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### ► To cite this version:

Ahlem Mnasri, Hatem Dhaouadi, Ramzi Khiari, Sami Halila, Evelyne Mauret. Effects of Deep Eutectic Solvents on cellulosic fibres and paper properties: Green “chemical” refining. *Carbohydrate Polymers*, 2022, 292, pp.119606. 10.1016/j.carbpol.2022.119606 . hal-03755448

**HAL Id: hal-03755448**

**<https://cnrs.hal.science/hal-03755448>**

Submitted on 23 Aug 2022

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# 1           **Effects of Deep Eutectic Solvents on cellulosic fibres and paper properties:**

## 2                           **Green “chemical” refining**

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### 13   **Abstract**

14   The treatment of cellulosic fibres using three Deep Eutectic Solvents “DES” of different pH  
15   (acid, neutral and alkaline), namely betaine hydrochloride-urea (BHCl-U), choline chloride-  
16   urea (CC-U) and choline chloride-monoethanolamine (CC-M) was studied. Cotton pulp  
17   (100% cellulose) and eucalyptus fibres (containing hemicellulose) were used to deeply study  
18   the effects of DES on both hemicellulose and cellulose. The treatment was conducted at  
19   100°C (1 or 4h) and the effects of these DES on the morphological, chemical, physical and  
20   thermal structure of cellulose fibres were investigated. Interestingly, during acidic DES  
21   treatment an esterification reaction occurred between BHCl and cellulose (DS=0.025). The  
22   hemicellulosic-xylan of eucalyptus fibres was partially eliminated after acidic and neutral  
23   DES treatment. The results showed that DES allow to promote an internal fibrillation  
24   (increase of the water retention value of treated fibres) and external fibrillation of the fibres  
25   (identified by scanning electron microscopy). Compared to conventional treatment (enzymatic  
26   hydrolysis), DES have significantly enhanced the mechanical properties of resulting papers  
27   because of exceptional retention of structural integrity of cellulose. To conclude, DES  
28   treatment could be considered as a soft and green “chemical” refining of cellulose fibres.

29   ~~Three Deep Eutectic Solvents “DES” of different pH (acid, neutral and alkaline) were used to~~  
30   ~~study their effects on cellulosic fibre structure (cotton and eucalyptus pulp), namely, betaine~~  
31   ~~hydrochloride urea (BHCl-U), choline chloride urea (CC-U) and choline chloride~~  
32   ~~monoethanolamine (CC-M). Interestingly, during acidic DES treatment an esterification~~  
33   ~~reaction occurred between BHCl and cellulose (DS=0.025). The hemicellulosic xylan of~~  
34   ~~eucalyptus fibres was partially eliminated after acidic and neutral DES treatment. Water~~

35 ~~retention value of treated fibres increased especially with CC-M, which reflects a specific~~  
36 ~~action of the DES on the structure of the fibre wall: DES seems to promote an internal~~  
37 ~~fibrillation. An external fibrillation was also identified by scanning electron microscopy.~~  
38 ~~Compared to conventional treatment (enzymatic hydrolysis), DES have significantly~~  
39 ~~enhanced the mechanical properties of resulting papers because of exceptional retention of~~  
40 ~~structural integrity of cellulose. To conclude, DES treatment could be considered as a soft and~~  
41 ~~green “chemical” refining of cellulose fibres.~~

42 **Key words:** Cellulose fibres, treatment, Deep Eutectic Solvents, fibrillation, chemical  
43 refining.

## 44 **1. Introduction**

45 Currently the development of “green” chemistry is one of the promising solutions to deal with  
46 environmental problems. This chemistry is particularly based on the use of non-toxic solvents  
47 and reagents. In this context, ionic liquids (ILs) have been widely studied. These solvents are  
48 made up of large organic cations and inorganic/organic anions (molecular ionic liquids). They  
49 are characterized by specific properties such as low vapor pressure, non-flammability,  
50 dissolving capacity, thermal and chemical stability and low melting point. Despite their  
51 advantages, ionic liquids must not be considered as green solvents due to their low  
52 biodegradability and their environmental toxicity. In addition, the use of these solvents on an  
53 industrial scale is limited for economic reasons related in particular to their preparation and  
54 purification which are time consuming (Kudłak, Owczarek, & Namieśnik, 2015).

55 ~~To replace ionic liquids, Deep Eutectic Solvents (DES) have been proposed by Abbott et al.~~  
56 ~~(2001). They have the same advantages as ionic liquids but are cheaper, easier to obtain, of~~  
57 ~~low toxicity and often biodegradable (Abbott et al., 2001).~~

58 Deep Eutectic Solvents “DES” were proposed by Abbott et al. (2001) as an alternative to  
59 ionic liquids. DES and Ilqs share some similarities such as low volatility, high tunability and  
60 capacity of dissolving organic and inorganic compounds. However, they are distinguished in  
61 the method of their preparation and the starting materials. DES exhibit many advantages than  
62 Ilqs, including low or non-toxicity, easy preparation, the use of cheap and sustainable  
63 compounds. Moreover, the components of DESs must not react with each other, while for Ilqs  
64 their synthesis requires many synthetic steps using various reagents and organic volatile  
65 solvents (Płotka-Wasyłka, de la Guardia, Andruch, & Vilková, 2020).

66 DES could be described as a mixture of acids and Lewis or Bronsted bases. They can be also  
67 defined as the mixture of hydrogen bond donors (HBD) and hydrogen bond acceptors (HBA).  
68 DES composed of a quaternary ammonium salt and a metal salt or hydrogen bond donor are  
69 the most studied systems. These systems (formed by salts components or by the mixture of  
70 solid and liquid) are characterised by a low lattice energy which increases system's stability  
71 that leads to charge delocalisation. This charge delocalisation is responsible for the decrease  
72 in the melting point of the mixture compared to the melting points of the individual  
73 components. DES are classified into four groups (Smith, Abbott, & Ryder, 2014): (I)  
74 combination of organic and metal salts; (II) mixture of organic salts and metal hydrates; (III)  
75 combination of organic salts and hydrogen bonding compounds and (IV) mixture of metal  
76 chlorides and hydrogen bond donor compounds. Many non-cationic DES have also been  
77 described in the literature. Therefore, a fifth class (type V) was proposed (Abranches et al.,  
78 2019; Moufawad, Gomes, & Fourmentin, 2021; Schaeffer et al., 2020). This type is obtained  
79 from the mixture of non-ionic species.

80 Eutectic mixtures are easily prepared by mixing the constituents of DES, while ensuring  
81 intimate contact between these constituents. This contact is achieved by different methods,  
82 either by stirring (Zahrina, Nasikin, & Mulia, 2017), grinding (Florindo, Oliveira, Rebelo,  
83 Fernandes, & Marrucho, 2014) or extrusion (Crawford, Wright, James, & Abbott, 2016). It is  
84 often necessary to couple this mechanical mixing operation to a heating usually in the range  
85 of 50 to 100°C but, for some systems, higher temperature is needed (Zahrina et al., 2017).

86 For these reasons, DES are attracting more and more interest in the scientific community.  
87 They are used in several fields such as organic synthesis (Ruesgas-Ramón, Figueroa-  
88 Espinoza, & Durand, 2017), polymer science (Tomé, Baião, da Silva, & Brett, 2018),  
89 electrochemical field (Fernandes, Campiña, Pereira, Pereira, & Silva, 2012; Hosu, Bârsan,  
90 Cristea, Săndulescu, & Brett, 2017), and waste water treatment (AlOmar et al., 2016). Some  
91 DES are known to be treatment media for lignocellulosic biomass as described in many  
92 reviews (Selkälä, Sirviö, Lorite, & Liimatainen, 2016; Zdanowicz, Wilpiszewska, & Spychaj,  
93 2018). In this case, lignocellulosic biomass delignification, cellulose treatment (modification,  
94 microfibrillation, nanocrystals production...) or hemicellulose extraction are the main  
95 objectives.

96 When applied to lignocellulosic biomass, DES must have a selective solubilisation capacity.  
97 This selective solubilisation depends on both DES components and the nature of the biomass.  
98 Several mixtures can be used for lignin extraction (Francisco, Bruinhorst, & Kroon, 2012)

99 such as CC-lactic acid, betaine-lactic acid mixtures and amino acid-based DES (lactic acid-  
100 proline, maleic acid-proline, maleic acid-lycine). On the other hand, as they do not solubilise  
101 cellulose, these DES make it possible to have a selective action. For the production of  
102 cellulose nanocrystals (CNC), when hydrolysis of the amorphous part of cellulose is targeted,  
103 choline chloride-oxalic acid dihydrate mixture is the most efficient system (Douard, Bras,  
104 Encinas, & Belgacem, 2021; Sirviö, Visanko, & Liimatainen, 2016). Other systems can be  
105 used for CNC production such as choline chloride-oxalic acid (anhydrous), choline chloride/  
106 *p*-toluene sulphonic acid monohydrate and choline chloride-levulinic acid (Laitinen,  
107 Suopajarvi, Österberg, & Liimatainen, 2017).

108 DES can also be used as pre-treatment media to enhance cellulose microfibrillation even  
109 though they are few articles related to this subject compared to the selective extraction of  
110 biomass components. For instance, choline chloride-urea (CC-U) mixture was the first system  
111 used for treated cellulose fibres before microfibrillation (Sirviö, Visanko, & Liimatainen,  
112 2015). Another study showed that urea ammonium thiocyanate or guanidine hydrochloride  
113 based DES can be used in wood pre-treatment to facilitate microfibrillation (Li, Sirviö,  
114 Haapala, & Liimatainen, 2017). These systems are able to swell the fibres and thus break the  
115 hydrogen bonds between the cellulose chains but without degrading the polysaccharide.

116 Furthermore, some studies reported the use of DES for microfibrillation with simultaneous  
117 modification. Cellulose cationization for instance can be carried out during DES treatment  
118 (Sirviö, 2018). In this work, a mixture of triethylmethylammonium chloride and imidazole  
119 was used as reaction medium for cellulose fibre cationization using betaine hydrochloride in  
120 the presence of *p*-toluenesulfonyl chloride. Cationized cellulose fibres were then mechanically  
121 disintegrated using microfluidisation. The resulting gels contained cationic individualised  
122 CNF together with larger aggregates.

123 Esterification reaction can also be performed in the presence of DES to produce esterified  
124 CNF (Liu, Zhang, Gou, Zhang, & Wang, 2021). Many carboxylic acid-based DES were used  
125 to pre-treat and esterify cellulose at the same time. This study showed that DES pre-treatment  
126 enables the swelling and esterification of cellulose fibres. Combining DES pre-treatment with  
127 mechanical treatment results in CNF having widths less than 100 nm which were used for  
128 composites (PLA) reinforcement.

129 For many years, one of the main topics of our group has been related to the reduction of the  
130 energy costs associated with cellulose microfibrillation (Banvillet, Depres, Belgacem, & Bras,

131 2021; Rol et al., 2017, 2020). Nowadays, various pre-treatments (biological or chemical) are  
132 used before the main mechanical treatment of the microfibrillation process (ultrafine grinding,  
133 homogenisation...) in order to reduce the energy consumption (Nechporchuk, Belgacem, &  
134 Bras, 2016; Rol, Belgacem, Gandini, & Bras, 2019). Indeed, conventional pre-treatments  
135 implemented for the production of MFC are expensive such as enzymatic hydrolysis and  
136 based on the use of toxic reagents like TEMPO-mediated oxidation. DES represent a solution  
137 to reduce the toxicity of chemical reagents and to protect the environment by the use of  
138 biodegradable components.

139 The use of DES as pre-treatment to promote microfibrillation was demonstrated as a novel  
140 and promising route to produce sustainable microfibrillated cellulose. This is due to their non  
141 or low toxicity, biodegradability, recyclability and their soft character. In addition, these  
142 solvents are sometimes nonreactive such as choline chloride-urea which can be an advantage  
143 especially for further modification (post-modification). It was demonstrated that DES  
144 containing quaternary ammonium salts with chloride anion are efficient for the treatment of  
145 cellulose thanks to the interaction of chloride anion with cellulose (Chen, Zhang, You, & Xu,  
146 2019).

147 In the light of promising results with DES pre-treatment, this work is part of the same strategy  
148 and aims investigating the influence of three different DES systems (acidic, neutral and basic)  
149 on the chemical and physical properties of cellulose fibres from eucalyptus and cotton. The  
150 choice of these biomasses is based on their different structure. Eucalyptus fibres (Kraft  
151 bleached pulp) are selected because they are widely used in the papermaking and the  
152 production of microfibrillated cellulose. In addition, they are composed from cellulose and  
153 hemicellulose which allows to see the effects of DES on both polysaccharides and how the  
154 presence of hemicellulose affects the microfibrillation process. On the other hand, cotton  
155 fibres are used as a model material because they contain only cellulose (almost 100%). The  
156 use of two different biomasses allows to give better understanding of the DES effects on  
157 cellulose and hemicellulose.

158 In such a context, betaine hydrochloride-urea BHCl-U (acidic), choline chloride-urea CC-U  
159 (neutral) and choline chloride-monoethanolamine CC-M (basic) were selected. The choice of  
160 DES is based on the fact that quaternary ammonium salts based-DES were shown as good  
161 systems that interact with cellulose (Chen et al., 2019). Based on this strategy, the selection of  
162 DES is focused on non-toxic and biodegradable DES having quaternary ammonium salts with  
163 chloride anion that were never used as pre-treatment for improving internal and external

164 fibrillation, in a first step, and enhancing cellulose microfibrillation in a second step (data to  
165 be published).

166 The selection was also oriented towards DES with different pH to investigate their acidity or  
167 basicity effects on cellulose fibres and whether their effects are similar to conventional  
168 solvents.

169 To the best of our knowledge, BHCl-U and CC-M were never used as pre-treatment media for  
170 cellulose microfibrillation. Indeed, BHCl-U was studied to produce cellulose carbamate from  
171 hardwood dissolving pulp (Willberg-Keyriläinen, Hiltunen, & Ropponen, 2018), while CC-M  
172 was tested for wheat straw delignification (Zhao et al., 2018). CC-U was selected as a  
173 reference because its wide use in cellulose pre-treatment to produce microfibrillated cellulose.  
174 DES treated fibres were compared to enzymatic-treated fibres and characterized according to  
175 the state-of-the-art techniques including infrared spectroscopy (FT-IR ATR), solid-state <sup>13</sup>C  
176 NMR, morphological analysis, water retention value (WRV), degree of polymerisation (DP),  
177 tensile tests of papers... So, the main purpose of this work is to evaluate the ability of these  
178 solvents, used as pre-treatment, to facilitate the fibrillation of the fibers and then their  
179 microfibrillation without modifying in a great extent the cellulose structure which can be an  
180 advantage, for instance for post-modification. This paper only presents the results of the first  
181 part of the study related to the effects of DES on cellulosic fibres. To the best of our  
182 knowledge, there is no published work focusing on the effects of DES of the fiber properties  
183 and those of the resulting papers. Understanding their effects and how they act on cellulose  
184 fibers could improve this pre-treatment to produce high quality MFC.

185 As DES treatment is going to be involved in a next work for the production of microfibrillated  
186 cellulose, a conventional pre-treatment (enzymatic hydrolysis) was chosen as a reference  
187 treatment. Based on literature, most of used DES for cellulose microfibrillation (i.e, choline  
188 chloride-urea...) allow to produce MFC without a great extent of chemical modification.  
189 Therefore, enzymatic hydrolysis is selected because it allows to facilitate the microfibrillation  
190 process without chemical modification.

## 191 **2. Materials and Methods**

### 192 **2.1 Materials**

193 Dry sheets of bleached hardwood pulp (eucalyptus, Fibria T35) and cotton pulp (CELSUR,  
194 CS 21 DHS), referenced as EF and CF respectively, were used as cellulose materials. These  
195 fibres were first disintegrated in water. The suspensions were then filtered, washed with

196 ethanol and oven dried at 60°C. Betaine hydrochloride (C<sub>5</sub>H<sub>12</sub>ClNO<sub>2</sub>) was supplied from  
197 VWR (Milliporesigma), urea ACS reagent (CH<sub>4</sub>N<sub>2</sub>O, 99.0-100.5%) from Merck-sigma  
198 Aldrich, monoethanolamine (C<sub>2</sub>H<sub>7</sub>NO, >99%) from ROTH, choline chloride (C<sub>5</sub>H<sub>14</sub>ClNO,  
199 >98%) from Alfa Aesar, bis(ethylenediamine)copper (II) hydroxide solution  
200 (Cu(H<sub>2</sub>NCH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>)<sub>2</sub>(OH)<sub>2</sub> 1.0 M in H<sub>2</sub>O, Sigma-Aldrich), sulfuric acid (H<sub>2</sub>SO<sub>4</sub>, 72%),  
201 acetic acid (CH<sub>3</sub>COOH), ethanol (CH<sub>3</sub>OH, 96%) and sodium acetate (C<sub>2</sub>H<sub>3</sub>NaO<sub>2</sub>) were  
202 obtained from Sigma Aldrich. Enzyme solution FiberCare (pH between 6 and 8) was  
203 purchased from Novozymes. Deionised water was used throughout the experiments.

## 204 **2.2 DES pre-treatment**

205 Three different DES were used. Acidic DES (BHCl-U) was obtained from the mixture of  
206 betaine hydrochloride and urea (1:4 molar ratio). Neutral DES (CC-U) was prepared by  
207 mixing choline chloride with urea (1:2 molar ratio) and alkaline DES (CC-M) from choline  
208 chloride-monoethanolamine (1:6 molar ratio). DES mixtures were heated in an oil bath at  
209 100°C (for BHCl-U) or at 80°C (for CC-M and CC-U) until a clear and transparent liquid is  
210 obtained.

211 The pH of each DES was measured by mixing 2mL of DES in 20mL of deionised water.  
212 Then, the pH was measured at 25°C using a pH meter. The pH values of BHCl-U, CC-U and  
213 CC-M are 1.89, 7.13 and 11.96, respectively.

214 Cellulosic fibres (EF or CF) were mixed with prepared DES in a reactor vessel (Pressure  
215 reactor-600 mL) with a mass ratio of 2.5:100 (for CC-U and BHCl-U) and 5:100 for CC-M.  
216 The mass ratio was adjusted depending on the used DES due to experimental needs. The  
217 suspension was left under mechanical stirring at 100°C for 1 or 4 hours. After treatment, the  
218 suspension was filtered and washed first with hot water and then cold water to remove the  
219 remaining DES. DES-treated fibres were finally stored at 4°C until further use. The yield after  
220 DES pre-treatment was between 90-95%.

## 221 **2.3 Enzymatic pre-treatment**

222 The enzymatic hydrolysis using cellulase enzyme is used as a reference pre-treatment in this  
223 study. A 2 wt % fibre suspension was used. The enzymatic treatment was performed in 12L  
224 reactor (büchiglasuster), at a temperature of 50°C and a pH adjusted at 5 using an acidic  
225 buffer solution (acetic acid and sodium acetate). Enzyme solution (FiberCare cellulase with an  
226 activity of 4948 ECU/g of solution) was added to the suspension with an enzyme's  
227 concentration of 300 ECU/g of cellulose. The reaction was left 2h and then enzyme was



228 disactivated by increasing the temperature to 80°C for 10 min. The obtained suspension was  
229 then filtered through a nylon sieve (10 µm of mesh) and washed with hot water and cold  
230 water. The washed and filtered pulp was finally stored at 4°C until further use. The yield after  
231 enzymatic hydrolysis was around 90%.

## 232 **2.4 Paper handsheet production**

233 Papers were prepared by filtration using a Rapid Köthen sheet former according to the  
234 Standard ISO 5269-2:2004). Untreated eucalyptus fibres, enzymatic treated fibres, and DES-  
235 treated eucalyptus fibres (EF-CC-M) were used at a concentration of 2 g/L. One litre of the  
236 suspension was introduced into the bowl of the former. After dilution with 5 to 7 L of water,  
237 the suspension was filtered on a metallic sieve. The wet sheet is pressed and collected on  
238 blotting papers, protected between two sheets and dried under vacuum at 80°C for 10 min. 10  
239 sheets were made for each kind of pulp. The weight of the sheets formed is generally around  
240 60 g.m<sup>-2</sup>.

241 Before any tests, the samples were placed in a conditioned atmosphere (T = 23°C and relative  
242 humidity = 50%) for 48h as recommended in ISO 187. For each pulp, 10 sheets were tested.

## 243 **2.5 FT-IR ATR spectroscopy**

244 FT-IR ATR analyses were done using a PerkinElmer spectrum 65. FT-IR ATR spectra were  
245 collected with a single reflection horizontal ATR sampling accessory having a diamond ATR  
246 crystal. A small piece of sample (air dried fibres) was placed directly on the ATR crystal and  
247 pressed gradually using sample accessory until a sufficient signal intensity was obtained. The  
248 pressure was then fixed and the IR spectrum was measured at the absorbance mode (16 scans)  
249 with a resolution of 2 cm<sup>-1</sup> in the frequency from 600 to 4000 cm<sup>-1</sup>.

## 250 **2.6 Solid-state <sup>13</sup>C NMR**

251 The NMR experiments were performed with a Bruker Avance III apparatus operating at 100.6  
252 MHz and using a CP-MAS probe of 4 mm. All the spectra were recorded at 295 K using the  
253 combination of cross-polarisation, high-power proton decoupling and magic angle spinning  
254 (CP/MAS) methods. Glycine was used as a reference, with a carbonyl signal fixed at 176.03  
255 ppm. The spinning speed was 12000 Hz with a contact time of 2000 µs and repetition times of  
256 2 s. The acquisition time was adjusted at 0.035 s and the sweep width at 29761.9 Hz.

257 Prior to performing NMR experiment, DES or enzyme treated eucalyptus fibres were air-dried  
258 and then grinded using a cryomill for 2 min. After grinding, sample was compacted in a 4 mm  
259 ZrO<sub>2</sub> rotors and then analysed. The analysis was done in the quantitative mode. The data were

260 proceeded using TopSpin software and crystallinity index was measured by integration on C4.  
261 The degree of substitution (DS) of esterified cellulose is obtained by the integration of the  
262 new carbonyl group appeared with respect to the C1. The integration is done thanks to the  
263 TOPSPIN software.

264 The DS is calculated according to the following formula:

$$DS = \frac{\text{Carbonyl group integral}}{C1 \text{ integral}}$$

## 265 **2.7 Chemical composition**

266 Chemical composition was analysed by ion chromatography (HPLC-dionex) according to the  
267 standard TAPPI T-249 cm-00. Fibres were first hydrolysed using sulfuric acid (72%) at 30°C  
268 for 1h. Then, an amount of water was added to the tube and heated at 120°C for 1h in  
269 autoclave. The mixture was then filtered and the filtrate was diluted and analysed by ion  
270 chromatography.

## 271 **2.8 Degree of polymerisation (DP)**

272 The DP was measured according to ISO 5351:2010 standard. Cellulose fibres were dissolved  
273 in a bis(ethylenediamine)copper(II) hydroxide solution. The intrinsic viscosity  $[\eta]$  was  
274 measured at 25°C with a capillary viscosimeter and the DP was deduced using the Mark–  
275 Houwink–Sakurada equation (Banvillet et al., 2021). Two measurements were made for each  
276 sample.

$$DP^{0.905} = 0.75 \times [\eta]$$

## 277 **2.9 X-ray Diffraction XrD**

278 X-ray Diffraction (XrD) was performed on paper samples deposited on a zero-background Si  
279 substrate. The measurements were carried out with a diffractometer X'Pert Pro MPD  
280 (PANalytical, Netherlands), equipped with a Bragg-Brentano geometry and a copper anode  
281 ( $K\alpha \lambda = 1.5419 \text{ \AA}$ ). The crystallinity index CrI (%) was calculated using Segal method:

$$CrI = \frac{I_{002} - I_{am}}{I_{002}} \times 100$$

282 where  $I_{002}$  represents the diffraction intensity of the main crystalline peak at  $2\theta \approx 22.5^\circ$  and  
283  $I_{am}$  is the intensity at  $2\theta \approx 18.7^\circ$ .

284           **2.10 Thermogravimetric analysis**

285 Thermogravimetric analyses (TGA) of cellulose fibres before and after DES or enzyme  
286 treatment were measured by a thermal analyser Mettler Toledo (TGA/DSC 3<sup>+</sup>) under air or  
287 nitrogen flow using a constant flow rate of 30 mL min<sup>-1</sup>. Approximately 6 mg of paper were  
288 heated from 25 to 700°C, at a rate of 10°C.min<sup>-1</sup>.

289           **2.11 Scanning electron microscopy (SEM)**

290 SEM is used to observe fibre surface aspect. This technique is based on the interaction  
291 between material and electrons. Dried fibre suspensions were coated with Au/Pd to make  
292 them conductive. Samples were analysed with SEM Quanta250 FEG using high voltage of  
293 2.5KV.

294 Cellulose fibres made of untreated, DES (after 4h of treatment) and enzymatically treated  
295 pulps were analysed.

296 SEM images were also performed on the papers produced from untreated EF, EF-CC-M and  
297 EF-enzyme with SEM Quanta 200 (FEI, USA) in ETD mode, with a working distance of 10  
298 mm and an acceleration voltage of 10.0 kV. Images were taken at an angle of 14°.

299           **2.12 Morphological analysis of the fibres**

300 Fibre morphology (arithmetic fibre length, fibre width, fine content...) was studied with a  
301 Morfi-Neo fibre analyser (techpap, France). 40 mg of dried pulp were diluted in 1L of  
302 deionised water. Tests were performed during 5 min with a limit fibre/fine elements fixed at  
303 80 µm. For each sample, analysis was triplicated and the mean value of the measured  
304 parameters was calculated.

305           **2.13 Water retention value (WRV)**

306 The WRV is used to determine the amount of water retained in a fibre when it is subjected to  
307 a certain centrifugal force. Centrifugation is used to remove the capillary water retained  
308 between the fibres of a wet pulp pad. A handsheet (basis weight of 60 g/m<sup>2</sup>) was made on an  
309 English former with a diameter of 159 mm and a water height of 350mm above the mesh.  
310 Pads of 0.2 g were centrifuged at 3000 g for 15 minutes.

311 WRV was calculated according to the following equation:

$$WRV = \frac{m_{wet} - m_{dry}}{m_{dry}} \times 100$$

312 where  $m_{wet}$  is the weight of the sample after centrifugation and  $m_{dry}$  is the sample weight  
313 after oven drying at 105°C for 24h.

#### 314 **2.14 Tensile tests**

315 Tensile tests were performed on the produced papers using an Instron 5965 machine (Instron,  
316 USA) in conditioned atmosphere (Temperature = 23°C and Relative humidity = 50%)  
317 according to ISO 1924-2 standard. Tests were done on samples with dimensions of 15×100  
318 mm<sup>2</sup> and a tensile speed of 10 mm/min. The Young's modulus of each sample was calculated  
319 using the thickness values of the paper. For each handsheet, 2 measurements were done. The  
320 average of 20 measurements was calculated.

#### 321 **2.15 Tear resistance**

322 Tear resistance of papers was measured using a tear tester equipped with a 4000 mN  
323 pendulum (Noviprofibre, France) according to ISO 1974 standard. Tests were done on  
324 samples with dimensions of 50x65 mm<sup>2</sup>. 2 measurements were made for each handsheet. The  
325 average of 20 measurements was calculated.

#### 326 **2.16 Internal bond strength**

327 The internal bond strength or delamination resistance of papers was determined using an  
328 Internal pendulum Bond Tester IBT-IDM. This device leads to determine the adhesion of  
329 internal layers in paper using a Scott-Bond method according to ISO 16260 standard. The  
330 sample was placed between two double-layered adhesive substrates under a pressure of 100  
331 psi for 30 s. The test sample was then subjected to a shear force by means of a pendulum. The  
332 internal bond strength is the energy required to delaminate the sample in the thickness. 10  
333 measurements were done for each sample.

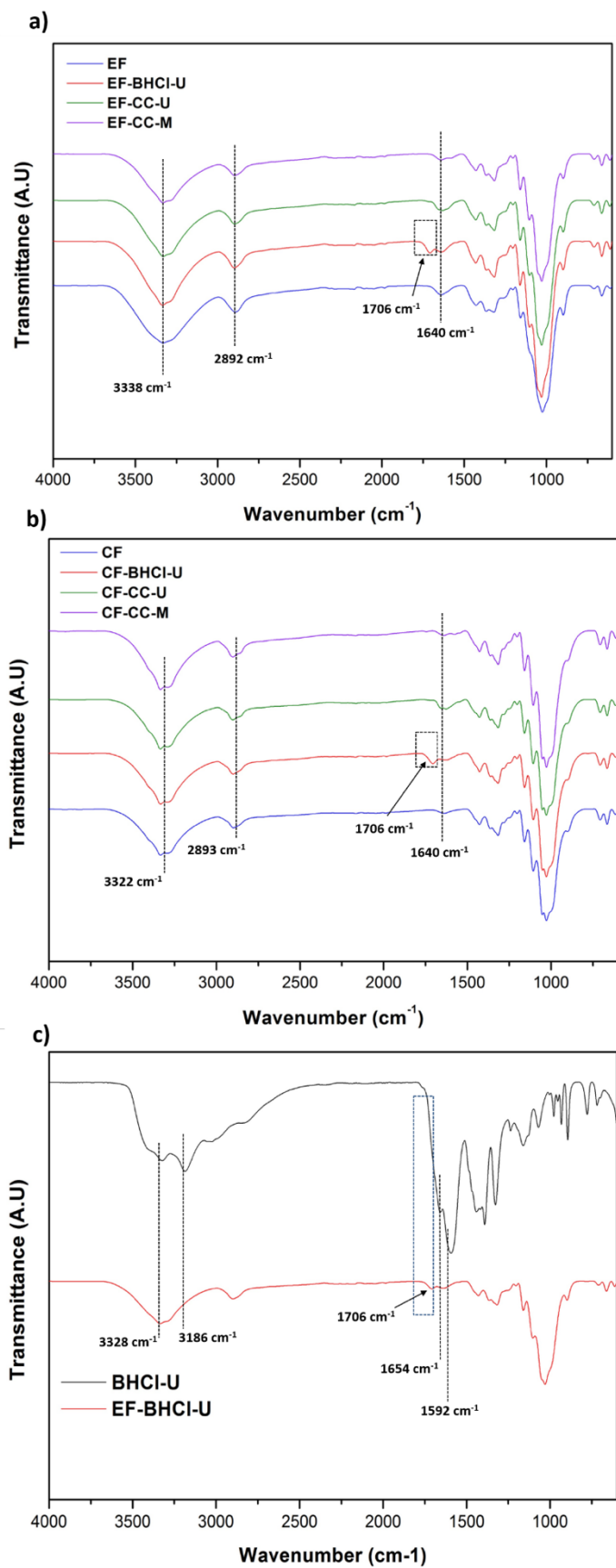
### 334 **3. Results and discussions**

#### 335 **3.1 Effect of DES treatments on the chemical structure of the fibres**

##### 336 **3.1.1 FT-IR ATR analysis**

337 Figure 1 presents the FT-IR ATR spectra of eucalyptus and cotton fibres before and after DES  
338 treatment (4h). On the spectra, many of the observed peaks are typical of cellulose (Chung,  
339 Lee, & Choe, 2004), such as a strong band around 3338 cm<sup>-1</sup> corresponding to O-H stretching  
340 and a peak at 2892 cm<sup>-1</sup> related to C-H stretching. The peak at 1640 cm<sup>-1</sup> is related to  
341 absorbed water. However, a new peak at 1706 cm<sup>-1</sup> was observed only with BHCl-U, for  
342 cotton and eucalyptus fibres, which is related to a carbonyl group (Figure 1a, red curve). The  
343 absence of this peak on the FT-IR spectrum of BHCl-U alone (see Figure 1c) leads to the

344 conclusion that this group is covalently linked to the cellulose through either an ester (-O-  
345 C(O)-) or a carbamate (O-C(O)-NH-) function. Indeed, as already mentioned, BHCl-U system  
346 was reported to produce cellulose carbamate with a low degree of substitution by reacting  
347 with urea (Willberg-Keyriläinen, Hiltunen, & Ropponen, 2018). On the other side, ester bond  
348 could be envisioned by acid-catalysed esterification between alcohol of cellulose and  
349 carboxylic acid of BHCl. In order to determine the chemical nature of this carbonyl group,  
350 solid-state  $^{13}\text{C}$  NMR have been performed.

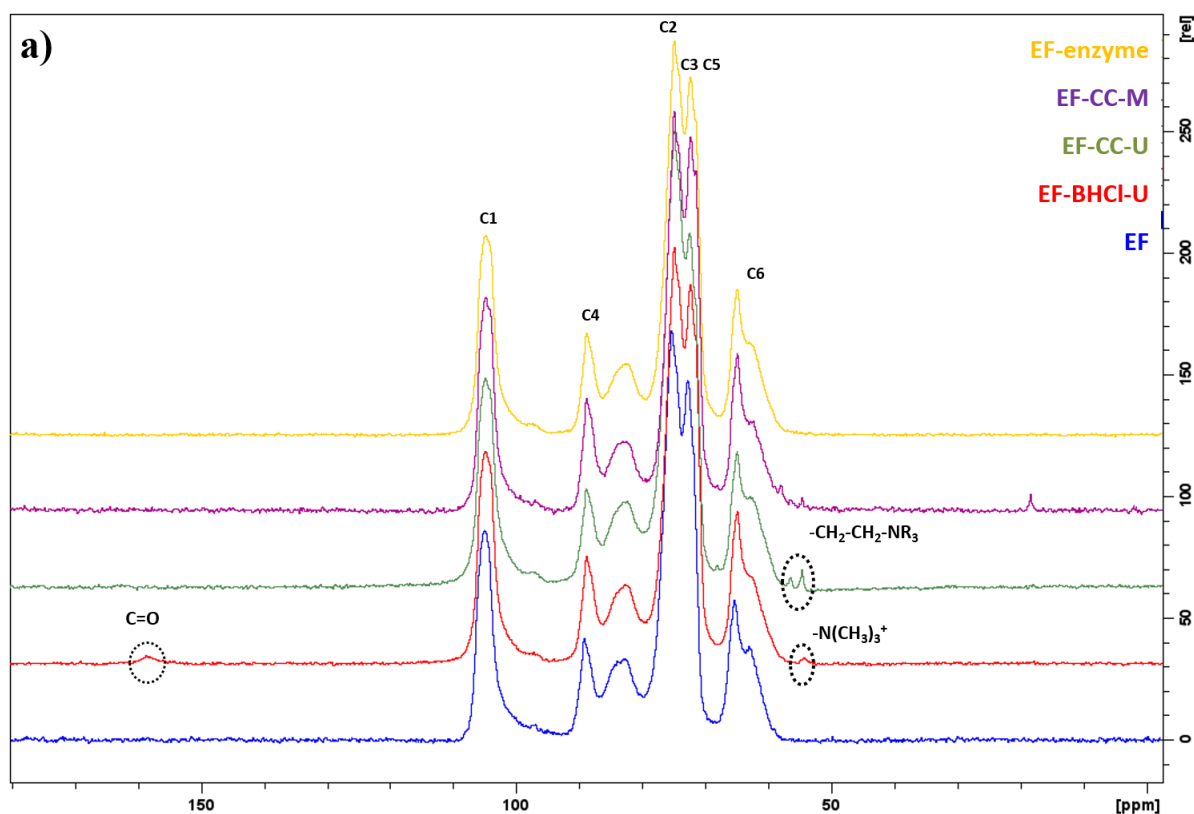


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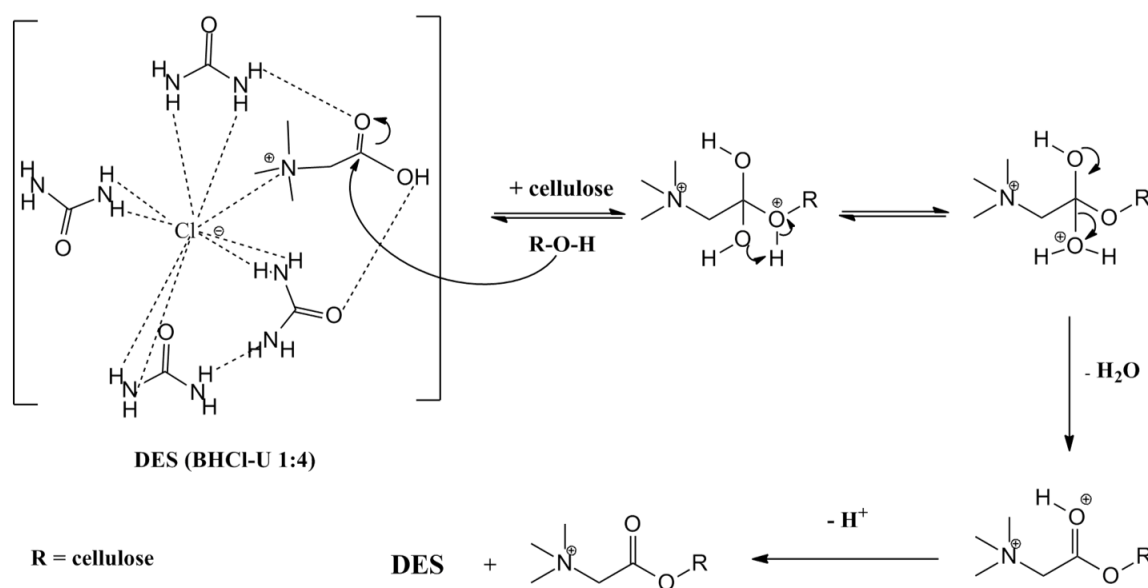
352 **Figure 1.** FT-IR ATR spectra of DES-treated eucalyptus(a), cotton fibres (b) and EF-BHCl-U  
 353 compared to BHCl-U (c).

354 **3.1.2 Solid-state <sup>13</sup>C NMR of DES/enzyme treated eucalyptus fibres**

355 The NMR spectra of DES or enzyme treated eucalyptus fibres are presented in Figure 2a and  
356 compared to that of the cellulose fibres before any treatment. Here again, all the carbon peaks  
357 related to cellulose structure reported in the literature (Johan Foster et al., 2018; Kono,  
358 Numata, Erata, & Takai, 2004) are observed. As expected, the chemical shifts are due to the  
359 contribution of C6 (62.8 ppm for amorphous cellulose, 65.3 for crystalline cellulose), C5  
360 (72.8 ppm), C4 (82.9 ppm for amorphous cellulose and 89 ppm for crystalline cellulose), C3  
361 (75.2 ppm), C2 (72.4 ppm) and C1 (104.9 ppm).



### b) Cellulose esterification mechanism



362

363 **Figure 2.** Solid-state  $^{13}\text{C}$  NMR spectra of DES or enzymatic treated eucalyptus fibres(a) and

364

the proposed mechanism of cellulose esterification (b).

365 On the other hand, additional peaks are observed with acidic and neutral DES-treatment. The

366 spectrum of acidic DES-treated fibres presents two new small peaks. The first peak (54.4

367 ppm) is attributed to the methyl of quaternary ammonium ( $-\text{N}(\text{CH}_3)_3^+$ ), while the second one

368 (158.7 ppm) is related to a carbonyl group. The presence of these two peaks clearly evidenced



369 that an esterification occurred between cellulose and BHC with a relatively low degree of  
370 substitution (DS = 0.025).

$$DS = \frac{1}{39} = 0.025$$

371 At the opposite of the work of Willberg-Keyriläinen et al. (2018) that reported only  
372 carbamation of cellulose with BHCl-U system, in our case we clearly demonstrated that an  
373 esterification occurred. The different reactivity of BHCl-U could be due to the experimental  
374 conditions since DES treatment was carried out during 1 or 4h in this study while for  
375 Willberg-Keyriläinen et al. (2018) the reaction time was about 20h. In conclusion, shorter  
376 duration of treatment seems to favour esterification. The proposed mechanism of the cellulose  
377 esterification is described in Figure 2b. Esterification mechanism can occur in three steps: (i)  
378 protonation of the carbonyl group of betaine hydrochloride; (ii) nucleophilic addition of the  
379 cellulose alcohol and (iii) deprotonation.

380 To the best of our knowledge, only one article reported cellulose esterification using BHCl-  
381 based DES but in the presence of *p*-toluenesulfonyl (tosyl) chloride as catalyst (Sirviö, 2018).

382 With neutral DES-treatment two small peaks also appeared around 54.7 and 56.4 ppm which  
383 can be assigned to the three methyl groups of the quaternary ammonium and the methylene  
384 group bounded to the alcohol of CC, respectively (Delso, Lafuente, Muñoz-Embid, & Artal,  
385 2019). However, we assumed that the peaks are rather CC contaminations, certainly by  
386 absorption, than an effective linkage onto cellulose because no reasonable coupling reaction  
387 could occur between cellulose and CC.

### 388 **3.2 Chemical composition of DES-treated eucalyptus fibres**

389 Bleached eucalyptus fibres (EF) are mainly composed of cellulose, a polymer of  $\beta$ -(1,4)-  
390 linked glucose, and xylan, a polymer of  $\beta$ -(1,4)-linked xylose, as major component of  
391 hardwood hemicelluloses (Spiridon & Popa, 2008). Indeed, the carbohydrate composition of  
392 EF studied in this work exclusively showed glucose and xylose, and no other sugar was  
393 detected (see table in additional data). The chemical composition of DES-treated EF reveals  
394 that hemicellulose content decreases for both acidic and neutral DES, from 22 to 17 and 18%,  
395 respectively. However, alkaline CC-M system does not affect hemicellulose content. These  
396 results are in line with other studies showing that a selective dissolution or reactivity can be  
397 achieved, depending on the pH and components of DES (Mäki-Arvela, Salmi, Holmbom,  
398 Willför, & Murzin, 2011; Suopajarvi, Ricci, Karvonen, Ottolina, & Liimatainen, 2020).

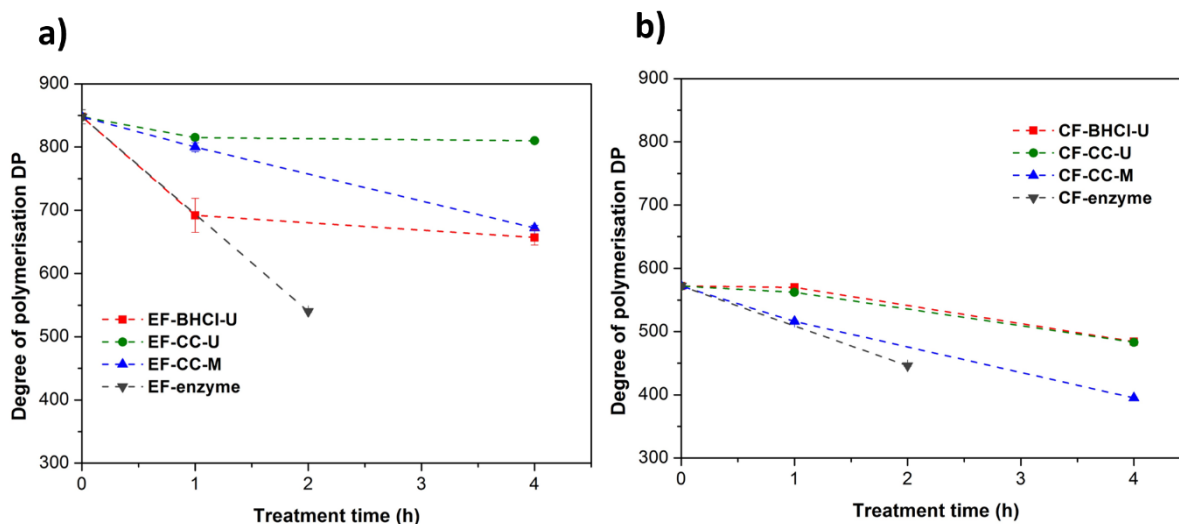
399 Generally speaking, the higher reactivity of hemicelluloses is due to their amorphous structure  
400 which facilitates their dissolution and also because of their chain lengths which are shorter  
401 than cellulose (Xiao, Sun, & Sun, 2001).

402 The chemical composition of cellulosic fibres after enzymatic hydrolysis were widely studied  
403 in the literature ((Mussatto, Fernandes, Milagres, & Roberto, 2008; Ruiz, Cara, Manzanares,  
404 Ballesteros, & Castro, 2008).

### 405 3.3 Degree of polymerisation (DP)

406 The DP of DES or enzymatic-treated cellulose fibres was determined from intrinsic viscosity  
407 in cupri-ethylene-diamine. The evolution of the DP with the treatment time for both  
408 eucalyptus and cotton fibres is presented in Figure 3. For eucalyptus fibres, the DP decreased  
409 significantly with acidic DES from 848 to 692 and 657 after 1 and 4h, respectively. This  
410 decrease is slightly less pronounced with alkaline DES treatment. However, DP is preserved  
411 after 1 and 4h of CC-U treatment, with final values of 815 and 810 respectively. DP of cotton  
412 fibres also decreased after DES treatment but not with the same trend. This behaviour is  
413 suggested to be related to the initial DP of the fibres which is low for cotton fibres. Moreover,  
414 it could be probably related to the fibre preparation which is different for the selected  
415 biomasses.

416 Indeed, depolymerisation occurs in all cases, with similar DP values for acidic and neutral  
417 DES, effect being more marked in alkaline conditions. Enzymatic hydrolysis leads to an  
418 expected decrease of the DP which is explained by enzyme's action on cellulose chains. Thus,  
419 the decrease of the DP is resulting from the reduce of the chain length caused by enzymatic  
420 hydrolysis from 848 to 540 and from 572 to 446 for eucalyptus and cotton fibres respectively.



421

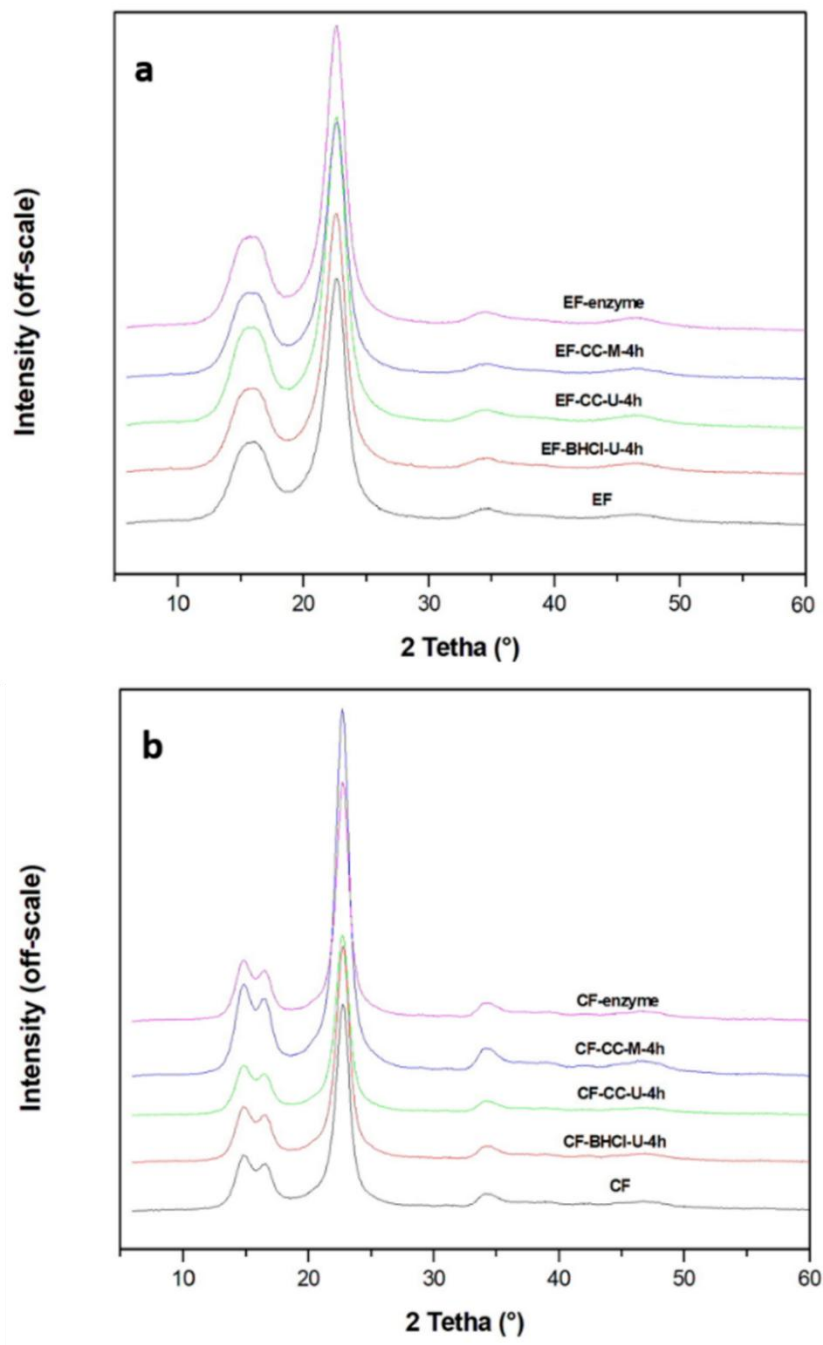
422 **Figure 3.** DP evolution of DES-treated eucalyptus (a) and cotton fibres (b).

423 It can be deduced that for eucalyptus fibres, acidic and alkaline DES are able to reduce  
424 cellulose chain, while neutral CC-U system allows a small reduction of DP especially with  
425 eucalyptus fibres which is in line with previous studies (Suopajarvi, Sirvio, & Liimatainen,  
426 2017; Tenhunen et al., 2018).

427 The decrease of the DP in acidic DES is discussed in the literature. This phenomenon results  
428 from glycosidic bond cleavage catalysed by acidic solvents and many acidic DES (formic  
429 acid, acetic acid, lactic acid-choline chloride) are known to lower the DP during  
430 delignification of lignocellulosic biomass (from 839 to 397, 367 and 323, respectively) (Tian  
431 et al., 2020). Thus, (Sirvio & Heiskanen, 2017) used acidic DES (dimethyl urea-ZnCl<sub>2</sub>), as a  
432 reactive solvent, to produce cellulose methyl carbamate and observed a significant decrease of  
433 DP from 1820 to 686 after 1.5h of treatment at 150°C.

### 434 **3.4 X-ray Diffraction and Crystallinity Index CrI**

435 X-ray diffraction patterns (Figure 4) of cellulose fibres before and after DES or enzymatic  
436 treatment are typical of cellulose I<sub>β</sub> crystalline structure. The diffraction peaks at 2θ angles of  
437 15.2, 16.5, 22.5 and 34.5° are related to the (1 $\bar{1}$ 0), (110), (200) and (004) lattice planes  
438 respectively (French, 2014; Park, Baker, Himmel, Parilla, & Johnson, 2010). The  
439 diffractograms show that cellulose crystalline structure remained the same as the original  
440 fibres (cellulose I<sub>β</sub>) for both eucalyptus and cotton fibres due to the absence of other  
441 crystalline allomorphs of cellulose. From these results, it can be deduced that there was no  
442 regeneration even with alkaline DES. These results are in line with other studies in the  
443 literature (Hong et al., 2020; Suopajarvi, Sirvio, Liimatainen, 2017). As expected, cotton  
444 fibres present a more crystalline structure than eucalyptus fibres: for the untreated fibres, CrI  
445 values of 87.0 and 80.2% were obtained from CF and EF respectively. In addition, the peaks  
446 at 15.2 and 16.5° of the diffractogram are more separated for cotton fibres which indicates  
447 that this structure is more crystalline. Indeed, the fact that this separation is not clearly  
448 observed with untreated eucalyptus fibres is due to the presence of more amorphous regions.



449

450 **Figure 4.** XrD diffractograms of treated-eucalyptus fibres (a) and cotton fibres (b)

451 The crystallinity index of both fibres calculated from XrD analysis (Segal method) showed  
 452 that the cellulose crystallinity is almost unchanged with DES-treatment, while a slight  
 453 increase with enzymatic hydrolysis was observed. In this case, the selective hydrolysis of  
 454 amorphous regions may explain this behaviour.

455 Moreover, the crystallinity index of DES or enzymatic treated eucalyptus fibres was also  
 456 analysed by  $^{13}\text{C}$  NMR solid-state using the integration on C4. The Table 1 gives the values

457 obtained from XrD and  $^{13}\text{C}$  NMR. CrI from  $^{13}\text{C}$  NMR are lower than those obtained from  
 458 XrD. This difference is explained by the better sensitivity of  $^{13}\text{C}$  NMR and the fact that Segal  
 459 method gives always higher values. Whereas CrI from XrD measurements are more or less  
 460 constant,  $^{13}\text{C}$  NMR allows observing a significant increase of CrI with alkaline DES, followed  
 461 by acidic DES and enzymatic hydrolysis. Neutral DES only exhibits a slight change.  
 462 Consequently, amorphous parts are probably removed from cellulose fibres by alkaline DES.  
 463 It is known that alkaline treatment allows increasing the crystallinity as observed with other  
 464 treatments such as ammonia treatment at higher temperature (Mittal, Katahira, Himmel, &  
 465 Johnson, 2011) or combination of NaOH (10 wt%) with enzymatic treatment (Banvillet,  
 466 Depres, Belgacem, & Bras, 2021). However, with these alkaline treatments, the initial  
 467 allomorph of cellulose is changed which is not the case with DES-treatment. For acidic DES,  
 468 its effect on crystallinity is comparable to enzymatic hydrolysis which could be probably due  
 469 to the acidic pH of both treatments. For concluding, DES can be considered as mild treatment  
 470 media for cellulose because it maintains the same cellulose allomorph during treatment.

471 **Table 1.** Crystallinity Index CrI of DES and enzymatic treated eucalyptus fibres and cotton  
 472 fibres

<b>Eucalyptus fibres</b>	<b>EF</b>	<b>BHCl-U</b>	<b>CC-U</b>	<b>CC-M</b>	<b>Enzyme</b>
<b>CrI-XrD (%)</b>	80.2	80.4	81.4	80.5	82.7
<b>CrI-<math>^{13}\text{C}</math> NMR (%)</b>	38.2	41.0	37.8	45.4	41.6
<b>Cotton fibres</b>	<b>CF</b>	<b>BHCl-U</b>	<b>CC-U</b>	<b>CC-M</b>	<b>Enzyme</b>
<b>CrI-XrD (%)</b>	87.0	87.7	86.6	88.7	88.9

473

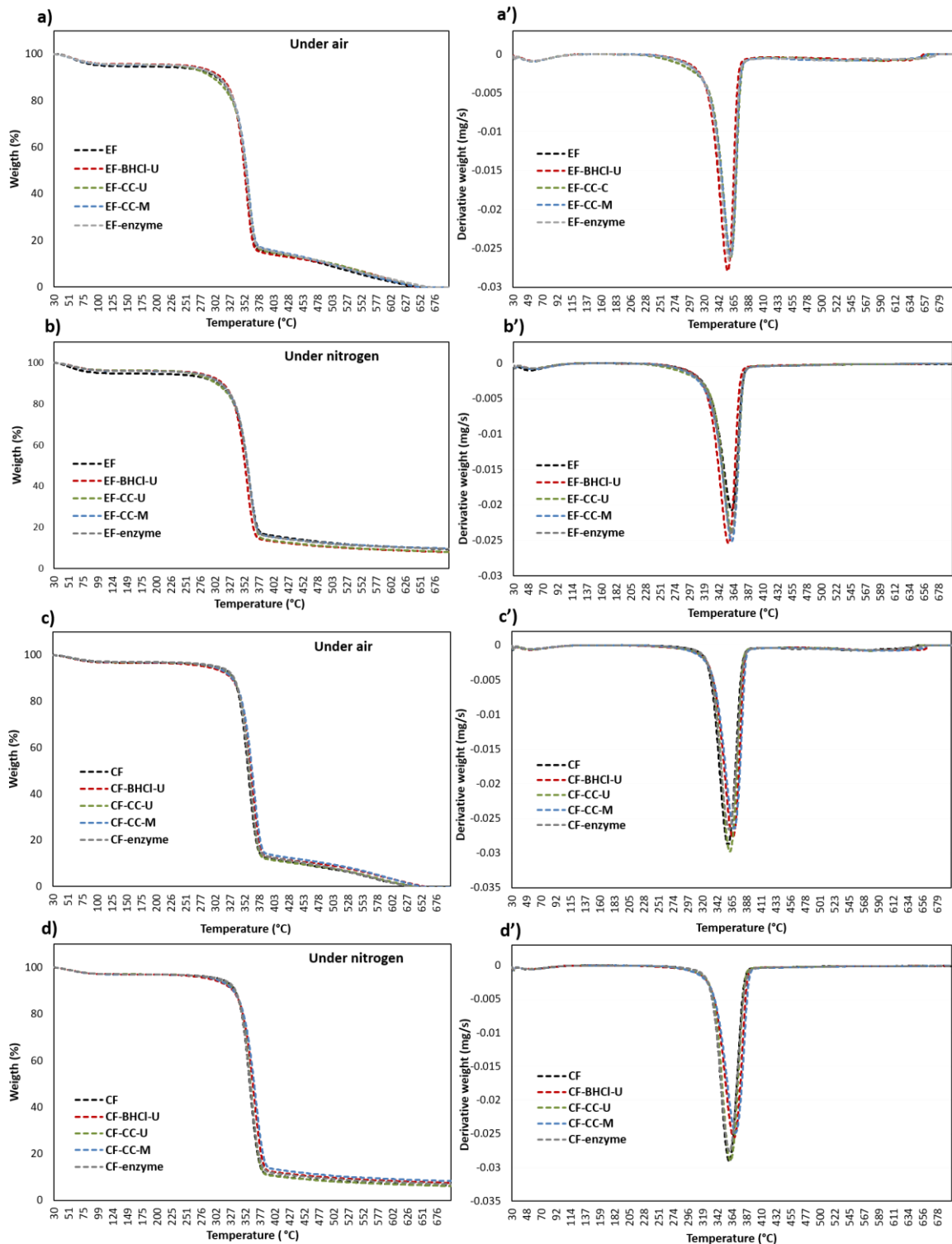
### 474 3.5 Thermogravimetric analysis

475 The thermogravimetric analysis TGA/DTG of eucalyptus and cotton fibres (Figure 5) before  
 476 and after treatment were analysed. All the curves showed the presence of two thermal  
 477 degradation patterns translated into weight loss (%). The first stage (35-100°C) is the water  
 478 evaporation. Stage 2 is related to hemicelluloses in the case of EF (200-300°C) and cellulose  
 479 decomposition (270-400°C).

480 Untreated EF were characterized by cellulose degradation onset temperature at 358°C and  
 481 362°C with air and nitrogen respectively. Cellulose decomposition onset temperature of  
 482 treated EF was not modified in the significant way. For cotton fibres, cellulose decomposition  
 483 onset temperature was 360°C with both gases. Here again, thermal stability was maintained

484 after the treatment, except for alkaline DES. In this case, decomposition onset temperature  
485 increased from 360 to 369°C with both gases.

486 These results demonstrate that DES treatment did not negatively impact the thermal stability  
487 of cellulose.



489 **Figure 5.** *TGA and DTG curves of DES/enzyme treated eucalyptus (a, a' and b, b') and*  
490 *cotton fibres (c, c' and d, d') under air and nitrogen respectively.*

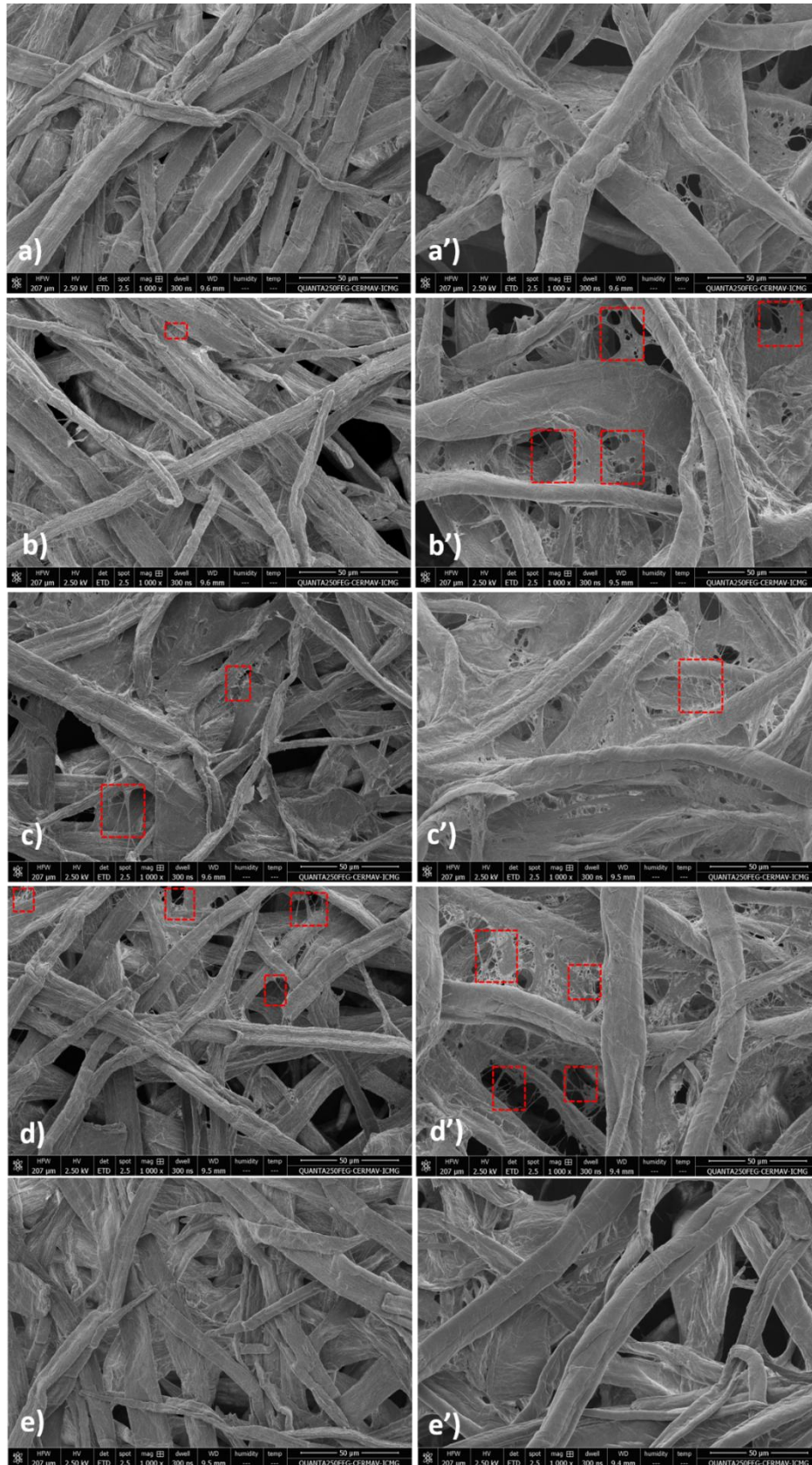
### 491 **3.6 Scanning Electron Microscopy (SEM)**

492 SEM images of eucalyptus and cotton fibres are presented in Figure 6. From these images, it  
493 clearly appears that the fibres have undergone changes during the treatment with neutral and  
494 alkaline DES. For neutral and alkaline treatments, the most remarkable observation is the  
495 appearance of macrofibrils partially detached of the fibres which create a network between the  
496 fibres. The effect of the DES treatment seems to be the same for the two fibres, although it is  
497 more pronounced for cotton fibres. Compared to neutral and alkaline DES, acidic solvent has  
498 much less effect on the production of macrofibrils. Oppositely, no visible macrofibrils were  
499 detected after enzymatic treatment and the fibres seem to be less bound to each other. The  
500 observation of a network of macrofibrils after DES per-treatment is similar to the refining  
501 effect as described in the literature (Banvillet, Depres, Belgacem, & Bras, 2021; Kang, 2007).

502

503



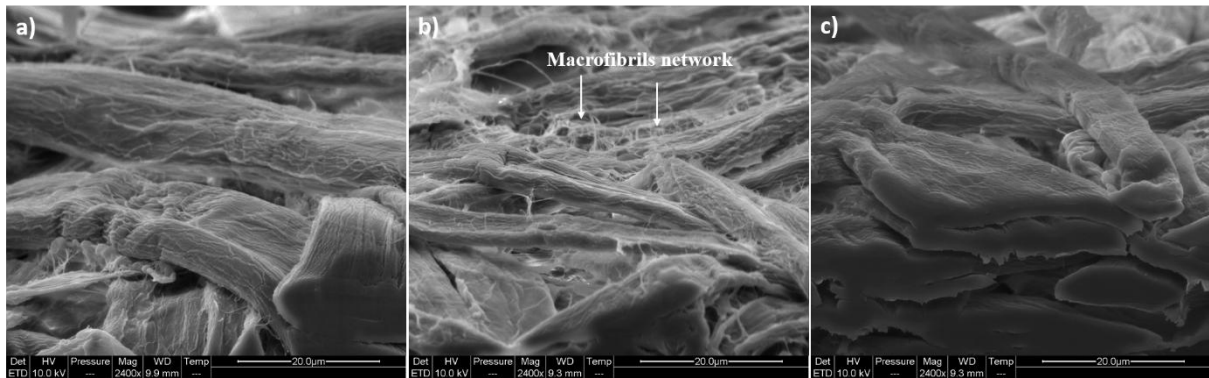


504

505 **Figure 6.** SEM images of eucalyptus and cotton fibres before (a, a') and after acidic DES (b,  
 506 b'), neutral DES (c, c') alkaline DES (d, d') and enzymatic hydrolysis (e, e') respectively.



507 The morphological properties of the produced papers from eucalyptus fibres, before and after  
 508 alkaline DES pre-treatment or enzymatic hydrolysis are presented in Figure 7. It is observed  
 509 that there is a network of macrofibrils created after DES pre-treatment indicating an external  
 510 fibrillation of the fibres (Kang, 2007). This fibrillation is not observed with enzymatic  
 511 hydrolysis. These observations confirm that effects of DES pre-treatment are similar to the  
 512 refining process. It can be concluded that DES pre-treatment can be considered as a green and  
 513 soft chemical refining of the cellulosic fibres.



514

515 **Figure 7.** SEM images of papers produced from untreated EF (a), EF-CC-M (b) and EF-  
 516 enzyme (c).

### 517 3.7 Morphological properties of fibres

518 Morphological properties were studied by analysing fibre length and width as well as fine  
 519 content. According to the results reported in Table 2, there was no significant change in length  
 520 and width of cellulose fibres (EF and CF) even after 4h of DES treatment. However, fine  
 521 content slightly increased for eucalyptus fibres from 13.1 to 18.5, 16.4 and 15.3% after 4h of  
 522 treatment in acidic, neutral and alkaline DES, respectively. For cotton fibres, it remained more  
 523 or less constant whatever the treatment which can be attributable to the initial high content of  
 524 fine elements in this pulp. Contrary to DES pre-treatment, enzymatic pre-treatment leads to a  
 525 slight decrease in fibre length. In this case, the fine content increased much more significantly  
 526 from 13.1 to 30.6% and from 35.8 to 56% for EF and CF, respectively. This increase was  
 527 expected and it is related to the hydrolysis of cellulose chain by enzymes that leads to fine  
 528 element liberation which is in accordance with literature (Banvillet, Depres, Belgacem, &  
 529 Bras, 2021).

530 **Table 2.** Morphological properties of DES/enzyme treated eucalyptus and cotton fibres

	EF	BHCl-U	CC-U	CC-M	Enzyme
<b>Mean arithmetic length</b>	523±4	511±1	508±1	512±3	446±3

	$\mu\text{m}$				
<b>Mean width <math>\mu\text{m}</math></b>	16.4 $\pm$ 0.1	16.3 $\pm$ 0	16.7 $\pm$ 0.1	17.3 $\pm$ 0.1	16.2 $\pm$ 0.1
<b>Fine content (% in length)</b>	13.1 $\pm$ 1.1	18.5 $\pm$ 0.2	16.4 $\pm$ 0.2	15.3 $\pm$ 0.1	30.6 $\pm$ 0.7
	CF	BHCl-U	CC-U	CC-M	Enzyme
<b>Mean arithmetic length <math>\mu\text{m}</math></b>	453 $\pm$ 3	480 $\pm$ 4	434 $\pm$ 3	481 $\pm$ 3	415 $\pm$ 1
<b>Mean width <math>\mu\text{m}</math></b>	19.3 $\pm$ 0.2	19.3 $\pm$ 0.1	19.4 $\pm$ 0.1	19.8 $\pm$ 0.1	18 $\pm$ 0
<b>Fine content (% in length)</b>	35.0 $\pm$ 1.1	35.8 $\pm$ 0.3	40.3 $\pm$ 0.6	35.8 $\pm$ 0.8	56 $\pm$ 0.2

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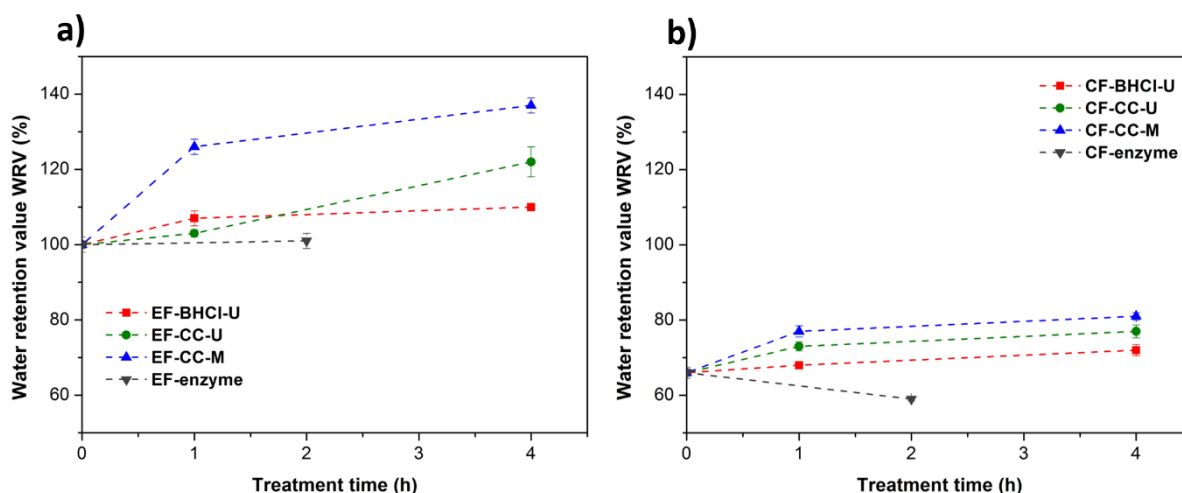
532

### 3.8 Water Retention Value WRV of DES-treated fibres

533

WRV of DES treated eucalyptus and cotton fibres are presented in Figure 8. These results show that WRV increased with DES treatment time. As expected, water swelling of eucalyptus fibres was more important than that of cotton fibres and the impact of DES treatment was also more pronounced. WRV increased after DES treatment from 100 to 110, 128 and 137% respectively with BHCl-U, CC-U and CC-M, for eucalyptus fibres, which corresponds to a maximum relative increase of 37%. In the case of cotton fibres, WRV varied from 66 to 81% with CC-M, the relative increase being of 23%. WRV, which is related to the swelling ability of fibres, depends on the accessibility of the cell wall to water. This accessibility is promoted by internal fibrillation, which usually results from mechanical refining of the pulp suspension. DES treatment, and more precisely neutral and alkaline DES, seem to have the same effect than a mechanical treatment (refining): internal fibrillation occurs (revealed by WRV) as well as external fibrillation (revealed by the presence of microfibrils) as observed by SEM. In contrast to what happens during a conventional refining process (Gharehkhani et al., 2015), the generation of fine elements is limited and the shortening of the fibres does not occur.

547



548

549 **Figure 8.** WRV evolution of cellulose fibres EF (image a) and CF (image b) according to  
 550 DES/enzyme treatment time.

551 After enzymatic pre-treatment, WRV remains unchanged for eucalyptus fibres and decreases  
 552 for cotton fibres. This decrease could be related to cellulose degradation by enzyme that  
 553 attack amorphous regions of cellulose, that have the greatest affinity with water.

554 **3.9 Mechanical properties of produced papers from DES treated eucalyptus**  
 555 **fibres**

556 The mechanical properties of papers produced from untreated eucalyptus fibres (EF),  
 557 enzymatic treated fibres (EF-enzyme) and alkaline-DES treated fibres (EF-CC-M) were  
 558 analysed. The evolution of the Young's modulus, density, internal bond strength, tensile  
 559 strength index, tear resistance index and elongation at break is depicted in Figure 9.

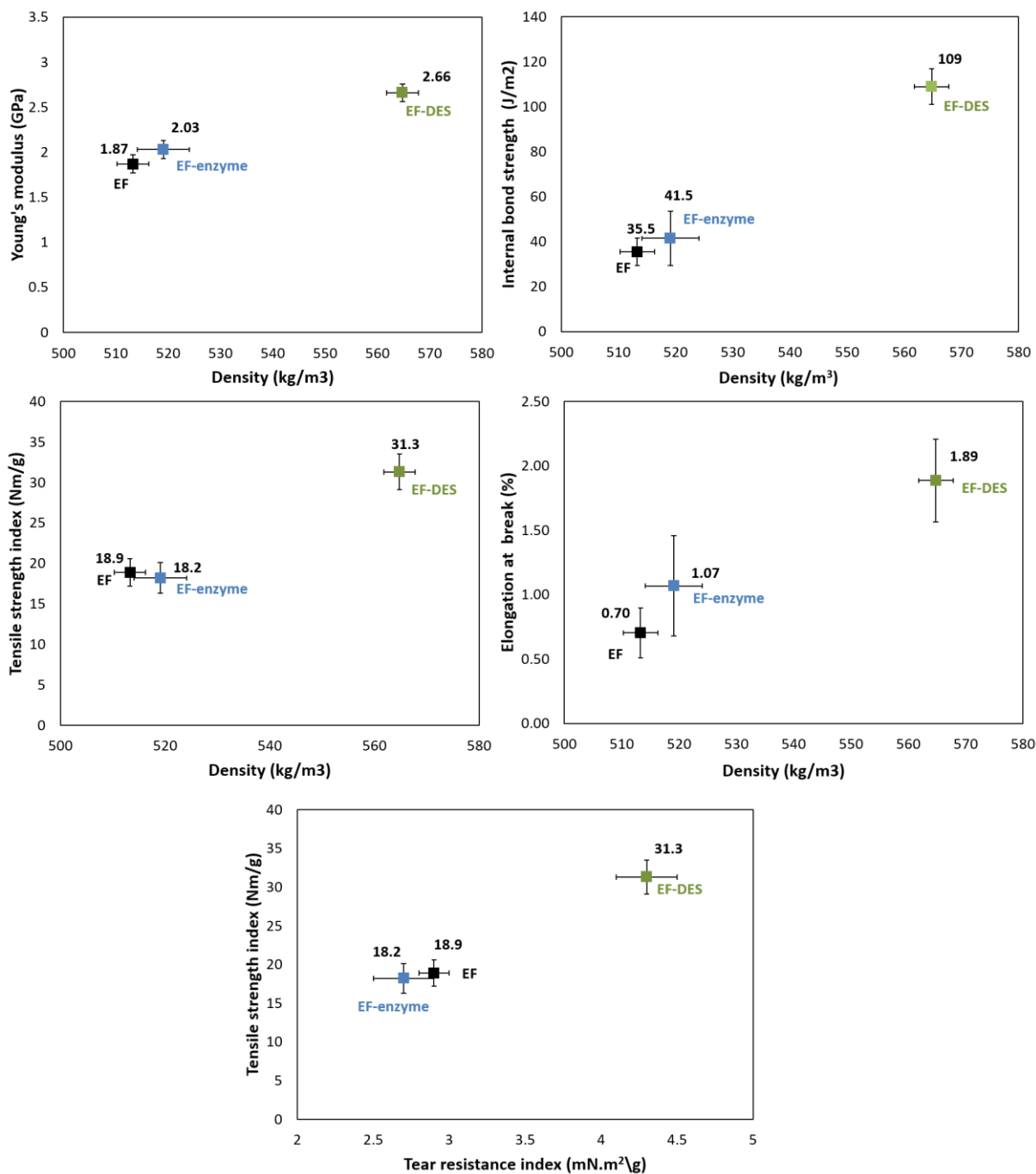
560 A first observation is the significant increase of the density after DES treatment: it rises from  
 561 514 to 565 kg/m<sup>3</sup>. Oppositely, after enzymatic treatment, the change is neglectable. This  
 562 increase, for papers made of DES-treated fibres, reflects a densification of the fibrous  
 563 network. This is in agreement with the results already discussed. The increase in external  
 564 fibrillation plays a role in this densification as well as the increase in fibre hydration, which  
 565 leads to a better flexibility and conformability of the fibres. All these phenomena favour a  
 566 larger contact surface area in the fibre network, more hydrogen bonds and a densification of  
 567 the structure.

568 As expected, the evolution of the microstructure of the paper leads to an improvement in its  
 569 mechanical properties. For DES-treatment, Young's modulus increases from 1.87 to 2.66 GPa.  
 570 In the same way, tensile strength index and internal bond strength increased by 66% and 207

571 %, respectively. Finally, it is worth noting that, as the length of the fibres is preserved, the tear  
572 strength was significantly improved after DES-treatment, varying from 2.9 to 4.3 mN.m<sup>2</sup>/g.  
573 Oppositely, the properties of the paper made from enzymatically treated fibres remain more or  
574 less constant.

575 To conclude, all these modifications, which are in accordance with the previously discussed  
576 results, show a very significant effect of DES on the properties of the papers produced. This  
577 effect could be similar to a "chemical" refining of the fibre suspensions, which is able to  
578 increase the bonding degree in papers in a great extent and to preserve certain properties such  
579 as tear strength.

580



581

582 **Figure 9.** Evolution of the mechanical properties of produced papers from eucalyptus fibres  
 583 before and after DES (CC-M) or enzymatic treatment.

584 **4. Conclusion**

585 In this work, three DES systems were used for the treatment of cellulose (eucalyptus and  
 586 cotton) fibres. The structural characterisation of DES-treated cellulose using solid-state <sup>13</sup>C  
 587 NMR reveals cellulose esterification with BHCl using acidic DES, while no modification was  
 588 observed with neutral or alkaline DES-treatment. The chemical composition of eucalyptus  
 589 fibres was modified by DES treatment only with acidic and neutral DES. They promote the

590 partial removal of xylan. Therefore, these findings highlight the reactive selectivity of these  
591 systems which depends on the DES components. DP and crystallinity index were influenced  
592 by the DES whereas thermal properties were not modified.

593 This work demonstrated that DES treatment causes both internal fibrillation (increase of the  
594 WRV) and external fibrillation (macrofibrils appearance). As a consequence, mechanical  
595 properties of the produced papers are enhanced in a very significant way by increasing the  
596 bonding degree and preserving fibre length. The obtained results are very interesting as the  
597 treatments proposed in this study may be similar to a chemical refining of fibre suspensions,  
598 in the case of neutral and alkaline DES. The perspectives to this work are to study the effect  
599 of these treatments for the production of microfibrillated cellulose and how they could  
600 facilitate the microfibrillation process. Moreover, studying the recycling of the DES could be  
601 interesting to evaluate the stability and efficiency of the used DES.

#### 602 **CRedit authorship contribution statement**

603 **Ahlem Mnasri:** Investigation, conceptualization, visualisation, writing - Original Draft.  
604 **Hatem Dhaouadi:** supervision. **Ramzi Khiari:** supervision. **Sami Halila:** Supervision,  
605 conceptualization, validation, Writing-Reviewing & Editing, Funding acquisition. **Evelyne**  
606 **Mauret:** supervision, conceptualization, validation, Writing-Reviewing & Editing, Funding  
607 acquisition.

#### 608 **Acknowledgements**

609 This work was financially supported by the “PHC Utique” program of the French Ministry of  
610 Foreign Affairs and Ministry of higher education, research and innovation and the Tunisian  
611 Ministry of higher education and scientific research in the CMCU project number TN  
612 18G1132// FR 39316VF. LGP2 is part of the LabEx Tec 21 (Investissements d’Avenir - grant  
613 agreement n°ANR-11-LABX-0030) and of PolyNat Carnot Institute (Investissements  
614 d’Avenir - grant agreement n° ANR-16-CARN-0025-01). This research was made possible  
615 thanks to the facilities of the TekLiCell platform funded by the Région Rhône-Alpes (ERDF:  
616 European regional development fund).

617 The authors would like to thank Isabelle Jeacomine and Christine Lancelon-Pin from  
618 CERMAV Grenoble, France for solid-state <sup>13</sup>C NMR analysis and SEM observations  
619 respectively. We would like also to thank Thierry Encinas from CMTC – Grenoble, France  
620 for the XRD analysis.

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