Silk "Quality" Revealed Using Dynamic Mechanical Thermal Analysis (DMTA)

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Abstract

Dynamic Mechanical Thermal Analysis (DMTA, en.wikipedia.org/wiki/Dynamic_mechanical_analysis OR www.oxfordsilkgroup.com/DMTA) is able to identify specific molecular signatures allowing us to differentiate 'good' from 'poor' silks. We propose DMTA as an effective tool for evaluating silk "quality" and grading silks. Moreover, DMTA can quantify the effect of industrial processing (temperature, humidity and loading) on the quality of silks, therefore, it helps the silk industry to achieve quality control in silk processing as well as to obtain optimum conditions for producing desirable silk products.

In the case study, we chose three cocoon silk grades (G1, G2 and G3) from the same region in China during the same period of production, and examined the three silk grades using DMTA. We observed statistical differences in the mechanical properties of three grades. We also discovered that lower grade silks display lower temperature transitions which are characteristic of more disordered molecular structure. Interestingly, the temperature annealing treatment under load can "heal" these poor silk structures and reduce the differences between the poor and good silks.

More DMTA based methods such as static-dynamic tests demonstrate how mechanical load and moisture affects the structure of silk, which has important consequences for other important practical properties such as optical transparency, reel-ability and dye-ability. This fundamental understanding can be crucial to silk production and processing.

Our study demonstrates that DMTA is a sensitive and very powerful technique that can be used to link rearing and production conditions to silk quality and silk properties. Importantly, we assert that the technique can be used on both mulberry and wild silks in both fundamental research and in quality control for commercial sericulture.

Keywords: Dynamic mechanical; thermal; glass transition

Introduction

Dynamic mechanical thermal analysis (DMTA) has been applied extensively in polymer science and engineering since the 1950s, and it is arguably the most important analytical tool to bridge between the microstructure and macroscopicl properties for amorphous or semicrystalline polymers [1]. Over the decades, this technique has moved into areas beyond fundamental research. For example, in pharmacology DMTA is used to evaluate the properties of polymer-based drug release systems [2].
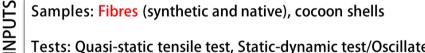
As illustrated in Figure 1, DMTA applies periodic deformations dynamically to the sample and measures the response as a function of time, temperature and frequency. As a result, modulus and loss tangent can be plotted with changing time, temperature or frequency. The modulus is a measure of stiffness (the resistance force per unit area); and the loss tangent is a measure of the damping properties, e.g. how much energy is absorbed or converted to internal heat. Modern DMTA is also capable of conducting quasi-static tensile testing and relaxation test (e.g. creep), which are important for engineering applications. With a tailored humidity accessory, DMTA can examine the moisture effect on the mechanical properties of biological samples. The above mentioned tests prove DMTA to be an incredibly powerful tool which could provide key structure and property information on silks and silk composites.
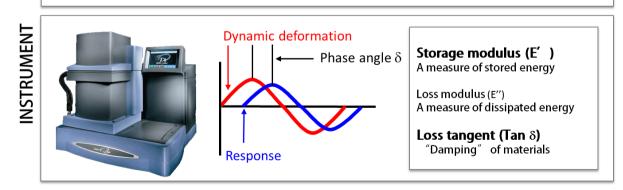
Sericulture in China has a history of over five thousand years. Historically it has been more or less an 'empirical' manufacturing process with respect to both the conditions of growing silkworms and the criteria of assessing the agricultural product, cocoons. The biggest silk-producing country, China, has recent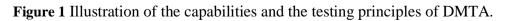ly established the 2008 standards of classifying fresh cocoons and the 2008 standards of evaluating raw silk quality [3, 4]. The Chinese standards of cocoon classification have evolved from the empirical 'look and feel' in the 1950s to a more machine-testing based procedure. However, the main characteristics remain empirical, e.g. the reelability and the maximum unravelled length [3]. In practice these methods can classify cocoons efficiently, nevertheless they do not lead to an understanding on the underlying science, e.g. what is the origin of the observed differences in the property of silks.

In this paper, we demonstrate that the technique DMTA can be applied in cocoon and silk classification, and DMTA is proposed to be a potential quality control tool for silk industry. In the case study, we chose three graded cocoons (G1, G2 and G3) from the same region in China during the same period of production. Focusing on mechanical properties of cocoon silks, both quasi-static tensile tests and dynamic mechanical thermal analysis were completed on DMTA. In the discussion, structural differences between graded B. mori silks are considered, which may shed light on the origin of silk quality and the role of silk farming and post-processing.

Figure 1 Illustration of the capabilities and the testing principles of DMTA.

Experimental section

Materials:

1. Cocoons

Three different quality cocoons (pupae removed) from Jiangsu Province, China were provided by Prof. Yaopeng Zhang from Donghua University, Shanghai. They were produced during the same period, June 2010; and pupae were removed immediately upon collection. Three cocoon samples (one for each grade, named as G1, G2 and G3) were randomly chosen for experiments and analysis. The rough outer layer of each cocoon was removed before taking the fibres from the middle of the cocoon shell.

2. Raw Silk Fibres

Silk fibres were manually gently pulled from the middle layer of cocoons and fixed to sample-holders for mechanical testing. The pulling of the fibres inevitably causes some breaking-up of the sericin coating. However, with care, minimal damage to the sericin-fibroin bonding was maintained. Sample-holders are card frames, designed for the DMA tension clamp and cut by laser cutter with precision.

Methods:

1. Physical-property Measurement of Cocoons

Photographs of the three cocoons were taken using a Panasonic camera in order to compare the features of their appearance such as colour and contamination. The size was measured from the photographs; and the weight was measured on a lab balance.

2. SEM Characterization of Silk Fibres

Images of the morphological features of both cross-sections and surfaces of raw silks were taken on a Scanning Electron Microscope (Jeol Neoscope JCM-5000). A magnification of 1,000 times was used for cross-sectional shots. Image analysis of the cross-sectional areas was done using ImageJ (protocol provided by lab members). The average area from this analysis for each grade was then used for mechanical testing analysis (calculating the stress and modulus).

3. (Quasi-static Mechanical) Tensile Testing

Quasi-static mechanical tests, or tensile tests, were conducted in a controlled-force mode on a TA Q800 instrument instead of Instron. 5mm gauge length was used for all mechanical tests, including dynamic tests. The engineering "end" effect is not taken into account here as the thickness/length ratio is very small for silk fibres. Tests were set up using the following parameters: force-ramp rate of 0.1 N/min; room temperature 25 °C; and relative humidity of 50%. Approximately 15 specimens were tested for each grade.

4. Dynamic Mechanical Analysis

Dynamic mechanical tests were conducted on the same instrument (TA Q800) as the tensile tests using the following settings: temperature ramps from -100 to 250 °C at 3 °C /min, 0.02 N static load for G1, 1 Hz frequency, 0.2% dynamic strain (equivalent of ~15 MPa dynamic stress at 25 °C). Specimens were all equilibrated at 30 °C for 10 mins under nitrogen purge (to remove the excess moisture).

A cyclical temperature test was conducted on G1 silks using the same other settings as above: the first scan was from -100 to 120 °C, and then the second scan was from -100 to 250 °C after cooling to -100 °C at -

10 °C /min. Another cyclical temperature scan (annealing test) was conducted on G3

silk with the first scan to a maximum temperature of 180 °C.

Results and discussion

1. Morphology of cocoons and raw silk fibres:

Colour/contamination: As shown in Figure 2, cocoon G3 is clearly discoloured (notice the dark brown spots). The colour of cocoons G1 and G2 is white, while cocoon G3 is yellowish.

Size: Cocoon G1 (4.3cm by 2.5cm) is much bigger than that of G2 (3.6cm by 2.0cm) and G3 (3.6cm by2.1cm).

Weight: The weights of the three cocoons are: G1, 0.51g; G2, 0.29g; G3, 0.33g. This suggests that cocoon G1 yields more silk. The size and weight are consistent in a way the top grade produces larger quantity of silk per cocoon.

As also shown in Figure 2 (middle column), silk fibres from G1 have more

regular cross-sectional shapes (triangular or bone-shaped), and the two brins are well wrapped by sericin with little exposure of single brins; while as G2 and G3 fibres are not coated evenly by sericin, and the spread or coating thickness of sericin varies a lot around and along the fibre, which can also be seen from the longitudinal view in the right column of Figure 2. In addition, for G2 and G3 silks, there are more cracks on the interfaces between sericin and fibroin and more holes in the fibre cross-sections. The 'cracks' in G2 and G3 are clearly defects and may cause early failure in mechanical testing. From the SEM cross-sectional measurements (data not shown here), it is found that the top grade G1 silks have the thickest average diameter; however, the variability (~10%) of the diameters does not differ between grades.
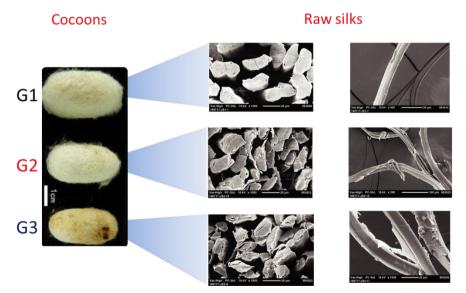

Figure 2 Photographs of the three cocoon grades are on the left, SEM images of the cross-sections are in the middle column, and surface/longitudinal views of the raw silks taken from the middle layer of the cocoons are shown on the right.

2. (Quasi-static) Tensile performances

Figure 3 shows three representative stress-strain curves with standard deviation bars for the breaking stress and breaking strain from about 15 silk fibre samples for each of the three grades. G3 has a larger variability in the breaking strain and stress compared with G1 and G2. It is also shown from a two-sample t-test that the initial moduli of G1 (4.95 GPa) and G2 (5.15 GPa) silk fibres are significantly higher than G3 (3.59 GPa); and the post modulus of G1 (796 MPa) silk fibres is significantly higher than G2 (664 MPa) and G3 (635 MPa). Interestingly, the breaking energy increases as the silk grade decreases, and the breaking energy of G3 is significantly higher than that of G1 at the 0.05 confidence level. This increased

3. Dynamic mechanical thermal analysis of silk fibres

How does temperature or heat affect the properties of silks? DMTA temperature scan is the most effective way of obtaining this information. Figure 4(a) shows the DMTA results of G1 silks in a cyclical temperature scan: storage modulus and loss tangent change as a function of temperature. In the first scan, as the temperature increases from -100 °C, the storage modulus decreases first with a lower gradient until 50 °C, where an increase in modulus is observed, which is attributed to water loss [5]. Then in the second scan (after the water is boiled off), the modulus decreases with increasing temperature from -100 °C to 100 °C. Starting from 150 °C, the modulus drops faster by a factor of about 10 and is accompanied by a Gaussian shape loss tangent peak centred at 212 °C. This loss

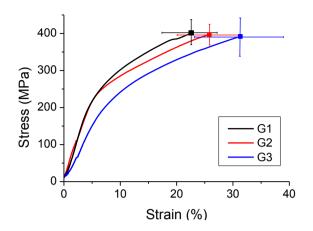breaking energy is usually a sign of increased disorder in the molecular structure.

Figure 3 Representative stress-strain curves of 3 grades of *B.mori* silks (from the middle layer of cocoons), error bars show the standard deviation of breaking strain and stress of 15 samples.

event is the glass transition of the "dry" silk structure [5].

How do the different silk grades behave in the DMTA profile? In Figure 4(b), for the major glass transition event, G2 and G3 silks show loss peaks between 151□C and 170°C which are absent for G1, although the three grades have similar loss tangent peaks below 100 °C (not shown here). The loss peaks in this temperature region are associated with more disordered structures, which also appear in the reconstituted silk fibroin films and other poorly reeled silks [6].

However, as shown in Figure 4(c), comparing the first and second temperature scans of G3 silks, these disordered structures can be annealed out through temperature treatment. The explanation is that the combination of heat and mechanical energy can effectively increase the mobility of the molecules and
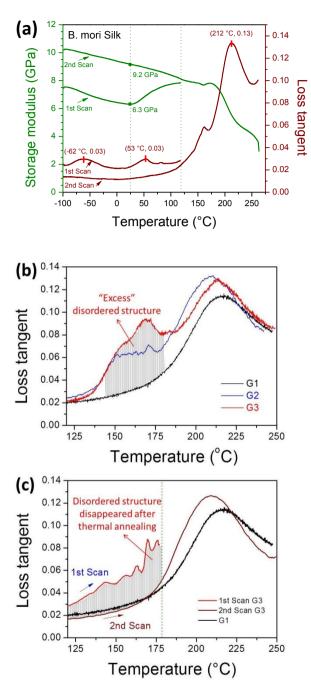
relax the structures into less disordered

Figure 4 (The figures are reproduced from [5]) Dynamic mechanical properties of silks from tests: DMTA cyclical temperature scan of G1 silk (a); DMTA temperature scans of the three grades G1, G2 and G3 (b); the annealing temperature scan to 180 °C of G3 (c).

4. The quality of silks

forms.

Instead of using the standard ways of assessing cocoons, we chose to test the properties of the raw fibres of different cocoon grade on DMTA. The link between cocoon grade and the raw silk quality has been established in this study: the raw fibres from better grade cocoon have more uniform fibre morphology, higher tensile modulus, more consistent tensile properties and better dynamic thermal mechanical properties. Further quantification of the relationship between silk quality and properties can be obtained using the methodology established in this paper.

The structural analysis on DMTA implies that lower grade silks have inferior silk structure compared with the normal or high grades. Because only raw silks were examined in our study, the differences in silks are most likely attributed to the production of the cocoons other than post processing, for example, whether a healthy diet was provided to silkworms or other factors such as environmental conditions which affect the growth of silkworms or the cocoon spinning process.

Furthermore, the thermal annealing study suggests that differences between the "bad" and "good" silks can be reduced through thermal mechanical treatments. In fact, it is known that the post-processing of silks has a major influence on the mechanical properties of silks [7].

The effect of static loading or mechanical stress on the properties of silks has been examined in our previous study [8]. It was shown that the loading history increases the storage modulus of silks. In other

words, higher loading or loading history makes stiffer silks.

There are two immediate options to apply DMTA in sericulture. Firstly, it could be used by breeders and/or rearers in order to monitor the quality of their 'product' i.e. the average quality of the fibres made by their worms. Secondly, DMTA would be used to monitor the effect, efficiency and efficacy of post-processing technologies such as thermal annealing or dying. For example, a reasonably quick scan of a specifically dyed silk would tell how the dyeing procedure changes the structures of silks and therefore how tightly the dye can bind to silks. Other post-processing procedures, such as throwing, degumming, washing, and even weaving can be interpreted using a set of a few physical parameters including temperature, mechanical stress and water permeation. As we discussed above, the effect of these treatments on silk fibres tend to be reasonably well understood on the scientific level. Perhaps commercially applied DMTA might prove to be equally useful in helping to improve sericulture practises and thus enhance the value chain.

Summary

Using both quasi-static tensile testing and dynamic mechanical thermal analysis, we examined the mechanical properties and the structures of raw silk fibres from three cocoon grades of the Chinese *B. mori* species. It is evident that the tensile modulus of the top grade is higher than the low grade and the tensile performance is more consistent for the top grade, but the breaking energy does not deteriorate with lower grades. The dynamic mechanical thermal properties of silks suggest that low grade silks have a more disordered molecular structure, which could be "annealed out" using thermal mechanical treatment, as shown in the thermal annealing tests.

We conclude that the structural differences in silk fibres from raw cocoons are due to the agricultural process; and DMTA is a sensitive tool for evaluating the quality of silks before or after post processing, as well as the effect of heat and mechanical treatment on silk quality.

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