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Dissolution, crystallilnity, and mechanical properties of silk sericin from Sericinjam silkworm cocoons

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Abstract

Recently, a silkworm strain (tentatively named Sericinjam) producing 100% sericin cocoons has been studied in South Korea. In this preliminary study, the crystallinity, mechanical properties, and dissolution conditions of sericin from Sericinjam cocoons were examined. The Sericinjam cocoon could be dissolved in water at high temperature (120°C) and high pressure (HTHP method) in an autoclave and in a $CaCl_2/H_2O/EtOH$ mixture (ternary solvent method), resulting in 82% and 97% dissolution after 30 min, respectively. The solution viscosity of the silk sericin formic acid (SSFA) solution obtained from sericin extracted using the ternary solvent method was higher than that obtained using the HTHP method; however, SSFA solutions obtained from sericin extracted from conventional Baekokjam cocoons yielded a higher solution viscosity. The crystallinity and breaking strength of the sericin film from Sericinjam cocoons were slightly lower, respectively, than those from Baekokjam cocoons. In contrast, the elongation at break of the Sericinjam sericin film obtained using the HTHP method was higher than that of the Baekokjam sericin film.

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Introduction

Silk sericin has attracted the attention of many researchers because it has the desirable properties of biocompatibility (Uebersax *et al*., 2013; Unger *et al*., 2004; Yang *et al*., 2007), cell adhesion and proliferation (Marelli *et al*., 2010; Minoura *et al*., 1995), wound healing (Aramwit *et al*., 2009; Nagai *et al*., 2009), high ultraviolet protection (Gulrajani *et al*., 2008), oxidation resistance (Suzuki *et al*., 2004), good water retention, wrinkle resistance (Padamwar *et al*., 2005), and cholesterol reduction (Limpeanchob *et al*., 2010; Seo *et al*., 2011). Owing to these unique properties, biomedical and cosmetic applications of silk sericin have been studied extensively (Aramwit *et al*., 2012; Siritientong *et al*., 2014; Zhang, 2002).

For such biomedical and cosmetic applications, the sericin is fabricated in various forms, including fibers (Zhang *et al*., 2012), gels (Jang and Um, 2017; Mase *et al*., 2006; Park *et al*., 2018; Teramoto *et al*., 2005), films (Jang and Um, 2017; Jo and Um, 2015; Nishida *et al*., 2011; Teramoto *et al*., 2008), sponges (Jang and Um, 2017; Park *et al*., 2018), particles (Cho *et al*., 2003), and beads (Oh *et al*., 2007). However, the sericin has low mechanical strength, and its molecules decompose in

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hot water during the extraction process from silk (Park *et al*., 2018).

Recently, the silkworms producing only sericin have attracted researchers' attention because a high production yield of sericin can be obtained by using these silkworms. Mase *et al*. (2006) labeled such silkworms the Sericin Hope silkworms, and their sericin product was labeled the Virgin Sericin. They reported that the corresponding sericin gel could be easily emulsified, firmed into a cream, or made into foam with 5% oil. Teramoto *et al*. (2005; 2008) fabricated elastic sericin gel and sericin gel films using the Virgin Sericin and reported that the sericin gel films exhibited morphological stability against swelling and good handling properties. Kweon *et al*. (2009) investigated the characteristics of silk sericin extracted from Sericinjam. They examined molecular weight, amino acid composition, molecular conformation, and thermal stability of sericin from Sericinjam. Kim *et al*. (2009) also examined the structure and thermal properties of sericin from Sericinjam using SDS-PAGE, UV-Vis spectroscopy, circular dichroism spectroscopy, and differential thermal scanning calorimetric analysis. Zhang *et al*. (2012) successfully fabricated an electrospun sericin web using the Sericin Hope silkworm cocoons.

Although various studies on sericin produced from Sericinjam and Sericin Hope silkworms have been conducted, the dissolution behavior, rheological properties and mechanical properties of sericin obtained from them have not yet been conducted in detail.

Therefore, in the present study, we examined the dissolution behavior of the Sericinjam cocoon under different dissolution conditions and prepared the sericin films to investigate their molecular conformation and mechanical properties by comparison with those of the sericin extracted from conventional silkworm cocoons (Baekokjam silkworm cocoons) comprising fibroin and sericin.

Materials and Methods

Materials

Bombyx mori Sericinjam silkworms that only produce sericin and Baekokjam silkworms were grown and their cocoons were harvested at the National Institute of Agricultural Science (Wanju, Republic of Korea).

Preparation of silk sericin powder

Silk sericin was extracted from Sericinjam silkworms using the following two methods: 1) extraction from water at high temperature and high pressure (HTHP method) and 2) dissolution in a mixed solution containing $CaCl_2$, H_2O, and ethanol (EtOH) (ternary solvent method). In the HTHP method, the Sericinjam silkworm cocoons were immersed in purified water and heated to 120 °C for 30 min in an autoclave (JSAC-60, JSR, Gongju, Republic of Korea). The purified water was obtained using a water purification system (RO50, Hana Science, Seongnam, Republic of Korea) equipped with a reverse osmosis membrane. The liquor ratio was 1:100. After the extraction, the sericin aqueous solutions were filtered through a polyester nonwoven fabric. In the ternary solvent method, the Sericinjam silkworm cocoons were dissolved in a ternary solvent containing $CaCl_2/H_2O/EtOH$ (1/8/2 molar ratio) at 85 °C for 5 min. The liquor ratio was 1:20. After the dissolution, the silk sericin solutions were dialyzed in a cellulose tube (molecular weight cut off = 12,000–14,000 Da) against circulating purified water for 5 days at 60 °C. For comparison, Baekokjam silkworm cocoons were immersed in purified water and heated to 120 °C for 30 min in an autoclave (Jang and Um, 2017; Jo *et al*., 2015). The liquor ratio was 1:25. The aqueous sericin solutions were poured onto petri dishes and solidified at 80 °C in a drying oven (WOF-50, Daihan Scientific, Wonju, Republic of Korea) to obtain silk sericin powders.

Preparation of silk-sericin formic acid solutions and films

To determine the rheological behavior of the sericin solutions, 0.3% (w/w) regenerated silk-sericin formic acid (SSFA) solutions were employed. The silk sericin powder was dissolved in a 98% (v/v) formic acid at 55 °C and stirred at 200 rpm for 30 min to prepare a SSFA solution. After the dissolution, the regenerated SSFA solutions were filtered through a polyester nonwoven fabric and stored at room temperature (25 °C) for 30 min. Thereafter, 20 mL of the 0.3% (w/w) regenerated SSFA solutions were poured onto petri dishes and dried in fume hood to obtain sericin films (Jo and Um, 2015; Park *et al*., 2018).

Characterization methods

The solubility of Sericinjam silkworm cocoons was measured at various dissolution times (5, 10, 20, and 30 min) for both dissolution methods (i.e., HTHP and ternary solvent methods). After the dissolution, the sericin solutions were filtered through a polyester nonwoven fabric to remove undissolved solids. The sericin solubility was calculated using Eq. 1.

$$\text{Solubility (\%)} = \frac{W_1 - W_2}{W_1} \times 100 \quad (1)$$

W_1: dry weight of Sericinjam cocoons

W_2: dry weight of undissolved Sericinjam cocoons

The complex viscosity of the 0.3% (w/w) SSFA solutions was measured using a rheometer (MARS III, Thermo Fisher Scientific, Karlsruhe, Germany) at 25 °C with a 60 mm cone and plate geometry with a 1° cone angle as a function of shear rate (0.01–100 s^{-1}) (Bae and Um, 2020; Jo and Um, 2015; Park and Um, 2015). The strain and frequency were controlled to 1% and 1 Hz, respectively.

The mechanical properties of the sericin films were evaluated using a universal test machine (OTT-003, Oriental TM, Ansan, South Korea). The tests were performed with a 3 kgf load cell at an extension rate of 10 mm min^{-1}. The gauge length was 30 mm. The sericin films casted from SSFA were cut into 5 mm × 50 mm pieces and preconditioned at 20 °C and 65% relative humidity. The mean and standard deviation of the mechanical test results of seven measurements were reported (Jo and Um, 2015; Lee *et al.*, 2016; Lee *et al.*, 2022).

The molecular conformation and crystallinity index of silk sericin films were examined using Fourier transform infrared spectroscopy (FTIR spectroscopy; Nicolet 380, Thermo Fisher Scientific, Waltham, MA, USA) with the attenuated total reflection (ATR, Smart iTR ZnSe) method. The scan range, scan number, and resolution were 4000–650 cm^{-1}, 32, and 8 cm^{-1}, respectively (Bae *et al.*, 2021; Choi *et al.*, 2020). The crystallinity index was calculated as the intensity ratio of the peaks occurring at 1620 and 1650 cm^{-1} in the FTIR spectrum, according to Eq. 2. The mean and standard deviation of the crystallinity index were reported for seven FTIR measurements.

$$\text{Crystallinity index (\%)} = \frac{A_{1620\,cm^{-1}}}{A_{1650\,cm^{-1}} + A_{1620\,cm^{-1}}} \times 100 \quad (2)$$

$A_{1620cm^{-1}}$: the absorbance at 1620 cm^{-1} caused by β-sheet crystallites (crystalline region)

$A_{1650cm^{-1}}$: the absorbance at 1650 cm^{-1} caused by random coils (amorphous region)

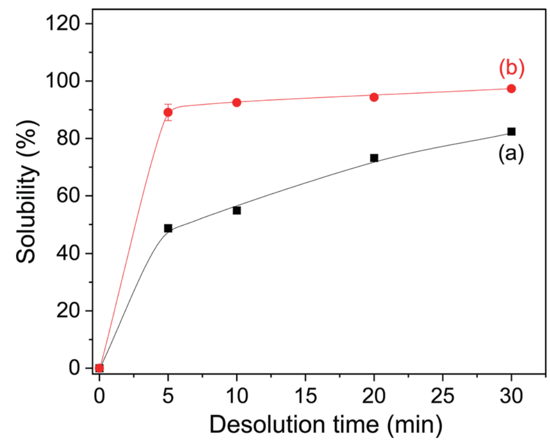

Fig. 1. Solubility of Sericinjam silkworm cocoons under various dissolution times and methods: (a) HTHP method and (b) the ternary solvent ($CaCl_2/H_2O/EtOH$) method.

Results and Discussion

Dissolution of Sericinjam silkworm cocoons

Two methods for the dissolution of Sericinjam silkworm cocoons were studied, and the solubility profiles were measured over time (Fig. 1). The amount of Sericinjam cocoons solvated using both dissolution methods increased rapidly and reached a plateau after approximately 5 min. The ternary solvent method (Fig. 1b) showed a higher overall dissolution of Sericinjam cocoons than the HTHP method (Fig. 1a). At a dissolution time of 30 min, 97.4% of Sericinjam cocoons were dissolved in the ternary solvent, whereas only 82.4% of Sericinjam cocoons were dissolved using the HTHP method. This result indicates that the ternary solvent method was a more effective dissolution method. For reference, when the Sericinjam cocoon was dissolved in LiBr solution at 70 °C, its solubility was reported to 75% (Kweon *et al.*, 2009). Considering that most of the sericin from Baekokjam silkworm cocoons was dissolved under the same HTHP conditions (120 °C, 30 min) (Jo *et al.*, 2015), it is noteworthy that 80% of Sericinjam cocoon were dissolved under the HTHP condition. Although the exact reason for this difference was not elucidated in this study, it is assumed that the sericin from Sericinjam cocoons has a different structure than the sericin from Baekokjam cocoons.

Rheological properties of the SSFA solution

Because the molecular weight (MW) of sericin determines its mechanical properties (Park *et al.*, 2018), it is important

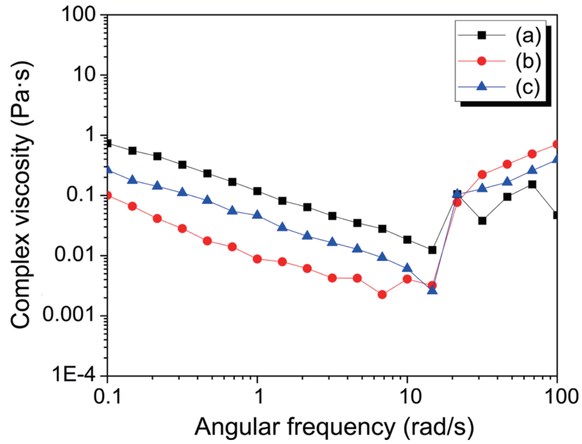

Fig. 2. Complex viscosity of the 0.3% (w/w) silk-sericin formic acid solutions obtained from various cocoons, using various dissolution methods, and for various dissolution times: (a) Baekokjam cocoon, HTHP method, 30 min; (b) Sericinjam cocoon, HTHP method, 30 min; and (c) Sericinjam cocoon, the ternary solvent method ($CaCl_2$/H_2O/EtOH), 5 min.

to measure the MW of sericin exactly. The measurement of rheological properties, such as solution viscosity, is a simple method to determine the MW of sericin owing to a strong correlation between the MW and solution viscosity (Oh *et al*., 2011; Park *et al*., 2018).

In this study, the complex viscosities of the Sericinjam SSFA solutions obtained using the HTHP and ternary solvent methods were measured and compared to that of the Baekokjam SSFA solution obtained using the HTHP method; the results are shown in Fig. 2. All the SSFA solutions showed shear thinning until 10 rad s^{-1} and shear thickening thereafter. This trend is consistent with previously reported studies (Jang and Um, 2017; Jo and Um, 2015; Park *et al*., 2018). The Baekokjam SSFA obtained using the HTHP method (Fig. 2a) showed the highest complex viscosity; the Sericinjam SSFA obtained using the HTHP method (Fig. 2b) showed the lowest viscosity. When Sericinjam sericin was dissolved into the ternary solvent (Fig. 2c), its complex viscosity was higher than that for the HTHP method. However, the Sericinjam SSFA solutions (Fig. 2b and 2c) showed lower viscosities than the Baekokjam SSFA solution (Fig. 2a), indicating that more Sericinjam sericin degradation occurred under the HTHP conditions than under the ternary solvent conditions. This order of viscosities implies that although Baekokjam sericin (i.e., >95%) is more dissolved under HTHP conditions (Jo *et al*., 2015) than Sericinjam sericin (~80%), Baekokjam sericin has a higher MW than that of Sericinjam sericin. While the exact reason could not be elucidated in this study, two logical hypotheses can be suggested. First, the original MW of Sericinjam sericin is higher than that of Baekokjam sericin. Second, although the original MW of Sericinjam sericin is similar to that of Baekokjam sericin, the Sericinjam sericin is more liable to decomposition under HTHP conditions than Baekokjam sericin. More detailed studies are necessary to further understand the differences observed.

Molecular conformation and crystallinity of sericin films

Fig. 3A presents the FTIR spectra of silk sericin films from Baekokjam and Sericinjam cocoons. All sericin films showed a strong infrared absorption peak at 1620 cm^{-1} that was attributed to the β-sheet conformation (Bae *et al*., 2021; Kim *et al*., 2022). This indicated that all sericin films were highly crystallized, as expected, because the sericin films were prepared with formic

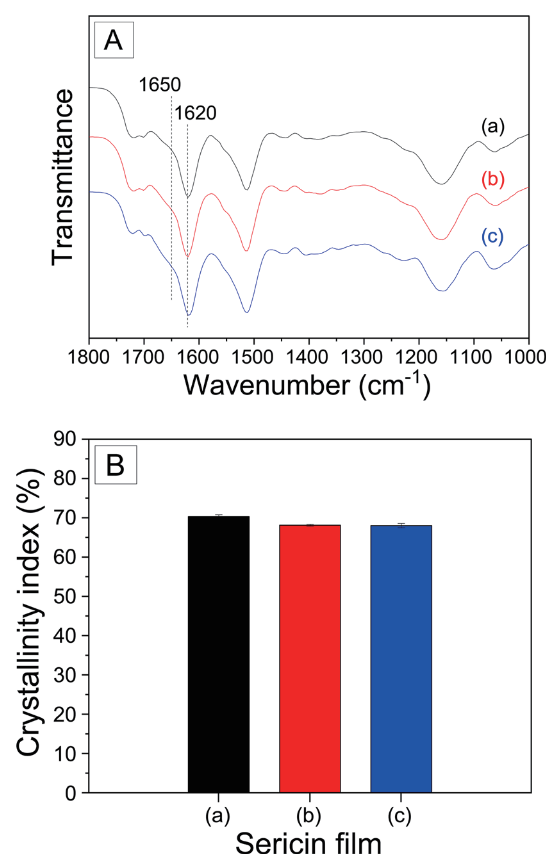

Fig. 3. (A) ATR–FTIR spectra and (B) crystallinity index of the sericin films obtained from various silkworm cocoons, using various dissolution methods, and for various dissolution times: (a) Baekokjam cocoon, HTHP method, 30 min; (b) Sericinjam cocoon, HTHP method, 30 min; and (c) Sericinjam cocoon, the ternary solvent ($CaCl_2$/H_2O/EtOH) method, 5 min (n = 7).

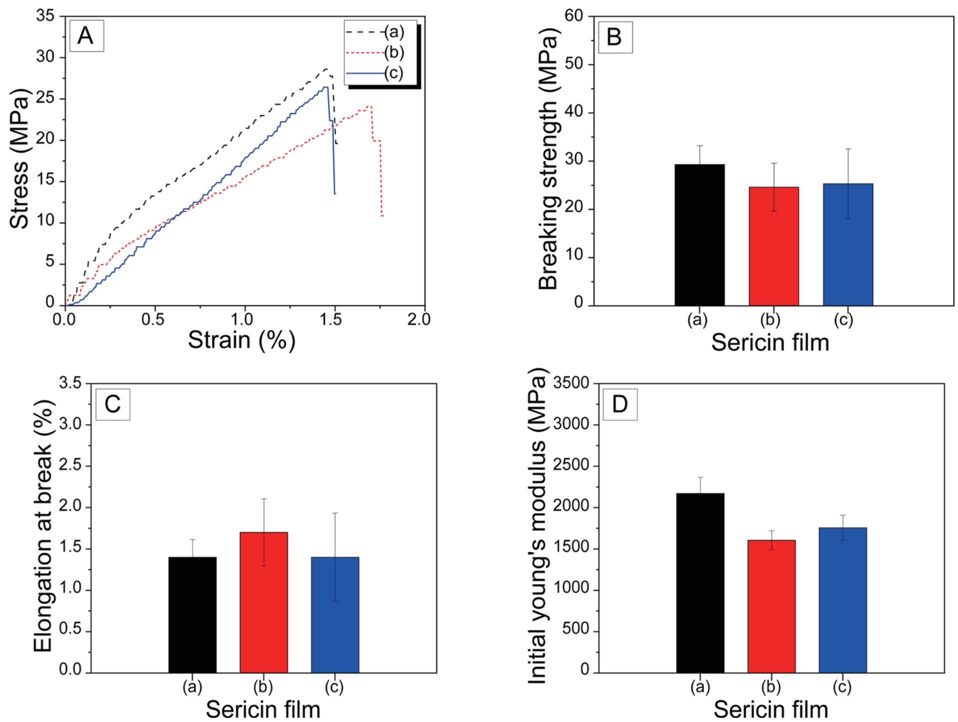

Fig. 4. (A) Representative stress–strain curve, (B) breaking strength, (C) elongation at break, and (D) initial Young's modulus of the sericin films obtained from various silkworm cocoons, using various dissolution methods, and for various dissolution times: (a) Baekokjam cocoon, HTHP method, 30 min; (b) Sericinjam cocoon, HTHP method, 30 min; and (c) Sericinjam cocoon, the ternary solvent ($CaCl_2/H_2O/EtOH$) method, 5 min (n = 7).

acid. Formic acid has been reported to induce the crystallization of silk fibroin (Um *et al.*, 2001; 2003) and sericin (Jo and Um, 2015).

To quantitatively examine the extent of molecular conformation of sericin, the crystallinity index was calculated (Fig. 3B). The Baekokjam sericin film (70.3%) showed a slightly higher crystallinity index than the Sericinjam sericin films (68.0%–68.1%). The slightly higher crystallinity index of the Baekokjam sericin film might be attributed to its higher MW compared with the Sericinjam sericin films (Fig. 2). That is, Park *et al.* (2018) reported the crystallinity of sericin film has a positive correlation with its MW.

Mechanical properties of the silk sericin films

Fig. 4 presents the mechanical strength test results for the silk sericin films obtained from Sericinjam and Baekokjam cocoons. Regardless of the silkworm variety and dissolution conditions, all sericin films showed a linear increase in stress with strain, and that resulted in breakage, indicating that all sericin films were brittle rather than ductile. Regarding breaking strength and initial Young's modulus, the Baekokjam sericin film [Fig. 4B(a) and 4D(a)] showed slightly higher values than the Sericinjam sericin films. For elongation at break, the Sericinjam sericin film obtained using the HTHP method [Fig. 4C(b)] showed the highest value. It can be concluded that the slightly higher strength of Baekokjam films over that of Sericinjam films could be attributed to the higher MW of Baekokjam sericin, as determined from the SSFA solution viscosity measurements (Fig. 2).

Conclusions

In this study, the dissolution behavior, structure, and mechanical properties of Sericinjam sericin extracted using various dissolution methods were examined and compared to those of Baekokjam sericin. Although the solution viscosity and film mechanical properties of the sericin extracted from Sericinjam cocoons were slightly lower than those of the sericin extracted from Baekokjam cocoons, those of Sericinjam sericin are still comparable to those of Baekokjam sericin. When Sericinjam cocoons were dissolved into the ternary solvent, higher sericin solubility and SSFA solution viscosity were observed compared to those of the Sericinjam sericin extracted using the HTHP method. Because a dialysis procedure is required to remove $CaCl_2$ and ethanol from the sericin obtained using the ternary solvent method, care should be taken in selecting the appropriate dissolution method for obtaining Sericinjam sericin. Based on these preliminary results, a more detailed study on the sericin extracted from Sericinjam cocoons is currently underway; the results will be reported in due course.

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