Chemical Pulping

The influence of the molecular weight of the added xylan on pulp properties

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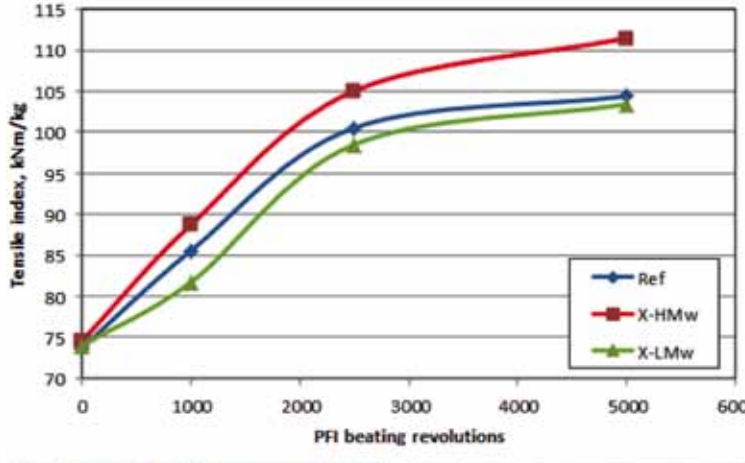

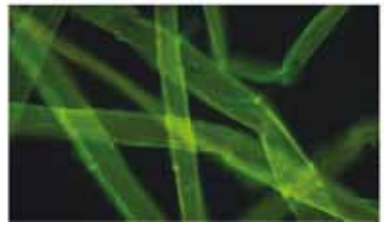

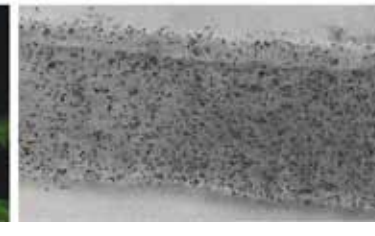

Intern rapport nr 9 (begränsad spridning)

CRUW
Centre for Research on Ultrastructure of Wood fibres
Centrum för forskning om Vedfiberns Ultrastruktur
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Summary

The aim of this study was to investigate if added beech xylan with low molecule weight (Mw) could penetrate into the fiber wall to a greater extent compared to xylan with high Mw. The high Mw (ca 11000) xylan was degraded using enzymes to obtain xylan with low Mw (ca 1800). The influence from the added xylan on the strength properties was evaluated. The cooks in this study were performed without mechanical damage introduced during the cooking process, thus any conclusions of the impact from adding low Mw xylan on the sensitivity towards mechanical damage was not possible.

The different microscopy analyses performed could not show any evidence for a higher penetration of xylan into the fiber wall when producing the pulp with addition of low molecular weight (low Mw) xylan.

The low Mw xylan did not contribute to any improved pulp properties, rather the opposite. Addition of low Mw-xylan did not result in straighter fibers compared to the Ref-pulp, which was the case for the high Mw-xylan-pulp. The tensile index-development (tensile vs PFI-beating and tensile vs density) for the low Mw-pulp was even worse compared to the Ref-pulp. The pulp produced with addition of the high Mw-xylan showed, as earlier seen, an improved tensile index development compared to the Ref-pulp.

Contents

1 Background

Several studies with added xylan have been performed within this program aiming to affect the sensitivity of fibers towards mechanical damage through affecting the strength properties (Daniel et al. 2010; 2011a,b; 2012). In all these investigations the xylan added had a high molecular weight (Mw) and the xylan was analyzed as primarily adsorbed onto the fiber surfaces; the outer fiber walls as well as on the fiber cell lumen walls. One reason for not achieving greater penetration of the xylan and affecting the sensitivity towards mechanical action could be the molecular size of the xylan used, being too large to penetrate into the fiber cell wall.

The aim of this study was to investigate if added xylan with low Mw could penetrate into the fiber wall to a greater extent compared to the xylan used in previous studies. The cooks in this study were performed without mechanical damages introduced during the cooking impregnation stage; therefore any conclusions of the impact from adding low Mw xylan on the sensitivity towards mechanical damage cannot be drawn.

2 Experimental

The pulps were produced from industrial produced chips from round wood spruce. The chips were laboratory screened according to SCAN 40:01 using a Chip Classifier model JWIIIA ll with fractions 2, 3 and 4 used in the cooking experiments.

Three different pulp variants were produced. Two variants with extra xylan added in the impregnation stage, one with high Mw and one with low Mw beech xylan added with a concentration of 10 g/L, as well as one reference pulp without any extra xylan added. In the pulp produced with addition of low Mw xylan, a few model chips were put in the digester for later sectioning and evaluation. Results from the model chip experiment will be presented in another report (Salmén et al. 2012). In Appendix 6, the distribution of lignin and xylan in the model chips for the pulp produced with low Mw xylan addition is compared with model chips produced in the same manner as the high Mw and Ref-pulp. The experimental data for these pulps are further described by Salmén et al. (2012).

Beech xylan (high Mw) was obtained from Sigma (X4252). The high Mw xylan was degraded using enzymes to achieve an Mw of about 1800 (= low Mw xylan). Degradation of xylan was also tested by treating the xylan in an alkaline solution at various pHs (10-13) at an elevated temperature. The degradation of high molecular weight xylan under alkaline conditions was difficult to control and therefore enzymes were used instead.

Data on carbohydrate composition, molecular weight and degree of substitution for the high and low Mw xylan are given in Appendix 1a.

The method used for degradation with alkali at high temperature respectively enzymes are described in more detail in Appendix 2.

Pulps were produced at Innventia in autoclaves. To obtain enough pulp, more than one cook was performed and merged. Primary data for all cooks are shown in Appendix 1b. All pulps were cooked to about the same kappa number of ca. 28.5. The cooking conditions used are shown in Table 1.

Table 1. Cooking condition

	EA charge %	Liquor/ wood	Cooking time at 165 ºC, min	H-factor	Residual OH^- g/L	Kappa no
Ref	22	4	162	- 1700	9.1	27.9
X-H-Mw	22	4	162	- 1700	8.6	29.6
X-L-Mw	22	4	162	- 1700	8.9	28.0

2.1 ANALYSIS

The chemical composition was analyzed on the pulps after acidic hydrolysis and HPLC-analysis using electrochemical detection. Analyses were performed by Stora Enso. Bulk and surface charge analysis were also performed by Stora Enso.

Fiber properties and strength properties were evaluated after PFI-refining. The analyses were performed by Eka Chemicals, except analysis of rewetted zero-span which was performed by Södra on sheets prepared at Eka.

Visualization of adsorbed xylan on whole fibers and sections was analyzed using SEM, immunofluorescence microscopy and using Transmission Electron Microscopy (TEM). More details can be found in Appendices 3, 4 and 5. Distribution of xylan and lignin was studied using FT-IR (Appendix 6).

3 Results and discussion

Three pulp variants were produced and evaluated (Table 2). Two of the variants were produced with added beech xylan having different molecular weight (Mw).

Table 2. Pulp variants included in the study

	Added xylan concentration g/L	Mw of added xylan	Description
Reference without xylan addition	-	-	Ref
Xylan with high Mw added	10	11100	X-HMw
Xylan with low Mw added	10	1800	X-LMw

3.1 INVESTIGATED PULPS

Results after cooking are shown in Table 3. All pulps were cooked to about the same kappa number of ca. 28.5. Although small, there were some differences in kappa number for the three pulp variants (Table 3). Possibly the slightly higher kappa number for the X-HMw-pulp may be a result of the lignin dissolution being obstructed by the high molecular weight xylan (see Appendix 6). A lower delignification rate, due to addition of xylan (high Mw-xylan), has been reported earlier (Daniel et al. 2011a). The pulp produced with added high Mw-xylan had about 1 % higher yield compared to the Ref-pulp whereas the yield for the pulp with added low Mw-xylan were similar as for the Ref-pulp. The difference in yield is also reflected by differences in the carbohydrate composition in the pulps (Table 4).

Table 3. Cooking results

	Residual OH^- g/L	Kappa no	Yield %	Shives %	Total yield %
Ref	9.1	27.9	46.9	0.70	47.6
X-H-Mw	8.6	29.6	47.7	0.92	48.6
X-L-Mw	8.9	28.0	47.2	0.88	48.1

3.2 CARBOHYDRATE COMPOSITION AND FIBER CHARGE

The pulp produced with high Mw-xylan addition contained a much higher xylan content compared (ca 20 %) to the Ref- and the X-LMw-pulp (Table 4**)**. The carbohydrate composition in the pulp produced with addition of low Mw-xylan was very similar to the Ref-pulp. The charge of the low Mw-xylan-pulp was however more similar to the high Mw-pulp, both having significantly higher total charge compared to the Ref-pulp (Table 4).

Table 4. Carbohydrate content and fiber charges in the investigated pulps

		Ref	X-HMw	X-LMw
Galactose	Rel. %	0.4	0.4	0.4
Glucose	Rel. %	83.6	81.8	83.2
Mannose	Rel. %	6.8	6.8	7.1
Arabinose	Rel. %	0.7	0.7	0.7
Xylose	Rel. %	8.5	10.3	8.6
Galactose anhydro	%	0.4	0.4	0.4
Glucose anhydro	%	80,7	79.1	80.9
Mannose anhydro	%	6.6	6.6	6.9
Arabinose anhydro	%	0.7	0.6	0.7
Xylose anhydro	%	8.0	9.8	8.2
Total anhydro sugar	%	94.1	94.2	94.3
Hydrorest	%	6.1	4.7	5.9
Total acid groups	mmole/kg	73	84	85
Surf. charge (neg)	meqv/kg	8	9	8

3.3 VISUALIZATION OF XYLAN ASSOCIATED WITH PULP FIBER CELL WALLS

Presence of xylan associated with the fiber cell walls of the 3 pulps was studied using specific antibody probes (LM10 and LM11) for detecting xylans (both poorly/unsubstituted and poorly/highly substituted xylans) using immunofluorescence microscopy of whole fibres and cross-sections (semi-thin sections ca 0,5 µm) and using TEM immunogold labeling of ultrathin fiber (ca 100 nm) cross-sections. The advantage of the TEM approach is the greater resolution and higher magnifications possible compared to the immunofluorescence approach.

With both the immunofluorescence and TEM approaches using cross-sections, a relatively even distribution of xylans was noted with both the beech X-HMw and X-LMw treated pulps as well as fibers from the reference pulp for both early- and latewood fibers (Appendix 4, Figs 1, 2; Appendix 5, Figs 3-5). The fact that the reference pulp fibers labeled (both early- and latewood fibers) were quite similar to those with the two types (X-HMw, X-LMw) of xylan added suggests that most of the fluorescence/immunogold labeling was partly derived from the native spruce xylan. Little difference in labeling was noted between the outer fiber (i.e. primary wall/S1 layer) and lumen walls with evidence of occasional precipitates/aggregates noted on the lumen wall. Immunofluorescence of whole fibers gave evidence for a variable presence of xylans on the surface of the fibers from the 3 pulps (Appendix 5; Fig. 2). However, it was not possible to distinguish major differences from

either the labeling patterns or intensities using immunofluorescence labeling that would provide evidence for the extra ca 20% xylan noted from the chemical analyses for xylan in the total X-HMw pulp. SEM observations on fibers from the 3 pulps showed a fairly similar and typical fiber surface ultrastructure with exposed cellulose macrofibrils (Appendix 3¸Figs 1-3). Little difference was noted between the beech xylan (X-HMw/X-LMw) treated and reference fibers with a surface structure composed of a variable primary cell wall and exposed S1 layer. Examination of the X-Hw fibers often showed however the microfibrillar structure to be coated with surface materials, which presumably was xylans and possibly adsorbed lignins (see below). Unlike in a previous study where considerable quantities of xylan precipitates/aggregates were noted on fiber surfaces (Daniel et al., 2012a) after 30 g/L xylan was introduced in the cooking/impregnation stage in the present study this was not found presumably due to the lower xylan (i.e.10 g/L) concentration added. From the paper testing, the X-LMw treated pulps had lesser or equivalent properties as the reference pulps thus we assume the morphological structure of the fibers should be similar as observed. The improved properties of the X-HMw treated pulps was most likely related to a higher amount of xylan remaining on the fiber surface, either through absorption of the added xylan or retention of native spruce xylan. The techniques used could not distinguish between the added and native xylans because the specific probes used can react with both the spruce and beech xylan thus knowledge on the possible penetrability of the low and high molecular xylan was not feasible. However, in some additional studies (Appendix 5; Daniel and Ander 2012; unpublished) with fully bleached spruce pulp fibers (i.e. impregnated for 1 hr with xylan at 120 C, pH 11) with solutions of either X-HMw or X-LMw xylan (at 10 g/L) showed similar results suggesting indicating that neither of the xylans despite their difference in molecular weight were able to penetrate easily into fiber structure. Presumably the situation was the same when the two xylans were introduced into the current kraft pulps where the porosity was presumably less than the bleached pulps. FTIR showed an increase signal for lignin in model chips in the treated pulps, particularly the X-HMw chips consistent with a higher absorption of xylans shown from the chemical analyses.

3.4 DEVELOPMENT OF STRENGTH PROPERTIES

The addition of high Mw-xylan had a straightening effect on the fibers, compared to the Ref-pulp, which was not observed by the addition of low Mw-xylan (Figure 1). The addition of high Mw-xylan also resulted in a pulp with higher xylan content which was not the case for the X-LMw-pulp.

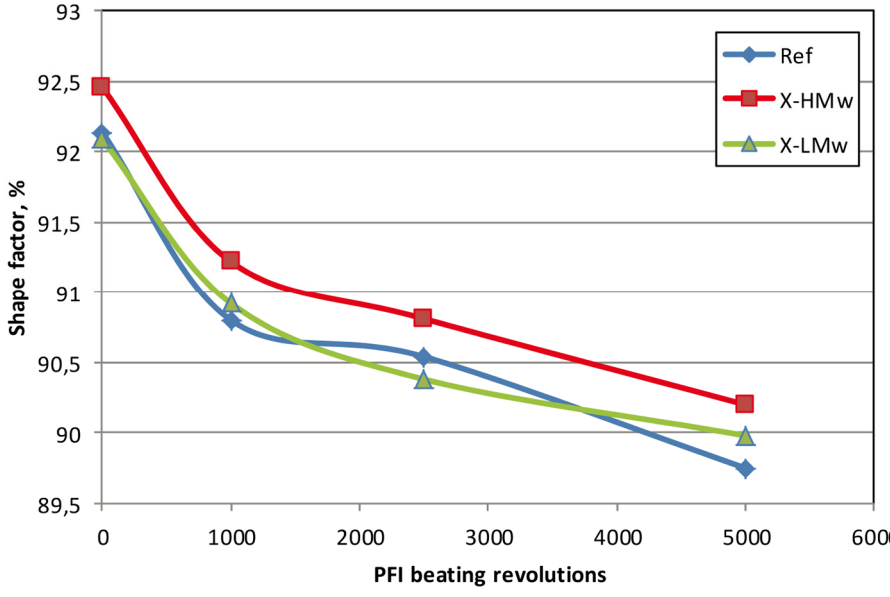

Figure 1. Shape factor vs. PFI beating revolutions.

The addition of high Mw-xylan also contributed to improved beatability, higher tensile index at a certain PFI beating revolution (Figure 2). When compared at a certain density the X-HMw-pulp had similar or slightly higher tensile index compared to the Ref-pulp (Figure 3). The X-LMw-pulp showed both impaired beatability and lower tensile index at a certain density compared to the Ref-pulp. Also when compared at a certain WRV, the tensile index was better for the X-HMw-pulp compared to the other two pulps (Figure 4).

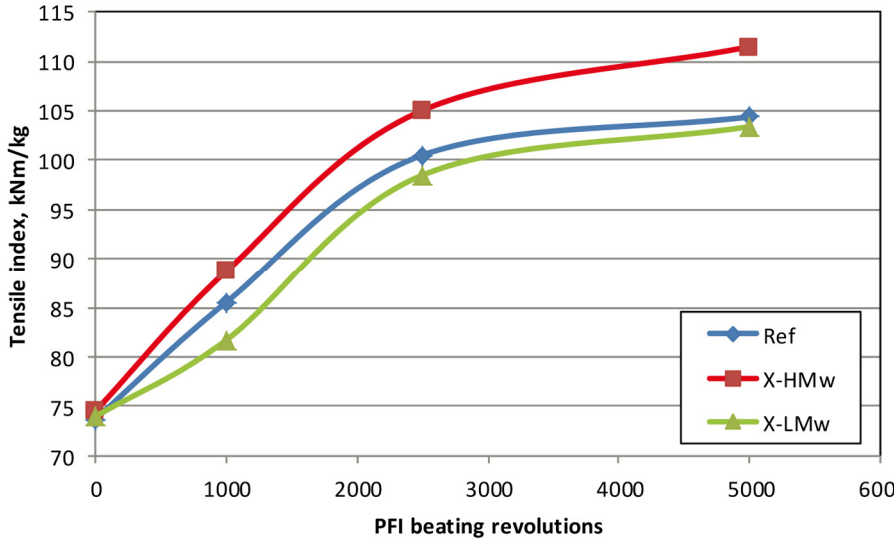

Figure 2. Tensile index vs. PFI beating.

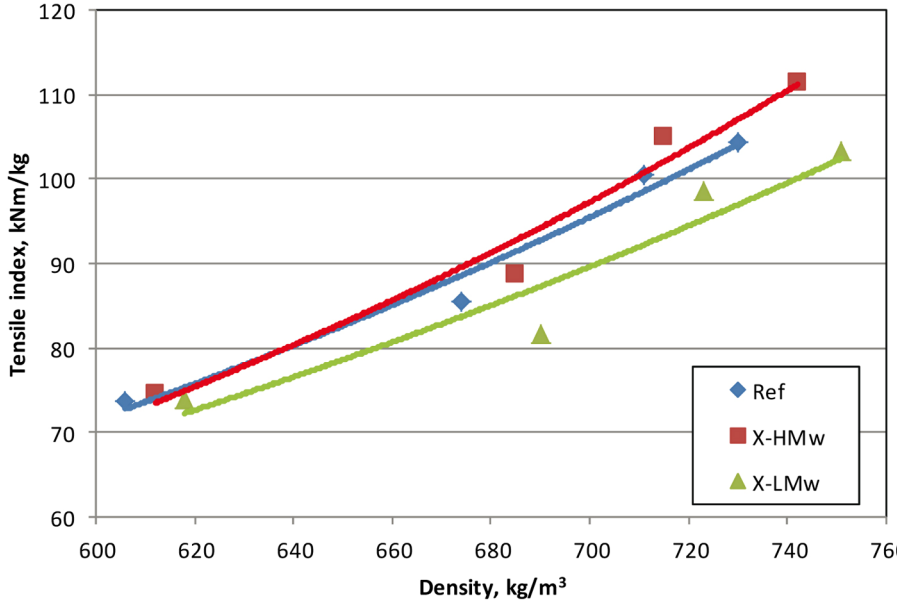

Figure 3. Tensile index vs. density.

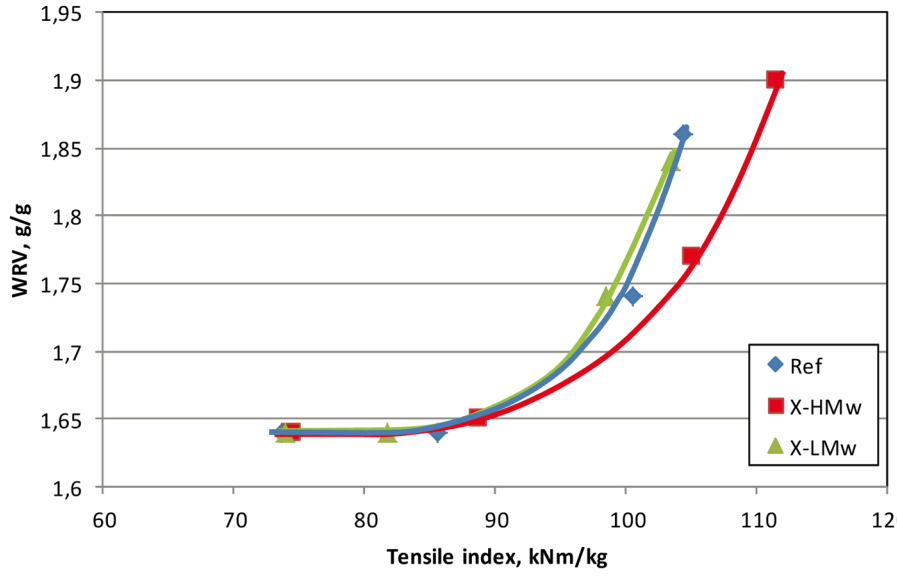

Figure 4. WRV vs. tensile index.

All fiber data for the investigated pulps are shown in Appendix 1c and all strength data are presented in Appendix 1d.

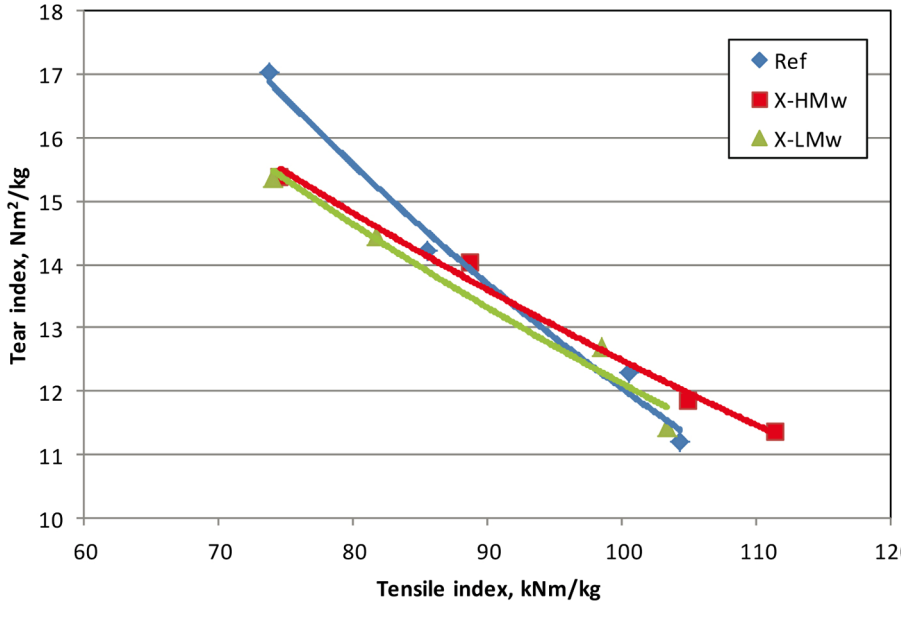

Figure 5. Tear index vs. tensile index.

4 Conclusions

The different microscopy analyses performed did not show any evidence for a greater penetration of xylan into the fiber wall when producing the pulp with addition of low molecular weight (low Mw) xylan.

The low Mw xylan did not contribute to any improved pulp properties, but produced a pulp with inferior properties compared with the reference pulp. . Addition of low Mw-xylan did not result in straighter fibers compared to the Ref-pulp, which was the case for the high Mw-xylan-pulp. Tensile index-development (tensile vs PFI-beating and tensile vs density) for the low Mw-beech xylan pulp was also lower compared to the Ref-pulp. The pulp produced with addition of the high Mw-xylan showed, as expected, an improved tensile index development compared to the Ref-pulp.

5 References

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APPENDIX 1A. ANALYSES OF THE BEECH XYLANS

Carbohydrate composition and molecular weight for the beech xylans used in the study.

		X-HMw	X-LMw
Galactose	Rel. %	1.3	7,65
Glucose	Rel. %	1.8	4,85
Mannose	Rel. %	<0.1	<0.1
Arabinose	Rel. %	0.1	2,2
Xylose	Rel. %	88.5	85,2
HexA	Rel. %	<1	-
GlcA	Rel. %	7.5	-
Rhamnose	Rel. %	0.8	-
Total anhydro sugar	%	100	100
Molecular weight	Mp	12570	-
	Mn	9310	1600
	Mw	11095	1800

APPENDIX 1B. COOKING RESULTS

	EA %	Cooking time at 165°C , min	H-factor	Yield %	Shives %	Tot. yield %	Residual OH-, g/l	Alkali cons. kg/t	Kappa no
Ref 1	22			46,6	0,82	47,5	8,58	185,7	28,31
Ref 2	22			47,2	0,58	47,8	9,66	181,4	27,47
MV[1] Ref	22	162	ca 1700	46,9	0,70	47,6	9,12	183,5	27,89
X-HMv 1	22						8,57	185,7	
X-HMv 2	22						8,74	185,06	
MV[1] X-HMv[2]	22	162	ca 1700	47,7	0,925	48,6	8,65	185,39	29,59
X-LMv 1	22			47,4	0,8	48,2	9,07	183,7	27,4
X-LMv 2	22			47,0	0,96	47,9	8,71	185,2	28,6
MV[1] X-LMv	22	162	ca 1700	47,2	0,88	48,1	8,89	184,4	28

[1]Mean value [2]X-HMw1 and X-HMv2 were merged before analyzing yield, kappa no etc.

APPENDIX 1C. FIBERMASTER RESULTS

	Beating	Fiber dimensions						
	rev.	Length	Width	Shape	Angle	Kink/fiber	kink/mm	Segment
		mm	µm	%	º			Length
Ref	0	2,317	32,4	92,14	53,88	0,224	0,119	2,166
	1000	2,300	31,8	90,8	53,83	0,279	0,150	2,096
	2500	2,301	31,8	90,54	54,28	0,279	0,150	2,114
	5000	2,232	32,0	89,75	55,73	0,332	0,186	2,006
X-H-Mw	0	2,250	32,1	92,46	52,59	0,192	0,110	2,123
	1000	2,212	31,6	91,22	53,49	0,232	0,129	2,042
	2500	2,176	31,6	90,81	55,15	0,244	0,138	2,014
	5000	2,188	32,0	90,2	56,76	0,284	0,160	1,993
X-L-Mw	0	2,216	31,7	92,09	52,61	0,116	0,204	2,066
	1000	2,179	31,2	90,92	52,59	0,15	0,260	1,087
	2500	2,184	31,2	90,38	54,74	0,16	0,276	1,996
	5000	2,168	31,4	89,98	55,1	0,178	0,306	1,962

APPENDIX 1D. STRENGTH PROPERTIES

	Beat rev.	SR	WRV g/g	Gram. g/m^2	Dens. kg/m^3	Tensile index Nm/g	Elong. %	Tens. energy abs. I J/Kg	Stiffn kNm/g	Tear index Nm^2/kg	Burst $kPam^2/g$	Zero-span kNm/kg
Ref	0	14,8	1,64	63,6	606	73,7	2	966	9,34	17,04	4,77	187,2
	1000	15,1	1,64	63,7	674	85,5	2,64	1484	9,33	14,22	6,33	187,5
	2500	17,0	1,74	62,8	711	100,5	2,89	1667	9,64	12,30	7,88	187
	5000	23,9	1,86	61,7	730	104,4	3,23	2177	9,46	11,20	8,56	180,7
X-H-Mw	0	14,5	1,64	62,9	612	74,5	1,95	941	9,13	15,37	5,21	185,6
	1000	15,5	1,65	64,8	685	88,7	2,46	1417	9,51	14,01	6,23	179,3
	2500	18,0	1,77	62,5	715	105,0	2,9	1967	10,00	11,84	7,68	182,3
	5000	24,7	1,9	61,6	742	111,5	3,13	2241	10,09	11,34	8,4	175
X-L-Mw	0	14,7	1,64	63,3	618	74,0	1,96	946	9,12	15,37	4,93	182,5
	1000	15,2	1,64	65,9	690	81,7	2,51	1341	8,84	14,45	6,19	176
	2500	17,2	1,74	65,5	723	98,5	2,81	1805	9,79	12,70	7,22	174
	5000	23,1	1,84	64,9	751	103,4	3,29	2199	9,47	11,42	7,76	175,7

APPENDIX 2. METHODS FOR XYLAN DEGRADATION

Paul Ander, SLU, Uppsala

The goal was to prepare low molecular weight beech xylan which can penetrate deeply into the fibre wall during cooking and give positive effects like better "Strength delivery". The work was done in the project "Localization of xylan (native and deposited) during cooking".

Beech xylan was purchased from Sigma-Aldrich (X4252). Endoxylanase from *Thermomyces lanuginosus* was produced by Novozyme and sold by Sigma-Aldrich (X2753; see Ates et al., 2009). The enzyme cleaves xylan chains randomly in the polymer background producing xylose and xylo-oligosaccharides. The centrifuge used was a Beckman Model J2-21M/E with 6 polycarbonate centrifuge tubes with maximum volume 415 ml each. The tubes were always weight balanced and closed before running. Tubes were not washed with acetone.

Initial experiments

DEGRADATION OF BEECH XYLAN AT HIGH TEMPERATURE

150 mg beech xylan was dissolved during stirring in thick glass flasks with plastic screw lids containing 50 ml 10 mM NaOH at pH 12 and incubated at 160°C for 2, 4 and 6h. With evaporation, additional water was added. The flasks were freezed at -20°C and placed in the freeze-drier and freeze-dried for 3 days. Xylan preparations were washed with 96% ethanol, absolute ethanol and acetone. Water control gave 121 mg, 128 mg and 124 mg xylan. NaOH gave 138 mg, 134 mg and 148 mg (2, 4 and 6h). No molar mass decrease was observed using size exclusion chromatography (SEC) at Innventia. Repetition of the treatment at pH 13 for 6h at 160°C did give some degradation. Increasing the pH to 13.43 using NaOH and to pH 13.74 using KOH and treatment for 6.5 h at 160°C gave strong degradation to monosaccharides. In addition, an undegraded part of xylan was detected.

Result: The results indicate that xylan degradation at high temperature at alkaline pHs is difficult to control.

Degradation of beech xylan using enzyme

SMALL SCALE

About 150 mg beech xylan was degraded in acetate, citrate and ammonium acetate buffers at pH 6 using endoxylanase at 60°C for 4h. Initially, the enzyme degraded xylan was freeze-dried and washed with 96% ethanol, absolute ethanol and acetone as above. Certain degradation was found using SEC, although a substantial part of

the xylan remained undegraded. Later a simplified technique using different concentrations of ethanol was developed in order to precipitate high molar mass beech xylan and then use a higher ethanol concentration to precipitate middle and low molar mass xylan. In this later development (without freeze-drying), the buffer solution containing the enzyme treated xylan was directly chilled at -18°C and precipitated with ethanol in two steps.

Result: Considerable beech xylan was still undegraded.

Preparative scale I. Determination of ethanol concentrations for precipitation
Beech xylan (3x 5 g in 5 cm wide-mouth 600 ml Erlenmeyer flasks) was dissolved in 500 ml 20 mM ammonium acetate buffer pH 6 (Pitkänen et al 2011) during stirring for 15 min. Then 33 mg endoxylanase suspended in 10 ml water was added to each of the flasks, stirred for 5 min and the flasks transferred to an incubation hood at 50°C and shaken at 130 rpm for 4h. The 3 flasks were then stirred for 15 min during cooling and chilled at -18°C for at least 2h. The cold solutions with the degraded xylans were transferred to 3 one litre Erlenmeyer flasks and precipitated by adding 214 ml cold absolute alcohol to obtain 30% alcohol in each flask. Precipitation was completed by storage overnight in the cold room at +8°C. This first precipitation representing high molar mass beech xylan was separated from the buffer solution by filtration through glass filter G3 and discarded. To the cold filtrate (*ca* 714 ml) was added to 1786 ml pure ethanol to give 80% ethanol for precipitation of remaining low and middle molar mass xylan. After storage at -18°C for 2h, the solutions with precipitated xylan (almost 2.5 L in three 3 L flasks) were centrifuged using 6 polycarbonate centrifuge tubes (maximum volume 415 ml/tube) at 10°C and 12000 rev/minute. After decanting and saving the slightly yellow 80% ethanol solutions containing small amounts of unprecipitated xylan, precipitated xylan was removed from the tubes and washed on 5 cm glass G3 filters using pure ethanol and acetone, drying starting at suction on the filter. The light brown degraded xylan was further dried at 105°C for 2h to constant weight and no smell of acetone. **Yield:** 1.664 + 1.528 + 1.574 = 4.771 gram. The 1.664 g part of this preparation was sent to Innventia for SEC analysis. The results are shown in *Table 1* and *Figure 1*.

Table 1. Xylan molar mass (mean of duplicate samples). Degradation of beech xylan by endoxylanase

Sample name	Peak no.	Mp	Mn	Mw	PD
Beech xylan 30-80% EtOH	1	9700	9900	10900	1,10
Beech xylan 30-80% EtOH	2	1800	1600	1700	1,05
Beech xylan 30-80% EtOH	3	700	700	700	1,02

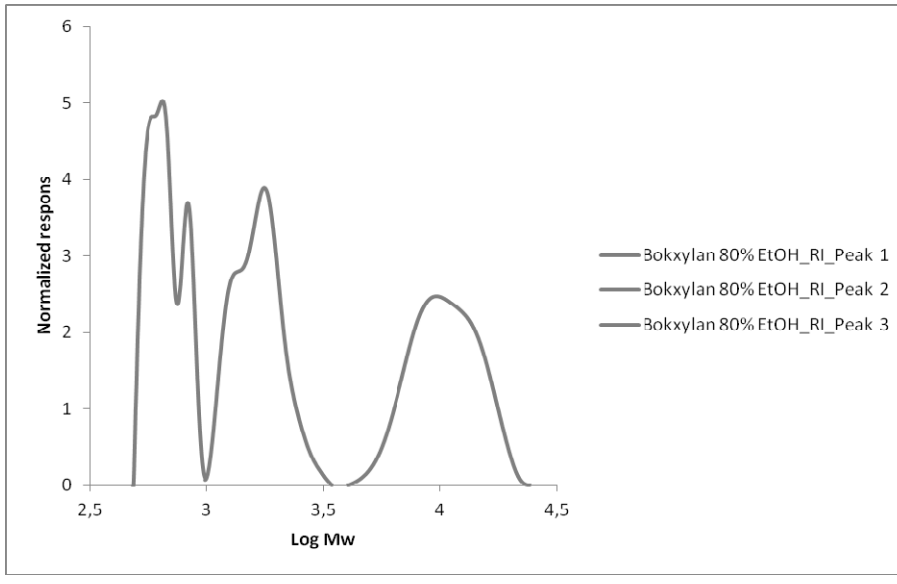

Figure 1. Normalized RI response versus Log Mw for enzyme degraded beech xylan precipitated with 80% ethanol after removal of some high molar mass xylan with 30% ethanol. Peaks 1, 2 and 3 in Table 1 are at approximately Log Mw = 4, 3.2 and 2.8 respectively.

Result

These results indicate some beech xylan degradation by the enzyme. However, the xylan in peak 1 at log Mw = 4 (Mw 10900) needs to be smaller and thus more degraded.

The above enzyme treatment was repeated but using 5.5 g beech xylan plus 464 ml buffer and stirring for 30 min. Then 10 ml enzyme (33 mg) was added and the three flasks incubated for 4h as above. After cooling, 250 ml reused 80% ethanol was added to obtain precipitation of high molar mass xylan by 28% ethanol. The precipitation was separated on glass G3 filters and the three filtrates transferred to three 5 L flasks and addition of 2906 ml pure ethanol/flask gave precipitation of low and middle molar mass xylan by 85% ethanol. After centrifugation, washing and drying of the precipitates the following quantities of degraded xylan were obtained: 1.707 + 1.774 + 1.709 + 3.1 (small amount of acetone in 3.1 prep.) = 8.29 grams. Sent to Innventia for SEC analysis.

Result: Insufficient degradation of high molar mass xylan.

Small scale

550 mg beech xylan was added to two 100 ml flasks containing 50 ml 20 mM

ammonium acetate buffer pH 6.1, giving final pH 6.0 after xylan addition. 3.3 mg enzyme was added and the flasks incubated at 50°C for 4h as above. After cooling, 50 ml and resp. 83 ml reused 80% ethanol were added to compare precipitation of high molar mass xylan by 40% and 50% ethanol. After separation of the precipitates by centrifugation, pure ethanol was added to the filtrates to obtain precipitation of low and middle molar mass xylan by 85% ethanol. Centrifugation, washing and drying of the degraded xylan preparation gave 115 mg and 131 mg respectively. The preparations were sent to Innventia for SEC analysis. The result is shown below in Table 2 and Figure 2.

Table 2. Beech xylan molar mass after degradation of beech xylan by endoxylanase and 1st precipitation by 40% or 50% ethanol and 2nd precipitation by 85% ethanol

Sample name	Peak no.	Mp	Mn	Mw	PD	Height %
40-85%	1	9300	8800	9300	1,06	21
40-85%	2	1300	1500	1500	1,05	48
40-85%	3	800	600	700	1,06	31
50-85%	1	8300	7800	8100	1,03	2,8
50-85%	2	1400	1500	1600	1,07	67
50-85%	3	800	600	700	1,06	30

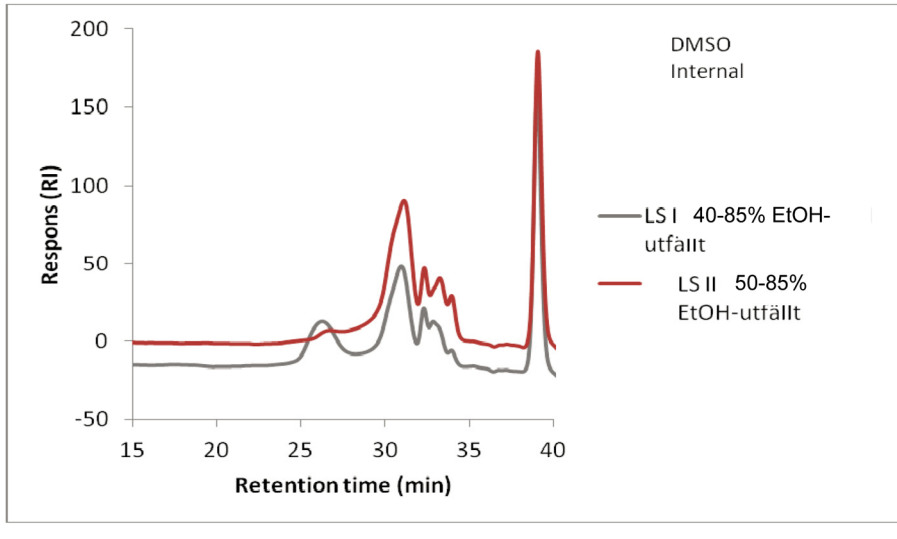

Figure 2. Xylan molar mass after degradation of beech xylan by endoxylanase and first precipitation by 40% or 50% ethanol and 2nd precipitation by 85% ethanol. Response (RI) versus Retention time.

Result: *Table 2* and *Figure 2* show that precipitation of high molar mass beech

xylan by 50% ethanol removes almost all high molar mass xylan from the preparation. In *Table 2* this is seen by the low relative height 2.8 % after 50 % ethanol precipitation compared with the height 21 % after 40 % ethanol precipitation. This high Mw is also seen in *Figure 2* at retention time 26.3 min.

Preparative scale II

Final preparation of degraded beech xylan was done in three different experiments with small modifications compared with earlier trials. Finally 13 g "return xylan" sent back to SLU from Innventia was degraded under the correct conditions (50-85% ethanol).

The first of these experiments will be described in some detail:
To 5.5 g beech xylan per three wide mouth 600 ml flasks containing 464 ml ammonium acetate pH 6.1 (gave pH 6.0 after xylan addition), 33 mg enzyme in 10 ml water was added and incubated for 4h at 50°C. Then the flasks were incubated at 160°C for evaporation and deactivation of the enzyme. After about 2 h, the temperature was shut off and the flasks left open in the heating chamber overnight. In the following morning, the liquid volumes were 370-385 ml, indicating that the amount of ethanol could be decreased and the centrifugation step facilitated. High molar mass xylan was now precipitated by adding 435-453 ml 85% ethanol giving 50% ethanol. After cooling at -18°C, the liquids were centrifuged and the liquid volumes collected (by decanting and filtration) and measured. Normally these precipitations were not analyzed. To the filtrates (765-790 ml) cold absolute alcohol (i.e. 1785-1843 ml) was added to obtain 85% ethanol for precipitation of low- and middle molar mass xylans. The resulting total volumes 2550-2633 ml in three large flasks was centrifuged (max volume 415 ml/tube) and the precipitations transferred from the centrifuge tubes to glass a G3 filter. After washing with absolute alcohol and acetone and drying (by suction and at 105°C), the resulting xylans were weighed.

The 2nd and 3rd runs were performed similarly with volumes after enzyme treatment and evaporation 331, 336 and 354 ml in the 2nd experiment and 320, 333, 336 ml in the 3rd experiment. After final precipitation with 85% ethanol as above, the degraded xylan was weighed.

Four different preparations of enzyme treated xylans returned from Innventia were mixed and 6.645 g added to two flasks with 500 ml buffer pH 6.1 (final pH 6.0) and stirred at about 35°C until the xylan was dissolved. Precipitation was achieved by adding 714 ml 85% ethanol to remove high molar mass xylan by 50% ethanol as before. To the resulting liquids (2 x 1140 ml) absolute alcohol was added and the precipitate by 85% ethanol was collected as before. This gave 4.685 gram.

Result: *In total, Preparative scale II gave 20.058 gram low- and middle*

molar mass xylans at a yield of 42%. Of this 19.317 gram was sent to Innventia for use in the Chemical Pulp project.

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APPENDIX 3. SEM OBSERVATIONS ON SPRUCE PULP FIBRES FROM KRAFT COOKS WITH EXOGENOUSLY ADDED LOW- AND NORMAL BEECH XYLANS

Geoffrey Daniel, SLU, Uppsala

Aims

The purpose of the study was to answer the following questions: *i)* Does exogenously added beech low- and normal molecular weight xylan added during the impregnation/cooking stage of kraft pulping remain on the surface of final spruce pulp fibres and is there any evidence for a reaction with the fibre macrofibrillar structure?; *ii)* If present, do the beech xylans show any morphological differences on the fibre surfaces?

Background

During kraft pulping, xylans are known to precipitate onto the surfaces of pulp fibres from the cooking liquor (Yllner and Enstrom, 1956, 1957) or when applied exogenously to the cook (Daniel et al, 2010, 2011). During the present study, beech xylans of different molecular weight (i.e. 11,100 Da; X-HMw xylans; 1800 Da, X-LMw xylans) were at the cooking impregnation stage of spruce chips to see whether penetration of the fibre walls could be attained and thereby give increase in fibre strength.

Scanning electron microscopy (SEM) was used as a means of observing the fibre surface and any changes associated with an effect of added/retained xylans. SEM has been used repeatedly as a means of observing changes in fibre surface structure during kraft processing and fibre wall delignification (e.g. Daniel and Duchesne, 1998; Duchesne and Daniel, 2000; Daniel et al., 2004; Daniel et al., 2010, 2011). While SEM can provide a 3-dimensional overview of any changes in the morphological structure of the fibres during processing it does not allow (i.e. without some form of marking) for distinguishing between native and exogenously added xylan. In the present work, a comparison was only possible by using reference samples without added xylan.

Materials and Methods

Fibre materials: These are described in detail in the report introduction and overview given in *Table 1*.

Table 1. Cooking conditions

	EA charge %	Liquor/ wood	Cooking time at 165ºC, min	H-factor	Residual OH^- g/L	Kappa no
Ref	22	4	162	- 1700	9.1	27.9
X-H-Mw	22	4	162	- 1700	8.6	29.6
X-L-Mw	22	4	162	- 1700	8.9	28.0

Table 2. Pulp variants included in the study

	Added xylan concentration g/L	Mw of added xylan	Description
Reference without xylan addition	-	-	Ref
Xylan with high Mw added	10	11100	X-HMw
Xylan with low Mw added	10	1800	X-LMw

Scanning Electron Microscopy (SEM) of fibres

Pulp fibres were processed according to Daniel and Duchesne (1998) and Daniel et al. (2004)) using ethanol dehydration and dried in an Agar E3000 critical point dryer (Agar Scientific Ltd, Stansted, UK) with CO_2 as the drying agent. Samples were thereafter coated with platinum gold (ca 6 nm) using an Agar high resolution coater and examined using Philips Environmental-SEM or Hitachi 4500 operated at variable kV. Images were digitalized using embedded software. For an overview of the effects of the different treatments, SEM observations were performed on the surface areas of at least twenty fibres including early- and latewood fibres at various magnifications.

Results and Discussion

General overview of pulp fibres; surface ultrastructural features of the different pulps

Representative SEM images at different magnifications from the 3 pulps *viz* X-LMw X-HMw and reference are shown in Figures 1-3. Approximately 30 images/pulp including early and latewood were made in order to make a representative appraisal. The two assumptions (Daniel, and Duchesne. 1998; Daniel et al., 2004; Duchesne and Daniel, 2000) used in previous SEM observations of changes in the surface ultrastructure of wood fibres during kraft pulping were also used in the present work namely:

i) That the surface ultrastructure of wood fibres during kraft pulping and delignification is reflected by a complex macrofibrillar structure of

cellulose macrofibrils (i.e. aggregates of cellulose microfibrils) *more or less* associated with hemicelluloses (i.e. xylan, glucomannans) and residual lignin. With removal of lignin and consequently some of the hemicelluloses during the kraft process, the macrofibrillar cellulose structure becomes apparent on the outer fibre wall and is composed either of the primary wall if present and the fibre S1 layer. The primary wall is often removed and even parts of the S1 layer sometimes thus revealing the S2 layer.

ii) If the macrofibrillar structure is not apparent, then it is usually covered with extraneous materials (e.g. hemicelluloses, lignin) from the cook or from that added externally to the cook *–in this case here it could be beech* X-LMw *or* X-HMw *xylans or native xylans together with any remaining lignins.*

Such changes in surface morphological ultrastructure are difficult to quantify by SEM and only trends can be realized. Also SEM cannot give any information of the penetration of extraneous added (or retained) xylan in the fibre wall or from the cell lumen. Therefore the following observations can be considered as trends.

General observations

X-LMw. *Figure 1a-f* shows typical views of the surface ultrastructure of fibres from pulps in which X-LMw (10 g/L) beech xylan were added. The most characteristic feature is the presence of the fibre surface macrofibrillar structure observed at high magnifications (*Fig. 1d-f*) and frequent presence of remaining primary wall (*Fig. 1d, f*). Most conspicuous was the apparent absence of precipitates/aggregates on the fibre surfaces at the magnifications used.

X-HMw. Figure 2a-f shows typical images of fibre surfaces from pulps in which the high molecular weight beech xylan was added during cooking. The images are at fairly similar magnifications as images from X-LMx to facilitate comparisons. Like X-LMw the surface structure at higher magnifications is shown consisting of a surface macrofibrillar structure of either the primary wall (Fig. 2c) or outer S1 layer (Fig. 2f). The surface ultrastructure of the fibre macrofibrils varied considerably and appeared covered with materials presumably xylans (Fig. 2d) in some places or appearing rather clean in other areas (Fig 2f). No conspicuous precipitates/aggregates were however noted on the fibre surfaces (Fig. 2a-f).

Reference pulp. Figure 3a-d shows images from the reference pulp. The surface ultrastructure appeared fairly similar to that observed on fibres from the beech X-LMw and X-HMw treated pulps with an outer wall composed of either the primary wall or S1 layer. No apparent precipitates/aggregates were apparent associated with fibre surface at the magnifications used.

Conclusions

1) From the present SEM observations and with the number of fibres observed, very little difference were apparent in the surface ultrastructure of fibres in which beech xylan had been added –both for X-LMw and X-HMw- compared with the reference pulp fibres. All 3 pulps were constituted with fibres with a surface ultrastructure composed of either remaining primary wall or exposed S1 layer.
2) All 3 pulps showed a surface ultrastructure lacking apparent large precipitates/aggregates that have been recognized during previous experiments and considered associated with precipitation of xylans/lignin (Daniel et al., 2012).

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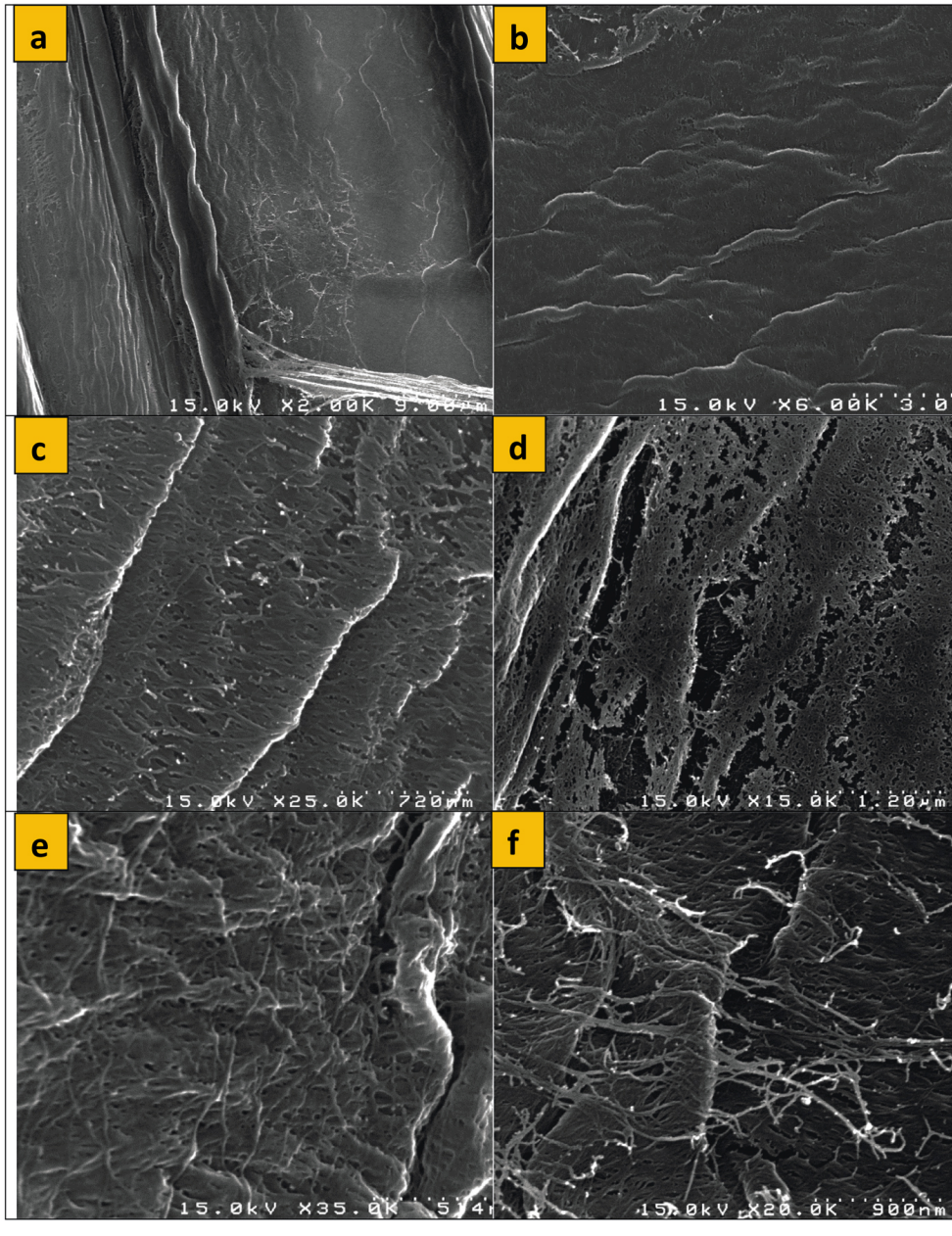

Figure 1. Surface ultrastructure of spruce pulp fibres with X-LMw beech xylan added.

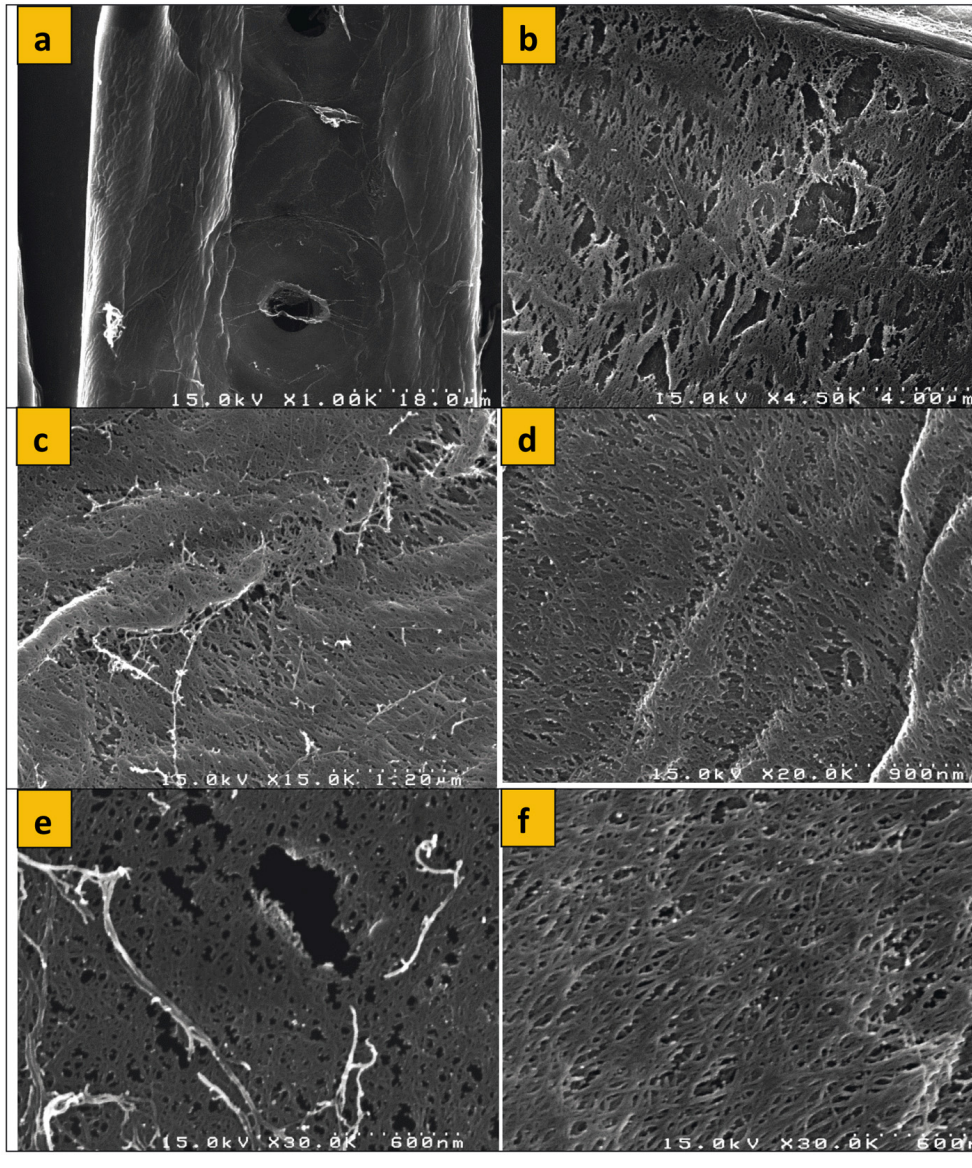

Figure 2. Surface ultrastructure of spruce pulp fibres with X-HMw beech xylan added.

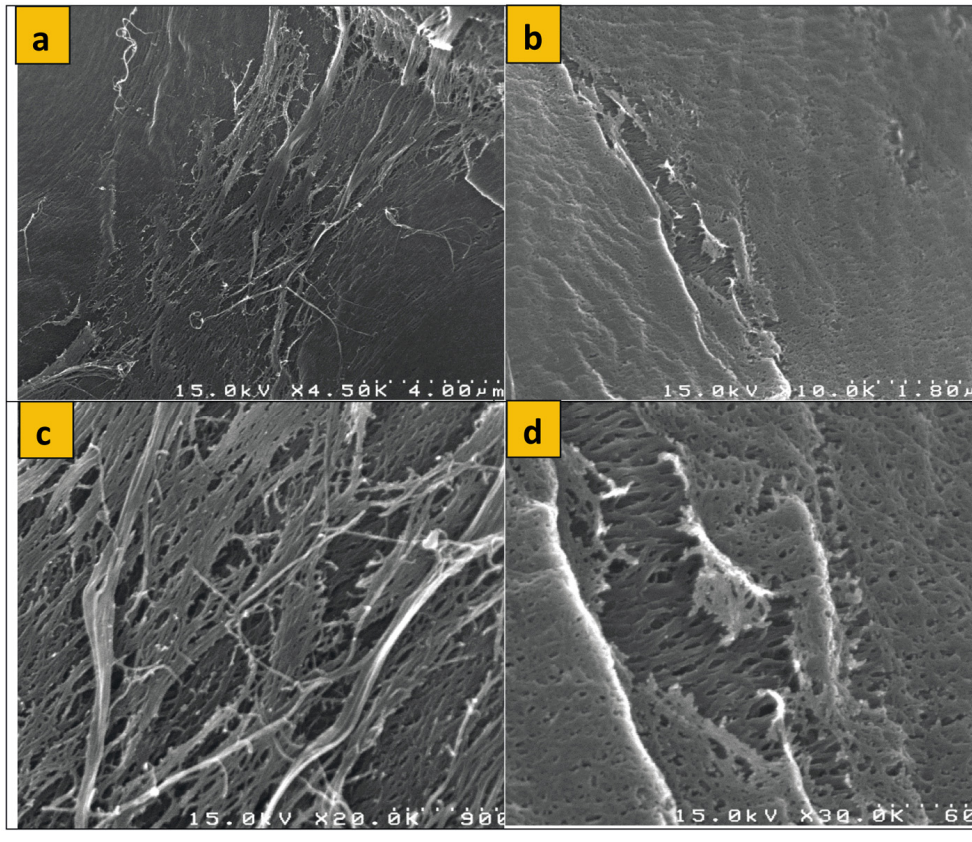

Figure 3. Surface ultrastructure of spruce pulp fibres from the reference pulp with no xylan added.

APPENDIX 4. TRANSMISSION ELECTRON MICROSCOPY (TEM) ON THE SPATIAL MICRODISTRIBUTION OF XYLAN IN KRAFT SPRUCE PULP FIBRES TREATED WITH EITHER LOW OR HIGH MOLECULAR WEIGHT XYLAN

Jong Sik Kim, Geoffrey Daniel, SLU

In an attempt to visualize the presence of xylan in the secondary cell walls of spruce fibres derived from cooks in which either low molecular weight, high molecular weight or no extra xylan was added, correlated Transmission Electron Microscopy (TEM) immunogold labeling was applied. The TEM immunogold approach complements the immunofluorescence technique but provides for improved resolution and higher magnification allowing for better overview of the spatial microdistribution and concentration (indirectly by intensity of labeling pattern) of the xylans when present. Transverse sections of fibres were labeled with antibodies primarily specific for low and highly substituted xylans (LM11) and low and unsubstituted (LM10) xylans (Daniel et al., 2012).

The aim of the work was to visualize the presence and spatial microdistribution of the xylans in *–both early- and latewood fibres-* to determine differences with respect to the different polymers possible penetration into the fibre cell walls. In a previous study (Daniel et al., 2012) using TEM immunogold, no convincing evidence was obtained to suggest that high molecular xylans can penetrate the fibre walls during the cooking process, thus in this particular study a comparison was made with low molecular weight xylan.

Materials and Methods

PULP FIBRE EMBEDDING AND IMMUNOGOLD LABELING

Spruce fibres treated with either low- or high molecular xylans and corresponding reference fibres were processed, embedded in London resin, sectioned and immunogold labeled with LM10 and LM11 as previously described (Daniel et al., 2004; 2012). Examination of labeled sections was by use of a Philips CM12 transmission electron microscope (TEM, USA) operated at 80 kV. Negative TEM films were scanned using an Epson Perfection Pro 750 film scanner. Observations were made on only cross-sections and care was made to make sure sections studied were perpendicular to the fibre axis and not oblique as this can affect both the intensity labeling patterns. In the following images, the black spots on the fibre walls indirectly show the localization and spatial microdistribution of xylans. In general, the greater the number of gold particles (av. size 10 nm) present, the greater the amount of xylan epitopes present and available for labeling. At least 20 images of different fibre cross-sections were taken in order to achieve representation.

Results

Compound TEM micrographs showing the microdistribution and intensity of labeling for low substituted and unsubstituted xylans (LM10) and low and high substituted (LM11) xylans in cross-sections of early- and latewood pulp fibres from cooks treated with low molecular weight and high molecular weight xylans are shown in *Figures 1C-F* and *2C-F*. Representative TEM images showing the microdistribution of substituted xylans in native reference spruce in early- and latewood fibres are shown *in Figs 1A, B* and *2A, B*. All TEM micrographs shown in *Figs 1-2* are of approximately the same magnification (Bar line bottom right corner = 500nm) and chosen to provide a high enough magnification to see the individual gold particles/labeling pattern and at the same time the largest part of the fibre wall as possible. Direct comparisons are therefore possible.

LM11 FOR HIGHLY SUBSTITUTED/LOW-SUBSTITUTED XYLANS (*FIG. 1*)

Very little difference can be seen in the labeling patterns between the spatial microdistribution and intensity of native xylan in spruce reference early- and latewood fibres (*Fig. 1A, B*) compared with fibres derived from cooks where either low molecular weight xylan (*Fig. 1C-D*) or high molecular weight xylan was added (*Fig. 1 E, F*). The fact that no difference is observed compared with the reference spruce in the early- and latewood fibre secondary cell walls testifies more against the penetration of the low- and high molecular weight xylans to penetration and replacement of native xylans. A feature observed with the low molecular weight treated xylans was the strong labeling for the presence of low molecular weight xylans in the cell lumen of the fibres (*Fig. 1D*). This would suggest that considerable xylan was present in the cell lumen either derived from the low molecular xylan used in the cook or from precipitated native xylan or a combination of both. Additional SEM studies should reveal if there are morphological differences in the types of precipitates reflecting xylan on the surfaces of the fibres between the native and the low- and high molecular weight treated pulps and thus give a better inference on the xylan distribution. The patterns and intensities for the high molecular xylans and reference spruce fibres were also similar to that found previously (Daniel et al., 2012).

LM10 FOR LOW SUBSTITUTED AND UNSUBSTITUTED XYLANS (*FIG. 2*)

Labeling with LM10 for low and unsubstituted xylans gave fairly similar results as for LM11. Again both the patterns and intensities were very similar between the low- and high molecular weight treated xylans (*Fig. 2C, D vs E, F*) with LM10. In some of the reference spruce latewood fibre secondary cell walls there was a suggestion for a weaker labeling pattern but this was not observed with the earlywood reference fibres. Thus this is thought most likely to result from some natural variation in the presence of xylan in the reference spruce material. Similar

and even lesser labeling was also observed with reference spruce pulps fibres – especially latewood fibres- that had been subjected to shearing (Daniel et al., 2012).

Conclusions

Since the labeling patterns and intensity using LM11 and LM10 were much the same as the spruce reference pulps we cannot infer penetration of the fibre walls by either the low- or high molecular xylans. An alternative explanation is that both the low- and high molecular weight beech xylan were penetrating the spruce pulp fibre walls but that because the xylans are similar to the native spruce xylans, we cannot visualize the differences using the probes applied. The poor results with the physical tests with the low molecular xylans would however indicate that if penetration was the case then it was not operating to increase the strength of the fibres and paper product.

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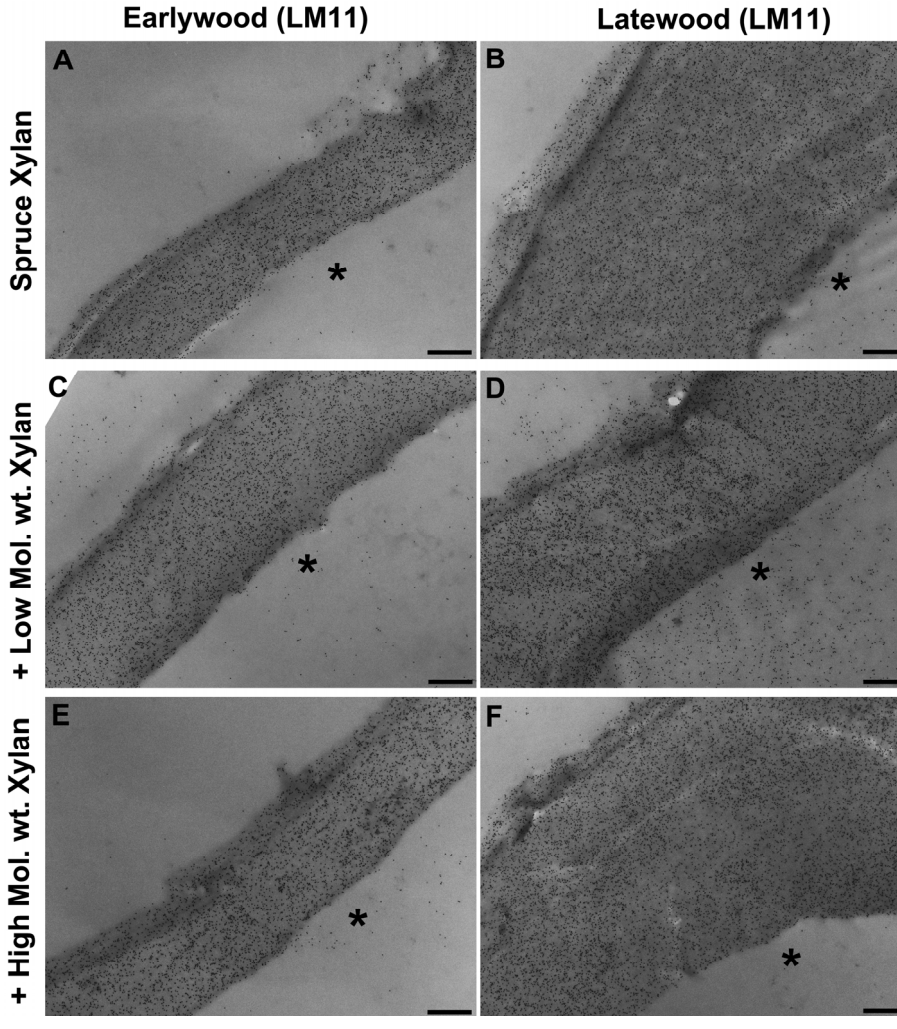

Figure 1A-F. Cell lumen side. Bars = 500 nm*

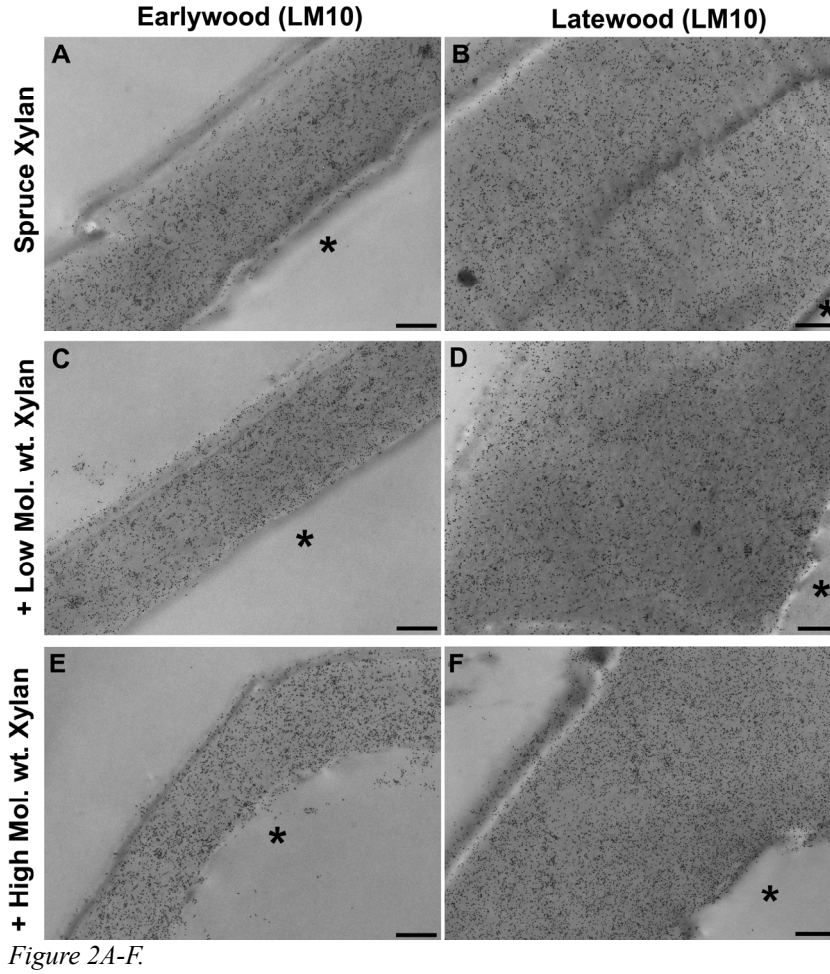
Earlywood (LM10)
Latewood (LM10)
Spruce Xylan
+ Low Mol. wt. Xylan
+ High Mol. wt. Xylan
A
B
C
D
E
F

Figure 2A-F.

APPENDIX 5. IMMUNOFLUORESCENCE OBSERVATIONS ON SPRUCE PULP FIBRES FROM COOKS WITH LOW- AND NORMAL BEECH XYLANS

Geoffrey Daniel, Jong Sik Kim, Lada Filonova, Paul Ander, SLU

Aims

The purpose of the study was: *i)* Visualize the presence of beech xylans (high X-HMw and low X-LMw molecular weight) on the surfaces of spruce fibres; and *ii)* visualize the presence of xylans in cross-sections of treated fibres. The fibres for the studies were derived from two cooks in which X-HMw and X-LMw ws added during the impregnation stage and were compared with a reference pulp in which no xylan was added

Background

To understand how hemicelluloses such as xylans can be used to retain (e.g. native xylans) or improve the strength of kraft pulps, it is imperative to know how xylans are associated with the fibres. This can be the native xylans and how they are retained or removed during processing or how xylans added associate with the fibre walls. One approach is to visualize the presence and spatial microdistribution of xylans on both the surfaces and that in the fibre wall. In the present study immunofluorescence in conjunction with specific antibodies to xylan was used.

2. MONOCLONAL ANTIBODIES

Presence of xylan was visualized using two rat monoclonal antibodies generated against unsubstituted/low–substituted (1-4)-β-D-xylan (LM10) xylans and poor and highly substituted xylans (McCartney et al., 2005). The antibodies were a generous gift from Prof. P. Knox (Leeds Univ., UK).

3. IMMUNOLABELING OF WHOLE FIBRES AND FIBRE CROSS-SECTIONS

i) Immunolabeling with LM10 and LM11 monoclonal antibody

Whole fibres were treated with anti-xylan in eppendorf tubes as described earlier by Daniel et al., (2010a).

ii) Immunolabeling of fibre cross-sections

Spruce fibres were processed and embedded in London resin (Daniel et al., 2010a) and fibre cross-sections cut and mounted on object glasses and labeled with the antibodies for immunofluorescence microscopy as described previously (Daniel et al. 2010b, Kim and Daniel, 2012). In contrast to previous studies the cross-sections were labeled over a two period at 4C.

iii) Positive and negative and substrate controls
Specificity of the anti-xylans was checked previously using a number of positive and negative substrate controls (Daniel et al. 2010a). Thus in the present study only the technical control where the antibody was omitted from the labeling procedure was adopted.

iv) Reference fibres for studying penetration of xylan
As a positive control on penetration, immunofluorescence observations were made on reference fully bleached spruce fibres (from Södra) in which X-LMw and X-HMw xylans were sorbed (Daniel et al., 2010). Spruce fibres (50 mg as dry wt) were treated in 8 ml sodium carbonate buffer (pH 10.5) containing 50 mg beech xylan (either X-LMw or X-HMw xylan in small beakers. Following swelling and mixing the fibres were autoclaved at 121C for 1h. The liquid was filtered off through glass filter G3. In the case of added X-HMw beech xylan (high MW), this took a long time (*ca* 20 min) and the fibers + xylan was finally blotted on a filter paper to remove the liquid buffer. The liquids were brown from added xylan. Following xylan treatment the fibres were embedded in London resin as described above, sectioned and immunolabeled.

4. Fluorescence microscopy
Whole fibres and fibre sections were placed on object glasses mounted in Fluorsave (Calbiochem) covered with coverslips and examined using a Leica DMRE fluorescence microscope fitted with a mercury lamp and I3-513808 filter-cube (Leica, excitation 450-490 nm, emission 515 nm) from Leica Microsystems, Wetzlar, Germany. Images were recorded using a Leica DC300F CCD camera and digital imaging system for professional microscopy (Leica Microsystems GmbH) at equal settings (exposure time 1s and gain 3.2).

Results and Discussion

1. WHOLE FIBRES SURFACES:

Immunolabeling of X-HMw and LMw fibres showed strong immunofluorescence and evidence for the presence of xylans (*Figure 2a-f*). Immunofluorescence showed large variability with some fibres labeling strongly, others weakly indicating a non-homogeneous spatial microdistribution of xylan in both the total fibre population as well as individual fibres (*Figures 2a-f)*. It was difficult to quantify any difference between the surface labeling of X-HMw and LMw fibres compared with the reference fibres (*Figure 1a-f*) although the treated fibres (i.e. X-HMw/X-LMw) gave an overall impression of a stronger fluorescence reaction. Similarly it was not possible to confirm any real difference between the labeling patterns of the two antibodies for unsubstituted and highly substituted xylans. Fibres not treated with the antibodies showed no immunofluorescence of their

surfaces or cross-sections thereby confirming the specificity of the antibody (*Figure 1a-c*).

2. FIBRE CROSS-SECTIONS:

Immunofluorescence of cross-sections showed labeling of both early- and latewood spruce fibres for the two treatments X-HMw and X-LMw xylans as well as the reference spruce fibres without extraneous added xylan (*Figures 3-5*). Overall a stronger fluorescence was shown by the LM11 for low and highly substituted xylans labeled fibres as shown in *Figure 5* where in some cases the serial sections of fibres have been labeled by either LM10 or LM11. Some variability of the labeling was noted for the inner and outer fibre wall suggesting stronger presence of xylan in both the treated and reference pulp fibres. No real difference however, was noted between fibres treated with X-HMw and those treated with LMw xylans (*Figures 3-5*).

Immunolabeling of fibre sections from the fully bleached pulps showed a similar pattern of immunofluorescence for both X-LMw and X-HMw pulps as observed kraft pulps (not shown). This no evidence for increased penetration of the xylans using this approach was noted.

The results with immunofluorescence are consistent with that achieved with the TEM immunogold labeling shown in *Appendix 4* of this report.

Conclusions

- Immunofluorescence using the anti-xylan monoclonal antibodies LM10 and LM11 showed strong positive evidence for the presence of beech X-HMw and X-LMw associated with both early- and latewood fibres when added during the impregnation stage of kraft cooking;
- Immunofluorescence of both whole fibres and fibre cross-sections gave complimentary information with whole fibres giving an overview of a larger population of fibres and reaction with different surface morphological structures on the fibre wall while the cross-sections gave information of the presence of xylan on both the outer fibre- and inner lumen walls surface as well as in the secondary fibre cell walls;
- Immunofluorescence and thereby beech xylan was generally unequally distributed over the fibre population and individual fibre surfaces suggesting variable presence;
- Immunofluorescence of cross-sections with LM11 appeared to give a slightly stronger reaction than LM10 indicating a greater presence of low- and highly substituted xylans

- Since the labeling patterns of fibre cross-sections were similar for the X-HMw, X-LMw pulps compared with the reference spruce pulp it is not possible to conclude penetration of either the low- or high molecular xylans. The alternative possibility is that the both the low and high molecular xylans are penetrating the spruce cell walls but because the xylans are similar to the native xylans it is not possible to distinuish between them. The results are however consistent with that achieved with the TEM-immunogold labeling studies (*Appendix 4*) which showed very little difference between the X-LMw and X-HMw and reference pulps.

References

Daniel G., Kaňuchová, A., Filonova L., Ander P. 2010a. Visualization and quantification of xylans on xylan treated kraft spruce pulp fibres using immunofluoresence microscopy, ELISA, HCL and xylanase assays. In: Proc. 11th Workshop on Lignocellulosics and Pulp, Hamburg, August 16-19, 33-37.

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Kim, J. S., Daniel, G. 2012. Immunolocalization of hemicelluloses in Arabidopsis thaliana stem. Part 1: temporal and spatial distribution of xylans. Planta DOI 10.1007/s00425-012-1686-y. 14pp.

Figure Legends

Figure 2a-f. Reference, X-LMw, and X-HMw (i.e. normal xylan) beech xylan showing positive immunofluorescence for xylan (green colour) on fibre surfaces. Labeling was typically non-homogeneous with the xylan frequently forming a thin outer layer on the fibre as shown with focus through the fibre wall (Figure b, c).

Figures 3-5. Immunofluorescence of fibre cross-sections (both early and latewood) from the X-HMw, X-LMw and reference spruce pulps labeled with either LM10 (for low and unsubstituted xylans) and LM11 (low and highly substituted xylans). Nomenclature of the type of treatment and antibody type is given directly on the individual images. Figure 5 gives a direct comparison between the two antibodies and treatments.

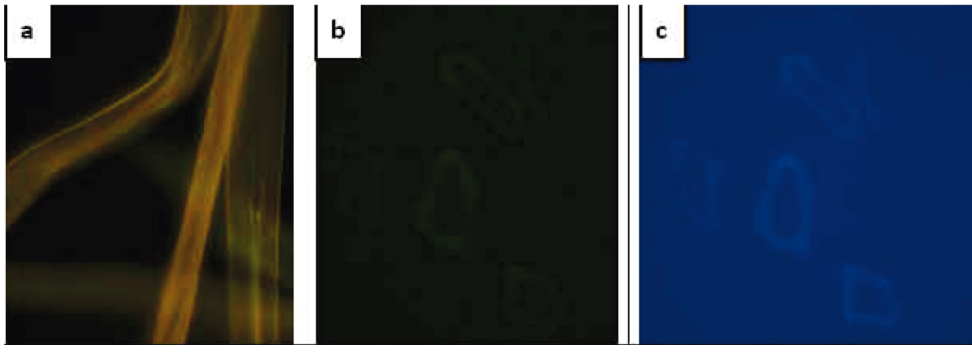


Figure 1a-c. Examples of whole fibres (a) and fibre cross-sections labeled with LM10 for unsubstituted and poorly substituted (b) xylans. Figure 1c shows the same cross-section as Figure 1b, but using UV-filters for presence of autoflourescence. Some indications for xylan are given in the outer cell layer (S1/primary wall?) of the lower earlywood fibre in the image.

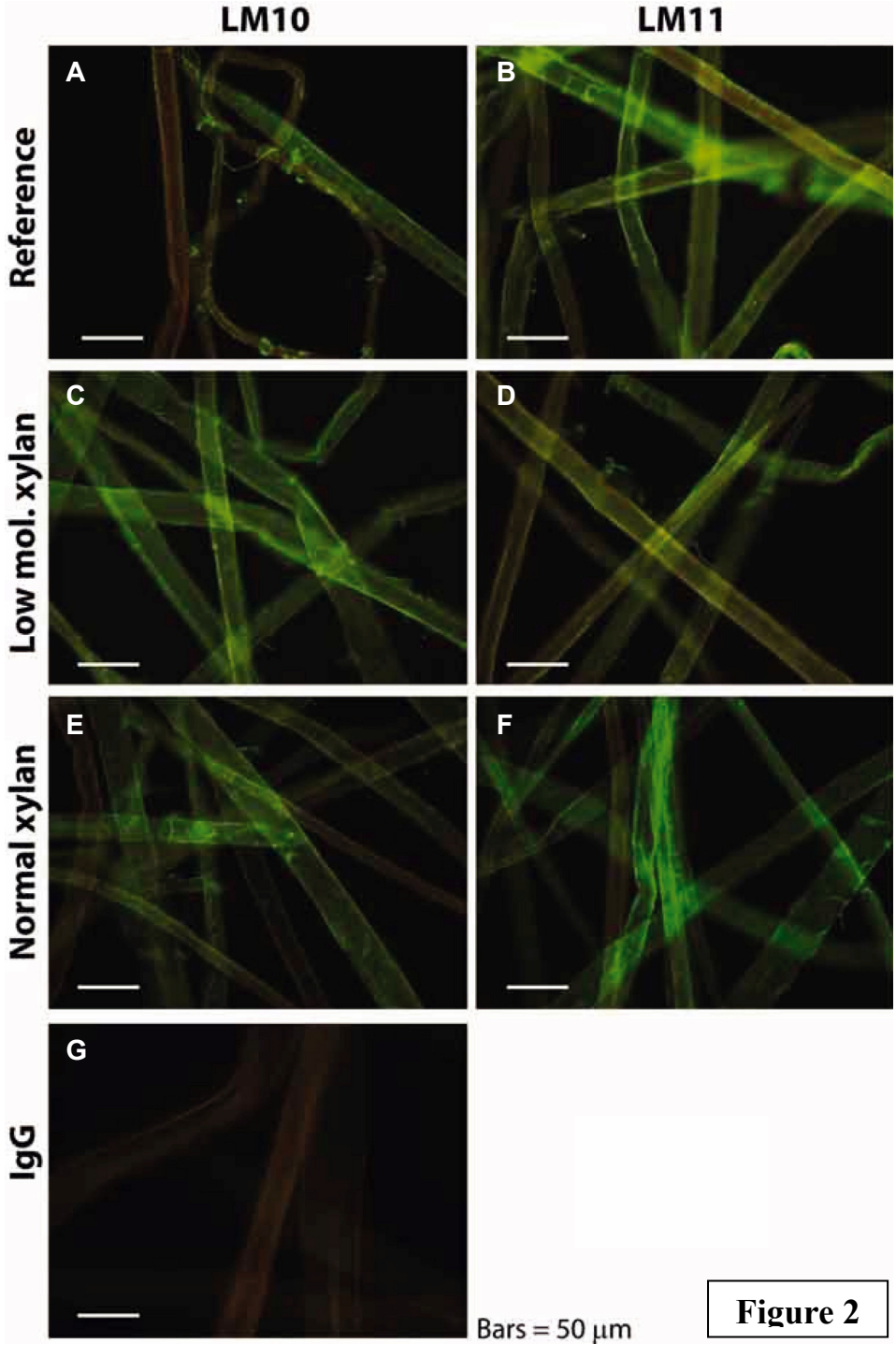
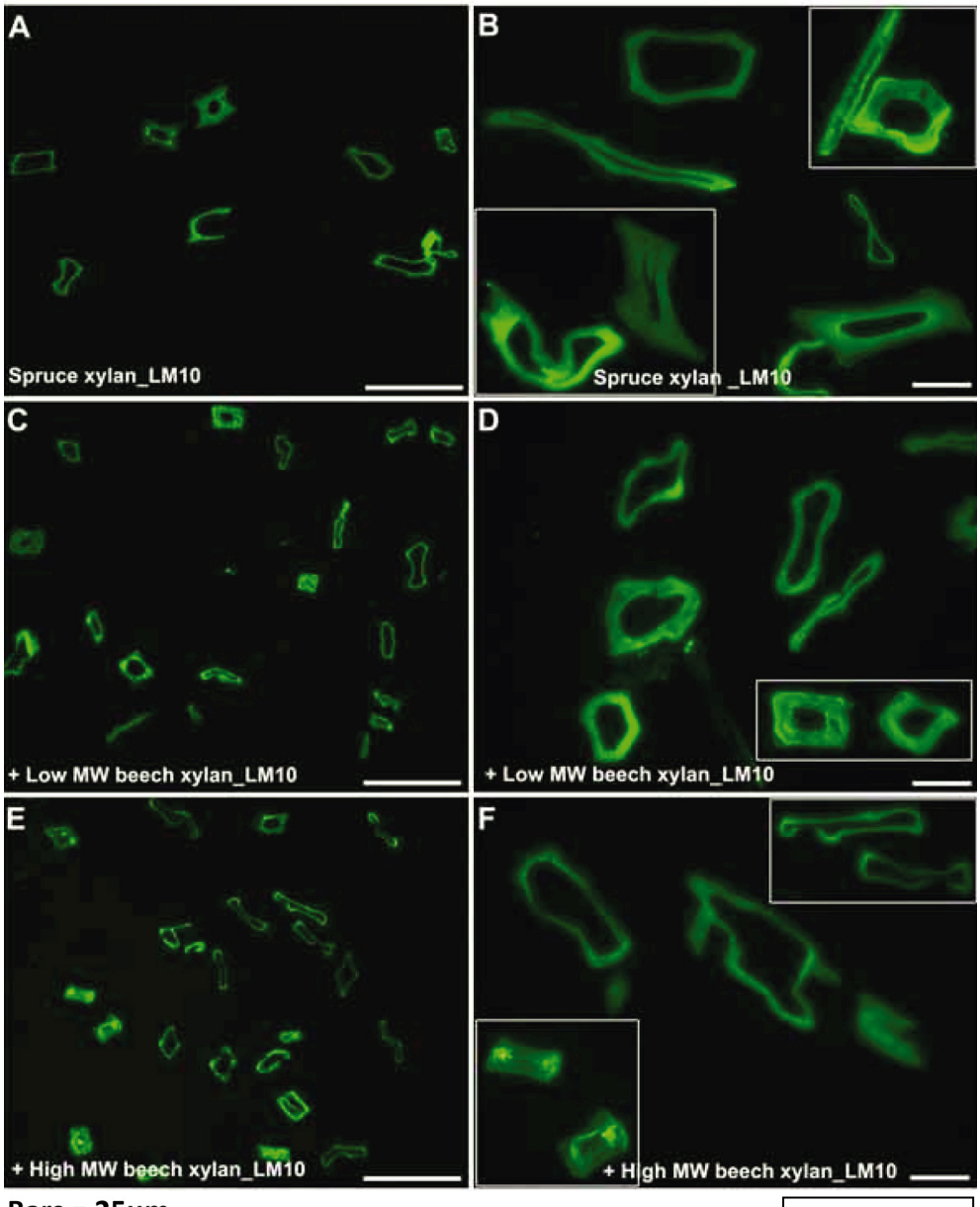

LM10
LM11
Reference
Low mol. xylan
Normal xylan
IgG
A
B
C
D
E
F
G
Bars = 50 µm

Figure 2

LM10



Bars = 25µm

Figure 3

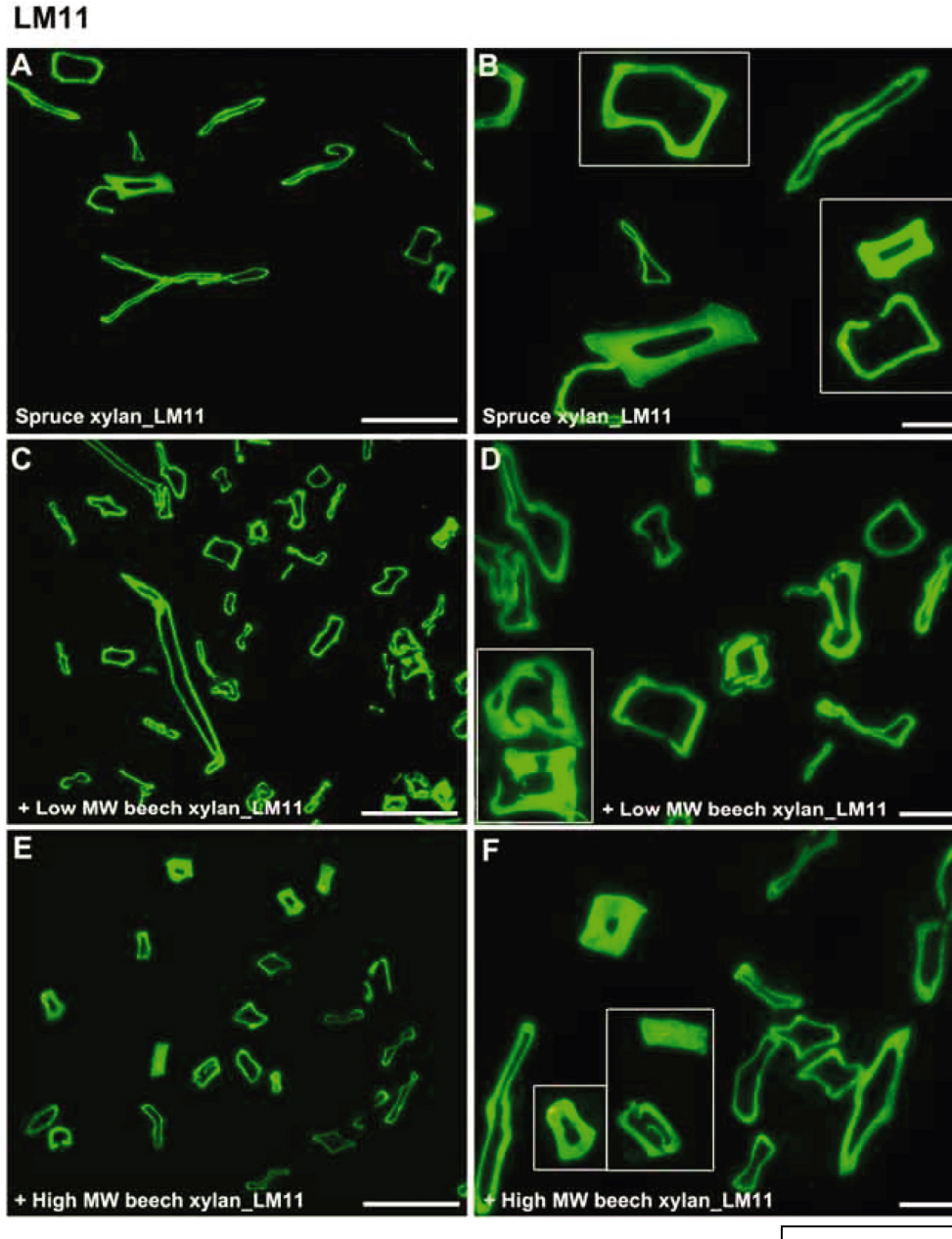

LM11
A
Spruce xylan_LM11
B
Spruce xylan_LM11
C
+ Low MW beech xylan_LM11
D
+ Low MW beech xylan_LM11
E
+ High MW beech xylan_LM11
F
+ High MW beech xylan_LM11

Figure 4

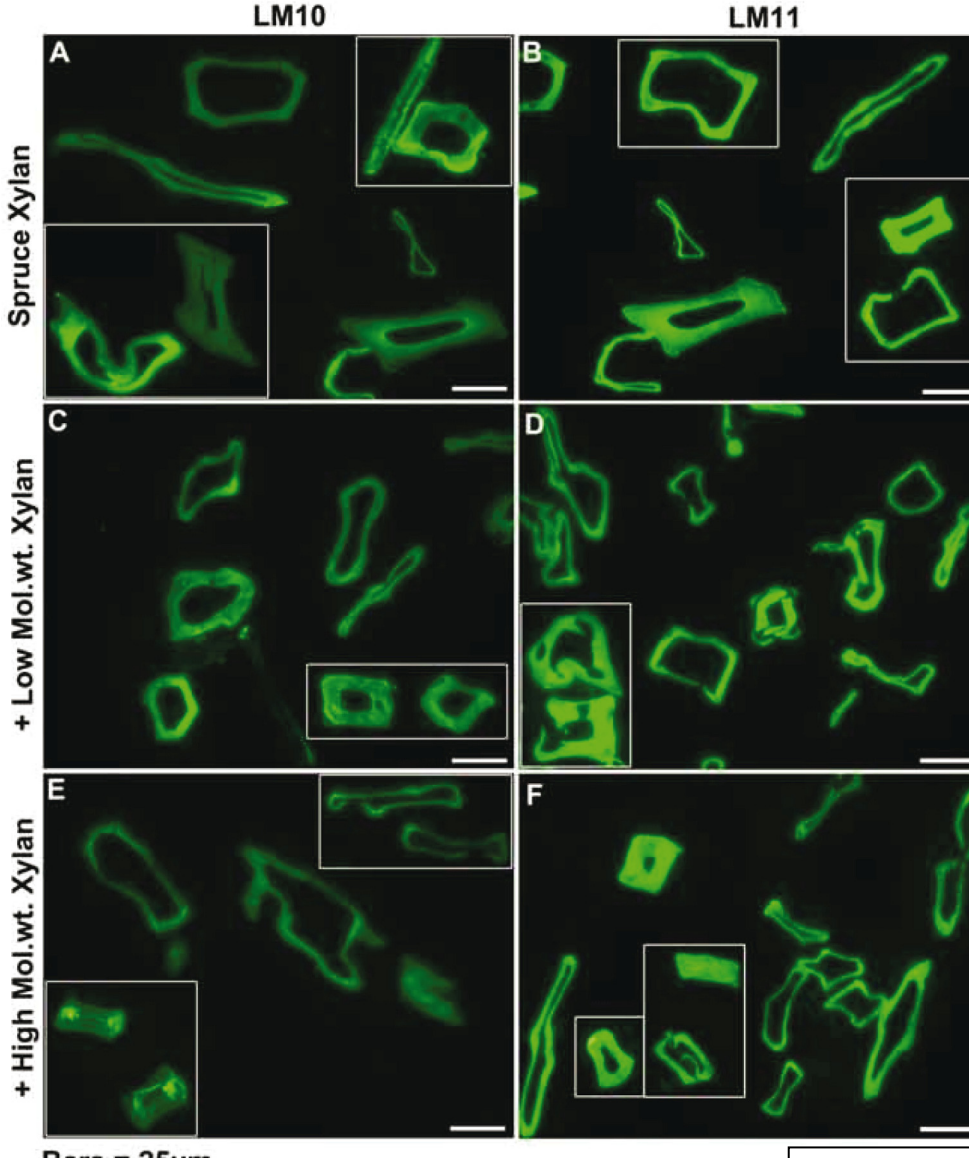

LM10
LM11
A
B
C
D
E
F
Spruce Xylan
+ Low Mol.wt. Xylan
+ High Mol.wt. Xylan
Bars = 25µm

Figure 5

APPENDIX 6. DISTRIBUTION OF LIGNIN AND XYLAN AFTER ADDITION OF XYLAN OF DIFFERENT MOLECULAR WEIGHTS

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Aims

The purpose of this study was to investigate if added beech xylan with low molecular weight could more easily penetrate into the cell wall than xylan wth high molecular weight and if such a penetration would affect lignin and/or xylan distribution within the chips after cooking. In order to monitor the local chemical changes in chips FTIR-spectroscopy utilised.

Background

It is well know that xylan is precipitated onto the fibres surfaces during cooking. With addition of additional xylan noticeable increase in the total xylan content of pulps are noticed. However it has been questioned if the added xylan may enter into the cell wall and if that could affect the dissolution of both lignin and xylan from the cell wall. With the addition of lower molecular weight xylan a higher possibility for xylan penetration into the cell wall would be envisaged. Thus such studies were here performed. In order to investigate the local composition of xylan as well as lignin from different positions of chips FTIR spectroscopy is a suitable technique. Small amounts of sample materials required.

Experimental

Wood material: Three cooks were performed; a reference, a cook with high molecular weight xylan added, a cook with low molecular weight xylan added, all with model chips of the size of 5*5*30 mm R*T*L added. For evaluation of the distribution of xylan and lignin samples were taken from the model chip as shown in *Figure 1*. The test pieces consisted of material from the innermost part of the chip and surface material from the outer part of the chip, positions **a** and **b**. Material from both the inner and outer parts was ground off. In order to be able to make an IR spectrum the powder was mixed with KBr salt, which is inert for the IR radiation, and pressed under high pressure to a tablet. As a comparison ground material from the upper surface of the chip was also evaluated, position **c**.

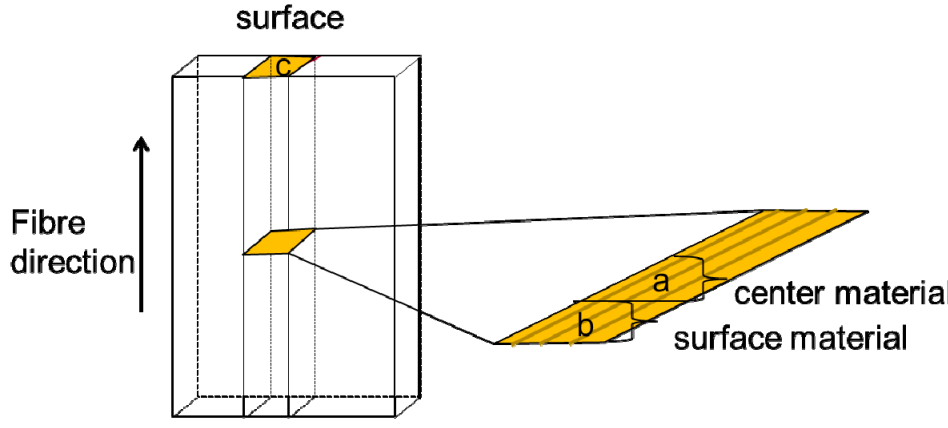

Figure 1. A model chip with the sample positions a, b, and c indicated.

FTIR analysis: The composition of the ground material was determined using FTIR spectra taken by a Varian 680-IR spectrometer (Agilent technologies, Santa Clara, CA, USA) in the range from 700 to 4000 cm^{-1} with a spectral interval of 1 cm^{-1} on KBr-tablets in transmission with a DTGS detector. The spectra were baseline corrected to zero at 813, 1525, 1845, 2505, 1981 and 3750 cm^{-1}. To compensate for different amounts of material in the tablet the spectra were normalized to an intensity of 1 at the peak at 2890 cm^{-1}, assigned to overall carbohydrates.

The lignin amount was evaluated as the peak height at 1508 cm^{-1} assigned to the C=C aromatic ring vibration. Xylan appear as a shoulder at 1460 cm^{-1} from the CH_2 bending. This value was difficult to determine why the xylan estimate is more uncertain than the lignin one. The peak heights has not been recalculated to an absolute content, therefore they must be seen as relative differences.

Results and discussion

The relative composition of lignin and xylan was studied from different positions on the chips using FTIR spectroscopy. Separately, FTIR spectra showed that a modification of the beech xylan, used as an additive in the cooks, occurs during preparation of the low molecular weight xylan. The endoxylanase treatment seemed to remove the charged groups occurring at 1734 cm^{-1}, *Figure 2*. Therefore, only the peak at 1460 cm^{-1} assigned to CH_2 wagging of xylan could be used to follow the xylan content of the cooked chips. Being a shoulder this peak is affected by surrounding peaks and therefore less accurate.

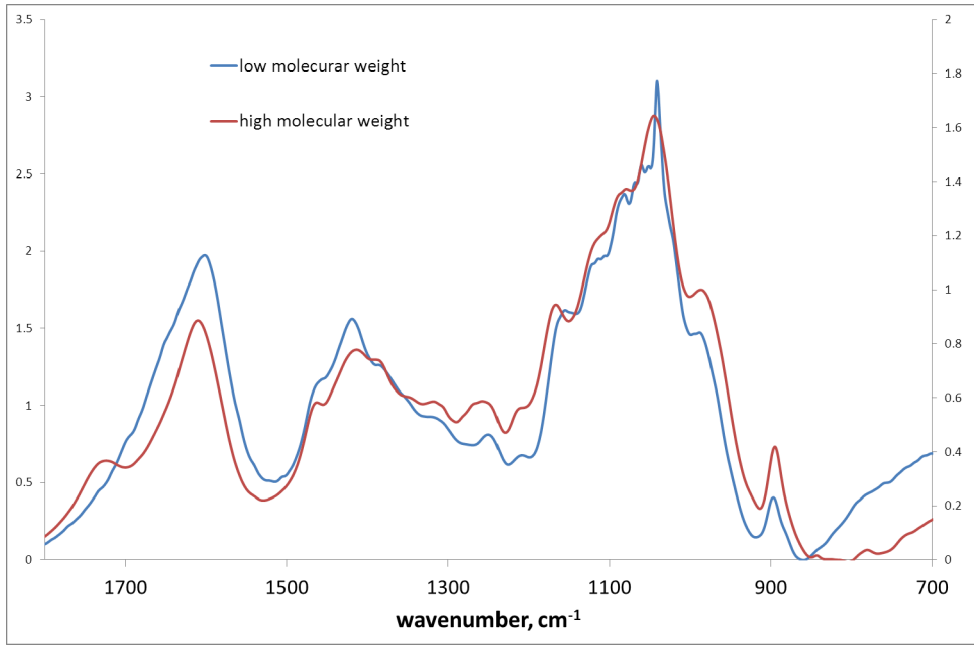

Figure 2. FTIR spectra for xylan of high and low molecular weight

Lignin distribution: *Figure 3* shows the relative peak height corresponding to lignin content for the centre of the model chip compared to the two surfaces b and c of the chips. Still at this point at the end of the cook with about 6% of lignin remaining in the pulp there was more lignin present in the center of the chip than at the surfaces. A slight indication of higher lignin content in cooks with added xylan, similar to what has previously been noted was seen. In general the same trend of lower surface lignin content was seen in the three cases. However there was some indication that the high molecular xylan hindered the dissolution of lignin from the surface of the chips to some extent. The scatter in the evaluation makes however the conclusions uncertain.

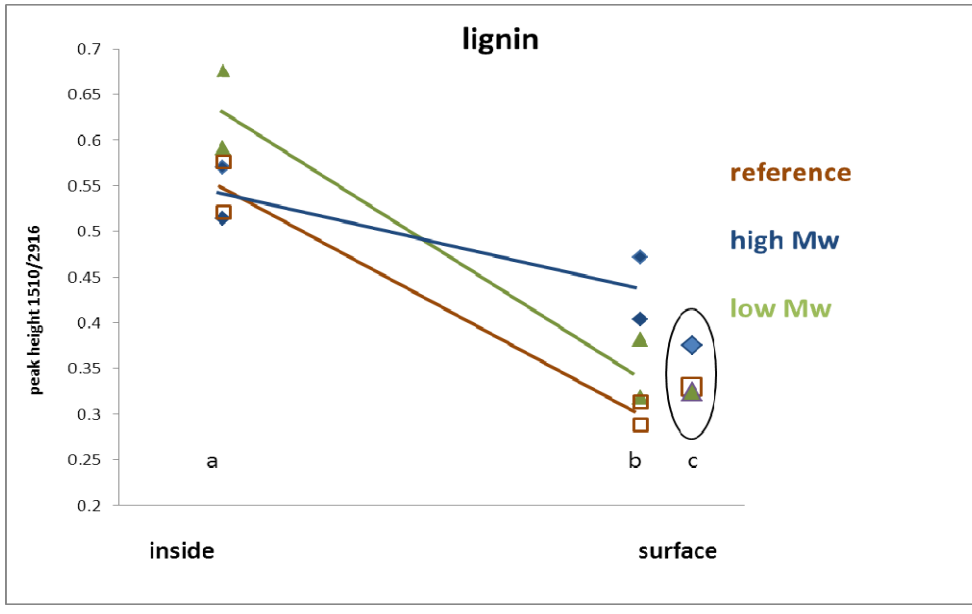

Figure 3. Relative height of the lignin peak at 1505 cm^{-1} in the centre (a) and in the surfaces (b) and (c) of model chips compared for the three cooks; reference, addition of high molecular weight beech xylan, addition of low molecular weight beech xylan.

Xylan distribution: In *Figure 4* the relative xylan distribution is indicated for the different cooks as taken from the height of the shoulder at 1460 cm^{-1}. The scatter in the evaluation is large and the difference between the samples small, especially for the surface measurements. The xylan distribution taken from the middle part of the chip seems to be rather uniform as also previously noted. For the end surface of the chip, position c, a slightly lower xylan content may be seen for all three cooks.

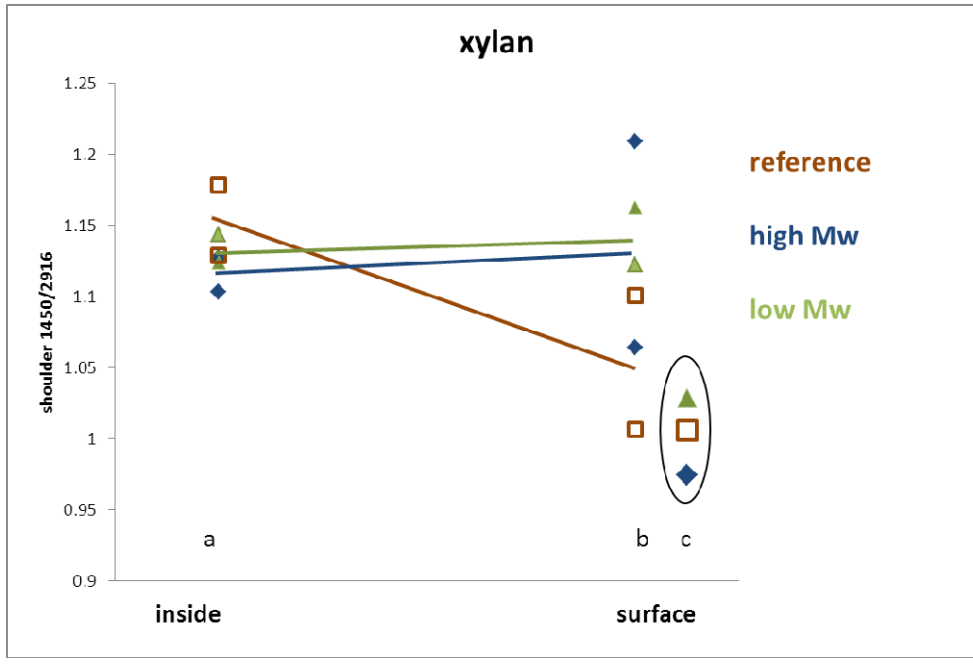

Figure 4. Relative height of the xylan peak taken as the height of the shoulder at 1460 cm^{-1} in the center (a) and in the surfaces (b) and (c) of model chips compared for the three cooks; reference, addition of high molecular weight beech xylan, addition of low molecular weight beech xylan.

Conclusions

With FTIR spectroscopy the detection and content of lignin from different positions in a chip during kraft cooking was possible determine while measurements on xylan content were more difficult. With the addition of xylan, the lignin content was found to slightly increase in the chips, particularly with added high molecular weight xylan. Possibly the lignin dissolution from the chip surfaces was restricted. For xylan, no clear differences between the different cooks were noted.

Collaborative Research on the Ultrastructure of Wood Fibres (CRUW)

CRUW represents a collaborative research program between the Swedish Forest Industries Akzo Nobel, Holmen, Smurfit Kappa Packaging, SCA, Stora Enso, Södra, SLU, Innventia, KTH and Mid Sweden University. The program is directed towards energy efficient processes for mechanical pulping and retention of the full fibre potential in chemical pulping. It is believed that research ideas based on insight into fibre ultrastructure can provide openings for breakthroughs in the applied area. The program forms part of the VINNOVA and Industry *"Branschforskningsprogram för skogs- och träindustrin"*.

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