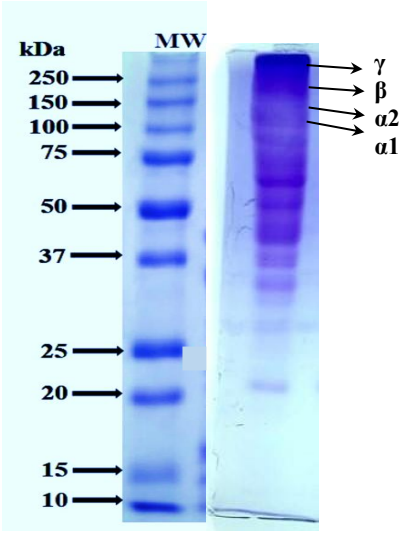

Fig. 3. SDS-PAGE profile of goat skin gelatin

The molecular weight distribution of goat skin gelatin is depicted in Fig. 3. The SDS-PAGE fractionation revealed the presence of distinct α-chains together with β- and γ-chains. It was estimated that the molecular weights of the α-1 and α-2 chains were between 80-125 kDa, β-chain, a dimer ranges from 160-250 kDa and γ-chain ranges from 240-375 KDa. The main component contributing to gelatin's enhanced functional qualities is its α-chain. The presence of very high molecular weight polymers from residual heat-stable cross-links in both forms of gelatin was depicted by the high-intensity bands at the top of the polyacrylamide gel. Significant amounts of proteins/peptides having molecular weights less than the α-chain were also discovered. The protein patterns obtained in the current study were in agreement with results of Mad-Ali et al. (2016) who reported the molecular weights of α-1 and α-2 chains to be 131 and 124 kDa respectively and β-chain had MW of 236 kDa in goat skin gelatin.

CONCLUSION

The present study revealed that pretreatment of goat skin with 0.1 M NaOH for 2 h and extraction temperature of 65°C for 6 h yielded gelatin with better physico-chemical properties in terms of yield, pH, colour and clarity. The structural and rheological properties demonstrated that the quality characteristics of goat skin gelatin were similar to bovine and porcine origin gelatin and may serve as potential alternative. Future studies are required to compare the quality and functionalities of goat skin gelatin with commercially available beef and pork skin gelatin.

COMPETING INTERESTS

The authors do not have any competing interests among themselves or other related to this research work.

ETHICS STATEMENT

The animal experiments conducted in this study were carried out in adherence to the approved protocols by the Institutional Animal Ethics Committee of ICAR-National Meat Research Institute (IAEC No. 007/NRCM/IAEC- 9).

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