Chemical Pulping

The influence of xylan on the sensitivity towards fiber damage analyzed on pulps cooked to high kappa numbers

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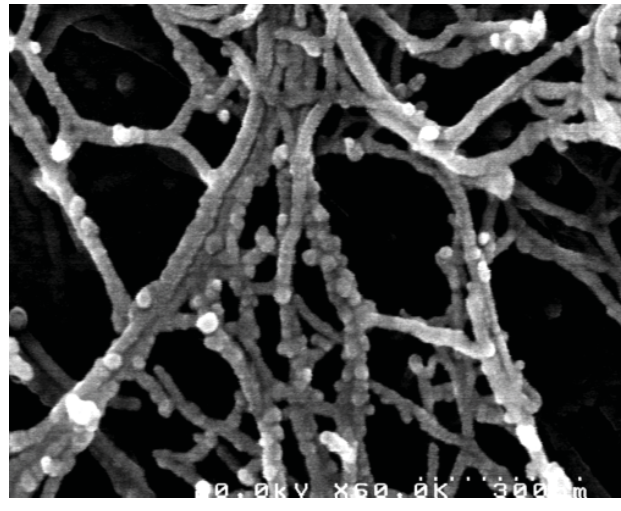

Intern rapport nr 5 (begränsad spridning)

CRUW
Centre for Research on Ultrastructure of Wood fibres
Centrum för forskning om Vedfiberns Ultrastruktur
Sveriges lantbruksuniversitet
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Uppsala 2011

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Summary

The aim of this study was to determine if the presence of xylan in the fiber wall was important for the degree of damage introduced in fibers during mechanical action in the cook for high kappa number pulps of ca. 80. The influence of the temperature on when the xylan was added and when mechanical treatment took place was also investigated. Kraft pulps from spruce with birch xylan addition were produced in the laboratory. At the end of the cook, fiber damages were introduced by subjecting the fibers to shear and compression forces.

Four different variants of pulps were produced, each with- and without mechanical treatment at the end of the cook. One variant was produced without added birch xylan at the end of the cook and three with birch xylan added. Prior to xylan addition, the cooking temperature was adjusted to different levels and the mechanical treatment was also performed at different temperatures. Two pulp variants had xylan added at a cooking temperature of 165 °C. The temperature was then adjusted to different levels before mechanical treatment; i.e. 120 °C and 80 °C. For the third variant with xylan addition, the temperature was adjusted to 120 °C before xylan addition and the mechanical treatment was also performed at that same temperature.

No direct indications were seen that the extra xylan added during the cook could protect the pulp from strength deterioration due to the introduced mechanical treatment during the cook. However, the temperature when the mechanical treatment took place was shown to be important. When performing the mechanical treatment at 80 °C instead of at 120 °C the strength properties were significantly improved.

The extra xylan added during the cook resulted in straighter fibers and, as expected, improved tensile strength development for these pulps.

Contents

1 Background

In a previous investigation (Daniel et al. 2010), the presence of xylan and the position of xylan in the fiber wall were studied to determine its importance for the degree of damage introduced in fibers during mechanical action in the cook. Kraft pulps from spruce with different amounts of xylan were produced in the laboratory, either by adding birch xylan at different positions in the cooking process or by redistribution of spruce xylan in the liquor. At the end of the cook, fiber damages were introduced by subjecting the fibers to shear and compression forces. No direct positive indications were obtained that xylan added during the cook resulted in a lower amount of introduced damaged areas although the added xylan led to less curled fibers. The surface adsorbed xylan also led to improved yield without impairing the tear-tensile relationship.

In this study the influence of temperature and the time of birch xylan addition and when mechanical treatment takes place was investigated for pulps cooked to high kappa numbers of ca. 80.

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 and fractions 2, 3 and 4 used in the cooking experiments.

Four different variants of pulps were produced, each with- and without mechanical treatment at the end of the cook. One variant was produced without added birch xylan at the end of the cook and three with birch xylan added. The temperature was adjusted to different levels before adding. The mechanical treatment was also performed at different temperatures. Two pulp variants had xylan added at a cooking temperature of 165 °C. The temperature was then adjusted to 120 °C or 80 °C before the mechanical treatment. For the third variant with xylan addition, the temperature was adjusted to 120 °C before xylan addition and then the mechanical treatment was performed at the same temperature. In *Appendix 1a* the difference between the three variants regarding xylan addition temperature and shearing temperature are illustrated in temperature-time-diagrams. Pulps were produced at Innventia, using a digester equipped with a device that can introduce mechanical forces, i.e. shear and compressive forces on the pulp. The digester is further described by Salmén and Lundqvist (Salmén, Lundqvist 2011). To obtain enough pulp, sometimes more than one cook per variant were performed and merged. Primary data for all cooks are shown in *Appendix 1b*. The pulps were cooked to about the same kappa number of ca 80 and the cooking conditions used shown in *Table 1*.

The birch xylan used was obtained from Sigma Aldrich. Data on carbohydrate composition, molecule weight and degree of substitution for the xylan can be found in *Appendix 1a* in Daniel et al. (2010). The birch xylan concentration was 10 g/L in the liquor used in the cooking stage.

Table 1. Cooking conditions

	Xylan addition at temp/time	Amount of xylan added g/L	Shearing temp	Compr %	EA %	H-factor	Cooking time[2] min	Res OH^- g/L	Kappa no
Ref-N[3]	-	-	-	-	20	780	67	5.0	83.0
Ref-S[4]	-	-	120	45.2	20	780	67	5.8	77.4
XY-N165	165/50	10	-	-	20	779	67	5.2	77.1
XY-S165	165/50	10	120	45.8	20	779	67	5.1	75.1
XY-N120	120/0	10	-	-	20	781	67	5.7	80.2
XY-S120	120/0	10	120	45.2	20	776	66	5.6	75.8
XY-N80	165/50	10	-	45.8	20	758	65	4.8	80.3
XY-S80	165/50	10	80	45.8	20	758	65	4.8	80.9

[2]*at 165 °C*
[3]*N cooks without mechanical treatment.*
[4]*S cooks with compression and shearing forces induced for 2 minutes 15 minutes before the end of the cook.*
All data are average values from respective cooks.

2.1 ANALYSIS

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

The surface chemical composition was analyzed on material produced from mechanical peeling in a disintegrator after 200,000 revolutions at 3% pulp concentration. More details on the procedure can be found in *Appendix 1d* in Daniel et al. (2010). These analyses were performed by Södra.

Quantification of birch xylan on fiber surfaces was done using ELISA. More details can be found in *Appendix 2*.

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

Visualization of adsorbed xylan was analyzed using immunofluorescence microscopy. More details can be found in *Appendix 3*.

Visualization of adsorbed xylan was also done using SEM. More details can be found in *Appendix 4*.

Distribution of xylan on fiber surfaces and overall composition was analyzed with FTIR microscopy *(Appendix 5)*.

Measurements on the number of weak points in fibers were analyzed using the HCl-method *(Appendix 6)*.

3 Results and discussion

The pulps were evaluated regarding introduced amount of damage and strength properties after the cook. When producing the pulps the variables included addition or absence of xylan with or without mechanical treatment in the digester as well as the temperature for birch xylan addition and when mechanical treatment took place. In total, eight pulps were included as outlined in *Table 2*. However, the results of the XY-N80-pulp indicated that something had happened during production of that pulp, leading to much lower strength properties compared to the other pulps produced without mechanical treatment in the cook. The results from that pulp have therefore not been included in the report. Instead XY-N165 will be used as a reference for the XY-S80-pulp as well.

Table 2. Pulp variants included in the study

	Description	
Reference without xylan added, mechanical treatment at 120 °C	Ref-N[1]	Ref-S[2]
Xylan added at 165 °C, mechanical treatment at 120 °C	XY-N165	XY-S165
Xylan added at 120 °C, mechanical treatment at 120 °C	XY-N120	XY-S120
Xylan added at 165 °C, mechanical treatment at 80 °C	XY-N80	XY-S80

[1] *N=without mechanical treatment*
[2] *S= with mechanical treatment 15 minutes before end of cook*

3.1 INVESTIGATED PULPS

Results after cooking are shown in *Table 3*. The pulps had kappa numbers of about 75-83. Pulps produced with mechanical treatment at the end of the cook (S) had a slightly higher dry matter content compared to their respective pulps produced without mechanical treatment (N), as analyzed after standardized centrifugation. The differences were however much smaller for these high kappa number pulps compared to pulps produced without or with mechanical treatment at kappa number levels around 30 (Daniel et al., 2010). Pulps produced with addition of extra birch xylan at 165 °C had a higher yield, about 1.7 %, compared to the pulp produced without xylan addition. The yield for the pulp produced when the xylan addition took place at 120 °C had about 1 % higher yield compared to the pulp produced without xylan addition (*Table 3*), i.e. lower then when the addition was made at 165 °C (variants XY-N165 and XY-N80). The time for the xylan present in the cook was also shorter for that variant.

Table 3. Cooking results

	Residual OH- g/L	Kappa no	Dry content %	Shives%	Tot. yield %	Cal. tot yield at Kappa no 80
Ref-N	5.0	83.0	23.1	1.2	56.3	55,8
Ref-S	5.8	77.4	23.9	1.3	56.6	-
XY-N165	5.2	77.1	23.2	0.7	57.3	57,7
XY-S165	5.1	75.1	23.4	1.1	57.1	-
XY-N120	5.7	80.2	21.2	1.1	56.8	56,8
XY-S120	5.6	75.8	23.4	1.3	56.5	-
XY-N80	4.8	80.3	23.1	1.3	57.3	57,3
XY-S80	4.8	80.9	24.7	1.3	57.3	-

3.2 XYLAN DISTRIBUTION WITHIN THE FIBER WALL; VISUALIZATION

Immunofluorescence observations on whole fibres and fibre sections and SEM observations studies on whole fibres were carried out to observe the presence of xylan associated with the fibres. Results emphasized the great heterogeneity of the fibre population (i.e. differences between early- and latewood fibres) with the microscopic visualization methods for the microdistribution of xylan on the fibre wall providing the complimentary background evidence to the ELISA results. Using specific xylan antibodies, immunofluorescence confirmed the presence of adsorbed birch xylan on the pulp fibre surfaces both on the outer and inner lumen walls (*Figure 1*). A similar trend was noted as in previous studies that only the highly processed fibres (e.g. sheared fibres) with very open structure allowed for strong xylan penetration. Otherwise the xylan was located as seen from cross-sections as forming a band on exposed surfaces. SEM observations confirmed the microfibrillar structure of the outer fibre wall (*Figure 1*). It further indicated that the birch xylan added to pulps was likely to be found in different binding forms; e.g. cellulose-xylan, xylan-xylan (e.g. aggregates). Aggregates of the order of 20-25 nm were recognized as a characteristic feature binding to macrofibrillar structure of the fibre walls in all pulps treated with the birch xylan.

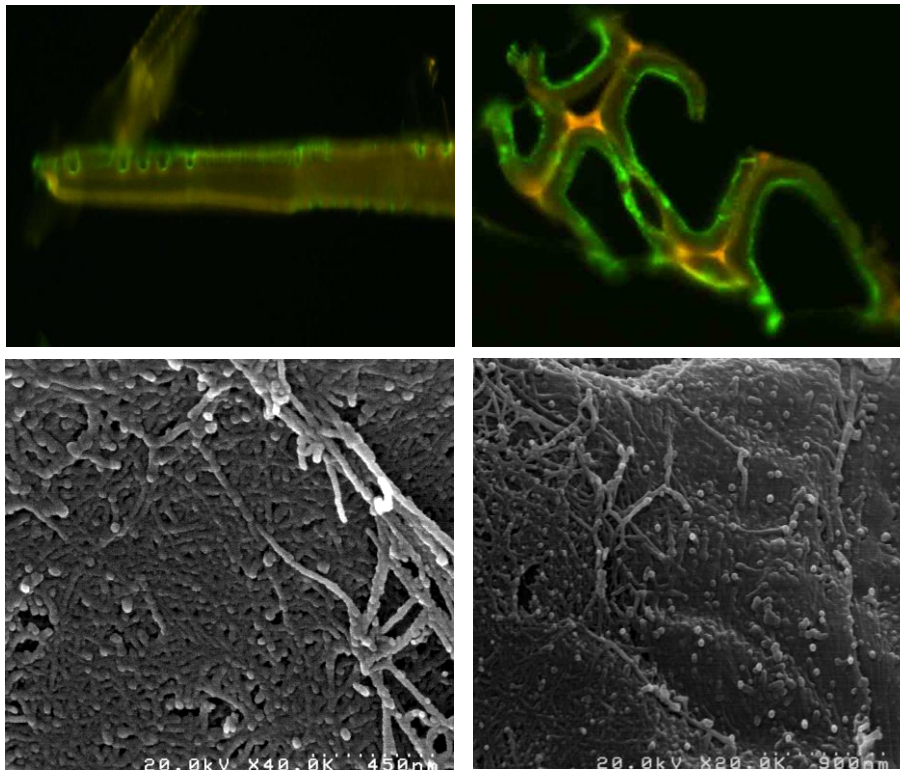

Figure 1. Visualization of adsorbed xylan on XY-165N using immunofluorescence and XY-165N (left) and XY-165S (right) using SEM. Xylan binding to the fiber surfaces was apparent by the green fluorescence of FITC secondary antibody label which was reflected by the presence of small agglomerates of the order ca 20-25 nm of the fiber surfaces using SEM.

3.3 CARBOHYDRATE COMPOSITION AND FIBER CHARGES

Birch xylan was added late in the cook at different temperatures. All pulps with added birch xylan had a higher relative xylan content compared to the Ref-pulp (*Table 4)*. When comparing the pulps with added xylan at different temperatures, no differences were seen between the pulps cooked without mechanical treatment. When comparing the pulps cooked with mechanical treatment at the end of the cook, the pulp where xylan addition was done at 120 °C had a lower xylan content compared to the pulps where the xylan addition was done at 165 °C. The time for the xylan present in the cook was also shorter for that variant. Two pulp variants with xylan addition at 165 °C were compared. Before the mechanical treatment, the temperature was adjusted to 120 respectively 80 °C for the two different variants (XY-N165

and XYS165 respectively XY-N80 and XY-S80). The pulp with adjusted temperature to 80 °C shows a higher total charge and also a slightly higher surface charge compared to the pulp with a higher temperature at the end of the cook. The carbohydrate composition analysed on the surface material is shown in *Table 5*. The scattering in the results was quite large for these high kappa no-pulps compared to previously results on kappa no-30-pulps (Daniel et al., 2010). The result for the Ref-N-pulp is obviously not correct. Maybe the higher scatter can be due to the low amount of carbohydrate material released during the mechanical treatment and thus be available for analysis.

Table 4. Carbohydrate content in the investigated pulps, as well as fiber charges

		Ref-N	Ref-S	XY-N165	XY-S165	XY-N120	XY-S120	XY-N80	XY-S80
Galactose	Rel. %	0.9	0.8	0.9	0.9	0.9	0.8	0.8	0.8
Glucose	Rel. %	81.1	81.2	79.5	78.9	79.9	79.9	80.0	79.4
Mannose	Rel. %	7.6	7.8	7.6	7.6	7.6	7.4	7.2	7.2
Arabinose	Rel. %	1.2	1.1	1.2	1.1	1.1	1.1	1.1	1.1
Xylose	Rel. %	9.3	9.1	10.8	11.6	10.5	10.8	10.9	11.5
Galactose anhydro	%	0.8	0.7	0.8	0.7	0.7	0.7	0.7	0,7
Glucose anhydro	%	67.8	68.2	64.4	63.0	65.8	67.2	67.9	67.5
Mannose	%	6.3	6.5	6.1	6.0	6.3	6.2	6.1	6.1
Arabinose anhydro	%	0.9	0.9	0.9	0.8	0.9	0.9	0.9	0.9
Xylose anhydro	%	7.6	7.5	8.6	9.1	8.4	8.9	9.0	9.6
Tot anhydo sugar	%	83.5	83.7	80.8	79.6	82.2	83.9	84.5	84.8
Hydrorest	%	13.1	12.9	12.8	14.4	13.4	12.9	12.1	11.5
Tot acid groups	mmole/kg	113	110	129	129	102	130	148	142
Surf. Charge (neg)	meqv/kg	10	10	10	13	10	12	12	14

Table 5. Surface carbohydrate composition, relative amount of anhydrosugar, %, analysed on the removed material from mechanical surface peeling

		Ref-N	Ref-S	XY-N165	XY-S165	XY-N120	XY-S120	XY-N80	XY-S80
Galactose anhydro	Rel. %	11.7	11.5	10.6	8.0	10.3	9.2	13.6	13.4
Glucose anhydro	Rel. %	14.4	35.2	32.2	41.3	23.6	33.9	16.1	16.3
Mannose	Rel. %	12.1	9.3	8.4	7.5	9.8	7.9	9.5	9.0
Arabinose anhydro	Rel. %	15.5	15.1	13.6	10.1	13.5	11.6	17.0	15.7
Xylose anhydro	Rel. %	46.2	28.9	35.3	33.0	42.8	37.3	43.9	45.7
Tot anhydro sugar	Rel. %	100	100	100	100	100	100	100	100

The ELISA analyses show a somewhat higher xylan content in the XY-S pulps compared to the XY-N-pulps (*Figure 2*) a results found with previous studies (Daniel et al., 2010) and also in accordance with the carbohydrate results in *Table 4*.

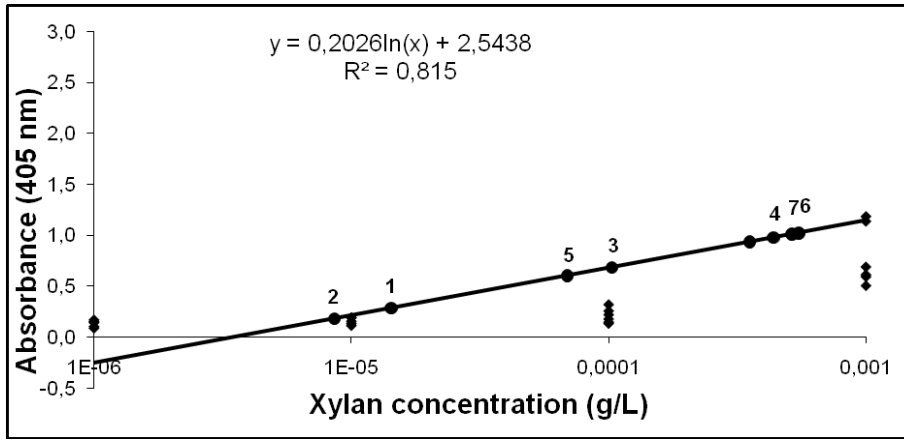

Figure 2. Estimation of xylan (g/L) binding with ELISA to the high Kappa pulps as shown from the standard curve: 1, Ref-N; 2, Ref-S; 3, XY-N165; 4, XY-S165; 5, XY-N120; 6, XY-S120; 7, XY-S80. Results reflect mean from 16 individual measurements of pulp samples.

3.4 INTRODUCTION OF FIBER DAMAGE

For all variants the pulp produced without mechanical treatment had straighter fibers, especially when unbeaten *Figures 3, 4*. The pulp produced with the lowest temperature when the mechanical treatment was applied (XY-S80) had the straightest fibers/lowest amount of kink/mm of all investigated pulps with applied mechanical treatment. In *Appendix 1c* FiberMaster data for all pulps after beating is shown.

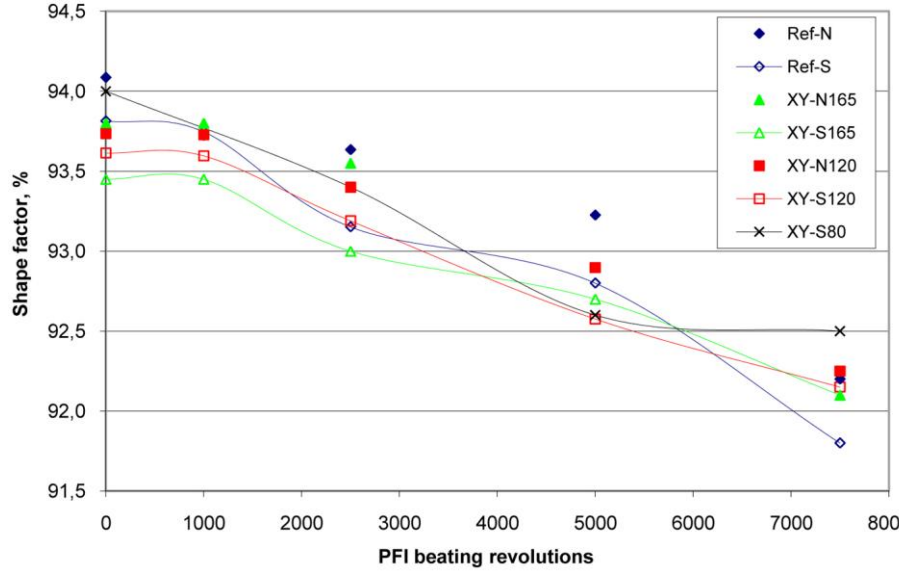

Figure 3. Shape factor vs PFI beating revolutions.

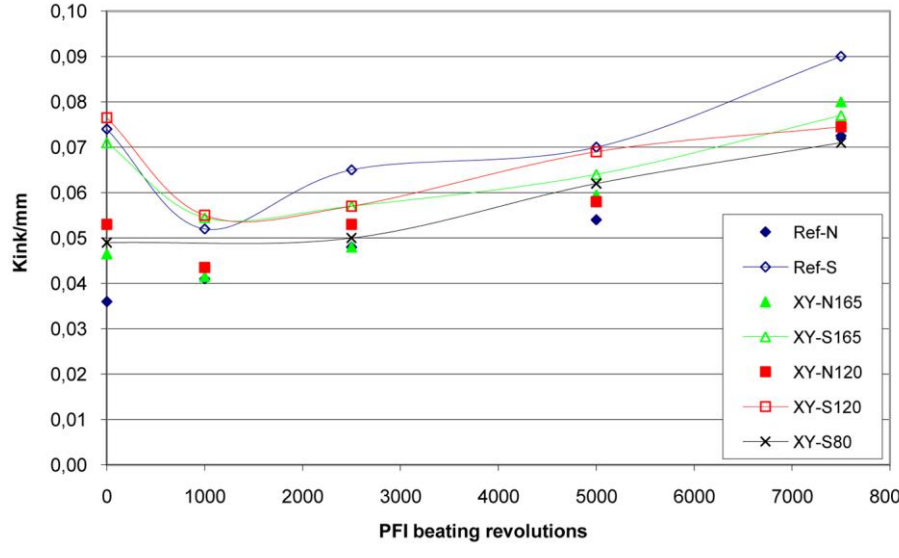

Figure 4. Kinks/mm vs PFI beating revolutions.

The acid sensitivity and the resulting fiber cleavage number for all investigated pulps are showed in *Table 5*. The pulp with lowest yield and lowest amount of surface xylan (XY-S120) had the highest cleavage number

compared to the other xylan variants after mechanical treatment. The cleavage number is about the same as for the Ref-S pulp (same fiber length after cleavage but higher before cleavage due to straighter fibers). When comparing the two pulps with xylan addition at 165 °C, the pulp where the mechanical treatment was introduced at 80ºC shows a lower sensitivity towards the acidic treatment compared to the pulp where the mechanical treatment was performed at 120 °C.

Table 6. Length weighted fiber lengths (LWFL) and cleavage per fiber by HCl (standard method: 4h at 80° C + 30 min final cleavage

		Ref-N	Ref-S	XY-N165	XY-S165	XY-N120	XY-S120	XY-S80
LWFL H2O	mm	2.28	2.29	2.30	2.33	2.27	2.33	2.25
LWFL after HCl	mm	1.86	1.21	1.88	1.32	1.90	1.21	1.37
Cleavage/fiber		0.195	0.883	0.225	0.765	0.195	0.927	0.641

The zero-span levels as a function of PFI beating revolutions is shown in *Figure 5*. No significant differences or any trends were observed.

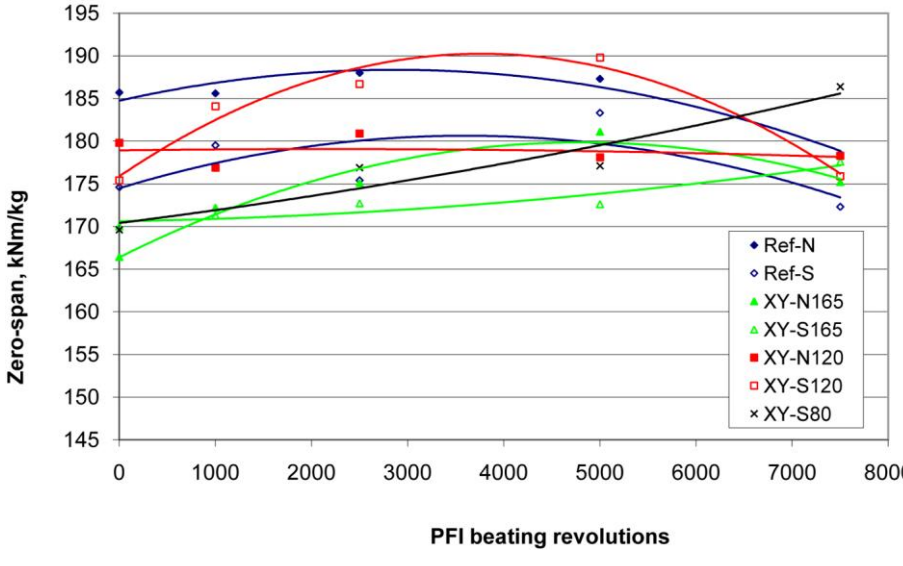

Figure 5. Zero-span vs PFI beating revolutions.

When introducing the shearing at 120 °C, the tear index at a certain tensile index drops significantly (XY-S165 and XY-S120). When the mechanical treatment is done at 80 °C the strength deterioration compared to the pulps produced without mechanical treatment is much lower (*Figure 6*). Of all the

pulps produced with mechanical treatment at the end of the cook, the pulp produced with mechanical treatment at 80 °C compared to 120 °C had the best tear-tensile relationship of all investigated pulp variants.

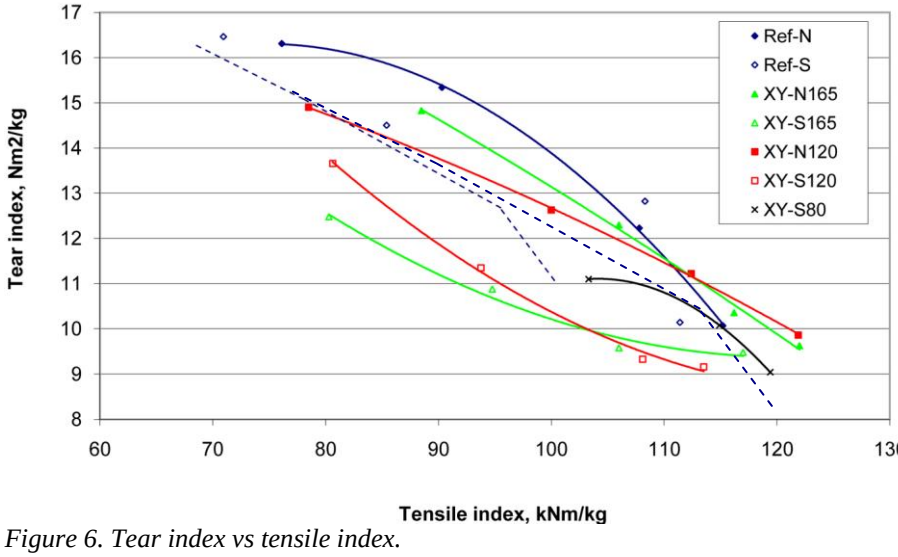

Figure 6. Tear index vs tensile index.

For all variants the tensile-development as a function of PFI-beating revolutions was better for the pulps produced without mechanical treatment. For the pulps produced with mechanical treatment, the pulp produced with mechanical treatment performed at 80 °C (XY-S80) showed the highest tensile index level (*Figure 7*). The tensile development for XY-S80 is even on the same level as the variants with xylan added and produced without mechanical treatment. All strength data are shown in *Appendix 1d.*

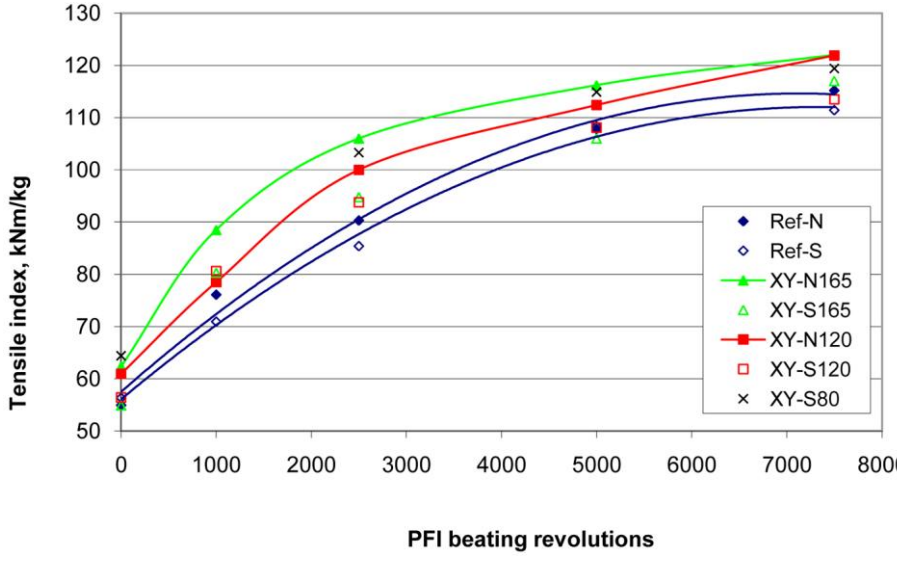

Figure 7. Tensile index vs PFI beating revolutions.

4 Conclusions

The extra birch xylan (i.e. 10 g/L) added during the cook resulted in straighter fibers and, as expected, improved tensile strength development for these pulps.

After xylan addition at 165 °C, a higher total charge and a somewhat higher surface charge was achieved for the pulp with the lowest temperature during the final part of the cook.

No direct indications were seen that the extra xylan added during the cook could protect the pulp from strength deterioration due to the introduced mechanical treatment during the cook. However, the temperature when the mechanical treatment took place was shown to be important. When performing the mechanical treatment at 80 °C instead of at 120 °C the strength properties were significantly improved.

6 References

Daniel, G., Olsson, A-M., Ander, P., Kaňuchová, A., Salmén, L., Filonova, L. Sjöström, K., Boussard, L., Berg, S., Heijnesson-Hultén, A. and Lindström, C. (2010): The influence ox xylan on the sensitivity towards fiber damage, CRUW, Intern rapport nr 2.

Salmén, L. and Lundqvist, F. (2011): Effects of mechanical forces for strength delivery in kraft cooking, Appita 64(1), 89.

7 Appendices

APPENDIX 1A.

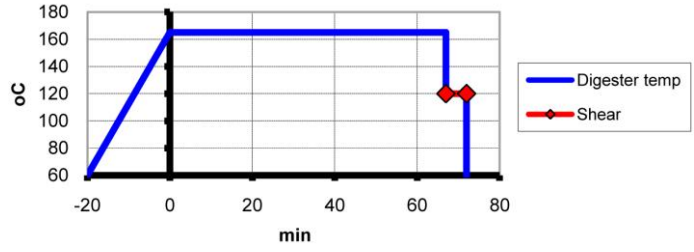

Figure A1a. Ref-N and Ref-S.

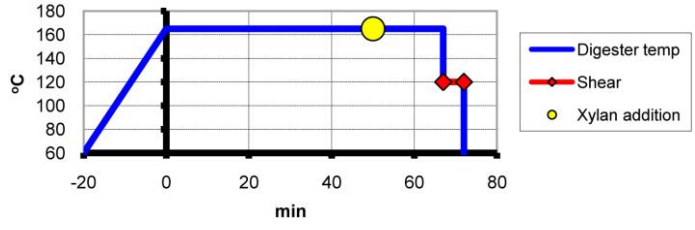

Figure A1b. XY-N165 and XY-S165.

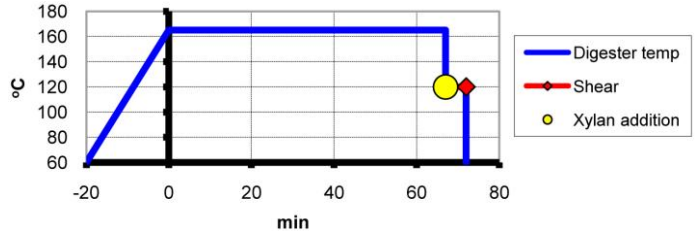

Figure A1c. XY-N120 and XY-S120.

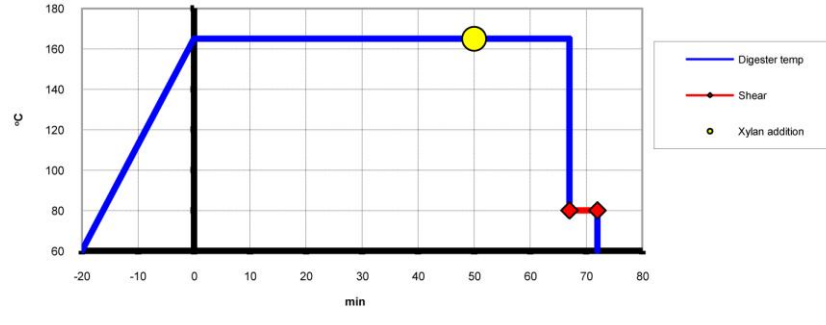

Figure A1d. XY-N80 and XY-S80.

APPENDIX 1B.

	Xylan addition at temp/time	Amount of xylan added g/L	Shearing temp	Compr %	EA %	H-factor	Cooking time[2] min	Res OH^- g/L	Kappa no	Dry content %	Tot shive[6] %	Tot yield %
Ref-N[3]	-	-	-	-	20	780	67	5.0	83.0	23.1	1.16	56.3
Ref-S[4]	-	-	120	45.0	20	782	67	5.9	76.6	23.1		
Ref-S	-	-	120	45.3	20	779	67	5.7	78.2	24.6		
XY-N165	165/50	10	-	-	20	779	67	5.2	77.1	23.2	0.7	57.3
XY-S165	165/50	10	120	44.8	20	778	67	5.1	76.0	23.3		
XY-S165	165/50	10	120	46.7	20	779	67	5.1	74.1	23.5		
XY-N120	120/0	10	-	-	20	781	67	5.7	80.2	21.2	1.1	56.8
XY-S120	120/0	10	120	45.5	20	771	66	5.6	78.2	22.5		
XY-S120	120/0	10	120	44.8	20	782	67	5.5	73.3	24.4		
XY-N/S80[5]	165/50	10	80	46.0	20	666	56	5.4	85.8/81.3	22.8/25.0		
XY-N/S80	165/50	10	80	45.5	20	758	65	4.8	81.7/82.4	23.9/24.7		
XY-N/S80	165/50	10	80	46.0	20	757	65	4.8	78.9/79.4	22.3/24.7		

[2]*at 165 °C*
[3]*N cooks without mechanical treatment.*
[4]*S cooks with compression and shearing forces induced for 2 minutes 15 minutes before the end of the cook.*
[5]*XY-N80 and XY-S80 was produced simultaneously, half of the pulp was mechanically treated half was not*
[6]*For the cooks producing mechanically treated pulp, the half of the pulp that was not mechanically treated was not used. That part had a different kappa no, therefore the yield calculation has been omitted for these pulp.*

APPENDIX 1C.

	Beating rev	Fiber length mm	Fiber width µm	Shape factor %	Kink angle °	Kink/mm	Kink/fiber	Mean segment length, mm
Ref-N	0	2,383	34,6	94,1	50,399	0,036	0,072	2,294
	1000	2,339	34,1	93,7	51,199	0,041	0,081	2,319
	2500	2,314	34,3	93,6	51,788	0,048	0,097	2,384
	5000	2,265	34,6	93,2	52,221	0,054	0,102	2,227
	7500	2,245	34,7	92,2	54,173	0,073	0,135	2,174
Ref-S	0	2,290	33,7	93,8	46,713	0,074	0,144	2,268
	1000	2,287	33,3	93,7	48,267	0,052	0,100	2,274
	2500	2,279	33,4	93,2	50,783	0,065	0,126	2,239
	5000	2,242	33,8	92,8	51,814	0,070	0,129	2,189
	7500	2,202	33,9	91,8	53,496	0,090	0,163	2,110
XY-N165	0	2,305	34,9	93,8	50,181	0,047	0,090	2,273
	1000	2,285	34,1	93,8	52,413	0,042	0,079	2,263
	2500	2,261	34,2	93,6	52,360	0,048	0,090	2,232
	5000	2,236	34,5	92,9	52,788	0,060	0,110	2,184
	7500	2,205	34,5	92,1	52,676	0,080	0,145	2,132
XY-S165	0	2,310	34,0	93,5	48,966	0,071	0,138	2,255
	1000	2,272	33,2	93,5	52,329	0,055	0,104	2,254
	2500	2,265	33,5	93,0	52,782	0,057	0,107	2,246
	5000	2,254	33,8	92,7	52,506	0,064	0,119	2,208
	7500	2,233	34,1	92,1	53,406	0,077	0,141	2,169
XY-N120	0	2,289	34,8	93,7	49,536	0,053	0,101	2,251
	1000	2,275	34,0	93,7	52,910	0,044	0,081	2,254
	2500	2,258	34,0	93,4	53,960	0,053	0,103	2,221
	5000	2,236	34,1	92,9	54,382	0,058	0,107	2,191
	7500	2,208	34,1	92,3	53,723	0,075	0,134	2,147
XY-S120	0	2,305	33,8	93,6	49,164	0,077	0,148	2,251
	1000	2,268	33,0	93,6	51,323	0,055	0,104	2,247
	2500	2,268	33,1	93,2	51,699	0,057	0,107	2,241
	5000	2,240	33,5	92,6	52,036	0,069	0,130	2,178
	7500	2,226	33,9	92,2	53,500	0,075	0,137	2,181
XY-S80	0	2,346	34,6	94,0	51,400	0,049	0,099	2,329
	2500	2,332	34,0	93,4	52,270	0,050	0,097	2,326
	5000	2,324	34,6	92,6	53,210	0,062	0,119	2,276
	7500	2,251	34,6	92,5	55,520	0,071	0,132	2,196

APPENDIX 1D.

	Beating rev	SR	WRV g/g	Density kg/m^3	Tensile I kNm/kg	Stretch %	Tens. energy abs. I, J/kg	Stiffness MNm/kg	Burst I kPam2/g	Tear I mNm2/g	Zero-Span kNm/kg
Ref-N	0	12,8	1,78	395	54,98	1,87	680	6,815	3,75	19,53	185,7
	1000	13,9	1,8	491	76,11	2,51	1	7,340	5,71	16,31	185,6
	2500	14,5	1,9	547	90,31	2,67	1572	8,416	6,68	15,34	188
	5000	16,0	2,01	646	107,80	3,19	2212	9,159	8,09	12,23	187,3
	7500	21,1	2,09	652	115,2	3,19	2370	9,6	8,21	10,07	178,5
Ref-S	0	12,5	1,64	437	56,37	1,91	713	6,859	3,53	17,66	174,6
	1000	13,5	1,7	488	70,94	2,20	1015	7,605	5,49	16,46	179,5
	2500	14,4	1,82	551	85,40	2,82	1563	7,717	6,66	14,50	175,4
	5000	15,6	1,97	642	108,30	3,00	2087	9,419	7,97	12,82	183,3
	7500	22	2,05	655	111,40	3,19	2297	9,423	8,22	10,14	172,3
XY-N165	0	15,2	1,79	454	62,35	2,01	825	7,277	4,1	15,90	166,4
	1000	15,4	1,82	548	88,50	2,32	1316	8,820	5,38	14,83	172,2
	2500	15,9	1,92	628	106,00	2,90	1974	9,360	7,25	12,30	175,1
	5000	18,2	2,06	676	116,20	3,06	2161	9,449	7,81	10,36	181,1
	7500	23,9	2,18	675	122,00	3,37	2538	9,708	8,59	9,63	175,2
XY-S165	0	15,1	1,69	410	54,90	1,91	691	6,562	4,15	14,71	170
	1000	15,5	1,77	517	80,29	2,52	1314	7,841	5,54	12,48	171,3
	2500	16,0	1,87	606	94,77	2,78	1697	8,604	7,02	10,88	172,7
	5000	19,6	2,06	638	106,00	3,14	2050	8,795	8,37	9,58	172,6
	7500	24,5	2,14	667	117,00	3,36	2654	9,902	8,75	9,48	177,6
XY-N120	0	14,3	1,86	438	60,95	2,10	838	6,745	4,21	15,50	179,8
	1000	15,2	1,87	531	78,50	2,56	1317	7,878	5,82	14,90	176,9
	2500	16,5	1,96	600	100,00	2,86	1858	9,097	7,03	12,63	180,9
	5000	19,2	2,07	667	112,40	2,88	2080	9,693	8,36	11,22	178,1
	7500	24,3	2,22	677	121,90	3,43	2673	9,726	8,83	9,86	178,3
XY-S120	0	14,3	1,71	426	56,43	2,04	765	6,774	3,95	14,31	175,4
	1000	14,8	1,77	505	80,62	2,35	1238	8,317	6,01	13,66	184,1
	2500	15,3	1,87	573	93,77	2,67	1623	8,865	7,17	11,35	186,7
	5000	18,4	2,05	651	108,10	3,20	2200	9,129	7,89	9,33	189,8
	7500	24,8	2,17	668	113,50	3,49	2530	9,232	8,72	9,16	175,9
XY-S80	0	15	1,73	474	64,42	2,13	907	7,296	4,27	13,71	169,6
	2500	16,4	1,91	621	103,30	2,81	1850	9,324	7,49	11,10	176,9
	5000	19,9	2,1	682	114,90	3,20	2358	9,578	8,23	10,08	177,1
	7500	27,6	2,27	708	119,40	3,10	2382	10,240	8,77	9,04	186,4

APPENDIX 2. QUANTIFICATION OF BIRCH XYLAN ADSORBED TO THE SURFACES OF SPRUCE PULP FIBRES COOKED TO HIGH KAPPA NUMBERS USING ELISA

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Aims: The aim of the study was to use the *Enzyme-Linked Immunosorbent Assay* (ELISA) to indirectly quantify the presence of birch xylan (BX) adsorbed to the surfaces of spruce kraft pulp fibres cooked to high kappa (*ca* 80) numbers.

Background: ELISA is an approach that can be adopted for the specific detection and quantification of biological components *in-situ* (e.g. Daniel & Nilsson, 1991). In previous studies (Daniel et al., 2010a, 2010b), we have adapted the method to use as a bioassay to detect and indirectly quantify the adsorption of birch xylan on spruce pulp fibres from various kraft cooks. Xylan quantification involves: *i)* Detection of the presence of xylan on the fibre surfaces using primary antibodies against BX, *ii)* Detection of the primary antibody using a specific enzyme-linked-FITC-conjugated secondary antibody; *and iii)* Determination of the amount of enzyme bound by addition of the enzyme substrate and measurement of colour development over a fixed period of time at 405 nm using spectrophotometry. The approach allows for the determination of unknown quantities of xylan binding to pulp fibres by comparison to a standard curve where different concentrations of pure birch xylan absorbed to the surfaces of microtitre plates have been determined in a pre-examination study. Advantage of the ELISA approach is that it allows determination of the presence of xylan on a relatively large population of fibres (i.e. *ca* 700-1000 fibres) thereby reducing the problems of substrate (fibre) variability from the true reactivity (e.g. differences in morphological structure, chemistry) when only a few fibres or fibre areas are analyzed as is often the case with microscopy approaches (e.g. fluorescence microscopy).

Material and Methods

Rat anti-xylan: A monoclonal antibody (LM10) as described previously (Daniel et al., 2010a, b) was used for the study.

Fibre materials: These are described in detail earlier in the experimental section (*Table 1.*) of this report.

Enzyme Linked Immunosorbent Assay: This involved the development of a standard using birch xylan and subsequent use of this standard for quantifying the presence of BX on the surfaces of the high Kappa pulps.

i) **Enzyme Linked Immunosorbent Assay-** ***Standard development***

The xylan standard was developed as described previously (Daniel et al., 2010a, b) and used in the present study.

ii) **Enzyme Linked Immunosorbent Assay-** ***High kappa pulps***

Presence of BX on the experimental pulps was determined as described previously (Daniel et al., 2010a). Briefly, 1.7 mg of never-dried pulp fibres taken from a filtered pulp fibre suspension suspended overnight in buffer were suspended in eppendorf tubes containing blocking agent in PBS and left for 1 hr at room temperature. Thereafter, the fibres were treated with rat-anti-xylan in PBS overnight at 4° C. Samples were washed and treated for 2 hr at RT in a shaker (or overnight in a cold room) with goat anti-rat secondary antibody IgG conjugated to alkaline phosphatase. After secondary labelling, the substrate p-nitrophenyl phosphate was added and samples incubated for 30 mins. Colour development was terminated with NaOH, the fibres centrifuged down and 100 µl transferred to ELISA plates for absorbance determination at 405 nm. Xylan concentration was determined from the 30 min. standard equation. The assay was repeated 16 times (i.e. repeated with 16 different fibre samples from the pulp) and results shown reflect mean absorbance values. Method controls included omission of the primary and secondary antibodies in the labeling procedure; all of which were negative.

Results and Discussion

Figure 1 shows the indirect quantification of xylan (principally BX and some native xylan) present and available for anti-xylan labeling on fibre surfaces of the different high Kappa pulps. The reaction is based on a relatively large number and mixed fibre population (i.e. early- and latewood) of fibres increasing the bioassays randomness and representativeness of the entire fibre population.

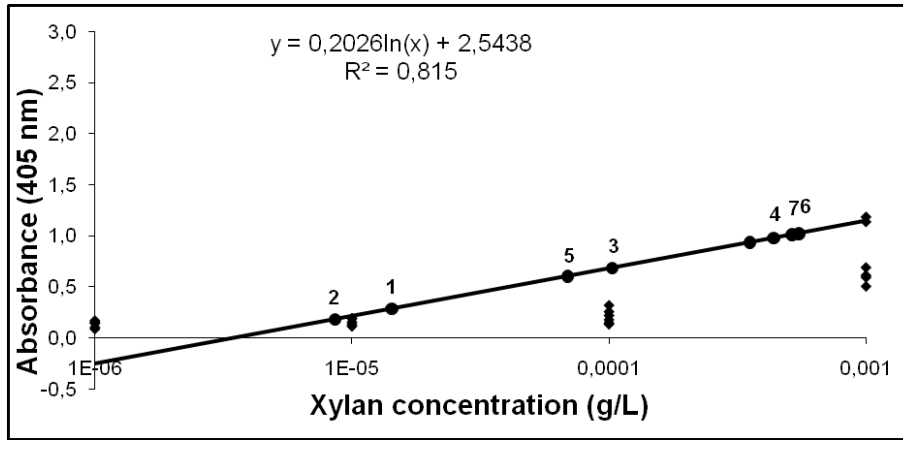

Figure 1. Estimation of xylan (g/L) binding to the high Kappa pulps as shown from the standard curve: 1, Ref-normal; 2, Ref-165C-sheared; 3, Xyl-Ref 165C-normal; 4, Xyl-165C sheared; 5, Xyl-Ref-120C-normal; 6, Xyl-120C-sheared; 7, Xyl-80C-sheared. Results reflect mean from 16 individual measurements of pulp samples.

Table 1. Results from ELISA together with bulk xylose, surface and acid groups (see Table 4. results and discussion)

Pulp Type	Number shown on Figure 1	Xylan g/L using ELISA	Bulk pulp xylose	Fibre surface charge	Total pulp acid groups
Ref-N	2	0,283	9,3	10	113
Ref-S	1	0,181	9,1	10	110
XY-N120	5	0,603	10,5	10	102
XY-N165	3	0,684	10,8	10	129
XY-S165	4	0,976	11,6	13	129
XY-S80	7	1,010	11,5	15	141
XY-S120	6	1,022	10,8	12	130

Results showed:

1) As expected the largest amounts of xylan present on the xylan treated pulps (i.e. pulps 3-7, *Figure 1*) in comparison to the reference untreated pulps. The antibody is thought to have some reaction with native softwood xylan thus the weak reaction with the reference pulps N and S. No studies with purified xylan from spruce have so far been carried out to characterize this aspect;
2) With the exception of the Ref-S pulps, all the sheared pulps with added xylan showed higher levels of xylan in comparison to their Normal unsheared counterparts. This result is consistent with that recorded previously for spruce fibres (Daniel et al., 2010a, b) and presumably reflects the opening-up of the fibre wall and greater surface area for xylan adsorption, the anti-xylan reaction and subsequent colour development. The reason for the slightly stronger reaction of the Ref-S compared with Ref-N is unknown;
3) As a group the sheared pulp showing the weakest reaction for xylan (XY-S165; 0,976 g/L) was greater than the strongest reacting normal unsheared xylan treated pulp (XY-N165; 0,684 g/L) (*Table 1*);
4) The unsheared normal xylan treated pulps showed the following order for presence of xylan: XY-N120 < XY-N165 and the sheared pulps: XY-S120 < XY-S80 < XY-S165. The values for pulps XY-S165 < XY-S80 < XY-S120 were in the range 0,976-1,022 g/L (*Figure 1; Table 2*) so the difference was not great; i.e. a range of 0,079 g/L;
5) On a general comparative basis, the Kappa pulps showing highest ELISA xylan levels showed also the highest pulp bulk xylose, surface charge and total acid groups apart from XY-S120 (*Table 1*);
6) On a ratio basis the greatest difference for the presence of xylan was seen between XY N120 and XYS120 (41%) followed by XY N165 and XYS165 (*ca* 30%);
7) In comparison to an earlier study with oxygen bleached pulps (*CRUW report 4*), the present high kappa pulps showed a much lower absorbance of birch xylan to the normal and sheared pulps suggesting a less open fibre surface structure (i.e. lied between 0,01-0,10 g/L).

References

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APPENDIX 3. VISUALIZATION OF THE SPATIAL MICRODISTRIBUTION OF BIRCH XYLAN ADDED TO SPRUCE PULPS COOKED TO HIGH KAPPA NUMBERS USING IMMUNOFLUORESCENCE MICRO-SCOPY OF WHOLE FIBRES AND FIBRE CROSS-SECTIONS

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Aims: The overall purpose of the study was to determine the influence of temperature on birch xylan addition and mechanical treatment using kraft pulps cooked to high kappa numbers (*ca* 80). The specific aims of the immunofluorescence work was: *i)* Visualize the presence of added birch xylan (BX) on the surfaces of whole fibres; *ii)* Determine whether there was any apparent differences in the spatial distribution of BX pulps caused through shearing; and *iii)* Visualize the spatial microdistribution of the xylan in cross-sections of the treated pulps to determine whether the xylan was penetrating into the fibre cell walls.

Background: In previous work (Daniel et al., 2010a, b), the influence of added BX on the degree of damage introduced into spruce fibres through the mechanical action of the cook was studied. Apart from less curled fibres and a positive increase in yield through adsorbed surface xylan, no particular reduction on the amount of damaged areas were observed. The overall aim of the present study was to determine the influence of temperature on birch xylan addition and mechanical treatment using spruce kraft pulps cooked to high kappa numbers (*ca* 80). An important part of understanding the mechanisms of fibre "healing" through carbohydrate (e.g. via xylan), addition or indeed the retention of xylan in the fibres during cooking, is to determine the distribution of xylan at the fibre level. Here, the spatial microdistribution of added xylan was visualized in order to help understand how the xylan may be used to improve/retain the strength of kraft pulps. Immunofluorescence microscopy in conjunction with specific antibodies against BX was used as a high precision technique to visualize the xylan associated with spruce fibre cell walls (both surface and penetrating) when added to high kappa pulps at different temperatures with and without shearing.

Materials and Methods

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

Table 1. Nomenclature for the eight pulp types used in the study

Pulp type	Xylan addition at temp/time	Amount of xylan added g/L	Shearing temperature	Kappa no.
Ref-N	-	-	-	83
Ref-S		-	120	77,4
XY-N165	165/50	10	-	77,1
XY-S165	165/50	10	120	75,1
XY-N120	120/0	10	-	80,2
XY-S120	120/0	10	120	75,8
XY-S80	165/50	10	80	80,9

2. Monoclonal antibody

Visualization of xylan was achieved using a monoclonal antibody generated against a low–substituted (1-4)-β-D-xylan. The antibody was a generous gift from Prof. P. Knox (Leeds Univ., UK).

3. Immunolocalization of xylans on whole fibres and fibre cross-sections

i) Whole fibres

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

ii) Fibre cross-sections

Fibres samples from the 8 pulps were processed dehydrated and embedded in resin as previously described (Daniel et al., 2010a, b). Semi-thin fibre cross-sections were cut and mounted on object glasses and labeled with the monoclonal antibody for immunofluorescence microscopy as described previously (Daniel et al., 2010a).

iv) Positive and negative and substrate controls

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

4. Fluorescence microscopy

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 1 s and gain 3.2).

Results and Discussion

1. Whole fibres:
As a general observation, whole fibres often showed rather weak immunofluorescence (i.e. green colour) and evidence for the presence of added birch xylan absorbed to the fibre surfaces (*Figures 1-3*). Thus it was difficult to distinguish qualitatively any major differences between the pulp types and with and without shearing. In comparison to the reference controls however, the treated fibres showed a stronger indication for the presence of xylan (*Figures 1-3 vs Figure 4*). As observed with previous studies, the fibre population shows fibres with strong labeling, others weakly and some hardly at all indicating a non-homogeneous distribution of BX in both the fibre population and individual fibres (*Figures 1-3).* With the ELISA study(*Appendix 2*) the xylan treated fibres formed two fairly close groups: 1) XY-S120; XY-S80; XY-S165 *and* 2) XY-N120, XY-N165. This is some ways reflected by the immunofluorescence labelling patterns in which it is very difficults to distinguish between the pulp types.

As a general trend, birch xylan was frequently found on the fibre surfaces associated with materials surrounding bordered pits (*Figures 1a, e; 2a, b, d)* and lying in the former regions of the middle lamella that previously joined the fibres together. It would appear the xylan was more easily bound/retained on these fibre regions which may suggest the presence of chemical groups/surface materials (i.e. cellulose, hemicelluloses) that react strongly or are able to bind the xylan.

2. Fibre cross-sections:
The advantage of using fibre cross-sections is that they can provide information on whether the birch xylan was able to penetrate into the cell walls of the fibres from the external surfaces, also allowing both early- and latewood fibres to be distinguished. Sections thereby provide complementing information on the fibre surface labeling achieved with the immunofluorescence of whole fibres and provide additional information on the presence of xylan in the fibre lumen and its ability to penetrate into the fibre from this direction.

Fibre cross-sections of the different pulp types generally showed strong labeling patterns for the presence of birch xylan (*Figures 5-7*). As observed with the whole fibres, the different pulp types showed heterogeneous

labeling with some fibres strongly labeled, others poorly labeled or even apparently non-reacting even though they were located in close proximity to each other (*Figures 5a, d; 6a; 7a*).

An interesting feature not noted previously was the strong immunofluorescence for xylan associated with the lumen walls of the fibres (*Figures 5-7*). This was apparent for both early- and latewood fibres and often discrete layers of strongly staining xylan were apparent (*Figures 5b, e; 6b, d; 7 b, c).* Examples were observed in all pulp types with and without shearing (*Figures 5-7*). Frequently, immunofluorescence was deeper inside the fibre wall indicating some penetration and binding of the xylan inside the secondary cell wall via the fibre lumen wall. The fact that the strong presence for xylan along the fibre lumen wall was noted in heterogeneous groups of fibres with other fibres unreactive testifies against unspecific labeling. Two aspects to consider here are: *i)* that the xylan was able in the processes to enter into the fibre lumen (i.e. presumably via pits, broken fibres) and react with fibre wall from the inside; and *ii)* that the xylan was not washed away later. Cross-sections of reference fibres untreated with birch xylan (Ref.-N, Ref.-S) showed no immunofluorescence under the conditions being used (*Figure 8a, b*).

Overall conclusion

It is difficult to speculate on any effect of temperature on shearing with the immunofluorescence results obtained. The results once again emphasize the heterogeneity of the fibre population and the great need to take this into account when using high resolution techniques of analysis. Nevertheless, the results prove once again a strong reaction of added birch xylan with the spruce pulp fibres but that the adsorption and penetrability are probably driven by different mechanisms. The binding of birch xylan to the fibre lumen wall and possible penetration from this direction is a novel observation.

References

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

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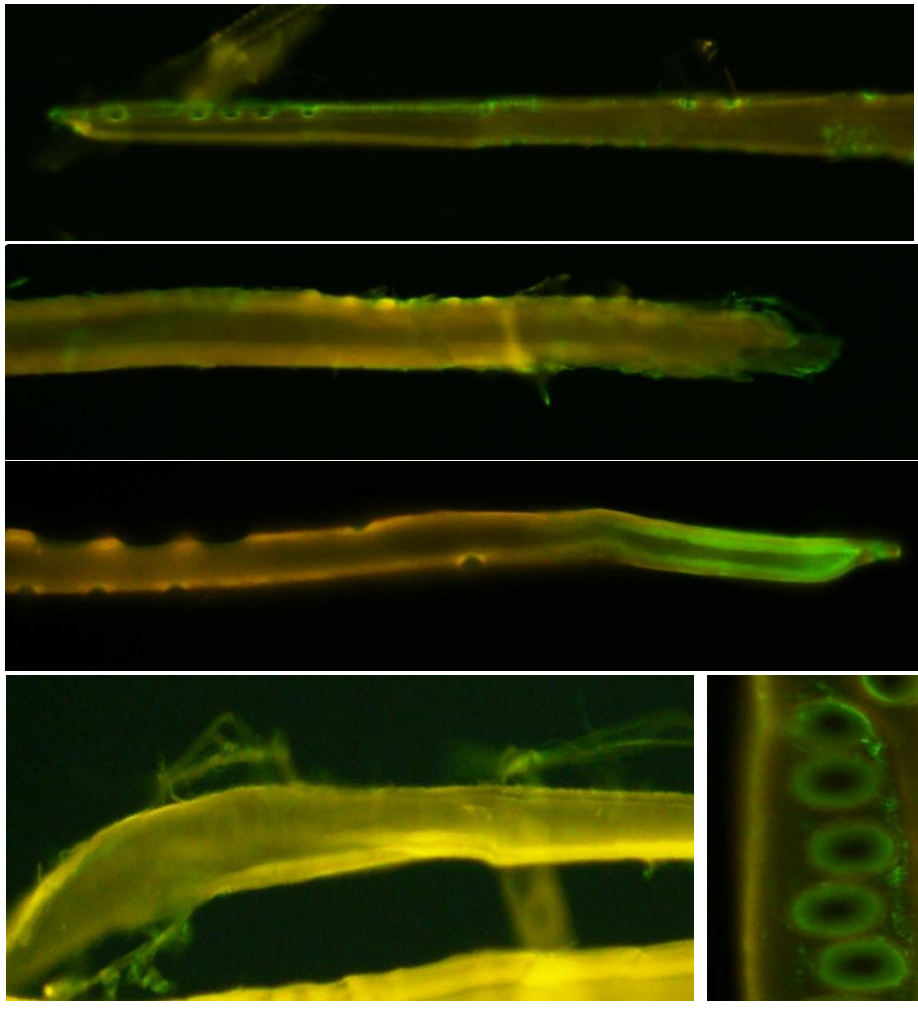

Figure 1. a,b, XY-N165 (top two images); c, d, e, XY-S165 (bottom three images).

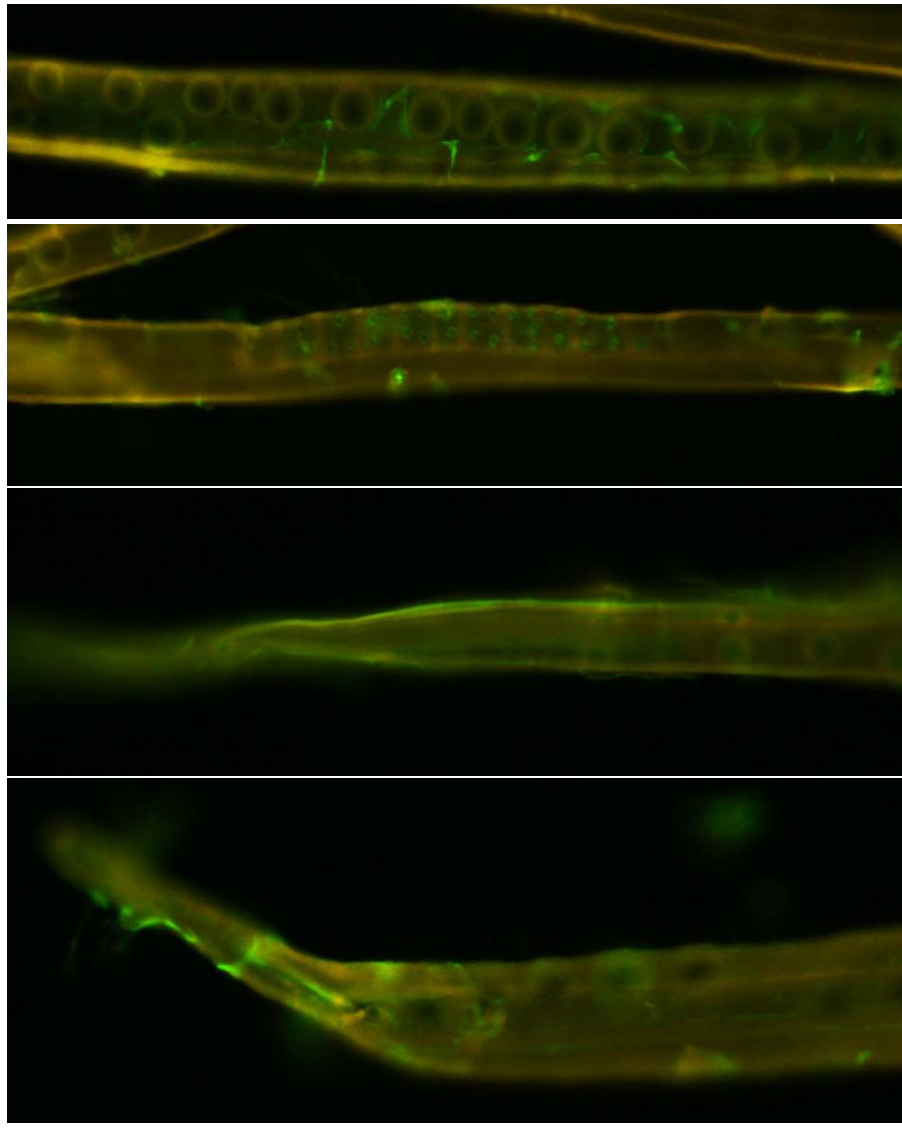

Figure 2. a, b, XY-N120 (top two images); c, d, XY-S120 (bottom two images).

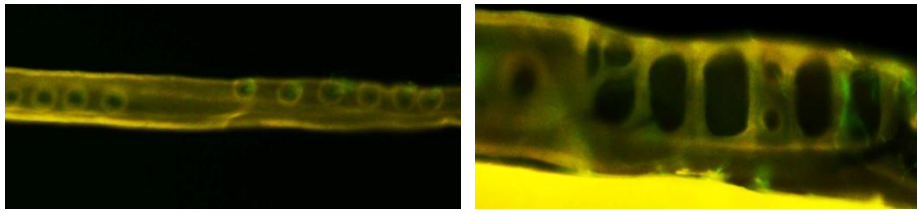

Figure 3. a, b XY-S80 (bottom two images).

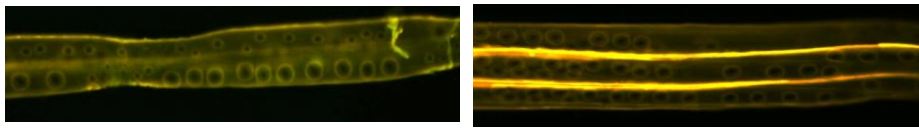

Figure 4. Reference-N (left) and Reference-S (right).

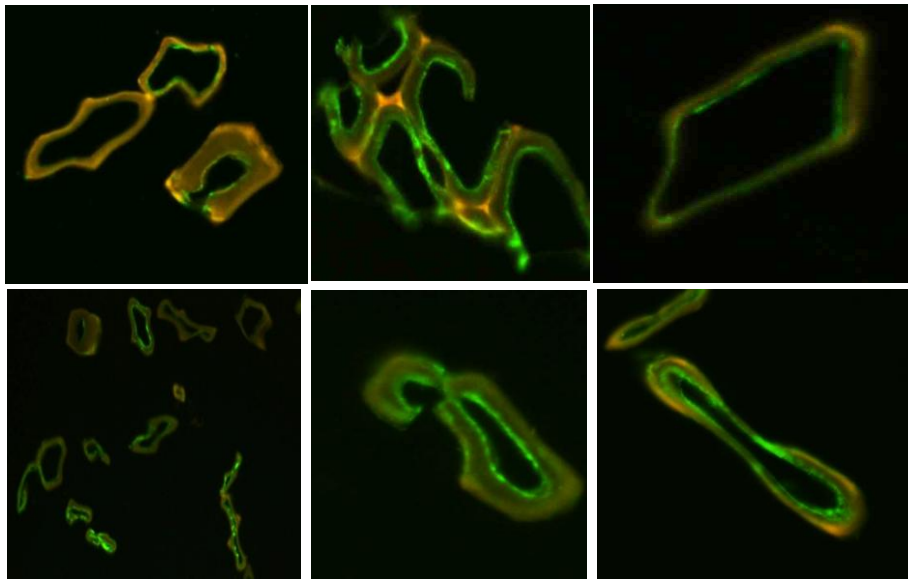

Figure 5. a, b, c XY-N165 (top three images); d, e, f, XY-S165 (bottom three images).

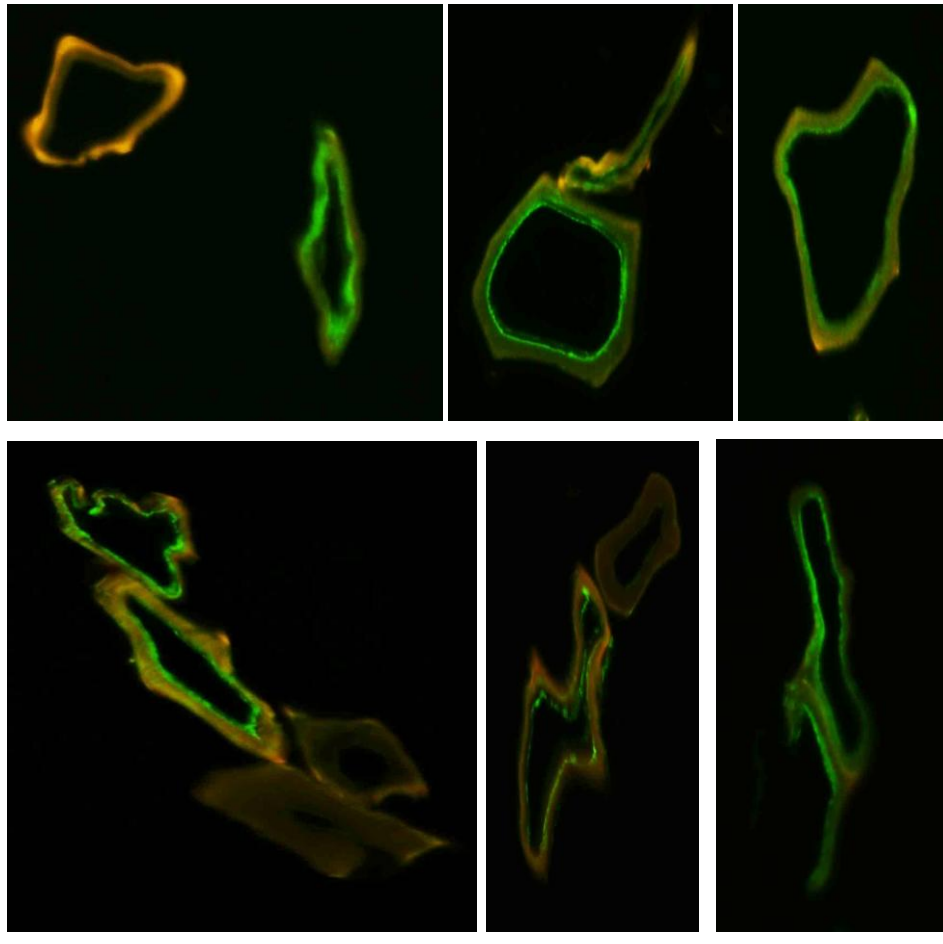

Figure 6. a, b, c, XY-N120 (top three images); d, e, f, XY-S120 (bottom three images).

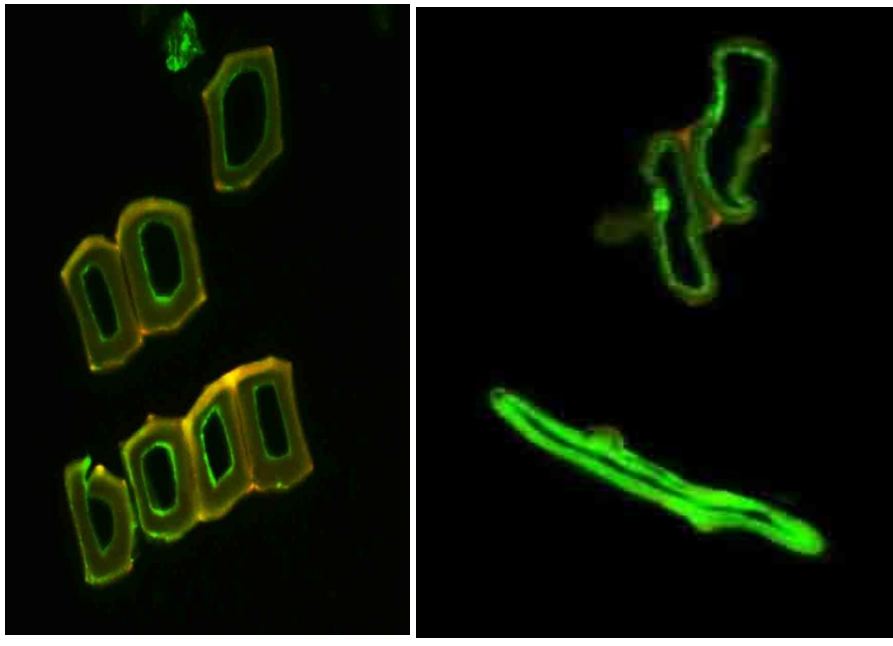

Figure 7. a, b, XY-S80 showing labelling of latewood and earlywood fibres.

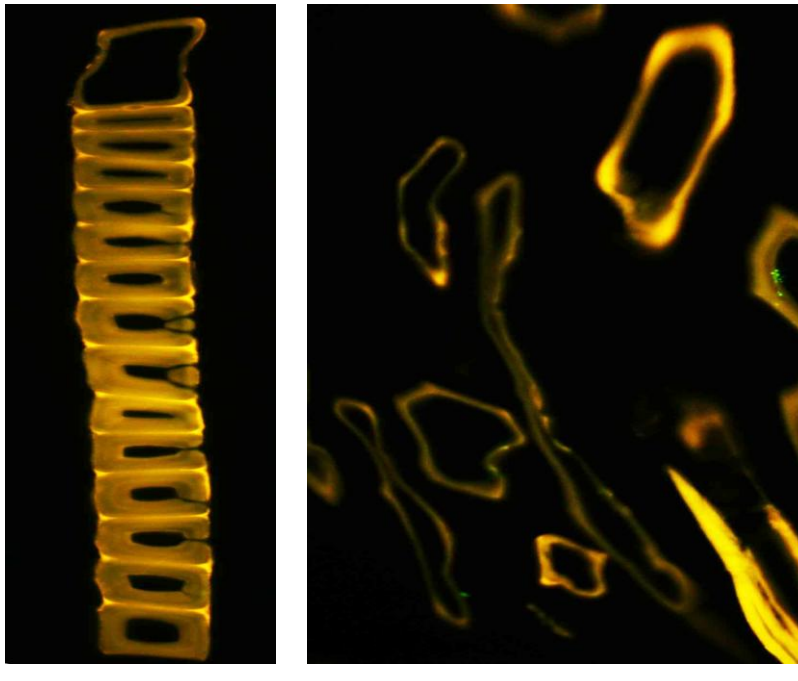

Figure 8. Reference-N (left); Reference-S (right).

APPENDIX 4. SEM OBSERVATIONS ON HIGH KAPPA 80 SPRUCE PULPS TREATED WITH BIRCH XYLAN

Geoffrey Daniel, SLU, Uppsala

Aims: The purpose of the study was to answer the following questions: *i)* Can exogenously birch xylan (BX) be observed on the surfaces of spruce pulp fibres cooked to high Kappa number (i.e. kappa 80) under different conditions?; *ii)* If present, does the xylan show any morphological form and concentration towards different fibre morphological features?; and *iii)* Can the affects of shearing be visualized and their affects interpreted with respect to BX addition?

Background: Xylans have been previously reported to precipitate onto the surfaces of pulp fibres directly from the kraft cooking liquor during standard cooking (Yllner & Enstrom, 1956) or when applied exogenously to the cook. This can be assessed conveniently by the use of scanning electron microscopy (SEM) which provides a 3-dimensional overview of the morphological structure of fibres. This was readily shown with a detailed SEM study on the changes in surface ultrastructure of Norway spruce fibres during kraft pulping (Duchesne & Daniel, 2000). However, the penetration of xylan into the fibre wall however cannot be observed using SEM when used in a conventional mode. In the present project on high kappa 80 pulps, the immuno-detection systems ELISA and immunofluorescence gave evidence for the presence of BX on the surfaces of both xylan treated normal (i.e. N-X) and sheared (i.e. S-X) treated fibres. SEM was used in the present study to visualize the birch xylan on the surface of treated fibres to ascertain its morphological state and microdistribution after addition at different times in the cooking process that was followed with- and without shearing.

Materials and Methods

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

Table 1. Fibre materials used from the different pulp treatments

	Xylan addition at temp/time	Amount of xylan added g/L	Shearing temp	Compr %	EA %	H-factor	Cooking time min	Res OH^- g/L	Kappa no
Ref-N	-	-	-	-	20	780	67	5,0	83,0
Ref-S	-	-	120	45,2	20	780	67	5,8	77,4
XY-N165	165/50	10	-	-	20	779	67	5,2	77,1
XY-S165	165/50	10	120	45,8	20	779	67	5,1	75,1
XY-N120	120/0	10	-	-	20	781	67	5,7	80,2
XY-S120	120/0	10	120	45,2	20	776	66	5,6	75,8
XY-S80	165/50	10	80	45,8	20	758	65	4,8	80,9

Scanning Electron Microscopy approach for fibre analysis: For Scanning Electron Microscopy (SEM), the kraft pulp samples (*Table 1*) were processed according to Daniel and Duchesne (1998) and Daniel et al. (2004a, 2004b) using ethanol dehydration and critical point drying. Fibres were placed on stubs sputtered with gold and subsequently examined using a Philips Environmental-SEM or Hitachi 4500 operated at variable kV. Images were digitalized using the embedded software.

Results and Discussion

General overview of pulp fibres:

In the present work both ESEM and FE-SEM approaches were applied thus giving an improved appraisal of the presence of the inherent cellulose micro- and macrofibrillar structure as revealed during the cooking process and the changes on pulp fibre ultrastructure brought about by the addition of xylan as well as the likely affects of shearing. Representative images of fibre areas from the different pulps are shown in *Figures 1-8*. In total over 300 images were taken i.e. roughly 40 images per sample, in order to make a representative appraisal.

Table 2. From the chemical studies showing analysis of xylan (as xylose), xylose anhydro and total anhydro sugars, total acid groups and surface charge on the different pulp types

		Ref-N	Ref-S	XY-N165	XY-S165	XY-N120	XY-S120	XY-S80
Xylose	Rel. %	9,3	9,1	10,8	11,6	10,5	10,8	11,5
Xylose anhydro	%	7,6	7,5	8,6	9,1	8,4	8,9	9,6
Total anhydo sugar	%	83,5	83,7	80,8	79,6	82,2	83,9	84,8
Hydrorest	%	13,1	12,9	12,8	14,4	13,4	12,9	11,5
Tot acid groups	mmole/kg	113	110	129	129	102	130	141
Surf. Charge (neg)	meqv/kg	10	10	10	13	10	12	15

Surface ultrastructural features of the different pulp types:

1) The fibre surface (both early- and latewood fibres) was comprised of the exposed outer secondary cell wall S1 layer and in some places the primary wall in all pulp types. The primary wall material left on the fibres was marginal compared to that of the S1 layer. This means that any increase in surface charge and acid groups likely reflects the presence of added and adsorbed birch xylan rather than that remaining from the primary wall materials (i.e. rich in pectin materials) (e.g. Reference pulps vs pulps treated with xylan, *Table 2*);
2) The fibre surface ultrastructure is composed of macrofibrillar structures (i.e. microfibrillar aggregates) of different sizes (e.g. widths 10-60 nm, i.e. representing from a few to many individual microfibrils aggregated together) reflecting the aggregation of the wall cellulose microfibrils (of order 3-4 nm) during the cooking process. In the reference pulps here (i.e. Ref N, Ref-S, *Table 1*), these macrofibrillar/microfibrillar structures are expected to have remaining xylan (i.e. 9,3, 9,1 %, *Table 2*) and other hemicelluloses (i.e. mannose, galactose, arabinose, chemical data, *Table 4* introduction) closely associated;
3) Considerable variability in fibre structure was recognized on a single fibre, between fibres from the same and different pulps and also between fibre types (i.e. earlywood vs latewood);
4) That in pulps with added birch xylan, xylan seemed to adsorb strongly to the fibre wall cellulose as suggested from the cellulose fibrils of the bordered pit membranes (both torus and margo) as shown in *Figure 5e, f* for the XY-N120 pulp;
5) Pulps treated with birch xylan during cooking have a surface ultrastructure that is slightly different than non-treated fibres. The fibre surface often appears composed of encapsulated cellulose micro/macrofibrils with adsorbed globular aggregates (*Figures 3f, 4f, 5c-f, 6e, f, 7e, f*). Some aggregates were also present on the reference-N and

S fibres but were not in the order of the pulp fibres treated with exogenously applied xylan. Presumably these aggregates consisted of solubilised lignins and hemicelluloses precipitated out in the residual part of the cook. Coverage however varies along individual fibres between fibres of the same pulp and between pulps. Differences between early- and latewood fibres are also apparent. In some places it seemed almost that the xylan formed a film on the fibre surface;

6) The shearing of pulps appears to open the surface structure (S1/primary wall) cell wall of the fibres revealing the cellulose micro/macrofibrils. Sheared pulps on the whole showed therefore a more open surface structure and presumably greater surface area for antibody labelling. This result is consistent with the ELISA findings that sheared pulps tend to show quantitatively slightly higher levels of xylan than non-sheared equivalents;
7) Examination of dislocations on fibres treated with xylan showed the presence of xylan aggregates associated with the depressions in wall structure (*e.g. Figure 8*). Observations of dislocations in sheared fibres showed their structure as variably open revealing the cellulose micro/macrofibrils; i.e. the shearing action had removed the surface xylan aggregates and presumably any xylan not bound strongly to the cellulose;
8) Using the SEM approach it is difficult to distinguish and quantify major differences between the pulps. The following general trends are suggested by the xylan treatments:

    a) Xylan is bound to the cellulose microfibrils at a *molecular level* and presumably adds to the thickness of individual microfibrils in the wall matrix (at least outer regions where the structure is open). An increase in width of the fibrils by the adsorption of xylan could prevent the strong cellulose-cellulose fibril binding. *This could be determined if the cellulose micro/macrofbrils widths were measured*.

    b) Xylan in the form of globules/aggregates of the order ca 20 nm were observed bound to the fibre micro/macrofibrils surface structure. Whether this represents crystalline or amorphous xylan or combination of both should be determined; *and*

    c) With further xylan aggregation/precipitation, the pores in the fibre structure (i.e. between micro/macrofibrils) become blocked and thereby the porosity is reduced.
9) In a previous study (Daniel et al., 2010) it was established that xylan can form amorphous and crystalline structures in-situ. In the present experiment is it likely that both xylan forms also exist and that the aggregates reflect the crystalline form while that filling the pores and binding to the cellulose fibrils maybe of both types. It is well known that xylan-xylan is preferred to xylan-cellulose binding. The presence of

bound xylan on the fibre surface is consistent with positive results on the straightening of fibres and extra surface charge.

Final overall conclusions:

1) Xylan treated fibres have xylan associated with the surface structure- *both normal and sheared fibres*;
2) The xylan adsorbed to the fibres is likely to be of composed of different binding forms; e.g. cellulose-xylan, xylan-xylan (e.g. aggregates) of which presumably the latter in the form of aggregates present in the pore structure is most easily removed and may not be adding to strength properties;
3) Dislocations (i.e. areas of fibre weakness) to have added xylan associated;
4) The presence of xylan as confirmed here associated with the surface fibre structure is consistent with the positive properties obtained with the pulp and paper testing;
5) The widths of the cellulose micro/macrofibrils should be quantified for the different pulps types;
6) Emphasis in further studies should be concentrated on penetrating the fibre wall with added xylan.

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Yllner, S. and B. Enstrom, 1956. Studies on the adsorption of xylan on cellulose fibres during the sulphate cook. Part 1. Svensk Papperstidn. 59, (6) 229.

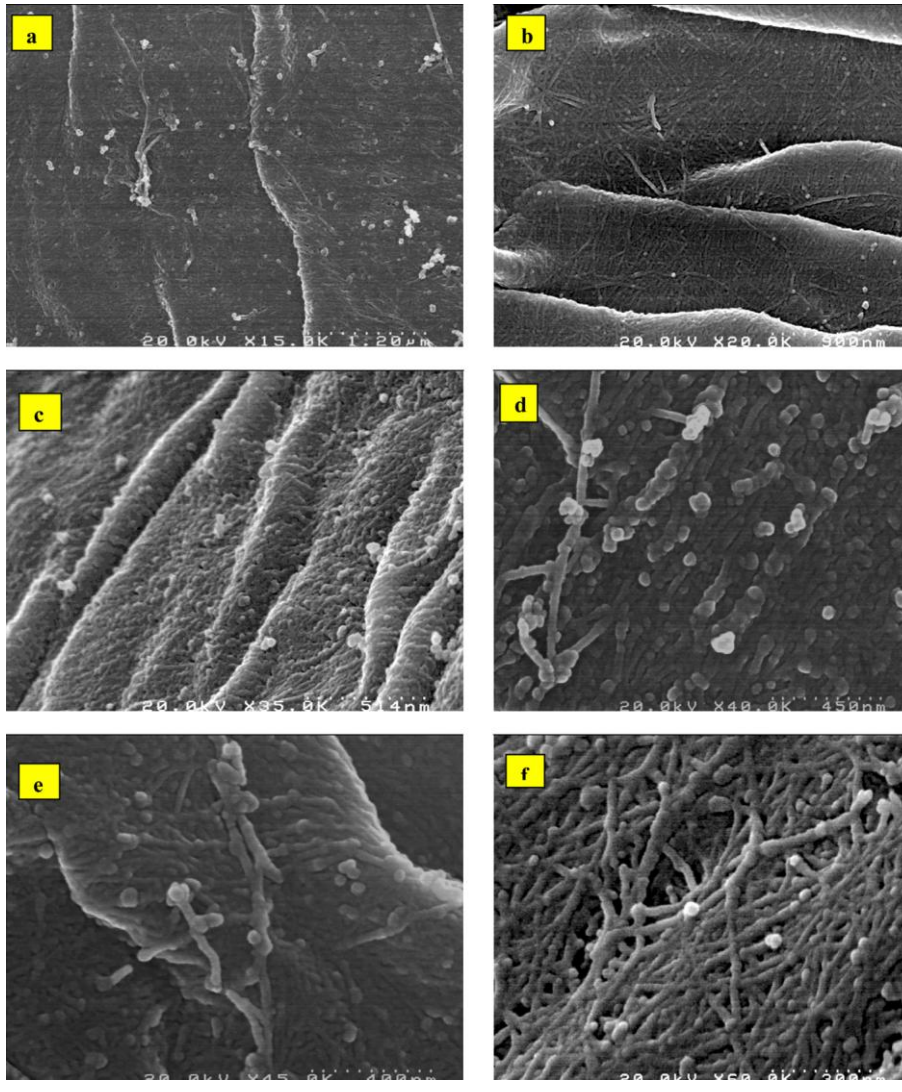

Figure 1. Reference-N pulps. Pulps were cooked at 165 °C without added xylan and were not sheared. Fibre surface has a heterogeneous nature and at higher magnification the cellulose microfibrils/macrofibrils are retained in a matrix material (a-e) or more exposed (f). Aggregates frequently found on the fibre walls (a, d, f) are likely composed of dissolved lignin and hemicelluloses that have reprecipitated on the fibre during the residual stage of the cook.

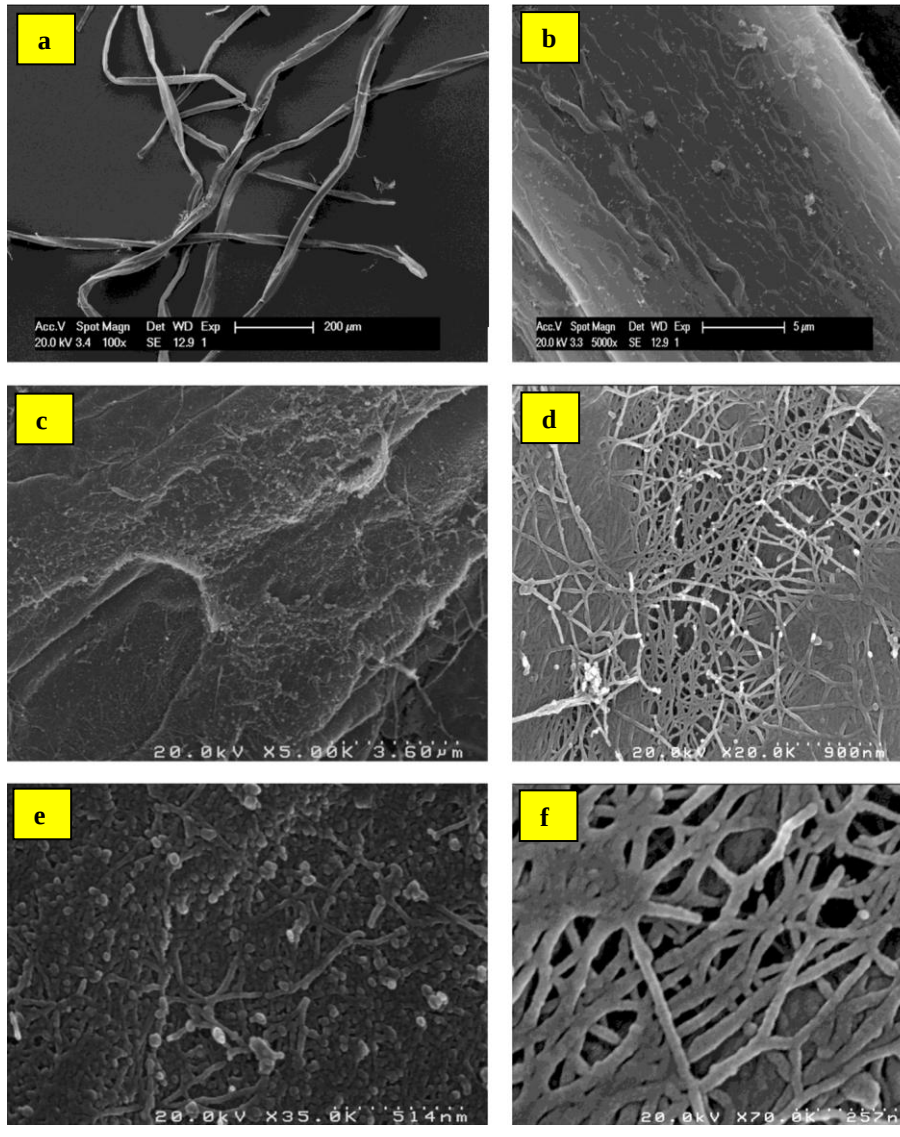

Figure 2. Reference-S pulps. Pulps were cooked at 165 °C without added xylan and sheared at 120 °C. Micrographs show the fibre surface structure at increasing magnifications. Fibre surface composed variably of areas in which the cellulose microfibrils/macrofibrils were still retained in a wall matrix (c, e) and released from the wall (d, f).

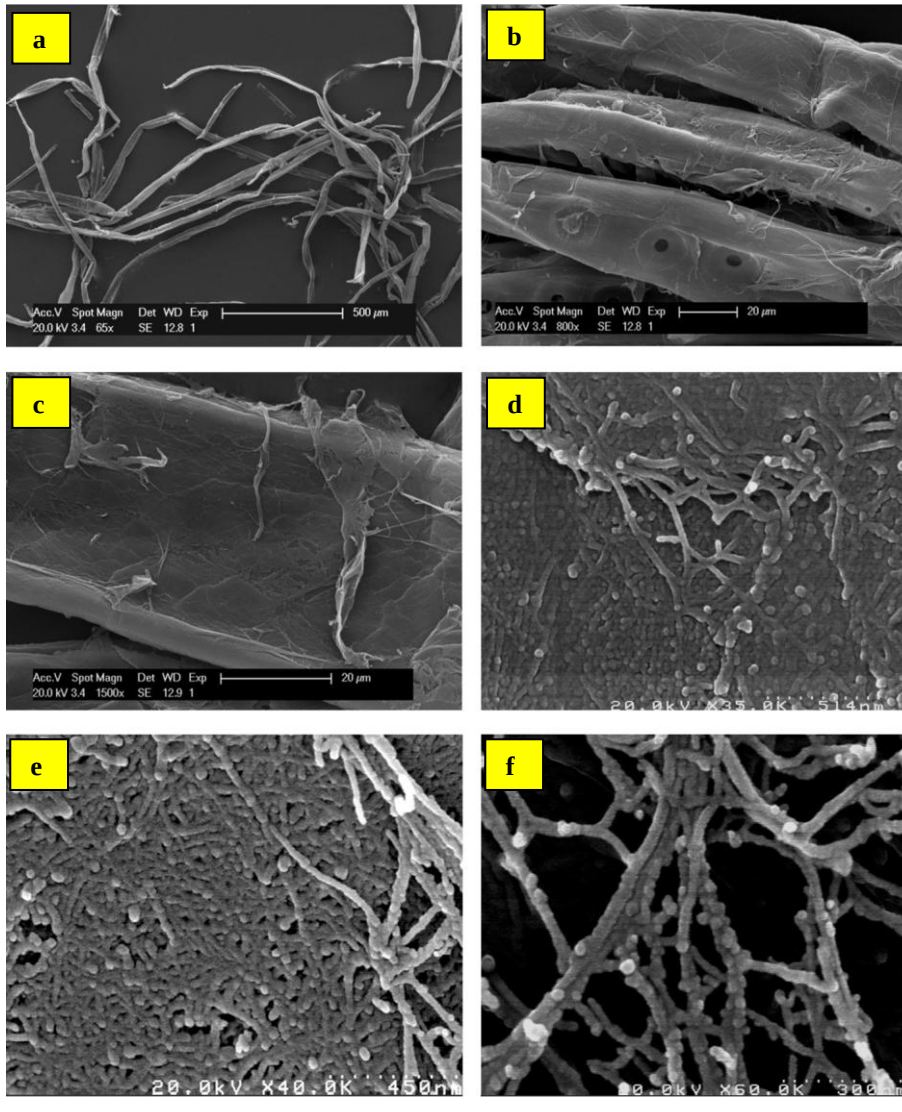

Figure 3. Pulp XY-N165. The pulp was cooked at 165 °C without shearing. Birch xylan was added during cooking at 165 °C. Micrographs at increasing magnification of the fibre wall structure. Cellulose micro-fibrillar/macrofibrillar structure appears enclosed in matrix material (presumably added xylan). At higher magnifications macrofibrils released from the fibre surface are covered with xylan aggregates (e, f)

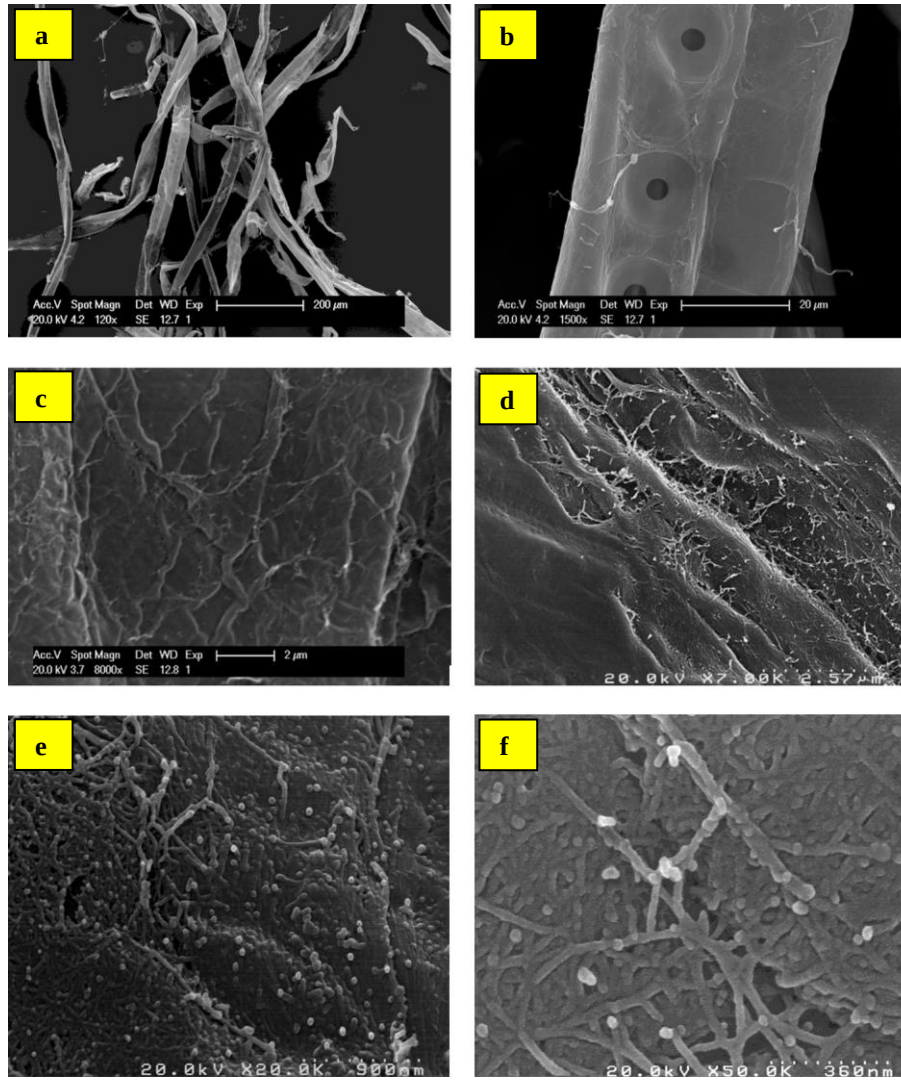

Figure 4. Pulp-XY-S165. The pulp was cooked at 165 °C and birch xylan added at 120C. Shearing was carried out at 120 °C. Shearing caused opening of the fibre wall in places (d). Fibre surface often covered in xylan aggregate structures (e, f).

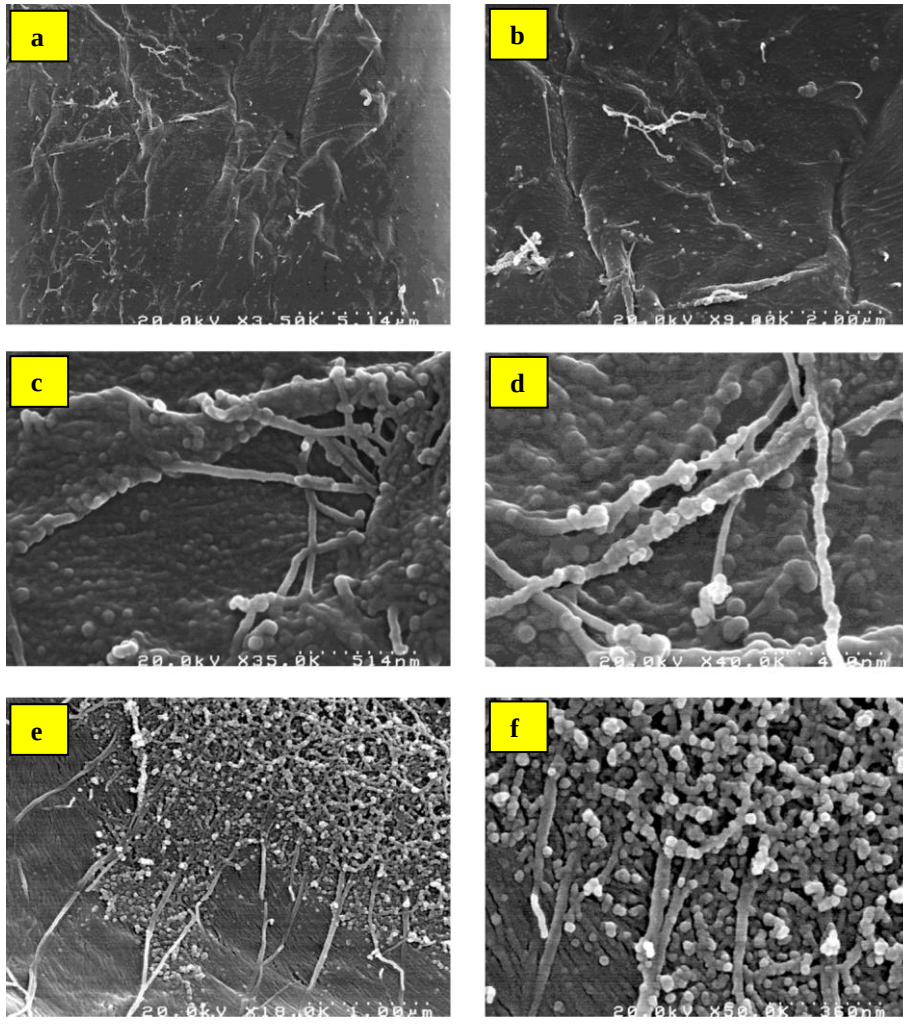

Figure 5. Pulp XY-N120. The pulp was cooked at 165 °C with birch xylan added later at 120 °C (i.e. in the residual cooking phase). Fibre surface appeared compact with xylan aggregates covering the cellulose microfibrils/macrofibrils (c, d). Bordered pit membranes (remaining cellulose, xylans and pectins (?) were coated with xylan aggregates (e, f) emphasizing the ready adsorption of xylan to free cellulose surfaces (e, f).

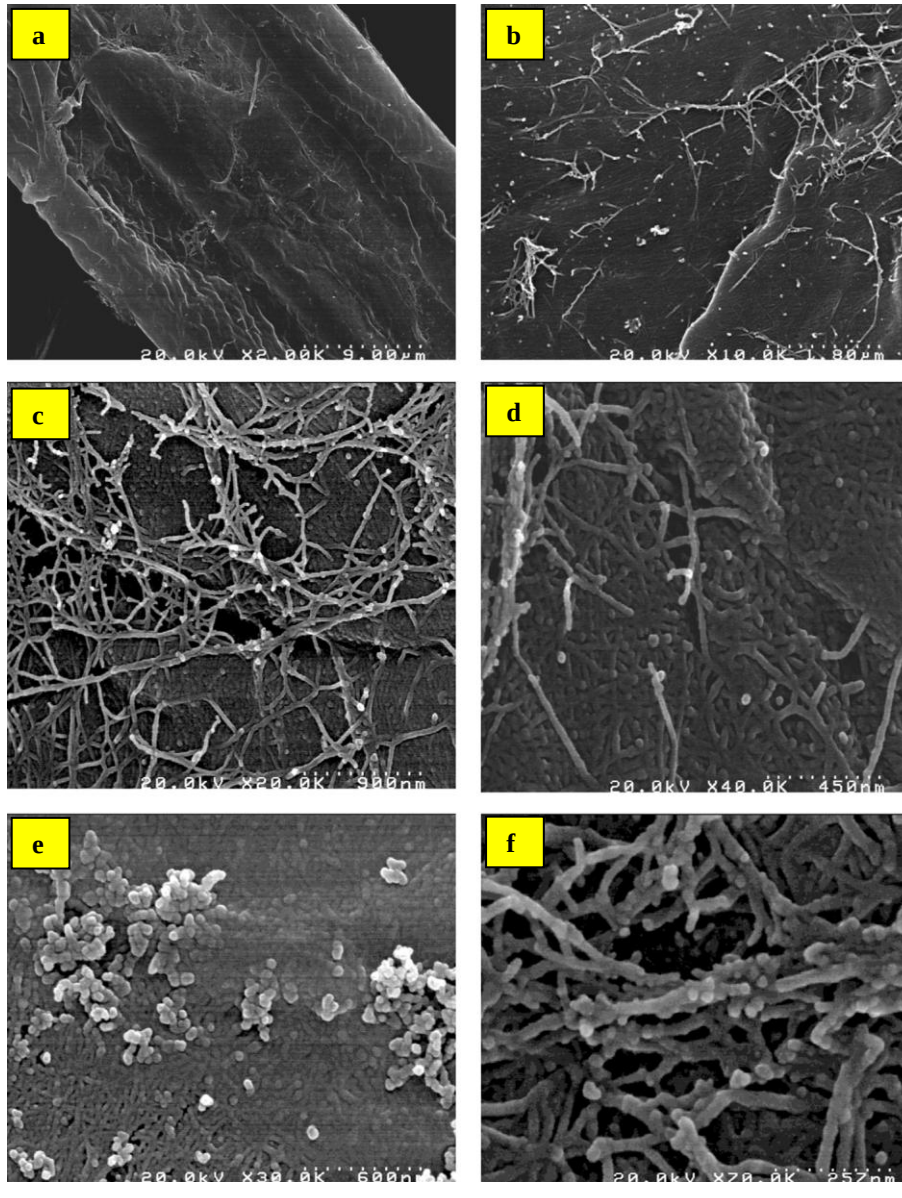

Figure 6. Pulp XY-S120. The pulp was cooked at 165 °C and birch xylan added later at 120 °C. Shearing was also carried out at 120 °C. Micrographs of fibre structure at increasing magnifications (a-f). Cellulose micro/macrofibrils appear embedded and coated with xylan. Exposed cellulose fibrils show encrustation with xylan aggregates (f). Larger agglomerates of xylan also found on the fibre surface (e).

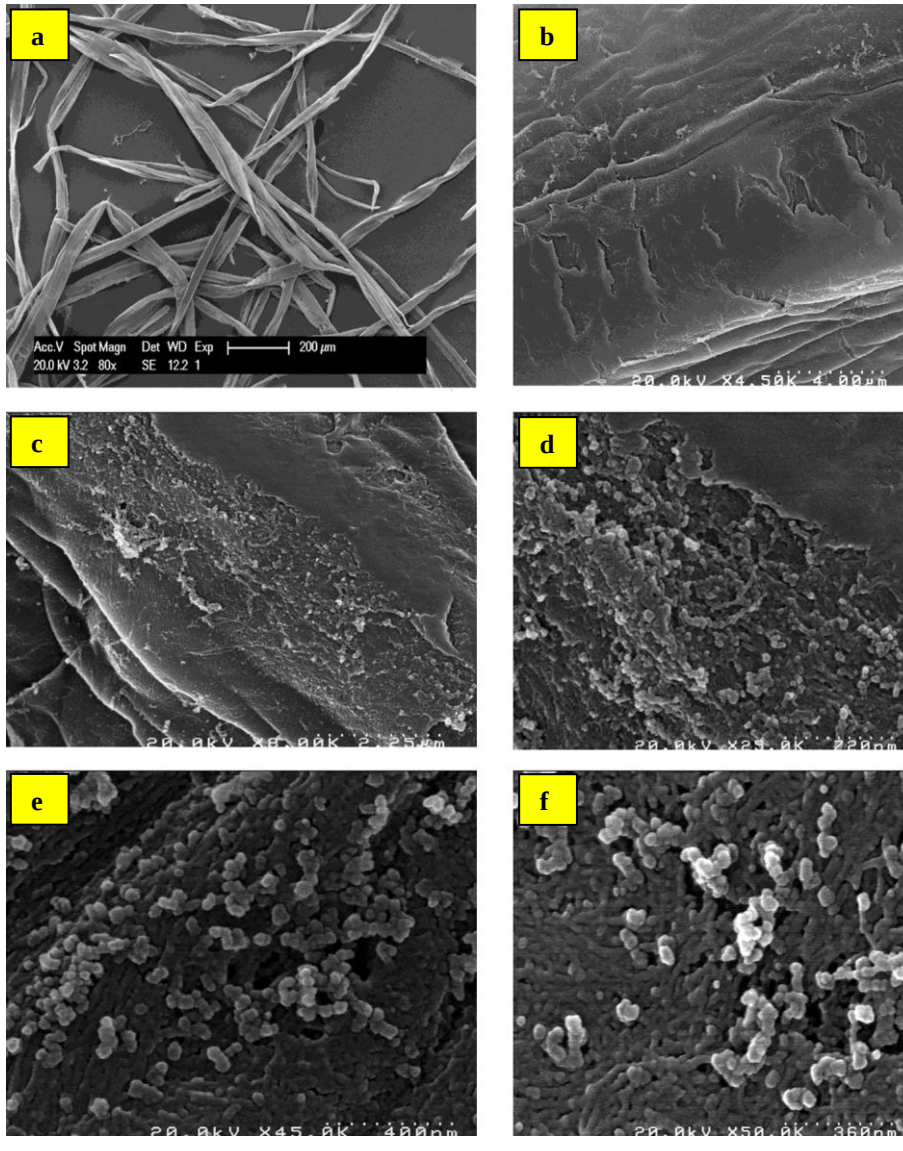

Figure 7. Pulp-XY-S80. The pulp was cooked at 165 °C with birch xylan added 50 mins during cooking. Shearing was carried out at 80 °C. Fibre structure at increasing magnification. Shearing opened the fibre structure (c-d). Cellulose micro/macrofibrils were encrusted with xylan (e, f). Xylan aggregates also remaining on the fibre surface (e, f).

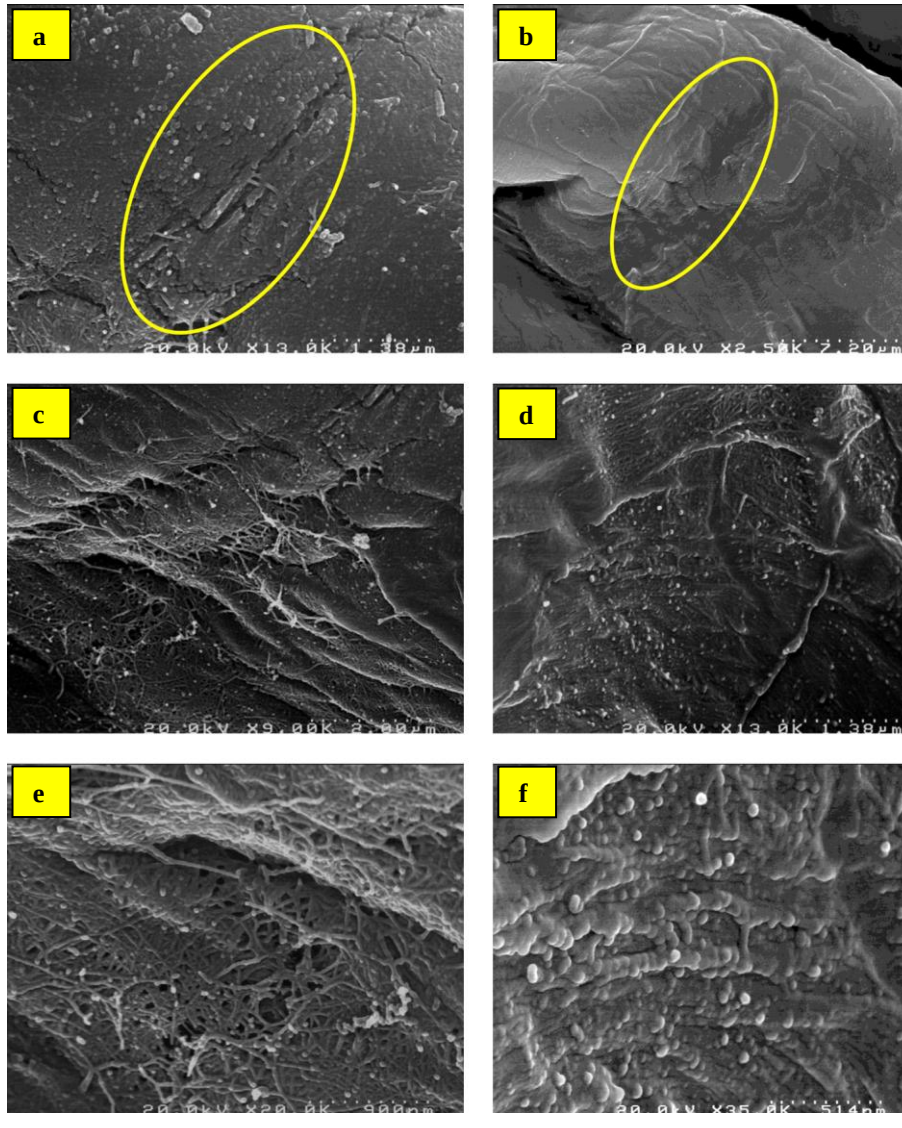

Figure 8. Typical examples of dislocations from pulp XY-165S. Dislocations from other pulps were similar. Some dislocations appear to be opened (a-c) during shearing revealing the cellulose micro/macrofibrillar network from the matrix materials. Xylan in the form of aggregates is present on the cellulose fibrillar structure. Other dislocations remain closed in the matrix. The left (a-c) and right columns (d-f) show the same dislocation at increasing magnifications.

APPENDIX 5. EVALUATION OF SURFACE COMPOSITION OF HIGH KAPPA PULPS EVALUATED BY FTIR MICROSCOPY

Anne-Mari Olsson, Lennart Salmén, Innventia, Stockholm

Objectives

The purpose of this work was to determine the surface composition of fibres from high kappa pulps with and without sorbed xylan. The goal was also to see if there was any difference between fibres subjected to shearing during pulping as opposed to those that had not been sheared.

Background

It is well known that xylan added in kraft cooking is deposited on the surfaces of the fibres. However, so far little is known with regard to the overall composition on the surface as well as to the form of the added xylan depositions. Imaging FTIR-microscopy offers here a possibility for investigating the spatial localisation of different components on the surfaces of fibres. Previous studies using surface imaging FTIR-microscopy have for unbleached pulp fibres indicated a somewhat uneven xylan distribution although that differences between different levels of addition was difficult to see (Daniel et al. 2010).

MATERIAL

Single fibres were prepared from kappa 80 pulps with and without xylan added, sheared at different temperatures compared to non-sheared. Five different pulps were examined;

- a reference pulp (no xylan added) not sheared
- a pulp with xylan added at 165 °C, not sheared
- a pulp with xylan added at 165 °C, sheared at 165 °C
- a pulp with xylan added at 165 °C, not sheared but compressed
- a pulp with xylan added at 165 °C, sheared at 80 °C

The fibres were dried and glued onto glass slides. Both earlywood and latewood fibres were prepared. 10 fibre samples were analysed for each pulp except for the pulps sheared at 80 °C where 5 fibres were tested.

Method

SURFACE MEASUREMENTS

The surface composition of the fibre surfaces were analysed by FTIR microscopy measurements using a Spectrum Spotlight 400 FTIR Imaging System (Perkin Elmer Inc, Shelton, CT, USA). FTIR ATR (Attenuated total reflection) was used. Over a length of 100 μm covering the total fibre width, spectra were acquired with a resolution of one spectrum from each subarea of 1.56 by 1.56 μm using an array MCT detector. A CCD camera was used to display the area of interest before it was irradiated with IR light. From these images the fibres were defined as earlywood or latewood fibres. In *Figure 1* the image before and after measurement on both a latewood and earlywood fibre is shown. The overall surface composition was achieved as an average spectrum calculated from all the sub-areas.

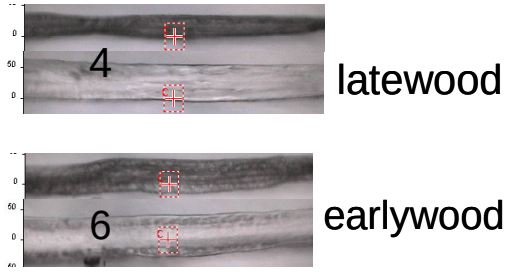

Figure 1. Image of latewood and earlywood fibres; before measurement (topimage) and after measurement (bottom image). The red square indicates the area of measurement.

EVALUATION

The IR Spectra were processed by the software Spotlight 1.5.1, HyperView 2.0 and Spectrum 6.2.0 (Perkin Elmer Inc., Shelton, CT, USA). The spectra were corrected by applying an atmospheric correction function to minimize the effects of CO_2 and H_2O. An ATR correction compensated for the effect of the ATR crystal. The whole matrix of spectra was automatically baseline corrected in the Hyper View programme.

The average spectra were analysed with multivariate analysis using SIMCA-P+ 11 (Umetrics). From a PCA analysis the principal components that separate the different pulps were identified in score plots, and in loading plots significant wavenumbers for this separation is shown.

Results

MULTIVARIATE ANALYSIS

In score plots grouping of samples can be identified. In *Figure 2 a* comparison is made between the present investigated high kappa 80 pulps and previously studied oxygen bleached kappa 30 pulps. The score plot for the principal components 1 and 2 shows a separation of fibres with expected high lignin content from fibres with low lignin content. This shows that differences in the composition of surface areas can be detected with the ATR-FTIR technique.

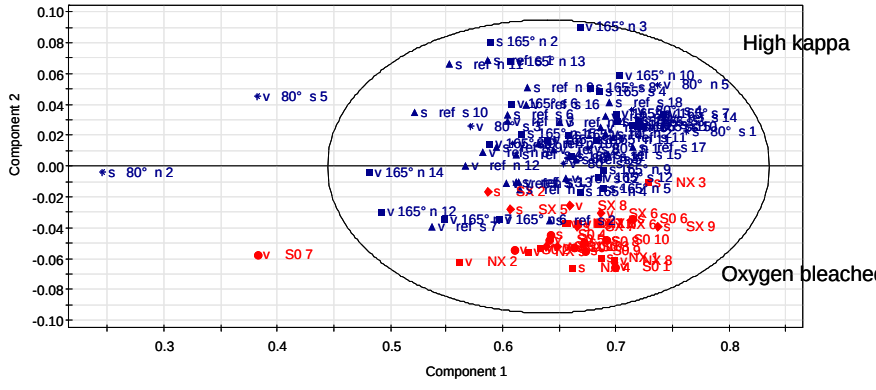

Figure 2. Score plot of principal components 1 and 2. Red marks correspond to oxygen bleached and blue marks correspond to the high kappa pulps.

For every principal component there is a loading plot showing the significant spectrum for this component. In *Figure 3* the loading plot for principal component 2 is shown together with one of the average spectra from a fibre. Higher lignin content pulps (kappa 80 pulps) appear as positive in the principal component 2, *Figure 2*. This means that in the loading plot positive values correlate with the high lignin content pulps. It is evident that in the loading plot of component 2 in *Figure 3* the high peaks at both 1500 cm^{-1} and 1600 cm^{-1} correspond to lignin. Thus it is the differences in lignin content on the surface that is responsible for the separation of the two different kind of pulps.

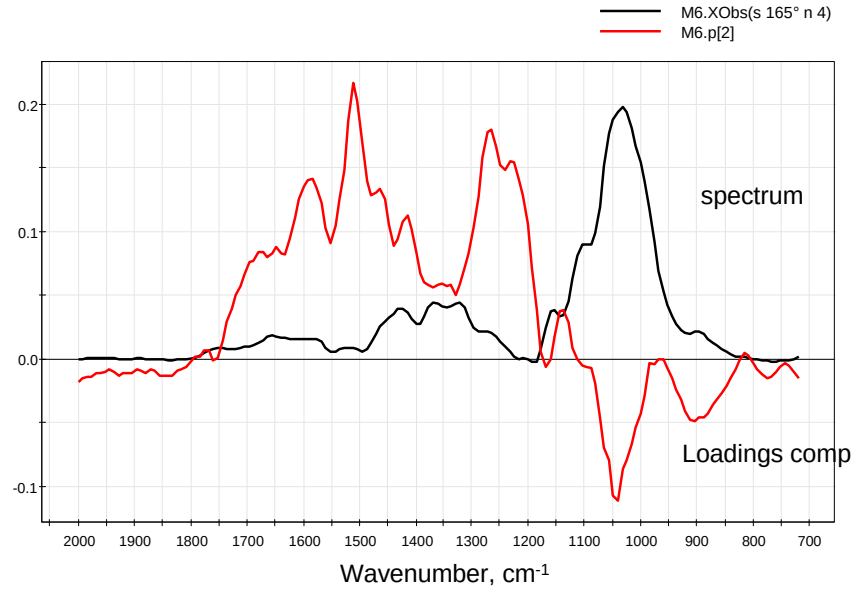

Figure 3. Loading plot for principal component 2 (red) and a spectrum for a high kappa 80 fibre (blue).

In the multivariate analysis of the high kappa fibres alone the differences between the samples are much smaller. The score plot in *Figure 4* shows that for component 2 all the fibres treated at 80 °C fall below zero. However some of the other fibres also have a negative value at component 2. No separate groups in the other components could be found either. Thus no separation between sheared and non- sheared, between earlywood and latewood or between xylan added or not added could be found.

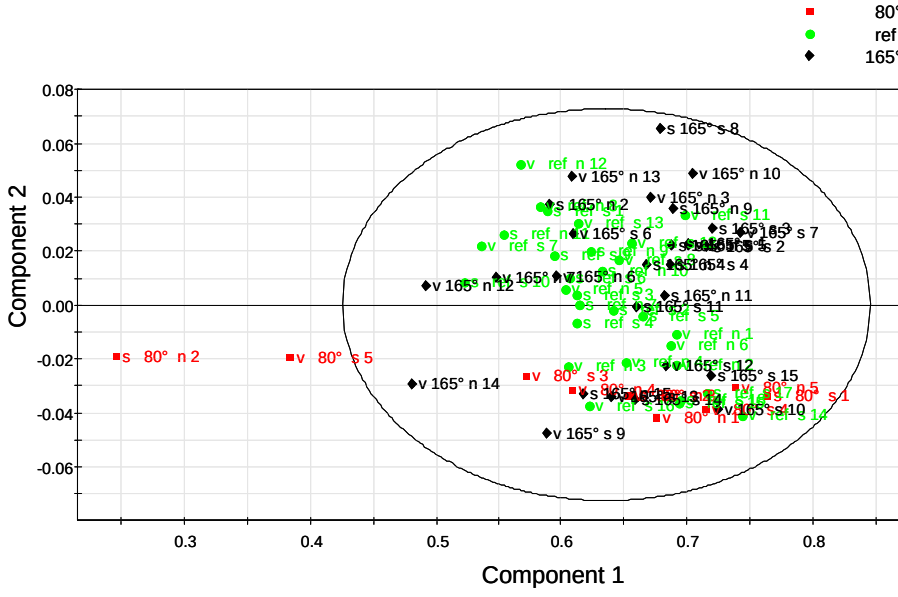

Figure 4. Score plot for components 1 and 2 for the high kappa fibres. Green marks correspond to the reference, black marks to fibres with xylan added at 165 °C and red marks to those with xylan added but treated mechanically at 80 °C.

The loading plot for component 2 is shown in *Figure 5*. The scores of component 2 were negative for fibres treated at 80 °C, *Figure 4*. This means that negative peaks in the loading plot is typical for these fibres, as for example the wavenumbers around 1600 cm^{-1} which corresponds to lignin. For xylan peaks 1730 cm^{-1} and 1460cm^{-1} in the loading plot shows positive values, thus these fibres have lower content of xylan. This higher content of lignin and lower content of xylan is however also true for some of the other pulp fibres treated at different conditions.

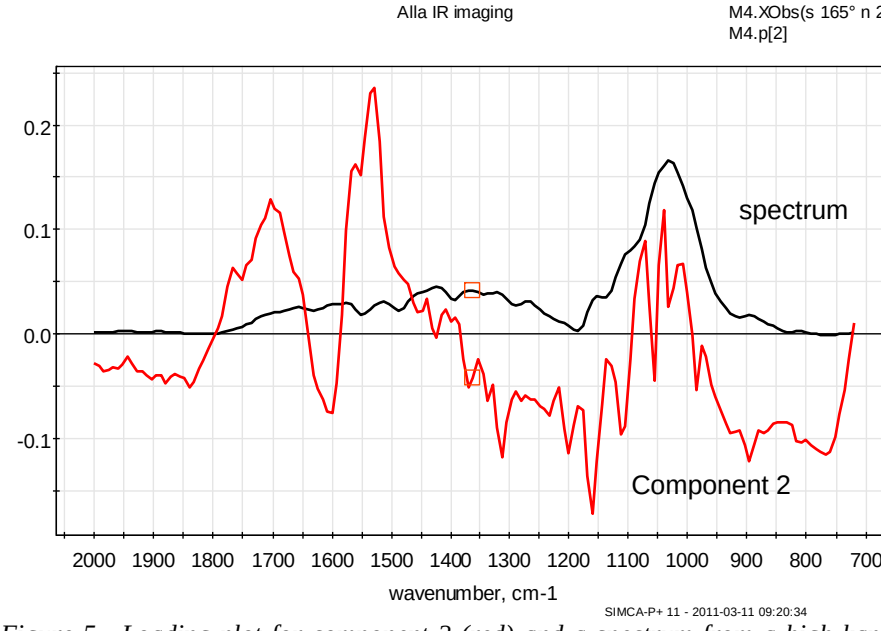

Figure 5. Loading plot for component 2 (red) and a spectrum from a high kappa fibre (black).

INTENSITY MAPS

Intensity maps for different wavenumbers can be made to visualise the distribution of components. *Figure 6* shows distribution maps for lignin using the area of 1590-1610 cm^{-1} divided by the area of the whole spectrum (800-1800 cm^{-1}). In comparison to the 165 fibres and the reference fibres the 80 fibres seem to display more lignin on the surface, in accordance with the score and loading plots of *Figures 4* and *5*.

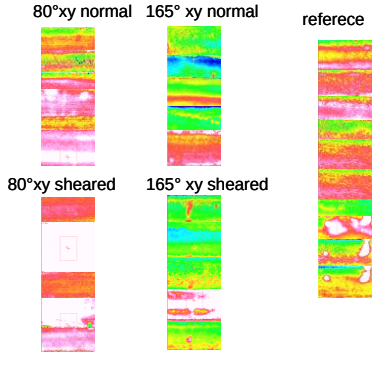

Figure 4. Distribution image of lignin (1590-1610/1800-800 cm^{-1}) for some of the fibres of the kappa 80 pulps.

Conclusions

The method of analysing the surface composition with ATR-FTIR and multivariate data analysis can detect large differences in surface composition, i.e. of the type of difference between bleached and unbleached fibres. However when differences in composition are small, as for the different high kappa pulps, the method is not able to make any discrimination, when using a reasonable amount of fibre measurements.

APPENDIX 6. HCL - METHOD USED ON SOFTWOOD KRAFT PULPS IN XYLAN EXPERIMENTS WITH HIGH KAPPA PULPS

Paul Ander, SLU, Uppsala

Spruce kraft pulps were cooked to rather similar kappa numbers (75-83, Table 1 Experimental) at temperatures 120, 165 and 80 °C, with or without added birch xylan or shearing and tested with the HCl-method (Ander et al 2008; Daniel et al. 2010; Heinemann and Ander 2011). The purpose was to find out if xylan addition could protect the fibres against shearing producing deformations and dislocations. The results are shown in Table 1 and in Figures 1 and 2.

Table 1. HCl-sensitivity of spruce kraft pulps with or without addition of birch xylan, or mechanical treatment (shearing). Ref-N was cooked at 165 ºC.

Kappa pulps	**Temperature °C at Xylan addition / during Shear**	**LWFL (mm) L0 H2O**	**LWFL (mm) L HCl**	**Clevage / fibre**
Ref-N	--/--	2.137/2.317 ***2.277***	1.859/1.866 ***1.863***	**0.195**
XY-N120	120/--	2.256/2.284 ***2.270***	1.874/1.924 ***1.899***	**0.195**
XY-N165	165/--	2.217/2.383 ***2.300***	1.923/1.832 ***1.878***	**0.225**
Ref-S	--/120	2.291/2.281 ***2.286***	1.092/1.335 ***1.214***	**0.883**
XY-S120	120/120	2.280/2.385 ***2.333***	1.278/1.143 ***1.211***	**0.927**
XY-S165	165/120	2.305/2.346 ***2.326***	1.230/1.405 ***1.318***	**0.765**
XY-S80	165/80	2.374/2.132 ***2.253***	1.331/1.414 ***1.373***	**0.641**

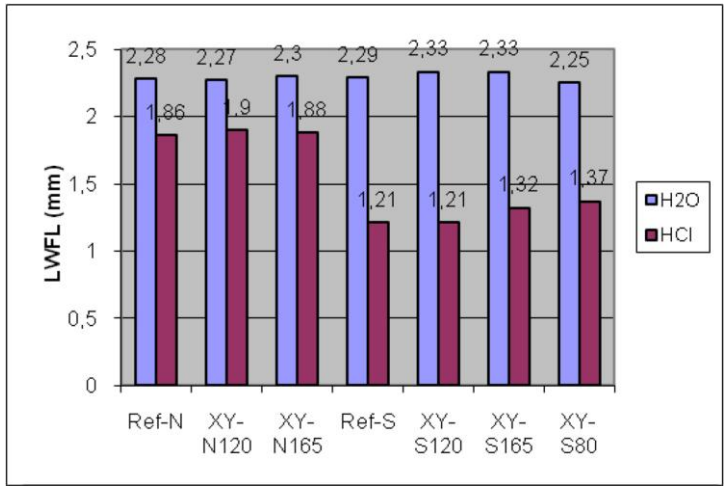

Figure 1. Fibre lengths of spruce kraft pulp fibres, with (10 g/L birch) and without added xylan at 120 ºC and 165 ºC, with and without shearing and cooked to kappa numbers 75-83. See also Table 1 above and Table 1 & 2 in Experimental part.

Results

In general, as shown in Figure 1, the pulp fibre lengths were very similar (2.25-2.33 mm) after cooking under the different conditions such as added xylan, different temperatures and shearing. However, after HCl treatment certain differences appeared. Pulp fibres without shearing (Ref-N; XY-N120 and XY-N165) had similar and shorter fibre lengths 1.86-1.90 mm, while with shearing (Ref-S, XY-S120 and XY-S165), the fibre lengths were only 1.21-1.32 mm. Thus at 120 and 165 ºC and with shearing, there was a clear decrease in length weighted fibre length, which did not increase by birch xylan addition. With shearing and HCl treatment, the XY-S80 pulps were slightly longer than the XY-S120 and XY-S165 pulps (1.37 mm compared with 1.21 and 1.32 mm).

Figure 2 shows that three normal pulps made at 120 and 165 ºC had cleavage per fibre: **0.195-0.225** with the strongest cleavage 0.225 for the pulp XY-N165 with the lowest kappa 77.1 (*cf* Experimental Table 1). Three sheared pulps had cleavage per fibre **0.765-0.927**, strongly indicating that shearing gives acid sensitivity, and that birch ylan could not protect against attack by the acid. The relationship between cleavage and kappa number was not so clear for the sheared pulps. The low temperature 80 ºC gave cleavage per fibre **0.641** for sheared pulps. The lower cleavage value 0.641 as compared with 0.765-0.927 at the higher temperatures is most probably due to the

lower temperature leaving more lignin in or on the fibres. The pulps sheared at 80 ºC were straighter and also had the lowest amount of kinks (Figures 3 & 4, Ch 3.4, page 11 and had good strength properties (Figures 6 & 7, Ch 3.4, pages 13, 14).

Due to the higher lignin content, the kappa pulps used here were less acid sensitive than the oxygen bleached pulps investigated in another report in this series (Daniel et al. 2011).

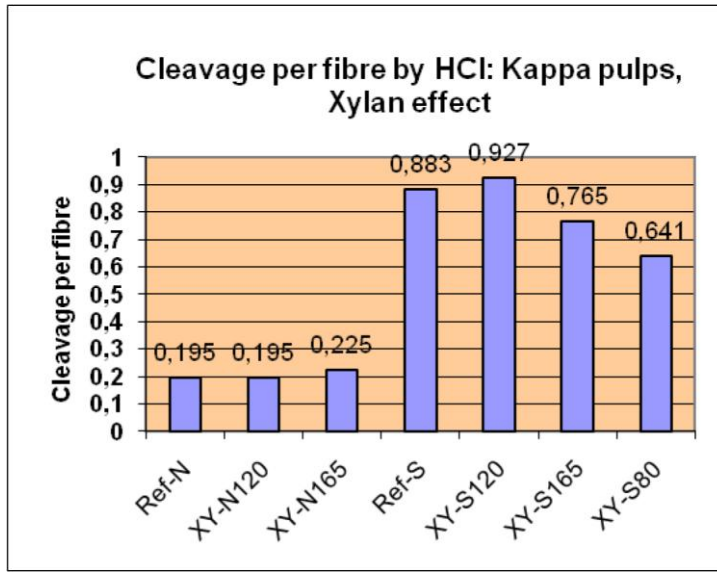

Figure 2. Cleavage per fibre for spruce kraft pulp fibres, with and without xylan added at 120 ºC and 165 ºC, with and without shearing and cooked to kappa numbers 75-83. See also Table 1 above and Table 1 & 2 in Experimental part.

Conclusions

Mechanical treatment (shearing) gives acid sensitivity and significantly shorter fibres and more fibre cleavage than normal fibres after testing with the HCl method. This indicates that xylan does not protect against attack by the acid. Pulps sheared at a lower temperature 80 ºC gives less fibre cleavage than fibres mechanically treated at 120 ºC and 165 ºC. This is reflected by less kinks and good tear-tensile relationship for pulps mechanically treated at 80 ºC.

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Collaborative Research on the Ultrastructure of Wood Fibres (CRUW)

CRUW represents a collaborative research program between the Swedish Forest Industries Eka Chemicals, 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"*.