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SILK FIBRE DEGRADATION AND ANALYSIS BY PROTEOMICS

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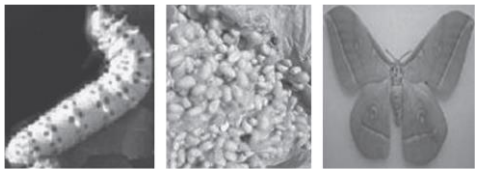
Abstract: *Silk is one of the promising natural fibres* and *has a long established history in textile production throughout the centuries. Silk is produced by cultured silk worms, spiders, scorpions, mites and flies. It is extracellular proteinaceous fibres which consist of highly crystalline and insoluble proteins, the fibroins glued with sericin and an amourphous protein. On the other hand, understanding and controlling the degradation of protein materials are important for determining quality and the value of appearance retention in textiles. Hence, for silk textiles, appearance retention is critical value for the quality. And this is one of the key properties directly related to the degree and nature of protein degradation. It is therefore necessary to understand the silk composition and damage to obtain good conservation treatments and long-term preservation especially for the historical silk fabrics. In this study, silk fibre and its properties are briefly introduced along with images on their fibre damages. Additionally, proteomics method which helps to understand the degradation at the molecular level in textiles is introduced. Finally, proteomic evaluation of silk is summarized according to the researchers carried out in the literature.*

Key words: *Silk, Properties, Damage, Proteomics, Mass spectrometry*

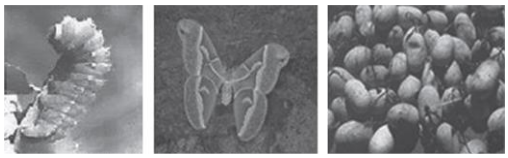
1. INTRODUCTION

Silk is an ancient and the only natural filament fibre which is used for thousands of years and nowadays the major producers are China, India and Japan. Silk is a fine, strong continuous fibroin filament with its lustre and excellent mechanical properties. It is produced by cultured silkworms and is a fibrous protein synthesized in specialized epithelial cells that line glands in the class of *Arachnida* and in several worms of the *Lepidoptera* larvae such as silkworms and insects as spiders, scorpions, mites and flies [1]. Some types of silk are suitable for commercial textiles due to the amount of silk that can be produced. These insects include the domesticated silkworm (*Bombyx mori*) and the wild silk worm i.e. *Eri* (Figure 1), *Tasar (Figure 2)* and *Muga (Figure 3)* [2].



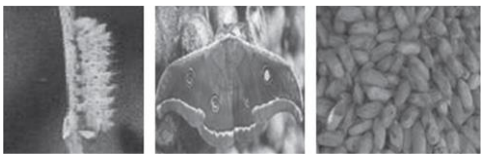
(a) (b) (c)

Fig.1: *Eri silk: (a) worm, (b) cocoons and (c) moth*



a) (b) (c)

Fig. 2: *Tasar silk: (a) worm, (b) moth and (c) cocoons*



a) (b) (c)

Fig. 3: *Muga silk: (a) worm, (b) moth and (c) cocoons*

The silk fibres of the *B.mori* silkworm are composed of a fibrous core twin thread of protein fibroin (70-80%) with sericin (20-30%) along with other impurities such as wax, colour pigments and inorganic components [3]. Whereas wild silk has higher fibroin content (80-90%) and lower sericin content (10-20%) than domesticated silk. Fibroin is the protein that forms the filaments of silkworm silk and gives unique physical and chemical properties to the fibre. On the other hand, sericin is consisting of group proteins which bring the fibroin filaments together and imparts strength to the cocoons. Silk sericin and other impurities cause hardness, coarseness and cover the lustre of silk fibroin. Hence, removing of sericin and other impurities is necessary because it provides soft, shiny and whitened silk fibres ready to be dyed.

For the silk textiles, appearance retention is critical value for the quality which is one of the key properties directly related to the degree and nature of protein degradation. It is therefore necessary to understand both silk properties and degradation which leads to a better conservation treatments and long-term preservation especially for the historical silk fabrics.

2. PROPERTIES OF SILK

As mentioned earlier, there are two main types of silk fibre: cultivated and wild. Both differ in diameter, cross-sectional shape and in fine structure. Its length is about 300-700 m; but generally is 300 m. and it has a fine diameter of 12-30 μm [4]. Because of its beauty, its handling and its high cost, silk is also known as a luxury fibre. Hence it remains its use both in various textiles i.e. fabrics, underwear, robes, socks, leggings, shirts, ties, blouses, formal dresses, high fashion clothes, folk costumes, furnishing applications, upholstery, rugs, beddings and more recently surgical sutures and etc. In the past, one of the elegant silk textiles was also used in caftans (see Figure 4) by several cultures in the world and these historical chic textiles must be well preserved for the next generations. And this can be achieved by understanding the fibre damages in textiles and advents of analysis methods, i.e. proteomics, for the degradation. Prior to the advent of proteomics method let's give a summary on the properties of silk, first.

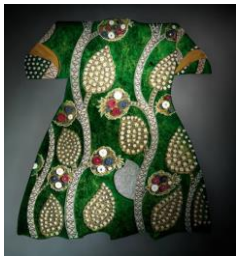


Fig. 4: *A silk caftan worn by Ottoman Sultan Süleyman [5]*

Silk fibres from the *B.mori* silkworm have a more or less irregular triangle cross-section with rounded corners (5–10 μm wide) and the density of *B.mori* silk is between 1.30-1.37 g/cm^3 [6]. Silk is more hygroscopic than cotton and can absorb up to 30% of its mass. Also, silk is one of the best natural fibres for its high strength and elongation at break result in higher fracture energy. It shows large initial modulus higher than that of nylon and wool but less than flax, polyester and aramid [7]. Although silk is a strong natural fibre, it loses up to 20% of its strength when wet. It has a good moisture regain of 11%. It can be weakened if exposed to too much sunlight and may also be attacked by insects, especially if left dirty. Silk is a poor conductor of electricity and thus susceptible to static cling. On the other hand, silk proteins consists of chain of amino acids, each of which is built of four groups: an amine group (-NH_2), a carboxyl group (-COOH), a hydrogen group (-H). These groups are bound to a carbon molecule designated as the α-carbon. The fourth group of each amino acid, the "R" group varies. The diversity of silk proteins is derived from these distinctive "R" groups [8]. Although acids and alkalis decompose warm silk easily, diluted acids revive the colour and increase the crispness of the silk. Diluted organic acids, tartaric acid and citric acid are used in finishing silk fabrics, whereas concentrated acids destroy silk. The concentrated solution of certain organic acids (i.e. formic acid) and 37% hydrochloric acid can cause dissolution and damage in proteins [9]. The sericin is soluble in hot soapy water or slightly alkaline solution while fibroin is insoluble. When silk fibre being heat-treated, it looses its weight gradually at 175^{o}C and its colour changes to black at 250^{o}C [10]. Silk is tolerant to burning, emitting a smell of burnt horn and leaving a black carbon residue. Silk resists heat better than wool and is a good thermal insulator.

3. SILK DEGRADATION

Understanding and controlling the degradation of protein materials are important for determining quality and the value of appearance retention in textiles such as silk materials. Protein oxidative damage is implication of decreased performance and degradation in proteinaceous materials such as silk, wool, even in reduced food quality, loss of enzyme activity etc [11-12]. For proteins, oxidative damage is generally attributable to the generation and attack of reactive oxygen species on both amino acid residue side chains and the protein backbone itself. Therefore for silk textiles, in particular the colour and mechanical properties of the material are critically influenced by the oxidative damage at the molecular level. Especially, both archaeological and historic silk fabrics that have survived to nowadays have been undergone some modifications in their physical appearance and chemical structure. Even though the damage cannot be entirely stopped, a good understanding of the textile composition and degradation can conduct to better conservation treatments and long-term preservation.

As silk is made of protein, it is at risk to damage by heat, humidity, light, microorganisms, chemicals and pollutants. And the processing of silk textiles involves degumming, weighting, dyeing and finishing which all these agents may break polymer molecules or cause other changes in their structure. Thus, all these processes weaken and degrade the fibre itself. Furthermore, fibre damage

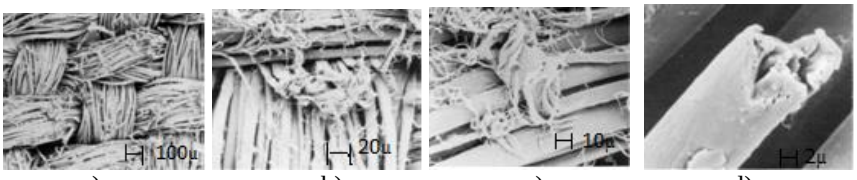
can be caused by light and other environmental factors as well. Microorganisms are also another problem for the archaeological silk textiles which must be understood to correlate damage of the fibre where it has been buried. Insects have also been a problem in museums and must be carefully monitored.

3.1 Examples on Degraded Fibres

As mentioned earlier that natural textile fibres are vulnerable to damage and degradation. Therefore, it is not surprising that in many archaeological contexts textiles do not survive. Fortunately attack by the majority of biological opponent is nearly eliminated if one or more of the following conditions endure: (1) absence of water; (2) temperature less than 5°C and (3) absence of air. Most of the significant collections of archaeological textiles have been preserved by such conditions. As a consequence, textiles rarely survive their useful lifetime. Even though, those textile collections in museums, where despite careful attention being taken are yet continue to degrade; because of on display they suffer photo-degradation and even suffer from insect damage. And in today's cities they become progressively more acidic.

In this section, fibres are in varying degrees subject to attack by light, heat and chemicals. So, differing forms of damage produce changes in morphology which are given below as some examples recognized by SEM images for fibre degradation.

In silk; the tensile strength, fibre cross-section and cocoon length are all influenced by the production condition and diet of the silk larva, and problems with either of these result in short cocoons and 'thin' filaments. Figure 5 shows wear damage in silk dress after 60 years; a) crown breakdown, b) fatigue breaks in weave, c) pilling and fibrillation, d) fibre fracture and rounding off.



a) b) c) d)

Fig. 5: *Wear damage in silk dress after 60 years [13]*

Some different archaeological environment has consistently surrendered with well-preserved textiles. The most widespread discovers have come from desert conditions, i.e. Egypt and Sudan, where the absence of water has prevented biological attack from the textile materials. The Northern European acid peat marshland has preserved many organic remains, including wood, animal and human cadavers, and textiles. Unfortunately acid conditions lead to acid-catalysed hydrolysis of cellulose, which eventually dissolves however consequently wool and silk survive, while linen, cotton, nettle and jute vanish [13].

4. PROTEOMIC EVALUATION OF SILK

In recent years “OMICS” technologies are one of the developing fields; before discussing proteomic method let us mention in brief what proteomics is: Genomics is a total practice for the description of information about genome and genes. Genomics studies provide assessment and interpretation of total genome of any cell or tissue. Proteomics is a discipline which obtains total information of proteins and its history goes back to 1975s. On the other hand, Marc Wilkins is the first PhD student who is working on the concept and suggested to use the “*Proteom”* term in a symposium in 1990’s and later he and his collegues [14] announced their work to scientists. The

word PROTEOM is a portmanteau of *protein* and *genome*. It can be defined as the entire set of proteins which are produced or modified by an organism or system. Proteomics is an interdisciplinary domain that has benefited greatly from the genetic information of the Human Genome Project [15]. While proteomics generally refers to the large range of experimental analysis of proteins, it is often and on the whole used for protein purification and mass spectrometry.

Silk degradation, especially photo-degradation has been studied for many years; however at the present the advent of the proteomic methods allows to examine the degradation in molecular level. Traditionally, fluorescence mapping and protein extractability [16] have been used to understand the silk degradation. But today, with the development and application of powerful new proteomic tools (i.e. mass spectrometry-MS) the protein photo-oxidation can be examined.

Proteomics, are known as an emerging field of life science research and can display, identify or characterize all the proteins in a given cell, tissue or organism. However in textiles, there are various researchers on proteomics; one which is Katagata [17] who separated the crystalline regions of fibroin by precipitation and digestion with α-chymotrypsin; 55% of the crystalline regions precipitate while the 45% remained in solution. Inoue et.al [18] characterized the glycoprotein P25 by SDS-PAGE (which is a technique to separate proteins by their molecular weight), Figure 6 shows the molecular markers in the left lane. In their work two bands were identified at 27 kDA and 24 kDa depending on the degree of glycosylation and N-linked oligosaccharide chains which were detected. When it was degummed, the heavy (350 kDa) and light fibroin (25 kDa) bands disappeared, showing only a smear at 100kDa. This indicates that degumming process damages the both heavy and light chains.

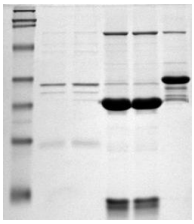


Fig.6: *Picture of an SDS (sodium dodecly sulfate)-PAGE (polyacrylamide gel electrophoresis) [19]*

MS techniques provide highly sensitive and powerful means to profile redox modifications at the molecular level within silk samples. MS has a distinct advantage over traditional tools. As a result; a redox proteomic approach consists of the following [20]:

- Digestion with a proteolytic enzyme to produce peptides,
- Separation of the peptides with appropriate liquid chromatography,
- Tandem mass spectrometric peptide fragmentation,
- Targeted bioinformatics evaluation for key redox products can provide detailed identification and localization of modification throughout the silk proteome.

In this way, oxidatively modified amino acid residues within the proteins can be accurately characterized through comparison of MS/MS data between native and degraded peptides.

5. CONCLUSION

The development and application of new technologies i.e. proteomic tools, may allow for identifying, mapping and tracking oxidative degradation at the protein amino acid residue level of the samples. Hence, implements in proteomics offer a better understanding of silk damage and degradation. We believe that, both with new technologies and advent of proteomics, textile fibres such as silk and other historical textile materials can be studied for their ageing and can help to identify their degradation.

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