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Effect of xylan structure on reactivity in graft copolymerization and subsequent binding to cellulose

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ABSTRACT Five xylans from hardwood and cereal sources were compared based on grafting
reactivity with glycidyl methacrylate (GMA). The structural property that best predicted the
reactivities of xylans with GMA was the fraction of 4-O-methylglucuronic acid (MeGlcA)
substitution. Comparatively high arabinose substitution was also positively correlated to
reactivity with GMA. The impact of MeGlcA and arabinose branching groups are likely

1 attributed to the solubilizing effect of these substituents. Consistent with this prediction, low
2 water solubility and high lignin content were found to hinder reactivity. Even though oligomeric
3 substrates have the advantage of water solubility, modified xylo-oligosaccharides were difficult
4 to purify. Accordingly, delignified and high molecular weight xylans that are soluble or
5 dispersable in water, are best suited for this type of backbone derivatization. Adsorption studies
6 with QCM-D indicated that grafting lowered the total adsorption of arabinoxylan, but did not
7 significantly affect the fraction of xylans adsorbed irreversibly on cellulose.

8 KEYWORDS xylan, biomass characterization, graft copolymerization, QCM-D

9 **Introduction**

10 Hemicelluloses are one of the three main components in plant biomass besides cellulose and
11 lignin, comprising 25-35% of lignocellulosic materials. So far it has been a relatively underutilized
12 side stream of pulp mills, especially in dissolving pulp production.^{1,2} Due to its abundance,
13 hemicellulose represents a highly promising raw material for bio-based chemicals and polymeric
14 materials.

15 Xylan comprises a particularly large fraction of the hemicellulose produced by vascular plants.
16 The backbone structure of xylan consists of β -D-(1 $\rightarrow$ 4)-Xylp, which can be substituted depending
17 of the plant resource (eg. cereal, hardwood, softwood). For instance, the xylan backbone in
18 secondary cell walls of hardwoods is substituted with α -(1 $\rightarrow$ 2)-linked 4-O-methyl-glucuronic acid
19 (MeGlcA) and can be acetylated at O2 and O3 positions of xylose. A typical ratio of
20 xylose:MeGlcA:acetyl groups in glucuronoxylan is 10:1:7.³⁻⁵ In conifers, xylan is primarily
21 composed of arabinoglucuronoxylan (AGX), where the xylan is substituted with both α -L-
22 arabinofuranose and 4-O-methyl-glucuronic acid residues at position O2 and O3 of xylose,

1 respectively, with a typical arabinose:glucuronic acid:xylose ratio of 1:2:8.⁶ Xylan from grasses
2 and straw is mostly glucuronoarabinoxylan (GAX), which differs to AGX by having lower
3 glucuronic acid content.¹ Finally, arabinoxylan, is the main xylan type in cereal grains, and is
4 characterized by arabinose substitutions at O2 and O3 positions of xylose; the extent of substitution
5 varies considerably, with up to 30% arabinose content in wheat arabinoxylan.^{1,7}

6 Efforts to valorize xylan include applications in both food and non-food products. Xylan-based
7 packaging and coating materials have also garnered renewed interest particularly as high-value co-
8 products from biofuel production. In this context, Hansen and Plackett⁸ reviewed the wealth of
9 literature on biomaterials from hemicellulose, including the strength characteristics and oxygen
10 barrier properties of xylan films and edible coatings, as well as swelling behaviors and rheological
11 properties of xylan hydrogels. As they note, the hydrophilic characteristics of xylans have been
12 modified through esterification, etherification and grafting reactions.⁸ Moreover,
13 hydroxyalkylation can assist the recovery as well as use of xylans in barrier coatings.⁹ Given its
14 natural affinity to cellulose, both native and derivatized forms of hemicellulose have been used to
15 coat cellulose fibrils and thereby improve the tensile strength and wettability of cellulose-based
16 materials,^{10,11} or to introduce functionalities suitable for click-chemistry reactions.¹² For such
17 modification it is important that the adsorption capability of xylan is not severely hindered by the
18 modification.

19 Due to having similar backbone structures, methods used to chemically modify cellulose are
20 often applicable to hemicellulose as well. Methods like graft copolymerization using free radical
21 or redox initiation,¹³⁻¹⁵ and controlled radical systems such as SET-LRP¹⁵ have been used
22 successfully with hemicelluloses. Nevertheless, it is anticipated that product yield and reaction
23 selectivity will vary according to the occurrence of different backbone sugars and branching

groups as they affect the solubility of xylan, and the number of hydroxyl groups available for the reaction. Although the structure of several hemicellulose types has been characterized, the possible differences in reactivity deriving from the structural characteristics have not yet been determined. This information could be helpful when choosing an optimal feedstock or isolation method to produce material for xylan derivatization.

The objective of the current study was to investigate the effect of xylan composition on the reactivity in chemical derivatization. Specifically, we analyzed five xylan sources (corn cob, oat spelt, wheat, birchwood, and beechwood), and modified them through graft copolymerization using identical conditions (concentrations of xylan, initiator, and monomer, temperature, reaction time) to establish correlations between xylan composition and reactivity. In particular, differences in graft yield and selectivity were correlated to the differences in sugar composition, molar mass, and presence of particular impurities, including cellulose, lignin, proteins, and inorganic compounds. Due to the lack of previously published information, we also studied the effect of polymer grafting on the adsorption of xylans to cellulose with a quartz crystal microbalance with dissipation (QCM-D).

A redox initiated free radical polymerization was performed through ceric ion initiation, a well-known method for modifying cellulose.^{16,17} According to the proposed mechanism, the initiator chelates with and oxidizes hydroxyl groups, creating radicals on the carbon backbone in the process.¹⁸ This mechanism ensures that the radicals are formed selectively on carbohydrates and minimal amount of homopolymerization occurs. In addition to being highly selective for graft copolymerization, the method works in aqueous solutions under mild conditions and low temperatures, making it environmentally benign.¹⁷

Glycidyl methacrylate (GMA) was selected as the monomer for the grafting experiments, since we have found it to be very reactive with nanofibrillated cellulose and therefore reasonable reactivity with other polysaccharides was also expected.¹⁷ GMA has also been grafted from macroinitiators¹⁹ and macromonomers²⁰ prepared from hemicelluloses. Additional benefit, considering our method, is that the monomer does not contain hydroxyl groups that could compete with the xylan as sites of initiation. GMA grafting also creates sites for further chemical modification. For instance, since epoxy groups readily react with amino and thiol groups, they can be used to introduce various functional groups²¹ or to immobilize enzymes²².

Experimental section

Materials

Birchwood glucuronoxylan was purchased from Sisco research laboratories (Mumbai, India), beechwood glucuronoxylan from Sigma-Aldrich (St. Louis, MO, USA), corn cob xylan from Intrade (Germany), oat spelt xylan from Molekula (UK), and wheat arabinoxylan from Megazyme (Ireland). Glycidyl methacrylate (GMA), cerium(IV) ammonium nitrate (CAN), sodium chlorite, and acetic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA), and methanol and tetrahydrofuran (THF) from VWR International (Radnor, PA, USA). Ethanol was purchased from Altia (Finland).

Water solubility

Water solubility of the xylans was determined by preparing a 5 wt% mixture of each xylan in deionized water. A relatively high concentration, compared to the grafting experiments, was selected in order to increase the amount of insoluble solids and thus make their separation easier. Mixtures were stirred at ambient temperature for 5 h and centrifuged for 30 min at 4000 rpm. The

precipitate was dried overnight in vacuum oven at 60 °C and weighed. The water solubility was then calculated from the weight of the insoluble fraction and the volume of added water.

Sugar composition analysis

The amounts of different sugars in the xylans were determined by acid methanolysis of the freeze-dried xylans.^{23,24} Two calibration solutions with equal amounts of analyzed monomeric sugars; arabinose, galactose, glucose, galacturonic acid, glucuronic acid, mannose, rhamnose, and xylose were used. Calibration solutions were used to calculate the response factor for each sugar, except 4-O-MeGlcA, whose response factor was calculated from glucuronic acid. Samples and calibration solutions were treated similarly during analysis. Samples were depolymerized with acid methanolysis, using 2 mL of 2 M HCl in anhydrous methanol and they were kept at 100 °C for 3 h. Samples were cooled to room temperature and were neutralized with 200 µL of pyridine. After neutralization, 1 mL of methanol solution containing 0.1 g L⁻¹ of sorbitol and 0.1 g L⁻¹ of resorcinol were added as internal standards to the samples. Samples were dried first under a nitrogen flow and then in a vacuum oven at 40 °C for 15 min. Samples were then silylated by adding 100 µL of pyridine, 150 µL of hexamethyldisilazane (HMDS), and 70 µL of trimethylchlorosilane (TMCS). After overnight silylation, the samples were analyzed using a Shimadzu GC-2010 (Shimadzu, Kyoto, Japan) gas chromatograph, equipped with a HP-1 column (25 m, 0.2 mm, 0.11 µm). The results were calculated as anhydrosugars by multiplying arabinose and xylose by 0.88; rhamnose by 0.89; glucose, mannose, and galactose by 0.9; and glucuronic acid, galacturonic acid, and 4-O-methylglucuronic acid by 0.91. Samples were analyzed four times.

NMR analysis

Approximately 5 mg of each xylan sample was dissolved in 0.7 mL of (CD₃)₂SO and then analyzed using a Bruker Avance III 400 MHz NMR spectrometer. The ¹H spectra were measured

at 70 °C, using 10 s relaxation delay and 32 pulses. The residual solvent peak at 2.50 ppm was used for calibration. The degree of acetylation was determined from ^1H spectra by using the ratio between peaks at 2.00 and 4.28 ppm, which were assigned to the acetyl group and the anomeric proton in xylose, respectively.²⁵

Identification of impurities

The cellulose, lignin, protein, and ash content in each xylan sample were evaluated to account for the impact of impurities on graft efficiency. Specifically, cellulose content was determined using the acid hydrolysis method as previously described.²⁶ Briefly, 10 mg of each sample or cotton linters calibration standard (Sigma Aldrich) were placed in 25 mL test tubes and hydrolyzed with 72 % sulfuric acid. After hydrolysis the hydrolysates were neutralized with BaCO_3 and 1 mL of sorbitol (5 mg mL^{-1}) was added to samples as an internal standard. Samples were silylated overnight and analyzed with a GC-2010 gas chromatograph (Shimadzu, Kyoto, Japan) using a HP-1 column (25 m, 0.2 mm, 0.11 μm). Samples were analyzed in duplicate, and the results were calculated as anhydrosugars. Since acid methanolysis can only cleave amorphous regions of cellulose, whereas acid hydrolysis can completely depolymerize cellulose to glucose, the amount of crystalline cellulose in each sample can be estimated by subtracting the amount of glucose determined through acid methanolysis from the amount of glucose determined through acid hydrolysis.

Protein content was evaluated using a CHN elemental analyzer (CHN-1000, LECO, USA). Between 100-150 mg of sample was weighed in a tin foil cup. Samples were then incinerated at 1050 °C in oxygen flow, and the released nitrogen was measured using a thermal conductivity cell. The percentage of nitrogen was converted to protein content by multiplying it by 5.7 for wheat xylan, and by 6.25 for all other xylans.²⁷

To estimate lignin content in the small sample quantities, 10 mg of each xylan sample was diluted in 0.1 M NaOH, and then the UV absorbance of the solubilized fraction was measured at 280 nm (Shimadzu UV-2401PC, Kyoto, Japan). The amount of lignin was calculated with an extinction coefficient $19.3 \text{ Lg}^{-1}\text{cm}^{-1}$, obtained from birch lignin extracted by pressurized hot water²⁸; analyses were done in duplicate.

The ash content in each sample was determined by thermogravimetric analysis (TGA) using a TA Instruments TGA Q500 (New Castle, DE, USA). Approximately 5 mg sample of each xylan was heated first to 105 °C in nitrogen atmosphere, and kept isothermal for 20 min to remove moisture. After drying, the sample was heated to 650 °C at $10 \text{ }^{\circ}\text{C min}^{-1}$, and kept isothermal in oxygen atmosphere for 20 min before cooling back to ambient temperature. The analysis was done in triplicate, and the ash content was calculated by dividing the residual mass with the dried mass.

Molecular mass

Molecular weights of xylans were determined by size exclusion chromatography (SEC) equipment including a single Ultrahydrogel 250 Å column from Waters (Milford, MA, USA). The effluent was monitored with a refractive index (RI) detector and Wyatt DAWN8+ multi-angle laser light scattering (MALLS) detector. Since most of tested xylans had limited solubility in water, aqueous sodium hydroxide solution (10 mM, pH 12) was used for elution at 1.0 mL min^{-1} , and the calibration curve was obtained with seven poly(ethylene oxide) (PEO) standards in the range of 25 to 1190 kDa. A sample of each xylan was dissolved in 10 mM NaOH solution at the approximate concentration of 1 g L^{-1} , and passed through a $0.45 \text{ }\mu\text{m}$ filter prior to the measurement. Light-scattering data was processed with Astra V software, using a dn/dc value of 0.145 mL g^{-1} obtained from literature.²⁹

1 Since the molecular weight of corn cob xylan was below the detection threshold of this SEC, it
2 was measured with a high-performance size exclusion chromatography (HPSEC) equipment using
3 an Agilent 1260 Infinity system (Agilent, Germany), equipped with three Ultrahydrogel (Waters)
4 size exclusion columns (500 Å, 250 Å, and 120 Å) in series. Column effluent was monitored with
5 an RI detector. The system was calibrated using xylose, raffinose, polyethylene glycol, and PEO
6 standards in the molar mass range of 0.15 to 115 kDa. Elution was performed at 30 °C column
7 temperature at 0.5 mL min⁻¹, using deionized water containing 0.02% NaN₃. The sample was
8 dissolved in deionized water and passed through a 0.45 µm PTFE filter prior to the measurement.

9 **Delignification**

10 Due to its comparatively high lignin content, the reactivity of oat xylan was also tested after
11 delignification by chlorine dioxide bleaching using a method adapted from elsewhere.³⁰ Briefly,
12 oat xylan (1.5 g) was dispersed in distilled water (125 mL). Sodium chlorite (0.5 g), and glacial
13 acetic acid (1 mL) were added, and the mixture was refluxed for 2 h at 70 °C. Bleached xylan was
14 precipitated with ethanol using 1:4 water-ethanol ratio, and washed three times by dispersion and
15 centrifugation in ethanol. The pellet was dispersed in water, frozen with liquid nitrogen, and
16 freeze-dried to yield off-white product.

17 **Graft copolymerization**

18 Xylan (0.32 g) was dispersed in deionized water (150 mL) in a round-bottom flask. The solution
19 was agitated by magnetic stirrer at 400 rpm, and nitrogen was bubbled through the solution for at
20 least 30 min to remove dissolved oxygen. Dissolved CAN (0.16 g, 0.30 mmol) was injected, and
21 the flask was heated with an oil bath to 35 °C. After 15 min, GMA (0.91 g, 6.4 mmol) was injected,
22 and the reaction was carried out for 60 min. After synthesis, the reaction medium was centrifuged
23 to obtain the non-soluble fraction, and precipitated with ethanol using 1:4 water-ethanol ratio to

obtain the water-soluble fraction. The separated solids were washed by dispersion and centrifugation in ethanol (repeated three times) and then THF (repeated three times). This THF solution was then precipitated with methanol to separate the homopolymer. The washed copolymer and homopolymer were dried in vacuum at 30 °C overnight and weighed. Each experiment was repeated at least once. The relative amount of grafted polymer (graft yield, G%) was calculated using formula 1.

$$G\% = \frac{m_{NS} + m_{WS} - m_X}{m_X} \times 100\% \quad (1)$$

where m_{NS} is the mass of the nonsoluble product, m_{WS} is the mass of the water-soluble product, and m_X is the mass of xylan.

Since some homopolymerization was expected, the selectivity of the polymerization reaction was evaluated by calculating the copolymer fraction of all formed polymer (i.e. the graft efficiency (GE%)) using formula 2.

$$GE\% = \frac{m_{NS} + m_{WS} - m_X}{m_{NS} + m_{WS} - m_X + m_{HP}} \times 100\% \quad (2)$$

where m_{HP} is the mass of the homopolymer.

The grafting was verified by Fourier transform infrared (FTIR) spectroscopy. Infrared spectra of the starting materials and all products were recorded with a Nicolet 750 Magna device as an attenuated total reflection (ATR) measurement to confirm the presence of new functional groups after modification.

QCM-D analyses

Adsorption of arabinoxylan (wheat) and glucuronoxylan (birch) on regenerated cellulose surfaces was studied before and after graft copolymerization. The model surfaces were prepared according to a method described elsewhere.³¹ Briefly, SiO₂ sensors (Biolin Scientific, Sweden) were spin-coated with trimethylsilylcellulose (TMSC, DS 2.3³¹) dissolved in toluene (10 g L⁻¹),

and the TMSC surfaces were then regenerated with 10% HCl vapor. Measurements by quartz crystal microbalance with dissipation (QCM-D) were performed with a Q-Sense E4 instrument (AB, Västra Frölunda, Sweden) with controlled flow. The water-soluble copolymer fractions were used to represent the grafted xylans. The samples were dissolved in deionized water at a concentration of 0.5 g L⁻¹, and pumped at constant rate of 0.1 mL min⁻¹ through the measurement chambers. The polysaccharide solutions were added after first acquiring a stable baseline with deionized water. The fundamental resonance frequency was 5 MHz, and the overtones at 15, 25, 35, 45, 55, and 65 MHz were recorded. Adsorbed masses were calculated from the 7th overtones with the Sauerbrey equation (3), and all experiments were done in triplicate.

$$\Delta m = -\frac{C\Delta f}{n} \quad (3)$$

where C is the device sensitivity constant (0.177 mg s m⁻², reported by manufacturer), Δf is the change in the sensor's resonance frequency, and n is the overtone number.

Results and discussion

Xylan composition

The sugar composition of xylan samples (Table 1) was determined by acid methanolysis to then correlate the type and extent of sugar branching with the chemical reactivity of each xylan source. Methanolysis was selected as it facilitates selective depolymerization of hemicellulose and less degradation of resulting monosaccharides compared to acid hydrolysis.²³ The type and extent of sugar branching within the commercial xylan samples used herein were consistent with previously reported values.^{4,5,32} Judging by the sugar composition, wheat xylan had superior purity compared to the other xylan sources containing less than 0.5% of carbohydrates unrelated to xylan, while the corn cob xylan contained approximately 15% of impurities from other polysaccharides. Most significant impurities were probably pectins (showing as rhamnose and galacturonic acid) and

starch (showing as glucose).²³ Oat xylan was re-analyzed after the delignification to determine possible effects on sugar composition. Apart from a slight increase in branching sugars, the chlorite delignification did not affect the sugar composition of oat xylan, which was in fact closer to the reference values than the composition of untreated oat xylan.

Table 1. Measured sugar compositions and acetylations of selected xylan sources compared to published reference values.

Measured	Birch	Beech	Corn	Oat	Bleached oat	Wheat
Sugars (mol%)						
Mannose	tr.	0.1	0.4	0.1	0.1	tr.
Glucose	1.4	2.1	11.1	0.9	0.8	0.4
Galactose	0.6	1.3	3.3	0.5	0.5	0.1
Xylose	94.6	89.8	81.1	89.3	85.2	65.4
Arabinose	0.1	0.5	3.8	6.9	9.4	34.2
Rhamnose	0.2	1.2	0.1	0.1	0.1	n.d.
Glucuronic acid	0.1	0.1	tr.	0.1	n.d.	n.d.
Galacturonic acid	0.1	1.3	tr.	0.1	1.1	n.d.
4-O-Me-GlcA	3.0	3.7	tr.	1.9	2.8	n.d.
Xylan% ^a	98	94	85	98	97	100
% Acetylated ^b	none	none	3.1	none	none	none
Literature values						
Arabinose	tr. ^c	1 ^c	3-32 ^d	10 ^e	N/A	34 ^f
4-O-Me-GlcA	4.5	4	0-10	4	N/A	0
% Acetylated ^b	38	40	low	low	N/A	low

tr, trace amount (<0.05%); n.d., not detected; N/A, not available.

^a Fraction of xylan-related sugars (Xyl, Ara, MeGlcA) of all detected sugars.

^b Number of acetyl groups per 100 xylose units.

^c Extracted with DMSO.⁵

^d Extracted with 5% NaOH.³³

^e Unknown extraction method.⁴

^f Alkali-extracted.⁴

The degree of acetylation (DA) of the xylans was determined by quantitative ¹H NMR measurements (Figure S1). Specifically, the DA was calculated from the ratio of peak areas corresponding to methyl protons of acetyl groups (1.95 to 2.05 ppm) and the anomeric proton of xylose (4.25 to 4.35 ppm). Acetylation was detected only in the corn cob xylan which had a DA similar to previously published analyses.³² Despite showing no acetylation, a strong singlet at 1.67 ppm, matching sodium acetate,³⁴ was present in the NMR spectra of oat and wheat xylans. This suggests that free acetyl groups released during the extraction of these xylans remained in the material; similar impurities are known to exist in crude alkaline extracted oat spelt xylan.³⁴ Although native forms of birchwood and beechwood glucuronoxylans have high DA,⁵ corresponding commercial preparations were completely free of acetyl side groups, typical of alkaline extracted xylan.²⁴

In addition to determining total sugar composition and extent of acetylation, the presence of impurities in each xylan sample was evaluated (Table 2). Assuming all sugars released through methanolysis originated from hemicelluloses, the combined fraction of all impurities was lowest in corn xylan (3.2%) and highest in oat xylan (10.3%). This range in purity likely reflects the extraction method used to recover xylan from respective plant sources.³³ The relatively high amount of lignin residue in oat xylan was successfully removed by chlorite bleaching, the remaining amount being undetectably low. Notably, methanolysis of delignified oat xylan gave

over 100% calculated yield, indicating complete depolymerization of the xylan. Ash content was also significantly lower after the delignification, showing that inorganics probably did not coprecipitate with xylan.

Table 2. Overview of xylan compositions.

Xylan type	Hemicelluloses ^a	Cellulose ^b	Lignin	Ash	Protein	Total analyzed
Birch	69.9	tr.	2.0	4.1	0.4	76.4
Beech	73.4	tr.	2.8	5.7	n.d.	81.9
Corn	89.0 ^c	1.8	1.1	0.3	n.d.	92.1
Oat	77.9	0.1	4.0	6.2	n.d.	88.2
Bleached oat	114	n.d.	n.d.	1.6	n.d.	115.6
Wheat	84.9	n.d.	0.3	3.5	2.3	91.2

^a Fraction depolymerized by methanolysis, including all non-cellulosic polysaccharides.

^b Presented as anhydrosugars.

^c Acetyl groups included.

Molecular mass and solubility

SEC confirmed that the molecular weight of corn xylan was significantly lower than the other xylans. In fact, 93% of the sample consisted of oligosaccharides having a degree of polymerization (DP) between 2 and 6. There was a small peak at approximately 8 kDa, representing DP of 63 and comprising 5.2% of the sample. Birchwood and beechwood samples had almost identical bimodal distributions, with the major peaks having M_w of 67 and 65 kDa, and the smaller peaks having respective values of 710 and 900 kDa. By contrast, the bimodal distribution of wheat xylan had a major peak at 530 kDa and a minor peak at 8.6 kDa. SEC analysis of oat xylan revealed a unimodal distribution with M_w of 65 kDa, and showed that the bleaching treatment caused a small shift in this main peak to 42 kDa. Notably, the bleaching treatment of oat xylan also led to a new peak in

1 the same region as birch and beech aggregate peaks, with M_w of 1010 kDa. This coincided with
2 the formation of an opaque supernatant during solubility measurements, and an overall twofold
3 increase in the water solubility of oat xylan. Together, these results are consistent with
4 delignification causing some chain scission as well as release of xylan aggregates that were
5 previously bound by lignin. MALLS analyses further confirmed the presence of high molecular
6 mass fractions in each xylan sample. However, in comparison with values measured by SEC, the
7 calculated M_w values obtained using MALLS were multiple times higher (11000 and 13000 kDa
8 for the hardwood xylans, 7500 kDa for bleached oat xylan, and 1800 kDa for wheat xylan),
9 presumably due to higher contributions from larger xylan aggregates.

10 Considering that grafting is likely to occur on all xylan fractions, Table 3 reports average
11 molecular weights calculated from entire xylan distributions determined by SEC. The weight
12 average molar masses of the main fractions from the hardwood xylans used herein were 4-8 times
13 higher than those previously reported.^{5,35} This could reflect the different extraction methods used
14 to isolate respective xylan samples. Alternatively, differences in weight average molar masses
15 could be partially explained by our use of PEO standards versus earlier assessments by mass
16 spectrometry. By contrast, the measured molar mass of corn xylan was significantly lower than
17 those previously reported: 87 kDa³⁶, 150 kDa³⁷, and 130-137 kDa³⁸. Though not specified by the
18 commercial provider, the low molecular weight of corn xylan used herein is consistent with having
19 been recovered from an acidic pretreatment.⁶ Results for oat spelt and wheat xylans were of the
20 same magnitude as the values reported by other groups: 90 kDa³⁹ and 167-290 kDa⁴⁰, respectively.

21 Finally, birchwood, beechwood, and wheat xylans had rather high polydispersity indices (PDI)
22 due to comprising two separate size fractions (Figure 1, Table 3). Oat xylan had a lower PDI due
23 to its unimodal character, and corn xylan was nearly monodisperse (PDI ~1) due to its very short

chain length. Even though the new aggregated fraction in bleached oat xylan significantly increased its PDI, the distribution of the main fraction was actually more narrow than before the treatment.

Figure 1. Molecular weight distributions of selected xyans measured by SEC. The RI detector responses (y axis) were normalized according to the highest peak.

Table 3. Molar mass parameters and water solubilities of selected xylan sources.

Xylan source	M _n (kDa)	M _w (kDa)	PDI	DP ^a	Solubility (g L ⁻¹) ^b	Appearance
Birch	43.6	204	4.7	330	21	Clear, brown
Beech	40.3	171	4.3	305	46	Cloudy, brown
Corn	0.892	1.20	1.3	7	>>50 ^c	Clear, faint yellow
Oat	25.5	65.1	2.5	193	12	Cloudy, brown
Bleached oat	33.2	273	8.2	251	24	Cloudy, white
Wheat	50.4	492	9.8	381	>50 ^d	Clear, faint yellow

M_n, number average molar mass; M_w, weight average molar mass.

^a Estimated for a pure xylose chain.

^b Dissolved amount in 5% mixture.

^c Multiple amount could be easily dissolved.

^d Added xylan dissolved without leaving visible residue.

The water solubility of each xylan sample was determined since it reflects the accessibility of individual xylan molecules to the reactants. Ideally, if the substrate dissolves completely, each xylan chain is available for the reaction. If the substrate is not totally soluble and forms a dispersion instead, the solubility can still be used to evaluate the relative accessibility of different xylans since higher solubility likely also indicates higher swelling of the dispersed particles. While there was no apparent increase in the viscosity of most xylan solutions, the wheat xylan solution was comparatively viscous and visibly shear-thinning, which is typical behavior for concentrated polymer solutions.⁴¹ The low viscosity of beech and birch glucuronoxylan solutions, despite their high molecular weights, supported our assumption that they were dissolved as aggregates rather than single molecules. The increased viscosity of polymer solutions is caused by chain entanglements, which are less likely to form between aggregates since they occupy significantly less volume than free chains at the same concentration.

When considering the role of solubility in the reaction, it is important to note that although much lower xylan concentration was used in the grafting reactions, oat xylan retained a clearly insoluble fraction and the high solubility of the corn xylan was facilitated by its comparatively low DP. With the other xylans, no clear correlation was observed between molar mass and water solubility.

Differences in xylan reactivity

All xylans were copolymerized in an aqueous system using identical reaction conditions. GMA was used as the monomer since it is known to react readily with nanofibrillated cellulose in similar conditions.¹⁷ The reaction was carried out in an aqueous environment to ensure good dispersion of the xylans and to avoid the use of organic solvents when possible. The yield of the grafting reaction was determined gravimetrically and the results were correlated to the structural features of the xylans described above.

The overall reactivities of xylans and their selectivities in copolymerization are shown in Table 4, represented by graft yield and efficiency, respectively. There were clear differences in the reactivity of the xylan samples, where the relative amount of copolymer formed ranged from 8% to 165% of the mass of the starting xylan. Beechwood xylan had the highest reactivity with GMA, while also birchwood and wheat xylans exhibited high reactivity with graft yields above 140%. Very little copolymer could be isolated from reactions with the corn xylan. Bleaching had a remarkable effect on the reactivity of oat xylan, resulting in graft yields that were nearly tripled. Furthermore, copolymerization was favored over homopolymerization of GMA with all xylan types except corn. The copolymers of birch, beech, and oat xylans were highly insoluble, more than 90% being insoluble in both water and THF. Our interpretation was that the grafted polymer rendered birch and beech glucuronoxylans sufficiently hydrophobic to precipitate out of water, whereas the polysaccharide component reduced copolymer compatibility with THF. Wheat xylan, which was highly soluble despite having comparatively high DP, retained its hydrophilicity even after modification with GMA, therefore yielding the soluble copolymer as the major product. Corn xylan produced roughly equal, yet extremely small, amounts of both copolymer types.

Figure 2. FTIR spectra of grafted wheat arabinoxylan products. NS = non-soluble copolymer, WS = water-soluble copolymer, HP = homopolymer (PGMA).

Successful grafting was confirmed by FTIR analysis of the products (Figure 2), which revealed a strong and characteristic absorption band for carbonyl groups at 1725 cm⁻¹ that was absent in the unmodified xylans. Grafted PGMA was detected in all THF-insoluble fractions, as well as in the water-soluble birch and wheat xylan products. Copolymer products from corn xylan could not be analyzed by FTIR due to insufficient yields.

Table 4. Graft yields and efficiencies, and distributions of the products in water-soluble and THF-insoluble fractions.

Xylan source	% Graft yield			% Graft efficiency			% Insoluble ^a	% Water-soluble
Birch	155	±	34	64	±	5	99.7	0.3
Beech	165	±	21	62	±	13	93.2	6.8
Corn	7.6	±	11	17	±	24	48.0	52.0
Oat	46	±	11	83	±	8	94.9	5.1
Bleached oat	131	±	7	78	±	5	98.4	1.6
Wheat	136	±	11	89	±	5	8.3	91.7

^a Insoluble in both water and THF.

Previous studies on redox initiated graft copolymerization of xylans are scarce, making it difficult to directly compare our results with other examples. However, similar synthesis routes have been applied earlier with other hemicelluloses and acrylic monomers. For example, graft yields of 122% and 77% were achieved when using a xylan-glucomannan mixture from larchwood with methyl acrylate and acrylonitrile, respectively. In the same study, a similar grafting experiment with a wheat arabinoxylan and acrylonitrile resulted in 43% graft yield.⁴² Graft yields are known to vary significantly depending on the monomer used,¹⁶ however to our knowledge, studies using redox initiation with GMA and hemicellulose have not been published. Still, graft yields of cerium initiated copolymerization between GMA and cellulose have been reported

1 between 60% on cellulose filter paper²² and 100% on cotton fabric¹⁶. In the former case, also graft
2 efficiency as high as 85% was reported.²² Our own previous study of cerium initiated
3 copolymerization between GMA and nanofibrillated cellulose (NFC), along with same reaction
4 conditions used herein, gave a graft yield of 150% and graft efficiency of 96%.¹⁷ The higher
5 reactivity of both NFC and xylans used herein compared to macroscopic cellulose fibers can be
6 explained by the high specific surface area of NFC and the solubility of xylans, which makes them
7 more accessible than macroscopic cellulose fibers.

8 **Impact of xylan composition on xylan reactivity with GMA**

9 When reviewing the reactivities of different xylans versus their substitution with different sugar
10 branches, a correlation was observed between increasing reactivity and branching degree. The
11 effect of MeGlcA branching (Figure 3) was especially clear and manifested already at a very low
12 DS. However, the high arabinose substitution of wheat xylan also correlated with its high
13 reactivity. We believe that the enhanced reactivity was due to the solubilizing effect of the
14 branching substituents, which effectively minimize stacking between xylan chains.⁴³ The negative
15 charge imparted by MeGlcA moieties in birch and beech glucuronoxylan may have increased graft
16 yield through two potential mechanisms. On the one hand, the presence of MeGlcA likely
17 promotes the swelling of xylan in water, and on the other hand MeGlcA groups may attract the
18 cationic initiator ions. The increased accessibility due to swelling would also explain the high
19 reactivity of birch, beech, and bleached oat xylans despite their limited solubilities.

Figure 3. Reactivity of different xylan sources as a function of the degree of 4-O-Me-glucuronic acid (a) and arabinose (b) substitution, and weight average molecular mass (c). Selectivity of the grafting is shown as a function of weight average molecular mass (d). Experimental error was evaluated by standard deviation.

Despite the variable amount of impurities detected in the starting materials, neither the purity nor the total carbohydrate content of the xylans could be correlated to xylan reactivity with GMA. An exception was the higher lignin content in oat xylan compared to the other xylan types. In addition to impacting solubility, lignin can decrease the solubility of xylans when present in significant amounts. Moreover, high lignin content has been shown to inhibit the cerium initiated

grafting on hemicelluloses.⁴² A plausible explanation for the inhibition is competition between the phenolic hydroxyl groups in lignin and carbohydrate hydroxyls for cerium oxidation. Unlike the highly reactive sugar chain radicals, the radicals formed on lignin are less likely to initiate polymerization since they are stabilized by delocalization on the aromatic ring.⁴⁴ To investigate this possibility, experiments were performed also with bleached oat xylan, resulting in increased graft yield while graft efficiency remained similar to unbleached oat xylan. Although significantly lower than the 11% reported by Fanta et al.⁴², the lignin content in oat xylan was evidently high enough to interfere with the graft copolymerization.

Impact of physical characteristics on xylan reactivity with GMA

A comparison of xylan solubility revealed the expected impact on reactivity only in the case of oat xylan. Instead, a clearer trend was observed between molar mass and grafting reactivity (Figure 3). The graft yield was clearly lowest with the oligomeric corn xylan, and increased with M_w before reaching a plateau at approximately 200 kDa. Although the molar mass of the substrate is not known to affect cerium oxidation, it may affect the separation efficiency of copolymer and homopolymer. Long polymer grafts on very short oligosaccharides may cause the product to behave more like the corresponding polymethacrylate rather than the carbohydrate. As a result, since the product separation was based on solubility differences, most of the corn xylan copolymer may have ended up in the homopolymer fraction, making the graft yield appear artificially low. Unreacted oligosaccharides most likely remained soluble in the ethanol precipitation and were discarded with the solvent. This prediction was supported by the additional correlation observed between molar mass and graft efficiency (Figure 3), and the unusually high amount of homopolymer isolated from corn xylan products (Table 4). Similar findings were reported in an earlier study, where the fraction regarded as homopolymer from wheat straw xylan / acrylonitrile

copolymerization was found to contain up to 57% hemicellulose.⁴² It is noteworthy that the correlations were not observed with the unaggregated molecular weights, suggesting that aggregates had an important role at facilitating the copolymer separation. Importantly, although the yield of copolymers containing oat xylan was low, the corresponding graft efficiency was above 80% and second only to wheat arabinoxylan. This suggests that while the low solubility of oat xylan hindered the copolymerization reaction, it may have facilitated copolymer recovery. The fact that graft efficiency remained high after bleaching may indicate that the effect of increased solubility was offset by the formation of large aggregates.

Association of modified xylans to cellulose

The effect of grafting on the adsorption of xylans to cellulose surfaces was determined by QCM-D measurements. The affinity to cellulose is a key property when evaluating the suitability of a polymer for fiber coating applications. Birchwood glucuronoxylan and wheat arabinoxylan before and after modification were chosen for comparison, and adsorbed masses (Table 5) were calculated using formula 3.

Table 5. Adsorbed masses and the fraction of unmodified and grafted birchwood and wheat xylans.

Xylan type	Adsorbed mass (mg m ⁻²)			
	initial adsorption		after rinsing	
Birch glucuronoxylan	0.93	± 0.11	0.64	± 0.11
Birch glucuronoxylan+PGMA	1.16	± 0.12	0.68	± 0.12
Wheat arabinoxylan	1.25	± 0.16	0.87	± 0.15
Wheat arabinoxylan+PGMA	0.89	± 0.17	0.56	± 0.09

1 Even though highly substituted arabinoxylan has been shown to adsorb onto cellulose less
2 efficiently compared to less substituted xylans,¹⁰ unmodified wheat arabinoxylan adsorbed most
3 readily on the regenerated cellulose surface, probably due to its lack of charge and comparatively
4 high molecular weight.⁴⁵ Moreover, the slight negative charge of cellulose in water may have led
5 to electrostatic repulsion between the cellulose surface and the less substituted glucuronoxylan. In
6 fact, it has already been established that the overall charge of xylan molecules influences affinities
7 to cellulose. For example, in a recent study, cationized birch glucuronoxylan was shown to adsorb
8 readily on regenerated cellulose, whereas the unmodified variety only adsorbed when salt was
9 added to mask electrostatic repulsions.⁴⁶ Significantly higher adsorption of birch glucuronoxylan
10 xylan to NFC surfaces was also observed in low pH buffer, which would reduce the fraction of
11 charged MeGlcA groups.⁴⁷

12 Grafting with PGMA decreased the total adsorption of wheat arabinoxylan on cellulose, possibly
13 due to grafting from arabinose side groups. By contrast, virtually no difference was observed
14 between the adsorption of the ungrafted and grafted birchwood xylan (Table 5). In fact, the slight
15 increase in adsorption by grafted birch xylan suggests that the occurrence of PGMA may have
16 reduced electrostatic repulsions between birch xylan and cellulose. Notably however, the
17 adsorption of both xylan types was approximately 70% irreversible before grafting and 60% after
18 grafting, indicating that grafting did not substantially affect the fraction of irreversibly adsorbed
19 glucuronoxylan or arabinoxylan.

Figure 4. Change in dissipation as a function of change in normalized frequency (7th overtone) for ungrafted and grafted xylans (0.5 g L⁻¹) adsorbed from water on regenerated cellulose surfaces.

The slope of change in dissipation (D) versus change in frequency (f) gives information about the viscoelastic properties of the adsorbed layer, where a higher slope indicates a more extended conformation of the adsorbed chains, resulting in a softer, more viscoelastic surface layer (Figure 4).⁴⁸ In this respect, the data as plotted in Figure 4 suggest that the wheat arabinoxylan formed a comparatively swollen surface layer. With the exception of untreated wheat arabinoxylan, rinsing reduced the dissipation values to below 1×10^{-6} , indicating that a rigid rather than soft and swollen adsorbed layer remained after washing.⁴⁸ Whereas the slope of data points before and after washing was similar for both grafted and untreated wheat arabinoxylan as well as grafted birch glucuronoxylan, the slopes depicting adsorption and desorption of untreated birch glucuronoxylan were clearly different, where the D to f ratio steadily decreased during the wash step. It appears then that while most of the xylan samples desorbed partially during rinsing while retaining their viscoelastic properties, the untreated birchwood glucuronoxylan rearranged during washing to form a denser layer. Although still speculative, outer layers of adsorbed glucuronoxylan may have been loosely attached given electrostatic repulsions imparted by the charged MeGlcA substituents.

1 This phenomenon would not occur to same degree with charge neutral wheat arabinoxylans, or the
2 birch glucuronoxylan with grafted PGMA which partially screens the charge, their adsorption
3 being primarily limited by steric hindrance.

4 This analysis shows that modification by GMA reduced wheat arabinoxylan adsorption on
5 cellulose, but not birch glucuronoxylan adsorption. This information could prove helpful in fiber
6 coating when properties like water repellence or improved water vapor barrier are desired, since
7 they are better accomplished using modified rather than native xylan.

8 **Conclusions**

9 Five different xylans sources were graft copolymerized with GMA by redox initiation. The
10 chemical composition, molar mass, and solubility of corresponding xylans were then correlated to
11 their reactivity. Glucuronoxylans and the highly substituted wheat arabinoxylan proved to be most
12 reactive. This was attributed to the swelling and initiator attracting effects of the charged, albeit
13 sparsely substituted MeGlcA groups on the former, and the water solubility of the latter. The low
14 apparent reactivity of corn xylan was explained by its oligomeric nature that made the separation
15 of homopolymer and copolymer difficult. The low reactivity of oat xylan, despite its moderate
16 degree of branching, was attributed to its relatively high content of lignin, which likely acted as a
17 radical scavenger, inhibiting the polymerization.

18 Even though grafting did not severely affect the fraction of xylan that was irreversibly adsorbed
19 to regenerated cellulose, it did decrease the affinity of wheat arabinoxylan to the cellulose surface.
20 However, only wheat arabinoxylan formed water-dispersable copolymer as the major product,
21 which is typically desired for fiber coating applications. An interesting possibility then, is to
22 harness enzyme selectivity to fine-tune the extent of arabinose branching in wheat arabinoxylan to
23 thereby maximize water solubility and stable cellulose adsorption. Meanwhile, the low overall

solubility of modified glucuronoxylan may be better suited in the production of bio-based thermoplastics where low water-solubility is often preferred.

Taken together, results from this study, including the similar graft efficiencies reported here for wheat arabinoxylan and earlier work using NFC, suggest the following criteria for selecting xylan sources for polymeric modification: i) high molar mass to facilitate efficient product separation and to retain beneficial polysaccharide properties in the final product, such as association to cellulose ii) high solubility or swelling in water to promote reactivity, and iii) low lignin content to maximize polysaccharide solubility and minimize interference during the radical polymerization.

Supporting information available. ¹H NMR spectra of the unmodified xylans. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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References

(1) Ebringerová, A.; Hromádková, Z.; Heinze, T. Hemicellulose. *Adv. Polym. Sci.* **2005**, *186*, 1-67.

- (2) Flickinger, P.; Lammi, L.; Ernerfeldt, B. Dissolving pulp PEERS. <http://www.tappi.org/content/events/11diss/flickinger.pdf> (accessed April 30, 2014).
- (3) Pettersen, R. The chemical composition of wood. In *The Chemistry of Solid Wood, Advances of Chemistry Series 207*; Rowell, R., Ed.; American Chemical Society: Washington, DC, 1984; pp 57-126.
- (4) Kormelink, F. J. M.; Voragen, A. G. J. Degradation of different (glucurono)arabino]xylans by a combination of purified xylan-degrading enzymes. *Appl. Microbiol. Biotechnol.* **1993**, *38*, 688-695.
- (5) Teleman, A.; Tenkanen, M.; Jacobs, A.; Dahlman, O. Characterization of O-acetyl-(4-O-methylglucurono)xylan isolated from birch and beech. *Carbohydr. Res.* **2002**, *337*, 373-377.
- (6) Sjöström, E. *Wood chemistry: fundamentals and applications*; Academic Press: San Diego, CA, 1993; pp 21-67.
- (7) Sun, R. *Cereal straw as a resource for sustainable biomaterials and biofuels: chemistry, extractives, lignins, hemicelluloses and cellulose*; Elsevier: Amsterdam, 2010; pp 300.
- (8) Hansen, N. M. L.; Plackett, D. Sustainable Films and Coatings from Hemicelluloses: A Review. *Biomacromolecules* **2008**, *9*, 1493-1505.
- (9) Laine, C.; Harlin, A.; Hartman, J.; Hyvärinen, S.; Kammiovirta, K.; Krogerus, B.; Pajari, H.; Rautkoski, H.; Setälä, H.; Sievänen, J.; Uotila, J.; Vähä-Nissi, M. Hydroxyalkylated xylans – Their synthesis and application in coatings for packaging and paper. *Ind. Crop. Prod.* **2013**, *44*, 692-704.
- (10) Köhnke, T.; Pujolras, C.; Roubroeks, J.; Gatenholm, P. The effect of barley husk arabinoxylan adsorption on the properties of cellulose fibres. *Cellulose* **2008**, *15*, 537-546.

(11) Parikka, K.; Leppänen, A.; Xu, C.; Pitkänen, L.; Eronen, P.; Österberg, M.; Brumer, H.; Willför, S.; Tenkanen, M. Functional and anionic cellulose-interacting polymers by selective chemo-enzymatic carboxylation of galactose-containing polysaccharides. *Biomacromolecules* **2012**, *13*, 2418-2428.

(12) Xu, C.; Spadiut, O.; Araújo, A. C.; Nakhai, A.; Brumer, H. Chemo-enzymatic Assembly of Clickable Cellulose Surfaces via Multivalent Polysaccharides. *ChemSusChem* **2012**, *5*, 661-665.

(13) El-Shinnawy, N. A.; El-Kalyoubi, S. F. Graft copolymerization of acrylonitrile onto hemicellulose using ceric ammonium nitrate. *J. Appl. Polym. Sci.* **1985**, *30*, 2171-2178.

(14) Soliman, A. Graft copolymerization of acrylonitrile onto hemicellulose and its effect on paper sheets. *J. Sci. Ind. Res.* **1997**, *56*, 545-552.

(15) Edlund, U.; Albertsson, A. SET-LRP goes "green": Various hemicellulose initiating systems under non-inert conditions. *J. Polym. Sci., Part A: Polym. Chem.* **2012**, *50*, 2650-2658.

(16) Zahran, M. K. Grafting of Methacrylic Acid and Other Vinyl Monomers Onto Cotton Fabric Using Ce (IV) Ion–Cellulose Thiocarbonate Redox System. *J. Polym. Res.* **2006**, *13*, 65-71.

(17) Littunen, K.; Hippel, U.; Johansson, L.; Österberg, M.; Tammelin, T.; Laine, J.; Seppälä, J. Free radical graft copolymerization of nanofibrillated cellulose with acrylic monomers. *Carbohydr. Polym.* **2011**, *84*, 1039-1047.

(18) Mishra, A.; Srinivasan, R.; Gupta, R. P. psyllium-g-polyacrylonitrile: synthesis and characterization. *Colloid Polym. Sci.* **2003**, *281*, 187-189.

(19) Edlund, U.; Albertsson, A. A controlled radical polymerization route to polyepoxidated grafted hemicellulose materials. *Polimery* **2014**, *59*, 60-65.

(20) Zhao, W.; Glavas, L.; Odelius, K.; Edlund, U.; Albertsson, A. A robust pathway to electrically conductive hemicellulose hydrogels with high and controllable swelling behavior. *Polymer* **2014**, *55*, 2967-2976.

(21) O'Connell, D. W.; Birkinshaw, C.; O'Dwyer, T. F. A chelating cellulose adsorbent for the removal of Cu(II) from aqueous solutions. *J. Appl. Polym. Sci.* **2006**, *99*, 2888-2897.

(22) Tyagi, C.; Tomar, L. K.; Singh, H. Surface modification of cellulose filter paper by glycidyl methacrylate grafting for biomolecule immobilization: Influence of grafting parameters and urease immobilization. *J. Appl. Polym. Sci.* **2009**, *111*, 1381-1390.

(23) Willför, S.; Pranovich, A.; Tamminen, T.; Puls, J.; Laine, C.; Suurnäkki, A.; Saake, B.; Uotila, K.; Simolin, H.; Hemming, J.; Holmbom, B. Carbohydrate analysis of plant materials with uronic acid-containing polysaccharides—A comparison between different hydrolysis and subsequent chromatographic analytical techniques. *Ind. Crop. Prod.* **2009**, *29*, 571-580.

(24) Kilpeläinen, P.; Leppänen, K.; Spetz, P.; Kitunen, V.; Ilvesniemi, H.; Pranovich, A.; Willför, S. Pressurised hot water extraction of acetylated xylan from birch sawdust. *Nord. Pulp Pap. Res. J.* **2012**, *27*, 680-689.

(25) Teleman, A.; Lundqvist, J.; Tjerneld, F.; Stålbrand, H.; Dahlman, O. Characterization of acetylated 4-O-methylglucuronoxylan isolated from aspen employing ¹H and ¹³C NMR spectroscopy. *Carbohydr. Res.* **2000**, *329*, 807-815.

(26) Sundberg, A.; Pranovich, A.; Holmbom, B. Chemical characterization of various types of mechanical pulp fines. *J. Pulp Paper Sci.* **2003**, *29*, 173-178.

- 1 (27) McDonald, C. Methods of Protein Analysis and Variation in Protein Results. *Farm Res.*
2 **1977**, *34*, 3-7.
- 3 (28) Kilpeläinen, P. O.; Hautala, S. S.; Byman, O. O.; Tanner, L. J.; Korpinen, R. I.; Lillandt,
4 M. K.; Pranovich, A. V.; Kitunen, V. H.; Willför, S. M.; Ilvesniemi, H. S. Pressurized hot water
5 flow-through extraction system scale up from the laboratory to the pilot scale. *Green Chem.* **2014**,
6 *16*, 3186-3194.
- 7 (29) Swenson, H. A.; Kaustinen, H. M.; Kaustinen, O. A.; Thompson, N. S. Structure of gum
8 arabic and its configuration in solution. *Journal of Polymer Science Part A-2: Polymer Physics*
9 **1968**, *6*, 1593-1606.
- 10 (30) Hallac, B. B.; Sannigrahi, P.; Pu, Y.; Ray, M.; Murphy, R. J.; Ragauskas, A. J. Biomass
11 characterization of *Buddleja davidii*: a potential feedstock for biofuel production. *J. Agric. Food*
12 *Chem.* **2009**, *57*, 1275-1281.
- 13 (31) Kontturi, E.; Thüne, P. C.; Niemantsverdriet, J. Cellulose model surfaces simplified
14 preparation by spin coating and characterization by X-ray photoelectron spectroscopy, infrared
15 spectroscopy, and atomic force microscopy. *Langmuir* **2003**, *19*, 5735-5741.
- 16 (32) Naran, R.; Black, S.; Decker, S. R.; Azadi, P. Extraction and characterization of native
17 heteroxylans from delignified corn stover and aspen. *Cellulose* **2009**, *16*, 661-675.
- 18 (33) Hromádková, Z.; Kováčiková, J.; Ebringerová, A. Study of the classical and ultrasound-
19 assisted extraction of the corn cob xylan. *Ind. Crop. Prod.* **1999**, *9*, 101-109.
- 20 (34) Hettrich, K.; Fischer, S.; Schröder, N.; Engelhardt, J.; Drechsler, U.; Loth, F. Derivatization
21 and Characterization of Xylan from Oat Spelts. *Macromol. Symp.* **2005**, *232*, 37-48.

- 1 (35) Jacobs, A.; Dahlman, O. Characterization of the Molar Masses of Hemicelluloses from
2 Wood and Pulps Employing Size Exclusion Chromatography and Matrix-Assisted Laser
3 Desorption Ionization Time-of-Flight Mass Spectrometry. *Biomacromolecules* **2001**, 2, 894-905.
- 4 (36) Ebringerová, A.; Hromádková, Z.; Alföldi, J.; Berth, G. Structural and solution properties
5 of corn cob heteroxylans. *Carbohydr. Polym.* **1992**, 19, 99-105.
- 6 (37) Dhimi, R.; Harding, S. E.; Elizabeth, N. J.; Ebringerova, A. Hydrodynamic characterisation
7 of the molar mass and gross conformation of corn cob heteroxylan AGX. *Carbohydr. Polym.* **1995**,
8 28, 113-119.
- 9 (38) Benkő, Z.; Andersson, A.; Szengyel, Z.; Gáspár, M.; Réczey, K.; Stålbrand, H. Heat
10 extraction of corn fiber hemicellulose. *Appl. Biochem. Biotechnol.* **2007**, 137-140, 253-265.
- 11 (39) Sarbu, A.; Gonçalves, F.; de Pinho, M. N. Oat spelts xylan molecular mass estimation by
12 size exclusion chromatography. *Carbohydr. Polym.* **2003**, 53, 297-304.
- 13 (40) Dervilly, G.; Saulnier, L.; Roger, P.; Thibault, J. Isolation of Homogeneous Fractions from
14 Wheat Water-Soluble Arabinoxylans. Influence of the Structure on Their Macromolecular
15 Characteristics. *J. Agric. Food Chem.* **2000**, 48, 270-278.
- 16 (41) Ferry, J. D. Illustrations of viscoelastic behavior in shear or simple extension. In
17 *Viscoelastic properties of polymers*; John Wiley & Sons: New York, 1980; pp 33-55.
- 18 (42) Fanta, G. F.; Burr, R. C.; Doane, W. M. Graft polymerization of acrylonitrile and methyl
19 acrylate onto hemicellulose. *J. Appl. Polym. Sci.* **1982**, 27, 4239-4250.
- 20 (43) Höije, A.; Sternemalm, E.; Heikkinen, S.; Tenkanen, M.; Gatenholm, P. Material Properties
21 of Films from Enzymatically Tailored Arabinoxylans. *Biomacromolecules* **2008**, 9, 2042-2047.

(44) Pan, X.; Kadla, J. F.; Ehara, K.; Gilkes, N.; Saddler, J. N. Organosolv Ethanol Lignin from Hybrid Poplar as a Radical Scavenger: Relationship between Lignin Structure, Extraction Conditions, and Antioxidant Activity. *J. Agric. Food Chem.* **2006**, *54*, 5806-5813.

(45) Evans, D. F.; Wennerström, H. Polymers in colloidal systems. In *The colloidal domain: where physics, chemistry, biology, and technology meet*; Wiley-Vch: New York, 1999; pp 351-394.

(46) Lee, S. H.; Lee, H. L.; Youn, H. J. Adsorption and viscoelastic properties of cationic xylan on cellulose film using QCM-D. *Cellulose* **2014**, *21*, 1251-1260.

(47) Eronen, P.; Junka, K.; Laine, J.; Österberg, M. Interaction between water soluble polysaccharides and native nanofibrillar cellulose thin films. *BioResources* **2011**, *6*, 4200-4217.

(48) Köhnke, T.; Östlund, Å; Brelid, H. Adsorption of Arabinoxylan on Cellulosic Surfaces: Influence of Degree of Substitution and Substitution Pattern on Adsorption Characteristics. *Biomacromolecules* **2011**, *12*, 2633-2641.

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