Changes in the cellulose fiber wall supramolecular structure during the initial stages of chemical treatments of wood evaluated by NMR and X-ray scattering

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Abstract The effect of initial stages of pulping of spruce, resembling prehydrolysis and alkaline cooking was studied using CP/MAS ^{13}C -NMR, X-ray scattering, FSP and carbohydrate composition in order to study the impact of the pre-treatments on the fiber wall nanostructure. Removal of fiber wall components, hemicellulose and lignin, increased the fiber wall porosity and induced cellulose fibril aggregation. The effect of temperature and pH in the treatment on cellulose fibril aggregate size appears to be secondary. It is the removal of hemicellulose that has a profound effect on the supramolecular structure of the cellulose fiber wall. As the amount of hemicellulose dissolved from wood increases, the fibril aggregate size determined by NMR increases as well, ranging from 16 to 28 nm. Specifically, a good correlation between the amount of glucomannan in the fiber wall and the fibril aggregate size is seen. The lower the amount of glucomannan, the larger the aggregate size. Glucomannan thus seems to prevent aggregation as it acts as a very efficient spacer between fibrils. Elemental fibril size determined by NMR, was quite similar for all samples, ranging from 3.6 to 4.1 nm. By combining measurement methods, a more well-resolved picture

of the structural changes occurring during was obtained.

Keywords Fibril aggregation · Fiber wall porosity · Hemicellulose · Lignin · FSP

Abbreviations

Ara	Arabinose
Cr	Crystallinity
d/rw	Dried and rewetted
FSP	Fiber saturation point
GGM	Galactoglucomannan
Glc	Glucose
LFAD	Lateral cellulose fibril aggregate dimension
LFD	Lateral cellulose fibril dimensions
Man	Mannose
nd	Never-dried
o.d.	Oven dry
SDD	Sample to detector distance
SAXS	Small angle X-ray scattering
SSA	Specific surface area
WAXS	Wide angle X-ray scattering
XRD	X-ray diffraction
Xyl	Xylose

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Introduction

The biopolymers in plant cell walls interact with each other through hydrogen bonds, van der Waals interactions and electrostatic forces, forming a supramolecular structure of the fiber wall. In this work two terms are used to describe the building blocks of the supramolecular structure of cellulose I β : fibrils and fibril aggregates. In cellulose isolated from e.g. spruce by kraft pulping a cellulose fibril has a lateral dimension of about 4 nm, fibrils can aggregate into bundles of fibrils with close-to co-linear main axes to form fibril aggregates. The lateral dimensions of fibril aggregates can range from bundles of a few fibrils up to widths of several tenths of nanometers. Within a fibril β -(1,4)-D-glucan polymers are tightly packed into fibrils with a cellulose I lattice at their core, and further arranged into fibril aggregates, surrounded by a matrix of hemicelluloses and lignin (e.g. Page 1976; Salmén and Olsson 1998; Fahlén and Salmén 2005). In addition to fibrils, the nanoscale supramolecular structure of the cell wall contains the empty spaces between the fibrils which form pores. The supramolecular structure and composition of the cell wall polymers can vary depending upon the biomass source, for example, the cellulose fibril width in spruce has been reported to be 4 nm (Hult et al. 2001), in cotton linters it is shown to be 7 nm while for tunicate cellulose it is 11 nm (Malm et al. 2010) and fibril aggregate sizes are 15, 40 and 38 nm respectively.

Thermal and chemical processes affect the supramolecular structure of the fiber wall. Hydrothermal treatments, such as autohydrolysis, dilute acid pretreatment and steam explosion, result in increased aggregate size (Inagaki et al. 2010; Driemeier et al. 2011; Nishiyama et al. 2014; Kaffle et al. 2015). Alkaline processes, such as kraft pulping, also lead to an increase of the fibril aggregate size (Hult et al. 2001; Fahlén and Salmén 2003, 2005). In both acidic and alkaline processes hemicelluloses are removed to different extent while lignin is redistributed by acidic hydrothermal treatments and dissolved in alkaline treatments. When the matrix material is dissolved, fibril surfaces are exposed, and aggregation of cellulose fibrils may occur. However, when matrix polymers remain on fibril surfaces, it can be assumed that they can act as spacers, preventing fibril aggregation (Hult et al. 2001). Fibril aggregation has also been

explained as an entropy driven process (Langan et al. 2014; Pingali et al. 2014).

The supramolecular structure of the cell wall has a significant importance in many conversion processes of biomass. In the production of biofuel by enzymatic hydrolysis of polysaccharides, the accessibility of the fiber wall to enzymes is crucial (Adani et al. 2011; Aldaeus et al. 2015; Peciulyte et al. 2015). Accessibility also affects the reactivity of cellulose in derivatization processes for example manufacturing of cellulose acetate (Chunilall et al. 2010) or viscose fibers (Wollboldt et al. 2010). The fiber wall nanostructure affects product properties as well, such as the paper strength (Larsson and Salmén 2014), quality of nanocellulose (Pääkkö et al. 2007) or mechanical properties of bio-composites of cellulose (Nilsson et al. 2012). It is therefore of immense economical interest to understand how different conditions affect the supramolecular structure to be able to modify the process conditions in order to obtain pulps with highly accessible fiber walls and highly reactive cellulose.

The bulk of previous studies on supramolecular structure have analyzed the effect of process conditions after completed treatment, for example after hydrothermal treatment or after pulping. Duchesne and Daniel (2000) and Hult et al. (2001) studied ultrastructural changes occurring during kraft pulping, also in the initial stages of the process. However, to get a fundamental understanding of what initiates aggregations and affects fiber wall porosity, the present study investigates the changes in fiber wall nanostructure at the very initial stages of both acidic and alkaline treatments, resembling the conditions in the most common pulping processes, i.e. kraft and prehydrolysis kraft cooking. Apart from using the well-established method of NMR (e.g. Larsson et al. 1997; Hult et al. 2001) to study the supramolecular structure of the cellulose fiber wall, the samples were also analyzed by small- and wide-angle x-ray scattering.

Methods and materials

Materials

Industrially produced never-dried spruce chips (*Picea abies*) were used in this study. The chips were stored at $-25\text{ }^{\circ}\text{C}$.

Chemicals

For wood chip treatment, stock solutions of NaOH and H₂SO₄ were made from ampoules (VWR International AB) and diluted with de-ionized water. For chlorite delignification, NaClO₂, NaOH pellets, and glacial acetic acid were delivered by Merck KGaA, Darmstadt, Germany. Dextran, from *Leuconostoc mesenteroides*, molecular mass approx. 1500–2800 kDa, CAS No. 9004-54-0; Sigma-Aldrich, Saint Louis, MO 63103, USA.

Methods

Treatment of wood chips

Before the chip treatments, the frozen chips were thawed at room temperature. The dry content was subsequently gravimetrically determined to be 52% by drying in oven at 105 °C. Batches of 50.0 g chips (oven dry content) were prepared and placed in steel autoclaves with a volume of 3 L. The air was removed from the autoclave by vacuum suction for 30 min after which the treatment liquor was sucked into the autoclaves to obtain a liquor-to-wood ratio of 6 L/kg. The conditions during the treatments are presented in Table 1. Treatment at the lower temperature was performed by mounting the autoclaves in a glycol bath holding 110 °C and after a heating time of 10 min, the treatment time was started. The alkaline extraction at the higher temperature was performed by starting the treatment at 100 °C and increasing the temperature with a rate of 3 °C/min. When reaching 170 °C, the treatment was stopped by cooling the autoclave in a water bath. The autohydrolysis at the higher temperature was performed by placing the autoclaves in the glycol bath at 170 °C and starting the treatment time

after heating for 10 min. The pH in the hydrolysate on completion of autohydrolysis was 4.1 at 110 °C, 3.5 at 170 °C, and 13.5 after the alkaline treatments. Residual alkali in liquor after alkaline treatments was 0.62 M at 110 °C and 0.59 M at 170 °C.

Chlorite delignification

The model to determine fibril and fibril aggregate size by NMR is developed for cellulose-rich samples. Thus, the samples after pre-treatment were chlorite delignified prior characterization by NMR and X-ray scattering and measurement of FSP. Batches of 20 g of treated wood chips were chlorite delignified using a sodium chlorite (NaClO₂) solution under acidic conditions (22 g of NaClO₂, 80%, was mixed with 2 mL of glacial acetic acid, 100%, and 1000 mL deionized water) at room temperature, mild stirring with magnetic stirrer for 12 h. After that the samples were washed with deionized water during the vacuum filtration using a 7 cm Büchner funnel and a Teflon wire mesh. The samples were then soaked in 0.1 M sodium hydroxide (NaOH) solution (4 g of NaOH pellets were mixed with 1000 mL deionized water) at room temperature, mild stirring with magnetic stirrer for 6 h. After that the samples were washed with deionized water during vacuum filtration using a 7 cm Büchner funnel and a Teflon wire mesh. This two-step procedure was repeated several times (7 times for samples NaOH170 and H₂O170, 10 times for sample NaOH110 and 13 times for sample H₂O110) until the pulps produced became bright in color. One half of these four pulps were analyzed as never-dried while the other half were analyzed as dried (oven dried at 105 °C for 19 h) and rewetted (soaked in deionized water for 24 h and vacuum filtrated using a 7 cm Büchner funnel and a Teflon wire mesh).

Table 1 Conditions for the treatment of wood chips. The liquor-to-wood ratio was 6 L/kg dry wood

	NaOH (M)	H ₂ SO ₄ (M)	Temperature (°C)	Retention time at temperature (min)	Sample ID
Alkaline extraction	1.1	–	110	30	NaOH110
	1.1	–	170	0*	NaOH170
Autohydrolysis	–	0.001	110	240	H ₂ O110
	–	–	170	30	H ₂ O170

*The autoclaves were removed after the heating time, which was from room temperature to 170 °C at a rate of 3 °C/min

Analysis

Carbohydrate composition

The chemical composition was analyzed in duplicate for each sample. Wood and treated wood chips were extracted with acetone in a Soxtec apparatus according to SCAN-CM 49:03. All samples were subsequently ground through a 40 mesh grid. The samples were hydrolyzed at 121 °C in an autoclave with 0.4 M H₂SO₄, according to SCAN-CM 71:09. The solubilized monosaccharides were quantified using an ion chromatograph coupled to a pulsed amperometric detector (IC-PAD). Acid-insoluble residue was determined gravimetrically according to TAPPI T222 om-11. The acid-soluble residue was measured by UV spectrophotometry at 205 nm according to TAPPI UM 250. MilliQ water was used as blank and for the dilution of hydrolysate. The content of acid-soluble residue was calculated using the absorptivity coefficient 110 L/g cm. The repeatability of the method has been determined on 10 duplicates. At 95% confidence level for total carbohydrates analysis, the repeatability is < 2.0%, which includes uncertainty in dry content determination, sample weight determination, hydrolysis, dilution and IC.

NMR

NMR spectra were recorded on four never-dried (nd) and four dried and rewetted (d/rw) samples packed uniformly in a zirconium oxide (ZrO₂) rotor with a 4 mm outer diameter. CP/MAS ¹³C-NMR spectra were recorded at 295 ± 1 K on a Bruker Avance III AQS 400 SB instrument operating at 9.4 T. NMR spectra were recorded on water swollen samples with a solids content of about 50% by weight. The MAS rate was 10 kHz. A 4 mm double air-bearing probe was used. Acquisition was performed with a CP pulse sequence, using a 2.95 microseconds proton 90° pulse, 800 microseconds ramped (100–50%) falling contact pulse and a 2.5 s delay between repetitions. A SPINAL64 pulse sequence was used for ¹H decoupling. Alpha-glycine (NH₂CH₂COOH) was used for the Hartmann-Hahn matching procedure, as well as an external standard for calibration of the chemical shift scale relative to tetramethylsilane ((CH₃)₄Si). The data point of maximum intensity in the alpha-glycine carbonyl (C=O) signal was assigned

a chemical shift of 176.03 ppm. Spectral fitting was performed using software, based on the Levenberg–Marquardt algorithm, developed at RISE (Larsson et al. 1997). From NMR spectra the lateral cellulose fibril dimensions (LFD) and the lateral cellulose fibril aggregate dimension (LFAD) can be determined (Larsson et al. 1997; Wickholm et al. 1998). In fiber samples with high cellulose content (> 90%) the specific surface area (SSA) of the fiber in a water swollen state can be determined using the LFAD obtained from spectra fitting and the density of cellulose I 1500 kg/m³ was used based on the dominating allomorph found in higher plants; cellulose Iβ (Chunilall et al. 2010). The treatments are not expected to change the allomorph as a much higher temperature is needed for this to happen (Yamamoto and Horii 1993; Kuribayashi et al. 2016).

Fiber saturation point measurements and fiber wall pore size

Fiber saturation point (FSP) measurements were conducted in a manner similar that of Stone and Scallan (1967) but using only one high molecular mass dextran. Water-swollen samples with a known solids content were mixed with a dextran solution of known concentration (approximately 1%, dextran mass/solution mass) in deionized water, approximately 1 mass unit of wet sample mass being mixed with 3 mass units of dextran solution. After mixing, the sample was stored in a sealed vessel at room temperature for 3 days to equilibrate. A liquid sample was subsequently taken and filtered through a Puradisc syringe filter (Whatman, Maidstone, UK) equipped with a 0.45 µm polytetrafluoroethylene membrane in a polypropylene housing (VWR International AB, Stockholm, Sweden). The concentration of dextran in the sample was determined using a calibration curve established for the optical rotation of polarized light measured using a Polartronic M100 Touch polarimeter (Schmidt + Haensch GmbH & Co., Berlin, Germany) operating at 589 nm, with a resolution of 0.001° (angular degree) and a precision of ± 0.005° at 589 nm. The calibration curve was computed using three dextran concentrations: approximately 0.5, 1.0 and 1.5% (dextran mass/solution mass), covering the range of all measurements. Dynamic light scattering was used to determine the hydrodynamic diameter of the dextran molecules (Dextran 1500–2800, CAS No.

9004-54-0) at high dilution in deionized water (Zeta-sizer ZEN3600; Malvern Instruments Ltd., Malvern, UK), using a He–Ne 4.0 mW, 633 nm laser and a detector angle of 178°. The hydrodynamic diameter was found to be 97 ± 2 nm with a polydispersity index of 0.2, measured at a dextran concentration of 0.15 g dextran per L solution. Based on the determined size of the dextran, the results obtained for the FSP were interpreted as representing liquid contained in pores smaller than approximately 97 nm in diameter. The FSP value is expressed as the dimensionless ratio of the mass of pore water to the mass of dry solids (g/g).

Since both the FSP measurements and solid-state NMR measurements were performed on water swollen pulp these results can be combined, and this was utilized for calculating estimates of average fiber wall pore sizes. Average fiber wall pore sizes were computed by combining estimates of the specific surface area estimates from NMR spectra with results from FSP measurements. Advantages of this approach is that average pore sizes can be estimated without the need for sample drying and no pore geometry needs to be assumed (Larsson et al. 2013).

The average fiber wall pore size was calculated from the following relation:

$$2t = 2(\text{FSP})/(\sigma \cdot \rho_L).$$

where $2t$ is the reported value for the average pore size, FSP is the fiber saturation point, σ is the cellulose specific surface area from solid state NMR, and ρ_L is the density of water. Although no particular pore geometry is assumed for the estimation of the average pore size, the reported value $2t$ should be comparable to average pore diameters as reported by other measurement methods (Larsson et al. 2013).

X-ray measurements

X-ray measurements were performed on an Anton Paar SAXSpoint 2.0 system (Anton Paar, Graz, Austria) equipped with a Microsource X-ray source (Cu $K\alpha$ radiation, wavelength 0.15418 nm) and a Dectris 2D CMOS Eiger R 1M detector with 75 μm by 75 μm pixel size.

All measurements were performed with a beam size of approximately 500 μm diameter, at a sample stage temperature of 25 °C with a beam path pressure at 1–2 mBar. For WAXS the sample to detector distance

(SDD) was 109.1 mm and, for SAXS the SDD was 561.9 mm.

All samples were mounted on a Multi-Paste Holder mounted on a Heated Sampler and a VarioStage (Anton Paar, Graz, Austria). The samples were kept between Kapton foils in hermetically sealed compartments and were not exposed to vacuum during measurement.

For each sample 5 frames each of 6 min duration were read from the detector, giving a total measurement time of 30 min per sample. For all samples the transmittance was determined and used for scaling of the scattering intensities. For wet samples, scattering data recorded on deionized water with same experimental setup was used for background subtraction.

The software used for instrument control was SAXSdrive version 2.01.224 (Anton Paar, Graz, Austria), and post-acquisition data processing was performed using the software SAXSanalysis version 3.00.042 (Anton Paar, Graz, Austria).

The distance between the center of adjacent fibrils (symbol d_{center}) was estimated from the maximum in the Kratky plot ($d = \frac{2\pi}{q_{\text{max}}}$) as suggested by Virtanen et al. (2015). A Kratky plot is a presentation of SAXS data where the observed scattering intensity $I(q)$ multiplied by the squared value of the scattering vector q , is charted against the scattering vector; a plot of $I(q)q^2$ vs. q . Kratky plots can be used as a tool to obtain information on the persistence length of polymers in solution (Cleland 1977), but it has also found use as a tool for characterizing the fiber wall structure in pulps. Virtanen et al. (2015), utilized Kratky plots for qualitatively monitoring changes in the average distances between fibrils in spruce pulps. The average distances determined by this method are here denoted d_{center} . As discussed by the authors (Virtanen et al. 2015) the determined d_{center} distances may not represent the exact distances between fibril center points, but can be used to establish correlations with exact distances, suitable for comparisons between similar samples. The interpretation of the d_{center} values as fibril center-to-center distances can be complicated in cases of compact cellulose fibril aggregate interiors, tightly packed fibrils may limit electron density contrast to the point where individual fibrils could not be distinguished. Further, changes of large-scale structures (outside the used SAXS detection range) can ‘tail’ scattering intensity into the detected region,

possibly contributing to a shift in the position of the Kratky plot maximum. For these reasons the determined d_{center} values were used mainly for qualitative comparisons. A fitting of the Kratky curve was made (see Fig. 6) to identify the position of the maximum, used to calculate d_{center} . The average crystallite size, $d_{\text{crystallite}}$, was determined by WAXS using the Scherrer equation applied to the (200) signal.

Results

Never-dried spruce chips were treated at 110 °C and 170 °C, at acidic and alkaline pH, resembling the conditions in the most common pulping processes, i.e. kraft and prehydrolysis kraft cooking. The aim was to study the initial effects of temperature and pH on the supramolecular structure of the fiber wall as well as the effect of drying. The treated wood samples were chlorite delignified prior to analysis. In Table 2, the chemical composition after treatment and after chlorite delignification is shown.

The supramolecular structure of the chlorite delignified samples are shown in Table 3, both before and after drying in oven at 105 °C. Never-dried wood chips were used to control the effect of the treatments and avoid the potential effect of drying on the supramolecular structure of the fiber wall. The fiber saturation point (FSP) was slightly reduced by a higher treatment temperature, both at alkaline and acidic conditions. At a given temperature, no significant difference was seen in FSP between alkaline and acid treatments. Drying reduced the FSP considerably. A higher pH in the treatment increased the pore size, whereas drying resulted in a decreased pore size.

Both higher temperature and pH during treatment decreased the specific surface area (SSA). The crystallinity was not affected by neither pH nor temperature. There was no significant effect on fibril thickness, the lateral fibril width (LFD), by treatment conditions or drying, the average was 3.8 nm. However, the fibril aggregate size, lateral fiber aggregate dimension (LFAD), was increased by both higher temperature and pH. Upon drying, LFAD remained on same level. The distance between adjacent fibril centers as estimated from the maximum of a SAXS Kratky plot, d_{center} , decreased with drying.

Table 2 Relative amount of monosaccharides, % of sample, after treatment of chips and after chlorite delignification of treated chips

Sample ID	Treatment yield (%)	Ara ± 0.01	Gal ± 0.01	Glc ± 0.01	Xyl ± 0.08	Man ± 0.16	Acid insoluble residue ± 0.41	Acid soluble residue ± 0.05	Hydrolysis yield (%)
Wood	100	1.2	1.8	45.6	5.4	11.5	34.1	0.5	90.9
<i>After treatment</i>									
NaOH110	85.7	1.2	1.6	49.3	5.7	8.0	33.7	0.5	88.0
NaOH170	76.0	1.2	1.1	51.7	6.0	6.1	33.5	0.5	89.9
H ₂ O110	92.2	0.9	1.9	47.2	5.9	12.5	31.2	0.5	88.3
H ₂ O170	91.6	0.2	1.6	49.2	5.4	9.5	33.8	0.4	89.3
<i>After chlorite delignification</i>									
NaOH110	–	1.2	1.2	68.1	7.2	10.9	8.4	3.0	88.7
NaOH170	–	1.2	0.9	72.7	7.6	8.5	6.5	2.6	92.2
H ₂ O110	–	0.6	1.7	65.9	6.0	16.9	5.4	3.6	88.0
H ₂ O170	–	0.1	0.6	74.5	5.3	11.8	5.0	2.7	91.6

Average standard error at 95% confidence level is given. For explanation of sample ID see Table 1

Table 3 Supramolecular properties of chlorite delignified fibers from NMR except d_{center} , the distance between adjacent fibril centers which is estimated from the maximum of a SAXS Kratky plot and $d_{\text{crystallite}}$, which is the crystallite size from WAXS

	FSP (g/g)	Pore size (nm)	SSA (m ² /g)	Cr (%)	LFD (nm)	LFAD (nm)	$d_{\text{crystallite}}$ (nm)	d_{center} (nm)
<i>Never-dried</i>								
NaOH110	3.54 ± 0.14	56.3 ± 5.3	126 ± 11	48 ± 3	3.7 ± 0.2	21.2 ± 1.8	2.6	9.9 ± 1.0
NaOH170	3.20 ± 0.12	66.6 ± 5.2	96 ± 7	49 ± 1	3.8 ± 0.1	27.8 ± 1.9	3.2	10.6 ± 1.1
H ₂ O110	3.50 ± 0.03	42.7 ± 4.0	164 ± 15	48 ± 4	3.7 ± 0.2	16.3 ± 1.5	2.5	9.0 ± 0.9
H ₂ O170	2.93 ± 0.15	46.2 ± 4.0	127 ± 8	52 ± 3	4.1 ± 0.2	21.0 ± 1.3	3.2	10.0 ± 1.1
<i>Dried and rewetted</i>								
NaOH110	1.52 ± 0.04	23.0 ± 1.3	132 ± 7	47 ± 1	3.6 ± 0.1	20.2 ± 1.0	2.5	8.2 ± 0.7
NaOH170	1.39 ± 0.06	27.4 ± 2.2	102 ± 7	50 ± 1	3.9 ± 0.1	26.2 ± 1.8	3.4	8.3 ± 0.8
H ₂ O110	1.48 ± 0.10	18.1 ± 2.0	164 ± 14	44 ± 1	3.4 ± 0.1	16.3 ± 1.4	2.7	8.1 ± 0.7
H ₂ O170	0.71 ± 0.15	12.7 ± 2.9	111 ± 10	52 ± 1	4.1 ± 0.1	24.0 ± 2.2	3.4	8.0 ± 0.5

Discussion

In Fig. 1 (top), the yield of the different compounds after treatment is shown. The amount of xylan, glucomannan, cellulose and lignin in the samples are calculated from the monosaccharides according to Janson (1974) using a mannose to glucose ratio of 4.2 in galactoglucomannan (GGM). The relative chemical composition in the treated samples is shown in Fig. 1 (bottom). The alkaline treatment dissolved a large part of the glucomannan. This is mainly due to the peeling reaction (e.g. Wigell et al. 2007; Paananen et al. 2013). The xylan content was decreased as well, primarily dissolved in polymeric form (e.g. Axelsson et al. 1962; Jansson and Brännvall 2011). In the acidic treatment, the content of arabinose, mannose and galactose was reduced, probably dissolved as mono- or oligosaccharides (e.g. Puls et al. 1985; Garrote et al. 1999; Chen et al. 2010; Borrega et al. 2011).

The increase in average pore size, correlates well with the amount of hemicellulose remaining, Fig. 2a, especially with glucomannan content in fibers, Fig. 2b. This is in accordance with previous studies showing that as wood matrix material is removed, new pores are generated and the size of existing pores increase in the fiber wall (Stone and Scallan 1967). Pores in pulp fibers have been reported to be in the range from 20 up to 50 nm, increasing with degree of delignification (Andreasson et al. 2003).

LFAD increased with pH and temperature, showing a correlation both between total hemicellulose content, Fig. 2c, and glucomannan content, Fig. 2d. An

increase in pore size with increased removal of glucomannan is thus accompanied by a decrease in specific surface area (increase in LFAD).

This is in accordance with earlier studies which have demonstrated that removal of hemicelluloses by hot water extraction results in a denser packing of fibrils and increased LFAD (Hult et al. 2001; Penttilä et al. 2013) and that increased glucomannan content results in smaller fibril aggregate size in pulp fibers (Duchesne et al. 2001; Virtanen et al. 2015). This might be due to the closer association between glucomannan and cellulose compared to xylan and cellulose (Åkerholm and Salmén 2001; Salmén and Olsson 1998). Jungnikl et al. (2008) saw that chlorite delignification had only small effect on the nanostructure of the cell wall whereas removal of glucomannan by alkali changed it significantly (Jungnikl et al. 2008).

Interestingly, effect of temperature and pH seems to be secondary. The total hemicellulose content after treatment with alkali at 110 °C for 30 min (NaOH110°C) and at acidic pH at 170 °C for 30 min (H₂O170°C) was similar, and the size of the cellulose fibril aggregates was 21 nm in both cases.

In literature, the reported aggregate sizes range from 15 to 28 nm (Penttilä et al. 2013; Leppänen, et al. 2009). The aggregate sizes of the treated materials in the present study are within this range, reaching as high as 28 nm. It has been shown previously that already during the heating phase of either alkaline pulping (Hult et al. 2001) or steam pre-treatment (Pingali et al. 2014) an increase in aggregate size is

Fig. 1 Top panel: Yield of different components, % on oven-dry (o.d.) wood after alkaline (NaOH) and acid (H_2O) treatment at 110 and 170 °C. Bottom panel: The relative sample compositions of GGM, xylan, cellulose and lignin after chlorite delignification

obtained. Matrix material acts as spacers between cellulose fibrils and when removed the cellulose chains on the surface of adjacent fibrils adjust to each other and form fibril aggregates, as schematically depicted in Fig. 3. The present study confirms that aggregation of fibrils occurs quite early in the process. Treatment of wood during short time, whether alkaline or acid and whether at relatively low temperature (110 °C) or high temperature (170 °C), leads to changes in the conformation of polysaccharide polymer chains to form structures of higher order.

Drying caused the largest decrease in pore size and pore volume (measured as FSP) for the H₂O170 sample, the reduction was 72% and 76% respectively. For the other samples, the reduction was 58–60% in pore size and 50–58% in FSP. The SAXS measurements confirm the effect of drying on pores in the fiber wall, Fig. 4. The largest difference between nd and

d/rw samples was seen for H₂O170. Interestingly, there was a difference between nd and d/rw for H₂O170 down to a q -value of about 2 1/nm (d -values about 3 nm), while no effect of drying was seen for other samples below about 10 nm.

The interpretation of the SAXS data is complicated by several factors. The structural nature of the fiber wall interior is composed of high aspect ratio cellulose fibrils that can form aggregates of different lateral dimensions, mixed with hemicellulose (and smaller amounts of lignin) which may change its spatial distribution within the fiber wall as a result of the nature of the pre-treatment. Partial removal of hemicellulose and lignin create pores with a size distribution that can cover the dimensional range probed in the SAXS measurements. The strategy for interpreting the SAXS data used here is to try establishing interpretations consistent with other measurement results

Fig. 2 Pore size as a function of **a** total hemicellulose content and **b** glucomannan content. Lateral fibril aggregate dimensions (LFAD) as a function of **c** total hemicellulose content and **d** LFAD as a function of glucomannan content. Filled symbols represent never-dried samples, unfilled symbols represent dried and re-wetted samples. The samples were chlorite delignified after treatment in alkaline (NaOH) or acidic (H_2O) pH. The treatment time at temperature varied from 0 min for NaOH170°C to 240 min for H_2O 110°C, see Table 1

Fig. 3 An illustration of a model for a cross-section of a 6-by-6 fibril aggregate, with lignin (brown) and hemicellulose (green). The blue squares represent the cross-sections of individual cellulose fibrils. During processing, the lignin and hemicellulose content is depleted, allowing for intimate contacts between

fibrils, leading to the formation of difficult-to-break-up aggregates of fibrils. The pattern of intimate contact between fibrils may vary in length direction (perpendicular to the depicted plane) yielding an overall cohesion for the fibril aggregate

(FSP, CP/MAS ^{13}C -NMR) and by doing so to obtain additional information on the fiber wall nano-structure.

Due to the differences in principles of operations between CP/MAS ^{13}C -NMR and SAXS, it was not expected that agreements in absolute values would be

found when measuring the same samples by the two techniques.

When comparing the SAXS data in Fig. 4 and the data in Table 3 some interesting features were observed. When never-dried and dried-and-rewetted samples are compared pairwise, the NMR data in

Fig. 4 The SAXS profiles recorded on the NaOH110, NaOH170, H₂O110 and H₂O170 sample. The intensity scale (y-axis) is arbitrary units and the q-range is expressed in reciprocal nanometers (1/nm) Each panel contains data recorded on pairs of samples, never-dried and after drying and rewetting. The SAXS data has been transmittance scaled and background subtracted. The small signal feature present in the data for the NaOH170 sample at about 1.5 1/nm corresponds to the position of the detector gap in the Eiger R 1 M detector and was a measurement artefact, confirmed by measurements using different SDD

Table 3 indicates that there were no statistically significant changes in LFAD because of drying and subsequent rewetting. The major changes were found in the FSP values, which reduced from 3.0 to 3.5 g/g in never-dried samples to 0.7–1.5 g/g after drying and rewetting. Also the average pore sizes, calculated from FSP and NMR data, decreased after drying, from 43 to 67 nm in never-dried samples to 13–27 nm. For three of the samples, NaOH110, NaOH170 and H₂O110, the average pore sizes decreased to roughly half their initial values after drying and rewetting. In the case of sample H₂O170 the average pore size decreased to about a quarter of its initial value after drying and subsequent rewetting.

The NMR data thus showed that drying and subsequent rewetting did not change the size of fibril aggregates. Based on these observations it was concluded that the main structural factors influencing the recorded SAXS data, in these cases, relates to the fiber wall pore structure. In the SAXS data for all four samples a significant decrease in signal intensity was observed in the low q-range, correlating with the decrease in FSP values. For the H₂O170 the difference in appearance of the SAXS profiles recorded before and after drying were the most pronounced, and particularly so at higher q-values. Consistent differences in SAXS profiles recorded on never-dried and dried-and-rewetted samples at q-values of about 1 1/nm and larger was only found in the SAXS data from the H₂O170 sample. This size range, about 6 nm and smaller, carries information about the internal structure of cellulose fibril aggregates. The acidic conditions in combination with a higher temperature could also explain the observed differences in the H₂O170 SAXS data by a more efficient removal of hemicellulose from within the cellulose fibril aggregate. This way, the high acidity and high temperature wood chip pre-treatment efficiently removes hemicelluloses also

from the more confined environments, allowing for a more profound pore collapse/aggregation when subsequently dried. This is in agreement with the observed differences in the FSP values.

Comparing the chemical composition of the samples, the H₂O170 and NaOH170 showed the largest relative cellulose content, 81% and 80% respectively. The different chemical nature of the pre-treatments, acidic versus alkaline, may explain the difference observed in SAXS results. The more efficient breakdown of hemicellulose by the acidic pre-treatment alleviate the removal of hemicellulose from more confined environments, i.e. in the H₂O170 sample. Spatial redistribution of hemicelluloses, due to higher solubility in alkali, does not occur for the more confined environments.

Using the method of Virtanen et al. (2015) for calculating an average distance between adjacent fibrils (the d_{center} -value reported in Table 3) from a Kratky plot of the SAXS data, gave a reasonable correlation with FSP values between the groups of samples. The d_{center} -values were larger in the group of never-dried samples (9–11 nm) compared with the group of dried-and-rewetted samples (8 nm). However, there were no statistically significant differences found among the d_{center} -values of the samples within each group. The d_{center} -values (Table 3) were in the range of these reported by Virtanen et al. (2015) estimated on a commercial spruce-pine dissolving sulphite pulp that was mechanically and enzymatically pre-treated. Also, even though the d_{center} -values did not represent the exact distances between adjacent fibrils, they could be used as a correlation to the true average distance and therefore suitable for comparisons among similar samples (Virtanen et al. 2015).

For the samples that were used in this study, an average distance between adjacent fibrils of 8–11 nm seems large. With an average lateral fibril dimension of about 4 nm, this would imply a separation of 4–6 nm between fibrils within a fibril aggregate, contradicting aggregation. However, if the fibrils within a cellulose fibril aggregate are partially aggregated, i.e. there may exist some small-scale pore system also within the aggregate, combined with regions of very intimate contact between neighboring co-axial fibrils, the observed distances may not contradict the occurrence of aggregation. As indicated in Fig. 3, partial aggregation of fibrils forming a fibril aggregate may introduce some ambiguity about lateral

Fig. 5 The correlation between average lateral fibril dimensions (LFD), determined by CP/MAS ¹³C-NMR and the average crystallite sizes as determined by XRD using the Scherrer equation on the (200) signal. The error bars for LFD was taken from the NMR spectra fitting results, and for the crystallite sizes calculated error from 3 to 5 determinations was used

particle dimensions for scattering measurements. Intimate contact between neighboring co-axial fibrils may decrease electron density contrast between adjacent fibrils to the point where the width of two fibrils effectively is observed as a single particle. Due to the differences in operating principle between X-ray scattering and CP/MAS ¹³C-NMR spectroscopy, it is not certain that the two methods operationally define measures such as particle sizes and interparticle distances in the same manner. Focus is put on trends, where both methods give results that supports that drying and subsequent rewetting results in a more extensive consolidation/aggregation of cellulose fibrils.

The LFD determined by CP/MAS ¹³C-NMR were compared with the so-called crystallite dimensions determined from XRD diffractograms recorded on the samples. The results are shown as a correlation graph in Fig. 5. It was found that the data grouped with respect to maximum treatment temperature. The scatter within each temperature group prevented any detailed conclusions to be drawn, a statistically significant difference was found between the groups, showing that both NMR and X-ray indicated that the samples that had been exposed to higher temperatures showed the larger dimensions.

There is a correlation between LFD determined by NMR and crystallite size determined by XRD, Fig. 5. As expected, the absolute numbers do not agree, but

the observed trends are similar. Interestingly, the major impact on LFD/crystallite size was due to the treatment temperature, where a higher temperature resulted in an increased internal fibril order. Increased internal fibril order as the result of hydrothermal treatments has been observed previously (Hult et al. 2001).

Within this tentative interpretation of combined NMR, FSP and SAXS data it became possible to address a size range of the fiber wall difficult to reach by other means, the internal structure of cellulose fibril aggregates. The close-to co-axial alignment of the 4 nm wide cellulose fibrils in the fiber wall makes packing and aggregation efficient building fairly, but not completely, compact structures. Accessibility to the interior of 10–40 nm wide fibril aggregates becomes an important controlling factor for e.g. chemical reactivity and cellulose-water interactions since most of the surface area is exposed by the 4 nm wide fibrils. Increasing accessibility to the fibril aggregates interior makes more surface area exposed and available.

Conclusions

Spruce chips subjected to different pre-treatments were studied using X-ray scattering, CP/MAS ^{13}C -NMR spectroscopy, FSP and carbohydrate composition in order to study the impact of the pre-treatments on the fiber wall nanostructure.

All samples were subjected to a mild chlorite delignification prior to structural analysis.

Removal of fiber wall components, hemicellulose and lignin, increased the fiber wall porosity and induced cellulose fibril aggregation.

A pronounced correlation between GGM removal and cellulose fibril aggregation was found.

The NMR results showed that no major changes were observed in the LFAD as the result of drying and re-wetting. However, drying and re-wetting had a significant impact on the SAXS results, covering the size range smaller than that characteristic for fibril

aggregate widths. Based on this an interpretation was made that the SAXS measurement gave information about changes in the interior structure of cellulose fibril aggregates, not necessarily associated with significant changes to the LFAD. A tentative explanation for these observations was that the internal aggregate structure is composed of intimately aggregated fibrils (partial aggregates) intermixed with small scale cavities between partial aggregates.

In summary, the effect of temperature and pH in the treatment on cellulose fibril aggregate size appears to be secondary. It is the removal of hemicellulose that has a profound effect on the supramolecular structure of the cellulose fiber wall. As the amount of hemicellulose dissolved from wood increases, the fibril aggregate size increases as well. Specifically, a good correlation between the amount of glucomannan in the fiber wall and the fibril aggregate size is seen. The lower the amount of glucomannan, the larger the aggregate size. Glucomannan thus seems to prevent aggregation as it acts as a very efficient spacer between fibrils.

By combining measurement methods, a more well-resolved picture of the structural changes occurring during wood chip pre-treatments was obtained.

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Availability of data and material Not applicable.

Declarations

Conflict of interest The authors declare no competing or conflicts of interest.

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Appendix

See Fig. 6.

Fig. 6 Kratky plots of never-dried (above) and dried and re-wetted samples (below). At the maximum, the fit is shown which has been used to identify the maximum (q position) when determining d_{center}

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