COMPARATIVE ANALYSIS OF STRUCTURE AND PROPERTIES OF STEREOSCOPIC COCOON AND FLAT COCOON

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Abstract:
Cocoon is a kind of natural biopolymer material with reasonable structure and various functions. However, its structure and functions are often destroyed in practical application. In this study, we took common Bombyx Mori as the research object, and provided different cocooning sites for single or multiple silkworms to construct common stereoscopic cocoons ("normal cocoons" [NC]) and flat cocoons ("single-silkworm flat cocoons" [SFC] and "multi-silkworm flat cocoons" [MFC]), respectively, and compared the morphological structure and basic properties of these cocoons. The study found that the flat cocoons have similar multi-layered variable structure and characteristics compared to those of the common cocoons; also, morphological characteristics and physical and chemical properties of silk fiber from outer layer to inner layer, such as sericin content, fiber fineness, and change rule of basic mechanical properties, are completely consistent with those of the common cocoons. It can be considered that the flat cocoons are constructed by silkworms in the same "procedural" process as that of common cocoons. Due to the expansion of cocooning space, the mechanical properties of fibers are significantly improved. By controlling the size of the cocooning space or the quantity of silkworms cocooning simultaneously, and the time of spinning, a cocoon material with controllable thickness, weight per square meter, porosity, and number of cocoon layers can be obtained as a composite material for direct application.

Keywords:
Stereoscopic cocoon, flat cocoon, structure, property, procedural, comparison

1. Introduction
Cocoon is a kind of natural biopolymer material, which is constructed by a silkworm through two continuous filaments (silk fibroin) with sericin on the surfaces in a procedural process, as an ideal place to adapt to the natural environment and resist predatory behavior of other organisms, which might be natural enemies that would prevent the silkworms from carrying out their normal life activities [1–3]. The spinning process of silkworms has undergone long-term natural selection and extensive evolution. Despite their small size and light weight, the cocoons have complex structure and unique functions, especially the cocoons of wild silkworms (such as eri-silkworms) living in a complex external environment, whose structure and morphology are more special [4, 5]. The cocoons are of obvious multi-layered structure, whose layers are connected in an orderly manner with significantly different morphological structure and mechanical properties [6, 7]. The content of sericin and calcium oxalate in the outer layer of cocoon is higher than that in the inner layer [8–10], and sericin possesses excellent UV and microbial resistance [11–13]. Structural difference of cocoon layers and the reasonable distribution of sericin and calcium oxalate result in unidirectional air conduction, so that the cocoons have obvious effect of temperature and humidity damping, ensuring the normal metabolic activities of silkworm larvae inside the cocoons [10, 14]. The study of the relationship among structure, properties, and functions of cocoons is conducive to the design of new functional protective materials. Although with special structure and functions, cocoons are difficult to be used directly as materials. At present, they are mostly used as miscellaneous biological raw materials through silk reeling and weaving or silk degradation [15], which destroys the natural structure of cocoons and fails to make full use of unique biological, physical, and chemical properties of silk.

In this paper, the common Bombyx Mori silkworm is taken as the research object, and common cocoons, single-silkworm flat cocoons, and multi-silkworm flat cocoons were obtained through different processes, and the differences between their structures and properties are studied, so as to provide a new idea for the direct application of cocoon materials.

2. Materials and methods
2.1 Cocoon material formation and specimen preparation
Bombyx Mori silkworms used in the experiment were provided by Sericultural Research Institute, Chinese Academy of Agricultural Sciences (Zhenjiang, China), and fed with folium

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mori under standard conditions until they started spinning. Fifty silkworms were taken and placed on a paper cocooning frame for normal cocoon formation, and the normal cocoons generated were marked as “normal cocoons” (NC). The above-mentioned 50 silkworms were taken and placed on a circular mold with a diameter of 8 cm to generate (single-silkworm) flat cocoons, which were marked as “single-silkworm flat cocoons” (SFC). Further, 300 silkworms were taken and placed on a 50 cm*100 cm flat spinneret plate to generate (multi-silkworm) flat cocoons, which were marked as “multi-silkworm flat cocoons” (MFC). The spinneret plate is equipped with a light control system and can be adjusted within the angle of 0°–30°, so as to prevent silkworms from gathering together to obtain flat cocoons with uniform thickness. A strip specimen with the width of 5 mm was taken along the direction of 20° relative to the axial direction of NC, and a strip specimen with the width of 5 mm was taken from SFC and MFC, respectively. The thickness and weight of each cocoon were measured by an electronic vernier caliper and an electronic balance, respectively. The silk on the three kinds of cocoons is peeled and loosened in the order of outer layer (cocoon coat), middle layer (cocoon layer), and inner layer (cocoon lining).

2.2 Morphological observation

Digital imaging equipment was used to obtain the appearance images of cocoons. SU8010 scanning electron microscope (Hitachi, Tokyo, Japan) was used for the analysis of morphological structure of gold-sprayed cocoons under the voltage of 5 kV.

2.3 Sericin content testing

The outer, middle, and inner fibers of each of the three cocoons were respectively soaked in 5 g/L sodium carbonate solution for sericin removal of 30 min at a bath ratio of 1:20 and at a temperature of 70–80°C, then washed with distilled water and dried at 60°C. The sericin content was calculated as \( \frac{W_0 - W_f}{W_0} \), where \( W_0 \) and \( W_f \) are the mass of specimen before and after the removal of sericin, respectively.

2.4 Porosity of cocoon

The density \( (\rho) \) of each cocoon shell specimen can be calculated based on the weight, area, and thickness of the cocoon strip specimen. The density \( (\rho) \) of the silk fiber is 1,300 kg/m³. The porosity of the cocoon shell specimen was calculated by the formula \( (P) = 1 - \rho / \rho_i \) [16].

2.5 Amino acid analysis

After vacuum sealing in 6 mol/L Hcl, the outer, middle, and inner layer fiber specimens of three kinds of cocoons (dried and accurately weighed) were hydrolyzed for 24 h at (110 ± 2)°C, filtered, adjusted to the preset volume, and tested for amino acid composition and content with a Hitachi 835-50 automatic amino acid analyzer.

2.6 Crystal structure analysis

The X-ray spectra of the cocoon materials were determined by a Bruker D8 advance X-ray diffractometer under the test conditions of: Ni filtering, Cu target Kα ray, tube voltage of 4.0 kV, tube current of 35 mA, scanning speed of 5(*)/min, and 2θ in the range of 10°–80°.

2.7 Mechanical property testing

Tensile testing instrument (YG065, Yantai, China) was used for tensile testing based on GB/T 3923.1-2013 (China), and all tests were carried out at room temperature with gage length of 50 mm and at a speed of 2 mm/min. The corresponding stress-strain curve was obtained by dividing the load and displacement by the cross-sectional areas and the gage length of the specimens, respectively.

XQ-2 fiber tensile tester was used to determine the mechanical properties of outer, middle, and inner cocoon filaments of each of the three cocoons based on GB/T 14337-2008 (China), under the test conditions of: tension speed of 200 mm/min, elongation range of 100%, gage length of 20 mm, and pre-tension of 0.05 cN/dtex. Each specimen was tested for 20 times.

3. Results and discussion

Figure 1 (a, b) clearly shows the appearance and microstructure of each of the cocoons (NC, SFC, and MFC) formed under different cocooning methods. Cocoons formed at different cocooning sites have different morphologies. The paper cocooning frame provides a sufficient stereoscopic cocooning space for silkworms, so the cocoons generated are stereoscopic (common). Neither the circular mold nor the flat spinneret plate has three-dimensional supporting points, so only flat cocoons can be generated. The size and shape of flat cocoons are closely related to the shape and size of the cocooning site, and the size of MFC is also related to the quantity of silkworms on the spinneret plate. Despite the difference in morphology, cocoons have developed similar non-woven composite structure, and all the cocoon layers are of the parallel multi-layered structure (cocoon coat, cocoon layer, and cocoon lining). Compared with NC, the relatively large cocooning space provides SFC and MFC with relatively loose structure, as a result of which the densities of fiber aggregates differ; however, the rules of cross distribution of fibers are basically the same, showing a Y or + type pattern, and the pores formed between silk fibers are uniform.

The weight of cocoon (without pupa) is related to the quantity of larvae. Based on the yield of each silkworm, the average weights of NC, SFC, and MFC specimens are ascertained as 0.37 g/silkworm, 0.38 g/silkworm, and 0.33 g/silkworm, respectively, indicating that the form of cocooning in this experiment does not affect the quality of silk spinning of a single silkworm, but the excessive quantity of silkworms cocooning simultaneously has a certain influence on the quality.
Figure 1. (a) Schematic illustrations for the fabrication process of silkworm cocoons (b) Generated cocoon (c) cross section of cocoon layer; SEM images of (d) cross-section; (e) outer layer structure; and (f) inner layer structure.
of silk spinning of silkworms; we thus infer that the influence of quantity of silkworms per unit area of spinneret plate on the structure and properties of flat cocoons needs further study. The thickness, weight per square meter, and porosity of flat cocoon are related to the size of cocooning tool and the quantity of silkworms cocooning simultaneously. It can be seen from Figure 2(d) that the thickness of NC, SFC, and MFC is 0.3 mm, 0.2 mm, and 2.6 mm, the weight per square meter is 135.5 g/m², 75.6 g/m², and 202.7 g/m², and the porosity is 65.5%, 71.3%, and 72.2%, respectively. The high porosity also indicates the relatively fluffy structure of cocoon, which has better thermal damping and impact resistance. The quantity of silkworms on per unit area of cocooning tool of MFC (600/m²) is three times that of SFC (200/m²). Therefore, the thickness, weight per square meter and porosity all vary accordingly, which can be used to control the size and structure of flat cocoons and generate materials that meet the requirements.

As can be seen from Figure 2(a), the average sericin content of NC, SFC, and MFC is close to each other, and the sericin content is gradually decreasing from outer layer to inner layer, which is related to the sericin’s good anti-ultraviolet property [5]. There are some differences in the time of silkworm spinning of MFC, so there is cross distribution of some fibers between the layers, which causes the sericin content of each layer of fibers to be slightly lower than that of NC and SFC. Since silkworms have larger space during the construction of SFC and MFC than that of NC, and the swing amplitude of spinning is larger than that of NC, the drawing distance of the same amount of sericin mixture is increased, and so the fiber fineness of SFC and MFC is greater than that of NC. The middle fibers of NC, SFC, and MFC are thicker than the inner fibers, and the inner fibers are thicker than the outer fibers. The mean radial sizes (μm) of the outer, middle, and inner layers of NC, SFC, and MFC were (13.13 ± 0.14, 13.55 ± 0.24, 12.41 ± 0.32), (12.35 ± 0.11, 12.75 ± 0.26, 11.71 ± 0.30), and (12.59 ± 0.25, 13.18 ± 0.30, 12.15 ± 0.29), respectively.

As can be seen from Figure 2(b, c), the stress-strain curves of SFC and MFC cocoon shells show a similar trend as that of common cocoons. In this case, the density of SFC and MFC is lower than that of NC (about 21% lower), and the stress of NC cocoon shell is higher than that of SFC and lower than that of MFC, mainly because the number of silk filaments per unit cross-sectional area of NC cocoon shell is larger than that of SFC, but the stress is close to that of MFC, and mainly because of the radial strip specimen of NC cocoon shell, the radius of NC cocoon layer decreases gradually from outside to inside, resulting in non-synchronous fracture during the stretching; the strains of SFC and MFC are roughly the same but greater than that of NC, and the fiber strengths of various layers of SFC and MFC are about 11.2% and 9.3% higher than that of NC, respectively. During the construction of NC, the internal space of cocoon is getting smaller and smaller, which reduces the swing amplitude of silkworm spinning, resulting in low degree of orientation of fibers but high elongation [17]. The middle fibers of NC, SFC, and MFC are stronger than inner fibers, and the inner fibers are stronger than outer fibers, which is in line with the change rule of fiber fineness.

Figure 2(e) shows that the amino acid composition of fibroin protein in each cocoon layer is the same (tryptophan is destroyed during acid hydrolysis and therefore not determined), but the content is different to some extent. Obviously, all alkaline amino acids have higher content in the outer layer, aromatic amino acids have the highest content in the outer layer, and hydroxyl amino acids have the highest content in the outer layer. NC, SFC, and MFC all show a similar rule.

As can be seen from Figure 3(a), XRD curves of inner, middle, and outer cocoon layers of NC, SFC, and MFC are similar, indicating that the main composition of each layer is not different. Crystallinity value of each cocoon layer can be obtained through peak-differentiating and imitating. It can be seen that the crystallinity of each cocoon layer decreases gradually from inside to outside.

The cocoon layers of NC, SFC, and MFC show similar rules in terms of morphological structure, content of sericin and amino acids, fiber fineness, strength and elongation, and crystallinity, indicating that during the construction of flat cocoons, silkworms also follow a certain natural law, which is a procedural process rather than an arbitrary spinning and cocooning procedure.

4. Conclusion

A series of cocoons with different shapes and structure and excellent properties (SFC and MFC) were produced through a natural process by providing different cocooning sites for silkworms, and the multi-layered morphological structure, composition, and mechanical properties of the new cocoons were characterized and compared with those of common cocoons. We found that SFC and MFC have excellent mechanical properties, and the multilayer structures and their performances of SFC, MFC, and NC have one-to-one correspondence, indicating that silkworms follow a natural procedure of normal cocooning during the construction of SFC and MFC. Flat cocoons with different sizes, thickness, and porosity can be obtained by applying this rule and reasonably controlling the quantity, start time of silk spinning, and size of spinneret plate, so that a new type of composite polymer material with novel structure, controllable density, excellent mechanical properties, and perfect functions can be obtained, and the cocoon can be used directly as a biomass material without destroying the structure of the cocoon, such as the substrate of facial mask, protective mask, and insulation and protective material. For some wild cocoons that have excellent silk properties but cannot be reeled for processing and utilization due to defects (for example, eri-silkworm cocoons cannot be reeled to develop high-grade products due to the existence oflosion holes), the utilization and industrialization prospects can be expanded by changing the silk spinning path. This study provides a reasonable and effective method for the direct application of cocoon, which is a natural biopolymer material, and has potential application prospects in development of green high-performance engineering materials.
Figure 2. Analysis of physical and chemical properties of cocoon layers of NC, SFC and MFC cocoon materials (a) fiber sericin content and fineness, (b) fiber stress-strain curve, (c) cocoon shell stress-strain curve, (d) cocoon shell thickness, weight per square meter and porosity, and (e) analysis of amino acid content of cocoon layer. MFC, multi-silkworm flat cocoons; NC, normal cocoons; SFC, single-silkworm flat cocoon.
Figure 3. XRD spectrograms, crystalline histograms and peak-differentiating and imitating curves of outer, middle, and inner fibers of NC, SFC, and MFC cocoon materials. (a) XRD spectrogram, (b) crystalline histogram, and (c)–(k) peak-differentiating and imitating curves. MFC, multi-silkworm flat cocoons; NC, normal cocoons; SFC, single-silkworm flat cocoon.
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Conflict of Interest

None.

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