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A Novel Approach towards Simultaneous Degumming and Dyeing of *Bombyx mori* Silk

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ABSTRACT

The present investigation focuses on reducing the cost of wet processing of silk fabric by saving energy and time through simultaneous degumming/dyeing of silk fibers followed by recovery of sericin from the dye effluent as a colored powder. Among other tried methods, degumming of silk with boiling water through three consecutive baths, 1 h each, was found to be the superior method for sericin extraction technically and economically. Degumming/ dyeing processes of silk were carried out concurrently using acid dyeing bath for the last degumming cycle. The colour strength values of the simultaneously degummed and dyed fibres are comparable with those fibres degummed and dyed consecutively. The obtained colored sericin powder was precipitated by adjusting the pH value of degumming effluent. The particle size of sericin powder was conducted using transmission electron microscopy. Scanning electron microscopic investigation of raw silk and degummed one was studied. The chemical composition of the said powder, raw silk and degummed silk was monitored by elemental analysis, and amino acid analysis. The molecular mass of the obtained sericin was determined using gel filtration system. The tensile properties of the degummed silk were evaluated compared with the raw silk.

Keywords: Silk, Sericin, Degumming, Dyeing, Isoelectric point

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INTRODUCTION

Silk is a proteinic biopolymer that can be spun into fibers by some *Lepidoptera larvae* [1,2]. The two main proteins of raw silk fibre are fibroin and sericin. Silk sericin is the second type of silk protein secreted in the mid-region of the silk gland. Sericine protein consists of different polypeptides, ranging in weight from 24 to 400 KDa depending on gene coding and post-translational modifications [3]. Sericin is composed of 18 amino acids, the major amino acids of its structure are serin (28%), aspartic acid (18%) and glycine (16%) [4]. Although, sericin exhibits high hydrophilicity, good antioxidant, UV-resistant, and anticoagulant, it was considered to be a waste water product of the silk processing industry [5]. Recently, it is being used in lots of applications, such as cosmetics and pharmaceutical uses [6]. Silk sericin hides the fibroin brightness and whiteness, moreover; it makes silk fibroin hard handling and difficult dyeing process [7]. Thus, sericin should be completely removed to obtain a luster, soft and typical shiny aspects of the fibre [8]. Removal of sericin is known as degumming process that is depending on the silk gum solubility, which acheived by the cleavage of the peptide chains of sericin protein into small molecules as amino acids and their oligomers [9]. Several methods were reported for degumming processes including extraction with water, boiling-off in soap [10], soda-ash method [11], degumming with alkalis and ultrasound method [12], degumming in acidic solutions [13], enzymatic method [14], plasma method [15] and microwave irradiation [16,17].

The present study with simultaeneous degumming and dyeing of *Bombyx mori* silk followed by recovery of soluble sericin through adjustment of the pH value of the degumming effluent around the isoelectric point of sericin which is 3.6-4.2.

EXPERIMENTAL

Materials

Raw silk yarns from Chinese silkworm *Bombyx mori* were used in this study. A commercial domestic soap of industrial grade [Marseille Soap (olive oil soap)] was used in this study. All other chemicals are of laboratory grade.

Degumming methods

Degumming with soap/soda ash mixture

Raw silk fibre was incubated in a bath containing 2 g/L Marseille Soap and 0.8 g/L soda ash with a liquor ratio (1:30) at 98°C for 1 h. The treated sample was removed and rinsed by warm distilled water for 10 min then placed in a bath containing 1.5 g/L of Marseille Soap and 1.6 g/L soda ash for further degumming. Finally, the sample was rinsed with warm and cold water and left to dry at atmospheric temperature [18].

Degumming with citric acid

Native silk fibres were inserted in a bath containing 30% citric acid (w/v) with a liquor ratio of 1:20 at 98°C for 30 min. The degummed sample was rinsed thoroughly with running tap water and left to dry at atmospheric temperature [19,20].

Degumming with Sodium carbonate

Raw silk sample was treated by sodium carbonate solution (10 g/L) with a liquor ratio of 1:20 at 98°C for 30 min. The degummed sample was rinsed with running tap water and left to dry at atmospheric temperature [11].

Degumming with water under pressure

Silk sample was incubated in water in presto cooker for 1 h. Unpleasant odour was observed in the degumming bath which implied the degradation of sericin under the used conditions of temperature and pressure.

Degumming with boiling water

Silk sample was placed in boiling water through three consecutive cycles, 1 h each (water is refreshed in each cycle). The degummed sample was rinsed with running tap water and left to dry at atmospheric temperature [21].

Simultaneous degumming/dyeing of raw silk

Silk sample was first degummed using boiling water through two cycles (1 h each) then the sample was removed from the degumming bath and thoroughly rinsed by running tap water. After that, the silk sample was cut into two pieces and subjected to the third cycle of degumming process. The third step of degumming was carried out in two baths containing 0.5% and 1% shade of acid dye (Acid Red 1), respectively. The degumming bath was adjusted at pH 5.5 and the dyeing process was performed for 1 h at 100°C.

Sericin recovery

Sericin was restored from the degumming bath by adjusting the pH value of the effluent at 4.0 using few drops of glacial acetic acid or 50 % (w/v) citric acid.

Analyses

Transmission Electron Microscopy (TEM)

The particle size and shape of sericin powder was investigated using TEM (JEOL, JEM-1230, and Japan, with an acceleration voltage of 120 kV). The sample subjected to TEM analysis, was prepared by placing a drop of the colloid dispersion onto a carbon-coated copper grid. The samples were dried at room temperature and examined using a TEM without further modification or coating.

Scanning Electron Microscopy (SEM)

The surface of raw, as well as the degummed silk fibres, was investigated using ZEISS LEO 1530 Gemini Optics Lens scanning electron microscopy. The sample was mounted on aluminum stubs, and sputter coated with gold in an S150A sputter (coated Edward, UK).

Elemental analysis

The amounts of carbon, hydrogen, nitrogen and sulphur in the raw silk, degummed sample and sericin powder were assessed using the Elementar CHNS Analyzer device, Model Vario EL III, Germany.

Amino acid composition

The amino acid composition of raw silk, degummed silk, as well as sericin powder was determined after hydrolysis in 6 N hydrochloric acid according to the method of Deveneyi et al. The amino acid composition of the hydrolysate was analyzed on an "Alpha Plus II" Amino Acid Analyser (Pharmaceutical/LKB, Freiburg, Germany).

Gel filtration chromatography on Sephacryl S-300 column of sericin

In typical experiment, sericin powder solution was dialyzed against 0.02 potassium phosphate buffer, pH 7.0. Three milliliters of

sericin solution was loaded on to a Sephacryl S-300 column (142 cm × 1.75 cm i.d.). The column was equilibrated and run with 0.02 potassium phosphate buffer, pH 7.0 (the equilibration buffer) at a flow rate of 30 ml/h. 2 ml fractions were collected and each fraction was analyzed for its absorbance at a wavelength of 220 nm [22]. This Sephacryl S-300 column was used for molecular weight determination of sericin according to the method of Andrews [23]**.** The equilibrated Sephacryl S-300 column was calibrated with β-amylase (200 kDa), alcohol dehydrogenase (150 kDa), bovine serum albumin (67 kDa), carbonic anhydrase (29 kDa) and cytochrome C (12.4 kDa) while the absorbance was monitored at 280 nm. The void volume was determined by the blue dextran (2000 kDa). The logarithms of the molecular weight of the marker proteins were plotted versus the ratios of their elution volume (Ve)/the void volume (V_o) and a calibration curve was constructed for molecular weight determination.

Determination of colour strength

The K/S of the dyed silk fibres was measured using a UV-Vis spectrophotometer with pulsed xenon lamps as light source (Ultra-Scan Pro, Hunter Lab, USA) 10° observer with D65 illuminant, d/2 viewing geometry and measurement area of 2 mm. The measurement of coloured fibres was occurred at λ_{525} nm wavelength. The corresponding color strength value (K/S) was assessed by applying the Kubelka Munk [24] (Equation 1).

$$K/S = \frac{(1-R)^2}{2R} \quad (1)$$

Where R is the decimal fraction of the reflection of the colored fabric, K is the absorption coefficient and S is the scattering coefficient.

Fastness to wash and light

The color washing fastness of the degummed and dyed silk fibers was assessed according to ISO standard methods. The specific tests were ISO 105-X12 (1987). The light fastness of the dyed fibres was measured according to ISO 105-BO2 (1988) using Xenotest 1200 apparatus [25].

RESULTS AND DISCUSSIONS

Effect of different degumming reagents on the sericin extraction

Different reagents were used for the degumming process of raw silk including domestic soap/soda ash mixture, citric acid, sodium carbonate, and boiling water. It has been observed that all the used reagents have the same effect on the degumming of silk, where the loss in weight of the raw silk sample was around 23-25%. However, degumming of silk with boiling water was found to be the most technically and economically feasible method from the point of view of easy recovery of sericin from the degumming bath, as sericin was recovered from the degumming or simultaneous degumming and dyeing effluent by adjusting the pH value at around the isoelectric point of sericin (3.6-4.2). This is an ecologically and economically acceptable method of degumming compared to the widely used method using large amounts of ethanol [21,23,26]. It is noteworthy to mention here that precipitation of sericin from the effluent by adjusting its pH at 4.0 is practically feasible only in case of degumming using boiling water.

Transmission Electron Microscopy (TEM)

Figure 1 shows the transmission electron micrograph of sericin powder recovered from the degumming effluent of raw silk degummed by boiling water for three cycles, 1 h each, and precipitated by adjusting the pH at 4.1–4.2, which is the isoelectric point of sericin. This Figure implies that the size of the obtained sericin powder ranged from 50 nm to 140 nm.

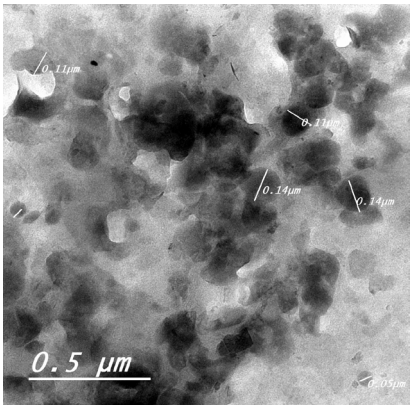

Figure 1: Transmission electron micrograph of sericin powder

Scanning Electron Microscopy (SEM)

The surface characteristics of raw silk as well as the degummed one were investigated using SEM. Figure 2a illustrates the bundles of silk fibres, where the fibroin fibers covered with the gum layer, sericin. On the other hand, Figure 2b shows the unpacked fibroin filament after the removal of sericin.

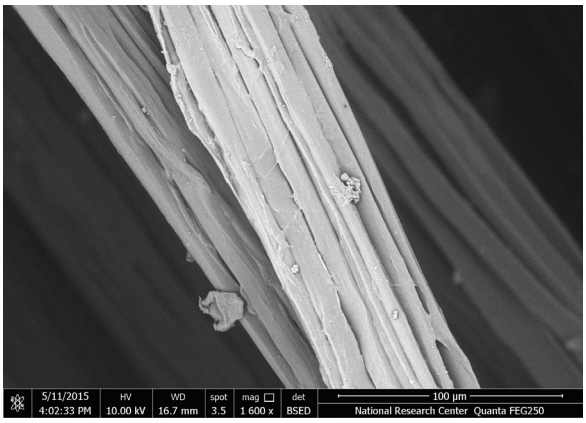

Figure 2a: Two bundles of raw silk

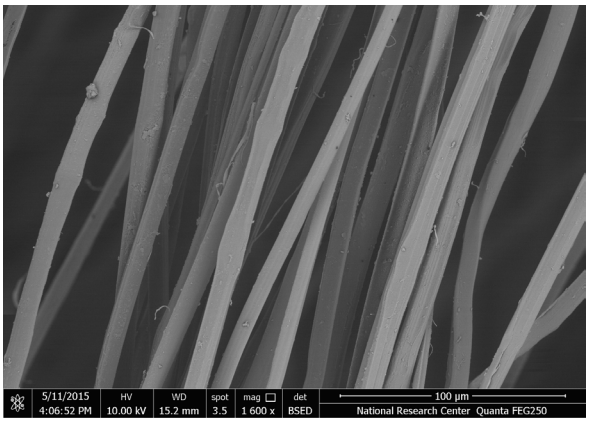

Figure 2b: Silk fibre (fibroin) after sericin removal

Elemental analysis

The elemental analysis of raw silk, degummed silk and sericin powder was assessed and the results is tabulated in Table 1. Data of Table 1 shows that the elemental content of blank sample, degummed silk (fibroin silk) as well as sericin powder are nearly similar to each other. These findings match well with the reported chemical composition of *Bombyx mori* Silk of 47.1% carbon and 16.52% nitrogen [27].

Table 1: The chemical composition of native silk, degummed silk and sericin powder

Sample Code	N %	C %	S %	H %
Blank	16.72	43.09	0.55	3.24
Degummed silk	17.03	44.08	1.15	4.05
Sericin	14.70	40.86	0.71	3.08

Amino acid composition

The amino acid composition of the raw silk yarn, silk degummed by boiling water and the removed sericin therefrom. Data of this experiment, summarized in Table 2, indicate that the content of the amino acids gylcine, alanine and tyrosine in the obtained sericin is lower than that in the raw silk and the degummed one. This ensures that the adopted conditions of degumming of silk by boiling water remove only sericin and does not have deteriorative action on the chemical composition of silk fibroin. This hypothesis is supported by the relatively high percent of serine, aspartic acid, glutamic acid and cystine in the removed sericin.

Table 2: Amino acid compositions of raw and degummed silk yarns (g/1000 g protein)

Amino acid	Raw silk	Degummed silk (fibroin)	Sericin
Glycine	40.9	43.1	12
Alanine	29.9	30.6	97
Valine	2.4	2.7	1
Leucine	0.8	0.9	0.3
Isoleucine	0.7	1	1
Serine	11.1	12.2	3.34
Threonine	1.3	1	0.9
Cystine	2	1	2
Aspartic acid	1.6	1.9	0.8
Glutamic acid	0.1	0.2	0.2
Arginine	0.4	0.6	0.4
Lysine	0.4	0.5	0.1
Phenylalanine	0.2	0.3	0.1
Tyrosine	4.9	5.3	4.2
Histidine	0.1	0.1	0.06
Proline	3	5	2
Tryptophan	1	2	0.6

Previous data of the amino acid amino composition of sericin demonstrate the considerable differences between sericin and fibroin. The amino acids which are the main structural components of fibroin (glycine, alanine, and tyrosine) are contained in sericin in much smaller amounts (alanine and tyrosine) or in exceedingly small quantities (glycine). On the other hand, sericin is characterized by a higher content of hydroxy amino acids, particularly of serine, dicarboxylic and diamino acids, and also by the presence of small amount of cysteine [28].

Molecular weight determination of sericin components

A typical elution profile of the chromatography of sericin on Sephacryl S-300 column (142 cm × 2.4 cm i.d.) is presented in Figure 3. The void volume (V_o) was determined by the dextran blue to be 132 ml. The sericin components were eluted from Sephacryl S-300 column with elution volumes (V_e) ranged from 150 ml to 188 ml and designated P1, P2, P3 and P4. The V_e/V_o ratio was calculated for sericin components and the molecular masses of sericin components were deduced from the calibration curve to be 316 kDa for P1, 178 kDa for P2, 158 kDa for P3 and 120 kDa for P4 respectively. These findings matches well with earlier investigations concerned with molecular weight determination of sericin [28,29].

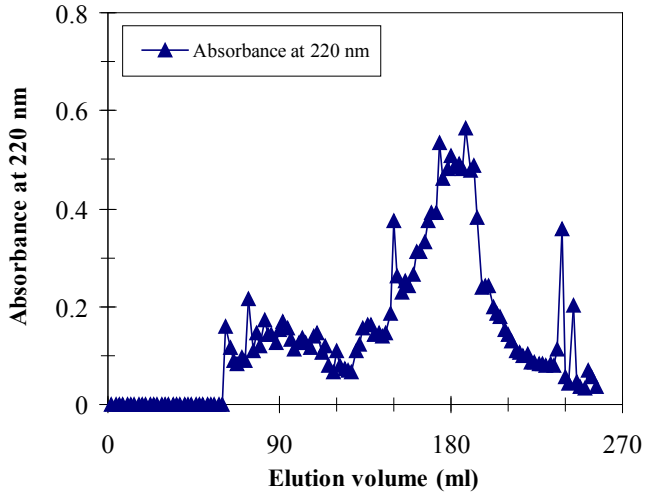

Figure 3: A typical elution profile for the chromatography of sericin on Sephacryl S-300 column (142 cm × 2.4 cm i.d.) previously equilibrated with 0.02 potassium phosphate buffer, pH 7.0. The fractions were eluted by the same buffer and 2 ml fractions were collected at a flow rate of 30 ml/ h.

Colour strength

The colour strength (K/S) values of simultaneously degummed and dyed silk sample (A & B), as well as degummed samples followed by dyeing in two consecutive steps (C & D), was recorded and tabulated in Table 3.

Data of Table 3 revealed that the values of colour strength of (C) and (D) samples are more than that of (A) and (B) samples. This can be explained in terms of the fact that, in case of samples (A & B), the discharged sericin competes with the degummed silk fibres in the dye uptake within the same bath. This hypothesis was supported by the colored sericin residue in the dye-bath. Based on these findings, researchers may start to think about utilization of sericin in dye removal from dyeing effluent.

On the other hand, degumming followed by dyeing of silk fibres (samples C & D) assures enhanced penetration of the dye molecules to the fibre interior, and hence higher colour strength of the dyed fibres.

Table 3: Colour strength values for simultaneously degummed and dyed silk samples and degumming followed by dyeing of silk in consecutive steps

Sample	Acid red 1 dye shade %	K/S (λ_{525})
Simultaneously degummed and dyed silk (A)	0.5	1.55
Simultaneously degummed and dyed silk (B)	1	1.83
Degumming followed by dyeing of silk in consecutive steps (C)	0.5	1.73
Degumming followed by dyeing of silk in consecutive steps (D)	1	2.13

Washing fastness

The wash and light fastness of the dyed silk fibres were evaluated. Results of this investigation were summarized in Table 4.

Data of Table 4 demonstrated that the washing and light fastness for all dyed samples are quite similar to each other except the staining on wool for both samples (C) and (D). Furthermore, it is clearly that all the dyed fabrics had good fastness to light.

Generally, from the color fastness results, it could be recognized that the simultaneously degumming/dyeing process had no effect on the fastness properties of the dyed samples.

Table 4: Wash and light fastness for simultaneously degummed/dyed silk and degumming followed by dyeing of silk in consecutive steps

Sample	Acid red 1 dye shade %	Wash fastness			Fastness for light
		St* cotton	St** wool	Alteration in colour	
Simultaneously degummed/dyed silk (A)	0.5	3-4	3	3	4-5
Simultaneously degummed/dyed silk (B)	1	3-4	3	3-4	4-5
Degumming followed by dyeing of silk in consecutive steps (C)	0.5	3-4	2-3	3	4-5
Degumming followed by dyeing of silk in consecutive steps (D)	1	3-4	2-3	3	4-5

St*: Staining on cotton; St**: Staining on wool washing fastness 1-5 grey scale Light fastness 1-8 scale

CONCLUSIONS

Boiling water was found to be the most technically and economically feasible method for degumming of silk. The pH value of the degumming bath plays a significant role in the sericin precipitation therefrom. The isoelectric point of sericin protein (around pH 4) was the appropriate value for the precipitation process. Simultaneous degumming and dyeing of raw silk is carried out in the third cycle of the degumming process; from which colored sericin powder were obtained. TEM reveals that the size of the obtained sericin powder ranged from 50 nm to 140 nm. Results of elemental analysis and amino acid composition clarify that there is no damage of both sericin and fibroin proteins, these results are confirmed by the molecular weight of sericin which is ranged from 316 to 120 KDa. The colorimetric data (K/S, wash and light fastness) of the simultaneously degummed/dyed fibres and those degummed and dyed in two consecutive bathes are comparable. One-bath degumming and dyeing of raw silk would reduce the cost of wet processing of silk fabric by saving energy and time.

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