

# High Molecular Sericin from *Bombyx mori* Cocoons: Extraction and Recovering by Ultrafiltration

Marcelino L. Gimenes, Vitor R. Silva, Melissa G. A. Vieira, Meuris G. C. Silva, and Agnes P. Scheer

**Abstract**—The present study focus on sericin extraction process from *Bombyx mori* cocoons using water in absence of chemical additives under different physical conditions aiming to obtain high molecular weight sericin. Separation and fractionation of sericin proteins to obtain high molecular weight material was also carried out using ultrafiltration process at different operating modes: batch, diafiltration and batch with back flushing water pulse. The molecular weight of extracted sericin protein using pure water showed in general a size distribution varying from 20 up to 400 kDa, with major peaks between 200 kDa and 100 kDa and peaks with fraction of sericin lower than 100 kDa, depending on conditions used in the extraction and concentration. The results demonstrated viability to separate sericin fractions having molecular size higher than 50 kDa, using the ultrafiltration process with 50 kDa cut-off membrane, however, a flux decline above 90 % was detected independent of operating mode. Increasing the concentration factor from 2 to 4 promotes the permeation of sericin molecules of high molecular weight, reducing the rejection coefficient of 71.1 % to 60.4 %.

**Index Terms**—Sericin, extraction, ultrafiltration, fouling.

## I. INTRODUCTION

Silk manufacturing is one of the textile industrial sector where the intensive water consumption cannot be avoid, therefore resulting in large volume of wastewater. In this manufacturing, the largest wastewater stage is the degumming process, which is used for elimination of external sericin prior to dyeing; generally synthetic soap solutions is used at 95 °C for 1 hour. As expected, 100 kg of silk produces almost 22 kg of residual sericin in wastewater [1]-[3]. By traditional process, sericin shows partial hydrolysis, reduces its natural molecular weight and loses some functional properties [4], [5].

Sericin is a highly hydrophilic macromolecular protein, comprising of 18 amino acids, with predominant polar amino acid groups, as serine (25 %), acids aspartic (17 %), threonine (6%) and acid glutamic (5 %) [3], [6], [7]. Due this natural composition, sericin can be applied in cosmetics formulations, including skin and hair care products. In food industry, sericin

peptides showed functional properties to development of new products with antioxidant properties and metal ion-chelating activity [7], [8]

The recovered sericin with high molecular weight can be applied in many uses for biomaterial and biofilms development. In tissue engineering, sericin can be used to induce the oriented crystallization from biomaterials based in hidroxyapatites and improve their biocompatibility due the presence of polar groups [9], [10]. Biodegradable materials with high molecular weight sericin can developed, due the presence of the large amount of polar side chain made of hydroxyl, carboxyl and amino groups, that can be used to enable easy cross-linking, copolymerization or blending with other polymers [1], [11], [12].

The study of alternative degumming methods to remove sericin from cocoons, and the methods to recovery and recycling sericin by-products from degumming stage could be significant economic and social benefit. Conventional degumming process, with soap and alkalis is effective to remove sericin, but promote the hydrolysis of the sericin chain structure, losing potential properties, while the soap removal from sericin solution is hard and expensive [13]. Sericin extraction by urea with 2-mercaptoetahnol showed high efficiency, however, a cost-consuming step of dialysis is necessary to purify the sericin solution before sericin application [14], [15]. Enzymatic degumming can be effective to remove sericin from silk cocoon; however, it is an expensive process and promotes the specific proteolytic hydrolysis of the primary sericin chain structure, which causes a molecular weight reduction [5], [13].

Recovery of valuable raw materials from wastewater, as biological molecules, has been well studied by ultrafiltration. Fabiani *et al.* [16] applied Ultrafiltration with cut-off of 15-30 kDa in silk degumming wastewater, where 95 % of rejection was reported for sericin volume concentraition factor of 4. However, a severe flux decline as high as 90 % was observed. Capar *et al.* [2] earned the complete recovery of the fraction of sericin with high molecular weight (180 KDa - 200 kDa) with polyethersulfone membrane of 20 kDa, but partial permeation was detected for fractions lower than 80 kDa and high flux decline was detected on the order of 87.6 %.

The present study focused on sericin extraction process *Bombyx mori* cocoons using water, in absence of chemical additives under different temperatures. Also, the extracted sericin was submitted at ultrafiltration process to promote the fractionation of sericin protein in order to obtain retentate with high molecular weight.

## II. MATERIAL AND METHODS

Sericin was extracted from cocoons of *Bombyx mori*

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silkworm provided from northwest region of Paraná Brazil. The cocoons were cut into small pieces (about 1 cm<sup>3</sup>) and then immersed in extracted solution. For each assay; 7.5 grams of chopped cocoon were deposited in 250 mL of extract solution in a Erlenmeyer of 500 mL. The extraction processes studied were hot water extraction and aqueous solution of Na<sub>2</sub>CO<sub>3</sub> (Anhydrous 99.5%, Vetec, Brazil) at 80 °C and 120 °C. The extraction yield *EY* was evaluated by gravimetric method [17], by (1), where: *EY* is the extraction yield (%), *M<sub>F</sub>* is the dried cocoon mass after the extraction (g) and *M<sub>I</sub>* is the initial dried cocoon mass before the extraction (g).

$$EY = \left[ 1 - \frac{M_F}{M_I} \right] \cdot 100\% \quad (1)$$

Surface morphology of silk degummed, i.e. the insoluble silk fibroin, was examined with a scanning electron microscope (Jeol<sup>TM</sup>, model JSM - 6360L, Japan) to observe the presence of sericin on the fibroin surface, after the extraction process.

The extracted solution with hot water process was treated in a ultrafiltration process (PAM Membranas<sup>TM</sup>, Brazil) with hollow fiber membrane of polyethersulfone (50 kDa and surface area of 520 cm<sup>2</sup>). Ultrafiltration was carried out under standard operating conditions: Transmembrane pressure of 0.05 MPa, feed flow of 1.0 L/min and initial sericin concentration of 3.0 g/L. The permeate flux was evaluated by gravimetric method [18], by (2), where: *J<sub>p</sub>* is the permeate flux (kg /m<sup>2</sup>h), *M<sub>p</sub>* is permeate mass (kg), *t* is time (h) and *S* is the membrane surface area (m<sup>2</sup>).

$$J_p = \frac{1}{S} \frac{dM_p}{dt} \quad (2)$$

The ultrafiltration system was operated by batch mode, diafiltration mode and batch + backwashing water pulse. The batch mode operation was the process where the permeate flow was continuous removed from the system (Fig. 1a), while in the diafiltration mode the permeate flow was continuous removed and the system was fed with water at the same flow of the permeate removed (Fig. 1b).

The batch mode + black flushing operation is the same procedure of the batch mode, however, each interval of 15 minutes the process is interrupted and subjected to a pulse of backwash with water for 3 minutes (reverse flux, from permeate channel, across membrane and recycling to the system by retentate channel), at pressure of 0.1 MPa. The water is fed by permeate channel and recycled to feed tank by retentate channel (Fig. 1c).

The sericin concentration in permeate and retentate flows were determined by Bradford method [19]. The rejection coefficient *CR* (%) and concentration factor *CF* were evaluated by (3) and (4), respectively, where: *C<sub>p</sub>* is the concentration of sericin in the permeate flow (g/L), and *CF* is the sericin concentration in feed stream (g/L); *M<sub>o</sub>* and *M<sub>F</sub>* are the initial and final mass solution (kg) [18].

$$CR = \left[ 1 - \frac{C_p}{C_f} \right] \cdot 100 \quad (3)$$

$$CF = \frac{M_o}{M_o - M_p} \quad (4)$$

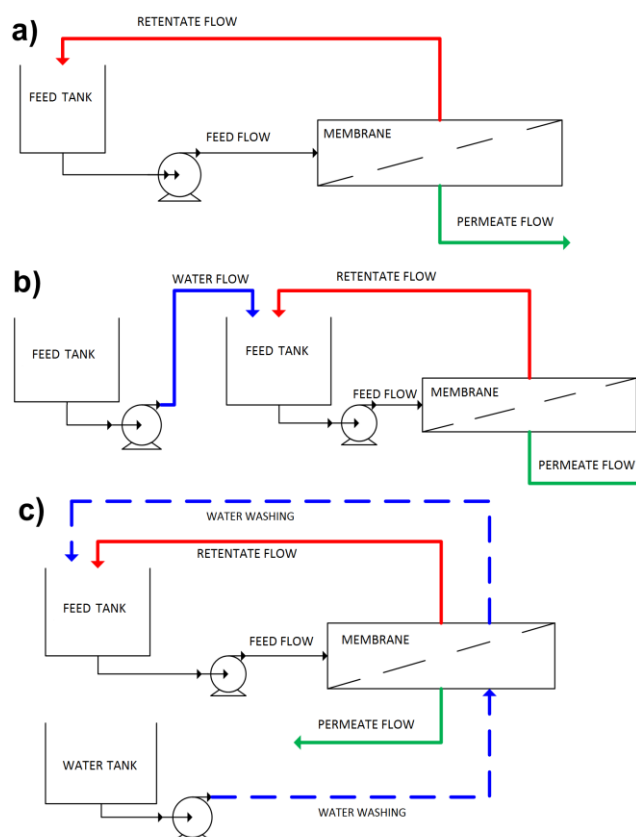


Fig. 1. Ultrafiltration modes operation. a) batch; b) diafiltration; c) batch + backflushing.

The fouling resistance effects were estimated using the resistance model [20], [21]. This model describes the effects that may interfere with the permeate flux as a function of the total resistance *R<sub>T</sub>*. The permeate flux can be described as (5), where  $\Delta P$  is the transmembrane pressure,  $\mu_{PERM}$  is the permeate viscosity.

$$R_T = \frac{\Delta P}{\mu_{PERM} \cdot J_p} \quad (5)$$

Fouling resistance *R<sub>F</sub>* is obtained by difference between the total resistance *R<sub>T</sub>* of sericin solution permeation and the hydraulic membrane resistance (6):

$$R_F = R_T - R_M \quad (6)$$

The hydraulic resistance *R<sub>M</sub>* was measured by (7) with deionized water, cleaning membrane at transmembrane pressure of 0.04 MPa and water temperature of 30 °C, where *J<sub>w</sub>* is the water permeate flux through the cleaning membrane and  $\mu_w$  is the water viscosity.

$$R_M = \frac{\Delta P}{\mu_w \cdot J_w} \quad (7)$$

The molecular weight distribution was measured with size exclusion chromatography HPLC (Merck<sup>TM</sup> Hitachi

LaChrom, with quaternary pump L-7100, autosampler L-7250, refractor index detector L-7490 and a module of heater column L-3000). The standard molecular weight used was the Pululan polysaccharide, with the standard of molecular weight: 20 kDa, 50 kDa, 100 kDa, 200 kDa, 400 kDa and 800 kDa.

All experiments were made in triplicate and the results were expressed in terms of means and standard deviations. The analysis of variance was then performed for the treatments. Tukey's test was used to compare means with 5% significance level. The software Statistica 7.0 (Statsoft™, USA) was used for statistical analysis.

### III. RESULTS AND DISCUSSION

#### A. Extraction Process

The extraction yields of sericin are shown in Table I. Sericin extraction with water at 120 °C extraction was extraction yield of  $21.99 \pm 0.96$  %, while at temperature process lower than boiling point of water the extraction yield is insignificant. Extraction yields obtained in this study without chemical was the same that obtained by Haggag *et al.* [22] with enzymatic process of 21.8 %.

According with Padamwar *et al.* [23], sericin can be classified in three groups: Sericin A, (outermost layer in cocoon), Sericin B (middle layer in cocoon) and Sericin C (the innermost layer and adjacent protein). Each one has different properties (molecular weight, amino acids composition and protein secondary structure) and since the sericin C has a higher proportion of the beta-sheet structure than the other groups, it has a lower degree of solubility in water. As illustrated in Fig. 2b, the residual sericin present on the surface of the silk sericin is due to group C, more strongly linked with the hydrophobic structure of fibroin.

High temperature decreases the hydrogen bonding stability, formed by the binding between the various hydroxyl groups, allowing the interaction of water with hydroxyl groups of the polar amino acid [24]. Due the predominant amount of hydrophobic amino acids in fibroin, only sericin is removed in water at high temperature.

Chemicals break all protein interactions, make easily the water permeation inside the protein and remove sericin, as showed in Fig. 1a. Extraction yield with aqueous solution of  $\text{Na}_2\text{CO}_3$  at 120 °C showed the highest extraction yield of  $30.04 \pm 0.83$  %, consistent with the amount of sericin present in *Bombyx mori* cocoons, according with the literature [1], [14].

TABLE I: EXTRACTION YIELD OF SERICIN BY *BOMBYX MORI* COCOONS

Extraction process	Temperature (°C)	Extraction yield EY (%)
Hot water	80	$0.75 \pm 0.68^D$
Extraction with $\text{Na}_2\text{CO}_3$ 0.5%	80	$23.68 \pm 0.85^B$
Hot water	120	$21.99 \pm 0.96^C$
Extraction with $\text{Na}_2\text{CO}_3$ 0.5%	120	$30.04 \pm 0.83^A$

\* means with different letters represent statistically difference according to the Tukey's test ( $p < 0.05$ )

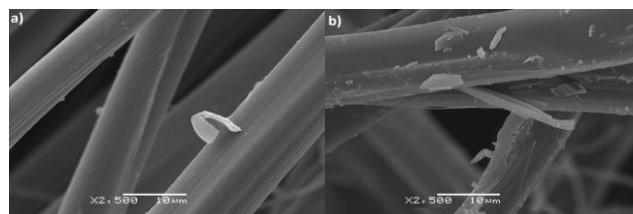


Fig. 2. Scanning Electronic Microscopy (SEM) of degummed cocoon, after the extraction process. a) Extraction with  $\text{Na}_2\text{CO}_3$  0.5 % at 120 °C; b) water extraction at 120 °C. Illustration of the residual sericin present on the surface of the silk sericin.

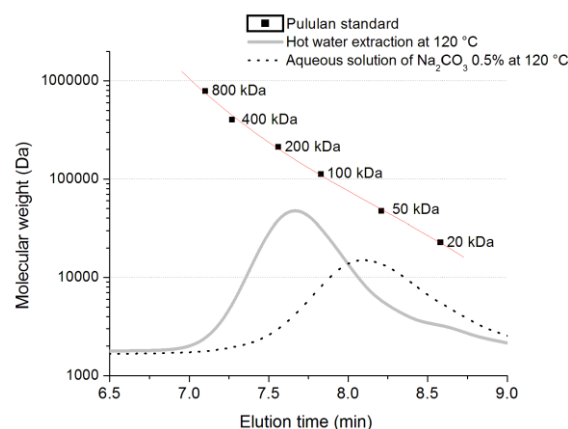


Fig. 3. Molecular weight profiles of sericin solution.

The molecular weight of extracted sericin protein using pure water showed in general a size distribution varying from 20 up to 400 kDa, with major peaks between 200 kDa and 100 kDa and sericin fractions lower than 100 kDa, while the extraction with  $\text{Na}_2\text{CO}_3$  showed molecular weight lower than 100 kDa (Fig. 3). Thus, the extraction with chemical indicates further chemical hydrolysis of sericin, while the hot water extraction preserves the primary structure and composition, which enables its use in the development of biomaterials.

#### B. Ultrafiltration Process

Sericin solution obtained by extraction with water at 120 °C was treated in ultrafiltration process under the following conditions set: initial sericin concentration of 3.0 g/L, feed flow of 1.0 L/min and transmembrane pressure of 0.05 MPa (optimal transmembrane pressure and feed flow were determined in previous study developed by Silva *et al.* [25]).

The flow profile of permeate in all operating modes evaluated showed an abrupt initial decrease due to the formation of the polarization layer and, after 10 minutes of process, reached the equilibrium between the polarized layer and renewal surface effects. After the equilibrium between the renewal effects and the polarized layer, fouling phenomena promote the gradual decay due the mechanical restriction, as the surface and internal pore blocking [21].

Initial permeate flux shown in Table II were statistically equal for all operation modes, indicated that chemical cleaning process was successful to recovery the membrane permeability. Steady state permeate flux obtained at the end of the process for diafiltration mode was statistically equal at batch mode and higher than the batch mode with back flushing water pulse, according with the Tukey's test at 5% significance.

TABLE II: FLUX DECLINE ANALYSIS AND FOULING RESISTANCE

Ultrafiltration modes operation	Initial Permeate flux (kg/m <sup>2</sup> h)	Final steady-state permeate flux (kg/m <sup>2</sup> h)	Flux Decay (%)	Hydraulic resistance - $R_M$ (m <sup>2</sup> kg) x 10 <sup>-9</sup>	Fouling resistance - $R_F$ (m <sup>2</sup> kg) x 10 <sup>-9</sup>
Batch	93.97 ± 0.94 <sup>A</sup>	8.48 ± 0.58 <sup>AB</sup>	89.7	2.24 ± 0.02 <sup>A</sup>	9.43 ± 0.81 <sup>AB</sup>
Diafiltration	95.43 ± 1.03 <sup>A</sup>	9.56 ± 0.67 <sup>A</sup>	89.9	2.21 ± 0.02 <sup>A</sup>	7.92 ± 0.73 <sup>A</sup>
Batch + back flushing	94.22 ± 3.92 <sup>A</sup>	8.02 ± 0.06 <sup>B</sup>	90.4	2.24 ± 0.09 <sup>A</sup>	10.24 ± 0.32 <sup>B</sup>

\* means with different letters represent statistically difference according to the Tukey's test ( $p < 0.05$ )

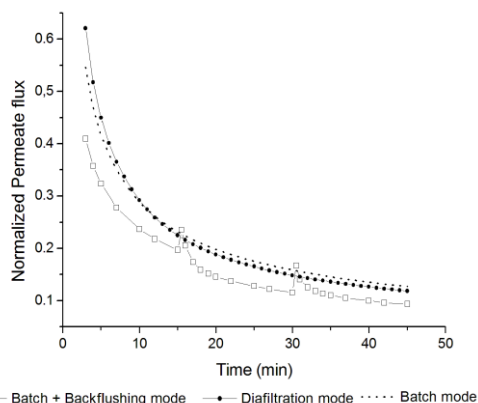


Fig. 4. Permeate flux profile for the operating modes evaluated.

Independent of the ultrafiltration operation mode adopted, the hydraulic resistance  $R_M$  was nearly the same and the permeate flux reduction was in the order of 90% (see Table II). This result indicates that physical process is not helpful to avoid the high flux decay due the polarized layer and gel layer precipitation. Same behavior is showed by Sonjui *et al.* [26], with flux decay in order of 90%, resulting in steady-state permeate flux in order of 20 L/m<sup>2</sup> h for treatment of aqueous solution of sericin with ultrafiltration system with flat sheet system and membrane of polysulfone.

The highest final steady-state permeate flux obtained with diafiltration (Table II) can be explained due the addition of water in system during the process, which control the concentration factor, reducing the viscosity. Back flushing water pulse applied in batch mode promoted a small increasing in permeate flux, followed by a sharp decay, as showed in Fig. 4.

As describe by Capar *et al.* [1], sericin can interact with surface membrane in adsorption uptake, make a monolayer resistance, and is followed by multi-layer protein build-up via intermolecular bonding. In this case, a cleaner membrane was possible to obtain with chemical treatment (24 hours of NaOH 0.1 mol/L).

TABLE III: SEPARATION/CONCENTRATION OF SERICIN USING MEMBRANE

CF	Sericin concentration in permeate (g/L)	Sericin concentration in retentate (g/L)	CR (%)
2	0.866 <sup>A</sup>	2.036 <sup>A</sup>	71.1 <sup>A</sup>
3	0.950 <sup>A</sup>	2.002 <sup>A</sup>	68.3 <sup>A</sup>
4	1.187 <sup>B</sup>	2.521 <sup>B</sup>	60.4 <sup>B</sup>

\* means with different letters represent statistically difference according to the Tukey's test ( $p < 0.05$ )

The separation process promoted by ultrafiltration allowed the removal of molecular weight below 50 kDa in the permeate stream (Fig. 5a). Retentate showed predominantly molecular weight distribution between 200 kDa and 100 kDa (Fig. 5b), while the permeate stream retains fractions of molecular weight lower than 50 kDa.

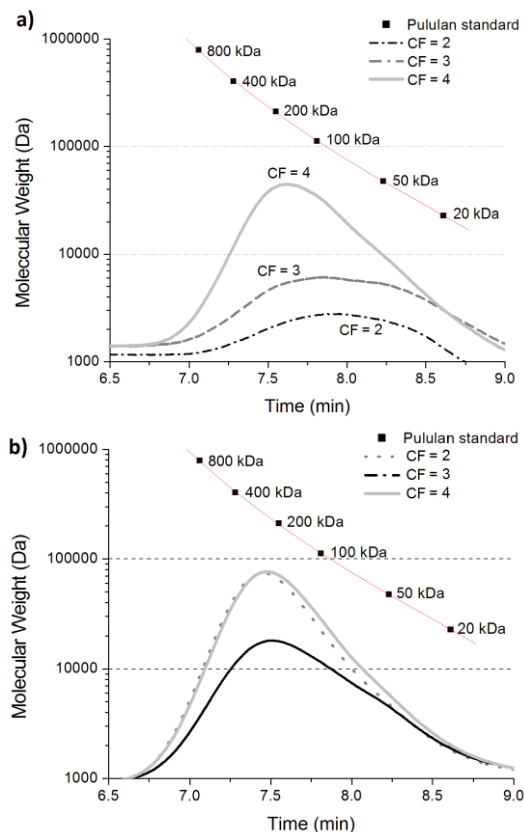


Fig. 5. Molecular weight profile in batch mode. a) permeate stream; b) retentate stream.

However, it was observed sericin permeation with molecular weight above 100 kDa which increases the solvent removal process, or as the increasing of the concentration factor  $CF$ , as illustrated in Fig. 5a. Table III shows the sericin concentration in retentate and permeate at different values of  $CF$ , and the coefficient of rejection  $CR$  was reduced due to the sericin lose in permeate with long time of operation.

For  $FC$  equal 2, same at batch volume reduction of 50 %, the sericin retention in ultrafiltration system was 71.1 %, while to  $FC$  equal 4, i.e. batch volume reduction of 75%, the sericin retention was 60.4 %. The results showed in this study (the reduction of the coefficient of rejection with the process time and the profile of molecular weight of sericin in the permeate stream showed in Fig. 5a) suggest that molecules with high molecular weight permeate the membrane after batch volume reduction of 50 %.

#### IV. CONCLUSION

Sericin extraction with water at 120 °C obtained yields of  $21.99 \pm 0.96\%$  and fraction with molecular weight between 200 kDa and 100 kDa. Carbonate helps the extraction process, but promotes the partial hydrolysis of sericin.



Ultrafiltration process with membrane of 50 kDa can be applied to sericin recovery with molecular weight above 100 kDa.

Permeate flux presented high decline, above 90 %, independent of the operating mode adopted. The steady-state permeate flux obtained varied between 8.02 at 9.56 kg/m<sup>2</sup> h.

Increasing the concentration factor from 2 to 4 promotes the permeation of sericin molecules of high molecular weight, reducing the rejection coefficient from 71.1 % to 60.4 %.

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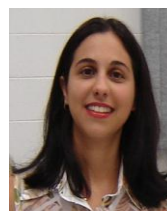


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