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Tailoring physical properties of transglutaminase-modified gelatin films by varying drying temperature

Fei Liu [a, b], Hamid Majeed [a], John Antoniou [a], Yue Li [a], Yun Ma [a], Wallace Yokoyama [b], Jianguo Ma [a], Fang Zhong [a, *]

[a] Key Laboratory of Food Colloids and Biotechnology, Ministry of Education, School of Food Science and Technology, Jiangnan University, Wuxi 214122, PR China

[b] Western Regional Research Center, ARS, USDA, Albany, CA 94710, United States

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ABSTRACT

Transglutaminase (TGase)-modified gelatin films containing different levels of physical and chemical networks were prepared by drying films above or below gelation temperature of gelatin. Differences in protein network structure were observed by optical microscopy analysis in freeze-dried film-forming solutions. The relative amount of triple helices decreased when drying temperature increased as observed by X-ray diffraction (XRD) and differential scanning calorimetry (DSC). The addition of TGase slightly inhibited triple helix formation. TGase-modified films exhibited stronger mechanical properties than blank films, and the highest tensile strength was observed in films dried close to the gelation temperature (25 °C) and the highest elongation at break above it (35 °C). TGase modification enhanced the water resistance and thermal stability of gelatin films by decreasing the water solubility, increasing the glass transition temperature and degradation temperature, respectively, which was further enhanced with increasing drying temperature. The cross-section (SEM) and surface (AFM) microstructure of gelatin films with TGase both appeared more compact and smooth, and improved when temperature increased. The results obtained show that drying temperature may be used to tailor the physical properties of TGase-modified gelatin films for specific applications.

1. Introduction

Gelatin, a biopolymer widely used in food, pharmaceutical, and photographic industries, is produced from the controlled hydrolysis of collagen, the main fibrous protein constituent in bone, skin, tendon and all other connective tissues (Gómez-Guillén, Giménez, López-Caballero, & Montero, 2011; Wisotzki, et al., 2014). Gelatin's excellent film-forming properties has been attributed to the protein conformation also responsible for its good gelling properties (Staroszczyk, Pielichowska, Sztuka, Stangret, & Kołodziejska, 2012). Gelatin chains undergo a conformational coil–helix transition when the temperature of a film-forming solution is lowered to the gelation temperature. The desirable physical properties of gelatin films are highly determined by the triple helix content (Chiou et al., 2009; Liu, Antoniou, Li, Ma, & Zhong, 2015; Staroszczyk et al., 2012). Although gelatin is edible and can form strong films, the high sensitivity of native gelatin to water and resulting changes in physical properties restrict its application as a food packaging material (Weng & Zheng, 2015).

Transglutaminase (TGase) produces inter- and/or intra-molecular bonds between γ-carboxamides of glutamine residues and ε-amine groups of lysine residues (Kieliszek & Misiewicz, 2014). The formation of intermolecular bonds between proteins by TGase decreases gelatin film solubility (Staroszczyk et al., 2012). Previous studies have shown that TGase and processing conditions can be used to tune the final solubility, mechanical properties and gelation kinetics of gelatin gels by combining different amounts of physical and chemical networks; terms of "physical gels", "chemical gels" and "physical-chemical gels" are used to differentiate between hydrogen-bonding or other noncovalent and covalent cross-linking or combinations of these two (Bode, da Silva, Drake, Ross-Murphy, & Dreiss, 2011; Sébastien, Dominique, Madeleine, & Véronique, 2004; Yung et al., 2007). According to Coimbra, Gil, and Figueiredo (2014), physical gelation is always present if the temperature is sufficiently low, irrespective of the reagents used to

* Corresponding author. Tel.: +86 510 85197876; fax: +86 510 85197876.
E-mail address: fzhong@jiangnan.edu.cn (F. Zhong).

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cross-link gelatin chains.

The properties of gelatin films might also be tuned by adjusting the relative amounts of triple helices, covalent bonds using TGase and thus physical networks in the protein matrix. Although the effect of TGase treatment on the properties of gelatin films has been previously investigated (de Carvalho & Grosso, 2004; Wang, Liu, Ye, Wang, & Li, 2015; Wang et al., 2015), the films were produced at only one casting temperature, usually room temperature, and their properties were not compared to their physical network counterparts. To our knowledge, it is still not known the effect of a combination of various relative amounts of physical and chemical networks, by varying drying temperature, on the properties of TGase-modified gelatin films in addition to gelatin gels.

In this study, TGase-modified gelatin films with varying physical and chemical networks were prepared by modulation of the film drying temperature. Corresponding films, without addition of TGase, were also produced as controls. The film-forming process was controlled by setting the temperature below or above the gelation temperature of 26.8 °C (Liu, Antoniou, Li, Ma, et al., 2015), in the absence or presence of TGase. Three different drying temperatures, which were respectively below (15 °C), around (25 °C), and above (35 °C) the gelation temperature of gelatin, were used to adjust the relative amount of triple helices of the film matrices. The amount of helices in the protein matrix was determined by X-ray diffraction (XRD) and differential scanning calorimetry (DSC). The morphology, thermal stability, water resistance and mechanical properties of the films were determined. The objective of the present study was to investigate the control of network structure and resulting physical properties by drying temperature in TGase-modified gelatin films.

2. Materials and methods

2.1. Materials

Gelatin (type B from bovine skin, ~200 Bloom) and glycerol were purchased from China Medicine (Group) Shanghai Chemical Reagent Co. (Shanghai, China). The gelation and melting temperature of gelatin used is respectively 26.8 °C and 31.9 °C, according to our previous report (Liu, Antoniou, Li, Ma, et al., 2015). Bacterial TGase, 3713 U/g powder, was purchased from Dongsheng Food Technology Co., Ltd. (Taizhou, China).

2.2. Preparation of films

Gelatin films were prepared according to our previously reported method with some modifications (Liu, Antoniou, Li, Yi, et al., 2015). Briefly, 2% (w/v) gelatin solutions were prepared by hydrating gelatin powders in distilled water for 1 h at room temperature and then dissolved by heating at 60 °C with continuous stirring. Glycerol (0.6%, w/v) was added as a plasticizer in order to reduce the brittleness and improve the manageability of the films. For TGase-modified films, TGase was added at a concentration of 0.6% (w/w) of gelatin according to the results of our preliminary experiment. The best physical properties of TGase-modified gelatin films were observed at the concentration of 0.6% (w/w). To study the effect of drying temperature, films were prepared by casting the 2% gelatin solutions (35 mL) in square Petri dishes (10 cm × 10 cm), drying at 15, 25 and 35 °C and 60% relative humidity (RH) until constant weight.

Resulting films were peeled off and conditioned at 25 °C and 60% RH for at least 48 h in a controlled environmental chamber (Shengxing Experimental Equipment Co., Ltd. Shanghai, China) prior to testing. For XRD, DSC, TGA, SEM and AFM studies, films were conditioned in a desiccator containing dried silica gel for 2 weeks at room temperature to obtain the most dehydrated films.

2.3. Optical microscopy of gels/solutions

After gelatin film forming solutions were incubated at various drying temperatures (15, 25, and 35 °C) for 6 h, the prepared gels/solutions were frozen immediately at −80 °C and freeze-dried. The optical micrographs of dried samples were captured by a VHX-1000 digital microscope (Keyence Int. Trading Co. Ltd., Japan).

2.4. X-ray diffraction (XRD)

XRD patterns of films were performed on an X-ray diffractometer (D2 Phaser, Bruker AXS Germany) using Cu Kα radiation (1.542 Å) operating at 2.2 kW. Data were recorded in the range (2θ) of 3–60° with a step size of 0.2°, and the scanning rate was 12° min^{-1}.

2.5. Differential scanning calorimetry (DSC)

The helix–coil transition/melting (T_m) and glass transition (T_g) temperatures of gelatin films were determined by differential scanning calorimetry (DSC) (Netzsch DSC 204 F1, Germany) according to Badii, MacNaughtan, Mitchell, and Farhat (2013), with minor modifications. Film samples (4–5 mg) were accurately weighed into aluminum pans, hermetically sealed, and scanned over the temperature range of −10 to 150 °C, with a heating rate of 10 °C/min. The empty aluminum pan was used as the reference. After the first heating, samples were cooled at the same rate (10 °C/min), and then the second heating cycle was performed. T_m was investigated as the peak temperatures obtained from the first heating scan and the corresponding melting enthalpy (ΔH_m) was determined from the area under the endothermic peak, while T_g was reported from the mid-point of the step-change due to the discontinuity of the specific heat of the sample in the second heating run.

2.6. Film thickness

Film thickness was measured at 8 random points on each specimen using a micrometer (Guilin, China) and mean values were calculated.

2.7. Mechanical properties

Mechanical properties of films were evaluated according to the ASTM standard method D882 (ASTM., 2001), with some modifications, by using a texture analyzer (TA.XT2i, Lloyd instruments, U.K.) equipped with a tension grip system A/TG at room temperature. Film strips of 10 × 50 mm were initially cut and tested at initial grip separation and crosshead speed of 30 mm and 0.5 mm/s, respectively. The curves of force (N) as a function of deformation (mm) were recorded using Texture Expert Exceed software (Version 2.64, Stable Micro Systems LTD., Godalming, UK). Tensile strength (TS) and elongation at break (EB) were calculated from the following equations (1) and (2):

$$TS = \text{Maximum force (N)}/(\text{Thickness (mm)} \times \text{Width(mm)}) \quad (1)$$

$$EB\% = 100 \times (L - L_0)/L_0 \quad (2)$$

where L_0 is the initial length of the film and L is the length of the film when it breaks. The reported values correspond to at least five