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Extraction, Structural and Functional Properties of Silk Sericin Biopolymer from *Bombyx mori* Silk Cocoon Waste

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Abstract

In the present investigation environment-friendly effective technique was used for silk sericin extraction from waste silk cocoon. Silk sericin powder was extracted from an only boiled water solution of silk cocoons without using any chemicals. Extracted sericin powder was characterized by UV spectrophotometer, Fourier transform infrared (FTIR) and Thermogravimetric Analysis (TGA). The crystalline index and crystallite diameter of silk sericin were investigated by X-ray diffraction (XRD). The crystalline index and crystallite diameters were found 39.66% and 2.179 nm respectively for silk sericin. The functional properties in terms of antimicrobial activity and antioxidant property were also evaluated. The antioxidant activity was evaluated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals. The results showed that silk sericin had a strong scavenging capacity for DPPH radicals.

Keywords: Silk sericin; Extraction; Crystalline index; Antibacterial activity; Antioxidant property

Introduction

Silk sericin is a natural glow like globular protein derived from *Bombyx mori* silk cocoon [1-3]. Its chemical structure [4] is shown in Figure 1. The *Bombyx mori* silkworm is composed mainly of a fibrous core of protein fibroin (70-80%) with sericin (20-30%) surrounding it with other impurities such as wax, color pigments, and inorganic components. Silk fibroin is covered by silk sericin layer. Three layers of silk sericin are available in the silk cocoon. The outer layer, middle layer and inner layer hold 15%, 10.5% and 4.5% sericin respectively [5] which is shown in the Figure 2. Sericin is insoluble in cold water. But soluble in hot water as the long protein molecules breaks down to smaller fractions in the hot condition which are easily hydrolyzed and dispersed [6,7]. It is estimated that 4, 00,000 tons of dry cocoons are produced worldwide per year. After degumming process at least 50,000 tons (12.5%) of sericin are going into wastewater [4]. Generally, the degumming process is accomplished by acid, alkali and enzyme. The effluent is released into the wastewater stream from the industry, leads to increase the chemical oxygen demand (COD) and biological oxygen demand (BOD) level. Therefore, the wastewater released by silk industry leads to contamination of water and environment [8]. If sericin is recovered, it could be used to develop value-added products and this would also be beneficial in terms of the economy and the environment [9].

Sericin is found to possesses various biological properties like antibacterial, antioxidant, antitumor activities, UV resistant and moisture absorbing properties, it can be used as finishing agent for natural or manmade fibres of textile industries, in cosmetics industries as skin care, and used for biomedical polymeric products [10-14].

Sericin can be extracted from silk by detaching it from the fibroin part. Currently, several methods are using to extract sericin. Degumming process done by using soap and alkali is very efficient to remove sericin completely from fibroin. But this method is not significant because the separation of soap and alkali from sericin is a very difficult process. As a result application of sericin becomes complicate [7,15]. Sericin can also be extracted using certain acids like citric, tartaric or succinic acid etc. Different types of acids have a degrading effect on proteins and sericin being proteinous nature can get damaged while extraction [16]. So extraction in urea solution with 2-mercaptoethanol becomes useful having a lesser degrading effect on sericin and damage can be downplayed. Using this process around 95% of the total sericin present

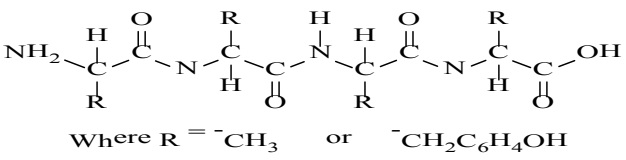

Figure 1: Chemical structure of silk sericin.

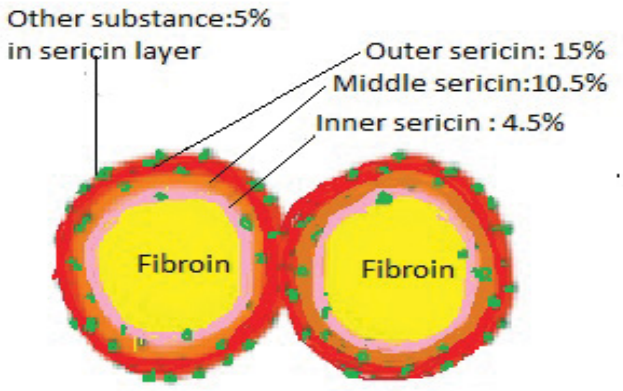

Figure 2: Schematic diagram of three layers sericin of silk cocoon.

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can be separated out without any damage [17]. But this process is costly and time-consuming because, before application, purification of sericin is required and is done by dialysis procedure. Enzymatic degumming for sericin extraction has also been anticipated. But it is expensive and sensitivity to degumming condition [18-20]. Due to the above mentioned constraints hot-water extraction by autoclave machine was considered for the present work. In this method silk cocoon is heated in hot distilled water adding no other chemicals. Time and temperature of extraction play a key role in the amount of sericin extracted. So it can be said that this extraction method will be very helpful in terms of environment and economic issues. The aim of the present work is to extract the silk sericin by autoclave machine without using any chemicals and investigated the different structural and functional properties of extracted silk sericin.

Experiment

Materials

Bombyx mori waste silk cocoons were collected from Bangladesh Sericulture Research Training Institute (BSRTI), Rajshahi. Silk cocoon waste means silk insects come out through the silk cocoon layer. These types of cocoon cannot be used to produce silk filament yarn. These types of cocoons are considered as wastage cocoon,

Methods

Silk sericin extraction: Extraction of silk sericin can be accomplished by the method with some modification according to Khalifaa et al. [21]. To do this, 50 gram silk cocoons were first cut into small pieces. Then, deionized water was added onto them and these were autoclaved at 120°C for 30 min. After autoclaving, the sericin and fibroin mixed solution was filtrated through Whatman filter paper. Silk fibroin is separated by this filtration process. After filtration, centrifuge machine is used to separate the sericin from sericin solution. Finally, sericin is dried at 100°C in an oven. This silk sericin extraction process was shown in the Figure 3.

Measurement of Different Structural and Functional Properties of Silk Sericin

UV absorption measurement

Sericin was dissolved in distilled water to obtain a very dilute solution. The UV absorption spectrum of the solution was recorded using UV-visible Spectrophotometer of Shimadzu 1650 model (Japan).

Fourier transform infrared spectrophotometer (FTIR)

The sericin sample and KBr (Potassium Bromide) were dried at 105°C for 10 hours. The sericin was mixed with potassium bromide (KBr) using mortar and pestle to make powder in the mass ratio of 1:100 (1 mg sericin powder and 100 mg KBr). The mixed sample was again dried at 105°C for 10 hours. Then the sample was analyzed with FTIR spectrophotometer (Spectrum-100, Perkin Elmer, USA) in the scanning range of 400-4000 cm^{-1}.

Thermogravimetric analysis

The thermal stability of the sericin was studied by a thermo gravimetric analyzer (Jupiter, Germany). The temperature range from 20°C to 600°C was used for analysis at a heating rate 10°C/min under a nitrogen atmosphere. The weight loss of the sample was continuously recorded as a function of temperature.

X-Ray diffraction (XRD) analysis

The XRD patterns were recorded using dry powder samples on PAN Analytical X Pert PRO X-ray diffractometer using Cu-Kα radiation of wavelength λ=1.5406 Å as the X-ray source. The measurement was carried out at a scanning rate of 8°/min in 2θ range of 10°-60°. The'd' spacing is determine by using Bragg's law.

According to the Bragg's law, $n\lambda = 2d \sin\theta$ ---------- (1)

Where, the X-ray wave length, 0.15406 nm is fixed and d is the plane spacing,

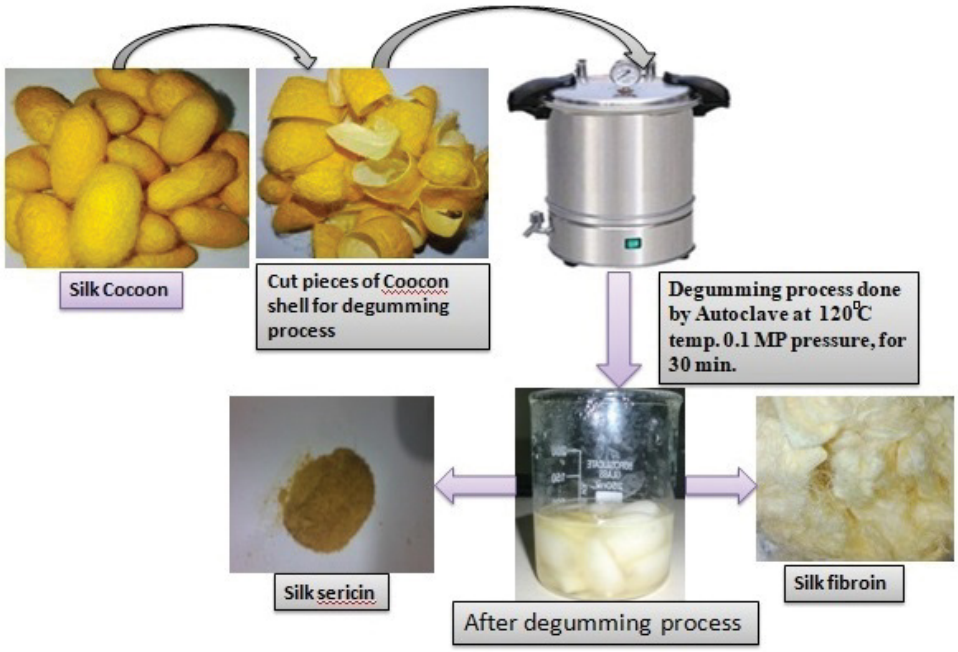

Figure 3: Schematic diagram of extraction process of sericin from silk cocoons.

The peak width at half maximum (FWHM) in the XRD was used to determine the crystal diameter as per the following Deby-Scherrer formula:

$$D=\frac{K\lambda}{\beta Cos\theta} \quad \text{.....} \quad (2)$$

Where, K= 0.9 is the Scherrer constant; the X-ray wave length, $\lambda = 0.15406$ nm; β the peak width of half maximum; and θ, the Braggs diffraction angle. β is the full width of the X-ray pattern line at half peak-height in radians. The crystalline index (CrI) of sericin can be calculated from the maximum intensity peak at 110 lattices (I_{110}, before 20° corresponding to maximum intensity) and the peak intensity of amorphous diffraction at 16°. The crystalline index (CrI) of sericin was calculated by the following equation [22,23].

$$CrI_{110}=\frac{I_{110}-I_{am}}{I_{am}}\times 100 \quad \text{.....} \quad (3)$$

Where, I_{110} is maximum intensity at 110 plane and I_{am} is amorphous diffraction at 16°.

Antibacterial activity test by qualitative method

Zone of Inhibition of silk sericin solution was determined by disc diffusion method [24]. 250 ml of nutrient agar medium was prepared and autoclaved. The autoclaved nutrient agar medium was poured into sterile petri dish under aseptic conditions. The petri dish was inoculated with 20 µl of *S. aureus* bacteria culture. Discs prepared by punching Whatman paper and autoclaved. Discs were dipped 5, 10 and 20 g/l concentration sericin solution. The discs were placed on the inoculated petri dish. The petri dish was incubated at 37°C for 24 hours and was observed for the formation of zone of inhibition to find the antibacterial activities of sericin.

Antioxidant activity measurement

Antioxidant activity was measured by using the 2.2-diphenyl-1-picryl-hydrazil (DPPH) reagent. DPPH is a stable free radical which characteristic absorption peak shown at 517 nm, was used to study the free radical scavenging effects of sericin by a method described by Wu et al. [3], with slight modification. Three different concentrations of 10 mg/ml, 20 mg/ ml, and 40 mg/ml silk sericin solution were prepared. 0.004% of DPPH solution was prepared in 0.1 M methanol and 3 ml of this solution was mixed with 1 ml of sericin solution and vortexed. The mixture solution was kept at the dark condition for 25 min at room temperature (25°C) to complete reaction. After reaction mixtures were centrifuged at 6000 rpm for 5 minutes. Consequently, absorbance of decolorized solution and control sample was taken by a UV visible spectrophotometer at 517 nm against a blank sample. The free radical scavenging activity was evaluated by the following equation [3].

$$\text{Radical scavenging activity (RSA)} = \left(1-\frac{\text{Sample absorbance}}{\text{Control absorbance}}\right)\times 100 \quad (4)$$

Where, control absorbance means methanol solution of DPPH and sample absorbance means 3 ml methanol solution of DPPH mixed with 1 ml sericin solution.

Results and Discussion

In this study silk sericin powder was extracted from silk cocoon waste. After extraction, the different structural and functional properties of silk sericin powder were investigated.

UV absorption

The main composition of silk sericin is protein. The amount of protein in sericin is almost 90% of its composition. The sericin contains 18 kinds of amino acids. Among the 18 kinds amino acids, serine, aspartic acid, and glycine are very important amino acids attributed to the physico-chemical and functional properties of sericin. In addition the 18 amino acids contain 70% hydrophilic amino acid which is responsible for good solubility and water absorbability of sericin. On the other hand, the amount of aromatic amino acids is only 6.6% of 18 kinds of amino acid which were identified by UV spectrum [3]. The proteins generally have two absorbance peaks in the UV region, one between 215-240 nm and other 260-290 nm. The peptide bonds of amino acid absorb 215-240 nm regions UV light. In the range of 260-290 nm UV radiation absorbed by aromatic amino acids like tryptophan, tyrosine and phenylalanine [25]. The silk sericin solution shows absorbance peak at 216 nm and 275 nm which indicates that sericin has UV resistance capacity (Figure 4).

FTIR analysis

Figure 5 shows the IR spectra of sericin powder. The peak at 3500-3000 cm^{-1} is associated with N-H stretching vibration. The O-H stretching band is located at 3600-3200 cm^{-1} that overlapped with N-H stretching vibration peak at 3500-3000 cm^{-1}. Sericin shows a peak between 1700 and 1600 cm^{-1} (1602 cm^{-1}) confirming the stretching vibration of the C=O. The Peak at 1580-1510 cm^{-1} (1512 cm^{-1}) confirms

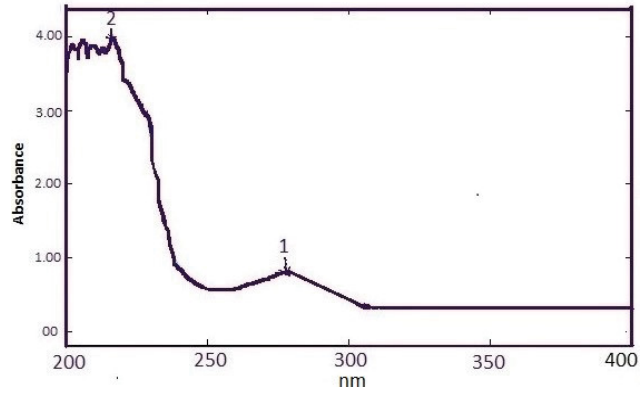

Figure 4: UV spectra of silk sericin.

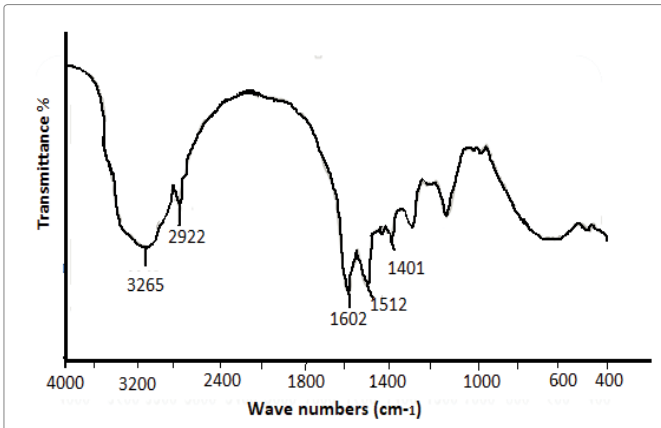

Figure 5: FTIR spectra of silk sericin powder.

the presence of N-H bending. C=O symmetry stretching is observed at about 1401 cm^{-1}. Similar findings of FTIR peaks of sericin powder obtained by Gulrajani et al. [26], Sarovart et al. [27] and Song & Wei [28].

Thermogravimetric analysis

From Figure 6, the mass loss was observed nearly 6%, at 100°C. This is due to vaporization of moisture, low molecular weight solvent and gas. The mass loss at 200°C was 14%. This loss occurs due to the degradation of peptide bond and amino acid of sericin. The 30% mass loss occurred at 300°C due to decomposition of the specimen. The significant mass loss 52% was observed at 400°C. 11% mass was lost between 400°C to 500°C temperature. Furthermore, a little amount of weight loss occurred between 500°C to 600°C. Eventually, 34% ash was found for sericin specimen. The similar ash content in sericin found by other researchers [29]. The DTG curve indicates the maximum decomposition point which was at 301°C. On the other hand, Tsukada found maximum decomposition temperature at 320°C [30].

X-ray analysis

Natural silk is composed of two kinds of proteins, namely, the crystalline fibroin and the amorphous sericin [8]. The XRD pattern of silk sericin reveals that sericin exhibit a broad diffraction peak at 2θ =18.86 and intensity about 1936 counts (Figure 7). Silva et al. [31], and Jo & Um [32], found the highest diffraction peak at 2θ=19.2. This peak indicates the conversion of the random coil structure into the β-sheet structure due to intermolecular hydrogen bonding between the hydroxyl groups of the amino acids present in sericin, indicating that the sericin powder obtained from the degumming and freeze-drying processes. The inter plane spacing 'd' is determined according to equation no.1. The calculated inter plane spacing value is 0.47 nm. The full width and half maximum value (FWHM) was determined with the help of origin pro software. The full width at half maximum (FWHM) value of sericin is 3.703 degree. The full width at half maximum (FWHM) value is inversely proportional to the crystallite diameter, and crystallite diameter also calculated according to the Deby-Scherrer formula (equation no. 2). After calculation, 2.179 nm crystallite diameters are found of silk sericin. When full width at half maximum value increases, the crystallite diameter of the specimen decreases. The maximum intensity was 1936 counts at 2θ =18.86 degree and the amorphous intensity was 1168 counts at 2θ =16 degree. The 39.66% of crystallinity index was found according to the formulae number 3.

Antioxidant activity

The antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. The DPPH is a stable free radical which gives characteristics absorption at 517 nm, was used to study the radical scavenging activity (RSA) of sericin solution. The absorbance of DPPH solution was 1.936 at 517 nm which indicates the control sample. On the other hand, the absorbance of 10, 20 and 40 mg/ml sericin solution were given 1.325, 0.928 and 0.661 respectively at 517 nm. These decreases in absorption were applied to assess the extent of radical scavenging according to the equation no. 4.

Table 1 shows that the 40 mg/ml of sericin solution was shown 66% radical scavenging activity (RSA). The UV absorption decreases when sericin concentration increases which indicate free radical scavenging activity increases. Other way absorption decreases gradually with increasing concentration of silk sericin solution as sericin donate protons to this free radical. In another research, it has been found that sericin completely inhibits lipid peroxidation showing sericin have antioxidant activity [10,33].

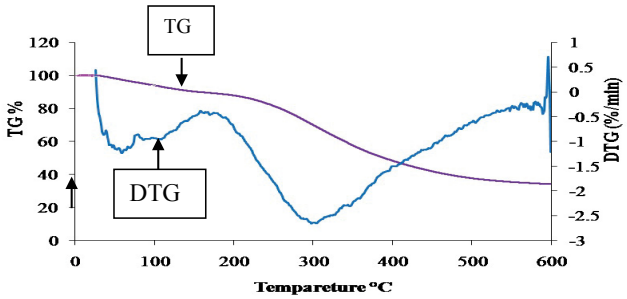

Figure 6: TG & DTG curve of silk sericin.

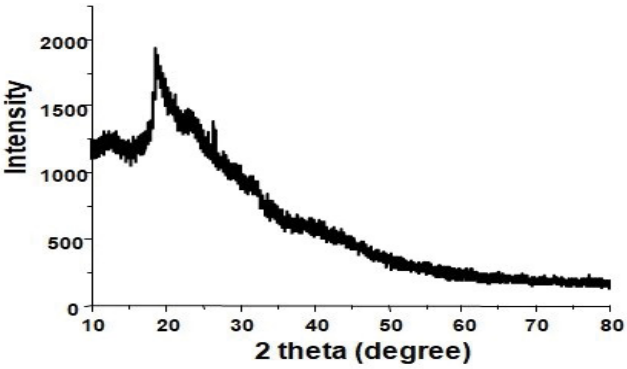

Figure 7: XRD pattern of silk sericin.

Sample	RSA (%)
10 mg/ml Sericin solution	32
20mg/ml Sericin solution	52
40 mg/ml Sericin solution	66

Table 1: Radical scavenging activity (RSA).

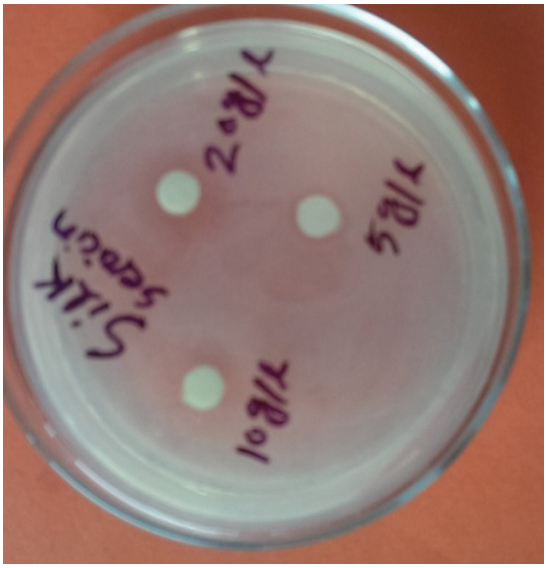

Figure 8: Antimicrobial activity of silk sericin solution against *S. aureus*.

Antimicrobial activity

The antibacterial activity of silk sericin solution was investigated by the disc diffusion assay. The antibacterial activity of silk sericin solution was evaluated with *S. aureus*, gram-positive pathogenic bacteria. It can be seen from Figure 8 that the circular inhibition zone increases with increasing concentrations of silk sericin solution against S. aureus gram-positive bacteria. The concentration of 5 g/l sericin solution was shown lowest antimicrobial activity and 20 g/l sericin solution was shown highest antimicrobial activity. It is assume that the antimicrobial activity of sericinis due to the interaction of the positively charged sericin with the negatively-charged residues at the cell surface of many fungi and bacteria, which causes extensive cell surface alteration and alters cell permeability. This causes the leakage of intracellular substances, such as electrolytes, UV absorbing materials, proteins, amino acids, glucose etc. As a result, sericin inhibits the normal metabolism of microorganisms and finally leads to the death of bacteria cells [34].

Conclusion

The sericin is obtained from silk cocoon waste through autoclave and centrifuge machine without using any chemicals. This technique could be helpful for the economy and environment. As sericin absorb UV range light, it may be used in sunscreen cream and can also be applied on textiles to produce UV protective garments. Sericin has been shown antibacterial activity which will be used to produce medical textiles such as surgical suture, hand gloves, pad, gauge, and bandage, apron and bed sheet. Sericin has been shown an antioxidant property. This valuable ingredient might be used as a food preservative, and in cosmetic and pharmaceuticals sectors.

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