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Flavonoid Insertion into Cell Walls Improves Wood Properties

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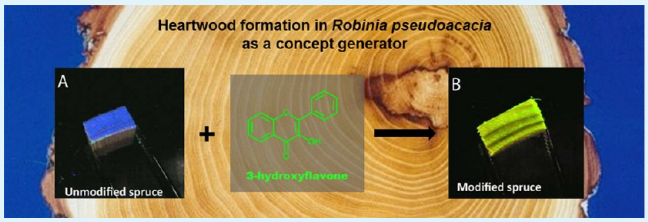
Supporting Information

ABSTRACT: Wood has an excellent mechanical performance, but wider utilization of this renewable resource as an engineering material is limited by unfavorable properties such as low dimensional stability upon moisture changes and a low durability. However, some wood species are known to produce a wood of higher quality by inserting mainly phenolic substances in the already formed cell walls − a process so-called heartwood formation. In the present study, we used the heartwood formation in black locust (*Robinia pseudoacacia*) as a source of bioinspiration and transferred principles of the modification in order to improve spruce wood properties (*Picea abies*) by a chemical treatment with commercially available flavonoids. We were able to effectively insert hydrophobic flavonoids in the cell wall after a tosylation treatment for activation. The chemical treatment reduced the water uptake of the wood cell walls and increased the dimensional stability of the bulk spruce wood. Further analysis of the chemical interaction of the flavonoid with the structural cell wall components revealed the basic principle of this bioinspired modification. Contrary to established modification treatments, which mainly address the hydroxyl groups of the carbohydrates with hydrophilic substances, the hydrophobic flavonoids are effective by a physical bulking in the cell wall most probably stabilized by π–π interactions. A biomimetic transfer of the underlying principle may lead to alternative cell wall modification procedures and improve the performance of wood as an engineering material.



KEYWORDS: *wood cell wall, heartwood formation, chemical modification, Raman spectroscopy, dimensional stability, nanoindentation*

INTRODUCTION

Wood is known for its excellent mechanical properties in view of a low density. However, wood also possesses some intrinsic features such as a low dimensional stability and durability that restrict its wider utilization in common applications.[1,2] The relevant structural unit which strongly determines the property profile of wood is the cell wall of the wood fibers. Wood cell walls are naturally fiber composites consisting of parallel-aligned cellulose fibrils embedded in a matrix made of hemicelluloses and lignin.[3,4] Because of the hygroscopic nature of amorphous cellulose and hemicelluloses wood cell walls shrink or swell upon changes in relative humidity which can lead to substantial deformations of construction elements.[5] Application of unprotected wood in the presence of high moisture almost inevitably results in degradation of cell walls by fungi and in consequence in a low durability.

Accordingly, there is a long history of research activities aiming at producing durable and stable wood timber by cell wall modification. Several reactive chemicals have been used so far such as; anhydrides, carboxylic acids, acid chlorides, isocyanides, epoxides, aldehydes, silicon-containing compounds, etc.[1,2,6,7] These chemical applications are mainly targeting to establish a single covalent bond with the hydroxyl groups of cell wall polymers or cross-links between several hydroxyl groups. The reduced availability of hydroxyl groups increases wood repellency against water and therefore such modifying treatments usually reduce equilibrium moisture content and increase dimensional stability. Besides this mode of action, an alternative treatment is to bulk the cell wall, i.e., to fill microvoids within the cell wall by incorporating materials, which may interact with cell wall components via weak hydrogen bonding or other physical interactions.[1]

Interestingly some tree species invented a process to subsequently modify the cell walls of dead wood fibers after they have been in function in the wood body for a few years. This so-called heartwood formation is a natural process that is driven by a variety of metabolic alterations in parenchyma cells at the sapwood−heartwood transition zone which lead to an insertion of heartwood substances into the cell walls.[8−14] Characteristics of heartwood tissue are determined by greater content of various organic substances classified as extractives,[9,10] thereof a major group are phenolic compounds.[8] Flavonoids belong to this class of phenolic compounds and can considerably affect wood quality and characteristics, such as durability and color in the course of heartwood formation.[9−11,15] A central-European wood species with a heartwood

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of high durability is black locust (*Robinia pseudoacia*) due to the insertion of flavonoids such as dihydrorobinetin, robinetin, etc.[15] Although the biochemical pathways that play a role during formation of flavonoids from storage materials such as starch have been investigated,[10−14] it is still not fully understood how flavonoids are deposited into the cell wall and how they interact with the cell wall components.[11]

In this study, we are using the heartwood formation in black locust as source of bioinspiration in order to improve the property profile of spruce wood (*Picea abies*), an extensively utilized wood species, which possesses a low dimensional stability and durability. By insertion of commercially available flavonoids into the cell walls we aimed at producing "artificial heartwood" with improved dimensional stability and at unraveling the basic principles of chemical and physical polymer interactions in order to derive general principles of bioinspired wood modification processes. A similar wood modification approach has been reported with different molecules such as simple or polycyclic phenolic compounds.[16−19] Using natural hydrophilic extractives (hematoxylin and catechin) Sakai and co-workers found that the dimensional stabilizing effect was hardly improved.[16]

EXPERIMENTAL SECTION

Para toluene sulfonyl chloride, dry pyridine, 3-hydroxy flavone (3-HF), organosolvent lignin, D-xylose, acetone (dried with 3-Å molecular sieves) were bought from Sigma-Aldrich and used as received. Cellulose fibers kindly provided by Borregaard company were used as received. Norway spruce wood samples (*Picea abies*) were cut parallel to grain direction and sawn into blocks of 1.0 × 0.5 × 0.5 cm^3 (tangential x radial x longitudinal).

Tosylation Reaction of Spruce Cell Walls. The Norway spruce (*Picea abies*) sapwood blocks were dried at 60 °C for 1 day and kept under vacuum for 1 more day in a round-bottom flask. Samples were weighed (0,78 g, 4,33 mmol, calculated as a glucopyranose equivalent). 40 mL of dry pyridine was added to the samples in the flask for swelling for 1 day. The flask was stored in an ice bath with the reactants. *p*-Toluenesulfonyl chloride (0,82 g, 4,33 mmol) was added to the solution and reacted for 1 day at 5 °C.

Impregnation of 3-Hydroxyflavone in the Cell Wall. Tosylated spruce wood blocks were washed with dry pyridine for 3 h to get rid of unreacted tosylates. 3-Hydroxyflavone (1,03 g, 4.33 mmol) dissolved in minimum amount of dry acetone (40 mL, dried over 3-Å molecular sieves) was added dropwise to the swollen and tosylated samples. Acetone was evaporated slowly by heating the flask to 45 °C to keep and precipitate the dissolved flavonoid molecules inside the cell wall. This temperature was used in order to avoid wood cell wall damage due to too high temperatures. After evaporation of acetone, samples were washed in a flask with distilled water while stirring to get rid of pyridine and flavonoid molecules that had not been impregnated in the cell walls but precipitated in the lumen.

Tosylation of Cellulose Fibers. Previously dried cellulose fibers (60 °C, 24 h) (0.23 g, 3.64 mmol of active OH-groups) were dispersed in 20 mL of dry pyridine and 2.35 g of *p*-toluene sulfonyl chloride (12.33 mmol, 3 equiv.) was added. The reaction mixture was stirred overnight at 5 °C. The reaction was stopped by the addition of an acetone/H_2O mixture (20 mL, 1:1), and the product was washed thoroughly with distilled water. Raman spectroscopy was used for characterization and band assignments of tosylated fibers (Figure 4A).

Tosylation of Lignin. Organosolvent lignin (0,5 g, 2,78 mmol − calculated as a coniferyl alcohol equivalent) was dispersed in 40 mL of dry pyridine and 1.06 g p-toluene sulfonic acid chloride (5,56 mmol, 2 equiv) was added. The reaction mixture was stirred overnight at 5 °C, stopped with 40 mL and washed with distilled water. ATR FT-IR spectroscopy was used for characterization and band assignments of tosylated lignin (Figure 4B).

Tosylation of D-Xylopyranoside. D-Xylose was methylated anomericly and tosylated as reported elsewhere.[20] Methyl β-D-xylopyranoside was prepared by refluxing D-xylose in anhydrous methanol, in the presence of acidic ion-exchange resin. The pure β-anomer was obtained by recrystallization in ethanol. Tosylation of the secondary hydroxyl groups in pyridine at 5 °C give the tritosylate. Raman spectrometry was used for characterization (Figure 4C).

Reaction of 3-HF and Individual Tosylated Cell Wall Polymers. Tosylated cellulose fibers, organosolvent lignin and D-xylopyranoside were reacted with 3-HF (1:1 ratio) in dry acetone at 45 °C in separate flasks. Isolated reaction products were characterized by Raman and ATR FTIR spectroscopies (Figure 4A-C).

Raman Spectroscopy and Imaging. For sample preparation (reference, tosylated, and modified spruce wood blocks), 40 μm thick slices were cut on a rotary microtome (LEICA RM2255, Germany) and kept between microscope slide and coverslip with a drop of water to maintain cell walls in wet condition. The modified spruce cell wall assembly was analyzed using confocal Raman microscopy. Spectra were acquired with a confocal Raman microscope (alpha300, WITec GmbH, Ulm, Germany) equipped with an objective (60X, NA = 0.8, 0.17 mm coverslip correction from Nikon Instruments, Amstelveen, The Netherlands). A 532 nm laser with k = 532 nm (Crysta Laser, Reno, NV, USA) was focused with a diffraction limited spot size of 0.61 λ/NA and the Raman light detected with an air cooled back-illuminated CCD (DV401-BV, Andor, Belfast, North Ireland) behind a spectrograph (UHTS 300, WITec) with a spectral resolution of 1 cm^{-1}. For mapping, an integration time of 0.2 s was chosen and every pixel corresponded to one scan and a spectrum (scan) acquired every 0.5 μm. Two-dimensional chemical images were measured by WitecProject software using the "basis analysis function". The algorithm of basis function fits each spectrum of the multispectral data set with a linear combination of the basis single spectra (four reference spectra; cellulose fibers, lignin, 3-hydroxyflavone, tosyl chloride) using the least-squares method. To solve the problem of variations in fluorescence background in various parts of the sample, the first derivative of both multispectral data set and basis single spectra was calculated and the distribution of various components (cellulose fibers, lignin, flavonoids, tosylated components) obtained from Raman spectral features were plotted.

Attenuated Total Reflection Fourier-Transform Infrared Spectroscopy (ATR FT-IR). To record attenuated total reflection (ATR) FT-IR spectra a FT-IR microscope (Hyperion 2000, Bruker Optics, Germany) equipped with a liquid nitrogen cooled MCT detector and connected to a Vertex 70 FT-IR spectrometer (Bruker GmbH, Germany) was used. The IR beam was focused to the sample surface through an ATR objective (Bruker Optics, Germany) characterized by the Ge internal reflection element with tip of 100 μm.

Thermal Analysis. Differential scanning calorimetry (DSC) was carried out on 10 mg samples using a Setaram TG/DSC Sensys Evo thermobalance equipment in the range 20−600 °C, with the temperature being increased at a rate of 5 °C/min.

XRD Analysis. The powder XRD patterns of all samples were measured in reflection mode (CuKα radiation) on a Bruker D8 diffractometer equipped with a scintillation counter.

Equilibrium Moisture Content (EMC). The modified and reference samples were equilibrated in a sealed system with a relative humidity (~80%) obtained with saturated solution of NaCl at room temperature. For the reference and each treatment 5 samples were measured. Weight equilibrium of the samples was recorded by weighing samples until constant weight. Afterward samples were oven-dried at 103 ± 2 °C and reweighed to determine final moisture content.

Cell Wall Shrinkage. Microtomed slices with a 40 μm thickness were observed under a Leica fluorescence microscope and photographed at wet (swollen) and dried (at room temperature for 1 day) conditions. Mean shrinkage of tangential cell walls was calculated by measuring radial dimensions of the same 40 cell walls (see Figure S2 in the Supporting Information).

Nanoindentation. The mechanical characterization of tracheid cell walls was carried out on a Dimension DI-3100 atomic force

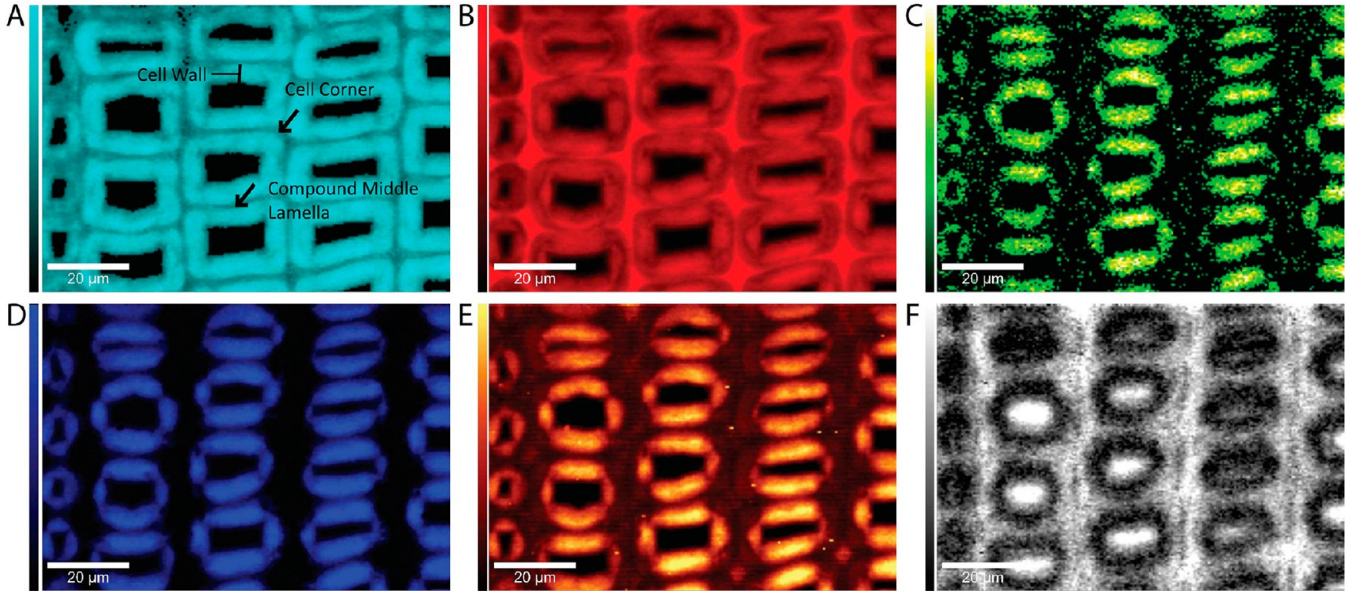

Figure 1. Confocal Raman microscopic images ($100 \times 70\ \mu m^2$) of of latewood cell wall tissue. (A–D) Plotted by fitting multispectral Raman data set with reference spectra of (A) cellulose fibers, (B) lignin, (C) flavonoid, and (D) tosyl chloride. (E) Basis analysis error indicating parts of the sample where the set of reference spectra was unsuccessful to fit original data set. (F) Water distribution (calculated by integrating from 3135 to 3735 cm^{-1} which is specific for hydroxyl stretching mode).

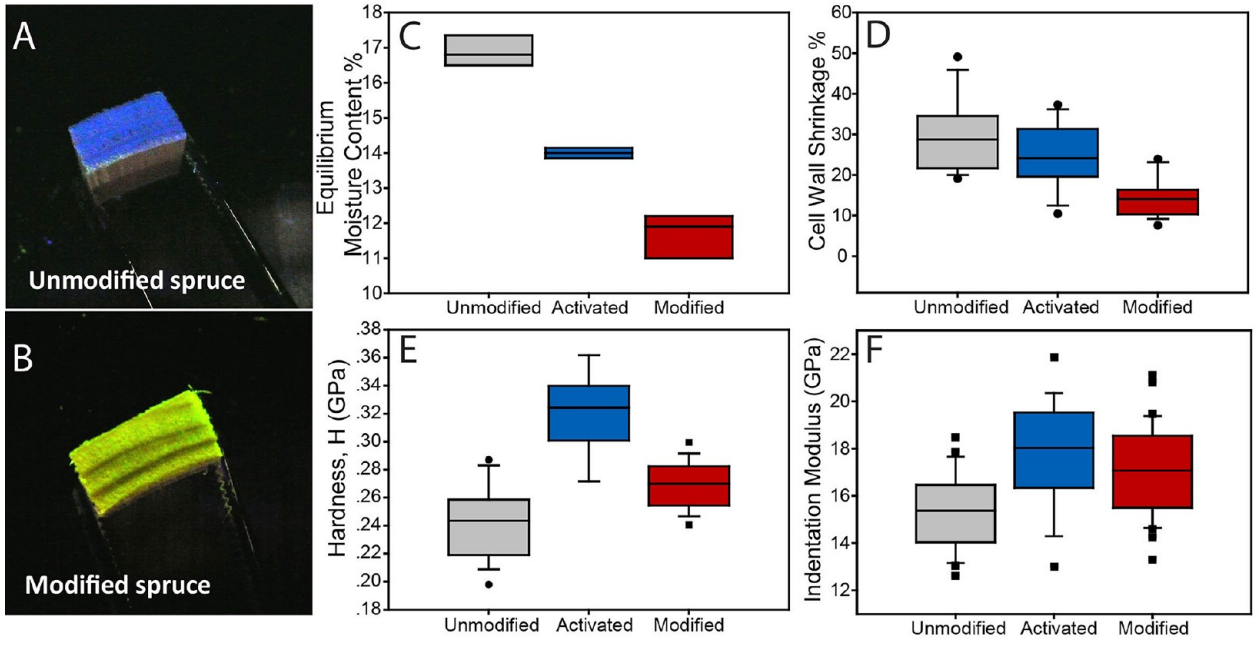

Figure 2. Physical properties of cell walls. (A, B) Unmodified and modified spruce blocks under 254 nm UV light. (C) EMC of unmodified, activated (tosylated), and modified samples. (D) Cell wall shrinkage of unmodified, activated (tosylated), and modified samples. (E , F) Hardness and indentation modulus of unmodified, activated (tosylated), and modified cell walls.

microscope (Digital Instruments, Veeco Metrology Group, Santa Barbara, CA) equipped with a Hysitron add-on force transducer for nanoindentation (Surface, Hueckelhoven, Germany). For this purpose, samples were dried overnight in an oven at 60 °C and subsequently immersed in AGAR resin (AGAR low viscosity resin kit, AGAR Scientific Ltd., Stansted, UK) to enable the resin to penetrate the wood samples. The impregnated specimens were glued onto metal discs (15 mm AFM specimen discs). Specimens impregnated with the embedding resin cured overnight in an oven at 60 °C. Then the samples surface was smoothed by polishing. Quasi-static indentation tests were performed in a force-controlled mode, the indenter tip (Berkovich-type triangular pyramid, radius of curvature ~150 nm) was loaded to a peak force of 250 μN at a loading rate of 100 μN/s, held at constant load for 15 s, and unloaded at a rate of 100 μN/s.[21] Middle regions of secondary cell walls of reference, activated (tosylated), and modified samples were chosen for nanoindentation (see Figure S3 in the Supporting Information). A minimum of 20 indents were made on a group of cell walls.

RESULTS AND DISCUSSION

Flavonoid Loading of Spruce Cell Walls. Small spruce wood samples were first activated by tosylation and then impregnated by 3-hydroxyflavone (3-HF) (see methods part).

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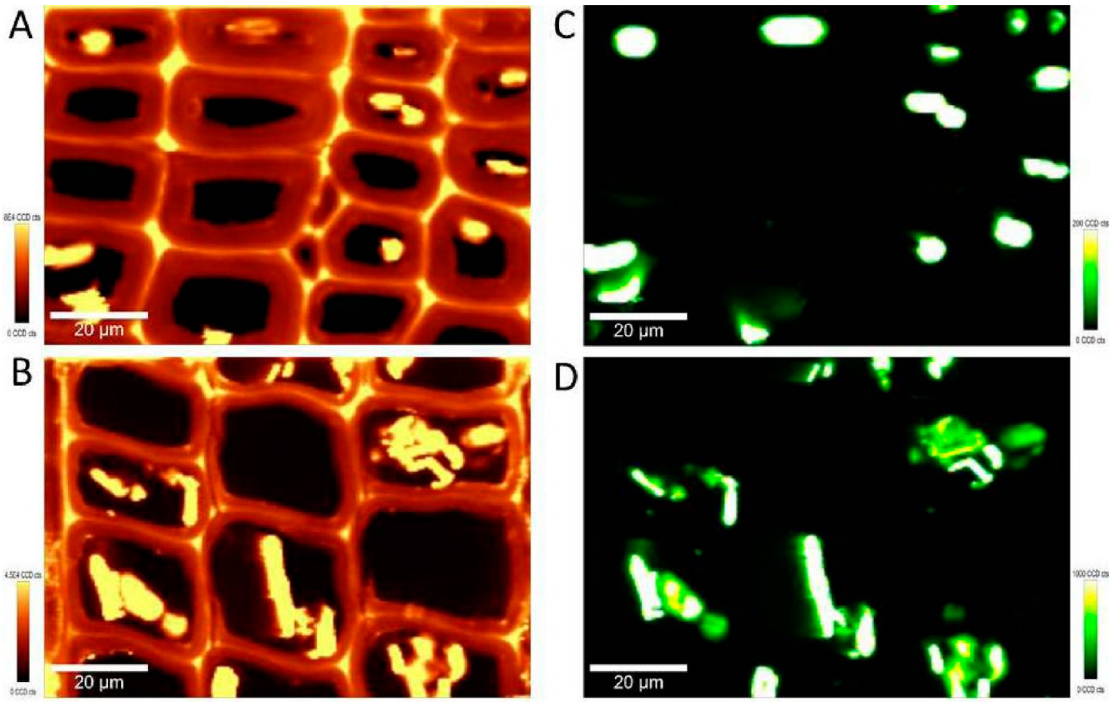

Figure 3. Confocal Raman microscopic images of impregnation of 3-hydroxy flavone in the cavities of nonactivated cell walls. (A, B) Raman images ($100 \times 70\ \mu m^2$) of latewood and earlywood cell wall regions that were calculated by integrating from 200 to 3600 cm^{-1}. (C, D) Raman images ($100 \times 70\ \mu m^2$) of flavonoid distribution after water leaching of cell walls that were calculated by integrating of flavonoid band between 665 and 685 cm^{-1} from the multispectral data set.

To gain detailed understanding of possible changes in the chemical structure after modification of spruce wood blocks, we applied confocal Raman imaging[22] on cross-sections of cell walls.

Images A and B in Figure 1 highlight the distribution of cellulose and lignin in the cell wall and further show that the chemical treatments did not result in cell wall degradation. Apart from the fiber-to-fiber interfaces, a homogeneous distribution of cellulose can be found (Figure 1A). In Figure 1B, cell corners (CC) and compound middle lamella (CML) are visible in brighter red color representing highly lignified regions.[23]

The distribution of 3-hydroxyflavone impregnated into the cell walls is shown in the Figure 1C. Clearly this process takes place from the lumen side of the cells because the inner cell wall region shows the major 3-hydroxyflavone loading. Careful analysis of Raman spectra indicates no reaction or impregnation in CC and CML regions. Interestingly impregnated flavonoid molecules were found exactly in the same region of tosylated tissue (Figure 1D).

Figure 1E shows the error from the basis analysis of the fitting procedure. Normally the basis analysis error should be almost constant for all analyzed regions. However, in this case, remarkable errors can be found in the impregnated regions suggesting spectral shifts due to chemical bonding and interactions in treated samples in comparison to reference spectra. Figure 1F shows a water distribution image which was extracted by integrating the hydroxyl stretching band (from 3135 to 3735 cm^{-1}). The darker region in the image is the modified region which coincides with the modified regions in Figure 1C. The image displays intensity difference in hydroxyl stretching band between modified and unmodified regions in the same cell wall. Comparison of average spectra proved that the intensity of the water band decreases in modified regions (see Figure S1 in the Supporting Information).

Improved Wood Performance. To quantify changes in the wood property profile due to the insertion of flavonoids, we conducted a variety of tests on the bulk material (Figure 2). The equilibrium moisture content (EMC) at 20 °C/80% relative humidity of flavonoid impregnated samples was around 11.7 ± 0.6% and of activated (tosylated) samples around 14.0 ± 0.2%, whereas the unmodified (reference) samples had an EMC of 16.9 ± 0.4%. Hence, the equilibrium moisture content of the modified spruce wood was only about 70% of the unmodified wood, whereas for simple activation (tosylation) it was about 83% of the reference (Figure 2C).

The anisotropic shrinkage of wood cell walls is the result of the cell wall contraction with the removal of water from the cell wall cavities. In our study we measured the tangential cell walls of the cross sections of wood fibers in unmodified, activated and modified cell walls before (wet) and after drying (dry) (see Figure S2 in the Supporting Information). In all cases, cell walls shrank by drying (Figure 2D). However, although shrinkage of the unmodified and activated cell walls was about 28 and 25% respectively, the shrinkage of the modified cell walls was found to be around 15%, which represents a significant improvement in dimensional stability (shrinkage values decreased of about 45% between untreated wood and modified wood).

Hardness and indentation modulus of reference, activated and modified cell walls were determined by nanoindentation (Figure 2E, F). Both, hardness and indentation modulus showed a significant ($P < 0.001$, U-test) increase of about 10% from unmodified cell walls to flavonoid loaded cell walls, which indicates an improvement of mechanical properties due to flavonoid insertion. Interestingly, the tosylated (activated) samples show the highest hardness and indentation modulus.

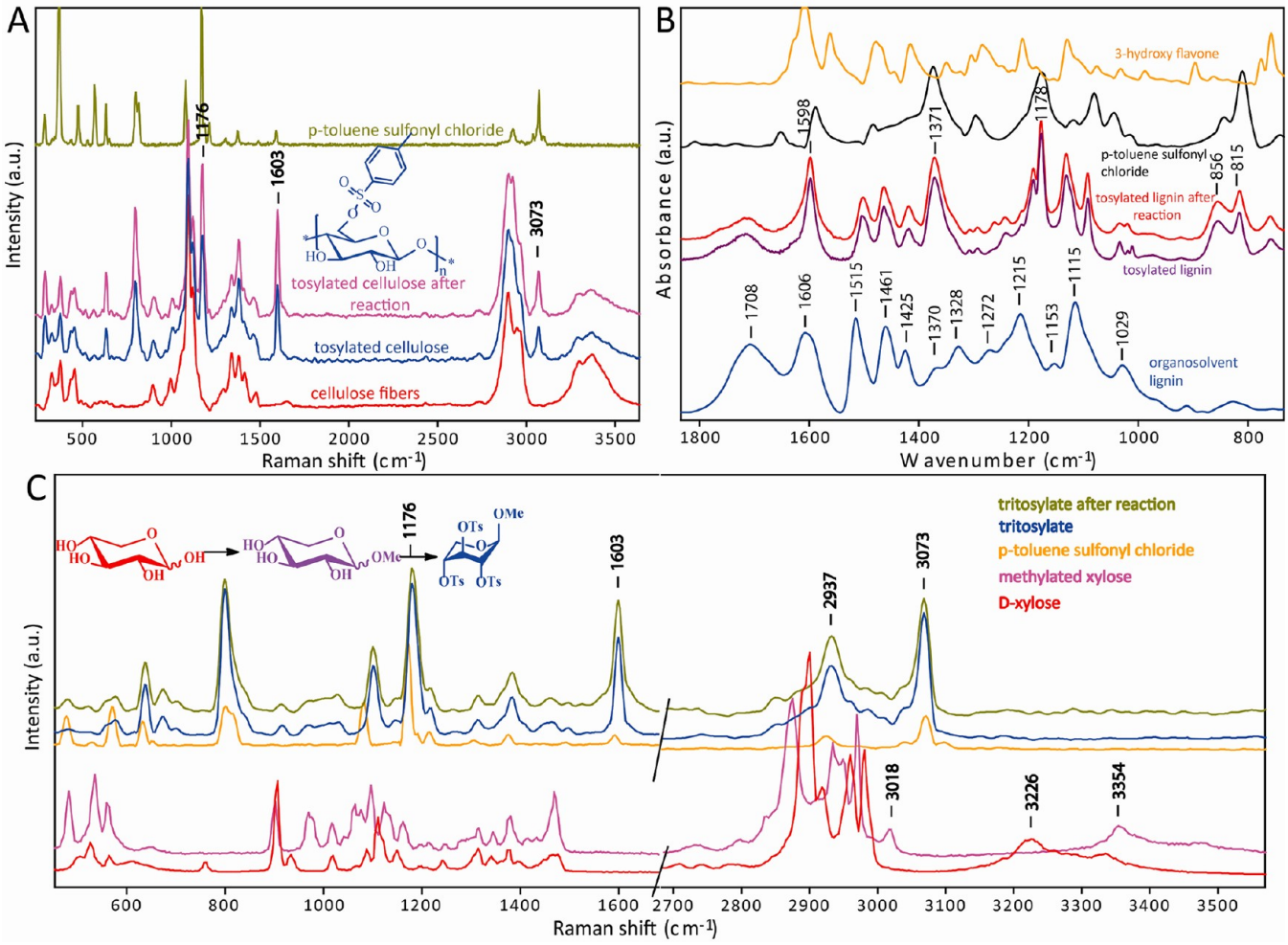

Figure 4. (A) Raman spectrum of cellulose fibers, tosylated cellulose fibers, tosylated cellulose fibers after 3-HF reaction and tosyl chloride. (B) ATR-FTIR spectrum of 3-hydroxy flavone, tosyl chloride, tosylated lignin after reaction, tosylated lignin, organosolv. lignin. (C) Raman spectrum of D-xylose, methylated xylose, tosyl chloride, tosylated methyl xylose, and tosylated methyl xylose after reaction.

The weight percentage gain (calculation based on the weight difference between untreated and modified wood samples) is a good indicator of an efficient impregnation (i.e., chemical bonding or interaction between the introduced chemical and wood cell wall polymers).[1] Activation by reaction of tosyl chloride with cell wall components increased weight of the samples by 19%. Subsequent flavonol impregnation increased the total weight percentage gain (WPG) to 23%. The flavonoids alone are therefore accountable for a 4% weight increase (data not shown). This nonleachable weight gain was interpreted as the result of a stable interaction between cell wall polymers and flavonoids.

Polymer Interactions in the Cell Wall. Raman spectroscopy demonstrated the ability of 3-HF to interact both with aromatic groups from lignin and tosyl groups inserted into the cell wall. The necessity of the activation reaction with tosyl chloride becomes obvious when comparing the results obtained on activated cell wall, with the impregnation of 3-HF without activation (Figure 3A–D). Interestingly, although 3-HF enters the cell wall of nonactivated samples (i.e., reference wood) using scrupulously identical conditions, the flavonoids content loaded in the cell wall is lower, and the molecules are more easily washed away (with most solvents, including water, Figure 3C, D), failing to establish any durable interaction with the cell walls.

To derive the basic principle of the modification process it was essential to understand how flavonoids interact with wood cell wall polymers and affect wood properties. As shown by Raman imaging (Figure 1C, F), there is a clear colocalization of tosyl groups and 3-HF in the secondary cell wall (S2). Although these results may immediately suggest a covalent bonding of 3-HF to cell wall polymers via a nucleophilic substitution reaction with the tosyl group, the evidence of an ether linkage formation is not trivial.

Indeed, vibrational spectroscopy of lignocellulosic materials yields spectra characterized by many overlapping signals from the main cell wall constituents (lignin, cellulose, and hemicellulose).[24] Therefore, we checked whether 3-HF can form ether bonds with individual cell wall polymers activated by tosylation. After reactions, Raman and IR spectra of modified polymers (cellulose, organosolv. lignin, and xylose (hemicelluloses)) clearly show the absence of characteristic 3-HF peaks (Figure 4).

The Raman spectrum of tosylated cellulose shows asymmetric stretching of sulfonyl group (ν_{as} SO_2) at 1372 cm^{-1}, and symmetric stretching of sulfonyl group (ν_s SO_2) at 1176 cm^{-1} (Figure 4A).[25] The C–H stretching band of aromatic ring at 3073 cm^{-1} also belongs to tosyl group covalently bonded to the cellulose. Same bands can be observed in the Raman spectrum of tritosylated xylose with the fingerprints of xylose at 800–

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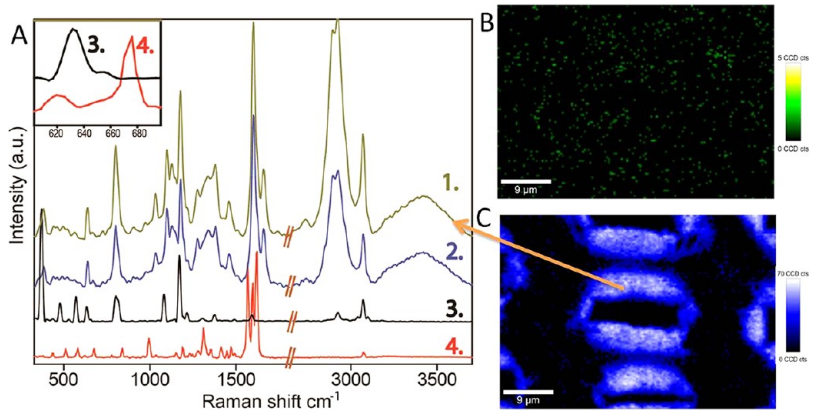

Figure 5. Raman spectroscopy and imaging measurements after acetone leaching. (A) Spectral band from 300 to 3600 cm^{-1} of acetone leached flavonoid inserted (modified) cell walls (1); tosylated cell walls (2); tosyl chloride (3); and 3-hydroxy flavone (4). (B) Raman image (50 × 35 μm^2) of 3-HF distribution in secondary cell walls after acetone leaching (calculated by integration of flavonoid band, 665−685 cm^{-1}). (C) Raman image (50 × 35 μm^2) of tosyl group distribution in secondary cell walls after acetone leaching (calculated by integration of tosyl band, 795−810 cm^{-1}).

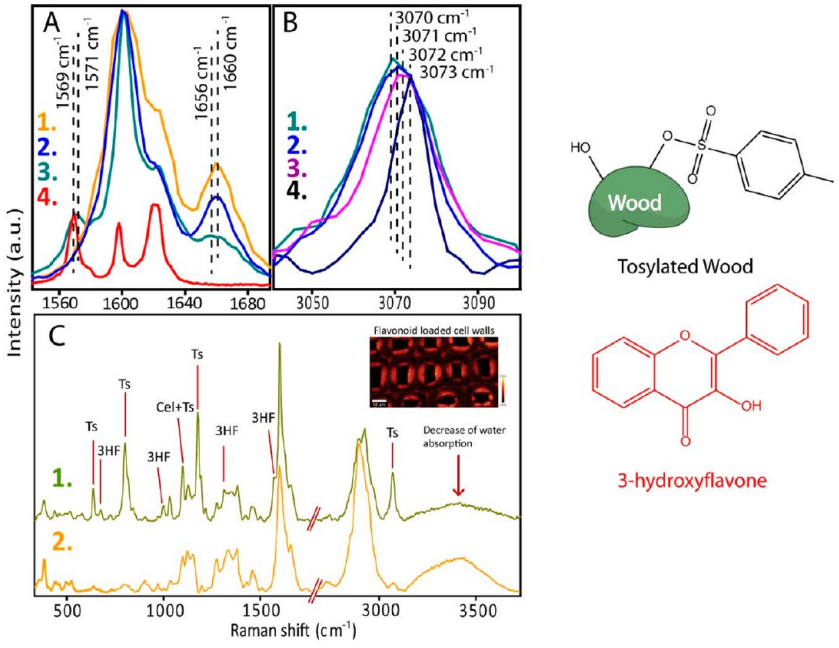

Figure 6. Chemical characterization of cell walls with Raman spectroscopy. (A) Spectral band from 1500 to 1730 cm^{-1} (aromatic region) of (1) untreated cell walls, (2) activated (tosylated) cell walls, (3) modified cell walls, (4) 3-hydroxy flavone. (B) Spectral band from 3000 to 3100 cm^{-1} (aromatic C−H stretching of tosyl group[26]) of (1) modified region of cell walls, (2) tosylated cell walls, (3) tosylated cellulose fibers, (4) tosyl chloride. (C) Raman spectrum of (1) modified region, (2) untreated cell walls. Schematic drawings on the right sight of the spectra show binding of the tosyl chloride to the cell wall hydroxyl groups and structural drawing of 3-hydroxyflavone.

1500 cm^{-1} region (Figure 4C). Tosylated lignin which has additional IR bands from tosyl group can clearly identified at (ν_{as} SO_2) 1372 cm^{-1}, (ν_s SO_2) 1178 cm^{-1}, 856−815 cm^{-1} C−H_{arom} symmetric and asymmetric bending (Figure 4B).[26]

Further, leaching tests performed on modified cell walls showed that 3-HF is easily washed out of the cell wall region (See Figure 5) using aprotic solvents (which are good solvents for 3-HF, such as acetone or acetonitrile), whereas it is hardly removed when using highly polar protic solvents such as methanol or water as shown in Figure 1C. This can be seen in Figure 5A where spectrum 1 belongs to acetone leached cell walls, whereas spectrum 2 represents the tosylated cell walls. In spectrum 1 Raman bands of tosyl groups can be found without any intensity decrease (compare to tosyl chloride (spectrum 3)), but no Raman band being characteristic for 3-HF can be found in the spectrum (compare to 3-hydroxy flavone (spectrum 4)). Raman spectra shown in this figure confirm that leached wood has a similar Raman signature when compared to activated wood. These results confirm that tosylation is unaffected by leaching, and that 3-HF is not covalently attached to wood (it is easily washed out of wood using acetone as solvent). This finding is also supported by Raman images B and C in Figure 5.

These results indicate the absence of covalent bonds between the cell wall polymers and 3-HF which could be a consequence of the mild reaction conditions which were chosen to avoid cell wall polymer destruction and cell wall deformation. This suggests that flavonoids most likely interact with aromatic groups present in the cell wall, i.e. lignin or tosyl groups linked to cell wall polymers. Hence the concomitant presence of

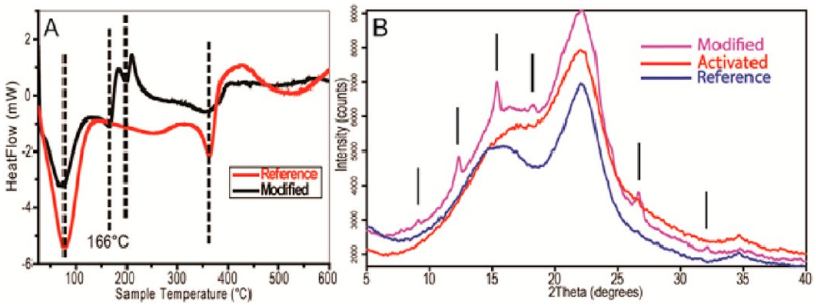

Figure 7. (A) Differential scanning calorimetry analysis data of modified and reference spruce samples. (B) X-ray powder diffraction profiles: modified, activated, and reference samples.

flavonoid molecules and tosyl groups in the S2 region (Figure 1C, D) has to be based on other types of interactions. Previous studies on the crystal structure of 3-HF demonstrated the presence of intramolecular H-bonding between the hydroxyl group and the ketone, and intermolecular interactions including stacking of conjugated aromatic rings, and H-bonding in between neighboring stacks.[27,28] Given the ability of 3-HF to enter the cell wall, and to form intra- and intermolecular associations via weak hydrogen bonding and π-stacking, the occurrence of such interactions with lignin polymers and tosylated cell wall polymers in the treated spruce samples is highly probable.

Evidence of π-stacking can be found in Raman spectra frequency shifts of the aromatic systems which were observed in the modified cell wall (Figure 6A). The Raman spectra provide information on aromatic vibration bands of 3-HF, and lignin (spectral region from 1500 to 1700 cm^{-1}, Figure 6A). Indeed, the most intense and not-overlapped Raman band of 3-HF, which belongs to $C_2{=}C_3$ and C=O conjugated stretching vibrations,[29] can be observed at 1569 cm^{-1}. Upon impregnation of 3-HF in the activated cell wall, we observed a slight blue shift of the flavonol band (Figure 6A, Spectra 3 and 4, 1569 to 1571 cm^{-1}), whereas the lignin aromatic bands red-shifted from 1660 to 1656 cm^{-1} (Figure 6A, spectra 1 and 3).

Considering a possible interaction between lignin and 3-HF conjugated systems, such an opposite frequency shift is characteristic of a donor−acceptor interaction[30] and is therefore supporting the existence of weak bonding via $\pi-\pi$ stacking. It is interesting to note that the lignin band is solely affected by the flavonol addition (with weak interactions), and not by the reaction with tosyl chloride. The effects of tosylation can be observed in the higher frequency spectral region where the band assigned to the aromatic C−H stretching of tosyl groups can be found (Figure 6A).[26] There is a clear red shift after reaction with the cell wall and addition of 3-HF (Figure 6B, spectra 1, 2, and 3). In the case of tosyl groups, characterized by the presence of both donor and acceptor substituents on the aromatic ring, the type of intermolecular interactions is more difficult to discern. The Raman shift observed may be caused by a CH or OH...π interaction or $\pi-\pi$ interactions with 3-HF.

Another important aspect of the assembly and interaction of cell wall constituents in the modified wood is whether 3-HF can built crystalline domains in the cell walls. This issue was addressed by differential scanning calorimetry and X-ray powder diffraction (Figure 7).

In case of modified cell wall, DSC revealed an endotherm at 166 °C (Figure 7A), corresponding to the melting of 3-HF. Therefore, it appears that the flavonol crystallized within small domains in the cell wall. This is confirmed by X-ray powder diffraction analysis as diffraction peaks of relevant crystalline 3-HF[31] can be observed in the modified sample (Figure 7B). Although 3-HF is known to form intermolecular interactions quite easily, to the point of forming polymerlike structures,[24] it is surprising to observe crystallization in such confined domains.

In line with our results, we propose that the aromatic groups provided by activation with tosyl chloride acts as a nucleation center for the growth of 3-HF crystals within the cell wall. In the case of nonactivated wood, the possibilities of weak interactions of such nature are present (with lignin), but considerably reduced, and explain the inability of 3-HF to stay in the cell wall in a durable manner.

In contrast to prior studies,[16,17] we used a rather hydrophobic flavonol in our study. In the cited studies, flavonoid molecules comprise up to five hydroxyl groups, explaining the easier impregnation of the cell wall. As shown here, loading of 3-HF in nonactivated cell wall was low, but tosylation (i.e., modification of OH groups) renders the cell wall slightly more hydrophobic, hence favoring the initial insertion of 3-HF into activated cell walls. Hence, inserting hydrophobic molecules into wood cell walls following a tosylation pretreatment provides an alternative and well-suited way to significantly reduce the water uptake of cell walls and improve the dimensional stability of wood.

CONCLUSIONS

We established a new method to facilitate cell wall insertion of hydrophobic polycyclic compounds via a pretreatment with tosyl chloride. The lesson to be learnt from nature is that it could be beneficial to impregnate wood cell walls with hydrophobic substances rather using treatments with hydrophilic flavonol compounds. The combined effects of hydrophobicity increase, and improved accessibility of aromatic moieties provided by the tosylation, contribute to a significant enhancement of the insertion and stability of flavonoids in the cell wall. Hence by overcoming the challenge of establishing the hydrophobic substances in an essentially hydrophilic environment of the cell wall, we were able to improve the dimensional stability of wood considerably at the expenses of a relatively small amount of the modifying flavonoid.

ASSOCIATED CONTENT

Supporting Information

Raman bands of cell wall components and 3-HF. Additional Raman images and spectra. Light microscopic images of wet and dry cell walls. AFM gradient images of nanoindented cell

walls. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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