Ultrasonics induced variations in molecular structure and tensile properties of silk fibers in a chemical free environment

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Abstract
Ultrasonics can be used as an alternative energy supply for a green silk wet processing and for enhancement of some chemical/physical properties of biopolymers. In this work, ultrasonically treated silk fabric were compared with that of untreated and were investigated for the changes in fiber conformation structure and subsequent tensile property. Experiments were conducted under different time duration in a chemical free environment. Fiber surface energy distribution and transformation of silk secondary structure were analyzed using X-ray photoelectron spectroscopy (XPS), energy dispersive spectroscopy (EDS), and Fourier transform infrared spectrum (FTIR). Results showed that subject to treatment time, a surface oxidation effect and a light transformation of random coil to \( \beta \)-sheet can be induced from ultrasonic irradiation. Tensile testing showed that under certain treatment time, ultrasonics can lead to silk fibers with a slightly increased strength and reduced extensibility, as a result of the transition of fiber secondary structure.

KEYWORDS
molecular structure, silk, surface oxidation, tensile properties, ultrasonics

INTRODUCTION
Silk has been used as textile material for centuries for its biodegradability, excellent moisture absorption, comfort, dye-ability, and good mechanical properties. Nowadays the application of silk has been extended to biomedical, cosmetics, functional membranes, optical applications, and food additives. Like other traditional textile wet processing, manufacturing of silk has undergone a heavy usage of water, chemical and power. In the past decades, application of ultrasound in silk wet processing, such as degumming, dyeing, laundering, drying,
have been broadly reported. In these studies, ultrasonics has been proved as an alternative energy supply in the process to reduce environmental impact.

In the sonic treatment, as cavitation bubbles explosively and abruptly collapsed in a liquid medium, there generates high temperature (instantly rise to 5000°C) and high pressure (reach up to 1000 atm).[12] The hot spot produced by collapsed bubble drives high-energy chemical reaction.

Silk is composed of amino acids, with hydrogen bond and salt linkages connecting between the polypeptide chains of the amino acids. Researches have demonstrated that ultrasonic irradiation can be involved in the transition of conformation structure of some protein fiber (such as wool), which can subsequently lead to changes in fiber mechanical properties.[13] A study has shown that the use of ultrasonics can enable the enhancement of chemical and physical properties of some bio-polymers.[14]

While much of the studies have focused on the green and environmentally friendly process of silk, little is reported on the effect of ultrasonic treatment on the mechanical properties of silk fibers. In terms of ultrasound induced changes in silk micro-structure, previous studies have been mainly conducted on the silk fibroin in the form of powder[15] and sol–gel.[16,17] Little is reported on using silk fiber/fabric, and on the way how changes in fiber molecular structure can affect fiber mechanical behavior. It is then decided to carry on the present work.

During silk wet processes, auxiliary chemicals are generally added to the processing bath which can to some extent, affect the silk conformation and mechanical properties. In this work, silk fabrics were treated with and without ultrasonics in a chemical free environment. Ultrasonic treatment at different time duration were investigated. XPS, EDS, and FTIR spectrum were used to examine fiber surface energy distribution and changes in secondary structure of silk protein as a result of ultrasonic treatment. Studies were also extended to fabric tensile behavior. Implications of the outcome of this study can be provided to the research into enhancement of chemical and physical properties of silk bio-polymer using ultrasonics, and to a green and energy sound silk wet process using ultrasonic technology.

### 2 EXPERIMENTAL SECTION

#### 2.1 Sample preparation

Undyed, scoured and degummed silk fabric (100% pure mulberry silk), weight 101 g m$^{-2}$, thickness 0.55 mm, ends and picks 106 × 61 per cm, satin weave, was sourced from local silk mill. Fabric was soaked and relaxed in water at room temperature for 1 hour and dried at 50°C for 1 hour before the experiment.

#### 2.2 Ultrasonic treatment

Ultrasonic treatment was conducted using 40 kHz digital control ultrasonic baths, with the capacity of 20 L and a maximum power of 130 W L$^{-1}$ (Haoshun Ultrasonic Instrument Co., China). Fabric samples with size of 260 × 220 mm were horizontally placed in bath without tension and with around 50 mm from the bottom to receive homogenized irradiation. Rising temperatures were recorded with treatment time in the ultrasonic bath (Table 1).

Fabric samples with the same size (used as a control) was treated at 45°C for 30 minutes in an electrical water bath tank (Jinyi Instrument Technology Co., Ltd, China). Deionized water was used in all the experiments. After treatment, samples were dried in oven at 50°C for 3 hours.

#### 2.3 Energy dispersive spectroscopy (EDS)

Surface elements of the sample were examined using Phenom Prox (Phenom Co., Netherlands). Working beam voltage of 10 kV, backscattered electron image and 1000× magnification were used in the measurement.

#### 2.4 X-ray photoelectron spectroscopy (XPS)

Surface element composition was obtained using an X-ray photoelectron (XPS-AXIS Ultra HAS, Kratos, Japan). A monochromatic Al Kα source (1486.6 eV) with a resolution of 0.1 eV was used to irradiate the samples. Surface charging phenomena was offset by means of centering C–C peak at 284.6 eV relating to binding energy in the Cis. The Shirley method was used to correct background of spectrum.

#### 2.5 Fourier transforms infrared–attenuated total reflectance (FTIR-ATR)

The infrared spectra of silk fabrics samples were evaluated with Spectrum Bu spectrometer (PE Co., USA) in the region of 4000–400 cm$^{-1}$. The FTIR spectra were scanned three times with a resolution of 2 cm$^{-1}$ at room temperature.

| TABLE 1 Processing time versus temperature within ultrasonic bath |
|---|---|---|---|---|---|
| Treatment time [minutes] | Starting | 15 | 30 | 60 | 90 |
| Temperature [°C] | 28 | 36 | 45 | 61 | 68 |
2.6 Tensile test

Fabric samples at size of 80 × 20 mm (warp × weft) were prepared. A MTS E44.104 (MTS Co., USA) tensile testing machine, with a gauge length of 50 mm and extension speed of 50 mm min⁻¹, was used in the testing. All tests were conducted under standard conditions of 20 ± 2°C and 65 ± 2% relative humidity. Five samples were measured for each group and results were averaged.

3 RESULTS AND DISCUSSION

3.1 Surface oxidation analyzed by XPS and EDS

Peak deconvolution of the XPS spectra is analyzed by Origin 9.0 software and presented in Figure 1. It can be seen that the Cls spectrum is divided into three distinct peaks of 284.8, 286.4, and 288.0 eV, which attribute to carbon atoms bound to carbon and hydrogen (C-CH), carbon with one oxygen bond or nitrogen bond (C-O or C-N), and carbon with two oxygen bonds (C = O).[18,19]

It can be seen from Figure 1A that the sample treated with ultrasound at a prolonged treatment time of 90 minutes has the largest percentage peak coverage area of 11.9% for C = O, followed by the treated with 60 minutes (9.7%) and then the control (8.5%). The observation suggests that ultrasonic cavitation is able to encourage carbon-oxygen double bonds of C = O in the peptide chain bounding towards the surface of the fiber during the process.

Measurement results of fiber surface elements by EDS is presented in Figure 2. It is shown that while percentage content of carbon (C) decreased with ultrasonic treatment, oxygen content (O) increased as a result of ultrasonic irradiation. From control sample to the ultrasonic treated for 90 minutes, oxygen content increased by 3.2%. The increased oxygen content indicates that there are increased number of oxygen-containing polar groups presented on fiber surface due to ultrasonic cavitation irradiation. It should be pointed out; however, this surface oxidation induced by ultrasound is at a small level.

Ultrasound induced oxidation, which has been reported on some chemical fiber and cellulose fiber,[20,21] was found on silk at a lower ultrasonic frequency where the cavitation intensity is higher.[22] The results of slightly increased carbonyl groups measured by XPS and slightly increased oxygen content by EDS from this study provided further support for this ultrasonic induced fiber surface oxidation effect.

3.2 Secondary structure analyzed by FTIR spectra

FTIR spectra of the samples, with Amide bands of Amide I (1630–1700 cm⁻¹), Amide II (1510–1555 cm⁻¹), and Amide III (1230–1265 cm⁻¹) are presented in Figure 3A. Amide I band is associated to stretching vibrations of the C = O bond, which is sensitive to the secondary structure in silk protein,[23] as is shown from the schematic diagram of silk secondary structure in Figure 3C. The peak at 1630, 1665, and 1700 cm⁻¹ in Amide I are attributed to random coil, β-sheet, and β-turn.[24,25] It is seen from Figure 3A a slightly increased absorbance at the wave number of around 1665 cm⁻¹ (attributed to β-sheet) for the samples treated for 60 and 90 minutes, suggesting the existence of a slight increase in β-sheet in the fiber secondary structure, compared with that of the control sample.

It can be seen from Figure 3 (A) that the control sample has relatively round and flat peak ends at 1630 cm⁻¹, compared with those ultrasonically treated (particularly at 60 minutes). A quantitative evaluation has been made by percentage change in peak intensity of 1630 cm⁻¹ and 1700 cm⁻¹ relative to the peak intensity of 1700 cm⁻¹ shown in Figure 3D. The percentage peak intensity is seen increased from the control sample to the treated for 30 and 60 minutes. This is expected because the peak shape can be associated with type and number of hydrogen bonds in the chemical structure, with sharper and more pointing peak ends indicating broken bonds.[26] The level of broken...
Figure 2  EDS analysis of silk with and without ultrasonic treatment; (A) images of EDS measurement, and (B) results of fiber surface element of Carbon and Oxygen.

Figure 3  FTIR analysis of silk with and without ultrasonic treatment; (A) spectra from 1800 to 1000 cm$^{-1}$, (B) deconvoluted FTIR spectra of Amide I band, (C) Amide I vibration and β-pleated sheet form of polypeptide chain configuration, (D) percentage change in peak intensity of 1630 and 1700 cm$^{-1}$, relative to the peak intensity of 1700 cm$^{-1}$, and (E) deconvolution results of percentage proportion of random coil and β-sheet in Amide I band.
bonds, however, reached its peak at 60 minutes as a result of cavitation implosion, and then slowed down when treatment time is extended to 90 minutes (Figure 3A and D). Hydrogen-bonding strengths in Amide I are variable due to their flexibility and tendency to twist or stretch, depending on the environment they are in and/or the treatment they receive. Details of Amide I in the FTIR spectra are presented as deconvoluted FTIR spectra shown in the Figure 3B, where Amide I area was fitted with three peaks by Gaussian method and calculated peak fitting area as the proportion of various secondary structures. It can be seen that the overall absorbance of ultrasonically treated samples were increased from that of the control, indicating that ultrasonic treatment had an effect on the changes in fiber secondary structure.

The spectra in Figure 3B are further quantitatively analyzed by Origin9.1 and results are shown in Figure 3E. It can be seen that when the sample treated for 30 minutes was compared with the control sample, there is slight decrease in random coil and increase in β-sheet. When the treatment time was extended from 30 to 60 minutes, a further decrease in random coil and an increase in β-sheet are seen, suggesting some of the random coil in the fiber molecular structure has transferred to β-sheet. The transformation is, however, at a small level. As treatment time prolonged from 60 to 90 minutes, the transforming rate is shown to have slowed down (Figure 3E). This observed random coil to β-sheet structure transformation resulted of ultrasonic treatment (up to 1 hour) is consistent with the previous analysis, and is in agreement with the results from a study using silk powder.[15]

Ultrasonic cavitation during the treatment process is responsible for the observed random coil to β-sheet transition. Acoustic cavitation is able to produce local intensive heat and energy necessary for the movement of peptide chains. While high pressure facilitated the rearrangement of the protein molecules, heat (hot water) could have an effect on conformation transition of peptide by causing relative area of β-sheet in the Amide I peak to increase and disorder conformation to decrease.[27] It was shown that conformation of silk fibroin to β-sheet structure can occur at the temperature of 60°C.[28]

The transition process from random coil to β-sheet is, however, a kind of exothermic process.[29] It is seen from Table 1 that the processing temperature has reached 60°C at ultrasonic treatment time of 60 minutes. Further raising of temperature produced by prolonged sonic treatment time would have restrained the transition process to some extent. This explained the reason why the transition has slowed down when the treatment time was extended from 60 to 90 minutes. This observed random coil to β-sheet transition (subjected to treatment time) is in agreement with the outcome from a previous study conducted on wool. Staple animal fiber of wool was found to have the same α-helix to β-sheet transition as a result of ultrasonic treatment.[13] No changes in fiber crystallinity, however, have been found as a result of ultrasonic treatment during the process, as has been reported previously.[13,22]

### 3.3 | Tensile strength

Fiber tensile strength was investigated in this work to verify the transformation of fiber molecular structure caused by ultrasonic treatment, results of which are shown in Table 2. It can be seen that among the samples tested, the control sample showed the lowest maximum load with the highest extension at break.

Conformation of fiber molecular structure is proved to be responsible for the fiber tensile properties, with strength determined by β-sheet and extension random coil.[30] It follows therefore that fiber strength increased with increasing proportion of β-sheet within fiber structure as a result of ultrasonic treatment, and that elasticity of the fiber descends with the decreasing proportion of random coil. These are consistent with the results shown in Table 2. Upon reaching breaking point, the highest maximum load is seen on the sample ultrasonic treated for 60 minutes, instead of that of 90 minutes. This is not unexpected. In the ultrasonic process, intense acoustic cavitation and thermal degradation acted as a co-effective factor when the treatment was prolonged from 60 to 90 minutes, during which time the transformation of random coil to β-sheet has slowed down, as is seen from the previous analysis. Ultrasonic treated samples showed lower extensibility than that of the control, with the 90 minutes treated sample showing the lowest extension rate (Table 2). This provides evidence that ultrasonic treated samples (with reduced amount of random coil) are tend to be less extensible than that of the sample without ultrasonic treatment.

### 4 | CONCLUSIONS

Silk, in fabric form, was investigated in this work for changes in secondary structure and tensile properties as a result of ultrasonic treatment. Ultrasonic induced surface...
oxidation was suggested by the measurement results of XPS and EDS, which showed increased carbonyl groups and oxygen content on the surface of fiber after ultrasonic treatment. FTIR analysis has shown that subjected to treatment time, a transformation of random coil to $\beta$-sheet within fiber secondary structure can be resulted from ultrasonic treatment. The transition rate first increased with increasing ultrasonic treatment time, and slowed down after the treatment time reached 60 minutes. Local intensive pressure and heat produced by acoustic cavitation during ultrasonic treatment are responsible for the random coil to $\beta$-sheet transformation. The transformation is, however, at a small level. Tensile testing showed that subjected to treatment time, ultrasonics can produce fibers with increased strength but reduced extensibility, as a result of random coil to $\beta$-sheet transformation within fiber molecular structure.

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