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Carbohydrate Polymers 63 (2006) 449–458

Carbohydrate Polymers

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The influence of surface chemical composition on the adsorption of xyloglucan to chemical and mechanical pulps

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Received 20 April 2005; received in revised form 9 September 2005; accepted 21 September 2005
Available online 9 November 2005

Abstract

Adsorption kinetics of xyloglucan (XG) onto bleached chemical and mechanical wood pulps were studied to better understand the application of this plant polysaccharide to the modification of key industrial cellulosic materials. Bleached pulps prepared by the kraft process adsorbed significantly larger amounts of xyloglucan than did thermomechanical pulps (TMP) and chemi-thermomechanical pulps (CTMP). The ability of pulps to adsorb xyloglucan was intimately related to the surface amounts of cellulose, lignin and extractives, as determined by electron spectroscopy for chemical analysis (ESCA). The distribution of absorbed XG on bleached pulp fibers was examined with an XG-fluorescein conjugate (XG-FITC), which was prepared by xyloglucan endotransglycosylase (XET, EC 2.4.1.207)-mediated incorporation of fluorescein-labeled xyloglucan oligosaccharides (XGO-FITC) into XG. Fluorescence microscopy revealed that XG-FITC preferentially adsorbed on fiber surfaces, and was adsorbed in higher amounts to chemical pulps than mechanical pulps, as observed for the unmodified parent polysaccharide. Addition of hybrid aspen XET (PttXET16A) and XGO-FITC to bleached and unbleached mechanical pulps indicated that the amount of enzyme-accessible xyloglucan presented on TMP and CTMP surfaces is low.

Keywords: Xyloglucan; Xyloglucan endotransglycosylase (XET); Chemical and mechanical pulp fiber; Surface chemical composition; Fluorescence microscopy; Cellulose; Wood pulp; Fiber modification; Surface chemistry; Green chemistry

1. Introduction

The cell walls of plant tissues are composite structures of neutral and anionic polysaccharides, structural proteins and glycoproteins, and aromatic hydrocarbon polymers (Carpita & McCann, 2000). Paracrystalline cellulose ($\beta(1\rightarrow4)$ glucan) is the primary load bearing component, while the extractable polysaccharides (i.e. hemicelluloses and pectins) confer flexibility and the ability to respond to dynamic loads. The main hemicellulose in the primary cell wall of dicotyledonous plants is xyloglucan (XG), which has a heavily decorated $\beta(1\rightarrow4)$ glucan (cellulosic) backbone. The pattern of substitution is species and tissue dependent (Vierhuis et al., 2001), but a common motif consists of an unsubstituted Glc residue followed by three bearing D-Xyl*p*($\alpha1\rightarrow6$) side chains, which may be further substituted by D-Gal*p*($\beta1\rightarrow2$) or L-Fuc*p*($\alpha1\rightarrow2$)-D-Gal*p*($\beta1\rightarrow2$) groups (York, Harvey, Guillen, Albersheim, & Darvill, 1993). Xyloglucan associates tightly with cellulose and tethers adjacent microfibrils to form a strong but compliant material (Chanliaud, Burrows, Jeronimidis, & Gidley, 2002; Steele, Sulova, Campbell, Braam, Farkas and Fry, 2001; Whitney, Gothard, Mitchell, & Gidley, 1999). Xyloglucan also accumulates as a storage polysaccharide in some seeds (Ren, Picout, Ellis, Ross-Murphy, & Reid, 2005, and references therein), such as those of the tamarind (*Tamarindus indica*) tree (York et al., 1993). As a result of this bulk availability, xyloglucan is used commerically as a gelling agent in foodstuffs and as a sizing agent for textiles (Rao & Srivastava, 1973; Shankaracharya, 1998).

The adsorption of xyloglucan onto cellulosic materials has been previously studied (Hayashi, Ogawa, & Mitsuishi, 1994; Hayashi, Takeda, Ogawa, & Mitsuishi, 1994) and the basis of the strong cellulose-xyloglucan interaction is postulated to be a train of $\beta(1\rightarrow4)$ glucopyranosyl units interacting with cellulose (Levy, Maclachlan, & Staehelin, 1997; Levy, York, Stuikeprill, Meyer, & Staehelin, 1991; Vincken, Dekeizer, Beldman, & Voragen, 1995). The high affinity of xyloglucan for cellulose is largely independent of the temperature of adsorption. Similarly, binding occurs equally well in water and buffers with different pH and ionic strength (Mishima,

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doi:10.1016/j.carbpol.2005.09.015

Hisamatsu, York, Teranishi, & Yamada, 1998). Binding capacity is affected by the molecular weight and precise sugar composition of xyloglucan, as well as the nature of the cellulose substrate (Hayashi, Marsden, & Delmer, 1987; Lima, Loh, & Buckeridge, 2004; Lima, Santos, Tine, Molle, & Buckeridge, 2001; Vincken et al., 1995).

The properties of xyloglucan can be tailored using endoglucanases (Vincken et al., 1995), β-galactosidase (Shirakawa, Yamatoya, & Nishinari, 1998), galactose oxidase (Lang et al., 1992), and xyloglucan endotransglycosylases (XET) (Brumer, Zhou, Baumann, Carlsson, & Teeri, 2004). XETs (EC 2.4.1.207) perform transglycosylation reactions of the xyloglucan backbone via a hydrolytically stable glycosyl-enzyme intermediate and are key enzymes affecting plant cell wall morphology (Campbell & Braam, 1999; Johansson et al., 2004). XETs can also use small xyloglucan oligosaccharides, even when chemically modified, as glycosyl acceptors. We have previously exploited the ability of XET to incorporate XGO conjugates into xyloglucan, together with the high affinity of xyloglucan for cellulose, to introduce novel functionality to cellulosic surfaces under mild conditions (Brumer et al., 2004). This method is extremely broad in scope, and has recently been used for the construction of biocomposite materials by elaboration of polymers from cellulose fibers (Zhou, Greffe, Baumann, Malmström, Teeri and Brumer, 2005). The demonstration that the conventional chemical syntheses of modified XGOs may be replaced by enzymic reactions further extends the 'green chemistry' aspects of the method (Gustavsson et al., 2005).

Recently, there has been a renewed interest in the use of xyloglucan as an additive in wood pulp and paper, which stems from observations that xyloglucan can positively affect sheet mechanical and formation properties (Christiernin et al., 2003; Lima, Oliveira, & Buckeridge, 2003), as well as provide a chemical handle to alter cellulose topochemistry (Brumer et al., 2004; Gustavsson et al., 2005; Zhou et al., 2005). Although the binding of xyloglucan to a number of pure cellulose materials has been examined in some detail (Brumer et al., 2004; Chanliaud et al., 2002; Hayashi, Baba, & Ogawa, 1994; Hayashi et al., 1987; Hayashi et al., 1994a,b,c; Lima et al., 2004; Mishima et al., 1998; Vincken et al., 1995), the industrially important wood pulps have received much less attention (Christiernin et al., 2003; Lima et al., 2003). In the context of its potential use in the pulp and paper industry, both in unmodified form and as a molecular anchor to introduce functional chemistry, we report here the adsorption properties and localization of xyloglucan on different industrial wood pulps in relation to the chemical composition of the fiber surface.

2. Materials and methods

2.1. General

Seven commercial chemical and mechanical pulps were kindly provided by Swedish pulp and paper companies (Table 1). Ultrapure water (Milli-Q, Millipore, $\rho > 18.2$ MΩ cm) was used in all experiments for dissolution of reagents. Xyloglucan (XG) from tamarind seed

Table 1
Industrial pulps used in this study

Mnemonic	Source	Pulping method	Bleaching sequence	Refined	Freeness[a]	Fiber length (mm)	Fines content[b]	Supplier
Chemical pulps								
Pine/spruce-kraft-unrefined	Mixed: pine and spruce	Modified kraft, Iso thermal cooking (ITC) to kappa number 24	$DDE_{o}D$[c] to 85% ISO brightness	No	14 °SR	2	4.5% K	Korsnäs
Pine/spruce-kraft-refined	Mixed: pine and spruce	Modified kraft, ITC to kappa number 24	$DDE_{o}D$[c] to 85% ISO brightness	Yes	n.a.[d]	2	n.a.[d]	Korsnäs
Birch-kraft	Birch	Modified kraft, ITC to kappa number 16	$DDE_{o}D$[c] to 87% ISO brightness	No	15 °SR	1	2.6% K	Korsnäs
Mechanical pulps								
Spruce-tmp-bleached	Spruce	Thermomechanical	dithionite, 4 kg/ton	No	50–60 mL CSF	1.4	35% BM	Holmen
Spruce-tmp-unbleached	Spruce	Thermomechanical	None	No	50–60 mL CSF	1.4	35% BM	Holmen
Aspen-ctmp-bleached	Aspen	Chemi-thermo-mechanical	n.a.[d]	No	450 mL CSF	0.8	8% K	M-real
Aspen-ctmp-unbleached	Aspen	Chemi-thermo-mechanical	none	No	450 mL CSF	0.8	8% K	M-real

[a] SR, Schopper-Riegler; CSF, Canadian Standard Freeness.

[b] K, Kajaani FS200; BM, Bauer McNett P200.

[c] D, chlorine dioxide stage; E_o, oxygen reinforced alkali extraction. Prior to the bleaching stage, two stage oxygen deliginification was performed to kappa number ca. 10.

[d] n.a., Not available-proprietary information.

(Xyl:Glc:Gal:Ara = 35:45:16:4) was purchased from Megazyme (Bray, Ireland) with weight average molecular mass (M_w) of 4.5×10^5 and polydispersity index (PDI, M_w/M_n) of 1.5. Xyloglucan endotransglycosylase (XET, EC 2.4.1.207) was obtained by heterologous expression of the *Populus tremula*×*tremuloides* (hybrid aspen) *Ptt*XET16A (Bourquin et al., 2002) in *Pichia pastoris* (Kallas et al., 2005). A mixture of xyloglucan oligosaccharides (XGOs, XXXG/XLXG/XXLG/XLLG ratio 15:7:32:46) was prepared from deoiled tamarind kernel powder (D.N. Palani, Mumbai, India) using an endoglucanase digestion essentially as described (York et al., 1993). Common XGO nomenclature (Fry et al., 1993) is used: X represents a Xyl*p*(α1→6)Glc*p* unit, L represents a Gal*p*(β1→2)Xyl*p*(α1→6)Glc*p* unit, and G represents a Glc*p* residue; when written sequentially, a β(1→4) linkage between the Glc*p* residues is implied, with the reducing end on the right. XGO-fluorescein conjugates (XGO-FITC, **2**, Fig. 1) were prepared via the aminoalditol derivatives (XGO–NH_2, **1**, Fig. 1) as described previously (Brumer et al., 2004). Gel permeation chromatography (GPC) measurements were performed with a Waters 616 HPLC system equipped with TSK gel G5000H_{HR} (7.8 mm×30 cm, pore size 1×10^5 Å, particle size 5 μm) and G3000H_{HR} (7.8 mm×30 cm, pore size 1500 Å, particle size 5 μm) columns connected in series. HPLC-grade dimethylsulphoxide (DMSO) was used as the eluent (flow rate of 1 mL/min, column temperature 60 °C) with evaporative light-scattering detection (Polymer Laboratories PL-ELS 1000). The use of the DMSO system (Sato, Norisuye, & Fujita, 1983) avoids experimental artifacts due to the well-known aggregation of xyloglucan in aqueous solution (Freitas et al., 2005; Yamatoya & Shirakawa, 2003). Pullulan polysaccharide standards (Polymer Laboratories) were used to calibrate the system over M_w range 180–788,000.

2.2. Preparation of laboratory sheets and electron spectroscopy for chemical analysis (ESCA)

Laboratory paper sheets were produced and characterized with ESCA by the Institute of Surface Chemistry (YKI), Stockholm, Sweden, as follows. Pulp samples (5 g, dry weight) were disintegrated in 2 L deionized water with 30 000 propeller revolutions, according to the SCAN-C 18:65 standard method, 'Disintegration of chemical pulp for testing.' Laboratory sheets were then prepared as described in SCAN-C 26:76 and SCAN-M 5:76, 'Preparation of laboratory sheets for physical testing.' Half of the volume from each disintegration was used for each sheet, i.e. the weight of each sheet was about 2.5 g. The sheets were dried overnight at room temperature (20 °C). For each sample, a small piece was cut from the laboratory sheet and subjected to Soxhlet extraction in acetone according to SCAN-CM 50:94, 'Determination of acetone-soluble matter'. The total number of extraction cycles was at least 30 (extraction for at least 5 h with at least 6 cycles per hour).

ESCA was performed with a Kratos AXIS HS X-ray photoelectron spectrometer (Kratos Analytical, Manchester, UK) using a monochromated Al Kα X-ray source. Survey scans and high-resolution regional spectra were recorded on an area of about 1 mm^2 at three different locations to account for paper surface heterogeneity. The measurement depth was 5–10 nm. The binding energies in the high-resolution spectra were charge-corrected using a tabulated value of 285.0 eV for the C 1s components. Relative amounts of C1 (carbon with no bonds to oxygen, C–C, C=C), C2 (carbon with one bond to oxygen, C–O, C–O–C), C3 (carbon with two bonds to oxygen, C=O, O–C–O) and C4 (carbon with three bonds to oxygen, O–C=O) were determined by deconvolution of high-resolution C 1s spectra using symmetric Gaussians. Relative peak widths and relative peak positions were fixed during the curve fitting process as described by Koljonen, Österberg, Johansson, & Stenius, 2003. Quantitative analysis of surface lignin and

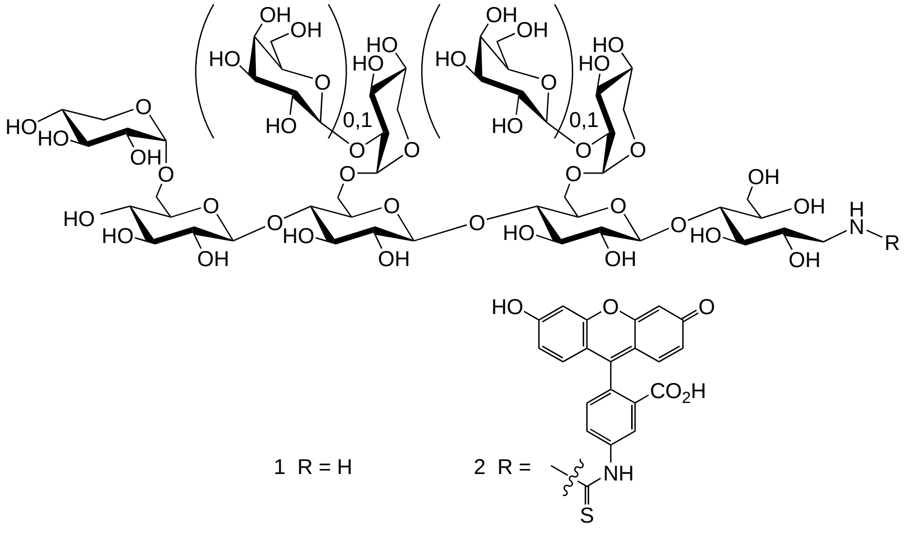

Fig. 1. Chemical structures of the aminoalditol xyloglucan oligosaccharide (XGO-NH_2) (**1**) and the fluorescein conjugate XGO-FITC (**2**).

extractives was performed using both the O/C ratio (atomic ratio of O 1s to C 1s, Eqs. (1) and (2)) and the intensity of the C1 carbon peak (Eqs. (3) and (4)), as previously described (Buchert, Carlsson, Viikari, & Ström, 1996; Koljonen et al., 2003; Maximova, Österberg, Koljonen, & Stenius, 2001; Stenius & Laine, 1994; Ström & Carlsson, 1992). Both models assume: (1) The extracted surface consists of regions of either lignin or carbohydrates; (2) On the unextracted surface, low molecular weight extractable organic material located on top of the carbohydrates and lignin; (3) The thickness of each of these three components is assumed to exceed the depth of analysis, about 10 nm.

The percentage of surface lignin, calculated using the O/C ratio, is given by Eq. (1),

$$\phi_{\text{lignin}} = \frac{\text{O/C}_{\text{extrated pulp}} - \text{O/C}_{\text{cellulose}}}{\text{O/C}_{\text{lignin}} - \text{O/C}_{\text{cellulose}}} \times 100\% \tag{1}$$

where $\text{O/C}_{\text{extractedpulp}}$ is experimentally determined and $\text{O/C}_{\text{lignin}}$ and $\text{O/C}_{\text{cellulose}}$ are the theoretical values 0.33 and 0.83, respectively (Table 2). The percentage of surface extractives using the O/C ratio is given by Eq. (2),

$$\phi_{\text{extractives}} = \frac{\text{O/C}_{\text{extracted pulp}} - \text{O/C}_{\text{pulp}}}{\text{O/C}_{\text{extracted pulp}} - \text{O/C}_{\text{extractives}}} \times 100\% \tag{2}$$

where $\text{O/C}_{\text{extractedpulp}}$ and O/C_{pulp} are experimentally determined and $\text{O/C}_{\text{extractedpulp}}$ is the theoretical value, 0.11 (Table 2).

The percentage of surface lignin, calculated using the C1 value, is given by Eq. (3),

$$\phi_{\text{lignin}} \frac{\text{C1}_{\text{extracted pulp}} - a}{\text{C1}_{\text{lignin}}} \times 100\% \tag{3}$$

where $\text{C1}_{\text{extractedpulp}}$ is the relative amount of the C1 component in the deconvoluted high-resolution C 1s spectrum of an extracted pulp sample, $\text{C1}_{\text{lignin}}$ is the theoretical value (49, Table 2), and a is the contribution to the C1 peak from surface contamination. A value of 2% was used for a, which was the lowest relative amount of C1 peak detected on the surface of fully bleached standard pulp fibers (pure cellulose contains only C2 and C3 carbons, however a small C1 peak is routinely observed due to adsorbed hydrocarbon contaminants). Using this analysis method, the percentage of surface extractives is given by Eq. (4),

$$\phi_{\text{extractives}} = \frac{\text{C1}_{\text{pulp}} - \text{C1}_{\text{extracted pulp}}}{\text{C1}_{\text{extractives}} - \text{C1}_{\text{extracted pulp}}} \times 100\% \tag{4}$$

where C1_{pulp} and $\text{C1}_{\text{extractedpulp}}$ are experimentally determined and $\text{C1}_{\text{extractives}}$ is the theoretical value, 94 (Table 2).

Table 2
Theoretical values of atomic composition and carbon deconvolution for cellulose, lignin and oleic acid

Sample	O/C[a]	C1	C2	C3	C4
Cellulose	0.83	0	83	17	0
Lignin	0.33	49	49	2	0
Oleic acid	0.11	94	0	0	6

[a] O/C, oxygen to carbon ratio; C1, percent carbon with no bonds to oxygen, C–C, C=C; C2, percent carbon with one bond to oxygen, C–O, C–O–C; C3, percent carbon with two bonds to oxygen, C=O, O–C–O; C4, percent carbon with three bonds to oxygen, O–C=O.

2.3. Binding isotherms of xyloglucan onto bleached pulps

0.1 g (dry weight) bleached pulp fibers were suspended in 5 mL of an aqueous solution containing 2.5, 5, 10 mg XG in glass vials and incubated at 20 °C with orbital shaking. At various time intervals, the pulp fibers were removed by centrifugation at 12,000×g for 10 min and the amount of XG bound was determined using a substractive method based on the colorimetric assay for XG devised by Kooiman (1960), as follows. 0.1 mL of the supernatant solution was mixed with 0.1 mL water, 0.8 mL of 20% (w/v) Na_2SO_4 and 0.2 mL of triiodide solution (0.5% I_2 +1% KI). The amount of bound XG was calculated from the difference in A_{620} of the solutions before and after the binding reaction using a standard curve derived from XG solutions of increasing concentrations. Three measurements were made and the average value was recorded for each pulp. Individual samples were used for each determination at 1, 5, 10, and 24 h. Control samples in which pulp was suspended in water not containing xyloglucan indicated that there was no contribution to the A_{620} measurements due to leachable components in the pulp.

2.4. Adsorption of fluorescein-modified xyloglucan (XG-FITC) onto bleached pulps

The xyloglucan-fluorescein conjugate, XG-FITC, was prepared by XET mediated incorporation of XGO-FITC into XG (Brumer et al., 2004). A sample (5 mL total volume) containing a mixture of XG (1 g/L), XGO-FITC (0.5 g/L) and XET (10 units) in sodium acetate buffer (20 mM, pH 5.5) was incubated at 30 °C for 60 min. The reaction was terminated by heating (80 °C, 10 min) and the denatured XET was removed by centrifugation at 12,000×g for 20 min. The XG-FITC produced in this manner had a M_w value of 1.9×10^4 and a PDI value of 1.4. 0.05 g (dry weight) bleached pulp fibers were suspended in the supernatant solution in glass vials and agitated in an end-over-end mixer for 24 h at 20 °C. The pulp fibers were then collected by filtration and washed with water (3×5 mL) in an end-over-end mixer. The amount of FITC incorporated into XG and subsequently bound to bleached pulp fibers was calculated from the loss of XGO-FITC in solution (including the wash solutions), as determined by A_{495} in 0.1 M sodium bicarbonate versus a standard curve of XGO-FITC ($\varepsilon_{495\text{nm}}$ = 43000 $\text{cm}^{-1}\,\text{M}^{-1}$ in 0.1 M $\text{NaHCO}_{3(\text{aq})}$). The adsorbed amount of XG-FITC was calculated from the number average molecular mass (M_n) and the amount of adsorbed FITC in moles. Microscopic analysis of fibers with adsorbed

XG-FITC was performed by MoRe Research Örnsköldsvik AB (Örnsköldsvik, Sweden). Fluorescence images were acquired at room temperature on a Carl Zeiss Axioplan 2 imaging microscope using Zeiss filter set 02 (maximum excitation λ 365 nm, low pass emission filter λ 420 nm). To obtain cross-sectional images, fibers were resin embedded and sectioned on an HM 355S microtome (Microm, Walldorf, Germany) with a diamond knife after heat treatment.

2.5. Localization of natural xyloglucan in mechanical pulps

The bleached and unbleached spruce TMP and aspen CTMP pulps (0.05 g dry weight, pre-washed with water and filtered) were incubated with 5 mL of XGO-FITC (0.5 g/L) and XET (200 units) in sodium acetate buffer (20 mM, pH 5.5) at 30 °C for 24 h. The reaction was terminated by filtration and the pulps were washed extensively with 20 mM sodium acetate buffer and ultrapure water. Control samples were treated in an identically, but without XET in the incubation buffer. Fluorescence microscopy of these fibers was performed as described in the Section 2.4.

3. Results and discussion

3.1. Surface chemical composition of the pulp fibers

The chemical composition of the pulp surface can vary dramatically from that of the bulk material. Moreover, hydrophobic materials present on surfaces of fibers and fines, in particular lignin and extractives, have an impact on strength properties of paper (Koljonen et al., 2003; Maximova et al., 2001). Results from ESCA of the contents of lignin and extractives on the surfaces of chemical and mechanical pulps are summarized in Table 3. The C1 carbon value decreased after extraction for all pulps and almost no lignin was detected for the bleached kraft pulp samples (pine/spruce-kraft-unrefined, pine/spruce-kraft-refined, and birch-kraft). Mechanical pulps had consistently higher amounts of surface lignin and extractives than the chemical pulps. In softwood TMP, surface lignin was slightly reduced and extractives were significantly reduced by bleaching (spruce-tmp-bleached and spruce-tmp-unbleached). Compared to the softwood TMPs, higher values of surface lignin and extractives were found in hardwood CTMP pulps (aspen-ctmp-bleached and aspen-ctmp-unbleached); bleaching caused a significant removal of both lignin and extractives in this pulp.

As mentioned previously, the models used to calculate the surface coverage of lignin and extractives from either the amount of C1 carbon or the O/C ratio assume that the extracted surface consists only lignin and carbohydrates, that low molecular weight extractable organic components in unextracted material reside on top of the carbohydrates and lignin, and that the thickness of each component is greater than the depth of analysis (ca. 10 nm). The last assumption is valid for lignin, which is present in patches thicker than 10 nm, but may not be valid for the extractable components such as fatty acids, resin acids, fatty alcohols, etc. (Koljonen et al., 2003). If this is the case, the surface coverage of extractable materials will be underestimated and should be viewed with some caution. Nonetheless, these values are useful for comparative purposes.

The results shown in Table 3 are in accordance with expected values, given the production methods used to produce each pulp. Chemical pulps produced using the kraft process are prepared by heating wood chips in a solution of NaOH and Na_2S. Kraft pulping effectively removes most of the lignin and

Table 3
Atomic composition, carbon deconvolution and surface coverage of lignin and extractives for the chemical and mechanical pulps measured by ESCA

Pulp	Extraction	O/C	C1	C2	C3	C4	ϕ_{lignin} (%)		$\phi_{\text{extractives}}$ (%)	
							C/O	C1	C/O	C1
Pine/spruce-kraft-unrefined	−	0.784	6.0	76.3	16.4	1.3	1	0	6	4
	+	0.825	2.1	77.4	19.6	0.9				
Pine/spruce-kraft-refined	−	0.822	3.1	77.0	19.1	0.8	0	0	1	2
	+	0.832	1.6	79.5	17.9	1.0				
Birch-kraft	−	0.799	4.0	76.1	19.0	0.9	1	0	4	3
	+	0.827	1.6	78.1	19.1	1.2				
Spruce-tmp-bleached	−	0.558	25.8	62.3	11.0	0.9	28	27	23	14
	+	0.690	15.2	71.0	12.6	1.2				
Spruce-tmp-unbleached	−	0.481	36.6	53.2	8.3	1.9	31	29	34	26
	+	0.673	16.4	69.5	13.0	1.1				
Aspen-ctmp-bleached	−	0.502	29.6	60.9	8.1	1.4	37	33	27	15
	+	0.643	18.1	69.0	11.6	1.3				
Aspen-ctmp-unbleached	−	0.372	45.1	45.9	6.4	2.6	42	38	48	34
	+	0.618	20.5	67.7	10.3	1.5				

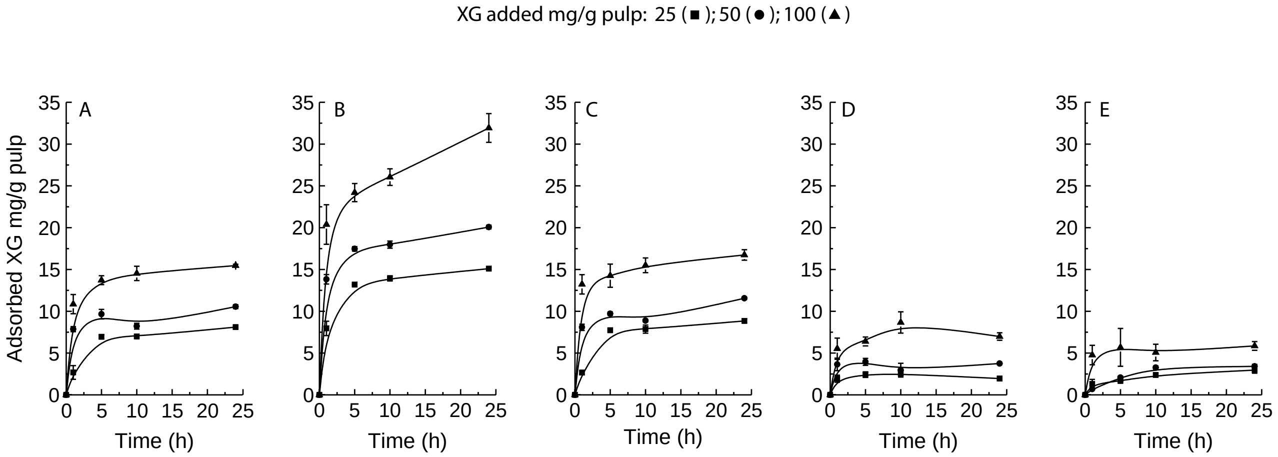

Fig. 2. Adsorption isotherms of xyloglucan on the bleached commercial chemical and mechanical pulps. (A) pine/spruce-kraft-unrefined; (B) pine/spruce-kraft-refined; (C) birch-kraft; (D) spruce-tmp-bleached; (E) aspen-ctmp-bleached.

hemicelluloses from the fibers, largely as a result of the destruction of the primary cell wall and middle lamella to leave the cellulose-rich secondary cell wall exposed. In contrast, mechanical pulps are produced by refining (grinding) wood chips after steam (TMP) or combined chemical/steam (CTMP) pretreatment. TMP and CTMP processes produce individual fibers by physically breaking fiber–fiber contacts mediated via the lignified middle lamella. Consequently, mechanical pulps retain much of the original lignin and hemicelluloses located on the outer structures of the wood cell wall. Bleaching of wood fibers after pulping is used to remove or decolor residual lignin by treatment with redox chemicals such as dichlorine, chlorine dioxide, hydrogen peroxide, ozone, and sodium dithionite.

3.2. Binding studies

To evaluate the binding of tamarind xyloglucan to different bleached commercial chemical and mechanical pulps, time-course binding experiments were performed at 20 °C; absorption isotherms are shown in Fig. 2. At different initial concentrations of xyloglucan, the amount adsorbed onto pulps from distilled water increased sharply during the first 5 h and continued to increase slowly over the next 19 h. The failure to reach equilibrium may be due to swelling of the pulp fibers in water, accompanied by a slow increase in available surface area. A second possibility is that this effect is due to the polydispersity of the xyloglucan ($M_w = 4.5 \times 10^5$, $PDI = 1.5$, Fig. 3), where quicker binding, low molar mass species are exchanged with species of higher molar mass. (Fleer, Cohen Stuart, Scheutjens, Cosgrove, & Vincent, 1993; Myers, 1999) The amount of xyloglucan bound to different pulps increased with increasing initial loading amount of xyloglucan (25, 50, and 100 mg XG/g cellulose). After 24 h absorption, bleached kraft pulp samples (Fig. 2(A)–(C)) demonstrated consistently higher adsorbtion capacity than bleached mechanical pulps (Fig. 2(D) and (E)). These observations are correlated with the surface coverage of lignin and extractives on the pulp fibers: Bleached kraft pulps, which present a cleaner cellulose surface, bind more xyloglucan than mechanical pulps, the cellulose microfibrils of which are extensively covered in lignin and hydrophobic extractives (Table 3). Beating of mixed pine/spruce bleached kraft pulp further increased the amount of xyloglucan bound (Fig. 2(A) and (B)), as would be expected due to increasing the accessible cellulose surface area by mechanical disruption of the fibers. Unrefined hardwood (birch, Fig. 2(C)) and softwood (pine/spruce, Fig. 2(A)) pulps had similar adsorption capacities for xyloglucan. Taken together, these results indicate that the amount of xyloglucan adsorbed onto pulp fibers is mainly affected by surface chemical compositions of the pulp fibers (cellulose, lignin and extractives) and fiber (accessible cellulose microfibril) surface area.

3.3. Surface distribution of adsorbed xyloglucan on bleached pulps

To characterize the distribution of adsorbed xyloglucan on pulp fiber surfaces, a xyloglucan-fluorescein conjugate

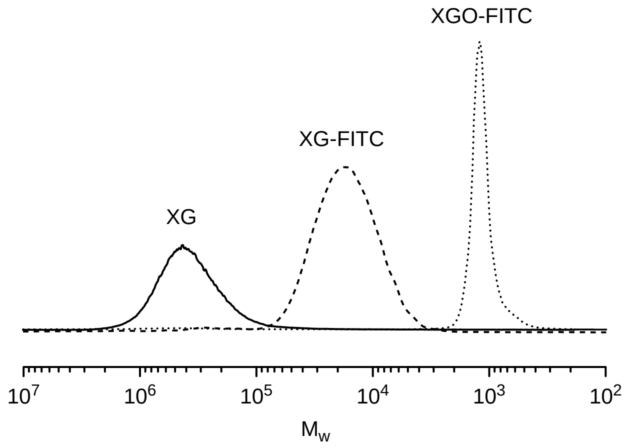

Fig. 3. GPC curves for unmodified xyloglucan (XG), XET-modified xyloglucan (XG-FITC) and xyloglucan oligosaccharide-fluorescein conjugates (XGO-FITC).

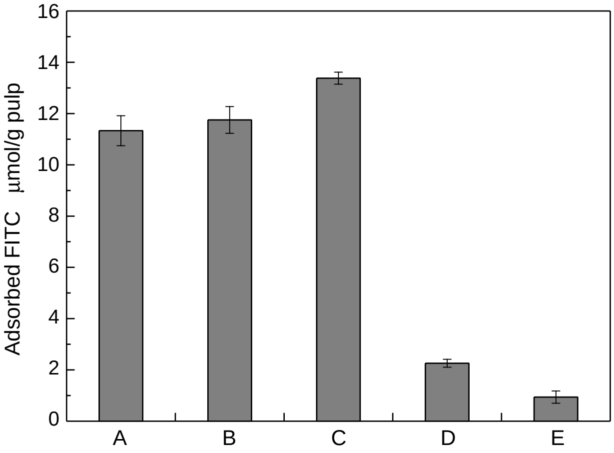

Fig. 4. Adsorption of fluorescein-modified xyloglucan (XG-FITC) on bleached commercial chemical and mechanical pulps. (A) PINE/SPRUCE-KRAFT-UNREFINED; (B) PINE/SPRUCE-KRAFT-REFINED; (C) BIRCH-KRAFT; (D) SPRUCE-TMP-BLEACHED; (E) ASPEN-CTMP-BLEACHED.

(XG-FITC) was prepared by incorporation of fluorescein-modified xyloglucan oligosaccharides (XGO-FITC) into high molecular mass xyloglucan using a XET. XG-FITC was subsequently applied to the pulp fibers at 20 °C at a loading ratio of ca. 150 mg XG-FITC/g pulp (cf. Fig. 2). Analogous to the results obtained for unmodified, high molecular mass xyloglucan, larger amounts of XG-FITC were adsorbed to chemical pulps (Fig. 4(A)–(C)) than to mechanical pulps (Fig. 4(D) and (E)). However, an interesting difference is observed in the adsorption of XG and XG-FITC among the chemical pulp samples. As shown in Fig. 2, PINE/SPRUCE-KRAFT-REFINED (Fig. 2(B)) binds ca. 2-fold more unmodified XG after 24 h (30 vs. 15 mg XG/g pulp) than either its unrefined counterpart, pine/spruce-kraft-unrefined (Fig. 2(A)) or the other KRAFT PULP, BIRCH-KRAFT (Fig. 2(C)), and ca. 6-fold more XG than either of the bleached mechanical pulps (Fig. 2(D) and (E)). Inspection of Fig. 4 indicates that PINE/SPRUCE-KRAFT-REFINED (Fig. 4(B)) similarly binds ca. 6-fold more XG-FITC conjugate after 24 h than the mechanical pulps (Fig. 4(D) and (E)), but that the level of XG-FITC binding is nearly identical among the kraft pulps (Fig. 4(A)–(C)). The binding of XG-FITC to the unrefined pulps is thus higher than expected, based on the data for unmodified XG. These results indicate that the low molar mass XG-FITC ($M_w = 1.9 \times 10^4$, PDI = 1.4, Fig. 3) may be better able to penetrate the more closed structure of unrefined pulp than high mass unmodified XG and that effectively the same available surface for binding is available to XG-FITC regardless of the level of refining of the pulp. In contrast, the increased binding capacity of refined pulp for unmodified XG indicates that mechanical disruption of the fiber structure exposes more surface area accessible to the higher mass polysaccharide. Near complete adsorption of XG-FITC was observed after 24 h (loaded: 150 mg XG-FITC/g pulp; bound: 130 mg XG-FITC/g pulp). These results are similar to those reported by Vincken et al., who noted a strong dependence of xyloglucan binding to crystalline Avicel cellulose on molar mass (Vincken et al., 1995), and imply that low molar mass xyloglucan is better able to penetrate and bind cellulose structures.

The XG-FITC-modified chemical pulp fibers exhibited a strong green fluorescence and an essentially homogenous distribution of the fluorescein group on the fiber surface (Fig. 5(A)–(C)). Preferential adsorption of XG-FITC to certain sites was observed on the mechanical pulp fiber surfaces, which generally exhibited lower fluorescence (Fig. 5(D) and (E)). As expected, mechanical pulp fibers were considerably more fibrillated than chemical pulp fibers. However, such damage does not increase the availability of cellulose which is capable of binding xyloglucan, as the fibrils still contain significant amounts of surface lignin (Table 3). Cross-sectional images of

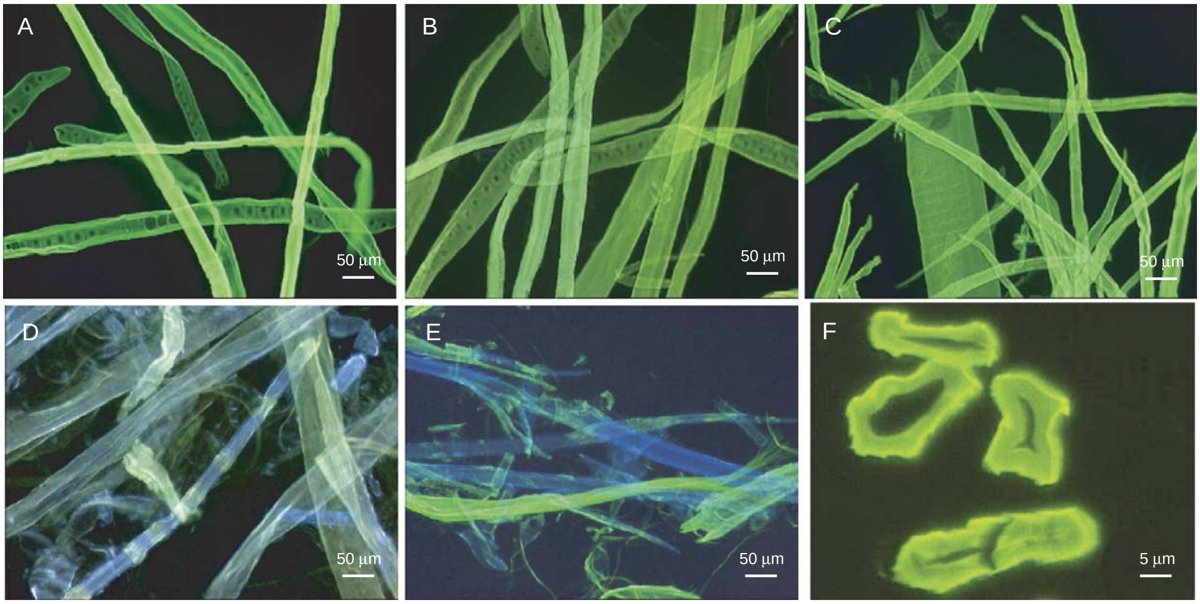

Fig. 5. Fluorescent images of fluorescein-modified xyloglucan (XG-FITC) adsorbed bleached pulps. (A) PINE/SPRUCE-KRAFT-UNREFINED; (B) PINE/SPRUCE-KRAFT-REFINED; (C) BIRCH-KRAFT; (D) SPRUCE-TMP-BLEACHED; (E) ASPEN-CTMP-BLEACHED; (F) CROSS-SECTION OF BIRCH-KRAFT.

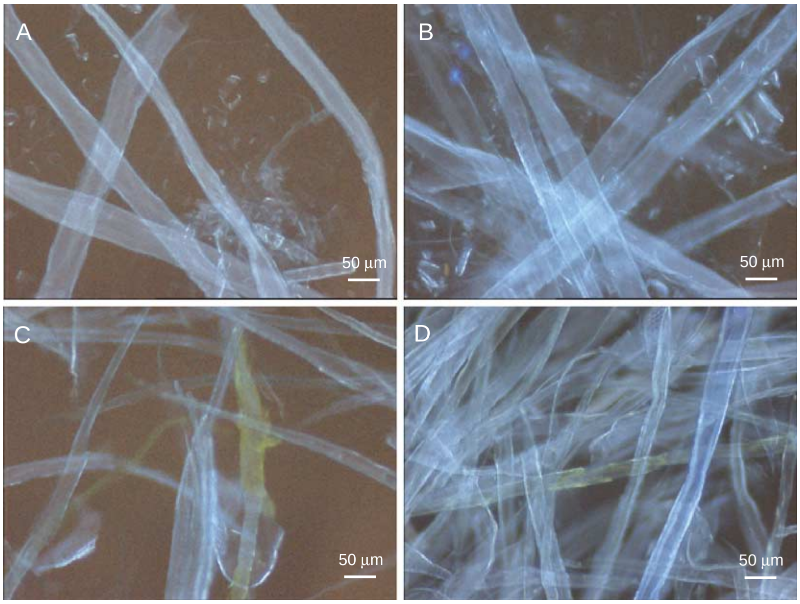

Fig. 6. Surface images of mechanical pulp fibers after incubation with XGO-FITC and hybrid aspen XET (PttXET16A). (A) SPRUCE-TMP-BLEACHED; (B) SPRUCE-TMP-UNBLEACHED; (C) ASPEN-CTMP-BLEACHED; D, ASPEN-CTMP-UNBLEACHED.

birch kraft fibers with adsorbed XG-FITC (Fig. 5(F)) indicate that xyloglucan binds across the fiber wall, with some preference for the surface. None of the pulps exhibited appreciable background green fluorescence due to the excitation of lignin at the wavelengths used for the analysis.

3.4. Natural xyloglucan on the surfaces of the mechanical pulps

It has been previously demonstrated that fluorescently labelled xyloglucan oligosaccharides can be used to co-localize XET enzyme activity and the substrate xyloglucan in the cell walls of developing wood fibers (Bourquin et al., 2002). In general, XGO conjugates, such as XGO-FITC, do not have a high affinity for cellulose and are readily removed by washing with water or aqueous buffers. However, once incorporated into an endogenous XG chain with a backbone of ca. 20 Glc units or larger by the action of a XET, the XGO effectively becomes permanently bound and can only be removed by extraction of the xyloglucan polysaccharide at high pH. This system thus provides a convenient method to localize the enzyme-accessible xyloglucan in plant material, including wood pulps. To determine whether there is a siginificant amount of xyloglucan accessible for in situ fiber modification using the XET technique, exogenous hybrid aspen XET (PttXET16A) (Bourquin et al., 2002; Kallas et al., 2005) and XGO-FITC were applied directly to mechanical pulp fibers. As shown in Fig. 6, no XET-accessible, natural xyloglucan was detected in bleached and unbleached spruce (softwood) TMP fibers (Fig. 6(A) and (B)), while only very small amounts were observed in bleached and unbleached aspen (hardwood) CTMP fibers (Fig. 6(C) and (D)). Indeed, the green fluorescence observed in the latter samples was very faint, as evidenced by comparison of the images in Fig. 6 with those in Fig. 5. It should be noted that non-optimal excitiation source was used for imaging all samples due to specific equipment limitations (See Section 2: source $\lambda_{\max}$ 365 nm, FITC $\mathrm{Ex}_{\max}$ 488 nm), so that the low or absent signal may be due in part to a poor detection limit. Nonetheless, the analysis implies that to achieve the highest possible density of modification of wood fiber surfaces using the XET/xyloglucan methodology (Brumer et al., 2004; Gustavsson et al., 2005; Zhou et al, 2005), the addition of exogenous xyloglucan is essential.

4. Conclusion

The present study expands current knowledge of the adsorbtion of the plant polysaccharide xyloglucan to a variety of industrially-important wood pulps used in paper and paperboard manufacture. Earlier work has demonstrated the effectiveness of xyloglucan as an additive to improve formation and increase various strength properties of sheets produced from northern softwood and tropical hardwood pulps (Christiernin et al., 2003; Lima et al., 2003). Here, the combination of surface chemical, adsorption kinetic, and fluorescence microscopic analysis has highlighted the importance of the surface properties of the pulp fibers on xyloglucan binding. In general, lower surface coverage of lignin and extractives is correlated with a potential to bind higher amounts of xyloglucan, presumably because of the presentation of a larger surface area of cellulose. It is anticipated that these studies will form a basis for the development of new materials based on wood fibers and xyloglucan derivatives. Indeed, the use of commerical pulps in this work, which are produced on multi-tonne scale, highlights the potential industrial applicability of xyloglucan-based cellulose modification.

Acknowledgements

This work was funded by the Swedish Agency for Innovation Systems (VINNOVA). The authors gratefully acknowledge helpful discussions with Cherryleen Garcia-Lindgren (M-real), Lars Sundström (Holmen), Fredrik Nilsson (Holmen), Mattias Johansson (Korsnäs). Additional financial contributions from M-real, Holmen, Korsnäs and SweTree Fiberzyme within the VINNOVA framework are also acknowledged. We thank Marie Ernstsson and Mikael Sundin (YKI, Ytkemiska Institutet AB) for performing ESCA and for assistance with data interpretation. Birgitta Lundgren (MoRe Research) is thanked for performing fluorescence microscopy and image analysis. M.J.B. is the recipient of pre-doctoral funding from the KTH Biofibre Materials Centre (BiMaC). Partial funding from the Swedish Foundation for Strategic Research (SSF) and the European Union (ECOTARGET, NMP2-CT-2004-500345) are also acknowledged.

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