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Biopolymer Characterization: Gelatin from Poultry Waste as a Sustainable Alternative

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ABSTRACT

The aim of this study was characterization of gelatin from chicken skin, head and feet blend by using two different food grade acids (Acetic acid and Citric acid). The extracted gelatins were analyzed for physico-chemical, proximate analysis, instrumental colour profile, textural profile analysis and determination of gel strength and viscosity. The gelatin treated with acetic acid has revealed higher protein percentage as well as gel clarity. No significant difference ($P \geq 0.05$) in the yield was observed between the two groups. A significant increase ($P \leq 0.05$) in pH and textural properties were observed in the gelatin treated with acetic acid. The hydroxyproline content, L^* and b^* values, gel strength and viscosity of acetic acid treated gelatin were significantly ($P \leq 0.05$) lower as compared to citric acid treated one. Gelatin extracted from chicken skin, head and feet (SHF) blended with 3% acetic acid represents a potential alternative to the mammalian gelatin.

Key words: Chicken gelatin, Acid treatment, Gel clarity, Gel strength, Viscosity

INTRODUCTION

In the slaughter flow of poultry-processing plants, by-products generated can represent up to 37% of the live weight of the chicken. Feathers, skin, bones, viscera, feet and head are the most abundant by-products in the carcass of broilers, representing 26.4% of the live weight of birds after slaughter. Valorization of poultry processing waste into high-value products will definitely promote the 'zero waste' concept and minimize their adverse impacts on the environment. Animal co-products are considered the most important sources of collagen and gelatin. Gelatin is a natural polymer derived from partial hydrolysis of collagen protein. Owing to its distinctive structure of amino acids and physico-chemical properties, gelatin exhibits good applicability in food as a texture modifying, water-binding, foaming, emulsifier and colloid stabilizer (Karim and Bhat, 2009), biodegradable packaging material and micro-encapsulating agents (Guillen et al. 2011).

The gelatin derived from poultry processing waste has been shown to contain amino acids, secondary structure and molecular weight similar to those of mammalian gelatin. Moreover, this novel source would further encourage efforts to exploit untapped available resources and recycle industrial waste (Sarbon et al. 2013). At the first stage of gelatin production, acidic pre-treatment yields type A gelatin, while an alkaline hydrolysis yields type B gelatin (Alipal et al. 2021). During the past decade, there has been an intensive trend in gelatin derived from non-mammalian sources. Chicken by-products acts as an an excellent source of gelatin that can be used for a wide variety of purposes in the industrial sector. Using the byproducts of chicken (skin, head and feet) together as raw material for the extraction of gelatin results in gelatin with good properties. The present study aimed to analyze the Physico-chemical, proximate analysis, instrumental colour profile, textural profile analysis and determination of gel strength and viscosity of gelatin extracted from the chicken by-products blend (chicken skin, heads and feet) by using two different food grade acids (acetic acid and citric acid).

MATERIAL AND METHODS

Procurement of chicken skin, head and feet sample

Poultry processing waste namely skins without feathers, head and feet of freshly slaughtered Indbro slow growing broiler chicken (6-8 weeks of age) was procured from the Poultry Processing Plant of ICAR-National Meat Research Institute (ICAR NMRI), Hyderabad as per standard protocols. The collected skin samples were washed thoroughly and after trimming of all separable fat, fascia, attached meat residues and skin was chopped into 1 × 1 cm^2 pieces. Chicken heads were washed under running tap water to remove impurities. The chicken feet was de-nailed manually and washed twice with potable water to remove dirt and blood. The chopped skin, head and feet all together was minced using commercial meat mincer and collected for storage at −20°C until use.

Experimental design

Gelatin extraction

Chicken gelatin was extracted from minced chicken skin, head and feet according to Gundem and Tarhan (2021), Ee et al. (2019) and Chakka et al. (2017) with some modifications. Thawed chicken skin was cut into pieces and then cleaned with potable tap water head and feet were minced with a meat mincer (LE/95 Meat Mincer Scharen, Germany). Chicken skin-head-feet (SHF) blend (1:1:1 w/w) was treated with 0.5 M sodium hydroxide (NaOH) (1/10) for 18 hrs to remove the non-collagenous proteins followed by removal of alkali material by filtration using a muslin cloth. The residue was then washed with distilled water till pH became neutral. After completing the pre-treatment with alkali, the obtained residue was treated for 18 hrs with 3% acetic acid (1:1 w/v) and/or 3% citric acid (1:1 w/v). Following the acid-hydrolysis process, the samples were washed under running tap water again until the pH reached neutral. The washed SHF blend sample were then subjected to thermo-hydrolysis (skin:distilled water, 1:2 w/v) in a water-bath (JEIO TECH BS-11, Korea) at 75°C for 6 hrs, filtered through muslin cloth to remove the residue. The gelatin solution was poured as a thin layer over a glass tray and dried in hot air oven at 60 °C for 24-26 hrs till dry gelatin sheets were formed followed by grinding with a mixer grinder to form the gelatin powder and packed in laminate pouches and stored at room temperature for further analysis.

Physico-chemical properties of gelatin

Gelatin Yield

The yield of dried gelatin was calculated based on the weight of chicken SHF using the following formula (Mulyani et al. 2021).

$$\text{Yield \%} = \frac{\text{(Dry gelatin weight)}}{\text{(Chicken SHF weight)}} \times 100$$

Determination of pH value

The pH value of dried gelatin samples was determined by the method of Aykin-Dincer et al. (2017) with minute modifications. One gram of dried gelatin sample was mixed in 10 ml of distilled water and then the solution was heated at 60^0 C for 10 min to dissolve properly and cool to 25 °C in a water bath and pH was measured.

Determination of gel clarity

Gel clarity was determined according to the method of Avena-Bustillos et al. (2006). The gelatin solution (6.67%) was heated at 60^0 C for 1 hr and the clarity of the obtained gel samples were assessed using spectrophotometrically by transmittance (%T) measurement at 620 nm using UV-VIS spectrophotometer. (Model: UV-1700 Pharma-Spec, SHIMADZU, Japan).

% Transmittance = Antilog (2-absorbance)

Estimation of hydroxyproline and collagen content

Hydroxyproline (HP) content of the dried gelatin samples was determined based on the procedure of Nueman and Logan (1950) with few modifications as suggested by Naveena and Mendiratta (2001). The hydroxyproline content of chicken SHF gelatin powder was determined by referring to a standard graph and was expressed as mg/g of skin. Collagen content was calculated by multiplying hydroxyproline content with 7.14 and was expressed in mg/g tissue (Dransfield and Sosnicki, 1999).

Estimation of collagen solubility

The collagen solubility was measured as per the method of Mahendrakar et al. (1989). A 250 ml beaker containing 5g of SHF blend was immersed in the water bath which was covered with a watch glass. The water bath was heated to reach the boiling point (100^{0}C) and maintained for 30 minutes without any interruptions. The sample was removed from the water bath and 30 ml of distilled water was added and homogenized for two minutes. Then the extracted sample was centrifuged at 4000 rpm for 30 minutes. Aliquots of cooked out juice and centrifugate were hydrolysed at 105 ^{0}C for 18 h and the soluble hydroxyproline was calculated.

Collagen Solubility (%) = 7.4×% HP solubilized

Proximate analysis

The moisture content was determined by hot air oven drying, protein by automatic Kjeldahl method, fat by Soxhlet extraction with petroleum ether and ash in muffle furnace as per AOAC (1995).

Instrumental colour profile

Instrumental colour 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. The CIE (Commission International d'Eclairage - International Commission on illumination's) L^* (Lightness), a^* (redness) and b^*(yellowness) values were measured and recorded.

Texture profile analysis

Texture profile analysis (TPA) of gelatin gel was carried out using the Texturometer (Model H1KF; Tinius Olsen, Redhill, England). About 30 ml of gelatin gel (6.67% w/v, 2.7 cm in height and 3.8 cm in diameter) were aged for 16–18 h at 7 °C. A spatula was used to carefully remove the samples from the beaker, and texture profile analysis was carried out immediately using the analyzer probe with a diameter of 75 mm (flat bottom). To simulate chewing, the test sample was put on a platform fixture and compressed through two cycles at a cross head speed of 0.5 mm/s to 80% of its original height. The TPA parameters were used to represent the textural characteristics from force and area measurements. The parameters for adhesion, chewiness, cohesiveness, gumminess, hardness, stickiness, resilience, and springiness were analysed.

Gel strength

Gel strength of gelatin was determined according to the method described by Montero et al. (2001). About 30 ml of 6.67 % (w/v) gelatin solution was prepared and kept at refrigeration temperature for 16-18 h. 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.

Determination of 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.

Statistical analysis

Statistical analysis was performed using SPSS (SPSS version 26.0 for windows; SPSS, Chicago, IL, USA). The experiments were conducted in six replications each and the collected data were subjected to independent t test analysis of variance for comparing the means to find the effects between samples at 5 % level.

RESULTS AND DISCUSSION

Physico-chemical properties of extracted gelatin

Yield

The yield of gelatin extracted from chicken skin-head-feet blend with different acid treatments were presented in Table 4.1. The yield of gelatin (dry weight basis) with alkali-acetic-acid and alkali-citric acid was 7.25 and 7.27 %. The yield of gelatin from chicken head with alkaline-acid pretreatment ranged from 7.67 – 10.04% (Ee et al. 2019). Acetic acid is considered one of the most popular solvents used in gelatin extraction because of the high solubility of collagen in acetic acid and its high extractability (Alemeida and Lannes, 2013).

pH

The pH of gelatin plays an important factor which influences the functional properties of gelatin (Bahar et al. 2020). The pH of gelatin extracted with different acid treatments were tabulated in Table 4.1. A significantly (P ≤ 0.05) higher pH was observed in alkali-acetic acid treated gelatin (5.97) compared to alkali-citric acid treatment (5.24). The conversion of collagen to gelatin induces molecular structural alterations in specific amino acids. Type B gelatin, characterized by a pH of 5, results from an alkaline process that deaminates glutamine to glutamic acid and asparagine to aspartic acid (Aykin-Dincer et al. 2017). The pH of chicken feet gelatin with different concentrations of acetic, citric, and lactic acid treatment was found in the range of 3.43-5.43 by Chakka et al. (2017).

Hydroxyproline content

The HP of gelatin extracted from chicken skin-head-feet blend with different treatments was presented in Table 4.1. The HP of gelatin treated with alkali-acetic acid and alkali-citric acid was 14.65% and 17.75. Similar results were reported by Ahmad et al. (2018), who reported that the HP content of bovine hide ranged between 15.99% to 17.21%. Muyonga et al. (2004) stated that increased purity levels such as higher viscosity and higher gel strength leads to the maximum hydroxyproline content.

Gel clarity

Gel clarity is an aesthetic attribute influencing consumer acceptability and delineating its prospective applications within the food industry. The gel clarity or transmittance (%) of gelatin obtained from the chicken skin-head-feet blend was tabulated in Table 1. The gel clarity was significantly (P ≤ 0.05) higher in gelatin extracted with alkali-acetic acid (4.5 %) compared to that of alkali-citric acid extracted gelatin (3.73 %). The gel clarity of chicken skin gelatin was found in the range of 1.45 to 1.95 % depending on the different extraction temperatures (Mrazek et al. 2019).

Instrumental colour profile

The colour of gelatin has the commercial importance as it affects the consumer acceptability, however, it does not affect other functional properties of gelatin (Aidat et al. 2023; Du et al. 2013). The L^*, a^*, and b^* values of the chicken skin, head and feet gelatin were presented in Table 1. The lightness (L^*) and yellowness (b^*) values of alkali-citric acid-extracted gelatin were significantly (P ≤ 0.05) higher (68.23 and 13.84) than acetic acid-extracted gelatin (64.45 and 12.25). There is no significant (P ≥ 0.05) differences in redness (a^*) of gelatin between acetic and citric acid treatment. Similarly, gelatin from chicken heads also showed higher values for L^* and b^* but lower a^* values in comparison with turkey head gelatins (Du et al. 2013).

Table 1: Yield and physico-chemical properties of chicken skin-head-feet blend gelatin

Parameters	*Alkali and Acetic Acid*	*Alkali and Citric Acid*
Yield (%)	7.25 ± 0.09	7.27 ± 0.02
pH	5.97 ± 0.18^a	5.24 ± 0.04^b
Hydroxyproline (%)	14.65 ± 0.14^b	17.75 ± 0.19^a
Protein (%)	83.93 ± 0.55^a	82.63 ± 0.28^b
Moisture (%)	11.02±0.06	10.88±0.07
Fat (%)	1.2±0.12	1.51±0.07
Ash (%)	2.22±0.12	2.17±0.15
Gel clarity	4.5 ± 0.14^a	3.73 ± 0.04^b
L*	64.45 ± 2.13^b	68.23 ± 0.4^a
a*	1.4±0.07	1.27±0.06
b*	12.25 ± 0.31^b	13.84 ± 0.37^a

Means with different superscripts in a row differ significantly (P<0.05).

Collagen solubility and collagen content of chicken skin, head and feet blend gelatin

The collagen solubilty and collagen content was 68.74% and 57.67% from raw chicken skin-head-feet blend. The collagen solubilty and collagen content was highly correlated to the yield which might be depends on the extraction process sarbon et al. (2013).

Table 2: Collagen solubility and collagen content of chicken skin-head-feet blend gelatin

Treatment	Collagen solubilty (%)	Collagen content (%)
Raw chicken skin-head-feet blend	68.74±0.42	57.67±0.59

Textural profile analysis

Texture profile analysis (TPA) was done by a double compression test, which determines the textural properties of food products Rather et al. (2022), and has been widely utilized for food characterization. The textural properties of chicken skin, head and feet blend gelatin of different treatments were tabulated in table 3.

The hardness is related to the strength of the gel which is extracted from chicken skin, head and feet structure under compression and corresponds to the maximum force during the first cycle of compression Chandra and Shamasundar, (2015). In the present study it has revealed that gelatin treated with acetic acid had shown highly (P ≤ 0.05) significant values than citric acid. Gum was the energy needed to break down a semi-solid food to swallow it (Yi et al. 2012). The results in the present study showed that the gelatin treated with citric acid had a higher gumminess value than acetic acid. The chewiness and springiness of the gelatin treated with acetic acid had shown higher ($P \leq 0.05$) significant values than citric acid. Cohesiveness indicates the intermolecular strength of the food and the degree to which a food can be deformed before breaking (Radocaj et al. 2011). There is no significant difference in the cohesiveness, stickiness and resilience.

Table 3: Texture profile analysis of gelatin treated with different acid groups

Texture Profile Analysis	*NaOH + 3% Acetic acid*	*NaOH + 3% Citric Acid*
Hardness (N)	4.1±0.25[a]	3.86±0.13
Gumminess (N)	5.94±0.29[b]	6.68±0.06[a]
Chewiness (N)	6.59±0.61[a]	6.52±0.39[b]
Cohesiveness (Ratio)	1.55±0.02	1.57±0.04
Springiness (cm)	0.98±0.01[a]	1.01±0.02[b]
Stickiness (N)	0.18±0.01	0.19±0
Resilience (Ratio)	0.85±0.04	0.64±0.18

Means with different superscripts in a row differ significantly (P<0.05).

Gel strength

The gel strength is one of the significant parameters to determine its quality which influences the commercial value of gelatin products (Sebastian, 2014). The standard gel strength of gelatins is evaluated by Bloom test and the values vary from 50 to 300 g. The gel strength of chicken skin, head and feet were presented in the table 3. The gelatin treated with acetic acid has shown higher ($P \leq 0.05$) significant values than citric acid. Saenmuang et al.

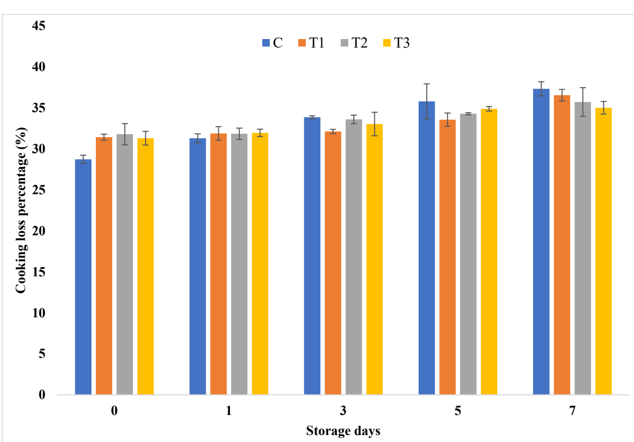

Fig. 1: Gel strength and viscosity of gelatin treated with different acid groups

(2020) reported gel strength of gelatin extracted from Black bone chicken skin and chicken feet as 239 and 263.5 g respectively.

Viscosity

Viscosity was considered as the second most important physical property of gelatin after gel strength. A gelatin solution with low viscosity usually yields a short and brittle textured gel, while a highly viscous gelatin solution yields a tough and extensible gel (Ahmad et al. 2018). The viscosity of chicken skin-head-feet gelatin was tabulated in figure 1. Traditionally extracted gelatin with acetic acid had significantly ($P \leq 0.05$) higher (38.92 mPa.s) viscosity than citric acid treated gelatin (29.4 mPa.s). Sarbon et al. (2013) reported that chicken gelatin exhibited a higher viscosity value than bovine gelatin, which corresponded well with the higher bloom values and rheological properties of gelatin.

CONCLUSION

The present study revealed that the chicken skin, head and feet blend treated with 3% acetic acid gelatin has shown better physico-chemical properties in terms of pH, colour and gel clarity. The textural properties, gel strength, viscosity and acceptable protein content demonstrated that the quality characteristics of chicken SHF blend gelatin were similar to the commercial porcine skin gelatin. Therefore, gelatin with chicken skin,head and feet blend may serve as a potential alternative in the food industries.

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