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**A low-cost environmentally friendly approach to isolate lignin containing micro and nanofibrillated cellulose from *Eucalyptus globulus* bark by steam explosion**

Saad NADER<sup>a,b</sup>, Nicolas BROSSE<sup>b,\*</sup>, Malek KHADRAOUI<sup>a</sup>, Cecilia FUENTEALBA<sup>c</sup>, Isabelle ZIEGLER-DEVIN<sup>b</sup>, Fabienne QUILÈS<sup>d</sup>, Sofiane EL-KIRAT-CHATEL<sup>d</sup>, Evelyne MAURET<sup>a</sup>

*a- Laboratoire de Génie des Procédés Papetiers (LGP2), Grenoble INP – Pagora, 461 rue de la papeterie, Saint Martin d'Hères, France.*

*b- Laboratoire d'Etude et de Recherches sur le Matériau Bois (LERMaB), Faculté des Sciences et Technologies, Université de Lorraine, Vandœuvre-lès-Nancy, France.*

*c- Unidad de Desarrollo Tecnológico (UDT), Universidad de Concepción, Coronel, Chile*

*d- Laboratoire de Chimie Physique et de Microbiologie pour les Matériaux et l'Environnement (LCPME), CNRS, Université de Lorraine, Villers-lès-Nancy, France.*

**\*Corresponding author:** Nicolas BROSSE

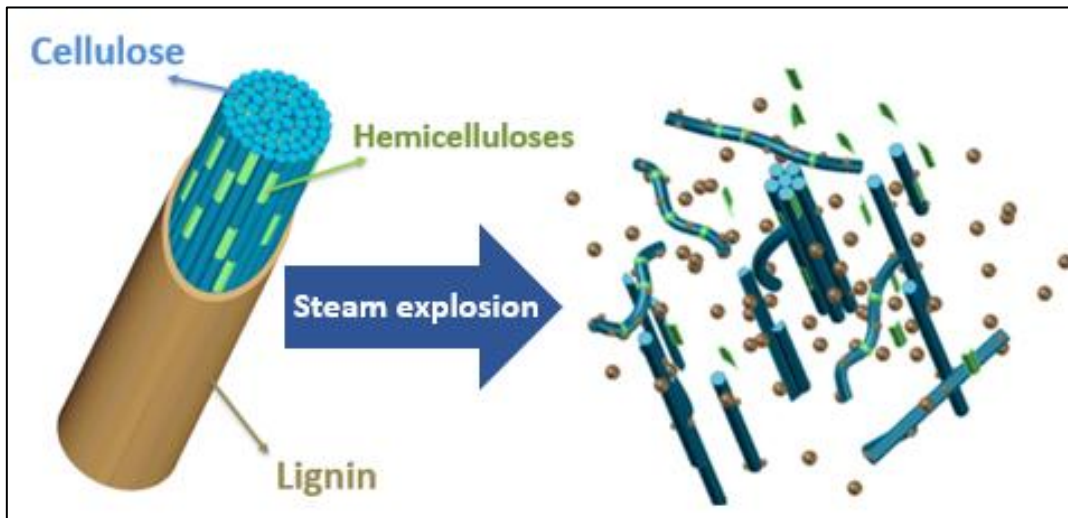
*Email: nicolas.brosse@univ-lorraine.fr*

**ABSTRACT**

Micro- and Nano-Fibrillated Cellulose (MNFC) have gained increasing attention due to their remarkable properties, but their production usually requires an intensive multi-step process. This study proposes to find a novel approach involving steam explosion for the production of lignin-containing micro- and nano-fibrillated cellulose (L-MNFC) using *Eucalyptus globulus* bark as a new lignocellulosic feedstock. *Eucalyptus globulus* bark was first pre-treated by steam explosion in alkaline conditions (200°C, 8 min) or by soda cooking in a rotating autoclave (170°C, 60 min), refined and then ground until gels formed. The chemical composition of the pulps was studied with ion chromatography and FTIR-ATR. The morphology of the products was studied with measurements of suspension turbidity and Morfi Neo, optical and atomic force microscopies. Nanopapers were produced from L-MNFC to investigate mechanical properties. Results obtained showed that steam explosion produced pulps with slightly higher lignin content ( $\approx 9\%$ ), containing shorter fibers ( $\approx 400\ \mu\text{m}$ ) and higher amounts of fines ( $\approx 86\%$ ) compared to soda cooking ( $\approx 5\%$ ,  $\approx 560\ \mu\text{m}$  and 66

%, respectively). AFM images of SteamEx L-MNFC gels showed a web-like structure containing lignin nanoparticles.

**Key words:** lignin-containing microfibrils; Steam explosion; *Eucalyptus globulus* bark; lignin; Alkali treatment.



**Graphical abstract**

## 1 INTRODUCTION:

Micro- and Nano-Fibrillated Cellulose (MNFC) constitutes a new bio-based, renewable, biodegradable and nontoxic material. MNFC width vary between approximately 10 and 100 nm or even more depending on the raw material and the production process used, and its length can reach a few micrometers (TAPPI WI 3021). Owing to their high specific surface area and aspect ratio, MNFC exhibits attractive physical and mechanical properties (165,166). As a result, it can be used in different applications, *i.e.* in paper reinforcement (Ahola et al. 2008; Dufresne 2012), nanocomposites (Siro and Plackett 2010), packaging (Lavoine et al. 2012), aerogels (Sehaqui et al. 2011), bio-medical (Lin and Dufresne 2014), cosmetics (Ullah et al. 2016), etc. MNFC is extracted from the wall of vegetal fibers and wood pulp was the first material used for the production (Herrick et al., 1983). Researchers focused on the production of fully bleached MNFC. Recently, isolation of lignin-containing micro and nanofibrillated cellulose (L-MNFC) was also studied: different lignocellulosic feedstocks have been experimented, such as banana, pineapple, jute (Abraham et al., 2011), different wood species (Park et al., 2017), Kraft wood pulp (Wang et al. 2012).

MNFC is generally produced by combining different processes (Nechyporchuk, 2016): (1) homogenization (Herrick et al., 1983), grinding (Gane et al., 2010), refining (Henriksson et al., 2007) and extrusion (Ho et al., 2015) being the most common studied processes, (2) chemical and biological pre-treatments such as carboxylation via TEMPO oxidation (Bäckström et al. 2012). Depending on the applications, post-treatments can be used to modify MNFC, *i.e.* surface modification (Missoum et al. 2013). The high energy consumption of the whole processes remains the most important obstacle to wide use of MNFC. It has been shown that the energy consumption of homogenization process significantly decreases with pre-treatments (from 70 MWh/t (Eriksen et al. 2008) to 2 MWh/t (Lindström et al. 2011)).

Steam explosion (SteamEx) is a thermomechanical process, which is known as one of the most efficient pre-treatments of lignocellulosic materials (Sun and Cheng 2002). A batch steam explosion facility is composed of a steam generator that supplies a reactor with vapor to heat the biomass under pressure for short times. A sudden release of pressure ejects the biomass from the reactor to the explosion tank. This process is constituted of two phases: (1) steam treatment where water vapor and high temperature act together to initiate the depolymerization of hemicelluloses and degradation of lignin, (2) explosive defibration where the sudden depressurization provokes a rupture of the fiber cell wall due to shear and compression forces caused by the evaporation of the condensed water in the biomass (Cherian et al., 2008; Jacquet et al., 2010; Nechyporchuk, 2016). An economic evaluation carried out by Avellar and Glasser in 1998 showed that SteamEx is a low-cost pretreatment, even at small scale. In this study, the cost of the steam explosion process was evaluated to 0.077 \$/Kg dry basis (in 1998). As a result, this technology is currently developed at the industrial scale for the pretreatment of biomass for commodity and low-cost cellulosic bioethanol.

It has been reported that SteamEx significantly impacts the cell wall by converting a large part of hemicelluloses into soluble sugars (Chadni et al., 2019) and by degrading the lignin through aryl-ether bond hydrolysis (Obame et al., 2019). During SteamEx treatment, the breakdown of lignin produces small fragments of melted lignin which may redeposit on the surface of the fibers. This lignin relocation has been extensively reported in the literature and microscopy images of lignin droplets have been published (Takada et al. 2019; Chandra et al. 2016). In contrast to the other cell wall components, cellulose is relatively unaffected

by the treatment. It has been shown that SteamEx primarily in alkali conditions (Sauvageon et al., 2018; Sutka et al., 2013) degrades the cell middle lamella allowing the isolation of cellulosic fibers and micro-fibrils from biomass. In a pioneering work, Kessler et al. 1995 reported the production of linen from soda impregnated flax fibers by SteamEx. More recently, in our group (Sauvageon et al., 2018), we described the cottonization of hemp fibers by alkali SteamEx and the production of individual fibers with a diameter of about 20  $\mu\text{m}$ . Damaged fibers were also observed due to the conjugated effect of alkali degradation and steam explosion.

SteamEx has also been studied in various experimental conditions by other authors for the production of MNFC from fibrous biomass. Cherian et al. 2010 produced MNFC from pineapple leaves by steam treatment performed in an autoclave (0.13 MPa during one hour) followed by bleaching ( $\text{NaClO}$ ,  $\text{KMNO}_4$ ). Kaushik et Singh 2011 applied SteamEx (2 MPa, 4h) on wheat straw followed by bleaching ( $\text{H}_2\text{O}_2$ ), sonification and homogenization. Abraham et al. 2011 obtained MNFC from three different biomasses (banana, jute and pineapple leaf fibers) by combining SteamEx to alkaline extraction, bleaching and acid hydrolysis. Tuzzin et al. 2016 used tobacco to extract MNFC using SteamEx (1.2 MPa) followed by bleaching ( $\text{NaClO}$ ,  $\text{H}_2\text{O}_2$  at  $50^\circ\text{C}$ ) and grinding. Nevertheless, because of technical limitations, most of the described experiments were performed at relatively low steam pressure (around 0.1 – 1.2 MPa) using an autoclave. In such conditions the explosion step may be quite less efficient because it is not well controlled and/or optimized. When SteamEx was applied in SteamEx apparatus, low pressures and long residence times have been used leading to a long energy consuming process. In addition, there were no consistency between mentioned pressures and cooking temperatures.

Economically, *Eucalyptus globulus* is one of the most important forest species in Chile. Harvesting of Eucalyptus generates about 30% of residues (15–25 t/ha year), 10–12% of the residue volume corresponding to barks (Sartori et al., 2016). There are only few publications on the valorization of Eucalyptus bark (EB) but, recently, this resource has been considered as a new raw material for the pulp and paper industry due to its high fiber content (Miranda et al. 2012; Romaní et al. 2019). Fuentealba et al. 2016 have also demonstrated that the morphological and the physical properties of EB allow its use as an innovative insulation material.

To the best of our knowledge, no study has been conducted until now to produce L-MNFC from *Eucalyptus globulus* bark. In addition, no comprehensive study has been conducted to thoroughly investigate the specific effect of the SteamEx treatment on MNFC morphology and composition.

The aim of this study is to find a novel way to valorize *Eucalyptus globulus* barks considered as a harvesting residue. To this purpose, the feasibility of the production of lignin-containing micro and nanofibrillated cellulose (L-MNFC) from this new lignocellulosic feedstock has been tested. A combinative process including SteamEx as a pre-treatment has been used to examine the impact of the explosive decompression on the fiber's morphology (optical microscopy, Morfi Neo, AFM, turbidity and nanosized fraction) and the chemical composition. L-MNFC gels have been produced and their quality index have been calculated. Nanopapers were also produced and tested.

## 2 Materials and methods:

### 2.1 Materials:

*Eucalyptus globulus* barks (EB) used in this work were provided by Unidad de Desarrollo Tecnológico (UDT) located in Coronel, Biobio, Chile. They were defibered, dried and dust was removed. Sodium hydroxide used for the pulping was purchased from VWR chemicals (Czech Republic).

### 2.2 L-MNFC Preparation:

EB were soaked, during one night at room temperature, in water (WS) or soda solution (SS, w:w = 1:100) to allow the fibers to swell and facilitate the penetration of chemicals during pulping.

SteamEx has been tested to study the effect of this pre-treatment on the production process. For this purpose, the treatment was carried out in a high pressure SteamEx apparatus. The equivalent of 100 g of barks was impregnated in 200 mL of NaOH solution (20% wt.) for one night. Barks were heated to reach 200°C in the reactor (real temperature, corresponding effective pressure of 1,45 MPa) by injecting vapor steam from a boiler (2 MPa, heating temperature around 220°C) and heating was maintained for 8 minutes. A sudden decompression ejects barks to the explosion tank. The obtained exploded material was filtered and washed on a 40 µm mesh.

In order to get a reference, bark pulping was also performed by soda cooking (SC) in electrically heated rotating autoclaves. The equivalent of 100 g of dry barks and a sodium hydroxide solution (20% wt.) were placed in the autoclave (liquid/solid ratio = 6). The autoclave was hermetically closed and heated while rotating to reach 170°C. This temperature was maintained for 60 minutes. Autoclave was then cooled, and the pulp was filtered and washed on a 40 µm mesh.

The equivalent of 30 g dry pulp from soda cooking or SteamEx was then refined using a PFI mill until a drainage index of 70°SR (Schopper Riegler degree). Refining was accomplished until 6500-7500 and 8500 revolutions of the PFI mill disk for fibers produced by soda cooking and SteamEx, respectively.

A 2 % suspension of refined fibers was then grinded using Masuko grinder (Model MKZA6-2, Disk model MKG-C 80, Masuko Sangyo Co., Ltd, Japan) as following: 10 passes at 0 (750 rpm), 10 passes at -5 (1200 rpm), 15 passes at -10 (1500 rpm), 5 passes at -15 (1700 rpm), 2 passes at -20 (1700 rpm). The whole process is summarized in figure 1.

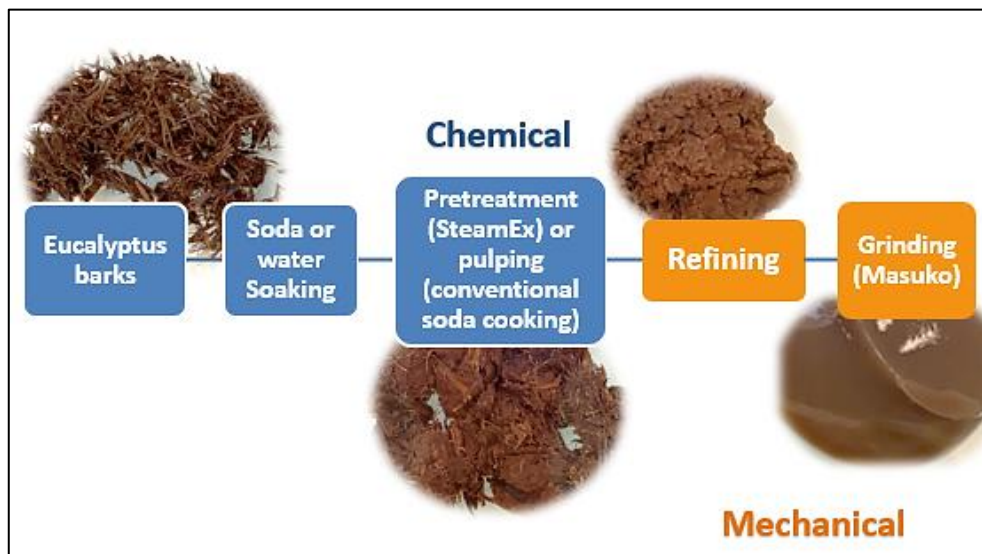


Figure 1: Schematic of the L-MNFC production process used in our study.

## 2.3 Characterizations:

Measurements in this part were at least triplicated except for soxhlet extraction and turbidity (1 and 10 measurements, respectively).

### 2.3.1 Morphological properties:

MorFi NEO is an analyzer of fiber morphology. This technology is based on image analysis of fiber suspension flowing through measuring cell. An optical system acquires images, which are processed by a computer. In this work, MorFi NEO was used to determine fibers length,

width and the percentage of fines obtained after refining and after Masuko grinding. Measurement time was fixed to 5 minutes and the adopted procedure considers fibers as elements with length higher than 200  $\mu\text{m}$  and fines as elements with length less than 200  $\mu\text{m}$ . For this purpose, different suspensions were prepared by dispersing 0.3 g (dry weight) of material in 1L of water (15 g of L-MNFC gel in 1L water).

### **2.3.2 Macrosized fraction:**

L-MNFC suspensions were diluted to 0.1% wt. (5 g of L-MNFC gel in 95 g deionized water) in then stirred by ultraturax (IKA®T-25) on speed 5 for 10 minutes. Images of each suspension were taken by a digital camera (AxioCam MRc 5) placed on an optical microscope (Carl Zeiss Axio Vert.A1, Germany). Images were analyzed by ImageJ to calculate average size of particles.

### **2.3.3 Nanosized fraction (NF):**

L-MNFC suspensions were diluted to 0.02% wt. (1 g of L-MNFC gel in 99 g deionized water) and stirred by ultraturax (IKA®T-25) on speed 5 for 6 minutes. 100g of suspension were centrifugated for 15 minutes at 4°C (velocity = 1000 g) using a Sigma 3-18 KS Germany centrifuge. To calculate NF, concentrations before ( $C_b$ ) and after ( $C_a$ ) centrifugation (in the supernatant phase) were measured:

$$Nf = 100 \times \frac{C_a}{C_b}$$

### **2.3.4 AFM:**

L-MNFC gels were observed using a BioScope Resolve atomic force microscope (Bruker) in Peak Force Tapping mode using SNL-C tips. For each sample, images were obtained on three different areas. L-MNFC suspensions were diluted to ( $10^{-5}$  % wt.) in deionized water. 200  $\mu\text{l}$  of each suspension were deposited on freshly cleaved mica substrates and let to dry overnight at 30°C before imaging.

### **2.3.5 Turbidity:**

The turbidity of L-MNFC suspensions (diluted to 0.1% wt. and stirred with ultraturax) was measured with a turbidimeter (Aqualytic, AL-250T-IT, wave-length 860 nm). This test is based on the determination of the scattered light at an angle of 90° to the incident light; values are directly linked to the shape, size, concentration and refractive index of the suspended matter.



### **2.3.6 Physical and mechanical properties of nanopapers**

Nanopapers were prepared with a handsheet former (Rapid Kothen, ISO 5269-2). The equivalent of 2g of dry L-MNFC (100 g of L-MNFC gel) was diluted to 0.5% in deionized water. Suspensions were filtered on 1  $\mu\text{m}$  nylon sieve under vacuum to remove water. The sheet was dried under vacuum at 85°C between two nylon sieves for 12 min. Nanopapers were stored for at least 48 hours in a conditioned room at 23°C and 50% RH before characterization.

Tensile properties were measured with a vertical extensometer (Instron 5965), following the standard NF Q03-004. Tensile tests were performed at 10 mm/min. The dimensions of the samples were 10 cm for the length and 15 mm for the width. The porosity (P) was calculated as following, considering that nanopaper is constituted of cellulose only:

$$P = 100 \times \left( 1 - \frac{\rho_{\text{sample}}}{\rho_{\text{cellulose}}} \right)$$

Where  $\rho_{\text{sample}}$  is the density (g/cm<sup>3</sup>) of the nanopaper and  $\rho_{\text{cellulose}}$  is equal to 1,5 g/cm<sup>3</sup>.

### **2.3.7 Simplified quality index:**

In a previous work, Desmaisons et al. 2017 developed an index that can be used as a quality control for MNFCs at the lab scale or industrial scale. This quality index was calculated according to the simplified formula:

$$QI = 0.30 x_1 + (-0.03 x_2) - 0.071 x_3^2 + 2.54 x_3 - 5.35 \ln x_7 + 59.9$$

with  $x_1$  representing the nanosized fraction (%),  $x_2$  the turbidity (NTU),  $x_3$  the Young's modulus (GPa) and  $x_7$  the macrosized fraction ( $\mu\text{m}^2$ ).

### **2.3.8 Chemical composition:**

Extraction of approximately 4 g of raw material or pulps was performed in a Soxhlet apparatus with toluene/ethanol mixture (2/1 v/v) under reflux, for 8 h. Extractives content was determined from the mass of the solid residue after drying at 105°C and reported as percent of the original sample.

In order to remove tannins and other polyphenols, alkaline lixiviation with 1% wt. NaOH was carried out in a stirred glass reactor under reflux using 1.0 g of the extractive-free material with a 1:50 solid:liquid ratio, at 100°C for 1h.

Klason lignin was determined on the extracted materials following an adapted method from National Renewable Energy Laboratory (NREL). Sulphuric acid (72%, 1.5 ml) was added to 0.175 g of the sample, and the mixture was placed in a water bath at 30°C for 1 h. Then, 42 mL of deionized water were added and the sample was hydrolyzed for 1 h at 120°C. The sample was vacuum filtered through a crucible and washed with ultrapure water until obtaining 100 mL of filtrate. Klason lignin was determined from the mass of solid residue dried at 105°C. The monomer sugar content in liquid fraction was analyzed using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (ICS-3000 Dionex).

### **2.3.9 Infrared spectroscopy:**

Fourier Transform Infrared (IR) spectra in Attenuated Total Reflexion mode (ATR) were recorded between 4000 and 700  $\text{cm}^{-1}$  on a Bruker Vertex 70v spectrometer equipped with a KBr beam splitter and a deuterated triglycine sulphate (DTGS) thermal detector. The resolution of the single beam spectra was 4  $\text{cm}^{-1}$ . A single-reflection diamond ATR accessory (Platinum ATR diamond, Bruker) was used for acquiring the spectra. The incidence angle was 45° and the refraction index of the crystal was 2.4. The number of bidirectional double-sided interferogram scans was 200, which corresponds to a two-minute accumulation. All the interferograms were Fourier processed using the Mertz phase correction mode and a Blackman-Harris three-term apodization function. No ATR correction was performed. IR-ATR spectra are shown with an absorbance scale corresponding to  $\log(R_{\text{reference}}/R_{\text{sample}})$ , where R is the internal reflectance of the device. Recording of the spectra, data storage and data processing were performed using the Bruker OPUS 7.8 software. The measurements were performed at  $21 \pm 1$  °C in an air-conditioned room. The obtained fibers after alkaline SteamEx and soda cooking in rotating autoclaves were laid on the ATR crystal and gently pushed on it to ensure a good contact between fibers and the diamond crystal. Water vapor subtraction was performed when necessary. The base line was corrected at 3600, 2750, 1800 and 800  $\text{cm}^{-1}$ . The spectra were normalized to one from region 1800-800  $\text{cm}^{-1}$ .

## **3 Results and discussion:**

The effect of SteamEx on the morphology and composition of lignocellulosic fibers has been extensively described in the literature. In a SteamEx treatment, the biomass is first impregnated and then treated with saturated steam at high pressure for a short period (only

few minutes). During the cooking step, a hydrolytic breakdown of the lignocellulosic complex occurs impacting primarily non-cellulosic polymers (hemicelluloses and lignin). The second step consists of a quick depressurization causing water expansion and physical degradation of the fibrous material. As a result, this process leads to a significant modification of the cell wall structure, degrading the cell middle lamella and allowing the extraction of cellulosic fibers from biomass. One of the most studied applications of SteamEx is the production of cellulose-rich residue bearing higher enzyme accessibility for bioethanol production. In this study, SteamEx has been experimented as a pre-treatment for L-MNFC production. Alkaline conditions have been used to promote the defibration by removing lignin cement. In order to identify the impact of SteamEx on the fibers, soda cooking performed in electrically-heated rotating autoclaves without explosive decompression step was conducted for comparison.

### **3.1 Pulping yield and chemical composition:**

Table 1 reports the pre-treatment and pulping conditions as well as the yield and the chemical composition of the different produced pulps.

In a surprising way, soda swelling had no visible effect on the chemical composition. It was expected that 1 %-NaOH impregnation will decrease the extractives content leading to a better response to treatments.

It appears that non-treated EB contains 15.1 % and  $19.9 \pm 1.4$  % toluene-EtOH and 1%-NaOH extractives, respectively. The lignin content is equal to  $14.5 \pm 0.5$  %. Cellulose and hemicellulose content are  $34.03 \pm 0.3$  % and  $14.7 \pm 0.0$  %, respectively.

The pulping yield after soda pulping was 58% and 52% for fibers soaked in water and 1% soda, respectively. Data in table 1 also shows that Toluene-EtOH and 1%-NaOH extractives content decreased to about 8 % and 12 %. After pulping, hemicellulose and lignin contents are close to 9 % and 6.5 %. Meanwhile, obtained pulps contains about 50 % of cellulose.

Chemical composition was also analyzed after alkaline SteamEx. Data in table 1 shows that pulps were obtained with a yield of 40-44 % wt. Toluene-EtOH and 1%-NaOH extractives content were close to 8 % and 10 %. Hemicellulose and lignin contents decreased to about 11 % and 9.0 %. Meanwhile cellulose content is 58.0-60.0 %.

Results thus show that alkaline SteamEx allowed the isolation of fibers with comparable chemical composition with those isolated with soda cooking method in autoclave (SC). The

advantage of SteamEx is the lower cooking time compared to SC (8 min and 60 min, respectively). Both approaches lead to hemicelluloses hydrolysis and partial delignification. Nevertheless, in the tested experimental conditions, the yield was higher for soda pulping even if delignification was greater. This observation is in accordance with the work of Lavoie et al. 2010 showing that the yield of kraft pulping was higher than that of SteamEx (34% and 30%, respectively).

NTEB, SC and SteamEx fibers were analyzed using IR-ATR to examine the changes in their chemical composition before and after the treatments (Figure 2). The bands at  $1750\text{ cm}^{-1}$  (Kaushik and Singh 2011), present in the spectrum of NTEB, is assigned to the vibrations of the carboxyl and acetyl groups of hemicelluloses. The absence of this band in the SC and SteamEx fibers is in accordance with the low hemicelluloses content previously observed (Table 1) and can also be justified by saponification reactions of acetyl moieties. Spectra also show a drastic decrease in intensity of the bands at  $1600\text{ cm}^{-1}$  and at  $1510\text{ cm}^{-1}$ , and can be attributed to the C=C stretching from the aromatic Guaiacyl (G), Syringyl (S) ring of the lignin, respectively (Carrillo et al., 2018). A decrease of the band at  $1260\text{ cm}^{-1}$  (Kaushik and Singh 2011) assigned to C-O stretching from lignin and hemicelluloses is also observed. All these observations confirm the hemicelluloses hydrolysis and the delignification during the soda pulping and the SteamEx. In region  $1200 - 1000\text{ cm}^{-1}$ , where mainly C-C and C-O stretching vibrations absorb, the general feature of the massif resembles to those of commercial cellulose (Richard et al., 2014). In addition, the anomeric band at  $896\text{ cm}^{-1}$ , specific for  $\beta$ -linkages in cellulose, is clearly resolved in the spectra of the treated samples.

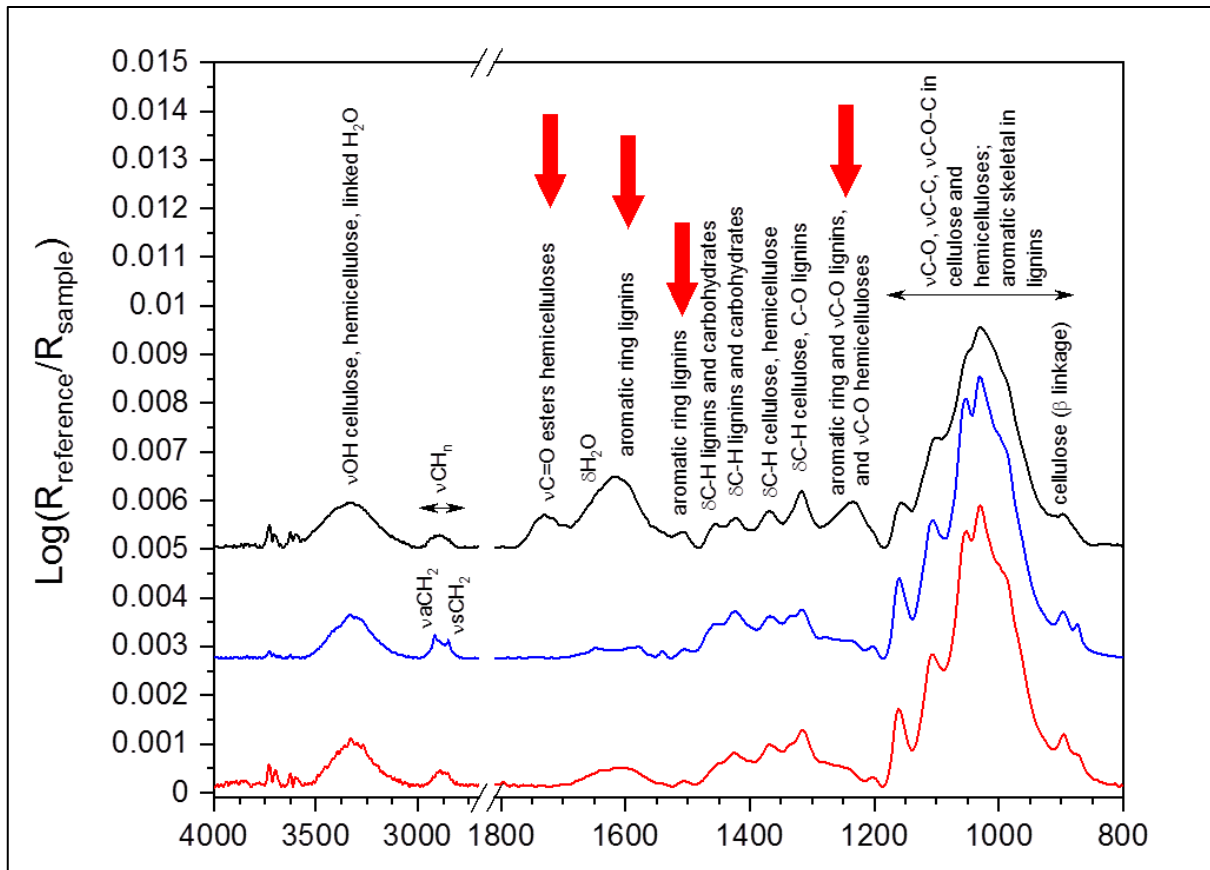


Figure 2: IR-ATR spectra of raw *Eucalyptus globulus* barks (dark), soda cooking in autoclave (red), and alkaline SteamEx (blue). Principal assignments are indicated. The spectra are normalized to one from region 1800-800  $\text{cm}^{-1}$ . For clarity, spectra were shifted vertically.

Key: n, stretching vibration; d, bending vibration.

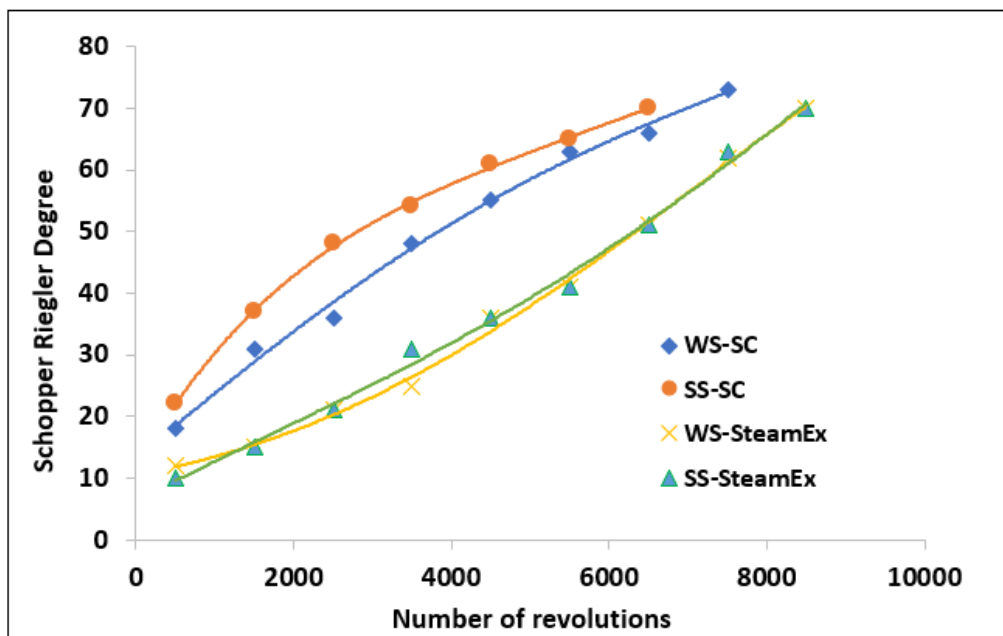
1 *Table 1: pulping conditions, yield and chemical composition of fibers obtained by alkaline SteamEx and soda cooking in rotating autoclaves*  
 2 *(values are given in wt% based on 100 g of pulp).*

Sample	Pretreatment	Temperature (°C)	Time (min)	Yield (%)	Chemical composition				
					Toluene-EtOH extractives	1%-NaOH extractives	Lignin	Hemicelluloses	Cellulose
EB		No treatment			15,1	19,9 ± 1,4	14,5 ± 0,5	14,7 ± 0,0	34,3 ± 0,3
WS-SC	soda cooking	170	60	58	8,0	12,9 ± 0,5	6,8 ± 0,4	8,2 ± 1,7	49,5 ± 5,1
SS-SC				52	8,4	11,7 ± 0,5	6,3 ± 0,6	10,9 ± 1,2	52,2 ± 3,4
WS-SteamEx	SteamEx	200	8	44	8,1	11,7 ± 0,5	8,6 ± 0,4	10,3 ± 0,4	58,0 ± 1,3
SS-SteamEx				40	8,2	8,0 ± 0,6	9,0 ± 0,4	11,8 ± 0,8	60,0 ± 4,3

### 3.2 Refining:

Figure 3 shows the evolution of the Schopper Riegler degree of the obtained pulps as a function of the number of revolutions of the PFI mill.

We can observe from that the four pulps do not display the same refining behavior. WS-SC and SS-SC pulps (soda cooking in autoclaves) were refined more easily than SteamEx pulps, a Schopper Riegler degree of 70 being reached after  $\approx 6000$  and  $\approx 8000$  revolutions respectively. These different behaviors can be explained, at least partially, by the slight difference in lignin content: lignin makes the separation of hemicelluloses and cellulose in the lignocellulosic matrix more difficult (Tuzzin et al., 2016), limits the swelling of fibers and thus negatively impacts the refining ability of the pulps.



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Figure 3: Evolution of Schopper Riegler degree for different pulps with the number of revolutions of the PFI mill disk. Key: WS, water swelling; SS, soda swelling; SC, soda cooking in autoclave.

### 3.3 Morphological properties:

Table 2 shows the data obtained from MorFi NEO analyzer. Before grinding, refined pulps exhibited important morphological differences: compared to SC fibers, SteamEx fibers were shorter ( $\approx 560 \mu\text{m}$  and  $\approx 400 \mu\text{m}$ , respectively) with higher fines contents ( $\approx 65\%$  and  $\approx 85\%$ , in length respectively). A higher number of fines (length  $< 200 \mu\text{m}$ ) was also detected  $5.3 - 6.3 \times 10^4$  and  $7.3 - 6.3 \times 10^4$  for SC and SteamEx, respectively. These results prove a most intense degradation of the fibers producing shorter fragments for the SteamEx process. The

23

24 mean total length was calculated for the refined samples. Results showed that the total  
 25 length was  $\approx 204\text{-}222 \mu\text{m}$  and  $\approx 77\text{-}90 \mu\text{m}$  for SC and SteamEx, respectively which revealed  
 26 that the remaining fibers after SteamEx were 2.5 times shorter than after SC. On the other  
 27 hand, the width of the SteamEx refined fibers is greater ( $\approx 19.5 \mu\text{m}$  and  $\approx 22.5 \mu\text{m}$ , for SC  
 28 and SteamEx respectively). This may be due to the higher content of lignin for SteamEx (see  
 29 table 1) leading to a lower separation of fibers.

30 Regarding the L-MNFC gels, grinding step had a levelling effect on the morphological  
 31 properties of the fibers produced but SteamEx residual fibers were still shorter ( $\approx 300 \mu\text{m}$   
 32 compared to  $360 \mu\text{m}$  for SC). As expected, the number of fibers (length  $> 200 \mu\text{m}$ ) detected  
 33 by MorFi decreased from  $\approx 12.2 - 13,5 \times 10^3$  in the refined pulps to  $\approx 0.6 - 3.0 \times 10^3$  in the gels  
 34 because of the grinding process which enhances the fragmentation of fibers into fines. Fines  
 35 content reached 98 % (in length) and SteamEx gels exhibited the highest proportion ( $21.7 -$   
 36  $24.9 \times 10^5$  fines/g of L-MNFC gel) compared to SC gels ( $5.27 - 5.85 \times 10^5$ ). Finally, the  
 37 difference in total mean length is also observed in the gels produced after grinding ( $\approx 33 - 38$   
 38  $\mu\text{m}$  and  $\approx 26 - 31 \mu\text{m}$  for SC and SteamEx, respectively). These observations are in  
 39 accordance with previously reported works : (Luo et al., 2018) found that length and fines  
 40 content of wind mill palm fibres were highly affected by SteamEx. (Sauvageon et al., 2018)  
 41 reported that alkali SteamEx performed from hemp fibres at high severity produced short  
 42 and damaged fibres.

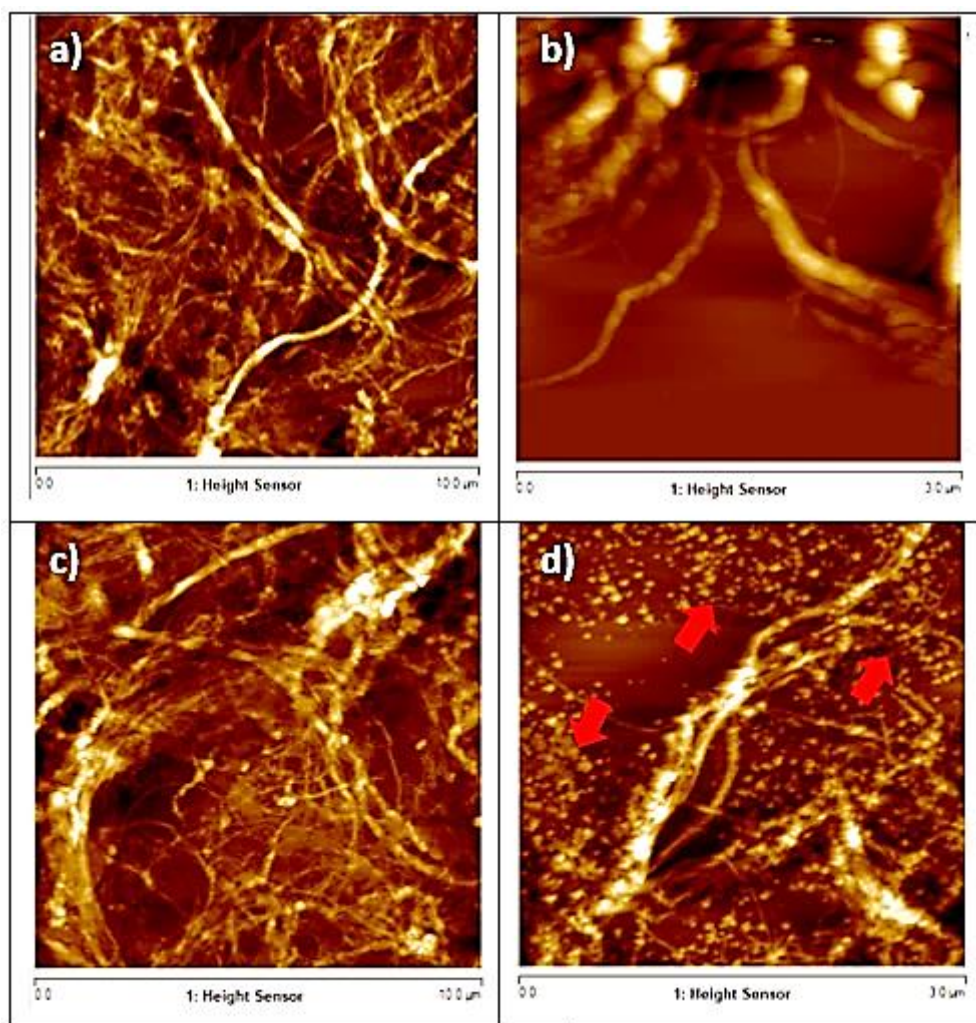
43 *Table 2: MorFi Analyzer results for refined pulps, and L-MNFC gels. Key: WS, water swelling;*  
 44 *SS, soda swelling; SC, soda cooking in autoclaves.*

Mechanical treatment	Sample	Fibers number (fibers/g)	Length ( $\mu\text{m}$ )	Width ( $\mu\text{m}$ )	Fines number (fines/g)	Fines content (%)	Fines length ( $\mu\text{m}$ )
Refining	WS-SC	$13.05 \times 10^3$	$558 \pm 2$	$19.8 \pm 0.1$	$6.26 \times 10^5$	67	31
	SS-SC	$12.74 \times 10^3$	$565 \pm 2$	$19.4 \pm 0.1$	$5.26 \times 10^5$	64	32
	WS-SteamEx	$13.51 \times 10^3$	$399 \pm 1$	$21.6 \pm 0.2$	$6.88 \times 10^5$	84	32
	SS-SteamEx	$12.27 \times 10^3$	$400 \pm 2$	$23.7 \pm 0.2$	$7.33 \times 10^5$	88	31
Masuko grinding	WS-SC	$0.61 \times 10^3$	$360 \pm 3$	$23,6 \pm 0.3$	$5.27 \times 10^5$	98	26
	SS-SC	$0.98 \times 10^3$	$355 \pm 3$	$23,0 \pm 0.3$	$5.85 \times 10^5$	97	28
	WS-SteamEx	$2.76 \times 10^3$	$307 \pm 2$	$21,7 \pm 0.5$	$24.9 \times 10^5$	99	27
	SS-SteamEx	$2.58 \times 10^3$	$293 \pm 4$	$22,0 \pm 0.6$	$21.7 \times 10^5$	99	25

45 AFM images of the samples (Fig 4a, b and d) showed a web like morphology with some  
 46 aggregates. The height of L-MNFC, calculated from AFM Images, varied between 5 and 100



47 nm and no significant difference was observed between SC and SteamEx samples. For the  
 48 SteamEx sample, dispersed globular particles are clearly visible (Fig. 4d). Based on literature,  
 49 they are most probably composed of lignin (Vignon et al. 1995; Ehman et al. 2020; Yuan et  
 50 al. 2021). In fact, during SteamEx, lignin is depolymerized through aryl-ether scission  
 51 producing small lignin fragments. The temperature of the SteamEx treatment being higher  
 52 than the glass transition temperature of lignin, the lignin becomes mobile and migrates  
 53 within the biomass sample. Due to its hydrophobic nature, lignin coalesces into spheres to  
 54 minimize its contact surface with water. This lignin translocation has been observed by  
 55 microscopic techniques in the form of droplets on the fiber surface.



56

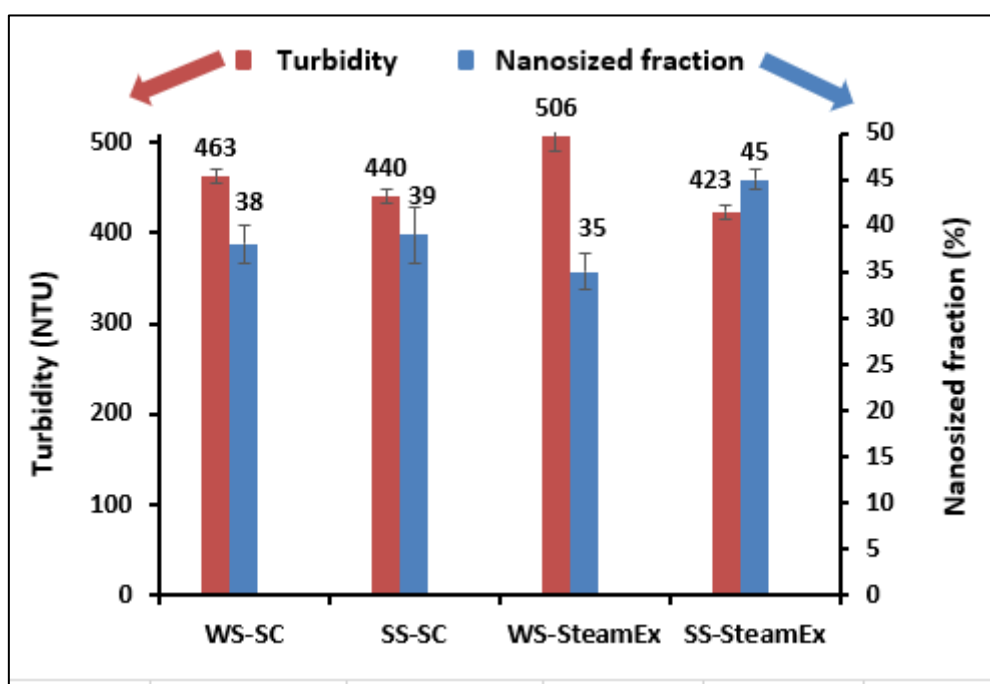
57 *Figure 4: AFM height images of (a) WS-SC 10 μm x 10 μm, (b) WS-SC 3 μm x 3 μm, (c), WS-SteamEx*  
 58 *10 μm x 10 μm and (d) WS-SteamEx 3 μm x 3 μm. Lignin droplets are marked by red arrows in d. Key:*  
 59 *WS, water swelling; SS, soda swelling; SC, soda cooking in autoclaves.*

60 The determination of the macrosized fraction was overestimated for the L-MNFC  
 61 suspensions obtained in our study, because of the broad size distribution of the samples

62 which was an obstacle for this measurement. However, there was no significant difference  
63 between the mean values ( $\approx 50 \mu\text{m}$ ) calculated for the different suspensions.

64 Figure 5 shows the turbidity and the nanosized fraction of the L-MNFC suspensions.

65 According to Nuopponen et al. 2013, the lower the turbidity value, the more nano-sized  
66 material the sample should contain. Accordingly, the turbidity results obtained agree with  
67 the proportion of the nanometric fraction: SS-SteamEx had the highest proportion of  
68 nanometric fraction and the lowest turbidity while the WS-SteamEx sample, had the lowest  
69 proportion of nanometric fraction and the highest turbidity. This higher turbidity value can  
70 be also explained by the higher amount of aggregates in WS-SteamEx samples. The lowest  
71 proportion of nanosized fraction is also in accordance with this observation. However, the  
72 differences between the samples remain low considering the standard deviations.



73

74 *Figure 5: Turbidity and nanosized fraction of different L-MNFC suspensions. Key: WS, water swelling;*  
75 *SS, soda swelling; SC, soda cooking.*

### 76 **3.4 Nanopapers**

77 Nanopapers have been prepared from L-MNFC gels produced in this study. Table 3 gives  
78 their physical and mechanical properties. The Young's moduli, quite similar for all the  
79 nanopapers (comprised between 8.42 and 9.45 GPa) are quite good when compared, for  
80 instance, to 6.3 GPa for nanopapers produced from lignin containing cellulose nanofibrils (L-  
81 CNF) obtained from rice straw (Taha et al. 2021). Nanopapers in this study were produced

82 from lignin containing cellulose nanofibrils obtained from rice straw. Young's moduli values  
83 of nanopapers obtained in our study are quite comparable to values obtained by Rojo et al.  
84 2015 (10 - 12 GPa). In their study L-CNF with different lignin contents (2 - 14%) were  
85 produced from Norway spruce using a combination of different mechanical processes. They  
86 showed that the produced nanopapers exhibited mechanical properties similar to fully  
87 bleached ones. The results also showed that the elongations at break for SteamEx L-MNFC  
88 nanopapers are considerably higher than those obtained by soda cooking. This was  
89 confirmed by the tensile strength results which were higher for SteamEx. This could be due  
90 to the higher proportion of fines which results in a larger surface area favoring hydrogen  
91 bonding between the fibrils (Kumar et al. 2014). Indeed, density of the SteamEx nanopapers  
92 is slightly higher even if the differences remain small. In the same way, nanopaper porosity  
93 varied between 20 % (SteamEx) and 26 % (SC). No significative correlation was found with L-  
94 MNFC composition in terms of cellulose, hemicelluloses and lignin. Interestingly, the  
95 relatively high lignin content remaining after SteamEx did not lead to the decrease of  
96 Young's moduli. This could be due to the relocation of the modified lignin during the  
97 explosive decompression previously discussed. In the literature, the effect of the presence of  
98 lignin in L-MNFC on the mechanical properties of nanopapers is still debated, the effect  
99 depending on the lignin content and the production process (Rojo et al., 2015; Albornoz-  
100 Palma et al., 2020). The presence of lignin nanoparticles in SteamEx L-MNFC could have  
101 increased the nanopaper density. In accordance with Bian et al. 2018, higher lignin content  
102 in SteamEx fibers facilitates the compaction during paper formation, leading to higher paper  
103 density and lower porosity. In our study, the dispersion of lignin nanoparticles, observed by  
104 AFM, could have also filled the voids within the cellulose fibrils, thus impacting nanopaper  
105 density. Moreover, analyses of the morphological properties (MorFi) showed that the  
106 number of fines is higher for SteamEx L-MNFC gels, which may also lead to an increase in the  
107 density. According to Henriksson et al. 2007, a less porous nanopaper results in a denser  
108 structure with higher mechanical properties. This trend was observed in our study for  
109 SteamEx L-MNFC where lignin nanoparticles filled the voids leading to a denser nanopaper  
110 with a higher tensile strength.

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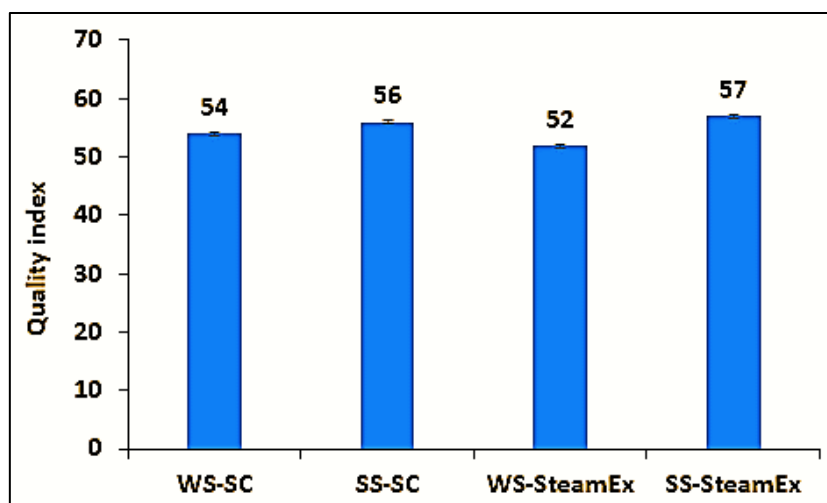
115 *Table 3: Mechanical and physical properties of nanopapers prepared from L-MNFC gels.*

M/NFC suspension	Young's modulus (GPa)	Strain at break (%)	Grammage (g/m <sup>2</sup> )	Tensile strength (MPa)	Density (Kg/m <sup>3</sup> )	Porosity (%)
WS-SC	8,67±0,09	1.6±0.1	65.21±0.44	78.4±0.56	1110±1.3	26
SS-SC	9,45±0,05	2.2±0.1	64.34±0.57	93.1±0.69	1110±1.7	26
WS-SteamEx	8,85±0,25	4.9±0.2	71.30±0.68	123±0.43	1220±1.7	19
SS-SteamEx	8,42±0,08	4.0±0.1	65.38±0.45	107±0.63	1180±1.3	21

116 **3.5 Quality index**

117 Figure 6 shows the quality index of the L-MNFC gels. This simplified quality index has been  
 118 defined as the sum of the marks of turbidity, nanosized fraction, macrosized fraction and  
 119 Young's modulus (see 2.3.7). The initial objective of this work was to propose an index,  
 120 based on relatively simple methods, and giving a quantitative evaluation of the quality of the  
 121 nanofibrillated cellulose gels at the macro- and microscales. In addition, the use of this index  
 122 is probably the most effective way to compare cellulose nanofibrils, in the absence of other  
 123 quantitative approaches.

124 Quality indexes were quite similar for all the produced gels and varied between 54-56 and  
 125 52-57 for SC and SteamEx, respectively. This result was expected since there was no  
 126 remarkable difference in the marks used in the calculations. The application of the same  
 127 ultrafine grinding conditions to different pulps (soda cooking or SteamEx) resulted in the  
 128 production of L-MNFC with comparable properties. The lower value for the WS-SteamEx  
 129 suspension is due to the highest turbidity value measured for this sample and to the lower  
 130 proportion of nanometric fraction. For the best of our knowledge, this index has been used  
 131 to compare bleached pulps and it has not been developed yet for lignin containing pulps.  
 132 Indexes calculated in this work are comparable to values of enzymatic commercial MNFCs  
 133 (quality index  $\approx$  60) (Desmaisons et al., 2017). This result showed that the proposed process  
 134 in this work leads to the production of good quality L-MNFC without bleaching or enzymatic  
 135 treatments.



136

137 *Figure 6: Quality index of L-MNFC suspensions. Key: WS, water swelling; SS, soda swelling; SC, soda*  
 138 *cooking.*

#### 139 **4 Conclusion:**

140 In this work, lignin-containing micro- and nano-fibrillated cellulose (L-MNFC) was produced  
 141 from Eucalyptus barks through a sequential process using steam explosion as pre-treatment.  
 142 Pulps were also obtained using soda cooking in rotating autoclaves to compare the different  
 143 production processes. In the tested experimental conditions, the obtained results showed  
 144 that the use of alkaline SteamEx allows to obtain pulps with comparable chemical  
 145 composition to conventional pulping methods but with a significantly lower cooking time. L-  
 146 MNFC exhibited web like morphology with fibrils width of 5-100 nm. AFM images showed  
 147 that SteamEx L-MNFC also contains lignin nanoparticles expelled during the explosive  
 148 decompression. The increase in density due to fines and lignin compensated the less ideal  
 149 fiber characteristics to achieve comparable properties. The results encourage the interest in  
 150 using alkaline SteamEx pre-treatment as an alternative way to produce L-MNFC comparable  
 151 to L-MNFC obtained by soda cooking, with respect to the quality index.

#### 152 **Authors contributions**

153 **Conceptualization:** Nicolas BROSSE, Evelyne MAURET and Saad NADER; **Methodology and**  
 154 **investigation:** Saad NADER, Malek KHADRAOUI, Isabelle ZIEGLER-DEVIN, Fabienne QUILES and  
 155 Sofiane EL KIRAT CHATEL; **Original draft preparation:** Saad NADER; **Review and editing:** Nicolas  
 156 BROSSE, Evelyne MAURET and Saad NADER; **Supervision:** Nicolas BROSSE and Evelyne MAURET;  
 157 **Funding acquisition:** Nicolas BROSSE, Evelyne MAURET and Cecilia FUENTEALBA

158 All authors have read and agreed to the published version of the manuscript.

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