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

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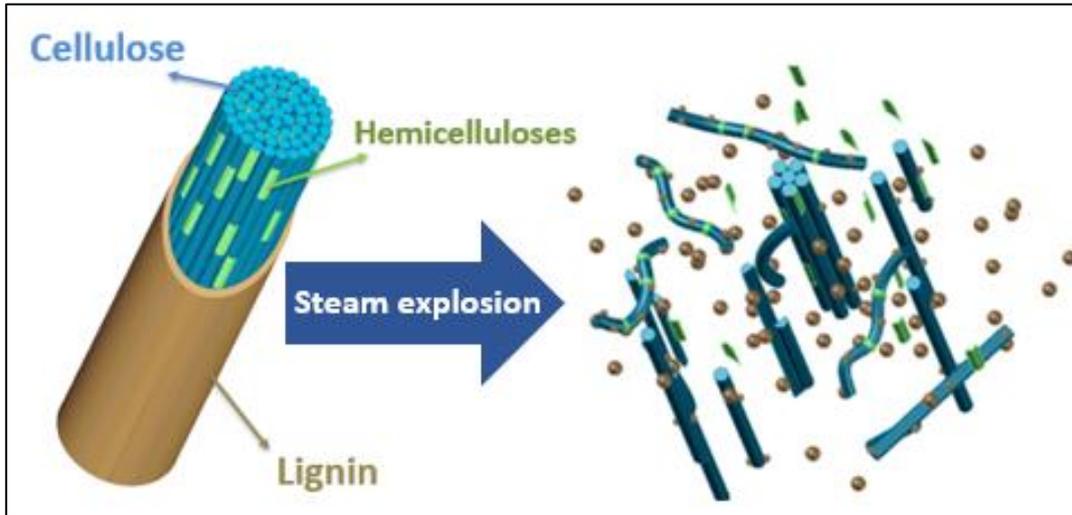
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15 **ABSTRACT**

16 Micro- and Nano-Fibrillated Cellulose (MNFCs) have gained an increasing attention due to
17 their remarkable properties, but their production usually requires an intensive multi-step
18 process. This study proposes to find a novel approach involving steam explosion for the
19 production of lignin-containing micro- and nano-fibrillated cellulose (L-MNFCs) using
20 *Eucalyptus globulus* bark as a new lignocellulosic feedstock. *Eucalyptus globulus* bark was
21 first pre-treated in alkali conditions by steam explosion in alkaline conditions (200°C, 8 min)
22 or by soda cooking in a rotating autoclave (170°C, 60 min), refined and then grinded until the
23 formation of gels. The chemical composition (Ionic chromatography, FTIR-ATR) of the pulps
24 and morphology of the products (Morfi Neo, Optical and Atomic Force Microscopies (AFM),
25 suspension turbidity) have been studied. Nanopapers were produced from lignin-containing
26 microfibrils to investigate the mechanical properties. Results obtained showed that steam
27 explosion produced pulps with slightly higher lignin content ($\approx 9\%$), containing shorter fibers
28 ($\approx 400\ \mu\text{m}$) and higher amounts of fine elements ($\approx 86\%$) compared to soda cooking ($\approx 5\%$,

29 $\approx 560 \mu\text{m}$ and 66 %, respectively). AFM images of SteamEx L-MNFC gels showed a web-like
 30 structure containing lignin nanoparticles.

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



33
 34

Graphical abstract

35 1 INTRODUCTION:

36 Micro- and Nano-Fibrillated Cellulose (MNFCs) constitute a new bio-based, renewable,
 37 biodegradable and nontoxic material. MNFCs width varies between approximately 10 and
 38 100 nm or even more depending on the raw material and the production process used, and
 39 their length can reach a few micrometers (TAPPI WI 3021). Owing to their high specific
 40 surface area and aspect ratio, MNFCs exhibit attractive physical and mechanical properties
 41 (165,166). As a result, MNFCs can be used in different applications, *i.e.* in paper
 42 reinforcement (Ahola et al. 2008; Dufresne 2012), nanocomposites (Siro and Plackett 2010),
 43 packaging (Lavoine et al. 2012), aerogels (Sehaqui et al. 2011), bio-medical (Lin and Dufresne
 44 2014), cosmetics (Ullah et al. 2016), etc. MNFCs are extracted from the wall of vegetal fibers
 45 and wood pulp was the first material used to produce them (Herrick et al., 1983).
 46 Researchers focused on the production of fully bleached MNFCs. Meanwhile, in the last
 47 years, isolation of lignin-containing micro and nanofibrillated cellulose (L-MNFC) was also
 48 investigated: different lignocellulosic feedstocks have been experimented, such as banana,
 49 pineapple, jute (Abraham et al., 2011), different wood species (Park et al., 2017), Kraft wood
 50 pulp (Wang et al. 2012), etc.

51 MNFCs are generally produced by combining different processes (Nechyporchuk, 2016): (1)
52 homogenization (Herrick et al., 1983), grinding (Gane et al., 2010), refining (Henriksson et al.,
53 2007), extrusion (Ho et al., 2015) being the most common studied processes, (2) chemical
54 and biological pre-treatments such as carboxylation via TEMPO oxidation (Bäckström et al.
55 2012). Depending on the applications, post-treatments can be used to modify MNFCs, *i.e.*
56 surface modification (Missoum et al. 2013). The high-energy consumption of the whole
57 processes remains the most important obstacle for MNFCs wide use. It has been shown that
58 the energy consumption of homogenization process significantly decreases with pre-
59 treatments (from 70 MWh/t (Eriksen et al. 2008) to 2 MWh/t (Lindström et al. 2011)).

60 Steam explosion (SteamEx) is a thermomechanical process, which is known as one of the
61 most efficient pre-treatments of lignocellulosic materials (Sun and Cheng 2002). A batch
62 steam explosion facility is composed of a steam generator that supplies a reactor with vapor
63 to heat the biomass under pressure for short times. A sudden release of pressure ejects the
64 biomass from the reactor to the explosion tank. This process is constituted of two phases: (1)
65 steam treatment where water vapor and high temperature acts together to initiate the
66 depolymerization of hemicelluloses and degradation of lignin, (2) explosive defibration
67 where the sudden depressurization provokes a rupture of the fiber cell wall due to shear and
68 compression forces caused by the evaporation of the condensed water in the biomass
69 (Cherian et al., 2008; Jacquet et al., 2010; Nechyporchuk, 2016). An economic evaluation
70 carried out by Avellar and Glasser in 1998 showed that SteamEx is a low-cost pretreatment,
71 even at small scale. In this study, the cost of the steam explosion process was evaluated to
72 0.077 \$/Kg dry basis (in 1998). As a result, this technology is currently developed at the
73 industrial scale for the pretreatment of biomass for commodity and low-cost cellulosic
74 bioethanol.

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

83 et al., 2018; Sutka et al., 2013) degrades the cell middle lamella allowing the isolation of
84 cellulosic fibers and micro-fibrils from biomass. In a pioneering work, Kessler et al. 1995
85 reported the production of linen from soda impregnated flax fibers by SteamEx. More
86 recently, in our group (Sauvageon et al., 2018), we described the cottonization of hemp
87 fibers by alkali SteamEx and the production of individual fibers with a diameter of about 20
88 μm . Damaged fibers were also observed due to the conjugated effect of alkali degradation
89 and steam explosion.

90 SteamEx has also been studied in various experimental conditions by other authors for the
91 production of MNFCs from fibrous biomass. Cherian et al. 2010 produced MNFCs from
92 pineapple leaves by steam treatment performed in an autoclave (0.13 MPa during one hour)
93 followed by bleaching (NaClO , KMNO_4). Kaushik et Singh 2011 applied SteamEx (2 MPa, 4h)
94 on wheat straw followed by bleaching (H_2O_2), sonification and homogenization. Abraham et
95 al. 2011 obtained MNFCs from three different biomasses (banana, jute and pineapple leaf
96 fibers) by combining SteamEx to alkaline extraction, bleaching and acid hydrolysis. Tuzzin et
97 al. 2016 used tobacco to extract MNFCs using SteamEx (1.2 MPa) followed by bleaching
98 (NaClO , H_2O_2 at 50°C) and grinding. Nevertheless, because of technical limitations, most of
99 the described experiments were performed at relatively low steam pressure (around 0.1 –
100 1.2 MPa) using an autoclave. In such conditions the explosion step may be quite less efficient
101 because it is not well controlled and/or optimized. When SteamEx was applied in SteamEx
102 apparatus, low pressures and long residence times have been used leading to a long energy
103 consuming process. In addition, there were no consistency between mentioned pressures
104 and cooking temperatures.

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

113 To the best of our knowledge, no study has been conducted until now to produce L-MNFCs
114 from *Eucalyptus globulus* bark. In addition, no comprehensive study has been conducted to

115 thoroughly investigate the specific effect of the SteamEx treatment on MNFCs morphology
116 and composition.

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

125 2 Materials and methods:

126 2.1 Materials:

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

131 2.2 L-MNFC Preparation:

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

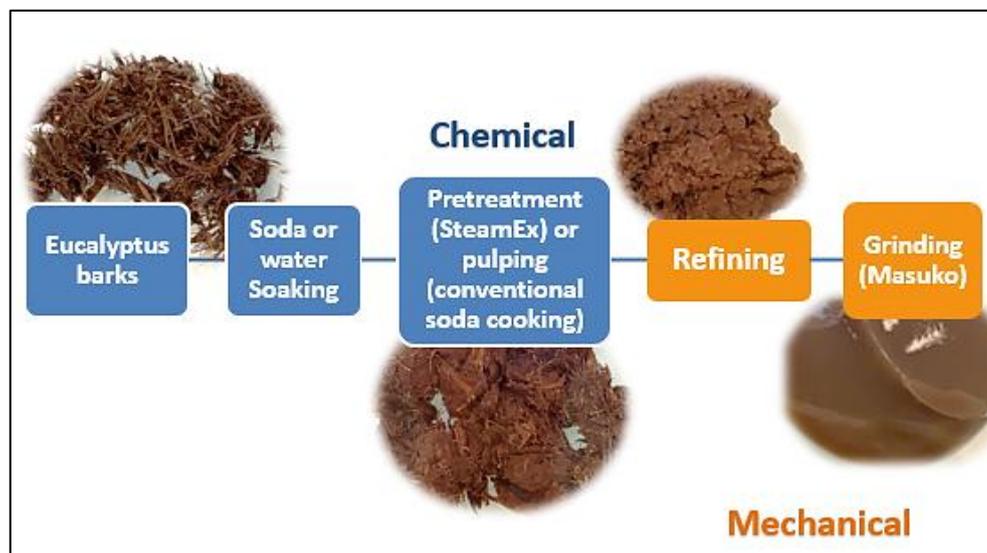
135 SteamEx has been tested to study the effect of this pre-treatment on the production
136 process. For this purpose, the treatment was carried out in a high pressure SteamEx
137 apparatus. The equivalent of 100 g of barks was impregnated in 200 mL of NaOH solution
138 (20% wt.) for one night. Barks were heated to reach 200°C in the reactor (real temperature,
139 corresponding effective pressure of 1,45 MPa) by injecting vapor steam from a boiler (20
140 bar, heating temperature around 220°C) and heating was maintained for 8 minutes. A
141 sudden decompression ejects barks to the explosion tank. The obtained exploded material
142 was filtered and washed on a 40 µm mesh.

143 In order to get a reference, bark pulping was also performed by soda cooking (SC) in
144 electrically heated rotating autoclaves. The equivalent of 100 g of dry barks and a sodium
145 hydroxide solution (20% wt.) were placed in the autoclave (liquid/solid ratio = 6). The

146 autoclave was hermetically closed and heated while rotating to reach 170°C. This
 147 temperature was maintained for 60 minutes. Autoclave was then cooled, and the pulp was
 148 filtered and washed on a 40 µm mesh.

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

153 A 2 % suspension of refined fibers was then grinded using a Masuko grinder (Model MKZA6-
 154 2, Disk model MKG-C 80, Masuko Sangyo Co., Ltd, Japan) as following: 10 passes at 0 (750
 155 rpm), 10 passes at -5 (1200 rpm), 15 passes at -10 (1500 rpm), 5 passes at -15 (1700 rpm), 2
 156 passes at -20 (1700 rpm). The whole process is summarized in figure 1.



157

158

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

159 **2.3 Characterizations:**

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

162 **2.3.1 Morphological properties:**

163 MorFi NEO is an analyzer of fiber morphology. This technology is based on image analysis of
 164 a fiber suspension flowing through a measuring cell. An optical system acquires images,
 165 which are processed by a computer. In this work, MorFi NEO was used to determine the
 166 fiber length, fiber width and the percentage of fine elements of the suspensions obtained
 167 after refining and after Masuko grinding. Measurement time was fixed to 5 minutes and the
 168 adopted procedure considers a fiber as an element of length higher than 200 µm and fine

169 elements as elements of length less than 200 μm . For this purpose, different suspensions
170 were prepared by dispersing 0.3 g (dry weight) of material in 1L of water (15 g of L-MNFC gel
171 in 1L water).

172 **2.3.2 Macrosized fraction:**

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

178 **2.3.3 Nanosized fraction (NF):**

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

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

184 **2.3.4 AFM:**

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

190 **2.3.5 Turbidity:**

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

196 **2.3.6 Physical and mechanical properties of nanopapers**

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

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

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

207 Where ρ_{sample} is the density (g/cm³) of the nanopaper and
 208 $\rho_{\text{cellulose}}$ is equal to 1,5 g/cm³.

209 **2.3.7 Simplified quality index:**

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

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

213 with x_1 representing the nanosized fraction (%), x_2 the turbidity (NTU), x_3 the Young's
 214 modulus (GPa) and x_7 the macrosized fraction (μm^2).

215 **2.3.8 Chemical composition:**

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

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

223 Klason lignin was determined on the extracted materials following an adapted method from
 224 National Renewable Energy Laboratory (NREL). Sulphuric acid (72%, 1.5 ml) was added to

225 0.175 g of the sample, and the mixture was placed in a water bath at 30°C for 1 h. Then, 42
226 mL of deionized water were added and the sample was hydrolyzed for 1 h at 120°C. The
227 sample was vacuum filtered through a crucible and washed with ultrapure water until
228 obtaining 100 mL of filtrate. Klason lignin was determined from the mass of the solid residue
229 after drying at 105°C. The monomer sugar content in liquid fraction was analyzed using high-
230 performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-
231 PAD) (ICS-3000 Dionex).

232 **2.3.9 Infrared spectroscopy:**

233 Fourier Transform Infrared (IR) spectra in Attenuated Total Reflexion mode (ATR) were
234 recorded between 4000 and 700 cm^{-1} on a Bruker Vertex 70v spectrometer equipped with a
235 KBr beam splitter and a deuterated triglycine sulphate (DTGS) thermal detector. The
236 resolution of the single beam spectra was 4 cm^{-1} . A single-reflection diamond ATR accessory
237 (Platinum ATR diamond, Bruker) was used for acquiring the spectra. The incidence angle was
238 45° and the refraction index of the crystal was 2.4. The number of bidirectional double-sided
239 interferogram scans was 200, which corresponds to a two-minute accumulation. All the
240 interferograms were Fourier processed using the Mertz phase correction mode and a
241 Blackman-Harris three-term apodization function. No ATR correction was performed. IR-ATR
242 spectra are shown with an absorbance scale corresponding to $\log(R_{\text{reference}}/R_{\text{sample}})$, where R
243 is the internal reflectance of the device. Recording of the spectra, data storage and data
244 processing were performed using the Bruker OPUS 7.8 software. The measurements were
245 performed at 21 ± 1 °C in an air-conditioned room. The obtained fibers after alkaline SteamEx
246 and soda cooking in rotating autoclaves were laid on the ATR crystal and gently pushed on it
247 to ensure a good contact between fibers and the diamond crystal. Water vapor subtraction
248 was performed when necessary. The base line was corrected at 3600, 2750, 1800 and 800
249 cm^{-1} . The spectra were normalized to one from region 1800-800 cm^{-1} .

250 **3 Results and discussion:**

251 The effect of SteamEx on the morphology and composition of lignocellulosic fibers has been
252 extensively described in the literature. In a SteamEx treatment, the biomass is first
253 impregnated and then treated with saturated steam at high pressure for a short period (only
254 few minutes). During the cooking step, a hydrolytic breakdown of the lignocellulosic complex
255 occurs impacting primarily non-cellulosic polymers (hemicelluloses and lignin). The second

256 step consists of a quick depressurization causing water expansion and physical degradation
257 of the fibrous material. As a result, this process leads to a significant modification of the cell
258 wall structure, degrading the cell middle lamella and allowing the extraction of cellulosic
259 fibers from biomass. One of the most studied applications of SteamEx is the production of a
260 cellulose-rich residue bearing a higher enzyme accessibility for bioethanol production. In this
261 study, SteamEx has been experimented as a pre-treatment s for L-MNFC gel production.
262 Alkaline conditions have been used to promote the defibration by removing lignin cement. In
263 order to identify the impact of SteamEx on the fibers, soda cooking performed in electrically-
264 heated rotating autoclaves without explosive decompression step was conducted for
265 comparison.

266 **3.1 Pulping yield and chemical composition:**

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

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

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

275 The pulping yield after soda pulping was 58% wt. and 52% wt. for fibers soaked in water and
276 1%-wt. soda, respectively. The data in table 1 also shows that Toluene-EtOH and 1%-NaOH
277 extractives content decreased to about 8 % and 12 %. After pulping, hemicellulose and lignin
278 contents are close to 9 % and 6.5 %. Meanwhile, obtained pulps contains about 50 % of
279 cellulose.

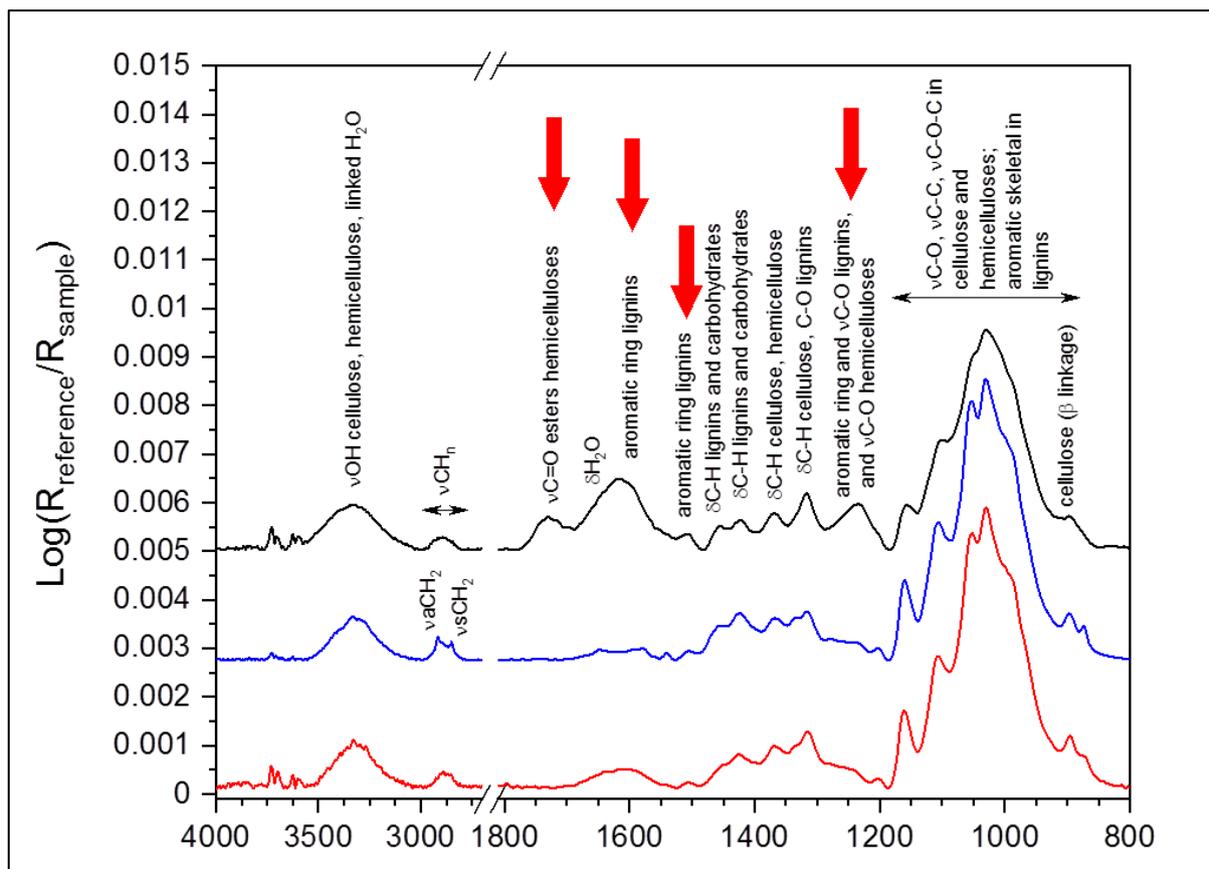
280 Chemical composition was also analyzed after alkaline SteamEx. Data in table 1 shows that
281 pulps were obtained with a yield of 40-44 % wt. Toluene-EtOH and 1% NaOH extractives
282 content are close to 8 % and 10 %. Hemicellulose and lignin contents decreased to about 11
283 % and 9.0 %. Meanwhile cellulose content is 58.0-60.0 %.

284 Results thus show that alkaline SteamEx allowed the isolation of fibers with comparable
285 chemical composition with those isolated with soda cooking method in autoclaves (SC). The
286 advantages of SteamEx is that the cooking time is lower compared to SC (8 min and 60 min,

287 respectively). Both approaches lead to hemicellulose hydrolysis and partial delignification.
288 Nevertheless, in the tested experimental conditions, the yield is higher for soda pulping even
289 if delignification is greater. This observation is in accordance with the work of Lavoie et al.
290 2010 showing that the yield of kraft pulping was higher than that of SteamEx (34% and 30%,
291 respectively).

292 NTEB, SC and SteamEx fibers were analyzed using IR-ATR to examine the changes in their
293 chemical composition before and after the treatments (Figure 2). The bands at 1750 cm^{-1}
294 (Kaushik and Singh 2011), present in the spectrum of NTEB, is assigned to the vibrations of
295 the carboxyl and acetyl groups of hemicelluloses. The absence of this band in the SC and
296 SteamEx fibers is in accordance with the low hemicelluloses content previously observed
297 (Table 1) and can also be justified by saponification reactions of acetyl moieties. Spectra also
298 show a drastic decrease in intensity of the bands at 1600 cm^{-1} and at 1510 cm^{-1} , and can be
299 attributed to the C=C stretching from the aromatic Guaiacyl (G), Syringyl (S) ring of the lignin,
300 respectively (Carrillo et al., 2018). A decrease of the band at 1260 cm^{-1} (Kaushik and Singh
301 2011) assigned to C-O stretching from lignin and hemicelluloses is also observed. All these
302 observations confirm the hemicelluloses hydrolysis and the delignification during the soda
303 pulping and the SteamEx. In region $1200 - 1000\text{ cm}^{-1}$, where mainly C-C and C-O stretching
304 vibrations absorb, the general feature of the massif resembles to those of commercial
305 cellulose (Richard et al., 2014). In addition, the anomeric band at 896 cm^{-1} , specific for β -
306 linkages in cellulose, is clearly resolved in the spectra of the treated samples.

307



308
 309 Figure 2: IR-ATR spectra of raw *Eucalyptus globulus* barks (dark), soda cooking in autoclave
 310 (red), and alkaline SteamEx (blue). Principal assignments are indicated. The spectra are
 311 normalized to one from region 1800-800 cm^{-1} . For clarity, spectra were shifted vertically.
 312 Key: n, stretching vibration; d, bending vibration.

313
314

Table 1: pulping conditions, yield and chemical composition of fibers obtained by alkaline SteamEx and soda cooking in rotating autoclaves (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 Fig. 3 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 ≈ 6000 revolutions and ≈ 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.

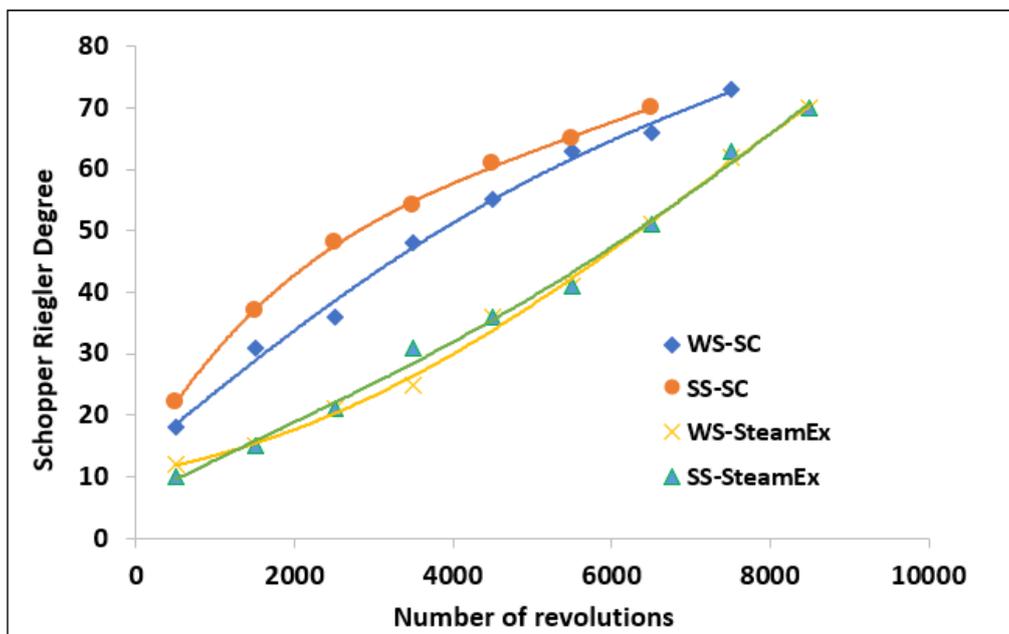


Figure 3: Evolution of Schopper Riegler degree for different pulps with the number of revolutions of the PFI mill disk. Key: WS, water swelling; SS, soda swelling; SC, soda cooking in autoclave.

3.3 Morphological properties:

Table 2 shows the data obtained from MorFi NEO analyzer. Before grinding, the 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 fine elements contents ($\approx 65\%$ and $\approx 85\%$, in length respectively). A higher number of fine elements (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 with a production of shorter fragments for the SteamEx process. The mean total length was calculated for the refined

samples. Results showed that the total length was $\approx 204\text{-}222 \mu\text{m}$ and $\approx 77\text{-}90 \mu\text{m}$ for SC and SteamEx, respectively which revealed that the remaining fibers after SteamEx were 2.5 times shorter than after SC. On the other 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 and SteamEx respectively). This may be due to the higher content of lignin for SteamEx (see table 1) leading to a lower separation of fibers.

Regarding the L-MNFC gels, grinding step had a levelling effect on the morphological properties of the fibers produced but SteamEx residual fibers were still shorter ($\approx 300 \mu\text{m}$ compared to $360 \mu\text{m}$ for SC). As expected, the number of fibers (length $> 200 \mu\text{m}$) detected 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 because of the grinding process which enhances the fragmentation of fibers into finer elements. The fine element content reached 98 % (in length) and SteamEx gels exhibited the highest number of fine elements ($21.7 - 24.9 \times 10^5$) compared to SC gels ($5.27 - 5.85 \times 10^5$). Finally, the difference in total mean length is also observed in the gels produced after grinding ($\approx 33 - 38 \mu\text{m}$ and $\approx 26 - 31 \mu\text{m}$ for SC and SteamEx, respectively). These observations are in accordance with previously reported works : (Luo et al., 2018) found that length and fine content of wind mill palm fibres were highly affected by SteamEx. (Sauvageon et al., 2018) reported that alkali SteamEx performed from hemp fibres at high severity produced short and damaged fibres.

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

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

AFM images of the samples (Fig 4a, b and d) showed a web like morphology with some aggregates. The height of L-MNFCs, calculated from AFM Images, varied between 5 and 100 nm and no significant difference was observed between SC and SteamEx samples. For the

SteamEx sample, dispersed globular particles are clearly visible (Fig. 4d). Based on literature data, they are most probably composed of lignin (Vignon et al. 1995; Ehman et al. 2020; Yuan et al. 2021). In fact, during SteamEx, lignin is depolymerized through aryl-ether scission producing small lignin fragments. The temperature of the SteamEx treatment being higher than the glass transition temperature of lignin, the lignin becomes mobile and migrates within the biomass sample. Due to its hydrophobic nature, lignin coalesces into spheres to minimize its contact surface with water. This lignin translocation has been observed by microscopic techniques in the form of droplets on the fiber surface.

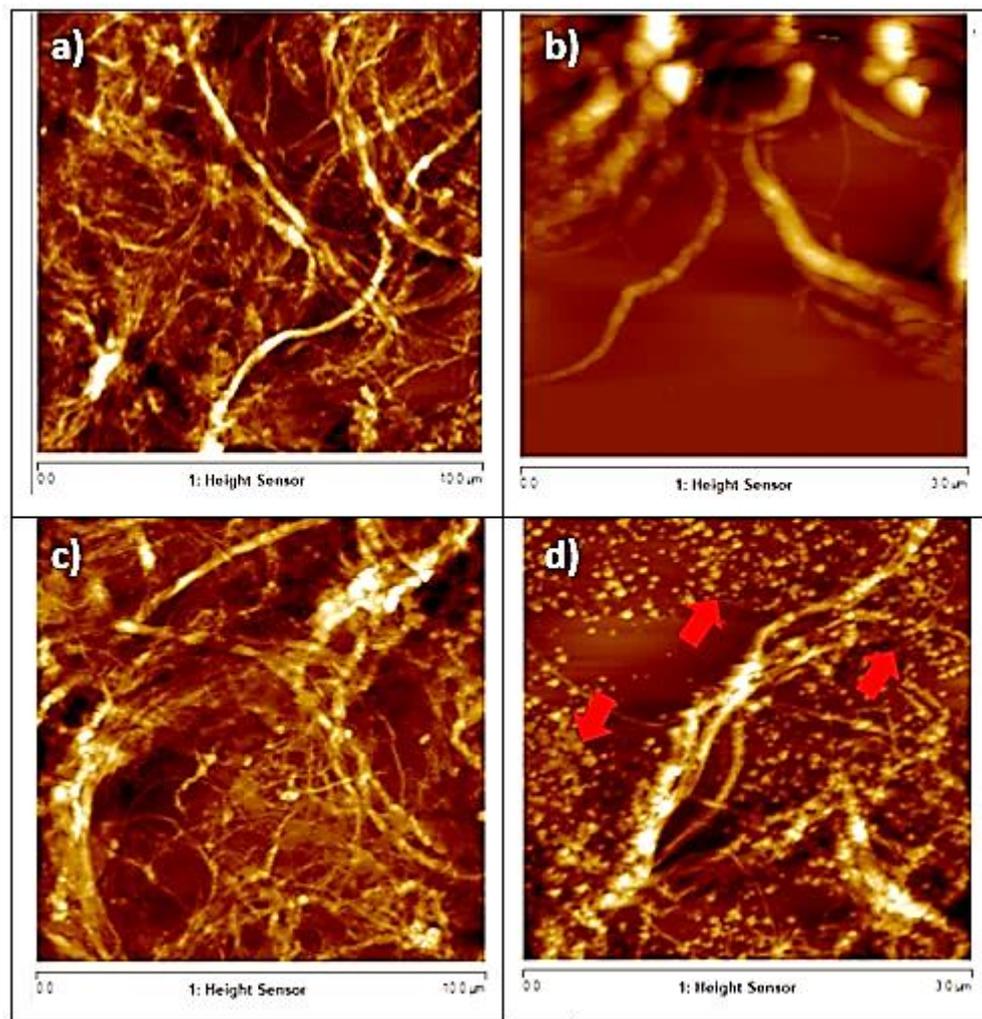


Figure 4: AFM height images of (a) WS-SC 10 $\mu\text{m} \times 10 \mu\text{m}$, (b) WS-SC 3 $\mu\text{m} \times 3 \mu\text{m}$, (c), WS-SteamEx 10 $\mu\text{m} \times 10 \mu\text{m}$ and (d) WS-SteamEx 3 $\mu\text{m} \times 3 \mu\text{m}$. Lignin droplets are marked by red arrows in d. Key: WS, water swelling; SS, soda swelling; SC, soda cooking in autoclaves.

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

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

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

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

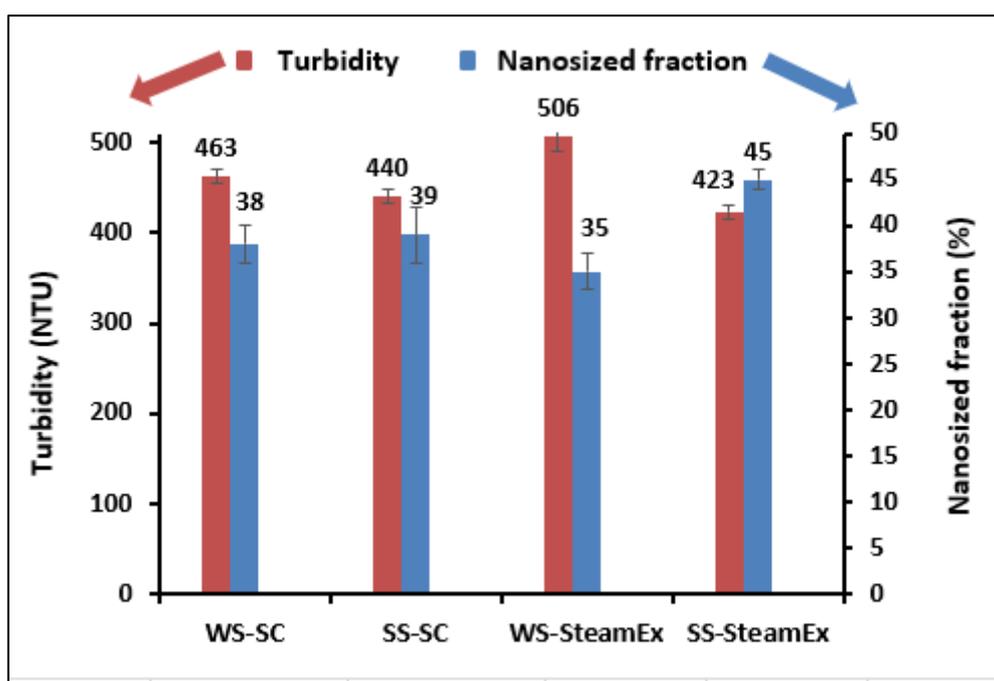


Figure 5: Turbidity and nanosized fraction of different L-MNFC suspensions. Key: WS, water swelling; SS, soda swelling; SC, soda cooking.

3.4 Nanopapers

Nanopapers have been prepared from L-MNFC gels produced in this study. Table 3 gives their physical and mechanical properties. The Young's moduli, quite similar for all the nanopapers (comprised between 8.42 and 9.45 GPa) are quite good when compared, for instance, to 6.3 GPa for nanopapers produced from lignin containing cellulose nanofibrils (L-

CNF) obtained from rice straw (Taha et al. 2021). Nanopapers in this study were produced from lignin containing cellulose nanofibrils obtained from rice straw. Young's moduli values of nanopapers obtained in our study are quite comparable to values obtained by Rojo et al. 2015 (10 - 12 GPa). In their study L-CNF with different lignin contents (2 - 14%) were produced from Norway spruce using a combination of different mechanical processes. They showed that the produced nanopapers exhibited mechanical properties similar to fully bleached ones. The results also showed that the elongations at break for SteamEx L-MNFCs nanopapers are considerably higher than those obtained by soda cooking. This was confirmed by the tensile strength results which were higher for SteamEx. This could be due to the higher proportion of fine elements which results in a larger surface area favoring hydrogen bonding between the fibrils (Kumar et al. 2014). Indeed, density of the SteamEx nanopapers is slightly higher even if the differences remain small. In the same way, nanopaper porosity varied between 20 % (SteamEx) and 26 % (SC). No significative correlation was found with L-MNFCs composition in terms of cellulose, hemicelluloses and lignin. Interestingly, the relatively high lignin content remaining after SteamEx did not lead to the decrease of Young's moduli. This could be due to the relocation of the modified lignin during the explosive decompression previously discussed. In the literature, the effect of the presence of lignin in L-MNFC on the mechanical properties of nanopapers is still debated, the effect depending on the lignin content and the production process (Rojo et al., 2015; Albornoz-Palma et al., 2020). The presence of lignin nanoparticles in SteamEx L-MNFC could have increased the nanopaper density. In accordance with Bian et al. 2018, higher lignin content in SteamEx fibers facilitates the compaction during paper formation, leading to higher paper density and lower porosity. In our study, the dispersion of lignin nanoparticles, observed by AFM, could have also filled the voids within the cellulose fibrils, thus impacting nanopaper density. Moreover, analyses of the morphological properties (MorFi) showed that the number of fine elements is higher for SteamEx L-MNFC gels, which may also lead to an increase in the density. According to Henriksson et al. 2007, a less porous nanopaper results in a denser structure with higher mechanical properties. This trend was observed in our study for SteamEx L-MNFCs where lignin nanoparticles filled the voids leading to a denser nanopaper with a higher tensile strength.

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/m²)	Tensile strength (MPa)	Density (Kg/m³)	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

3.5 Quality index

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

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

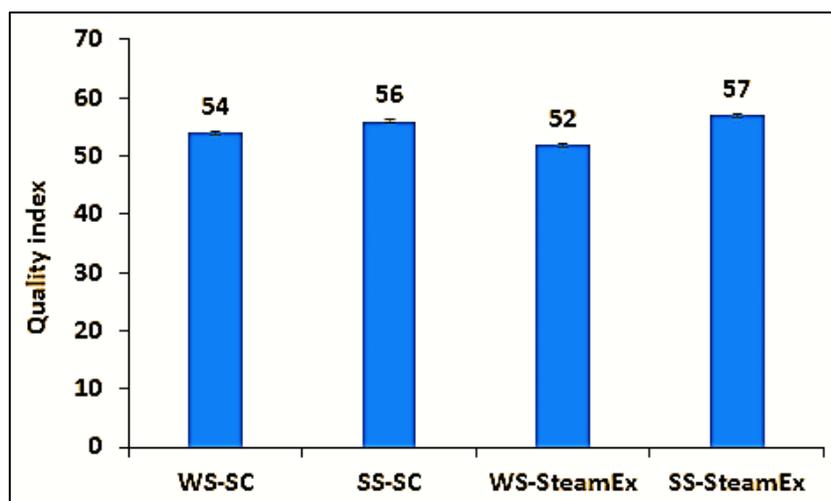


Figure 6: Quality index of L-MNFC suspensions. Key: WS, water swelling; SS, soda swelling; SC, soda cooking.

4 Conclusion:

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

Authors contributions

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

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

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