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Isolation and characterization of cellulose nanocrystals from spruce bark in a biorefinery perspective

Myriam Le Normand, Rosana Moriana, Monica Ek*

Division of Wood Chemistry and Pulp Technology, School of Chemical Science and Engineering, KTH Royal Institute of Technology, Teknikringen 56-58, SE-10044 Stockholm, Sweden

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ABSTRACT

The present study reports for the first time the isolation of cellulose fibers and cellulose nanocrystals (CNCs) from the bark of Norway spruce. The upgrading of bark cellulose to value-added products, such as CNCs, is part of the "bark biorefinery" concept. The removal of non-cellulosic constituents was monitored throughout the isolation process by detailed chemical composition analyses. The morphological investigation of the CNCs was performed using AFM and showed the presence of nanocrystals with an average length of 175.3 nm and a diameter of 2.8 nm, giving an aspect ratio of around 63. X-ray diffraction (XRD) analyses showed that the crystallinity index increased with successive treatments to reach a final value greater than 80% for CNCs. The thermal degradation of the isolated bark CNCs started at 190 °C. Spruce bark appeared to be a new promising industrial source of cellulose fibers and CNCs.

1. Introduction

The development of the next generation of materials, products, and processes is currently guided by the emphasis on sustainability, industrial ecology and green chemistry. At the same time, the pulp and paper sector is facing a deep market change and must identify new innovative strategies. In this sense, the forest biorefinery presents an attractive alternative for producing novel added-value products from lignocellulosic side-streams and improving the competitiveness of the pulp and paper industry.

Norway spruce (*Picea abies*) is the most abundant coniferous tree growing in Northern Europe and it is extensively used in the Scandinavian pulp and paper industry. The bark of Norway spruce represents about 10% of the weight of the tree trunk (Fengel & Wegener, 1983). Due to its resistance to pulping and its high content of extractives, bark is considered to be an undesirable material in the pulp and paper production. In most pulping processes, bark is therefore removed from the logs before they undergo chipping and used as a fuel to produce heat and electrical energy. According to the Swedish Forest Agency, the annual bark yield in Sweden is estimated to be more than 1.5 million tons (dry weight). Part of this considerable amount of bioresidue might be upgraded in a bark biorefinery where high-value components from the bark could be fractionated, prior to combustion of the residue (Le Normand, Edlund, Holmbom, & Ek, 2012). For instance, tannins could be used for their adhesive properties (Feng, Cheng, Yuan, Leitch, & Xu, 2013), hydrophilic extractives for their potential bioactivity (Co et al., 2012; Nilsson et al., 2011; Pietarinen, Willför, Ahotupa, Hemming, & Holmbom, 2006), and non-cellulosic polysaccharides for their immunomodulating properties (Le Normand et al., 2014) or for ethanol production (Kemppainen, Inkinen, Uusitalo, Nakari-Setälä, & Siika-aho, 2012). However, little has been published regarding the utilization of the cellulose which is present in bark in non-negligible quantities. The cellulose content in the bark of Norway spruce was reported for the first time by von Dietrichs, Garves, Behrensdorf, and Sinner (1978) who showed that 19% of the bark consisted of cellulose. More recently, we have shown that the whole bark of Norway spruce collected just after the debarking process consisted of 15% cellulose (Le Normand et al., 2012). A parallel study performed on inner and outer bark separately showed a cellulose content of 23% in the inner bark and 11% in the outer bark (Krogell, Holmbom, Pranovich, Hemming, & Willför, 2012).

In the present work, a novel way to upgrade spruce bark through the exploitation of its cellulose content and the isolation of cellulose nanocrystals (CNCs) is presented. CNCs have attracted great interest as a novel nanostructured material due to their exceptional mechanical properties (high specific strength and modulus), large specific surface area and high aspect ratio (Azizi Samir, Alloin, & Dufresne, 2005). The isolation of CNCs from wood and plants involves two main stages: extraction of cellulosic fibers and removal of the amorphous regions of cellulose. In the present study, pre-treatment with hot water was preferred instead of common

* Corresponding author. Tel.: +46 8 7906000/+46 8 7908104; fax: +46 8 7906166.
E-mail address: monicaek@kth.se (M. Ek).

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pulping or steam explosion processes for the extraction of cellulosic fibers. This method allowed a first recovery of extractives and non-cellulosic polysaccharides, which could be used for added-value applications, leaving cellulose in the insoluble fraction. The removal of the amorphous regions of cellulose is commonly based on controlled acid hydrolysis. During the hydrolysis stage, the amorphous domains are preferentially hydrolyzed, since the crystalline regions have a greater resistance to acid attack. The dimension, morphology and degree of crystallinity of the CNCs depend on the cellulosic raw material as well as on the pretreatment and hydrolysis conditions. In the past decade, research dealing with the production of CNCs from industrial wastes has mostly been focused on agricultural residues such as mango seeds (Henrique, Silvério, Flauzino Neto, & Pasquini, 2013), rice husk (Johar, Ahmad, & Dufresne, 2012), corn cobs (Silvério, Flauzino Neto, Dantas, & Pasquini, 2013) and cassava bagasse (Pasquini, Teixeira, Curvelo, Belgacem, & Dufresne, 2010). Recently, Moriana and Ek (2013) have also shown that it is possible to recover CNCs from forest residues. Potential applications of CNCs include reinforcing agents in polymer matrices (Dufresne, 2010), barrier films (Belbekhouche et al., 2011), flexible displays (Nakagaito, Nogi, & Yano, 2010), drug delivery excipients (Jackson et al., 2011), security paper (Revol, Godbout, & Gray, 1998) and templates for electronic components (Azizi Samir, Alloin, Sanchez, & Dufresne, 2004).

The present paper describes, for the first time to our knowledge, the preparation and detailed characterization of CNCs isolated from one of the largest side-streams of the Scandinavian forest industry, i.e. the bark of Norway spruce. The objective of this work was to isolate the CNCs from the bark residue after partial extraction of the non-cellulosic constituents i.e. extractives, tannins, hemicellulose, pectins. The chemical composition, morphological structure, and thermal properties of the CNCs were evaluated and compared with those of other nanocellulosic materials to estimate their suitability for use as reinforcements in composites.

2. Materials and methods

2.1. Materials

Bark of Norway spruce was sampled from a fresh 30-year-old tree cut in Gävleborg County (Sweden) in July 2009. It was stored in the dark at −20 °C. The inner and outer bark were separated manually using a scalpel. The inner bark was ground with a hand blender to a particle size of approximately 5 × 2 mm. The dry content of the ground bark was 56%. Analytical grade chemicals used for extraction, bleaching, hydrolysis and carbohydrate analysis were purchased from Sigma-Aldrich and used without further purification.

2.2. Isolation of CNCs from the spruce bark

2.2.1. Pre-treatment of the inner bark

The ground inner bark was sequentially extracted in an Accelerated Solvent Extractor (ASE) (Dionex, California) as described by Le Normand et al. (2012) by treating wet inner bark (dry content of 56%) with acetone at 100 °C followed by hot water at 100 °C and 140 °C. This method led the partial extraction of non-cellulosic constituents (extractives, tannins, hemicellulose, pectins). The solid residue was freeze-dried and used for the isolation of cellulose nanocrystals (CNCs).

2.2.2. Isolation of CNCs from the bark residue

The residue after the extraction was bleached in a mixture of 1% sodium chlorite, acetate buffer pH 4.8 and water in the proportions 1:1:1. The concentration of the sample in the solution was 4% w/w. The bleaching procedure was performed at 80 °C for 4 h and repeated four times. The bleached fibers were washed repeatedly with distilled water and dried in an oven at 40 °C. The fibers were then ground in a Wiley mill (20 mesh screen) and hydrolyzed in sulfuric acid solution (60% w/w) at 50 °C for 60 min. The hydrolysis was stopped by pouring the solution into ice. The suspension was then centrifuged at 13,000 rpm and 4 °C for 10 min, and the precipitate was resuspended in milliQ water and subsequently centrifuged. This process was repeated until the supernatant reached pH 5. The suspension was then dialyzed against MilliQ water during one week. A CNC suspension was obtained after sonication for 10 min at an amplitude of 27% and centrifugation.

2.3. Characterization of the material at different stages of the extraction

2.3.1. Chemical composition

The content of neutral carbohydrates and Klason lignin were determined after acid hydrolysis with 72% H_2SO_4, following the standard procedures of Tappi test method T222 om-06 (TAPPI, 2006). The samples were analyzed with high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). A combination of methanolysis and TFA hydrolysis according to De Ruiter, Schols, Voragen, & Rombouts, 1992 was used to determine the uronic acid composition of the samples. Briefly, 2–5 mg of dried sample were heated for 16 h at 80 °C with 1 ml of 2 M HCl in methanol in duplicate experiments. The methanolic HCl was evaporated by a stream of air at 25 °C and the remaining carbohydrates were further hydrolyzed with 1 ml of 2 M TFA for 1 h at 121 °C. The TFA was removed by evaporation and the samples were dissolved in water before analysis by HPAEC-PAD. The HPAEC-PAD Dionex ICS-3000 was equipped with a CarboPac® PA1 4 × 50 mm precolumn, a CarboPac® PA1 4 × 250 mm column and a CarboPac® PA100 4 × 250 mm postcolumn. The eluent flow of water was 1 ml min^{-1}.

The ash content was determined after pyrolysis of the dry sample in a furnace at 525 °C during 6 h, following the standard procedure of Tappi test method T211 om-02 (TAPPI, 2012).

The sulfur content of the CNC sample was measured by inductively coupled plasma optical emission spectroscopy (ICP-OES) using a spectrometer iCAP 6000 Series (Thermo Scientific). The instrument was calibrated with a standard sulfur solution (Merck KgaA, Germany) at different wavelengths: 180.7 nm, 182.0 nm and 182.6 nm.

2.3.2. Size exclusion chromatography (SEC)

About 1 mg (dry weight) of wet bleached fibers and CNC were subjected to an activation treatment by solvent exchange, which consisted of one swelling step in 1 ml deionized water for 2 h, followed by three consecutive exchanges of 30 min with 1 ml of methanol, and ending with three consecutive exchanges with 1 ml *N*,*N*-dimethylacetamide (DMAc). The first DMAc exchanges lasted 30 min and the third was prolonged overnight. After the last DMAc exchange, 127 μl of 8% LiCl/DMAc were added to the activated sample and the mixture was stirred until complete dissolution. The solution was then diluted to 0.5% LiCl/DMAc and filtered through a 0.45-μm polytetrafluorethylene syringe filter before injection. SEC analyses were performed on a Shimazu instrument which consisted of a DGU-20A3 degasser, an LC-20AD liquid chromatograph, a CT-20A column oven equipped with a Rheodyne 7725i fixed loop (100 μl) and a RID-10A refractive index detector. The separation system comprised three Mixed-A columns (7.5 × 300 mm, Polymer Laboratories) packed with 20 μm particle diameter, preceded by a Mixed-A guard column (7.5 × 50 mm, Polymer Laboratories) with 20 μm particle diameter. The mobile phase was 0.5% LiCl/DMAc with a flow of 0.5 ml min^{-1}. The separation was carried out at a

temperature of 80 °C. The data were acquired and processed with the LC Solution software (Shimadzu).

2.3.3. Scanning electron microscopy (SEM)

SEM micrographs were obtained from the bark, extraction residue and bleached fibers using a JEOL JSM-5400 scanning electron microscope (JEOL Ltd., Japan) after sputtering the samples with gold/palladium on a Cressington 208HR Sputter coater.

2.3.4. Atomic force microscopy (AFM)

The morphology of the CNCs was imaged in the dry state with tapping-mode AFM (Multimode V, Bruker, Santa Barbara, CA). Images in height, amplitude and phase modes were recorded with an E-scanner. RTESP silica cantilevers (Bruker) having a tip with a radius of 8 nm and a spring constant of 20–80 N m^{-1} oscillated at its fundamental resonance frequencies between 306 and 366 kHz. Lengths and diameters were obtained from printouts of several height mode AFM images, using the section analysis tool of the NanoScope Analysis software (Bruker, version 1.40). More than a hundred CNCs were randomly selected and measured to determine their average length and diameter.

2.3.5. Fourier transform infrared spectrometry (FTIR)

Fourier transform infrared spectrometry (FTIR) was carried out on a Perkin-Elmer Spectrum 2000 FTIR with an attenuated total reflectance (ATR) crystal accessory (Golden Gate). Spectra of the bark, extraction residue, bleached fibers and CNCs were obtained by means of 16 individual scans at 2 cm^{-1} resolution in the 4000–600 cm^{-1} interval. All spectra were smoothed and fitted to an automatic base line correction by OMNIC 7.0 software.

2.3.6. Wide angle X-ray scattering (WAXS)

The bark, the extraction residue, bleached fibers and CNCs were analyzed at ambient temperature in an X-ray diffractometer (X'Pert PRO MPD PANalytical, The Netherlands) using a monochromatic Cu $K\alpha$ radiation ($\lambda = 1.54$ Å) in the range of $2\theta = 10$ to 50° with a scanning rate of 1.0 min^{-1}. X-ray diffraction data were processed and analyzed using HighScore Plus 3.0 software (PANalytical, Inc.). The crystalline index (CI) of the bark after the different treatments was determined using the peak height method and calculated by the Segal empirical method (Segal, Creely, Martin, & Conrad, 1959) after subtraction of the background signal:

$$\mathrm{CI}(\%) = \frac{(I_{002} - I_{\mathrm{AM}})}{I_{002}} \times 100 \quad (1)$$

where I_{002} is the peak intensity corresponding to crystalline cellulose I at 2θ of 22.5°, and I_{AM} is the intensity of the amorphous fraction at 18°.

The average thickness of the cellulose crystallites was estimated from the XRD patterns using Scherrer equation:

$$D_{hkl} = \frac{K\lambda}{\beta_{1/2}\cos\theta} \quad (2)$$

where D_{hkl} is the crystallite dimension in the direction normal to the ($h\,k\,l$) lattice planes, K is the Scherrer constant usually taken to be 0.9, λ is the radiation wavelength ($\lambda = 0.154$ nm for Cu Ka), θ is the diffraction angle and $\beta_{1/2}$ is the peak width at the half-maximum intensity in radians. The crystal size was determined using the diffraction pattern obtained from 0 0 2 lattice planes.

2.3.7. Thermogravimetric analysis (TGA)

The thermal behavior of the material at different stages of extraction was studied by thermal gravimetric analyses (TGA), using a Mettler Toledo TGA/SDTA 851e. The samples were heated from 25 to 800 °C at a rate of 10 °C min^{-1} in an inert atmosphere (50 ml min^{-1} N_2 flow). The ignitions were performed in duplicate for each sample. The data were collected and processed by Mettler-STARe Evaluation software. The onset, maximum decomposition temperature and mass loss for each thermal decomposition process were obtained.

3. Results and discussion

The isolation of cellulose nanocrystals (CNCs) from the inner bark of Norway spruce required a sequence of chemical treatments. The materials obtained after each treatment were characterized with respect to their chemical composition, morphological structure, thermal behavior and crystallinity.

3.1. Chemical composition

The chemical compositions of the bark, extraction residue, bleached fibers and CNCs are summarized in Table 1.

The bark contained 21.0% lignin, 6.7% ash and 61.6% carbohydrates. The glucose content of the bark was 43.4% and included cellulose, starch, stilbene glucosides and tannins (Krogell et al., 2012; Le Normand et al., 2012). In order to remove the non-cellulosic components, a successive extraction method based on pressurized acetone and water at different temperatures was performed. Acetone was used to remove hydrophilic extractives from the bark while hot water was used to extract the non-cellulosic polysaccharides. Sequential extraction was preferred to alkali treatment because it allowed the partial recovery of non-cellulosic constituents under rather mild conditions while preserving the cellulose intact. The extraction method as well as the chemical composition of the extracted components were described in previous studies (Le Normand et al., 2012; Le Normand et al., 2014). In these studies, it was also shown that the glucose content of the residue was entirely due to the presence of cellulose. In the present study, the removal of starch, stilbene glucosides and tannins resulted in a residual material containing 53% cellulose, 10% hemicelluloses and 29% Klason lignin. In order to remove the lignin residues, four bleaching steps with $NaClO_2$ were applied. After bleaching, the lignin content was significantly reduced from 28.7% to 3.5%. The hemicellulose content did not decrease during this step but, after hydrolysis with sulfuric acid, the hemicellulose content was significantly reduced to less than 3%.

During the whole process of extraction of CNCs, the glucose content gradually increased to finally reach 94.5% after the hydrolysis step, which showed that the purification process to obtain cellulose from the inner bark of Norway spruce was successful. The hydrolysis yield was about 32%, which was similar to the value reported for the hydrolysis of microcrystalline cellulose from Norway spruce (Bondeson, Mathew, & Oksman, 2006). The overall

Table 1
Yields (% w/w) obtained after each chemical treatment and chemical compositions (% w/w) of the bark, residue, bleached fibers and CNC. The yields and compositions are calculated on a dry weight basis.

	Bark	Residue	Bleached fibers	CNC
Yield	–	51.5	64.6	31.8
Ara	5.6 ± 0.4	1.0 ± 0.2	0.6 ± 0.0	0.0
Rha	0.7 ± 0.1	0.2 ± 0.0	0.0	0.0
Gal	1.7 ± 0.1	1.2 ± 0.1	0.8 ± 0.1	0.0
Glc	43.4 ± 1.6	53.2 ± 1.2	78.6 ± 1.0	94.5 ± 2.9
Xyl	2.1 ± 0.2	4.0 ± 0.2	6.0 ± 1.0	0.5 ± 0.1
Man	1.4 ± 0.1	2.0 ± 0.1	3.4 ± 0.1	0.3 ± 0.0
GalA	6.5 ± 0.3	1.5 ± 0.7	<0.1	0.0
GlcA	0.2 ± 0.0	0.4 ± 0.1	<0.1	0.0
Total	61.6 ± 2.8	63.5 ± 2.6	89.4 ± 2.2	95.3 ± 3.0
Klason lignin	21.0 ± 1.0	28.7 ± 1.2	3.5 ± 1.0	N/A
Ash	6.7	7.8	7.1	4.7
Sulfur content	N/A	N/A	N/A	0.93 ± 0.03

yield of CNCs, with respect to the initial amount of inner bark, was close to 11%. CNCs produced by sulfuric acid hydrolysis are electrostatically stabilized in aqueous suspension by anionic sulfate ester groups introduced during the reaction. The sulfur content of the bark CNC was estimated to be about 0.93% which is similar to the sulfur content of CNCs extracted from wood Kraft pulp under similar conditions (Beck-Candanedo, Roman, & Gray, 2005).

3.2. Morphological structure

SEM and AFM images were used to follow the morphological changes in the materials during the whole isolation process. Fig. 1 shows the surface morphology of the bark and the residual fibers after sequential extraction and bleaching. The bark fibers appeared to be homogeneous with smooth and orientated surfaces (Fig. 1a). After partial extraction of the non-cellulosic components from the bark, the matrix opened, liberating hemicelluloses, pectins and tannins, which acted as cementing agents around the fiber bundles in the bark (Fig. 1b). This pre-treatment step was essential to promote swelling of the structure and enhance the penetration of the bleaching agents. As can be seen in Fig. 1c, bleaching resulted in an opening of fiber bundles. The bleached bark fibers had a diameter of approximately 20 μm and a length of more than 2 mm. These dimensions were close to the values generally reported for the fibers of Norway spruce wood, where the tracheids are 2–4 mm in length and 20–40 μm in diameter (Sjöström, 1981).

The isolation of bark CNCs was verified by means of AFM images, which are presented in Fig. 2. The bark CNCs had a rod-like aspect, individualized or agglomerated. CNCs have the tendency to agglomerate due to their high specific area and the strong hydrogen bonds established between crystallites.

Table 2
Average molar mass (*Mn*, *Mw*), polydispersity (*Mw*/*Mn*) and degree of polymerization (DP) of cellulose in the bleached fibers and CNC isolated from spruce inner bark.

	Mn (kDa)	*Mw* (kDa)	*Mw*/*Mn*	DP
Bleached fibers	407	1526	3.7	9420
CNC	14	43	3.1	266

AFM images obtained in the height mode were used to measure the diameter and length of more than hundred particles. Fig. 3 shows the histograms corresponding to these measurements. The isolated CNCs had a broad polydispersity, with a length ranging from 60 to 340 nm and a diameter between 1.5 and 4.5 nm. Similar polydispersity profiles were observed for wood CNCs (Beck-Candanedo et al., 2005) and for other sources such as mulberry bark (Li et al., 2009), mango seeds (Henrique et al., 2013) and Capim Dourado (Siqueira, Abdillahi, Bras, & Dufresne, 2010). The average diameter (d) and length (l) of spruce bark CNCs were 2.8 ± 0.8 nm and 175.3 ± 61.8 nm, respectively. These values were slightly different from the dimensions observed for nanocrystals isolated from softwood under approximately the same conditions. Bondeson et al. (2006) obtained larger cellulose nanocrystals from microcrystalline cellulose derived from Norway spruce, with a length between 200 and 400 nm and a width less than 10 nm. On the other hand, the CNCs isolated from black spruce showed smaller and wider crystals, with a length of around 120 nm and a diameter of 4.9 nm, giving an aspect ratio of 24 (Beck-Candanedo et al., 2005). In the case of CNCs isolated from the bark of Norway spruce, the average aspect ratio (l/d) was about 63, which is much higher than that of CNCs isolated from wood. This morphological characteristic was nevertheless reported for nanocrystals isolated from several cellulosic agricultural wastes such as mango seeds

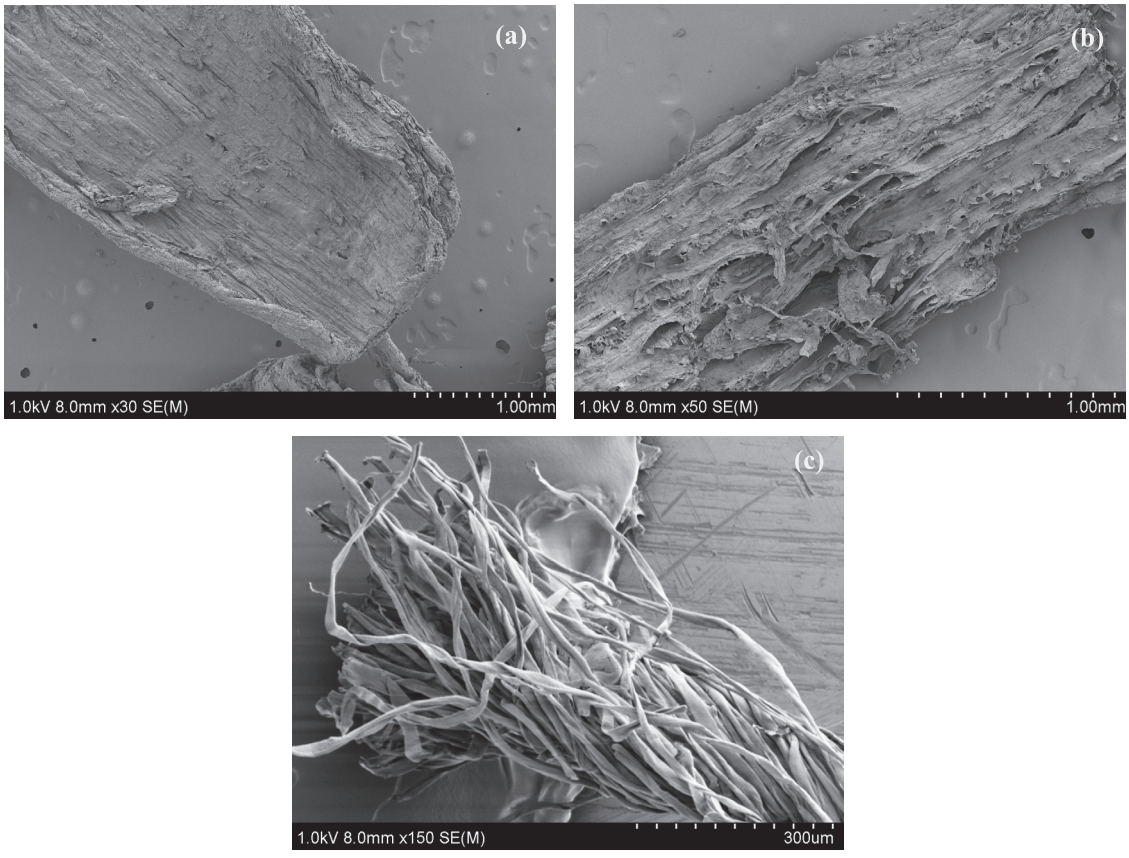

Fig. 1. Scanning electron micrographs of (a) the original bark fibers, (b) the residue after acetone and hot-water extraction and (c) the bleached fibers.

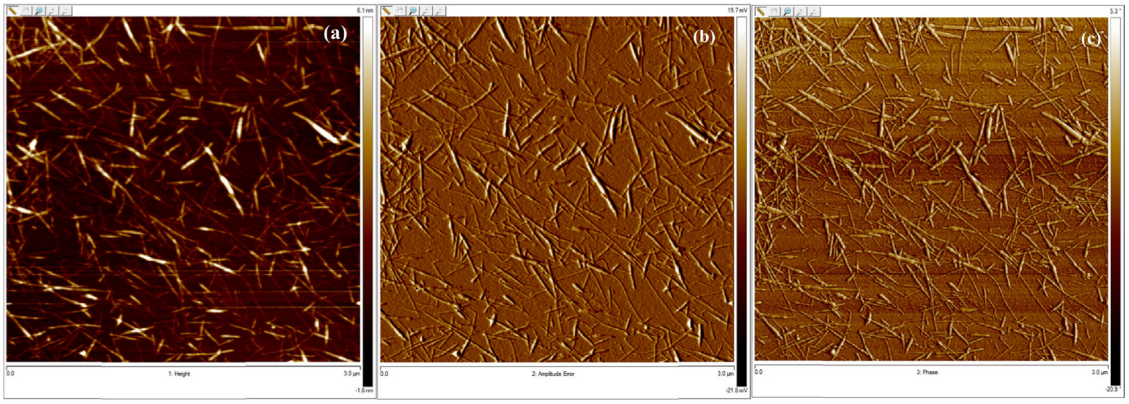

Fig. 2. AFM images in (a) height, (b) amplitude and (c) phase modes of CNCs.

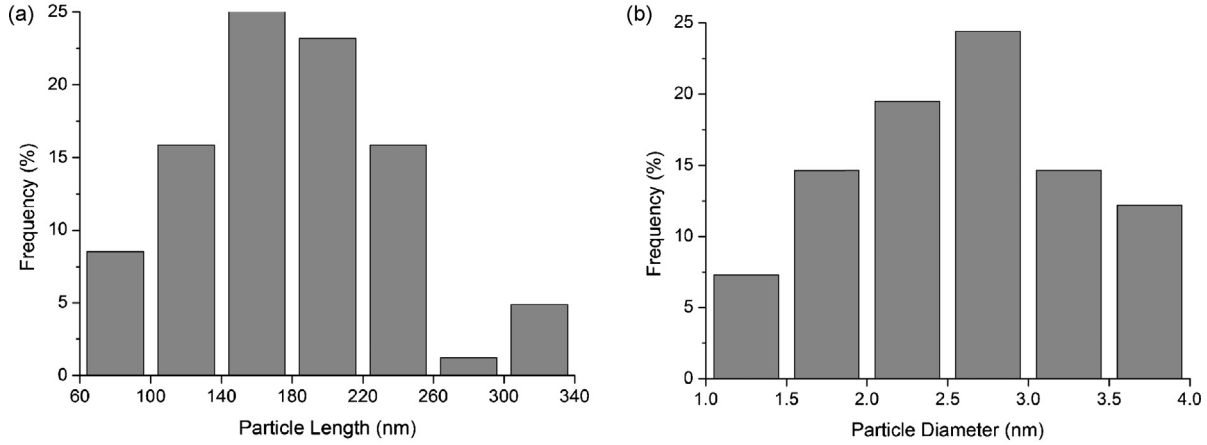

Fig. 3. Distribution of (a) particle length and (b) diameter of bark CNCs.

(Henrique et al., 2013), coconut husk fibers (Rosa et al., 2010) and sugar bagasse (Teixeira et al., 2011). In general, nanocrystals with a high aspect ratio have a good reinforcing effect, resulting in mechanical improvement at low loads.

Size-exclusion chromatography was used to evaluate the molar mass distribution and the degree of polymerization of the cellulose in the bleached fibers and CNCs. These values are reported in Table 2 and the profiles of molar mass distributions for each material are provided in supplementary data.

The DP of spruce bark cellulose was consistent with the typical values reported for wood cellulose in its native state, around 10000 (Sjöström, 1981), and of the same order of magnitude as those observed in the bark of white birch (7500), amabilis fir (7200), Engelmann spruce (7100) and lodgepole pine (10300) (Mian and Timell, 1960; Timell, 1961). The molecular size of the cellulose was tremendously reduced during the hydrolysis step. In fact, the molecular mass of the CNC was 35 times smaller than that of the cellulose present in the bleached fibers. Similar DP were reported for cellulose crystals obtained after partial hydrolysis of bleached wood (Battista, Coppick, Howsmon, Morehead, & Sisson, 1956).

3.3. Fourier transform infrared spectrometry (FTIR)

The efficiency of each chemical treatment of the bark fibers was confirmed by FTIR. The spectra of the materials were recorded (Fig. 4) and several characteristic bands were identified. These are summarized in Table 3.

The FTIR spectra of the bark and residue were typical of lignocellulosic biomass while the spectra of the bleached fibers and CNC showed a pattern more characteristic of cellulose fibers (Pandey, 1999). All the spectra were characterized by a dominant O—H stretch band (3306–3330 cm^{-1}) and a C—H stretch band (2897–2923 cm^{-1}) corresponding to the aliphatic moieties in polysaccharides. The top of the band in the O—H stretching region was shifted to lower wavenumbers (from 3329 cm^{-1} for bark to 3306 cm^{-1} for CNCs) indicating an average increase in

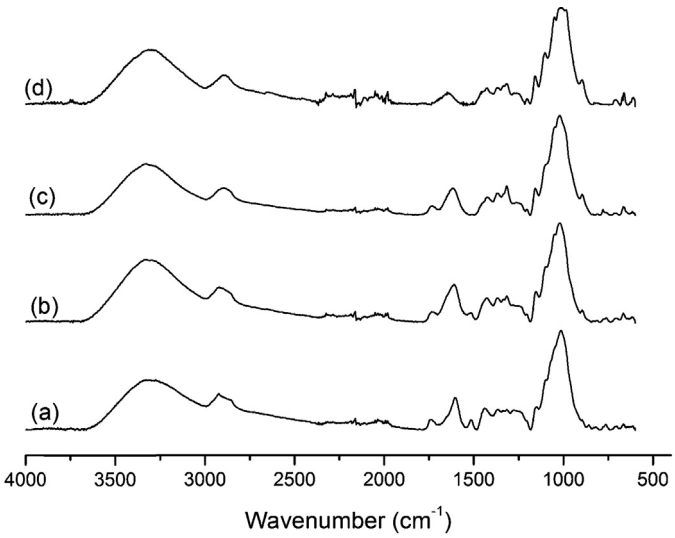

Fig. 4. FTIR spectra of the (a) bark. (b) residue, (c) bleached fibers and (d) CNC.

Table 3
Vibrational frequencies (cm^{-1}) of bark, residue, bleached fibers and CNCs.

Bark	Residue	Bleached fibers	CNCs	Peak assignment[a]
3329	3330	3322	3306	O—H stretching
2923	2922	2898	2897	C—H stretching
1738	1737	1733		C=O stretching
			1644	O—H bending of absorbed water
1603	1607	1614		C=C aromatic skeletal vibration and C=O stretching
1515	1516			C=C aromatic skeletal vibration
1435	1428	1424	1427	C—H bending
1367	1368	1368	1367	C—H bending
1338	1338		1332	C—H bending
1316	1316	1316	1315	C—H wagging
1279	1281	1267	1277	C—O stretching and C—H bending
1235	1243	1247	1251	C—O stretching
1205	1205	1204	1203	O—H in-plane bending
1152	1154	1157	1160	C—O—C asymmetric vibration
1100	1100	1102	1104	O—H bending
1049	1050	1051	1051	C—O stretching
1017	1023	1022	1025	C—O stretching
			984	C—O stretching
897	897	897	897	C—H wagging
667	665	667	662	O—H out of plane bending

[a] Based on literature data (Baeza & Freer, 2000; Pandey, 1999).

hydrogen bond strength (Kondo, 1997; Miya, Iwamoto, & Mima, 1984). A significant reduction in intensity of the main bands associated with lignin and non-cellulosic polysaccharides, at 1515 and 1735 cm^{-1}, was noticed during the isolation of the CNCs. The band at 1515 cm^{-1}, assigned to the aromatic C=C vibration in lignin, disappeared after the residue was bleached. The band at 1735 cm^{-1} was attributed to either the acetyl and uronic ester groups of hemicelluloses or the ester linkage of the carboxylic group in lignin (Kačuráková, Capek, Sasinková, Wellner, & Ebringerová, 2000; Sakakibara & Sano, 2001). This band was observed for the bleached fibers, which confirmed the presence of hemicelluloses, pectins and lignin in the sample after bleaching. It was however almost absent in the spectrum of the CNCs, which indicated the extensive removal of the lignin and hemicellulose residues during the hydrolysis step. This observation was in good agreement with the results of the chemical analyses discussed earlier.

3.4. Wide angle X-ray scattering (WAXS)

The diffraction patterns of the bark, residue, bleached fibers and CNC samples are shown in Fig. 5. In all the samples, major peaks were obtained at 16.2° and 22.5°, which are characteristic of the 1 0 1 and 0 0 2 lattice planes, respectively, in cellulose type I (Park, Baker, Himmel, Parilla, & Johnson, 2010). The small and broad peak at $2\theta = 34.5°$ represents the contribution of the 040 plane (Cave, 1997). The peak intensity corresponding to the 002 lattice planes increased and became sharper as a result of the chemical treatment, which was related to an increase in crystallinity of the material.

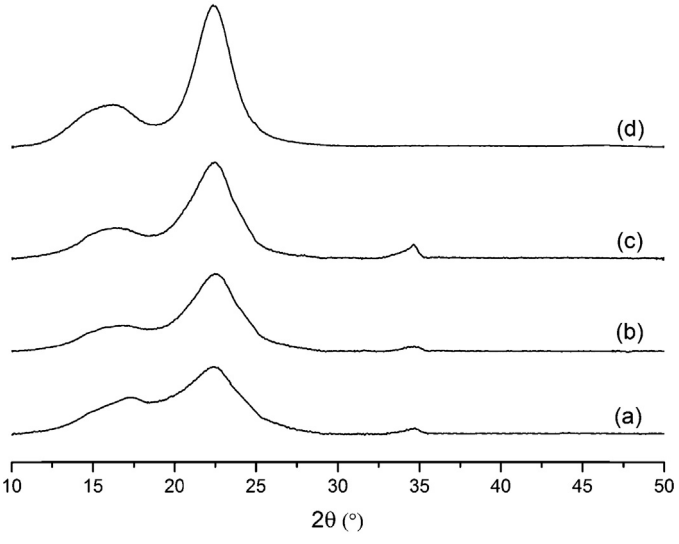

Fig. 5. X-ray diffraction patterns of the (a) bark, (b) residue, (c) bleached fibers and (d) CNC.

Table 4
Crystallinity index (CI) of the bark, residue, bleached fibers and CNC.

	CI (%)
Bark	46
Residue	68
Bleached fibers	78
CNC	84

The crystallinity index (CI) of the materials was obtained by the peak height method and is summarized in Table 4.

Several authors have mentioned the limitations of this method (Park et al., 2010; Thygesen, Oddershede, Lilholt, Thomsen & Ståhl, 2005), particularly due to the underestimation of the contribution of the amorphous region, resulting in an overestimation of the CI. Despite its limitation, the peak height method has been extensively used for the calculation of the CI and it was therefore used here to enable a comparison with reported data. The CI clearly increased as a result of the chemical treatment, from 46% for the bark to 84% for the CNCs. This increase could be explained by the progressive removal of the amorphous regions from the fibers, i.e. the non-cellulosic components, through the CNC isolation process. The CI of the bark CNCs was comparable with the values in the range of 70–90% reported for nanocrystals isolated from e.g. mango seeds (Henrique et al., 2013), wheat straw (Kaushik, Singh, & Verma, 2010), alfa fibers (Mabrouk, Kaddami, Boufi, Erchiqui, & Dufresne, 2012), sisal fibers (Morán, Alvarez, Cyras, & Vázquez, 2008), corn cobs (Silvério et al., 2013) and Capim Dourado (Siqueira et al., 2010). The crystallite size of the CNCs calculated using the Scherrer equation was around 3.03 nm. This value was in the same range as the average diameter found on the AFM images. The crystallite size of the cellulose in the bark appeared to be similar to that in the wood of Norway spruce, where the average thickness of the crystallites has been reported to be between 2.5 and 4 nm (Andersson, Serimaa, Paakkari, Saranpää, & Pesonen, 2003; Hult, Larsson, & Iversen, 2000; Leppänen et al., 2009).

Table 5
Thermogravimetric parameters for the thermal degradation processes of the bark, residue, bleached fibers and CNC samples.

	(25–150) °C		(150–600) °C			Residue
	Mass loss (%)	T_1 (°C)	Onset (°C)	Mass loss (%)	T_{max} (°C)	Mass (%)
Bark	5.1 ± 0.2	71.5 ± 0.5	208.7 ± 1.6	67.7 ± 0.5	322.4 ± 1.1/349.8 ± 0.2/390.0 ± 2.0	23.3 ± 0.2
Residue	6.0 ± 0.1	66.6 ± 0.6	238.2 ± 1.2	73.4 ± 0.5	357.7 ± 0.2	18.2 ± 0.3
Bleached fibers	5.1 ± 0.1	66.8 ± 2.2	249.7 ± 1.3	70.7 ± 0.1	336.3 ± 1.5	21.7 ± 0.2
CNC	4.4 ± 0.2	68.6 ± 0.8	191.5 ± 1.4	64.5 ± 0.1	276.9 ± 0.1/356.3 ± 0.1	28.6 ± 0.1

3.5. Thermogravimetric analyses

Thermogravimetric (TG) and derivative thermogravimetric (DTG) curves of the bark, the residue, the bleached fibers and CNCs are shown in Fig. 6. The thermal decomposition process was further characterized by extrapolating the onset temperature and the maximum degradation temperatures of the main mass loss regions (Table 5).

Several mass-loss regions could be observed during the pyrolysis of each sample. All the samples had a small mass loss (<6%) in the low temperature range (<150 °C), corresponding to the evaporation of absorbed water. In the high temperature range (150–600 °C), where the mass loss is due to thermal degradation, the samples behaved differently. The DTG of the bark and CNC showed at least three main overlapping peaks, but this profile was not observed for the DTG curves of the residue and bleached fibers. These differences were linked to the variations in chemical composition since extractives, hemicelluloses, cellulose, and lignin decompose at different temperatures (Moriana, Vilaplana, Karlsson, & Ribes-Greus, 2011). Hemicelluloses and pectins are usually rapidly degraded, with a pyrolysis focused at 210–350 °C (Moriana et al., 2011). The pyrolysis of cellulose happens later, around 350–400 °C whereas lignin degradation has been reported to cover the whole temperature range (Yang, Yan, Chen, Lee, & Zheng, 2007).

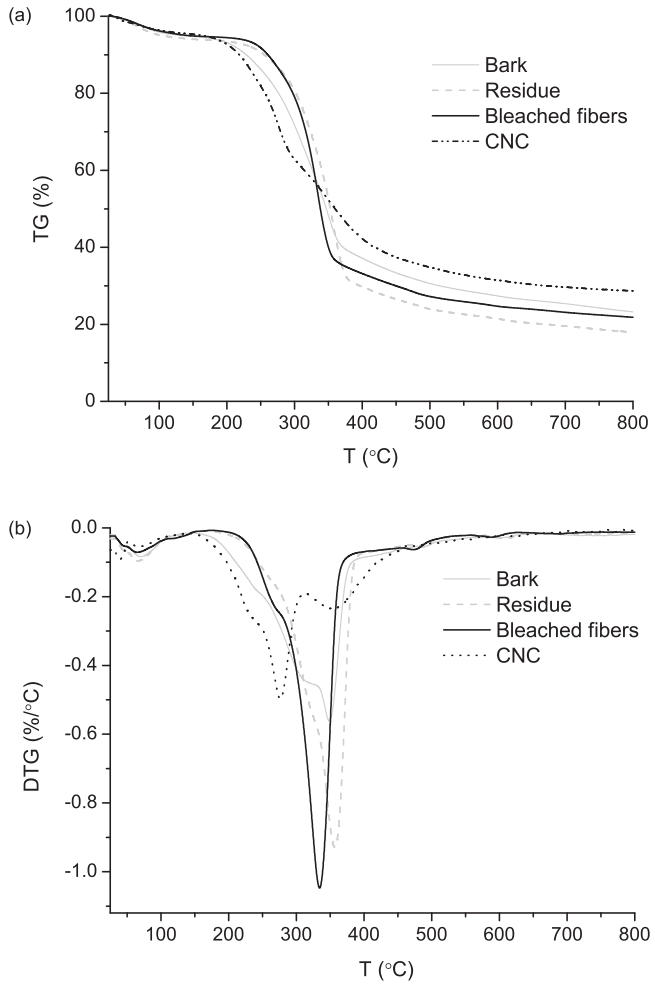

Fig. 6. (a) TG and (b) DTG curves of the bark, residue, bleached fibers and CNC.

After partial removal of the extractives, tannins, hemicelluloses and pectins with acetone and hot water, the extraction residue started to degrade at a higher temperature (ΔT = 30 °C). This increase in thermal stability after elimination of extractives has also been observed for different wood species (Shebani, van Reenen, & Meincken, 2008). The partial removal of hemicelluloses in the residue and bleached fibers could be observed by the diminution of the shoulder at 275 °C on the DTG curve. The bleached fibers degraded within a narrower temperature range and showed better thermal stability than the bark itself. This improvement in thermal stability could be due to an increase in crystallinity and intermolecular hydrogen-bonded domains after extraction of the non-cellulosic components (Antal, Várhegyi, & Jakab, 1998). However, acid hydrolysis of the bleached fibers led to a significant decrease in thermal stability. The early thermal degradation of CNCs, around 190 °C, is often correlated to the presence of sulfate groups that catalyze the dehydration process of cellulose (Fahma, Iwamoto, Hori, Iwata, & Takemura, 2011). The degradation of the CNCs occurred over a wide temperature range and the degradation profile showed two main pyrolysis processes in the DTG curves. A similar profile was reported for CNCs isolated from kenaf bast fibers (Kargarzadeh et al., 2012). The first pyrolysis process (between 150 °C and 300 °C) occurred with a mass loss of 35% and included two overlapping peaks at 243 °C and 277 °C. Roman and Winter (2004) assigned the lower temperature peak to the thermal degradation of the sulfated amorphous region and the second peak to the breakdown of the more accessible region in the crystal interior. The second pyrolysis process (between 300 °C and 600 °C) displayed one main peak at 356 °C with a mass loss of 30%, which was assimilated to the thermal degradation of the least accessible crystal interior. The amount of residue in the CNCs was significantly higher (30%) than in the other samples and could be explained by the presence of sulfuric acid which promoted the dehydration reactions (Julien, Chornet, & Overend, 1993; Kim, Nishiyama, Wada, & Kuga, 2001) and acted as a flame retardant (Roman & Winter, 2004). Considering their thermal properties, bark CNCs have a potential for use as reinforcing filler in thermoplastic composites, since typical processing temperatures are around 200 °C (Glasser, Taib, Jain, & Kander, 1999).

4. Conclusion

Cellulose nanocrystals (CNCs) were isolated from the inner bark of Norway spruce. The interest in a pretreatment with acetone and hot water was two-fold: (1) to recover part of the non-cellulosic components for further upgrading within the bark biorefinery concept and (2) to promote swelling of the bark fibers for better penetration of the bleaching agents. Bleaching with sodium chlorite reduced the lignin content by more than 85%, resulting in fibers with a high content of cellulose (78.6%) suitable for the isolation

of CNCs. Hydrolysis of the holocellulose yielded CNCs with a high crystallinity index. The thermal stability of the fibers was decreased after hydrolysis due to the introduction of sulfate groups in the crystals which catalyzed the degradation of CNCs. Nevertheless, their thermal resistance was still good enough for processing together with other polymers under typical melting temperatures. The yield of CNCs, with respect to the initial amount of inner bark, was 11%. The CNCs presented rod-like aspects with a diameter and length in the range of 2.8 nm and 175.3 nm, respectively, giving an aspect ratio greater than 60. Due to their high aspect ratio and good thermal stability, CNCs obtained from spruce bark have a great potential for use as reinforcement agents in the manufacture of nanocomposites.

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