Structure and properties of flat cocoon silk after silk reeling

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Abstract
Silk is obtained mostly from oval cocoons. In this paper, the flat cocoons were obtained by changing the silking environment of Bombyx mori silkworms. Then the appropriate method was used to reel the flat cocoons. The structure, thermal and mechanical properties of flat cocoon silk (FCS) after silk reeling and degumming were studied. The experimental results have shown that flat cocoon silk has the same main composition and similar thermal performance as that of common cocoon silk (CCS), but the sericin distribution on the surface of FCS is more uniform, the crystallinity degree of the FCS (53.77%) is slightly higher than that of the CCS (50.02%), and the cross-sectional areas of the FCS before and after degumming are smaller than those of the CCS. Before degumming, the stress of FCS is about 1% higher than that of CCS, the initial modulus is about 4.7% higher, and the strain is about 10.7% lower. After degumming, the stress of FCS is about 2.7% higher than that of CCS, the initial modulus is about 7.8% higher, and the strain is about 31.3% lower. The results have shown that FCS after silk reeling has application performance close to or even better than that of CCS.

1. Introduction

Beside the traditional textile industry, silk has become a new material that can be used in many high-tech industries [1–5]. Results of proteomic studies show that silk is a complicated protein complex, and contains many biologically active protein components besides fibroin and sericin. Natural sericin protein has good biocompatibility, and has been used in beauty, skin care, anti-inflammatory and health care products. Sericin films, tissue engineering scaffold materials, etc made from sericin protein have also been used in the field of biomedicine [6]. Modification of sericin can improve the properties of sericin films and further increase their applicability [7–11].

Fibroin, of which is in high concentration in silk, also has a wide range of applications. Fibroin protein has long been used in the field of cosmetics. Fibroin protein is also a good optical material, and its immobilized enzyme can be used to make enzyme sensors. Fibroin can be widely used in the field of medicine and the preparation of biological materials [12, 13]. Fibroin protein can also inhibit the growth of microorganisms [14].

Mature Bombyx mori silkworms spin silk in a three-dimensional space, forming oval cocoons. Silk is obtained mostly from oval cocoons. However, due to the differences in varieties of Bombyx mori silkworms, the amount of silk production will be different. The way of body movement and the change of place during silk spinning will have an impact on the shape and the properties of cocoons [15–18]. If the silking place is limited to two-dimensional space, mature Bombyx mori silkworms can hardly find the best place of cocooning and will spin out a piece of silk on a flat surface, which is the so-called flat cocoon. After silk spinning, Bombyx mori silkworms will transform into pupa above the flat cocoons. Van Der Kloot, W G et al systematically studied the rule of silk spinning of the Cecropia silkworms and found that the silkworms spin silk into outer, middle and
inner layers, and the amount of silk spinning and non-repeatability of each layer of flat cocoons were the same as those of oval cocoons [19]. Bin Zhou et al verified that flat cocoons had the multi-layer structure and characteristics similar to those of common oval cocoons. The fiber characteristics and performance change rules of the flat silk from the outer layer to the inner layer were completely consistent with those of common oval cocoons. They believed that flat cocoons were also constructed by the silkworm according to the same ‘programmed’ process as common oval cocoons [20]. Zongpu Xu et al combined flat cocoons with resin to form films as flexible and transparent reinforcing materials for substrates on electronic devices, with a significant increase in tensile strength and modulus compared with pure resin, while maintaining flexibility and transparency [21].

In a traditional silk production process, such methods as cocoon drying, cocoon boiling, silk reeling and skein finishing are used to obtain raw silk from dry cocoons [22]. However, this process is complicated. Therefore, a fresh cocoon reeling process is developed, that is, fresh cocoons are directly reeled after vacuum hot water osmotic treatment without pupae killing and drying, and then processed into raw silk [23, 24]. If the fresh cocoons can’t be reeled immediately, they need to be stored or frozen in the cold storage [25–28]. If flat cocoons could be used for silk reeling, processes such as cocoon drying, cocoon boiling and cold storage will be eliminated.

There have been no previous studies on silk reeling of flat cocoons. In this paper, flat cocoons with uniformly distributed silk were obtained through experiments, and were reeled using improved traditional silk reeling method. The microstructure, and mechanical and thermal properties of the FCS were tested and compared with those of CCS, which will provide theoretical support for further development and utilization of FCS. The method of flat cocoon reeling in this study can also provide a new way to obtain and use other kinds of cocoons that cannot be reeled, such as Eri-silkworm cocoons.

2. Experiments

2.1. Materials

100 Bombyx mori silkworms (Sericultural Research Institute, Chinese Academy of Agricultural Sciences), 40 smooth-surfaced round disks with a diameter of 8 cm, and a wood cocooning frame.

2.2. Preparation of flat cocoons and common cocoons

The mature Bombyx mori silkworms were divided equally into two groups, one group was placed on the wood cocooning frame for normal silk spinning and cocooning, and the other group was placed on round disks, with one mature Bombyx mori silkworm on each disk to make flat cocoons. A plastic case was placed below each disk, so that the disk was about 15 cm from the table, and a mature Bombyx mori silkworm was placed on each disk after urination. After 3 to 4 days, the mature Bombyx mori silkworms finished spinning. Then the pupae on surfaces of flat cocoons were taken away, and the flat cocoons were reserved. The processes of silk spinning for making flat cocoons and common cocoons are shown in figure 1.

2.3. Preparation of silk fibers

2.3.1. Silk reeling

Flat cocoons and common cocoons were placed in hot water at about 85 °C simultaneously. 30 min later, the flat cocoons were fully soaked, and the common cocoons could be reeled smoothly. Silk reeling of flat cocoons in water will result in intertwined silk, so that the fully soaked flat cocoons need to be taken out of the water bath environment, placed on the disks upside-down, and reeled, with the help of a magnifying glass, by extracting a
strand of silk from the top layer. In order to ensure that the CCS and the FCS were always in the same environment, when the flat cocoons were taken out of the water bath environment, the common cocoons were also taken out for silk reeling. The two kinds of silk were wrapped on a bottle with the diameter of 5 cm respectively. Repeat this experiment, 40 groups of flat cocoons and common cocoons were reeled into silk fibers for later use. The process of silk reeling is shown in figure 2.

2.3.2. Degumming
10 meters silk from each group of FCS and CCS obtained by reeling were placed in a beaker respectively, and then were treated with 5 g l\(^{-1}\) Na\(_2\)CO\(_3\) solution at 100 °C for 3 times, 30 min each time, during which the solution was unceasingly stirred with a glass rod, and then the silk was rinsed with distilled water and placed on absorbent paper until it was completely dried [29–33].

2.4. Property tests
2.4.1. Cocoon and silk specifications
Twenty common cocoons that had pupae removed and twenty flat cocoons were taken and tested for thickness using a fabric thickness instrument (YG(B)141D, Wenzhou Darong Textile Instrument Co., Ltd.) in accordance with GB/T 3820–1997 Determination of Thickness of Textiles and Textile Products, with pressure weights of 25 cN and pressing time of 10 s. An electronic balance (ESJ30–5 A, Shanghai Precision Instrument Co., Ltd.) was used to measure the weight of flat cocoons and common cocoons. A microscope equipped with CU fiber fineness software was used to obtain the cross-sectional area data of 200 silk fibers from different samples.

2.4.2. Scanning electron microscope
A scanning electron microscope (SEM, Japan/SU8010) was used to observe the microscopic morphology of gold-sprayed common cocoon, flat cocoon and dried degummed cocoon silk at a voltage of 5 kV.

2.4.3. Thermogravimetric analysis
A thermogravimetric analyzer (NETZSCH STA 449F3) was used to test the thermogravimetric properties of the two kinds of cocoon silk at a heating rate of 10 °C min\(^{-1}\) and a nitrogen flow of 20 ml min\(^{-1}\).

2.4.4. Infrared spectra
A US Nicolet iS5 Fourier Transform Infrared Spectrometer (FT-IR) was used to test the two kinds of cocoon silk samples under the following test conditions: room temperature, ATR test method, wave number range of 500–4000 cm\(^{-1}\), scanning per acquisition of 32 and spectral resolution of 4 cm\(^{-1}\). Absorption spectra were obtained by transformation using OMNIC 6.0 (Nicolet Instrument Co., USA) software.

2.4.5. X-ray diffraction
The two kinds of degummed cocoon silk samples were cut into powder and tested using an x-ray diffractometer (Smartlab 9kw) at a wavelength of 0.15406 nm, a voltage of 40 KV, an electric current of 40 mA, a speed of 2° min\(^{-1}\), and a 2θ scanning area of 10°–40°.
2.4.6. Mechanical property test
A fiber stretcher (YG004, Changzhou Dahua Electronic Instrument Co., Ltd.) was used to test the mechanical properties of FCS and CCS under the following test conditions: environment temperature of 22 °C, and relative humidity of 50%. Before the test, the FCS and CCS both before and after degumming were balanced in the test environment for 24 h. The tensile speed was 10 mm min⁻¹ and the gauge length was 20 mm. Samples were taken from 40 groups of silk for testing. SPSS software was used to test the abnormal value and analyze the coefficient of variation of the test data.

3. Results and discussion

3.1. Structure comparison of flat cocoons and common cocoons
The appearance comparison of flat cocoon and common cocoon is shown in figure 3, and the specification comparison is shown in table 1. It can be seen from figure 3 that there is an obvious difference in shape between the flat cocoon and the common cocoon. The common cocoon is an ellipsoid, while the flat cocoon is a circular flat. The comparative data in table 1 show that the thickness of the flat cocoon is about one-third of that of the common cocoon, and its weight is slightly lighter. This is because the Bombyx mori silkworms were not placed on the disks until the urination was completed. At this time, the Bombyx mori silkworms had spun out some or even all of the outer layer cocoon silk. The common cocoons are relatively hard and difficult to deform, while the flat cocoons are soft and easy to bend, but they also have enough strength to keep circular.

The microstructure comparison of a flat cocoon and a common cocoon is shown in figures 4(a) and (b). It can be seen from the SEM image that the silk of both the flat cocoon and the common cocoon is stacked in a regular cross-layer shape. The comparison shows that the silk of common cocoon is more closely connected. The cross angle of FCS is smaller than that of CCS, and the straightness is higher than that of CCS, which is due to larger movement area when the Bombyx mori silkworms make flat cocoons. Both the FCS and CCS are formed by two parallel fibroins wrapped by outer sericin. The sericin on the FCS is evenly distributed, while there is multiple obvious sericin protrusions on the CCS.

Figures 4(c) and (d) show the microstructures of FCS and CCS after degumming. It can be seen from the figures that both FCS and CCS after degumming are free of sericin bond and are in a good monofilament state. The comparative data in table 1 show that the cross-sectional areas of FCS before and after degumming are both smaller than those of CCS, which may be due to the large cocooning space and the large swing amplitude of the head when the silkworm is cocooning the flat cocoon, so that the silk is subject to greater stretching, and the

![Figure 3. Appearance Comparison of Flat Cocoon and Common Cocoon.](image_url)

| Specimen          | Thickness (mm) | Weight (g) | Cross-sectional area of silk(μm²) |
|-------------------|----------------|------------|----------------------------------|
|                   | Before degumming | After degumming |
| Flat Cocoons      | 0.16 ± 0.03   | 0.27 ± 0.05 | 136.5 ± 6.2                      |
| Common Cocoons    | 0.5 ± 0.04    | 0.30 ± 0.04 | 162.9 ± 9.3                      |

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silkworm can maintain such a spinning posture for most of the cocooning time, so that the stretching force of the FCS is more uniform, and the silk has a better straight state. However, during the formation of common cocoons, due to the influence of the internal structure of cocoons, the swing amplitude of silk is not uniform, resulting in a large difference in silk elongation.

3.2. Thermal property comparison of FCS and CCS

Figure 5 shows the thermodynamic analysis curves of FCS and CCS. The thermal decomposition can be roughly divided into three stages along the temperature axis: in the first stage (0 °C–100 °C), the termination temperatures of the two kinds of silk fibers are both 100 °C, and the weight loss rate is about 5% for CCS and about 10% for FCS. The weight loss rate in this stage may be caused by the release of the water bound by physical
adsorption in the silk fibers. In the second stage (100 °C–320 °C), the decomposition rates of both silk fibers are the highest in the range of 250 °C to 320 °C, which is of great significance for evaluating the heat resistance and thermal stability of the silk fibers. At the end of this stage, the weight loss rate of FCS is about 18%, while that of CCS is about 20%, indicating that there was no significant difference between the two fibers. Compared with FCS fibers, CCS fibers have slightly poor arrangement and stacking structure between macromolecular bonds, therefore, the thermal stability of FCS is slightly better than that of CCS. The extrapolated onset temperature of weight loss rate is related to the β-sheets structure of protein. According to figure 5, the extrapolated onset temperature of weight loss rate is about 293 °C for CCS and 301 °C for FCS. Therefore, it can be inferred that the β-sheets structure of protein of CCS is less than that of FCS. In the third stage (320 °C–620 °C), the decomposition rates of both silk fibers are slowed down.

3.3. Infrared spectrum comparison of FCS and CCS
Figure 6 shows that the infrared spectra of FCS and CCS are similar in peak shapes and peak positions on the whole, both have some typical characteristic peaks, and no characteristic peak displacement has occurred, indicating that basic structures of the two kinds of silk have not changed. Both of the infrared spectra have characteristic absorption peaks at 1620 cm⁻¹, 1508 cm⁻¹ and 1226 cm⁻¹. The absorption peak near 1620 cm⁻¹ is an amide I α-helix structure caused by C=O vibration, the absorption peak near 1508 cm⁻¹ is a protein amide II β-sheets structure caused by C–N stretching vibration and N–N vibration, and the protein amide III band absorption peak near 1226 cm⁻¹ is caused by N–H and O–C–O vibration in the protein peptide bond.

3.4. X-ray diffraction comparison of FCS and CCS
Figure 7(a) shows the x-ray diffraction curves of FCS and CCS of Bombyx mori. It can be seen from figure 7 that the diffraction angles of the principal peaks of the FCS and CCS are very close, which are respectively 20.496° and 20.438°, indicating that the main structures of the two are similar without significant changes, but the diffraction intensity of FCS is higher than that of CCS. Software Jade was used for full spectrum fitting of diffraction peaks within the test range, as shown in figures 6(c) and (d), pseudo-Voigt function was used to describe diffraction peaks, and finally the integral area values of crystalline peaks and non-crystalline peaks were obtained and the crystallinity degrees were calculated. The crystallinity degree of FCS is 53.77% and that of CCS is 50.02%, as shown in figure 6(b). The calculation results show that the crystallinity degree of FCS is slightly higher than that of CCS, but there is little difference. The reason for the difference may be that the silk fibers of the flat cocoon have a higher relative content of β-sheets and a lower relative content of α-helix, thus affecting the regularity of macromolecular arrangement.

3.5. Mechanical properties
The stress-strain curves are shown in figure 8, and the tensile property comparison of FCS and CCS before and after degumming is shown in table 2. Compared with those of the CCS, the average stress of FCS before degumming is about 1% higher, the average initial modulus is about 4.7% higher and the average strain is about 10.7% lower. The stress difference between FCS and CCS is not obvious, and the variations range of stress error
of FCS is smaller than that of CCS. Presumably, the main reason is that when a *Bombyx mori* silkworm spins silk on a flat surface, the space becomes larger and the cocoon thickness decreases, silk of the cocoon layers is subjected to a more uniform stretching force, and silk straightness is higher; or the sericin on the FCS is more evenly distributed, reducing the weak link phenomenon during the stretching. Before degumming, there is no significant difference in the strain of FCS and CCS, and the variations of strain of FCS is still smaller than that of CCS. After degumming, the average stress of FCS is about 2.7% higher than that of CCS, the average initial modulus is about 7.8% higher and the average strain is about 31.3% lower. The stress and initial modulus of degummed FCS are higher than those of CCS, and the strain is lower than that of the CCS. This is because the crystallinity degree of fibroin part of FCS is slightly higher, the molecular arrangement is more regular, the bond between molecules is stronger, and molecular chain segment is not easy to slide when stretched by external force. In addition, the cross-sectional area of single FCS after degumming is smaller than that of the CCS, which also leads to an increase in its stress.

4. Conclusion

After the environment in which *Bombyx mori* silkworms spin silk is changed, the properties of silk will be affected. In this paper, *Bombyx mori* silkworms were placed on disks with smooth surfaces to obtain flat cocoons. Through SEM observation, it was found that the silk of both flat cocoons and common cocoons was interlaced and layered, and the silk fibers were evenly distributed; the cross angle of FCS is smaller than that of CCS, and the straightness is higher than that of CCS; the sericin on the surface of FCS was evenly distributed, while there were some unevenly distributed sericin on the CCS. The flat cocoons and common cocoons were reeled and degummed in the same way, and the properties of the two cocoons before and after degumming were compared. The thermogravimetry experiment showed that the thermal stability of FCS was similar to that of CCS. The infrared spectrum and x-ray diffraction experiments showed that the main components of FCS were the same as those of CCS, but the crystallinity degree of FCS was slightly higher than that of CCS. The results of mechanical experiments showed that FCS showed higher stress and initial modulus and lower strain compared with CCS after degumming. The results of this study have shown that FCS after silk reeling has application performance close to or even better than that of CCS. In the later research, a more suitable method can be attempted to reel the flat cocoons, or silk membrane with a wider range of area and thickness can be obtained by changing the area of silk spinning place, increasing the number of mature *Bombyx mori* silkworms in the silk spinning place.
study may provide a new way for industrial development and utilization of silkworm cocoons that have structural defects and can’t be continuously reeled silk to manufacture silk products, such as *Eri-silkworm* cocoons.

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**Data availability statement**

All data that support the findings of this study are included within the article (and any supplementary files).
Conflicts of interest

The authors declare no conflict of interest.

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