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

Green “chemical” refining

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Abstract

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

~~Three Deep Eutectic Solvents “DES” of different pH (acid, neutral and alkaline) were used to study their effects on cellulosic fibre structure (cotton and eucalyptus pulp), namely, betaine hydrochloride urea (BHCl-U), choline chloride urea (CC-U) and choline chloride monoethanolamine (CC-M). Interestingly, during acidic DES treatment an esterification reaction occurred between BHCl and cellulose (DS=0.025). The hemicellulosic xylan of 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 ¹³C
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 (C₅H₁₂ClNO₂) was supplied from
197 VWR (Milliporesigma), urea ACS reagent (CH₄N₂O, 99.0-100.5%) from Merck-sigma
198 Aldrich, monoethanolamine (C₂H₇NO, >99%) from ROTH, choline chloride (C₅H₁₄ClNO,
199 >98%) from Alfa Aesar, bis(ethylenediamine)copper (II) hydroxide solution
200 (Cu(H₂NCH₂CH₂NH₂)₂(OH)₂ 1.0 M in H₂O, Sigma-Aldrich), sulfuric acid (H₂SO₄, 72%),
201 acetic acid (CH₃COOH), ethanol (CH₃OH, 96%) and sodium acetate (C₂H₃NaO₂) 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⁻².

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⁻¹ in the frequency from 600 to 4000 cm⁻¹.

250 **2.6 Solid-state ¹³C 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 ZrO₂ 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:

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

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⁺) under air or
287 nitrogen flow using a constant flow rate of 30 mL min⁻¹. Approximately 6 mg of paper were
288 heated from 25 to 700°C, at a rate of 10°C.min⁻¹.

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/m²) 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 mm² 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 mm². 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.

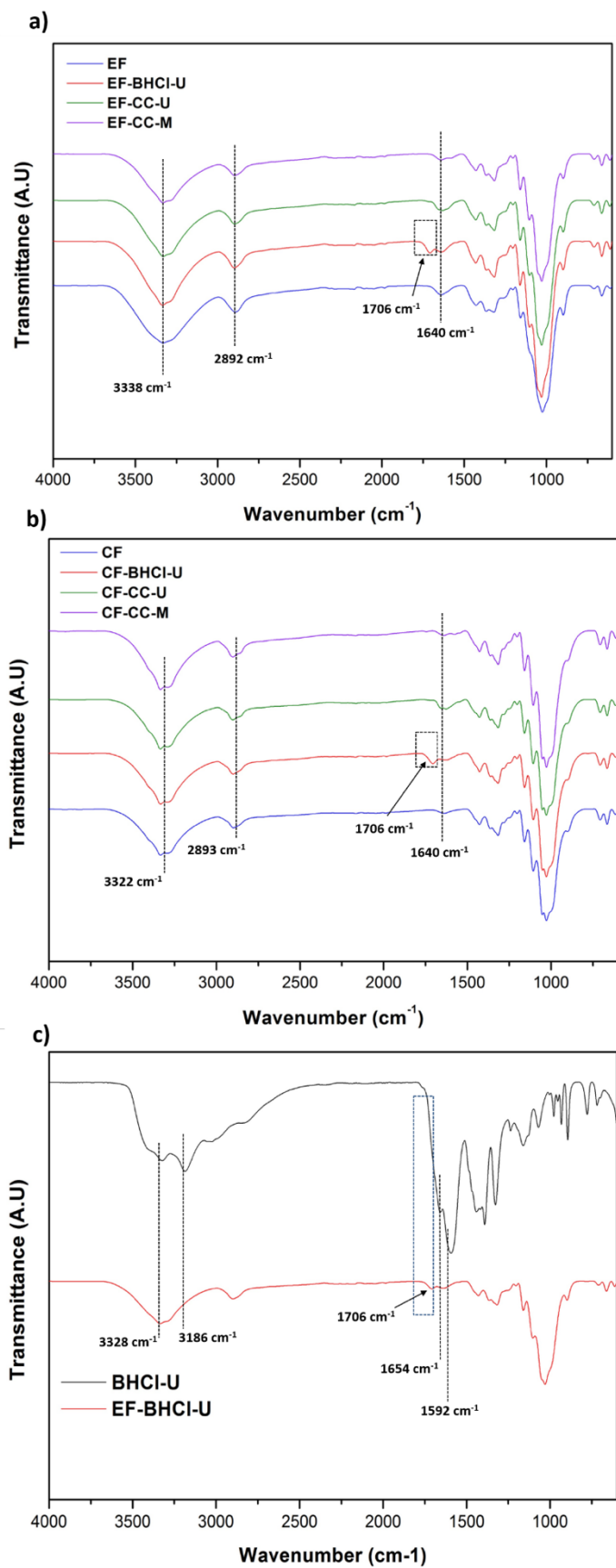
334 **3. Results and discussions**

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⁻¹ corresponding to O-H stretching
340 and a peak at 2892 cm⁻¹ related to C-H stretching. The peak at 1640 cm⁻¹ is related to
341 absorbed water. However, a new peak at 1706 cm⁻¹ 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}C NMR have been performed.

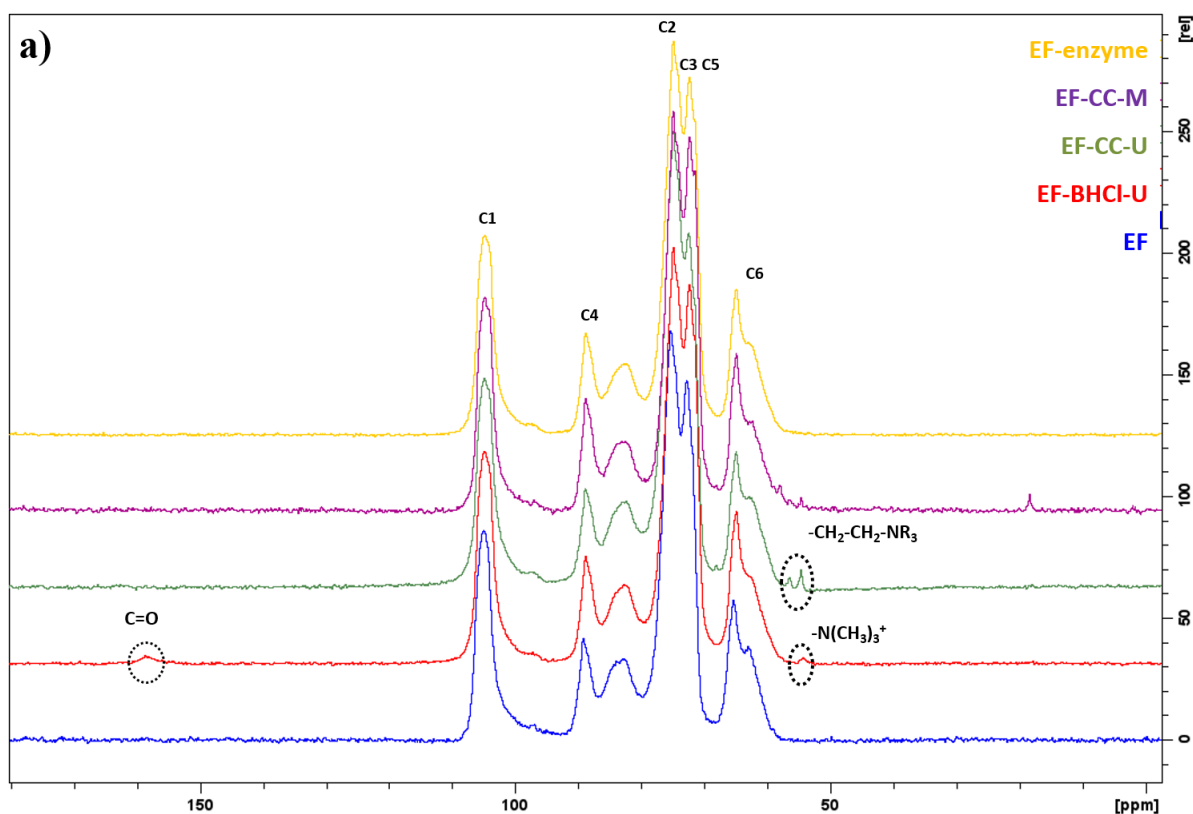


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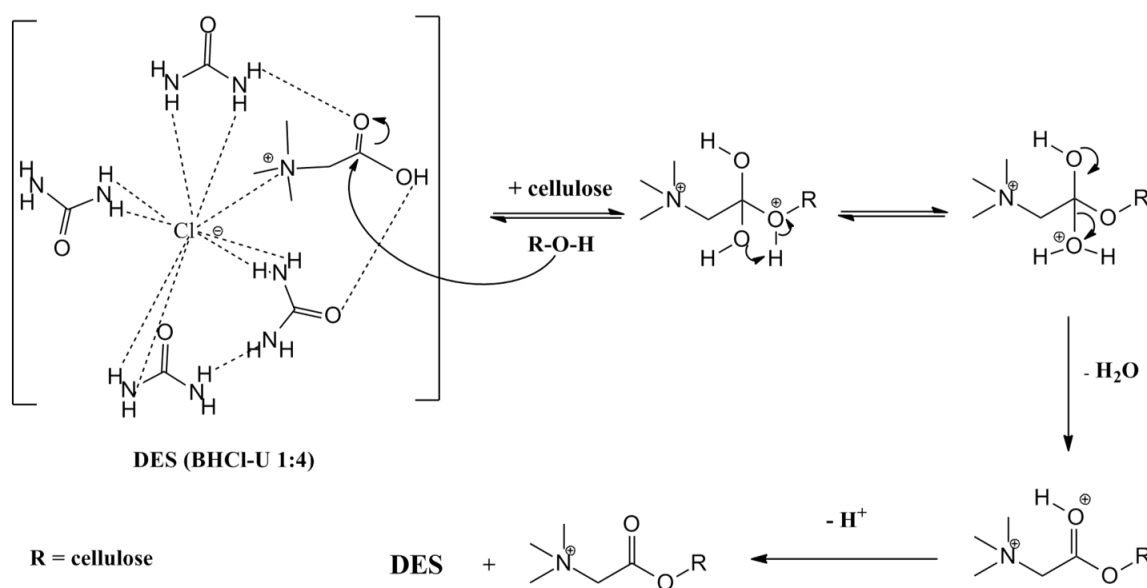
352 **Figure 1.** FT-IR ATR spectra of DES-treated eucalyptus(a), cotton fibres (b) and EF-BHCl-U
 353 compared to BHCl-U (c).

354 **3.1.2 Solid-state ¹³C 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}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 β -(1,4)-
390 linked glucose, and xylan, a polymer of β -(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).

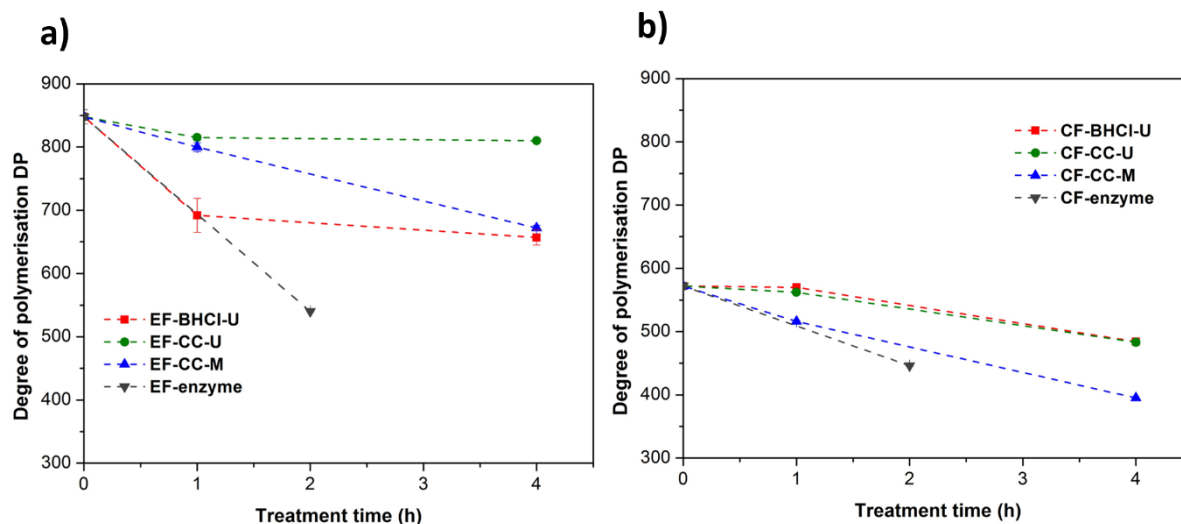
399 Generally speaking, the higher reactivity of hemicelluloses is due to their amorphous structure
400 which facilitates their dissolution and also because of their chain lengths which are shorter
401 than cellulose (Xiao, Sun, & Sun, 2001).

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

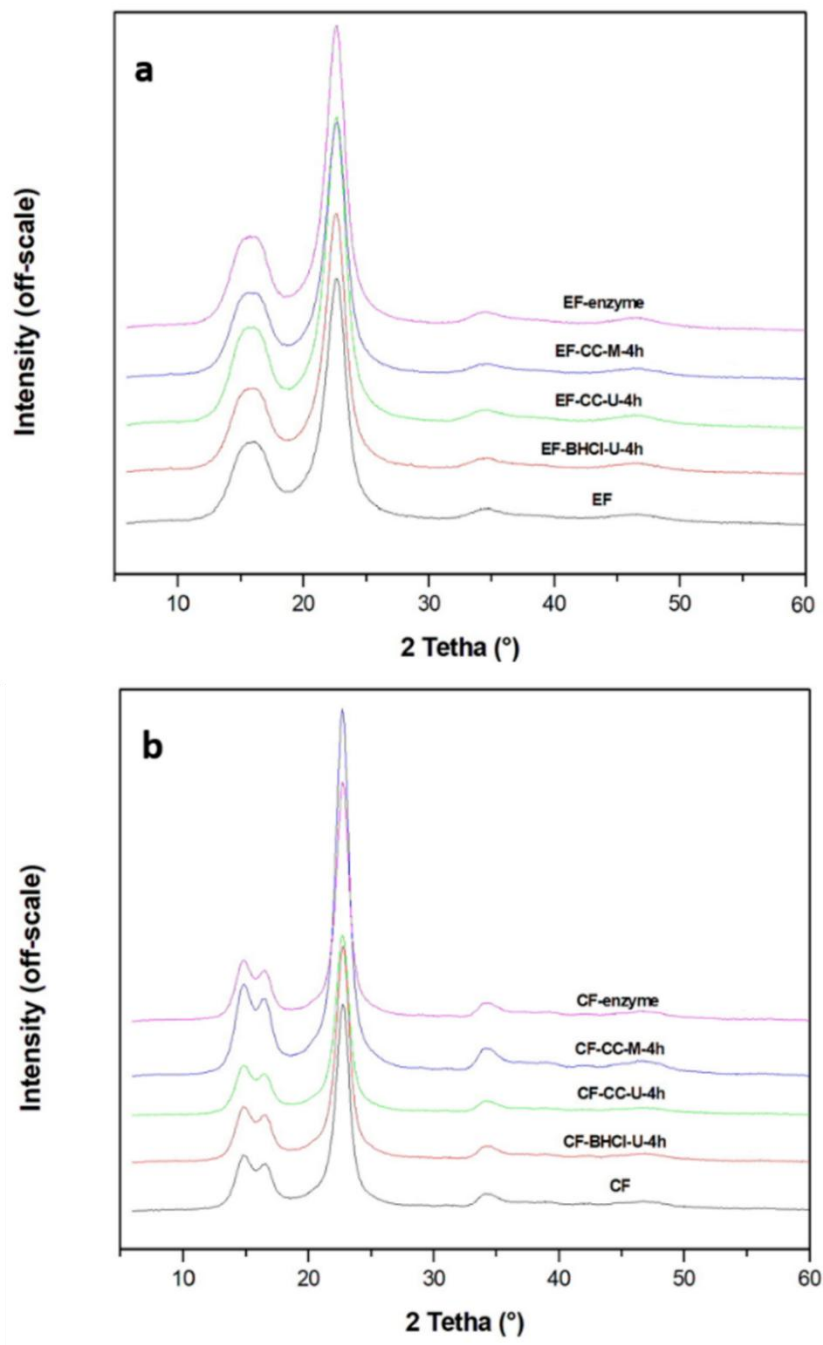
422 **Figure 3.** DP evolution of DES-treated eucalyptus (a) and cotton fibres (b).

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-ZnCl₂), 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_β 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_β) 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}C NMR solid-state using the integration on C4. The Table 1 gives the values

457 obtained from XrD and ^{13}C NMR. CrI from ^{13}C NMR are lower than those obtained from
 458 XrD. This difference is explained by the better sensitivity of ^{13}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}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

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

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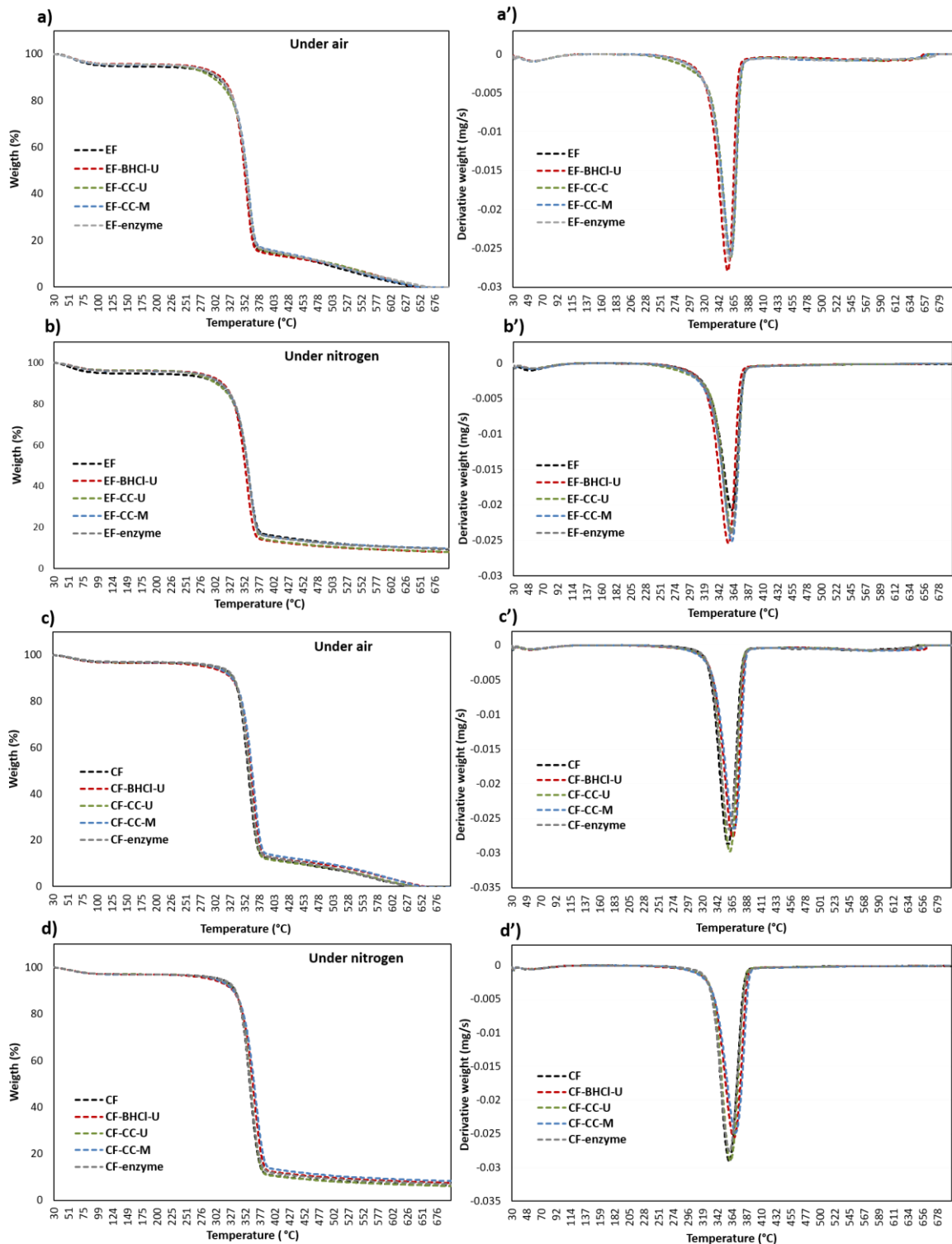
474 3.5 Thermogravimetric analysis

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.



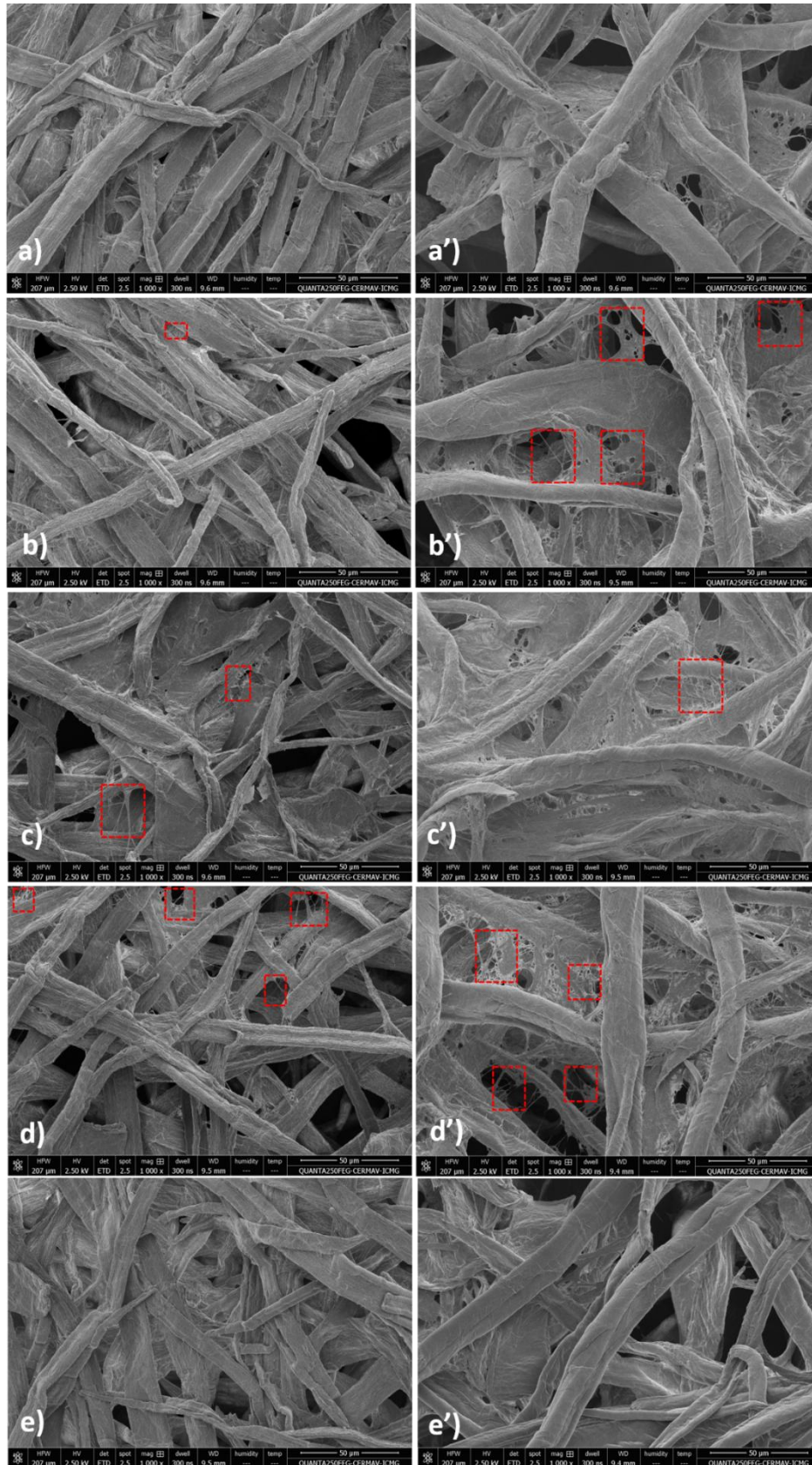
489 **Figure 5.** TGA and DTG curves of DES/enzyme treated eucalyptus (a, a' and b, b') and
490 cotton fibres (c, c' and d, d') under air and nitrogen respectively.

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

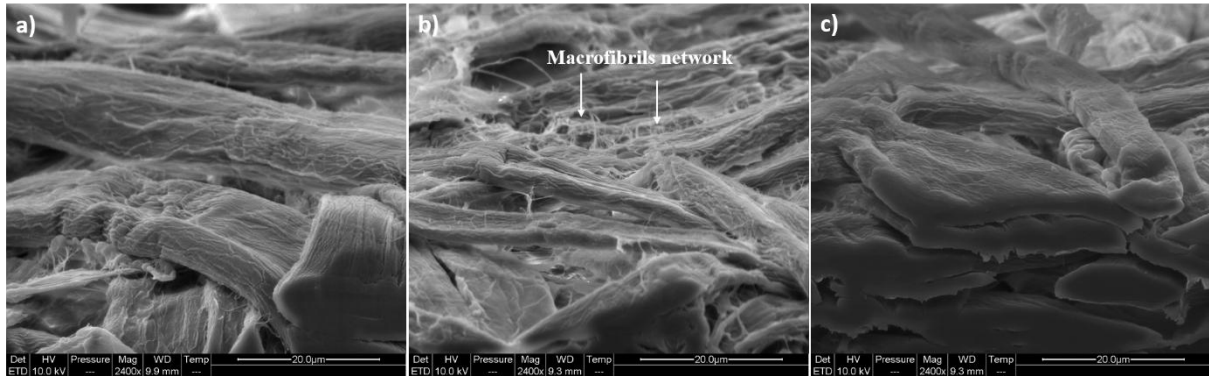
503



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

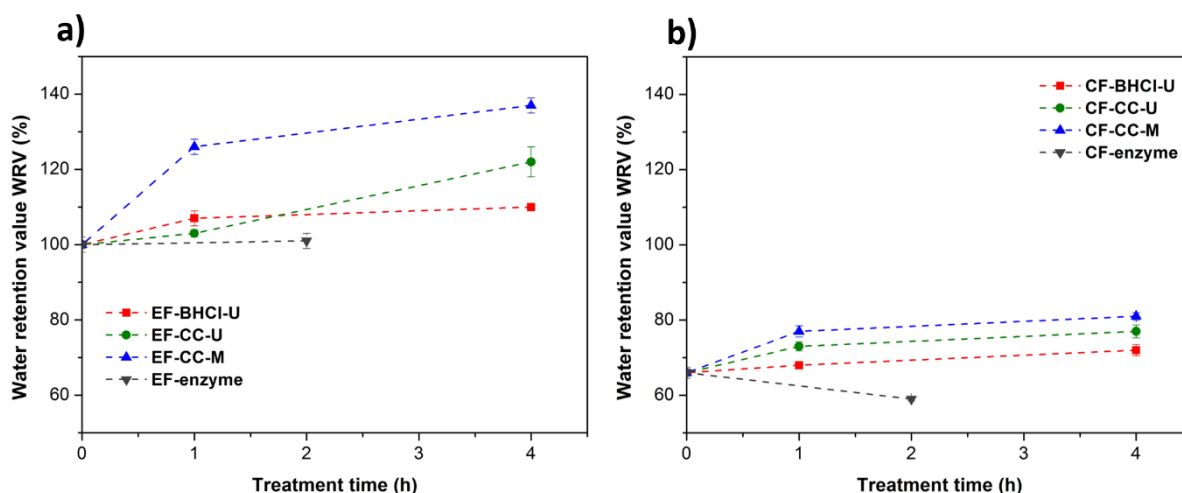
	EF	BHCl-U	CC-U	CC-M	Enzyme
Mean arithmetic length	523±4	511±1	508±1	512±3	446±3

	μm				
Mean width μm	16.4±0.1	16.3±0	16.7±0.1	17.3±0.1	16.2±0.1
Fine content (% in length)	13.1±1.1	18.5±0.2	16.4±0.2	15.3±0.1	30.6±0.7
	CF	BHCl-U	CC-U	CC-M	Enzyme
Mean arithmetic length μm	453±3	480±4	434±3	481±3	415±1
Mean width μm	19.3±0.2	19.3±0.1	19.4±0.1	19.8±0.1	18±0
Fine content (% in length)	35.0±1.1	35.8±0.3	40.3±0.6	35.8±0.8	56±0.2

531

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
534 show that WRV increased with DES treatment time. As expected, water swelling of
535 eucalyptus fibres was more important than that of cotton fibres and the impact of DES
536 treatment was also more pronounced. WRV increased after DES treatment from 100 to 110,
537 128 and 137% respectively with BHCl-U, CC-U and CC-M, for eucalyptus fibres, which
538 corresponds to a maximum relative increase of 37%. In the case of cotton fibres, WRV varied
539 from 66 to 81% with CC-M, the relative increase being of 23%. WRV, which is related to the
540 swelling ability of fibres, depends on the accessibility of the cell wall to water. This
541 accessibility is promoted by internal fibrillation, which usually results from mechanical
542 refining of the pulp suspension. DES treatment, and more precisely neutral and alkaline DES,
543 seem to have the same effect than a mechanical treatment (refining): internal fibrillation
544 occurs (revealed by WRV) as well as external fibrillation (revealed by the presence of
545 macrofibrils) as observed by SEM. In contrast to what happens during a conventional refining
546 process (Gharehkhani et al., 2015), the generation of fine elements is limited and the
547 shortening of the fibres does not occur.



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.

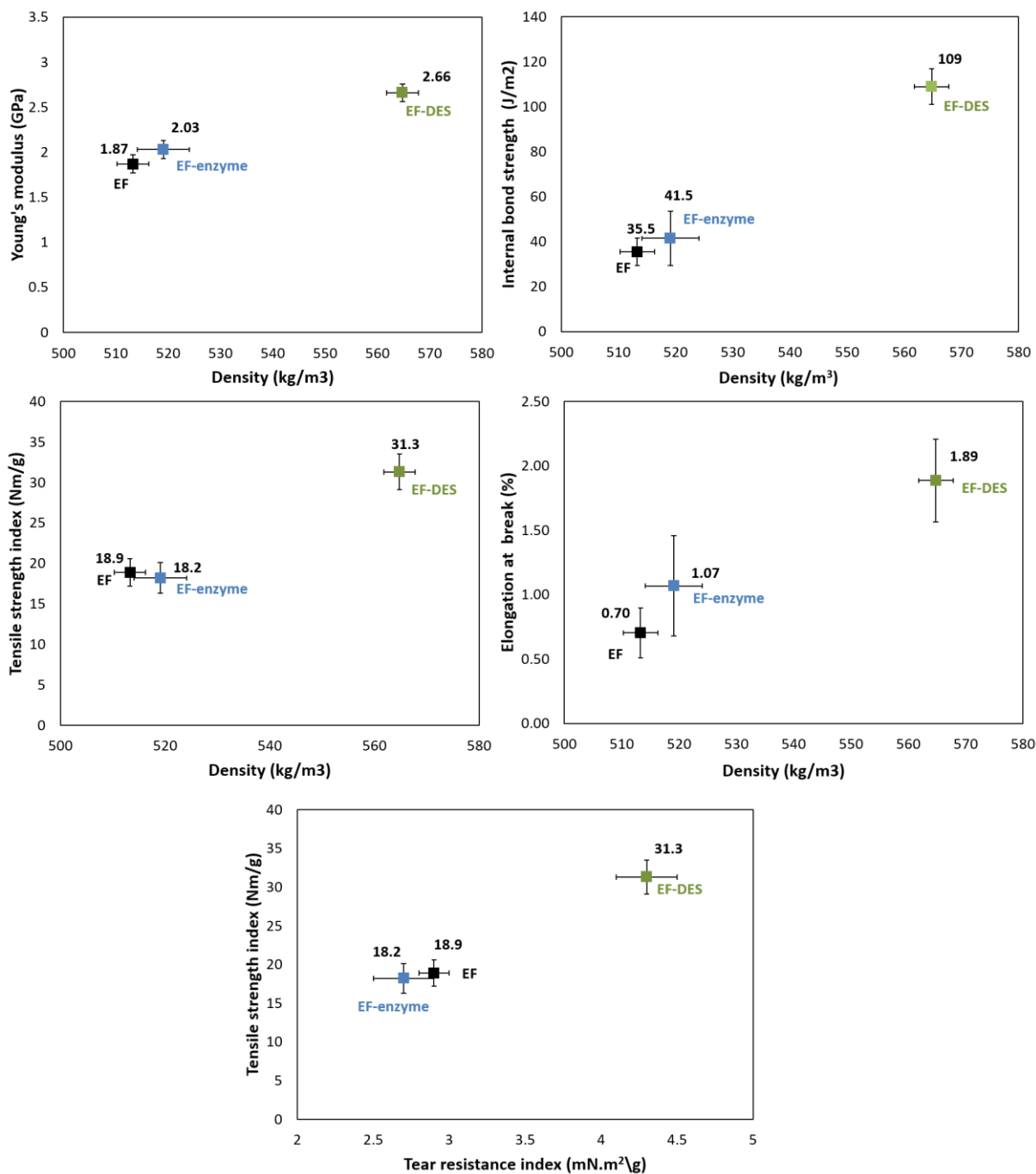
560 A first observation is the significant increase of the density after DES treatment: it rises from
 561 514 to 565 kg/m³. Oppositely, after enzymatic treatment, the change is neglectable. This
 562 increase, for papers made of DES-treated fibres, reflects a densification of the fibrous
 563 network. 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.m²/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 ¹³C
 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.

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621 **References**

- 622 Abbott, A. P., Capper, G., L. Davies, D., L. Munro, H., K. Rasheed, R., & Tambyrajah, V.
623 (2001). Preparation of novel, moisture-stable, Lewis-acidic ionic liquids containing
624 quaternary ammonium salts with functional side chains. *Chemical Communications*, 0,
625 2010–2011.
- 626 Abranches, D. O., Martins, M. A. R., Silva, L. P., Schaeffer, N., Pinho, S. P., & Coutinho, J.
627 A. P. (2019). Phenolic hydrogen bond donors in the formation of non-ionic deep
628 eutectic solvents: The quest for type V DES. *Chemical Communications*, 55, 10253–
629 10256.
- 630 AlOmar, M. K., Alsaadi, M. A., Hayyan, M., Akib, S., Ibrahim, R. K., & Hashim, M. A.
631 (2016). Lead removal from water by choline chloride based deep eutectic solvents
632 functionalized carbon nanotubes. *Journal of Molecular Liquids*, 222, 883–894.
- 633 Banvillet, G., Depres, G., Belgacem, N., & Bras, J. (2021). Alkaline treatment combined with
634 enzymatic hydrolysis for efficient cellulose nanofibrils production. *Carbohydrate*
635 *Polymers*, 255, 117383.
- 636 Chen, Y.-L., Zhang, X., You, T.-T., & Xu, F. (2019). Deep eutectic solvents (DESs) for
637 cellulose dissolution: A mini-review. *Cellulose*, 26, 205–213.
- 638 Chung, C., Lee, M., & Choe, E. K. (2004). Characterization of cotton fabric scouring by FT-
639 IR ATR spectroscopy. *Carbohydrate Polymers*, 58, 417–420.
- 640 Crawford, D. E., James, L. A., & Abbott, A. P. (2016). Efficient continuous synthesis of high
641 purity deep eutectic solvents by twin screw extrusion. *Chemical Communications*, 52,
642 4215–4218.
- 643 Delso, I., Lafuente, C., Muñoz-Embid, J., & Artal, M. (2019). NMR study of choline
644 chloride-based deep eutectic solvents. *Journal of Molecular Liquids*, 290, 111236.

645 Douard, L., Bras, J., Encinas, T., & Belgacem, M. N. (2021). Natural acidic deep eutectic
646 solvent to obtain cellulose nanocrystals using the design of experience approach.
647 *Carbohydrate Polymers*, 252, 117136.

648 Fernandes, P. M. V., Campiña, J. M., Pereira, N. M., Pereira, C. M., & Silva, F. (2012).
649 Biodegradable deep-eutectic mixtures as electrolytes for the electrochemical synthesis
650 of conducting polymers. *Journal of Applied Electrochemistry*, 42, 997–1003.

651 Florindo, C., Oliveira, F. S., Rebelo, L. P. N., Fernandes, A. M., & Marrucho, I. M. (2014).
652 Insights into the Synthesis and Properties of Deep Eutectic Solvents Based on
653 Cholinium Chloride and Carboxylic Acids. *ACS Sustainable Chemistry &*
654 *Engineering*, 2, 2416–2425.

655 Francisco, M., Bruinhorst, A. van den, & Kroon, M. (2012). New natural and renewable low
656 transition temperature mixtures (LTTMs): Screening as solvents for lignocellulosic
657 biomass processing. *Green Chemistry*, 14, 2153–2157.

658 French, A. D. (2014). Idealized powder diffraction patterns for cellulose polymorphs.
659 *Cellulose*, 21, 885–896.

660 Gharekhani, S., Sadeghinezhad, E., Kazi, S. N., Yarmand, H., Badarudin, A., Safaei, M. R.,
661 & Zubir, M. N. M. (2015). Basic effects of pulp refining on fiber properties—A
662 review. *Carbohydrate Polymers*, 115, 785–803.

663 Hong, S., Yuan, Y., Li, P., Zhang, K., Lian, H., & Liimatainen, H. (2020). Enhancement of
664 the nanofibrillation of birch cellulose pretreated with natural deep eutectic solvent.
665 *Industrial Crops and Products*, 154, 112677.

666 Hosu, O., Bârsan, M. M., Cristea, C., Săndulescu, R., & Brett, C. M. A. (2017).
667 Nanostructured electropolymerized poly(methylene blue) films from deep eutectic
668 solvents. Optimization and characterization. *Electrochimica Acta*, 232, 285–295.

669 Johan Foster, E., J. Moon, R., P. Agarwal, U., J. Bortner, M., Bras, J., Camarero-Espinosa, S.,
670 ... Youngblood, J. (2018). Current characterization methods for cellulose
671 nanomaterials. *Chemical Society Reviews*, 47, 2609–2679.

672 Kang, T. (2007). *Role of external fibrillation in pulp and paper properties*. Helsinki
673 University of Technology.

674 Kono, H., Numata, Y., Erata, T., & Takai, M. (2004). ¹³C and ¹H Resonance Assignment of
675 Mercerized Cellulose II by Two-Dimensional MAS NMR Spectroscopies.
676 *Macromolecules*, 37, 5310–5316.

677 Kudlak, B., Owczarek, K., & Namieśnik, J. (2015). Selected issues related to the toxicity of
678 ionic liquids and deep eutectic solvents—A review. *Environmental Science and*
679 *Pollution Research*, 22, 11975–11992.

680 Laitinen, O., Suopajärvi, T., Österberg, M., & Liimatainen, H. (2017). Hydrophobic,
681 Superabsorbing Aerogels from Choline Chloride-Based Deep Eutectic Solvent
682 Pretreated and Silylated Cellulose Nanofibrils for Selective Oil Removal. *ACS Applied*
683 *Materials & Interfaces*, 9, 25029–25037.

684 Li, P., Sirviö, J. A., Haapala, A., & Liimatainen, H. (2017). Cellulose Nanofibrils from
685 Nonderivatizing Urea-Based Deep Eutectic Solvent Pretreatments. *ACS Applied*
686 *Materials & Interfaces*, 9, 2846–2855.

687 Liu, Zhang, Gou, Zhang, & Wang. (2021). Esterification of cellulose using carboxylic acid-
688 based deep eutectic solvents to produce high-yield cellulose nanofibers. *Carbohydrate*
689 *Polymers*, 251, 117018.

690 Mäki-Arvela, P., Salmi, T., Holmbom, B., Willför, S., & Murzin, D. Yu. (2011). Synthesis of
691 Sugars by Hydrolysis of Hemicelluloses- A Review. *Chemical Reviews*, 111, 5638–
692 5666.

693 Mittal, A., Katahira, R., Himmel, M. E., & Johnson, D. K. (2011). Effects of alkaline or
694 liquid-ammonia treatment on crystalline cellulose: Changes in crystalline structure and
695 effects on enzymatic digestibility. *Biotechnology for Biofuels*, 4, 41.

696 Moufawad, T., Gomes, M. C., & Fourmentin, S. (2021). *Solvants eutectiques profonds—Vers*
697 *des procédés plus durables*. 27.

698 Mussatto, S. I., Fernandes, M., Milagres, A. M. F., & Roberto, I. C. (2008). Effect of
699 hemicellulose and lignin on enzymatic hydrolysis of cellulose from brewer's spent
700 grain. *Enzyme and Microbial Technology*, 43, 124–129.

701 Nechyporchuk, O., Belgacem, M. N., & Bras, J. (2016). Production of cellulose nanofibrils: A
702 review of recent advances. *Industrial Crops and Products*, 93, 2–25.

703 Park, S., Baker, J. O., Himmel, M. E., Parilla, P. A., & Johnson, D. K. (2010). Cellulose
704 crystallinity index: Measurement techniques and their impact on interpreting cellulase
705 performance. *Biotechnology for Biofuels*, 3, 10.

706 Płotka-Wasyłka, J., de la Guardia, M., Andruch, V., & Vilková, M. (2020). Deep eutectic
707 solvents vs ionic liquids: Similarities and differences. *Microchemical Journal*, 159,
708 105539.

709 Rol, F., Belgacem, M. N., Gandini, A., & Bras, J. (2019). Recent advances in surface-
710 modified cellulose nanofibrils. *Progress in Polymer Science*, 88, 241–264.

711 Rol, F., Karakashov, B., Nechyporchuk, O., Terrien, M., Meyer, V., Dufresne, A., ... Bras, J.
712 (2017). Pilot-Scale Twin Screw Extrusion and Chemical Pretreatment as an Energy-
713 Efficient Method for the Production of Nanofibrillated Cellulose at High Solid
714 Content. *ACS Sustainable Chemistry & Engineering*, 5, 6524–6531.

715 Rol, F., Sillard, C., Bardet, M., Yarava, J. R., Emsley, L., Gablin, C., ... Bras, J. (2020).
716 Cellulose phosphorylation comparison and analysis of phosphate position on
717 cellulose fibers. *Carbohydrate Polymers*, 229, 115294.

718 Ruesgas-Ramón, M., Figueroa-Espinoza, M. C., & Durand, E. (2017). Application of Deep
719 Eutectic Solvents (DES) for Phenolic Compounds Extraction: Overview, Challenges,
720 and Opportunities. *Journal of Agricultural and Food Chemistry*, *65*, 3591–3601.

721 Ruiz, E., Cara, C., Manzanares, P., Ballesteros, M., & Castro, E. (2008). Evaluation of steam
722 explosion pre-treatment for enzymatic hydrolysis of sunflower stalks. *Enzyme and*
723 *Microbial Technology*, *42*, 160–166.

724 Schaeffer, N., Conceição, J. H. F., Martins, M. A. R., Neves, M. C., Pérez-Sánchez, G.,
725 Gomes, J. R. B., ... Coutinho, J. A. P. (2020). Non-ionic hydrophobic eutectics –
726 versatile solvents for tailored metal separation and valorisation. *Green Chemistry*, *22*,
727 2810–2820.

728 Selkälä, T., Sirviö, J. A., Lorite, G. S., & Liimatainen, H. (2016). Anionically Stabilized
729 Cellulose Nanofibrils through Succinylation Pretreatment in Urea–Lithium Chloride
730 Deep Eutectic Solvent. *ChemSusChem*, *9*, 3074–3083.

731 Sirviö, J. A. (2018). Cationization of lignocellulosic fibers with betaine in deep eutectic
732 solvent: Facile route to charge stabilized cellulose and wood nanofibers. *Carbohydrate*
733 *Polymers*, *198*, 34–40.

734 Sirviö, J. A., & Heiskanen, J. P. (2017). Synthesis of Alkaline-Soluble Cellulose Methyl
735 Carbamate Using a Reactive Deep Eutectic Solvent. *ChemSusChem*, *10*, 455–460.

736 Sirviö, J. A., Visanko, M., & Liimatainen, H. (2015). Deep eutectic solvent system based on
737 choline chloride-urea as a pre-treatment for nanofibrillation of wood cellulose. *Green*
738 *Chemistry*, *17*, 3401–3406.

739 Sirviö, J. A., Visanko, M., & Liimatainen, H. (2016). Acidic Deep Eutectic Solvents As
740 Hydrolytic Media for Cellulose Nanocrystal Production. *Biomacromolecules*, *17*,
741 3025–3032.

742 Smith, E. L., Abbott, A. P., & Ryder, K. S. (2014). Deep Eutectic Solvents (DESs) and Their
743 Applications. *Chemical Reviews*, *114*, 11060–11082.

744 Spiridon, I., & Popa, V. I. (2008). Chapter 13 - Hemicelluloses: Major Sources, Properties
745 and Applications. In M. N. Belgacem & A. Gandini (Eds.), *Monomers, Polymers and*
746 *Composites from Renewable Resources* (pp. 289–304). Amsterdam: Elsevier.

747 Suopajarvi, Sirviö, & Liimatainen. (2017). Nanofibrillation of deep eutectic solvent-treated
748 paper and board cellulose pulps. *Carbohydrate Polymers*, *169*, 167–175.

749 Suopajarvi, T., Ricci, P., Karvonen, V., Ottolina, G., & Liimatainen, H. (2020). Acidic and
750 alkaline deep eutectic solvents in delignification and nanofibrillation of corn stalk,
751 wheat straw, and rapeseed stem residues. *Industrial Crops and Products*, *145*, 111956.

752 Tenhunen, T.-M., Lewandowska, A. E., Orelma, H., Johansson, L.-S., Virtanen, T., Harlin,
753 A., ... Tammelin, T. (2018). Understanding the interactions of cellulose fibres and
754 deep eutectic solvent of choline chloride and urea. *Cellulose*, *25*, 137–150.

755 Tian, D., Guo, Y., Hu, J., Yang, G., Zhang, J., Luo, L., ... Shen, F. (2020). Acidic deep
756 eutectic solvents pretreatment for selective lignocellulosic biomass fractionation with
757 enhanced cellulose reactivity. *International Journal of Biological Macromolecules*,
758 *142*, 288–297.

759 Tomé, L. I. N., Baião, V., da Silva, W., & Brett, C. M. A. (2018). Deep eutectic solvents for
760 the production and application of new materials. *Applied Materials Today*, *10*, 30–50.

761 Willberg-Keyriläinen, P., Hiltunen, J., & Ropponen, J. (2018). Production of cellulose
762 carbamate using urea-based deep eutectic solvents. *Cellulose*, *25*, 195–204.

763 Xiao, B., Sun, X. F., & Sun, R. (2001). Chemical, structural, and thermal characterizations of
764 alkali-soluble lignins and hemicelluloses, and cellulose from maize stems, rye straw,
765 and rice straw. *Polymer Degradation and Stability*, *74*, 307–319.

766 Zahrina, I., Nasikin, M., & Mulia, K. (2017). Evaluation of the interaction between molecules
767 during betaine monohydrate-organic acid deep eutectic mixture formation. *Journal of*
768 *Molecular Liquids*, 225, 446–450.

769 Zdanowicz, M., Wilpiszewska, K., & Szychaj, T. (2018). Deep eutectic solvents for
770 polysaccharides processing. A review. *Carbohydrate Polymers*, 200, 361–380.

771 Zhao, Z., Chen, X., Ali, M. F., Abdeltawab, A. A., Yakout, S. M., & Yu, G. (2018).
772 Pretreatment of wheat straw using basic ethanolamine-based deep eutectic solvents for
773 improving enzymatic hydrolysis. *Bioresource Technology*, 263, 325–333.

774