

Production of cellulose nanostructures from Chilean bamboo, *Chusquea quila*

P.E. Oliveira^{1,*}, X. Petit-Breuilh², O.J. Rojas³ and W. Gacitúa⁴

¹Department of Industrial Process Engineering, Nucleus of Research in Bioproducts and Advanced Materials, Catholic University of Temuco, Manuel Montt Street, Postal Code CL4813302 Temuco, Chile

²School of Engineering and Business, Viña del Mar University, Diego Portales Street, Postal Code CL2580022, Chile

³Department of Bioproducts and Biosystems, Aalto University School of Chemical Engineering, P.O. Box 16300, 00076 Aalto, Finland

⁴Postgraduate Department of Wood Engineering, Biomaterials and Nanotechnology Center, Bío-Bío University, Collao Avenue 1202, Postal Code CL4051381 Concepción, Chile

*Correspondence: poliveira@uct.cl

Abstract. In Chile, bamboo bushes of *Chusquea quila* genus (or popularly known as ‘quila’) have brought economic and ecological problems for decades in the south-central part of this country. On the other hand, this plant species was studied as a raw material for the production of nanofibrillated cellulose (CNF) and nanocrystalline cellulose (CNC), presenting an opportunity for sustainable and environmentally friendly management, positioning Chile as a Latin American country at the forefront of studies with nanotechnological approaches. The methodology applied to generate these nanostructures contemplated hydrolysis with sulfuric acid and oxalic acid, in addition to an intermediate stage of microfluidization to generate nanofibrillated cellulose. The products obtained showed morphological and topographic homogeneity in the scanning electron microscopy (SEM) and atomic force microscopy (AFM) images. The diameter values of the particles ranged from 10 to 20 nanometers for the CNCs. Through Fourier transformed spectrophotometry (FTIR) it was found that the processes of microfluidization and acid hydrolysis did not affect the molecular shape of the nanostructures and X-ray diffraction (XRD) was important to determine crystallinity index (IC), presenting values higher than 80%.

Key words: *Chusquea quila*, Chilean bamboo, cellulose nanofibrils, cellulose nanocrystals, acid hydrolysis.

INTRODUCTION

Bamboo is a naturally occurring composite material which grows abundantly in most tropical countries and has been used widely for household products and industrial applications, thanks to advances in processing technology and increased market demand. In Asian countries, bamboo has been used for a multitude of household utilities such as containers, chopsticks, woven mats, fishing poles, cricket boxes, handicrafts, chairs, etc.

It also finds a role in a variety of building applications, such as flooring, ceiling, walls, windows, doors, fences, roofs and trusses (Banga et al., 2015).

This species is considered a composite material because it consists mainly of cellulose fibres imbedded in a lignin matrix. Apart from these two constituents, hemicelluloses and other minor components are also present. In this composite system, cellulose fibres are aligned along the length of the bamboo, providing maximum tensile and flexural strength and rigidity in that direction (Wang & Shen, 1987). So far, over 1,200 bamboo species have been identified globally (Banga et al., 2015). Bamboo offers several advantages as a promising alternative feedstock to substitute for wood: fast growth and development, high productivity per hectare, low management costs and capital investment, and minimal energy expenditure in processing (Canilha et al., 2012). Based on these advantages, it is already being used in more than 4,000 different areas. Bamboo does not suffer from cracks or knots, which leads to far more evenly distributed stresses throughout the length of pieces.

In Chile, the genus *Chusquea* is represented by 11 species, of which *Chusquea quila* is among those with the greatest potential as a source of cellulose (Oliveira et al., 2016). Traditionally, this native species has been regarded as brush-wood. Its main habitat is the forest of the provinces of Cautín, Valdivia, Llanquihue and Chiloe, with an altitude distribution from sea level up to 800–900 m.

Oliveira et al., 2018 reports a delignification process which can be applied to ‘quila’ to produce crystalline cellulose, an important resource for obtaining cellulose nanofibres or nanocrystals. Alternatively, the crystalline cellulose can then be subjected to fermentation processes, making it a useful raw material for biofuel production. The treatment studied is an oxidative treatment at low concentration (15% v/v), for short time periods (10, 20 min) and at temperatures between 100 and 140 °C, which produces alpha-cellulose fibres with high crystallinity (over 70%) and low residual lignin content (< 2%). The morphology of the cellulose fibres was analysed by scanning electron microscope, revealing a smooth surface containing many fibrils. Infrared spectroscopy was used to identify functional groups, showing that almost 99% of the lignin was removed.

Biodegradable plastics and biocompatible compounds from lignocellulosic sources are promising materials which could replace synthetic polymers and reduce dependence on fossil fuels (González et al., 2013). Among these, nanostructured cellulose has unique characteristics which are of interest for the development of new materials, including high strength and rigidity; mechanical and barrier properties, biocompatibility and low density (Lin et al., 2015).

In Federal University of Lavras (Department of Agricultural Engineering), Ferraz et al (2020) study use of lignocellulosic materials residue in cement composites has one of the rises as sustainable building materials in most developing countries. Besides, this alternative is seen as a good option for new cement panels formulations for indoor applications. In this case, the research aims to evaluate de chemical properties of five potential lignocellulosic materials residues to be used for cement panels reinforcement: Eucalyptus, sugarcane bagasse, coconut fibre, coffee rusk, and banana pseudostem. The physical properties of the lignocellulosic materials were evaluated such as lignin, extractives, ash, and holocellulose.

Today, the most frequently source of cellulose nanocrystals is wood. However, there is growing interest in alternative sources with rich lignocellulosic composition. In the last 10 years, there has been an increase in the number of reports using other resources, for example, from biomass derived from the agri-food industry. Meanwhile in tropical countries (in Asia, Africa and the Americas) interest has focused on non-wood forest species such as shrubs and bamboos.

Some of the common non-wood resources used to produce cellulose nanofibers include bamboo (Li et al., 2015), wheat straw (Chen et al., 2011), New Zealand flax *Phormium tenax* (Fortunati et al., 2013), banana skins (Khawas & Deka, 2016), orange peel (Hideno et al., 2014) and palm oil industry waste (Fatah et al., 2014). Bamboo, the species selected for this study, grows in abundance in most tropical countries and is a material with a complex cell structure. It has been widely used in products for home use and industrial applications, owing to advances in processing technologies and increased market demand (Banga et al., 2015).

Among these, *Chusquea* is a genus of bamboo found in Chile, with eleven species, with *Chusquea quila*, or simply quila, being one of the most abundant. For years, quila has created environmental and economic problems for farmers in southern Chile. During flowering, it promotes an increase in the mouse population (Schlegel, 1993) and in summer it presents a high fire risk due to the accumulation of dry biomass (CONAF, 2012). In addition, it forms a dense scrub which occupies land that could otherwise be used for farm production (Pinto & Barrientos, 1993).

According to preliminary studies by Oliveira et al., 2016, its chemical composition, bamboo, quila, contains 54.7% cellulose; 13.8% lignin; 4.6% extractable compounds and 2.2% ash. Its particularly high cellulose content, together with the structural characteristics of the lignocellulosic matrix, is a promising resource from which cellulose nanomaterials can be obtained. The objective of the present study was to investigate the potential of *Chusquea quila* as a source of cellulose nanofibrils, CNF and cellulose nanocrystals. CNCs were obtained following two protocols: via acid hydrolysis to produce nanocrystals directly using the quila cellulose and physical microfluidization to obtain CNF which was then finally hydrolysed to CNC.

MATERIALS AND METHODS

Preparation of cellulose nanostructures from Chusquea quila

Quila samples presented colour variations from opaque to shiny green; and were generally covered with algae, lichens and mosses. According to previous studies, the best age to harvest the canes is 1.5 to 2 years, when they are mature, and their structure is strongest. Samples were selected following the methodology of Campos et al. (2003). The bamboo was collected from local farmers in Temuco and Freire (towns in the Araucanía Region, Chile). Samples approximately 2 metres in height were cut 20 cm above ground level. They were prepared by eliminating impurities from the surface by washing (maintaining the outer skin) and then converted to chips for analyses and reactive oxidation.

The process for obtaining cellulose fibres from quila followed Oliveira et al., 2018. Briefly, the bamboo was oxidised with a low concentration of peracetic acid (15% v/v) for short periods of time (10 and 20 minutes) at 100 and 120 °C. The produced cellulose

fibres had an apparent crystallinity index of alpha-cellulose of 78% and residual lignin content less than 2% (Kappa number 12.34).

The proposed methods for isolation of cellulose nanostructures is shown in Fig. 1. Quila cellulose fibres were first reduced to particles (150 to 80 μm) in an ultra-centrifugal mill (ZM200 Ultra-Centrifugal Mill, Retsch GmbH, Haan, Germany). The first method for obtaining nanocrystalline cellulose followed Brinchi et al., 2013 for obtaining cellulose nanocrystals using sulphuric acid. The second method used involved acid hydrolysis with oxalic acid, following Abraham et al., 2011.

Treatments were divided into the following stages: (1) Acid hydrolysis of pure cellulosic material (15 g of quila cellulose) under strictly controlled conditions which included temperature in a thermo-regulated bath (45 °C), time (45min), shaking (35 rpm), acid concentration 55% v/v sulphuric acid and 5% w/v oxalic acid hydrolysis. The same ratio of acid solution/cellulose, 1:20 (gm L⁻¹), was used in both cases; (2) Dilution with distilled water to stop the reaction and repeated washing by successive centrifugation; (3) Extensive dialysis to eliminate any free acid (72 hours repeated exchange with distilled water); (4) Mechanical sonication (20 minutes) to disperse the nanocrystals as a stable, uniform suspension; (5) Ultra-centrifugation to obtain a concentrated CNC suspension.

The second method to obtain CNC included an intermediate quila cellulose fibre microfluidization stage (Junka et al., 2014). In this stage, cellulose was diluted in ultra-pure water (Water for chromatography, LC-MS Grade, LiChrosolv®. CAS 7732-18-5), at a concentration of 1% w/v, for 24 hours. Then the suspension was subjected to high pressure fluidization (Microfluidics Corporation, model M-110P, Newton, Massachusetts, USA) at operating pressures of 69 MPa (10,000 psi), 138 MPa (20,000 psi) and 207 MPa (30,000 psi). The suspension obtained was centrifuged to eliminate excess water and then stored as a hydrogel (5% dry weight). Finally, the quila CNF was subjected to acid hydrolysis under the same conditions as in the first method to obtain quila CNC.

Final yield (Y) of CNC was determined by calculating the total nanocrystalline cellulose mass obtained compared to the initially hydrolyzed fiber mass as demonstrated in Eq. 1 (Beltramino, 2016). In brief, 25 mL (in triplicate) of each suspension was measured in tared beakers. The CNC suspensions were evaporated at 50 °C until constant weight; and, after cooling in a desiccator, total mass was determined.

$$Yield (\%) = \left(\frac{(a - b) \cdot total\ volume(mL)}{dried\ volume\ (mL) \cdot fibers\ mass(g)} \right) \cdot 100 \quad (1)$$

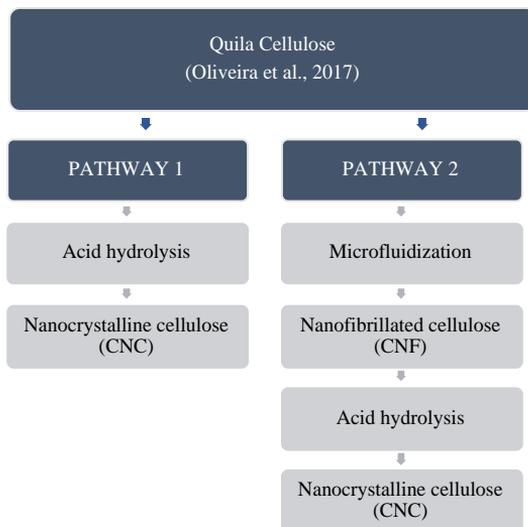


Figure 1. Synthesis methods to obtain nanostructures (nanocrystals and nanofibrillated cellulose) from *Chusquea quila*.

Characterization of the cellulose nanostructures

Concentration of carboxyl groups in CNC was determined by conductimetric assessment following Katz et al. (1984). Briefly, a dry sample was dispersed in 15 mL HCl (0.01M) to exchange the sodium cations bound to the COO⁻ groups for protons (H⁺). This suspension was shaken for 10 minutes and conductivity was determined in a nitrogen atmosphere. The assessment was completed by adding 0.1 mL of a solution of NaOH (0.01M) and recording the conductivity value. This operation was repeated until constant conductivity was reached. The titration profile revealed the presence of a strong acid (HCl) and a weak acid, the carboxyl content.

Morphology of the nanofibrillated structures derived from the quila fibres was characterised by scanning electron microscopy (SEM, Zeiss Sigma VP, Carl Zeiss Microscopy Ltd, Cambridge, UK). Operating voltage was 2 kV to 3 kV, and the working distance was between 2.5 mm and 2.6 mm. Sample drops of CNF at a consistency of approximately 0.1% were dried on polished aluminium supports and gold-coated to provide the required conductivity.

Fourier transform infra-red spectrophotometry (FTIR) was used to identify the characteristic functional groups of quila CNC and CNF. Samples were measured using a Bio-Rad FTS 6000 spectrophotometer (Cambridge, MA, USA) with a photo-acoustic detector and a constant mirror velocity of 5kHz, resolution 8 cm⁻¹ and 1.2 kHz filter.

X-ray diffraction (XRD) was used to determine the crystallinity of the nanocrystalline cellulose. X-ray diffraction analysis was carried out on fibres dried at 60 °C for 24 h in an ED53 chamber stove (Binder GmbH, Germany). Samples weighing approximately 1 g were compressed in a 25 mm diameter matrix at 4 kPa for 5 min. The discs were mounted on the rotating sample holder (15 rpm) of a D2 Phaser diffractometer (Bruker, Germany). The equipment used filtered Ni-Cu radiation (30 kV and 10 mA), 1 mm divergence slit, 1 mm anti-dispersion slit, 2.5° Soller slits and a LYNXEYE™ detector. The alignment was checked regularly with the NIST SRM1976 alumina plate standard. The patterns were collected over a range of 5–45°, counting 5 seconds per 0.01° pass. The crystallinity index (CI) was calculated according to the method of Segal et al., 1959. In order to ensure minimum statistical validity, each analysis was repeated with at least three different samples.

Atomic force microscopy (AFM, NanoScope IIIa Multimode, Digital Instruments Inc., Santa Barbara, CA, USA) was used to assess nanoscale topography of the fibrils and crystals. The recorded images were adjusted using NanoScope Analysis 1.2 software.

RESULTS AND DISCUSSION

We tested hydrolysis reactions with a mineral acid (sulphuric acid – H₂SO₄), and an organic acid (oxalic acid). Fig. 2 includes a schematic illustration of both methods.

Method 1, acid hydrolysis with oxalic acid, yielded no nanocrystals. This result was due to the format of the molecular structure of cellulose, which is characteristic of bamboo, i.e., oriented along the length of the bamboo fibre, giving strength, flexion and rigidity in that direction (Chattopadhyay et al., 2011).

The CI obtained was 75.56%, close to the value for cellulose fibre prior to hydrolysis. The X-ray diffraction pattern in Fig. 3 shows the similarity between the structures of hydrolysed and unhydrolyzed cellulose. The amorphous cellulose predominates close to the macrofibrillated surface, leading to the exposure of the bundles

of microfibrils (Zhao et al., 2007). The loss of this amorphous cellulose surface did not significantly alter the apparent crystallinity of the cellulose, since the amount was very small (CI almost 15%) when compared with the volume of amorphous cellulose.

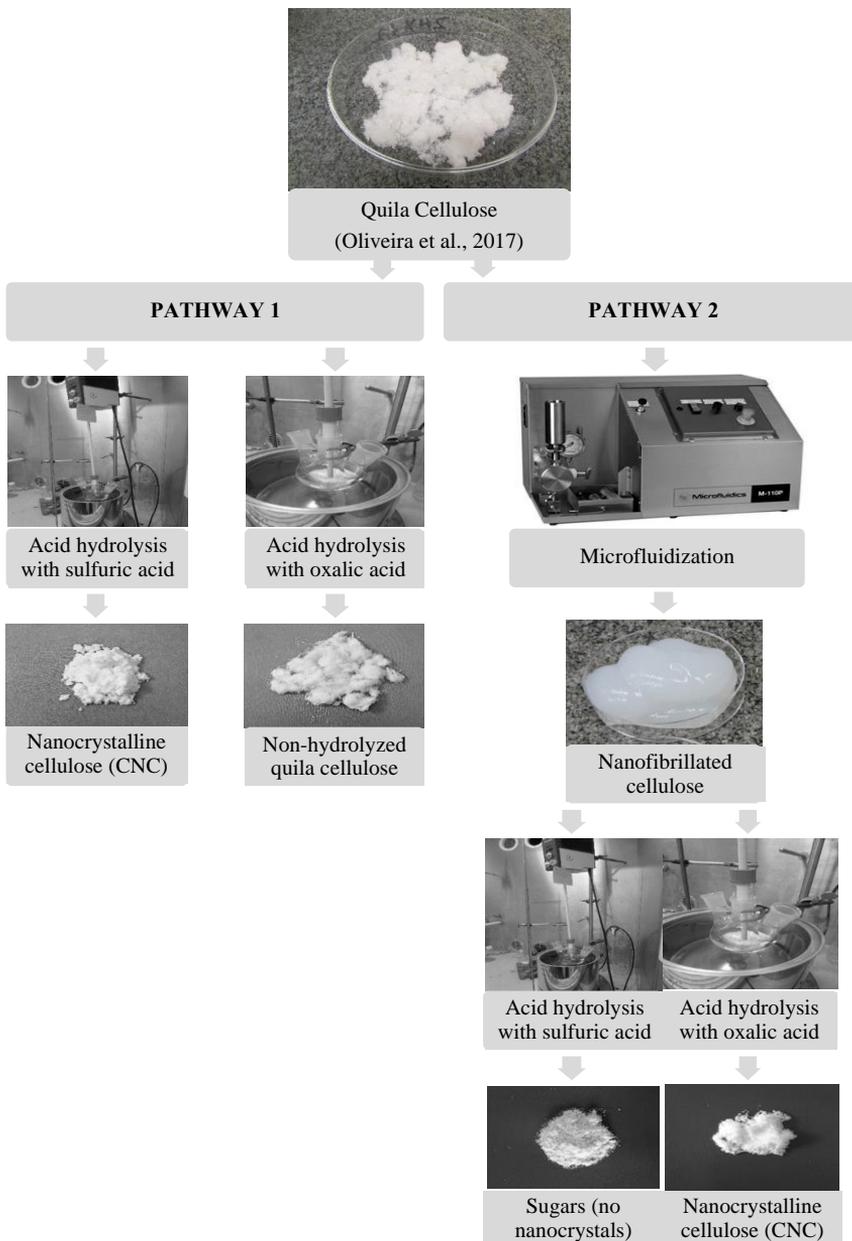


Figure 2. Photographic diagram of pathways 1 and 2 used in this study.

Most cellulosic materials consist of crystalline and amorphous domains, in varying proportions, depending on both source and history. The physical properties of cellulose, as well as their chemical behavior and reactivity, are strongly influenced by the arrangement of the cellulose molecules with respect to each other and to the fiber axis,

as well. Most of the reactants penetrate only the amorphous regions and it is only in these regions with a low level of order and on the surface of the crystallites that the reactions can take place, leaving the intracrystalline regions unaffected (Ciolacu et al., 2011).

After dialysis and sonication, the supernatant of the hydrolysed sample was examined by atomic force microscopy (AFM) to identify the presence of nanoparticles, however no nanocrystals were found.

Were found no nanocrystals in the product obtained by hydrolysis of nanofibrillated cellulose with sulphuric acid (Method 2). According to Kumar et al. (2009), this process may have generated mainly monomers from fermentable sugars. Minerals acids first attack the amorphous regions in cellulose and nanocellulose, since they are more susceptible to degradation. In the methodology applied, we considered the possibility that sugars were generated (degradation of nanofibers).

In the event that in the future is necessary to determine what types of sugars were formed as a result of this hydrolysis process, the study by Meile et al., 2018 could be used as a guide. Their work consisted of obtaining quantitative results from the determination of sugar in birch wood hydrolyzates using two iodometric titration methods and UPLC-ELSD. Both analytical methods showed acceptable precision with a relative standard deviation < 5% and xylose recovery from an enriched sample of approximately 90% and could be used to control the performance of sugars in the wood pretreatment process.

After the hydrolysis stage, we recovered the supernatant from the hydrolysed sample for examination via atomic force microscope (AFM), however no suspension could be recovered from the dialysis process. The alternative was to bring the solution to neutral pH and lyophilise the mixture.

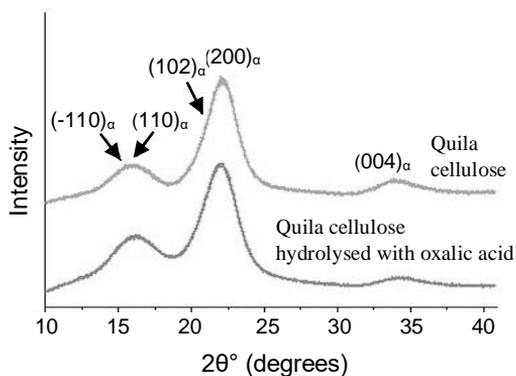


Figure 3. XRD diffractogram of the sample hydrolysed by oxalic acid (Method 1).

Table 1. Yields of CNC and CNF

Raw material	CNC (Y%)	References	Raw material	CNF (Y%)	References
Banana waste	27	Bolio-López et al., 2011	Bleached eucalyptus pulp	> 95	Beltramino, 2016
Residues of <i>Furcraea bedinghausii</i>	2	Espitia, 2010	Banana waste	64	Bolio-López et al., 2011
Bamboo pulp	21.8	Hong et al., 2013	Poplar cellulose kraft pulp	86	Qinghua et al., 2013
C. quila Route 1	32 ± 0.3	This work	Hibiscus cannabinus (kenaf)	67	Safwa et al., 2015
C. quila Route 2	1.2 ± 0.3	This work	C. quila cellulose	91 ± 0.5	This work

The treatments which formed cellulose nanocrystals, Method 1, included acid hydrolysis with sulphuric acid, and Method 2, acid hydrolysis of nanofibrillated cellulose with oxalic acid. Table 1 shows, in the first column, the empirical results obtained in this *Chusquea quila* study compared with other lignocellulosic sources reported in different manuscripts. It can be seen that, related to yields, the treatment with Chilean bamboo has better yields, which can be verified by the study by Abraham et al., 2011 when it refers to the need to study different raw materials.

Concentrations of the carboxyl groups were determined by conductivity titration of the CNC samples. Results for the product of hydrolysis with sulphuric acid (Method 1) was 1.47 mmol g^{-1} , indicating that the carboxyl groups on the CNC surfaces were not affected by acid hydrolysis with H_2SO_4 (reference value, 1.50 mmol g^{-1} , Salajková et al., 2016). The nanocrystalline cellulose produced by hydrolysis with sulphuric acid was accompanied by the formation of sulphate esters on the surface. One of the main problems was that the sulphate remnants on the surfaces of the nanocrystals were very unstable, however they were able to be eliminated by alkaline treatment. To improve dispersion in water, oxidation reactions were used to convert primary hydroxyls (C6) into carboxylic acid groups, which are more chemically stable than sulphate esters (Habibi et al., 2010). The product of acid hydrolysis of CNFs by oxalic acid (Method 2) presented a concentration of 1.63 mmol g^{-1} .

The morphology and topography of cellulose nanofibers were investigated with scanning electron microscopy (SEM) and atomic force microscopy (AFM). Fig. 4 shows that microfluidization produced a fine, homogeneous, uniform CNF network structure. Although it is difficult to measure the width and length of the nanofibers, 10–30 nm and 2,000–3,000 nm were estimated in the present case, respectively.

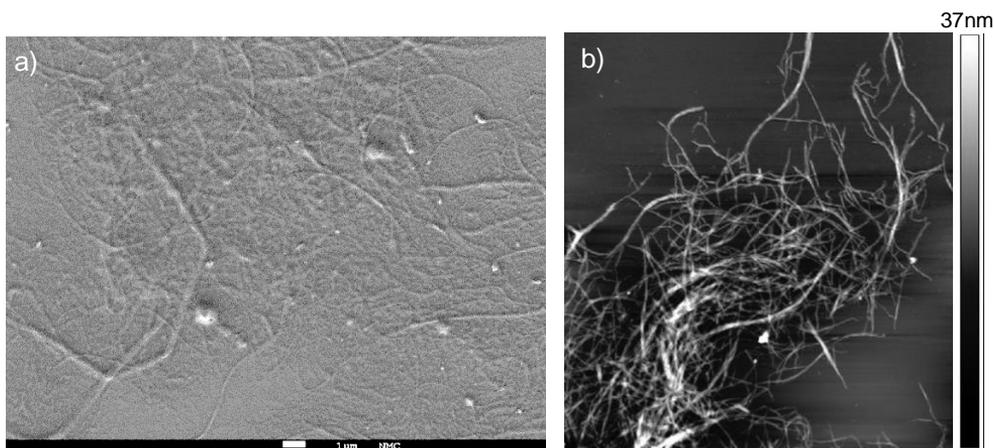


Figure 4. a) Scanning electron microscopy (SEM) and b) atomic force microscopy (AFM) of CNF from quila cellulose.

Fig. 5 shows size differences of the crystals isolated by Method 1 (acid hydrolysis with sulphuric acid) and Method 2 (acid hydrolysis of CNF with oxalic acid). The crystals in Fig. 5, a are heterogeneous in both size and shape (more sharply pointed). Meanwhile, the crystals in Fig. 5, present a more homogeneous form and suggest a trend towards regular size. It should be noted that CNC produced with mineral acids have a

greater tendency of degradation, depending on the type of cellulose used and reaction time, suggesting the formation of smaller crystals (Wang et al., 2014). In fact, the presence of sulphate groups on the NCC results in negatively charged surfaces that repel each other and do not allow the aggregation of the NCC by hydrogen bonding (Marchessault et al., 1961).

The sulphate esters produced during acid hydrolysis can decompose as the temperature increases, forming an organic sulphate that causes the dehydration of the cellulose due to the low energy required to remove the acid sulphate from the sulphated anhydroglucose units, then the sulfuric acid considered as a catalyst for dehydration. Then, the decomposition involves both the dehydration reaction and the increase in the formation of gas, carbonyl compounds, carboxyl and carbon residues (Wei et al., 1017).

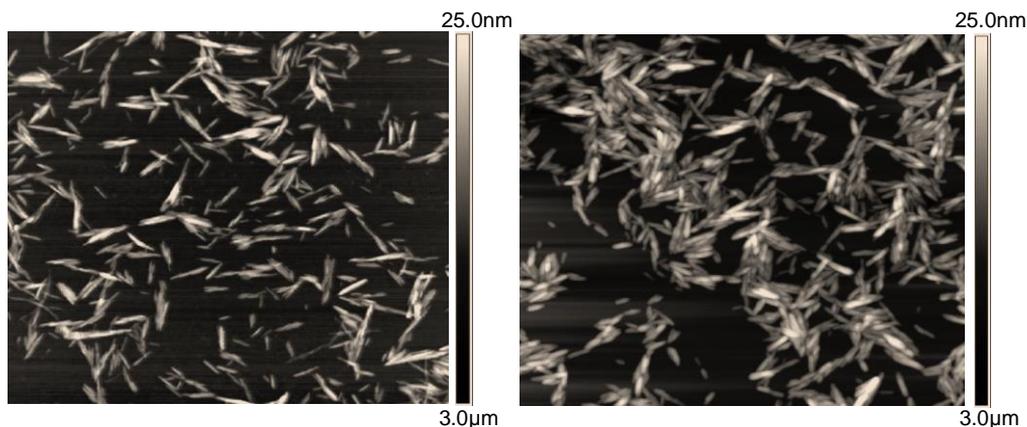


Figure 5. Atomic force microscopy images of CNC obtained by Methods 1 and 2.

Width of the cellulose nanocrystals was measured with ImageJ processing software (IJ 1.46) using images obtained AFM. Twelve AFM images were used to measure the size distribution. Histograms for width distribution are presented in Fig. 6. The diameters of the product of Method 1, Fig. 6(A) presented variable frequency, with a maximum distribution range between 10 and 15 nm. Fig. 6(B) shows a more uniform diameter distribution, between 10 and 25 nm.

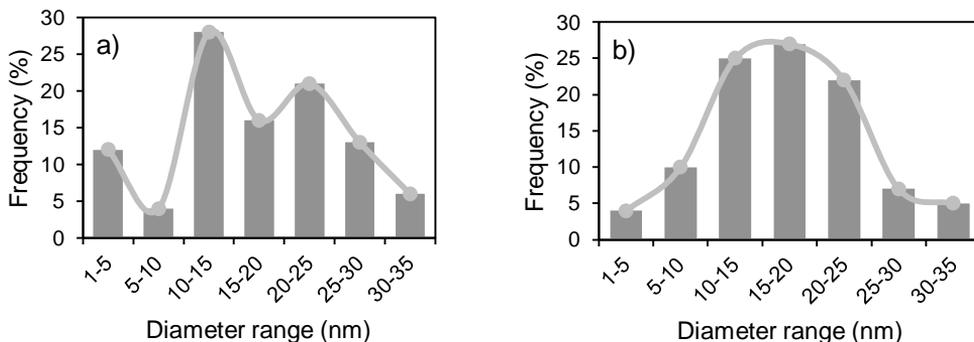


Figure 6. Width distribution histograms for nanocrystalline cellulose produced by Methods 1 (a) and 2 (b).

The study carried out by Oliveira et al. (2018), the processes of delignification and bleaching of cellulose used in this research are considered in detail. The methodology details the oxidation reactions using 15% (v/v) peracetic acid. During the acid hydrolysis that we estimate here, most of the amorphous part of cellulose was eliminated, remembering the successful processes: Method 1 hydrolysis using sulfuric acid and Method 2 using oxalic acid.

The crystalline parts of cellulose have a high resistance to acid hydrolysis (Vanhatalo & Dahl 2014). Fig. 7 shows the FTIR spectrum of nanofibrillated quila cellulose and the nanocrystalline cellulose of the two proposed Methods. As indicated by Rosli et al. (2013), the peak $1,735\text{ cm}^{-1}$ belonged to C=O and C-O of the acetyl groups, uronic ester and the aryl group in hemicellulose and lignin, respectively. During the delignification phase, carbohydrates are very stable and in the bleaching phase, there was an increase in carbohydrate degradation when about 90% of the lignin was oxidized (Zimmermann et al., 1992). The peaks $3,440\text{--}3,300\text{ cm}^{-1}$ belong to the free stretch of OH of the hydroxyl group in the cellulose molecules. In addition, the peak at $2,887\text{ cm}^{-1}$ belongs to the C-H stretch (Wang et al., 2007).

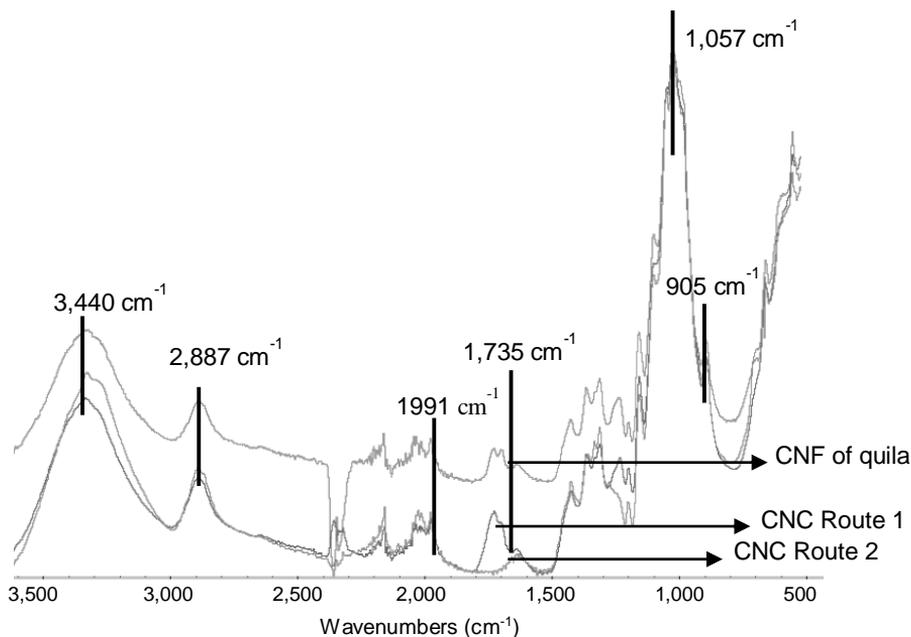


Figure 7. FTIR spectrum of CNC and CNF from quila cellulose.

The C-O-C bond of the pyranose skeletal ring can be observed at $1,057\text{ cm}^{-1}$, which increases the intensity of this bond due to the increases in the crystallinity of the samples. The most significant absorption bond of 905 cm^{-1} has been related to the CH bond of glycoside deformation and OH flexion, these properties are characteristic of the β -glycosidic bond between the anhydroglucose units in cellulose (Razalli et al., 2017). No significant difference was found in the FTIR spectrum between CNF and CNC, suggesting that their molecular chemical structures remained practically unaltered during microfluidization and acid hydrolysis.

Cellulose nanofibers presented a clear, characteristic peak at diffraction angle $2\theta = 22.7^\circ$ (Fig. 8). X-ray diffraction analysis showed that the nanofibers analysed in this study had a crystallinity index $> 80\%$. The CI value for nanofibrillated quila cellulose was 85%, while the nanocrystals isolated by Methods 1 and 2 presented indices of 92 and 88%, respectively. The slightly lower crystallinity in the sample from Method 2 could be explained by the fact that the size reduction of the crystalline cellulose structure (CNF) leads to a lower volume of crystals (Method 2, acid hydrolysis of nanofibrillated cellulose using oxalic acid), and therefore, a reduction in crystallinity (Qinghua et al., 2013).

It is important to remember that hemicellulose and lignin exist naturally in an amorphous form in the biomass, while the cellulose macromolecule is a pure crystalline structure. For cellulose, changes in crystallinity reflect changes in the composition and structure of plant fibers (Li et al., 2014). This study showed that the crystalline form of cellulose did not change during the treatment, but the crystallinity of the fiber was substantially improved. From the raw material (quila cellulose) the crystallinity index increased regardless of the treatment method since the non-crystalline region in the cellulose was gradually eliminated in each stage. It is noted that the bleaching process did not destroy the cellulose structure by fractionating hemicellulose and lignin.

From the diffraction pattern obtained from CNC, the high-intensity peak was noted at 2θ reflection assigned to the crystallographic plane = 22.7° (arising due to diffraction from the plane (200)), with additional double peak signals at $= 15^\circ$ and 16.9° due to the reflections of (110) and (110) diffraction, respectively. The higher value of the given index shows that the test material has a high degree of crystallinity with an adequate order structure. The treatments with chemical products can alter the order in the structure and, in the case of CNC, reach a higher value due to the elimination of the amorphous state of cellulose (Theivasanthi et al., 2018).

From another perspective, prior milling can help the action of hydrolysis in amorphous regions of cellulose fibres, leading to crystal separation. This process has been reported with positive results in fibres of *Pinus banksiana* (jack pine) (Rambabu et al., 2016).

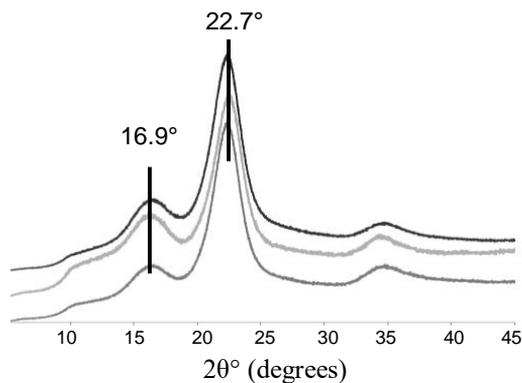


Figure 8. XRD diffractogram of quila cellulose CNC and CNF.

CONCLUSIONS

Chilean bamboo *Chusquea quila* was used as source of nanocrystalline and nanofibrillated cellulose. Two alternative methods were introduced for obtaining these nanostructures. Methods involved acid hydrolysis of the cellulosic fibres with an intermediate microfluidization stage. Method 1 used direct hydrolysis of quila cellulose fibre using sulphuric acid; and Method 2 involved an alternative stage of fibre

microfluidization to produce CNF, followed by hydrolysis with oxalic acid. The yield was higher for Method 1.

Morphological analysis of the nanofibrillated quila cellulose showed a fine, homogeneous, uniform network structure. The AFM images of the nanocrystalline celluloses showed small differences for Method 1 and Method 2. These nanocrystals had widths between 10 and 20 nm. FTIR analysis indicated that the microfluidization and acid hydrolysis processes did not affect the molecular form of the nanostructures. X-ray diffraction showed crystallinity values higher than 80%.

Our results indicate the potential of quila to open up new opportunities as a lignocellulosic species for promoting sustainable, environmentally friendly products.

ACKNOWLEDGEMENTS. This study was supported by the Wood Engineering Department of Universidad del Bío-Bío (Chile) and the Biomaterials and Nanotechnology Centre from the same university. We are grateful to the support of members of the Bioproducts and Advanced Materials Nucleus of Catholic University of Temuco (Chile) and our special thanks goes to the Biobased Colloids and Materials, BiCMat research group led by Dr. Orlando Rojas, in University of Aalto, Finland.

REFERENCES

- Abraham, E., Deepa, B., Pothan, L.A., Jacob, M., Thomas, S., Cvelbar, U. & Anandjiwala, R. 2011. Extraction of nanocellulose fibrils from lignocellulosic fibres: A novel approach. *Carbohydrate Polymers* **86**, 1468–1475. doi: 10.1016/j.carbpol.2011.06.034
- Banga, H., Singh, V.K. & Choudhary, S.K. 2015. Fabrication and Study of Mechanical Properties of Bamboo Fibre Reinforced Bio-Composites. *Innovative Systems Design and Engineering* **6**(1), ISSN 2222-2871.
- Beltramino, F. 2016. *Enzymatic-assisted preparation of nanocrystalline cellulose from non-wood fibers*. Pages 4–1 to 4–7. PhD Tesis. Programa de Doctorat d'Enginyeria Tèxtil i Paperera. Universidad Politècnica de Catalunya – España. (in Spanish).
- Bolio-López, G.I., Valadez-González, A., Veleza, L. y Andreeva, A. 2011. Cellulose whiskers from agroindustrial residues of banana: obtaining and characterization. *Revista Mexicana de Ingeniería Química* **Vol. 10**, No. 2 291–299. ISSN 1665-2738 (in Spanish).
- Brinchi, L., Cotana, F., Fortunati, E. & Kenny, J.M. 2013. Production of nanocrystalline cellulose from lignocellulosic biomass: technology and applications. *Carbohydrate Polymers* 2013 Apr 15; **94**(1), 154–169. doi: 10.1016/j.carbpol.2013.01.033
- Campos Roasio, J., Peñalosa, R., Kahler, C., Poblete, H. & Cabrera, J. 2003. Bamboo in Chile. Book published by the Technological Research Corporation of Chile, FONDEF Project D98I1002, pp. 41–51. Santiago, Chile (in Spanish).
- Canilha, L., Chandel, A.K., Milessi, T.S., Antunes, F.A., Freitas, W.L., Felipe, M.G. & Silva, S.S. 2012. Bioconversion of sugarcane biomass into ethanol: an overview about composition, pretreatment methods, detoxification of hydrolysates, enzymatic saccharification, and ethanol fermentation. *Journal of Biomedicine and Biotechnology* Volume **2012**, Article ID 989572. doi: 10.1155/2012/989572
- Chattopadhyay, S.K., Khandal, R.K., Uppaluri, R. & Ghoshal, A.K. 2011. Bamboo fiber reinforced polypropylene composites and their mechanical, thermal, and morphological properties. *Journal of Applied Polymer Science* **119**, 1619–1626. doi:10.1002/app.32826
- Chen, W.S., Yu, H.P., Liu, Y.X., Hai, Y.F., Zhang, M.X. & Chen, P. 2011. Isolation and characterization of cellulose nanofibers from four plant cellulose fibers using a chemical-ultrasonic process. *Cellulose* **18**, 433–442. doi: /10.1007/s10570-011-9497-z

- Ciolacu, D., Ciolacu, F. & Popa, V.I. (2011). “Amorphous cellulose – structure and characterization”. *Cellulose Chemistry and Technology* **45**(1–2), 13–21.
- (CONAF) National Forestry Corporation. 2012. Manual with Measures for the Prevention of Forest Fires (*Technical Report*), Ministry of Agriculture, Santiago, Chile (in Spanish).
- Espitia, H.M. 2010. *Isolation of cellulose nanofibers from agro-industrial residues of sugar cane and sugarcane, with potential application in thermoplastic polymer reinforcement* Master's thesis. Universidad Nacional de Colombia, Facultad de Ciencias, Escuela de Química, Sede Medellín – Colombia. <https://repositorio.unal.edu.co/handle/unal/69993> (in Spanish).
- Fatah, I.Y., Khalil, H.P., Hossain, M.S., Aziz, A.A., Davoudpour, Y. & Dungani, R. 2014. Exploration of a chemo-mechanical technique for the isolation of nanofibrillated cellulosic fiber from oil palm empty fruit bunch as a reinforcing agent in composites materials. *Polymers* **6**, 2611–2624. doi: 10.3390/polym6102611
- Ferraz, P.F.P., Mendes, R.F., Ferraz, G.A.S., Rossi, G., Conti, L. & Barbari, M. 2020. Chemical analyses of lignocellulosic materials residue for cement panels reinforcement. *Agronomy Research* **18**(S1), 815–822. doi: 10.15159/AR.20.155
- Fortunati, E., Puglia, D., Monti, M., Peponi, L., Santulli, C., Kenny, J.M. & Torre, L. 2013. Extraction of cellulose nanocrystals from Phormium tenax fibres. *Journal of Polymers and the Environment* **21**, 319–328. doi: 10.1007/s10924-012-0543-1
- González, Y., Meza, J., González, O. & Córdova, J. 2013. Synthesis and biodegradation of polyhydroxyalkanoates: plastics of microbial origin. *Revista Internacional de Contaminación Ambiental* **29**(1), 77–115. ISSN 0188-4999 (in Spanish).
- Habibi, Y., Lucia, L.A. & Rojas, O.J. 2010. Cellulose Nanocrystals: Chemistry, Self-Assembly, and Applications. *Chemical Reviews* **110**, 3479–3500, 3479. doi: 10.1021/cr900339w
- Hideno, A., Abe, K. & Yano, H. 2014. Preparation using pectinase and characterization of nanofibers from orange peel waste in juice factories. *Journal of Food Science* **79**, 1218–1224. doi: 10.1111/1750-3841.12471
- Hong, B., Chen, F. & Xue, G. 2013. Preparation and characterization of cellulose nanocrystals from bamboo pulp. *Cellulose Chemistry and Technology* **50**(2), 225–231.
- Junka, K., Guo, J., Filpponen, I., Laine, J. & Rojas, O.J. 2014. Modification of cellulose nanofibrils with luminescent carbon dots. *Biomacromolecules* **15**(3), 876–881. doi: 10.1021/bm4017176
- Katz, S., Beatson, R.P. & Scallan, A.M. 1984. The determination of strong and weak acidic groups in sulfite pulps. *Svensk Papperstidning* **6**, R48–R53.
- Khawas, P. & Deka, S.C. 2016. Isolation and characterization of cellulose nanofibers from culinary banana peel using high-intensity ultrasonication combined with chemical treatment. *Carbohydrate Polymers* **137**, 608–616. doi: 10.1016/j.carbpol.2015.11.020
- Kumar, P., Barrett, D.M., Delwiche, M.J. & Stroeve, P. 2009. Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production. *Industrial & Engineering Chemistry Research* **48**(8), 3713–3729. doi: 10.1021/ie801542g
- Li, J., Wang, Y. & Wei, X. 2014. Homogeneous isolation of nanocelluloses by controlling the shearing force and pressure in microenvironment. *Carbohydrate Polymers* Volume **113**, 26 November 2014, pp. 388–393. doi.org/10.1016/j.carbpol.2014.06.085
- Li, W., Zhang, Y.C., Li, J.H., Zhou, Y.J., Li, R.S. & Zhou, W. 2015. Characterization of cellulose from banana pseudo-stem by heterogeneous liquefaction. *Carbohydrate Polymers* **132**, 513–519. doi: 10.1016/j.carbpol.2015.06.066
- Lin, R., Li, A., Zheng, T., Lu, L. & Cao, Y. 2015. Hydrophobic and flexible cellulose aerogel as an efficient, green and reusable oil sorbent. *RSC Advances* **5**, 82027–82033. doi:10.1039/C5RA15194E

- Lu, Q., Cai, Z., Lin, F., Tang, L., Wang, S. & Huang B. 2016. Extraction of Cellulose Nanocrystals with a High Yield of 88% by Simultaneous Mechanochemical Activation and Phosphotungstic Acid Hydrolysis. *ACS Sustainable Chemistry & Engineering* **4** (4), 2165–2172. doi: 10.1021/acssuschemeng.5b01620
- Marchessault, R., Morehead, F. & Koch, M.J. 1961. Some hydrodynamic properties of neutral suspensions of cellulose crystallites as related to size and shape. *Journal of Colloid Science* **16**(4), 327–344.
- Meile, K., Zhurinsk, A., Briede, L. & Viksna, A. 2018. Investigation of the sugar content in wood hydrolysates with iodometric titration and UPLC-ELSD. *Agronomy Research* **16**(1), 167–175. doi: 10.15159/AR.17.076
- Oliveira, P.E., Cunha, A., Reyes, G., Gacitúa, W. & Petit-Breuilh, X. 2016. *Chusquea quila*, a natural resource from Chile: its chemical, physical, and nanomechanical properties. *BioResources* **11**(4), 10057–10069. doi: 10.15376/biores.11.4.10057-10069
- Oliveira, P.E., Cunha, A., Pesenti H., Gacitúa, W. & Petit-Breuilh, X. 2018. Exploring an oxidative bleaching treatment for Chilean bamboo: a source of cellulose for biofuel generation and the nanotech industry. *European Journal of Wood and Wood Products* **76**, 1009–1019. doi: 10.1007/s00107-017-1237-0
- Pinto, E. & Barrientos, J. 1993. *Flowering Quila (XI Region) /Florecimiento de la Quila (XI Región)*. Technical Report, Corporación Nacional Forestal, Ministerio de Agricultura, Santiago, Chile (in Spanish).
- Qinghua, X., Yang, G., Menghua, Q., Kaili, W., Yingjuan, F. & Jian, Z. 2013. Nanocrystalline cellulose from aspen kraft pulp and its application in deinked pulp. *International Journal of Biological Macromolecules* **60**, pp. 241–247. doi: 10.1016/j.ijbiomac.2013.05.038
- Rambabu, R., Panthapulakkal, S., Sainb, M. & Dalai, A.K. 2016. Production of nanocellulose fibers from pinecone biomass: Evaluation and optimization of chemical and mechanical treatment conditionson mechanical properties of nanocellulose films. *Industrial Crops and Products* **83**, 746–754. doi: 10.1016/j.indcrop.2015.11.083
- Razalli, R.L., Abdi, M.M., Tahir, P.M., Moradbak, A., Sulaiman, Y. & Heng, L.Y. 2017. Polyaniline-modified nanocellulose prepared from Semantan bamboo by chemical polymerization: preparation and characterization. *RSC Advances* **7**(41), 25191–25198.
- Salajková, M., Berglund, L.A. & Zhou, Q. 2016. Hydrophobic cellulose nanocrystals modified with quaternary ammonium salts. *Journal of Materials Chemistry* **22**, 19798–19805. doi: 10.1039/C2JM34355J
- Schlegel, F. 1993. The problem of flowering. Informe Técnico (*Technical Report*); Revista Chile Forestal **206**, 35–37 (in Spanish).
- Segal, L., Creely, J., Martin, Jr.A. & Conrad, C. 1959. An Empirical Method for Estimating the Degree of Crystallinity of Native Cellulose Using the X-Ray Diffractometer. *Textile Research Journal* **29**(10), 786–794. doi: 10.1177/004051755902901003
- Safwa, S., Mokhtar, M.N., Naim, M.N., Baharuddin, A.S., Salleh, M.A.M. & Sulaiman, A. 2015. Study on the Preparation of Cellulose Nanofibre (CNF) from Kenaf Bast Fibre for Enzyme Immobilization Application. *Sains Malaysiana* **44**(11), 1541–1550. ISSN 0126-6039
- Tang, J., Sisler, J., Grishkewich, N. & Chiu Tam, K. 2017. Functionalization of cellulose nanocrystals for advanced applications. *Journal of Colloid and Interface Science* Volume **494**, 15 May 2017, pp. 397–409. doi: 10.1016/j.jcis.2017.01.077
- Theivasanthi, T., Anne Christma, F.L., Toyin, A.J., Gopinath C.B. & Ravichandran, R. 2018. Synthesis and characterization of cotton fiber-based nanocellulose. *International Journal of Biological Macromolecules* **109**, 832–836. doi: 10.1016/j.ijbiomac.2017.11.054
- Vanhatalo, K.M. & Dahl, O.P. 2014. Effect of mild acid hydrolysis parameters on properties of microcrystalline cellulose. *BioResources* **9**(3), 4729–4740.

- Wang, D. & Shen, S.-J. 1987. *Bamboos of China*. Hardcover, August 1, Timber Press, pp 19–21.
- Wang, H.Y., Li, D.G., Yano, H. & Abe, K. 2014. Preparation of tough cellulose II nanofibers with high thermal stability from wood. *Cellulose* **21**, 1505–1515. doi: 10.1007/s10570-014-0222-6
- Wang, L., Han, G. & Zhang, Y. 2007. Comparative study of composition, structure and properties of Apocynum venetum fibers under different pretreatments. *Carbohydrate Polymers* **69**(2), 391–397.
- Wei, L., Luo, S., McDonald, A. G., Agarwal, U.P., Hirth, K.C., Matuana, L.M., Sabo, R.C. & Stark, N.M. 2017. "Preparation and Characterization of the Nanocomposites from Chemically Modified Nanocellulose and Poly (lactic acid)". *Journal of Renewable Materials* **5**(5), 410–422, 2017.
- Zhao, H., Kwak, J.H., Zhang, Z.C., Brown, H.M., Arey, B.W. & Holladay, J.E. 2007. Studying cellulose fiber structure by SEM, XRD, NMR and acid hydrolysis. *Carbohydrate Polymers* **68**, 235–241.
- Zimmermann, M., Patt, R. & Kordsachia, O. 1992. ASAM pulping of Douglas-fir followed by a chlorine-free bleaching sequence. *TAPPI J* **74**(11), 129–134.