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**Lignin containing micro and nano-fibrillated cellulose obtained by Steam explosion:
comparative study between different process**

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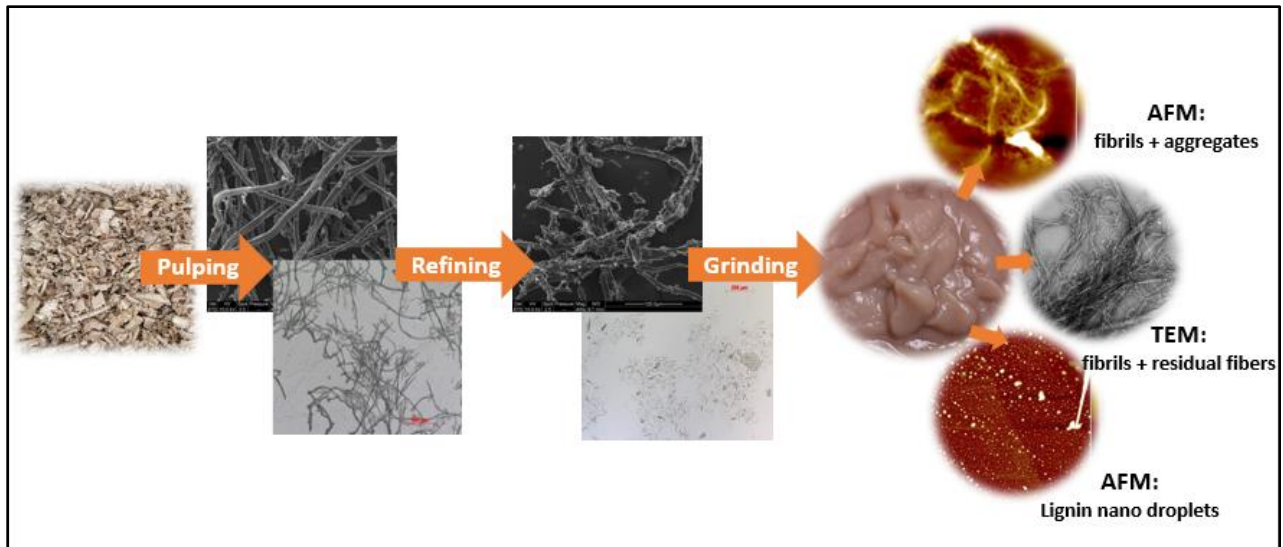
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ABSTRACT

The isolation of lignin containing micro and nanofibrillated cellulose (L-MNFCs) requires a multistep process. In this study, beech wood was pre-treated by steam explosion (SE), refined or pretreated by 3 SE and grinded until gel formation. A conventional cooking in an autoclave (SC) was applied in order to get a control sample. The effect of bleaching of a SE pulp was studied also. The chemical composition of the pulps was assessed as well as morphology to compare the effect of the different process. Quality index and energy consumption during the production process were studied. Results showed that SE can replace SC for the production of pulps with higher lignin content = 8-12 % wt. and 3 % wt., respectively. Gels contains micro and nanofibrillated cellulose and residual fibers with a dispersion of lignin nanoparticles and some nanocrystals. Optimization of the ultrafine grinding step was required to minimize the energy consumption.

Key words: lignin containing micro and nano-fibrillated cellulose; Steam explosion; Beech wood; ultrafine grinding.



Graphical abstract

1 INTRODUCTION:

With the growth of environmental concerns, researchers are focusing on the replacement of oil-based products by bio-based ones. Cellulose is the most abundant renewable polymer on earth. Owing to its high specific surface area, nanocelluloses, which are extracted from the wall of vegetal fibers, presents important physical and mechanical properties at the nanoscale (Dufresne, 2012; Klemm et al., 2011). Nanocellulose is composed of two main grades: cellulose nanocrystals (CNCs) and cellulose nanofibers (CNFs) known also as micro and nanofibrillated cellulose (MNFCs). MNFCs are a relatively new biobased, renewable, biodegradable and non-toxic material, ranging from 10 to 100 nm in width and a few micrometers in length. They are of great interest for many innovative applications (Nechyporchuk 2016; Rol et al. 2019). Researchers have mainly focused over the years on the production of fully bleached MNFCs. More recently, isolation of lignin containing micro and nano-fibrillated cellulose (L-MNFC) has been studied. This new field of research has the advantage of being straighter forward, minimizing the use of chemicals. L-MNFCs are extracted from the wall of vegetal fibers and different lignocellulosic feedstocks have been experimented, such as banana, pineapple, jute (Abraham et al., 2011), barks (Chen et al., 2019; Nader et al., 2022), different wood species (Park et al., 2017), etc. Wang et al. (2012) used kraft wood pulps to produce L-MNFCs samples with 5 and 10 % lignin content by acid hydrolysis followed by homogenization. Herrera et al. (2018) produced L-MNFCs with 23% lignin content from Eucalyptus pulp using catalyzed chemical oxidation followed by high-

pressure microfluidization. Wen et al. (2019) worked on the isolation of L-MNFCs with lignin content varying between 15.5 and 23.15% from a poplar pulp using TEMPO oxidation followed by homogenization. It has been concluded from these different works that the produced L-MNFCs present hydrophobic properties due to the presence of lignin. In the manufacture of composites, the hydrophobic property gave a better compatibility with the polymer matrix resulting in better mechanical properties.

Due to their remarkable properties, L-MNFCs can be used in different applications such as paper industry (Delgado-Aguilar et al., 2016), MNFC films (Horseman et al., 2017; Rojo et al., 2015), reinforcing fillers in composites (Sun et al. 2014; Wang et al. 2014), wood adhesive reinforcement (Nader et al., 2022).

Steam explosion (SE) is one of the most efficient pre-treatments of lignocellulosic materials (Sun and Cheng 2002). It is a thermomechanical process constituted of two phases (Cherian et al., 2008; Jacquet et al., 2010):

1. Steam treatment for which the lignocellulosic biomass is placed in a reactor and heated with water vapor at high temperature. During this phase, high temperature and pressure primarily initiate the depolymerization of hemicelluloses.
2. Explosive defibration corresponding to a sudden depressurization which provokes a rupture of the fiber cell wall by shear force caused by the evaporation of the condensed water in the biomass.

Due to the short residence time of the cooking period (few minutes), SE is commonly presented as less energy intensive than conventional processes. It has been reported in the literature that the SE treatment of Eucalyptus produced a high quality pulp with a lower energy consumption comparing to chemical-mechanical and chemical-thermo-mechanical pulps (Kokta and Ahmed 1993). SE has been previously experimented for the isolation of MNFCs from various lignocellulosic biomass pineapple leaves *i.e.* (Cherian et al. 2010), wheat straw (Kaushik et Singh 2011), tobacco (Tuzzin et al. 2016), etc. However, in these works the effect of SE on the morphology of MNFCs have never been studied in detail. Previously in our team, Nader et al., (2022) isolated L-MNFCs from *Eucalyptus globulus* bark pulps obtained by conventional soda cooking or SE followed by refining and ultrafine grinding. Obtained L-MNFCs have lignin content of approximately 4%, with a diameter 5-100 nm. Wood adhesive formulations were reinforced with L-MNFCs. It has been shown that the addition of 1 % of L-MNFC significantly increased the adhesive performance.

SE use for the production of MNFCs is described in the literature but is mainly restricted to the treatment of non-woody plants. Only few works use relevant operating conditions, a major part of the published works being conducted at low pressure (2 – 12 bars) in autoclave. At these pressure conditions, the explosive defibration step could not be controlled. When SE apparatus were used, long resident times have been applied leading to high energy consumptions.

Supermasscolloider grinder (Masuko Sangyo Co. Ltd., Japan) is one of the most common process for the microfibrillation step. Fiber suspension is passed between two disks (static and rotating) and the gap between them can be adjusted. Due to the resulting shearing forces, cell walls are destructured producing MNFCs (Nechyporchuk et al. 2016).

This mechanical treatment was used by different authors for the production of fully bleached MNFCs. Thus, Taniguchi and Okamura (1998) produced MNFCs from fully bleached wood pulps. They passed fiber suspensions 10 times between the disks of Masuko and they obtained MNFCs with a width between 20 and 90 nm. Spence et al. (2011) produced MNFCs from bleached kraft pulps using refining followed by Masuko (9 passes at 1500 rpm). Bulota et al. (2012) produced MNFCs from bleached kraft pulp. For this purpose, a fibrous suspension of 3 % wt. was ground with Masuko (7 passes) for a duration of 5 h. They obtained MNFCs with diameters ranging from 10 to 20 nm with a length ranging from 200 nm to 2-3 μm . Jonoobi et al. (2015) investigated the effect of changing the rotation speed of Masuko disks on energy consumption. They prepared 3% wt. cellulose fibrous suspension by dispersing the fibers with an electric blender for 10 min. The suspensions were then grinded by Masuko at 1440 or 3200 rpm until gel formation. 60 % of the obtained MNFCs had diameters between 20 and 50 nm. In addition, they found that using a lower speed (1440 rpm) results in lower energy consumption, i.e. 1.3 KWh/Kg compared to 3.1 KWh/Kg for 3200 rpm. To the best of our knowledge, a fix rotation speed has been applied to the grinding process in the literature. The effect of the variation of the ultrafine grinding conditions on the quality of L-MNFCs has never been clearly described.

The aim of this study is to produce Lignin containing micro and nano-fibrillated cellulose (L-MNFC) from beech wood by a combinative process including alkaline SE as the pulping step, followed by a mechanical pre-treatment of the obtained pulps (refining or 3 consecutive SE) and, finally, by ultrafine grinding as the main mechanical treatment. The originality of this work is the use of SE treatment performed in a pilot that allows applying high pressures to

produce L-MNFCs with different lignin contents from beech wood. The application of SE to produce L-MNFCs from this lignocellulosic biomass has never been tested. Conventional soda cooking will be also used to obtain reference pulps without the explosive defibration step and to compare the exploded and non-exploded materials in order to evaluate the possible benefit of the explosive expansion. The substitution of refining by 3 consecutive Se in order to facilitate the production process and to study the effects on the fibers has been tested. We also studied the impact of the rotation speed of the disks during the ultrafine grinding treatment on the energy consumption and on the quality index of L-MNFCs suspensions. For this purpose quality index of the L-MNFCs gels has been calculated in order to make quantitative comparison.

2 Materials and methods:

2.1 Materials:

Particles of 2 cm of beech wood (BW) were used to prepare L-MNFCs. Sodium hydroxide, sodium chlorite and glacial acetic were purchased from VWR chemicals (Czech Republic).

2.2 L-MNFC and MNFC Preparation:

SE apparatus was used for the isolation of the fibers from wood particles (pulping). For this purpose, the equivalent of 100 g of BW was impregnated in 200 mL of sodium hydroxide solution (20 or 30% wt.) for one night. Impregnated BW was then heated to reach 200°C by injecting vapor steam in the pressure reactor and heating was maintained during 8 minutes. This step was followed by a sudden decompression ejecting BW to the explosion tank. The obtained pulp (SE20 and SE30 depending on the soda concentration used) was filtered and washed on a 40 µm mesh.

Pulping by conventional soda cooking (SC), was also applied to BW samples. The equivalent of 200 g of dry BW and a sodium hydroxide solution (20 or 30% wt.) was placed in the autoclave (liquid/solid ratio = 6 Kg/Kg). The autoclave was hermetically closed and heated while rotating until reaching 170°C. This temperature was maintained during 60 minutes. Autoclave was then cooled and the pulp (SC20 and SC30) were filtered and washed on a 40 µm mesh.

To study the effect of lignin content, BWSE30 was bleached using a chlorite method. Pulp was placed in a reactor with deionized water (83 ml/g fiber), glacial acetic acid (2 ml/g fiber) and sodium chlorite (2 mg/g fiber). The mixture was heated to 70°C for 2 h. The addition of

sodium chlorite and glacial acetic acid was repeated once. A whitish solid residue consisting essentially of holocellulose (cellulose and hemicelluloses) was obtained. The bleached fibers were then filtered under vacuum and washed with deionized water until the pH of the filtrate is neutralized. Bleached sample was named SE30B.

30 g of pulp (dry weight) were then refined using a PFI mill until a drainage index of 70°SR (Schopper Riegler degree). 6500-7500 and 8500 revolutions were required for pulps produced by SC and SE, respectively. Refined pulps were named SC20R, SC30R, SE20R, SE30R and SE30BR. For comparison, in order to study the possibility of replacing refining, 3 consecutive SE were applied to SE pulps. Obtained SE pulp were filtered and washed on a 10 µm mesh and samples were named SE203SE and SE303SE.

A 2 % wt. suspension of the pre-treated pulps (refined or exploded 3 times) was then grinded using a Masuko ultrafine grinder (Model MKZA6-2, Disk model MKG-C 80, Masuko Sangyo Co., Ltd, Japan) as following: 10 passes at 0 (500 rpm), 10 passes at -5 (1000 rpm), 10 passes at -10 (1500 rpm), 5 passes at -15 (1500 rpm). Due to a long grinding time and heating problems, Masuko conditions were then adapted for each suspension by varying the rotation speed of the disks to minimize the process duration, maintain a more stable flow of the suspensions and avoid their heating. The grinding conditions are reported in table 1. Method 1 refers to constant conditions during grinding. Method 2 corresponds to variable conditions of grinding, each of one being adapted to the different fiber suspensions. The whole process is resumed in figure 1.

Table 1: ultrafine grinding applied conditions and process duration.

Disks Gap	Method 1				Method 2			
	SC20R	SC30R	SE203SE	SE303SE	SC20R	SC30R	SE203SE	SE303SE
	Disk speed							
0			500		750	500	500	750
-5			1000		1100	1000	1000	1100
-10			1500		1700	1350	1500	1500
-15			1500		1800	1700	1500	1700
-20			N/A		1800	1700	1500	1700
Total grinding time (min)	194	183	296	239	87	118	145	111

2.3 Characterizations of pulps, MNFCs and L-MNFCs:

Measurements in this part were at least triplicated. Given values represent the mean and the deviation was calculated as following: $Deviation = 0.5 \times (maximum\ value - minimum\ value)$.

2.3.1 Chemical composition of pulps:

Extraction of 4 g of the sample (BW, SC20, SC30, SE20 and SE30) was performed in a Soxhlet apparatus with a toluene/ethanol mixture (2/1 v/v) under reflux, for 8 h. The extractives were determined by the difference from the mass of the solid residue after drying at 105°C, and reported as percent of the original sample.

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. After this, 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 as the mass of the solid residue after drying at 105°C. The monomer sugar content in liquid fraction was analyzed using HPAE-PAD (ICS-3000 Dionex).

2.3.2 Morfi NEO

To analyze fiber morphology, MorFi NEO was used. It is based on the analysis of the images of a fiber suspension through a measuring cell. Images were acquired by an optical system and then processed by a computer. In this work, Morfi NEO was used to determine the fiber length, width and the percentage of fine elements of the suspensions obtained after refining, 3 consecutive SE and after ultrafine grinding. Measurement time was fixed to 5 minutes. According to this method, a fiber was considered as an element of length higher than 80 µm and fine elements as elements of length less than 80 µm. Suspensions were prepared by dispersing 0.3 g of fibers in 1L of water before the analysis.

2.3.3 Macrosized fraction:

L-MNFC and MNFC suspensions were diluted to 0.1% wt. in deionized water and stirred for 10 minutes by ultraturax (IKA®T-25) on speed 5. Images of the suspensions were taken with a camera placed on an optical microscope (Zeiss Axio Vert.A1, Germany). Images were then analyzed by ImageJ to calculate average size of microparticles.

2.3.4 Nanosized fraction (NF):

L-MNFC and MNFCs suspensions were diluted to 0.02% wt. and stirred for 6 minutes by ultraturrax (IKA®T-25) on speed 5. 200g of suspension were centrifugated for 15 minutes at 4°C and a velocity of 1000 g. Nanosized fraction (NF) was the calculated after the measurement of the concentrations before (Cb) and after (Ca) centrifugation (in the supernatant phase):

$$Nf = 100 \times \frac{Ca}{Cb}$$

2.3.4 Atomic force microscopy (AFM):

L-MNFC were observed using a Dimension Icon atomic force microscope with an OTESPA cantilever. L-MNFC suspensions were diluted to (10⁻² % wt.) in deionized water. 200 µl of each suspension were deposited on freshly cleaved mica substrates and let to dry overnight at 30°C before imaging. For each sample, images were obtained on three different areas.

2.3.5 Scanning electron microscopy (SEM):

Fiber suspensions were diluted to 0.1 % wt. and dried at room temperature on a carbon adhesive, then coated with a 5 nm Au/Pd layer. Images were acquired with an ESEM Quanta 200 (FEI, Japan) in ETD mode. A working distance of approximately 10 mm and an acceleration voltage of 10.0 kV were used. Images were acquired with magnifications between ×25 and ×3000.

2.3.6 Transmission electron microscopy (TEM):

Droplets of dilute aqueous L-MNFC and MNFC suspensions were deposited onto glow-discharged carbon-coated TEM grids. After a few minutes, the liquid in excess was removed with a blotting paper and, prior to drying, the preparation was negatively stained with 2 % wt. uranyl acetate. Stain in excess was blotted and the specimen allowed to dry. Images were recorded with a JEOL JEM-2100-Plus microscope operating at 200 kV equipped with a Gatan Rio 16 digital camera.

2.3.7 Turbidity:

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

2.3.8 Young's modulus of nanopapers

A handsheet former (Rapid Kothen, ISO 5269-2) was used to prepare nanopapers. The equivalent of 2g of dry L-MNFC and MNFC was diluted to 0.5 % wt. in deionized water. Water was then removed by filtering the suspension under vacuum. The sheet was dried under vacuum at 85°C between two nylon sieves for 20 min. Before characterizations, nanopapers were stored for 48 hours in a conditioned room at 23°C and 50% RH.

An extensometer (Instron 5965) was used to measure the tensile properties, following the standard NF Q03-004. Tests were performed at 10 mm/min, on 15 mm width samples with a distance between jaws of 100 mm.

2.3.9 Quality index of MNFCs and L-MNFCs:

Desmaisons et al. (2017) developed an index that can be used as a quality control for MNFCs at the lab scale or industrial scale. 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 MNFCs gels at the macro- and microscales. In addition, the use of this index is probably the most effective way to compare cellulose nanofibrils because there is no other quantitative method to do this comparison. 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 (μm^2).

3 Results and discussion:

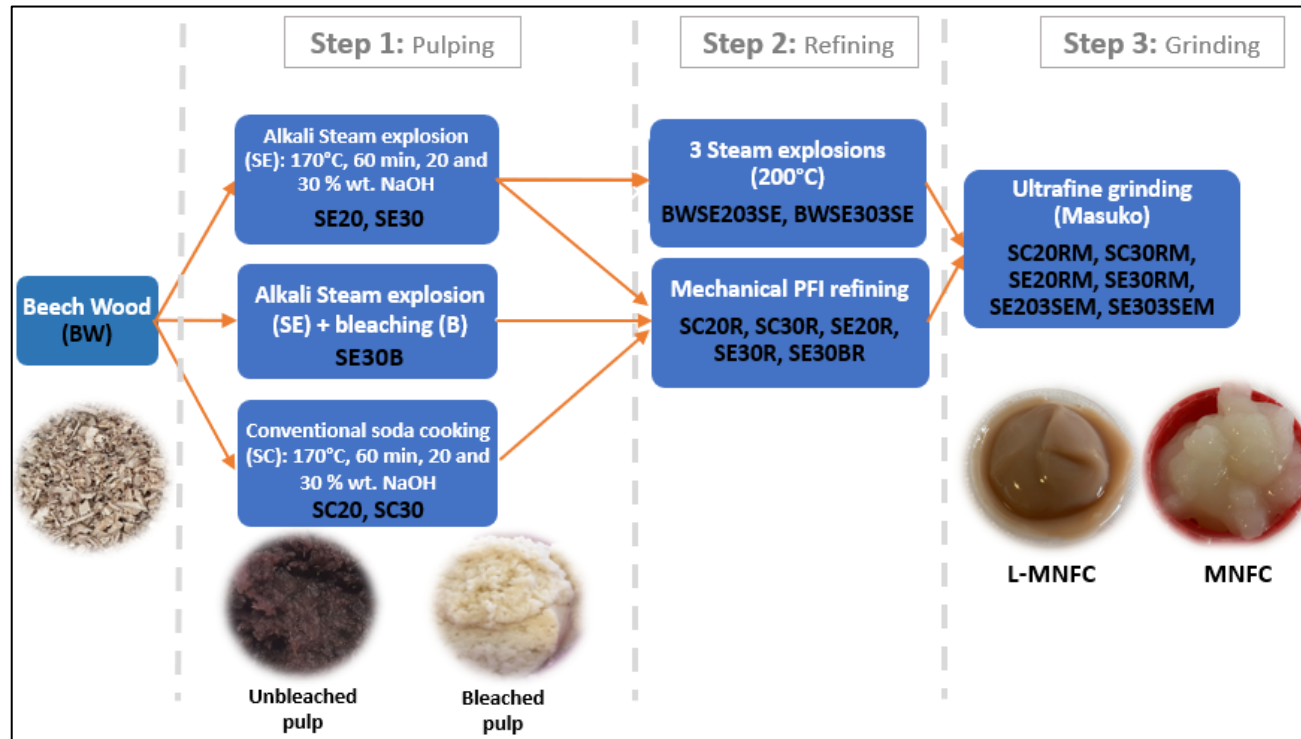
3.1 Pulping yield and chemical composition:

In this study, L-MNFC gels have been produced by combining steam explosion (SE) with conventional grinding. A classical soda cooking (SC) performed in an autoclave has been also used to produce pulps without any explosive defibration. The benefits of the SE process include a significantly shorter residence time and greater flexibility in the biomass to be used (Nader et al., 2022). Figure 1 resumes the production process used for L-MNFC and MNFC production. Table 2 shows the pulping conditions, the yield and the chemical composition of the different lignocellulosic pulps.

1

Table 2: pulping conditions, yield and chemical composition of different pulp. All data are yields (g) per 100g of dry weight BW

Sample	Pulping	Temperature (°C)	Time (min)	Yield (%)	Chemical composition (%)				
					Toluene-EtOH extractibles	Lignin	Hemicelluloses	Cellulose	Total
BW		No treatments			6.53	21.88 ± 0.15	22.67 ± 0.92	39.96 ± 1.61	91.04
SC20	Autoclave cooking	170	60	40	0.40	1.23 ± 0.06	6.67 ± 0.06	29.28 ± 0.37	37.58
SC30				31	0.36	0.99 ± 0.03	5.12 ± 0.14	22.15 ± 0.21	28.62
SE20	SteamEx	200	8	52	1.76	6.05 ± 0.18	7.92 ± 0.19	31.92 ± 0.47	47.65
SE30				49	0.06	4.16 ± 0.19	5.33 ± 0.30	35.81 ± 0.34	45.37



2

3

Figure 1: Schematic of the L-MNFC and MNFC production process used in this work.

The chemical composition of the pulps show that hemicellulose contents decreased from ~23 % in the non-treated BW to ~7 and ~5 % (for SC20 and SC30, respectively) and ~8 and ~5 % (for SE20 and SE30, respectively). Lignin content strongly decreased from ~22 % for BW to ~1 % (for SC20 and SC30) and ~6 and ~4 % (for SE20 and SE30, respectively). Toluene-EtOH extractives content decreased also from ~7 % in the BW to ~0-1 %. These results confirm that during pulping, a hydrolytic breakdown of the lignocellulosic complex occurs impacting the non-cellulosic polymers (hemicelluloses and lignin) which are more affected than cellulose due to its crystallinity. As expected, for the same pulping method (SC or SE), a lower hemicellulose and lignin content was obtained for a higher soda concentration. For SC, hemicellulose hydrolysis was slightly more pronounced, meanwhile lignin dissolution was strongly higher which is in line with the low yields obtained for these experimental conditions. In the same way, cellulose content decreased from ~40 % in the BW to ~29 and ~22 % (for SC20 and SC30, respectively) and ~32 and ~36 % (for SE20 and SE30, respectively). This is probably due to the peeling reactions promoted for longer duration and the higher removal of lignin during SC pulping making cellulose is more exposed while pulping (Martin-Sampedro et al. 2014). Degradation by peeling reactions can also explain the lower yield obtained for SC (31-40 %) compared with SE (49-52 %) (see table 2). It is worth noting that the pulping yields obtained after the conventional pulping are quite low (40 and 31% for SC20 and SC30 respectively).

3.2 Refining:

Schopper Riegler test is used in pulp and paper industry to measure the refining degree of a suspension of fibers in water. The test is designed to measure the drainability of water from a dilute pulp suspension. 1°SR represents 10 mL of drained water. Figure 2 shows the evolution of the Schopper Riegler degree (°SR) of the pulps as a function of the number of revolutions of the PFI mill. It can be seen that the pulps obtained by SC (S20R and SC30R) displayed similar refining behaviors, reaching 70°SR after 3500 revolutions. These two pulps contain the lower lignin content (about 3 %). The refining behavior of these two pulps is comparable to the bleached one. This is due to the comparable lignin content in these pulps (see supplementary materials). Meanwhile, the two SE pulps did not show comparable refining kinetics: SE20R and SE30R reached 70°SR after 4500 and 3000 revolutions, respectively. Tuzzin et al. 2016 showed that a higher lignin content makes it more difficult to

separate hemicelluloses and cellulose in the lignocellulosic matrix, thereby decreasing the refining ability of the resulting pulps. Moreover, pulps having a high lignin content present a hydrophobic behavior which could decrease the fiber swelling. This can explain the refining behavior of SE20 containing 12 % lignin. Moreover, SE30 presenting 8 % lignin, which is higher than SC pulps, had the best refining behavior. That can be explained by the impact of the SE at 30 % NaOH on the morphology of this pulp featuring the shortest fibers and the highest number of fine elements (see table 3). The well-known lignin deconstruction during the SE treatment through the extensive beta aryl-ether breakdown (mentioned in the following section) may also justify this increased refining capacity despite a relatively high lignin content.

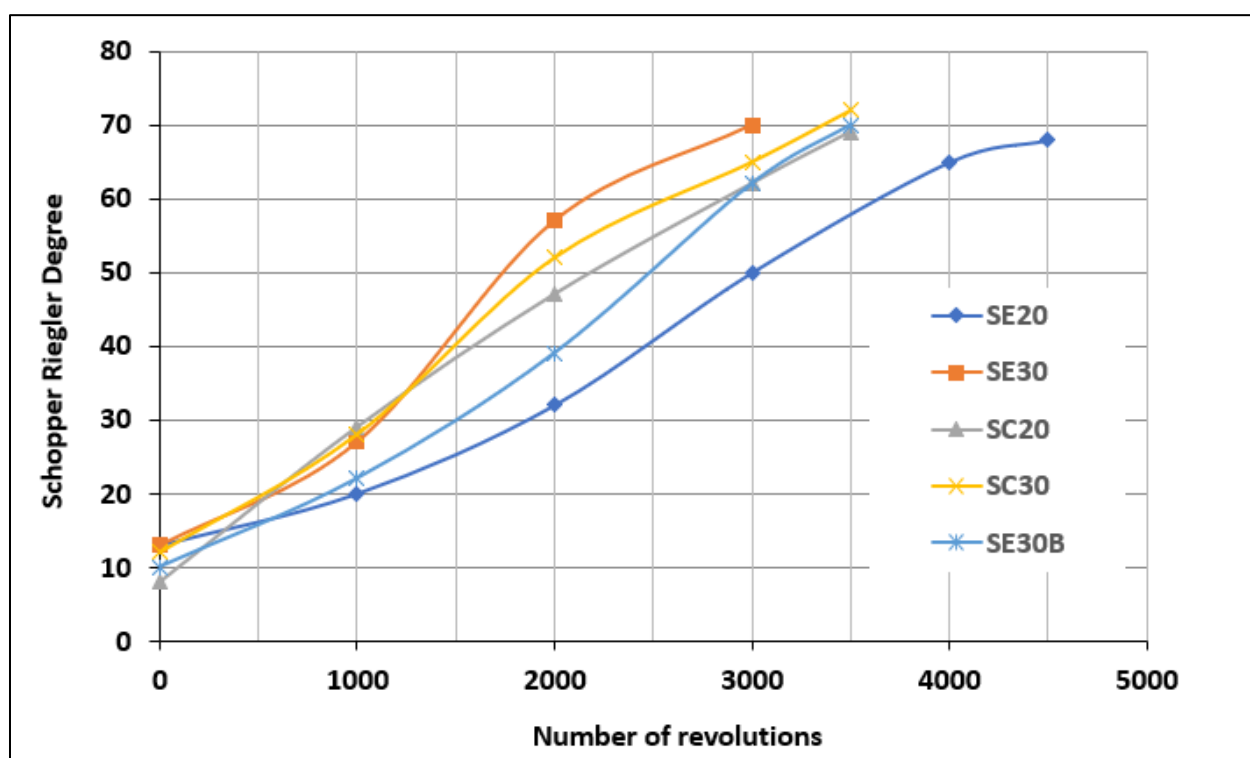


Figure 2: Evolution of Schopper Riegler degree for different pulps with the number of revolutions of the PFI mill disk. Key: SC, conventional soda cooking in autoclave; SE, steam explosion, B, bleaching, 20 and 30, 20 and 30 % wt. NaOH.

3.3 Morphology:

Table 3 shows the data obtained from MorFi NEO analyzer, including number, length and width of fibers. After the refining stage the pulps produced were composed of $\approx 50\%$ of fibers with a length of $\approx 250 \mu\text{m}$ and a width of $\approx 19 \mu\text{m}$. As expected, SE pulps contained significantly shorter fibers than SC pulps, 215-238 μm and 246-248 μm , respectively. These results agree with previously reported works. Nader et al. 2022 and Luo et al. 2018 showed

that the length and the fine elements were affected by the explosive decompression of SE. The widths of the fibers remain stable and comprised between 18 and 20 μm .

It is noted that the substitution of the refining step by 3 successive SE (SE203SE and SE303SE) led to fibers with comparable characteristics (length \approx 255-270 μm). In these conditions the lower amounts of fine elements (\approx 30 %) can be explained by the loss of fine particles during pulp washing and filtration steps of the three successive SE. It can also be seen that steam explosion significantly impacted the thickness of the fibers. Thus, SE303SE have the smallest widths (17.0 μm) after 3 successive SE. This observation can be justified by previous studies which showed by scanning electron microscopy that the middle lamella and the primary cell wall were impacted and denuded by the SE (Sauvageon et al. 2018). Similar observations can be done by comparing SC30 fibers and SE30 (Figure 3 A and B) micrographs. The damage on the surface of the exploded fibers are clearly visible (Figure 3 B).

Bleaching effect resulted in the width decrease of the fibers and a lower fine elements content (18.2 μm and 18.7 μm , 55% and 60% for SE30BR and SE30R, respectively). After grinding, the bleached gel has lower residual fibers 1.09×10^3 fibers/g compared to 9.31×10^3 fibers/g.

As expected, ultrafine grinding had a leveling effect on the morphologies of the samples resulting in a five- to ten-fold decrease in the number of fibers and about a five-fold increase in the number of fine elements. The length of the residual fibers in the gels (\approx 140-160 μm) is much lower than that of the initial fibrous suspensions, as well as their width (\approx 15-17 μm), which also reflects the effect of grinding. Expressed as a length fraction, fine element contents are all above 95%. Nechyporchuk et al. 2015 showed that the percentage of fine elements increased reaching a plateau at 97 % indicating the conversion of the fibers to fines and nanofibrils. SE303SE gels have again a particular behavior and present abnormally low contents of fine elements. Finally, it can be pointed out that there are no significant differences between the gels in terms of morphological properties, except, as mentioned above, for SE303SE gels produced by 4 successive SE. We can therefore conclude that ultrafine grinding has a levelling effect on the morphological properties of the samples determined by Morfi NEO and that there are no clear differences between the samples, if we

85 consider the residual fibers and the visible fine elements still remaining in the suspensions
 86 after grinding.

87

88 **Table 3:** Morfi Analyzer results for refined and 3SE pulps, L-MNFC and MNFC. Key: SC, soda
 89 cooking; SE, steam explosion; R, refining; B, Bleaching and M, Masuko grinding.

Method	Sample	Number of fibers (fibers/g)	Mean fiber arithmetic length (μm)	Mean fiber width (μm)	Number of fine elements (fines/g)	Content of fine elements (%), in length
SC + R	SC20R	4.64×10^4	248 ± 3	19.3 ± 0.12	4.34×10^5	48
	SC30R	5.02×10^4	246 ± 1	19.1 ± 0.21	4.46×10^5	47
SE + R	SE20R	4.41×10^4	238 ± 2	20.3 ± 0.35	3.78×10^5	48
	SE30R	3.71×10^4	215 ± 1	18.7 ± 0.15	4.80×10^5	60
SE + B + R	SE30BR	4.44×10^4	251 ± 1	18.2 ± 0.32	6.05×10^5	55
SE + 3 SE	SE203SE	3.73×10^4	270 ± 3	18.1 ± 0.05	1.61×10^5	27
	SE303SE	4.61×10^4	256 ± 1	17 ± 0.00	2.21×10^5	32
SC + R + M	SC20RM	5.67×10^3	143 ± 2	16.4 ± 0.06	2.32×10^6	98
	SC30RM	1.66×10^3	157 ± 5	16.9 ± 0.30	1.01×10^6	98
SE + R + M	SE20RM	6.54×10^3	165 ± 3	15.4 ± 0.75	2.61×10^6	98
	SE30RM	9.31×10^3	159 ± 2	15.8 ± 0.25	2.15×10^6	96
SE + B + M	SE30BRM	1.09×10^3	150 ± 3	15.6 ± 0.40	7.01×10^5	98
SE + 3 SE + M	SE203SEM	4.3×10^3	163 ± 2	17.5 ± 0.30	1.88×10^6	77
	SE303SEM	7.2×10^3	139 ± 3	15.2 ± 0.10	4.83×10^6	84

90 It can be seen in the SEM images (Figure 3 A) that SC fibers have less damaged surface
 91 compared to SE fibers (Figure 3 B), which confirms the destructive effect of the steam
 92 explosion on the cell wall. Figure 3 C also shows that refining induced great modifications of
 93 the fibers and a partial detachment of the macrofibrils. This phenomenon is particular visible
 94 in this case with a high refining degree of the pulps.

95 It can be seen from TEM images (Figure 3 D and E) that the samples presented a web like
 96 morphology with the presence of aggregates. Nechyporchuk et al. (2015) also showed some
 97 non-fibrillated flocculated structures after ultrafine grinding, even for gels produced from
 98 bleached pulps. For the SE sample, we notice the presence of some regular shaped particles
 99 on the surface and between the fibrils (Figure 3 E). Their nature and composition are not
 100 clearly understood but they have been already observed but only in the case of MNFC gels
 101 produced from hardwood species (unpublished data). The most probable hypothesis is that
 102 these particles result from the crystallisation of xylenes after treatments. Considering the
 103 higher cellulose content compared to hemicelluloses (cellulose/hemicelluloses ratio about

104 5), we also cannot totally exclude the formation of cellulose nanocrystals during the
105 treatments.

106 Moreover, AFM images (Figure 3 F) show the presence of other particles, dispersed in the
107 sample with a spherical shape. Indeed, in the literature, it has been reported that lignin is
108 depolymerized through aryl-ether scission producing small lignin fragments during SE
109 process (Obame et al., 2019). The use of temperatures higher than the Tg of lignin makes the
110 fragments mobile and facilitates their migration within the biomass sample. Lignin may then
111 coalesce into spheres to minimize its surface area due to its hydrophobic nature (Vignon et
112 al. 1995; Ehman et al. 2020; Yuan et al. 2021, Nader et al. 2022). Thus, based on these
113 previous works for which such nanodroplets have been observed by AFM, it has been
114 concluded that the globular particles present in our samples were composed of lignin.

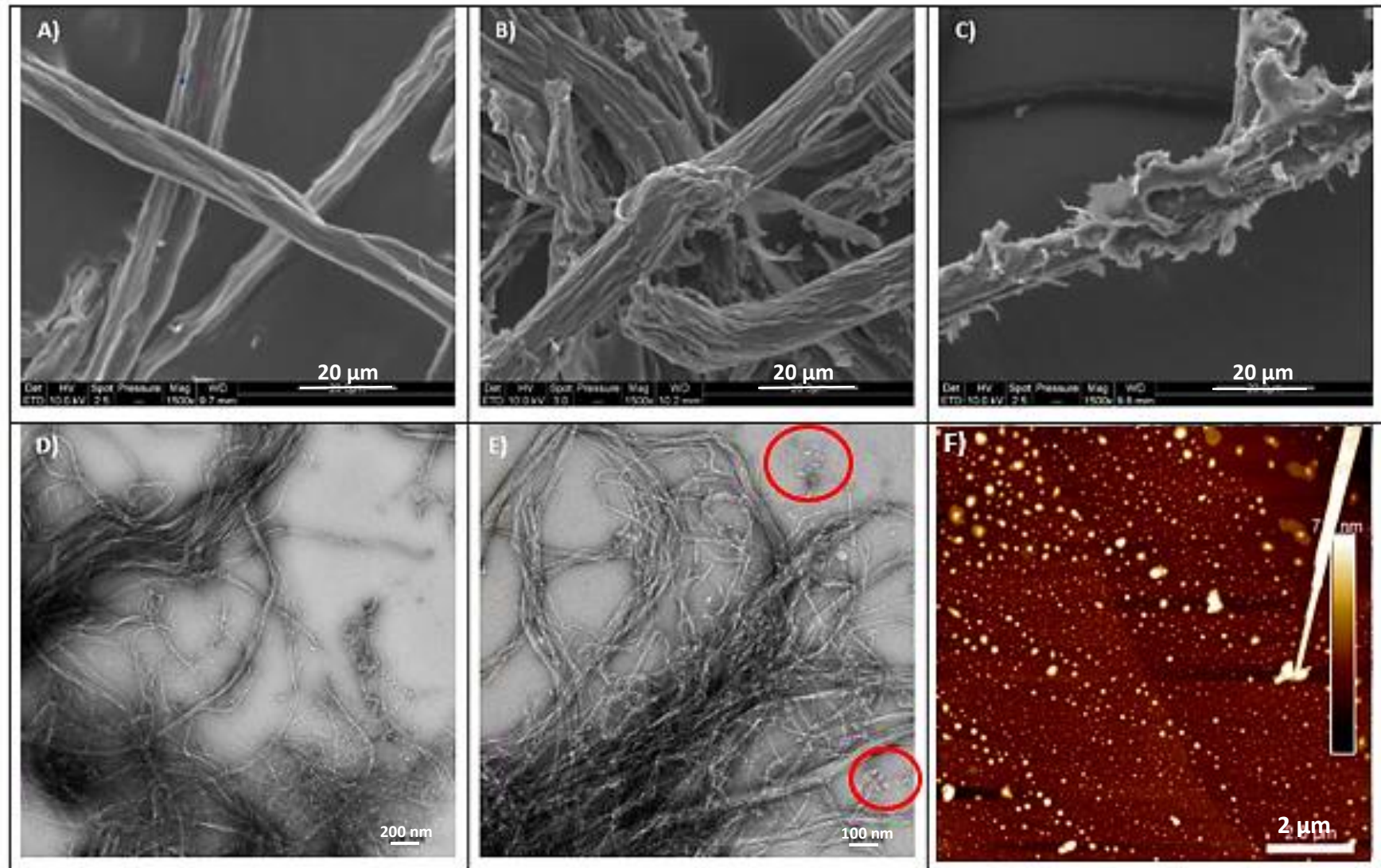


Figure 3: SEM images of (A) SC30, (B) SE30, (C) SE30R, TEM images of (D) SC30RM, (E) SE30RM and AFM image of (F) SE30RM.

3.4 Quality index and energy consumption:

During ultra-fine grinding process, the application of the same conditions (Method 1, see table 1) to all the fibrous suspensions was not adapted. For certain suspensions, grinding needed a very long-time leading to an important increase of the disk temperature and a very high energy consumption. It has been decided to adapt the disk rotation velocity of the Masuko grinder to decrease the grinding duration and the related energy consumption (Method 2, see table 1).

Figure 4 shows the quality index for all the gels obtained either after a conventional cooking in autoclaves, or after alkaline steam explosion, considering the two methods of grinding, as well as gels for which refining has been replaced by 3 steam explosions and finally for gels produced from bleached fibers. For these last two gels, method 1 was used.

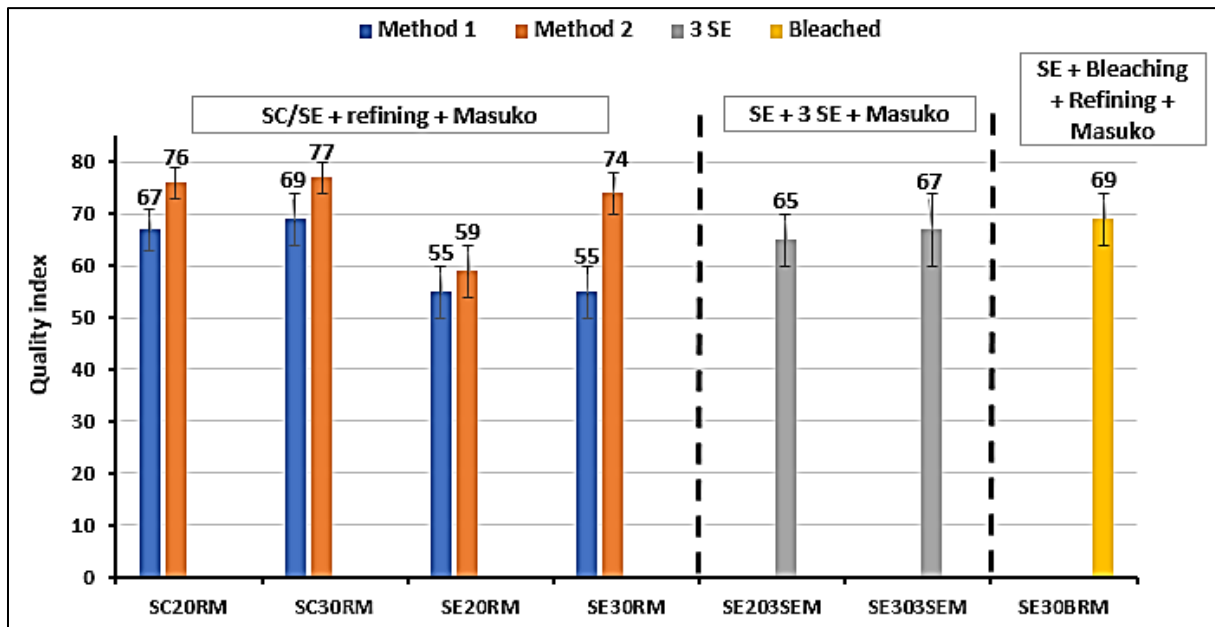


Figure 4: Quality index of the different L-MNFC and MNFC gels (3SE and bleached samples were produced according to Method 1).

From these results, it appears that the produced L-MNFC have a QI ranging between 55 and 77. These results are comparable to the QI values of industrial MNFCs produced from bleached pulps by enzymatic treatments (Desmaisons et al., 2017). It also appears that the L-MNFCs produced by conventional cooking in autoclave have higher QI values than SE. That may be due to the higher removal of lignin during conventional cooking compared to SE. The application of 3 consecutive SE instead of the refining improved the QI of L-MNFCs even with the loss of fine elements (QI increased from ~55 to ~65). This loss of fine elements can be

explained by the washing and the filtration steps. As it has been demonstrated, steam explosion process produced a higher content of fine elements. During the washing and the filtration between 4 consecutive steam explosions, more fine elements were lost. The optimization of this step will be necessary to further improve the L-MNFCs quality. The MNFCs produced from bleached SE pulp shows also an improvement of the QI. This result further demonstrates the relationship between QI and lignin content in L-MNFCs. It can also be noted that the modification of the speed to obtain a stable flow out of the Masuko (applying the method 2), increased the QI values for all the gels. This quality improvement is particularly noticeable for SE L-MNFCs for which the QI values are comparable to those produced by conventional cooking in autoclave and the bleached MNFCs. These results indicate that the process control of the grinding step is a crucial parameter for the quality of the L-MNFCs.

Figure 5 shows the correlation between the quality index and the specific energy consumption during refining and ultrafine grinding steps. For the SC pulps, the same specific energy was consumed during refining (= 300 kWh/t). Meanwhile, for SE pulps, the specific energy consumption during refining was 333 kWh/t and 133 kWh/t for SE20 and SE30, respectively. These results are in accordance with the evolution number of the PFI mill discussed before.

Nevertheless, almost all the specific energy consumption takes place during the ultrafine grinding step. Jonoobi et al. (2012) showed that grinding at low speed consumes less energy compared to high speed. Results present in figure 5 shows that the energy consumed greatly decreased when the grinding method was modified, especially for SE20RM for which the energy decreased from 32667 kWh/t to 14000 kWh/t. These results show that when we increased the grinding speed we successfully produced L-MNFCs and MNFCs with a lower energy consumption during the grinding step.

3.5 Influence of lignin:

It was shown that during refining, soda cooking pulps (SC20 and SC30) exhibited better behavior than steam explosion pulps (SE20). This behavior was comparable to fully bleached pulps (SE30B). This is due to the lower lignin content for SC pulps (see table 2) leading to a better separation of hemicelluloses and cellulose decreasing the response during refining.

However, SE30 pulps showed better refining behavior even with a higher lignin content. This easier ability of refining may be explained by the depolymerization and the detachment of lignin which makes cellulose more accessible to refining.

It has been shown also that soda cooking L-MNFCs quality was better than steam explosion ones (Method 1). This quality was comparable to fully bleached L-MNFCs. That may also be explained by the lower lignin content of soda cooking L-MNFCs. The removal of lignin in soda cooking process enhanced the formation of hydrogen bonds between the fibrils resulting an increase in the Young's moduli of the L-MNFCs gels (see table 6 in supplementary materials). The lower lignin content decreased the turbidity of the soda cooking L-MNFCs gels. The modifications of these two parameters resulted in a better quality of these L-MNFCs.

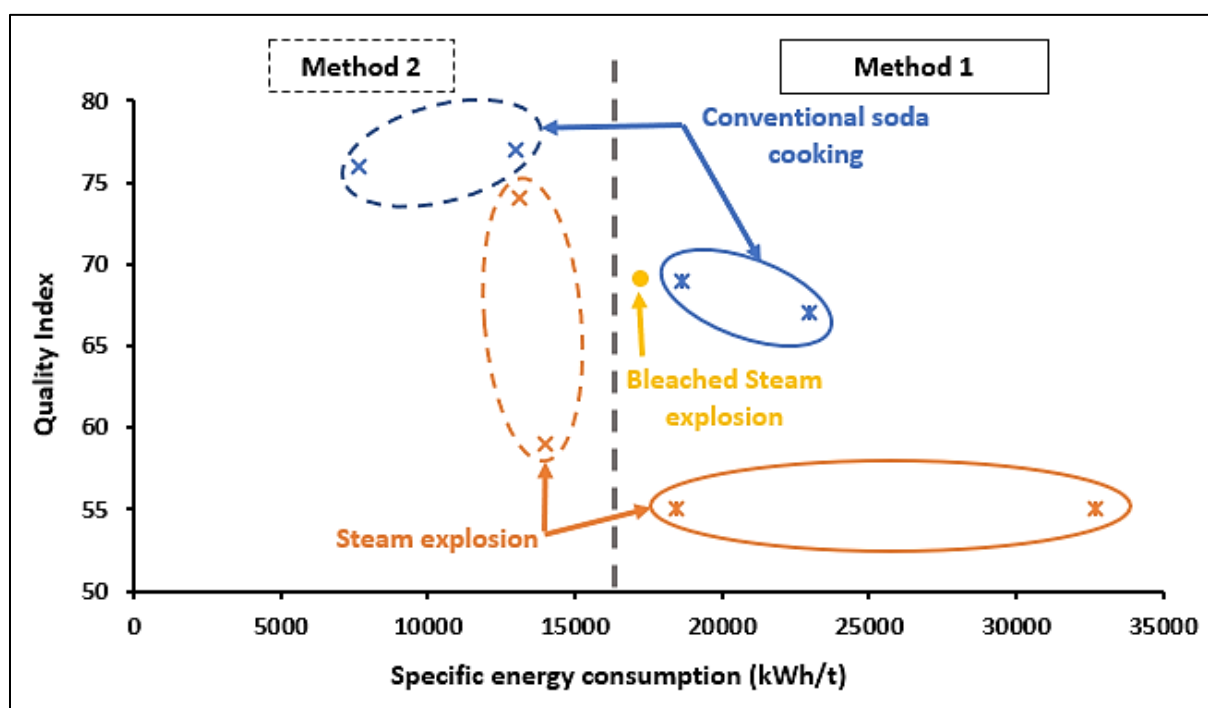


Figure 5: Relationship between quality index of L-MNFCs and MNFCs and specific energy consumption for the two methods used for grinding.

4 Conclusion:

In this work lignin containing micro and nano-fibrillated cellulose (L-MNFC) were produced from beech wood pulps through a sequential process starting by pulping using steam explosion or conventional soda cooking followed by a pretreatment (refining or 3 consecutive steam explosions) and a main mechanical treatment (ultrafine grinding). Obtained results showed that the steam explosion pulping leads to a higher yield pulps

containing more lignin than conventional cooking in autoclave. SE fibers were more damaged due to the explosive defibration step. L-MNFCs gels contains in the two cases residual fibers, micro and nanofibrils. TEM images showed that some nanocrystals (cellulose or xylanes) were present on the surface and between the fibrils, meanwhile AFM images shows lignin nanodroplets dispersed in the SE L-MNFCs. Quality index values showed that the quality of the gels not only depends on the lignin content but also on the ultrafine grinding used method. These results suggest that SteamEx can advantageously replace conventional Alkali pulping and refining by reducing the cooking time from 60 minutes to 8 minutes and by simplifying and facilitating the production process. Optimization of SE conditions is necessary to better understand the effect of lignin content on the quality of L-MNFCs. Finally, it has been demonstrated that the bleaching step was not required to obtain L-MNFCs with good quality.

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