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Performance measurement of sericin-coated silks during aging

ZHANG XiaoMei[1*] & WYETH Paul[2]

[1]*School of Archaeology and Museology, Peking University, Beijing 100871, China*
[2]*c/o Centre for Textile Conservation and Technical Art History, University of Glasgow, Glasgow G12 8QH, UK*

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Silks are among the most precious ancient and historic artefacts worldwide. While washing removes the natural gum from the fibres during the fabrication of most silk textiles, for a small proportion of historic silks some or perhaps all of the sericin still remains. This paper investigated the effect of sericin coating on the aging of silk fibroin by means of ATR and tensile testing. The results show that sericin can provide some extent of protection from light and heat aging. However, in high humidity environments degummed and ungummed silk aged at the same rate because of leaching of sericin. Silk degraded at faster rate and more extensively in a moist environment. ATR could give very useful information about the aging of silk. The ATR-derived crystallinity index is good at tracing the aging factor and extent of silk deterioration. Alanine and tyrosine within fibroin, as estimated by ATR spectroscopy, are very sensitive to light, but not to heat and water. The ATR absorbance intensity ratio $I_{\text{amide III}}/I_{\text{amide I}}$ is very useful for deterioration evaluation of archaeological silk objects. As a high humidity resulted in the leaching of silk, it is suggested for sericin-coated silk in collections, that not only wet cleaning is harmful, but also that storage or display in a high RH environment would be detrimental.

attenuated total reflectance infrared spectroscopy, archaeological silk, sericin

1 Introduction

Silk is an animal fibre, produced by caterpillars belonging to the genus *Bombyx*. A single silk filament is the product of a series of stages derived from the cultivation of mulberry trees for feed to the propagation of the domesticated silkworm, *Bombyx mori.* During the caterpillar phase, the two large glands of worm secrete two triangular-shaped filaments proteinaceous fibroin, held together by sericin, a gumlike protein. Degumming is the process of removing the sericin, or silk gum, from silk. Removing the gum improves the sheen, color, hand, and texture of the silk. In some cases, the fabric is woven to completion without degumming, for example Chinese Juan, which was used as recording materials for documents, calligraphy and drawing before paper was invented, and also mounting pictures since ancient times. The U.S. First Ladies gowns in Smithsonian Institution collection revealed sericin-rich materials [1]. The analyses of the same archaeological fibres from Mongolian tomes gave one sample containing binary mixture of fibroin and sericin in the ratio 82/18, while the other two 100/0 [2].

It needs to be made clear what kind of effect the sericin has on fibroin, what deteriorated sericin looks like, how to distinguish the natural aged sericin, and how such textiles should be handled, especially in the choice of wet cleaning or dry cleaning.

In a former paper of Zhang and Wyeth, FTIR spectroscopy was found to be very useful for the detection of sericin in historic silks [3]. Research by means of ESEM by Zhang *et al*. [4] showed that silk fibres with different amounts of sericin deteriorated to different extent and showed different characteristics after light and heat aging, such as the shapes of fissures, lacunae and holes resulting from aging in fibers, which is very useful in studying the deterioration mechanism, factors and extents.

*Corresponding author (email: zxm@pku.edu.cn)

Becker *et al* [1] investigated the degradative changes in silk after light exposure by means of amino acid analysis and the solubility under denaturing conditions. The study suggests the protective effect of sericin against light damage. The changes of solubility and tyrosine contents were useful indicators of deterioration at the molecular level.

This paper discusses the effect of a sericin coating on silk fibroin aged under the conditions of heat, light, 100 °C, 100% RH environment. Attenuated total reflectance infrared spectroscopy and tensile testing were used for the research of the performance and the deterioration of silk.

2 Experimental

2.1 Aging of silk surrogates

Commercially ungummed and degummed *Bombyx mori* silk textiles were used for the experiments. The samples were cut into 2.5 cm wide strips along weft for artificial aging.

2.1.1 Thermal aging

Conditions were selected to degrade the test fabric to a range of strengths after reasonable exposure times. The fabric test strips were placed in a forced convection laboratory oven preheated to 125 °C on racks covered with an enamel plate and exposed up to 16 days.

2.1.2 Light aging

Ultraviolet radiation over the wavelength range 290–400 nm can initiate the chemical reaction of most macromolecules, resulting in degradation and crosslinking. A 375 W ultraviolet lamp at 365 nm was selected as light source. The temperature of the sample chamber was maintained at 38 °C and relative humidity (RH) at 10 ± 5%. The irradiance reaching the specimens was measured with an UV Irradiance Meter to 2000 μW/cm^2. Samples were aged up to 16 days.

2.1.3 100 °C, 100% RH

Silk strips were put in bottles with a small test tube that contained 2 mL water in each bottle to give high moisture. The bottles were sealed with caps. Put the bottles in a forced convection laboratory oven preheated to 100 °C. Silk samples were aged in 100 °C, 100% RH for up to 16 days.

2.2 Characterization of physical properties

Tensile strength analysis can measure quantitatively the reduction in strength resulting from aging. Test strips were prepared series measuring 2.5 × 5 cm, with the long dimension parallel to the weft. The samples were conditioned for at least 72 h at 20 °C, 55 ± 5% RH, and then were measured at a gauge length of 2.0 cm and a crosshead speed of 2 mm/min on an Instron 5544 fitted. Six replicates from each sample were analyzed, and average values calculated.

2.3 Microstructural and molecular characterization

Using ATR-FTIR can quantitatively estimate the changes of silk crystallinity. Absorbance spectra were recorded using a Perkin Elmer 'Spectrum One' FTIR spectrometer, fitted with a PE 'Universal ATR' accessory; spectra were captured over the range 4000–400 cm^{-1}, using 32 scans and with a resolution of 4 cm^{-1}. Spectra were subsequently manipulated with Galactic Industries 'GRAMS/32 AI (6.00)' software. ATR spectra were recorded from the bulk fabric specimens, laid square on the 'UATR' accessory.

3 Results and discussion

3.1 Tensile strength

3.1.1 Heat aging

Changes in the tensile strength of fabric samples with heating time at 125 °C are shown in Figure 1. With the exposure time the tensile strength decreases nearly linearly. The degummed samples change at a little bit faster rate than ungummed samples.

3.1.2 Light aging

Changes in the tensile strength of the fabric samples exposed to light irradiation are shown in Figure 2. The

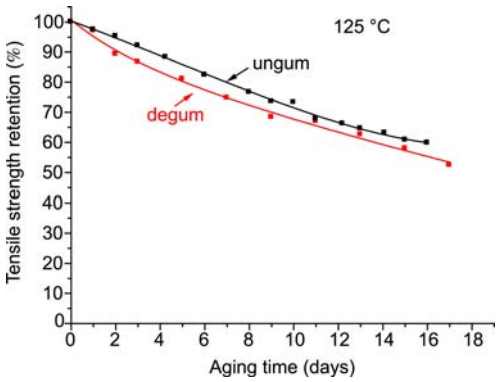

Figure 1 Tensile strength retention as a function of heating time at 125 °C.

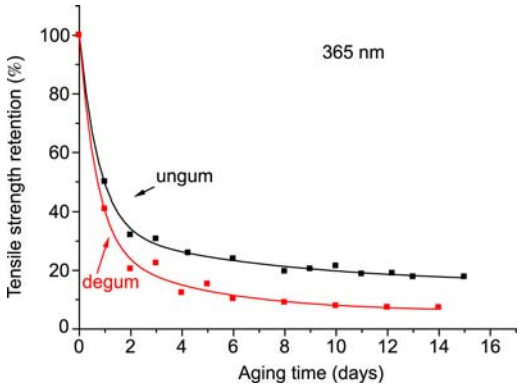

Figure 2 Tensile strength retention as a function of irradiation time.

strength decreased very faster in 2 days. After 2 days of aging the strength loss rate tends to slow down. The degummed samples were degraded faster and heavier than ungummed samples.

3.1.3 100 °C, 100% RH

Changes in the tensile strength of fabric samples after exposure to 100 °C 100% RH are shown in Figure 3. The strength of degummed samples decreased at the same rate as those of ungummed samples. Sericin is likely to have been leached from silks in very high humidity environments.

Compared with Figure 1, Figure 3 clearly shows that because of the effect of water vapor the silk was degraded at a faster rate to a heavier extent.

3.2 ATR

The silk spectra are typical polypeptide backbone amide absorption bands. The conformations of the polypeptide backbone include the α-helices, β-pleated sheets, turns and random coil conformations, with β-pleated sheets corresponding to crystalline components of silk [5]. The amide I band is at around 1620 cm^{-1}, with β-pleated sheets at around 1615 cm^{-1} and α-helices/random coil around 1650 cm^{-1}. The amide II band is at around 1520 cm^{-1}, with β-pleated sheets at around 1520 cm^{-1} and α-helices/random coil around 1545 cm^{-1}. The amide III band is at around 1230 cm^{-1}, with β-pleated sheets at around 1260 cm^{-1} and α-helices/random coil around 1230 cm^{-1}. Band around 1448 cm^{-1} is assigned as CH_3 modes of alanine. Phenol of tyrosine is at around 1160 cm^{-1} [6–9].

According to the work of Paul Garside *et al*. [10], the most reliable and satisfactory proved to be the direct measurement of intensities within the amide I band, at points corresponding to the theoretical maxima for the β-sheet (1615 cm^{-1}) and α-helix/random coil (1655 cm^{-1}) motifs, above a baseline drawn from 1730 to 865 cm^{-1}, giving the intensities I_β and I_α respectively. Spectral deconvolution showed an intensity measured at 1615 cm^{-1} (I_β) to be composed largely (50%–70%) of the β-sheet contribution, and similarly the intensity at 1655 cm^{-1} (I_α) to arise predominantly (50%–60%) from the α-helix component. It is then possible to define an ATR crystallinity index, X, as the nominal ratio of these intensities: $X = I_\beta/I_\alpha$.

Similarly, for amide II, crystallinity index $X_{(\text{amide II})}$ could be obtained by exploiting intensities measured at 1510 and 1545 cm^{-1}(assigned to the β-sheet and α-helix motifs, respectively); and for amide III, crystallinity index $X_{(\text{amide III})}$, at 1260 and 1230 cm^{-1} (assigned to the β-sheet and α-helix motifs, respectively).

In looking for microstructural information it is appropriate to measure the intensity of a band associated with a particular structural motif, comparing this with band intensity independent of the secondary or tertiary protein structure. Amide I was used as the reference band [6] to observe the changes of particular structural motif.

3.2.1 Heat aging

For silk samples with and without sericin aged under 125 °C, only $I_{\text{amide III}}/I_{\text{amide I}}$ changed with aging time, as illustrated in Figure 4.

Degummed samples decrease linearly at rate 0.00128, while ungummed samples at rate 0.000806.

3.2.2 365 nm aging

There is no evident change in $X_{\text{amide III}}$ for both ungummed and degummed silk. $X_{\text{amide I}}$, $X_{\text{amide II}}$, $I_{\text{amide II}}/I_{\text{amide I}}$, $I_{\text{ala}}/I_{\text{amide I}}$, $I_{\text{tyr}}/I_{\text{amide I}}$ and $I_{\text{amide III}}/I_{\text{amide I}}$ decrease with the exposure time for both ungummed and degummed silk.

For $X_{\text{amide I}}$, $X_{\text{amide II}}$ and $I_{\text{amide II}}/I_{\text{amide I}}$, the decrease extents and rates of degummed and ungummed silk samples are almost same, as is shown in Figure 5. The smaller crystallinity index is indicative of a change in short-range order, a disruption of the local environment of the atoms responsible for the infrared peaks. The decreases in crystallinity indices show the harmful light could damage the crystalline part which is usually think relatively stable.

For $I_{\text{ala}}/I_{\text{amide I}}$, $I_{\text{tyr}}/I_{\text{amide I}}$ and $I_{\text{amide III}}/I_{\text{amide I}}$, the degummed silk samples decrease faster than ungummed silk samples,

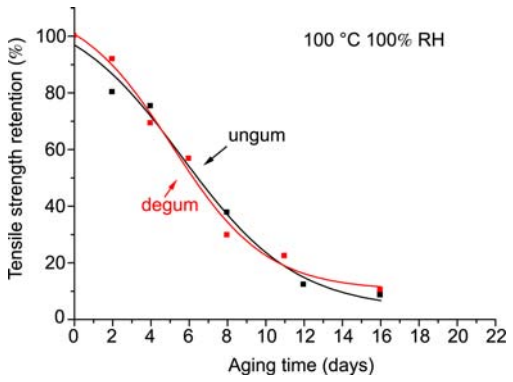

Figure 3 Tensile strength retention as a function of aging time in 100 °C 100% RH.

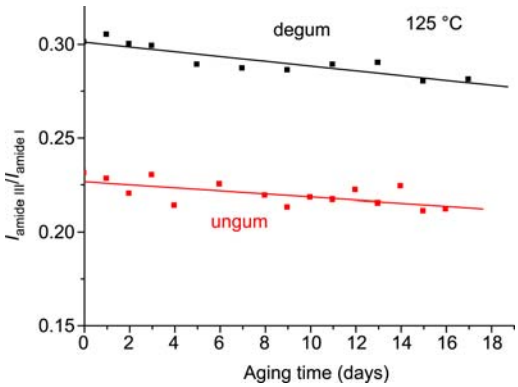

Figure 4 $I_{\text{amide III}}/I_{\text{amide I}}$ of samples heated at 125 °C.

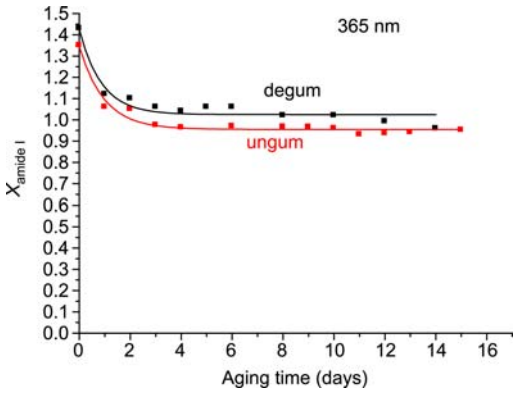

Figure 5 $X_{amide\ I}$ of samples exposed to UV-light.

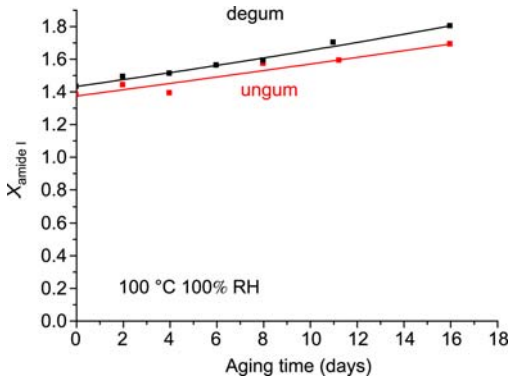

Figure 8 $X_{amide\ I}$ of samples exposed to 100℃ 100% RH.

as is shown in Figure 6 and Figure 7. The content of tyrosine in sericin is 4.9%, while in fibroin 11.8% [11]. So for degummed silk samples, it is expectable a significantly decrease in light-sensitive tyrosine. Apparently, sericin provides some extent of protection from light aging.

3.2.3 100 ℃ 100% RH

$X_{amide\ I}$, $X_{amide\ II}$ and $X_{amide\ III}$ increase with aging time at almost the same rate for both degummed and ungummed samples, as is shown in Figure 8. This means that the amorphous parts of silk samples were affected in high moisture environments and conformational changes in the molecular chains occurred, resulting in the increase in crystallinity.

For $I_{amide\ II}/I_{amide\ I}$ and $I_{amide\ III}/I_{amide\ I}$, the degummed silk samples decrease with aging time, while ungummed samples change slightly, as is shown in Figure 9.

The resolving of sericin that contains less alanine and tyrosine in water vapors results in the increase of alanine and tyrosine in ungummed samples. After 8 days, the contents of alanine and tyrosine in ungummed samples are almost same as those of degummed samples, and keep unchangeable, as is shown in Figure 10.

Comparing the results of light, heat and high moisture

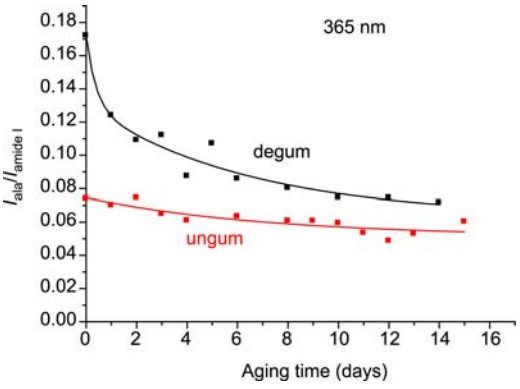

Figure 6 $I_{ala}/I_{amide\ I}$ of samples exposed to UV-light.

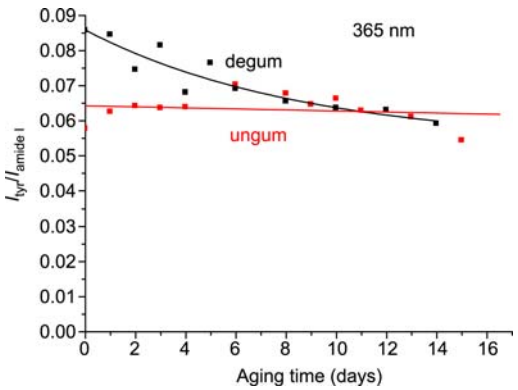

Figure 7 $I_{tyr}/I_{amide\ I}$ of samples exposed to UV-light.

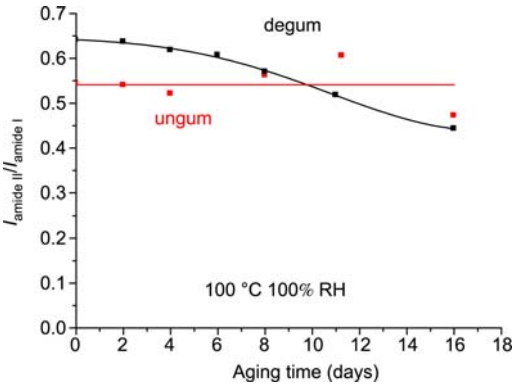

Figure 9 $I_{amide\ II}/I_{amide\ I}$ of samples exposed to 100 °C 100% RH.

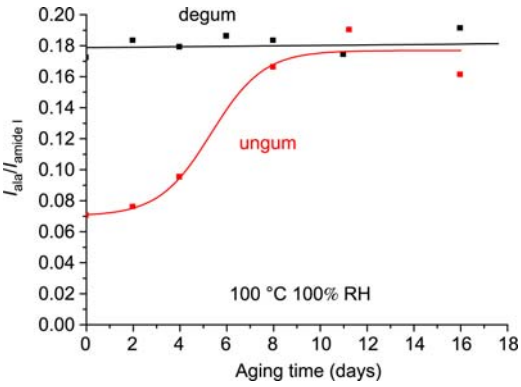

Figure 10 $I_{ala}/I_{amide\ I}$ of samples exposed to 100 °C 100% RH.

aging, it is found that alanine and tyrosine very sensitive to light, but not to heat and water.

The amide I band arises principally from the –C=O carbonyl stretch with a small contribution from N–H in-plane bending of the peptide group. The amide II band arises principally from N–H bending with a minor contribution from the C–N stretching vibration. The amide III band results from a combination of N–H bending and C–N stretching of the peptide group. So the decrease of $I_{\text{amide II}}/I_{\text{amide I}}$ and $I_{\text{amide III}}/I_{\text{amide I}}$ means the damage of peptide chemically. While increase of $I_{\text{amide III}}/I_{\text{amide I}}$ for ungummed silk samples aged in 100 °C 100% RH may be indicative of water effect on –C=O and N-H because of forming hydrogen bonds.

Most archaeological silk objects usually were unearthed from environments without light but with high humidity. The ATR results suggest that the crystallinity index of amide I, amide II, and amide III can be used as evaluation of conservation condition for objects without light damaging. Of course they also could be used as tracing the storage condition of silk without harmful light damages.

If the crystallinity indices ($X_{\text{amide I}}$, $X_{\text{amide II}}$ and $X_{\text{amide III}}$) are plotted against breaking load, for the high moisture aged silk samples, it can be seen that there is a good correlation, with crystallinity indices increasing as the fibres age and become physically weakened, as illustrated in Figure 11.

In fact most silk objects, including both archaeological and historical ones, are degummed. For degummed silk $I_{\text{amide III}}/I_{\text{amide I}}$ is very useful for deterioration evaluation, as is shown in Figures 12 and 13. Plots of the logarithm of the strength retention as a function of $I_{\text{amide III}}/I_{\text{amide I}}$ are shown in Figure 13. The plots are linear suggesting that the degradation is first order. Thus, the loss in strength is proportional to the $I_{\text{amide III}}/I_{\text{amide I}}$. There is a very good correlation between tensile strength and $I_{\text{Amide III}}/I_{\text{Amide I}}$.

3.3 The effect of sericin on silk fiber

Clearly, sericin can provide some extent of protection to silk fiber from light and heat aging.

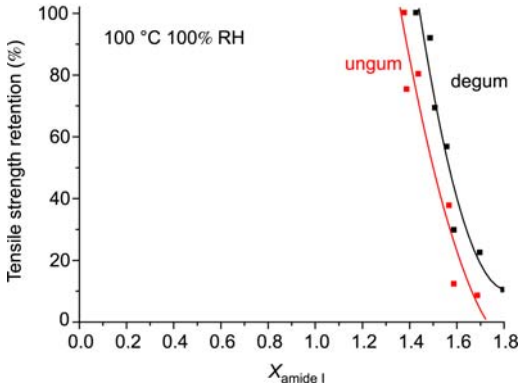

Figure 11 Relationship between $X_{\text{amide I}}$ and tensile strength for samples exposed to 100 °C 100% RH.

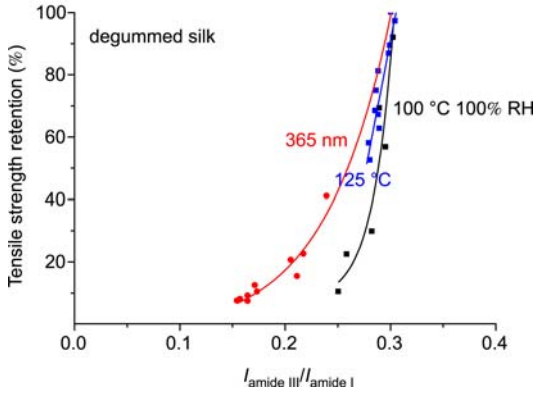

Figure 12 Relationship between $I_{\text{amideIII}}/I_{\text{amideI}}$ and tensile strength for degummed silk.

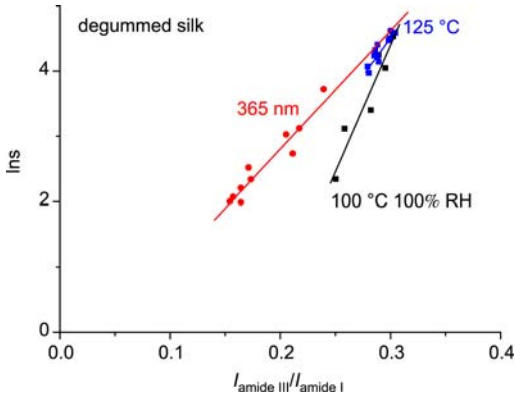

Figure 13 Logarithm of tensile strength retention as a function of $I_{\text{amide III}}/I_{\text{amide I}}$ for degummed silk.

According to researches [12, 13], sericin has a high antioxidation, antibacterial cryoprotective activity. Sericin functions as an antioxidant defense barrier against oxidative stress in order to protect the cocoon and silkworm and, as do other antioxidants such as ascorbic acid and kojic acid, inhibits the activity of tyrosinase, which is responsible for the browning of various foods and the biosynthesis of skin melanin. It is speculated that the abundant hydroxy groups in sericin might be involved in its antioxidant action by stopping the oxidation reaction of free radical and inhibit microorganisms growth leading to numerous diseases.

So sericin's antioxygenic property should also contribute to the protective effect of sericin besides its screening effect, especially in the condition of heat aging.

High moisture could result in the reduction of sericn because of dissolving. So for sericin-coated silk, not only wet cleaning is harmful, but high RH environment also detrimental.

4 Conclusions

Sericin can provide some extent of protection from light and heat aging for silk fibroin. In high humidity environments,

however, degummed and ungummed silk aged at the same rate because of leaching of sericin. Silk fibroin degraded at faster rate and more extensively in a moist environment.

ATR gave very useful information about the aging of silk. Crystallinity index is good at tracing the aging factor and conservation extent of silk. For silk samples with and without sericin aged under 125 °C, $I_{\text{amide III}}/I_{\text{amide I}}$ decreased with longer aging time. For light aging, $X_{\text{amide I}}$, $X_{\text{amide II}}$, $I_{\text{amide II}}/I_{\text{amide I}}$, $I_{\text{ala}}/I_{\text{amide I}}$, $I_{\text{tyr}}/I_{\text{amide I}}$ and $I_{\text{amide III}}/I_{\text{amide I}}$ decreased with increased exposure time for both ungummed and degummed silk.

In a high humidity environment $X_{\text{amide I}}$, $X_{\text{amide II}}$ and $X_{\text{amide III}}$ increased with aging time at almost the same rate for both degummed and ungummed samples. The amorphous parts of silk samples were affected in high moisture environments and conformational changes in the molecular chains occurred, resulting in the increase in crystallinity. The intensity ratios $I_{\text{amide II}}/I_{\text{amide I}}$ and $I_{\text{amide III}}/I_{\text{amide I}}$, decrease with aging time for the degummed silk samples, but change little for the ungummed samples change. The crystallinity indices of amide I, amide II, and amide III could be used to evaluate the conservation condition for objects without light damage.

The silk fibroin content of the aminoacids alanine and tyrosine, as determined by ATR spectroscopy, was very sensitive to light, but not to heat and water.

$I_{\text{amide III}}/I_{\text{amide I}}$ is potentially very useful for evaluating the state of deterioration of archaeological silk objects.

Sericin is quite easily leached from silk by moistuire, so wet-cleaning is inappropriate for historic sericin-coated silks, and, furthermore, storage and display in a high RH environment will also prove detrimental.

This work would not have been possible without the support of all our colleagues at the Textile Conservation Center and the School of Archaeology and Museology, especially Prof. YUAN SiXun, who was an invaluable mentor.

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