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Effects of solvent on the solution properties, structural characteristics and properties of silk sericin

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ABSTRACT

Sericin films have attracted much attention from researchers in biomedical and cosmetic fields because of its unique properties, including good cytocompatibility and its promotion of wound healing. However, poor mechanical properties of sericin films have restricted its application in these fields. In this study, a new solvent, formic acid, was used to fabricate sericin solutions and films. The effects of formic acid on the structural characteristics and mechanical properties of the sericin solutions and films were examined and compared with water. The sericin/formic acid solution showed fewer aggregated sericin molecules, resulting in a lower turbidity than that of the sericin/water solution. In addition, the gelation of the sericin solution was retarded in formic acid compared to that of water. Sericin films cast from the formic acid solution exhibited a much higher crystallinity index than that produced from water. The tensile strength and elongation of the sericin films cast from the formic acid solution were more than double that of the sericin films cast from water. It is expected that the more stable sericin solution and high-crystallinity sericin films, which have significantly improved mechanical properties, produced by using formic acid as the solvent could be utilized in biomedical and cosmetic applications.

1. Introduction

Silk is a naturally occurring material that consists of fibroin and sericin. Sericin has been removed from silk in the textile industry to improve the luster and feel of silk textiles. That is, sericin is considered an impurity and a useless material in silk. However, sericin has a high resistance against oxidation [1], antimicrobial properties [2], UV resistance [3], a moisturizing effect on skin [4], and can lower cholesterol [5,6]. Recent studies have also revealed sericin has additional valuable properties for biomaterial applications. Tsubouchi et al. [7] reported that sericin enhances the attachment of cultured human skin fibroblasts. Aramwit et al. [8] and Nagai et al. [9] also reported that sericin promotes the healing of wounds. Dash et al. [10] reported that silk sericin from the tasar silkworm inhibits ultraviolet-B (UVB)-induced apoptosis in human skin keratinocytes. Therefore, because of these unique properties, sericin has recently been considered as a good candidate for biomedical and cosmetic applications [11–13].

For these applications of sericin, it should be processed into film form. However, it is difficult to make sericin films because of its brittleness. That is, when the sericin solution is dried, the sericin solid is easily split, preventing film formation. Even though it is possible to fabricate a sericin film through careful control, it is unsuitable for biomedical applications because the sericin film has poor mechanical properties. Therefore, many studies have been conducted to improve the mechanical properties of sericin [14–17]. In addition, various solvent systems have been considered as alternatives to the conventional aqueous system. Oh et al. [18] used a LiCl/dimethyl sulfoxide (DMSO) solvent system to prepare sericin beads, and Zhang et al. [19] utilized trifluoroacetic acid to electro-spin a sericin solution.

Formic acid has been utilized to improve the solution stability and processability of silk fibroin (SF) during film preparation with wet spinning, electro-spinning, etc. Um et al. [20] reported that an SF/formic acid solution is transparent and there was no molecular aggregation of SF, resulting in no gelation occurring during storage. This greatly improves the processability of SF solutions. On the other hand, an SF/water solution is turbid. The molecular aggregation and gelation of SF easily occurs, resulting in the poor processability of SF solutions. It has also been reported that SF crystallizes when formic acid is removed from a SF/formic acid solution [20]. Many previous studies have proved that the SF/formic acid solution has good fiber formation properties for wet spinning [21–23] and electro-spinning processes [24–26].

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In recent studies, the sericin was not entirely removed from regenerated silk, with the residual sericin content varied by controlling the degumming process. The wet spinning and electrospinning of regenerated silk containing residual sericin have been studied and it was found that residual sericin improves electrospinnability [27], the electro-spinning rate [28], and the wet spinning performance [22,23]. In these studies, the residual sericin was dissolved in formic acid, which improved the processability of regenerated silk (i.e., wet- and electro-spinning of regenerated silk). At the very least, the processability of regenerated SF did not deteriorate with the addition of sericin, and previous studies imply that sericin/formic acid solutions may be utilized to improve the sericin solution stability (i.e., no gelation) and the processability of sericin, like SF/formic acid solutions.

In this study, sericin solutions and films were prepared with two solvents: water and formic acid. The effects of the solvents on the solution properties, structural characteristics, and mechanical properties of sericin were comparatively examined.

2. Experimental

2.1. Preparation of the sericin/water solutions and sericin films

Baekokjam silkworm cocoons were used for the silk sericin extraction. The extraction of sericin has been discussed in detail in the literature [29,30]. In brief, the silk cocoons were immersed in the degumming liquor at 100 °C for 30 min. The ratio of raw silk to degumming liquor was 1:25. After the hot water treatment, the sericin/water solution was filtered with a nonwoven fabric. Finally, a 0.3% w/w sericin/water solution was prepared. The sericin film was prepared by drying the sericin/water solution at 40 °C. The sericin film was immersed in a 70% v/v ethanol/water solution for 1 h to crystallize it.

2.2. Preparation of the sericin/formic acid solutions and sericin films

The sericin/water solution was dried at 80 °C to obtain solid sericin powder. To prepare the sericin/formic acid solution, the solid sericin powder was dissolved in 98% formic acid at 45 °C for 30 min. To fabricate the sericin films, the solid sericin powder was dissolved in 98% formic acid at 55 °C for 30 min and the sericin/formic acid solution was dried in a fume hood. The sericin films cast from the formic acid were immersed in a 70% v/v ethanol/water solution for 1 h to crystallize them.

2.3. Measurement and characterization of the sericin solutions and films

The turbidity of the solutions was measured to examine the solution properties of the sericin solutions. The turbidity was calculated after measuring the transmittance of the solutions at 700 nm with a UV–Vis spectrophotometer (Evolution 201, Thermo Fisher Scientific, USA) as a function of the sericin concentration. The following equation was used for the calculations: $\tau = -(\ln T)/c$, where τ is the turbidity, T is the transmittance at 700 nm, and c is the cell length.

The molecular/particle size distribution of sericin in the solutions was measured with a dynamic light scattering spectrophotometer (ELSZ-1000, Otsuka Electronics, Japan). However, molecular aggregates and insoluble particles of sericin may exist in the solutions. The sericin aggregates and insoluble particles of the 0.1% sericin/water and sericin/formic acid solutions were removed by centrifugation (relative centrifugal force (RCF) = 3000 × g, for 10 min) and the supernatant sericin solution was collected and used for the measurements.

The rheological properties of the sericin solutions were determined with a rheometer (MARS III, Thermo Fisher Scientific, Germany) using a 60 mm cone in the plate geometry with a 1° cone angle at 25 °C. Frequency sweep tests were conducted to evaluate the complex viscosity of the sericin solutions. The angular frequency ranged from 0.1 to 100 rad/s with a strain of 0.1%. In addition, axial tests were performed to investigate the gelation behavior of the sericin solutions. The 35-mm-diameter plate of the rheometer compressed the sericin samples at a speed of 0.18 mm/s.

Scanning electron microscopy (SEM) was used to examine the cross sections of the sericin films. The sericin films were Pt-Pd-coated for SEM imaging (FE-SEM, S-4800, Hitachi, Japan).

Fourier transform infrared spectroscopy (FTIR, Nicolet 380, Thermo Fisher Scientific, USA) was performed using the attenuated total reflection method to examine the molecular conformation of the sericin films. As shown in Fig. 1, the crystallinity index of a sericin film was calculated as the intensity ratio of the 1645 and 1616 cm^{-1} absorptions of the amide I band using Eq. (1) [30]:

$$\text{Crystallinity index}\,(\%) = \frac{A_{1616\,cm^{-1}}}{A_{1616\ cm^{-1}} + A_{1645\ cm^{-1}}} \times 100 \quad (1)$$

where $A_{1616\,cm^{-1}}$ and $A_{1645\,cm^{-1}}$ are the absorbances at 1616 cm^{-1} and 1645 cm^{-1}, respectively.

To examine the crystalline structure and crystallinity of the sericin films, small-angle X-ray scattering (SAXS) with a general area detector diffraction system (GADDS, Bruker-AXS, Germany) using Cu K_α radiation was used for the X-ray diffraction (XRD) analysis. The irradiation conditions were 45 kV and 40 mA with a measurement time of 10 min. The XRD patterns were obtained by scanning the 2θ angles in the fiber diagram.

The water content of the sericin films was determined with Eq. (2).

$$\text{Water content}\,(\%) = \frac{\text{Intial weight} - \text{dry weight}}{\text{dry weight}} \times 100 \quad (2)$$

The dry weight of the sericin films was measured with a moisture balance (XM60, Precisa, Switzerland). The sericin films were conditioned at the standard conditions (20 °C and 65% relative humidity) for more than 1 day to reach the equilibrium state. After that, the weight of sericin films was measured with the moisture balance to obtain the weight of the sericin films under standard conditions.

To evaluate the mechanical properties of the sericin films, stress–strain curves were obtained with a universal testing

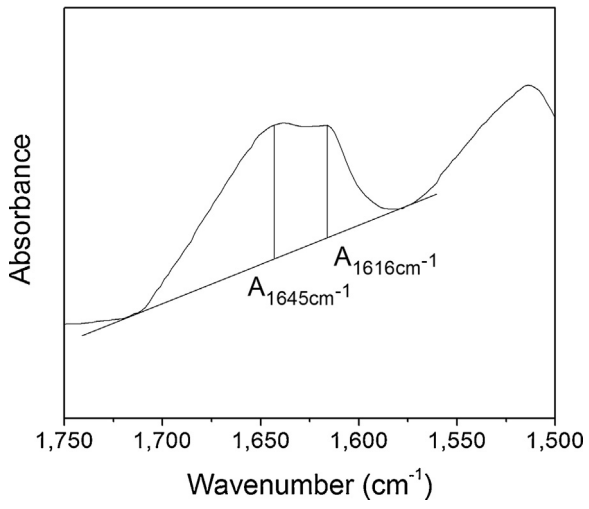

Fig. 1. Determining the crystallinity index of sericin from the FTIR peaks of the amide I band.