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1 Plant Cell wall inspired Xyloglucan/Cellulose

2 Nanocrystals Aerogels Produced By Freeze-Casting

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19 HIGHLIGHTS

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- Non-directional and directional freezing of CNC/xyloglycan aerogels is reported

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- Addition of xyloglycan (XG) changes aerogel morphology from lamellar to alveolar

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- Directional freeze-casting leads to oriented pores enhancing mechanical properties

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- Increasing CNC/XG ratio improves mechanical properties of aerogels

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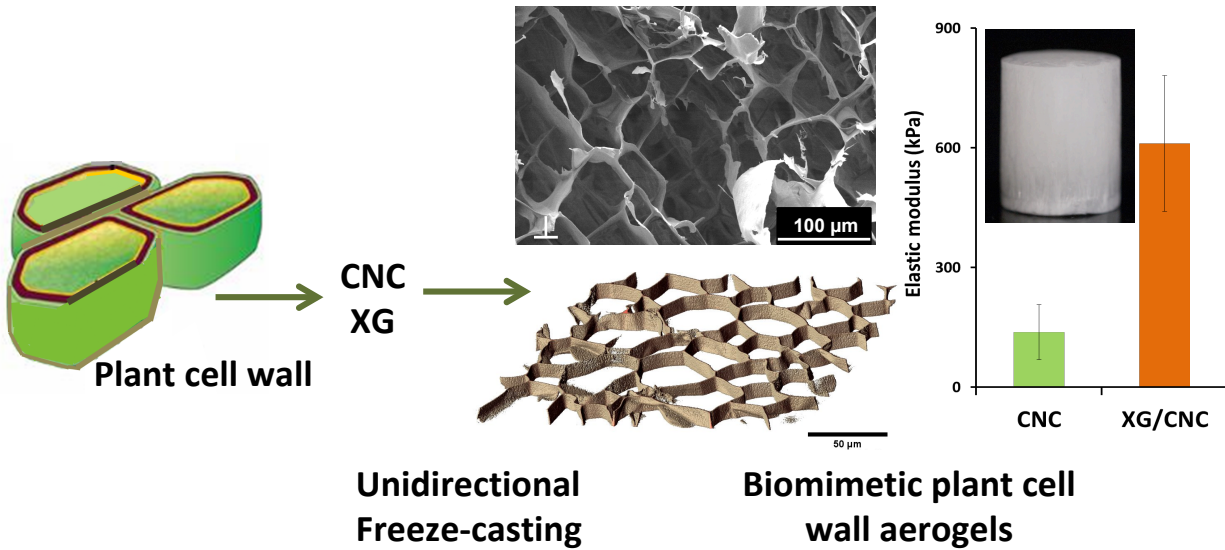
- Without chemical cross-linking, such CNC/XG aerogels do not dispersed in water

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34 GRAPHICAL ABSTRACT:



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37 ABSTRACT

38 Cellulose nanocrystals (CNC) and xyloglucan (XG) were used to construct new aerogels inspired
39 by the hierarchical organization of wood tissue, i.e., anisotropic porous cellular solid with pore
40 walls containing oriented and stiff cellulose nanorods embedded in hemicellulose matrix. Aerogels
41 with oriented or disordered pores were prepared by directional and non-directional freeze-casting
42 from colloidal dispersions of XG and CNC at different ratios. XG addition induced a clear
43 improvement of the mechanical properties compared to the CNC aerogel, as indicated by the
44 Young modulus increase from 138 kPa to 610 kPa. The addition of XG changed the pore
45 morphology from lamellar to alveolar and it also decreased the CNC orientation (the Hermans'
46 orientation factor was 0.52 for CNC vs 0.36-0.40 for CNC-XG). The aerogels that contained the
47 highest proportion of XG also retained their structural integrity in water without any chemical
48 modification. These results open the route to biobased water-resistant materials by an easy and
49 green strategy based on polymer adsorption rather than chemical crosslinking.

50 KEYWORDS: xyloglucan (XG), cellulose nanocrystal (CNC), directional freeze-casting, aerogel,
51 mechanical property

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60 1 INTRODUCTION

61 Plant stem tissues are highly organized, hierarchical structures. At the tissue level, cells form an
62 anisotropic and porous cellular structure that is oriented along the stem (Cosgrove, 2005; Svagan,
63 Jensen, Dvinskikh, Furó, & Berglund, 2010). Cell walls are themselves nanostructured since they
64 consist of oriented cellulose nanofibers along the long axis of the fibers and organized in a
65 helicoidal manner. This structural hierarchy is largely responsible for the remarkable mechanical
66 properties of plant cell walls that also display low density and resistance to pathogens and other
67 stresses (Burgert, 2006; Lakes, 1993). The main cell wall components, namely cellulose,
68 hemicellulose and lignin, form a complex network in which they act cooperatively and thereby
69 determine the mechanical behavior of the entire cell wall (Burgert, 2006; Cosgrove, 2005). The
70 whole structural role of hemicelluloses in the cell wall is not fully established, but hemicelluloses
71 are considered to contribute to load transfer and connecting cellulose fibers (Lopez-Sanchez et al.,
72 2015).

73 Cellulose nanocrystals (CNC) are rod-like nanoparticles prepared by the removal of disordered
74 parts of cellulose fibers (Klemm et al., 2011). Because of their impressive mechanical properties
75 and nanometric dimensions, they have recently been used as reinforcement materials in composite
76 films, hydrogels and aerogels (Karaaslan, Tshabalala, Yelle, & Buschle-Diller, 2011; Saxena,
77 Elder, Pan, & Ragauskas, 2009). Xyloglucans (XG) are hemicelluloses displaying a large
78 structural variability present in the primary plant cell wall. XG is believed to contribute to the

79 mechanical properties of the plant cell walls (Cosgrove, 2014; Scheller & Ulvskov, 2010), since
80 XG has a high affinity to cellulose and adsorbs irreversibly on cellulosic surfaces (Cosgrove,
81 2014). It has been shown that XG adopts different conformations when adsorbed on CNC,
82 depending on the CNC/XG concentration ratios: at low ratios, almost all XG chains bind as trains
83 to the CNC surface, whereas at high ratios, XG forms more loops and tails (Dammak et al., 2015;
84 Villares, Moreau, Dammak, Capron, & Cathala, 2015). Here, we hypothesize that such
85 conformational variations might affect the properties of cellulose/XG assemblies.

86 Aerogels are materials derived from hydrogels in which the liquid phase has been replaced by a
87 gas in mild conditions. They are characterized by a very high porous volume (> 90%) and display
88 interesting properties (Fricke & Tillotson, 1997). Among the different techniques used to create
89 porous materials, freeze-casting is a versatile and easy to implement technique. Freeze-casting
90 involves unidirectional freezing of a colloidal suspension or a solution following a predefined
91 thermal gradient (Deville, 2010). In water, due to the limited solubility of most compounds in ice,
92 most solutes and/or suspended particles are segregated from the growing ice front into the
93 interstitial space defined by ice crystals. Subsequent solvent sublimation leads to the formation of
94 aerogels whose porosity reproduces the ice crystals' morphology (Deville, 2008). Bioaerogels, *i.e.*
95 aerogels prepared from natural materials, with homogeneous and well-defined architectures have
96 been a subject of primary interest because of their potential applications in areas such as thermal
97 insulation (Wicklein et al., 2015), tissue engineering (Ghorbani, Nojehdehian, & Zamanian, 2016;
98 Yin, Divakar, & Wegst, 2019), drug delivery (Szepes, Ulrich, Farkas, Kovács, & Szabó-Révész,
99 2007) and automotive components (Köhnke, Lin, Elder, Theliander, & Ragauskas, 2012; Lee &
100 Deng, 2011). It was found that the distribution of pore size, pore shape and pore connectivity is
101 the result of the shape of ice crystals formed during freezing (Jiménez-Saelices, Seantier, Grohens,

102 & Capron, 2018). Hence, different microstructures and associated functional properties can be
103 obtained by controlling operating parameters such as the freezing rate (or ice front velocity), the
104 interfacial free energy between the particles and the liquid, the interaction of the particles with
105 themselves, as well as the size, distribution and concentration of the particles (Deville, Saiz, &
106 Tomsia, 2006).

107 A number of CNC aerogels prepared by freeze-casting have been reported in the literature (Chau
108 et al., 2016; Chu, Qu, Zussman, & Xu, 2017; Dash, Li, & Ragauskas, 2012; Munier, Gordeyeva,
109 Bergström, & Fall, 2016). However, only few studies have focused on CNC-hemicelluloses
110 hydrogels (Karaaslan et al., 2011; Köhnke, Elder, Theliander, & Ragauskas, 2014; Lopez-Sanchez
111 et al., 2015) or aerogels (De France, Hoare, & Cranston, 2017; Köhnke et al., 2014; Köhnke et al.,
112 2012). These examples, however, rely on chemical modification of CNC or hemicelluloses in order
113 to promote polymer-polymer interactions. In contrast, the present study is devoted to the use of
114 XG and CNC without any chemical pretreatment, taking advantage of their physical interactions
115 and of the different conformations of XG adsorbed on CNC (Dammak et al., 2015). These
116 conformations were controlled by varying the CNC/XG concentration ratios (Dammak et al.,
117 2015) in order to develop bioaerogels with cellular structures that mimic the structural and
118 mechanical properties of the plant cell wall (Kam et al., 2019; Pan et al., 2016). In order to elucidate
119 the interplay between CNC/XG interactions and the properties of freeze-cast materials, they were
120 compared to reference aerogels of the same composition obtained by non-directional freezing.
121 With the aim of providing an integrated vision of these CNC/XG aerogels, we combined a
122 multiscale (from nano- to macro-scale) structural approach using X-ray diffraction (XRD) to
123 assess the orientation of the CNCs, scanning electron microscopy and confocal microscopy to
124 investigate the porous structure, mechanical testing and water immersion conditions to study their

125 physical and chemical stability. On this basis, it was possible to unveil the multifaceted influence
126 of XG on the macroscopic properties of biobased and biological materials.

127

128 2 MATERIALS AND METHODS

129 2.1 Chemicals

130 CNCs obtained from acid hydrolysis of bleached softwood kraft pulp were provided by
131 CelluForce (Montreal, Canada). Product specifications are the following : crystalline fraction =
132 0.88 (by XRD), surface charge density = 0.023 mmol/g (by conductivity), lateral dimension = 2.3-
133 4.5 nm (by AFM), length = 44-108 nm (by AFM). Aqueous CNC suspension (60 g.L⁻¹) was
134 prepared by dispersing the CNC powder in deionized water (18.2 MΩ.cm⁻¹, Millipore Milli-Q
135 purification system) under vigorous stirring with a magnetic stirring bar overnight until it appeared
136 to be well-dispersed according to visual inspection. Afterwards, the dispersion was sonicated for
137 10 min in an ice bath to avoid overheating, using a QSonica Q700 sonicator (20 kHz, QSonica
138 LLC, Newtown, USA) with a 12.7 mm in diameter ultrasound probe (50 W with 50% amplitude).

139 Xyloglucan from *Tamarindus indica* was provided by Megazyme (Bray, County Wicklow,
140 Ireland). Monosaccharide composition (dry weight %): Glucose 49.1 ± 1.1; Xylose 32.1 ± 2.3;
141 Galactose 16.4 ± 0.6; Arabinose 2.4 ± 0.1. The mass average molar mass was $\overline{M}_w = 840 \times 10^3$
142 g.mol⁻¹ and the number average molar mass was $\overline{M}_n = 677 \times 10^3$ g.mol⁻¹ (dispersity $D = 1.24$). A
143 XG solution with a 10 g.L⁻¹ concentration was first prepared. It was then concentrated to 20 g.L⁻¹
144 by osmosis in a dialysis membrane (Espectra/Por®, cutoff 6–8 kDa) immersed in a 10 wt.%
145 aqueous dextran solutions (10⁵ g.mol⁻¹).

146

147 2.2 Aerogel preparation

148 The final concentration of the CNC/XG suspensions was fixed at 2 wt.% dry matter and this
149 concentration was kept constant independently of the CNC:XG ratio. CNC-XG complexes were
150 prepared by mixing dispersed CNC and XG at two different concentration ratios: 1.9/0.1 wt.% and
151 1.6/0.4 wt.% CNC/XG. The mixtures were respectively denoted CNC1.9 XG0.1 and CNC1.6
152 XG0.4 (Figure 1) and they were left under stirring for 18 h. CNC and CNC/XG aerogels were
153 prepared using unidirectional (UF) and non-directional (NF) freeze-casting methods. The UF
154 device was built in our laboratory according to the literature^{34,35} and consisted of a liquid nitrogen
155 Dewar, a copper bar, a heating element and a polypropylene tube partially inserted in the hot end
156 of the copper bar to hold the sample prior to freezing. It was assembled in such a way that half of
157 the copper bar was plunged into liquid N₂ to create a heat sink. In a typical experiment, 3 mL of
158 the 2 wt.% CNC-XG mixture was poured in the polypropylene tube, in direct contact with the
159 copper surface. After a 5-min equilibration time at 20 °C, the sample was cooled down to -60 °C
160 at a cooling rate of -1 °C.min⁻¹, and then removed from the device and placed at -20 °C before
161 freeze-drying for 24 h. NF experiments were examined by placing dispersions in a freezer (-20 °C)
162 for approximately 3 h. All the samples were cylinders with a mean diameter (D) of ≈ 10 mm and
163 a mean initial height (h) of ≈ 20 mm.

164 Ice sublimation was conducted in a Christ Alpha 2-4 LD freeze dryer. The temperature of the
165 freeze dryer condenser was kept below -60°C and the internal pressure stabilized within few
166 minutes to approximately 5.10⁻⁵ bar. The freeze-drying process was left to proceed for 24 h,
167 allowing for the recovery of a dried lightweight solid.

168 The bulk density $\rho_{aerogel}$ of the aerogels was calculated by dividing their weight by their
169 volume. The weight of aerogels was determined with an analytical balance (Mettler Toledo XS),
170 and their volume was measured with a digital caliper. The void fraction (%) of the aerogel was

171 calculated using the equation below where the density of cellulose is: 1500 kg.m³.

172
$$\Phi = \left(1 - \frac{\rho_{aerogel}}{\rho_{CNC}}\right) * 100 \quad (1)$$

173 To visualize the structure of the aerogels, the foams were manually cut with new scalpel blades.
174 Scanning electron microscopy (SEM) observations were performed on a Thermo Fisher Quattro
175 S. The samples were sputter-coated with 5 nm of platinum and observed under 3-4 kV acceleration
176 and 30 μ A probe current.

177

178 2.3 Fluorescent labeling of XG and CNC and confocal microscopy

179 Labeling was achieved according to the protocol reported by Berder *et al.* (de Belder & Granath,
180 1973). Briefly, XG or CNC (1 g) were dissolved/dispersed under stirring in 40 mL of
181 dimethylsulfoxide containing a few drops of pyridine at 65°C, then 10 mg of fluorescein
182 isothiocyanate (FITC) or rhodamine B isothiocyanate (RBITC) were added with 50 μ L of
183 dibutyltin dilaurate. The mixture was heated during 2 hours at 95°C. The solution/suspension was
184 poured in ethanol and the precipitate was resuspended several time in acetone and precipitated
185 again to remove the unbound dye. The precipitate was then dissolved in water and dialyzed against
186 ultra pure water until no UV signal of free dye was detected in the washing solutions. CNC and
187 XG suspensions/solutions were then concentrated by osmosis as described above. The aerogels
188 were also prepared according to the same protocols as described above.

189 Confocal microscopy was performed on a Leica SP5 upright microscope using a Leica HPX CL
190 Apochromat 63x 1.4 oil objective. Samples were sliced perpendicular to the ice growth direction
191 (thickness inferior to 1mm) followed by immersion in oil before imaging. The immersion of
192 samples in oil enabled refractive index matching between the objective, the glass coverslips and
193 the sample porous volume thus minimizing optical refraction and maximizing resolution. No

194 dissolution or deformation of the foams could be noticed when performing the oil immersion.
195 Sequential images were acquired in 300 nm z steps. Image analysis was conducted in FIJI software
196 using the Volume Viewer and Coloc2 plugins (Schindelin et al., 2012).

197

198 2.4 Mechanical testing

199 Longitudinal compression measurements were performed on the dry aerogels. Before testing, all
200 the aerogels were stored for at least one day in the measurement room in order to guarantee the
201 same conditions of temperature (20 °C) and humidity (RH= 35%). The mechanical properties were
202 characterized by uniaxial compression experiments in an MTS SYNERGIE 100 machine equipped
203 with a 100 N load cell. The compression tests were performed up to the maximum load and the
204 compression force was parallel to the temperature gradient used for the manufacturing of the UF
205 samples. The cylindrical samples were compressed between two steel plates. A deformation rate
206 of 3 mm.min⁻¹ was applied on the top surface of each specimen until the deformation of the sample
207 exceeded 70%. The corresponding stress-strain curves were obtained for five replicas per sample.

208 2.5 Determination of CNC orientation by X-ray diffraction

209 The diffractograms were recorded on a Bruker-AXS D8 Discover diffractometer. The X-ray beam
210 was produced in a sealed copper tube at 40 kV and 40 mA. The 500- μ m beam with a CuK α 1
211 wavelength (1.5405 Å) was collimated and parallelized using two crossed-coupled Göbel mirrors.
212 The X-ray diffraction data were collected using a Vantec 500 two-dimensional detector in the 3-
213 40° 2 θ range. The samples were placed perpendicular or parallel to the X-ray beam.

214 The azimuthal intensity profiles were used in the calculation of the Herman's orientation factor
215 defined by (Hermans, Hermans, Vermaas, & Weidinger, 1946):

216
$$f = [3 (\cos^2\chi) - 1]/2 \quad (2)$$

217 Where f is the crystal chain axis orientation factor and χ is the angle between the chain axis and
218 the reference direction. The value of $\cos^2\chi$ is computed from the azimuthal angular distribution
219 of XRD intensity profile by:

220

$$221 \quad \cos^2(\chi) = \frac{\int_0^{\pi/2} I(\chi) \sin(\chi) \cos^2(\chi) d\chi}{\int_0^{\pi/2} I(\chi) \sin(\chi) d\chi} \quad (3)$$

222

223 Where $I(\chi)$ Is the angular intensity profil from the XRD pattern. The degree of orientation f is the
224 first term in the expansion of an orientation distribution function which depends on the angle
225 between the chain axis and the reference direction. f is equal to 0 for random orientation and to 1
226 for a perfect alignement (Kim, Oh, & Islam, 2012).

227

228 2.6 Evaluation of dispersibility in water

229 The aerogels were immersed in deionized water containing one drop of blue ink for enhancing
230 the visualization of the wet aerogel. The immersed aerogels were kept at 4°C and dispersion was
231 visually evaluated every 12 hours. Photographs were taken at different characteristic times.

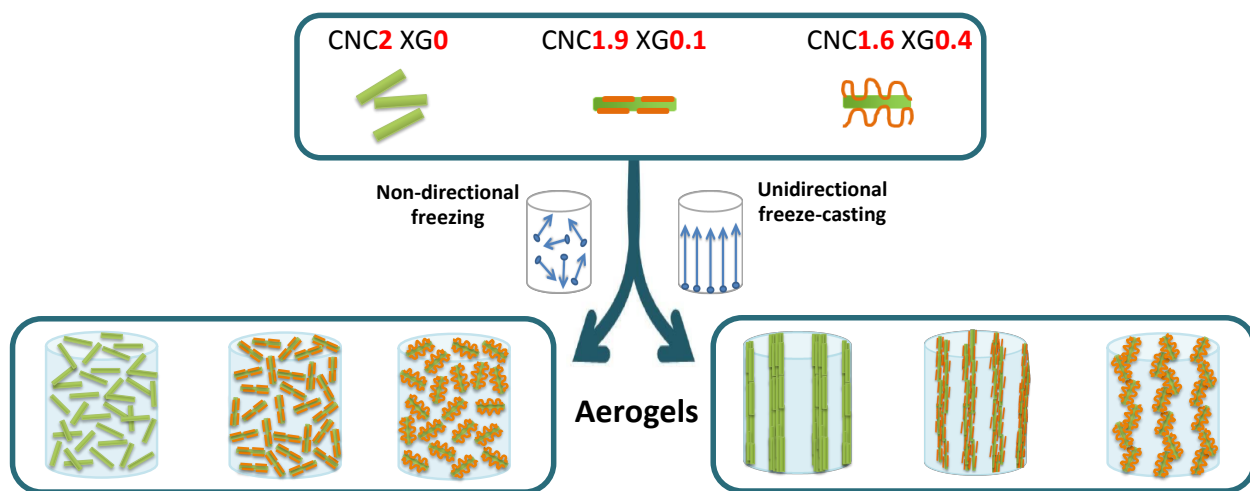
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233 3 RESULTS

234 3.1 Aerogel preparation

235 Figure 1 gives a schematic description of the different compositions and freezing routes of the
236 aerogels. All the aerogels were prepared by ice templating from suspensions of 2 wt% total
237 polymer content. Two different CNC/XG concentration ratios, 1.9/0.1 wt% and 1.6/0.4 wt%, were
238 selected corresponding to different adsorption regimes, below and above the required ratio to
239 saturate the CNC surface with XG molecules, respectively (Dammak et al., 2015). Previously

240 reported saturation range is between 20-30 mg XG /g of CNC. Thus, for CNC1.9 XG0.1, the
 241 amount of XG is lower than that needed for CNC surface saturation. XG will stretch to cover all
 242 the CNC surface available and adsorb in the train conformation. For the CNC1.6 XG0.4 mixture,
 243 XG is close to the saturation concentration. The CNC surface is fully covered, increasing the
 244 polymer crowding that will thus adsorb with more loops and tails. (Dammak et al., 2015) CNC2
 245 XG0 aerogels were used as references. Aerogels were prepared by both NF where the ice crystals
 246 nucleate and grow randomly in diverse directions and UF where the suspension is exposed to a
 247 controlled cooling element inducing the growth of ice crystals along the generated thermal gradient
 248 (Figure 1) (Deville, 2008). Monolithic and homogeneous aerogels were successfully obtained from
 249 CNC/XG mixtures with the two freezing methods.



250
 251
 252 Figure 1: Schematic description of the different aerogels with distinct compositions CNC2 XG0;
 253 CNC1.9 XG0.1 and CNC1.6 XG0.4, obtained from both non-directional (left) and unidirectional
 254 (right) freezing techniques. In the case of CNC1.9 XG0.1, XG is adsorbed as trains on the surface
 255 of CNCs, while CNC1.6 XG0.4 has the XG adsorbed in the form of loops and tails. Blue arrows

256 show directions of ice crystals growth. XG and CNC dimensions were not scaled for clarity reasons
 257 (the gyration radius of XG in solution is larger than the CNC lateral dimensions).

258 Table 1 summarizes the composition and the characteristics of the aerogels. The aerogels
 259 based on 2% CNC or 2% CNC-XG mixtures have densities of *ca.* 19 kg.m⁻³ when prepared by UF,
 260 and *ca.* 23 kg.m⁻³ when prepared by NF. This difference can be correlated with the observed slight
 261 decrease in the volume of the aerogels prepared by NF, indicating a small shrinkage of the structure
 262 during the drying step of the hydrogel.

263
 264 Table1: Characteristics of the different aerogels (CNC2 XG0, CNC1.9 XG0.1 and CNC1.6 XG0.4)
 265 prepared with a final concentration of 2 wt%, by unidirectional freezing (UF) or non-directional
 266 freezing (NF).

267

Aerogel	Composition		Method	Density (kg.m ⁻³)	Void fraction (%)	Elastic modulus (kPa)	Yield stress (kPa)	Herman's orientation factor <i>f</i>
	CNC	XG						
CNC2 XG0	2%	0%	UF	19±1	98.7	138±69	7.8	0.52
CNC1.9 XG0.1	1.9%	0.1%	UF	18±2	98.8	361±171	28.9	0.40
CNC1.6 XG0.4	1.6%	0.4%	UF	17±1	98.8	610±246	53.3	0.36
CNC2 XG0	2%	0%	NF	23±1	98.5	10±1	–	0.15
CNC1.9 XG0.1	1.9%	0.1%	NF	23±1	98.5	136 ±12	19.67	0.10
CNC1.6 XG0.4	1.6%	0.4%	NF	23±1	98.5	237±91	9.83	0.18

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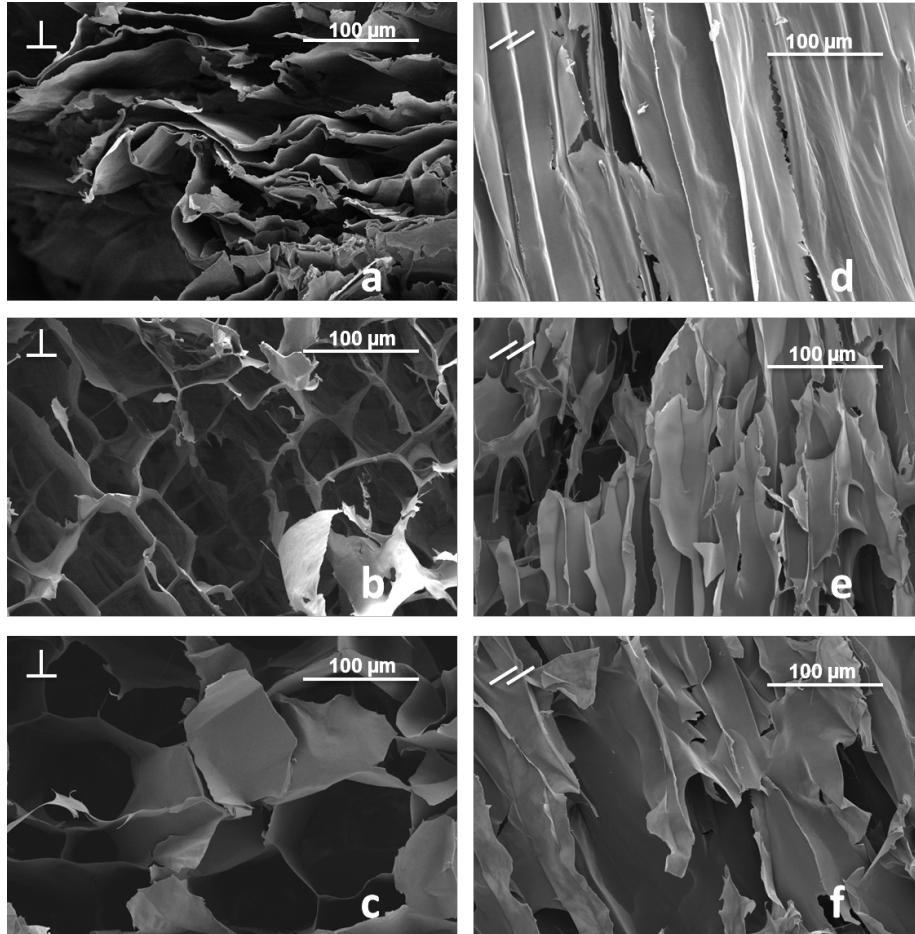
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272 3.2 Aerogels morphology

273 The morphologies of the aerogels were investigated by scanning electron microscopy
274 (SEM). Aerogels prepared by NF showed random, or disordered, structures and no significant
275 morphological difference could be evidenced between the different compositions (Fig. SI1).
276 Indeed, when solutions were frozen in the absence of a controlled temperature gradient, the crystals
277 tend to grow with no preferred direction, resulting in isotropic pore structures (Köhnke et al.,
278 2012). Figure 2 shows representative SEM images of the aerogels prepared by UF at increasing
279 CNC/XG concentration ratios (*i.e.* CNC2 XG0, CNC1.9 XG0.1 and CNC1.6 XG0.4).
280 Perpendicular and parallel sections (Fig. 2) of the cylindrical monolith show oriented structures of
281 the aerogels along the temperature gradient direction. UF-generated CNC aerogels exhibited a
282 lamellar pore structure (Fig. 2a), a common morphological feature found in the literature for
283 freeze-cast CNC aerogels (Chau et al., 2016; Dash et al., 2012; Kam et al., 2019; Munier et al.,
284 2016). In contrast, the XG-CNC aerogels displayed alveolar elongated pores (Figs. 2b and 2c),
285 similar to a honeycomb structure (Pan et al., 2016) reminiscent of the morphology reported for
286 cellulose nanofiber (CNF) aerogels (Donius, Liu, Berglund, & Wegst, 2014; Munier et al., 2016;
287 Pan et al., 2016). Increasing XG concentration induced an increase in pore size. Assuming that the
288 alveoli shape is almost circular, based on SEM images, the average cell dimension was estimated
289 at $46 \mu\text{m}$ ($\pm 15 \mu\text{m}$) and $127 \mu\text{m}$ ($\pm 23 \mu\text{m}$) for CNC1.9 XG0.1 and CNC1.6 XG0.4 aerogels,
290 respectively. In both cases, the pores have been found to be open since windows between the pores
291 are clearly visible, as commonly obtained for ice-templated materials. This was also confirmed by
292 the confocal images as discussed in the next section. The change from lamellar to alveolar
293 morphology in the presence of XG has been related to an increase in the viscosity of the CNC/XG
294 colloidal dispersion.⁴⁰ Accordingly, we recently reported that simple mixing of XG and CNC leads

295 to an increase of the viscosity of the dispersion and the formation of hydrogels (Talantikite,
296 Gourlay, Gall, & Cathala, 2019). We have proposed that gelation is due to steric stabilization when
297 the CNC surface are crowded while cross-linking might occur at lower CNC/XG to form microgels
298 in agreement with our previous reports (Dammak et al., 2015; Villares et al., 2015). These
299 variations in the physicochemical environment may change ice nucleation as well as the growth of
300 ice crystals. The full understanding of the link between CNC/XG mixture rheology properties and
301 ice nucleation and growth would require a dedicated and in-depth study that is beyond the scope
302 of the current work (Zhang & Liu, 2018). This finding is reminiscent of a recent study on the
303 preparation of XG-CNC aerogels by 3D printing coupled with UF (Kam et al., 2019) showing that
304 higher relative amounts of XG increase the viscosity of the CNC/XG suspensions and induce a
305 morphological evolution of the ice crystals, yielding to a transition from a lamellar aerogel
306 morphology a to tubular morphology or to a disordered morphology at the highest concentrations
307 (Buchtova & Budtova, 2016; Kam et al., 2019).



308

309

310 Figure 2: SEM micrographs of CNC and CNC/XG aerogels prepared by UF. Compositions of the
 311 aerogels are given as: (a, d) CNC2 XG0; (b, e) CNC1.9 XG0.1; (c, f) CNC1.6 XG0.4. Slices are
 312 cut perpendicular (a, b, c) and parallel (d, e, f) to the freezing direction.

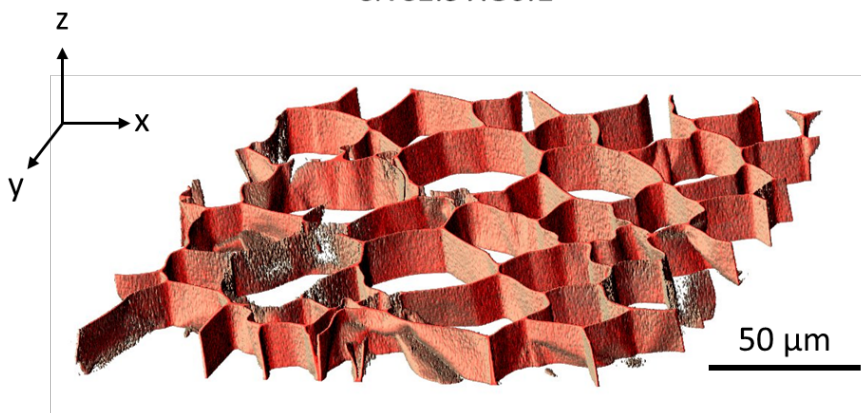
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314 Aerogel morphologies were also investigated by confocal microscopy and XG was tagged
 315 with rhodamine B isothiocyanate (RITC) and CNC with fluorescein isothiocyanate (FITC).
 316 Typical morphologies of the aerogels prepared by UF are depicted in Figure 3. The lowest XG
 317 ratio sample (CNC1.9 XG0.1) yields more disordered pores than the high XG ratio (CNC1.6
 318 XG0.4) aerogels in terms of both pore section dimensions and pore alignment. In particular, the

319 CNC1.6 XG0.4 sample displays polyhedral pore cross-sections suggesting the growth of uniform
320 ice columns during ice templating. The CNC1.9 XG0.1 sample displays a more disordered
321 structure suggesting that the ice growth phenomenon is less controlled by the thermal gradient.
322 Confocal microscopy images are in good agreement with SEM observations since larger pores are
323 observed in the case of CNC1.6 XG0.4 samples, in contrast with pores observed at lower CNC/XG
324 ratios. The samples were prepared from a representative zone of the monolith and connections
325 between pores can be observed in both samples. Pore connections are sometimes visible, indicating
326 that the pores are not fully closed. However, it can be stated qualitatively that the CNC1.6 XG0.4
327 sample has the most continuous wall structure of all samples observed.



CNC1.9 XG0.1



CNC1.6 XG0.4

328

329

330 Figure 3: 3D reconstruction of CNC/XG UF aerogels from confocal microscopy of the XG-RITC
331 channel (top, CNC1.9 XG0.1; bottom, CNC1.6 XG0.4). Aerogel sections were imaged after
332 lyophilization, slicing with a scalpel normal to the ice growth axis and placed in immersion oil for
333 observation. Confocal images of $180 \times 180 \times 15 \mu\text{m}^3$ volume sections were acquired in 300 nm z-
334 steps. Reconstruction was conducted in FIJI software using the Volume Viewer plugin (Schindelin
335 et al., 2012).

336 The segregation of solutes from the initial solution is inherent to ice templating. Since the
337 solubility of each polysaccharide may differ during the freezing-induced segregation, it is critical
338 to assess whether both polymers are co-localized after ice-templating or if some phase separation
339 occurs, and thus to ensure that they reproduce locally the global CNC/XG ratios. Figure SI2 depicts
340 the analysis of the colocalization between the two fluorescence channels for samples CNC1.9
341 XG0.1 and CNC1.6 XG0.4. In both cases a positive correlation between the intensities of the two
342 channels, as defined by the Pearson's R value can vary from -1 for perfect negative correlation to
343 +1 for perfect correlation. Here, the Pearson's R value was 0.74 and 0.84 for high- and low-XG
344 ratios, respectively indicating that CNC and XG are distributed homogeneously throughout the
345 imaged sample section and that no segregation occurs.

346

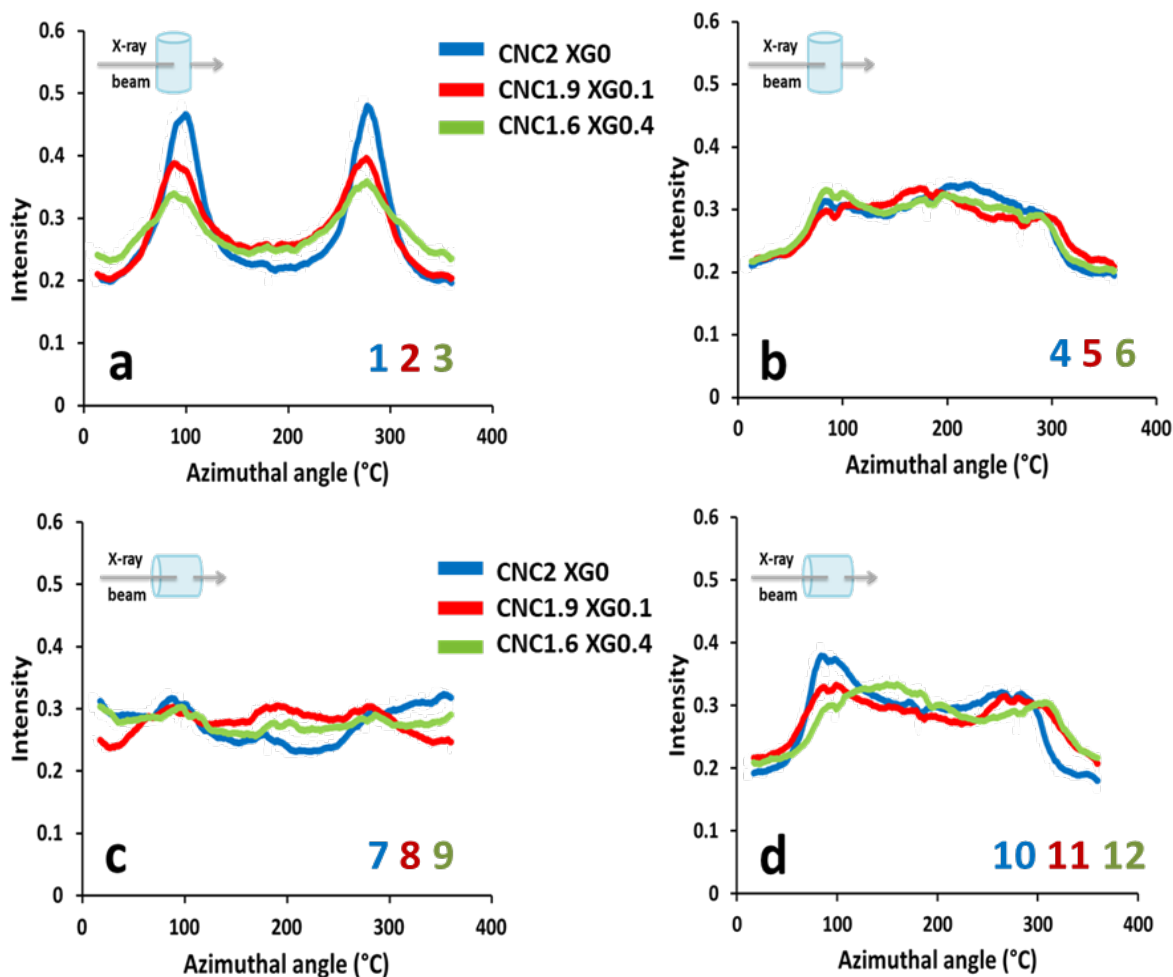
347 3.3 CNC orientation

348 X-Ray diffraction (XRD) was used to determine the azimuthal intensity profiles of the
349 (200) ring of crystalline cellulose I (Fig. SI3 and Fig. 4). When the X-ray beam is perpendicular
350 to the freezing direction, aerogels prepared by UF show diffraction patterns with a typical fiber
351 diffraction profile due to preferred orientation. This is observed as two distinct peaks at
352 approximately 90° and 270° in the azimuthal intensity profiles (Fig. 4a). This indicates that CNC

353 are oriented along the freezing direction when the samples are produced by UF. When the X-ray
354 beam is parallel to the aerogel's axis, an almost flat azimuthal profile is observed (Fig. 4c). Similar
355 results were reported in previous studies for 0.5% CNF and 1.5% CNC aerogels prepared by
356 directional freeze-casting, showing anisotropic profiles of X-ray diffraction patterns and indicating
357 that cellulose nanoparticles are also highly oriented along the freezing gradient (Munier et al.,
358 2016). These diffractograms strongly contrast with the quasi-isotropic diffractograms of non-
359 directional freeze-cast NF aerogels that display minimal azimuthal intensity variations (Fig. 4b
360 and d).

361 The degree of orientation of CNC can be quantified by Herman's orientation factor, f (Hermans,
362 Hermans, Vermaas, & Weidinger, 1946). f is calculated and presented in Table 1 for the different
363 aerogels. The aerogels prepared by NF are characterized by very low Herman's factors compared
364 to aerogels prepared by UF (Table1), confirming that the UF aerogels bear oriented CNC within
365 their walls. It has been proposed that CNC can align through compaction during freeze-drying or
366 by the growing ice front during unidirectional freezing (Han, Zhou, Wu, Liu, & Wu, 2013). Indeed,
367 during freezing of aqueous suspensions of CNCs, water is frozen into ice crystals and most CNC
368 above a critical size are trapped by the moving ice front and confined in interstitial spaces between
369 the ice crystals (Deville, Saiz, Nalla, & Tomsia, 2006; Han et al., 2013). CNC aerogels prepared
370 by UF display a higher alignment than XG/CNC aerogels. We correlate the presence of XGs in
371 these aerogels to their less-aligned microstructure. In a previous study dedicated to CNC and CNF
372 aerogels prepared by UF, it was shown that cellulose particles were oriented when the
373 concentration of the dispersion was above a critical value (0.2 wt% for CNC and 0.08 wt% for
374 CNF) (Munier et al., 2016). CNF were slightly less oriented compared to CNC in the freeze-cast
375 aerogels, which was attributed to the rod-like shape of the rigid CNC compared to the more flexible

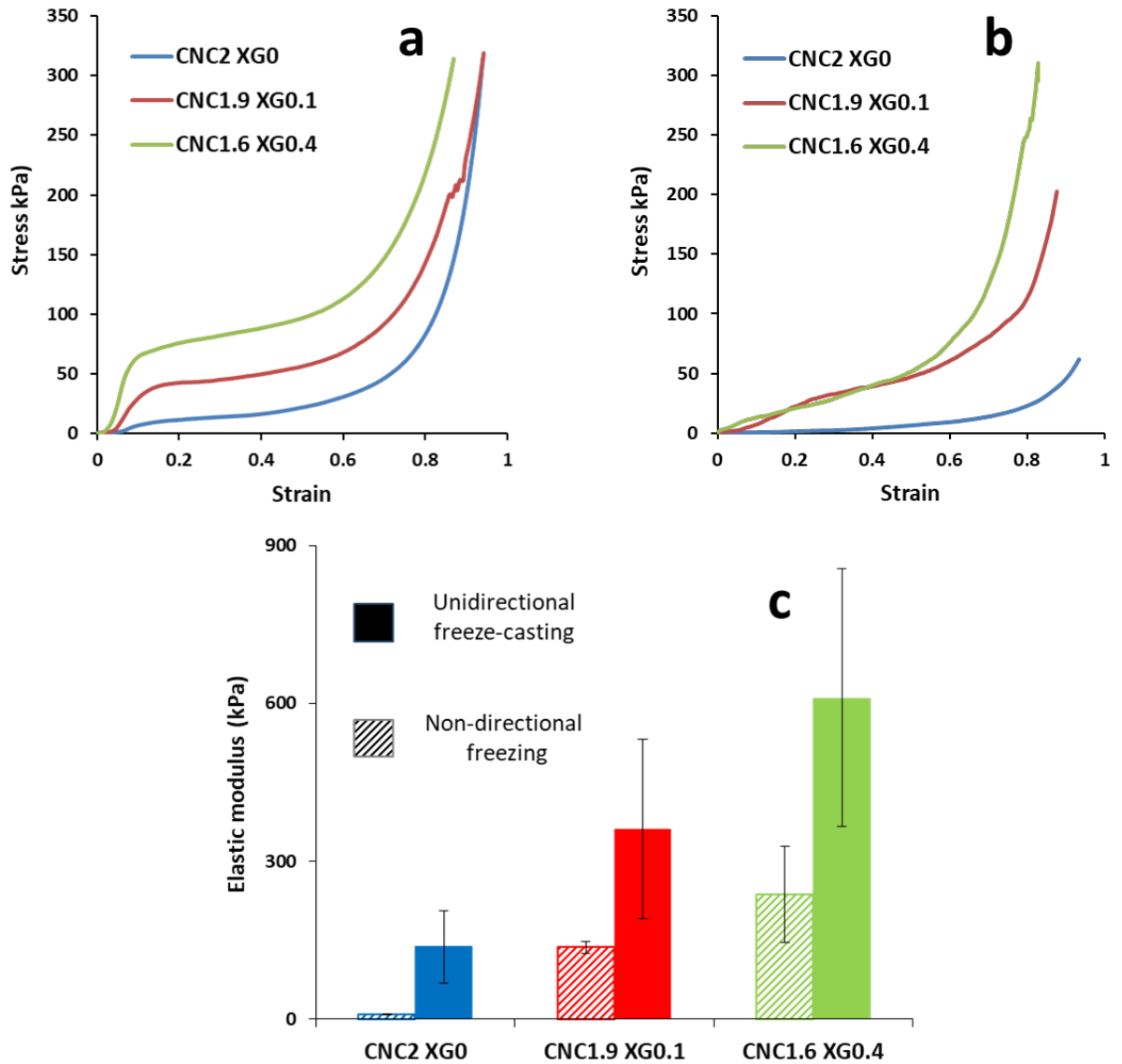
376 CNF (Munier et al., 2016). Similar assumptions can be made here. Rigid CNC can easily reorient
 377 due to a local concentration increase or shearing during ice growth, as opposed to a CNC/XG
 378 network that likely forms a less mobile assembly, with collective motion.
 379



380
 381
 382 Figure 4: X-ray diffraction investigation showing azimuthal intensity profiles of the aerogels
 383 (CNC2 XG0, CNC1.9 XG0.1 and CNC1.6 XG0.4) prepared by UF (a, c) or NF (b, d), where the
 384 X-ray beam is perpendicular (a, b) or parallel to the freezing direction (c, d). 2D detector images
 385 (Figure S13) of the aerogels are given above the azimuthal intensity profiles.

386 3.4 Mechanical properties

387 The mechanical properties of the aerogels were studied by uniaxial compression in the
388 longitudinal direction both on UF and NF samples. Figure 5 shows representative compression
389 curves for the aerogels prepared by UF (Figure 5a) and NF (Figure 5b). The aerogels prepared by
390 UF (Figure 5a) show typical compressive stress–strain curves where three regions can be
391 distinguished (Gibson & Ashby, 1999). The first linear region at low strains results from the elastic
392 deformation from which the elastic modulus can be determined. A yield stress corresponding to
393 the elastic limit at the end of the linear region is determined (Table 2). The second region with a
394 gradual increase in the stress value corresponds to the cell collapse plateau. Finally, a steep
395 increase in the stress at strains larger than *ca.* 60% is observed in the densification region where
396 the pore cell walls merge. For aerogels prepared by NF (Figure 5b), the stress-strain curves present
397 a less defined elastic region at low strains (the determination of yield stress is not possible for
398 CNC2 XG0), after which it progressively increases until it reaches a densification region.



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400

401 Figure 5: Typical stress-strain uniaxial compression curves for aerogels (CNC2 XG0; CNC1.9

402 XG0.1; CNC1.6 XG0.4) prepared by UF (a) and NF (b). A rod diagram comparing the longitudinal

403 elastic moduli (kPa) resulting from the uniaxial compression of the aerogels (CNC2 XG0; CNC1.9

404 XG0.1; CNC1.6 XG0.4) prepared by UF and NF (c).

405 The values measured for UF-generated samples are in the same range (100-600 kPa) as

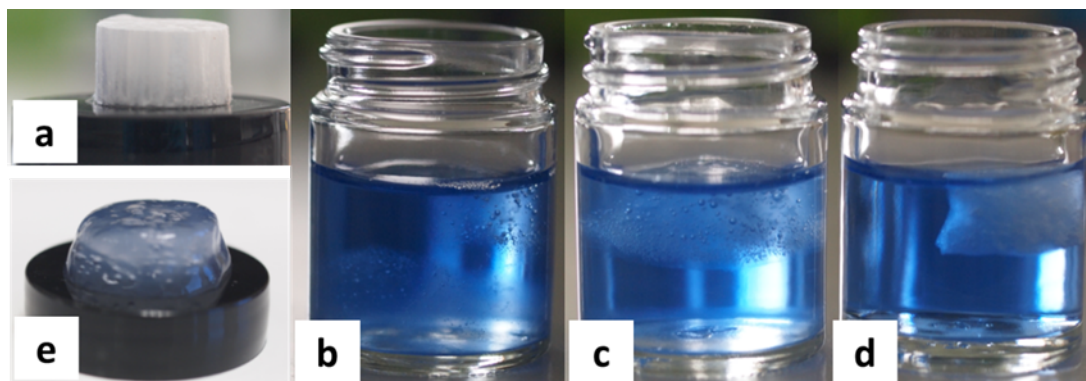
406 those reported in the literature and larger than for those prepared by NF(Pääkkö et al., 2008;

407 Sehaqui, Salajková, Zhou, & Berglund, 2010). In particular, CNC2 XG0 aerogels prepared by UF
408 have a longitudinal elastic modulus 14 times higher than CNC2 XG0 prepared by NF (Table 1 &
409 Fig. 4). However, the influence of the freezing method is less pronounced with CNC1.9 XG0.1
410 and CNC1.6 XG0.4 aerogels, with only a 2.5 factor between longitudinal elastic moduli of UF and
411 NF samples. Our results clearly demonstrate that the addition of XG to CNC improved the rigidity
412 of the aerogels due to XG adsorption. This effect is magnified when pores are oriented in the
413 direction of the compression, due a high lineic relative density on UF samples compared to NF
414 samples. In fact, for aerogels prepared by UF, an elastic modulus of 610 kPa is reached for CNC1.6
415 XG0.4, which is almost two times higher than CNC1.9 XG0.1 (360 kPa) and four times higher
416 than CNC2 XG0 (138 kPa). Concomitantly, the addition of XG increases the yield stress 7 times,
417 from 7.8 kPa for CNC2 XG0 to 53.3 kPa for CNC1.6 XG0.4. This result indicates that XG
418 increases the resistance to the deformation before irreversible damage. This result is noteworthy
419 since it highlights the importance of XG for the cell wall mechanical resistance, a resistance that
420 is often assumed to be provided by cellulose alone. As a comparison, the incorporation of XG in
421 microfibrillated cellulose (MFC) aerogels (2/8 XG/MFC), with an equivalent total dried mass
422 fraction of 2 %, increased their elastic modulus from 440 kPa to 970 kPa (Xu et al., 2013). This
423 result is in the same range as CNC1.6 XG0.4 whereas MFC have considerably higher aspect ratios
424 than CNC. It is also important to point out that the highest modulus is obtained for the sample
425 where CNC orientation degree is the lowest, suggesting that such this alignment is detrimental to
426 compression properties, in accordance with the current knowledge on compression wood (high
427 microfibril angle) with respect to tension wood (low microfibril angle, strong longitudinal
428 alignment).

429

430 3.4 Dissolution in water

431 Aerogels prepared by UF were examined after immersion in ink-colored water (Fig. 6).
432 The cylindrical shape of pure CNC aerogels was quickly lost upon swelling (Fig. 6b). The
433 dispersion took 5 h for CNC1.9 XG0.1 (Fig. 6c), whereas CNC1.6 XG0.4 retained its structural
434 cohesion for up to two weeks after immersion (Fig. 6 d, e). This experiment, despite its simplicity,
435 clearly demonstrates the efficiency of the CNC/XG cross-links. These cross-links have a similar
436 effect on the water stability of the aerogels than covalent cross-links. For instance, a previous
437 report on xylan/CNC aerogels prepared by UF showed that xylan oxidation was necessary to create
438 covalent bonds during drying and therefore to obtain water-stable aerogels, whereas unmodified
439 xylan led to composite aerogels that were easily dispersed in water (Köhnke et al., 2014) Similar
440 results can therefore be obtained without the use of specific chemicals.



441
442
443 Figure 6: Photo illustrating an example of the freeze-cast aerogel before immersion in water (a)
444 and after 4 hours (b, c, d) and 2 weeks (e). The aerogels used in this experiment were prepared by
445 UF and consist of CNC2 XG0 (b), CNC1.9 XG0.1 (c) and CNC1.6 XG0.4 (d, e).

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449 4 DISCUSSION

450 Aerogels of CNC/XG mixtures have been successfully prepared by employing the
451 unidirectional freeze-casting technique, where the pore morphology of the material was controlled
452 by the freezing conditions. It has been shown that the addition of XG changed the morphology of
453 the CNC aerogels from lamellar to alveolar. The pore size was larger when higher XG amounts
454 were present in the aerogel. Uniaxial compression tests showed that UF provided stronger aerogels
455 than non-directional freezing. Non-directional freezing resulted in random unordered porous
456 structures. This result is in agreement with previous works showing that aligning cellulose or chitin
457 nanofibers can enhance both the stiffness and the strength of dense films (Gindl & Keckes, 2007;
458 Kvien & Oksman, 2007; Sehaqui et al., 2012) or filaments (Hakansson et al., 2014; Torres-
459 Rendon, Schacher, Ifuku, & Walther, 2014). It has been also shown that the freeze-cast aerogels
460 with the highest relative amount of XG (CNC1.6 XG0.4) bore the largest pores. These pores were
461 alveolar. These samples also had the highest elastic modulus compared to the other aerogels with
462 lower amounts of XG. Hence, the distinct mechanical behaviors of the aerogels prepared by UF
463 and NF can be explained by the difference in structural morphologies of the aerogels. It has to be
464 noticed that the freezing rate, that is markedly different for UF and NF methods, may impact the
465 setting of CNC-XG interactions and therefore contribute to the difference in mechanical properties.
466 Our results are consistent with literature results related to xylan-CNC aerogels, showing that the
467 strong anisotropy of the porous structure of the UF-generated structure induced greater mechanical
468 properties than random porosity obtained by NF, and that samples possessing larger pores were
469 stronger and stiffer than samples containing smaller pores (Köhnke et al., 2012).

470 X-ray diffraction investigation showed that XG has a negative effect on CNC orientation in the ice
471 crystal growth direction. According to Herman's factor, CNC are less aligned in CNC1.6 XG0.4,

472 while the best mechanical properties are obtained with this composition. The alignment of CNC is
473 therefore not the major factor of the improved mechanical properties of XG-CNC aerogels
474 prepared by freeze-casting. The formation of a co-continuous network of rigid nanocrystals relying
475 on hydrogen bonding and van der Waals interactions between XG and CNC could be the reason
476 for the observed mechanical improvement, since relatively isotropic aerogels were affected that
477 way. This co-continuous network is also efficient at providing a much better resistance to water
478 immersion as illustrated by the CNC1.6 XG0.4 sample. In contrast, simply percolated CNC
479 aerogels without any XG addition are prone to water redispersion due to the lack of intimate
480 binding between the rigid CNC rods alone, probably due to steric effects.

481 It is known that the high stiffness of CNC reinforced composites results from the hydrogen-
482 bonding forces that hold the percolating network of fibers together (Buchtova & Budtova, 2016;
483 Favier, Chanzy, & Cavaille, 1995); (Capadona, Shanmuganathan, Tyler, Rowan, & Weder, 2008).
484 However, this stiffness increase comes at the price of interparticle binding in wet environments, a
485 function that the matrix provides. The influence of a co-continuous polymer network on moisture
486 resistance was also observed when cellulose was used instead of XG (Lourdin et al., 2016).
487 However the ease of use of XG constitutes a breakthrough in that this process is water-based and
488 doesn't require to use hazardous ionic liquids or chemical reagents.

489 This behavior of XG is well consistent with previous results on XG and bacterial nanocellulose
490 hydrogel as a cell wall analogues, where tethers of XG between the cellulose fibers have been
491 evidenced (Lopez-Sanchez et al., 2015). In addition, these XG segments were still present after
492 extensive washing of the samples, indicating that XG interacts strongly with the cellulose fibers in
493 water-saturated environments. It was also shown that XG and bacterial nanocellulose hydrogels
494 were approximately half the thickness of bacterial nanocellulose-only samples produced after the

495 same fermentation time, likely due to the effect of xyloglucan crosslinks bringing the cellulose
496 fibers closer and leading to a more compact structure (Lopez-Sanchez et al., 2015).
497 In fact, the challenge of creating aerogels from CNCs relies on the ability to form effective water-
498 stable bonds between individual rigid particles. CNC aerogels based on hydrogen bonding and
499 physical cross-links may suffer in mechanical performance and may permanently collapse or
500 redisperse in water. Thus, in the most of the previous studies that examine CNC aerogels, CNCs
501 were used as a nanofiller within a polymer matrix to give a reinforced gel (Coulibaly et al., 2013;
502 Xu et al., 2013; Yang, Bakaic, Hoare, & Cranston, 2013) or used alone to form a networked
503 structure by chemical or physical cross-linking (Dash et al., 2012; Fumagalli, Sanchez, Boisseau,
504 & Heux, 2013; Heath & Thielemans, 2010; Yang & Cranston, 2014). In this work, CNC were
505 cross-linked with XG in water, without any chemical treatment. Moreover, freeze-cast XG-CNC
506 biomimetic aerogels were obtained and showed well-defined architecture and improved
507 mechanical properties. These results provided a better insight into the contribution of XG to the
508 mechanical properties of the plant cell wall and provided promising strategies to design new fully
509 bio-based materials in green synthesis and processing conditions

510

511 5 CONCLUSIONS

512 Novel bioinspired CNC/XG aerogels processed by the UF were successfully prepared
513 without chemical modification of the biopolymers. The addition of XG changed the morphology
514 of CNC aerogels from lamellar to alveolar, resulting in a honeycomb-like microstructure, and
515 increased both the elastic modulus and the aqueous stability of the aerogels. This can be explained
516 by the interactions between XG chains and CNC rigid nanocrystals, that not only increased the
517 viscosity of the starting mixture but also allowed the formation of strongly interacting CNC/XG

518 network. Therefore, the CNC/XG ratio has a profound impact of the properties of the aerogels.
519 Increasing the CNC/XG ratio enhances the mechanical properties of the aerogels but decreases the
520 orientation of CNCs within their walls. It is likely that the high relative XG amounts increased the
521 interactions between the CNC, thus limiting their mobility and ability to orient during UF. Such a
522 strengthening of inter-CNC interactions would also explain the enhanced stability of aerogels in
523 water. Importantly, these interactions are mediated by the XG molecules and therefore depend on
524 their conformation on the CNC surface. Based on the different concentration regimes studied here,
525 a loop and tail XG conformation seems more favorable than a train conformation. Altogether the
526 influence of XG on the functional properties of the aerogels is multifaceted and needs to be
527 considered at different scales. These results therefore highlight the possibility to design
528 mechanically-robust and water-stable bioaerogels with well-defined architectures without
529 chemical treatment but relying only the optimal setting of intermolecular forces.

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534 **ASSOCIATED CONTENT**

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