Supplementary Information for Article

Revealing the influence of the degumming process in the properties of silk fibroin nanoparticles.

Guzmán Carissimi1§, A. Abel Lozano-Pérez2§, Mercedes G. Montalbán3, Salvador D. Aznar-Cervantes4, José Luis Cenis2 and Gloria Víllora1*.

1 Department of Chemical Engineering, Faculty of Chemistry, University of Murcia (UMU), Campus de Espinardo (Murcia), 30100, Spain;
2 Department of Biotechnology, Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA). La Alberca (Murcia), 30150, Spain.
3 Department of Chemical Engineering, University of Alicante, Apartado 99, 03080, Alicante, Spain.
§ Authors contributed equally.
* Correspondence: gvillora@um.es; (G.V.)
Received: date; Accepted: date; Published: date

Supplementary Information for Article

2. Materials and Methods

2.5. Morphological, physicochemical and structural characterization of SF fibers and SFNs

![Graph showing Amide III absorption band for crystallinity index calculation](image-url)

**Figure S1.** Example of base line traced in Amide III absorption band for the crystallinity index calculation.
Secondary structure assignation by ATR-FTIR

The secondary structure of the SF samples was also assigned by band fitting of the Amide I region, (1600–1700 cm\(^{-1}\)) of the spectra by using OMNIC V9.9.471 software following the approach described by Hu et al. [46]. Firstly, a baseline and advanced ATR corrections for one bounce of the incident light at an angle of 45° and a refractive index of 1.60 for the sample were applied to the original spectrum. Then, Fourier Self-Deconvolution (FSD) was applied to the Amide I region as a band narrowing technique. The parameters for the FSD were chosen on the basis of the resolution of the overlapping spectral features. The minimum values of the second derivative of the spectrum obtained by the FSD were used to center the initial bands of Gaussian shapes. Then, the iterative fitting algorithm implemented in the software was used to minimize the residual value between the spectrum and the sum of the deconvoluted bands without baseline.

Table S1. Assignment of the vibration bands in Amide I.

| Secondary structure feature | Wavenumber range (cm\(^{-1}\)) |
|----------------------------|---------------------------------|
| Aggregate beta-strand/beta-sheets (weak)a | 1616-1621 |
| β-Sheet (Strong)a | 1622-1627 |
| β-Sheet (Strong)b | 1628-1637 |
| Random coil/extended chains | 1638-1646 |
| Random coil | 1647-1655 |
| α-Helix | 1656-1662 |
| Turns | 1663-1670 |
| Turns | 1671-1685 |
| Turns | 1686-1696 |
| β-Sheets (weak)a | 1697-1703 |

a Intermolecular β-Sheet; b Intramolecular β-Sheets

![Figure S2. FSD of Amide I absorption band and example of band fitting.](image-url)
2.5. Morphological, physicochemical and structural characterization of SF fibers and SFNs

**Calculation of the surface charge density (σ) of a spherical colloidal particle.**

For a nanoparticle with constant surface charge density, the surface charge density is a more characteristic quantity than the Z-potential, \( \psi_0 \), because for such particles the Z-potential is not a constant and depends on the electrolyte concentration. The method is based on the following equation:

\[
\sigma = \frac{2e_\varepsilon_0 \kappa kT}{ze} \sinh \left( \frac{2e_\varepsilon_0 \psi_0}{2kT} \right) \left[ 1 + \frac{1}{\kappa a \cosh^2(z e_\varepsilon_0 / 4kT)} + \frac{1}{(ka)^2} \frac{\sinh(z e_\varepsilon_0 / 4kT)}{\cosh(z e_\varepsilon_0 / 4kT)} \right]^{\frac{1}{2}} \tag{S1}
\]

where \( \kappa \) is the Debye-Hückel parameter defined by:

\[
\kappa = \left[ \frac{1000e^2 N_A (2I)}{\varepsilon kT} \right]^{1/2} \tag{S2}
\]

and \( k \) is the Boltzmann constant, \( T \) is the absolute temperature, \( \varepsilon \) is relative permittivity of the solvent, \( \varepsilon_0 \) is the permittivity of the vacuum, \( \varepsilon \) is the permittivity, \( N_A \) is the Avogadro’s number, \( e \) is the elementary electric charge, \( I \) is the ionic strength; \( a \) is the radius of the spherical nanoparticles, \( \psi_0 \) is the Z-potential and \( z \) is the electrolyte valence. All the parameter values used for the calculation of the surface density charge are shown in the Table S2.

**Table S2.** List of parameters used to calculate the surface charge density

| Parameter                           | Value      | Units  |
|-------------------------------------|------------|--------|
| Relative permittivity 25°C, \( \varepsilon \) | 78.30      | adim.  |
| Permittivity of a vacuum, \( \varepsilon_0 \)  | 8.85E-12   | C/(J·m) |
| Permittivity, \( \varepsilon \)              | 6.93E-10   | C/(J·m) |
| Boltzmann constant, \( k \)            | 1.38E-23   | J/K    |
| Avogadro’s number, \( N_A \)          | 6.02E+23   | mol⁻¹  |
| Elementary electric charge, \( e \)    | 1.60E-19   | C      |
| Temperature, \( T \)                 | 298.15     | K      |
| Ionic strength, \( I \)               | 1.00E-03   | M      |
| Debye-Hückel parameter, \( \kappa \)   | 1.04E+08   | m⁻¹    |
| Electrolyte valence, \( z \)          | 1          | adim.  |
3. Results and Discussion

3.1. Degumming results

Table S3. Degumming efficiency expressed in terms of mass loss and Crystallinity index (CI) of SF samples: D1) Autoclave, D2) Na2CO3 30´, D3) Na2CO3 120´ and D4) Ultrasounds.

| Sample | Mass loss (wt. %)\(^a\) | CI (%)\(^a\) |
|--------|-----------------------------|--------------|
| SF-D1  | 31.3 ± 0.5                  | 59 ± 2       |
| SF-D2  | 32.4 ± 0.8                  | 56 ± 3       |
| SF-D3  | 44.4 ± 1.0                  | 59 ± 2       |
| SF-D4  | 25.9 ± 1.2                  | 52 ± 1       |

\(^a\)Results are shown as mean ± standard deviation, \(n = 3\).
3.2. SF Secondary Structure Analysis

Figure S3. ATR-FTIR spectra of silk fibroins (SF) and silk fibroin nanoparticles (SFN) degummed by: D1) Autoclave, D2) Na$_2$CO$_3$ 30’, D3) Na$_2$CO$_3$ 120’ and D4) Ultrasound Amide III absorption band for the crystallinity index calculation. Insets with magnification of the Amide I region of the spectra, used for determination of the secondary structures are included. (Please refer the online version for the color representation of the figure)
Table S4. Relative contribution of secondary structure features of the Amide I in the different stages of the process. SC) Silk Cocoons, internal or external faces, SF) Degummed silk fibroin and SFN) Silk fibroin nanoparticles prepared from SF degummed by: D1) Autoclave, D2) Na₂CO₃ 30’, D3) Na₂CO₃ 120’ and D4) Ultrasound obtained by FSD analysis of the Amide I infrared absorption band. n = 3, average ± standard deviation.

| Sample  | β-Sheet    | Random Coil | α-Helix | Turn     |
|---------|------------|-------------|---------|----------|
| SF-D1   | 53.6 ± 2.6 | 21.5 ± 1.0  | 9.7 ± 0.6 | 15.2 ± 0.7 |
| SF-D2   | 51.2 ± 1.1 | 22.4 ± 0.3  | 9.9 ± 0.4 | 16.5 ± 0.7 |
| SF-D3   | 50.0 ± 1.0 | 24.5 ± 1.1  | 10.0 ± 0.2 | 15.5 ± 0.7 |
| SF-D4   | 46.7 ± 0.8 | 21.6 ± 0.1  | 11.2 ± 0.6 | 20.5 ± 0.4 |
| SFN-D1  | 59.2 ± 0.6 | 20.4 ± 0.1  | 8.2 ± 0.2 | 12.2 ± 0.5 |
| SFN-D2  | 58.9 ± 1.0 | 21.3 ± 0.7  | 8.1 ± 0.3 | 11.7 ± 0.5 |
| SFN-D3  | 60.2 ± 1.0 | 20.4 ± 1.0  | 7.1 ± 0.8 | 12.2 ± 0.7 |
| SFN-D4  | 55.4 ± 0.7 | 21.9 ± 0.4  | 8.4 ± 0.5 | 14.3 ± 0.8 |
Table S5. Analysis of variance (ANOVA) of the relative contribution of secondary structure (n = 3).

| Tukey's multiple comparisons test | Mean Diff. | 95.00% CI of diff. | Significant | Adjusted P Value |
|----------------------------------|------------|---------------------|-------------|------------------|
| **β-Sheet**                      |            |                     |             |                  |
| SF-D1 vs. SF-D2                  | 2.4        | -0.6805 to 5.480    | No          | 0.2661           |
| SF-D1 vs. SF-D3                  | 3.6        | 0.5195 to 6.680     | Yes         | 0.0098           |
| SF-D1 vs. SF-D4                  | 6.9        | 3.820 to 9.980      | Yes <0.0001 |                  |
| SF-D2 vs. SF-D3                  | 1.2        | -1.880 to 4.280     | No          | 0.9579           |
| SF-D2 vs. SF-D4                  | 4.5        | 1.420 to 7.580      | Yes         | 0.0004           |
| SF-D3 vs. SF-D4                  | 3.3        | 0.2195 to 6.380     | Yes         | 0.026            |
| SFN-D1 vs. SFN-D2                | 0.2837     | -2.797 to 3.364     | No >0.9999  |                  |
| SFN-D1 vs. SFN-D3                | -1.02      | -4.101 to 2.060     | No          | 0.9855           |
| SFN-D1 vs. SFN-D4                | 3.784      | 0.7032 to 6.864     | Yes         | 0.0052           |
| SFN-D2 vs. SFN-D3                | -1.304     | 4.385 to 1.776      | No          | 0.9305           |
| SFN-D2 vs. SFN-D4                | 3.5        | 0.4195 to 6.580     | Yes 0.0137  |                  |
| SFN-D3 vs. SFN-D4                | 4.804      | 1.724 to 7.885      | Yes         | 0.0001           |
| **Random Coil**                  |            |                     |             |                  |
| SF-D1 vs. SF-D2                  | -0.9       | -3.980 to 2.180     | No          | 0.9941           |
| SF-D1 vs. SF-D3                  | -3         | -6.080 to 0.08047   | No          | 0.0628           |
| SF-D1 vs. SF-D4                  | -0.1       | -3.180 to 2.980     | No >0.9999  |                  |
| SF-D2 vs. SF-D3                  | -2.1       | -5.180 to 0.9805    | No          | 0.453            |
| SF-D2 vs. SF-D4                  | 0.8        | -2.280 to 3.880     | No          | 0.9976           |
| SF-D3 vs. SF-D4                  | 2.9        | -0.1805 to 5.980    | No          | 0.0824           |
| SFN-D1 vs. SFN-D2                | -0.8918    | -3.972 to 2.189     | No 0.9945   |                  |
| SFN-D1 vs. SFN-D3                | 0          | -3.080 to 3.080     | No >0.9999  |                  |
| SFN-D1 vs. SFN-D4                | -1.492     | -4.572 to 1.589     | No          | 0.8555           |
| SFN-D2 vs. SFN-D3                | 0.8918     | 2.189 to 3.972      | No          | 0.9945           |
| SFN-D2 vs. SFN-D4                | -0.6       | -3.680 to 2.480     | No 0.9998   |                  |
| SFN-D3 vs. SFN-D4                | -1.492     | -4.572 to 1.589     | No 0.8555   |                  |
| **α-Helix**                      |            |                     |             |                  |
| SF-D1 vs. SF-D2                  | -0.2       | -3.280 to 2.880     | No >0.9999  |                  |
| SF-D1 vs. SF-D3                  | -0.3       | -3.380 to 2.780     | No >0.9999  |                  |
| SF-D1 vs. SF-D4                  | -1.5       | -4.580 to 1.580     | No 0.8515   |                  |
| SF-D2 vs. SF-D3                  | -0.1       | -3.180 to 2.980     | No >0.9999  |                  |
| SF-D2 vs. SF-D4                  | -1.3       | -4.380 to 1.780     | No 0.9317   |                  |
| SF-D3 vs. SF-D4                  | -1.2       | -4.280 to 1.880     | No 0.9579   |                  |
| SFN-D1 vs. SFN-D2                | 0.06327    | -3.017 to 3.144     | No >0.9999  |                  |
| SFN-D1 vs. SFN-D3                | 1.02       | -2.060 to 4.101     | No 0.9855   |                  |
| SFN-D1 vs. SFN-D4                | -0.2367    | -3.317 to 2.844     | No >0.9999  |                  |
| SFN-D2 vs. SFN-D3                | 0.9571     | 2.123 to 4.038      | No 0.9908   |                  |
| SFN-D2 vs. SFN-D4                | -0.3       | -3.380 to 2.780     | No >0.9999  |                  |
| SFN-D3 vs. SFN-D4                | -1.257     | -4.338 to 1.823     | No 0.944    |                  |
|        |        |        |        |
|--------|--------|--------|--------|
| **β-Turn** |        |        |        |
| SF-D1 vs. SF-D2 | -1.3 | -4.380 to 1.780 | No | 0.9317 |
| SF-D1 vs. SF-D3 | -0.3 | -3.380 to 2.780 | No | >0.9999 |
| SF-D1 vs. SF-D4 | -5.3 | -8.380 to -2.220 | Yes | <0.0001 |
| SF-D2 vs. SF-D3 | 1 | -2.080 to 4.080 | No | 0.9874 |
| SF-D2 vs. SF-D4 | -4 | -7.080 to -0.9195 | Yes | 0.0024 |
| SF-D3 vs. SF-D4 | -5 | -8.080 to -1.920 | Yes | <0.0001 |
| SFN-D1 vs. SFN-D2 | 0.5449 | -2.536 to 3.625 | No | 0.9999 |
| SFN-D1 vs. SFN-D3 | 7.105e-15 | -3.080 to 3.080 | No | >0.9999 |
| SFN-D1 vs. SFN-D4 | -2.055 | -5.136 to 1.025 | No | 0.4846 |
| SFN-D2 vs. SFN-D3 | -0.5449 | -3.625 to 2.536 | No | 0.9999 |
| SFN-D2 vs. SFN-D4 | -2.6 | -5.680 to 0.4805 | No | 0.1733 |
| SFN-D3 vs. SFN-D4 | -2.055 | -5.136 to 1.025 | No | 0.4846 |