

## *Supplementary Material for*

# **Influence of different silk-based fillers on the thermal and mechanical properties of polylactic acid composites**

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**Figure S10.** Micrographs of the samples stained with Coomassie blue. (Scale bar: 500  $\mu\text{m}$  (5 $\times$ ) or 100  $\mu\text{m}$  (10 $\times$ )).

## **References**

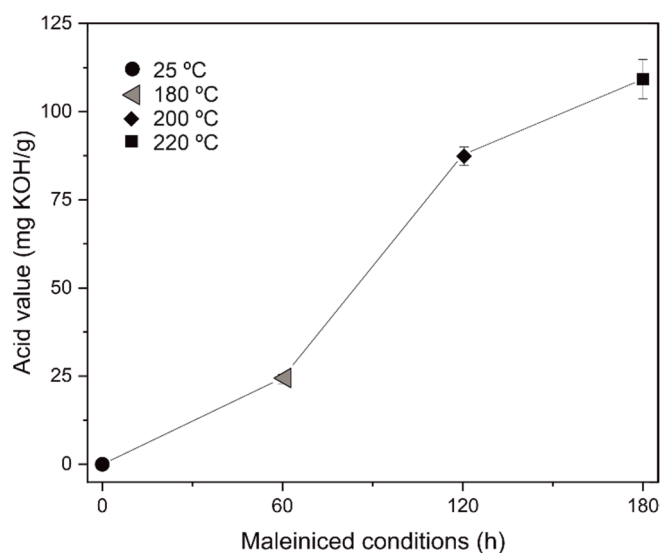
### S1. Determination of the degree of maleinization

The degree of maleinization was determined following the guidelines set by the ISO 660:2009 standard [1] by using the following expression:

$$\text{Acid Value} = \frac{56.1 \times c \times V}{m} \quad (1)$$

Where  $c$  is the exact concentration of the KOH standard solution used ( $\text{mol} \cdot \text{L}^{-1}$ ),  $V$  is the volume of the KOH standard solution used (expressed in mL) and  $m$  the analyzed mass (g).

Figure 2 shows the evolution of the acid number values at the end of each of the stages at different temperatures, 180, 200 and 220 °C. Initially the AV is  $0.15 \text{ mg KOH g}^{-1}$  and at the end of the first hour, at 180 °C, it is observed that it increases to  $24.4 \text{ mg KOH g}^{-1}$ , verifying that the maleinization reaction is occurring. After the second hour, at 200 °C, another large increase is observed until reaching an AV value of  $87.8 \text{ mg KOH g}^{-1}$  and, finally, after the last stage, at 220 °C, the AV reaches  $109.2 \text{ mg KOH g}^{-1}$ . These results are in total agreement with the values obtained by A. Perez-Nakai *et al.* With an AV of 105 for the maleinized hemp seed and 130 for the maleinized brazil nut at the end of the epoxidation process [2]. Furthermore, Quiles Carrillo *et al.* indicate that commercial grade maleinized linseed oil has a VA of between 105-130  $\text{mg KOH g}^{-1}$  [3].



**Figure S1.** Evolution of the acid value in the different stages of the process of maleinization of corn oil.

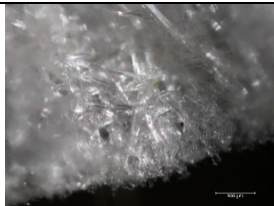
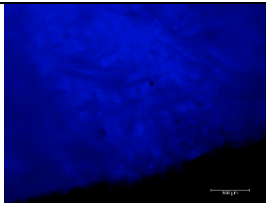
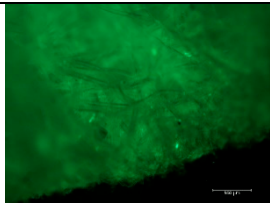
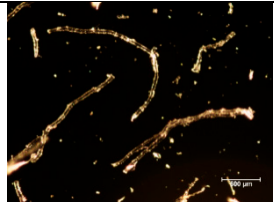
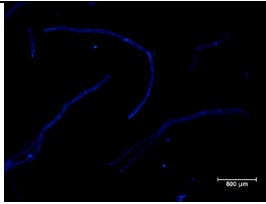
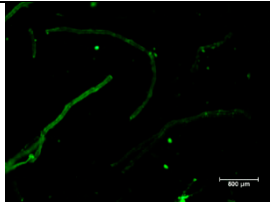

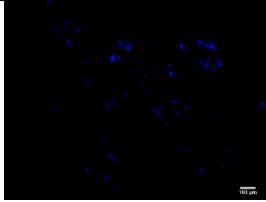
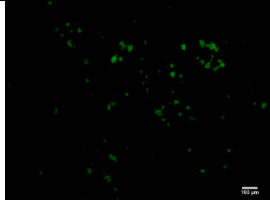
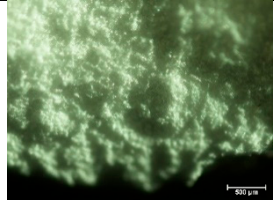
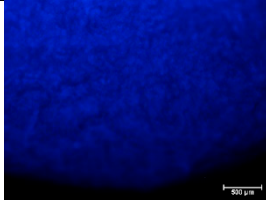
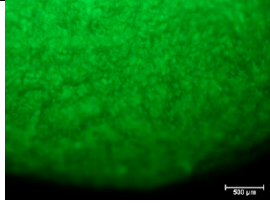
## S2. Silk Fillers

### *S2.1 Characterization.*

Microscopic analysis of the samples was also performed by using a Nikon Eclipse 50i microscope (Nikon Instrument Inc., Melville, NY, USA) equipped with Plan Fluor objectives (5×, 10×, 20×, 40×, 50× and 100×), fluorescence filters for DAPI (UV-2E/C), FITC (B-2E/C) and TRITC (G-2E/C), and a 100 W mercury lamp as light source. Images were captured by a Nikon DS-Fi1-U2 camera controlled by NIS-Elements F software (Ver. 3.00, SP7, Build 547).



**Figure S2.** White silk fibroin cocoons used for the study under white light lamp (top) and UV lamp ( $\lambda=365\text{nm}$ ) (down).

| ADDITIVE                          | Bright field   | UV+DAPI Filter  | UV+FITC Filter   |
|-----------------------------------|--|---|--|
| -Raw cocoon<br>(Scale bar: 500μm) |   |   |   |
| CS<br>(Scale bar: 100 μm)         |   |   |   |
| SFM<br>(Scale bar: 100 μm)        |   |   |   |
| SFN<br>(Scale bar: 500 μm)        |  |  |  |

**Figure S3.** Micrographs of the silk fillers under white light and UV light and fluorescence filters.

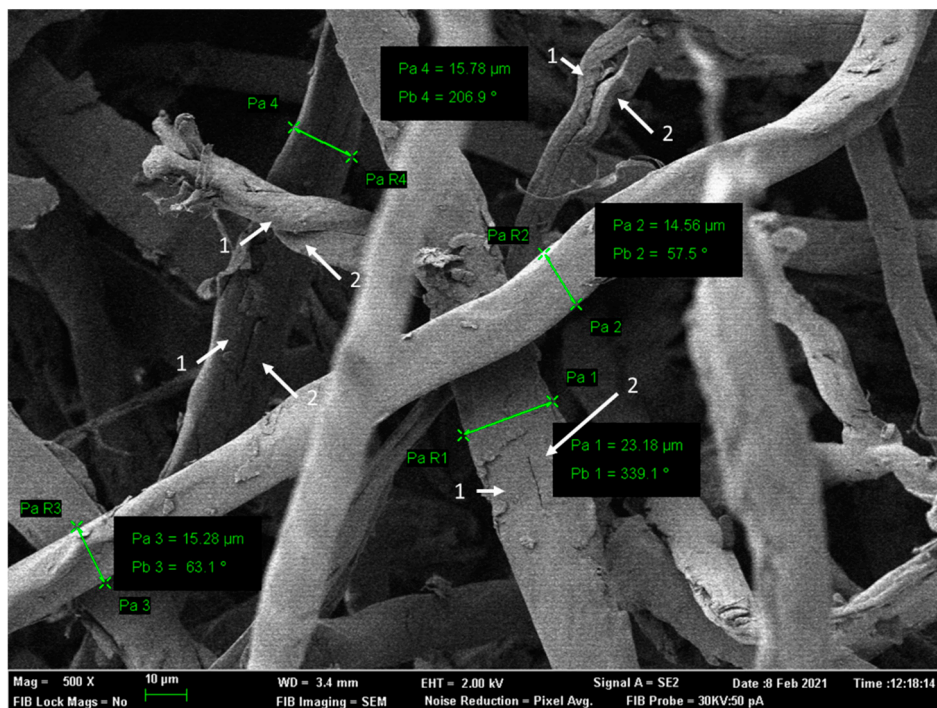


Figure S4. SEM micrograph of the crushed silk fibres.

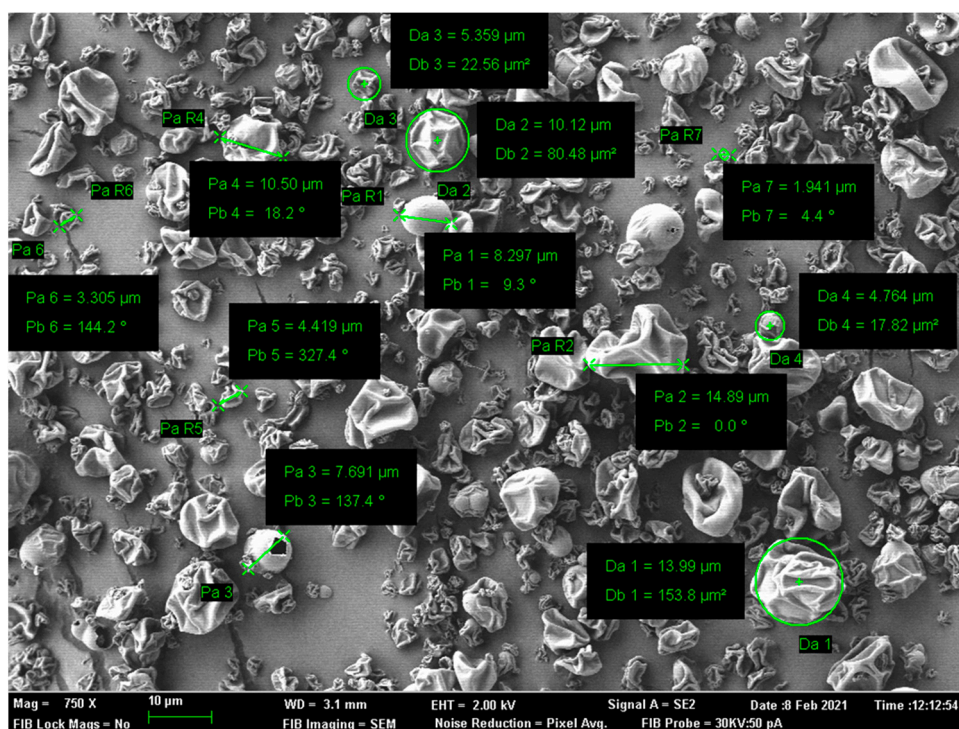
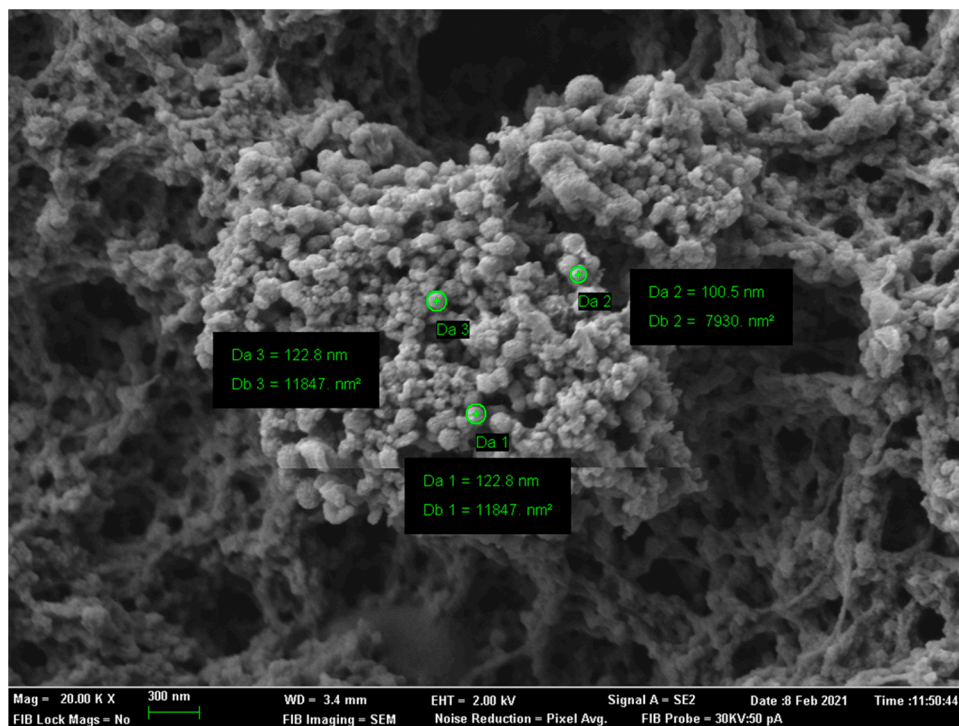


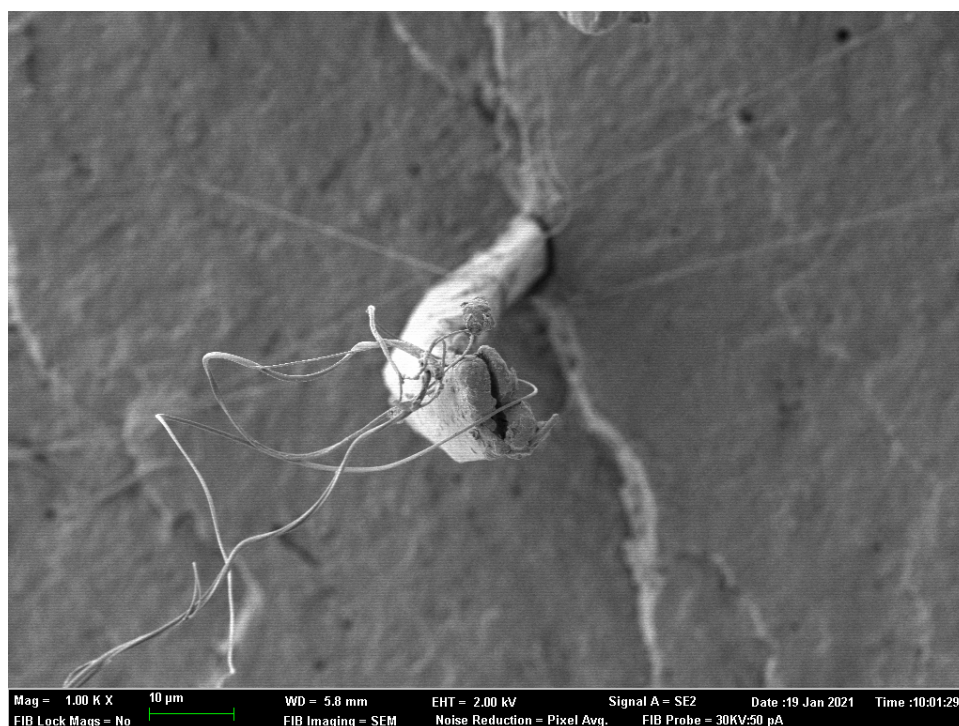
Figure S5. SEM micrograph of the silk microparticles.



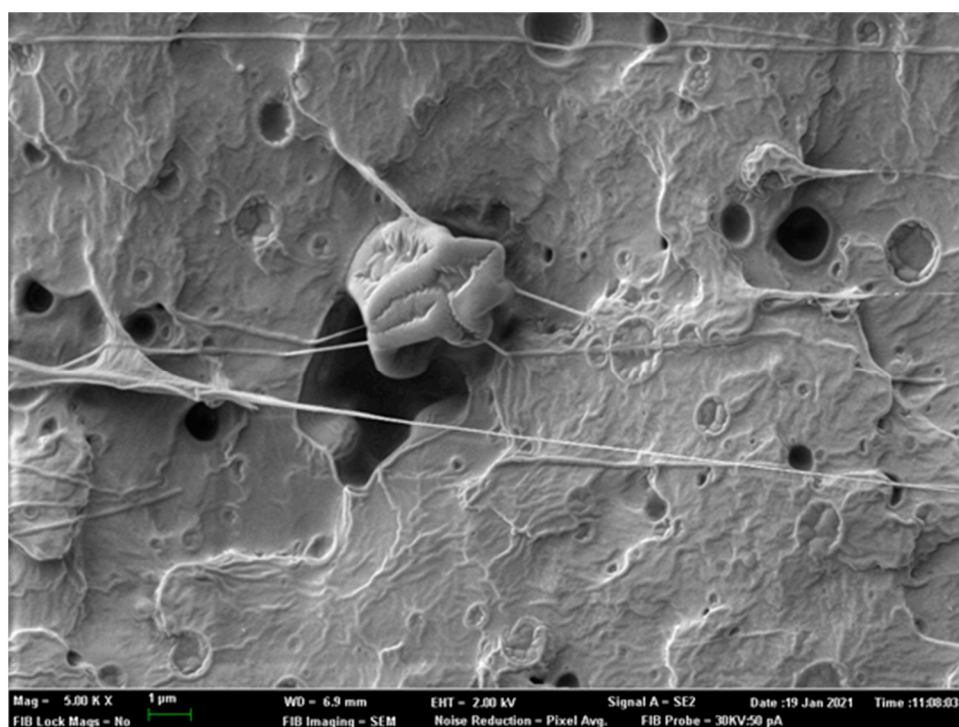


**Figure S6.** SEM micrograph of the silk nanoparticles.

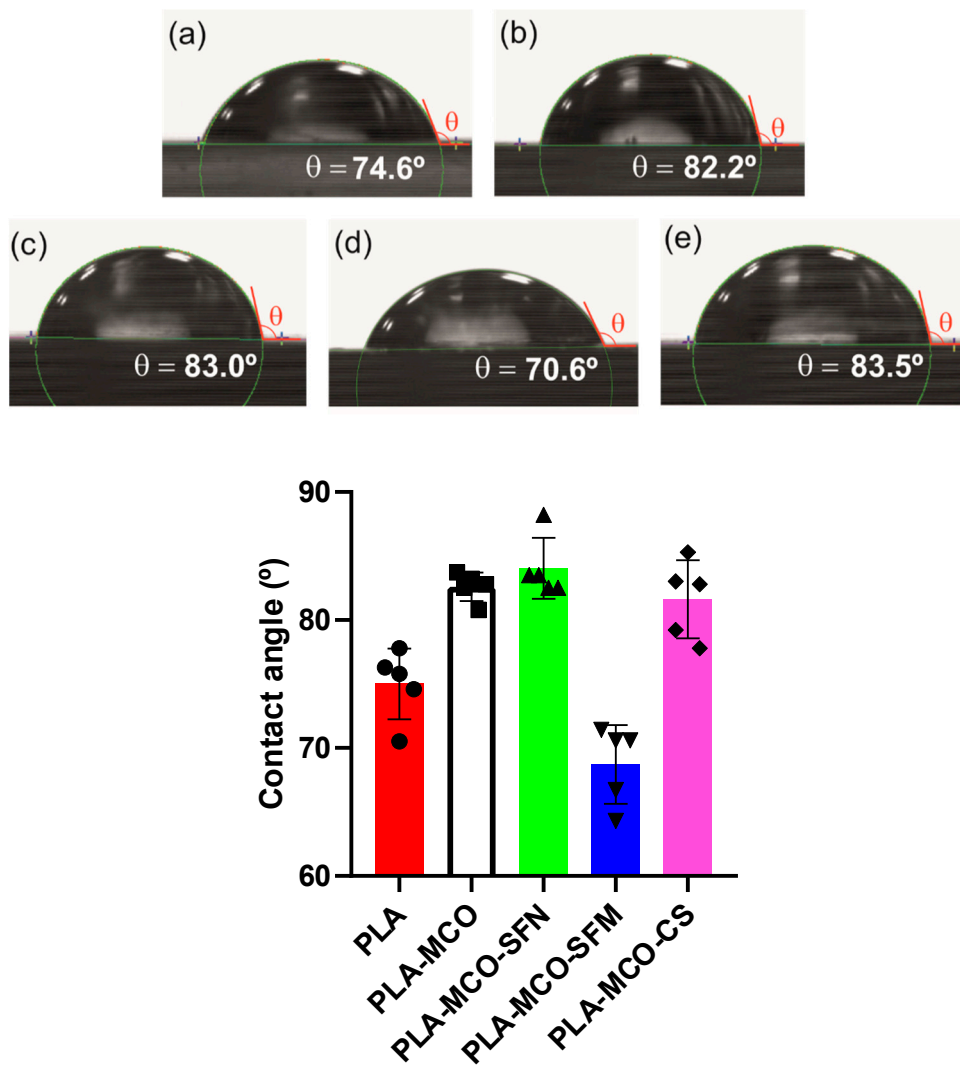
### S3. Characterization of PLA-MCO-silk composites



**Figure S7.** SEM micrograph of a silk fiber included in the PLA-MCO matrix.





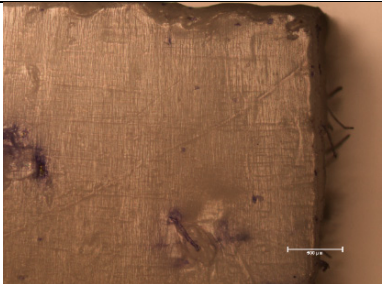
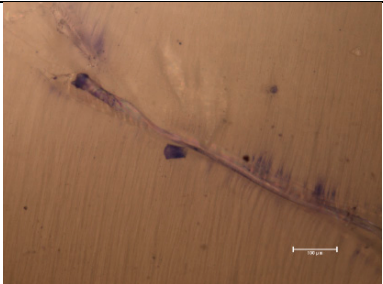
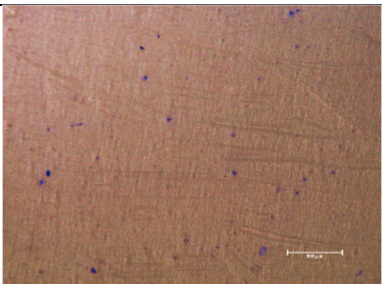
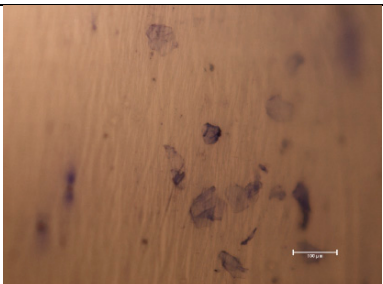
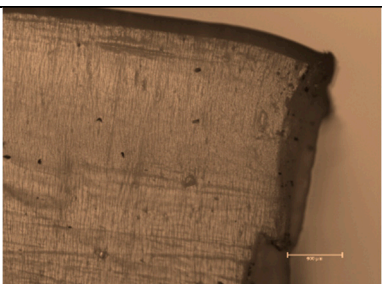
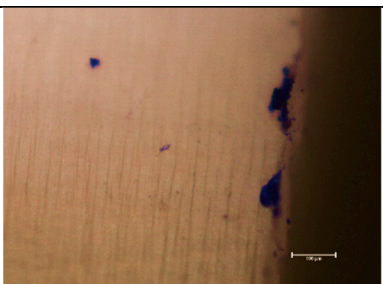
**Figure S8.** SEM micrograph of silk microparticles included in the PLA-MCO matrix.



**Figure S9.** Micrographs used for the measurement of the water contact angle ( $\theta_w$ ) of the samples: (a) PLA, (b) PLA-MCO, (c) PLA-MCO-CS, (d) PLA-MCO-SFM and (e) PLA-MCO-SFN. Values are inserted.



Fractured samples were stained with 0.25% Coomassie Brilliant Blue (Acros Organics, Geel, Belgium), fixed and discolored in ethanol/water mixture, following the established protocol for staining proteins in the electrophoresis polyacrylamide gels as described by Laemli *et al.* [4] and photographed for analysis.

| Sample      | General view  | Detail   |
|-------------|---|--|
| -PLA-MCO    |    |    |
| PLA-MCO-CS  |   |   |
| PLA-MCO-SFM |  |  |
| PLA-MCO-SFN |  |  |

**Figure S10.** Micrographs of the samples stained with Coomassie blue. (Scale bar: 500 µm (5×) or 100 µm (10×)).

## References

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