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Effects of pressure on gelatinization of collagen and properties of extracted gelatins

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ABSTRACT

Ultra-high pressure (UHP) was applied as a pretreatment to extract gelatins with 1% (w/v) hydrochloric acid as the transmission medium at a skin/solution ratio of 1:1.5 (w/v). The effects of various pressures (ranging from 0.1 to 500 MPa) on the gelatinization of collagen and the properties of the extracted gelatins were investigated. The thermostability of the pressurized collagens and the yield, molecular-weight distribution, gel strength and rheological properties of the extracted gelatins were evaluated. UHP pretreatment could decrease the thermostability and enhance the extent of gelatinization of the collagen. The temperature at which the major endothermic peak was observed (T_m) decreased as the pressure level increased from 100 to 400 MPa but increased as the pressure reached 500 MPa, whereas the opposite trend was observed for the gelatins yields. The UHP gelatins contained more subunit components than traditional gelatin, which increased in content with increasing pressure up to 300 MPa; the amount of subunit components remained nearly constant when the pressure was further increased. The gel strength and rheological properties of the UHP gelatins were better than those of traditional gelatin, and they exhibited similar tendencies to those of high-molecular-weight gelatins with increases in the pretreatment pressure.

1. Introduction

Gelatin is derived from collagen, which is a heterotrimer composed of three α chains in a triple-helix structure (Wynnyckyj et al., 2011). The triple-helix collagen molecule, which is stabilized by hydrogen bonds, aggregates with other collagen molecules to form a staggered array that is stabilized by covalent intermolecular crosslinks (Wang, Li, Bank, & Agrawal, 2002; Wang et al., 2000). The transformation of collagen into gelatin is interpreted as the disintegration of the helical structures into random coils. During traditional gelatin manufacturing, native collagenous material is treated with dilute acid or an alkali, resulting in the partial cleavage of the hydrogen bonds and crosslinks; the structure is degraded to such an extent that "warm-water-soluble collagen", which is gelatin, is formed (Karim & Bhat, 2009). Collagen with this type of structure is known as gelatinized collagen.

However, the traditional method of manufacturing gelatin has several limitations, such as serious acid/alkaline pollution, long processing times and excessive consumption of water and electricity. Furthermore, excessive acid/alkaline treatments could even break a number of peptide bonds, leading to reductions in the molecular weight and the quality of the resulting gelatin (Yang, Wang, Zhou, & Regenstein, 2008). Therefore, it is important to improve traditional gelatin manufacturing procedures and establish a clean and efficient manufacturing method.

The UHP technology used to modify the conformations and functional properties of proteins is garnering increasing interest. It has been reported that UHP can induce protein denaturation by disturbing the balance of the non-covalent interactions that stabilize the native conformations of many proteins (Cheret, Delbarre-Ladrat, De Lamballerie-Anton, & Verrez-Bagnis, 2005; Collins, Kim, & Gruner, 2011; Qin et al., 2013; Torrezan, Tham, Bell, Frazier, & Cristianini, 2007). It is speculated that the triple-helix structure of collagen can be destabilized during UHP treatment because the hydrogen bonds that stabilize it could become damaged. Moreover, collagen could swell as a result of the acid/alkaline process during UHP treatment because water could favorably penetrate into the interiors of proteins with increasing pressure (Sarupria, Ghosh,

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Garcia, & Garde, 2010). Therefore, the UHP treatment could substitute for the traditional acid/alkaline process to produce a gelatin that exerts consistent effects on proteins.

Gómez-Guillén, Gimenez, and Montero (2005) applied UHP to produce gelatin, and concluded that the application of UHP was a useful alternative to the conventional procedure for extracting gelatin from fish skins, essentially because the longest phase of treatment could be dramatically shortened, thus making it possible to produce gelatins with high gelling qualities in only a few minutes. Zhang, Ma, and Shi (2011) observed that gelatin with high extraction yields and qualities could be obtained when pretreated at 300 MPa for 10 min. Chen et al. extracted gelatins from pig skin using a UHP pretreatment and observed that using hydrochloric acid as the transmission medium could induce collagen gelatinization in a short time during UHP pretreatment and produce gelatin with a high gel strength (Chen, Zhang, Zhou, Han, & Ma, 2012). Hence, UHP could be used in gelatin manufacturing. However, little is known about the effects of different pressures on the gelatinizing extent of collagen and the characteristics of gelatin during UHP pretreatment.

The objective of the present work was to compare the thermostabilities of collagens obtained from pig skin via UHP treatment performed at different pressures and to investigate the effects of different pressures on the extraction yield, molecular-weight distribution, gel strength and rheological characteristics of the extracted gelatins.

2. Materials and methods

2.1. Materials

Fresh pig skin was purchased from a local market in Beibei, Chongqing, China. L-hydroxyproline was obtained from Shanghai Kayon Biological Technology Co., Ltd. (Shanghai, China). Sodium dodecyl sulfate (SDS), tris, ammonium persulfate (APS), β-mercaptoethanol (2-ME), Coomassie Blue R-250 and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) were procured from Bio-Rad Laboratories (Hercules, CA, USA). Glycine and bromophenol blue (BPB) were obtained from Sangon Biotech Co., Ltd. (Shanghai, China), and 30% acrylamide (mass ratio of acrylamide to bisacrylamide was 29:1) was obtained from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). PageRuler™ Unstained Protein Ladder was purchased from Fermentas (Beijing, China). Gelatin (food grade) was obtained from Kelon Chemical Reagent Factory (Chengdu, China). All other chemicals were of analytical grade.

2.2. Pig skin preparation

The pig skin used was cleaned and washed. The subcutaneous fat layers were scraped off using a knife. The prepared skin was presteamed at 95 °C for 8 min and subsequently cut into small pieces ($1 \times 3 \times 5$ mm^3) and dried for 2 h at 105 °C. The pieces were then defatted by the Soxhlet method using petroleum ether as the solvent. To remove non-collagenous proteins, the skin was mixed with five volumes of 1% NaCl (w/v). The mixture was stirred for 6 h, and the NaCl solution was changed every 2 h. After removing the supernatant, the samples were washed three times with distilled water, placed in polyethylene bags and stored at −20 °C until use.

2.3. Gelatin extraction

Gelatin was prepared using acid- and UHP-assisted extraction techniques. Traditional acid-assisted extracted gelatin was used as the control gelatin to which to compare the UHP-assisted extracted gelatin. The prepared collagen was swollen using 1% (w/v) hydrochloric acid at a skin/solution ratio of 1:10 (w/v) for 20 h at room temperature with discontinuous stirring. The samples were then washed thoroughly with distilled water until the washing water was nearly neutral. The swollen skin was mixed with distilled water at 60 °C for 6 h in a skin/water ratio of 1:3 (w/v) in a SHZ-B thermostat water bath oscillator (Jiangren Experimental Equipment Co., Ltd., Shanghai, China) to extract the gelatin. The mixture was then filtered and centrifuged at 12,800 × *g* for 20 min using a 5810 Centrifugal Machine (Eppendorf AG, Hamburg, Germany). The supernatant was dried under vacuum (0.07 MPa) at 50 °C until the moisture level was below 15%.

For the UHP-assisted extractions, the prepared pig skin was sealed in a polyethylene bag with 1% (w/v) hydrochloric acid in a skin/solution ratio of 1:1.5 (w/v) and subsequently placed under different pressures, 0.1, 100, 200, 300, 400 and 500 MPa at ordinary temperature, for 15 min using a high-pressure vessel (Huataisenmiao Biological Technology Co., Ltd., Tianjin, China). Subsequently, the skin was washed thoroughly with distilled water until the wash water was nearly neutral, and the gelatin was then extracted and dried as mentioned above. The gelatins extracted from skins pretreated at ordinary pressure (0.1 MPa) and at high pressure (from 100 MPa to 500 MPa) are referred to as ordinary gelatins and UHP gelatins, respectively. Triplicate extractions were performed.

2.4. Yield of extracted gelatin

The hydroxyproline content of the gelatin and pig skin samples was measured according to ISO 3496:1994 (Meat and Meat Products − Determination of Hydroxyproline Content), and the extraction yields of gelatin were calculated using the following equation:

Yield (%) = hydroxyproline content in gelatin (mg) × 100/hydroxyproline content in pig skin (mg) × 100%.

2.5. Differential scanning calorimetry (DSC)

The thermal properties of collagen were investigated using a S-300N differential scanning calorimeter (NETZSCH-Gerätebau GmbH, Selb, Germany). The collagen submitted to UHP treatment at different pressures and acid-treated collagen were prepared as mentioned above but without extraction. A 20 mg sample was weighed, loaded into the sample cells and placed in the DSC together with an empty reference cell. DSC scans were recorded from 15 °C to 120 °C at a heating rate of 5 °C/min. The temperature at which the maximum peak height occurred in the scan was measured using the Origin 8.0 software package (available in Micro-Cal).

2.6. Electrophoretic analysis (SDS-PAGE)

The molecular weight distributions of the extracted gelatins were determined by SDS-PAGE. Dry gelatin was dissolved in distilled water at 60 °C to create a 1.5 mg/ml solution. The sample solution was mixed in a 4:1 (v/v) ratio with a 5-fold-concentrated loading buffer containing β-mercaptoethanol. The mixed solution was heated in boiling water for 5 min and analyzed by SDS-PAGE using 5% stacking gels and 6% resolving gels in a miniature electrophoresis unit (Bio-Rad Laboratories, Hercules, CA) at a constant current of 15 mA/gel; the current was increased to 25 mA/gel when bromophenol blue ran into the resolving gel. Fifteen-microliter gelatin samples were loaded into the gel together with 10 μl of the protein marker. Following electrophoresis, the gel was stained with 0.1% (w/v) Coomassie Blue R-250 in 25% (v/v) isopropanol and 10% (v/v) acetic acid for 2 h and destained with 5% (v/v) alcohol and