

# ARRANGEMENT OF CELL-WALL CONSTITUENTS IN CHEMICALLY TREATED NORWAY SPRUCE TRACHEIDS

*Tanja Zimmermann*

Research Scientist

*Klaus Richter*

Senior Scientist and Head of Wood Laboratory

*Nico Bordeanu*

Research Scientist

Swiss Federal Laboratories for Materials Testing and Research (Empa)

Ueberlandstrasse 129

8600 Duebendorf, Switzerland

and

*Jürgen Sell*

Professor, former Head of Wood Laboratory (Empa)

Robaenkli 22

8607 Aathal-Seegraeben, Switzerland

(Received May 2006)

## ABSTRACT

The cell-wall of tracheids in conifer wood has evolved to provide both water conduction and mechanical strength to the standing tree. However, its structure at the nanometer level is not yet accepted beyond doubt, and little is known about the interactions between the cell-wall components. In the present study, the fracture pattern of the S2 layer of Norway spruce tracheids was observed by field emission scanning electron microscopy (FE-SEM) after pretreatment of the cell wall with various alkali solutions, acetic and nitric acid, and ASAM delignification. The resulting cell-wall arrangements were also studied in ultra-thin sections of unfractured samples with transmission electron microscopy (TEM). In the case of untreated samples (reference), radial fracture patterns—perpendicular to the compound middle lamella—were regularly observed. A treatment with 10% and 18% NaOH or 24% KOH at room temperature—associated with a slight decrease of glucomannan—resulted in the disappearance of these radial fracture formations. As the severity of the alkali treatment increased and acid and ASAM delignification was applied, concentric alignments in the cell wall became more and more discernable. The increasing loss of hemicelluloses and lignin therefore led to distinct changes in the fragmentation patterns of the cell walls. In addition, reduction in strength and stiffness were determined for all chemically treated cell walls. It is concluded that even slight changes in cell-wall constitution influence the interactions of the cell-wall components and thus fracture mechanics and ultrastructural appearance of wood cell walls.

*Keywords:* Cell-wall structure, interactions of cell-wall components, S2 layer, chemical treatment, FE-SEM, TEM.

## INTRODUCTION

The complex architecture of the wood cell wall determines the strength and stability of the standing tree, and therefore also the mechanical properties of solid wood. The structure of wood

at all length scales makes it possible for tons of plant biomass to be supported by astonishingly slim stems to heights of sometimes more than 50 meters. The tree can withstand large static and dynamic forces of gravity and wind-loads. Moreover, the wood structure efficiently con-

ducts water from the roots to the crown (Booker and Sell 1998). Natural selection has ensured that tree and wood structure at the cellular level are optimized to satisfy these engineering requirements (Mattheck 1991). However, the structure at the nano level and the specific molecular mechanistic phenomena are not yet fully understood (Fratzl et al. 2004). The organization, as well as the interactions of the stiff cellulose fibrils and the softer matrix polymers lignin and hemicelluloses in the thickest secondary two wall layer, the S2, is still open to debate.

Microscopic studies on the ultrastructure of wood cell-wall transverse sections revealed a lamellar (Daniel and Nilsson 1984; Fahlén and Salmén 2002; Kerr and Goring 1975; Ruel and Goring 1978), radial (Schwarze and Engels 1998; Sell and Zimmermann 1993) or random (Donaldson and Frankland 2004; Zimmermann et al. 2006) distribution of cell-wall components in the S2. It has been suggested that different organization patterns do coexist (Sell and Zimmermann 1998; Singh and Daniel 2001). Another hypothesis is that the wood cell-wall components rearrange into various structural patterns under different stress conditions and preparation methods (Zimmermann et al. 2006). Indeed, a morphological rearrangement process following plastic deformation of wood has been discovered by Keckes et al. (2003). They found evidence for a molecular stick-slip mechanism similar to the motion of dislocations in crystalline materials. In detail, a re-formation of the amorphous matrix between the cellulose fibrils within the cell wall was discussed. A similar mechanism may explain the formation of different ultrastructural patterns observed in the past.

Further studies are required to resolve the underlying interactions of the cell-wall constituents. In this context, our study investigates the influence of chemical pretreatment on fractured or ultra-thin tracheid cross-sections. For this purpose, spruce cell walls were modified by dissolving chemical components, mainly hemicelluloses and lignin using alkaline and acetic treatment. Possible changes in the supermolecular structure of cellulose due to alkali treatment were evaluated by using X-ray diffraction. The

resulting morphological structures were then studied by field emission scanning electron microscopy (FE-SEM) and transmission electron microscopy (TEM).

## MATERIALS AND METHODS

### *Chemical pretreatment*

Three-mm-thick panels were prepared from a spruce board (*Picea abies* Karst) by planing and cut with a circular saw into single sticks. Prior to preparation, the wood was stored outside for months under cover and afterwards for several weeks in a climate chamber at 65% RH and 23°C. For each treatment, 50 small sticks of sound wood (cross-section 3 mm × 3 mm, each containing early- and latewood, 70 mm in length) were shaken in various solutions. The treatments were designed to dissolve distinct parts of the binding components of the cell wall (hemicelluloses and lignin) with increasing severity. Fifty sticks were retained as reference material.

Alkaline treatment was used for hemicellulose degradation:

1. Sodium hydroxide, NaOH (10% w/v at room temperature, 5 h)
2. Sodium hydroxide, NaOH (18% w/v at room temperature, 5 h)
3. Potassium hydroxide, KOH (24% w/v at room temperature, 5 h)
4. Hot water extraction with addition of NaOH (10% w/v, 100°C, 5 h) in a rotary evaporator

Acetic treatment and ASAM pulping were designed for intensive degradation of both hemicelluloses and lignin, leaving the cellulose generally unaltered. To represent the effect of ASAM-pulping, industrial wood chips of spruce were used instead of small sticks:

5. Acetic acid, CH<sub>3</sub>COOH (80% v/v) and nitric acid, HNO<sub>3</sub> (65% v/v) = 10/1 (by volume) during 30 min at 120°C
6. Steaming with hot water vapor during 30 min at 1 bar pressure and 100–120°C and ASAM pulping with sodium sulfite, Na<sub>2</sub>SO<sub>3</sub>/sodium hydroxide, NaOH = 80/20 (by volume), heating time 105 min, until T<sub>max</sub> = 180°C is reached

After treatment with the various chemicals, all samples were washed in distilled water until the washing solutions became neutral. The wood was then conditioned to an equilibrium moisture content of about 10%.

Samples of all treatments were submitted to total hydrolysis with sulphuric acid; the hydrolyzed carbohydrates were separated by borate complex ion exchange chromatography, and detected photometrically with copper-2,2-bicinchonate reagent according to Uremovic et al. (1994).

#### *X-ray diffraction studies*

To assess the possible influence of the alkali treatments on the supermolecular structure of cellulose, XRD measurements were carried out. The tangential surfaces of the sample sticks treated with 10% (cold and hot water extraction) and 18% NaOH as well as 24% KOH were prepared by microtoming the surface and thoroughly washing with deionized water. The samples were then investigated with a diffractometer (X'Pert Pro, Panalytical, Netherlands) using Ni-filtered Cu K $\alpha$  radiation ( $\lambda = 0.15418$  nm). Lattice recognition was done by comparison with literature data.

#### *Mechanical tests*

Three-point bending tests were applied to loosen the cell-wall structure of the sticks on the fracture surface. For each combination of chemical treatment, at least 5 wood samples were loaded to failure (44 N load cell, loading speed 20  $\mu\text{m/s}$ ). Therefore, special equipment for the bending testing of small samples was used (Fig. 1). From the results, the bending strength and the modulus of elasticity (MOE) of the samples were calculated. The wood chips (ASAM delignification) were manually broken without measuring strength and stiffness.

#### *Structural analysis*

*SEM studies.*—Samples of the transverse fracture surfaces were extracted with razor blades in

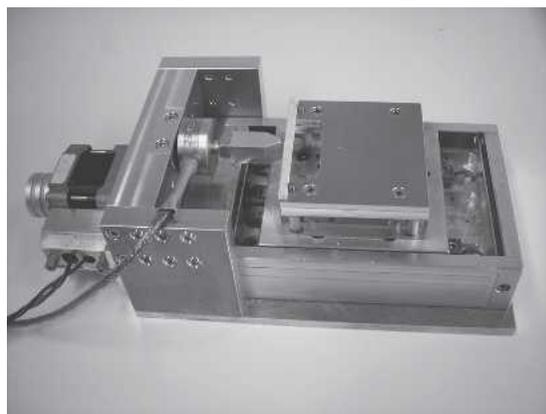


FIG. 1. The bending tests were carried out using special equipment for small samples.

close proximity to the outermost tension zone. These were then prepared for SEM by drying in a vacuum oven at 40°C and 10 mbar for 12 h, glueing on a specimen holder using carbon-adhesive and sputtering with a platinum layer of approximately 10 nm. The samples were investigated in a Field Emission SEM (Jeol 6300F) at an acceleration voltage of 5 kV and working distance of 24 mm.

*TEM studies.*—From each combination of chemical treatment, three samples were embedded following the methodology proposed by Spurr (1969). Ultra-thin sections (approximately 100 nm) of the samples were produced using a diamond knife (microtome type LKB Ultratome, 4801 A) and placed on Formvar coated copper grids. Half of the sections were stained for 4 minutes with a solution of KMnO<sub>4</sub> (1% w/v) in sodium citrate (0.1% w/v). The remaining sections were stained with uranyl acetate (1% w/v) and lead citrate (Reynolds 1963). After staining, the sections were washed in double-distilled water. Finally, the sections were examined with a Philips STEM CM30 transmission electron microscope.

## RESULTS AND DISCUSSION

### *Chemical pretreatment*

The effects of the various chemical treatments applied are shown by the results of total hy-

drolisis, presented as relative percentages in Table 1.

It is well known that the hydrolysis residue represents a rough approximation of the Klason lignin content (Fengel and Wegener 1989). Thus, our own data are in good agreement with results cited in the literature and confirm that an increase in the severity of alkali treatment did not lead to lignin degradation, while the relative amounts of sugars, typical for the hemicelluloses did, as expected, decrease. A treatment with 10% w/v and 18% w/v NaOH or 24% w/v KOH at room temperature led only to slight changes in the carbohydrate composition of the cell wall. Only small proportions of the hemicellulose glucomannan were solubilized, indicated by a decrease of mannose and simultaneously an increase of glucose. In contrast, the hot water extraction with NaOH (10% w/v) caused a sharp decline in mannose. As intended, the treatment with acetic acid and nitric acid or ASAM pulping (with Na<sub>2</sub>SO<sub>3</sub> and NaOH) led to a distinct degradation of both hemicelluloses and lignin.

#### *X-ray diffraction studies*

Different authors have reported that the lattice structure of cellulose changes due to mercerization by sodium hydroxide treatment (e.g. Borysiak and Doczekalska 2005; Mansikkamäki et al. 2005; Nishiyama et al. 2000; Okano and Sarko 1984). This might have an influence on the interactions of the cell-wall constituents. The path from cellulose I (parallel structure) to cel-

lulose II (antiparallel structure) during mercerization goes by way of Na-cellulose I. If a cellulose sample is treated with an alkali solution, the cellulose swells to various extents depending on the type and the concentration of alkali, and also on the temperature (Fengel and Wegener 1989). At low concentrations, only the large pores in the cellulose structure are occupied. With increasing concentration, the smaller cation Na<sup>+</sup> (Na<sup>+</sup> = 0.276 nm) can advance more easily into smaller pores. Na<sup>+</sup> seems to have a favorable diameter that is able to widen the smallest pores down to the space between the lattice planes and advance into them. During intensive washing, the linked Na-ions are removed and another lattice is formed, the cellulose II lattice.

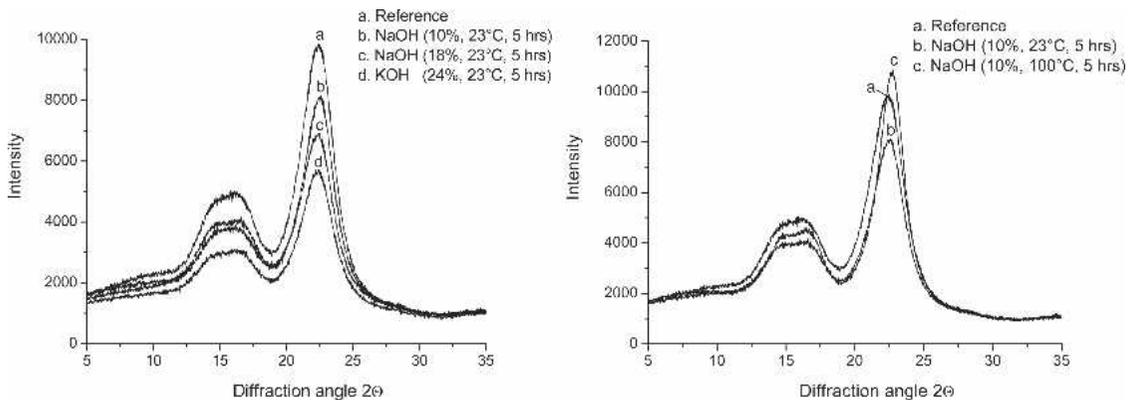
In our study, for all NaOH and KOH treatments in different concentrations, no lattice conversion of cellulose (transformation of cellulose I to cellulose II) was observed (Figs. 2a, 2b). This is also true for the samples treated with 10% NaOH at 100°C although these samples showed distinct losses in glucomannan. The diffraction peaks found are typical for cellulose I (Borysiak and Doczekalska 2003; Mansikkamäki et al. 2005). The results are in accordance with different studies reported in literature. Kim (2005) found for the sapwood of sound oak wood that the cellulose was converted more slowly to Na-cellulose I during mercerization than in delignified wood and that very little Na-cellulose was converted to cellulose II. During

TABLE 1. Determination of carbohydrates and unhydrolyzed residues obtained from spruce wood submitted to various chemical treatments.

	Relative composition of the hydrolysates (by weight)						Unhydrolyzed residue <sup>2</sup> (%)
	Glucose (%)	Xylose (%)	Galactose (%)	Arabinose (%)	Mannose (%)	4-O-Me-GluA <sup>1</sup> (%)	
Reference	71.37	6.97	1.03	1.35	18.32	0.95	27.48
10% NaOH	70.48	7.07	1.80	1.35	18.35	0.77	28.77
18% NaOH	73.38	6.21	1.70	1.32	16.50	0.89	28.13
24% KOH	74.17	5.73	1.34	1.60	16.23	0.94	28.20
10% NaOH 100°C	80.00	7.41	1.06	1.30	9.77	0.46	31.82
CH <sub>3</sub> COOH/HNO <sub>3</sub>	86.19	4.08	—	—	9.05	0.69	10.27
ASAM pulping	84.4	7.8	—	—	7.5	—	10.5

<sup>1</sup> 4-O-Me-GluA: 4-O-methyl-glucuronic acid.

<sup>2</sup> Related to absolutely dry wood.



Figs. 2a, 2b. X-ray diffraction patterns of alkali treated spruce wood showing the typical peaks for cellulose I.

washing and drying, Na-cellulose I of sound wood was reconverted to cellulose I.

Revol and Goring (1981) reported only a partial conversion of cellulose I to cellulose II although they impregnated 0.5- × 0.5-mm<sup>2</sup> samples under vacuum for 24 h. In our study, larger 3- × 3-mm<sup>2</sup> samples were alkali treated for only 5 h but not with vacuum.

Lonikar et al. (1984) found no lattice conversion at all for white birch treated with 23% aqueous NaOH. On the other hand, wood subjected to a pretreatment that results in the loosening of its morphological texture showed varying degrees of lattice conversion during mercerization.

The authors explained the fact that alkali treatment caused only little or no transformation from cellulose I to cellulose II by the chemical composition of wood. Hemicelluloses and lignin are deposited in between the adjacent cellulose fibrils. Intramolecular as well as intermolecular interactions are present within and among these wood components so that none of the physical or physicochemical characters of an individual wood component is displayed independent of the other components. Even chemical bonds exist between wood components as detected in lignin-carbohydrate complexes. Thus, wood displays a very tough and compact morphological texture that restricts either the penetration of the caustic solution or the subsequent swelling of cellulose, and hence no lattice conversion is observed.

As no degradation of lignin could be observed in our study for all alkali treated samples, we con-

clude that lignin prevented the alkali swelling of the cellulose to some extent and therefore the transformation from cellulose I to cellulose II.

Recent results of Jungnickl (2006) support our conclusion. In her study, the influence of lignin on the formation of cellulose II was assessed. Semi-thin tangential slices of spruce earlywood were treated with 10% and 20% NaOH at 90°C for 10 h with and without prior delignification. A pronounced formation of cellulose II was observed from WAXS (wide angle X-ray scattering) patterns of the delignified samples after treatment with 10% and 20% NaOH. A conversion to cellulose II in the samples treated with 10% NaOH without prior delignification could not be observed.

Nevertheless, mercerization and a transformation from parallel cellulose I to antiparallel cellulose II occur at another hierarchical level than the rearrangements observed in the presented microscopic study. It is not likely that changes in the lattice structure affect the arrangement of wood cell-wall components.

### Mechanical tests

Figures 3a and 3b show the results of the 3-point bending tests. The highest strength and stiffness were determined for the untreated samples (reference). The MOE values are in accordance with those known from the literature for sound wood (Sell 1989), while the calculated bending strength was even higher. The lowest

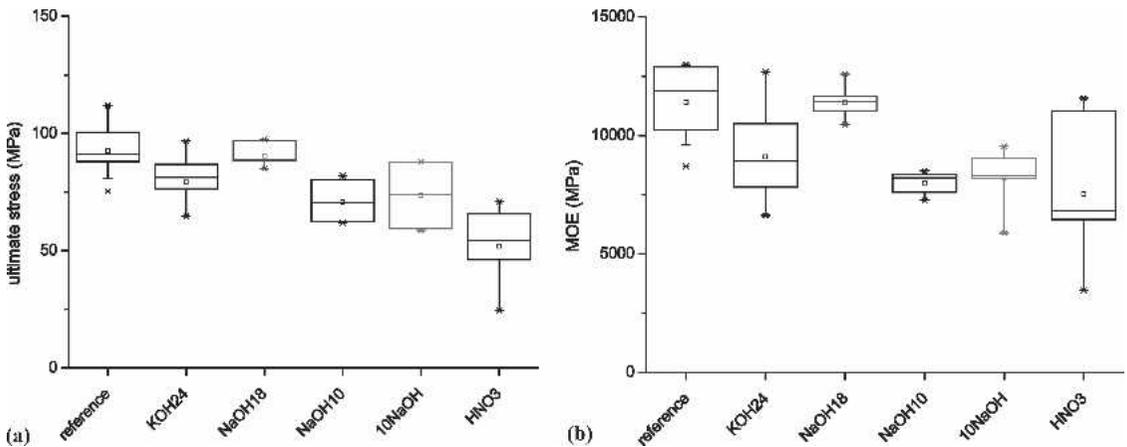


FIG. 3a. Bending strength of chemically treated Norway spruce samples.

FIG. 3b. Modulus of elasticity of chemically treated Norway spruce samples.

values were obtained for the samples treated with acetic acid ( $\text{CH}_3\text{COOH}$ ) and nitric acid ( $\text{HNO}_3$ ). These samples showed an intensive decrease in hemicelluloses and lignin residue (see Table 1) and were strongly deformed. Köhler and Spatz (2002) obtained similar results for the strengthening tissues of *Aristolochia macrophylla Lam.* with chemically altered cell-wall assembly. They showed that a chemical extraction of hemicelluloses and lignin respectively, led to significant changes in the stress-strain behavior of the samples. Removing the lignin or the hemicelluloses reduced the initial stiffness in the linear deformation stage. In addition, the samples where hemicelluloses were extracted lost their high toughness.

Although alkaline treatment (with NaOH or KOH), extraction at room temperature) led only to a slight hemicellulose degradation (see Table 1), it had an influence on the measured MOE and bending strength. Generally, alkali treated samples showed lower values compared with the reference samples. One exception was the treatment with 18% NaOH: The MOE and strength values are apparently not different from those determined for the reference samples. Fratzl et al. (2004) showed that the macroscopic mechanical properties depend to a large extent on the strength of the interface between cellulose fibrils and matrix polymers. A weakening of the

interface might be caused by the permanent swelling of the cell wall during the alkali treatment and the starting solubilization of hemicelluloses. Consequently, molecular interaction between fibrils and the matrix polymers altered, bonds were solved and eventually new bonds were constituted. Recently, Keckes et al. (2003) observed cell-wall recovery mechanisms that lead to the re-formation of the amorphous matrix between the cellulose fibrils within the cell wall. It is conceivable that similar phenomena play a role when fracture mechanics change in chemically swollen wood.

Although only small proportions of glucomannan have been solubilized by alkaline treatment at room temperature, it is also possible that the degree of polymerization (DP) of the residual polymers changed, and as a consequence influenced strength and stiffness of the respective samples.

The samples extracted in hot water with 10% NaOH had an intensive reduction in mannose (approximately 9%) and showed a similar deformation as the specimens treated with  $\text{CH}_3\text{COOH}$  and  $\text{HNO}_3$ . Surprisingly, these samples showed slightly higher MOE and strength values than those treated with 10% NaOH at room temperature without an apparent change in carbohydrate composition. A possible explanation might be derived from the respective X-ray diffraction patterns (Figs. 2a and 2b). The X-ray diffraction

experiments with samples treated with alkali at room temperature showed a decreasing peak intensity (cellulose I peaks) with increasing alkali concentration (Fig. 2a). This indicates a decrease in crystallinity due to the alkali treatment. In contrast, the peak intensity of samples treated with 10% NaOH at 100°C was higher than for all the other alkali treated samples and even higher than for the reference samples. It is possible that due to a higher degradation of glucmannan, the crystallinity of the remaining matrix increases and therefore no further losses in strength and stiffness occurred. However, as it was very difficult to determine the correct dimensions of the deformed small samples, required for the calculation of MOE and bending strength, slight measuring irregularities can also not be excluded.

In summary, it was evident that even a slight change in carbohydrate composition (e.g. 2% reduction in mannose) resulted in strength and stiffness losses. A distinct degradation of all hemicelluloses and lignin (e.g. decrease of unhydrolyzed residue of about 18%, compare Table 1) caused sample deformations as well as a sharp decline in MOE and bending strength.

### Structural analysis

*Reference samples.*—In the case of untreated samples, radial fracture patterns (perpendicular to the compound middle lamella) were apparent on transverse surfaces of the cell-wall layer S2 (Fig. 4). This result is in good agreement with earlier studies (Sell and Zimmermann 1993). As cellulose, hemicelluloses, and lignin have similar electron optical densities, it is not possible to distinguish between these cell-wall components. Consequently, the aligned features in the S2 of fractured samples will hereafter generally be described as fracture or fragmentation patterns.

In ultra-thin TEM sections of reference samples, no consistent orientation of the cell-wall components could be discerned. Lamellar as well as disordered and, in few cases, moderate striations perpendicular to the compound middle lamella were visible (Fig. 5).

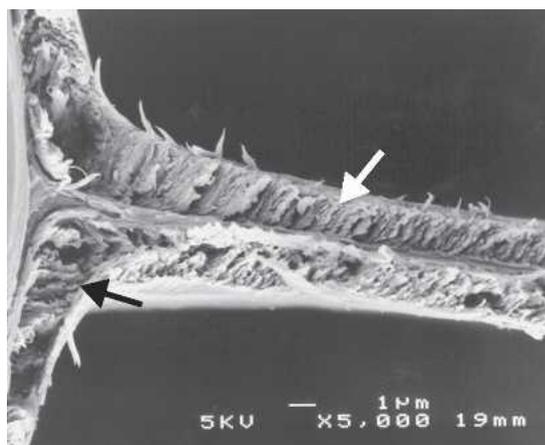


FIG. 4. FE-SEM micrograph: Transverse fracture surface (tension zone) of an untreated tracheid cell wall of spruce (earlywood). Distinct preferentially radial (perpendicular to the compound middle lamella) fragmentation of the S2 (arrows).

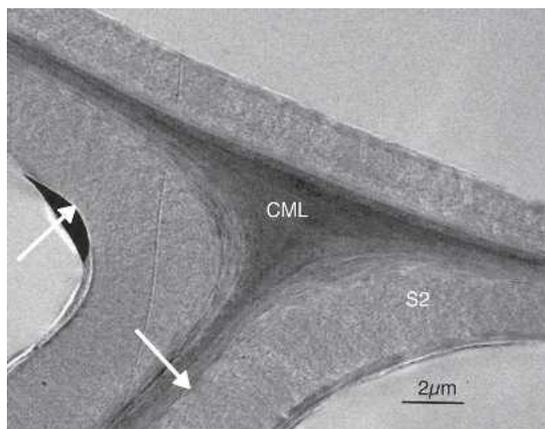


FIG. 5. TEM micrograph: Transverse ultra-thin section of a cell wall of spruce (transition zone early-/latewood, reference sample). Stained with  $\text{KMnO}_4$ . No distinct preferential orientation of the cell-wall components (arrows).

### Chemically treated samples

Figures 6 to 11 show FE-SEM micrographs of transverse fracture surfaces and a TEM micrograph of an ultra-thin section of chemically treated wood cell walls. Chemical treatment was associated with changes in the fragmentation of the thick S2 layer.

Swelling of the cell wall and a slight reduction in hemicelluloses after treatment with NaOH

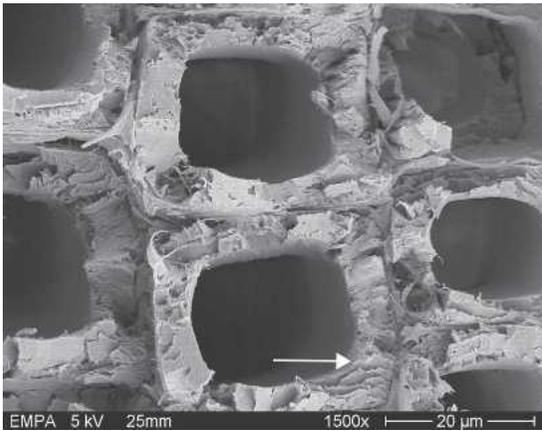


FIG. 6. FE-SEM micrograph: Transverse fracture surface (tension zone) of cell walls of spruce (transition zone early-/latewood); treated with KOH (24%, room temperature). Relatively brittle fracture surfaces, no preferential orientations in the S2 are visible. In exceptional cases radial fracture patterns were found (arrow).

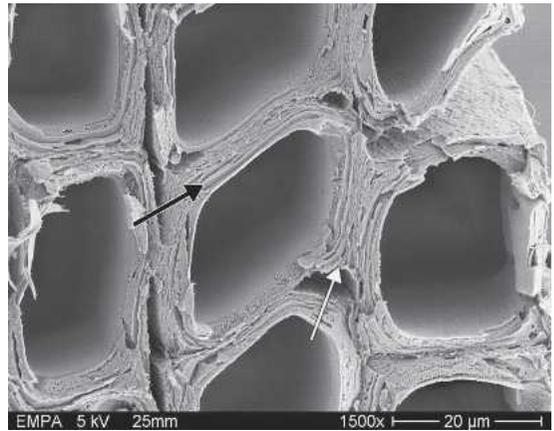


FIG. 8. FE-SEM micrograph: Transverse fracture surface (tension zone) of cell walls of spruce (transition zone early-/latewood); boiled for 30 min at 120°C with acetic acid (80%) and nitric acid (65%) in a ratio of 10:1. Concentric lamellas within the S2 are clearly visible (arrows).

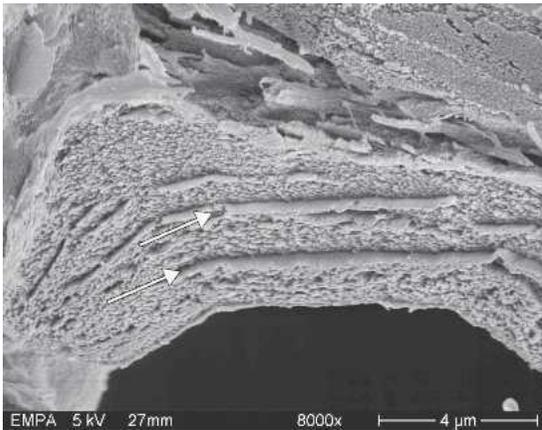


FIG. 7. FE-SEM micrograph: Transverse fracture surface (tension zone) of a cell wall of spruce (latewood); treated with NaOH (10%, 100°C). Within the S2 layer distinct concentric fracture patterns are discernible (arrows).

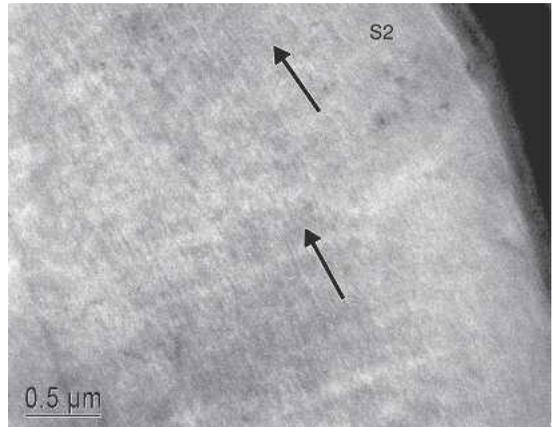


FIG. 9. TEM micrograph: Transverse ultra-thin section of a latewood cell wall of spruce; boiled for 30 min at 120°C with acetic acid (80%) and nitric acid (65%) in a ratio of 10:1. Stained with  $\text{KMnO}_4$ . Concentric lamellar striations (light and dark areas parallel to the other layers) within the S2 are distinguishable (arrows).

(10% w/v and 18% w/v) or KOH (24% w/v) led to the disappearance of radial structures and resulted in a disordered fragmentation (Fig. 6).

On transverse fracture surfaces of samples treated with hot water and NaOH (10% w/v), concentric layers within the S2 became apparent (Fig. 7).

Substantial degradation of hemicelluloses and lignin after treatment with  $\text{CH}_3\text{COOH}$  and

$\text{HNO}_3$  or  $\text{Na}_2\text{SO}_3$  and NaOH, respectively, was linked to increasing formation of distinct lamellar arrangements (concentric rings parallel to each other) within the S2. This could be observed both on fractured samples by FE-SEM as well as in ultra-thin sections of non-fractured samples (Figs. 7–11).

For the alkali treated samples, it was remark-

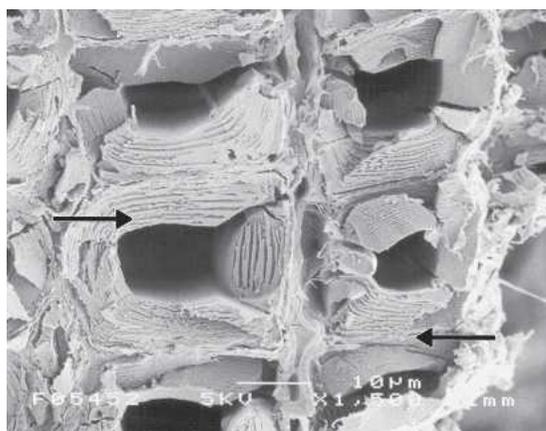


FIG. 10. FE-SEM micrograph: Transverse fracture surface (tension zone) of latewood cell walls of spruce; treatment with chemicals of ASAM pulping (sodium sulfite and sodium hydroxide). Concentric lamellas within the S2 are recognizable in almost every cell wall (arrows). No radial or disordered fracture patterns could be found.

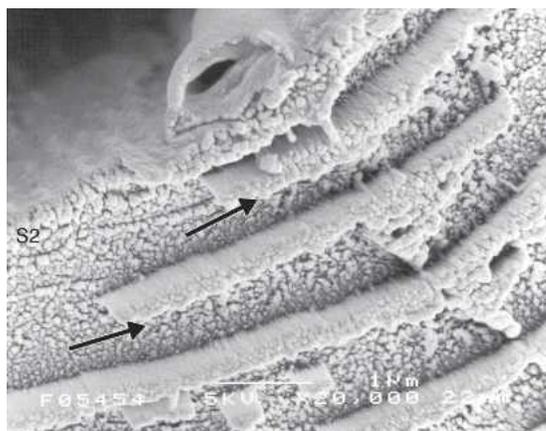


FIG. 11. FE-SEM micrograph: Transverse fracture surface (tension zone) of the S2 layer of a latewood cell wall of spruce; same sample as in Fig. 8. With high magnifications, a graduation of agglomerated cellulose fibrils (slippage planes) in the circumferential direction (parallel to the compound middle lamella) is discernible (arrows).

able that a swelling without an apparent change in the carbohydrate composition had an influence on the mechanical interactions of the cell-wall components during the fracture process. This is also reflected in the reduction of strength and stiffness (see Fig. 3). The decomposition of small portions of the hemicellulose glucomannan, indicated by a decrease of mannose and

simultaneously an increase of glucose (compare Table 1), results in the disappearance of formerly visible radial fracture patterns. It is well established that cellulose fibrils are surrounded with hemicelluloses forming larger units which are embedded in the hemicellulose/lignin matrix (Fengel and Wegener 1989). According to the observations of Salmén and Olsson (1998) on softwoods, the hemicellulose glucomannan is closely associated with cellulose, whereas xylan seems to appear in combination with lignin. Slight solubilization of glucomannan may influence the interactions between the cellulose fibril agglomerates (visible by SEM) and the matrix constituents of the cell wall and thus the cell-wall assembly. Thus, fracture mechanics and as a consequence the fracture pattern will alter. According to Fratzl et al. (2004) a tight binding between matrix and cellulose fibrils is required for wood to be strong and tough. They postulated that hemicelluloses could play a role as special interface polymers, capable of binding to the cellulose fibrils and forming aqueous networks between them.

The samples with a significant loss in hemicellulose and/or lignin proportion are strongly deformed and show a significant decrease in strength and stiffness. The increasing losses of binding components result in a complete rearrangement of initially radial to lamellar oriented cell-wall components (Fig. 11). The severe chemical treatments applied loosen the tracheid cell-wall structure. A distinct proportion of hemicelluloses and lignin is dissolved, whereas the cellulose remains probably unaltered. It is known that the cellulose fibrils are aligned at an angle of 5–30 degrees (microfibril angle) to the longitudinal axis in the secondary wall layer, e.g. Liese (1970). As the cellulose fibrils are spirally arranged in this layer, its deformation due to the chemical treatment and the fracturing process may cause the structural rearrangement in concentric slippage planes.

#### CONCLUSIONS

- Depending on the applied chemical treatment, transverse fracture surfaces and ultra-thin sec-

tions of the S2 layer of Norway spruce tracheids showed radially, randomly, or concentrically arranged cell-wall constituents.

- A slight change in carbohydrate composition resulted in strength and stiffness losses. A distinct degradation of all hemicelluloses and lignin caused sample deformations as well as a sharp decline in MOE and bending strength.
- Hemicelluloses and lignin appear to be important components that are associated with fracture mechanics and the resulting fracture patterns. The fracture process and the resulting fragmentation pattern of the cell wall are very sensitive to alterations of the chemical composition of the wood cell wall.
- The hemicellulose glucomannan seems to play a special role for the interactions between the cell-wall constituents. Even small losses of glucomannan influence their interactions and therefore the ultrastructural appearance of transverse sections.
- For all NaOH and KOH treatments in different concentrations, no lattice conversion of cellulose (transformation of parallel cellulose I to antiparallel cellulose II) was observed. The X-ray diffraction patterns for all alkali treated samples were typical for cellulose I. Thus, mercerization and therefore changes in the lattice structure of cellulose are not the reason for the observed rearrangements of cell-wall constituents.
- The natural arrangement of the structural components in unaffected wood could not be derived from these studies.

For future studies it is of foremost importance to investigate underlying interactions of cell-wall components and to understand the mechanisms of re-orientations of cell-wall constituents.

#### ACKNOWLEDGMENTS

The authors wish to thank U. Klotz (Empa, Laboratory for Joining and Interface Technology) and R. Wessicken (ETH Zürich) for support with the TEM operation. We are also grateful to D. Eckstein for critical reading as well as to O. Kordsachia and J. Puls for carrying out the

total hydrolysis and analysis of the sugar composition of the hydrolysates (Federal Research Centre for Forestry and Forest Products, Hamburg, D). Thanks are due to I. Burgert and M. Eder (Max-Planck Institute of Colloids and Interfaces, Golm, D) for performing the bending tests on a special equipment for small samples. I am also grateful to P. Lienemann and U. Gfeller (Empa, Laboratory of Solid State Chemistry and catalysis) for helping me with the X-ray diffraction measurements. The support of E. Strub (Empa, Wood Laboratory) on the chemical treatment of the samples is gratefully acknowledged. The idea for the chemical treatments arised from a collaboration with Prof. J. Fromm, Institute for Wood Research, TU München, D.

#### REFERENCES

- BOOKER, R. E., AND J. SELL. 1998. The nanostructure of the cell wall of softwoods and its functions in a living tree. *Holz Roh-Werkst.* 56:1–8.
- BORYSIK, S., AND B. DOCZEKALSKA. 2005. X-ray diffraction study of pine wood treated with NaOH. *Fibres & Textiles in Eastern Europe* 13(5):87–89.
- DANIEL, G., AND T. NILSSON. 1984. Studies on the S2 layer of *Pinus sylvestris*. Report 154, Department of Forest Products, Uppsala, Sweden. 34 pp.
- DONALDSON, L., AND A. FRANKLAND. 2004. Ultrastructure of iodine treated wood. *Holzforchung* 58(3):219–225.
- FAHLÉN, J., AND L. SALMÉN. 2002. On the lamellar structure of the tracheid cell wall. *Plant Biology* 4(3):339–345.
- FENDEL, D., AND G. WEGENER. 1989. Wood-chemistry, ultrastructure, reactions. De Gruyter, Berlin, Germany. 613 pp.
- FRATZL, P., I. BURGERT, AND H. S. GUPTA. 2004. On the role of interface polymers for the mechanics of natural polymeric composites. *Physical Chemistry Chemical Physics* 6(24):5575–5579.
- , ———, AND J. KECKES. 2004. Mechanical model for the deformation of the wood cell wall. *Zeitschrift für Metallkunde* 95(7):579–584.
- JUNGNICKL, K. 2006. Personal Communication.
- KECKES, J., I. BURGERT, K. FRÜHMANN, M. MÜLLER, K. KÖLLN, M. HAMILTON, M. BURGHAMMER, S. VON ROTH, S. STANZL-TSCHEGG, AND P. FRATZL. 2003. Cell-wall recovery after irreversible deformation of wood. *Nature Materials* 2:810–812.
- KERR, A. J., AND D. A. I. GORING. 1975. Ultrastructural arrangement of the wood cell wall. *Cellulose Chem. Technol.* 9:563–573.
- KIM, N.-H. 2005. An investigation of mercerization in de-

- cayed oak wood by white rot fungus (*Lentinula edodes*). *J. Wood Science* 51:290–294.
- KÖHLER, L., AND H.-C. SPATZ. 2002. Micromechanics of plant tissues beyond the linear-elastic range. *Planta* 215: 33–40.
- LIESE, W. 1970. Elektronenmikroskopie des Holzes. Pages 109–170 in H. Freund, *Handbuch der Mikroskopie in der Technik*. Umschau-Verlag, Frankfurt, Germany.
- LONIKAR, S. V., N. SHIRAIISHI, AND T. YOKOTA. 1984. Effect of the loosening of the wood texture on the mercerization of cellulose in wood. *J. of Wood Chem. Technol.* 4(4): 483–496.
- MANSIKKAMÄKI, P., M. LAHTINEN, AND K. RISSANEN. 2005. Structural changes of cellulose crystallites induced by mercerisation in different solvent systems; determined by powder X-ray diffraction method. *Cellulose* 12:233–242.
- MATTHECK, C. 1991. *Trees, the mechanical design*. Springer, Berlin, Heidelberg, New York.
- NISHIYAMA, Y., S. KUGA, AND T. OKANO. 2000. Mechanism of mercerization revealed by X-ray diffraction. *J. Wood Science* 46:452–457.
- OKANO, T., AND A. SARKO. 1984. Mercerization of cellulose. I. X-ray diffraction evidence for intermediate structures. *J. Appl. Polym. Sci.* 29:4175–4182.
- REVOL, J.-F., AND D. A. I. GORING. 1981. On the mechanism of the mercerization of cellulose in wood. *J. Appl. Polym. Sci.* 26:1275–1282.
- REYNOLDS, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biology* 17:208.
- RUEL, K. F., AND D. A. I. GORING. 1978. Lamellation in the S2 layer of softwood tracheids as demonstrated by scanning transmission electron microscopy. *Wood Sci. Technol.* 12:287–291.
- SALMÉN, L., AND A. M. OLSSON. 1998. Interaction between hemicelluloses, lignin and cellulose: Structure-property relationships. *J. Pulp Paper Sci.* 24(3):99–103.
- SCHWARZE, F. W. M. R., AND J. ENGELS. 1998. Cavity formation and the exposure of peculiar structures in the secondary wall (S-2) of tracheids and fibres by wood degrading basidiomycetes. *Holzforschung* 52(2):117–123.
- SELL, J. 1989. *Eigenschaften und Kenngrößen von Holzarten*. Baufachverlag AG, Zürich. 80 pp.
- , AND T. ZIMMERMANN. 1993. Radial fibril agglomerations of the S2 on transverse-fracture surfaces of tracheids of tension-loaded spruce and white fir. *Holz Roh-Werkst.* 51:384.
- , AND ———. 1998. The fine structure of the cell wall of hardwoods on transverse- fracture surfaces. *Holz Roh-Werkst.* 56(5):365–366.
- SINGH, A. P., AND G. DANIEL. 2001. The S2 layer in the tracheid walls of *Picea abies* wood: Inhomogeneity in lignin distribution and cell wall microstructure. *Holz-forschung* 55(4):373–378.
- SPURR, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastructure Res.* 26(1–2):31 ff.
- UREMOVIC, A., T. DOKK GLAWISCHNIG, J. SCHUSEIL, B. SAAKE, A. BORCHMANN, A. HERRMANN, AND J. PULS. 1994. Chromatographische Untersuchungen zur quantitativen Bestimmung der Holzzucker. *Holz Roh-Werkst.* 52:347–354.
- ZIMMERMANN, T., V. THOMMEN, P. REIMANN, AND H. J. HUG. 2006. Ultrastructural appearance of embedded and polished wood cell walls as revealed by Atomic Force Microscopy. *J. Structural Biol.* 156:363–369.