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Effect of centrifugation on the structure and properties of silk sericin

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Abstract

Recently, silk sericin has been studied extensively for biomedical and cosmetic applications because of its unique properties, including UV resistance and wound healing ability. For use in applications, sericin is fabricated in various forms including films and gels. However, the mechanical properties of sericin are too weak. In this basic study on improving the mechanical properties of sericin, a silk sericin aqueous solution was separated into two layers by centrifugation. The solution viscosity, molecular conformation, and mechanical properties of each separation layer of the sericin were examined. Sericin from the lower layer had a higher solution viscosity and film mechanical properties (strength and strain) than that from the upper layer, implying that sericin from the lower layer had a higher molecular weight than that from the upper layer. The molecular conformation of the sericin films varied depending on the casting solvent. In aqueous solution, the sericin film from the lower layer showed a β-sheet conformation, whereas that from the upper layer displayed a random coil conformation. All the sericin films showed a highly β-sheet-crystallized state when cast in formic acid, regardless of the separation layer.

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Introduction

Silk is a natural composite fiber in which sericin covers two strands of fibroin. The sericin has been removed from silk fiber to improve the luster and feel of silk textiles (Jo and Um, 2015). However, useful properties of sericin as a biomaterial have been reported recently. Specifically, sericin is highly resistant to oxidation (Suzuki *et al*., 2004), is UV resistant (Sarovart *et al*., 2003), and has a moisturizing effect on skin (Padamwar *et al*., 2005). In addition, Tsubouchi *et al.* (2005) reported that sericin improves the attachment of cultured human skin fibroblasts. Nagai *et al.* (2009) revealed that sericin can promote wound healing. Dash *et al.* (2008) reported that tasar silk sericin inhibits ultraviolet-B-induced apoptosis in human skin keratinocytes.

However, sericin has undesirable physical properties that limit its use in biomedical and cosmetic applications. For instance, sericin films are too brittle. Therefore, they are difficult to fabricate because they split easily during the casting process. In addition, the mechanical properties of prepared sericin films are too low.

Therefore, researchers have tried to improve the mechanical properties of sericin. Jo and Um (2015) used formic acid as a casting solvent for sericin and found that the mechanical properties of sericin could be remarkably improved. Chung *et al.*

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(2015) extracted sericin from silk cocoons of different silkworm varieties and found that the mechanical properties of sericin film can be enhanced by choosing the cocoons of the optimal silkworm variety.

Despite this enhancement, it is necessary to further improve the mechanical properties of sericin films. In this basic study on improving the mechanical properties of sericin, a silk sericin aqueous solution was centrifuged to obtain two different layers. The differences in the viscosity of the sericin solution as well as the molecular conformation and mechanical properties of sericin films depending on the separation layer were examined.

Materials and Methods

Preparation

To extract the sericin aqueous solution, Baekokjam silk cocoons were treated in 120 °C water for 30 min using an autoclave (JSAC-60, JSR, Japan). The sericin aqueous solution was centrifuged by a centrifugal separator (VS-150Fi, Vision, South Korea) at 20 °C for 120 min. The centrifugal force was 17,000*g*. After centrifugation, the silk sericin was separated into two layers: upper and lower layers. The sericin in each layer was extracted separately and dried at 80 °C to obtain sericin films and powders. The sericin powders were dissolved in 98% formic acid at 55 °C for 30 min to prepare 0.3% (w/w) sericin–formic acid solutions. The sericin–formic acid solutions were cast in a fume hood to fabricate sericin films.

Measurement and characterization

The rheological properties of the sericin–formic acid solutions were examined at 25 °C using a rheometer (MARS III, Thermo Fisher Scientific, Germany) with a 60 mm cone and a plate geometry with a 1° cone angle. To evaluate the complex viscosity of the sericin–formic acid solutions, a frequency sweep test was conducted. The strain was 0.1%, and the angular frequency was controlled from 0.1 to 100 rad/s.

To examine the crystalline structure of the sericin films, a Fourier transform infrared (FTIR) spectrometer (Nicolet 380, Thermo Fisher Scientific, USA) was used. The crystallinity index was calculated from the FTIR spectrum as the intensity ratio of the 1616 and 1645 cm^{-1} lines using eq. 1 (Jo and Um, 2015).

$$\text{Crystallinity index (\%)} = \frac{A_{1616\mathrm{cm}^{-1}}}{A_{1645\mathrm{cm}^{-1}} + A_{1616\mathrm{cm}^{-1}}} \times 100 \qquad \text{(eq. 1)}$$

$A_{1616\mathrm{cm}^{-1}}$: Absorbance at 1616 cm^{-1}

$A_{1645\mathrm{cm}^{-1}}$: Absorbance at 1645 cm^{-1}

To evaluate the mechanical properties of the sericin films, a universal test machine (OTT-03, Oriental TM, South Korea) was used. Tensile tests were conducted using a 3 kgf load cell at an extension rate of 10 mm/min. The dimensions of the sericin film samples prepared for measurement were 5 mm (width) × 50 mm (length), and the gauge length was 30 mm. All the samples were preconditioned under the standard condition (20 °C and 65% relative humidity); five samples of each sericin film were tested under the standard condition, and the average and standard deviation of the measurement results were used.

Results and Discussion

Rheological properties of sericin solution

The rheological properties of polymer solutions are studied because they provide information on the structural characteristics of the polymer and polymer solution states. Because the silk sericin solution easily becomes a gel in the aqueous state, the rheology of the silk sericin–formic acid solution was studied (Jo and Um, 2015). The sericin–formic acid solutions from the upper and lower layers were used in the rheological measurement, and the result is shown in Fig. 1.

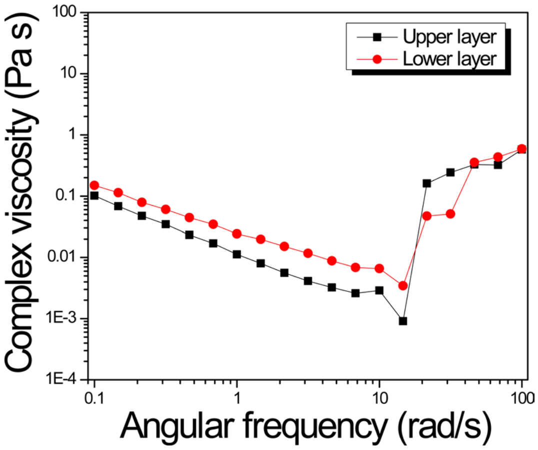

Fig. 1. Complex viscosity of 0.3% (w/w) silk sericin–formic acid solutions containing sericin from upper and lower layers.

Regardless of the separation layer, all the sericin–formic acid solutions exhibited shear thinning until 10 rad/s, and the complex viscosity increased above 10 rad/s. This result is in good agreement with that of a previous report (Jo and Um, 2015). The sericin–formic acid solution from the lower layer showed slightly higher viscosity at lower angular frequencies (<10 rad/s) than that from the upper layer. Considering that the same sericin concentration (0.3%) was used to fabricate the sericin–formic acid solutions, the higher viscosity of the sericin solution from the lower layer might be related to a difference in the molecular weight (MW) of the sericin. That is, the higher-MW sericin molecules may move to a lower separation layer under the centrifugal force. As a result, the higher-MW sericin molecules are located in the lower layer rather than the upper layer.

Molecular conformation and crystallinity index of sericin films

The molecular conformation of sericin was examined using FTIR spectroscopy because it affects the gelation of sericin and the mechanical properties of sericin films (Jang *et al.*, 2015; Jo *et al.*, 2013; Jo and Um, 2015; Jo *et al.*, 2015). To examine the molecular conformation of the sericin films obtained from different separation layers, FTIR measurements were conducted, and the results are shown in Fig. 2.

The sericin film cast from the upper layer of the aqueous solution showed IR absorption at 1645 cm^{-1}, which is attributed to the random coil conformation (Jo and Um, 2015; Jo *et al.*, 2015). On the other hand, the sericin film from the lower layer exhibited an IR absorption peak at 1616 cm^{-1} and a shoulder peak at 1645 cm^{-1}. These are due to the β-sheet conformation and random coil conformation, respectively. This result indicates that the sericin film from the upper layer has a random coil conformation, and the film from the lower layer contains mainly the β-sheet conformation with some random coil conformation. The difference in the crystallization of sericin from the upper and lower layers is confirmed by the crystallinity index, as shown in Fig. 3. The crystallinity index of the sericin film from the lower layer was 51%, whereas that of the film from the upper layer was 41.2%.

In this study, the sericin films were fabricated by drying sericin aqueous solution at 80 °C. Sericin reportedly adopts a random coil conformation when a sericin aqueous solution is dried at 80 °C (Jo *et al*., 2015). The reason is that the high temperature (80 °C) prevents hydrogen bond formation between sericin molecules and β-sheet crystallization of sericin.

However, β-sheet crystallization occurred in the sericin film from the lower layer. This is tentatively attributed to the centrifugal force because, after centrifugal separation, the sericin in the lower layer was found to be in a semi-gel state. That is, the sericin aqueous solution was converted to the gel state after centrifugal separation. The reason might be that hydrogen bonds between sericin molecules were formed by the centrifugal force. As a result, crystallization and gel formation of sericin occurred. Because some of the sericin from the lower layer became a gel before drying at 80°C, it is thought that part of the resultant sericin film contains the β-sheet conformation.

To exclude the effect of gelation of the sericin aqueous solution, the sericin aqueous solutions from different layers

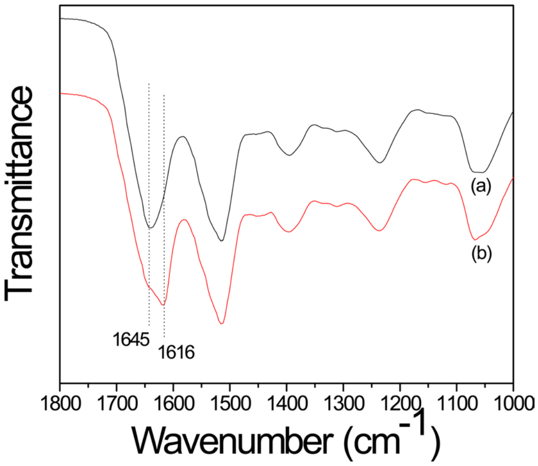

Fig. 2. FTIR spectra of silk sericin films cast from (a) upper layer and (b) lower layer of sericin aqueous solutions.

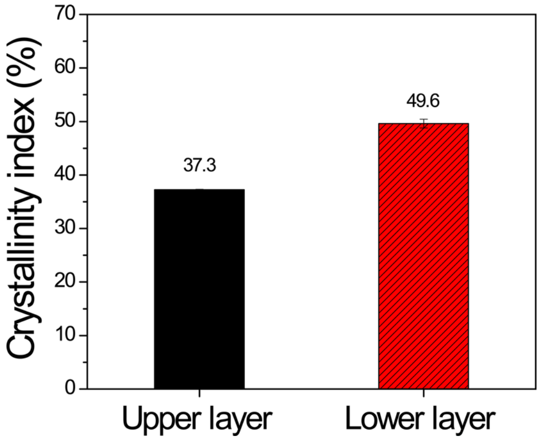

Fig. 3. Crystallinity index of silk sericin films cast from different separation layers of sericin aqueous solutions.

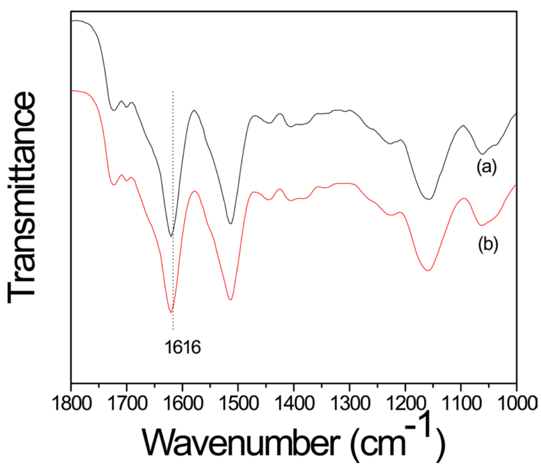

Fig. 4. FTIR spectra of silk sericin films cast from sericin–formic acid solutions containing sericin from (a) upper layer and (b) lower layer.

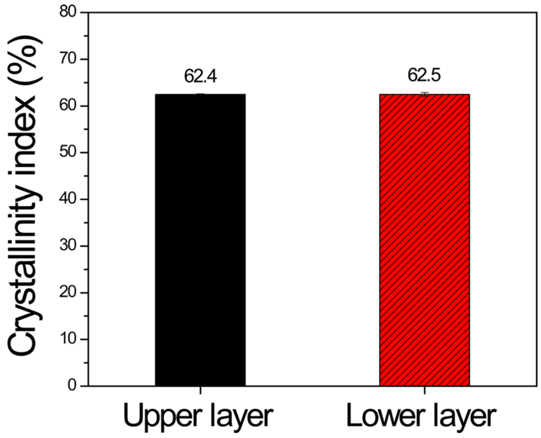

Fig. 5. Crystallinity index of silk sericin films cast from sericin–formic acid solutions containing sericin from different separation layers.

were dried to prepare sericin powder, which was dissolved in formic acid to obtain the sericin–formic acid solutions. These solutions were cast to obtain sericin films, which were examined by FTIR measurement. Figs. 4 and 5 show the FTIR spectra and crystallinity index, respectively, of sericin films cast from the sericin–formic acid solutions from the upper and lower layers. Regardless of the separation layer, all the sericin films showed a strong IR peak at 1616 cm^{-1}, which is attributed to the *β*-sheet conformation. The IR peaks and crystallinity indices of the upper and lower layers do not differ, indicating that the separation layer does not affect the molecular conformation and crystallinity index of the sericin films cast from the formic acid solution. The highly crystallized sericin films cast from formic acid are consistent with a previous report (Jo and Um, 2015).

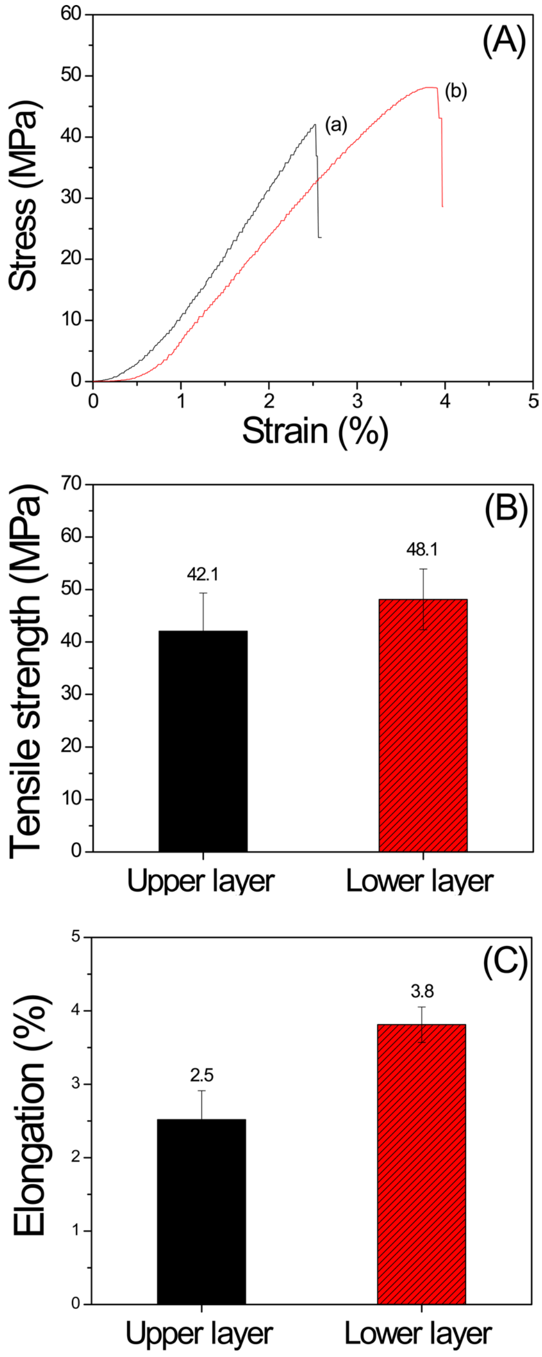

Fig. 6. (A) Representative stress-strain curve, (B) tensile strength, and (C) elongation of silk sericin films cast from sericin–formic acid solutions containing sericin from (a) upper layer and (b) lower layer.

Mechanical properties of sericin films

Fig. 6 shows the mechanical properties of sericin films cast from sericin–formic acid solutions from different layers. The sericin film from the lower layer showed higher tensile strength (48.1 MPa) and elongation (3.8%) than that from the upper layer

(42.1 MPa, 2.5%). As shown in Fig. 5, the crystallinity indices of the sericin films cast from formic acid are almost the same. Therefore, the difference in the mechanical properties of the sericin films might be due to a change in the MW of sericin. That is, as revealed in Fig. 1, the sericin from the lower layer has a higher MW (viscosity) than that from the upper layer. Therefore, it is thought that the higher-MW sericin in the lower layer contributes to its better mechanical properties compared to the upper layer.

Conclusions

In this study, a sericin aqueous solution was separated into upper and lower layers by centrifugation, and the differences in the solution viscosity, film molecular conformation, and film mechanical properties of sericin from each layer were examined. The sericin from the lower layer exhibited a higher solution viscosity and better film mechanical properties. This indicates that the MW (solution viscosity) of sericin is important for fabricating stronger sericin films and that centrifugal separation might be a suitable method for extracting useful sericin and excluding useless sericin to fabricate sericin films with good mechanical properties.

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