RESEARCH ARTICLE

European Journal of Advanced Chemistry Research
www.ej-chem.org

Extraction and Characterization of Sericin from Cocoon of Four Different Silkworm Races *Bombyx Mori* L.

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ABSTRACT

The removal and use of sericin could have a strong economic, social, and environmental impact, particularly in countries where sericulture is practiced, especially in Bangladesh. Because in our country Bangladesh, sericin is a waste material in the sericulture industry. Several techniques have been adopted for sericin extraction, but maintaining its chemical properties after extraction and environment-friendly extraction methods are still a major challenge. Sericin is fully or partially hydrolyzed and solubilized during the degumming of the cocoon. Consequently, it is important to create sericin extraction procedures that require less energy, don't release any chemicals, and don't harm the environment. Numerous research has been done to extract sericin but the differences in sericin content and chemical properties of Bivoltine and Multivoltine silkworm cocoon have not been studied yet. In this work, sericin was extracted from the silkworm cocoon of four different silkworm races at two different temperatures and durations. Significant differences were observed in yield% with different silkworm races and different treatments. Extracted sericin was characterized through SDS PAGE, FTIR & UV spectroscopy, and TGA.

Keywords: Cocoon, Characterization, Extraction, Natural Polymer, Sericin, Silk Gland, Sericulture, Silkworm (*bombyx mori* L.).

Published Online: June 01, 2023

ISSN: 2684-4478

DOI: 10.24018/ejchem.2023.4.3.134

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I. Introduction

The main primary components of silk are fibroin and sericin, with minor amounts of waxy materials, mineral salts, and coloring matter. These two main components are fully natural proteins, which make up roughly 75% and 25% of the total weight of silk, respectively [1]. Sericin is mainly amorphous and more water-soluble, which serves as a gum binder to keep the cocoon's structural integrity. In sericin, random coil and β sheet structures are present. The random coil shape transforms into a β sheet structure when the temperature drops and forms into a gel. Random coil structure dissolves in hot water. Sericin has excellent gelling capabilities since it dissolves readily in water at a temperature of 50 to 60 ºC and returns to gel upon cooling [2]. Its chemical structure contains a significant concentration of hydroxyl groups due to its serine (approximately 33%), aspartic acid (16.8%), and glycine (8.8%) content [3].

Many studies are exploring novel uses for sericin because of its features, which include corrosion resistance, antibacterial activity, UV protection, moisture absorption, and release [4]. Numerous studies are seeking new uses for this protein. Sericin is now a feasible source for the creation of many natural goods, and there is a huge opportunity to use it in the food, cosmetics, and pharmaceutical industries [5]. The search for options to make use of waste materials from the manufacture of silk fabric has increased significantly in recent years. Sericin, a protein obtained from silk fiber during the degumming process, is also a waste material. Sericin should be eliminated throughout the silk textile manufacturing process to produce silk fibers with a lustrous appearance, a soft handle, and an attractive glance [6]. Sericin can be extracted from silk using a variety of techniques, including autoclaving [7], hot sodium carbonate aqueous solution [8], and aqueous urea solution at 85 °C [9]. All of these techniques are dependent on the solubility of proteins in water. The extraction procedures greatly influence the raw materials used for extraction and these methods also significantly affect the sericin's properties [10]. Cold water cannot dissolve sericin. Yet, the lengthy protein molecules are soluble in hot water because they break down into smaller portions in the hot environment that are easily hydrolyzed and distributed [11], [12]. 400,000 tons of dry cocoons are produced annually worldwide. At least 50,000 tons (12.5%) of sericin are discharged into wastewater during degumming [13]. The three main components used in the degumming process are acid, alkali, and enzyme. The effluent from the industry is discharged into the wastewater stream, which causes the chemical oxygen demand (COD) and biological oxygen demand (BOD) levels to rise. As a result, the environment and water are contaminated by the effluent that the silk industry releases [14].

If sericin is recovered, it might be used to produce different types of goods for value-addition. Sericin has recently been discovered to possess many significant and beneficial qualities, including antioxidant [15], anti-tyrosinase [16], UV absorption, and moisture-absorbing capabilities [9]. Sericin is a finishing chemical that can be applied to both natural and synthetic textiles. Sericin is a skincare ingredient used in the cosmetics industry. In the food sector, it may also be a valued natural component. Biomedical polymeric applications have been used with fibroin and sericin [17], [18]. Degumming of silk has been accomplished using a variety of techniques, including high-temperature, high-pressure (HTHP) water extraction [19], boiling-off in soap [20], alkalis [21], acidic solution [22], aliphatic amines [23], enzymes [1] and bio-surfactants [24]. Attempts have been made to recover sericin from soap alkali baths using micro, ultra, and nanofiltration techniques [25]. Spray drying, freeze drying, or tray drying have all been used for drying [26]-[28]. Degumming using soap and alkali to extract sericin from fibroin is a very effective procedure. The extraction of soap and alkali from sericin is a highly challenging operation; hence this method is insignificant and its application became more difficult as a result [12], [29]. Precipitation by ethanol for lab-scale applications is one of the additional purification techniques [30]. Some acids, such as citric, tartaric, or succinic, can also extract sericin. Various acids degrade proteins in various ways because sericin's protein structure makes it susceptible to destruction during extraction. As a result, damage can be minimized because extraction in urea solution with 2-mercaptoethanol has a less damaging effect on sericin. Around 95% of the total sericin present may be sorted out using this method without causing any harm [31]. However, this approach is expensive and time-consuming since a dialysis procedure must first purify sericin before administration. Sericin extraction with enzymatic degumming has also been anticipated. Nonetheless, it is pricey and delicate to degumming conditions [1], [32], [33]. Hot-water extraction by autoclave machine was considered for the current task due to the abovementioned restrictions. This technique uses only distilled water to heat the silk cocoon. The amount of sericin extracted is significantly influenced by the temperature and time of extraction. The sericin extraction and recovery technique determines its properties [34]. Sericin is fully or partially hydrolyzed and solubilized in the degumming liquid during degumming. Consequently, it's important to create sericin extraction procedures that require less energy, don't release any chemicals, and don't harm the environment. In the present study, an attempt was made to extract sericin from the cocoons of four different silkworm races of Bangladesh Sericulture Research and Training Institute using the HTHP method. The yield of sericin obtained was compared. The structure and properties of the sericin obtained were also investigated.

II. Materials and Methods

A. Materials

Silkworm cocoons were collected from the silkworm section of Bangladesh Sericulture Research and Training Institute, Rajshahi, Bangladesh. Tris-HCl, mercaptoethanol, glycerol, Potassium Bromide, and all chemicals and reagents were analytical grade and were supplied by Invent Technologies Ltd, Dhaka, Bangladesh.

B. Extraction of Sericin from Silkworm Cocoon

Sericin was extracted through High Temperature and High Pressure (HTHP) method. 4 g of dry and fresh silk cocoon were cut into small pieces and taken in a 500 mL steal autoclave, 100 mL of deionized water was added and the autoclave was sealed. The mixture was then autoclaved for 15 minutes and 30 minutes at two different temperatures, 90 and 110 ºC. After autoclaving, the sericin and fibroin mixed solution was subjected to filtering using Whatman filter paper. The filtrate was collected and sericin powder was finally obtained through the freeze-drying method. The whole extraction process is shown in Fig. 1.

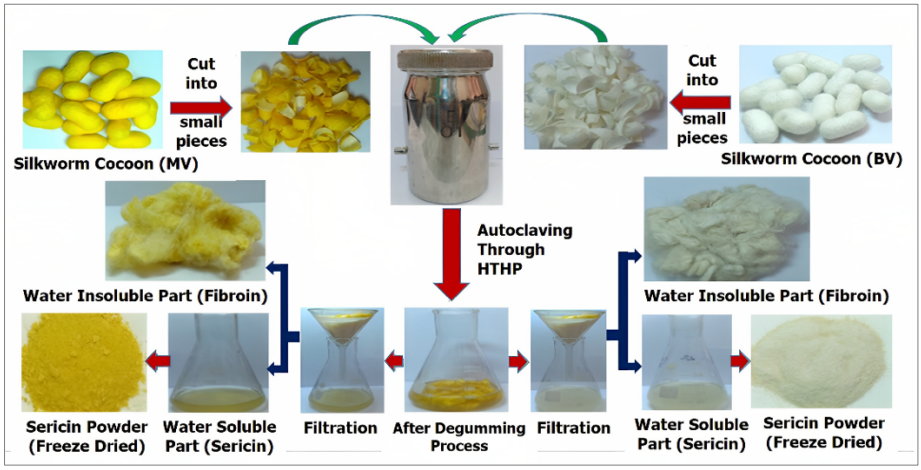

Fig. 1. Schematic diagram of sericin extraction.

Sericin extraction from four silkworm races at two different temperatures with two different durations was considered as treatment which is shown in Table I.

TABLE I: DIFFERENT SILKWORM RACES AND TREATMENTS FOR SERICIN EXTRACTION

Treatments	Silkworm races
T_1: 90 °C with 15 minutes duration.	Race-1: BSRB 01(P) [Bivoltine]
T_2: 90 °C with 30 minutes duration.	Race-2: BSR 92/2. [Multivoltine]
T_3: 110 °C with 15 minutes duration.	Race-3: Nistary [Multivoltine]
T_4: 110 °C with 30 minutes duration.	Race-4: Urboshi [Multivoltine]

C. Molecular Weight Determination through SDS-PAGE

Laemmli's instructions were followed while performing the sericin SDS-PAGE analysis [34]. The sample was combined with an equivalent volume of SDS-PAGE sample buffer (0.5M Tris-HCl, pH 6.8, 2.5% SDS, 5% 2-mercaptoethanol, 10% glycerol), heated for five minutes, and then removed from the heat. The stacking gel was 5%, while the resolving gel was 12%. Using an Enduro electrophoresis machine, the electrophoresis was performed for 4 hours at a constant voltage of 90 V. (Labnet International Inc., USA). After electrophoresis, the gel was stained with Coomassie Brilliant Blue R-250. The gels were stained for 30 minutes, and then de-staining was done. A molecular weight marker (MWM) purchased from Genni-India was used to estimate molecular weights.

D. Fourier Transform Infrared Spectrophotometric Analysis (FTIR).

Sericin was analyzed using Fourier transform infrared spectrophotometric analysis (FTIR). To completely remove all moisture, the corresponding samples and KBr (potassium bromide) were dried at 105 °C for 10 hours. Using a mortar and pestle, the materials were combined in a mass ratio of 1:100. The combined sample was further dried for 10 hours at 105 °C. The material was then subjected to FTIR spectrophotometer analysis in the 400–4000 cm-1 scanning range (Spectrum-100, Perkin Elmer, USA).

E. UV Spectroscopy

To create a highly diluted solution, sericin was dissolved in distilled water. Using a UV-visible Spectrophotometer, the UV absorption spectrum of the solutions was measured throughout the wavelength at the range of λ 280 to 330 nm (Shimadzu, 9000i, Japan). Before performing the spectral scanning, distilled water was used to perform the baseline zero.

F. Thermogravimetric Analysis (TGA)

The temperature stability of the sericin was evaluated using a thermal gravimetric analyzer (Perkin Elmer, STA 8000, USA). At a heating rate of 10 o C/min and a nitrogen environment, the temperature range of 20 ºC to 800 ºC was used for analysis. Regarding its temperature, the sample's weight loss was continuously tracked.

III. RESULT AND DISCUSSION

A. Extraction of Sericin from Silkworm Cocoon

Cocoons from four different silkworm races were collected and their sericin content was estimated are shown in Table I and Table I. From Table II, sericin extraction yield% was found to be 15.32%, 21.43%, 25.68% and 24.98% for silkworm race-1 with T_1, T_2, T_3 and T_4 respectively. For silkworm race-2 sericin content was found to be 14.19%, 14.59%, 29.54% and 27.93% with T_1, T_2, T_3 and T_4 respectively. In the case of silkworm race-3, sericin content was observed as 12.00%, 14.09%, 28.82%, and 29.96% whereas silkworm race-4 showed sericin content as 16.53%, 17.23%, 32.96% and 33.84% with T_1, T_2, T_3 and T_4 respectively. Average extraction yields from cocoon were 21.85, 21.56, 21.21, and 25.14 percent for silkworm race-1, 2, 3 & 4 respectively.

TABLE II: EXTRACTION OF SERICIN FROM SILKWORM COCOON (*BOMBYX MORI* L.)

Treatment	Sericin Extraction Yield %			
	Race-1	Race-2	Race-3	Race-4
T_1	15.32 ± 0.53^{b}	14.19 ± 1.12^{b}	12.00 ± 1.09^{b}	16.53 ± 0.74^{b}
T_2	21.43 ± 0.37^{a}	14.59 ± 0.65^{b}	14.09 ± 1.30^{b}	17.23 ± 0.75^{b}
T_3	25.68 ± 1.30^{a}	29.54 ± 0.44^{a}	28.82 ± 0.74^{a}	32.96 ± 1.69^{a}
T_4	24.98 ± 0.71^{a}	27.93 ± 0.99^{a}	29.96 ± 1.01^{a}	33.84 ± 2.27^{a}

Data present Mean ± SEm, n=3, Means with different letters represent statistical differences according to Tukey's HSD test ($P < 0.01$).

It was observed that when sericin was extracted from silkworm cocoon, the highest sericin yield% 33.80 was found for silkworm race-4 with 110 °C and 30-minute duration, and the lowest sericin yield% 12.00 was found for silkworm race-3 with 90 °C and 15-minute duration.

Sericin extraction yield% was increased when the temperature, as well as the extraction duration, was increased for all silkworm races except race-1 and race-2.

Several researchers studied the extraction of sericin from silkworm cocoons. Gupta *et al.* extracted sericin from four different sources; silk fabric, cocoons, silk flats, and silk waste, and reported that the effect of temperature is much more prominent in high-temperature high pressure (HTHP) as compared to that in IR heating bath and reported that the yield was found to increase with time as well as the temperature of extraction, and the highest yield% from the cocoon was estimated 25% [35]. In another study, Wang *et al.* reported the highest yield at 220 °C (36.50% ± 0.4%) among the three temperatures and the yield was low at lower temperatures [36]. Another researcher also indicated that the extraction yield was 25 to 30% of the silk cocoons. The extraction yield% of sericin obtained from this research was very much relevant to the previously reported results.

B. Molecular Weight Determination through SDS-PAGE

The molecular weight distribution of the different sericin samples was determined using the sodium dodecyl sulfate-polyacrylamide gel electrophoresis SDS-PAGE method as shown in Fig. 2. Sericin obtained from races 1, 2, and 3 showed a diffused band in the molecular weight range of 15-250 kDa. The heat treatment of the cocoon suspensions attacked the peptide bonds of the primary structure of the protein. Therefore samples showed broader bands due to the mixture of different molecular weights of polypeptides. According to other researchers, the molecular weight distribution of sericin is ranged from 20 to 400 kDa.

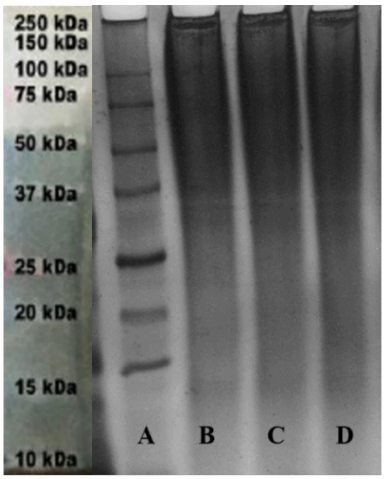

Fig. 2. SDS-PAGE Analysis of sericin samples: A) Marker; B) Race-1; C) Race-2; D) Race-3, 10 mg/ml.

The molecular weight of sericin has been reported as 309, 177, 145, 134, and 80 kDa [34], while other researchers noted that the presence of at least 15 distinct polypeptides in the anterior region of the middle silk gland with molecular weights ranging from roughly 20 to 200 kDa [37]. Sericin can be shown in a continuous distribution between 97 and 14 kDa, while some bands can also be seen above and below those two values [38]. The quality of sericin is highly influenced by the chemicals used in the extraction process and the physical conditions imposed during the extraction process.

The physical and chemical changes during the extraction process not only randomly cleave the peptide bonds but also effects the covalent and non-covalent interaction between silk fibroin and silk sericin, resulting in the peptides of dissimilar lengths and wide molecular weights (10–400 kDa) [39], [40]. During extraction from cocoons, the high temperature under a high-pressure technique might lead to dissolution. Previous research reported that the ionization of amino acids is altered by a change in the pH of the protein microenvironment (using acid or base combined with heat energy), which subsequently leads to variable lengths of silk sericin with varying percentages of α-helix, β-sheets, random coils, and turns [41]. Moreover, the percentage of β-sheets and random coils reflects SS's crystalline or amorphous nature, respectively [42], [43]. In the present study, electrophoresis analysis (with different temperatures and duration) demonstrated the breakage of sericin into different-length peptides, which showed wide-ranging molecular weights (15-250 kDa). Extraction through high temperature and high-pressure methods exhibited a high molecular weight of sericin, indicating the large percentage of β-sheets structure and liable for its crystallinity. Gupta et al. also reported the molecular weight distribution of sericin extracted from fabrics as a diffused band in the molecular weight range 6.5-205 kDa [35], which was similar to this result.

C. Fourier Transform Infrared Spectrophotometric (FTIR) Analysis

The FTIR spectra of sericin isolated from several silkworm cocoons are shown in Fig. 3. O-H stretching vibration is linked to the peak at 3700-3584 cm^{-1}. There are free hydroxyl groups present because sericin exhibits a peak at 3550.49 cm^{-1}.

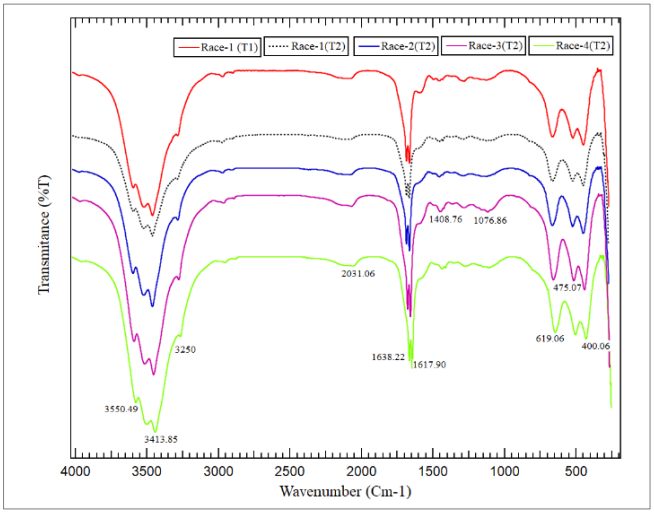

Fig. 3. FTIR spectra of sericin extracted from different silkworm cocoons with different temperature and duration.

While sericin exhibits this peak at 3413.85 cm^{-1}, the N-H stretching band is situated between 3500 and 3400 cm^{-1}. The protein's structure, such as its random coil and beta-sheet structures for the primary amide, is confirmed by the observation of overlapping peaks at 1645 and 1616 cm^{-1}, which are likely indicative of the presence of the C=O group of the amide linkage. No significant differences were observed for different silkworm races with different temperatures and extraction duration.

To enhance the properties of the air filter, sericin was extracted from three different waste species and coated the filter with silk sericin and characterize the extracted sericin through FTIR, and the peaks of that sericin were similar to the above findings [49]. Gulrajani *et al.* [44], and Song and Wei [45] also got similar FTIR spectra of sericin powder.

D. UV Spectroscopy

The UV spectrum of sericin derived from different silkworm races is shown in Fig. 4. Sericin contains 18 different kinds of amino acids; among them, serine, aspartic acid, and glycine are very important amino acids attributed to the physicochemical and functional properties of sericin. In addition, these different amino acids contain 70% hydrophilic amino acid, which is responsible for good solubility of sericin and water absorbability. On the other hand, the amount of aromatic amino acids is only 6.6% of the 18 kinds of amino acids that were identified by the UV spectrum [38]. Proteins absorb light strongly in the ultraviolet region mainly due to peptide bonds and aromatic acids. The proteins generally have two absorbance peaks in the UV region, one between 215-240 nm and the other 260-290 nm. The peptide bonds of amino acid absorb 215-240 nm regions of UV light. The aromatic amino acids like tryptophan, tyrosine, and phenylalanine are known to absorb UV radiation in the range of 260-290 nm due to $\pi \rightarrow \pi^*$ transition, and these absorptions can be used to assess the quality of sericin protein [35].

All samples of sericin obtained showed the characteristic peak between 282-285 nm for treatment T_1 and 283-284 nm for treatment T_3 which are assigned to the aromatic amino acids absorption wavelength. The shape of the absorption spectrum was found to be different for different samples.

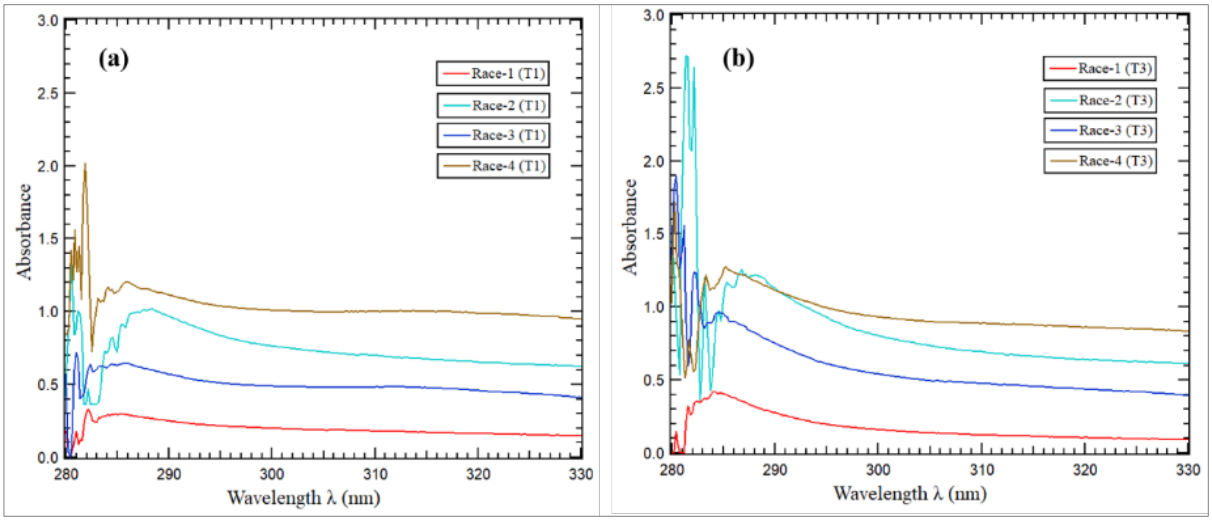

Fig. 4. UV-Spectra of sericin extracted from cocoons of different silkworm races Bombyx mori L. with different treatments: a) 90 °C and 15 minutes; b) 110 °C and 15 minutes.

Although the peaks for all sericin samples have similarities, variations were observed in peak intensities due to the changes in concentration. In some cases, the broadening of the peak indicates some changes in protein properties due to different temperatures and extraction duration. Capar *et al.* [48] reported that the reference sericin showed a distinct peak at λ=275 nm.

E. Thermogravimetric Analysis (TGA)

Under an inert environment, the samples were heated to a linear temperature increase of about 20 °C up to 800 °C at a constant heating rate of 10 °C/min. Fig. 12 displayed the thermo-gravimetric (TG) curves of sericin samples taken from silkworm cocoons at various temperatures and times. The TG curves of sericin samples were presented in the same pattern. Around 8% of weight loss at approximately 100 °C due to the dehydration of absorbed water in sericin samples. 50% weight loss i.e. T50 for all sericin samples was observed at the range of 400-460 °C. The final decomposition of the samples was in the range of 490-650 °C.

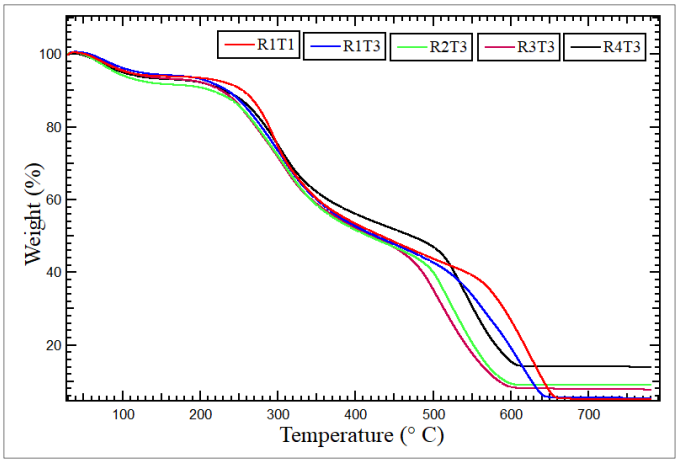

Fig. 6. Thermo gravimetric analysis of sericin samples.

Significant differences were observed in thermal stability among the sericin derived from different silkworm races. Sericin derived from silkworm race-1 showed the highest thermal stability with both 90 °C (15 min) and 110 °C (15 min) among the four different silkworm races. From the above TG curves, it was identified that the lowest extraction temperature and duration (R1T1) showed the highest thermal stability.

The decomposition temperature of all sericin samples was in the range of 470-580 °C which is similar to the other's observations [50]. Saha *et al.* also reported the thermal properties of sericin as a significant mass loss of 52% was observed at 400 °C. 11% mass was lost between 400 °C to 500 °C temperature [46]. Tsukada [47] also reported similar outcomes.

IV. Conclusion

A crucial component of the textile business is the silk cocoon. However, the sericin found in silk cocoons is typically discarded in our country. Due to its exclusive chemical properties, sericin has drawn attention to its use in skin care products. The results of the present study indicate that the quantity of the sericin varies with the different races of silkworms as well as with temperature and extraction duration. The multivoltine silkworm cocoon was found to contain more sericin compared to the bivoltine silkworm cocoon. Spectroscopic and other analyses showed that sericin did not change chemical properties during the extraction process. Significant differences were observed in thermal stability with different treatments for sericin extraction. The lowest extraction temperature and duration ($R1T_1$) showed the highest thermal stability. The UV absorption spectrum of sericin indicated the UV resistance capacity of sericin samples which may lead to use in pharmaceuticals, UV protective garments, and cosmetics products.

Acknowledgment

The authors thank the Director, BSRTI, and the head of the silkworm & mulberry and post cocoon technology sections of BSRTI for their heartiest assistance during this research. The authors also gratefully thank the Director of the Institute of Biological Sciences (IBSc), University of Rajshahi, for her valuable advice and logistic support.

FUNDING

The authors are thankful to the Director of the National Agricultural Technology Program- Phase II (NATP-2) Project, Bangladesh Agricultural Research Council, Dhaka, Bangladesh, for providing a Ph.D. scholarship and other financial support.

CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest.

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