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# Effect of extraction conditions on the stability and safety of sericin

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## Abstract

To assess the feasibility of silk sericin for non-textile application, the storage stability and biological safety of sericin were examined. It was extracted at 37°C, 70°C, 100°C, and 121°C for 1, 3, and 5 h to elucidate the effect of extraction condition on the stability and safety of silk sericin. The solubility was increased till approximately 26% with extraction temperature of 121°C for 1 h. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) showed that the molecular weight distribution depended on the extraction conditions. Extracted sericin displayed typical UV absorption bands upon spectrometric analysis. To examine the reproducibility of its obtained conformation, sericin was extracted thrice and its circular dichroism (CD) spectra was measured each time. Most CD spectra showed reproducibility regardless of temperature and time except under 100°C extraction condition. The diversity of CD spectrum showed gradual reduction and was finally coincident with extraction time from 1 to 5 h. Notably, sericin has a negative peak of approximately 200 nm attributed to random coil conformation, regardless of extraction condition. However, at the 100°C extraction condition, sericin showed both bands to be negative bands of approximately 200 and 220 nm, respectively. Sericin was centrifuged to determine the stability of storage conditions. The sericin extracted at 100°C and 121°C for 1 h was found to form gel rapidly within 1 h, but at 121°C condition, the gel fraction was approximately 20% within 1 h which retained its phase regardless of storage time. The gel fraction of sericin extracted at 100°C for 5 h increased with time, however at the 121°C for 5 h condition, the gel fraction was measured to be less than 10% regardless of increase in storage time. Petriflim™ AC plates test showed that sericin was safe from aerobic bacteria activity by extraction under high temperature.


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## Introduction

Silk sericin is one of major component of cocoon filament and accounts for approximately 25% of the cocoon protein. Sericin acts as a binder during silkworm spinning process (Arango *et al*., 2021; Kweon *et al*., 2012). In the textile industry, sericin was removed during the making of the silk fabric by degumming process and is considered as a pollutant in industrial wastewater.

The structural characteristics including the amino acid compositions of sericin using degummed methods have been

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recently studied (Lee *et al*., 2001; Kweon *et al*., 2009; Kim *et al*., 2009). Rheological characteristics and mechanical properties of sericin including breaking tensile strength, elongation, and Young's modulus were examined, which revealed that they depend on the genetic resources of the silkworms (Chung *et al*., 2015). Moreover, the conformational transition was adjusted by various methods including blending and using structure transition materials (Kweon and Cho, 2001; Kweon *et al*., 2000; Lee *et al*., 2003).

Sericin is traditionally known as a skin-compatible materials in Asian countries. The amino acid composition of sericin is similar to that of the plasma amino acid in infants (Steginк *et al*., 1982). The applications of sericin have been investigated as soap, bath preparation, cosmetics, toothpaste, and oral gargle ingredients (Lee *et al*., 2001; Kang *et al*., 2019). Recently, sericin has been studied for use as dietary resources, ointment, biological and tissue engineering materials including fetal bovine serum (FBS) substitutes (Yeo *et al*., 2006; Kang *et al*., 2000; Jo *et al*., 2021; Hong *et al.* 2021; Kim *et al*., 2020; Jo *et al*., 2019; Kim *et al*., 2021).

To further the application of silk sericin in non-textile usage, its quality control is important. The phase stability and shelf period of sericin were also a key characteristic to commercialize sericin usages.

In this study, sericin was extracted at various temperatures and times period conditions from cocoons of Korean commercial silkworm varieties, and their molecular weight distribution was measured using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), molecular conformation using spectrometric method including UV and CD, storage stability using centrifugation, and biological safety using aerobic bacteria detection method.

## Materials and methods

### Sample preparation

The cocoons of commercial silkworm varieties were obtained from the Industrial Insect and Sericulture Division, National Institute of Agricultural Sciences, RDA, Korea. Silk sericin was extracted at several temperatures (37°C, 70°C, 100°C, and 121°C) for varied hours (1, 3, and 5 h).

### SDS-PAGE

The extracted sericin 15 uL was added to 15 uL 8M urea (Sigma) and then 10 uL SDS-PAGE sample buffer (4× buffer, Bio-Rad Laboratories, Hercules, CA, USA). The mixture was heated at 50°C for 30 min till complete decomposition of silk sericin protein. SDS-PAGE was performed using a readymade 4-15% gradient gel (Bio-Rad laboratories, USA) according to the manufacturer's instructions. Next, the gel was stained using a silver staining kit (Invitrogen, Carlbad, CA, USA).

### Spectrometric analysis: UV and CD

UV spectrum was obtained using UV/VIS spectrometer (Lambda 10, Perkin Elmer, USA). CD spectra were obtained using CD spectrometer (J-1500, Jasco, Tokyo, Japan) equipped with a quartz cell of 10 mm path length at room temperature. The silk sericin solution was adjusted to a concentration of 0.01 wt.% and the scanning was performed within the range of 190-260 nm with 3 scans of accumulation.

### Storage stability: Centrifugation

The extracted sericin was centrifuged at 15,000 G and at room temperature several times. The supernatant and precipitate were separated and the weights of dried samples were measured, and then calculated sediment ratio (%).

Sediment ratio (%) = dried sediment weight / (dried supernatant weight + dried sediment ratio) × 100.

### Biological safety

The number of general bacteria was counted using the 3M™ Petrifilm™ AC medium (3M Petrifilm AC, 3M, St. Paul, MN, USA) method with serially diluted solution after being cultured at 35°C for 48 h to calculate the total aerobic bacteria.

## Results and Discussion

### Extraction and yield percentage

Sericin was extracted with various temperatures at 37°C, 70°C, 100°C, and 121°C. The extraction percentage was calculated from the weight difference of the cocoon weight at 1, 3, and 5 h treatment and then shown in Fig. 1. The extraction percentage was less than 10% at the low extraction temperatures (37°C and 70°C). At 100°C extraction condition, most sericin was extracted within 1 h and then increased slowly with time. At 121°C extraction condition, extraction percentage was increased upto around 26% within 1 h and then maintained similar value with time.

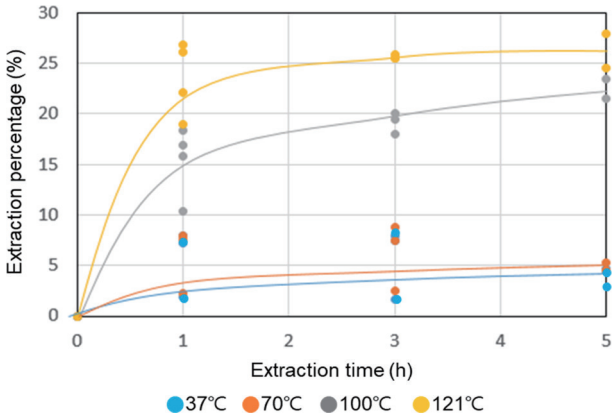


**Fig. 1.** Extraction percentage of sericin with the extraction temperature at 37°C, 70°C, 100°C, and 121°C.

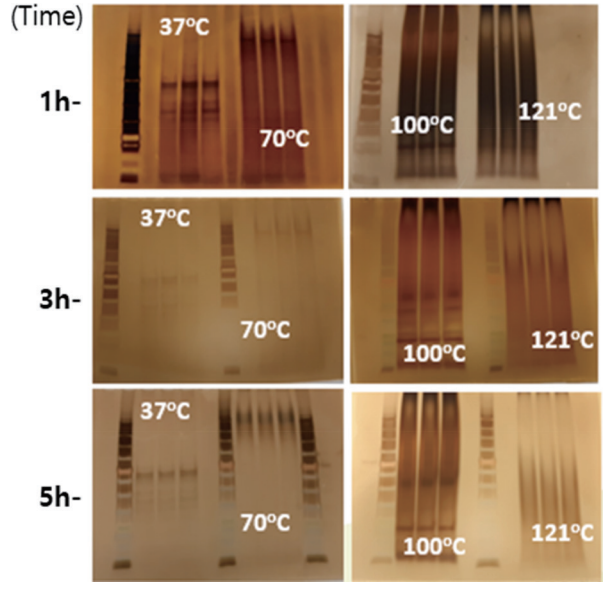


**Fig. 2.** SDS electrophoresis patterns of sericin according to extraction time and time.

## Molecular weight distribution

SDS-PAGE was used to examine the molecular distribution of silk sericin. Fig. 2 showed the molecular weight distribution pattern of sericin extracted at various temperatures. The main molecular weight of silk sericin obtained at 37°C and 70°C was measured to be approximately 70 kDa and 250 kDa, respectively. The molecular weight distribution of sericin extracted at high temperature condition showed a relatively broader range than that at low temperature condition. Especially, at 121°C extraction condition, the molecular weight distribution was decreased with extraction time.

Several researchers reported electrophoretic pattern of silk sericin extracted at various extraction conditions. Gamo *et al*. (1977) reported that sericin is a complex mixture of 5-6 polypeptides ranging in size between 40 and 400 kDa. Takasu *et al*. (2002) showed that sericin without heat treatment exhibited three main components at >250, 180, and 100 kDa. Kweon *et al*. (2009) reported that lithium bromide (LiBr) was used for extraction of sericin from various Korean silkworms, which showed 5 peptides of approximately 250, 120, 90, 70, and 40 kDa. Guo *et al*. (2022) reported 7 peptides of 400, 280, 260, 250, 230, 150, and 120 kDa. Sericin extracted at high temperature showed a tailed and broad electrophoresis pattern (Kim *et al*., 2009; Aramwit and Sangeakul, 2007). According to these results, the molecular weight and distribution of sericin varied with the denaturation method.

## UV spectra

Sericin is a protein secreted by silkworms. It exhibits characteristic absorption bands at specific wavelength region. UV absorption characteristics were measured as per the extraction temperatures and are shown in Fig. 3. Sericin showed a peptide band at 216 nm and an aromatic band at 278 nm.

Protein shows absorption in the range 180 to 230 nm almost entirely due to $\pi \rightarrow \pi^*$ transitions in the peptide bonds. Absorption in the range of 230-300 nm is dominated by the aromatic side-chains of tryptophan, tyrosine, and phenylalanine residues, with weak contribution by disulphide bonds near 260 nm (Saraiva, 2020). Sericin showed typical protein absorption bands.

## CD characteristics

The effects of extraction temperature and duration on the conformation of silk sericin were examined using CD spectrometer. As shown in Fig. 4, CD spectrum was measured thrice to elucidate its reproducibility according to the extraction temperature and duration. Except in the 100°C extraction condition, CD spectra showed similar pattern regardless of temperature (121°C, 70°C, and 37°C) and duration (1, 3, and 5 h). In this condition, the diversity of CD spectrum was found to reduce and finally be coincident with extraction time of 1 to 5 h. Notably, sericin exhibited a negative peak at approximately 200 nm attributed to random coil conformation, regardless of extraction condition. However, at the 100°C extraction condition, sericin exhibits another negative band at approximately 220 nm attributed to the β-sheet structure. The reproducibility of CD spectra at was increased with extraction time from 1 h to 5 h at

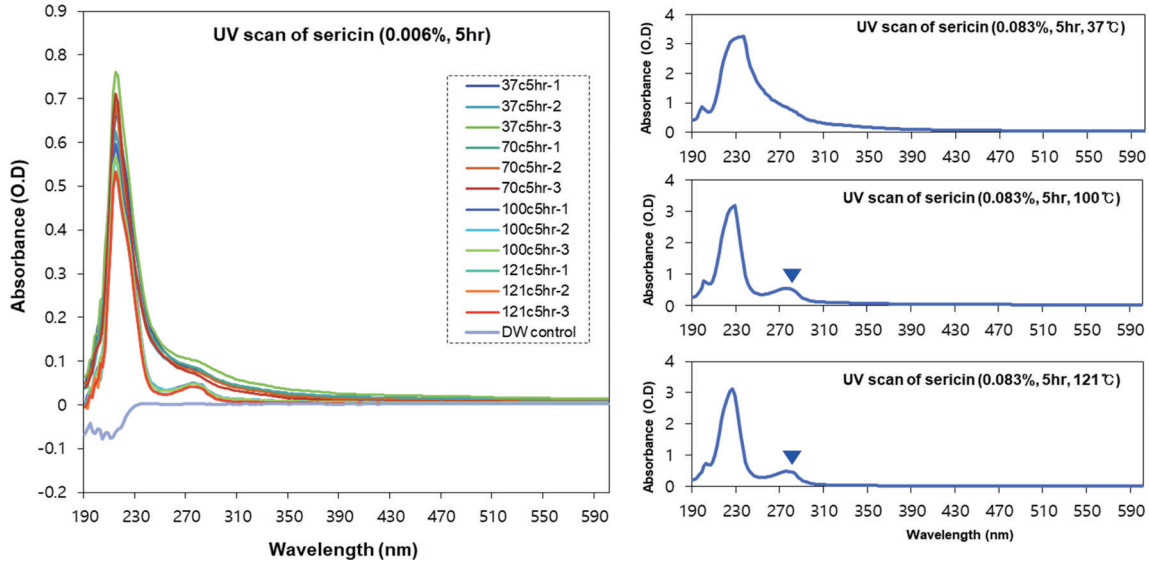


**Fig. 3.** UV spectra of sericin according to the extraction temperature and time.

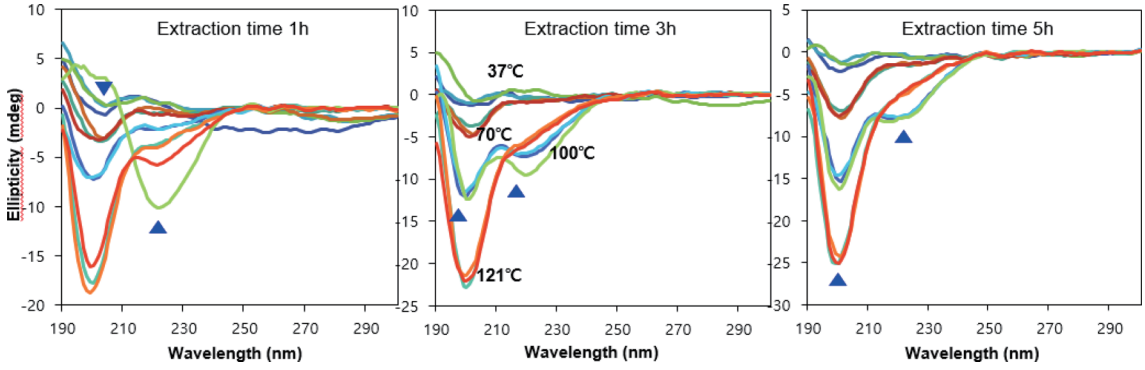


**Fig. 4.** CD spectra of sericin according to the extraction temperature and time.

the 100°C extraction condition.

Lee *et al*. (2021) studied the conformational transition of silk polymer using CD spectroscopy. By treating with ethanol, which is a structure transition material, CD spectra showed that the random coil characteristics band was decreased and β-sheet structural characteristics band was increased. The solution conformation of sericin was found to depend on the extraction conditions.

## Stability of sericin solution

Silk polymer including sericin and fibroin molecule disperses in water and slowly loses its solution stability to become gel-like during storage. In this experiment, sericin solution was centrifuged to separate the gel and sol phases during storage and the solid weight was then measured (Fig. 5). The sericin extracted at 100°C and 121°C for 1 h was observed to form gel rapidly within 1 h, but the gel fraction was till approximately 20% within 1 h at 121°C, which it retained regardless of storage duration. The gel fraction of sericin extracted at 100°C for 5 h increased with time; however, at the 121°C for 5 h condition, the gel fraction was measured as less than 10%, regardless of increase in storage duration.

Sericin solution extracted at 100°C was relatively unstable as compared to that in other extraction conditions and transitioned to gel-like over time. Sericin extracted at high temperature for 5 h showed a certain value during the storage duration of 3 d; therefore, this can be an optimum extraction condition for non-textile application. The centrifuge technique can also be a valuable technique to examine the stability of sericin solution.

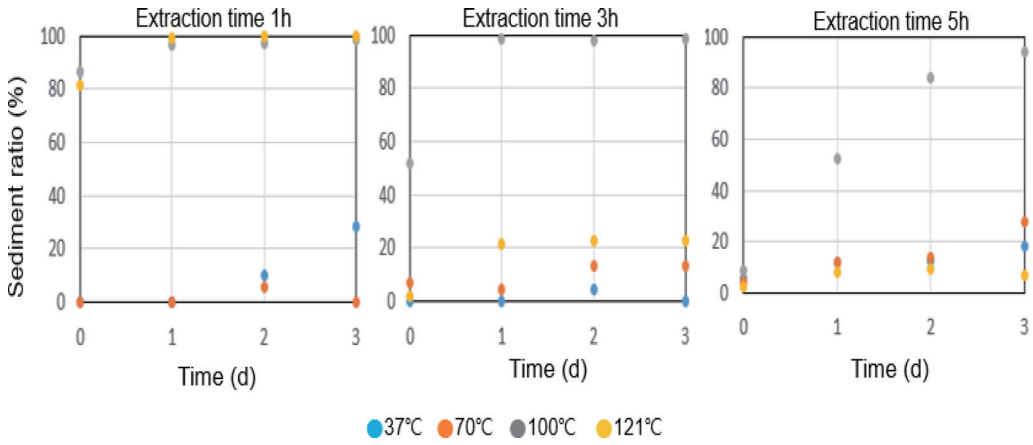


**Fig. 5.** Effect of extraction conditions on the sediment ratio(%) of sericin according to the storage time.

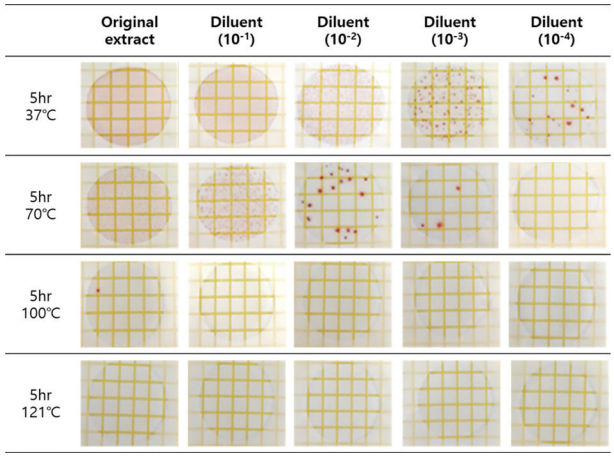


**Fig. 6.** Petriflim™ AC plate analysis of sericin with the various extraction temperatures for 5 h.

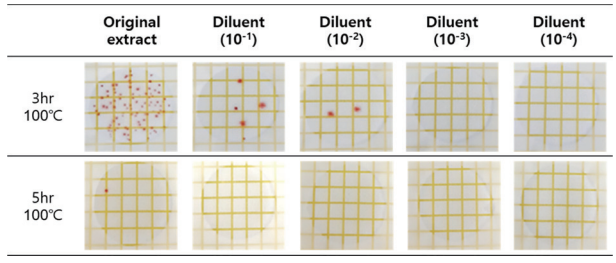


**Fig. 7.** Petriflim™ AC plate analysis of sericin extracted at 100°C for 3 and 5 h.

## Biological safety of sericin

To evaluate the relationship between the extraction conditions and aerobic bacteria counts, Petriflim™ AC plates were used (Fig. 6). Photographs of the plates confirmed numerous bacteria associated with sericin extracted at low temperature (37°C and 70°C) for 5 h , and biological safety associated with sericin extracted at high temperature (100°C and 121°C) for 5 h.

The effect of extraction duration on the 100°C extraction condition is shown in Fig. 7. The bacteria colony of sericin solution extracted in 100°C extraction condition were decreased with increase in the extraction duration.

Petriflim™ AC plates have been used several researchers to confirm the biological safety of food (Byrne Jr. and Bishop, 1991; Schraft and Watterworth, 2005; Yuji *et al.* 2021) because they are designed specifically for enumeration of aerobic bacteria in the food and dairy industry containing Standard Methods nutrients. According to findings of this study, sericin was found to be safe from the aerobic bacteria by extraction with high temperature.

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