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Extraction and characterization of silk sericin

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Study compares the quality and quantity of sericin obtained from four sources, namely mulberry silk cocoons, silk flats, reeling silk waste and woven silk fabric. Sericin has been extracted using the conventional HTHP machine as well as IR heating machine. Results show that among the four sources, the maximum yield of sericin (28%) is obtained from silk fabric followed by 25% sericin obtained from silk flats. IR machine extraction gives ~13 times higher yield of sericin as compared to that obtained by HTHP at 100^0C for 60 min. Sample obtained from IR extraction is also compared with the standard sample in terms of its physical properties, morphological structure, protein quality, protein structure, molecular weight and thermal behaviour using various analytical methods. The study proposes a protocol for assessing the quality of sericin protein. Since the source as well as the method of extraction can affect the properties of sericin, this protocol can be used to assess and determine the quality parameters of various sericin samples.

Keywords: Infrared spectroscopy, Mulberry silk, Protein analysis, Sericin, Silk

1 Introduction

Fibroin and sericin, along with very small amounts of waxy substances, mineral salts and coloring matter, are the main constituents of silk[1]. They both are proteins, accounting for about 75% and 25% of total silk weight respectively[2]. Sericin is primarily amorphous, more water soluble and acts as gum binder to maintain the structural integrity of the cocoon[3]. Sericin is rich in serine (about 32%), aspartic acid (16.8%) and glycine (8.8%), thus has a high concentration of hydroxyl groups[4].

Till recently, sericin has been considered as a waste by-product and usually discarded. But there have been continual efforts to recover and reuse it as a natural biopolymer in various applications. Recently, it has been found that sericin shows several important useful properties, such as antioxidant[5], anti-tyrosinase[6], UV absorbing and moisture absorbing properties[7]. Sericin can be used as a finishing agent for natural or manmade textiles. The cosmetics industry is using sericin in skin care products. It can also be a valuable natural ingredient in food industry. Fibroin and sericin have been used for biomedical polymeric applications[8,9].

Several processes like extraction with water at high temperature and high pressure (HTHP)[10], boiling-off in soap[11], alkalis[12], acidic solutions[13], aliphatic amines[14], enzymes[2] and bio-surfactants[15] have been used for degumming of silk. Recovery of sericin from soap alkali bath has been attempted using micro, ultra and nano filtration methods[16]. Drying has been carried out using spray drying[17,18], freeze drying or tray drying[19]. Other methods of purification include precipitation by ethanol for lab scale applications[20]. The properties of sericin obtained depend upon the method used for its extraction and recovery[21]. During degumming process, sericin is fully/partially hydrolyzed, and solubilized in degumming medium. Therefore, there is a need to develop extraction processes for sericin that are energy efficient, produce no chemical discharge and cause minimum damage to sericin.

Recently, different forms of energies have become available for laboratory applications in textile industry. Infrared dyeing and drying machine works on the principle of radiation heating where heat is transferred in the form of electromagnetic waves directly to the center of material without heating the surrounding air[21]. It is reported[22] that IR heating has higher thermal efficiency and faster heating rate as compared to convection heating, so it is gaining popularity.

A comprehensive analysis of the extraction conditions is important because these conditions can affect the final properties of sericin. In case of sericin, the extraction conditions should be carefully controlled since it is highly susceptible to heat. Extraction parameters such as temperature and time of treatment are known to affect the extraction efficiency and quality of sericin. Yield is important because it will determine the economic value of the sericin extraction method. In this study, sericin has

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been extracted from four different sources of mulberry silk and the effects of various parameters on extraction of sericin using infrared dyeing machine and HTHP machine are studied. Extracted sericin is characterized and compared with standard sericin sample.

2 Materials and Methods

2.1 Materials

Various sources of silk, namely silk cocoon, flat silk, reeling silk waste and woven silk fabric were used for extracting sericin. These sources represent various stages of processing of silk and thus possess varying levels of purity and quantity of sericin. Silk cocoon is the purest form of silk. However, cocoons are rare to find and are very expensive. Silk flats are produced by a special method where mature silk worms are kept on a spinning bed instead of a cocooning frame. The inclined spinning bed is rotated every few minutes to prevent the worm from spinning till it starts extruding silk on a flat surface thus flat sheets of silk are produced. They also yield a relatively pure form of silk. Silk waste is the silk left over after most silk has been reeled from the cocoon. The part that cannot be reeled is known as silk waste. It contains insect parts, is difficult to clean and is pre-processed, so quality of silk is poor. Silk waste is a by-product of the silk processing industry, relatively cheap and abundantly available. All silk fabrics are subjected to a degumming process for removing sericin from it. Therefore, this is the most obvious and abundant source for collection of sericin.

Mulberry silk cocoons and silk flats were procured from Seribiotech, Bangalore, India. Silk waste was procured from Central Silk Technological and Research Institute, Central Silk Board, Bangalore, India. Commercial silk fabric of 110 ends per cm, 40 picks per cm and weight of 61.60 GSM was procured locally.

The chemical reference substance, sericin (S 5201), extracted from *Bombyx mori*, was procured from Sigma Aldrich, USA. Analytical grade chemicals, sodium carbonate (Qualigen), sodium hydroxide (Qualigen), copper sulphate (Merck), sodium potassium tartarate (Merck), bovine serum albumin (Spectrochem) and Folin Ciocalteau's phenol reagent (Merck) were used for protein estimation. Deionized water was used for all extractions.

2.2 Methods

2.2.1 Extraction of Sericin

Degumming was carried out in an aqueous medium using two machines, namely high temperature high pressure (HTHP) beaker dyeing machine (R.B. Electronic and Engg. Pvt. Ltd, India) and IR dyeing machine (DLS 7000, Daelim Scarlet, Korea) at 100°C for 40 min as per the method developed by authors[23]. Extraction was carried out at different time and temperature. IR dyeing machine was used for extraction at 90, 100, 110 & 120°C for 15, 30, 60, 90, 120 & 150 min separately. HTHP dyeing machine was used for extraction at 100, 110, 120°C for 15, 30, 60, 90, 120 & 150 min separately.

2.2.2 Determination of Quality and Quantity of Sericin

Qualitative estimation of sericin was done by recording the ultraviolet spectra on D-2750 UV-Vis spectrophotometer (Shimadzu, Singapore) and then determining the A-ratio. A-ratio, signifying the purity of proteins, was determined by calculating the ratio of absorbance of the sample at 280 nm and 260 nm. Since nucleic acids absorb UV radiation at 260 nm and proteins absorb UV radiation at 280 nm, A-ratio above 1.8 typically corresponds to a sample that is free of contamination[24]. The quantity of sericin in the extracted liquor was calculated by determining the protein content by Lowry's method using bovine serum albumin (BSA) as a standard.

2.2.3 Preparation and Characterization of Sericin Powder

The sericin liquor extracted by using optimized conditions was converted into powder using a laboratory spray dryer LU-227 Advanced (Labultima, India), keeping inlet temperature at 110°C and the atomization pressure at 3-4 kg/ cm^2.

Moisture content was calculated using AOAC method (AOAC, 2000). Nitrogen content of sericin powder was determined by Kjeldahl method (IS: 7219 – 1973) and converted to protein content by a factor of 6.25. Ash content was determined according to AOAC method (AOAC, 2000)[25].

2.2.4 X-ray Diffraction (XRD)

XRD was conducted at ambient temperature with X'pert PRO X-ray diffractometer (PANalytical, Netherlands) having an X-ray tube producing monochromatic CuKα radiation. Powdered samples were mounted onto sample stage to record the crystallinity index. Sample stage was mounted on horizontal axis and the diffracted beam optics and the detector were mounted on 2θ axis. The scanning rate was 0.5°/min at 2θ = 5° - 35° under the acceleration voltage of 30 KV and 20 mA.

2.2.5 Spectroscopic Analysis

Fourier transform infrared (FTIR) spectroscopy

The FTIR spectra of sericin samples were recorded on BX FTIR spectrophotometer (PerkinElmer, US) in order to determine the functional groups present in

sericin. KBr pellet method was used and the spectra were recorded in the region of 4000 – 400 cm^{-1}.

Fluorescence spectroscopy

The fluorescence spectra were recorded at 25^0C on a Cary Eclipse, Varian Spectrofluorimeter. Typically, 1.0 – 2.0 µM protein in 10 mM Tris-HCl, *p*H 7.5 was used and the fluorescence emission spectra were recorded from 300 nm to 400 nm upon excitation at 295 nm. The excitation and emission slit widths were kept at 2 nm and 5 nm respectively. The fluorescence spectra were normalized and corrected for buffer contributions.

Circular dichroism (CD) spectroscopy

Far-UV CD spectra were recorded on a JASCO J-815 spectropolarimeter (Jasco Corporation, Tokyo, Japan) equipped with a Peltier-type temperature controller and a thermostated cell holder, interfaced with a thermostatic bath at 25°C using a cell with a path length of 0.1 cm. Typical spectral accumulation parameters were a scanning rate of 50 nm/min with a 2 nm bandwidth over the wavelength range 200–250 nm with six scans averaged for each far-UV spectrum using a protein concentration of 10–15 µM in 10 mM Tris–HCl and *p*H 7.5. The CD data are presented in terms of mean residue ellipticity (MRE, expressed as deg cm^2 $dmol^{-1}$) as a function of wavelength, using the following equation:

$$[\theta]_{\mathrm{MRE}} = \frac{MRW \times [\theta]_{obs}}{10 \times d \times c} \quad \ldots(1)$$

where $[\theta]_{\mathrm{MRE}}$ is the calculated mean residue ellipticity (deg cm^2 $dmol^{-1}$); *MRW*, the mean residue weight for the peptide bond [*MRW* is calculated as MRW = M/N^{-1}, where M is the molecular mass of the polypeptide chain (Da), and N is the number of amino acids in the chain]; θ_{obs}, the observed ellipticity (expressed in degrees); *d*, the path length (cm); and *c*, the protein concentration (gL^{-1}). All CD spectra were corrected for buffer contributions and secondary structures were calculated by using web based K2d neural network software package.

2.2.6 Thermogravimetric and SEM Study

TGA analysis of sericin powder was carried out on TGA-7 (PerkinElmer, US). The thermograms were obtained under nitrogen atmosphere at a uniform heating rate of 10^0C/ min in the temperature range 50 - 900°C.

Surface morphology of the sericin powder was observed under the scanning electron microscope EVO50 (Zeiss, Germany) at a magnification of 5 KX. Surface of specimen was coated with gold before analysis.

2.2.7 SDS-PAGE Analysis

The SDS-PAGE analysis of sericin was performed according to Laemmli[34]. Sample was mixed with an equal amount of SDS-PAGE sample buffer (0.5M Tris-HCl of *p*H 6.8, 2.5% SDS, 5% 2-mercaptoethanol, 10% glycerol) and boiled for 5 min. The resolving gel was 12% and stacking gel was 5%. The electrophoresis was carried out at constant voltage of 90V for 4h using an Enduro electrophoresis system (Labnet International Inc., USA). After electrophoresis, the gel was stained with Coomassie Brilliant Blue R-250. After staining the gels for 30 min, de-staining was carried out. Molecular weights were estimated using molecular weight marker (MWM) procured from Genni-India.

3 Results and Discussion

Sericin was extracted from different sources, namely cocoons, flats, silk waste and woven fabric. The liquor obtained from these extractions was subjected to qualitative assessment by spectroscopy and quantitative assessment by determining protein content. The results were benchmarked against standard sericin obtained from Sigma.

3.1 UV Spectroscopy

UV spectra of standard sericin and sericin extracted from various sources are presented in Fig. 1. Characteristic peak, attributed to the absorption of amino acids is observed at 275.40 nm in the standard sericin sample (A) as well as in the test samples (B – E). Proteins absorb strongly in the ultraviolet region mainly due to peptide bonds and aromatic acids. 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

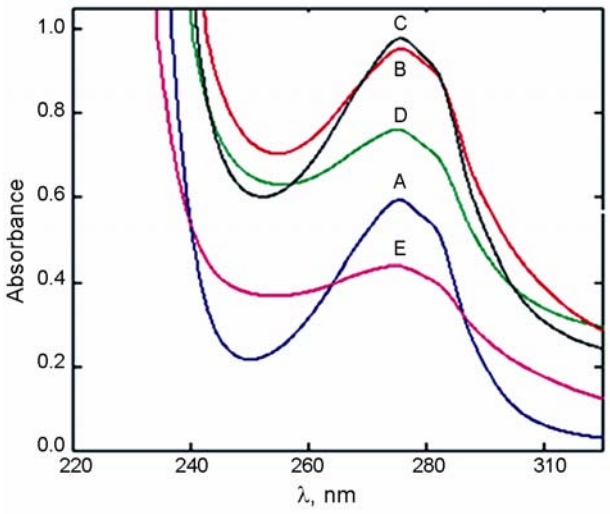

Fig. 1— UV spectra of sericin extracted from different sources (100^0C, 40 min) [A - Standard sericin, B - Cocoons, C - Fabric, D – Flats and E – Waste]

quality of sericin protein. Although all samples of sericin show the characteristic protein peak at 275.4 nm, the shape of the absorption spectrum is found to be different for different samples.

Sericin obtained from the cocoons (B), silk flats (D) and silk waste (E) show a broadening of the peak, as compared to the standard sericin, indicating some changes in the properties of protein. Sample C extracted from fabric shows a spectrum closest to that of the standard. This trend is endorsed by the values of yield (%) and A-ratio (A_{280}/A_{260}) for various samples given in Table 1.

Maximum yield of ~ 28% is observed with silk fabric. Slightly less yield is obtained with cocoons and silk flats. Minimum yield of ~ 22% is obtained from silk waste. It is observed that A-ratio of sericin extracted from different sources at same extraction conditions is different, indicating that the source has a great impact on the quality of sericin. The order of source based on A-ratio is as follows:

Standard sericin > fabric > cocoons > flats > waste

Standard sericin sample shows the highest A-ratio of 1.73, which is close to the ideal value of 1.8. The higher the A-ratio, the better is the quality of sericin. The next highest value of 1.35 is obtained for sericin extracted from silk fabric. The value of sericin extracted from cocoons also didn't match the ideal one. Sericin extracted from silk waste shows the lowest A-ratio of 1.09, indicating that some changes or damage occur in sericin during the process of silk reeling, which gives a broad peak in UV and a corresponding low value of A - ratio. Thus, silk fabric is found to give the maximum yield as well as highest quality of protein amongst all the sources.

3.2 Protein Content

Protein content of the sericin extracted from various sources is given in Table 1. Standard sericin is available in powder form, whereas other samples are available in the form of liquor, therefore, the results cannot be correlated. The protein content in liquor extracted from silk fabric is found to be maximum (31.62 mg/mL), while liquor extracted from fabric and cocoons show protein content of 29.12 mg/mL and 28.2 mg/mL respectively. Protein content in the liquor extracted from silk waste is found to be minimum (23.2 mg/mL).

Table 1 — A-ratio and protein content of various sericin samples

Source of sericin	A-ratio	Protein content mg/mL	Yield, %
Standard	1.73	-	-
Silk fabric	1.35	31.62	28
Cocoons	1.25	28.20	25
Silk flats	1.12	29.12	26
Silk waste	1.09	23.10	22

Thus, keeping in mind both the quality and quantity of sericin extracted from various sources, fabric is found to be the best source. Since silk fabric is the most easily and abundantly available source as compared to the others, it is decided to conduct all further studies with this source.

3.3 Extraction of Sericin using Different Sources of Energy

Conventionally, silk degumming is carried out with the help of soap and alkali. Alkali leads to degradation of sericin protein, however the industry continues to use this method as it is cheap and recovery of sericin is not a consideration for most processors. This method is highly polluting since it leads to addition of large amounts of soap and alkali into the effluent stream. More critically, it is extremely difficult to recover sericin from these effluent streams. In order to maximize the recovery of sericin and minimize the damage to protein, extraction by water is the best option. Conventionally, HTHP machines are available in process houses and have been used by some researchers to separate sericin from the fabric[26]. However, some recent studies on extraction of sericin from silk waste show that HTHP extraction is not efficient and damages the protein[27]. In the current study, infrared heating is used for the extraction of sericin from silk fabric and results are compared with HTHP method.

Figure 2 shows the yield of sericin obtained from extraction of fabric for different time and temperature of extraction, using IR and HTHP machine.

Yield of sericin at 15 min of extraction time in IR method at 100^0C is found to be ~12.6%. Yield is found to increase slightly with increase in time with maximum reaching upto ~14.6% at 150 min. Further increase in temperature to 120^0C does not result in increase in yield. Trend observed in HTHP extraction is, however, quite

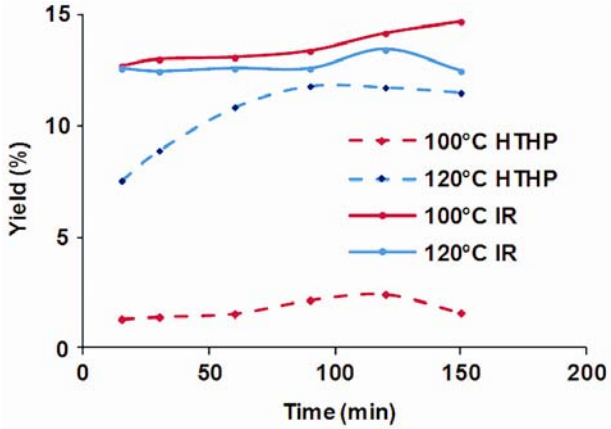

Fig. 2 — Effect of time and temperature

different. Yield in all cases is much lower than that obtained in IR method. Effect of temperature is much more prominent in HTHP as compared to that in IR heating bath. Yield at 100^0C is very low (~3%) even after 120 min of extraction. Yield is found to increase with time as well as temperature of extraction. Maximum yield of ~12% is obtained at 120^0C for 90 min of extraction. These observations clearly show that IR heating is a much more efficient method of sericin extraction as compared to HTHP heating. The yield is higher and time and temperature required are also less. This finding is similar to results reported earlier[23].

The difference between yield from IR and HTHP method is likely due to the principle of heating employed in the two cases. It can also be seen that IR is much more efficient than HTHP in removing sericin from fibroin since the bulk of sericin is removed within 15 min. Since sericin, like all proteins, is prone to degradation at high temperatures, 15 min at 100°C is taken as the suitable procedure for the extraction of sericin from silk fabric.

The UV spectra of standard sericin and of that extracted by HTHP and IR machine show the characteristic peak at 275.4 nm. However, slight peak broadening is observed for samples B and C, thus suggesting some changes in the sample. Also, higher A-ratio (1.35) which is indicative of protein quality is observed for the sericin sample extracted in IR machine as compared to that extracted in HTHP (Table 2). The results suggest that the source of energy i.e. the method of heating has a very important role in the quality of protein obtained.

Since, a lower temperature is preferable to prevent denaturation of proteins, it can be concluded from these results that extraction in IR machine at 100^oC for 15 min is the most optimum method of extraction.

3.4 Characterization of Sericin

Sericin extracted from fabric at 100°C for 15 min using infrared heating is found to give the better quality and quantity of sericin. Therefore, this liquor is converted to powder and the characterization data for its composition, spectroscopic and thermal properties and molecular weight are generated. Results are benchmarked against the standard sericin sample and presented in the following paragraphs.

Table 2 — A-ratio and yield of sericin extracted from fabric

Source of energy	A-ratio	Yield , %
Standard	1.73	--
HTHP (120°C, 90 min)	1.29	11.78
IR (100°C, 15 min)	1.35	12.68

The sericin powder extracted from fabric and the standard sericin sample are characterized in terms of moisture, ash, nitrogen and protein content and the results are compiled in Table 3. Moisture content of the standard sericin sample is found to be slightly higher than the sericin extracted from fabric. The ash content of standard sample is also found to be higher than the sericin sample recovered from fabric. This may be because a different method of extraction may have been used for the standard, resulting in higher residual matter. Gulrajani *et al.*[18] have reported that ash content can vary from 0.8% to 5.2% in sericin extracted from cocoons using HTHP and alkali method respectively. Wu *et al.*[28] report an ash content of 4.2% in sericin extracted from cocoons using HTHP method.

The nitrogen content of the sericin powder is also estimated and the protein content is calculated by multiplying the value by 6.25. Sericin sample obtained from the fabric shows higher nitrogen content (14.13%) and hence higher protein content (88.31%) as compared to the standard sericin sample. Similar values (14.65% nitrogen) have been reported earlier[28].

3.5 X-ray Diffraction Analysis

X-ray diffraction has been carried out to study the crystalline structure of sericin samples. Three types of conformations have been proposed for silk protein[28,29]. The glandular state prior to crystallization is called Silk I. Silk II is the spun silk state which consists of the β-sheet secondary structure and Silk III (an air/water assembled interfacial silk) is a helical structure. The main diffraction peaks of Silk I are present at around $2\theta = 12.2°$ and 28.2°, while Silk II are present at about $2\theta = 18.9°$ and 20.7°.

The XRD curves of sigma sericin and sericin extracted from fabric exhibit a strong diffraction peak around $2\theta = 20.5°$. It is observed that the peaks are broad, indicating that the powders are amorphous. XR diffractograms are found to be similar for all sericin samples including the reference sample. Similar results have been reported in earlier studies[28].

3.6 FTIR Analysis

In FTIR spectra, proteins show characteristic vibration bands in the range 1630 - 1650 cm^{-1} for amide I (C-O stretching), 1540 cm^{-1} –1520 cm^{-1} for amide II (secondary N–H bending) and 1270 cm^{-1} –1230 cm^{-1} for

Table 3 — Composition of sericin powder

Characteristic	Sigma sericin	Sericin from silk fabric
Moisture content, %	17	10.4
Nitrogen, %	13.59	14.13
Ash content, %	6	3
Protein, %	85	88.31

amide III (C–N and N–H functionalities). In addition, the positions of these bands conform the protein materials, such as 1650 cm^{-1} (random coil) and 1630 cm^{-1} (β-sheet) for amide I; 1540 cm^{-1} (random coil) and 1520 cm^{-1} (β-sheet) for amide II, and 1270 cm^{-1} (β-sheet) and 1230 cm^{-1} (random coil) for amide III.

Sericin extracted from fabric shows absorption between 1600 cm^{-1} and 1700 cm^{-1}, confirming amide I absorption which arises predominantly from the C=O stretching vibration and is most useful for determining proteins secondary structure. The peak of sericin at 1540 cm^{-1} belongs to amide II which arises because of the random coil structure. Signature peak for sericin at 1400 cm^{-1} is observed in case of both samples. In addition, the amide III characteristic peak, which arises mainly from the C-N stretching vibration coupled to the N-H plane bending vibration, is found to shift in the range 1240 cm^{-1} - 1250 cm^{-1} corresponding to a change from random coil conformation to β-sheet structure. No major difference is observed in IR spectra of standard and test samples extracted from different sources and prepared from different methods. Results are similar to those reported by Teramoto and Miyazawa[30] and Zhang and Wyeth[31].

3.7 Fluorescence Spectra

Fluorescence spectra of the standard and test samples have been recorded to determine if the extraction parameters affect the conformational state of sericin in any way. Spectra are shown in Fig. 3(a). It can be seen that standard sericin (A) gives a distinct peak at 355 nm, while sericin obtained from fabric (B) shows a minor peak at around 355 nm and a distinct peak at 380 nm.

The fluorescence of a folded protein is a mixture of the fluorescence from individual aromatic residues. Most of the intrinsic fluorescence emissions of a folded protein are due to excitation of tryptophan residues, with some emissions due to tyrosine and phenylalanine. Typically, tryptophan has the excitation wavelength of 295 nm and an emission peak that is solvatochromic, ranging from 300 nm to 350 nm depending on the polarity of the local environment[32]. In Fig. 3(a), the extracted sericin sample shows a fluorescence pattern which is different from that of the standard sample. This indicates that some changes do take place in the sericin, during extraction processes that affect the conformational state of sericin protein. The effect of these changes on the biological and performance properties such as cell regeneration, antioxidant property and moisture absorption needs to be established to understand the relevance of the change in structure.

3.8 Circular Dichroism (CD) Spectra

CD spectroscopy measures the differences in the absorption of the left handed polarized light versus a right handed polarized light and can be used to determine the proteins secondary structure in the far-UV spectral region (190 – 250 nm). At these wavelengths, a chromophore is a peptide bond, and a signal arises when it is located in a regular, folded environment, α-helix, β-sheet and random coil structures that provides characteristic shape and magnitude of a CD spectrum. The negative peak at 208 nm is characteristic of an α-helix protein and a negative peak at 214 nm is characteristic of β-sheet of protein[27]. CD curves (Fig. 3b) of the IR extracted sericin sample in this study show a negative band at 206–208 nm suggesting α-helix conformation. Gulrajani *et al.*[18] found that sericin recovered from HTHP degumming liquor shows a strong negative band at 198 nm and a weak band at 218 nm suggesting random coil and β-sheet configuration respectively. On the other hand, sericin recovered from alkaline degumming shows a negative peak at 201 and 216 nm, revealing the presence of α-helix structure[18]. Wu *et al.*[28] also reported similar findings for the secondary structure of sericin

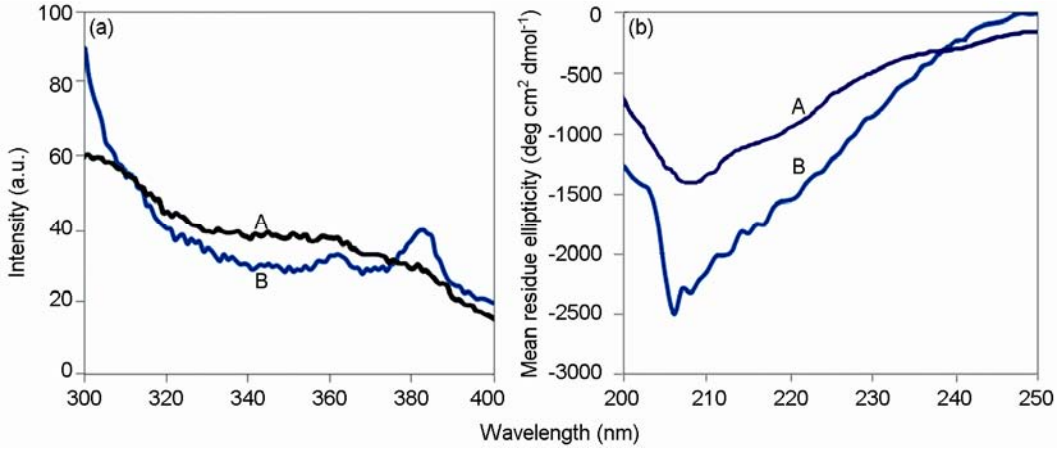

Fig. 3 — (a) Fluorescence spectra and (b) CD spectra of sericin samples [A - standard sericin, B - sericin from silk fabric]

prepared by ethanol precipitation. Low molecular weight sericin recovered from *A. mylitta* also shows similar peaks in its CD spectra[33]. From these observations it can be concluded that sericin can show both α- helix and β- sheet structure depending on the method of extraction.

3.9 Molecular Weight

The molecular weight distribution of the different sericin samples was determined using SDS-PAGE method and the results are shown in Fig. 4. The sericin recovered from fabric (D) shows a diffused band in the molecular weight range 6.5 - 205 kDa, while standard sericin (B&C) shows bands in the low molecule weight range, 3.5 - 43 kDa.

The molecular weight of standard sericin is found to be much lower than that of extracted silk. When the two samples are dissolved in water, their solubility is also found to be different. While standard sericin with lower molecular mass (< 50 kDa) is found to be readily soluble in cold water, extracted sericin with a higher molecular mass between 50 kDa and 200 kDa could only be dissolved in hot water (90°C). This large difference in molecular weight can have a significant effect on the properties and applications of sericin.

It has been reported that sericin consists of a group of protein molecules of molecular weight ranging from 20 kDa to 400 kDa. Gamo *et al.*[34] estimated the molecular mass of sericin to be 309, 177, 145, 134 and 80 kDa, whereas Speragues[35] reported at least 15 different polypeptides having molecular weight ranging from about 20 kDa to 200 kDa in the anterior portion of middle silk gland. Wu *et al.*[28] showed that sericin appears in a continuous distribution between 97 kDa and 14 kDa, while some bands are observed above and below 97 kDa and 14 kDa respectively.

3.10 Thermogravimetric Analysis

Thermal behavior of sericin samples was examined by TGA. The weight loss trace indicates that the initial weight loss occurred because of the evaporation of water. Thereafter, an abrupt decrease in weight is detected in the wide temperature range from 220°C upward for both the samples. Similar weight loss pattern has been reported for sericin by Tsukada[36]. It is clear from the weight loss pattern in the thermograms that sericin extracted from the fabric exhibits higher weight loss as compared to the standard sericin, thus indicating that the sericin prepared from fabric is relatively unstable to temperature. Higher residue is obtained in standard sericin powder (9%) as compared to the sericin powder recovered from fabric (5%). These results are in a good agreement with per cent ash content as reported above.

3.11 Morphological Studies

The morphology of sericin powders was studied using a scanning electron microscope. As can be seen from the micrographs (Fig. 5), the particle shape is

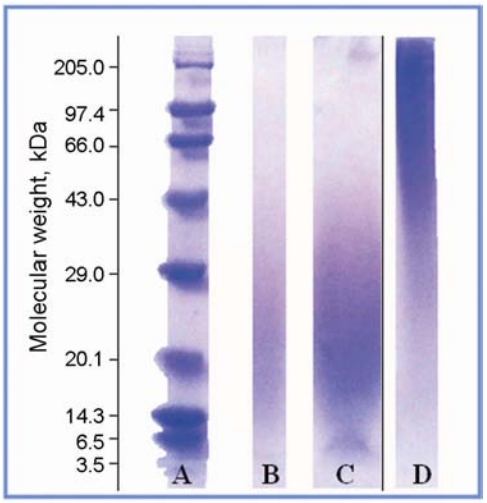

Fig. 4 — SDS-PAGE analysis of sericin samples [A - Marker, B - Standard, 20 mg/mL, C - Standard, 60 mg/mL and D - Sericin from fabric, 10 mg/mL]

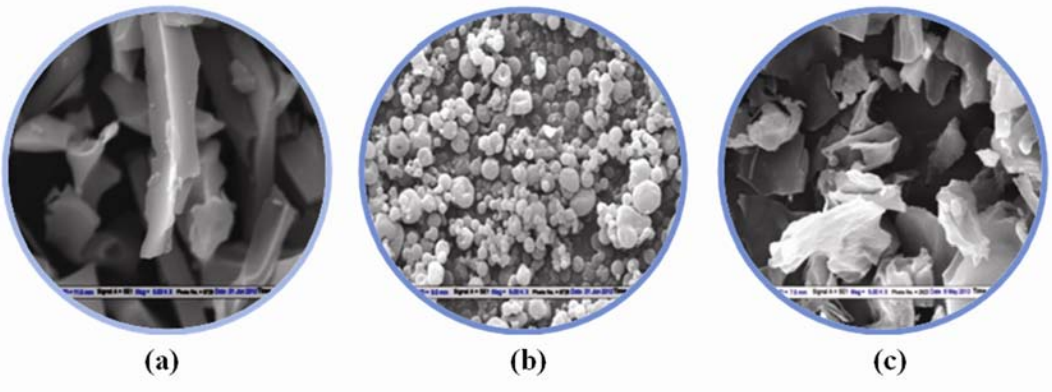

Fig. 5 — SEM images of sericin samples (× 5K magnification) [A - standard, B - spray dried sample, and C - freeze dried sample]

found to be very different in the two cases. The standard sample is found to be made up of long planar size particles, the test sample has spherical globules. Another significant observation is that the spherical globules have a very wide size distribution and appear to be highly aggregated. Such particles generally have problems with solubility. To study if the particle shape is indeed characteristic of a drying technique, another test was conducted where the extracted liquor was freeze dried instead of spray drying. The SEM of the powder obtained by freeze drying is given in Fig. 5(c).

Planar shaped particles are obtained indicating that particle shape is a function of the method of drying. All other properties of the new powder are found similar to the globular particles.

4 Conclusion

Results of this study show that the quality of sericin varies with the source as well as the method of extraction. Woven silk fabric yields the best quality as well as quantity of sericin while silk waste yields the poorest quality. IR heated machine is much more efficient than HTHP machine as extraction is higher and faster with IR. Surface morphology is affected by the method of drying. Spray drying yields globular aggregated particles while freeze drying gives planar sheet like particles. Molecular weight of sericin could vary from 20 kDa to 200 kDa depending on the source or method of extraction. Higher molecular weight sericin is soluble only in hot water while low molecular weight sample dissolves easily in water. Spectroscopic and other analyses shows that sericin does undergo some degree of damage during extraction process. The effect of this degradation on the performance properties needs to be determined. These findings can be used to draw up a protocol for assessing the quality of sericin protein obtained from various sources or various methods of extraction and processing.

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