Supplementary Information for Article

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

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**Figure S1**. Example of base line traced in Amide III absorption band for the crystallinity index calculation.

**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.

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

|  |  |  |
| --- | --- | --- |
| Parameter | Value | Units |
| Relative permittivity 25oC, *Ɛr* | 78,30 | *adim.* |
| Permittivity of a vacuum, *Ɛ0* | 8,85E-12 | *C2/(J·m)* |
| Permittivity, *Ɛ* | 6,93E-10 | *C2/(J·m)* |
| Boltzmann constant, *k* | 1,38E-23 | *J/K* |
| Avogadro’s number, *NA* | 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-Hüuckel parameter, | 1,04E+08 | *m-1* |
| Electrolyte valence, *z* | 1 | *adim.* |

**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 |

aResults are shown as mean ± standard deviation, *n* = 3.

**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 |
| Int | 37.2 ± 3.3 | 32.1 ± 2.2 | 12.3 ± 1.4 | 18.4 ± 1.3 |
| Ext | 35.9 ± 2.6 | 30.6 ± 2.1 | 13.0 ± 0.3 | 20.5 ± 0.1 |
| 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 |