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Chemical modification of New Zealand hoki (*Macruronus novaezelandiae*) skin gelatin and its properties

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ABSTRACT

Chemical modifications of gelatin from New Zealand hoki (*Macruronus novaezelandiae*) skins were carried out using three different cross-linking agents, namely, genipin, glutaraldehyde and caffeic acid, at different concentrations. The chemically modified gelatins exhibited better physical properties, such as higher gel strength, melting point, and rheological properties than did the uncross-linked gelatin. Gelatin cross-linked with glutaraldehyde had higher gel strength and melting point (231 g, 21.9 °C) than those cross-linked with caffeic acid (229 g, 21.6 °C) and genipin (211 g, 20.5 °C) at concentrations of 0.133, 0.111, and 0.044 M, respectively. The elastic modulus (G') and the loss modulus (G'') of chemically cross-linked gelatins were higher than those of the uncross-linked ones. These improved physicochemical properties of gelatin could lead to the development of products in the food industry that meet consumer demands.

1. Introduction

The skin gelatin derived from hoki (*Macruronus novaezelandiae*) exhibits lower gel strength and melting point than do bovine and porcine gelatins (Mohtar, Perera, & Quek, 2010). Hoki gelatin gels tend to melt and behave as a viscous liquid at room temperature, which limits their potential use in food applications. One possibility for improving its physiochemical properties is through chemical cross-linking. Cross-linked pollock and salmon gelatins were reported to have higher gel strength at room temperature than had uncross-linked gelatins (Chiou et al., 2006). Thus, this work deals with the modification of hoki gelatin using three chemical cross-linking agents, namely, genipin, glutaraldehyde and caffeic acid, at various concentrations.

Genipin is an aglycone derived from the glycoside, geniposide. It is isolated from the fruits of *Genipa americana* and *Gardenia jasminoides Ellis* (Akao, Kobayashi, & Aburada, 1994; Djerassi et al., 1961). Almost 4–6% of the dry mass of the above dried fruits consists of genipin, having a molecular weight of 226 Da. It is an excellent natural cross-linking agent for protein, and it has a low cytotoxicity. Its cytotoxicity is known to be lower than that of glutaraldehyde (Nimni, Cheung, Strates, Kodama, & Sheikh, 1988; Sung, Chang, Chiu, Chen, & Liang, 1999; Sung, Huang, Huang, & Tsai, 1999), formaldehyde (Nimni et al., 1988), dialdehyde starches (Rosenberg, 1978), and epoxy compounds (Imamura, Sawatani, Koyanagi, Noishiki, & Miyata, 1989). The cross-linking reaction of genipin and proteins involves two possible mechanisms, as shown in Supplementary Fig. S1A. The first mechanism is when the genipin molecule undergoes a nucleophilic substitution by the primary amines of proteins, resulting in the heterocyclic linking of genipin to the amine in the protein (Butler, Ng, & Pudney, 2003). The second reaction takes place when the ester group on genipin undergoes a nucleophilic substitution by the secondary amide linkage (Butler et al., 2003). The occurrence of covalent cross-links between the primary amine residues leads to minimal residual toxicity of the materials (Liang, Chang, Liang, Lee, & Sung, 2004; Tsai, Huang, Sung, & Liang, 2000).

Glutaraldehyde, a linear 5-carbon dialdehyde, is known to be one of the most widely used chemical cross-linking agents. It reacts with amine groups in protein and is relatively inexpensive. It is commonly used to cross-link proteins through the reaction of the aldehyde functional groups (Farris, Song, & Huang, 2010). The mechanism of cross-linking is illustrated in Supplementary Fig. S1B, which shows a nucleophilic addition-type reaction of aldehyde functional groups with free nonprotonated ε-amino groups (—NH_2) of lysine. The initial reaction involves the nucleophilic addition of the ε-amino groups to the carbonyl groups of the aldehyde. This is followed by the formation of an intermediate called carbinoalamine. Formation of Schiff base occurs after the protonation of the hydroxyl group, followed by the loss of a water molecule, as shown in Supplementary Fig. S1B (Farris et al., 2010).

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Caffeic acid (3,4-dihydroxycinnamic acid) is a plant-derived phenolic compound like genipin, which is commonly found in coffee beans, tea leaves, potatoes, cell walls of fruits, and hulls of cereal grains. It is a good substrate for polyphenol oxidase and it undergoes an oxidation process in plant tissues. Caffeic acid is also used to cross-link amine-containing polymers (Butler et al., 2003; Nickerson, Patel, Heyd, Rousseau, & Paulson, 2006). The cross-linking reaction of caffeic acid is initiated by its conversion to an intermediate quinone (Strauss & Gibson, 2004). Subsequently, lysine groups from proteins react reversibly with the quinone group itself to form quinonimines (Cheftel, 1979). Further reactions lead to the formation of cross-linkages and complexes between lysine and polymerised quinones, as illustrated in Supplementary Fig. S1C. Several studies on cross-linking of proteins, using caffeic acid, have been previously conducted (Isenburg, Karamchandani, Simionescu, & Vyavahare, 2006; Strauss & Gibson, 2004). The results have demonstrated that protein-caffeic acid cross-linking leads to the formation of covalent bonds, thus improving the mechanical properties of the protein-caffeic acid complexes.

To the best of our knowledge, there is no available information on the chemical cross-linking of hoki gelatin modified by genipin, glutaraldehyde and caffeic acid. Therefore, the scope of the current work was to investigate the effect of concentration of these three chemical cross-linking agents on the gel strength and melting point of gelatin gels. Further characterisation of the properties of chemically cross-linked gelatins was also conducted by their molecular weight distribution and rheological measurements.

2. Materials and methods

2.1. Materials

Frozen hoki skins were thawed at room temperature (15 °C) overnight. The adherent flesh and scales from the skin were removed using a sharp knife. The skin was washed in tap water to remove obvious impurities and was then cut into approximately 40 × 40 mm pieces. The cleaned skin was minced without water, using a Waring blender (Waring Commercial®, New Hartford, CT, USA) at the lowest speed setting for 15 min. The minced skins were packed into 150 × 90 mm snap-lock plastic bags (GLAD, Clorox New Zealand Ltd., Auckland, New Zealand), sealed after the headspace air was removed manually, and stored at −20 °C until used within the next 1–2 weeks. All chemicals and reagents used were of analytical grade.

2.2. Extraction of fish gelatin

Gelatin from hoki skin was extracted according to Mohtar et al. (2010). Briefly, the frozen minced skins were thawed at room temperature (15 °C) and were rinsed in tap water. The rinsed and minced skins were pre-treated in 0.75 M NaCl solutions, after which they were rinsed again in tap water. These steps were repeated twice. The minced skins were gently stirred in Milli-Q water (Millipore Corporation, Billerica, MA, USA) for 60 min at a controlled temperature of 49.3 °C in a shaking water bath (Ratek Instruments, Boronia, Victoria, Australia). The samples were centrifuged using Sorvall® RC-28S Centrifuge (Sorvall®, Newton, CT, USA) at 10,000×*g* for 30 min at 15 °C. The clear extract obtained was filtered using Whatman filter paper (No. 5) (Whatman International Ltd., Kent, UK), and the filtrate obtained was dialysed against three changes of Milli-Q water before freeze-drying using a FreeZone Plus (Labconco, MO, USA). The freeze-dried gelatin samples were kept in sealed containers, wrapped in aluminum foil to avoid direct exposure to light and stored in a vacuum desiccator prior to further use within 1–6 months.

2.3. Preparation of gelatin gels

2.3.1. General

In this experiment, gels were prepared using 5% (w/w) hoki gelatin with concentrations of 0.022, 0.044, 0.066, 0.111, 0.133, and 0.177 M of the different chemical cross-linking agents. A sample of 5% (w/w) hoki gelatin was used in these experiments, as reported by different authors for pollock (Chiou et al., 2006), salmon (Chiou et al., 2006), and porcine gelatins (Chiou et al., 2006; Strauss & Gibson, 2004). Gelatin gel solutions, with and without the added cross-linking agents, were prepared according to the procedures described below.

2.3.2. Preparation of gelatin gels without added chemical cross-linking agent

5% (w/w) gelatin solution was prepared by mixing 5 g of dry gelatin in 95 g of Milli-Q water. The solution was left at room temperature for 10 min to form a visibly homogeneous gelatin suspension, and then heated at 45 °C for 30 min until the gelatin was completely dissolved. The gelatin solution was cooled to room temperature before maturing in a refrigerator at 10 °C for 18 h, prior to gel strength analysis. In addition, 5% (w/w) samples of bovine and porcine gelatins were prepared in the same way as for hoki gelatin gels and used as controls.

2.3.3. Preparation of gelatin gels with added genipin

5% (w/w) of gelatin solution with added genipin was prepared according to Chiou et al. (2006), as given in Section 2.3.2. The appropriate amount of genipin (Prod. No. G4796, Sigma–Aldrich, Auckland, New Zealand) was added to the gelatin solution and was stirred for an additional 20 min at 45 °C. The concentrations of genipin in the gelatin samples were set at 0.022, 0.044, 0.066, 0.111, 0.133, and 0.177 M. The gelatin solutions with added genipin were cooled to room temperature before maturing in a refrigerator at 10 °C for 18 h, prior to gel strength analysis.

2.3.4. Preparation of gelatin gels with added glutaraldehyde

The same procedure as in Section 2.3.2 was followed, except that the appropriate amount of glutaraldehyde (Prod. No. G5882, Sigma–Aldrich, Auckland, New Zealand) was added to the gelatin solution and stirred for 5 min at 25 °C. The concentrations of glutaraldehyde in the gelatin samples were set at 0.022, 0.044, 0.066, 0.111, 0.133, and 0.177 M. The gelatin solutions with added glutaraldehyde were cooled to room temperature before maturing in a refrigerator at 10 °C for 18 h, prior to gel strength analysis.

2.3.5. Preparation of gelatin gels with added caffeic acid

A 5% (w/w) gelatin solution with added caffeic acid was prepared according to Kosaraju, Puvanenthiran, and Lillford (2010), as given in Section 2.3.2. The appropriate amount of caffeic acid (Prod. No. C0625, Sigma–Aldrich, Auckland, New Zealand) was added to the gelatin solution and was stirred for 20 min at 60 °C. The concentrations of caffeic acid in the gelatin samples were set at 0.022, 0.044, 0.066, 0.111, 0.133, and 0.177 M. Oxygen gas (BOC Gases Ltd., Auckland, New Zealand) was bubbled through the solution for 20 min to initiate the oxidation reaction and cooled to room temperature before maturing in a refrigerator at 10 °C for 18 h, prior to gel strength analysis.

2.4. Determination of gel strength

The gel strengths of 5% (w/w) hoki gelatin gels (with and without added chemical cross-linking agents) were determined according to the British Standard Institution method (British Standard Institution, 1975). The samples (prepared as given before) were used immediately after they were removed from the refrigerator