Xylan Localization on Pulp and Viscose Fiber Surfaces

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A method for the immunological visualization of plant polysaccharides in native plant tissues was adopted for the histological investigation of xylan on kraft pulp and xylan-enriched viscose fibers. The method consisted of the selective labeling of xylan structures through the binding of specific monoclonal primary antibodies and fluorescein isothiocyanate (FITC)-carrying secondary antibodies. This indirect immunolabeling method was adapted for pulp and viscose fibers through the blockage of unspecific binding sites with bovine serum albumin (BSA), which allowed a selective localization of xylan. The combination of this technique with high resolution confocal laser scanning microscopy (CLSM) rendered a parallel detection of morphological changes of pulp fibers alongside various processing steps possible. Within this study, kraft pulps from birch, beech, and eucalyptus were investigated throughout a purification process that enabled an upgrade from paper pulps to dissolving pulps by caustic hemicellulose extraction and xylan-enriched viscose fibers. The method demonstrated its potential for gaining novel insights into pulp purification, as well as fiber modification through the application of an immunolabeling method.

Keywords: Confocal scanning immunofluorescence microscopy; Hemicellulose extraction; Kraft pulp fiber; Scanning electron microscopy; Xylan antibody labeling; Viscose; Rayon

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INTRODUCTION

Dissolving pulps mainly consist of wood-derived cellulose prepared by the acidic sulfite or alkaline prehydrolysis kraft (PHK) cooking processes. The production of these pulps is characterized by low process yields due to cellulose degradation reactions under acidic conditions. A process concept that could benefit from higher yields is "modified kraft cooking" (Sixta 2007; Gehmayr *et al.* 2011). This concept requires an alkaline pulp treatment step for removing hemicellulose, residual lignin, and extractives, followed by totally chlorine-free (TCF) bleaching sequences for further reducing residual lignin content and adjusting the degree of polymerization (DP) of cellulose macromolecules. The near quantitative removal of hemicellulose is desired, especially for hardwood xylan, as it is known to interfere with viscose processing (Treiber 1983).

To evaluate the extraction efficiency of each process step, the xylan content of the pulp must be monitored. A suitable strategy includes the quantification of sugar monomers released by total hydrolysis followed by HPLC detection (Wright and Wallis 1996). This information describes the average concentration but gives no picture of the distribution of hemicellulose at the outer surface.

The appearance of xylan in native hardwood cells is not homogeneous across the cell wall cross-section. Concentration averages among fiber cross-sections were determined by Sjöberg *et al.* (2005), who applied enzymatic peeling. For studying the macroscopic distribution of xylan in pulps, depending on the structural and morphological changes in pulp production, advanced microscopic techniques are needed to complement chemical analysis.

To produce regenerated fibers with new interesting properties, xylan could be used as an additive during viscose preparations (Schild and Liftinger 2014). This strategy would additionally result in an increase in the economic value of the xylan and high yields in viscose manufacturing. The adsorption of xylan onto paper pulps has been successfully implemented, and it was shown to improve yield (Köhnke and Gatenholm 2007; Ramirez *et al.* 2008; Silva *et al.* 2011) and strength properties of the resulting paper products (Westbye *et al.* 2006; Saake *et al.* 2008). The sorption kinetics of xylan in alkaline media have been extensively studied (Most 1957; Yllner and Enström 1957; Meller 1965; Hansson 1970), though Coulomb interactions were found to be the driving force (Ribe *et al.* 2010a, 2010b). The fiber surface has an important impact on the difference in xylan uptake between pulp and regenerated fibers. To use xylan as a blend for viscose fibers and to avoid its degradation, it was added during the dissolving step right after alkalization and xanthation in viscose preparation (Schild and Liftinger 2014). Field emission scanning electron microscopy (FE-SEM) pictures of the corresponding fibers revealed a smooth surface, indicating a good blending of both xylan and cellulose. To investigate the xylan distribution over the fiber cross-section, enzymatic peeling according to Sjöberg *et al.* (2005) was applied. To clarify the macroscopic distribution of xylan on the regenerated fibers, advanced microscopic techniques are required.

For the specific visualization of plant polysaccharide structures, indirect labeling with monoclonal primary antibodies in combination with secondary antibodies that carry a reporter molecule is a feasible method. The probe molecules are, in most cases, represented by a variety of fluorescent dyes, which can be visualized through fluorescence microscopic techniques.

Secondary antibodies are also available with bound colloidal gold, which can be localized by transmission electron microscopy (TEM). Several studies have focused on the immunofluorescence labeling of hemicellulosic and pectic structures in intact as well as chemically and enzymatically-treated plant cell systems (Sugimoto *et al.* 2000; McCartney 2005; Altaner *et al.* 2007; Talbot *et al.* 2007; Arend 2008; Kim *et al.* 2010; Marcus 2010; Kim and Daniel 2012; Wang *et al.* 2012; Fu 2014; Hell *et al.* 2015; Sandquist *et al.* 2015). Hemicellulose localization has also been accomplished using immuno-gold electron microscopy for intact cell systems (Baba *et al.* 1994; Awano *et al.* 2000; Kim *et al.* 2010; Kim and Daniel 2012) and for kraft pulps (Duchesne *et al.* 2003).

The aim of this study was to adapt immunofluorescence microscopy to visualize xylan structures on kraft pulp and xylan-enriched viscose fibers by bypassing the problem of unspecific binding to cellulose. The method was further used to visualize xylan on fibers under various pulp processing conditions. Pulp samples from three different hardwood species (birch, beech, and eucalyptus) were investigated by confocal microscopy after cooking, alkaline post-extraction, and bleaching. Xylan-enriched viscose fibers were also investigated by confocal microscopy.

EXPERIMENTAL

Materials

Starting pulps

An industrially processed oxygen-delignified never-dried birch (*Betula papyrifera*) kraft pulp was studied (supplied by a collaboration partner). Additionally, a never-dried oxygen-bleached eucalyptus (*Eucalyptus globulus*) kraft pulp was supplied by ENCE, Huelva, Spain. The oxygen-delignified beech (*Fagus sylvatica*) kraft pulp (Lenzing AG, Lenzing, Austria) was prepared using a pilot scale digester applying a continuous batch cooking (CBC) protocol. All pulps were never-dried before microscopy. The parameters of the corresponding pulps are illustrated in Table 1 (Results and Discussion section).

Pulp preparation

Oxygen-delignified kraft paper pulps were treated with alkaline solutions according to the method used by Wallis and Wearne (1990) for 30 min. To simulate industrial pulp upgrading processes, white liquor was used as an alkali source, because it is the predominant alkali source used in kraft pulp production sites. The synthetic white liquor used consisted of sodium hydroxide, sodium sulfide, and sodium carbonate, reflecting a typical white liquor composition with 30% sulfidity and a causticizing degree of 90%. Extraction temperatures varied between 20 °C and 80 °C and effective alkalinities (EA) between 40 g L^{-1} and 120 g L^{-1}, representing feasible variation in large-scale operations. Pulps were separated from the hemicellulose-containing lyes by filtration through a glass frit. Their parameters are displayed in Table 2 (Results and Discussion section).

Further pulp processing was completed with totally chlorine-free (TCF) pulp bleaching. This was done by an ozone treatment followed by hydrogen peroxide bleaching. Ozone bleaching was conducted in a medium-consistency high-shear mixer at 50 °C for 10 s at pH 2.5 and 10% volumetric consistency. Subsequent peroxide bleaching was performed by applying 6 kg sodium hydroxide (NaOH) t^{-1}odp, 5 kg hydrogen peroxide (H_2O_2) t^{-1}odp, and 1 kg magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$) t^{-1} odp at 70 °C for 120 min at 10% consistency.

Preparation of xylan-enriched viscose fibers

Xylan-enriched viscose fibers were produced from a beech sulfite dissolving pulp according to the method described by Schild and Liftinger (2014). The resulting fibers contained 10.4% xylan.

Methods

The Kappa number was determined according to TAPPI T 236 cm-85(1993a), the brightness according to ISO 2470-1 (2009), and the intrinsic viscosity [η] according to SCAN-C 15:99 (1999). The carbohydrate content of pulps and xylan-enriched viscose fibers was measured after a two-stage total hydrolysis by high performance anion exchange chromatography with pulsed amperometric detection according to the method described by Wright and Wallis (1996). The water retention value (WRV) was measured according to the method described by Zellcheming Merkblatt IV/33/57 (1957). The specific surface area (BET surface area) determination was conducted with a Belsorp mini II (MicrotracBEL Corp., Osaka, Japan) using N_2 gas according to the method

described by Brunauer *et al.* (1938). The carboxyl group content (COOH) was determined according to the method described by Philipp *et al.* (1965). The degree of crystallinity (CrI) and the cellulose II content (Cell II) were determined through Fourier transform Raman spectroscopy (FT-Raman) (Bruker, Billerica, USA) according to the method described by Röder *et al.* (2006). The molar mass distribution of pulps was measured by size exclusion chromatography (SEC) with multi-angle light scattering detection (MALLS) (Wyatt Corp., Santa Barbara, USA) in a LiCl/DMAc (lithium chloride/dimethylacetamide) solution according to the method described by Schelosky *et al.* (1999).

Scanning electron microscopy

For field emission scanning electron microscopy (FE-SEM), samples were atmospherically dried at room temperature, put on the object holder, and sputtered with a thin layer of Au/Pd. The samples were examined by high-resolution scanning electron microscopy up to a magnification of 10.000× with a Tescan Vega 2 LMU using an acceleration voltage of 5 kV and a working distance of 10 mm (Tescan, Kohoutovice, Czech Republic). An Everhart Thornley secondary electron detector was used.

Immunofluorescence microscopy-labeling

Prior to immunolocalization preparations, pulps and xylan-enriched viscose fibers were washed with a sodium acetate buffer with pH 7.0. Fibers were decollated on the slide and washed 5 times with 0.1 M phosphate buffered saline (PBS). Afterwards, unspecific binding sites were blocked by incubation with 10% BSA solution in 0.1 M PBS for 30 min. Unbound BSA was removed through the washing of pulps and fibers 5 times with 0.1 M PBS.

An undiluted primary rat monoclonal antibody hybridoma cell culture supernatant LM10 (PlantProbes, Leeds, UK) was added to the microscopic preparations and incubated for 2 h at room temperature (RT). This antibody preparation was kindly provided cost-free by PlantProbes, Leeds, UK. The preparations were subsequently washed 5 times with 0.1 M PBS after incubation to remove the unbound primary antibody. The primary antibody was labeled with an FITC-labeled secondary antibody (goat anti-rat IgM, IgG, and IgA from Sigma Aldrich (Schnelldorf, Germany) at a 1:100 dilution in PBS for 2 h at RT under light exclusion.

The preparations were then washed 5 times with 0.1 M PBS to remove the unbound secondary antibody, and finally mounted with water. Control samples were measured without the addition of the primary antibody.

Fluorescence microscopy

The samples were studied using the upright fluorescence microscope Leica DM5500B equipped with the L5 filter cube including the following ranges: excitation range: blue; excitation filter: BP 480/40; Dichromatic Mirror: 505; and suppression filter: BP 527/30 (Leica Microsystems CMS GMBH, Mannheim, Germany).

Confocal laser scanning microscopy

Images were captured by line-average (34) scan using the Leica SP5 confocal laser scanning microscope with the following filter settings: excitation wavelength 488 nm, emission wavelength 508 nm to 544 nm (Leica Microsystems CMS GMBH, Mannheim, Germany). The images were processed using Leica confocal software version

2.61 (Leica Microsystems CMS GMBH, Mannheim, Germany) and ImageJ (Open Source).

RESULTS AND DISCUSSION

The extraction of hemicellulose from hardwood kraft paper pulps by alkaline treatments below 80 °C is a merely physical process, as it is influenced by swelling, the diffusion of alkaline ions, and the subsequent dissolution of biopolymers. All of these effects are triggered by ion concentration and the temperature of extraction as well as xylan concentration and residence time. Because hardwood xylan exhibits weaker intermolecular hydrogen bonds compared to cellulose due to uronic acid substituents, the lack of one hydroxyl group, and shorter chain lengths, they are easily dissolved in the alkaline solutions. Both the effective alkalinity (EA) and the treatment temperature were varied in experiments prior to microscopy. All of these variations for modifying substrate pulps (parameters presented in Table 1) led to pulps with different residual xylan contents as presented in Table 2 for birch kraft paper pulps exemplarily. Pulp property parameters of alkali-treated hardwood pulps are illustrated exemplarily for birch as they are very similar to those from eucalyptus and beech pulps due to chemical as well as structural similarities.

Table 1. Parameters of Oxygen-delignified Kraft Pulps Used

Pulp Property	Birch	Eucalyptus	Beech
Kappa Number	7.6	7.4	5.2
Brightness (%ISO)	66.4	67.7	61.6
Intrinsic Viscosity (CUEN viscosity) [η] (mL g^{-1}odp)	925	880	925
Water Retention Value (WRV) (%)	103	98	81
Carboxyl Group Content (µmol g^{-1})	109.6	126.7	66.7
Glucan (%)	76.0	77.5	83.7
Xylan (%)	23.8	22.1	16.1
Mannan (%)	0.2	0.0	0.2
Arabinan (%)	0.0	0.0	0.0
Rhamnan (%)	0.0	0.0	0.0
Galactan (%)	0.0	0.2	0.0
Weight Average Molar Mass (kg mol^{-1})	420	514	540
Crystallinity (%)	57	56	52
Cellulose II Content (%)	10	10	17

Table 2. Parameters of White Liquor-treated Oxygen-delignified Birch Kraft Pulp

Pulp Treatment (Temp./ EA)	20 °C / 40 g L^{-1}	20 °C / 80 g L^{-1}	20 °C / 120 g L^{-1}	80 °C / 40 g L^{-1}	80 °C / 80 g L^{-1}	80 °C / 120 g L^{-1}
Kappa Number	4.28	2.95	3.03	4.25	3.18	2.48
Brightness (%ISO)	72.1	73.4	74.0	74.7	76.1	76.2
[η] (mL g^{-1}odp)	1010	1020	902	915	980	868
Water Retention Value (%)	94	93	88	96	88	79
Carboxyl Content (µmol g^{-1})	49.3	27.5	31.7	55.9	32.9	21.7
BET Surface Area (m^2 g^{-1})	0.55	0.44	0.41	0.56	0.74	0.55

Xylan (%)	10.1	4.7	5.2	12.9	6.5	3.2
Weight Average Molar Mass (kg mol^{-1})	454	443	435	449	489	425
Crystallinity (%)	54	50	46	59	56	49
Cellulose II Content (%)	19	39	86	10	14	50

Before the immunolabeling experiments, field emission scanning electron microscopy was performed, which is a suitable technique for visualizing the influence of the alkali treatment on fiber morphology and cell wall structure. Figure 1 shows birch pulp fibers after treatment with varying alkalinities of the white liquor used at 20 °C and 80 °C. After kraft paper pulp cooking, the outer cell wall layer was largely preserved. Even after oxygen-bleaching and alkaline treatment, a sound structure was observed, underscoring the gentleness of the post-extraction of hemicellulose with this process modification. The substantially higher cellulose yield compared to prehydrolysis kraft and sulfite dissolving pulps was reflected in the intact cell wall structure. In Fig. 1A, a smooth surface indicated at least a partially preserved primary wall (P) due to the mild conditions of alkaline post-extraction. The primary wall is the thinnest layer with approximately 0.1 µm thickness, and is characterized by a loosely arranged network of cellulose microfibrils (Sixta 2006). The fiber contained approximately 10% residual xylan (Table 2). A FE-SEM picture of an untreated oxygen-delignified birch kraft pulp is not displayed as differences to low-alkali treated pulps (Fig. 1A) are not visible. When the temperature increased to 80 °C at the same alkali level of 40 g L^{-1}, a xylan concentration of approximately 13% was achieved. At low effective alkalinity (below 80 g L^{-1} of the pulp suspension), the performance of xylan removal strongly depended on the treatment temperature, indicating decreasing extraction efficiency with rising temperature due to hindered pulp swelling (Hutterer *et al.* 2016b). Pulp swelling enabled the accessibility of xylan by alkali and therefore facilitated dissolution.

Within the FE-SEM image, the steeper angles of the cellulose microfibrils of the S1 layer cannot be distinguished from wrinkles due to shrinkage that originated from drying during the sample preparation. Hence, the exact cell wall layer could not be precisely identified. Nevertheless, it was observed that a certain degradation of the outer cell wall occurred. The high temperature during alkaline treatment led to degradation reactions of the cellulose matrix, indicated by fiber bundles that stuck out of the cell wall. The fiber shown in (Fig. 1C) contained a much lower xylan content of approximately 5%. Due to a rupture of the cell wall, the shape of the fibril bundles became visible. Clearly, fibril bundles aggregated during sample preparation and hemicellulose extraction, and were preserved in an orientation almost perpendicular to the fiber axis. A steep angle of 70° to 90° was assigned to the thickest of all cell wall layers—secondary layer 2 (S2) (Brändström *et al.* 2002). Figure 1D illustrates the beginning of the fibrillation of the pulp treated with alkali at 80 °C, that contained approximately 3% xylan. At alkalinities above 80 g L^{-1}, the extraction was facilitated by an increased diffusive flow of alkali ions, which were less temperature dependent (similar extraction efficiencies above 40 °C), which resulted in a residual xylan concentration in pulps of approximately 3% w/w at 120 g L^{-1}. The decrease in specific surface induced by alkaline treatments was heavily influenced by the alkalinity used. The use of high alkali concentrations led to an irreversible closure of pores and an aggregation of cellulose fibrils, as indicated earlier by the FE-SEM pictures. This reduction of the accessible surface area can be confirmed by porosity measurements according to the method described by Brunauer *et al.* (1938) and the water retention value (WRV). The latter is widely used to measure the swelling capacity of pulp in water,

indirectly giving information about the amount of accessible hydroxyl groups therefrom. The accessibility directly reflects the reactivity of pulps in subsequent derivatization processes, as it shows the ability of pulps to take up chemicals (Gehmayr *et al*. 2011). For birch, heightening the effective alkali concentration from 40 g $L^{-1}$to 120 g L^{-1} led directly to a decrease of the BET surface area from 0.55m^2 $g^{-1}$to 0.41 m^2 g^{-1} for pulps treated at 20 °C (BET data of untreated pulps are not shown as they do not differ from those treated by 40 g L^{-1}alkali). In parallel, WRV values decreased from 94% to 88% for pulps treated with lyes at 20 °C and from 96% to 79% at 80 °C (Table 2). Similar trends were examined for eucalyptus and beech pulps. These trends, which are derived from the collapsed macropores, are well known as pulp hornification, hindering the production of highly reactive dissolving pulps by alkaline post extractions (Gehmayr *et al*. 2011). The WRV was additionally influenced by the xylan as well as the cellulose II content. Xylan shows a higher swelling potential than cellulose through its content of hydrophilic groups, such as uronic acids.

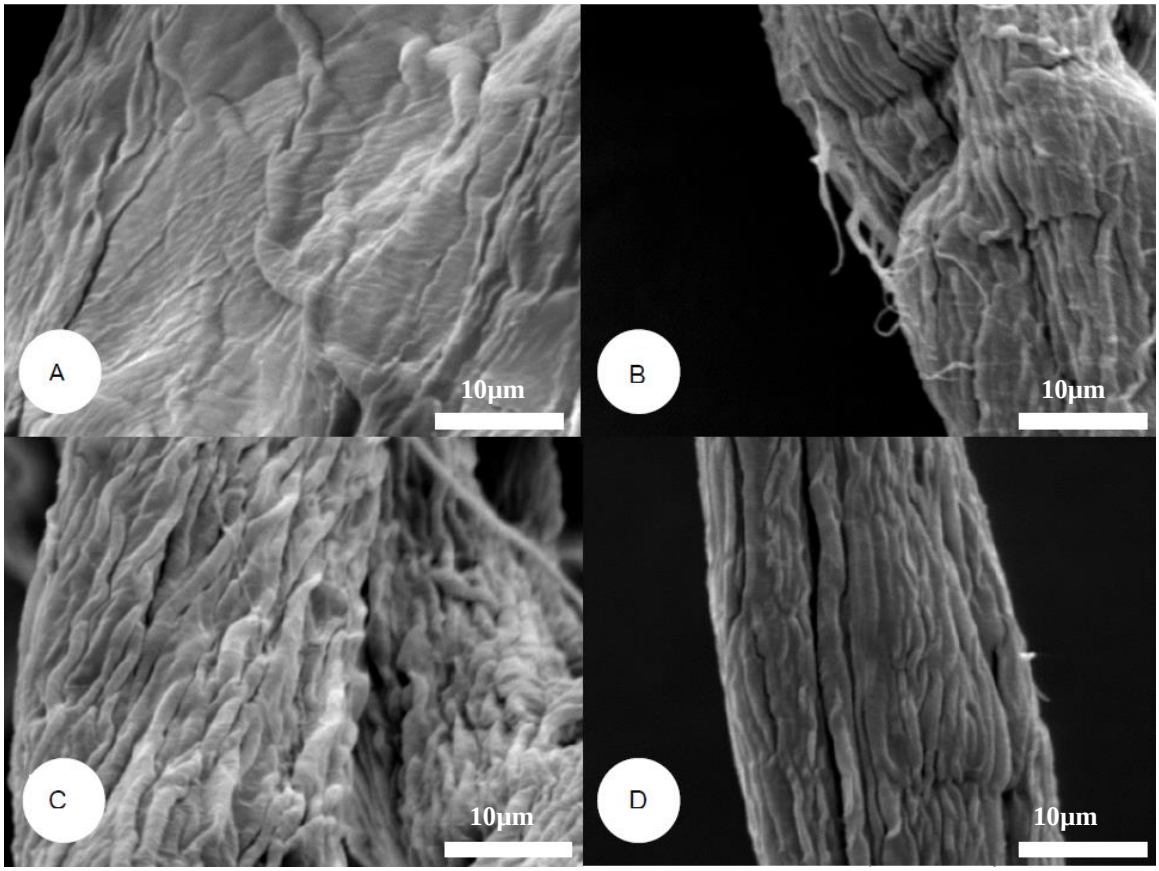

Fig. 1. FE-SEM pictures of alkali-treated birch paper pulps with varying process temperatures and alkalinity using synthetic white liquor as the alkali source with the following conditions: (A) 20 °C and 40 g L^{-1} EA; (B) 80 °C and 40 g L^{-1} EA; (C) 20 °C and 120 g L^{-1} EA; (D) 80 °C and 120 g L^{-1} EA

Hence, FE-SEM provided a suitable tool for studying the influence of alkali treatment on fiber morphology and cell wall structure, but had limitations. More detailed structures, such as microfibrils, could only be identified by microscopic techniques with higher resolution, such as atomic force microscopy (AFM). Fluorescence microscopy has

been introduced to study the interaction of cellulose and cellulase (Moran-Mirabal 2013). To selectively identify xylan structures on pulp surfaces, labeling with specific markers in combination with fluorescence and confocal microscopy was a promising technique.

The immunohistological visualization of plant polysaccharides in plant tissues, in their native and chemically and enzymatically-treated form, has been established, as cited above. The disruption of the wood tissue during pulp production by alkaline cooking led to a separation of the major polymer constituents and to severe morphological changes. For a successful labeling of xylan by specific monoclonal antibodies, the selective binding from immunoglobulins to epitope structures must be guaranteed. Through chemical pulping, structural changes within the O-acetyl-(4-*O*-methylglucurono)xylan (Sjöström 1981; Fengel and Wegener 1984) macromolecules occur that include the following reactions at high temperatures and alkalinities: deacetylation (Rydholm 1965; Malinen and Sjöström 1975), the breakage of the α-glycosidic bond between xylose and methylglucuronic acid (Hamilton and Thompson 1960) or the conversion of the latter into 4-deoxy-β-L-threo-hex-4-enopyranosyluronic acid (HexA) side groups (Buchert *et al.* 1995; Teleman *et al.* 1995), the degradation of the xylan backbone by peeling (Kolmodin and Samuelson 1973; Lindström and Samuelson 1975; Johansson and Samuelson 1977), and chain depolymerizations (Prey *et al.* 1953; Collier 1960). These reactions may change the structural appearance of the xylan macromolecules completely; thus it would hinder the recognition of xylan epitopes by the antibodies. Furthermore, pulp tissues exhibit a far higher specific cellulosic surface area than wood sections, which enable the unspecific binding of the immunoglobulins to cellulose macromolecules.

All of these difficulties made the adaptation of the immunostaining method to the authors' application challenging. To block unspecific binding sites on the pulp fiber surface, pulps were incubated with a bovine serum albumine (BSA) protein solution prior to antibody addition. As the proteins bind to the cellulosic surface by the same mechanisms as antibodies do, the subsequent addition of the primary antibodies still allows binding to the xylan epitopes due to their higher affinity towards these structures than BSA. To test the successful adaptation of the method, xylan was labeled with the LM10 primary antibody and fluorescein isothiocyanate (FITC) that contained the secondary antibody, followed by fluorescence microscopy. Figure 2 illustrates the pulp fibers of an oxygen-delignified birch kraft paper pulp with a xylan content of approximately 24% (Table 1). Figure 2A shows pulps immunolabeled with the whole immunoconjugate, and Fig. 2B shows the negative control without the addition of the primary antibody against the xylan epitope. The negative control illustrated a weak fluorescence of the non-specific bound secondary antibody, which confirmed a successful blocking of unspecific binding sites through BSA. The addition of the primary LM10 antibody led to the specific labeling of agglomerated xylan visible as green dots on the fiber surface. During alkaline conditions in pulping and oxygen-bleaching, dissolved xylan is present as both a single molecule and in aggregated form (Linder 2003). Aggregates are formed through the association of linear, unsubstituted regions of the chains. Furthermore, the xylan contains small amounts of covalently bound lignin residues that can promote association by hydrophobic interaction (Hutterer *et al.* 2016a). The interactions of neighboring chains are hindered through the repulsive coulomb interactions of dissociated carboxyl groups of uronic acids in xylan under alkaline conditions. When the pH was lowered during the final cooking phase and in subsequent washing steps, xylan aggregates grew and adsorbed onto pulp fiber surfaces. For producing high-quality pulps with low hemicellulose content, this xylan precipitation is

an unwanted effect and can be bypassed by process modifications in cooking or additional caustic extractions. The picture additionally illustrates the green fluorescence of pulp fibers that could be a consequence of unspecifically bound secondary antibodies or evenly distributed xylan over the entire fiber surface. Nevertheless, the successful recognition of epitopes within the processed and hence chemically changed xylan molecules by antibodies was visualized by fluorescence microscopy.

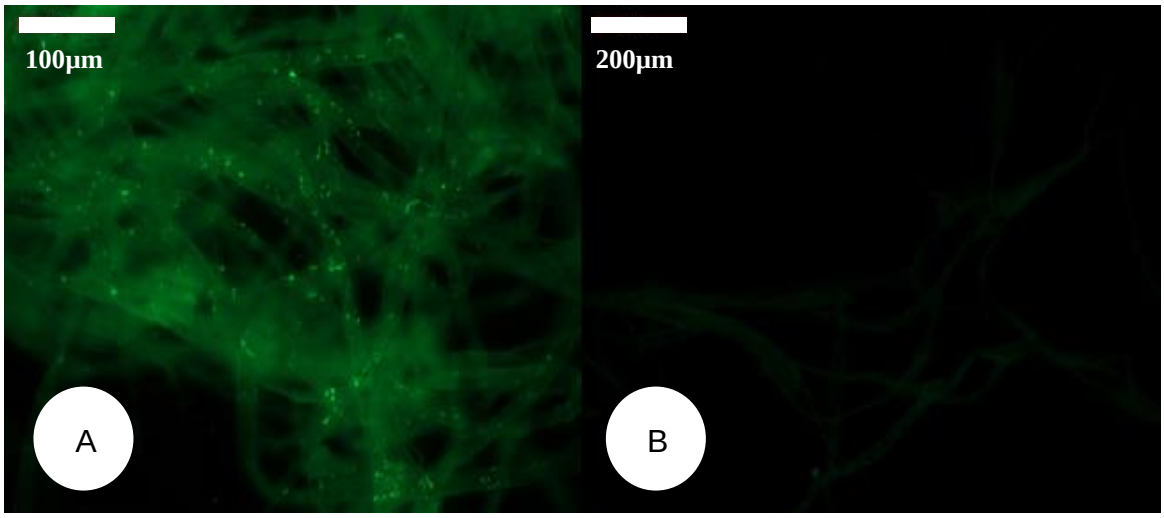

Fig. 2. Immunofluorescence images of a birch kraft paper pulp after oxygen-delignification; A) Xylan was labeled with a specific rat LM10 primary antibody, which was then labeled with a goat anti-rat IgG secondary antibody that carries FITC; B) Reflects the negative control without the addition of the primary antibody

Figure 3 depicts birch kraft pulp fibers after oxygen delignification with a 24% xylan content. The green fluorescence visible in Fig. 3A shows the excited FITC from the primary/secondary antibody conjugate specifically bound to xylan on the fiber surface. The surface structure of pulp fibers with and without excitement of the fluorophore revealed an outer cell wall layer with a high xylan content almost evenly distributed. Agglomerates of re-precipitated xylan could not be identified.

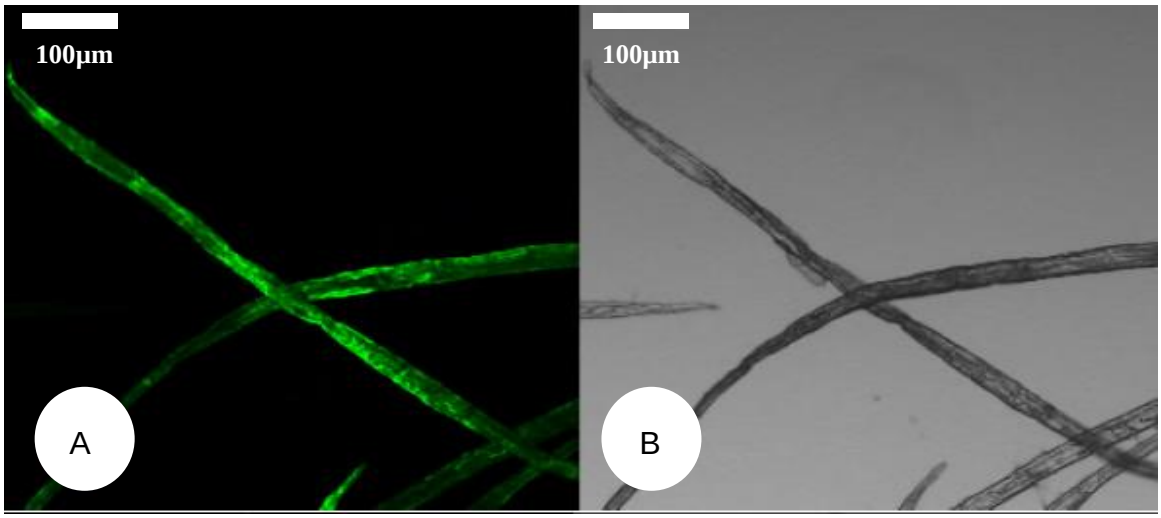

Fig. 3. Immunofluorescence images of a birch kraft paper pulp after oxygen-delignification using CLSM; A) Xylan on the pulp surface, which was labeled with the FITC-containing immunoconjugate after excitation with an argon laser; B) The bright field picture.

Clearly, the fluorescence can be attributed to residual xylan in the outer cell wall. Similar structures were observed in Fig. 1A, a eucalyptus kraft paper pulp after oxygen-delignification that contained approximately 22% xylan. Short vessel elements with a bigger diameter can be distinguished from long and narrow fiber tracheids. Regions with different xylan concentrations were observed. The beech kraft pulp (Fig. A2) showed similar fiber geometry with native xylan accumulated at certain regions on the fiber surface represented by conserved regions. This pulp contained approximately 16% xylan.

To benefit from the alkali-solubility of hardwood xylan, pulps were treated with an 80 gL^{-1} process white liquor at 80 °C, which led to a xylan concentration of approximately 6% in the resulting pulp. The fibers were curly and fibrillated (Fig. 4B). The corresponding immunolabeled fibers of the birch pulp (Fig. 4A) showed only spots of residual xylan on the fiber surface. Figure 4A indicated that the presence of xylan was unevenly distributed, and therefore concentrated in some regions of the pulp fibers. This behavior might be a consequence of alkali-resistant xylan molecules being preserved to a certain extent (Rydholm 1965). When looking at the fiber geometry in Fig. 4B, the alkali-treated fibers revealed a lower diameter and therefore denser structures compared to the untreated fibers (Fig. 3B). This was in agreement with the FE-SEM pictures and the WRV and BET data. This clearly indicates that the removal of xylan from kraft pulp fibers allows the cellulose microfibrils to form a more aggregated structure (Wallis and Wearne 1990). This behavior correlates with the decreased accessibility of the cellulose toward derivatization reagents, directly affecting their processibility (Jayme and Schenck 1949; El-Din and El-Megeid 1994).

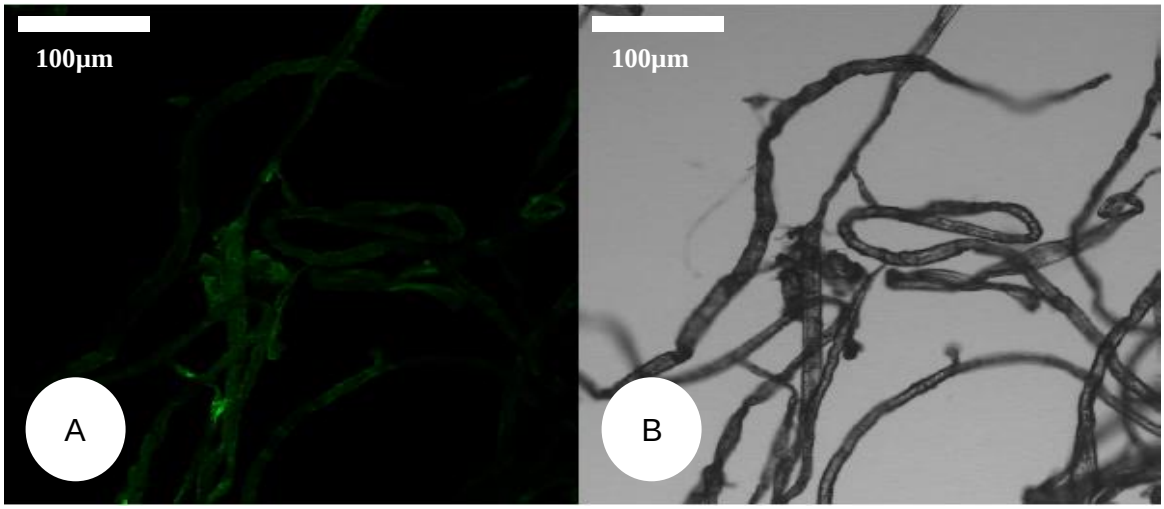

Fig. 4. Immunofluorescence images of an oxygen-delignified birch kraft paper pulp using CLSM; A) after alkaline post-extraction at 80 °C and 80 g L^{-1} effective alkalinity; B) The bright field picture

The additional processing of paper pulps to obtain high-purity dissolving pulps included subsequent totally chlorine-free (TCF) bleaching, applying ozone and hydrogen peroxide bleaching. These treatments lead to the removal of chromophore structures and the adjustment of the degree of polymerization (DP). They do not lead to further xylan

removal. Solely the depolymerization of glucan chains by ozone could lead to the liberation of low amounts of xylan that could then be dissolved in the alkaline hydrogen peroxide bleaching step.

Microscopic images of the resulting birch pulp (Fig. 5) using confocal laser scanning microscopy (CLSM) showed the fibers in excitation (Fig. 5A) and in bright field mode (Fig. 5B). These images confirmed the expected behavior, as similar fluorescing areas were visible before and after TCF bleaching. Hence, a certain fraction of xylan could not be removed by simple alkaline extractions, which is referred to as the previously mentioned alkali-resistant xylan. These macromolecules are thought to be preserved in the pulp matrix as a consequence of their long-chain structure and fewer functional groups (Schild *et al.* 2010). Consequently, they are thought to form molecular aggregates with cellulose fibrils upon intense dewatering and drying. These aggregates may play a key role in the relation of hornification and pulp reactivity. Residual hemicellulose from prehydrolysis kraft (PHK) pulp is of low molecular weight and contains many functional groups that are more easily extracted by alkaline post-extractions. Hence, the immunofluorescence images directly indicated the suitability of the pulps for subsequent fiber manufacture and could enable process optimizations. The bright field picture showed fibers having a dense structure and cellulose fibrils that stuck out of the pulp fibers due to fiber destruction by bleaching chemicals. This dense structure correlated hand-in-hand with pulp hornification and a decreased pulp reactivity, which caused a hindered sorption as indicated by the previously mentioned decreased BET and WRV values.

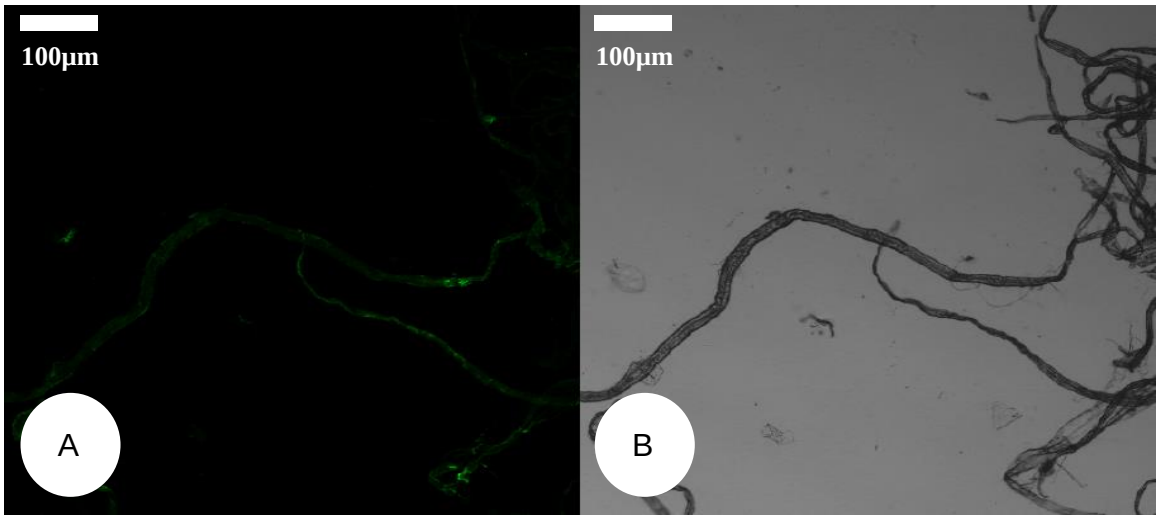

Fig. 5. Immunofluorescence images of an oxygen-delignified birch kraft paper pulp after alkaline post-extraction at 80 °C and 80 g L^{-1} effective alkalinity and subsequent totally chlorine-free bleaching using CLSM; A) Excited FITC immunoconjugate bound to xylan; B)The bright field picture.

Figure 6 illustrates an antibody-labeled xylan-enriched viscose fiber with 10.4% xylan using CLSM in excitation (Fig. 6A) and in bright field mode (Fig. 6B). It illustrated the successful implementation of the immunolabeling method through the visualization of xylan agglomerates on the fiber surface visible as green dots. These agglomerates arose from the association of linear, unsubstituted regions of the xylan chains and the reduced coulomb repulsion of the uronic acid substituents in acidic conditions during viscose fiber

manufacture. Hence, the picture clearly indicated that the xylan was not taken up into the fiber homogeneously during cellulose regeneration. Conclusions about the xylan distribution within the fiber could not be drawn by this method.

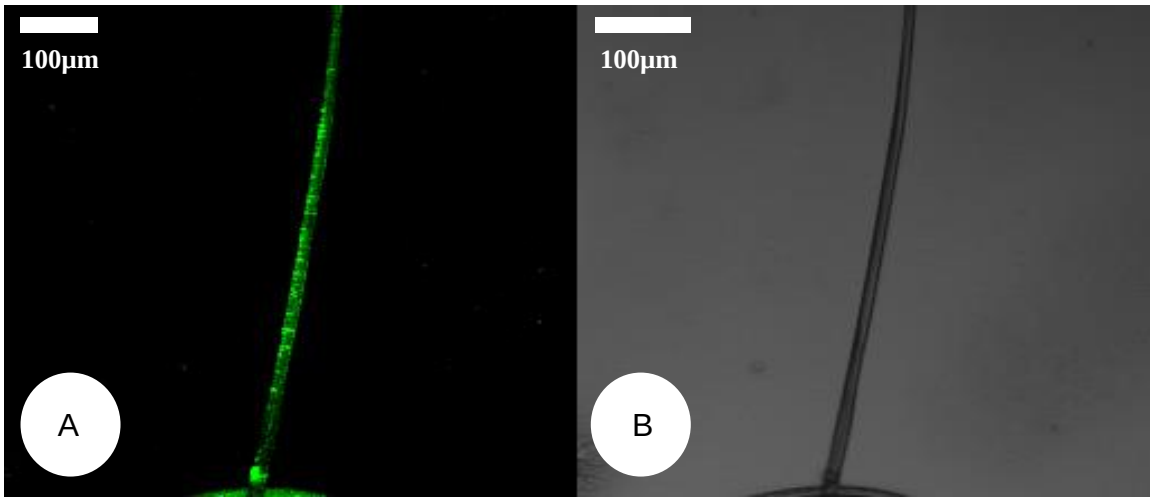

Fig. 6. Immunofluorescence images of a xylan-enriched viscose fiber with 10.4% xylan using a CLSM; A) Excited FITC immunoconjugate bound to xylan; B) The bright field picture.

CONCLUSIONS

1. The immunofluorescence microscopy technique that is normally used to visualize pectic and hemicellulosic structures in intact wood tissues was successfully adapted to track xylan structures on lignocellulosic pulp substrates throughout a process for hardwood kraft pulp purification and xylan-enriched viscose fibers.
2. The morphological changes of the pulp fibers were also illustrated through this microscopic method.
3. The data depicted areas on fiber surfaces with increased xylan content and the presence of evenly distributed alkali-resistant xylan in the bleached pulps.
4. The method was successfully applied to also visualize xylan on the surface of xylan-enriched viscose fibers, which directly indicated xylan agglomerates on the fiber surface.

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APPENDIX

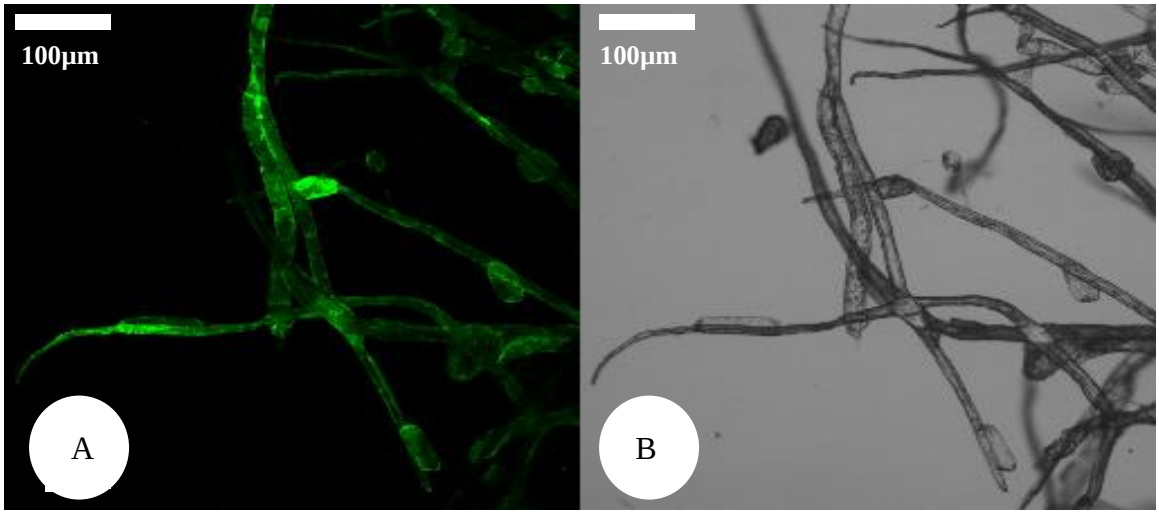

Fig. A1. Immunofluorescence images of an oxygen-delignified eucalyptus Kraft paper pulp using a CLSM; A) Excited FITC immunoconjugate bound to xylan; B) The bright field picture

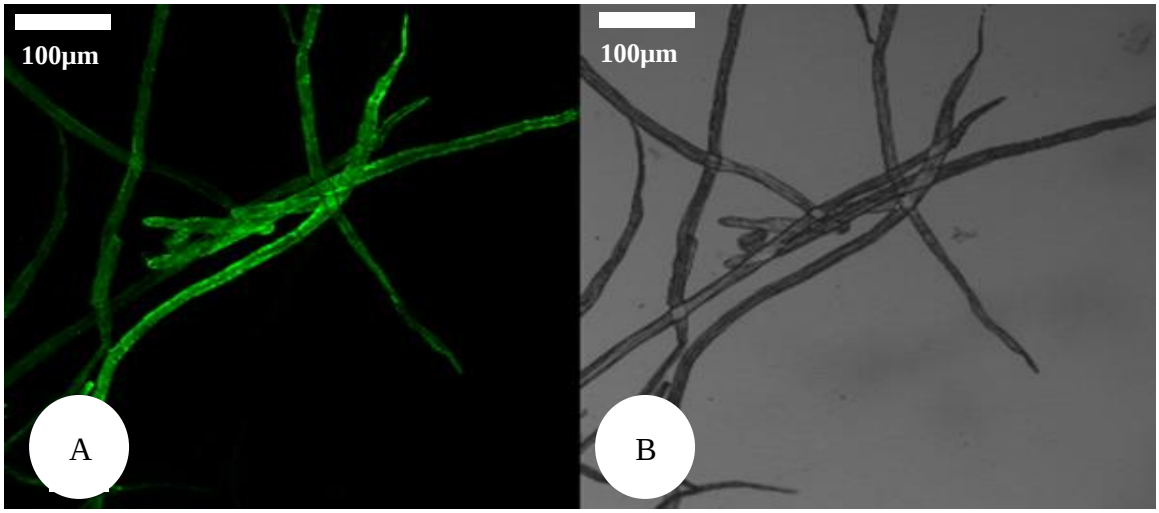

Fig. A2. Immunofluorescence images of an oxygen-delignified beech Kraft paper pulp using a CLSM; A) Excited FITC immune conjugate bound to xylan; B) Bright field picture