Effects of Ultrasonic and Salt-shrinking Treatments on Structure and Physical Properties of Bombyx mori Silk Fibroin Fiber

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The silk fibroin (SF) fiber from the Bombyx mori silkworm was treated with ultrasonic and salt-shrinking treatments, and its structure and physical properties were investigated to elucidate the effects of the two treatments.

In conclusion, the weight of the SF decreased after the ultrasonic treatment. The SEM images of the SF that underwent partial ultrasonic treatment showed fractures on the vertical surface and micropores on the cross section surface of the silk fibers. The tensile strength and elongation percentage of ultrasonically treated SF decreased considerably. The silk fiber crinkled and shrunk when the ultrasonically treated SF was treated with calcium salt, and many micropores appeared inside the SF. There were also changes in the aggregation structure; some of the molecule chains changed from a $\beta$-sheet structure to an unregulated structure after the salt-shrinking treatment.

Keywords: Silk fibroin; salt-shrunk silk fibroin; structure; properties.

INTRODUCTION

The silk fiber is a natural protein macromolecule material composed of numerous juxtaposed fiber bundles. Silk fibroin (SF) produced by the Bombyx mori silkworm has been used commercially in biomedical sutures for decades (Altman, et al., 2003). Because of their impressive biological compatibility and mechanical properties, SFs have also been used in many other biomedical applications including osteoblast, hepatocyte, and fibroblast cell support matrices and for ligament tissue engineering (Lv, et al., 2005; Unger, et al., 2004 and Altman, et al., 2002). On the other hand, there is a growing interest in several technological tools for the functionalization of natural polymers with an aim to create a new range of high-performance, environment-friendly processes and materials for traditional and innovative applications. Attention was first focused on proteases, whose ability to impart some positive properties on silk (shrink resistance, softness, and so on) have been demonstrated (Freddi, et al., 2006).

Ultrasonic homogenizers, also known as disintegrators, are based on the piezoelectric effect while generating the high energy or ultrasonic wave, interacting with the sample. Energy, resolved after the explosion/implosion of gas microbubbles, effectively destroys solid particles such as cells.
During operation, the immersed horn or probe tip vibrates longitudinally, creating a powerful shearing action caused by the expansion and collapse of millions of microscopic vapor bubbles, known as the cavitation phenomenon. This activity produces miniature shock waves throughout the sample, which causes the molecules in the liquid to become intensely agitated to create the desired effect (Bodzon-Kulakowska, et al., 2007). Ultrasonic devices are widely used for homogenizing, emulsifying (Martinac, et al., 2005), dispersing (Pacheco, et al., 2003), and extracting or suspending mixtures (Townsend, et al., 2004). For sample preparation, ultrasonic disintegrators are successfully used to homogenize the cells, after cell culturing or isolation from the organism. However, investigations have been very limited and little attention has been paid to the characterization of the structure and properties of ultrasonically treated SF fibers.

The mechanism whereby a silk fiber crinkles and shrinks when treated with calcium salt correlates with the amino acid composition and the advanced molecular structure of the silk fiber. In the treatment process, the calcium ions coordinate with the amino acid side chains of large silk molecule chains of non-crystal sections, thus forming complex structures. During the process, damage to some hydrogen bonds and Van der Waals forces among molecule chains results in the swelling of the fibers, which leads to the shrinkage of the silk fibers (Kato, 1990 and Hirabayashi, et al., 1974).

From the cavitation effect, we thought in the ultrasonic treatment process, this appears to be the result of the preliminary damage, expansion and splitting of SF fibers. Further, this effect will be beneficial to the calcium ion to enter the internal silk fiber structure effectively in the salt-shrinking treatment.

We aim to find a new approach that can improve the natural shortcomings of silk fibers, such as poor shrink resistance. In this study, we investigate the effects of ultrasonic and salt-shrinking treatments on the structure and properties of SF fibers using scanning electron microscopy (SEM), wide-angle X-ray diffraction (WAXD), FT-IR spectra, and tensile measurements.

**MATERIALS AND METHODS**

**Materials**

A *Bombyx mori* raw silk fiber (47 dtex) was first degummed to remove sericin. The degumming process was performed by using the standard Marseille soap/soda ash method (Kato, 1968). In the degumming method, the sericin was removed (the degumming ratio of the SF fiber was 27.25%). The degummed SF fiber was soft and smooth with a fine sheen. Ultrasonic homogenizers (Misonix Astrason Model XL2020, Wakenyaku Co. Ltd., Japan; maximum output: 550 W, frequency: 19.8 kHz) were used for treating the SF (Fig. 1.).

**Method of Ultrasonic Treatment**

![Fig. 1. Schematic diagram of the reactor with the ultrasonic generator (1), SF samples (2), double jacket (3), and samples stator (4).](image-url)
The SF fiber was treated with ultrasonic equipment at different treatment times (30 s, 60 s, 5 min, 10 min, 15 min, 20 min) and normal temperature, washed with clean water, dried at 80°C for 2~3 h, and weighed after stabilizing in air for 24 h.

**Method of Salt-shrinking Treatment**

The ultrasonically treated SF was immersed in a calcium nitrate solutions with concentrations of 1.41 and 1.44 for 10 min at a rate of 1:100 at 80°C.

**Measurements**

The fiber morphology was examined with a Hitachi S-2380N scanning electron microscope at an acceleration voltage of 15 kV.

A WAXD profile was obtained by a Rigaku Rotaflex RU-200B diffractometer using Ni-filtered CuKα radiation generated at 40 kV and 150 mA.

FT-IR spectra were measured in the ATR mode with a Nexus spectrometer Thermo Nicolet, equipped with a ZnSe ATR cell mod. Smart Performer.

The tensile properties were measured with a Tensilon Model RTC 1250 A (Orientec Corporation, Japan), using a standard technique at 22°C and 65% RH and a gauge length of 40 mm and strain rate of 40 mm/min.

**RESULTS AND DISCUSSION**

**Weight change rate analysis**

**Fig. 2.** Weight loss of ultrasonically treated SF fibers at different treatment times.

**Fig. 3.** Weight loss of SF fibers subjected to ultrasonic and salt-shrinking treatments.

Fig. 2 shows the weight loss values of the SF fibers obtained by immersion at different ultrasonic treatment times (from 30 s to 20 min). With the increase in the ultrasonic treatment time, the weight decrease shows a tendency toward a linear loss. The weight lost is approximately 1.4 wt% after an immersion time of 20 min with ultrasonic treatment.

Fig. 3 shows the weight loss of the ultrasonically treated SF fibers that were immersed in the 1.41 calcium nitrate concentrated solution for 10 min. It should be noted that in the process of shrinking the silk with calcium salt, the destructive effects of calcium on the noncrystal regions of the silk fiber
were significant (Hirabayashi, et al., 1974); therefore, the average weight loss value was approximately 3–5% after calcium salt treatment. It is also clear that with the increase of the ultrasonic treatment time from 30 s to 20 min, the weight decrease shows a tendency toward a linear loss after calcium salt treatment.

**Fiber Morphology**

From Fig. 4(a, b), the surface and cross section of *Bombyx mori* SF appeared considerably plain and smooth, showing only very fine longitudinal striation attributable to the fibrillar structure of the fiber as degummed SF fiber (Khan, et al., 2007).

![Fig. 4](image)

**Fig. 4.** Surface and cross-sectional morphology of untreated SF fiber: (a) surface of untreated specimen and (b) cross section of untreated specimen.

![Fig. 5](image)

**Fig. 5.** Surface and cross-sectional morphology of ultrasonically treated SF fiber: (a) surface of treated specimen and (b) cross section of treated specimen.

Fig. 5(a, b) shows the surface and cross section of ultrasonically treated SF fiber. Compared to Fig. 4(a, b), this figure shows some shallow vertical lines on the surface of the ultrasonically treated SF fiber in the vertical morphology. Fractures and cracking appear in the cross-sectional morphology after ultrasonic treatment because of cavitation.

![Fig. 6](image)

**Fig. 6.** Surface and cross-sectional morphology of (1.41) SF fiber subjected to the ultrasonic and salt-shrinking treatments: (a) surface of treated specimen and (b) cross section of treated specimen.

![Fig. 7](image)

**Fig. 7.** Surface and cross-sectional morphology of (1.44) SF fiber subjected to the ultrasonic and salt-shrinking treatments: (a) surface of treated specimen; (b) cross section of treated specimen.

Effects are mainly due to the destruction and
damage of the inter-structure of the ultrasonically treated SF fibers. When the ultrasonically treated SF is dipped in the calcium salt solution, the calcium salt ions easily destroy the amorphous region of the SF fibers and create splits on the surface of the fibers; hence, the structure becomes loose and expands.

Fig. 7(a, b) shows the surface and cross section of the SF fiber after ultrasonic and salt-shrinking treatments (concentration of calcium nitrate solution: 1.44). After ultrasonic and high concentrations of salt-shrinking treatment, part of the silk fibers began to dissolve and split; one monofilament of the SF fiber (10–15 m) split into almost 10 small monofilaments (2–3 µm) after the treatments. In the figure, it is clear that the cross section of the SF fiber swelled and expanded, and the amorphous area of the SF fiber was seriously damaged. A large crack (about 2–3 µm) and many micropores accrue on the surface of the samples.

Mechanical Properties

Tensile properties are the most important factors for evaluating the performance of fibers for proper applications. Table 1 shows the tensile strength and elongation percentage of the untreated and ultrasonically treated SF fibers as a function of different treatment times. The strength and elongation rate of the ultrasonically treated SF fiber decreased directly with the increase in the ultrasonic treatment time from 0.5 min to 20 min, which proves the cavitation function of ultrasonic treatment. In the ultrasonic treatment process, the acoustic vibration and pressure reaches a certain value and then creative liquid microbubbles in the ultrasonic process, the bubble will quickly become expansion and closure. The closure of the bubble generated a large shock wave around 1,000 atm. The large instantaneous pressure and miniature shock waves throughout the sample caused the rapid destruction of the surface of the SF fibers (Gonze, et al., 2003).

Table 2 shows the tensile strength and elongation percentage of SF fibers subjected to ultrasonic and salt-shrinking treatments as a function of different treatment times. In the tables, after comparing the untreated sample and the sample subjected only to the salt-shrinking treatment with the SF fiber subjected to the ultrasonic and salt-shrinking treatments, it is clearly observed that the decrease in the SF fiber subjected to the ultrasonic and salt-shrinking treatments was significant. After 20 min of ultrasonic and salt-shrinking treatments, the tensile strength and elongation obtained were

<table>
<thead>
<tr>
<th>Table. 1. Strength and elongation at the breaking point of ultrasonically treated SF fibers at different treatment times.</th>
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<td><strong>Solution</strong></td>
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<tr>
<td>---</td>
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<tr>
<td>Strength (cN/dtex)</td>
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<tr>
<td>Elongation (%)</td>
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</table>
Table. 2. Strength and elongation at the breaking point of SF fibers subjected to the ultrasonic and salt-shrinking treatments at different treatment times.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Initial</th>
<th>0.5 min</th>
<th>1 min</th>
<th>5 min</th>
<th>10 min</th>
<th>15 min</th>
<th>20 min</th>
<th>Salt shrinking treatment only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strength (cN/dtex)</td>
<td>2.99</td>
<td>1.76</td>
<td>1.69</td>
<td>1.65</td>
<td>1.62</td>
<td>1.58</td>
<td>1.48</td>
<td>1.79</td>
</tr>
<tr>
<td>Elongation (%)</td>
<td>20.00</td>
<td>19.90</td>
<td>19.90</td>
<td>17.10</td>
<td>16.70</td>
<td>14.10</td>
<td>14.00</td>
<td>19.50</td>
</tr>
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</table>

approximately 1.48 cN/dtex and 14%, respectively; the value of the tensile strength was less than that of the samples subjected only to the salt-shrinking treatment (1.79 cN/dtex) and only equivalent to 50% of the tensile strength of the untreated SF fibers. The elongation value of the SF fibers subjected to the ultrasonic and salt-shrinking treatments also decreased by approximately 30% compared with that of the untreated SF fibers.

The decline in the tensile strength and elongation occurs when the dissolution of silk creates holes within the fibers because of the cavitation function of the ultrasonic treatment. Then, the calcium salt ions can easily enter the silk fibers, and the destructive effects of calcium on the non-crystal regions of the silk fiber are significant.

**Structural characteristics**

X-ray diffraction was used to characterize the structure of the SF fiber. Generally, the X-ray diffraction patterns were determined as follows: 24.0° (d = 3.70 Å) for an α-helix structure, and 16.7° (d = 5.30 Å) and 20.3° (d = 4.37 Å) for a β-sheet structure (Wang, et al., 2005). There is a major diffraction intensity peak around 20.3° in the XRD curve of the Bombyx mori SF fiber (Fig 8; curve (a)), which indicates a β-sheet structure and high crystallinity of pure SF. It can be seen from curve(b) that the sample prepared by the ultrasonically treated SF fiber shows the peak of a β-sheet structure changed to 20.38°, and the peaks of the SF fibers subjected to the ultrasonic and salt-shrinking treatments (Fig 8; curve (c)) appear at around 20.52°. After treatment, the positions of the major diffraction peak were almost similar to those of the untreated SF, which means that there is no radical change in the SF fiber’s microstructure after the treatment.

According to curves (a), (b), and (c), the peak intensity of the pure SF fiber is the highest, while that of the SF fiber subjected to the ultrasonic and salt-shrinking treatments is the lowest, indicating that the β-sheet crystalline structure in the treated
SF fibers was smaller than that in the pure SF fiber. In conclusion, these results indicate the lack of crystalline structure of the SF fibers after ultrasonic and salt-shrinking treatments. Compared with the results of the test of tensile properties, the decrease in the crystallite orientation demonstrates the damaging function of both ultrasonic and salt-shrinking treatments.

The structural and conformational properties of SF substrates were further investigated by FT-IR. The samples showed the typical IR pattern of SF materials with a $\beta$-sheet molecular conformation (Fig. 9. a–c), as evidenced by the position and intensity of the amide I, II, and III bands at 1616, 1510, and 1228 cm$^{-1}$, respectively (Sampaio, et al., 2005). However, sample (c), the SF fiber subjected to the ultrasonic and salt-shrinking treatments, showed amide II at 1514 cm$^{-1}$ (pure SF at 1508 cm$^{-1}$), and amide III at 1230 cm$^{-1}$ (pure SF at 1228 cm$^{-1}$). The change in wavenumbers indicated that some of the molecule chains changed from the $\beta$-sheet structure to a disordered structure after ultrasonic and salt-shrinking treatments.

**CONCLUSIONS**

The structure and properties of *Bombyx mori* SF fibers subjected to the ultrasonic and salt-shrinking treatments were investigated with SEM, WAXD, FT-IR, and mechanical measurements to elucidate the effects of the treatments on the SF fibers.

The weight of pure SF decreased by approximately 1.4wt% after 20 min of ultrasonic treatment. The tensile strength and elongation percentage of ultrasonically treated SF decreased considerably. The fiber morphology of the SF was greatly influenced by ultrasonic treatment, which showed fractures on the vertical surface of SF and micropores on the cross-sectional surface of the silk fibers. The WAXD patterns of the SF fibers suggested that ultrasonic treatment did not directly affect the crystalline regions but caused a minor decrease in the molecular orientation in the amorphous regions.

The SF fiber crinkled and shrunk when the ultrasonically treated SF was treated with calcium salt, and many micropores appear inside the SF. There were also changes in the aggregation structure; some of the molecule chains changed from a $\beta$-sheet structure to an unregulated structure after salt-shrinking treatment.

The paper adopted the first ultrasonic then salt-shrunk experimental process, and the inverse experimental treatment, first salt-shrunk then ultrasonic treatment, was also done in the total experimental process. During the process of shrinking the silk with calcium salt, the destructive effects of calcium on the SF fiber were significant, therefore, the inter-structure of the SF fiber became loose and damaged. Many SF samples were fractured when the SF fibers were subjected to ultrasonic and salt-shrinking treatments, so there
was no significance in the practical application of the experiment.

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REFERENCES