Studies on Structural and Functional Properties of Sericin Recovered from Silk Degumming Liquor by Membrane Technology

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ABSTRACT: This article describes the recovery of silk sericin from three different degumming liquors, that is, high temperature high pressure (HTHP), alkaline, and soap plus alkali (SPA), using membrane filtration technology. The recovery of sericin by membrane technology results in the reduction of about 78–85% chemical oxygen demand as well as biological oxygen demand values in the final discharge liquor. The sericin powders produced from the purified degumming liquors have been characterized in terms of color, nitrogen content, protein content, ash content, and thermogravimetric analysis and compared. It has been found that the sericin recovered from HTHP degumming liquor has about 98% protein content as compared to that recovered from alkaline (92%) and SPA (67%) degumming liquors. The molecular weight distribution and secondary structure of the recovered sericin powders have been determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and circular dichroism (CD) spectroscopy, respectively. The molecular weight range of sericin recovered from HTHP, alkaline, and SPA degumming liquor was 20–>205 kDa, 20–97 kDa, and 20–43 kDa, respectively. The secondary structure of sericin recovered from HTHP degumming showed random coil conformation with some β sheet structure. The sericin recovered from alkaline and SPA degumming liquors showed denaturation with some random coil, β sheet, and α helix conformation. The functional properties of the three different recovered sericin powder samples in terms of moisture content and ultra protection factor (UPF) have also been evaluated and compared. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 113: 2796–2804, 2009

Key words: silk sericin; recovery; membrane technology; molecular weight; CD analysis

INTRODUCTION

Sericin is a kind of hot water-soluble macromolecular globular protein that envelops the fibroin fibre with successive sticky layers. It helps in the formation of cocoon and contributes about 20–30% of the total cocoon weight. It is made of 18 amino acids most of which have strongly polar side groups such as hydroxyl, carboxyl, and amino groups.[1–4] Sericin is being used in cosmetics because it inhibits the tyrosinase activity and this enzyme is responsible for biosynthesis.[5] Masahiro et al.[6] reported that consumption of sericin enhances bioavailability of Zn, Fe, Mg, and Ca in rats and suggested that sericin is a valuable natural ingredient for food industry. Use of sericin as a finishing agent for natural and synthetic textiles enhances their moisture absorption, antistatic, softness, and comfort properties.[7] Filters made of polyamide or polyester fibers coated with sericin gave antioxidation and antimicrobial activity, suggesting their potential use as indoor air filters to reduce free radicals and fungi and bacteria contamination.[8] Sericin can be crosslinked, copolymerized, or blended with other polymers to produce a new range of biodegradable materials with improved properties.[4] Thus because of its varied properties, sericin can be used as an additive in food, cosmetics, textiles, and pharmaceutical products.

To generate luster and soft feel on silk textiles, the sericin protein is removed through a process known as degumming, prior to dyeing. The degumming of silk is usually carried out by using chemical or biochemical systems or with water alone under pressure. The processing of raw silk produces about 50,000 tons of sericin, worldwide each year. Major part of it is discarded into the waste water stream, which leads to high chemical oxygen demand (COD) and biological oxygen demand (BOD) level.[4] Therefore, the waste water released by silk industry leads to contamination of water and environment.

Over the last decade, due to its valuable properties such as antioxidant, UV protection, moisture absorption, antibacterial activity, etc, various methods have been developed and patented to recover

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this protein material from degumming liquor.[9–15] These methods are based on adsorption, precipitation, coagulation, evaporation, chromatography, and ultrafiltration. The recovery of this protein substantially reduces the pollution load in the waste water.

In the recent years, several studies have focused on the structural and functional properties of sericin recovered from the degumming liquor. Kurioka et al.[16] have studied the morphology and biochemical properties of sericin powder recovered from different degumming liquors through alcohol precipitation method. In this study, the sericin powder obtained from acid degumming liquor showed strong trypsin inhibitor activity as compared to sericin obtained from boil-off degumming liquor. The sericin obtained through alkaline degumming had no trypsin inhibitor activity. Wu et al.[9] have found that sericin recovered from high temperature high pressure (HTHP) degumming waste liquor by alcohol precipitation has wide range of molecular weight distribution and random coil conformation. The recovered powder showed the antioxidant and tyrosinase inhibition activity.

Most of the structural functional studies are based on the biological properties of the sericin and there is limited literature available on its moisture absorbance[17] and UV protective properties. Moreover, limited studies report the properties of sericin recovered using membrane technology, which is a physical separation process and is carried out at ambient condition without use of any chemicals.

The aim of this study was to compare the structural and functional properties of spray dried sericin powder produced from various degumming liquors such as that from HTHP, alkaline, and SPA purified by membrane filtration technology.

MATERIALS AND METHODS

Materials

Mulberry (*Bombyx mori*) silk cocoons were procured from Central Silk Research Institute of Technology, Central Silk Board, Bangalore, India. HTHP and alkaline degumming liquors were prepared in the laboratory. The HTHP degumming liquor was prepared by boiling the silk cocoons at 115°C for 30 min, keeping material to liquor (M : L) ratio 1 : 40 using lab scale HTHP machine. The alkaline degumming liquor was prepared by treating the chopped and cleaned silk cocoons in the bath containing sodium carbonate (1.06%), sodium bicarbonate (0.84%) buffer at 100°C for 60 min, keeping material to liquor (M : L) ratio 1 : 40.[1] SPA degumming liquor was obtained from silk processing industry, Nath Brothers Exim, Noida, India. Electrophoresis grade acrylamide, *N*,*N*′-methylenebisacrylamide, TEMED (*N*,*N*,*N*′,*N*′-tetramethylethylenediamine), ammonium persulfate, Bromophenol Blue, Coomassie Brilliant Blue-250, and 2-mercaptoethanol, were procured from Sisco Research Laboratory, Mumbai, India. Electrophoresis grade glycine (Merck), sodium dodecyl sulfate (BDH), urea (Qualigen), Tris (Merck), and glycerol (Merck) was used. The reagent grade chemicals sodium carbonate (Qualigen), sodium bicarbonate (Qualigen), sulfuric acid (Merck), Bromocresol Green (Rankem), Methyl Red indicator (Renkem), acetic acid (Merck), methanol (Merck), sodium hydroxide (Merck), and sodium azide (Merck) were used for analytical purpose.

Methods

Recovery of sericin from various degumming liquors

The HTHP or alkaline degumming liquor was prefiltered with Whatman filter paper grade 1 (11 μm pore size). The filtrate was subsequently filtered and concentrated on ultrafiltration membrane system (Amicon 8000, Millipore) having membrane of 10 kDa molecular weight cut off. A schematic diagram of the process is shown in Figure 1(a).

The SPA degumming liquor was centrifuged at 9000 rpm for 60 min. After centrifugation, the soap and large suspended particle were removed in the form of pellet. The supernatant liquor containing sericin was taken out and filtered with Whatman filter paper grade-1 (11 μm pore size). The filtrate was again microfiltered (pore size 0.22 μm) to remove the remaining soluble soap. The microfiltrate was simultaneously filtered and concentrated on 10-kDa molecular weight cut-off ultrafiltration membrane system. A schematic diagram of the process is shown in Figure 1(b).

Preparation of sericin powder

The recovered sericin solution obtained from different degumming liquors was converted into the powder form on laboratory spray drier (Model-LU–227 Advanced, Labultima, Mumbai, India), keeping inlet temperature 180°C and the atomization pressure 3.00 kg/cm^2.

Environmental parameters

The chemical oxygen demand (COD) and biological oxygen demand (BOD) of the initial degumming liquor and the final discharge water were evaluated according to APHA standard test methods.[18]

Characterization of sericin powder

The ash content of the recovered sericin powder samples was determined according to the AOAC

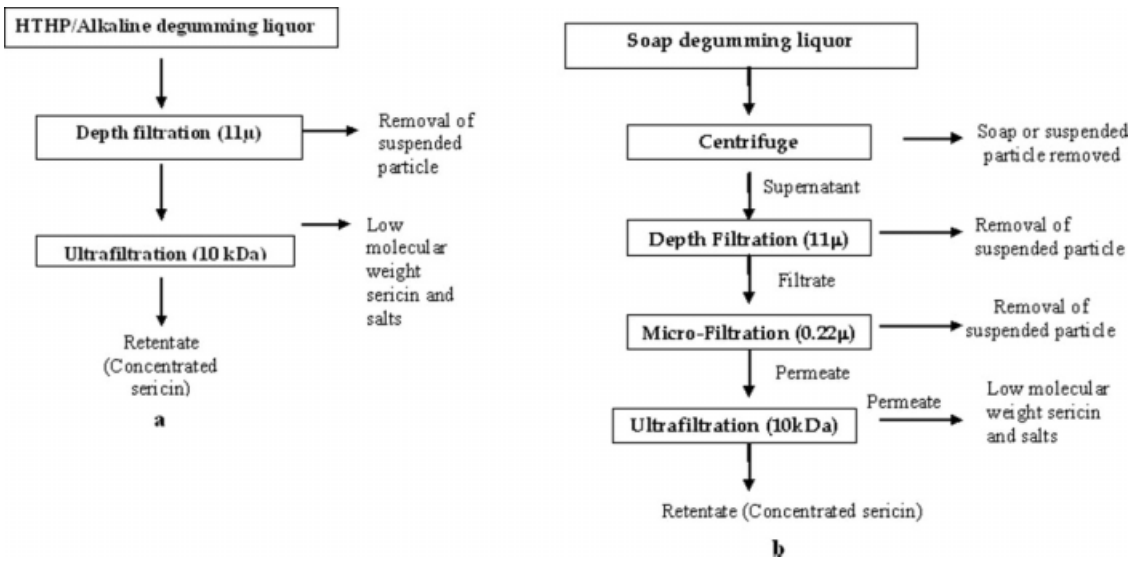

Figure 1 A schematic diagram of the recovery of sericin from (a) high temperature high pressure (HTHP)/alkaline degumming liquor and (b) soap plus alkali (SPA) degumming liquor.

900.02 method.[19] Nitrogen content and crude protein content ($N \times 6.25$) of sericin powders was estimated by Kjeldahl method AOAC 2000.[19] The sodium content of the sericin powder samples was determined according to ASTM D 4291-03 method.

Thermogravimetric analysis

Thermogravimetric (TGA) studies of sericin powder were carried out on Perkin-Elmer TGA-7 system (MA). The thermograms were obtained under nitrogen atmosphere at a uniform heating rate of 10°C/min in the temperature range 50–900°C.

Molecular weight distribution

The sericin solution (1 wt %, 1 mL) was mixed with 960 mg urea and adjusted to 2 mL with deionized water. The 8*M* urea-denatured solution was mixed with an equal amount of SDS-PAGE sample buffer (0.1*M* Tris-HCl of pH 6.8, 4% SDS, 12% 2-mercaptoethanol, 20% glycerol) and boiled for 5 min. SDS-PAGE was performed according to the procedure followed by Laemmli[20] using 15% resolving gel and 5% stacking gel. The electrophoresis was carried out at 8 mA for 1 h and subsequently at 15 mA for 2 h. After electrophoresis, the gel was stained with Coomassie Brilliant Blue R-250. Molecular weights were estimated using Molecular Weight Marker (MWM) procured from Genni-India.

Circular dichroism

The circular dichroism (CD) studies of sericin samples were performed on a JASCO J-810 spectropolarimeter (Easton, MD), using 0.1 cm path length quartz cell at room temperature. The UV spectrum was collected at protein concentration of 1 mg in 10 mL hot deionized water. The data points were recorded with a step resolution of 1 nm, time constant of 4 s, sensitivity 10 mdeg, scan speed 50 nm/min, and spectral bandwidth of 1 nm. To reduce random error and noise each spectrum was an average of three scans in the range 250–190 nm.

Infrared analysis

The infrared (IR) spectra of the sericin samples were recorded between 400 and 4500 cm^{-1} on a Perkin-Elmer Spectrum-BX FTIR system (MA) using KBr pellet technique. The KBr pellets were prepared by grounding 1 part of the sample with 9 parts of spectral-grade KBr and pressed in an evacuated die under suitable pressure to get pellets.

Morphology of the sericin powder

The appearance and shape of the sericin powder samples were investigated by placing the powder on aluminum stubs using double-sided adhesive tape. The samples were then coated with silver and examined with scanning electron microscope (ZEISS EOV 50, Oberkochen, Germany) operating at 0.3–30 kV accelerating voltage.

Functional properties of sericin

Moisture content

The sericin powder samples were conditioned (65% RH and 25°C ± 2°C) for 48 h prior to performance testing. The moisture content of the sericin powder

was measured using Sartorius Moisture Analyzer (MA 51, 98648-002-76, Goettingen, Germany) where,

$$\text{Moisture content}\,(\%) = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

UV absorption

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 Perkin-Elmer (MA) Lambda 25 software.

Uniform thickness films of 5% sericin solution were prepared. Mean ultra protection factor (UPF) and Rated UPF of the sericin films were measured as per Australia/New Zealand standards in Sun Penetration and Protection Measurement System of SDL, ATLUS, UK. UPF is defined as the ratio of the average effective UV irradiance calculated for unprotected skin to the average effective UV irradiance calculated for skin protected by the test specimen. According to this method, the UPF of each film is calculated as follows:

$$\text{UPF} = \frac{\sum_{\lambda=290}^{400} E_\lambda S_\lambda \Delta_\lambda}{\sum_{\lambda=290}^{400} E_\lambda S_\lambda \Delta_\lambda T_\lambda}$$

where E_λ is the CIE relative erythemal spectral effectiveness; S_λ is the solar spectral irradiance; T_λ is the spectral transmittance of the sericin film; Δ_λ is the wavelength step in nm; and λ is the wavelength in nm.

RESULTS AND DISCUSSION

In this study, sericin recovered from various degumming liquors, using membrane filtration technology followed by spray drying, has been characterized and compared. The advantage of membrane technology is that, it is primarily based on the physical separation and minimizes physical damage to biomolecules from shear effects.[21]

It is well known that the structural and functional properties of the sericin are dependent on its molecular weight distribution and secondary structure. The molecular weight distribution and secondary structure of recovered sericin depends on two major factors namely (1) method of degumming and (2) method of recovery. Sericin is easily degraded by heat or chemical treatment, during its separation from fibroin threads. Depending on the method used, sericin removal from the fibroin is a combination of dispersion/solubilization and hydrolysis process, which produces a wide range of the soluble sericin polypeptides.[22] The recovery of sericin from the degumming liquor thus affects its structural and functional properties. The addition of chemical or exposure to heat (high temperature) causes the denaturation of sericin polypeptides during degumming as well as recovery process.

Environmental impact

The COD and BOD of degumming liquor were measured initially and after the recovery of sericin from the various degumming liquor. The results are summarized in the Table I. Data suggests that, in case of HTHP degumming liquor, the concentration of sericin in permeate is highly reduced after ultrafiltration which results in about 78% reduction in COD and BOD value in the discharged waste water. In case of SPA degumming, recovery of sericin reduced the COD and BOD load by about 85%. However, the COD value (1123–2772 mg/L) of final discharge liquor is higher than the standard COD value (200–1000 mg/L) of the final discharge of waste water. Similar results have been reported by the other researchers where the recovery of sericin from the degumming liquor using ultrafiltration technique reduced the COD and BOD values in the range of 85–95%.[14,15] The recovery of sericin from the degumming liquor using membrane filtration technology thus has significant environmental benefit, as it reduces the effluent load in the silk industries.

TABLE I
Environmental Parameters of Degumming Liquor Before and After the Recovery of Sericin

Degumming process	Parameters	Initial degumming liquor	Final discharge liquor	% Reduction
HTHP	COD (mg/L)	8750	1900	78
	BOD (mg/L)	4700	1050	78
Alkaline	COD (mg/L)	7645	1123	85
	BOD (mg/L)	2367	894	62
Soap plus alkali	COD (mg/L)	19404	2772	86
	BOD (mg/L)	8220	1092	87

TABLE II
Analytical Parameters of Sericin Powder Recovered from Different Degumming Liquors

S. no.	Parameters	HTHP	Alkaline	SPA
1	Color	Light yellow	Brown	Cream
2	Nitrogen content (%)	15.8	9.2	14.7
3	Protein content (%)	99.0	92.0	58.0
4	Ash content (%)	0.8	5.2	22.0
5	Sodium content (ppm)	–	698	89474

Characterization of sericin powder

The sericin powder recovered from different degumming liquors were characterized in terms of color, nitrogen content, protein content, ash content, and sodium content and the results are summarized in the Table II. The sericin powder obtained from different degumming liquors had a different color, namely, cream for sericin powder recovered from SPA degumming liquor, pale yellow for sericin powder recovered from HTHP degumming liquor, and brown for sericin powder recovered from alkaline degumming liquor. Yellow color of sericin may be due to flavanol which is originally present and the change in color may be attributed to the modification of flavanol due to action of heat and chemicals. The color of sericin powder also depends on heat exposure during degumming process. Kurioka et al.[23] have also reported that increase in the temperature of HTHP degumming process from 115 to 125°C, the color of sericin changed from dull yellow to dark brown. Tsukada[24] have found that the color of sericin cocoon (Nds/Nd-S) is changed from white to light yellow, deep yellow, light brown, and black on heating from 170 to 210°C.

The ash content of the sericin powder was determined according to the AOAC 900.02 test method. Sericin powder produced from SPA degumming liquor showed highest amount of ash content (i.e., 22.03%) followed by that produced from alkaline degumming liquor (i.e., 5.25%). However, sericin powder obtained from HTHP degumming liquor had only 0.8% ash content. This may be due to the higher residual salt in the sericin powder. During alkaline and SPA degumming, alkali is absorbed by sericin and the COOH groups get converted into $COO^{-}Na^{+}$. These bounded sodium ions are difficult to remove from the sericin during membrane filtration. An estimation of the sodium content by atomic adsorption spectroscopy in the sericin powder recovered from alkaline and SPA degumming liquor indicated that the sodium content was higher in sericin recovered from SPA degumming liquor (i.e., 899473 ppm) as compared to the sericin recovered from alkaline degumming liquor (i.e., 698 ppm).

The thermal behavior of the sericin powders was examined by TGA and the results are shown in Figure 2. All the different sericin powder samples showed a continuous weight loss that may be due to degradation of sericin at higher temperature. The percent residue of the powders indicated that there was no residue left in powder recovered from HTHP degumming liquor. The percent residue in powder recovered from alkaline degumming liquor and SPA degumming liquor was 8 and 22%, respectively. These results are in a good agreement with percent ash content as reported above.

The nitrogen content and the protein content of the sericin powders were also estimated and the results are shown in Table II. The HTHP sericin powder is purer (nitrogen content 15.8%, protein content 98.7%) as compared to sericin powder obtained from alkaline and SPA degumming liquors. Wu et al.[9] have reported a 90% protein content and 4.2% ash content in their study on recovery of sericin from HTHP degumming liquor. Similar results

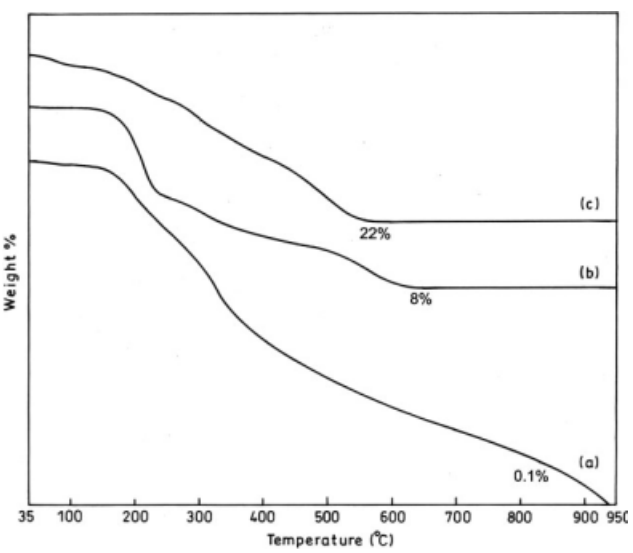

Figure 2 TGA thermograms of sericin powder recovered from (a) high temperature high pressure (HTHP), (b) alkaline, and (c) soap plus alkali (SPA) degumming liquors.

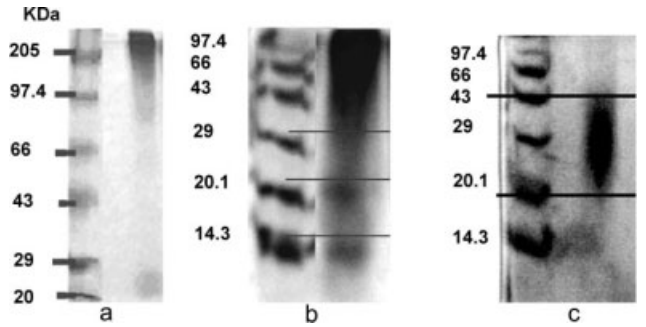

Figure 3 Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of sericin powder recovered from (a) high temperature high pressure (HTHP), (b) alkaline, and (c) soap plus alkali (SPA) degumming liquors along with molecular weight markers.

have been reported by Vaithanomsat and Kitpreechavanich[14] for the sericin powder recovered from SPA degumming liquor using tray drying, freeze drying, and membrane technology.

Molecular weight distribution

In general, sericin consists of group of protein molecules ranging from 20 to 400 kDa.[4] Gamo et al.[25] estimated the molecular masses of sericin to be 309, 177, 145, 80, and 134 kDa. Whereas Sperage reported at least 15 different polypeptides ranging from about 20 to 200 kDa in the anterior portion of middle silk gland.[26] Wu et al.[9] conducted the SDS-PAGE analysis and results indicated that sericin appeared in a continuous distribution between 97 and 14 kDa, and there were some bands above 97 kDa and also some bands below 14 kDa. Silk sericin peptides with higher molecular mass between 50 and 400 kDa are soluble in hot water, while those having lower molecular mass of less than 50 or 20 kDa are easily soluble in cold water.

In the present study, the molecular weight distribution of the different sericin powder samples was determined by using SDS-PAGE method and the results are shown in Figure 3. The sericin recovered from HTHP degumming liquor showed the distinct bands at 25, 66, and 90 kDa and a broad smear band accompanied by distinct bands above 205 kDa. The sericin recovered from the alkaline degumming liquor showed distinct band at 14 and 20 kDa, and a diffuse band between 43 and 97 kDa. The sericin recovered from SPA degumming liquor showed diffuse band between 43 and 20 kDa. The native sericin showed three distinct bands at above 250, 180, and 100 kDa in SDS-PAGE analysis.[27] During HTHP degumming, on exposure to high temperature (115°C, 30 min), the sericin polypeptides of 180 and 100 kDa may be converted in to the lower fraction of polypeptide of 25, 66, and 90 kDa. The addition of chemicals also affects the molecular weight distribution of sericin. The above results indicate that degumming of silk with alkaline and SPA liquors results in further hydrolysis of sericin protein resulting into the formation of low molecular weight fractions.

Secondary structure of sericin powder

The secondary structure of the sericin powders was assessed by far UV CD spectroscopy. The CD spectrum of sericin powder samples (Fig. 4) shows that the sericin recovered from HTHP degumming liquor has a strong negative band at 198 nm suggesting a random coil structure. A slight negative band at 218 nm reveals the presence of β sheet. These results are similar to those obtained with the native sericin.[27] These results indicate that the high temperature does not affect the conformation of the sericin.

The sericin powder recovered from alkaline and SPA degumming liquor showed three negative bands at 196, 201, and 206 nm. Band at 196 nm suggests random coil conformation and the bands at 201 and 206 nm suggest a small amount of α helix conformation. The negative peak intensity of band at 196 nm is lower for sericin recovered from alkaline and SPA degumming liquor, as compared to the sericin obtained from HTHP degumming indicating some denaturation of sericin polypeptides.[28,29] The addition of alkali and soap affects the conformation of the sericin. Sericin is a kind of globular protein. During degumming, the addition of soap and alkali hydrolyze the sericin and result in a low molecular weight fraction (Fig. 3). These low molecular weight polypeptide chains may extend in presence of soap and alkali. The diffuse bands of sericin powders recovered from alkaline and SPA degumming in SDS-PAGE (Fig. 3) also suggested the denaturation of sericin. It

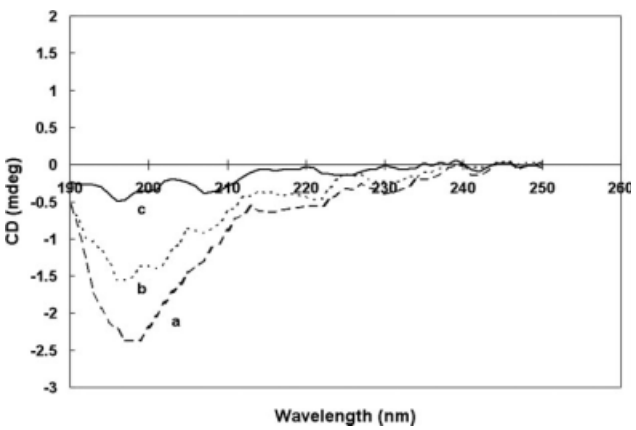

Figure 4 Far UV CD spectra of sericin powder recovered from (a) high temperature high pressure (HTHP), (b) alkaline, and (c) soap plus alkali (SPA) degumming liquors.

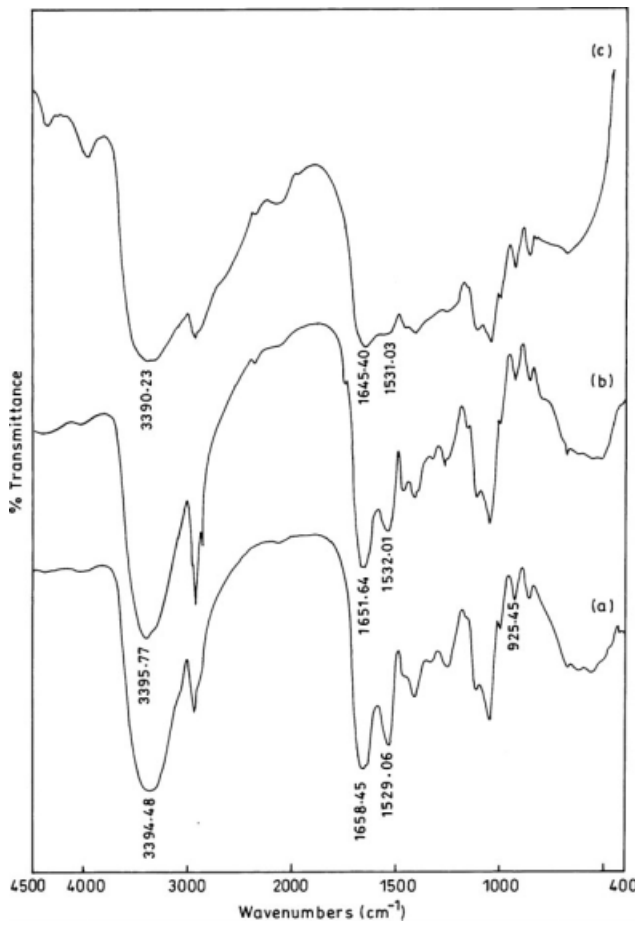

Figure 5 Infrared spectra of sericin powder recovered from (a) high temperature high pressure (HTHP), (b) alkaline, and (c) soap plus alkali (SPA) degumming liquors.

has been reported that, in the case of sericin recovered through alcohol precipitation method, the addition of alcohol to sericin solution also shifted the negative peak from 198 to 205 nm, which indicates that the molecular environment of silk sericin is affected by the addition of methyl alcohol.[30]

IR spectroscopy is another useful technique for the characterization of protein secondary structure. The absorption band of amide I is sensitive to hydrogen bonding interaction and therefore responsive to differences in protein secondary structure. Figure 5 shows the IR spectra of sericin recovered from HTHP, alkaline, and SPA degumming liquors. The IR spectra of the sericin recovered from HTHP degumming liquor [Fig. 5(a)] exhibited amide I adsorption band at 1658 cm^{-1}, which is a characteristic of random coil conformation.[30] The above result is in good agreement with CD analysis reported above.

The sericin recovered from alkaline and SPA degumming liquor showed adsorption band at 1651 and 1645 cm^{-1}, respectively. Amide I band between 1640 and 1648 cm^{-1} is a characteristic of unordered structure.[31] The above results suggest that addition of soap and alkali during degumming hydrolyzed the sericin in a random manner and disrupted its secondary structure.

Morphology of sericin powder

The surface morphology of the recovered sericin powder was examined under a scanning electron microscope at different magnification ranging from 6400 to 100,000×, as shown in Figure 6. It is seen that the sericin powder is mostly in the agglomerated form which may be due to the hydrophilic nature of the sericin. The sericin exposed to atmosphere picks up moisture immediately and gets agglomerated. The sericin powder recovered from HTHP degumming liquor has a collapsed sphere shape with dents of various depths and size and the surface looks smooth. The collapsed structure of the particle may be due to the removal of higher amount of water from the low solids liquid droplets during the dehydration process resulting in higher shrinkage of the droplets that tend to form the dents. The particles of sericin powder recovered from alkaline degumming liquor appeared as glued to neighboring particles. The particles have rough surface with porous structure. The powder produced from the SPA degumming liquor has collapsed spherical shape with dents and have rough

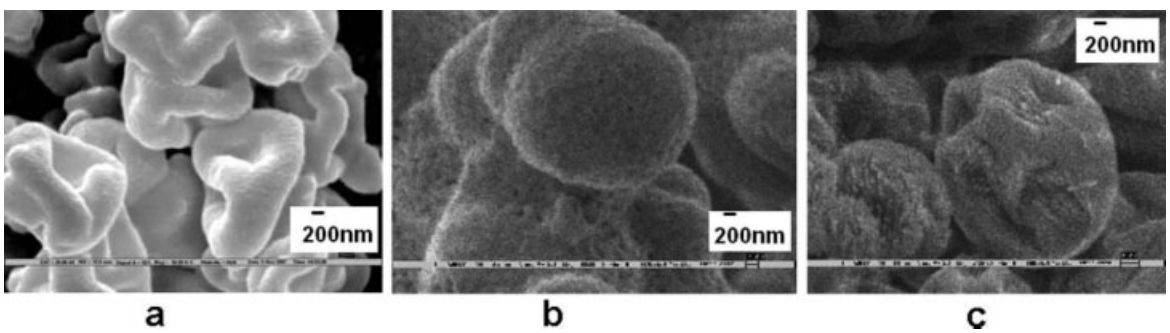

Figure 6 SEM photographs of sericin powder recovered from (a) high temperature high pressure (HTHP), (b) alkaline, and (c) soap plus alkali (SPA) degumming liquors.

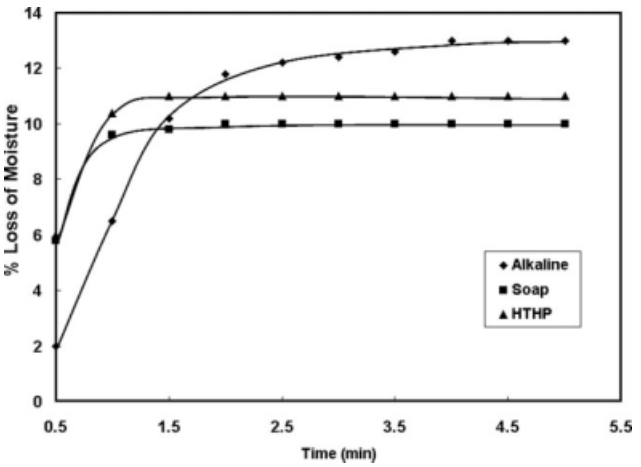

Figure 7 Loss of moisture content with time in sericin powder.

surface without any pores. The rough surface of particles may be due to the presence of sodium salts in the powder. Lee et al.[30] have studied the effect of methyl alcohol on the morphology of silk sericin and found that the structure of sericin changed from sponge to spherical fine particles. Kurioka et al.[16] have studied the morphology of silk sericin recovered from acid and alkaline degumming liquor using alcohol precipitation technique. Sericin recovered from acid degumming liquor showed thin-film-like structure of 10–100 μm with good dispersity, whereas sericin recovered from alkaline degumming liquor has much larger thin film structure (< 500 μm).

Functional properties of sericin powder

Moisture content

The moisture absorption capacity of the sericin powder was measured in terms of moisture content using Sartorius Moisture Analyzer. Figure 7 shows the percent loss of moisture with time for sericin powder recovered from various degumming liquors. The results indicate that rate of evaporation is similar for all the three samples of sericin powder. Sericin powder recovered from alkaline degumming liquor showed higher moisture content (13%) as compared to sericin recovered from HTHP (11%) and SPA (12%) degumming liquor. Sericin has moisture absorption property due to high hydroxyl-amino acid content. Sericin powder recovered from HTHP degumming liquor has high molecular weight distribution (i.e., low end amino groups), while the sericin recovered from alkaline and SPA degumming processes have low molecular weight distribution (i.e., high end amino groups). The sericin recovered from alkaline and SPA degumming liquors therefore absorbs more moisture as compared to that recovered from HTHP.

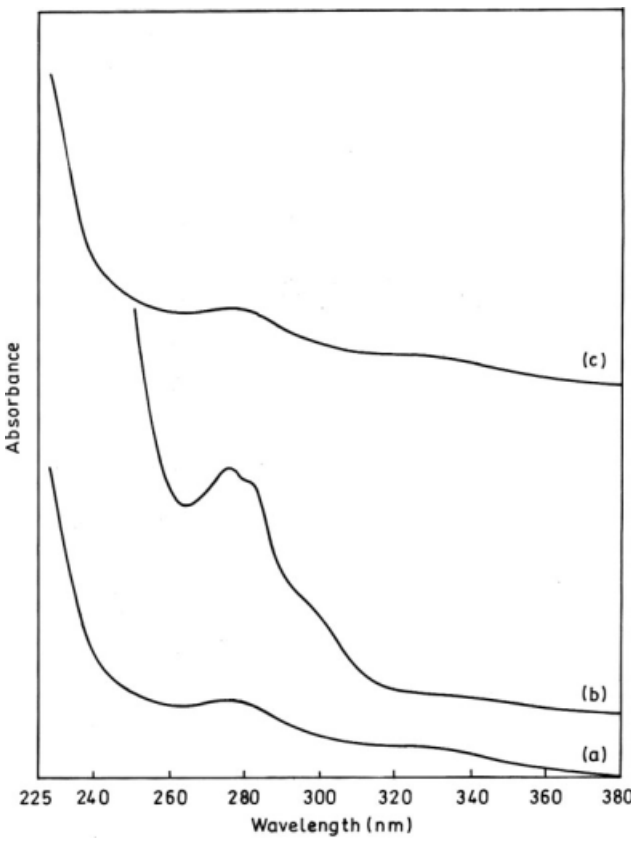

Figure 8 UV absorption spectra of sericin powder recovered from (a) high temperature high pressure (HTHP), (b) alkaline, and (c) soap plus alkali (SPA) degumming liquors.

UV protection property

The UV spectrum of the 0.1% aqueous sericin solutions is shown in Figure 8. It is observed that sericin recovered from different degumming liquors absorbs the UV light in the range of 200–360 nm. Since sericin absorbs UV light in the range of 300–320 nm, it may act as a UV protective agent.[32] Uniform thickness films (76 μm) of recovered sericin were prepared to measure the mean UPF as per Australia/New Zealand standards and the data is summarized in Table III. It has been found that all the sericin samples recovered from different degumming liquor have mean UPF value of 420 ± 2. The above result also suggests that the UV protection property of sericin does not get affected by the molecular weight distribution and the secondary structure of sericin.

TABLE III
UPF Factor of Sericin Films Prepared from Powder Recovered from Different Degumming Liquors

Properties	HTHP	Alkaline	SPA
Mean UPF	420 ± 12	431 ± 7	406 ± 8

CONCLUSION

The sericin powder recovered from the three different degumming liquors had different color, purity, and molecular weight range. The powder recovered from HTHP degumming liquor has a pale yellow color, 98% protein content, 0.8% ash content, and 20 to > 205 kDa molecular weight range, whereas that recovered from alkaline degumming liquor has brown color, 92% protein content, 5% ash content, and 20–97 kDa molecular weight range. However, the sericin powder recovered from SPA degumming liquor has cream color, low protein content (67%), high ash content (22%), and molecular weight in the range of 20–43 kDa.

The secondary structure of sericin recovered from HTHP degumming has random coil conformation with some β sheet structure. The sericin recovered from alkaline and SPA degumming liquors has denatured secondary structure with some random coil, β sheet, and α helix conformation. The addition of alkali and soap during degumming hydrolyzes the sericin to low molecular weight fraction and disrupts its secondary structure.

SEM micrographs showed that all the three sericin powder prepared through spray drying technique had collapsed sphere shape with dents of various depth and sizes. They were mostly in the agglomerated form which may be due to its hydrophilic nature. The sericin powder recovered from HTHP degumming liquor has smooth surface, whereas that recovered from alkaline and SPA degumming liquor show rough surface. This may be due to the presence of residual salts in these samples.

The moisture content of the sericin depends on its molecular weight distribution. Low molecular weight range of sericin obtained from alkaline and SPA degumming liquor absorbs more moisture as compared to the high molecular weight sericin obtained from HTHP degumming liquor. The recovered sericin powder films show a high UV absorption property and this property does not get affected by its molecular weight range.

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