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# Cellulose nanofibrils prepared by twin-screw extrusion: Effect of the fiber pretreatment on the fibrillation efficiency

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1                    **Cellulose nanofibrils prepared by twin-screw extrusion:**  
2                    **Effect of the fiber pretreatment on the fibrillation efficiency**

3  
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5  
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9  
10                    **Highlights**

- 11  
12  
13                    - Cellulose nanofibrils from eucalyptus pulp were produced by twin-screw extrusion (TSE)  
14                    - A chemical pretreatment by oxidation or carboxymethylation was necessary for the TSE.  
15                    - The chemical pretreatment strongly affects the swelling degree of the fibers  
16                    - The TSE contributed to facilitate the production of CNFs at a high solid content

19

## 20 **Abstract**

21 Twin-screw extrusion (TSE) is a rather recent method to produce cellulose nanofibrils (CNFs)  
22 at a high solid content under continuous feeding. Here, never-dried commercial eucalyptus  
23 pulp was used as starting material to produce CNFs by TSE after a chemical pretreatment to  
24 introduce carboxylic groups via TEMPO-mediated oxidation and carboxymethylation. Five  
25 samples with a carboxyl content ranging from 800 to 1300  $\mu\text{mol.g}^{-1}$  were produced to explore  
26 how the carboxyl content affects the aptitude of cellulose fibers to be broken down to  
27 nanoscale. The properties of the resulting CNFs in terms of nanosized fraction, morphology  
28 and rheological properties were investigated. A critical carboxyl content of 700  $\mu\text{mol.g}^{-1}$  was a  
29 prerequisite for the successful conversion of cellulose fibers into a CNF gel by TSE,  
30 regardless the pretreatment method. The degree of swelling of the fibers was put forward to  
31 account for this critical parameter.

32

33 *Keywords:* cellulose nanofibrils, oxidation, twin-screw extrusion

34

## 35 **1. Introduction**

36 During the last decade, cellulose nanofibrils (CNFs) have gained widespread scientific and  
37 commercial interest with promising potential applications in different fields including high-  
38 volume industries such as paper and board and packaging where CNFs have demonstrated  
39 their strong strengthening effect when added in bulk as well as coating layers (Boufi et al.  
40 2016, Lavoine et al. 2012). CNFs also find numerous uses in low-volume application as  
41 improved rheology additive of food, cosmetics, pharmaceuticals and paints, lightweight  
42 reinforced agent in composites for automotive, and sporting goods.

43 However, despite the progress achieved in reducing the energy cost related to the  
44 mechanical disintegration of cellulose fibers along with the diversification of production  
45 methods and of CNFs using wide range of available feedstock cellulose resources, their  
46 widespread use at the industrial scale is still limited and beyond expectation. One of the

47 obstacles is the low consistency of CNF slurries, typically in the range of 1-3 wt%, making  
48 the transportation expense decisive in the final cost of CNFs. In addition, the high water  
49 content generates additional problems, such as excessive dilution effects when CNFs are used  
50 as additive, necessity to handle large volumes of CNF slurries and longer time for water  
51 removal.

52 The concentration of CNF slurries via pressure filtration or water evaporation is a possible  
53 solution to make up for transport and storage. However, this solution is highly energy  
54 consuming and capital investment is needed with additional economic impact on the final  
55 cost of CNFs. Another alternative would be the use of a high-consistency route to produce  
56 CNFs, among which twin-screw extrusion (TSE) has recently been reported as a promising  
57 method to break down cellulose fibers into CNFs with a solid content up to 20 wt% and a  
58 notably lower energy consumption (about 60% and more) than high-pressure homogenizer or  
59 grinder widely used to produce CNFs as has been highlighted in recent papers (Rol et al.  
60 2019, Baati, Magnin&Boufi2017,Rolet al. 2017).

61 Despite the numerous advantages of TSE as a new route to produce CNFs, so far, only  
62 seven papers have been reported in the literature using the terms TSE and cellulose  
63 nanofibrils. The first work reporting the possible production of CNFs by TSE dates back 2014  
64 when untreated never-dried refined pulp (needle-leaf bleached kraft pulp) had been extruded  
65 by TSE at a solid content ranging from 30-40% (Ho, Zimmermann & Yano 2015). Then, in  
66 2017, two papers were published concerning the successful disintegration of cellulose fibers  
67 into CNFs via TSE at a high solid content ranging from 10 to 17%. In the first one, CNFs with  
68 a diameter around 5 nm and a yield in nanosized material exceeding 70% were obtained after  
69 30 min of recirculation through a twin-screw minicomponent, using a TEMPO-mediated  
70 oxidized holocellulose (Baati, Magnin&Boufi2017). In the second paper, enzymatic  
71 pretreatment of TEMPO-oxidized eucalyptus pulp was used as starting material (Rolet al.

2017). After 7 passes through the TSE, CNFs with a width around 20-30 nm were produced with a yield in nanosized fraction in the range of 35 to 65%, depending on the fibers pretreatment. More recently, cationized cellulose fibers with a degree of substitution (DS) around 0.3 were extruded in TSE at a solid content of 17% to produce cationized CNFs with a width of  $43 \pm 20$  nm estimated by AFM (Rol et al. 2019). The fraction of nanosized material evaluated by centrifugation test was found to be around 50%, meaning that TSE did not ensure the effective breakdown of cellulose fibers into nanofibrils and a high fraction of residual fibers was still present after TSE even after 7 passes. It was hypothesized that the presence of microsized fibers was due to a slipping effect of fibers into the TSE. The same approach has been used to produce phosphorylated CNFs with good flame-retardant properties. The phosphorylation was performed by treating cellulose fibers in a solution of urea and ammonium phosphate, drying at 105°C and curing at 150°C for 1h to ensure the grafting of phosphate moiety on cellulose. However, the phosphorylated CNFs were composed of large microsized fiber fragments and very small nanofibers.

In our previous works (Batti, Magnin&Boufi 2017, Baati, Mabrouk, Magnin&Boufi 2018), the successful disintegration of cellulose fibers into CNFs by TSE with a high yield exceeding 80% was demonstrated. However, it was shown that the pulping routes as well as the carboxyl content strongly affect the efficiency of the disintegration process, and only oxidized holocellulose fibers could be broken down into nanofibrils with a lateral dimension lower than 5 nm and a length within the micrometer scale. In the present work, we pursue our investigation concerning the production of CNFs at high solid content by TSE using commercial pulp instead of lab-scale pulped cellulose fibers. The main emphasis is to gain better knowledge on the effect of the chemical pretreatments (TEMPO-mediated oxidation and carboxymethylation) on the capacity of commercial bleached cellulose pulps to be disintegrated into CNFs and how the resulting CNFs are affected by the chemical

97 pretreatment in terms of morphology, nanosized fraction, mechanical properties and  
98 rheology.

99

## 100 **2. Experimental section**

### 101 *2.1 Materials*

102 Never-dried eucalyptus pulp (NDP) kindly provided by Torraspapel with a water content of  
103 50% was used for the production of CNFs. Sodium chlorite ( $\text{NaClO}_2$ ), acetic acid (AA)  
104 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO), sodium bromide (NaBr), isopropanol,  
105 sodium chloroacetic acid are from SigmaAldrich and used as received. 4% sodium  
106 hypochlorite solution ( $\text{NaClO}$ ) was a commercial product. Its concentration was checked by  
107 volumetric titration with sodium thiosulfate.

108

### 109 *2.2 TEMPO-mediated oxidation*

110 The TEMPO-mediated oxidation was carried out at pH 7 and 10, using  $\text{NaClO}_2$  and  $\text{NaClO}$   
111 as oxidizing agents, respectively. The oxidation protocol is as follows:

#### 112 TEMPO- $\text{NaClO}$ -, $\text{NaClO}_2$ (neutral oxidation)

113 Cellulose fibers (5 g) were dispersed in a 0.05 M sodium phosphate buffer (500 mL, pH 7)  
114 solution, containing TEMPO (200 mg). Sodium chlorite (from 2.5 to 4 g) and 20 mL  $\text{NaClO}$   
115 solution were added to the beaker and the fiber suspension was kept under magnetic stirring  
116 during 60°C for 12h. Then, fibers were recovered by filtration were washed three times with  
117 deionised water and the consistency was adjusted at 10% by vacuum filtration of the fiber  
118 suspension and water addition at appropriate level. Two samples of oxidized fibers were  
119 prepared using 2.5 and 4g of  $\text{NaClO}_2$ . They were labeled Neut-500 and Neut-800,  
120 respectively.

121 TEMPO-NaBr-NaClO (basic oxidation)

122 Cellulose fibers (4 g) were suspended in 500 mL water. TEMPO (200 mg) and NaBr (0.8 g  
123 were added to the suspension. Then the appropriate volume of hypochlorite solution was  
124 added dropwise to the cellulose suspension at a temperature around 5°C, kept constant  
125 throughout the oxidation reaction. The pH was maintained around 10 by the continuous  
126 addition of a 0.1 M aqueous solution of NaOH. The fibers were then recovered by filtration  
127 and copiously washed with water until neutral pH. Three samples of oxidized fibers were  
128 prepared using 300, 400 and 600 mL of hypochlorite solution. They are referred to as Bas-  
129 800, Bas-1000 and Bas-1300, respectively.

130

### 131 *2.3 Carboxymethylation*

132 The carboxymethylation was performed in isopropanol (IPA) as follows: 5g of pulp was  
133 added to 300 mL IPA and the suspension was kept under magnetic stirring for 3h after  
134 addition of 4 mL of 30% (w/v) sodium hydroxide. The carboxymethylation reaction was  
135 started by adding sodium chloroacetic acid in 50 mL IPA to the reaction mixture placed in a  
136 water bath thermostated at 60°C. The reaction was stopped after 6 h by cooling the mixture to  
137 room temperature and the pH of the suspension was adjusted to neutrality with acetic acid.  
138 The mixture was then filtered and copiously washed with water until the conductivity of the  
139 suspension decreased below 200 $\mu$ S/m. Two samples with carboxyl content 580 and 800,  
140 referred to as Car-600 and Car-800, were prepared by adding appropriate amounts of sodium  
141 chloroacetic acid (0.65 g and 0.8 g for Carb-600 and Carb-800, respectively).

142

### 143 *2.4 Carboxyl content*

144 The carboxyl content of the oxidized cellulose was determined using conductometric  
145 titration, as described elsewhere (Besbes, Alila & Boufi). Briefly, around 100 mg of fibers were  
146 dispersed in 15 mL water and 5 mL 0.01 M HCl was added to bring the pH to 3 and turn all

147 carboxyl groups in their undissociated form. Then, the suspension was titrated by NaOH  
148 solution (0.01 M) using conductivity measurement.

149

### 150 *2.5 Water retention value (WRV)*

151 The WRV was evaluated by centrifugation according to the method of Okubayashiet  
152 al.(Okubayashi, Griesser& Bechtold 2004). In brief, about 0.3 to 0.5 g of fiber saturated in  
153 water was centrifuged at 4000 g for 10 min, and the dry weight of the fiber was measured. The  
154 WRV was calculated from Eq. 1.

$$155 \quad WRV (\%) = (W_w - W_d) / W_d * 100 \quad (\text{Eq. 1})$$

156 where  $W_w$  and  $W_d$  are the weight of wet and dry samples.

### 157 *2.6 Preparation of CNF films*

158 A CNF suspension with a 0.5 wt% consistency was prepared by dilution of the CNF gel and  
159 dispersed with an Ultra Turrax during 20 s at 15000 rpm. 50 mL of the suspension was then  
160 cast into a Petri dish and left in a circulated oven at 40°C for 24h. The CNF films were stored  
161 at 25 °C and 50% relative humidity (R.H.) to reach moisture equilibrium absorption.

162

### 163 *2.7Twin-screw extrusion of fibers*

164 Samples were processed in a laboratory scale co-rotating conical twin-screw DSM-  
165 Xplore15cc Micro-extruder, comprising a clamshell barrel with a conical twin-screw extruder,  
166 which can be operated in batch and continuous modes ensured by a recirculation channel and  
167 a control valve built into the barrel (Supplementary Material **FigureS1**). Pulps with a solid  
168 content of 10 wt% were fed in to the barrel and continuously extruded at a constant screw-  
169 speed of 200 rpm via recirculation for 20 min.

170

### 171 *2.8 Nanosized fraction*



172 The nanosized fraction was evaluated by centrifugation as follows: a dilute suspension with  
173 about 0.2 wt% solid content ( $S_c$ ) was centrifuged at 4500 rpm for 20 min to separate the  
174 nanofibrillated material (in supernatant fraction) from the unfibrillated fibers which settled  
175 down. The nanosized fraction in % corresponds to the suspension concentration after  
176 centrifugation against the initial suspension concentration. The test was repeated at least twice  
177 and the average is reported.

## 178 179 *2.9 Mechanical properties*

### 180 Tensile tests

181 The mechanical properties of CNF films were investigated using an ARES rheometer (TA  
182 Instruments). Rectangular film specimen (30 mm  $\times$  5 mm) with a thickness of about 30  $\mu$ m  
183 were used and a cross-head speed of 5 mm.min<sup>-1</sup> was applied. Specimens were equilibrated at  
184 23°C and 50% R.H. for at least 2 days to reach moisture equilibrium and five specimens were  
185 tested for each composition.

### 186 Dynamic mechanical analysis (DMA)

187 DMA experiments were conducted in tension mode using an ARES rheometer (TA  
188 Instruments) in tension mode. Temperature scans were run from 20 to 60°C at a heating rate of  
189 2°C.min<sup>-1</sup>, a frequency of 1 Hz and an amplitude of 10  $\mu$ m.

## 190 191 *2.10 Microscopy*

### 192 Field-emission scanning electron microscopy (FE-SEM)

193 Drops of the CNF suspensions with a solid content of about 0.05 wt% were deposited on the  
194 surface of a silicon wafer and coated with a 2-3 nm-thick carbon layer by ion sputtering.  
195 Images of the film surface were recorded using an in-lens secondary electron detector in a  
196 ZEISS Gemini SEM 500 microscope equipped with a field-emission (FE) gun and operated at  
197 a low acceleration voltage (2-5 kV).

198 Transmission electron microscopy (TEM)

199 Droplets of 0.001 wt% CNF suspensions were deposited on freshly glow-discharged  
200 carbon-coated films supported by copper TEM grids. Prior to drying, a drop of 2 wt% uranyl  
201 acetate negative stain was deposited on the specimens. After 1 min, the stain in excess was  
202 blotted with filter paper and the remaining liquid film allowed to dry. The specimens were  
203 observed with a JEOL JEM 2100-Plus microscope operating at a voltage of 200 kV. The  
204 images were recorded with a Gatan Rio 16 digital camera.

205 Optical microscopy

206 Fibers were dispersed by gentle mixing in water at a concentration of about 0.5 wt% and a  
207 drop of the suspension was deposited on a glass slide covered with a slip. The optical  
208 microscopy images were taken using a Carl Zeiss Axio optical microscope in transmission  
209 mode equipped with an AxioCamMRc 5 digital camera.

210

211 *2.11 Shear rheometry*

212 The shear rheological measurements were made on DHR3 rheometer (TA Instruments). A  
213 plate-plate cell with a diameter of 25 mm was used. Its surface was rough to prevent slippage  
214 to the wall. The temperature was 25 °C maintained by Peltier heating. Dynamic mode tests  
215 under small strains were performed by measuring firstly storage modulus  $G'$  and loss modulus  
216  $G''$  vs. strain to determine the linear behavior domain. Then, frequency sweep at a fixed strain  
217 in the linear domain was performed.

218

219 *2.12 Transmittance measurements*

220 The optical properties of transmittance and haze of the nanopaper was obtained with a UV-  
221 Vis Lambda 35 Spectrometer (PerkinElmer, USA), and the transmittance was measured  
222 between 700 and 400 nm. Haze is a measurement of the amount of light that is diffused or

223 scattered when passing through a transparent material and is experimentally quantified by  
224 Eq. 2:

$$225 \quad \text{Haze} = \frac{T_d}{T_t} \cdot 100 \quad (\text{Eq. 2})$$

226 Where  $T_d$  is the scattered transmitted light and  $T_t$  is the total transmitted light.

### 227 228 2.13 X-ray diffraction (XRD)

229 One millimeter-large strips of nanopaper specimens were cut and fixed with tape on 0.2  
230 mm collimators. The films were X-rayed in a Warhus vacuum chamber using a Philips  
231 PW3830 generator operating at 30 kV and 20 mA (Ni-filtered  $\text{CuK}\alpha$  radiation,  
232  $\lambda = 0.1542$  nm), during 1-h exposures. Two-dimensional diffraction patterns were recorded on  
233 Fujifilm imaging plates, read offline with a Fujifilm BAS 1800-II bioimaging analyzer.  
234 Profiles were calculated by rotational averaging of the 2D patterns. The crystallinity index  
235 (CrI) was estimated using Segal's equation (Segal, Creely, Martin & Conrad 1959):

$$236 \quad \text{CrI} = \left( \frac{I_{\text{Crs}} - I_{\text{am}}}{I_{\text{Crs}}} \right) \quad (\text{Eq. 3})$$

237 where  $I_{\text{Crs}}$  is the intensity of the 020 peak at  $22.3^\circ$  and  $I_{\text{am}}$ , the intensity of the minimum  
238 between the 020 and 110 peaks at  $18^\circ$ .

239

## 240 3. Results and discussion

241 NDP was used as starting material to produce CNFs by TSE. This choice was motivated by  
242 the easier fibrillation aptitude of NDP compared to dried commercial pulps as will be  
243 demonstrated in the following. Without any chemical pretreatment, it was impossible to  
244 extrude NDP because of the compression of fibers at the end of the screw obstructing any  
245 possible recirculation for multiple passes. Photos showing this phenomenon are given in  
246 Supplementary Material **Figure S3**. The same effect was observed for oxidized fibers with a

247 carboxyl content lower than 600  $\mu\text{mol.g}^{-1}$ . For samples with a carboxyl content exceeding 750  
248  $\mu\text{mol.g}^{-1}$ , a successful extrusion of fibers without any clogging has been performed and a thick  
249 gel was obtained even after 10 min extrusion. Five samples of chemically treated fibers have  
250 been extruded, encompassing: three samples of fibers oxidized under basic condition having a  
251 carboxyl content of 750, 1000 and 1350  $\mu\text{mol.g}^{-1}$  (referred as Bas-800, Bas-1000, Bas-1300),  
252 one oxidized under neutral condition with a carboxyl content around 800  $\mu\text{mol.g}^{-1}$  (referred as  
253 Neut-800) and one carboxylated fibers with a carboxyl content 820  $\mu\text{mol.g}^{-1}$  (referred to as  
254 Carb-800) The aim of the variation of the carboxyl amount was to investigate how this  
255 parameter affects the extrusion aptitude of fibers and properties of the resulting CNF  
256 dispersions. It is worth noting that under neutral condition, it was impossible to go over a  
257 carboxyl content of 800  $\mu\text{mol.g}^{-1}$ , even though the amount of the oxidizing agent ( $\text{NaClO}_2$ )  
258 was doubled as well as the TEMPO catalyst. The only possibility to tailor the carboxyl content  
259 was to operate under basic condition and to adjust the concentration of  $\text{NaClO}$  to the desired  
260 level of oxidation. One possible reason accounting for this effect might be the difference in the  
261 pH during the oxidation reaction. The basic pH in the oxidation in the presence of  $\text{NaClO}$   
262 favors the swelling of the fibers and facilitates the diffusion of reagents inside the inner  
263 micropores of the cellulose fibers while this phenomenon is less favored at pH 7. In fact, while  
264 the TEMPO-mediated oxidation as pretreatment in nanocellulose production proved popular,  
265 the oxidation under neutral pH is not widely reported in the literature, and to our knowledge  
266 only reported in three papers (Saito et al. 2009, Hirota, Tamura, Saito & Isogai 2009, Besbes,  
267 Alila & Boufi 2011). The leveling off of the carboxyl content was observed in two of them  
268 (Saito et al. 2009, Besbes, Alila & Boufi 2011).

269 The properties of CNFs produced by TSE were evaluated by measuring: (i) the nanosized  
270 fraction, (ii) the mechanical performance of film of CNFs produced by casting and drying at  
271 40 °C and (iii) the morphology of cellulose nanofibrils and CNF films. From the data collected

272 in **Table 1**, we can note an enhancement in the extent of fibrillation yield of extruded fibers  
 273 with increasing carbonyl group content. This effect was expected and was pointed out when  
 274 other disintegration methods were used such as high pressure homogenization (Benhamou et  
 275 al. 2014) or grinding (Saito, Kimura, Nishiyama & Isogai 2007). It was inferred that the  
 276 presence of ionic groups within the cellulose fibers generated an osmotic pressure that favored  
 277 the expansion of the fibers and reduced the interaction between cellulose fibrils by hydrogen  
 278 bonding. However, with TSE, in addition to these two effects, the carboxyl groups render  
 279 fibers more flexible and highly hydrated to be dragged at the end of the screw where fibers  
 280 were submitted to a high compression degree.

281

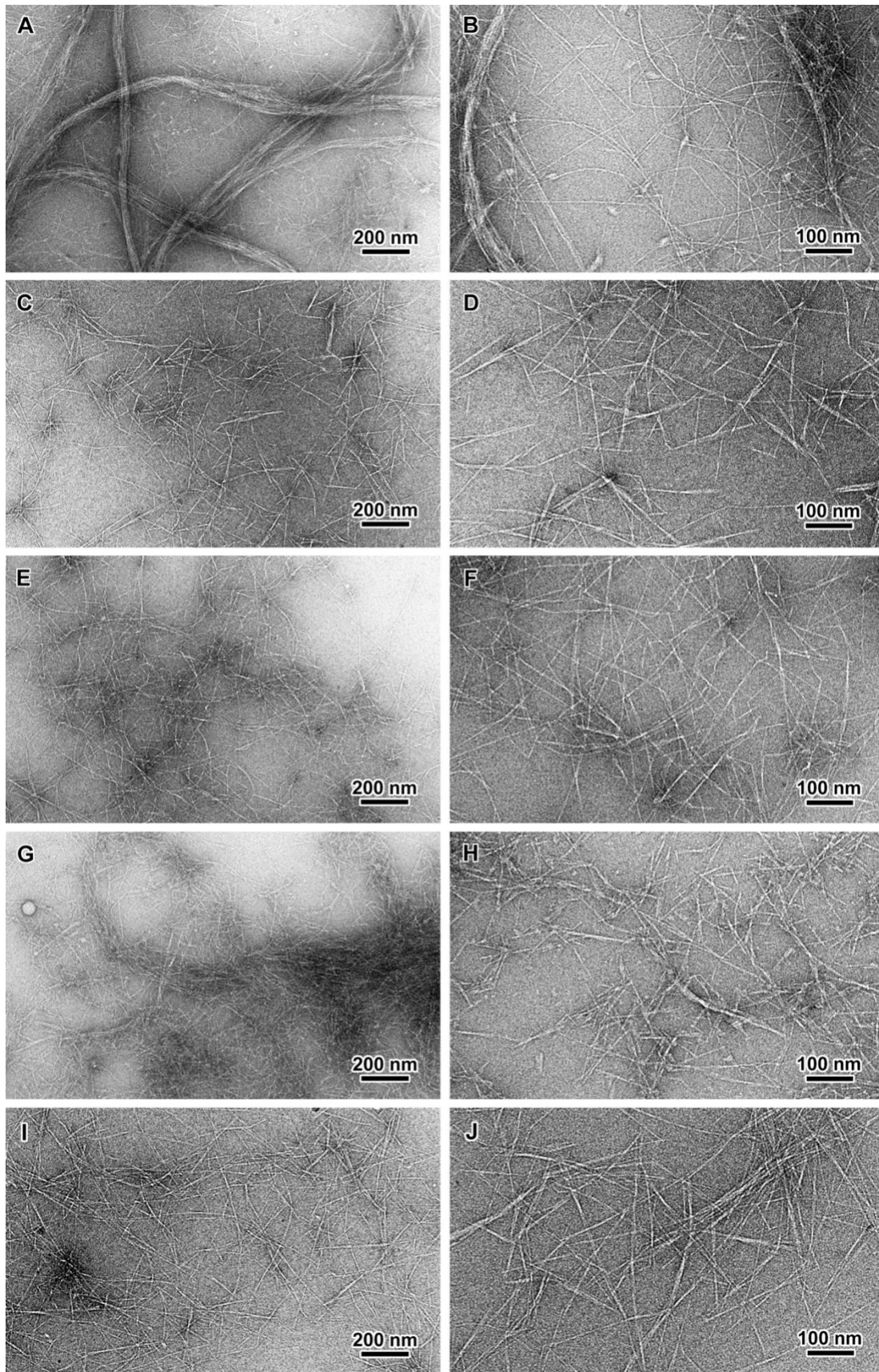
282 **Table 1.** Different pretreated fibers and properties of CNFs and nanopapers produced from the  
 283 resulting CNFs. CrI is the crystallinity index.

Treated fibers	Carboxyl content ( $\mu\text{mol.g}^{-1}$ )	Tensile strength [MPa]*	Tensile strength [MPa]**	Storage modulus E' (GPa)	CrI	Nanosize d fraction [%]	T at 600 nm	WRV (%)
Bas-450	450	-	-	-	-	-	-	224
Bas-800	750	42 $\pm$ 4	13 $\pm$ 1	2.8 $\pm$ 0.2	0.73	45 $\pm$ 5	44	323
Bas-1000	1000	48 $\pm$ 4	17 $\pm$ 2	5.6 $\pm$ 0.3	0.77	64 $\pm$ 5	61	454
Bas-1300	1350	52 $\pm$ 5	21 $\pm$ 2	6.8 $\pm$ 0.4	0.72	92 $\pm$ 5	78	491
Neut-500	500	-	-	-	-	-	-	245
Neut-800	780	71 $\pm$ 5	18 $\pm$ 2	3.3 $\pm$ 0.2	0.71	56 $\pm$ 5	67	347
Carb-600	580	-	-	-	-	-	-	-
Carb-800	820	53 $\pm$ 5	21 $\pm$ 5	4.2 $\pm$ 0.2	0.57	58 $\pm$ 5	57	486

284 \*nanopaper

285 \*\* paper from treated fibers

286



287

288

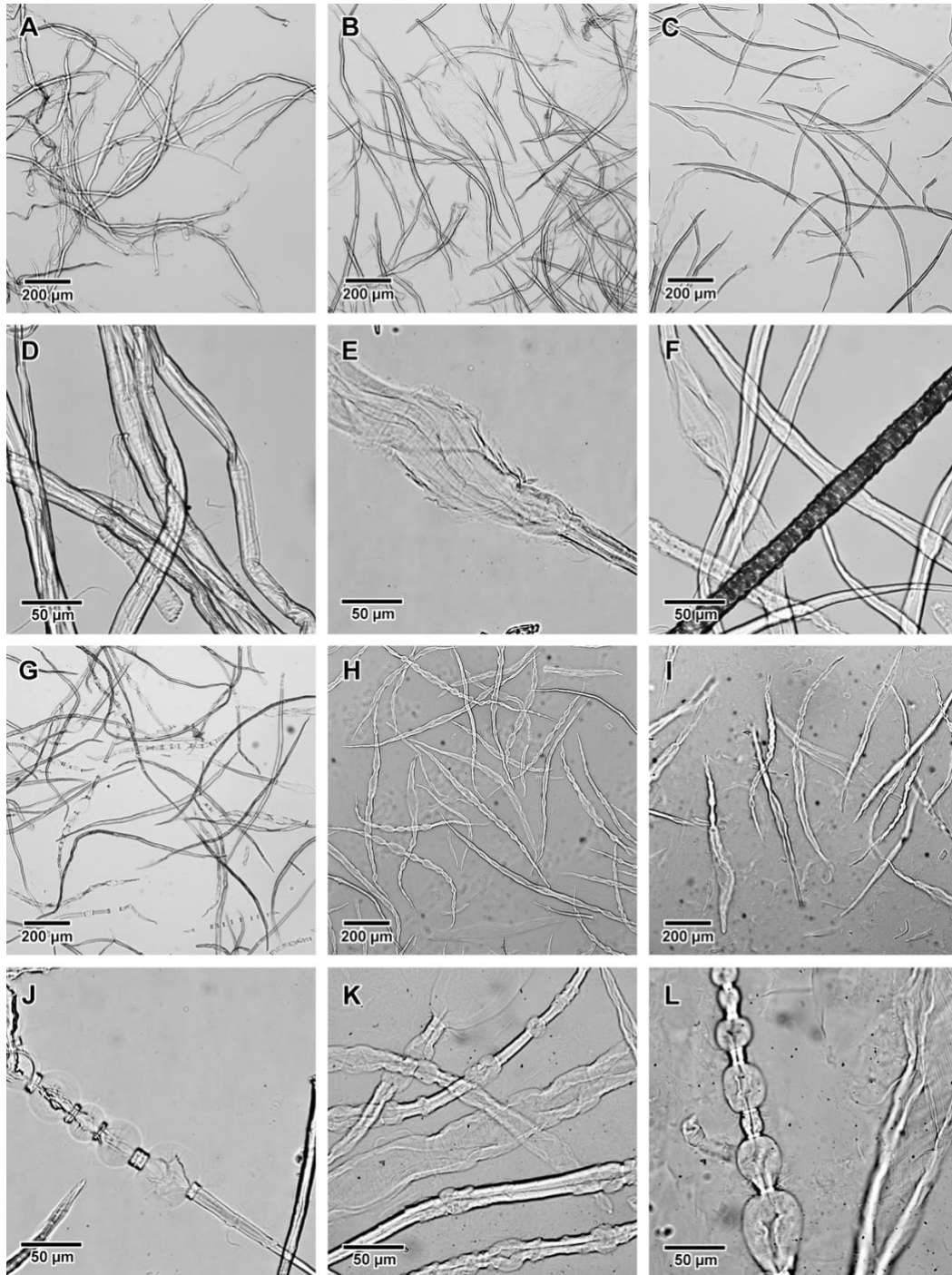
289

**Figure 1.** TEM images of negatively stained preparations from Bas-800 (A,B), Bas-1000 (C,D), Bas-1300 (E,F), Carb-800 (G,H) and Neut-800 (I,J).

290

291 The supernatant fraction of the centrifuged suspensions of extruded fibers was observed by  
292 TEM. As seen in the images of **Figure 1**, all preparations revealed entangled networks of fairly  
293 well individualized CNFs, thus validating the successful breakdown of pretreated cellulose  
294 fibers into nanofibrils. However, the Bas-800 suspension still contained a significant number of  
295 fibrils that were only locally disrupted (Fig. 1A and 1B). Additional details of the morphology  
296 of the resulting CNFs were provided by AFM observation (**Figure S2**), where indication about  
297 the lateral section of the CNFs was given from the height profile. The lateral section of Bas-  
298 800 and Bas-1000 CNFs was in the 3-9 nm range, while a lower width, around 3-4 nm, was  
299 found for Bas-1300.

300 Two possible reasons might explain the unsuccessful disruption of fibers by TSE with a  
301 carboxyl content lower than  $700\mu\text{mol.g}^{-1}$ : the first one is the insufficient density of ionic  
302 groups to overcome the cohesion of cellulose fibrils through hydrogen bonding. The second  
303 one might be related to the morphology of the treated cellulose fibers. To access this effect,  
304 optical microscopy observation of the neat and treated cellulose fibers was performed (**Figure**  
305 **2**). Neat NDP fibers are composed of different cell elements including long fibers, about 15  
306  $\mu\text{m}$ -wide vessel elements, collenchyma lamellar elongated cells and tracheids.



307

308 **Figure 2.** Optical micrographs of virgin fibers (A,D), Neut-800 (B,E), Bas-800 (C,F),  
 309 Carb-800 (G,J), Bas-1000 (H,K), and Bas-1300 (I,L) treated fibers.

310

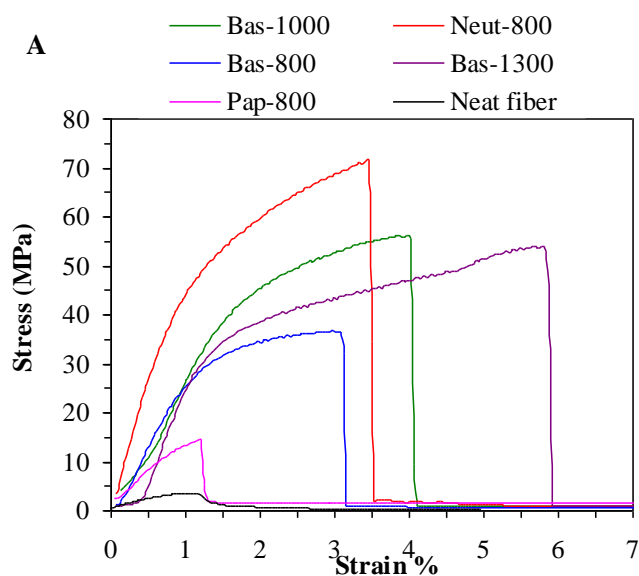
311 Oxidized fibers with a carboxyl content around 700-800  $\mu\text{mol.g}^{-1}$  exhibit a heterogeneous  
 312 morphology. Swollen fibers with loose cell walls can be seen next to long fibers without any  
 313 visible change in their aspect. In carboxymethylated fibers Carb-800, some of the fibers exhibit



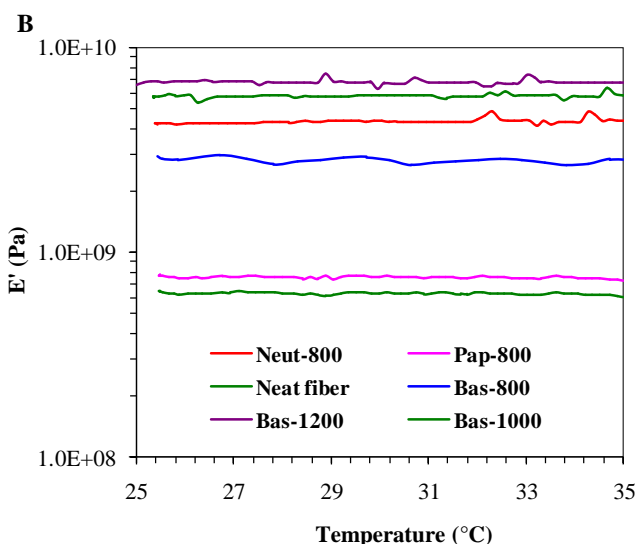
314 balloon-like swollen regions. This phenomenon was commonly observed during dissolution of  
315 cellulose fibers in N-methylmorpholine-N-oxide / water mixtures (Cuissinat&Navard 2007), or  
316 cellulose fibers swollen by alkali and carbon bisulfate (Ott, Spurlin&Grafflin 1954). This  
317 morphology was explained by the radial expansion of the cellulose in the secondary wall  
318 causing the primary wall to burst. However, the swelling was not homogeneous and unswollen  
319 sections remain, restricting the uniform expansion of the fiber and promoting the formation of  
320 balloons. With increasing carboxyl content, the fraction of swollen fibers increased. In  
321 oxidized fibers with a carboxyl content over 1000  $\mu\text{mol.g}^{-1}$ (Bas-1000 and Bas-1300), most  
322 fibers were swollen and many of them showed a balloon structure (**Fig. 1H, K and 1I,L**). The  
323 heterogeneous morphology in oxidized or carboxymethylated fibers might be due to a  
324 difference in the accessibility of cellulose fibers to chemical reagents (NaClO for oxidation and  
325 sodium chloroacetic acid for carboxymethylation). We infer that the successful defibrillation of  
326 chemically-treated fibers by extrusion is favored by the swelling of fibers, thus facilitating the  
327 breakdown of cell walls and the individualization of the cellulose nanofibrils. The fiber  
328 expansion also increases its flexibility and reduces the risk of clogging by accumulation of  
329 compressed fibers at the extremity of the screw. This might explain the easier extrusion of  
330 oxidized or carboxylated cellulose fibers as their carboxyl content exceeded a critical  
331 threshold. The expansion of fibers after oxidation or carboxymethylation is further highlighted  
332 from WRV measurement. The WRV of neat NDP was 52% and markedly grew after oxidation  
333 treatment, reaching about 323% and 490% for Bas-800 and Bas-1300 treated fibers  
334 respectively (**Table 1**). The WRV also increased with increasing carboxyl content, confirming  
335 the tendency of the notable expansion of the fibers revealed by optical microscopy observation  
336 (**Figure 2**).

337 The mechanical properties of CNF films prepared by drying CNF suspensions and storage  
338 at 50% R.H. were evaluated by tensile tests. Typical stress–strain curves for CNF nanopaper

339 and fiber films prepared by casting and drying at 40°C are shown in **Figure 3A** from which the  
 340 tensile strength, tensile modulus, and strain at break were collected and given in **Table 1**. For  
 341 comparison purpose, the mechanical properties of films from pretreated and neat fibers were  
 342 included (Neat fibers, Pap-800) and their tensile curve added in **Figure3A**. For CNF films, the  
 343 tensile curve shows an elastic region with a limit deformation up to about 1% followed by a  
 344 strain-hardening plastic region extending from 1 to 2-4%.



345



346

347 **Figure 3.** (A) Typical stress-strain curves for cellulose films and (B) storage modulus ( $E'$ ) vs.  
 348 temperature of cellulose films prepared from extruded fibers. Pap-800: film from basic  
 349 oxidized fibers with a carboxyl content of  $800 \mu\text{mol.g}^{-1}$  without extrusion.

350

351 The strength of film from neat (Neat fiber) and pretreated fibers with carboxyl content 800  
352 (Pap-800) was quite low, around 3.6 and 14.5 MPa, respectively. The enhancement observed  
353 after the oxidation treatment is presumably due to improved bonding between fibers as a result  
354 from the carboxylic groups generated and hydration of the cellulose fibers that improve the  
355 contact between cellulose fibers. After TSE and for samples with carboxyl content exceeding  
356 700  $\mu\text{mol.g}^{-1}$ , a strong enhancement of the tensile modulus and strength is observed which is  
357 explained by the formation of CNFs generating entangled network held through hydrogen  
358 bonding and to form homogenous and compact structure (Henriksson et al. 2008). For films of  
359 fibers oxidized under basic condition, the tensile strength at carboxyl content of 750, 1000 and  
360 1350 attained 42, 48 and 52 MPa and the tensile modulus was 2.8, 5.6 and 6.8 GPa,  
361 respectively. This steady increase in mechanical performance of extruded films could be  
362 explained by the increase in the nanosized fraction of cellulose fibrils, which is responsible for  
363 the strong reinforcing effect of extruded cellulose films. Given their nanoscale, flexibility  
364 resulting from the coexistence of disorganized and crystalline domains within the cellulose  
365 fibrils, CNFs are known to form tightly interconnected networks held by a strong hydrogen-  
366 bonding between neighboring CNFs. This led to a dense nanopaper structure with a low  
367 porosity and high capacity to transfer stress from fibril-to-fibril (Henriksson et al. 2008, Isogai,  
368 Saito & Fukuzumi 2011). Moreover, albeit their similar carboxyl content, the strength and  
369 tensile modulus of nanopaper of Neut-800 were higher than that of Bas-800. Two reasons might  
370 account for the disparity in the mechanical performance of the CNF film: (i) the higher  
371 nanosized CNF fraction from Neut-800 in comparison of Bas-800, and (ii) the presence of  
372 large bundles of fibrils from Bas-800 that were not observed in CNFs from Neut-800,  
373 suggesting a poor fibrillation efficiency in Bas-800 fibers.

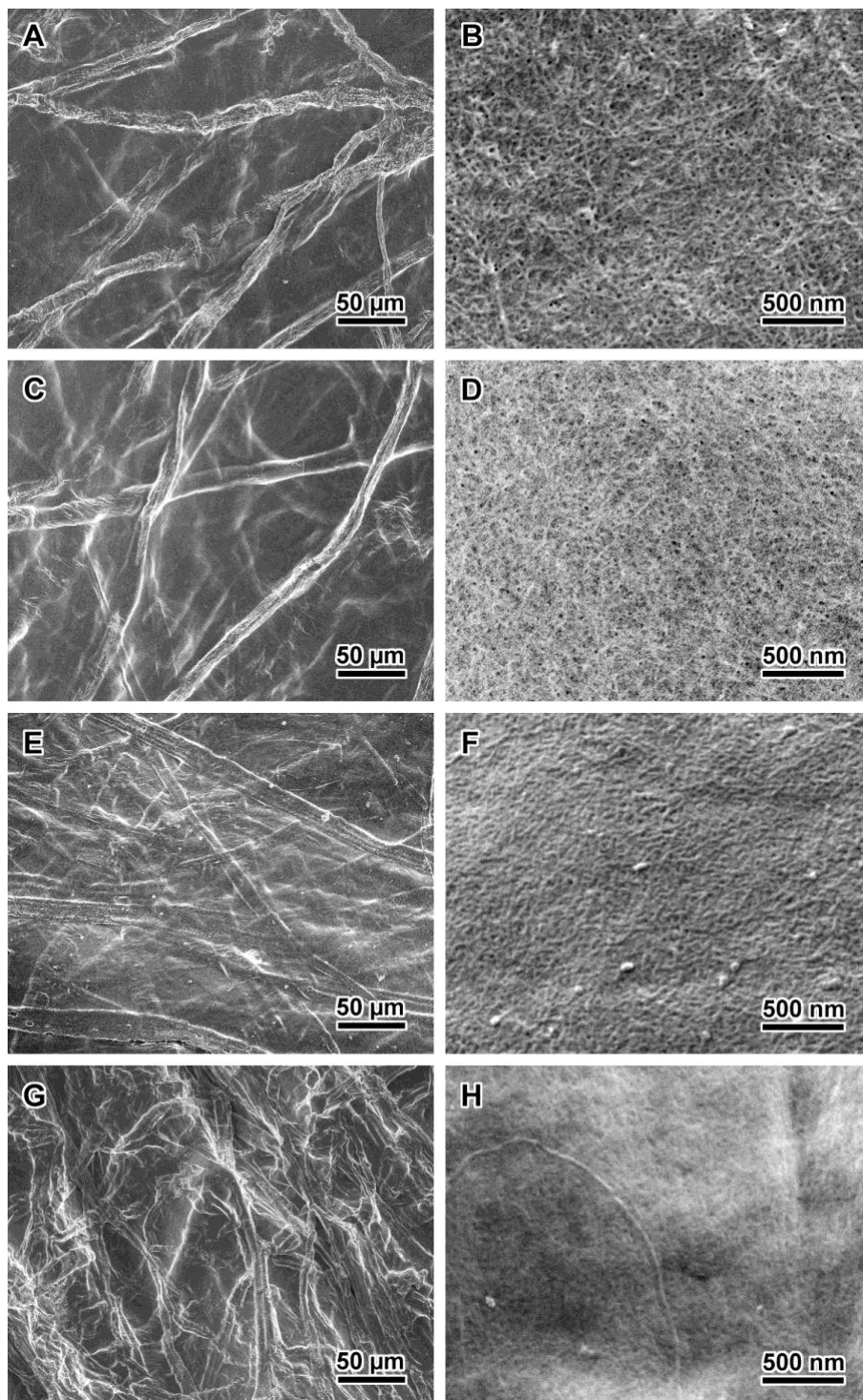
374 However, referring to literature data, it worth to note that the strength of the films from  
375 CNFs produced by TSE was lower in comparison with those from CNFs produced by HPH  
376 (Fujisawa et al. 2011) even though the same method of preparation of the CNF film was  
377 adopted (i.e. casting-drying without pressing) and a TEMPO-mediated oxidized fibers were  
378 used. Strength within the range of 120 to 150 MPa was reported along with a tensile modulus  
379 between 7 to 12 GPa (Henriksson et al. 2008). One possible reason justifying this difference is  
380 the lower fibrillation efficiency of TSE compared to HPH and the difference in the morphology  
381 of the cellulose fibrils produced from the two methods. The later led to better individualization  
382 of the cellulose nanofibrils attaining the level of the elementary fibrils with diameter around 3-  
383 5 nm, especially when a chemical pretreatment was adopted (Besbes, Alila & Boufi 2011).

384 DMA was also performed on cellulose films to evaluate the tensile modulus ( $E'$ ) of films  
385 within the linear domain with good accuracy (**Fig. 3B**). For all tested films,  $E'$  remained nearly  
386 constant in the temperature domain 20-60°C with a magnitude depending on the carboxyl  
387 content. A strong increase in  $E'$  by about one order of magnitude was noted for films produced  
388 from extruded fibers. The magnitude of  $E'$  increased with increasing carboxyl content of  
389 extruded fibers, indicating an enhancement in the stiffness of the nanopaper. This effect is in  
390 line with tensile test data and is presumably due to the increase in the fraction of nanofibrils.

391 The morphology of the different cellulose films produced from extruded and pretreated  
392 fibers was studied by FE-SEM observation of surface and cross-section fracture of films  
393 (**Fig. 4**). At low magnification, micron-sized cellulose fibers dispersed within a web-like tight  
394 network can be seen which corresponds to the fraction of intact and partially fibrillated fibers.  
395 At high magnification, a random-in-plane web-like network structure formed by entangled  
396 CNFs tightly bound to each other can be seen. This interconnected network structure with a  
397 low porosity in the range 10-30 nm accounts for the high density of CNF films and their higher  
398 strength and stiffness compared to films from non-extruded fibers. The width of CNFs ranges

399 from 10 to 30 nm which is higher than that revealed by TEM. A possible reason is the  
400 aggregation of individual cellulose nanofibrils during the drying process.

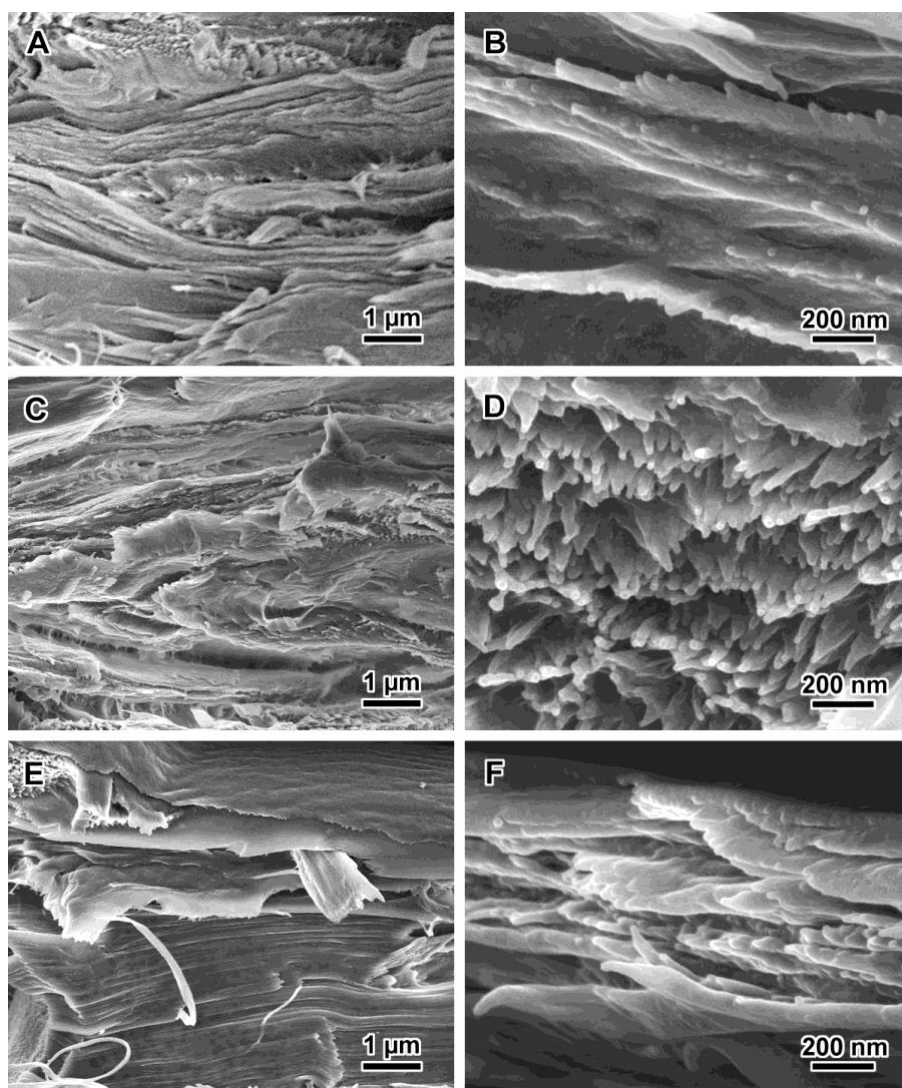
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402

403 **Figure 4.** FE-SEM images of CNF films from extruded treated fibers: A,B) Bas-800;

404 C,D) Bas-1300; E,F) Neut-800; G,H) Carb-800.



406

407 **Figure 5.** FE-SEM images of cross-sections of freeze-fractured CNF films from extruded  
408 treated fibers: A,B)Bas-800; C,D) Bas-1300; E,F) Neut-800.

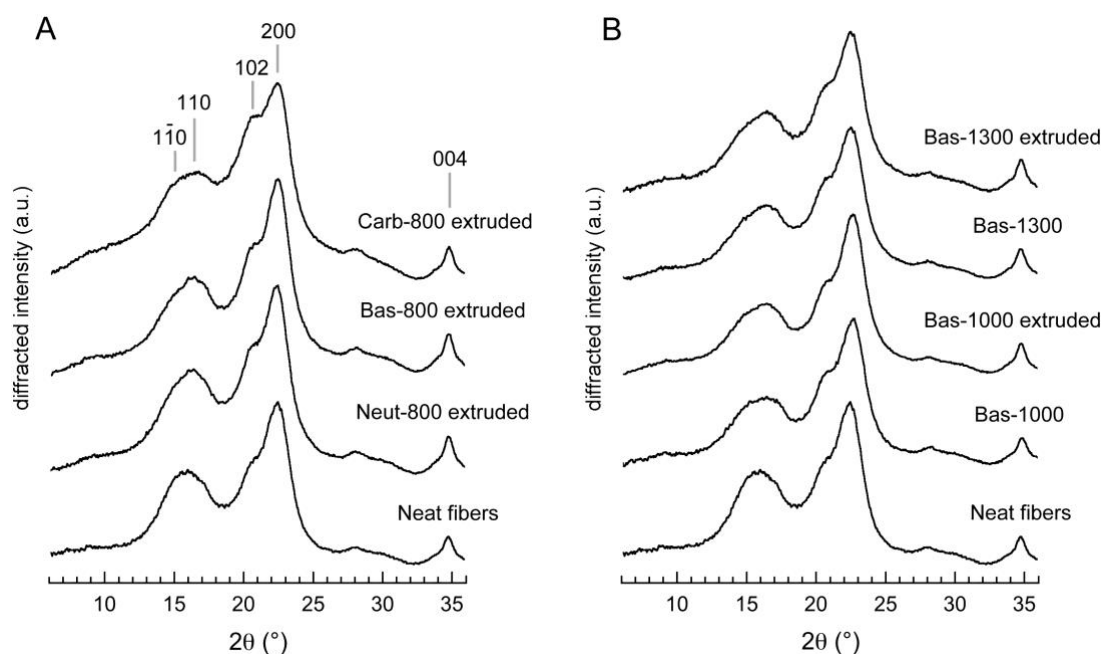
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410 The cross-section of the CNF films (**Fig. 5**) reveals a layered structure which is the typical  
411 structure observed in nanopapers from CNFs produced by high pressure homogenization or  
412 microfluidization (Henriksson et al. 2008). This layered structure has been ascribed to the  
413 deposition of successive layers of CNFs during filtration. However, this explanation cannot  
414 be presently supported considering the mode of preparation of CNF film based on a simple  
415 evaporation of a 0.2 wt% CNF suspension in a Petri dish. The hypothesis of a concentration-

416 induced aggregation and floc formation at a later stage of water removal has been also  
417 suggested to explain this organization (Benítez et al. 2013).

418 The chemical pretreatment of cellulose fibers and their mechanical disintegration can  
419 affect the crystalline degree of the material leading to decrease in the reinforcing potential of  
420 CNFs. The effect of the chemical treatments and extrusion on the crystallinity of the different  
421 samples was analyzed by X-ray diffraction (**Fig. 6**). All diffraction patterns shows reflections  
422 of cellulose I at  $2\theta= 16.2, 22.4$  and  $34.6^\circ$  associated to  $(1\bar{1}0)$ ,  $(020)$  and  $(004)$  crystal planes,  
423 respectively (Sassi, & Chanzy, 1995)

424



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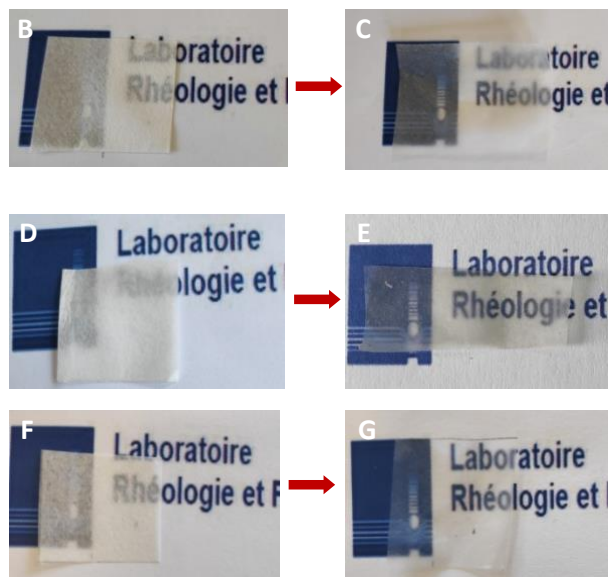
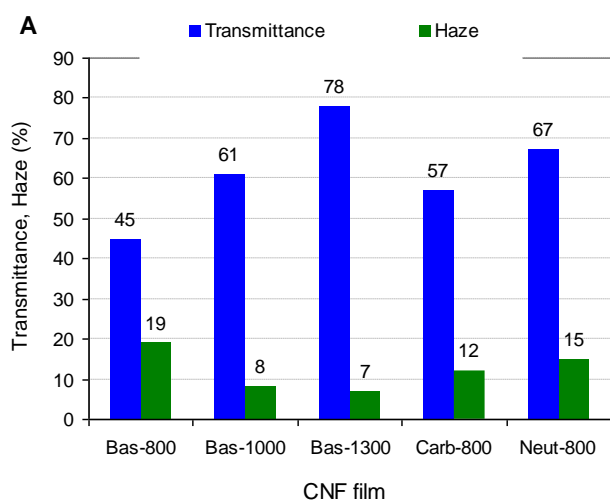
426 **Figure 6.** X-ray diffraction profiles for neat, oxidized and carboxylated fibers before and after  
427 extrusion.

428

429 For oxidized fibers, the CrI remained within the 0.74-0.77 range - over a carboxyl content  
430 between 800 and 1300  $\mu\text{mol.g}^{-1}$  and did not change after the extrusion of treated fibers,  
431 implying that the crystalline structure was not significantly affected neither by the oxidation  
432 treatment nor by the extrusion process. However, in carboxymethylated fibers, the CrI for  
433 sample with a carboxyl content around 800 decreased to 0.57 indicating a decrease in the

434 crystallinity of cellulose fibers after the carboxymethylation. This phenomenon has already  
435 been reported in the literature and explained by the breakage of hydrogen bonds in the  
436 crystalline regions of cellulose chains during the carboxymethylation reaction (Eyholzer et al.  
437 2010).

438



439

440 **Figure 7.** (A) Transmittance and haze at 600 nm of CNF films produced by extrusion of  
441 pretreated fibers at different carboxyl content. Visual appearance of film Bas-800 (B) before  
442 and (C) after extrusion; Bas-1000 before (D) and after (E) extrusion of fibers; Bas-1300  
443 before (F) and after (G) extrusion of fibers.

444

445 The transparency degree of thin films produced from cellulose fibers or nanofibers without  
446 any additive or pigment depends on a number of parameters including thickness, fiber or fibril  
447 width, porosity and surface roughness. Due to the mismatch between the refractive index of  
448 air ( $n=1$ ) and cellulose ( $n=1.5$ ) light is scattered at the fibril/air interface during propagation  
449 through the film, which results in the reduction of transmitted light and consequently in the  
450 transparency of the film. The scattering intensity strongly depends on the particle size and  
451 wavelength and, below a critical size of about 40 nm, the material turned transparent to light  
452 without any scattering effect. The transmittance and haze at 600 nm are shown in **Figure 7.**



453 Haze is a measurement of the light scattering characteristics of a material and is responsible  
454 for the difficulty to clearly see objects through a film. The light transmittance for all CNF  
455 films from extruded fibers with thickness around 30  $\mu\text{m}$  is above 45% (at 600 nm) with a haze  
456 being lower than 20 which is indicative of that a high fraction of incident light can cross over  
457 the film without being scattered. At a higher carboxyl content, the transmittance further  
458 increased up to 80% at a 1300  $\mu\text{mol.g}^{-1}$  carboxyl content and with a haze around 7. The drop  
459 in haze corresponds to a decrease in scattered light which is due to the lower porosity,  
460 enhancement in the nanosized fraction and reduction in surface roughness of CNF films. This  
461 result is in agreement with literature data concerning the correlation between  
462 transparency/haze of nanopaper films and the CNF morphology (Hsieh, Koga, Suganuma,  
463 & Nogi 2017, Zhu et al. 2013). The visual appearance of CNF films supported the  
464 transmittance measurement, where it can clearly be seen that cellulose film from oxidized  
465 non-extruded fibers looks white and rather opaque and turned to translucent-transparent films  
466 when the film was produced from extruded fibers.

467

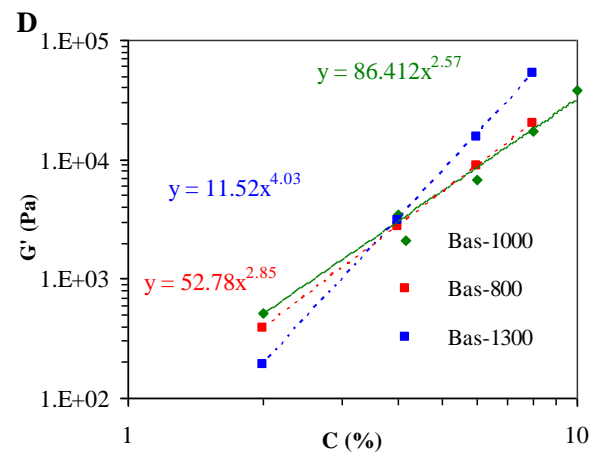
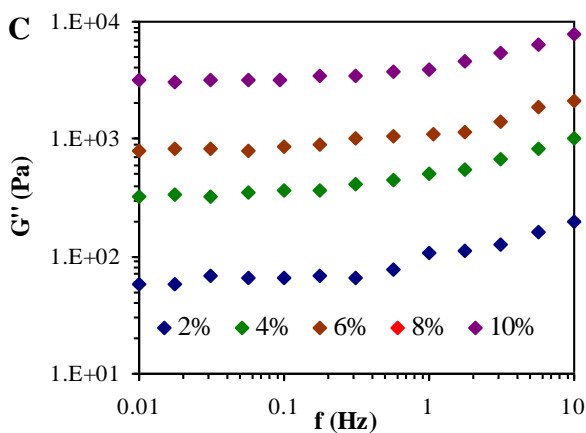
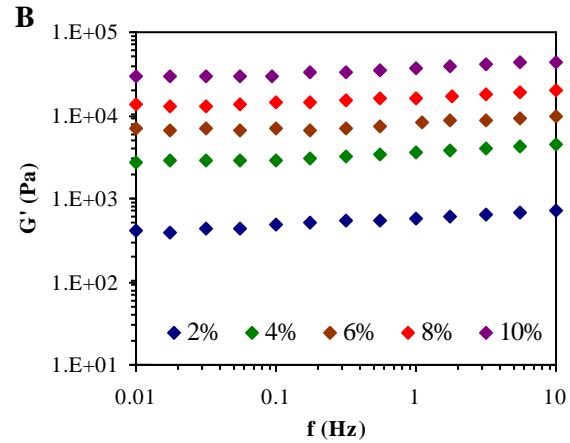
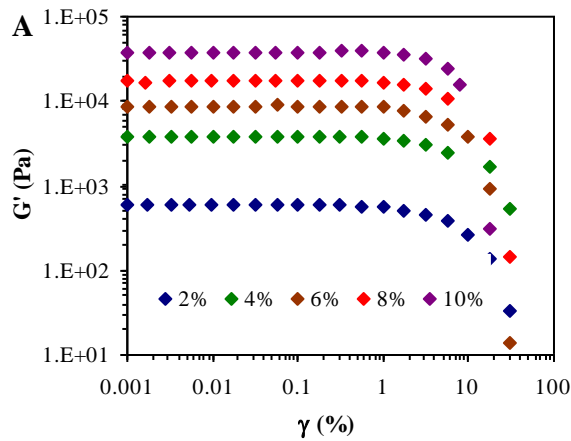
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476 **Figure 8:** (A) Storage modulus  $G'$  vs. strain amplitude, (B) storage modulus  $G'$  and (C) loss  
 477 modulus  $G''$  vs. frequency for Bas-1000 CNFs, and (D) storage modulus  $G'$  at 1Hz vs. solid  
 478 content  $C$ . The lines correspond to the fits of the power law  $G'=KC_n$  on experimental  
 479 measurements.

480

481 The rheological behavior of the CNF suspension at different solid contents was  
 482 investigated by oscillatory sweep measurements of the storage modulus ( $G'$ ) and loss modulus  
 483 ( $G''$ ) as a function of strain amplitude ( $\gamma$ ) or frequency ( $f$ ). Examples of oscillatory sweep  
 484 measurements for Bas-1000 CNF gel are given in **Figure 8A-C**. For all CNF suspensions, a  
 485 transition from a linear to nonlinear viscoelastic behavior was observed over a critical strain  
 486  $\gamma_c$  in the range of 2–4% with an abrupt drop of the magnitude of  $G'$  which is the result of the  
 487 breakdown of the CNF network under the effect of deformation. In addition, all of the CNF

488 suspensions produced by TSE exhibited a gel-like character over a solid content in the range  
489 of 1 to 10%, as attested by the dominance of  $G'$  over  $G''$  in the measured frequency range with  
490 ( $G'$  being about one decade higher than  $G''$  over the whole frequencies domain). For all  
491 studied samples and with the solid content ranging from 2 to 10%, both  $G'$  and  $G''$  were  
492 nearly independent of frequency and carboxyl content as seen in Figure S4. This property is  
493 consistent with the gel-like behavior typically observed for CNFs produced by other  
494 disintegration methods such as high pressure homogenization or grinding (Nechporchuk,  
495 Belgacem, & Pignon 2014). The origin of this effect was explained by the tendency of CNF  
496 to form an interconnected three dimensional network, even at low concentration ( $C < 0.5\%$ )  
497 thanks to the flexibility of the cellulose nanofibrils along with their high aspect ratio (Pääkkö  
498 et al. 2007, Nechporchuk, Belgacem & Pignon 2016). The upholding of the gel character of  
499 all the CNFs produced by TSE in the domain of solid content between 1 to 10%, regardless of  
500 the carboxyl content and the fibrillation extent, suggested that the rheological properties of the  
501 CNFs were dominated by the presence of nanosized fibrils.

502 A decrease in the magnitude of  $G'$  as the solid content  $C$  is getting down is observed,  
503 which is expected due to the weakening of the network resulting from the decrease in the  
504 number of fibril–fibril contact upon dilution of the CNF gel. A power-law dependence of  $G'$   
505 with CNF concentration ( $G' = KC_n$ ) in the linear domain was noted for the different gel, where  
506  $C$  is the weight concentration of CNFs and the  $K$  factor and  $n$  power are characteristic of the  
507 individual nanofibril characteristics and the structural property of the CNF suspension,  
508 respectively. The fit of the power laws is shown in **Figure 8D** for basic oxidation. The  $K$   
509 factor attained about 52, 86 and 11 Pa for Bas-800, Bas-1000 and Bas-1300 respectively,  
510 while the  $n$  power index is about 2.85, 2.57 and 4.03, for Bas-800, Bas-1000 and Bas-1300,  
511 respectively. The regression coefficients  $R^2$  are higher than 0.99. Based on theoretical  
512 consideration, the value of  $n$  in the power-law relation between  $G$  versus solid content of

513 polymer gel should be 2-2.5 (MacKintosh, Kaä & Janmey 1995). However, referring to  
514 literature data, the wide range of the  $n$  values from 1 to 5 has been reported without soundly  
515 clarifying the origin of this variation (Nechyporchuk, Belgacem & Pignon 2016). A similar  
516 power-law behavior was also reported for carbon nanotube suspension with  $n$  varying from 2  
517 to 7 depending on the aspect ratio and surface treatment. The highest value was observed for  
518 nanotubes with the shortest length while for the long fibrils the  $n$  value is close to 2-2.5  
519 (Rahatekar et al. 2009). Recently, a rheological investigation on chitin nanofibrils with  
520 different lengths and degrees of aggregation has shown a strong dependence of the  $n$  value on  
521 their aspect ratio, degree of dispersion and self-interaction (Yokoi, Tanaka, Saito & Isogai  
522 2017). The  $n$  value was found to increase from 2.7 to 3.8–3.9 as nanofibrils self-organized  
523 from a randomly oriented structure into a nematic ordered arrangement. One possible reason  
524 explaining the higher value of  $n$  power in for Bas-1300 is the lower length of these CNFs in  
525 comparison with CNFs from Bas-800 and Bas-1000. The decrease in CNF length would  
526 result from secondary reaction, mostly  $\beta$ -elimination, accompanying the oxidation reaction  
527 under basic condition inducing cellulose chains breaking (Shinoda, Saito, Okita & Isogai  
528 2012). The decrease in the nanofibril length at high carboxyl content in TEMPO-mediated  
529 oxidation of cellulose was also reported by Isogai et al. (Isogai, Saito & Fukuzumi 2011) where  
530 a correlation between the carboxyl content, the length and the degree of polymerization (DP)  
531 was established (Shinoda, Saito, Okita & Isogai 2012). The AFM observation of CNFs from  
532 Bas-800, Bas-1000 and Bas-1300 supported also the hypothesis of a decrease in the CNF  
533 length as their carboxyl content is increasing (**Figure S2A-C**). However, the contribution of  
534 the carboxyl content should not be disregarded. In fact, with the increase in carboxyl content,  
535 the CNFs became more surface-charged and less inclined to interact with neighboring CNFs  
536 due to the electrostatic repulsion effect. The contribution of this effect is probably more  
537 marked at low CNF concentration, which might explain the lower magnitude of  $G'$  for Bas-

538 1300 at a solid content lower than 3 wt%. At higher concentration, the morphology of CNFs,  
539 especially their length, will prevail over the other effects. This could explain the difference  
540 observed in **Figure 8D**.

541 The steady-state viscosities as a function of the shear rate for CNF suspensions displayed  
542 a typical shear-thinning behavior of CNF gel which is attributed to the disentanglement and  
543 alignment of CNFs along the shear direction under the low shear force, causing a gradual  
544 decrease in viscosity, which is in agreement with literature data (Nechporchuk,  
545 Belgacem&Pignon 2016). An example of the steady flow curves for Bas-1000 CNFs at  
546 different solid contents is given in **Figure S5**.

547

#### 548 **4. Conclusion**

549 Commercial never-dried cellulose pulp was used as starting material to produce CNF gels  
550 at high consistency (10wt% solid content) by TSE. Three approaches of chemical  
551 pretreatment consisting in a TEMPO-mediated oxidation at basic and neutral condition and  
552 carboxymethylation with sodium chloroacetic acid were adopted to generate carboxyl groups  
553 at controlled amount. Without any chemical pretreatment, it was impossible to extrude NDP  
554 because of the clogging of fibers impeding recirculation for multiple passes. The successful  
555 disintegration of cellulose fibers into CNF gels was observed only for samples with a  
556 carboxyl content exceeding 700  $\mu\text{mol.g}^{-1}$ , irrespective of the method of chemical  
557 pretreatment. Based on optical observation, it was proposed that the chemical pretreatment led  
558 to a swelling of the fibers turning them more flexible to be recirculated through TSE without  
559 any risk of clogging. The swelling of fibers facilitates also their breakdown by reducing the  
560 interfibrillar interaction through hydrogen bonding.

561 The increase in carboxyl content led to an increase in the nanosized fraction and  
562 enhancement in the tensile modulus and strength of nanopaper from CNF gels. FE-SEM

563 observation of CNF films revealed a random-in-plane web-like network structure, exhibiting a  
564 layered structure when cross-section observed.

565 The successful disintegration of commercial eucalyptus pulp into cellulose nanofibrils by  
566 TSE at a solid content of 10% contributed to further develop this relatively new processing  
567 method to produce CNFs. This work has contributed to identify some of the parameters  
568 controlling the breakdown of cellulose fibers into nanoscale objects when a chemical  
569 pretreatment based on TEMPO-mediated oxidation or carboxymethylation. Although this  
570 chemical pretreatment was necessary to run the process, the high solid content of the  
571 produced CNFs without any risk of clogging and the use of conventional commercial TSE  
572 constitutes the main advantage of this processing route. The lower energy consumption of  
573 TSE is another merit of this method as has been reported in two recent papers<sup>4,5</sup>. Work is  
574 under progress to scale up the production of CNFs via TSE using oxidized fibers with  
575 carboxyl content around 1000  $\mu\text{mol.g}^{-1}$  and using a pilot twin-screw extruder.

576

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