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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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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~~

~~retention value of treated fibres increased especially with CC-M, which reflects a specific action of the DES on the structure of the fibre wall: DES seems to promote an internal fibrillation. An external fibrillation was also 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.~~

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

1. Introduction

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

2. Materials and Methods

2.1 Materials

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

ethanol and oven dried at 60°C. Betaine hydrochloride ($C_5H_{12}ClNO_2$) was supplied from VWR (Milliporesigma), urea ACS reagent (CH_4N_2O, 99.0-100.5%) from Merck-sigma Aldrich, monoethanolamine (C_2H_7NO, >99%) from ROTH, choline chloride ($C_5H_{14}ClNO$, >98%) from Alfa Aesar, bis(ethylenediamine)copper (II) hydroxide solution ($Cu(H_2NCH_2CH_2NH_2)_2(OH)_2$ 1.0 M in H_2O, Sigma-Aldrich), sulfuric acid (H_2SO_4, 72%), acetic acid (CH_3COOH), ethanol (CH_3OH, 96%) and sodium acetate ($C_2H_3NaO_2$) were obtained from Sigma Aldrich. Enzyme solution FiberCare (pH between 6 and 8) was purchased from Novozymes. Deionised water was used throughout the experiments.

2.2 DES pre-treatment

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

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

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

2.3 Enzymatic pre-treatment

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

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

2.4 Paper handsheet production

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

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

2.5 FT-IR ATR spectroscopy

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

2.6 Solid-state ^{13}C NMR

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

Prior to performing NMR experiment, DES or enzyme treated eucalyptus fibres were air-dried and then grinded using a cryomill for 2 min. After grinding, sample was compacted in a 4 mm ZrO2 rotors and then analysed. The analysis was done in the quantitative mode. The data were

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

The DS is calculated according to the following formula:

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

2.7 Chemical composition

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

2.8 Degree of polymerisation (DP)

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

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

2.9 X-ray Diffraction XrD

X-ray Diffraction (XrD) was performed on paper samples deposited on a zero-background Si substrate. The measurements were carried out with a diffractometer X'Pert Pro MPD (PANalytical, Netherlands), equipped with a Bragg-Brentano geometry and a copper anode (Kα λ = 1.5419 Å). The crystallinity index CrI (%) was calculated using Segal method:

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

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

2.10 Thermogravimetric analysis

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

2.11 Scanning electron microscopy (SEM)

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

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

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

2.12 Morphological analysis of the fibres

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

2.13 Water retention value (WRV)

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

WRV was calculated according to the following equation:

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

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

2.14 Tensile tests

Tensile tests were performed on the produced papers using an Instron 5965 machine (Instron, USA) in conditioned atmosphere (Temperature = 23°C and Relative humidity = 50%) according to ISO 1924-2 standard. Tests were done on samples with dimensions of 15×100 mm² and a tensile speed of 10 mm/min. The Young's modulus of each sample was calculated using the thickness values of the paper. For each handsheeet, 2 measurements were done. The average of 20 measurements was calculated.

2.15 Tear resistance

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

2.16 Internal bond strength

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

3. Results and discussions

3.1 Effect of DES treatments on the chemical structure of the fibres

3.1.1 FT-IR ATR analysis

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

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

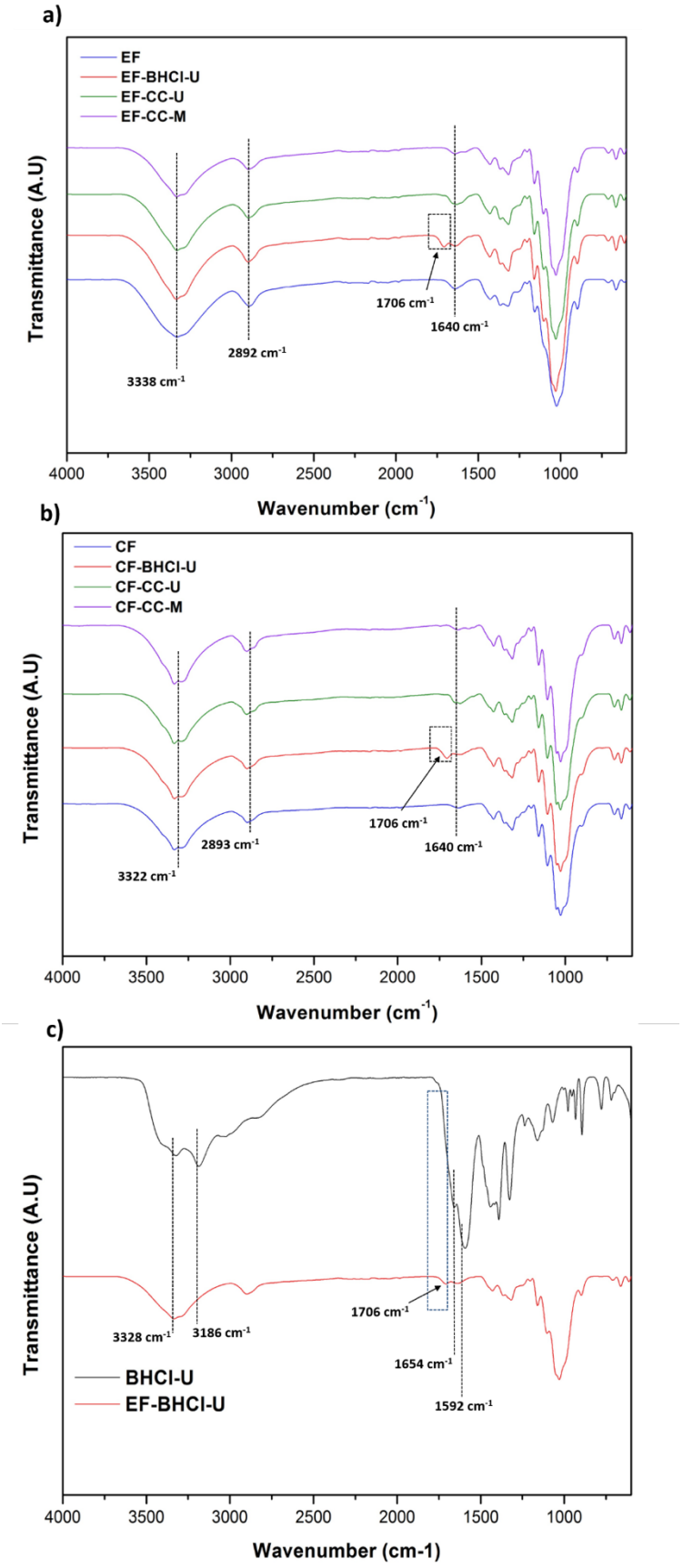
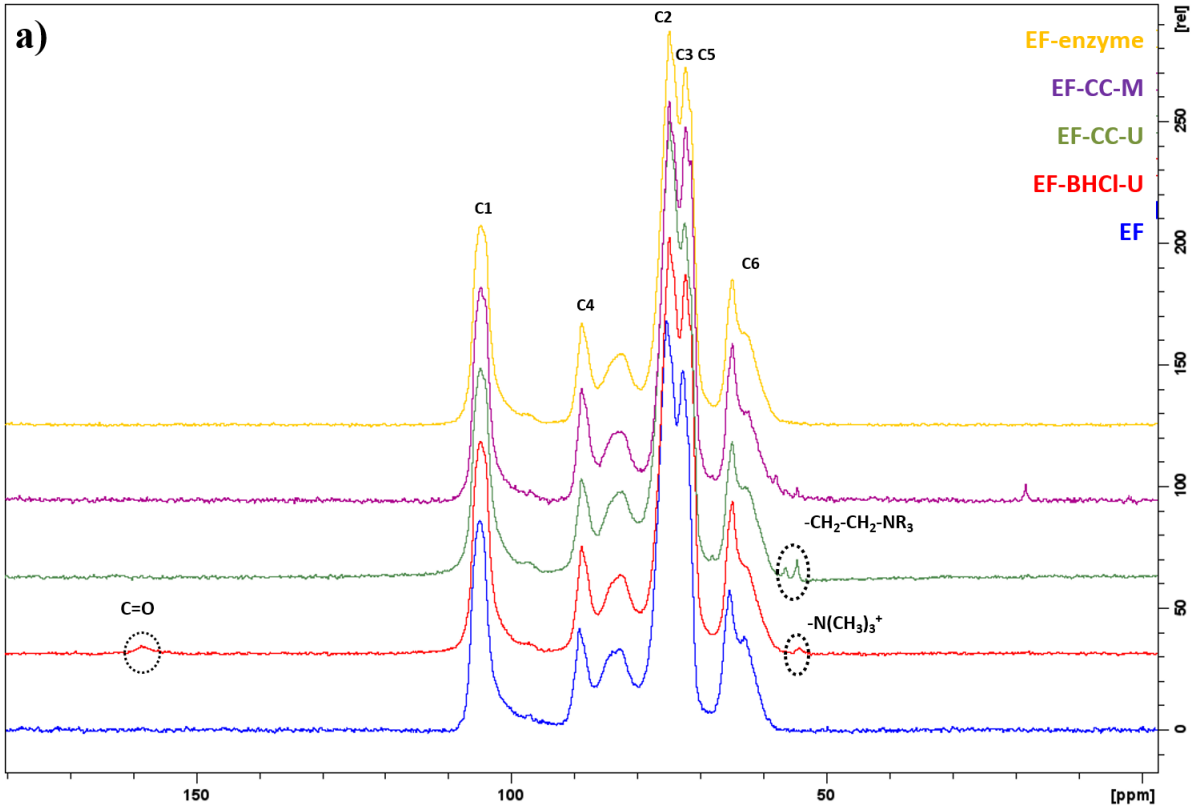


Figure 1. *FT-IR ATR spectra of DES-treated eucalyptus(a), cotton fibres (b) and EF-BHCl-U compared to BHCl-U (c).*

3.1.2 Solid-state ^{13}C NMR of DES/enzyme treated eucalyptus fibres

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



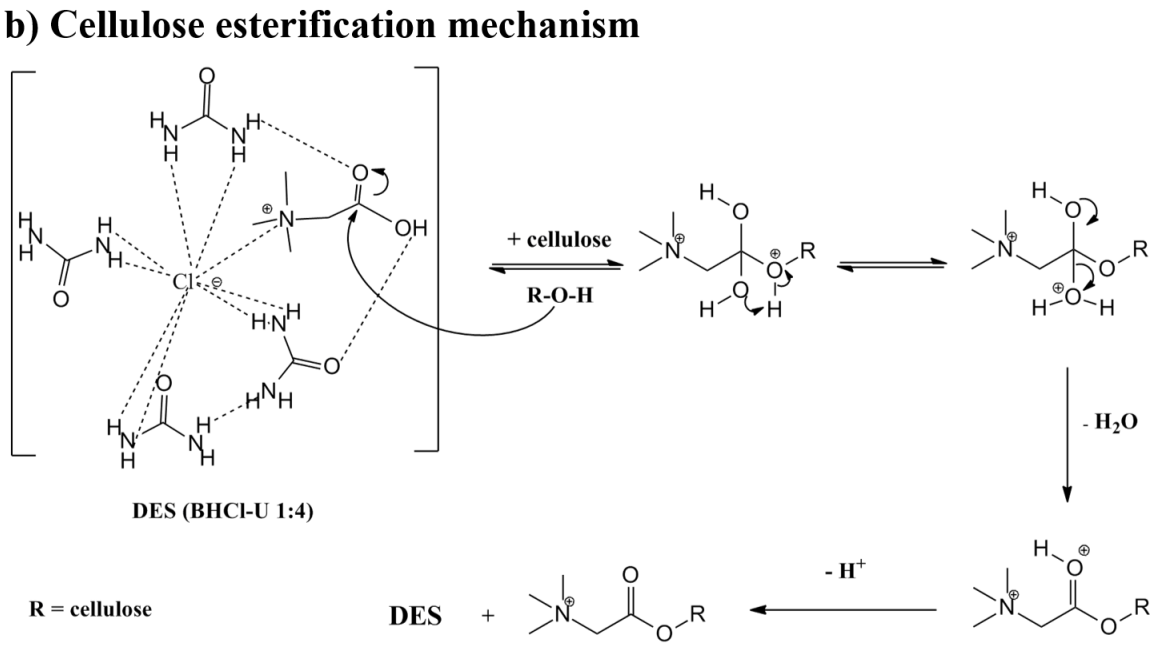


Figure 2. Solid-state ^{13}C NMR spectra of DES or enzymatic treated eucalyptus fibres(a) and the proposed mechanism of cellulose esterification (b).

On the other hand, additional peaks are observed with acidic and neutral DES-treatment. The spectrum of acidic DES-treated fibres presents two new small peaks. The first peak (54.4 ppm) is attributed to the methyl of quaternary ammonium ($-N(CH_3)_3^+$), while the second one (158.7 ppm) is related to a carbonyl group. The presence of these two peaks clearly evidenced

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

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

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

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

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

3.2 Chemical composition of DES-treated eucalyptus fibres

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

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

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

3.3 Degree of polymerisation (DP)

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

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

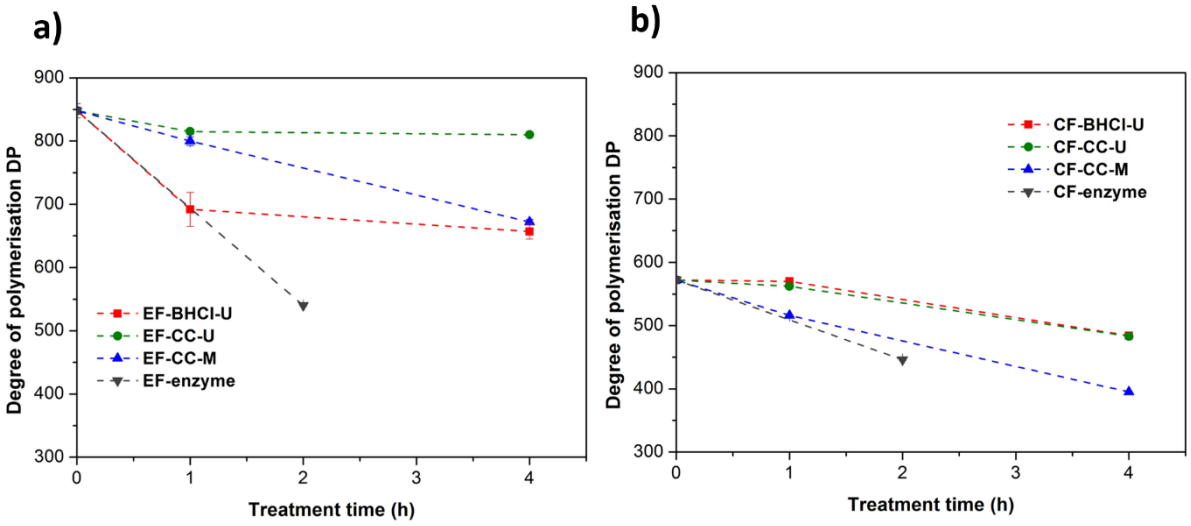


Figure 3. *DP evolution of DES-treated eucalyptus (a) and cotton fibres (b).*

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

The decrease of the DP in acidic DES is discussed in the literature. This phenomenon results from glycosidic bond cleavage catalysed by acidic solvents and many acidic DES (formic acid, acetic acid, lactic acid-choline chloride) are known to lower the DP during delignification of lignocellulosic biomass (from 839 to 397, 367 and 323, respectively) (Tian et al., 2020). Thus, (Sirviö & Heiskanen, 2017) used acidic DES (dimethyl urea-$ZnCl_2$), as a reactive solvent, to produce cellulose methyl carbamate and observed a significant decrease of DP from 1820 to 686 after 1.5h of treatment at 150°C.

3.4 X-ray Diffraction and Crystallinity Index CrI

X-ray diffraction patterns (Figure 4) of cellulose fibres before and after DES or enzymatic treatment are typical of cellulose I_β crystalline structure. The diffraction peaks at 2θ angles of 15.2, 16.5, 225 and 34.5° are related to the $(1\bar{1}0)$, (110), (200) and (004) lattice planes respectively (French, 2014; Park, Baker, Himmel, Parilla, & Johnson, 2010). The diffractograms show that cellulose crystalline structure remained the same as the original fibres (cellulose I_β) for both eucalyptus and cotton fibres due to the absence of other crystalline allomorphs of cellulose. From these results, it can be deduced that there was no regeneration even with alkaline DES. These results are in line with other studies in the literature (Hong et al., 2020; Suopajärvi, Sirviö, Liimatainen, 2017). As expected, cotton fibres present a more crystalline structure than eucalyptus fibres: for the untreated fibres, CrI values of 87.0 and 80.2% were obtained from CF and EF respectively. In addition, the peaks at 15.2 and 16.5° of the diffractogram are more separated for cotton fibres which indicates that this structure is more crystalline. Indeed, the fact that this separation is not clearly observed with untreated eucalyptus fibres is due to the presence of more amorphous regions.

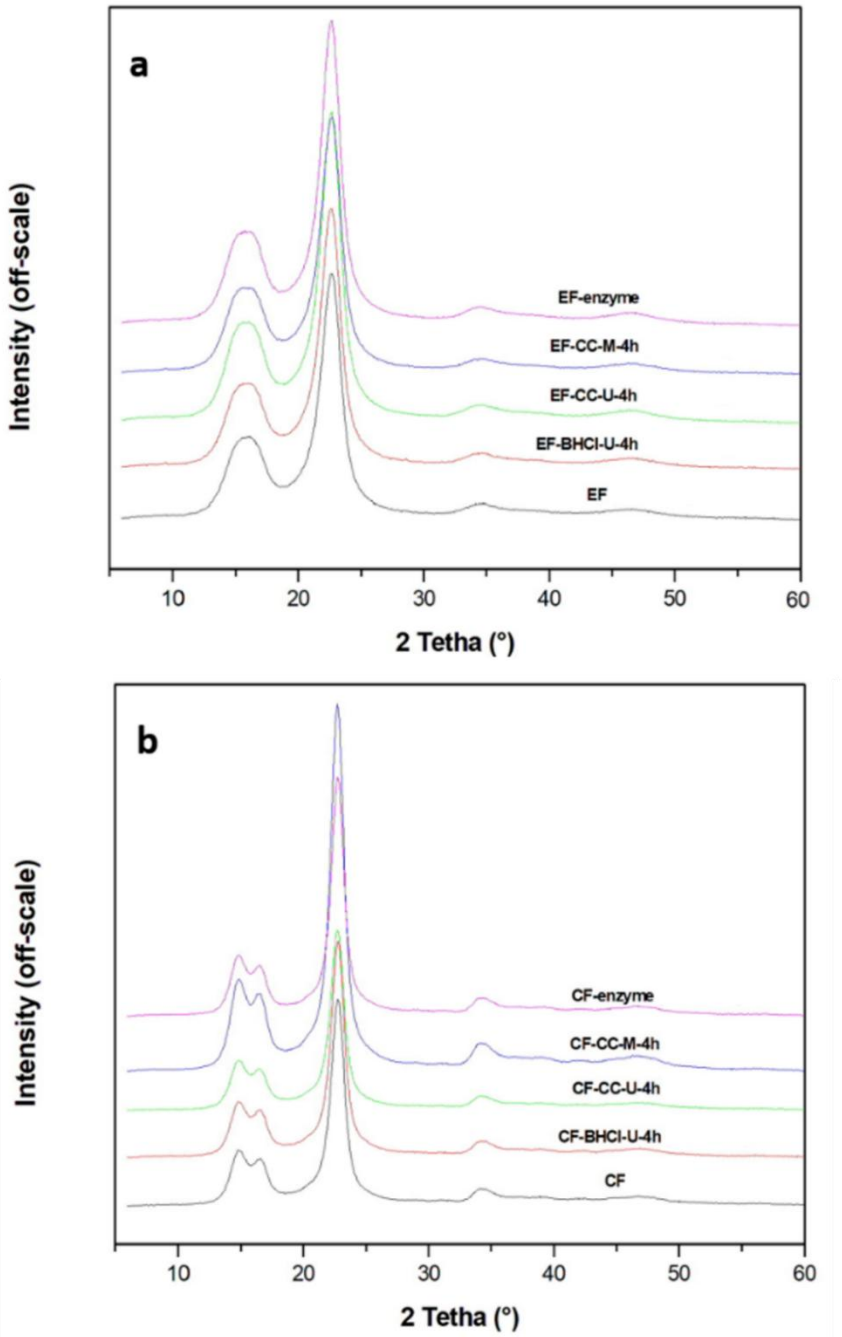


Figure 4. *XrD diffractograms of treated-eucalyptus fibres (a) and cotton fibres (b)*

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

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

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

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

3.5 Thermogravimetric analysis

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

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

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

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

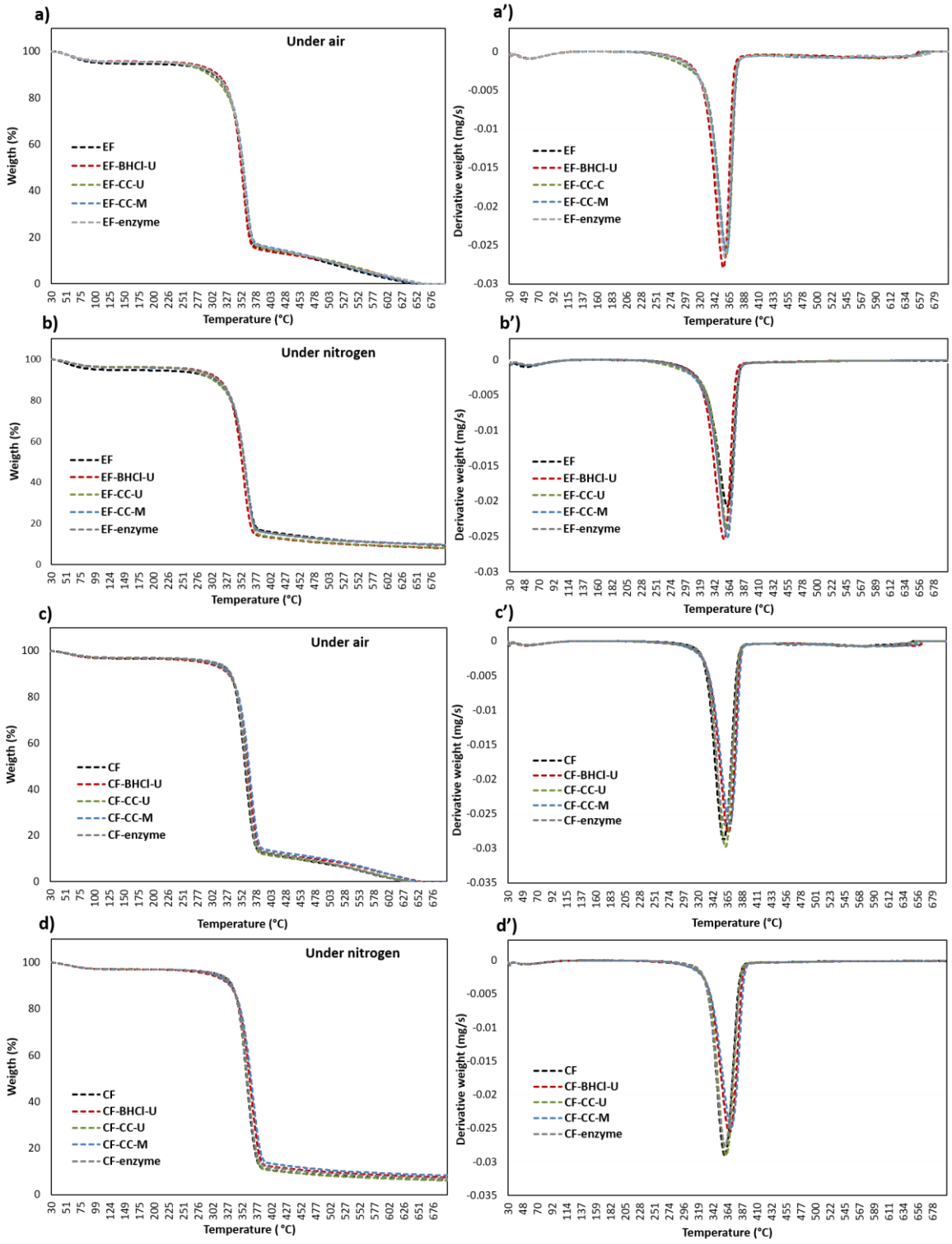


Figure 5. *TGA and DTG curves of DES/enzyme treated eucalyptus (a, a' and b, b') and cotton fibres (c, c' and d, d') under air and nitrogen respectively.*

3.6 Scanning Electron Microscopy (SEM)

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

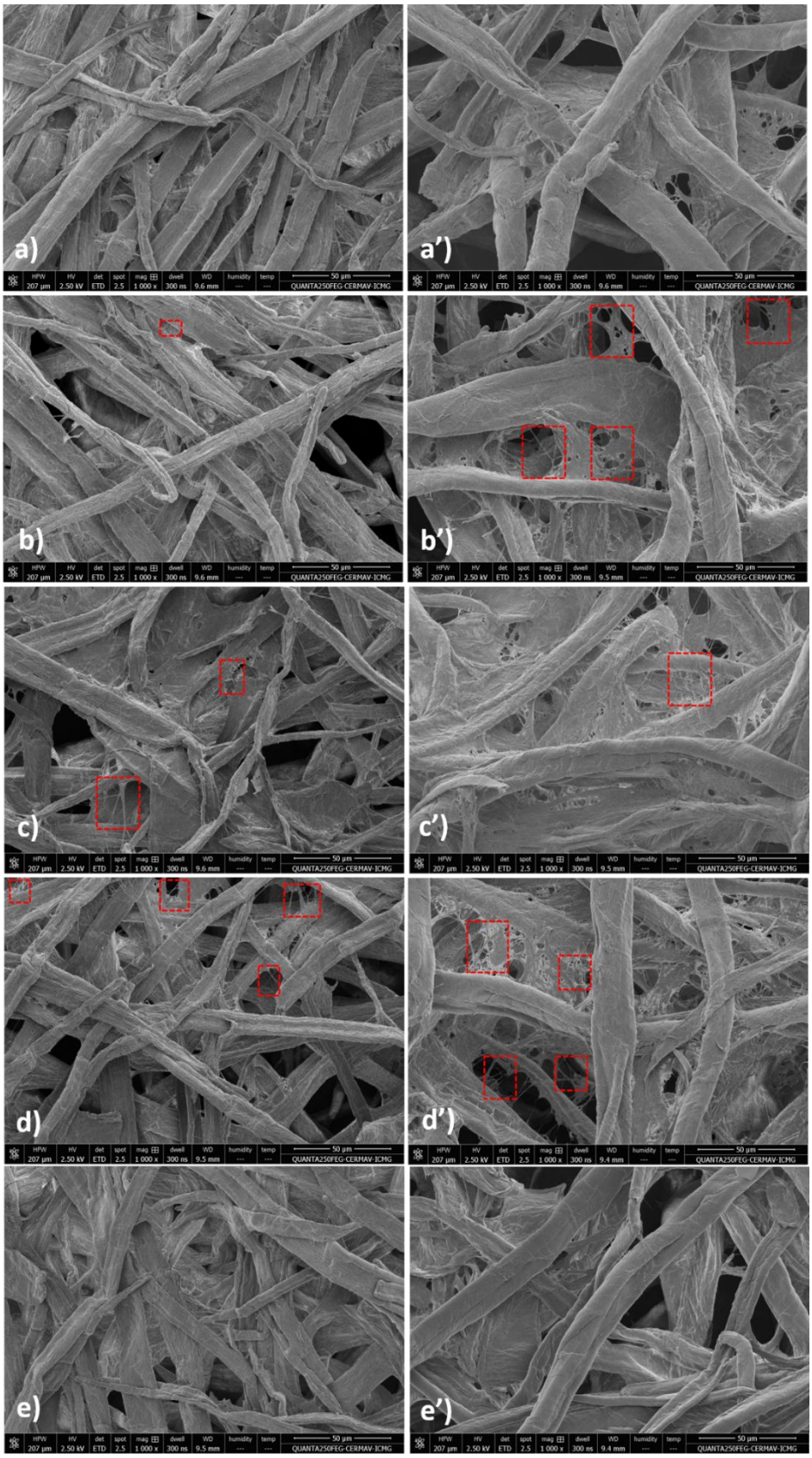


Figure 6. *SEM images of eucalyptus and cotton fibres before (a, a') and after acidic DES (b, b'), neutral DES (c, c') alkaline DES (d, d') and enzymatic hydrolysis (e, e') respectively.*

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

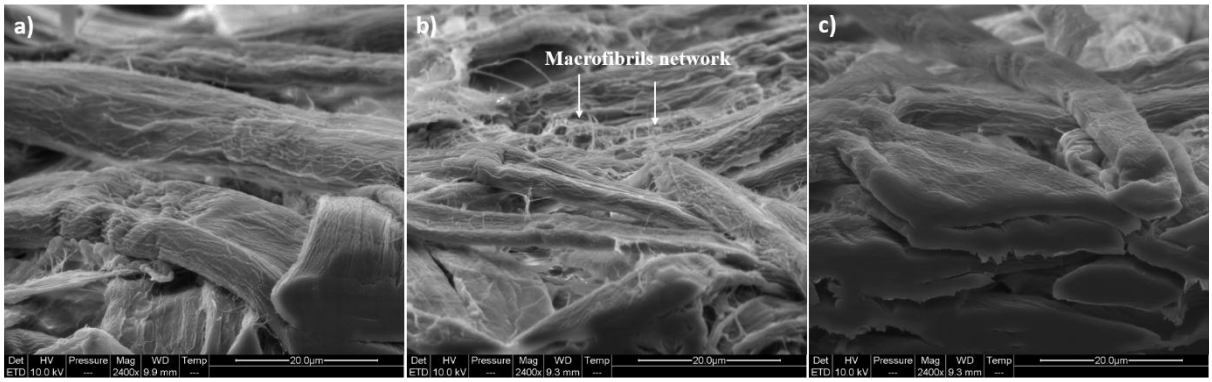


Figure 7. *SEM images of papers produced from untreated EF (a), EF-CC-M (b) and EF-enzyme (c).*

3.7 Morphological properties of fibres

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

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

3.8 Water Retention Value WRV of DES-treated fibres

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 macrofibrils) 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.

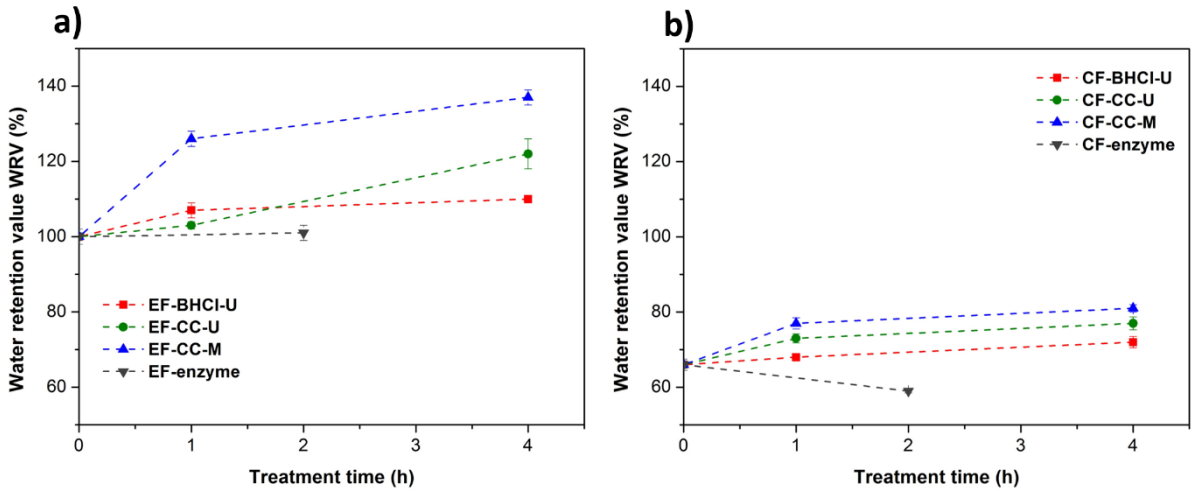


Figure 8. *WRV evolution of cellulose fibres EF (image a) and CF (image b) according to DES/enzyme treatment time.*

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

3.9 Mechanical properties of produced papers from DES treated eucalyptus fibres

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

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

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

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

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

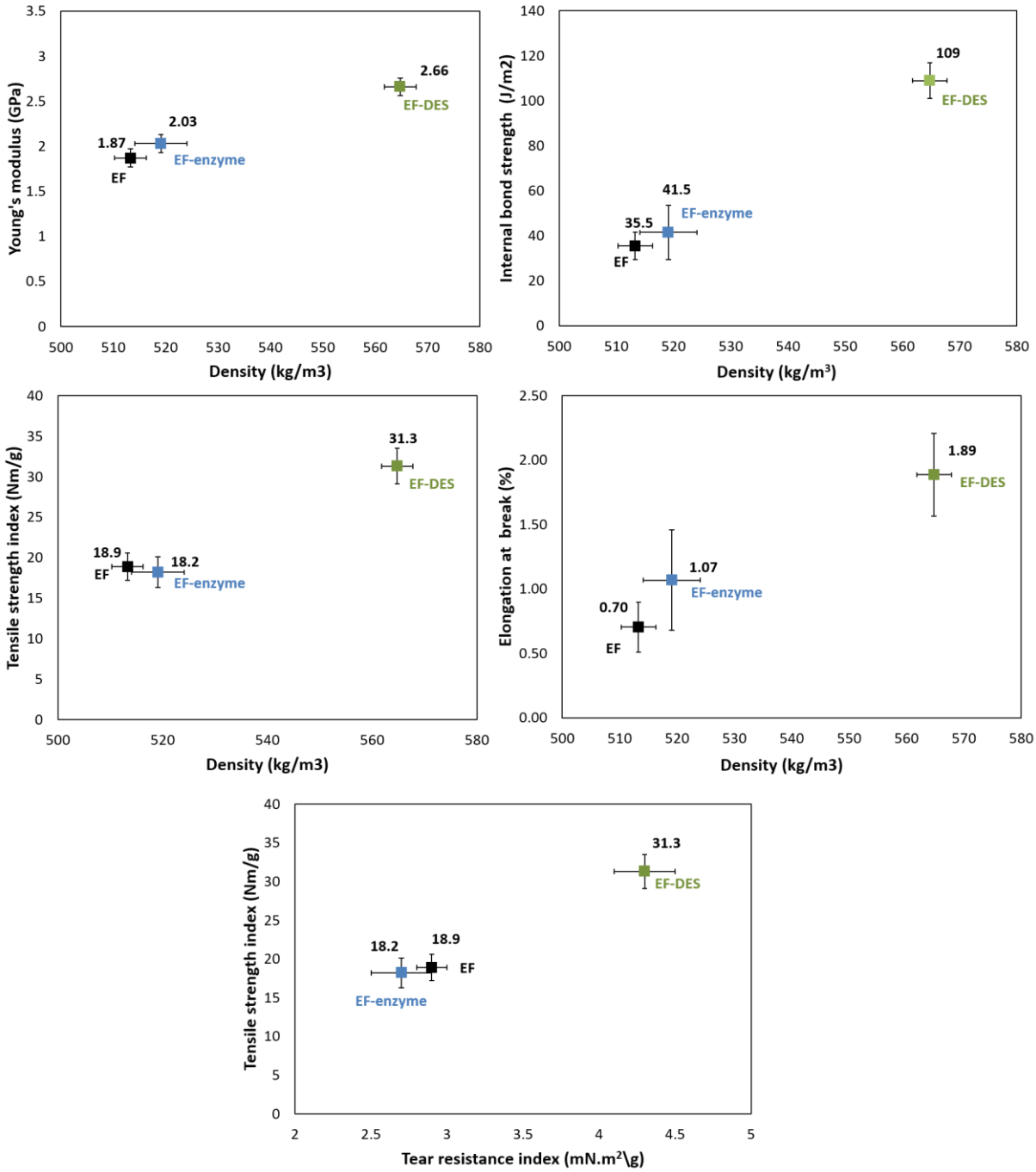


Figure 9. *Evolution of the mechanical properties of produced papers from eucalyptus fibres before and after DES (CC-M) or enzymatic treatment.*

4. Conclusion

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

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

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

CRedit authorship contribution statement

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

Acknowledgements

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

The authors would like to thank Isabelle Jeacomine and Christine Lancelon-Pin from CERMAV Grenoble, France for solid-state ^{13}C NMR analysis and SEM observations respectively. We would like also to thank Thierry Encinas from CMTC – Grenoble, France for the XRD analysis.

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