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Extraction and characterization of pig skin gelatin compared to commercial porcine gelatin

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ABSTRACT

This study was conducted to extract gelatin from pig skin and to evaluate and compare its physicochemical properties with those of commercial porcine gelatin. Whitish yellow-coloured gelatin powder was extracted from pig skin through acetic acid pre-treatment, heating, filltration and drying steps and the average yield was 5.98 % (w/w). The protein, moisture, fat and ash content of PSG were 90.24, 8.13, 0.60 and 0. 56 %, respectively. The gel (6.67%) prepared from PSG showed a transmittance of 24.71 % and a pH of 6.45. The electrophoretic pattern of the gelatin samples showed the presence of both α1 and α2 chains with the highest molecular weight band observed at around ~ 200 kDa. The L^*, a^*, and b^* values of PSG were recorded as 79.28, 0.58 and 5.79, respectively. Compared to commercial porcine gelatin, PSG had lower hydroxyproline content but a higher pH ($p < 0.05$) value. Our findings suggest that good quality gelatin with optimal properties can be extracted from low-value pig skin which can provide a suitable alternative to traditional gelatin derived from pork bones or beef bones and skin.

Keywords: *Gelatin, Pig skin, Hydroxyproline content, Proximate composition, Colour*

INTRODUCTION

Gelatin is a complex mixture of polypeptides usually produced by partially hydrolyzing collagen which is abundantly found in the skin, bones, blood vessels, cartilage, tendons, ligaments and connective tissues of mammals as well as poultry and fish. It is a biodegradable and biocompatible versatile biopolymer with unique water binding, thermo-reversible gel forming, film forming, stabilizing, foaming, and with emulsifying ability (Vidal et al. 2020). The cost-effectiveness and numerous functionalities of gelatin make it irreplaceable and widely used in various food, pharmaceutical, and cosmetic applications. The primary industry to use gelatin with the highest growth expectations is the food sector. The need for gelatin is being driven by the expanding functional food and beverage industry,

together with the nutraceuticals and sports nutrition sectors. Gelatin from land animals is more stable and has greater rheological properties than that from aquatic or marine species (Norland 1990). Mammalian gelatin has typically been used because of its high melting, gelling, and thermo-reversibility points (Gudmundsson 2002). Several investigators have tried to extract and characterize gelatin from various natural sources like fish skin (Tkaczewska et al. 2018; Kittiphattanabawon et al. 2016); chicken skin (Chand et al. 2021; Tümerkan et al. 2019 and Aykin-Dincer et al. 2017); buffalo hide (Mulyani et al. 2017); bovine skin (Ahmad et al. 2018 and Raja Mohd Hafidz et al. 2011) and pig skin (Sompie et al. 2015). Globally, majority of commercial porcine gelatin are being prepared from pig bones. But, only limited investigators have tried to extract and characterize gelatin from pig skin till date. Hence, the current study was designed to produce high-quality gelatin from pig skins and compare its physicochemical characteristics to those of commercial porcine gelatin.

MATERIALS AND METHODS

Source and pre-treatment of the raw materials

Commercial porcine gelatin (G1890-100G, Type A, Bio reagent and Batch N -SLCN0074) was procured from Sigma Aldrich for use in this study.

Pig skin

Pig (Large white Yorkshire of 6-7 months) skins without hair were collected from the retail meat shop, Hyderabad and were washed thoroughly under running potable tap water to remove all the visible dirt, hair, blood clots and stains for further extraction of gelatin. All separable fat, fascia and attached meat residues were trimmed off and skin were packed in LDPE bags and frozen at -20 °C till further use.

Extraction of gelatin from pig skin

Extraction of pig skin gelatin (PSG) was carried out according to Sompie et al. (2015) with slight modifications. Frozen pig skins were thawed at refrigeration temperature (4 ± 1 °C) for 24 h prior to use. Then skins were washed with potable tap water thoroughly and cut into small pieces of sizes approximately 0.5×0.5 cm^2. Skin pieces were soaked in 0.5 M acetic acid solution of 4 % (v/v) concentration (1:4 w/v) for 24 h with intermittent mixing. Then the acid solution was drained out and skin pieces were filtered with the help of a four layered muslin cloth and then washed several times with tap water till the wash water pH became 7.0. Later hot water extraction of gelatin was carried out with distilled water (1:3 w/v) at 60 °C for 6 h with intermittent stirring. After extraction the liquid part was separated from the residual skins by filtering through a four layered muslin cloth. Then the extracted gelatin was kept at refrigeration temperature for 10-12 h to observe the gelling behaviour of the gelatin. Also, the solidified fat layer present at the top portion of the liquid gel was removed manually with the help of a spatula. Then gelatin extract was heated at 60 °C in a hot water bath for few minutes to get it liquefied before keeping for drying. The gelatin solution was poured in the form of a thin layer over a glass tray and dried in hot air oven at 60 °C for 24-26 h followed by removal of dried sheets and grinding with a home mixer grinder to form the gelatin powder. The dried gelatin powder was packed in air tight PET bottles and stored at room temperature for further analysis.

Physico-chemical properties of gelatin

Yield of extracted gelatin

The yield of dried gelatin was calculated based on weight of fresh skin using the following formula (Mulyani et al. 2017):

$$\text{Yield (\%)} = \frac{\text{Weight of dried gelatin (g)}}{\text{Weight of fresh hide/skin (g)}} \times 100$$

pH value

The pH was evaluated by mixing one g of powdered gelatin with distilled water (10 mL) and heating at 60 °C for 10 min followed by cooling and measuring pH (HANNA instruments, HI 2216, Europe).

Colour measurement

According to Al-Hassan (2020), the colour of gelatin powder was assessed using a colour reader CR-20 (Konica Minolta, Inc.) using the Hunter system, and colour was expressed in terms of brightness (L*), redness (a*), and yellowness (b*). Using a white calibration cap, the equipment was calibrated. The values of L vary from 100 = white to 0 = black, while the values of a and b are respectively -50 = green and +50 = red and -50 = blue and +50 = yellow.

Gel clarity

Clarity of the gelatin samples was determined by the method of Avena–Bustillos et al. (2006). A UV spectrophotometer (model UV-1800, Shimadzu) was used to measure the transmittance (% T) at 620 nm against distilled

water after heating the gelatin solution (6.67%) at 60 °C for 1 hour.
% Transmittance = Antilog (2-absorbance)

Proximate composition

According to methods outlined by the Association of Official Analytical Chemists (AOAC 1995), dried gelatin powders' moisture, crude fat, protein, and ash levels were measured using a hot air oven, the Soxhlet apparatus, the Kjeldhal apparatus, and the Muffle furnace, respectively.

Estimation of Hydroxyproline content

The method of Nueman and Logan (1950) was used to determine the dried gelatin powder's hydroxyproline content, with a few adjustments recommended by Naveena and Mendiratta (2001).

Water activity

A water activity metre (Hygrolab-3R, Rotronic, Switzerland) was used to measure the water activity. The sample container was placed inside the chamber after being filled to the 3/4 mark. After the beep, the water activity was noted in quick mode.

Determination of sensory quality of gelatin gel

In test tubes with screw-on lids, gelatin solutions of 6.67% (w/v) concentration were made by combining 0.5 g of dried gelatin powder with 7 mL of distilled water. The tubes were then lightly closed with the screw caps in a hot water bath maintained at 50 °C until the gelatin got dissolved. A group of 21 educated experts, including postgraduate students and scientists from the NMRI, ICAR, Hyderabad, India, evaluated the odour and colour parameters of the gelatin sample. Participants were instructed to remove the screw caps, inhale the odours of the contents and assess the colours, and rate the intensity of the odours and colours on a six-point descriptive scale that includes, 0: no odour, transparent and clear, 1: very light, can be sensed when carefully evaluated, slightly whitish 2: mild, easily detectable, whitish 3: strong but not offensive, very light yellowish 4.: strong and offensive, light yellowish 5: very strong and offensive and yellowish.

SDS-PAGE fractionation of gelatin

For powdered gelatin, samples (10 mg) were dissolved in distilled water (1 mL) maintained at 60 °C. Powdered gelatin extracts were mixed with 1x sample buffer solution in 1:1 (v/v) ratio to have a final concentration of 10 mg/mL and heated at 100 °C for 5 min and the final solution (10 µL) and high molecular weight protein marker (4 µL) were loaded onto the 12% gel and SDS-PAGE fractionation was performed as per the procedure outlined by Laemmli (1970) with mini electrophoresis apparatus (Mini-PROTEAN® 3, BioRad Laboratories, Hercules, CA, USA) at a constant voltage of 80 V for about 2 h or till the dye front reached 0.5 cm from the lower edge of the gel. Staining of the gel was done with Coomassie brilliant blue for 1 h followed by 2 h of destaining.

Statistical analysis

The physico-chemical properties of the PSG and commercial porcine gelatin were studied on a three different occasion (n=3). The data obtained were analysed by analysis of variance (ANOVA) using SPSS 22.0 software (Chicago, II, USA). Duncan's post-hoc test was used to determine significant differences at a significance level of $P < 0.05$. The results were expressed as mean ± standard error.

RESULTS AND DISCUSSION

Yield

The average yield of PSG was 5.98 % on a wet weight basis. In contrast, the yield obtained in the current study were lower than those observed by Sompie et al. (2012) for pig skin (10.22- 12.67 %); Hasdar et al. (2019) for sheep skin (23.10-23.33 %) and Ahmad et al. (2018) for bovine hide (7.09 - 19.71 %) gelatins. During the gelatin extraction process, non-collagenous protein is removed by the alkali and acid treatment, and the sample swells in the acid solution. The thermo-hydrolysis process used in the hot water extraction solubilizes the gelatin, which is subsequently separated. In this study, it was observed that pig skins tend to swell less during acid treatment. Lower yields were produced from pig skins, possibly because the cross links weren't properly opened during swelling (Shyni et al. 2014). The gelatin yield amongst various raw materials have reportedly varied, mostly due to variations in the extraction time, pretreatment method, washing phase, collagen content, and skin components (Sinthusamran et al. 2014).

pH

The extracted pig skin and commercial porcine gelatins both comes under the Type B group with pH values of 6.45 and 5.07, respectively (Table 1). Type B gelatin has the lower viscosity and stronger gel strength at pH 5 (Cole 2000),

which evidenced the importance of pH on rheological characteristics of gelatin. The earlier studies have reported the pH values of 5.62 to 5.73 in camel bone gelatins (Al-Hassan 2021), 2.40- 2.51 in bromelain treated bovine hide gelatin (Ahmad et al. 2018) and 5.52- 7.45 in duck foot gelatin (Abedinia et al. 2017). The various acid and alkali pre-treatments employed during the extraction process may be the cause of the variations in pH values of the gelatin samples.

Proximate composition

The proximate composition of commercial porcine gelatins and PSG was presented in Table 1. The PSG was found to have 90.24, 8.13, 0.56, and 0.59 % of protein, moisture, ash, and fat, respectively. These results were concurred with the information provided by Sompie et al. (2015), wherein PSG protein was reported to be 88.52 %. The protein content in both PSG and commercial porcine gelatin samples were remained comparable to each other. The low-fat level suggested that fat was removed from the skin effectively. In comparison to commercial porcine gelatin, the PSG showed higher ($p < 0.05$) moisture and fat levels. However, the moisture content of PSG was lower than the maximum (15%) permitted for edible gelatin (GME 2008). In light of the fact that the permitted top limit for edible gelatin is 2.6% (Jones 1977), the PSG's relatively low ash level demonstrated their exceptional quality. There may have been a difference in the amount of ash in PSG compared to commercial porcine gelatin due to the formation of inorganic compounds during the extraction procedure utilising acid solutions.

Table 1: Yield, pH, hydroxyproline, proximate analysis and water activity of PSG and commercial porcine gelatin

Parameter	PSG	Commercial porcine gelatin
Yield	5.98 ± 0.14	NA
pH	6.50 ± 0.17 [a]	5.07 ± 0.02 [b]
Hydroxyproline (%)	18.85 ± 0.33 [b]	24.27 ± 0.08 [a]
Protein (%)	90.24 ± 0.50 [a]	91.96 ± 0.23 [a]
Moisture (%)	8.13 ± 0.26 [a]	6.77 ± 0.05 [b]
Fat (%)	0.59 ± 0.34 [a]	0.45 ± 0.01 [b]
Ash (%)	0.56 ± 0.04 [b]	0.83 ± 0.02 [a]
Water activity (a_w)	0.46 ± 0.02 [a]	0.35 ± 0.01 [b]

NA: Not applicable; Mean ± S. E with different superscripts in a row differ significantly ($p < 0.05$), n=3

Colour and gel clarity

The PSG and commercial porcine gelatin samples had significantly different L*, a*, and b* colour values ($p < 0.05$) (Table 2). The difference between the L* value of PSG and commercial porcine gelatin was significant ($p < 0.05$). Positive a* values were present in both gelatin samples, and PSG's values were significantly ($p < 0.05$) lower. In comparison to commercial porcine gelatin, the b* value of PSG was significantly ($p < 0.05$) lower, indicating that PSG was less yellow. These findings demonstrated how variables like source material and extraction settings affected the colour of extracted gelatin.

Transmittance of the gelatin is essential for food applications because gelatin with a high transmittance has no negative effects on the colour or opacity of the final product (Jamilah et al. 2011). The PSG had a much lower transmittance (%) than commercial porcine gelatin (Table 2), which had good transmittance (%). This might be because, during extraction inorganic, proteinaceous, and mucosubstance impurities were either added or weren't eliminated. Accordingly, turbidity in gelatin solution could be caused by non-settling, unfilterable particulate matter. Clarity of gelatin solutions is directly influenced by the efficiency of filtration during extraction (Muyonga et al. 2004). Depending on its intended usage, the colour and gel clarity of gelatin are significant aesthetic qualities.

Table 2: Colour and transmittance (%) values of PSG and commercial porcine gelatin

Parameter	PSG	Commercial porcine gelatin
L*	79.28 ± 0.22 [b]	93.72 ± 0.09 [a]
a*	0.58 ± 0.03 [b]	1.10 ± 0.04 [a]
b*	5.79 ± 0.08 [b]	13.98 ± 0.06 [a]
Transmittance (%)	24.71 ± 0.42 [b]	90.40 ± 0.27 [a]

Mean ± S. E with different superscripts in a row differ significantly ($p < 0.05$), n=3

Hydroxyproline content

The hydroxyproline is one of the main amino acid in gelatin in addition to glycine, proline, alanine and glutamic acid (Atma 2017). As mentioned in Table 1, the hydroxyproline content of commercial porcine gelatin (24.27 %) was significantly ($p < 0.05$) higher than PSG (18.85 %). The higher hydroxyproline content suggested that the commercial porcine gelatin may exhibit better rheological properties by structurally stabilising the triple helix of collagen and developing a strong gel structure (Ktari et al. 2014). This is because the hydroxyl groups of hydroxyproline form hydrogen bonds with available water molecules. Hydroxyproline content of PSG samples were higher compared to previously reported values by Sarbon et al. (2013). This might be because different extraction processes produce gelatin with varying degrees of purity (Tümerkan et al. 2019).

Water activity

One of the most significant elements influencing microbial development is the water in food, including its location and availability. Water can be viewed as a physical component of the food as well as a chemical molecule required for microbial development and their enzymatic activity (Frazier 1991). The activity coefficient, also referred to as water activity, is the ratio of the vapour pressure of water in food (p) to the vapour pressure of pure water (p_0) at the same temperature. (Scott 1957). The a_w values below 0.7 are typical for dried foods (Lewicki 2004). Both PSG and commercial porcine gelatin samples used in the current study exhibited water activity < 0.5. Although water content and water activity are not directly proportional, the lower the water content, the lower the water activity (Mishra et al. 2015). Food stability may depend more on active water than on the total amount of water present (Rahman and Labuza 2007). The findings are in line with those of Thomas (2007), who noted that a decrease in moisture content was accompanied by a decrease in water activity.

Sensory evaluation

Table 3 represents the comparison between the odour and colour parameters of gelatins extracted from pig skin and commercial porcine gelatin. Visual sensory analysis could not identify any notable ($p > 0.05$) aroma differences between PSG and commercial porcine gelatin (Table 3). But, both gelatins were found to have a very mild odour because the hedonic score was around 1.0 to 1.05. PSG was slightly whitish to whitish in colour. It can be deduced that the odour and colour of the PSG examined in this study did not have an impact on consumers and were comparable to those of commercial porcine gelatin products sold on the market. Choi and Regenstein (2000) claimed that adding activated carbon treatment right before extraction can further lessen odour and improve customer acceptance of the gelatin.

Table 3: Sensory properties of PSG and commercial porcine gelatin

Parameters	PSG	Commercial porcine Gelatin
Odour	1.05±0.05 [a]	1.00±0.00[a]
Colour	0.09±0.07[b]	1.43±0.11 [a]

Mean ± S.E with different superscripts in a row differ significantly ($p < 0.05$), *n*=21. PSG: pig skin gelatin

SDS-PAGE fractionation and Molecular weight distribution of gelatins

The molecular weight distribution of PSG and commercial porcine gelatin is given in Fig. 1. Both PSG and commercial porcine gelatin had distinctive protein patterns, as can be seen in Fig. 1. The primary components of both the samples of gelatin gels were found to be α1 and α2 chains. It was claimed that gelatins with increased α chain content have better functional qualities (Gomez-Guillen et al. 2002) and allow for a better stabilised and more structured triple helix (Tümerkan et al. 2019). According to Silva et al. (2014), type I collagen is distributed in the protein patterns in bands with a molecular weight of about 100 kDa (α1). Also, β component (covalently bonded α chain dimer) with molecular weight ~ 200 kDa was found in both PSG and commercial porcine gelatin. PSG samples showed evidence of the presence of peptides with molecular weights under 100 kDa. The excessive heat-induced hydrolysis of collagen and peptide inter- and intramolecular linkages during the manufacture of gelatin may be the cause of the low molecular weight peptides (Muyonga et al. 2004). These fragments were also found to be responsible for gelatin's poor viscosity, low melting and setting point, prolonged setting time, and diminished gel strength (Nagarajan et al. 2012). However, both gelatins contained very heavy polymers (245 kDa) (γ-chain), which could be leftover cross-linked proteins that are heat stable. These findings suggested that PSG exhibited good molecular stability and contained a large proportion of α- and β-chains.

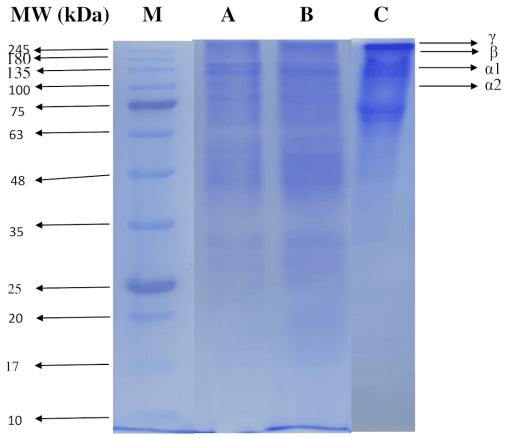

Fig. 1: SDS PAGE image of gelatin. M: Molecular weight protein markers; A-B: Pig skin gelatin; C: Commercial porcine gelatin

CONCLUSION

The results of the current study suggest that pig skins may be a potential source of gelatin, which may be recovered from these skins using methods such as heating, filtration, drying, and pre-treating with acetic acid. The recovered gelatin possessed similar physical, chemical, and sensory qualities to commercial pig gelatin. Future studies should

look into more intensive extraction conditions in order to see if they can further boost the yield of PSG, which was 5.98% on a fresh skin weight basis. The protein composition and molecular weight distribution characteristics of the gelatin that was isolated from pig skin resembled those of commercial porcine gelatin. The findings of the sensory evaluation and physicochemical attributes indicated that PSG might be a suitable replacement for commercial gelatin made from beef or pork bones and skin in a number of food applications.

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