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The Production Process and Characterization of Spore Free Sericin

Jun-ichi Miyakawa, Toshio Kubozuka, Kayo Ohya*, and Shoji Takigami*
Somedono,Yano Co. Ltd., 3-2112-1 Hishimachi, Kiryu, Gunma, 376-0001, Japan
Fax: 81-276-30-3349, e-mail: somedono@kkyano.co.jp
*Center for Material research by Instrumental Analysis, Gunma University, 1-5-1 Tenjincho, Kiryu, Gunma, 376-8515, Japan
Fax: 81-276-30-1141, e-mail: takigami@chem-bio.gunma-u.ac.jp

Sericin is a water-soluble protein and composed of hydrophilic amino acids and acidic amino acids. The key amino acids in sericin are serine, threonine, aspartic acid, and glutamic acid. Sericin has the antioxidative effect, the tyrosinase inhibitory effect, and the amelioration effect of the atopic dermatitis and the contact dermatitis as well as the moisture retention effect. It is rarely known that sericin contains the spore forming bacteria which are parasitic on the mulberry leaves. Here, sericin was extracted from the cocoon by changing of the extraction conditions and the effects of the conditions were investigated. All sericin can be extracted from the cocoon within 1 h with water when the extraction temperature is higher than 120°C. The sericin molecule was hydrolyzed randomly during the extraction but denaturation of sericin hardly occurred. The extraction temperature is the important factor to obtain high molar mass sericin. The ultrafiltration is the effective method to exterminate the spore forming bacteria and the pore size of 0.45 μm is enough for the filtration.
Key words: sericin, spore forming bacteria, molar mass, extraction

1. INTRODUCTION

The silk cocoon is formed with two kinds of protein to be referred to as "fibroin" and "sericin". Fibroin is the base of the silk thread and amounts to 70~75% of the cocoon. Sericin is water-soluble protein. It is obtained by extraction of the cocoon and the floss, moreover from silk processing waste water. Sericin is composed of hydrophilic amino acids and acidic amino acids. The key amino acids in sericin are serine, threonine, aspartic acid, and glutamic acid [1]. Sericin has the antioxidative effect and the tyrosinase inhibitory effect as well as the moisture retention effect [2,3]. In addition, the sericin is used as a surface preparation agent of the synthetic fibers [4]. It was reported that the sericin fixed underwear shows the amelioration effect of the atopic dermatitis and the contact dermatitis [5].

Recently, sericin is used as additives of cosmetics such as skin care and hair care agents. However, it is rarely known that sericin contains the spore forming bacteria which are parasitic on the mulberry leaves. The spore forming bacteria decomposes the sericin aqueous solution. The spore forming bacteria generate spores at the sterilization treatments such as the autoclaved sterilization and the ultraviolet irradiation. Accordingly, extermination of spore forming bacteria is extremely difficult and the effective method is still not known.

In this study, sericin was extracted from the cocoon by changing of the extraction temperature and time and pH of the solvents. The effect of the extraction conditions on the yield, molar mass, and chemical structure of the extracted sericin were investigated. The elimination method of the spore forming bacteria from the sericin extract was also examined.

2. EXPERIMENTAL

2.1 Extraction of sericin

Silk cocoon used was Gunma 200. After having removed inside skin of the cocoon, sericin was extracted with distilled water and water adjusted to pH 9 by sodium carbonate. The extraction was carried out from 100°C to 140°C for 1 and 2 h using an infrared heating-type rotary pot dyeing test machine (Texsam Co. Ltd.). The sericin extract was filtered by a filter paper and the filtrate was lyophilized. The extracted sericin was obtained by the freeze-drying method.

The extraction yield of sericin was determined as follows;

$$\text{Extraction yield (\%)} = (W_0 - W_1)/W_0 \times 100$$

where W_0 and W_1 are the weights of cocoon before and after extraction,respectively.

2.2 SEM measurement

After the extraction, the silk threads were evaporated with gold and palladium. Scanning electron microscopic observations were carried out using a Hitachi S-3000N scanning electron microscope (SEM) at an accelerating voltage at 10 kV.

2.3 Molar mass measurement

The extracted sericin samples were dissolved into purified water and filtered with 0.22μm omnipore hydrophilic PTFE membrane filters (Millipore). Molar mass of the samples was determined by a Prominence gel permeation chromatograph (GPC) system equipped with a UV detector (Shimadzu) at 30°C. The GPC column used was a Protein KW-802.5 (Shodex) and 0.1M phosphate buffer (pH 6.8) was used as eluent at 0.6 ml/min. The chromatogram was recorded at wavelength of 220 nm. The calibration curve for the molar mass was prepared by using commercial

water-soluble proteins (BIO-RAD).

2.4 FT-IR measurement

Fourier transform infrared (FT-IR) measurements of the sericin were carried out using a Magna 560 FT-IR spectrometer equipped with a Continuμm infrared microscope (Nicolet). The FT-IR spectra of the samples were analyzed by the attenuated total reflection (ATR) method.

2.5 Spore free sericin

The sericin extract was filtered by a filter paper then the filtration was repeated twice using ultrafiltration membranes with the pore size of 0.45 μm and 0.22 μm. The filtrate was freeze-dried and the spore free sericin was thus obtained.

The spore free sericin aqueous solution was spread on the agar medium containing beef extract and bacto peptone and it was incubated at 37°C for overnight. The elimination of the spore forming bacteria was judged by counting viable count on the culture medium.

3. RESULTS AND DISCUSSION

Sericin was extracted from cocoons with distilled water. Figure 1 shows relationships between the yield of sericin and the extraction temperature and time. When the extraction time was 1 h, the yield increased with increase of the extraction temperature to 120°C, then reached a constant value of 30%. The yields determined at 100°C and 110°C increased with increasing the extraction time. However, the sericin extracted at more than 120°C showed the constant yield regardless of the extraction time. Sericin content of the cocoon was reported as about 30% [6]. When the extraction is carried out at more than 120°C, all sericin can be extracted by 1 h.

Sericin is usually extracted with a weak alkali aqueous solution. To investigate the effect of pH on the extraction, sericin was extracted with water adjusted to pH 9 by sodium carbonate. Figure 2 shows the relationships between the yield and the extraction temperature and time. As seen in the figure, the increment behavior of the yield shows similar trends observed for the extraction with distilled water. Accordingly, it was found that the sericin extraction is scarcely influenced by pH of the solvent.

To examine the state of sericin on the silk thread, SEM observation was performed for water extracted threads. Figure 3 shows the scanning electron micrographs of the extracted threads at various temperatures together with the original thread. The extraction time of the samples was 1 h. As shown in Figure 3(a), the silk thread is formed by two fibroin filaments glued together by sericin. For the silk thread extracted at 100°C, most of sericin which covered up the thread was removed but two fibroin filaments were still glued together. For the thread extracted at 110°C, the fibroin filament was separated but quite a small amount of sericin was remained on the filament (Figure 3(c)). As seen in Figures 3(d) to 3(f), it was found that sericin was

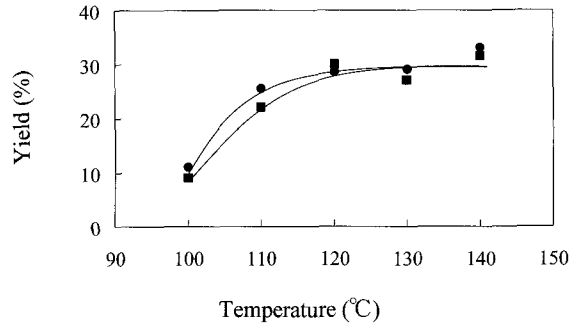


Figure 1. Relationships between yield of sericin and extraction temperature with distilled water. Extraction time: (■) 1h, (●) 2h.

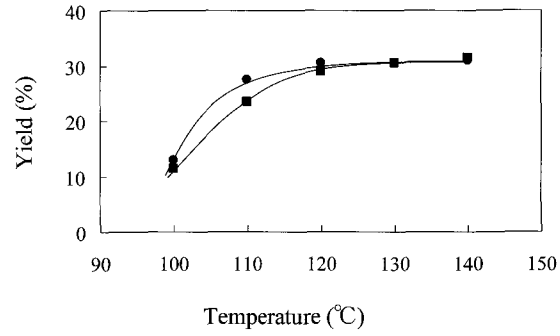


Figure 2. Relationships between yield of sericin and extraction temperature with water adjusted to pH 9 by sodium carbonate. Extraction time: (■) 1h, (●) 2h.

completely extracted from the fibroin filaments of the silk threads extracted at more than 120°C. These results accord with the relationship between the yield and the extraction temperature (see Figure 1).

The molar mass of the sericin extracted with distilled water was measured by GPC. Figure 4 shows typical GPC chromatograms of the extracted sericins for 2 h at various temperatures. Two broad peaks were observed for the sericin extracted at 100°C. It is deduced that the dominant peak appeared at high molar mass region is due to the original sericin and the weak peak corresponds to degraded sericin by the extraction. The main peak shifted to low molar mass side and the peak width increased with increasing the extraction temperature. This means that the sericin molecule is hydrolyzed randomly during the extraction.

Figure 5 shows relationships between the weight average molar mass (Mw) of the sericin and the extraction temperature. The extraction was performed for 1 h and 2 h. In the case of 1 h extraction, the Mw of the sericin obtained at 100°C was 273 kDa. The Mw decreased remarkably with the increase of the temperature and the Mw decreased to 37 kDa at 130°C. In addition, the Mw decreased with increasing the

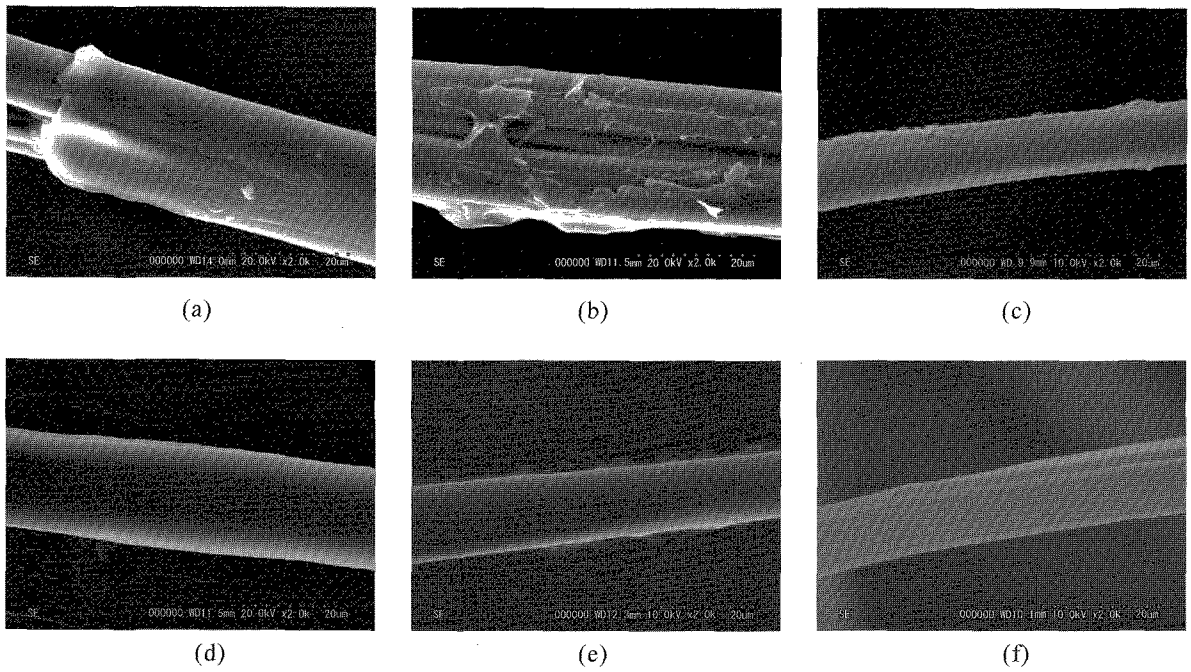


Figure 3. Electron micrographs of extracted silk threads at various temperatures for 1 h: (a); original, (b); 100°C, (c); 110°C, (d); 120°C, (e); 130°C, (f); 140°C.

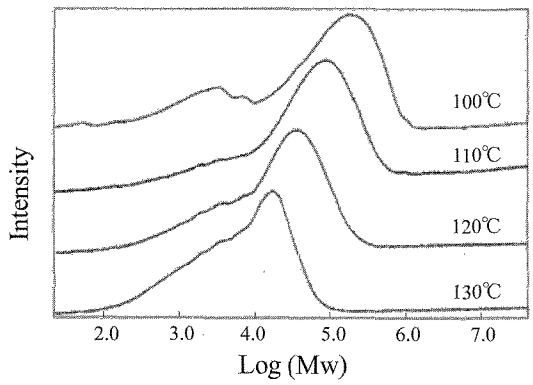


Figure 4. Gel permeation chromatograms of extracted sericin at various temperatures for 2 h.

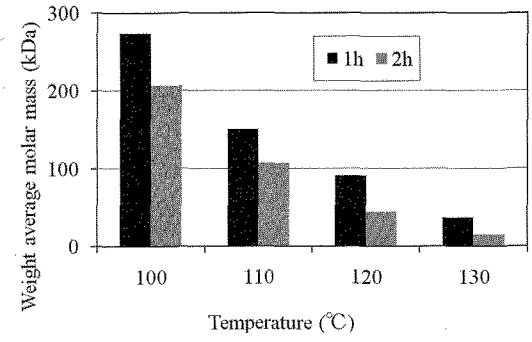


Figure 5. Relationships between weight average molar mass and extraction temperature. Extraction was performed for 1 h and 2 h.

extraction time. The decrement of Mw at high temperature is remarkably bigger than that at low temperature; the values of decrement are 27% at 100°C and 63% at 130°C. It is found that the extraction temperature is the important factor to obtain high molar mass sericin.

To investigate chemical structure change by the extraction conditions, FT-IR spectra of the sericin extracted at various temperatures were measured using the FT-IR microscope. Figures 6 and 7 show FT-IR spectra of the sericin extracted for 1 h and 2 h, respectively. All the extracted sericin showed characteristic features of sericin [7,8], i.e., amide I (~1650 cm^{-1}), amide II (~1530 cm^{-1}), amide III (~1245 cm^{-1}), $-CH_2-$ scissoring vibration (~1400 cm^{-1}) and $-CH_2-$ rocking vibration (~1070 cm^{-1}). As shown in Figure 6, in the case of 1 h extraction, the profile of the IR spectrum hardly changed with increasing the extraction temperature. Similar tendency was observed for the extracted sericin for 2 h (Figure 7). The difference due to the extraction time is not recognized on the IR spectra, too. Accordingly, it is thought the denaturation of sericin hardly occurs by changing of the extraction conditions, except for the hydrolysis of the main chain.

The sericin in aqueous solution is decomposed easily by the spore forming bacteria. We used the extracted sericin at 130°C for 1.5 h and tried to remove the spore forming bacteria from the sericin aqueous solution by using the ultrafiltration method. The pore size of the membranes was 0.45 μm and 0.22 μm. Figure 8 shows the culture mediums, which the sericin aqueous

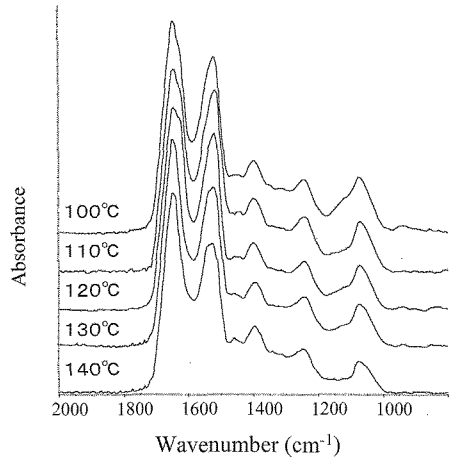


Figure 6. FT-IR spectra of extracted sericin at various temperatures. Extraction time is 1 h.

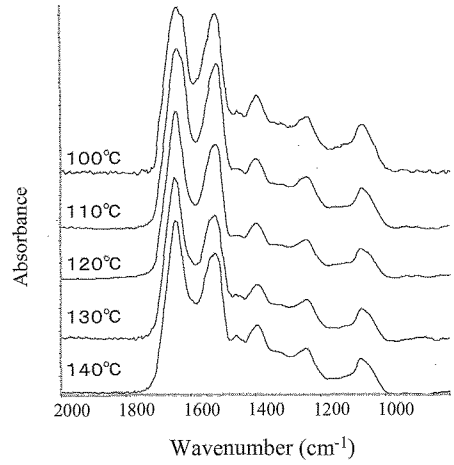
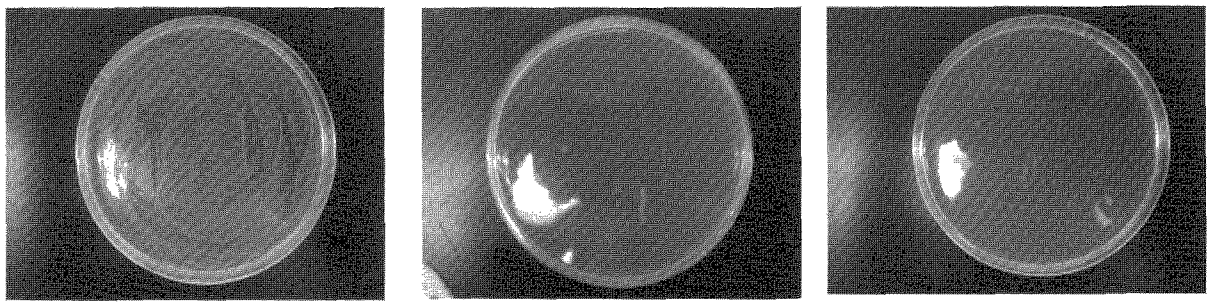


Figure 7. FT-IR spectra of extracted sericin at various temperatures. Extraction time is 2 h.



(a) (b) (c)

Figure 8. Culture state of spore forming bacteria. Cultivation was performed at 37 °C for overnight: (a); sericin without filtration, (b); sericin filtered by 0.45μm filter, (c); sericin filtered by 0.22μm filter.

solutions with and without filtration were spread on and were incubated at 37°C for overnight. As shown in Figure 8(a), the colonies of the spore forming bacteria were observed on
the medium and the viable count was 3.15 × 10^8/ml. However, any colonies were not observed in both sericin solutions filtered through the membranes with 0.45 μm and 0.22 μm of pore size. When the filtrates were kept for a long time, they were not decomposed. Therefore, it was found that the ultrafiltration is the effective method to remove the spore forming bacteria and the pore size of 0.45 μm is enough for the filtration.

4. CONCLUSIONS

The characterization of the sericin extracted at various conditions was investigated and the following results are clarified.

1) When the extraction temperature is more than 120°C, all sericin can be extracted within 1 h with water.

2) The extraction of sericin is scarcely influenced by pH of the solvent.

3) The sericin molecule is hydrolyzed randomly but denaturation of sericin hardly occurs. The extraction temperature is the important factor to obtain high molar mass sericin.

4) The ultrafiltration is the effective method to exterminate the spore forming bacteria and the pore size of 0.45 μm is enough for the filtration.

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