Pepsin-Aided Extraction of Gelatin from Goat Skin

Nishanthika Jesslyn, C.M.*, V. Leela, G. Suganya, K.G. Tirumurugaan and H. Pushkin Raj

Dept. of Veterinary Physiology, Madras Veterinary College (TANUVAS), Vepery, Chennai – 600 007

*Corresponding author's Email id: njesslyn@gmail.com

Received: September 2024 *203/24* *Accepted: October 2024*

ABSTRACT

The study was designed to evaluate the effect of pepsin on the extraction of gelatin from goat skin. The goat skin was treated with pepsin at levels of 5 and 10 U/g of tissue, and its effect on gelatin yield, pH and bloom strength was studied. The yield of gelatin was 10.12 % for the control group and 12.6% and 16.5% for the pepsin pretreated groups at 5 and 10 U, respectively. There was no significant difference in the pH of gelatin between the control and pepsin-treated groups. However, there was a significant decrease ($P<0.01$) in bloom strength in the enzyme-pretreated group when compared to the control group. Thus, goat skin can be considered a potential source of commercial gelatin. Pepsin pretreatment can be used to increase the yield of gelatin for industrial and tissue engineering applications.

Keywords: Gelatin, goat skin, pepsin

INTRODUCTION

Gelatin is a natural biopolymer that is obtained by partial hydrolysis of collagen. Gelatin has multifunctional applications in the food, pharmaceutical and cosmetic industries (Alipal *et al.,* 2021). In the food industry, gelatin is predominantly utilized in stabilizing emulsions, to enhance the texture of food, and in meat preservation (Lu *et al.*, 2022). As gelatin is derived from collagen, which is the major type of protein found in the body, it also serves as an excellent biomaterial. Gelatin has low immunogenicity and good biocompatibility and is widely used in various tissue engineering applications (Lukin *et al.,* 2022). Furthermore, gelatin is widely used in the pharmaceutical industry for the encapsulation of drugs and can act as carrier for the release of active ingredients of drugs. The major sources of global gelatine production are contributed by bovine and porcine sources (Samatra *et al.*, 2022). However, the availability of the aforementioned sources is limited in India due to various religious and cultural constraints. Hence, goat skin is an attractive alternative for the traditional sources of gelatin. Enzymatic pretreatments are often used to increase gelatin yield and reduce extraction time. The studies exploring the influence of enzymes in gelatin extraction from goat skin is limited. In this context, the present study aims to develop a protocol for the enzymatic pretreatment of goat skin with pepsin in order to extract gelatin.

MATERIALS AND METHODS

The study was performed at the Department of Veterinary Physiology, Madras Veterinary College, Chennai - 07. The steps involved in gelatin extraction in the present study are given in Figure 1.

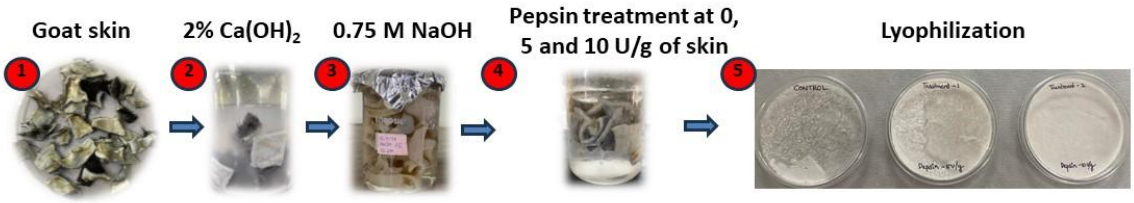

Fig. 1. Steps involved in the extraction of gelatin from goat skin

Preparation of skin

Goat skin was procured from Villivakkam slaughterhouse, Chennai, immediately after slaughter and transported to the laboratory using ice packs. The skin was then thoroughly washed with running tap water to remove blood and dirt. The skin was then treated with 2 % lime for 24 hrs for depilation. The depilated skin was then cut into small pieces of approximately 2 x 2 cm. The prepared skin was placed in zip-lock bags at – 20 ^{o} C until use.

Removal of non-collagenous proteins

The prepared skin was soaked in 0.75 M NaOH for 6 hrs at room temperature with skin to solution ratio of 1:4. This facilitated the removal of non-collagenous proteins from the skin. The skin was then washed extensively several times until the pH of the wash water turned neutral.

Gelatin extraction

Gelatin extraction was done according to Ahmad *et al*. (2021) with slight modification. The skin was immersed in 1% HCl for 2 hrs at room temperature with a skin-to-solution ratio of 1:4. After acid treatment, the skin was then washed thoroughly for complete removal of acid until the pH returned to neutral. This was followed by pretreatment of skin with pepsin. The swollen skin samples were treated with pepsin at the level of 5 and 10 U/g of skin with a skin-to-enzyme solution ratio of 1:4 (w/v). It was then transferred to a water bath maintained at 37 ^{o}C for 18 hrs. The enzyme activity was then inactivated by adjusting the pH to 7 and keeping the mixture at 4 ^{o}C for 1 hr. Gelatin was then extracted by thermal hydrolysis at 60 ^{o}C for 6 hrs with skin to skin-to-water ratio of 1:5. The solution mixture was then filtered with double-layered cheesecloth to separate the skin remnants from the extracted gelatin. This was followed by further filtration of the extracted gelatin using a Whatman filter. The filtered gelatin extract was then freeze-dried in petri plates using VirTis Genesis Pilot Lyophilizer to obtain dry gelatin.

Yield

The yield of gelatin was calculated using the following formula (Mad-Ali et al., 2017)

$$Yield\ (\%) = \frac{Freeze\ dried\ weight\ of\ gelatin\ (g)}{Wet\ skin\ weight\ (g)}\ x\ 100$$

pH

The pH of gelatin was measured as described by Ahmad *et al*. (2019). 1% gelatin solution was prepared by mixing 0.2g of the sample with 20 ml of distilled water and heating the mixture at 50^{o}C until complete dissolution of gelatin. pH was measured after cooling the solution to room temperature using Cyber Scan EC-Ph Tutor, Eutech Instruments.

Gel strength

Gel strength was measured using a Texture Analyser (Model TA-XT). A 6.6%

gel solution was prepared in distilled water using a 50 mL glass beaker. The mixture was then transferred to a water bath at 60^{o}C until complete dissolution of gelatin. The diameter and height of the samples were 3.8 and 2.7 cm, respectively. The measurements were taken using a ½ inch spherical probe (P/0.5S), and the plunger speed was kept at 0.5 mm/s to pierce a depth of 4 mm. (Ahmad *et al.*, 2021).

RESULTS AND DISCUSSION

Yield

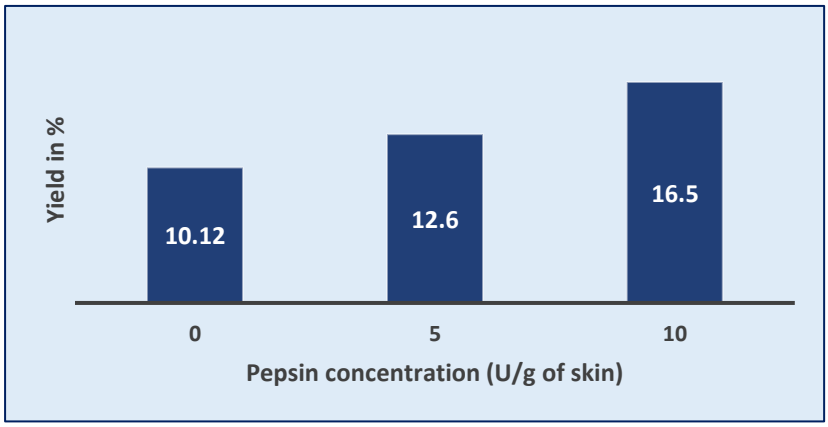

Figure 2: Yield of gelatin (%)

The yield of gelatin obtained in the present study are given in Fig.2. The yield of gelatin was higher in the enzyme pretreated group when compared to the control group. Lassoued *et al.* (2014) reported a significantly higher yield of gelatin from thornback ray fish with inclusion of pepsin at 5 U/G of tissue during acid treatment. Similarly, Ahmad *et al.* (2021) demonstrated an increase in the yield of gelatin from bovine skin with increasing concentration of pepsin. Pepsin cleaves peptide bonds located in the telopeptide region of native collagen, which enhances the solubilization of collagen during the acid swelling process, resulting in a higher yield. concentration of pepsin. Pepsin cleaves peptide bonds located in the telopeptide region of native collagen, which enhances the solubilization of collagen during the acid swelling process, resulting in a higher yield.

Table I. The mean ± SE values of pH and bloom strength of gelatin

Pepsin concentration (U/g of skin)	pH (n = 3)	Bloom strength (n = 3)
0	5.31 ± 0.01	337 ± 6.74
5	5.28 ± 0.03	122 ± 6.00
10	5.32 ± 0.02	67 ± 4.41

pH

The pH value of gelatin is crucial for determining its quality, as it influences the viscosity and gel strength of gelatin. The pH values of gelatin obtained in the present study are given in Table I. The pH values were within the reference range of 3.8 to 5.5 specified for commercial gelatin

(GMIA, 2012). The pH of the extracted gelatin did not differ significantly (P<0.05) between the control and treatment groups. Similar results were also obtained by Yahdiana *et al*. (2018) wherein a pH of 5.03 was obtained from goat skin gelatin by acid pretreatment.

Gel strength

The gel strength determines the mechanical properties of gelatin. It greatly varies depending on the molecular weight distribution, amino acid composition and extraction procedure of gelatin. The gel strength of the control and pepsin-pretreated gelatin is given in Table 1. There was a significant decrease in the gel strength in pepsin pepsin-pretreated group when compared to the control group. Similar findings were reported by Ahmad *et al.* (2021) in the extraction of gelatin from bovine skin, wherein the gel strength of gelatin decreased with an increase in pepsin concentration. In a concurrent finding by Ahmad *et al.* (2020), the bloom strength of gelatin decreased significantly with enzymatic pretreatments using the enzymes zingibain and papain. Thus, enzymatic treatment may result in a more intense breakdown of the polypeptide chain, leading to the formation of low molecular weight fragments, which reduces the bloom strength of gelatin formed.

SUMMARY

Goat skin can be considered a valuable source of gelatin. Pepsin pretreatment significantly increased the yield of gelatin when compared with the control. However, there was a significant decrease in gel strength with increasing concentration of pepsin.

REFERENCES

Ahmad, T., Ismail, A., Ahmad, S.A., Khalil, K.A., Teik Kee, L., Awad, E.A. and Sazili, A.Q., 2019. Physicochemical characteristics and molecular structures of gelatin extracted from bovine skin: effects of actinidin and papain enzymes pretreatment. International Journal of Food Properties, 22(1), pp.138-153.

Ahmad, T., Ismail, A., Ahmad, S.A., Khalil, K.A., Kee, L.T., Awad, E.A. and Sazili, A.Q., 2020. Extraction, characterization and molecular structure of bovine skin gelatin extracted with plant enzymes bromelain and zingibain. Journal of food science and technology, 57, pp.3772-3781.

Ahmad, T., Ismail, A., Ahmad, S.A., Abdul Khalil, K., Awad, E.A., Akhtar, M.T. and Sazili, A.Q., 2021. Recovery of gelatin from bovine skin with the aid of pepsin and its effects on the characteristics of the extracted gelatin. Polymers, 13(10), p.1554.

Alipal, J., Pu'Ad, N.M., Lee, T.C., Nayan, N.H.M., Sahari, N., Basri, H., Idris, M.I. and Abdullah, H.Z., 2021. A review of gelatin: Properties, sources, process, applications, and commercialisation. Materials Today: Proceedings, 42, pp.240-250.

GMIA (Gelatin Manufacturers Institute of America) (2012). Gelatin Handbook. version January 2012, New York: GMIA.

Lassoued, I., Jridi, M., Nasri, R., Dammak, A., Hajji, M., Nasri, M. and Barkia, A., 2014. Characteristics and functional properties of gelatin from thornback ray skin obtained by pepsin-aided process in comparison with commercial halal bovine gelatin. Food hydrocolloids, 41, pp.309-318.

Lu, Y., Luo, Q., Chu, Y., Tao, N., Deng, S., Wang, L. and Li, L., 2022. Application of gelatin in food packaging: A review. Polymers, 14(3), p.436.

Lukin, I., Erezuma, I., Maeso, L., Zarate, J., Desimone, M.F., Al-Tel, T.H., Dolatshahi-

Pirouz, A. and Orive, G., 2022. Progress in gelatin as biomaterial for tissue engineering. Pharmaceutics, 14(6), p.1177.

Mad-Ali, S., Benjakul, S., Prodpran, T. and Maqsood, S., 2017. Characteristics and gel properties of gelatin from goat skin as affected by extraction conditions. Journal of Food Processing and Preservation, 41(3), p.e12949.

Samatra, M.Y., Noor, N.Q.I.M., Razali, U.H.M., Bakar, J. and Shaarani, S.M., 2022. Bovidae-based gelatin: Extractions method, physicochemical and functional properties, applications, and future trends. Comprehensive Reviews in Food Science and Food Safety, 21(4), pp.3153-3176.

Yahdiana, H., Irwandi, J. and Effionora, A., 2018. Characterization and functional properties of gelatin extracted from goatskin. International Food Research Journal, 25(1), pp.275-281.