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Ready-to-use silkworm sericin: finding the best starting point

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INTRODUCTION

Silk sericin (SS) was until recently considered as a by-product in the field of sericulture. However, the potential of this natural protein for biomedical engineering is being unraveled over the past few years owing to its sol-gel transition properties and excellent biological characteristics [1,2]. SS is usually concentrated using different procedures such as rota-evaporation, lyophilization, dialysis and evaporation. Although evaporation in a heating plate is challenging to control, this technique is simple and allows to obtain high concentrations [3]. Lyophilization has been used in different works to improve control over the concentrations used. However, this process is reported to induce β-sheet conformation which can cause a blockage of hydrogel permeability and mechanical constrain [4]. Rotavapor is also used to concentrate silk-sericin, nevertheless studies do not usually report all the relevant experimental parameters used such as precise evaporation temperature and pressure [5]. Similarly, only limited information about dialysis is available in the literature [4]. In the present study, the abovementioned concentration techniques were optimized based on the best available evidence and compared.

METHODOLOGY

SS was obtained using extraction in boiling water. Afterwards, different concentration techniques were performed: 1) SS.E: controlled evaporation; 2) SS.RV: rota-evaporation (BUCHI Rotavapor™ RII) at 40°C, 500 mbar and 120 rpm; 3) SS.L: lyophilization, in which the SS solution was frozen in liquid nitrogen (LN2) and freeze-dried under vacuum; 4) SS.D: SS concentrated against a 20 wt.% PEG solution (Sigma-Aldrich, 20 KDa), using a dialysis membrane (Spectra/Por™ pre-wetted RC tubing MWCO: 3.5 kD) (**Figure 1**). The concentrated SS solutions were analyzed by Circular Dichroism (CD), FTIR (Spectrum Two FTIR PerkinElmer), XRD (D8 Advance de Bruker) and Cryo-SEM (JEOL JSM 6301F/ Oxford INCA Energy 350/ Gatan Alto 2500).

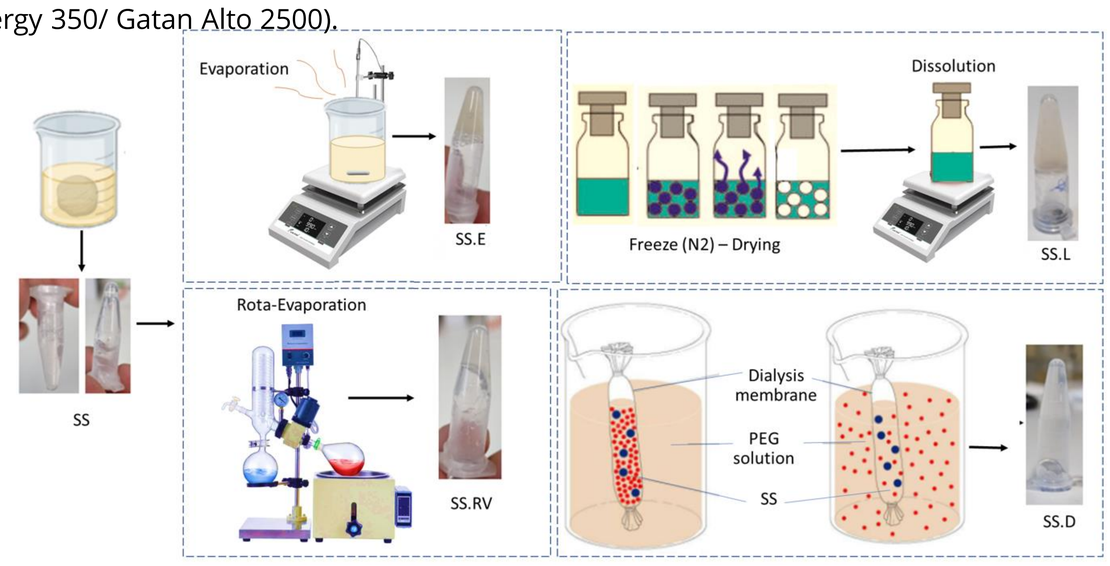

Figure 1. Experimental set-ups used for SS processing.

The presence of different types of porosity was also verified for this condition in Cryo-SEM, one of them apparently with a higher orientation degree. The longer concentration period during dialysis has favored the molecular self-assembly of silk sericin, which explains the differences registered. On the other hand, the proposed method of lyophilization using cryogenic temperature was able to freeze and dry the silk maintaining its amorphous structure. In this particular case it was possible to obtain a ready-to use dry material which was easy to re-dissolve (**Figure 4**).

RESULTS AND DISCUSSION

The CD spectra of the SS samples obtained have strong negative bands around 200 nm assigned to the random coil conformation and a weak negative band around 220 assigned to the β-structure, characteristic of SS extracted in boiling water [6]. The presence of these conformations was corroborated by FTIR through the deconvolution of amide I peak. The FTIR spectrum of SS.D resulted in a change in the amide I/II ratio **(Figure 2)**.

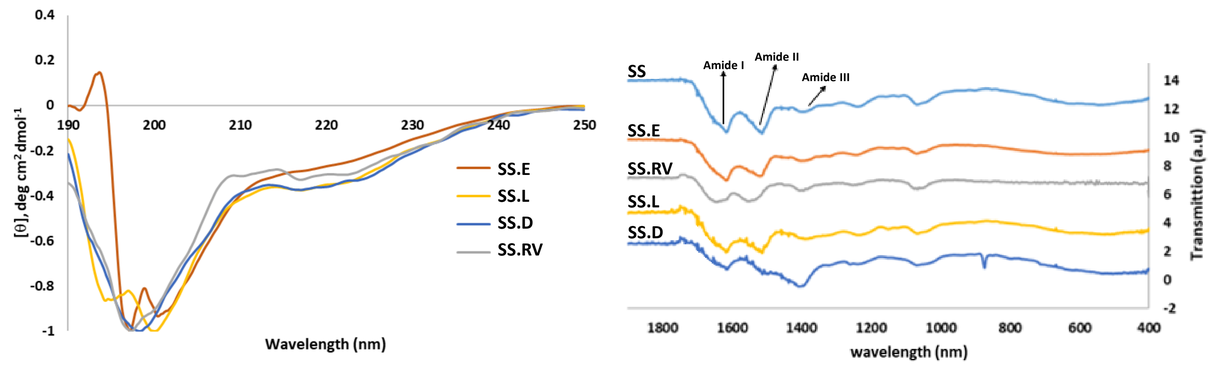

Figure 2. CD and FTIR spectra.

This was associated with a change in secondary structure due to the increase in β-sheet conformation. In XRD, a diffraction peak with broad intensity at 2θ = 28 and at 40 indicates the poor crystallinity of the SS-solutions, characteristic of random coil conformations. Again, peaks for SS concentrated by dialysis were slightly sharper, indicating an increase in crystallinity when compared to the other concentration methods, which corroborates the presence of higher β-sheet content (**Figure 3**).

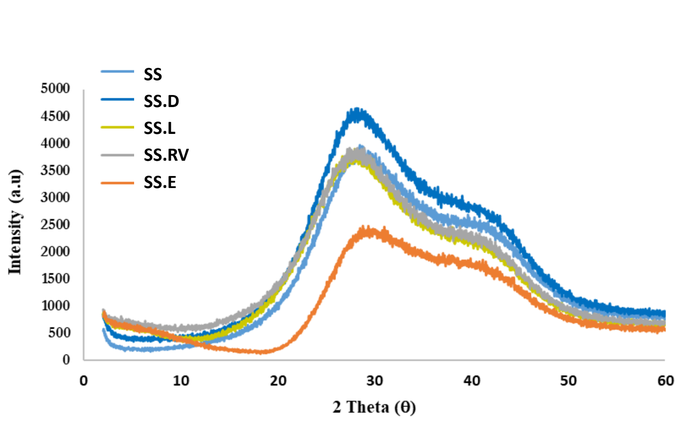

Figure 3. XRD spectra.

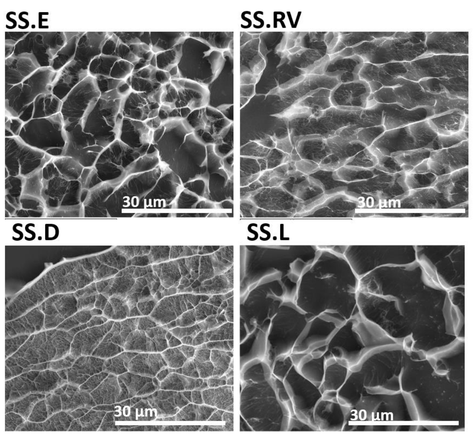

Figure 4. Cryo-SEM images.

CONCLUSIONS

For the first time, a study was carried out on comparing different SS concentration methods on its physicochemical characteristics and further processability. The results show that our so called cryo-lyophilization does not change the properties of the protein. Thus, this methodology in particular has high potential to generate a ready-to-use powder which can be easily sterilized and processed. Concentration by dialysis, on the other hand, leads to the formation of a more rigid structure with higher β-sheet amount. This study constitutes an important step to ensure the best silk characteristics at the starting point before further development, according with the processing goal.

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