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Original article

Heat and moisture promoted deterioration of raw silk estimated by amino acid analysis

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ABSTRACT

Silks are amongst the most precious artifacts of our textile heritage. While most silk fabrics are made from degummed silk, some in collections, such as ancient Chinese Juan, are composed of raw or just partially degummed silk. Such silks with remnant sericin gum may require special consideration for their optimum preservation. Conservators and curators then need to know which of the silks in their collections are in this category, and what effect the sericin has on the ageing of silk fibres. In the study reported here, the artificial heat and moisture ageing of raw silk was investigated by means of amino acid analysis. Amongst various amino acid molar ratios, Asp/Gly proved a valuable indicator of residual sericin gum on aged silk. Measurement of the tyrosine content was also useful for gauging the conservation state of silk in some circumstances. The results of the amino acid analyses when combined with those for tensile tests suggested that sericin did not inhibit fibroin deterioration under the ageing conditions employed.

1. Research aims

Silk is an animal fibre, produced by caterpillars belonging to the genus *Bombyx*, and much commercial silk comes from the domesticated form *Bombyx mori*. Before the pupal stage, the silkworm wraps itself in a protective protein secretion from two large glands in its head. This liquid crystalline fibroin hardens upon extrusion and exposure to the air. The resulting filaments are cemented together by a second protein secretion, sericin, forming the cocoon. [1] In sericulture, the pupa is killed in the cocoon by steam or hot air before metamorphosis. Subsequent boiling of the cocoon serves to soften the hardened sericin so that the silk filaments can be unwound [2].

The majority of raw silk is then subjected to a degumming process in which the sericin gum is removed. This involves treatment with aqueous alkali (calcium oxide or plant ash) or lytic enzymes (e.g. from hog pancreas, white gourd) [2]. The fabric woven from degummed silk has a much improved sheen, colour, handle, and texture, and the majority of the silk artefacts in textile collections (costume, banners, furnishing, etc.) were constructed from it. However, some historic fabric was also woven from raw silk. A case in point is Chinese Juan [3], which was a substrate for written documents and drawings before paper was invented, and which was also used for mounting pictures. While relatively uncommon, some archaeological silk fibres and pseudomorphs excavated from tombs in South China [4] and Mongolia [5], for example, have been shown to be sericin rich, and probably came from raw silk fabric. There are also a few more recent examples of sericin-coated silks in collections, such as the US. first ladies gowns in the Smithsonian institution [6].

Besides its historical significance, the presence of the remnant sericin gum may have a consequence for the preservation of silk artefacts. Becker et al. [6], monitoring the increasing solubility and decreasing tyrosine content of light-aged silk, concluded that sericin can protect silk fibroin against light-induced damage. Zhang (Y.H.) and co-workers, through the application of environmental scanning electron microscopy, have confirmed that sericin coated fibres behave differently upon light ageing, adopting unique morphologies which are characterised by the distinct shapes of fissures and lacunae [7]. Heat ageing also seemed to induce this singular surface b damage. Until now, though, there appear to have been no detailed reports concerning the condition and molecular composition of raw silk fibres after controlled heat and moisture promoted deterioration. This is of particular relevance, as these factors may be dominant in promoting the ageing of silk within historic collections. Furthermore, the results of such analyses could suggest the burial conditions under which archaeological raw silk might best survive.

Following on from our previous work on the detection of sericin by instrumental methods [5,8], we have now investigated the heat

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and moisture ageing of raw silk, in comparison to light ageing. Elsewhere we report the results of tensile testing and FTIR-ATR spectroscopy of the artificially aged raw silk, [9] and here present the outcomes of complementary amino acid analyses.

2. Experimental

2.1. Ageing of silk surrogates

Commercial raw *B. mori* silk fabric and the same degummed fabric were obtained from the Suzhou Shanshui Silk Co., China. The samples were cut into 2.5 × 10 cm strips, with the weft along the length, for artificial ageing.

2.1.1. Dry thermal ageing

Conditions were selected to degrade the raw and degummed test fabrics to a range of strengths. The fabric test strips were placed on enamel plates in a forced convection oven, and exposed for up to 16 days at 125 °C, with samples removed at zero, two, four, eight, 12 and 16 days.

2.1.2. High humidity thermal ageing

Silk strips were put in glass bottles with a small test tube containing 2 ml water in each. The bottles were sealed with caps and put in a forced convection oven. The silk samples were aged at 100 °C, 100% RH, with samples removed at zero, two, four, eight and 16 days. Using the rate data and assumptions recently presented by Luxford, at 16 days this artificial ageing regime would equate to around 30 years under the conditions generally encountered for textiles on open display [10].

2.1.3. Light ageing

Silk strips were exposed to ultraviolet radiation in a light-ageing chamber maintained at 38 °C and RH 10 ± 5%. The 365 nm irradiance at the specimens was 20 W/m^2/nm. Samples were removed at zero, one, three, nine, and 16 days. At 16 days, the exposure is equivalent to around one year under continuous intense sunlight.

2.2. Tensile strength measurements

The protocol for tensile testing was adapted from BS EN ISO 13934-1:1999 for textile strips. The samples were conditioned for at least 72 h at 20 ± 2 °C and 55 ± 5% RH. Tests were then performed with strip gauge lengths of 2.0 cm and a crosshead speed of 2 mm/min on an Instron 5544 instrument. Six replicates for each sample were analyzed, and average values calculated.

2.3. Amino acid analysis

Calibrated amino acid analysis at the sub-microgram level was performed according to the procedure reported previously [5]. Microgram samples of the silks were hydrolysed with HCl vapour for 20–24 hours at 110–120 °C in a custom made vessel. Subsequently each sample was dried and the residue dissolved in 100 μl 0.1 N HCl. Amino acid analysis, by the Waters AccQ.Tag method, was then carried out using a Waters high-performance liquid chromatography system with fluorescence detection of the amino acid derivatives.

3. Results and discussion

3.1. Tensile testing

The tensile strengths of the unaged raw and degummed silk strips were 321 ± 9 N and 236 ± 3 N respectively. The degumming process can be quite deleterious, damaging silk fibroin [11]. The lowered tensile strength of degummed silk probably reflects this damage, rather than a significant contribution from sericin in raw silk. The further effects of the three ageing regimes on the deterioration of the silks, as measured by the residual tensile strengths, are shown in Fig. 1. As we have already noted, [9] in each case the two types of silk show comparable changes in performance, although for light ageing the residual strength of raw silk tails off to around twice that for degummed silk. This may be related to the initial partially cleaved state of fibroin in the unaged degummed silk.

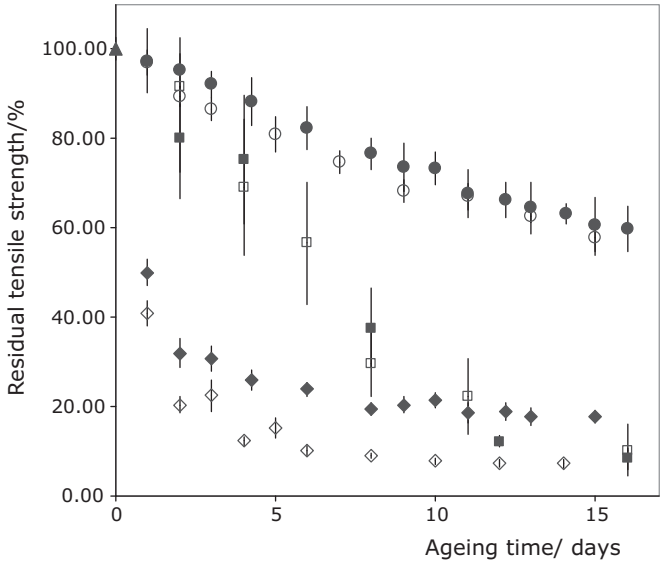

Fig. 1. The residual tensile strengths of raw (solid symbols) and degummed (open symbols) silk during dry thermal (circles), high humidity thermal (squares) and UV light (rhombi) ageing. For each silk the tensile data is expressed relative to the value for the unaged silk (triangles). The standard error bars reflect the higher variability for the tensile data from the silks subjected to high humidity thermal ageing.

3.2. Amino acid analysis

3.2.1. Detection of sericin on silk

The amino acids found in raw and degummed silk are tabulated (Table 1). The experimental data for the unaged silks are entirely consistent with previous literature reports [5,12]. Glycine, alanine, serine and tyrosine predominate in silk fibroin, while the hydrophilic residues serine, threonine, aspartic acid and glutamic acid are the main components of sericin [12]. The 20% sericin content then moderates the overall amino acid composition of raw silk [8].

We have suggested before that the relative molar content of glycine and alanine can be used to infer the presence of sericin on silk [5]. These two amino acids were chosen as they are generally considered the more stable. For the various silks, the molar ratios of alanine, serine, tyrosine, threonine, aspartic acid and glutamic acid to glycine are also tabulated (Table 2). While the Ala/Gly ratio differentiates the unaged silks, it does not appear to be the best sericin marker for silk subjected to the artificial ageing regimes. Rather the Ser/Gly, Thr/Gly and Asp/Gly ratios are the more sensitive and somewhat better indicators. Although within the bounds of experimental error, both dry thermal ageing and UV light ageing of raw silk resulted in consistent increases in the Ala/Gly ratio over the ageing time courses (perhaps due to the transformation of the side chains of some other amino acids to -methyl), whereas the Ser/Gly, Thr/Gly and Asp/Gly ratios were almost constant. No diminution in the initial sericin content would be expected under these ageing conditions. In contrast, the Ser/Gly, Thr/Gly and Asp/Gly ratios sug-

Table 1
Amino acid compositions of both unaged and aged, raw and degummed silks. Data is given for the final point in each ageing time course; the standard errors are ± 6% of the values.

Silk/ ageing regime		Amino acid content/ mole %							
		Asp	Ser	Glu	Gly	His	Arg	Thr	Ala
Raw	Unaged	4.1	14.2	1.8	40.8	0.8	0.9	2.2	24.3
	A	4.2	13.7	1.8	40.3	0.8	0.8	2.2	26.0
	B	1.4	9.6	1.0	45.6	0.6	0.3	0.8	31.1
	C	4.0	14.0	1.7	41.1	0.7	0.9	2.0	26.4
Degummed	Unaged	1.8	10.5	1.4	43.8	0.7	0.4	1.0	29.9
	A	1.9	10.6	1.4	43.7	0.6	0.4	0.9	30.6
	B	0.8	9.5	0.8	46.6	0.5	0.3	0.6	32.0
	C	1.8	10.4	1.3	44.5	0.5	0.5	0.9	31.6
		Pro	Tyr	Val	Met	Lys	Ile	Leu	Phe
Raw	Unaged	0.6	4.9	2.5	0.1	0.7	0.7	0.7	0.7
	A	0.7	4.4	2.5	0.1	0.5	0.7	0.7	0.6
	B	0.7	4.7	2.3	0.1	0.2	0.6	0.5	0.5
	C	0.7	3.2	2.5	0.2	0.6	0.7	0.6	0.6
Degummed	Unaged	0.8	5.1	2.2	0.1	0.3	0.7	0.5	0.7
	A	0.7	4.6	2.3	0.1	0.2	0.8	0.6	0.7
	B	0.7	4.7	2.2	0.1	0.1	0.5	0.3	0.4
	C	0.8	3.3	2.3	0.1	0.2	0.7	0.5	0.6

Ageing regimes: A – thermal, B – high humidity thermal, C – UV light.

Table 2
Selected amino acid molar ratios for both unaged and aged, raw and degummed silk. Data is given for the final point in each ageing time course; the standard errors are ± 12% of the values.

Silk	Ageing	Amino acid molar ratios					
		Ala/Gly	Ser/Gly	Tyr/Gly	Thr/Gly	Asp/Gly	Glu/Gly
Raw	Unaged	0.60	0.35	0.12	0.05	0.10	0.04
	A	0.65	0.34	0.11	0.05	0.10	0.04
	B	0.68	0.21	0.10	0.02	0.03	0.02
	C	0.64	0.34	0.08	0.05	0.10	0.04
Degummed	Unaged	0.68	0.24	0.12	0.02	0.04	0.03
	A	0.70	0.24	0.11	0.02	0.04	0.03
	B	0.69	0.20	0.10	0.01	0.02	0.02
	C	0.71	0.23	0.07	0.02	0.04	0.03

Ageing regimes: A – thermal, B – high humidity thermal, C – UV light.

gest that sericin is completely lost over the course of high humidity thermal ageing. This is consistent with our FTIR-ATR studies which also indicate that sericin is gradually leached from raw silk under these conditions [9]. In comparison, although the outcomes of the parallel spectroscopic studies allowed confident predictions of the sericin content for unaged silks, [8] in the case of aged silks the amino acid data is far more definitive.

3.2.2. Deterioration of silk

The tabulated molar ratios also highlight the particular susceptibility of tyrosine to oxidative thermal and UV light ageing. The latter, for instance, effected decreases of the tyrosine content from 4.9 ± 0.3 to 3.2 ± 0.2 mole %, and from 5.1 ± 0.3 to 3.3 ± 0.2 mole %, for raw and degummed silk respectively. This result is contrary to that deduced from the FTIR-ATR spectra, which suggested that the tyrosine level of raw silk changed little [9]. The amino acid analysis would seem to be incontrovertible, and again appears to be the more reliable technique for this particular investigation.

Simple calculation shows that the resultant levels of tyrosine determined in raw silk by amino acid analysis are just those that would be expected if it was modified at the same rate as in degummed silk. So, although sericin has been shown by others to have antioxidative action [13,14], according to the analytical and tensile data presented here, it does not seem to markedly moderate the deterioration of fibroin under the oxidative artificial ageing conditions employed in this research. This may be because the ageing times are extreme in relation to those relevant to the natural protection of the silkworm cocoon.

The tyrosine content of raw silk is plotted against tensile strength retention in Fig. 2, and there is a good correlation, which parallels that for degummed silk. The mole percentage tyrosine content decreases as the silk fibres become physically weakened, with the trend for the dry thermal and UV light ageing regimes apparently being the same. This may suggest a common oxidative process, in which tyrosine modification precedes condition-related peptide cleavage [1]. So tyrosine could be a useful marker for the conservation state of raw silk as well as degummed silk, especially when the silk has been kept in an environment where the main ageing factors are heat and light.

The interpretation of the data from moist thermal ageing in Fig. 2 is complicated by the gradual leaching of sericin gum from raw silk. Nonetheless, it is obvious that there is a quite different trend. In this instance hydrolytic cleavage of the proteins is dominant and performance drops rapidly relative to small changes in the tyrosine content. For degummed silk changes in the acidic, basic and hydroxylated amino acid residues are the most significant, presumably consequent upon peptide hydrolysis and leaching of amino acids and small peptides from the amorphous regions of fibroin. The changes observed in the aspartic acid content for all the silks are plotted against the residual tensile strengths in Fig. 3. The plot emphasises the value of aspartic acid for detecting sericin on silk, with a level of around 4 mole % for raw silk in the absence of a

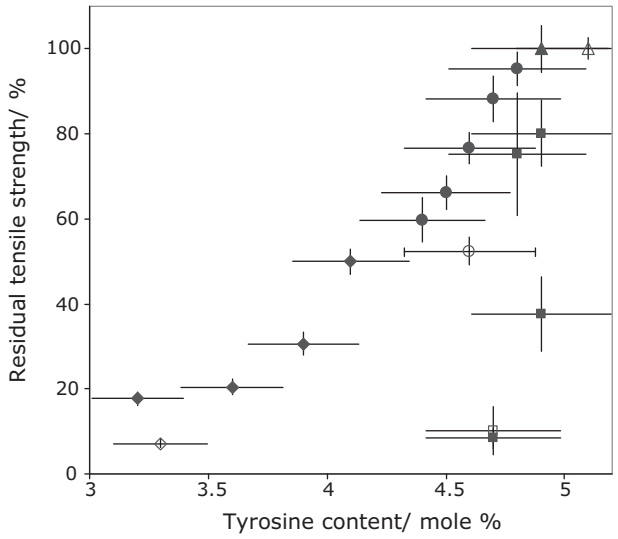

Fig. 2. Plot showing the changes in the tyrosine content (mole %) for raw silk (solid symbols) and degummed silk (open symbols) versus the residual tensile strength upon dry thermal (circles), high humidity thermal (squares) and light (rhombi) ageing. The data points for the unaged silks are triangular. Standard error bars are shown.

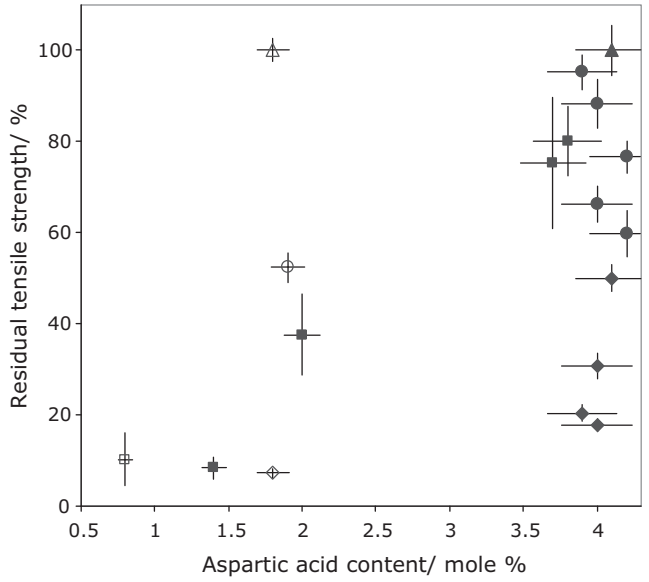

Fig. 3. Plot showing the changes in the aspartic acid content (mole %) for raw silk (solid symbols) and degummed silk (open circles) versus the residual tensile strength upon dry thermal (circles), high humidity thermal (2,4,8,16 days - squares) and light (rhombi) ageing. The data points for the unaged silks are triangular. Standard error bars are shown.

moist environment and just below 2 mole % for degummed silk. The data suggests that after eight days thermal ageing at high humidity nearly all the sericin has been removed.

Furthermore, for raw silk, during high humidity thermal ageing, the level of remaining aspartic acid correlates remarkably well with the residual performance. This may be a fortuitous result though, the values for both parameters perhaps simply reflecting the exposure time. At the extreme time point the aspartic acid level for raw silk is significantly higher than that for degummed silk, although the tensile strengths are similar. The levels of serine, threonine and glutamic acid tell a similar story. Under such an environment then, sericin seems not to protect fibroin against condition-dependent hydrolytic cleavage, as reflected by the drop in tensile strength, but it may reduce amino acid or peptide loss.

4. Conclusions

In ancient times raw silk cloth was used as a precursor to paper, and some silk fibres excavated in China have been shown still to contain sericin gum. Even more recent fabrics in historic textile collections may have been fabricated from unprocessed or just partially degummed silk.

Amino acid analysis of sub-microgram samples of the silks can serve to identify those fibres with a sericin coating, and offers a more reliable approach for categorising aged silks than FTIR-ATR spectroscopy. The aspartic acid to glycine molar ratio is a particularly useful marker for aged raw silks. This parameter confirms that sericin may be leached from silk under high humidity conditions, suggesting that ancient fibres with an intact sericin coating can only be expected to be recovered from arid burial environments.

For such silks, which have aged under dry conditions by thermally driven oxidation, the tyrosine content is a useful indicator of their conservation state, where moisture driven hydrolysis is the dominant deterioration mechanism. However, this is not a reliable indicator.

There is no definitive evidence from this research for a protective role for sericin on raw silk. The fibroin in raw silk appears to be cleaved as readily as that in degummed silk upon artificial dry thermal, high humidity thermal and UV light ageing.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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