Effect of Degumming on the Tensile Properties of Silkworm (*Bombyx mori*) Silk Fiber

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ABSTRACT: Forcibly reeled silkworm (*Bombyx mori*) silk was used to study how exposure to a degumming treatment (boiling in distilled water for 30 min) affects tensile properties. Because forcibly reeled and naturally spun fibers exhibit comparable mechanical behavior, the results can be generalized to material obtained conventionally from cocoons. The effects of degumming include: a decrease in the initial elastic modulus, a decrease in the stress at the proportional limit (yield strength), a change in the qualitative shape of force-displacement curves, and significant qualitative and quantitative variability in force-displacement data from samples subjected to nominally identical degumming histories. Immersion in water at room temperature or heating in air at 100°C for 30 min are both qualitatively equivalent to a 30-min degumming treatment in boiling water, in terms of the effect on silk tensile properties.
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INTRODUCTION

Silkworm (*B. mori*) silk has been used as a textile fiber for over 5000 years.[1] Besides several highly desirable physical characteristics (good mechanical properties, soft texture, dyeability), the convenience of reeling long (300–1200 m) continuous fibers from the cocoon has certainly contributed to its success as a textile fiber. This situation contrasts with the currently negligible economic importance of spider silk: although the tensile properties of spider drag line exceed those of silkworm silk, the drag line cannot yet be produced in sufficient quantity to support any industrial process.

The silkworm cocoon is built at the end of the larval stage and protects the pupa during metamorphosis to an adult moth. The as-spun fiber is composed of two cores of fibroin surrounded by a layer of sericin in a structure known as a *bave* (each individual fibroin core is known as a *brin*).[2] Although both fibroin and sericin consist of protein, their compositions are widely different. Silkworm fibroin is a single protein and contains the motif -Gly-Ala-Gly-Ala-Gly-Ser- repeated along its sequence, while sericin is a mixture of proteins and contains a large number of amino acids with hydroxyl groups (Ser, Thr, and Tyr).[3,4] The microstructures and biological functions of the two materials are also different: the -Gly-Ala-Gly-Ala-Gly-Ser- motif in fibroin forms a high volume

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fraction of β-sheet microcrystallites[5,6] that act as a reinforcement and contribute to the strength and stiffness of the silk. Sericin, on the other hand, is primarily amorphous[7] and acts as an adhesive binder to maintain the structural integrity of the cocoon.

Unfortunately, bave can only be reeled from the cocoon after the adhesive sericin coating has been removed. Sericin removal requires thermochemical treatment of the cocoon in a process conventionally known as *degumming*. A degumming treatment consists of submerging the cocoon in boiling water, sometimes with salt or detergent to increase the efficiency of the process.[8,9] Because degumming imposes a markedly nonnatural environment on the silk, we must consider the possibility that changes could occur in the fibroin structure and mechanical properties. In other words, the properties of conventionally retrieved silk may not accurately reflect those of the native as spun material.

The possible effect of degumming on silkworm silk has been studied by microstructural characterization techniques that do not depend on the isolation of *individual* fibers: X-ray diffraction,[10,11] and a combination of differential scanning calorimetry and birefringence measurements.[12] Although these techniques did not detect any significant microstructural consequences of degumming, it would be instructive—and more conclusive—to perform tensile tests on isolated bave to check directly whether degumming has an effect on the mechanical properties. It has also been noted[12] that changes in the orientational order of the amorphous fraction in fibroin would be extremely difficult to detect with the above-referenced microstructural characterization techniques; however, such changes could affect tensile properties. Therefore, it is especially convenient to have the capability to obtain testable bave samples without degumming a cocoon, so that the effect of a subsequent degumming treatment on tensile behavior can be monitored unambiguously.

The first step towards meeting this objective has recently been achieved, with the report[13] that bave can be reeled directly from silkworms. The larvae were reared to the fifth instar stage, and silk was reeled carefully by pulling with tweezers as soon as the silkworm started to spin fiber. In this way, it was possible to acquire samples of bave that were long enough to use for tensile tests. The results presented in ref. 13 were concerned with characterizing the tensile properties of these untreated bave samples, and focussed on the reproducibility of the properties. In the present article, based on this initial work, the effect of a degumming treatment on the tensile properties of the forcibly reeled silk is ascertained.

MATERIALS AND EXPERIMENTAL METHODS

Bombyx mori silkworms were reared to the fifth larval stage on a diet of mulberry leaves. Immediately after they stopped feeding, they were subjected to constant surveillance to detect the onset of cocoon spinning. When silk was first observed, the worm was placed on a horizontal, flat, black vinyl surface and allowed to spin a short length of fiber. (Silkworms placed on a flat horizontal surface, away from other objects, are unable to construct a cocoon, and so leave a trail of bave. The black background makes the silk easier to see, and vinyl is useful to prevent the silk from sticking.) The spun fiber was grasped with tweezers and reeled from the worm by hand at a nominal speed of 1 cm/s, equivalent to the rate at which silkworms spin their fiber naturally.[14] The value of the average force exerted on the fiber during reeling was estimated as 2–3 gf (20–30 mN). Bave of a length of 25–30 cm was obtained at each attempt. Also, some limited lengths of silk were retrieved entirely from the collecting surface instead of being reeled, to minimize possible changes to their native properties. These fibers will be referred to as "naturally spun" as distinct from "forcibly reeled" fibers (The term "forcibly reeled" is borrowed from the spider silk literature where it is commonly used to refer to silk fiber that has been collected from spiders onto a mechanical winding device).

The degumming treatment proceeded as follows: samples 7 cm long were cut from forcibly reeled fiber and mounted on an aluminium foil support frame as described elsewhere.[15] The mounted samples were introduced in 15 cm^3 polypropylene tubes, and enough water was added to immerse the sample completely. The polypropylene tubes were heated in a boiling water bath for 30 min, to reproduce the conditions of degumming as described previously.[16] In addition, some samples were degummed for 110 min to ensure maximum sericin removal[12] and enable more accurate determination of the fibroin core cross-section. All samples were allowed to dry in air for at least 24 h before testing.

Forcibly reeled samples, 5 cm long, both degummed and control (as collected) were cut and mounted across the hole in a cardboard card frame as described elsewhere,[2,16] so that the gauge length for tensile tests was $L_0 = 30$ mm. Tensile tests were performed with an Instron 4411 machine, allowing the strain rate to be controlled accurately. All tensile tests were conducted at a strain rate of 0.0002 s^{-1}, under nominal environmental conditions: 20°C, 60% relative humidity. A balance (Precisa 6100C, resolution ±10 mg) attached to the lower end of the sample was used instead of a conventional load cell.[16] The displacement of the crosshead was taken as equal to the deformation of the sample, because the compliance of silk is approximately 1000 times greater than that of any other part of the system.[16] After tensile testing to failure, selected samples were metallized with gold and examined in a scanning electron microscope (JEOL 6300; observation conditions V = 10 kV, I = 0.6 nA) to characterize their cross-sectional geometry.

In describing mechanical test data, we will refer to the following parameters: m, the initial slope of the force–displacement curve; the "proportional limit" (i.e., the point where a force–displacement curve intersects a straight line that passes through the origin and has a slope equal to 95% of the initial slope of the curve); σ_p, the stress at the proportional limit; ϵ_p, the strain at the proportional limit; σ_u, the tensile strength; ϵ_u, the strain at breaking.

RESULTS AND DISCUSSION

Forcibly Reeled vs. Naturally Spun Fibers

The forced reeling process enables the collection of isolated silkworm silk bave, so that the effect of a subsequent degumming treatment on tensile properties can be studied easily. However, we need to be confident that the material acquired by forced reeling is representative of naturally spun fiber. For example, a higher (lower) stress during the manual reeling process might lead to a more (less) pronounced alignment of the chains along the fiber axis, with consequent effects on how the material behaves in a tensile test. We addressed this issue by collecting some naturally spun bave as described above to be compared with the forcibly reeled fibers.

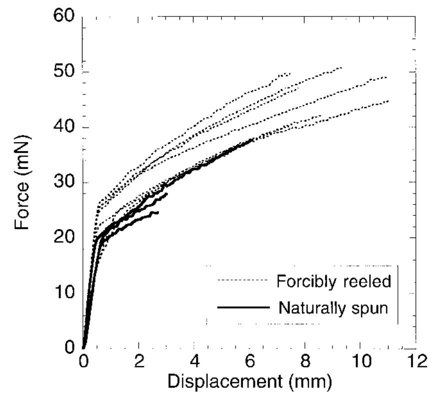

Figure 1 Force–displacement curves of forcibly reeled and naturally spun *B. mori* silk obtained from the same silkworm.

Figure 1 compares force–displacement curves of seven forcibly reeled and three naturally spun samples; all the material was obtained from the same silkworm. As has been proven in our previous work,[13] silk obtained from the same silkworm yields very similar force–displacement curves. Consequently, comparison of force–displacement curves of silk obtained from the same silkworm is equivalent to comparison of stress–strain curves. However, the use of force instead of stress allows us to avoid errors related to the uncertainty in the determination of the fiber cross-section. The two data sets overlap significantly, showing that the forcibly reeled material conveys the behavior of the naturally spun bave, and so can be used to study the effect of degumming. Forcibly reeled material exhibits, on average, a slightly higher load at the proportional limit, suggesting that some of the forcibly reeled samples have been "strain-hardened" by inadvertent stretching during the collection procedure. Stretching has a similarly small (and conventionally neglected) effect on the proportional limit when silk is reeled from cocoons.[16]

It is also apparent from Figure 1 that forcibly reeled material shows more reproducible forces and displacements at breaking, compared with naturally spun silk. The same can therefore be said for the reproducibility of the tensile strengths and the strains at breaking,[13] consistent with the higher Weibull modulus of the forcibly reeled material.[13] It is for this reason that we use forcibly reeled silk in our present study of how degumming affects tensile properties—even though we have no clear idea of a *mechanism* for its superior reproducibility.

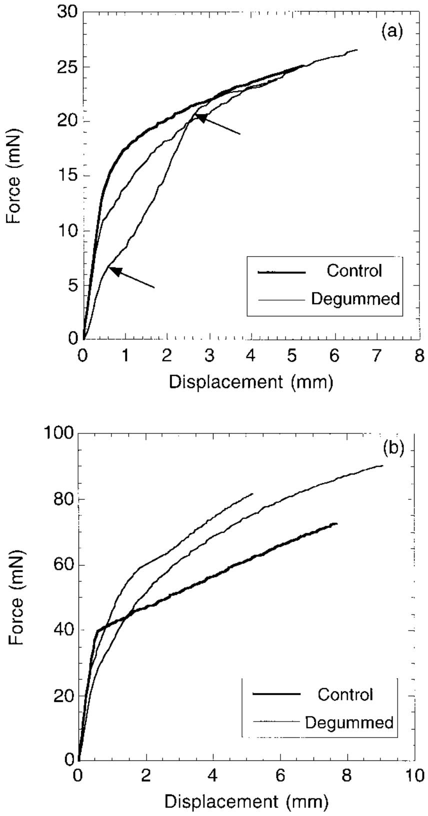

Figure 2 Force–displacement curves of forcibly reeled bave, comparing control and successive adjacent degummed samples. The samples in (a) and (b) were obtained from different silkworms. The very different quantitative behavior of the controls in (a) and (b) is consistent with the previously characterized variability in silk reeled from different worms.[13]

Effects of Degumming

The best way to compare samples before and after degumming, to minimize spurious sources of scatter, is to work with *adjacent samples* (i.e., samples cut from the same original bave). The similarity of the tensile properties of adjacent samples was first established for spider drag line[17] and subsequently confirmed for silkworm silk.[16]

Tensile Parameters

Figure 2(a) is an example of the tensile behavior of a control sample and two successive adjacent degummed samples, and Figure 2(b) repeats this exercise with bave from a different silkworm. Degumming has two principal quantitative effects on the force–displacement plots: both the initial slope and the proportional limit are decreased. Table I summarizes the tensile parameters of a larger number of degummed samples: averages were compiled from 18 degummed samples (successions of six degummed samples and adjacent controls were obtained from each of three different silkworms). For properties that depend on sample cross-section, we performed a normalisation relative to the corresponding control sample.[15]

Shape of Force–Displacement Curves

Besides these quantitative differences, the qualitative shape of the force–displacement curves is also changed considerably by degumming. Forcibly reeled fibers prior to degumming are characterized by simple force–displacement curves: an initial straight segment extending up to the proportional limit, and a second almost straight segment with a different slope extending from the proportional limit to the breaking point [see Figs. 1, 2(a) and (b), and results in ref. 13]. On the other hand, degummed samples may exhibit two yield points [marked with arrows in Fig. 2(a)]. This more complex shape has been described previously for silkworm silk obtained by reeling degummed cocoons.[18]

Variability

We have shown previously that silk forcibly reeled under similar conditions from the same silkworm gives reproducible force–displacement curves.[13] Figures 2(a) and 2(b) each show that nominally identical degumming treatment induces

Table I Tensile Properties of Forcibly Reeled *B. mori* Bave after Degumming

m/m_R	σ_p/σ_{pR}	ε_p	σ_u/σ_{uR}	ε_u
0.65 ± 0.04 ($m_R \approx 16$ GPa)	0.64 ± 0.02 ($\sigma_{pR} \approx 230$ MPa)	0.015 ± 0.001	1.03 ± 0.04 ($\sigma_{uR} \approx 530$ MPa)	0.22 ± 0.02

Parameter symbols are defined under *Materials and Experimental Methods*.
Subscript R indicates values obtained from control (reference) samples.
The numbers in parentheses are representative values of reference untreated samples, and have been estimated from the data presented in ref. 13.

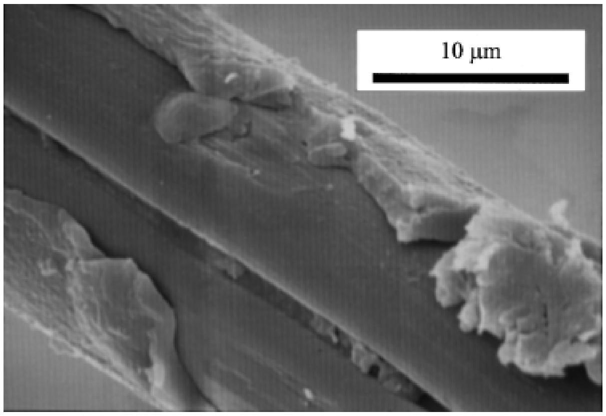

Figure 3 SEM image of *B. mori* cocoon silk showing partial removal of the sericin coating after a 30-min degumming treatment. Fibroin cores can be distinguished, but they are not bare.

significant qualitative and quantitative variability in such curves. Material obtained from a degummed *B. mori* cocoon exhibits a similarly large variability.[16] In the latter case, it was shown that the variability was not simply an artefact of nonuniform cross-sectional area, because measuring the cross-section and rescaling data into the form of stress–strain curves only reduced the initial scatter slightly. We hypothesized that the remaining scatter could be the result of incomplete sericin removal by the degumming treatment. Sericin would contribute to the cross-section as measured in the SEM but not to the mechanical performance of the fiber. Moreover, the degree of sericin removal might vary considerably from fiber to fiber after a degumming treatment of 30 min. An example of the incomplete degumming achieved by a 30-min treatment is presented in Figure 3.

Maximum degumming of forcibly reeled fibers offers an opportunity to test whether the large variability of treated material is due to retained sericin, or microstructural changes introduced by the degumming procedure, or a combination of both. Thus, four lengths of forcibly reeled fiber from the same silkworm were degummed for 110 min. (The duration was chosen with reference to a study that showed that detectable sericin removal does not continue beyond this time,[12] although we emphasize that 100% removal is not necessarily achieved.) To compare the mechanical properties of bave obtained from the cocoon with the properties of forcibly reeled and maximally degummed bave, it is necessary to consider the respective stress–strain data, because these materials were not provided by the same silkworm. It therefore was necessary to determine the cross-sectional area of the samples after the 110 min degumming process. The diameter of each sample was determined from SEM micrographs and used to calculate cross-sectional areas in a procedure that is described in detail elsewhere.[13] The mean value of the diameter for the maximally degummed fibers was $D = 6.9 \pm 0.2$ μm, and this value was used to rescale force–displacement data to give the stress–strain curves presented in Figure 4. Tensile parameters gleaned from each curve are collated in Table II.

The values presented in Table II are comparable, in both their magnitude and their scatter, to the results obtained from conventionally degummed cocoon silk.[16] This similarity indicates that the large scatter in the stress–strain curves of degummed silkworm silk cannot be assigned entirely to the incomplete removal of the sericin coating. Instead, it appears that the degumming treatment can variably modify the intrinsic mechanical properties of the fibroin cores. Also, because the variability after a 110-min treatment is similar to that after a 30-min treatment, this modification already occurs in the earlier stages of degumming.

Consistency with Previous Studies

Previous comparisons of native and degummed material[10–12] have not detected any significant changes in physical properties such as birefringence or X-ray diffraction. The absence of significant microstructural changes was taken as an indication that tensile properties would remain

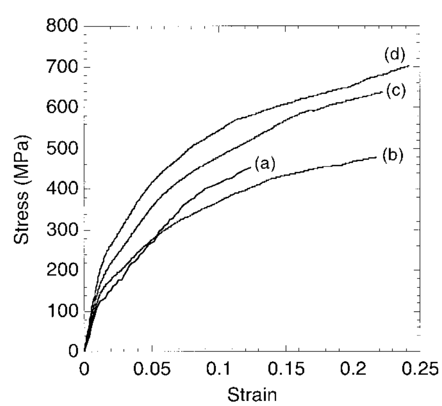

Figure 4 Stress–strain curves of four nonadjacent samples of bave that were forcibly reeled from the same silkworm. The cross-sectional areas were characterized after the samples were subjected to a maximal degumming process.

Table II Tensile Parameters of Maximally Degummed Samples of Forcibly Reeled *B. mori* Bave

Curve (in Fig. 4)	E (GPa)	σ_p (MPa)	ε_p	σ_u (MPa)	ε_u
(a)	12	120	0.011	450	0.12
(b)	13	160	0.014	480	0.22
(c)	15	190	0.015	640	0.22
(d)	17	210	0.014	700	0.24
Ref.16	9–17	110–230	0.010–0.016	300–600	0.10–0.20

The values obtained from ref. 16 are presented to facilitate comparison.

largely unaffected by degumming.[12] However, tensile tests were not performed in those studies, although it was recognized that some microstructural changes—such as the degree of molecular alignment in amorphous regions—could go undetected by the techniques used. The present work, in which tensile properties are characterized directly, is not open to such ambiguity.

The Separate Effects of Water and Heat

A degumming treatment imposes two conditions on the silk: immersion in water, and heating to 100°C. It is instructive to consider their separate contributions to altering the mechanical behavior of *B. mori* silk.

To study the influence of submerging the forcibly reeled fiber in water, we followed an experimental procedure similar to that described above for degumming, except that the water was maintained at room temperature (approx. 20°C). The duration of immersion was 30 min, and the samples were air dried at room temperature for at least 24 h before testing.

To study the influence of heat, reeled fibers were introduced into an oven at 100°C. The duration of the treatment was 30 min, and the samples were stored in air for at least 24 h before testing. Samples were free to undergo dimensional changes in either case, as the aluminium foil support frames offer little resistance to out-of-plane bending.

Figure 5 shows the force–displacement curves of degummed samples from two different silkworms, in each case comparing these with data from adjacent samples that had either been immersed in water at room temperature and then dried or been treated at 100°C in air. On this evidence, either of the separate conditions is qualitatively equivalent to a degumming treatment regarding its effect on the tensile properties of the fiber. Although the water must be hot if it is to degrade and remove the sericin, the mere presence of water suffices to alter the tensile properties of the fibroin core, as does the application of heat alone.

The effect of water can be understood from previously published results,[15] where *B. mori* silk was immersed in water or ethanol (a desiccant) throughout the course of a tensile test, and a significant decrease in the initial modulus was observed. This sensitivity to test environment chemistry (composition) highlights the important contribution of protein–protein hydrogen bonds to the stability of the polymer microstructure. Water acts as a plasticiser for the silk: by penetrating amorphous regions and disrupting the internal distribution of hydrogen bonds, it increases the freedom for relative displacements of protein chain segments to occur in response to any driving force for microstructural change. In contrast, heating to 100°C is unlikely to significantly disrupt the pattern of hydrogen bonding in the dry fiber. This temperature provides a thermal energy equivalent of 3.1 kJ/mol, which is below the

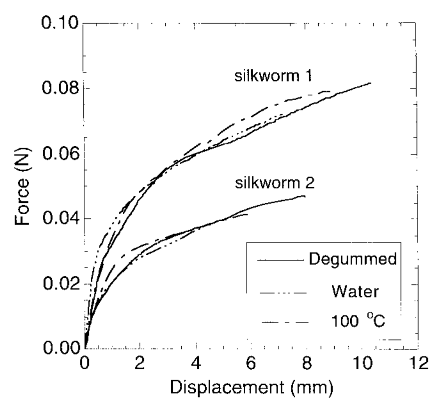

Figure 5 Force–displacement curves of degummed samples, compared to data obtained from adjacent samples that had either been immersed in room-temperature water and then dried, or been heated at 100°C in air. Results for material reeled from two different silkworms are presented.

range of typical hydrogen bond energies (10 kJ/mol, similar to the energy of the van der Waals interactions, to 40 kJ/mol[19]). However, increasing the temperature will enhance the *drive* for conformational disorder; any consequent loss of molecular alignment will be concentrated in the amorphous regions.

In the present study, no external stress is applied when forcibly reeled material is immersed in water or heated to 100°C. Therefore, the resultant irreversible changes in tensile properties indicate that (1) chain segments have rearranged into a lower energy microstructure, and (2) the rearrangement must occur in response to an *internal* driving force. These considerations imply in turn that the microstructure of as-spun fiber is metastable—there is a thermodynamically more stable arrangement of the protein chains, but this is not kinetically accessible in the as-spun material. Silk fibroin has an especially high molecular weight (of the order of 350 kDa[7]), and it is unlikely that these molecules have the opportunity to anneal into low-energy arrangements during spinning.

CONCLUSIONS

1. The tensile properties of “forcibly reeled” *B. mori* silkworm bave are similar to those of “naturally spun” bave. Therefore, forcibly reeled bave is a representative material to be used in studies of how the tensile properties of silkworm silk are changed by the conditions imposed during a degumming treatment.
2. Forcibly reeled material shows more reproducible values for (a) tensile strength and (b) the strain at breaking, compared with naturally spun silk.
3. Degumming has two principal quantitative effects on force–displacement plots: both the initial slope and the proportional limit are decreased.
4. The qualitative shape of force–displacement curves is changed significantly by degumming.
5. The large scatter in the tensile behavior of degummed silkworm silk cannot be assigned entirely to incomplete removal of the sericin coating. Instead, the degumming treatment can variably modify the intrinsic mechanical properties of the fibroin cores. This modification occurs in the earlier stages of degumming.
6. The effect on fiber tensile properties of (a) immersion in water at room temperature for 30 min, or (b) heating the fiber at 100°C in air for 30 min, or (c) degumming in boiling water for 30 min are qualitatively similar.
7. The microstructure of as-spun *B. mori* bave is metastable.

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