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Preparation and characterisation of chicken skin gelatin as an alternative to mammalian gelatin

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ABSTRACT

The aims of this study were to report for the first time, the extraction and physico-chemical properties of chicken skin gelatin compared to bovine gelatin. Extracted chicken skin gelatin 6.67% (w/v) had a higher bloom value (355 ± 1.48 g) than bovine gelatin (259 ± 0.71 g). The dynamic viscoelastic profile of chicken gelatin exhibited higher viscous and elastic modulus values compared to bovine gelatin for a range of concentrations and frequencies. Thermal properties studied by differential scanning calorimetry (DSC) showed that the melting temperature of 6.67%, chicken skin gelatin was significantly greater ($p < 0.05$) than that of bovine gelatin, indicating lower stability of bovine gelatin compared to chicken skin gelatin. Results obtained in this study showed that Gly (33.70%), Pro (13.42%), H.Pro (12.13%) and Ala (10.08%) were the most dominant amino acids in chicken skin gelatin which contributed to the higher gel strength and stability. Raman spectra of chicken skin and bovine gelatin were similar and displayed typical protein spectra. Chicken gelatin showed strong hydrogen bonding compared to bovine gelatin as the tyrosine doublet ratio (I_{855}/I_{830}) of chicken gelatin was significantly lower than that of bovine gelatin. Significantly, the alpha helix and β-sheet type structures were higher for chicken skin gelatin compared with bovine gelatin. The average molecular weight of chicken gelatin was 285,000 Da. These findings, obtained for the first time for chicken skin gelatin, show that it has high potential for application as an alternative to commercial gelatin.

1. Introduction

Gelatin is a polypeptide produced by the partial hydrolysis of collagen derived from animal skin, connective tissue and bones (Morrison, Clark, Chen, Talashek, & Sworn, 1999). Gelatin has gelling, foaming and emulsifying properties that contribute to a wide range of applications in the food, pharmaceutical, photographic and cosmetic industries. The unique properties of gelatin are the ability to form thermo-reversible gels with a melting temperature close to body temperature and solubility in water (Zhou, Mulvaney, & Regenstein, 2006). The estimated world usage of gelatin is reported at about 200,000 metric tonnes per year (Choi & Regenstein, 2000). Most available gelatins are manufactured from mammalian resources such as pig skin, cattle bones and cattle hide. However, other sources of gelatin are becoming increasing relevant, such as fish bone, scales and skin.

Gel strength or bloom value including low (<150), medium (150–220) and high bloom (220–300) determines the quality of gelatin and viscoelastic properties such as gelling and melting. Rheological data are required for the analysis of flow conditions in different food processing operations and the measurement of texture (Binsi, Shamasundara, Dileepa, Badii, & Howell, 2009). The quality of gelatin depends on its physicochemical properties, which is influenced by the species, tissue and the processing method. The rheological properties of thermo-reversible gelatin gels are primarily a function of temperature and the concentration of gelatin for a given gelatin type.

The development of gelatin alternatives has gained importance in recent years as the demand for non-bovine and non-porcine gelatin has increased due to the BSE (bovine spongiform encephalopathy) crisis and for religious and social reasons. Since then, there has been much concern about using gelatin derived from possibly infected animal parts. Pig skin gelatin is not acceptable for Judaism and Islam and beef gelatin is acceptable only if it has been prepared according to religious requirements (Badii & Howell, 2006). Therefore, the development of gelatin alternatives is highly desirable to food processors as the global market for food certified halal is growing rapidly (Karim & Bhat, 2009).

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To date however, few alternatives for mammalian gelatin are available. A number of studies on developing gelatin alternatives to mammalian gelatin have been reported including from fish skin such as cod skin (Gudmundsson & Hafsteinsson, 1997), horse mackerel skin (Badii & Howell, 2006), sin croaker and shortfin scad skin (Cheow, Norizah, Kyaw, & Howell, 2007), black and red tilapia skin (Jamilah & Harvinder, 2002) and hake skin (Gomez-Guillen et al., 2002). Similarly, in South Korea investigations on the feasibility of using chicken feet to replace cowhides for jokpyun (traditional Korean gel-type food) have been undertaken (Jun, Lee, Lee, & Kim, 2000). Additionally, there is growing interest in developing alternative substitutes of raw materials such as chicken bone and fishery by-products (Lim, Oh, & Kim, 2001). To our knowledge, there are no reported studies on the production of gelatin from chicken skin and detailed physicochemical and rheological studies. As skin is a wasted by-product of poultry processing, it may be possible to replace mammalian sources of gelatin with gelatin extracted from chicken skin.

Therefore, the objectives of the present study were to prepare gelatin from chicken skin and to compare the physicochemical, thermal and rheological properties, of the extracted gelatin with commercially available bovine gelatin. The hypothesis is that avian gelatin may have comparable properties to mammalian gelatin based on the imino acids, secondary structure and molecular weight, that are important indicators for high bloom strength. Moreover, this novel research may also lead to maximizing the usage of under-utilised resources and industrial waste.

2. Material and methods

2.1. Materials

Fresh chicken skins were obtained from a local market, Guildford, Surrey, UK and chilled in ice while transporting them to the laboratory, Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, UK. Upon arrival at the laboratory, the visible fat was mechanically removed, the skin was washed and weighed (wet weight) before storage at −80 °C until used for further experiments. Chemicals for amino acids analysis including acetonitrile HPLC grade (BDH), sodium acetate, mixed resin (amberlite MB-6113), sodium hydroxide, sulphuric acid and Bloom jars (SCHOTTGLAS Mainz. Bloom test vessel, product no. 2112501) were obtained from Fisher Scientific, Loughborough, UK. Triethylamine (TEA), phenylisothiocyanate (PITC), amino acid standards for food analysis, silicone oil, citric acid, and commercial bovine gelatin (Type B) from bovine skin were obtained from Sigma–Aldrich Company Ltd., Poole, Dorset, UK. All reagents used were analytical grade.

2.2. Methods

2.2.1. Chicken skin preparation

Frozen chicken skins were thawed in a cold room (4–5 °C) overnight. After thoroughly rinsing in excessive water to remove impurities, the skins were cut into 2–3 cm pieces and freeze-dried for about 4–5 days. Completely dry skins were ground before being defatted using the Soxhlet method (AOAC, 2006).

2.2.2. Gelatin extraction

Gelatin was extracted from chicken skin according to the method of Badii and Howell (2006) with slight modification. To extract gelatin, 14 g defatted dried chicken skin was mixed with 200 ml sodium hydroxide (0.15% w/v). The mixture was shaken well and slowly stirred at room temperature (22 °C) for 40 min before centrifuging at 3500 × *g* for 10 min. This step was repeated three times. The alkaline solution was changed every 40 min to remove non-collagenous proteins and pigments. The alkaline treated pellets were rinsed with distilled water and then mixed with 200 ml 0.15% (v/v) sulphuric acid. Again, the resulting pellets were mixed with 200 ml of 0.7% (w/v) citric acid solution. The mixture was shaken well and stirred gently at room temperature for 40 min before centrifuging at 3500 × *g* for 10 min. The acid solution was changed every 40 min to denature the collagen in the chicken skin matrix. Each treatment was repeated three times and each treatment took about 2 h to complete. The pellets were washed with distilled water to remove any residual salts followed by centrifuging at 3500 × *g* for 15 min. The final extraction was carried out in distilled water at a controlled temperature (45 °C) overnight without stirring. The resultant mixture was filtered in a Büchner funnel with a Whatman filter paper (no.4) and deionised, using an Amberlite mixed bed resin (MB-6113) according to the GME Monograph Version 2000 (GME, 2000). The solution ionic strength was checked with a conductivity meter to obtain 50 μ Siemens/cm. The pH was adjusted to 6.0 with 0.1 M sulphuric acid. The volume was reduced to 1/10 by evaporation under vacuum (using a rotary evaporator) at 45 °C and then kept in the freezer overnight before freeze-drying. The dry matter was referred to as 'gelatin powder'.

2.2.3. Characterisation of gelatin

2.2.3.1. Proximate analysis. The moisture, ash and fat content of extracted dried gelatin was determined according to the AOAC (2006). The crude protein content was determined by estimating its total nitrogen content by the Kjeldahl method (AOAC, 2006). A factor of 5.55 was used to convert the nitrogen value to gelatin protein. The yield of gelatin was calculated based on dry weight of fresh skin using the following formula:

$$\text{Yield of gelatin}(\%) = \frac{\text{Weight of freeze} - \text{dried gelatin}}{\text{Weight of dried skin}} \times 100$$

2.2.3.2. Determination of bloom strength of gelatin gels. Bloom value was determined according to the method described by the Gelatin Manufacturers of Europe Monograph version 1 July 2000. Gelatin (7.5 g) was weighed into a Bloom jar (SCHOTTGLAS Mainz. Bloom test vessel, product no. 2112501) to which 105 ml deionised water was added. The solution (6.67%) was swirled with a glass rod, covered and allowed to stand at room temperature for 3 h to allow the gelatin to swell. The Bloom jars were then transferred and heated in a beaker of water at 60 °C, on a magnetic heater stirrer for 20 min to dissolve the gelatin completely. The jar was covered and allowed to cool for 15 min at room temperature. Bloom jars were kept in a refrigerated water bath at 10 ± 0.1 °C overnight (16–18 h) for gel maturation and tested on a TA-XT2 texture analyzer (Stable Microsystem, Godalming, UK) by penetration with a standard radius cylinder (P/0.5R) probe, to a depth of 4 mm at 0.5 mm/s. The standard glass Bloom jar was placed centrally under the plunger and the maximum force reading (the resistance to penetration) was obtained and is the Bloom strength (*g*) of the gel. The analysis was undertaken in triplicate and bloom value of chicken skin gelatin was compared to that of a commercial bovine gelatin.

2.2.4. Amino acid analysis

2.2.4.1. Preparation of samples and standards. The amino acid content of chicken muscle hydrolysate was determined according to using HPLC (Waters: Alliance, Waters, UK, Hertfordshire, UK) with an integrated detector (Dual λ absorbance-Waters 2487) and separation module (Waters 2695). Chicken skin gelatin and